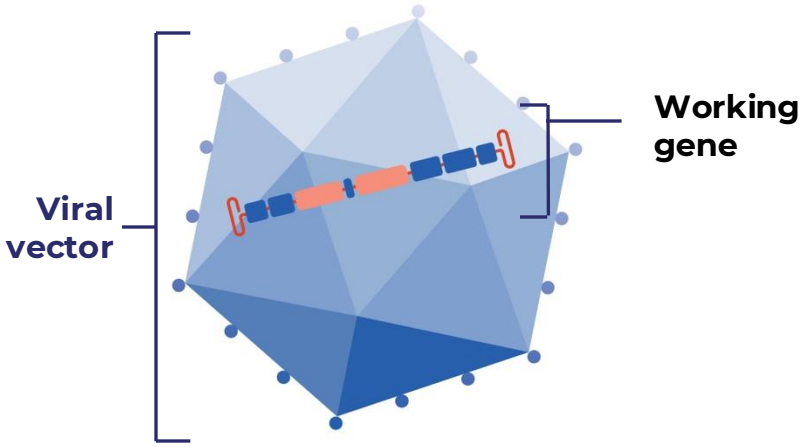
Adeno-Associated Virus (AAV) in Gene Therapy

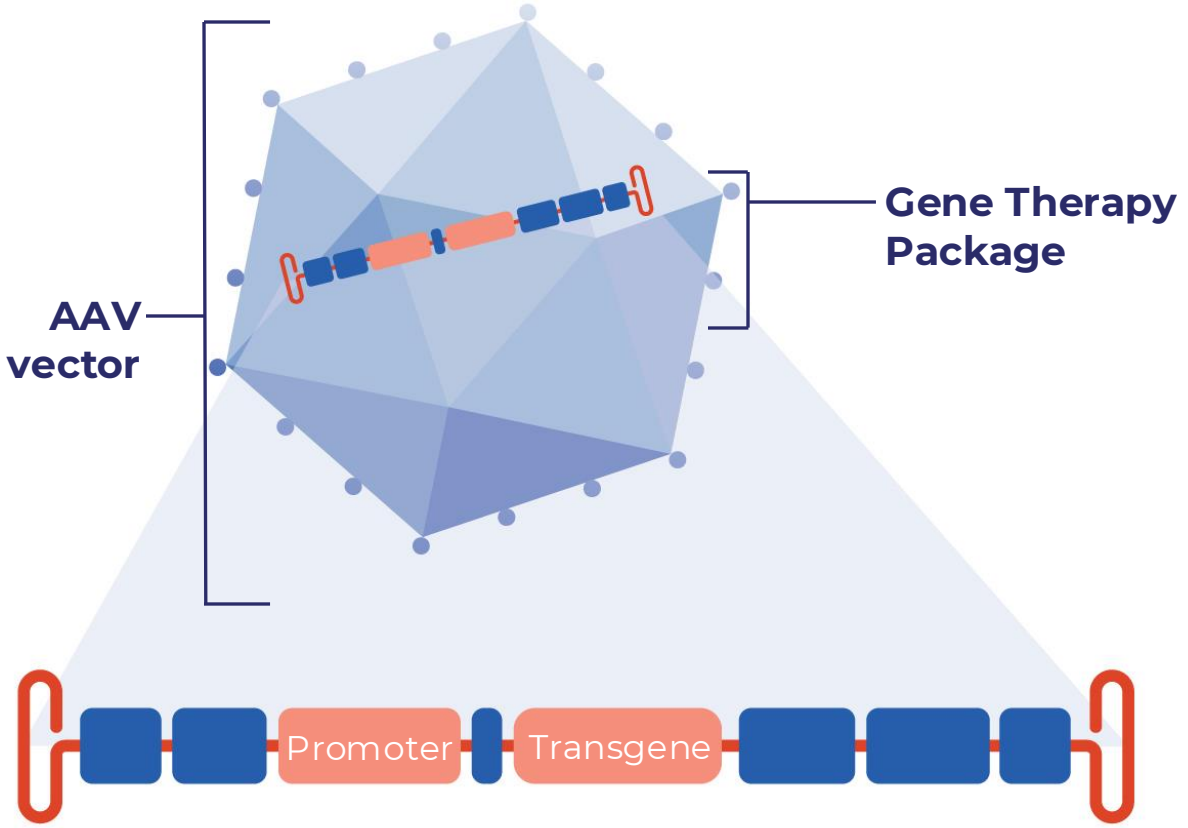
- What are AAVs?
  - Viruses that occur naturally in the environment[2]
  - Do not cause symptoms or disease in people[2]
- Why are they used in gene therapy?
  - Efficient at delivering new genes to cells[2]
  - Different types of AAVs exist and can be tailored to target specific types of cells[2]
- How are AAVs (vectors) used for gene therapy?
  - Viral gene is removed from the AAV vector
  - New working gene inserted
  - AAV vectors deliver the working gene to target cells[1]



