DEPARTMENT OF

MEDICAL BIOCHEMISTRY

AND MICROBIOLOGY

ANNUAL REPORT

2013

Potential pathways to antibiotic resistance via point mutation and gene duplication.

In bacteria, low-level resistance to several classes of antibiotics can evolve via a number of different pathways. The specific evolutionary trajectories and endpoints observed are determined by the order of appearance of resistance mutations and the chromosomal location of the *lon* gene (encoding Lon protease).

Hervé Nicoloff and Dan I. Andersson (2013). Lon protease inactivation, or translocation of the *lon* gene, potentiate bacterial evolution to antibiotic resistance. Molecular Microbiology. 90(6):1233-1248.

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INTRODUCTION

I would like to begin by welcoming Åke Lundkvist and his group to our Department. Åke has been recruited from the "Smittskyddsinstitutet" today renamed as "Folkhälsomyndigheten" – Public Health Agency of Sweden. Much to our satisfaction Åke has accepted the position as a Professor in Virology at our University. Åke will be an important addition to our staff since he is the successor of Göran Magnusson who has done, and still does, a fantastic job at our Department, now as a Senior Professor. Since Åke and his collaborators work with highly pathogenic viruses the enrolment of his group to Uppsala also has involved a major challenge to secure funding to build a security laboratory (BSL3 laboratory) for work with pathogenic microorganisms. Much to our satisfaction Åke recently received the necessary funding and we hope that the construction of this BSL3 laboratory will be completed towards the end of 2014.

IMBIM also congratulates Aristidis Moustakas who was promoted to Professor in Medical Biochemistry with a special interest in Tumor Biology during 2013. Aris who is the vice-director of the Ludwig Institute for Cancer Research in Uppsala is a welcome addition to our excellent group of scientists working with tumor biology.

It is also with a great pleasure I have been watching the remarkable speed at which the construction the new SciLife building (Navet) has been completed. The building was expected to be finished before the end of 2013, and much to my satisfaction they succeed with this ambitious project plan. Thus, the first groups moved into the Navet building during November-early December. Now with the infrastructure completed IMBIMs role as the host for the SciLife project has come to an end. Therefore, from January 2014 SciLife Uppsala has become an independent unit with the administrative functions uncoupled from IMBIM. However, IMBIM with its highly competent administrative staff will always be there to help if the need comes up.

Prizes and Awards:
Several scientists at IMBIM have received prestigious awards during 2013. The Royal Swedish Academy of Sciences awarded Professor Kerstin Lindblad-Toh the Göran Gustafsson prize in Molecular Biology for her studies on the identification of functional parts of mammalian genomes. The Göran Gustafsson Prizes are awarded annually since 1991 in the fields of mathematics, physics, chemistry, molecular biology and medicine to young Swedish researchers. The prize consists of a 4,5 Mkr grant and a small personal prize. Kerstin was also awarded the Karl Johan Öbrink Lecturer Award for 2013 for her work on dogs as a model system to study human disease. For those who do not know, Karl Johan Öbrink is regarded as the founding father for the Biomedical Centre (BMC). He started this work already during the 60-ties and the project was not completed until his retirement in the middle of the 80-ties. In honor of this great man the Karl Johan Öbrink Lecturer Award is annually presented to a merited scientist working at the BMC. We also congratulate Professor Leif Andersson who was awarded the Olof Rudbeck Prize for 2013 for his outstanding work in the field of functional genomics. The Olof Rudbeck prize is one of the most prestigious prizes that are awarded to scientists working in Uppsala and is presented by the "Upsala Läkareförening"

Teaching:
Teaching of undergraduate and graduate students is a primary undertaking for IMBIM. During 2013 a total of 56 students were registered as PhD students and 5 students received their doctoral degree and 3 students a licentiate degree. The teachers at IMBIM does an excellent job something that is illustrated by the fact that Linus Sandegren received the so called Limbic prize from the Medical and Biomedical students during 2013. The most remarkable thing is that Linus is a two times winner of this prestigious pedagogic prize (2011 and 2013). I do not know whether anyone else has received this prize more than once. IMBIM congratulates Linus for the well-deserved recognition of his work as a teacher and expects him to win the prize again in 2015 ☺.

Scientific Highlights:
The work at IMBIM has also been the focus in numerous press releases and newspaper articles describing the people and the research they have done at the Department. I would especially like to mention the media attention given to Erik Fries who as a post doc in James Rothmans laboratory during the late 70-ties carried out the ground breaking experiment that set the stage for Professor Rothmans Nobel Prize in Medicine or Physiology in 2013. This type of media attention is important to increase the public understanding of basic research and to improve the attitude towards science in general. During 2013 more than 80 scientific articles were published with scientists from IMBIM involved many that were published in the top rank journals.

Ongoing research projects at IMBIM are summarized later in this annual report. Below I have picked out four examples of studies that illustrate the wide range of research carried out at our Department.

Kerstin Lindblad-Toh's group has sequenced the coelacanth genome and compared it to tetrapods. The study definitively demonstrates that the lungfish, and not the coelacanth, is the closest living relative of the fish that first came on to land. Innovation in genes and regulatory elements involved in immunity, nitrogen excretion, and the development of fins, tail, ear, eye, brain and smell are found to be important for the vertebrate adaptation to land (*Nature* 496, 311-316).

Glioblastoma multiforme (GBM) is a brain cancer with very poor prognosis and is characterized by extreme heterogeneity in the tumor cell phenotype. The bone morphogenetic protein (BMP) pathway suppresses GBM tumor progression by regulating tissue differentiation via unknown pathways. Aristidis Moustakas group has shown that the transcription factor Snail mediates BMP signals provides a new molecular explanation behind the high heterogeneity of this cancer (*Oncogene* 32, 5409-5420).

Rifampicin-resistance is caused by single mutations in rpoB, but clinical isolates of M. tuberculosis usually carry multiple mutations in the RNA polymerase. Diarmaid Hughes group has shown that resistant strains evolve to reduce fitness costs of resistance, by acquiring compensatory mutations in different subunits of the polymerase, explaining the genetic complexity of the clinical isolates. (*Antimicrob. Chemother.* 68:2493-2497).

ZBED6 is a transcription factor unique to placental mammals that previously has been shown to regulate muscle growth. Now, Leif Andersson's group in collaboration with Nils Welsh have shown that ZBED6 also affects gene expression, proliferation and cell death in

pancreatic beta cells and may be a target for treating diabetes patients (*Proc Natl Acad Sci USA* 110:15997-16002)

Birgitta Heymans group have shown *in vivo* that IgM induces deposition of complement factor C3 on antigen in the blood within 10 seconds after immunization. These complexes cause much higher antibody and germinal center responses than antigen without IgM/complement on its surface (*PLoS One* 8:e81299).

The administrative and technical staff at IMBIM is of top-notch quality and does a brilliant job to support the researchers working at IMBIM. The service they provide is of highest standard making life bearable for the chairman of this large unit. Without the effective support functions at IMBIM, the output in terms of research and teaching would rapidly deteriorate. We are fortunate that we have been able to fill the vacancies of three excellent staff members that retired during 2013 (Marianne Wigenius, Barbro Lowisin, Kerstin Lidholt). We are looking forward to see the Department continue to prosper during the coming years.

Finally, I would like to thank all of those who left IMBIM during the past year for your involvement 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 contribute to the further development of this Department.

Uppsala March 2014

Göran Akusjärvi
Head of the Department

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SCIENTIFIC PRESENTATIONS

COMPARATIVE GENOMICS

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

Comparative genomics is of crucial importance to unravel gene function and regulation. We are using domestic animals and other model organisms (*Schizosaccharamyces pombe* and mouse) 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.

Genome evolution. We use comparative analysis to identify functional elements in the human genome and those of model organisms to study the evolution of these elements and other genomic sequences. For example, comparison across 29 mammals identifies 3.6 million elements of which we can suggest a function for ~60%. Evolutionary analysis also identifies lineage-specific selection and innovation of both protein coding and regulatory elements. Furthermore, analysis of genetic variation within species enables us to identify regions targeted by selection, and to understand the mechanisms and evolution of recombination.

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. More specifically, 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 understand the complex relationship between the spatial organisation of the cell nucleus and the regulation of genome function.

Host – retrovirus evolution. Retroviruses have successfully colonized vertebrate genomes for millions of years as endogenous proviruses (ERVs). This genomic record provides a unique perspective on host-virus relationships. We deploy a bioinformatics approach to identify ERVs in genomic sequences of domestic animals and other vertebrates to further our understanding of retrovirus evolution and effects of retroviruses on host genome function.

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 a unique 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 2013

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Rajesh Gupta, reseracher
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Marta Promerova, post-doc (until September 2013)
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Shady Younis, PhD student
Khurram Maqbool, PhD student, (associated with Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences)
Doreen Schwochow, PhD student, (associated with Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences)

International exchange during 2013

Miguel Carneiro, University of Porto, Portugal (visiting researcher during two months)
Congying Chen, Jiangxi Agricultural University, Nanchang, China (visiting researcher during two months)

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Agencies that support the work

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

Rajesh Gupta, Lin Jiang, Mårten Larsson, Ola Wallerman, Elisabeth Sundström, 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 C2C12 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*. Genes associated with ZBED6 binding sites showed a highly significant enrichment for certain GeneOntology classifications including development and transcriptional regulation. 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. More recently we have revealed that ZBED6 has a significant role for transcriptional regulation and differentiation in pancreatic beta cells. A broad research program involving functional assays and mutation screenings in humans has been initiated to study the biological and medical significance of ZBED6. This includes the generation of *Zbed6* knock-out.

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

DETECTING SIGNATURES OF SELECTION USING WHOLE GENOME RESEQUENCING

Alvaro Martinez Barrio, Miguel Carneiro, Ulla Gustafson, Sangeet Lamichhaney, Khurram Maqbool, Jessica Pettersson, Marta Promerova, Nima Rafati, Markus Sällman Almén, Leif Andersson

Next generation sequencing offers the possibility to carry out whole genome resequencing of large genomes, like the human. We have pioneered the use of using pooled samples to detect signatures of selection when comparing different populations that has 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 was very successful and resulted in (i) the detection of more than 7 million single nucleotide polymorphism, (ii) 38 loci with strong signatures of selection and (iii) almost 1300 deletions with a high frequency in at least one population. We have also applied the method in pigs and Atlantic herring. We are currently applying this approach to the Atlantic and Baltic herring, wild and domestic rabbit, and Darwin's finches. The project is 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

Freyja Imsland, Susanne Kerje, Jessica Pettersson, Doreen Schwochow, Elisabeth Sundström, Markus Sällman Almén, 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 an 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* tumor suppressor gene; (ii) the patterning phenotype in chicken; (iii) inhibition of gold in chicken; (iv) white spotting in dogs controlled by *MITF*, encoding a transcription factor of crucial importance for pigment cell development and function; (v) white spotting in pigs controlled by the *KIT* locus encoding a tyrosinase kinase receptor; (vi) roan coat colour in horses, controlled by a regulatory mutation in the *KIT* gene; (vii) 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; (viii) variant red in cattle. In all these projects our ambition is to nail down the causal mutation(s) and explain the mechanism of action for the detected mutations.

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 Kerje, Ulla Gustafson, Jessica Pettersson, Leif Andersson

We have intiated 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 identification of disease-related genes will lead to a better understanding of pathogenesis, as well as of general mechanisms underlying autoimmune diseases, thus facilitating the development of better diagnostic, prognostic and therapeutic tools. The work is carried out in collaboration with Drs. Olov Ekwall and Olle Kämpe at Department of Medical Sciences and Dr. Örjan Carlborg at SLU.

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

Anna Golovko, Elisabeth Sundström, Lin Jiang, Leif Andersson

Grey is a dominant coat colour mutation that is common in horses and found in a variety of breeds including Arabian horses, Lippizzaner 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. 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

Susanne Kerje, Khurram Maqbool, Alvaro Martinez Barrio, 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. Using these resources we have identified the genes causing dominant white colour, dominant black colour, silver plumage colour, yellow skin and Pea-comb. More recently we have employed next-generation sequencing to resequence the chicken genome from different populations with the aim to reveal loci that have been under strong selection during chicken domestication.

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.

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Members of the group 2013
Erik Axelsson, Assistant Professor

Project workers during 2013
Jessika Nordin (June-August)

In addition Maja Arendt (see Kerstin Lindblad-Toh) and Abhi Ratnakumar (see Kerstin Lindblad-Toh and Matthew Webster) have been involved in this project during 2013.

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. We study two aspects of chromatin dynamics, changes in histone modifications and the influence of subnuclear localisation on the expression status of a gene. 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.
Moreover, we have initiated a project with the long-term goal of developing new drugs against yeast infections, 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 patients 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 improved formulas would be of great importance.

Members of the group during 2013
Pernilla Bjerling, group leader
Vladimir Maksimov, post-doc
Alejandro Rodriguez, post-doc
Daniel Steinhauf, PhD student

Projects students during 2013
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Publications 2011 to 2013

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FORMATION OF REPRESSIVE CHROMATIN

Vladimir Maksimov, Alejandro Rodriquez, Daniel Steinhauf

In *Schizosaccharomyces pombe* there are several regions where a special form of transcriptionally repressed chromatin, named heterochromatin, is formed. 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, Clr2 is crucial for heterochromatin formation in *S. pombe*, yet very little is known about the function of Clr2 (Bjerling et al, 2004). However, a bioinformatics approach revealed three conserved motifs in the Clr2 protein. Conserved amino acids were mutated at the endogenous *clr2*$^{+}$ locus, and the resulting mutant strains have silencing defects. Moreover, several of the mutant strains display unstable silencing phenotypes indicating deficiencies in either establishment or maintenance of heterochromatin (Steinhauf et al PlosOne, 2014). The different phenotypes of the mutated strains provide us with an excellent tool for further analysis of the Clr2 protein in heterochromatin formation. Of particular interest will be to elucidate the indicated involvement of the Clr2 protein in heterochromatin establishment or maintenance.

MOLECULAR FUNCTION OF ZBED6

Daniel Steinhauf

ZBED6 is a recently discovered transcription factor unique to placental mammals. It was discovered because it acts as a repressor at the *IGF2* locus. A point mutation disrupting its target site in *IGF2* in domestic pigs leads to a three-fold upregulation of *IGF2* expression and increased muscle growth. Interestingly, ZBED6 has evolved from a domesticated transposon and belongs to a family of related transcription factors. However, the molecular mechanism of this family of transcription factors is more or less uncharacterised. In this project the aim is to find interacting partners to ZBED6 using the yeast 2-hybrid system and biochemical purifications. The project is carried out in collaboration with Leif Andersson and his group.

CHROMATIN DYNAMICS DURING NITROGEN DEPLETION

Alejandro Rodriguez

By expression profiling we have identified 118 genes that are upregulated after 20 minutes of nitrogen starvation. In addition, we find that nucleosomes are lost from all of the induced genes both at the promoter and in the coding region (Kristell et al. 2010). Moreover, some of

the highly affected genes are found together in clusters in the genome and these gene clusters display a drastic loss of nucleosomes during induction. We have labelled two of these loci using the *lacO*/LacR-GFP strategy, allowing for live cell analysis. During normal growth conditions the gene clusters localise to the nuclear periphery. Already 20 minutes after nitrogen depletion drastic changes in subnuclear localisation of the two loci are observed, away from the nuclear membrane towards the nuclear interior (Alfredsson-Timmins et al. 2009). We are currently investigating whether the movement is crucial for induction of the genes by targeting the gene clusters to the NM preventing the movement.

HiSTIDINE KINASES AS DRUG TARGETS IN CANDIDA

Pernilla Bjerling

Several species of the pathogenic yeast *Candida* normally grow on the skin of humans and only patients with a lowered immuneresponse, like immunosuppressed patient undergoing transplantation or AIDS patients, suffer from *Candida* infections. The drugs against *Candida* frequently give strong side effect so improved formulas would be of great importance. There are primarily two species that causes infections, *Candida albicans* and *Candida glabrata*. Bacteria, plant and yeast have on their cell surface histidine kinases that act as environmental sensors not found in higher eukaryotes and therefore they are promising drug targets. In *C. albicans* the histidine kinases are important for the transition between yeast (unicellular) to hyphal (multicellular) growth and hence an important virulence factor, since this transition need to occur in order for *C. albicans* to penetrate the human skin. Therefore it is of interest to get drugs that prevent the formation of the virulent form of the yeast, rather then drugs that would just inhibit growth. This project aims to set up a drug screen against the histidine kinases in the two *Candida* species.

EVOLUTIONARY BIOINFORMATICS AND COMPUTATIONAL BIOLOGY

Manfred G. Grabherr

In the wake of novel sequencing technologies, the past decade has brought with it a wealth of genomic data, providing an in-depth view on the genetic basis for life. However, many important questions remain unanswered, both with respect to the evolutionary forces shaping the coding regions of genomes as well as the functional relevance of non-coding and intergenic regions. The Grabherr Group is thus centered on developing and applying computational algorithms and methods to explore sequence data and answer a broad range of biological questions.

One of the corner stones of our work is the use of *transcriptomics*, i.e. the analysis of the repertoire of transcripts that are expressed in a cell or sample and/or under certain conditions. Such data can provide valuable insights into the function of genes as well as help guiding the search for unknown genes or understudied genomic regions. In addition, we are using *comparative genomics* to study the variation within populations as well as across species to understand how speciation may occur on a genetic level or to trace the evolutionary trajectory of genes, such as those implicated in diseases. Perhaps most importantly, we work in close collaboration with a number of other research groups to explore the interplay between regulatory mechanisms and expression, functional characterization of transcripts, and meta-transcriptomics studies.

To help us in our research, we are developing algorithms and methods for whole-genome comparisons, protein alignments, detecting local changes in genomic phylogeny, and gene annotation and characterization. Since 2013, the group is host to the BILS Genome Annotation Centre, funded by the Bioinformatics Infrastructure for Life Sciences.

Members of the group during 2013
Manfred G. Grabherr (group leader)
Neda Zamani (post-doc)
Görel Sundström (researcher)
Marc P. Höppner (BILS Uppsala Genome Annotation Centre)
Henrik Lantz (BILS Uppsala Genome Annotation Centre)

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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 that 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.

WHOLE GENOME COMPARATIVE PLATFORM

Comparative genomics provides a powerful means for establishing relationships between gene function and location in a variety of different organisms and gaining insight into conservation of functional elements and evolutionary histories. Related species often preserve the order and orientation of genes and other features over long genomic regions, which, in absence of large-scale duplications and deletions, allows for identifying orthologous sequences. While pairwise synteny aligners exist, the fast growing pool of available genomes, brought about by the increasing rate at which new genomes are being sequenced, prohibits the generation of all-to-all pairwise mapping.

We have thus designed and implemented a rapid universal mapper, called *Kraken*, which infers sequence correspondence by walking along a graph of alignments. We thus perform a re-alignment in two steps: (i) a fast heuristic search using cross-correlation, which finds the approximate coordinates, and (ii) an optimal local alignment to determine the exact boundaries of genomic features. Subsequently, entire gene annotations from many species can be accurately mapped over without the need to re-compute genome-wide alignments, even if the pairwise alignments between these genomes do not exist. In only a few hours of runtime, this platform allows for mapping millions of genomic features onto a mammalian-sized genome

TYPE I DIABETES GENE EXPRESSION STUDY

Görel Sundström

In this collaboration with Olle Korsgren, Oskar Skog, Sofie Ingvast (Uppsala University), and Lars Krogvold (Oslo University Hospital), we examine the differences in gene expression patterns in pancreatic islets between patients with early onset type I diabetes and non-diabetic controls. While insulin production is reduced due to down-regulation of the *INS* gene in the diabetics, we also found two upstream regulators expressed at lower levels, as well as parts of the *MAPK* pathway. Interestingly, genes in the class II major histocompatibility complex genes show significantly higher activity, but not class I or class III genes, indicating a rather narrow and specific immune response, albeit the cause to which the immune system responds remains unclear. In addition, we find differences in a number of other genes linked to inflammatory response. Further analysis on a pathway level promises to shed light on the biological programs that differentiate diabetic pancreatic islets from non-diabetic tissue.

BILS UPPSALA GENOME ANNOTATION CENTRE

Marc Höppner, Henrik Lantz

Funded by the Bioinformatics Infrastructure for Life Sciences (BILS), the Uppsala Genome Annotation Centre was started in the summer of 2013 with the mission to provide high quality genome annotations to Swedish genome sequencing projects. This group both utilizes state-of the art annotation software, as well as developing novel algorithms and exploring new routes to best accommodate experimental data from large-scale RNA sequencing efforts. Prediction- and evidence-based annotations include both protein coding genes and non-coding transcripts, with the latter emerging as new and promising candidates for explaining disease or phenotypes in regions that were identified in whole-genome association studies. The annotation process includes both automated algorithms, as well as a graphical web user interface for manual inspection and curation accessible to the research groups that generated the data.

Pilot projects include the genomes of the European crow, the Atlantic herring, and the Surirella diatom.

The European crow is a model for speciation, with two differently colored subspecies being currently recognized (black vs. grey/black). The genome has been sequenced and assembled by the group of Jochen Wolf (Uppsala University) in an effort to investigate the genetic basis of this speciation. The annotation procedure chosen uses the wealth of RNA-seq data available to produce an annotation that is not overly biased by genes known from other species and is also able to recover novel genes.

The Atlantic herring is a commercially important species that occurs both in the Atlantic as well as in the Baltic Sea with pronounced morphological and physiological differences between these populations. The genome is sequenced and assembled by the group of Leif Andersson (Uppsala University) with the aim of studying population genomics and the genetic adaption of this species to different salinity and feed resources among other factors. High quality proteins from closely related species are lacking, and the annotation therefore relies heavily on RNA-seq to compute accurate gene models.

The diatom *Surirella brebissonii* is a species that is studied in the IMAGO Marine Genome project at Gothenburg University (PI Anders Blomberg). Living inside the cells of this species are symbiotic bacteria, and there has been a number of transfers of bacterial genes to the nuclear genome over evolutionary time. This means that the annotation procedure not only needs to deal with an assembly consisting of mixed diatom and bacterial sequences but also that the genes in the nuclear genome can be heterogeneous and of different origin. Methods to deal with this are currently being developed.

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 colonized host genomes for millions of years, leaving traces as ERVs in their genetic make-up, and thereby providing a unique resource for understanding the biology and evolution of virus-host relationships. We principally employ bioinformatics to study retrovirus-host relationships in two main lines of research:

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 2013
Patric Jern, associate professor
Alexander Hayward, researcher

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RETROVIRUS AND TRANSPOSABLE SEQUENCE EVOLUTION

Alexander Hayward, Patric Jern

The expanding catalogue of re-sequenced genomes and reference assemblies permits detailed comparative studies across the genomes of diverse organisms. We take advantage of this resource 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 combine a phylogenetic approach to construct evolutionary hypotheses of relationship with bioinformatics methodology. 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. In addition, RNA-Seq analysis of many mammals and vertebrates is under way to get a more complete picture of the coding and non-coding transcriptome in these organisms. 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 coelacanth genome allowing the study of genome evolution linked to how vertebrates crawled onto land and 2) the cichlid genome projects examining the diverse changes linked to the dramatic phenotypic diversity and species radiations seen in African lakes. A key project in Uppsala has also been the dog domestication analysis revealing that changes in genes involved in brain development and starch metabolism have accompanied dog domestication.

