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Turku and Oulu – you made it!

It is my great pleasure and joy to congratulate Development&Communication editors in Oulu and Turku. Right here, in front of our eyes, we have the very first non-Helsinki made volume of D&C. This is perfect way to start the fourth year in the history of our webjournal now really reaching beyond of the old limits in geography and linking together the whole Finnish Society of Developmental Biologists. In this volume we go back to Hyytiälä, both in science and fun. Additionally, two very informative stories written by Matti Poutanen and Seppo Vainio tell how research is organised in Turku and Oulu. A new insight for what brings a foreigner to Finland is given by Leon Brokken, who also educates the readers about hedgehogs in reproduction.

Now that winter finally arrived Finland it is time to turn the thoughts towards the coming spring and summer. The major developmental biology meeting organised by ISDB is held this year in September in Sydney, Australia (http://www.isdb2005.com/). To get some preface of Australian style symposiums some Finnish kidney researchers, undersigned among others, participated in the 9th International Workshop in Developmental Nephrogenesis last August in Barossa Valley, relatively small region also famous for excellent wineries producing half of the country’s annual wine amount. Florence Naillat is telling about the workshop itself elsewhere in this volume but here I can ensure you that Australia is worth of travelling 30 hours to get there; both for the science and atmosphere! Before going to Australia or anywhere else, I want to congratulate our head of Developmental Biology Center of Excellence Irma Thesleff for being nominated as a Professor of the Year 2004. Now, enjoy about the journal.

Satu Kuure
editor-in-chief
Hyytiälä 2004 Meeting

Pictorial by Leon Brokken

Prize for the Best Developmental Biology Publication 2003

Posters by Developmental Biologists from Oulu and Turku
Was it a good or a bad sign that this year the meeting did not ring in the winter season? Global warming? Nevertheless, the atmosphere was as good as ever!

Leon Brokken
ACTE DE PRÉSENCE!
Experimental design
16 semi-voluntary scientists are nonrandomly divided over 4 research-group teams, each group existing of 2 females and 2 males. Add 4 oranges which should be carried over without using hands, and enjoy the spectacle!

Conclusion
Collaboration not only works well in science, it also pays off when handling fruit!

The winners, with joined forces:

Oulu & Turku!!!
It seems every year more band members show up...

Poetry with Jorma Wartiovaara

"Keep on playing that rock ‘n roll!!"

...a LOT of dancing!!!

...yeah right, serious stuff too...

...last defenders of the floor!

Rumour has it that there even was an afterparty somewhere... the details of which are, unfortunately, highly confidential :-( 
I was asked by the Board of the Society to assess the papers proposed by 24 June 2004 and pick the winner of the Annual Publication Reward for Young Scientists given by the Finnish Society for Developmental Biology.

The winner was to be chosen from:
1. All papers published in 2002-2003 in international scientific journals;
2. with a young scientist being an author and a member of the society.

This time the accepted papers submitted to the contest were from:

1. **Ilpo Huhtaniemi’s ranks:**
   - Fu-Ping Zhang

2. **Juha Partanen’s hockey team:**
   - Ulla Pirvola, Nina Trokovic and Ras Trokovic

3. **Irma Thesleff’s band:**
   - Johanna Laurikka, Tuija Mustonen, Ritva Rice and Mark Tummers

4. **Seppo Vainio’s racing team:**
   - Petri Itäranta

The standard of the papers was very good or even excellent - in the majority of cases. Almost all papers were published in journals of high ranking, in their respective fields. The journals included *Genes & Development, Development, Developmental Biology, EMBO Journal, Neuron* and *PNAS.*

But forget the impact factors!

For the finals, I found myself mostly pondering on the products of six candidates, papers headed by Johanna, Ulla, Ritva, Nina, Mark, and Fu-Ping, here listed in the alphabetical order of their last name. Then some of them began to rise above others.

But what is the most beautiful, what is the best?
Maybe a rhyme will solve the test!

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**The Reward**

The yearly fashion contest is at hand.
And the referee was asked to stand
On the bench in front of ye all,
Without intent to appall.

So I say,
If I may:

This candidate has glamour and charm.
But some lack in form does her harm.
One can ask where: Where is the chord?
That one there has plenty of wit and credits
As she presents her delicious merits.
But alas! Too meekly, Oh My Lord!

In this again,
You can see his goal,
And the sharp mind and pen,
But where, where is the soul?

Then, something modest to adore.
Notice how simple her plane dress,
But it brings something new to the floor,
And with elegance does us all impress.
So where do I stand
With the nine and fine,
Which form this year's brand,
Are eligible, and seek the sublime.

They come from four ambitious labs, it seems,
Ilpo’s, Irma’s, Juha’s, and Seppo’s prospering teams.
Their name,
In journals of fame,
Glow in black ink,
And their song
Will long,
Into the learned public,
In this republic
Sink, I think.

But who is the best?
That is the quest.
But what is the test?
At the end we will see
Might one of the following glee.

To challenge a dogma outright
Fu-Ping seems to dare.
The knockout of LHR is an honor to fight
If only the testis maintains
And spermatogenesis sustains.
So to his delight tonight
The spotlights flare
And many honors declare.

But Mark has tasted a delicious quiche.
He has his tooth on the stem cell niche.
The topic is hot like a shot.
The fingers can burn.
The FGF and BMP,
Both in turn, work and churn.
And we’ll just have to see,
If that’s the whole lot to be.

Johanna loves enamel knots and their domains,
And other ectodermal signaling centers that ectodin ordains.
Such gene products are fun to identify
That inhibit or enhance and make things fly
Along the signaling pathways that marvel us.
That’s our life and work, sometimes so sweet and marvelous.
This feat of Johanna deserves a roaring applaud,
It comes next to the top; it’s no fraud.

Developmental patterns have been an enigma,
Yet a fascinating landscape for Nina’s adventure.
Experimental hypomorphs are not a stigma,
They enlarge our marvel of Mother Nature. Oh! what wonderful creatures those cells of neural crest,
But tissue-specific mutagenesis can these nomads arrest.
Mutants of Fgf receptor number one, I presume
Gave the clue to, guess whom?
They revealed the functional fissure,
The altered gene expression in ectodermal tissue,
And maybe not to your surprise,
Has given Nina the illustrious Prize

Nina Trokovic

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Papers submitted for the Annual Publication Reward for Young Scientists given by the Finnish Society for Developmental Biology in 2004:


Posters

In utero and in vitro effects of flutamide and diethylstilbestrol on prenatal testicular testosterone production in rat

A. Adamssson, L. Broksen, J. Paranko, and J. Toppari

Department of Anatomy, Department of Physiology, Department of Paediatrics, University of Turku, Finland

Introduction

In male rat fetuses, maximal testosterone production is reached by the age of ED18.5-19.5. This prenatal testosterone surge is considered crucial for the physiological masculinization of male progeny. To gain more information about xenohormone effects on foetal male steroidogenesis, we have analysed in utero and in vitro effects of flutamide (FLU) and diethylstilbestrol (DES) on testosterone (T) and progesterone (P4) production, and steroidogenic acute regulatory protein (StAR) and androgen receptor (AR) expression in 19.5-day-old foetal Sprague-Dawley rat testes.

Materials and Methods

Exposure in utero:

Daily oral exposures to flutamide (25 mg/kg in DMSO - corn oil) were carried out on EDs 13-17 and subsequent exposures to diethylstilbestrol (DES, 0.02 mg/kg in DMSO - corn oil) on EDs 13, 15, and 17. Analysis of testosterone and progesterone and expression of StAR and AR proteins were carried out on the ED 19.5.

Exposure in vitro:

ED 19.5 testes were exposed for 3 hours under tissue culture conditions to FLU (0.1-100 mg/L) and DES (0.1-100 mg/L). Testosterone and progesterone production, StAR and AR protein levels were assayed. Real-time RT-PCR was carried out for StAR mRNA. Immunohistochemistry was used to analyse the changes in testicular AR-expression.

Results

1. In vitro-expression of StAR correlates with steroid hormone profile in the developing foetal rat testes.

2. In utero-exposure to flutamide and DES. No impact on StAR protein, progesterone, and testosterone levels were observed on ED 19.5.

3. Developmental pattern of androgen receptor (AR) protein expression level in EDs 15.5, 17.5, 19.5, and in neonates (1 Dpn).

4. In utero-exposure to FLU 25 mg/kg or DES 0.02 mg/kg had no effects on AR protein expression on ED 19.5.

5. 3h culture of 19.5 ED testes in the presence of flutamide (FLU) increases the expression of StAR protein dose-dependently.

6. 3h culture of ED 19.5 testes in the presence of diethylstilbestrol (DES) increases StAR protein levels, but instead of testosterone, enhances the accumulation of progesterone.

7. Down-regulation of AR protein during a 3h tests culture (19.5 ED) in the presence of flutamide and diethylstilbestrol.

