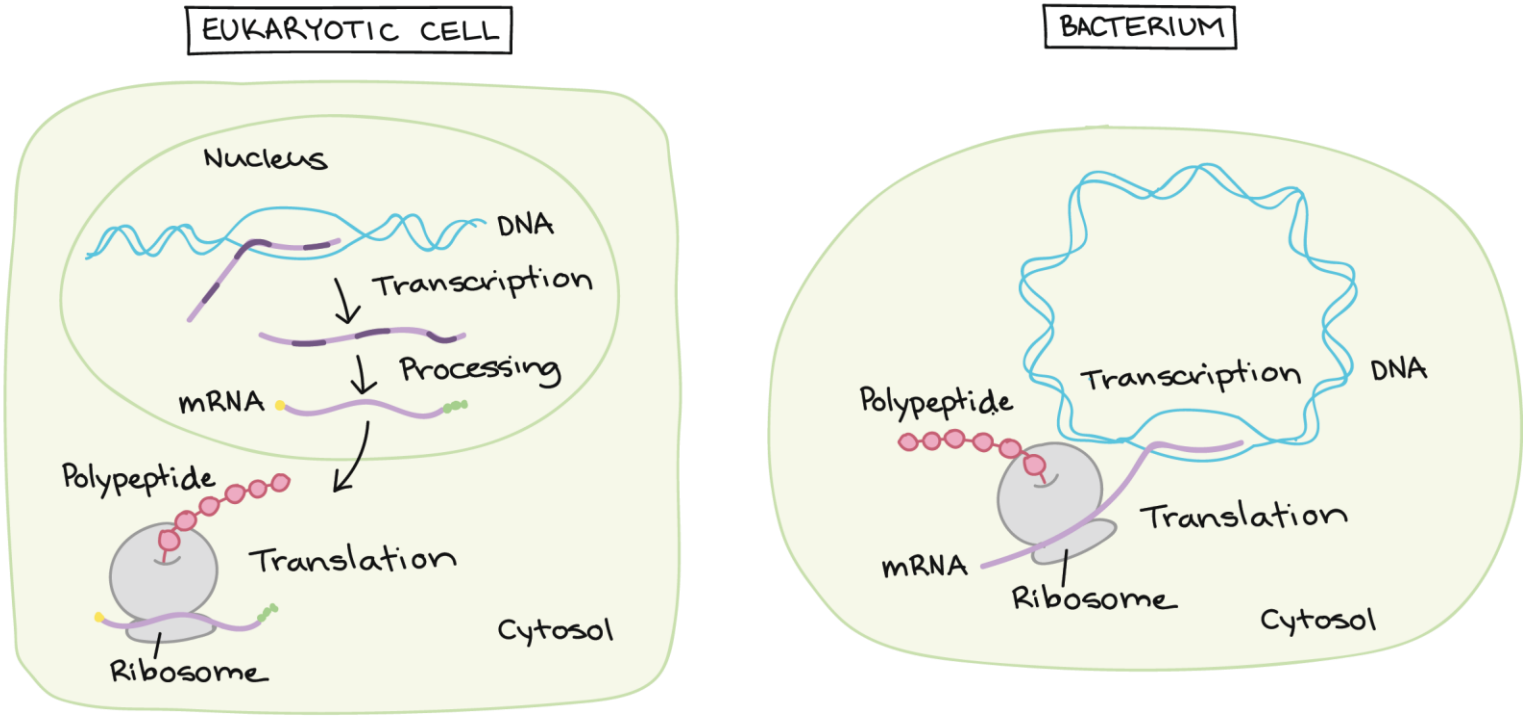
Translation

Eukaryotic pre-mRNA processing

- **Key points:**
- When an RNA transcript is first made in a eukaryotic cell, it is considered a **pre-mRNA** and must be processed into a **messenger RNA** (**mRNA)**.
- A **5' cap** is added to the beginning of the RNA transcript, and a 3' **poly-A tail** is added to the end.
- In **splicing**, some sections of the RNA transcript (**introns**) are removed, and the remaining sections (**exons**) are stuck back together.
- Some genes can be **alternatively spliced**, leading to the production of different mature mRNA molecules from the same initial transcript.

