3rd Trans-Tech-Meeting

Karolinska Institute

Stockholm

June 18 - 20

Trans-Tech Meeting 2001
Karolinska Institutet

Dear Colleagues

Sorry that it took so long time to have the Trans-Tech Meeting Abstracts as PDF-file, but the month after the meeting were quite busy for us. And we are also only human and can forget some things.

I would like to thank all people that helped to make this meeting success and also the lecturers for their very good talks.
A special thank goes to Biborka Bereczky-Veress who was the "good soul" of this meeting. Without her, I would have been lost.

Thanks also to all that cheered up my days during this meeting, especially the Skansen gang Oscar and Tom with his wife Kay.

I wish you all the best for the future and hope to see you at the next meeting. Please watch out for the information put out in the tg-list.

Cheers and see you,

Johannes Wilbertz

Program

3rd Trans-Tech-Meeting
Stockholm
18 – 20 June 2001

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1. Day

08.30 – 10.00 **Registration for all Participants**

10.15 **Welcome and Introduction**

a) Karolinska Institutet
b) Johannes Wilbertz / Organizing Board

Chairman: Stephan Teglund / Karolinska Center for Trangene Technologies, Sweden

Basics in transgenic research (10.30 – 12.30)

10.30 – 11.15	Setting up a transgenic lab (Anna B. Auerbach, / Skirball Inst., New York, USA)
11.15 – 12.00	Basics in construct design (tg – ko) (Achim Plum / Mice&More, Germany)

Lunch

13.00 – 14.00 Coffee Break, Network and Opportunity to view trade exhibition

Poster should be mounted

Chairman: Nelson Khoo / Umeå Transgenic Core Facility, Sweden

Methods and problems in tg research (14.30 – 17.00)

14.00 – 14.45	New microinjection methods for mammalian transgenesis (Anthony Perry / Advanced Cell Technology Inc., USA)
14.45 – 15.30	Injection of large constructs - Use and problems. (Luis Montoliu / Centro Nacional de Biotecnologia (CNB-CSIC), Madrid, Spain)
15.30 – 16.15	Behavioral phenotyping of mice (Alexander Kuzmin / Div. of Behavioral Neuroscience, KI, Sweden)
16.15 – 17.00	Knockouts of APP-Family members: Gene redundancies and influence of genetic background on phenotypes (Ulrike Müller / MPI Brain Research, Germany)

Poster Session + Coffee (17.00 – 18.00)

Presenting authors should be present at their posters

1. Award of the genOway Price for Transgenic Technologies (18.15 – 19.30) (Open to all public)

Introduction of the price

Introduction of the price winner

Awarding of the price and lecture of the prize winner Teruhiko Wakayama

20.00 Get together

Lunch buffet

2. Day

Chairwoman: Marcela Pekna / Gothenburg Transgenic Core Facility, Sweden

Public concerns about and alternatives to laboratory animals in transgenic research
(09.00 – 12.30)

09.00 – 09.45	Refinements in the production, management and care of genetically modified rodents (Vicky Robinson / RSCPA, UK)
09.45 – 10.30	Targeted oncogenesis in transgenic mice: A strategy for cell immortalization (Alain Vandewalle / INSERM / Faculté de Médecine Xavier Bichat, Paris, France)

Coffee Break

11.00 – 11.45	Horizontal gene transfer (Alfred Pühler / University Bielefeld, Germany)
11.45 – 12.30	Going public – How to present your research to the public (Robin Lovell- Badge / National Institute for Medical Research, Mill Hill, UK)

Lunch

Chairman: Ulrich Märki / Provimi Kliba AG, Switzerland

Use of transgenics in research I (14.00 – 17.30)

14.00 – 14.45	Xenografting (Ejvind Kemp / Denmark)
14.45 – 15.30	Gene pharming (Mathias Müller / Vienna University, Austria)

Coffee Break

16.00 – 16.45	The use of transgenic techniques in the characterization of genes involved in growth factor signaling in Drosophila (Udo Häcker / Lund University, Sweden)
16.45 – 17.30	Chloroplasts of higher plants as a tool of genetic engineering (Iwona Adamska / Stockholm University, Sweden)
17.30 – 18.30	Happy Hour and Poster Session II

3. Day

Chairman: Anne Plück / University Cologne, Germany

Use of transgenics II (9.00 – 11.30)

09.00 – 09.30	Transgenics in cancer research (Lars Holmgren / CCK, KS, Sweden)
09.30 – 10.00	Prion diseases: insights from mouse models (Eckhard Flechsig / MRC Prion unit/Neurogenetics, Imperial College School of Medicine, UK)

Coffee Break

Poster should be dismounted!

10.30 – 11.00	Strategies to Control NR1(N598Q/R) Mutant NMDA Receptor Expression in the Mouse Brain (Frank N. Single / MPI Med. Research Heidelberg, Germany)
11.00 – 11.30	Transgenics in developmental biology (Urban Lendahl / CMB, KI, Sweden)

Final summary

11.30	Johannes Wilbertz

LECTURE ABSTRACTS

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Setting up a transgenic lab

Anna B. Auerbach

NYU SM, Skirball Institute of Biomolecular Medicine,
New York

An important step in setting up a new Transgenic Service Facility is to carry out a careful evaluation of future needs. A survey of potential “clients” can be very helpful. It is important to determine what genetic backgrounds scientists want to utilize. What are their levels of experience with transgenic/ES-cell targeting technologies? Are they planning a single experiment, or do they want to dedicate a major part of their lab’s work to transgenic experiments? How many clients have access to other rodent facilities that could threaten the health of animals in the transgenic facility?
If the goal is to organize production to cover the needs of only a single lab, of course all of the above is simpler.

In a new facility every room and experiment should have a **Standard Operating Procedure** developed beforehand and updated with time. This will help with training and interactions with researchers. Having protocols available e.g. during initial meeting with PI or postdoc, will be very beneficial. We always hand out a “DNA Transgenic Fragment Purification Protocol”, which in our opinion delivers the most consistent results.
If possible, the transgenic facility should be located in a SPF animal colony to produce mice of known pathogen free status. It is important to note that animals coming from the transgenic lab may end up in several other animal rooms or other colonies. For this reason, and the fact that surgery will be performed on the foster mothers and cell lines injected into embryos, the Transgenic Facility represents one of the greatest risk areas for initiating the spread of pathogens. Therefore, the highest standard of an **infection prevention program** should be applied during transgenic production.

What **services** will be offered by a new facility? Many transgenic facilities offer chimera production service along with DNA microinjection. ES cell chimeras can be produced both by ES cell-morulae aggregation using outbred mice as a host and by injection of ES cells into blastocysts. The aggregation technique is less expensive, but it is not ideal for all experiments and only effective with certain ES cell lines. Other services often offered are rederivation of pathogen free mice and cryopreservation of mouse germplasm.

A very helpful tool to organize such complex work is to establish an extensive **database** for weekly planing, up to date record keeping, reporting, reviewing and summarizing results, and billing. I will show an example of such a database.

Even if from the results of the survey it looks like there will be a need to manipulate embryos of many **mouse strains**, initial efforts should be made to create a solid and simple base. For example, begin with mastering microinjections into eggs of one inbred strain. FVB/N is an excellent inbred strain for DNA microinjection into zygotes. FVB/N females usually respond well to superovulation, embryos have prominent pronuclei and tolerate the procedure well, and finally they implant and develop well after manipulation. Expansion into new genetic backgrounds and new services should not be done until satisfactory results of controls for culturing conditions and implantation of non-manipulated embryos are achieved, and few full-scale experiments have produced good numbers of founders with a line like FVB/N.

