

LECTURE ABSTRACTS

Wednesday May 17

The laboratory mouse in transgenic research

Michael FW Festing

MRC Toxicology Unit, University of Leicester, PO Box 138, Lancaster Road,
Leicester, LE1 9HN, UK.
mfwf1@leicester.ac.uk

The mouse is the most widely used vertebrate in biomedical research, and has made immense contributions to human health and the understanding of fundamental biological processes.

Modern research based on genetic markers now recognises twelve species or subspecies of the genus *Mus*. Eight of these are "aboriginal" wild species that do not live in close association with humans. In contrast, the four subspecies *Mus musculus domesticus*, *M.m. musculus*, *M. castaneus* and *M. bactrianus* are commensal or feral species living in close association with humans, and as a result have become widely distributed throughout the world. The eight aboriginal species apparently inhabit different ecological niches, and their ranges overlap with each other and with the four commensal subspecies. On the other hand, the latter subspecies have different geographical distributions with *M.m. musculus* being confined to Northern Europe and Asia, *M. m. domesticus* living in Western Europe, Africa, the Americas and Australia, *M. castaneus* living in S.E. Asia and *M. bactrianus* being confined to the Indian sub-continent. In Japan *M. m. musculus* and *M. castaneus* have apparently hybridised to produce what was originally assumed to be a separate subspecies name *M. molussinus*, but is now recognised as a hybrid species. In contrast, in Eastern Europe *M. m. domesticus* and *M. m. musculus* do not interbreed in the wild, though all four of the subspecies are fully fertile and produce fertile offspring in the laboratory.

Mice are agricultural pests, but they have also been domesticated as pets and "fancy" animals for several thousand years both in Asia and Europe. The laboratory mouse is largely derived from *M. m. domesticus*, but with some genes from the other comensal subspecies. However, studies of mtDNA show that all the "old" inbred strains of laboratory mice can be traced back to a single female within the last 200 or so years. Laboratory mice also differ from wild mice at many immunologically-defined loci.

Mice have been used in research since at least 1664 when Robert Hooke used mice in studies of the properties of air. Modern research began soon after the rediscovery of Mendel's laws in 1900 in the laboratory of William Castle. One of the most significant events has been the development of inbred strains by 20 or more generations brother x sister mating, with the first of these, strain DBA, being developed by C.C. Little, who went on to found the Jackson Laboratory in Bar Harbor, Maine in 1929. This laboratory has played a central role in developing strains, preserving mutants, training investigators in mouse biology, and performing cutting-edge research ever since then.

Inbred strains have played a key role in biomedical research, with at least seventeen Nobel prizes being awarded for work which was partly or wholly dependent on these strains. Their most obvious contribution has been their use in dissecting the various components of the immune system, but their use in cancer research has been equally important. Oncogenes and other regulatory molecules have largely been discovered and characterised through studies of mouse tumours, mouse oncogenic viruses and viral oncogenes.

Two major technical developments have depended on some unique strain characteristics. Strain BALB/c develops myelomas when injected with mineral oil, and this has formed the basis for the development of monoclonal antibody production. Transgenic mice have now even been "humanised" so as to produce human monoclonal antibodies. The development of embryonic stem cells from

strain 129 mice has also been of great significance as these can be manipulated in-vitro, but remain totipotent so that live young can be produced from them. This has led to the development of "knockout" mutant mice produced by homologous recombination, and a whole series of new transgenic techniques.

There are some problems with strain 129, which has a very unsatisfactory genealogy involving a number of outcrosses which were designed to study the genes responsible for the development of testicular teratomas. As a result, the strain is highly heterogeneous, and recently it has been necessary to rename the various branches in such a way that there should be less confusion in the future.

The mouse genome will be fully sequenced within the next two or three years, and it is clearly destined to play a major role in unravelling the function of many human genes whose existence is only now being revealed, many of which undoubtedly will have an important impact on human health.

Production of congenic mice / speed congenics

K. Reifenberg

University Mainz, Germany

Congenic strains are produced by transferring specific alleles referred to as differential alleles, from undesired donor strains to desired recipient inbred strains. The breeding scheme commonly used for establishment of “traditional” congenic strains comprises an initial outcross between the donor and recipient strain followed by 9 consecutive backcrosses of carrier individuals of the differential allele to the recipient strain. The considerable time factor associated with the generation of congenic strains suggested to reduce the number of backcrossing generations by using additional selection criteria. The rationale behind this idea was to not only select for transmission of the desired donor-derived differential region but also to select against the transmission of unwanted genomic material of donor origin.

Microsatellites consisting of tandem repeats of basic DNA-motifs comprised of 2 to 4 single base pairs are found with high frequency in all mammalian genomes and exhibit an extreme intraspecific polymorphism, characterized by the number of repetitions of the underlying motif. Importantly, microsatellite polymorphisms can easily be detected by PCR-amplification of the entire locus using primers homologous to the specific flanking DNA sequences and separation of the amplified PCR products by electrophoresis.

It has been demonstrated recently that by using microsatellite-based selective breeding strategies congenic strains can be generated after as few as four backcrosses to the recipient strain. The resulting variants are referred to as “speed” congenic strains. This article provides relevant information about the generation of “speed” congenic strains of mice.

Derivation of ES cells from strains other than 129

L. Schoonjans

Thrombogenics, Herestraat 49 n, 3000 Leuven , Belgium
luc.schoonjans@med.kuleuven.ac.be

At the moment virtually all ES cell lines for gene targeting are made from substrains of 129 mice. Since it is an arduous task to develop each knockout, investigators rely heavily on technologies with proven success, and 129-based ES cell lines have an impressive record of success. The universal use of 129 ES cells is also largely due to the fact that ES cell derivation of other strains has been shown to be much more difficult, inefficient and even impossible.

Very often the lack of ES cells of other strains results in considerable additional costs of animal housing, since an inbreeding period of 1- 1.5 year is required to transfer the gene manipulation phenotype into the desired background. Therefore, the scientific community would benefit greatly from the development of ES cell from other strains than 129 that remain pluripotent under extended culture conditions.

Recently we developed improved ES cell culture medium and ES cell derivation-methods. It has already allowed us to derive ES cells with germline transmission capability from 9 different inbred strains and 1 outbred strain. The availability of ES cells from mice strains other than 129 will allow studying the loss of function mutation phenotype directly in the favorite mouse strain. The time and money consuming backcrossing can be omitted.

The improved ES cell culture medium and ES cell derivation-methods also enabled the establishment of ES cell lines from 5 different homozygous knockout mice with an embryonic or early postnatal lethal phenotype. These $-/-$ ES cells can be used for *in vitro* differentiation experiments or tissues specific rescue experiments.

The availability of ES cells of other strains also generates the possibility of over-expressing a transgene directly in any desired background via non-homologously recombination and thus creates a time and money saving alternative for pronuclear zygote injection and the subsequent backcrossing in the desired background.

Conditional Gene Targeting in Mice

Ralf Kühn

Artemis Pharmaceuticals GmbH, Cologne, Germany

Gene targeting in mice has been extended in the last years by conditional, somatic mutagenesis utilising site specific recombination systems which allows to determine the cell type specificity and timing of gene inactivation. The growing resource of transgenic lines which express recombinase constitutively in various cell types demonstrates the feasibility of conditional gene targeting and reflects the need of this approach. However, the best way to characterise gene function in vivo would allow to switch from wildtype to a mutant allele by induction through inducible recombinase expression. Although the parameters of a useful inducible system, low background and high and rapid inducibility, are simple to define, only a few examples of inducible knockout mice have been so far reported. The common approaches to control recombinase activity are fusion proteins with mutant steroid receptors or systems for transcriptional regulation like the Tet-regulated system. By reducing the basal activity of the Tet system and uncoupling from promoter strength we are attempting to generate a collection of inducible recombinase mice covering all major cell types as a tool for genetic analysis. In particular, inducible gene targeting is advantageous to validate the utility of potential drug screening targets since the induced inactivation of a target gene mimics the inhibition of the corresponding protein by a drug.

