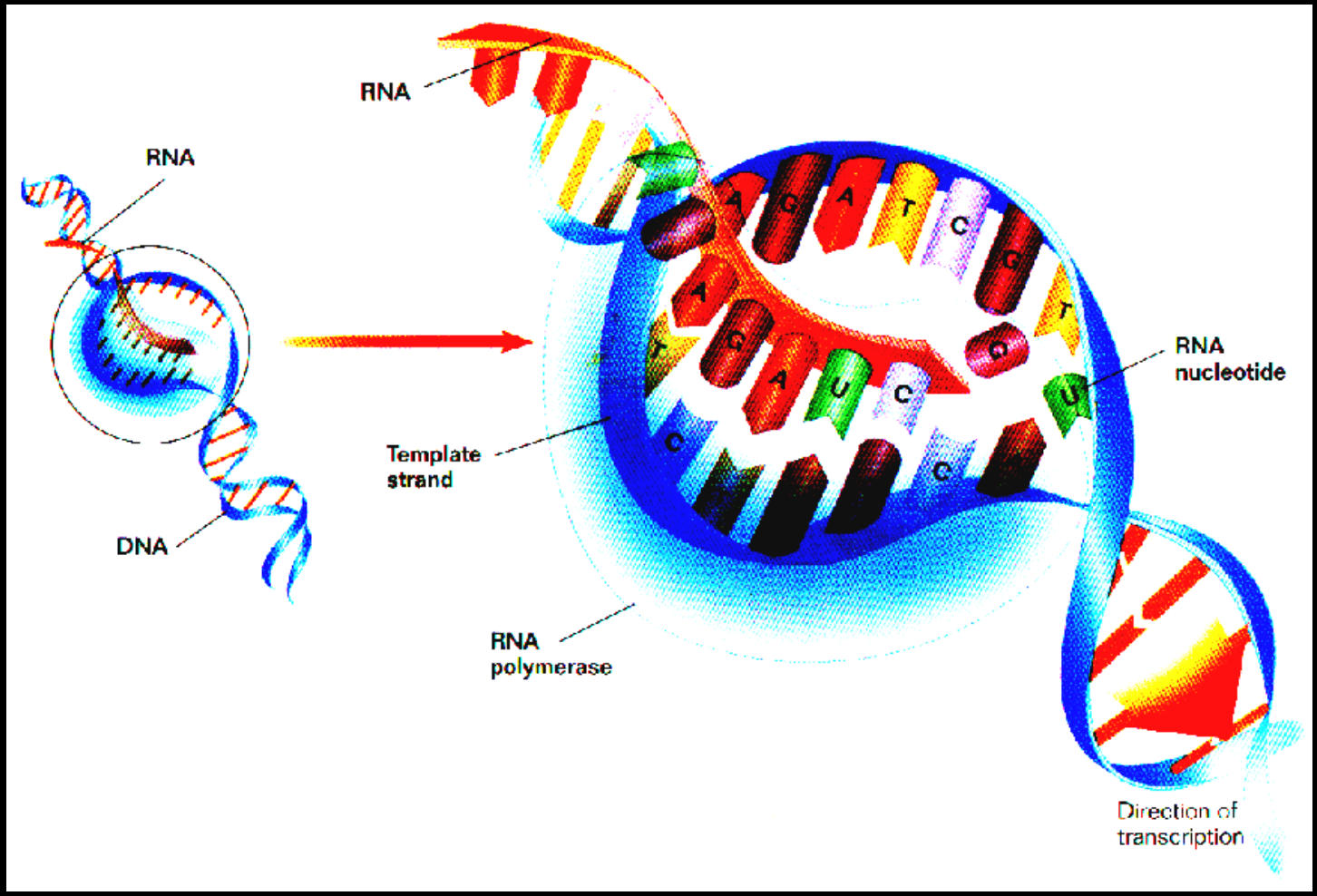
Transcription



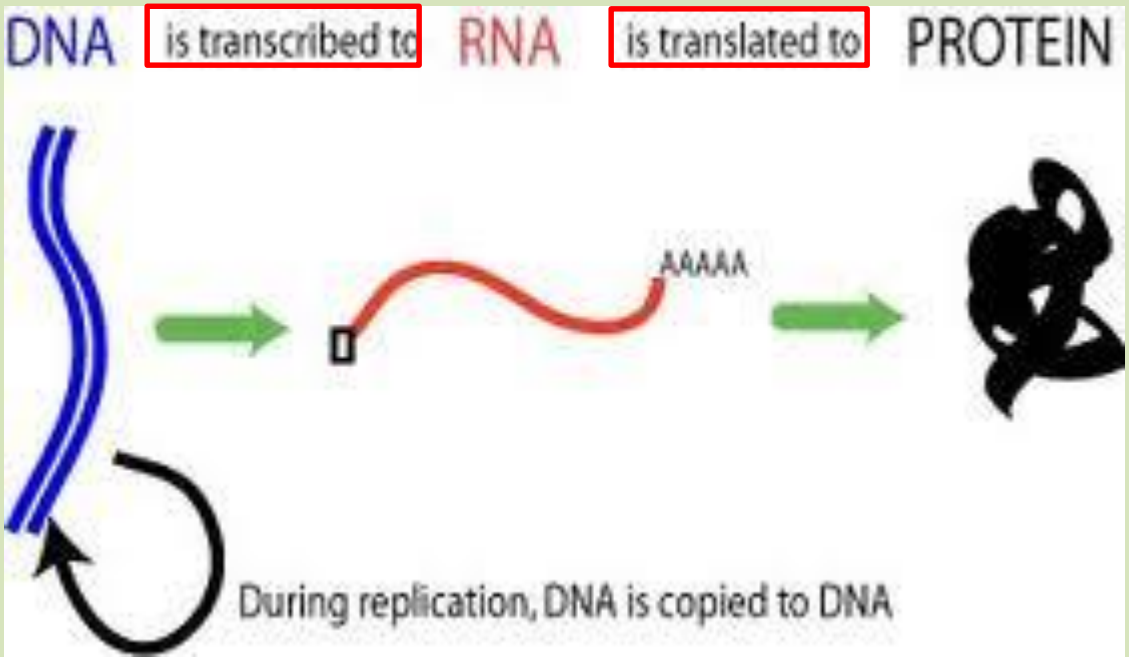
https://www.dnalc.org/view/16360-Animation-16-One-gene-makes-one-protein-.html

Beadle & Tatum experiment

- showed that the mutants had lost use of a specific gene that ordinarily facilitates one particular enzyme necessary to the production of arginine
- conclusion: one gene-one enzyme hypothesis

