

# Analysis of Gene Function

Constructing Animal Models

Lecture 1

Jackson Labs Course September, 2005

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**Search for Orthologues and  
Paralogues by Database  
Mining**

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## Search for Genes with Known Functions that Share Conserved Domains with the Newly Identified Gene and thus Allow Functional Predictions

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### Examples:

- Gly-X-Ser-X-Gly motif found in the catalytic centers of esterases and lipases
- His-Glu-X-Gly-His motif in Zn-proteases
- Basic Helix-Loop-Helix motif in transcription factors
- Presence of Protein Interaction Domains, e.g. leucine zipper, WD domains, SH2 and SH3 domains, PTB domains, NPXY or YXXL motifs, etc.
- Presence of recognizable catalytic domains, e.g. kinase domains, protease domains, homology to P450 enzymes, etc.
- Presence of other functional domains, e.g. immunoglobulin domains, fibronectin repeats, lectin domains, ligand binding repeats, etc.
- Presence of posttranslational modification sites, e.g. farnesylation, geranyl-geranylation, myristoylation, palmitoylation, glycolipid anchors, etc
- Presence of membrane spanning segments as they are found in signal-transducing 7-transmembrane receptors, ion channels, membrane tyrosine kinase receptors, etc.

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**Formulate Hypotheses and Devise *In Vitro* and *In Vivo* Assays to Determine the Biological Function of the Gene**

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**Assemble a Molecular Toolkit**

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- Cloning of expressible cDNA
- Production of antipeptide antibodies
- Purification of the protein from natural tissue sources or by recombinant expression (e.g. in *E.coli*, insect cells, or in mammalian cells using adenovirus or tissue culture cell transfection)
- Production of more antibodies against the complete and native protein
- And all the other tools Shelly you already heard about

## Generate **General** Biological Data That Are Specific for Your Gene of Interest

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- Tissue-specific expression pattern at different stages of life (e.g. during embryonic development, before and after sexual maturation, lactation, senescence, during the cell cycle, etc.)
- Subcellular expression pattern, e.g. nuclear, cytoplasmic, cell surface, endoplasmic reticulum, Golgi, mitochondrial, lysosomal, etc.
- Test for post-translational modifications (e.g. unusual glycosylation, lipid modification, phosphorylation)
- Overexpress the protein in cultured cells and see what happens (i.e. is the cell cycle affected, does the cytoskeleton rearrange, survival, etc.)
- Test for interactions with other proteins (i.e. proteomics), using for instance yeast-two-hybrid screening
- CHIP-IT (i.e. test for changes in gene transcription using available data sets or probe for transcriptional regulation using suitable systems based on your specific hypotheses)

## Generate **Specific** Biological Data for Your Gene of Interest

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- Does the protein have enzymatic activity?
- Is the protein an ion channel - what is the specificity?
- Is the protein a receptor - what is the ligand?
- Etc., etc., etc.

Depending on your hypothesis and the outcome of the preliminary experiments you would devise more specific experimental approaches

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- Frog oocyte injection to analyze the role of the gene in development or to measure ion conductance properties
- Site-specific mutagenesis to characterize functional domains in the gene
- Generation of dominant negative mutants (including siRNA)
- Viral Gene Transfer in vivo
- Generation of genetically altered mice

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Transgenic Mice Are Made By  
Pronuclear Injection of DNA  
Constructs

