Mouse Knock-out by Homologous Recombination

Transgenic animals by DNA Transfer

Embryonic Stem Cell Biology and Somatic Cell Nuclear Transfer
## HISTORICAL ADVANCES: KNOCKOUT TECHNOLOGY

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td><strong>1960s</strong></td>
<td>Teratocarcinomas containing many cell types identified</td>
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<td></td>
<td>Embryonal carcinoma (EC) cells injected into blastocysts are shown to contribute to the formation of many tissues</td>
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<td><strong>1981</strong></td>
<td>Embryonic stem cells (ESC) identified and shown to be more totipotent than EC cells</td>
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<td><strong>1980s</strong></td>
<td>Homologous recombination between plasmid DNA and chromosomes of cultured mammalian cells documented</td>
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<td><strong>1988</strong></td>
<td>Plus-minus selection strategy developed</td>
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<tr>
<td><strong>1989</strong></td>
<td>ES cells with targeted mutation transmitted through germ line</td>
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CONSTRUCTION OF A KNOCKOUT MOUSE

1. Embryonic Stem (ES) cells
2. Targeting vector transfection
3. Rare targeted cells
4. Clone selection/screening
5. Pure targeted population
6. Blastocyst injection
7. Chimeric offspring of pseudopregnant mother
8. Breeding with +/- animals
9. Germ-line transmission of modified ES cell genome
After plus-minus selection, 0.05% to 50% of surviving ES cells have desired homologous recombination event.
INJECTION OF ES CELLS INTO BLASTOCYSTS

NORMAL CHROMOSOME

TARGETED MUTATION

ES CELLS FROM BROWN MOUSE

BLACK FEMALE

BROWN MOUSE

BLASTOCYST-STAGE EMBRYO

ALTERED EMBRYO

EMBRYO

SURROGATE MOTHER

NEWBORN CHIMERIC MALE (CARRYING CELLS FROM TWO MOUSE STRAINS)
CHIMERIC MICE ARISING FROM ES CELL INJECTION
GERM LINE TRANSMISSION OF KO MUTATION

+ = Wild type
- = Knockout mutation

1:2:1 Segregation
SALIENT FEATURES OF KNOCKOUTS

- Few mouse strains only, failed to date in other species
- Involves manipulation of the target gene in cultured cells followed by their injection into very early mouse embryos (blastocyst), and implantation of the modified embryos into foster mothers
- Manipulations alter expression of an endogenous gene
- Diverse changes can be introduced at the DNA level (e.g., deletions, insertions, replacements, point mutations)
- Useful for the generation of animal models of human disease and the determination of gene function
PMS + HCG to superovulate

1-cell pronuclear stage mouse embryo.

Injection of 100-200 copies transgene

2-cell stage embryo

Oviduct Transfer

Pregnant Foster Mother

≤ 30%
GENERATION OF TRANSGENIC MICE

• Superovulation by injection of reproductive hormones, mating

• Fertilized egg collection and culture

• Microinjection of transgene (100-200 copies, nanoliter volume)

• Transfer of injected embryos to recipient mothers

• Development in utero to term

• Labor and delivery
TRANSGENE DESIGN

Homologous transgene

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Intron</th>
<th>Coding region, exons</th>
<th>Terminator</th>
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- Employs gene’s own regulatory sequences

Heterologous transgene

<table>
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<th>Coding region, exons</th>
<th>Terminator</th>
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- Employs another gene’s regulatory sequences
MICROINJECTION OF FERTILIZED MOUSE EGG
EXPERIMENTAL USES OF TRANSGENESIS

• Overexpress or ectopically express transgene as part of a gain-of-function study

• Define regulatory elements in a gene that dictate tissue-specific or cell type-specific expression patterns

• Express toxic transgene that ablates a specific cell type

• Define new host genes that are disrupted upon transgene integration
Transgenic sheep expressing a human gene for a clotting factor in mammary glands
“GOLDEN” AND WILD TYPE RICE
Somatic Cell Nuclear Transfer

- Donor somatic cells
- Isolate and Culture Cells
- Capture donor nuclei
- Inject Donor Nuclei into Recipient Cell
- Enucleated cell
- Establish hESCs for disease studies and potential tissue regeneration
- Renucleated Cell

Isolate recipient oocyte
Remove nucleus & chromosomes
Major Points

1. Development of multiple molecular tools for gene replacement: homologous recombination; embryonic stem cells; micro-injection of blastocysts and selectable marker strategies

2. Construction of KO mice: generation of KO ES cells, inject ES cells into blastocysts and propagation of chimeric animals

3. Germline transmission of KO genes and homozygous KO progeny

4. KO mice are critical for generating animal models of human disease and for testing drug therapies targeting specific genes
5. Transgenic animals carry copies of recombinant genes expressed under the control of synthetic promoters

6. Uses of transgenic organisms include: over-expression of desired gene products; map cis-regulatory elements in whole animals; express foreign genes in specific cell types or tissues; disrupt endogenous genes upon insertion of transgene at ectopic site

7. Examples of useful transgenic organisms: sheep that overproduce human blood clotting factors; rice that produce vitamins or specific amino acids as food supplements for the Third world
Major Points

8. Emerging area of human embryonic stem cell biology

9. Hope to use hESC’s to generate specific cell-types for both basic research and novel tissue regeneration therapeutics

10. Somatic cell nuclear transfer as one potentially important strategy for generating specific differentiated cell lineages with an exact genetic match to individual patients