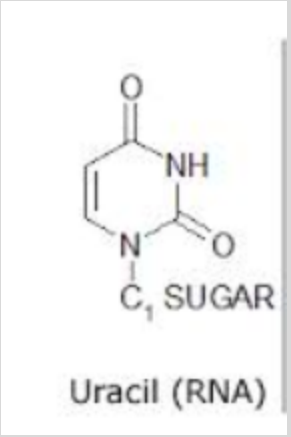
Central Dogma - Watson

- DNA flows in one direction in all living organisms
  - DNA → RNA → protein

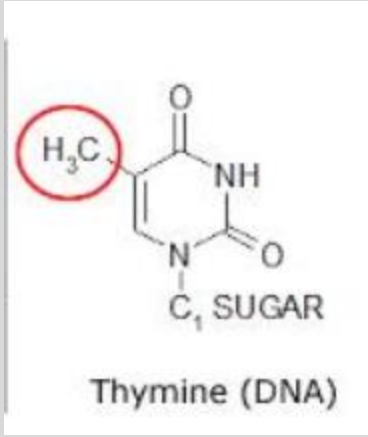


Overview of Transcription

- "making a working copy"
  - makes a disposable copy of DNA → mRNA
    1. The mRNA will be sent to the *construction site* (ribosomes) *for building* the protein.
    2. RNA nucleotides use Ribose *instead* of Deoxyribose
       - This makes the RNA *less stable* than DNA
    3. In RNA, Uracil replaces Thymine.
       - Thymine can't exit nuclear pores



Uracil (RNA)



Thymine (DNA)

Transcription

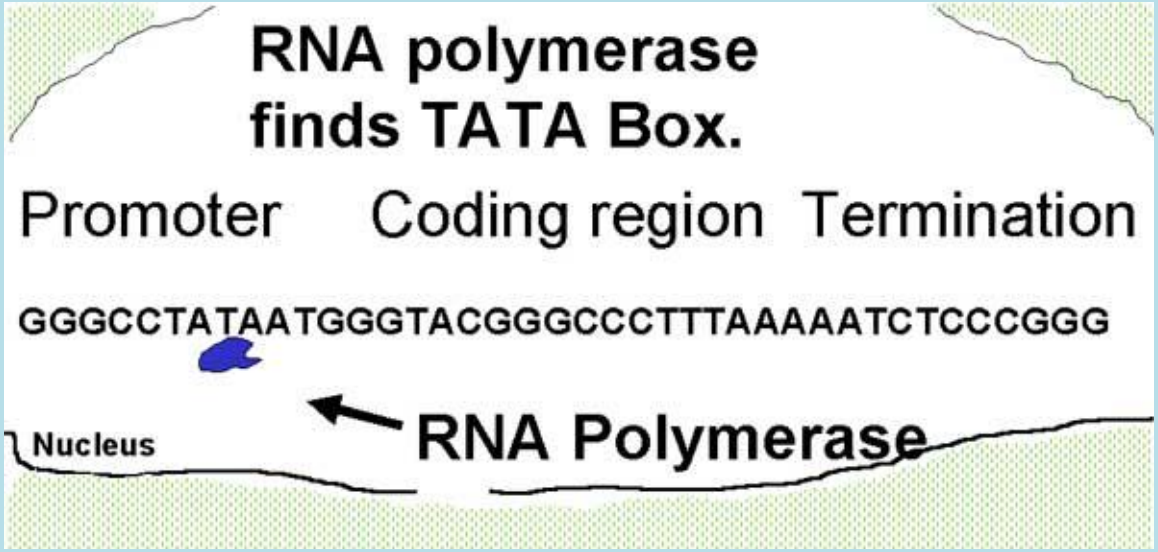
- process of making mRNA
- occurs in the nucleolus
- one DNA strand serves as a template for ordering the nucleotides
  - called the template strand
- 3 Steps:
  1. Initiation
  2. Elongation
  3. Termination

1. Initiation – building the factory

- transcription factors (proteins) attach to the TATA box to *determine* the *direction* the "factory" will proceed
  - the TATA box is part of the promoter sequence

1. Initiation – building the factory

- Then additional transcription factors (proteins and enzymes) are added to the “factory”.
- Finally**, RNA Polymerase II** joins to *complete* the factory. The whole “factory” is called a **Transcription Initiation Complex**.

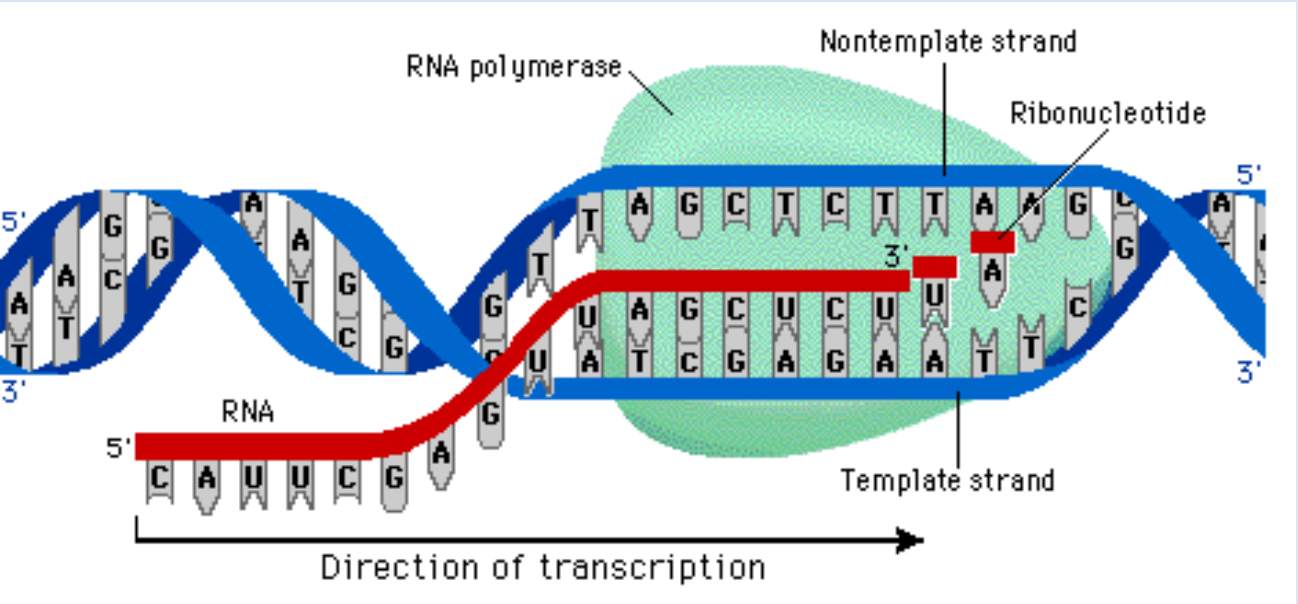


2. Elongation – making mRNA

- This *must* be made in the 5' → 3' direction!
  - will add the nucleotide to the 3' end of the growing strand (just like in replication)
- RNA Polymerase II *separates* the double helix to *make room to work*.
- RNA Polymerase II also *adds nucleotides* to the growing molecule.
- After RNA Polymerase II has past the DNA transcription point, the DNA reforms the helix.

