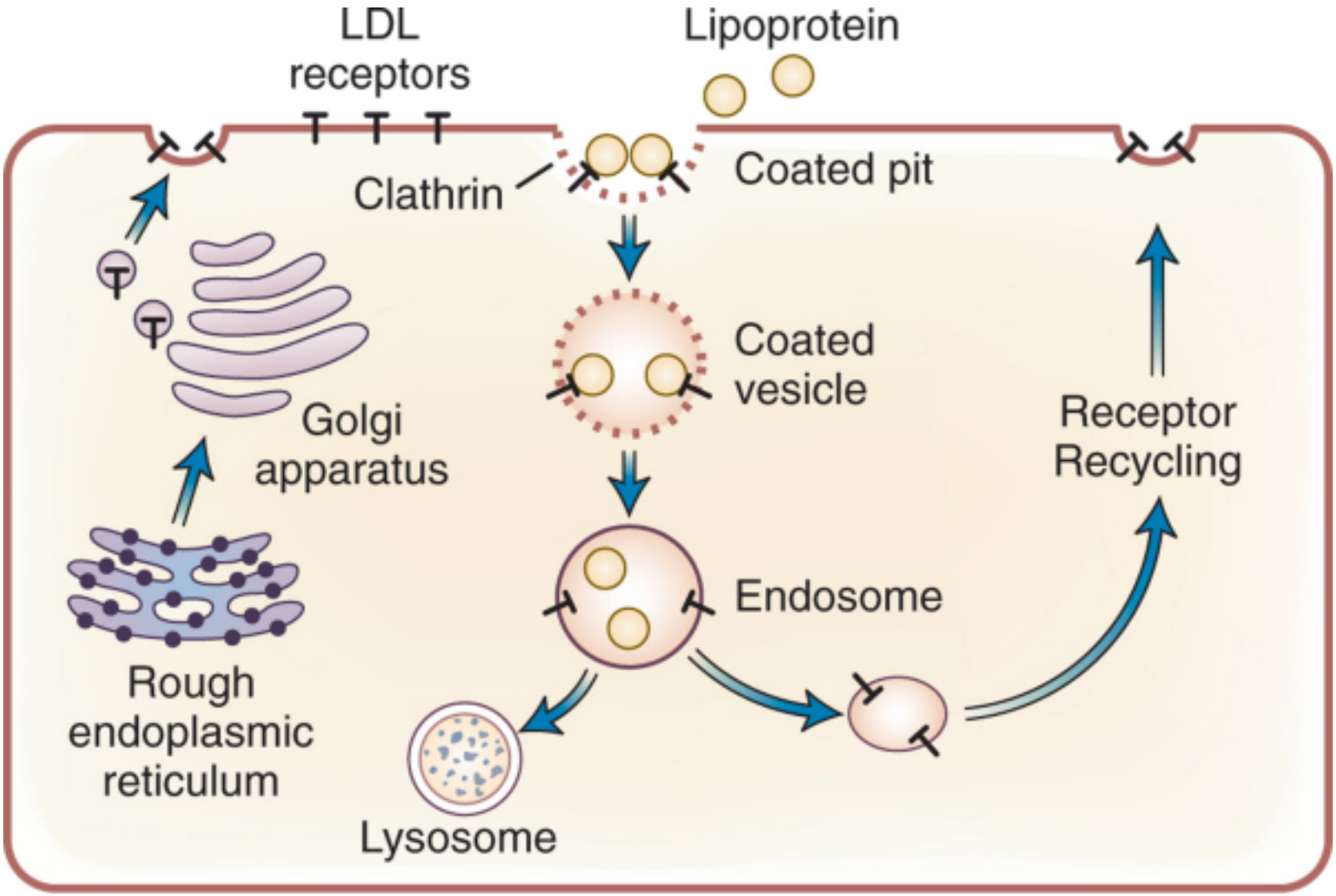
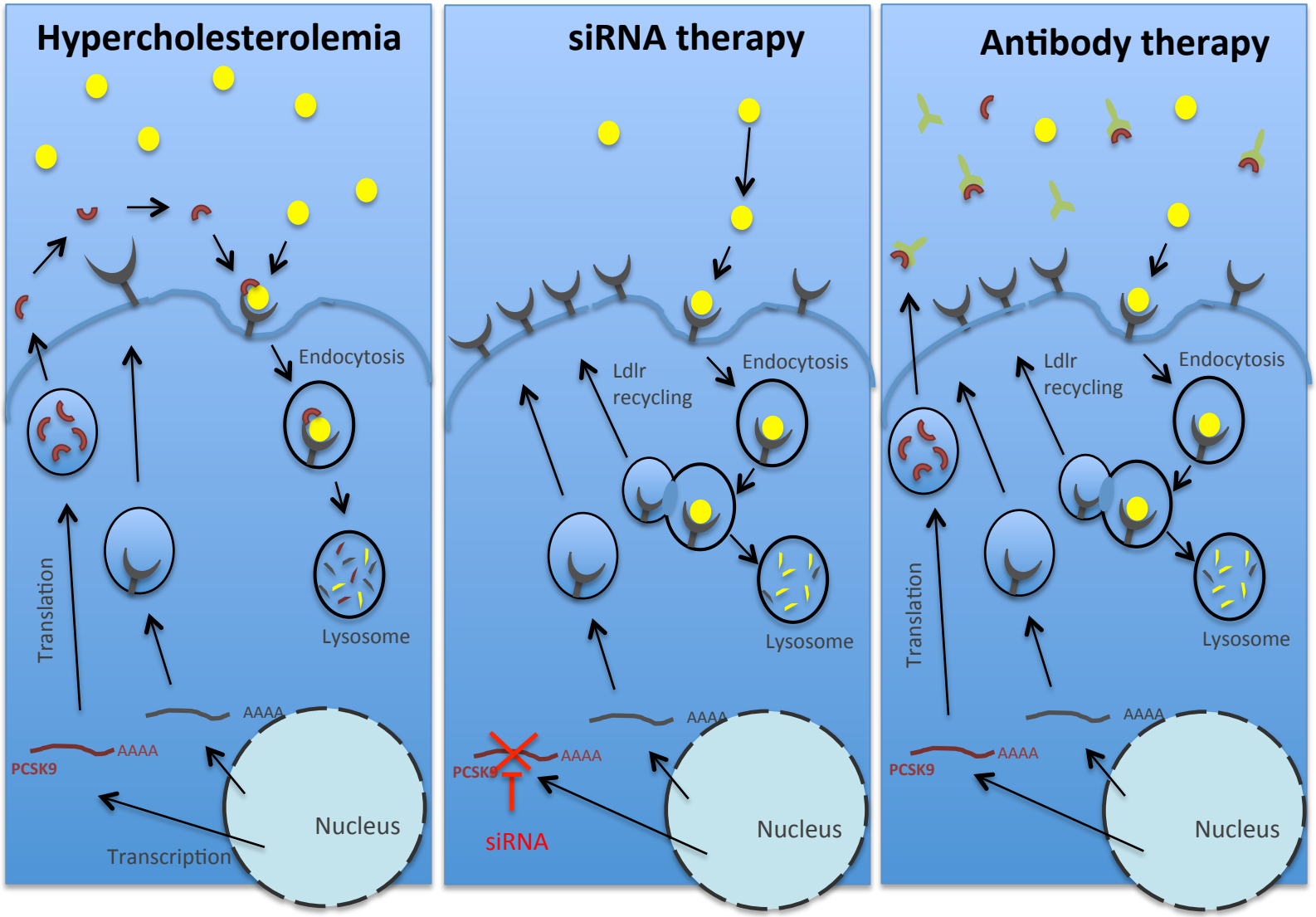
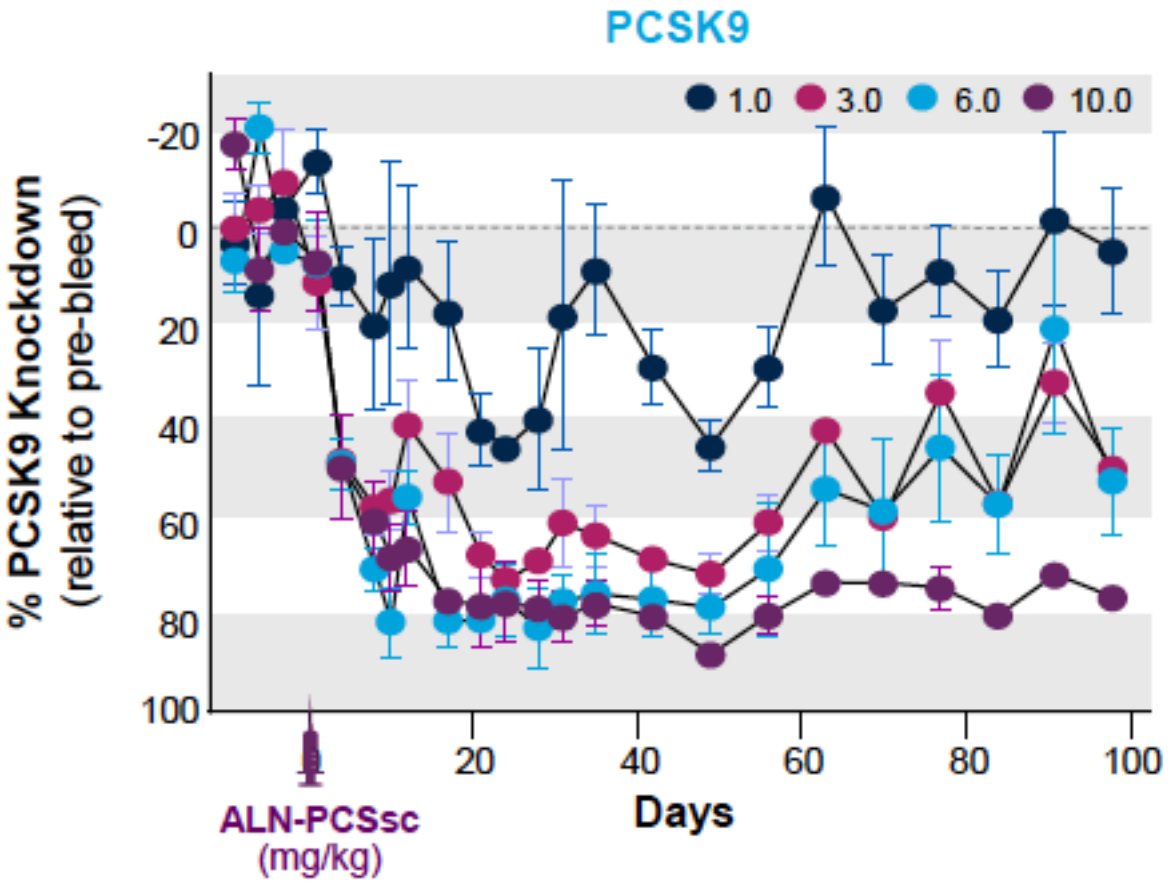
Hypercholesterolemia