1 Vectors 101. American Society of Gene + Cell Therapy. 2021. https://patienteducation.asgct.org/gene-therapy-101/vectors-101. Accessed September 14, 2022.
2 Glossary. American Society of Gene + Cell Therapy; 2022. https://asgct.org/education/more-resources/glossary. Accessed September 14, 2022.

AAV Vectors Contain the Working Gene

- Inside the AAV vector is the **Gene Therapy Package**
  - A **transgene** which is the new working gene that will be delivered
  - A **promoter** which directs the transgene to “turn on” in selected cells – like heart muscle cells



Domenger, C & Grimm, D. 2019. Next-Generation AAV Vectors – Do not Judge a Virus (Only) by its Cover. Human Molecular Genetics, 28(R1). https://doi.org/10.1093/hmg/ddz148

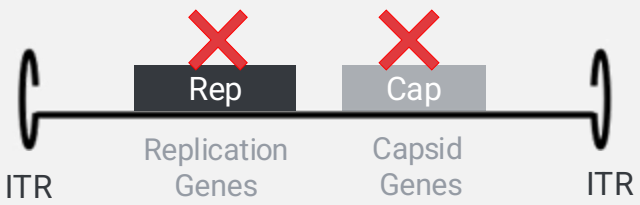
Content courtesy of TENAYA THERAPEUTICS

Steps to Constructing Recombinant AAV Vectors

rAAV vectors have been designed to be non-pathogenic[1]

1

Delete natural or WT viral genes Virus becomes replication incompetent



AAV Virus - WT Genome

The virus has a linear single strand of DNA inside a protein capsid, and the DNA has two native (WT) genes that are used for virus replication[2]

2

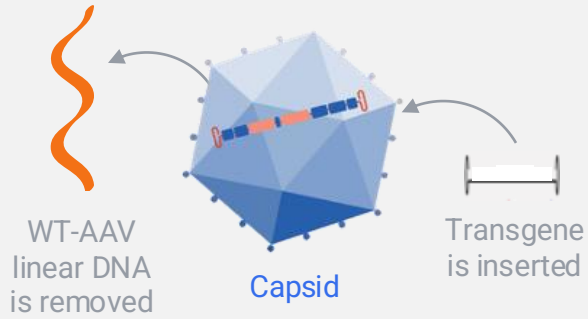
Replace with a transgene cassette

Transgene Cassette

The native genes are replaced with a transgene cassette that contains the healthy or working version of the gene and a promoter to direct expression in specific cell types[2]