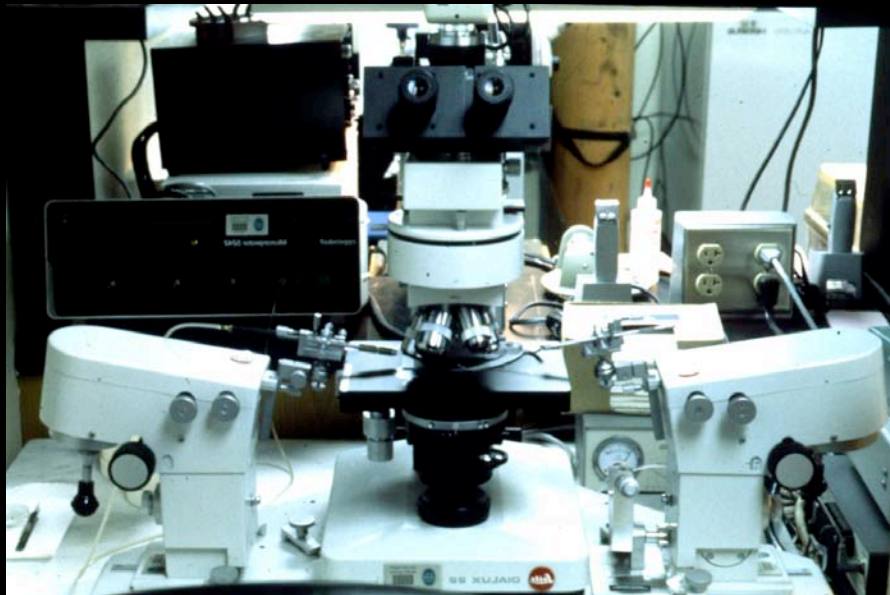
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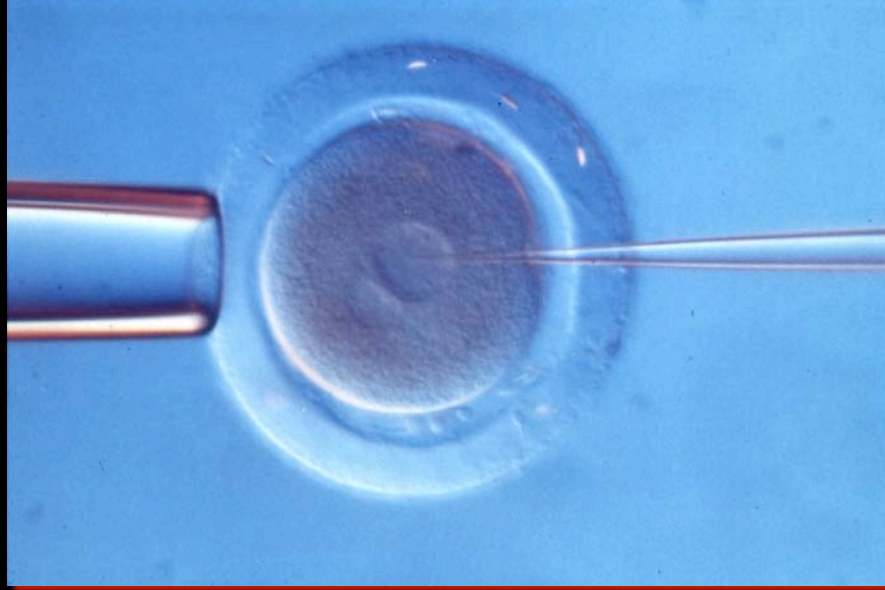
## Blastocyst or Mouse Egg Collection Setup



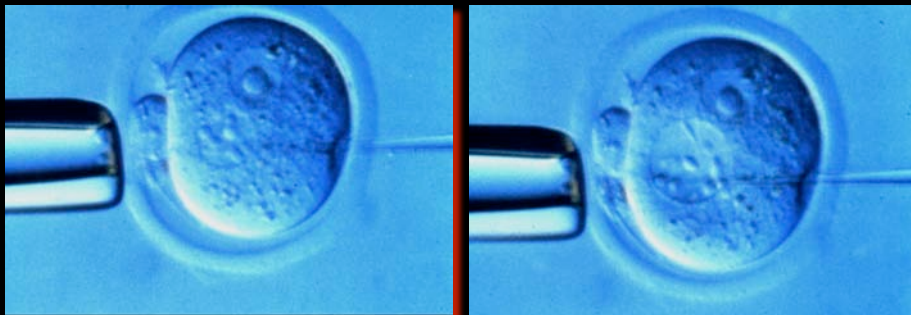
## Microinjection Setup



## Pronuclear Injection



## Microinjection of Fertilized Mouse Egg Pronucleus



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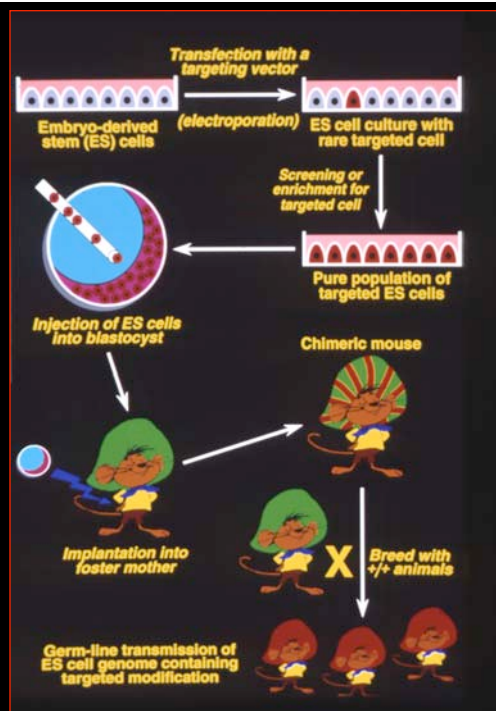
## Gene Knockouts (and Knockins) Are Made in Embryonic Stem (ES) Cells