Reporter Genes

S. Aparicio

Induced mutagenesis in mice (ENU)

Martin Hrabe de Angelis

GSF National Research Center for Environment and Health, Institute of Experimental Genetics, Ingolstaedter Landstr. 1, D-85764 Neuherberg, Germany

Mutants are the most important tool to obtain insight into biological function of genes. Due to the similarity in genomes, developmental, biochemical and physiological pathways, the mouse has become the model of choice for the study of inherited diseases in man. We have established a research center that carries out a large scale dominant and recessive ENU-mouse mutagenesis screen. A number of participating research groups screen the mice for specific abnormalities, i.e. congenital malformations, clinical-biochemical alterations, immunological defects and complex traits.

Emphasis is on phenotypes that are relevant for the pathogenesis of human diseases. Base line levels for the selected parameters/phenotypes have been established for C3HeB/FeJ and C57BL/6Jlco mice. Over 180 mutant lines have been already isolated and are in the process of further characterisation.

The mutant lines are analysed by backcross mapping and genome wide microsatellite typing in order to chromosomally map the mutations.

Conserved synteny between mouse and human genome is used to check for the potential homologous human disease. Several lines are currently used in the scientific community for further analysis of gene function. The screen will be continued for the next 3-5 years in order to isolate several hundred new mouse lines. The project is partially funded within the German Human Genome Project.

For further information please see: <http://www.gsf.de/isg/ENU.index.html>

Thursday May 18

Immunology

C. Sentman

Transgenic animals models for the functional analysis of vasoactive peptides

Michael Bader

Max-Delbrück-Center for Molecular Medicine (MDC), Robert-Rössle Strasse 10,
D-13122 Berlin-Buch, Germany
e-mail: mbader@mdc-berlin.de

The interplay of vasoactive peptide systems is an essential determinant of blood pressure regulation in mammals. While the endothelin and the renin-angiotensin systems raise blood pressure by inducing vasoconstriction and sodium retention, the kallikrein-kinin and the natriuretic-peptide systems reduce arterial pressure eliciting vasodilatation and natriuresis. For the functional analysis of vasoactive peptide systems transgenic technology has proven to be very useful. As an outstanding example, transgenic rats overexpressing the mouse *Ren-2* renin gene in several tissues become extremely hypertensive. Several other transgenic rat and mouse strains with genetic modifications of components of the renin-angiotensin system have been developed in the past decade. Moreover, in recent years gene-targeting technology was employed to produce mouse strains lacking these proteins. The established animal models as well as the main insights gained by their analysis are summarized in this review.

Genetic

S. Aparicio

The SCP3 protein is required for synaptonemal complex formation, homologous chromosome synapsis and meiotic cell cycle progression.

Li Yuan, Jian-Guo Liu, Jian Zhao, Eva Brundell, Bertil Daneholt and Christer Höög

Meiotic cell divisions ensure that haploid cells can be produced in diploid organisms. During the first meiotic division, the homologous chromosomes (each consisting of two sister chromatids) pair and recombine. These chromosomal activities are accompanied by the assembly and disassembly of the synaptonemal complex (SC), an evolutionary conserved meiosis-specific protein structure. We have previously characterized a structural component of the axial core of the SC, a protein called SCP3. We have now generated a null mutation in the SCP3 gene in mice and show that homozygous mutant males become sterile due to massive and synchronous apoptotic cell death at the zygotene stage of meiotic prophase (Yuan et al., (2000) Mol. Cell 5: 73-83). We show that the SCP3 deficient mice fail to form axial cores in meiotic cells and that the homologous chromosomes in the mutant spermatocytes fail to pair. We find, however, no evidence that the axial core is required for sister chromatid cohesion. We observe residual SC structures in the SCP3-deficient spermatocytes formed by the transverse filament protein SCP1, suggesting that a residual chromatin structure remains in the mutant spermatocytes. In conclusion, we show for the first time in a mammalian organism that the SC is required for homologous synapsis of meiotic chromosomes and for meiotic cell cycle progression. We also show that failure to synapse results in massive cell death, suggesting that the chromosome pairing process in meiotic cells is monitored by a cell cycle checkpoint.

Avian Embryonic Stem Cells: isolation and perspectives

Bertrand Pain

Vivalis SA

CHU Hotel-Dieu, Pl. A. Ricordeau – 44093 Nantes cedex 1

Tel : 0 240 084 925 ; Fax : 0 240 084 926 ; E-mail : bertrandpain@vivalis.com

Embryonic Stem (ES) cells were first isolated from mouse during the 1980's. These cells have unique properties making them receptive to genetic modifications. The relative ease with which to manipulate these cells *in vitro* have made them a powerful tool to target endogenous genes, and this has led to a dramatic increase in the number of targeted transgenic mice throughout the 90's. The challenge has been to isolate and characterize cells with similar properties from other species. A number of years passed before ES and/or EG cells were isolated from non-murine, mammalian species like the medaka, rabbit, pig, monkey and human, but mammalian germ line transmission has only been successfully demonstrated in mouse. With the application of nuclear transfer technology to large farm animals, progress towards widespread animal transgenesis appears to be in a revolutionary period.

Other than mouse, chicken is the only species that has shown successful germ-line transmission of transgenes. Modification of the avian genome, although difficult, has important implications for three areas of research. First, the avian model is a preferred developmental model for basic and fundamental research: early embryo development is easy to follow and the embryo itself is easy to manipulate. Moreover, comparisons between different species help to define general or specific biological mechanisms. Second, the ability to produce poultry resistant to disease or harboring specific traits should be of interest to agronomists. And third, the chicken should be viewed as a powerful bioreactor. The ability to modify protein expression in the egg may prove fruitful in producing molecules with therapeutic interests. In this prospect, the white egg appears to be a candidate of choice for overexpressing exogenous molecules. With 6 to 7 g of dry matter per egg, the three first molecules, ovalbumin,

conalbumin and lysozyme represent more than 4,5 g per egg. By just modifying one of them, the yield of production could be expected as important for the production of therapeutic molecules. In order to modify such endogenous gene, a cellular and molecular vectors have to be well mastered. We tried to control these two levels by developing the Chicken Embryonic Stem (CESs) cells and specific vectors.

We succeeded in isolating and cultivating chicken ES cells as permanent lines in 1996 (Pain et al., 1996). Based on the previous observations made by Petite et al., (1990), we demonstrated that blastodermal cells with ES cell features could be isolated, maintained and amplified *in vitro*. These chicken embryonic stem (CESs) cells were characterised by the presence of different antigens (mainly ECMA-7, EMA-1 and SSEA-1) on their surface, known to be specific to ES cells. We were also the first to detect telomerase activity in these chicken ES cells. Thus criteria which has recently been used to characterise human ES cell lines appears to be a very sensitive one to discriminate between ES cells and more differentiated cells. A major limitation has however remained: How to efficiently introduce genetic modifications into these cells for the generation of transgenic birds.

More recently, we also succeeded in stably modifying CES cells at the genetic level. Different techniques were chosen: one used a test vector containing a CMV promoter and the EGFP reporter, another involved a gene trap vector. The results presented here will mainly refer to this vector and to the new identified trapped gene. Finally at last will be developed by using homologous recombination techniques.

Transgenesis in fish: transposon mediated enhancer trapping using *Sleeping beauty* in medaka

Thorsten Henrich and Jochen Wittbrodt

Developmental Biology Programme, European Molecular Biology Laboratory, Meyerhofstrasse 1, P/O Box 10.2209, 69012 Heidelberg Phone: +49 6221 387 576, Fax: +49 6221 387 166, E-mail: Wittbrodt@EMBL-Heidelberg.de

Laboratory fish such as zebrafish and medaka are well-established model systems for genetic and developmental research and thus manipulating the fish genome was a compelling task.

First experiments to generate transgenic fish were designed corresponding to methods used in mouse by DNA microinjection into the germinal vesicle of the oocyte or into the cytoplasm of fertilized eggs. This leads to transgenesis at rates comparable to those obtained in mice. As tools for insertional mutagenesis or enhancer trapping however, this random integration is not sufficiently efficient.

This problem was overcome in the lab of Nancy Hopkins by using pseudotyped retroviruses that were initially developed for human gene therapy. These vehicles do stably integrate and cause mutations that can easily be traced, however expression from the elements was not sufficiently strong to allow direct enhancer trapping.

A promising new tool was generated by the reconstruction of an active TC class transposase from several mutated fragments by Zoltan Ivics and colleagues. The synthetic transposon that is able to catalyze transposition in different tissue culture cells was termed *Sleeping Beauty*, awakened from a long evolutionary sleep.