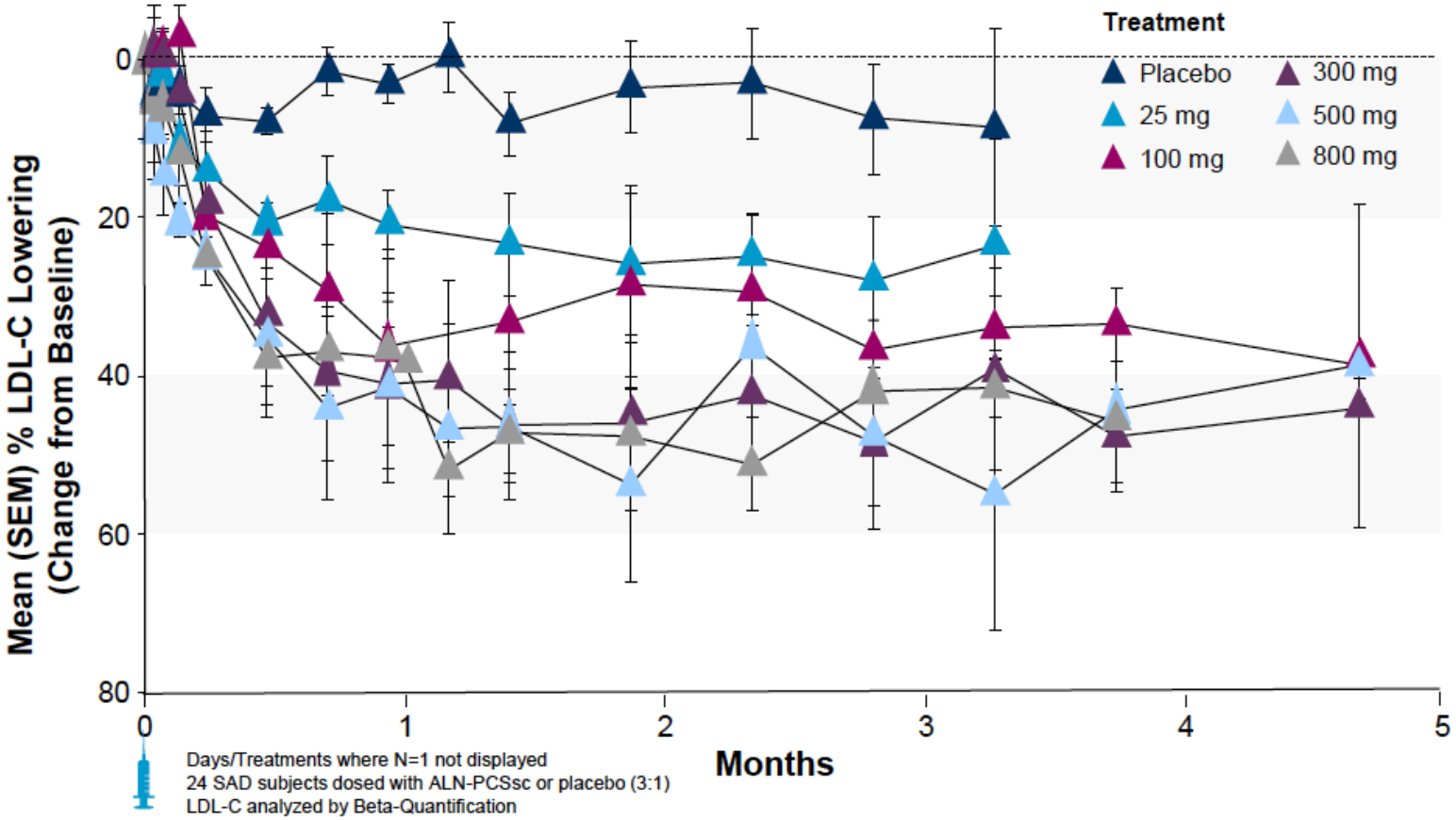
Hypercholesterolemia



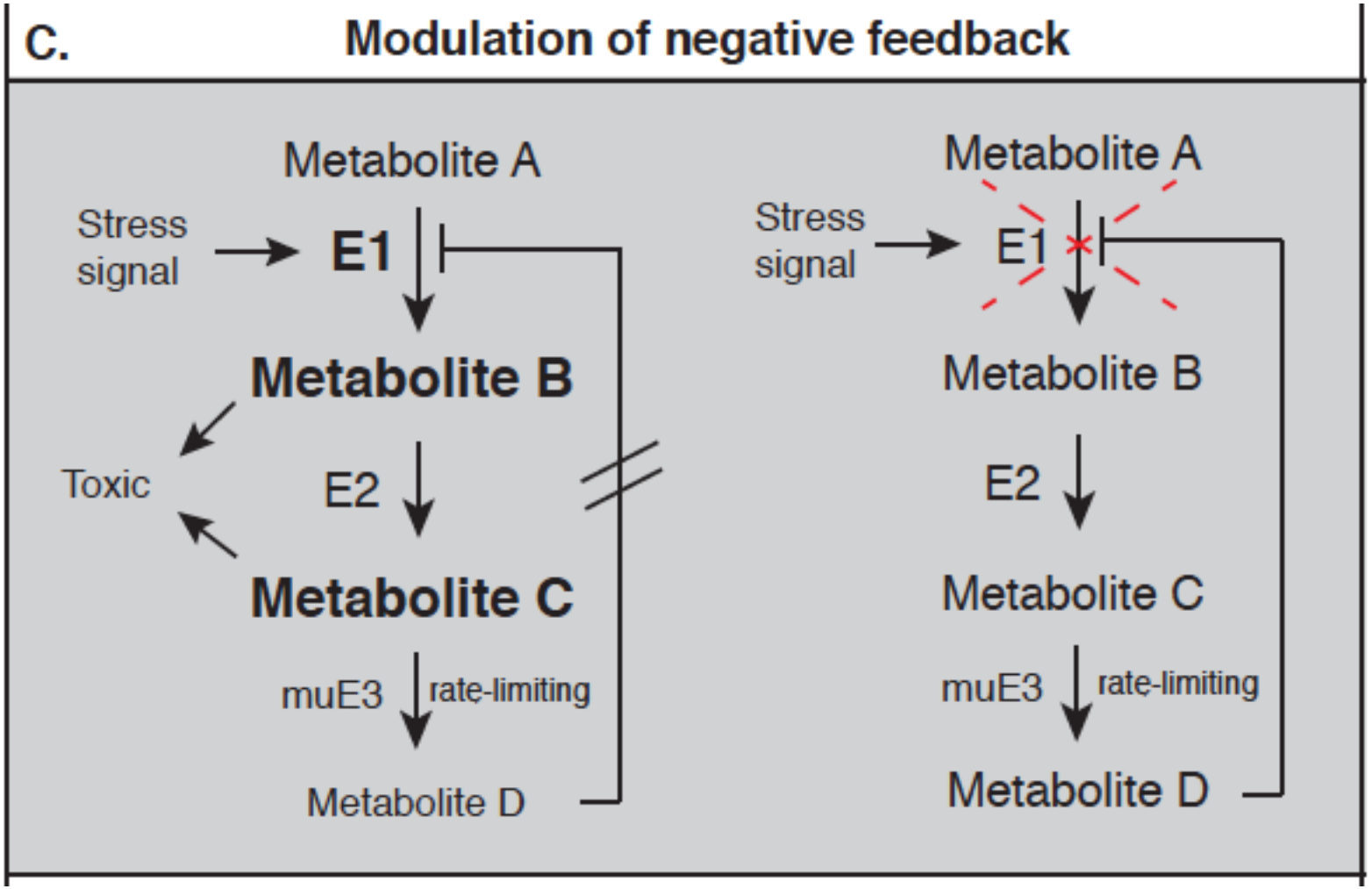
Long-term knockdown of hepatic PCSK9 