UPPSALA
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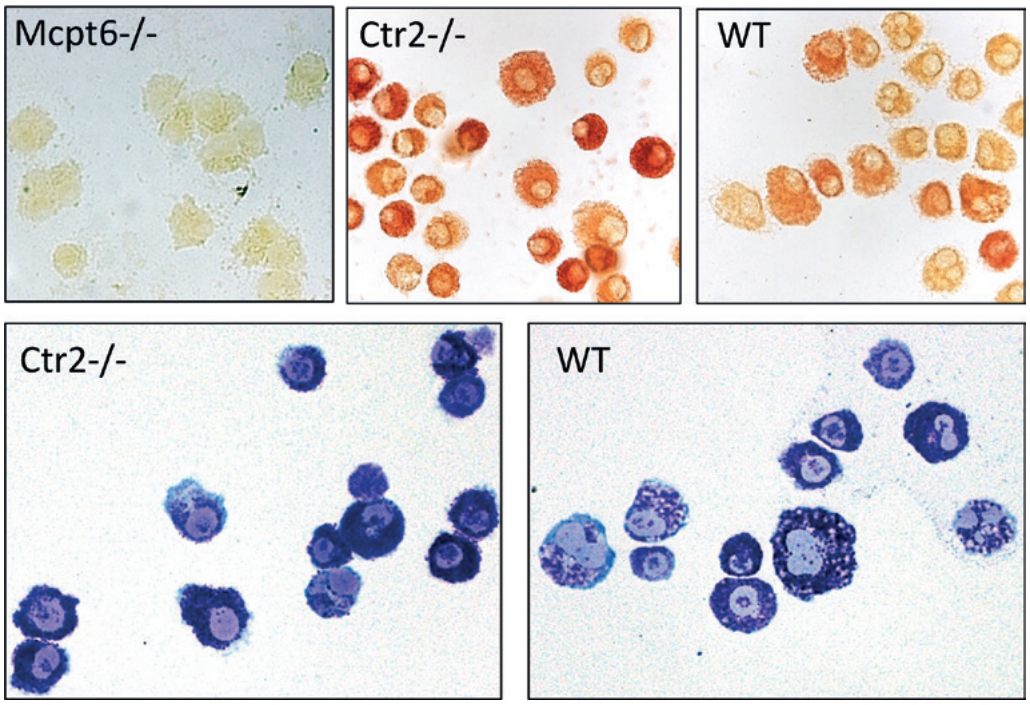
Department of
Medical Biochemistry
and Microbiology

IMBIM
ANNUAL REPORT
2015



DEPARTMENT OF

MEDICAL BIOCHEMISTRY

AND MICROBIOLOGY

ANNUAL REPORT

2015

Pictures taken by Helena Öhrvik

The role of copper in mast cell granule homeostasis

Ctr2 is a protein involved in cellular transport of copper. Upper panels: staining for tryptase, a mast cell granule protease, in tryptase (Mcpt6)-deficient (negative control), Ctr2-/- and wild type (WT) mast cells. Note that the absence of Ctr2 causes upregulated expression of tryptase. Lower panels: staining of Ctr2-/- and WT mast cells with toluidine blue, a proteoglycan-binding dye. Note increased toluidine blue staining of Ctr2-/- mast cells, indicating increased proteoglycan content of granules.

Öhrvik, H., Logeman, B., Noguchi, G., Eriksson, I., Kjellén, L., Thiele, D.J., Pejler, G. (2015) Ctr2 regulates mast cell maturation by affecting the storage and expression of tryptase and proteoglycans. *J. Immunol*. 195, 3654-364.

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PREFACE

Another year has passed with both small and large successes for the laboratory. The Department has a stable staff consisting of some 150 people. Including project workers, post doc etc we are around 250 persons that spend our daily work hours at the Department. Fortunately the granting situation for the IMBIM researchers improved considerably during 2015. Many of the small and medium sized research groups received grants that will help them continue to excel during the coming years. In addition Dan Andersson, Leif Andersson, Per Jemth, and a couple of scientists at ICM, received a hefty 47 milj kr grant from "Knut och Alice Wallenbergs Stiftelse" to support their research on the evolution of new genes and proteins. We also congratulate Dan Andersson who was elected a member of "The Royal Swedish Academy of Sciences", class for medical sciences. Further, we note that Leif Andersson received the 2015 Nilsson-Ehle medal from the Royal Swedish Academy of Forestry and Agriculture with the motivation "for outstanding research in molecular genetics with applications in animal genetics". Also we congratulate Kerstin Lindblad-Toh who was elected a member of the Swedish Governments Science Advisory Board.

It was also with great pleasure that we saw that the BSL3 laboratory for work with pathogenic organisms finally was delivered to BMC. Thanks to economic support from the faculty of medicine and pharmacy we could custom design a BSL3 laboratory in the form of a container that was assembled in France and delivered to us in early September. With some anxiety the container was finally lifted on top of the roof next to corridor A9:3 on September 11. After a period of testing everything appear to be functioning and we hope that the container should be in full operation during spring 2016. The media attention, in terms of press releases and newspaper articles that the Zoonotic laboratory has received during 2015 has been colossal. Much attention has been given to the building of the new BSL3 laboratory and the research activities of the zoonoses group. Their reports on highly pathogenic microorganisms carried by city rats, and campylobacter in ducks in Svandammen, are some of the well-cited examples. This type of media attention is most valuable to increase the public understanding of research and to improve the attitude towards science in general.

While writing this section we were all saddened by the message that our long time coworker Barbro Lowisin has passed away after a long time struggle with cancer. Barbro was a remarkable woman that we all miss and never will forget.

Scientific Highlights:
Ongoing research projects at IMBIM are summarized later in this annual report. Below I have picked out a few examples of studies that illustrate the high quality and wide range of research carried out at our Department.

Jenny Hallgren Martinssons group has, as the first group in the world, identified mast cell progenitors in human blood. Individuals with a reduced lung function had a higher frequency of blood mast cell progenitors than individuals with a normal lung function. These newly identified blood mast cell progenitor population provides a new therapeutic target for diseases were increases in tissue mast cells aggravate the disease (Dahlin et al., Blood).

Darwin's finches and the evolution of their beaks is an iconic model for evolutionary biology. In a collaborative effort with the Princeton University Leif Anderssons group sequenced the entire genome of 120 birds representing all species of Darwin's finches. The study demonstrated that genetic variation at the ALX1 locus, encoding a transcription factor with a pivotal role during craniofacial development, has a major effect on variation in beak shape. Thus, this locus has contributed to diversification of beak morphology among Darwin's finches which has lead to an expansion of food utilization (Lamichhaney et al., Nature 518, 371-275).

Anna-Karin Olssons group has found new clues to how cancer causes organ failure. They show that Neutrophil Extracellular Traps (NETs) acts to impair vascular function and to promote systemic inflammation. Removal of these tumor-induced NETs restored the vascular function in distant organs to levels seen in healthy mice and also suppressed the inflammation, demonstrating that the condition is reversible (Cedervall et al., Cancer Res. 13, 2653-2662).

Malignancy in cancer depends on the ability of tumors to disseminate cells from primary sites of growth to other organs. In Aris Moustakas research group they study the molecular nature of cancer cell invasiveness with a hope to contribute to the development of new treatment modalities of cancer. The research focuses on the TGFβ (transforming growth factor β) and bone morphogenetic protein (BMP) families of signaling proteins. During 2015 Aris and his coworkers published a new study on regulation of BMP signaling by the tumor suppressor kinase LKB1, a mechanism that has relevance to developmental and cancer biology (Raja et al., Oncotarget).

Teaching:
Teaching of undergraduate and graduate students is a primary undertaking for IMBIM. During 2015 a total of nine students received their doctoral degree and two student a licentiate degree. The teachers at IMBIM does an excellent job something that is best illustrated by the fact that Linus Sandegren in 2015 received the "Limbiska priset" for the third time. The prize was awarded to Linus for his dedicated teaching in bacteriology. IMBIM congratulates Linus for this unheard-of achievement and expect to be able to use copy paste in the preface to the IMBIM book in 2017.

The beginning of something new:
During the coming year(s) the administrative and technical staff at IMBIM will have to set up mechanisms to handle an expanding Department. Thus, the Ludwig Institute for Cancer Research will merge with IMBIM in August 2017. This will create a major change in the research environment at IMBIM. The administrative and technical staff at IMBIM is of superb quality and does a fantastic job to support the research teams working at IMBIM. Without these effective support functions, the output in terms of research and teaching would rapidly deteriorate and the life for me as the head of the Department would become excruciating.

Finally, I would like to thank all of those who left IMBIM during the past year for your participation in building up this fantastic scientific environment. I wish you all the best for the future and hope that you only have fun memories from your time at IMBIM. At the same time I welcome all new students/scientists etc. to this exciting environment and hope that you will actively take part in the future development of this Department.

Uppsala March 2016

Göran Akusjärvi
Head of the Department

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Birgitta Tomkinson	teacher representative, deputy
Matthew Webster	teacher representative, deputy
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Jessica Pettersson	repr for technical/administrative personnel, deputy (from June)
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SCIENTIFIC PRESENTATIONS

COMPARATIVE GENETICS AND GENOMICS

Leif Andersson, Kerstin Lindblad-Toh, Matthew Webster, Jennifer Meadows, Carl-Johan Rubin, Patric Jern, Gerli Rosengren-Pielberg Pernilla Bjerling, Manfred Grabherr, Erik Axelsson

Comparative genomics is of crucial importance to unravel gene function and regulation. We utilise specific human cohorts, domestic animals, natural populations and model organisms to study genotype-phenotype relationships, gene regulation, chromatin organization and epigenetics.

Functional genomics in domestic animals. Domestic animals constitute a unique resource of genetic diversity due to their long history of selective breeding. We use a variety of domestic animals, including chicken, dogs, horse, pig, and honeybee, for in-depth studies of genes underlying both monogenic and multifactorial traits, as well as diseases of human relevance. We use both pedigree-based analysis and genome-wide association studies combined with high-throughput genomics and functional studies to achieve these goals.

Genetic and genomic investigation of human cohorts. We have leveraged our knowledge of population genetics to delve into the genetics underpinning heritable disease with relevance to the health of Sweden. The specific population structure and bottlenecks of Sweden mean that smaller scale studies are likely to be able to reveal rare mutations or large effect on disease and at the same time allow for efficient burden analyses to be performed. Our current focus examines multiple diseases including the continuum of autoinflammatory - autoimmune diseases.

Genome evolution. We use comparative analysis to identify functional elements in the human genome and other organisms to study the evolution of these elements and other genomic sequences. For example, comparison across 29 mammals identifies 3.6 million elements, 60% of which we can suggest a function. Evolutionary analysis also identifies lineage-specific selection and innovation of both protein coding and regulatory elements. Analysis of genetic variation within species also enables us to identify regions targeted by selection, and to understand the mechanisms and evolution of recombination. Here we also employ whole genome sequencing in natural populations to understand the speciation process, population history, and the genetic basis of adaption, using a variety of species including birds (Darwin's finch, ruff), bees, fish (herring), and canids (wolves, coyote).

Chromatin organization and epigenetics. We investigate how transcription factors and silencer proteins influence the epigenome, both by changes in chromatin modifications and organisation of the chromatin within the cell nucleus. For example, we are investigating the newly identified human transcription factor ZBED6, its mechanism of action and its possible role in human diseases. In addition, we are using the *S. pombe* model system to get a deeper understanding of the molecular mechanism behind chromatin dynamics.

Retrovirus-host evolution. Retroviruses have colonized vertebrate hosts for millions of years, leaving traces in their genetic makeup as endogenous retroviruses (ERVs). This genomic ERV record provides a unique perspective on the long-term coevolution of retroviruses and their hosts. We use mainly bioinformatics to identify ERVs in genomic

sequences of domestic animals and other vertebrate hosts to better understand retrovirus evolution and the effects of ERVs on host genome function and evolution.

Computational biology. Computational biology plays a key role for all of the above research areas. To support novel research questions and new data types we develop new algorithms and analysis methodologies, and we make the software publicly available for researchers around the world. These include tools to align entire genomes to each other to determine their relationships on a highly localized level, to *de-novo* assemble transcripts from RNA-Seq data, and to identify signals of selection within populations on a genome-wide scale.

FUNCTIONAL GENOMICS IN DOMESTIC ANIMALS AND NATURAL POPULATIONS

Leif Andersson

The overall objective of the group is to use genetics and genomics to advance our understanding of the genetic mechanisms underlying phenotypic variation. We are primarily using domestic animals models because domestication and animal breeding have caused major changes in many phenotypic traits. This gives an excellent opportunity to unravel the genes underlying phenotypic variation. The research includes genetic studies of both monogenic traits, like inherited disorders and coat colour, and multifactorial traits, like muscle development, fat deposition, autoimmune disorders and general disease resistance. The research projects involve genetic studies in chicken, horses, pigs, rabbit, Darwin's finches and Atlantic herring. The ultimate goal of the research is to identify the genes and mutations affecting a certain trait and thereafter to study the mechanism as well as the biological and medical significance of the genes and regulatory elements affected by the observed mutations. An important tool in the current research is to use whole genome resequencing to detect signatures of selection.

Members of the group during 2015
Leif Andersson, professor, group leader
Sam Barsh, project assistant (until Sep)
Susanne Bornelöv, researcher (until Sep)
Xiaofang Cao, research engineer
Chungang Feng, post-doc
Fan Han, PhD student
Anna Hjälm Golovko, researcher (until March)
Ulrika Gunnarsson, researcher
Ulla Gustafson, technician (associated with Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences)
Freyja Imsland, PhD student
Susanne Kerje, researcher
Sangeet Lamichhaney, PhD student
Mårten Larsson, first research engineer
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Jessica Pettersson, research engineer
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Elisabeth Sundström, researcher
Ola Wallerman, post-doc (associated with Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences)
Chao Wang, post doc
Shady Younis, PhD student

International exchange during 2015

Dr. Miguel Carneiro, University of Porto, Portugal (visiting researcher during one months)
Ms. Angela Fuentos Pardo, Department of Biology, Dalhousie University, Halifax, Canada (visiting student during two months)
Dr. Miriam Friedman-Einat, Agricultural Research Organization, Volcani Center, Israel (visiting researcher during 6 months)

Publications 2013 to 2015

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Freyja Imsland: Monogenic phenotypic traits associated with structural variants in chicken and horse, September 25

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ZBED6 – A NOVEL MAMMALIAN TRANSCRIPTION FACTOR ORIGINATING FROM A DNA TRANSPOSON

Xiaofang Cao, Mårten Larsson, Rakan Naboulsi, Jessica Pettersson, Daniel Steinhauf, Elisabeth Sundström, Ola Wallerman, Shady Younis, Leif Andersson

A single nucleotide substitution in intron 3 of *IGF2* in pigs abrogates a binding site for a repressor and leads to a three-fold upregulation of *IGF2* in skeletal muscle. The mutation has major effects on muscle growth, size of the heart and fat deposition. We have identified this repressor and found out that the protein, named ZBED6, is a previously unknown protein, specific for placental mammals and derived from a domesticated DNA transposon. ChIP-sequencing using mouse C2C12 myoblast cells identified about 2,500 ZBED6 binding sites in the genome and the deduced consensus motif gave a perfect match with the established binding site in *IGF2*. We have also shown that ZBED6 contributes to transcriptional regulation in pancreatic islet cells as well as human colorectal cancer cells. The phenotypic effects in mutant pigs, the extreme sequence conservation, its nucleolar localization, the broad tissue distribution and the many target genes with essential biological functions suggest that ZBED6 is an important transcription factor in placental mammals affecting development, cell proliferation and growth. A broad research program involving functional assays has been initiated to study the biological and medical significance of ZBED6. This includes the generation of *Zbed6* knock-out mice which are undergoing phenotypic characterization and transcriptome analysis.

The project is carried out in collaboration with researchers at Uppsala University, Swedish University of Agricultural Sciences, Karolinska Institutet and Texas A&M University.

DETECTING SIGNATURES OF SELECTION USING WHOLE GENOME RESEQUENCING

Sam Barsh, Susanne Bornelöv, Miguel Carneiro, Chungang Feng, Ulla Gustafson, Fan Han, Sangeet Lamichhaney, Jessica Pettersson, Mats Pettersson, Nima Rafati, Chao Wang, Leif Andersson

Next generation sequencing offers the possibility to carry out whole genome resequencing of large genomes. We have pioneered the use of pooled samples to detect signatures of selection when comparing different populations that have been exposed to different selection pressures. Our first application of this approach involved whole genome resequencing of eight different populations of domestic chicken (four broiler populations and four layer populations), a pool of red junglefowl birds and the single red junglefowl female that was previously used to produce a draft genome assembly for the chicken. The project resulted in the detection of more than 7 million single nucleotide polymorphism and 38 loci with strong signatures of selection.

More recently we applied the approach to a comprehensive comparison of wild and domestic rabbits with the aim to reveal the genetic basis for domestication. The study is an advance in understanding animal domestication. We demonstrated that (i) rabbit domestication has a highly polygenic basis as signatures of selection was associated with a large number of genes, (ii) that changes in non-coding sequences have been more prominent than changes in coding sequences, (iii) that there were no example that gene inactivation played a prominent role, and (iv) that domestication were associated with changes in allele frequencies at many loci rather than fixed changes at a few major "domestication genes" with large effects.

Whole genome sequencing of individual birds have been applied to study the evolution of the Darwin's finches. We have sequenced 120 birds representing all species of Darwin's finches including the Cocos island finch, and two closely related tanager species. This has allowed us to (i) revise the phylogeny of this radiation, (ii) to demonstrate extensive interspecies gene flow during the evolution of these birds and (iii) to identify a gene controlling variation in beak shape within and between species of the Darwin's finches.

Another major research program concerns the Atlantic herring. The Atlantic herring is one of the most abundant vertebrates on earth and constitutes an enormous biomass in the North Atlantic and associated waters including the Baltic Sea. We have now made a genome assembly of the herring genome and have carried out whole genome resequencing of 20 population samples from the Baltic Sea, Kattegat, Skagerrak, North Sea, Atlantic Ocean and Pacific Ocean, the latter sample represents the closely related Pacific herring. This study has revealed a large number of genes related to the adaptation to the Baltic Sea as well as to variation in spawning time (spring vs. autumn spawners). The Atlantic herring has turned out to be an outstanding model to study the genetic basis for adaptation due to the huge populations size and the lack of genetic differentiation at selectively neutral loci. We demonstrate that both sequence polymorphisms in coding and non-coding sequences contribute to ecological adaptation in herring.

Some of the projects are carried out in collaboration with Drs. Carl-Johan Rubin, Matthew Webster and Kerstin Lindblad-Toh at IMBIM and with several external collaborators.

MOLECULAR COAT COLOUR GENETICS

Susanne Bornelöv, Freyja Imsland, Susanne Kerje, Jessica Pettersson, Doreen Schwochow, Ann Staiger, Elisabeth Sundström, Leif Andersson

Coat colour variation has been extensively used during the history of genetics to study how genes act and interact in shaping phenotypic variation. This is because the phenotypic read-out is so straightforward making it possible to establish high-resolution genotype-phenotype relationships as well illustrated by our track record in this field. A hallmark of domestic animals is extensive coat colour diversity. We have taken advantage of this and characterized a large number of mutations causing coat colour phenotypes in various domestic animals. At present, we are working with the following phenotypes: (i) Sex-linked barring in chicken, which is controlled by mutations in the *CDKN2A* tumour suppressor gene; (ii) the patterning phenotype in chicken; (iii) inhibition of gold in chicken; (iv) roan coat colour in horses, controlled by a regulatory mutation in the *KIT* gene; (v) dun coat colour in horses, dun is the wild-type colour in horses and is characterized by dilution of pigmentation, a dorsal black stripe and occasional zebra-like leg stripes. The ambition is to nail down the causal mutation(s) and explain the mechanism of action for the detected mutations. We have recently discovered that the non-dun colour in horses is caused by regulatory mutations affecting the expression of the TBX3 transcription factor in the hair follicle in growing hair (Nature Genetics in press). The dilution of pigment in hair from Dun horses is caused by asymmetric expression of TBX3 that excludes pigment cells from one side of the hair.

These projects are carried out together with numerous collaborators in Sweden and abroad.

GENETIC ANALYSIS OF THREE CHICKEN MODELS FOR AUTOIMMUNE DISORDERS IN HUMANS

Susanne Bornelöv, Miriam Friedman-Einat, Ulrika Gunnarsson, Ulla Gustafson, Susanne Kerje, Mårten Larsson, Rakan Naboulsi, Jessica Pettersson, Leif Andersson

We have initiated cross-breeding experiments and genome scans for three lines of chickens representing novel models for three autoimmune disorders in humans, Hashimoto's thyroiditis, systemic sclerosis and vitiligo. The Obese strain (OS) chickens develop a spontaneous autoimmune thyroiditis closely resembling Hashimoto's thyroiditis in human. The strain was established in the 1960's and has been widely used as an animal model to reveal various aspects of the disease. The University of California at Davis line 200 (UCD200) chickens develop an inherited syndrome with features very similar to human systemic sclerosis including fibrotic destruction of the skin and internal organs. Finally, the Smyth line (SL) represents an animal model for vitiligo in which 70-90% of the birds express a post-hatch autoimmune destruction of melanocytes leading to feather de-pigmentation at 6–14 weeks of age. Interestingly, the incidence of vitiligo is dramatically increased (from ~15% to ~85%) after immunization with a Herpes virus vaccine. Virus infections are generally believed to trigger autoimmune disorders in humans. The intercross pedigrees are used for genome scans with the ultimate goal of identifying genes underlying these autoimmune disorders. The work is carried out in collaboration with Dr. Olle Kämpe at Karloinska Institutet and Dr. Örjan Carlborg at SLU.

MOLECULAR CHARACTERIZATION OF THE MUTATION AND MECHANISM CAUSING GREYING WITH AGE IN HORSES

Anna Hjälm Golovko, Elisabeth Sundström, Leif Andersson

Grey is a dominant coat colour mutation that is common in horses and found in a variety of breeds including Arabian horses, Lipizzaner horses, Thoroughbreds, Swedish Warmblood and Icelandic horses. A grey horse is born coloured (e.g. bay, black or chestnut) but for each year it becomes gradually greyer and eventually all hair become completely white. A remarkable feature of this coat colour variant is that there is a very high incidence of melanomas in old grey horses. It has been estimated that ~80% of grey horses older than 15 years have melanomas whereas this is a very rare condition in horses with other colours. Thus, the identification of the *Grey* mutation provides an opportunity to generate new basic knowledge about tumour development of melanocytes. The causal mutation is a 4.5 kb duplication located in intron 6 of *Syntaxin17*. We also demonstrated that this is cis-acting regulatory mutation that upregulates both *Syntaxin17* and the neighbouring gene *NR4A3* encoding an orphan nuclear receptor. We are currently exploring the mechanism leading to premature greying and melanoma development. We have established a mouse knock-in mouse where the horse mutation (a 4.5 kb duplication) has been introduced at the orthologous position in the mouse genome. The work is carried out in collaboration with researchers at University of Natural Resources and Applied Life Sciences, Vienna.

GENETIC ANALYSIS OF DIVERGENT INTERCROSSES OF CHICKEN

Ulrika Gunnarsson, Susanne Kerje, Leif Andersson

We have in collaboration with Prof. Per Jensen (Linköping) and Prof. Paul Siegel (Blacksburg, USA) developed two unique resource pedigrees for genetic dissection of multifactorial traits. One of these pedigrees was generated by crossing a single Red junglefowl male with females from a line of White Leghorn selected for egg production. The populations differ markedly in growth, fertility (number of eggs and size of eggs), behaviour and body composition (fat vs. protein content). The other intercross involves two lines (High and Low) that have been divergently selected for growth for more than 40 generations. The selection response has been remarkable and the 8-week weight shows approximately a 9-fold difference between lines, about 1.8 kg for the H line in contrast to about 0.2 kg for the L line. The two lines show a dramatic difference in appetite (hyperphagia vs. anorexia) and the body composition has been altered with the H line developing obesity. The HxL intercross is a unique resource for understanding the genetic regulation of growth, appetite and obesity. Both pedigrees comprise almost 1,000 animals from three generations. This experimental design gives an excellent power in the genetic analysis. A broad collection of phenotypic data and DNA samples has been obtained from all animals. Our strategy is to combine genetic marker and gene expression analysis for gene discovery. The analysis of the Red Junglefowl intercross has localized more than 40 quantitative trait loci (QTL) controlling growth, body composition, fertility, egg weight, behaviour and bone density.

The project is carried out with collaborators at Uppsala university, Linköping university, Swedish University of Agricultural Sciences, INRA (France) and Virginia Polytechnic Institute (Blacksburg, USA).

GENETIC AND FUNCTIONAL CHARACTERISATION OF DOG DOMESTICATION

Erik Axelsson

The dog may have been the first animal to be domesticated and has since been an integral part of human culture. Up until recently our understanding of the genetic basis of traits separating the dog from its wild ancestor the wolf was limited. Our group has now completed a survey of genetic diversity in dog and wolf using light-coverage whole genome resequencing. This data has allowed us to shed new light on the molecular basis of early dog domestication by identifying and functionally characterising genomic regions that were selected for during the initial, pre-breed, phase of the domesticating process. Our results show that (1) dogs have adapted to cope with a starch-rich diet and (2) that selection during dog domestication affected mutations in nervous system development genes - changes that are likely to underlie several of the behavioural differences between dogs and wolves. We now plan to build on this work in three ways.

First, by screening both contemporary, and ancient, dog and wolf populations for mutations that alter the efficiency of starch digestion we aim at determining how variable this trait is among dog breeds, whether it is associated with metabolic disorders and when selection for this trait may have started.

Secondly, by testing if candidate mutations that affect nervous system development genes have led to functional differences between dog and wolf we aim at identifying mutations that underlie behavioural changes during dog domestication.

Finally, to further increase our understanding of the molecular basis of dog domestication we will extend our survey of genetic diversity in dog and wolf populations by additional whole genome resequencing.

Members of the group during 2015
Erik Axelsson, group leader

Project worker during 2015
Caroline Johansson, Initial characterisation of an adaptive duplication during dog domestication.

Publications 2013 to 2015

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EPIGENETICS AND NEW ANTIFUNGAL DRUGS

Pernilla Bjerling

The basic unit of chromatin is the nucleosome, consisting of a core of histone proteins that the DNA is wrapped around. The chromatin is constantly undergoing dynamic changes adjusting the transcriptome during development and as a response to environmental stimuli. We study both transient and stable, or epigenetic, changes. Epigenetic changes in the genome is believed contribute significantly to several diseases like cancer, diabetes type II and obesity. Still, very little is known about how to reverse disease-causing changes in the epigenome. By using fission yeast, *Schizosaccharomyces pombe* as a model system we can learn more about what determines the epigenome and how switches between different types of chromatin can occur.

We have also initiated a project with the long-term goal of developing new drugs against yeast, since severe systemic yeast infections is a growing problem in the health care. Several species of the pathogenic yeast *Candida* normally grow on the skin of humans and only people with a lowered immune response, for example immunosuppressed patient undergoing transplantation or AIDS patients, suffer from *Candida* infections. The drugs against *Candida* frequently give strong side effect and resistance to the drugs is increasing, so new drugs would be of great importance.

Members of the group during 2015
Pernilla Bjerling, group leader
Vladimir Maksimov, post doc
Daniel Steinhauf, PhD student

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FORMATION OF REPRESSIVE CHROMATIN

Vladimir Maksimov, Daniel Steinhauf

In *Schizosaccharomyces pombe* there are several regions where a special form of transcriptionally repressed chromatin, named heterochromatin, is formed. The formation of heterochromatin results in transcriptional silencing of reporter genes inserted into that region of the chromosome. The molecular characteristic of heterochromatin is conserved between fission yeast and human with low acetylation levels of the histones and methylation of lysine 9 at histone H3 (H3K9Me2/3). The methylation is made by histone methyltransferase, primarily SUV39H in humans, and Clr4 in *S. pombe*. The H3K9Me2/3 modification creates a binding site for chromodomain proteins of the HP1 subfamily. Moreover, the SNF2-HDAC repressor complex (SHREC) is crucial for heterochromatin formation in *S. pombe*. SHREC contains a nucleosome remodeller, Mit1, the histone deacetylase Clr3, the HP1 homologue Chp2, the uncharacterised Zn-finger containing protein Clr1 and Clr2. The function of the three last proteins in the SHREC complex remains to be elucidated. We have together with collaborators made strains that carry point mutations of critical, conserved amino acids in these three proteins. The effect on silencing in the strains with point mutations in the Clr2 protein has been characterised (Steinhauf et al 2014). Several of the Clr2 mutant strains display unstable silencing phenotypes indicating deficiencies in either establishment or maintenance of heterochromatin. We are now investigating whether these point mutations in Clr2, as well point mutations in Chp2 or Clr1, affects the integrity of the SHREC complex or the SHREC complex ability to bind to chromatin. We think that these will help to clarify the role of the SHREC complex in the establishment as well as the maintenance of heterochromatin. This will then advance our knowledge how switches between different epigenetic states can occur.

HiSTIDINE KINASES IN S. POMBE AND CANDIDA ALBICANS

Pernilla Bjerling, Vladimir Maksimov

Bacteria, plant and yeast have on their cell surface histidine kinases that act as environmental sensors. *S. pombe* has three histidine kinases, Mak1, Mak2 and Mak3, that are known to be important for the response to nitrogen starvation, which results in mating between cells with different mating-types, P or M, and subsequent sporulation. Our group has previously investigated the effect on gene regulation and chromatin changes during nitrogen starvation in *S. pombe*, and our findings are published in two papers (Alfredsson-Timmins et al JCS 2007 and Kristell et al GR 2011). We have now uncovered distinct functions for the three histidine kinases; Mak1 alone or Mak2 and Mak3 together are sufficient for the repression of the meiotic cycle when nutrients are available. Moreover, strains lacking histidine kinase genes are sensitive to various types of stress conditions in an auxotrophic strain background. Finally, the stress sensitivity of a *S. pombe* strain that lacks endogenous histidine kinases is complemented by the ectopic expression of the Chk1 histidine kinase from *C. albicans*. This finding opens up for the possibility to perform a drug screen with a biological readout to find molecules that inhibit the activity of the Chk1 histidine kinase. Inhibition of Chk1 is predicted to prevent the transition from yeast to hyphal growth and thereby infection of *C. albicans*.

EVOLUTIONARY BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

The Manfred G. Grabherr Group

Every corner of this world is populated by living beings. Recent advances in sequencing technology allow for a deeper glimpse into how these organisms function, where they are, what they do, and how they evolve. Of particular interest to our group is RNA sequencing, since it produces both the sequences that are transcribed, as well as the abundance at which they are expressed. We have thus developed a number of novel algorithms to assemble transcripts, characterize them, and quantify their expression.

In analyzing RNA-Seq data with our collaborators, we do not limit ourselves to particular organisms, but apply our methods to species as diverse as aspen and spruce trees and their associated microbial communities; cultured and environmental bacteria; humans in context of disease onset and progression; and a variety of birds and other reptiles. Since every new project is unique, we find the need for novel or improved bioinformatics software, which we subsequently develop to answer the fundamental biological questions behind each experiment.

Since 2013, the group is host to the BILS Genome Annotation Centre, funded by the Bioinformatics Infrastructure for Life Sciences (BILS). In 2015, the group was expanded into the BILS Uppsala Genome Assembly and Annotation Centre. In addition, we organize a number of bioinformatics workshops and courses open to all levels of academia and all Swedish research institutions, which proved very popular and are highly oversubsrcibed.

Members of the group during 2015
Manfred G. Grabherr, group leader
Neda Zamani, researcher
Görel Sundström, researcher
Thomas Källman, bioinformatician
Henrik Lantz, bioinformatician
Marc P. Höppner, bioinformatician
Jacques Dainat, bioinformatician
Martin Norling, bioinformatician
Mahesh Panchal, bioinformatician
Lucile Soler, bioinformatician
Torgny Karlsson, master student

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DNA AND RNA SEQUENCE ASSEMBLY

Neda Zamani

Next generation sequencing technologies allow for generating large amounts of RNA or DNA data at low cost. However, the sequenced fragments are short and require assembly into larger, continuous sequences. Powerful existing sequence assemblers are built on the concept of the *de Bruijn* graph, in order to compress the data so that it can be processed in finite amounts of computation time. This method, however, is limited by the graph complexity, which hampers accurate reconstruction of closely related gene paralogs, either stemming from

duplications within a single species, or a metagenomics or metatranscriptomics data set from mixed species. We have developed an alternative algorithm and software, *Ananas*, which efficiently computes possible read-read overlaps dynamically and without the need for hash- or k-mer-tables, which typically consume significant amounts of computer memory. In addition, we eliminated the use of fixed-size entities or sub-sequences, as required by *de Bruijn* graph based methods, resulting in optimal specificity and minimal complexity. Thus, our algorithm is suitable for deployment on third-generation sequencing technology that produce longer reads, but at higher average error rates. The modular architecture of the software allows for assembling both genome sequences as well as transcriptomes, with initial results indicating that we accurately recover complex sequences that were missed by existing assembly programs, both on DNA and RNA data sets.

PLANT AND MICROBIAL GENOMICS AND TRANSCRIPTOMICS.

Görel Sundström

We analyzed, as part of the aspen genome sequencing project, genomic differences within and between populations of Poplar, European and American aspens. We utilized several bioinformatics tools developed in the group, and were able to identify a genomic region in American aspen that differed between populations at different geographic locations. Using the conserved synteny between aspen and the fully assembled poplar, it was possible to place the genomic region on chromosome 2. The region consists of 0,1% of the genome and holds over 150 genes, with several interesting candidates among them.

The spruce genome project generated a transcriptome intended to aid gene annotation. However, besides spruce transcripts, we also identified transcripts from fungi and lichen. This gave us the opportunity to investigate the complex phyllosphere associated with different spruce tissues. We took advantage of the difference in GC-content in fungi and plant and reassembled RNA-reads originating from fungi, and were able to classify many of them both taxonomically and functionally. It was also possible to detect some expression differences between tissue samples.

BILS

Thomas Källman

I was giving active support in analysis of 12 different projects studying gene expression dynamics in species with already available genome sequence and annotation. Most projects were from human cell lines, but I also worked on bacteria and plants. I also started to develop an R analysis pipeline for analysis of protein expression data. I have over the year been teaching at both PhD and Master level.

BILS UPPSALA GENOME ASSEMBLY AND ANNOTATION CENTRE

Henrik Lantz (Team Leader), Jacques Dainat, Martin Norling, Mahesh Panchal, Lucile Soler, Marc Höppner

The annotation service successfully completed 13 projects last year, and still experiences a strong inflow of new projects. The types of organisms annotated are diverse, including yeasts, plants, birds and butterflies. Advances include refinement of structural annotation and better determination of orthology based on synteny. Marc Höppner left the group during 2015 for a new position in Germany and was replaced by Lucile Soler in October. Lucile brings expertise in particular for annotation of fish but also in functional genomics to the team.

The assembly service was in a build-up phase during 2015, with in particular a lot of pipeline development needed. Seven projects were completed, including chloroplast assemblies of Lycophytes and non-assembly projects such as pipeline development for a CRISPR/CAS9 system. Mahesh Panchal joined the group in March having earlier worked on assembly of mostly vertebrates.

RETROVIRUS-HOST EVOLUTION

Patric Jern

The overall aim is to better understand the evolutionary interactions among retroviruses and their host species. Retroviruses, such as HIV in humans, must become part of the host cell's genome to produce new viruses. When a germline cell is infected there is a chance for the retrovirus to be passed on to the host's offspring as an inherited endogenous retrovirus (ERV). Consequently, retroviruses have invaded host genomes for millions of years, leaving traces as ERVs in their genetic make-up, which constitute some 8% of human DNA. These ERVs represent retroviruses that were around at the time of infection and provide unique insights into the biology and co-evolution among viruses and hosts. We mainly use bioinformatics along two lines of research to generate new knowledge about long-term retrovirus-host associations:

I. How did our ancestors deal with their pathogens?
We perform comparative studies across the genomes of diverse host species to construct evolutionary hypotheses of relationship and explore retrovirus features, dynamics and transmission for insights into evolutionary retrovirus-host interactions.

II. What are the evolutionary effects of retrovirus integrations on host biology?
We characterize ERVs and other transposable genetic sequences across diverse host genomes in order to elucidate the contributions that they have had on host genomic variation and innovation, and to evaluate their contributions to host biology and phenotypic evolution.

Members of the group during 2015
Patric Jern, group leader
Jonas Berglund, researcher (from July)

Publications 2013 to 2015

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Serendipitous Meta-Transcriptomics: the fungal community of Norway spruce (Picea abies). PLoS ONE 10(9): e0139080.

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RETROVIRUS AND TRANSPOSABLE SEQUENCE EVOLUTION

Jonas Berglund, Patric Jern

Retroviral spread among different hosts can be estimated from the genomic ERV record, and their associations with host cellular factors provide important insights into virus-host interactions during evolution. Thus, sequenced genomes are a valuable resource for comparisons across many species to establish connections among ERVs and host evolution. We take advantage of the growing sequence catalogue to characterize ERVs and other transposable genetic sequences in order to identify novel broad-scale patterns and processes of evolutionary importance. Specifically, we seek to elucidate retroviral spread during evolution and contributions that retroviruses and transposable genetic sequences have had on the phenotypic evolution of their hosts. To this end, we develop bioinformatic methodology to screen for sequence patterns of selection and interaction among viruses and hosts. We further use a phylogenetic approach to construct evolutionary hypotheses of relationship. Since genetic divergence is often great among infectious retroviruses, ERVs or other transposable sequences, an additional part of our research concerns developing improved means of extracting informative phylogenetic signal from these sequences.

COMPARATIVE GENOMICS AND GENETICS

Kerstin Lindblad-Toh

The overall research focus is on identification of disease genes and mutations of relevance for canine and human disease. Once genes, mutations and biological pathway causing disease have been identified, these can be used to develop better diagnostics and treatment options. Two major areas of research are utilized together to accomplish this; i) all the functional elements in the human and mammalian genomes need to be identified to understand what the functional mutations may be, ii) domestic animals allow us to more easily find disease mutations, genes and pathways and then translate these findings to human medicine.