Transgenic production is a complex process consisting of sequential steps. Each step of the production can and should be evaluated for its efficiency. Many different factors contribute to the efficiency of every step and thus, influence the overall efficiency of the whole process. The factors include the following: response of egg donor females to superovulation, male fertility, purity of DNA, embryo culture conditions, ability of manipulated embryos to implant and to continue to develop to term. All of these factors seem to have a different influence depending on the strain of embryo donor. Does the embryo donor strain influence embryo transfer time (same day or the next day) best for your procedure? I will share my experience with manipulating embryos of different genetic backgrounds and strain dependent influence on the efficiency of the production. In this context, I will discuss transgenic production **efficiency measures.**

Results ought to be reviewed often. A solid database is essential to pinpoint the step(s), which requires attention. Also, depending on the embryo donor strain, the manipulation procedure might need to be modified to overcome strain dependant characteristics.

In my final comments, I will discuss helpful **breeding techniques** to make transgenic production more efficient.

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Basics in Construct Design

Achim Plum

mice & more GmbH & Co. KG, Martinistr. 52, 20251 Hamburg, Germany

Vector construction, especially for gene targeting in embryonic stem cells, is a tedious and time consuming business. Yet, good design and high quality of the vector are crucial to the success of the whole experiment and mistakes made in this step often only become apparent after a lot of time and money were put into the project.

The talk will review and compare various strategies that can be employed to approach the analysis of gene function *in vivo* and will try to give some guidelines to make the right choice to create the mutant mouse strain desired. Constitutive and conditional gene ablation strategies, knock-in approaches and inducible systems will be covered.

Once a decision for a general strategy has been made, careful construction of the vector is essential for efficient homologous recombination in ES cells as well as for effectiveness of the mutation introduced. Some general rules as well as hints to possible pitfalls in the construction process will be provided.

Finally an outlook on new developments will be given that will possibly speed up and facilitate the construction of gene targeting vectors and transgenes.

New microinjection methods for mammalian transgenics

Anthony Perry

Advanced Cell Technology Inc. / USA

(not submitted at time of print)

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INJECTION OF LARGE CONSTRUCTS. USE AND PROBLEMS.

Lluís Montoliu

Abstract

(taken from**: Giraldo P. & Montoliu L. (2001). Size matters: use of YACs, BACs and PACs in transgenic animals.** ***Transgenic Research*** **10(2): 83-103)**

In 1993, several groups, working independently, reported the successful generation of transgenic mice with yeast artificial chromosomes (YACs) using standard techniques. The transfer of these large fragments of cloned genomic DNA correlated with optimal expression levels of the transgenes, irrespective of their location in the host genome. Thereafter, other groups confirmed the advantages of YAC transgenesis and position-independent and copy number-dependent transgene expression were demonstrated in most cases. The transfer of YACs to the germ line of mice has become popular in many transgenic facilities to guarantee faithful expression of transgenes. This technique was rapidly exported to livestock and soon transgenic rabbits, pigs and other mammals were produced with YACs. Transgenic animals were also produced with bacterial or P1-derived artificial chromosomes (BACs / PACs) with similar success. The use of YACs, BACs and PACs in transgenesis has allowed the discovery of new genes by complementation of mutations, the identification of key regulatory sequences within genomic loci that are crucial for the proper expression of genes and the design of improved animal models of human genetic diseases. Transgenesis with artificial chromosomes has proven useful in a variety of biological, medical and biotechnological applications and is considered a major breakthrough in the generation of transgenic animals. In this report, we will review the recent history of YAC/BAC/PAC-transgenic animals indicating their benefits and the potential problems associated with them. In this new era of genomics, the generation and analysis of transgenic animals carrying artificial chromosome-type transgenes will be fundamental to functionally identify and understand the role of new genes, included within large pieces of genomes, by direct complementation of mutations or by observation of their phenotypic consequences.

Behavioral phenotyping of transgenic and knockout mice.

A.Kuzmin and S.O. Ögren

Division of Behavioral Neuroscience, Department of Neuroscience, Karolinska Institutet

One of the most important aspects in the studies on the role of genes in behavioral function is the selection of relevant behavioral techniques, which provide the researcher with appropriate information (in a time efficient manner) about the impact of the genetic manipulation on behaviors. This point is particularly relevant in the case of behavioral and neurological battery of tests, which are often extremely labor intensive and require well trained personal. Genetically modified animals create new opportunities for evaluating the determinants of behavior and the role of various neurotransmitters and receptor subtypes. Furthermore, they also give an opportunity for validation of pharmacological hypothesis. Concerning attempts to model psychiatric and neurological disorders, genetic manipulation offers a possibility to identify genetic determinants of the disease, and to develop models to mimic more closely clinical symptoms.

However, attempts to interpret phenotypical changes, which arise in the genetically engineered animals, are a subject of several caveats. The ability to disrupt a single gene might lead to the compensational changes in other genes and systems, which might profoundly confound the analysis of behavioral phenotype. In addition, ignoration of the genetic background might lead to misinterpretation of the results. Mouse strains show remarkable genetic differences in motor and operant activity, memory and learning, basic anxiety and sensitivity to stress and reward. Therefore, the genetic background of mice with a targeted mutation may interact with strain specific behaviors. Without proper controls, one can thus never be sure, whether an observed impairment in behavior of a targeted mutation is caused by the engineered mutation or by a flanking natural mutation of the129 sub strains, which are established as donors of ES cells. Therefore, knowledge about the behavioral phenotype of the parental strains is a must in order to select the most appropriate strain for the genetic manipulation.

Behavioral phenotyping of mutant mice requires rigorous and stepwise analyses. Well-characterized paradigms must be chosen from the established behavioral techniques. Rigorous experimental design can minimize the high risk of false positives and false negatives in the behavioral phenotyping of the mutant mice. Use of well-established, quantitative, reproducible behavioral tasks, correct statistical methods, and attention to litter and gender issues will maximize the efficiency of comparison between -/-, +/- and +/+ genotypes.

In the sequential analysis of the behavior of mutant mice the following general scheme is proposed. It is important to start with behavioral and neurological tests, which have the minimal stressful influence. A battery of observational tests (without interfering with the animals) including measurement of food and water intake is preferable in the beginning of the analysis. This is preferably followed by a battery of relatively quick neurological and behavioral tests for measurement of unconditioned behavior. In the final stage of analysis a series of specific behavioral tests are introduced, clustered by category, including multiple paradigms for each category (eg. learning and memory tests, tests for anxiety like behavior, tests for depression like behavior…).

Historically, most of the behavioral tests have been designed for rats. However, the step from rat to mice in behavioral experiments has turned out to be quite complicated and, probably, require different experimental approaches, especially with respect to methodology and design of the experiments.

Finally each form of complex behavior is characterized by multifactor dependence (i.e. disruption of different higher nervous functions may lead to convergent behavioral phenotype). Therefore, a multifactorial approach by performing analysis of the single animals’ behavior in a battery of tests is required. For each test several parameters of the behavior must be recorded in order to find correlations between different aspects of the behavioral repertoire and to exclude the influence of nonspecific factors in the interpretation of genetic impact on behavioral performance.

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Knockouts of APP-Family members: Gene redundancies and influence of genetic background on phenotypes

U. Müller

Max-Planck-Institute for Brain Research, Frankfurt Germany

The amyloid precursor protein APP and it's metabolism play a crucial role in the pathogenesis of Alzheimer's disease (AD). Understanding of the physiological functions of APP and homologous proteins will be essential to unravel the misregulation and pathogenic mechanisms that lead to Alzheimer's disease. APP is a member of a larger gene family including the closely related APP-like proteins APLP1 and APLP2. To determine the physiological role of APP we have previously generated by gene targeting APP knockout mice that showed retarded growth and sensorimotor development, reduced grip strength, were behaviourally impaired and showed increased susceptibility to epileptic seizures. As the only anatomical abnormality APP knockout mice displayed a high incidence of agenesis of the corpus callosum.To determine to what degree the APP mutation interacts with genetic background alleles that predispose for forebrain commissure defects in some mouse lines, we have assessed the size of the forebrain commissures in a sample of 298 mice. Lines with mixed genetic background were compared with congenic lines obtained by backcrossing to the parental strains C57B/6 and 129/SvEv. Both APP knockout mice and a line of mice expressing a modified form of APP (with a deletion of exon 2 and at only 5% of normal levels) revealed a drastically increase in the frequency and severity of callosal agenesis in mouse lines with 129-SvEv and 129-OLA background.