Conclusion

- During the foetal development of rat testis, StAR expression correlates with the increase in steroid hormone synthesis and expression of androgen receptors.
- Flutamide and DES at the doses introduced in utero did not considerably alter any of the parameters measured. This may be a matter of dosing.
- In vitro exposure to flutamide caused an increase in StAR-protein and testosterone levels. The changes, however, included a rapid down-regulation of androgen receptors.
- For DES, increased accumulation of progesterone seems to be involved in the steroidogenic inhibition of testosterone synthesis. Also DES causes a rapid in vitro depression of androgen receptors.
- There were no statistical differences in StAR mRNA-levels after 3 h cultures in the presence of FLU and DES.
- In vitro testis assay is a promising model for mechanistic studies of male reproductive toxicology and for the identification of potential endocrine disruptors.
UNIVERSAL EXPRESSION OF HUMAN 17β-HSD1 RESULTS IN UNDEVELOPED VAGINA AND ANOVULATION IN TRANSGENIC FEMALE MICE

Taija Saloniemi, Tarja Lamminen, Kaisa Huhtinen and Matti Poutanen

Department of Physiology, University of Turku, Kiinanmyllynkatu 10, FIN-20520 Turku, Finland

Human 17β-HSD1 is steroid-activating enzyme catalyzing the interconversion of low-active estrone (E₁) to more potent estrogen 17β-estradiol (E₂) in tissues such as human endometrium, trophoblasts of placenta, granulosa cells of ovary, and normal and malignant breast tissue. To obtain more information about the role of 17β-HSD1 in the regulation of estrogen action, we have generated transgenic mice universally expressing human h17β-HSD type 1.

Materials and Methods
In present study, five transgenic (TG) mouse lines expressing h17β-HSD1 under chicken β-actin promoter were generated. The different mouse lines were compared for their h17β-HSD1 expression. Furthermore, for the mouse line with highest transgene expression, both histological and macroscopical analysis were performed at the ages of 0.5 days, 2 months and 4 months and compared to wild type (WT) mice.

Results
H17β-HSD1-transgene expression in different mouse lines correlated with the phenotype and increased 17β-HSD1 activity.

Mouse lines
| WT 016 012 020 050 013 | Strong phenotype |

Correlation between h17β-HSD1 expression level and phenotype

Universal h17β-HSD1 expression lead to reproductive phenotype in TG female mice. However, circulating E₂ levels were not significantly different between WT and TG mice at ages of 2 and 4 months.

Circulating estradiol levels were not significantly different in WT and h17β-HSD1-TG mice at 2 and 4 months

At birth, TG female mice had undeveloped vagina, which lacked opening. Anourethral distance was longer than in WT females due to differential positioning of urethral opening. TG females also lacked nipples.

Different positioning of urethral opening and exceptional vaginal morphology at birth in h17β-
HSD1 TG-females

WT neonatal

TG neonatal

Lack of nipples and opening of undeveloped vagina in h17β-HSD1 -TG mice

Ur = urethra V = vagina R = rectum

Histology in the ovaries in adults referred to anovulation indicated by the lack of corpora lutea.

Lack of corpus luteum in the h17β-HSD1 TG ovary (4 mo)

CL = corpus luteum

AF: antral follicle

Conclusions
Universal h17β-HSD1-expression results to serious morphological disturbances in female mice, including undevelopment of vagina and lack of nipples, and affects gonadal function in adult mice. Circulating E₂ levels of TG females were normal at adult age while the phenotype existed at birth, indicating that TG mice have possibly been exposed to sex steroid excess during embryonic development.
Tamoxifen induced geneactivation-studies using Cre/loxP-system
Tina Jokela and Seppo Valnio
Biocenter of Oulu and Department of Biochemistry, University Of Oulu, Finland

Introduction
Cre is a 38 kDa recombinase protein from bacteriophage P1 which mediates intramolecular and intermolecular site specific recombination between loxP-sites. In recent years Cre-recombinase has become an essential tool for conditional gene activation or inactivation in mouse.

The aim of my research is to apply Cre-loxP technology to study inducible gene activation in the mouse. This is done by breeding two mouse strains together. The Cre-ERT strain contains fusion protein between Cre and an estrogen binding domain, which makes Cre inactive in all cells unless nonsteroidal estrogen analog 4-hydroxytamoxifen is introduced. The reporter Rosa26-strain contains an inactive LacZ-gene, which is flanked by two loxP-sites. When the Cre-ERT strain are crossed with Rosa26-mice the LacZ-gene is activated upon Cre-mediated genomic recombination process.

Materials and methods
Embryos (11.5 and 12.0 dpc) from the crossing Rosa26 and Cre-ERT were dissected and kidneys were taken for cultures. Various concentrations of tamoxifen were added and the kidneys were cultured 24 h or 48 h. After culturing the kidneys were fixed and stained using X-gal as a substrate. LacZ-gene activation leads generation of blue color in the cultured kidneys.

Results
LacZ-gene activation was successful in the cultured kidneys (fig. 1). Low concentrations of tamoxifen gave less signal but as the concentration of tamoxifen raised, the signal became much stronger. Control kidneys had some LacZ-gene activation (maybe due to some leaking of the fusion protein).

Conclusion
Cre/loxP-system can be used as a tool to study different geneactivation-patterns. In the future the same system is going to be tested with adenocre-viruses.
Mechanisms of Wnt-II signal transduction

Antti Railo, Pekka Kilpeläinen, Petri Iitäranta and Seppo Vainio
Biocenter Oulu and Department of Biochemistry, University of Oulu

Introduction
Wnt-II is an important multifunctional mediator of many different and crucial events during animal development. It regulates convergent extension movements during Xenopus and zebrafish gastrulation and is essential inductive signal in mouse heart and kidney development. However, its signaling mechanisms are poorly understood especially in vertebrate tissues and cells. We have addressed Wnt-II signaling in CHO (Chinese hamster ovary) cell line. Our initial hypothesis was that Wnt-II may signal through some of non-canonical Wnt signaling pathways.

Methods and Results
CHO cells were co-transfected with Wnt-II, TopFlash and beta-galactosidase plasmids. After 24 hours cells were lysed, luciferase activities assayed and normalized to transfection efficiencies. We showed that Wnt-II inhibits both endogenous (Fig. 1) and LcI activated (Fig. 2) canonical Wnt signaling pathway.

Latter experiment indicates that the inhibition mechanism lies downstream of GSK-3 since Li+ activates the cascade at the level of GSK-3.

The role of Wnt-II in the regulation of JNK pathway was studied by co-transferring Wnt-I1 into CHO cells with AP-1 luciferase reporter and beta-Gal plasmids. Results were surprising, since Wnt-II inhibits also JNK pathway (Fig. 4). Equally, JNK pathway activated by the overexpression of MEKK was inhibited by Wnt-II (Fig. 5). Finally, we measured directly the activity of JNK by using SAPK/JNK assay kit (Cell Signalling Technology). From this assay it could be concluded that the inhibition of the cascade most likely is at the level of JNK (Fig. 6).

Conclusion and Hypothesis
Wnt-II appears to inhibit both the canonical beta-catenin pathway and the non-canonical JNK pathway in CHO cell line. The inhibition of JNK cascade could be tissue and cell line specific phenomenon given that Wnt-II has been proposed to activate JNK signaling in certain zebrafish and Xenopus tissues and cells.

Based on our studies the mechanism of canonical pathway inhibition by Wnt-II have to lie at least partly downstream of GSK-3 given that Wnt-II is capable of inhibiting LiC activation signaling. Tak-1/Nik mitogen activated protein kinase cascade may serve as one potential mechanism of canonical pathway downregulation. Others have shown that NIK can phosphorylate TCF preventing beta-catenin/TCF complex from interacting with DNA and that the cascade can be activated at least by Wnt-3a (a counterpart of Wnt-11). Interestingly phosphorylation of TCF homolog TCF-3 by NIK is known to potentiate the expression of a certain Wnt target genes. It has been reported that NIK strongly enhances the convergent/divergent phenotypes associated with Wnt-11 signaling in zebrafish. This possibility have to be studied by measuring possible Tak-1 activation by Wnt-II.

Mechanism of observed JNK downregulation by Wnt-II is still unclear. We propose that the inhibition is at the level of JNK, since inhibition of JNK activation could be demonstrated in cells where cascade was activated by MEKK. Others have reported an evidence that Par-1 could play a role in Wnt based repression of JNK, Par-1 is able to bind dsh (dishevelled), and the inhibition could be mediated by that way. However, this interaction has been suggested also to potentiate canonical signaling. Dsh homolog dsh-3 may play a role in Wnt based inhibition of JNK. Furthermore, there could be mechanism independent of dsh. Finally, we don’t know which frizzleds and other downstream components are present in CHO cells. It could be that CHO cells express only frizzleds able to repress JNK. There are at least three different dsh and JNK homologs. In addition, JNK have many different splice variants and role of those variants is very poorly known. Our hypothesis of Wnte-
Sprouty signalling regulates FGF9 signalling in Wolffian duct controlling migration of mesonephric cell into developing testis

Lijun Chi, Shaobing Zhang, Retta Vuolteenaho, Sirpa Kontusaari and Seppo Vainio
Biocenter Oulu and Department of Biochemistry, Oulu University, Finland

Introduction
Sprouty (Stry) function as an antagonist receptor tyrosine kinase, such as fibroblast growth factor (FGF) and epidermal growth factor (EGF) receptors (Casci et al, 1999). Four vertebrate Stry genes have been identified and are expressed in the developing mouse (Zhang et al., 2001). The function of Stry is open at the moment.