The comparative genomics work is part of an ongoing collaboration with my group and other colleagues at the Broad Institute to find functional elements in the human genome and that of model organisms. This includes analysis of large numbers mammalian genomes to identify common constraint elements, of which two-thirds fall outside coding genes, and contain other functional signatures such as non-coding RNAs and associated RNA structures, potential enhancers and insulators. We have just started sequencing an additional 150 mammals to reach a total of 200 mammals and single base constraint resolution. The majority of the sequencing will be performed in Uppsala. We also study genome evolution across vertebrates to understand how genomes change and allow organisms to adapt to novel environments. This year a lot of emphasis has been on 1) the rabbit genome allowing the study of genes involved in rabbit domestication revealing thousands of non-coding allele frequency differences affecting brain development and function and 2) the cichlid genome projects examining the diverse changes linked to the dramatic phenotypic diversity and species radiations seen in African lakes. This project points to the importance of regulatory variants and standing variation for adaptation to novel environments.

The unique breeding history of the domestic dog offers an unparalleled opportunity to map genes important in disease susceptibility, morphology, and behaviour. The breed structures where certain genetic risk factors have been enriched within specific populations and where recent bottlenecks have generated long haplotypes makes the dog excellent for trait mapping. The dog is also a unique animal to use for comparative analysis since; dogs spontaneously get diseases with the same aetiology as humans, they share largely the same environment and have roughly the same gene content. The past years our group has worked actively to map genes for both monogenic and complex traits including Amyotrophic Lateral Sclerosis, Obsessive Compulsive Disorder and Cardiomyopathy and Systemic Lupus Erythematous like syndrome, an Auto Inflammatory Disease as well as multiple cancers. Currently identified mutations show a spectrum of variation types from point mutations and deletions within coding regions to regulatory insertions and duplications. For several other diseases we have identified strong candidate loci and are in the process of evaluating these genetically and functionally. In parallel, we are now studying patient cohorts to identify disease mutations in genes found in dogs.

Members of the group during 2015
Kerstin Lindblad-Toh, professor, group leader[†]
Cecilia Johansson, project coordinator
Eva Murén, senior research engineer
Åsa Karlsson, research engineer
Sergey Kozyrev, researcher, group leader
Maja Arendt, researcher
Marcin Kierczak, researcher
Brita Ardesjö-Lundgren, researcher
Voichita Maricu, researcher
Lina Hultin-Rosenberg, bioinformatician
Johanna Dahlqvist, post doc
Fabiana Farias, post doc
Ingegerd Elvers, post doc[†]
Nina Oparina, post doc
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Katherine Megquier, graduate student[†]
Katarina Tengvall, graduate student
Jessika Nordin, graduate student
Sharadha Sakthikumar, graduate student
Sergei Abramov, graduate student
Michael Boyle, master student[†]
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International exchange during 2015
Kerstin Lindblad-Toh (Broad Institute)
Ingegerd Elvers (Broad Institute)

Publications 2013 to 2015

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Dissertations 2015

Katarina Tengvall: Genetic Studies in Dogs Implicate Novel Genes involved in Atopic Dermatitis and IgA Deficiency, October 6

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CANCER

Maja Arendt, Ingegerd Elvers, Voichita Marinescu, Argyri "Iris" Mathoudaki, Katherine Megquier, Sharadha Sakthikumar

Cancer is one of the most prevalent diseases in both humans and dogs. Moreover, specific breeds often show a predilection for certain tumour types. The molecular basis of the increased cancer risk within breeds is mostly unexplained and knowledge regarding susceptibility genes may enable improved diagnosis and treatment. We study cancer from two angles; the identification of predisposing genetic risk factors and the analysis of tumour mutations. We have focused on investigating a selection of tumour types, including:

- Mammary tumours
- Osteosarcoma
- Lymphoma
- Mast cell tumours
- Glioma
- Hemangiosarcoma

A few high-risk breeds have been chosen for initial investigations of each tumour type. To study predisposing factors, in conjunction with collaborators we have collected a large set of case-control genetic material from both Europe and the US. With this we have performed genome-wide association studies (GWAS) on a few hundred dogs per tumour type and breed using the Illumina CanineHD array (>170,000 SNPs). For each tumour type we identified multiple loci significantly associated with tumour presence. Targeted resequencing and fine-mapping revealed a large number of candidate mutations that are currently being validated and assessed for function. We are also sequencing matched DNA samples from tumour and normal tissues in order to identify somatic mutations and commonly mutated pathways in the canine tumours. This will provide important information on distinct and/or shared cancer pathways and therapeutic targets.

We published two articles on predisposing risk factors associated with canine cancer. Arendt *et al.* studied mast cell tumors in golden retrievers from Europe and the US, identifying mutations in separate loci in the two populations but with a common effect on hyaluronan. The study also identified a predisposing variant in the GNAI2 gene, resulting in a truncated protein. Tonomura *et al.* showed that hemangiosarcoma and B-cell lymphoma in American golden retrievers share two predisposing loci. Differential gene expression studies of tumors show that the risk haplotypes at both loci are associated with an effect on immune system regulation.

In addition, we published a pilot canine cancer exome study. Elvers *et al.* showed that B-cell lymphomas from two predisposed breeds share their most significantly mutated genes, while T-cell lymphomas from two predisposed breeds have very little overlap in significantly mutated genes and pathways. The study identified genes implicated in human lymphoma and leukemia, genes recurrently mutated in other human cancers, as well as novel genes that could allow new therapeutic options.

BREAST CANCER

Breast cancer (BrCa) ranks worldwide as the most common cancer type among women, and is the second highest cause of death among women cancer patients. Due to the many similarities of canine mammary tumor (CMT) and BrCa, dogs have emerged as a complementary model for investigating the genetic basis of this cancer in humans, and for understanding further the biology of tumor progression. Together with Tobias Sjöblom, UU and Annika Lindblom, KI, we are performing a comparative genetic study aiming at extending the benefits of the genetic studies carried on in dogs to humans. In the search for human mutations associated with BrCa, we have performed targeted sequencing with an in-house liquid capture design targeting the exons, UTRs and evolutionary conserved regions from known BrCa genes plus the most associated loci from the CMT GWAS. The study focuses both on germ-line mutations in Swedish BrCa patients, and on tumor/normal comparison for somatic cancer mutations that may affect carcinogenesis, metastasis and disease prognosis. Our ultimate goal is to identify new genetic risk factors in human disease that could be used as more effective and reliable screening tests before the disease manifests, or as novel diagnostic and/or prognostic targets.

AUTOIMMUNE AND INFLAMMATORY DISEASES

SYSTEMIC LUPUS ERYTHEMATOSUS

Sergey Kozyrev, Fabiana Farias, Hanna Bremer, Johanna Dahlquist, Nina Oparina, Lina Hultin-Rosenberg

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disorder characterized by dysregulation of the immune system, which results in production of autoantibodies, generation of toxic immune complexes, increased rate of apoptosis, defective clearance and complement activation. This leads to persistent inflammation and damage of peripheral organs and tissues. The human disease is more frequent in women of childbearing age than in men (9:1). While SLE was first described in human patients, it is also observed in other species including dogs with similar clinical manifestations. We have identified eleven genes associated with SLE in Nova Scotia duck tolling retrievers, a dog breed strongly predisposed to an SLE-like disease called immune-mediated rheumatic disease (IMRD). Further, we found that particular sub-phenotypes of IMRD, based on homogenous and speckled immunofluorescent antinuclear antibodies staining pattern, are associated with different but overlapping sets of genes, suggesting not only immunological but also genetic differences associated with various disease manifestations.

In parallel we study genetics of human SLE by targeted resequencing of candidate genes in a large cohort of human SLE patients, where phenotypic sub-classification is available (see abstract below).

Finally, the Kozyrev's group is focused on the analysis of exomes of Icelandic patients with severe highly penetrant familial form of SLE. We also identified a novel SLE susceptibility gene *ABHD6* coding for a protein acting in the endocannabinoid signaling pathway, and study the gene functions related to autoimmunity and the role of endocannabinoid pathway in the immune system responses.

SUB-CATEGORIZING INFLAMMATORY DISEASE BY MOLECULAR PATHWAYS

Fabiana Farias, Johanna Dahlquist, Åsa Karlsson Sergey Kozyrev, Argyri "Iris" Mathoudaki, Jennifer Meadows, Eva Muren, Jessika Nordin, Gerli Rosengren-Pielberg, Lina Hultin-Rosenberg

We have selected a set of 1900 genes comprising of genes from our canine immunological disease models and the genes and pathways implicated by corresponding human disease studies. We are sequencing these genes and the non-coding conserved elements within 100 kb in different human disease cohorts. Our goal is to identify both common and rare disease variants. By looking at carefully phenotyped human patient cohorts and a distinct and comprehensive gene set we expect to start to link diseases and sub-phenotypes to mutations in specific genes and pathways, possibly allowing a more comprehensive view of the molecular pathogenesis.

In a pilot study targeting a subset of the genes in SLE patients, we detected a large number of novel SNPs. Three SNPs in three genes were selected as the best regulatory candidates based on predictions of the SNP altering transcription factor binding. None of those three genes have previously been associated with SLE in genetic studies. The variants were associated to specific clinical sub-phenotypes of SLE indicating possible roles of specific pathways in development of certain manifestations. Functional analysis showed differential binding between the reference and mutant alleles as well as differential luciferase expression between reference and mutant allele for all three candidate SNPs. Overall the data suggests a potential function of these SNPs in specific cell types related to certain sub-phenotypes of SLE.

In the full study, thousands of samples have been sequenced and variants have been called and quality controlled. For each of the disease cohorts investigated around 200 000 – 300 000 variants remain after stringent quality filters, among which a large proportion seem to be novel regulatory variants. On going association analysis aims to identify key variants and pathways important for the pathogenesis of autoimmune disorders, as well as their relation to clinical sub-phenotypes. Further on, we will perform functional studies to establish the cellular and molecular effects of the identified risk alleles.

ATOPIC DERMATITIS

Katarina Tengvall, Marcin Kierczak, Sergey Kozyrev, Brita Ardesjö-Lundgren, Fabiana Farias, Eva Murén

Canine Atopic Dermatitis (CAD) is defined as a genetically predisposed allergic skin disease. The characteristic clinical features are most commonly associated with IgE antibodies directed towards environmental allergens. Typical signs of CAD are pruritus of the face, ears, paws, extremities, and ventrum. Previously, we performed a GWAS of ~200 German shepherd dogs (GSDs) and by measuring the IgA levels in the serum of the same individuals we detected a high correlation between serum IgA and CAD. The GWAS, using IgA levels and age at sampling as a covariate, generated a genome wide significant association to a locus on CFA27 and fine-mapping of this 1.5 Mb region suggested the candidate gene *PKP2* encoding the protein Plakophilin 2. As a follow-up of these results, we collected skin biopsies and evaluated *PKP2* expression in the skin using immunohistochemistry and mRNA sequencing. In addition, we performed additional fine-mapping, which resulted in candidate variants that were functionally evaluated by using Luciferase assays.

ADDISON'S DISEASE

Katarina Tengvall, Jennifer Meadows, Maria Johansson, Helene Hamlin-Hansson (SLU), Jeanette Hansson (SLU)

Addison's disease is an organ-specific disease and is generally caused by an immune-mediated destruction of the adrenal cortex tissue leading to glucocorticosteroid and mineralcorticoid deficiencies. Autoimmunity occurs when the central immunological tolerance is broken and the immune system fails to recognise its own tissue as self. The diagnosis of Addison's disease is diagnosed routinely by an ACTH stimulation test where artificial ACTH is injected and the cortisol levels in the sera are measured before and after the injection. We aim to identify the genetic risk factors in the high-risk breeds Standard Poodles, Bearded collies and Portuguese Waterdogs. Whole genome association mapping has been conducted in Swedish and US Standard Poodles. Analysis and additional phenotypic characterisation of cases and controls is ongoing.

We have also performed two epidemiological studies of canine adrenocortical insufficiency (AI). The first is a novel breed specific analysis of Survival in Standard Poodles diagnosed with naturally occurring adrenocortical insufficiency (AI). This work is expected to impact the treatment and survival outcome in this and other breeds. The second represents the largest epidemiological study based on ~500,000 Swedish dogs insured by AGRIA. In this study we present data supporting the presence of breed-specific differences in AI regarding incidence rates, gender distribution and survival fractions, indicating the existence of different subtypes of AI in the dog, in analogy to what is known in people.

METABOLIC AND CARDIOVASCULAR DISEASE

DIABETES

Maja Arendt

The pathogenesis of gestational diabetes is complex and not well understood. Female dogs can develop hormone induced diabetes post oestrous, or during pregnancy which is similar to human gestational diabetes. Certain dog breeds have a relatively high incidence of hormone related diabetes compared to others, indicating a genetic predisposition in these breeds.

We have performed genome wide association studies (GWAS) comparing healthy and diseased dogs in two high risk dog breeds, the border collie and the Swedish elkhound in order to identify regions in the genome associated with disease risk. We have also performed whole genome sequencing in a subset of Swedish elkhound cases (18) and controls (12) and identified many variants in candidate genes. We are currently expanding our GWAS to increase the power of our study and to investigate the relevance of identified germline variants.

Long term we are hoping that identified disease associated genes will help us achieve a better understanding of the development of diabetes in general. We hope that this can lead to better prophylactic measures and also better treatment options for the disease which could benefit human as well as dogs.

DILATED CARDIOMYOPATHY

Jennifer Meadows, Göran Andersson (SLU)

To date, at least 19 genes have been implicated in familial forms of human dilated cardiomyopathy (DCM). The majority of these have been shown to encode structural proteins essential to the heart muscle's contractile strength, however this still leaves many hereditary and idiopathic cases of disease without known genetic cause. There are several large- and giant dog breeds, which are also predisposed to DCM and the current study considered both purebred Great Danes (GD) and Newfoundlands (NF). The disease in these breeds is described histopathologically as the attenuated wavy fibre type, in which the myocytes appear thinner than normal and are separated by oedematous fluid. As opposed to the other form of canine DCM, this fluid space is generally free from fatty cell infiltrates.

GWAS results from 182 GDs and 133 NFs has allowed for the identification of associated regions. Whole genome sequencing was performed and the role structural variants play in disease is currently being assessed. Genes of interest from the canine cardiac project are being carried forward into a human targeted sequencing program where the collection of Swedish cases and controls relative for Sudden Cardiac Death is ongoing.

NEUROLOGICAL AND BEHAVIOURAL TRAITS

DEGENERATIVE MYELOPATHY

Emma Ivansson, Katherine Megquier, Sergey Kozyrev, Eva Muren

Canine degenerative myelopathy (DM) is a severe neurodegenerative disease of late onset. Signature features of the disease are axonal and myelin degeneration of the spinal cord causing progressive ataxia and paresis. We have mapped and identified a mutation in the *SOD1* gene as the major cause of DM in at least five dog breeds using genome-wide association analysis (GWAs) in the Pembroke Welsh Corgi (PWC) breed and further validation in other breeds. A mutation in human *SOD1* causes a similar neurodegenerative disease, amyotrophic lateral sclerosis (ALS), suggesting that this is a good animal model for human ALS.

The identified *SOD1* E40K missense mutation appears to encode a major risk factor for the disease but is not fully penetrant, suggesting that additional modifier loci may be involved. We have performed additional GWAs in carriers of the SOD1 risk allele in the PWC breed. We identified a novel modifier gene, which affects both risk of disease and age of onset. Several newly identified variants within this gene have regulatory potential, altering transcription factor binding and/or overall isoform balance and gene expression. We are collaborating with Ingela Nygren at Akademiska Hospital and Peter Andersen at Umeå University to follow up genes and pathways identified in the canine breeds in human ALS patients.

INVESTIGATING GENETICS UNDERLYING BEHAVIORAL TRAITS IN DOGS

Marcin Kierczak, Katarina Tengvall, Fabiana Farias, Sharadha Sakthikumar

The Domestication of wolves and the creation of modern breeds are important events in the history of the domestic dog. Via natural and directed selection on certain phenotypes, including particular types of behaviour important in, e.g. herding or guarding, each event is reflected in the structure of canine genome.

Since 1989 the Swedish Working Dog Association has been performing Dog Mentality Assessment (DMA) tests, in which different aspects of behaviour are measured, e.g. intensity of social contact, playfulness or eagerness to chase. We have genotyped nearly 500 German shepherds with DMA data available.

Following data collection, we developed some novel statistical methods, and enhanced others already existing, and applied these to identify loci associated with several different aspects of canine behaviour. The loci we identified as being associated behaviour that, in general, these traits are controlled in an oligo- and polygenic manner. One of the most interesting findings pinpoints two families of cell membrane proteins and one family of receptors as being involved in controlling playfulness and the ability to chase. Members of these families are involved in the development of the central nervous system and in transmission of signals at the nervous cell-effector cell junction. Detailed haplotype analyses followed by whole-genome sequencing of selected individuals allowed us to fine-map associated regions and to identify a number of candidate polymorphisms causing non-synonymous substitutions and/or potentially affect gene-expression regulation.

Our current efforts are directed towards fuctional *in vitro* validation of our findings and whilst we also actively collect samples from additional German shepherd breeds to allow

for multi-breed analyses.

While the studied traits describe normal variation within dog breeds, a number of human behavioural diagnoses such as anxiety, autism, ADHD or depression may be caused by similar mechanisms. Thus, our study will potentially contribute to the development of diagnostic tools and novel treatments from which also we, humans, will benefit.

COMPARATIVE GENOMICS

A single base pair resolution map of mammalian evolutionary constraint

Voichita Marinescu, Eva Murén

Access to high-resolution genomic information for the evolutionarily conserved functional elements is instrumental in identifying and prioritizing candidate causative mutations and in evaluating their potential biological consequences. In fact, it is now known that mammalian conservation is the annotation of the human genome that most highly enriches for functional variants. Previously the Lindblad-Toh group sequenced, analyzed and compared the genomes of 29 placental mammals, enabling the identification in the human genome of 3.6 million constraint elements at a resolution of 12 bp. Using this as a baseline, it was estimated that reaching single base pair resolution would require the comparative analysis of approximately 200 sequenced mammalian genomes. To date, high-quality assemblies are already available for 50 mammalian genomes, and the current work aims to add another 150 genomes to this in collaboration with the Uppsala University SNP&SEQ Technology Platform. This task will be made more efficient with the improved *de novo* genome assembler, DISCOVAR using sequencing data from a single PCR-free Illumina libraries. This work will not only provide essential information on evolutionary constraint and a basis for refining the human genome annotation, but will also make available to the scientific community high-quality genome assemblies for many mammalian species that are non-standard model organisms for human disease but for which the genomic sequence is currently unavailable.

GENETIC DISSECTION OF AUTOINFLAMMATORY DISEASE

Jennifer Meadows

The long-term research goal is to identify the genes and molecular variants that underpin traits of importance to both the health and well being of companion animals. Paired with this is a comparative genetics aspect, where through international collaborative efforts, research findings are translated to aid human patients with orthologous disease sets.

Our current projects leverage knowledge gained from studying the purebred form of the domestic dog. The dog represents an excellent model organism for human comparative analyses since they share roughly the same gene set, develop spontaneous disease with similar aetiologies and generally share the same environment. The true benefit to using purebred dogs lies in the exploitation of the genetic structure honed through selection bottlenecks, where long within breed haplotypes and the enrichment of genetic risk factors mean that the trait mapping of heterogeneous diseases can be investigated with both fewer markers and fewer individuals than is possible for human genetics.

The two main projects investigate the molecular genetics of canine and human forms of autoinflammatory disease (AID). These diseases are characterised as unprovoked episodes of inflammation associated with abnormal regulation of innate immunity (i.e. absence of high-titre autoantibodies or antigen-specific T cells).

Members of the group during 2015
Jennifer Meadows, researcher, group leader
Argyri "Iris" Mathioudaki, PhD student
Jessika Nordin, PhD student

Project worker during 2015
Maria Johannson, project worker

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CANINE GENETICS AND GENOMICS

SHAR-PEI AUTOINFLAMMATORY DISEASE (SPAID)

Jennifer Meadows

Autoinflammatory disease results from the dysregulation of the innate immune system: the body's first line of defiance against infection. The clinical picture of SPAID in purebred Shar-Pei dog is typically 6-72 hours-long attacks of high fever and other signs of inflammation (especially around the hocks). During these attacks the animal shows reluctance to move and is generally indolent. Within 24 hours, the dog is again alert, remaining asymptomatic between episodes. Shar-Pei with SPAID have consistently elevated levels of the cytokine IL-6 and can be treated with blockers of IL-1B, such as colchicine.

Persistent inflammation in affected Shar-Pei can lead to reactive amyloidosis, the accumulation of aberrantly produced acute phase proteins in multiple organs. These aggregates are particularly damaging to the kidney and can result in organ damage and ultimately organ failure. The clinical picture is varied. Some individuals may be susceptible to amyloidosis without having other symptoms of SPAID and vice versa.

Our understanding of the genetics that underpin Shar-Pei health has rapidly evolved over the past five years. Since our 2011 publication, which reported that a shared locus was linked to both Familial Shar-Pei Fever (FSF) and the dogs' classical thickened and wrinkled skin, we now understand that Fever is only one of the signs of Shar-Pei Autoinflammatory Disease (SPAID). We have used discrete genome wide association analyses to show that a single genetic locus predisposes the breed to many types of persistent inflammation, in addition to recurrent fevers, including arthritis, Shar-Pei specific secondary dermatitis (hyaluronan filled vesicles affecting the skin, termed vesicular hyaluronosis), otitis and systemic reactive amyloidosis. Our most recent research has been geared towards using whole genome sequencing to resolve the difference between breed subtypes and disease associated alleles. We have also been refining a genetic test for SPAID and investigating the potential genetic signatures of early and late onset renal amyloidosis.

ADDISON'S DISEASE

Jennifer Meadows, Katarina Tengvall, Maria Johansson, Helene Hamlin-Hansson (SLU), Jeanette Hansson (SLU)

Addison's disease is an organ-specific disease and is generally caused by an immune-mediated destruction of the adrenal cortex tissue leading to glucocorticosteroid and mineralcorticoid deficiencies. Autoimmunity occurs when the central immunological tolerance is broken and the immune system fails to recognise its own tissue as self. The diagnosis of Addison's disease is diagnosed routinely by an ACTH stimulation test where artificial ACTH is injected and the cortisol levels in the sera are measured before and after the injection. We aim to identify the genetic risk factors in the high-risk breeds Standard Poodles, Bearded collies and Portuguese Waterdogs. Whole genome association mapping has been conducted in Swedish and US Standard Poodles. Analysis and additional phenotypic characterisation of cases and controls is ongoing.

DILATED CARDIOMYOPATHY

Jennifer Meadows, Göran Andersson (SLU)

To date, at least 19 genes have been implicated in familial forms of human dilated cardiomyopathy (DCM). The majority of these have been shown to encode structural proteins essential to the heart muscle's contractile strength, however this still leaves many hereditary and idiopathic cases of disease without known genetic cause. There are several large- and giant dog breeds, which are also predisposed to DCM and the current study considered both purebred Great Danes (GD) and Newfoundlands (NF). The disease in these breeds is described histopathologically as the attenuated wavy fibre type, in which the myocytes appear thinner than normal and are separated by oedematous fluid. As opposed to the other form of canine DCM, this fluid space is generally free from fatty cell infiltrates.

GWAS results from 182 GDs and 133 NFs has allowed for the identification of associated regions. Whole genome sequencing was performed and the role structural variants play in disease is currently being assessed. Genes of interest from the canine cardiac project are being carried forward into a human targeted sequencing program where the collection of Swedish cases and controls relative for Sudden Cardiac Death is ongoing.

HUMAN GENETICS AND GENOMICS
PATHWAYS OF HUMAN INFLAMMATORY DISEASE

Jennifer Meadows, Argyri "Iris" Mathoudaki, Jessika Nordin

Ankylosing Spondylitis (SpA) is one of a growing number of human polygenic autoinflammatory diseases. It is manifested by chronic spinal and sacroiliac joint arthritis, which in time will result in the loss of mobility due to spinal fusion and potentially, restrictive lung disease. Inflammation may also affect peripheral joints and nonarticular structures, presenting clinically as enthesitis or uveitis. SpA is highly heritable (>90%) and with an affliction rate of 1/200 within Europe, is one of the most common forms of inflammatory arthritis. The genetics of disease have long implicated HLA-B27, however 10% of the European population carries this subtype, but only 1-5% of those develop SpA.

In 2015, we completed the sequencing of ~400 carefully phenotyped SpA patients and a similar number of matched controls using the 1900 gene ImmunoArray. This “array” is a targeted liquid capture library which, with a combination of coding and regulatory regions, covers ~32Mb of the human genome. Illumina paired end sequencing was used to sequence each individuals to an average depth of 30x depth. A robust bioinformatic pipeline moving from fastq raw reads to vcf formatted annotated variants has been designed and implemented at UPPMAX. Functional validation of our candidate variants shows the power of this analysis pipeline to find rare and low frequency variation which impact the regulation of disease. This success of this analysis is in part due to the homogeneity of our region-specific Swedish population, but also due to the added power of aggregation in association testing. The disease associated variants identified by this project may prove useful in both the diagnostic and treatment fields.

COMPARATIVE GENETICS OF IMMUNOLOGICAL DISEASES TOWARDS FUNCTIONAL GENOMICS

Gerli Rosengren Pielberg

The overall aim of the research group is to take advantage of information from comparative genetics and provide functional genomics data both in model organisms as well as humans. We are working with dog as a model organism and more precisely breeds predisposed to immunological and immune-mediated diseases with comparative value to human diseases.

Dogs, as other domestic animals, have a genomic structure suitable for gene mapping. In addition, dogs also present the same spectrum of diseases as humans and nowadays even share our living-environment. Therefore, taking advantage of those characteristics may provide us knowledge of genetic risk factors lying behind human diseases. In general our approach goes from identifying risk loci in a dog breed all the way to providing functional evidence of an identified mutation contributing to the disease development. Our goal is to provide information necessary for the future development of genetic testing, diagnostics and therapy for the dogs. Furthermore, the ultimate goal is to provide functional genomic data of the same mutations, genes and pathways being involved in the development of human homologous diseases.

Members of the group during 2015
Gerli Rosengren Pielberg, assistant professor, group leader
Matteo Bianchi, PhD student
Daniel Eriksson, visiting PhD student from Karolinska Institutet

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CHARACTERIZATION OF GENETIC RISK FACTORS BEHIND CANINE HYPOTHYROIDISM

Matteo Bianchi, Gerli Rosengren Pielberg

Hypothyroidism is one of the most frequent endocrinopathies in dogs, affecting multiple breeds. The disease is most often characterized by autoimmune destruction of the thyroid gland resulting in functional failure of the thyroid. The homologous disease in humans is called Hashimoto's Thyroiditis, resembling most clinical aspects of the disease in dogs.

The overall aim of this project is to use dog as a model organism to identify mutations, genes, and pathways potentially contributing to development of human Thyroiditis. We have performed a genome-wide association analysis and identified several candidate loci in different dog breeds. Furthermore, by performing a meta-analysis we have identified a hypothyroidism risk locus shared among three dog breeds. Currently we are in the process of identifying potential candidate mutations by next generation sequencing of key individuals representing the risk/protective haplotypes. Identified candidate mutations will be screened in larger sample cohorts and functionally evaluated.

The results from this study may lead to development of genetic tests and better diagnostic methods as well as new alternative therapies for treatment and breeding guidelines of dogs.

IDENTIFICATION AND CHARACTERIZATION OF GENETIC RISK FACTORS BEHIND HUMAN AUTOIMMUNE POLYENDOCRINE SYNDROMES

Matteo Bianchi, Daniel Eriksson, Gerli Rosengren Pielberg

Human autoimmune polyendocrine syndromes are a heterogeneous group of diseases characterized by autoimmune activity against more than one endocrine organ. In collaboration with Prof. Olle Kämpe (Karolinska Institutet), we have started a project for characterizing genetic risk factors contributing to the development of these syndromes.

We are taking advantage of knowledge gained from comparative genetics (more specifically canine genetic risk factors and involved pathways). By using modern high-throughput techniques, such as hybrid capture and next-generation sequencing, we will perform a thorough screening of mutations in about 1900 candidate immune genes and their regulatory elements in Swedish Addison patient cohort of more than 700 individuals.

The results from this study will provide us knowledge about the sharing of genetic risk factors behind immunological diseases in different species, as well as potentially identify new genes and pathways important in development of such diseases in humans.

FUNCTIONAL GENOMICS

IDENTIFICATION AND CHARACTERIZATION OF GENES AND MECHANISMS CONTROLLING PHENOTYPIC TRAITS IN HORSE AND SALMON

Carl-Johan Rubin

The overall aims are to explain how genetic variation impacts diseases and phenotypic traits and to explore molecular processes affecting how the genetic code is processed depending on environmental factors. In one project, massively parallel DNA sequencing is utilized in order to identify regions of the genome that have responded to selection during horse domestication or the subsequent diversification into specific breeds. In addition we use the sequencing data to reveal genotype/phenotype associations for diseases and other traits such as pigmentation and performance. The most important findings made during the course of this project are pursued and validated in collaboration with breeders and/or veterinarians. In another project we investigate the contribution of genetic and environmental factors on phenotypic variation in Atlantic salmon using genetic-, epigenetic- and gene expression profiling. Phenotypes assessed include time of sexual maturation, growth, and various behavioral traits. For this project we will include clonal lines as well as wild salmon in order to study the impact of environmental difference on traits with and without confounding effects added by genetic variation. Furthermore, the integration of genetic, epigenetic and transcriptome profiling gives a first opportunity of deep exploration of Atlantic salmon genome biology; including gene regulatory and epigenetic mechanisms.

Members of the group during 2015
Carl-Johan Rubin, group leader
Markus Sällman Almén, PhD

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GENETICS OF DISEASE, MORPHOLOGY AND PIGMENTATION IN HORSES

Carl-Johan Rubin

Millennia of human-imposed selective breeding for desired traits has altered the phenotypic repertoire of the horse for traits such as size, body conformation, behavior, and color, with such variation being conferred by changes in frequencies of alleles at mostly unknown genetic loci. Lately, new methods for DNA sequencing have emerged and it is now possible to determine near-complete sequences of large numbers of mammalian genomes in parallel.

The major aims are to generate a fine-scale map of genetic variation in the horse (*Equus caballus*) genome, including structural variation such as inversions, duplications, CNVs and large deletions. We then analyze patterns of genetic variation in various contrasts in order to detect loci affected by selection and to detect genetic variants contributing to specific traits and diseases. To achieve these aims we have sequenced DNA samples from diverse horse breeds and populations, selected to represent distinct disease/trait classes. Samples were subjected to whole genome resequencing (WGS) and obtained sequences were used in genome scans to detect signatures of selection and functional polymorphisms/mutations. We predicted functional genetic variants using bioinformatics methods and screen for alleles uniquely/preferentially observed in individuals expressing certain diseases or traits. For such candidates we proceed with association analysis in larger cohorts of horses to investigate whether identified candidate alleles are significantly associated with the traits.

The project is carried out in collaboration with Sofia Mikko at the Swedish University of Agricultural Sciences

EPIGENETICS AND OTHER MEANS OF ALTERING GENOME UTILIZATION IN RESPONSE TO ENVIRONMENTAL VARIATION

Carl-Johan Rubin, Markus Sällman Almén

GENETIC AND EPIGENETIC CONTRIBUTION TO MALE SEXUAL MATURATION AND BEHAVIOR

Farmed Atlantic salmon (*Salmo salar*) has been the subject of intense selection for increased growth, but selection alone has not sufficed to counteract frequent occurrence of reduced growth in response to early puberty. Reduced growth due to premature maturation has been mitigated by specific light regimens. It is assumed that maturation is also modulated by variation in water temperature, which could be detrimental to commercial Salmon breeding as sea temperature is expected to rise due to global warming. One mechanism by which environmental factors can influence the expression of phenotypic traits is through epigenetic modifications of DNA in animal cells, thereby affecting the activity of genes.

In this project we use massively parallel sequencing (genome sequencing, whole genome bisulfite sequencing, Reduced Representation Bisulfite Sequencing (RRBS), RNA-sequencing and micro-RNA sequencing) in order to, in clonal individuals, investigate changes in epigenetic marks and gene expression signatures accompanying exposure to environmental variation, including different light- and temperature regimens and stress tests during different life stages in the Atlantic salmon. Furthermore, wild salmon are known to differ for their time of sexual maturation and we recently identified that genetic variation at a single locus, the *vgll3* locus, acts as a major determinant for early- vs. late sexually maturation by sequencing the genomes of late- vs. early maturing individuals from rivers along the Norwegian coast. We are now following up on this locus to investigate the mechanism of action of the identified *vgll3* maturation sweep and how it performs under variable environmental conditions. The combination of global genetic-, epigenetic- and transcription profiling provides an integrated view of Atlantic salmon genome biology and makes it possible not only to study factors driving behavior and maturation, but also to provide a first functional context in terms of gene expression and regulation.

In order to evaluate the contribution of genetic- as well as epigenetic factors on a trait as complex as behavior we are utilizing lines exhibiting behavioral differences as well as clonal fish, since the latter makes it easier to study epigenetic effects without genetic variation acting as a confounder. To obtain relevant phenotypic read-outs we are utilizing PIT-tags, i.e. sensors capable of determining spatial location of individual fish in tanks or sea-cages. Data from these sensors can be used to approximate traits such as activity, feeding, swim depth etc. and these traits will be correlated with life history events as well as with read-outs from genetic- and epigenetic screens, i.e. alleles or epi-alleles.

These projects are conducted in collaboration with researchers at the Institute of Marine Research in Bergen, Norway.

GENOME EVOLUTION

Matthew T Webster

We study evolution on the molecular level by analysing patterns of genetic variation on the whole-genome scale, using bioinformatic and statistical approaches. We aim to identify genetic variants that have been affected by natural selection and analyse the phenotypes that they produce. We are also interested in understanding the mechanisms and evolutionary consequences of genomic variation in meiotic recombination.

We are currently using massively-parallel sequencing to characterise global patterns of genetic variation in the honeybee. A major goal of this project is to identify genes and genetic variants important for adaptation to climate and disease, which could be vital to protect this important species from colony losses. We are using transgenic fruit flies to directly measure the functional effects of these variants. We are also using genome sequencing to investigate the causes and consequences of extremely high recombination rates in honeybees.

Members of the group during 2015
Matthew Webster, group leader
Andreas Wallberg, post doc
Martin Schmid, post doc
Ronald Nelson, post doc
Anna Olsson, lab technician

International exchange during 2015
Dora Henriques, visiting PhD student (Polytechnic Institute of Bragança, Portugal)

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MOLECULAR BASIS OF ADAPTATION IN THE HONEYBEE, *APIS MELLIFERA*

Andreas Wallberg

The honeybee is vital for maintaining levels of biodiversity and agricultural production through its role in plant pollination. However, it is threatened by several factors, including pathogens, biological invasions, climate change and pollution. Honeybees, and the plants that rely on them, are in decline, incurring major ecological and economic costs. The native range of honeybees spans a large geographic area across Europe, Africa and the Middle East. Natural selection has resulted in populations becoming adapted to their local environments. More recently, the management of colonies by humans has resulted in artificial selection for desirable traits.

Our goal is to uncover the molecular basis of these adaptive and beneficial traits. To achieve this, we have sampled populations drawn from several honeybee subspecies, and from populations of honeybees specifically selected for disease resistance and are surveying genetic variation across the entire genome in these populations using next-generation sequencing. We then analyse these fine-scale patterns of genetic variation for the characteristic footprints of "selective sweeps" which indicate genes or genomic regions that are responsible variation in traits of interest. We have recently uncovered specific genetic variants that control reproductive strategy and the ability to survive at high altitudes. In addition, we are mapping the genetic basis of parasite resistance and hygienic behaviour, traits that are important for honeybee health and viability.

FUNCTIONAL CHARACTERISATION OF GENETIC POLYMORPHISMS IN THE HONEYBEE

Martin Schmid

We are using the fruit fly *Drosophila melanogaster* as a model to investigate the functional effects of specific genetic variants of evolutionary importance that we identify using selection scans in the honeybee. This is possible to due the vast array of transgenic techniques and mutant lines that are available in *Drosophila* and due to the levels of homology between this species and other insects such as honeybees. We aim to introduce honeybee gene variants into knockout flies and characterise phenotypes using a variety of assays. We are currently focussing on genes involved in metabolism and morphology.

RECOMBINATION AND GENOME EVOLUTION

Ronald Nelson

Meiotic recombination is a fundamental biological process, which maintains genetic variation within populations and is essential for chromosomal segregation. In many taxa, the genomic distribution of recombination events is localized to specialized sites known as hotspots, but the mechanisms controlling this variability are unclear.

The honeybee has the highest levels of meiotic recombination measured in a sexual eukaryote. The reasons for this are unknown, but it is likely related to their highly developed sociality. Recombination may also have damaging effects on the genome, either because it causes structural mutations or due to a process known as biased gene conversion, which alters the frequency of mutations in a population. We are analysing genomic variation in recombination rates in honeybees and other insects in order to understand the evolutionary forces responsible for increasing recombination rates and their effects on genome evolution. These studies will shed light on how recombination events are controlled in invertebrates.

MEDICAL BIOCHEMISTRY

GLYCOBIOLOGY

PROTEOGLYCANS - BIOSYNTHESIS AND BIOLOGICAL FUNCTIONS

Lena Kjellén, Jin-ping Li, Dorothe Spillmann, Maria Ringvall, Ulf Lindahl, Cecilia Annerén

The IMBIM groups active in this area study proteoglycans and elucidate functional aspects of these glycoconjugates in relation to embryonic development and during pathophysiological conditions such as amyloidosis, inflammation and tumor progression. In addition, mouse and human embryonic stem cells are studied focusing on molecular mechanisms that regulate self-renewal as well as roles of proteoglycans in differentiation into different lineages. Many collaborations between the groups create a strong unit.

A majority of the projects concerns heparan sulfate proteoglycans. Heparan sulfate modulates growth factor and cytokine action and participates in the generation and maintenance of morphogen gradients and is therefore of particular importance both during embryonic development and in different pathologies. Biosynthesis of heparan sulfate and its regulation is one important focus. Recent projects also address the question of functional overlaps between heparan sulfate and chondroitin sulfate proteoglycans. Model systems include mice, zebrafish, C. elegans and most recently Drosophila melanogaster.