To assess potential functional redundancies within the gene family we have recently generated mice lacking individual or all possible combinations of APP family members. Mice deficient for the nervous system-specific APLP1 protein showed a postnatal growth deficit as the only obvious abnormality. In contrast to the minor phenotype of single mutants, APLP2$^{o/o}$/APLP1$^{o/o}$- and APLP2$^{o/o}$/APP$^{o/o}$-mice proved lethal early postnatally. Surprisingly, APLP1$^{o/o}$/APP$^{o/o}$-mice were viable, apparently normal and showed no compensatory upregulation of APLP2 expression. These data corroborate a key physiological role for APLP2 and indicate redundancy between APLP2 and both other family members. This view gains further support from the recent observation of haploinsufficiency of APLP2 in $APLP2^{+/o}/APLP1^{o/o}/APP^{o/o}$-mice: about 90% of these animals die postnatally and the surviving animals show strongly impaired mating behavior. None of the lethal double mutants displayed, however, obvious histopathological abnormalities in the brain or any other organ examined. Moreover, cortical neurons from single or combined mutant mice showed normal survival rates under basal culture conditions and unaltered susceptibility to glutamate excitotoxicity in vitro.

Refinements in the production, management and care of genetically modified rodents

Vicky Robinson on behalf of the BVA(AWF)/FRAME/RSPCA/UFAW Joint Working Group on Refinement.

Since the results of the first GM mice were reported in 1980, GM rodents have been used extensively in a wide range of biological and biomedical disciplines. Their use is however of increasing concern to animal welfare organisations not least because of the numbers of animals used; the surgery and other invasive procedures involved; and the deleterious effects that genetic modification can have on animal welfare.

With any use of animals it is critically important to apply the principles of the three Rs namely the replacement of animals with humane alternatives; the reduction in the number of animals used; and the refinement of procedures to minimise any suffering that may be caused. The production and use of GM rodents however poses new challenges and obstacles to the application of the principles of the 3R's. This is due in part to the limitations of the current technology. Nonetheless, there is a lack of awareness of the animal welfare issues and best practices associated with the production and care of GM rodents that needs to be overcome if the principles of reduction and refinement are to be consistently applied. With this in mind the sixth BVA(AWF)/FRAME/RSPCA/UFAW Joint Working Group on Refinement was convened to address various scientific, practical and animal welfare issues associated with generation of GM rodents.

This paper summarises the areas of concern and how the Working Group is addressing them.

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Targeted oncogenesis in transgenic mice : A strategy for cell immortalization

A. Vandewalle

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In recent years, targeted oncogenesis in transgenic mice (1) has been used in many laboratories to derive a variety of new types of differentiated nonepithelial and epithelial cell lines. This strategy has given rise to strains of transgenic mice harboring an oncogen, generally the t and T antigens (Tag) of the simian virus 40 (SV40), under the control of the regulatory sequences of a cell-specific promoter. As a result, the transgene is present in all cells, but is regulated and activated only in those cells in which the regulatory sequences used are functionally regulated by their appropriate transcription factors, and in some cases by hormones and/or diet. SV40-positive tumors can be produced under certain conditions, but the induction of the transgene expression does not necessarily imply tumor formations. The major advantage of transgenes harboring the immortalizing agent under the control of the regulatory sequences of a given gene is that they are regulated like the corresponding endogenous gene. The ***in vivo*** integration of Tag in cells also present the advantage to avoid the procedures of infection or transfection of primary cultured cells with immortalizing agents, which are often responsible for rapid dedifferentiation processes.

In our laboratory we have used two models of transgenic mice carrying either wild-type or temperature-sensitive (TsA_{58}) Tags placed under the control of the 5' regulatory sequences of the human vimentin promoter or different constructs of the rat L-type pyruvate kinase (L-PK) promoter to derive lines of renal, liver and intestine cell lines (for review see 1-3).

Transgenic mice carrying the TsA_{58} Tag under the control of the vimentin promoter have been used to establish lines of renal glomerular and tubule epithelial cells. These cell lines were used to analyze the sequential appearance (or disappearance) of cytoskeleton elements in cells grown either at permissive (33°C) or restrictive (39.5°C) temperatures mimicking the ***in vivo*** mesenchyme-to-epithelial cell conversion (4).

Transgenic mice carrying Tag under the control of a 3.2 kb region of the L-PK promoter, which can be highly stimulated ***in vivo*** by carbohydrate-rich diet or ***ex vivo*** by D-glucose-enriched medium, allowed us to establish lines of fetal hepatocyte-like cells and tumoral liver cells (5, 6), intestinal crypt cells (7), and highly differentiated renal proximal (8) and collecting duct cells (9). Interestingly, the recently cloned cell line derived from microdissected cortical collecting duct (mpkCCD$_{cl4}$ cells) which have maintained sodium transport stimulated by corticosteroid hormones has also been used to clone two new murine genes (mCAP1 and Nedd4-2) involved in the regulation of the amiloride-sensitive Na^+ channel (ENaC) and to identify the candidate genes specifically induced or repressed by aldosterone and vasopressin (10).

These overall results provide now lines of evidence that targeted oncogenesis represents a powerful strategy to derive differentiated cell lines suitable for ***ex vivo*** analyses. In next future, transfection of the highly differentiated mpkCCD$_{cl4}$ collecting duct cells with mutated transporters or ion channels should represent unique cell systems for the analysis of ionic transport dysfunctions observed in human diseases.

(1) Briand et al., 1995, 47: 388-394. (2) Vandewalle, A. Exp. Nephrol. 1999, 7: 386-393. (3) Vandewalle et al. Curr. Opin. Nephrol. Hypertens. 1999, 8: 581-587. (4) Cluzeaud et al. J. Cell. Physiol. 1996, 167: 22-35. (5) Antoine et al. J. Biol. Chem. 1997, 272: 17937-17943. (6) Courjault-Gautier et al. Exp. Cell Res. 1997, 234: 362-372. (7) Bens et al. Am. J. Physiol. Cell Physiol. 1996, 270: C1666-C1674. (8) Cartier et al. J. Cell Sci. 1993, 104: 695-704. (9) Bens et al. J. Am. Soc. Nephrol. 1999, 10: 923-934. (10) Robert-Nicoud et al. Proc. Natl. Acad. Sci. USA. 2001, 98: 2712-2716.
65. Cluzeaud, F et al. J. Cell. Physiol. (1996) 167: 22-35.

Horizontal gene transfer – Analysis of broad host range plasmids carrying interesting gene loads

Alfred Pühler

Department of Genetics, Faculty of Biology, University of Bielefeld

The lecture will start with the sequence analysis of the antibiotic resistant plasmid pTP10 originally identified in the clinical isolate *Corynebacterium striatum*. It was found that this plasmid is composed of DNA segments initially identified in soil bacteria and in plant, animal, and human pathogens. In order to demonstrate horizontal gene transfer conjugative antibiotic resistance plasmids isolated from bacterial communities of activated sludge were analyzed as a next step. It could be demonstrated that most of the plasmids analyzed belonged to the incompatibility group IncP. The specific IncP plasmid pB4 carried among other resistance genes a gene region encoding a tripartite multidrug efflux system. The occurrence of broad host range plasmids was also demonstrated in a microbial population residing in the rhizosphere of alfalfa. The mercury resistance plasmid pSB102 was further analyzed. It was found that the transfer region of plasmid pSB102 encoded a type IV secretion system closely related to the *virB* gene cluster found on the chromosome of the mammalian pathogen *Brucella* and the Ti-plasmid of *Agrobacterium tumefaciens*.