Three general male-specific changes are known to involve testis: epididymis cell proliferation of the coelomic epithelium, cell migration from mesonephric into gonad and organization of testicular cords (Martinezou et al., 1997). Mesonephric cell migration plays a critical role in the formation of testicular cords and differentiation of XY gonad (Tilmann and Capel, 1999). During this process, the rapid morphological changes initiated by Stry require likely coordinate integration of many signaling pathways, but very little is known about signals induced. We report that Stry has a role in Wolffian duct development and organogenesis of testis.

Materials and Methods
Pax2 promoter was used to drive human sprouty2 cDNA with enhanced green fluorescent protein (GFP) in line marked as transgenic Pax2GFP mice. Whole mount, radioactive section in situ hybridization and gonad with mesonephric recombinants organ culture were used as techniques to analysis function of Stry.

Results

1. Stry genes are expressed in unique patterns in early gonad

2. The transgene is expressed in the Wolffian duct, mesonephric tubules, testis and epididymis

3. Cystic, polycystic epididymis and vas deferens in Pax2GFP transgenic testis

4. Downregulated of FGF9 in Wolffian duct, disturbed Sertoli cells and Leydig cells differentiation and testicular cord formation Pax2GFP transgenic XY gonad

5. Stry signalling inhibits mesonephric cell migration into testis

Conclusion
1. Stry didn’t have an effect on sex determination of XY gonad.
2. Stry may antagonise FGF9 signaling in Wolffian duct of testis.
3. Stry signaling inhibits migration of mesonephric cells into testis.
4. Reduced the number of Sertoli/Leydig cells and testicular cords.

References
Screening of Wnt-4 mutant kidney to identify candidate genes for nephron development

Florence Naihatu, Lasse Vääriskoski, Jussi Vuoristo & Seppo Vainio
Department of Biochemistry, Biocenter Oulu, University of Oulu, Finland

1 Introduction
Inductive interactions between epithelial and mesenchymal tissues. In the kidney these interactions occur between the epithelial ureteric bud and the kidney mesenchyme, and they control development of the nephrons. Wnt-4 is a critical signal to regulate this process as there is a deficiency in the nephron formation in its absence (Stark et al. 1994). Wnt-4 deficient mice die within 24 hours after the birth apparently due to kidney failure (Figure 1). Wnt-4 is also important in human kidney since changes in Wnt-4 gene expression has been recently associated to renal tubular injury (Saureman et al. 2000).

In addition to the kidney Wnt-4 has been implicated in the development of the gut, adrenal, mammary glands and gonadogenesis. Wnt-4 function is also important in the mesonephros and its derivatives as the female is masculinized (Figure 1); the Wolffian duct develops but the Müllerian duct and most of the ovaries degenerate (Vainio et al. 1989).

Purpose of the study:
The aim of this project was to identify some genes that associate to Wnt-4 regulated nephrogenesis by using microarray technology.

2 Materials and methods
Wild type and Wnt-4 deficient kidneys were collected at E12.5 and E14.5, and the total RNA was extracted. The isolated RNA was applied to 12,000 mouse genes by using an Affymetrix oligonucleotide chip. MsP140 Targt Av2. The analysis was based on the comparison between the E14.5 and E12.5 wild type embryos and the E12.5 and E12.5 wild type samples.

3 Analysis of the genes

4 Results
After analysing the 12,000 genes on the chip, around 40 genes have been revealed whose expression has changed significantly more or less than two log ratio. From the 40 genes, 22 were chosen for further studies (graph 1). GeneSpring 8.1 and GeneStar programs were used to group the 22 genes into clusters (Figure 2) and six distinct groups were obtained. These are transcription factors, cell adhesion molecules, hormones and genes encoding cytoskeletal components.

From the pool of the 22 genes, we have focused on the expression of six genes, (Lim-1, FGF-8, Fox-1, Dcr1, Dcr2 and Erb1). Their changes in expression were confirmed. The Reverse Transcript PCR and whole mount in situ hybridization analyses

As expected Wnt-4 gene expression was decreased in the Wnt-4 deficient embryonic kidney, and this was confirmed with whole mount in situ hybridization and RT-PCR experiments. These were consistent with the microarray results (data not shown).

At E12.5, FGF-8, Dcr1, Lim-1, Fox-1, Dcr2 and Erb1 are expressed in the wild type while in the Wnt-4 deficient embryonic kidney expression was clearly decreased. At E14.5 no Erb-1 expression was observed. At the same stage FGF-8, Lim-1 and Fox-1 were expressed strongly in the developing nephrons in the wild type but in the Wnt-4 deficient kidneys their expression was weaker. Dcr1 and Dcr2 were expressed weakly as well as in the wild type while their expression is not found in the Wnt-4 deficient embryonic kidney.

5 Conclusion
Wnt-4 has been speculated to regulate nephrogenesis by controlling expression of components of the cell adhesion machinery (Stark et al.). Our data now support the idea that Wnt-4 signaling involves several genes and induce receptors, components of the cell adhesion transcription and translation factors. A hypothetical model of some identified factors in the Wnt-4 signaling cascade are presented in Figure 3.

6 Reference

7 Acknowledgement
The ERS-1 plasmid is a kind gift from Prof. Arangela (Italy).

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Graph 1: Different levels of expression of the selected genes at two developmental stages based on microarrays. Analysis of expression of some selected genes by whole mount in situ hybridization. The gene expression was analysed at E12.5 and at E14.5 in Wnt-4 mutant and wild type embryonic kidney. WT: Wild type, MT: mutant

Graph 2: Grouping genes to clusters based on function. The 22 genes were clustered into six groups.

Figure 3. Hypothetical association of the identified genes to Wnt-4 signaling in the embryonic kidney.
Wnt-pathway as a mediator of fertilization

Ilkka Pietilä and Seppo Vainio
Biocenter Oulu and Department of Biochemistry, Oulu University, Finland

Introduction:
Fertilization has been studied for a long time, but still it’s not known what happens at the molecular level when sperm fuses into the oocyte. In my studies I have tried to find out if "Wnt"-pathway is mediating fertilization and oocyte activation. Wnt-pathway components have been found from oocytes which are at the late 3-cell stage. This gives the idea that Wnt might play a crucial role also in fertilization. In vertebrates unfertilized oocytes have to make mRNA, enzymes, substrates and cell organelles to the cytoplasm before fertilization, because in vertebrates the fertilized eggs own genetic transcription starts at the late 2-cell stage.
Our hypothesis was that sperm has some "Wnt"-like protein that binds to the frizzled receptor on the oocytes membrane. This would trigger the oocytes activation and already made mRNA translation. One of the first detectable event in fertilized eggs is the cyclic release of calcium ions into the cytoplasm. These calcium waves prevent polyspermy by releasing the cortical granules into the perivitelline space. These Ca²⁺ waves also drive the early development forward and starts the translation.

Methods and Results:
CD-1 mouse line was used and the females were 7-8 weeks old. Superovulation was done to the females using 2,5 U PMSG and 48 h later 5,0 U HCG. 13 h later mice were killed and the oocytes were collected. Cumulus cells were detached using hyaluronidase (0,3 mg/ml). After hyaluronidase treatment cells were incubated for at least an hour before IVF or activation experiment were started, so that the cells had some time to adapt to the new environment.
Males were older and before collecting the sperm they had to mate at least once and produce offspring. One week before sperm collection they were not allowed to mate. IVF was done in different culture media and in the end we used HTF and KSM-1AA media. Different LIF and EtOH concentrations were tested and also different activation times. After the experiment the done cells were placed in 4% PFA and kept over night.

Conclusions:
After these studies it’s still hard to say what is the mechanism in fertilization and oocyte activation, but these results gives two possible way how these events might proceed.
1) It can go through canonical Wnt-pathway. This hypothesis is supported by the results from Wnt3a, BIO and RT-PCR experiments. Wnt3a and BIO both activate the canonical Wnt-pathway and fertilized-1 is known to be a canonical pathway component in at least in some cell lines.
2) It can also go through non-canonical Wnt/Ca²⁺ pathway. Support for this hypothesis comes from the fact that Ca²⁺ ions are released soon after fertilization and LiP, is known to be present in these calcium releases. RT-PCR results also showed that fertilized-1 is expressed in unfertilized oocytes. It’s possible that fertilized-3 works through non-canonical Wnt pathway.
Germ cells in Wnt-4/- mice
Prunskaitė R, Oikarinen A, Sormunen R, Shan J and

Wnt-4 are important secreted signaling molecules. It is proven that Wnt-4 is important for female development (Vanttila S. et al., 1999). It is expressed in the ovary. The absence of Wnt-4 signaling leads to female-to-male sex reversal and ectopic testosterone production. Wnt-4/- mice ovaries morphologically mimic testes (Fig.1) by forming structures similar to fat body (bold arrows in Fig.1) and tunica albuginea (Fig.2, arrows), normally existing only in males.

