GGSB Prelim Q2 – Hang Chen

Gene therapy: treating genetic disorders using genetic approaches

- Major class of diseases that are conducive to gene therapy:
  - Monogeneic: easy to target
  - Loss-of-function or haploinsufficiency: easy to rescue
  - Many are also hematological diseases: easy to access
  - Lower-level restoration: easier to molecularly fix cells than tissues

Dx	Cause	Gene	Gene changes	Location	Vector
X-linked Severe Combined Immunodeficiency (SCID)	Lymphocytes development deficiency	*IL2RG*	Mutations in *IL2RG* gene	Ex vivo	RV
Thalassemia	Less alpha or beta hemoglobin	*HBA1/2* and/or *HBB1/2*	Mutations in *HBA1/2* and/or *HBB1/2 genes*	Ex vivo	AAV
Sickle cell anemia	Hemoglobin polymerization	*HBB*	A mutation in both *HBB* alleles	Ex vivo	AAV
Congenital hemophilia	Blood does not clot properly	*F8* and/or *F9*	Mutations in *F8* or *F9* genes	In vivo	AAV
Vision Loss	Impaired retinoid cycle	*RPE65*	Mutations in *RPE65* gene	In vivo	AAV
Spinal muscular atrophy (SMA)	Insufficient survival motor neuron protein (SMN)	SMN1	Mutations in *SMN1* gene	In vivo	AAV

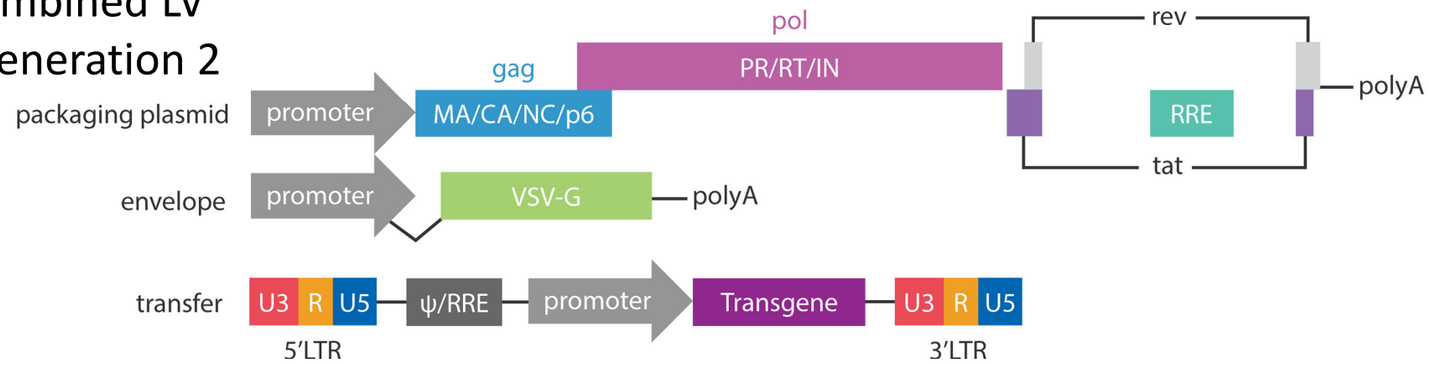
High et al., 2019

Lentivirus (LV)

