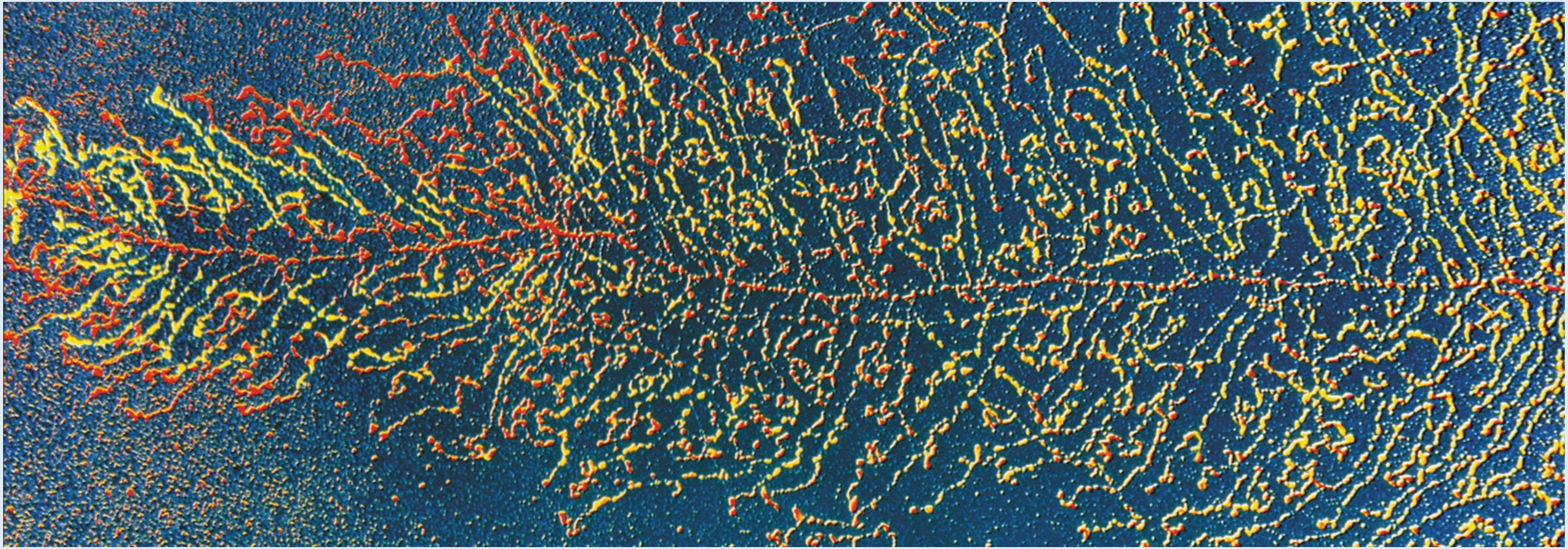
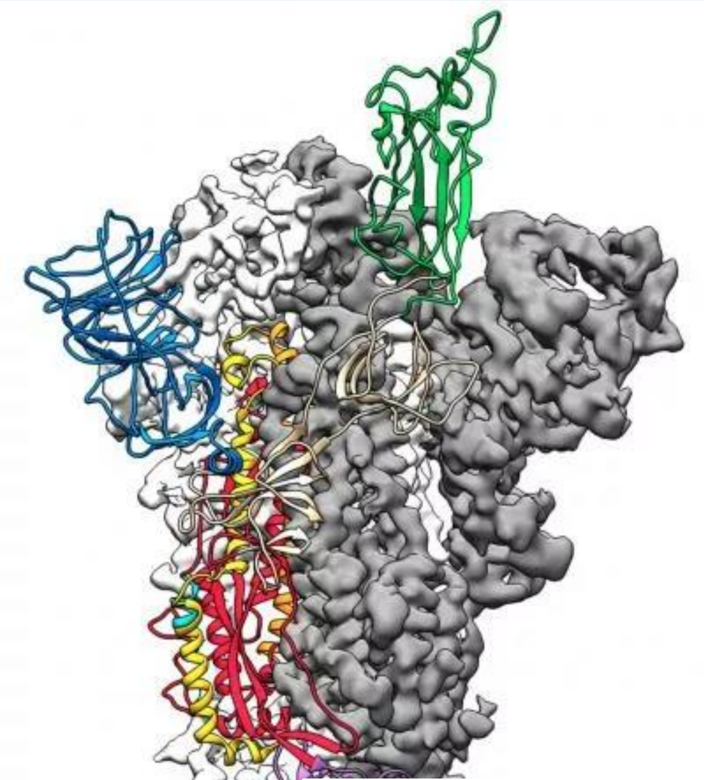
TRANSCRIPTION, RNA PROCESSING, AND TRANSLATION

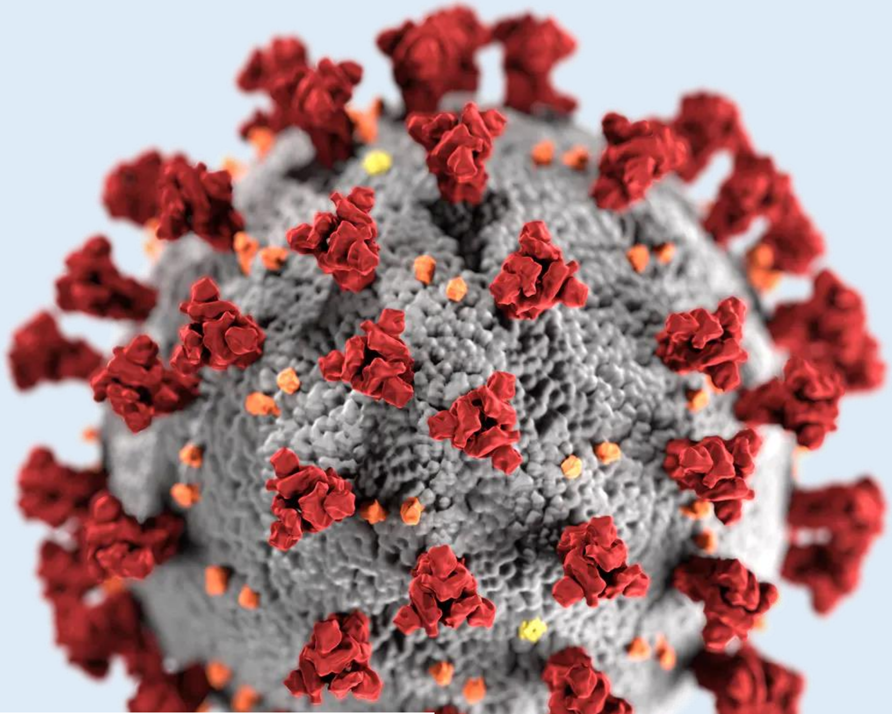
BIOL 1115, Ch. 17

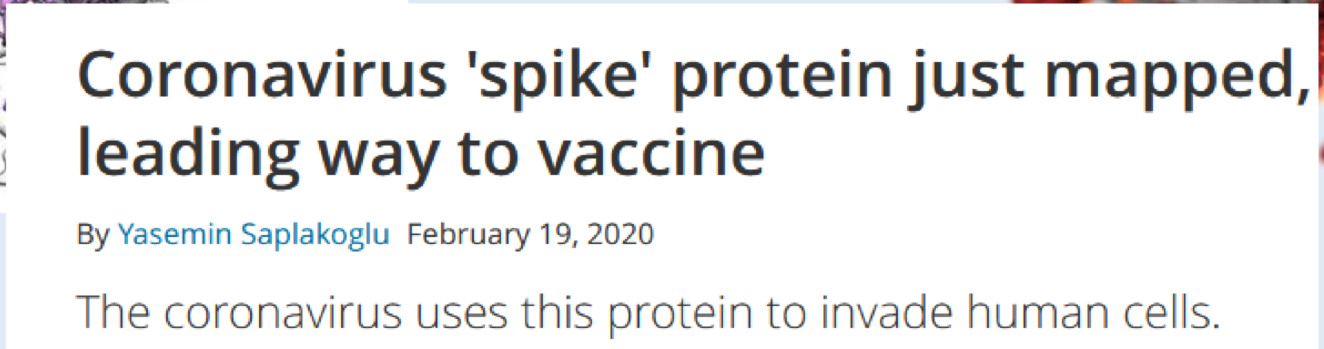
Guest: David Moulton (dmoulton@alumni.ubc.ca)



Importance of transcription, mRNA, and translation







Coronavirus 'spike' protein just mapped, leading way to vaccine

By Yasemin Saplakoglu February 19, 2020

The coronavirus uses this protein to invade human cells.

About Me

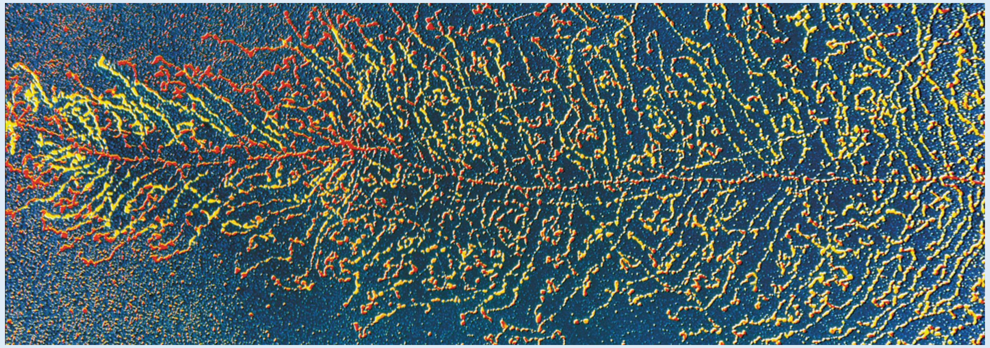
David Moulton (dmoulton@alumni.ubc.ca)

- PhD Candidate: Pacific Salmon Ecology and Conservation Lab (UBC)
- Researching stressors affecting sockeye salmon spawning migrations (fishing nets, rising water temperatures, infections)
- Prior experience: coastal sportfish, coral reef fish, and pelagic (open ocean) fish including sharks, tuna, and billfish
- Instructor training: Advanced Teaching and Learning Certificate Program at UBC

Voluntary Surveys (pre- and post-lesson)

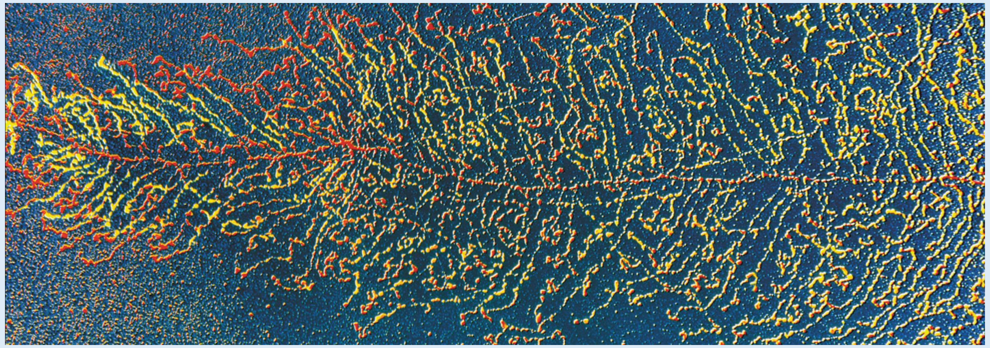
- Scholarship of Teaching and Learning (SoTL) pilot project
- Learning experience for my teaching program – practice conducting educational research
- Participation appreciated – thank you!!
- Please mark each with 4 digit code identifier (e.g. last 4 digits of phone number)

Lesson Outline



1. Transcription
2. RNA Processing in Eukaryotes
3. Translation

Learning Outcomes



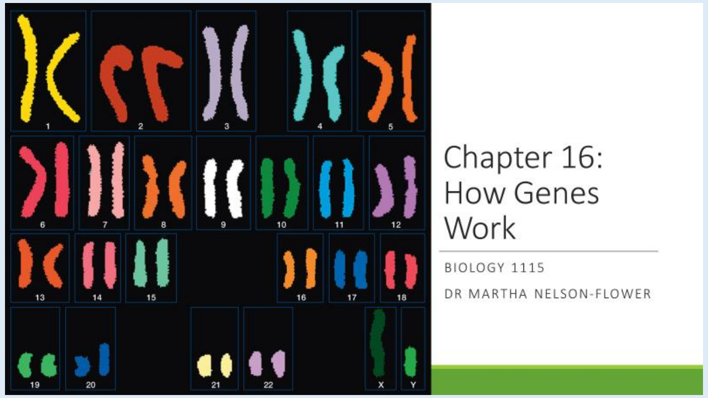
1. Identify the main phases and macromolecules of **transcription** and describe the steps involved. Create a pre-mRNA from a DNA code (17.1).

