Overview of Gene-Based Therapeutics

Hope in Action – CTNNB1 and MED13L Conference 2025

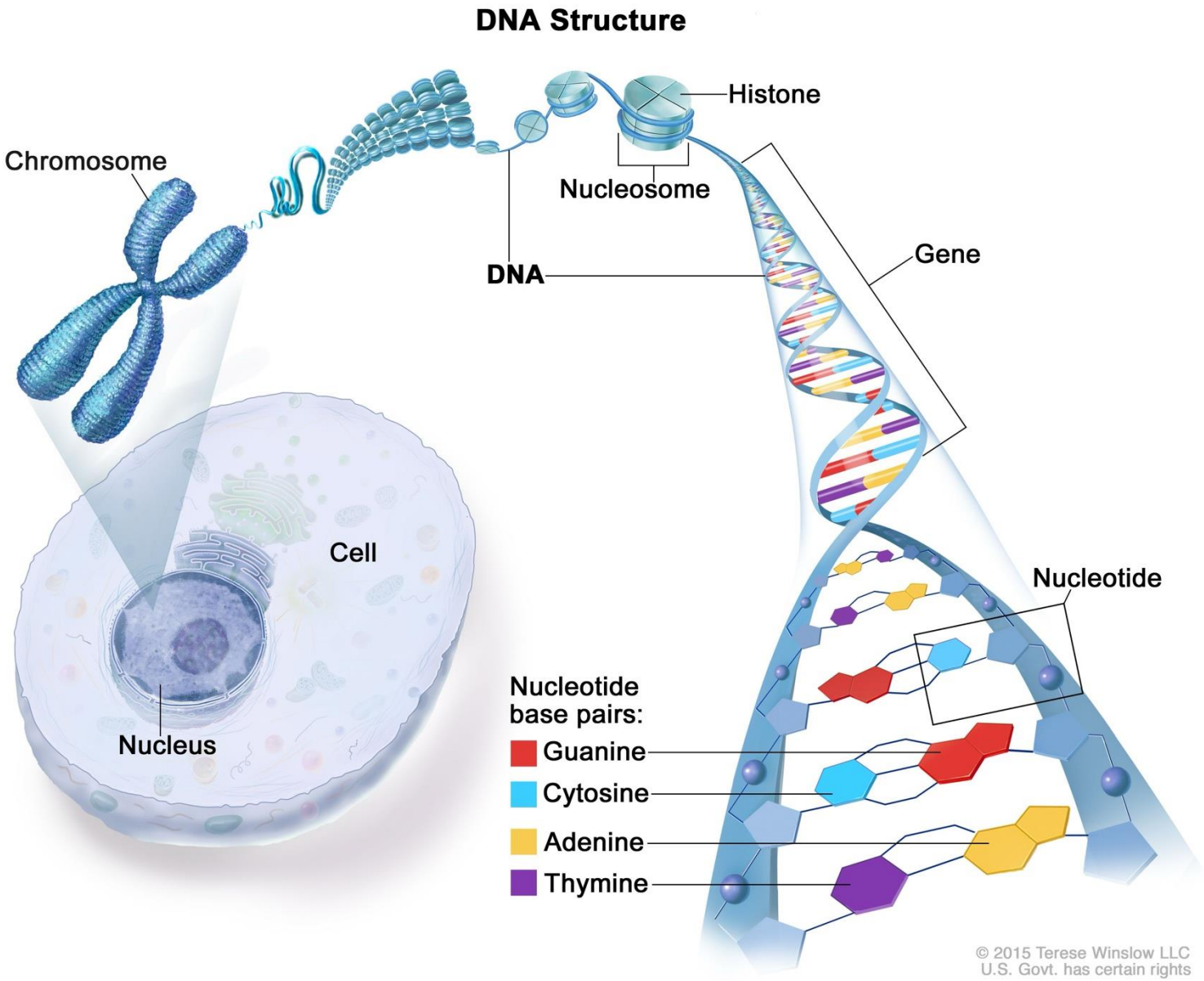
July 12th, 2025

Matthew Fuller, PhD
Vice President, Head of Gene Therapy Research – Ultragenyx
mfuller@ultragenyx.com

Disclaimer

- Matthew Fuller is an employee of Ultragenyx
- The opinions stated here are solely mine and do not necessarily reflect the views of Ultragenyx