3

Place cassette into empty viral capsid



AAV Vector

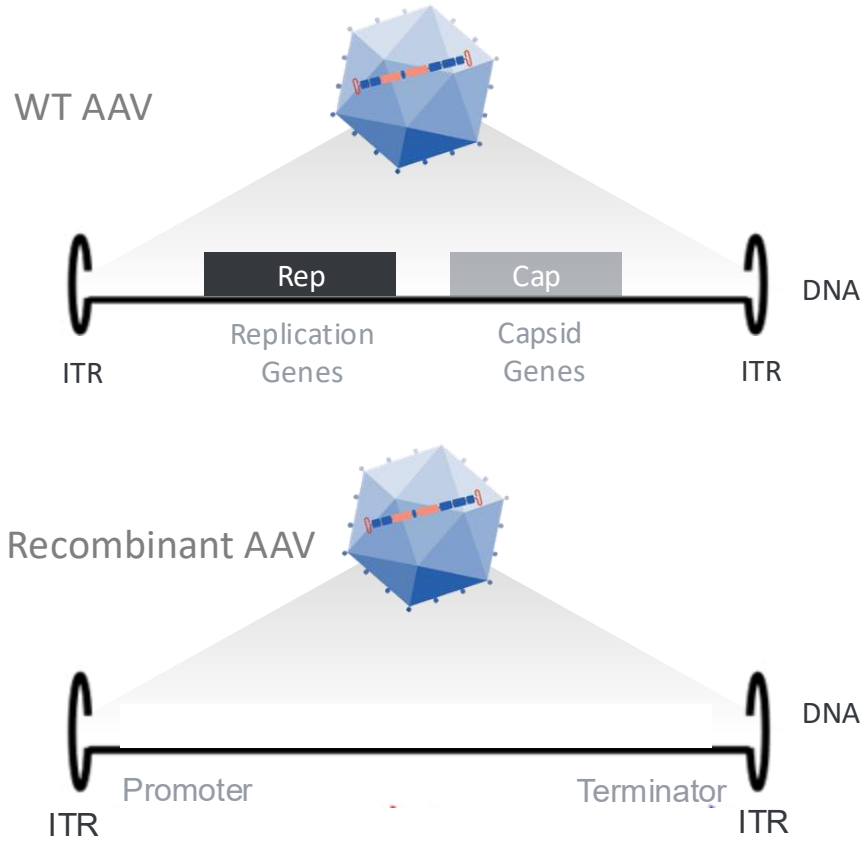
The payload capacity of AAV vectors is limited to ≈4.5kb of gene sequence (4.7kb with regulatory elements)
The small packaging size limits the delivery of a large therapeutic genes[2]

rAAV, recombinant adeno-associated virus; WT: Wild Type; ITR: Inverted Terminal Repeat; kb, kilobase.

1. Kantor B, et al. *Adv Genet*. 2014;87:125; 2. Wang D, Gao G. *Discov Med*. 2014;18:67–77.

From Wild Type AAV to Recombinant AAV Vector

- rAAVs are composed of the same capsid sequence and structure as found in WT AAVs
- However, rAAVs encapsidate genomes that are devoid of all AAV protein-coding sequences and have therapeutic gene expression cassettes designed in their place
- The only sequences of viral origin are the ITRs



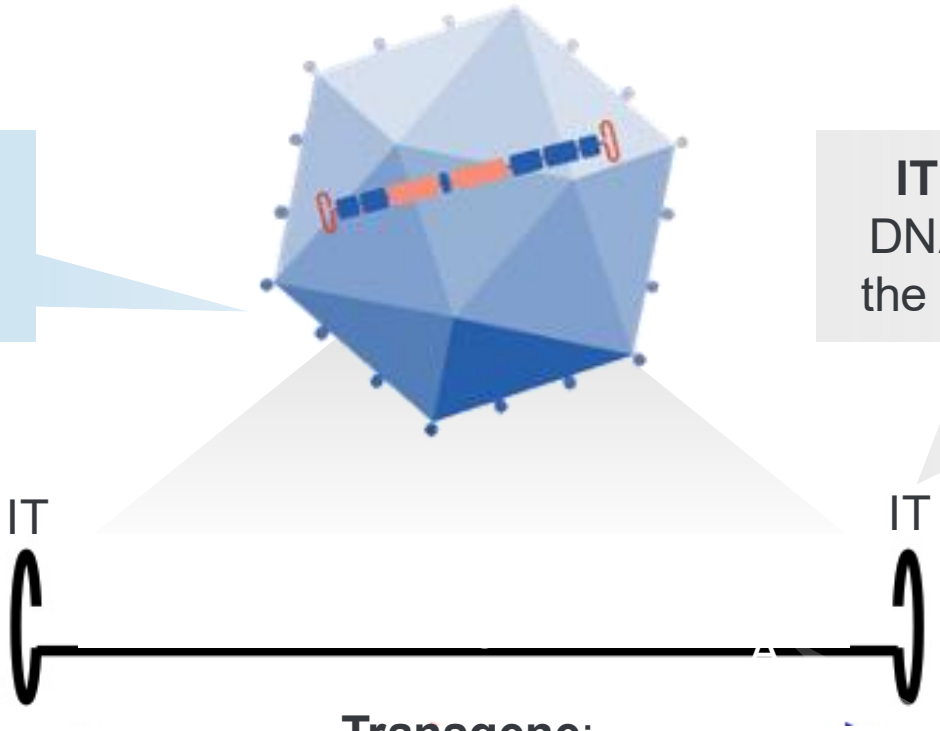
The complete removal of viral coding sequences maximizes the packaging capacity of rAAVs and contributes to their low immunogenicity and cytotoxicity