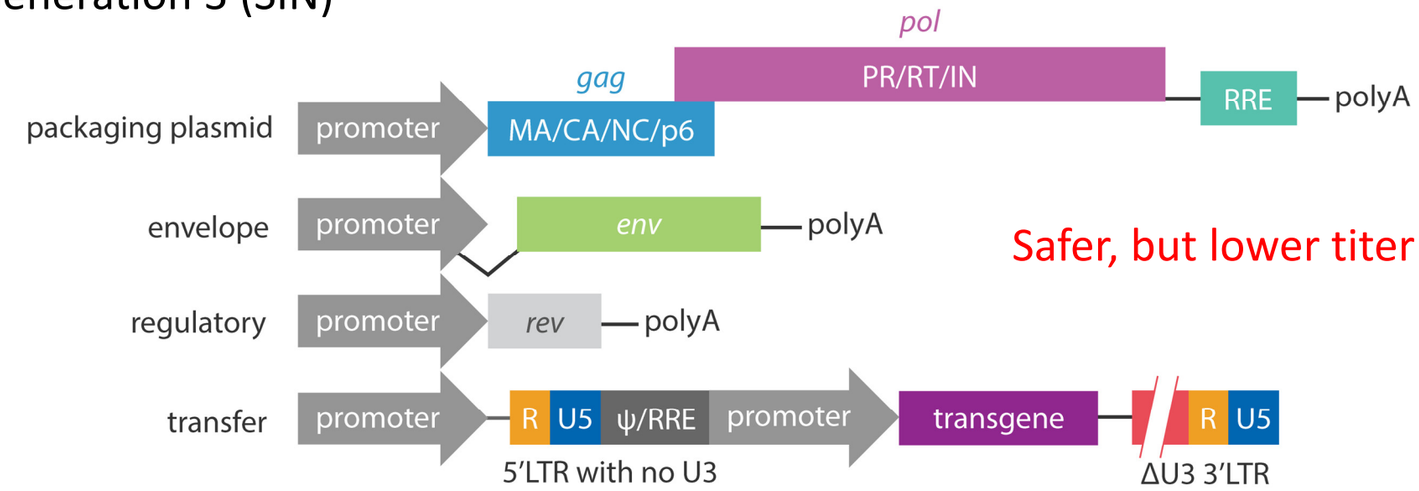
- Type
  - Retrovirus (enveloped)
- Genome
  - Single stranded positive sense RNA
  - ~10kb
  - 5’ and 3’ LTRs: required for transcription and reverse transcription
  - **gag: core**
  - **pol: reverse transcription**
  - **env: surface protein**
  - **rev/tat: regulatory**
  - some other replication related genes

Recombined LV

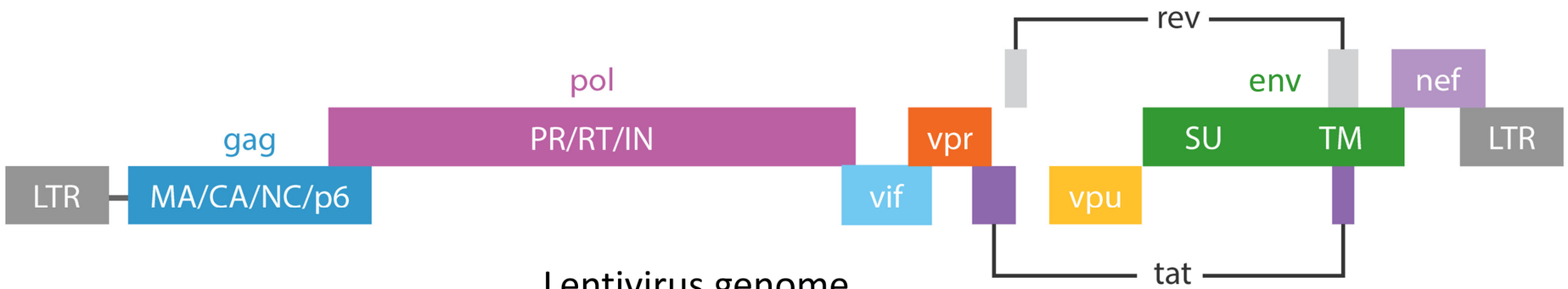
- Generation 2



- Generation 3 (SIN)



Safer, but lower titer



Lentivirus genome

Abm Inc.