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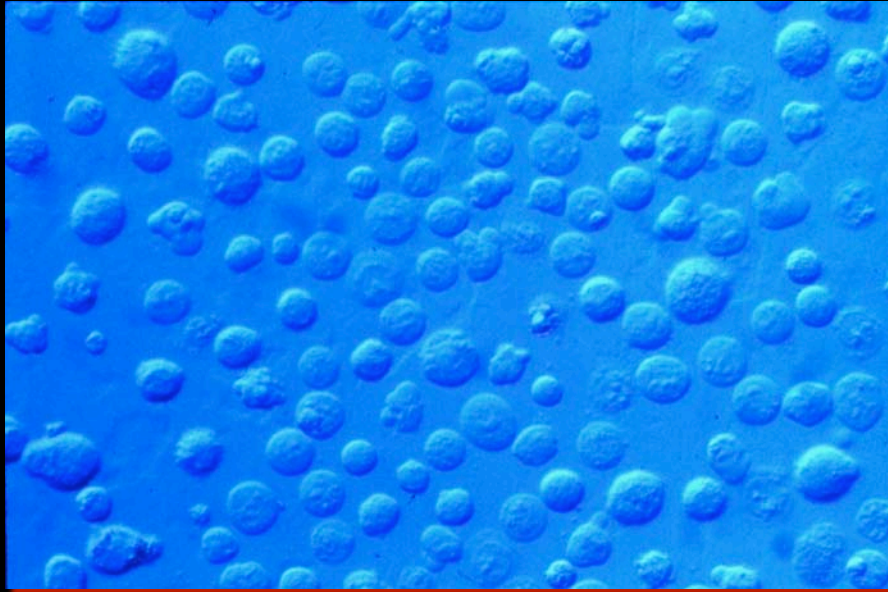
## How Embryonic Stem Cells Are Selected and Used for the Production of Chimeric Mice

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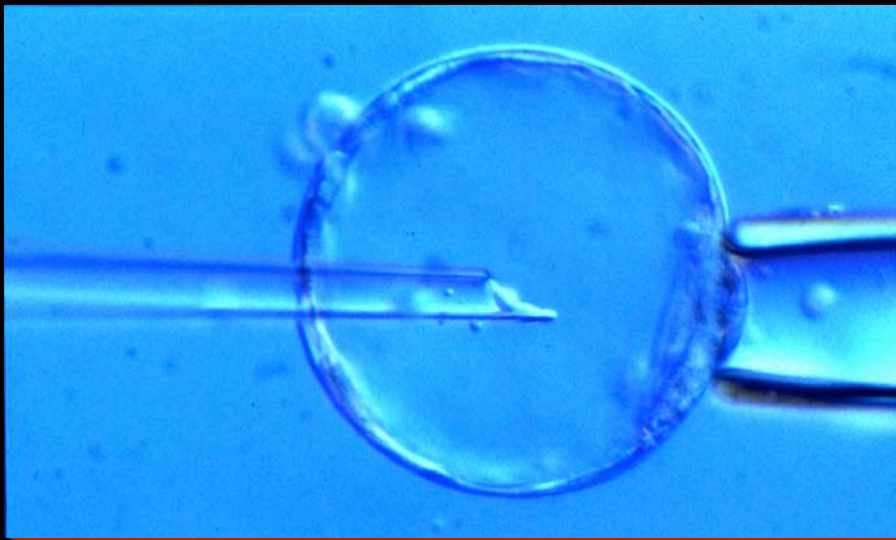
## **Trypsinized ES Cells Ready for Blastocyst Injection**

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## **Injection of Mouse Blastocyst**