using GalNAc-conjugated siRNAs



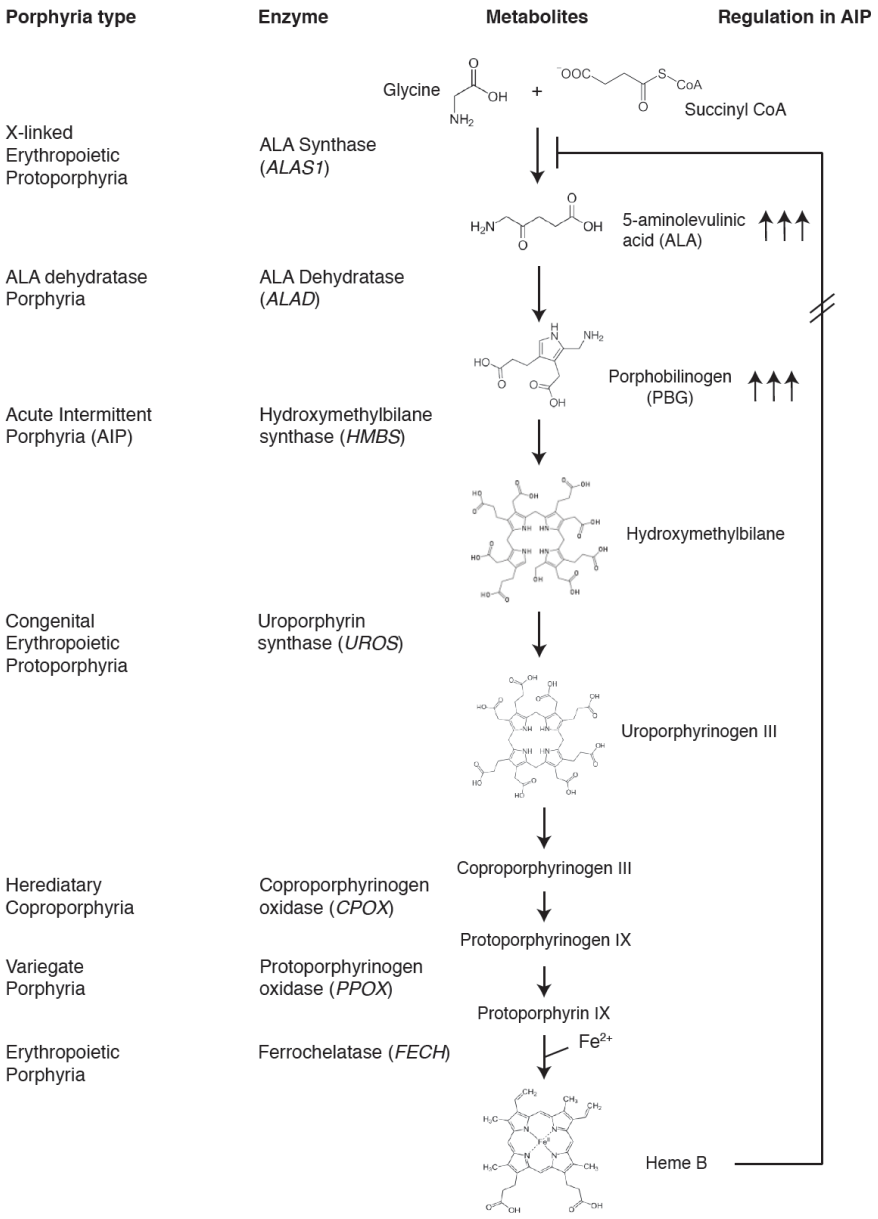
Long-term knockdown of hepatic PCSK9 using GalNAc-conjugated siRNAs