2. Identify the steps involved in **processing** eukaryotic pre-mRNA, and explain the implications of alternative splicing for the proteome (17.2).

3. Identify the phases and macromolecules of **translation** and describe the steps involved. Create a polypeptide from an mRNA transcript (17.3-17.5).

Review: setting the stage

- Central dogma: DNA -> RNA -> protein
- Genotype -> phenotype
- DNA: code of life – genotype
- Proteins: do most of the functional work! – phenotype
- RNA is the intermediate messenger
- Transcription: RNA transcribed (copied) from DNA code
- Translation: protein translated (decoded) from RNA code



Chapter 16: How Genes Work

BIOLOGY 1115

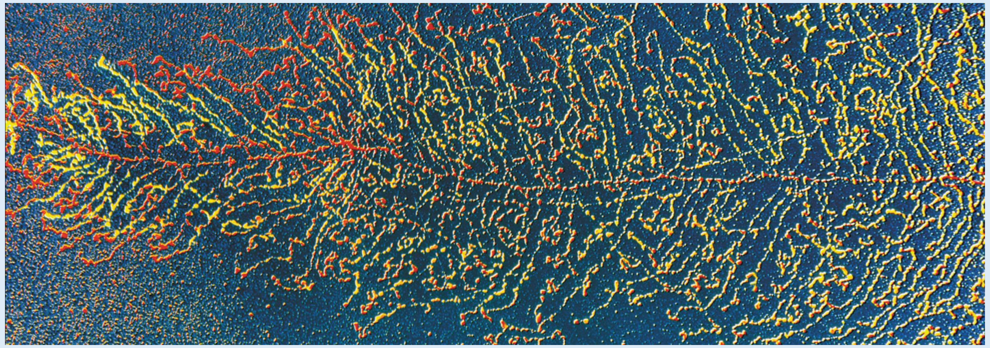
DR MARTHA NELSON-FLOWER

Genotype to Phenotype – Firefly Vid

- https://learn.genetics.utah.edu/content/basics/firefly/

- Genotype: gene codes for luciferase protein
- Transcription, translation, and processing occur
- Phenotype: light emitted from tail (avoid predation, attract mate)
- These traits play a role in natural selection (future module)

Lesson Outline

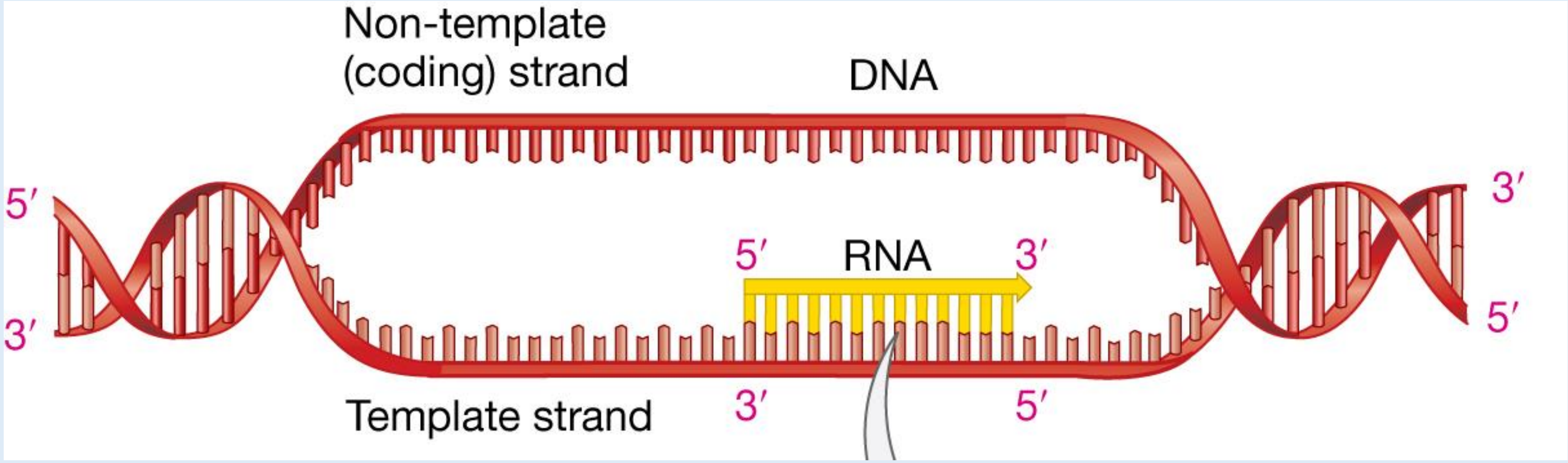


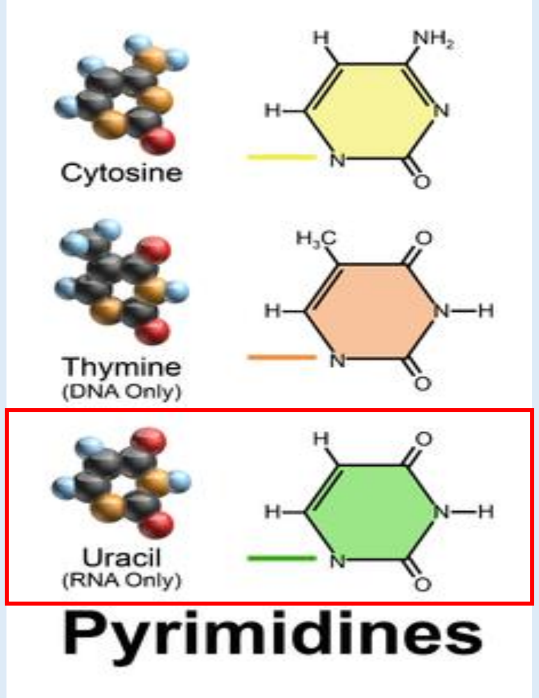
1. Transcription (nucleus)

LO1: Identify the main phases and macromolecules of transcription and describe the steps involved. Create a pre-mRNA from a DNA code (17.1).

Review of transcription basics

- RNA polymerases synthesize an RNA transcript of the instructions stored in DNA
- Only one strand of DNA is copied (template strand)
- Ribonucleotides (NTPs) are used to produce an RNA transcript that is complimentary and antiparallel to the template strand
  - The NTPs used for RNA synthesis are **ATP, GTP, CTP, UTP**





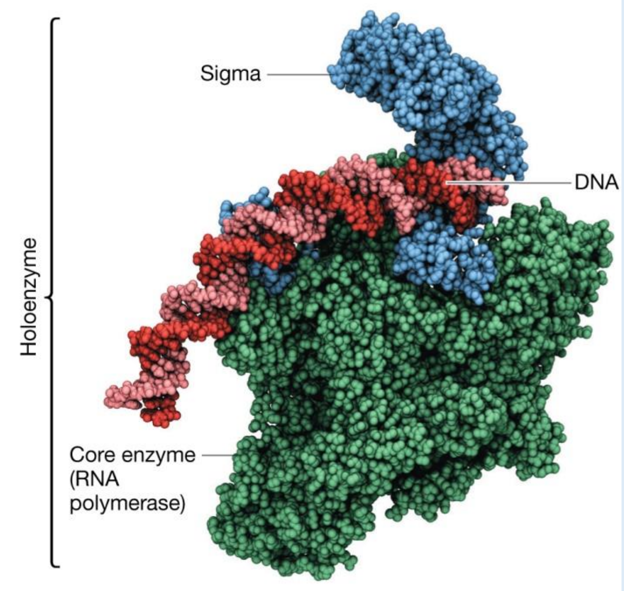
3 major phases of transcription:

1. Initiation
2. Elongation
3. Termination

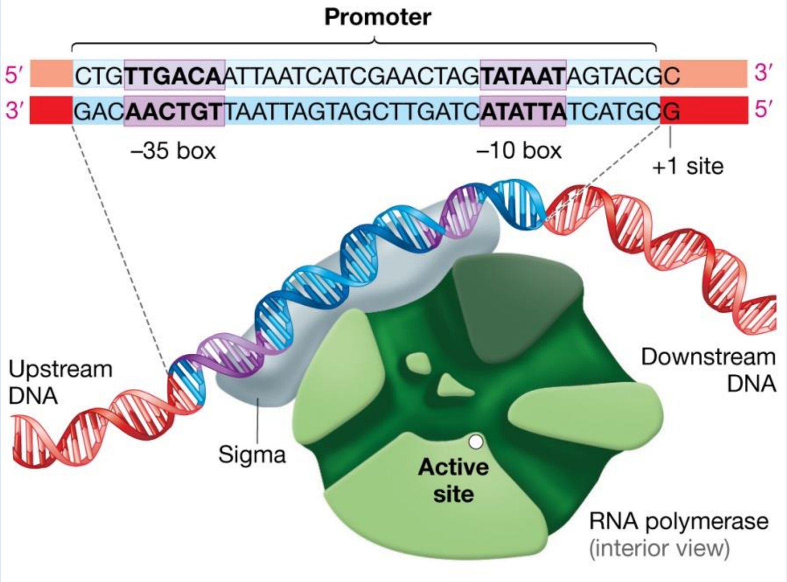
Initiation of Transcription

- RNA polymerase and basal transcription factor proteins (eukaryotes) or sigma protein (bacteria) bind to DNA at promotor sequence
- Eukaryotes have 3 kinds of RNA polymerases (pol I, pol II, pol III)
  - mRNA is transcribed by pol II
- Promotor sequence indicates where a gene starts, and which DNA strand is the template strand
- Holoenzyme: complex of RNA polymerase and other required proteins that binds to promotor

(a) RNA polymerase and sigma form a holoenzyme.

