

WELCOME TO THE INTERNATIONAL SYMPOSIUM 'CELLULAR MECHANISMS OF DEVELOPMENT'

Developmental biology has become a science with mutually beneficial interactions with several other disciplines, such as genetics, cell biology, neurobiology and cancer biology. Its techniques offer ways to manipulate the genome and gene functions and follow the effects of these manipulations in the context of developing embryo – a stereotypic pattern of cell division, migration, differentiation and death. Recently, stem cells for various organs have been identified. Detailed knowledge of the mechanisms that guide these cells in their development can be expected to offer new avenues for therapy of human disease.

The current meeting 'Cellular Mechanisms of Development' brings together 250 people with various backgrounds to discuss recent advances in our knowledge of molecular and cellular mechanisms which drive embryonic development. Our invited speakers are leading scientists of multiple fields with diverse model organisms and with diverse research interests within the theme of the meeting.

Reflecting the wide scope of the meeting, it has been supported by many different organizations: Life 2000 Research Programme (Academy of Finland and Tekes), Helsinki Graduate School in Biotechnology and Molecular Biology, Viikki Graduate School in Biosciences, Helsinki Biomedical Graduate School and Neuroscience Center.

We want to thank all the participants and contributors and hope you will have an enjoyable and stimulating event in the new premises of Biomedicum Helsinki!

On behalf of the organizing committee

Juha Partanen

Seppo Vainio

PROGRAMME

Thursday May 8

13.00 Registration and poster set-up

KEYNOTE LECTURES

14.00 Opening of the meeting

14.10 **Eddy De Robertis** (*HHMI/UCLA, USA*): Extracellular regulation of BMP signaling in the *Xenopus* embryo

15.00 **Janet Rossant** (*Samuel Lunenfeld Research Institute and the University of Toronto, Canada*): Cell fate specification and axis patterning in the peri-implantation mouse embryo

15.50 Coffee break

SESSION 1: Cellular architecture and cell fate (*Chair: Tapio Heino*)

16.20 **Lynn Cooley** (*Yale University, USA*): Genetic and genomic analysis of *Drosophila* oogenesis: cell biology on parade

17.05 **Christos Samakovlis** (*University of Stockholm, Sweden*): Control of apical membrane growth and tube size by Bnl/FGF signalling

17.50 Break

18.00 **Tom Gridley** (*The Jackson Laboratory, USA*): Notch signaling in development and disease

18.45 **Gudrun Wahlström** (*University of Helsinki, Finland*): Non-muscle specific alpha-actinin localizes to ovarian ring canals in *Drosophila*

19.00 Get-together and refreshments

Friday May 9

SESSION 2: Cell adhesion and migration (*Chair: Hannu Sariola*)

9.00 **Lou Reichardt** (*UCSF, USA*): Adhesive signaling in brain development

9.45 **Heikki Rauvala** (*University of Helsinki, Finland*): Amphoterin (HMGB1) and amphoterin-induced genes: from development to disease

10.30 Coffee break

11.00 **Irma Thesleff** (*University of Helsinki, Finland*): Ectodysplasin signaling and the formation of ectodermal organs

11.45 **Ras Trokovic** (*University of Helsinki, Finland*): FGFR1 is independently required both in developing mid- and hindbrain for sustained response to isthmus signals

12.00 **Anna Popsueva** (*University of Helsinki, Finland*): GDNF promotes tubulogenesis of GFR α 1-expressing MDCK cells by Src-mediated phosphorylation of Met receptor tyrosine kinase

12.15 Lunch

SESSION 3: Nuclear control of development (Chair: *Juha Partanen*)

- 13.15 **Susan Mango** (*University of Utah, USA*): Eating disorders: Organogenesis of the *C. elegans* pharynx
- 14.00 **Sven Enerbäck** (*Göteborg University, Sweden*): Inner ears and sensorci ganglions - two sites for forkhead gene action
- 14.45 Coffee break
- 15.15 **Matthias Hammerschmidt** (*Max Planck Institut für Immunobiologie, Germany*): Bone morphogenetic proteins (Bmps) during dorsoventral (D-V) patterning of the zebrafish embryo
- 16.00 **Madis Jacobson** (*University of Helsinki, Finland*): Transcription factor GATA3 regulates GDNF signaling in migrating Wolffian duct cells
- 16.15 **Matti Poutanen** (*University of Turku, Finland*): Insulin-like factor 3 is an estrogen sensitive gene involved in gender independent gubernaculum development
- 16.30 **SESSION 4: Posters & refreshments**
- 18.00 Evening party at the Hall of Department of Otorhinolaryngology

Saturday May 10

SESSION 5: Intercellular communications (Chair: *Marjo Salminen*)

- 9.00 **David Wilkinson** (*National Institute of Medical Research, UK*): Patterning segments in the hindbrain
- 9.45 **Sarah Millar** (*University of Pennsylvania, USA*): WNT signals in hair follicle, tooth and mammary gland
- 10.30 Coffee break
- 11.00 **Annette Neubüser** (*Research Institute of Molecular Pathology, Austria*): The function of FGF signaling during vertebrate craniofacial development
- 11.45 **Abigail Tucker** (*King's College London, UK*): Development of the primary jaw joint
- 12.00 **Erika Gustafsson** (*University of Lund, Sweden*): Perlecan maintains microvessel integrity *in vivo* and modulates vascular formation *in vitro*
- 12.15 Lunch

SESSION 6: Proliferation and differentiation (Chair: *Kirsi Sainio*)

- 13.15 **Paul Martin** (*University College London, UK*): Morphogenesis and wound healing in embryos
- 14.00 **Saverio Bellusci** (*Childrens' Hospital LA, USA*): Fibroblast growth factor 10 plays multiple roles during mouse organogenesis
- 14.45 Coffee break
- 15.15 **Seppo Vainio** (*University of Oulu, Finland*): Cell signaling in the control of urogenital system development
- 16.00 **Manuel Selg** (*University of Lund, Sweden*): The embryoid body as a model system for lymphangiogenesis
- 16.15 **Marika Kärkkäinen** (*University of Helsinki, Finland*): Critical role of VEGF-C in lymphatic vascular development

GENERAL INFORMATION

REGISTRATION DESK

Registration desk at the entrance floor of Biomedicum Helsinki will be open and occupied at 12-4 p.m. on May 8, 2003 and 8.30-11.00 a.m. on May 9, 2003. At other times self service based registration is located next to the lecture hall 1 at the ground floor.

INSTRUCTIONS FOR POSTER PRESENTATIONS

Poster setup is possible from Thursday May 8, 2003 from 12 o'clock onwards. The poster session will be held on Friday May 9, at 4.30-6 p.m. We wish that the posters would be available until Saturday May 10, 3.15 p.m. The posters should be removed at the end of the meeting.

COFFEE BREAKS

Coffee is served at the ground floor near the lecture hall 1 at Biomedicum Helsinki and is free for conference attendants.

LUNCH

Lunch is not provided by conference organization. We advice conference attendents to have lunch at nearby restaurants (at Biomedicum and at Unicafe Meilahti (Haartmaninkatu 3))

PARTY INFO

On Thursday May 8, a get-together party is arranged immediately after Session 1 at the Biomedicum ground floor. On Friday May 9 an evening party takes place at the nearby 'Hall of Department of Otorhinolaryngology' at the same campus at 6 p.m. (right after poster session).

FIBROBLAST GROWTH FACTOR 10 PLAYS MULTIPLE ROLES DURING MOUSE ORGANOGENESIS

SAVERIO BELLUSCI

Developmental Biology Program, Childrens Hospital Los Angeles, 4650 W. Sunset Blvd, Room 804 SRT mail stop 35, Los Angeles, CA 90027, USA

Our research project focuses on the localization, migration and mechanism of differentiation of the smooth muscle progenitor cells during embryonic lung development. Fibroblast growth factor 10 is one of the earliest genes expressed in a regionalized manner in the distal mesenchyme of the embryonic lung. Our preliminary data indicate that a novel, but incompletely characterized, mouse transgenic line expressing LacZ under the transcriptional control of Fgf10 regulatory sequences can be used to follow Fgf10 expression at the cellular level during lung development. LacZ expression pattern in the developing lung suggests that a pool of Fgf10-positive cells in the distal mesenchyme can migrate toward the distal lung epithelium and differentiate into bronchial smooth muscle cells. We provide evidence that this new transgenic line can be used to generate allelic series to analyze the effect of decreasing Fgf10 expression on smooth muscle cell differentiation. We show that alpha-smooth muscle actin (SMA) expression around the bronchi is reduced in Fgf10LacZ⁻ compound heterozygote embryos. We finally show that over-expression of Bone Morphogenetic Protein 4 (Bmp4), a downstream epithelial target of FGF10, can induce ectopic expression of alpha-SMA in the mesenchyme.

GENETIC AND GENOMIC ANALYSIS OF *DROSOPHILA* OOGENESIS: CELL BIOLOGY ON PARADE

LYNN COOLEY

Yale University, USA

In many animals, the earliest steps of embryonic development are directed by maternal factors deposited within eggs during oogenesis. Studies of oogenesis in the fruit fly *Drosophila melanogaster* have provided extensive insight into the earliest steps of embryogenesis. In *Drosophila* nurse cells that are connected to each other and the oocyte by intercellular junctions called ring canals carry out the production of most maternal components. Ring canals are derived from arrested cleavage furrows in mitotic germline cells, and similar structures are present during the development of most animal gametes. Using a combination of forward and reverse genetics, our lab has identified and characterized several genes required in nurse cells for cytoplasm transport to the oocyte. These genes have begun to reveal key roles of specialized cytoskeletal structures and their regulation during oogenesis. For example, ring canals are rich in actin filaments and the dilation of the ring canal lumen during development requires dynamic regulation of filament cross-linking by the Kelch protein. In a new approach to discovering proteins required for oogenesis, we are carrying out a large-scale protein-trapping screen in which fusions between Green Fluorescent Protein and endogenous proteins are generated. The cellular and subcellular patterns of GFP-fusion protein expression provide instant insight into protein function. The expression patterns along with rapidly identified gene sequences give a powerful starting point for genetic and functional analyses of proteins used during development.

NEURAL INDUCTION IN *XENOPUS*

**EDDY M. DE ROBERTIS, HIROKI KURODA, BRUNO REVERSADE,
ATSUSHI IKEDA, LISE ZAKIN AND CATHERINE COFFINIER**

*Howard Hughes Medical Institute & Department of Biological Chemistry, University
of California, Los Angeles, CA, USA*

Dorsal-ventral patterning in vertebrate and invertebrate embryos is controlled by a system of interacting secreted proteins that include BMP, Chordin, Xolloid, Tolloid and Twisted gastrulation. Chordin, the molecule that generates the pattern is a BMP antagonist that contains four cysteine rich (CR) domains that bind to BMP, blocking its binding to the receptor. Many proteins contain CR-like repeats, and several of them have been found to regulate TGF-beta or BMP signalling. Examples include CTGF, Neuralin, Crossveinless-2, procollagen II and CRIM-1, all of which induce secondary axes in *Xenopus*. Experiments with an activated form of xTsg show that it is a very potent pro-BMP agent that functions as an inhibitor of multiple CR proteins. This indicates that CR-containing proteins play an essential role in the metabolism of BMP signaling.

Knockdown of Chordin in *Xenopus* caused mild ventralized phenotypes similar to those of the zebrafish chordino mutant. A strong requirement for Chordin in dorsal development was revealed by experimental embryology assays. First, dorsalization by lithium chloride treatment was completely blocked by Chd-MO. Second, Chd-MO inhibited elongation and muscle differentiation in Activin-treated animal caps. Third, Chd-MO completely blocked the induction of central nervous system (CNS), somites and notochord by organizer tissue transplanted to the ventral side of host embryos. Unexpectedly, transplantations into the dorsal side revealed a cell-autonomous requirement of Chordin for neural plate differentiation. This led us to analyze the early expression of Chordin at the blastula stage. Expression of Chordin in a novel signaling center, called the pre-organizer, is required for neural induction. The discovery of the blastula pre-organizer center helps explain the old problem of planar versus vertical signals in neural induction.

NOTCH SIGNALING DURING DEVELOPMENT AND DISEASE

TOM GRIDLEY

The Jackson Laboratory, Bar Harbor, Maine 04609, USA

The Notch signaling pathway is an evolutionarily conserved, intercellular signaling mechanism essential for proper embryonic development in organisms as diverse as insects, nematodes, echinoderms and mammals. Perturbations in the Notch signaling pathway contribute to the pathogenesis of several inherited human diseases, including Alagille syndrome, Spondylocostal dysostosis, and Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL). We have been studying the role of the Notch pathway in mice by creating targeted mutations in a number of genes encoding Notch pathway components. These studies have revealed essential roles for the Notch pathway in vascular and heart development, liver and kidney development, somitogenesis, and sensory cell differentiation.

We have recently shown, in collaboration with Dr. Hiroshi Hamada's laboratory, that the Notch signaling pathway plays a primary role in the establishment of left-right asymmetry in mice by directly regulating expression of the Nodal gene. Embryos mutant for the Notch ligand Dll1 or doubly mutant for the Notch1 and Notch2 receptors exhibit multiple defects in left-right asymmetry. Notably, Dll1^{-/-} embryos do not express the Nodal gene in the region around the node. Analysis of the enhancer regulating node-specific Nodal expression (termed the NDE) revealed the presence of binding sites for the RBP-J protein. Mutation of these sites destroyed the ability of the NDE to direct node-specific gene expression in transgenic mice. These results demonstrate that Dll1-mediated Notch signaling is essential for generation of left-right asymmetry, and that the Notch pathway acts upstream of Nodal expression during left-right asymmetry determination in mice.

BONE MORPHOGENETIC PROTEINS (BMPS) DURING DORSOVENTRAL (D-V) PATTERNING OF THE ZEBRAFISH EMBRYO

MATTHIAS HAMMERSCHMIDT AND JEROEN BAKKERS

MPI für Immunbiologie, Stübeweg 51, 79108 Freiburg, Germany

Based on mutants isolated in large scale screens, Bmp2b and Bmp7, their type I receptor Alk8, the downstream transcription factor Smad5, and the secreted Bmp antagonist Chordin were identified as essential components regulating zebrafish D-V patterning. Analyses of mutant embryos suggest that D-V patterning occurs in three distinct phases. During the first two phases a D-V gradient of Bmp activity is set up, which in the third phase leads to differential cell fate specifications along the D-V axis.

Using a subtractive approach, we identified DNp63, a member of the p53/63/73 family of tumor suppressors, as a direct transcriptional target of Bmp2b/7 signaling. DNp63 acts as a transcriptional repressor, mediating the effect of Bmp signaling during dorsoventral patterning of the ectoderm to block neural development in the presumptive epidermal ectoderm. Comparing this early role with the later role of DNp63 to promote the maintenance of skin stem cells, we suggest that Bmps and DNp63 have a similar, specification-blocking function in the non-neural ectoderm of the gastrulating embryo.

In addition to cell fate determination, Bmps have been shown to regulate differential cell movement behavior along the D-V axis. Here, we dissect the two roles, showing that Hyaluronan synthase 2 (Has2) interacts with Bmps during cell movements, but not during dorsoventral patterning. Loss of Has2 function leads to a block of dorsal convergence as in *bmp* mutants, however, in contrast to *bmp* mutants, *has2* morphants are not dorsalized. In contrast to DNp63, Has2 does not act downstream of Bmps. Rather, Has2 and Bmps act in parallel, with Has2 regulating the migratory potential of cells, and Bmps determining the direction of the migration.