Fig. 2. Sections of Wnt-4/- and wild type mice gonads. Arrows are pointing male specific structure tunica albuginea.

Germ cells. Our studies have showed that germ cell migration from hind gut to ovary in Wnt4/- mice is normal (Fig 3. E12.5), but later on majority of the germ cells undergo apoptosis. There are finally remaining only 10% of the germ cells found in the wild type gonads at the same time of development (Fig 3. E17.5).

Fig 3. Germ cells are abundant at E12.5 in Wnt-4/- and wild type ovary. In later stages are remaining only 10% of germ cells compare to the wild type mice E17.5.

Ultrastructure of primordial germ cells shows disrupted contacts between cells (Fig.4), that is making cell-cell interaction almost impossible. The Wnt4/- germ cells are clearly smaller, both, at the most narrow and the most wide points; P<0.0001 (Fig.5).

The Wnt4/- germ cells have less cytoplasm, however the nucleus remains the same size as in wild type.

The Wnt-4/- germ cells have less mitochondria (Fig.6).

Fig. 5. The germ cells size in Wnt-4/- mouse ovary is smaller compare to wild type mice

Fig 6. The amount of germ cells is decreased in the Wnt-4/- germ cells.

In the Wnt-4/- germ cells is hard to see Golgi apparatus, however it is clear in WT mice (Fig 7). There are few adherent junctions between primordial follicles and somatic cells (Fig 8) in Wnt-4/-.

Fig 7. Golgi apparatus in wild type mice

Fig 8. Adherent junctions in WT

Conclusions:
Putative functions of Wnt-4:
Crucial for gonad survival and differentiation
Important for the normal formation of primordial follicles
Impacts the amount of oocytes
Contributes to cell adhesion
Teratoma/tumor induction

Future prospects:
Germ cell cultures
Rescue experiments with Wnt-4 expressing cells
Center for Reproductive and Developmental Medicine (CREDE)
Matti Poutanen, Director of CREDE

Background

CREDE is a research program of BioCity Turku and of the Medical Faculty of University of Turku. It is an umbrella organization that has actively promoted research in the areas of reproductive, developmental and skeletal research at the University of Turku for ten years. Also all the students and research fellows and principal investigators that have participated the Annual Hyytiälä meetings from Turku belong to CREDE research program.

Currently, the research program is composed of 18 research groups. The program comprises basic science, clinical and social science projects, thus, giving us a good perspective for translational research and integration of activities with different scientific backgrounds. This combination of three orientations has been one of the specific characteristics of CREDE activities from the beginning. We are committed to further strengthening the interaction between basic and clinical sciences in the future, and keep the modern medical care and its development in the focus of our research.

The major research topics of the program fall into three categories 1) Reproductive health and the mechanisms of hormone action, 2) Developmental mechanisms, and 3) Skeletal research. However, the basic science projects under the different research topics are highly overlapping in themes such as the mechanisms of development, differentiation and hormone action.

Research projects in CREDE

Role of estrogens and estrogen receptors in prostate and mammary gland, Sari Mäkelä: The major goal is to clarify the role of estrogens and estrogen receptors (ERs) in mammary gland and prostate development, growth and tumorigenesis.

Hormonal differentiation of mammalian testis and ovary – sensitivity to endocrine-active substances, Jorma Paranko: The aim of the project is to determine the effects of exogenous hormones and hormone-like chemicals on the prenatal rat testicular testosterone surge and gonadotropin production, and on the postnatal maturation of Leydig cells.

A. Cell and molecular biological regulatory mechanisms in the differentiation of the embryonic testis and ovary, B. Cell biological collaborative research of embryonic development, biotechnology, and diseases, Lauri J. Pelliniemi: A. Research objects are developmental events and respective regulatory mechanisms in the differentiation of the gonads and other genital organs in embryos and young animals, especially the differences between the two sexes. B. Development of embryonic heart and eye are studied especially in view of apoptosis as developmental mechanism in mice. Human stem cell clusters will be analysed for cell replacement therapies.

Research program on male reproductive health, Matti Poutanen, Ilpo Huhtaniemi: The goal is to address key challenges in the field of male reproductive health, in particular to better understand: 1) The basic mechanisms underlying the endocrine regulation of male reproductive functions and sex steroid target
tissues. 2) Endocrine pathophysiology of male infertility. 3) Pathophysiology of hormone-dependent cancers. 4) Development and testing of novel contraceptive strategies for the male. 5) Endocrine physiology and pathophysiology of male ageing. In the research both basic science methodology and clinical studies are applied. The basic science studies are largely based on genetically modified mouse models.

**Developmental disorders of male genital organs, semen quality and regulation of spermatogenesis, Jorma Toppari:** The aim of our studies is the maintenance of good male reproductive health. We focus our research to 1) the regulation of spermatogenesis, 2) genetic and environmental influence on semen quality, and 3) pathophysiology of cryptorchidism.

Other interesting (but less developmental biology-related) research projects, are:

Novel treatments for improved reproductive health, *Antti Perheentupa.*

Estrogen-related prostatitis, *Risto Santti.*

Health and sexuality education, *Ansa Ojanlatva.*

Use of mesenchymal stem cells in bone healing and tissue engineering of bone, *Risto Penttinen.*

Generation of transgenic mouse models for skeletal diseases and development of new approaches for their treatment, *Eero Vuorio.*

Bone biology research, H. Kalervo Väänänen, Teuvo Hentunen, Tiina Laitala-Leinonen.

Growth mechanisms of hormonal cancer, *Pirkko Härkönen.*

The role of the NF1 gene in intercellular communication and tumoral cell biology, *Juha Peltonen.*

Molecular genetics of inherited disorders, *Helena Kääriäinen, Kirsi Huoponen.*

Kinetochore-microtubule interaction and chromosome instability, *Marko J. Kallio.*

Mitochondrial DNA sequence variation as a risk factor for neurodegenerative diseases, *Kari Majamaa.*

Development and functioning in very low birth weight infants from infancy to school age (PIPARI), *Liisa Lehtonen, Helena Lapinleimu, Leena Haataja.*

The Finnish family competence study, *Matti Sillanpää, Päivi Rautava.*

**Research training in CREDE**

Participation in graduate school training is one of the central activities of CREDE indicated by the fact that the nation wide Drug Discovery Graduate School (DDGS) is directed by a CREDE group leader (Härkönen). Furthermore, CREDE is actively participating at the Boards of DDGS, Turku Graduate School of Biomedical Sciences (TuBS, Poutanen), and the Finnish Graduate School in Musculoskeletal Diseases (TULES).

The CREDE researchers have actively initiated and planned a weekly campus seminar series together with the other BioCity Turku research programs. Also, CREDE has co-sponsored several national and international meetings in various areas of biomedical science. Symposia sponsored or co-sponsored by CREDE in 2004 were: Regulation of spermatogenesis, Key genes in differentiation, In vivo imaging of animal models for drug discovery and action, CREDE-IRDB meeting (in London), Endocrine regulation of gene expression, CREDE-DDGS Symposium on target identification and validation. CREDE members have actively participated in BioCity Symposia organizations as well.
Scientific activities in CREDE

During the period of 2000-2004, CREDE research groups reported total of 605 original publications in peer reviewed international journals. At least 53 publications have been published as a result of active collaboration between two or more CREDE research groups. There were 182 publications with IF>5 (30% of the total number), of them 17 were collaboration studies between CREDE research groups.

CREDE research groups have largely been responsible for initiating and maintaining the Transgenic Mouse Facility (TMF) in Turku, and continue to provide a major contribution to the development of TMF activities, by covering partially the salaries of the TMF personnel. An external reviewer evaluated the TMF activities in 2003 and provided a very positive outcome. We are strongly devoted to supporting these activities further. As an outcome of this, the CREDE research groups have actively participated in a proposal to establish the Turku Center For Disease Models, aimed at strengthening the expertise and training in the usage of *in vivo* animal models in biomedicine and drug discovery.

The CREDE research groups have also played an important part in establishing a Stem Cell Laboratory as a mutually beneficial project between the Institute of Biomedicine and Institute of Microbiology and Pathology. The laboratory is focusing on studies on the regulation of mesenchymal stem cell differentiation to bone and cartilage cells, and the development of applications in skeletal repair. Activities will be later extended to other stem cell applications.

