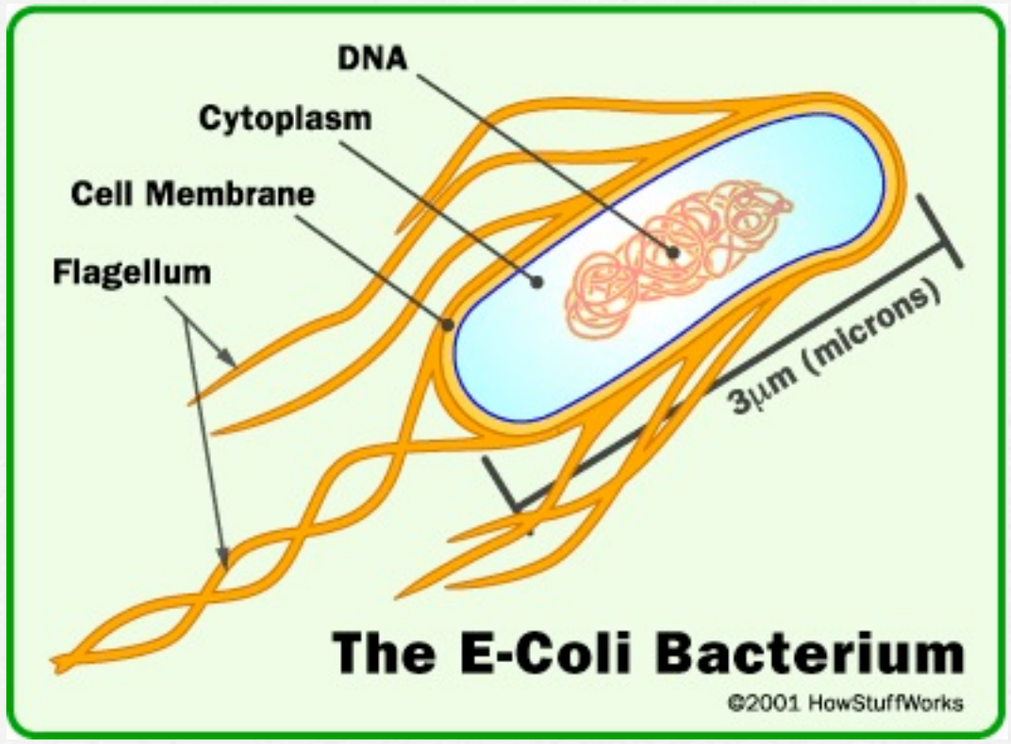
Synthetic Biology for

iGEM

Making Protein from DNA in E. coli

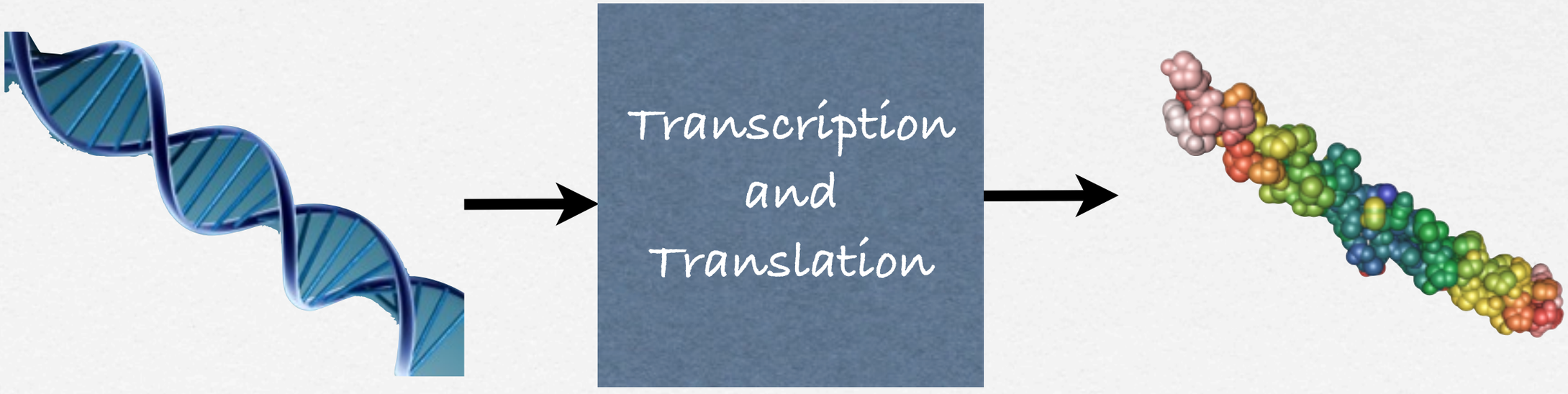
The Organism

- Main organism used in synthetic biology
  - Alternates include yeast
- Easy to work with
  - reproduces fast
  - Easily accepts and expresses new DNA