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, Cardiomyopathy, Systemic Lupus Erythematous (SLE) 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. Key findings this year include a novel gene for atopic dermatitis and 33 novel loci in three breeds with osteosarcoma

Members of the group during 2013
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Pedro Sousa, Master student
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International exchange during 2013
Kerstin Lindblad-Toh (Broad Institute)
Ingegerd Elvers (Broad Institute)

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CANCER

Malin Melin, Emma Ivansson, Maja Arendt, Ingegerd Elvers and Argyri Mathoiudaki

Cancer is one of the most prevalent diseases in both humans and dogs and specific breeds often show a predilection for certain tumour types. The molecular basis of the increased cancer risk in the breeds is mostly unexplained and knowledge about the susceptibility genes may enable improved diagnosis and treatment. We have focused on identifying predisposing genetic risk factors for a selection of tumour types, including:

- Mammary tumours (breast cancer)
- Osteosarcoma (bone cancer)
- Lymphoma
- Mast cell tumours
- Glioma
- Hemangiosarcoma

A few high-risk breeds have been chosen for initial investigations of each tumour type. We have collected large case-control materials both in Europe and the US by extensive collaborations and have performed genome-wide association studies (GWAS) in a few hundred dogs per tumour type and breed using 170,000 SNPs. For each tumour type we identify multiple loci significantly associated with tumour development. Targeted resequencing and fine-mapping has revealed a large number of candidate mutations that are currently being validated and assessed for functionality. We are also performing tumour-normal and mRNA sequencing to identify somatic and expression level alterations in the canine tumours. By our analyses we have identified a large number of candidate genes for canine cancer and the genes are now being investigated further in dogs and human cancer patients. By translating the results to human cancer, this could provide a unique opportunity to improve diagnosis and treatment of cancer in both dogs and humans.

In 2013 we published an osteosarcoma GWAs in three breeds identifying many different risk factors explaining a large portion of the heritability. The study demonstrated that each breed has its own risk genes, but these genes converge in common disease mechanisms such as bone formation and cell growth. We also studied one of the risk factors in more detail and found a new regulatory signal that leads to increased gene expression in bone cancer cells (Karlsson *et al* Genome Biology 2013).

AUTOIMMUNE AND INFLAMMATORY DISEASES

SYSTEMIC LUPUS ERYTHEMATOSUS

Sergey Kozyrev, Fabiana Farias, Hanna Bremer (SLU) and Maria Wilbe (SLU)

We use three different approaches in order to uncover mechanisms predisposing individuals to Systemic Lupus Erythematosus (SLE). SLE is a complex autoimmune disorder characterized by dysregulation of the immune system, which results in production of

autoantibodies, generation of toxic immune complexes (ICs), 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. Moreover, SLE has complex multigenic inheritance in both humans and dogs. We have identified four loci 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). Resequencing of the associated loci in the patient dogs revealed novel genetic variants that regulate gene expression, splicing and protein functions. Further, we identified genes with stronger association to particular sub-phenotypes of IMRD differed by the immunofluorescent staining pattern, homogeneous and speckled, suggesting not only immunological but also genetic differences associated with various disease manifestations. Interestingly, among the canine SLE genes we found was *BANK1,* previously strongly associated with human SLE. This finding may support the hypothesis that both species not only have shared symptoms, but also genes and pathways involved in the development of autoimmune diseases.

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

Finally, the Kozyrev group also performed deep analysis of exomes in patients with severe highly penetrant familial form of SLE. We have identified novel non-synonymous variants in the key genes and study their effect on protein function and cell signaling.

SUB-CATEGORIZING INFLAMMATORY DISEASE BY MOLECULAR PATHWAYS

Jennifer Meadows, Fabiana Farias, Sergey Kozyrev, Argyri Mathoudaki, Eva Murén, Åsa Karlsson, Mia Olsson and Gerli Rosengren Pielberg

Together with human geneticist and clinicians working on different immunological diseases we have selected a set of 1900 genes which encompass the genes and pathways found so far by our canine immunological disease models as well as the genes and pathways implicated by corresponding human disease studies. We are now in the process of sequencing these genes and the non-coding conserved elements within 100 kb of these genes to allow detection of mutations both in coding and regulatory sequences. Our goal is to identify rare variants of slightly stronger effect than those found by GWAs studies and to ascribe a potential candidate function to some of these using the available RNA-Seq, epigenomics and 29 mammals conservation data sets. By looking at carefully phenotyped human patient cohorts and a distinct and comprehensive gene set we expect to start to link diseases and subphenotypes to mutations in specific genes and pathways, possibly allowing a more comprehensive view of the molecular pathogenesis. In a pilot study, performed in collaboration with Lars Rönnblom, targeting a subset of the genes we detected a large number of novel SNPs (not in 1000genomes/db SNP) of which a third were found only in cases. SNPs present in genes enriched for novel case-only variants were screened for regulatory marks such as histone marks, DNaseI hypersensitivity, conservation and transcription factor binding (ENCODE data and by prediction). Ten SNPs in 6 genes were selected as the best regulatory candidates where the SNP may alter transcription factor binding, only one of those genes has been previously associated with human SLE. Patient history was analyzed in conjunction with SNP and gene

information, and in some cases point to a correlation with specific sub-phenotypes. Functional studies are ongoing to determine the functional relevance of these candidate mutations.

ATOPIC DERMATITIS

Katarina Tengvall, Fabiana Farias, Marcin Kierczak (SLU), Brita Ardensjö (SLU)

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. We have performed a GWAS of ~200 German shepherds. In addition, serum IgA was measured in the same individuals. Due to its high correlation with the CAD phenotype, IgA levels were included as a covariate in the GWAS of CAD. The GWAS generated a genome wide significant association to a locus on CFA27. Fine-mapping of this 1.5 Mb region pinpointed the candidate gene *PKP2* encoding the protein Plakophillin 2, important for maintaining strong skin structure. Currently, we are collecting skin biopsies from case and control individuals to be used for RNA studies and immunohistochemistry with the specific aims of evaluating the *PKP2* expression in the skin and the overall immunephenotype of atopic dogs versus control dogs.

ADDISON'S DISEASE

Katarina Tengvall and 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.

METABOLIC AND CARDIOVASCULAR DISEASE

DIABETES

Maja Arendt, Abhi Ratnakumar, Tove Fall (Medicinska Vetenskaper)

Hormone induced diabetes can develop in female dogs in relation to estrous or pregnancy. This is similar to diabetes developing in pregnant women. Certain dog breeds have a relatively high incidence of hormone related diabetes compared to others, indicating a genetic predisposition. We have performed genome wide associations studies 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. In addition we have also

looked for regions with reduced homozygocity within each breed to find genetic patterns, which are nearly or partly fixed within each breed. In the long term we are aiming to identify disease-associated genes leading to better understanding of the diabetes in general as well as improved manors of treatment and prevention which could benefit both human as well as dogs.

DILATED CARDIOMYOPATHY (DCM)

Jennifer Meadows, Suzana Steila (SLU), Susanne Björnerfeldt (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 the identification of associated regions. Targeted genome sequencing was performed for each breed across eight candidate loci, four for GD and NF respectively. Genes of interest from each cardiac project will be carried forward into a human targeted sequencing program.

NEUROLOGICAL AND BEHAVIOURAL DISEASE

DEGENERATIVE MYELOPATHY

Emma Ivansson and Eva Murén

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 breed and further validation in other breeds. A mutation in human *SOD1* causes a similar neurodegenerative disease, amyotrophic lateral sclerosis (ALS), suggesting that 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. In humans the identified *SOD1* mutations account for only 20% of the familial ALS also suggesting other genetic variations to be involved in disease development. We have performed additional GWAs and resequencing in carriers of the SOD1 risk allele to identify genetic modifiers that can predict disease severity and onset as well as identifying other genetic variations that can cause disease. We are also collaborating with Ingela Nyström at Akademiska Hospital to follow up genes and pathways identified in the canine breeds with targeted resequencing of human ALS patients.

INVESTIGATING GENETICS UNDERLYING BEHAVIORAL TRAITS IN DOGS

Marcin Kierczak (SLU), Katarina Tengvall and Fabiana Farias

Dogs have been accompanying humans for more than two thousand years. Our common history dates back to the domestication of wolves and continues to-day. From the point of view of genetics, two events in this common history are particularly important: the domestication and the creation of breeds. Both the events involved selection of certain traits and features. Several dog breeds have been created, often by favouring individuals displaying certain types of behaviour. Examples include selection for herding, hunting or friendliness.

Sweden has a long and rich history of well-organized dog breeding and extensive records of pedigrees, health and behavioural data are available. Since 1989 the Swedish Working Dog Association has been carrying Dog Mentality Assessment (DMA or MH in Swedish) test, which consists of several standardized situations, e.g. exposure to a sudden metallic noise. Behaviour of a tested dog is evaluated by a professional judge and several traits are measured, e.g. intensity of social contact, playfulness, eagerness to chase, etc.

Our current study is primarily focused on German shepherds (GSD), which are used as both companion and as working dogs. Importantly, a sufficient degree of within-breed behaviour variation is present in this breed, which allows us to carry on genome-wide association studies in order to unravel the genetics of behaviour. Our pilot study, based on nearly two hundred genotyped GSDs, revealed several genomic regions that show interesting associations with behavioural traits such as chasing, aggression, curiosity, playfulness and sociability. Several of the regions have been implicated in the development and functioning of the nervous and the neuroendocrine system.

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 may contribute to the development of diagnostic tools and novel treatments from which also we, humans will benefit.

GENETIC DISSECTION OF AUTOINFLAMMATORY DISEASE

Jennifer Meadows

The long-term research goal is to indentify 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 heterogenous 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 2013
Jennifer Meadows, Researcher, group leader
Iris Mathioudaki, PhD student

Project worker during 2013
Jessika Nordin, Master student
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SHAR-PEI AUTOINFLAMMATORY DISEASE (SPAID)

Jennifer Meadows

Autoinflammatory disease results from the dysregulation of the innate immune system: the body's first line of defence 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 indicated that there is a second region of the genome, separate to the SPAID locus, which may act as a modifier for amyloidosis. This secondary peak could harbor variants that influence the rate of amyloid deposition and ultimately animal mortality.

PATHWAYS OF HUMAN INFLAMMATORY DISEASE

Jennifer Meadows, 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 conjunction with collaborators, we have selected ~400 carefully phenotyped SpA patients and a similar number of matched controls to run on 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 has been used to sequence each individual to a minimum of 10x depth. Part of the implementation of this experiment has involved the design and refinement of a robust bioinformatic pipeline capable of dealing with large amounts of data in a computational and time efficient manner. We now stand ready to evaluate our identified variants in the context of those common globally (1000 genomes/dbSNP) and those common to our patients' specific region of Sweden (using our control set). The rare 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 2013
Gerli Rosengren Pielberg, Assistant Professor, group leader
Matteo Bianchi, PhD student
Nils Landegren, PhD student at Dept. of Medical Sciences

Project worker during 2013
Hannes Hällgren, SoFoSko student

Publications 2011 to 2013

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Agencies that support the work
The Swedish Research Council Formas
Agria/SKK

CHARACTERIZATION OF GENETIC RISK FACTORS BEHIND CANINE LYMPHOCYTIC THYROIDITIS

Matteo Bianchi, Gerli Rosengren Pielberg

Lymphocytic Thyroiditis is one of the most frequent endocrinopathies in dogs, affecting multiple breeds. The disease is 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. 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, Nils Landegren, Gerli Rosengren Pielberg

Human autoimmune polyendocrine syndromes are a heterogeneous group of diseases characterized by autoimmune activity against more than one endocrine organ. In particular, autoimmune polyendocrine syndrome type 2 (APS-II), is characterized by presence of Addison's disease, hypothyroidism and Diabetes mellitus. In collaboration with Prof. Olle Kämpe (Dept. of Medical Sciences), we have started a project for characterizing genetic risk factors contributing to the development of this syndrome.

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

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 utilized depending on its environment. In one project massively parallel DNA sequencing is utilized in order to identify genes underlying phenotypic variation and disease in the horse; a somewhat overlooked species in studies aimed at genetic contribution to phenotypic traits. The most important findings made during the course of the project will be further pursued in spin-off projects.
In another effort we investigate how the genetic code is utilized depending on its environment by exploring two mechanisms in Atlantic salmon; DNA methylation and temperature dependent gene regulation. For this project we will include clonal lines of salmon in order to dissect the extent of environmental impact on traits without confounding effects of genetic variation.

Members of the group during 2013
Carl-Johan Rubin

Agencies that support the work
The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning
The Research Council of Norway

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 Deoxyribonucleic acid (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, and to analyze patterns of genetic variation in horse breeds in order to detect loci affected by selection and to detect genetic variants underlying specific traits and diseases. To achieve these aims we will analyze DNA samples from diverse horse breeds and populations, selected to represent distinct disease/trait classes Samples will be subjected to whole genome resequencing (WGS) and the resulting data will be used in order to scan the genome for signatures of selection. We will predict functional genetic variants using bioinformatics methods and will screen for alleles uniquely/preferentially observed in individuals expressing certain diseases or traits. For such candidates we proceed with association analysis in large well-characterized cohorts of horses to investigate whether certain alleles coincide with phenotypic variation and disease.

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

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 has been mitigated by specific light regimens and growth can also be further plastically modulated by variation in water temperature. It is well known that one avenue by which environmental factors can influence 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 in order to investigate epigenetic and transcriptomic changes accompanying exposure to different light regimens and water temperatures during different life stages in the Altantic salmon.

This project is partly conducted in collaboration with with researchers at the Institute of Marine Research in Bergen, Norway.

GENOME EVOLUTION

Matthew T Webster

We study evolution on the molecular level by comparing genomes of different species and analysing patterns of genetic variation on the whole-genome scale, using bioinformatic and statistical approaches. We are interested how molecular forces such as meiotic recombination interact with natural and artificial selection to shape the evolution of genomes and the phenotypes they produce.

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 important for adaptation, which could be vital to protect this important species from colony losses. Using the domestic dog as a model, we are performing a whole-genome analysis of recombination hotspots and copy number variation, in order to understand the molecular mechanisms that lead to genome instability. We have performed an analysis of molecular footprints of artificial selection in the dog genome, and are currently attempting to characterise mutations with important functional effects for dog domestication.

Members of the group during 2013
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Jonas Berglund, PhD student

Agencies that support the work
Vetenskapsrådet
Formas

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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. Honeybees are grouped into a number of subspecies, which are estimated to have diverged and spread across Africa and Eurasia around one million years ago. Natural selection resulted in each of these subspecies becoming adapted to its local environment. 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 traits. These include traits common in certain races, such as cold adaptation and gentleness. In addition, certain traits, such as parasite resistance and hygienic behaviour are important for honeybee health and viability. 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.

RECOMBINATION AND GENOME EVOLUTION

Jonas Berglund

Meiotic recombination is a fundamental biological process, which maintains genetic variation within populations and is essential for chromosomal segregation. However, recombination may also have damaging effects on the genome, either as a cause of structural mutations or due to a process known as biased gene conversion, which alters the frequency of mutations in a population. The genomic distribution of recombination events is localized to specialized sites known as hotspots, but the mechanisms controlling this variability are unclear.

The dog genome is unusual among mammals in that it lacks a key protein that initiates recombination in other species. We have used genomewide genetic variation data to map the locations of recombination hotstpots in dogs and uncovered unique features that may reflect novel mechanisms of the control of recombination. We are now using comparative genomic approaches to investigate how these mechanisms might affect the human genome. Furthermore, we are analysing array comparative genomic hybridisation data from various dog breeds in order to identify copy number variants and understand the role of recombinogenic mechanisms in their generation.

GENOME SCANS FOR SELECTION IN DOGS

Abhi Ratnakumar

There are hundreds of dog breeds that exhibit massive differences in appearance and behaviour sculpted by tightly controlled selective breeding. This large-scale natural experiment has provided an ideal resource that geneticists can use to search for genetic variants that control these differences. In collaboration with Illumina and the LUPA consortium for dog disease mapping, we have developed a high-density genotyping array (canineHD) that surveys variable sites at more than 170,000 positions in the dog genome. This array has been now been used to analyse genetic variation in thousands of dogs. We have analysed these data to identify chromosomal regions that are extremely variable between breeds and are likely to control many of the traits that vary between them. We are characterising one such region in more detail, which associates with differences in body size and ear type between breeds. We are using "next-generation" sequencing technology to identify candidate mutations that may control these traits. Our results suggest that artificial selection has targeted genes involved in development and metabolism and that it may have increased the incidence of disease in dog breeds. Knowledge of these regions will be of great importance for uncovering the genetic basis of variation between dog breeds and for finding mutations that cause disease.

MEDICAL BIOCHEMISTRY

GLYCOBIOLOGY

PROTEOGLYCANS - BIOSYNTHESIS AND BIOLOGICAL FUNCTIONS

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

The IMBIM groups active in this area study proteoglycans and elucidate functional aspects of these glycoconjugates in relation to embryonic development, angiogenesis 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 and zebrafish and most recently C. elegans.

MOLECULAR MECHANISMS REGULATING PLURIPOTENCY AND SELF-RENEWAL OF EMBRYONIC 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 or 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 large-scale 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
Cecilia Annerén, Ph.D., Adjunct Senior Lecturer
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Publications 2011 to 2013

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Patents 2013
Method for cell culture. 2013. Patent application number: 135400-6

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

Christoffer Tamm, Sara Pijuan Galitó

Most stem cell laboratories still rely on old culture methods to support the expansion and maintenance of mouse embryonic stem (ES) cells. These involve growing 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 media. 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 ES 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 cells in a spheroid suspension culture in a defined medium containing LIF and bFGF herein called ESN2 medium. Two feeder-dependent mouse ES (mES) cell lines and two cell lines adapted to feeder-independent growth were used in the study. In addition to compare self-renewal and differentiation capacity, we also assessed ease-of-use and cost efficiency. We showed that mES cells when grown adherently proliferate much faster than when grown in suspension as free-floating spheres, independent of media used. Our data confirms previous reports showing that the 2i medium generates purer stem cell cultures with negligible signs of spontaneous differentiation, as compared to standard mES media. Furthermore, we show that this medium effectively rescues and cleans up cultures that have started to deteriorate, as well as allows for effective adaption of feeder-dependent mES cell lines to be maintained in feeder-free conditions. A drawback that we observed with the 2i medium, is that the mES cells are much harder to transfect with standard reagents such as Lipofectamine™ 2000, compared to cells grown in standard serum containing medium. We are therefore currently evaluating different transfection reagents for mES cells grown in 2i medium.

ROLE OF A NOVEL SERUM PROTEIN 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 have shown 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 foetal 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 have identified and isolated a serum protein (herein named SP), which is capable of activating TEAD2-dependent transcription. So far we have found that purified SP induces TEAD2-dependent transcription in a dose dependent manner and that SP, similar to LIF, induces activation of cYes and YAP nuclear trans-localization. We have also found that addition of FBS or SP to the media promotes mouse ES cell attachment under serum-free media conditions. Interestingly, this also seems to

be the case for human ES and iPS cells. The exact mode of action for SP-induced activation of the Yes/YAP/TEAD2 pathway is yet to be shown and is currently under investigation. Long-term culture of mouse and human ES cells in medium containing purified SP is also on-going in order to study the long-term effects of SP on the ES culture *in vitro,* as well as the mechanism through which the protein induces attachment to the tissue-culture treated plastic-ware.

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 and zebrafish where we study 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.

Sulfation, obviously important in heparan sulfate biosynthesis, also regulates protein and steroid hormone action. New projects in the lab include characterization of sulfate metabolism in zebrafish and in cancer metastasis. Heparan sulfate biosynthesis in mucopolysaccharidoses is also a new area studied in the group.

Members of the group during 2013
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REGULATION OF HEPARAN SULFATE BIOSYNTHESIS/ IN SEARCH FOR THE GAGOSOME

Audrey Deligny, 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. Our recent finding of altered heparan sulfate biosynthesis in Hurler syndrome will be 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 fetures of their heparan sulfate chains. Mice with targeted mutations in the core protins identified will be used to study functional aspects of the cell surface proteoglycans.

SYNTHESIS AND TRANSPORT IN ZEBRAFISH OF THE SULFATE DONOR PAPS

Beata Filipek-Górniok

PAPS, 3'-phosphoadenosine-5'-phosphosulfate, is the general sulfate donor, needed in all sulfation reactions. It is synthesized from ATP and sulfate by PAPS synthases, located in the cytoplasm and, as recently shown, also in the nucleus. Specific PAPS transporters carry the sulfate donor into the Golgi compartment where sulfation of glycosaminoglycans as well as proteins take place. We have identified three PAPS synthases and two PAPS transporters in the fish and studied their expression. Using morpholino knockdown of gene expression we identified an important role for one of the synthases in muscle development. This phenotype is further investigated in collaboration with Jonas von Hofsten in Umeå.

SULFATE METABOLISM IN CANCER

Anders Lundequist, Magnus Rosling, Beata Filipek-Górniok

Altered PAPS metabolism in the context of cancer development and metastasis is a previously almost unexplored area. In addition to its important role in heparan and chondroitin sulfate biosynthesis, PAPS is also the donator of sulfate when sex hormones are sulfated, a substitution which results in inactivation of the hormones. Zebrafish will be used as a model to study the impact of PAPS concentration on invasion and metastasis of breast cancer and prostate cancer cell lines. Mutant fish with lowered PAPS concentration as well as cell lines where PAPS production has been manipulated will be used in the studies.

FUNCTIONAL OVERLAP BETWEEN HEPARAN SULFATE AND CHONDROITIN SULFATE

Tabea Dierker

Recent results suggest that chondroitin sulfate sometimes can substitute for heparan sulfate in physiologically important interactions. To understand and characterize this overlap is particularly important in light of the increased interest of the development of glycosaminoglycan mimetics for therapeutic purposes. The nematode *C. elegans* synthesizes heparan sulfate which in all structural aspects is similar to the mammalian polysaccharide. In contrast, only chondroitin without any sulfate substitution is produced by the nematode. *C. elegans* mutants with defective heparan sulfate biosynthesis show a strong misrouting of motor axons. To investigate if chondroitin sulfate can substitute for heparan sulfate we will, in collaboration with Andrea Hinas at IMBIM, introduce different zebrafish chondroitin sulfate sulfotransferases into these mutants, confirm that the worm now is able to produce sulfated chondroitin and investigate how this affects the routing of the motor axons.

FUNCTIONAL STUDIES OF BLOOD VESSEL GUIDANCE

Johan Kreuger

The overall objective of our research is to increase our understanding of how instructive concentration gradients of signaling proteins control blood vessel formation. In this context, we also study the contribution of inflammatory cells to the angiogenic process. Detailed information on how multiple instructive gradients together steer blood vessel growth is currently lacking. The rationale for our research is that increased understanding of how gradients control cell migration events central to angiogenesis ultimately will lead to new approaches to treat pathological angiogenesis, occurring for example in association with cancer. The research activities in our group are cross-disciplinary. We use advanced cell culture systems, various disease models, whole-genome sequencing and biochemical approaches to study directional angiogenesis. We collaborate closely with Prof. Pär Gerwins at IMBIM/Uppsala University Hospital to identify mutations that cause vascular malformations.