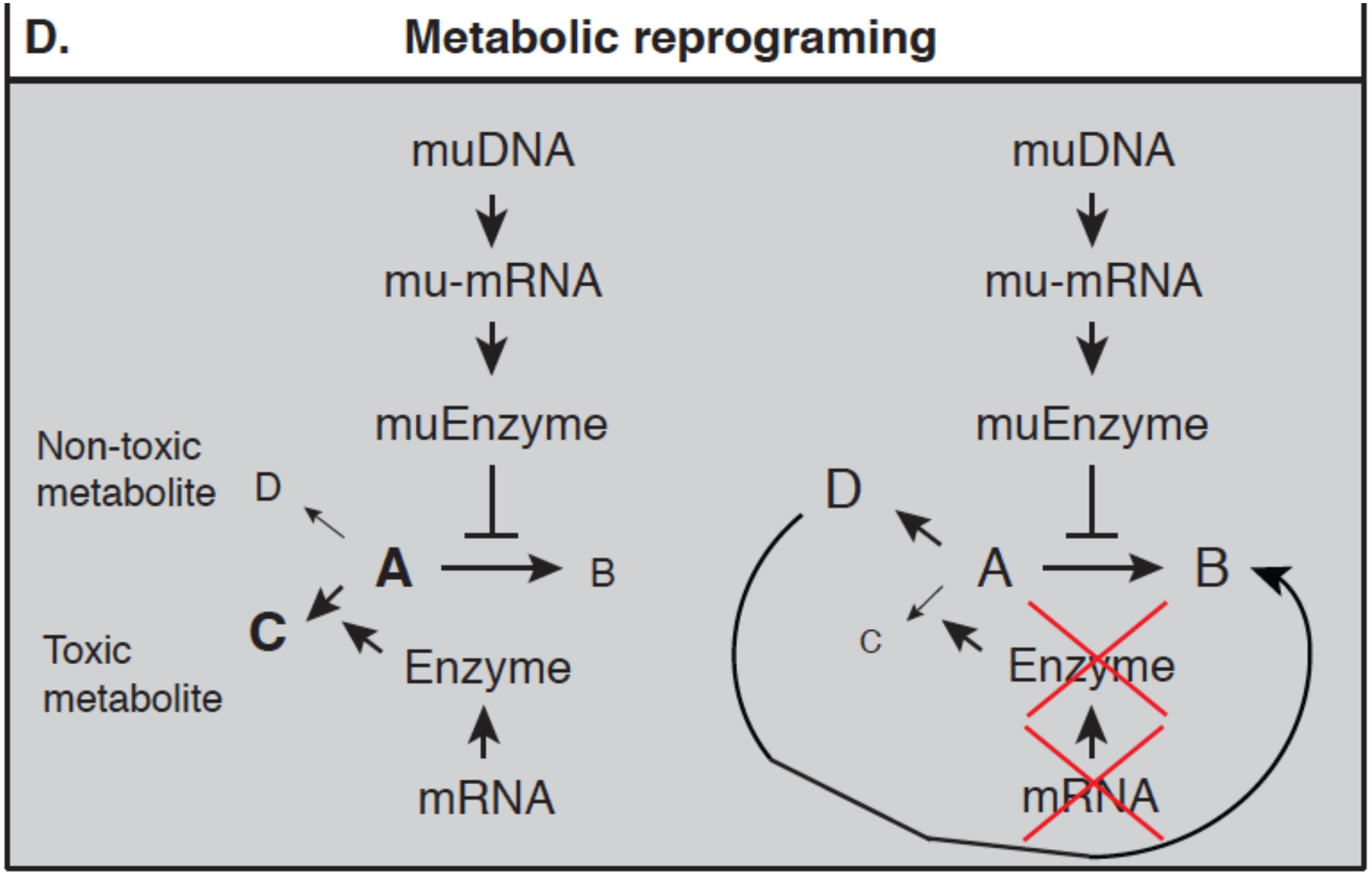
Applications of RNA Therapeutics



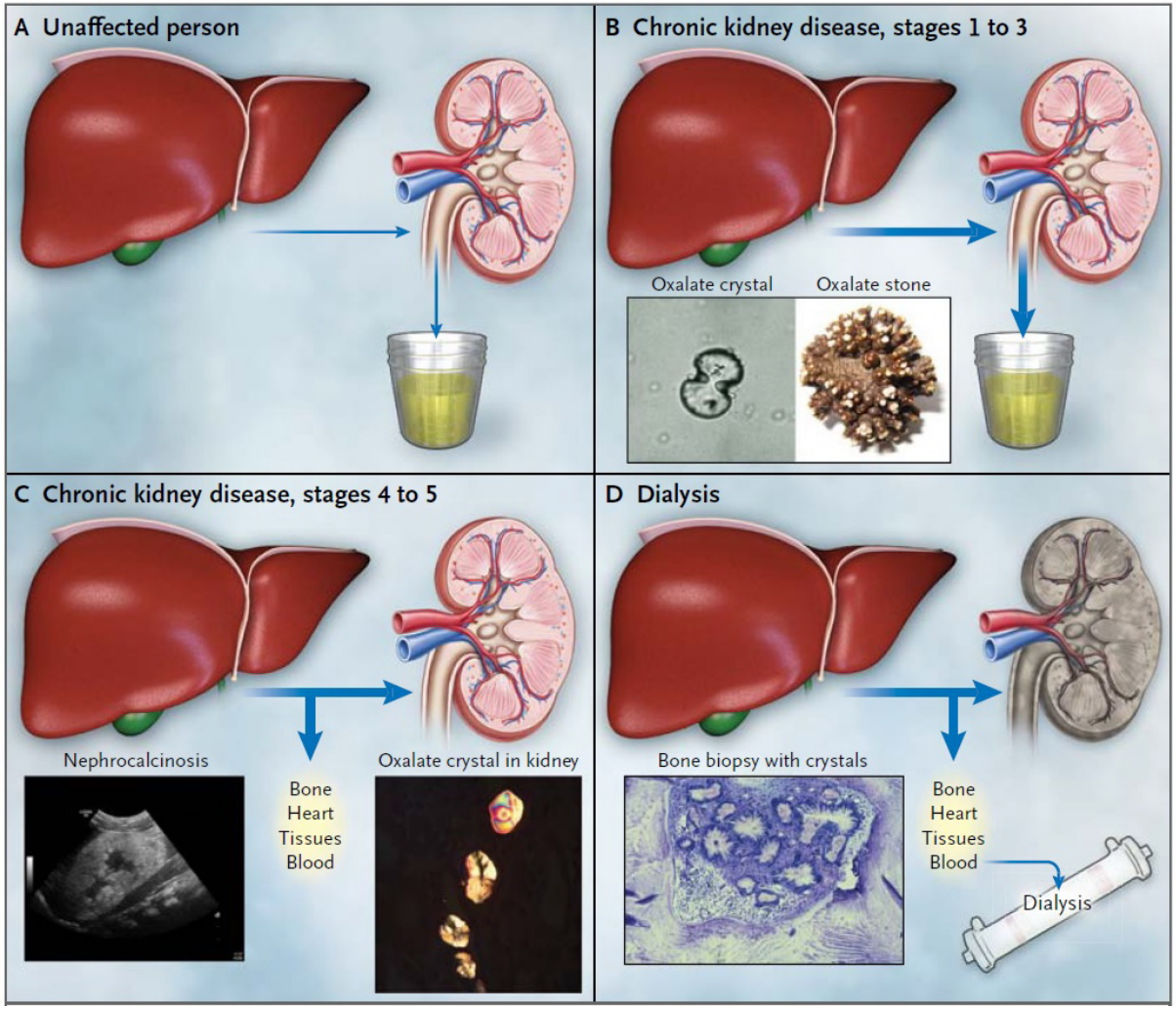
Porphyrias


Porphyria type
Enzyme
Metabolites
Regulation in AIP
Glycine
Succinyl CoA
X-linked Erythropoietic Protoporphyria
ALA Synthase (ALAS1)
5-aminolevulinic acid (ALA)
ALA dehydratase Porphyria
ALA Dehydratase (ALAD)
Porphobilinogen (PBG)
Acute Intermittent Porphyria (AIP)
Hydroxymethylbilane synthase (HMBS)
Hydroxymethylbilane
Congenital Erythropoietic Protoporphyria
Uroporphyrin synthase (UROS)
Uroporphyrinogen III
Coproporphyrinogen III
Hereditary Coproporphyria
Coproporphyrinogen oxidase (CPOX)
Protoporphyrinogen IX
Variegate Porphyria
Protoporphyrinogen oxidase (PPOX)
Protoporphyrin IX
Erythropoietic Porphyria
Ferrochelatase (FECH)
Fe2+
Heme B