METHODS FOR MAINTANENCE AND GENETIC MANIPULATION OF PLURIPOTENT STEM CELLS

Cecilia Annerén

Pluripotent stem (PS) cells e.g. embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, offer novel cell sources for basic research, drug toxicity studies, *in vitro* modeling of genetic disorders or therapeutic cell replacement. However, realization of the full potential of stem cells is currently hampered by the difficulty in genetically manipulating as well as routinely culturing these cells. The overall aim of our research is to improve long-term propagation, genetic manipulation and large-scale expansion of PS cells. More specifically, we are: 1) identifying best practices by benchmarking novel and commercially available cell culture media, matrices/surfaces and transfection reagents, 2) delineating the mechanisms involved in stem cell self-renewal and cell attachment by characterizing a newly identified serum protein and identifying its role in these processes and, 3) applying our knowledge to long-term culture using chemically defined reagents. Translation of our findings into commercially available products or methods may potentially be realized via close collaboration with GE Healthcare BioSciences AB (GEHC).

Members of the group during 2015
Cecilia Annerén, PhD, adjunct senior lecturer, group leader
Sara Pijuan Galitó, PhD student/post doc
Sandeep Kadekar, post doc

Publications 2013 to 2015
1. Tamm C, Pijuan-Galitó S, Annerén C. A comparative study of protocols for mouse embryonic stem cell culturing. PLoS One, 2013. 8(12):e81156
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Dissertations 2015
Sara Pijuan Galito: Novel Culture Strategies and Signal Transduction Pathways of Pluripotent Stem Cells, May 15

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ROLE OF INTER-α-INHIBITOR AND THE cYES/YAP/TEAD2 PATHWAY FOR SELF-RENEWAL AND ATTACHMENT OF MOUSE ES CELLS

Sara Pijuan Galitó, Christoffer Tamm

We have previously shown that a novel kinase pathway activated by LIF is involved in the maintenance of self-renewal and pluripotency of mES cells (Annerén *et al,.* 2004, Tamm *et al,* 2011 and Tamm *et al,.* 2012). Briefly, we demonstrated that LIF activates the Src kinase family member Yes, which in turn activates the Yes Associated Protein (YAP). YAP then enters the nucleus and forms an active transcription complex with TEAD2, inducing transcription of other well-described self-renewal and pluripotency factors such as Oct3/4 and Nanog. During our experiments we also found that fetal bovine serum (FBS) can activate Yes and induce TEAD2-dependent transcription in a dose- and time-dependent manner. Through a set of serum fractionations techniques we identified and isolated Inter-α-Inhibitor (IαI). IαI activates Yes/YAP/TEAD pathway by inducing Yes auto-phosphorylation, YAP nuclear localization and TEAD-dependent transcription. The cleaved heavy chain 2 (HC2) sub-component of IαI, was demonstrated to be responsible for this effect. We also found that addition of IαI or HC2 to the culture promotes mouse and human PS cell attachment under serum-free media conditions, and that PS cells seeded in the presence of IαI can successfully be cultured on uncoated, standard tissue-culture treated plastic. We then developed a new cell culture protocol for human PS cells. The medium consists of the Essential 8 (E8) formulation supplemented with soluble IαI. Seven different PS cell lines (both embryonic and induced hPS cells) were adapted to the new media formulation, and 4 different human PS cell lines were cultured in the medium for over 20 passages and tested for maintenance of pluripotency and genetic integrity. Moreover, IαI successfully supports single cell passaging and clonal growth of human PS cells even without pre-treatment with ROCK inhibitor. This time-efficient and simplified culture method paves the way for large-scale, high-throughput hPS cell culture, and will be valuable for both basic research and commercial applications. A revised version of a manuscript, presenting the work is currently pending acceptance in *Nature Communications*.

STUDY OF DIFFERENT PROTOCOLS FOR MOUSE EMBRYONIC STEM CELL CULTURE AND TRANSFECTION

Christoffer Tamm, Sandeep Kadekar, Sara Pijuan Galitó

Most stem cell laboratories are still growing mouse PS cells on mouse embryonic fibroblast feeder cells or on gelatin in media supplemented with fetal bovine serum and leukemia inhibitory factor (LIF). However, these techniques have several drawbacks including the need for feeder-cells and/or use of undefined components. Culture of stem cells under undefined conditions can induce spontaneous differentiation and reduce reproducibility of experiments. In a recent study (Tamm et al, 2013), we compared standard PS cell culture protocols with two newly described ones: 1) growing cells in semi-adherence in a medium containing two small molecule inhibitors (CHIR99021, PD0325901) herein called 2i medium and; 2) growing ES cells in a spheroid suspension culture in a defined medium containing LIF and bFGF herein called ESN2 medium. Our data confirms previous reports showing that the 2i medium generates purer stem cell cultures with negligible signs of spontaneous differentiation, as compared to traditional, FBS-based, mouse PS media. A drawback that we

observed with the 2i medium, is that the mouse ES cells are much harder to transfect using standards protocol as compared to cells grown in standard serum-containing medium. We therefore performed a side-by-side comparison of commercially available, non-viral transfection reagents with regard to their ability to deliver plasmid DNA and/or siRNA into adherent or trypsinized mES cells cultured in 2i medium and assessing transfection rates, plasmid gene expression, and siRNA mediated knockdown of Oct4 and viability. We developed a fast and efficient method for plasmid DNA transfection of trypsinized mES cells using the liposomal-based Lipofectamine 2000. With only a five-minute long transfection time we obtained close to 90% transfected cells with 80% maintained viability. This protocol saves up to one day experimental time since the cells are in suspension at time of transfection which allows for immediately re-plating into the appropriate format. This fast, simplified and highly efficient transfection method will be valuable for both basic research and high-throughput applications.

CELLULAR DESIGN OF HEPARAN SULFATE

Lena Kjellén

Heparan sulfate structure varies greatly during embryonic development and differs also when heparan sulfate isolated from different tissues and cell types of an adult animal are compared. Biosynthesis takes place in the Golgi compartment and relies on the action of a multitude of enzymes. Our main goals are to find out how the cell decides on a particular heparan sulfate design and to characterize the molecular machinery responsible for its biosynthesis. Our model systems are mouse, zebrafish and *C. elegans* where we study biological effects of mutations in biosynthesis enzymes. Embryonic stem cells and embryonic fibroblasts derived from mutant mice as well as mammalian cell-lines overexpressing or lacking selected biosynthesis enzymes are important tools. A sensitive method to determine glycosaminoglycan concentration and structure is available in the lab, enabling analysis of cultured cells as well as small tissue samples. Our focus has been on the biosynthesis enzyme glucosaminyl *N*-deacetylase/*N*-sulfotransferase, NDST, which has a key role in heparan sulfate design during biosynthesis in the Golgi compartment. NDST removes acetyl groups from glucosamine residues and replaces them with sulfate groups. These *N*-sulfate groups are important for further modifications including *O*-sulfation in various positions and epimerization of glucuronic acid to iduronic acid. Four NDST isoforms, transcribed from four genes, have been identified.

Members of the group during 2015
Anders Dagälv, post doc
Tabea Dierker, post doc
Inger Eriksson, research engineer
Beata Filipek-Górniok, graduate student
Lena Kjellén, professor, group leader
Anders Lundequist, postdoc
Catherine Merry, guest researcher

Project workers during 2015
Parisa Missaghian (SOFOSKO)
Amanda Åhman (biomedical student)

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Disserations 2015

Beata Filipek-Górniok**:** Glycosaminoglycans Biosynthesis in Zebrafish, November 27.

Agencies that support the work

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The Leverhulme Trust

REGULATION OF HEPARAN SULFATE BIOSYNTHESIS

Catherine Merry, Tabea Dierker, Parisa Missaghian, Amanda Åhman, Anders Lundequist, Inger Eriksson

Our previous results support a GAGosome model where biosynthesis enzymes are assembled into modifying units and the composition of the unit determines the outcome of biosynthesis.

This model is now being challenged and potential interactions between biosynthesis enzymes are being explored. The role of alternative splicing in regulation of enzyme activity and outcome of biosynthesis is an important part of the project. In addition, we study the altered heparan sulfate biosynthesis in rare diseases caused by mutations in biosynthesis enzymes. Our finding of altered heparan sulfate biosynthesis in Hurler syndrome is also the basis for a more general characterization of this process in other mucopolysaccharidoses, a group of lysosomal storage diseases caused by mutations in glycosaminoglycan degradative enzymes.

MAST CELL PROTEOGLYCANS

Anders Dagälv, Inger Eriksson

Previously, serglycin was the only proteoglycan characterized in mast cells. This proteoglycan is found inside the cells in the granulae where it is essential for the storage of inflammatory mediators. Our preliminary results indicate that mast cells also synthesize cell surface proteoglycans. We are now characterizing these proteoglycans with regard to core protein identity and structural features of their heparan sulfate chains. Mice with targeted mutations in the core proteins identified will be used to study functional aspects of the cell surface proteoglycans.

ZEBRAFISH MODELS FOR HEPARAN SULFATE BIOSYNTHESIS

Beata Filipek-Górniok

The CRISPR-Cas9 technology (clustered regularly interspaced short palindromic repeats) is a powerful method allowing site targeted mutagenesis. In collaboration with Johan Ledin (Zebrafish platform, SciLife) and Shawn Burgees group (NIH, US) we have created zebrafish mutants for the NDST enzymes as well as for two of the PAPS synthases which are now phenoypically characterized.

FUNCTIONAL OVERLAP BETWEEN HEPARAN SULFATE AND CHONDROITIN SULFATE

Tabea Dierker

In collaboration with Andrea Hinas, Department of Cell and Molecular Biology, we use the nematode *C. elegans* to study potential functional overlap between heparan sulfate and chondroitin sulfate. Mutants with defective heparan sulfate biosynthesis show a strong misrouting of motor axons. By introducing chondroitin sulfate sulfotransferases into the mutants we investigate if the routing can be corrected. The nematode synthesizes large amounts of non-sulfated chondroitin, which was the reason for us to select it as a model. However, we have also recently shown the presence of chondrotin sulfate, which has been missed by previous investigators. We are now identifying the responsible sulfotransferases and the core proteins to which the chondroitin sulfate chains are attached.

HEPARAN SULFATE AND HEPARANASE: FUNCTIONS IN HOMEOSTASIS AND DISEASES

Jin-ping Li

The research of this group aims at elucidating functional properties of heparan sulfate (HS) and heparanase in animal development and homeostasis, as well as under pathological conditions. To study the functions of HS in development, we have generated transgenic mice by interfering expression of genes involved in HS biosynthesis and degradation. To study the impact of HS under pathological conditions, we apply these transgenic mice to models of diseases such as inflammation, Alzheimer disease (AD), atherosclerosis and cancer. We also collaborate with clinical researchers to correlate our findings from animal models with human diseases. We focus on two key enzymes involved in HS biosynthesis (glucuronyl C5-epimerase) and modification (heparanase). We use various techniques including biochemical, cellular and immunohistological tools. Animal models, e.g. mouse and *Drosophila,* are employed to study the molecular mechanisms of these enzymes and HS in the disease models.

Members of the group during 2015
Tahira Batool, graduate student
Hao Cui, PhD, post doc (until Feb)
Andreas Digre, graduate student
Jianping Fang, PhD, post doc (until July)
Jin-ping Li, MD, PhD, group leader
Ulf Lindahl, PhD, professor emeritus
Shilpashree Mallesh, master student (until June)
Hiroshi Nakato, PhD, visiting professor (from Aug)
Tianyi Song, graduate student
Ganlin Zhang, post doc (from July)

Project worker during 2015
Anton Forsell (Oct – Dec)
Annelie Barrueta (4 weeks in Oct)
Martina Kerndl, trainee (since Nov)

International exchange during 2015
Jin-ping Li, visited Oncology Department of Beijing Hospital of traditional Chinese Medicine, one week in April, one week in June.
Prof. Guowang Yang (and his 3 colleagues Dr. Jie Yu; Dr. Weiru Yu; Dr. Lin Yang) from Oncology Department of Beijing Hospital of traditional Chinese Medicine visited my lab in September for 2 weeks.

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STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE ENZYMES INVOLVED IN HEPARAN SULFATE BIOSYNTHESIS

Jianping Fang, Tianyi Song

Biosynthesis of HS is a complex process; concerted action of at least 11 different enzymes results in HS polysaccharide chains with a high degree of heterogeneity. Though same enzymes are expressed in all cells, the structure of HS is highly tissue/cell specific. Our primary interest is to find out how the biosynthesis is regulated *de novo*. Approaches to understanding the organization of HS biosynthesis involve characterization of the enzyme complex in the Golgi, denoted as "gagosome".

In this project, particular attention is given to the interactions between enzymes, e.g. GlcA C5-epimerase and O-sulfotransferases by three lines. 1) Overexpression of the enzymes in cell models: The genes coding for GlcA C5-epimerase, 2-O-sulfotransferase and 6-O-sulfotransferases will be introduced into a cell model, individually or in combined form. 2) Using authentic cells isolated from transgenic mice defect in the enzymes: In more complex biological systems, tissues, cells or sub-cellular organelles (membranes, Golgi fractions) derived from transgenic mice (GlcA C5-epimerase KO, heparanase KO and heparanase overexpression) will be used for identification of enzyme complexes (the "gagosome"), using various analytical approaches. 3) *In vitro* studies: recombinant enzymes (GlcA C5-epimerase, HexA 2-O-sulfotransferase and GlcN 6-O-sulfotransferase) are applied to modify polysaccharide substrates for investigation of substrate specificity of the individual enzymes, interaction/regulation of the enzymes in their separate or concerted action towards various substrates and kinetics of the enzymatic reactions.

FUNCTIONS OF HEPARN SULFATE AND HEPARANASE IN CELL SIGNALING AND PROLIFERATION

Tahira Batool, Jianping Fang

Heparanase is an endo-glucuronidase that specifically cleaves HS and heparin polysaccharide chains. Modification of the molecular structures by the action of heparanase modulates the functions of HS. Expression of heparanase is upregulated in most human tumor tissues, correlating with increased metastatic potential, tumor vascularity and poor postoperative survival of cancer patients. A direct role of heparanase in tumor progression was demonstrated by increased tumor angiogenesis and metastasis following overexpression of heparanase in the cells, and by marked decrease in the pro-metastatic and pro-angiogenic potentials of cells subjected to heparanase gene silencing. These findings indicate that heparanase is causally involved in cancer progression.

Our earlier studies revealed that the HS chains isolated from tissues overexpressing heparanase had higher activity to assemble FGF2-FGFR complex, suggesting that heparanase promotes functions of the mitogenic growth factors. In this project, we will continue the studies by examination of heparanase effect on cell activities. Tumor cells expressing high or low levels of heparanase are examined for signaling activities of growth factors, e.g. FGF2, VEGF and TGF, as well as cell proliferation and migration. HS structures expressed in the cells will be characterized.

DEVELOPMENT OF HEPARANSE INHIBITORS FOR TREATMENT OF TUMOR METASTASIS

Ganlin Zhang, Tahira Batool

Up-regulated expression of heparanase has been detected in a number of cancers, including lung cancer, breast cancer, mesothelioma, glioma, gastric, pancreatic cancer, arguing for the enzyme as a reactant protein involved in pathogenesis of tumor development and metastasis. Clinical observations confirm that the level of heparanase expression of heparanase is associated with enhanced tumor metastasis and poor prognosis, making heparanase a promising target for treatment of cancer. Several HS mimetics have been developed and are under clinical evaluation.

Traditional Chinese Medicine, e.g. herbal extracts, has been used for treatment of various cancers for years in China. Application of such herbal drugs along with chemotherapy has significantly improved survival rate and life quality of cancer patients. As Glycans are major components in the water extract of herbs, our hypothesis is that the glycans in the herbal medicines may though either inhibition of heparanase or interfering with HS-chemokine interactions. This project aims: 1) to explore the implications of heparanase in tumor metastasis models (primarily Louise lung carcinoma and breast cancer) using our transgenic mouse models; 2) to evaluate effect of herbal medicines and other HS-mimetics in the tumor metastasis model.

IMPLICATIONS OF HEPRANASE IN THE PATHOLOGY OF RHEUMATOID ATHRITIS

Andreas Digre

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by aggressive proliferation of synovial tissue (ST), which leads to destruction of bone and cartilage in joints. Early changes in the synovia are characterized by revascularization, a marked infiltration of inflammatory cells, and associated synoviocyte hyperplasia. Recent study revealed that heparanase level is dramatically increased (more than 100-fold) in the synovial fluid of rheumatoid arthritis (RA) patients. However, the underlying mechanisms are unknown. Our major question is: *what is the role of heparanase in RA pathology?* We will address this question by applying our unique transgenic mice that are either overexpressing or lacking heparanase for collagen II-induced RA mouse model.

HEPARAN SULFATE AND HEPARANASE IN LIPID METABOLISM AND ATHEROSCLEROSIS

Tianyi Song, Ganlin Zhang

Atherosclerotic cardiovascular disease is one of major cardiovascular diseases affecting more and more people globally. The disease is pathologically characterized by formation of atherosclerotic lesions that are typically asymmetric focal thickenings of the vessel wall intima. This pathological process involves a complex interplay between lipid metabolism, vessel injuries and inflammation, resulting in the lesions consisting of inflammatory and immune cells, lipids, endothelial and smooth muscle cells as well as proteoglycan complex, e.g. HSPG. Contradictory suggestions have been reported for the roles of HSPG in this pathophysiological process; some studies found HSPG being anti-atherogenic through inhibition of monocytes adhesion and smooth muscle cell (SMC) growth; while others reported HSPGs having pro-atherogenic effects in mouse models. Thus, more studies are needed for clarification of the pathophysiological functions of HSPG in atherosclerosis.

Our earlier study found increased expression of heparanase in symptomatic carotid atherosclerosis. It is known that acute coronary syndrome and stroke are associated with 'vulnerable plaques' that tend to rupture under certain circumstances. A key aspect of stabilizing these plaques is to have an intact and stable ECM structure that is mainly composed of collagens and HSPG. Our hypothesis is that heparanase degrades HS in the ECM structure of plaques can lead to formation of vulnerable plaques. First, we will examine the effect of heparanase on lipid metabolism by feeding mice with high fat diet and in primary cultured adipocyte cells.

STRUCTURE AND FUNCTIONS OF HEPARAN SULFATE IN AMYLOIDOSIS

Andreas Digre

"Amyloidosis" refers to a clinical condition encompassing a group of more than 20 post-secretory protein-misfolding diseases. In these diseases, proteins that are normally soluble undergo aggregation to form insoluble fibrils and are accumulated in the extracellular space (also intracellular) of affected tissues or organs. A common feature of all amyloidosis diseases is the presence of HS-proteoglycans (HSPGs) in the deposited amyloid plaques. HS and HSPGs appear not to be merely passive components of amyloid deposits but rather play functional roles in the pathophysiological process of amyloidosis. Two types of amyloid diseases that have a broad clinical and social impact are Alzheimer's disease (AD) and type 2 diabetes.

We primarily focus on Alzheimer's disease (Aβ deposition in the brain), type II diabetes (IAPP deposition in the pancreas) and inflammation associated amyloid A (SAA) deposition in the spleen/liver/kidney. Approaches taken include: **a)** *in vitro* studies to investigate the effects of HS and heparin in aggregation of the amyloid peptides, with regard to HS/heparin chain length and sulfation pattern; **b)** cellular studies to find out the roles of cell surface HS for internalization and toxicity of the amyloid peptides; different cell models with distinct HS property are used; **c)** animal models to address the *in vivo* functional roles of HS in amyloidosis.

DROSOPHILA MODELS FOR INVESTIGATION OF ALZHEIMER DISEASE

Hiroshi Nakato, Martina Kerndl

Alzheimer's disease (AD) is **the most common form of dementia, which causes problems with memory, thinking and behavior.** Neuropathological hallmarks of AD include extracellular amyloid plaques and intracellular neurofibrillary tangles. Plaques are primarily composed of Amyloid-β peptides (Aβ) generated by proteolytic cleavage of Amyloid Precursor Protein (APP). Aβ is a heparin/heparan sulfate (HS)-binding protein, and HS is believed to affect multiple aspects of AD pathogenesis.

Drosophila genetics has been used as a powerful model for AD research. *There are established fly lines in which secreted* Aβ peptides are expressed in a specific tissue/organ. Neuronal expression of Aβ42 caused its intra- and extracellular accumulation, leading to neurotoxicity, locomotion defects and reduced lifespan. To elucidate the molecular mechanisms by which HS affects AD pathogenesis, we will use molecular genetic tools available in *Drosophila* to manipulate HS structures in vivo, and determine their effects on the phenotypes produced by Aβ expression. The hypothesis to be tested is that specific HS fine structures play critical roles at different steps of AD pathogenesis.

THE INVOLVEMENT OF PROTEOGLYCANS AND GLYCOSAMINOGLYCANS IN CANCER AND ANGIOGENESIS

Maria Ringvall

The establishment of a tumor and further progression into a malignant cancerous lesion is dependent on several processes such as dysregulated proliferation, inflammation and angiogenesis. An array of proteins whereof some are proteoglycans are involved in these processes. Proteoglycans are a group of molecules with glycosaminoglycan (GAG) sugar chains attached to a core protein and alterations in expression, structure and glycanation status have been seen in relation to cancer. With the exception of hyaluronan, all GAGs are sulfated and the degree and pattern of sulfation can regulate the capacity to bind to other proteins. Proteoglycans are found in the extracellular matrix, cell surface or intracellularly and are expressed by virtually all cel types. Heparan sulfate (HS) is the most abundant GAG and binds proteins such as growth factors and cytokines in the exracellular matrix and is known to be involved in regulation of cell surface ligand-receptor interactions and activation. Chondroitin sulfate (CS) is another abundant GAG, also with the capacity to bind many different proteins, although the role of these interactions are less well studied than for HS.

Members of the group during 2015
Maria Ringvall, PhD, associate professor
Andrew Hamilton, PhD, post doc
Vladimir Basic, PhD, post doc
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Stiftelsen för forskning om proteoglykaner

THE INVOLVEMENT OF PROTEOGLYCANS AND GLYCOSAMINOGLYCANS IN CANCER AND ANGIOGENESIS

Maria Ringvall

The establishment of a tumor and further progression into a malignant cancerous lesion is dependent on several processes such as dysregulated proliferation, inflammation and angiogenesis. Anarray of proteins whereof some are proteoglycans are involved in these processes. Proteoglycans are a group of molecules with glycosaminoglycan (GAG) sugar chains attached to a core protein and alterations in expression, structure and glycanation status have been seen in relation to cancer. With the exception of hyaluronan, all GAGs are sulfated and the degree and pattern of sulfation can regulate the capacity to bind to other proteins. Proteoglycans are found in the extracellular matrix, cell surface or intracellularly and are expressed by virtually all cel types. Heparan sulfate (HS) is the most abundant GAG and binds proteins such as growth factors and cytokines in the exracellular matrix and is known to be involved in regulation of cell surface ligand-receptor interactions and activation. Chondroitin sulfate (CS) is another abundant GAG, also with the capacity to bind many different proteins, although the role of these interactions are less well studied than for HS.

EFFECTS OF HEPARAN SULFATE MIMETICS ON ANGIOGENESIS

Andrew Hamilton, Maria Ringvall

Angiogenesis, the formation of new blood vessels, is an important element during embryo development and wound healing but is additionally a highly active process in different pathological states such as cancer and rheumatoid arthritis. Several signaling systems involved in angiogenesis, such as fibroblast growth factor, vascular endothelial growth factor and platelet derived growth factor can be regulated by interactions between HS and the ligand, or formation of a ternary complex consisting of the ligand and its receptor. The binding properties of HS to other proteins are dependent on the sulfation status of the polysaccharide chain where both total amount of sulfation and sulfation pattern along the glycosaminoglycan chains are of importance. The potential for heparan sulfate to regulate different systems that act during pathological conditions has put forward small, synthetic heparan sulfate mimetics as interesting for drug design. We are now using a group of HS mimetics to evaluate their effect on regulation of the angiogenic process. The design of these mimetics is based on exact positioning of defined sulfated domains separated by a spacing linker molecule. Preliminary results show that such mimetics can have an effect on physiological angiogenesis and we are now continuing to study this aspect together with their effects on pathological angiogenesis.

THE ROLE OF SERGLYCIN IN CANCER

Andrew Hamilton, Vladimir Basic, Maria Ringvall

Serglycin, predominantly a CS-proteoglycan, is the only proteoglycan with a manifested intracellular function where it is known to aid in storage of compounds such as proteases and amines. Another recently discovered function for serglycin is at the cell surface where it can block effector molecules from reaching their targets at the plasma membrane. Serglycin is

mainly expressed by different immune cells such as mast cells, neutrophils and macrophages and expression of serglycin has also recently been noted in some cancer cell types. The expression level seems to affect the behavior of these tumor cells and a high expression correlates with a more aggressive phenotype.

We use different *in vivo* and *in vitro* model systems to gain more information about the role of serglycin in tumorigenesis and have seen that serglycin affects both tumor cell behavior and angiogenesis.

WHAT ARE GLYCOSAMINOGLYCANS GOOD FOR?

Dorothe Spillmann

Our main focus addresses questions how glycosaminoglycans (GAGs), negatively charged, long carbohydrate chains protruding from all cell membranes and intercalated in extracellular matrices, affect diverse cellular processes. GAGs can serve as adhesion sites, co-receptors, stabilizers of molecular interactions, protectors against proteolytic degradation and many more functions. Each cell and tissue produces distinct collections of them with a sophisticated set of enzymes. Absence of GAGs will lead to developmental failure and death during embryogenesis, while structural alteration may be encountered in parallel to disturbed homeostasis in pathologic conditions in adult organisms. Thus, one may wonder whether alterations are cause or result and whether and how GAGs can affect the organism in health and disease.

We recognize GAGs, predominantly heparan sulfate (HS) and chondroitin sulfates/dermatan sulfate (CS/DS), as tuners of molecular interactions at cell surfaces and in the matrix to allow for robust cellular interplay. To approach our hypotheses we analyze structural features of GAGs from different sources, during various physiological and pathological conditions and correlate structural with functional properties apparent in these situations. We also deliberately modulate the expression of GAGs in model systems to check the influence of qualitative and quantitative structural changes on cellular functions. Such we can analyze how cells are affected by altered structures and how these changes translate at organism level during specific phenomena, *e.g.* regeneration processes in invertebrates.

Thus, our goals are to elucidate the underlying mechanisms how HS and CS/DS structures modulate cellular behavior and communication, of critical importance to understand the control of physiological and pathological processes in multicellular organisms.

Members of the group during 2015
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Ulf Lindahl, professor emeritus
Ramesh Babu Namburi, graduate student

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Agencies that support the work
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GLYCOSAMINOGLYCANS IN LIMB REGENERATION

Ramesh Babu Namburi, Ulf Lindahl, Dorothe Spillmann

Many brittle stars, stellate marine invertebrates found in most parts of the world, have the capacity to autotomize their arms upon predator action followed by regeneration of the lost limb. This regeneration process resembles at least in part a recapitulation of developmental processes. We therefore analyze what role GAGs play for the regenerative capacity of these animals [collaboration with S. Dupont and O. Ortega-Martinez, Kristineberg, GU]. Brittle star species produce remarkably highly sulfated CS/DS chains correlated with an exceptional limb regeneration capacity. GAG structures change during regeneration after induction of experimental autotomy in arm tissue. During the process GAG sulfation is increased, and conversely, regeneration experiments with interference in biosynthetic sulfation results in a dramatic impairment of arm regeneration by severely affecting cell proliferation. We could demonstrate that several evolutionary conserved morphogens/growth factors interact with these polysaccharides and we therefore aim to identify the corresponding biosynthetic genes in the brittle stars to study their regulation.

MICROBIAL INTERACTION WITH GLYCOSAMINOGLYCANS

Ramesh Babu Namburi, Dorothe Spillmann

As GAGs are prominently exposed on every cell in the body it is not surprising that microbes also make use of these structures to interact with their hosts. During the past years we have mainly focused on studying the effect of host-microbe interactions in order to develop antagonists for GAG based receptors and prevent or reverse microbial attack while avoiding overt interference with endogenous processes leading to *e.g.* the development of a treatment approach to reverse symptoms of severe malaria [collaboration with A. Leitgeb, Dilaforette, and M. Wahlgren, KI, Stockholm].
Recently we have shifted focus and started to characterize enzymes used by symbiotic bacteria [collaboration with O. Berteau, INRA, Jouy-en-Josas, France, and M. Rossi, University of Helsinki, Finland]. Sulfatases and lyases are among enzymes that commensal bacteria need for their survival. The main goal to characterize these types of enzymes is to improve our understanding of successful host-microbe symbiosis, but also to identify potential pathological twists that may go along their use by these microbes. In addition we also gain valuable tools that are of analytical value for characterization of GAGs of different origins.

CHARACTERIZATION OF GLYCOSAMINOGLYCANS

Dorothe Spillmann

The possibility to analyze GAG structures from different sources is a crucial requirement to correlate structure/function aspects of GAGs in different context. It is therefore important to have the analytical tools to characterize cells or tissues for their GAG production under different conditions. We aim at improving our high-throughput analysis technique for compositional analyses of GAGs to further applications and optimize for diverse sample sources. As complementation of our analytic possibilities we set up a quick, semi-quantitative screening method for large sample numbers based on mass spectrometry together with M. Ramström Jonsson and J. Bergquist at the Dept. of Chemistry, UU.
On collaborative basis we isolate and characterize GAGs from a wide spectrum of sources for different projects.

MEDICAL PROTEIN CHEMISTRY

Per Jemth, Birgitta Tomkinson, Leif Andersson

Proteins are essential to all life. They catalyse virtually all chemical reactions in the cell and they govern scaffolding and signalling. Protein chemistry is therefore central to life sciences. In essence, results generated in fields such as genetics, cell biology and bacteriology can only be understood at a molecular level if we understand the structure and function of the proteins involved. Thus, for a profound understanding of any biological phenomenon a solid knowledge in protein science is imperative. Such basic knowledge is not only vital to gain through research but also crucial to convey to students in life sciences.

Here at IMBIM three groups pursue fundamental research as well as teaching on both enzymes and non-catalytic proteins. The Jemth group looks at protein folding and protein ligand interactions and tries to unravel basic and general concepts about the action and evolution of proteins. In a second programme, the group focuses on proteins from human papillomavirus with the long term goal of preventing cancer caused by the virus. The Tomkinson group works on a huge and enigmatic enzyme, tripeptidyl-peptidase II, to reveal the molecular details of the catalysis as well as its physiological role. This enzyme is ubiqutious among eukaryotes and bigger than the ribosome! Finally, Leif Andersson, professor in functional genomics at IMBIM uses state-of-the-art proteomics to follow up findings from their genomic work.

The three groups ask different questions on various biological systems but share the common goal of understanding protein function at the level of molecular and atomic resolution. They also share the common goal of teaching undergraduate students fundamental biochemical principles and mechanisms. The aim is to make students in three different programmes (medicine, biomedicine and biomedical laboratory science) understand complex biological phenomena through basic concepts.

STRUCTURE-FUNCTION RELATIONSHIPS OF PROTEINS

Per Jemth

The ultimate goal of our research is to better understand fundamental structure-function and structure-reactivity relationships in proteins. Our research focuses on the molecular details and evolution of protein-protein interactions, protein folding and allostery. We use protein engineering and biophysics to dissect the chemical reactions of proteins.

We use a number of model systems to address fundamental questions. These model systems are small protein domains from modular proteins, with special focus on intrinsically disordered protein domains. The lab is also running a project on proteins from human papillomavirus, in particular E6. Certain strains of human papillomavirus cause cancer, for example cervical cancer, and this discovery was awarded the Nobel prize in physiology or medicine in 2008. The E6 protein is a so-called oncogene, and a major culprit in the carcinogenesis. We want to inhibit its interaction with cellular proteins with the long-term goal of treating persistent infection.

Members of the group during 2015
Per Jemth, associate professor, group leader
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Emma Åberg, PhD student
Eva Andersson, research assistant
Fulvio Saccoccia, post doc
Jakob Dogan, post doc
Mikael Malmqvist, post doc
Nicole Lin, student

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Agencies that support the work
The Swedish Research Council
The Cancer Society

PROTEINS: FOLDING, STABILITY, INTERACTIONS AND EVOLUTION

Proteins govern all of life's chemical reactions and they generally do so by first folding into precise three dimensional (3D) structures dictated by their amino acid sequences. But, to great surprise for most scientists, we have learned during the last 15 years that as much as 25% of our proteome is not folded, instead these proteins are disordered. Such intrinsically disordered proteins (IDPs) lack, in part or completely, a well-defined 3D structure. There is now a tremendous interest in understanding structure, function and dynamics of IDPs. However, despite this intense interest of the last couple of years, there is still a marked paucity of experimental data regarding the many aspects of how this disorder influences the function of IDPs and how functional disorder has evolved.

We address these questions using a combination of biophysics, protein engineering and phylogenetic methods. We use different model systems, but up to now most work has been done on domains from transcriptional co-regulators such as the nuclear co-activator binding domain of CREB binding protein (NCBD). A new and exciting line of research that we are currently pursuing is resurrection of ancient proteins, in an effort to understand how intrinsically disordered regions have evolved.

HUMAN PAPILLOMAVIRUS AND CANCER: DESIGN OF A PROTEIN DRUG

The role of human papillomavirus (HPV) in cervical cancer was demonstrated by Harald Zur Hausen who was awarded the Nobel prize in physiology or medicine in 2008. It is now clear that other cancers are also caused by HPV. HPVs are classically divided into two groups: “low risk” and “high risk”. The “high risk” is based on prevalence ratio in cancer, with HPV16 being the most common cause of cervical cancer. HPV utilizes mainly two proteins to immortalize infected cells, and they are called E6 and E7. These two proteins bind to a number of proteins involved in cell cycle regulation, for example p53 and retinoblastoma tumor suppressor. We are looking at the molecular mechanisms of interactions between the HPV E6 and cellular targets and how this can be utilized to design a protein drug. We hope that our basic research will open up new routes for treating HPV infection.

STRUCTURE, FUNCTION AND PHYSIOLOGICAL ROLE OF TRIPEPTIDYL-PEPTIDASE II

Birgitta Tomkinson

Intracellular protein degradation is as important for regulating the concentration of specific proteins in the cell as protein synthesis, but much less well characterized. Protein degradation is malfunctioning in a number of diseases such as cancer, muscle wasting and Alzheimers disease. Tripeptidyl-peptidase II (TPP II) is an important player in intracellular proteolysis, and our ultimate goal is to determine the specific physiological role of the enzyme in this process. TPP II is a huge enzyme complex with a widespread distribution in eukaryotic cells and the ability to cleave oligopeptides into tripeptides. Our main focus is a biochemical characterization of TPP II, in order to investigate how its substrate specificity is determined and how oligomerization is regulated. We are also investigating how expression of this enzyme varies in different cell types. These investigations will provide a basis for future drug discovery efforts. Since TPP II appears to be important for inactivation of the neuropeptide cholecystokinin and also for tumour progression, it is a potential drug target.

Members of the group during 2015
Birgitta Tomkinson, professor, group leader
Emma Vianden, research assistant

Project workers during 2015
Emma Vianden, "Characterization of a missense mutation in Tripeptidyl-peptidase II."
Matilda Widerström: Tripeptidyl-peptidase II: studies of an interesting enzyme that takes part in intracellular protein turnover (SOFOSKO, part 2).

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Agencies that support the work
The Medical Faculty

CHARACTERIZATION OF TRIPEPTIDYL-PEPTIDASE II AND INVESTIGATION OF STRUCTURE AND FUNCTION OF TPP II

Birgitta Tomkinson, Emma Vianden, Matilda Widerström

This project focuses on the relationship between structure and function in TPP II.

These studies are important not only for understanding the physiological role of the enzyme, but also in designing drugs targeting TPP II.

Previously, the endopeptidase activity and the pH-dependence of TPP II was investigated. The results have given some insights into the structure of the active site, and have been expanded with experiments using enzyme variants with point mutations. More importantly, we have established tools to be used for investigations of structure-function relationships.

Various collaborations have also been instigated, exploiting the current knowledge of structure-function relationships for TPP II. Thus, in collaboration with Eric Reits, University of Amsterdam, a specific irreversible inhibitor of TPP II has been characterized and in collaboration with Alexander Zimprich, Medical University of Vienna, a mutant enzyme with a potential role in a neurodegenerative disease has been partly characterized. This work will be continued and investigations aimed at examining the oligomerization of TPP II and if this is a way of regulating enzyme activity in vivo are also under way.

TPP II, CANCER AND PROTEIN-PROTEIN INTERACTIONS

Matilda Widerström, Birgitta Tomkinson

TPP II activity is increased in some cancer cells e.g. Burkitt´s lymphoma, and an overexpression of TPP II increases the risk for chromosomal damage as the enzyme appears to protect tumour cells from apoptosis. Therefore TPP II could be a potential marker for malignant tumours. In order to investigate this, a screening method for the expression of TPP II will be developed. A real-time PCR method has been developed in order to measure the amount of mRNA encoding TPP II in different tumour cells. In addition, an assay measuring enzyme activity has been evaluated and used for comparison of results. The different methods have been compared to determine if the amount of mRNA, protein or active enzyme is correlated to tumour malignancy. In a pilot experiment, the amount of TPP II was quantified in blood samples from healthy individuals and patients with different haematological diagnoses. These results will now be used as a starting point for an investigation with a lager number of patient samples.

Interactions between TPP II and the proteasome is also investigated, in order to determine if the increase in TPP II amounts is a secondary effect, for example as a result of decreased degradation or impaired proteasome-function.

Furthermore, a former member of the group, Jarmila Nahalkova, has performed a protein-protein interaction study to identify the involvement of TPP II in known signalling pathways of human cells. The results of co-immunoprecipitation assays (co-IP) and/or Proximity Ligation Assays (PLA) showed that TPP II interacted physically with the tumour suppressor MYBBP1A, a protein having an activating effect on p53, and the cell cycle regulator CDK2. Interactions between TPP II, p53 and SIRT7 were also detected.

Thus, interactions of TPP II were detected with proteins having functions in tumour suppression and neuroprotection, which could suggest that TPP II has potential regulatory effect on cell cycle, apoptosis and senescence, beside its proteolytic function. The mechanism for this is not known and merits further investigations.

TUMOR BIOLOGY

Aristidis Moustakas, Staffan Johansson, Anna-Karin Olsson, Maria Ringvall, Christian Sundberg, Kristofer Rubin

IMBIM researchers that staff the cluster known as Tumor Biology focus on cancer, its manifestations at the basic cellular level and at the pre-clinical, more translational level. Cancer research permeates all areas of the biological sciences; however, the Tumor Biology unit specializes on mechanistic studies of cell communication and on the organization of the tumor tissue in diverse types of cancer. Architectural tumor tissue organization, signal transduction and cell differentiation, tumor cell invasiveness and tumor stromal constituent functions, including platelets, blood vessels and extracellular matrix (ECM), are the principal areas that the constellation emphasizes in its research. Clear priority is given to molecules that empower us with prognostic and therapeutic potential for the future analysis and treatment of cancer.