Members of the group
Johan Heldin, PhD student
Paul O'Callaghan, postdoc
François Binet, postdoc
Rodrigo Hernández Vera, postdoc
Yvette Zarb, masters student
Anna Nieto Esteve, Erasmus student

Agencies that support the work
The Swedish Cancer Society
The Foundation for Proteoglycan Research
Gösta Naeslund's Foundation
The Swedish Research Council
The Medical Faculty at Uppsala University

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Patents 2013

New use of a fluidic device - SE 1350861-9

Describes a fluidic device to determine responses of cells to test substances for diagnostic purposes.

VASCULAR DEVELOPMENT IN RESPONSE TO GROWTH FACTOR GRADIENTS

Paul O'Callaghan, Johan Heldin, Yvette Zarb, Anna Nieto Esteve, Francois Binet, Rodrigo Hernández Vera

Directional migration of vascular cells is important for angiogenesis during normal development and during many diseases such as cancer, atherosclerosis and proliferative retinopathies. Our goal is to better understand the basic mechanisms behind regulated cell chemotaxis, and in this context we study endothelial cells, mural cells and immune cells. We have identified a set of genes, including the exocyst complex component *exoc3l2* that is selectively expressed by growing blood vessel sprouts. We are now further characterizing the roles of *exoc3l2* and the exocyst complex, as well as a relatively uncharacterized guanine nucleotide exchange factor, in directional angiogenesis.

PROTEOGLYCANS REGULATING TISSUE DEVELOPMENT

Paul O'Callaghan, Francois Binet, Rodrigo Hernández Vera

Proteoglycans are critical for vascular development as they modulate and potentiate VEGF-receptor mediated angiogenesis. We focus our study on the mechanisms of proteoglycan secretion and the roles of proteoglycans in tyrosine kinase receptor signaling. We have also identified several genes and miRNAs that are selectively expressed either in actively sprouting blood vessels, mural cells, or inflammatory cells. We are now knocking down these candidate genes and miRNAs to identify their roles in the formation, patterning and pathfinding of growing blood vessels.

IN SEARCH FOR GENETIC CAUSES OF PARKES WEBER SYNDROME- A SEVERE VASCULAR ANOMALY

Pär Gerwins, Johan Kreuger, Paul O'Callaghan, François Binet

Congenital malformations in different components of the vascular system give rise to conditions known as vascular anomalies. Within this group there are some rare conditions that cause severe pain, ulcerations, cardiac failure, amputations and substantial morbidity. The purpose of this project is to reveal mutations associated with Parkes Weber syndrome, a complex disorder of the vascular system. Whole-genome sequencing of a family of four individuals where the two children but not their parents have been diagnosed Parkes Weber has been performed, and the search for the disease-causing mutations is currently ongoing. Today there are no effective treatments for Parkes Weber. This project represents the beginning of a larger effort to increase our basic understanding of the molecular mechanisms behind this rare but very severe disease, with the ultimate goal of finding new pharmacological treatment strategies.

HEPARAN SULFATE AND HEPARANASE: IMPLICATIONS ON ANIMAL DEVELOPMENT AND PATHOPHYSIOLOGICAL PROCESSES

Jin-ping Li

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

Members of the group during 2013
Andreas Digre, graduate student
Jin-ping Li, MD, PhD, group leader
Ulf Lindahl, PhD, professor emeritus
Elin Grahn, research engineer (April – November)
Hao Cui, post-doc (since April)
Jianping Fang, post-doc (since August)

Project worker during 2013
Erika Manlig, summer student (June-July)

International exchange during 2013

Visitors to my lab
Robert Kisilevsky (Canada), Prof. emeritus, one week in May
Ganlin Zhang (Beijing, China), Post-doc, one week in June

Group member to visit other lab
Jin-ping Li, visited Oncology Department of Beijing Hospital of traditional Chinese Medicine, one week in January, one week in October

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HEPARAN SULFATE IN ANIMAL DEVELOPMENT

Jianping Fang

Heparan sulfate (HS) is an essential molecule in animal development, as demonstrated by early embryonic lethality of mice defect in HS synthesis. Glucuronyl C5-epimerase, one of the enzymes involved in HS biosynthesis, catalyzes the conversion of D-glucuronic acid (GlcA) to its C5-epimer, L-iduronic acid (IdoA), at the HS polymer level. The IdoA units are believed to promote binding of HS chains to protein ligands, due to the marked conformational flexibility of these residues. Therefore, the reaction catalyzed by the C5-epimerase is crucial for many biological functions of HS.

Targeted disruption of the GlcA C5-epimerase gene in mouse resulted in neonatal lethality of the animals, with a severely disturbed developmental phenotype, such as renal agenesis, lung hyperplasia and multiple skeletal malformations. Analysis of HS isolated from mutant animals revealed a perturbed structure completely lacking IdoA residues, but with increased *N*- and 6-*O*-sulfation contents. The alteration of the HS structure apparently affected functions of a number of cytokines, e.g. FGF-2, that require HS as co-receptors. Recently, we have found that the GlcA C5-epimerase is involved in lymph organ development (in collaboration with Prof. S. Pals, the Netherlands).

To continue the study, we are examining the mechanisms underlying the defects in kidney, lung and skeletal systems.

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

Jianping Fang, Elin Grahn

Biosynthesis of HS is a complex process; the action of at least 11 different enzymes results in polysaccharide molecules with a high degree of heterogeneity. As 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 "gagosome", *i.e.* the complex of (largely) membrane-bound enzymes in the Golgi. Particular attention will be given to the interactions between enzymes, e.g. GlcA C5-epimerase and O-sulfotransferases. Recombinant enzymes (GlcA C5-epimerase, HexA 2-O-sulfotransferase and GlcN 6-O-sulfotransferase) are applied to modify polysaccharide substrates for investigation of: 1) substrate specificity of the individual enzymes; 2) interaction/regulation of the enzymes in their separate or concerted action towards various substrates; 3) kinetics of the enzymatic reactions. In more complex biological systems, tissues, cells or sub-cellular organelles (in particular 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.

Another line of this project is to illustrate the molecular structures of the enzymes by X-ray crystallographic technique. The recombinant enzymes, especially GlcA-epimerase, are used for crystallization. Further plan is to co-crystallize the enzymes with oligosaccharide substrate to define the catalytic site through mutations of critical amino acid residues in the proteins.

STRUCTURE AND FUNCTIONS OF HEPARAN SULFATE IN AMYLOIDOSIS

Andreas Digre, Hao Cui

"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 selective organ deposition of disease-specific fibrillar proteins along with HS-proteoglycans (HSPGs). HS and HSPGs appear not to be merely passive components of amyloid deposits but rather play functional roles in the pathophysiology of amyloidosis. Two types of amyloid diseases that have a broad clinical and social impact are Alzheimer's disease (AD) and type 2 diabetes.

As HS is pertinently found in all types of amyloid deposits in different patients, it is of importance to find out the functions of HS in these diseases. We primarily focus on the studies on few-selected amyloidosis, e.g. inflammation associated amyloid A (SAA) deposition in the spleen/liver/kidney; type II diabetes (IAPP deposition in the pancreas) and Alzheimer's disease (Aß deposition in the brain). 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.

HEPARANASE – A MODULATOR IN BLOOD COAGULATION?

Hao Cui

Heparanase is an endo-glucuronidase that cleaves HS and heparan. The enzyme was discovered first in a mast cell tumor in 1975, believed to be specific for cleavage of heparin. Later the same enzymatic activity was detected in all tissues. At normal conditions, the enzyme is expressed at a relatively low level in most of organs, essentially non-detectable by Western blotting technique, with the exception of platelet and placenta. However, the enzyme is significantly upregulated at several pathological conditions, such as inflammation and cancers. This project aims to find out the functional roles of heparanase in platelets, with regarding to thrombosis.

Several *in vivo* thrombosis models will be applied in our unique transgenic mice, the heparanase-overexpression mice that overexpress human heparanase (Hpa-tg) and the heparanase knockout mouse (Hpa-KO) that is generated by targeted interruption of the gene.

IMPLICATIONS OF HEPRANASE IN 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 the level of heparanase 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 the collagen II-induced RA mouse model on our unique transgenic mice that are either overexpressing or lacking heparanase.

THE INVOLVEMENT OF PROTEOGLYCANS AND GLYCOSAMINOGLYCANS IN CANCER AND ANGIOGENESIS

Maria Ringvall

Proteoglycans consist of a core protein to which gycosaminoglycan chains are attached. Proteoglycans are produced by virtually all cell types and are grouped accordingly as cell surface, extracellular matrix and intracellular molecules. One family of cell surface proteoglycans, syndecans, have been extensively studied and have for a long time been recognized as important regulators both in physiological and pathological cell-signaling systems, including tumor development. Serglycin is the only intracellular proteoglycan and has been the focus of relatively few studies. *In vivo* studies has this far shown that serglycin has a regulatory function in the immune system upon infection.

Establishment of a tumor and its further progression into a malignant phenotype is dependent on many different factors, such as dysregulated cell division, angiogenesis and inflammation. These processes are supported by the production of excessive amounts of different pro-tumorigenic factors from both the tumor cells and from non-malignant cells, such as endothelial and immune cells, within the tumor. Angiogenesis, which describes the formation of new blood vessels, is a highly active process during development, and is an important element in different pathological states such as rheumatoid arthritis and cancer. A number of the factors produced in a tumor facilitate angiogenesis, which is necessary for tumor growth and metastasis. Many of these pro-angiogenic, and thereby also pro-tumorigenic, signaling systems can be regulated by proteoglycans and their glycosaminoglycans, which interact with growth factors and their receptors. The most abundant and most studied glycosaminoglycan is heparan sulfate, the molecular binding properties of which are regulated by variations in the amount and pattern of sulfate groups attached to it.

To gain a clearer understanding of how serglycin and glycosaminoglycans contribute to angiogenesis and to the progression of a tumor into a malignant state we use several *in vitro* and *in vivo* models including mouse and zebrafish.

Members of the group during 2013

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Ananya Roy, PhD, postdoc
Nashwan Asmail, PhD student
Benedikt von der Heyde, International master student
Sandra Andersson, International master student
Tereza Brachtlova, Assistant
Alice Haux, SOFOSKO student

Publications 2011 to 2013

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Agencies that support the work
The Swedish Cancer Foundation
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The Medical Faculty, Uppsala University
Magnus Bergvall's Foundation

THE EFFECT OF SERGLYCIN ON PRIMARY TUMOR DEVELOPMENT AND THE METASTATIC PROCESS

Andrew Hamilton, Nashwan Asmail, Sandra Andersson, Ananya Roy and Maria Ringvall

Serglycin is mainly expressed by different immune cells where it has a major function for retention of a plethora of effector molecules within storage granules and vesicles. However, it has quite recently been shown that serglycin also can be expressed by carcinoma cell lines, where a high expression correlates to a more aggressive tumor cell phenotype. Serglycin has also been located *in situ* in human primary tumor tissue and metastases. Most of the interactions between serglycin and other molecules are dependent upon the glycosaminoglycan moiety of this proteoglycan, although the interaction between serglycin core protein and other proteins has also been reported.

We are studying how seglycin affects carcinoma development, and by what mechanisms this may occur. To this end, we use mouse models for primary tumor growth and metastasis. We have seen that serglycin has an effect on both the growth and aggressiveness of the primary tumors in a serglycin deficient mouse model for spontaneous formation of carcinoma. This may possibly be conferred by an alteration of the tumor vasculature and thus we are expanding these studies with the use of additional *in vivo* and *in vitro* methods to elucidate some of the mechanisms by which serglycin may affect parameters of importance for cancer progression.

THE EFFECT OF HEPARAN SULFATE AND HEPARAN SULFATE MIMETICS ON PHYSIOLOGICAL AND PATHOLOGICAL ANGIOGENESIS

Andrew Hamilton, Benedikt von der Heyde and Maria Ringvall

The most abundant polysaccharide in the mammalian body is the sulfated glycosaminoglycan heparan sulfate. Several signaling pathways involved in tumor progression and angiogenesis (e.g. fibroblast growth factor and vascular endothelial growth factor) are regulated by heparan sulfate that binds the ligand and/or acts as a co-receptor.

The degree, spacing and pattern of sulfate groups on heparan sulfate regulate its capacity to bind different proteins. We are therefore investigating how impaired sulfation of

endothelial heparan sulfate affects tumor progression in a mouse model for spontaneous development of tumors. Of particular interest is how the onset of vascular growth in dysplastic premalignancies is affected by under-sulfated endothelial heparan sufate. We are also investigating how small, specifically tailored, synthetic heparan sulfate mimetics affect developmental and pathological angiogenesis in zebrafish with the aim to translate these findings into a mouse model of spontaneous tumor development. In another part of the project, we investigate the functionality of the interaction between the endogenous angiogenesis regulator histidine-rich glycoprotein and heparan sulfate for regulation of angiogenesis.

WHAT ARE GLYCOSAMINOGLYCANS GOOD FOR?

Dorothe Spillmann

We are interested in how glycosaminoglycans (GAGs), negatively charged, long carbohydrate chains protruding from all cell membranes and intercalated in extracellular matrices affect diverse cellular processes. These GAGs can serve as adhesion sites, co-receptors, stabilizers of molecular interactions, protectors against proteolytic degradation and many more functions. Each cell and each tissue produces distinct collections of them with a sophisticated set of enzymes. When GAGs are totally absent, organisms cannot develop beyond a few days of embryogenesis. When these carbohydrates are structurally altered due to changes in their biosynthetic production, serious consequences can be seen as *e.g.* failed organogenesis. In pathological situations altered structures may be encountered in parallel to disturbed homeostasis. 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 (CS), 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 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 2013

Anna Eriksson, graduate student (defense May, 17 2013)
Ulf Lindahl, professor emeritus
Ramesh Babu Namburi, graduate student
Rashmi Ramachandra, post doc (until March)
Dorothe Spillmann, group leader

Publications 2011 to 2013

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* shared first author

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Thesis UU

Anna S. Eriksson: Syndecan – Regulation and Function of its Glycosaminoglycan Chains, May 17, 2013

Agencies that support the work

Foundation for Proteoglycan Research at Uppsala University
The Swedish Cancer Society

GLYCOSAMINOGLYCANS AND CELLULAR PROPERTIES

Anna Eriksson, Dorothe Spillmann

One of our main foci is to understand how the propagation of an extracellular stimulus generated outside the cell, *e.g.* by a growth factor or the cellular contact to the surrounding, is affected by GAGs. How does the presence of GAGs affect the reception and propagation of stimuli from outside to inside? Are these features affected by how the GAGs are presented, where and how they are localized at the cell surface or in the matrix, attached to a core protein or released as short oligosaccharides by the action of degrading enzymes as for instance used by cancer cells that pave their way to be able to metastasize? We have been able to show a direct role of structural features of HS chains when cells are stimulated by a growth factor, *e.g.* fibroblast growth factor (FGF) and soluble chains to rescue HS-deficient cells. These effects may in turn be different when chains are attached to their core protein anchored in the plasma membrane or the matrix. We have therefore developed a cellular model system to characterize these effects: With different isoforms of the core proteoglycan syndecan expressed in various ‘backgrounds’ of GAG biosynthesis we now study the impact of the core protein, the role of different types of GAG chains and their structural features, on the

propagation of extracellular stimuli (growth factor stimulation, adhesion) on the intracellular signals developed and the resulting cellular activities (*e.g.* proliferation, migration, contraction).

GLYCOSAMINOGLYCANS IN LIMB REGENERATION

Rashmi Ramachandra, 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 use this model to study what role GAGs play for the regenerative capacity of these animals [collaboration with M. Thorndyke, S. Dupont and O. Ortega-Martinez, Kristineberg, GU]. One of the brittle star species produces a remarkably highly sulfated type of CS chains correlated with an exceptional limb regeneration capacity and therefore we sought to study the structure/function relationship of these GAGs in the process of limb recovery. We can induce experimental autotomy in arms and characterize GAG production during the regeneration process. During regeneration 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 now to identify the corresponding biosynthetic genes in the brittle stars to study their regulation during these processes.

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 symbiontic bacteria [collaboration with O. Berteau, INRA, Jouy-en-Josas, France]. Sulfatases are among such enzymes that commensal bacteria need for their survival and to digest host GAGs. The main goal to characterize these types of enzymes is to improve our understanding of successful host-microbe symbiosis, to identify potential pathological twists but also to gain valuable analytical tools. We have been able to characterize three exo-sulfatases specific for either HS or CS structures, and one rare CS-specific endo-sulfatase.

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. We thus have a major interest to be able to characterize cells or tissues for their GAG production under different conditions. Therefore we continuously develop 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 collaborate with J. Bergquist and M. Ramström Jonsson at the Dept. of Chemistry, UU, to also set up mass spectrometry-based analytic tools for GAGs. So far we have been able to establish a quick semi-quantitative screening method for large sample numbers that should be a valuable help in deciding further processing of samples before more tedious and time consuming approaches are taken.

On collaborative basis we isolate and characterize GAGs from a wide spectrum of sources for different projects.

MEDICAL PROTEIN CHEMISTRY

Pia Ek, Åke Engström, Per Jemth, Birgitta Tomkinson

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 all 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 four groups pursue teaching as well as fundamental research on both enzymes and non-catalytic proteins. The Ek group studies a key mechanism in cellular function, namely regulation by phosphorylation. In particular, they examine histidine phosphorylation and the enzymes involved, a field where data is scarce but potential impact high. The Engström group provides a state-of-the-art proteomics facility as part of the technology platform for SciLifeLab in Uppsala. The power of their analyses lies in the extraordinary precision and accuracy of mass spectrometry, the highest in life sciences. The Jemth group looks at protein folding and protein ligand interactions and tries to unravel basic and general concepts about the action 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. Finally, 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!

The four 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.

CHARACTERIZATION OF MAMMALIAN 14-kDa PHOSPHOHISTIDINE PHOSPHATASE

Pia Ek

Protein phosphorylation is a central mechanism of signal transduction in eukaryotic cells involved in all inter- and intracellular functions. The O-phosphorylation is performed by specific protein kinases, which transfer the γ-phosphate from ATP to an acceptor group of the selected amino acid residue in target proteins. Protein phosphatases make this process reversible.

The presence of phosphohistidine phosphate in eukaryotic cells has long been recognized but its physiological importance not yet understood. Phosphohistidine has been estimated to amount to as much as 6 % of the total phosphoamino acid pool in nuclei, indicating important functions. At least in slime mould, histidine phosphorylation is several times more abundant than tyrosine phosphorylation, although not as widespread as serine phosphorylation. However, histidine phosphorylation is much less studied than that of serine, threonine and tyrosine, and only a few phosphohistidine proteins have hitherto been identified. The major reason for this may have been methodological difficulties: phosphohistidine, which exists as one of two isomeric forms (3-phosphohistidine and 1-phosphohistidine) is labile at acid and neutral pH. Histidine phosphorylation would therefore easily escape detection, for instance with standard SDS-PAGE.

Published phosphohistidine kinases were not completely purified and there are some evidences that histidine is phosphorylated by other mechanisms, for instance by NDP kinase.

The mammalian 14-kDa phosphohistidine phosphatase, also denominated PHPT1, which we found more than 10 years ago by probing pig liver extracts with a phosphohistidine-containing peptide, has been further investigated. The localization of PHPT1-mRNA by Northern blot analysis revealed high expression in heart and skeletal muscle. An extended immunohistochemical analysis in mouse and human tissues using a PHPT1-specific polyclonal antibody was essentially consistent with the previously reported expression of corresponding mRNA of a few human tissues. In addition, several other tissues, including testis displayed a high protein expression. A salient result of the present investigation was the ubiquitous expression of the PHPT1-protein and its high expression in continuously dividing epithelial cells.

Phosphorylation of histone H4 has been described, and histone H4 histidine kinase has been isolated from different eukaryote cells; yeast, slime mould and from mammalian cells. Protein histidine phosphorylation has been described in cells that are highly proliferative and in regenerating cells. In pilot experiments in our laboratory we have phosphorylated recombinant histones H1 (one type), H2A, H2B, H3 and H4, using phosphoramidate, i.e. a chemical phosphorylation. We observed that all phosphohistones were dephosphorylated by PHPT1 and some of them more rapidly than the phosphorylated peptide succinyl-Ala-His(P)-Pro-Phe-*p*-nitroanilide used in our previous PHPT1-activity studies. Besides the recombinant histones, we have used histone H4 purified from commercially available sources. The corresponding phosphorylated H4 was dephosphorylated at a similar high rate.

We have developed an assay of PHPT1 using a histidine phosphorylated peptide from a newly described physiological target for PHPT1 – ion channel KCa3. This method can be used for assay of phosphohistidine phosphatase and kinase activity and makes it possible to search for proteins that are responsible for histidine phosphorylation.

Two mammalian splice variants of PHPT1 have been cloned and expressed in E coli and yeast and only the wild type had phosphohistidine phosphatase activity. No expressed variants had kinase activity.

An earlier finding by me from research on phosphorylation in and by prostasomes, resulted in a promising method for determination of prostate cancer malignancy.

Members of the group during 2013
Pia Ek, Professor
Örjan Zetterqvist, Professor em

Publications 2011 to 2013

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Agencies that support the work
The Swedish Agricultural Research Council
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EXPRESSION PROTEOMICS

Åke Engström

This laboratory gives the scientific community an opportunity to at a low cost make use of recent developments in techniques and instrumentations for the analysis and identification of proteins. The facility for Expression Proteomics is equipped with 2-D electrophoresis systems, systems for post or pre-gel labeling of proteins, visible and UV light scanners, software for image analysis, spot picking systems, semi automated spot processing and MALDI-TOF/TOF instrumentation. The facility has expertise for 2-D analysis, mass spectrometry, image analysis, data base searches and general protein chemistry.

Our area of work is analysis and comparison of proteomes, identification of proteins in protein spots/bands by mass spectrometry, analysis of expressed proteins for quality control and analysis of proteins for post-translational modifications. The facility is open for all scales of problem solving or analysis, although the capacity for 2D gels might be a limiting factor for very large undertakings.

The service is primarily intended for identification of proteins from species with large numbers of genes or proteins characterized. The facility has in addition a limited capacity for de novo sequencing of proteins from any species. The service includes straightforward methods for characterization of expressed recombinant proteins. Considering the low cost for analysis this is highly recommended to avoid the potential risk of doing experiments with the "wrong" or modified protein. An MS analysis of intact expressed protein and a peptide mapping with MS give much better confidence than a simple SDS-gel analysis. If suitable for our techniques and knowledge we provide analysis of any type of sample

Members of the group during 2013
Åke Engström, Laboratory manager
Eva Andersson, Research engineer

Publications 2011 to 2013

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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 nature and specificity of protein-ligand and protein-protein interactions, and also on protein folding, stability 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 2013
Andreas Karlsson, PhD student
Emma Åberg, PhD student
Greta Hultqvist, postdoc
Gustav Sundell, MSc student
Jakob Dogan, postdoc
Josefin Jonasson, MSc student
Josefin Orre, SOFOSKO student
Maria Friberg, MSc student
Per Jemth, Associate professor

International exchange during 2013:
Rait Kivi, Tartu

Publications 2011 to 2013

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Agencies that support the work
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PROTEINS: FOLDING, STABILITY, INTERACTIONS AND ALLOSTERY

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 intense interest in disordered protein during 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 two domains from transcriptional co-regulators: "activator domain from thyroid hormone and retinoid receptors" (ACTR) and "nuclear co-activator binding domain of CREB binding protein" (NCBD).