EATING DISORDERS: FORMATION OF THE *C. ELEGANS* FOREGUT

JEB GAUDET¹, JIM KENT² AND SUSAN E. MANGO¹

¹*Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, 84112 and*

²*Genome Bioinformatics Group, University of California at Santa Cruz, 95064, USA*

How do embryos form complex organs such as liver or eyes? We use a simple animal, *C. elegans*, to understand how the foregut, or pharynx, is established during development. One goal of the lab is to decipher the transcription regulatory network that governs cascades of gene expression in the developing pharynx. Previously we demonstrated that many, perhaps all, genes selectively expressed in the pharynx were under direct control of the FoxA transcription factor PHA-4 (1). Moreover, the affinity of PHA-4 for its DNA binding site influenced the timing of the onset of expression. For example, mutation of a high affinity site to a low affinity site delayed the onset of expression, and vice versa (1). However, the affinity of PHA-4 for its DNA binding site does not predict that absolute timing of expression, suggesting that other transcription factors cooperate with PHA-4. We are using a genomics approach to discover the regulatory circuit that governs temporal control of pharyngeal expression. Our strategy is to i) identify genes selectively expressed in the pharynx using microarray, ii) cluster pharyngeally-expressed genes into subgroups according to their gene expression profiles, and iii) search the subgroups for potential cis regulatory elements using the Improbizer algorithm (2). Improbizer searches for motifs in DNA or RNA sequences that occur with improbable frequency using a variation of the expectation maximization algorithm. Using this strategy, we identified three control sites that match elements bound by known pharyngeal transcription factors and thirteen candidate new regulatory sequences. Our analysis of these elements has defined those that regulate early vs. late pharyngeal expression and allowed us to construct ‘rules’ for temporal regulation

MORPHOGENESIS AND WOUND HEALING IN EMBRYOS

PAUL MARTIN

University College London, UK

Embryos heal wounds very rapidly and efficiently and without leaving a scar. Studying how they do this can tell us much about the natural morphogenetic movements of embryogenesis as well as suggesting ways in which we might make adult tissues repair more efficiently. Using live confocal imaging of transgenic *Drosophila* embryos expressing *gfp-actin* in epithelial tissues we have revealed the key actin machineries that drive the paradigm morphogenetic process of dorsal closure which appears to bear striking analogy with re-epithelialisation of a vertebrate skin wound. Using embryos expressing mutant forms of the various small GTPases, we have tested the function of each of these actin-based elements - the actin cable and dynamic filopodia and lamellipodia – in both dorsal closure and the repair of laser-generated wound holes. Our experiments in embryonic chicks and mice and in the neonatal PU.1 null mouse, which is genetically macrophageless, suggest that an inflammatory response is not essential for repair and may indeed be causal of fibrosis in post-embryonic animals. Consequently, we have used a microarray approach with this mouse in order to identify a portfolio of candidate inflammation/fibrosis genes. Finally, by taking advantage of the transparency of the zebrafish larval tail we have begun to make DIC movies of the inflammatory response and to dissect the genetics of this process by screening for mutants that fail to recruit leukocytes to the wound site and by morpholino knockdown of candidate “inflammation” genes.

WNT SIGNALS IN THE DEVELOPMENT OF HAIR FOLLICLES, MAMMARY GLANDS AND TEETH

SARAH E. MILLAR

Departments of Dermatology and Cell and Developmental Biology, University of Pennsylvania, USA

The development of epidermal appendages, such as hair, mammary glands and teeth, relies on epithelial-mesenchymal interactions, implicating intercellular signaling in appendage formation. Mutations in effector genes of the canonical WNT signaling pathway cause defective morphogenesis of all of these appendages, suggesting that paracrine WNT signals play key roles in appendage development. To test this hypothesis we ectopically expressed the potent endogenous secreted WNT inhibitor Dickkopf 1 (DKK1) in the surface epithelium of transgenic mouse embryos. Transgenic embryos expressing high levels of DKK1 lack hair follicles, teeth and mammary buds. None of the tested molecular markers for early stages of hair follicle placode formation showed patterned expression in transgenic embryo skin, indicating that the initiation of hair follicle placode development is blocked by DKK1.

To investigate whether DKK1 also blocks the initiation of mammary placode formation, we identified molecular markers that show specifically elevated expression in the epithelial mammary line and placodes. These markers include the WNT pathway genes *Wnt10b*, *Wnt6* and *beta-catenin*. None of these genes showed localized expression in the mammary region of DKK1-transgenic embryos. However, expression of *Fgf10*, an early regulator of mammary placode formation that is expressed in the underlying dermamyotome, was unaffected by DKK1. These results suggest that *Fgf10* may cooperate with one or more broadly expressed *Wnt* genes to initiate mammary placode formation, and indicate that WNT signals are required for the initiation of several different types of appendage.

In contrast, analysis of tooth development in DKK1-expressing embryos revealed that initial, localized changes in the epithelium, visible histologically and by expression of the early dental lamina marker *Pitx2*, occur in transgenics, while subsequent downgrowth of the epithelium to form a tooth bud is blocked. Thus the mechanisms of tooth induction may differ from those regulating the initiation of hair and mammary placode development.

THE FUNCTION OF THE NASAL PLACODES FOR DEVELOPMENT OF THE VERTEBRATE FACE

ANNETTE NEUBÜSER

Research Institute of Molecular Pathology, Austria

Development of the midfacial region begins with the appearance of the nasal placodes, bilateral ectodermal thickenings at the ventrolateral sides of the forebrain that will give rise to the olfactory epithelium. Subsequently, tissue around the nasal placodes grows out to form the nasal processes. How the early facial region is patterned and how the areas of mesenchymal outgrowth are established is largely unknown.

We have examined the function of the nasal placodes for patterning of the midfacial region using the chick as an experimental organism. In a series of ablation experiments we demonstrate that the nasal placodes are required for normal outgrowth and patterning of the nasal region. After ablation of a nasal placode at stage 16-17 outgrowth of the nasal mesenchyme on the operated side was severely reduced and the nasal capsule and nasal bone failed to form. These defects could be rescued by back transplantation of a placode after ablation. Transplantation of a nasal placode into ectopic positions within the face resulted in the formation of an ectopic nasal capsule indicating that the nasal placodes are sufficient to induce nasal capsule development.

In order to gain insight into how the nasal placodes may control development of the facial mesenchyme, the expression of the signalling molecules (*Bmp2*, *Bmp4*, *Bmp7*, *Fgf4* and *Fgf8* and *Shh*) in the developing face was analyzed and the effect of nasal placode ablation on the expression of molecular markers was characterized. Based on this analysis we propose a model how the nasal placode may pattern the early facial region.

AMPHOTERIN (HMGB1) AND AMPHOTERIN-INDUCED GENE EXPRESSION: FROM DEVELOPMENT TO DISEASE

HEIKKI RAUVALA

*Neuroscience Center and Institute of Biotechnology and Department of Biosciences,
University of Helsinki, Finland*

Amphoterin was isolated as a heparin/heparan sulfate-binding protein that enhances neurite outgrowth in forebrain neurons. In addition, amphoterin was found to bind both plasminogen and its activators, resulting in a ternary complex that strongly enhances plasmin formation. Based on its biochemical and cell-biological properties, amphoterin was suggested to regulate invasive migration of growth cones during neurite outgrowth and invasive migration of tumour cells in an autocrine or paracrine manner.

Amphoterin has a sequence characteristic of high-mobility group (HMG) proteins (hence the designation HMGB1), a protein family that has been classically implicated in DNA binding. Although no forms of amphoterin have been found that would contain a secretion signal, amphoterin secretion can be induced *in vitro* and *in vivo* upon cell activation, e.g. by many cytokines. After secretion, amphoterin binds to RAGE (receptor for advanced glycation end products), an immunoglobulin superfamily member related to N-CAM (neural cell adhesion molecule). Ligation of RAGE by amphoterin activates the small GTPases Cdc42 and Rac, which is an essential step in amphoterin-induced migration. Regulation of invasive migration of tumour cells by the amphoterin/RAGE/GTPase pathway has been studied in some detail. A C-terminal RAGE-binding motif of amphoterin is required for transendothelial tumour cell migration *in vitro* and metastasis *in vivo*.

Amphoterin displays pronounced effects on gene expression, which is probably important in its mechanism of action. On this line of work, AMIGO (**amphoterin-induced gene and orphan receptor**) was very recently cloned using ordered differential display. Based on the AMIGO sequence, two homologous proteins (AMIGO-2 and AMIGO-3) were also cloned. AMIGO, AMIGO-2 and AMIGO-3 form a novel protein family characterized by a tandem array of LRR (leucine-rich repeat) sequences and an immunoglobulin domain followed by a transmembrane and a cytosolic sequence. Like amphoterin, AMIGO also promotes neurite outgrowth and is implicated in fasciculation of neurites. We suggest that AMIGO plays a role in the construction of fiber pathways of brain through a homophilic interaction mechanism.

ADHESIVE SIGNALING IN BRAIN DEVELOPMENT

LOUIS F. REICHARDT

University of California, San Francisco, USA

I will discuss recent data using genetic approaches on the roles of the focal adhesion kinase in CNS development. At early stages of CNS development, absence of FAK results in phenotypes in brain, eye and muscle very similar to the phenotypes of several congenital muscular dystrophies—type II lissencephaly in the CNS; dysplasias in lense and retina, dystrophic fibrosis in muscle. When lost at later times, absence of FAK affects axonal branching and synapse formation. In collaboration with the laboratories of W. Birchmeier (Berlin) and B. Lu (Bethesda), we have shown that deletion of beta-catenin in differentiated neurons destabilizes synapses *in vivo* and in cell culture. Results indicate that beta-catenin is functioning primarily as a scaffolding protein, linking cadherins with synaptic scaffolding proteins, independent of the F-actin cytoskeleton.

CELL FATE SPECIFICATION AND AXIS DEVELOPMENT IN THE PERI-IMPLANTATION MOUSE EMBRYO

JANET ROSSANT, CLAIRE CHAZAUD, DAN STRUMPF, TILO KUNATH,
LAURA CORSON AND YOJIRO YAMANAKA

*Samuel Lunenfeld Research Institute and Department of Molecular and Medical
Genetics, University of Toronto, Mount Sinai Hospital, 600 University Avenue,
Toronto, Ontario, CANADA, M5G 1X5*

The mouse blastocyst, at the time of implantation, has three distinct cell lineages: epiblast, trophoblast and primitive endoderm. Interactions between these three lineages and their directional growth and migration are critical for establishing the initial asymmetries that result in anterior-posterior patterning of the embryo proper. We have used a combination of lineage tracing, *in situ* gene expression analysis and genetic studies in both embryos and stem cell lines to investigate the molecular basis of lineage specification at the blastocyst stage. We propose a model in which a combination of cell division order, signal transduction differences between inner and outer cells and segregation of key transcription factors can produce a blastocyst in which all three lineages are normally set up in an ordered, lineage-dependent manner, but which can also reconstruct a blastocyst when division order or cell interactions are disturbed. The FGF signaling pathway may be critical for trophoblast and primitive endoderm lineage specification and proliferation, while the Wnt signaling pathway needs to be locally activated for initiation of anterior-posterior asymmetries leading to primitive streak formation.

CONTROL OF APICAL MEMBRANE GROWTH AND TUBE SIZE BY BNL/FGF SIGNALING

CHRISTOS SAMAKOVLIS

*Department of Developmental Biology, Wenner-Gren Institute, Stockholm University,
Sweden*

The cellular architecture of tubular organs suggests striking similarities in the mechanisms of tubulogenesis between species. The formation of the *Drosophila* respiratory organ (trachea) highlights the basic principles of branch patterning and tube growth that generate a highly elaborate, but stereotyped epithelial tubular network. Oriented cell migration, changes in cell shape, selective growth of the apical cell membrane and intracellular lumen formation are essential events in the creation and growth of the tracheal network. These morphogenetic processes are employed in different genetic plots to build four structurally distinct classes of tubes that facilitate optimal airflow and gas exchange with target tissues. The molecular players in these plots include attractant and repellent signals, differentiation factors resulting in a high diversity of cell fates within the epithelium, and determinants of tube formation and dimensions.

ECTODYSPLASIN SIGNALING AND THE FORMATION OF ECTODERMAL ORGANS

IRMA THESLEFF, MARJA L. MIKKOLA, JOHANNA PISPA, TUIJA
MUSTONEN, JOHANNA LAURIKKALA, AAPO KANGAS,
MARITTA ILMONEN AND RISTO JAATINEN

*Developmental Biology Reserch Program, Institute of Biotechnology, University of
Helsinki, Finland*

Ectodysplasin (Eda) is a signaling molecule belonging to the tumor necrosis factor family. It is required for the normal development of several, perhaps all, organs developing as ectodermal appendages. Mutations in *Eda*, its receptor *Edar*, or other components of the signaling pathway cause ectodermal dysplasias in humans and mice. We have analysed the roles of *Eda*-*Edar* signaling in wild type and mutant mice either lacking functional *Eda* protein or overexpressing *Eda* or *Edar* in the ectoderm. We have shown that this pathway mediates interactions between ectodermal cell compartments during tooth and hair follicle morphogenesis. It regulates the initiation as well as morphogenesis and differentiation of ectodermal organs. It controls the formation and function of ectodermal placodes and is integrated with Wnt and activin signaling. It acts upstream of many signal molecules expressed in ectodermal placodes and regulates the patterning of hair and teeth.

This work has been supported by the Academy of Finland and the Sigrid Jusélius Foundation.

CELL SIGNALING IN THE CONTROL OF UROGENITAL SYSTEM DEVELOPMENT

SEPPO VAINIO

*Department of Biochemistry and Biocenter Oulu, Linnanmaa, 90570 Oulu,
University of Oulu, Finland*

The urogenital system plays an important role in morphogenesis of several organs in mammals. These include the adrenal gland, gonad and the metanephros. In addition to this the embryonic hematopoietic stem cells are also thought to be born in this transient embryonic organ system, in the aorta-gonad-mesonephros (AGM) region. Experimental evidence points that members of the Wnt family of secreted inductive signals play a key role in ontogenesis of the urogenital system. For example Wnt-4 regulates development and function of the adrenal, gonad, metanephros and maintains the female germ line. Wnt-4 is the first identified signal that specifies the female sex upon sex determination. Wnt-4 also acts as a sexually dimorphic anti-angiogenic signal in the ovary. In the metanephric kidney Wnts control ureteric bud and stromal signaling and by that way ureteric bud grow development and mesenchymal tubulogenesis to build the nephron. Recent studies suggest that type XVIII collagen is a component in Wnt signaling. Its domain endostatin may effect Wnt signal transduction and the frizzled domain may bind Wnts and regulate their signaling. For example in the kidney expression of this collagen is induced in the tubules and it plays a role in pattern formation in the kidney mesenchyme possibly to specify sites where nephrogenesis is initiated. Taken together, the urogenital system is a good organ to be used to study several key embryonic processes. Multiple Wnt signals take part in urogenital system development. The Wnts may function by controlling migration and differentiation of the stem cells in this specific embryonic organ.

PATTERNING SEGMENTS IN THE HINDBRAIN

YI-CHUAN CHENG, MARC AMOYEL, MARISA COTRINA,
ANDREA PASINI, ALEXEI POLIAKOV AND DAVID WILKINSON

*Division of Developmental Neurobiology, National Institute for Medical Research,
London NW7 1AA, UK*

The establishment of organised patterns of cell types at appropriate locations in the nervous system is achieved by its initial subdivision into regional domains, each specified to form a distinct set of derivatives. An important question is how these regional domains are formed and maintained as discrete structures, despite the potential for them to become scrambled by cells intermingling during tissue growth and cell proliferation. One mechanism by which sharp interfaces are maintained between adjacent cell populations is to specifically inhibit cells from intermingling across the boundary. In some cases, these boundaries then form a distinct cell population that acts to organise local pattern. However, little is known regarding the formation and roles of boundaries in the vertebrate hindbrain.

Our previous work has shown that bi-directional activation of Eph receptor tyrosine kinases and transmembrane ephrinB proteins at the interface of complementary domains of expression restricts the intermingling of cells across the interface. We also found that Eph receptors and ephrins act upstream of the formation of distinct hindbrain boundary cells. This talk will discuss progress in dissecting the roles of other receptor systems in the control of cell movement and formation of hindbrain boundaries.

PERLECAN MAINTAINS MICROVESSEL INTEGRITY IN VIVO AND MODULATES VASCULAR FORMATION IN VITRO

ERIKA GUSTAFSSON¹, CATARINA CRAMNERT^{1,2}, WILHELM BLOCH³,
MERCEDES COSTELL⁴, KLAUS ADDICKS³, RUPERT TIMPL² AND
REINHARD FÄSSLER²

¹Department of Experimental Pathology, Lund University, SE-221 85 Lund, Sweden,

²Max Planck Institute for Biochemistry, Department of Molecular Medicine, D-821 52 Martinsried, Germany, ³Institute for Anatomy, University of Cologne, D-509 31

Cologne, Germany and ⁴Department of Biochemistry and Molecular Biology, University of Valencia, E-460 71 Valencia, Spain

Perlecan is the major heparan sulfate proteoglycan of basement membranes (BMs). Its location and ability to bind and present growth factors suggested an important role in vascular formation. In the present study we have investigated vascular development in mice, teratomas and embryoid bodies (EBs) lacking perlecan. We report that blood vessels formed normally in perlecan-null embryos. At embryonic day (ED) 12.5, however, the mutant microvasculature in brain and skin developed dilations leading to bleedings at ED17.5. The ultrastructure of BMs in affected microvessels was abnormal suggesting that perlecan is essential for the assembly of distinct BMs. Perlecan-null embryonic stem (ES) cells gave rise to teratomas of normal size. The ES cell-derived, mutant endothelial cells contributed to the tumor vasculature that displayed no abnormalities. In EBs, however, mutant ES cells differentiated into endothelial cells that assembled significantly less efficient into a vasculature when compared to controls. Addition of FGF-2 rescued the *in vitro* deficiency of the mutant ES cells. This modulatory role is not obvious *in vivo* where other angiogenic factors not present *in vitro*, may compensate. These findings suggest that perlecan is essential for the maturation of a subpopulation of blood vessels and that it promotes vasculo- and angiogenesis by modulating FGF-2 function.