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## Chimeric Mice

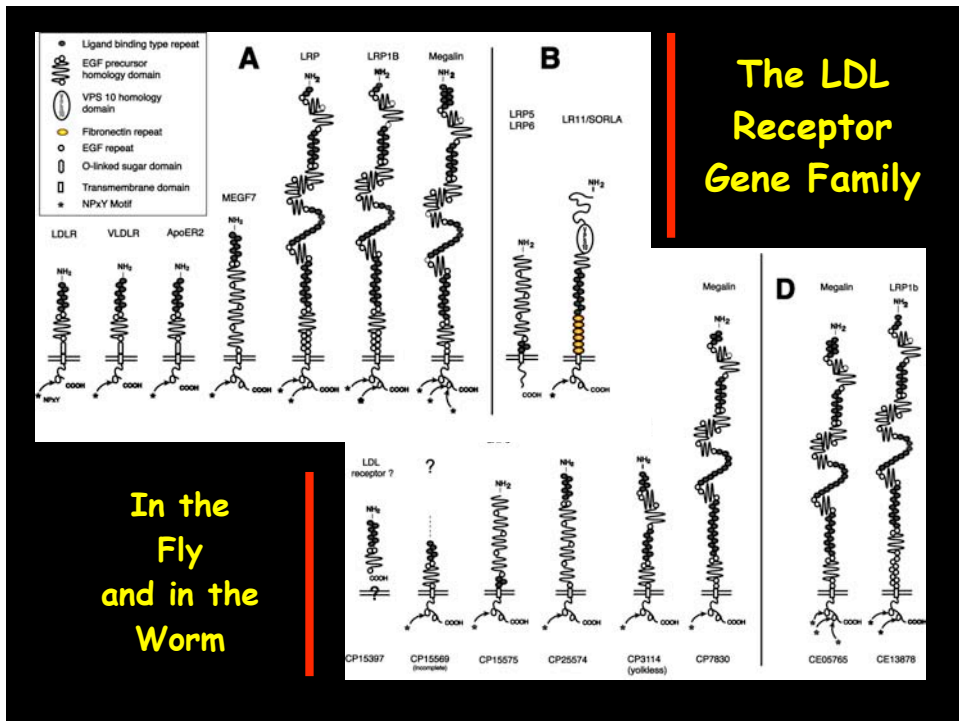
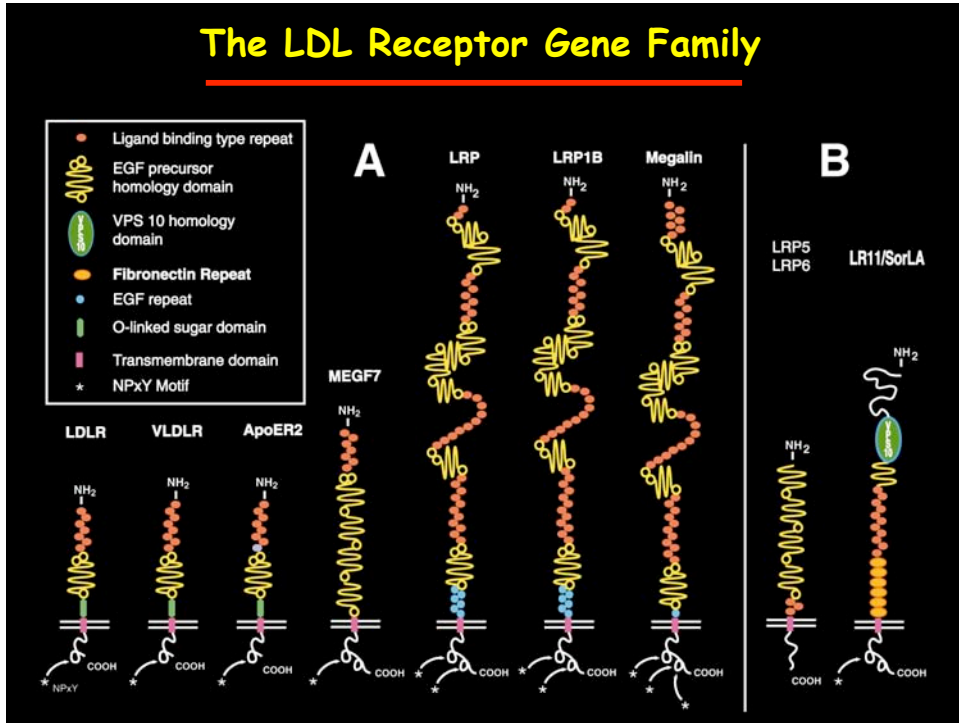


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**Now to Some Practical Applications...**

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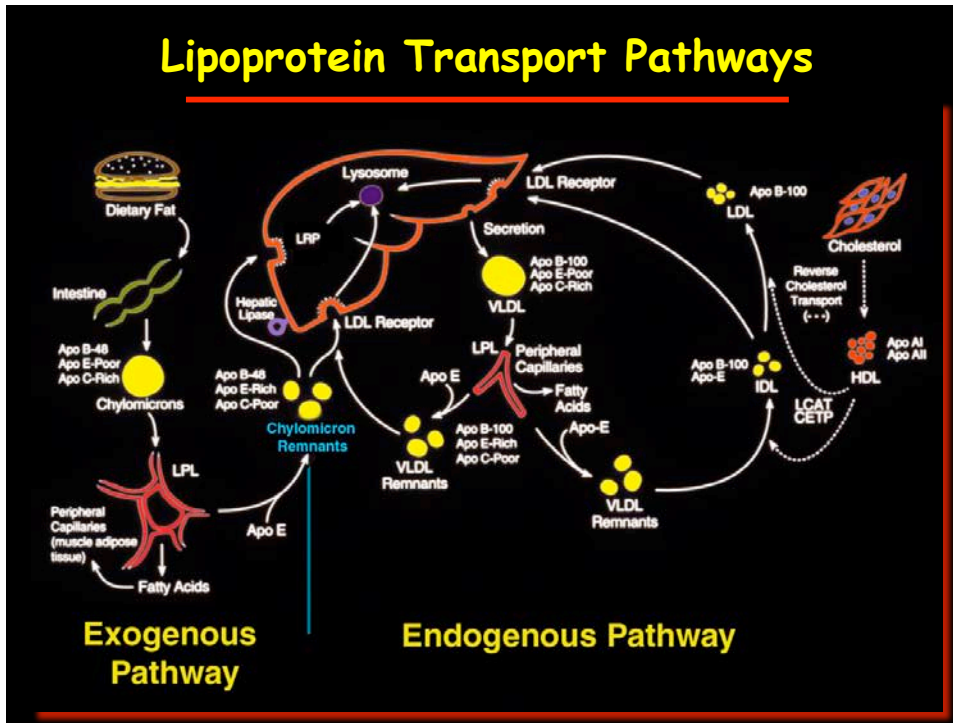
# The LDL Receptor Gene Family



