Generation and Application of Genetically Modified Mouse Models of Human Disease.

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The Plan

• Techniques to manipulate the mouse genome - when to use which one.

From Gene to Physiology.

• Some recent exciting applications in neuroscience:
  1) Neuronal pathways regulating body weight homeostasis.
  2) Mouse Model of Rett Syndrome

• Helpful mouse databases.
Genome Manipulations: Somatic vs Germline

Genetically modified mice:
• **germline** manipulations, ie every cell in the body harbors the DNA modification

Somatic manipulations:
• anatomically localized to injection site

virus etc.
Why do we need to manipulate the mouse genome at all?

The post-Human Genome Project Era:

<table>
<thead>
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<th>Assembly:</th>
<th>NCBI 36, Oct 2005</th>
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<tbody>
<tr>
<td>Genebuild:</td>
<td>Ensembl, Sep 2008</td>
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<tr>
<td>Known protein-coding genes:</td>
<td>21,649</td>
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<tr>
<td>Novel protein-coding genes:</td>
<td>65 (since oct 2007)</td>
</tr>
<tr>
<td>Base Pairs:</td>
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Of those 21,649 genes we only know the function of roughly half.

It is thought that only ~1% of human genes have no mouse counterpart.
Monogenic disorders - Leptin deficiency

Mice

Humans

Weight (kg)

Age
Monogenic disorders - MC4R mutations

**Mice**
- WT
- MC4R KO

**Humans**

Prevalence: 2.5% of BMI>30
How do we study the physiological role of genes?

• monogenic disorders in humans are very helpful in understanding the physiological function of specific genes, but extremely rare

• limited access to humans and limited experimental design....

• tissue culture, cell lines (‘simple’ systems) can give us some information on specific functions of a protein, but no information on its physiological function is revealed - effect on body weight regulation???

    → rodents (rats and mice)
How do we study the physiological function of genes in the CNS?

**Neuroanatomy:**
- where in the brain are specific genes expressed (co-localisation)
- which neurons project where

**Lesions:**
- which brain areas have which functions

**Pharmacology:**
- what is the physiological effect of specific drugs/agents?

What is the physiological role of specific genes in specific areas of the brain?  

**Neuronal subset-specific genetic modifications.**
How do we study the physiological function of genes?

What is the physiological role of specific genes?

Alter a gene’s expression level in mice and analyze the physiological outcome \textit{in vivo}.

\begin{itemize}
  \item \textbf{deletion, ie knock-out mice}
\end{itemize}
How do we study the physiological function of genes?

What is the physiological role of specific genes?

→ Alter a gene’s expression level in mice and analyze the physiological outcome \textit{in vivo}.

- **over-expression, ie transgenic mice**

endogenous gene: \begin{center}
\begin{tabular}{c}
\hline
\textbf{‘weak’ promoter,} \textbf{i.e. low expression levels} \\
\hline
\end{tabular}
\end{center}

DNA transgenic construct:

\begin{center}
\begin{tabular}{c}
\hline
\textbf{strong (e.g. \(\beta\)-actin) promoter} \\
\hline
\end{tabular}
\end{center}
**Targeted**

- modify an **endogenous** gene
- targeted alteration
- via ES cells

**Transgenic**

- add an ‘extra’ gene under it’s own promoter control
- random integration of transgenic construct
- via injection of DNA transgene construct in embryos

DNA transgenic construct:

```
ATG XYZ cDNA
```

```
ATG STOP
```

ATG

endogenous gene:

```
1 2 3 4 5
```

modified gene:

```
neo
```
Generating transgenic mice

**construct:**

POMC-promoter ie tissue-specificity ATG eGFP STOP

random integration of an ‘extra piece of DNA’

Transgene DNA is microinjected into the male pronucleus of a fertilised murine oocyte

Injected oocytes are transferred to a 0.5-day pseudopregnant recipient mouse

Offspring are screened for the transgene by DNA analysis
Transgenic techniques

Promoter DNA

Temporal and spatial expression:
• tissue-specific
• cell-type specific
• ubiquitous
• inducible

DNA gene of interest

Gene of interest:
• over-expression
• mutant
• dominant-negative
• fluorescent reporter
• cell killer

DNA Transgene construct
Transgenic techniques

POMC-promoter
ie tissue-specificity
ATG eGFP STOP

Leptin (100 nM)

Potential (mV)

Time (s)
Transgenic techniques

POMC-Kir6.2[ΔN2–30,K185Q]–GFP BAC DNA construct

>45 kb POMC 5ʹ sequences

Kir6.2[ΔN2–30,K185Q]–GFP

ATG  Stop

Exon 1  2  3

ATG  Stop

Glucose-excited WT neuron

1 2

0 1 2 3 4 5

5 mM 3 mM glucose 5 mM

Time (min)

Firing rate (AP s⁻¹)

5 10 15 20 25 30 35

mut-Kir6.2 neuron

3

0 1 2 3 4 5

5 mM 3 mM glucose 5 mM

Time (min)

Firing rate (AP s⁻¹)

0 100 200 300 400

Blood glucose (mg dL⁻¹)

0 30 60 90 120 150 180

Time after glucose injection (min)

* **

POMC-mut-Kir6.2

Wild type
What controls gene expression?

- characterized promoters?
- integration site effects?
Targeted modification - Generating genetically modified mice using ES cells

Deletion of POMC:

Wild-type

Mutant allele

→ alter the endogenous gene!
Targeted modifications - Generating genetically modified mice using ES cells

**Modifications:**

- deletion
- point mutation
- cell killers

![Diagram of POMC gene modifications](image)

![Graph showing weight change](image)
Deletion of AgRP neurons

**Agrp locus**

Targeting construct

$hDTR$ - $\text{Sv-Neor}$ - $\text{Agrp}$

$\text{Agrp promoter}$ - $\text{hDTR}$ - $\text{Agrp}$

Injection of DT → AgRP neuron-specific ablation

Coil death

WT

DMN

VMN

Arc.

hDTR + diphtheria toxin injection

G

H
Deletion of AgRP neurons

**Neonatal:**

**Adult:**
Generating genetically modified mice using ES cells

Modifications:

- deletion
- point mutation
- cell killers

- embryonic lethal? ie no pups
- compensation? ie no phenotype
Targeted vs. Transgenic

- **Targeted**
  - modify an endogenous gene
  - targeted alteration
  - via ES cells

- **Transgenic**
  - add an ‘extra’ gene under its own promoter control
  - random integration of transgenic construct
  - via injection of DNA transgene construct in embryos

DNA transgene construct:

- strong (e.g., β-actin) promoter
- \( XYZ \) cDNA
The Cre-\textit{loxP} system

- Deletion of gene in cells expressing Cre \textit{and} ‘floxed’ gene

\begin{itemize}
  \item cell type-specific promoter
  \item Cre recombinase
  \item Gene/region to be knocked out
\end{itemize}
What is the physiological role of LepR in specific areas of the brain?

Leptin → fertility

Leptin → bone mass

Leptin → food intake

Leptin → energy expenditure

Leptin → glucose homeostasis

LepR mRNA in the CNS

db/db mice (lacking all LepR)
POMC neuron-specific LepR KO

LepR ‘floxed’ mice

Lepr

Exon 16

loxP

17

17’

18a

18b

transmembrane domain

JAK docking site

Lepr-c last exon

Lepr-a last exon

Lepr-b last exon

Cre-recombinase

non-signaling LepR

Lepr

Exon 16

loxP

17’

18a

18b

transmembrane domain

Lepr-c last exon

Lepr-a last exon

Lepr-b last exon
POMC neuron-specific LepR KO

POMC-Cre mice

Diagram showing the genomic region with exon 1, Exon 2, and Exon 3, with ATG and STOP codons. The diagram highlights >45kb Pomc 5' sequences and >70kb Pomc 3' sequences.
POMC neuron-specific LepR KO

POMC-Cre mice × LepR ‘floxed’ mice

POMC-neuron specific LepR deletion,
i.e. mice lacking LepR on POMC neurons
Mice lacking LepR on POMC neurons are mildly obese

Males lacking LepR on POMC neurons are mildly obese.

=> LepR on POMC neurons are required for normal body weight regulation!
MC4R Regulate Body Weight in Man and Mouse

Targeted Disruption of the Melanocortin-4 Receptor Results in Obesity in Mice

Dennis Huszar,† Catherine A. Lynch,† Victoria Fairchild-Huntress,† Judy H. Dunmore,† Qing Fang,† Lucy R. Berkemeier,† Wei Gu,† Robert A. Kesterson,‡ Bruce A. Boston,‡ Roger D. Cone,‡‡ Francoise J. Smith,§ L. Arthur Campfield,§ Paul Burn,§ and Frank Lee*†

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Nutley, New Jersey 07110

A frameshift mutation in MC4R associated with dominantly inherited human obesity

Giles S.H. Yeo†*, I. Sadaf Farooqi*,∥,† Shiva Aminian†, David J. Halsall†, Richard G. Stanhope* and Stephen O’Rahilly†

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∥Institute of Child Health and Great Ormond Street Hospital, London, U.K.
*G.S.H.Y. and I.S.F. contributed equally to this work. Correspondence should be addressed to S.O.R. (e-mail: sorahili@hgrp.mrc.ac.uk)

Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity

Christian Vaisse,1,2,3 Karine Clement,1,2 Emmanuelle Durand,2 Serge Herberg,4 Bernard Guy-Grand,2 and Philippe Froguel1,2

1Centre National de la Recherche Scientifique, UPR EA 8090, Institute of Biology of Lille, Lille, France
2Laboratoire de Nutrition et Service de Médecine et Nutrition, Hôtel-Dieu, Paris, France
3Department of Medicine and Diabetes Research Center, University of California San Francisco, San Francisco, California, USA
*Conservatoire National des Arts et Métiers, Paris, France


Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency

I. Sadaf Farooqi,1 Giles S.H. Yeo,1 Julia M. Keogh,1 Shiva Aminian,1 Susan A. Jebb,2 Gary Butler,3 Tim Cheetham,4 and Stephen O’Rahilly†

1University Departments of Medicine and Clinical Biochemistry, Addenbrooke’s Hospital, Cambridge, United Kingdom
2Medical Research Council, Human Nutrition Research, Cambridge, United Kingdom
3Leeds General Infirmary, Leeds, United Kingdom
4Royal Victoria Infirmary, Newcastle, United Kingdom
MC4R expression in several areas of the CNS

MC4R mRNA

Endocrine
- e.g. growth, reproduction

Behaviour
- e.g. feeding

Autonomic
- e.g. Thermogenesis, Insulin secretion, Cardiovascular control
Generating loxTB MC4-R mice

WT allele

disrupted, null allele

re-activated allele after Cre recombination
Why the PVH?

• lesions of the PVH cause obesity

• high MC4R expression levels in the PVH
Generating loxTB MC4-R mice

WT allele

disrupted, null allele

re-activated allele after Cre recombination

HinDIII
+1
ATG
MC4-R
loxP
HinDIII
TB=Stop
MC4-R
loxP
loxP
HinDIII
+1
ATG
MC4-R
loxP
NotI
NotI
Sim1-Cre transgenic mice

Sim1 5’ sequences

PVH

SON

NLOT

ATG

STOP

Cre

1

2

3

4

5

6

7

8

ATG

Sim1-Cre transgenic mice
Sim1-Cre                 x               loxTB MC4R
PVH-specific Cre-expressing mice

mice expressing MC4R in PVH neurons only
MC4Rs in the PVH play a key role in body weight regulation

MC4R mRNA *in situ* hybridization

**WT**

Sim1-Cre x loxTB MC4R

**MPOA**

**PVH**

**NLOT**

**PAG**

**Raphe**

**DMV**

**bodyweight [g]**

**age [weeks]**

**male**

- loxTB MC4R
- Sim1-Cre, loxTB
- WT
Stereotaxic injections of AAV-Cre in the PVH of loxTB MC4R mice

Combining virus and genetically modified techniques:

**AAV-Cre**

**Cre immunohistochemistry**

**Mc4r mRNA *in situ* hybridization**

breed vs inject, neuronal subpopulation vs. neuranatomy
Inducible Cre-recombinase CreER

Cre fusion protein with a tamoxifen-responsive Estrogen Receptor ligand binding domain

CreER is sequestered in the cytoplasm by heat shock protein. The administration of tamoxifen confers a conformational change on CreER that releases it from Hsp 90 and allows it to be translocated to the nucleus, where it induces recombination between loxP sites.
**Inducible Cre-recombinase - an example in mouse models of Rett syndrome**

**Rett syndrome:**

- severe autism spectrum disorder with delayed onset (loss of mobility and stalling of cognitive development at 6-18 months of life)
- mutation in MeCP2 gene
- X-chromosome-linked; affects 1:10000 girls
- abnormal neuronal morphology, but not neuronal death

→ neurodevelopmental disorder rather than neurodegenerative disorder

**Question:**

As MECP2-mutant neurons are viable, could re-expression of MeCP2 reverse the condition? Or is the developmental damage irreversible and permanent?
lox-STOP-MeCP2 mice:

Cre-ER + Tamoxifen injection

WT

lox-STOP-MeCP2 x Cre-ER

lox-STOP-MeCP2 X Cre-ER + Tamoxifen
MeCP2 is required to stabilize and maintain mature neurons.
Developmental absence of MeCP2 does not irreversibly damage neurons.
Neurological disorders in this disease are not irrevocable.
Inducible Cre-recombinase - Tet on/off system

**Tet off**

- tTA
- TSP
- tetR
- VP16
- pA

- -Dox
- +Dox

- tetO7
- TATA
- Target ORF
- pA

- tetO7
- TATA
- Target ORF
- pA

- Dox
- Dox

- X
Inducible Cre-recombinase - Tet on/off system

**Tet on**

- **rtTA**
- TSP
- rtetR
- VP16
- pA

When **Dox** is present (+Dox):
- rtTA binds to rtetR
- VP16 activates transcription
- Target ORF is transcribed

When **Dox** is absent (−Dox):
- rtTA cannot bind to rtetR
- Target ORF is not transcribed

**tetO7** TATA Target ORF pA

**tetO7** TATA Target ORF pA
Databases of mouse models for the biomedical research community:

http://jaxmice.jax.org/ - Database and repository of commercially available mice.
http://www.informatics.jax.org/imsr/index.jsp - International Mouse Strain Repository
http://www.informatics.jax.org/ - Mouse Genome Informatics: Phenotype database
http://www.eucomm.org/htgt/welcome - European Conditional Mouse Mutagenesis Program
Search Results

JAX Mice Database - 005622 B6.Cg-Shh<tm1(EGFP/cre)Cjt>/J
Stock# - 005622 Strain name - B6.Cg-Shh<tm1(EGFP/cre)Cjt>/J JAX® Mice Database
Site Section: JAX® Mice and Services
jaxmice.jax.org/strain/005622.html - 61Kb

JAX Mice Database - 005628 B6.129S2-Emx1<tm1(cre)Krj>/J
Stock# - 005628 Strain name - B6.129S2-Emx1<tm1(cre)Krj>/J JAX® Mice Database
Site Section: JAX® Mice and Services
jaxmice.jax.org/strain/005628.html - 30Kb

JAX Mice Database - 006302 B6.SJL-Slc6a3<tm1.1(cre)Bkmn>/J
Stock# - 006302 Strain name - B6.SJL-Slc6a3<tm1.1(cre)Bkmn>/J JAX® Mice Database
Site Section: JAX® Mice and Services
jaxmice.jax.org/strain/006302.html - 29Kb

JAX Mice Database - 004128 B6.Cg-Tg(Tek-cre)12Flv/J
Stock# - 004128 Strain name - B6.Cg-Tg(Tek-cre)12Flv/J JAX® Mice Database
Site Section: JAX® Mice and Services
jaxmice.jax.org/strain/004128.html - 30Kb

JAX Mice Database - 005975 B6.Cg-Tg(Plp1-cre/ESR1)3.16Pop/J
Stock# - 005975 Strain name - B6.Cg-Tg(Plp1-cre/ESR1)3.16Pop/J JAX® Mice Database
Site Section: JAX® Mice and Services
jaxmice.jax.org/strain/005975.html - 31Kb

JAX Mice Database - 007684 STOCK Tg(Atoh1-cre/ESR1)14Fsh/J
Stock# - 007684 Strain name - STOCK Tg(Atoh1-cre/ESR1)14Fsh/J JAX® Mice Database
Site Section: JAX® Mice and Services
jaxmice.jax.org/strain/007684.html - 31Kb

JAX Mice Database - 005965 STOCK Tg(Pomc1-cre)16Lowl/J
Stock# - 005965 Strain name - STOCK Tg(Pomc1-cre)16Lowl/J JAX® Mice Database
Site Section: JAX® Mice and Services
jaxmice.jax.org/strain/005965.html - 45Kb

Done
EU COMM Genes with Targeted / Trapped ES Cells

Below are links to lists of genes with available conditional gene traps and conditional targeted ES cells.

- Click here to view all 2828 EU COMM Conditional trapped and targeted genes.
- Click here to view all 254 ES COMM Conditional trapped genes only.
- Click here to view all 442 EU COMM Conditional targeted genes only.

Genes in the EU COMM Gene Targeting Pipeline

We invite the scientific community to help prioritize the genes for targeted mutagenesis in ES cells by emailing us at eucomm@sanger.ac.uk.

- Click here to view pipeline status of all 442 EU COMM genes selected for conditional targeted mutagenesis as of 30 June 2008.
- Click here to view all pipeline status explanations.

Summary

<table>
<thead>
<tr>
<th>Stage</th>
<th>Totals</th>
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<tr>
<td>Gene Trap Annotated</td>
<td>2514</td>
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<tr>
<td>ES Cells: Targeting Confirmed</td>
<td>443</td>
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<td>Intermediate Vector Complete</td>
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</table>
Genetically modified mouse models as powerful tools to further our understanding of gene function in health and disease.

BUT mice are mammals, expensive, breed ‘slowly’, aren’t see-through….

- flies
- fish
- worms
HOME READ:

Guy J, Gan J, Selfridge J, Cobb S, Bird A.
Reversal of neurological defects in a mouse model of Rett syndrome.