TRANSCRIPTION FACTOR GATA3 REGULATES GDNF SIGNALING IN MIGRATING WOLFFIAN DUCT CELLS

MADIS JAKOBSON, ILLAR PATA, KIRSI SAINIO, FRANK GROSVELD,
HANNU SARIOLA AND ALAR KARIS

*Developmental Biology laboratory, Department of Biomedicine, Biomedicum
Helsinki, PO Box 63, FIN-00014 University of Helsinki, Finland*

During the early stages of urogenital development Wolffian duct arises from the intermediate mesoderm, migrates posteriorly under the paraxial mesoderm and fuses with the cloaca. Normal development of the Wolffian duct is essential for all further steps of kidney morphogenesis. It induces development of the mesonephros and gives rise to the ureteric bud that initiates the permanent kidney differentiation. Wolffian duct and its derivatives give also rise to components of the male genital tract. It has been shown that GDNF signalling through its co-receptor GFRa-1 is sufficient to direct pathfinding of migrating Wolffian duct in axolotl embryos. It is now suggested that Wolffian duct elongation is accomplished by migration up on a gradient of GDNF. Transcription factor GATA3 plays essential role in multiple developmental processes and is expressed also in the Wolffian duct-derived epithelia. Here we demonstrate a novel role for GATA3 during early steps of kidney and genital tract morphogenesis. We have discovered that mice homozygous for targeted inactivation of Gata3 gene have Wolffian duct migration defect, the duct is misguided during caudal elongation and never reaches the cloaca. Expression of GDNF receptor molecules, Ret and GFRa1, is initially down-regulated and subsequently lost from the mutant duct. However, GDNF expression in the nephrogenic cord appeared similar between wild type and Gata3 mutant embryos. We show also that the mutant Wolffian duct has lost its developmental potential to form ectopic ureteric buds in response to the natural inducer, GDNF. Bud formation, however, can be partially rescued by recombinant GFRa1, thus indicating that defective migration of the Wolffian duct in Gata3 mutant embryos may be caused by lack of GFRa1 or related molecules. Our data show that transcription factor GATA3 regulates expression of GDNF receptor molecules during kidney development and is essential for migration and guidance of the Wolffian duct and ureteric budding.

CRITICAL ROLE OF VEGF-C IN LYMPHATIC VASCULAR DEVELOPMENT

MARIKA J. KÄRKKÄINEN¹, PAULA HAIKO¹, JUHA PARTANEN²,
KAISA KARILA¹, MARJA JÄNNE², HEIKKI RAUVALA² AND
KARI ALITALO¹

¹Molecular/Cancer Biology Laboratory and Ludwig Institute for Cancer Research, Haartman Institute and Helsinki University Hospital, Biomedicum Helsinki, PO Box 63, 00014 University of Helsinki, Finland and ²Institute of Biotechnology, PO Box 56, 00014 University of Helsinki, Finland

Lymphatic vessels have an essential role in transporting tissue fluid, extravasated plasma proteins and cells back to the blood circulation. The lymphatic system also contributes to the immune surveillance of the body and it has an important function in fat adsorption from the digestive tract. Abnormal function or growth of the lymphatic vessels is involved in various diseases such as tumor metastasis and lymphedema. We have shown that vascular endothelial growth factor C (VEGF-C) induces proliferation, migration and survival of lymphatic endothelial cells by binding to the tyrosine kinase receptors VEGFR-2 and VEGFR-3. However, the mechanisms regulating lymphangiogenesis during development have been unclear. We have produced mouse embryos lacking *Vegfc*, and show that VEGF-C is essential in the early differentiation of the lymphatic endothelium from the veins. The *Vegfc*^{-/-} embryos are devoid of lymphatic vasculature and they die before birth due to insufficient fluid drainage from critical tissues. Interestingly, in *Vegfc*^{+/-} mice, haplo-insufficiency of VEGF-C results in transient hypoplasia of the lymphatic vessels in several organs. These mice show accumulation of chylous fluid into the abdomen after birth. This phenotype resembles that of the Chy lymphedema mice which have a heterozygous inactivation of the VEGFR-3 tyrosine kinase activity. While the lymphatic vessels develop postnatally in most organs of the *Vegfc*^{+/-} mice, the defects in the skin persist. These results indicate that VEGF-C is essential in embryonic lymphangiogenesis and that the VEGF-C expression levels need to be finely tuned to sustain normal lymphatic vascular development. The *Vegfc*^{+/-} mice should be useful in studies of potential therapeutic agents for lymphedema and lymphatic dysfunction.

GDNF PROMOTES TUBULOGENESIS OF GFR α 1- EXPRESSING MDCK CELLS BY SRC-MEDIATED PHOSPHORYLATION OF MET RECEPTOR TYROSINE KINASE

ANNA POPSUEVA¹, DMITRY POTERYAEV², ELENA ARIGHI¹, XIAOJUAN MENG¹, ALEXANDRE ANGERS-LOUSTAU³, DAVID KAPLAN³, MART SAARMA² AND HANNU SARIOLA¹

¹*Developmental Biology, Institute of Biomedicine, Biomedicum Helsinki, P.O. Box 63, FIN-00014 University of Helsinki, Finland,* ²*Institute of Biotechnology, Program for Molecular Neurobiology, Viikki Biocenter, PO Box 56, FIN-00014 University of Helsinki, Finland and* ³*Brain Tumour Research Center, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4*

Glial cell line-derived neurotrophic factor (GDNF) and hepatocyte growth factor (HGF) are multifunctional signaling molecules in embryogenesis. HGF binds to and activates Met receptor tyrosine kinase. The signaling receptor complex for GDNF typically includes both GDNF family receptor alpha1 (GFRalpha1) and Ret receptor tyrosine kinase. GDNF can also signal independently of Ret via GFRalpha1, although the mechanism has remained unclear. We now show that GDNF partially restores ureteric branching morphogenesis in *ret*-deficient mice with severe renal hypodysplasia. The mechanism of Ret-independent effect of GDNF was therefore studied by the MDCK cell model. In MDCK cells expressing GFRalpha1 but no Ret, GDNF stimulates branching but not chemotactic migration, while both branching and chemotaxis are promoted by GDNF in the cells co-expressing Ret and GFR α 1, mimicking HGF/Met responses in wild-type MDCK cells. Indeed, GDNF induces Met phosphorylation in several *ret*-deficient/GFRalpha1-positive as well as GFRalpha1/Ret co-expressing cell lines. However, GDNF does not immunoprecipitate Met, making a direct interaction between GDNF and Met highly improbable. Met activation is mediated by Src-family kinases. The GDNF-induced branching of MDCK cells requires Src activation, whereas the HGF-induced branching does not. Our data show a mechanism for the GDNF-induced branching morphogenesis in non-Ret signaling.

INSULIN-LIKE FACTOR 3 IS A ESTROGEN SENSITIVE GENE INVOLVED IN GENDER INDEPENDENT GUBERNACULUM DEVELOPMENT

MILLA SUVANTO¹, PASI KOSKIMIES^{1,2}, ELINA NOKKALA¹, ANKE MCLUSKEY³, AXEL P.N. THEMMEN³, ILPO HUHTANIEMI¹ AND MATTI POUTANEN¹

¹Department of Physiology and ²Turku Graduate School of Biomedical Sciences, University of Turku, Kiinamyllynkatu 10, FIN-20520 Turku, Finland, ³Department of Internal Medicine, Erasmus Medical Center, PO Box 1738, 3000DR Rotterdam, The Netherlands.

The mouse knockout studies indicated that *Insl3* is involved in the gubernaculum formation in males, essential for normal testicular descent. To determine further functions of *Insl3* we have generated transgenic (TG) mice ubiquitously expressing *Insl3*. In the TG females the ovaries are descended to the base of abdominal cavity during fetal period, as a consequence of the formation of male-like gubernaculum structures. At adult age the ligaments were also formed between the lower and upper part of the uterine horns, and these ligaments forced the uterus to form coiled structure. However, the TG females retain their reproductive functions, indicating that neither the location of ovaries nor the macroscopic structure of the uterus is vital for reproduction. In addition, *Insl3* expression caused inguinal hernia in females, suggesting that combination of estrogen and *Insl3* action disrupts proper development of the muscular and connective tissue structures of abdomen. The lack of phenotype in other tissues indicates that gubernaculum formation is the most sensitive biological response for *Insl3*, and also female fetuses respond to *Insl3*. Interestingly, estrogenic exposure in fetal life leads to cryptorchidism in mice, and it has been shown that estrogens down-regulate *Insl3* expression. SF-1 is one of the major regulators of *Insl3*, but estrogens have no effect on SF-1 expression. Hence, we have analyzed the putative interplay between SF-1 and estrogen receptors (ERa and ERb) on *Insl3* promoter. The data suggest that both ERa and ERb down-regulate the SF-1 dependent *Insl3* promoter activity in transfected Leydig and HEK 293 cells. The nucleotide sequence of the consensus binding sites for SF-1 and ERs are highly similar suggesting that these transcription factors compete of the same *cis*-acting elements at the 5' region of the *Insl3* promoter. The putative protein-protein interactions between SF-1, ERs and some other transcription coregulators remain to be studied.

THE EMBRYOID BODY AS A MODEL SYSTEM FOR LYMPHANGIOGENESIS

MANUEL SELG, MICHAEL SIXT, NATHALIE HORN, THOMAS SAMSON,
MICHAEL DICTOR AND LYDIA SOROKIN

*Lunds Universitet, Avdelning för Experimentel Patologi, Sölvegatan 25, 22185 Lund,
Sweden*

In mammals the blood and lymphatic vasculature develop into two independent circulatory systems. Whereas the blood vascular system is a closed circulatory network and is in charge of delivering oxygen and nutrients to tissues, the lymphatic vascular network is blind ending and responsible for removing lymph by means of the thoracic duct to the venous blood stream. Although in the adult these two systems are morphologically and molecularly distinct, they are in close association with each other during development: During embryonic development angioblasts proliferate and differentiate to form the primary capillary plexus, ultimately giving rise to the blood vascular system. A subpopulation of endothelial cells in the embryonic veins then promotes lymphangiogenesis by budding and sprouting of the anterior cardinal vein, eventually forming the entire lymphatic network. However, despite this close embryological association between the vascular and the lymphatic systems, it is still not clear whether they originate from a common precursor cell. We describe a novel system to study the early steps in development of lymphatic endothelium and its organization into a network. Embryoid bodies (EB) derived from mouse ES cells develop a network of Lyve-1 positive lymphatic vessels that is distinct from a PECAM-1 positive vascular network. The EB lymphatic vessels resemble their *in vivo* counterparts morphologically and in their expression of Flt-4 and Lyve-1. Furthermore, we can show that day 6 EBs, grown in suspension, contain cells that are PECAM-1+/Lyve-1+, suggesting the two vascular systems are derived from the same precursor cell in this *in vitro* system.

FGFR1 IS INDEPENDENTLY REQUIRED BOTH IN DEVELOPING MID- AND HINDBRAIN FOR SUSTAINED RESPONSE TO ISTHMIC SIGNALS

RAS TROKOVIC¹, NINA TROKOVIC¹, SANNA HERNESNIEMI¹, ULLA PIRVOLA¹, DANIELA M. VOGT WEISENHORN^{4,5}, JANET ROSSANT², ANDREW P. MCMAHON³, WOLFGANG WURST^{4,5} AND JUHA PARTANEN¹

¹Institute of Biotechnology, University of Helsinki, PO Box 56, 00014-University of Helsinki, Finland, ²Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario M5G 1X5, Canada, ³Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138, USA, ⁴GSF- Research Centre for Environment and Health, Technical University Munich, Institute of Mammalian Genetics, Ingolstaedter Landstrasse 1, D-85764 Neuhenberg, Germany and ⁵Max Planc Institute of Psychiatry, Kapelinstrasse 2-16, D-80804 Munich, Germany

Fibroblast growth factors (FGFs) are signaling molecules of the isthmic organizer, which regulates development of the midbrain and cerebellum. Tissue-specific inactivation of one of the FGF receptor genes, *Fgfr1*, in the midbrain and rhombomere 1 of the hindbrain of mouse embryos results in deletion of the inferior colliculi in the posterior midbrain and vermis of the cerebellum. Analyses of both midbrain-hindbrain and midbrain specific *Fgfr1* mutants suggest that, after establishment of the isthmic organizer, FGFR1 is needed for continued response to the isthmic signals, and that it has direct functions on both sides of the organizer. In addition, FGFR1 appears to modify cell adhesion properties critical for maintaining a coherent organizing center. This may be achieved by regulating expression of specific cell-adhesion molecules at the midbrain-hindbrain border.

DEVELOPMENT OF THE PRIMARY JAW JOINT

ABIGAIL TUCKER, J. WILSON AND A.S. TUCKER

*Craniofacial Development, Floor 28 Guy's Tower, Guy's Hospital, London Bridge,
London, SE1 9RT, UK*

The development of the primary jaw joint has recently been shown to involve the gene Bapx1 (Nkx3.2), the vertebrate homolog of the *Drosophila* gene Bagpipe. Morpholinos against Bapx1 in zebrafish embryos lead to fusion of the jaw joint. Bapx1 is expressed in the developing mesenchyme of the mandible in two distinct caudal, proximal patches on either side of the head. From the zebrafish work, the signalling molecule endothelin has been indicated as a possible inducer of Bapx1 expression. Endothelin, however, is expressed in the epithelium throughout the mandible and therefore its expression pattern can not account for the restricted expression pattern of Bapx1. We have investigated the mechanisms that result in the restricted expression of Bapx1 in the mandible, and conclude that expression is repressed by other signalling molecules originating in the overlying epithelium. By manipulating these signals the expression pattern of Bapx1 can be altered and the development and position of the resulting jaw joint changed.

NON-MUSCLE SPECIFIC ALPHA-ACTININ LOCALIZES TO OVARIAN RING CANALS IN *DROSOPHILA*

GUDRUN WAHLSTRÖM, VESA-PEKKA LAHTI, CHRISTOPHE ROOS
AND TAPIO HEINO

Institute of Biotechnology, PO Box 56, 00014 University of Helsinki, Finland

Alpha-actinin is an actin cross-linking protein belonging to the spectrin superfamily of proteins. It exists as a dimer with an N-terminal actin-binding domain, four spectrin-like repeats, and a C-terminal calcium binding domain. Different isoforms of alpha-actinin have been isolated from both muscle and non-muscle cells. In *Drosophila* a single gene encodes two muscle specific and one non-muscle specific isoform through alternative splicing, which results in differences in the region between the actin binding domain and the first central repeat. We have isolated a mutant that specifically lacks the non-muscle specific alpha-actinin isoform. These flies are viable and fertile with no obvious phenotype. Using a monoclonal antibody that recognizes all isoforms, we have compared the alpha-actinin distribution in wild type and mutant flies. We found that non-muscle specific alpha-actinin is present ubiquitously in young embryos and in the embryonic central nervous system. In ovaries, non-muscle specific alpha-actinin is present in the subcortical cytoskeleton, actin cables and ring canals of the nurse cells. Ring canals are actin based structures that have been well characterized both morphologically and genetically. Now alpha-actinin can be added to the list of known ring canal components. Remaining antibody staining in the mutant, which arise from either one of the muscle specific isoforms of alpha-actinin, was seen in muscles, but also in some epithelial cells in both embryos and ovaries. These results show that non-muscle specific alpha-actinin is redundant in *Drosophila*, and that muscle specific alpha-actinin expression is not limited to muscle cells.

EXPRESSION OF LAMININ ISOFORMS AND THEIR RECEPTORS IN VASCULAR ENDOTHELIUM OF DEVELOPING MOUSE BRAINS

SMRITI AGRAWAL, MICHAEL SIXT AND LYDIA SOROKIN

*Department of Experimental Pathology Lunds Universitet, Sölvegatan 25,
Lund 223 62, Sweden*

Laminins are one of the major components of basement membranes. Twelve distinct laminin chains (a, b,g) have been identified which combine to form 15 distinct isoforms with tissue specific distributions. Cellular receptors for laminins include integrins, a-dystroglycan, and Lutheran blood group antigen.

Work in our laboratory has shown that endothelial cell basement membranes contain laminins 8 (a4b1g1) and 10 (a5b1g1)^{1,2}: Laminin 8 is present in all endothelial basement membranes from embryonic day 8 (E8) in the mouse when the first blood vessels form, and is maintained at this site throughout development. Laminin 10, in contrast, is first detectable in endothelial basement membranes only 3 weeks after birth², suggesting that laminins that contain these two a chains have different functions. Further, *in vivo* studies³ have suggested that laminin a4 and a5 play a role in the barrier function of brain blood vessels in inflammatory situations.