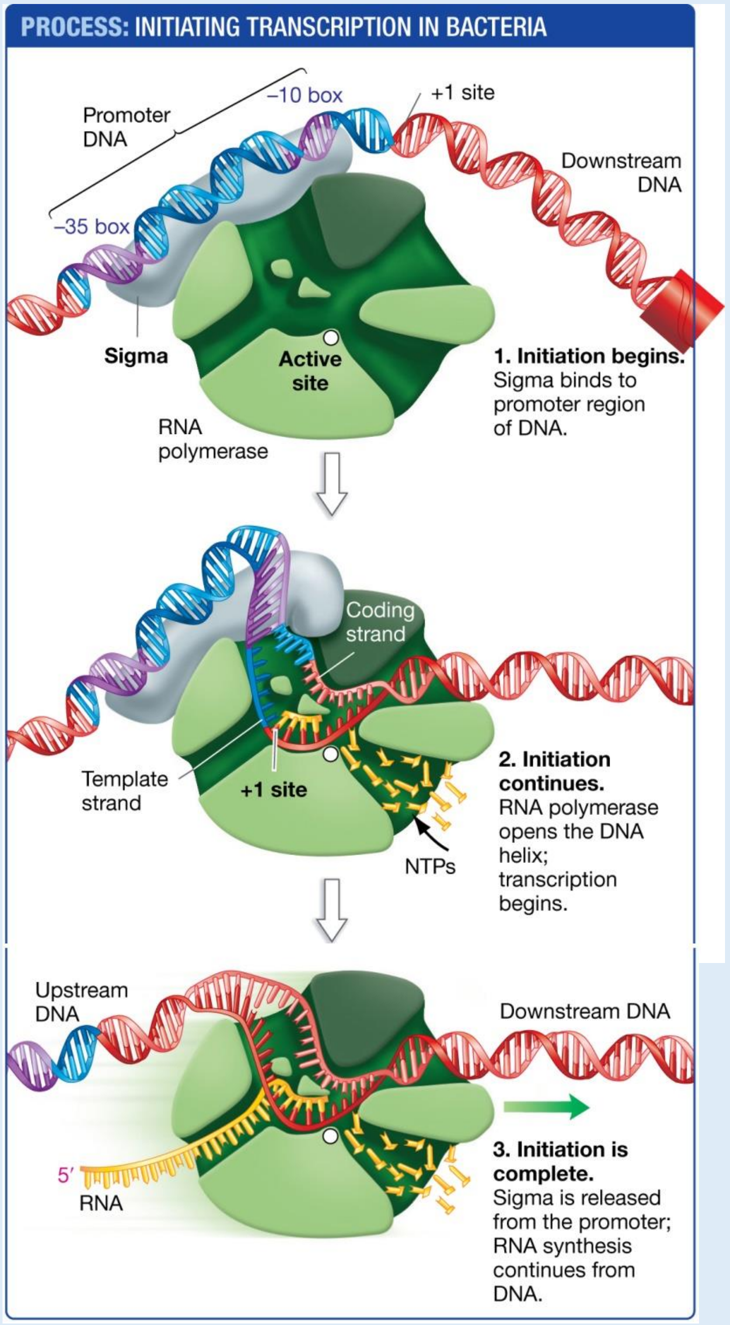


(b) Sigma recognizes and binds to the promoter.



Initiation of Transcription

- Holoenzyme binds to the promotor sequence
- RNA polymerase opens the DNA double helix, creating a transcription bubble
- The template strand is threaded through the RNA polymerase active site
- Ribonucleotides (NTPs) enter the enzyme through a channel and diffuse to the active site



PROCESS: INITIATING TRANSCRIPTION IN BACTERIA

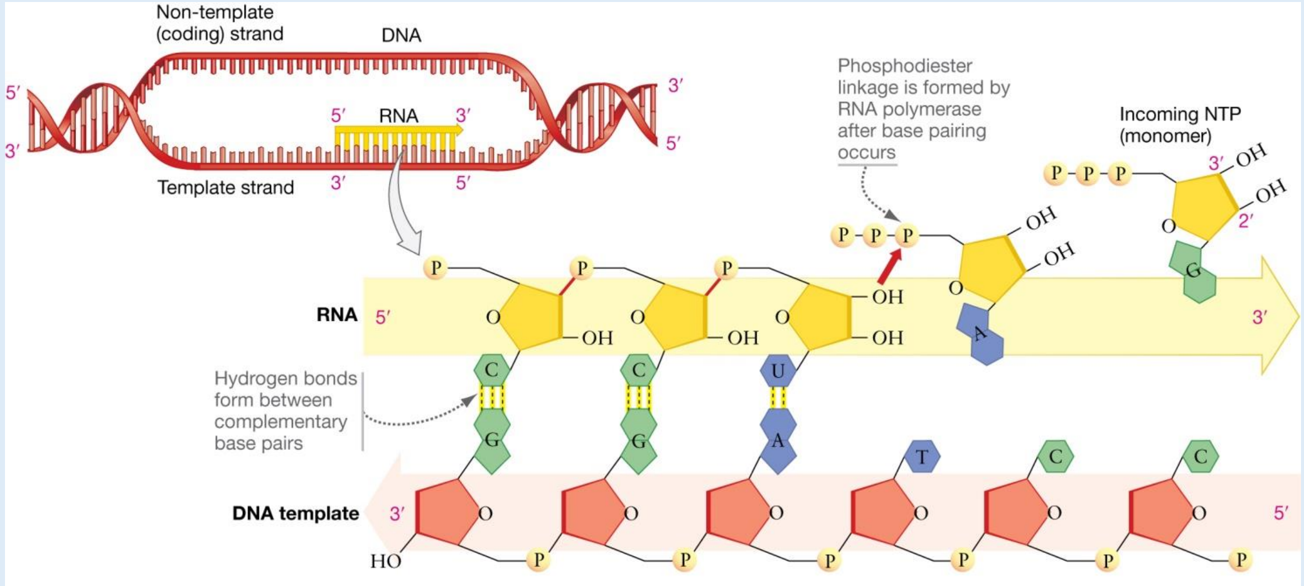
1. Initiation begins. Sigma binds to promoter region of DNA.

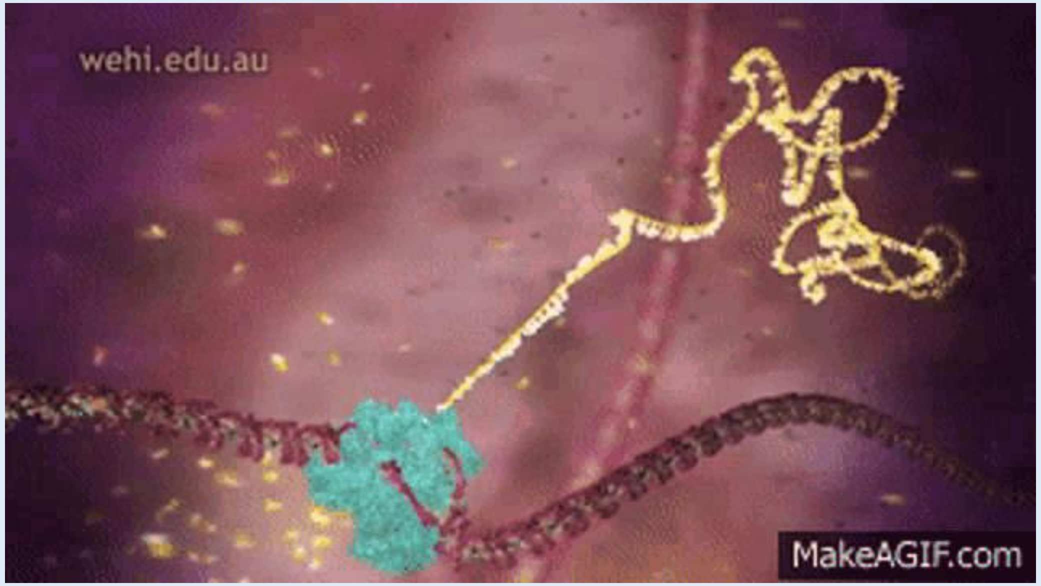
2. Initiation continues. RNA polymerase opens the DNA helix; transcription begins.

3. Initiation is complete. Sigma is released from the promoter; RNA synthesis continues from DNA.

Elongation of the Transcript

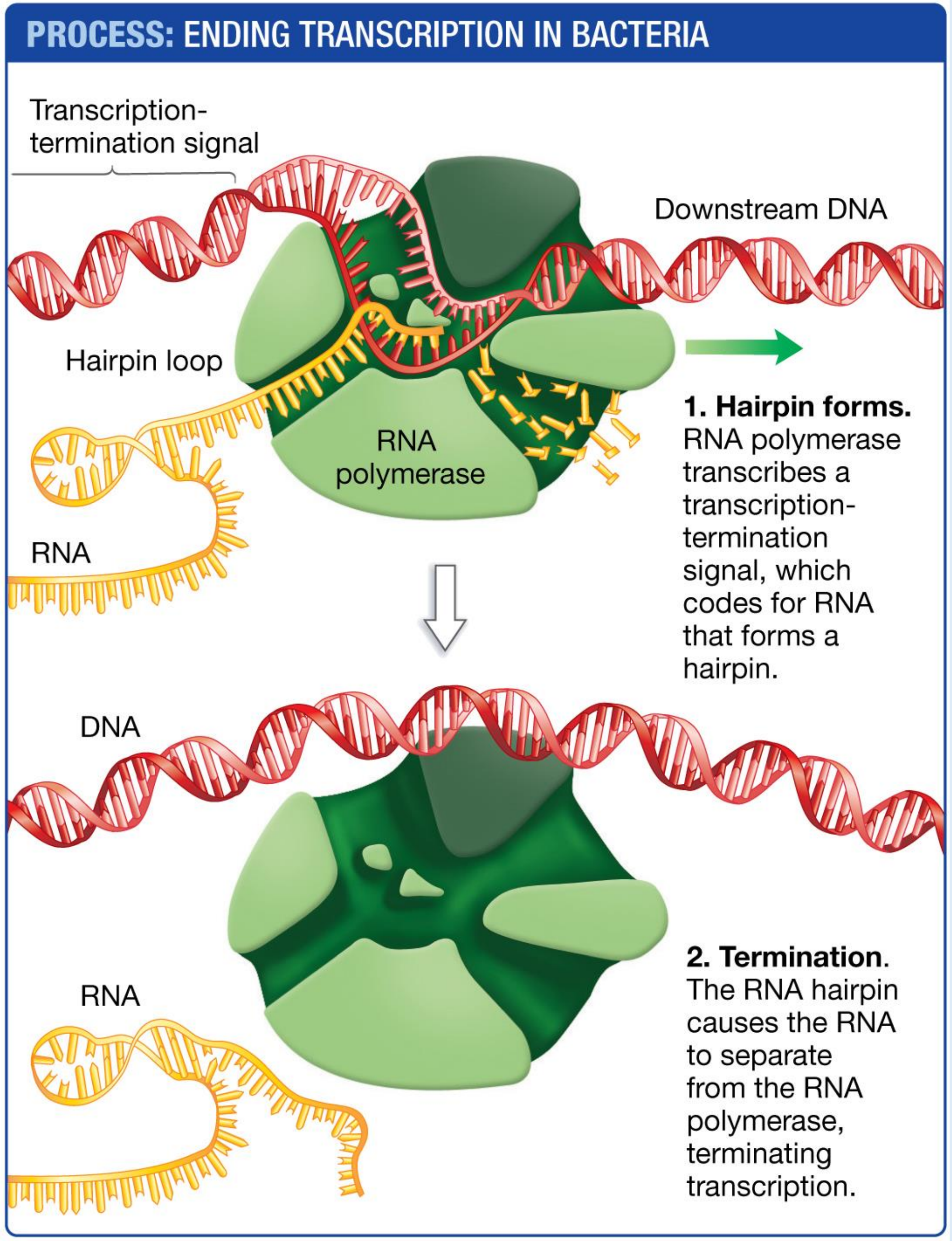
- NTPs are recognized by RNA polymerase and added to the growing transcript according to base pairing: C-G and A-U
- Reactions are spontaneous due to potential energy of ribonucleotides (NTPs – 3 phosphate groups)
- Rate: ~ 50 nucleotides per second





Termination of Transcription

- Termination: RNA polymerase transcribes the transcription-termination signal
- Bacteria: code for hairpin loop causes transcript to separate
- Eukaryotes: poly(A) signal is transcribed, causing enzyme to cut the RNA transcript ~10-35 nucleotides downstream
- Product: primary transcript in need of processing to become mature mRNA



1. Hairpin forms. RNA polymerase transcribes a transcription-termination signal, which codes for RNA that forms a hairpin.