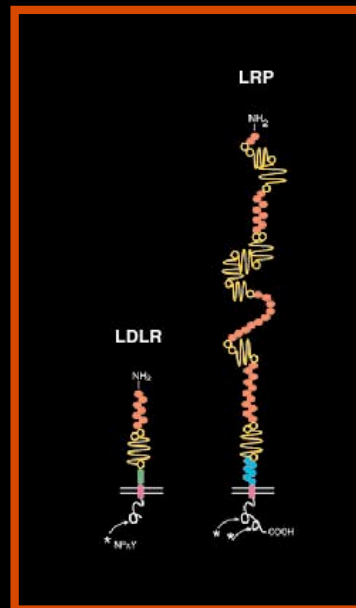
# The LDL Receptor Gene Family

In the Fly and in the Worm

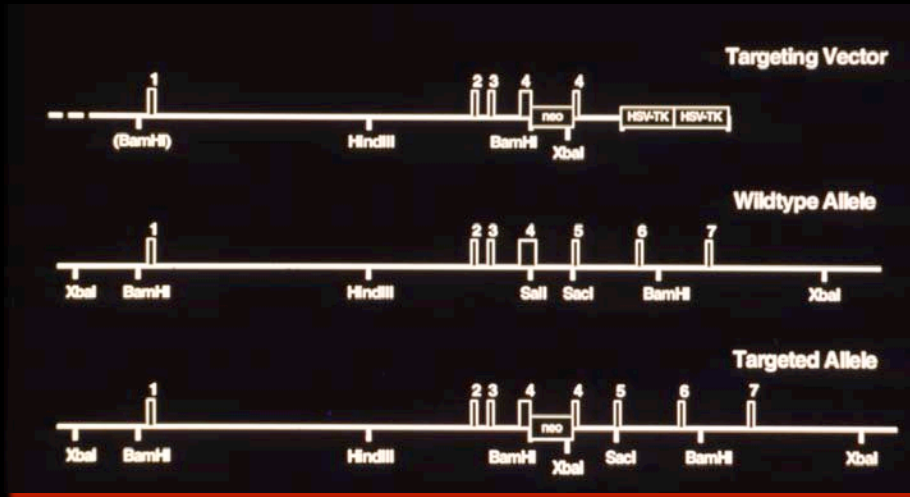
# Lipoprotein Transport Pathways



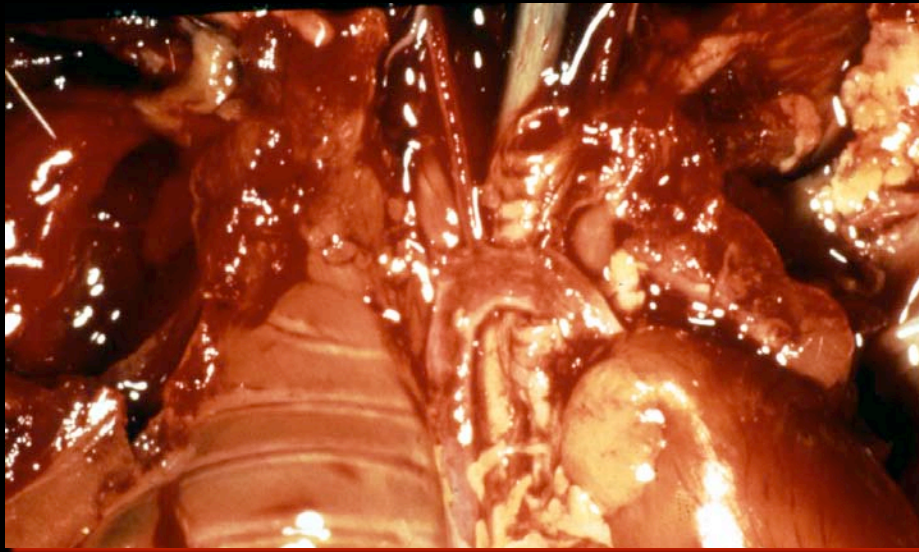
**LDL Receptor**  
and  
**LRP**  
Cooperate  
in the  
Uptake  
of  
Remnant Lipoproteins  
by the  
Liver



