What‘s wrong with my mouse?

Generation and analysis of genetically engineered rodents

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Culturing preimplantation embryos and reimplantation in foster mice developed in the 1950s

Generation of genetically modified mice

- chimeric mice
- classical KO mice
- knock-in mice
- classical transgenic mice
- „targeted“ transgenics
- conditional „transgenics“ in space and time

Behavioural analysis of mice

- Sensory abilities
- Motor performance
- Learning and memory
- Emotions
Chimeric mice: 
Mouse mosaics of the pre-Cre era

- Early 1960s by Tarkowski and Mintz

- Generation of mice consisting of cells with at least two different genotypes

- Purpose: Cell lineage and development
  Pigmentation
  Cell Autonomy

- Degree of chimerism detected by glucose phosphate isomerase (GPI) isoenzymes
Generation of chimeric mice

**Generation of chimeric mice**

![Diagram of generation of chimeric mice]

SOD1\textsuperscript{G85R}  
SOD1\textsuperscript{G37R}

R1/129 (E-YFP)  
ES cells

SOD1\textsuperscript{G37R}  
SOD1\textsuperscript{G93A}

wt (hNF-L)  
wt

SOD1\textsuperscript{G37R}; wt (hNF-L); SOD\textsuperscript{G93A}  
+ Littermate controls

Blastocyst injection  
Morula aggregation

G85R  
Chimera

**NF**  
**E-YFP**  
**hSOD**
Asymmetric distribution of wild type cells

Clement et al, 2003
Mutant SOD1 neurons are protected in a wild type environment

All motor neurons express mutant SOD1

Clement et al., 2003
1980s – The birth of transgenic mice

1980: Gordon et al., PNAS 1980 „Genetic transformation of mouse embryos by microinjection of purified DNA“

1981: Generation of first transgenic mice
Costantini and Lacy; Nature 1981 „Introduction of rabbit beta-globin gene into the mouse germ line“

Gordon and Ruddle; Science 1981 „Integration and stable germ line transmission of genes injected into mouse pronuclei“

Wagner TE et al.; PNAS 1981 Microinjection of a rabbit beta-globin gene into zygotes and its subsequent expression in adult mice and their offspring

First „functional“ transgenic mouse

Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes

Palmiter et al.; Nature 1982
Transgenic mice

Injection of DNA into fertilized oocyte

Transfer into recipient mother

Transgenic

Non-transgenic

mod. Bockamp et al, 2002
Establishment of mouse ES cells and homologous recombination

**Martin GR.** Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *PNAS* 1981.


Knock-out cloning: Homologous recombination in ES-cells

nach Bockamp et al, 2002
How to generate a KO-mouse

Homologous recombination in ES cells

Injection of ES cells into blastocyst

Transfer into recipient mother

Chimeric mouse

Knock-out with recombined locus  Wildtype

Resources:
http://www.jax.org

Bockamp et al, 2002
The members of the International Knockout Mouse Consortium (IKMC) are working together to mutate all protein-coding genes in the mouse using a combination of gene trapping and gene targeting in C57BL/6 mouse embryonic stem (ES) cells.

Knockout Mause Project (KOMP) (USA)

CSD, a collaborative team at the Children's Hospital Oakland Research Institute (CHORI), the Wellcome Trust Sanger Institute and the University of California at Davis School of Veterinary Medicine, led by Pieter deJong, Ph.D., CHORI, along with K. C. Kent Lloyd, D.V.M., Ph.D., UC Davis; and Allan Bradley, Ph.D. FRS, and William Skarnes, Ph.D., at the Wellcome Trust Sanger Institute.

Regeneron, a team at the VelociGene division of Regeneron Pharmaceuticals, Inc., led by David Valenzuela, Ph.D. and George D. Yancopoulos, M.D., Ph.D

European Conditional Mouse Mutagenesis Program (EUCOMM) (Europe)

North American Conditional Mouse Mutagenesis Project (NorCOMM) (Canada)

Texas A&M Institute for Genomic Medicine (TIGM) (USA)
International Gene Trap Project (www.genetrap.org): towards gene-driven saturation mutagenesis in mice


Wiles et al, 2000
Identification of trapped genes: 5' RACE PCR

From Human Molecular Genetics 2: T. Strachan, A.P. Read
Caveats of conventional transgenics and KO mice

<table>
<thead>
<tr>
<th>Transgenics</th>
<th>KO - mice</th>
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<tbody>
<tr>
<td>- Random integration</td>
<td>- Embryonal lethality</td>
</tr>
<tr>
<td>- Levels of expression</td>
<td>- Systemic KO</td>
</tr>
<tr>
<td>- cDNA constructs</td>
<td></td>
</tr>
<tr>
<td>- Systemic expression</td>
<td></td>
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Tightly controlled expression in time and space, which is ideally reversible.
Tools for generating conditional mice:
Tissue restricted expression