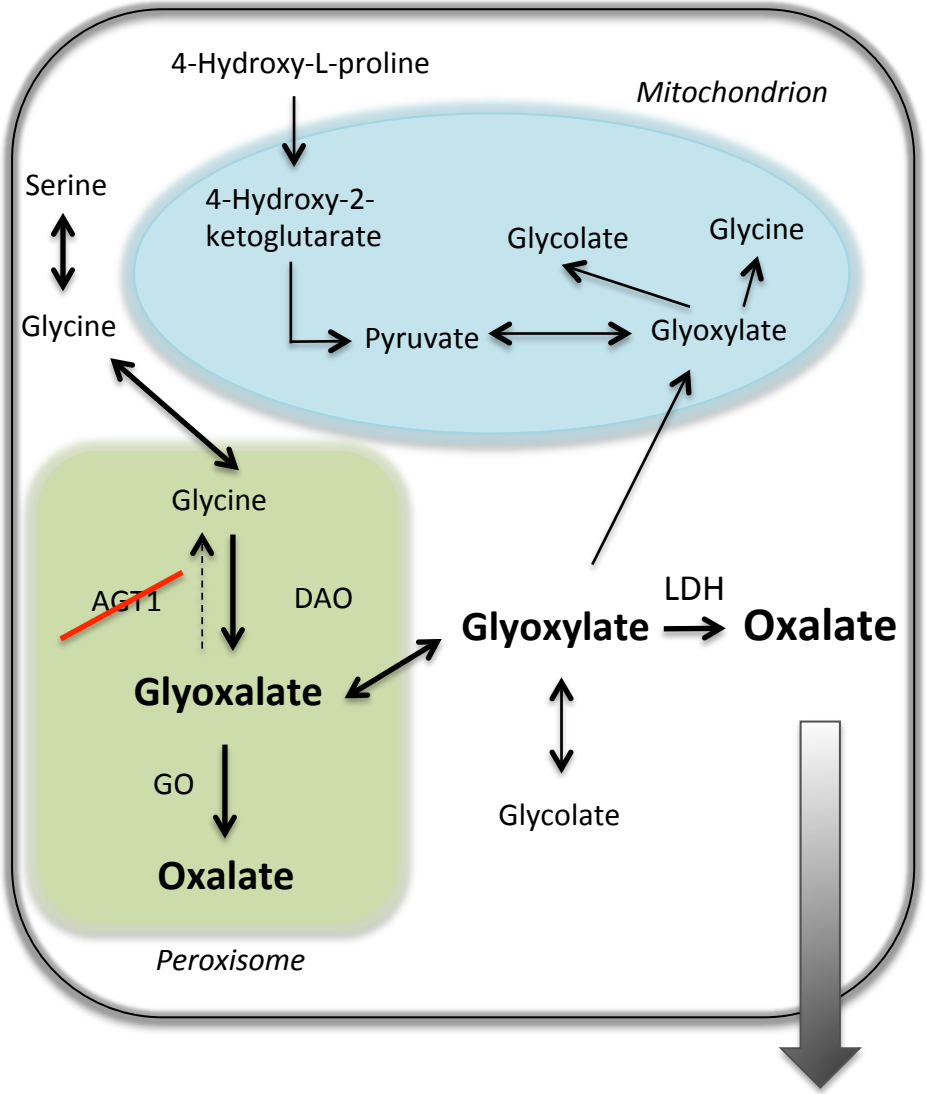
Applications of RNA Therapeutics

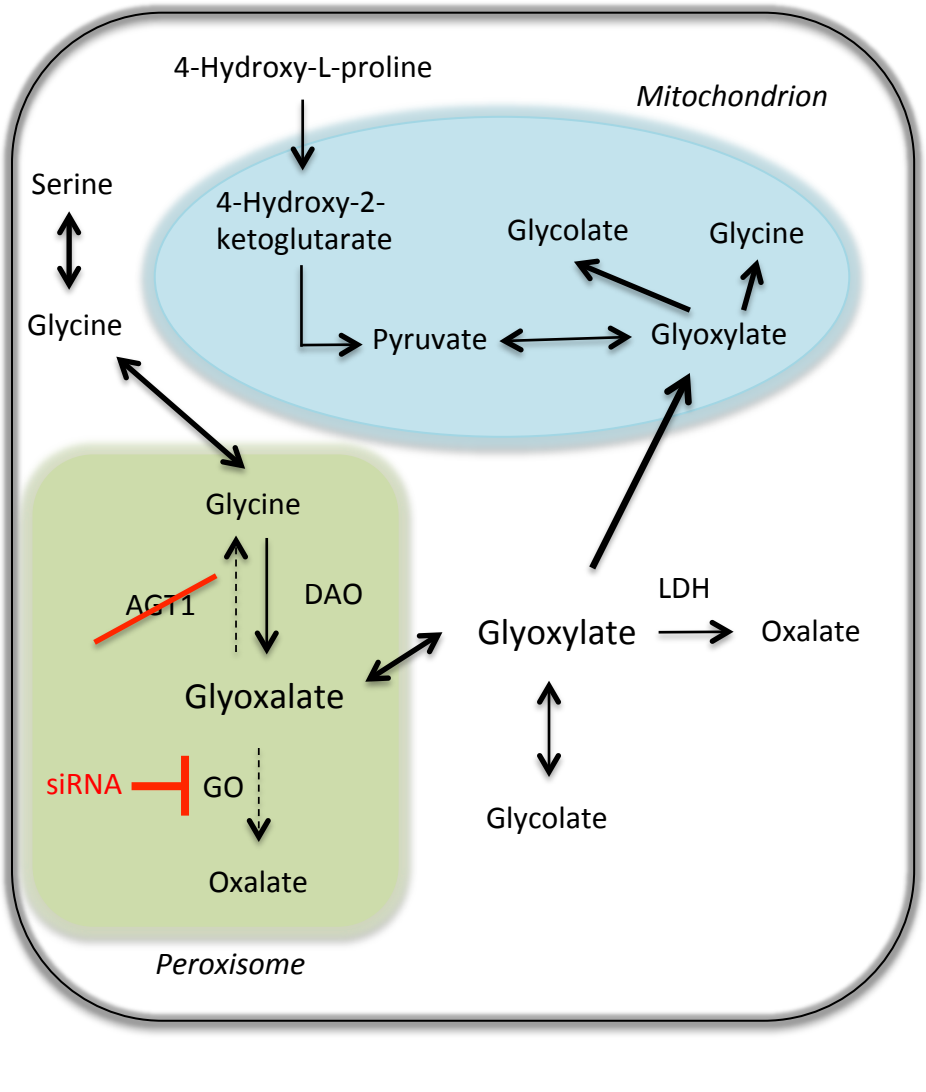


Primary Hyperoxaluria



Primary Hyperoxaluria





Calcium oxalate
Kidney stones

Non-viral delivery systems for DNA/RNA

- Methods of non-viral gene delivery have also been explored using **physical** (carrier-free gene delivery) and **chemical** approaches (synthetic vector-based gene delivery).

Physical methods

- **Physical approaches,** including
  - Needle injection
  - Electroporation
  - Gene gun
  - Ultrasound
  - Hydrodynamic delivery

- employ a physical force that permeates the cell membrane and facilitates intracellular gene transfer

Naked DNA

- The simplest method of non-viral transfection. Clinical trials carried out of intramuscular injection of a naked DNA plasmid have occurred with some success; however, the expression has been very low in comparison to other methods of transfection.

