2nd Workshop on Innovative Mouse Models

Thursday 17 June 2004

Leiden University Medical Center
Albinusdreef 2, 2333 ZA Leiden

Program, Abstracts and Participants of the

2nd Workshop on Innovative Mouse Models

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Organized by:

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Program

2nd Workshop on Innovative Mouse Models, Thursday 17 June 2004

8.30	Coffee and registration	30′
9.00	Opening *Sjef Verbeek*	10′
9.10-10.30	**Session 1: In vivo RNAi, F1 ES cells, and lentiviral transgenesis** *Chair: Werner Müller*	
9.10	Keynote Lecture – Conditional RNAi in mice *Ralf Kühn*	20′
9.30	Speeding up conditional gene targeting: RNAi and ES mouse technology *Branko Zevnik*	10′
9.40	Lentiviral transgenesis in lifestock *Barbara Kessler*	15′
9.55	Inducible lentiviral vectors for transgenesis *Jurgen Seppen*	10′
10.05	In vivo silencing of CCR2 by lentiviral siRNA transfer to bone marrow cells leads to persistent loss of CCR2 function *Ilze Bot*	10′
10.15	Short presentation *Werner Müller*	5′
10.20-10.50	Coffee Break	
10.50	**Forum discussion on Session 1** *Moderator: Ralf Kühn*	60′
11.50-12.50	Lunch	

12.50-14.15	**Session 2: Conditional mutagenesis and gene trapping** *Chair: Paul Krimpenfort*	
12.50	Keynote Lecture – Spatio-temporally controlled targeted somatic mutagenesis during mouse development and adulthood *Daniel Metzger*	30′
13.20	A gene trap strategy for the large scale induction of conditional mouse mutants *Frank Schnütgen*	10′
13.30	5-Minute presentations:	35′
	Development of DiCre, a rapamycin-regulatable fragment complementation system for Cre *Jean-Paul Herman*	
	Melanoma induction via melanocyte-specific Ink4a/Arf inactivation and Hras activation by a self-inactivating CreERΔD transgene *Ivo Huijbers*	
	Use of a self-deleting CreERt2 cassette in conditional transgenic mouse models to evaluate key signaling mediators of skeletal muscle hypertrophy and atrophy *Ka-Man Lai*	
	Generation of kidney-epithelial cell-specific Cre mice *Irma Lantinga*	
	Inducible renal-specific Cre recombinase activity in GGT-CreER^{T2} mice *Bernd Dworniczak*	
	Fate mapping of hematopoietic stem cells during mouse development *Aneta Oziemlak*	
	Time- and tissue-restricted lineage ablation using a conditional diphtheria toxin receptor transgenic mouse strain *Thomas Wunderlich*	
14.05	Questions on 5-minute presentations	10′
14.15-14.45	Coffee break	

14.45-16.10	**Session 3: Recombineering and inducible systems** *Chair: Marian van Roon*	
14.45	Keynote Lecture – Multi-purpose alleles; construction by recombineering and use for conditional analyses *Francis Stewart*	30′
15.15	In vivo imaging of digestive tumors using a novel intestinal pathogen-based vectorization approach *Silvie Robine*	10′
15.25	5-Minute presentations:	35′
	Strategies for efficient and predictable generation of Tet-controlled animal models *Kai Schönig*	
	Generation of ES cells showing position-independent Doxycyclin-regulated gene expression *Jaap Jansen*	
	Towards an optimal docking site in the mouse genome *Cor Breukel*	
	Functional analysis of an rtTA-GFP operon in the ROSA26 locus *Simone Freese*	
	Generation of antigen presenting cell-restricted tTA and rtTA transgenic mouse lines *Hisse Martien van Santen*	
	Tet-dependent conditional immortalization as a new tool for molecular analysis of mouse mutants *Tobias May*	
	Knock-in mouse models for human XPD disorders, relevance of gene expression *Jaan-Olle Andressoo*	
16.00	Questions on 5-minute presentations	10′
16.10	**Forum discussion on Sessions 2 and 3** *Moderator: Francis Stewart*	60′
17.10	Drinks & snacks	

Presentations

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CONDITIONAL RNAi IN MICE

Ralf Kühn, Christiane Hitz, Wolfgang Wurst
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Mice transgenic for shRNA expression vectors exhibit permanent and bodywide gene silencing, comparable to conventional knockout mice. Conditional RNAi would allow inducible or cell type-specific gene silencing in mice to avoid embryonic lethality and dissect gene function in vivo at high precision. We developed an approach to activate shRNA vectors through Cre recombinase mediated deletion of a loxP-flanked transcriptional stop cassette. Using transient transfection in ES cells we tested the efficiency of RNAi to reporter genes upon insertion of loxP into four positions within the H1/U6 promoter or the transcribed region. We found that two of the latter configurations retain gene silencing activity in combination with the U6 but not the H1 promoter, pointing to a difference in transcriptional processivity. To demonstrate conditional gene silencing in mice we are in the process to insert these constructs into hybrid ES cells derived from cell type-specific or inducible Cre transgenic mice. Since Cre/loxP is widely used as versatile system for gene targeting we expect it to provide also a useful tool for conditional gene silencing in the mouse.

LENTIVIRAL TRANSGENESIS IN LIFESTOCK

Barbara Kessler[1], **Andreas Hofmann**[2], **Alexander Pfeifer**[2], **Eckhard Wolf**[1]
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[2]*Department of Pharmacy, Institute for Pharmacology, Center for Drug Research, Ludwig-Maximilians University Munich*

Microinjection of DNA is now the most widespread method for generating transgenic animals, but transgenesis rates, achieved this way in higher mammals, are extremely low. To address this longstanding problem, we used lentiviral vectors carrying a ubiquitously active promoter (phosphorylglycerate kinase, LV-PGK) to deliver transgenes to porcine embryos. Of the 46 piglets born, 32 (70%) carried the transgene DNA and 30 (94%) of these pigs expressed the transgene (green fluorescent protein, GFP). Direct fluorescence imaging and immunhistochemistry showed that GFP was expressed in all tissues of LV-PGK transgenic pigs, including germ cells. Importantly, the transgene was transmitted through the germ line.
Tissue-specific transgene expression was achieved by infecting porcine embryos with lentiviral vectors containing the human keratin K14 promoter (LV-K14). LV-K14 transgenic animals expressed GFP specifically in basal keratinocytes of the skin.
In cattle, the efficacy of lentiviral gene transfer was depending on the time of virus injection during in vitro production of embryos. Best results were achieved when virus injection took place before oocyte fertilization. All 4 calves derived from LV-GFP injection into oocytes were transgenic and exhibited green fluorescence.

INDUCIBLE LENTIVIRAL VECTORS FOR TRANSGENESIS

David Markusic, Marian van Roon and Jurgen Seppen
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Lentiviral vectors are retroviral vectors based on HIV and can be used for the stable transduction of a wide variety of cells and tissues. One of the features that distinguish lentiviral vectors from murine oncoretroviral vectors is their ability to transduce non-dividing cells. This property makes these vectors useful in gene therapy and basic research.
We have previously shown that lentiviral vectors can be used for sustained expression of therapeutic levels of erythropoietin (Epo) in rats. However, for clinical application, Epo gene expression will have to be regulated. We therefore developed a novel doxycycline (dox) regulatable system that fits in a single lentiviral vector and has many advantages over previously published systems.
The reverse tetracyclin transactivator (rtTA) binds Tet operator sequences (TetO) in the presence of dox. Because rtTA contains a VP16 activation domain, a basal promoter containing TetO will be switched on after binding of rtTA. Constitutive expression of rtTA is detrimental because the protein is toxic, basal binding of rtTA to TetO will lead to background expression and an immune response to rtTA might occur. We developed a lentiviral vector in which the therapeutic gene and rtTA are linked by an IRES. In this bi-cistronic transcript both genes are under control of the same dox inducible promoter. This creates a "feed forward" loop, which has very low basal expression of the therapeutic gene and rtTA in the absence of dox. Upon dox administration, the basal amount of rtTA present will bind to the TetO and increase expression of both the therapeutic gene and rtTA, establishing the feed forward amplification. The disadvantage of this system is relative insensitivity to dox, at low dox concentrations the loop is not activated because the basal levels of rtTA are very low. We solved this problem by using a novel rtTA mutant (Das et al., JBC 2004), which is more sensitive. Using this rtTA, 50 nM dox already fully activates the system.
Experiments with lentiviral vectors have to be done under C1 conditions, this makes microinjection of these vectors in the perivitelline space of fertilized oocytes cumbersome, as the microinjector and microscope cannot be easily fitted into a flow cabinet. We therefore transduced denuded fertilized mouse oocytes with lentiviral vectors capable of constitutive and inducible GFP expression. Transductions were performed in a volume of 20-30 µl with 106 transducing lentiviral particles per oöcyte. After 3 days, all oocytes that had divided were expressing GFP and dox dependent GFP expression in blastocysts could be shown by fluorescence microscopy after 5 days. However, the denuding procedure makes the oocytes "sticky" and transplantation the day after transduction could be problematic because the cells might not travel trough the oviduct efficiently. In vitro development of oocytes to blastocysts and transplantation in the uterus could circumvent this problem. In preliminary experiments we established that transduction of fertilized oocytes does not change the frequency by which they develop to blastocysts. Generation of transgenic animals from transduced oocytes by our lab currently depends on permission from the department of agriculture.
Conclusions. We developed a novel lentiviral vector that might be useful for regulation of gene expression in gene therapy and transgenic animals. Lentiviral vectors efficiently transduce fertilized oocytes, but legal requirements can make lentiviral transgenesis difficult to implement.

SPEEDING UP CONDITIONAL GENE TARGETING: RNAI & ES MOUSE TECHNOLOGY

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ES-mice are completely derived from embryonic stem cells and can be obtained at high efficiency upon injection of hybrid (F1) ES cells into tetraploid blastocysts. This method allows the immediate generation of targeted mouse mutants from genetically modified ES cell clones, in contrast to the standard protocol that involves the production of chimaeras and several breeding steps. Extensive phenotypic characterization of ES mice via morphological, physiological and behavioral parameters revealed elevated body weight as the only major difference of ES mice that exhibit an otherwise normal phenotype. By using specifically designed parental ES cell lines for gene targeting, e.g reequipped with an inducible Cre recombinase, the ES mouse technology allows the production of conditional mouse mutants within the time frame of 8 to 9 months.