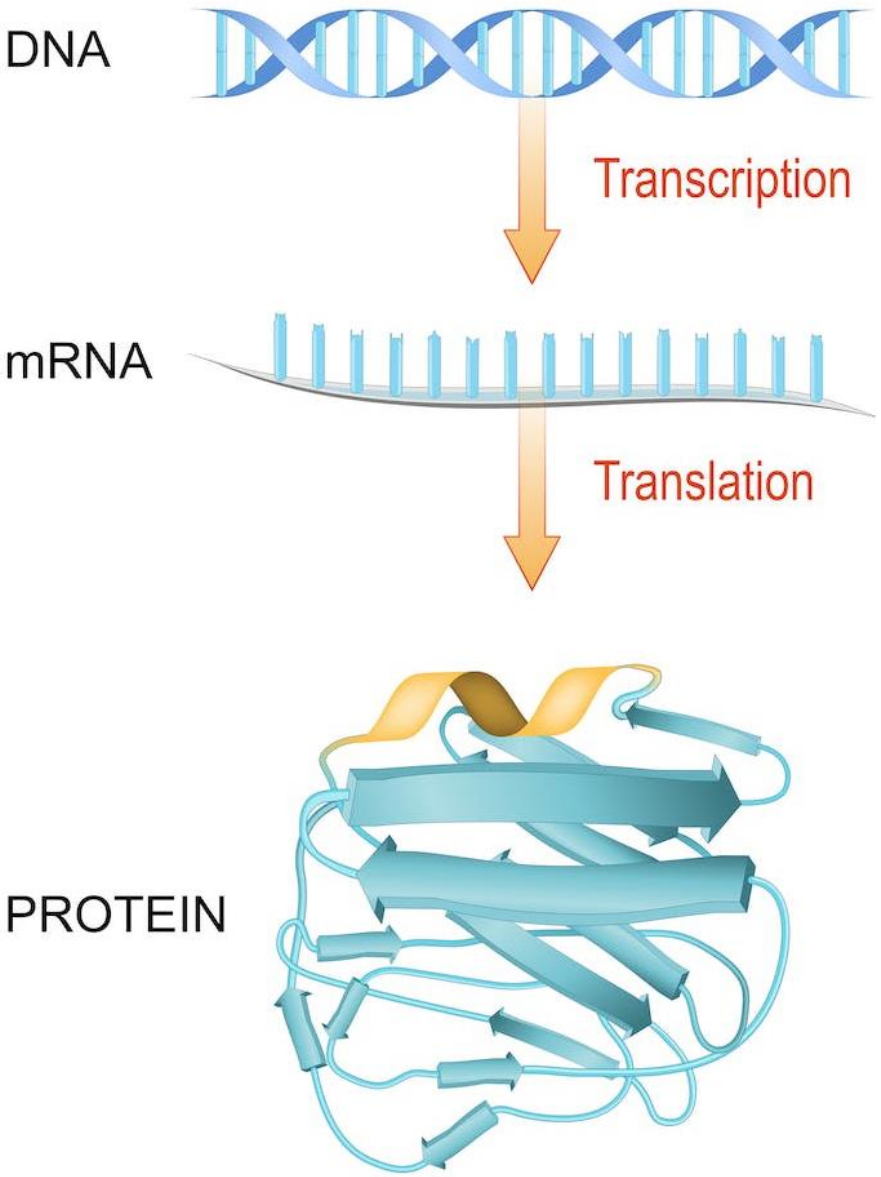
Basic Architecture of Genes & DNA



- **Gene – A basic genetic unit encoding a function**
  - Located in a specific section of DNA on a specific chromosome
  - Encoded by the DNA nucleotide "alphabet" – A, G, T & C
  - Genes encoding overt functions typically code for proteins
  - *Alterations* in genes can, but do not always, cause dysfunction
- **CTNNB1 gene is located on Chromosome 3 [3p22.1]**
- **MED13L gene is located on Chromosome 12 [12q24.21]**