This has prompted investigation of laminin isoforms, with emphasis on laminin a4 and a5 chains, and their receptors in vascular endothelium during formation of the blood-brain-barrier in the mouse. Brains of mouse embryos were examined by immunofluorescence and *in situ* hybridization for the expression of laminins (laminin a1-5, b1-2, g1 chains) and laminin receptors (integrins, a-dystroglycan, and Lutheran blood group antigen) before (E14) and after formation of the blood-brain-barrier (E17, new born and 2 weeks post natal).

1. Frieser et al., 1997. Eur. J. Biochem. 246, 727-735.
2. Sorokin et al., 1997. Develop. Biol. 189, 285-300.
3. Sixt et al. JCB

LEYDIG CELL HYPERPLASIA AND HYPERTROPHY IN TRANSGENIC MALE MICE EXPRESSING HUMAN CHORIONIC GONADOTROPIN

PETTERI AHTIAINEN, SUSANA RULLI, JORMA TOPPARI,
MATTI POUTANEN AND ILPO HUHTANIEMI

*Department of Physiology, Institute of Biomedicine, University of Turku, FIN-20520
Turku, Finland*

Activation of LH receptor (LHR) before puberty for example through activating LHR-mutations leads in humans to precocious puberty and even Leydig cell adenomas. Our aim was to investigate the effects of chronic excessive levels of hCG/LH on male reproductive functions, with special reference to prepubertal testicular development. For this purpose, two TG mouse lines were developed. The hCGbeta+ transgenic mice express the hCGbeta subunit with moderately elevated levels of bioactive LH/hCG (5-fold), and the hCG+ transgenic mice express both alfa and beta subunits with highly elevated levels of LH/hCG bioactivity (2000-fold). At the age of 10 days, the testes weights and FSH-levels were significantly decreased in hCG+. Furthermore, at the age of 21 days, the testes of the hCG+ males were 50% smaller than in WT males. At the age of 6 months, both of the TG mouse lines had clearly smaller testes and lower FSH-levels than WT mice. Histological examination of 1- and 10-day-old hCG+ male testes demonstrated severe Leydig cell hyperplasia and hypertrophy with mitotic figures and Leydig cell adenomas. At these ages, no histological changes were seen in the seminiferous tubules or in spermatogenesis. Leydig cell hyperplasia in the adult hCG+ male testes was less prominent than at younger ages and no adenomas were detected. However, the testes had vacuoles in the basal compartment of the seminiferous epithelium, despite qualitatively full spermatogenesis. In contrast to the hCG+ mice, the histology of the hCGbeta+ testes was normal at all ages studies. The hCGbeta+ and hCG+ mouse models demonstrate that only highly elevated LH levels affect the normal reproductive functions of male mice, leading to enhanced steroidogenesis and Leydig cell hyperplasia, hypertrophy and adenomas.

CORRELATION BETWEEN EXPRESSION OF COLLAGEN XVIII IN EMBRYONIC HEARTS AND DOWN SYNDROME

LORENZA CARVALLHAES^{1,2}, R. HELJASVAARA², M. ILVES³,
R. SORMUNEN⁴, T. PIHLAJANIEMI² AND G.T. KITTEN¹

¹Department of morphology, Federal University of Minas Gerais, Belo Horizonte, Brazil, ²Biocenter of Oulu and Department of Medical Biochemistry and Molecular Biology, ³Department of Physiology and ⁴Department of Pathology, University of Oulu, Finland

A novel subgroup of collagens, named the MULTIPLEXINS, has been recently described and includes collagens XV and XVIII. Cleavage of the C-terminal portion of collagen XVIII gives rise to endostatin, a peptide fragment which has been shown to block angiogenesis by inhibiting the proliferation and migration of endothelial cells. It has also been shown that endostatin inhibits tumor progression in vivo mouse models. The gene encoding collagen XVIII is localized to chromosome 21. Down Syndrome (DS, Trisomy 21) is caused by the presence of a third copy of chromosome 21. DS individuals have higher levels of endostatin in their blood, a lower incidence of solid tumors than the general population. Approximately 40% of newborn DS individuals possess congenital heart defects that can be directly associated with a malformation of the cardiac valves. During the initial stages of cardiac valve formation, endothelial cells in the atrio-ventricular canal region undergo an essential epithelial-mesenchymal transformation (EMT) which gives rise to a population of cells that migrate and proliferate in the extracellular matrix between the endothelium and myocardium. We hypothesize that an over-expression of collagen XVIII in DS perturbs the EMT and may be directly related to the cardiac malformations, which occur in these individuals. As a starting point to determine if there is any correlation between the expression of collagen XVIII-endostatin and DS, we decided to analyze embryonic mouse hearts collected from different stages of development. Immunofluorescence, immuno-electron microscopy, quantitative Real-Time PCR, and in situ hybridization methods were used to investigate the distribution and quantity of collagen XVIII protein and mRNA present in these hearts. We determined that collagen XVIII is localized at high levels in the regions directly responsible for the formation of the atrioventricular valves and that its pattern of expression changes in the valve tissues during heart development.

mRNA EXPRESSION OF *FIBROBLAST GROWTH FACTOR RECEPTOR* ISOFORMS DURING MURINE CRANIOFACIAL BONE AND CARTILAGE DEVELOPMENT

DAVID PC RICE^{1,2}, RITVA RICE^{1,2}, ELAINE CONNOR¹ AND IRMA THESLEFF¹

¹*Institute of Biotechnology, PO Box 56, 00014 University of Helsinki, Finland and*

²*Dept of Craniofacial Development, Kings College London, SE1 9RT, UK*

Mutations in *fibroblast growth factor receptors (FGFRs)* are known to cause both chondrodysplasias as well as craniosynostoses, both of which can cause abnormalities in the growth and development of the craniofacial region. Although *Fgfrs* have been isolated at numerous locations during early mouse development including the craniofacial area, relatively little is known about the expression of *Fgfr* splicing alternatives during craniofacial bone development, a process which involves a combination of both endochondral and intramembranous ossification. In an attempt to address this, we have performed a detailed in situ hybridisation analysis to detect these genes in the developing mouse craniofacial region. We concentrated on areas of major growth, namely the synchondroses of the cranial base, the bones and sutures of the skull vault, as well as the mandibular body and condyle.

Fgfr1c was detected weakly in osteoblastic cells in both the developing calvarial and mandibular bones. *Fgfr3b* and *Fgfr3c* were found in proliferating chondrocytes of the synchondroses and the mandibular condyle. *Fgfr2b* transcripts were most notably detected in the perichondria of the mandibular condyle and the cranial base cartilages. While also being weakly expressed in the parietal and frontal bones. *Fgfr2c* transcripts were detected with high intensity in differentiating osteoblasts at the sutural osteogenic fronts of the calvarial bones. In addition, *Fgfr2c* was also expressed in the perichondria of the mandibular condyle and the cranial base cartilage. The *Fgfrs* are compared to bone and cartilage markers. This data forms the foundation upon which the disturbances in craniofacial growth exhibited by both patients and by mutant mice carrying mutations in *Fgfrs* can be understood.

CHARACTERIZATION OF ADULT MOUSE NEURAL STEM CELLS

JANNE HAKANEN, SÉBASTIEN DUPRAT AND MARJO SALMINEN

*Institute of Biotechnology, Developmental Biology Program, PO Box 56, 00014
University of Helsinki, Finland*

Neural stem cells are self-renewing, multipotent cells that generate neurons, astrocytes, and oligodendrocytes in the developing nervous system. Neurogenesis occurs also in the adult mammalian brain. There are two neurogenic areas in the adult mammalian central nervous system (CNS): the olfactory bulb and the dentate gyrus of the hippocampus, where neurogenesis has been shown to occur throughout adulthood. In this work we have studied adult neural stem cells (ANSCs) which reside in walls of the lateral ventricles in the adult mouse brain. New neurons are born in this zone and they migrate through the rostral migratory pathway to the olfactory bulb, where they mature into local interneurons. To investigate the differentiation capacity of ANSCs *in vivo*, we isolated ANSCs from adult mice lateral ventricles and spinal cord, propagated the cells by culturing them with presence of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). After culturing ANSCs were transplanted into the telencephalic vesicle of E14.5 embryos by microinjections and were analyzed at E18.5. We noticed, that transplanted ANSCs are able to integrate in various brain region from fore- to hindbrain in the host tissue and majority of these integrated cells seem to have a tendency to differentiate into astrocytes. Netrin family of secreted molecules participate in both attraction and repulsion of the axonal growth cone. Our experiments show an expression of netrin-1 in specific areas of the CNS, known to contain neural stem cells. Following an injury in the CNS, an increasing netrin-1 expression has also been observed along the scar. Therefore we investigate the role of netrin-1 in neuronal regeneration. We also propose that netrin is a relevant marker to identify and purify adult neural progenitor cells.

CORRELATION BETWEEN EXPRESSION OF COLLAGEN XVIII/ENDOSTATIN IN EMBRYONIC HEARTS AND THE CARDIAC MALFORMATIONS WHICH OCCUR IN DOWN SYNDROME

L.S. CARVALLHAES¹, R. HELJASVAARA², M. ILVES³, R. SORMUNEN⁴,
T. PIHLAJANIEMI² AND G.T. KITTEN¹

¹Department of Morphology, Federal University of Minas Gerais, Belo Horizonte, Brazil (kitten@icb.ufmg.br), ²Biocenter and Department of Medical Biochemistry and Molecular Biology, ³Department of Physiology and ⁴Department of Pathology, University of Oulu, Oulu, Finland

A novel subgroup of collagens, named the MULTIPLEXINS (multiple triple-helix domains with interruptions), has been recently described and includes collagens XV and XVIII. Cleavage of the C-terminal portion of collagen XVIII gives rise to endostatin, a peptide that has been shown to block angiogenesis by inhibiting the proliferation and migration of endothelial cells. It has also been shown that endostatin inhibits tumor progression in vivo mouse models. The gene encoding collagen XVIII is localized to chromosome 21. Down Syndrome (DS, Trisomy 21) is caused by the presence of a third copy of chromosome 21. DS individuals have higher levels of endostatin in their blood and a lower incidence of solid tumors than the general population. In addition, approximately 40% of newborn DS individuals possess congenital heart defects that can be directly associated with a malformation of the cardiac valves. During the initial stages of cardiac valve formation, endothelial cells in the atrio-ventricular canal region undergo an essential epithelial-mesenchymal transformation (EMT) which gives rise to a population of cells that migrate and proliferate in the extracellular matrix between the endothelium and myocardium. We hypothesize that an over-expression of collagen XVIII in DS perturbs the EMT and may be directly related to the cardiac malformations that occur in these individuals. As a starting point to determine if there is any correlation between the expression of collagen XVIII-endostatin and DS, we decided to analyze embryonic mouse hearts collected from different stages of development. Immunofluorescence, immunoelectron microscopy, quantitative Real-Time PCR, and in situ hybridization methods were used to investigate the distribution and quantity of collagen XVIII protein and mRNA in these hearts. We show that collagen XVIII is expressed at high levels in the regions directly responsible for the formation of the atrioventricular valves and that its expression pattern changes in the valve tissues during heart development.

SYMPATHETIC CHOLINERGIC TARGET INNERVATION REQUIRES GFRA2

PÄIVI H. HILTUNEN AND MATTI S. AIRAKSINEN

*Program of Molecular Neurobiology, Institute of Biotechnology, Viikki Biocenter,
FIN-00014 University of Helsinki, Finland*

Target innervation by many cholinergic parasympathetic and a subpopulation of enteric neurons requires neurturin (NRTN) signaling through GDNF family receptor GFRA2 and Ret receptor tyrosine kinase. In contrast, NRTN signaling is apparently not needed for the development of sympathetic noradrenergic neurons *in vivo*. Yet, a subpopulation of sympathetic neurons are cholinergic. Targets of these neurons include the sweat glands in footpads that produce some factors inducing the cholinergic phenotype. NRTN is expressed postnatally in sweat glands, making it a candidate target-derived factor for the development of sympathetic cholinergic neurons. In this study, we show that the sympathetic cholinergic target innervation requires GFRA2. In postnatal (P10) sympathetic stellate ganglia, which innervate the forepaw sweat glands, the cholinergic but not the noradrenergic neurons expressed GFRA2. In the sweat glands of adult GFRA2-deficient mice, the density of cholinergic nerve terminals immunolabeled for VIP was reduced by more than 80%, as compared to wild type littermates. Very similar deficits were observed using other cholinergic markers VAcHT or AChE. In contrast, noradrenergic sympathetic innervation around blood vessels in the footpads or in other sympathetic target areas was not affected. The results indicate that the cholinergic but not the noradrenergic subpopulation of sympathetic neurons require NRTN signaling via GFRA2 for terminal innervation *in vivo* but apparently not for the transmitter phenotype. Thus, NRTN may be a general target innervation factor for cholinergic axons in all parts of the autonomic nervous system. Ongoing studies will assess (i) when the innervation deficit happens and (ii) whether the number of sympathetic cholinergic neurons in adult stellate ganglion is reduced.

COLLAGEN XVIII EXPRESSION IN KIDNEY MESENCHYME IS INDUCED AND MAY FUNCTION TO PATTERN FOCI WHERE TUBULOGENESIS IS INITIATED

Y. LIN¹, HENRIKA HONKANEN¹, S. ZHANG¹, RITVA HELJASVAARA²,
TAINA PIHLAJANIEMI² AND SEPPO VAINIO¹

¹*Biocenter Oulu and Department of Biochemistry, University of Oulu, Linnanmaa, FIN-90570 Oulu, Finland and* ²*Collagen Research Unit, Biocenter Oulu and Department of Medical Biochemistry, University of Oulu, FIN-90220 Oulu, Finland*

Tubule formation in the kidney mesenchyme is induced in vivo by the ureteric bud and in vitro by various inducers, for example by spinal cord and Li⁺-ions. Wnt-4 which is expressed in the condensed mesenchyme and pretubular aggregates is an essential mesenchymal inducer for kidney tubulogenesis. Extracellular matrix is also an important component in morphogenesis. It plays active role in inductive tissue interactions by controlling for example activities of growth factors. Type XVIII collagen is a basement membrane component which has a frizzled domain and could function in controlling Wnt signalling. We report here that collagen type XVIII is expressed in differentiating kidney tubules, where Wnt-4 is also present. Expression of type XVIII collagen in mesenchyme depends on inductive signals for tubules. We speculate that type XVIII collagen may bind and by that way limit the diffusion of Wnt-4 to the tubules that are differentiating. Preliminary data suggest that blocking of type XVIII collagen with specific antibody does not perturb Wnt-4 or spinal cord induced kidney tubulogenesis but prevent formation of pretubular foci as a response to LiCl as judged morphological and molecular criteria. Interestingly, induction of type XVIII collagen expression with LiCl lead to induction of endothelial cells that expressed type XVIII collagen and these cells project into induced tubules raising the possibility that endothelial cells may be involved in type XVIII collagen action and tubule induction. We can conclude the type XVIII collagen may play role in induced tubulogenesis possibly on patterning kidney mesenchyme, localizing diffusion of Wnt-4 signals to the pretubular cells.

THE EXPRESS AND LOCALIZATION OF TRANSPORT PROTEINS GLUT-4, SGLT-1, TGN-38 AND AQP-2 IN RATS RENAL CORPUSCLE AND TUBULES

PIRET HUSSAR¹, TOIVO SUUROJA² AND ÜLO HUSSAR¹

¹*Department of Anatomy, Tartu University, Estonia and* ²*Department of Morphology, Estonian Agricultural University, Estonia*

Two types of transepithelial glucose transporters have been identified: facilitated- diffusion glucose transporters (GLUT family), and Na(+)-dependent glucose co-transporters (SGLT family). These transporters play important roles in the sugar reabsorption in renal tubular cells (Takata, 1998). GLUT-4 is mainly localized in the distal tubules, connected with JGA. SGLT-1, an isoform of Na(+)-dependent glucose transporters, is localized at the apical plasma membrane of the thin segment of Henle's loop. TGN-38 play a role as a cargo transporter in cells, also a role of evaluation of the glucose transport. Comparable routine histology, transmission EM and fluorescence immunohistochemistry of renal corpuscle and tubules epithel was made in young adult male Wistar rats to observe the location and activity of transepithelial transport proteins for glucogen, sodium and water ultrafiltration and reabsorption in renal corpuscles and tubules accordingly. An intensive GLUT-4 expression in proximal tubules and, especially in convoluted segment of distal tubules, connected with JGA has been observed. The intensive SGLT-1 expression was marked in all renal tubules, and also in the visceral layer of capsule (on the line of the secondary cytopodies contact with capillary wall). TGN-38 was expressed mainly in the S1 of proximal tubules (elevation of the glucose reabsorption). The most intensive AQP-2 expression in the thin part of Henle's loope has been detected. In some cases aquaporin-2 expression in the collecting tubules has been noticed. Various technique of immunomorphology have been applied to elucidate the transport-protein localization in rats renal corpuscle and tubules. These new data suggest that glucose and water transepithelial transporter proteins expression have been observed with different intensity in all part of renal tubules; SGLT-1 expression also in the visceral layer of corpusculum renis, lining the glomerulus endothel, and still AQP-2 expression in collecting tubules.