RNA interference through the expression of small hairpin RNA (shRNA) molecules has become the most promising tool in reverse mouse genetics as it may allow inexpensive and rapid gene function analysis in vivo. However, the prerequisites for appropriate shRNA expression are not well defined. We demonstrate that a single copy shRNA inserted into the rosa26 locus under the control of the U6 or H1 promoter mediates ubiquitous mediated gene knock down when integrated in the rosa26 locus.

IN VIVO SILENCING OF CCR2 BY LENTIVIRAL SIRNA TRANSFER TO BONE MARROW CELLS LEADS TO PERSISTENT LOSS OF CCR2 FUNCTION

Ilze Bot, Jian Guo, Miranda Van Eck, Peter J. Van Santbrink, Reeni B. Hildebrand, Pieter H.E. Groot[‡], Jurgen Seppen[#], Theo J.C. Van Berkel, Erik A.L. Biessen
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Progress in genomic research is considerably hampered by the laborious generation of knockout mice and subsequent backcrossing to an appropriate disease background for analysis of gene function. In this study we have explored the potential of a novel lentiviral gene silencing approach on bone marrow cells for the *in situ* generation of knock-downs of a given target gene.

As model gene we have chosen CC-Chemokine Receptor 2 (CCR2), which was previously shown to play a central role in the recruitment of monocytes to sites of inflammation and during atherogenesis. A small interfering RNA (siRNA) construct was developed against CCR2 (siCCR2), which potently and dose-dependently reduced CCR2 mRNA (>80%, $P<0.001$) and protein levels (>66%, $P<0.001$) in HEK293 cells overexpressing murine CCR2. Also, siCCR2 fully abolished CCR2 signal transduction in response to its ligand JE, as indicated by calcium influx measurements.

Subsequently, bone marrow cells were isolated from C57Bl/6 donor mice and transduced at high efficiency (85-90%) with lentivirus containing siCCR2 under control of the H1 promoter or with H1.Empty control virus (MOI=15). After 16 hours, the transduced bone marrow cells were injected into irradiated recipient mice, while, as positive control, one group received $CCR2^{-/-}$ bone marrow. Six weeks after transplantation, thioglycollate (3%) was injected intraperitoneally and macrophages were isolated from the peritoneal cavity 5 days later and analyzed for cell count and CCR2 expression. Interestingly, the number of macrophages, migrated into the peritoneal cavity, was 70% reduced from $27\pm11*10^6$ in control mice to $8\pm8*10^6$ cells ($P=0.03$) in mice that had received siCCR2 lentivirus transduced bone marrow, which is essentially similar to that in $CCR2^{-/-}$ transplanted mice ($8\pm1*10^6$ cells). Moreover, CCR2 mRNA levels in peritoneal macrophages were 73% lower in siCCR2 treated than in control mice ($P=0.002$).

In conclusion, transplantation of siRNA lentivirus transduced bone marrow cells proofs to be a very rapid and effective method for the *in situ* generation of haematopoietic knock-downs and thus for investigating the role of key genes in inflammatory disorders, as illustrated in our studies using CCR2 as model gene.

SHORT PRESENTATION

Werner Müller

SPATIO-TEMPORALLY-CONTROLLED TARGETED SOMATIC MUTAGENESIS DURING MOUSE DEVELOPMENT AND ADULTHOOD

Daniel Metzger
Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Université Louis Pasteur, BP10142, 1 rue Laurent Fries, 67404 Illkirch-Cedex, France, and Institut Clinique de la Souris (ICS), BP 10142, 1 rue Laurent Fries, 67404 Illkirch-Cedex, France

Methods based mainly on the properties of the bacteriophage P1 site-specific Cre recombinase have been developed over the last years to achieve conditional gene targeting in the mouse. As the Cre recombinase can efficiently excise a DNA segment flanked by two loxP sites (floxed DNA) in animal cells, spatially or temporally controlled somatic mutations can be obtained in mice by placing the Cre gene under the control of either a cell-specific or an inducible promoter, respectively. However, these conditional gene targeting systems have intrinsic limitations, as they are either only spatially or temporally controlled. We thus developed strategies and techniques to create spatio-temporally-controlled somatic mutations of target genes, based on cell-specific expression of the chimeric Cre-ERT2 recombinase, which results from the fusion of the Cre recombinase with a mutated ligand binding domain of the human estrogen receptor a (ERa), and whose activity is induced by anti-estrogens such as Tamoxifen (Tam) (1). Transgenic mice cell-specifically expressing Cre-ERT2 has allowed us, upon Tam treatment, to efficiently induce Cre-mediated recombination at various loci flanked by LoxP sites (2 – 10). Examples of spatio-temporally-controlled site-specific somatic mutations generated through this approach will be presented to illustrate successful application and some of its limitation during mouse development and throughout its post-natal life.

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A GENE TRAP STRATEGY FOR THE LARGE SCALE INDUCTION OF CONDITIONAL MOUSE MUTATIONS

Frank Schnütgen, Petra Van Sloun, Joachim Altschmied, Patricia Ruiz, Ralf Kühn, Thomas Floss, Wolfgang Wurst, and Harald von Melchner

As human disorders are almost always the result of a late onset gene dysfunction, conditional mutagenesis in the mouse is essential for the creation of relevant disease models. For most available mouse mutant strains the significance for human disease is uncertain since mutations involve the germ line and can thus reveal only the earliest, non-redundant role of a gene. While conditional mutagenesis has been performed on a single gene basis and clearly shown to be more informative than conventional germ-line mutations, there is no technology available for the large-scale production of conditional mouse mutant strains. Since one major aim of the post-genomic era is to understand the function of every single gene in the context of an adult organism, we have developed a conditional gene trap approach suitable for large-scale mutagenesis in mouse embryonic stem (ES) cells. The approach is based on the combined use of two site-specific recombination systems such as FLPe/frt and Cre/loxP in one retroviral gene trap vector named FlipROSAβGeo. To investigate whether this gene trap would effectively disrupt genes expressed in ES cells, we isolated 8064 FlipROSAβGeo infected clones by selecting in G418 and recovered their GTSTs by 5' RACE and inverse PCR. Up to now we have obtained 3631 high quality sequences, which will be made publicly available (http://www.genetrap.de/).

DEVELOPMENT OF DICRE, A RAPAMYCIN-REGULATABLE FRAGMENT COMPLEMENTATION SYSTEM FOR CRE

Jean-Paul Herman and Nicolas Jullien

Cre recombinase is extensively used to engineer the genome of experimental animals. However, its usefulness is still limited by the lack of an efficient temporal control over its activity. To overcome this, we have developed DiCre, a regulatable fragment complementation system for Cre. The enzyme was split into two moieties that were fused to FKBP12 (FK506-binding protein) and FRB (binding domain of the FKBP12-rapamycin associated protein), respectively. These latters can be efficiently hetero-dimerized by rapamycin. Several variants based on splitting Cre at different sites and using different linker peptides, were tested in an indicator cell line. The fusion proteins, taken separately, had no recombinase activity. Stable transformants, co-expressing complementing fragments based on splitting Cre between Asn(59) and Asn(60), displayed low background activity affecting 0.05-0.4 % of the cells. Rapamycin induced a rapid recombination, reaching 100% by 48-72 h, with an EC_{50} of 0.02 nM. Thus, ligand-induced dimerization can efficiently regulate Cre, and should be useful to achieve a tight temporal control of its activity, such as in the case of the creation of conditional knockout animals and we have begun investigating the applicability of DiCre for this purpose.

MELANOMA INDUCTION VIA MELANOCYTE-SPECIFIC INK4A/ARF INACTIVATION AND HRAS ACTIVATION BY A SELF-INACTIVATING CREERΔD TRANSGENE

Ivo Huijbers

Immunotherapy represents an attractive approach for the treatment of cancer, in particular melanoma. Preclinical studies of various strategies of immunotherapy rely on model systems where tumor cells grown *in vitro* are inoculated into syngeneic mice. However, this does not recapitulate the long-term host-tumor relationship that occurs in patients during tumor development. In order to have a model system more relevant to the human situation, we are trying to develop a mouse strain in which we can induce melanomas expressing a tumor antigen of interest.
One of the most common sites of genetic lesions in human melanoma is the *INK4A/ARF* locus, which encodes two distinct tumor suppressor proteins $p16^{INK4A}$ and $p14^{ARF}$. Genetic disruption of this locus predisposes mice to the formation of various tumor types, but is not sufficient to induce melanoma unless the Ras-pathway is specifically activated in melanocytes. In order to have a fully controlled model system for melanoma, we generated transgenic mice in which the deletion of the *Ink4a/Arf* genes and the melanocyte-specific expression of both activated Harvey-Ras^{G12V} and a well-characterized antigen is spatially and temporally regulated by either $CreER^{\Delta D}$ or CreER-T2.
Several transgenic lines and one Rosa-26-knock-in line were generated, harboring a construct of respectively the tyrosinase promoter, a CreER fusion gene flanked by loxP sites, a V12 mutated H-Ras gene, an *IRES* and the P815A-antigen encoding gene, P1A. The expression and regulation of the CreER fusion gene was analyzed by crossing these mice to a Rosa-26 Cre reporter strain. In three transgenic lines specific blue staining could be observed in melanocytes after topical treatment of the ear by 4-hydroxytamoxifen. In order to determine whether the transgene was still intact and functional after Cre-recombination, these transgenic lines were crossed with a CMV-Cre-deleter strain. In two lines H-ras and P1A were still detected after Cre-recombination implying that the transgene was arranged in such a way that the tyrosinase-promoter was now driving the expression of genes H-ras and P1A. These two lines have been crossed to a conditional *Ink4a/Arf* background and in one line melanomas could be induced with a 25% incidence after subcutaneous injections with 4-hydroxytamoxifen.