2. Termination. The RNA hairpin causes the RNA to separate from the RNA polymerase, terminating transcription.

Differences During Transcription

	Bacteria	Eukaryotes
RNA polymerase types	One	Three (pol I, pol II, pol III)
Holoenzyme proteins	Sigma	Basal transcription factors
Promotor sequences	-35 and -10 box	TATA box (-30)
Termination	Hairpin loop	Cut after poly A

* Additional note: T&T is spatially separated for eukaryotes (nucleus and then ribosomes in cytosol), while bacteria often begin translation before transcription is even complete (nucleus)

Check-in Question

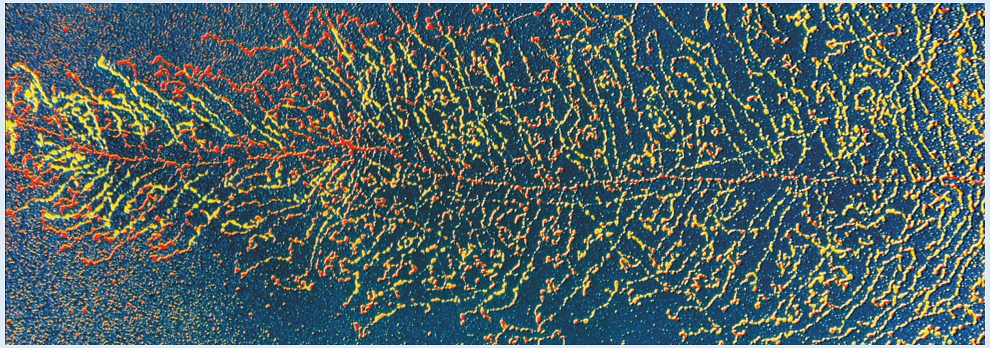
- What is the mRNA sequence complementary to this DNA sequence?
- Label the direction they are read (DNA) and created (mRNA) with an arrow

DNA

3’ TCTAGATCCAA 5’

RNA

Lesson Outline



2. Eukaryotic RNA Processing (nucleus)

LO2: Identify the steps involved in processing eukaryotic pre-mRNA, and explain the implications of alternative splicing for the proteome (17.2).

Processing of the Primary Transcript

Bacteria

- Primary transcript *is* mature mRNA

Eukaryotes

- Chemical modifications needed to turn primary transcript (pre-mRNA) into mature mRNA ready for translation:

   1. RNA splicing
   2. Addition of a 5’ cap
   3. Polyadenylation (Poly A tail)

RNA Splicing

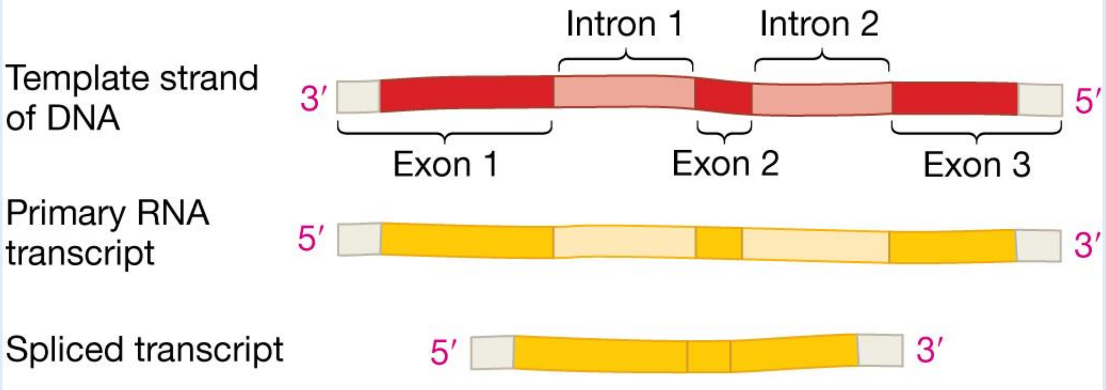
- RNA splicing: removal of introns; the sequences that remain are called exons

“<u>Ex</u>ons” because they are “<u>Ex</u>pressed”

“<u>In</u>trons” because they are “<u>In</u>tervening”

- ~90% of human genes contain at least one intron

(a) Introns must be removed from eukaryotic RNA transcripts.



- **Alternative splicing** of the same primary transcript leads to different mRNAs that code for different polypeptides
  - This allows one gene to code for multiple (related) proteins
  - Results in the human proteome being larger than the human genome!

RNA Splicing

- Performed by spliceosomes, complexes made of molecules called small nuclear ribonucleoproteins (snRNPs – “snurps”)
- snRNPs consist of single nuclear RNA (snRNA) and proteins
- Introns cut and recycled, exons linked by phosphodiester bonds

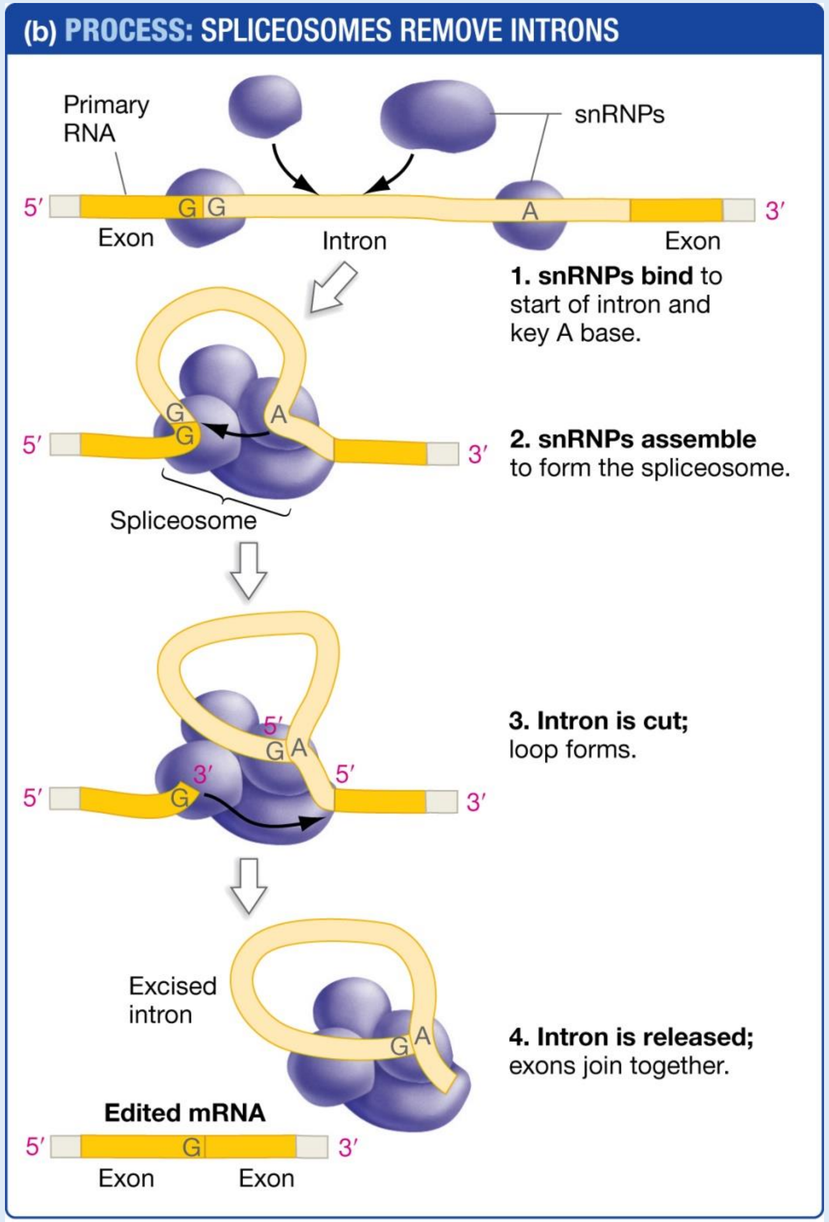


Figure 17.6 **Introns Are Spliced Out of the Primary Transcript.**

Addition of Caps and Tails

1. 5’ cap – modified guanine + 3 phosphates
    - enables ribosomes to bind, and protects the 5’ end from enzymatic degradation
2. 3’ poly(A) tail – 100-250 adenine nucleotides
    - Also required for translation, and protects the 3’ end from enzymatic degradation

- The region that codes for a polypeptide is flanked by untranslated regions (UTRs), which help stabilise the mature mRNA and regulate translation

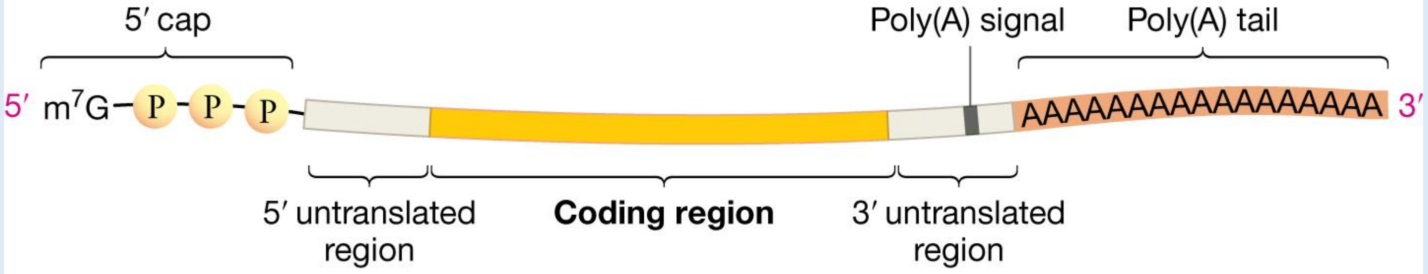
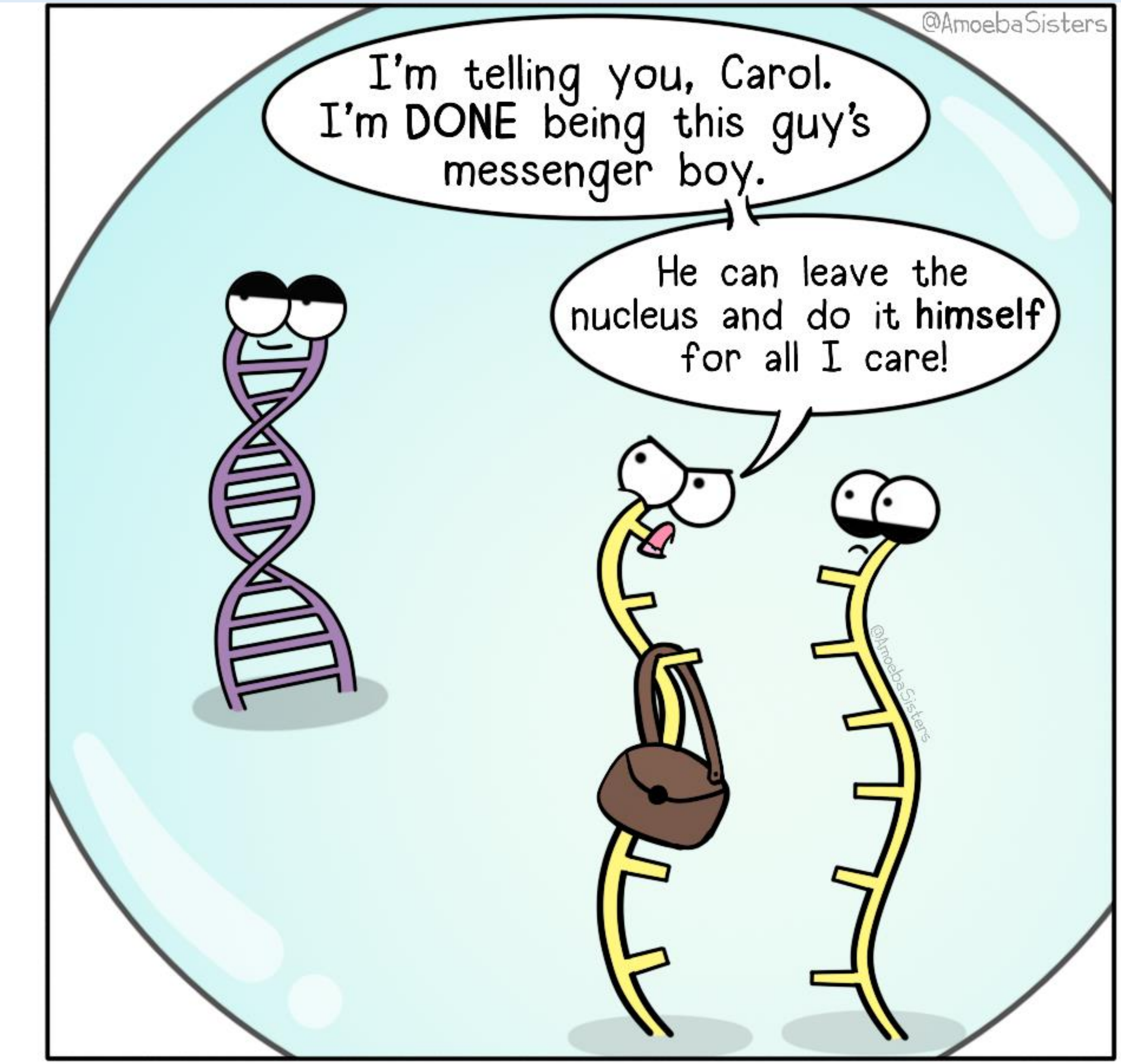