3. Termination – stop

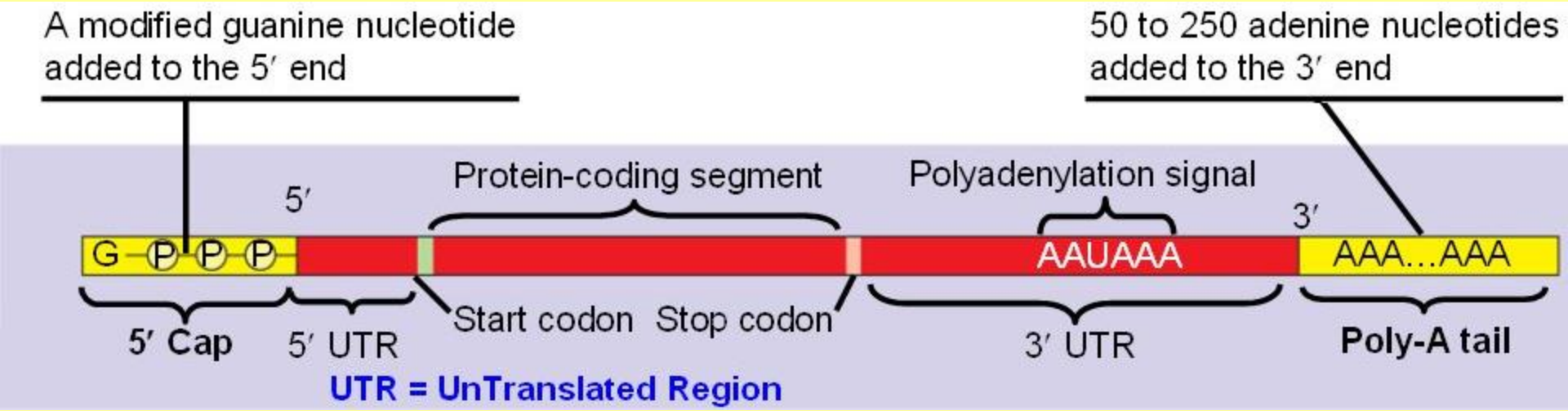
- A stop codon is made (for the ribosome) and the “factory” molecule slows down.
- RNA Polymerase II slows down *until* it stops transcription by forming an AAUAAA sequence and is then released from the DNA.

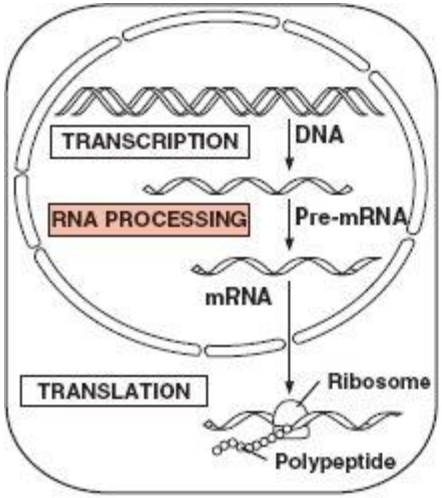


LabBench

RNA Processing- fixing the ends

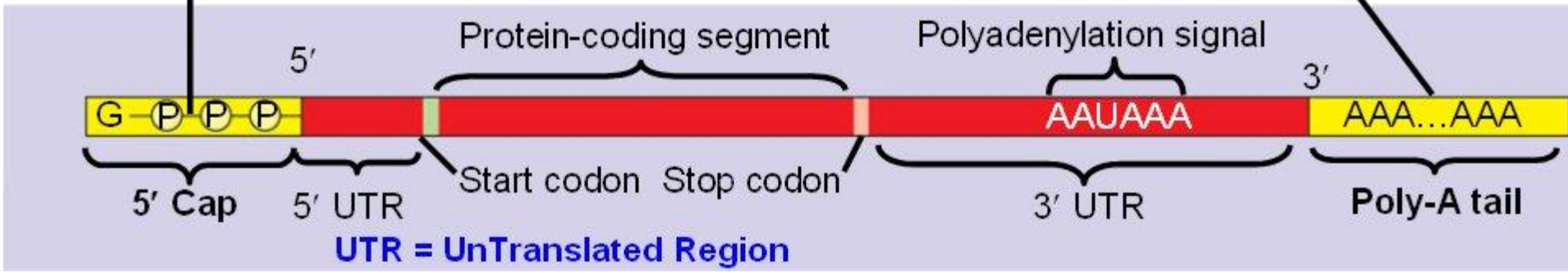
- mRNA must be modified prior to leaving the nucleus
- the 5’ and 3’ ends are fixed
  - 5’ end receives a guanine cap
  - 3’ end receives a polyadenine tail





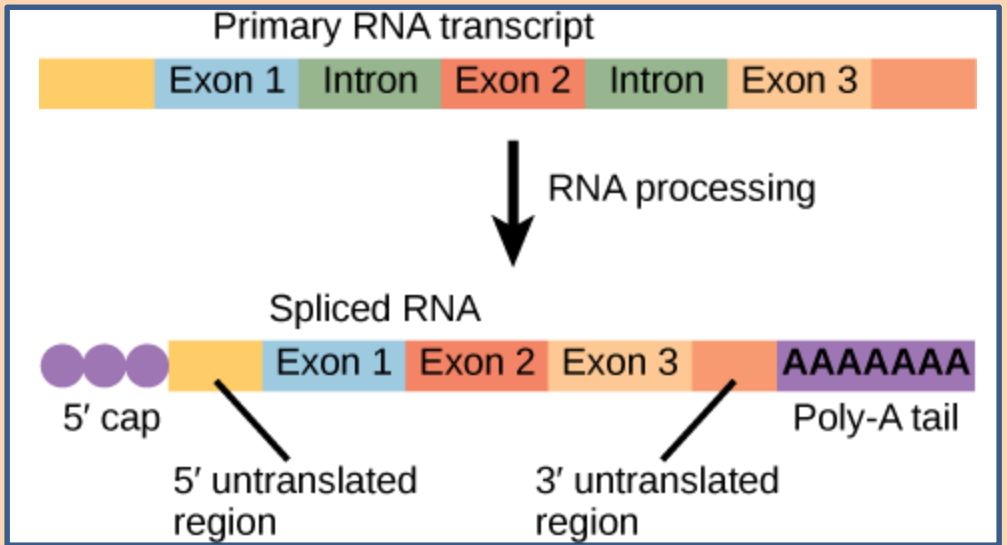
A modified guanine nucleotide added to the 5′ end