USE OF A SELF-DELETING CREERT2 CASSETTE IN CONDITIONAL TRANSGENIC MOUSE MODELS TO EVALUATE KEY SIGNALING MEDIATORS OF SKELETAL MUSCLE HYPERTROPHY AND ATROPHY

Ka-Man Lai, Michael Gonzalez, William Poueymirou, Elizabeth Zlotchenko, Erqian Na, William Kline, Frank Panaro, Roy Bauerlein, Lawrence Miloscio, Robin Cuevas, Trevor Stitt, Aris Economides, George D. Yancopoulos, David J. Glass
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Skeletal muscle atrophy is a severe morbidity caused by a variety of conditions, including cachexia, cancer, AIDS, prolonged bedrest, and diabetes. One strategy in the treatment of atrophy is to induce the pathways normally leading to skeletal muscle hypertrophy. In particular, we are interested in the Akt/mTOR pathway, which has been implicated as an important mediator of hypertrophy.

To facilitate the in vivo functional analysis of Akt involved in muscle atrophy and hypertrophy, we have developed a system to simplify the production of transgenic mice that allows the conditional activation of a transgene in one single targeting step without crossbreeding. In particular, we utilize a Tamoxifen-inducible fusion of Cre recombinase with a mutated estrogen receptor ligand-binding domain (CreERt2). The targeting vector is designed to incorporate a cassette that contains a floxed CreERt2-stop sequence upstream of an EGFP-tagged constitutively activated Akt (caAkt-EGFP) from a muscle-specific promoter, such that expression of caAkt-EGFP only occurs in the presence of tamoxifen. We show that caAkt-EGFP expression is undetectable in non-induced transgenic mice. Administration of tamoxifen mediates the self-deletion of CreERt2-stop cassette, which results in the production of caAkt-EGFP specifically in adult muscles. We further demonstrate that acute activation of caAkt-EGFP is sufficient to induce rapid and significant skeletal muscle hypertrophy *in vivo,* accompanied by activation of the downstream Akt/p70S6 kinase protein synthesis pathway. These findings suggest that pharmacologic approaches directed towards activating Akt will be useful in inducing skeletal muscle hypertrophy. Our data also demonstrates for the first time that a targeting strategy of an inducible self-deletion of CreERt2 could be used to generate conditional transgenic mouse models suitable for the validation of genes for pharmaceutical drug development.

GENERATION OF KIDNEY-EPITHELIAL CELL-SPECIFIC CRE MICE

Irma S. Lantinga-van Leeuwen[1], Wouter N. Leonhard[1], Annemieke van de Wal[2], Ramon Roozendaal[1], Sjef Verbeek[1], Martijn H. Breuning[1], Emile de Heer[2] & Dorien J.M. Peters[1]
[1]Department of Human Genetics, and [2]Department.of Pathology, Leiden University Medical Center, Leiden, The Netherlands

We are developing Cre mice that facilitate inducible gene targeting exclusively in kidney epithelial cells. We are using two inducible systems: 1. the CreER^{T2} -system, in which Cre-recombinase becomes activated upon tamoxifen treatment, and 2. the Tet-On system (M2 variant), in which Cre-recombinase becomes expressed upon doxycycline administration. We placed Cre-recombinase under the control of a 1.3 kb promoter fragment of the kidney-specific (Ksp)-cadherin gene, which has been reported by others to drive gene expression restricted to renal tubular epithelial cells, with the highest expression in collecting ducts and loops of Henle (JASN 13 (2002): 1837-1847). We confirmed the inducibility and promoter specificity *in vitro* using a luciferase reporter construct. For each system we have generated 7 transgenic lines. We are now evaluating Cre expression in kidneys and other tissues by RT-PCR analysis and by crossbreeding to the Z/EG reporter mouse line.

INDUCIBLE RENAL-SPECIFIC CRE RECOMBINASE ACTIVITY IN GGT-CREER^{T2} MICE

Bernd Dworniczak, Petra Pennekamp

Conventional gene knockout strategies by homologous recombination in embryonic stem (ES) cells have resulted in a wealth of developmental, physiological, and pathophysiological information. Despite this, whole animal gene disruption often results in embryonic lethality. But conditional knockout technology combined with inducible transgenes provides a valuable improvement for further analysis of gene function in vivo.
That far only few kidney specific Cre recombinase expressing mouse lines have been described, mainly because of lack of appropriated renal cell specific promoters. Here we report the generation and characterization of a mouse line expressing the improved variant of the tamoxifen-inducible Cre recombinase, CreER^{T2} specifically in the kidney. Renal specific expression of the Cre protein was obtained by fusion of the CreER^{T2} hybrid gene - the recombinase is fused to a mutated ligand-binding domain of the human estrogen receptor - with a truncated form of the mouse gamma-glutamyl transpeptidase type II (GGT) promoter. Using the GGT-CreER^{T2} transgene, we generated mice that show specific inducible Cre function, as analyzed by cross-breeding experiments into the Rosa26 Cre-LacZ reporter line, in the S3 segment of adult nephrons in the kidney. Of specific importance is the fact that the transgene is active from very early on (16-17dpc) up to at least 6 month of age of the animals. This broad time window of activity will give us the opportunity to delete floxed target genes specifically in renal cells either already within the pregnant mothers or in adult mice. We will use these mice to investigate cyst formation in the kidneys and to work on the improvement of therapeutic strategies for polycystic kidney disease.

FATE MAPPING OF HEMATOPOIETIC STEM CELLS (HSCS) DURING MOUSE DEVELOPMENT

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Despite many years of research on the origin of blood cells, the precise embryonic site from which the adult blood system is derived remains unclear. One hypothesis suggests that adult hematopoietic system or its precursors are generated in yolk sac, and then colonizing different hematopoietic compartments such as the fetal liver and adult bone marrow. According to the second hypothesis, which is currently favored, embryonic and adult hematopoietic systems originate independently. Adult blood precursors and hematopoietic stem cells (HSCs) are generated in a part of the embryo called the aorta-gonad-mesonephros (AGM) region, while the embryonic blood arises in the yolk sac. This model suggests that AGM generates the HSCs that are later found in the fetal liver and adult bone marrow.

To study the origin of the adult hematopoietic stem cells, we are using classical and more advanced recombination Cre/loxP fate mapping methods. For the classical fate mapping experiments we use the vital dyes (DiI) or a fluorescent marker (GFP) to mark specific groups of cells and follow them through embryo / adult development.

Using the Cre / loxP recombination system first we generated and characterized a transgenic mouse line with high expression of Cre recombinase, fused to the mutated ligand – binding domain of estrogen receptor (CreLBD), under the control of the Sca-1 promoter. Sca-1 is one of the surface markers using in HSC enrichment.

To perform fate-mapping experiments we have crossed this transgenic Cre line with different lox reporter lines: Rosa 26 lox lacZ and Z/EG line, which upon Cre-mediated recombination express ß-galactosidase and eGFP respectively. To induce recombination we injected tamoxifen directly into pregnant females. Results of these ongoing experiments will be presented.

TIME- AND TISSUE-RESTRICTED LINEAGE ABLATION USING A CONDITIONAL DIPHTHERIA TOXIN RECEPTOR TRANSGENIC MOUSE STRAIN

Thomas Wunderlich and Thorsten Buch

Lineage ablation is a powerful approach for the analysis of cell function. A new ablation system in the mouse uses transgenic expression of diphtheria toxin receptor (DTR) and injection of Diphtheria toxin (DT), requiring, however, the cumbersome generation of DTR transgenic mouse strains. To make this approach more generally applicable, we generated a mouse strain (iDTR) in which expression of the DTR gene is controlled by the ubiquitously expressed ROSA26 promoter and Cre mediated excision of a STOP cassette, rendering a cre expressing cell type sensitive to DT. We tested the iDTR system by breeding to the CD4-cre and CD19-cre strains and injection of DT. Efficient ablation of T and B cells, respectively, was observed in double transgenic mice confirming that the iDTR strain facilitates the rapid analysis of cell function by DT mediated ablation.

MULTI-PURPOSE ALLELES; CONSTRUCTION BY RECOMBINEERING AND USE FOR CONDITIONAL ANALYSES

Francis Stewart

The alteration of an allele from one state to others by FLP and Cre recombinases permit the design of multi-purpose alleles. Recombineering simplifies the construction of these alleles. A variety of uses are possible in mice and ES cells.

IN VIVO IMAGING OF DIGESTIVE TUMORS USING A NOVEL INTESTINAL PATHOGEN-BASED VECTORIZATION APPROACH

Klaus-Peter Janssen[1,2], Thomas Falguières[1], Danijela Vignjevic[1], Guilhem Bousquet[1], Luc Fetler[3], Mohamed Amessou[1], Christophe Rosty[4], François Amblard[3], Daniel Louvard[1], Sylvie Robine[1], and Ludger Johannes[1]

with the contribution of Mauna Kea Technologies for Cell-viZio imaging www.maunakeatech.com

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Efficient methods for tumor targeting are eagerly awaited and must satisfy several challenges: molecular specificity, transport through physiological barriers, and capacity to withstand extra- or intracellular degradation and inactivation by the immune system. The method proposed here exploits the non-toxic B-subunit of Shiga toxin, produced by enteropathogenic bacteria, that has naturally evolved to fulfill these requirements. We show that the cellular B-subunit receptor, the glycosphingolipid Gb3, is specifically expressed in human and murine digestive tumors. After force-feeding mice, the B-subunit accumulates in, and remains associated with these tumors, due to its intracellular trafficking via the retrograde route. This approach can serve to vectorize contrast agents for in vivo imaging, as demonstrated here using confocal fiber optics endoscopy (Cell-viZio) and two-photon imaging. In conclusion, we have laid the groundwork for a novel vectorization technology that, in addition to non-invasive tumor imaging, could also be used for targeted tumor therapies.

STRATEGIES FOR EFFICIENT AND PREDICTABLE GENERATION OF TET-CONTROLLED ANIMAL MODELS

Kai Schönig, David Kentner, Tina Baldinger, Katrin Welzel, Andreas Vente, Dusan Bartsch and Hermann Bujard

The establishment of functional random transgenic animals is often limited by problems which can be attributed to the influence of the integration site on the transgenic DNA construct. These problems can be sometimes circumvented by using large DNA fragments for transgenesis which carry all major important elements required for expression. Tetracycline (Tet)-controlled transcription units most commonly used in conditional expression approaches in mice are strongly influenced by the genomic surrounding, which can result in non-inducible or constitutive active transgenic lines. We developed a strategy to minimize integration effects on conditional expression constructs based on a 70kb genomic fragment. This fragment was derived from the LC-1 mouse line, which previously was shown to have outstanding regulation properties. Cloning and identification of the integration site of the Tet-regulated transcriptional unit of the LC-1 mouse line has led to the construction of a bacterial artificial chromosome (BAC) vector, which allowed efficient generation of position independent Tet-controlled transgenic mouse lines. These results suggest that targeting of the identified genomic locus by homologous recombination in ES cells could be an alternative for the generation of predictable and comparable mouse models. In addition, it opens the possibility to use this construct in other animals.