https://www.cancer.gov/publications/dictionaries/cancer-terms/def/gene

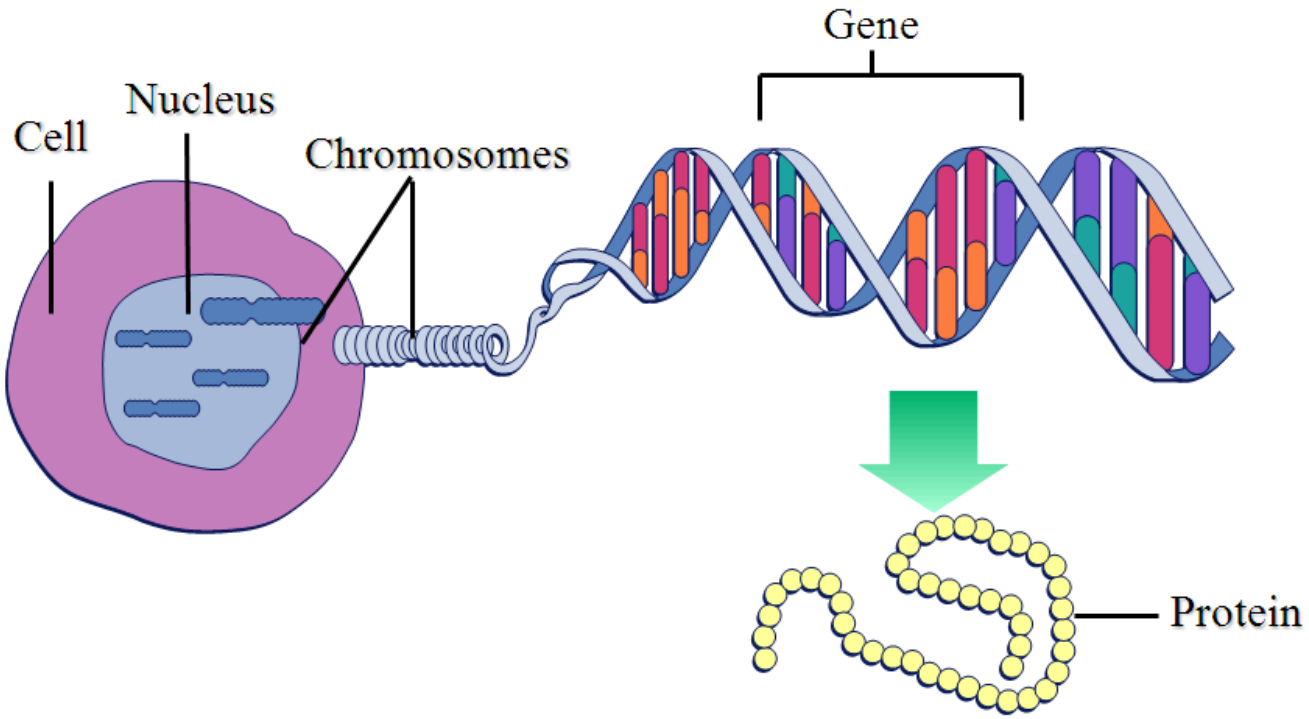
DNA Encodes for Proteins with Specific Functions



- DNA encodes ~20,000 genes with discrete cellular functions utilized by our cells & bodies for our day-to-day existence
- These functions are facilitated by pathways that utilize the 'DNA -> mRNA -> Protein' program to provide the necessary elements to carry out these day-to-day tasks
  - Examples of protein functions include processing of metabolites, muscle contraction, DNA replication, movement of other proteins, activation or inhibition of other proteins, much more…
- Gene-based therapies seek to replace or repair these specific pathways & molecules when typical function is not being provided due to mutation(s)

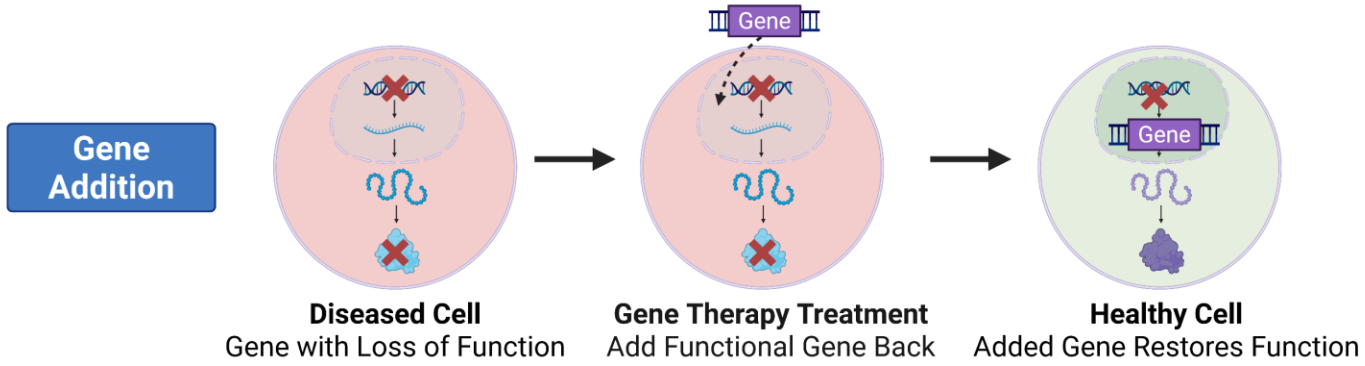
Basic Definition of Gene Therapy

Treatment or prevention of a [genetic] disease *via* introduction of genetic material expected to provide a necessary function