FGFR1 SIGNALLING IS ESSENTIAL FOR THE UROGENITAL DEVELOPMENT

MARJO HYTÖNEN, T. IMMONEN, N. TROKOVIC, H. SARIOLA,
J. PARTANEN AND K. SAINIO

Institute of Biomedicine, PO Box 63, FIN-00014 University of Helsinki, Finland

FGFR1 is one of the four receptor tyrosine kinases that bind more than 20 different FGF ligands. A null mutant of FGFR1 is early embryonic lethal and to dissect genetically the functions of *fgfr1* during development, a hypomorphic mutant was generated. The reduced amount of the receptor leads to somitogenesis and neural tube defects, and posterior homeotic vertebral transformations. (Partanen et al., 1998). Therefore we have now analysed the possible defects in the urogenital organs of these animals. First, the kidneys were hypoplastic or absent. However, when present, the renal tissue differentiates, since the few nephrons had normal tubules and glomeruli. Moreover, all the essential tubulogenic factors were present. Also the mesonephroi differentiated.

Occasional hydronephroses or large cysts in kidneys suggested ureteric branching morphogenesis defect. Indeed, whole-mount immunohistochemistry showed delayed or blocked ureteric branching, but no obvious defects in the forming nephrons. Ectopic ureteric buds, even multiple kidneys were produced in culture and mesonephric area was posteriorly expanded. FGFR1 has been shown to regulate Hox gene activity during A/P patterning (Partanen et al., 1998), and it was recently reported that Hox genes are involved in the regulation of ureteric branching morphogenesis (Patterson et al. 2001). Our results show that FGFR1-mediated signalling regulates branching morphogenesis in the metanephric kidneys and urogenital patterning, possibly by regulating posterior Hox gene activity.

DICKKOPF-1 BLOCKS KIDNEY TUBULOGENESIS AND HAS AN EFFECT ON URETERIC BUD BRANCHING

PETRI ITÄRANTA¹ AND SEPPO VAINIO^{1,2}

¹*Biocenter Oulu and Department of Biochemistry, Oulu University, Finland and*

²*Faculties of Science and Medicine, Oulu University, Finland*

Dickkopf proteins (Dkk) are secreted antagonists of Wnt signaling implicated in many important processes during development in several animal species. The antagonistic effect of Dkks is thought to be mediated by their ability to prevent the formation of active Wnt –Frizzled receptor–LRP5/6 co-receptor complexes. This occurs when Dkk is binding and sequestering the LRP5/6 co-receptor from the cell surfaces with another cell surface receptor, Kremen. We have earlier shown that Wnt signaling is essential for kidney development indicating a critical role for the Wnt pathway in the process. To get further insight into the transmission pathway we analysed expression of Dkks in the developing kidney. Of these Dkk1 gene expression is detected at E13.5 in developing nephron which is consistent with the possible function of Dkk1 to control action of Wnt-4 that correlates with Dkk1 expression. Consistent with this hypothesis recombinant hDkk1 protein was sufficient to inhibit experimentally induced kidney tubulogenesis that was mediated by heterologous inducers such as cells that express a Wnt or spinal cord pointing that that the block may be mediated by modulating Wnt-4 function. Hence, the findings are in line with known requirement of Wnt and LRP function in kidney tubule induction. When Dkk1 was applied to the culture preceding its normal activation, changes in ureteric bud were noted. The branches of ureteric bud swelled and the tips made abnormal branches and some fused to each other. These results suggest that ectopic Dkk1 affects ureteric bud development directly possibly by having an effect on ureteric bud Wnt signaling such as those of Wnt-6, 7b or 15. To further target Dkk1 function in vivo, in the ureteric bud signaling, its expression will be directed to this tissue with the Pax2/HoxB7 promoters. Taken together the data suggests a role for Dkk1 in kidney tubulogenesis at the level of Wnt-4 and suggests also a role for Wnt signaling in controlling patterning of ureteric bud for generating its branches.

WNT-4 IN THE CONTROL OF VASCULARIZATION OF THE UROGENITAL SYSTEM

P. ITÄRANTA¹, T. SEPPÄNEN¹, M. NIKU¹, J. TUUKKANEN²,
H. PELTOKETO¹ AND S. VAINIO¹

¹*Biocenter Oulu and Department of Biochemistry, Faculties of Science and Medicine, University of Oulu, Finland and* ²*Biocenter Oulu, Department of Anatomy and Cell Biology, Faculty of Medicine, University of Oulu, Finland*

The secreted signal from the Wnt gene family, Wnt-4 is necessary for nephrogenesis and is also sufficient to induce formation of kidney tubules in the classic transfilter model system of tubulogenesis where nephrogenic induction is typically analysed. In the mesonephros Wnt-4 signalling is important for female sex organogenesis and the *Wnt-4* deficient female embryos obtain male characteristics. Analysis of the gonads of the *Wnt-4* knock out embryos indicate that Wnt-4 has anti-angiogenic property in the ovary. In the kidney the developing nephron has been suggested to express angiogenic factors to promote vascularization of the organ. As Wnt-4 is specifically expressed in the assembling tubule Wnt-4 may serve also as an angiogenic signal to promote endothelial differentiation. We tested the role of Wnt-4 in controlling assembly of the vessels. In the kidney at E12 the number of proliferating cells was reduced 10% from the wild type controls accompanied with induced apoptosis suggesting a role for Wnt-4 in early growth control. Vascularization of the *Wnt-4* deficient kidney was, however initially normal and markers of the endothelial cells were expressed. In tissue recombination studies the kidney mesenchyme had vasculogenic potential but this potential was independent of Wnt-4 signalling. Closer studies revealed that the Wnt induced pretubular mesenchymal cell aggregates are associated to the differentiating endothelial cells that are typically seen in close proximity to the assembling tubules. Moreover, in lithium induced kidney mesenchymes endothelial cells also survive and project to foci where tubulogenesis is initiated suggesting roles in induction. Taken together signals from the developing nephron regulated by Wnt-4 signalling is not necessary for vascularization of the kidney but rather endothelial cells may differentiate also *in situ* from metanephric mesenchyme and could promote tubulogenesis. Wnt-4 may initially have anti angiogenic property and regulate timing of endothelial assembly with the developing ovary and the nephron.

PROFILING OF TABBY MUTANT MICE - A MODEL FOR ECTODERMAL DYSPLASIA SYNDROME

RISTO JAATINEN AND IRMA THESLEFF

Institute of Biotechnology, PO Box 56, FIN-00014 University of Helsinki, Finland

Inactivation of two tumor necrosis factor (TNF) family pathway genes, namely ectodysplasin ligand, encoded by the Tabby gene, and its receptor, edar, encoded by the downless gene have been shown to result in ectodermal dysplasia syndromes in mice and humans. Several epithelial/mesenchymal derived organs such as teeth, hair, and exocrine glands develop defectively in these syndromes. Previously we have found that the ectodermal expression of ectodysplasin is up-regulated by the Wnt pathway, whereas edar is induced by the activin pathway in tissue culture experiments. However, the downstream targets and response genes of ectodysplasin signaling are still largely unknown. Therefore we have initiated a search for target genes by profiling the differential gene expression pattern between wild type and Tabby mice in both skin and tooth tissues. First, a cDNA library was generated by the suppression subtractive hybridization method from embryonic control and Tabby mice skin and a taylor-made cDNA microarray was generated from these clones. Our second approach to screen the differential gene expression has been to hybridize the Affymetrix microarrays. Currently, we have started to analyse the candidate clones including putative TNF pathway genes and other hair follicle growth regulating genes as well as genes encoding structural proteins of developing follicles with various *in silico* and wet-laboratory methods.

MICROARRAY SCREEN TO IDENTIFY DOWNSTREAM TARGETS OF RUNX2 IN TOOTH DEVELOPMENT

MARTYN JAMES AND IRMA THESLEFF

Institute of Biotechnology, PO Box 56, FIN-00014 University of Helsinki, Finland

Humans carrying a defective copy of the human Runx2 (Cbfa1) transcription factor suffer from cleidocranial dysplasia. This syndrome leaves patients with severe bone and dental defects including persistently open skull sutures and underdeveloped or absent clavicles (collarbones). Similarly Runx2-deficient mice completely lack bone formation (Komori et al, 1997) and have abnormal hypoplastic teeth (D'Souza et al, 1999). Runx2, therefore plays a crucial role during mouse development. The tooth phenotype is particularly interesting because Runx2 acts around the bud (E13) to cap (E14) stage of tooth development and thus represents a defect at a specific stage of tooth morphogenesis. We have carried out microarray analysis on RNA isolated and amplified from wild type and Runx2-deficient E14 molars to investigate which molecular pathways or events may be acting downstream of the Runx2 gene during tooth development. Preliminary data suggests that certain signalling pathways may be defective in the Runx2-deficient mice and special emphasis is being applied to validate this data through a mixture of real-time PCR and in situ hybridization experiments.

GENE PROFILING OF TRANSGENIC MICE WITH INACTIVATED FGFR1 USING GENECHIP (AFFYMETRIX) TECHNOLOGY

TOMI JUUKOLA, RAS TROKOVIC AND JUHA PARTANEN

Institute of Biotechnology, PO Box 56, 00014 University of Helsinki

Fibroblast growth factor (FGF) signalling has been implicated in patterning, cellular proliferation and differentiation in many organs, including the developing midbrain-hindbrain. Gene expression studies have revealed that signalling through fibroblast growth factor receptor 1 (FGFR1) might play an important role in the midbrain-hindbrain patterning and development of this region into superior and inferior colliculi as well as cerebellum.

This study aims to apply gene expression profiling to gain insight into the molecular regulation of mid-hindbrain patterning. In order to unravel the FGFR1 signalling in the mid-hindbrain tissue a conditional mutant mouse model was employed. In these FGFR1 mutants the Cre recombinase is expressed under the control of the Engrailed-1 transcription factor. When these mice are crossed with *Fgfr1* Flox/Flox mice in which the *Fgfr1* gene is flanked by Cre recombination sequences (loxP) some of the offspring will have inactivated FGFR1 in the midbrain-hindbrain tissue. In adult mutant mice, defects in cerebellum and midbrain are detected. Studies in our laboratory suggest that the MHB is established but FGF signals cannot be interpreted in tissues lacking the *Fgfr1* expression.

Microarray analysis of gene expression provides a powerful tool that can aid in the understanding of the molecular status of tissue. In this study the aim is to use the gene profiling to discriminate differences between mutant and wild type tissues. We hope that microarray analysis will clarify the cascade of molecular events maintaining cell differentiation in MHB tissue. In addition, aim is to characterise novel genes that are involved in cellular processes (e.g. signal transduction, cell adhesion and cellular proliferation) in mid-hindbrain patterning.

INDUCIBLE AND TISSUE-SPECIFIC CRE EXPRESSION UNDER REGULATION OF MOUSE HOXB7 PROMOTER

KAIA KALA AND ILLAR PATA

University of Tartu, Institute of Zoology and Hydrobiology, Vanemuise St 46, Tartu 51014, Estonia

To study genetic regulation of kidney development, we aim to create transgenic mice that express Cre recombinase in kidney and also in temporally controlled fashion. For this purpose we placed Cre coding sequence under the control of the mouse Hoxb7 promoter, which has been shown to target reporter gene expression to the Wolffian duct-derived epithelia. To achieve temporal control we combined Cre and Hoxb7 promoter with reverse tet transactivator (rtTA) system. Founder animals obtained from microinjections are currently analysed for Cre expression pattern by crossing to Rosa26 LacZ reporter mice (The Jackson Laboratory, stock no 003310). To induce Cre expression we administered doxycycline for four days either in drinking water (2 mg/ml dox supplemented with 5% sucrose) or by intraperitoneal injections (80 micrograms dox/g body weight). Both these methods appear to induce Cre expression effectively. We have observed interesting Cre expression patterns in these mice and established several lines with kidney and nervous system-specific expression. Described lines are useful tools in developmental studies, especially for studying genes that have an embryonic lethal phenotype when knocked out.

ROLE OF NEURAL CREST IN KIDNEY DEVELOPMENT AND DIFFERENTIATION OF THE STROMAL CELL FATE

MARIA KUURE, KEIJO VIIRI, RIIKKA KNUUTI AND SEPPO VAINIO

Biocenter Oulu and Department of Biochemistry, Linnanmaa, PO Box 3000, FIN-90014 University of Oulu, Finland

The metanephric mesenchyme has a central role in the generation of nephron but the origin of stromal cells remains unclear. The finding that *Wnt-1* is expressed in the dorsal spinal cord and is sufficient to induce kidney tubulogenesis, raised the possibility that neural crest cells may carry this protein to the kidney during crest migration and contribute possibly to induction of tubules, and the crest may generate the stromal cells of the kidney. We addressed these possibilities by taking the advantage of the *Spotch* (*Sp*) mouse line that carries a mutation in the *Pax3* gene. *Sp/Sp* embryos survive until around E13.5-14.5. They develop posterior spina bifida and their spinal cord remains open at the level of the metanephros. We used a mouse line where LacZ protein is driven by the *Wnt-1* promoter to study the migration of the neural crest cells into the kidney mesenchyme. It has been shown that neural crest migration is perturbed in the *Sp/Sp* embryos whereas in wild type embryos LacZ positive cells are detected at the area where kidney development is initiated. Irrespective of the defective neural crest migration, the kidneys of *Sp/Sp* embryos appear morphologically normal. The *Sp/Sp* kidneys also develop well in culture. Ureteric bud branches and kidney tubules appear. Moreover, analysis of stromal markers indicated the presence of stromal cells. We can conclude that *Wnt-1* does not play a role in the kidney as a tubulogenic inducer carried to the kidney by the innervating cells, and stromal cells are not of neural crest in origin.

INACTIVATION OF THE EGFR SIGNALLING IMPAIRS KIDNEY BRANCHING MORPHOGENESIS

TARJA KOIVISTO AND PÄIVI J. MIETTINEN

*Program for Developmental and Reproductive Biology, Biomedicum Helsinki, PO
Box 63, FIN-00014 University of Helsinki, Finland*

EGFR is tyrosine kinase receptor strongly expressed in the lung, salivary gland, pancreas and kidney, all of which develop through branching morphogenesis. EGFR ligands have been implicated in pancreas and lung development but in kidney direct evidence lacks. We have used the EGFR deficient mice (EGFR $-/-$) to elucidate the role EGFR signalling in the differentiation of the metanephric kidneys. The EGFR $-/-$ mice are born alive, but die soon after birth from respiratory failure and intestinal problems. We analyzed wild-type and EGFR $-/-$ newborn and embryonic kidneys for morphology, cell proliferation, apoptosis and extracellular matrix remodelling proteinase (MMP) activities. The differentiation of EGFR $-/-$ kidneys was delayed. At E17 they contained fewer glomerulal and the newborn kidneys contained more undifferentiated cortical area. The early events of the differentiation of the metanephric kidney were studied by culturing E12 kidney rudiments. In serum free conditions the EGFR $-/-$ ureter bud branched less than the control one ($p < 0.004$). There were no difference in the MMP-9 and MMP-2 activities studied by zymography. Caseinolysis assay revealed that the serine proteinase urokinase plasminogen activator (uPA) activity was decreased in the EGFR $-/-$ kidneys. Although no difference was detected in cell differentiation or proliferation, apoptosis was enhanced both in the embryonic and postnatal EGFR $-/-$ kidneys, particularly in the epithelium of the collecting ducts and in the ampulla. Our results suggest that functional EGFR signalling is needed for the branching morphogenesis of the ureter bud. Inactivation of the EGFR also accelerates programmed cell death of the kidney epithelium. The role of decreased uPA activity in these processes is under investigation.