The rat has remained the system of choice in many areas of biomedical research, but an experimental strategy, which permits the efficient generation of conditional mutants with these animals has still been lacking. Considering the high sequence homologies between rat and mouse, we assumed that the BAC technique will be applicable in the rat. With this technology we are currently developing transgenic rat models for conditional brain specific overexpression of reporter genes and L-type calcium channels, which have been shown to be involved in age-related working memory impairment.

GENERATION OF ES CELLS SHOWING POSITION-INDEPENDENT DOXYCYCLIN-REGULATED GENE EXPRESSION

Jaap Jansen[1], Cor Breukel[2], Kai Schönig[3], David Kentner[3], Hermann Bujard[3], Sjef Verbeek[2] and Niels de Wind[1]
(1) Department of Toxicogenetics and (2) Department of Human Genetics, Leiden University Medical Center, and (3) Zentrum fuer Molekulare Biologie (ZMBH) Universitaet Heidelberg.

Tight control of the transcriptional activity of target genes using the Tet-off system depends strongly on (i) the high and stable expression of the tTA transactivator-repressor and (ii) the genomic locus where the Tet-responsive target gene is integrated. Here we describe a novel approach that fulfils these criteria in mouse Embryonic Stem (ES) cells. Recently, a genomic locus, LC-1, was identified that appears to be an excellent site for stringent control of transcription of marker genes via the Tet regulatory system. tTA-2-expressing ES cells were obtained by gene-targeting the *ROSA26* locus in *Hprt*-defective ES cells using a *ROSA26* targeting vector comprising a promoterless zeocine selection marker and the tTA-2 gene under control of a CAG promoter (a fusion of the CMV-enhancer and the chicken β-actin promoter). ES cells with a targeted integration of the tTA-2 gene at the *ROSA26* locus were subsequently transfected with a construct that included an *Hprt* minigene preceded by the Tet-responsive promoter, embedded within LC-1 sequences. Hprt-expressing cell clones were obtained using HAT selection, showing that the *Hprt* minigene is functional when flanked with LC-1 sequences. Eighty-four independent HAT-resistant clones were cultured for 7 days in absence or presence of the tetracycline analog doxycyclin, to allow repression of *Hprt* transcription. To test whether *Hprt* transcription was switched off by doxycyclin, each cell population was cultured in medium containing 6-thioguanine (for selecting *Hprt*-deficiency) or HAT (selecting *Hprt* proficiency). All cell clones became resistant to 6-thioguanine and sensitive for HAT after culture in the presence of doxycyclin, strongly suggesting a tight and locus-independent tTA-2-dependent control of *Hprt* transcription. As expected, cells cultured in the absence of doxycyclin remained resistant to HAT and sensitive to 6-thioguanine.
In conclusion, this system shows great promise for the reproducible and locus-independent expression of Tet-responsive elements in ES cells and in the mouse.

TOWARDS AN OPTIMAL DOCKING SITE IN THE MOUSE GENOME

Cor Breukel, Kai Schonig, Hermann Bujard, Michelle Calos and Sjef Verbeek

We are developing a procedure for the generation of transgenic mice that facilitates: (a) efficient highly reproducible single copy integration (b) within a ("inert") site of the mouse genome in which the expression of the transgene is not influenced by flanking endogenous regulatory sequences.
Our approach is based on two elements: a. phage integrase PhiC31, an unidirectional recombinase (two sites aatB and aatP recombine to form a aatL or aatR site, which are not functional in recombination any more, (J.Mol.Biol. 335, 667(2004). b. The mouse LC-1 locus (recently published to be "inert", Nucl.Acid Res. 30, e134(2002) for the following two reasons:
a. Recombinases as Cre and Flp provide suitable means to "shuttle in" sequences, but a drawback of these enzymes is their bi-directionallity (with the equilibrium very much towards excision, not integration). Moreover the presence of LoxP sites within the transgenic locus restricts the application in Cre expressing conditional KO models. b. Although highly efficient in targeting procedures by homologous recombination in ES cells the frequently used ROSA26 locus does not provide the ideal "inert" docking site in the mouse genome.
Recently Belteki at all. (Nature Biotech. 21, 321 (2003) published an elegant PhiC31based strategy which we modified. Belteki inserted the aatP site randomly in the genome, whereas we choose the LC1 locus. Basically the docking site in the genome consists of the following elements: a Frt site, a PGK promotor, an aatP site, the PhiC31 gene plus NLS, an IRES Puro, a PGK TK gene and finally the second aatP site. This cassette is flanked by 7 and 3 Kb LC1 homologous arms generated by long range PCR on E14 ES cell. This construct will be targeted to the LC1 locus in ES cells. Once the docking site is correctly integrated, sequences can be shuttled in via a shuttle vector that contains an aatB site, a Neo gene without promoter, a Frt site, a MCS and the second aatB site. Upon transfection of the shuttle vector into the ES cell line with the PhiC31 integrase expressing LC1 docking site recombination will take place resulting in insertion of Neo downstream of the PGK promotor while PhiC31-IRES-Puro-PgkTK will get lost (selected with GANC). The end product will be the integration of a FRT flanked pgkNeo and a single copy of the gene of interest into the LC1 locus. Subsequently the pgkNeo can be removed with Flp.
The described PhC31 cassette was functionally tested in human cells and proved to be expressing PhiC31 integrase. The PhiC31cassette was co-transfected with a marker plasmid (containing the LacZ gene, flanked by an aatB and an aatP site in the same direction). If PhiC31 is expressed, the LacZ gene will be excised, and the isolated plasmids will become white after transformation. In our first pilot we obtained 15% white colonies. Targeting of the PhC31 cassette to the LC1 locus in E14 ES cells is currently being performed.

FUNCTIONAL ANALYSIS OF AN rtTA-GFP OPERON IN THE ROSA26 LOCUS

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The tTA/rtTA system has been proven as a powerful tool for reversible controlled gene expression in rodents and other species. We investigated the functionality of an rtTA-GFP operon after its insertion into the ubiquitously expressed ROSA26 gene locus. Doxycycline activated rtTA, expressed from the endogenous ROSA26 promoter, controls GFP transcription that could be detected by immunoblots in all peripheral tissues tested of mice heterozygous for the rtTA-GFP operon. However, GFP expression levels varied strongly among tissues analysed, with highest expression in thymus and liver. Mice homozygous for the rtTA-GFP operon show a similar expression pattern with exception of the skeleton muscle where we observed a non-proportional increase of GFP expression. Similar, in a few distinct neuronal populations of the amygdala, striatum and hypothalamus GFP expression was detected immunhistochemically, whereas heterozygous mice lack GFP expression in the brain. Currently, we are investigating the silencing mechanism of the ROSA26 controlled rtTA-GFP operon in the brain of adult mice.

GENERATION OF ANTIGEN PRESENTING CELL-RESTRICTED tTA AND rtTA TRANSGENIC MOUSE LINES

Hisse Martien van Santen

We generated two transgenic mouse lines in which an agonistic T cell epitope is expressed under control of the tetracycline (tet)-regulated gene expression system. In one line, expression of the tTA driver (Tet-off) is under control of a murine MHC class II promoter (Witherden et al., 2000). The driver in this line is predominantly expressed in radio-resistant cells of the thymus (Witherden et al., 2000; Van Santen et al., submitted). RNA expression of the reporter in the thymus can be regulated over four decades by treating animals with graded amounts of tet in the drinking water. High doses of Tet completely shut off expression, as shown by completely normal differentiation of agonist-specific TCR-transgenic T cells in mice treated with such doses of Tet.

The other line carries a reverse transactivator (SRTA, Tet-on) under control of the murine invariant chain promoter (Obst, Van Santen, Benoist and Mathis, ms in preparation). In this line, expression of the reporter gene can be detected in thymus as well as peripheral lymphoid organs. Interestingly, expression of the reporter in the periphery is predominant in CD11c+ dendritic cells (DCs), with little expression in B cells and macrophages.

These lines have been used to study the role of the dose and time of availability of a defined peptide antigen in differentiation and activation of T cells. The lack of leakiness, the ability to titrate the amount of expression and to switch expression on and off with little manipulation allow us to study these quantitative aspects of the immune response in a physiological setting.

TET-DEPENDENT CONDITIONAL IMMORTALIZATION AS A NEW TOOL FOR MOLECULAR ANALYSIS OF MOUSE MUTANTS

Tobias May, Hansjörg Hauser and Dagmar Wirth

Primary cells derived from mouse mutants or knockouts represent an important tool for a detailed molecular characterization of the respective phenotype. Protocols to reproducibly immortalize cells of different tissues are of great importance. In particular, a conditional, reversible immortalization would avoid any interference of the immortalizing gene with cellular signaling pathways.
We developed a vector system employing the tet-on system that allows the conditional immortalization of primary cells in a single transduction step. The vector is auto-regulatory and the expression of the transactivator as well as the expression of the immortalizing gene e.g. SV40 large T antigen depends on doxycycline. As a proof of principle we have immortalized mouse embryo fibroblasts with this vector and characterized the resulting clones. These clones showed highly regulated expression of the oncogene. The ability of this vector to control proliferation was monitored. Cell growth and clonogenicity were also strictly dependent on doxycycline. Furthermore the vector enabled us to alter the cell cycle profile of the immortalized cells. In the repressed state cells arrested in G0/G1 while in the induced state a profile typically seen for proliferating cells was observed. Furthermore, expression profiling studies correlated the phenotypic analysis. Finally, soft agar colony formation was analyzed and revealed that the immortalized cells were not tumorigenic.
Taken together these results clearly demonstrate that the expression of the immortalizing protein can be reverted to a level that is not sufficient to induce proliferation. Therefore this vector is the ideal tool to elucidate the molecular phenotype of a given knockout.