http://igbiologyy.blogspot.com/2014/03/chromosomes-dna-genes-and-alleles.html

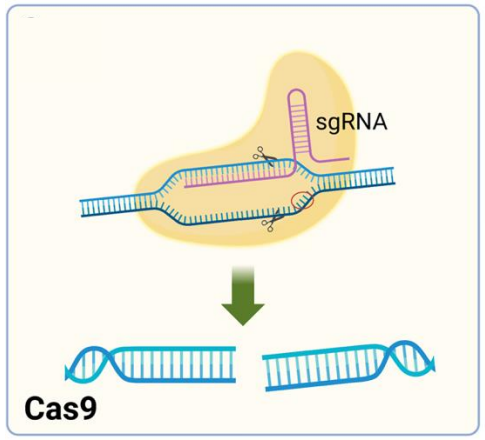
Gene Therapy Approaches



- **Gene therapy approaches include the delivery of a package/cargo designed to:**
  - Add back a functional or wildtype copy of a mutated or missing gene that is causing disease
  - Inhibit, inactivate, or "knock out," a mutated or overexpressed gene that is functioning improperly
  - Edit a mutated gene back to a functional or wildtype copy of that gene

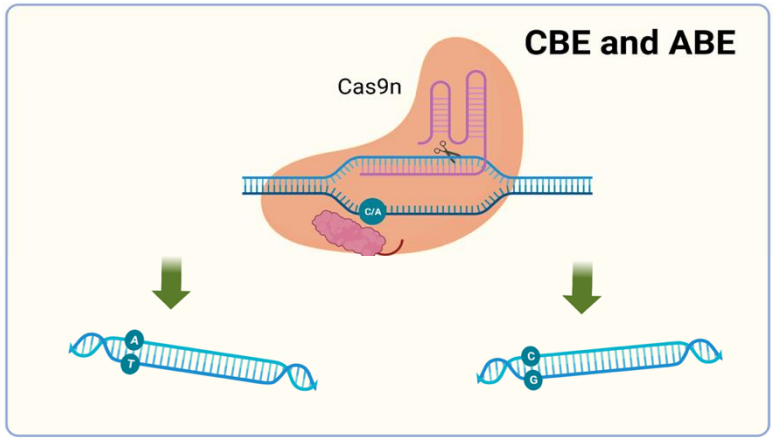
Gene Editing Therapeutic Approaches

Gene Editing



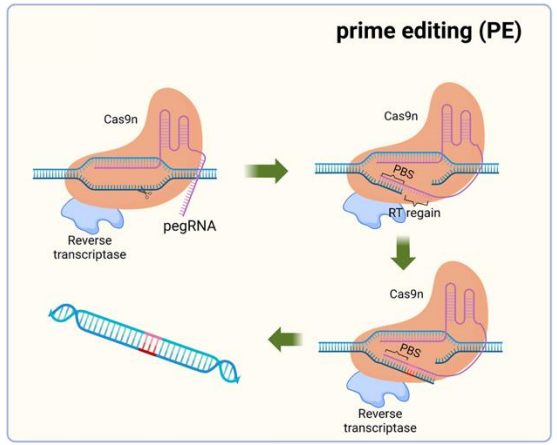
DNA Removal or
DNA Insertion

Base Editing



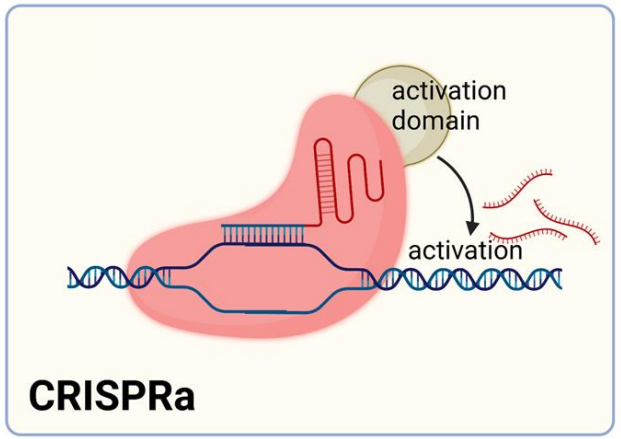
Change DNA Base “Letter”

Prime Editing



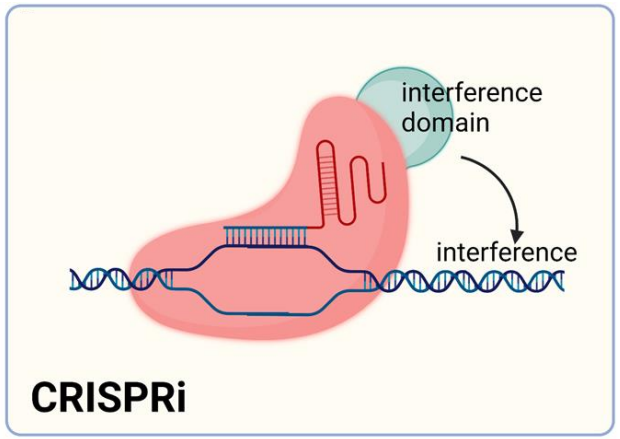
Longer DNA Insertion

CRISPR Activation



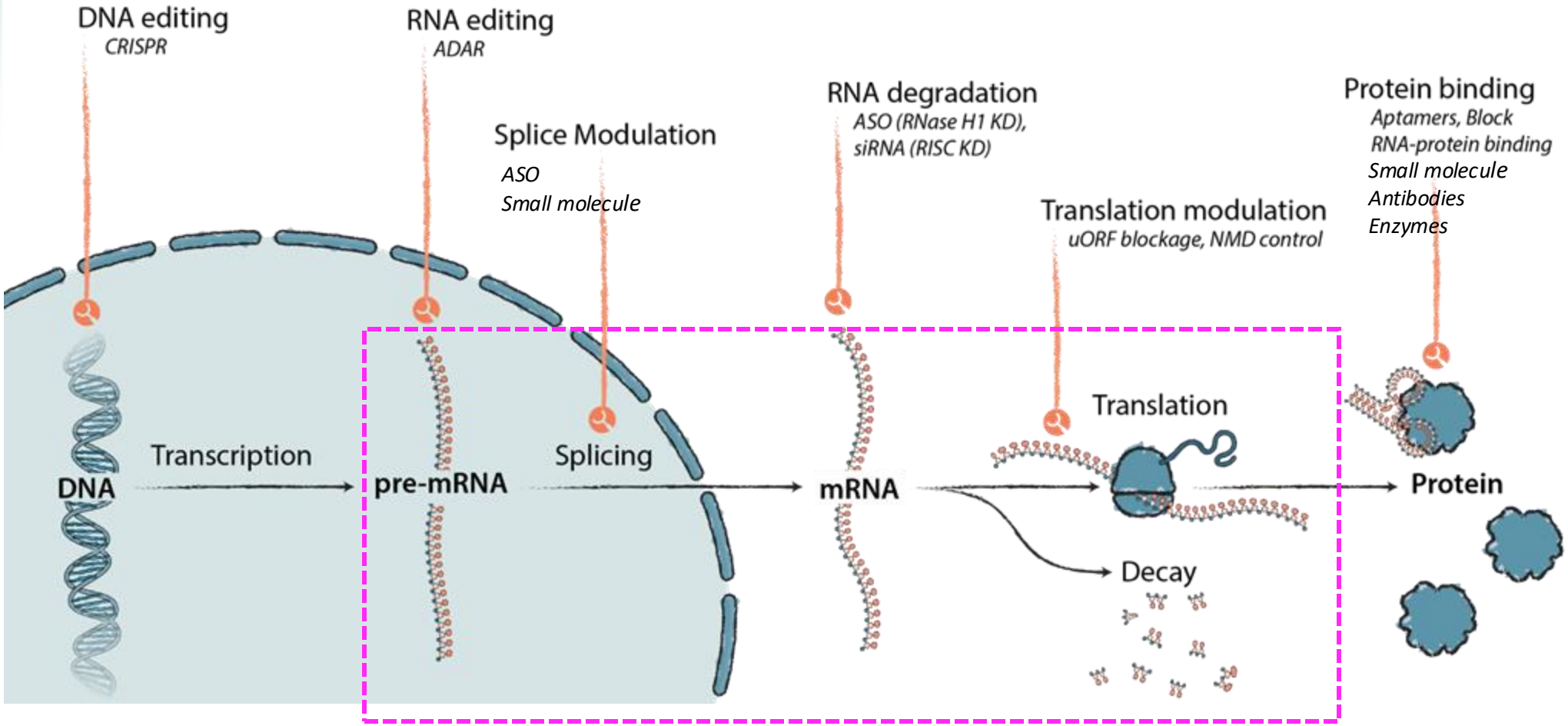
Turn On a Gene

CRISPR Interference



Turn Off a Gene

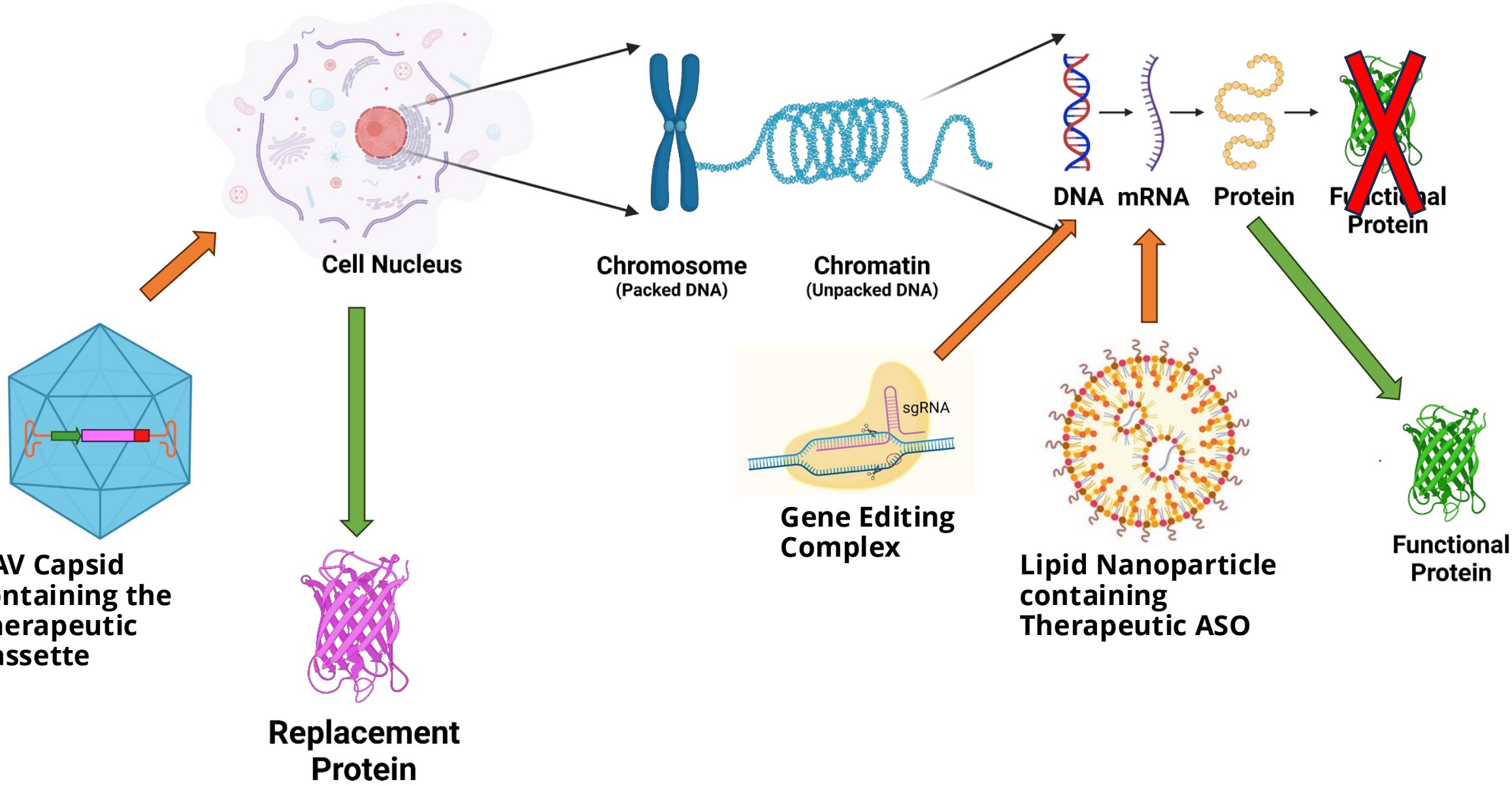
Overview of Gene-based Therapeutic Approaches



Key consideration of different modalities is where to intervene to correct the genetic processes in the most efficient way

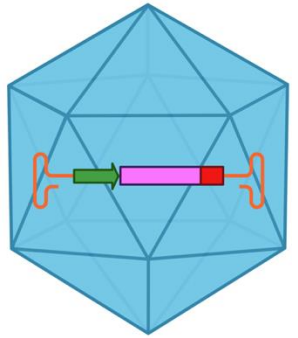
Different pathogenic variants within the same rare disease may be amenable to different modalities

Gene-based Therapy Mechanisms

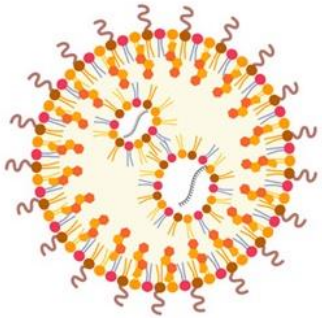


Considerations for CTNNB1 Syndrome Therapies

- **Gene Addition Therapy**
  - The CTNNB1 coding region is ~2346 nucleotides, which is small enough to fit into the AAV capsid with some real estate left for additional required elements
  - Will need to understand target tissues and cell types that must be treated for therapeutic benefit (CNS, Muscle?, Others?)
  - Will likely need to characterize regulatory elements to limit CTNNB1 overexpression

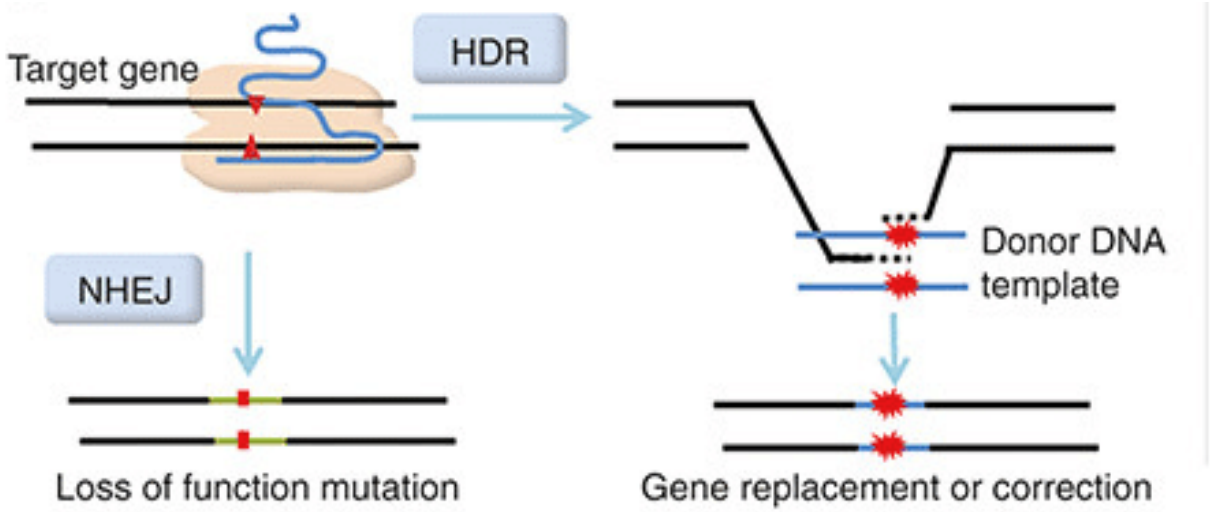


- **RNA-based Therapies**
  - CTNNB1 syndrome is autosomal dominant/haploinsufficient
    - In theory, the 'healthy' gene can be upregulated
  - ASO approach could possibly be used to increase expression of β-Catenin
  - ASO approach could possibly be used to target exon 3 of β-Catenin for exon skipping



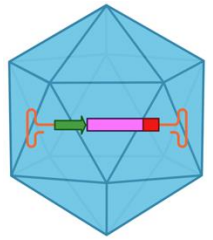
Considerations for CTNNB1 Syndrome Therapies

- **Gene Editing Therapy**
  - Potential to directly “fix” underlying genetic cause
  - Will require identification of clinically relevant ‘editable’ regions to correct mutation(s)
  - Newer modality; CRISPR only successfully used in clinic for *ex vivo* treatments so far

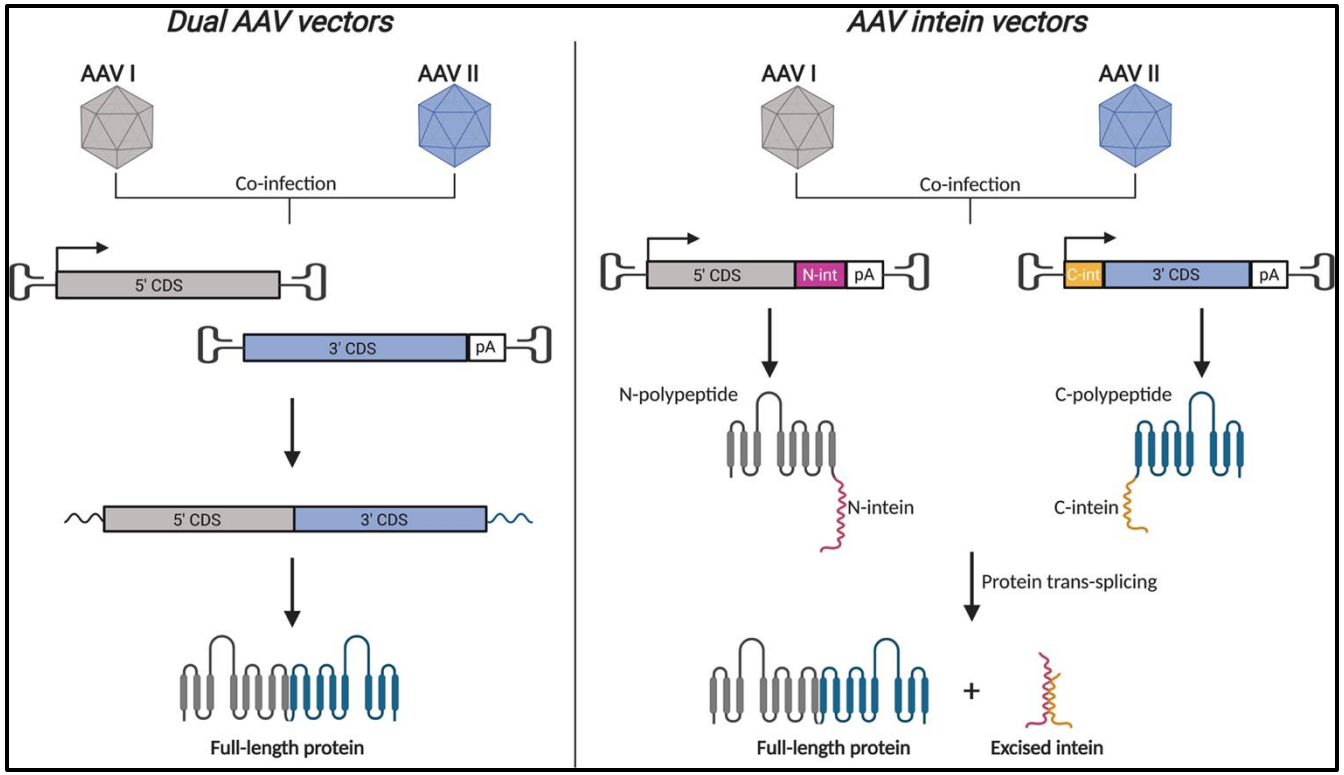


  - Will need to understand target tissues, cell types and number of cells that must be treated for therapeutic benefit
  - *In vivo* brain gene editing technology still being developed
    - Efficiency of gene editing is currently low and is likely lower in CNS cells with current generation of technology
    - *In vivo* delivery of gene editing components to CNS is challenging

Considerations for MED13L Syndrome Therapies



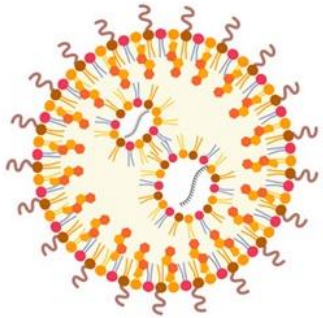
- **Gene Addition Therapy**
  - The MED13L coding region is ~6,633 nucleotides, which is ~2,000 nucleotides too large to fit in the AAV capsid.
- This would require utilizing unique approaches to facilitate the delivery of the full MED13L protein or generation of a “mini-gene”
  - Dual vector approach
  - Use of ‘trans-splicing’
  - Use of ‘inteins’
  - Identification of a MED13L mini-gene and testing of its function *in vitro* and *in vivo*



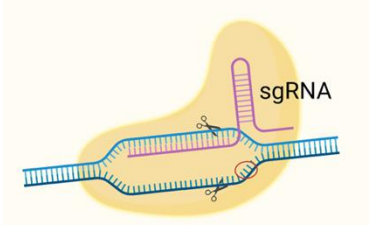
- These approaches are currently *low efficiency* and might be even lower efficiency with brain delivery

Considerations for MED13L Syndrome

- **RNA-based Therapies**
  - Therapeutic options for MED13L syndrome will be driven by an individual's genetic diagnosis.
    - For autosomal dominant/haploinsufficient cases, the 'healthy' gene could be upregulated
  - ASO approach could increase expression of MED13L
  - Exon skipping via ASO could be considered for exons 7, 15 or 25



- **Gene Editing Therapy**
  - For microdeletions, gene addition or prime editing could be considered
  - Will require identification of clinically relevant 'editable' regions/hot spots to correct mutation(s)
  - Efficiency of prime editing is currently very low
  - *In vivo* delivery of prime editing components to CNS is challenging at present time



Additional Considerations for CTNNB1 and MED13L

- **Continue supporting *in vitro* (cell) and *in vivo* (mouse model or similar) studies to fully understand target cell types and target expression levels**
  - Current data suggests that excitatory neurons could be a key target for CTNNB1
  - Current data suggests that cortical and hippocampal neurons could be a key target for MED13L
  - Currently unclear if CTNNB1/MED13L delivery to muscle or other tissues will be *necessary* for therapeutic benefit
  - Target expression levels for therapeutic benefit will need to be determined, which will inform therapeutic design
    - Levels of expression between haploinsufficiency and wild-type likely required
    - Unclear where the threshold of minimal expression required for therapeutic benefit lies. Overexpression would likely be detrimental.
  - Therapeutic treatment window will need to be determined
- **Invest in multiple modalities if financially feasible**
  - Some therapeutic approaches work better than others based on disease-specific mechanisms, which are not always predictable.

Key Takeaways

- **Success is *critically dependent* upon solid basic and clinical science knowledge, a targetable cellular/tissue target choice, and knowledge of the therapeutic window**
- **Each therapeutic strategy has pros and cons**
  - Strategy must be matched to the biology of the disease and target cells
- **Invest in multiple therapeutic strategies/modalities, as possible**
  - Disease biology is not always predictable, and it is important to invest in several treatment strategies to improve probability of technical success

Questions?