Figure 17.7 In Eukaryotes, a Cap and a Tail Are Added to mRNAs. As part of eukaryotic pre-mRNA processing, a cap consisting of a modified guanine (G) nucleotide (symbolized as m^7G) is added through an unusual linkage to the 5’ end, and a tail made up of a long series of adenine (A) residues is added to the 3’ end after cleavage of the primary transcript downstream of the poly(A) signal.

mRNA is ready!

- After splicing and the addition of a cap and tail, the end product is a mature mRNA!



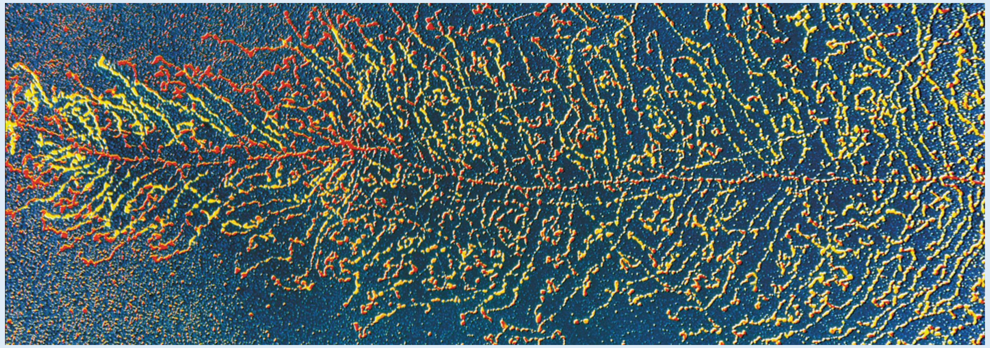
Arthur, the mRNA, was at the end of his strand at work.

Check-in question:

- Given the same number of genes, which is likely to have a bigger proteome?

A) Bacteria
B) Eukaryote
C) Neither, one gene -> one polypeptide

Lesson Outline



3. Translation (Cytoplasm)

LO3: Identify the phases and macromolecules of translation and describe the steps involved. Create a polypeptide from an mRNA transcript (17.3-17.5).

RNA Review

Recall that the three major types of RNA are:

1. mRNA (messenger RNA)
2. tRNA (transfer RNA)
3. rRNA (ribosomal RNA)

All three are involved in translation!

Overview of Translation

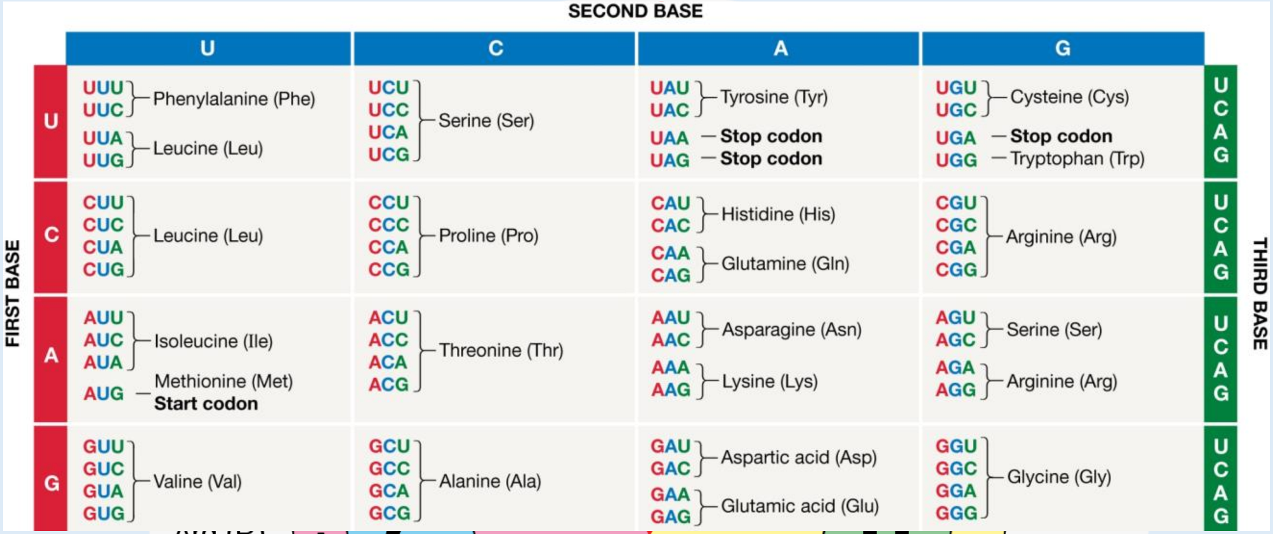
Instructions in one language (nucleic acid) **translated** to another language (amino acid) to create proteins

Major macromolecules involved:

- mRNA
- Amino acids
- tRNA
- Ribosome: proteins and rRNA

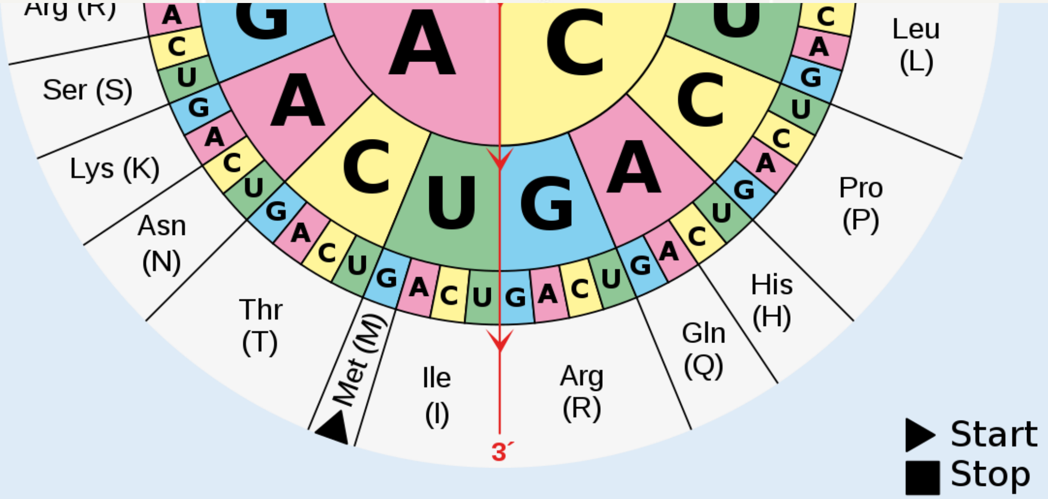
Codons code for amino acids

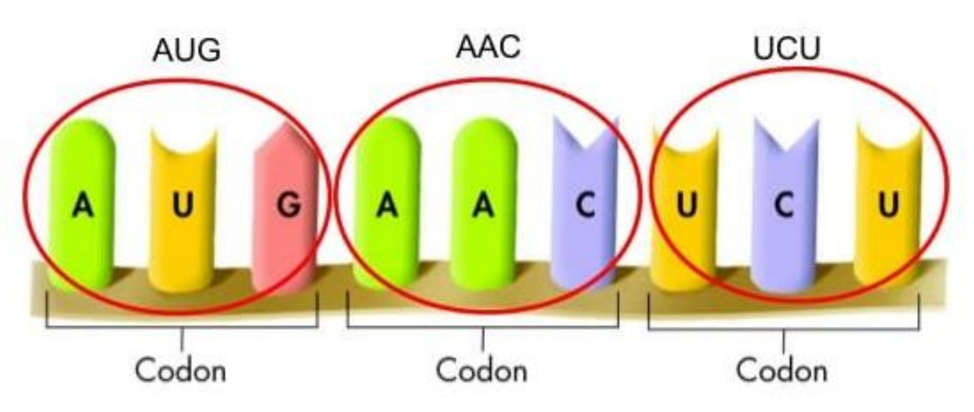
- mRNA are grouped into nucleotide triplets called codons
- Each codon specifies the amino acid (of 20 possible) to be added



SECOND BASE

FIRST BASE	U	C	A	G	THIRD BASE
U	UUU Phenylalanine (Phe)	UCU Serine (Ser)	UAU Tyrosine (Tyr)	UGU Cysteine (Cys)	U
	UUC	UCC	UAC	UGC	C
	UUA Leucine (Leu)	UCA	UAA — **Stop codon**	UGA — **Stop codon**	A
	UUG	UCG	UAG — **Stop codon**	UGG — Tryptophan (Trp)	G
C	CUU Leucine (Leu)	CCU Proline (Pro)	CAU Histidine (His)	CGU Arginine (Arg)	U
	CUC	CCC	CAC	CGC	C
	CUA	CCA	CAA Glutamine (Gln)	CGA	A
	CUG	CCG	CAG	CGG	G
A	AUU Isoleucine (Ile)	ACU Threonine (Thr)	AAU Asparagine (Asn)	AGU Serine (Ser)	U
	AUC	ACC	AAC	AGC	C
	AUA	ACA	AAA Lysine (Lys)	AGA Arginine (Arg)	A
	AUG — Methionine (Met) **Start codon**	ACG	AAG	AGG	G
G	GUU Valine (Val)	GCU Alanine (Ala)	GAU Aspartic acid (Asp)	GGU Glycine (Gly)	U
	GUC	GCC	GAC	GGC	C
	GUA	GCA	GAA Glutamic acid (Glu)	GGA	A
	GUG	GCG	GAG	GGG	G