KNOCK-IN MOUSE MODELS FOR HUMAN XPD DISORDERS, RELEVANCE OF GENE EXPRESSION

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Alterations in the XPD helicase subunit of the basal transcription/DNA repair factor TFIIH trigger multiple photosensitive disorders, with symptoms ranging from cancer predisposition (Xeroderma Pigmentosum, XP) to segmental progeria (XP combined with Cockayne Syndrome, XPCS; trichothiodystrophy, TTD). Origin of enormous clinical variation within and between each disorder has largely remained unknown.
We have used cDNA fusion and/or inclusion of artificial Poly(A) signal strategy in mimicking XPD point-mutations in the mouse Xpd locus. Different targeting constructs with minimal difference in sequence, resulted in mouse Xpd alleles with variable, up to 25 fold difference in Xpd mRNA level; with homozygous phenotypes ranging from pre-blastocyst stage embryonic lethality to truthful phenocopy of the given human disease. Application of LoxP-Cre based system yielding in Xpd alleles with native Xpd Poly(A) signal resulted in normal Xpd mRNA expression. Although we were able to elucidate several aspects of XPD related human disease from our mouse-models, such as define XPD as a cancer-progeria locus and reveal interallelic complementation between two disease causing alleles, our experience suggests strong caution in using cDNA fusion-artificial poly(A) targeting strategy, particularly for genes which mRNA-protein levels may be crucial in normal and pathological conditions.

Additional abstracts

Andrej Alendar

Retroviral insertional mutagenesis is a powerful approach to identify genes that confer a selective advantage to cells in vitro and in vivo in the multistep process of tumorigenesis. Induced tumorigenesis in cells of hematopoietic system has allowed for the identification of genes that collaborate in lymphoid transformation- Myc, Bmi1 and Pim. Pim-1 is one of the most commonly mutatated gene in mouse lymphomas induced by MuLV. Three members of Pim oncogene family have been identified - Pim-1, Pim-2 and Pim-3, respectively. The Pim-1 proto-oncogene encodes a serine/threonine protein kinase and proviral insertion near Pim-1 results in the overexpression of the wild type PIM1 protein. In search for physiological functions of PIM proteins, we have generated Pim deficient mice (Pim-1, Pim-2 and Pim-3). Using MuLV proviral complementation tagging in Pim-1;Pim-2 double knockout mice carrying Em-Myc transgene, has enabled us to identify genes that could complement for PIM-1 function. Thus far, Pim-3, Kit, CyclinD2 and Tpl2 have been identified by their specific expression in Pim-deficient tumors. None of these genes were activated by insertional mutagenesis in Pim-1;Pim-2 proficient background. In addition, ten novel genes have been identified with putative PIM complementing function. To further elucidate thier function in vivo and their role in lymphomagenesis we will develop conditional knock-out and transgenic mouse models.

NEW CONDITIONAL GENE TRAP VECTORS BASED ON CASSETTE EXCISION/INVERSION

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We have constructed a new set of gene trap vectors for large-scale conditional mutagenesis in mice. The functions of both the plasmid and retroviral vector are based on site-specific recombination events catalyzed by the enzymes FLP and CRE. Upon integration of the vectors into the host genome a neomycin-phosphotransferase gene fused to an adenoviral splice acceptor traps cellular transcripts such that ES cells with gene trap integrations can be selected in G418. This selector cassette, which inactivates one allele of the trapped gene, can be excised with the recombinase FLP leaving a splice acceptor-GFP fusion in a non-mutagenic orientation behind. Mice obtained from these ES cells should show no phenotype as the function of the mutagenized allele is restored and these animals can be bred to homozygosity with regard to the gene trap insertion. Reactivation of the gene trap by CRE-mediated inversion of the splice acceptor-GFP mutator cassette will then lead to inactivation of the trapped gene in the tissues where CRE has been expressed allowing for the creation of spatially and temporally restricted knock-outs. We are currently testing the vectors in a pilot experiment in mouse ES-cells.

Carlos G. Arques

The ability to induce a genetic alteration in a random cell and its descendants during organism development has held a central role in the progress of developmental biology for decades. In practice, however, this approach has been restricted mainly to a few of the currently used model systems. The development of inducible recombinases and 3D imaging now appears to enable the use of this fruitful approach in the mouse embryo. We are studying the extent to which this is possible employing current biological and bioinformatic tools, to be applied to the study of vertebrate limb patterning.

CONDITIONAL FcγR KO MOUSE MODELS

Peter Boross, Cor Breukel, Ramon Roozendaal, Jill Claassens, Jos van der Kaa en Sjef Verbeek

Fcγ receptors are receptors for the Fc (constant) part of IgG immunoglobulins and link the humoral and cellular branches of the immune system. Three types of FcγR exists in mice: FcγRI, II and III. FcγRI and III mediate activatory signals whereas FcγRIIb is an inhibitory receptor. FcγRIIb is expressed on various immune cell types and hence plays a crucial regulatory role throughout the entire immune response. Targeted disruption of the FcγRIIb gene results in various enhanced immune responses resulting in higher susceptibility to autoimmunity in a strain dependent fashion.
To study the contribution to the immune regulation of FcγRIIb expressed on the different cell types we are generating a conditional knockout mouse model. We use the Cre/LoxP system in order to achieve temporal and spatial control of the gene function. Conventional FcgRIIb knock out mice spontaneously develop autoimmune glomerulonephritis on C57Bl6 background. For that reason we generated the targeting construct from C57Bl6 BAC library and we performed the targeting to Bruce4 (C57Bl6-derived) ES cells.
We are also generating mice expressing Cre recombinase in a tissue specific and inducible manner to study FcγRIIb function on the diverse cell types. We use the CreERT2 construct driven by the CD19 promoter to provide B lymphocyte specific expression. To abolish the modifying effect of the integration site on the expression characteristics of the system we placed our transgenic construct into the E11 BAC and used the linearized BAC to generate transgenic mice. This BAC contains the LC1 locus previously reported to be inert in mice.

Peter Bouwman

Targeted deletion of several components of the TGFβ signaling cascade (e.g. TGFβ receptors I and II) in the mouse results in midgestational lethality due to abnormal yolk sac blood vessel formation.
To enable the identification of primary TGFβ target cells during development we are generating TGFβ reporter mice using a TGFβ/Activin specific promoter coupled to a β-galactosidase reporter gene. At the moment we are testing several independent transgenic lines for reporter activity.
In addition, we and others are further investigating the role of TGFβ signaling during blood vessel formation using tissue specific mouse mutants. For the analysis of the function of TGFβ signaling in vascular smooth muscle cells we have crossed the floxed TGF□RII strain (Leveen *et al.*, Blood 2002) with the Sm22αCreERT2(ki) strain (Kuhbandner *et al.*, Genesis 2000). Induction of recombination *in utero* was verified using the Gtrosa26^{tm1Sor} Cre reporter (Soriano, Nature Genetics 1999), but we did not any abnormalities in Sm22αCreERT2(ki)$^{+/-}$ TGFβRII$^{floxed/floxed}$ embryos. Currently, we are planning experiments in adult smooth muscle TGFβRII mutant mice.

Rita Carvalho

Hereditary Hemmorrhagic Telangietasia (HHT) is an autosomal dominant disorder in humans characterized by multisystemic vascular dyplasia and recurrent hemorrhage. Germline mutations in one of two different genes, endoglin or ALK-1 can cause HHT. Both are members of the transforming growth factor (TGF)-β receptor family of proteins, and are expressed primarily on the surface of endothelial cells. Mice lacking endoglin or activin receptor like kinase (ALK)-1 die at mid-gestation as a result of defects in the yolk sac vasculature. Here we analyzed TGFβ signaling in yolk sacs from endoglin knockout mice and from mice with endothelial-specific deletion of the TGFβ type II receptor (TβRII) or ALK-5. We showed that TGFβ/ALK5 signaling from endothelial cells to adjacent mesothelial cells is defective in these mice, as evidenced by reduced phosphorylation of Smad2. This results in the failure of vascular smooth muscles to differentiate and associate with blood vessels so that they remain fragile and become dilated. These effects can be rescued by culture of the yolk sac with exogenous TGFβ1. The data show that disruption of TGFβ signaling in vascular endothelial cells results in reduced availability of TGFβ for recruitment and differentiation of smooth muscle cells and provide a possible explanation for weak vessel walls associated with HHT.

SOMATIC INACTIVATION OF E-CADHERIN AND P53 LEADS TO METASTATIC BREAST CANCER

Patrick Derksen[1], Xiaoling Liu[1-2], Bastiaan Evers[1], Hanneke van der Gulden[1-2], Hans Peterse[3], Jacqueline Vink[2], Paul Krimpenfort[2], Anton Berns[2] and Jos Jonkers[1-2]
Divisions of Molecular Biology[1], Molecular Genetics[2] and Pathology[3], Netherlands Cancer Institute, Amsterdam, The Netherlands

E-cadherin is a calcium-dependent membrane-bound receptor, mediating homophilic interactions and controlling the formation of catenin-containing complexes, which link E-cadherin to the actin cytoskeleton. Progression to a malignant tumour requires the loss of adhesion to neighbouring cells, and the subsequent invasion of the surrounding tissues. In breast cancer, loss of E-cadherin coincides with an epithelial to mesenchymal transition, tumour invasiveness, metastasis, and poor prognosis. To address the question whether loss of E-cadherin is causal to breast cancer progression and metastasis, we generated conditional knock out mice in which E-cadherin and/or p53 are inactivated in stratified epithelial tissues, including skin and mammary gland. We show that animals carrying conditional *Trp53* alleles develop mammary and skin tumours with a median latency of 334 days. Whereas inactivation of E-cadherin alone did not result in tumour formation, combined inactivation of E-cadherin and p53 led to a dramatic decrease in the onset of mammary and skin tumours, resulting in a latency of 194 days. This suggests that E-cadherin acts as a tumour suppressor in the absence of p53 and that concurrent inactivation of E-cadherin and p53 efficiently synergize in tumorigenesis. Furthermore, while conditional loss of p53 in the mammary gland induced well-circumscribed, non-invasive ductal carcinoma, concomitant loss of E-cadherin provoked the formation of invasive metastatic mammary tumours reminiscent of human invasive lobular carcinoma. Our results show that somatic inactivation of E-cadherin in the absence of p53 results in enhanced tumorigenesis, and progression and metastasis of tumour cells.