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 2013:
Birgitta Tomkinson, professor
Jarmila Nahalkova, Ph. D., Researcher

Project workers during 2013
Daniel Andersson, "Evaluation of correlation between mRNA and protein expression of tripeptidyl-peptidase II: Possible future use as a biomarker for cancer?"

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

Birgitta Tomkinson

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.

The major focus of the structure/function project has been on the endopeptidase activity of the enzyme. This activity is very slow compared to the exopeptidase activity (i.e. the release of tripeptides). Further studies include the pH-dependence of TPP II from three species with two different substrates. The results so far have given some insights into the structure of the active site, and have been expanded with experiments on point mutations. The continued investigations will be aimed at examining the oligomerization of TPP II and if this is a way of regulating enzyme activity *in vivo*.

INVESTIGATION OF TPP II AS A POTENTIAL TUMOUR MARKER

Daniel Andersson, 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.

PROTEIN-PROTEIN INTERACTIONS OF TPP II

Jarmila Nahalkova

TPP II has beside its proteolytic function downstream of the ubiquitin-proteasome system also a potential regulatory effect on cellular cycle, apoptosis and senescence.

Since the mechanisms of this regulatory effect of TPP II was not previously elucidated, a protein-protein interaction study was performed 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. A mutual protein-protein interaction was also detected between MYBBP1A and CDK2 using combination of co-IP with LC-MS/MS identification of eluted proteins. PLA using HEK293 cells overexpressing TPP II showed that the interaction of TPP II with the tumour suppressor MYBBP1A was TPP II dependent and the interaction was reversible under serum-free cell growth conditions by the specific inhibitor of TPP II, butabindide, exclusively in the cytoplasm, but not in the nucleus. The MYBBP1A-CDK2 interaction did not depend on the inhibition of CDK2 kinase activity by using CDK2 inhibitor II at the cell growth condition of serum arrest, however the presence of foetal bovine serum in the medium significantly decreased the frequency of the interaction monitored by PLA. The interacting proteins studied: TPP II, MYBBP1A and CDK2 have cellular functions in protein degradation, tumour suppression, regulation of the cell cycle and apoptosis and they have been previously suggested as targets for development of tumour suppressing agents. Further in vivo studies should be performed to investigate the biological function of these interactions.

Additional interactions of TPP II were detected with proteins having functions in tumour suppression and neuroprotection, which are also included in our current research.

TUMOR BIOLOGY

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

A constellation of scientists within IMBIM focus their research on basic and translationally oriented questions of Tumor Biology. The major focus of this constellation is the behavior of tumor cells, their signal transducing pathways and the ways they communicate with other cell types within the tumor microenvironment. In other words, less emphasis is given on the more established role of genetic mutations as causal elements of tumorigenesis and more attention is paid on the architecture of the tumor tissue, its cellular and molecular constituents and the plasticity that governs cell behavior, blood vessel patterning and extracellular matrix adaptation during cancer progression. A major aim of the Tumor Biology unit is to generate knowledge for new prognostic markers and molecular targets that can be used for therapy.

The research projects of the Tumor Biology unit aim at understanding how cancer progression and plastic changes in the tumor microenvironment are controlled by distinct infiltrating cell types such as platelets for example, and how this impacts on blood vessel differentiation within the tumor (*Olsson, Ringvall, Sundberg*). Key molecular players in such processes are cell surface receptors such as integrins and extracellular matrix proteglycans such as serglycin, which co-ordinately regulate tumor tissue tension and remodelling (*Gerwins, Johansson, Ringvall*). In addition, the differentiation of tumor cells seems to undergo cyclical and alternate changes under the influence of specific growth factors and their signaling pathways, an example of which is the process of epithelial-mesenchymal transitions, which is also targeted by members of the unit (*Moustakas, Olsson, Rubin*). Such basic research is coupled to pre-clinical studies on anti-cancer drug uptake based on manipulation of the cancer microenvironment, and to studies where the tumor vasculature and cancer cell plasticity is manipulated by specific chemical or biological agents (Gerwins, *Moustakas, Olsson, Rubin*).

MECHANISMS OF TISSUE VASCULARIZATION

Pär Gerwins

Neovascularization is a prerequisite for normal physiological processes and for development of human disease. The goal for the reseach group is to define mechanisms that regulate angiogenesis and tissue vascularization in order to understand disease development and to define new therapeutic targets. A combination of in vitro models and in vivo animal experiments are used which allows validation of basic findings in more complex models. We have developed a novel in vivo angiogenesis assay and discovered a potentially important and novel mechanism of tissue vascularization. Tensional forces generated by myofibroblast mediated contraction of wounds mediate and direct translocation of neovessels which are pulled from the pre-existing vasculature as loops with functional circulation within the expanding tissue. This new mechanism, that has been termed looping angiogenesis, is the basis for the future research in the group.

Group members 2013
Pär Gerwins, professor, group leader
Peder Fredlund Fuchs, post doc
Ewa Kolosionek, post doc
Femke Heindryckx, post doc
Francois Binet, post doc

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FIBRIN DEGRADATION PRODUCTS AS REGULATORS OF NEOVASCULARIZATION AND FIBROSIS

Peder Fredlund Fuchs

Fibrinogen is a central protein in the haemostatic pathway that forms a provisional fibrin matrix in wounds, tumors and at sites of inflammation. Fibrin is continuously degraded by plasmin, which generates fibrin degradation products in the form of fragment E (FnE) and D-dimer. FnE has biological functions and stimulates proliferation and migration of endothelial and smooth muscle cells as well as induces angiogenesis by an unknown mechanism. Our hypothesis is that FnE released from the provisional fibrin matrix stimulates migration and differentiation of resident fibroblasts into myofibroblasts. In our model of looping

angiogenesis the myofibroblast has a central role in generating biomechanical forces that in turn mediates neovascularization. The fibrin matrix would in this model be a natural source of stimuli for cell migration and neovascularization by forming a relatively stable gradient of FnE.

Preliminary results indicate that FnE is as potent as PDGF-BB in stimulating fibroblast migration. We also find that although FnE did not alter αSMA levels by itself it potentiated TGFβ induced myofibroblast differentiation as determined by a doubling of αSMA expression compared to TGFβ alone. We have now initiated in vivo experiments using the chorioallantoic membrane assay and mice lacking fibrinogen to investigate if our in vitro findings can be translate to the in vivo situation. We are also purifying the putative FnE receptor.

These results have the potential not only to define fundamental mechanisms of angiogenesis in normal as well as pathological angiogenesis associated with diseases such as cancer, but also to provide novel therapeutic targets.

A LINK BETWEEN COAGULATION, TUMOR STROMA AND ANGIOGENESIS IN TUMOR GROWTH

Femke Heindryckx

An association between cancer and the haemostatic system has since long been recognized. There is a significant contribution of the coagulation system (fibrinogen, tissue factor, thrombin, factor X), the fibrinolytic system (plasminogen) and platelets to tumor growth and metastasis. Local deposition of fibrinogen/fibrin around tumor cells seems to be an early and universal event in most solid tumors. Fibrin(ogen) regulates proliferation, migration, apoptosis and expression of inflammatory mediators in tumor cells, fibroblasts, endothelial cells, and inflammatory cells through either integrin or non-integrin receptors. Furthermore, both fibrin and fibrin degradation products (FDPs) have been shown to support angiogenesis, consistent with the prevailing hypothesis that fibrin and its derivatives may promote tumor stroma formation by mechanisms that are comparable to those employed in normal tissue repair. Experiments in mice lacking fibrinogen have shown that the metastatic potential of tumor cells is greatly reduced in fibrinogen deficient animals. We are using fibrinogen knockout mice and inhibitors of coagulation and platelet function in xenograft and autochthonous (colon and liver cancer) tumor models to investigate the influence of fibrin(ogen) and and the coagulation system on tumor growth and metastasis. We have found that fibrin fragment E (FnE) stimulates migration and differentiation of myofibroblasts. Multiple monoclonal antibodies targeting FnE are under production with the goal to generate an antibody that binds and blocks the biological effects of FnE. Blocking antibodies will be administered to mice and its effects on angiogenesis, tumor growth, metastasis and stroma formation and wound healing analyzed. If successful, this could be the starting point for development of a novel treatment strategy.

BIOMECHANICAL FORCES AS REGULATORS OF NEOVASCULARIZATION AND LOOPINGANGIOGENESIS

Francois Binet

Biomechanical forces are important for embryo development as well as for reparative and pathological conditions in the adult. We have recently shown that tractional force generated during wound contraction directs and mediates angiogenesis and wound vascularization through a mechanism that has been termed looping angiogenesis. An important goal for our current research is to further explore the role of biomechanical regulation of neovascularization. To achieve this goal we use the mouse cornea as model system where sutures are placed in the cornea, which induces ingrowth of neovessels into the normally avascular cornea. By manipulating biomechanical forces surgically or by photochemical cross-linking of the cornea we will be able to further study how biomechanical forces regulate tissue vascularization.

Time-lapse imaging has provided a great deal of information on vasculogenesis and angiogenesis during embryo development e.g. in Zebra fish embryos. However, since the mechanisms of angiogenesis in postnatal life likely are different from embryo development there is a need for time lapse studies in the adult during e.g. wound healing. The cornea model provides a suitable model system since the cornea is avascular and almost two-dimensional. We will use the suture model and follow the neovascular ingrowth using a contact endoscope, which will provide details of neovascularization and increase our understanding of looping angiogenesis.

NOVEL TGFβ REGULATED GENES IN MYOFIBROBLASTS

Ewa Kolosionek

TGFβ is considered to be one of the major inducers of αSMA expression and collagen 1 secretion in myofibroblasts and cancer associated fibroblasts through activation of the canonical Smad pathway that regulates transcription of a large number of genes. The objective is to analyze TGFβ regulated genes in primary human fibroblasts in a non-biased manner using cDNA micro array. As expected we find that TGFβ increase expression of e.g. αSMA, NOX4 and N-cadherin. However, we also find highly induced expression of genes not previously known to be TGFβ regulated. One of these new genes is up-regulated more than 50 times after addition of TGFβ. Preliminary results suggest that knock-down using RNAi impair cell migration. Little is known on the function of the protein but high expression has been observed in malignant glioma.

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. As the main adhesion and migration receptors of cells, integrins are potential targets for regulation of 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 2013
Staffan Johansson, professor
Anjum Riaz, PhD student (until 130420)
Kathrin Zeller, PhD postdoc (until 130430)
Xiaofang Cao, postdoc
Siamak Kamranvar, postdoc (from 130501)
Nicolette Jansen, project worker (130201-130831)

Publications 2011 to 2013

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Agencies that support the work
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REGULATION OF SURVIVAL, MIGRARATION, AND CYTOKINESIS BY INTEGRINS

Anjum Riaz, Kathrin Zeller, Xiaofang Cao, Siamok Kamranvar

A. Adhesion-dependent survival. A key 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 p110   as the catalytic isoform of the PI3 kinase family that is activated by   1 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   1 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.

B. Mechanosignaling. A main function of integrins is to serve as “mechanoreceptors”. We study the role of integrins for signaling responses to external forces acting on the cell as well as to actomyosin-based intracellular force. 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 particular stretch-responsive signal.

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   1 integrin stimulation. The latter reaction is monitored as lamellipodia protrusion during cell attachment with TIRF microscopy.

C. Cytokinesis. Cytokinesis of normal adherent cells requires signals from integrins, and the lack of such signals in detached cells cause 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 appear to divide successfully, the uncoordinated karyokinesis-cytokinesis results in increased numbers of permanently binucleated cells, known to cause aneuploidy and chromosomal instability. The significance of transient cell detachment for chromosomal instability will be further studied. We have also shown that the cytokinesis block in suspended cell occurs at a late step, after the recruitment of CEP55 to the midbody. We aim to identify the integrin signal regulating this step.

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

Our research program covers several aspects of signal transduction and basic cancer biology. We are interested in 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 want to explain how the EMT process contributes to the maintenance of cells that carry tumor-initiating and metastasis-initiating capacities. We also test novel chemicals that perturb EMT and try to move our research into more applied medical science.

From the signal transduction perspective, the lab focuses on the Transforming Growth Factor β (TGF-β) and bone morphogenetic protein (BMP) pathways and on nuclear events such as regulation of transcription. In addition, we study cytoplasmic signaling events and in particular mechanisms of cell polarity regulation by the tumor suppressor kinase LKB1 and its downstream effectors, the AMP-regulated kinase (AMPK) family.

TGF-β and BMP regulate cellular processes such as cell growth, differentiation, and tumorigenesis via a group of proteins known as Smads and via several intracellular kinase and GTPase pathways. The Smads transmit signals from type I and type II TGF-β receptors on the cell surface and into the cell nucleus, where they regulate gene expression. TGF-β and BMP signaling have a complex impact on tumorigenesis. The pathways suppress the growth of early-stage tumors by inhibiting cell growth or by prompting cells to undergo apoptosis, but nevertheless drive tumorigenesis in late-stage tumors. We investigate the function and regulation of various TGF-β/BMP-responsive genes by combining functional experiments with genome-wide expression and location analysis in several cell models of epithelial cells. This approach has allowed us to decipher key steps in the genetic programs that mediate tumor suppression or tumor progression in response to TGF-β and BMP.

Members of the group during 2013
Claudia Bellomo, PhD student
Laia Caja, post-doc
Mahsa Shahidi Dadras, PhD student
Aristidis Moustakas, professor

Project workers during 2013
Gad Hatem, project worker (from Sep 2013)
Kalliopi Tzavlaki, project worker (from Aug 2012)

International exchange 2013
Katia Chourlia, visiting student, Aristotelian Univ. of Thessaloniki, Greece.

Satellite group at the Ludwig Institute for Cancer Research (LICR) during 2013
Members of the group
Andries Blokzijl, post-doc (from May 2013)
Jonathon Carthy, post-doc
Ulla Engström, technician
Kaoru Kahata, post-doc
Varun Maturi, PhD student

Anita Morén, technician
Panagiotis Papoutsoglou, PhD student (from Jan 2013)
Erna Raja, PhD student (till May 2013)
E-Jean Tan, PhD student
Yukihide Watanabe, post-doc (till Aug 2013)

Project workers 2013
Angelos Heldin, project worker (from Jun to Aug 2013)
Oskar Idås, project worker (from Sep 2013)

International exchange 2013
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REGULATION OF TGF-β/BMP RECEPTOR SIGNALING BY PROTEIN KINASES

Mahsa Shahidi Dadras and Kalliopi Tzavlaki

We are interested in two members of the AMP-regulated kinase (AMPK) family, widely known as being substrates of the master kinase and tumor suppressor LKB1. These are the salt-inducible kinase (SIK) and the Nuak2 kinase, whose genes are immediate-early targets of TGF-β signaling. SIK regulates turnover of the TGF-β receptor after ligand binding by cooperating with Smad7 and the Smurf ubiquitin ligases. Peutz-Jeghers Syndrome (PJS) patients develop benign hamartomatous polyps in early age and are predisposed to intestinal or other forms of cancer in adult life. PJS is caused by loss-of-function mutations in the LKB1 gene. PJS hamartomas resemble those in Juvenile Polyposis Syndrome (JPS) patients, who inherit inactivating mutations in the Smad4 or the BMP type I receptor, BMPR1A genes. We try to uncover the molecular links between TGF-β, BMP and LKB1/AMPK signaling by focusing on mechanisms of receptor function and trafficking. The model that we have generated so far shows that LKB1 negatively regulates the BMP type I receptor ALK2, a process important during Drosophila organogenesis and lung cancer progression. Negative regulation of receptor stability requires the inhibitory Smad7 and formation of a ternary complex between the receptor, Smad7 and LKB1.
This work has been partially carried in collaboration with Anita Morén, Drs. Erna Raja and Carl-Henrik Heldin (LICR-Uppsala University), Dr. Patrick Micke (Department of Immunology, Genetics and Pathology, Uppsala University), Dr. George Pyrowolakis (Freiburg University, Germany) and Dr. Ola Söderberg (Department of Genetics and Pathology, Uppsala University).

MOLECULAR MECHANISMS OF EPITHELIAL-MESENCHYMAL TRANSITION (EMT)

Claudia Bellomo and Mahsa Shahidi Dadras

EMT is an important process during cancer dissemination and contributes to the generation of cancer stem cells. In our recent work, we analyze the role of LKB1 and SIK kinases in regulating critical aspects of the EMT process, including cell polarity. LKB1 promotes epithelial differentiation, while TGF-β by inducing SIK promotes the mesenchymal transition. We identified new substrates of SIK as proteins that regulate the cytoskeleton and epithelial polarity. Our model suggests that SIK, via phosphorylation, provides signals for proteasomal degradation of its substrates. Inhibitors of the SIK kinase would perturb the EMT response. In a new project under the ITN “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 TGF-β. We characterize two specific molecular pathways that may mediate such responses of the liver cancer cells.

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

LINKS BETWEEN INVASION AND SELF-RENEWAL OF TUMOR INITIATING CELLS

Laia Caja and Kalliopi Tzavlaki

The chromatin regulator high mobility group A2 (HMGA2) protein mediates EMT in response to TGF-β by facilitating the transcriptional induction of the transcription factors Snail and Twist. HMGA2 regulates the ability of normal brain stem cells to self renew. We analyze the role of TGF-β and HMGA2 in the survival and stemness of tumor-initiating cells of the breast and brain (glioblastoma multiforme, GBM). In addition, we performed a genome-wide screen for mRNAs expressed under the control of BMP-7, which suppresses GBM tumorigenesis. We found that BMP signaling induces expression of the transcription factor Snail, which then causes astrocytic differentiation of the GBMs, enhanced invasiveness and loss of self renewal capacity by the GBM cancer stem cells. We also developed mouse xenograft models based on human breast cancer cells and human GBMs that have been engineered to express the EMT regulator, Snail or downregulate HMGA2. Our current work focuses on two axes: a) identification of a double positive feedback loop between BMP signaling and Snail transcription. b) Analysis of the transcriptional regulation of oligodendrocytes differentiation in the context of GBM.

This work has been partially carried in collaboration with Drs. Kaoru Kahata, E-Jean Tan and Carl-Henrik Heldin (LICR-Uppsala University), Drs. Jessica Cedervall and Anna-Karin Olsson (Department of Medical Biochemistry and Microbiology, Uppsala University), Drs. Lene Uhrbom, Bengt Westermark and Karin Forsberg-Nilsson (Department of Immunology, Genetics and Pathology, Uppsala University).

SUMMARY OF ACTIVITIES AT THE LICR

We focus on the role of the ribosyl-transferases PARP-1 and PARP-2 and the dePARylating enzyme PARG, which regulate nucleosome assembly and transcriptional initiation and elongation. We want to understand how such nuclear enzymes organize integrated biological responses by modulating the activity of TGF-β signaling. We also attempt to understand how post-translational modifications of Smads may change between normal and cancer cells. Using in situ proximity ligation we can detect the endogenous protein interactions and post-translational modifications. We also analyze genome-wide the 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. Based on our HMGA2 work on EMT we have deciphered a new mechanism by which HMGA2 represses the E-cadherin gene via DNA methylation on its promoter. We have also identified novel compounds that affect myofibroblast activation in response to TGF-β and analyze the role of nuclear receptors in

this process. Finally, in a recent collaborative project we work on the signaling pathway by the TGF-β family member GDF-15 (growth differentiation factor 15).
This work is carried in collaboration with Ulla Engström, Varun Maturi, Anita Morén, Panagiotis Papoutsoglou, Drs. Andries Blokzijl, Jonathon Carthy, Kaoru Kahata, E-Jean Tan, Yukihide Watanabe and Carl-Henrik Heldin (LICR-Uppsala University), Dr. Ulf Landegren (Department of Immunology Genetics and Pathology, Uppsala University), Dr. Michael Hottiger (University of Zurich, Switzerland) and Dr. Takeshi Imamura (Ehime University, Japan).

TUMOR VASCULAR BIOLOGY

Anna-Karin Olsson

The overall aim of the research group is to increase our understanding of how tumor vascularization is regulated and how tumor vessels can be targeted to treat cancer. Deregulated angiogenesis (formation of new capillary blood vessels) has been implicated in a number of pathological processes, for instance rheumatoid arthritis, retinopathy and tumor growth, and contribute to progression of the disease. To prevent or reduce angiogenesis in these situations is therefore of clinical interest.

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.

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.

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

Members of the group during 2013
Jessica Cedervall, post-doc
Julia Femel, graduate student
Else Huijbers, post-doc
Anna-Karin Olsson, Assoc Prof, group leader
Falk Saupe, post-doc
Yanyu Zhang, graduate student

Project students during 2013
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Argyro Kalogeropoulou, UGSBR, Uppsala University

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TARGETING TUMOR VESSELS BY THERAPEUTIC VACCINATION

Julia Femel, Else Huijbers, Falk Saupe, Danielle Verboogen

A small number of molecules have been identified as specifically expressed by tumor blood vessels, either by the endothelial cells themselves or by the adjacent stroma. These molecules are interesting targets for therapeutic vaccination against cancer, since they are absent in normal tissue and not expressed by the actual tumor cells, which often escapes the immune system. So far we have managed to efficiently break tolerance against these self-antigen and shown that prophylactic vaccination could reduce tumor size by 70%. Recently we have also found that vaccination using this approach could significantly reduce growth of already established tumors, which is the clinically relevant situation. These data show that tumor vascular antigens are highly interesting candidates for development of therapeutic vaccines targeting solid tumors. We are now continuing this work by immunizing against additional tumor vascular targets, alone or in combinations, to see if the efficacy of the vaccine can be enhanced.

HISTIDINE-RICH GLYCOPROTEIN IN PHYSIOLOGICAL AND PATHOLOGICAL ANGIOGENESIS

Jessica Cedervall, Yanyu Zhang

Histidine-rich glycoprotein (HRG; alternatively, HRGP/HPRG) 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. A model that we use for this purpose is the RIP1-Tag2 mouse that develops orthotopic insulinoma through a multistep process. One of these steps is characterized by

induction of angiogenesis, the so called "angiogenic switch", which renders the RIP1-Tag2 mouse an excellent model for studies of tumor angiogenesis. Another transgenic model in our lab is the MMTV-PyMT model for breast cancer. These mice closely resemble the human disease and accordingly develop metastases. 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. Using these models we are currently investigating the mechanism of action of HRG in physiological and pathological angiogenesis, as well as the impact of HRG on tumor growth and metastasis.

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

Jessica Cedervall, Yanyu Zhang, Argyro Kalogeropoulou

Platelets are anuclear cellular fragments, which play a crucial role in regulating blood hemostasis as well as non-hemostatic processes such as immunity, tumor metastasis and angiogenesis. Our research is focused on the mechanisms by which platelets affect tumor vascularization, with a special focus on the role of HRG in this process. 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. We are therefore interested in the mechanisms by which platelets promote tumor angiogenesis and EMT. Using *in vitro* as well as *in vivo* assays we have identified a number of molecules involved in the platelet-induced effects in the pre-tumorigenic environment.