Cre / loxP - System
Flp / FRP - System

Changing expression on the genomic level
Cre-recombination in transgenic mice

**Transgene Switch-on**


**Transgene Switch-off**

Transgene Switch-off in disease models

Boilleé et al. 2006, Yamanaka et al., 2008

Motoneurone

Astrozyten

Microglia

Boilleé et al. 2006, Yamanaka et al., 2008
Utilizing the Cre-sytem: Knockin-first

B B B

Ubiquitous Cre expression

Ubiquitous excision

Liver specific Cre expression

Liver specific excision

Bockamp et al, 2002
Utilizing the Cre-sytem: Knockout-first

Cre-Database:
http://nagy.mshri.on.ca/cre/

Testa et al., 2004
Tools for generating conditional mice: Temporally regulated expression

**Tet-off**

- tTA binds to TRE
- Transcriptional induction

- + Dox
  - tTA unable to bind TRE
  - No transcription

- - Dox
  - tTA binds to TRE
  - Gene of interest
  - pA

**Tet-on**

- rtTA
- + Dox
  - rtTA unable to bind TRE
  - No transcription

- - Dox
  - rtTA binds to TRE
  - Transcriptional induction

- + Dox
  - rtTA binds to TRE
  - Gene of interest
  - pA

Other inducible systems:
- Progesteron and estrogen receptor
- Ecdysone receptor
- Cytochrome C
- IPTG (lactose operon)

Gossen M and Bujard H., 1992, Bockamp et al, 2002
Temporal regulation of expression

Tet-mouse database
http://www.zmg.uni-mainz.de/tetmouse/

Furth et al., 1994
Temporal, spacial and reversible transgene expression

Sun et al., 2007
Temporal, spacial and reversible gene silencing

Sun et al., 2007
All inclusive: Temporal, spacial and reversible gene silencing – and therapy


Have a mouse – have a……?

Converting behaviour into numbers

- Sensory and motor performance is rather objective
- Learning and memory are less objective
- Emotions: measurable? In mice?
- Feeding; Reproduction; Social; Reward
Motor performance: Gait

- Gait length
- Order

A. BOSIO, E. BINCZEK, AND W. STOFFEL*; Membrane in central and peripheral nervous system by disrupted galactocerebroside synthesis; PNAS 1996
Motor performance: Grip strength

Source: TSE Systems
Motor performance: Rotarod

- Speed (rpm)  -  Time

Neymotin et al., 2009
Motor performance: Running wheel

Rhodes et al., 2003
Motor performance: Open field

- Distance
- Speed
- Rearing: number and time
- Sector
- Rotation

Video tracking system

Source: TSE-Systems
Learning and memory

Explicit Memory: remembering a specific item of information.
(What is a memory?)
e.g. telephone number, keys

Implicit Memory: learning skills, habits and complex reflexes
(What is memory?)
e.g. using a phone, open a lock

From Milner et al., 1998
Morris water maze task (1982) - Acquisition

Set up

Protokol

Day 1 to 4 →
Four different positions
Maximum 1 min

3 Minutes Acclimatisation

1 Minutes without platform

Day 1 to 4
Day 1
Day 2
Day 3
Day 4
Day 5

Videos courtesy of Noldus
Morris water maze task - Analysis and flaws

Analysis

- Time finding the platform
- Swim time and distance
- Speed
- Time in quadrant

Flaws

- memory vs. anxiety
- swim lovers
- genetic background
- blind rats learn task
Morris water maze task - APP-mice

Probe trial 2
- after 12 trials with platform

Probe trial 3
- after 18 trials with platform

Moran et al., 1995
Barnes maze

- cued and spacial versions
- physically not challenging
- primarily not anxiety driven
- analysis of search strategy

Source: Biobserve
Cued and contextual conditioning
Association between an aversive experience and environmental cues

Ataxin-KO

Duong et al., 2009
Extinction of aversive memories

Sensitivity to foot shock not changed in CB1-KO mice

Tone exposure 24h post conditioning

Tone exposure 6d post conditioning

Marsicano et al., 2002
T- and Radial-Mazes

**Basis:**
- Food or water restriction
- Avoidance of foot shock

Source: Noldus
Measuring fear, depression and other emotions

Validity of the model system:

- Robust and reproducible
- Simply enough for routine
- Quantitative and automated
- Replicates at least one symptom of human disease
- Responsive to treatment used in humans
- Unaffected to ineffective treatments in humans
- Conceptual analogy to multiple components of human disease

- Behavioral symptoms
- Neuroanatomy
- Neurochemistry
- Temporal progression
- Precipitating event
Measuring Anxiety: Elevated plus maze

- Time on open bars
- Number of entries
- Distance
- Time rearing
- Head dips
- Freezing
- Streching postures

- Panlab / Harvard Biosciences
- Komada et al., Journal of Visualized Experiments

Other tests: Light-dark exploration; social interaction in open field
Measuring Depression: Porsolt forced swim test

Par-4 ΔLZ: Interaction with dopamine receptor

Source: Stoelting

Depression

Forced swim test

Tail suspension

Anxiety

Park et al., 2005
## Factors affecting behavioural analysis

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Factors affecting behavioural analysis

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Where do mouse strains come from?