EXPRESSION OF THE INSULIN SIGNAL TRANSDUCTION AND GLUCOSE METABOLISM PRODUCTS AT THE PROTEIN LEVEL IN THE RAT TESTIS

**KERSTI KOKK, E. VERÄJÄNKORVA, X.K. WU, H.TAPPER
AND P. PÖLLÄNEN**

Institute of Anatomy, Biomeedikum, Ravila19, 50411 Tartu, Estonia

Insulin Receptor Substrate (IRS) proteins are key mediators in insulin signaling from the insulin receptor and play a central role in maintaining basic cellular functions such as growth, survival and metabolism. It takes place through receptor-mediated tyrosine phosphorylation of the IRS proteins. IRS-1 and IRS-2 genes have been considered plausible candidates involved in the pathogenesis of Type2 diabetes. A family of glucose transporters (GLUT) mediates the cellular uptake of glucose. The aim of present study is to demonstrate the distribution of Insulin Receptor Substrates 1-3 (IRS 1-3), glucose transporters 1-4 (GLUT 1-4), SIRP1α, PKB kinase and PI3 kinase in the rat testis to see if signal transduction mediated by these proteins is active in testicular cells. Wistar rats were used as donors of testis tissue. Expression of these genes was studied at the protein level using immunohistochemistry and Western blotting. IRS-1, IRS-2, GLUT 1, GLUT 2, GLUT 3 and SIRP1α were strongly expressed in the different types of cells in all the testes investigated by immunochemistry. Positive immunoreactions for IRS-3, GLUT 4, PKB kinase and PI3 kinase were not found in the rat testis. Immunoblotting experiments demonstrated the presence of about 26-67 kD reactive with anti- IRS-1, IRS-2, GLUT 1, GLUT 2, GLUT 3, PKB kinase and SIRP 1α, but no proteins reactive with IRS-3, GLUT 4 and PI3 kinase antibodies were detected in the rat testis. The present result suggest that proteins like insulin and certain cytokines using IRS-1, IRS-2, GLUT 1, GLUT 2, GLUT 3, PKB kinase and SIRP1α in their signal transduction can have effects on the epithelial cells of the male reproductive tract in the rat.

JAGGED1 OVER-EXPRESSION DISRUPTS KIDNEY MORPHOGENESIS

SATU KUURE¹, KIRSI SAINIO¹, SEPPO VAINIO² AND HANNU SARIOLA¹

¹Developmental biology laboratory, Institute of Biomedicine, Biomedicum Helsinki and ²Biocenter Oulu, Department of Biochemistry, University of Oulu

Notch signalling pathway is crucial for different cell fate decisions during embryogenesis. We have analysed this pathway during kidney development. In the expression screen Notch2 receptor was found to be expressed in embryonic kidney in tubulogenic mesenchyme and in the branching ureter whereas its probable ligand Jagged1 localised to early pretubular structures and to ureteric bud tips. This indicates two possible roles for Jagged1-activated Notch signalling in the early kidney development: the regulation of ureteric branching morphogenesis and control of epithelial differentiation of the tubulogenic mesenchyme. To test the function, we utilised Hoxb7 promoter to drive Jagged1 expression to Wolffian duct and its derivative, the ureter. The renal phenotype in these animals is variable but correlates with the transgene copy number. In the severe cases transgenic mice die postnatally either due to renal aplasia or severe hypoplasia. Animals with 5 or more copies of the transgene show the most severe phenotype, a complete renal aplasia already at E13. At E11 these animals have a metanephric mesenchyme but the ureteric bud fails to invaginate the mesenchyme leading to apoptotic degeneration of the kidney rudiment. Mice with 2-3 copies of the transgene show hydroureters with dilated pelvis and often unilateral hypoplasia. In vitro such Jagged1 transgenic kidneys develop poorly and show a severe ureter branching defect or supernumerary budding from the Wolffian duct. The mildest phenotype is characterised by hypoplastic kidneys with some disorganisation in the overall structure such as mislocated glomeruli in the medulla and a thin differentiation zone in the cortex. All different renal phenotypes are possible consequences of defective ureter branching. This conclusion is supported by the alterations in GDNF and Bmp expression patterns in the Jagged1-overexpressing kidneys. However, at this point we cannot completely rule out the possible disruption of mesenchymal differentiation in Jagged1 transgenics.

IDENTIFICATION OF A NOVEL SECRETED BMP ANTAGONIST AND ITS REGULATION BY BMP, FGF AND SHH DURING ECTODERMAL ORGAN DEVELOPMENT

JOHANNA LAURIKkala, YOSHIAKI KASSAI, LEILA PAKKASJÄRVI,
IRMA THESLEFF AND NOBUYUKI ITOH

Institute of Biotechnology, PO Box 56, 00014 University of Helsinki, Finland

More than 30 BMPs are known and they regulate embryonic development in all animals and practically in all tissues and organs. It has become apparent that the fine tuning of BMPs is critical for a variety of their functions for example in neural induction, limb morphogenesis and skeletal development. The functions of BMPs are regulated at many levels and include intracellular, cell surface associated as well as extracellular modulators. An increasing number of secreted BMP antagonists have been described during the last ten years. In vertebrates such proteins include noggin, chordin, chordin-like, follistatin, FSRP and DAN/ Cerberus protein family. We have identified a mouse cDNA encoding a novel secreted BMP inhibitor, which shows a striking expression pattern in developing ectodermal organs including teeth, vibrissae and hair follicles. This inhibitor is most homologous (~37% amino acid identity) to sclerostin involved in bone formation. Gene transcripts were observed at all stages of tooth and hair development analysed. Interestingly, expression was intense in the tooth germ at E14, but in its center there was a round completely negative area with sharp boundaries. This area did not correspond to anatomical tissue boundaries but it included the enamel knot in the middle and several surrounding epithelial and mesenchymal cell layers. We also analysed the regulation and function of the inhibitor in cultured tooth germs. The recombinant protein antagonized the BMP-mediated induction of *Msx2* expression. BMP2 and -7 were capable of affecting the transcription of their own antagonist and this was prevented by SHH and FGF4 but not by WNT6. We conclude that the BMP inhibitor integrates BMP signalling with the SHH and FGF signal pathways and contributes in defining the exact spatiotemporal domain of BMP target field around the ectodermal signalling centers.

P38 PATHWAY SUPPRESSES CELL SURVIVAL BY INDUCING DEPHOSPHORYLATION OF MEK1,2

SONG-PING LI, M.R. JUNTILA, J. HAN, VELI-MATTI KÄHÄRI AND J. WESTERMARCK

University of Turku, Finland

Recent studies have provided evidence for opposite roles of the ERK1,2 and p38 mitogen-activated protein kinase (MAPK) pathways in the regulation of cellular survival. However, the molecular mechanism how these pathways oppose each other in the regulation of cell survival is not fully understood. Here, we have examined the mechanisms of p38 MAPK-mediated apoptosis induced by cell stress. The activation of p38 by arsenite was followed by immediate inactivation of MEK1,2→ERK1,2 survival pathway in both human skin fibroblasts and rat primary neurons. Inhibition of p38 activity by SB203580 and by adenoviral expression of dominant negative forms of p38 α and p38 β ; potently inhibited arsenite-elicited dephosphorylation of MEK1,2 and apoptosis. Activation of p38 by adenovirally expressed constitutively active MKK3b resulted in potent dephosphorylation of MEK1,2 and subsequently apoptosis. In contrast, activation of p38 by MKK6b had no effect on MEK1,2 activity or on cell viability, indicating distinct roles for MKK3b and MKK6b in the regulation of ERK1,2 pathway and cell survival. Interestingly, p38-mediated MEK1,2 inactivation was not detected in a series of cancer cell lines suggesting that this inhibitory mechanism of ERK1,2 signaling pathway is suppressed in the course of malignant transformation to promote cell survival. Taken together, the results of this study provide novel mechanistic insight to explain the opposing effects of ERK1,2 and p38 pathways in the regulation of apoptosis.

TRANSCRIPTION FACTORS GATA2 AND GATA3 IN INNER EAR DEVELOPMENT

KERSTI LILLEVÄLI^{1,2}, TANJA MATILAINEN², ILLAR PATA¹,
ALAR KARIS¹ AND MARJO SALMINEN²

¹*Institute of Zoology and Hydrobiology, Tartu University, Vanemuise 46, Tartu
Estonia and* ²*Institute of Biotechnology, University of Helsinki, PO Box 56, Helsinki,
Finland*

Transcription factors GATA2 and GATA3, members of the GATA family have been shown to play an important role during early stages of vertebrate development. Targeted mutations in these GATA-genes in mice result in embryonic lethality at embryonic stage 10.5. Both genes are expressed from early on during inner ear development. The inner ear phenotype in GATA2 mutant mice has not been analyzed yet. In GATA3 mutant embryos the morphological development of the ear is blocked at a very early stage. The molecular basis of this developmental arrest is not understood since we do not know the target genes regulated by GATA3 factor. In addition to morphogenesis, GATA3 is thought to control hair cell differentiation and we have shown previously that cochlear and vestibular efferent nerve fibres, with cell bodies in rhombomere 4, fail to target the ear in the absence of GATA3. Mutations in the human GATA3-gene result in the HDR syndrome with multiple developmental disorders including deafness. In this study we describe the spatio-temporal expression of the gene encoding transcription factor GATA2 compared with GATA3 and also show the epigenetic relationships between these genes as well as between these and the other genes in the course of inner ear development.

THE BIOLOGICAL SIGNIFICANCE OF U12-DEPENDENT SPLICING IN MOUSE

XIAOJUAN MENG, HELI PESSA AND MIKKO FRILANDER

Institute of Biotechnology, PO Box 56, 00014 University of Helsinki, Finland

Most genes in higher eukaryotes are split and contain non-coding intron sequences that separate the information-containing exon sequences. A large molecular machine called a spliceosome removes introns from the pre-mRNA molecules following transcription. Intron removal is not only absolutely necessary for the formation of functional, protein-coding mRNA molecules but also provides an important post-transcriptional mechanism to regulate eukaryotic gene expression. Majority of introns are excised by the U2-dependent spliceosome. Spliceosome is composed of five small nuclear RNAs (snRNAs) and numerous proteins. In addition to the standard U2-dependent spliceosome, many multicellular organisms contain a parallel U12-dependent spliceosome. This spliceosome specifically removes a minor subset of noncoding intron sequences during pre-mRNA processing phase of the eukaryotic gene expression pathway. The significance of this machinery, which affects the expression of less than 1% of all genes, is unknown.

The key question in our research is why the metazoans maintain two parallel pre-mRNA splicing machineries. Recent studies have suggested a regulatory role for the U12-dependent spliceosome, possibly during the development of an organism. We use mouse as a model system to study the U12-dependent spliceosome. Our very recent data suggest that the expression of protein components of this machinery vary dramatically in different tissues. Additionally, we observed a moderate variation with one of the snRNA species (U4atac). The most interesting tissue in this sense is testis in which the proteins of the U12-dependent system are most highly expressed of all the tissues examined. Further more, they are specifically localized in pachytene spermatocytes, suggesting a possible role in the regulation of spermatogenesis during meiotic phase. This study provides the first clue of the possible functions of U12-dependent spliceosome in a whole organism, and also suggests that mouse germ cell lines or testis can serve as the model systems to study the regulation of gene expression by the U12-dependent spliceosome.

EMX2 IS REQUIRED FOR PROPER WOLFFIAN DUCT FORMATION

MAXIM MOSHNYAKOV, KIRSI SAINIO AND HANNU SARIOLA

*Developmental Biology Program, Biomedicum Helsinki, PO Box 63, FIN-00014
University of Helsinki, Finland*

We have studied development of the Wolffian ducts (WD) in the *Emx2*-deficient mice during embryonic days E9 to E14. From the very early stages of its development Wolffian duct is abnormal, as shown by whole mount immunohistochemistry. We therefore analysed more closely WD of *Emx2* deficient mice by electron microscopy, BrdU incorporation, and apoptosis test (TUNEL staining). We found that the WD phenotype in *Emx2* ^{-/-} mice is due to overproliferation and possibly shortage of apoptosis in the WD cells. WD in the mutants is a thick, blebbing tube, where cells are unpolarised, and no lumen is formed. Mutant WD is not able to send out a proper ureteric bud and hence, to induce metanephric mesenchyme. The data suggest that *Emx2* might be one of regulators of cell proliferation and/or apoptosis.

STIMULATION OF ECTODERMAL ORGAN DEVELOPMENT BY ECTODYSPLASIN-A1

TUIJA MUSTONEN, J. PISPA, M. MIKKOLA, M. PUMMILA, A. KANGAS,
L. PAKKASJÄRVI, R. JAATINEN, AND I. THESLEFF

Institute of Biotechnology, PO Box 56, 00014 University of Helsinki, Finland

Ectodysplasin, a member of TNF superfamily, is required for normal development of ectodermal organs. Overexpression of ectodysplasin-A1 (Eda-A1) under K14 promoter resulted in alterations in a variety of ectodermal organs. Hair development was initiated continuously from E14 until birth and in addition the transgenic mice had supernumerary teeth and mammary glands, phenotypes not reported previously in transgenic mice. Also hair composition and structure was abnormal and the cycling of hairs was altered so that the growth phase (anagen) was prolonged. Both hairs and nails grew longer than normal. Molar teeth were of abnormal shape and enamel formation was severely disturbed in incisors. Furthermore, sweat gland function was stimulated and sebaceous glands were enlarged. We conclude that ectodysplasin-Edar signaling has several roles in ectodermal organ development controlling their initiation, as well as morphogenesis and differentiation (Mustonen et al., 2003).

STRATEGY TO IDENTIFY TARGET GENES OF THE CANONICAL WNT SIGNALING PATHWAY

PETRI ITÄRANTA^{1,2}, FLORENCE NAILLAT^{1,2}, ANTTI PAJUNEN¹ AND
SEPPÖ VAINIO³

¹Department of Biochemistry, ²Biocenter Oulu, P.O.Box 3000, FIN-90014 University of Oulu, Finland and ³Department of Biochemistry & Biocenter Oulu, Faculty of Science and Medicine, PO Box 3000, FIN-90014 University of Oulu, Finland

Embryonic development depends on cell-cell and tissue interactions that are mediated in part by secreted signals such as the Wnts. Substantial experimental evidence points to a critical role for Wnts at all key developmental steps. Wnt signal transduction appears complex and has both canonical and non-canonical pathways. The canonical signaling involves a complex of β -catenin and transcription factor TCF-1 that regulates expression of target genes. To date little is known about the downstream genes that are regulated by the Wnts. To identify such genes, we generated a cell line that expressed a dominant positive TCF. This was made as a fusion protein between TCF activating domain of β -catenin and TCF-1. The fusion protein localized to the nucleus and bound to the consensus TCF-1 site A/TA/TCAAAG as revealed by electrophoretic mobility shift assay. Chromatin immunoprecipitation with an antibody against β -catenin lead to the identification of a number of fragments with the TCF binding site and they were partially sequenced. Several cDNAs were generated by RT-PCR to analyze changes in gene expression as a response to Wnt pathway mediated induction. Current studies revealed partial correlation to the expression of TCF-1 in organs such as embryonic lung. Taken together, we have identified a panel of genes that have a conserved TCF binding sequence and these may be considered as putative Wnt target genes of the canonical signal transduction pathway.

CIRCULATING CELLS CONTRIBUTE LITTLE TO THE DEVELOPMENT AND MAINTENANCE OF NONHEMATOPOIETIC BOVINE TISSUES, BUT MAY BE IMPORTANT IN REGENERATION

M. NIKU, T. PESSA-MORIKAWA, L. ILMONEN AND A. IIVANAINEN

Department of Basic Veterinary Sciences, FIN-00014 University of Helsinki, Finland

Many recent reports suggest that surprisingly multipotent cells exist in the bone marrow and other tissues of adult mammals. However, the physiological significance of this is unclear. We have used chimeric freemartin cattle to investigate the integration of circulating cells in developing and adult nonhematopoietic tissues in an unmanipulated large mammal. A freemartin is the female of sex-mismatched bovine twins. In cattle twin pregnancies, placental anastomoses typically cause an exchange of circulating cells, including bone marrow stem cells. This results in persistent blood chimerism. In this study, we combined Y-chromosome specific in situ hybridization with pan-leukocyte labeling (CD45 immunohistochemistry and mistletoe lectin I histochemistry) to visualize the donor-derived (Y^+) nonhematopoietic cells in freemartin tissues.

We detected Y^+ cells in all tissues examined in 12 freemartins. However, most Y^+ cells were leukocytes. In 11 of 12 freemartins, the donor contribution to nonhematopoietic lineages was minor, with less than 1% of all nonhematopoietic cells being donor-derived in most tissues, and majority of these probably representing fibroblasts and other interstitial cells. In cardiac and smooth muscle, renal epithelia, and epidermis the average frequency was 0.1% or less, and in brain, less than 0.001%. No cell clusters indicating local expansion were seen. However, significantly greater numbers of Y^+CD45^+ cells were detected in granulation tissue in methylcellulose pads implanted subcutaneously in one freemartin. In 1 of 12 freemartins, major donor contribution was seen in several nonhematopoietic lineages (19% in intestinal epithelium).

In conclusion, circulating cells do not appear significant in the development and physiological turnover of nonhematopoietic bovine tissues, but may be important in regeneration and other special conditions.