We used the *Sleeping Beauty* (SB) transposon system to establish transposon mediated germ line integration and enhancer trapping in fish (medaka, *Oryzias latipes*). The short generation time, the extrauterine development and the clarity of the embryos combined with the use GFP as a marker allow direct access to study developmental processes in medaka, that is free of endogenous TcEs. Thus no cross interactions between the transgene and endogenous autonomous as well as non-autonomous elements are to be expected.

We show that reporter genes are efficiently integrated into the genome (40% efficiency) and stably transmitted expressing the reporter gene to the subsequent generations (up to F6). Integration occurs in a SB dependent fashion and once integrated elements can be remobilized (precisely and imprecisely) by SB RNA injection. In addition to expression patterns controlled by the promoter used in the reporter construct, we observe position effects leading to very specific expression patterns. This occurs at a frequency that makes SB a useful tool for enhancer trapping in medaka.

SPERM-MEDIATED GENE TRANSFER

Corrado Spadafora

CNR Institute of Biomedical Technology, Rome Italy

Sperm cells from virtually all species have the spontaneous ability to take up foreign DNA molecules and to further transfer them into nuclei. Exogenous DNA binding and nuclear internalization is a wide spread phenomenon that can be induced in the majority of sperm cells. We have identified a network of receptors and regulatory factors that specifically mediate these events, suggesting that they take place as part of a well-regulated process. The binding of foreign DNA molecules to sperm cells appears to trigger the activation of genetic functions that are otherwise silent in mature spermatozoa. Internalized DNA sequences are found in tight association with the sperm nuclear scaffold and can undergo recombination with the sperm chromosomal DNA, resulting in the integration of foreign DNA sequences into the sperm genome.

Recently, we have also identified an active reverse transcriptase (RT) activity associated with the sperm nuclear matrix: spermatozoa challenged with foreign RNA molecules synthesize cDNA copies that are transferred to embryos after fertilization. DNA-loaded spermatozoa retain their fertilizing capability. Hence, our and other laboratories have generated genetically transformed animals in a variety of species by exploiting such capability. As yet, however, not all the molecular mechanism(s) underlying sperm-mediated transgenesis are completely understood, and a common aim of several studies is the identification of critical parameters to be adapted to gametes from different species. Different experimental approaches that have been assessed in recent studies will be discussed, including conditions for the direct interaction between sperm cells and the exogenous DNA, lipofection or electroporation of spermatozoa, the direct injection of DNA in the male reproductive tract and Intracytoplasmic Sperm Injection (ICSI) of DNA-loaded sperm cells. Together, these studies are revealing a useful role of spermatozoa as genetic tools in animal biotechnology, while

disclosing unsuspected biological functions in these cells that have long been regarded as metabolically inactive.

Embryo Aggregation and Tetraploid Blastocyst Injection

Hans-Christian Theussl and Erwin F. Wagner

Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7,
A-1030 Vienna, Austria

Embryonic stem (ES) cells provide a unique tool for producing mutations in mice. Aggregation of manipulated ES cells with wild-type morulae or injection into wild-type blastocysts results in chimaeric mice with different contributions of the two cell types (manipulated and wild-type) in their somatic tissues as well as in the germ-line.

"ES mice" completely derived from manipulated ES cells can be generated by aggregation of tetraploid morulae with diploid ES cells or injection of ES cells into tetraploid blastocysts (1). Tetraploidy of embryos is achieved by electrofusion of diploid two-cell stage embryos and culturing them to the morula- or blastocyst-stage. It is known that when tetraploid embryos are aggregated with diploid embryos the differentiation of tetraploid cells is mostly restricted to primitive endoderm and trophoctoderm that will form extraembryonic tissues, whereas the diploid cells can form the embryo proper. Dependent on the ES cell line used, "ES mice" can develop to term and sometimes also to adulthood.

In many knock-out studies, the absence of a specific gene leads to embryonic lethality. For example, lack of JunB, an early gene product and member of the AP-1 transcription factor family, causes embryonic lethality between embryonic day 8.5 and 10. This is due to multiple defects in extra-embryonic tissues, mainly the yolk sack and placenta (2). Injection of JunB ^{-/-} ES cells into tetraploid wild-type blastocysts can lead to a partial rescue, in which the ES cell derived fetuses are no longer growth retarded and display a normal placental labyrinth. Other examples, where the tetraploid technique was applied to define the functions of a gene are the aggregation and blastocyst injection of ES cells either lacking the EGFR (epidermal growth factor receptor, ref. 3) or the transcription factor Fra-1 (unpublished data).

Recent results from these studies will be reported, which illustrate the power of the tetraploid technique for functional studies in mice.

(1) Wang, Z.-Q., Kiefer, F., Urbánek, P. and Wagner, E.F. (1997) Generation of completely embryonic stem cell-derived mutant mice using tetraploid blastocyst injection. *Mech. of Development* 62, 137-145.

(2) Schorpp-Kistner, M. Wang, Z.Q., Angel, P. and Wagner, E.F. (1999) JunB is essential for mammalian placentation, *EMBO J.*18, 934-948.

(3) Sibilio, M., Steinbach, J.P., Stingl, L., Aguzzi, A. and Wagner, E.F. (1998) A strain-independent postnatal neurodegeneration in mice lacking EGF-receptor. *EMBO J.* 17, 719-731.

Cloning mice by nuclear transfer

Peter Mombaerts

The Rockefeller University
New York, NY, USA

Cloning allows the asexual reproduction of selected individuals such that the offspring have an essentially identical nuclear genome. Cloning by nuclear transfer has thus far only been reported with freshly isolated cells and cells from primary cultures. In 1998 Wakayama and Yanagimachi developed a method of cloning mice from adult somatic cells following nuclear transfer by microinjection. We have applied this method to clone mice from widely available, established embryonic stem (ES) cell lines at late passage. With the ES cell line R1, 29% of reconstructed oocytes developed *in vitro* to the morula/blastocyst stage, and 8% of these embryos developed to live-born pups when transferred to surrogate mothers. We thus cloned 26 mice from R1 cells. Nuclei from the ES cell line E14 were also shown to direct development to term. We present evidence that the nuclei of ES cells at G1- or G2/M-phases are efficiently able to support full development. Our findings demonstrate that late-passage ES cells can be used to produce viable cloned mice, and provide the first link between the technologies of ES cells and animal cloning. It may thus be possible to clone from a single cell a large number of individuals over an extended period.

Friday May 19

Characterization of Progressive Neurological Deficits in Mice Transgenic for the Human Huntington's Disease Mutation

Dr A. J. Morton

Department of Pharmacology, University of Cambridge, Tennis Court Road,
Cambridge CB2 1QJ, UNITED KINGDOM
ajm41@cam.ac.uk

Transgenic mice expressing exon 1 of the human Huntington's disease (HD) gene carrying a 141 – 157 CAG repeat (line R6/2), develop a progressive neurological phenotype resembling many of the motor symptoms of HD. Overt symptoms appear in these mice between 8 and 10 weeks of age, and progress rapidly until the death of the mice between 14-18 weeks of age. We have characterized the progression of motor and cognitive deficits in the R6/2 transgenic mouse line using a battery of five motor tests (swimming tank, rotarod, raised beam, fore- and hind-paw footprinting and acoustic startle / prepulse inhibition) and four cognitive tests (Morris water maze, visual cliff avoidance, two-choice swim-tank and T-maze). On all of the tests, R6/2 transgenic mice showed a progressive deterioration in performance, compared with wild-type littermates, over the 10 week testing period. However, R6/2 transgenic mice show significant difficulty on some of the motor tests as early as 5 – 6 weeks of age, several weeks prior to the onset of an obvious phenotype. Further between R6/2 mice displayed progressive deterioration in specific aspects of learning between 3.5 and 8 weeks of age, with the age of onset and progression of the deficits in the individual tasks depending upon the particular task demands. Our studies show that R6/2 mice provide not only a model for studying cognitive and motor changes in trinucleotide repeat disorders, but also a framework within which the functional efficacy of therapeutic strategies aimed at treating such diseases can be tested.

We have investigated a number of factors that significantly improve the general well being and life expectancy of R6/2 mice. Data showing that changes in feeding regimes and environmental stimulation improve general well-being and life expectancy of R6/2 mice will be presented. The fact that environmental stimulation improves health and life expectancy in R6/2 mice not only enables the mice to serve as more useful research tools, but also suggests that environmental stimulation may have a beneficial impact on the progression of HD in patients.