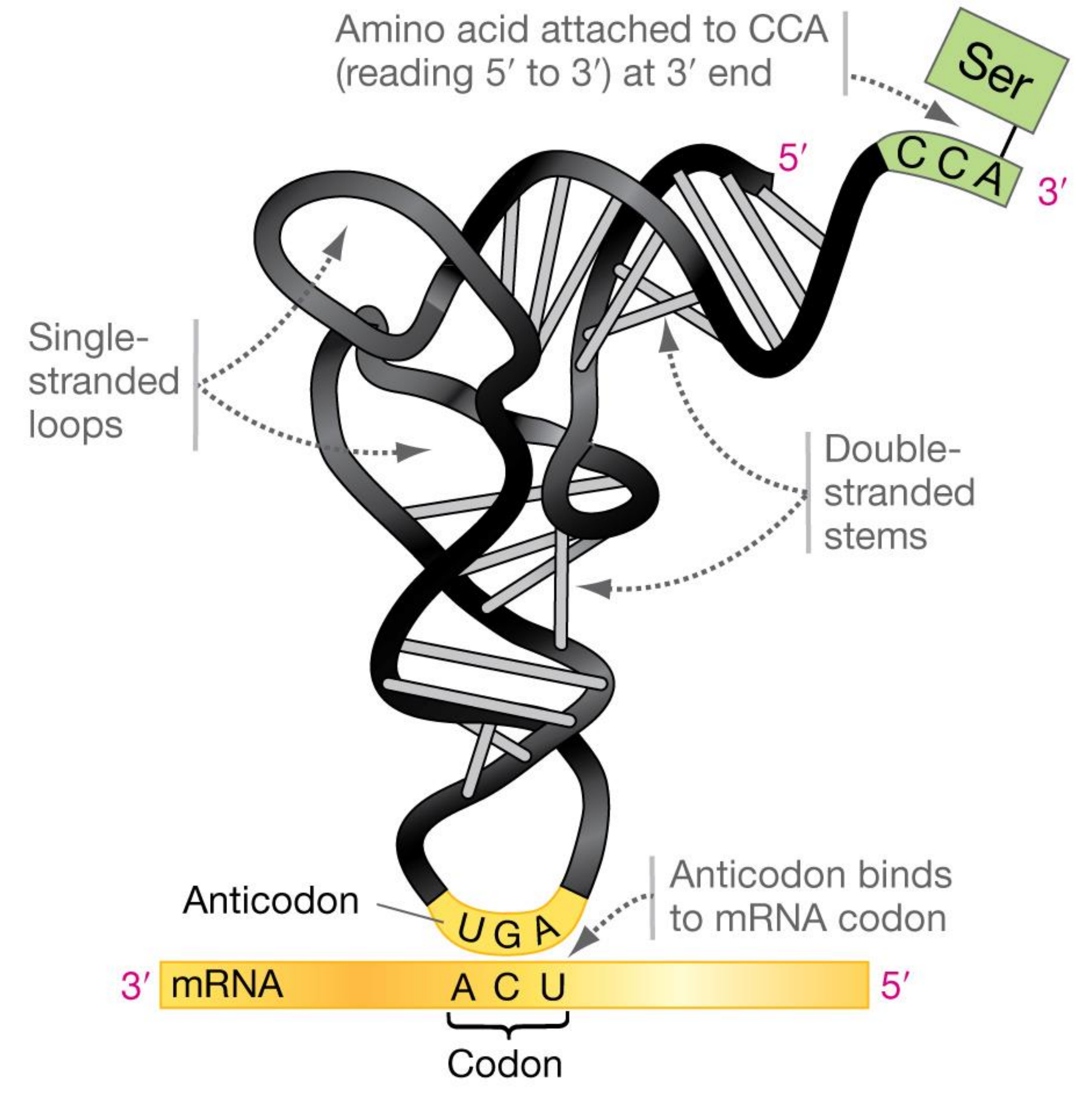




Transfer RNA (tRNA)

- tRNAs transfer amino acids to growing polypeptide
- The 3’ end of tRNA contains a CCA sequence that binds to an amino acid
- Aminoacyl tRNA: tRNA linked to an amino acid
- The loop at the opposite forms an anticodon:
  - Sequence of 3 nucleotides that base-pairs with an mRNA codon (complementary and antiparallel)

(b) Tertiary structure of aminoacyl tRNA



Attachment of Amino Acids to tRNA

- Enzymes called aminoacyl-tRNA synthetases catalyze reaction to charge tRNAs

- For each amino acid there is a different aminoacyl tRNA synthetase, and <u>one or more</u> tRNAs

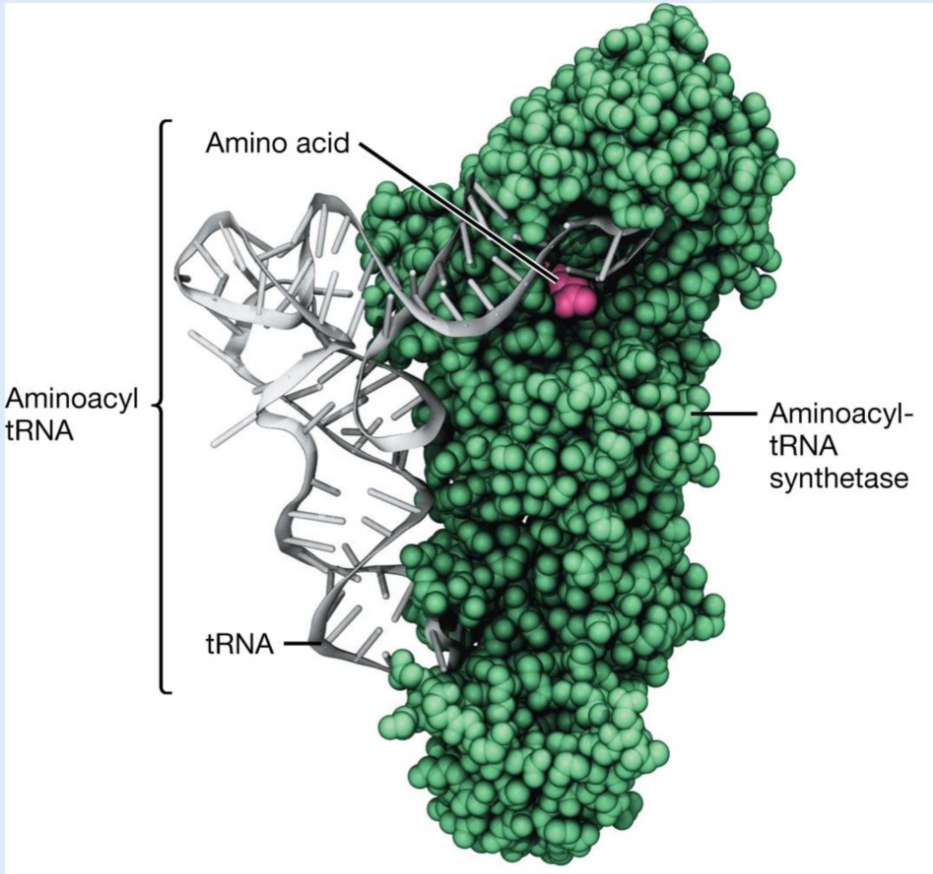


Figure 17.12 Aminoacyl-tRNA Synthetases Couple the Appropriate Amino Acid to the Appropriate tRNA.

tRNA and the Wobble Hypothesis

tRNA and the Wobble Hypothesis

- There are 61 different codons, but only about 40 tRNAs in most cells
- Wobble Hypothesis (proposed by Francis Crick):
  - States that while the first 2 positions of a tRNA anticodon must be complementary to the codon it binds to, the third position can accommodate a nonstandard base pairing (it can “wobble”)
  - This allows for a given tRNA to base-pair with more than one codon

tRNA with the anticodon GUU can bind to both codons for glutamine

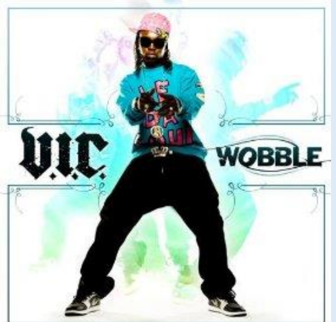
GUU →

Standard

CAA

CAG

Nonstandard

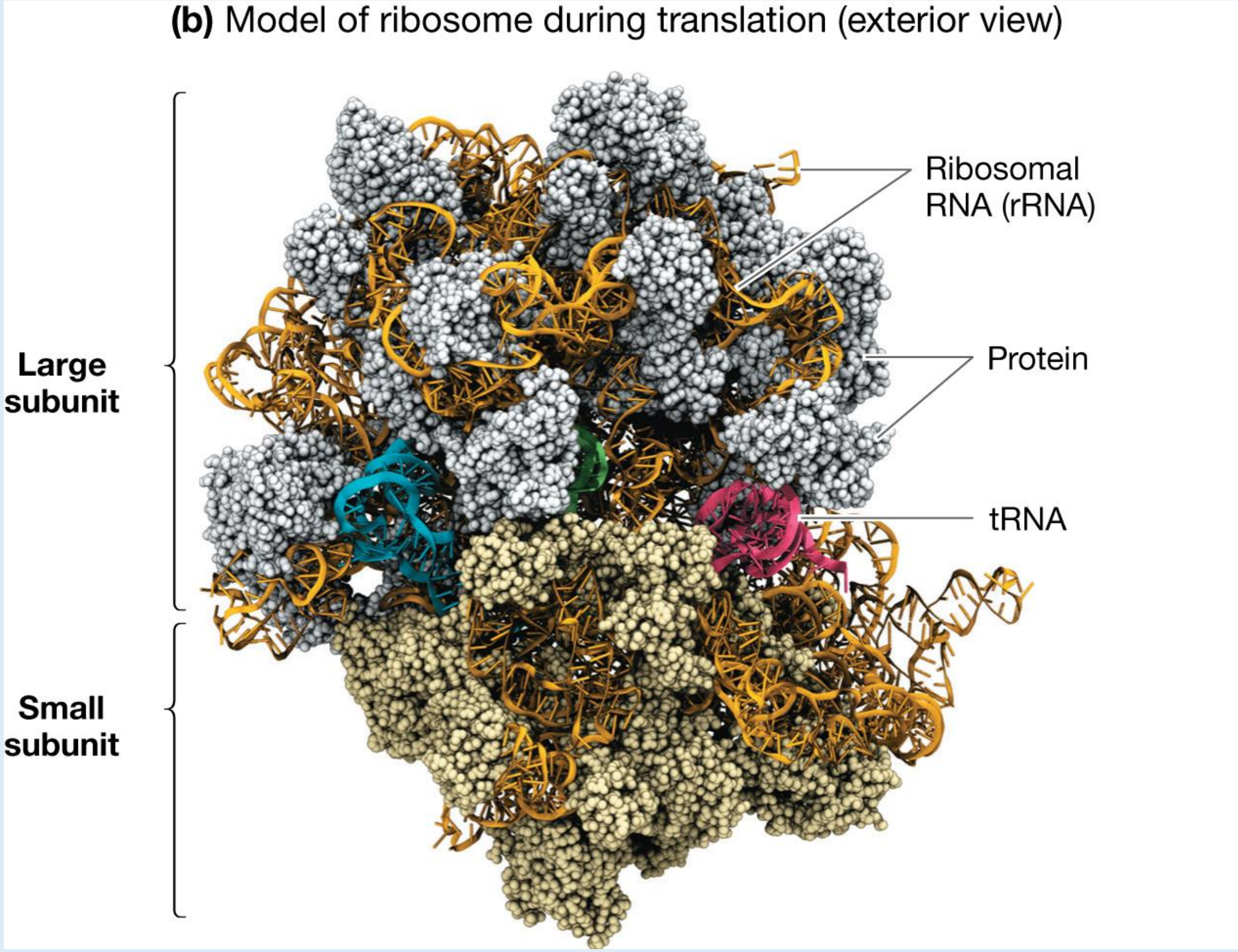


Ribosome Structure

- Ribosomes**:** contain proteins and ribosomal RNA (rRNA)
- rRNA catalyzes protein synthesis ("ribozyme" – <u>ribo</u>nucleic en<u>zyme</u>)

- Ribosomes have two subunits:
    1. The small subunit holds mRNA in place as translation occurs
    2. The large subunit is where peptide bonds form, and contains three tRNA binding sites