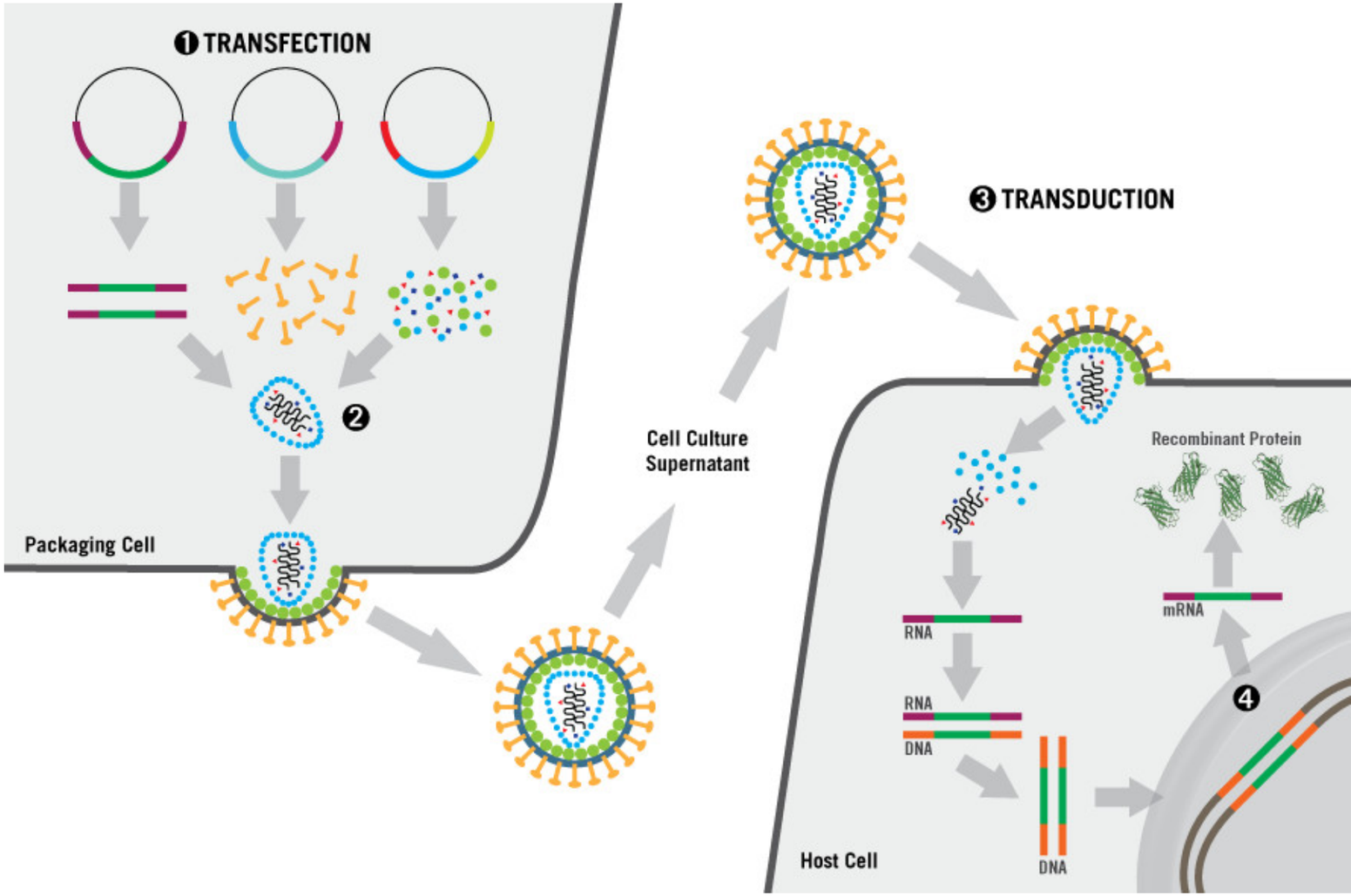
Recombined Lentivirus (LV)

- Infection
  - Tropism: VSV-G, for most cells
  - Entry: membrane fusion and endocytosis
  - Dividing cells (hard to cross nuclear membrane)
  - Genome integration