Overview of pre-mRNA processing in eukaryotes

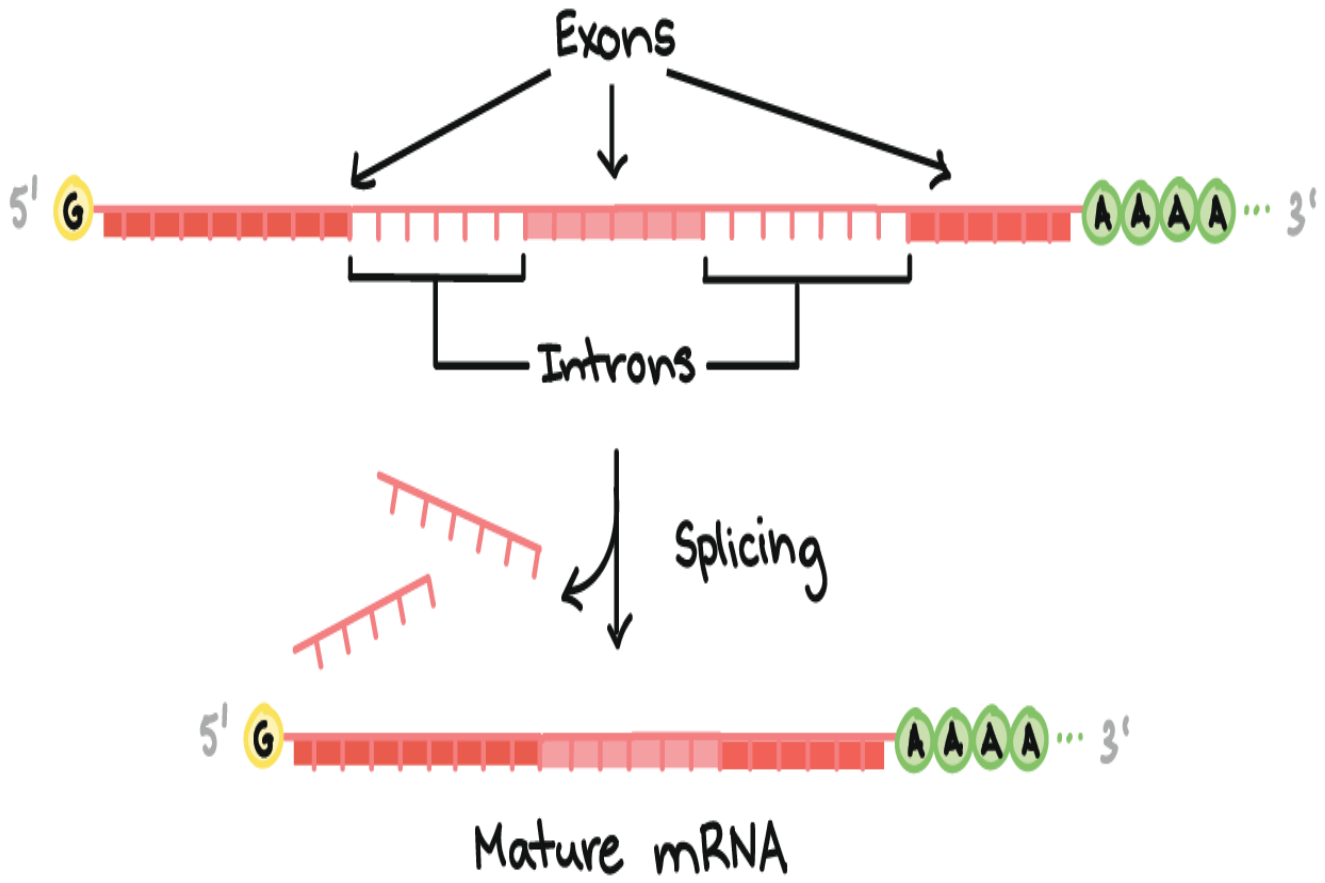


- In bacteria, RNA transcripts are ready to act as messenger RNAs and get translated into proteins right away. In eukaryotes, things are a little more complex, though in an pretty interesting way. The molecule that's directly made by transcription in one of your (eukaryotic) cells is called a **pre-mRNA**, reflecting that it needs to go through a few more steps to become an actual messenger RNA (mRNA). These are:
- Addition of a **5' cap** to the beginning of the RNA
- Addition of a **poly-A tail** (tail of A nucleotides) to the end of the RNA
- Chopping out of **introns**, or "junk" sequences, and pasting together of the remaining, good sequences (**exons**)
- Once it's completed these steps, the RNA is a mature mRNA. It can travel out of the nucleus and be used to make a protein.

5' cap and poly-A tail

- Both ends of a pre-mRNA are modified by the addition of chemical groups. The group at the beginning (5' end) is called a cap, while the group at the end (3' end) is called a tail. Both the cap and the tail protect the transcript and help it get exported from the nucleus and translated on the ribosomes (protein-making "machines") found in the cytosol^11start superscript, 1, end superscript.
- The **5’ cap** is added to the first nucleotide in the transcript during transcription. The cap is a modified guanine (G) nucleotide, and it protects the transcript from being broken down. It also helps the ribosome attach to the mRNA and start reading it to make a protein.
- Image of a pre-mRNA with a 5' cap and 3' poly-A tail. The 5' cap is on the 5' end of the pre-mRNA and is a modified G nucleotide. The poly-A tail is on the 3' end of the pre-mRNA and consists of a long string of A nucleotides (only a few of which are shown).
- How is the poly-A tail added? The 3' end of the RNA forms in kind of a bizarre way. When a sequence called a **polyadenylation signal** shows up in an RNA molecule during transcription, an enzyme chops the RNA in two at that site. Another enzyme adds about 100100100 - 200200200 adenine (A) nucleotides to the cut end, forming a **poly-A tail**. The tail makes the transcript more stable and helps it get exported from the nucleus to the cytosol.

- **RNA splicing**
- The third big RNA processing event that happens in your cells is **RNA splicing**. In RNA splicing, specific parts of the pre-mRNA, called **introns** are recognized and removed by a protein-and-RNA complex called the **spliceosome**. Introns can be viewed as "junk" sequences that must be cut out so the "good parts version" of the RNA molecule can be assembled.
- What are the "good parts"? The pieces of the RNA that are not chopped out are called **exons**. The exons are pasted together by the spliceosome to make the final, mature mRNA that is shipped out of the nucleus.