Studies of Physiological Functions in Genetically Altered Mice

Professor Peter Thoren

Department of Physiology and Pharmacology, Karolinska Institutet, Sweden

The mouse is by not means an ideal animal for experimental research compare with larger animals such as rats and rabbits. Thus, the mouse has a blood volume of about 1.8 ml and only 10% of this volume can be withdrawn at the same time without hemodynamic effects. In addition, the mouse is more difficult to anaesthetise, stabilise body temperature and perform extensive surgery. However, despite this the mouse will no doubt become our most important experimental animal within a few years, because it is an ideal animal to perform transgenic manipulations and all these new animal models will no doubt be a very important source of new information in the future.

During the last 3 years my laboratory has been involved in an extensive development of new methods in order to study complex changes in cardiovascular and pulmonary functions in the mouse. It is indeed possible to study the same parameters in the mice as in larger animals.

I will briefly describe the following methods:

1. Anaesthesia.

We have tried many different types of anaesthetic combinations in the mice. In our opinion inhalation-anaesthesia with isoflourane (Forene) is by far the best method, with moderate effects on arterial blood pressure and respiration. This form of anaesthesia is also safe and easy to use.

2. Arterial blood pressure.

Arterial pressures can be obtained with the tail cuff method. The technique is easy, but the animals are exposed to stress, which no doubt will influence the data. Cannulation of the carotid artery is a useful technique in the mice and the

catheters can be kept patent for several days. The best technique is to use telemetric measurements of arterial pressure in undisturbed mice. However, this technique is very expensive and requires good surgical skill.

3. ECG and heart rate.

Standard techniques can be used in anaesthetised mice. The mouse myocytes have very short action potential duration. Thus, T-wave diagnostics is difficult. Telemetry of ECG, heart rate, temperature and activity is easy to perform and very useful.

4. Cardiac function studies.

Modern echocardiographic equipment is surprisingly useful in order to study cardiac dimensions and function in intact anaesthetised mice. The machine is very expensive. Changes in cardiac inotropi can also be studied with catheter tip transducers with high accuracy. However, this is an invasive technique and the animal has to be sacrificed after the experiment.

5. Isolated heart preparations.

The retrograde Langendorff technique is very useful to study cardiac function, metabolism ischemic responses and coronary flow in the mice. Advantages and drawbacks will be discussed.

Food For Transgenic Rodents

Frank Günther

Charles River Deutschland, Stolzenseeweg 32-36, D-88353
Kisslegg

Every rodent strain has its own demands in nutrient requirements. Especially mutants like nude or hairless rodents, disease models for obesity and diabetes, gnotobiotics or transgenic animals seem to have different requirements in energy, protein and vitamins. Unfortunately it is difficult to find representative data about the different mouse and rat strains. Often just general recommendations for feeding rats and mice are given. A good base for discussions about average and special nutrient requirements is the latest issue of Nutrient Requirements Of Laboratory Animals (National Research Council 1995, Washington DC, USA).

Due to the fact that the nutrient requirements vary during the lifetime of a laboratory animal we differ between maintenance, breeding and experimental diets. Maintenance diets are normally given to grown up animals, to animals in longterm toxicity studies or aging studies. Breeding diets are given to breeders, males and females, and to raise pups. Experimental diets are especially designed for your study and can be of chow or purified origin.

Whether transgenic mice and rats have higher demands in nutrients than others depends very much of the result of the genetic manipulation. If the animal is in a good condition then there is a good reason to feed it like any other. If the animal is weak special precautions have to be taken into account. A good approach is to use breeding diets. If further steps are necessary it is recommendable to begin with the energy and protein content of the diet.

Influence of Infections on Research

Colin Dunn BVMS PhD MRCVS

Director Veterinary Affairs, Charles River UK Limited, Margate, Kent CT9 4LT,
UK. *c.dunn@criver.co.uk*

The health status of animals is frequently cited in terms of their association with pathogenic and opportunistic infectious agents. Sophisticated research models require animals of a defined health status to help ensure that adventitious agents do not interfere with research results. Infectious agents can affect animals in numerous ways some of which are readily apparent but other mechanisms are more subtle. Although veterinarians, scientists and facility managers are familiar with the wide array of viruses, bacteria and parasites which may be associated with laboratory animals, it can be difficult to link the presence of an agent with a mechanism which alters the behaviour of the research model.

In microbiology the pathogenicity or virulence of an infectious agent is determined by the host-pathogen interaction. In laboratory animal science the effect on research can also be rationalised in these terms. This can be particularly helpful given the growing number of genetically manipulated rodents used as models in research and the growing list of potential infectious agents. Specific information on the influence of a particular infectious agent on any one research model may be absent in the literature, so it can help to consider basic principles. A knowledge of host biology, the nature of the pathogen and the objectives of the research is essential but allows a logical approach to be taken if unexpected findings are made at routine health monitoring.

The two major host factors which influence whether an infection has an adverse effect are immunodeficiency and age. Many animal models for studying immunology are genetically manipulated and lack either functional cell types or receptors and ligands. Such models will be more susceptible to tissue damage by an infectious agent as the immune system is less able to control or eliminate

infection. Similarly, neonatal animals do not have fully functional immune systems so infections are always more severe than for adults.

Viral agents are non-living obligate intracellular parasites and since viruses do not possess their own metabolism, replication is effected by redirecting cell function to produce viral nucleic acid and proteins rather than the cellular equivalents. Therefore even subclinical acute infection can alter metabolic pathways but viruses also stimulate strong cellular immune responses and provoke the production of numerous cytokines. Many bacteria may reside on a surface and only provoke an inflammatory response when invading damaged tissue as secondary agents or producing toxins. Compared to viruses, there is a greater role for non-specific immune mechanisms in host defence against bacterial invasion. Parasites of laboratory species vary from single cell flagellates to complex helminths or mites, so host defence mechanisms are more varied as is pathogenesis.

Factors to consider as being likely to contribute to an adverse impact on research results following accidental introduction of an infectious agent will be discussed.

Germ-free transgenic animals

Jane Miller

Dept of Haematology, University of Cambridge, CIMR, Cambridge

As the benefits of keeping animals clean has been covered, my talk will concentrate on the practical aspects of keeping animals germ free, with particular reference to moving animals from one site to another.

Our main method for ensuring clean animals is embryo rederivation, and I shall use examples from our own lab to highlight the advantages and disadvantages of this technique.

Another method is straightforward quarantine and again I shall use our experience to explain this technique.

Each of these techniques has their uses at certain times and by concentrating on the practical issues involved, I hope to show planning ahead and choosing the most appropriate method is essential when moving animals into a germ-free site.

Cryoconservation of Embryos and Gametes

Peter Glenister

(Frozen Embryo & Sperm Archive), Medical Research Council, Mammalian Genetics Unit, Harwell, Oxfordshire, OX11 0RD, UK

Mouse genetics is set to play a pivotal role in the key post-genome challenge in the study of mammalian gene function. The explosion of new mouse models resulting from transgenesis and mutagenesis programmes threatens to overwhelm existing animal facility space and places an intolerable load on the infrastructural resources needed to maintain these animals as conventional breeding colonies.

Cryopreservation of embryos is widely employed for the efficient conservation of mouse stocks. Recent advances in technology for successful cryopreservation of mouse spermatozoa has considerably widened the scope for the creation of large archives, particularly from mutagenesis programmes. Moreover, frozen oocytes and ovaries may offer a valuable addition to current cryopreservation approaches.

Frozen embryos, gametes and ovarian tissue provide great opportunities for vastly increasing the available worldwide genetic resource and also provide a convenient means for disseminating and exchanging stocks between laboratories. Cryopreserved tissues probably remain viable indefinitely.

In addition, in vitro fertilisation with fresh or frozen spermatozoa is invaluable for producing large numbers of mice of a particular genotype in a short space of time. This proves extremely useful for the generation of speed backcrosses for genetic mapping and for building large numbers of a new stock of particular interest.

Running an animal facility: Conventional open unit, strict barrier conditions or IVCs?

J. Weiss, Center for Molecular Biology, Univ. of Heidelberg, Germany
&

G.T. Taylor, Dept. of Psychology, Univ. of Missouri-St. Louis, USA

Since the 1960s extensive work in the field of experimental animal science has established a comprehensive literature on the importance of standardizing the genetic background, microbiological status and housing conditions of research animals. Today there is widespread recognition that adoption of laboratory standards is essential for achieving reliable and reproducible experimental results.