More specifically, the impact of tumor-induced platelet and neutrophil activation on tumor progression, vascular function and metastasis is investigated (*Olsson*). The contributions of major constituents of the tumor surface and extracellular milieu, such as integrin receptors, the proteoglycan serglycin, collagens and fibrin, is explained based on research of the cluster (*Johansson, Ringvall, Rubin*). Such extracellular molecules but also growth factors orchestrate changes in tumor cell differentiation that facilitate invasiveness and cooption of the tumor vasculature, helping the progression of tumor malignancy (*Moustakas, Olsson, Sundberg*). Inspired by basic findings, the cluster engages also into pre-clinical activities by targeting fibronectin, a major matrix protein, by targeting processes of tumor cell differentiation using chemical inhibitors, and tumor blood vessels and their surrounding ECM within a given tumor tissue (*Moustakas, Olsson, Rubin*).

ADHESION-DEPENDENT CELL SIGNALING

Staffan Johansson

Adhesion of cells to specific proteins in the extracellular matrix contributes to the organization of tissues. In addition, it provides the cells with information about the molecular and physical properties of the surrounding environment, which is important for their migration, differentiation, and proliferation. Integrins are a family of cell adhesion and migration receptors of crucial importance for several adhesion-related events of clinical relevance, such as wound healing, angiogenesis, thrombus formation, leukocyte extravasation to inflammatory sites, and tumor metastasis. Our work is focused on integrin signals involved in adhesion-dependent cell survival and proliferation.

Members of the group during 2015
Staffan Johansson, professor, group leader
Xiaofang Cao, postdoc (until March)
Deepesh Gupta, PhD student
Ying Huang, post doc
Siamak Kamranvar, post doc

Project worker during 2015
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Agencies that support the work
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REGULATION OF SURVIVAL, MIGRARATION AND CYTOKINESIS BY INTEGRINS

Xiaofang Cao, Deepesh Gupta, Ying Huang, Siamak Kamranvar

A. Adhesion-dependent survival. A central step in integrin-mediated signaling is the activation of PI3 kinase, a key reaction for adhesion-dependent cell survival, actin polymerization, as well as several other signaling pathways. We identified p110α as the catalytic isoform of the PI3 kinase family that is activated by β1 integrins and in detail characterized the downstream pathway to the activation of AKT1/2. Analysis of the requirement of RICTOR, ILK, PAK1/2, FAK, PYK and SRC for AKT Ser473 phosphorylation downstream of β1 integrins, LPA receptors (G protein-coupled), PDGF and EGF receptors (tyrosine-kinases) revealed that the mechanism is more complex than commonly depicted and varies depending on the stimulated receptor. A yet unidentified receptor for the enzyme heparanase was shown to closely cooperate with and depend on integrins for the activation of the PI3 kinase/AKT pathway. We also have indications that TGFβ-induced activation of AKT may depend on integrin-mediated adhesion, and presently we try to characterize these connections.

B. Mechanosignaling. Another important function of integrins is to serve as "mechanoreceptors" for forces acting on the cell. Our results show that the signaling responses to physical force during cell stretching are not the same as the responses to ligand-binding during cell adhesion. Thus, events generally referred to as "integrin signals" are actually composed of separate sets of reactions triggered by different types of integrin stimulation, i.e. integrin clustering by ligands and unfolding of integrin-associated proteins by force. The phosphorylation of ERK1/2 appears to be a particularly stretch-responsive signal, and further studies are required to understand the mechanism for this force-induced ERK activation.

In collaborative studies we analyze the signaling pathways resulting in inflammation and water accumulation in lung alveoli during mechanical ventilation in intensive care situations (in a pig model). In particular, the importance of integrin stimulation and TGFβ activation for the clinical problem is investigated.

Syndecan 4 is known to work together with integrins to organize focal contacts and actin filaments. We investigate the possible role of syndecan 4 in the generation of signals during cell attachment or cell stretching using syndecan 4 knockout MEFs. The absence of syndecan 4 affects several phosphorylation reactions and strongly reduces the rate of actin polymerization induced by β1 integrin stimulation. The latter reaction is monitored as lamellipodia protrusion during cell attachment with TIRF microscopy. The mechanisms underlying these observations are investigated with a focus on the role of the syndecan-regulated Ca^{2+} channel TRPC7.

C. Cytokinesis. Cytokinesis of normal adherent cells requires signals from integrins, and the lack of such signals in detached cells causes binucleated cells. Our data shows that a new round of the cell cycle still will proceed in the absence of cytokinesis, and that cytokinesis will resume uncoupled from karyokinesis if such cells reattach. Although most of the reattached cells divide successfully an increased number of permanently binucleated cells are formed, a feature known to cause aneuploidy and chromosomal instability. We have also shown that the cytokinesis block in suspended cell occurs at a late step, impairing the recruitment of ALIX and TSG101 to CEP55 at the midbody. We are presently defining the failing step further and try to clarify the integrin signaling pathway regulating this reaction. So far we have found that integrin signaling affects CEP55 localizaion through a pathway from FAK-Src to PLK1, a kinase known to regulate CEP55 function,

D. Adenoviral tumor therapy. See Catharina Svensson for a collaborative project on a mouse model for human adenovirus in tumor therapy.

SIGNAL TRANSDUCTION AND EPITHELIAL PLASTICITY

Aristidis Moustakas

Introduction
Our research program concentrates on novel aspects of signal transduction and basic cancer biology. We study the developmental process of epithelial-mesenchymal transition (EMT) and its links to tumor metastasis and cancer stem cell biology. EMT confers upon cancer cells capacities that are required for metastasis. We aim at explaining how Transforming Growth Factor β (TGFβ) can promote tumor invasiveness by inducing EMT, and how EMT or local invasiveness contributes to tumor growth. This work is complemented with attempts to generate new drugs against EMT, and to develop new biomarkers with clinical significance.

The lab focuses on major developmental signaling pathways including those of TGFβ and bone morphogenetic protein (BMP). We also specialize on studies of regulation of gene expression at the transcriptional and post-transcriptional level. These signaling pathways are regulated at multiple points inside the cell, and for this reason we also investigate how cytoplasmic signaling events, such as mechanisms of cell polarity regulation by the tumor suppressor kinase LKB1 and its downstream effectors, the AMP-regulated kinase (AMPK) family, control the activities of the TGFβ and BMP pathways.

TGFβ and BMP regulate cell growth, differentiation, and tumorigenesis via a group of proteins known as Smads and via protein kinase and GTPase pathways. After activation from the type I and type II TGFβ receptors on the cell surface, the Smads and protein kinases accumulate into the cell nucleus, where they regulate gene expression. TGFβ suppresses the development of early stage tumors, but promotes progression of advanced tumors. A relatively deep understanding of the mechanisms that mediate the tumor suppressor actions of TGFβ has been achieved; however, the mechanisms whereby TGFβ promotes tumor progression and metastasis demand further investigation. We investigate the function and regulation of various TGFβ/BMP-responsive genes by combining functional experiments with genome-wide expression and location analysis in several tumor models. This approach has allowed us to decipher key steps in the molecular programs that mediate tumor suppression or tumor progression in response to TGFβ and BMP.

Members of the group during 2015
Claudia Bellomo, PhD student
Laia Caja Puigsubirà, post doc
Mahsa Shahidi Dadras, PhD student
Aristidis Moustakas, professor
Kalliopi Tzavlaki, PhD student

Project workers during 2015
Anna Webb, thesis project and SOFOSKO summer student (Jan - Aug 2015).

International exchanges 2015
Georgios Divolis, visiting PhD student (from Sep 2015).
Kalliopi Tzavlaki, visiting PhD student at Tsukuba Univ., Japan (Aug - Oct 2015)

National exchange 2015
Claudia Bellomo, visiting PhD student at Alligator Bioscience AB, Medicon Valley, Lund (Sep - Nov 2015)

Satellite group at the Ludwig Cancer Research (LCR) during 2015
Members of the group
Jonathon Carthy, post doc (until May 2015)
Ulla Engström, technician
Kaoru Kahata, post-doc (until Mar 2015)
Constantinos Kolliopoulos, PhD student (50% with Evi Heldin's group)
Varun Maturi, PhD student
Anita Morén, technician
Panagiotis Papoutsoglou, PhD student
Yutaro Tsubakihara, post doc (from Apr 2015)

International exchange at LCR
Paris Pallis, visiting Master's student student, Erasmus program, Univ. of Vienna, Austria (Jan - June 2015).
Natasa Sopaki-Valalaki, visiting Master's student, Erasmus program, Univ. of Patras, Greece (Oct 2015 to Jan 2016).

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Agencies that support the work

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REGULATION OF TGFβ/BMP RECEPTOR SIGNALING

Mahsa Shahidi Dadras, Kalliopi Tzavlaki

We study the tumor suppressor kinase liver kinase B1 (LKB1) and two of its downstream protein kinases, members of the AMP-regulated kinase (AMPK) family. These are the salt-inducible kinase 1 (SIK1) and the Nuak2 kinase, whose genes are immediate-early targets of TGFβ signaling. We gradually uncover the molecular links between TGFβ, BMP and LKB1/SIK1/Nuak2 signaling by focusing on mechanisms of receptor function and trafficking. LKB1 negatively regulates BMP receptors, a process important during Drosophila organogenesis and lung cancer progression. The LKB1 kinase forms complexes with the BMP type I receptors and together with the inhibitory Smad7 causes ubiquitination of the receptors. Similar to LKB1, SIK1 regulates turnover of the TGFβ receptor after ligand binding by cooperating with Smad7 and ubiquitin ligases of the Smurf family.
This work has been partially carried in collaboration with Anita Morén, Drs. Erna Raja and Carl-Henrik Heldin (LCR-Uppsala Univ.), Dr. Patrick Micke (Dep. of Immunology, Genetics and Pathology, Uppsala Univ.), Dr. George Pyrowolakis (Freiburg Univ., Germany) and Dr. Ola Söderberg (Dep. of Genetics and Pathology, Uppsala Univ.).

MOLECULAR MECHANISMS OF EPITHELIAL-MESENCHYMAL TRANSITION (EMT)

Claudia Bellomo, Mahsa Shahidi Dadras, Kalliopi Tzavlaki

EMT is an important process during cancer dissemination and contributes to the generation of cancer stem cells. In our recent work, we analyzed the role of LKB1 and SIK1 kinases in regulating critical aspects of the EMT process, including cell polarity. We identified new substrates of SIK1 as proteins that regulate the cytoskeleton and epithelial polarity. SIK1, via phosphorylation, causes proteasomal degradation of its substrates and promotes EMT. Inhibitors of the SIK1 kinase would perturb the EMT response. We also study the role of LKB1 in controlling epithelial differentiation by knocking out the LKB1 gene in three dimensional, polarized epithelial cell models, using CRISPR/Cas9 technology. In our project under the European Marie Curie ITN project “IT-Liver”, we study liver cancer cells at different stages of differentiation for sensitivity or resistance to a panel of compounds that either affect the differentiation of these cells or cause synthetic lethality together with TGFβ. We characterize new molecular pathways that may mediate such responses of the liver cancer cells and have been focusing on the nuclear receptor superfamily of transcription factors and the control of enzymes of lipid biogenesis.

This work has been partially carried in collaboration with Drs. Jonathon Carthy and Carl-Henrik Heldin (LCR-Uppsala Univ.), Drs. Andrew Shiau and Timothy Gahman (LCR-San Diego, USA), Dr. Martin Stöter (Max Planck Institute, Dresden, Germany), Dr. Steven Dooley (Clinical Medicine at Mannheim, Heidelberg Univ., Germany), Dr. Isabel Fabregat (Institute for Biomedical Investigations at Bellvitge, Barcelona, Spain), Dr. Wolfgang Mikulits (Medical Univ. of Vienna, Austria) and with Drs. Christer Busch, Patrick Micke and Fredrik Pontén (Dep. of Immunology, Genetics and Pathology, Uppsala Univ.).

LINKS BETWEEN INVASION AND SELF-RENEWAL OF TUMOR INITIATING CELLS

Laia Caja Puigsubirà, Mahsa Shahidi Dadras, Georgios Divolis, Kalliopi Tzavlaki

In addition to carcinoma (epithelial) tumors, we study brain tumors, such as glioblastoma multiforme, GBM. We try to explain why BMP signaling suppresses stemness of tumor-initiating cells in GBM, and we examine whether BMP signaling affects tumor cell invasiveness. Our strategy is based on genome-wide screens for mRNA expression under the control of BMP7 or transcription factors acting downstream e, such as Snail1, and mouse xenograft models of GBM. Our work showed that Snail1 enhances astrocytic differentiation and invasiveness and suppresses the self-renewal capacity of GBM cancer stem cells. In addition, we identified feedback regulatory loops between BMP, TGFβ signaling and Snail1 transcription. Three specific target genes of Snail1 that contribute to GBM cell invasiveness or GBM cancer stem cell maintenance are studied functionally. Finally, we analyze the roles of the LKB1 kinase and the Par3 polarity protein in GBM cell invasiveness.

This work has been partially carried in collaboration with Drs. Jessica Cedervall and Anna-Karin Olsson (Dep. of Medical Biochemistry and Microbiology, Uppsala Univ.), Drs. Lene Uhrbom, Bengt Westermark and Karin Forsberg-Nilsson (Dep. of Immunology, Genetics and Pathology, Uppsala Univ.).

SUMMARY OF ACTIVITIES AT THE LCR

We completed our studies on ADP-ribosyl-transferases PARP-1 and PARP-2 and the deribosylating enzyme PARG, which regulate nucleosome assembly and transcriptional initiation and elongation. We have shown that such nuclear enzymes organize integrated biological responses by modulating the activity of TGFβ and BMP signaling during cell differentiation. We also analyze the genome-wide location of major EMT transcription factors in breast cancer cells, while analyzing the role of specific long non-coding RNAs on EMT and breast cancer cell stemness. New breast cancer cell models that can probe EMT in vivo have been generated using molecular beacons. The chromatin regulator high mobility group A2 (HMGA2) protein mediates EMT in response to TGFβ by facilitating the transcriptional induction of the transcription factors Snail1 and Twist1, and by guiding DNA methyl-transferases to the promoter of the *E-cadherin* gene. Our new work demonstrates how HMGA2 regulates micro-RNA biogenesis in breast cancer stem cells. We have also identified novel compounds that block EMT or affect myofibroblast activation in response to TGFβ and analyzed the role of nuclear receptors in this process. Finally, in a recent collaborative project we work on signaling pathways that link TGFβ receptors and neurotrophic factors in tumors and in neurodegenerative disorders.

This work is carried in collaboration with Ulla Engström, Varun Maturi, Anita Morén, Panagiotis Papoutsoglou, Drs. Andries Blokzijl, Jonathon Carthy, Yutaro Tsubakihara and Carl-Henrik Heldin (LCR-Uppsala Univ.), Dr. Ulf Landegren (Dep. of Genetics and Pathology, Uppsala Univ.), Dr. Michael Hottiger (Univ. of Zurich, Switzerland) and Dr. Takeshi Imamura (Ehime Univ., Japan).

TUMOR VASCULAR BIOLOGY

Anna-Karin Olsson

Cancer is a systemic disease and mortalities are mainly caused by metastasis, deep vein thrombosis and organ failure. These complications all involve an interplay between the tumor cells and the vasculature, both in the primary tumor as well as in peripheral organs. We aim to get a better understanding of this tumor-vessel interface, with the ultimate goal to improve current cancer therapy and to reduce adverse events caused by the disease or current treatment strategies. We also explore how tumor vessels can be targeted to treat cancer.

Cancer patients commonly display elevated platelet activation and suffer from increased risk of thromboembolic complications due to tumor-induced platelet activation. In turn, the platelets can promote angiogenesis, tumor progression and metastasis. This highlights the potential benefit of keeping platelet activity as low as possible in cancer patients, without increasing the risk of bleedings. A main interest in our lab is the mechanism by which platelets promote tumor vascularization and metastasis.

We are also interested in the genetic and molecular mechanisms responsible for deregulated blood vessel formation. We have previously identified histidine-rich glycoprotein (HRG) as en endogenous regulator of tumor vascularization. Using various genetic models we address the mechanism(s) of action of this plasma protein.

One of our projects is focused on the development of therapeutic cancer vaccines directed specifically at molecules expressed by tumor vessels. A main advantage of targeting the tumor vessels is that they have not developed the same immune escape mechanisms as the tumor cells. In addition, the vasculature has a more stable genetic composition and is easily accessible for the immune system.

Members of the group during 2015
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Anna-Karin Olsson, associate professor, group leader
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Project students during 2015
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The Medical Faculty, Uppsala University

SYSTEMIC EFFECTS OF CANCER

Jessica Cedervall, Yanyu Zhang

Cancer mortality is mainly connected to systemic effects induced by the primary tumor such as metastasis, deep vein thrombosis and organ failure. Studies of tumor-induced effects on distant organs have primarily focused on tissues that represent metastatic sites. Surprisingly little is however known about the situation in organs that are not targets for metastasis or affected by the primary tumor. For obvious reasons human biopsy material from these tissues is rare and mouse models therefore become important tools for such investigations. Using the two orthotopic and metastasizing models RIP1-Tag2 for insulinoma and MMTV-PyMT for breast cancer, both believed to closely resemble disease development in the human situation, we have found a dramatic impairment of the vascular function in kidney and heart compared to healthy littermates of the same genetic background. We have identified the neutrophil and its ability to form neutrophil extracellular traps (NETs) as a major component in the reduced vascular function in peripheral organs in individuals with cancer. Moreover, our data indicate that systemic inflammation and endothelial activation takes place in peripheral organs as a consequence of cancer. Our current activities aim to identify the tumor-derived molecules and mechanisms involved in this process. Moreover, we are addressing if this tumor-induced systemic inflammation and endothelial activation promotes metastasis.

THE ROLE OF PLATELETS IN TUMOR VASCULARIZATION, EPITHELIAL-MESENCHYMAL TRANSITION AND METASTASIS

Jessica Cedervall, Yanyu Zhang, Gerleen Wayenberg

Platelets are central players in maintaining hemostasis of the blood. At sites of blood vessel injury, platelets are activated to induce blood coagulation and form aggregates at the site of the damaged endothelium to prevent hemorrhage and thereby protect us from fatal bleedings. Besides their role in hemostasis, platelets have been shown to contribute to non-hemostatic processes such as wound healing, immunity, angiogenesis, cardiovascular disease, epithelial-to-mesenchymal transition and metastasis. Our research is focused on the mechanisms by which platelets regulate these processes, with a special focus on the role of HRG (described below). Using *in vitro* as well as *in vivo* assays we have identified a number of molecules potentially involved in the platelet-induced effects in the pre-tumorigenic environment.

HISTIDINE-RICH GLYCOPROTEIN IN PHYSIOLOGICAL AND PATHOLOGICAL ANGIOGENESIS

Jessica Cedervall, Yanyu Zhang

Histidine-rich glycoprotein (HRG; alternatively, HRGP/HPRG) is a heparin-binding plasma protein that has been identified as an angiogenesis inhibitor *in vitro* and *in vivo*. HRG has the capacity to reduce tumor growth and vascularization in mice. We are presently addressing the role of HRG in physiological and pathological angiogenesis using HRG-deficient mice, which are cross-bread to transgenic tumor models. We have demonstrated that mice lacking HRG have an elevated angiogenic switch and display increased tumor growth, a finding that firmly establishes HRG as an endogenous regulator of pathological angiogenesis. Moreover,

epithelial-mesenchymal transition (EMT) as well as metastasis is accelerated in HRG-deficient mice. Mice lacking HRG display enhanced coagulation and increased platelet activation and we have found that several features of the accelerated tumorigenesis in HRG-deficient mice are mediated by platelets.

TARGETING TUMOR VESSELS BY THERAPEUTIC VACCINATION

Julia Femel, Falk Saupe, Matthias Reichel

One of the major success stories in human and veterinary medicine during the past 100-150 years is vaccines targeting various infectious diseases. Vaccines have together with antibiotics likely been more important for human and companion animal health than any other part of human or veterinary medicine. Due to the success of vaccines the interest in using vaccine technology for the treatment of non-infectious diseases like allergies, autoimmunity and cancer is increasing. Therapeutic vaccination targeting self-molecules could provide a cost-efficient alternative to monoclonal antibody-based therapies for cancer and various inflammatory diseases. The development of cancer vaccines has, however, so far not been successful enough to qualify as a standard therapy in the clinic. The reason for this is probably multifaceted, but one complicating factor is that tumor cells have developed strategies to escape recognition by the immune system. Antigens specifically expressed by the tumor vasculature can therefore provide alternative targets.

A small number of molecules have been identified as specifically expressed by neoplastic vasculature, either by the endothelial cells or by the surrounding matrix. The reported lack of expression of these molecules in healthy tissue renders them highly interesting for targeted cancer therapies. Examples are the alternatively spliced extra domains-A and –B (ED-A and ED-B) of fibronectin (FN). These extra-domains of FN are expressed during vasculogenesis in the embryo but essentially undetectable under normal conditions in the adult. Both ED-A and ED-B are highly expressed around angiogenic vasculature in various tumor types and show a strong conservation between species. We have in two recent publications shown that it is possible to compromise tumor vessel function and as a consequence suppress growth of aggressive pre-clinical tumors by immunization against ED-A or ED-B, both in a prophylactic and a therapeutic setting. Furthermore, we found that in mice with anti-ED-A antibodies the number of metastases was reduced in a transgenic model of metastatic mammary carcinoma. These data suggest that tumor vascular antigens are highly interesting candidates for development of therapeutic vaccines targeting solid tumors. Currently we are working on design of multi-targeting vaccines to achieve a broad coverage of many tumor types simultaneously and to further increase the efficacy of the vaccine.

LOOSE CONNECTIVE TISSUES – POTENTIAL TARGETS FOR THERAPIES IN CANCER AND INFECTIOUS DISEASES

PI Kristofer Rubin

Loose connective tissue elements are present in all organs outside the central nervous system. They embed blood vessels and underlie mucosal surfaces and also constitute the stroma of carcinoma. During inflammatory processes leukocyte leave the blood vessels and enter the surrounding loose connective tissues. The composition of the interstitial matrix, *i.e.* the amount (concentration) and type of the fibrous scaffolding and ground substance, in concert with connective tissue cells determine the physical properties for convective and diffusive movement of molecules in the tissue. The loose connective tissue surrounding blood vessels was commonly thought of as a "passive" framework in the sense that its physical properties such as diffusivity, hydraulic conductivity, compliance and interstitial fluid pressure (IFP) remain fairly constant. This concept of a static and passive loose connective tissue has been challenged by recent research suggesting that IFP is "actively" controlled and thereby also fluid content and possibly fluid fluxes through tissues.

Together with prof. Rolf Reed at Bergen University in Norway we have proposed a mechanism for control of IFP *in vivo*. Our proposed mechanistic model holds that connective tissue cells apply tensile forces on ECM-fibers that in turn restrain the under-hydrated ground substance from taking up fluid and swell. A decrease in cellular tension on the ECM fibers allows the ground substance to swell and form edema. During this process negative IFP values can be recorded if refilling of the tissue with fluid is inhibited. Dermal IFP lowered after anaphylaxis can be normalized by instilments of platelet-derived growth factor (PDGF) BB or insulin. Our data suggest that whereas β_1-integrins participate in maintenance of fluid homeostasis, β_3-integrins participate in PDGF BB-induced IFP-recovery after inflammation-induced lowering of dermal IFP.

One obstacle in the pharmaceutical treatment of carcinomas is the poor uptake of anti-cancer drugs into the tumor tissue. We have shown that the IFP in carcinoma reflects conditions that form a barrier for penetration of low-molecular compounds such as chemotherapy into the carcinoma tissue. Lowering of carcinoma IFP by local treatment with prostaglandin E_1 or by systemic treatment with inhibitors of the PDGF or TGF-β systems lower carcinoma IFP. This lowering of IFP is paralleled by increases of the efficacy of conventional chemotherapy. We have spent considerable efforts in elucidating the mechanisms by which carcinoma IFP is controlled. Microarrays for gene expressions and other techniques such as immunohistochemistry, real-time PCR, imaging and cell analyses, all combined with physiological measurements in carcinoma grown in wild-type or transgenic animals have been adopted. We have found a correlation between inflammatory processes and the architecture of the collagen network of the stroma during regulation of IFP.

Group members during 2015
Kristofer Rubin, PhD, professor, (leave of absence), group leader
Vahid Reyhani, PhD, post doc
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Publications 2013 to 2015

1. Kelkka, T., Pizzolla, A., Laurila, J.P., Friman, T., Gustafsson, R., Källberg, E., Olsson, O., Leanderson, T., Rubin, K., Salmi, M., Jalkanen, S. and Holmdahl, R. (2013) Mice Lacking NCF1 Exhibit Reduced Growth of Implanted Melanoma and Carcinoma Tumors. PLoS ONE. 8(12):e84148.
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Agencies supporting the work

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The Swedish Science Council

SIGNALING PATHWAYS INVOLVED IN PDGF-INDUCED MATRIX CONTRACTION

Vahid Reyhani

Cell-mediated matrix contraction plays a crucial role in regulation and maintenance of the IFP. The contraction process is stimulated by PDGF-BB and inhibited by pro-inflammatory factors such as prostaglandin E_1 and interleukin-1. Recently, others and we have identified at least two mechanisms for cell-mediated collagenous matrix contraction. The collagen-binding integrins, $\alpha_1\beta_1$, $\alpha_2\beta_1$ and $\alpha_{11}\beta_1$ all mediate rapid contraction that proceeds in serum-free media. Contraction mediated by these integrins requires integrin-elicited signaling. When the collagen-binding β_1 integrins either are absent or their signaling and/or activity perturbed, a second mechanism can become operative. This mechanism depends on the RGD-dependent integrin $\alpha_V\beta_3$ and presence of RGD-containing fibrous ligands such as fibrin or fibronectin. Presently, this work is concentrated on the signaling events downstream of PDGF-Rβ during cell-mediated matrix contraction. More specifically, to study signaling pathways downstream of PDGF-Rβ, which lead to actin cytoskeleton turnover and generation of cellular contractile forces.

PRO-INFLAMMATORY ROLE OF FIBRIN BY INDUCTION OF INTERFERON RESPONSE

Vahid Reyhani

The functional significance of fibrin deposits typically observed in inflammatory sites, carcinomas and in healing wounds is not fully understood. Recently we described a novel biological significance of fibrin in such pathologies, where the collagen-binding β_1 integrin signaling is impaired. The extravasated fibrin provides an interface between the collagenous matrix and cells to allow them to re-exert contractile forces on the matrix. This process can potentially be part of an edema clearance program *in vivo*. We also, for the first time, characterized the direct binding of fibrin to collagen type I, and showed that this binding plays an essential role in the stability of the interface fibrin network. Currently our focus is to investigate other possible impacts of fibrin deposition at inflammatory sites. Based on our microarray data analysis fibrin induced interferon response. Currently, in this project we study the molecular details of this observation.

MECHANISMS OF OPTIMAL TISSUE REGENERATION VERSUS FIBROSIS AND THE ROLE OF THE MICROVASCULATURE

PI Christian Sundberg

The main focus of this group is to understand the biology of blood vessels and their role in tumor formation and fibrosis. Fibrosis is a common denominator in a wide variety of diseases characterized by chronic inflammation including stroma formation in solid tumors, rheumatoid arthritis and inflammatory bowel disease, connective tissue diseases, atherosclerosis, heart failure, transplant rejection and wound healing to name a few. The progression of fibrosis in these diseases leads to the derangement of tissue architecture and subsequent failure of the organ. In many of these diseases current therapeutic approaches have only marginally contributed to cure and must be seen as approaches that delay the progression of the disease. However, in certain circumstances in the adult, diseased organs (for instance the kidney in glomeruloid nephritis, the liver after hepatitis, and the heart during ventricular hypertrophia) are capable of healing themselves with minimal damage to the tissue and its function. Tissue regeneration following damage to an organ during embryogenesis and infancy is also an example of tissue repair with minimal functional sequel. Thus, the body has mechanisms by which to adequately repair damaged organs. Why the body does not always achieve this, and what causes progression in one instance, and healing in another, is largely unknown and is one of the main subjects of study in the laboratory.

Members of the group during 2015
PI: Christian Sundberg, MD, PhD, associate professor, Senior Scientist at the Swedish Scientific Research Council, Medical Branch.

Post doc, position open
Project worker, position open

Publications 2013 to 2015
1. Rodriguez A, Friman T, Gustavsson R, Kowanetz M, van Wieringen T and Sundberg C. (2013) Phenotypical differences in connective tissue cells emerging from microvascular pericytes in response to over-expression of PDGF-B and TGF β1 in normal skin in vivo. Am J. Pathol. Jun;182(6):2132-46.

1. DEFINING THE PERICYTE-FIBROBLAST LINEAGE AND THEIR COMMON STEM CELLS

We have published the novel concept that microvascular pericytes have the ability to differentiate into collagen type I producing fibroblasts, thereby coupling the process of angiogenesis and fibrosis in a previously unrecognized way. Our research involves the isolation and study of stem cells that define the pericyte-fibroblast lineage, and to identify different stages of this differentiation process. Preliminary results show that five different stages are involved in this process. We will study differences in gene expression and gene products during this differentiation process using cDNA microarray techniques as well as proteomics (2-D gel electrophoresis). By defining this new lineage novel insights into the process of fibrosis and potential modulation will be identified.

2. INHIBITING THE PERICYTE-FIBROBLAST DIFFERENTIATION PROCESS

As a follow up project to point 1 we will devise an assay to study the differentiation of pericytes to collagen type 1 producing fibroblasts in a high throughput assay system. We will use a chemical library to attempt to identify compounds that modify this differentiation process. Furthermore, conditioned medium from certain tumor cell lines have an inhibitory effect on the differentiation process. We would like to identfy what this component in conditioned medium is.

3. GENE THERAPEUTIC APPROACH FOR STUDYING FIBROSIS AND BLOOD VESSEL FORMATION IN THE BODY

We have previously published a novel animal model by introducing VPF/VEGF into normal tissues by adenoviral vectors. These studies have led to the discovery of three previously unknown modes of angiogenesis. We will further study cell progression and events that occur during blood vessel formation and fibrosis in the body. To this effect gene therapy techniques using adenoviral vectors will be used in order to induce genes for growth factors that are believed to modulate the development of the tumor stroma. Growth factors will be introduced into normal and diseased tissues, both individually and in combination. Effects of these growth factors will be studied using advanced morphological and physiological techniques which are being developed. This approach might be used for treatment of heart disease as well as diseases in other arteries in the body resulting from arteriosclerosis and diabetes.

MEDICAL MICROBIOLOGY

IMMUNOLOGY

Birgitta Heyman, Jenny Hallgren Martinson, Gunnar Pejler

The cellular and humoral components of the immune system are crucial in our defense against foreign microorganisms. The central themes in our work is to try to understand how B cells and antibodies, complement, mast cells and their progenitors, dendritic cells and T cells are operating in concert to achieve an optimal immune response and what goes wrong when allergies develop. In addition, we are studying how mast cells can be targeted and how mast cells affect disorders of non-allergic type. We are primarily working in mouse models using different transgenic, knock-out and knock-in strains but, in addition, in vitro techniques and human studies are used.

MAST CELLS AND THEIR PROGENITORS IN ALLERGIC AIRWAY INFLAMMATION (ASTHMA) AND RESPIRATORY INFECTIONS

Jenny Hallgren Martinsson

Mast cells contribute to many features of allergic asthma and express the high affinity receptor for IgE, FcεRI. Cross-linking of FcεRI-bound IgE with specific antigen degranulates mast cells and release pro-inflammatory mediators such as tryptase and histamine. Mast cells mature in tissues from committed mast cell progenitors that even though they are rare can be quantified by multi-colour flow cytometry. The mouse lung contains few mast cell progenitors, but allergic inflammation or respiratory infection increases the numbers dramatically. The increase in mast cell progenitors leads to higher numbers of mature lung mast cells and resembles the mast cell hyperplasia that occurs in asthmatic patients. Asthmatic patients with reduced lung function have more mast cell progenitors. We study the mechanisms behind the mast cell increment in the lung and the role of mast cells and their progenitors in allergic asthma and respiratory infections.

Members in the group during 2015
Joakim Dahlin, post doc
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Maya Salomonsson, PhD student
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Publications 2013 to 2015
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Agencies that support the work
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HUMAN MAST CELL PROGENITORS

Maya Salomonsson, Joakim Dahlin, Jenny Hallgren Martinsson

This project was aimed at identifying and quantifying human mast cell progenitors with flow cytometry. The development of mast cells from early progenitors to a committed mast cell progenitor has been studied in the mouse. We hypothesized that we would identify a mast cell progenitor population in human blood by evaluating the mast cell potential in sorted cell populations from human blood. During 2015 we have identified such a mast cell progenitor population in human blood that is characterized as lineage^{-} CD34^{hi} CD117$^{int/hi}$ FcεRI^{+} cells. We are now analyzing asthma patients and healthy controls for their frequency of blood mast cell progenitors to investigate whether this parameter reflect a certain phase, severity or type of asthma. We are also characterizing the human mast cell progenitors in terms of activation and growth capacity under different culture conditions.

WHAT ARE THE MECHANISMS BEHIND THE INCREASE IN LUNG MAST CELL PROGENITORS SEEN IN RESPIRATORY VIRUS INFECTIONS?

Behdad Zarnegar, Annika Westin, Erika Mendez Enriquez, Kjell-Olov Grönvik, Jenny Hallgren Martinsson

The increase in lung mast cell progenitors in an experimental asthma model is rapid and can be inhibited by antibody blocking or genetic deletion of molecules involved in endothelial transmigration. This suggests that the increase in mast cell progenitors is largely due to recruitment. Preliminary results suggest that infection of mice with influenza virus induce increased numbers of mast cell progenitors in the lung but since this occurs around one week after virus inoculation there is a possibility that the mast cell progenitors increase due to in situ cell division. We are currently investigating how much of the increase in lung mast cell progenitors that is due to recruitment and how much that is caused by cell division in situ upon influenza infection. Moreover, we are investigating if the influenza-induced increase in lung mast cell progenitors is translated into an increase in mature lung mast cells.

THE ROLE OF MAST CELLS IN ALLERGIC AIRWAY INFLAMMATION

Erika Mendez Enriquez, Behdad Zarnegar, Jenny Hallgren Martinsson

Previous studies of the role of mast cells using mouse models of allergic airway inflammation have been performed in different mouse strains with mutations in the White-spotting locus (W), which codes for the stem cell factor receptor, c-kit. Since stem cell factor is a critical growth and maturation factor for mast cells, these strains lack mast cells. However, stem cell factor is also a critical growth factor during the early haematopoiesis for normal development of other lineages making these strains having other deficiencies. In this project we aim to validate the findings associated with mast cells observed in c-kit dependent models using a new kit-independent model of mast cell deficiency. In collaboration with Thorsten Feyerabend and Hans-Reimer Rodewald we use

their new mast cell deficient mice strain. This strain has a targeted insertion of Cre-recombinase into the mast cell carboxypeptidase A3 locus (CPA3-Cre mice), which induces a deletion of mast cells by a genotoxic Trp53-dependent mechanism. To model allergic airway inflammation, we use house dust mite, a common allergen that also induces human asthma.

ARE MAST CELLS INVOLVED IN THE EXACERBATIONS OF ALLERGIC ASTHMA SEEN AFTER RESPIRATORY VIRUS INFECTIONS?

Erika Mendez Enriquez, Annika Westin, Behdad Zarnegar, Kjell-Olov Grönvik, Jenny Hallgren Martinsson

We are using experimental models of allergic asthma and respiratory infections to mimic viral induced exacerbations. We will first investigate which features of virus-induced exacerbations of asthma that are mast cell dependent by using the mast cell deficient CPA3-Cre mice described in the project above. We hypothesized that mast cells are involved in the exacerbations of allergic asthma seen after respiratory infections via a combination of mechanisms: 1) Virus-infections stimulate pathways that lead to recruitment of mast cell progenitors, which in itself may force exacerbations upon allergen challenge since more mast cells will be activated by antigens 2) Mast cells express pattern recognition receptors that may be triggered upon the viral infection. Hence, mast cells may release more mediators if they are activated by a combination of allergen that crosslinks the IgE on the FcεRI receptors and viral products that activate pattern recognition receptors. By quantifying the degree of mast cell activation and mast cell numbers, we aim to find out if mast cells are more activated by the combination of virus infection and allergic asthma or if the worsening are due to higher numbers of mast cells that are equally activated.

ANTIBODY FEEDBACK REGULATION

Birgitta Heyman

Antibodies in complex with their specific antigen can feedback-regulate antibody responses against this antigen. Depending on antibody class, affinity and type of antigen, complete suppression or 10-1000-fold enhancement of the in vivo immune response can be seen. Both passively administered and actively produced antibodies are effective, suggesting a biological role. One of the most successful clinical applications of modern immunology is Rhesus prophylaxis, where administration of suppressive IgG anti-RhD prevents Rh-immunization in Rh negative mothers carrying Rh postive fetuses. This antibody treatment has almost eradicated hemolytic disease of the newborn in industrialized countries. In spite of this successful treatment, the mechanisms behind feedback regulation are poorly understood. An immune complex is composed of antigen/antibody/complement (if the antibody is able to activate complement). Such immune complexes can bind to the B-cell receptor, Fc-receptors (FcR) and complement receptors (CR). Ligation and co-ligation of these receptors on the B cell surface can negatively or positively regulate the B cell. Increased uptake of complexed antigen by antigen-presenting cells via FcRs or CRs can enhance T helper cell activation. Follicular dendritic cells (FDC) do not express MHC-II molecules and do not present antigen to T cells. They are interspersed in the B cell follicles of the spleen and lymph nodes and interact closely with B cells. Since FDC express both FcR and CR, they may capture immune complexes and act as a concentration device, facilitating antigen recognition by the B cells. A novel interesting function of antibodies is to transport antigen into the optimal locations in secondary lymphoid organs. The main objective of our research is to clarify the mechanisms behind antibody feedback regulation, both during a normal immune response and in autoimmune diseases and allergies.