Martijn Dollé

To study carcinogenesis, aging and mutagenesis, our laboratory is involved in designing, creating and analyzing various transgenic, knockout and knock-in mice. Examples of these are a transcribed lacZ-plasmid transgene targeted to an endogenous mouse gene (mRad23a) to study genome stability, mouse models with DNA repair deficiencies and mouse models with various point mutations in the tumor suppressor p53. The latter comprise mutations associated with the heritable cancer syndrome Li-Fraumeni, mutations frequently found in somatic human cancers, mutations affecting regulatory residues and a common human polymorphism (R72P). Expression of these mutations is under control of a (triple) stop-cassette flanked by loxP-sites, allowing tissue specific analysis of the mutant phenotypes. The effects of the gene modifications in response to genotoxic stresses are studied both in vivo and in vitro.

David Engblom

The glucocorticoid receptor (GR) regulates transcription through DNA-binding and through cross-talk with transcription factors, such as STAT5. Using hepatocyte-specific deletion of GR it was recently shown that GR, acting as a co-activator for STAT5, is essential for post-natal growth. However, the general impact of GR-STAT5 interactions on transcriptional regulation has not been assessed. We have performed genome-wide expression profiling, comparing liver-transcriptomes from mice with hepatocyte-specific deletion of GR to those from mice with deletion of STAT5. The striking similarities between the two profiles indicate that cross-talk between GR and STAT5 may be responsible for a substantial part of the transcriptional activation executed by the two respective transcription factors, and that such cross-talk induces coordinated expressional changes of genes involved in functions such as ribosome biogenesis, growth control, male-predominant gene expression and endopeptidase activity.

SOMATIC MUTATION OF TUMOR SUPPRESSOR GENES IN THE MAMMARY GLAND FAT-PAD TRANSPLANTATION SYSTEM: A NOVEL MODEL OF P53 AND BRCA2 INTERACTION

Bastiaan Evers[1], Matthew Smalley[2], Xiaoling Liu[1], Patrick Derksen[1], Jos Jonkers[1]
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Although the penetrance of mutations in *BRCA2* clearly indicates its importance in suppressing tumor formation in the mammary gland, data showing the effect of conditional loss of these genes in vivo are still not available. Since germ line deletion of *Brca2* induces embryonic lethality and no suitable promoter for high and specific cre-expression in mammary epithelial tissue is available, we made use of a Mammary Epithelial Cell (MEC) transplantation system to study the effects of conditional inactivation of *Brca2* and/or *Trp53* in the mouse mammary gland. Switching of the floxed alleles was mediated by using a 4-hydroxytamoxifen (4-OHT) inducible cre allele, cre-ERT. Following isolation of the conditional MECs, a cre-pulse was given in vitro by addition of 4-OHT, after which the cells were harvested and transplanted into cleared fat pads of 21-days old virgin females. Mammary glands can then be harvested at any given stage of development, enabling us to study both early and late effects of inactivation of Brca2 and p53. Our results show that transplantations of switched $Brca2^{F/F};Trp53^{F/F}$ donor cells lead to the formation of tumors in 45% of the recipients, 10-weeks after transplantation (n=15). Additionally, our results show a two-fold reduction in successful outgrowths when transplanting switched $Brca2^{F/F}$ cells as compared to transplanting non-switched cells. We are currently in the process of setting up a control group with $Trp53^{F/F}$ cells. Taken together, our observations suggest that while loss of Brca2 alone leads to developmental arrest, additional loss of p53 may lead to a rescue of this effect. Alternatively, the switched $Brca2^{F/F};Trp53^{F/F}$ cells giving rise to tumors, may have gained a third hit that cooperates with p53 loss, alleviating the growth arrest resulting from loss of Brca2.
Finally, to extend the flexibility of the MEC transplantation system, we are currently employing lentivirus-based gene transduction in order to overexpress putative oncogenes or silence candidate tumor suppressor genes. Thus, we hope to create a very versatile and flexible model system to quickly test and study new genes involved in mammary tumorigenesis.

Sarah Francoz

In cells exposed to various forms of stress, the p53 protein is stabilized and consequently elicits either a cell cycle arrest or apoptosis, thereby protecting organisms from developing cancer. In our laboratory we study molecules that control p53 biological activities. We have recently focused our attention on two structurally related molecules, Mdm2 and Mdm4. We have shown that *Mdm4* disruption leads to an embryonic lethality at 10.5dpc. The mutant embryos exhibit an overall cell proliferation block, particularly obvious in the fetal liver, and increased spontaneous apoptosis in the neuroepithelium. The disruption of *Mdm2* also leads to an embryonic lethality but as early as 4.5dpc. Interestingly, both phenotypes are completely rescued on a *p53*-null background. Therefore these two molecules function as negative regulators to restraint p53 activity during embryonic development.
The aim of our current work is to gain further mechanistic insights on how these two proteins modulate p53 activity *in vivo*. To this end, we use different mouse model systems allowing us to inactivate *Mdm2* and/or *Mdm4* in a *p53* wild type background and thus in various cell types or at different stages of differentiation in a given tissue.
In a first approach, *Mdm2* and *Mdm4* conditional knockout mice (kindly provided to us by Dr G. Lozano, University of Texas, USA) will be bred to different transgenic mouse lines expressing constitutively the cre recombinase in specific tissues and expressing cre ubiquitously in an inducible manner (actin-creER).
The second approach takes advantage of a particular p53 mutant mouse line harboring a stop translation cassette flanked by loxP sites upstream the first coding exon and therefore rendering the p53 locus inactive (provided by Dr T. Jacks, Center for Cancer research, Cambridge, USA). This mutation has been transferred into *Mdm2*, *Mdm4* and *Mdm2* and *Mdm4*-null backgrounds. Using retroviral-based approaches, cre will be expressed in various primary cell types (mouse embryonic fibrobasts, bone marrow hematopoietic progenitors) from these compound mice and the consequences of p53 reactivation will be studied and compared. Finally, in order to highlight a specific role for Mdm4 and/or Mdm2 depending the proliferation/differentiation state of the cells, the latter mice have also been crossed with transgenic lines expressing constitutively cre in neural progenitors (Nestin-cre) or in post-mitotic neurons (Math2-cre).

STRATEGIES TO SWITCH OFF THE EXPRESSION OF THE TRANSGENIC SPI-1/PU.1 ONCOGENE IN LEUKEMIC PROERYTHROBLASTS

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The proto-oncogene Spi-1/PU-1 encodes a transcription factor of the ETS family. It is transcriptionally activated during the Friend erythroleukemia. However, it is not necessary for the normal development of the red blood cells. The PU.1 protein is a major player of the B lymphopoiesis and myelopoiesis. Using a transgenic mouse model for Spi-1/PU.1, we have shown that PU.1 overexpression participates as an early event to the erythroleukemic process by blocking the proerythroblast differentiation. Our aim is to get insights into the cellular and molecular events modified by PU.1 and responsible for the blocking of erythroid differenciation. To do so, the cellular behaviour and the transcriptional and proteomic contents in cells that express spi-1 or not will be compared. Normal proerythroblasts, which are devoid of spi-1 expression, do not proliferate. Consequently, it is necessary to elaborate a cellular system that allows to switch off spi-1 expression in the spi-1 transformed transgenic proerythroblasts.
Two strategies are in progress:
1) The first one is based on the cre-lox methodology. The loxP site will be introduced into the genomic DNA of the transformed transgenic proerythroblasts by somatic homologous recombination at the integration site of the spi-1 transgene. The integration site of the transgene has been cloned. The spi-1 knocking out is triggered by the inducible expression of the cre recombinase. So far, no homologous recombinant has been obtained, probably because of the inefficiency of the somatic homologous recombination.
2) The second strategy is based on the use of small interfering RNA. The inhibition of spi-1 is done using stably integrated inducible siRNA vector (van de Wetering et al., 2003, EMBO Reports). This vector allows the transcription of siRNA from H1 gene promoter upon doxycycline induction. In the transgenic proerythroblasts, spi-1 is down regulated by 70% after doxycycline treatment. The analysis of cellular and molecular events following this inhibition is under investigation.

Peter Hohenstein

Kidney development is a dynamic process, regulated by complex communication patterns between different tissues in the developing kidney, like mesenchyme and tubules. The condensation of mesenchyme and subsequent epithelialisation to form nephrons is an essential step in the development of functional kidneys. Moreover, it is believed that disturbance of this process leads to the formation of Wilms' tumors, pediatric kidney tumors which are found with an incidence of 1:10.000 and are associated with different familial syndromes.
For decades kidney organ culture has been used to study kidney development *in vitro*. We have recently shown that siRNA-mediated knockdown of genes can be used to study the role of genes in the mesenchyme. This use of siRNA provided the first easy, fast and reproducible means of studying some aspects of kidney development *ex vivo* with genetic approaches. Using this system, we showed that loss of WT1, which has been implicated as a tumor suppressor gene in the development of Wilms' tumors, leads to disturbance of nephrogenesis, providing the first functional link between WT1 and the cellular origin of Wilms' tumors.
We are now trying to dissect the roles of WT1 and other genetic aberrations that have been described in Wilms' tumors, in first instance the oncogenic activation of β-catenin, in the development of these tumors. For this we will be using transgenic lacZ reporter mice and mice with dox-inducible expression of oncogenic β-catenin. In addition, we are testing lentiviral expression systems for the possibility to target other tissues in kidney organ culture than the mesenchyme, either for overexpression studies or expression of siRNAs. We hope this will enable us to characterize many different aspects of kidney development in an *ex vivo* setting. Finally, we are currently starting the use of lentiviral vectors to generate transgenic mice, so that interesting findings from the *ex vivo* experiments can easily be translated into an *in vivo* situation.