- Pros:
  - Large capacity for transgene
  - Easy to design (transfer plasmid)
  - SIN system for safety
  - Integrated into genome, permanent expression

- Cons:
  - Insertional mutagenesis risks
  - Copy number issues

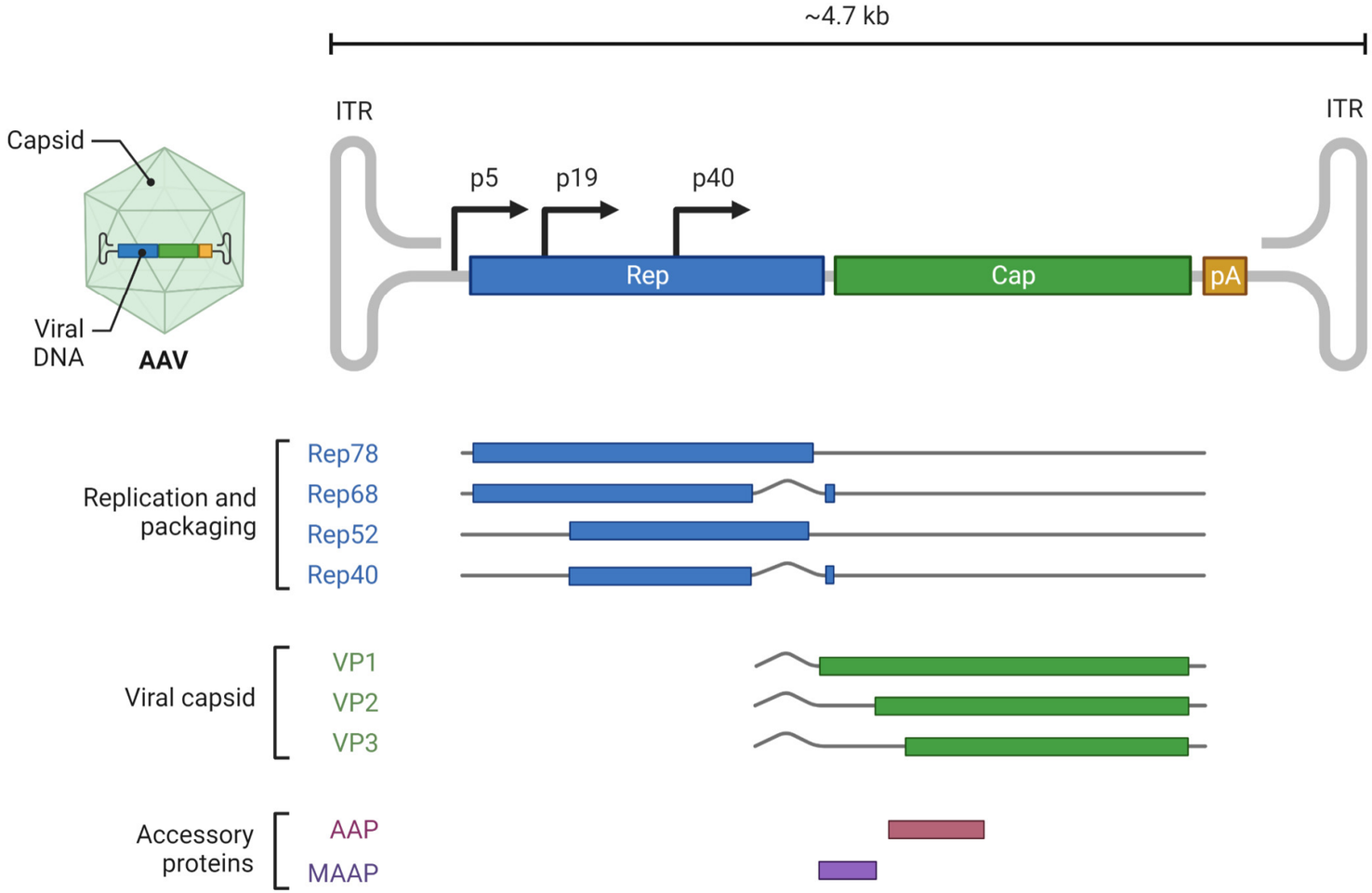
- Suited for:
  - Dividing cells
  - Permanent correction
  - E.g., hematopoiesis diseases (ex vivo treatment)