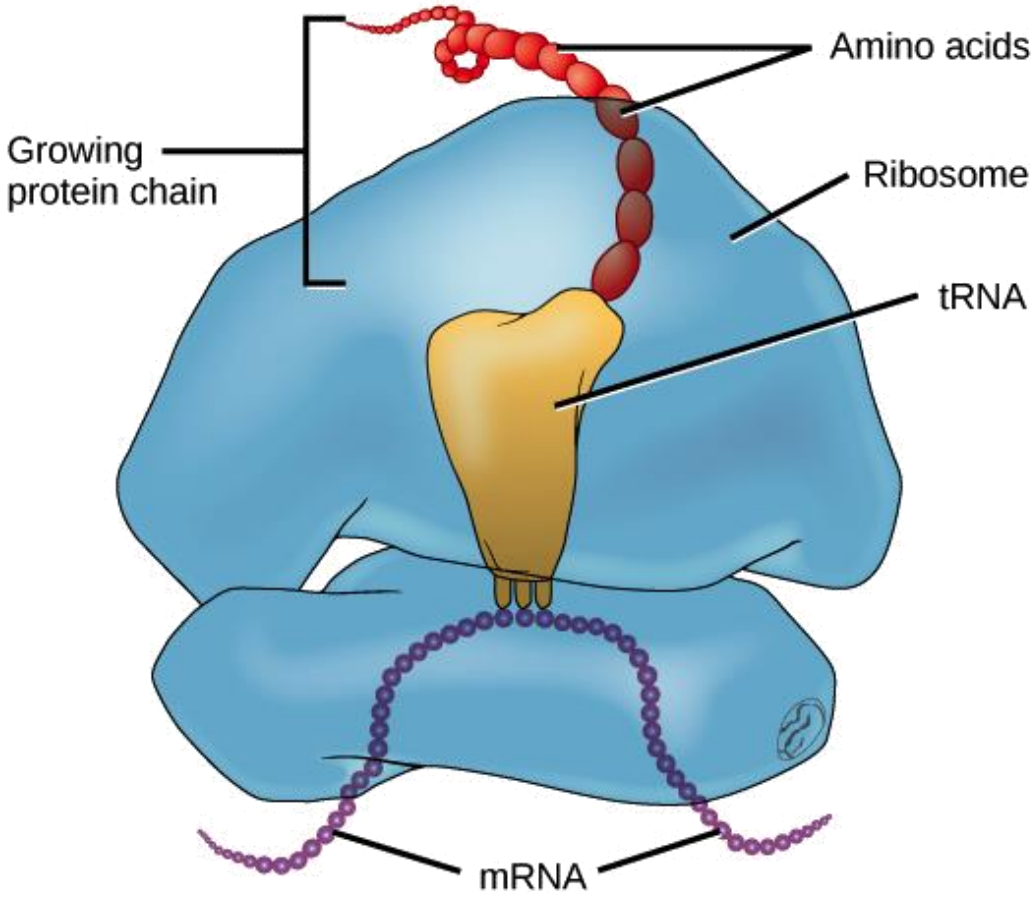

Exons
5'
G
A A A A
3'
Introns
Splicing
5'
G
A A A A
3'
Mature mRNA

- A key point here is that it's *only* the exons of a gene that encode a protein. Not only do the introns not carry information to build a protein, they actually *have* to be removed in order for the mRNA to encode a protein with the right sequence. If the spliceosome fails to remove an intron, an mRNA with extra "junk" in it will be made, and a wrong protein will get produced during translation.

tRNAs and ribosomes

- **Introduction**
- Translation requires some specialized equipment. Just as you wouldn't go to play tennis without your racket and ball, so a cell couldn't translate an mRNA into a protein without two pieces of molecular gear: ribosomes and tRNAs.
- **Ribosomes** provide a structure in which translation can take place. They also catalyze the reaction that links amino acids to make a new protein.
- **tRNAs** (**transfer RNAs**) carry amino acids to the ribosome. They act as "bridges," matching a codon in an mRNA with the amino acid it codes for.

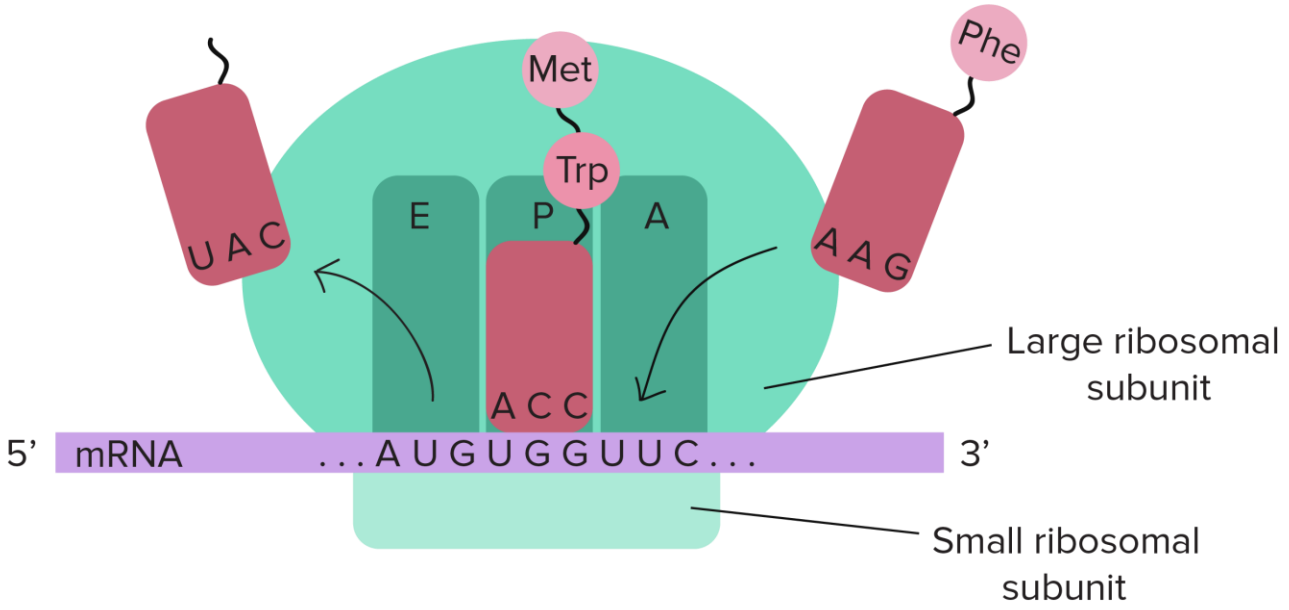
Ribosomes: Where the translation happens



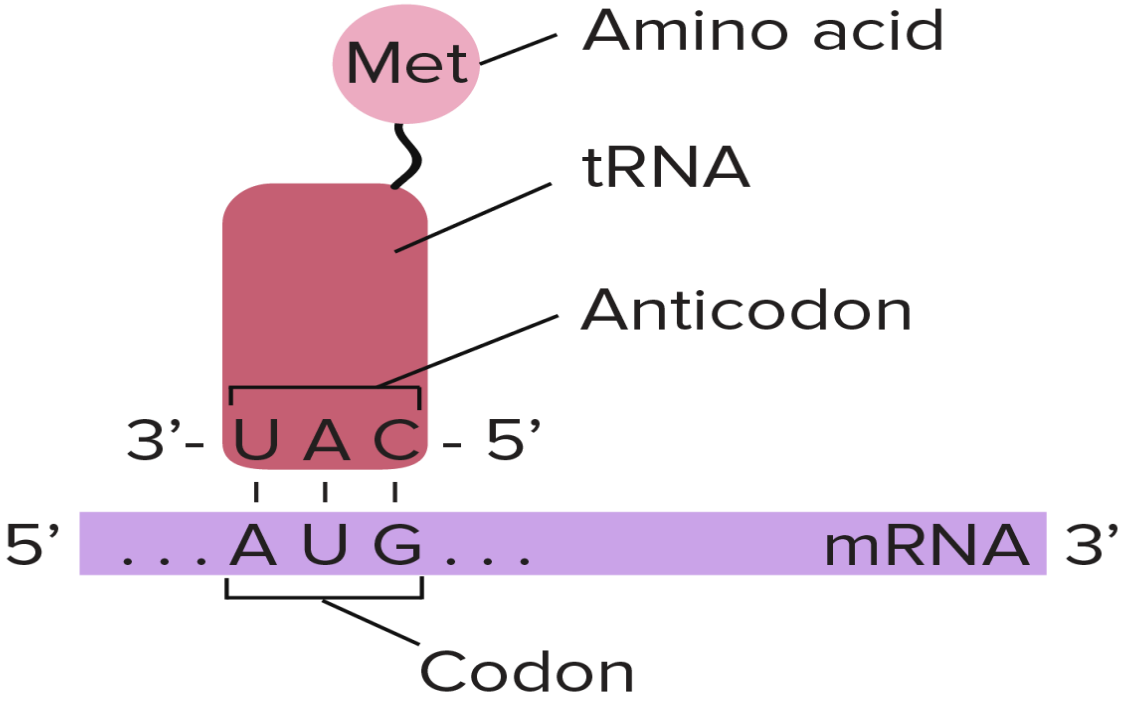
- Translation takes place inside structures called **ribosomes**, which are made of RNA and protein. Ribosomes organize translation and catalyze the reaction that joins amino acids to make a protein chain.