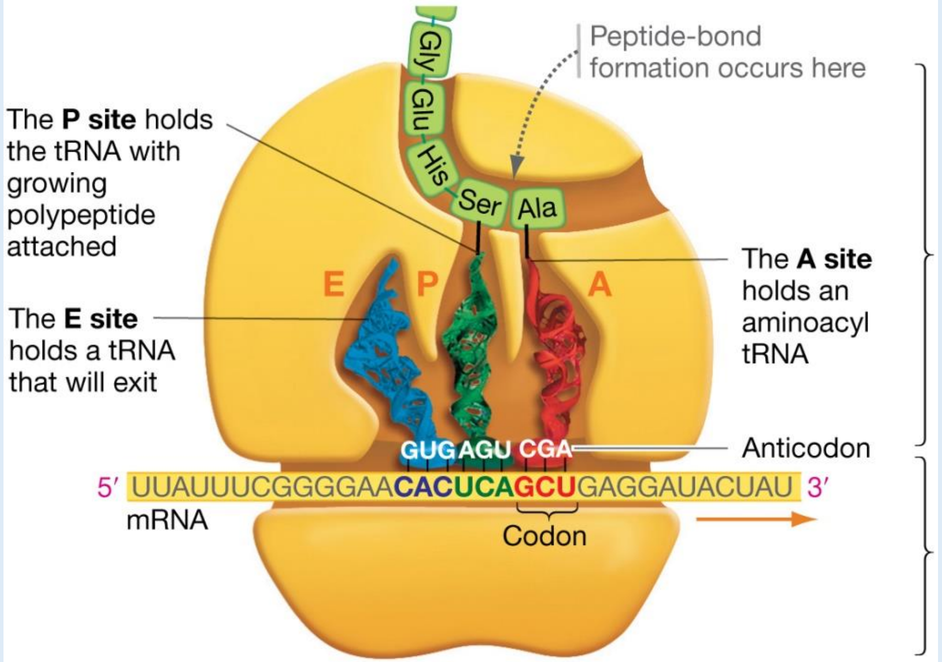
(b) Model of ribosome during translation (exterior view)



tRNA Binding Sites

- During translation, three tRNAs can enter the ribosome at a time
- A tRNA can only fit when its anticodon binds to the corresponding mRNA codon
- Three distinct binding sites:
    1. The **A site** is the acceptor site for an aminoacyl tRNA
    2. The **P site** is the peptidyl site where a peptide bond will form
    3. The **E site** is where tRNAs without amino acids exit the ribosome

(a) Diagram of ribosome during translation (interior view)



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3 major phases of translation:

1) Initiation
2) Elongation
3) Termination

Initiation of Translation: Eukaryotes

1. Initiation factor proteins bring an initiator tRNA with a methionine to the small ribosomal subunit

2. Scanning: The whole complex binds to the mRNA at the 5’ cap, and moves down the mRNA towards the 3’ end until it finds the AUG start codon

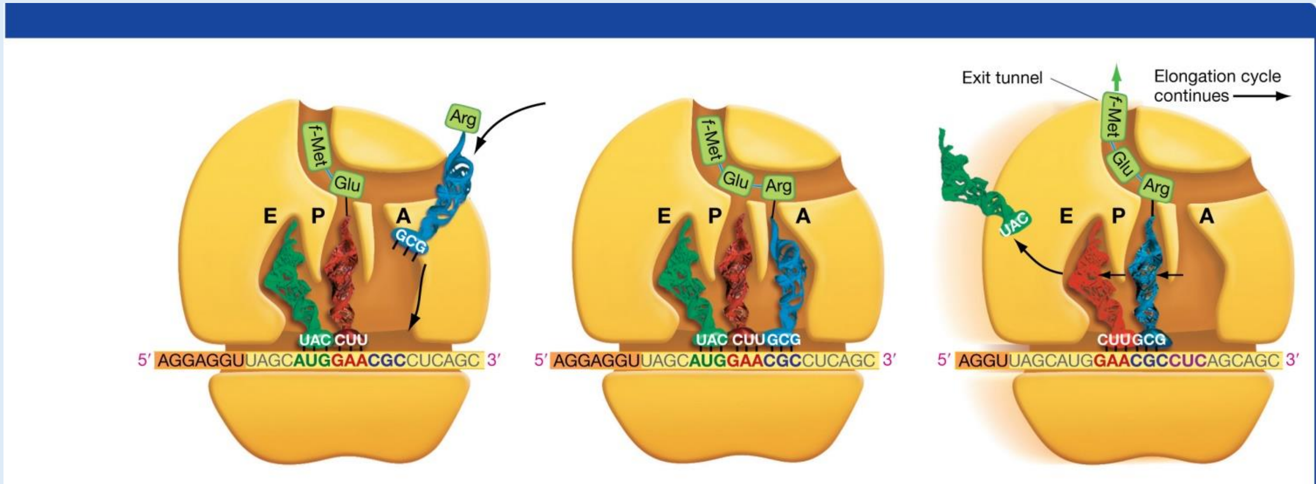
3. The initiation factors leave, the large ribosomal subunit binds, and translation can begin

Elongation of the Polypeptide

- At the start of elongation, the initiator tRNA is in the P site , and the E and A sites are empty. Next:

1. An aminoacyl tRNA binds to the codon in the A site
2. The amino acid on the P site tRNA forms a peptide bond with the amino acid on the A site tRNA
3. <u>Translocation</u> occurs, where the ribosome slides one codon towards the 3’ end of the mRNA

  - This repeats at each codon of mRNA
  - Elongation factors help move the ribosome along



4. Incoming aminoacyl tRNA
New tRNA moves into A site, where its anticodon base pairs with the mRNA codon.

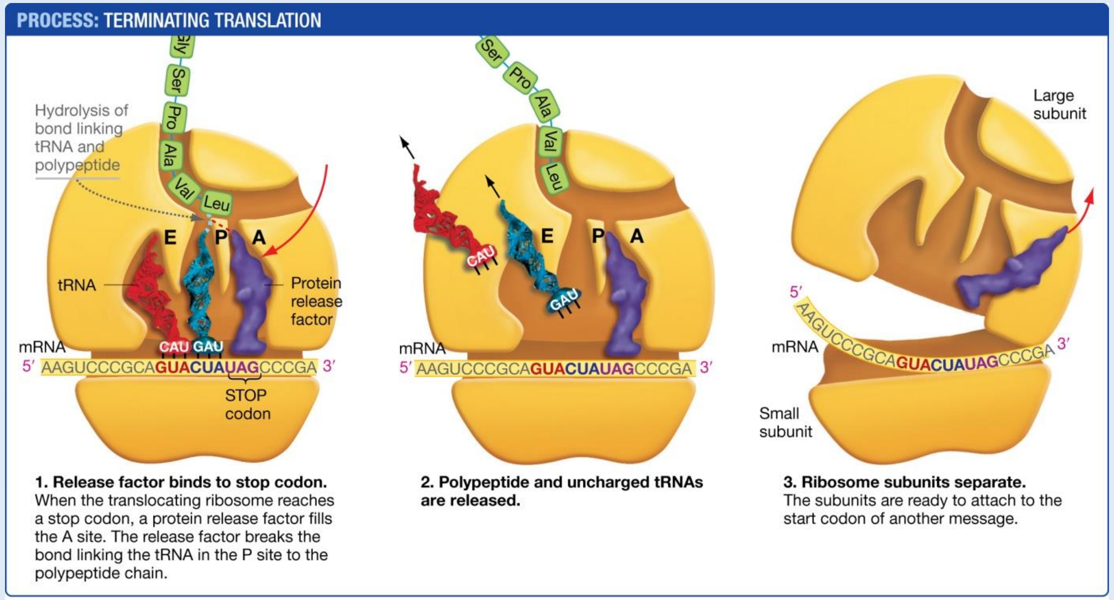
5. Peptide-bond formation
The polypeptide chain attached to the tRNA in the P site is transferred to the aminoacyl tRNA in the A site.

6. Translocation
The ribosome once again moves one codon down the mRNA. The tRNA attached to polypeptide chain moves into P site. Empty tRNA from P site moves to E site, where tRNA is ejected. The A site is empty again.

Termination of Translation

- Termination occurs when the A site encounters a stop codon (UAA, UAG, or UGA)
- Release factor protein enters the A site and binds
- Release factors resemble tRNAs in both size and shape, but they do not carry an amino acid – instead they are surrounded by water molecules

- The bond linking the P site tRNA to the polypeptide chain is hydrolyzed
- Polypeptide and tRNAs are released and the ribosomal subunits separate



PROCESS: TERMINATING TRANSLATION

1. Release factor binds to stop codon. When the translocating ribosome reaches a stop codon, a protein release factor fills the A site. The release factor breaks the bond linking the tRNA in the P site to the polypeptide chain.

2. Polypeptide and uncharged tRNAs are released.

3. Ribosome subunits separate. The subunits are ready to attach to the start codon of another message.

Post-Translation Modifications

- Most proteins go through an extensive series of processing steps called post-translational modifications before they are completely functional

- **Folding**: determines a protein's <u>shape and function</u>
- Molecular chaperones speed up this process

- Chemical modifications: enzymes may also add sugar, lipid, or phosphate groups

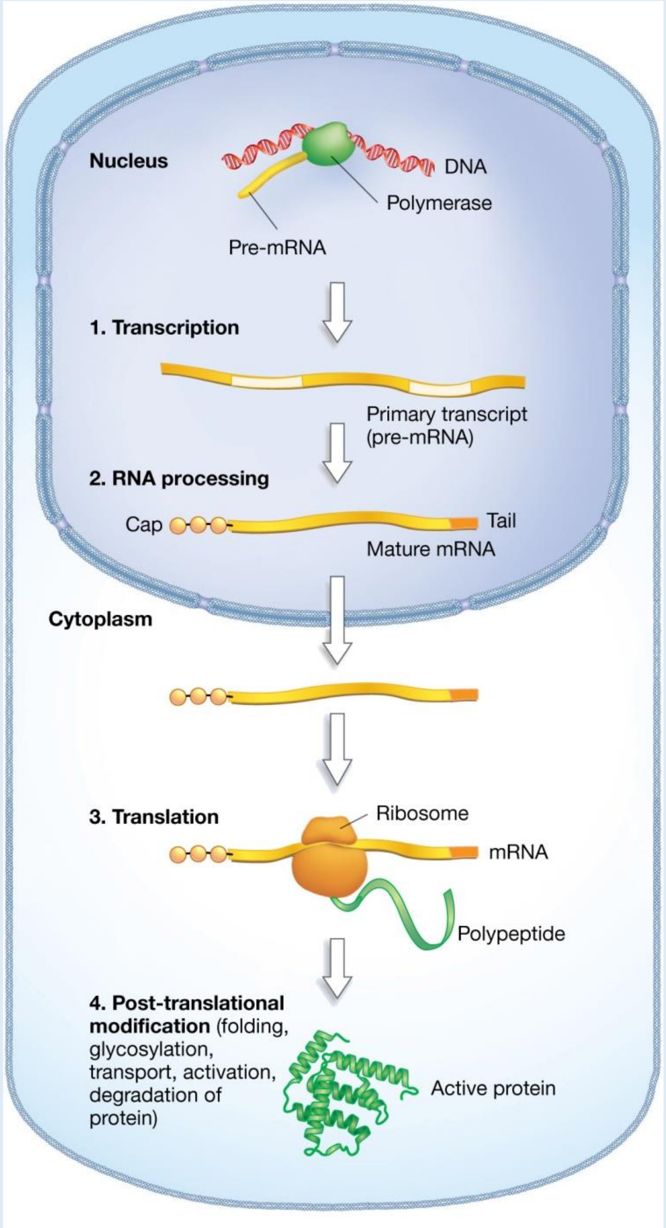


Figure 17.17 **The Major Steps of Gene Expression in a Eukaryotic Cell.**

Check-in Question: Can you identify each macromolecule and event involved in translation?