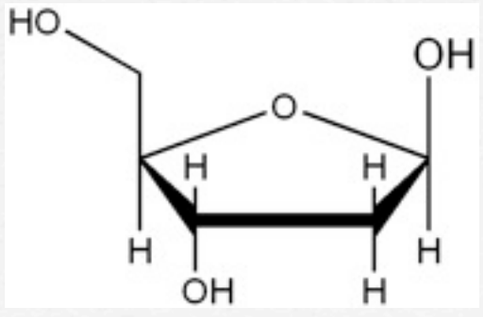
What does it mean to express DNA?

- DNA stores the information that encodes for proteins
- Proteins are the drivers of cells' response to their environment



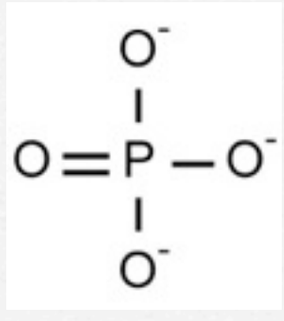
DNA: Base of Life

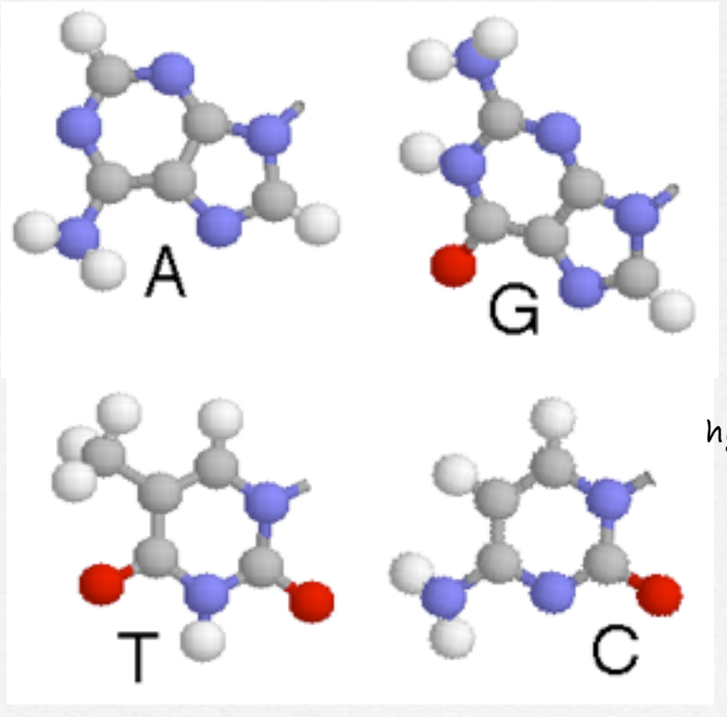
- Composed of three parts: sugar (deoxyribose), phosphate, and a base
- 4 different bases: Adenine, Guanine, Cytosine, and Thymine