- **Structure of the ribosome**
- A ribosome is made up of two basic pieces: a large and a small subunit. During translation, the two subunits come together around a mRNA molecule, forming a complete ribosome. The ribosome moves forward on the mRNA, codon by codon, as it is read and translated into a polypeptide (protein chain). Then, once translation is finished, the two pieces come apart again and can be reused.
- Overall, the ribosome is about one-third protein and two-thirds **ribosomal RNA (rRNA)**. The rRNAs seem to be responsible for most of the structure and function of the ribosome, while the proteins help the rRNAs change shape as they catalyze chemical reactions^11start superscript, 1, end superscript.

The ribosome has slots for tRNAs



What exactly is a tRNA?



Some tRNAs bind to multiple codons ("wobble")

- Some tRNAs can form base pairs with more than one codon. At first, this seems pretty weird: doesn't A base-pair with U, and G with C?
- Well...not always. (Biology is full of surprises, isn't it?) Atypical base pairs—between nucleotides other than A-U and G-C—can form at the third position of the codon, a phenomenon known as **wobble**.
- Wobble pairing doesn't follow normal rules, but it does have its own rules. For instance, a G in the anticodon can pair with a C or U (but not an A or G) in the third position of the codon, as shown below^44start superscript, 4, end superscript. Rules like this ensure codons are read correctly despite wobble.
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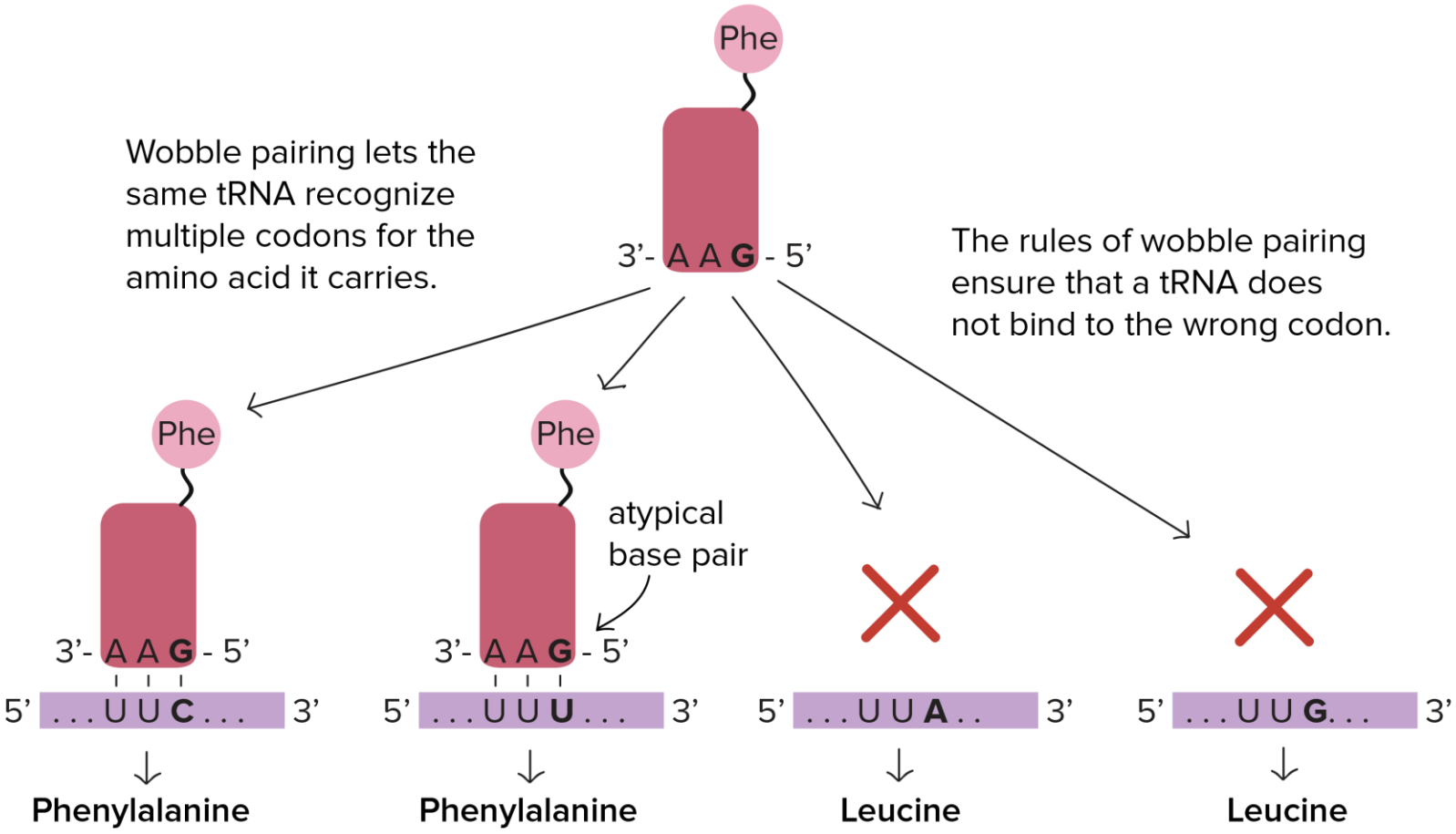
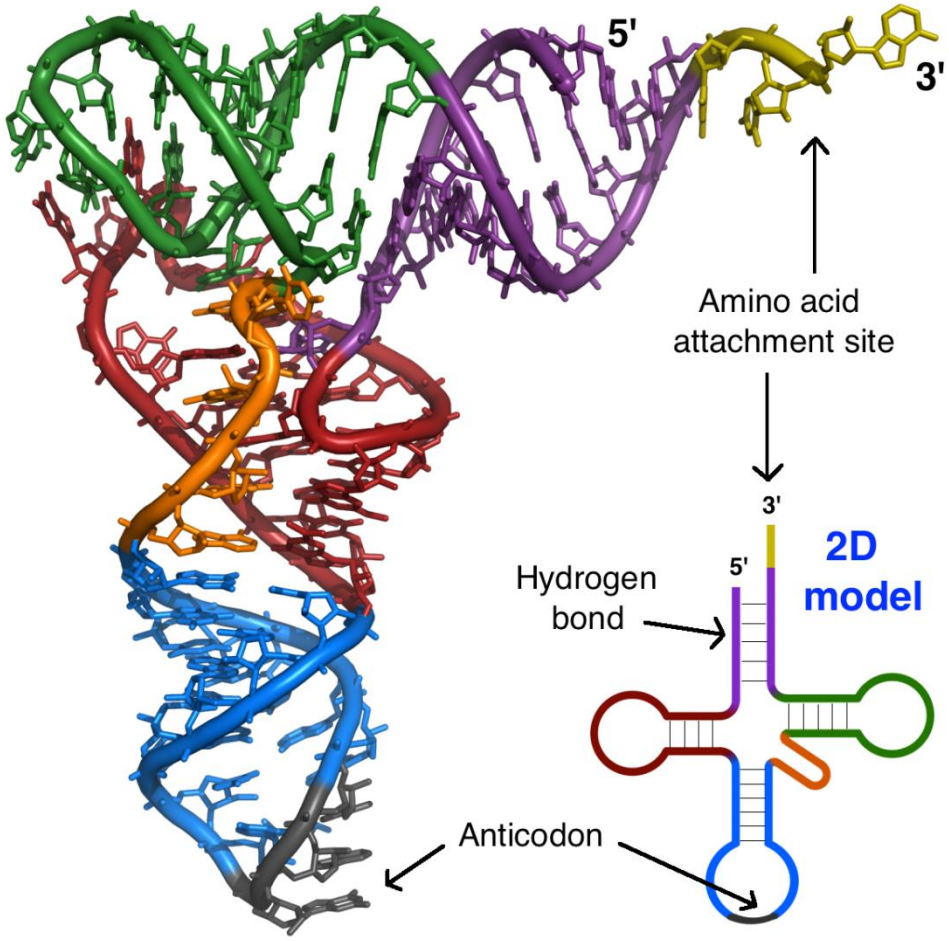
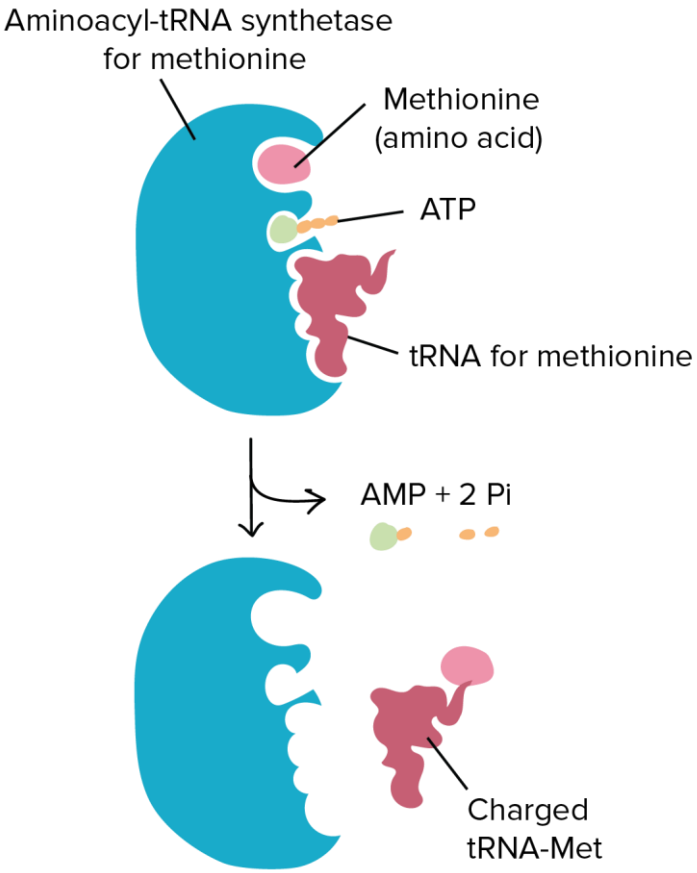

Phe
3'- A A G - 5'
Wobble pairing lets the same tRNA recognize multiple codons for the amino acid it carries.
The rules of wobble pairing ensure that a tRNA does not bind to the wrong codon.
Phe
3'- A A G - 5'
5' . . . U U C . . . 3'
Phenylalanine
Phe
atypical base pair
3'- A A G - 5'
5' . . . U U U . . . 3'
Phenylalanine
5' . . . U U A . . 3'
Leucine
5' . . . U U G. . . 3'
Leucine

The 3D structure of a tRNA



Loading a tRNA with an amino acid

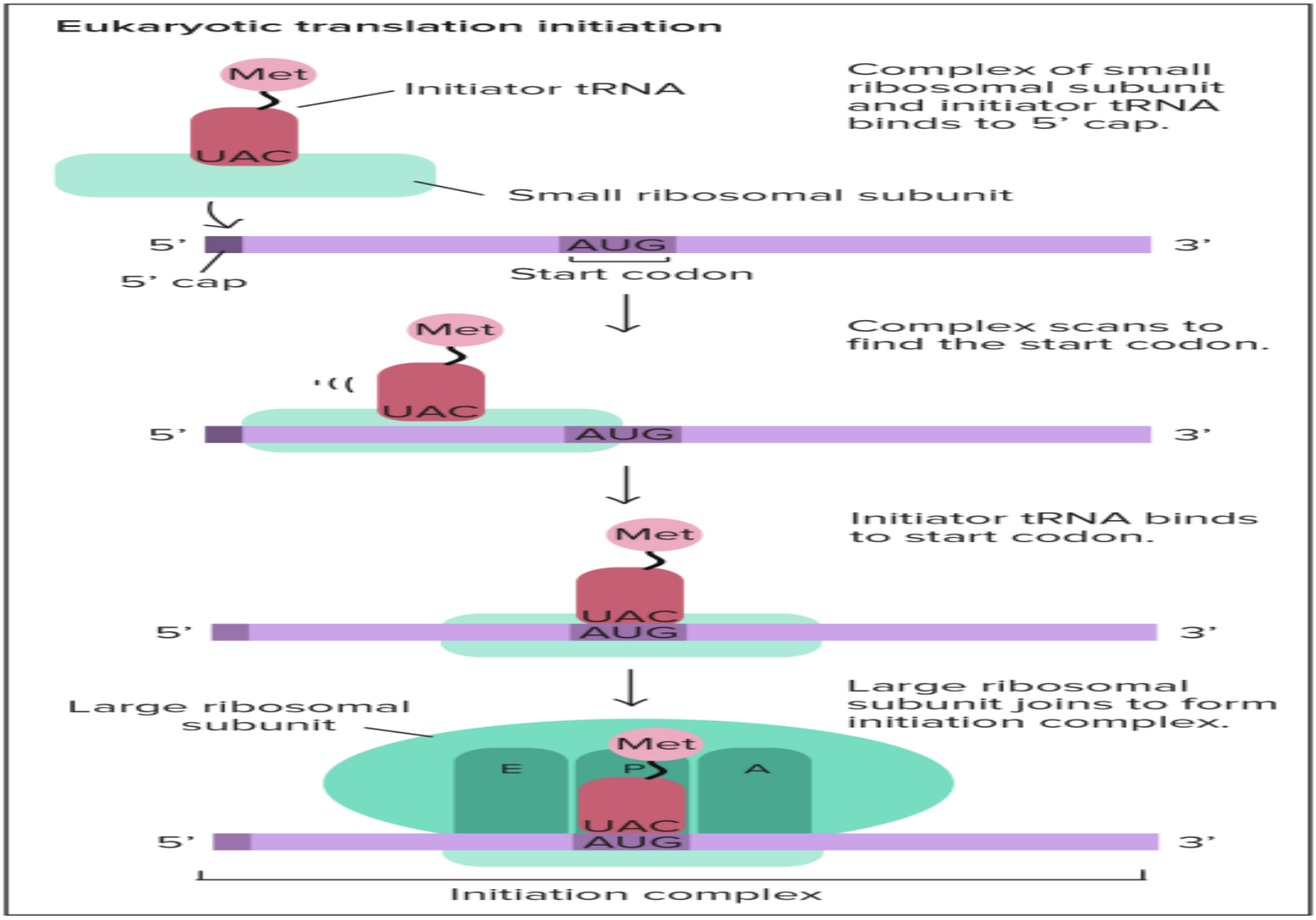
- How does the right amino acid get linked to the right tRNA (making sure that codons are read correctly)? Enzymes called **aminoacyl-tRNA synthetases** have this very important job.
- There's a different synthetase enzyme for each amino acid, one that recognizes only that amino acid and its tRNAs (and no others). Once both the amino acid and its tRNA have attached to the enzyme, the enzyme links them together, in a reaction fueled by the "energy currency" molecule adenosine triphosphate (ATP).


Aminoacyl-tRNA synthetase
for methionine
Methionine
(amino acid)
ATP
tRNA for methionine
AMP + 2 Pi
Charged
tRNA-Met