CHEMICAL METHODS THAT ENHANCE THE DELIVERY OF GENE THERAPY

- lipoplexes
- polyplexes

Lipoplexes

- DNA must be protected from damage & its entry into the cell must be facilitated
- Plasmid DNA can be covered with lipids in an organized structure like a micelle or a liposome
- complexed with DNA it is called a lipoplex

3 types of lipids:

- anionic (negatively charged)
- neutral
- cationic (positively charged)

Lipoplexes

Anionic and neutral lipids:

- were used for the construction of lipoplexes for synthetic vectors.
- used to the delivery of other therapeutic macromolecules
- but, there is toxicity associated with them,
- they are compatible with body fluids
- there was a possibility of adapting them to be tissue specific

Cationic lipids, due to their positive charge,

- naturally complex with the negatively charged DNA.
- their charge they interact with the cell membrane
- endocytosis of the lipoplex occurs
- DNA is released into the cytoplasm.
- The cationic lipids also protect against degradation of the DNA by the cell.

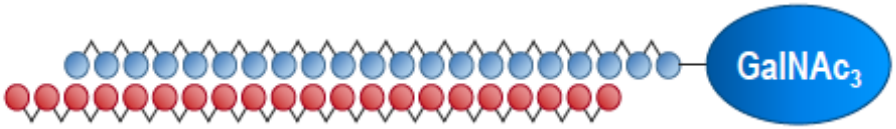
Common used of lipoplexes

- In gene transfer into cancer cells, where the supplied genes have activated tumor suppressor control genes in the cell
- decrease the activity of oncogenes.
- useful in transfecting respiratory epithelial cells, so they may be used for treatment of genetic respiratory diseases such as cystic fibrosis.

Inorganic nanoparticles

- Inorganic nanoparticles, such as gold, silica, iron oxide (ex. magnetofection) and calcium phosphates have been shown to be capable of gene delivery.
- Some of the benefits of inorganic vectors is in their storage stability, low manufacturing cost and often time, low immunogenicity, and resistance to microbial attack.
- Nano-sized materials less than 100 nm have been shown to efficiently trap the DNA or RNA and allows its escape from the endosome without degradation.
- Inorganics have also been shown to exhibit improved in vitro transfection for attached cell lines due to their increased density and preferential location on the base of the culture dish.

GalNAc-siRNA Conjugates
Subcutaneous RNAi Therapeutics



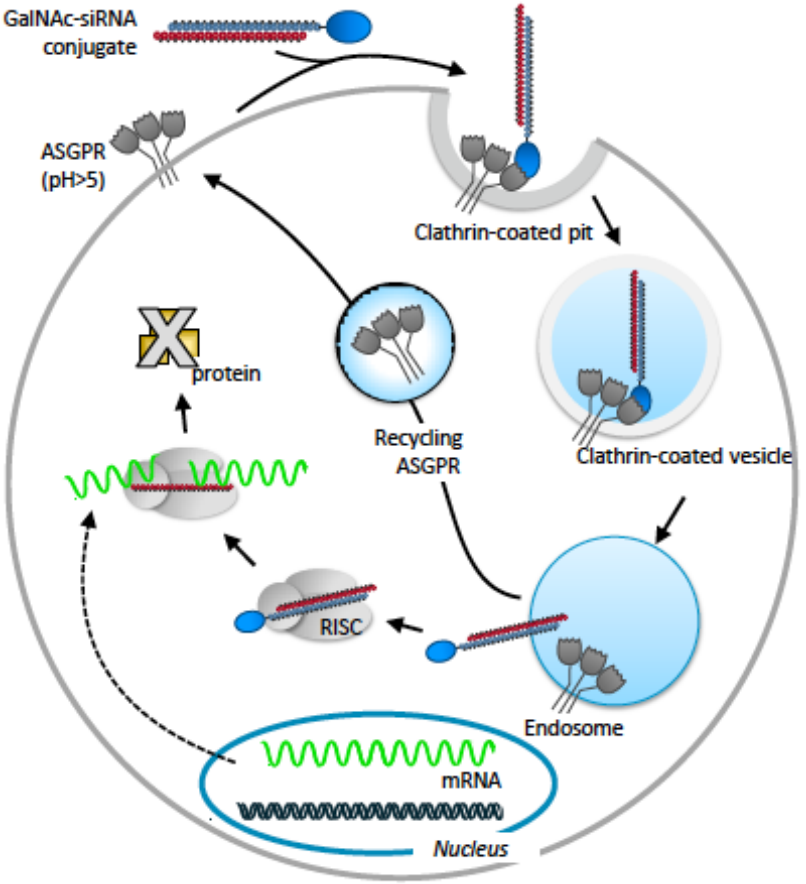
Asialoglycoprotein Receptor (ASGPR)

- Highly expressed in hepatocytes
- High rate of uptake
- Recycling time ~15 minutes
- Conserved across species

ALN-PCSsc

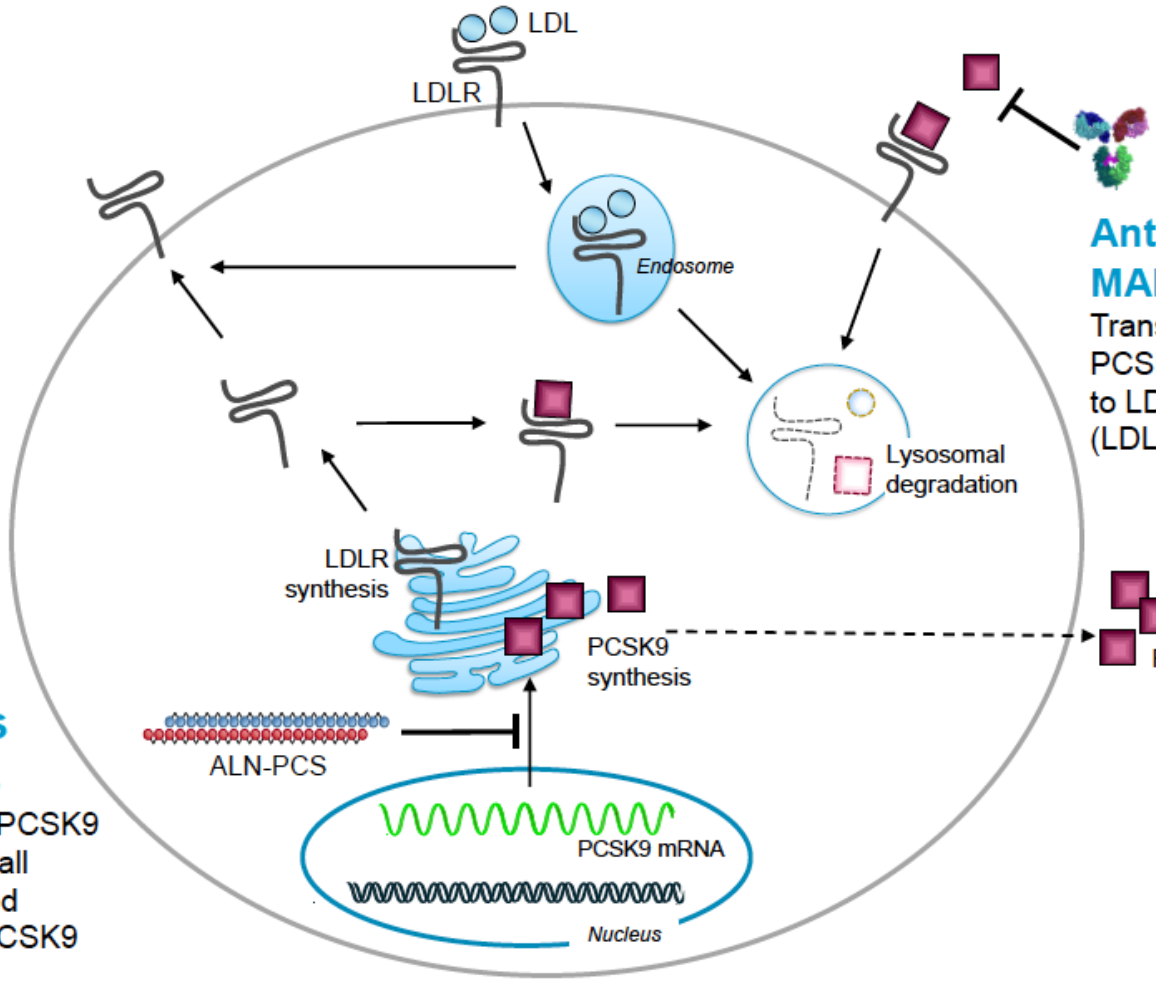
siRNA conjugated to N-acetylgalactosamine (GalNAc) ligand