Deoxyribose

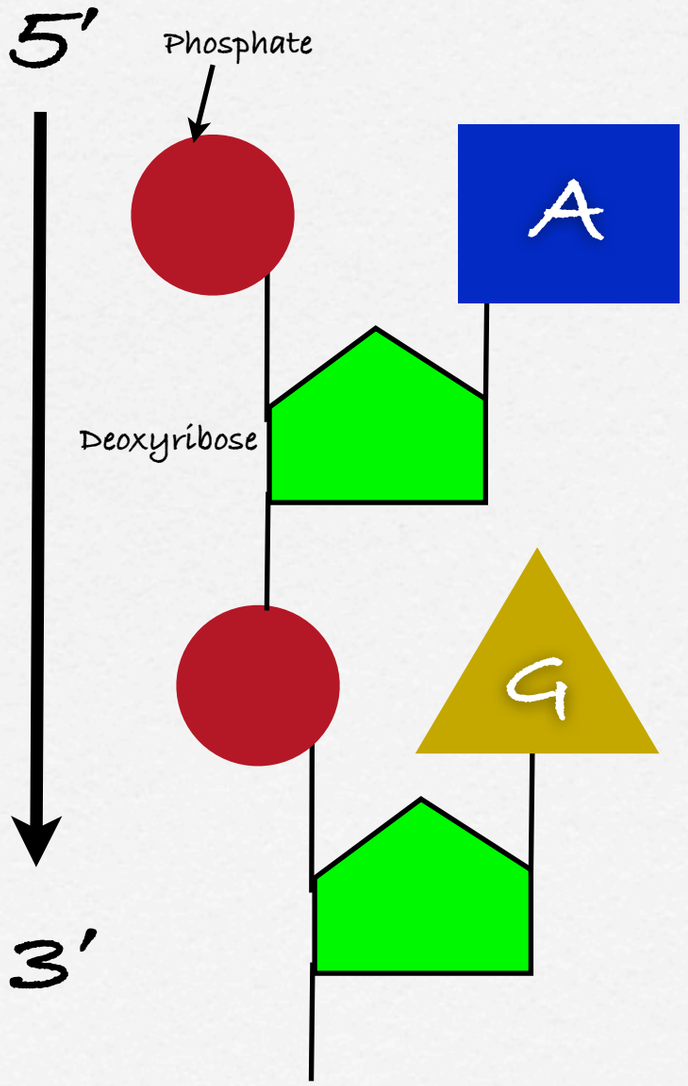
Phosphate





The four bases. Gray beads indicate carbon, white beads hydrogen, blue beans nitrogen, and red beads oxygen.

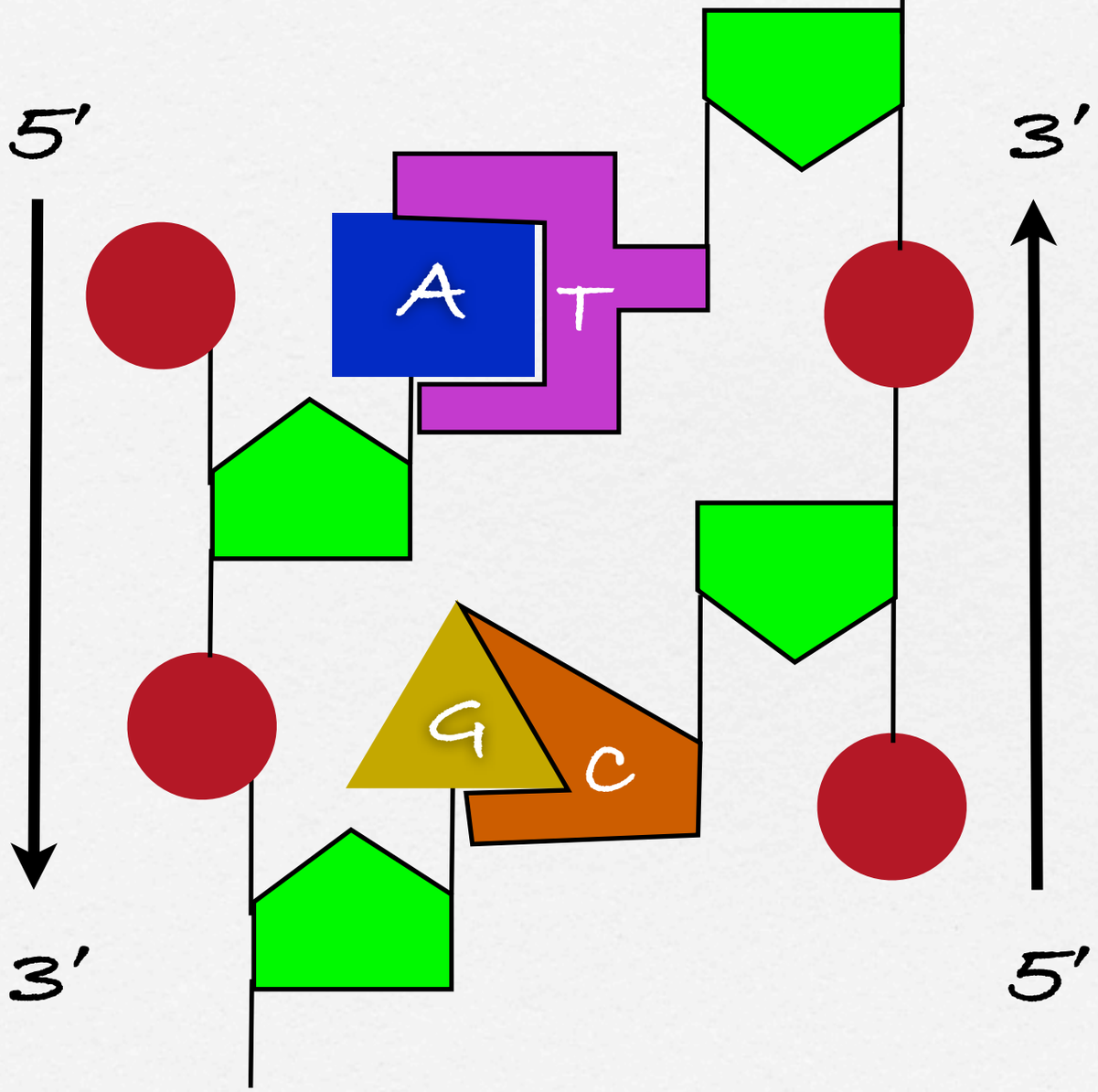
DNA Structure: Linear



- Sugar and phosphate form the backbone
- Together a sugar, a phosphate, and one of the four amine bases make a nucleotide
- A DNA sequence is a long, linear chain of nucleotides, read 5' to 3' (see diagram)

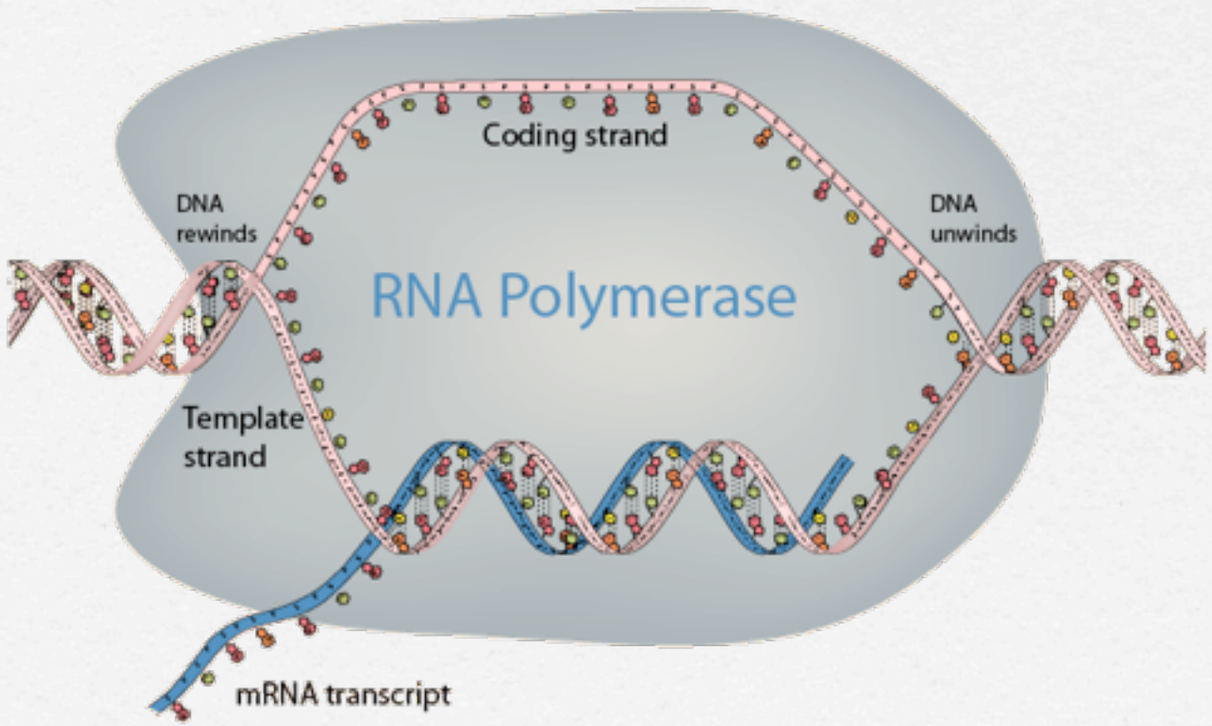
DNA Structure: The Double Helix

- Two DNA strands wind together to make a double helix
- The DNA strands run in <u>opposite</u> directions
- Bases pairs match to their complementary partners
  - A to T; G to C