50 to 250 adenine nucleotides added to the 3′ end



RNA Processing- fixing the middle

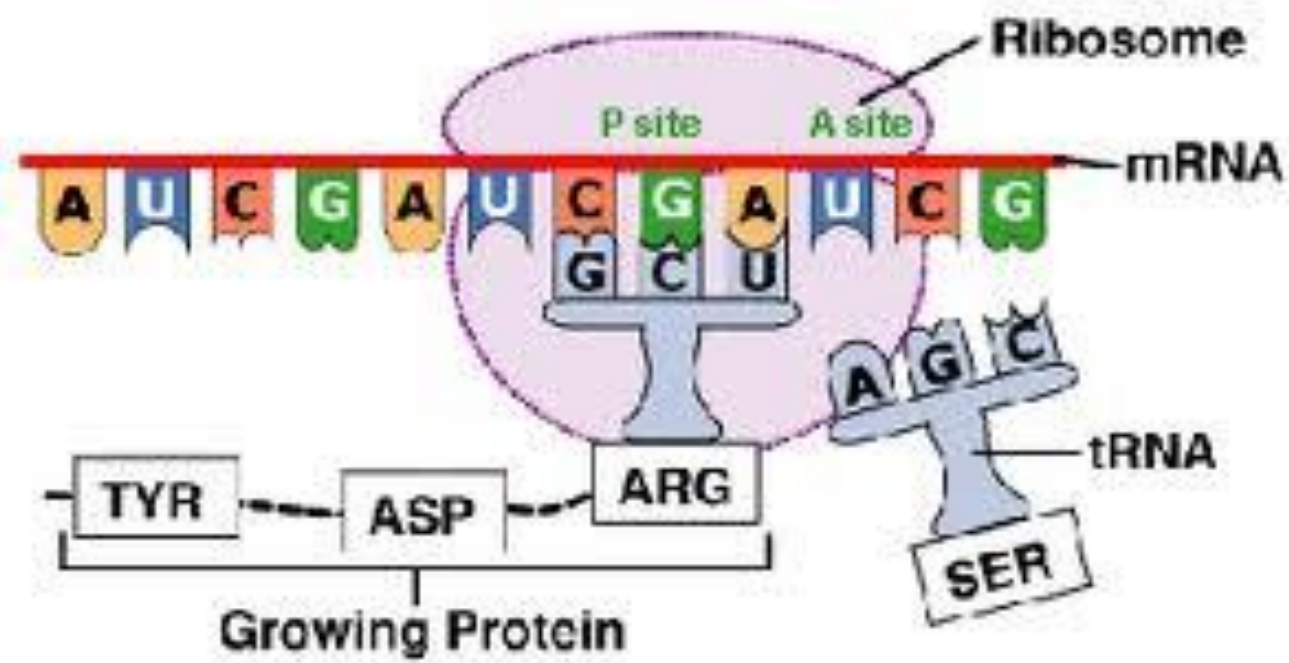
- also known as RNA alternative splicing
- introns (noncoding regions) are cut out of the mRNA and exons (coding regions) are glued together
  - spliceosomes perform this function



Transcription Review

1. Which enzyme unwinds the DNA?
2. Which enzyme adds new nucleotides?
3. Which direction does transcription occur?
4. What is made at the end of transcription?
5. Where is the mRNA made? Where does it go?
6. What happens to the mRNA before it goes to the ribosome?
7. What is spliced out of the mRNA?

Translation



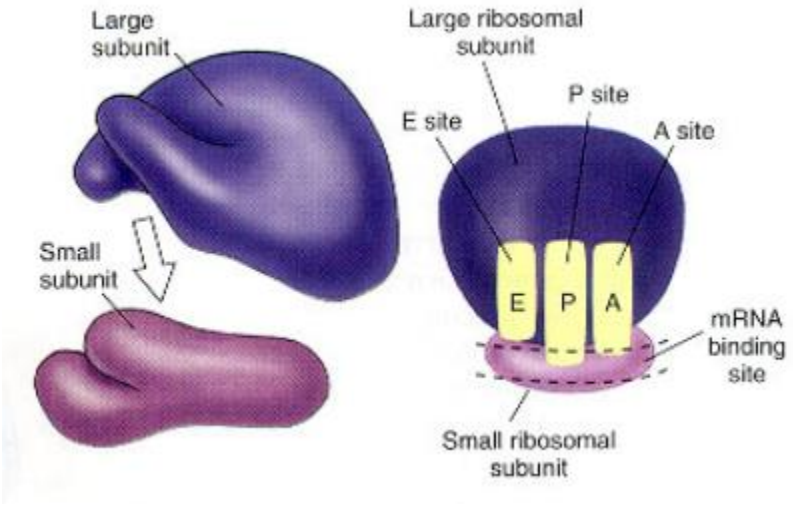
Translation Overview

- occurs at the ribosome
- turns mRNA into a sequence of amino acids
- needs tRNA to transfer *free* amino acids from the cytoplasm, to the ribosome
- more than one ribosome complex may attach to the mRNA – called a polyribosome
  - allows many proteins to be made at once

Ribosome Structure

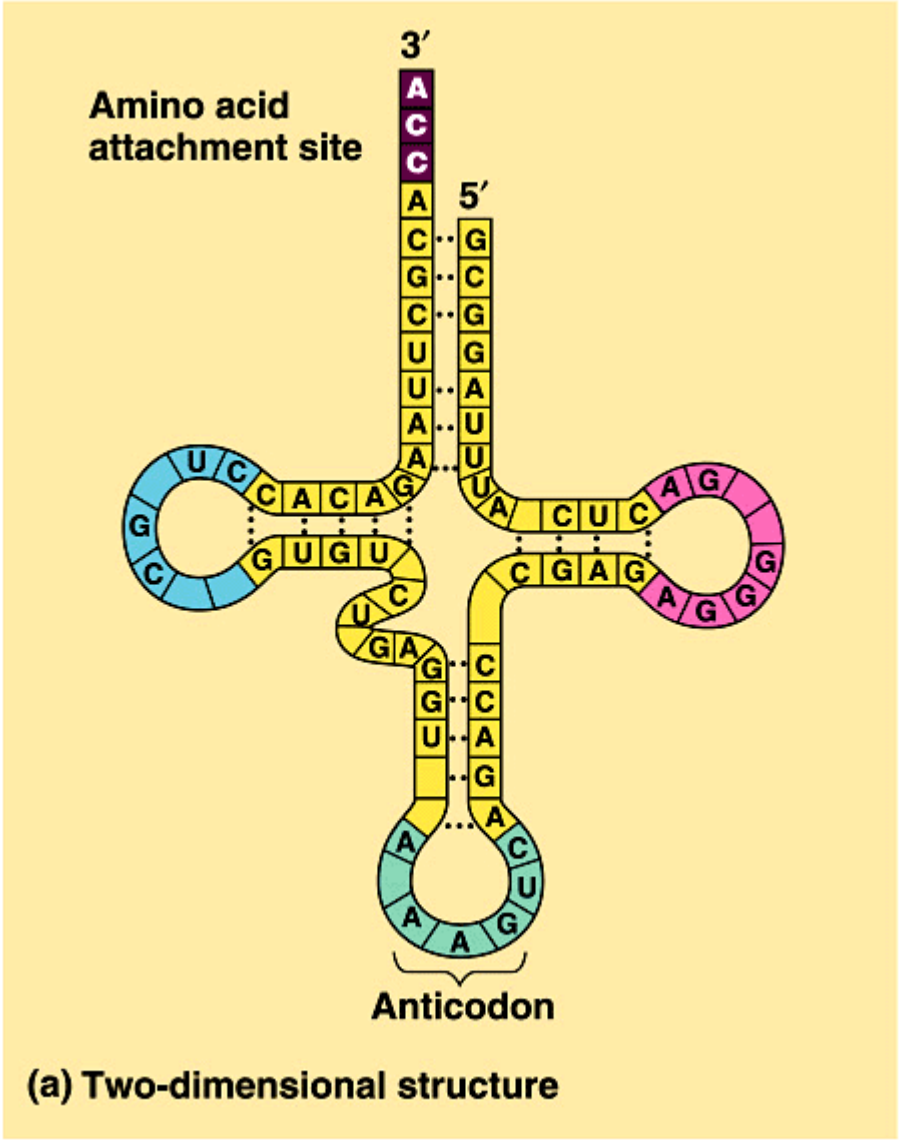
2 parts:

1. small sub-unit: platform for work
2. large sub-unit: factory
   - A site: where the next tRNA is added
   - P site: protein attachment
   - E site: where tRNA exits



Transfer RNA (tRNA)

- Each tRNA molecule is different and is made from a DNA template in the nucleus.
- tRNA has a three-dimensional shape. The 3'end of the molecule holds a specific amino acid.
- The opposite end has the compliment to an mRNA codon called the **anticodon**.



Transfer RNA (tRNA)

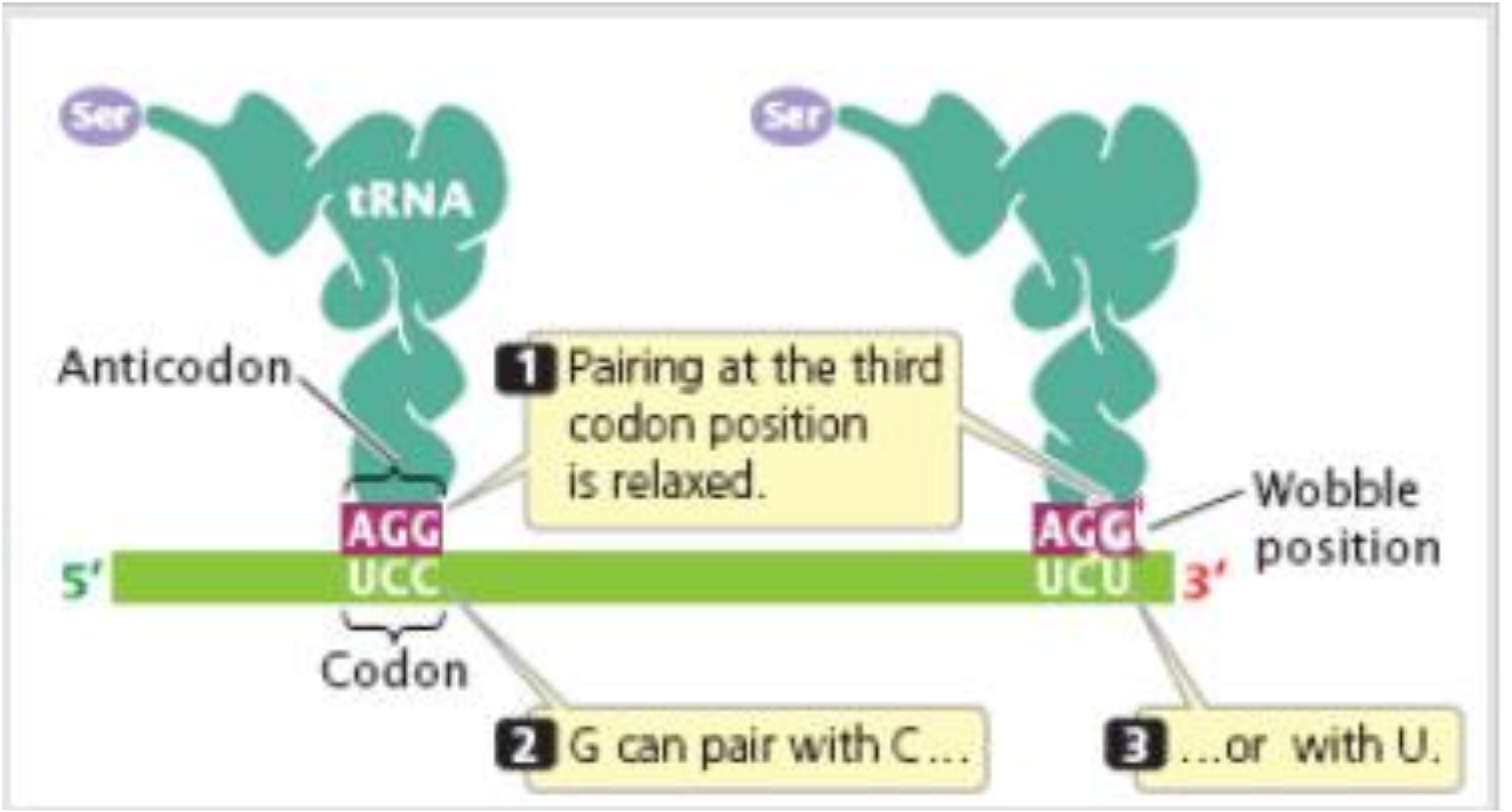
- There are 45 different tRNA molecules for 61 possible codon combinations.

First Letter	Second Letter				Third Letter
	U	C	A	G	
U	phenylalanine	serine	tyrosine	cysteine	U
	phenylalanine	serine	tyrosine	cysteine	C
	leucine	serine	stop	stop	A
	leucine	serine	stop	tryptophan	G
C	leucine	proline	histidine	arginine	U
	leucine	proline	histidine	arginine	C
	leucine	proline	glutamine	arginine	A
	leucine	proline	glutamine	arginine	G
A	isoleucine	threonine	asparagine	serine	U
	isoleucine	threonine	asparagine	serine	C
	isoleucine	threonine	lysine	arginine	A
	(start) methionine	threonine	lysine	arginine	G
G	valine	alanine	aspartate	glycine	U
	valine	alanine	aspartate	glycine	C
	valine	alanine	glutamate	glycine	A
	valine	alanine	glutamate	glycine	G