Previously, the issue of horizontal gene transfer has become important in the context of biosafety. In particular, the occurrence of antibiotic resistance genes in transgenic plants turned out to become a phenomen of public concern. However, considering the transfer frequency of resistance genes between plants and microorganisms it becomes evident that there is only a very low risk of an undesirable spread of antibiotic resistance determinants.

Going public - How to present your research to the public

Robin Lovell-Badge

National Institute for Medical Research, Mill Hill, UK
(not submitted at time of print)

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Xenografting

Ejvind Kemp

Denmark

Xenografting means transplantation of organs or tissue between species .

The purpose is to transplant organs or tissue from say pig to man with end stage organ failure .

Thousands of patients die today without transplantation due to a catastrophic shortage of organs .

Many attempts have been done in order to make xenotransplantation useful in the clinic – but so far without great success .

On the other hand our understanding of the process is growing . It is now possible to keep monkeys alive with xenotransplanted kidneys from pigs in 3 – 4 months .

These animals survive due to manipulation with the complement system via introduced transgenes.

The alfa-gal knock out pig is a future goal for further progress .

Many problems remained to be solved before going to the clinic , but the threat of creating virus disease after xenografting is dominating . It seems to be conquered and then clinical testing will follow soon. Many countries are preparing for clinical xenografting .

In the meantime living kidney donors are used more and more and artificial organs are doing better and better .

But the need for successful xenotransplantation of all organs and tissues is still the ultimate goal for treating many severe disease.

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Gene Farming

Mathias Müller[1] and Gottfried Brem[2]

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The classical method to isolate pharmaceutical proteins for humans was by extracting them from a variety of sources, including plant, animal and human tissues or organs. Current approaches for the production of high value proteins focus on the use of recombinant DNA technology, i.e. genetic engineering or transgenesis.

The generation of transgenic farm animals was a milestone initiating intense research aiming at the efficient, cost effective production of complex proteins in livestock species. The use of domestic animals (or plants) as bioreactors was termed 'gene farming'. The expression of genes encoding proteins of high value is directed to body liquids or organs of transgenic animals. Milk, serum, urine and semen fluid has been successfully tested as production sites for foreign proteins. An advantage of gene farming in domestic animals is the capability of mammalian cells to produce proteins carrying all post-translational modifications which are required for biological activity. In addition, animal bioreactors are considered more cost effective than tissue culture systems.

The mammary gland is the most interesting organ for the production of recombinant proteins. It has an enormous physiological potential for the daily production of proteins. Milk is easy to collect and usually has a high hygienic standard. For some recombinant proteins other organs or tissues are of advantage if their post-translational modification is only possible in specific somatic cells. The selection criteria to choose the most suitable species for gene farming are usually based on the quantity of protein needed per year. A simplified rule for gene farming in the mammary gland is: The production of a protein in tons should be carried out by cows, in hundreds of kg by sheep or goats and in kg per year by rabbits. Other important points to be considered include the reproduction performance (generation interval, number of offspring), the availability of tools for genetic engineering (cloning or gene targeting, see below), the susceptibility to potential anthropozoonoses (e.g. transmissible spongiform encephalopathies, TSE) and the cost of animal husbandry (e.g. specific pathogen free, SPF conditions).

Rabbits appear more and more to be an intermediate animal well adapted for the production of limited amount of proteins (see above). Rabbit husbandry can be done under SPF conditions and the species seems to be free of TSE. Rabbits have a short generation interval and transgenic founders can be generated with a reasonable efficiency. Milking can be performed semi-automatically resulting in a milk yield of 10 kg per rabbit and year. Thus considering both economical and hygienic aspects this species is suitable for gene farming.

We used alpha-S1-casein and immunoglobulin regulatory sequences for transgene expression in the mammary gland and in blood, respectively. Produced proteins include human insulin-like growth factor 1 (IGF-1) (Zinovieva et al., 1998), human nerve growth factor beta (NGF-_) (Coulibaly et al., 1999), bovine follicle stimulating hormone (FSH) (Brem, Müller et al., in preparation) and antibodies (Weidle et al., 1991). Bioassays have demonstrated the biological activity for all expressed transgenes.

Key experiments for the further development and exploitation of gene farming are based on the nuclear transfer (cloning) technique (Wilmut et al. 1997) and were the generation of transgenic farm animals (Schnieke et al., 1997) and the first reports on successful targeting of specific genes in sheep (MacCreath et al., 2000; Denning et al., 2001). These milestone in gene transfer technology together with the growing knowledge on the design of transgenes will lead to an even more intensive and effective use of farm animals for novel production purposes.

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The use of transgenic techniques in the characterization of genes involved in growth factor signaling in Drosophila.

Erica M. Selva[1], Kyoungja Hong[2], Gyeong-Hun Baeg[1], Salvatore J. Turco[2], Norbert Perrimon[1] and Udo Häcker[1,3]

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Secreted growth factors of the Wnt/Wingless (Wg), Hedgehog (Hh) and Fibroblast Growth Factor (FGF) families play important roles in patterning the body plan in both ***Drosophila*** and vertebrates. Recently it has become clear that the proper function of these molecules depends on the integrity of proteoglycan and glycosaminoglycan biosynthesis. In ***Drosophila***, mutations in enzymes involved in the biosynthesis of heparan sulfate proteoglycan (HSPG) side chains, or in proteoglycan core proteins, lead to defects very similar to those seen in mutations that affect the Wg, FGF, or Hh signaling pathways. In addition, the specificity of growth factor receptors may be regulated by the differential addition of polysaccharide side chains (see review by Perrimon and Bernfield[1]). In ***Drosophila*** the specificity of the Notch receptor for its ligands Serrate (Ser) and Delta (Dl) during determination of the dorsal/ventral boundary in the wing and eye imaginal discs appears to be modified by the activity of the Fringe (Fng) protein (reviewed in [2]), an N-acetylglucosaminyltransferase [3,4]. Here we show that mutations in the ***fringe connection*** (***frc***) gene, which encodes a nucleotide sugar transporter, affect not only Wg, Hh and FGF-dependent pathways but also the activity of Fng/Notch-dependent pathways. We propose that Frc transports several crucial UDP-sugars into the Golgi apparatus, an essential process for the Fng mediated modification of the Notch receptor, as well as for the synthesis of HS chains involved in Wg, Hh and FGF signaling. Consistent with this, we find that Frc transports UDP-N-acetylglucosamine and UDP-glucuronic acid.

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Trans-Tech Meeting 2001
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Chloroplasts of higher plants as a tool of genetic engineering

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Plant cells have three gene-containing compartments: the nuclear-cytoplasmic system, mitochondria and plastids. Plastids are intracellular organelles where photosynthesis, synthesis of fatty acids, lipids, plant hormones, amino acids, vitamins, nucleotides and secondary metabolites take place. Recently, the ability to introduce foreign genes at specific locations into a plastid´s genome has been acquired. It turned out that plastids are important alternatives to the cytoplasm as sites to engineer enzymatic reactions or to store proteins or enzyme products. There are several advantages of using plastids for genetic engineering of plants. A chimeric protein might be more stable in the plastid than in the cytoplasm because of differences in proteases and ions. A specific type of plastid (chlorophyll-containing chloroplasts, carotenoid-containing chromoplasts, starch-storing amyloplasts, oil-containing elaioplasts or dark-grown etioplasts) might be a good place to accumulate chimeric proteins that would be harmful if they were present in large amounts in the cytoplasm or in a plastid of a different type. It might also be advantageous to place energy-intensive biochemical reactions close to the site of photosynthesis. The maternal inheritance of the chloroplast genome may eliminate the risk of the escape of a foreign gene through pollen dispersal from transplastomic plants. Another advantage of chloroplast engineering is a very high level of transgenic expression that is attributed to the presence of 50-100 chloroplast per cell and 60-100 DNA copies per plastid. Thus, each leaf cell contains between 3.000 and 10.000 copies of each gene present once in each plastid chromosome. These advantages of plastid engineering are currently used for designing metabolic pathways for production of tailor-made plant polymers, improvement of food quality, production of vaccines and antibodies in plants or for increasing plant resistance toward pathogens and pesticides.