GENERATION AND ANALYSIS OF GENETICALLY MODIFIED MOUSE MODELS FOR THE ANALYSIS OF THE BIOLOGICAL FUNCTION OF NEPHRIN

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Nephrin, a transmembrane protein belonging to the Ig-supergene family, is detected on podocytes in the kidney, on the β-cells of the pancreas and on Purkinje cells in the cerebellum. Children suffering from CNF (congenital nephrotic syndrome of the Finnish type) proved to be homozygous for a non-functional nephrin gene resulting in impaired podocyte function causing death within a year after birth. Also KO mice died within days after birth and the podocytes within their kidneys showed similar morphological abnormalities as noticed from CNF patients (Rantanen M, 2002). Because of the lethality of nephrin deficiency in the kidney shortly after birth, little is known about:

1) The functional consequences of loss of nephrin later in life.
2) The phenotypic consequences of nephrin deficiency in β-cells of the pancreas, Purkinje and granular in the cerebellum, and hematopoeitic cells

Conditional nephrin KO mice: In order to study the phenotypic consequences of nephrin deficiency in β-cells of the pancreas, in the cerebellum by passing lethality from deficiency in the kidney, a conditional KO strategy was designed. Two loxP sites were introduced in the nephrin gene by homologous recombination in two sequential steps, one with hygromycin selection in intron 4 and the other with neomycin selection in intron 28. Breeding between mice with a *floxed nephrin gene* and mice expressing the recombinase Cre in a particular cellular subset will result litters in which the main part of the nephrin gene - between the two loxP sites - is removed in a tissue specific manner. Transgenic mice expressing recombinase Cre exclusively in particular cerebellar cells, β-cells of the pancreas and particular haematopoietic cells are available from the scientific community.

Podocyte specific inducible recombinase Cre mouse: Because of the lethality of nephrin deficiency in the kidney shortly after birth, nothing is known about the functional consequences of loss of nephrin later in life. Therefore a podocyte specific inducible system was designed. In a *binary system* a podocyte specific promoter (best candidate: *podocin promoter*) drives the expression of the recombinant inducible transcription factor *rtTA* (effector). In the presence of doxycyclin (inducer) rtTA binds to the tetO7 element linked to a minimal CMV promoter driving the expression of recombinase Cre. Breeding between the mice with the *floxed nephrin gene* and the mice with the binary inducible recombinase Cre will result litters in which the nephrin gene can be deleted specifically in podocytes later in life by doxycyclin administration.

Podocyte specific inducible nephrin mouse: Very little is also known about the regeneration capacity of nephrin deficient flattened podocytes upon reintroduction of functional nephrin. As *a proof of principle* for the opportunity to restore kidney function by *gene therapy* the following strategy was designed. In the previously described *binary system* the recombinase Cre will be replaced by nephrin cDNA. Within two generations of breeding between mice with one disrupted allele of the nephrin gene (the mice generated with the ES cells from the gene trap library) and the transgenic mice with the binary inducible nephrin gene, a litter is obtained without a functional endogenous nephrin gene but with exclusively the inducible podocyte-specific nephrin transgene. In the presence of doxycyclin these mice will survive. In this viable litter, nephrin can be switched off later in life, when doxycyclin is no longer administered. When doxycyclin administration is started again after while and if litter is survived, this will serve as a proof of possibility to use gene therapy in the treatment of CNF.

USING THE GENESWITCH SYSTEM TO STUDY THE ROLES OF WNT GROWTH FACTORS AND THEIR ANTAGONISTS DURING DEVELOPMENT

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To understand the What, When and Where of Wnt signaling during mouse development, we are focusing on signaling occurring in the extracellular space, as it is the primary source of information used by embryonic cells to define their fate and ultimately the formation of the embryo. First, a gain-of-function study will be conducted by generating transgenic mouse lines containing inducible Wnt transgenes. Second, loss-of function experiments of Wnt signaling will be carried out by controlled overexpression of secreted Wnt antagonists.
We plan to use the GeneSwitch system to trigger the overexpression of Wnts or Wnt antagonists at specific time points during the process of mesoderm induction and patterning. We have already generated several lines containing the regulatory construct flanked by insulator sequences as well as lines containing the response construct containing an inducible mWnt-1 cDNA sequence. Crosses are now being performed to determine the best driver and responding lines as well as to study the phenotype after the induction (using mifepristone) of Wnt-1.

ANALYSIS OF LONG RANGE TRANSCRIPTIONAL CONTROL OF PAX6 IN TRANSGENIC MICE WITH ENGINEERED YACS

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Aniridia is a congenital eye malformation characterised by severe hypoplasia of the iris, usually accompanied by foveal hypoplasia, cataracts and corneal opacification. It is caused by haploinsufficiency of the PAX6 gene at chromosome 11p13 as shown by deletion cases, and through loss-of-function point mutations (van Heyningen and Williamson, 2002). In addition a number of aniridia patients with translocation breakpoints mapping downstream of the PAX6 gene have been described (Fantes et al., 1995). Detailed mapping of these breakpoints placed them at various positions downstream from PAX6, with the furthest located 125 kb beyond the final exon, within an intron of an adjacent, ubiquitously expressed gene, ELP4. Even though all breakpoints map within the final intron of this gene, presumably interfering with its activity, it was shown through transgenic YAC rescue of a deletion mutant of the murine Pax6 locus that heterozygosity of Elp4 is unlikely to be the cause of the eye phenotype (Kleinjan et al, 2002). A naturally occurring Pax6 mutant carrying a truncating mutation, the Smalleye (Sey) mutant, was employed to further study the PAX6 'position effect'. A 420 kb human PAX6 YAC, containing sequence extending a further 80 kb beyond the position of the most distal patient breakpoint was shown to rescue Sey lethality and give full phenotypic correction in both heterozygous and homozygous Sey mice (Schedl et al, 1996). In contrast, a shorter YAC extending only as far as the breakpoint failed to rescue or correct the mutant phenotype. Essential regulatory elements were identified in the sequence between the ends of the shorter and longer YACs using DNaseI hypersensitive site (HS) mapping and evolutionary sequence comparison. Transgenic reporter studies revealed that some of these elements direct tissue-specific expression in eye and brain (Kleinjan et al., 2001), while others may serve a different function. To further study the role of these elements in PAX6 gene regulation we have inserted a GFP marker into the PAX6 gene in the 420 kb YAC and flanked the distal control region containing the hypersensitive sites with LoxP sites.

Laurent Massip

Hox homeotic genes encode homeodomain transcription factors implicated in embryonic patterning. Among them, *Hoxa2* is transiently expressed in the second branchial arch and confers their identity to its skeletal derivatives. Although the functional significance of the gene's expression pattern is progressively better understood, nothing is known yet about the meaning of its temporal expression dynamic. Aiming to address this question, we generated a transgenic mouse line for a conditional *Hoxa2* misregulation. Based on the prokaryotic Cre/loxP recombination system, this line allows to specifically direct the homeogene's expression in any tissue and at any time during mouse development.
Hoxa2 expression is normally turned off in differentiating branchial skeleton and, to better understand the meaning of this precise temporal dynamic, we used our transgenic line to force the homeogene's expression in all differentiating cartilages. Here, we show that transgenics for a *Collagen type II* directed Hoxa2 expression suffer of lethal chondrodysplasia probably due to an impaired prechondroblasts differentiation. Consistently, although no major patterning or proliferation defects were observed, each cartilaginous differentiation step was severely affected in mutant mice. In the last third of gestation, while a strong cartilaginous skeleton is clearly visible in wild type embryos, mutants only harbour coastal and cervical rudiments. Later on, cartilages hypertrophy, mineralization and ossification are all delayed in such individuals. Ongoing experiments intend to identify the precise molecular causes to the reported chondrodysplasia. For the first time here, we clearly uncoupled a Hox factor's patterning function from its antidifferentiating potential. This study shows that an important function of Hoxa2 during skeletogenesis, is to keep precursor cells undifferentiated until they reach their final embryonic location. There, Hoxa2 must be necessary turned off for differentiation to proceed.

ANIMAL MODELS TO STUDY FMR1 AND ITS HOMOLOGUES FXR1 AND FXR2 *IN VIVO*

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FXR1 and *FXR2* are the two known vertebrate autosomal homologues of the X-linked *FMR1* gene. Mutations in the *FMR1* gene cause the most common hereditary form of mental retardation in humans, the fragile X syndrome. The three genes are highly expressed in brain and testes. In addition, Fxr1 expression is also present in striated muscle. To study the function of the fragile X gene (*FMR1*) and its homologues *FXR1* and *FXR2 in vivo*, homologous recombination in ES-cells was used to generate knockout models for the three genes in mice. In the case of *Fmr1* and *Fxr1*, we now have conditional knockout mouse strains in which the first exon of the respective gene is flanked by loxP sites. The Fmr1 knockout mouse displays some behavioral abnormalities and crosses have been set up between the conditional Fmr1 knockout mice and brain specific Cre expressers such as the L7/pcp2-Cre mice that limit the Cre expression to the Purkinje cells. *Fxr1* knockout mice die shortly after birth due to a strong muscle phenotype. We have recently acquired a striated muscle-specific Cre expressor, which we will employ to confirm if indeed the neonatal lethality is due to poor muscle development. In addition these mice will also be crossed with brain specific Cre lines such as the L7/pcp2-Cre and CaMKII-cre to avoid the muscle phenotype and to determine if these mice exhibit behavioral abnormalities.

BMP SIGNALING IN MOUSE DEVELOPMENT

Rui Monteiro

Signaling by Bone Morphogenetic Proteins is essential for a wide variety of developmental processes. Receptor-regulated Smad proteins, Smads 1 and 5 are intracellular mediators of Bone Morphogenetic Protein signaling. Together with Smad4, these proteins translocate to the nucleus and modulate transcription by binding to specific sequences on the promoters of target genes. We sought to map transcriptional Smad1/5 activity in development by generating embryonic stem cell lines carrying a Smad1/5-specific response element derived from the Id1 promoter coupled to β-galactosidase or luciferase as reporters. Three independent lines (BRE-lac1, BRE-lac2 and BRE-luc) have shown the existence of an autocrine Bone Morphogenetic Protein signaling pathway in mouse embryonic stem cells. Reporter activity was detected in chimaeric embryos, suggesting sensitivity to physiological concentrations of Bone Morphogenetic Protein. Reporter activity in embryos from transgenic mouse lines was detected in tissues where an essential role for active Bone Morphogenetic Protein signaling via Smads 1 or 5 had been previously established. We have thus generated, for the first time, an in vivo readout for studying the role of Smad1/5 mediated transcriptional activity in development.