Members of the group during 2015
Joakim Bergström, PhD student
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Zhoujie Ding, PhD student/postdoc
Malin Eriksson, master degree project student
Birgitta Heyman, professor, group leader
Jennifer Peterson, bachelor project student
Anna Sörman (fd. Bergman), PhD student/postdoc
Annika Westin, laboratory engineer
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Lu Zhang, PhD student

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Dissertations 2015
Zhoujie Ding: Feedback enhancement of Immune Responses by IgE, IgM, and IgG3 Antibodies, February 12
Anna Sörman (fd. Bergman): IgM and complement in regulation of antibody responses, November 19

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MECHANISMS FOR COMPLEMENT-MEDIATED REGULATION OF IMMUNE RESPONSES

Anna Sörman, Zhoujie Ding, Lu Zhang, Hui Xu, Birgitta Heyman

Complement receptors 1 and 2, CR1/2 are important for the production of antibodies since CR1/2-knock-out mice have very poor antibody responses. Activation of complement via the classical pathway explains the influence of complement on antibody responses. Antibodies, and in particular IgM, are the activators of the classical pathway. We have shown that although mice lacking the first factor in the classical pathway, C1q-knockout mice, have abrogated antibody responses, knock-in mice which produce IgM with a point-mutation making the IgM-molecule unable to activate complement, have near-normal antibody responses. This is a surprising finding, demonstrating that something else than antibodies must activate the classical pathway. We are now trying to identify which substance does activate C1q in these situations and where in the body the antigen becomes covered with activated complement factors.

The mechanism by which complement is required for antibody responses is not known. CR1/2 expressed on B cells play a central role in responses to antigens administered alone. In addition we study the ability of IgM and IgG3 antibodies to upregulate antibody responses. We have shown that both antibody classes induce enhanced antibody-, but not T cell-responses, enhanced germinal center reaction and are dependent on CR1/2 expressed both on B cells and follicular dendritic cells. IgG3 causes antigen to be deposited in splenic follicles. We will now investigate the effects of induction of immunological memory.
In a collaboration with professor Ji-Yang Wang, Fudan University in Shanghai, we will study the relative role of complement and FcμR in immune regulation by IgM antibodies. For this project, we have received a Sweden-China network grant from VR.

MECHANISMS FOR IgG-MEDIATED SUPPRESSION OF IMMUNE RESPONSES

Joakim Bergström, Hui Xu, Birgitta Heyman

Another project aims at understanding the mechanisms behind IgG-mediated suppression of antibody responses. Purified IgG, prepared from serum of mice hyperimmunized with sheep erythrocytes (SRBC), is administered together with SRBC. This results in more than 99% suppression of the IgM response. We are now investigating the suppressive effect of IgG on IgG responses, on the development of germinal centers in the spleen and on development of immunological memory and longlived plasma cells.

MECHANISMS FOR IgE-MEDIATED ENHANCEMENT OF IMMUNE RESPONSES

Zhoujie Ding, Hui Xu, Birgitta Heyman

IgE, passively administered to mice together with its specific antigen, will bind to circulating B cells via their low affinity receptor for IgE, CD23. After 30 minutes the antigen has been transported to the follicular areas in the spleen, where the fine tuning of antibody responses takes place. There, antigen is delivered to dendritic cells which internalize and present the antigen to T cells which in turn help B cells to produce antibodies. The result is a potent T cell proliferation followed by a several 100-fold enhanced antibody response. We will now try to detect the antigen in vivo in various cell types using flow cytometry. We will investigate which subgroup of dendritic cells that actually present the peptides to T cells using cell sorting, in vitro proliferation assays as well as confocal microscopy. The possibility of one dendritic cell type presenting IgE-antigen to cytotoxic T cells will be investigated.

THE ROLE OF MAST CELLS IN DISEASE

Gunnar Pejler

Mast cells are traditionally thought of as being the main cause of the symptoms associated with allergic reactions, including allergic asthma. When mast cells become activated they may respond by degranulation, which is associated with the release of a panel of inflammatory substances, and it is these substances that cause the typical allergic symptoms. Although mast cells certainly are main players in allergy, more recent research has revealed that they cause damage also in a large panel of other diseases, including arthritis, multiple sclerosis, cancer and atherosclerosis. On the other side, it has been discovered that mast cells in addition possess a number of beneficial functions, including a role in host defense towards bacteria, parasites and even snake venom. In this research group we are studying the biological function of mast cells. In particular, we are studying the mechanisms by which mast cells contribute to various pathological conditions and how they contribute to host defense. We also study the mechanisms behind formation of the mast cell secretory granules as well as the function of individual granule compounds, in particular the mast cell proteases and proteoglycans. Moreover, our research aims at identifying novel ways to target the detrimental activities of mast cells.

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Gianni Garcia Faroldi, post doc
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Publications from the group 2013 to 2015

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NOVEL STRATEGIES FOR LIMITING HARMFUL MAST CELL ACTIVITIES

Aida Paivandy, Fabio Rabelo Melo, Elin Rönnberg, Gianni Garcia Faroldi, Mirjana Grujic, Gunnar Pejler

Mast cells are implicated as detrimental players in numerous pathologies, including asthma, cancer and inflammatory skin disease. The aim of this project is to evaluate novel strategies for inhibiting mast cell function. One part of the project focuses on selective induction of mast cell apoptosis, mainly by investigating the ability of lysosomotropic agents to induce mast cell apoptosis. We are also investigating the effect of compounds that interfere with mast cell gene induction and/or degranulation. In this project we use both cultured mouse mast cells and primary human lung mast cells.

TRYPTASE AND EPIGENETICS

Fabio Rabelo Melo, Aida Paivandy, Gunnar Pejler

We have found that tryptase, a secretory granule protease, can be found in the nucleus of mast cells. Moreover, we have found that nuclear tryptase can cleave off N-terminal ends of core histones, thereby removing sites for epigenetic modifications. Our hypothesis is that such activities of tryptase can lead to epigenetic effects in mast cells. We are currently evaluating this hypothesis.

MAST CELLS AND CANCER

Mirjana Grujic, Helena Öhrvik, Fabio Rabelo Melo, Gianni Garcia Faroldi, Gunnar Pejler

Mast cells have been implicated in cancer, and in this part of the project we are studying this issues, using a melanoma model. We also use mice deficient in mast cells or deficient in various mast cell-restricted proteases.

MAST CELLS AND COPPER

Jun Mei Hu Frisk, Anne-Marie Gustafson, Gunnar Pejler, Helena Öhrvik

In this project we are studying the effect of copper on immune mechanisms, mainly by focusing on mast cells. We have recently shown that mice deficient in a protein involved in the regulation of copper uptake exhibit marked alterations of their mast cell population. We are currently pursuing this research by studying how cellular copper status affects mast cells as well as other immune cells.

MAST CELLS AND BACTERIAL INFECTION

Carl-Fredrik Johnzon, Elin Rönnberg, Anne-Marie Gustafson, Gunnar Pejler

Mast cells have previously been implicated to play a role in bacterial infection. However, the experimental systems used to support this notion have been questioned. We are currently addressing this issue by using a new-generation mast cell-deficient mouse model.

THE ROLE OF MAST CELLS IN ANGIOGENESIS

Gianni Garcia Faroldi, Gunnar Pejler

Mast cells have been implicated to regulate angiogenesis, mainly due to their ability to secretie various pro-angiogenic factors. However, there is still little formal evidence to support this notion. We are currently addressing this issue by using mast cell-deficient mice, and by using mice deficient in individual mast cell compounds.

MOLECULAR BACTERIOLOGY

Dan Andersson, Diarmaid Hughes, Linus Sandegren, Göte Swedberg, Joakim Näsvall, Lionel Guy

The area of molecular bacteriology at IMBIM is made up of six independent research groups. Overall our research is aimed at understanding how pathogenic and commensal bacteria genetically adapt and evolve in response to various external (e.g. antimicrobial drugs, biocides, exposure to a host organism and other novel environments) and internal (e.g. different types of deleterious mutations) selective pressures. A particular focus is to understand which biological factors determine the rates and trajectories of adaptive evolution when bacteria are exposed to antibiotics and other antimicrobial compounds. Using a combination of genetics, experimental evolution, biochemistry and mathematical modeling we have shown that mutation, recombination and horizontal gene transfer rates, fitness costs of resistance and strength of the selective pressure are the main determinants of how rapidly resistant bacteria emerge and spread in a population. The long-term goal of this research is to increase our understanding of fundamental adaptive evolutionary processes and how we can slow down the emergence of drug resistant microbes by rational choices of drug targets, antibiotic use patterns and drug dosing. Ultimately this will allow us to treat infectious diseases in a more efficient way.

MECHANISMS AND DYNAMICS OF BACTERIAL ADAPTATION AND EVOLUTION

Dan Andersson

We study the mechanisms and dynamics of molecular evolution in bacteria and are interested in how factors such as the extent and type of genetic variation, strength of selection pressures, compensatory mutations and population dynamics affect the tempo and mode of adaptive evolution. Our research is focused on two different areas: A) analysis of the various genetic factors that affect genome stability and variability in bacteria and B) analysis of the factors that influence the dynamics of the evolution of antibiotic resistance development. We study these problems in several bacterial species (Salmonella enterica, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus and Mycobacterium tuberculosis) using a combination of methods, including experimental evolution, bacterial genetics, molecular biology, biochemistry, whole-genome sequencing and mathematical modeling.

A. Genome variability and stability. The long-term goal of this project is to examine the evolutionary and mechanistic factors that influence genome stability and variability and how these factors influence the tempo and mode of bacterial evolution. We use the bacteria S. enterica and E. coli as model systems to: 1. Examine the role of gene amplification in adaptive responses to antimicrobial drugs and in the evolution of novel genes. 2. Examine the fitness effects and constraints on horizontal gene transfer. 3. Examine the distribution of fitness effects of random mutations in different types of proteins and its impact on adaptive evolution. 4. Examine the mechanisms by which synonymous mutations can affect bacterial fitness and growth rates. 5. Experimentally study de novo evolution of new genes.

B. Mechanisms and dynamics of the evolution of antibiotic resistance. The overall objective of this project is to understand how antibiotic resistance affects the fitness, virulence and transmission of various pathogenic bacteria and which factors determine how rapidly resistance develops in bacterial populations. Our main aims are to: 1. Determine how various types of resistance mechanisms affect bacterial fitness and virulence. 2. Determine how bacteria can compensate for resistance-conferred fitness costs. 3. Examine the importance of genetic epistasis on the rate and trajectory of multi-drug-resistance development and compensatory evolution. 4. Identify mechanisms that confer resistance to antimicrobial peptides and determine the impact of these mechanisms on bacterial fitness and virulence. 5. Examine how very low levels of antibiotics can enrich for and select de novo resistant mutants in various types of laboratory and natural environments.

Members of the group during 2015
Dan I. Andersson, Professor, group leader
Marlen Adler, Researcher (with Dr. Linus Sandegren)
Erik Gullberg, researcher
Karin Hjort, researcher
Peter Lind, researcher
Herve Nicoloff, researcher
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Ulrika Lustig, research engineer
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Michael Knopp, PhD student
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Marius Linkevicius: Evolution and mechanisms of tigecycline resistance in Escherichia coli, September 25

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SELECTION OF ANTIBIOTIC RESISTANCE IN COMPLEX MIXTURES OF ANTIBIOTICS AND METALS

Lisa Albrecht

Complex mixtures of antibiotics and biocides exist in the environment as a consequence of release of sewage water as well as agricultural and industrial runoff. Different antibiotics are present in the mixtures, together with metals that are used agriculturally and industrially. Metals of interest in this study are copper, used in pig farming, arsenic, a component in poultry growth promoters and in pesticides, and silver, an antimicrobial used especially in the health care setting. These metals are combined with antibiotics of various classes like aminoglycosides, tetracycline and rifampicin. The resultant mixtures are evaluated for their potential to select for antibiotic resistance using competition assays. Resistant strains with either chromosomally encoded or plasmid encoded resistance genes are included, and the competitive ability of the resistant strain is measured by flow cytometry, using blue fluorescent protein and yellow fluorescent protein for detection of the strains. Both synergistic and antagonistic actions have so far been observed, demonstrating that the effect varies depending on the specific components in the mixtures. The exact mechanisms behind the interactions are currently being investigated.

ANTIBIOTIC SELECTIVE PRESSURE AT SUB-MIC CONCENTRATIONS

Erik Gullberg

When bacterial populations are exposed to antibiotics, bacteria carrying mutations giving them a higher resistance to the antibiotic will have a selective advantage over sensitive bacteria, despite the fitness costs these mutations often cause. Not all resistance mutations have a fitness cost, there are also cases where fitness neutral mutations confer a high level of antibiotic resistance. The use of antibiotics in human and veterinary medicine can cause contamination of external sites, and many environments like sewage plants, farm run-off water and lake water can contain low levels of antibiotic residues. This project investigates how low, sub-MIC levels of antibiotics cause selection for resistant mutants. By performing competition experiments where a defined mix of resistant and susceptible bacteria is grown at different concentrations of antibiotics, the level that provides enough selective pressure for the resistant bacteria to take over can be determined. We also investigate the enrichment of de novo resistant mutants at sub-MIC concentrations of antibiotics and identify the resistance mutations of these mutants using whole genome sequencing. Reconstruction of the strains with the candidate mutations in an isogenic background show what mutations or combination of mutations are responsible for the resistance and which fitness costs they confer.

IDENTIFICATION OF DE NOVO RESISTANT MUTANTS AT SUB-MIC CONCENTRATIONS OF COLISTIN.

Karin Hjort

Bacteria can increase their resistance and develop de novo resistance against antibiotics at sub-MIC (minimal inhibitory concentration) levels. This can become a challenging environmental problem since sewage water, lake water and farm run-off contain low levels of

antibiotics. The measured amount for specific antibiotics in these environments are in some instances within the range known to increase the frequency of pre-existing antibiotic resistant bacteria and the generation of antibiotic de novo resistance. In this project I am studying the antibiotic colistin, clinically used for treatment of multiresistant bacteria such as extended-spectrum β-lactamases (ESBL) producing bacteria. The focus of this project is to examine the ability of colistin to enrich for de novo resistant mutants of E. coli and Salmonella typhimurium during cycling at sub-MIC levels. Our results show that for E. coli de novo mutations in pmrA and pmrB, which are well known to cause colistin resistance, were selected.

However, the S. typhimurium mutants showed no stable resistance, but rather heteroresistance. Heteroresistance is a phenotype characterized by the presence of cells within the same supposedly isogenic population with different sensitivity/resistance to an antibiotic. In this case the heteroresistance was unstable and could be increased or decreased depending on the strength of colistin selection, making it difficult to detect with Etests. The whole genome sequencing of nine mutants from different cycled linages showed no pmrA or pmrB mutations, instead most of them (seven out of nine) had amplifications of the genomic region containing pmrD and arnBCADTEF-pmrE. PmrD function as a connector between the two-component system phoPQ (sensing antimicrobial peptides and low levels of Mg^{2+}, Ca^{2+}) and pmrAB (sensing low pH and low levels of Al^{3+} and Fe^{3+}) making it possible to transcriptionally regulate genes under the regulation of PmrAB through the PhoPQ signal pathway. Genes transcriptionally regulated by PmrA are pmrCAB and arnBCADTEF-pmrE, involved in synthesis and transferring of phosphoethanolamine (PEtN) and 4-amino-4-deoxy-L-arabinose (L-Ara4N) additions to lipid A, rendering the cells colistin resistance. The level of pmrD amplification determined if the bacteria was sensitive, heteroresistant or resistant towards colistin. These results show that de novo resistance develops at sub-MIC and that there is a difference between sub-MIC and above MIC resistance in E. coli and S. typhimurium. It also gives a genetic explanation for heteroresistance.

EVOLUTION OF COMPLEMENT RESISTANCE IN BORRELIA

Jon Jerlström-Hultqvist

This project will investigate the potential of Borrelia strains to resist complement-mediated lysis by the mechanism described in the innovation-amplification-divergence (IAD) model. The IAD model predicts the creation of new genes often happens by selection on an already weak preexisting promiscuous activity (the innovation) in a protein. If the weak activity becomes under selection it may initially be compensated by amplification of the gene. Amplification creates a larger mutational target and might lead of new variants by accumulation of mutations. Mutations that improve the weak activity will lead to the collapse of the amplified gene array and the birth of a novel gene. Predictions of this model include that evidence of positive selection should be found in the newly evolved gene copy.
The Pfam54 gene family of Borrelia is interesting because there is evidence of positive selection acting on certain amino acid positions and only some family members have the capacity to bind to human fH and FHL-1 exclusively. The non-monophyly of hfH and FHL-1 binding among CRASP-1 homologs points to accidental promiscuous activity that has arisen in some members of this family. Most B. garinii isolates have limited to no serum resistance. In line with this the BgCRASP-1 proteins bind only weakly to FHL-1 and (not at all) to fH. Further, the expression of BbCRASP-1 in serum sensitive B. garinii has been shown to endow

the cells with complement resistance. Non-immune human serum (NHS) will be applied to select a serum sensitive B. garinii isolate to acquire the ability to survive the serum challenge by amplification of weak binding activity of its BgCRASP-1 homologs and eventual divergence of the amplified gene copies within the amplified array will be determined by sequencing the evolved strains. This project is performed in collaboration with Prof. Sven Bergström at Umeå University.

FISHING FOR GENES WITH PROMISCUOUS ACTIVITIES

Jon Jerlström-Hultqvist

The evolution of new genes is a central question in biology that might help to explain the large variability observed in living organisms today. It is clear that new genes may evolve along many different routes, in bacteria gene duplication and horizontal gene transfer appears to be especially important mechanisms. The genetic underpinnings that lead to the establishment of novel genes is incompletely understood but may involve incremental improvement of protein function through selective pressures. The existence of weak enzymatic activities provides a mechanism whereby new genes may evolve through innovation (the weak activity), amplification and divergence (IAD) model where positive selection drives the generation of novel gene variants with increased activities. In this project we aim to screen for novel genes effecting metabolic innovation through weak promiscuous activities. We have investigated the evolutionary potential of phage proteins by screening metagenomic phage libraries from several different environments (coral, riverine, pond, mucus) in single-gene knock-out auxotrophs. We have identified candidate metagenomics gene fragments that rescue auxotrophic strain and the mechanism and adaptive potential behind the observed phenotypes is now under study.

DE NOVO GENE BIRTH FROM RANDOMIZED NUCLEOTIDE SEQUENCES

Michael Knopp, Jon Jerlström-Hultqvist, Omar Warsi

The origin and evolution of genes has been subjected to many studies, mainly focusing on gene acquisition via horizontal gene transfer or gene duplications where pre-existing genes serve as substrates for evolution of new functions. However the “Holy Grail” problem in the field of how the first genes arose from non-coding sequences has been much less studied.
To address this question we have generated highly diverse plasmid libraries (10^8-10^9) encoding randomized nucleotide sequences of different length ranging from 30 to 300 nucleotides. These random sequences were incorporated between transcriptional and translational initiation and termination signals to enable high-level expression of random mRNAs and peptides, respectively. The libraries were subsequently used in extensive screens for rescue of a) auxotrophic mutants in E. coli MG1655 and b) thermosensitive mutants in S. typhimurium LT2. After screening of over 60 strains with five libraries and an average transformation efficiency of ~10^8 we were able to isolate two sequences that were able to complement a serB deletion mutant allowing growth in M9 minimal media. To elucidate the mechanism of these potential ‘de novo generated genes’ we are conducting genetic screens for improved functions and cross-complementation as well as structural analysis and in vitro activity assays.

STRAIN-SPECIFICITY AND EPISTATIS OF DIFFERENT RESISTANCE MECHANISMS

Michael Knopp

The phenotypic expression of resistance mechanisms is thought to be largely independent of the genetic background. To investigate the strain specificity of antibiotic resistance mechanisms we constructed five characterized resistance mutations (rpsL, rpoB, fmt, fusA and gyrA) in four different strains of Salmonella enterica. Our results show that the phenotypic expression of the five investigated resistance mutations is independent of the strain context: thus, the effect on fitness and resistance is similar in all four strains. To investigate if the robustness is a general feature of resistance mutations we are extending our study to include different groups of resistance mutations associated with import/export (marR, acrR, ompR), stress responses (spoT) or metabolism (cysB). Another important aspect of the influence of the genetic background on the phenotype of a resistance mutation is potential epistatic interactions between the phenotypic expression of different resistance mechanisms. Two mutations have negative, neutral or positive epistasis, if the fitness of a strain carrying both mutations is lower, equal or higher than the product of the fitness of the two individual mutants. In the case of positive epistasis the double mutant has a higher fitness than expected. This could lead to a selection against the loss of resistance in a multi-resistant strain, because the loss of any resistance mechanism would lower the fitness of the bacterium. To test epistatic interactions between the investigated resistance mutations we are constructing combinations of all five target alteration mutations in S. enterica serovar typhimurium LT2. Assuming all combinations are viable this would yield five single, ten double, ten triple, five quadruple and one quintuple mutant. The so far constructed combinations show a pervasive additive effect, partly contradicting recent publications.We are investigating the cause of this discrepancy, which is possibly due to the use of different resistance mutations, different organisms or a different method of fitness determination.

RAPID AND EFFICIENT COMPENSATION OF PORIN-LOSS IN ESCHERICHIA COLI

Michael Knopp

The emergence and spread of antibiotic resistances has lead to the loss of many therapeutic options and represents a major public health concern. The molecular mechanisms of resistances often impose severe fitness costs to the resistant bacterial clones. The success of resistance mechanisms is strongly dependent on their influence on growth and survival, and it is therefore of importance to understand which factors ameliorate the fitness burden and increase the stability of antibiotic resistance mechanisms. Many studies have focused on the compensation of fitness costs that are based on resistance mechanisms due to target alterations and where typically the mechanism of compensation involves point mutations that restore function of the affected target molecule or process. We recently identified two pathways involving the porins PhoE and ChiP that can compensate the growth defect associated with the loss of OmpC and OmpF in E. coli. In a separate study we are now investigating how the loss of ompR, a global regulator involved in ompCF expression, can be compensated. Surprisingly, we identified a completely different spectrum of compensatory mutations in an ompR-deficient background compared to an ompCF-deficient background. This indicates that the deleterious effect imposed by removal of OmpR is only partly due to

an ompCF-downregulation. We are conducting transcriptome analysis of the unevolved and evolved mutants to elucidate the biological cost of ompR loss and the mechanisms involved in the compensation of this cost.

COMPENSATING THE FITNESS COSTS OF SYNONYMOUS MUTATIONS

Anna Knöppel, Joakim Näsvall

Although synonymous mutations do not change the sequence of the polypeptide, an increasing number of studies have shown that they often confer a cost. In this study we have investigated why four synonymous mutations in rpsT, encoding ribosomal protein S20, in Salmonella enterica are costly, and how this cost can be compensated for. The synonymous mutants were found to have low levels of both rpsT mRNA and S20 protein. Previous studies have showed that 30S ribosomal subunits lacking S20 are impaired in mRNA binding and docking of the 30S initiation complex to the 50S subunit, and it is likely that the cost of these synonymous mutations is due to production of a defective subpopulation of 30S subunits lacking S20. The mechanistic cause of the impairments of the synonymous mutants was tentatively found to be on the level of translation for three of the mutants and at least partially on transcript levels for one of the mutants. In an adaptive evolution experiment, these impairments were compensated by up-regulation of S20 though increased gene dosage (duplications), increased rpsT transcript levels (rpoD mutation and intragenic rpsT mutations), or increased translation from the rpsT transcript (other intragenic rpsT mutations). Our results demonstrate how individual synonymous mutations can have very large effects on fitness, which may have implications for the use of dN/dS ratios as signature for selection and how these costs can be ameliorated by many different compensatory mutations in several types of target genes.

FIS AND RPOA MUTATIONS COMPENSATE FOR LOW S20 LEVELS BY DOWN REGULATING RIBOSOMES

Anna Knöppel, Joakim Näsvall

As a follow up of the project described above “Compensating the fitness costs of synonymous mutations” we are studying the compensatory effect by an additional six mutations that also ameliorated the cost of the four deleterious synonymous mutations in rpsT. Five of these were shown to down-regulate the global regulator and nucleoid-associated protein Fis and one was an amino acid change in the C-terminal domain of the α subunit of RNA-polymerase, next to a known Fis interaction site. These mutations had either none or inconclusive effects on rpsT mRNA and S20 protein levels. So, how can these mutations compensate for the fitness costs associated with reduced S20 levels in the synonymous mutants? Fis together with UP-elements are known to increase expression from rrn P1 promoters during exponential growth in rich medium for E. coli. One appealing explanation for the compensatory effect of the fis mutations is that they could compensate for the deleterious effects caused by production of a subpopulation of ribosomes lacking S20. Thus, down-regulation of rRNA would bring the ratio S20:rRNA closer to 1:1 and result in a larger proportion of functional 30S subunits. Any deleterious effects of having fewer ribosomes would be outweighed by the improved quality of the 30S pool. Subsequent rrn-yfp fusions and LC-MS/MS analyses indicated that synonymous mutants with additional fis or rpoA compensatory mutations have lower rrn-

transcription than both wild-type and the synonymous mutants alone, and that ribosomal protein concentrations are lower than in the wild-type, and sometimes even lower than in the single synonymous mutants. These results suggest that down-regulation of fis and an amino acid substitution in RpoA reduce ribosome concentration (by virtue of reducing rRNA transcription) and that this effect is beneficial in S20 deficient strains.

ADAPTATIONS TO LABORATORY CONDITIONS IN ESCHERICHIA COLI AND SALMONELLA ENTERICA

Anna Knöppel, Joakim Näsvall, Lisa Albrecht, Michael Knopp, Erik Lundin, Ulrika Lustig

Microorganisms that are used as laboratory model organisms have evolved in conditions that are different and far more varying than the common laboratory conditions. As a result, when grown in the lab, mutants that are better adapted to the lab environment and growth media are selected. We are characterizing mutants that were selected in four different growth media in Escherichia coli K12 strain MG1655 and Salmonella enterica ssp. enterica ser. Typhimurium strain LT2. Mutations in cultures passaged in the same media for between 500–1000 generations were identified by whole genome sequencing. If mutations were found in the same gene or pathway in multiple lineages evolved in the same medium they were considered potential adaptive mutations. These candidate adaptive mutations were genetically re-constructed and their fitness was tested by growth rate measurements and competitions. We found little overlap between which mutations were selected in the two organisms. Most of the candidate adaptive mutations conferred an increase in fitness as compared to the non-evolved wild-types. In addition, some mutations occur frequently enough to be a significant source of biological variation.

STAPHLYOCOCCUS AUREUS RESISTANCE TO ANTIMICROBIAL PEPTIDES

Jessica Kubicek-Sutherland, Hava Lofton, Karin Hjort, Hanne Ingmer, Martin Vestergaard

Antimicrobial peptides are in clinical development for treating multi-drug resistant pathogens, including methicillin-resistant Staphylococcus aureus (MRSA). However, many of these peptides are highly similar to essential components of the human innate immune system. We investigated the ability of a human clinical MRSA isolate to evolve resistance to such peptides derived from human, plant and pig (LL-37, wheat germ histones and PR-39, respectively) while grown in conditions reflecting a host environment. Stable resistance to these peptides developed rapidly along with cross-resistance to human β-defensins, which serve as our own first line of defense against this pathogen. Antimicrobial peptide resistant mutants of S. aureus were fully virulent in a mouse model of sepsis and also less susceptible to clinically prescribed antibiotics (teicoplanin, daptomycin, gentamicin), indicating that these pathogens would be even more difficult to treat. These findings suggest that widespread clinical use of antimicrobial peptides should be avoided in order to prevent the selection of bacterial resistance to the innate immune system. This project is performed in collaboration with Martin Vestergaard and Hanne Ingmer at Univ. of Copenhagen.

HORIZONTAL TRANSFER OF ANTIBIOTIC RESISTANCE GENES IN BACTERIA

Jessica Kubicek-Sutherland, Anna Knöppel, Chantal Weibel

The soil has been shown to serve as a reservoir for antibiotic resistance genes. Horizontal gene transfer of these genes between soil commensal bacteria and coincidental human pathogens is thought to play a significant role in the spread of clinical resistance to antibiotics. However, the diversity in resistance genes identified in the soil environment far exceeds that found in human pathogens. In actuality, the exchange of antibiotic resistance genes between natural soil bacteria and clinical pathogens occurs at a relatively low frequency, largely thought to be hampered by physical mobilization barriers. However the possibility that these genes are unfit in human pathogens has not been evaluated. Thus, we are interested in assessing whether resistance genes with no homology to those identified in clinical isolates are even capable of conferring resistance in a human pathogen, and how expression of these genes influence bacterial fitness. We obtained a collection of resistance genes derived from soil samples taken at wastewater treatment plants, a location where soil bacteria would often transiently encounter human pathogens. Resistance genes from a variety of antibiotic classes are represented including nucleic acid inhibitors (trimethoprim, sulfamethoxazole, rifampicin), protein synthesis inhibitors (amikacin, azithromycin, erythromycin, gentamicin, spectinomycin, tetracycline) and cell wall synthesis inhibitors (amoxicillin, ampicillin, carbenicillin, ceftazidime, piperacillin). These genes have been synthesized and cloned into an inducible expression system. We are now analysing the stability, fitness cost, and level of resistance conferred by these genes as a function of the level of gene expression in Escherichia coli and Salmonella, both bacterial species capable of causing disease in humans.

DYNAMICS OF ANTIBIOTIC RESISTANCE GENE TRANSFER IN Escherichia coli DURING GUT COLONIZATION

Jessica Kubicek-Sutherland

The human gut is a complex environment home to trillions of bacteria. Human pathogens often encounter these seemingly innocuous local microbiota during the course of an infection. Transfer of antibiotic resistance genes between commensal bacteria in the gut and human pathogens has been observed showing that the gut can serve as a reservoir for antibiotic resistance. However, little is known about the dynamics of these gene transfer events in the host environment. A study that followed E. coli colonization in the gut of a human baby from birth throughout the first year of its life isolated an E. coli strain that acquired a virulence-associated and antibiotic-resistant plasmid. This plasmid was not present in this strain at birth but was identified around 11 days later and was stably maintained in the child's gut throughout the course of the study (over a year). In order to investigate the role this plasmid plays in the ability of this E. coli strain to colonize and survive in the gut, we are using a mouse model. By competing this E. coli isolate with and without the plasmid, we aim to determine the plasmid's role in establishing and maintaining gut colonization as well as assess the dynamics of plasmid transfer over time. These findings will help to elucidate the selective pressures imposed by the host that propagate the transfer of both antibiotic-resistance and virulence-associated genes between bacterial species. This project is performed together with Morten Sommer at DTU, Denmark.

FITNESS EFFECTS OF RANDOM MUTATIONS

Peter Lind

How and why randomly introduced mutations affect cellular function are fundamental questions in evolutionary biology and medical genetics. We utilize a bacterial model system where highly sensitive fitness assays are used to assess the function of randomly mutated gene variants in comparison with an isogenic wild type strain. Our results demonstrate that both the distribution of fitness effects and the mechanistic causes of them are dependent on which functional class the gene belongs to. Ribosomal proteins had a unimodal DFE whereas mutations in AraC (transcription regulator), AraD (enzyme) and AraE (transporter) had bimodal DFEs with most mutations either being neutral or deleterious. Thus, the AraCDE and ribosomal proteins differ drastically from each other with regard to the shape of the DFEs and robustness. Mathematical modelling suggests that the higher average robustness of ribosomal proteins is due to selection to reduce the deleterious effects of mistranslation. Selection strength is expected to depend on the mass of the functional unit, copy number and error rate.

MECHANISMS of TIGECYCLINE Resistance IN Escherichia coli

Marius Linkevicius

Tigecycline is the main representative of the new class of antibiotics named glycylcylines. It is active against multidrug resistant gram-positive bacteria like MRSA, VRE and gram-negative pathogens producing extended spectrum ß-lactamases. However, resistance against tigecycline has been reported and overexpression of unspecific RND or MATE family transporters is the reason for the resistance to tigecycline. This study focused on determination of resistance mechanisms to tigecycline and the consequential fitness costs in E. coli. Two main groups of spontaneous E. coli mutants with low-level resistance to tigecycline were identified. Genes involved in LPS biosynthetic pathway (rfaC, rfaD, rfaE, rfaF and lpcA) were found in one group. It is likely that these mutations affect the uptake of tigecycline, though the actual influx mechanism is not fully elucidated. Another group of mutations (lon, acrR and marR) was linked to bacterial efflux and its regulation. Some of these mutations are present in Enterobacteriaceae strains clinically resistant to tigecycline. The selected low-level tigecycline resistant mutants had increased MICs for hydrophobic antibiotics and reduced MICs for SOS inducing antibiotics. In addition, a fitness cost of these mutations was observed. Oxidative stress, bile sensitivity, serum sensitivity and acid tolerance were examined for the reconstructed E. coli mutants. While LPS mutants demonstrated increased sensitivity to bile, none of the other in vitro tests revealed any major differences from the wild-type. Similarly, only LPS mutants were cleared out in some in vivo competition experiments. Collectively, these data indicate that the majority of parameters that can be important during infection are not affected by the mutations leading to the reduced susceptibility to tigecycline. However, efflux regulator network mutations seem to have a lower overall fitness cost and a higher tigecycline resistance level compared to LPS mutations, poviding a possible explanation for why upregulation of efflux systems is the main tigecycline resistance mechanism reported in clinics.

TIGECYCLINE RESISTANT TET PROTEINS

Marius Linkevicius

Tigecycline overcomes major resistance mechanisms that render previous two generations of tetracyclines non-usable. It is not transported out of the cell by Tet efflux pumps, as the transport proteins cannot recognise the antibiotic as a substrate due to its bulkier chemical structure. In addition, higher affinity to ribosome and the 9-t-butylglycylamido side chain prevents the dissociation of tigecycline from the A site in the 30 S subunit even in the presence of Tet ribosomal protection proteins. In this study, we were interested whether evolution of tigecycline resistance conferred by Tet proteins was possible. DNA sequence libraries of Tet efflux, ribosomal protection and enzymatic modification proteins were generated using error-prone PCR. Selections of tigecycline resistant mutants harbouring mutagenised Tet efflux proteins revealed that it is possible to select protein variants, with increased minimal inhibitory concentration of tigecycline. Mutations leading to elevated tigecycline MIC accumulated in periplasmic loops and transmembrane domains of Tet(A) efflux protein. These mutations most likely are affecting the architecture of the pump to accommodate the bulky tigecycline molecule. All Tet(M) mutant proteins contained at least one mutation in loop III of domain IV. This loop interacts with C1054 in 16S rRNA, the nucleotide important for tetracycline binding to the ribosome and chases out tetracycline molecule from its binding site. Amino acid substitutions or deletions present in this region increased tigecycline MIC. Interestingly, both Tet(A) and Tet(M) mutants with elevated tigecycline resistance level demonstrated collateral sensitivity to earlier generations of tetracyclines. Contrary to the above observations, this was not the case for the modification enzyme Tet(X). The Tet(X) mutants with higher tigecycline MICs also improved the activity against earlier tetracyclines. These findings combined with a recent report of Tet(X) horizontal transfer to human pathogens lead to worrisome predictions that Tet(X) mutants might compromise the therapeutic potential of tetracycline antibiotics in the future.

DISTRIBUTION OF FITNESS EFFECTS OF MUTATIONS IN THE BIOSYNTHETIC ENZYME HISA

Erik Lundin, Joakim Näsvall

The distribution of fitness effects (DFE) of mutations is of fundamental importance to understand major evolutionary questions regarding, for example, disease development, the maintenance of healthy population sizes of endangered species and understanding the evolution of antibiotic resistance. Little is known about the DFE as it rarely has been studied using sensitive experimental procedures. The effect of mutations can be classified into three major categories: beneficial, neutral and deleterious. Beneficial mutations are rare and tend to be exponentially distributed whereas deleterious mutations tend to show a bimodal U-shaped distribution with most mutations being lethal/highly deleterious or close to neutral. We used Salmonella typhimurium LT2 to study the DFE of random nucleotide mutations in HisA, an enzyme that catalyzes the fourth step of the L-histidine biosynthetic pathway. The fitness effects a large set of unique mutants were measured and shown to span the whole fitness landscape. The DFE was analyzed in relation to number of mutations, type of amino acid substitution and the surface exposure of the mutations. DFE was also compared between synonymous and nonsynonymous substitutions to assess the fitness constraints at the messenger RNA level.

DOES NEUTRAL GENETIC VARIATION AFFECT EVOLVABILITY?

Erik Lundin, Joakim Näsvall

Mutations can affect the function of a gene in many different ways and may also affect later evolution of new functions by allowing the evolving enzyme to traverse fitness valleys and reach new, distant peaks in the fitness landscape. The hisA gene will be placed under the control of a strong promoter so that the activity of the HisA enzyme is not limiting for growth and mutations will be accumulated through rounds of mutagenesis. A collection of mutants containing one or several neutral mutations will be used as starting points for experimental evolution towards TrpF activity.

FUNCTIONAL TRADE-OFFS DURING EVOLUTION OF NEW FUNCTIONS

Erik Lundin, Joakim Näsvall

When a specialist enzyme (with a weak secondary activity) accumulates mutations and evolves towards a new specialist enzyme with a new function (with weak or no original activity) it will at some intermediate time points be a generalist enzyme with some original and some new activity. The mutations introduce or increase a new beneficial activity in a gene and may have one of three different effects on the original activity: 1) the original activity is unaffected or only slightly decreased. 2) the original activity is lost proportionally to the gain in new activity. 3) the original activity is completely lost or severely decreased. The nature of the functional relationship between the new and original activity is likely to determine which paths evolution can take when both the new and old function is selected. The aim of this project is to study the intermediate generalist enzymes occurring through evolution towards new gene function and determine the nature of the trade-offs when acquiring a new function and loosing an old. To test which of the trade-offs are present and assess the effects on protein stability we are setting up a model system based on mutations that confer TrpF activity to the hisA gene product (see above). The hisA gene will be mutagenized and variants with TrpF activity will be selected. The growth rates of strains carrying these mutant alleles in the absence of histidine or tryptophan will be used as a measurement of the different activities to see which trade-off(s) exists in this system.

DISTRIBUTION OF MUTATIONS AND FITNESS EFFECTS IN SALMONELLA TYPHIMURIUM LT2 EVOLVED WITH STRONG GENETIC DRIFT

Ulrika Lustig

In order to examine the distribution of randomly accumulated mutations in the bacterial genome during growth, we passaged Salmonella typhimurium LT2 on solid rich media without selection pressure. The bacteria were grown for 2000 generations under conditions expected to result in high genetic drift (one-cell bottlenecks). The 110 evolved lineages of S.typhimurium were whole genome sequenced (MiSeq) which revealed a distribution of 0 to 5 mutations per genome. Fitness measurements are performed by competing the evolved lineages to the original wild-type, using Magnetic-Activated Cell Sorting (MACS).