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Transgenics in cancer Research

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Angiogenesis is the formation of new capillary blood vessels by a process of sprouting from pre-existing vessels and occurs during development as well as in a number of physiological and pathological settings (Folkman 1995). Angiogenesis is necessary for tissue growth, wound healing, and female reproductive function and is a component of pathological processes such as hemangioma formation and ocular neovascularization. However, much of the longstanding interest in angiogenesis comes from the notion that for solid tumors to grow beyond a critical size, they must recruit endothelial cells from the surrounding stroma to form their own endogenous microcirculation. One emerging biological principle is the interrelationship between tumor angiogenesis and modulation of cell death This was first shown in a Lewis lung carcinoma model where the primary tumor is angiogenic but generates a circulating angiogenesis inhibitor, angiostatin, that inhibits vascularization of distant metastases (O'Reilly et al 1994). Systemic treatment with angiogenesis inhibitors maintains dormancy of these metastases after the removal of the primary tumor. This state of dormancy is characterized by a state of no growth where cell proliferation is balanced by an equal rate of cell death by apoptosis (Holmgren et al 1995). The generation of transgenic mice designed to overexpress activated oncogenes has enabled studies that link angiogenesis to specific stages in tumor progression. One example is that of islet cell carcinomas in RIP-Tag transgenic mice where the Large T oncogene drives the progressive formation of ß-islet carcinomas in mice. When the RIP-Tag mice were treated with a combination of angiogenesis inhibitors tumor growth was dramatically impaired and vessel density significantly reduced (Parangi et al., 1996). The same pattern was repeated in this model-system, treatment with angiogenesis inhibitors did not affect the percentage of cells in S-phase but increased significantly the incidence of apoptosis in the tumors. Together, these two models implicate the vasculature as a paracrine regulator of apoptosis. Elevating apoptosis in tumors may, however, have its side effects. We have recently shown that DNA may be transferred between tumor cells in an horizontal manner by the uptake of apoptotic bodies. (Bergsmedh et al 2001) These results suggest that lateral transfer of DNA between eukaryotic cells may result in aneuploidy and the accumulation of genetic changes that are necessary for tumor formation.

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Prion diseases: insights from mouse models

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Prion diseases are fatal brain disorders such as Creutzfeldt-Jakob disease (CJD) and bovine spongiform encephalopathy (BSE). The prion, the transmissible agent, is believed to be a modified form of a host-derived protein, called prion protein (PrP), which propagates by converting the cellular form into a likeness of itself. Mice devoid of PrP behave normally, but are resistant to scrapie and fail to replicate the agent. Introduction of PrP transgenes into such mice restores susceptibility to scrapie, opening the way for reverse genetics.

A set of PrP knockout mice transgenic for PrP molecules with increasing amino proximal deletions were challenged with mouse prions. PrP with deletions up to residue 93, but not to residue 106, still restored scrapie susceptibility. Mice transgenic for PrP devoid of residues 32 to 93 developed disease after prolonged incubation period and their brains showed no scrapie-like pathology. However, in spinal cord, infectivity and pathological changes were as in scrapie-infected wild-type mice. Uninfected PrP knockout mice expressing PrP with deletions extending to residues 121 or 134, exhibited severe ataxia and neuronal death limited to the granule cell layer of the cerebellum, as early as 1-3 months after birth. The defect was completely abolished by a single wild-type PrP gene. We suggested that these truncated PrPs may be non-functional and compete with some other molecule with PrP-like function for a common ligand.

The physiological function of PrP remains elusive, although the murine *Prnp* gene is one of the rare loci for which several knockout lines have been generated differing in the design of the gene disruption on chromosome 2. Lines in which only the coding sequence was targeted remained healthy, whereas more extensive gene deletions resulted in ataxia and Purkinje cell degeneration, which has been attributed to ectopic expression in the brain of Doppel (Dpl). Dpl shows similarity to the pathogenic PrP truncated to position 134 and the pathogenic effect, which is also abrogated by expression of wild-type PrP, may be due to the same mechanism postulated for truncated PrP.

Prions are far more resistant to sterilisation procedures than common pathogens. Concerns that surgical instruments might transmit variant CJD, the human form of BSE, have been raised by the finding of prions not only in neurons, but also in lymphatic tissue. As a model for contaminated instruments, we used thin stainless steel wire segments exposed to prions. Wire exposed to prion-infected mouse brain is as efficient in causing disease in indicator mice as injection of standard inoculum. This model enables studies on sterilisation of surface-bound prions that more closely mimic real-life conditions than those with solutions and can be used to assess sterilisation procedures for BSE and vCJD prions, using appropriate indicator mice.

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Strategies to Control NR1(N598Q/R) Mutant NMDA Receptor Expression in the Mouse Brain

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In the mammalian central nervous system the family of ionotropic glutamate receptors mediate the majority of excitatory synaptic transmission. Moreover, some forms of long term changes in synaptic strength – mechanisms which might underlie memory, learning and neuronal development – are induced by the activation of the NMDA receptor subtype of the ionotropic glutamate receptor family. At resting potential of the postsynaptic membrane the NMDA receptor is impermeable for cations since the channel pore is blocked by extracellular Mg^{2+}, but after membrane depolarization Mg^{2+} is released and the receptor becomes permeable for Ca^{2+}. Thus the NMDA receptor functions as a coincidence detector for simultaneous activity of presynaptic (glutamate release) and postsynaptic (membrane depolarization) cells and the Ca^{2+} influx serves as an intracellular signal.

As indicated by site directed mutgenesis *in vitro* the voltage regulated Ca^{2+} influx is controlled by a single asparagine (N598) residue in the channel lining domain M2 of the NMDA receptor subunit NR1. Voltage dependend Mg^{2+} block and Ca^{2+} permeability are reduced by the change of this asparagine to a glutamine (N598Q) and abolished when changed to an arginine (N598R).

To improve our understanding of the voltage-controlled Ca^{2+} influx by the NMDA receptor on plasticity changes at the level of the whole organism, we use modern transgenic mouse technologies as the platform of choice to evaluate altered gene expression *in vivo.*

NMDA receptors in gene targeted mice with substitutions of asparagine (N) in position 598 of the NR1 subunit to glutamine (Q) and arginine (R) die soon after birth (Single et al., 2000), and thus preclude studies of the effect of the altered channel parameters caused by these mutations in the mature brain. To overcome the lethal phenotype during development we now search for strategies to restrict mutant NMDA receptor expression.

In the above mice, the mutant *NR1* alleles are silenced by the insertion of a floxed neomycin selection marker cassette into intron 18 (*NR1*^{Qneo}, *NR1*^{Rneo}), which is reverted upon Cre-mediated elimination (*NR1*^{Q}, *NR1*^{R}). This enables us to exploit different Cre-loxP systems to regulate mutant NMDA receptor expression at the regions and time periods of interest.

With the use of mice that express Cre recombinase under the control of the α−CamKII promotor (Mayford et al., 1995), either directly or via the tetracycline-sensitive transcriptional transactivator tTA and a tTA-sensitive promotor, we generated mice that express the *NR1*^{Q} or *NR1*^{R} alleles restricted or inducible to forebrain areas. These mice are able to overcome the prematurely lethal phenotype, and thus allow us to investigate plasticity changes induced by the altered channel parameters.