TOWARDS A MOUSE BRCA1 CONDITIONAL KNOCKOUT MODEL

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A mouse model with a targeted mutation in the 3' end of the endogenous Brca1 gene, Brca1-1700T, was generated (Hohenstein et al., Oncogene, 2001, 20:2544-50). Mice heterozygous for the Brca1-1700T mutation do not show any predisposition to tumorigenesis, whereas in homozygous state this mutation is embryonic lethal. However, in contrast to Brca1-null models, homozygous Brca1-1700T embryos do not undergo growth arrest leading to a developmental block at 6.5 dpc, but continue to proliferate and differentiate until 9.5 dpc, after which they die due to massive apoptosis throughout the embryo. These results indicate that a C-terminal truncating Brca1 mutation removing the last BRCT repeat has a different effect on normal cell function than does the complete absence of Brca1. To be able to study the phenotypic effects of this allele in adult mice, we are aiming to make our existing Brca1-1700T model conditional. Ideally, we would like to cross our mice with the conditional null allele of Brca1. Upon cre activation the conditional allele would be lost, leaving one copy of the Brca1-1700T only. The other possibility is to use the floxed copy of the human genomic BRCA1 gene carried on BAC, which is capable of completely rescuing the embryonic lethality of the Brca1-1700T mutation. We want to manipulate Cre expression by employing the bicistronic Cre-rtTA construct. To drive the rtTA expression, we have decided to use the promoter of the rat prostatic steroid binding protein c3(1). In a published transgenic mouse model, this promoter has been shown to direct the expression of the large T antigen exclusively to the mammary tissue of the female transgenic mice. Furthermore, this promoter seems to work during all stages of postnatal life, not only during pregnancy and lactation, as is the case with the MMTV or WAP promoters. This will allow us to study the phenotypic effect of Brca1 inactivation in any desired stage of mammary gland development, not only during pregnancy.

Haydn Prosser

Recessive ENU screens in the mouse, and subsequent positional cloning of the mutated genes is complicated by the difficulty of maintaining the mutant pedigrees. In order to facilitate this process balancer chromosome are being created in the mouse. These tools are balanced inversions of a defined chromosomal region, which act to suppress meiotic recombination so that mutant genes can be maintained in the heterozygous state. In addition the use of coat colour markers linked to the inversion and SNiP genotyping of the backcrossed strain can distinguish homozygous from heterozygous mutant mice.
A series of balancer inversions are being created for mouse chromosome 2. To this end 5' HPRT insertion vectors have been targeted into AB2 ES cells (HPRT negative) at approximately 41 and 132 Mb of chromosome 2. ES clones have been tested for germline transmission and a second round of targeting with the insertion of 3' HPRT vectors has created cell lines appropriate for inversion of chromosome 2 regions: 3.4Mb to 41Mb, 41Mb to 85Mb, 85Mb to 133Mb, 133Mb to 178Mb. These cell lines will now be transfected with Cre recombinase and HAT selected for isolation of ES cell clones containing the appropriate chromosomal inversion.
It is intended that a combination of meiotic mapping and the use of balancer chromosomes and BAC transgenesis for complementation will be used to identify the location of ENU induced mutations.

Sven Rottenberg

Resistance to chemotherapy is the central unsolved problem in cancer therapy. In recent years, a new hypothesis to explain resistance has gained popularity. Indications that drugs kill cells by inducing apoptosis or senescence led to the proposal that „genetic defects in these effector mechanisms could confer chemoresistance" (Schmitt C.A., 2003). However, this creates a paradox since there is abundant evidence that the average tumor cell has shut off apoptotic and senescence pathways to allow survival in the presence of abnormal cell proliferative stimuli. Hence, realistic model systems in which resistance mechanisms can be studied under controlled conditions in a homogeneous genetic background, are required to tackle this controversy. „Spontaneous" epithelial mouse tumors, which are induced by directed genetic alterations resemble human tumors more and may provide a better starting point for studying drug resistance mechanisms than transplantable mouse tumors or human xenografts. Using conditional and inducible mouse models of lung cancer and mammary gland cancer, we will treat mice harboring these neoplasms with different anti-cancer drugs currently used in chemotherapy regimens for human patients. We hope that some of the tested conditions will provide resistant tumors, and thus establish a murine drug resistance model. In such a model, important *in vivo* mechanisms of drug resistance can be analyzed and targeted to be reversed.

DEVELOPMENT OF A GENOME-WIDE CGH-ARRAY DEDICATED TO CANCER GENOME ANALYSIS OF MOUSE TUMOR MODELS

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The ability to generate mouse mutants developing malignancies had a major impact in oncogenesis. These mutants are instrumental to demonstrate the role of oncogenes and tumor suppressor genes, and are potentially models to investigate human tumors pathogenesis and new therapeutical strategies. The public availability of the mouse genome draft reinforces the interest of these mouse mutants, by facilitating the identification of new recurrent gene alterations and by evaluating the pertinence of these models for human cancers, according to similarities and differences in human and mouse tumor genetics.
We have developed a first generation genome-wide cancer dedicated CGH-array that contains 746 BACs. The collection of BACs was assembled from the Korenberg set (149 BACs), from BACs containing known oncogenes and tumor-suppressor genes (240 BACs). The genome coverage was completed by 347 BAC clones providing an average resolution of 6 Mb.
Each clone was isolated, validated by end sequencing, and amplified by Multiple Displacement Amplification. The collection is spotted in quadruplicate, in 75% formamide, on Corning Ultragaps slides. Experiments were conducted in duplicate, with a dye-swap process. Bioinformatics, in addition of standard processes of normalization and analysis, allow directly comparison of human and mouse tumor CGH array results using syntenic conversion of BAC positions.
A series of mouse models of human tumors, selected by the French national CIT2 program, is analyzed using this array. Single nucleotide polymorphism (SNP) allelotype in F1 (C57BL6 x CAST) tumor samples is used to confirm gains and losses identified by the CGH array and to validate the array.
Results obtained in leukemia in MTPC1 transgenics and hepatocarcinoma in WHV-MYC transgenics will be presented.

A CONDITIONAL KNOCK-OUT APPROACH WITH MINIMAL INTERFERENCE IN A COMPACT GENE

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The dramatic increase in the occurrence of insulin resistance and metabolic syndrome requires appropriate animal models for the study of the underlying mechanisms. The *Cpt1b* gene for muscle-type carnitine palmitoyltransferase (M-CPT I) is an attractive target, i.e. to create models of extra-hepatic insulin resistance. This gene of 10 kb contains 18 introns (average size 365 bp) and is highly expressed in heart, muscle and testes. In a standard Cre-Lox strategy to target *Cpt1b*, the removal of the Neo-TK cassette was obstructed (gancyclovir titrations suggested that the TK gene had become inactive). Still, a targeted ES-line with this 3.5 kb cassette remaining in a 550 bp intron was used, and 11 chimaeras were obtained by blastocyst injection. Only 2 male chimaeras were fertile, and their offspring (2 x 100 pups) were all of wild type phenotype. We therefore concluded that leaving a relatively large insert in a small intron mimicked a classical knockout model, and that M-CPT I function may be crucial for sperm formation. A novel design has now been developed to minimally modify mouse *Cpt1b*. The latter strategy includes only one *Lox*P-site in an intron, the other *Lox*P-site and a Neo^R gene are downstream of the transcribed region. Since immediately upstream of *Cpt1b* another gene is located, this approach seems to be the best option to obtain a conditional KO with minimal interference.

HOX GENE EXPRESSION AND SPECIFICATION AND EARLY POLARIZATION OF THE LIMB FIELD

Johan van Nes, Wim de Graaff and Jacqueline Deschamps

Initiation of limb development in vertebrates is still not well understood. Classical experiments on embryos and molecular studies by several laboratories have made it clear that a strong link exists between patterning of axial structures and local limb induction. *Hox* expression patterns along the main axis, which are set from gastrulation on, are likely to be involved in early determination of the limb field. However the molecular mechanism underlying the coupling between the axial "*Hox* code" and the patterning molecules shown to play a role in limb induction, such as Wnts and FGF/FGFRs is still elusive.
In addition, early *Hox* gene expression may induce a posterior genetic cascade, ending up with the establishment of the Zone of Polarizing Activity and proximodistal outgrowth of an anterior-posterior polarized limb. Previous experiments have shown that early ectopic expression of *Hoxb8* in anterior limb tissue results in the activation of posterior markers in anterior limb tissue.
Deciphering the genetic interactions between the Hox genes and the early determinants of limb field specification (such as FGF/FGFRs, Wnts) and A-P polarization (dHAND, Gli3 and gene products from the anterior and posterior cascades) should shed light on the relationship between axial patterning and appendage development. As an approach we induce altered expression of *Hox* genes and their *Cdx* regulators in transgenic embryos. We analyze the resulting changes in limb patterning, and assay gene expression at early stages of limb development.

SEVERE AFFECTION OF GI-TRACT AND LETHALITY FOLLOWING INDUCIBLE AND SMOOTH MUSCLE SPECIFIC DELETION OF THE P53 INHIBITOR MDM2 IN MICE

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The tumor suppressor gene p53 has been implicated to play a role in vascular disease. To dissect the role of p53 we generated mice that allow inducible and smooth muscle cell (SMC)-specific deletion of the p53 inhibitor MDM2. ApoE deficient mice that develop atherosclerotic vascular disease were combined with mice that carry a tamoxifen-inducible Cre-recombinase under control of the SMC-specific SM22 promoter (SM-CreER^{T2}(ki) mice) and mice that carry a floxed allele of MDM2 (FM mice), thereby creating SM-CreER^{T2}(ki)/FM/ApoE$^{-/-}$ mice. Daily intra-peritoneal tamoxifen administration to these mice resulted in deletion of MDM2 in all SMC-rich tissues, including the GI-tract, coinciding with decreased health status and concurrent lethality. Macroscopically a severely dilated stomach and small intestines were observed, while colon was not affected. Histological analyses revealed massive presence of atrophy, vacuolisation, pyknotic and fragmented nuclei (apoptosis as confirmed by TUNEL) in the SMC-layers, coinciding with upregulation of p53, p21 mRNA and protein and absence of MDM2 mRNA and protein. Control FM/ApoE$^{-/-}$ mice were not affected. The early death that followed SM-MDM2 deletion hampered mouse studies on the role of the p53 pathway in vascular disease. Future studies should aim at local arterial SMC-specific deletion of MDM2. In conclusion, our data might indicate a role for MDM2 in survival of non-stressed SMCs.

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