tRNA – wobble effect

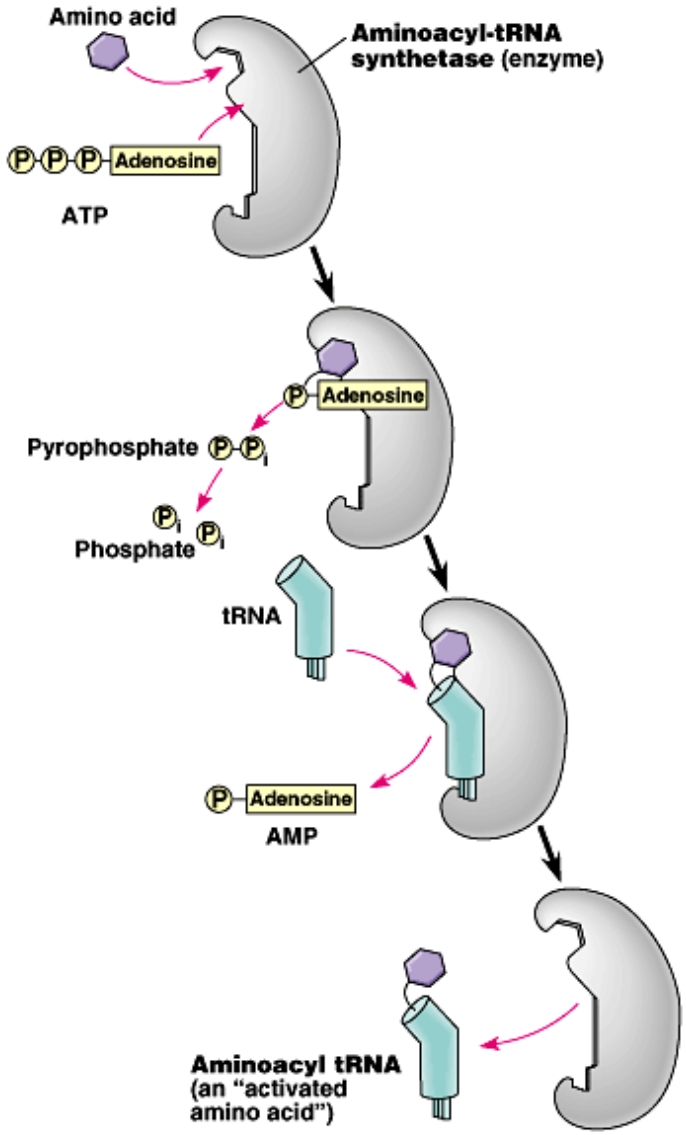
- Remember: there are 3 bases per anticodon!
  - Anticodons are complimentary to codons to ensure the correct amino acid is brought to the ribosome.
- However, the 3rd base does not adhere to base-pairing rules!
  - U on tRNA can bond with an A or G on mRNA. Hence, the reason the third base pair on a codon can be different but code for the same a.a.
  - Some tRNA has a special kind of base called Inosine (I) which can bond with U, C, or A.

Wobble Effect



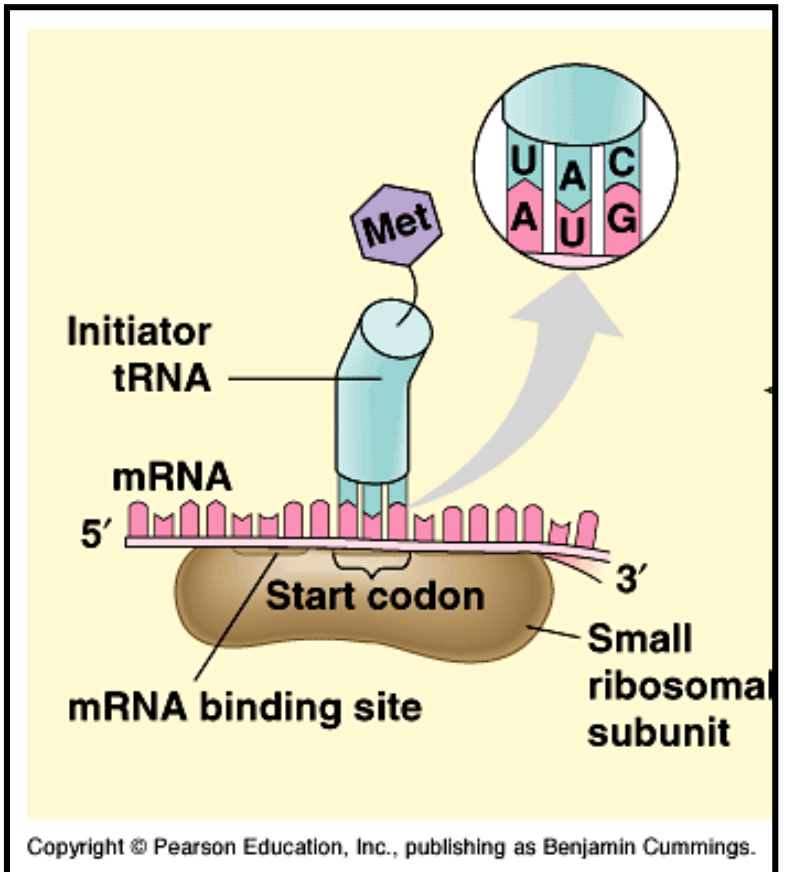
Amino Acid Pick-Up

- An enzyme called <u>aminoacyl-tRNA synthetase</u> is used to join amino acid to tRNA in the cytoplasm.
- The addition is driven by ATP.