- Efficient delivery to hepatocytes following subcutaneous administration
- Wide therapeutic index
- "Enhanced stabilization chemistry" (ESC) used with all programs after revusiran
  - Significantly improved potency and durability



Targeting PCSK9 for the treatment of hypercholesterolemia

PCSK9 Therapeutic Hypothesis



Anti-PCSK9 MAbs
Transiently block PCSK9 binding to LDL receptor (LDLR)

PCSK9 Synthesis Inhibitors
Durably block PCSK9 synthesis and all intracellular and extracellular PCSK9 functions

Genome editing

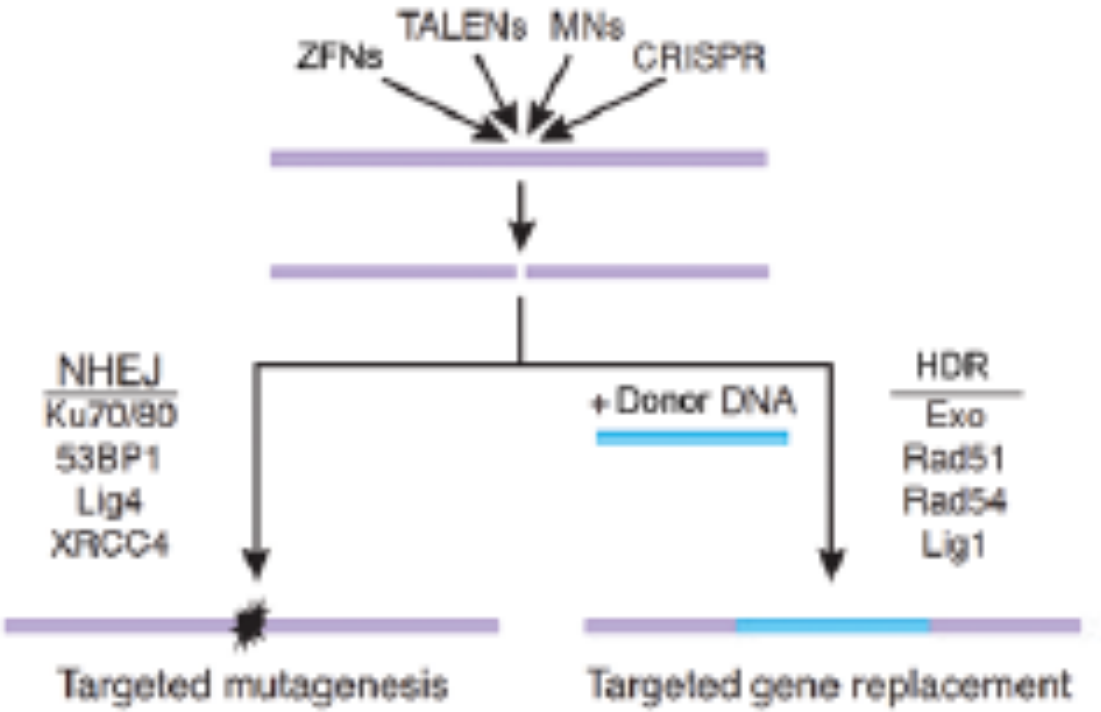
Genetic engineering in which DNA is inserted, replaced, or removed from a genome using artificial engineered nucleases

Mechanism:
Nucleases create specific double stranded breaks (DSBs) at desired locations in the genome and harness the cell's endogenous mechanisms to repair the induced break
By natural processes of homologous recombination (HR) and non-homologous end-joining (NHEJ)

Four families of engineered nucleases being used:

1. Zinc finger nucleases (ZFNs)
2. Transcription factor-Like Effector Nucleases (TALEN)
3. Engineered meganucleases
4. Crispr/Cas

Zinc finger nucleases



Zinc finger nucleases

Zinc finger nucleases (ZFNs)

Artificial restriction enzymes generated by fusing a zinc finger DNA binding domain to a DNA cleavage domain.
Can be designed to target specific DNA sequences in complex genomes.

Contain 2 domains:

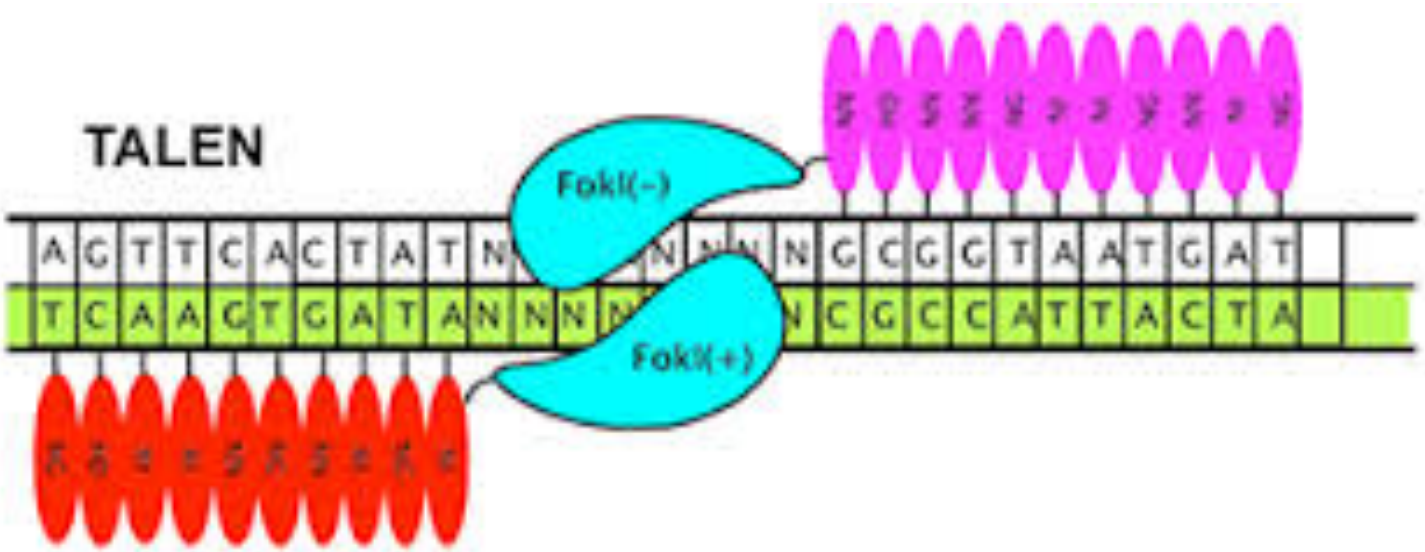
1. **DNA binding domain**

Typically contain between three and six individual zinc finger repeats and can each recognize between 9 and 18 basepairs. If the zinc finger domains are perfectly specific for their intended target site, even 18 bp can in theory target a single locus in a mammalian genome

2. DNA cleavage domain

Non-specific cleavage domain from the restriction endonuclease FokI is typically used as the cleavage domain in ZFNs. This cleavage domain must dimerize in order to cleave DNA and thus a pair of ZFNs are required to target non-palindromic DNA sites

Talens



Talen

Transcription activator-like effector nucleases (TALENs)

Tales are similar in architecture to ZNFs except that they use a different DNA binding domain They consist of arrays of single protein modules that each recognize a single DNA base pair and that are derived from transcription activator-like effectors (TALEs), factors encoded by plant pathogenic bacteria (Xanthomonas bacteria).