Other core activities sponsored by CREDE are: whole animal imaging (pQCT, CT), live cell imaging, confocal microscopy, electron microscopy, tumour laboratory and DNA sequencing.
Short history of the Vainio lab in University of Oulu 1997-2004

Seppo Vainio

I was lucky to get to work with Irma Thesleff during my years of making a PhD. After making a PhD I was ready to realize my dream to make a post doc in USA which took place 1993-1996. These years were stimulating indeed and I was very happy to work among 20 other post docs. The time came to make a new move in my life. I was getting a chance to have the opportunity to interview with group leader positions in USA, Sweden and Finland. At those days the Viikki biocenter had just started and Irma Thesleff had been selected as the program leader. I was initially committed to Irma´s program to start my lab in Helsinki. However, during the years in USA I had started to look the world in an other perspective. In addition to Irma’s program I got offers from USA and Sweden. However, when the process was on I also met the scientific leaders Karl Tryggvason and Taina Pihlajaniemi from Biocenter Oulu. Karl and Taina advertised the opportunities also in Oulu.

During my PhD work I acted as a freelance radio interviewer to the local radio station in Helsinki, Radio City. This work gave me good opportunities to participate to congresses in Helsinki. I also had a change to interview top scientists in the meetings. Among those I met Dr. Erkki Ruoslahti, a leading scientist in the integrin field in USA. When I asked his opinion about the quality of science done in Finland he said that “some where far in the woods there is interesting things happening and soon a lot of good stuff will come out from Oulu”!

Karl and Taina invited me to come for a visit to Oulu and look around the groups over there. I visited Oulu January 1996. It was very cold and in the moon shine I was driving around the small city. It turned out that many of the labs were working in the areas that I thought might be good for our research. For example Pirkko and Reijo Vihko were working with hormones, Juhani Leppäluoto and Olli Vuolteenaho with pituitary gland peptides, Heikki Ruskoaho with heart physiology. Taina Pihlajaniemi had identified a new collagen that was a candidate to operate in the Wnt signalling pathway, an area close to my interests. Kalervo Hiltunen´s work focused to aspects of lipid metabolism, a topic that I though might be interesting in Wnt signalling and embryonic development. There was also no developmental biology, which was good and bad for me. If I would accept the offer from Oulu I would need to initiate pretty much everything from the scratch. However, Oulu sounded an attractive opportunity.

It was big step to me to consider Oulu as a place to work. The Helsinki School of developmental biology was already well established and after working with Sulo Toivonen, Lauri Saxén, Hannu Sariola and Irma Thesleff an idea to work all by myself in Oulu was a hard choice. Also my PhD boss Andy McMahon said that it would be a scientific suicide to consider Oulu seriously.

However, the change in Oulu looked very
nice at the end. There was a good enough animal facility in the department of biochemistry and it was very easy to make agreement with the local scientific community in Oulu. After USA I also found it very attractive to vision myself in an interactive scientific environment. Comparing a very international Boston to Oulu where the people were more homogenous. The cultural opportunities and other choices outside the lab bothered my mind for quite sometime. I though that to date the internet, e-mail telephone and good airplane connections had made a difference and one could also do good science outside the exact centers of science. Also living expenses were much less in Oulu. There were several internationally recognized labs in Oulu.

University of Oulu attracts students from rather large area in Finland and the city has motivation to invest to Biotechnology. I was also impressed with the nice winter in Oulu region and the darkness was compensated with good public lights. Summing all the aspects together I decided to take the change of moving from Harvard to Biocenter Oulu.

We started 1997. I got initially Reetta Vuolteenaho and Marika Uusitalo to work in the lab. The new program of Developmental Biology attracted well students and soon there were several people in the lab. After supervising some pro gradu work few students wanted to go on for their PhD. To manage a lab asked lot of new skills but I was motivated and ready to take the change. I was really lucky to get few Chinese persons to the lab and the collaboration with Taina Pihlajaniemi started also very well. Soon we got some results that I considered interesting in the field of developmental biology. The students Yanfeng Lin and Shaobing Shan were durable, very hard working and learned rapidly the techniques to be productive in the lab.

Yanfeng was very talented in the classical microsurgical operations and Shaobing together with post doc Reetta Vuolteenaho from Karl Tryggvason lab started more long term projects to study Sprouty signalling in kidney development using the transgenic mouse facility. We also initiated conditional mutagenesis work to assay roles of Wnt-4 signalling during development with the Cre-LoxP technology. I was also lucky to have a post doc Hellevi Peltoketo from Reijo Vihko’s lab to help me to conduct the lab and supervise the pro gradu and PhD students. Hellevi was a great help to get for the writing of the manuscripts.

While some pro gradu students found science a challenge and wanted to move to work in biotech companies Shaobing and Yanfeng continued and made their PhD. Minna Heikkilä (now Komu) was a student from Leena Peltonen lab and she put light to the project of sex determination. With her energetic input we could finalize the work that I had initiated in USA and we were lucky to get a full article in Nature. Minna was also a committed student and she graduated for a PhD. Shaobing and Yanfeng are now post docs at Harvard University. Minna was lucky to experience the embryological miracle and after her PhD it was a time for her to establish a family.

Petri Itäranta has been a person that has been in the laboratory from the beginning. He has very large repertoire of skills and he is a knowledgeable student. He has helped me to guide the PhD students and Petri has several good projects. He is aiming to complete his PhD 2005. Shaobing and Yanfeng got help from an other Chinese medical student Lijun Chi. When Lijun came to the laboratory she hardly spoke any English and had not much experience in the lab. During the years she has turned out to be a very skillful experimentalist and she is very talented in making the micro dissections. She carried the transgenic Sprouty work to its completion and this year she was awarded of her Development paper as a Discovery of the year in Biocenter Oulu of the best scientific article. Yanfeng’s paper in Development was selected as the developmental biology article of the year by the Society of Finnish developmental biology and also Minna
Heikkilä’s article in Nature was awarded as a discovery of the year in Biocenter Oulu.

The laboratory has grown now in size to be over ten people and November this year we got fancy new laboratory space in the new main building of the medical campus area. Since I moved from USA to Oulu one of my jobs was also to be involved in planning a barrier mouse facility for the university and run the transgenic mouse microinjection and embryonic stem cell facilities. This has now been finished and the facility is operating very well. We were also lucky to get Raija Soininen from Karl Tryggvason laboratory from Sweden to take over the transgenic mouse facility. Raija has done excellent work to establish the embryonic stem cell and transgenic core in Biocenter Oulu.

I have now worked in Biocenter Oulu seven years. During these years I have learned that the scientific atmosphere in the Biocenter Oulu is very good. The organization is composed of scientists that love to do their work and look the wonders of nature. The organization is evaluated every four years to keep the quality at the international level. Oulu is a small but dynamic city that has realized that the only way for visibility is to go for innovations in research and science based industrialization. The electronics has been a strong tradition but Oulu is also recognized from for example of its research in extra cellular matrix and collagens.

University of Oulu has been absolutely instrumental in keeping the brain power in the north. Without the university the city would look very different. The culture was formerly based on developing products from wood and tar. There has been a long tradition to send ships out to the world. Oulu is far enough from Helsinki, Turku and Tampere to also geographically justify as a university city. Oulu is clearly the center of northern Finland.

Even though I am still looking the world as a place for opportunities I am also motivated to develop the school of developmental biology in the northern climate. To set up a lab and to initiate a program from the scratch is a hard work but life is to take new challenges. With some durability, motivation, willingness to invest to the work and motivated and talented students science has a lot to offer to develop a research career in interesting places in the world such as Oulu.
Hedgehogs and reproduction

Leon Brokken
(Turku)

“How do hedgehogs reproduce? –Very, very carefully!” That’s an old joke. But what do hedgehogs have to do with reproduction?

Hedgehog (Hh) proteins were first discovered in Drosophila where they are critical in establishing segment polarity during embryogenesis, and got their name because flies mutant for Hh developed bristles all over their body, resembling a hedgehog.

To date, three Hh homologues have been identified in mammals: Sonic (Shh; named after a comic book character, which also seemed to have inspired a Finnish rock band!), Indian (Ihh) and Desert Hedgehog (Dhh). Shh establishes cell fate in the developing limb, somites and neural tube, and Ihh is involved in chondrocyte development, and Dhh plays a key role in germ cell development. With the exception of the gut, in which both Shh and Ihh are expressed, the expression patterns of the hedgehog family members do not overlap.

The Hh proteins act through the 12-transmembrane Patched receptor (Ptc) that, when not bound by Hh, represses the action of the seven-transmembrane-protein Smoothened (Smo). Upon binding of Hh to Ptc, the inhibitory effect of Ptc on Smo is relieved, allowing Smo to transduce the Hh signal across the plasma membrane.

Currently two Ptc homologues have been characterised in vertebrates – Ptc1 and Ptc2. Whereas Ptc1 is broadly expressed, Ptc2 is expressed at high levels in the skin and spermatocytes. Interestingly, the chromosomal region 1p32-36, which encompasses the Ptc2 gene, is lost in 36% of germ cell tumour cases.