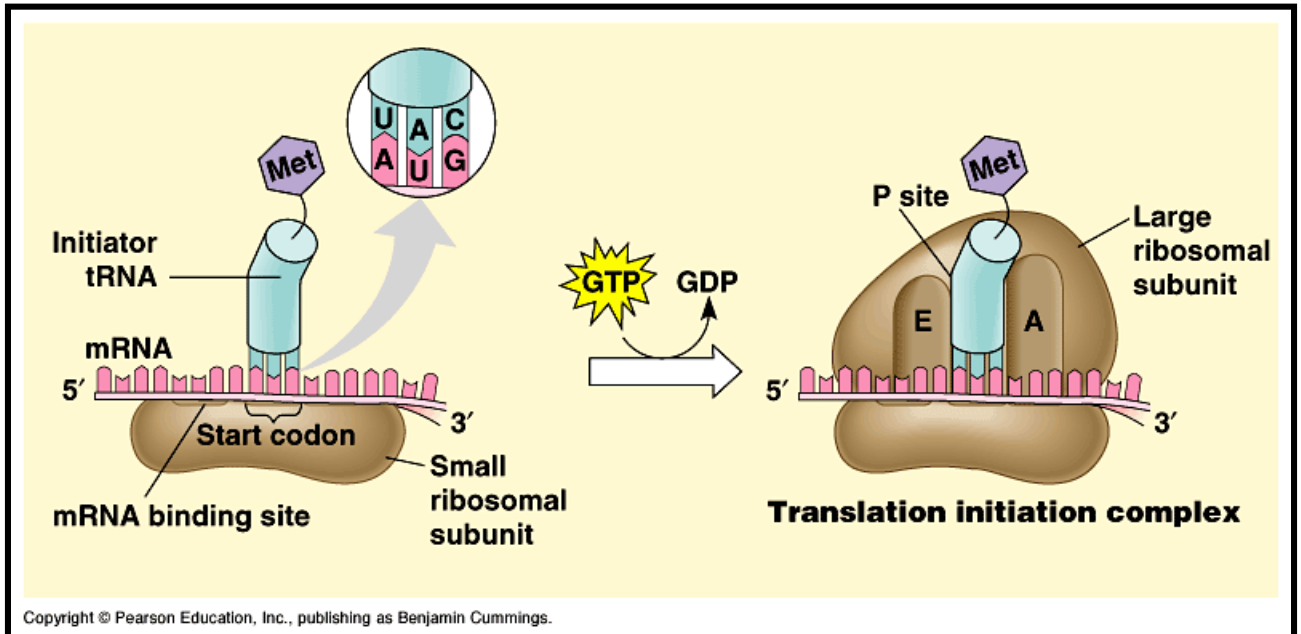
Initiation – building the factory

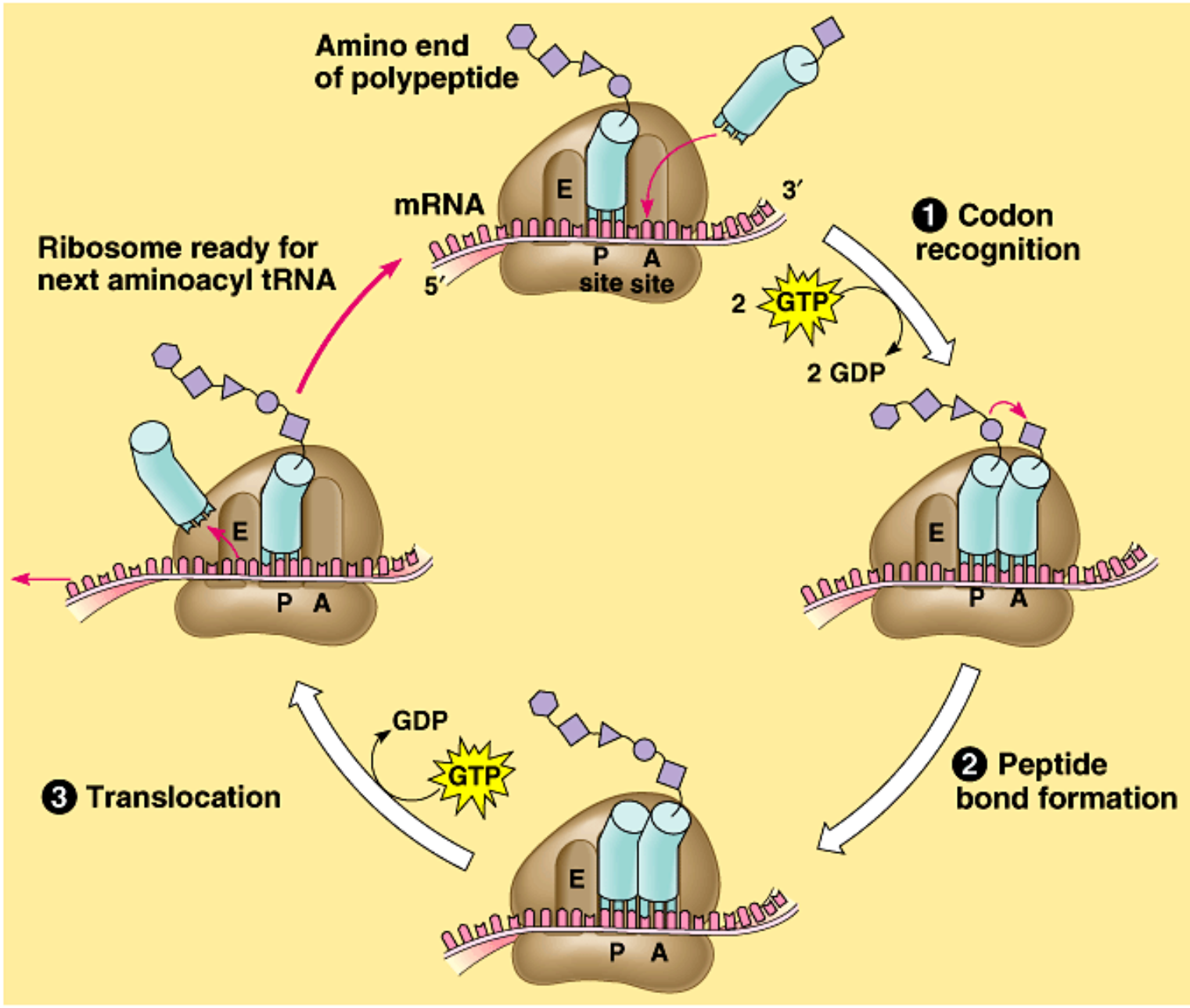
1. small sub-unit attaches to 5’ cap of mRNA
2. start codon (AUG) brings the tRNA molecule with methionine attached
   – this starts production of the protein



Initiation – building the factory

3. large sub-unit will bind using enzymes called <u>initiation factors</u>
   - uses GTP for energy
4. large sub-unit aligns methionine to P site
   - A site is now open