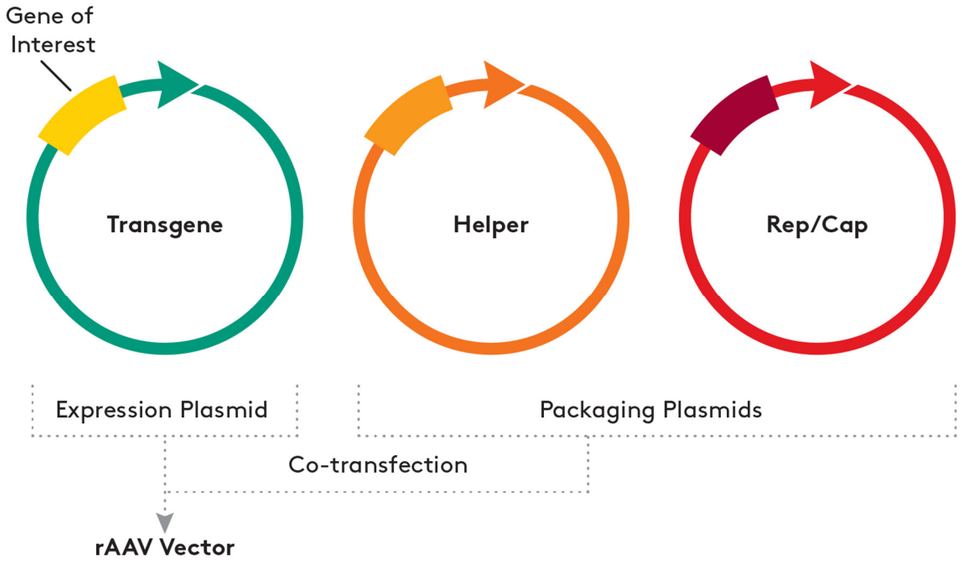
Mirus Bio LLC

Adeno-associated virus (AAV)

- Type
  - Dependovirus (non-enveloped, rely on other viruses to help replicate)
- Genome
  - ssDNA
  - ~4.7kb
  - 5’ and 3’ ITRs: required for the synthesis of the complementary DNA
  - **rep: replication genes**
  - **cap: capsid genes**



Dyno Therapeutics



Aldevron

Recombined AAV (rAAV)

- transfer plasmid: ITR and transgene
- helper plasmid: required for virus replication
- rep/cap plasmid: may require special design for targeted cells