Recent publications have proposed an interesting feature of the Ptc receptors. Whereas classical signal transduction is initiated by ligand-receptor interactions, Ptc appears to belong to the ‘dependence receptors’, a specific class of receptors that induce apoptosis in the absence of ligand. Thus, at least two distinct pathways exist for these receptors: a classical signal transduction pathway that leads to an antiapoptotic response, and a nonclassic pathway that results in a proapoptotic signal. This cellular dependence ties cells to specific contexts in which the dependent ligands are available and thus provides a strategy to prevent tumour growth beyond specific ligand fields, thus blocking metastasis. For an excellent review on dependence receptors see Bredesen et al.

Now, on to reproduction. Dhh is expressed by Sertoli cells in the foetal and adult testis. Ptc1 is expressed by interstitial Leydig cells and peritubular myoid cells that line the seminiferous tubules. Ptc2 is expressed by germ cells. This reflects another characteristic of Hh signalling: typically, Hh is secreted by epithelial cells which induces proliferation and differentiation of bordering mesenchymal cells that express the Ptc receptor. In response, mesenchymal-derived signals promote epithelial proliferation. Dhh is expressed in Sertoli cells shortly after Sry around 11 days post coitum (dpc) in the rat, and is one of the earliest signs of male sex differentiation. Dhh-/- female mice develop normally, but males display discrete defects
of testicular organisation, including abnormal development of peritubular myoid cells, apolar Sertoli cells, absence of basal lamina, and anastomotic testis cords.\(^9\) Furthermore, in Dhh-/- XY gonads the foetal type Leydig cells fail to differentiate properly, as indicated by absent or low levels of P450 side chain cleavage enzyme (P450scc) and steroidogenic factor 1 (SF1) expression.\(^10\) Rats that have been exposed in utero to the antiandrogen flutamide typically have undescended testes later in adult life. We have shown that during development these exposed foetuses have suppressed levels of Dhh mRNA from 17 dpc until delivery (Brokken et al., manuscript in preparation). Ptc1, which is positively regulated by Dhh, is also strongly reduced. Furthermore, mRNA levels of SF1 and the steroidogenic enzymes 3ß-hydroxysteroid dehydrogenase type I and P450scc were suppressed. SF1 is the major regulator of steroidogenic enzymes. Lastly, insulin-like factor 3 (Insl3), a growth factor indispensable for proper testicular descent, was also reduced. Insl3 is also regulated through SF1. These results are in line with the observations in the developing Dhh knock out mice and, more importantly, they indicate that developmental exposure to antiandrogens can disrupt proper Dhh signalling. Sertoli cells only start to express androgen receptors (AR) a few days after birth, which means that the effect of the AR antagonist flutamide on Dhh expression in Sertoli cells must be indirectly mediated through another cell type. Leydig cells do express AR during foetal development, which suggests that there is an intimate cross talk between these cells and the Sertoli cells. An androgen dependent factor secreted by foetal Leydig cells would then stimulate Dhh expression in Sertoli cells, which in turn regulate Leydig cell gene expression. Another possibility is that the effect on Sertoli cells is mediated through the peritubular myoid cells, the only other cell type in the foetal testis that expresses AR. Autocrine and paracrine regulation in the (foetal) testis is largely unknown, and especially the role of the peritubular myoid cells has hardly received any attention.

Currently we are studying the link between Dhh signalling and its target genes in Leydig cells more closely. Our preliminary data show that when Hh signalling is inhibited by cyclopamine in 14 dpc foetal testis cultures for 3 days, a disturbed differentiation of Leydig cells is reflected by reduced expression levels of Insl3 and steroidogenic enzymes, and testosterone production is markedly suppressed. On the other hand, when Hh signalling is stimulated with recombinant Shh, Insl3 expression is enhanced. Furthermore, when Hh signalling is inhibited the developing Wolffian duct expresses high levels of the apoptotic marker cleaved caspase 3 and remains closed, whereas in controls there is no apoptosis and the duct is open after 3 days in culture. Whether this apoptosis in the Wolffian duct is caused directly by a lack of Hh signalling, rather than by insufficient intratesticular testosterone levels remains to be studied.

Hedgehog proteins are typically expressed at epithelial-mesenchymal boundaries in developing organs. In some cases however, they are also expressed during postnatal life in tissues that are essential for reproduction and that depend on continuous cell renewal, such as gonads, uterus, prostate and mammary gland.\(^11\) Sertoli cells maintain the expression of Dhh throughout adult life, and primary and secondary spermatocytes express Ptc2 receptor.5 Currently we are studying what the role of this pathway is in proliferation and differentiation during spermatogenesis. Come all to the next Hyytiälä meeting where we will hopefully be able to present exiting results!

References:

Student life

Developmental thinking – how it’s achieved?

Antti Railo (Oulu)

Florence and Renata asked me to write something for the next issue of Development and Communication. Well, it was quite difficult to figure out what to write until I just decided to note down something that comes to my mind about me, and about few thoughts that I have got during my short career as a developmental biologist.

I have been wondering lately that I may have a small problem. I tend to stuck thinking certain things too much and too deeply in situations where it is not very convenient and necessary. I watched the movie called Erin Brochovich weeks ago. In the movie, Erin got herself a job as a secretary in an attorney office. Then she revealed quite soon that a big corporation had used hexavalent chromium in preventing corrosion in their factories. This chromium had made its way to the drinking water in nearby areas. Hexavalent chromium is a serious carcinogen, and it had caused different kinds of cancers and other diseases for people. Then in the end, they sue the corporation and of course won the case and got millions of dollars. It was a quite good movie, but during the watching, I found myself wondering the basis of hexavalent chromium to be so carcinogenic and I remembered basic chemistry things (I studied also chemistry for a while) such as oxidation and reduction events. Well as it is obvious, that kind of (useless) thinking makes following the movie a bit difficult when it is actually not necessary to understand these things to be able to appreciate the movie.

It has been about a year and half of developmental thinking for me and I have to say that I have enjoyed doing it. I feel that I am working at the right place now. I mean, I guess that it does not do any harm in Science if you try to think deeper for example Wnt signaling. I am just not sure if the behavior that I described above is caused because I am trying to become a researcher or my character has driven me to this kind of life. I actually hope that the latter is the case but I think that it is the other way around. Maybe I just need to learn not to start thinking too deeply in a situation where it makes more harm than good and leave that hard thinking to work issues. It would be nice to be able to read a novel again without the reading feeling as a studying experience.

Anyway, thinking of developmental events and cell signaling cascades is interesting and rewarding; when I have understood, learnt or discovered something, it feels good. I think that it is important for your motivation to do research work that you are allowed and supported to think independently. It is also essential that you can plan your own experiments and work at least at certain extent from the beginning of your researcher carrier. Of course, it is necessary to have guidelines and more experienced researchers to give instructions and hints. At least for my motivation it is essential that at least I get the impression that I may have been doing my own discoveries based on my own thinking and planning. If you just do something, which have been thought and planned fully by some body else, it does not feel very interesting even though the plan and the possible results would be great. Therefore it is very important to have own ideas and plans supported by your supervisors and that students are allowed to test them even though the results are not necessary the very best ones at the beginning. Appropriate guidance and quite clear project should of course be the basis of students work at least for the beginning.
The Hyytiälä meetings, which I have been very happy to attend two times now, have been also very motivating for me. I hope that several other times are ahead. At the first time last year, I had just started my Master thesis at Seppo Vainio’s group. All those presentations were new to me and it was actually quite difficult to follow all of them (particularly Saturday☺). Many details went little bit over my head. Anyway, those talks were interesting and people were presenting them with enthusiasm. It was also nice to meet other people working in the same field and to notice how casual people are. It is quite important that you can work with people you like and in an atmosphere that you feel comfortable. The evening party was fantastic. When we left Hyytiälä, I was in very good mood and I thought that I have to come again next year. I think that the meeting serviced its purpose very well also for the beginner.

This year meeting was a good mixture of stress, Science, outdoor activities and partying. I had a little bit of stress because Seppo asked me to give a talk at Hyytiälä. That was my first scientific talk in that kind of meeting. Well, the bus trip to Hyytiälä went largely by concentrating for the presentation. Girls, actually, noticed that I was not talking as much as usually and looking a little bit thoughtful. I have been always quite nervous when I had presentations, but now I learnt that by preparing and concentrating well I am able to perform better. I felt that the talk went quite well and got a good feeling about it. I think that the Hyytiälä meeting is a very good place for practicing to give talks for young scientists, because it is a real scientific meeting with casual atmosphere.

As an outdoor activity, we had football game and I have to say that it was great to have it and I hope that it will become a tradition. It is also an excellent way to meet people from other places. I think that we should schedule more time for outdoor activities for the coming years. The evening party was again really nice and spontaneous as it was last year. I am waiting forward to see you all again next year!
Opinions

Why Finland?