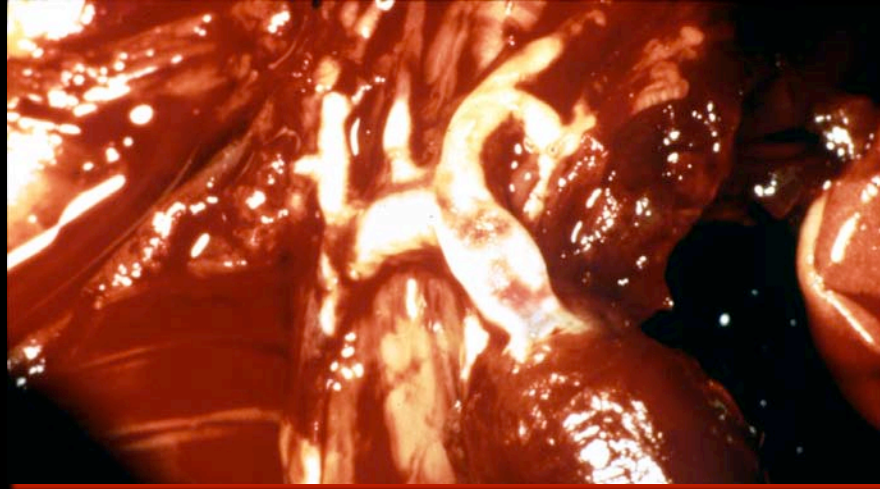
## Gene Targeting Strategy for the LDL Receptor Locus



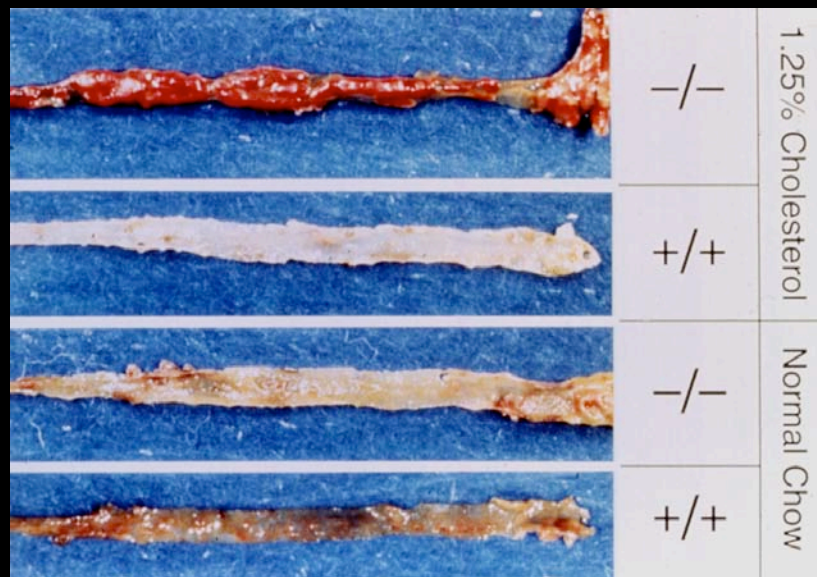
## Protection from Atherosclerosis in Wild Type Mice



## Diet-Induced Atherosclerosis in the LDL Receptor Knockout Mouse



## Diet-Induced Atherosclerosis in Wild Type and LDL Receptor Knockout Mice



**Protection from Diet-Induced Atheroma in Wild Type Mice**

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**Atheroma in the LDL Receptor Knockout Mouse**

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## Xanthelasma in LDL Receptor Knockout Mice



## LDL Receptor

and

## LRP

Cooperate

in the

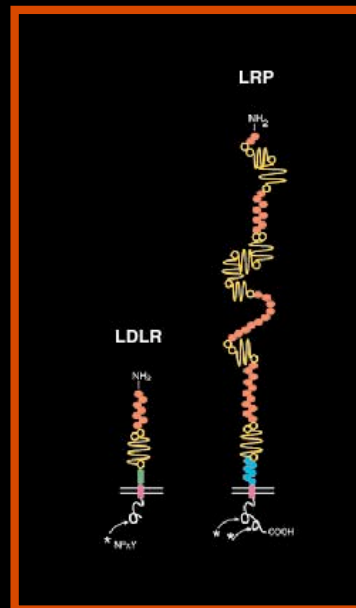
Uptake

of

Remnant Lipoproteins

by the

Liver



## Wild Type and LRP Knockout Embryos

E9.5



wt



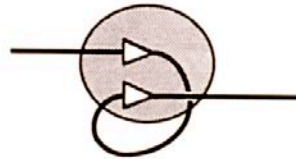
ko

E12.5



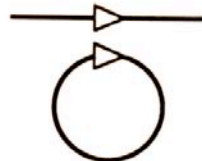
**loxP site:**

ATAACTTCGTATA	ATGTATGC	TATACGAAGTTAT
TATTGAAGCATAT	TACATACG	ATATGCTTCAATA



**Cre recombinase:**

- 38 kDa protein from phage P1
- site-specific recombination at loxP site
- does not require ATP