Management decisions for an animal facility determine the extent of standardization, particularly of the microbiological standards. The conventional facility is an open animal house with insufficient, or nonexistent, regulations to prevent the introduction of microorganisms into the colony, including those that are pathogenic to experimental animals. Also, there is often little or no health monitoring of the animals and the facility. Under these circumstances one must expect an ongoing threat of contamination with pathogens. At worst, this situation may result in the deaths of a large percentage of the colony animals.

An opposite concept is management of an animal house as a strictly isolated barrier system. This requires a certain type of construction within the physical facility and a number of well defined regulations and SOPs (Standard Operating Procedures) to manage the facility. Such a system governs access to the unit by personnel and experimenters, as well as supervises admission of new animals and supplies being brought into the barrier-unit. It is critical that such regulations are in place and that they are religiously followed. The result is a SPF facility within the recommendations for the health monitoring made by FELASA (1994) that can be maintained over years.

Ventilated cage racks (IVC) are an interesting alternative to barrier units that also can maintain stable, hygienic conditions for experimental animals. However, successful implementation of an IVC system requires detailed and thoroughly observed SOPs. Of particular concern is an appropriate procedure for changing of cages that ensures their segregation from other cages and the outside environment. Health monitoring is an extensive procedure in IVC-housed animals. A barrier unit is one single hygienic unit, although usually the unit consists of a series of animal rooms, whereas every single cage in an IVC system represents a distinct hygienic unit. One consequence is that complete health monitoring necessitates sampling periodically from every cage. Although representing a promising newer technology for an animal facility, a final judgement of the IVC systems can be made only after careful assessment of their effectiveness over long periods of time.

The Four Levels of Science in Ventilated Caging Systems (VCS)

Mats Spångberg, DVM, PhD

As Ventilated Caging Systems (VCS) have developed, so increasingly more scientifically based evidence is emerging both on the operation of the various systems, and what is desirable for the clients, remembering that there are, in effect, three clients – the scientists, the care staff and the animals. This presentation will explore the Four Levels at which this evidence can be acquired, and will review some of the findings already available. The first level of evidence is easiest to gather but is largely hypothetical.

Level 1. Theoretical data: Hypothetical data, usually taken from computer modelling, e.g. Computational Fluid Dynamics (CFD) which may hypothesise the way air will move through the system and allow this to be theoretically optimised. This can be very useful during the design of novel systems. However it is largely irrelevant once the first prototypes are built and real data can be measured. The second and third levels are based upon real test data and therefore can be considered to be very relevant to specific aspects of operation.

Level 2. Engineering data: Once the VCS has been built, a range of tests can be performed to either confirm the theoretical data, or to advise adjustments which are needed in the face of real data e.g. a) Noise levels, in the cage and adjacent to the rack. b) Velocity of air movement at all points within the cage where animals may be exposed. c) Effectiveness of air exchange using tracer gases or similar. d) Evidence of absence of cross contamination between cages using physical markers. e) Heat generation, and the effect on air movement within the system and on room heat gain.

Level 3. Biological data: A range of biologically relevant factors can be measured on the system in a laboratory environment to ensure that the system minimises undesirable effects e.g.: a) The effect of air change rates on the microenvironment in the cages – NH₃, CO₂, RH, Temp, etc. b) Behavioural responses of animals to the environment within the system, such as preferences

and evidence of aversions. However, evidence at Level 4 is necessary to truly understand the effectiveness of the equipment in addressing the needs of all three clients

Level 4. Operational data: Once the system is in routine use, data can be generated which is relevant to its operation in research animal facilities. This takes into consideration, for example, the effect of different genetic strains, the working procedures of animal care staff, the variable environmental contaminations, which can occur, and the response of the system to such contaminations. These data provide the truly valuable evidence of whether the system works. They will demonstrate, for example, whether the noise levels and in-cage air velocities measured at Level 2 are acceptable to the animals, or whether the system succeeds in containing contaminated animals or protecting very clean animals, e.g.: a) Do animals (especially difficult and sensitive strains such as nudes, scids, and certain knockouts) breed and rear their litters effectively in the system? Do offspring grow well? Is there behavioural or other evidence that the environment is not stressful? b) Does the system effectively prevent cross contamination between cages under normal working conditions (especially using gnotobiotic animals as test animals)? Can clean animals be maintained in a “dirty” environment under normal working conditions? Can allergens and toxic chemicals be contained within the system under normal working conditions? c) How comfortable is the system to work with, and what working practices (e.g. frequency of cage-changing) are optimal for all three clients?

These areas of research are potentially extremely fruitful for Laboratory Animal Scientists and Veterinarians to generate high quality data of enormous utility to colleagues. The presentation will review some of the scientific evidence already available within this four level structure – and identify some of the gaps in our knowledge.

Marking of animals

M. Schröder

POSTER ABSTRACTS

A1

Applying the Principles of Replacement, Reduction and Refinement to the Production and Use of Transgenic Animals in Scientific Research

Elizabeth S. Jenkins and Robert D. Combes

FRAME, Russell & Burch House, 96-98 North Sherwood Street, Nottingham, NG1 4EE.

Since the generation of the first transgenic rodents, the technology of transgenesis has developed rapidly and it is now possible to produce genetically modified (GM) animals by several different methods. The process of transgenesis is inherently inefficient, and can have a negative impact on animal welfare, both in terms of the procedures used (often), and as a consequence of the genetic manipulation (sometimes).

Until recently, little attention has been given to applying the concept of the Three Rs, namely replacement, refinement and reduction, to the process of transgenesis. However, recent scientific advances are now providing new opportunities for more humane research. This presentation considers options for the refinement of procedures used in transgenesis, reduction in the number of animals required to generate and produce transgenic rodents and replacement of *in vivo* studies with *in vitro* assays. This study is being undertaken as part of a larger investigation by the BVA/WF/FRAME/RSPCA/UFAW Joint Working Group on Refinement, which is currently considering the welfare of GM rodents. This group aims to make recommendations for best practice, and to identify suitable approaches to reduce the number of GM rodents used and minimise any pain, suffering and distress that may be caused by the procedures involved.

A2

THE MGU FROZEN EMBRYO AND SPERM ARCHIVE (FESA)

Peter Glenister, Claire Thornton

The Frozen Embryo and Sperm Archive (FESA) at the Mammalian Genetics Unit, Harwell is the largest in Europe and one of the largest in the world. At present, FESA contains more than 250,000 embryos comprising almost 1000 stocks. In addition, spermatozoa are being archived from all F1 males generated in the Harwell ENU mutagenesis programme. Currently, more than 2,500 F1 males have been banked. Many of the embryo and sperm stocks are potential models of human genetic disease. The bank was founded in the mid 1970s after extensive studies into the feasibility of embryo freezing as a reliable and cost effective means of storing mouse strains for long periods of time. FESA now offers researchers the opportunity to have stocks frozen and archived at Harwell. This securely funded archive is the major UK centre for the deposition and distribution of mouse mutants and stocks that are pivotal to much of today's scientific research. We offer a free service for cryopreservation, storage and promotion of mouse embryos or sperm. Charges are levied for withdrawals from the archive. FESA is one of six nodes that constitute EMMA (European Mouse Mutant Archive). Contact: fesa@har.mrc.ac.uk

Visit <http://www.mgu.har.mrc.ac.uk/>

A3

Rapid purification of genomic DNA for identification of transgenic mice by analysis of feces

Emanuelle Gilbert

Transgenic Unit, Genos Developpement, 49033 Angers, France

To identify transgenic mice for routine genetic monitoring procedures, tail biopsies or blood samples are commonly used as sources of DNA. With a DNA extraction kit, mouse fecal samples collected contain enough colorectal epithelial cells to yield sufficient DNA for polymerase chain reaction (PCR). This technique is much less traumatic to the mouse, there is no surgical intervention or wounding, and sampling can be repeated as often as needed. To our knowledge, no similar method using DNA binding matrix enables PCR amplification of DNA from fecal samples. This technique is reliable, requires no organic solvents, and no ProteinaseK. Adapted on the Easy Prep System, the DNA extraction can process up to 24 samples in 90 minutes, without requiring centrifugation. The method makes high quality DNA rapidly available for all current molecular techniques such as PCR. The method is particularly suitable for DNA extraction from large variety of complex samples (soaked seeds and vegetal tissues, food samples, biological fluids), in view of detection of micro-organisms, identification of molecular markers and genetically modified organisms.