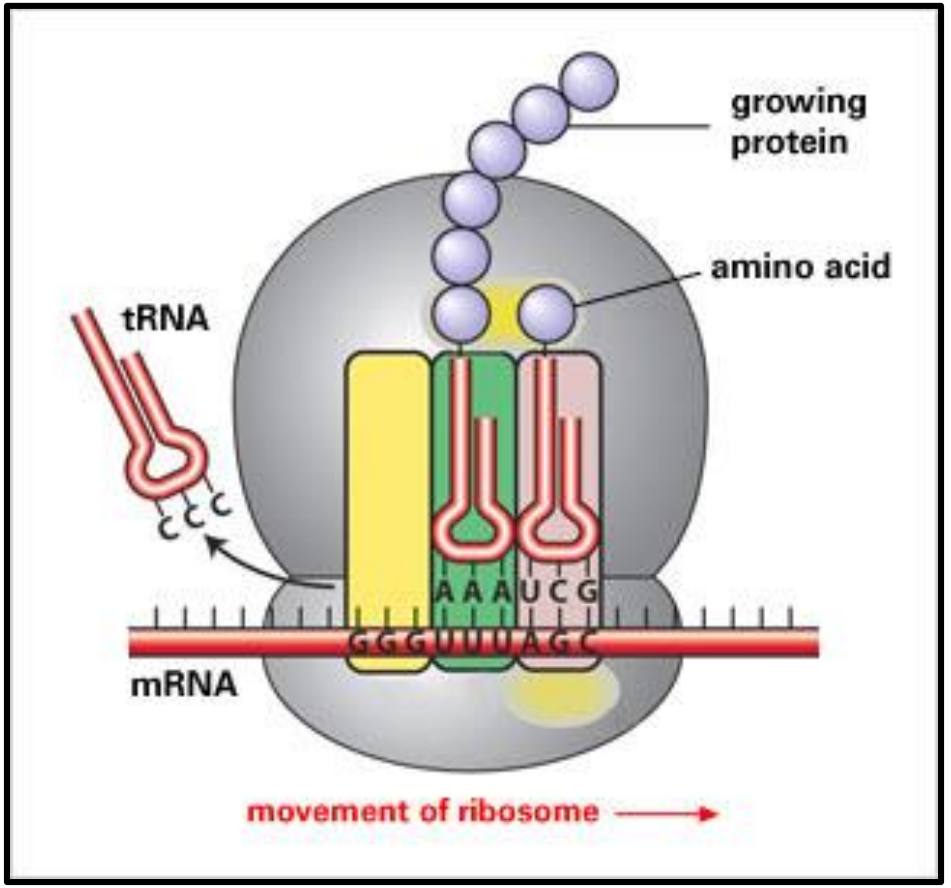


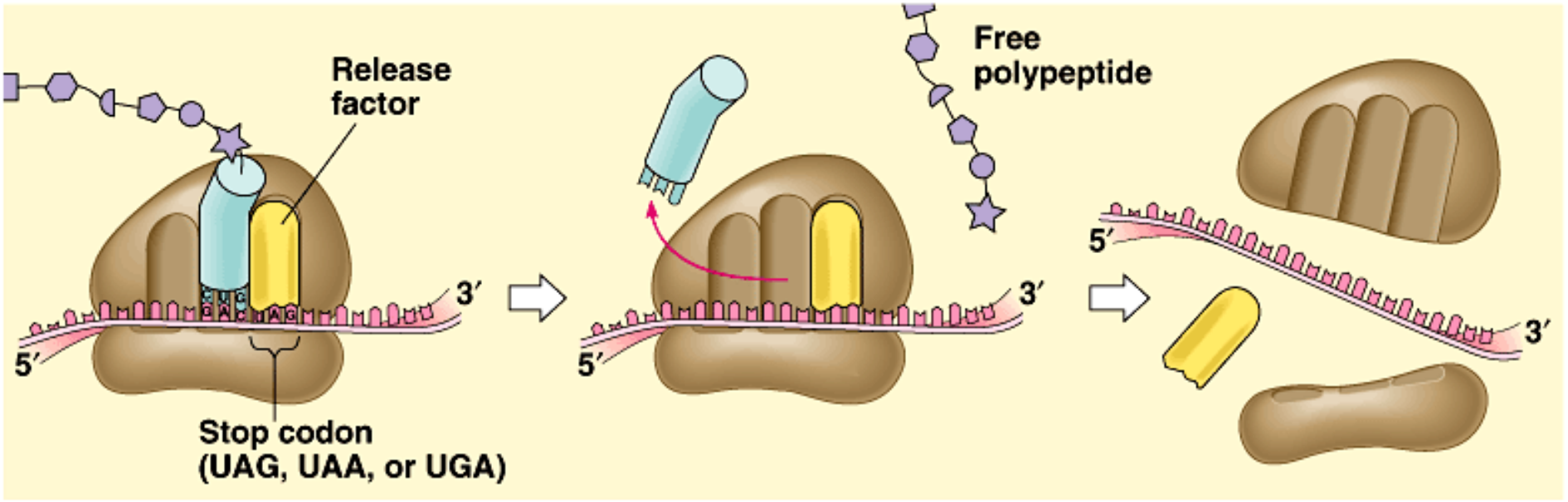


Elongation – actual making of a.a.

1. ribosome translocates ("walks") down the mRNA *one codon* at a time using GTP
   - tRNA adds a single amino acid to the open A site
2. another GTP is used to make the peptide bond between each amino acid

*tRNA's move from A to P to E site= moving APE!



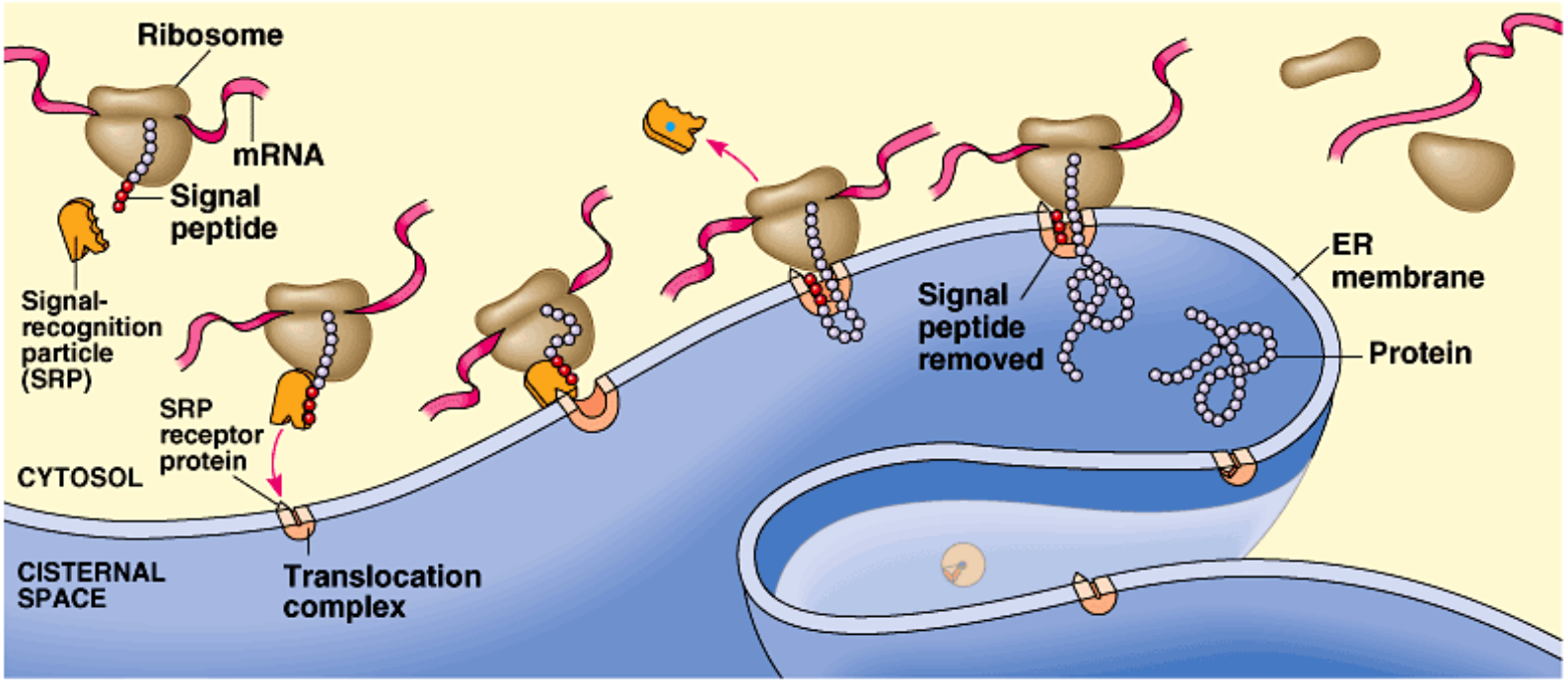


Termination – the end

1. termination codon reaches the A site
   - UAA, UAG or UGA
2. release factors (enzymes) enter the A site causing a *hydrolysis* reaction
   - releases protein from tRNA
3. ribosomal sub-units detach & separate
4. mRNA will either be recycled or reused

Post-Translation Modification

- If the sequence enters a chaperonin – the protein will stay in the cell
  - these proteins are made by free ribosomes



Post-Translation Modification

- If the sequence enters the RER – the protein will be exported out of the cell
  - these proteins are made by bound ribosomes
  - will have a signal peptide attached that will be recognized by the RER