rAAV, recombinant adeno-associated virus; WT, wild-type

1. Wang D et al. Nature Reviews Drug Discovery 2019; 18:358; 2. Genetic Science Learning Center. (2018, August 7) Learn.Genetics. Retrieved May 15, 2019, from https://learn.genetics.utah.edu/

The Key Components of Recombinant AAV

Capsid: Vehicle by which the transgene is delivered to the target cell



ITRs: At the extremities of the vector DNA, allow encapsidation and promote the synthesis of a double-stranded DNA genome

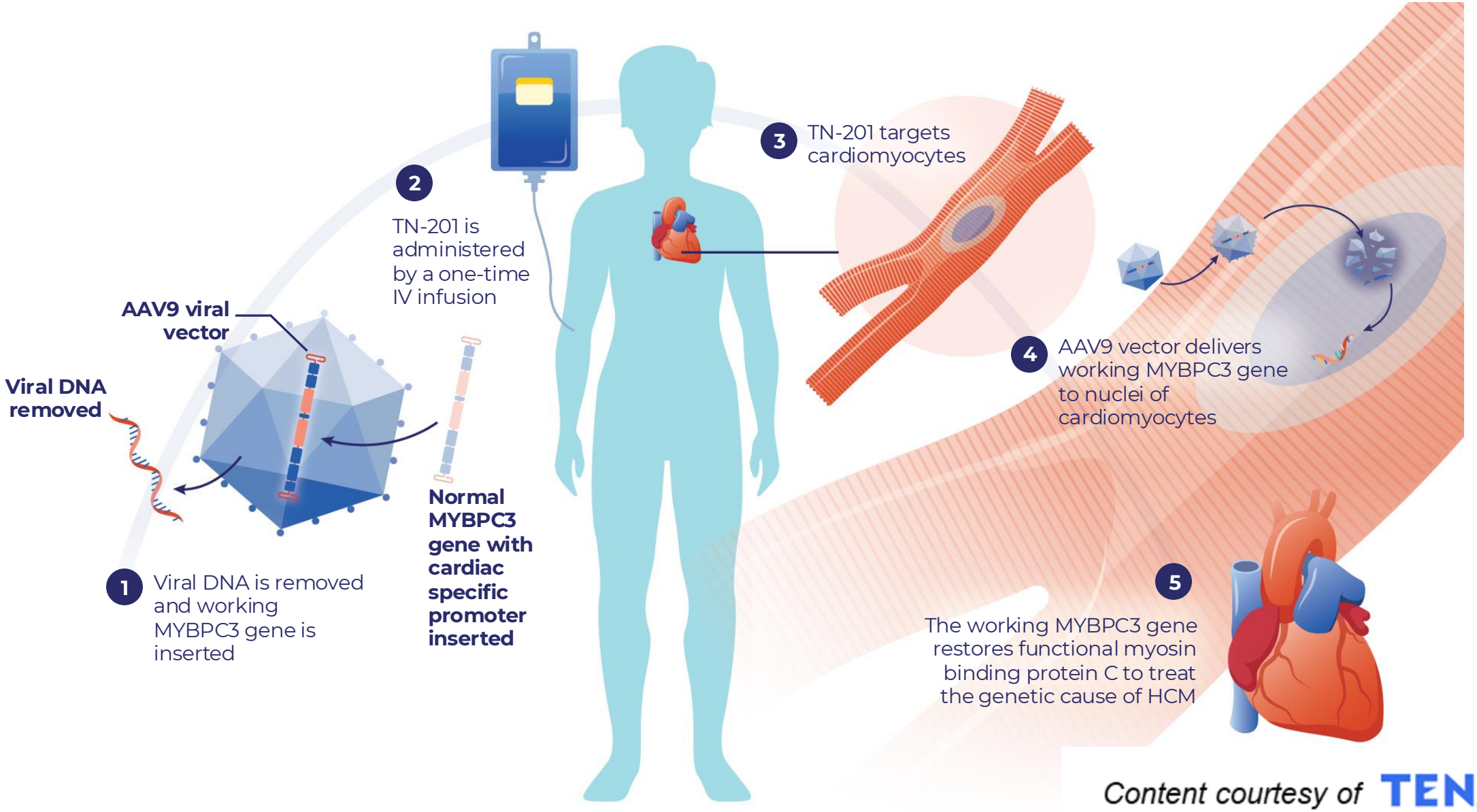
Promoter: Switch that initiates the expression of the transgene, can be tissue-specific