Each module is about 34 amino acids long, an they are nearly identical except for the identities of amino acids at positions 12 and 13, which together are known as “repeat variable di-residues”.

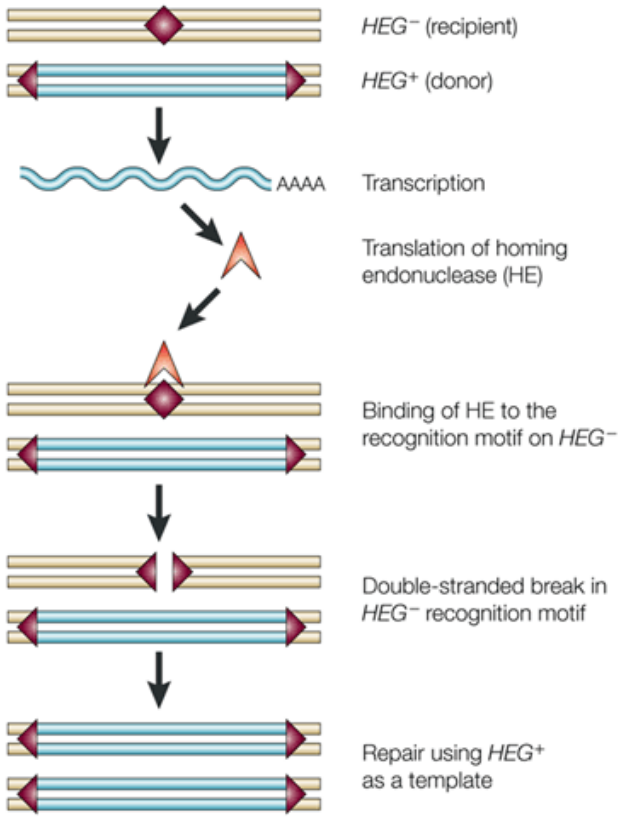
Can be engineered to bind practically any desired DNA sequence combining such an engineered TALE with a DNA cleavage domain (which cuts DNA strands), one can engineer restriction enzymes that are specific for any desired DNA Sequence introduced into cells, they can be used for genome editing.

Engineering of specific DNA-binding domains by selecting a combination of repeat segments containing the appropriate RVDs

Non-specific DNA cleavage domain from the end of the FokI endonuclease

If a TAL effector nuclease is not specific enough for its target site or does not target a unique site within the genome of interest, off-target cleavage may occur.

Homing endonucleases



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Homing endonucleases

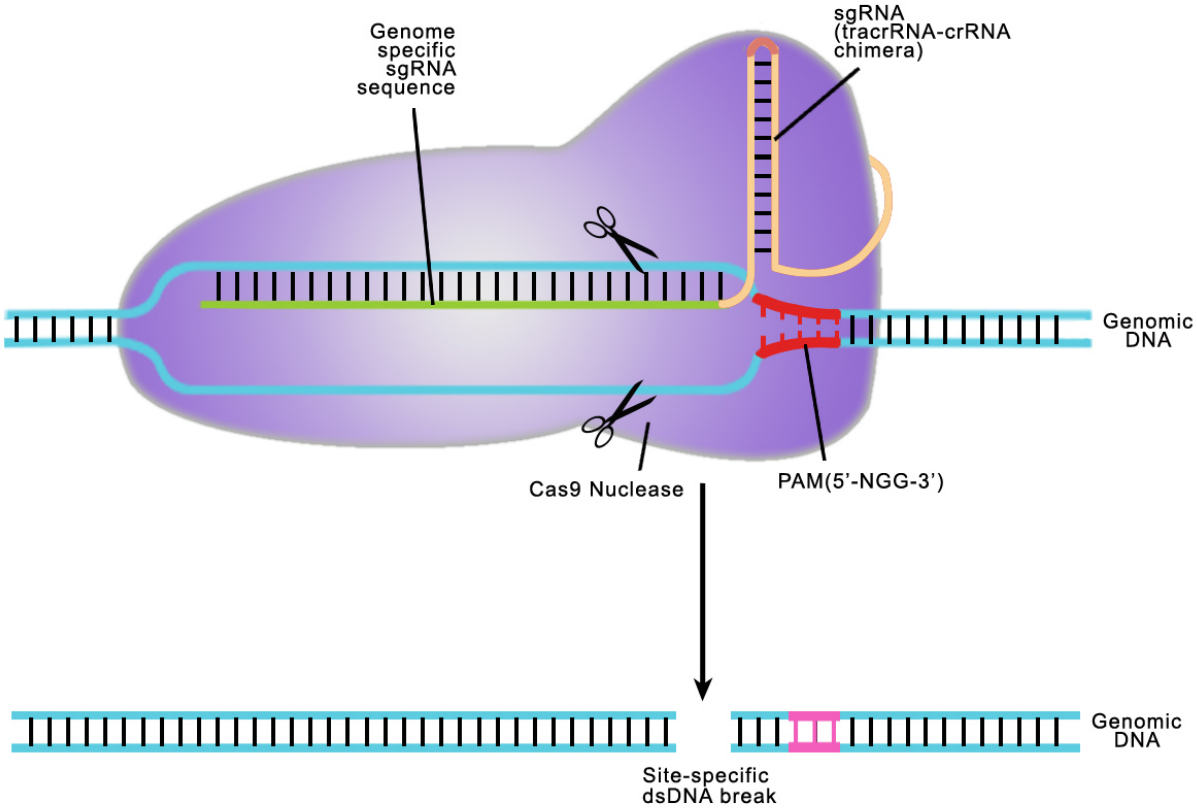
The **homing endonucleases** are a collection of endonucleases encoded either as unique Genes within introns, as fusions with host proteins, or as self-spicing inteins.

They catalyze the hydrolysis of genomic DNA within the cells that synthesize them.

Cleavage events are rare, often at singular locations.

One hypothesis considers them as selfish genetic elements, similar to transposons. They facilitate the perpetuation of genetic elements that encode them .

Cripsr/Cas9


Genome
specific
sgRNA
sequence
sgRNA
(tracrRNA-crRNA
chimera)
Genomic
DNA
Cas9 Nuclease
PAM(5'-NGG-3')
Genomic
DNA
Site-specific
dsDNA break

Cripsr/Cas9

Is distinct from engineered endonucleases in that it uses an RNA-guided system to perform DNA editing

Platform is derived from an innate bacterial immune system

Recognition of the target DNA sequence is mediated between the genomic DNA target and by a 20 nucleotide sequence in the crRNA.

crRNA hybridized with a transactivating RNA (tracrRNA) and the RNAs form complexes with the Cas9 protein.

Cas9 protein is directed to cleave the complementary target DNA sequence id it is adjacent to a short sequence known as 'protospacer adjacent motif' (PAM, NGG, NAG)

crRNA and tracrRNA can be combined in a single molecule known as the guide RNA (gRNA)

Cripsr/Cas9

Two YouTube movies on Crispr/Cas9 discovery and applications:

Jennifer Doudna (UC Berkeley / HHMI):
Genome Engineering with CRISPR-Cas9

//youtu.be/0dRT7slyGhs