Leon Brokken

That’s the question a foreigner in Finland gets asked invariably. Well, here it goes. Three years ago I was finishing my PhD studies on thyroid physiology in Amsterdam, The Netherlands. In other words, time to think about the inevitable question: What next? Around the same time I read about a vacancy for a post-doc at the Department of Physiology in Turku, Finland. I had never heard about Turku, and Finland didn’t ring much bells either. All I knew, and I think I speak for most foreigners outside of Scandinavia, was that it was a country with few people and lots of trees and lakes. Pretty accurate so far! The project fitted me perfectly, as it dealt with reproduction and developmental biology, two of my main interests. After giving it some thoughts I decided that my grave lack of knowledge about Finland was a good reason to go and ‘check it out’. And so I did. In February 2002 I packed my bags and moved to Turku.

Initially I lived in the Student Village. I could walk to the lab in 10 minutes, which was a relief after commuting between Utrecht and Nijmegen, and later between Utrecht and Amsterdam for the last 7 years. Since there was plenty of snow I didn’t even miss my motorbike (yet). The lab was highly organised and everywhere were notes on what to do and what to don’t. Nothing like the lab I came from, but fine with me. I’ve escaped joining the army so a little lesson in discipline wouldn’t hurt.

The project (more details about the scientific stuff elsewhere in this issue) I was appointed to was carried out in cooperation with the Department of Animal Physiology. There the ‘lab culture’ resembled more the situation in Amsterdam and since most of the work was initially carried out there, I could get used to the new work ethos bit by bit.

The reason for the question “Why Finland?” appears to be the climate. Too wet and too long and dark winters. But to me it all seemed highly exaggerated. Coming from The Netherlands I knew all too well what it was to live in a rainy country. I also knew that people tend to complain a lot about the weather. But as my grandmother used to say: if people complain about the weather they means they don’t have any serious problems. Moreover, back home winter means even more rain, with some occasional snow if one is lucky. And then it stays for a few days before it turns to sludge and disappears. Here in Finland, the winter was dry and it was freezing constantly! Now that’s what I call a decent climate! Then spring came. To me it seemed to take about one week. A bit of rain, and then the sun came out exactly on the Easter weekend. Everything turned green and the Finns came out of hibernation! And the sun stayed until October! I was convinced that this was my kind of climate! All the Finns told me that I was exceptionally lucky with the weather this year. Of course I didn’t (want to) believe them. It’s just the typical complaints I told myself.

After 3 months I had to go back to Amsterdam to defend my thesis. By then life had become unbearable without a motorbike so I planned to return to Finland by bike. Due to the condition of the bike, and the lack of time to repair it for the 1500-km journey I got myself another motorbike (for the intimae: a 1977 Honda GL1000-K2). As a EU citizen I thought to be allowed to import a vehicle free of tax. However, this only applies when the vehicle has been owned for at least half a year. Bummer! I filed a complaint about the amount of the taxation since a similar case was in the
EU court, and last week (!) I got a reply: my taxation was within the law so no refund. Bummer-2!

Nevertheless, with the bike I was now fully equipped to go and see what Finland was all about. That summer I explored the beautiful scenery of the Archipelago and most of southwest Finland.

It’s a biker’s paradise: beautiful winding roads in good condition, no traffic and hardly any traffic control! Quite the opposite of the situation back home. The few cameras take your picture from the front, which is no problem on a bike ;-) The question “Why Finland?” became more and more incomprehensible!

Fig. 1 The unique Finnish Archipelago, you’ve gotta love it! (photo by Tomi Pakarainen)

Like spring, autumn also seemed to happen within a week. Again, a bit of rain and then the snow came. How different from the Dutch climate, where spring and autumn torture the country for at least 4 months, each! That winter I started snow boarding. Although I’d been ice-skating since early childhood, this was my first encounter with down hill winter sports. It turned out to be a steep and painful learning curve. My very first descend down that curve hit me with a whiplash that would last half a year, but much to my surprise after a few trials I got the idea. Now heading for my third season, I consider myself almost a pro… So another reason-why could be added!

Last summer I explored the rest of Finland, and my girlfriend and me headed for the North Cape (by bike, of course) and back along the Norwegian coast. For more on this magnificent trip see http://users.utu.fi/~leobro/northcap2.htm

Fig. 2 Kevo Subarctic Research Institute, University of Turku, Utsjoki.

By now, after the summer of 2004, I have to admit that indeed summer can be a lot worse, and spring and autumn seem to be a lot wetter indeed. Nevertheless, I still think that Finland has a lot to offer.

At the moment, after almost three years as a post-doc it is again time to think what will be next. Hopefully somewhere in The Netherlands. I start to miss the traffic jams.
Meeting report

The 9th international workshop on developmental nephrology
(Adelaide, Australia)

Florence Naillat

When I heard that the American Society of Nephrology will organize a conference on kidney development I just ran for it. The list of speakers was impressive that I definitely tried to go there. The location of the conference was not bad as well, Australia, time to discover the country.

The abstract of the poster was sent and as a post graduated student I needed to get some money, I applied to grants from the Faculty. After some month of waiting, I finely got both answers; YES I was on the way to go to Adelaide. The Finnish delegation was small but the quality was there with Professors Hannu Sariola and Seppo Vainio. Two post graduate students Satu Kuure and I were happy to see again, to share a room and discuss about work…well not always.

One of the persons who impressed me a lot was Professor Marelyn Wintour from Monash University (Australia). She presented her results on kidney development more specifically on nephrogenesis on cow and sheep. That was different from what I was usually hearing. It was the first time for me to hear a kidney research on sheep than on mouse or zebra fish. Her project is to define if cardiovascular diseases would be correlated to the environment during pregnancy. She demonstrated factors like intra uterine (genetics) and extra uterine (environment such as vitamin A, glucocorticoids) would influence the health of the future individual by a decrease of nephron formation. They might cause hypertension and/or renal diseases in adult life when these factors appear at the beginning of the pregnancy.

Break after intensive day.

Through three working days, two nice evenings were settled one scientific on Intravital and three-dimension imaging of the kidney and one for tasting Australian wine.

Do you think they are talking about work or just about the aromas of the wine? Which berry?

Movie time: how the kidney develops in three dimensions with the feeling of understanding the real process “in vivo”.

Finnish delegation!!
Dear friends,

Best wishes to each of you! I returned from Alex Karavanov's (Sasha's) funeral, and I thought I would write down some of my observations and reflections on the day. So I've put below some of my thoughts about Sasha's funeral. I will get back to you about other matters after the weekend.

With every best wish,

Scott Gilbert

I arrive early at the Russian Orthodox Cathedral of St. John the Baptist. It must be the right church. It has an Orthodox cross, a golden onion dome, and the Romanoff two-headed eagle. Besides, the black woman cab driver told me it was the right place. She is an Ethiopian Orthodox believer. She knew all the back roads to get there.

I am invited in by the priest, and the inside of the church is beautiful in its gold, red, and blue paint and gold leaf. Every inch is covered with remarkable pictures of Jesus, the Holy family, saints, and patriarchs. Some of the icons are incredibly detailed and jeweled. The church has no lights on, and all the paintings are seen in sunlight from the roof windows and from the numerous candles. In the center of the church is Sasha's body in an open casket, his feet facing the alter. Flowers and candlesticks surround the casket. One of the flower displays is from Ira's laboratory. There is no music, but the assistant priest starts chanting by Alex's head. The side of the casket frames Alex's profile, making his face look like a coin or a medallion. He looks very noble but disfigured around the eyes by the disease. There is a band on Alex's forehead and a cross in his hand.

People start to come into the church. Most look a bit intimidated, like they had never been there before and don't want to do anything wrong. Others seem to know exactly what to do and where to go. There are no chairs. The assistant priest gives each person a thin golden candle, and some of the candles have cups to catch the falling wax. Many of the people are well dressed, but many are wearing clothes as if they just happened to walk off the street. The assistant priest is not wearing anything special, although the priest as on a beautiful white vestment with gold embroidery. Ira, Anja, and Anja's husband enter. Anja's husband is carrying a baby, about 8 months old. They are not wearing anything special, nor is there any ceremony for their entrance. We all go over to them. I am happy that the baby's name is Alexandra and is called Sasha. Alex got to know his grand daughter before he was dying. He had once told me that the "secret of life" is life itself. So I am very glad that he saw his continuation. Anja lights her candle from mine, and I am honored to play this minor role in the ceremony. About 50 people are present.

The priest chants in Russian, all minor keys, with an assistant priest giving a bass counterpoint. The singing is quite good, and everything seems to be repeated three times. Every so often, there is an English translation, asking Jesus to accept his servant into eternal happiness. The incense smelled very solemn, reminding me of Japanese temples. The priest gave a sermon, saying that every believer lives his life in order to die properly and meet Jesus. He aid that despite the horrors of Communism and secular humanism, some brave people kept the faith, and Alex was one of them. He died in the absence of the pain of sin. According to Ira,
though, his physical pain was quite real, and Ira hoped that Alex would find peace physically as well as spiritually.

Then each person was allowed a final viewing of Alex. Ira went first, kissing his face. She then left the casket in tears and immediately embraced her granddaughter. I think this was the most moving and important moment of the entire ceremony. Then Anja and everyone else placed their candles in the candle-holders and gave their last respects to Sasha. Some kissed him, some touched his arm, others simply silently said goodbye to him. The priest then showered holy water in and on the casket, put a document of some sort into the casket, placed a shroud over the body, and closed the casket. It was wheeled out and into a car.

We left the church and went out into a clear, cold, sunny day. I asked Igor Dawid, Alex's mentor when he came to the United States, if I could have a ride with him; so Igor drove Jean-Pierre Saint-Jeanette and I to the graveside. The grave was in a "Russian" area of the cemetery (near Walter Reed Army Hospital). Every person was given a rose or carnation, and after the casket was lowered into the ground, we were each invited to pour a handful of dirt onto the casket and to throw our flowers into the grave.

Jean-Pierre and I are driven to Ira's house by one of Alex's colleagues from Cyphergen, a biochemist named Joe. Jean-Pierre and Joe know each other from Igor's lab, where they first had met Alex. We tell funny stories about Sasha. I mention how Alex would go "Oh, Shit" when things didn't workout as he had hoped or when someone was saying something that he didn't like at a meeting. We also recalled how he would shake his head, look down, and exclaim something like, "Noooooo! No, Scott, you are wrong!"

We mill around in Ira's house, while Ira's friends are putting wine, vodka, blinis, smoked salmon, cold cuts, and sandwiches on a large table. There are about 30 of us who sit down at the table. I meet Maria M., who worked with Alex and is from Venezuela. We have several friends in common and she has the office next to Rocky Tuan's. Many people here have known Alex from when they were in Russia. Some people know Alex from his time in America. I am the only one who knows him from Helsinki. I am wearing the University of Helsinki tie in commemoration of our time there and the feeling that I am somehow "representing" those friends who knew him during that time of his life. The Russian men said how he was a brother or best friend to them. The main themes of the toast were: (1) Alex gathered around him interesting people wherever he was-in Russia, in Finland, and in America. He formed the nucleus of many such groups. (2) Alex loved people. Ira said that he would come home beaming, and when she asked him what was making him so happy, he would say that some friend got a grant or paper accepted. (3) Everything was important to him. He was passionate about the world, whether it be scientific, political, or cultural. (4) He never stopped being a scientist, even when he was out of the office or the laboratory. (5) He had definite opinions and acted upon them. He was a remarkably brave, intelligent and caring person.
The Professor of the Year - Irma Thesleff

The Professor of the Year is elected each year by the Board of the Finnish Union of University Professors. This year the Union wanted to focus on the significance of current research in developmental biology.

Professor Irma Thesleff is the Research Director at the Institute of Biotechnology’s Developmental Biology Research Program. The Program employs approximately 60 scientists and is one of the Centres of Excellence nominated by the Academy of Finland.

By its selection, the Finnish Union of University Professors wanted to focus on the significance of current research in developmental biology.

In her acceptance speech at the Science Festival (Tieteen päivät 2005, Helsinki), Irma stressed the importance of adequate tenures for research scientists. According to her, reaching the top of the international level and staying there is more than a fulltime job.

Congratulations!! editorial board of D&C

PhD Dissertations in Developmental Biology

Elena Arighi, Institute of Biomedicine Molecular characterisation of ret tyrosine kinase signalling. 
Opponent: Professor Massimo Santoro, Department of Biology and Cellular and Molecular Pathology, University Federico II of Naples, Italy
Supervisor: Professor Hannu Sariola

Tuija Mustonen, Institute of Biotechnology Ectodermal Organ Development: Regulation by Notch and Eda Pathways.
Opponent: Professor Annette Neubüser University of Freiburg, Germany
Supervisor: Professor Irma Thesleff

Mark Tümmers, Institute of Biotechnology To The Root of the Stem Cell Problem – The evolutionary Importance of The Epithelial Stem Cell Niche During Tooth Development
Opponent: Professor Thimos Mitsiadis King’s College London, England
Supervisor: Professor Irma Thesleff

Xiu-Ping Wang, Institute of Biotechnology Molecular Mechanisms underlying Tooth Morphogenesis and Cell Differentiation
Opponent: Docent Amel Gritli-Linde Department of Oral Biochemistry Göteborg University, Sweden
Supervisor: Professor Irma Thesleff

Annual Tvärminne retrait

Time: 28. – 29. of April

All people in Developmental Biology Program in Helsinki are encouraged to participate in two-day seminar organised in Tvärminne Biological Station in Hanko. Details about registration and schedule will be given by e-mail later, but now it is time to reveal your great ideas concerning the program – both for scientific and fun parts. The organising committee is “under construction” but will be headed by Satu Kuure, to whom you can tell your suggestions (satu.kuure@helsinki.fi).
10 Questions

What’s your name? Florence Naillat

Where are you from? From south of France near the Mediterranean Sea, a town called Nîmes.

What did you do before you come here? Long story but to be short I was studying in Montpellier for the “Maitrise de Biochimie” equivalent of Master, then worked in Finland and at the same studied for a second Master in Finland.

What is your favorite animal and why? I would choose an animal which is active and take few hours for resting. Any idea? A black panther may be.

What do you like to do? I like many things and it is difficult to peak only one in particular: Walking in the nature when it is not too cold or reading books and new papers to be in touch with the outside world of sciences.

What don’t you like to do? Cleaning the whole flat… I would rather do something else. I guess I am not the only one.

Is your glass half full, half empty or always empty? Better to be half full or even more if you want to enjoy life as I do. No time to stop, life is too short and need to be appreciated each second.

What would you be doing if you weren’t a scientist? I love flying and traveling. I always wanted to be a pilot but I am too small for reaching the pedals!!

What is your favorite food? As French I will choose everything but I love cheeses and wine which can not be disconnected from each other.

Did you ever get into a fight and why? Well I don’t remember if you are talking about physical one. Word fight more probably.

What is the best day of the week? All the week days if you always see and find the positive signs from it.

What would you rather be, a worm or a tiger, and why? I think a tiger it is better than to be a worm.

10 Questions

What’s your name? Renata

Where are you from? Vilnius, Lithuania

What did you do before you come here? Besides of studying I was doing lots of interesting things as well as I’m doing also here.

What is your favourite animal and why? As a pet dog as they are truly great friends

What do you like to do? It depends when, other vice starting with reading, travelling, spending time with friends…handcrafting and even science ;)

What don’t you like to do? I just happen to dislike ironing, but still doing it

Is your glass half full, half empty, or always empty? Mostly half full

What would you be doing if you weren’t a scientist? Once I was about to become a psychologist luckily science has rescued me

What is your favourite journal and why? It is still not issued
What is your favourite food? Lithuanian speciality - Cepelinai (kind of potato dumplings with various filings) and dark chocolate with nuts.

Did you ever get into a fight and why? If I ever did so do not recall it.

What’s the best day of the week? Wednesday.

What would you rather be, a worm or a tiger, and why? Tiger as they have more chances to enjoy the sun.

How long are your toenails? I cut them often, so they are not long. Maybe 1.5 cm.

As we all know, Turku is a lovely town (the best in Finland). What is the best thing here? My favourite things in Turku are the sea, the forest and the cottages. My favourite pub is Börs. I like Turku because the size of the city is good: not too big, which makes me feel stressful. And it also has a very good communication system: Internet and mobile phones are widely used here. I like the Turku summer, which is fantastic, but I am a bit afraid of the winter, quite dark but luckily not cold in Turku.
What is your favourite method/technique/equipment in the lab?
I like different microscopy techniques, because they enable scientists to visualise things that would otherwise be invisible.

What is your favourite animal and why?
In the lab I prefer mice, but generally cats and horses.

What would you rather be, a worm or a tiger, and why?
A tiger, because it’s bigger, more beautiful and it can fight.

What do you like to do?
I like music, dancing, horse-back riding and reading, but most of all I love sleeping…

How long are your toenails?
Very short because of my ballet lessons. Those are really painful with long nails!

As we all know, Turku is a lovely town (the best in Finland). What is the best thing here?
Nice people, great friends, Ruissalo and everything…!

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10 Questions

What’s your name?
Petra Sipilä.

Where are you from?
Kuopio.

What are you researching and what is your goal in that?
I want to learn how small sperm learn to swim in the epididymis.

What would you be doing if you weren’t a scientist?
I would dream about being a scientist.

What is your favourite method/technique/equipment in the lab?
ET-recombination.

What is your favourite animal and why?
Lab mouse – does it need to be explained?

What would you rather be, a worm or a tiger, and why?
We all want to be worms. Worms can mind their own business without others bothering them. Tigers are hunted to extinction.

What do you like to do?
I would like to win in a lotto.

How long are your toenails?
Short.

As we all know, Turku is a lovely town (the best in Finland). What is the best thing here?
The river side with lots of nice happenings!