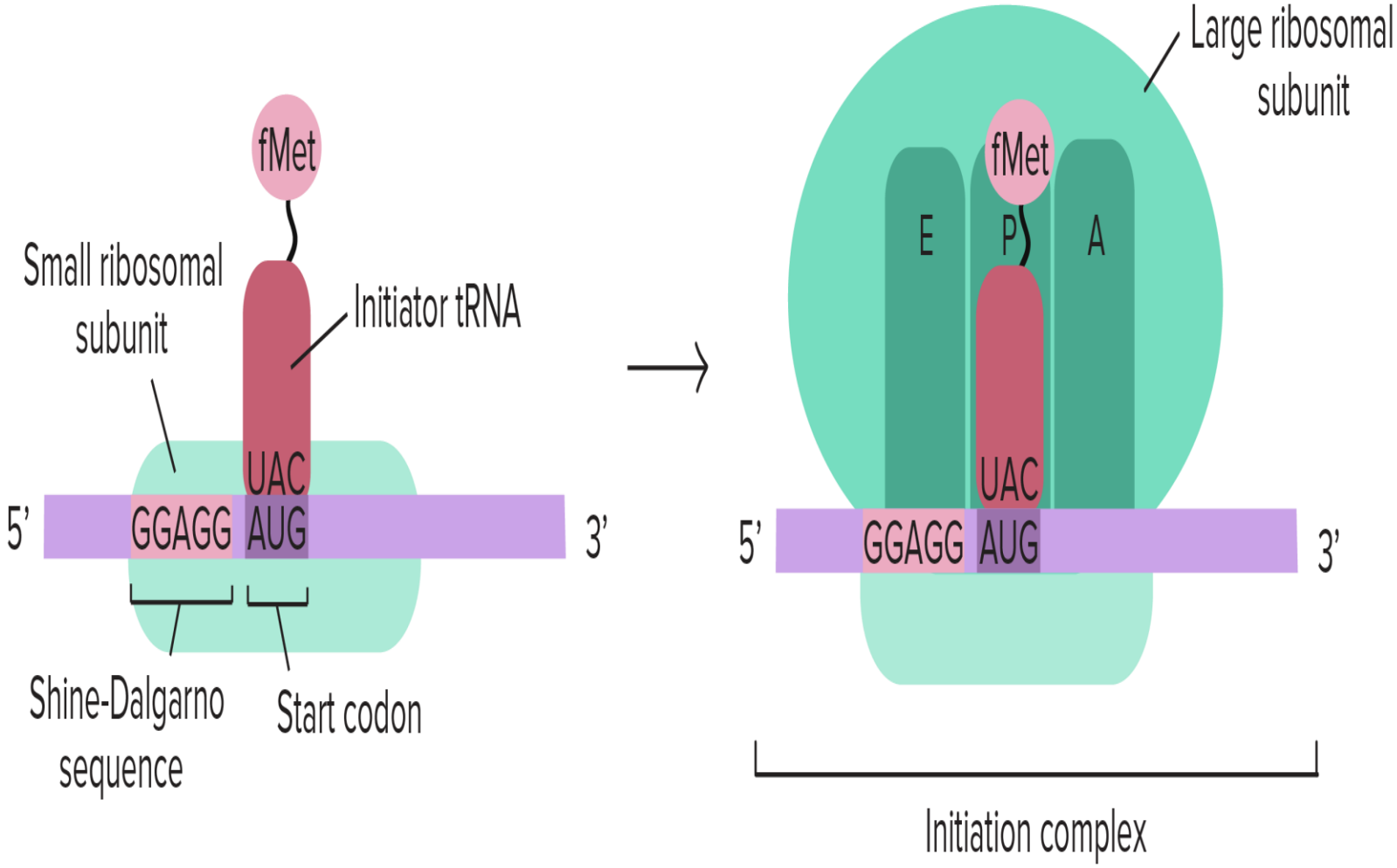
Translation: The big picture

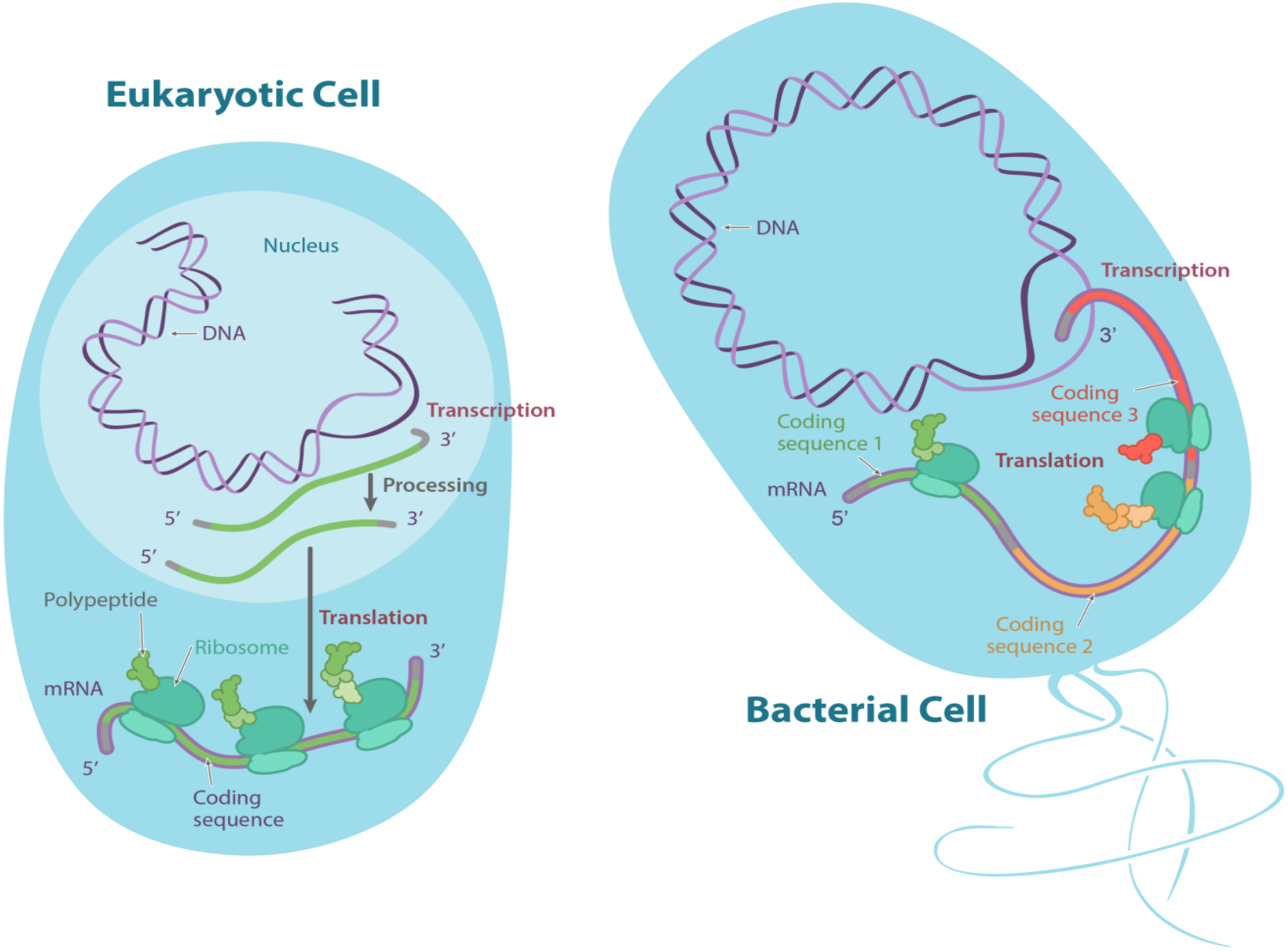
- **Translation** involves “decoding” a messenger RNA (mRNA) and using its information to build a **polypeptide**, or chain of amino acids. For most purposes, a polypeptide is basically just a protein (with the technical difference being that some large proteins are made up of several polypeptide chains).
- **The genetic code**
- In an mRNA, the instructions for building a polypeptide come in groups of three nucleotides called **codons**. Here are some key features of codons to keep in mind as we move forward:
- There are 616161 different codons for amino acids
- Three “stop” codons mark the polypeptide as finished
- One codon, AUG, is a “start” signal to kick off translation (it also specifies the amino acid methionine)