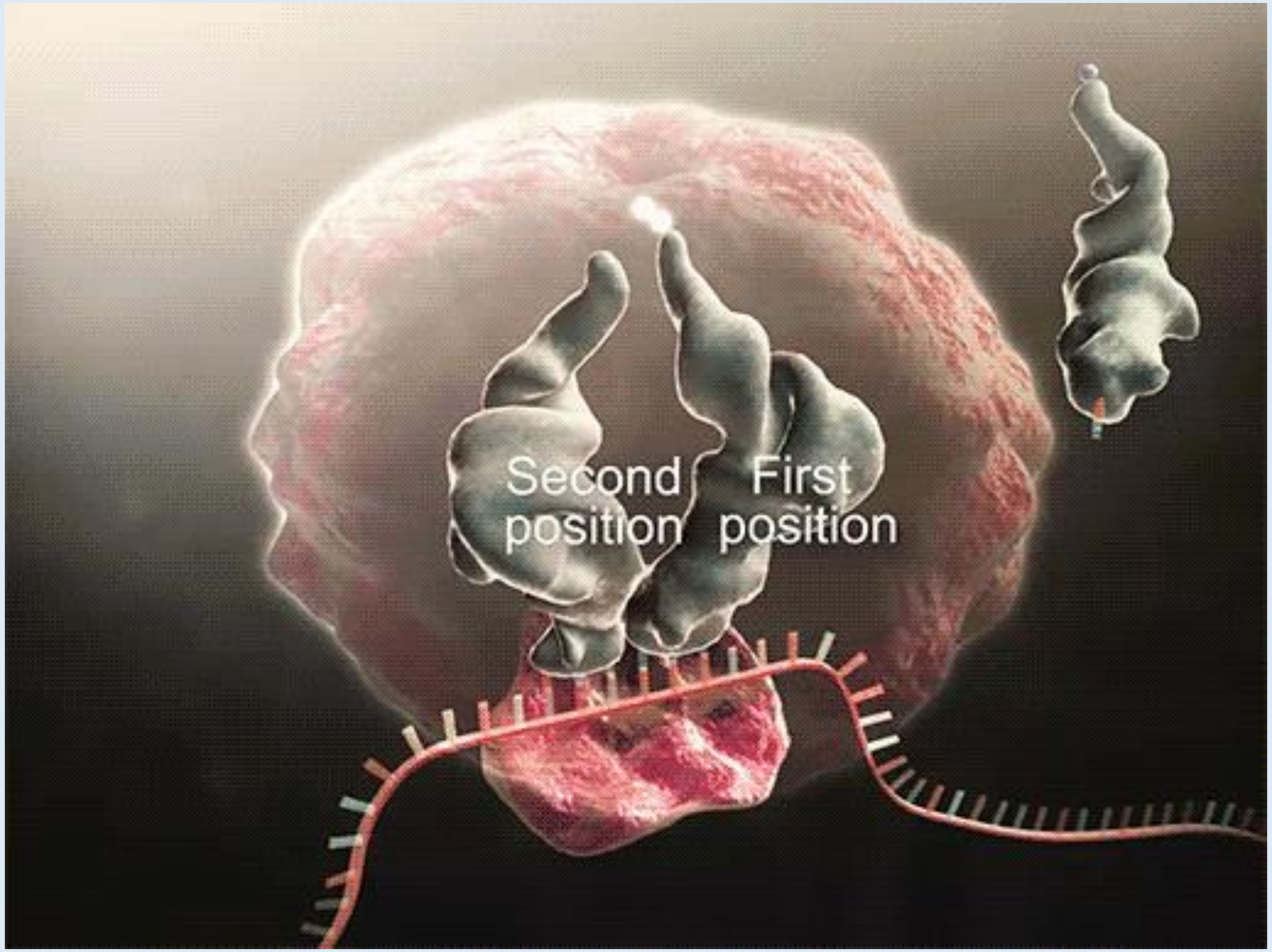
Macromolecules:

1.
2.
3.
4.
5.
6.

Hint: what types of RNA?

Event :

1.
2.
3.

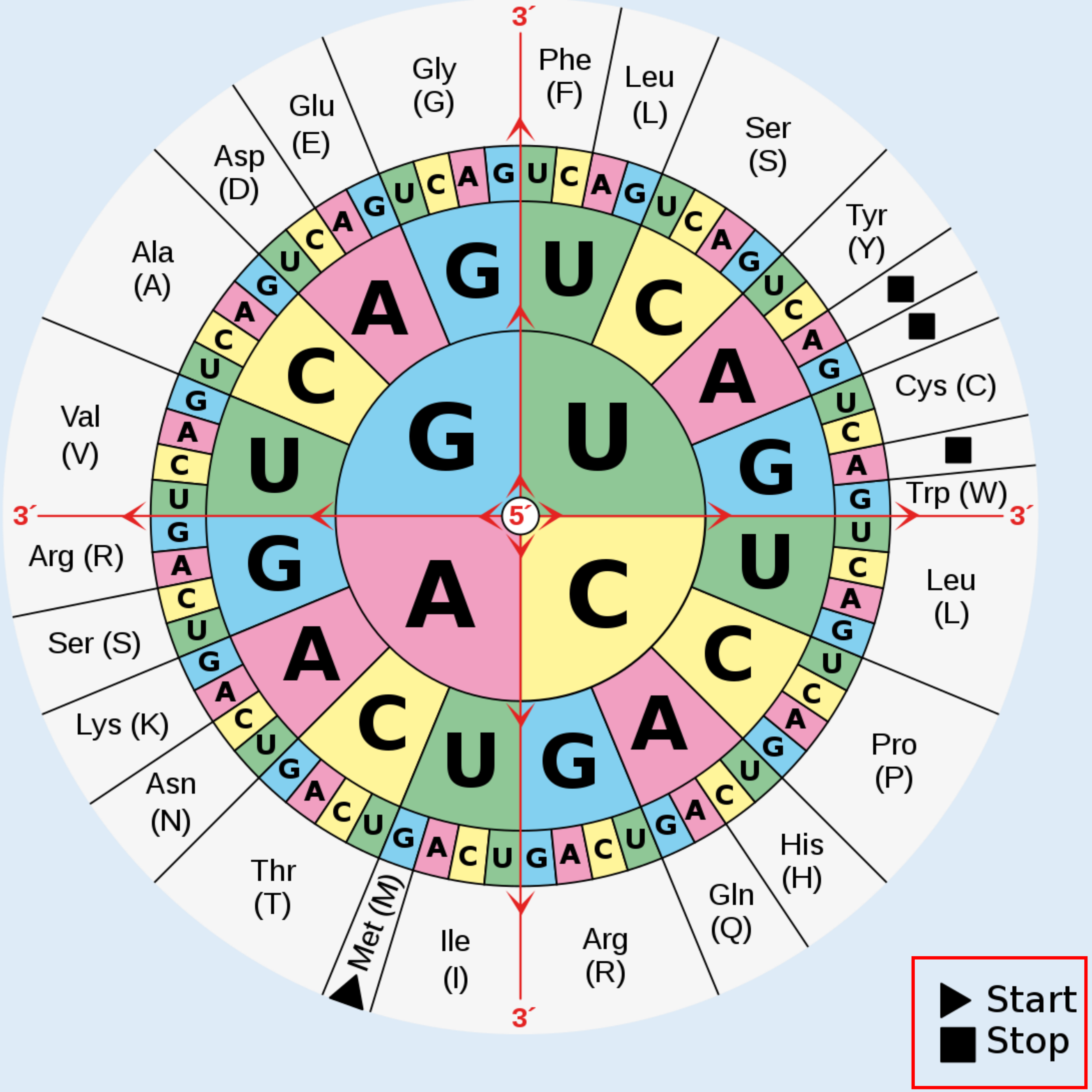


Check-in Question:

- What polypeptides are created from the following mRNA sequences?

1) 5’ UCAUGUGCGUUCAUUUAAA 3’

2) 3’ GGUAGUAUUGCCGUAG 5’



From DNA to protein: building a house comparison

- Work with partner(s) to determine the role you think each of these would play in the house building process:

- DNA: master blueprint
- mRNA: working blueprint / engineer / boss
- Ribosome: construction site / workers
- tRNA: supply trucks / drivers
- Amino acids: building supplies
- Protein: house

Conservation application: salmon physiology and infection

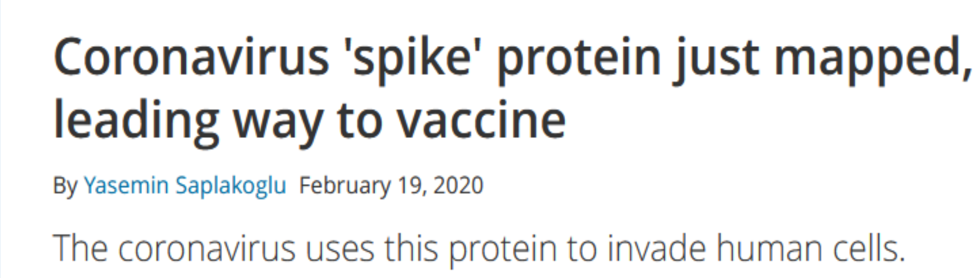
- Effect of stressors? Injuries from fishing nets, high temperatures, infections
- Quantify mRNA to determine what body functions are most active and what pathogens are replicating
- Injury, infection, and stress can take energy away from things like producing eggs

Brighter yellow = more mRNA

- Greater gene expression
- More severe infection

Health application: mRNA vaccines for COVID-19

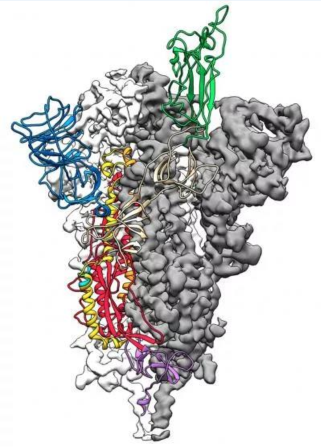
- mRNA created by laboratory (does not use live virus)
- mRNA translated to produce spike protein (found on surface of SARS-CoV-2)
- mRNA broken down and removed (~ 1 week)
- Spike protein piece displayed on cell surface, recognized by immune system
- Triggers immune system to produce antibodies and activate other immune cells to fight what it perceives as infection
- If COVID-19 contracted, immune response will fight infection

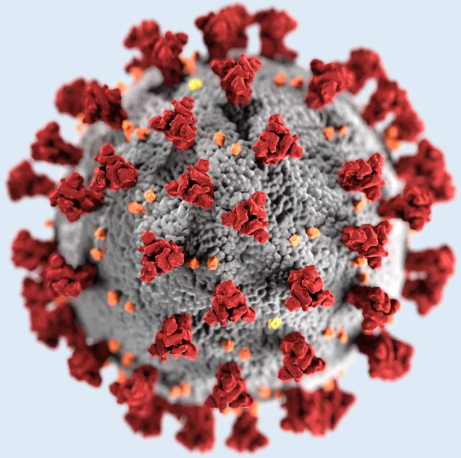


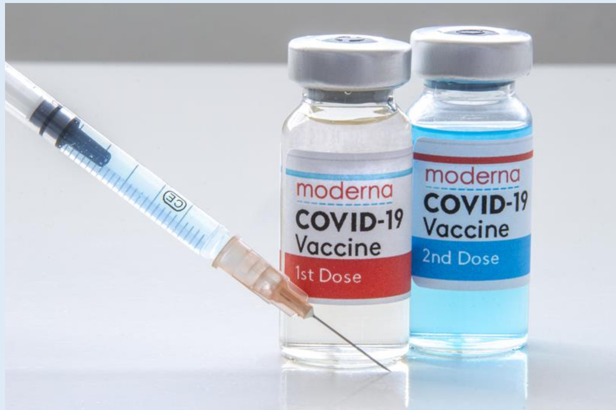
Coronavirus 'spike' protein just mapped, leading way to vaccine

By Yasemin Saplakoglu February 19, 2020

The coronavirus uses this protein to invade human cells.







Thank you!