**Recombination:**

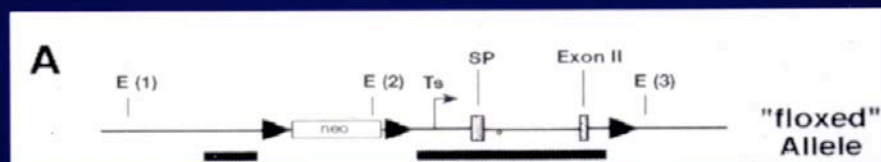
- sequence deletion between loxP sites
- circularized excision product

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## Inactivation of LRP in the Liver By Inducible Cre Expression

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### Inducible Cre-Lox-Mediated Recombination of the LRP Allele



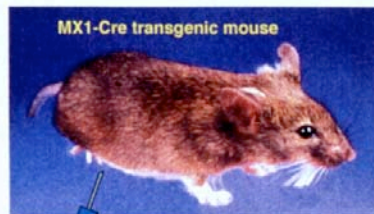
'floxed' LRP mice

X



Transgenic mice carrying Cre under control of the IFN-sensitive MX1 promoter

## How Inducible Cre-Expression With the MX1-Promoter Works



Inject i.p. with pl:pC  
(double stranded RNA)



Activated macrophages secrete interferon

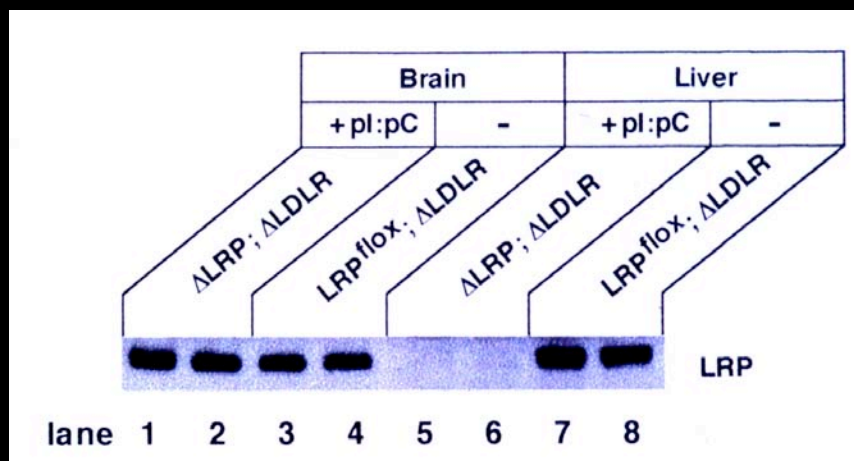
IFN $\alpha/\beta$

Cre-Expression  
 Induction of MX1-Promoter  
 Interferon Receptor Signaling

Liver  
 Kidney  
 Heart

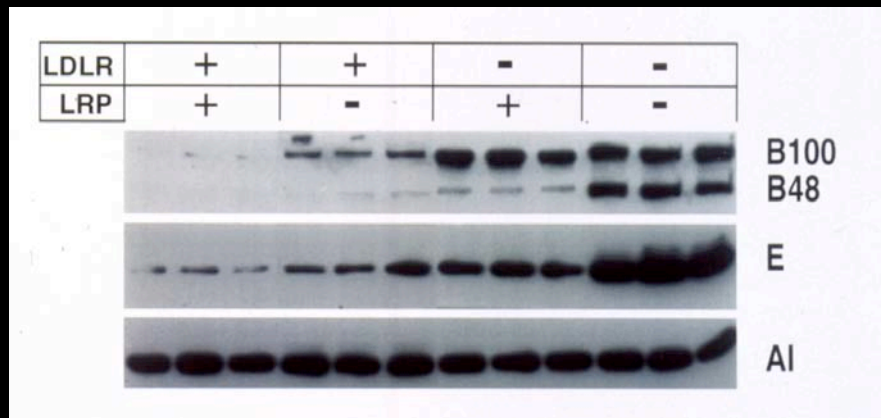
Circulation

## Immunoblot of LRP in pI:pC-Treated Mouse Livers



## Immunoblot Analysis of Mouse Apoproteins

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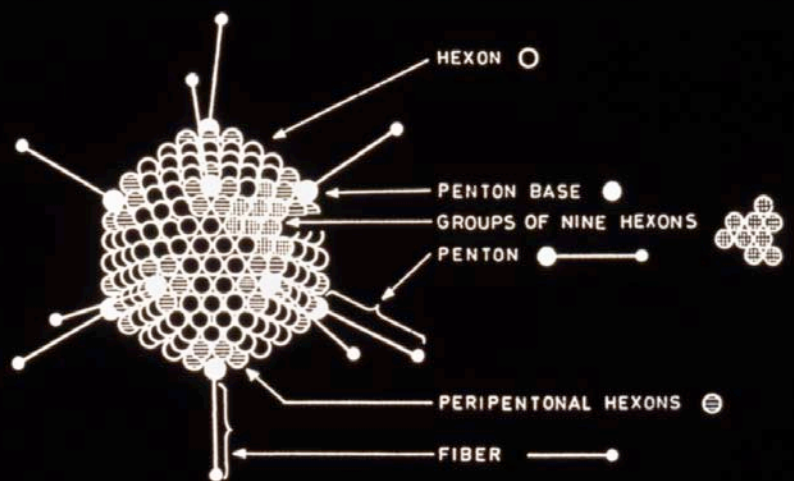
## Inactivation of LRP in the Liver By Gene Transfer