Basis: segregation of coat color

M. Coladon observed Mendelian genetics in mice….

…..36 years before Mendel (1866)

First inbred strain: 1909 by C.C. Little: non agouti brown dilute

1919 by Miss Lathrop: black offspring of female 57 (C57BL/6)
Genetic background: inbred strains

Alcohol tolerance


Acoustic reflexes

Paylor and Crawley, 1997
### Naturally occurring mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>Übersetzung</th>
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<tbody>
<tr>
<td><em>Plp</em>&lt;sub&gt;ip&lt;/sub&gt;</td>
<td>jimpy</td>
<td><em>Gimpy</em>: untauglich</td>
</tr>
<tr>
<td><em>Pmp22</em>&lt;sub&gt;Tr-J&lt;/sub&gt;</td>
<td>trembler Jackson</td>
<td>zittern</td>
</tr>
<tr>
<td><em>Prop1</em>&lt;sub&gt;df&lt;/sub&gt;</td>
<td>Ames dwarf</td>
<td></td>
</tr>
<tr>
<td><em>Prph2</em>&lt;sub&gt;Rd2&lt;/sub&gt;</td>
<td>retinal degeneration 2</td>
<td></td>
</tr>
<tr>
<td><em>Ptch</em>&lt;sub&gt;mes&lt;/sub&gt;</td>
<td>mesenchymal dysplasia</td>
<td></td>
</tr>
<tr>
<td><em>Rab27a</em>&lt;sub&gt;ash&lt;/sub&gt;</td>
<td>ashen</td>
<td>aschfahl</td>
</tr>
<tr>
<td><em>Reln</em>&lt;sub&gt;rl&lt;/sub&gt;</td>
<td>reeler</td>
<td><em>to reel</em>: schwanken</td>
</tr>
<tr>
<td><em>Slc45a2</em>&lt;sub&gt;uw-d&lt;/sub&gt;</td>
<td>underwhite dense</td>
<td></td>
</tr>
<tr>
<td><em>Sgsh</em>&lt;sub&gt;mps3a&lt;/sub&gt;</td>
<td>mucopolysaccharidosis</td>
<td>IIIA</td>
</tr>
<tr>
<td><em>Tcirg1</em>&lt;sub&gt;oc&lt;/sub&gt;</td>
<td>osteosclerotic</td>
<td></td>
</tr>
<tr>
<td><em>Tgn</em>&lt;sub&gt;cog&lt;/sub&gt;</td>
<td>congenital goiter</td>
<td>angeborener Blähhals</td>
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<tr>
<td><em>Tshr</em>&lt;sub&gt;hyt&lt;/sub&gt;</td>
<td>hypothyroid</td>
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Quelle: [www.jax.org](http://www.jax.org)
Maintainance vs. experimental breeding

Maintainance

Experiment

10 generations to be >99% homologous
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<td>It‘s all about motivation: Working in the red light milieu</td>
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Mouse circadian rhythm

by Franz Halberger, 1994

Rhodes et al., 2003
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<td>Gender</td>
<td>Like in real life: We have equal rights but are not equal</td>
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Urocortin 2-Deficient Mice Exhibit Gender-Specific Alterations in Circadian Hypothalamus–Pituitary–Adrenal Axis and Depressive-Like Behavior
Chen et al., 2006

**Gender matters – for statistics**

**Forced swimm test: Depression**
Factors affecting behavioural analysis

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<tr>
<td>Age</td>
<td>Neonatal: 0 - 3 weeks</td>
</tr>
<tr>
<td></td>
<td>Juvenile: 3 - 8 weeks</td>
</tr>
<tr>
<td></td>
<td>Adult: 3 - 12 month</td>
</tr>
<tr>
<td></td>
<td>Aged: 13 - 24 month</td>
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Bringing everything together

CaMKII and memory

[Diagram showing the transition from Inactive to Active form of CaMKII with the addition of Ca^2+.

Conventional transgenic mouse

- WT-CaMKII
- Asp-295-Transgen

Barnes maze

- Hippocampus dependent
- Hippocampus independent

Mayford et al., 1995

Bach et al., 1995
Dox-dependent repression of CaMKII-ASP286 expression

Expression of CaMKII-ASP296 in double transgenics

Mayford et al., 1996
Correlation of tissue distribution of CaMKII-ASP286 and memory

Spatial Barnes maze: B22 line

Fear conditioning

Mayford et al., 1996
The beauty of the Tet-System: Studying temporal effects

Experimental set up

Mayford et al., 1996
Despite the advances in „mouse technology“ and some implications for human disease:

A mouse is a mouse is a mouse is a mouse ..... 

......and not human
Resources

Mouse resources
http://www.jax.org
http://ww.criver.com

KO-Databases
http://www.genetrap.org
http://www.knockoutmouse.org

Cre-Database:
http://nagy.mshri.on.ca/cre

Tet-mouse database
http://www.zmg.uni-mainz.de/tetmouse/

Web sites of companies distributing behavioural research apparatuses
TSE; Noldus; San Diego Instruments, Panlab and many others

„What’s wrong with my mouse“ by Jaqueline N. Crawley, Wiley Press