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 $_1$-integrins participate in of maintenance fluid homeostasis, $_3$-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 a found a correlation with inflammatory processes and the architecture of the collagen network in the stroma.

Group members during 2013

Vahid Reyhani, MSc, PhD-student

Lars Rask, PhD, professor in Medical Biochemistry

Kristofer Rubin, PhD, professor in Connective Tissue Biochemistry

Publications 2011 to 2013

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FIBROBLAST-MEDIATED COLLAGEN GEL CONTRACTION

Vahid Reyhani

Fibroblast-mediated collagen gel contraction is stimulated by PDGF-BB and inhibited by pro-inflammatory agents such as prostaglandin E_1 and interleukin-1. Recently, others and we have identified at least two mechanisms for cell-mediated collagen gel contraction. The collagen-binding integrins, $\alpha_1\ _1$, $\alpha_2\ _1$ and $\alpha_{11}\ _1$ 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 is stimulated by *e.g.* PDGF-BB. Presently the work is concentrated on the role of fibrin deposits for $\alpha_V\beta_3$-directed contraction and cell phenotype regulation.

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.

Group members
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 2011 to 2013

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2. 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, Frida Henningson Johnson, Jenny Hallgren Martinson, Kjell-Olov Grönvik

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 antibodies, complement, mast cells, dendritic cells and T cells are operating in concert to achieve an optimal immune response and what goes wrong when allergies develop. 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.

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 in the group during 2013
Anna Bergman, PhD student
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Frida Henningson Johnson, assistant professor
Birgitta Heyman, professor, group leader
Annika Westin, technician
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Lu Zhang, PhD student

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MECHANISMS FOR COMPLEMENT-MEDIATED REGULATION OF IMMUNE RESPONSES

Anna Bergman, 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 the 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.

MECHANISMS FOR IgG-MEDIATED SUPPRESSION OF IMMUNE RESPONSES

Joakim Bergström, Anna Bergman, 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 and the development of germinal centers in the spleen. Also the importance of complement activation and Fc-receptors will be investigated.

MECHANISMS FOR IgE-MEDIATED ENHANCEMENT OF ANTIBODY RESPONSES

Zhoujie Ding, Hui Xu, Frida Henningson Johnson, 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 areas in the spleen where the fine tuning of antibody responses takes place, the follicles. There it 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 severel 100-fold enhanced antibody response. We will now try to detect the antigen in vivo in various cell types using flow cytometry. Mice will be immunized with antigen conjugated to fluorophores that can be detected intracellularly. After various times, the cells from the spleen are analyzed for antigen content. To explore the possitility of using CD23-mediated antigen transport without having to involve IgE antibodies (which are dangerous because they induce anaphylaxis, and which are also difficult to produce) we will chemically conjugate anti-CD23 monoclonal antibodies to various antigens. Hopefully, these conjugates will reach CD23 and behave in the same way as IgE-antigen immune complexes.

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 proinflammatory mediators such as tryptase and histamine. Mast cells mature in tissues from committed mast cell progenitors that are rare but can be quantified by limiting dilution assay or 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. 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.

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HUMAN MAST CELL PROGENITORS

Joakim Dahlin, Jenny Hallgren Martinsson

This project is 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. Recently, we identified a committed mast cell progenitor in mouse blood. However, nobody has yet found a committed human mast cell progenitor. We hypothesized that we will identify a committed mast cell progenitor population in human blood by evaluating the mast cell potential in sorted cell populations from human blood.

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

Behdad Zarnegar, Annika Westin, Joakim Dahlin, Kjell-Olov Grönvik, Jenny Hallgren Martinsson

The increase in lung mast cell progenitor 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 also causes increased numbers of mast cell progenitors in the lung but since this occurs around one week after virus inoculation there is a possibility that mast cell progenitors while likely being recruited from the blood also divide in situ. We are currently investigating how much of the increase in mast cell progenitor numbers in the lung that is due to recruitment and how much that is caused by cell division in situ in these two experimental disease models.

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

Annika Westin, Behdad Zarnegar, Malin Castelius, Kjell-Olov Grönvik, Jenny Hallgren Martinsson

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. We are using experimental models of allergic asthma and respiratory infections to mimic viral induced exacerbations. 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 mast cells that are equally activated but higher in numbers.

PROPHYLACTIC TREATMENT WITH IgY ANTIBODIES AGAINST INFLUENZA VIRUSES DOES NOT INTERFERE WITH THE NORMAL DEVELOPMENT OF ADAPTIVE IMMUNITY AGAINST INFLUENZA

Kjell-Olov Grönvik

Aim : To develop new forms of intranasal-oral immuno-therapy by using IgY antibodies to control seasonal and pandemic influenza.
Methods: Laying hens were immunized into the breast muscle with HPAI H5N1 inactivated influenza viruses emulsified in Freund's adjuvant. Starting two weeks after last injection eggs were collected and IgY antibodies in egg yolks were purified by extraction with super Q water. Mice were treated intranasally with IgY plus influenza viruses and were scored for loss of weight as a sign if disease.
Results: Prophylactic treatment with IgY antibodies protected mice against lethal infection of H5N1 avian influenza virus. Such IgY also blocked virus invasion by H1N1 PR8 influenza virus during *in vitro* and *in vivo* challenges, demonstrating cross protection against different strains of viruses.

Three months later mice initially protected by IgY antibodies, were challenged with a lethal dose of PR8 virus. No signs of disease were observed indicating the development of a protective adaptive immunity to influenza. *In vitro* challenge of T cells from these mice with APCs from infected lung rapidly induced strong production of pro-inflammatory cytokines associated with protective memory T cells.
Conclusions: The heterogeneity of the IgY response to viruses in chickens generates antibodies with broadly protective activity against influenza viruses that can be used to control acute influenza virus infection without interfering with the development of adaptive immunity due to quiescent infection or conventional vaccination.

STUDIES ON THE IMMUNE RESPONSE AGAINST INFLUENZA VIRUS IN MICE

Objectives: To study phenotype, function and localization of influenza virus antigen presenting cells and virus specific immune T cells.
Methods: Mice were infected intranasally with a sublethal dose of live H_1N_1 PR8 influenza virus. After three months animals were challenged with a lethal dose of the homologous virus and three months post challenge T cells were isolated from sacrificed mice. APCs were isolated from naive and immune syngeneic mice at 36 hours post infection and virus RNA was detected by RT-PCR. Purified APCs and T cells were co-cultured *in vitro* and cytokines were determined by immunoaffinity with Gyrolab Bioaffy and with FACS.
Results: After primary infection virus RNA was detected in lungs and in mediastinal lymph nodes but not in spleen or in inguinal lymph nodes. Virus challenge of protected mice showed a low or no PCR signal in lungs and in lymph nodes similar to primary infected mice treated intranasally with homologous influenza immune bronchoalveolar lavage. Challenge of immune mice rapidly induced IFN-γ containing T cells in lung and spleen and stimulation *in vitro* with $CD11c^+$ dendritic antigen presenting cells, APCs, of primary virus infected lungs resulted in poor proliferation of immune $CD4^+CD44^+$ T helper cells while retaining a high production of IFN-γ, IL-17 and IL-13 cytokines. On the contrary APCs of infected autologous immune lungs induced significantly less cytokine production by immune T cells compared to

APCs of primary infected lungs indicating a decreased uptake of virus or a tolerogenic function of $CD11c^+$ lung APCs of immune mice.
Conclusions: Anti-influenza antibodies present in bronchoalveolar lavage of immune mice inhibit excess virus uptake by airway associated APCs and, in contrast to primary infected APCs, dendritic lung APCs of immune mice may induce a local tolerance in immune, influenza specific T cells in order to protect the lungs from repeated inflammation.

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MOLECULAR BACTERIOLOGY

Dan Andersson, Andrea Hinas, Diarmaid Hughes, Linus Sandegren, Göte Swedberg

The area of molecular bacteriology at IMBIM is made up of five 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 and biocides) and internal (e.g. 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. 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 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, RATES AND TRAJECTORIES OF BACTERIAL EVOLUTION

Dan Andersson

Our research addresses the mechanisms and dynamics of molecular evolution in bacteria and 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 (S. typhimurium, E. coli, K. pneumoniae, S. aureus and M. 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 Salmonella typhimurium and E. coli as model systems to: 1. Examine how the extent and type of genetic variation affects bacterial fitness and rates of adaptive evolution. 2. Examine the role of gene amplification in adaptive responses to antimicrobial drugs and in the evolution of novel genes. 3. Examine the mechanism, physiological effects and evolutionary constraints on deletion formation. 4. Examine the functional role of ribosomal proteins and the mechanisms by which their absence can be genetically compensated. 5. Examine the fitness effects and constraints on horizontal gene transfer. 6. Examine the distribution of fitness effects of random mutations in different types of proteins and its impact on adaptive evolution. 7. Examine the mechanisms by which synonymous mutations can affect bacterial fitness and growth rates. 8. Experimental study of 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 (e.g. S. typhimurium, E. coli, S. aureus and M. tuberculosis) 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. Examine the feasibility of reversion of resistance by determining if reduced antibiotic use in community settings may result in a reduced frequency of resistance. 5. Identify mechanisms that confer resistance to antimicrobial peptides and determine the impact of these mechanisms on bacterial fitness and virulence. 6. Examine how very low levels of antibiotics can enrich for and select de novo resistant mutants in various types of laboratory and natural environments.

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PLASMID SELECTION IN THE PRESENCE OF SUB-MIC LEVELS OF ANTIMICROBIAL METALS

Lisa Albrecht

Plasmid carrying resistance genes to multiple antibiotics is a growing concern. It is of interest to elucidate the modes of maintenance of these plasmids in bacterial populations. Since metal resistance genes are sometimes also found together with antibiotic resistance genes on plasmids, it is possible that the use and presence of metals could co-select for antibiotic resistance. Metals of interest are arsenic, a component in poultry growth promoters and in pesticides, and silver, an antimicrobial used especially in the health care setting. The focus of this project is on positive selection conferred by plasmid encoded metal resistance genes, and how the these genes may enable a plasmid to be maintained in a population that is exposed to even very low levels of metals. The plasmid investigated is pUUH239.2 which was isolated from Uppsala University Hospital. It carries resistance genes to several antibiotics, and in addition it harbors arsenic and silver resistance operons. The competitive ability of the plasmid strain in a metal containing environment is measured by a Fluorescence Activated Cell Sorting (FACS) using the fluorescent tags blue fluorescent protein and yellow fluorescent protein for detection of the strains. It has so far been found that arsenic levels well below the minimum inhibitory concentration (MIC) confers positive selection on the plasmid. Subsequent steps will involve combinations of arsenic and antibiotics in order to asses the affect on selection in more complex environments. Similar approaches will be taken with regards to silver.

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 doing 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. Besides chromosomal resistance mutations or genes, we will investigate how low levels of antibiotics affect the selection, conjugation and maintenance of conjugative resistance plasmids in bacterial populations. We also study 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 will show what mutations or combination of mutations are responsible for the resistance and which fitness costs they confer.

A NEW METHOD FOR IDENTIFICATION OF ANTIBIOTIC RESISTANCE IN BACTERIA IN CLINICAL MICROBIOLOGY

Erik Gullberg

When a patient comes to a hospital with a urinary tract infection or another infection that could be caused by bacteria, a sample is sent for analysis to a clinical microbiological laboratory. To identify the bacterium and to determine the antibiotic resistance profile, several growth steps and tests must be performed, many of these requiring overnight incubations. Because of this, the response time back to the physician is 2-3 days, and if the patient has a severe infection they will usually have been started on a broad-spectrum antibiotic or the default antibiotic for the suspected type of infection. Not only does this lead to over-use of antibiotics in case of virus infections, but the patient might also be given an antibiotic that has no effect in case of infection with resistant bacteria.In this project, a method based on a combination of genotypic and phenotypic screening is being developed. The sample is grown for a few hours in the presence or absence of different antibiotics, and a sensitive probing technique allows for the simultaneous detection of both the species of bacteria and the resistance pattern. Padlock probes, which are single stranded DNA molecules designed for circularization when bound to target DNA, are utilized. The padlock probes target species-specific regions of the 16S rRNA in the bacteria. The ligated circles are used as templates for so-called rolling circle amplification (RCA), which gives amplification of the target. This method is sensitive, fast, and probes can be designed against the relevant species for different kinds of infections, which means that contaminating bacteria such as skin normal flora will not interfere with the detection.

SYNTHETIC CRISPR SYSTEMS TARGETING RESISTANCE GENES

Robin Hagblom and Erik Gullberg

Horizontal gene transfer, HGT, is an important process in microbial evolution. In some cases, the transferred genes can provide the receiving bacteria with competitive advantages such as antibiotic resistance but in many cases they can be costly or even lethal. A CRISPR array (Clustered Regularly Interspaced Short Palindromic Repeats) along with CRISPR associated (cas) genes comprise the CRISPR/cas system which is a form of bacterial adaptive immunity against mobile genetic elements such as conjugative plasmids and phages. Although shown to be of the *Escherichia coli* type, little is known of the CRISPR/cas system in *Salmonella typhimurium* LT2 so further characterization is necessary. Once better understood, the native *Salmonella* system could be reprogrammed to target a given mobile element. For example, extant clinical plasmids that carry antibiotic resistance genes can be targeted in the hopes of protecting bacteria from the uptake of such a plasmid and thus preventing the spread of antibiotic resistance in a given bacterial population.

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

Karin Hjort

Bacterial populations 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 multiresistant bacteria such as extended-spectrum β-lactamases (ESBL) producing bacteria. The focus of this project is to examine colistin's ability to enrich for *de novo* resistant mutants of *E. coli* and *Salmonella typhimurium* during cycling at sub-MIC levels. Our results shows that resistant populations of *E. coli* and *S. typhimurium* are generated at sub-MIC concentrations of colistin and that the level of antibiotic resistance increased during the cycling experiment. Whole genome sequencing of colistin resistant strains showed different resistance mutations in *E. coli* and *S. typhimurium*. For *E. coli* the resistant strains contained mutations in the *pmrA* and *B* genes part of the two-component system involved in sensing low pH, AL^{3+} and Fe^{3+}. Mutations in these genes are known to generate resistance in both *E. coli* and *S. typhimurium* at high concentrations of colistin. In the colistin resistant *S. typhimurium* genomes no known resistance genes were mutated. Instead genes (*arnT* and *prmD*) known to be involved in colistin resistance were amplified in seven out of nine strains. Along with the amplifications a mutation in the *arcA* gene was also present. Mutations in the *arcA* gene are not known to generate resistance and in absence of gene amplifications the *arcA* mutations did not generate resistance. These experiments will give us a better understanding of the spread of antibiotic resistance at sub-MIC levels of antibiotics in the environment and the implications of heteroresistance due to gene amplification/segregation.

ESTABLISHING AN *IN VIVO* METHOD FOR COMPETITION ASSAYS OF ANTIBIOTIC RESISTANT MUTANTS

Karin Hjort

Most experimental setups to study evolution and fitness cost of antibiotic resistant strains involve *in vitro* experiments using defined media and antibiotic concentrations. The establishment of an *in vivo* model mimicking environments such as natural water and sewage plants will increase our understanding of fitness costs of antibiotic resistant populations. In these environments nutrients are limited, bacterial populations are competing and phages are common which changes the generation time of bacterial populations and probably also the fitness cost of an antibiotic resistant mutant. In addition these environments can contain low concentrations of antibiotics that can increase the frequency of antibiotic resistant bacteria and also select for *de novo* generated mutants. Bacterial fitness cost can be analyzed with competition experiments between antibiotic resistant mutants and wild type bacteria. The changes of bacterial populations are determined with fluorescently labeled bacterial populations in a flow cytometer. Our preliminary data indicates that wild type *E. coli* can grow with a reasonable generation time and population size in autoclaved and filtered sewage water from the inlet of Kungsängens sewage plant. Competition experiments in the modified

sewage water with antibiotic resistant mutants versus wild type bacteria will determine if the fitness cost of antibiotic resistant mutants are different from a defined medium. These results will increase our understanding of the development and spread of antibiotic resistance in the environment.

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 to 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 done in collaboration with Sven Bergström's group 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. This variation also includes the ability of microorganisms to evolve resistance to antibiotics and contribute to virulence. 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. Promiscuous catalytic activity is a common feature of many enzyme/substrate systems. 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 conferring resistance to antibiotics through weak

promiscuous activities. The screen will be conducted using an *E. coli* proteome overexpression library to judge the potential of endogenous genes. The second part will investigate the evolutionary potential of phage proteins by employing metagenomic phage libraries from several different environments (coral, riverine, pond, mucus) to simulate the potential from horizontal gene transfer. The frequency and identity of protective gene fragments will be determined and their adaptive potential will be investigated.

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. This is a necessary assumption to adapt research based on laboratory strains to the clinical situation. 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 completely independent of the strain context. The effect on fitness and resistance is constant in all four investigated strains. Another important aspect of the influence of the genetic background on the phenotype of a resistance mutation is potential epistatic interactions between 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 five investigated resistance mutations we constructed all possible combinations of these mutations in *S. enterica* serovar typhimurium LT2. Assuming all combinations are viable this would yield five single, ten double, ten triple, four quadruple and one quintuple mutant. The double-mutants in LT2 showed a clear additive effect of the fitness cost. In contrast to other recent publications our results show that the combined effects of resistance mutations on fitness are independent without any indication of epistatic interactions. Combinations of three or more resistance mechanisms are being constructed and may reveal epistatic interactions in more complex situation.

RAPID AND EFFICIENT COMPENSATION OF LOW-FITNESS MUTANTS

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. Using long-term evolution experiments we were able to minimize the associated fitness cost of several resistance-causing mutations to four clinically important antibiotics: ertapenem, meropenem, fosfomycin and colistin. The resistance mechanisms include alterations in transport functions and two-

component systems. By regular screening of growth rates and resistance levels we determined the rate of compensation. In addition, we measured the correlation between bacterial fitness and susceptibility to these antibiotics. Our results show that compensation of fitness costs is very rapid and efficient. While the compensation for constructed knockout strains with defects in porins was only partial, compensated fosfomycin resistant strains increased their growth rate above wild type levels. Additionally, some of the evolved strains were able to tolerate even higher concentrations of the corresponding antibiotic than the unevolved parental strain. These findings demonstrate the high adaptability and competitiveness of resistant mutants and underline the importance of preventing the initial development of antibiotic resistances.

CAUSES OF FITNESS COSTS OF SYNONYMOUS MUTATIONS

Anna Knöppel

Synonymous mutations are sometimes deleterious, although they do not change the sequence of the polypeptide. Fundamental selective forces seem to underlay the selected base composition but very little is know about what levels the main fitness constrains operate (e.g. mRNA secondary structures or translation effectiveness). To investigate the mechanistic causes of the fitness reduction I use 6 synonymous single random base pair substitutions in a non-essential ribosomal protein, S20, in *Salmonella typhimurium* that have previously been shown to confer high fitness costs during rapid growth in rich medium. Eight independent lineages out of each strain have been evolved until compensatory mutations appeared that ameliorate the fitness costs. Whole genome sequencing analysis indicates that mutations in *fis*, *rpoA*, *rpoD*, and *rpsT* (coding for S20), as well as large duplications all including *rpsT*, can compensate for the fitness costs of the synonymous mutations. We hypothesise that the synonymous mutations in *rpsT* lowers the S20 levels in the cells and that the loss of function mutations found in *fis* (activator of rRNA transcription) as well as the mutations found in *rpoA* and *rpoD* (both important for activating rRNA transcription) could be a compensation for toxic non-functional ribosomes formed that lacks S20. Downregulation of rRNA would bring the ratio rRNA:S20 closer to 1:1 in the cells. Accordingly, the duplications seen would increase S20 levels, which could also be the case with the compensatory mutations found in *rpsT* itself. Reconstructed compensatory mutants are currently being evaluated in terms of fitness and S20 protein levels compared to wild type. I am also planning to compare *rpsT*, *fis*, *rpoA* and *rpoD* mRNA levels for wild type and mutant S20, and to examine if the fitness costs can be compensated for by increasing the concentrations of the tRNAs reading the affected codons. In addition, I want to mutagenize the defective *rpsT* alleles with the intention to further understand the fitness costs caused by the synonymous mutations.

MINOR FITNESS COSTS IN AN EXPERIMENTAL MODEL OF HORIZONTAL GENE TRANSFER IN BACTERIA

Anna Knöppel, Joakim Näsvall and Ulrika Lustig

Horizontal gene transfer (HGT) plays a major role in bacterial evolution and is known to be a key mechanism in the spread of pathogenicity determinants and antibiotic resistance. Apart from ecological and mechanistic constrains, the fixation of HGT events is greatly determined by selection and fitness effects. In this project we experimentally test the causes of fitness

constrains of HGT. Random DNA fragments varying in size from 0.45 to 5 kb of *Bacteroides fragilis*, *Proteus mirabilis* and human intestinal phage were inserted into a neutral position of the *Salmonella typhimurium* chromosome and the fitness costs of the inserts determined and correlated to size and origin of insert, GC content, gene type, and the expression level of inserts. We found that 8 inserts were deleterious and ninety inserts did not have any detectable fitness effects. When inducing transcription from a P_{BAD} promoter located at one end of the inserts, 16 transfers were deleterious and 82 did not have any detectable fitness effects. In conclusion, a major fraction of the inserts had minor effects on fitness implying that extra DNA transferred by HGT, even though it does not confer an immediate selective advantage, could be maintained at transfer-selection balance and serve as raw material for the evolution of novel beneficial functions. We could not detect any correlation between fitness costs and origin of insert, GC content or gene type. The fitness costs, however, correlated positively with expression level and the number of inserts with detectable deleterious effects increased under inducing conditions.

MECHANISMS OF TIGECYCLINE RESISTANCE IN *ESCHERICHIA COLI*

Marius Linkevicius

Tigecycline is the main representative of the new class antibiotics glycylcylines and it has been used in medical practice since 2005. It is active against multidrug resistant gram-positive bacteria like methicillin resistant *Staphylococcus aureus*, vancomycin resistant enterococci and gram-negative pathogens producing extended spectrum ß-lactamases. However, resistance against tigecycline has been recently reported. Overexpression of unspecific RND or MATE family transporters was suggested as the reason for the resistance to tigecycline. This study focuses on determination of resistance mechanisms to tigecycline and the consequential fitness costs in *Escherichia coli*. Two main groups of spontaneous *E. coli* mutants with low-level resistance to tigecycline were identified. Genes involved in LPS biosynthetic pathway 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 was linked to bacterial efflux and its regulation. Some of these mutations are present in strains of *Enterobacteriaceae*, 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 from growth assays, as reconstructed *E. coli* mutants with reduced susceptibility to tigecycline grew 3 to 18 percent slower than the wild-type *E. coli* strain. In future, *in vitro* compensatory evolution experiments will help us identify the targets restoring the growth of the mutants and *in vivo* competitions will show if the mutants can establish a successful infection.