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TRANSGENICS IN DEVELOPMENTAL BIOLOGY

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The aim of this seminar is to discuss how transgenic techniques can be used to study questions in developmental biology. Special emphasis will be placed on discussing embryonic lethality in genetically modified mice. Targeting or overexpression of many genes in the mouse leads to embryonic lethality, which gives new and sometimes unanticipated insights into developmental processes. I will briefly discuss which organ systems are most vulnerable to malformations caused by targeting/overexpression during embryogenesis. Finally, I will provide examples of embryonic phenotypes caused by targeting mutations in two classes of genes: the presenilins and Notch receptor genes. I will describe how analysis of presenilin and Notch knock out mice has led to a better understanding of the interplay between presenilin and Notch function, and how this relates to the molecular basis of familial Alzheimer's disease.

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GEN0WAY PRIZE FOR TRANSGENE TECHNOLOGIES

AWARDED TO:

Dr. Teruhiko Wakayama

For outstanding achievements in the field of cloning of mice

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Cloning mice and embryonic stem (ES) cells by nuclear transfer

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The absence of a model organism has impeded progress in understanding mammalian cloning by nuclear transfer (nt). Although the mouse is the pre-eminent candidate model due to our grasp of its biology, the cloning of mice from adult somatic cells has proved elusive. We recently developed a new method to generate cloned mice based on piezo-actuated nuclear transfer. Mice have been cloned from cumulus cells, tail-derived cells (most cells were fibroblast), adult/fetus males and females, and extensively passaged embryonic stem (ES) cell lines. ES cells have now been derived from cloned embryos produced by nt from somatic cells (ntES cells); ntES cells show full capacity for differentiation, including gametogenesis, after chimera production by blastocyst injection. The rate of full term development in cloning from adult somatic cell of hybrid strains, over all is invariably low, with only approximately 2% of reconstructed oocytes developing to term. When inbred strains were used as nuclear donor, the success rate of cloning is extremely low (0-0.3%) except 129 strains. All cloned mice are associated with abnormal placentae, often dying perinatally of unknown causes with a strain-dependent frequency. However, clones surviving to adulthood exhibit normal fertility, and re-cloned mouse which repeated 6 times also had a healthy body. In attributing causes to cloning phenomena, distinctions should be made between technical limitations, nuclear “reprogramming”, somatic mutation, genomic imprinting and incompatible cell cycle effects as contributing factors.

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POSTER ABSTRACTS

01/1

A human yeast artificial chromosome containing the preprotachykinin-A gene is able to express substance P in mice and drives appropriate marker-gene expression during embryonic development and in the adult.

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The PreprotachykininA gene (PPTA) encodes for several neuropeptides including neurokinin A (NKA) and substance P (SP). PPTA is expressed in many areas of the central nervous system, the dorsal horn of the spinal cord and in dorsal root ganglia (DRG). Gene knockout studies have shown that SP is involved in a wide variety of processes including nociception and inflammation. Aetiologies associated with altered PPTA/SP expression include neuropathic pain, Parkinson’s disease, Huntington's Chorea, Crohn’s disease, rheumatoid arthritis, Epilepsy, and Dementia. However, little is currently known about the mechanisms influencing human PPTA transcriptional regulation.

As part of an ongoing study into elucidating the mechanisms responsible for controlling the human PPTA gene we produced a transgenic model of human PPTA expression in mice using a human yeast artificial chromosome (YAC) containing the PPTA gene tagged with a LacZ marker gene. This experiment initially demonstrated that, despite over 100million years of divergent evolution, the human YAC could produce SP within the mouse. These models also successfully generated the vast majority of the expression patterns previously described for PPTA/SP both during embryonic development and in the adult. Furthermore, The YAC could also generate expression of LacZ in areas of the brain where PPTA/SP are not known to be expressed in the mouse but are known to express PPTA/SP in humans. These areas include the cerebellum, the mammiliary bodies of the hypothalamus and dentate gyrus. In the case of the dentate gyrus (DG) others have demonstrated that the expression of SP in the rodent is strongly associated with susceptibility to status epilepticus. Paradoxically, it has also been shown that humans have naturally high levels of PPTA expression within the DG. We believe that our human PPTA expression models will be invaluable in the proper understanding of such apparent contradictions.

This data is consistent with a high degree of conservation not only within the transcriptional machinery driving the expression of the PPTA gene but also of the mechanisms for proper translational and post-translational processing of one of its neuropeptide products. We believe that the determination of this degree of functional conservation of the PPTA gene throughout divergent mouse and human evolution, in addition to the highlighting of several species specific differences, removes important obstacles in determining the mechanisms behind the modulation of the human PPTA gene *in vivo*.

01/2

GENERATION OF YAC/BAC TRANSGENIC MOUSE MODELS TO STUDY THE REGULATION OF *PDGFRA* EXPRESSION

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The major goal of the current project is to develop transgenic mouse models which will enable us to study the normal *in vivo* regulatory mechanisms of the platelet-derived growth factor α receptor gene (*PDGFRA*), and the possible defects involving these regulatory mechanisms that may occur in certain brain tumors.

Previously we have generated transgenic mice containing a 2 kb piece of *PDGFRA* promoter region ligated to a LacZ reporter gene to evaluate the *in vivo* promoter function. These studies indeed demonstrated the functionality of the *PDGFRA* promoter in many tissues. However, we also encountered areas of ectopic or lack of transgene expression. In addition, transgene expression was highly variable among different transgenic lines.

To improve our transgenic mouse model, we have intitiated the generation of transgenic mice from *PDGFRA* containing YAC DNA. These large pieces of DNA usually provide all the necessary regulatory elements to obtain the correct, copy number dependent, and integration independent transgene expression. However, the transgene expression in mice generated from the *PDGFRA* YAC still did not recapitulate completely the endogenous *Pdgfra* expression pattern. This was most likely due to an unanticipated fragmentation of the YAC within the *PDGFRA* promoter region.

Although the *PDGFRA* YAC transgenic mice still not gave the perfect transgene expression, they did express the transgene (in contrast to the 2 kb promoter-LacZ) in the oligodendrocyte precursor cells, which are of major interest for our project. We have recently modified the YAC by homologous recombination in yeast, and incorporated a lacZ reporter gene within the *PDGFRA* locus, and we also reduced the length of the YAC by targeted fragmentation. In addition, we are currenty characterizing and modifying *PDGFRA* containing BACs to furthr improve our transgenic mouse model.

01/3

Use of coisogenic host blastocysts for efficient establishment of germline chimeras with C57BL/6J ES cells.

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Currently germline-competent ES cell lines are available from only a limited number of mouse strains and inappropriate ES cell/host blastocyst combinations often restrict efficient production of gene-targeted mice. Here, we describe the derivation of C57BL/6J (B6) ES lines and compare the effectiveness of two host blastocyst donors, FVB/NJ and the coisogenic strain C57BL/6-Tyr$^{c\text{-}2J}$ (c^{2J}), for the production of germline chimeras. We found that when B6 ES cells were injected into c^{2J} host blastocysts a high rate of coat-color chimerism was detected and germline transmission could be obtained with few blastocyst injections. In all but one case highly chimeric mice transmitted to 100% of their offspring. Injection of B6 ES cells into FVB/NJ (FVB) blastocysts produced some chimeric mice. However, the proportion of coat-color chimerism was low, with many more blastocyst injections required to generate chimeras capable of germline transmission. Our data support the use of the coisogenic albino host strain, c^{2J}, for the generation of germline-competent chimeric mice when using B6 ES cells.

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01/4

In vitro and *in vivo* study of the oncogenic function of the pleomorphic salivary gland adenoma gene *PLAG1.*

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PLAG1, a novel developmentally regulated C_2H_2 zinc finger gene, is consistently rearranged in pleomorphic adenomas of the salivary glands with 8q12 translocations. This results in the replacement of the *PLAG1* promoter, inactive in adult salivary glands, by a strong promoter derived from the translocation partner leading to an ectopic *PLAG1* expression in tumoral cells [1, 2]. The *PLAG1* gene is a member of a subfamily of zinc finger proteins containing *PLAGL1* and *PLAGL2* [3]. *PLAGL1* is also identified independently by other groups and called *LOT1* and *ZAC1 [4, 5]*. Previously, *PLAGL1* has been shown to prevent the proliferation of tumour cells by inducing cell cycle arrest and apoptosis [5, 6].

To investigate the role of *PLAG1* in cellular transformation and tumorigenesis, we first determined the oncogene capacity of *PLAG1 in vitro*. Our analyses were extended to *PLAGL2*, the third member of the family. NIH-3T3 cells overexpressing *PLAG1* or *PLAGL2* express the typical markers of neoplastic transformation; they loose their cell-cell contact inhibition, show anchorage independent growth and induce tumour formation in nude mice. This transformation is accompanied with an upregulation of *IGF-II* for which we proved to be a direct target of *PLAG1* and *PLAGL2*. These results strongly suggest that *PLAG1* and *PLAGL2* play an important role in the development of neoplasias probably by activating the *IGF-II* mitogenic pathway. As the results obtained from the *in vitro* NIH-3T3 cell system cannot be completely transferred to *in vivo* tumours, we try to analyse this aspect by generating transgenic mice. A first attempt to develop a transgenic mouse containing a *PLAG1* transgene under the control of a modified actin promoter fused to the CMV enhancer was unsuccesfull. Characterisation of the fifty descendants obtained after microinjection revealed that only one transgenic founder mouse contained the *PLAG1* transgene. Moreover Northern Blot analysis revealed that the transgene was not expressed, suggesting that overexpression of *PLAG1* during embryonic development could have a toxic effect. To ensure that the transgene is completely silenced during development, we decided to utilise the bacteriophage P1 derived Cre/LoxP system. We have cloned a neo cassette that is flanked by two LoxP sites with the same orientation between the modified actin promoter and *PLAG1*. The stop codon of the neo cassette should terminate translation and the cassette can be excised simply by breeding with Cre recombinase transgenic mice. The transgenic mice obtained will be bred with the MMTV-Cre expressing mouse which gives high Cre expression in the salivary gland and the mammary gland.

The first results will be discussed on the meeting.

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01/5

Physological functions of heparin and heparan sulfate

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Heparin and heparan sulfate (HS) are polysaccharides of the glycosaminoglycan (GAG) type that are formed in the Golgi apparatus attached to core proteins forming proteoglycans. The variability (e.g. sulfation) in the structure of GAGs is large but the functional consequences poorly understood. However, evidence is emerging that structural variability is of great physiological importance e.g. in growth factor signaling, lipid metabolism and thrombosis.

We want to understand the function of heparin and heparan sulfate *in vivo*, and to understand the mechanism for biosynthesis of these GAGs. Our approach is to knock-out enzymes that are important in the production of heparin and heparan sulfate. Different isoforms of N-deacetylase/N-sulfotransferase (NDST) are key regulatory enzymes in the modifying machinery of the polymerized sugar backbone.

We have, in our lab, established two mouse strains lacking NDST-1 and NDST-2 respectively. NDST-2$^{-/-}$ mice completely lack heparin and show a severe mast cell defect and an altered degradation of fibronectin. Lack of NDST-1 lowers the sulfation degree of HS and most NDST-1$^{-/-}$ mice die neonatally due to respiratory failure. A smaller fraction, however, die earlier during development. NDST-1/NDST-2 double deficient embryos have been achieved by crossing the two other strains. Lack of both enzymes leads to embryonal death before gastrulation is completed. We are also constructing a conditional targeting vector for inactivation of the heparin/HS polymerase EXT-2.

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01/6

AUTOCRINE PDGF A MICE ARE EMBRYONIC LETHAL

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Growth factors and their receptors are expressed in a strictly tissue specific way during embryonic development. Expression of ligands and receptors in adjacent cell layers allows for paracrine stimulation to occur. In normal mouse embryogenesis, platelet-derived growth factor A (Pdgf A) and its receptor α (Pdgfrα) can form a paracrine loop. Pdgf A is expressed in the ectoderm and epithelium, while Pdgfrα is expressed in the adjacent mesenchymal tissue. In human tumors, such as malignant glioma both PDGF and PDGFRα are overexpressed in the same tissue indicating that an autocrine PDGF loop is generated in the tumors.

Here we report the generation of autocrine PDGF A transgenic mice, which express human PDGF A chain ectopically in the Pdgfrα expressing mesenchymal cells. The expression of a human PDGF A cDNA was driven by a 6kb mouse Pdgfra promoter and transient transgenic mice were created by pronucleus injection. Embryos were collected at E11.5 to E 14.5 and incorporation of the transgene and subsequent PDGF A mRNA expression were detected by PCR and in situ hybridization, respectively. We found that autocrine PDGF A mice exhibited variant phenotypes. Those with severe phenotypes died at about E12.5 with defects in midline fusion of the head and overgrowth or disturbed formation of cranial facial mesenchyme. A minor phenotype included body size variation and subepidermal blebs. Routine histological studies, neural crest cells identification and determination of proliferation and apoptosis levels in the embryos are ongoing.

01/7

Notes from a trangenic Lab: Using Transgenics In Screening Novel Genes for Biological Activity In Vivo

Haugen, H., Grossmann, A., Waggie, K., Chapman, S., Stamm, B., Durnam, D., LeCiel, C. and Blumberg, H.

ZymoGenetics Inc, Seattle, USA

At ZymoGenetics we elucidate biological activity of novel genes, discovered through bioinfomatics, using in vitro and in vivo procedures, including the analysis of transgenic mice. We have performed over 200 pronuclear injections, expressing 62 different proteins, using 15 different promoters. Approximately 60% of our constructs use the metallothionein promoter which expresses the genes during development and in multiple tissues,. We have analyzed more than 1000 founders for expression, physiological parameters, and in many instances for reproductive capacity. In our hands, approximately 3% of the injected embryos become transgenic, 25% of the founders end up with a significant expression level, 75% of the founders are capable of breeding, and 70% of the breeders transmit the gene to the next generation. We found that 20% of our novel genes showed definite signs of biological activity, and 40% of the genes were difficult to characterize due to low numbers of founders, perinatal mortality, or lack of significant expression. We believe that getting a low proportion of founders which lack significant expression may indicate a biologically active gene.

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01/08

CONDITIONAL TRANSGENIC MOUSE WITH DESENSITIZATION-RESISTANT BETA ADRENERGIC RECEPTOR AS A MODEL OF GENE THERAPY FOR HEART FAILURE

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Congestive heart failure is a widespread disease for which there is no other cure than heart transplantation. The aim of this project we is to create a conditional transgenic animal model of gene therapy for heart failure. Several evidences suggest that continuous desensitization of adrenergic receptors in hypertrophic hearts may be a primary factor in initiating the pathological changes that lead to overt heart failure (1,2,3,4). To challenge this hypothesis we generated a mouse strain in which a transgene encoding for desensitization-resistant beta adrenergic receptors can be switched on in response to withdrawal of doxycyclin from diet (5). In this animal model, following experimentally-induced heart failure, it will be possible to study whether the transient expression of the modified receptor at varius stages of the pathological progression can prevent or rescue the heart from failure. This study has two main aims. First, to assess if receptor desensitization has a critical role in starting the pathological changes that destroy the cardiac muscle during the disease. Second, it will explore a novel possible strategy of therapy for heart failure based on the introduction in myocardiocytes of genetically engineered adrenergic receptors.

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01/09

SENTINEL MICE ARE IMPORTANT TOOLS FOR MONITORING ANIMALS HEALTH STATUS IN A TRANSGENIC MICE BREEDING FACILITY.