A4

MEASUREMENT OF LIGHT INTENSITY WITHIN CAGES MADE OF DIFFERENT PLASTIC MATERIALS

Edoardo Bernardini

During the last few years the number of plastic materials used for transparent cages for laboratory animals has increased and now includes new plastic materials such as polysulfone or polyetherimide representing the modern evolution of polycarbonate.

The need for measuring the light intensity in cages is even more important today after the release of the “Report for the revision of the Council of Europe Convention ETS 123 Appendix A for rodents and rabbits”, Issued by the Council’s Working Group for Rodents and Rabbits (Strasbourg, 21 February 2000).

The working group underlines that...albino rats have been shown to prefer areas with a light intensity of less than 25 lux (Schlingmann, 1993) and that “The effects of different cage materials, e.g. fully or partially “tinted” polycarbonates, should be studied” (pag. 26 of the Proposal).

We have measured the light intensity in two groups of 48 SEALS SAFE Tecniplast cages all with bedding, bottles with water and card holders. The first group was the Ultem™ Polyetherimide amber-coloured cages and the second was the H-Temp™ Polysulfone cages.

We measured a light intensity of 220-230 lux 100 cm from the floor and 65 cm from the front of the rack. The light intensity never exceeded 43 lux in Ultem™ cages (group one) and 54 lux in H-Temp™ (Polysulfone) cages (group two). Even when the intensity was increased to 360 lux 100 cm from the floor the maximum lux value was 63 in Ultem™ cages and 70 in H-Temp™ cages.

From our measurements we can also see that the light intensity in each cage varies from lighter areas to darker areas where the intensity is lower than the maximum values stated above.

To have a more comprehensive comparison study similar data have been collected in transparent polycarbonate cages and will be presented on the poster.

A5

Optimisation of cryopreservation procedures for rat embryos

Séverine MENORET, Béatrice CHARREAU, Miguel JEAN*, Laurant TESSON, Jean-Paul SOULILLOU and Ignacio ANEGON

INSERM U437 and Institut de Transplantation et de Recherche en Transplantation, 30 bd Jean-Monnet. * Hôpital de la Mère et de l'Enfant, CHU, France.

The purpose of this study was to determine optimal media for cryopreservation of 1- and 2-cell rat embryos. Sprague-Dawley rat females, 22 – 28 day-old, were superovulated with 20 IU of pregnant mare's serum gonadotropin followed 48h later with 1 µg LHRH. Females were mated to males of the same strain. Embryos were recovered from the oviducts at the pronuclear stage (20 – 22h after the LHRH injection). 2-cell rat embryos were obtained following overnight culture in M16 medium containing 10% FCS, 100 IU/ml penicillin, 100µg/ml streptomycin, 2mM glutamine. Cryopreservation was carried out in 8 different media:

Medium **A**: 10M sucrose + 1.5M propylene glycol + 20% heated fetal calf serum (FCS) in PBS. Medium **B**: 3.5M dimethylsulfoxide (DMSO) + 0.5M sucrose in M16. Medium **C**: 1.5M propylene glycol in FCS. Medium **D**: 1.5M propylene glycol in M16. Medium **E**: 0.7M DMSO in FCS. Medium **F**: 1.5M DMSO in FCS. Medium **G**: 0.7M DMSO in M16. Medium **H**: 1.5M DMSO in M16.

One or 2-cell stage embryos were placed in different media and cooled from 20°C to -7°C at 1.5°C/min and then from -7°C to -70°C at -0.3°C/min before being plunged into liquid nitrogen. To assess viability, rat embryos were thawed by rapid warming and washed two times in M16 medium, cultured in this medium at 37°C in 5%CO₂ for 24 hours and analyzed microscopically for morphological appearance and passage of embryos from 1- to 2-cells.

Table 1: Effect of cryopreservation on survival rate of rat embryos following thawing

	Media	A	B	C	D	E	F	G	H
1-cell	% viability n	8.70% (8/92)	0% (0/105)	68.30% (97/142)	69.30% (86/124)	85.40% (76/89)	46.90% (45/96)	26% (32/123)	50% (47/94)
2-cell	% viability n	21% (28/133)	3.50% (3/85)	62.10% (59/95)	90.70% (49/54)	80.60% (108/134)	32.30% (40/124)	24.80% (29/117)	ND ND

To evaluate the ability of frozen embryos to give rise to live animals, embryos cryopreserved in Medium E were transferred to pseudopregnant females allowing to obtain rates of newborn animals comparable to these obtained with non frozen embryos (37 vs 27%). Similar results have been obtained with inbred and transgenic rat strains.

We demonstrate that composition of cryopreservation medium deeply affect embryo survival. Our data shown that a high percentage of embryo recovery was achieved by using 0.7M DMSO as a cryoprotectant in FCS as diluent. In this condition, embryos transferred into pseudopregnant females after thawing successfully develop to newborn. In conclusion, these results indicate that it is possible to freeze rat embryos and that cryopreservation is an effective mean to preserve inbred, outbred or transgenic rat strains. Additionally, cryopreserved rat embryos could be a convenient source of large quantities of stage matched eggs for production of transgenic rats by microinjection.

B1

ANTI- AND PRO-INFLAMMATORY PROPERTIES OF ALPHA-1-ACID GLYCOPROTEIN: STUDIES IN RAT-ALPHA1-AGP TRANSGENIC MICE

Hoche pied, T., Wielockx, B., Staelens, J., Van Molle, W., Berger, F., Baumann, H. and Libert, C.

Department of Molecular Biology, University of Ghent, 9000 Ghent, Belgium

Alpha-1-acid glycoprotein (AGP) is a highly glycosylated acute phase protein of 43 kDa. AGP is mainly produced by hepatocytes. During an acute phase condition, the concentration rises 2 – 5 times, making AGP one of the predominant proteins in the serum. The biological function of this protein is uncertain: depending on the system, AGP has anti-inflammatory or pro-inflammatory properties. We are interested in finding clues about the pathophysiological role of AGP. Therefore, we study the response of rat-AGP-transgenic mice in several systems and are generating AGP-deficient mice.

We have shown that rat-AGP-transgenic mice are significantly protected in a *K. pneumoniae* model of Gram-negative infection and systemic inflammation. When a dose of $1 - 5 \times 10^5$ CFU *K. pneumoniae* was injected intramuscularly, AGP-transgenic mice were significantly protected compared to control mice. When tissue sections of spleen, liver, kidney and lung of control and transgenic mice were compared histologically, we found bacteria in all tissues of control mice and not in transgenic mice. Moreover, the spleen of control mice exhibited large necrotic spots, filled with bacteria, whereas such necrotic spots were not found in the transgenic mice. The data support our findings that exogenously injected AGP protects in this model.

In another model of acute inflammation, induction of acute colitis, by administering 2% dextrane sodium sulphate in the drinking water for 5 days, we have shown that homozygous transgenic mice die significantly faster from the

treatment compared to wild-type mice, probably because the inflammatory reaction is aggravated by the transgenic AGP.

In conclusion, rat-AGP-transgenic mice are protected against a lethal challenge of *K. pneumoniae*, pointing to an anti-inflammatory role for AGP in systemic inflammation. In contrast, rat-AGP-transgenic mice are sensitized in a model of acute colitis, suggesting a pro-inflammatory role for AGP in this model of local inflammation.

B2

***In vivo* expression of a truncated form of BMPR-II utilizing an internal ribosome entry site (IRES) in the TH locus**

Henrik Bengtsson, Dmitry Usoskin, Stine Söderström, Annika Kylberg, Jonas Lindeberg and Ted Ebendal

Department of Neuroscience, Uppsala University, BMC Box 587, 751 23 Uppsala, Sweden

A truncated form of bone morphogenetic protein receptor type II (trBMPR-II) was constructed using a PCR strategy. The entire intracellular region was excised, leaving the extracellular and transmembrane domains intact, rendering the receptor unable to propagate signals into the cell upon ligand binding. In addition, a c-myc tag was added to the carboxy terminal of the trBMPR-II. To express trBMPR-II *in vivo*, the 3' end of the mouse tyrosine hydroxylase (TH) gene was isolated and characterized. An internal ribosome entry site (IRES) was added to the trBMPR-II together with an ftr-flanked neomycin resistance gene cassette for introduction downstream of the stop codon in exon 13, but upstream of the poly A signal of TH.