TIGECYCLINE RESISTANT TET PROTEINS

Marius Linkevicius

Tigecycline overcomes major resistance mechanisms that render previous two generations of tetracyclines non-usable. It is believed that due to its bulkier chemical structure, tigecycline is not transported out of the cell by Tet efflux pumps, as the transport proteins cannot recognise the antibiotic as a substrate. In addition, a higher tigecycline affinity to ribosome and the

bulky 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 are interested whether evolution of tigecycline resistance conferred by Tet proteins is possible. DNA sequence libraries of Tet efflux and ribosomal protection proteins have been generated using error-prone PCR. According to the pilot selection of tigecycline resistant mutants harbouring mutagenised Tet efflux proteins, it is possible to select protein variants, with increased minimal inhibitory concentration for tigecycline. Larger scale selection experiments have been performed and the mutations in Tet proteins that are responsible for the decreased susceptibility to tigecycline are being characterized.

SALMONELLA MUTANTS RESISTANT TO ANTIMICROBIAL PEPTIDES

Hava Lofton

Antimicrobial Peptides (AMPs) are listed as promising new drug candidates. They are part of the innate immunity of all living organisms. Many have intense, broad-spectrum antimicrobial activities and have been shown to have many other immune system roles, such as chemotactic response and modification of the host gene expression. We set out to test bacterial resistance development to AMPs and the resulting effects (i.e. fitness costs) in bacteria. By daily passaging small amounts of *Salmonella typhimurium* in periodically increasing concentrations of three different AMPs, CNY100HL, LL-37 and Wheat Germ Histones, we obtained mutants that tolerated much higher peptide exposure than parental strains. Whole genome sequencing identified several mutations, from which a subset was chosen for further investigation: *rfaY*, *pmrB* and *phoP* (all three genes are involved in modifying LPS) and were subsequently reconstituted in a wild type background. The mutation in *rfaY* (phosphorylates HepII in the LPS core) confers much of the resistance against all three AMPs. The fitness cost measured for all of the reconstituted mutants ranged from +0.8% to -16%. Significantly, the concentrations of AMPs used in our experiments are comparable to the levels found in human tissues, and that mutations such as *rfaY* could be selected de novo and possibly maintained by contact with host defense peptides though direct resistance and/or cross-resistance.

DOES CRYPTIC GENETIC VARIATION AFFECT EVOLVABILITY?

Erik Lundin and Joakim Näsvall

Neutral mutations, i.e. mutations that do not affect the original function of a gene, may 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 weak promoter so that the activity of the HisA enzyme is limiting for growth. Neutral mutations will be accumulated through rounds of mutagenesis and selection for maintained HisA activity. A collection of mutants containing one or several neutral mutations will be used as starting points for experimental evolution towards TrpF activity.

FUNCTIONAL TRADE-OFF DURING EVOLUTION OF NEW FUNCTIONS

Erik Lundin and 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.

STRATEGIES FOR ANTIBIOTIC DEVELOPMENT TO REDUCE RESISTANCE

Ulrika Lustig and Cao Sha

Antibiotic resistance in clinical settings and the decline of antibiotic drug development are increasing problems. The volume and pattern of antibiotic use influences the rate of resistance development and one idea is that dosing strategies in clinical settings can be optimized such as to minimize the emergence of antibiotic resistance while still maintaining efficacy. In order to collect *in vitro* data of bacterial growth rates and killing at different concentrations of antibiotics, we perform time-kill experiments on susceptible and well-characterized antibiotic resistant mutants of *E. coli*. By using the time killing data of MG1655 (a well-characterized laboratory wild type strain), an *in silico* model was developed. The model has been tested on 11 isogenic laboratory strains carrying mutations relevant to clinical ciprofloxacin-resistance. We also study how bacterial inoculum size, growth phase, and medium, affect the rate of bacterial killing by antibiotics. Ciprofloxacin time-kill experiments were also conducted on a set of clinical urinary tract infection (UTI) isolates. Data from these experiments were used to test the relevance of the extensive experiments on laboratory strains, and to further develop the *in silico* model. The *in silico* modeling is a tool to support predictions on how to dose one or several antibiotics in combination to optimize the effectiveness of therapy. The models can also be used to forecast the resistance potential of new drug candidates. This project is a collaboration between the groups of Diarmaid Hughes and Dan Andersson (IMBIM), Lena Friberg and Mats Karlsson (FarmBio) and Otto Cars (Med Sci).

STUDY OF ANTIBIOTIC RESISTANT *E.COLI* IN MALLARDS (*Anas platyrhynchos)*

Ulrika Lustig, Marie Nykvist and 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 0,43-43-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 and Björn Olsén (MedSci) and Linus Sandegren and Dan Andersson (IMBIM).

RESISTANCE DEVELOPMENT TO 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 typhimurium* and 4 independent lineages of *Escherichia coli* were cycled in increasing concentrations of cyO2 for 100 or 150 cycles (600-700 or 900-1050 generations). Clones were isolated from the populations evolving under this selective pressure. A number of mutations were identified by whole genome sequencing. We are examining the phenotypic effects of individual mutations using a combination of genetic and biochemical tools. Characterization of these effects may hint about the mechanism(s) of action of cyO2. In addition, studies of fitness costs may provide data based on which rate and trajectory of evolution and spread of resistance can be predicted. This project is a collaboration Ulf Göransson at Uppsala University.

LON PROTEASE INACTIVATION, GENOME ARCHITECTURE AND ORDER OF APPEARANCE OF MUTATIONS POTENTIATE BACTERIAL EVOLUTION TO ANTIBIOTIC RESISTANCE

Hervé Nicoloff

Resistance to low concentrations of antibiotics can serve as stepping-stones towards higher resistance. However, the evolutionary paths towards low resistance are not well characterized. A previous study revealed the frequent co-selection of *lon* mutations along with mutations causing low multidrug resistance. The *lon* mutations alone do not cause growth on the selective plates, suggesting that low antibiotic resistance might preferentially evolve within *lon*$^-$ subpopulations of bacteria. In this work, we show that a *lon* mutation causes a very low multidrug resistance by inducing the AcrAB-TolC pump via the stabilization of the *acrAB* transcriptional activators MarA and SoxS, which are substrates of the Lon protease. We found that the fast evolution of *lon*$^-$ mutants towards higher resistance involves selection of common next-step resistance mutations consisting of large duplications including *acrAB* and the *lon* locus. The high frequency of *acrAB* duplications is due to the presence of several sets of identical IS sequences present around the *acrAB* locus and involved in duplication formation. Growth on the selective plates results from the combined effects of *acrAB* duplication and *lon* mutation. By opposition to first step *lon* mutants, we found that the frequent first-step *acrAB* duplication mutants lack access to frequent next-step mutations and fail to evolve faster towards higher resistance than a wild type strain. Furthermore, when *acrAB* duplication occurs as the first step mutation, *lon* co-duplication increases Lon activity, which reduces the effect of *acrAB* duplication on resistance. As predicted, when the functional *lon* gene is relocated far from *acrAB* to prevent their co-duplication, first-step *acrAB* duplication confers a higher resistance, which then allows selection of frequent next-step mutations and results in faster evolution towards higher resistance. In conclusion, we demonstrate how the genome architecture and the order of appearance of mutations can influence the rate of antibiotic resistance evolution.

MONITORING BACTERIAL POPULATIONS AND FITNESS USING GENETIC TAGS AND DEEP SEQUENCING

Hervé Nicoloff

Genetic tags consist of short chromosomal DNA sequences that are unique to the cells they are cloned into. By mixing populations of cells carrying different tags, one can measure the fate of each population by measuring the fate of its tag. In this project, we are using small 15 nucleotides-long random tags to differentiate bacteria, and deep amplicon sequencing (Illumina) or qPCR to monitor the fate of each tag (and therefore population) during growth. Two sets of 49 *Escherichia coli* and 46 *Salmonella typhimurium* carrying different tags were constructed. In these sets, all the *E. coli* or *S. typhimurium* strains are genetically identical with the exception of their non-transcribed and untranslated tag. By opposition to translated markers typically used to differentiate bacterial populations (e.g. fluorescence or antibiotic-resistance markers), we do not expect the tags to cause any fitness cost by themselves. We intend to use these sets of tagged bacteria in an innovative, cost-efficient and highly sensitive approach to measure the effect on fitness of known mutations, especially those with an expected low impact on fitness. Because bacterial population dynamics affects the rate of evolution and fixation of genotypic and phenotypic traits, understanding and characterizing

factors affecting population dynamics is of great interest for bacterial evolution studies. Using large libraries of tagged bacteria (in the order of 10^5 different tags), we intend to directly measure the effect of growth conditions on bacterial diversity, such as the effect of genetic drift or the effect of shifting carbon sources during growth. We expect that the size and diversity of the tagged populations used in combination with the depth of the analysis (based on Illumina amplicon deep sequencing) will allow us to efficiently detect very small changes in population dynamics.

DE NOVO GENES FROM RANDOM PEPTIDES

Hervé Nicoloff, Karin Hjort, Michael Knopp and Joakim Näsvall

De novo gene birth results from random nucleotide sequences acquiring transcription and translation abilities. However, the vast size of the sequence landscape makes it highly improbable to find a specific biological function in a random sequence of amino acids. Consequently, appearance of the first biological functions on earth would have resulted from extremely improbable events. One way to circumvent this dilemma could be if the same function can be found in many different sequences or structures but that life, which must have originated from a relatively small set of *de novo* genes, would only use a small subset of those sequences/structures for any given function or activity. To test this hypothesis, we intend to experimentally select for biological functions encoded by random peptides. For this, we have designed five libraries of random genes encoding random peptides differing in size and amino acid content. By varying amino acid content, we intend to sample specific regions of the sequence landscape (e.g. sampling for sequences depleted in hydrophobic residues to favor disordered peptides, or sampling for sequences enriched in “primordial” amino acids). Random peptides potentially encoding specific biological activities will be positively selected using a large collection of auxotrophic and conditionally lethal mutants of *Escherichia coli* and *Salmonella typhimurium*. Further analysis will be performed to determine the exact activity encoded by the random peptides selected.

EVOLUTION OF NEW GENES THROUGH INNOVATION, AMPLIFICATION AND DIVERGENCE

Joakim Näsvall

It is a generally accepted idea that new genes can evolve from a duplication of an ancestral gene, freeing one copy from the constraints of purifying selection. Through random mutations and natural selection one of the copies may acquire a new function, while the other copy retains the original function. Several models for this process have been proposed, differing in the timing of the initial duplication, acquirement of the first beneficial mutation that leads to the new function, and the onset of selection. One model (Innovation, Amplification, Divergence; IAD) is based on the observation that many enzymes have weak secondary activities. A change in the environment, such as the presence of a toxic compound, a new nutrient or fixation of a deleterious mutation, can make such a minor activity beneficial, leading to a selective pressure to increase the activity. We have developed a genetic model system to study the early stages of evolution of new genes. We isolated mutants in *hisA* that can partially substitute for *trpF* while still retaining some of the original activity. These bifunctional *hisA* alleles were placed on the plasmid F’128 in a *Salmonella enterica* strain

lacking the chromosomal *hisA* and *trpF* genes, and were allowed to evolve during serial passages in medium lacking both histidine and tryptophan. Amplifications started accumulating within the first few tens of generations, and dominated most lineages throughout the experiment. During 3,000 generations of continuous selection, some lineages accumulated additional mutations in *hisA*. In several of the lineages clones carrying two different *hisA* alleles within an amplified array appeared, each showing functional specialization towards one of the enzymatic activities. This model system (evolution of TrpF activity in HisA) will be used to study several different factors influencing the evolution of new genes (see below). A follow-up to this study will determine the structure, stability, expression and specific activities of the evolved enzymes.

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 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 appears to be essential, unless the strain also has a duplication of a mutant *prfB* gene known to confer some recognition of UAG stop codons. Such a strain is very slow growing, especially on rich medium, leaving plenty of room for adaptive evolution by selection for faster growth. We are currently allowing this mutant to undergo adaptive evolution by serial passage of multiple populations in both rich and minimal medium. In only a few tens of generations of growth under selection, all 32 populations increased their growth rates by amplifying the mutant *prfB* gene to high copy numbers, but after a few hundred generations of growth there are still no additional mutations in *prfB*.

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

Annika Söderholm

In the study ”Real-time evolution of new genes by innovation, amplification, and divergence” by Näsvall *et al.* it was demonstrated how new genes can evolve by the innovation-amplification and divergence evolutionary model. In the study, a mutated *hisA* gene (*S. enterica*) which had acquired a low level of TrpF activity while retaining some original

activity was isolated and placed in a *hisA*- and *trpF* lacking strain. Through continuous selection for both activities amplification of the gene was promoted and the amplified gene products diverged so that different mutants 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 my 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. I work together with Xiaohu Guo (Maria Selmer's group, ICM) on determining the structures of the different mutants by using X-ray crystallography. So far we have solved approximately 10 structures including wild type HisA and structures from every catalytic category. The structures vary in resolution and the mutants display differences with regard to stability, expression and crystallization behavior. In general, the success rate for the wild type enzyme and the wild-type like mutants has been higher (higher yield, higher stability and more crystallizable) as compared to the highly mutated variants. A persistent problem has been the occurrence of two flexible active-site loops that have not been visible in the previous structures. These loops are very important for the structural analysis. However, we had a recent breakthrough in this matter. Our latest structure (a TrpF specialist) is a complex structure with a TrpF-product analogue bound to the active site. The binding of the ligand made the flexible loops stabilize, thereby making them visible in this structure. Our current priority is to get more complex structures, both with the TrpF-product analogue as well as with the HisA substrate. This project is a collaboration with Maria Selmer, Uppsala University.

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 the resistance development to mecillinam has remained low, meaning that it is now used more widely for UTI treatment. The goal of this project is to understand the dynamics of resistance development in order to minimize it. The project focus on the genetics, physiology and evolution of mecillinam resistance, examining mutants isolated in the laboratory as well as clinical isolates. This is achieved by identifying different mecillinam resistance mechanisms, how they arise and how they influence bacterial fitness and virulence. Mecillinam resistant mutants of *Salmonella typhimurium* and *Escherichia coli* have been selected in the lab. Characterization of the mutants and identification as well as reconstruction of the resistance mutations has been done for both species. We found several novel mecillinam resistance genes. In an evolution experiment mutants that have compensated for the fitness loss (decreased growth rate) associated with mecillinam resistance have been selected. These will be sequenced to find which mutations are responsible for the compensation in growth rate. 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 appeared in all the clinical strains. These mutations contribute to low-level resistance, but cannot alone explain very high resistance. 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 developing higher resistance in the clinical setting. 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 epistasis in mecillinam resistance and identifying mutations in the fitness compensated mutants (both in vitro selected and possible clinical ones). The future plans for the project are; set up of new experiments to get a better understanding of the impact of ppGpp (stringent response) on mecillinam resistance and examination of mecillinam together with other antibiotics.

THE ROLE OF ENDOSOME-ASSOCIATED PROTEINS IN SMALL RNA PATHWAYS AND INTERCELLULAR RNA TRANSPORT IN THE NEMATODE CAENORHABDITIS ELEGANS

Andrea Hinas

RNA interference (RNAi), RNA-induced sequence-specific degradation of mRNA, has emerged as a major mechanism of gene regulation in most eukaryotes and has important implications in biomedical research and drug development. Extensive research has led to a relatively detailed understanding of this process in the short time since its discovery. However, a much less explored aspect of RNAi is the uptake and transport of RNAi silencing signals between cells in animals. Intriguingly, recent studies suggest that this represents a new means of cell-cell communication. Increased knowledge about spreading of RNAi is also of importance for the development of RNA-based drugs in order to efficiently deliver the drug to the location of the target mRNA. In some organisms, such as the nematode *Caenorhabditis elegans*, RNAi uptake and spreading occurs with high efficiency. By taking advantage of the many molecular and genetic tools available for *C. elegans,* a number of proteins required for RNA transport has been discovered.

From our previous studies, we know that the SID-5 protein is required for cell-cell transport of RNAi silencing signals in *C. elegans* and that it localizes to late endosomes/multivesicular bodies (MVBs). Studies in *Drosophila* and mammalian cells have shown that MVBs are required for efficient cell autonomous RNAi whereas intact lysosomes seem to limit the RNAi efficiency. The co-localization of SID-5 and MVBs indicates that the RNAi transport pathway could also rely on endocytosis. However, the function of SID-5 in RNAi uptake and spreading is still unclear. We therefore performed a membrane yeast-two-hybrid screen, which identified several putative SID-5 interacting proteins. Current research is focused on further analysis of these proteins and their potential interactions with SID-5. We are also investigating whether SID-5 and its associated proteins affect microRNA-mediated silencing. Together, these experiments will give us clues to how endolysosomal compartments regulate small RNA pathways and transport of RNA between cells in animals.

Members of the group 2013
Andrea Hinas (group leader)
Yani Zhao (PhD student)
Benjamin Holmgren (PhD student)
Dirui Li (postdoctoral fellow)

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THE CONSERVED SNARE PROTEIN SEC-22 IS A NEGATIVE REGULATOR OF RNA INTERFERENCE IN C. ELEGANS

Yani Zhao, Benjamin Holmgren

As described above, the endolysosomal machinery has proven important for regulation of silencing by RNAi and microRNA pathways, both within cells and for transport of RNA molecules between cells. Soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins are key effectors in vesicle fusion and thus in intracellular trafficking and exocytosis. In spite of this, no SNARE protein has thus far been reported to affect RNAi or RNA transport. In a yeast-two-hybrid screen using the *C. elegans* RNA transport protein SID-5 as a bait we identified the highly conserved SNARE protein SEC-22 as a potential SID-5 interactor. Interestingly, and in contrast to SID-5, we could show that loss of SEC-22 results in more efficient RNAi whereas overexpression inhibits RNAi. Genetic analysis demonstrated that the enhanced RNAi phenotype of *sec-22* mutants requires *sid-5*, suggesting that SEC-22 acts at least in part through SID-5. To our knowledge, SEC-22 is the first SNARE protein with demonstrated function in RNAi and the first example of a protein that inhibits intercellular transport of RNAi silencing signals. We are currently investigating the subcellular localization of SEC-22 as well as whether it affects cell autonomous RNAi and/or intercellular RNA transport.

QUANTITATIVE MICROSCOPY AS A TOOL TO IDENTIFY GENES AFFECTING RNAI EFFICIENCY

Benjamin Holmgren

In addition to SEC-22 (see above), a number of potential SID-5 interacting proteins were identified in our yeast-two-hybrid screen. To quickly identify proteins that affect RNAi silencing efficiency, we deviced a two-step silencing method followed by quantitative microscopy. With this method, a gene of interest – in this case from the yeast-two-hybrid screen – is first silenced by RNAi. Then, a transgene expressing green fluorescent protein (GFP) is targeted by RNAi. Finally, the GFP expression level is quantified using fluorescence microscopy and the CellProfiler software. Using this approach, we have so far found three (out of 15 tested) putative SID-5 interacting proteins that are required for efficient RNAi. These three proteins are now being studied in more detail regarding localization, genetic interaction with *sid-5* etc. We will also test the remaining ten candidates from the yeast-two-hybrid screen using the quantitative microscopy approach.

FUNCTION OF THE ENDOSOME-ASSOCIATED PROTEIN SID-5 IN MICRORNA-MEDIATED SILENCING

Dirui Li

As shown previously in *Drosophila* and mammals, late endosomes/MVBs are also sites of micro (mi)RNA-mediated silencing. In addition to this cell-autonomous function, miRNAs are abundant in extracellular vesicles in humans. Therefore, we are interested in investigating the role of the SID-5 protein in miRNA silencing in *C. elegans*. We are approaching this by crossing a *sid-5* deletion allele into two different genetically sensitized strains, a method that has proven useful to identify proteins involved in miRNA processing or function. One of these sensitized strains lacks two miRNAs (miR-241 and miR-48) from the let-7 family, resulting in aberrant development of epithelial seam cells, whereas the other one carries a partial loss of function allele affecting the miRNA lsy-6, leading to about 20% loss in left-right symmetry of the sensory ASE neurons. By looking for enhanced phenotypes when genes of interest (in this case *sid-5*) are knocked down/mutated, both strains can be used to identify proteins that promote miRNA function. In addition, the lsy-6 strain can be used to identify proteins that inhibit miRNA function, by looking for suppression of the 20% asymmetry phenotype. This is a collaborative project with the group of Prof. Victor Ambros, University of Massachusetts, Worcester, USA. If *sid-5* is found to enhance or suppress the miRNA phenotypes in the sensitized strains, we will continue analysis by standard methods, for example tissue-specific rescue of SID-5 to determine whether the observed phenotypes result from non-cell autonomous function of miRNAs or from a previously uncharacterized cell-autonomous effect of *sid-5*.

BACTERIAL RESPONSES TO STRESS AND SELECTION

Diarmaid Hughes

Our main research interest is in bacterial genetics and evolution, specifically where it concerns the development of resistance to antibiotics and bacterial microevolution. 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. In this context we must look beyond just aiming for discovering new drugs or drug targets. It is critical to develop approaches to maintain and increase the therapeutic effectiveness of drugs already available.

We are studying the development of resistance to antimicrobial drugs, with a particular focus on the fluoroquinolones and rifampicin. Particular questions include how resistance development impacts on bacterial fitness in different environments and how bacteria respond to growth inhibition by compensatory evolution. The step-wise nature of antibiotic resistance evolution, and the co-evolution of resistance to multiple antibiotics are being studied. 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.

We also study bacterial microevolution in response to growth limitation caused either by genetic defects or physiological limitations imposed by the growth environment. These studies relate bacterial genetics and growth physiology with transcription, translation and gene expression regulation. Among the specific questions are 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. In addition we have a long-standing interest in bacterial mutation rates and the mechanisms and rates of gene conversion and genome rearrangements associated with repetitive sequences.

Members of the group during 2013
Diarmaid Hughes, Professor
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Cao Sha, Postdoc (on parental leave most of 2013)
Jessica Bergman, PhD student
Gerrit Brandis, PhD student
Eva Garmendia, PhD student
Franziska Pietsch, PhD student
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Project workers during 2013
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Dina El Khalifa (Research practice, Autumn term)
Yinyu Wu (Research practice, Autumn term)

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TRANSLATION FACTOR EF-Tu AND GROWTH REGULATION

Jessica Bergman and Disa Hammarlöf

This project concerns growth regulation in *Salmonella enterica* serovar Typhimurium, with a focus on starvation responses. As a model system we are using a strain with a mutation in *tufA*, the gene for the translation elongation factor EF-Tu. This mutant EF-Tu is a weak binder of aminoacyl-tRNA. Bacteria that rely on a single, mutant *tuf* gene show an extreme slow growing phenotype. Using a combination of a screen for multi-copy suppressors of this slow growth, mutational studies, and biochemical assays, we discovered that the mutant bacteria overproduce the global transcriptional regulator ppGpp. ppGpp is known to accumulate during to starvation, more specifically as a response to an uncharged tRNA in the ribosomal A-site. We have confirmed that all tRNA isoacceptors for at least one amino acid have a lower level of aminoacylation in the slow-growing strain than in the wild-type, thus providing an explanation for the increased levels of ppGpp. This suggests that the mutant bacteria experience constant starvation even when they are grown in rich medium. During real starvation, ppGpp is produced and used to shift transcription towards biosynthetic operons and away from production of ribosomal proteins and ribosomal RNA. This is also what happens in the slow growing *tuf* mutant, but instead of surviving real starvation it gets trapped in a vicious circle where the mutant bacteria produce less EF-Tu, experience reduced translation activity, and grow extremely slowly. By introducing genetic alterations affecting ppGpp production we can break this vicious circle and re-direct transcription activity so that it is more appropriate for a rich medium. This increases the levels of EF-Tu in the cell and also the probability of successful translation elongation, and improves growth rate.

EXTRAGENIC SUPPRESSORS OF RNase E MUTANTS

Disa Hammarlöf and Jessica Bergman

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.

IMPORTANCE OF ACETATE UTILIZATION FOR THE GROWTH OF MUTANT SUB-POPULATIONS ON AGING BACTERIAL COLONIES

Marie Wrande and Jessica Bergman

Spontaneous mutations in either *rpoB* or *rpoS* were found to confer a significant growth advantage to bacteria in aging wild-type colonies of *Salmonella enterica*. We have constructed a series of isogenic strains carrying mutations in *rpoB*, *rpoS* or both genes, and tested these for growth advantage in the context of colonies that are predominantly made of wild-type cells and find that the mutants have a very large growth advantage on aging wild-type colonies. We asked if acetate, which is excreted during exponential growth on rich medium and re-imported during the transition to stationary phase, played any role in the continued growth of these mutants. By combining the mutations in *rpoB* and *rpoS* with mutations important for acetate incorporation and utilization we are testing this hypothesis. The tests are carried out as growth competitions on aging colonies, by radioactive tracking of acetate uptake and by proteome analysis of wild-type as well as *rpoB* and *rpoS* mutants.

COMPENSATORY EVOLUTION AND RIFAMPICIN RESISTANCE

Gerrit Brandis

Multiple drug resistant *Mycobacterium tuberculosis* is causing serious problems in the treatment of tuberculosis. Many mutations in the ß subunit of the RNA polymerase are known to cause resistance to rifampicin, a first line drug against tuberculosis, but only little is known about the compensation of the fitness loss due to these mutations. In a previous study we have evolved a *Salmonella enterica* serovar Typhimurium strain harbouring a rifampicin resistance mutation, which causes a very strong fitness cost, to increased growth rate and compensatory mutations have been identified within the -subunit, the ß-subunit, and the ß'-subunit of RNA polymerase. Due to its high fitness cost this particular mutation is not clinically relevant and has not been observed in clinical rifampicin resistant *M. tuberculosis* isolates. In the present study a *Salmonella* strain harbouring the rifampicin resistance mutation *rpoB* S531L, which is found in up to 75% of all clinical rifampicin resistance *M. tuberculosis* isolates, was evolved by serial passage to increased growth rate. Compensatory mutations have been identified in the same regions as previously observed, showing the importance of these types of compensatory mutations in clinical tuberculosis cases.

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.

EFFECTS OF CODON USAGE BIAS ON BACTERIAL GROWTH RATE

Gerrit Brandis

All organisms utilize a universal genetic code in the translation of genetic information from messenger RNA into proteins. The genetic code is a triplet code with combinations of four different nucleotides. Thus, there are 64 different triplet combinations. These 64 codons encode only 20 different amino acids, which means that the genetic code is redundant. Despite the fact that the codon usage has no effect on the sequence of the translated protein bacteria have developed a strong bias towards the usage of certain codons over others. This codon bias is especially strong in highly expressed genes. The *tufA* and *tufB* genes, encoding for elongation factor EF-Tu, are among the most highly expressed genes in *Salmonella* and have an extreme codon usage bias. Growth rate and translational accuracy are very sensitive to changes in the concentration or activity of EF-Tu, which makes the *tuf* genes a perfect candidate to study the effects of codon usage. In this project the codon usage of the tuf genes in *Salmonella* will be altered to a less biased version. The physiological consequences of changing the codon usage will be assessed and the constructed strains will be experimentally evolved to improve fitness. Consequently fitness improving mutations will be determined and phenotypic effects of single mutations assessed. The results hopefully help to understand the physiological significance of codon usage bias in highly expressed genes and how rapidly codon usage bias evolves under selection.

AMELIORATION MECHANISMS OF 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. They found that the serial passage of *Escherichia coli* strains carrying classical drug-resistance plasmids reduced the cost of the plasmid; furthermore, they were able to show that the serial passage 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.

IMPORTANCE OF LOCATION AND ORIENTATION OF HIGHLY EXPRESSED 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 the DNA is replicated. The current hypothesis is that genomic-scale organization reflects selection pressure for maximum growth rate, by increasing the relative copy number of highly transcribed genes and minimizing the frequency of clashes between DNA and RNA polymerases. This study focuses on testing the significance of position and orientation of a gene whose product is directly liked to growth rate, the elongation factor EF-Tu. EF-Tu is, under normal circumstances, the single most abundant cytoplasmic protein in both *Escherichia coli* and *Salmonella typhimurium*. The gene encoding EF-Tu 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 those factors and how this relates to position and orientation. Evolution experiments will then address whether sub-optimal location and/or orientation can be compensated and by which mechanisms.

RNA POLYMERASE MUTATIONS ARE SELECTED DURING EVOLUTION OF CIPROFLOXACIN RESISTANCE IN *E. COLI*

Franziska Pietsch

E. coli is naturally sensitive to the DNA gyrase inhibitor ciprofloxacin. There is no single mutation or genetic resistance determinant known that can raise the level of resistance above the clinical breakpoint. Hence, resistance towards ciprofloxacin arises through several mutational steps. To study the development of resistance to ciprofloxacin, independent lineages of the laboratory strain MG1655 were cycled in progressively higher concentration of ciprofloxacin and a number of end-point strains were analyzed by whole genome sequencing. In addition to mutations in known resistance-associated genes, we also found mutations in *rpoB* and *rpoC*, coding for the β –and β'-subunit of RNA polymerase at high frequencies. This suggests that mutations affecting RNA polymerase arise commonly under these selective conditions. To determine the order in which the successive mutations had occurred in the genetic evolution of resistance, clones of earlier cycling steps were locally sequenced for the mutations found in their according end-point strain. Based on this knowledge, strains representing the successive steps in the resistance evolution were constructed and characterized for susceptibility to ciprofloxacin and their impact on the bacterial fitness. In order to better quantify the selective advantage conferred by the identified mutations, competition experiments between successive pairs of constructed strains were performed at different ciprofloxacin concentrations. Each of the successive mutations in each lineage provided a competitive advantage relative to the previous genotype, as a function of increasing drug concentration.

FITNESS COSTS AND COMPENSATION IN FLUOROQUINOLONE RESISTANCE DEVELOPMENT

Lisa Praski and Douglas Huseby

Fluoroquinolones are synthetic broad-spectrum antibiotics frequently prescribed to treat UTIs, gastrointestinal infections, respiratory tract infections, sexually transmitted diseases, and skin and bone related infections. Resistance to fluoroquinolones in pathogens can arise through mutations that affect the targets of fluoroquinolones (gyrA, gyrB, parC, parE); mutations that affect the transcriptional repressors of the AcrAB-TolC multi-drug efflux system (marR, acrR); or acquisition through horizontal transfer of genes conferring a protective effect on the antibiotic targets (qnr). Many of these resistance-conferring mutations have a fitness cost to strains carrying them. We have experimentally evolved lineages of *Escherichia coli* that are highly resistant to the fluoroquinolone antibiotic ciprofloxacin by subjecting strains to increasing concentrations of the antibiotic over hundreds of generations of growth. The resulting highly antibiotic resistant strains have been subjected to whole-genome sequencing. In addition to mutations that are known to confer antibiotic resistance, a variety of other mutations were also observed in these strains. During the process of these laboratory evolution experiments, it is expected that there will be selection for both mutations that enhance resistance to the antibiotic selected against, and mutations that compensate for the fitness cost of previously generated antibiotic resistance mutations. Using these experimentally evolved strains as test cases, we will retrace the mutational steps that led to the final, highly-antibiotic resistant strains. By determining the contribution of mutations that are not in genes that are known mutational targets for fluoroquinolone resistance, whether directly to the antibiotic resistance or to the strains' ability to mitigate the fitness cost of harboring antibiotic resistance mutations, we hope to identify and characterize the contribution of mutations in genes outside of the standard set typically assayed. These genes may prove relevant for the clinical evolution of highly-antibiotic resistant bacteria.

OSMOTIC EFFECTS ON SMALL COLONY VARIANTS (SCV) OF STAPHYLOCOCCUS AUREUS

Cao Sha

SCVs of *S. aureus* are associated with reduced susceptibility to aminoglycosides and enhanced persistence in mammalian cells. In the previous work we showed that FusE SCVs comprised three sub-groups. All FusE SCVs have mutations in *rplF*. The FusE-hem and FusE-men sub-group carry in addition mutations in genes coding for hemin or menadione biosynthesis, respectively. When these SCVs were selected for faster growth mutants, in most cases, they acquired compensatory mutations (or reversions) in the *rplF*, *hem*, or *men* genes. However, in some exceptional cases the original SCV-mutations were retained unchanged even though the growth rate was significantly improved. By whole genome sequencing, we identified mutations in four different genes among these growth-compensated SCV mutants. Each of the four genes could be associated by database annotation with transport across the cell membrane, and some were predicted to affect the accumulation of osmoprotectants. The addition of osmoprotectant chemicals to the growth media significantly improved the growth rate of several of the SCV mutants. To confirm and test these results, a new collection of SCVs was selected for aminoglycoside resistance. This collection of mutants is currently undergoing testing for osmosensitivity-related phenotypes. Considering the function of

osmoprotectants, we propose that many SCVs are hypersensitive to osmotic stress and have a different optimum to the wild-type strain. These phenotypes could be relevant to the ability of SCVs to survive and grow in particular human microenvironments.

STRATEGIES TO REDUCE ANTIBIOTIC RESISTANCE DEVELOPMENT

Ulrika Lustig and Cao Sha

Antibiotic resistance in clinical settings and the decline of antibiotic drug development is an increasing problem. The volume and pattern of antibiotic use influences the rate of resistance development and the idea is that dosing strategies in clinical settings can be optimized such as to minimize the emergence of antibiotic resistance while still maintaining efficacy. In order to collect *in vitro* data of bacterial growth rates and killing at different concentrations of antibiotics, we perform time-kill experiments on susceptible and well-characterized antibiotic resistant mutants of *E. coli*. By using the time killing data of MG1655 (a well-characterized laboratory wild type strain), an *in silico* model was developed. The model has been tested on 11 isogenic laboratory strains carrying mutations relevant to clinical ciprofloxacin-resistance. We also study how bacterial inoculum size, growth phase, and medium, affect the rate of bacterial killing by antibiotics. Ciprofloxacin time-kill experiments were also conducted on a set of clinical urinary tract infection (UTI) isolates. Data from these experiments were used to test the relevance of the extensive experiments on laboratory strains, and to further develop the *in silico* model. The *in silico* modeling is a tool to support predictions on how to dose one or several antibiotics in combination to optimize the effectiveness of therapy. The models can also be used to forecast the resistance potential of new drug candidates. This project is made as a collaboration beetween the groups of Diarmaid Hughes and Dan Andersson (IMBIM), Lena Friberg and Mats Karlsson (FarmBio) and Otto Cars (Med Sci).

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 CRISPR-based targeting of resistance genes be a way to eliminate resistance plasmids 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 be accelerated 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 aim to explore a novel system 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.

Publications 2011 to 2013

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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. Furthermore, we find two regions on the plasmid matching chromosomal genes, one from *E. coli* and one from *Ralstonia spp*., indicating that mobilization of genes from several different bacterial species has occurred. 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 are currently looking into how the plasmid has changed during the outbreak and what factors influences plasmid stability and loss/gain of genes on the plasmid.

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

Erik Gullberg, Christoffer Karlsson, Amanda Sahlin

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 segregational stability properties of plasmids in different genetic backgrounds to understand why some plasmids are very stably inherited in one host even though they infer a fitness cost on the 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, that are needed to counter-select the fitness cost and balance the stability in the population.

CARBAPENEM RESISTANCE IN *E. COLI*

Marlen Adler, Mehreen Anjum

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. However there is a clear carbapenem-resistance seen for all mutations.

In collaboration with the Department of Medical Sciences at Uppsala University we have used an *in vitro* dynamic system to study the effect of ESBL-production in *E. coli* on the

effect of treatment with ertapenem. This system allowed us to mimic free drug concentrations that are achieved during the course of conventional ertapenem treatment. Mutants were detected in 55% of all experiments with ESBL-producers. Our experiments also showed that doubling of the conventional ertapenem dose would not prevent enrichment of these mutants. These findings may directly help to decide treatment regimes in order to minimize selection of resistant mutants.

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. pneumonia* 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 more selective steps to reach comparable resistance levels. 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, Mehreen Anjum

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, leading to 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 cost of carrying extra gene copies is 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. Very little is known about development of resistance against this new class of antibiotics but clinical resistance has been reported, mainly through efflux pumps.
We are looking at 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 or proteins that prevent binding of the antibiotic to the ribosome) can evolve to also provide resistance to tigecycline.
We find that in addition to the overexpression of AcrAB efflux system, spontaneous *E. coli* mutations in LPS biosynthesis also lead to reduced susceptibility to tigecycline. Both groups of mutations (efflux and LPS) come with a fitness cost and further *in vivo* experiments are needed to evaluate mutant ability to establish a successful infection. Additionally, the *in vitro* compensatory evolution experiments will show if the observed fitness cost can be alleviated.

SYNTHETIC CRISPR SYSTEMS TARGETING RESISTANCE GENES

Robin Hagblom, Erik Gullberg

The rapid development of antibiotic resistance in bacteria is one of the most serious current threats to human health, and this resistance is often spread through the exchange of mobile genetic elements such as plasmids. A CRISPR array (Clustered Regularly Interspaced Short Palindromic Repeats) along with CRISPR associated (cas) genes comprises the CRISPR/cas system, which is a form of bacterial adaptive immunity against mobile genetic elements such as conjugative plasmids and phages. The capacity of the CRISPR system to specifically and efficiently degrade DNA solely based on the spacer sequences can be used to inactivate any unwanted genetic element. Could this adaptive immune system of bacteria be reprogrammed to target antibiotic resistance genes? In this study, clinical plasmids such as the pUUH239.2 will be targeted using synthetic CRISPR arrays in the hope of protecting bacteria from the uptake of such a plasmid and thus preventing the spread of antibiotic resistance in a given bacterial population.

STUDY OF ANTIBIOTIC RESISTANT *E. COLI* IN MALLARDS (*ANAS PLATYRHYNCHOS*)

Ulrika Lustig, Marie Nykvist

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 0,43-43-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 and Björn Olsén (MedSci) and 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 2013

Göte Swedberg, associate professor
Nizar Enweji, PhD student
Catherine Lwanira, PhD student (Makerere University, Kampala, Uganda)
Lemu Golassa, PhD student (Addis Ababa University, Ethiopia)

Project workers during 2013

Abrar al-Azawi: Genetisk analys av resistensplasmiden pLMO20
Salima Bellamine: HPPK-DHPS som målenzym vid behandling av malaria
Asha Kayima: Prevalence of G6PD mutation in a population of children in the Iganga region, Uganda
John Samuelsson: Pseudomonas putida och dess förmåga att använda sulfonamider som kolkälla
Matilda Stureson: Prevalence of the sickle cell trait in the population of Iganga, a malaria endemic district in eastern Uganda
Lovisa Andersson: Genetisk kartläggning av plasmiden pLMO20 med kanamycinresistens
Ali Abdulwahab: Hög prevalens av *Pfmdr1* vildtypssekvens i isolat från asymptomatiska och symptomatiska *P. falciparum* bärare i södra och centrala Oromia, Etiopien
Toqa Jasem: Kloning av genen *folT* från *Streptococcus mutans*
Omar Kamil: Screening studie om förekomst av resistensmarkörer för klorokin i Etiopien
Ravin Selo: Genetisk polymorfism hos *Plasmodium vivax* i Etiopien
Katia Soria Vasquez: Analys av mitokondrie-DNA för att påvisa asymptomatiska bärare av *Plasmodium vivax*
Angel Choy: Variation of genetic markers for chloroquine resistance in *Plasmodium falciparum* in Tanzania

International exchange during 2013
Lemu Golassa, Addis Ababa University, Ethiopia, worked in the lab Feb-May.
Erasmus Kamugisha and Karol Marwa, Mwanza, Tanzania, worked in the lab Sep-Oct.
Göte Swedberg visited partners in Uganda and Ethiopia November 18-30.

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Agencies that support the work
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Indevelop
VR (Swedish Research Links)

RESISTANCE TO ANTIMALARIAL DRUGS AND EVALUATION OF NEW DRUG TARGETS

Catherine Lwanira, 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.

EVOLUTION OF DRUG RESISTANT PLASMODIUM FALCIPARUM IN EASTERN SUDAN

Nizar Enweji

The project addresses two related questions:
1) Are drug resistant *Plasmodium falciparum* genotypes in Sudan independent lineages or similar to those originally selected in south east Asia and later appearing in east and southern Africa?
2) Do mutant *P. falciparum* genotypes that persist during the lengthy dry and anti-malarial drug free period have lower fitness (ability to multiply and produce transmissible stages), compare to drug sensitive ones?

So far a number of patients with PCR positive samples from a full year has been recovered and they will form the basis for continued analysis of stability of parasite markers. Remarkable variation has been seen in samples collected during the dry season, showing that parasites in asymptomatic carriers are not just resting, but constantly growing without causing malaria symptoms. Some carriers show the same parasites each month, and our interpretation is that these individuals have just a single clone infection, while those showing variation have multiple clones. Most focus has been on investigating microsatellite markers, which are independent of drug pressure, but show the variety of parasite clones present in the area. With the help of these markers, we can see different patterns of clone fluctuation throughout the dry period in the asymptomatic carriers.

MOLECULAR VIROLOGY AND VIRAL ZOONOSES

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

Most human infections are zoonotic, which means that they can cross species barriers and pass from animals to humans, or vice versa. A large new area of research at our Department is focused on zoonotic viruses where we use an interdisciplinary approach based on molecular virology, immunology, genetics, molecular epidemiology and virus diagnostics to study viral zoonoses. The present work is focused on several medically important virus families like hantaviruses, flaviviruses, Sinbis virus, Rift Valley fever virus and avian influenza virus.

In a second line of research 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.

ADENOVIRUS IN BASIC AND MEDICAL RESEARCH

Göran Akusjärvi, Tanel Punga, 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, 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 remodeling of the host cell transcription and RNA splicing machinery during an adenovirus infection
- The structure and function of adenoviral miRNAs
- Establishment and maintenance of long-term persistent/latent adenovirus infections
- Biochemical functions of the histone-like adenoviral core proteins
- Novel functions of the adenoviral E1B oncoproteins
- Adenovirus control of Alu RNA expression and cholesterol metabolism
- Non-coding RNA mediated chromatin modification
- Function of exosomes in adenovirus infections
- Viral vectors in cancer therapy
- Gene regulation in neurological disease Friedreich’s Ataxia (FRDA)

Members of the groups during 2013
Göran Akusjärvi, professor, group leader
Göran Magnusson, professor emeritus
Tanel Punga, assistant professor, group leader
Daniel Öberg, researcher, group leader
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Helen Bergquist post doc (from July)
Roberta Biasiotto, post doc
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Alexis Fuentes, researcher (from June)
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Sara Östberg, PhD student

Project workers during 2013
Tereza Brachtlova (7 months)
Sebastian Kapell (7 months)
Kwang-Chol Mun (2 months)
Johan Paulsson (4 months)
Julia Pickl (3 months)

Maria Leonor Segurado Gouveia (6 months)

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MAPPING OF THE DNA-PK AND PKA PHOSPHORYLATION SITES IN THE PHOSPHORYLAYION OF THE ADENOVIRUS L4-33K SPLICING ENHANCER PROTEIN

Mohammad Feraz Ahsan

The late genes of adenovirus are transcribed from the major late transcription unit (MLTU), giving rise to five different families of mRNA ranging from L1 to L5. The L4-33K protein is a virus encoded RNA splicing factor required to activate the early to late switch in adenovirus major late L1 alternative splicing. The L1-52,55K mRNA is produced both early and late after infection, whereas the L1-IIIa mRNA is restricted to the late phase of infection. The activation of splicing takes place in transcripts with a weak 3’ splice site context. The L4-33K protein plays a key role in spliceosome assembly and is sufficient to convert nuclear extract prepared form uninfected HeLa cells to extracts with splicing properties almost identical to that of nuclear extracts prepared from infected cells.

We have previously shown that two cellular protein kinases, DNA-PK and PKA, phosphorylates the L4-33K protein and have opposite effects on L1 alternative RNA splicing. Thus, DNA-PK phosphorylation has an inhibitory effect whereas PKA phosphorylation has an enhancer effect on L1-IIIa splicing. Collectively, our results suggest a possible regulatory role of reversible L4-33K protein phosphorylation as a regulator of adenovirus alternative RNA splicing in vivo. The aims of the project is to identify the residues phosphorylated by the two regulatory protein kinases, DNA-PK and PKA, and establish their relevance for L1 alternative RNA splicing during a lytic adenovirus infection.

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.

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

Roberta Biasiotto

Human adenoviruses (Ad) have a double strand linear DNA genome, encoding for several early, intermediate and late proteins, subjected to a fine-tuned regulation at the transcriptional and post-transcriptional level, through differential polyadenylation and alternative RNA splicing.

The late protein L4-22K was previously shown to play a key role in the regulation of late viral gene expression: it stimulates transcription from the major late promoter by a feed-forward mechanism, regulates late gene expression and is specifically involved as an enhancer of L4-33K pre-mRNA splicing. Also, L4-22K has been implicated in the control of early gene expression and has been shown to be required for the packaging of Ad genome during virus assembly.

The aim of the present project is to characterize the potential role of the L4-22K protein as a regulator of alternative splicing of the early gene E1A, a transcriptional activator responsible for the activation of the other viral early during a lytic infection. By alternative splicing, the primary E1A transcript produces 5 mRNA species (13S, 12S, 11S, 10S, and 9S) that are differentially expressed during the infection cycle.

By using a semi-quantitative PCR assay we demonstrate that 10S transcript expression specifically increases in the presence of L4-22K. Preliminary experiments performed with L4-22K deletion mutants and L4-22K of different Ad serotypes suggests that the conserved C-terminal domain of L4-22K is essential for this stimulatory effect on 10S mRNA splicing. By testing E1A mutants for the different splicing sites and for the promoter region and by performing in vitro splicing assays we aim to elucidate the mechanism through which L4-22K regulates E1A expression.

MOLECULAR MECHANISMS AND EPIGENETIC REGULATION OF ADENOVIRUS GENOME STRUCTURE IN PERSISTENT INFECTION

Sibel Ciftci

Human adenoviruses (Ad) generally cause lytic infection in gastrointestinal tract, mucoepithelial cells in respiratory tract and in cornea. However, early evidences have shown that adenoviruses, subgroup C in particular, can also establish persistent infection mainly in lymphocytes of the human tonsils and adenoids. With this atypical life cycle of adenovirus, it enters to quiescent stage following the primary infection and can be maintained as episomal in its host cell for extended time periods. However, due to the lack of reliable cell systems, very little progress has been made on the molecular details in adenovirus persistent infection. Therefore, I have reconstituted Ad5 persistent infection in BJAB cells (B lymphocytes). With this system, I have been dissecting molecular mechanisms that may play significant roles during long-term infection of adenoviruses. Furthermore, to understand the long-term maintenance of adenovirus genome, the potential molecular mechanisms that might be involved in epigenetic signaling are also elucidated in this project. Indeed, the preliminary data have shown that adenovirus distinctly targets and regulates several cellular pathways and use different strategies in order to persist to B cells. My ongoing study also aims to enlighten the regulatory mechanisms of the viral and host cell chromatin-remodeling during the persistent infection. The ultimate goal of this project is to provide a better understanding towards adenovirus persistent infection and the molecular mechanism behind it.

ADENOVIRUS PERSISTENT INFECTIONS

Alexis Fuentes

Persistent viral infections have a high prevalence in the human population; some are asymptomatic whereas others can cause disease or even death. A better understand of the mechanisms behind the establishment and maintenance of persistent infections is essential to curb the medical implications of persistency. Human adenoviruses, generally cause lytic infection in the gastrointestinal tract, epithelial cells in the respiratory tract and in the cornea. However, human adenovirus subgroup C can also establish persistent infections in lymphocytes of the human tonsils and adenoids.

We are using an established B-lymphocyte cell culture model system to study persistent adenovirus infections. The aim of our work is to understand the significance of the virus associated RNAs (VA I and II) in establishment and maintenance of persistent infections. We have previously speculated that VA RNAII has a critical function in persistence. This question will be addressed by using viral mutants defective in VA RNAI and/or VA RNAII expression. Further, we are investigating whether the low amount of viral particles that has to be produced to support the persistent infection are masked from immune recognition. For example, the virus, or the viral DNA, might spread in the lymphocyte population through exosomes, which are vesicles normally secreted from cells to shuttle cargo between cells in an organism.

CHARACTERIZATION OF VA RNA-DERIVED MIRNAS FROM DIFFERENT ADENOVIRUS SEROTYPES.

Wael Kamel and Anette Carlsson

VA RNAI is a 160 nucleotides long non-coding RNA, accumulating at high levels during the late phase of an adenovirus infection (approximately 10^8 copies 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, VA RNAI also suppresses the RNAi machinery in adenovirus-infected cells at three different levels. Firstly, it blocks the Exportin5 mediated nuclear export machinery, inhibiting the transport of per-microRNA to the cytoplasm. Secondly it it acts as a competitive substrate hijacking the Dicer enzyme to process the VA RNAI into small viral miRNAs (the so-called mivaRNAs). Finally these mivaRNAs saturates and block the RNA-Inducing Silencing Complexes (RISCs) with virus derived small RNAs. However the importance of the VA RNA derived mivaRNAs for the virus growth still remains large unresolved. It has been demonstrated that mivaRNAs incorporate into RISC and direct the cleavage of complementary targets both in vivo and in vitro. However, recently we showed that the significance of such interactions might be unessential during lytic adenovirus infection, as adenovirus with mutated mivaRNA seed sequences replicates as the wild type virus in different cell lines. So far all available information about the functional significance of the VA RNAs are obtained from Adenovirus group C. We are therefore extending our knowledge by investigating whether the VA RNAs from different adenovirus subgroups can be processed into small RNAs, which are capable of incorporating into RISC.

FUNCTIONAL CHARACTERIZATION OF THE ADENOVIRUS PVII PROTEIN

Raviteja Inturi

The adenovirus major core protein VII (VII) is a histone-like protein and is responsible for structural stability, functional organization and transcriptional regulation of viral DNA. It 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 synthesized from the precursor pVII (~21.8K) protein, by adenovirus protease proteolytic cleave during the final stage of virion maturation. The presence of precursor pVII and subsequent cleavage to form mature VII may be important for the functional and temporal regulation of adenovirus infection. As part of the study, we are characterizing the significant function of precursor pVII and mature pVII during a lytic adenovirus infection. We have identified specific residues of pVII and a cellular protein ubiquitin E3 ligase Cullin-3 regulating the protein stability of pVII. Our results clearly indicated the differences in stability and localization between the precursor and mature pVII proteins. Our ongoing goals were 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.

CHARACTERIZATION OF ADENOVIRUS L4-22K PROTEIN RESPONSIVE ELEMENTS IN THE MAJOR LATE PROMOTER

Xin Lan

The adenovirus L4-22K protein is multifunctional in different aspects of viral infection. In this project we focus on its effect on transcription from the major late promoter (MLP). Based on previous results, the main activating elements within the MLP are the so-called downstream elements (DE), the upstream element (UPE) and the CAAT box. We have also identified an intronic inhibitory element spanning the 5' splice site of the first tripartite leader segment. Mutations within this element enhances L4-22K activation of MLP transcription.
Recent data show that the L4-22K protein binds to this inhibitory element albeit with lower affinity compared to the DE element in gel shift assays. In this element, the L4-22K appears to bind to a 5'-TTTG-3' motif, which is a characterized L4-22K binding site in the packaging domain and the DE elements. Whether the L4-22K binds to the mRNA transcribed from this element will be studied (L4-22K binds to both the double-stranded and single-stranded DNA form of the element), which could further investigate the role of the L4-22K in regulation of viral gene expression at the posttranscriptional level (e.g. splicing).

Along with our previous results showing that the L4-22K induces the accumulation of 30-40 bp long pre-terminated RNA products of the MLP, the recent data indicate that the L4-22K can bind to this inhibitory element thus blocking the transcription elongation from the MLP.

REGULATION OF CHOLESTEROL CYTOCHROME P450 SUPERFAMILY MEMBER, CYP51A1, IN ADENOVIRUS INFECTED CELLS: THE ROLE OF A cis-ACTING ALU ELEMENT RNA

Julia Pickl, Wael Kamel, Sebastian Kapell

Alu elements are the most abundant repetitive DNA elements in the human genome, present in approximately 1.4 million copies. In general, Alu elements are transcriptionally silent. Under some stress conditions, such as an adenovirus infection, Alu RNA expression becomes stimulated. We have found that in Ad37-infected cells expression of a single unique Alu RNA, AluCYP51A1, is selectively enhanced resulting in an approximately 75% share of all Alu RNAs expressed in this infection. Our experiments further indicate that the accumulation of AluCYP51A1 controls expression of a key enzyme required for cholesterol biosynthesis in Ad37-infected cells. Since Ad37 is known to cause ocular inflammation and cholesterol is a vital constituent of cell membranes such a regulation suggests a potential connection between Ad37 regulation of Alu RNA expression and the development of serious eye infections in humans.

VIROTHERAPY AGAINST CANCER

Daniel Öberg

Adenovirus Onyx-015 was the pioneering agent in the field of cancer gene therapy using cancer selective vectors. This approach was based upon the fact that many tumour 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 its replication in cancer cells. As it turned out the viral E1B protein had several additional functions. This made the approach severely restricted in tumour targets and potency therein. The aim with my work is to decipher the intricate gene expression of adenovirus E1B 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. The lab is now verifying the oncoselectivity in several alternative models and also working on the initiation of 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 wanted to study whether the function of the two proteins also are conserved, and therefore set out to test 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.

VIRAL ZOONOSES

Åke Lundkvist, Karin Sundström, Tanja Strand, Jenny Verner-Carlsson

Viruses have been with us since ancient times. They will also be our "companions" in the future, for as we have been able to defeat some diseases, new ones 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, immunology, genetics, molecular epidemiology and diagnostic aspects of zoonoses, especially emerging zoonotic viruses. We are at present focusing on the following agents: hantaviruses, flaviviruses (TBE, Dengue and West Nile viruses), Sindbis virus, Rift Valley fever virus, and avian influenza virus.

Our hantavirus program has generated important results concerning novel animal models (monkey and rodents), vaccine candidates, virus-host interactions, pathogenesis, apoptosis 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. We have developed a number of new methods for identification and characterization of genetic markers responsible for infectivity/pathogenicity and new techniques for studies on how hantaviruses infect their rodent reservoirs.

Our research on TBE virus has focused on molecular epidemiology of the virus in the Nordic countries and in the Baltic states. The recent increase of clinical cases in Sweden encouraged us to investigate the mechanisms behind and to create hypotheses explaining such emergence. 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.

Dengue virus constitutes of four quite distinct virus types, dengue 1-4. Unique clinical virus strains from Cambodia, isolated from patients suffering of classical dengue, dengue hemorrhagic fever and dengue shock syndrome, respectively, have been characterized for phenotypic and genotypic differences in vitro and in vivo.

The awareness of highly pathogenic avian influenza virus 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 and dramatic changes in virulence. A similar project on West Nile virus has recently been initiated.

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ADENOVIRUS TYPE 12 INDUCED INTERFERON RESPONSE

Catharina Svensson

Adenovirus expresses a multitude of regulatory proteins to ensure efficient replication during a lytic virus infection. The immediate early E1A gene encodes two primary regulators, which are essential for transcriptional activation and forced entry of the host cell into the S-phase and for blocked induction of interferon (IFN) and IFN signalling. Despite many similarities, non-oncogenic and highly oncogenic adenovirus demonstrate differences in their productivity and cytopathic activity, where the highly oncogenic HAdV12 is less virulent and shows significantly less impact on host cell gene expression compared to the non-oncogenic HAdV2. We have shown that HAdV12 induces a specific activation of the IFN pathway during the later stage of infection. The inability of HAdV12 to completely evade the first line antiviral defence might explain the relatively low virulence of this virus compared to HAdV2, but an inadequate expropriation of the biosynthetic machinery of the host cell is also likely to play an important role. Preliminary results show a specific phosphorylation of PKR and a subsequent decrease in the accumulation of viral proteins. Our hypothesis is that the inability to counteract PKR activation is the result of poor expression of the HAd12V VA RNA.

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EFFICIENT PRODUCTION OF HUMAN ADENOVIRUS IN NORMAL MOUSE EPITHELIAL CELLS

Catharina Svensson and Staffan Johansson

The development of modified human adenovirus (HAdV) for oncotherapy is hampered by the lack of suitable immunocompetent mouse model systems where the oncolytic efficacies can be determined. The reason is that 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 has not been obtained. We have identified a non-transformed mouse cell line where the infection by HAdV2 is rapid and results in efficient production of new virus as determined by the ability of the recovered virus to superinfect standard human cell lines. Since there is a great interest for the possibility to use adenovirus vectors of less prevalence compared to type C HAdV, we have extended our analysis to members of all human HAdV types. Our results show that the here identified mouse cells also support growth of HAdV of types D and E, but not of types A, B or F. In this 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.

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DISSERTATIONS 2013

Anjum Riaz: Adhesion Dependent Signals: Cell survival, receptor crosstalk and mechanostimulation, April 12, 2013

Peder Fredlund Fuchs: Myofibroblasts and the vascular endothelium. Impact of fibrin degedration products and miRNA on vascular motility and function, May 3, 2013

Anna Eriksson: Syndecan - Regulation and function of its glycosaminoglycan chains, May 17, 2013

Joakim Dahlin: Mast Cell Progenitor Trafficking in Allergic Airway Inflammation October 17, 2013

Abhirami Ratnakumar: Detecting signatures of selection within the dog genome, December 4, 2013

LICENTIATE THESIS 2013

Ding Zhoujie: Antibody and complement in immune regulation, January 24, 2013

Sara Pijuan Galito: Regulation of pluripotency and self-renewal in pluripotent stem cells, June 10, 2013

Hava Lofton Tomenius: Bacterial Resistance to Antimicrobial Peptide: Mechanisms and Biological Costs, November, 15, 2013

ECONOMY
(kSEK)

	2012	**2013**
Undergraduate Education Grant	24.325	22.852
Faculty Grant	64.954	69.561
External Grants	72.563	80.723
Others	684	1.042
Total	**162.526**	**174.178**

PRIZES AND AWARDS 2013

1) The Göran Gustafsson Prize in Molecular Biology and Medicine

The Göran Gustafsson Prizes are awarded annually in the fields of mathematics, physics, chemistry, molecular biology and medicine by the Göran Gustafsson Foundation for Scientific and Medical Research.

Kerstin Lindblad-Toh received this prize 2013 for "her studies of mammalian genomes, which led to the identification of the parts of the genome that is functional."

2) The Olof Rudbeck Prize (Olof Rudbeckpriset in Swedish)

Leif Andersson was awarded this prize for his outstanding contributions in basic research that also has a clinical significance.

3) The Karl Johan Öbrink Lecturer Award

One Karl Johan Öbr+ink Lecturer Prize is awarded annually to an active researcher who works according to the tradition and spirit of Öbrink himself.

Kerstin Lindblad-Toh received this award 2013 for her outstanding work on dogs as a model system to study human disease. She upholds a dual position where she shares her time between her research groups at Uppsala University and the Broad Institute in Boston. She has also been instrumental in the development of the SciLife Uppsala laboratory at BMC. Taken together all this activities demonstrates that she truly embraces Öbrink's ideal of "integrated integrity".

4) The Limbic Prize (Limbiska priset in Swedish)

The students of the biomedical program has for a second time (2011 and 2013) awarded assistant professor **Linus Sandegren** the Limbic Prize for his dedication to teaching bacteriology and immunology.

From the Prize motivation: *"Linus Sandegren is a dedicated course leader and an inspiring lecturer who makes a brilliant effort for the students in the Biomedical program. He has, using both basic research related and clinical examples in his teaching, managed to couple together his research area with the focus of the Biomedical program. Only the best teacher gets comments on the course evaluation like "The course leader made the course the best course on the whole biomedical program!" and "Very interesting and fun lectures, I did not fall asleep even once!". The students on the biomedical program think this makes You a worthy recipient of the Limbiska priset 2013."*

UNDERGRADUATE TEACHING AT IMBIM

IMBIM has about 20 full professors and associate professors as well as 6 assistant professors and research fellows who contribute to the undergraduate teaching. Additionally, there are some 35 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 a master programme in Infection biology. In all of these, laboratory work is an important part and IMBIM has about 400 m^2 dedicated to 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 subject of a department - and the teachers come from different departments. Thus, teachers from IMBIM take part in courses covering topics like ”Energy and food stuff balance”, ”Homeostasis and endocrine regulation” and ”Attack and defense”. Teaching is done through regular lectures, study groups and practicals. 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. Some 100 students are enrolled in this programme every semester.

Pharmacy

This 5-year programme 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. IMBIM is responsible for the teaching of microbiology. In this programme 90 students are enrolled every semester.

Dispensing pharmacy

This 3-year programme leads to a Dispensing Pharmacist Degree which prepares the students for work in retail and hospital pharmacies. IMBIM is responsible for the teaching of microbiology. Some 40 students are enrolled every semester.

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, genetical 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 the teaching of biochemistry, cell biology and microbiology.

Biomedical Laboratory Sciences

This 3-year programme leads to a Bachelor of Medical Science (Major in Biomedical Laboratory Science) which 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

UGSBR

IMBIM manages a one year postgraduate programme allowing the students to rotate between different lab projects.

INFECTION BIOLOGY

IMBIM is in charge of a two year master programme in infection biology.

THE PhD PROGRAM AT IMBIM

During 2013 the department had 56 students registered for postgraduate studies. Five students defended their PhD theses and three students obtained a licentiate degree. New students are required to take a short introductory course in safety and general practice at the laboratory. In addition, 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 help new students with practical matter like help with employment, lodging and financial issues and good advice with what to expect from the department contra the responsibility of the student. IPhAB also organizes regular social events during the semesters to increase the interaction between students and employees at IMBIM. The department arranges several PhD courses that are aimed at broadening the knowledge of the research conducted at IMBIM. A monthly seminar series with the group leaders at IMBIM presenting their research area are arranged. After this lecture IPhAB arranges a social gathering with food to further stimulate interactions between PhD students and researchers working within the different disciplines at IMBIM. Further, four different weekly subgroup specific research seminars in Bacteriology, Genomics, Immunology, and Tumor Biology are arranged. These seminars give credit points in proportion to attendance. In summary, the PhD students have a collection of seminars within multiple disciplines to choose from which gives them a great opportunity to further expand their scientific expertise.

RESOURCE CENTERS 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 (UU), Örjan Carlborg (SLU)

The aim of the center, funded by Formas, is to establish a world-leading Centre that uses domestic animals 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 and regional resequencing of identified regions is ongoing. The candidate loci identify both previously known cancer genes but also new interesting pathways. 2) **Autoimmune and inflammatory disease**. 3) The **metabolic and cardiovascular disease** projects span a large set of disorders including models for muscle growth, diabetes and several cardiovascular projects in multiple species and 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.

PROTEOMICS RESOURCE CENTER

Åke Engström and Eva Andersson

This laboratory gives the scientific community an opportunity to at a low cost make use of recent developments in techniques and instrumentations for the analysis and identification of proteins. The facility for Expression Proteomics is equipped with 2-D electrophoresis systems, systems for post or pre-gel labeling of proteins, visible and UV light scanners, software for image analysis, spot picking systems, semi automated spot processing and MALDI-TOF/TOF instrumentation. The facility has expertise for 2-D analysis, mass spectrometry, image analysis, data base searches and general protein chemistry.

Our area of work is analysis and comparison of proteomes, identification of proteins in protein spots/bands by mass spectrometry, analysis of expressed proteins for quality control and analysis of proteins for post-translational modifications. The facility is open for all scales of problem solving or analysis, although the capacity for 2D gels might be a limiting factor for very large undertakings.

The service is primarily intended for identification of proteins from species with large numbers of genes or proteins characterized. The facility has in addition a limited capacity for de novo sequencing of proteins from any species. The service includes straightforward methods for characterization of expressed recombinant proteins. Considering the low cost for analysis this is highly recommended to avoid the potential risk of doing experiments with the "wrong" or modified protein. An MS analysis of intact expressed protein and a peptide mapping with MS give much better confidence than a simple SDS-gel analysis. If suitable for our techniques and knowledge we provide analysis of any type of sample

Organization

The expression proteomics facility is organized for running samples for researchers or for the researcher to use the equipment after approval. The latter is recommended for longer series of experiments. The facility is a part of the Proteomics platform at the Science for Life Laboratory in Uppsala.

Location

Equipment for handling and performing 2-D gel electrophoresis experiments and MALDI-Tof/Tof instrumentation are located at the Dept. of Medical Biochemistry and Microbiology, Biomedical Center (building D9 floor 3), Uppsala.

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UPPSALA GRADUATE SCHOOL IN BIOMEDICAL RESEARCH, UGSBR

Uppsala Graduate School in Biomedical Research (UGSBR) started in January 1997 as one of originally six local biomedical preparatory research schools initiated through support by the Foundation for Strategic Research (SSF). The school has as its prime objective to give university students, heading for a research education, a deeper knowledge about research and development, increasing possibilities to make active choices among the multitude of biomedical research fields and to establish productive national and international contacts.

As of Dec 2013, approximately 280 students have been accepted to UGSBR. The vast majority of UGSBR students have continued with PhD studies and of these, most have continued with their research career after receiving a PhD degree.

Management 2013:
Staffan Johansson, program director
Susanne Lundgren and Alexis Fuentes, program administrators

SCIENCE FOR LIFE LABORATORY IN UPPSALA

SciLifeLab is internationally competitive based on its combination of platforms for large-scale bioscience and applications of these resources to both large-scale and hypothesis driven smaller research projects. Since the 1st of July, SciLifeLab in Uppsala has merged with SciLifeLab in Stockholm in a National SciLifeLab Center.

In 2013 SciLifeLab Uppsala has continued to expand. The center in Uppsala now includes 850 people, more than 200 of which are platform staff (a ~25% increase in 2013). To accommodate this and a more interactive environment, a new building has been constructed and taken into use. The genomics and bioinformatics platforms have moved into the adjoining space to be near each other and the interactive space at the new ”hub” building.

Scientific Resources

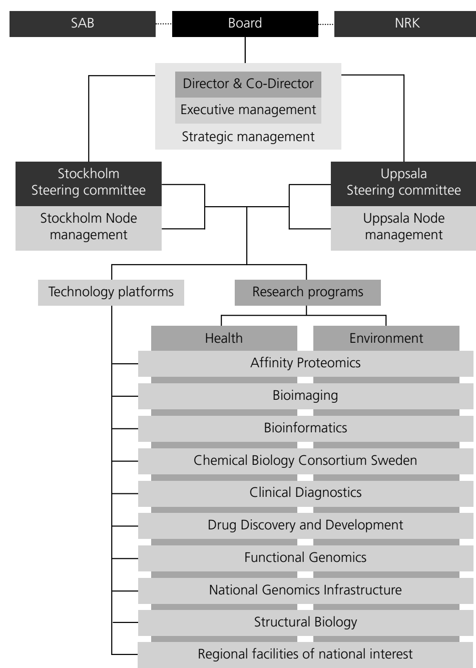
The technology platforms of SciLifeLab Uppsala have performed 1293 research projects, > 35% of which were led by a principal investigator from outside of UU. This is a substantial increase over the ~700 project performed in 2012, ~ 500 projects conducted in 2011 and ~300 in 2010.Furthermore, the projects have increased in size, with expanding numbers of samples analyzed per project. The largest platforms have been bioinformatics (521 projects) and genomics (328 projects). Other platform facilities have also performed a large number of projects and performed extensive training including for both external and internal users.

A Research Community generating research results of high international quality

The research community of SciLifeLab Uppsala has now surpassed 850 members and activities related to interactions with the clinic and industry continue to increase. In fact, the number of companies interacting with the SciLifeLab environment increased to more than 44 in 2013. The number of publications produced by the community as a whole surpassed 850 this year and external funding topped 440 million SEK. SciLifeLab Uppsala scientists have published 234 publications specifically citing SciLifeLab, an 80% increase from 2012. This includes 95 papers published in journals with an impact factor >6 and 15 with an impact factor >30 including Nature, Science and Nature Genetics. Highlights from these papers include 1) genome sequencing to find adaptations underpinning the domestication of dogs (Nature), 2) a better understanding of the genetic basis for obesity (Nature Genetics), 3) comparative genome sequencing and analysis of self fertilizing plants shedding light on how this trait influence their evolution (Nature Genetics), 4) new methods to sequence transcripts in histologically preserved cells (Nature Methods) and 5) tools to measure movements and binding of biomolecules in living single cells (Nature Methods).

Collaborations between academia and industry

SciLifeLab has continued to develop concepts for collaborations between academia and industry. The AIM Day concept has been further expanded and utilized in 2013 with AIMdays in ‘Diagnostics and Biomarkers’ and ‘CNS Disorders’ resulting in 6 new collaborations, possibly more. As an alternative method for fostering collaboration, UU Innovation and KI Innovation has developed a new concept ‘SciLife Innovation’, to generate new collaborative projects. A pilot with two projects shared between academia and industry has received funding from Vinnova.



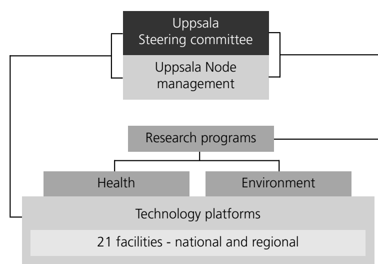


Fig. 1 and 2

Since 1st of July 2013, SciLifeLab in Uppsala is of one of two nodes of the National SciLifeLab. The Figures show the organization of the National SciLifeLab and the organization of the Uppsala node.

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