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In recent years transgenic mice have assumed an increasing importance as an experimental system in biomedical research. Normal gene function, altered gene expression, and the mechanisms responsible for tissue-specific patterns of gene expression are some of the types of studies made possible by transgenic mouse technology. Among the major tasks that a good transgenic breeding facility should provide are, a centralised service to efficiently breed transgenic mice, the capability to genotype the offspring, maintaining transgenic lines, and establishing animal health control systems. The cost and time to establish a transgenic mouse is high, therefore control or limiting the spread of infection in a transgenic breeding facility is of primary importance. This could be achieved by complying with established restrictions pertaining of working behind barriers, and with strict control on the reception of animals from other facilities. In addition, foreign animals should not be contaminated with unwanted pathogens or removed by rederivation. An important measure to be taken in a transgenic mouse breeding facility is establishing a sentinel system as an indicator of changes in the health status of the animals in the facility. One method is to use a group of pre-selected mice as sentinels. Sentinel mice are immune-competent, and housed indirect contact with the colony of mice to be used in experiments or bred for other purposes (Waller T et al. 1997, *Scand. Lab. Anim. Sci*. No. 3:24, 118-120). By exposing these mice to potential infective agents harboured by colony mice, one can effectively monitor the health of the entire population. Commonly, this can be achieved by the regular transfer of bedding material from the cages of the regular colony mice to the sentinel cages. In our studies a handful of bedding was transferred, twice a week, from the cages of colony mice to be investigated or breed to cages containing sentinel mice. All mice were kept in the same animal room in a microisolater (IVC racks), with a microenvironment of 21^{o}C - 24^{o}C, ~50% relative humidity, and a 14/10-hour light/dark cycle. Following 10-12 weeks of exposure sentinel mice were subjected to an established health monitoring program, as recommended by FELASA (Kraft V et al. 1994, *Lab. Anim*., 28:1-12). In conclusion, the use of a sentinel system offers the ability to control or limit the spread of infections in a highly valuable transgenic mice breeding facility.

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01/10

Non invasive monitoring of NF-κB activity in glowing transgenic mice

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Abstract

A wide range of human disorders involves inappropriate regulation of NF-κB including cancers and numerous inflammatory conditions. Toward our goal to define mechanisms through which NF-κB leads to the development of disease we have developed transgenic mice that express luciferase under the controle of NF-κB enabling real-time *in vivo* imaging of NF-κB activity in intact animals. We show that in the absence of extrinsic stimulation, strong luminescence is evident in lymph nodes in the neck region, thymus and Peyer's patches. treating mice with TNFα, IL-1α or LPS increase the luminescence in a tissue specific manner, with the strongest activity observed in skin, lungs, spleen, Peyer's patches and the wall of the smaller intestine. Liver, kidney, heart, muscle and adipose tissue display less activities. Also, exposure of skin to a low dose of ultraviolet radiation increased the luminescence in the exposed areas. Furtermore, induction of chronic inflammation resembling rheumatoid arthritis produce a strong NF-κB activity in the affected joints as revealed by *in vivo* imaging. Thus, we have developed a versatile model for monitoring NF-κB activation *in vivo*.

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01/11

Studies on the behavioral phenotypes of mouse strains using different experimental models
(with a special attention for a model of spatial learning and tests for anxiety like behavior)

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Different inbred mouse strains were compared with regard to locomotor activity, anxiety levels and behavior in the Morris water maze task. The work focussed on the differences between mouse strains used as the parenteral strains for the generation of the genetically modified animals.

In the first study NMRI, C57Bl/6J, CBA and 129/OlaHsd inbred strains and two F2 hybrids (C57/CBA and C57/129) were analysed for their performance in the Morris water maze task.The results show that some mouse strains can easily learn the Morris water maze task and can preferably be used in such tasks (C57Bl/6J, NMRI, B6/CBA-F2 and B6/129-F2). However, only NMRI and B6 mice displayed a clear spatial learning strategy in this task, whereas other strains developed goal oriented seach strategies, which may involve mechanisms others than hippocampally dependent learning. The large interstrain differences raise an important question of the proper selection of the parental strains in learning/memory related studies with genetically modified mice. It is concluded that the differential performance in learning tasks by mouse strains probably interacts with other strain differences in response to various pharmacological and genetic manipulations. Therefore, the genetic background must be taken into account in studies aimed to evaluate both cognition enhancing and impairing manipulations. The second important conclusion of the present study is that the poor performance displayed by some strains (129/OlaHsd and CBA), probably depends on motivational and motor aspects and on non-cognitive behavioral strategies. Finally, due to the multifactor dependence (motor capability, memory function, anxiety level, motivational background) it is preferable to use the water maze task in combination with other tests (a battery of at least two-tree) for a reliable assessment of learning and memory functions.

The results of another study suggest that four studied inbred strains of mice NMRI, CBA, C57Bl/6J and 129/OlaHsd display different phenotypes according to the behavioral effects observed in a battery of tests believed to reflect basal “anxiety” levels and levels of locomotor activity. The rank order of “anxiety” levels and locomotor/exploratory activity between strains varied depending on the test used . This suggested that different tests of “anxiety” probably “measure” different aspects of the particular behavioral manifestations defined as “anxiety”. Although light-dark test, open field test and elevated plus maze test are based on the same principle of unconditioned behavior and exploit a similar conflict between the tendency of mice to explore the novel environment and the fear produced by new environment, the main stressful stimulus of each test clearly differs. These findings have important implications for the analysis of behavioral phenotypes in mice. These results reinforce the conclusion that a battery of tests is needed in order to exclude premature and non-validated conclusions of behavioral phenotypes.

01/12

Core facility functions at The UNIT FOR EMBRYOLOGY AND GENETICS Karolinska Institutet and Huddinge University Hospital

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The Unit for embryology and genetics (EG Unit) deals with research and education within the field of experimental pre-implantation embryology. A murine embryo bank has been established with the purpose to safeguard unique genetic material. The Unit is a node of the European Mouse Mutant Archive (EMMA).

By direction of the Huddinge University Hospital and the Karolinska Institutet, the Unit also performs core facility functions in the following areas:

* Mouse mutant banking
* Pathogen decontamination
* Rederivation service (import/export of mouse stocks)
*** Generation of transgenic animals (as part of KCTT)**
* Molecular biology service regarding vector design and strategy for the generation of transgenic mice
* Supply of pre-implantation embryos
* Micro-manipulative techniques in assisted reproduction

In addition, in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), sperm and ovary cryopreservation, ovary transplantation and vasectomy are offered as core facility service.

The EG Unit has a close collaboration with the Unit for Morphological Phenotype Analysis, KI. This unit assists with mouse mutant analysis regarding pathological changes caused by introduction of genetic changes or due to strain-specific background lesions.

The EG unit is also organizing theoretical and practical courses in embryology and related techniques (please see http://www.ki.se/kfc/meg/egedu_en.html).

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01/13

IMPROVED EFFICIENCY FOR THE GENERATION OF TRANSGENIC CATTLE BY NUCLEAR TRANSFER COMPARED TO MICROINJECTION

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Until recently, transgenic cattle, producing human pharmaceuticals in milk, were generated through microinjection of recombinant DNA into the pronucleus of fertilized oocytes, resulting in transgenesis rates no higher than 5%. Previously, we have conducted studies to improve transgenesis rates, such as alternative methods for recombinant DNA isolation, collection of high-quality oocytes via ovum pick-up, early transgene detection via PCR of amniotic fluid isolated between Days 78 and 85 of gestation and early termination of non-transgenic pregnancies. Nevertheless, generation of large numbers of non-transgenic pregnancies and, when succesfull, the time consuming process of establishing a production herd from a single (mosaic) founder remain a limitation of the microinjection approach. To circumvent these shortcomings, a different strategy was implemented: genetic manipulation of female totipotent cells and generation of transgenic animals via nuclear transfer. By screening the transfected cells prior to nuclear transfer, only transgenic non-mosaic female calves with desired number of intact transgenes are born. In fact, inserting transgenes into predetermined chromosomal locations (targeting) also becomes a possibility eliminating the

chromosomal position efeects on protein expression levels. Finally, by repeating nuclear transfer, herds consisting of any number of genetically identical transgenic cows can be established instantly, rather than be dependent on the time consuming breeding process.

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