Through homologous recombination, mouse ES-cell clones resistant to G418 were isolated. ES-cells carrying the altered TH allele then were injected into blastocysts and chimeric mice produced. The F1 offspring from the chimeras are viable and reproductive. Such mice, heterozygous for the TH^{IRES/trBMPR-II/ftrNeo} locus, were mated with Flp-deleter mice and pups genotyped. Four animals were found to have the altered TH locus and in one of these four pups the Flp recombinase had removed the neomycin resistance gene.

Further breeding will give rise to TH^{IRES/trBMPR-II} / TH^{IRES/trBMPR-II} mice, expressing bicistronic mRNAs coding for the TH as well as the truncated, dominant negative BMPR-II receptor protein transcribed from the TH locus.

Studies are in progress to reveal gene dosage effects, mRNA and protein levels of TH and trBMPR-II in dopaminergic neurons of the mutant mice. It will also be examined whether the normal expression pattern of TH is maintained despite the

downstream addition of the IRES-trBMPr-II construct and how the mRNA processing is affected before and after removal of the ftr-flanked neo cassette. Conclusions will thus be reached regarding the general applicability of bicistronic knock-in strategy in mice, previously shown by us to be effective in cell lines (Lindeberg and Ebendal, 199, Nucl Acids Res, 27:1552-1554).

B3

Genetic analysis of GDF10 and ALK-2 signaling in the brain

Susanna Althini, Stine Söderström, Jonas Lindeberg, Annika Kylberg,
Dmitri Usoskin, Henrik Bengtsson and Ted Ebendahl

Department of Neuroscience, Uppsala University, BMC Box 587, 751 23
Uppsala, Sweden

The aim of this project is to study GDF10 and ALK-2 signalling in the mouse brain. Both molecules are members of the TGF- β signalling system. GDF10 is a ligand, closely related to Bone Morphogenetic Protein (BMP9-3b. Its receptor and therefore actual signalling pathway is still unknown. Activin Like Kinase (ALK)-2 is a type I serine-threonine kinase receptor. It has recently been shown to activate the BMP- β induction.

A conventional GDF10 knock out has been created by replacement of a ~ 120bp fragment, including the start ATG-codon, with the neomycin selection marker. An external probe has been used for identification of positive ES-cell clones and genotyping Southern Blot. Two positive clones were injected into blastocysts and resulted in one male chimera. The mutation has gone germline and analysis of GDF10-mutants is now proceeding.

A truncated ALK-2 has been created by a PCR-based strategy. We have shown that it has a dominant negative effect on BMP-7 signalling in HepG2 cells and that it is expressed at the cell surface in COS cells. We have also constructed targeting vectors to knock in ALK-2 Δ into the Tyrosine Hydroxylase and the Brain Factor-1 loci. The TH construct will target dopaminergic cells in substantia nigra and locus coeruleus, while the BF-1 construct will disturb ALK-2 signalling in hippocampus and cerebral cortex.

These mutant mice will be used as tools for *in vivo* studies of the relevance of GDF10 and ALK-2 signals for neural plasticity and repair.

B4

Improved technique for detection of enhanced green fluorescent protein (EGFP) in transgenic mice

Ramin Shariatmadari, Petra Lahti, Ilpo Huhtaniemi and Matti Poutanen

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

One of the most exciting recent advances in the cell biology is the possibility to use the green fluorescent protein and its various mutated forms as a genetic reporter protein in studies in vitro and in vivo. In the present study, several detection techniques for enhanced green fluorescent protein (EGFP) were compared. Different tissue preparation techniques (squash preparations, vibratome sections, frozen sections) were evaluated using fluorescence- and confocal microscopy. As a model we used transgenic mice expressing the EGFP protein under control of a 5.0-kb long 5'-flanking region of the epididymis-specific glutathione peroxidase isoenzyme 5 protein (GPX5) promoter. In these mice, a low level of EGFP expression is present in the distal caput epididymis. Among the various preparation procedures used, best morphological and histological intactness and reproducibility were obtained using frozen sections after slow tissue freezing. This tissue handling combined with confocal microscopy gave the highest sensitivity and resolution for EGFP detection. In addition, confocal microscopy had the advantage to allow detection of EGFP fluorescence from cells in different layers which cannot be achieved using normal fluorescence microscopy. Slow tissue freezing and storing the specimens at -70°C for up to six weeks did not affect EGFP fluorescence. Hence, the method developed offers the possibility to collect and store material of interest for several weeks without loss of EGFP fluorescence.

B5

Cre-recombinase expression and deletion of *LoxP*-flanked gene segments in transgenic mice utilizing the 6-kb inhibin- α subunit promoter

Fu-Ping Zhang, Tomi Pakarinen, Pasi Koskimies, Ilpo Huhtaniemi,
Matti Poutanen

Department of Physiology, Institute Biomedicine, University of Turku,
Kiinamylynkatu 10, FIN 20520 Turku, Finland

To delete genes expressed specifically in gonadal tissues, we have established transgenic mice expressing the Cre recombinase under a 6 kb segment of the inhibin α -subunit promoter (*Inh-Cre*). Seven *Inh-Cre* mouse lines were produced, and based on the level of Cre expression, three of them were characterized further. RT-PCR shown that the CRE-recombinase was specifically expressed in the testis and ovary, while there was no expression in the other tissues analyzed, including liver, kidney, lung, heart, adrenal, pituitary, intestine, muscle and brain. To monitor the efficiency of the Cre-mediated recombination, we crossed the *Inh-Cre* mice with a transgenic mouse line carrying a reporter gene construct, CAG-CAT-*lacZ*. The data from the double positive transgenic mice indicated that the excision of the CAT gene was mainly found in the testis, ovary, and faintly in the epididymis, but not in other tissues. The cellular localization of the Cre-recombinase in the testis was studied further in the double transgenic mice by analyzing the *lacZ* activation with a histochemical X-gal staining. As expected, the staining was localized in testicular Sertoli cells and Leydig cells, but it was also found in developing spermatogonia. Due to endogenous X-gal staining in the ovary, we could not identify the exact location of Cre-recombinase in ovary. In conclusion, we have established a mouse model for a gonad specific expression of Cre-recombinase. These mice will be useful in analyzing the function of a variety of genes of interest in the testis and ovary.

B6

Development of *in vivo* models to test the effectiveness of specific enzyme inhibitors in the prevention of diabetic vascularpathies

Stolen, C. M., Bono P., and Jalkanen, S.

MediCity Research Laboratories, University of Turku, FIN-20520 Turku, Finland

Advanced stage diabetes is associated with both microvascular and macrovascular changes leading to damage in the kidneys, eyes, and peripheral nerves as well as to other cardiovascular disorders. Although the mechanisms behind the diabetes related vascular changes are incompletely understood the oxidative glycation process is clearly associated with vascular damage. Accumulating new research suggests that the deamination of substrates, such as aminoacetone and methylamine, by semicarbazide-sensitive amine oxidase (SSAO) produces substances (e.g. aldehyde, ammonium, and hydrogen peroxide) that can directly cause cytotoxicity and indirectly enhance the formation of advanced glycation end products. In human diabetes and in animal models of diabetes the plasma levels of SSAO are increased, and in diabetic patients a significant positive correlation between SSAO activity and the degree of vascular damage (e.g. nephropathy and retinopathy) exists. The discovery of SSAO activity in the recently characterized and cloned human and mouse vascular adhesion protein-1 (VAP-1) now provides us with an opportunity to test SSAO biological function with controlled experiments. VAP-1 is a novel endothelial sialoglycoprotein having at least two different cellular roles; it functions both as an enzyme with amine oxidase activity and as a lymphocyte-endothelial cell adhesion protein. By using transgenic and knockout mice, both gain of function and loss of function experiments are being performed to investigate the relationship between SSAO activity and vascular pathology. Together with specific inhibitors of VAP-1, these mice will be used as models to aid in the assessment of various vascular pathology treatment modalities. The production of these animal models are at multiple stages of preparation ranging from the

generation of transgene constructs to the existence of homozygous transgenic mice.