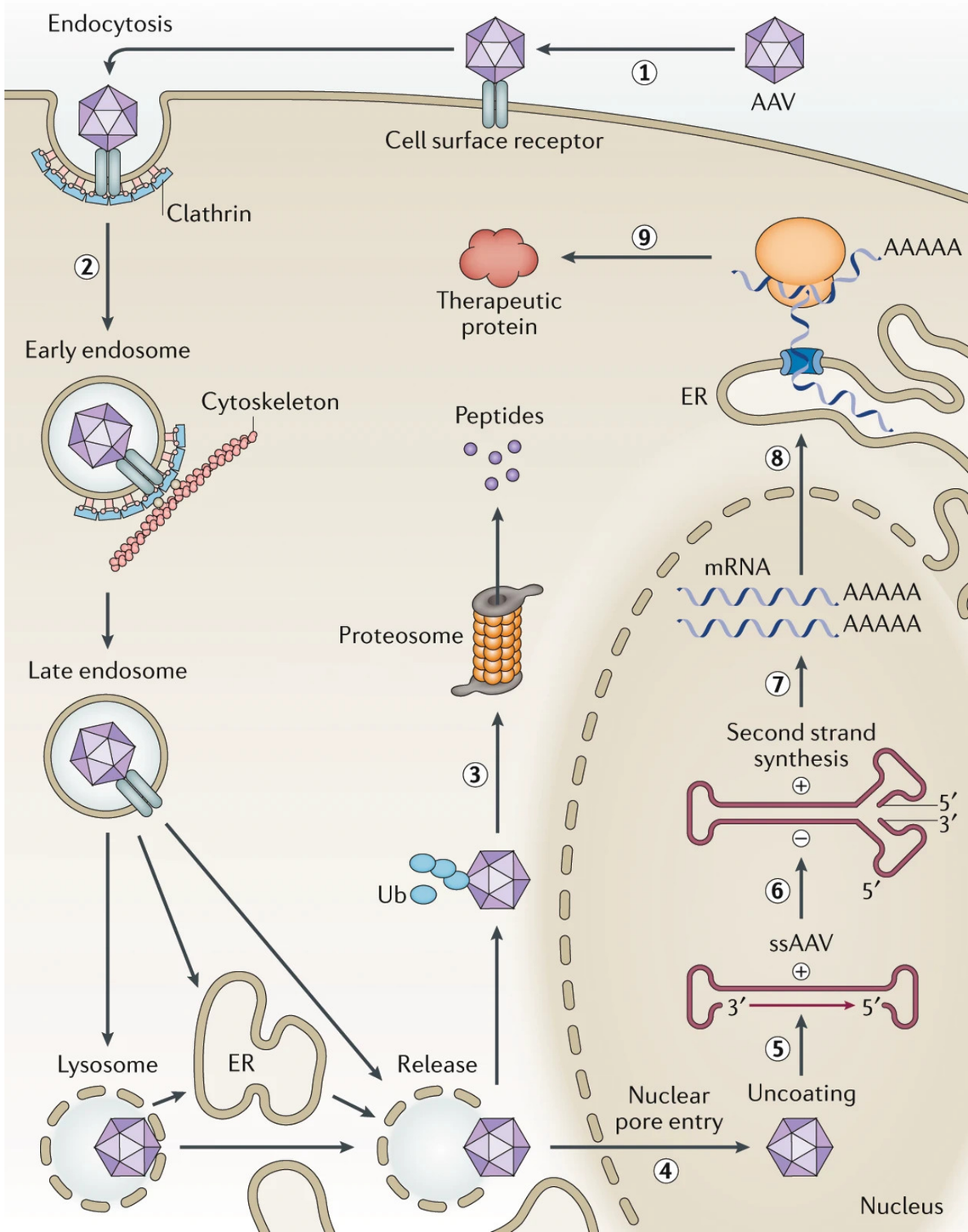
Recombined Adeno-associated virus (AAV)

- Infection
  - Tropism: more specific (may require optimization, e.g., directed evolution, *in silico* design)
  - Entry: endocytosis
  - Both dividing and non-dividing cells
  - Very low genome integration

- Pros:
  - Tissue specificity
  - Very low insertional mutagenesis risks (although it could happen if administrated in long term)
  - AAV itself does not cause disease

- Cons:
  - Small capacity for transgene
  - Complicated to design, hard to manufacture in large scale
  - Some people may have immunity against naturally existing AAV, which can decrease the efficacy

- Suited for:
  - Non-dividing cells
  - Transient expression
  - E.g., neurological disorders (in vivo treatment)



Challenges

- Ethics
  - Bottomline: no germline editing
- Safety
  - Genotoxicity (insertional mutagenesis, copy number issues)
- Efficacy
  - Delivery (tropism, immunity, cell migration)
- Cost
  - Up to $1.2 billion per patient
- Some other directions:
  - ASO (RNAi issue)
  - CAR-T CAR-NK (exhaustion issue)
  - Maybe SARS-CoV-2 as a vector in the future?
    (~30Kb capacity, strong infection ability)

Special thanks to Xiaochang Zhang group.