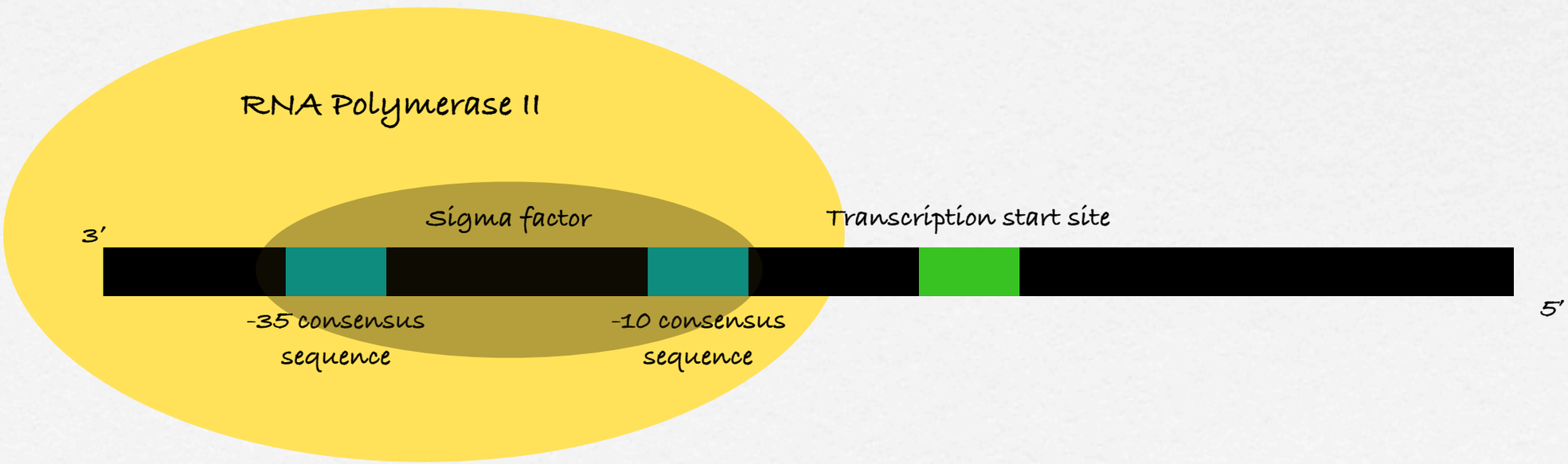
DNA to Protein: Transcription

- First step to make protein from DNA
- Overall: Converts DNA to RNA (usually mRNA)
- Three phases
  - Initiation
  - Elongation
  - Termination