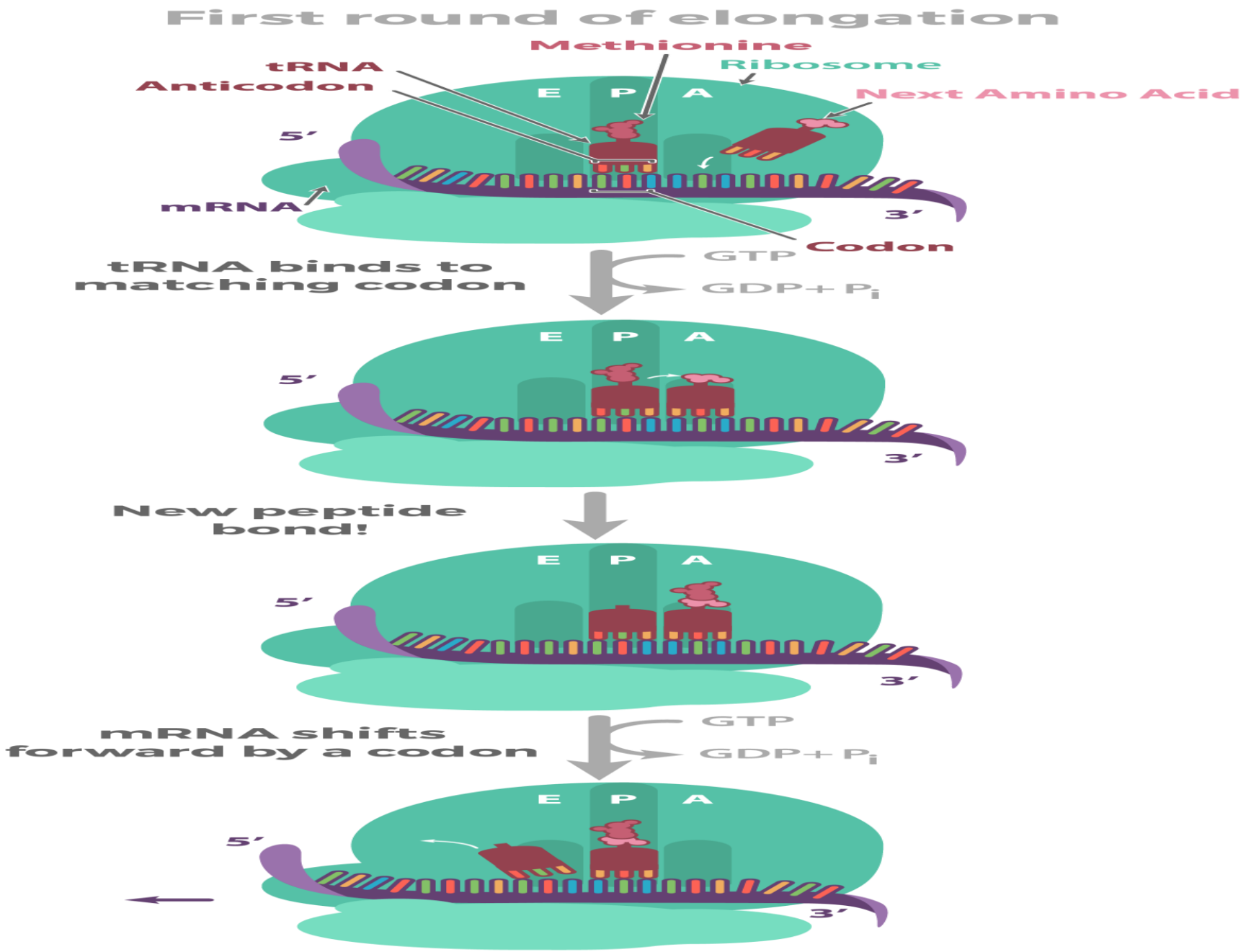
- **Translation: Beginning, middle, and end**
- A book or movie has three basic parts: a beginning, middle, and end. Translation has pretty much the same three parts, but they have fancier names: initiation, elongation, and termination.
- **Initiation** ("beginning"): in this stage, the ribosome gets together with the mRNA and the first tRNA so translation can begin.
- **Elongation** ("middle"): in this stage, amino acids are brought to the ribosome by tRNAs and linked together to form a chain.
- **Termination** ("end"): in the last stage, the finished polypeptide is released to go and do its job in the cell.


Eukaryotic translation initiation
Met
Initiator tRNA
UAC
Complex of small ribosomal subunit and initiator tRNA binds to 5' cap.
Small ribosomal subunit
5'
AUG
3'
5' cap
Start codon
Met
UAC
Complex scans to find the start codon.
5'
AUG
3'
Met
Initiator tRNA binds to start codon.
UAC
AUG
5'
3'
Large ribosomal subunit
Large ribosomal subunit joins to form initiation complex.
Met
E
P
A
UAC
AUG
5'
3'
Initiation complex

Bacterial translation initiation




Eukaryotic Cell
Nucleus
DNA
Transcription
3′
Processing
5′
3′
5′
Polypeptide
Translation
Ribosome
3′
mRNA
5′
Coding sequence
DNA
Transcription
3′
Coding sequence 3
Coding sequence 1
Translation
mRNA
5′
Coding sequence 2
Bacterial Cell


First round of elongation
Methionine
tRNA
Anticodon
Ribosome
Next Amino Acid
E
P
A
5′
mRNA
3′
Codon
tRNA binds to
matching codon
GTP
GDP+ Pi
E
P
A
5′
3′
New peptide
bond!
E
P
A
5′
3′
mRNA shifts
forward by a codon
GTP
GDP+ Pi
E
P
A
5′
3′

Termination

- Polypeptides, like all good things, must eventually come to an end. Translation ends in a process called termination. Termination happens when a stop codon in the mRNA (UAA, UAG, or UGA) enters the A site.
- Stop codons are recognized by proteins called **release factors**, which fit neatly into the P site (though they aren't tRNAs). Release factors mess with the enzyme that normally forms peptide bonds: they make it add a water molecule to the last amino acid of the chain. This reaction separates the chain from the tRNA, and the newly made protein is released.
- What next? Luckily, translation "equipment" is very reusable. After the small and large ribosomal subunits separate from the mRNA and from each other, each element can (and usually quickly does) take part in another round of translation.

Epilogue: Processing

- Our polypeptide now has all its amino acids—does that mean it's ready to do its job in the cell?
- Not necessarily. Polypeptides often need some "edits." During and after translation, amino acids may be chemically altered or removed. The new polypeptide will also fold into a distinct 3D structure, and may join with other polypeptides to make a multi-part protein.

- Thank you