Transgene: cDNA coding for the desired gene

Polyadenylation signal: Allows synthesis of a mRNA transcript with a poly-A tail

cDNA, complementary DNA
Wang D, Gao G. Discov Med 2014;18:67–77

Figure 1: TN-201 Construct and Mechanism of Action



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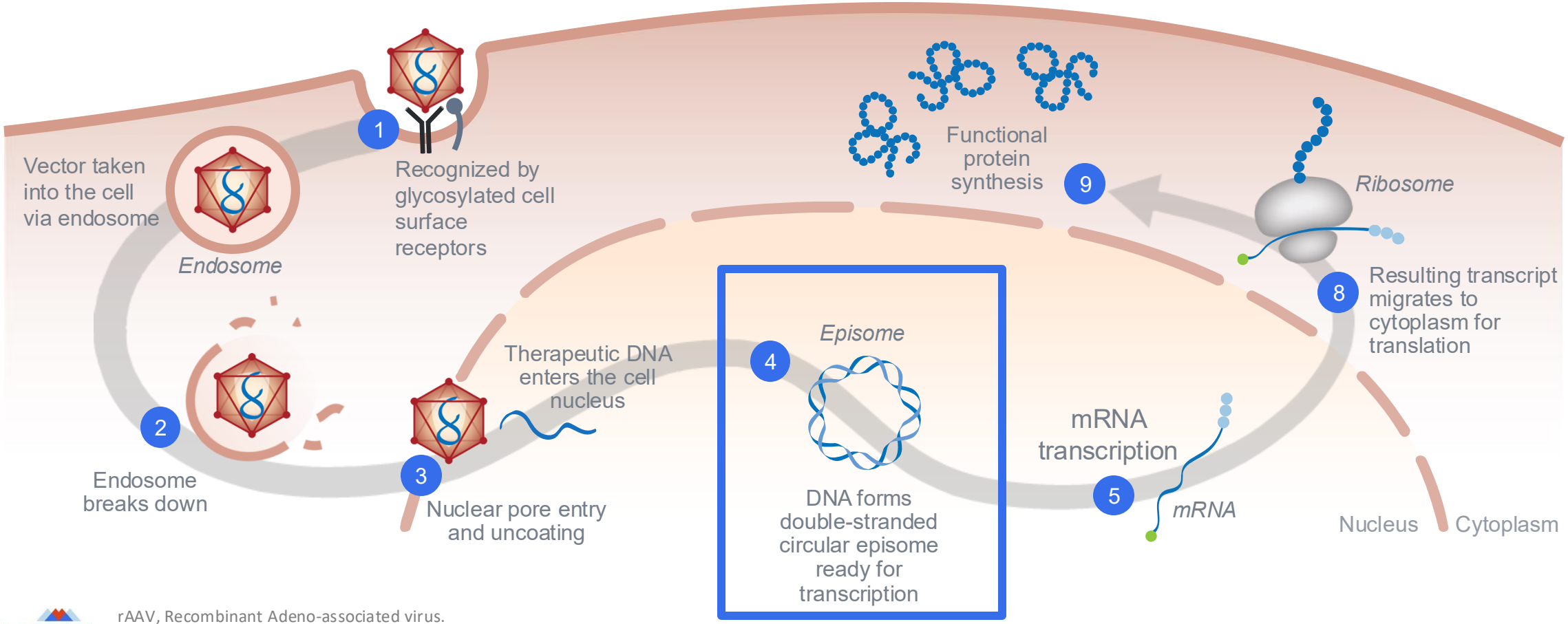
Gene Therapy Using AAV