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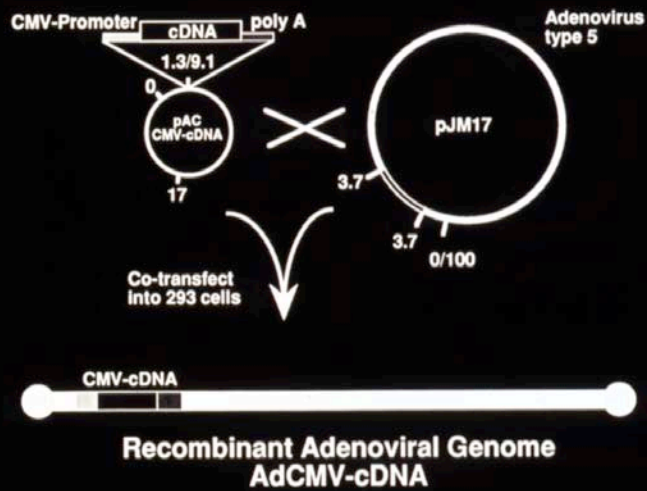
## Adenovirus Particles



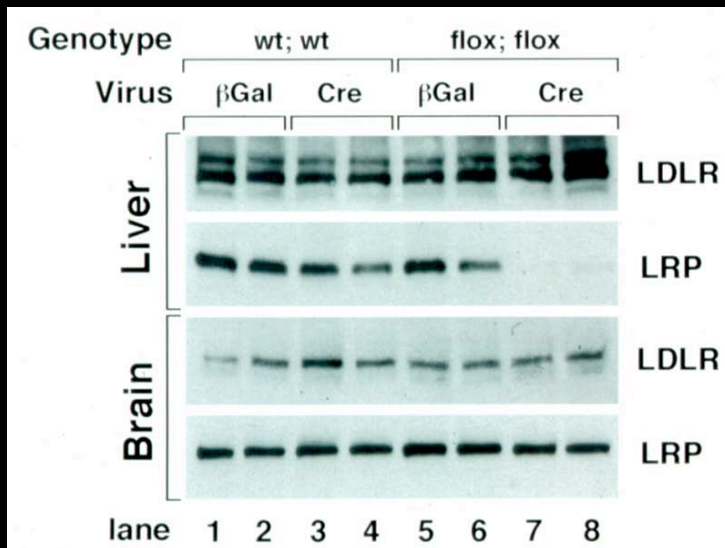
## Structure of Adenovirus



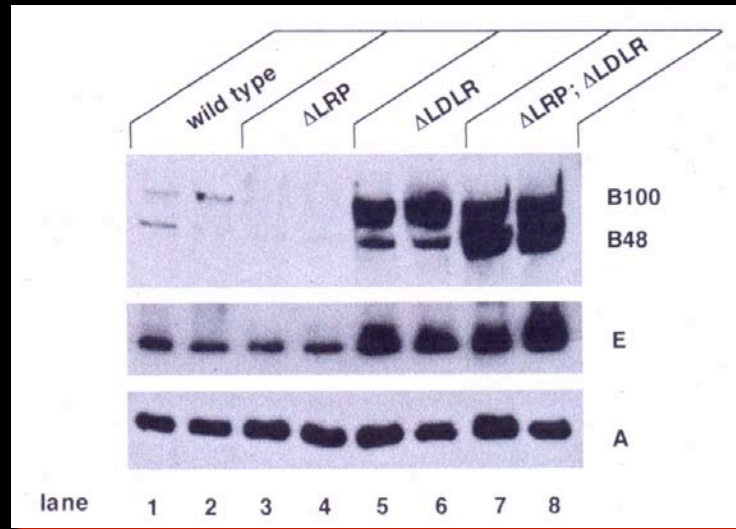
## Principle of Adenovirus Construction



## Gene Knockout by Adenovirus



## Immunoblot Analysis of Plasma Apoproteins in AdCre-Injected Mice



## Receptor Associated Protein (RAP) Is a Chaperone for LRP and Blocks Receptor Binding

Chylomicron Remnants



RAP



Studying the *In Vivo* Role of LRP using and RAP-Adenovirus



**Adenovirus-Mediated Gene Transfer to LDL  
Receptor Knockout Mice Induces Hyperlipemia**

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