B7

Transposon mediated germline integration in medaka

Thorsten Henrich

Developmental Biology, EMBL-Heidelberg

Recently the reconstruction of a transposable element (**Sleeping beauty**) of the TcE family has been reported. Its ability to catalyse the integration of plasmid DNA into the genome and to remobilize the element to a new location in tissue culture was shown.

To investigate whether Sleeping beauty can be used to stably integrate foreign DNA into the genome of medaka fish, the Sleeping beauty transposase RNA was coinjected with a DNA reporter construct into one and two cell stage embryos. This reporter construct contains the specific targets for the Sleeping beauty transposase flanking a GFP gene driven by an ubiquitously active cytoskeletal actin promoter.

20 stably GFP expressing fish lines were generated. Germline transmission and unaltered GFP expression was observed up to the F5 generation. One line (SV) shows strong ubiquitous GFP expression during development. Five lines however, show remarkable position effects. In one line, Mr. Green, the strongest expression can be found in the lens. Also single cells in the retina express GFP. Another line (VV) shows GFP expression in a row of single cells in the tectum. The fourth line (DE) shows differential GFP expression in the diencephalon. Enhancing as well as silencing effects were observed in this line. A fifth line (Yes) expresses GFP specifically in the ventricle of the heart. The fluorescent ventricle is visible even through the bodywall of adult fish. Interestingly two recessive mutants affecting heart development were detected in crosses of this line. One mutant (*Kleines Herz, kh*) has reduced head and eye structures and a weak tubular heart. The other mutant (*Spiegelfisch, spf*) shows a *situs inversus totalis* phenotype.

Southern blot hybridisation in the SV transgenic line revealed the presence of the GFP insert flanked by the IR/DRs in the absence of any vector backbone

sequences, indicating a *Sleeping Beauty* mediated integration event. This was confirmed by sequencing the regions flanking the insertion site. It was demonstrated that the insert was cut at the predicted position, that it integrated into the characteristic TA-target site and that this target site was duplicated upon integration. In the Yes-line a concatemer of head-to-tail plasmid copies was the substrate for the *SB* transposase, such that three repeats were cut out of the concatemer at the IR/DRs and integrated into the genome.

Sleeping Beauty RNA injection into a stable line (Yes) resulted in the excision of the reporter construct. The region of excision was sequenced and revealed the presence of precise as well as imprecise excision footprints.

B8

Knockout targeting of the mouse gene *MXTH* for the second AP-endonuclease in mammalian cells

B.E. Johnsen, E. Seeberg and A. Klungland

Department of Molecular Biology, Institute of Medical Microbiology, University of Oslo, The National Hospital, Oslo

Abasic (AP for apurinic or apyrimidinic) sites in the DNA arise by spontaneous loss of purine bases or through the enzymatic removal of damaged bases during base excision repair. Specific and abundant enzymes exist for AP-site recognition and incision (AP-endonucleases) and these include ExoIII in *E. coli*, Apn2 in *S. cerevisiae* and HAP1 in humans. We have recently identified and characterised a second gene family for enzymes with putative AP-endonuclease activity in eukaryotic cells, termed APN2 in yeast and Hxth in human cells. To investigate the biological importance of this gene in a mammalian organism we have made gene constructs for targeting the corresponding gene in the mouse. Mouse *XTH* was isolated from a mouse spleen genomic library by screening with a probe from the human XTH2. The genomic clone containing the mouse gene was characterised by restriction enzyme analysis and sequencing. The deduced amino acid sequence of the mouse gene has 64% sequence identity with that of human XTH and both genes show similarity to members of the exoIII/HAP1 family of AP-endonucleases. The knockout vector pKO1904 was used to construct an *MXTH* targeting vector, containing the neomycin marker flanked by homologous sequences of 3 kb on one side and 1,2 kb on the other. Counterselection of nontargeted integration was obtained by insertion of the thymidine kinase gene and selection with gancyclovir. AB2.2-Prime embryonic stem (ES) cells were used for the targeting event, and more than 500 clones with putative homologous integration of the construct have been isolated. These clones are now being analysed to identify cells where the targeting construct has been correctly inserted by homologous recombination. ES cells heterozygous for

MXTH will be microinjected into blastocysts, and homozygous knockout mice will hopefully be generated by appropriate breeding and selection.

B9

AN EASY AND SIMPLE WAY TO REMOVE THE *NEO* CASSETTE FROM THE MOUSE GENOME

Pei-Zhang, Zan Gu, Sasha Wang and William Chen

**Department of Molecular Genetics, University of Illinois at Chicago,
Chicago, IL60607-7170**

Using Cre-loxP system for conditional knockout, there is one step that requires to delete the *neo* cassette from the loxP-loxP-*neo*-loxP in the mouse genome. The *neo* cassette was used as a positive selection marker, but it has been reported that leaving it in the genome exhibited as a null mutation by blocking the normal splicing. Here we report an efficient and novel way to excise only the *neo* cassette but leaving the third loxP site untouched, without manipulating the ES cells or generating a new strain of mouse. We used this method for the conditional knockout for EGF receptor gene. The targeting vector contains exon 1 of EGF receptor gene flanked by two loxP sites with a *neo* cassette also flanked by a loxP site (loxP-loxP-*neo*-loxP). The targeting vector was introduced into ES cells by electroporation, and the heterozygous mouse containing the targeting vector was crossed with E11a-Cre mouse. Our studies of the offspring from this match show that we have deleted the *neo* gene cassette successfully.

B10

Pdgfr α , a target gene in the Shh signaling pathway? Comparison with Gli, Patched and Pax1 expression pattern

Zhang X-Q., Afink, GB. and Nistér, M.

Department of Genetics and Pathology, Unit of Pathology, University Hospital,
Uppsala, Sweden

In situ hybridizations were performed to investigate the expression patterns of Gli, Ptc and Pdgfr α during early mouse embryonic development. During the axial and limb bud bone development, Gli, Ptc and Pdgfr α are coexpressed in the precartilaginous condensation. Pax1 is also expressed but only in the axial bone. Ptc and Gli expression continues to be expressed until the early cartilage stage while Pdgfr α mRNA expression is terminated before cartilage formation. In these areas of epithelio-mesenchymal interactions, Gli, Ptc and Pdgfr α expression partly overlapped but in the expression of these components is found in different layer (e.g. mainly in the mesenchyme adjacent to epithelium) while Pdgfr α in the layer most far away from the epithelium and is present only in the mesenchyme tissue.

B11

Generation of a Friedreich's ataxia conditional knock out mouse model

Ahern, A., Chamberlain, S. and Pook, M.

Hereditary Ataxia Research Group, Department of Molecular Genetics, Sir Alexander Fleming Building, Imperial College School of Science Technology and Medicine, Exhibition Road, London SW7 2AZ, UK

Friedreich's ataxia (FRDA) is an autosomal recessive progressive neurodegenerative disorder which presents at an early stage of onset with symptoms of gait ataxia, dysarthria, loss of tendon reflexes, and results in premature death from hypertrophic cardiomyopathy. The disorder is caused by mutation of the FRDA gene, which encodes frataxin, a highly conserved protein involved in mitochondrial iron haemostasis. The predominant mutation is a pathological expansion of a non-coding GAA triplet repeat motif located within intron 1 of the frataxin gene. This GAA expansion interferes with transcription, possibly by the formation of a triplet helix, producing reduced levels of frataxin expression, which results in mitochondrial iron accumulation and damage to oxidative phosphorylation processes.

In order to provide further insight into the FRDA pathological mechanism and also to provide an effective means to study drug and gene therapies, an FRDA mouse model would be invaluable. However, as a conventional FRDA knock out model has already shown homozygous embryonic lethality, we are employing gene-targeting technology in conjunction with the Cre/lox system to generate a FRDA conditional knockout mouse model.

We have isolated genomic clones of the mouse frataxin gene and have generated a targeting construct, which comprises 10 kb of mouse genomic sequence homology flanking floxed exon 1 and floxed neo^R sequences. The neo^R cassette will be excised in vitro in targeted ES cell clones to exclude the possible outcome of a hypomorphic allele, and the floxed exon 1 clones will be

used to produce chimeras. The resulting targeted mice will be crossed with tissue-specific cre-expressing mice, and possibly also inducible cre-transgenic mice, to obtain temporal and spatial control over the loss/reduction of frataxin expression.