Module 2: Proteins and mRNAs as Medicines
Summary

Medicines: Small molecules and proteins

- Small molecule medicines
  - Most target proteins to change their function
  - Advantages
    - Can be formulated into solid, stable forms
    - Can be taken by mouth
    - Can go everywhere in the body
  - Disadvantages
    - Lengthy screening process
    - High rate of failure
    - Expensive development process
- Protein medicines
  - Also known as therapeutic proteins or biologics
  - Advantages
    - Very effective, especially monoclonal antibodies
  - Disadvantages
    - Cell culture and purification processes are expensive
    - Each protein requires a unique formulation
    - Difficulty entering cells

What makes mRNA suitable as a medicine

- Classic and ideal medicines have 3 attributes
  - Their effect is dose-dependent
  - Their effect is time-limited
  - They can be dosed repeatedly
- Both proteins and mRNAs also have these attributes
  - Proteins and mRNAs are both inherently transient
    - Proteins have a median half-life of 30 hrs
    - mRNAs have a short ~5 hour median half-life
- Other mRNA medicine advantages include:
  - mRNA can direct the body to make any type of protein, including intracellular and transmembrane proteins
  - mRNAs have smaller manufacturing infrastructure requirements than proteins
  - mRNA medicines are a platform technology
    - Allows for different mRNA sequences to be swapped in to create new medicines of the same type
  - mRNA medicines are easily multiplexed
    - More than 1 mRNA can be delivered in a single medicine

Making and delivering mRNA medicines

- mRNA is made outside of the body from a DNA template
  - Bacteria are used to make copies of DNA
  - Then, the DNA template is cut, linearized, and transcribed via T7 RNA polymerase
- Difficulties with mRNA delivery include:

  - Naked mRNA is rapidly destroyed
  - mRNA alone has no tissue or cell selectivity
  - mRNA cannot easily cross cell membranes
- For these reasons, mRNA medicines require a delivery vehicle

Anatomy of a lipid nanoparticle

- LNPs are the most commonly used delivery vehicle for mRNA medicines
- LNP anatomy
  - LNPs are made up of lipids, a form of fat, which don't mix well with water
    - As a result, they form a sphere and protect the mRNA inside
  - "Ionizable lipids" are used to get mRNA inside the LNPs
    - These lipids coat mRNA under acidic conditions, but not under physiological conditions
  - A phospholipid bilayer is then added around the ionizable lipids and mRNA
    - This is another layer of protection for the mRNA
- PEG is used to coat LNPs and prevent them from fusing together
- Once an LNP has reached its target cell, it is taken up via endocytosis
  - The LNP fuses with the cell membrane and releases the mRNA into the cell

mRNA medicines and the innate immune system

- Our adaptive immune system protects us against specific infectious agents
  - Antibodies and T cells recognize and eliminate specific pathogens and infected cells
  - However, it can take days to months to mount an adaptive immune response to an infection
- Our innate immune system is our first line of defense
  - It recognizes common pathogen features, including LPS
- Viruses that infect humans can look similar to LNPs used in mRNA medicines
- Innate immune sensors trigger an immune response when they find:
  - The U nucleotide inside endosomes
  - Long stretches of double-stranded RNA
- mRNA medicines are engineered to evade these sensors
  - They use modified versions of U (1mU)
  - Specific transcription reaction conditions and strict purification protocols reduce double-stranded RNA byproducts