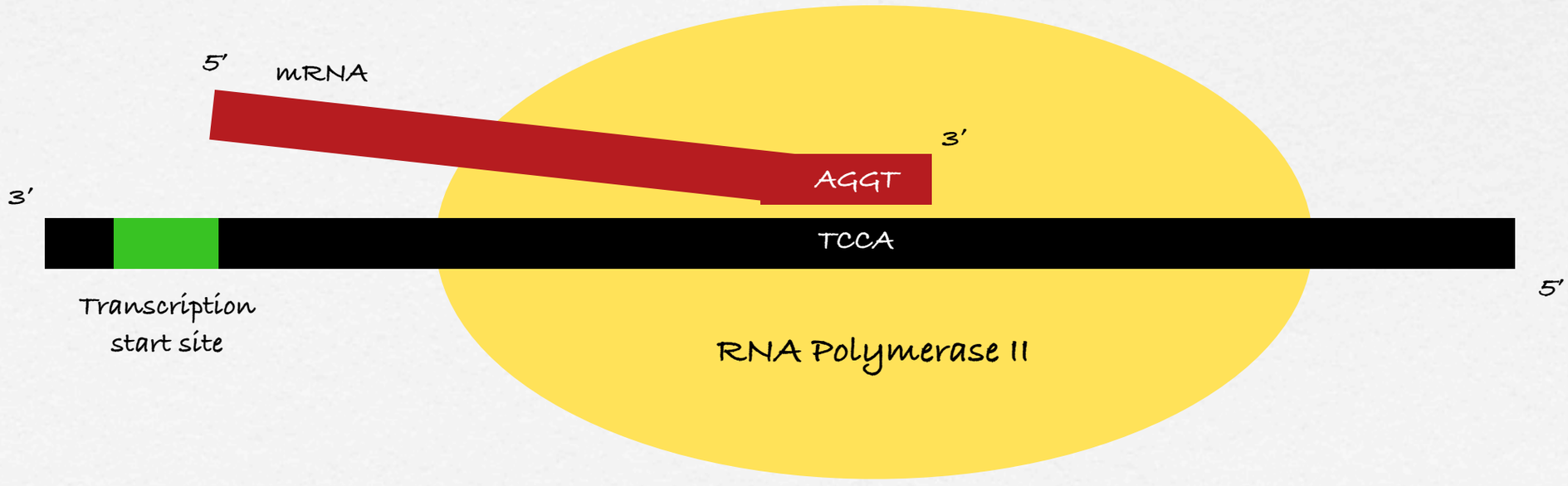
Transcription I: Initiation

- RNA polymerase II binds to promoter sequence of DNA
  - Sigma, a transcription factor, assists binding
- Promoter sequences are usually -10 and -35 bases upstream of transcription start site (called consensus sequences)



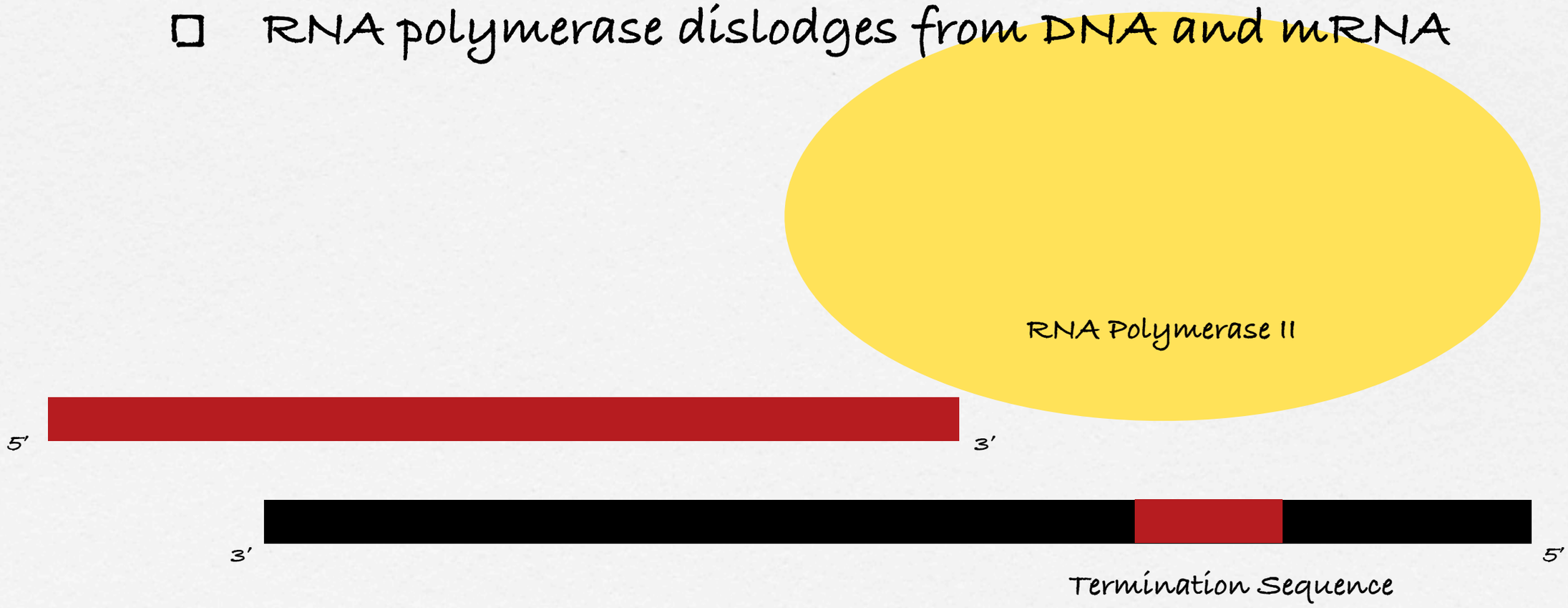
Transcription 2: Elongation

- RNA polymerase II moves along ssDNA, called the template strand
- RNA polymerase II uses complementary base pair matching to synthesize new strand of mRNA



Transcription 3: Termination

- Transcription ends when termination sequence is reached
- RNA polymerase dislodges from DNA and mRNA