PECAM-1 (CD31) IS EXPRESSED IN THE HUMAN ADENOID CRYPT EPITHELIUM

MERVI PAJUSTO, AARO MIETTINEN, JUSSI TARKKANEN AND
PETRI MATTILA

Department of Otorhinolaryngology, Helsinki University Central Hospital, Finland

Background: Transport of foreign antigens to immune cells in the adenoids and tonsils is thought to occur via specialized epithelial cells residing in the follicular crypts. Anatomically, the location of the follicular crypt between lymphoid follicles resembles the location of the lymph node efferent vessel, which conveys antigens and inflammatory cells to the lymph node.

Object of the study: To search for antigens characteristic for the crypt and that might be related to function of the crypt. The expression of various adhesion molecules was studied in human adenoid epithelium.

Methods: Human adenoidal paraffin embedded tissue sections were stained with mouse monoclonal antibodies to cytokeratin-5/6 and cytokeratin-8. Frozen tissue sections were stained with mouse monoclonal antibodies to V-CAM, PECAM-1 and rabbit anti-pancytokeratin antibodies.

Results: Immunohistochemical staining of the follicular crypt epithelium with anti-cytokeratin antibodies was characteristic for simple epithelia: the basal layer of the epithelium was positive for cytokeratin-5/6 and the apical layer for cytokeratin-8 antibody. Some epithelial cells residing in the outer opening of the follicular crypt were positive for anti-V-CAM antibody whereas follicular crypt epithelial cells residing at the base of the crypt were positive for anti-PECAM-1 antibody. Double immunofluorescence staining revealed that the cells positive for anti-PECAM-1 antibody were also positive for anti-pancytokeratin antibody.

Conclusion: The ordered expression of V-CAM and PECAM-1 in the follicular crypt epithelium suggests that the orifice and base of the follicular crypt may have specialized functions. Further, as PECAM-1 and V-CAM function as adhesion molecules, their expression in the follicular crypt epithelial cells suggests that they may have a role in the infiltration of antigen presenting leucocytes in the crypt epithelium.

EARLY SPONTANEOUS CYTODIFFERENTIATION IN HUMAN EMBRYONIC STEM (ES) CELLS INTO EPITHELIAL-STROMAL AGGREGATES AS REVEALED BY ELECTRON MICROSCOPY

LAURI PELLINIEMI¹, MILLA MIKKOLA², ANNE-MARIE STRÖMBERG²,
JOSE INZUNZA² AND OUTI HOVATTA²

¹*Laboratory of Electron Microscopy, University of Turku, FIN-20520 Turku, Finland*
and ²*Department of Obstetrics and Gynaecology, Karolinska Institutet, Huddinge
University Hospital, S-14 86 Stockholm, Sweden*

To study stem cell viability and differentiation under culture conditions we prepared human ES cells from a blastocyst, which could not be used in clinical in vitro fertilisation treatment. The experiments were started after approval of Ethics Committee and informed consent from the couples. The line HS181 was derived using human post-natal fibroblasts as feeder cells. The inner cell mass of a 5-day-old blastocyst was isolated immunologically, and cultured on the feeder layer in medium Knock-out D-MEM (Gibco) containing 15% foetal calf serum for an initial period of nine days. The growing cells were then passaged to new plates at 5-7-day intervals after mild enzymatic and partially mechanical disaggregation. These cells have the characteristics of ES cells, and for the time being they have been in continuous culture for 10.5 months. At passage level 12, we took cell aggregates showing partial early differentiation, as observed on an inverted light microscope, for electron microscopic analysis of cellular and subcellular differentiation. The aggregates were initially fixed in 2 % glutaraldehyde fixation in 0.1 mol/L Na-cacodylate-HCl buffer pH 7.3, which contained 0.1 mol/L sucrose. After postfixation in aqueous 1 % OsO₄ containing 1.5 % K-ferrocyanide, the specimens were dehydrated in ethanols and embedded in epoxy resin according to standard methods. Some of the aggregates had clearly organised into two different tissues: the surface epithelium and internal stroma. The epithelium consisted of pseudotrified columnar epithelial cells, which had ordinary cytoplasmic organelles including groups of darkly stained glycogen granules. The apical cytoplasm formed several small irregular microvilli and adherens-type intercellular junctions along the side of the cell apex. The basal surface was also irregular, but there was no basement membrane, which is typical of early developing embryonic epithelia in vivo. The stromal cells inside the aggregates were large and contained ordinary cytoplasmic organelles, especially abundant was the granular endoplasmic reticulum, which is a sign of active protein synthesis. The extracellular stromal space was filled with extracellular material organised into a meshwork of irregularly arranged fibers. Inside and outside the aggregate there are small residual bodies, which consisted of disorganised cytoplasmic material and represent thus remnants of dead cells. The aggregate as a whole resembles histologically the bilaminar blastocyst, where similar epithelial and stromal cells have differentiated into two different tissues. The present culture conditions provide thus a good basis for further development on goal-oriented regulation of ES-cell differentiation.

THE ROLE AND SIGNALLING MECHANISMS OF PLEXINS DURING MOUSE EMBRYOGENESIS

NINA PERÄLÄ AND HANNU SARIOLA

Developmental Biology, Institute of Biomedicine, PO Box 63, FIN-00014 University of Helsinki, Finland

Plexins are large transmembrane proteins that are receptors for semaphorins, either alone or as a complex with neuropilin-1 or -2. Seven different mouse plexin genes have been found so far: plexins A1, A2, A3, B1, B2, B3 and C1. Plexins have mostly been studied in the context of the developing nervous system, where they mediate the chemorepulsive signals of secreted semaphorins and cause the collapse of axon growth cones. However, the broad expression of plexins in different embryonic tissues indicates functions in the development of other organs as well. We have studied the expression of plexin mRNAs during the development of a mouse embryo using automated non-radioactive in situ hybridisation, both on slides and on whole mounts. The expression of the A-subfamily plexins is mainly restricted to the developing nervous system, as is the expression of Plexin-C1. The expression of the three plexin-B genes is mainly outside of the nervous system. For example, Plexin-B2 mRNA is expressed in the kidney of both E16 and E18 embryos, as well as in developing bone structures and undifferentiated mesenchyme (E14). The expression pattern of Plexin-B2 and the fact that plexins of the B-subfamily interact with Met receptor tyrosine kinase, make it an interesting candidate for the unidentified receptor in Ret-independent GDNF (glial cell line-derived neurotrophic factor) signalling. GDNF is a crucial signalling molecule for the development of the kidney, and it typically activates a complex of GFRA1- and Ret-receptors. In non-Ret signalling, GDNF activates Met through the GPI-linked GFRA1, Src and a yet unidentified receptor.

NOTCH SIGNALING IN DIFFERENTIATION OF NEURONAL PRECURSOR CELLS

KATJA PILTTI, SATU KUURE, SEPPO VAINIO, HANNU SARIOLA AND
KIRMO WARTIOVAARA

*Developmental Biology, Institute of Biomedicine, Biomedicum Helsinki, PO Box 63,
FIN-00014 University of Helsinki, Finland*

Notch signaling is essential for the maintenance of neuronal progenitor pools in developing and adult central nervous system (CNS). Notch pathway also inhibits neurogenesis and oligodendrogenesis by directing differentiation into astroglial direction. However, the mechanisms by which Notch signaling activates astrocyte specific gene expression are not yet known. We have studied *in vitro* neuronal progenitor cells derived from brains of Jagged1 transgenic embryos. In our culture system we have found differences in cell proliferation, renewal (ability to form colonies) and differentiation between transgenic and wildtype littermates. Initially transgenic neuronal progenitors proliferate faster than controls but in long term culture the proliferation of Jagged1 progenitors decreases when compared to controls. Beside this, single transgenic progenitor cells diminish their ability to form colonies during prolonged culture. Our results show that overexpression of Jagged1 promotes astroglial and inhibits neuronal differentiation of neuronal precursors *in vitro*.

ECTODYSPLASIN, EDAR AND TNFRSF19 ARE EXPRESSED IN COMPLEMENTARY AND OVERLAPPING PATTERNS DURING MOUSE EMBRYOGENESIS

JOHANNA PISPA, MARJA L. MIKKOLA, TUIJA MUSTONEN AND IRMA THESLEFF

Developmental Biology Programme, Institute of Biotechnology, University of Helsinki, 00014 Finland

Ectodysplasin (Eda), a member of the tumor necrosis factor (TNF) superfamily, and its receptor Edar are necessary components of ectodermal organ development. Malfunction of the Eda or Edar gene causes hypohidrotic ectodermal dysplasia (HED) in humans, and in mice the Tabby or downless mutants respectively. These disorders are characterised by deficient hair development, hypodontia, and abnormal formation of several glands, e.g. the sweat glands. Analysis of the ectodysplasin and Edar expression patterns has shown that during mouse hair and tooth development they may be involved in signalling between separate epithelial compartments. Here we have analysed ectodysplasin and Edar expression in other embryonic mouse tissues, and show that they are expressed in separate epithelial or epithelial/mesenchymal compartments in the developing brain, the lacrimal gland, and the salivary gland. We also studied the expression pattern of a related TNF receptor, TNFRSF19, and show that it is expressed in an overlapping domain with Edar in the tooth, mammary gland, whiskers, and limb bud suggesting a potentially redundant role. In addition, transgenic mice expressing a reporter gene under the ectodysplasin promoter were generated. Analysis of the reporter gene expression revealed that the promoter is insufficient for reproduction of the endogenous expression pattern.

**INVOLUTION OF MAMMARY GLAND IN P450
AROMATASE EXPRESSING TRANSGENIC MALE MICE
IS ASSOCIATED WITH INACTIVATION OF MITOGEN-
ACTIVATED PROTEIN KINASE CASCADE**

MATTI POUTANEN, XIANGDONG LI, SARI MÄKELÄ AND
RISTO SANTTI

*Department of Physiology, Institute of Biomedicine, University of Turku, 20520
Turku, Finland*

We have previously shown that the mammary glands of AROM+ males undergo ductal and at 9 months the alveolar development morphologically resembling that of terminally differentiated female mammary glands. Interestingly, the mammary gland development is strongly suppressed by a 6 week-long aromatase inhibitor treatment (started at 4 months of age), and the mammary gland also undergone involution in aging mice analyzed at 12 month-old AROM+ males. The involution is marked by the disappearance of alveolar structures and the majority of the tertiary side branches of the ducts. However, estrogen receptors were still expressed in the epithelial cells despite of the declined estrogen stimulus in both of these models. However, in contrast to the inhibitor-treated AROM+ mice, androgen receptor was not expressed in the involuted breast epithelium of the aged AROM+ males. The Stat 5 (signal transducer and activator of transcription 5) proteins are activated both in 4 months and 12 months old AROM+ male mammary gland, but not in the inhibitor treated mice. A common feature for the mammary gland involution/regression both in inhibitor treated mice and old AROM+ mice is the decrease in estrogen stimulus and the reduced activation status (phosphorylation) of the p42/44 MAPK (ERK 1/2) signaling cascade, demonstrated immunohistologically. Our data, thus, indicate that the involution of mammary gland in AROM+ male mice is associated with a decline in estrogen action, and with the inactivation of MAPK pathway. The AROM+ model is a valuable tool for better understanding the cellular and molecular mechanisms involved in the involution of mammary gland in male, and to understand the signaling mechanisms essential for mammary gland development.

PROGRESSION OF CALVARIAL BONE DEVELOPMENT REQUIRES FOXC1 REGULATION OF MSX2 AND ALX4

RITVA RICE, DAVID P. C. RICE, BJORN R. OLSEN AND IRMA THESLEFF

*Craniofacial Development, King's College London, Guy's Tower, floor 28, London,
SE1 9RT, United Kingdom*

Calvarial bones form by direct ossification of mesenchyme. This requires condensation of mesenchymal cells which then proliferate and differentiate into osteoblasts. Congenital hydrocephalus (ch) mutant mice lack the forkhead/winged helix transcription factor Foxc1. In ch mutant mice, calvarial bones remain rudimentary at the sites of initial osteogenic condensations. In this study we have localized the ossification defect in ch mutants to the calvarial mesenchyme which lacks the expression of transcription factors Msx2 and Alx4. This lack of expression is associated with a reduction in the proliferation of osteoprogenitor cells. We have previously shown that BMP induces Msx2 in calvarial mesenchyme (Development 125, 1241-1251, 1998). Here we show that BMP also induces Alx4 in this tissue. We also show that BMP-induced expression of Msx2 and Alx4 requires Foxc1. We therefore suggest that Foxc1 regulates BMP-mediated osteoprogenitor proliferation and that this regulation is required for the progression of osteogenesis beyond the initial condensations in calvarial bone development.

MALE GERM CELL-SPECIFIC TRANSCRIPTION OF THE MURINE PIASX GENE IS GOVERNED BY A SHORT GC-RICH PROXIMAL PROMOTER REGION

HENRIKKI SANTTI, LAURA MIKKONEN, SIRPA HIRVONEN-SANTTI,
JORMA TOPPARI, OLLI A. JÄNNE, AND JORMA J. PALVIMO

*Institute of Biomedicine, University of Helsinki, PO Box 63, FIN-00014 Helsinki,
Finland*

PIASx (protein inhibitor of activated STAT) acts as an E3 ligase in the protein sumoylation pathway. The PIASx gene is highly expressed in the testis, and the encoded protein may thus be involved in the regulation of spermatogenesis. To examine the factors governing the regulation of PIASx gene expression in the testis, we have isolated the promoter region of the murine PIASx gene and shown that a fragment comprising nucleotides from -168 to +76 relative to transcription start site is sufficient for basal promoter activity in reporter gene assays. Even though *in vitro* assays failed to reveal differences in promoter activity between testis- and non-testis-derived cell lines, the proximal promoter encompasses the elements necessary for a strong male germ cell-specific expression in transgenic mice *in vivo*. The proximal promoter is GC-rich, lacks a TATA box and contains a GC box as well as a potential binding site for Ets proteins. The GC box forms *in vitro* a major DNA-protein complex with testicular nuclear extracts, but not with liver nuclear extracts. This complex contains Sp1, Sp2, and Sp3 transcription factors. In conclusion, we have characterized the minimal PIASx promoter that can be used for highly specific targeting of transgene expression to male germ cells.

FGFR1 REGULATES PATTERNING OF THE PHARYNGEAL REGION

NINA TROKOVIC¹, RAS TROKOVIC¹, PETRA MAI,
ANDREW P. MCMAHON² AND JUHA PARTANEN¹

¹*Institute of Biotechnology, PO Box 56, University of Helsinki, 00014, Helsinki, Finland,* ²*Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Avenue Cambridge, MA 02138, USA*

Hypomorphic fibroblast growth factor receptor 1 (Fgfr1) mutants were produced by targeting insertion of neo cassette into the intron 7 of Fgfr1 gene. This insertion results in lowered expression level of Fgfr1. Mutants die neonatally and exhibit multiple defects, including those in the craniofacial region. They have underdeveloped second branchial arch (BA) and deficiencies in skeletal structures deriving from the neural crest cells (NCCs) of the first and second BAs. We have shown that the initial segmentation and patterning of the hindbrain is unaffected in hypomorphs. Large number of NCCs migrate from rhombomere 4 but fail to enter the arch. Increased apoptosis was also observed in this region. To answer the question whether Fgfr1 is required cell autonomously in the NCCs, we took advantage of the Cre-recombinase technique. We inactivated conditional Fgfr1flox allele specifically in NCC progenitors with Cre recombinase expressed by Wnt1-Cre transgene. To our surprise, Wnt1-Cre/+; Fgfr1Flox/Flox mice did not show BA defects. In addition, using Wnt1-Cre transgene, we tried to rescue Fgfr1n7 allele (where neo cassette is flanked by loxP sites) specifically in NCCs. Wnt1-Cre/+; Fgfr1n7/n7 mice could not be distinguished from the Fgfr1 n7/n7 hypomorphs. This strongly suggests that Fgfr1 is needed for BA development in a cell type other than NCC. Importantly, we found that Fgf3 is downregulated in ectoderm covering the presumptive second BA of hypomorphic Fgfr1 n7/n7 mutants at a stage prior to NCC entry. These results suggest that Fgfr1 patterns the pharyngeal region to create a permissive environment for neural crest cell migration.