Gene therapy aims to provide sufficient gene expression cardiomyocytes to ameliorate or correct the disease phenotype[1]



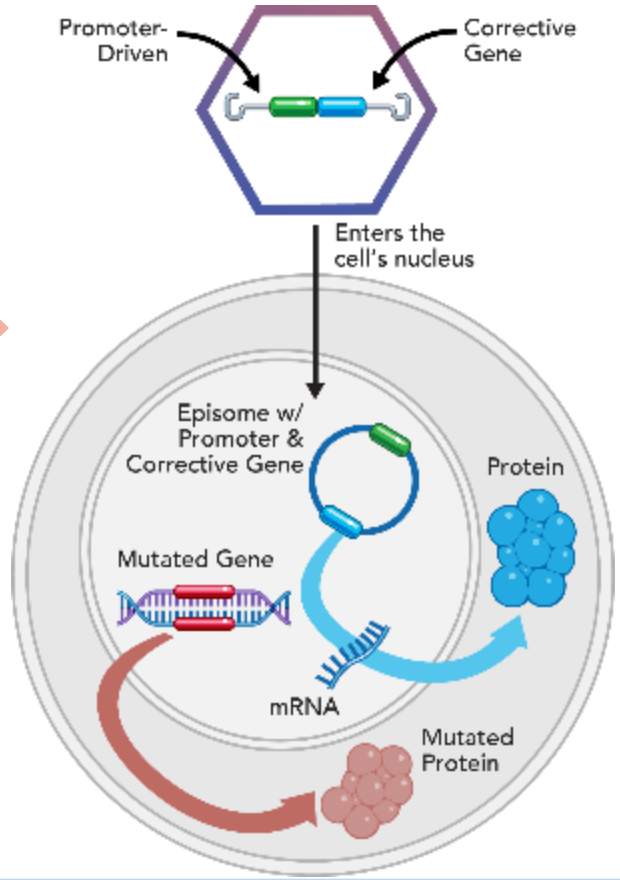
rAAV, Recombinant Adeno-associated virus.

1. Collins M, Thrasher A. Proc. R. Soc. B. 2015;282:20143003; 2. Naso MF, et al. BioDrugs. 2017;31:317–334; 3. Wang D et al. Nature Reviews Drug Discovery 2019; 18:358.

TN-201: Gene Therapy Construct for MYBPC3-Associated HCM

TN-201 delivered via a one-time intravenous infusion

Gene Therapy
(Gene Transfer Method)



- *MYBPC3* transgene forms episomal DNA
- *MYBPC3* transgene does not integrate into host genome
- *MYBPC3* transgene encodes cardiac myosin binding protein C (MyBP-C)
- Durable expression of MyBP-C anticipated due to nondividing cardiomyocytes

DNA → RNA → protein