RESISTANCE DEVELOPMENT AND MODE OF ACTION OF CYCLOVIOLACIN O2

Sohaib Z. Malik

Cyclotides are a family of plant proteins with a cyclic backbone and three disulfide bonds that tie them into the so-called cyclic cystine knot. The extreme stability of cyclotides to chemical, thermal and enzymatic degradation makes them a promising scaffold for drug design applications. We have previously shown that the cyclotide, cycloviolacin O2 (cyO2) has a killing effect on Gram-negative bacteria in low micro-molar concentrations. In the present study, we have explored the mechanisms of resistance development to cyO2. For this purpose, 14 independent lineages of Salmonella enterica and 4 independent lineages of Escherichia coli were serially passaged in increasing concentrations of cyO2 for 100 or 150 cycles (600-700 or 900-1050 generations) to select for mutants with reduced cyclotide susceptibility. Clones were isolated from the populations evolving under this selective pressure and whole genome sequenced. Whole genome sequencing identified a number of mutations that conferred resistance. Mutations that appeared in more than one resistant clone were reconstituted in a wild type background. All but one of these mutations reduced susceptibility to cycloviolacin O2. Cross-resistance to other antimicrobial peptides was tested. Growth rates of these mutants, relative to congenic wild type strains, were determined. As the next step, the effects of combinations of resistance mutations will be tested. In another part of this project: a) interaction of cyO2 with the outer membrane of Gram-negative bacteria was investigated using hydrophobic probes and organisms with a tighter permeability barrier, b) the effect of different culture conditions (bacterial growth phase, effect of bacteriostatic drugs, pH, inoculum size) was explored, c) antibodies were raised against cycloviolacin O2, which will be used in quantification of cyclotide binding to bacterial cells. This project is a collaboration with Prof. Ulf Göransson at Uppsala University.

ANTIMICROBIAL EFFECTS OF CYCLOTIDES

Sohaib Z. Malik

Cyclotides possess many positive attributes from a drug development perspective. Extreme stability to thermal, chemical and biological degradation, together with potent activity of the prototype bracelet cyclotide, cycloviolacin O2, against Gram-negative bacteria; warrants an in-depth exploration of their potential as a family of antimicrobial peptides. We isolated sixteen (16) cyclotides from three different plants; Oldenlandia affinis, Viola odorata and Viola arvensis. An initial screen in E. coli, revealed two cyclotides with comparable inhibitory activity to cycloviolacin O2. Another three peptides, were moderately active while the rest were inactive. NMR was done on one of the most active and one moderately active peptide. Cross-resistance to cycloviolacin O2 resistant mutants was tested. We will broaden the screen, by adding more cyclotides (natural and synthetic) and by testing against a broader set of bacteria. These studies would be a stepping-stone for the attempts to engineer cyclotides as antimicrobial therapeutics. This project is a collaboration with Prof. Ulf Göransson at Uppsala University.

PASSIVE ANTIBIOTIC RESISTANCE

Hervé Nicoloff

Passive resistance describes the protective effect that an antibiotic-resistant population can exert on a sensitive population. It is observed during coinfections involving both β-lactam-resistant and β-lactam-sensitive bacterial populations. During these, β-lactamases in resistant cells efficiently decrease the antibiotic concentration in the cells surrounding environment over time, allowing nearby β-lactam-sensitive bacteria to resume growth. The unique localization of β-lactamases in the periplasm combined with the efficient water-dependent drug-inactivation mechanism led to the speculation that passive resistance could be specific to β-lactams and β-lactamases. In this project, we investigated whether passive resistance could be detected in other situations. We tested passive resistance to thirteen antibiotics from seven different classes using fifteen resistance markers. We observed passive resistance to several antibiotics and with antibiotic-modifying enzymes active in the cytoplasm and requiring cofactors such as acetyl-CoA. Thus, the unique characteristics of β-lactamases were not required for passive resistance to those drugs. Rather, we found that passive resistance was dependent on the drug diffusion rate through the cell membranes. While the concentration of efficiently-diffusing antibiotics, which require efficient drug-modifying enzymes for resistance, could decrease over time in presence of a resistant strain and lead to a passive resistance phenotype, slow-diffusing drugs could not. This work revealed that co-infections might be a greater cause of antibiotic treatment failures than previously thought.

HETERORESISTANCE

Hervé Nicoloff, Karin Hjort

Heteroresistance is the ability of bacterial isolates to grow as mixed populations characterized by different antibiotic resistance phenotypes. While the main population remains relatively sensitive to an antibiotic, a small unstable subpopulation presents a higher resistance. Heteroresistance has been described in clinical settings for a few bacterial species and antibiotics and is a great cause of concern during antibiotherapy. It is difficult to detect and its mechanism remains unknown or unclear. In this project, we wanted to assess the frequency of heteroresistance and describe the mechanisms leading to heteroresistance. To assess heteroresistance frequency, fourty clinical isolates from four different species (E. coli, S. enterica, K. pneumoniae and A. baumannii) were tested for heteroresistance against more than twenty antibiotics, most of which are of clinical interest. All strains tested presented a heteroresistance phenotype to at least one antibiotic, and heteroresistance was found for most antibiotics. The instability and the frequency of the resistant subpopulations observed (typically around $10^{-4}/10^{-5}$) could be compatible with genetic duplications/amplifications events. Therefore, to find the mechanisms of heteroresistance, subpopulations with increased resistance were selected, tested for instability of the resistance, and sent for whole genome sequencing. Potential mutations involved in heteroresistance will be detected by comparing the sequences of isolates under antibiotic selection to sequences of the same isolates grown without antibiotic selection. Eight isolates from three different species and with unstable heteroresistance phenotypes to five antibiotics were sequenced so far. This project revealed high frequencies of heteroresistance phenotypes and ongoing work will hopefully lead to the description of heteroresistance mechanisms.

PAN-GENOME AND ANTIBIOTIC RESISTANCE

Hervé Nicoloff, Anna Knöppel

Most of our knowledge on antibiotic resistance derives from studies performed with a single or a limited number of bacterial strains (e.g. the Escherichia coli laboratory strain MG1655). However, the number of genes that can be found in a species (the pan-genome) far exceeds the number of genes found in individual strains/isolates. For example, the E. coli pan-genome is estimated to be potentially as large as >60,000 genes although individual isolates typically carry only about 5,000 genes. Thus, studies performed with a very limited number of isolates likely fail to identify many genes and mutations that could be involved in antibiotic resistance. A better understanding of a species ability to develop antibiotic resistance – both in terms of frequency and ability to develop resistance, and in terms of antibiotic resistance mechanisms and their effects on fitness - is of outmost importance to fully grasp the threat caused by antibiotic resistance, predict development of resistance and combat it efficiently.
In this project we aim to (i) determine whether using a large set of E. coli isolates (35 isolates) could lead to the discovery of new resistance mechanisms that are either encoded on the part of the pan-genome that is not common to all isolates (the variable genome) or are encoded on the core genome (common to all strains) but are affected by the variable genome by epistasis effects; and (ii) measure the impact that the genetic background plays on the development of a resistance phenotype and on the fitness of bacteria carrying chromosomal mutations or expressing genes known to increase resistance.

INSIGHTS INTO ENZYME EVOLUTION BY CRYSTALLOGRAPHIC STRUCTURE DETERMINATION OF HISA MUTANTS POSSESSING TRPF ACTIVITY

Annika Söderholm

Näsvall et al. 2012 demonstrated how new genes can evolve by the innovation-amplification and divergence evolutionary (IAD) model. In the study, a mutated hisA gene from S. enterica which provided a low level of TrpF activity while retaining some original activity was isolated and placed in a strain that lack functional hisA- and trpF genes. Through continuous selection for both activities in the absence of histidine and tryptophan amplification of the dual function hisA gene was promoted and the amplified gene products diverged so that different mutant enzymes evolved. The evolved mutants could be divided into three different categories related to their catalytic characteristics; HisA specialists, TrpF specialists or generalists. The aim of this project is to provide a structural explanation of how the different mutations affect the two catalytic activities and to understand what is the basis for generalist and specialist activities. An additional aim of the project, that arose due to discrepancies in previously published literature regarding the catalytic mechanism of HisA, is to establish the correct mechanism of this enzyme. I have been working together with Xiaohu Guo (Maria Selmer's group, ICM) on determining the structures of the wild type and mutant HisA enzymes by X-ray crystallography and the stability by using circular dichroism spectroscopy. Our structure and stability data is analysed together with enzyme kinetic data provided by Matilda Newton in Wayne Patrick's group (University of Otago, New Zealand).. We have solved structures of 11 different S. enterica HisA mutants, including wild type enzyme, and for some of these we also have ligand structures with the HisA substrate ProFAR. We also attempted solving structures with the TrpF product analogue rCdRP but unfortunately the

affinity for this ligand is too weak to observe clear electron density. However, as indication for binding we do observe ordering of the active-site loops when soaking with the ligand. Earlier this year we published our structural and kinetics results on the wildtype HisA enzyme. From this study, we could establish the catalytic mechanism of HisA and provide new insight into its mode of catalysis. For example, we showed that substrate binding occurs in two distinct steps where enzyme and substrate undergoes coupled conformational changes. We are currently working on the manuscript covering the study on the mutant enzymes, the evolutionary aspects of adaptation and how the kinetic parameters of the enzymes are correlated to the growth phenotypes of the evolved mutant genes. Our structures have allowed us to understand how HisA evolved TrpF activity and suggest what the roles of the different mutations are in changing the phenotype. In summary, our hypothesis suggests that the innovation mutation yielding TrpF activity, a duplication of a VVR sequence in active-site loop 1, does so by allowing the loop to, in the closed state, put a positive charge (arginine) in position for binding the negatively charged TrpF substrate in the active site. The extended loop 1 competes for the active site space with loop 5, essential for HisA activity. The mutations leading to TrpF specialist phenotype will either disfavour HisA substrate binding by decreasing the active site or act by stabilizing the conformational state where loop 1 is closed. The mutations promoting HisA specialist phenotype will instead favour loop 5 closing. The generalist phenotypes have increased loop dynamics leading to the conformational freedom of adopting both "closed loop 1" (TrpF) state and "closed loop 5" (HisA) state. Since a few months back I am supervising a project student, András Erdelyi, who is also involved in the HisA project, to make a better TrpF using a HisA mutant from Erik Lundin's screening as a starting point and modify it by a protein engineering approach with the bifunctional HisA homologue PriA as a model structure. He will rationally design mutations, carry out mutagenesis and then test the mutants by binding assays as well as in vivo and in vitro activity assay. He will also try to solve complex structures with the ligand rCdRP.

STRUCTURAL STUDIES OF DE NOVO EVOLVED ENZYMES

Annika Söderholm

The aim of this project is to structurally characterize three hits complementing auxotrophs from the project of Jon and Michael (see "De Novo genes from random sequences**")**. From the random screening performed by Michael two 150 bp inserts complementing a SerB knockout were found. From the phage-library screening performed by Jon one hit encoding a 162 amino acid polypeptide complementing a ilvA knockout was picked up. Expression tests have shown that all three constructs have solubility problems. I have not been able to obtain pure and soluble protein for any of them. The constructs are his-tagged and I use IMAC as purification method. I have tried various buffer conditions including denaturing conditions but always end up with a high fraction of contaminating proteins. A possible future strategy to try could be co-expressing with a fusion protein that can be utilized for another type of affinity chromatography.

MECILLINAM RESISTANCE

Elisabeth Thulin

Many of the traditional antibiotics used for treatment of urinary tract infections (UTIs) have been rendered useless due to the resistance development in the UTI-causing pathogens. But resistance development to mecillinam has remained low and it is now used more widely for treatment. The goal of this project is to understand the dynamics of mecillinam resistance development using genetics, physiology and experimental evolution of mecillinam resistance, and examining mutants isolated in the laboratory as well as clinical isolates. Mecillinam resistant mutants of Salmonella typhimurium and Escherichia coli have been selected in the laboratory. Characterization of the mutants and identification as well as reconstruction of the resistance mutations was done for both species. We found several novel mecillinam resistance genes. The mecillinam resistant clinical isolates were whole genome sequenced and compared to 20 reference strains (both clinical and isolated in the lab). The clinical isolates have significantly higher fitness compared to the in vitro mutants both in media with and without mecillinam added. Mutations in one particular mecillinam resistance gene (cysB) appeared in all the clinical strains. These mutations confer resistance above the clinical break point for mecillinam, but cannot alone explain the very high resistance shown in a few of the clinical isolates. The clinical isolates might have this mutation because it has a low fitness cost, and since it gives an intermediate resistance it might be important as a stepping stone for development of higher resistance in clinical settings. Several other mutations that might contribute to higher resistance were also found in the clinical isolates. Presently we are comparing growth of in vitro and clinical mutants in urine (clinical isolates are from UTI patients), examining several different types of epistasis in mecillinam resistance and working on getting a better understanding of the impact of ppGpp (stringent response) on mecillinam resistance. We are also trying to find the common denominator of all or most of the mecillinam resistance genes. At least 40 mecillinam resistance genes are known (including the novel ones fond in this project), many of which act through the stringent response. By comparison of ten resistant mutants with different mecillinam resistance genes, we may uncover the mechanism that unifies the seemingly different resistance mutations.

FITNESS COMPENSATION OF MECILLINAM RESISTANT MUTANTS

Elisabeth Thulin, Michael Knopp

In a serial passage evolution experiment mecillinam resistant mutants (obtained from the previously described project) that had compensated for the fitness loss (decreased growth rate) associated with mecillinam resistance were selected and sequenced to identify the mutations responsible for the growth compensation. Reconstruction of the compensatory mutations to determine their effects on fitness and resistance is ongoing. In addition, we have found mecillinam resistant clinical isolates that appear to have acquired compensatory mutations. These have been whole genome sequenced and we are comparing them to clinical isolates from the same patient as well as to the laboratory selected compensated strains to identify the compensatory mutations and to determine whether they are the same in clinical isolates and laboratory selected mutants. Preliminary results show that the same type of compensatory mutations can be found in at least some of the laboratory selected strains and clinical isolates, implying that compensatory evolution has occurred in the resistant clinical isolates.

MECILLINAM RESISTANCE IN URINE FROM DIFFERENT DONORS

Elisabeth Thulin, Karin Hjort

In previous studies, when growing mecillinam resistant mutants in urine, we found that the susceptibility of a strain depends on the donor of the urine. Thus, a strain that is mecillinam resistant in common laboratory growth medium can be susceptible during growth in urine of certain individuals. To examine this phenomenon closer we will grow mecillinam resistant strains in mecillinam-supplemented urine donated from 50-100 volunteers. We expect to find that in urine from some people the strains will be susceptible and in urine from some the strains will be resistant. Urine from these two different groups will be sent for metabolome analysis to identify the metabolic differences in urine from the two groups.

E. COLI ADAPTATION TO GROWTH IN URINE

Elisabeth Thulin, Ulrika Lustig

The growth rate in urine of clinical E. coli isolates from patients with urinary tract infections (relative growth rates ranging between 1.2 and 1.7) is significantly higher than the growth rate in urine of the laboratory reference strain E. coli MG1655 (relative growth rate set to 1). To determine which mutations can confer growth advantages in urine we serially passed four lineages of E. coli MG1655 in urine for 500 generations. The evolved lineages grow faster in urine and the mutant populations are currently being whole genome sequenced to identify the adaptive mutations. The mutations found in these strains will be compared to whole genome sequenced clinical E. coli isolates from patients with urinary tract infections, to determine if the same mutations are present in clinical urine-adapted isolates. Additionally the clinical E. coli isolate with the lowest relative growth rate (1.2) was also serially passaged to determine if the relative growth rate would increase further in the adapted lineages. The growth rate measurements for the adapted lineages are yet to be performed.

RESCUE OF TEMPERATURE-SENSITIVE SALMONELLA TYPHIMURIUM L2 MUTANTS USING METAGENOMIC ENVIRONMENTAL PHAGE LIBRARIES

Omar Warsi, Jon Jerlström-Hultqvist

Evolution of new gene functions have been central to major evolutionary transitions in life forms, and is the primary contributor to the process of adaptive radiation. Studies on this topic have primarily been theoretical or comparative. Early studies including those done by Haldane (1932), Fisher (1935) and Muller (1935) were based on population genetics principles, while more recent ones include phylogenetic comparisons between sister species. To empirically investigate this question, our experimental design consists of rescuing temperature-sensitive Salmonella mutants using metagenomic phage libraries. Environmental phage DNA is extracted from different locations (lake, sewage, cystic fibrosis patients) and is cloned into the vector pCA24N; each of the phage DNA is also under the control of an inducible promoter. The temperature sensitive mutants used in the study were previously generated by chemically mutagenizing Salmonella typhimurium LT2. Sixty temperature sensitive mutants have been screened so far using twelve different phage libraries. Different non-permissive temperature boundaries have been established for each of these mutants, and

the screenings have been done at different temperatures, wherever appropriate. Rescue of these mutants is seen by aid of different tRNAs, metabolic enzymes and fused proteins, suggesting that rescue can occur by several types of mechanisms.

SELECTION OF SALMONELLA TYPHIMURIUM LT2 MUTANTS WITH THE ABILITY TO METABOLIZE NON-NATIVE CARBON SOURCES

Omar Warsi, Erik Lundin, Ulrika Lustig

Adaptation to novel nutrient sources is one of the central aspects of evolutionary innovation, which allows the organism to occupy new niche space. To investigate the genetic changes that accompany this form of evolutionary innovation we evolve Salmonella typhimurium LT2 to utilize new carbon sources. Salmonella typhimurium LT2 was allowed to grow under the effect of an inducible error prone polymerase, with the increased mutation rate measured to be in the order of 10^{-7}/bp/generation/individual. These mutated populations were then screened for their ability to grow on 103 non-native carbon sources that Salmonella typhimurium LT2 is unable to metabolize. Mutant clones that have the ability to grow on 13 of these carbon sources have been identified. Whole genome sequencing of these clones suggest cryptic operons (celD for metabolizing cellobiose), regulator proteins (fucR for metabolizing arabinose) and enzymes from gluconeogenesis pathways (pmgI for metabolizing leucine) to be key targets for these novel phenotypes.

EVOLUTION OF HOST-ADAPTATION

Lionel Guy

Our research explores how microorganisms, particularly bacteria, adapt to (eukaryotic) hosts, and how their relationships evolve over time. The overall goal of our research is to better understand the long- and short-term evolution of host adaptation, and to identify strategies and critical genetic innovations that allow free-living bacteria to adapt to, and then profit from, or live in symbiosis with, eukaryotic hosts.
For their wide variety in host-adaptation strategies and their ecological success, we currently focus on the bacterial order *Legionellales*, which includes two human pathogens, *Legionella* and *Coxiella*. So far, all described species of *Legionellales* can replicate intracellularly, although their degree of reliance on their host and their host spectrum varies, which makes them especially interesting to study.
Our research is divided in four axes:

1. Identifying human-specific adaptations in *Legionella pneumophila*, which could occur during the course of an infection and increase *Legionella*'s virulence.
2. Reconstructing the last common ancestor of *Legionellales*, by sequencing uncultured novel genera with deep metagenomics methods, and tracing the emergence and fate of host-adaptation genes.
3. Setting up a long-term experimental evolution experiment with *Legionella pneumophila* in three different hosts, to (i) identify crucial host-adaptation genes and (ii) test whether adaptation trade-offs that are postulated in generalist host-adapted bacteria actually exist.
4. Understanding the environmental factors shaping the diversity and life-style of amoeba-resisting bacteria (ARBs), in particular of *Legionella*.

Using both experimental microbiology and bioinformatics, we expect to shed some light on those fundamental processes underlying the evolution of the genomes of host-adapted bacteria.

Members of the group during 2015
Lionel Guy, assistant professor, group leader

International exchange during 2015
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SHORT-TERM ADAPTATION TO THE HUMAN HOST IN *LEGIONELLA PNEUMOPHILA*

Dennis Leenheer, Lionel Guy

- Can short-term adaptation in outbreaks reveal adaptations to human?

By sequencing *Legionella pneumophila* isolates from legionellosis outbreaks and environmental sources, we identify short-term mutations that occur inside the accidental human host. By tracing independent mutations in identical genes (convergent evolution), we point to adaptations specific to the accidental human host.
We have sequenced the genomes of 20 pairs of isolates (collaboration with the Public Health Agency of Sweden), one from a patient and the other the presumed environmental source, and 30 isolates from two separte outbreaks (collaboration with the Spanish Reference Center for Legionellales). We have identified a handful of genes that are mutated several times in our sample, but more samples will be gathered and sequenced in the future to increase the statistical support and identify more genes.

EMERGENCE AND EVOLUTION OF HOST-ADAPTATION IN *LEGIONELLALES*

Lionel Guy

- Is the current diversity of *Legionellales* resulting from a single event of host-adaptation? Was the ancestor of *Legionella* and *Coxiella* living inside amoebae?
- What systems, crucial for host-adaptation, were invented or recruited that paved the way to its current ecological success?

To answer these questions, we are using a comparative genomics approach, tapping into the unexplored diversity of *Legionellales*. We use state-of-the art metagenomics and single-cell genomics to harvest organisms that are uncultivable, and reconstruct full genomes. We aim at reconstructing the ancestors of *Legionellales* and establish the flow of host-adaptation systems.
We have started by sequencing the genomes of the only two *Legionellales* isolates that hadn't been sequenced, *Aquicella lusitana* and *Aquicella siphonis*. We are also currently identifying the most interesting types of environments where to sample novel *Legionellales* by analyzing published metagenomes.

INSIDE THE AMOEBAL TROJAN HORSE: ENVIRONMENTAL FACTORS SHAPING THE DIVERSITY OF INTRA-AMOEBAL BACTERIA

Lionel Guy

The overall goal of this project is to better understand the environmental factors shaping the diversity and life-style of amoeba-resisting bacteria (ARBs), in particular of *Legionella*. Amoebae play an important in the ecological success of *Legionella*, protecting them from bactericidal products, and providing them with a "melting-pot of evolution" where they are exposed to a continuous flow of potentially beneficial genes. Co-evolution between *Legionella* and its host is thought to have paved the way to the success of *Legionella* as a human pathogen.

- What is the genetic diversity inside one single amoebal host? In a population of hosts?
- What environmental factors influence the diversity of *Legionella* and other amoeba-resisting bacteria?
- What is the role of the amoebal host? Are them all equally likely to harbor and disperse intracellular bacteria? How do they respond to environmental factors?
- What genes and host-adaptation systems are frequently exchanged? Is it possible to identify these transfers as they happen?

In this context, it is very interesting to investigate the effects of the environment on the microbial diversity occurring inside single amoebal hosts and communities of hosts, and to monitor the prevailing gene flux inside them.
This project is one of the SciLifeLab National Projects for 2015.

LONG-TERM EXPERIMENTAL EVOLUTION IN INTRACELLULAR BACTERIA

Christian Dobre-Lereanu, Lionel Guy

The overall goal of this project is to better understand the precise molecular mechanisms underlying the evolution of host adaptation, to understand the specifics of evolution in small populations and with frequent bottlenecks, and to identify critical genetic innovations that drive host selection and range in intracellular bacteria.
Main questions:

1. Are there adaptation trade-offs? Does adaptation to a specific host reduces the fitness of adaptation to other hosts? Does alternating between hosts reduce the adaptation to a specific host but preserves (or increases) the ability to adapt to novel hosts?
2. Are convergent adaptive mutations, clonal interference and genetic interactions – all salient features of Lenski's Long-Term Experimental Evolution (LTEE) with *E. coli* – also common in intracellular bacteria that have small population sizes and frequent bottlenecks?
3. What are the genes or systems that are most crucial for specific or generic host-adaptation in *Legionella*? Are there more human-specific adaptations that can be identified?

These three questions can be answered by setting up a LTEE with *Legionella pneumophila* in different hosts or host ranges, comparing the fitness of the evolved strains to the ancestor and to each other, and by performing whole-population sequencing.
Currently, we are setting up the different infection models in the lab, testing semi-automated ways to measure growth and constructing *Legionella pneumophila* mutants with genes for fluorescent proteins integrated in the chromosome.
This project is a collaboration with Dan Andersson (IMIBIM), Elisabeth Kay (International Center for the Research on Infectious Diseases, Lyon, France), Fredrik Söderbom (ICM) and Patrik Ellström (IMBIM).

BACTERIAL RESPONSES TO STRESS AND SELECTION

Diarmaid Hughes

Our research interests are focused on bacterial genetics and evolution, specifically where it concerns the development of resistance to antibiotics and the significance of bacterial genome organisation. In studying the important phenomenon of antibiotic resistance, an in-depth understanding of the selection processes and evolutionary principles behind fitness/resistance trade-offs is required for developing methodologies capable of suppressing the growth and spread of resistant bacteria. We also lead the microbiology effort in the IMI ND4BB ENABLE project, a public-private partnership involving over 30 different groups in Europe (large pharmaceutical companies, small to medium enterprises, hospitals, research institutes, and universities), with the aim of developing novel antibiotics active against Gram-negative organisms and taking at least one into phase 1 clinical trials.

We are studying the development of resistance to antimicrobial drugs, with a particular focus on the fluoroquinolones and rifampicin. Questions include the step-wise nature of antibiotic resistance evolution, how resistance impacts bacterial fitness in different environments, and how bacteria respond by compensatory evolution. Antibiotic dosing strategies to minimize the risk of resistance development or selection are an important area of research where we collaborate with other groups within Uppsala University.

Within ENABLE we are involved in testing the antibacterial activities of novel compounds targeting Gram-negatives, and with testing resistance frequencies and resistance mechanisms, employing whole genome sequencing and genetics.

We also study bacterial microevolution and the significance of bacterial genome organisation. These studies relate bacterial genetics and growth physiology with transcription, translation and gene expression regulation. Specific questions include the regulation of bacterial growth rate and ability to sense starvation, the importance of codon usage as a regulatory device and the possibility to evolve codon usage in response to demand, and the rates and physiological significance of gene conversion and genome rearrangements

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Franziska Pietsch**:** Evolution of Antibiotic Resistance, December 11

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EXTRAGENIC SUPPRESSORS OF RNase E MUTANTS

Disa Hammarlöf, Jessica Bergman, Eva Garmendia

Why is the RNA processing enzyme RNase E essential? Bacterial cells need to process tRNA and rRNA and to degrade old or damaged mRNA transcripts in order to keep the transcription and translation machinery and processes in balance and attuned to growth requirements. In these processes, RNase E plays a central role, but the reason for its essentiality is unknown. Using a set of temperature-sensitive *rne* mutants in *Salmonella enterica* serovar Typhimurium, we selected and isolated extragenic suppressors that restored viability. Since these double mutants grow at the non-permissive temperature where mutant RNase E does not carry out its essential function, each of the suppressor mutations must somehow reduce the requirement for, or bypass, the essential function of RNase E. We mapped and identified a number of extragenic suppressors that are all related to translation or degradation of mRNA. Based of this we are exploring the hypothesis that the essential function of RNase E is to degrade mRNA, possibly to rescue ribosomes trapped on defective messages. This work was published during 2015 (reference 13 above).

COMPREHENSIVE PHENOTYPIC CHARACTERIZATION OF RIFAMPICIN RESISTANCE MUTATIONS

Gerrit Brandis, Franziska Pietsch

Mutations in the ß-subunit of RNA-polymerase, encoded by *rpoB*, are responsible for rifampicin resistance. Although many different mutations can cause rifampicin resistance, only a few different resistance mutations are predominantly found among clinical *M. tuberculosis* (MTB) isolates. It has been suggested that there is a correlation between the fitness costs caused by the resistance mutations and their respective clinical frequency but so far no comparable fitness cost measurements have been conducted for these mutations. We tested this hypothesis using *Salmonella* as a model organism. Our results suggest that rifampicin resistance mutations in clinical MTB isolates are primarily selected for high-level resistance (rather than low fitness cost), and that fitness-compensatory mutations are subsequently selected that reduce the fitness cost caused by the resistance mutation. This work was published during 2015 (reference 12 above).

THE SELECTIVE ADVANTAGE OF SYNONYMOUS CODON USAGE BIAS IN *SALMONELLA*

Gerrit Brandis

The genetic code in mRNA is redundant, with 61 sense codons translated into 20 different amino acids. Individual amino acids are encoded by up to six different codons but within codon families some are used more frequently than others. This phenomenon is referred to as synonymous codon usage bias. The genomes of free-living unicellular organisms such as bacteria have an extreme codon usage bias and the degree of bias differs between genes within the same genome. The strong positive correlation between codon usage bias and gene expression levels in many microorganisms is attributed to selection for translational efficiency. However, this putative selective advantage has never been measured in bacteria and theoretical estimates vary widely. By systematically exchanging optimal codons for synonymous codons in highly expressed genes we quantified the selective advantage of biased codon usage to range over an order of magnitude, from 0.2 - 4.2 x 10^{-4} per codon per generation. These data quantify for the first time the potential for selection on synonymous codon choice to drive genome-wide sequence evolution, and in particular to optimize the sequences of highly expressed genes. This quantification may have predictive applications in the design of synthetic genes and for heterologous gene expression in biotechnology.

POST-TRANSCRIPTIONAL REGULATION OF THE *TUFB* OPERON IN *SALMONELLA*

Gerrit Brandis, Jessica Bergman

In *Salmonella enterica* and related species, translation elongation factor EF-Tu is encoded by two widely separated but near-identical genes, *tufA* and *tufB*. Two thirds of EF-Tu is expressed from *tufA* with the remaining one third coming from *tufB*. Inactivation of *tufB* decreases total EF-Tu in the cell by one third. In contrast, inactivation of *tufA* is partly compensated by a doubling in the amount of EF-TuB. How the cell senses a shortfall in EF-Tu, and how it regulates a response by increasing expression from *tufB* is unknown. We have used genetics to address the mechanism by which *tufB* expression is regulated in *Salmonella*. By experimental evolution of a strain with an inactive *tufA* gene we selected six different non-coding or synonymous point mutations close to the *tufB* start codon. Based on these results we constructed a total of 122 different non-coding and synonymous point mutations around the *tufB* start codon and measured *tufB* expression using a *tufB-yfp* fusion protein. The expression data show the presence of two competing structures of stems and loops that can form in 5'-end of the *tufB* mRNA. Formation of the 'closed' structure leads to rho-dependent transcriptional termination of the *tufB* mRNA. We propose a model in which translational speed is used as a sensor for EF-Tu concentration and the expression of *tufB* is post-transcriptionally regulated to regulate cellular EF-Tu levels.

REDUCING THE COST OF PLASMID CARRIAGE IN *ESCHERICHIA COLI*

Eva Garmendia

The horizontal transfer of plasmids and other pieces of foreign DNA into naïve bacteria may impose a fitness cost that must be ameliorated by genetic changes. This type of evolutionary change is very relevant in medical microbiology where the acquisition of plasmids is associated with antibiotic resistance, and in biotechnology, where the introduction of expression vectors may disturb the physiological balance of the engineered strains. Previous work addressed the question of whether fitness cost and compensation are associated with plasmid acquisition and carriage. It was found that during serial passage of *Escherichia coli* strains carrying classical drug-resistance plasmids, that the costs of carriage were rapidly reduced; furthermore, it was shown that the cost reduction was associated with evolution occurring on both the chromosome and the plasmid. This project aims to map and identify the specific alterations that had occurred in those strains using whole genome sequencing analysis, and if they are of interest, perform new evolution experiments to study by what mechanisms and at what rate the fitness compensation can be achieved.

LOCATION AND ORIENTATION OF CRITICAL GENES IN BACTERIA

Eva Garmendia

One important feature of bacterial genomes is that the organization of genes on the chromosome is often highly skewed. Thus, highly expressed genes are often preferentially located close to the origin of replication and transcribed in the same direction as DNA is replicated. The current hypothesis is that genomic-scale organization reflects selection pressure for maximum growth rate, by increasing the copy number of highly transcribed genes and minimizing the frequency of clashes between DNA and RNA polymerases. This study is testing the significance of position and orientation of *tuf*, a gene whose product, EF-Tu, is directly liked to growth rate. EF-Tu is the single most abundant cytoplasmic protein in both *Escherichia coli* and *Salmonella typhimurium*. The *tuf* gene is normally present in two copies, equidistant from the origin of replication. By constructing strains with one gene at the normal position and the other in different locations around the chromosome (and also in both orientations) I will investigate the physiological consequences of this change, and if the growth rate varies systematically depending on position and orientation. Evolution experiments will address whether sub-optimal location and/or orientation can be compensated and by which mechanisms.

RNA POLYMERASE MUTATIONS CONTRIBUTE TO THE EVOLUTION OF CIPROFLOXACIN RESISTANCE IN *E. COLI*

Franziska Pietsch, Jessica Bergman, Gerrit Brandis, Douglas Huseby

Escherichia coli was experimentally evolved in the presence of increasing concentrations of ciprofloxacin (CIP) up to the clinical resistance breakpoint. At the end-point, mutations in *rpoB* were found at a frequency of 20-30% in 3 out of 10 independent lineages. The mutations map outside of the rifampicin-resistance determining region in *rpoB*. End-point clones had a total of 4-5 mutations and in addition to CIP-selected *rpoB* they carried mutations in drug

target and efflux regulator genes. In each of these lineages the temporal order of mutation occurrence was (i) target, (ii) efflux regulator, (iii) *rpoB*, (iv) target or efflux regulator. Using isogenic strains that recapitulated evolutionary trajectories, CIP-selected *rpoB* mutations were shown to confer a competitive growth advantage in the presence of ciprofloxacin. CIP-selected *rpoB* mutations also increased MIC for other fluoroquinolones, and in two out of three cases, for the unrelated antibiotic chloramphenicol. Quantitative RT-PCR analysis showed that the mRNA levels of components of the major AcrAB-TolC efflux pump were increased in two of the three strains carrying CIP-selected *rpoB* mutations. The remaining CIP-selected *rpoB* mutation significantly increased *gyrA* and *gyrB* mRNA levels. These data identify mutations in RNA polymerase as a novel contributor to the evolution of resistance to fluoroquinolones and suggest that this phenotype may be mediated by altered expression levels of genes that are functionally important in determining susceptibility to the drug.

MUTATION SUPPLY AND RELATIVE FITNESS SHAPE THE GENOTYPES OF CIPROFLOXACIN-RESISTANT *ESCHERICHIA COLI*

Franziska Pietsch, Douglas Huseby, Gerrit Brandis, Angelica Tegehall, Linnéa Garoff

Resistance to ciprofloxacin (CIP) in clinical isolates of *Escherichia coli* is strongly associated with a small number of mutant genotypes. The most frequent resistance genotype is triple mutation combination *gyrA* S83L D87N, *parC* S80I, alone, or together with additional target or efflux regulator mutations. Other genotype variants and combinations are found among resistant isolates but at much lower frequencies. This strong bias suggests that the high frequency resistance genotype has a selective advantage over other viable allele combinations. To address the nature of this advantage we made experimental measurements of drug susceptibility, mutation rates, and competitive fitness using a set of isogenic mutant strains. Experimentally generated data was used to model trajectories of resistance evolution under different conditions of drug exposure and population bottlenecks. We could identify rate-limiting steps in the evolution to high-level resistance, and mapped the trajectories leading to the most common genotypes found among resistant clinical isolates. The experimental data and modelling support the hypothesis that the resistance genotypes frequently observed in clinical isolates can be enriched under conditions of weak drug selection pressure where the relative fitness of mutants is of paramount importance but also under conditions where population size and mutation supply are not limiting.

EXPERIMENTAL EVOLUTION IDENTIFIES A NEW CLASS OF GENES SELECTED DURING THE DEVELOPMENT OF CIPROFLOXACIN RESISTANCE IN *ESCHERICHIA COLI*.

Franziska Pietsch, Linnéa Garoff, Douglas Huseby, Tua Lilja, Gerrit Brandis

Independent lineages of *Escherichia coli* were evolved in liquid culture and on agar in the presence of increasing concentrations of ciprofloxacin to above the clinical resistance breakpoint. Deep sequencing was used to analyze the genetic composition of populations at different stages during evolution in liquid. A consistent wave-like pattern of mutation occurrence and accumulation was observed in independent lineages. At each stage in evolution multiple mutant alleles of genes were present but these were filtered as selection pressure increased. The initial wave of mutations affected primarily the drug target gene *gyrA*

with one mutation typically going to fixation early in evolution. This was followed by a wave of mutations affecting regulators of drug efflux (*marR. acrR, soxR*) with one mutation typically going to fixation late in evolution. Third and fourth waves of mutations affected genes with roles in transcription and translation, and additional drug target genes (*gyrB, parC, parE*), respectively. Mutations affecting transcription and translation were selected both in liquid culture evolution (transfer bottleneck $3x10^8$ cfu) and agar evolution (single cell bottleneck). Whole genome sequencing of individual clones from liquid and agar evolution experiments confirmed the linkage of these different mutational classes on individual genomes. The data suggest that mutations affecting transcription and/or translation functions play a role in ciprofloxacin resistance development, and that the trajectory of evolution, while variable at the allele and gene level, follows a predictable path to resistance when viewed at a higher level of function.

SPONTANEOUS FRAMESHIFT MUTATION SUPPRESSION

Douglas Huseby, Lisa Praski Alzrigat, Gerrit Brandis

Frameshift mutations alter the translational reading frame in coding sequences, and are not unusual mutational events. Frameshifts are practically always inactivating mutations, since all codons downstream of the mutation will be incorrect. During an experimental evolution, we isolated an unusual frameshift mutation in an essential gene in *Escherichia coli*. Defying our expectations, a strain carrying this mutation is able to survive without any additional frameshift-suppressing mutations. Our investigations have determined that the sequence surrounding the site of the mutation spontaneously suppresses the frameshift, allowing the ribosome to slip back into the correct reading frame with extraordinarily high frequency. Our model for this suppression, for which we have accumulated considerable evidence, invokes a hungry codon preceded by a codon that is prone to slipping, both of which must be present in a gene that is highly expressed. This mechanism of frameshift suppression has been observed before, but only in artificial constructions. Subsequent experimental evolutions have identified other mutations that disrupt the reading frame of different essential genes. This suggests to us the tantalizing possibility that spontaneous suppression of frameshift mutations may not be as unusual as believed.

FITNESS COSTS AND COMPENSATION IN FLUOROQUINOLONE RESISTANCE DEVELOPMENT

Lisa Praski Alzrigat, Douglas Huseby

Resistance to fluoroquinolones can arise through mutations that affect the drug targets; by mutations that inactivate repressors of the AcrAB-TolC multi-drug efflux system; and by acquisition of genes conferring a protective effect on the antibiotic targets. Resistance-conferring genetic alterations could a fitness cost to strains carrying them. We have experimentally evolved lineages of *Escherichia coli* that are highly resistant to ciprofloxacin, by subjecting strains to increasing concentrations of the antibiotic over hundreds of generations of growth. The antibiotic resistant mutants have been subjected to whole-genome sequencing. In addition to mutations known to confer resistance, a variety of non-canonical mutations were also observed in these strains. During the process of these laboratory evolution experiments, it is expected that there will be selection for resistance, and also

mutations that reduce the fitness cost of resistance mutations. We are analyzing the contribution of non-canonical mutations to the final phenotype to determine whether they contribute directly to resistance or whether they mitigate the fitness costs of resistance mutations. This analysis will contribute to a fuller understanding of resistance evolution.

MUTATIONAL BIAS IN EFFLUX MUTANTS

Lisa Praski Alzrigat, Douglas Huseby, Gerrit Brandis

AcrAB-TolC is the major efflux pump associated with resistance to ciprofloxacin in *E. coli* and mutations causing overexpression increase the MIC. Expression of AcrAB-TolC is regulated by the local repressor *acrR* (*acrA* and *acrB*) and the global transcriptional regulator *marA*, which in turn is regulated by the repressor *marR*. Knockout mutations in either *acrR* or *marR* cause increased expression of the AcrAB-TolC efflux pump. Because neither *marR* nor *acrR* is essential, knock out mutations in these genes are expected to arise at a higher frequency than specific single amino acid substitutions. However, a surprisingly high frequency of amino acid substitutions is found in these regulator genes in resistant isolates. We are assaying the phenotypes of individual *acrR* and *marR* mutations observed in clinical isolates by creating isogenic mutant strains and measuring MICs and fitness costs. This is to test the hypothesis that knockout mutations are relatively costly and that the specific mutations selected in clinical isolates successfully balance fitness costs with the advantage of increased resistance. Across a range of drug concentrations there are no significant differences in exponential growth rates between mutants, but in growth competition assays amino acid substitution mutations have a significant advantage over the isogenic *marR* deletion. qPCR analysis shows that different amino acid substitution mutations in *marR* cause significant upregulation of *marA*, *acrA*, *acrB*, and *tolC*. In contrast, the *marR* deletion mutation upregulates *marA* but not the structural components of the efflux pump. The mechanism of this activity is currently being investigated.

FUNCTIONAL SIGNIFICANCE OF tRNA SYNTHETASE MUTATIONS IN EVOLUTION OF RESISTANCE TO CIPROFLOXACIN IN *E. COLI*

Linnéa Garoff, Douglas Huseby

Ciprofloxacin is a commonly used fluoroquinolone that targets the essential enzymes DNA gyrase and topoisomerase IV. Resistance to fluoroquinolones in *E. coli* is known to arise by mutations altering the target protein genes and by mutations increasing drug efflux, however resistance above the clinical breakpoint requires multiple mutations. In an *in vitro* evolution experiment selecting for increasing resistance to ciprofloxacin, mutations in tRNA synthetases arose frequently. Isogenic *E. coli* strains were created and tested for phenotypes of these mutations affecting resistance or bacterial fitness. Mutations in *leuS* increased MIC and reduced growth rate when in combination with a known resistance mutation. When grown in the presence of ciprofloxacin, strains with a *leuS* mutation in combination with a resistance mutation had a significant growth advantage that was dependent on their ability to activate the stringent response. Taken together, our findings suggest that the *leuS* mutations may have been selected as compensatory mutations that increase the fitness of *E. coli* strains carrying primary resistance mutations, and that the increased fitness is due to an induction of the stringent response via activation of RelA.

IDENTIFICATION OF SUPPRESSORS OF THE SCV PHENOTYPE IN STAPHYLOCOCCUS AUREUS

Sha Cao, Douglas Huseby

Small colony variants (SCV's) of *S. aureus* are associated with persistent infections and may be selectively enriched by antibiotic therapy. The SCV phenotype is most commonly associated with mutations in the genes of the hemin or menadione biosynthesis pathways. SCVs are phenotypically very unstable and are easily overgrown by faster-growing secondary mutants. In this project, we are interested in how SCVs are compensated and if the compensatory mutants keep the antibiotic resistance. Compensatory mutants were selected from menadione and hemin auxotrophic SCVs. Whole genome sequencing (WGS) of showed that SCVs evolved to faster growth rates by three different genetic paths: (i) reversion or second-site mutations in the mutant *hem* or *men* genes; (ii) translational suppression of *hem* or *men* mutations by mutant tRNAs or ribosomes; (iii) mutations affecting the *srrAB* global transcriptional regulation system. The fast-growing mutants in groups (i) and (ii) lost antibiotic resistance and had restored cross membrane potential. In contrast, srrAB suppressors keep the antibiotic resistance and did not recover cross membrane potential. RNA-seq data shows that compared to the parent strains, the suppressors with srrAB mutations had greatly increase their ability to import and ferment specific amino acids. This was supported by growth rate datashowing that the addition of serine and threonine increased the growth rate of srrAB supressors strains. This class of suppressor bypass the SCV defect by opening up an alternative pathway to support fast growth.

ENABLE GRAM-NEGATIVE DRUG DISCOVERY PLATFORM

Sha Cao, Karin Hjort, Kavita Yadav, Douglas Huseby

As part of the Uppsala University-led ENABLE consortium we are carrying out *in vitro* microbiology assays on novel drug hits and leads, including MIC assays involving a hierarchy of different strain panels, hemolysis assays, time-to-kill assays, measurements of the frequency of resistance, resistance-fitness assays, and whole genome sequencing together with genetic reconstruction to identify resistance mechanisms. We have recently set up the capability to perform MMS (MacroMolecular Synthesis) assays assist in the identification of drug targets. The project is set up to efficiently process a pipeline of novel compounds with the ultimate aim of identifying one or more candidate drugs for continuation into Phase 1 clinical trials. We are working on multiple programmes at both Hit-to-Lead and Lead-to-Candidate stages, in addition to carrying out Pre-ENABLE assays on programmes seeking entry into ENABLE.

DESIGNING OF A TOOL FOR TUNABLE EXPRESSION OF GENES WITH CONSTITUTIVE AND INDUCIBLE PROMOTERS

Kavita Yadav

Tunable expression of genes would help in experimental studies of function, essentiality and development of genes. We need to express the genes under promoters of different strenght to study the minimum or maximum expression level of a gene required to maintain its cellular

function. We have developed a tool with constitutive and inducible arabinose promoter in *E. coli* MG1655. Constitutive promoters of the J23100 series were integrated in the *galK* region of *E. coli* MG1655. We have constructed an *E. coli* strain in which sYFP is under the control of 9 different constitutive promoters. sYFP expression ranges from 2 to 500 fluorescence units. Tunable expression with an arabinose-inducible promoter was achieved by (i) deleting the arabinose utilising genes (araBAD), (ii) deleting the high affinity arabinose transporter (araFGH), and (iii) replacing the native promoter of the low-affinity arbinose transporter (araE) with the constitutive J23106 promoter. Using this system we have constructed an *E. coli* strain in which sYFP is under the control of the tunable arabinose promoter and we get an exponential increase (up to 200 fold) in expression with increasing amount of arabinose in this strain. These genetically engineered *E. coli* strains containing constitutive and inducible arabinose promoter can be used as a genetic tool to study the function of genes and in particular to mimic the effects of functional inhibition by antimicrobial drugs.

EXPRESSION AND EVOLUTION STUDIES OF TETRACYCLINE RESISTANCE RIBOSOMAL PROTECTION PROTEINS IN *E. COLI* FROM DISTANTLY RELATED BACTERIA

Kavita Yadav

The widespread use of tetracyclines has led to an increase in tetracycline resistance in clinically relevant pathogenic bacteria, which could limit the medical utility of this important antibiotic class. Drug efflux and ribosome protection are the most common tetracycline resistance mechanisms acquired by bacteria. There are 12 distinct classes of ribosome protection proteins (RPPs) that confer resistance to tetracycline. TetO and TetM are most prevalent and best characterized. The different classes of RPPs have high homology with one another. RPPs confer resistance to tetracycline by binding to the ribosome and chasing the drug from its binding site. These genes can be transferred horizontally on plasmids and/or transposons. We want to study the physiological consequences when these genes are transferred horizontally, either in the same genera or to the distantly related bacteria. We are asking whether their resistance phenotypes are identical in different genetic backgrounds, whether they impose fitness costs on recipients, and whether they are subject to evolution to balance resistance and fitness costs. We have selected 6 ribosomal protection proteins from distantly related bacteria for expression in *E. coli*. We will measure resistance level, fitness costs, and evolution of these proteins. Expression studies can give an idea about how the genes are adapted and evolved with time. This study will give insights in the factors that limit or promote the transfer of different tetracyline resistance genes across species boundaries.

EVOLUTION OF NEW GENES

Joakim Näsvall

Our research focuses on a very fundamental question in evolutionary biology; how new genes come into existence and how they evolve. A new gene can appear in a genome by three different routes: (1) Horizontal (lateral) gene transfer, where a gene is transferred from another organism. (2) *De novo* evolution, where a previously non-functional DNA sequence acquires a function. (3) Duplication-divergence, where a copy of a duplicated ancestral gene acquires a new function. Our current research mainly deals with duplication-divergence.

We have developed a genetic model system to study the early stages of evolution of new genes, based on evolution of a new activity (TrpF, in tryptophan biosynthesis) in the *Salmonella enterica* HisA enzyme (normally active in histidine biosynthesis). We use this model system (evolution of TrpF activity in HisA) to study how various factors influence the evolution of new genes.

We use experimental evolution and genetic methods in bacterial model systems for studying how gene duplication and amplification, neutral mutations, functional trade-offs and other factors influence the appearance of new functions and their further evolution. We are also interested in other questions like evolution of the components of the translational apparatus and the genetic code.

Members of the group during 2015
Joakim Näsvall, assistant professor, group leader
Erik Lundin, PhD student (with professor Dan I. Andersson)

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EVOLVABILITY OF DIVERGING ORTHOLOGOUS GENES

Joakim Näsvall

This project aims to simulate what could happen during divergence of orthologous genes by "speeding up" the molecular clock. A gene (*hisA*) will be taken through rounds of relaxed purifying selection by mutagenesis and screen for partial loss of activity, followed by another

mutagenesis and selection for restored activity. The mutants collected from several lineages of *hisA* genes evolved through multiple rounds under this regime will be analysed with regard to their evolvability towards a new function (TrpF enzymatic activity). The individual deleterious and compensating mutations, as well as combinations of only compensating mutations will be tested both with regards to their effect on the original function (HisA enzymatic activity) and the evolution of the new activity. The effects on structure, stability and enzymatic function will be studied in collaboration with other groups.
The stringency of the regime can be varied by varying the expression of the *hisA* gene during the screens for loss of activity. Low expression (when the activity of the wild-type enzyme is limiting growth) will detect relatively mildly deleterious mutations, while high expression will detect only large losses in function.

With this project we may test several hypotheses:
1. Some sequences are more evolvable than others, despite having the same function.
2. Mutations that introduce a new function are by necessity de-stabilizing.
3. Mutations that stabilize a protein ("global suppressors") make it more evolvable by allowing later de-stabilizing mutations.

EVOLUTION OF BACTERIAL CLASS I PEPTIDE RELEASE FACTORS

Joakim Näsvall

Protein synthesis on the ribosome is terminated by class I peptide release factors, which specifically recognize stop codons in the ribosomal A-site and trigger hydrolysis of the peptidyl-tRNA. Eukaryotes and archaea have only one class I release factor (eRF1 and aRF1, respectively) that recognize all three stop codons, while most bacteria have two release factors (RF1 and RF2) that only recognizes two stop codons each. RF1 (encoded by the *prfA* gene) recognizes UAA and UAG, RF2 (encoded by the *prfB* gene) recognizes UAA and UGA. It has previously been assumed that both of the bacterial Class I release factors are essential, but recent studies have demonstrated that RF1 is not essential in Escherichia coli if RF2 is fully functional. Experimental adaptive evolution of a bacterium lacking one of the release factors can be used in the context of studying evolution of new genes, e.g. evolution of a "new RF1" from a duplicate of the *prfB* gene or evolution of an eRF1/aRF1-like release factor that recognizes all three stop codons efficiently. It can also be used to study evolution of the genetic code itself, e.g. codon reassignments, and give clues to why almost all bacteria have two class I release factors when archaea and eukaryotes suffice with only one, omnipotent, release factor. We have constructed a *Salmonella enterica* strain lacking the *prfA* gene. In *Salmonella* the *prfA* gene is essential, unless the strain also has an amplification of the *prfB* gene. Such a strain is extremely slow growing, leaving plenty of room for adaptive evolution by selection for faster growth. We have allowed this mutant to undergo adaptive evolution by serial passage of multiple populations. In only a few tens of generations of growth under selection, all 16 populations increased their growth rates by amplifying the *prfB* gene to high copy numbers. During 500 generations of adaptive evolution, several populations acquired additional mutations in *prfB*. Mutations in other components of the translational machinery, including Release Factor 3, ribosomal protein S9, tmRNA and three 16S rRNA modification enzymes accumulated in several populations. Currently we are re-constructing strains with these candidate adaptive mutations to determine which mutations compensate for the loss of RF1. It is notable that the deleterious effects caused by lack of a release factor can be compensated by such an extensive variety of mutations in the protein synthesis machinery.

DISTRIBUTION OF FITNESS EFFECTS OF MUTATIONS IN THE BIOSYNTHETIC ENZYME HISA

Erik Lundin, Joakim Näsvall, Dan I Andersson

The distribution of fitness effects (DFE) of mutations is of fundamental importance to understand major evolutionary questions regarding, for example, disease development, the maintenance of healthy population sizes of endangered species and understanding the evolution of antibiotic resistance. Little is known about the DFE as it rarely has been studied using sensitive experimental procedures. The effect of mutations can be classified into three major categories: beneficial, neutral and deleterious. Beneficial mutations are rare and tend to be exponentially distributed whereas deleterious mutations tend to show a bimodal U-shaped distribution with most mutations being lethal/highly deleterious or close to neutral. We used *Salmonella enterica* to study the DFE of random nucleotide mutations in HisA, an enzyme that catalyzes the fourth step of the L-histidine biosynthetic pathway. The fitness effects a large set of unique mutants were measured and shown to span the whole fitness landscape. The DFE was analyzed in relation to number of mutations, type of amino acid substitution and the surface exposure of the mutations. DFE was also compared between synonymous and nonsynonymous substitutions to assess the fitness constraints at the messenger RNA level.

DOES NEUTRAL GENETIC VARIATION AFFECT EVOLVABILITY?

Erik Lundin, Joakim Näsvall

Mutations can affect the function of a gene in many different ways and may also affect later evolution of new functions by allowing the evolving enzyme to traverse fitness valleys and reach new, distant peaks in the fitness landscape. The *hisA* gene will be placed under the control of a strong promoter so that the activity of the HisA enzyme is not limiting for growth and mutations will be accumulated through rounds of mutagenesis. A collection of mutants containing one or several neutral mutations will be used as starting points for experimental evolution towards TrpF activity.

FUNCTIONAL TRADE-OFFS DURING EVOLUTION OF NEW FUNCTIONS

Erik Lundin, Joakim Näsvall

When a specialist enzyme (with a weak secondary activity) accumulates mutations and evolves towards a new specialist enzyme with a new function (with weak or no original activity) it will at some intermediate time points be a generalist enzyme with some original and some new activity. The mutations introduce or increase a new beneficial activity in a gene and may have one of three different effects on the original activity: 1) the original activity is unaffected or only slightly decreased. 2) the original activity is lost proportionally to the gain in new activity. 3) the original activity is completely lost or severely decreased. The nature of the functional relationship between the new and original activity is likely to determine which paths evolution can take when both the new and old function is selected. The aim of this project is to study the intermediate generalist enzymes occurring through evolution towards new gene function and determine the nature of the trade-offs when acquiring a new function and loosing an old. To test which of the trade-offs are present and assess the effects on protein

stability we are setting up a model system based on mutations that confer TrpF activity to the *hisA* gene product (see above). The *hisA* gene will be mutagenized and variants with TrpF activity will be selected. The growth rates of strains carrying these mutant alleles in the absence of histidine or tryptophan will be used as a measurement of the different activities to see which trade-off(s) exists in this system.

EVOLUTION OF CAPABILITIES TO UTILIZE NEW CARBON SOURCES

Erik Lundin, Ulrika Lustig, Omar Warsi, Joakim Näsvall

Environments are often fluctuating and the access to nutrients can change over time. The ability to adapt to new environments and utilize new nutrients is central to all bacteria. In this project, we aim to evolve *Salmonella enterica* to utilize new carbon sources. A previous study identified several hundred compounds that *S. enterica* cannot use as a source of carbon. Using a *S. enterica* strain with a high mutation frequency (due to an inducible error prone DNA-polymerase, DinB) we screened for compounds that the bacterium can use as a new carbon source. After growth of bacteria with the induced error prone DNA-polymerase in rich media, the bacteria are spread on agar plates supplemented with the compound of interest. Slow growing *Salmonella enterica* are collected after incubation up to 14 days on previously non-usable carbon sources. After a whole genome sequencing, point mutations have been identified in genes related to biochemical pathways involved in utilization of the new compounds.

DYNAMICS OF PLASMID-BORNE ANTIBIOTIC RESISTANCE

Linus Sandegren

We study fundamental aspects of how resistance plasmids are maintained and disseminated between pathogenic bacteria and how they serve as platforms for evolution of antibiotic resistance. The main focus is to understand how factors such as stability, mobility, positive selection and fitness costs influence the evolutionary success of plasmids. The experimental systems used are based on clinically isolated multi-resistance plasmids encoding extended spectrum β-lactamases (ESBLs) in enteric bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) that pose an increasing clinical problem by providing bacteria with resistance to the most used antibiotics today, β-lactams such as penicillins and cephalosporins.

Four main themes are of particular interest in these studies:

1. What impact do low levels of antibiotics have on spread, selection and maintenance of multi-resistance plasmids?

2. What plasmid factors cause a fitness-cost on the host cell and can the fitness-cost of plasmid carriage be alleviated by the bacterium in the absence of antibiotics?

3. How common are gene amplifications during treatment, how do they affect the efficacy of antibiotics and does the dynamics of gene amplification on plasmids accelerate evolution of new resistance?

4. Can targeting of resistance plasmids be a way to eliminate resistance genes from a specific bacterial population?

From these studies we expect to gain new knowledge of how bacterial cells and plasmids co-evolve and how selection of new resistance can accelerate through gene amplification and different antibiotic concentrations. Such knowledge can be used to design antibiotic treatment regimens that limit selection of resistance and minimize the potential for new resistance to evolve. We also explore novel systems for targeted eradication of multi-resistance plasmids from defined bacterial populations without killing the bacteria (anti-resistance therapy). In the future such treatment might be a way to clear the resistance determinants from the bacterial flora of patients.

Group members during 2015
Linus Sandegren, associate professor
Marlen Adler, post doc
Fredrika Rajer, PhD student
Erik Gullberg, PhD student (see also Dan Andersson)
Marius Linkevicius, PhD student (see also Dan Andersson)

Project students during 2015
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ESBL-PLASMID EVOLUTION

Linus Sandegren

During 2005-2007 there was a large outbreak of a multi-resistant, ESBL-producing *Klebsiella pneumoniae* clone at the Uppsala University Hospital. We have been involved in the characterization of the outbreak both with respect to the bacterium and the resistance plasmid. Ongoing projects are dealing with further characterization of how the outbreak clone and the resistance plasmid have changed over time with different selective pressures and how it contributes to evolution of resistance against other antibiotics.

The multi-resistance phenotype of the *Klebsiella pneumoniae* that caused the outbreak at the Uppsala University Hospital was due to a large multi-resistance plasmid. We have determined the complete sequence of the plasmid using massive parallel sequencing. Analysis of the plasmid shows that it consists of a backbone that is highly similar to a previously sequenced *Klebsiella* plasmid but has a resistance cassette comprising 45-kbp that instead is highly similar to the resistance cassette from plasmids associated with *E. coli* belonging to the international outbreak lineage ST131. This combination of a backbone and a resistance cassette from another plasmid have occurred through direct homologous recombination, in part mediated through homology in shared mobile insertion sequences between the two plasmids. We have also detected conjugational transfer of the plasmid from the outbreak *Klebsiella* to *E. coli* of the patient's own intestinal microflora. However, the plasmid is only stable in *Klebsiella* with an increased loss-rate in *E. coli* and no further spread of the *E. coli* transconjugants could be detected during the outbreak. We have now also completed the genome sequence of the outbreak clone and are performing comparative analysis of 110 isolates of the outbreak clone looking into how both the plasmid and the chromosomal sequence has changed during the outbreak.

STABILITY AND SELECTION OF RESISTANCE PLASMIDS AT VERY LOW LEVELS OF ANTIBIOTICS.

Fredrika Rajer, Erik Gullberg

The evolutionary success of a plasmid is largely determined by its potential to be stably maintained in the host population. Resistance plasmids are widespread among clinically important bacteria due to the beneficial resistance genes encoded on the plasmids. However, plasmids usually confer a fitness cost on the host cell under conditions when the beneficial factors encoded are not needed (i.e. when antibiotics are not present). Why plasmids pose a fitness cost on the bacterium is still unclear. Under such non-selected conditions plasmid-bearing cells will be at a disadvantage and loss of the plasmid will result in more fit segregants that may out-compete the plasmid-containing cells. Stable plasmid maintenance in a bacterial population can therefore only be achieved if the rate of plasmid loss (by segregational loss and/or fitness costs) is balanced by the rate of plasmid gain (by horizontal transfer and/or fitness advantages).

In this project we study the fundamental properties of plasmid fitness costs and how they can be compensated for. We also study segregational stability properties of plasmids in different genetic backgrounds to understand why some plasmids are very stably inherited in one host while they are relatively unstable in a closely related host. We also measure how low

antibiotic concentrations of different antibiotics, for which the plasmid gives resistance, can counter-select the fitness cost and balance the stability in the population.

CARBAPENEM RESISTANCE IN E. COLI

Marlen Adler

The use of last resort antibiotics such as carbapenems has increased in response to the worldwide spread of extended-spectrum β-lactamase (ESBL) producing pathogens. Within several projects we are studying the mechanisms by which bacteria can spontaneously increase their tolerance to carbapenems, the involvement of β-lactamases in further resistance development and how different carbapenems and treatment regimens affect resistance development.

The main spontaneous cause of carbapenem resistance or increased tolerance in *E. coli* is through changes in the expression of outer membrane proteins OmpC and OmpF. Loss of expression or down-regulation of the expression of OmpC and F can occur by mutational change in several regulatory proteins. Our studies show that the spectra of mutations and the final resistance levels (minimal inhibitory concentration MIC) differ between carbapenems. Furthermore, the production of β-lactamases increases the carbapenem MICs and allows for a wider spectrum of mutations.

To study the effects of less frequent mutations on the development of carbapenem resistance we conducted a study with mutants that already lacked OmpC and OmpF and serially passaged them at increasing carbapenem concentrations. All resulting mutations affected the AcrAB-TolC efflux system and targeted the penicillin-binding protein (PBP) specific for each carbapenem. Ertapenem resistance mutations targeted PBP3 while meropenem resistance mutations targeted PBP2 or induced the stringent response rendering PBP2 non-essential.

EVOLUTION OF CARBAPENEM RESISTANCE IN KLEBSIELLA PNEUMONIAE

Marlen Adler, Cecilia Strömhielm

We are interested in the detailed genetic mechanisms by which Klebsiella pneumoniae can develop resistance to carbapenems. Previous reports show that the mechanisms employed by *K. pneumoniae* and *E. coli* are similar, but porin deficient ESBL-producing *K. pneumoniae* are much more common in clinical settings than their *E. coli* counterparts. We are especially interested in the differences that allow *K. pneumoniae*'s more frequent associations with nosocomial outbreaks.

The outbreak of ESBL-producing *K. pneumoniae* at the Uppsala University Hospital gives us the opportunity to study the evolution and spread of carbapenem-resistance in over 100 clinical isolates over a time of five years. We also performed step-wise selections for increased carbapenem-resistance with sensitive reference strains and clinical outbreak strains. Characterisation of these mutants showed rapid and strong resistance development for the outbreak strains, whereas the reference strain needed additional selective steps to reach comparable resistance levels.

Just as with *E. coli* decreased expression of porins serves as the first line of defence but for *K. pneumoniae*, loss of one porin alone is sufficient for significantly lowered drug susceptibility.

The sensitive strain did not employ any of the previously seen mutations to decrease its susceptibility and whole genome sequencing will be necessary to discover the exact mutations involved.

Initially we hypothesised that the reason for the more frequent *Klebsiella* infections would be a lower biological cost for the same mutations as compared to *E. coli*. This proved not to be the case and more experiments will be needed to learn why carbapenem resistance develops more frequently in K. pneumoniae.

INSTABILITY AND COST OF GENE AMPLIFICATION REDUCE RATES OF EVOLUTION OF NEW GENES

Marlen Adler

Gene duplication and amplification (GDA) is a very frequent mechanism of adaptation in bacteria and has been reported to contribute to antibiotic resistance. We have found that amplifications of plasmid-encoded β-lactamases with trace catalytic activity against carbapenems are selected when cells are exposed to carbapenems and that the amplifications elevate the tolerance to the antibiotics. These amplifications also increase the probability to accumulate mutations that would allow better catalytic activity towards carbapenems, the evolution of a new gene.

A major mechanism for generation of new genes is by duplication-divergence. Here an existing gene is first duplicated and later diverges through accumulation of neutral mutations into different genes. Duplication-divergence includes several different sub-models: i) subfunctionalization where the original function is distributed between two partially functional and complementary genes and ii) neofunctionalization where a new function evolves in one of the duplicated copies while the old function is maintained in another copy. The likelihood of these mechanisms depends on the stability of the duplicated state, which in turn depends on the fitness cost and genetic stability of the duplications.

We experimentally determined the fitness cost and stability of defined gene amplifications of beta-lactamase genes on our ESBL-plasmid. Our results show that the costs of carrying extra gene copies are substantial and that each additional kbp of DNA reduces fitness by approximately 0.15%. Furthermore, gene amplifications are highly unstable and rapidly segregate to lower copy numbers in absence of selection. Mathematical modelling shows that the fitness costs and instability strongly reduces the likelihood of both sub- and neofunctionalization, but that these effects can be off-set by positive selection for novel beneficial functions such as resistance in the presence of antibiotics.

EVOLUTION OF TIGECYCLINE RESISTANCE

Marius Linkevicius

Tigecycline is one of the very few new antibiotics that target Gram-negative bacteria. It is the first compound belonging to the glycylcyclines, a group specifically designed to circumvent the prevailing resistance mechanisms against tetracycline antibiotics. Little is known about development of resistance against this new class of antibiotics but clinical resistance has been reported, mainly through efflux pumps.

We were interested in how spontaneous tigecycline resistance develops in *E. coli* and also if the dominant and wide-spread plasmid-mediated resistance mechanisms against tetracycline (specific efflux pumps, proteins that prevent binding of the antibiotic to the ribosome or modification enzymes) can evolve to also provide resistance to tigecycline.

We found that in addition to the overexpression of the AcrAB efflux system, spontaneous *E. coli* mutations in LPS biosynthesis also led to reduced susceptibility to tigecycline. Both groups of mutations (efflux regulation and LPS) came with a fitness cost and while efflux regulation mutants behaved similarly to wild-type in the mice model systems tested, the LPS mutants were cleared out from some *in vivo* infection models, suggesting difficulties establishing themselves within the host. Additionally, the *in vitro* compensatory evolution experiments showed that the observed fitness cost could be alleviated by acquiring mutations within the resistance gene (efflux) or in other targets (LPS).

Horizontally disseminated *tet* resistance determinants could also evolve to cause reduced susceptibility to tigecycline. We found accumulation of such mutations in the Tet(A) pump, the Tet(M) ribosomal protection protein and the Tet(X) modification enzyme. While the Tet(A) mutations most likely led to changes in channel architecture to fit the bulkier tigecycline structure, the Tet(M) mutations affected the loop interacting with the tigecycline binding site within the ribosome. Tet(A) and Tet(M) mutations causing increased tigecycline resistance level also resulted in collateral sensitivity to earlier tetracyclines. The opposite was observed for the Tet(X) enzyme. Tet(X) mutants not only acquired improved tigecycline activity, but also became better at inactivating earlier tetracyclines. The spread of such mutant variants might have negative implications for the use of tigecycline in the future.

STUDY OF SPREAD AND SELECTION OF ANTIBIOTIC RESISTANT E. COLI IN MALLARDS (ANAS PLATYRHYNCHOS)

Ulrika Lustig, Marie Nykvist, Clara Atterby

It has been shown *in vitro* that very low concentrations of antibiotics, more than a hundred times lower than the minimal inhibitory concentration (sub MIC), can select for antibiotic resistant bacteria. Such low concentrations of antibiotics can be found in various natural environments. This leads to the question if resistance can be selected for in bacteria that are exposed to sub MIC concentrations of antibiotics in the environment, and if resistant bacterial strains can be spread long distances by migrating birds.

We have used four different ESBL (Extended Spectrum Beta Lactamase) producing *E. coli* strains isolated from gulls to infect a set of mallards. With this *in vivo* model we confirmed that mallards can be infected by gull ESBL *E. coli* strains and the different ESBL strains were readily transmitted between birds within the group. The infection persisted in some cases for four weeks, which would allow spreading of resistant strains long distances by migrating birds.

We have also studied if plasmid conjugation occurs between bacteria within the mallards and how different concentrations of antibiotic selects for antibiotic resistant bacteria in the gut of mallards. The birds were infected with an equal amount of two isogenic ESBL producing *E. coli* strains, one of them resistant to ciprofloxacin. During the study the mallards were exposed to concentrations ranging from 0-43-fold MIC of ciprofloxacin in the drinking water. In this *in vivo* competition we observed that ciprofloxacin resistant *E. coli* were selected for at

a concentration of about 0,86-fold MIC in the water, corresponding to a much lower concentration within the bird. Transconjugant bacteria that had acquired plasmids from other strains were also detected.
This project is a collaboration between the groups of Josef Järhult, Linus Sandegren and Dan Andersson (IMBIM).

MUTATIONS AND GENETIC TRANSFER CONTRIBUTE TO EVOLUTION AND STABLE PERSISTENCE OF DRUG RESISTANT MICROORGANISMS

Göte Swedberg

Asexually reproducing microorganisms mainly rely on mutations for genetic variation. However, bacteria have evolved a variety of genetic transfer mechanisms that enhance genetic exchange and evolution of new traits like antibiotic resistance. My main interest has been sulfonamide drugs that act by inhibition of folate synthesis, thereby interfering with biosynthesis of nucleotides and some amino acids. In malaria treatment, sulfonamides are still important for combination therapy. The rapid development of resistance to antimalarial drugs in Africa is a serious problem and we follow the development by field studies. One aim of the project is to explain the evolution of drug resistance on a molecular level and hopefully point the way towards design of better inhibitors both for bacterial and malarial infections. This is done by biochemical analysis of the target enzyme, dihydropteroate synthase, and the biochemical pathway, folate biosynthesis, where this enzyme has its function.

Members of the group during 2015
Göte Swedberg, associate professor, group leader
Nizar Enweji, post doc
Catherine Lwanira, PhD student (Makerere University, Kampala, Uganda)
Lemu Golassa, PhD student (Addis Ababa University, Ethiopia) Graduated June 8, 2015
Getachew Tadesse, PhD student (Addis Ababa University, Ethiopia)
Carol Marwa, PhD student (Catholic University of Health and Allied Sciences, Mwanza, Tanzania)
Boniphace Sylvester, PhD student (Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania)

Project workers during 2015
Ema Vilic: **Relation between Mega and ICE in macrolide resistant *Streptococcus* spp.**
Inan Asa: **Prevalensen av malaria och dess koppling till polymorfismen hos hemoxygenas och haptoglobin.**
Saba Mehrabkhani: **Nedbrytning av ciprofloxacin hos *Micrococcus* bakterier.**
Bilall Al-Shakargi: **Genetisk polymorfism hos cytokrom- generna; CYP3A4*1B, CYP3A5*3 och CYP2B6*6 mellan en population i Uganda och Tanzania.**
Songul Hamdiye Kalyun: **Analys av sulfonamidnedbrytning hos *Pseudomonas putida***
Claude-Bernard Sekuberwa: **Development of highly sensitive methods of detection for malaria parasites**
Vian Hasan: **Makrolidresistens i *Streptococcus* spp. och insättning av MEGA i kromosom.**
Nawres Haidar: **Artbestämning av Streptokocker och undersökning av MEGA omgivning.**
Safa Hiba: **Motverka resistensutveckling av sulfadoxin genom att hitta "svagheter" i enzymet (HPPK) som kan utnyttjas för att hitta nya hämmare.**
Madle Sirel**: Effects of internal deletions of hydroxylmethylpteridine pyrophosphokinase-dihydropteroate synthase from *Plasmodium falciparum***

International exchange during 2015
Boniphace Sylvester and Erasmus Kamugisha, Tanzania, worked in the lab April to June
Getachew Tadesse, Ethiopia, worked in the lab October to November
Göte Swedberg visited partners in Tanzania and Uganda in April and November

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Agencies that support the work
SIDA, VR (Swedish Research Links)

RESISTANCE TO ANTIMALARIAL DRUGS AND EVALUATION OF NEW DRUG TARGETS

Nizar Enweji, Lemu Golassa

Several antimalarial drugs act on the folate metabolism affecting synthesis of DNA precursors, especially dTTP. This project involves further characterization of one already known drug target, the bifunctional enzyme HPPK-DHPS. An expression clone giving good

amounts of bifunctional enzyme has been constructed. The plasmodial enzyme contains long stretches of amino acids that do not align with the corresponding bacterial enzymes. We are generating deletions in these stretches and have found both sequences that can be removed without losing enzyme activity as well as sequences that seem to be necessary for function. Another approach is to evaluate different treatment strategies by genotyping of malaria parasites exposed to antimalarial drugs. This is done by PCR-based analysis of parasite DNA in blood samples from patients undergoing anti-malaria therapy. The project is based on collaboration with Uganda, Tanzania and Ethiopia. Both countries are now switching from using antifolates to a drug combination with artemisinin and lumefantrine (coartem). The project is aimed at analysing the genetic changes in the parasites that result from this change in drug use. No signs of artemisinin resistance were detected. In Ethiopia malaria due to *Plasmodium vivax* is common, and the first choice for treatment is chloroquine. This may explain our findings that one main marker for chloroquine resistance in *Plasmodium falciparum* has a low frequency in Tanzania while the frequency is still high in Ethiopia. In Ethiopia we could also detect a large number of asymptomatic carriers for both parasites, a finding that has implications for control of malaria. The parasite population in Tanzania shows much greater variation than in Ethiopia by a number of indicators, including microsatellite markers, as well as variations in potential drug resistance genes.

HOST FACTORS INVOLVED IN SUSCEPTIBILITY TO MALARIA INFECTIONS

Catherine Lwanira, Boniphace Sylvester, Carol Marwa

An infection is an interplay between parasite and host. While most of our previous work has been focused on the parasite, some new projects focus on host factors. These include genetic polymorphisms in blood groups, other blood related variations and immune system factors. These factors are investigated by Catherine Lwanira with the purpose of linking host polymorphisms to susceptibility to infection in a cohort of children living in a village in Eastern Uganda, where malaria is still very prevalent.

Sylvester focus his work on malaria during pregnancy, and the impact on the mothers immune status on the development of immunity and susceptibility to malarial infections in newborn children. So far, only mothers with acute malarial infections has been studied, but a more thorough investigation of the role of asymptomatic infections in pregnant women is planned.

Still another host factor of interest is polymorphisms in cytochrome P450 enzymes involved in drug metabolism. There are substantial genetic differences between people from different countries. Since the arsenal of antimalarial drugs is limited, the same or very similar drugs are used in all malaria afflicted areas, but drug metabolism can be quite different in Africa as compared to South East Asia and South America. Therefore, aspects of drug metabolism are important for choice of drugs and the dosing during treatment. A complicating factor is interactions between antimalarials and antiretroviral drugs. Especially in Tanzania there is a substantial overlap in HIV and malaria infections. The PhD project of Carol Marwa, Mwanza, Tanzania will address this issue.

MOLECULAR VIROLOGY AND VIRAL ZOONOSES

Göran Akusjärvi, Åke Lundkvist, Tanel Punga, Catharina Svensson, Daniel Öberg, Göran Magnusson

We are using viruses as model systems to study gene expression both in normal cells and under stress conditions and disease. Viral reprogramming of cellular processes sometimes goes haywire causing disease and under extreme conditions malignant transformation and/or death. To understand how viruses can have such a profound effect on human health and regulatory networks at the cellular and organism level we are using human viruses to study basic mechanisms in gene expression, viral interaction with the innate immune response, the molecular mechanisms of viral latency, structure and function of virus-encoded microRNAs. A thorough characterization of host-pathogen interactions is crucial to be able to understand the significance of the basic cellular processes of life for a virus survival and to develop novel strategies to use viruses in medical applications.

Most human infections are zoonotic, which means that they can cross species barriers and pass from animals to humans, or vice versa. In a second line of research we are focusing on zoonotic viruses where we use an interdisciplinary approach based on molecular virology, immunology, genetics, molecular epidemiology and ecology to study viral zoonoses. The present work is focused on several medically important virus families like hantaviruses, flaviviruses, Sindbis virus, and avian influenza virus.

ADENOVIRUS IN BASIC AND MEDICAL RESEARCH

Göran Akusjärvi, Daniel Öberg

Viruses typically encode for a few potent regulatory proteins that have the capacity to rapidly and efficiently disarm host cell gene expression, resulting in a selective synthesis of virus specific gene products in the virus-infected cell. The great advantage with viruses is that they are small and therefore offer a simple genetic system that is easy to manipulate *in vitro*. Further, viruses typically need to manipulate crucial regulatory nodes in cells to reprogram them into virus-producing factories. In fact, the mechanisms discovered in viral model system often recapitulates what life does in general. Thus, there have been, and still are, several lessons to be learned from studies of our viruses. Our current work is focused around several areas covering basic mechanisms in virus gene expression with an ultimate goal to use our gained knowledge for design of safer viral vectors for medical applications.

We study:

- The structure and function of adenoviral miRNAs
- Regulation of RNA splicing and transcription by the viral L4-22K protein
- Long-term persistent/latent adenovirus infections in human tonsils
- Novel functions of the adenoviral E1B oncoproteins
- Viral vectors in cancer therapy

Members of the groups during 2015
Göran Akusjärvi, professor, group leader
Göran Magnusson, senior professor
Daniel Öberg, researcher, group leader
Farzaneh Assadian, post doc
Roberta Biasiotto, post doc
Cecilia Nordfors, post doc
Anette Carlsson, technician
Wael Kamel, PhD student
Xin ”Susan” Lan, PhD student
Sara Östberg, PhD student

Project workers during 2015
Martin Holmer

Publications 2013 to 2015

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Dissertations 2015

Sara Östberg: Functional Characterization of the Evolutionarily Conserved Adenoviral Proteins L4-22K and L4-33K, February 13

Agencies that support the work

The Swedish Cancer Society
Uppsala RNA Research Center (URRC)

CONTRIBUTION OF ADENOVIRUS INFECTIONS IN RECURRENT AND CHRONIC TONSILLITIS

Farzaneh Assadian

Surprisingly little is known about the pathogenesis of tonsillitis. To study the contribution of adenovirus infections in tonsillar diseases, I have established a patient-derived tonsil biobank (at present 100 patients), which allows us to classify particular clinical material according to the diagnosis, age and sex of the patients. This biobank is developed in collaboration with the clinicians at the Department of Surgical Sciences, unit of Otolaryngology, Uppsala University Hospital (Prof. Göran Laurell). During the last year I have set up efficient tonsil processing methods, which allow me to prepare the high quality single-cell suspensions from tonsillar tissues. Thus, current experimental approach makes it possible to isolate pure fractions of B and T cells from tonsillar mononuclear cells (Assadian *et al*., 2015).

To assess the contribution of adenovirus infections in recurrent and chronic tonsillar diseases, I planned the following experiments. First, purified B and T cell fractions were characterized for the presence of adenovirus genomic DNA by qPCR. This approach defined the distribution and molecular characterization of adenovirus infections in lymphocyte populations. Second, I plan to analyze the presence and abundance of the viral derived miRNAs (the mivaRNAs) in the mentioned cell populations. For this experiments I have established a modified small RNA isolation protocol, which recovers high-quality RNA from the isolated cell fractions. To analyze the mivaRNAs, I will use a state-of-art RNA-sequencing method, which will reveal the origin and biochemical characteristics of the viral mivaRNAs in the T and B cell subpopulations. Further, the mivaRNA profiles will be correlated to the presence of viral DNA in the B and T cell populations. Finally, the quantitative data will be correlated to the patient's medical records.

REGULATION OF ADENOVIRUS EARLY PROTEIN E1A EXPRESSION BY THE VIRAL LATE PROTEIN L4-22K

Roberta Biasiotto

The Adenovirus (Ad) late protein L4-22K was shown to play a key role in the regulation of the viral gene expression: it stimulates the transcription from the major late promoter by a feed-forward mechanism and it is specifically involved in the splicing of the L4-33K mRNA. On the other hand, it affects the early genes expression and it is required for the packaging of the Ad genome.

The aim of the present project is to characterize the potential role of the L4-22K protein as a regulator of the alternative splicing of the early gene E1A, a transcriptional activator responsible for the activation of the other viral early and several cellular genes. By alternative splicing, the primary E1A transcript produces five mRNA species (13S, 12S, 11S, 10S, and 9S) that are differentially expressed during the infection cycle. By using a semi-quantitative PCR assay I have shown that 10S transcript expression specifically increased in the presence of L4-22K. A similar effect was found also in the presence of L4-33K, suggesting that the shared N-terminal region of these two proteins might be essential for this effect. This hypothesis is also supported by preliminary results obtained by analysis of N- and C-terminal deletion mutants of the L4-22K protein.

Furthermore, results obtained by testing an E1A promoter mutant showed that the packaging signal (containing multiple binding sites for the L4-22K protein) is not involved in the L4-22K-mediated regulation of E1A isoform accumulation. On the other hand, the analysis of E1A splice site mutants suggests that the minor intron of E1A might be the target region for the L4-22K-dependent regulation. Further experiments aiming at investigating the role of the minor intron region and at elucidating the mechanism through which L4-22K regulate E1A expression are ongoing.

FUNCTIONAL CHARACTERIZATION OF VIRAL NON-CODING SMALL RNA FROM DIFFERENT ADENOVIRUS SEROTYPES

Wael Kamel, Anette Carlsson

VA RNAI is a 160 nucleotides long non-coding RNA, accumulating at high levels during the late phase of the viral infection cycle (approximately 10^8 molecules per cell). Adenovirus utilizes the VA RNAI molecule as a tool to silence the Interferon-induced Immune response by binding to dsRNA-activated protein kinase (PKR), This binding leads to PKR inactivation, thereby sustaining a high translational efficiency in late adenovirus-infected cells. On the other hand the VA RNAs also suppresses the RNAi machinery in adenovirus-infected cells at multiple levels. Most importantly, the VA RNAs are processed by the cellular Dicer enzyme into virus-specific miRNAs, the so-called mivaRNAs. The mivaRNAs are efficiently incorporated into the RNA-Inducing Silencing Complexes (RISCs) in late virus infected cells. I have created the first model system where the predicted miRNA-like function can be specifically investigated without the interference of the other VA RNA functions. This was accomplished by construction of recombinant adenoviruses, in which the seed sequence of the mivaRNAI was mutated. The results showed that late viral protein synthesis as well as new virus progeny formation were essentially unaffected by the mivaRNAI seed sequence mutations under lytic growth conditions in HeLa or HEK293 cells. Thus, the result suggested that either strand of the mivaRNAI duplex does not have target mRNA interactions that are critical for the establishment of virus growth under lytic conditions in cell culture.

In addition, we have also utilized a phylogenetic conservation strategy to study the function of the mivaRNAs in different human adenovirus subgroups. We have shown that the terminal stem of the VA RNAs originating from human serotypes Ad4, Ad5, Ad11 and Ad37 all undergo Dicer dependent processing into mivaRNAs. We further demonstrated that the mivaRNA duplexes are subjected to a highly asymmetric RISC loading with the 3´-strand from all VA RNAs being the favoured strand, except for the Ad37 VA RNAII, where the 5´-mivaRNAII strand was preferentially assembled into RISC. Although the mivaRNA seed sequences are not fully conserved between the Ads a bioinformatics prediction approach suggests that a large fraction of the VA RNAII-, but not the VA RNAI-derived mivaRNAs are still able to target the same cellular genes. Future work will focus on experimentally validating the conserved targets between different serotypes, and investigations on the significance of these for the adenovirus life cycle

THE ADENOVIRUS L4-22K PROTEIN REGULATES MAJOR LATE TRANSCRIPTION AND RNA SPLICING THROUGH A SEQUENCE-SPECIFIC BINDING TO SINGLE-STRANDED RNA

Xin "Susan" Lan

The adenovirus major late promoter (MLP) is responsible for synthesis of essentially all mRNAs encoding the structural proteins of the viral capsid. The L4-22K protein is multifunctional and participates in different aspects of the viral infection. The adenovirus L4-22K protein binds to multiple elements in the MLP. Binding to the so-called downstream element (DE element) stimulates transcription from the MLP whereas binding to the so-called R1 region, which includes the major late first leader 5´splice site has a suppressive effect on MLP transcription. L4-22K binding to the R1 region promotes recruitment of the cellular transcription factor Sp1 to the first leader 5' splice site region. Recruitment of Sp1 has a negative effect on L4-22K-mediated activation of MLP transcription.

Recent results have shown that the L4-22K protein binds to the single-stranded R1 RNA of the same polarity as the nascent MLP transcript. Further, L4-22K binds to same sequence motif in both single-stranded RNA and double-stranded DNA. L4-22K binding to the single-stranded RNA has dual effects. It recruits U1 snRNP to the major late first leader 5' splice site. This L4-22K-mediated recruitment of U1 snRNA stimulates major late first intron splicing and at the same time inhibited the efficiency of MLP transcription. Collectively, the data indicates that the L4-22K protein regulates adenovirus late gene expression both at the level of transcription and RNA splicing.

STRUCTURE AND FUNCTION OF A NOVEL ADENOVIRAL TRANSCRIPTIONAL START SITE MIRNA

Cecilia Nordfors

During an adenovirus infection, colossal amounts of so-called virus-associated (VA) RNAI and VA RNAII, accumulate (more than 10^8 copies/infected cell). These multifunctional non-coding RNAs are required for efficient virus multiplication by targeting the host cells innate immune response. Previous studies have demonstrated that the VA RNAs are processed into viral miRNAs, the so-called mivaRNAs that target the cellular RNAi/miRNA pathways in infected cells.

During the extensive studies of the biogenesis of the mivaRNAs a novel non-canonical viral miRNA was discovered, the 31nt long MLP-TSS-miRNA. This miRNA has several unique features and is very similar in length to the family of Piwi-interacting RNAs, the so-called piRNAs (26 - 31nt in length). piRNAs are produced by a distinct mechanism compared to the classical miRNAs, where pre-piRNAs are bound to Piwi proteins and processed in a Dicer independent fashion. Together with piRNA, Piwi-proteins form so-called piRISC-complexes which functions to repress expression from transposable elements in order to maintain genomic stability in germ cells. Recent data suggest that Piwi protein expression is activated in some cancer cells and some virus infections.

The aim of this project is to characterize the expression pattern and function of the newly discovered MPL-TSS-miRNA and its association with the Ago/Piwi family of effector proteins. We have so far demonstrated that the MLP-TSS-miRNA binds at least one protein of the Piwi-family. Next step will be to evaluate possible binding to other members of the Piwi-family and to study production of MLP-TSS-miRNA by an siRNA knockdown

approach. Potential candidate genes involved in miRNA and piRNA production will be the initial targets.

VIROTHERAPY AGAINST CANCER

Daniel Öberg

The adenovirus Onyx-015 virus was the pioneering agent in the field of cancer gene therapy using cancer selective vectors. The Onyx-015 approach was based on the fact that many tumor types have a dysfunctional p53 pathway. A deletion in the virus gene E1B, responsible to counteract the cellular p53 response to unscheduled DNA replication, was supposed to inhibit the virus in normal cells but allow it to replicate in cancer cells. As it turned out the viral E1B protein had several additional functions. This made the approach severely restricted in tumor targets and potency therein. The aim with my work is to decipher the intricate gene expression pattern of the adenovirus E1B transcription unit in order to understand the additional functions of the E1B protein and thereby be able to engineer viruses that have enhanced tumour selectivity. As such my work consists of basic research with the possibility of clinical application. Current work has generated an Oncolytic Virus patent, which has resulted in an early start-up company. The laboratory is now verifying the oncoselectivity of several alternative viral vectors in different models and also working on the initiation of human clinical trials.

THE FUNCTION OF L4-22K AND L4-33K PROTEINS EXPRESSED FROM DIFFERENT ADENOVIRAL SEROTYPES

Sara Östberg

Our group has previously shown that the closely related L4-22K and L4-33K proteins are integral parts of the early to late switch of adenovirus 5 gene expression. While the L4-22K protein enhances transcription from the major late promoter, L4-33K induces alternative RNA splicing of transcripts with a weak 3' splice site context. We are studying this by looking at the major late transcription unit 1 (L1). Early in infection only 52,55K mRNAs are produced from L1, but through an L4-33K mediated activation of a distal 3' splice site both 52,55K and IIIa mRNAs are accumulating in the late phase.

Both L4-22K and L4-33K are conserved in sequence between different adenovirus serotypes. We want to study whether the function of the two proteins also are conserved. For this experiment we are testing the activity of the L4-22K and L4-33K proteins encoded by adenovirus serotypes 3, 4, 9, 11, 12 and 41 (representing different adenoviral subgroups) for activity in splicing and transcription. Our preliminary results suggest that while the function of the two proteins are conserved between most serotypes, there are some remarkable exceptions which will be investigated further.

ZOONOSES

Åke Lundkvist, Björn Olsen, Josef Järhult

Viruses, bacteria and parasites have been with us since ancient times. They will also be our "companions" in the future, although we have been able to defeat many diseases, new ones continuously emerge, or old ones re-emerge. Most human infections are zoonotic, meaning that they occur mainly in animals but also have the capacity to cross species-boundaries and attack humans.

Our research is based on an interdisciplinary approach between molecular virology, bacteriology, ecology, immunology, genetics, molecular epidemiology and diagnostic aspects of zoonoses, especially emerging zoonotic viruses and multidrug resistant bacteria in wild-life. We are at present focusing on the following agents: hantaviruses, avian influenza virus, flaviviruses (TBE, Dengue and West Nile viruses), Sindbis virus and Campylobacter.

IMBIMs new BSL-3 container lab was delivered during fall 2015 and is now connected, fully equipped, and functioning. The remaining validations are hopefully finalized during the spring 2016.

Members of the group during 2015
Åke Lundkvist, professor, group leader
Björn Olsen, professor, group leader
Josef Järhult, associate professor, group leader
Patrik Ellström, associate professor, researcher
Tanja Strand, co-ordinator, researcher
Erik Salaneck, researcher
Jan Lundström, researcher
Olga Katargina, post doc
Michelle Wille, post doc (from fall 2015)
Jenny Hesson, post doc (spring 2015, now in UK)
Irina Golovljova, post doc (mainly in Estonia)
Jenny Verner-Carlsson, microbiologist (spring 2015)
Jenny Isaksson, microbiologist
Anna Gillman, PhD student
Clara Atterby, PhD student
Marie Nykvist, PhD student
Tove Hoflund, PhD student (from fall 2015)
Per Eriksson, PhD student (from fall 2015)
Erik Skog, PhD student (from fall 2015)
Olivia Borg, project-student (spring 2015, research assistant since fall 2015)
Jenny Olofsson, project-student (Jan - Sep)
Victor Lorente Leal, project student (Jan - July)
Atalay Tok, project student (spring 2015)
Jon Hessman, project student
Evangelos Mourkas, project student
Refath Farzana, project student
Sadika Rahman, project student

Publications 2013 to 2015

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RODENT-BORNE ZOONOSES

Åke Lundkvist

Our hantavirus program has generated important results concerning novel animal models (monkey and rodents), vaccine candidates, virus-host interactions, pathogenesis, and innate immunity. We have also found valuable results on how, and under which circumstances, various Bunyaviruses are transmitted and survive outside their vectors and hosts. The recent awareness of Seoul hantavirus present in Swedish pet rats made us initiate a broad investigation of rats as carrier of various microorganisms pathogenic to man. The "Rat project" has now been extended abroad and we have during 2015 proved the circulation of Seoul hantavirus in wild rats in The Netherlands. We have developed a number of new methods for identification and characterization of genetic markers responsible for infectivity/pathogenicity. Most recently, we found Puumala hantavirus, the causative agent of nephropathia epidemica ("Sorkfeber") in rodents trapped in Uppsala and outside Stockholm (Bogesundslandet), i.e. far outside the previously known endemic areas in northern Sweden. Our rat-project has further revealed the presence of the most severe variant of *Leptospira (interrogans* serovar Icterohaemorrhagiae) in Swedish city rats.

TICK-AND MOSQUITO-BORNE ZOONOSES

Åke Lundkvist

Cell-mediated matrix contraction Our research on TBE virus has focused on molecular epidemiology of the virus in the Nordic countries and in the Baltic states. The focus on tick-borne pathogens has been extended to include also Borrelia, Rickettsia, Anaplasma, and Neoehrlichia. During the winter 2015/2016, we have initiated an NGS-project on ticks in collaboration with SLU and SciLifeLab on tick-borne pathogens (viral and bacterial). The recent increase of clinical TBE cases in Sweden encouraged us to investigate the mechanisms behind, to create hypotheses explaining such emergence, and to develop improved diagnostics

(10-min rapid tests and Luminex-based serology). The different virulence and pathogenesis of the three distinct substrains of TBEV (Western, Siberian and Far Eastern) has recently been investigated and confirmed in a novel bank vole model. Ticks from Swedish migratory birds were analyzed by PCR for the newly described human disease-associated intracellular pathogen Candidatus Neoehrlichia micurensis. We found that a few percent of the ticks carried Neoehrlichia, suggesting that migratory birds can be involved in the epidemiology.
Our work on the mosquito-borne Sindbis virus (the only pathogenic mosquito-borne virus endemic in Sweden) defined Culex torrentium as the major enzootic vector. We further revealed significant trapping biases when comparing various mosquito-traps (CDC-traps, Ovitraps and Gravid traps).

AVIAN INFLUENZA VIRUS

Josef Järhult

The awareness of highly pathogenic avian influenza virus (AIV) repeatedly infecting man prompted us to establish efficient surveillance systems based on wild birds, and to initiate basic research aiming for a better understanding of the transmission, the dramatic changes in virulence, and the development of drug resistance.

The avian influenza virus (AIV)-mallard model has generated a number of important results during 2015; a) Oseltamivir-resistant AIV (H1N1), with an H274Y mutation in the neuraminidase, was shown to persist without any drug pressure in infected mallards, b) AIV (H7N9) acquired resistance related to a neuraminidase I222T substitution when infected mallards were exposed to only low levels of Oseltamivir in the surrounding water, c) The Oseltamivir-resistance caused by a R292K substitution in the neuraminidase in AIV (H6N2) was not maintained without the drug pressure in infected mallards. The degradation and formation of transformation products of three anti-influenza drugs by wastewater ozonation has been studied in detail. Isolation and genetic changes of AIV in embryonated mallard, as compared to chicken, eggs have been investigated.

CAMPYLOBACTER AND ANTIBIOTIC RESISTENCE

Björn Olsen

Our work on Campylobacter revealed the same MLST-types of C. jejunii in samples collected from broilers in 2000 and in 2008. Further, these MLST-types were shown to be prevalent in human clinical cases. Our results further refined the role of capsule features associated with invasive disease and accentuated the possibility of methylation and restriction enzymes in the potential of C. jejuni to establish invasive infections.
Despite 150 years since the introduction of Blackbirds to the Australian continent, their C. jejunii showed an extremely high degree of genetic similarities to C. jejunii carried by their European counterparts. This indicated that C. jejunii in Blackbirds are extremely host-specific and that their genetic drift is very low. The abundant free-living amoeba, Acanthamoeba polyphaga, was proven to increase the survival of Campylobacter jejuni in milk and orange juice, suggesting a potential transmission route to humans.
Fecal samples were collected from the opportunistic bird House Crows in Bangladesh. The same MLST-clones of E. coli were found in patients and in the crows, suggesting a spread from hospitals to the environment.

By analyzing soil and fecal samples from Antarctica we were able to isolate the fungus Penicillum nalgiovense Laxa. Further, we could, for the first time, prove that these fungi produced Amphotericin B. A Chlamydiophila-like bacterium was isolated from Antarctic sea birds. The bacterium was related to, but not identical to, the bird-associated C. psittaci.

EPIGENETIC CONTROL DURING ADENOVIRUS INFECTION

Tanel Punga

The role of infectious agents in the pathogenesis of human disease has received an increased awareness over the past decades. In particular, virus infections appear to be associated with a number of malignant and metabolic disorders.

Epigenetics is a rapidly growing research field that investigates alterations in gene expression caused by mechanisms other than changes in DNA sequence. Virus infections usually induce various epigenetic modifications to ensure optimal viral replication in the recipient cells. Therefore, our group is interested in understanding how a virus infection alters cellular gene expression patterns by introducing epigenetic changes. We use human adenovirus as a model system for our studies. Using this model system we aim to understand what kind of epigenetic changes associate with lytic and persistent virus infections both in the virus genome as well as in the host cell genome. Our special interests are concentrated on the adenovirus "histone-like" protein pVII and virus-associated RNA I named as VA-RNAI. Therefore, we are characterizing the biochemical properties and biological functions of the pVII protein and VA-RNAI in different adenovirus infections. In addition, we study the epigenetic mechanisms involved in the onset of a devastating neurological disease Friedreich ataxia.

Characterization of the causative disruption or dysregulation of normal epigenetic signaling pathways in virus-infected cells will broaden the general understanding of virus pathogenesis and may also lead to the innovation of novel therapeutic applications.

Members of the group during 2015
Tanel Punga, group leader
Kwangchol Mun, PhD student (from Sep)
Raviteja Inturi, PhD student
Helen Bergquist, post doc

Project workers during 2015
Elizabeth Melissa Navarro Garcia (6 months)

Publications 2013 to 2015

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DISECTING EPIGENETIC CHANGES IN NEURODEGENERATIVE DISEASE FRIEDREICH ATAXIA (FRDA)

Helen Bergquist, Tanel Punga

Friedreich ataxia (FRDA) is a monogenic neurodegenerative disease caused by expanded GAA repeats in the frataxin (FXN) gene. The majority of FRDA patients (95%) have a pathogenic expansion of a trinucleotide GAA repeat within the first intron of the FXN gene. Generally, healthy individuals have up to 38 GAA repeats, whereas FRDA patients have most commonly 600-900 GAA triplets on both alleles of the FXN gene. The expanded GAA repeats correlate with a specific enrichment of repressive chromatin (heterochromatin) within the first intron of the FXN gene. This particular epigenetic modification pattern correlates with reduced expression of the FXN protein, which has been considered as the underlying cause for FRDA.

Our ongoing studies are focused on the interplay between different chromatin modifications and expanded GAA repeats on FXN locus. Our ultimate aim of the project will be to specifically modify epigenetic pathways by novel chemical compounds and thereby enhance expression of the FXN protein in FRDA cells.

FUNCTIONAL CHARACTERIZATION OF THE ADENOVIRUS pVII PROTEIN

Kwangchol Mun, Raviteja Inturi

The adenovirus major core protein VII is a histone-like protein and is responsible for structural stability, functional organization and transcriptional regulation of viral DNA. The VII protein tightly complexes with DNA to form compact repeating structures termed ´adenosomes´ by analogy with the nucleosomes observed in nuclei of mammalian cells. Mature polypeptide VII (~19.4kDa) is generated from the precursor pVII (~21.8K) protein, by adenovirus protease proteolytic cleavage during the final stage of virion maturation. The presence of precursor pVII and subsequent cleavage to the mature VII protein may be important for the functional and temporal regulation of adenovirus infection. Therefore, we are interested in molecular and functional characterization of the precursor pVII and mature VII proteins in lytic and persistent adenovirus infections. In our recent study, we characterized a novel interplay between the pVII/VII proteins and the cellular ubiquitin-proteasome pathway. We have identified a cellular ubiquitin E3 ligase Cullin-3-based complex as the regulator of the precursor pVII protein stability. Further, our results clearly indicated the differences in stability and localization between the precursor and mature pVII/VII proteins. Our ongoing goals are to elucidate the molecular function of pVII and its cellular partners in adenovirus gene expression as well as their general role in eukaryotic gene transcription.

MOLECULAR CHARACTERIZATION OF THE ADENOVIRUS VIRUS-ASSOCIATED RNA I FUNCTIONS

Raviteja Inturi, Helen Bergquist

Human adenoviruses (HAdVs) encode for multifunctional non-coding virus-associated (VA) RNAs, which function as powerful suppressors of the cellular interferon (IFN) and RNA interference (RNAi) systems. This is in a striking contrast to other mammalian and plant viruses, which rely on the suppressor proteins, and not on non-coding RNA, to alter the functions of the IFN and RNAi systems. Therefore, we have tested the ability of various plant and animal virus encoded RNAi and IFN suppressor proteins to functionally substitute for the HAdV-5 VA-RNAI. Our results revealed that only the Vaccinia virus (VACV) E3L protein was able to substitute for the HAdV-5 VA-RNAI functions in virus-infected cells. Interestingly, the E3L protein rescues the translational defect but does not stimulate viral capsid mRNA accumulation observed with VA-RNAI. We further show that the E3L C-terminal region containing the dsRNA-binding domain is needed to enhance VA-RNAI mutant virus replication. Additionally, we show that the HAdV-4 and HAdV-37 VA-RNAI are more effective than the HAdV-5 VA-RNAI in rescuing virus replication. Our ongoing studies are concentrated on structural characterization of the HAdV-4 VA-RNAI with an ultimate aim to define VA-RNAI structural determinants controlling its proper function.

ADENOVIRUS TYPE 12 INDUCED INTERFERON RESPONSE

Catharina Svensson

Human adenovirus type 12 (HAdV-12), in contrast to HAdV-2, displays a relatively low virulence and slow replication in cultured human cells, which is manifested by premature death of HAdV-12-infected cells. Whereas HAdV-2 induction of IFN-β expression is transient, HAdV-12-infected cells maintain high levels of IFN-ß expression, protein kinase R (PKR) activation and eIF-2α phosphorylation throughout the infectious cycle. The failure of the HAdV-12 virus-associated RNA (VA RNA) to prevent PKR activation appears to be a major underlying cause of the poor growth of HAdV-12. The importance of the IFN-inducible PKR kinase in restriction of HAdV-12 is supported by the enhanced growth in HeLa cells where PKR is knocked down. Finally, ectopic expression of HAdV-2 VA RNAI increases HAdV-12 hexon protein expression, further suggesting that the restricted growth of HAdV-12 is due in part to the inability of the virus to evade the antiviral host response because of insufficient VA RNA expression.

The strong antiviral response against HAdV-12 might be important for the host to prevent the establishment of long-lasting/persistent infections in humans. In a separate project, our group is analyzing establishment of persistent HAdV-5 infections in cultured B-cells, as well as the presence of adenovirus in lymphoid cells isolated after tonsillectomies (see project description by Tanel Punga). So far, HAdV-12 has not been found in human tonsills. In our current work, preliminary results indicate that HAdV-12 is unable to establish a persistent infection in B-cells. Upcoming experiments aims to determine whether this is due to inability to evade the antiviral defense of the infected B-cells.

Members of the group during 2015
Catharina Svensson, professor, group leader
Wu Chenjun, researcher
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Publications 2013 to 2015

1. Chengjun Wu, Daniel Öberg, Asif Rashid, Rajesh Gupta, Marco Mignardi, Staffan Johansson, Göran Akusjärvi and Catharina Svensson (2013) A mouse mammary epithelial cell line permissive for highly efficient human adenovirus growth. Virology 435, 363-371.
2. Wu Chengjun, Lufeng Bai, Zhiqun Li, Charles E. Samuel, Göran Akusjärvi and Catharina Svensson (2015) Poor growth of human adenovirus-12 compared to adenovirus-2 correlates with a failure to impair PKR activation during the late phase of infection. Virology, 475, 120-128
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ONCOLYSIS OF TUMORS CAUSED BY T-ANTIGEN TRANSFORMED NORMAL MOUSE EPITHELIAL CELLS BY HUMAN ADENOVIRUS

Catharina Svensson, Staffan Johansson

Human adenovirus (HAdV) is severely defective for growth in rodent cells and although some viral gene expression occasionally has been detected, efficient production of new progeny virus cannot be observed. Thus, the development of HAdV for oncotherapy is hampered by the lack of suitable immunocompetent mouse model systems where the oncolytic efficacies can be determined. We recently identified a non-transformed mouse cell line (NMuMG) where the infection by HAdV2 is rapid and results in efficient production of new virus. Our results also showed that the NMuMG cells support growth of HAdV of types D and E, but not of types A, B or F. In the follow-up project we aim to determine the cellular prerequisite for multiplication of HAdV in mouse cells and also the molecular reason for the observed selectivity among HAdV types. Preliminary results demonstrate that HAdV efficiently enters into both the cytoplasm and the nucleus, but that the expression of the immediate early gene E1A is undetectable in cells infected with restricted HAdV types.

The NMuMG cell line is derived from normal mouse mammary epithelial cells and retains the growth characteristics of non-transformed primary cells. Since NMuMG cells cannot form tumors in mice, we have established a tumorigenic NMuMG cell line expressing the T antigen of SV40 virus (NMuMG-T). Infection of NMuMG-T-induced tumors in mice by HAdV-2 efficiently reduced tumor growth and correlated with the ability of the virus to infect and replicate. So far, the oncolytic properties have been investigated in SCID mice. The current aim is to identify the origin of NMuMG cells to be able to repeat the analysis in immunocompetent, syngenic mice.

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DISSERTATIONS 2015

Ding Zhoujie: Feedback enhancement of Immune Responses by IgE, IgM, and IgG3 Antibodies, February 12, 2015

Östberg Sara: Functional Characterization of the Evolutionarily Conserved Adenoviral Proteins L4-22K and L4-33K, February 13, 2015

Pijuan Galito Sara: Novel Culture Strategies and Signal Transduction Pathways of Pluripotent Stem Cells, May 15, 2015

Linkevicius Marius: Evolution and mechanisms of tigecycline resistance in Escherichia coli, September 25, 2015

Imsland Freyja: Monogenic phenotypic traits associated with structural variants in chicken and horse, September 25, 2015

Tengvall Katarina: Genetic Studies in Dogs Implicate Novel Genes involved in Atopic Dermatitis and IgA Deficiency, October 6, 2015

Sörman Anna: IgM and complement in regulation of antibody responses, November 19, 2015

Filipek-Górniok Beata: Glycosaminoglycans Biosynthesis in Zebrafish, November 27, 2015

Pietsch Franziska: Evolution of Antibiotic Resistance, December 11, 2015

LICENCIATE THESIS 2015

Bergström Joakim: Feedback suppression by IgG and the impact on germinal center formation, January 23, 2015

Shahidi Dadras Mahsa: Regulation of cell polarity and invasion by TGF-B and BMP Signaling, March 18, 2015

PRIZES AND AWARDS 2015

1) The Limbic Prize (Limbiska priset in Swedish)
The students of the biomedical program has for the third time (2011, 2013 and 2015) awarded Linus Sandegren the Limbic prize for his dedication to teaching in bacteriology and immunology.

From the prize motivation: ”på ett pedagogiskt sätt åter och åter igen lyckas motivera, inspirera och stimulera studenter till djupare studier inom bakteriologin. Det är omöjligt att somna till på en morgonföreläsning med Linus och det märks verkligen att studenterna och deras åsikter står i centrum för all undervisning. Att lyckas med allt detta på ett så pass heterogent program som Biomedicinprogrammet kräver sannerligen stort engagemang och tålamod”

2) Nilsson-Ehle medal
Leif Andersson received the 2015 Nilsson-Ehle medal for his outstanding molecular genetic research with applications to livestock breeding.

UNDERGRADUATE TEACHING AT IMBIM

IMBIM has ~20 full professors and associate professors as well as around 20 assistant professors and research fellows who contribute to the Department's undergraduate teaching. Additionally, there are some 50 PhD students who act as teaching assistants in the practical course work.

IMBIM participates in four different undergraduate programmes - Medicine, Pharmacy, Dispensing Pharmacy, Biomedicine and Biomedical Laboratory Science - as well as two master programmes, one in Infection Biology and the other in Medical Research. In all of these programmes, laboratory work is an important part and IMBIM has ~400 m^2 lab space dedicated for this purpose; some 20 different practicals are given by IMBIM each year, some of which are common to two or three of the programmes.

Medicine
In the Medical programme, which is 11 semesters long, each course focuses on a specific medical topic - rather than the specialisation of a department – and therefore teachers from different departments share the education duty in different courses. Thus, teachers from IMBIM have major responsibilities in courses covering topics like "Energy and nutrition balance", "Homeostasis and endocrine regulation", or "Attack and defence", while many IMBIM teachers are also engaged in other medical courses at preclinical and clinical level. Teaching is done through regular lectures, study groups and labs. The overall objective of these courses is to provide basic knowledge of the biological function of the human body and to create a basis for later clinical studies and future work in the medical profession. The major part of the Department´s contribution is in the field of metabolism and microbiology. Around 115 students are enrolled in this programme every semester.

Pharmacy and Dispensing Pharmacy
The 5-year programme of Pharmacy leads to a Master of sciences in Pharmacy and is designed to prepare the students for work in retail and hospital pharmacies, pharmaceutical industry, government agencies and academic institutions. In this programme 90 students are enrolled every semester. A shorter, 3-year programme leads to a Dispensing Pharmacist degree, which prepares the students for work in retail and hospital pharmacies. Some 40 students are enrolled at that programme every semester. For both pharmaceutical programs IMBIM is in charge for the microbiology part of the curriculum.

Biomedicine
This 3-year programme aims to give the students a sound understanding of the physiological and pathological processes occurring in humans. It contains different courses describing these processes from a molecular, cellular, genetic and medical perspective. Through practical sessions throughout the programme the students obtain experience in techniques used in current biomedical research. The programme aims at providing training for future activity in research, development and information. About 50 students are enrolled each year and the staff of IMBIM takes part in teaching of biochemistry, cell biology, immunology and microbiology.

Biomedical Laboratory Sciences
This 3-year programme leads to a Bachelor of Medical Science (Major in Biomedical Laboratory Science) that prepares the students for work as biomedical scientists in diagnostic and research laboratories. Placements at external laboratories constitute a substantial part of the curriculum allowing the students to specialize within the programme. The major part of the Department's contribution to this programme is in the field of biochemistry. Some 40 students are enrolled in this programme every year.

POSTGRADUATE TEACHING

Medical research
This two-year master programme aims to provide research-oriented students a sound preparation for PhD studies. During the first year students follow master courses offered by the medical faculty. The second year is dedicated to individual research practice carried out by rotation through different lab projects in parallel to following theoretical research-oriented topics. IMBIM manages the students during their rotation between different lab projects and the theoretical programme part.

Infection biology
IMBIM is in charge of a two-year master programme in infection biology. During this period the student will meet a thorough education on microbes that surround us all the time and may endanger but potentially also help to treat diseases. With start from a molecular perspective the student will learn about diagnosis and therapies, development of resistance and surveillance. IMBIM is in charge of the programme, while some of the course modules are given in collaboration with other departments and faculties.

THE PhD PROGRAM AT IMBIM

During 2015 the department had 49 students registered for postgraduate studies. Nine students defended their PhD theses and two students obtained a licentiate degree. Six new students were registered during 2015. New students are required to take a short introductory course in safety and general practice at the laboratory. The graduate students at IMBIM participate in the teaching at various courses. The “older” PhD students take a great responsibility in helping the newcomers. Thus, the PhD students at IMBIM have formed an organization, the IMBIM PhD association board (IPhAB), which helps new students with practical matters like employment, lodging and financial issues and to clarify what to expect from the department contra the responsibilities of the students. IPhAB also organizes regular social events during the semesters to increase the interaction between students and employees at IMBIM. The monthly IMBIM seminar series, which has been running for many years, has been substituted with two "IMBIM days", starting 2014. To broaden the knowledge of research conducted at IMBIM, researchers at the department will present their work for the whole department. Attendance at the IMBIM days is highly recommended for PhD students since they will generate the credit points needed to fulfil the requirements of a PhD. Further, the different research constellations at IMBIM have arranged seminar series specific for their respective scientific interests and during 2015, seminars in Bacteriology, Genomics, Immunology, Virology and Tumor Biology have been arranged. These seminars give credit points in proportion to attendance.

ECONOMY
(kSEK)

	2014	**2015**
Undergraduate Education Grant	21.507	20.084
Faculty Grant	42.105	39.397
External Grants	99.786	108.081
Others	1.167	1.241
Total	**164.565**	**168.803**

IMBIM PHD STUDENT ASSOCIATION (IPHA)

The main purpose of IPhA is to look after student-related issues and to support its members in questions concerning education, teaching, economy etc. IPhA also works for increased social relationships between students and employees at IMBIM.

We arrange the pubs, where also postdocs and group leaders are welcome. We also arrange several social events such as the annual crayfish party, the cheese and wine party and the Lussepub during Christmas time.

Questions IPhA have worked on in the past are:

- In cooperation with MDR we have worked on improving the employment situation for PhD students.
- We are continuously working on improving the Introduction to Scientific Research course, which is being held twice a year in English now.
- Questions regarding the teaching at the Department.
- The situation for project students. Clarify lengths of projects.
- Creation of a Ph.D. handbook with relevant information.
- Approval and creation of a room for writing for students.
- Acquisition of 3 doctoral hats to rent out to students for the conferment ceremony.

Every graduate student and unregister undergraduate student on scholarships at the Department is member of IPhA, with the option to withdraw their membership if desired. We have a meeting once a month and each corridor should have at least two representatives.

If you have any questions regarding the IPhA activities or any other student-related issue do not hesitate to contact us at: ipha@imbim.uu.se

RESOURCE CENTER AT IMBIM

CENTRE FOR COMPARATIVE DISEASE GENETICS AND GENOMICS

PI: Kerstin Lindblad-Toh
Co-PI: Leif Andersson (UU), Åke Hedhammar (SLU), Göran Andersson (SLU), Olle Kämpe (KI), Örjan Carlborg (SLU)

The aim of the center, funded by Formas, is to establish a world-leading Centre that uses natural model organisms to study the genetics of multi-factorial disease. Genetic studies of domestic animals will provide insights into the molecular basis for phenotypic diversity and disease susceptibility that is difficult to obtain by studies in human or experimental organisms. The research focus will be on dogs, but we will exploit emerging opportunities in any domestic animal, such as chicken, pig and horse, as they appear. Four major disease areas will be covered within the center;

1) Cancer; We have performed genome wide association analysis on a large number of cancers including mammary tumours, osteosarcoma, lymphoma, hemangiosarcoma and mast cell tumours as well as targeted and whole genome sequencing to identify causative variants. Our newest research focuses on tumour and normal tissue sequencing to identify cancer signatures as well as systems pathway analyses to gain insight into tumor progression.

2) Autoimmune and inflammatory disease; our canine models of disease have revealed previously unknown pathways involved in the diseases spanning the innate and acquired immune systems. This work has been translated to a human targeted liquid capture and sequencing program for a coordinated cohort of Swedish control individuals plus patients (with one of the following complex diseases, Systemic lupus erythematosus, Myositis, ANCA-associated vasculitis, lymphocytic thyroiditis, Sjögren syndrome and Ankylosing spondylitis).

3) Metabolic and cardiovascular disease; projects span a large set of disorders including models for muscle growth, diabetes and several cardiovascular projects in multiple species

4) neurological and behavioural disease where we have demonstrated the potential of mapping neurological disease in dogs by identifying major genes for canine Amyotropic lateral sclerosis (ALS) and Obsessive Compulsive Disorder. For a number of the projects we are currently performing functional characterization of the susceptibility genes identified in the dog model as well as the role of the same genes in human patient samples.

LIST OF AUTHORS

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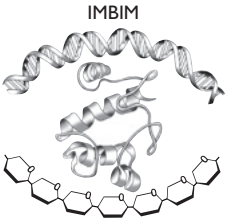

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