**EVOLUTIONARILY CONSERVED *DROSOPHILA*
TMB PROTEIN: A POSSIBLE COMMON PLAYER IN
PRODUCTION OF CYTOPLASMIC AND
MITOCHONDRIAL RIBOSOMES**

TIMOFEY TSELYKH, CHRISTOPHE ROOS AND TAPIO I. HEINO

*Institute of Biotechnology, University of Helsinki, PO Box 56, 00014 University of
Helsinki, Finland*

The nucleolus and mitochondrion are two cellular sub-compartments with distinct functions and are physically separated by nuclear and mitochondrial membranes. We report the discovery of a novel gene *tom thumb* (*tmb*) in *Drosophila*, which is highly conserved in multicellular animals and shows no homology to any protein with known function. It encodes a protein of 107 amino acids which is localized within the cell both in nucleolus and mitochondria. The bovine homologue of Tmb has been reported to be a component of the large subunit of mitochondrial ribosome, which supports our results in subcellular localization of the protein. Null *tmb* mutants die as small larvae and move slowly. This phenotype is related to the possible function of the *tmb* gene in the synthesis of cytoplasmic ribosomes and mitoribosomes, which is essential for the viability of the organism. Nucleolar distribution of the protein in larval tissues appears to be dependent on the metabolic state of the cell: in proliferating imaginal cells Tmb is not localized to nucleoli opposite to transcriptionally highly active cells in such tissues as salivary glands where the protein is found exclusively in nucleoli. The central region of the Tmb protein sequence shows high similarity to RNA-binding KOW-motif. This indicates a possible interaction of Tmb with rRNAs which might play a crucial role in the synthesis of both cytoplasmic and mitochondrial ribosomes.

**THE FLEXIBLE REGULATION OF THE EPITHELIAL
STEM CELL NICHE IN THE TOOTH RESULTED IN THE
EVOLUTION OF DIFFERENT TOOTH TYPES**

MARK TUMMERS AND IRMA THESLEFF

*Developmental Biology Program, Institute of Biotechnology, PO Box 56, FIN-00014
University of Helsinki, Finland*

The rodent incisor grows continuously throughout its lifetime. The epithelial stem cell niche is located at the apical end of the tooth and its progeny gives rise to the ameloblasts that form the hard enamel. Mesenchymal FGF10 supports the stem cell niche, and epithelial notch signaling is involved in the differentiation of the progeny. The molar of the sibling vole also grows continuously unlike the molar of the mouse. The vole molar has a similar regulatory system as the rodent incisor, whereas Notch and FGF10 signaling is mainly absent in the mouse molar, which stops growing and develops roots. By simply altering the regulation of the stem cell niche evolution had the possibility to generate different tooth types. In continuously growing teeth the stem cells the stem cell niche is maintained forever, in high crowned molars its lifetime is extended and in low crowned molars quickly ended.

PARTIAL FEMALE-TO-MALE SEX REVERSAL IN WNT-4 DEFICIENT MICE IS DUE TO ECTOPIC TESTOSTERONE PRODUCTION IN FEMALE GONAD

M. HEIKKILÄ¹, R. PRUNSKAITE¹, H. PELTOKETO¹, J. LEPPÄLUOTO²
AND SEPPO VAINIO³

¹*Biocenter Oulu and Department of Biochemistry,* ²*Department of Physiology, PO Box 3000, FIN-90014 University of Oulu, Finland and* ³*Biocenter Oulu and Department of Biochemistry, Faculty of Science and Medicine*

Phenotype of Wnt-4 knock out mice indicates that this secreted signal is essential for development of several steroidogenic tissues such as the pituitary gland, ovary and adrenal gland. Our recent data showed that Wnt-4 is expressed in the cortex of the presumptive adrenal gland, and Wnt-4 deficiency leads to a dysfunctional gland due to reduced aldosterone production consistent with the diminished P450 aldosterone synthase gene expression. The pituitary-adrenal axis is enhanced as concentrations of ACTH and corticosterone are also enhanced in the mutants. Expression of Cyp17 suggests that the adrenal gland of the female Wnt-4 deficient embryos is masculinized. Wnt-4 is the first identified key signal for female development. Both female and male Wnt-4 deficient mice lack the female Müllerian ducts and females have ectopic Wolffian ducts at birth. Masculinization of the female Wnt-4 knock out mice appears also as ovotestes, surrounded by epididymis, fat body and primitive seminiferous tubules similarly to testes. The gonads of female Wnt-4 deficient mice express the enzymes of testosterone biosynthesis pathway. Our results now show that the ectopic testosterone is a likely reason for the masculinization of the Wnt-4 deficient females. Testosterone can be measured from the plasma and the mutant ovary and anti-androgen, flutamide treatment results in eased phenotype of the mutant females. Such treated Wnt-4 deficient female embryos lack Wolffian ducts and their derivatives or they are more weakly developed than untreated mutant female mice at birth. We may conclude that Wnt-4 functions in the ovary by maintaining the female phenotype by repressing the formation of testosterone producing cells and/or testosterone biosynthesis.

RUNX2 IS REQUIRED FOR FGF AND SHH SIGNALING DURING TOOTH MORPHOGENESIS

XIUPING WANG, THOMAS ÅBERG, TAKASHI YAMASHIRO AND IRMA THESLEFF

*Developmental Biology Programme, Institute of Biotechnology, PO Box 56,
University of Helsinki, Finland*

Runx2 (Cbfa1) is a runt domain transcription factor that is essential for osteoblast differentiation and tooth morphogenesis. Mutations of one allele of Runx2 gene in humans are responsible for cleidocranial dysplasia, a syndrome characterised by hypoplasia of intramembranous bones and supernumerary teeth. Runx2 knockout mice completely lack bone formation. Their teeth arrest at late bud stage. Our previous studies have shown that Runx2 is expressed in the dental mesenchyme and is regulated by epithelial signals, like Fgfs (D'Souza et al. 1999). In order to clarify the role of Runx2 during tooth development, we have performed extensive in situ hybridization to search for the downstream targets of Runx2 gene. We found that expression of Fgf3 and Shh was downregulated in Runx2 mutant teeth. Bead Induction experiments have shown that Runx2 is required in the dental mesenchyme mediating epithelial signals and involved in both FGFs and SHH signaling pathways regulating expression of Fgf3 and Patched (Ptc1), respectively. In addition, we found differences in mutant upper and lower molars. Most of enamel marker genes are expressed normally in mutant upper molars, while reduced or missing in lower ones. Expression of Ptc1 and Gli3 was downregulated only in mutant lower molars, but remained normal in upper teeth. In contrast, Runx3 was upregulated in upper molars, which may compensate for Runx2 in the mediation of Shh signals. We conclude that Runx2 functions in the dental mesenchyme and mediates both FGF and Shh signaling pathways during tooth morphogenesis. We propose that the absence of Runx2 may have disturbed the balance between different signaling pathways that is critical for the transition of tooth bud to cap stage.

WNT-11 KNOCK OUT IS EMBRYONIC LETHAL POSSIBLY DUE TO CARDIAC DYSFUNCTION

LASSE VÄÄRISKOSKI, JUHA RÄSÄNEN, HANNA LESKINEN,
HEIKKI RUSKOAHO, ANDY MCMAHON AND SEPPO VAINIO

*Department of Biochemistry, PO Box 3000, University of Oulu, FIN-90014, Oulu,
Finland*

Several lines of evidence point to an important role for Wnt signaling in heart development. Wnts contribute to the specification of the presumptive heart primordia and determine the field in which organogenesis is initiated. Wnt pathway is also implicated in pathophysiological conditions of the heart but the nature of the endogenous Wnts involved are not known. Wnt-11 has been implicated in heart development of vertebrates. In the frog and chick embryos Wnt-11 gene is expressed in cells that form the heart and in explants Wnt-11 signaling induces contractile behavior.

To get an insight into Wnt-11 function its expression was analyzed and function studied by generating a knock out mouse. Wnt-11 expression is detected in the early cardiac region, suggesting a developmental role. Wnt-11 deficient embryos revealed a role in kidney development (manuscript submitted). In 129SV genetic background Wnt-11 knock out is embryonic lethal and indicates other essential functions for Wnt-11. Our hypothesis is that Wnt-11 is functional in the heart. Preliminary ultrasonic analysis of knock-out mouse line embryos revealed defects in endocardial cushion tissue and function. Interestingly, in the CD-1 background the Wnt-11 knock-out phenotype is alleviated and we obtain 17% homozygous null individuals. Hence, there appears to be genetic modifiers of Wnt-11 function. The survived null individuals live through adulthood. However, ultrasonic and histological studies revealed a protrusion in the ventricle of the heart, and also hypertrophy of the heart muscle was evident if compared to wild type control litter mates. Taken together, the lack of Wnt-11 signaling leads to prenatal lethality possibly due to a cardiovascular defect.

IDENTIFICATION OF DEVELOPMENTAL CONTROL GENES REGULATED BY WNT-4 SIGNALLING

LASSE VÄÄRISKOSKI¹, FLORENCE NAILLAT¹, JUSSI VUORISTO²
AND SEPPO VAINIO¹

¹*University of Oulu, Department of Biochemistry and Biocenter oulu, Linnanmaa, 90570 Oulu, Finland and* ²*University of Oulu, Department of Internal Medicine & Biocenter oulu, PO Box 5000, FIN-90014 University of Oulu*

Wnt-4 is a signal that is critical for development of the ovary, the metanephros, pituitary-, mammary- and adrenal glands but very little is known about the target genes that are regulated by it. In the mouse kidney *Wnt-4* knock out leads to very specific phenotype; a lack of nephrons and kidney development fails likely due to this reason. In the gonad *Wnt-4* knock out is manifested by partial female to male sex reversal and the Müllerian duct does not develop and oocytes degenerate. These studies suggest that the *Wnt-4* knock out mouse line serves as an excellent tool to identify genes that are associated to nephrogenesis and female development, a process in which Wnt-4 acts as the first identified sex determinant. We have collected wild type and mutant tissues of gonads and kidneys, isolated RNA and analyzed changes in the expression of 12 000 genes by using the Affymetrix chips. By using this approach we have been able to identify close to 40 genes whose expression is changed significantly with out Wnt-4 signalling. The genes regulated by Wnt-4 appear to be different between the gonad and the kidney. Changes in expression will be analyzed further with real time PCR and *in situ* hybridization to obtain selected ones for further functional studies. Taken together, the Affymetrix approach has been successful and given a number of candidate genes that may regulate female and kidney development.

EPIDERMAL GROWTH FACTOR CONCENTRATIONS IN AMNIOTIC FLUID OF WOMEN WITH PRE-ECLAMPSIA OR FETAL GROWTH RESTRICTION

KATJA-ANNELI WATHÉN, ULF-HÅKAN STENMAN, HENRIK ALFTHÁN,
ERJA HALMESMÄKI AND PIIA VUORELA

Background and objective: Pre-eclampsia affects about 5 % of all pregnancies. The etiology of this multiorgan failure is not known. Neither are there any preventive nor therapeutic treatments available. Fetal well-being is often compromised by intra-uterine hypoxia and growth restriction. Pathophysiological features include both maternal and placental vascular endothelial dysfunction. Similar placental changes are found in the presence of fetal growth restriction (FGR). Epidermal growth factor (EGF), a mitogen for various tissues, is also expressed in the placenta and needed for placental growth in mice. The objective of this study was to determine whether the amniotic fluid (AF) concentrations of EGF would differ between healthy women and those with pre-eclampsia or FGR.

Methods: AF samples, collected for assessment of fetal lung maturity, were analysed for EGF concentrations by an immunofluorometric assay (IFMA) from women with pre-eclampsia (n=20, 31 ± 1 yrs), FGR (n=11, 29 ± 2 yrs) and healthy women (n=9, 29 ± 1 yrs) at gestational age 28-35 weeks.

Results: EGF level concentrations in AF of women with pre-eclampsia were higher (24,5 ± 12 ng/l) than those of healthy mothers (13,4 ± 7 ng/l, p<0,05). EGF concentrations in AF of women with FGR (20,2 ± 16 ng/l) did not differ from those of healthy or pre-eclamptic women.

Conclusions: Pre-eclampsia, but not FGR, is associated with increased amniotic fluid EGF concentrations. EGF may be associated with the pathophysiology of pre-eclampsia. It remains to be seen whether EGF provides a marker for assessing the risk of pre-eclampsia, or possibly a tool in predicting the prognosis of manifest disease.

COMPLETION OF SPERMATOGENESIS IS POSSIBLE IN THE ABSENCE OF LUTEINIZING HORMONE STIMULATED ANDROGEN PRODUCTION

FU-PING ZHANG, TOMI PAKARAINEN, MATTI POUTANEN,
JORMA TOPPARI AND ILPO HUHTANIEMI

*Institute of Biomedicine/Physiology, PO Box 63, FIN-00014 University of Helsinki,
Finland*

Luteinizing hormone (LH) and its cognate receptor (R), LHR, play a key role in the regulation of testicular androgen production, considered indispensable for spermatogenesis. In the present study, we analyzed the spermatogenesis in testes of mice with targeted disruption of the LHR gene (LuRKO mice) at different ages (2 and 12 months). Spermatogenesis was arrested at round spermatids of stage 7-8 at 2 months of age. Surprisingly, when the LuRKO mice were studied at the age of 12 months, spermatogenesis had developed further, until elongated spermatids at late stage 13-16. The testes at both ages remained cryptorchid, and the accessory reproductive organs were undeveloped. The number and size of Leydig cells in (-/-) testes was dramatically decreased at both ages studied in comparison to age-matched wild-type (wt) mice. Likewise, serum and testicular testosterone (T) levels were dramatically (by > 98%) decreased, though still measurable, and the basal and forskolin-stimulated rates of T production were very low or undetectable in cultured testis slices from 2 and 12 month-old LuRKO mice. Due to defective feedback regulation, the serum LH levels were 30-fold, and those of FSH 2-fold elevated in the (-/-) mice. Compared with reports on gonadotropin-deficient hpg and common α -subunit knockout mice, where spermatogenesis proceeds up to the diplotene stage, this process was clearly more advanced in aged LuRKO mice, reaching elongated spermatids. RT-PCR analyses demonstrated that 17 β -hydroxysteroid dehydrogenase (17 β -HSD) III mRNA was expressed, and that of thrombospondin (TSP) 2 was absent in both (+/+) and (-/-) testes, indicating that the Leydig cells present in the 12 month-old (-/-) testes were of the adult type. When the mice were treated with flutamide between 9-12 months of life, spermatogenesis was arrested at the round spermatid stage, indicating that the very low levels of T were responsible for the advancement of spermatogenesis to the elongated spermatid stage. Taken together, our study provides evidence for the first time that LH/LHR and high intratesticular androgen level are not obligatory for qualitatively complete spermatogenesis in the mouse testis. The prolonged elevation of FSH together with the very low level of T, even in cryptorchidism, may compensate for the defective LH and androgen action. Likewise, the development and differentiation of adult Leydig cells can occur independent on LH/LHR signalling. These findings, if extrapolated to the human, emphasize the necessity of complete blockage of testicular androgen production in the attempts to achieve azoospermia upon hormonal contraception for men.

EPITHELIAL *HSPROUTY* PATTERNS KIDNEY DEVELOPMENT BY COORDINATING SIGNALING BETWEEN THREE TISSUE COMPARTMENTS *IN VIVO*

SHAOBING ZHANG¹, YANFENG LIN¹, LIJUN CHI¹, RENATA PRUNSKAITE¹, REETTA VUOLTEENAHO¹, SIRPA KONTUSAARI¹, HELLEVI PELTOKETO¹ AND SEPPO VAINIO^{1,2}

¹ *Biocenter Oulu and Department of Biochemistry, University of Oulu, Oulu University, Finland* and ² *Biocenter Oulu and Department of Biochemistry, Faculties of Science and Medicine, Oulu University, Finland*

The mammalian kidney is an excellent model system to study mechanisms of organogenesis. As many other organs it develops through signaling between epithelium and mesenchyme such as ureteric bud and metanephric mesenchyme but the signals involved are still poorly known. Sprouty (Spry) proteins are factors that regulate receptor tyrosine kinase function and antagonize signaling of EGR and FGF receptors. We addressed the function of Spry in kidney development by expressing human *Spry2* cDNA in ureteric bud with the *Pax2* promoter that drives gene expression to the ureteric bud *in vivo* where *mSpry2* is normally expressed. Embryos transgenic for the *hSpry2* had small, ectopic or cystic kidneys. In embryos derived from crosses between two transgenic carriers developed of the kidney was perturbed completely and embryos had in such cases only one reduced kidney. *Hspry2* deregulated *FGF2* expression in ureteric bud and kidney mesenchyme, and this was accompanied by reduced expression of mesenchymal genes such *FGF7*, *FGF8*, *FGFR1* and *Gdnf* and stromal marker *BF-2* suggesting a central role for Spry signaling in patterning kidney development. In culture the ureteric bud of the transgenic kidney branched less then the wild type. This defect was partially rescued by ectopic FGF7 and Gdnf consistent with the role of Spry controlling expression of these signals. Interestingly in organ culture FGFs and Gdnf induced supernumerary bud formation from the Wolffian duct, not observed in their wild-type littermates. Together the data points to an important role for Spry in regulating kidney development and suggests that Spry may affect the epithelial branching pattern by coordinating expression of signals between major tissue compartments, the epithelium, mesenchyme and stroma.

Notes: