Transgenic animal models:
Impact on Biomedical Research

Margit Mahlapuu 2007-12-06

Advantages of Using Mice as Model Organisms

Advantages:

✓overall physiological similarity to man

✓small size: easy housing and handling, relatively inexpensive

✓short gestation time (20 days)

✓short generation time (attains sexual maturity 4-6 weeks)

✓availability of inbred strains

✓established embryonic stem cell lines

✓high resolution genetic, physical, linkage maps, sequence in mouse available :

-similar number of genes in mouse and human: ~ 30,000; haploid mouse and human genome: ~ 3 x 10^9 bp

- comparative maps between mouse and humans (**http://www.informatics.jax.org**)

Disadvantages:

✓ short life span (less than 2 years) does not allow diseases of ageing to be studied effectively (Alzheimers' disease, cancer etc)

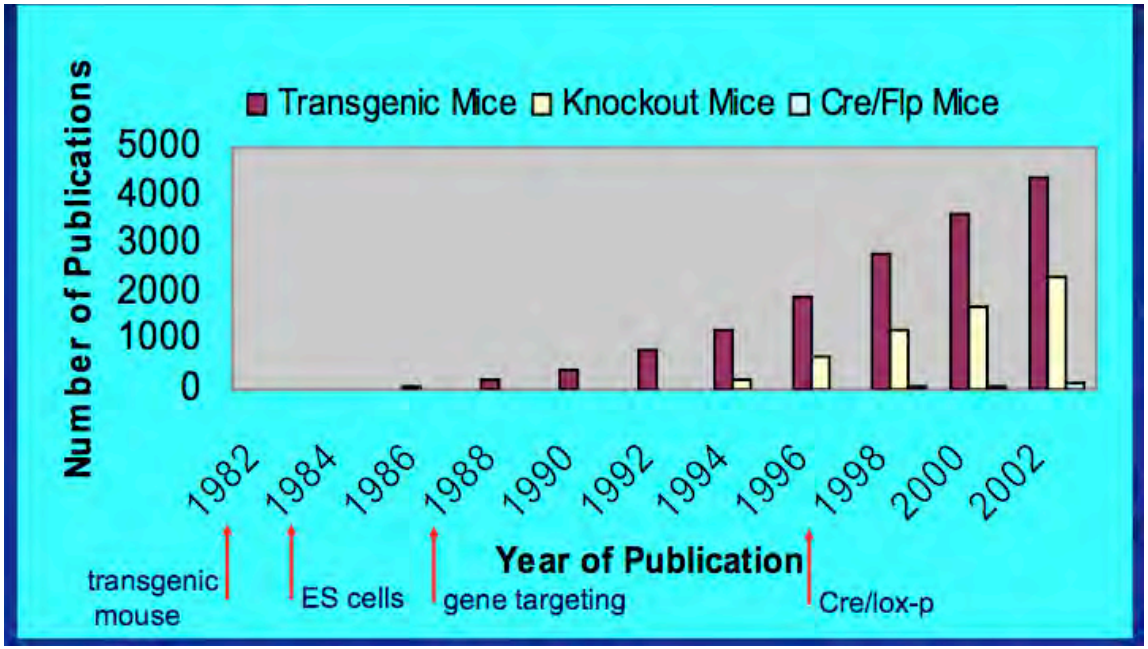
✓ certain difference in in functional role of genes, gene expression, physiology, etc. comparing mouse to man (evolutionary distance of 75 Myears between mouse and man)

Genetically modified mouse models: Historic overview

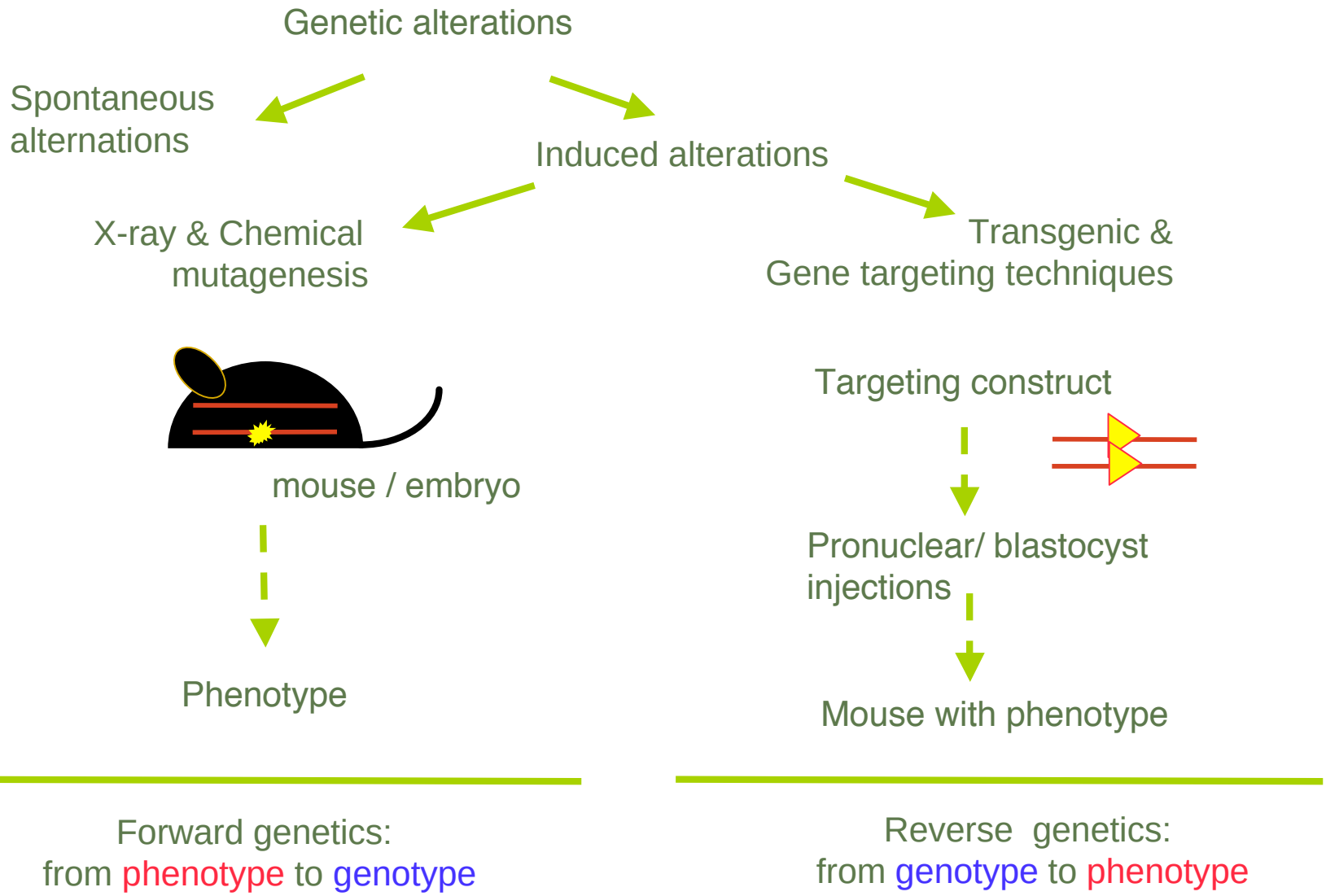
Mid-1970's -several labs, including those of Dr. R.Jaenisch and Dr. R. Mulligan at MIT, infected mouse embryos with retroviruses and demonstrated that viral DNA integrated into the genome and was passed to subsequent generations.

1980-81: Several groups (Gordon et al., Brinster, Constantini et al, Lacy et al.) reported the development of transgenic mice by microinjecting genes into the pronucleus of a fertilized egg.

1982: The first visible phenotype was shown by Dr. R.Palmiter and colleagues in mice overexpressing rat growth hormone.



Genetic alterations in mice



Spontaneous mutations: examples

✓In 1966, a new 'hairless' phenotype in mice that had spontaneously arisen in an albino stock was described (Flanagan SP, *Genet Res.* 1966).

✓**Nude mice** also suffer from an agenesis of the thymus and are severely immuno-compromised.

✓Today, Nude mice are widely used as hosts for transplanted human tumors.

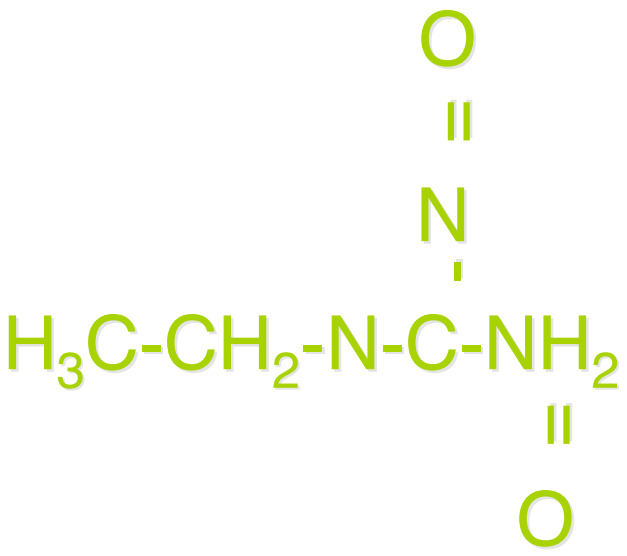
✓In 1994, the molecular nature of the nude defect was characterized: Foxn1 was identified as the product of the *nude* gene (Nehls M *et al*., *Nature* 1994).

Induced mutagenesis: Mouse supermutagen ENU

✓Most powerful mutagen in the mouse

✓Single base changes that define single gene function

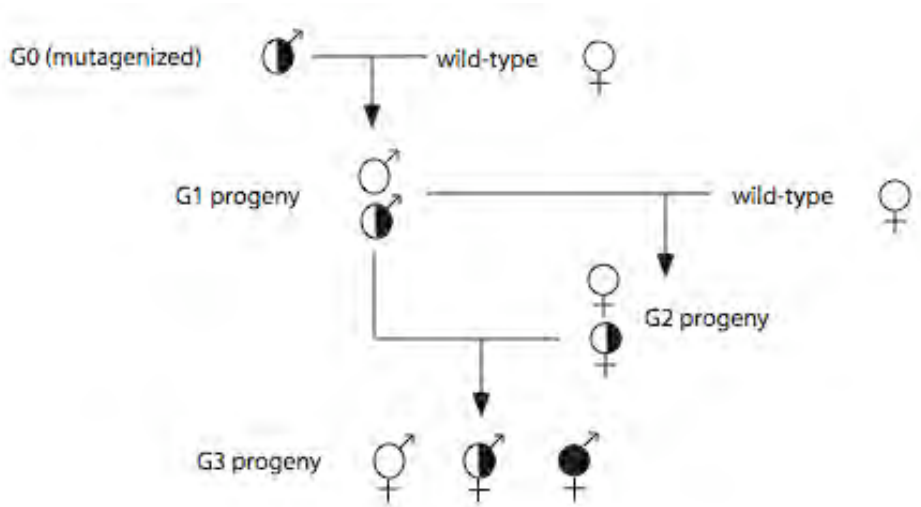
✓Alterations can affect the function of the protein product in many ways



N-ethyl-N-nitrosourea

ENU mutagenesis

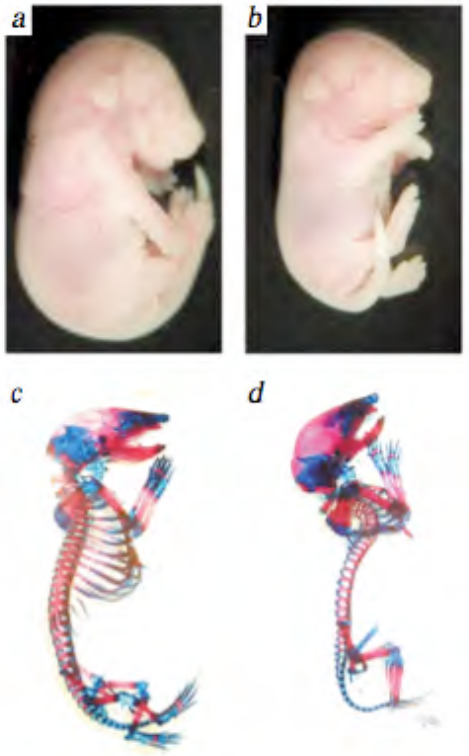
- ✓ Adult male mice are injected with ENU to mutagenize spermatogonial stem cells
- ✓ Germ cells are depleted after treatment, causing temporary sterility
- ✓ After regaining fertility, males are bred in different breeding schemes designed to recover recessive mutations



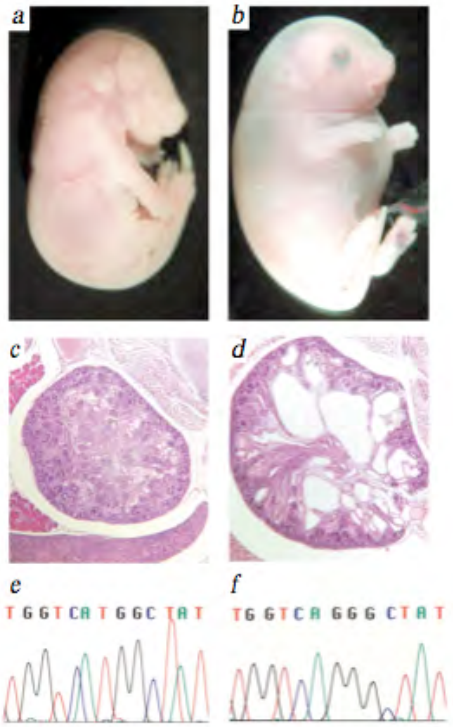
Breeding scheme for recovering recessive mutations

ENU mutagenesis: examples

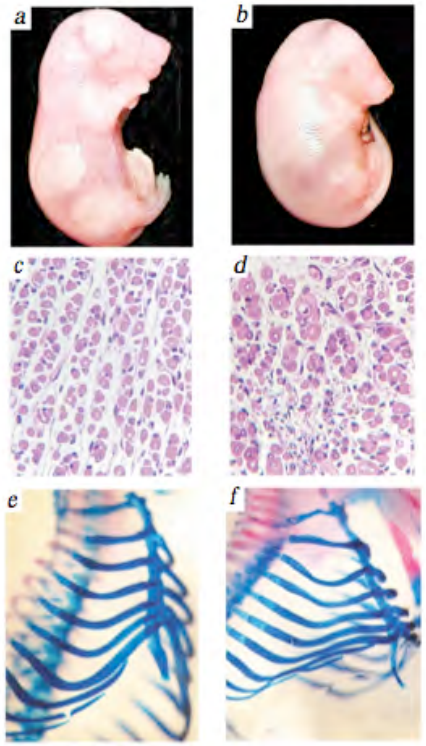
Herron BJ *et al.*, Nature Genetics 2002: the progeny of ENU mutagenized mice was screened at E18.5 for abnormalities in organogenesis:



Line 104: Shortened A-P axis, skeletal abnormalities.



Line 175: severe edema, large cysts in kidneys. Caused by substitution in *Pkd1* gene.



Line 156: edema, abnormal muscle morphology and rib structure. Caused by mutation in *Ryr1 gene.*

Genetic modifications of mouse embryos:

✓Transgenic techniques

✓Knock-out techniques

Transgenic mice: applications

Transgenic mice: Foreign DNA introduced into the germ line of mice by pronuclear injection; random insertion into the mouse genome

Applications:

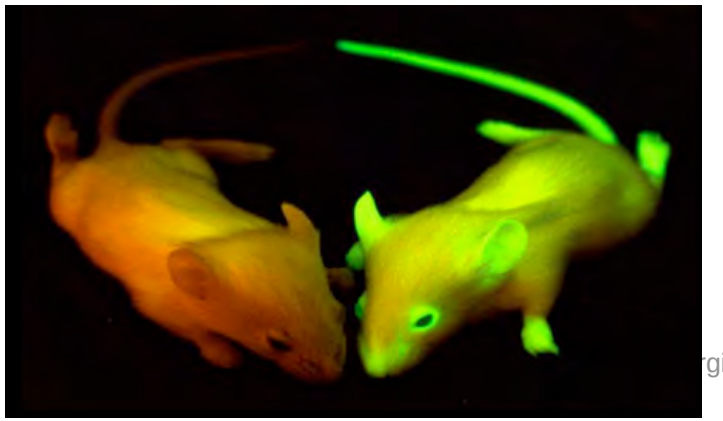
✓Over-expression of an endogenous gene
✓Ectopic expression of an endogenous gene
✓Over-expression of a mutant version of a gene
✓Expression of genes from other species

Limitation:

✓ Integrate at variable copy number into random sites
✓ Subject to position effects

rat GH transgenic mice
(Palmiter RD *et al.*, Nature 1982)

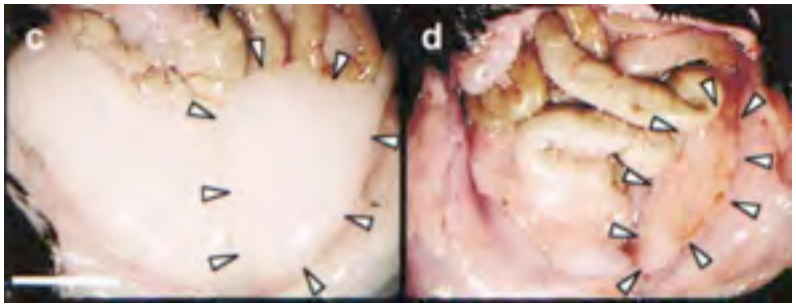
jellyfish GFP transgenic mice
(Hadjantonakis AK *et al.*, Mech. Dev. 1998)

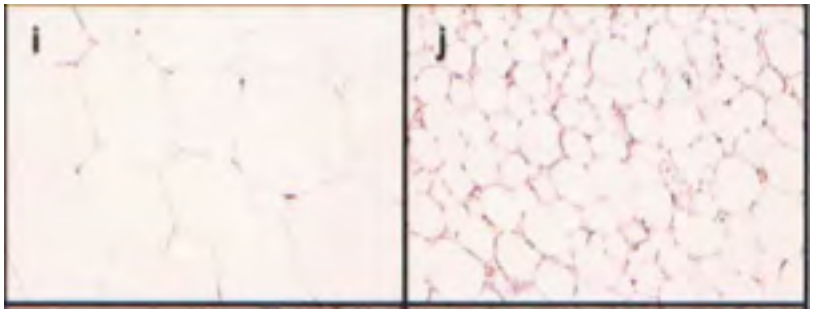


Conventional transgenes: targeting construct

Conventional transgenes - specific promotor sequence is used to drive expression of target transgene to a certain tissue/cell type

Example: increased expression of *Foxc2* in WAT & BAT (Cederberg A. *et al.,* Cell 2001) leads to a lean and insulin sensitive phenotype





c, i wt; d, j Foxc2 tg
Cederberg *et al* Cell 2001

Inducible transgenes: activate or silence a transgene where and when we want

Advantages:

✓Expression can be controlled in time

✓Expression levels proportional to inducer concentrations

✓Expression of transgene is reversible

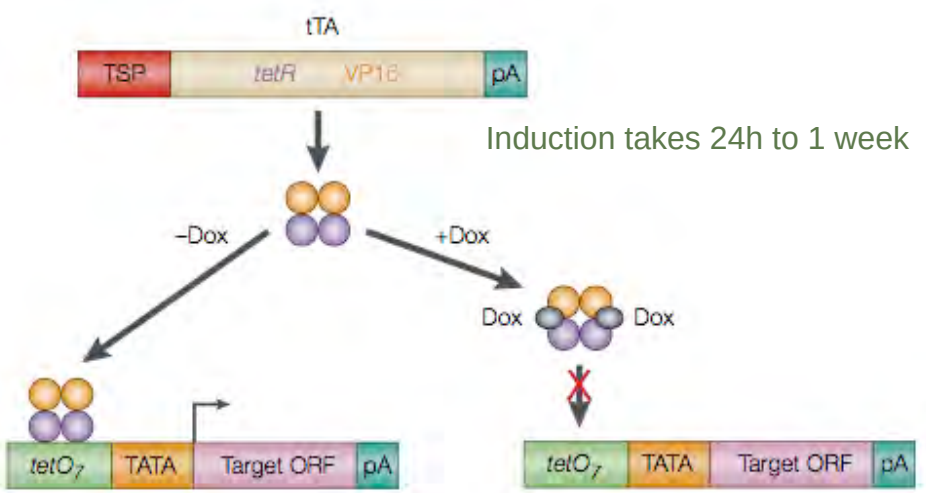
✓Distinguish role in embryogenesis and adulthood

Historic overview:

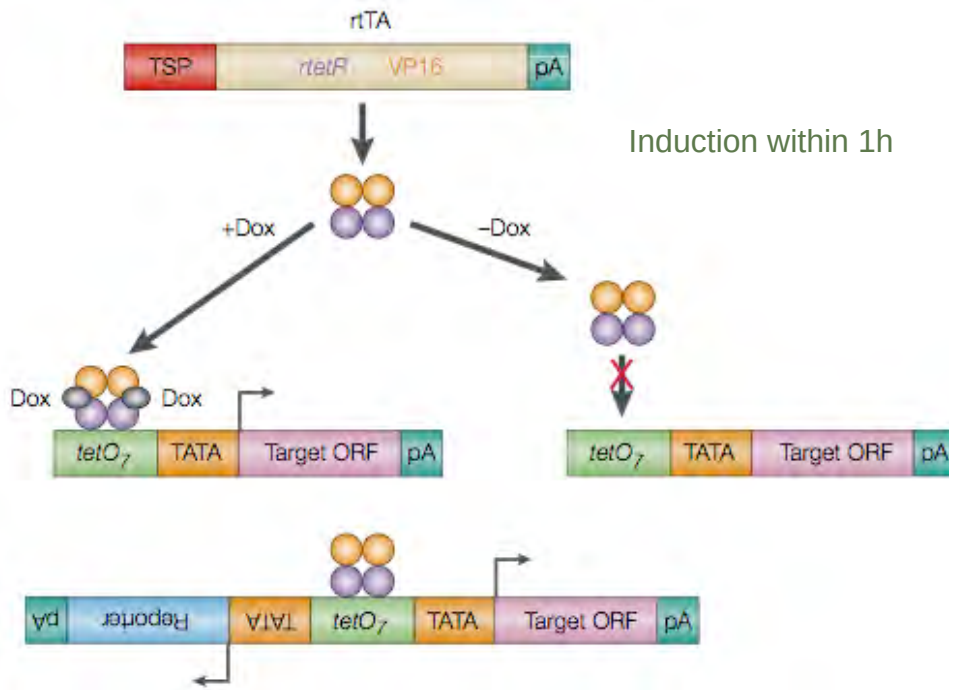
Single transgenes with promoters that could be induced by heavy metals, heat shock, interferon or steroids were unsuccessful. Binary systems (“effector transgene” acting on “target transgene” with components derived from systems evolutionarily distant from mouse) turned to be a success.

Inducible transgenes: tTA-based systems

tTA (tet-off)



reverse tTA (tet-on)

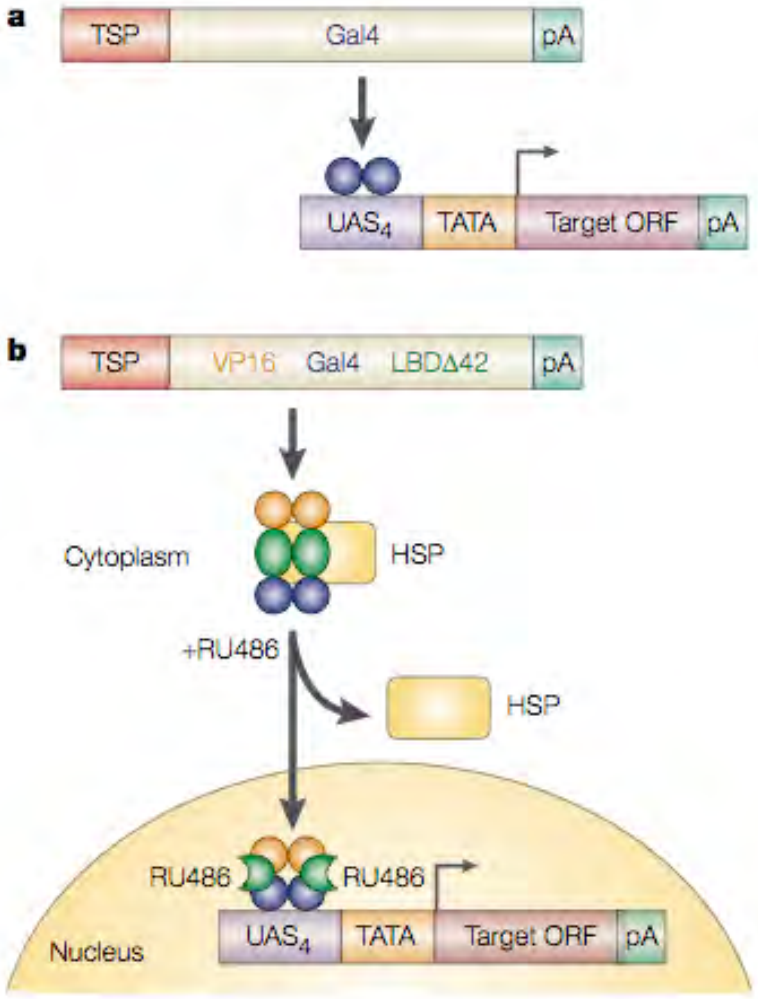


M. Lewandoski Nature Reviews 2001

TetR-based systems (Gossen & Bujard PNAS 92):

Effector: fusion of VP16 (HSV) transactivation domain and *E. coli* tetracycline repressor (TetR), which binds both tetracycline and 19-bp operator sequences (tetO) of the tet operon in the target transgene. tTA, does not bind DNA when inducer is present, rtTA binds DNA only when inducer is not present.

Inducible transgenes: Gal4-based systems



A) In *S. cerevisiae*, transcription factor Gal4 directs transcription of Gal4-responive genes by binding to 17 bp upstream activating sequences (UAS).

B) HSV VP16 activation domain and Gal4 DNA-binding domain and the ligand-binding domains of the progesterone receptor, mutated such that it fails to bind progestone but can bind antiprogestins, are fused. The complex remains in the cytoplasm due to the binding of heat-shock proteins, unless antiprogestins are added.

M. Lewandoski Nature Reviews 2001

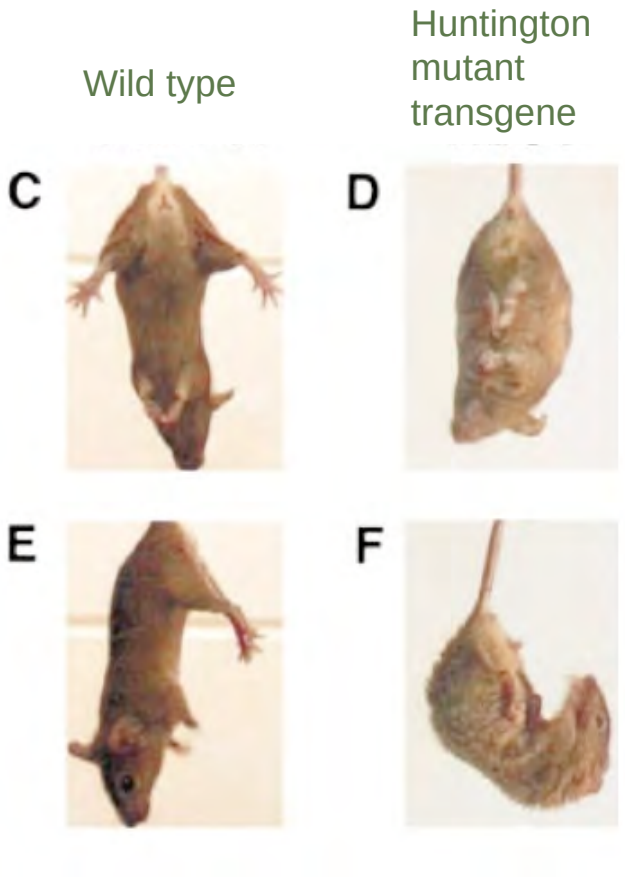
Conditional transgenes: examples (1)

✓Inducing and repression of the expression of oncogenes using tTA or rtTA in solid tumours and leukemias has shown that oncogene that induces tumorigenesis in also required for its maintenance.

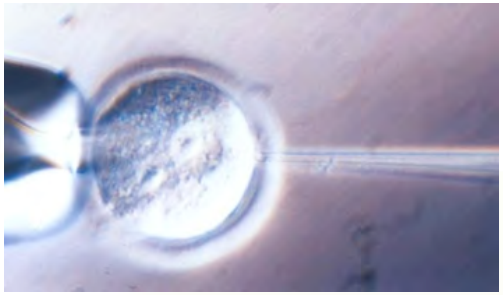
This indicates that therapeutic strategies that target the activity of a single molecule might be clinically effective.

Inducible transgenes: examples (2)

In a **mouse model of Huntington disease**, the neuropathology and behavioral abnormalities are reversed when the mutant huntingtin transgene is repressed by Dox (Yamamoto A *et al.*, Cell 2000).



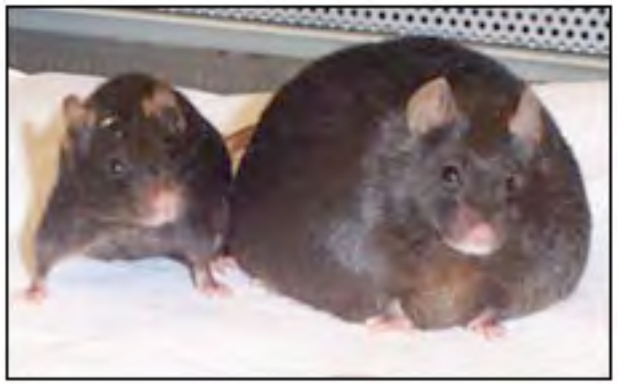
Pronuclear microinjections: making a transgenic mouse



PMSG+hCG injections, mated with vasectomized males

Gene targeting

Gene targeting: introduce specific mutations into endogenous genes.



Mice without the **leptin** are morbidly obese (right).

Mice lacking **Scd-1** stay skinny compared to wild type littermates (Ntambi JM *et al*., PNAS 2002).

Gene Targeting: overview

Gene targeting: Gene knock-outs/knock-ins introduce specific mutations into endogenous genes. Mutations are created by homologous recombination in embryonic stem cells (ES) which contribute to all cell lineages when injected into blastocysts.

Gene targeting approaches can be divided:

- ✓ General (conventional)
- ✓ Tissue-specific (conditional)

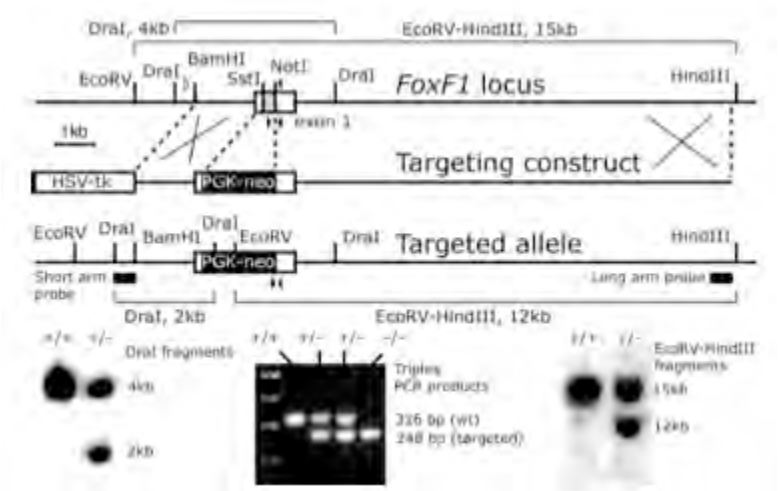
Applications:

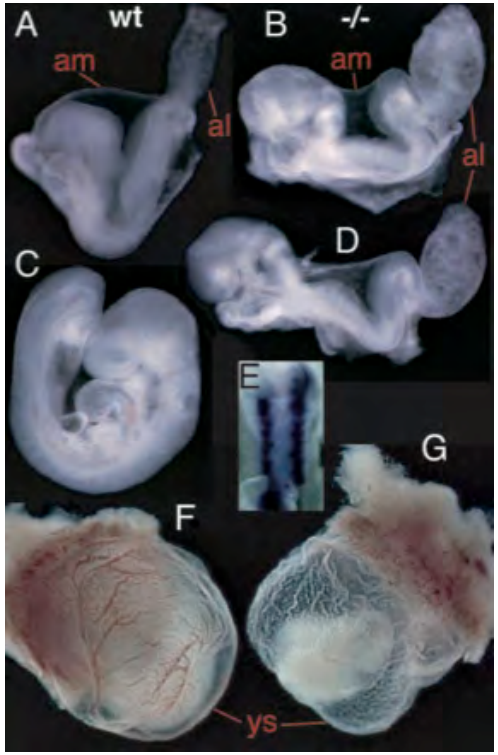
- ✓ Knock-in
- ✓ Knock-out

Constitutive gene-inactivation

Complete knock-out of a gene by:
- ✓Exon deletion, deletion of functional domains
- ✓Introduction of STOP codons
- ✓Introduction of frameshift mutations
- ✓Knock-out of enhancer/silencer elements

Targeting vectors can be either:
- ✓replacement-type gene targeting vectors
- ✓insertion-type gene targeting vectors





Mahlapuu *et al* Development 2001

Conditional gene inactivation: applications and limitations

Conditional gene knock-out allows gene inactivation in a specific cell population at desired time.

Requires a two component system:

✓A gene knock-out line insertion of loxP/FRT sites into a “neutral” region surrounding critical exon/s

✓Transgneic recombinase line: expression of Cre/FLP recombinase

Applications:

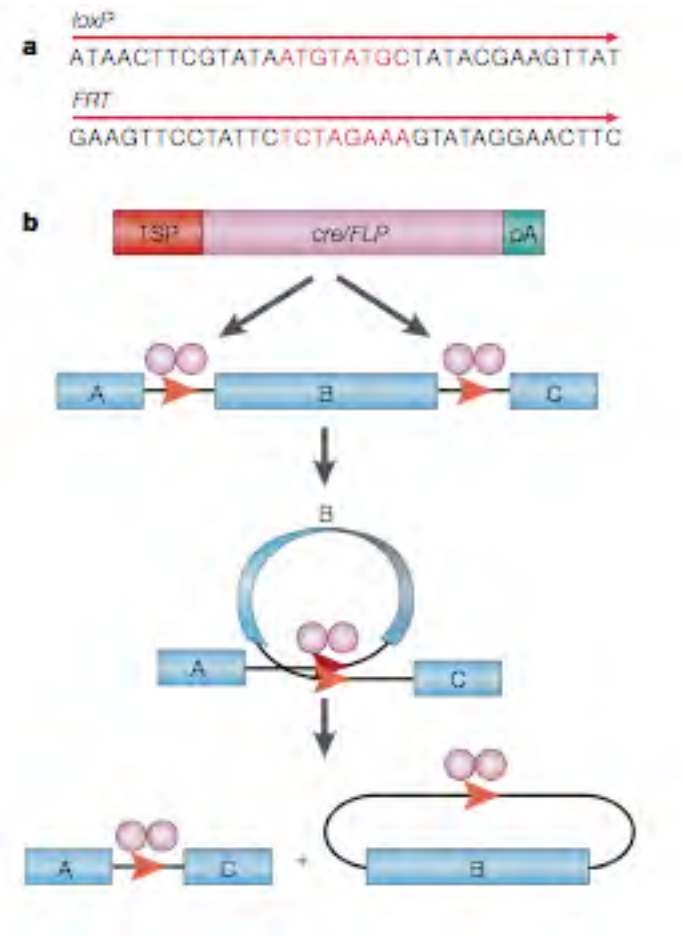
✓a widely expressed gene can be tested for function in a particular cell lineage without being influenced by gene loss in adjacent tissues, as the rest of the embryo is genetically wild-type

✓ used to investigate gene function at late embryonic stages or in the adult if null mutations lead to a severe or lethal phenotype during embryonic

Limitations (summarized by Schmidt-Supprian & Rajewsky, Nature Immunology, 2007):

✓recombination of the target locus occurs efficiently in the designated cell lineage, otherwise excision will be mosaic so that mutation is induced in only a fraction of the desired cells.

Conditional gene targeting: targeting construct



✓ Cre from bacteriophage P1 and Flp from *S. cerevisiae*, catalyze DNA recombination events between two 34-bp recognition sites (loxP and FRT, respectively).

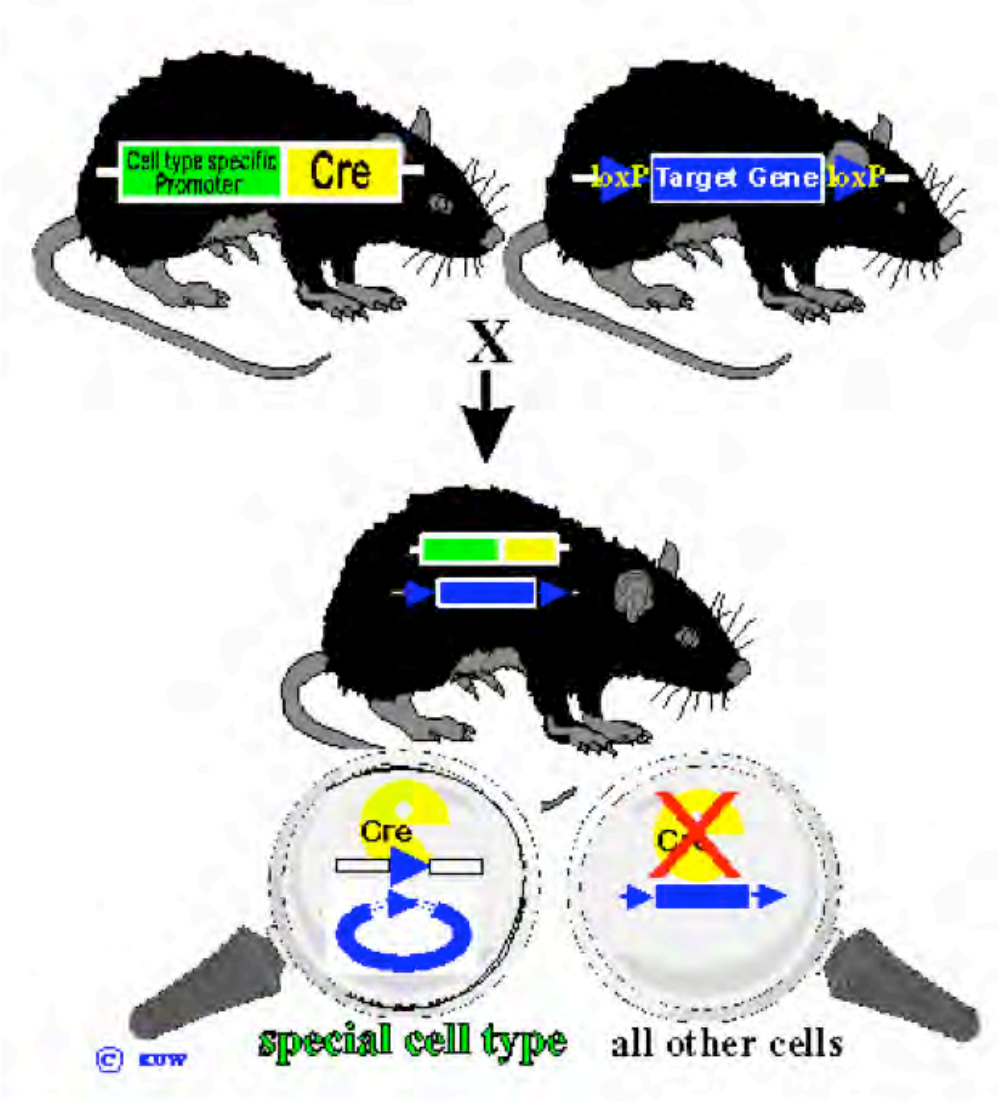
✓Expression of these recombinases leads to deletion of DNA fragments that have been flanked by directly repeated loxP or FRT sites (“flowed” or “flrted” alleles, respectively).

✓Before recombination, the conditional allele should have wild-type activity, so in most cases, loxP sites are placed into introns.

✓Cre/Flp can be temporaly controlled by Cre transgenes conrolled by TetR-based system.

Conditional gene targeting

By crossing a mouse line with a conditional allele to an effector mouse line that expresses Cre in TSP manner, progeny are produced in which the conditional allele is inactivated only in those tissues or cells that express Cre.



Examples of different Cre mice available

Table 2 | **Examples of tissue-specific *cre*-expressing mice**

Tissue-specific promoter	Tissue/cell of expression	Floxed target gene
Lck	T cells	DNA polymerase-β
Cryaa	Eye lens	*SV40* (activated)
Ins2	Pancreatic β-cells	*Gck*
Alb	Liver	*Gck*
Myog	Skeletal muscle	Diphtheria toxin A[i]
KRT5	Epidermis	β1-integrin
Nes	Neuronal cells	*Mecp2*
Ins2	Pancreatic β-cells	*hGH*[ii]
Gcg	Pancreatic α-cells	*hGH*[ii]
Ppy	Pancreatic β-cells	*hGH*[ii]
Pdx1	Pancreatic α-cells	*hGH*[ii]
En2	Mid/hindbrain	*lacZ*[iii]
Wnt1	Neural crest	*lacZ*[iii]
Camk2a	Forebrain	*Hdh*
Nes	Branchial arch	*Fgf8*
Msx2	Apical ectodermal ridge of limb bud	*Fgf8*
Pax6	Retina	*Pax6*

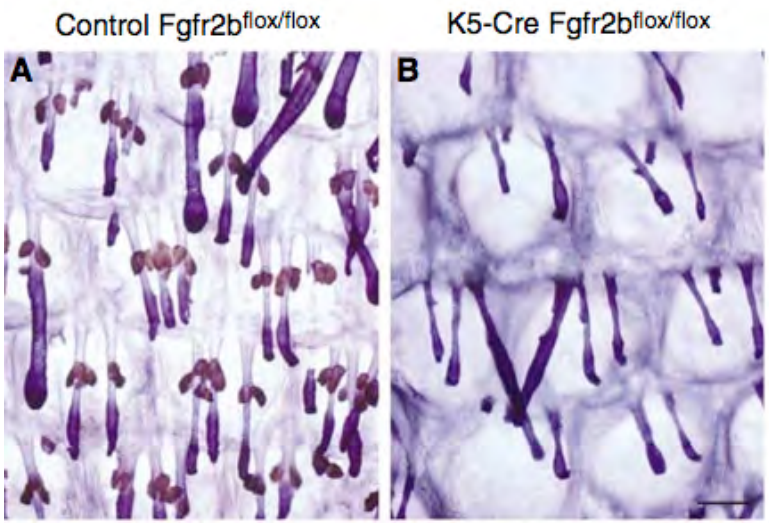
Conditional gene targeting: examples

✓Fgfr2b knock out mice die at birth due to multiple developmental defects (Revest JM *et al.*, Dev. Biol. 2001).

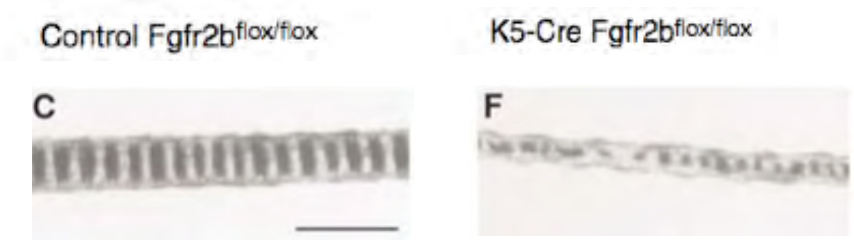
✓Using Cre-Lox strategy, Fgfr2b gene was deleted only in the epidermis starting from E15.5.

✓The mice lacking Fgfr2b in epidermis display abnormalities in hair and sebaceous gland development (Grose R *et al.*, EMBO J. 2007).

Sebaceous gland atrophy



Disorganized hair structure



Knock-in: applications

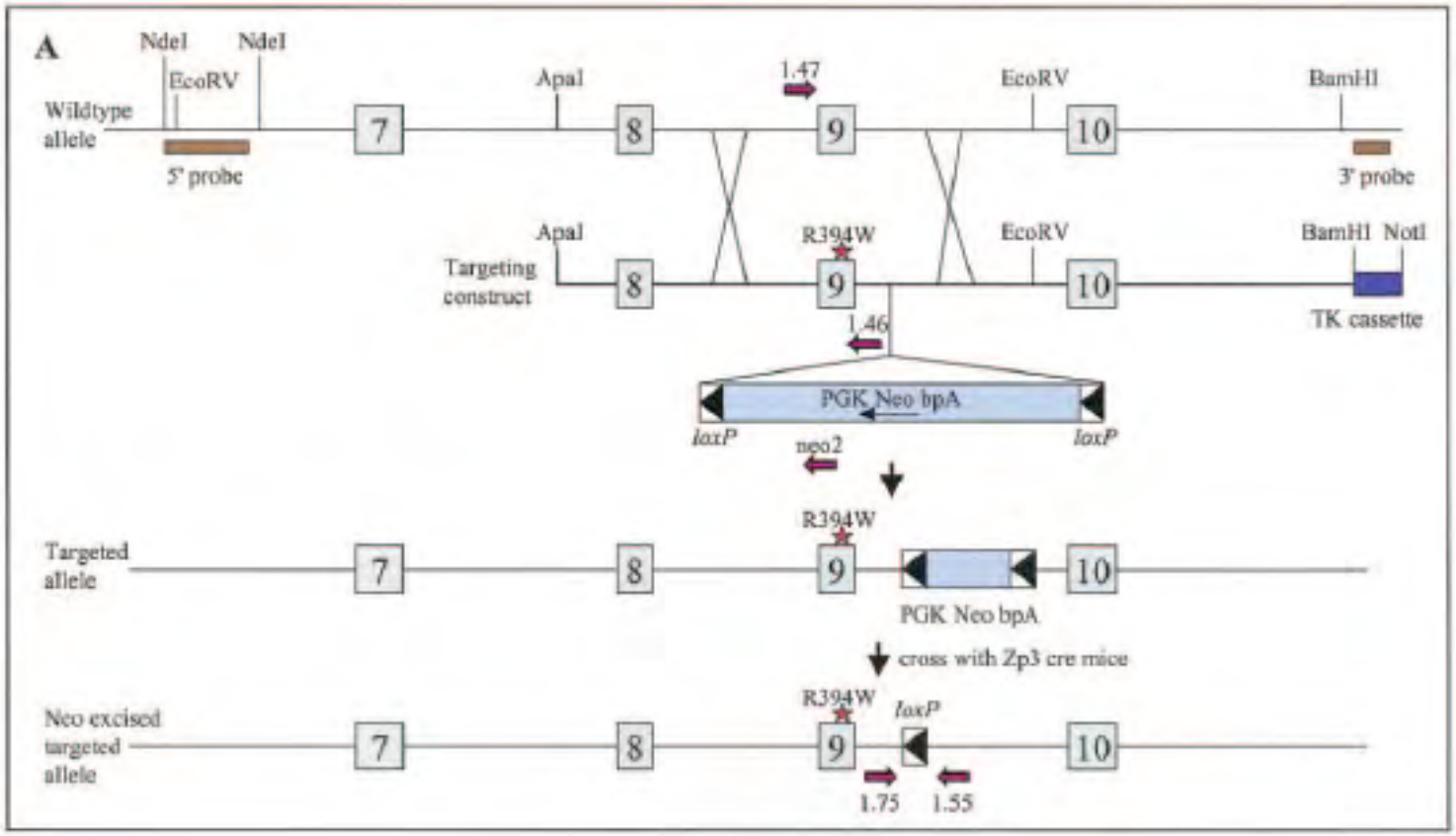
Applications:

✓Introduce a specific mutation

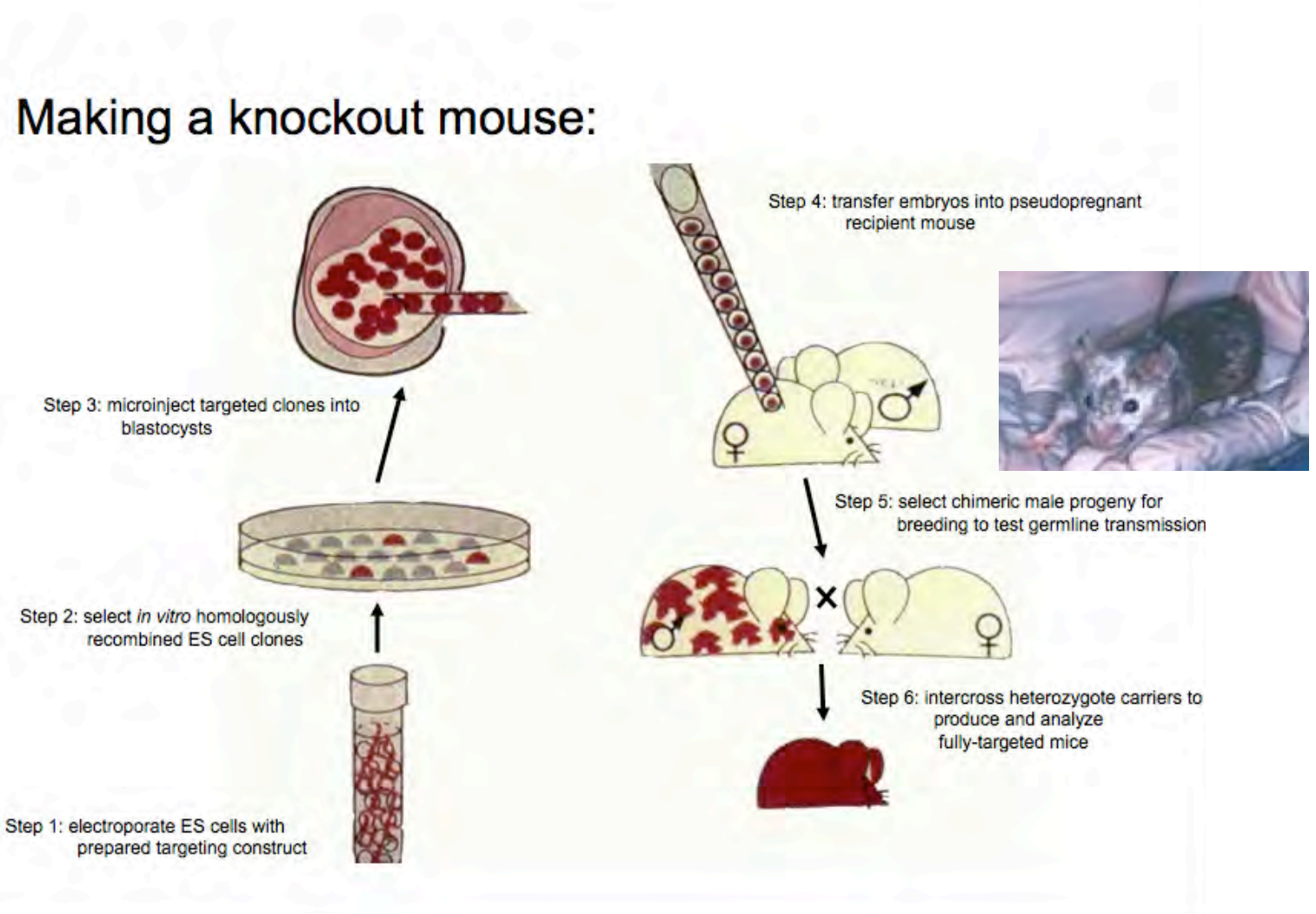
✓Incorporate lac Z reporter to follow tissue expression

Targeting vector: knock in

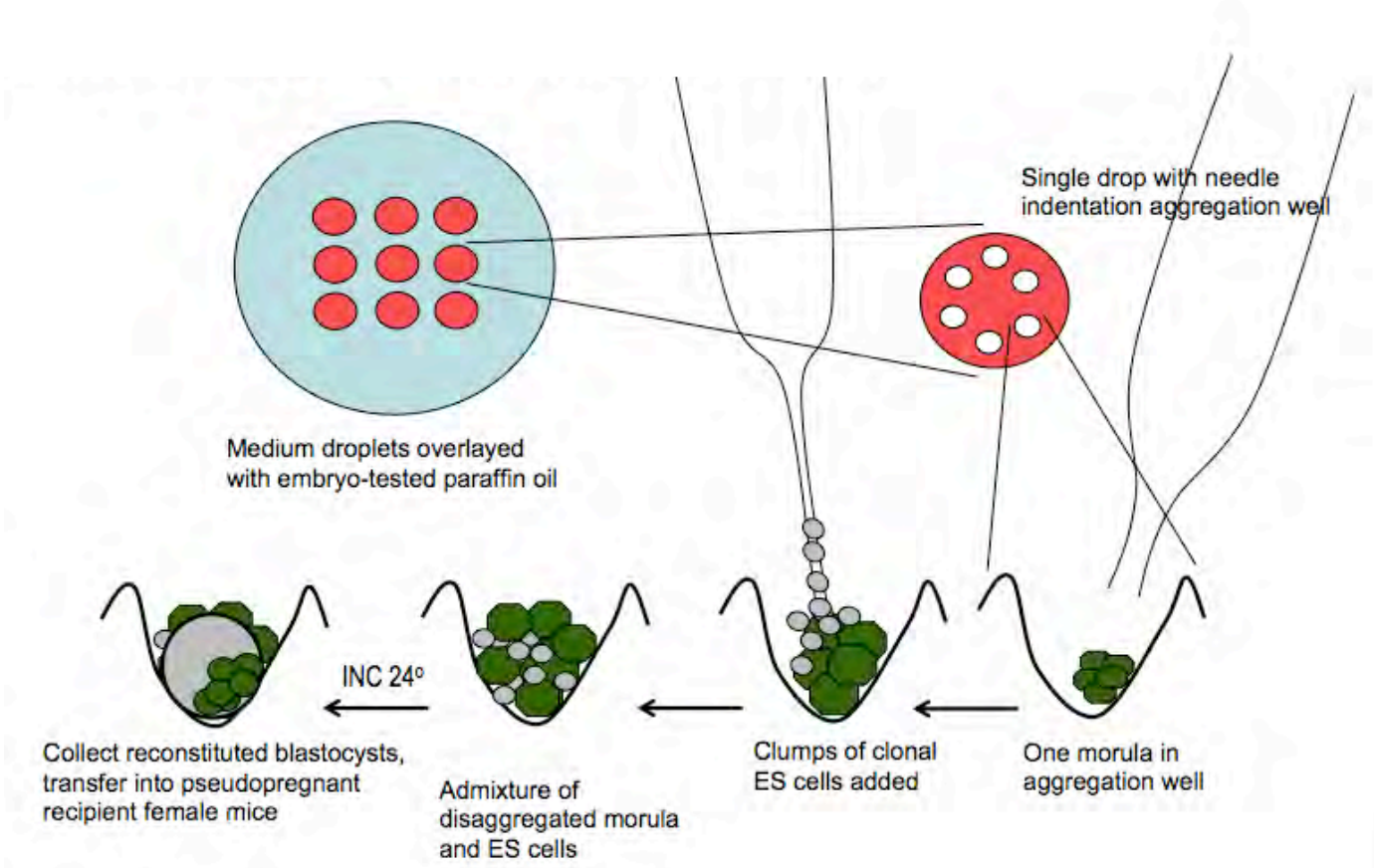
Example of knock in : mouse strain carrying missense mutation in Wilms tumor gene (Wt1 R394W) -
early onset renal failure (Denys-Drash Syndrome)
Gao *et al.* Mol. Cell. Biol. 24:9899 (2004)



Blastocyst injection with targeted ES cells



Morula aggregation

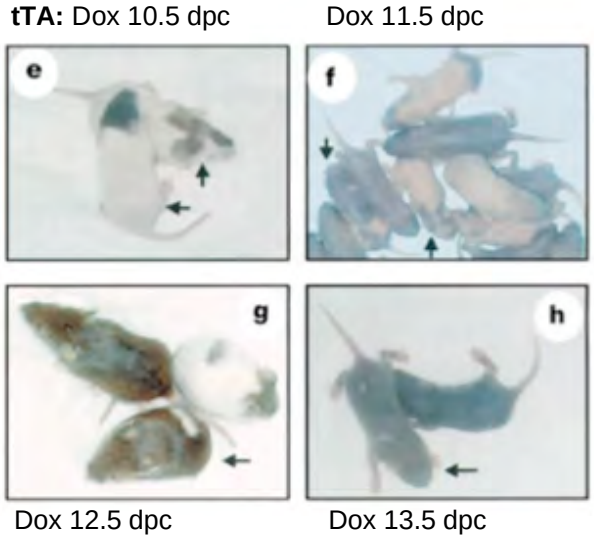


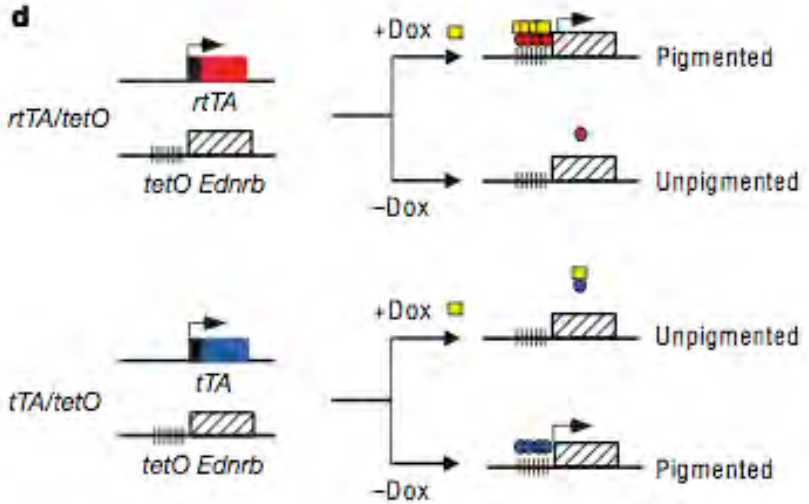
Combination of different targeting events in one mouse

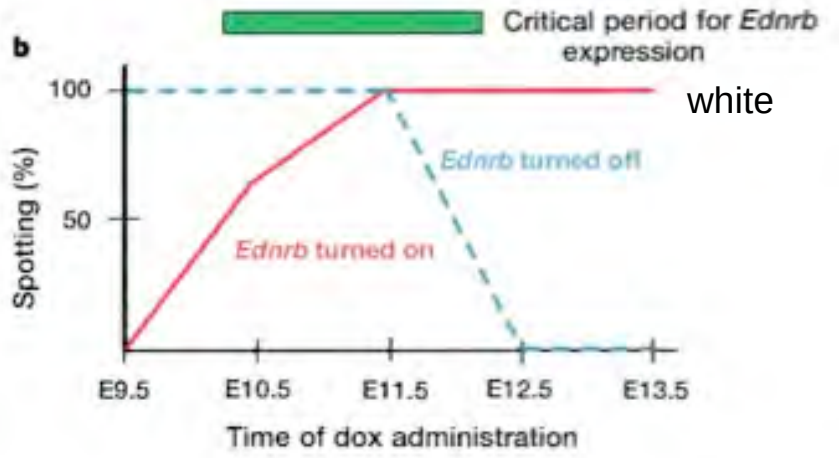
Possibility to combine multiple mutations within the same mouse to delineate molecular pathways; gene interaction, rescue of phenotype, etc.

Inducible gene targeting: Examples

•Endothelin receptor B (EDNRB) is a GPC required for the development of melanocytes and enteric neurons.
•*Ednrb*-/- mice are white and die as juveniles from megacolon.
•To determine when EDNRB signaling is required during embryogenesis, strains of mice in which the endogenous *Ednrb* locus was under the control of tTa or rtTA were generated (Shin et al., *Nature*, 1999): EDNRB is required during a restricted period of between 10 and 12.5dpc.







Supporting techniques

- ✓IVF
- ✓Embryo transfer
- ✓Cryopreservation

In Vitro fertilizations

Applications:
Production of offspring from non-mating mice

Procedures:
Superovulation of female mice
Sperm recovered from epididymis of male mice
Mix together 13.5-15 h after hCG administration
Incubate 4 h, 37 C
Embryo transfer

Embryo transfer

Oviduct Transfer
✓1-cell embryo to morulae
✓Ampulla of 0.5 day postcoitum pseudopregnant recipient

Uterine Transfer
✓3.5 day blastocyst
✓uterine horns of 2.5 day postcoitum pseudopregnant recipient

Cryopreservations

Applications:
To archive the exponentially growing number of strains resulting from transgenic and targeted mutation technologies

Procedure:
✓Collection of embryos (1-8 cell)

✓Freezing and storage of embryos

✓Derivation by embryo transfer

Transgenic models in drug design & development:

- ✓ Gene regulation studies
- ✓ Mechanism of action studies
- ✓ Off-target effects
- ✓ Disease models
- ✓ Transgenic animals as bioreactors

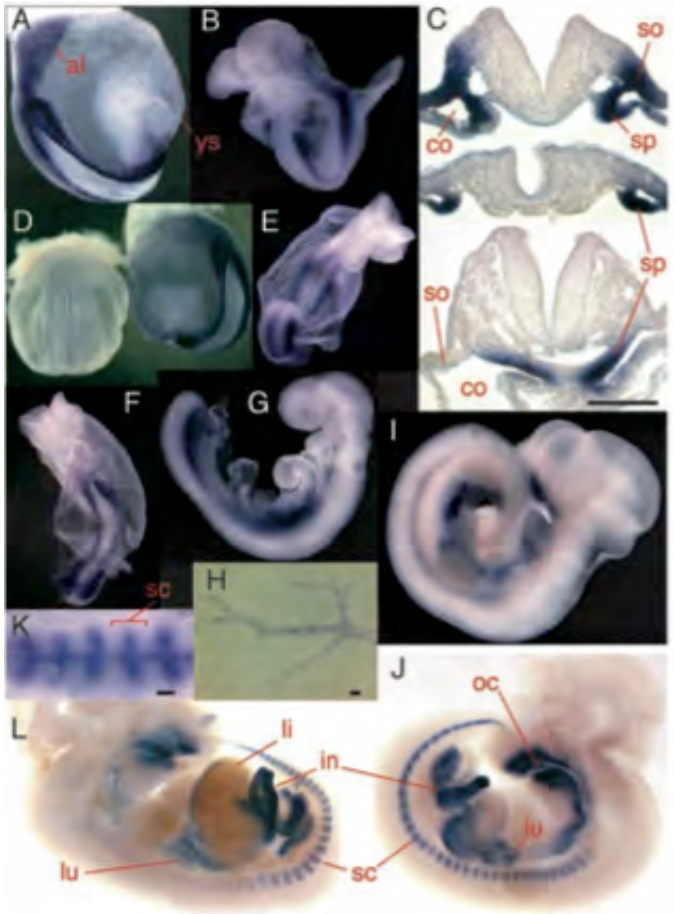
Gene regulation studies:

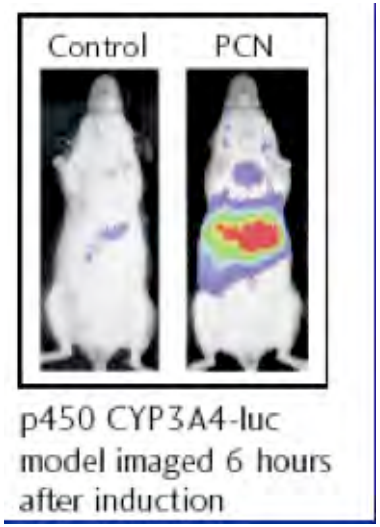
Applications:

- ✓ Site of expression
- ✓ Intensity of expression
- ✓ Modulation during development process
- ✓ Response to stimuli (drug challenge) etc.

Reporter genes:

- ✓ β-galactosidase encoded by the bacterial gene ***lacZ*** cleaves lactose into glucose and galactose. Also, β-galactosidase cleaves the colorless substrate X-gal into galactose and a blue insoluble product.
- ✓ GFP from green jellyfish
- ✓ Luciferase from firefly





p450 CYP3A4-luc model imaged 6 hours after induction

Mechanism of actions studies:

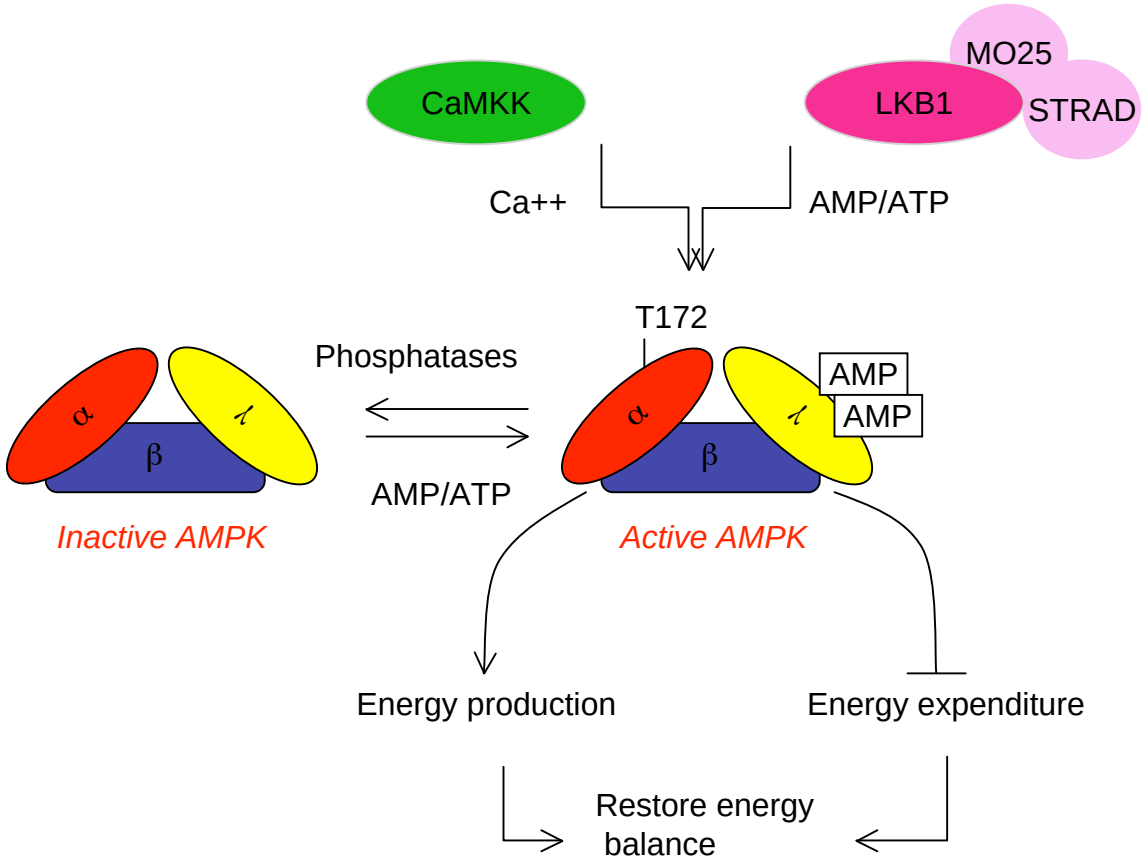
Applications

- ✓ Identification of new potential targets for therapeutic intervention
- ✓ Predicting potential side effects
- ✓ Evidence for a role of a gene in disease

Example 1:
Mouse models for AMPK γ3

Example 2:
MCHR1-/- mice

AMPK – an intracellular sensor by which organisms regulate energy status



AMPK γ3 - potential target in T2DM?

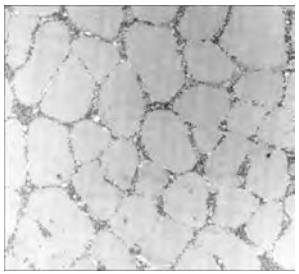
RN$^-$ phenotype affecting glucose and fat metabolism in skeletal muscle of pigs is caused by a R225Q mutation in γ3 subunit of AMPK

A Hampshire pig

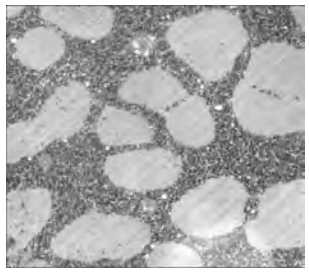
RN$^-$ phenotype is characterized by:

- Increased glycogen content by about 70% in skeletal muscle but not in liver or heart!
- Higher oxidative capacity in white skeletal muscle.
- No negative effects on health.

rn+/rn+



RN-/rn+ or RN-/RN-



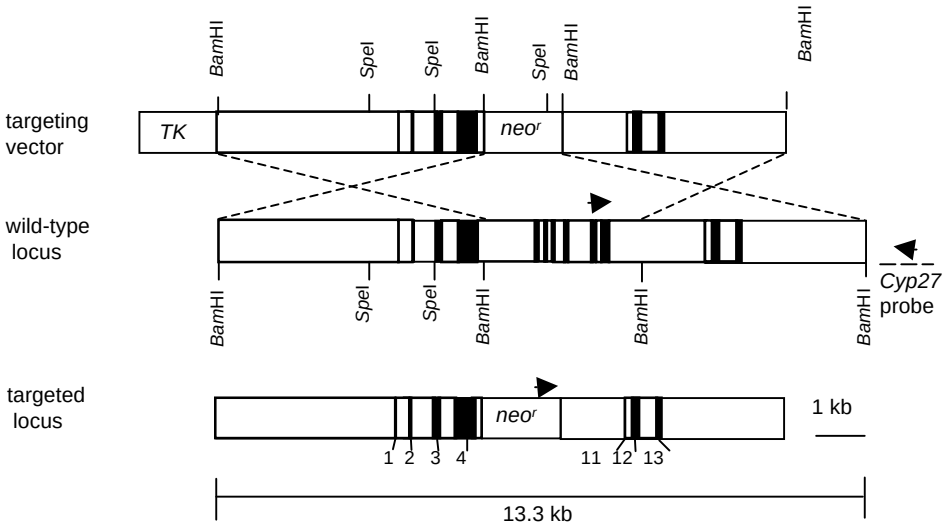
Glycogen content in skeletal muscle fibers

L. Andersson, Science 2000

Mouse models of AMPK γ3: targeting constructs

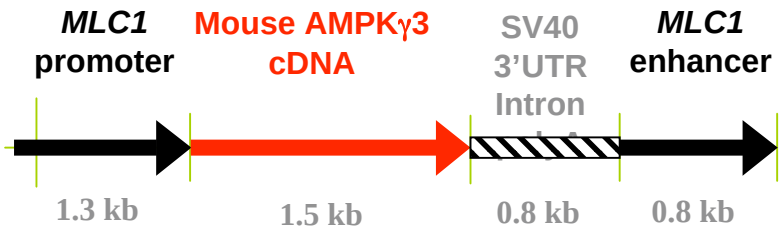
✓transgenic mice with skeletal muscle-specific overexpression of wt AMPK γ3
✓transgenic mice with skeletal muscle-specific overexpression of mutant AMPK (R225Q) γ3
✓AMPK γ3 knock out mice

•γ3 knock out construct generated by conventional gene targeting strategy replaces exons 5-10 with the neomycin resistance gene.

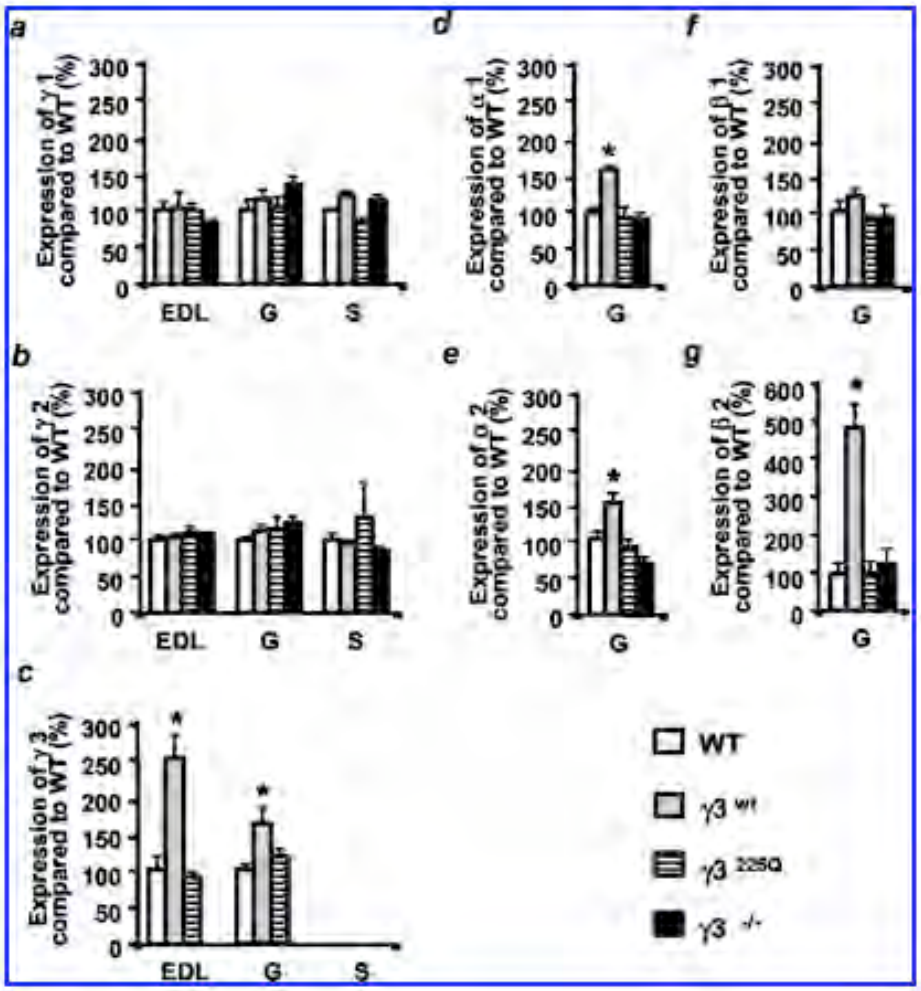


Barnes JBC 2004

•Transgenic mice expressing wild type (γ3 ^{WT}) or mutant form of γ3 (γ3 ^{225Q}) were generated using a myosin light chain (MLC1) promoter and enhancer, which result in the expression of the transgene in white skeletal muscle.



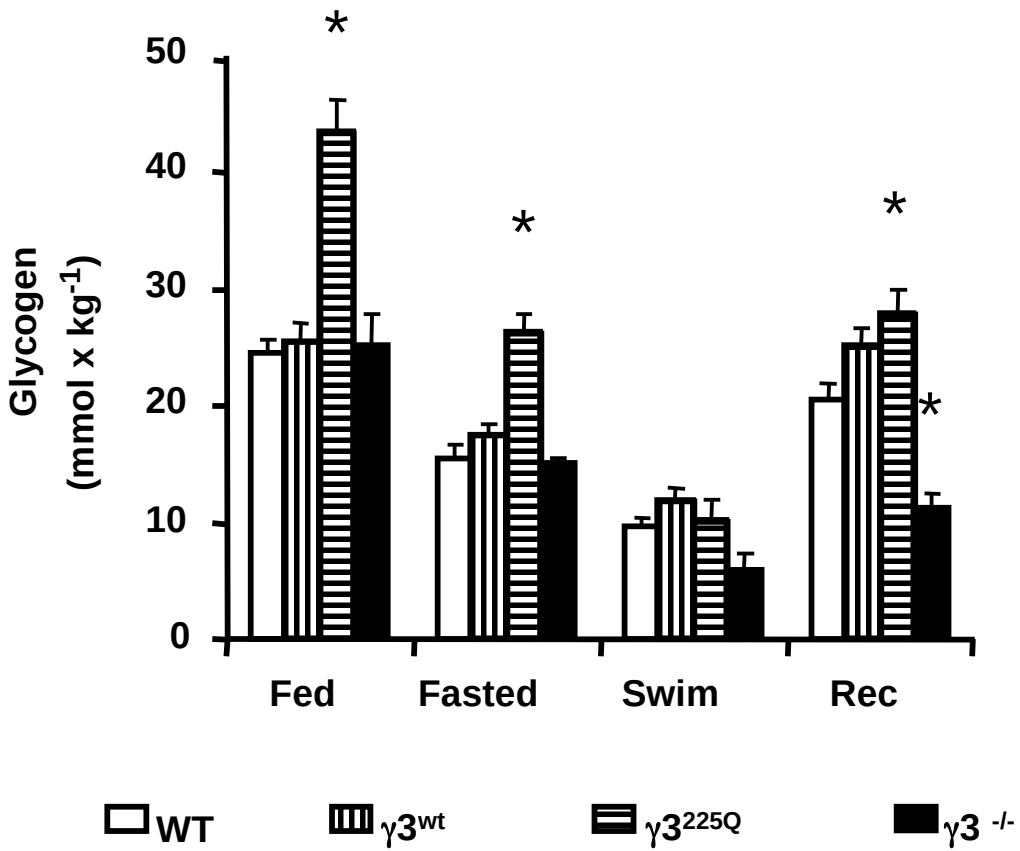
Mouse models of AMPK γ3: expression analysis



Protein expression analysis (Western blot) indicates that R225Q mutant transgenic line can be though of as “functional knock in” model: R225Q substituted γ3 replaces the endogenous γ3 .

AMPK R225Q γ3 mice replicate RN- phenotype in pigs

Phenotypic analysis of mouse models: the role of AMPK γ3 in glycogen storage

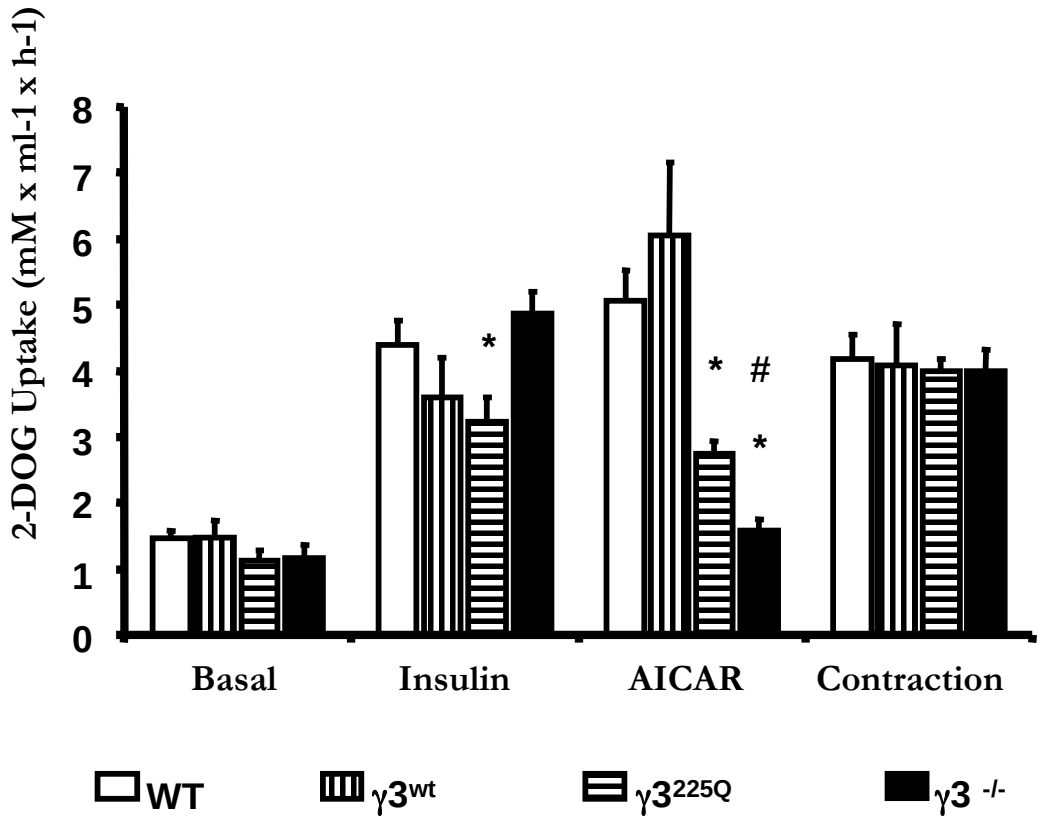


- Transgenic mutant mice show excessive glycogen accumulation in skeletal muscle thus replicating the RN- phenotype in pigs.
- Glycogen content after swimming (Swim) was similar between all genotypes indicating that AMPK γ3 is not required for glycogen utilization.
- Glycogen levels after recovery (Rec) were reduced in AMPK γ3 knock out and markedly enhanced in transgenic mutant mice suggesting that γ3 isoform is important for glycogen resythesis after exercise.

Barnes JBC 2004

AMPK γ3 knock out mice indicate the role of this target in glucose uptake

Phenotypic analysis of mouse models: the role of AMPK γ3 in glucose uptake

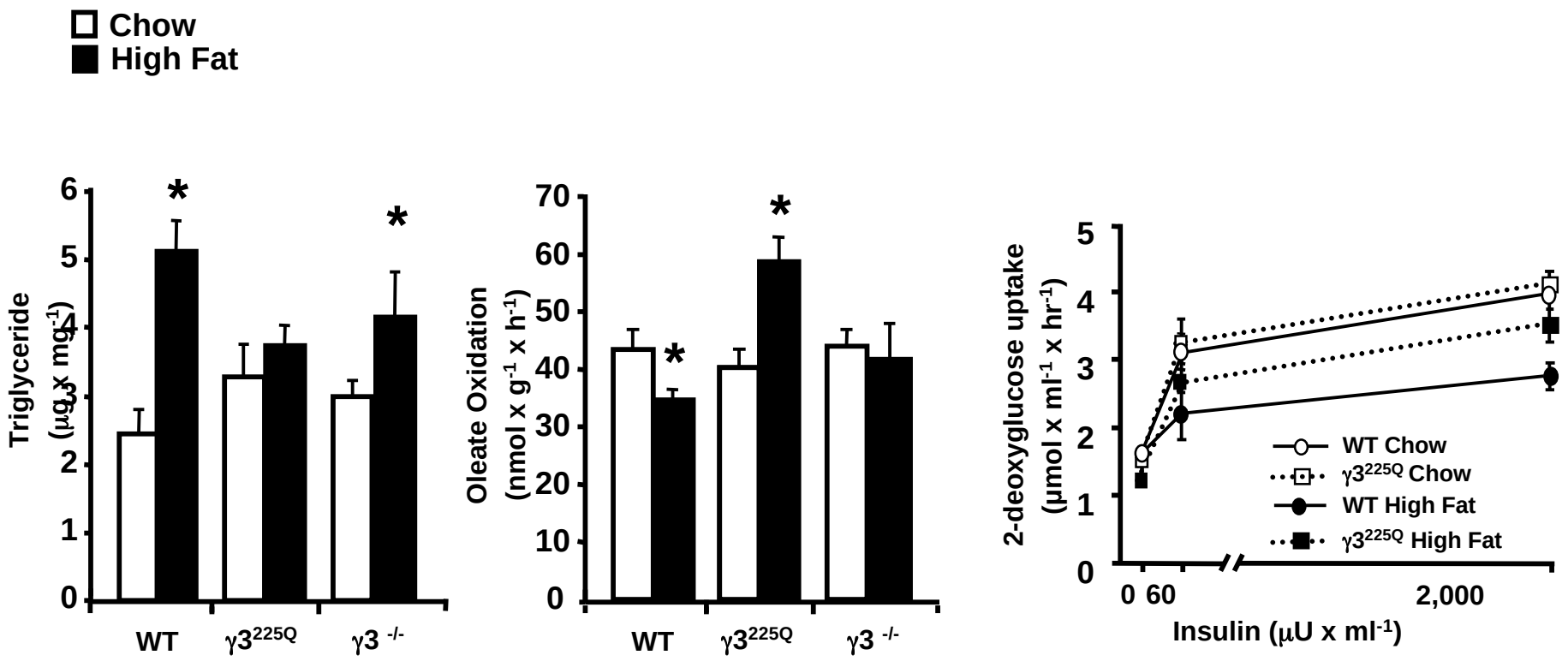


Barnes , JBC 2004

- Basal and contraction stimulated glucose uptake was similar between all genotypes.
- The partial AICAR and insulin resistance in mutant transgenic mice could be explained by inhibition of AMPK activity because of excessive glycogen content in the muscle.
- AICAR-treatment of EDL muscle did not increase glucose transport in knock out animals. Thus, AMPK γ3 subunit is essential for AICAR-induced glucose transport and other γ isoforms cannot compensate for the loss of γ3 function.

AMPK γ3 mut TG mice indicate the role of this gene in fat oxidation

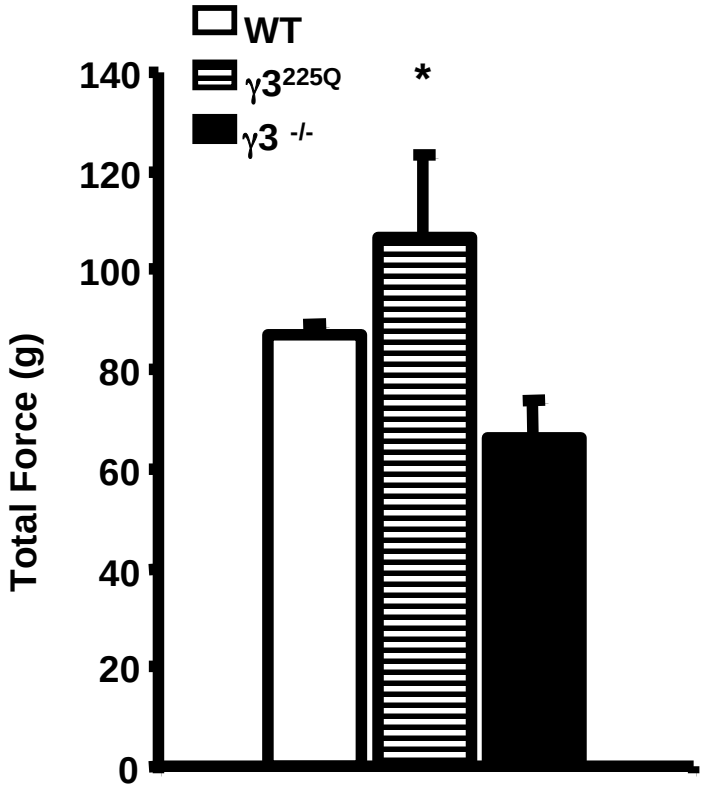
•When challenged with a high fat diet, transgenic mutant mice, in contrast to wild type and knock out mice, were protected against triglyceride accumulation and insulin resistance in skeletal muscle, due to increased levels of beta oxidation. Thus, AMPK γ3-containing trimers are essential for fatty-acid oxidation in the skeletal muscle after a fat-rich diet and by targeting these complexes one could prevent dietary induced insulin resistance.



Barnes , JBC 2004

Role of AMPK γ3 in muscle ergogenics

Phenotypic analysis of mouse models: the role of AMPK γ3 in muscle fatigue



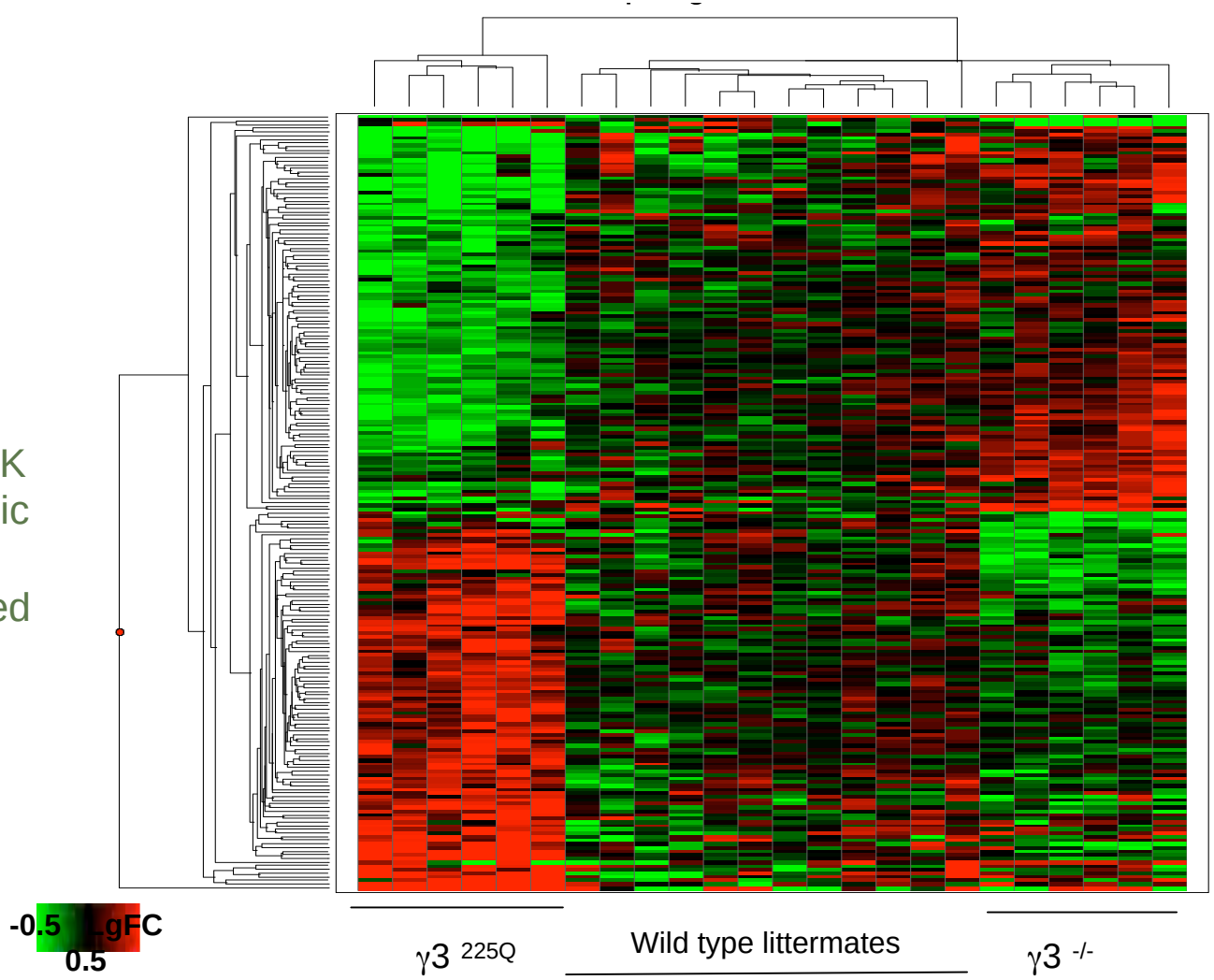
•EDL muscles from transgenic mutant mice were fatigue resistant, while muscle from knock out mice showed increased fatigability indicating that γ3 isoform influences force generation during muscle exercise.

Barnes , JBC 2004

R225Q: activating mutation or dominant-negative one?

Global transcriptome analysis of γ3 R225Q transgenic *versus* knock out muscle using Affymetrix gene arrays:

Reciprocal regulation of gene expression in AMPK γ3-/- and mutant trangenic mice: R225Q is an activating mutation judged by its biological effect.



Nilsson , JBC 2006

AMPK γ3 R225Q transgenic *versus* knock out muscle: pathway analysis

Coordinated and reciprocal expression pattern suggests a role of AMPK γ3 in glucose and fat homeostasis of skeletal muscle

Figure 3

Mutations in AMPK γ3 subunit

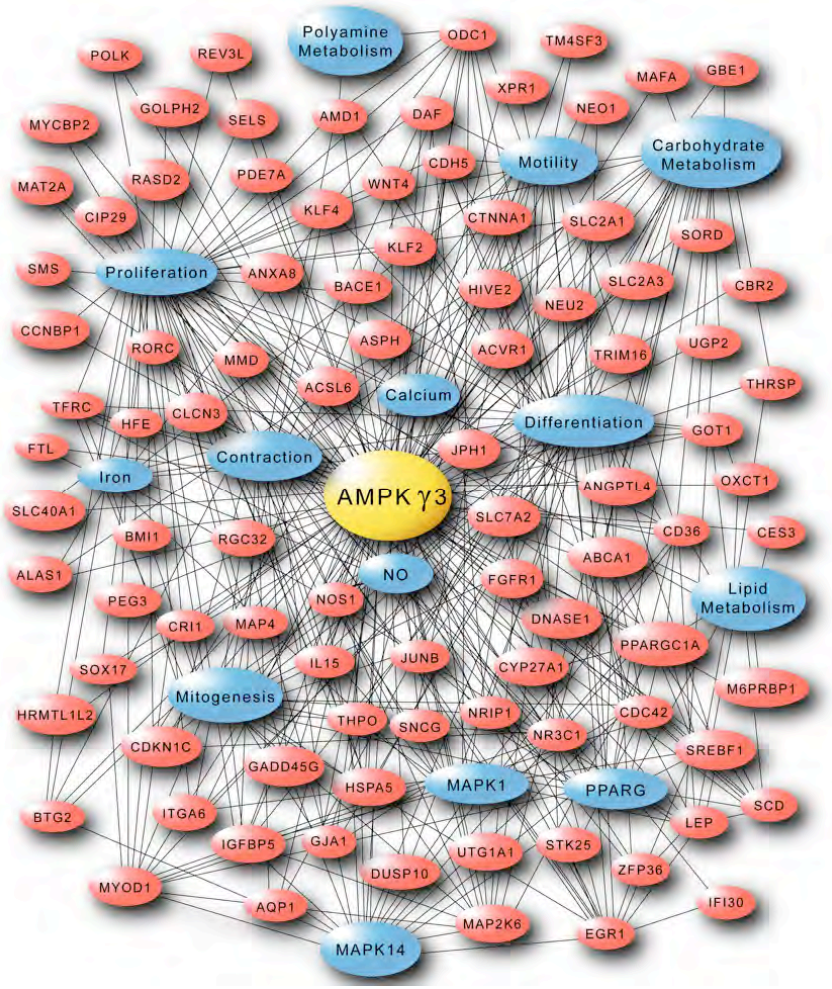
Prkag3$^{225Q/+}$ *versus* *Prkag3*$^{-/-}$

Transcriptional responses (A)

- **Glycogen synthesis** (*Ugp2* ↑↓, *Gbe1* ↑↓)
- **Activation of Mapk14** (*Map2k6* ↑↓, *Dusp10* ↓↑)
- **Mitochondrial biogenesis** (*Ppargc1a* ↑↓)
- **Fatty acid uptake** (*Cd36* ↑↓)
- **Utilization of fat-derived energy** (*Oxct1* ↑↓, *Ces3* ↑↓)
- **Iron storage and utilization** (*Tfrc* ↓, *Ftl1*↑↓, *Alas1* ↑↓)
- **Nitric oxide metabolism** (*Nos1* ↑↓)

Predicted physiological responses (B)

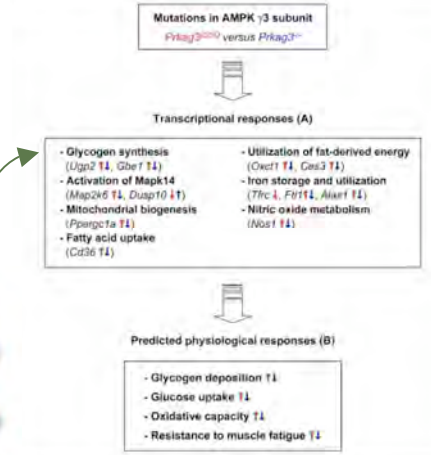
- **Glycogen deposition** ↑↓
- **Glucose uptake** ↑↓
- **Oxidative capacity** ↑↓
- **Resistance to muscle fatigue** ↑↓



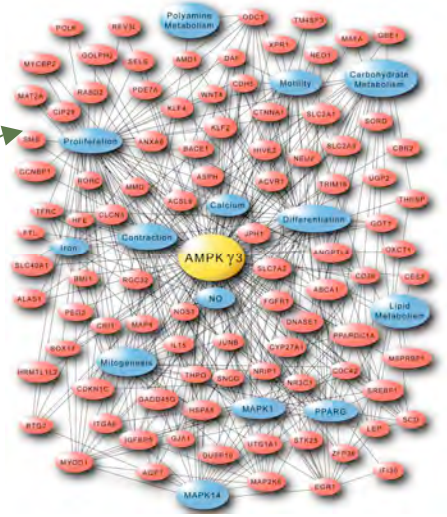
Nilsson , JBC 2006

AMPK γ3 R225Q transgenic *versus* knock out muscle: systems biology

Functional genomics: global transcriptome analysis in combination with pathway mapping discloses molecular pathways behind the phenotype observed (Nilsson *et al*., JBC 2007).



Molecular explanation for physiological changes



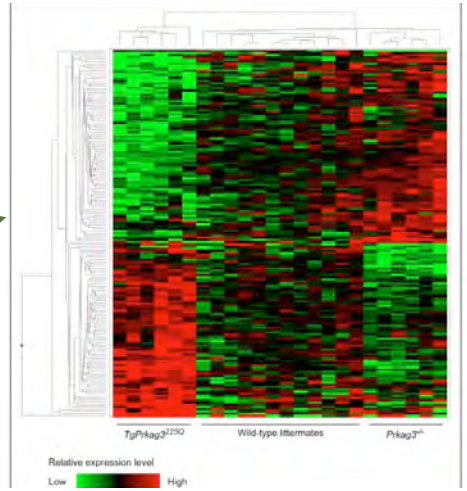
… and coordinated changes in gene expression

AMPK γ3−/−

Wild type

AMPK γ3 ^{225Q}

Mouse models

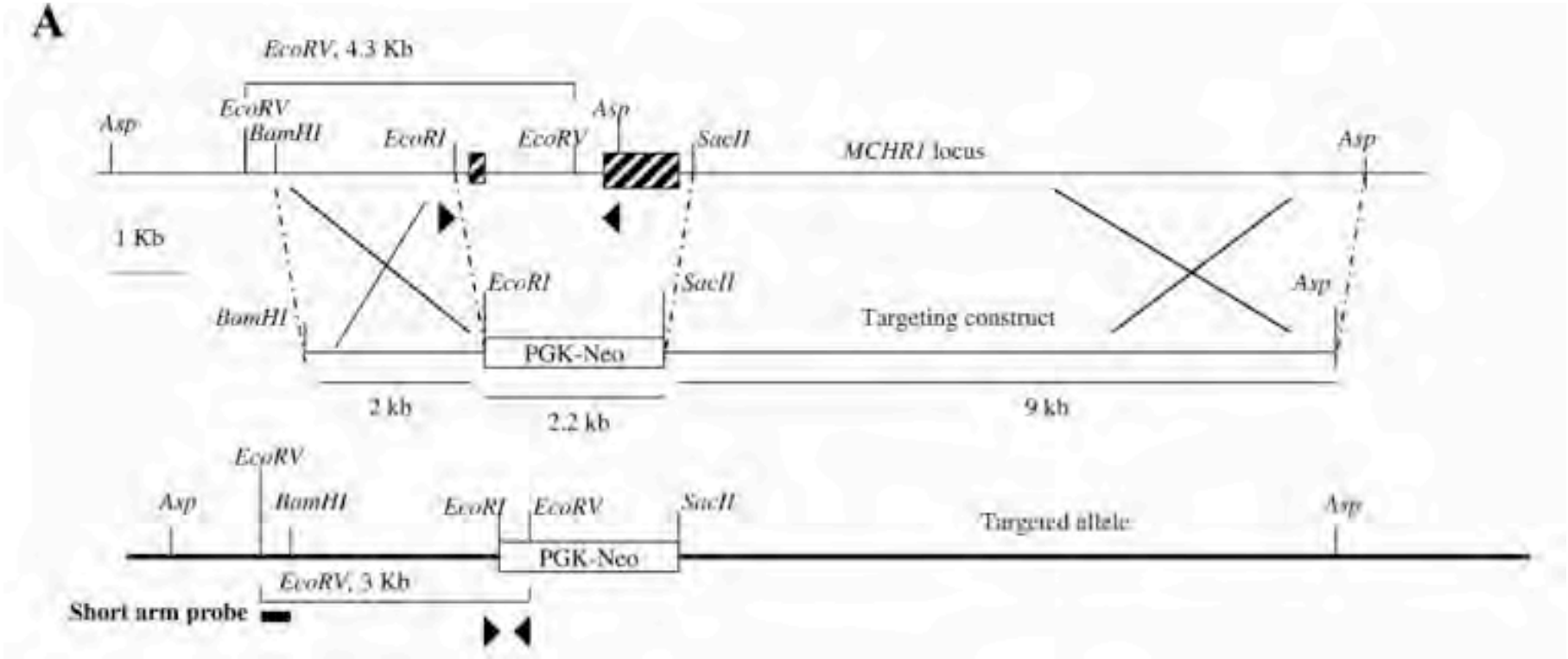


Reversed …

Nilsson , JBC 2006

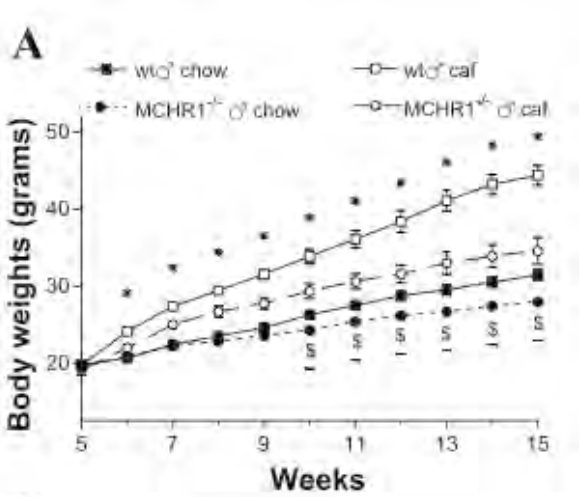
MCHR1 : knock out mice

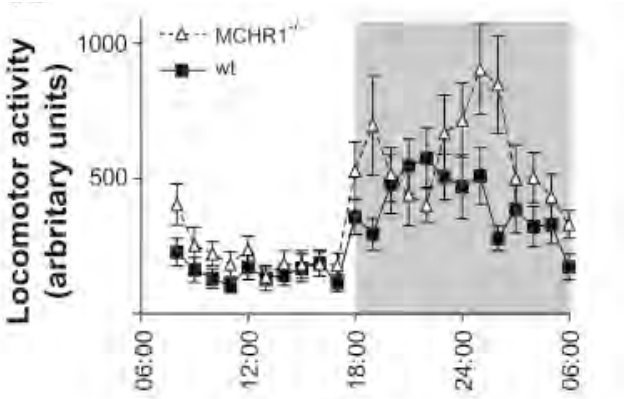
Conventional targeting construct, Neo under the control of constitutive promoter



Åstrand Am J Physiol 2001

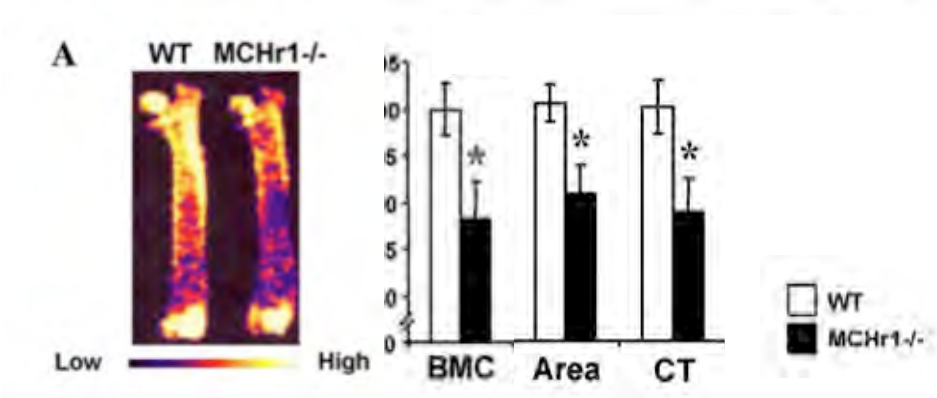
Mice lacking melanin concentrating hormone receptor 1 are lean and osteoporotic





Mchr1-/- mice have decreased body weight and reduced fat mass. They are hyperphagic and show increased spontaneous activity (Åstrand *et al*., Am J Physiol. 2004).

Mchr1-/- mice develop osteoporosis as indicated by reduced bone mineral content (BMC), cross-sectional area and cortical thickness (CT) of the femur (Bohlooly *et al*., BBRC, 2004).



Off target effects

One can administrate a cmpd to the knock out mice to observe possible side effects due to the effect on other targets (one would not expect any effects in the knock out mice in case the cmpd is specific for its target)

Disease models: To develop an animal model to test therapeutic strategies

- ✓ Induced: The condition to be studied is induced into healthy animals

  Disease models based on naturally occurring polymorphic variation in genes between mouse strains
  - > 465 Inbred mouse strains available
  - differ in susceptibility to disease, eg. cancer, type II diabetes

- ✓ Disease models based on introduction of specific mutation or defined genetic lesion into endogenous genes

Induced model: often the disease is induced on a certain genetic background

- ✓ Post surgical adhesions: rat sidewall model, rat multiple abrasion model
- ✓ T2DM: cafeteria or High fat diet induces the condition on C57Bl6 mice
- ✓ CIA (collagen induced arthritis) in DA rats

Disease models based on introduction of defined genetic lesion into endogenous genes

Examples in the field on cancer:

Transgenic mice (expression of oncogenes):

- c-myc oncogene + MMTV sequences - breast cancer
- Int-2 oncogene + viral promoter - prostate cancer

Knock out mice (targeting of tumor supressor genes):

- p53 knockout mouse lymphomas, breast cancer and osteosarcomas

Note: important to know what kind of mutation causes the disease
(i.e null mutation, dominant negative mutation)
and introduce the same kind of mutation in the mouse gene

Example: point mutation in the collagen type X, a gene (COL10A1)
– causes Schmid metaphyseal chondroplasia in humans
- transgenic mice with analogous Col10a1 mutations have similar phenotype as in humans
however, mice homozygous null for Col10a1 are phenotypically normal
therefore dominant negative mutation of the Col10a1 gene implicated,
not overall deficiency of collagen gene

Human genes can be expressed in mouse to generate more reliable disease models

The ability to express normal or mutant human genes in mice has greatly expanded their use as models for human disease.

Example: Polio Virus·Normal mice lack the polio virus receptor found in humans.
Transgenic mice expressing the human polio virus receptor gene can be infected by polio virus and even develop paralysis and other pathological changes characteristic of the disease in humans

A sampling of mouse models distributed by The Jackson Laboratory (www.jax.org)

Cystic Fibrosis (CF) - The ***Cftr* knockout mouse** has helped advance research into cystic fibrosis, the most common fatal genetic disease in the United States today, occurring in approximately one of every 3,300 live births. Scientists now know that CF is caused by a small defect in the gene that manufactures CFTR, a protein that regulates the passage of salts and water in and out of cells. Studies with the *Cftr* knockout have shown that the disease results from a failure to clear certain bacteria from the lung, which leads to mucus retention and subsequent lung disease. These mice have become models for developing new approaches to correct the CF defect and cure the disease.

Cancer - The **p53 knockout mouse** has a disabled *Trp53* tumor suppressor gene that makes it highly susceptible to various cancers, including lymphomas and osteosarcomas. The mouse has emerged as an important model for human Li-Fraumeni syndrome, a form of familial breast cancer.

Type 2 Diabetes - A metabolic disorder also called Non-Insulin Dependent Diabetes Mellitus (NIDDM), this is the most common form of diabetes and occurs primarily after age 40. The leading mouse models for NIDDM and obesity research were all developed at The Jackson Laboratory: ***Cpe^{fat}, Lep^{ob}, Lepr^{db}*** **and *tub.*** ·

Heart Disease - Elevated blood cholesterol levels and plaque buildup in arteries within three months of birth (even on a low-fat diet) are characteristics of several experimental models for human atherosclerosis: the ***Apoe*** knockout mouse.

Margit Mahlapuu 2007-12-06

Mouse models for T2DM

Strain	Inheritance	Protein Encoded	Type of defect
Obese (ob)	Recessive	Leptin	Defect in leptin
Diabetes (db)	Recessive	Leptin receptor	Defect in leptin receptor
Agouti yellow (ay)	Dominant	Agouti	Ectopic expression of melanocortin receptor antagonist
Tubby	Recessive	Phosphodiesterase	?
Fat	Recessive	Carboxypeptidase E	Carboxypeptidase E activity abolished

Agouti mice

- ✓Obesity and yellow coat color, hyperphagia, hyperinsulinemia, hyperglycemia, increased rate of lipogenesis and decreased rate of lipolysis, slight increase in linear growth.

- ✓The agouti locus was positionally cloned in 1993 (Miller et al, Genes Dev). It encodes the secreted 131 residue agouti protein that normally antagonizes the melanocortin 1 receptor in peripheral hair follicles to control pigmentation.The obesity of A^g mice results from ectopic expression of agouti in the CNS, which antagonizes the melanocortin-4 receptor in the hypothalamus.

- ✓ Deletion of the MCR4 phenocopies A^g, Huszar et al, *Cell* 1997.

- ✓Human agouti is expressed in adipose tissue (unlike in mice). To mimic human agouti mice that overexpress murine aguti in adipose tissue were generated, but these were not obese (Mynatt, PNAS, 97).

Agouti and wt littermates

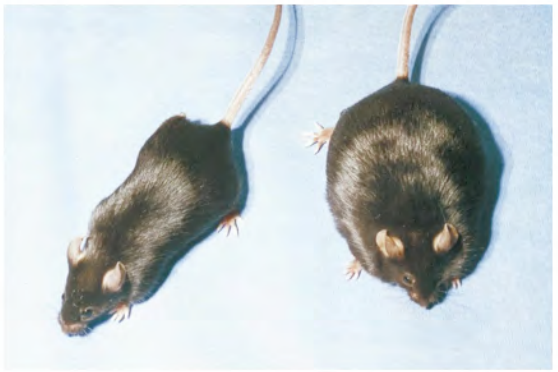
Ob/ob and Db/db mice

- ✓Both ob/ob and db/db mutations were known well before gene targeting era (reviewed by Bray and York, Physiol Rev, 79). Mutations leading to obesity in these mice have been defined (Zhang, Nature 1994, Tartaglia, Cell 1995, Chen Cell 1996).

- ✓Leptin is a hormone produced in adipose tissue which regulates body energy homeostasis by binding to its receptors in hypothalamus. ICV of leptin reduces body weight and fat mass through reduction of food intake and increased energy expanditure in rodents (Murphy PNAS 97).

- ✓Ob/ob (leptin deficiency) & Db/Db (leptin receptor deficiency) phenotypes are close to identical: The mice are morbidly obese (weigh 300% more than normal) , hyperphagic, hypothermic, hyperinsulinemic and hyperglycemic.

- ✓ Human Ob/ob and Db/db are also hyperphagic & obese.



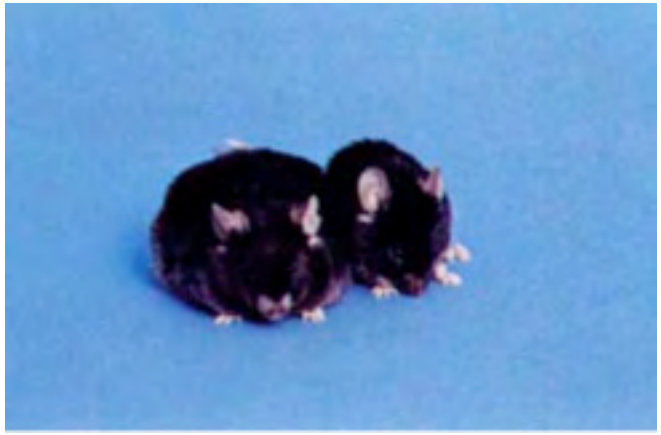
Murphy PNAS 97: ob/ob were photographed 6ʌweeks after being treated with rAAV-leptin (*Left*) or saline vehicle (*Right*)

Fat/Fat mice

- ✓Mice homozygous for the *fat* mutation develop obesity and hyperglycaemia that can be suppressed by treatment with exogenous insulin. *Fat* mutation results in more slowly developing obesity compared to ob/ob and db/db mice.

- ✓ The *fat* mutation maps to mouse chromosome 8, very close to the gene for carboxypeptidase E (*Cpe*), which encodes an enzyme that processes prohormone intermediates such as proinsulin.

- ✓Naggert et al (1995) demonstrate a defect in proinsulin processing associated with the virtual absence of CPE activity in extracts of *fat/fat* pancreatic islets and pituitaries. A single Ser202Pro mutation distinguishes the mutant *Cpe* allele, and abolishes enzymatic activity *in vitro*.



Fat/fat and wt littermates at 6 months of age Naggert et al., *Nature Genetics* 95

- ✓Relationship between the hyperproinsulinaemia and obesity remains unclear. It is possible that obesity may develop as a result of widespread defect in exopeptidase processing of neuroendocrine hormontes other than proinsulin.

Transgenic animals as bioractors:

Examples of producting human proteins in transgenic animals:

- ✓Human lactoferrin in cows' milk
- ✓Alpha-1-antitrypsin in sheep
- ✓HGH in mouse urine (uroplakin promoters)
- ✓Human antibodies in mice (H and L chain tgenics à hybridomas)