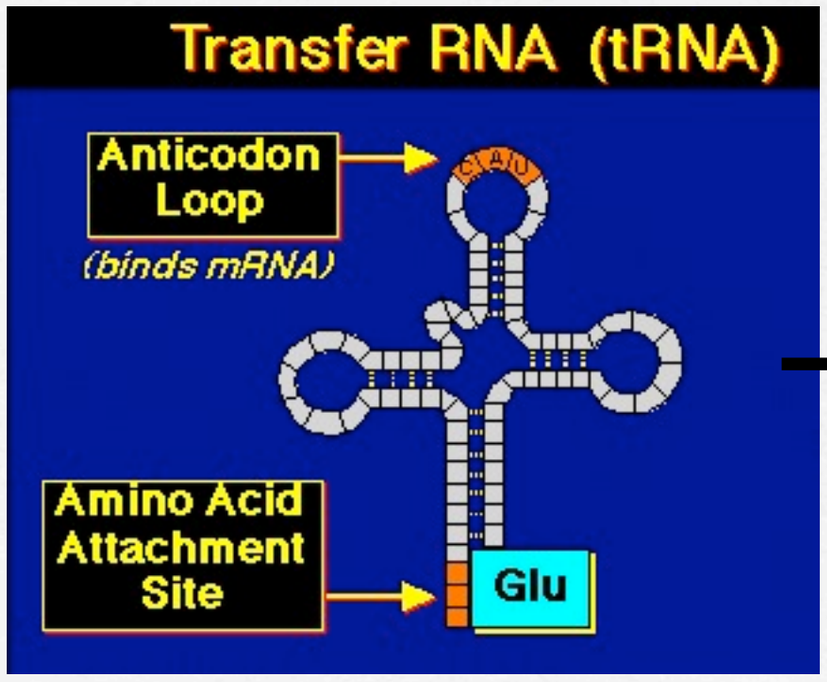


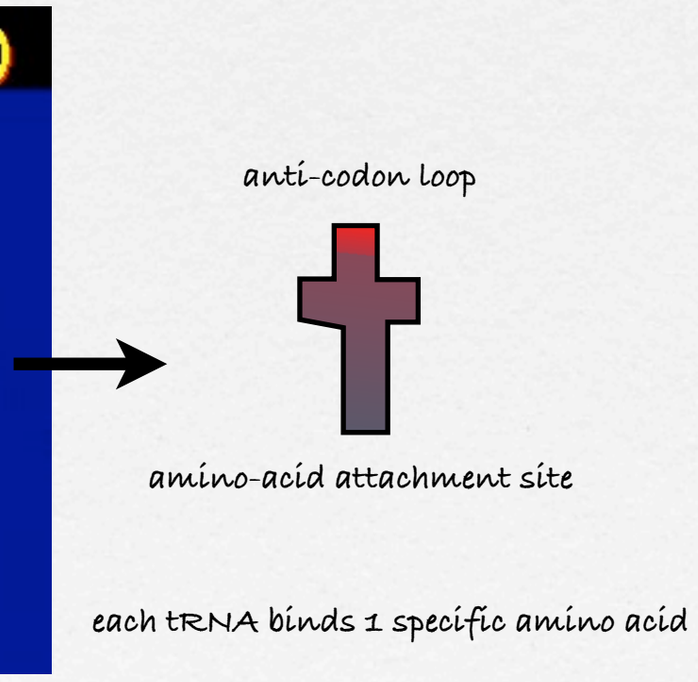
The mechanism of termination is a bit more complex, and may involve a protein called rho factor or bending of the RNA molecule, but for the purposes of iGEM, just know that when RNA polymerase transcribes the termination region, transcription is stopped.

Translation: mRNA to Protein

- The next stage in making protein is translation
- Translation involves conversion of mRNA transcripts to proteins
- This is done using Ribosomes and tRNA

Ribosome

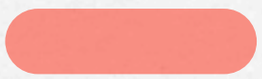




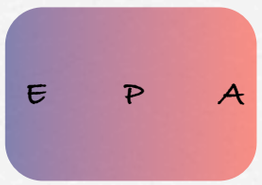
each tRNA binds 1 specific amino acid

The Ribosome

- The ribosome is made of rRNA (ribosomal RNA)
- Bacterial ribosomes are composed of two different parts, called the 30s subunit (the little one) and the 50s subunit (the big one)



30s subunit



50s subunit

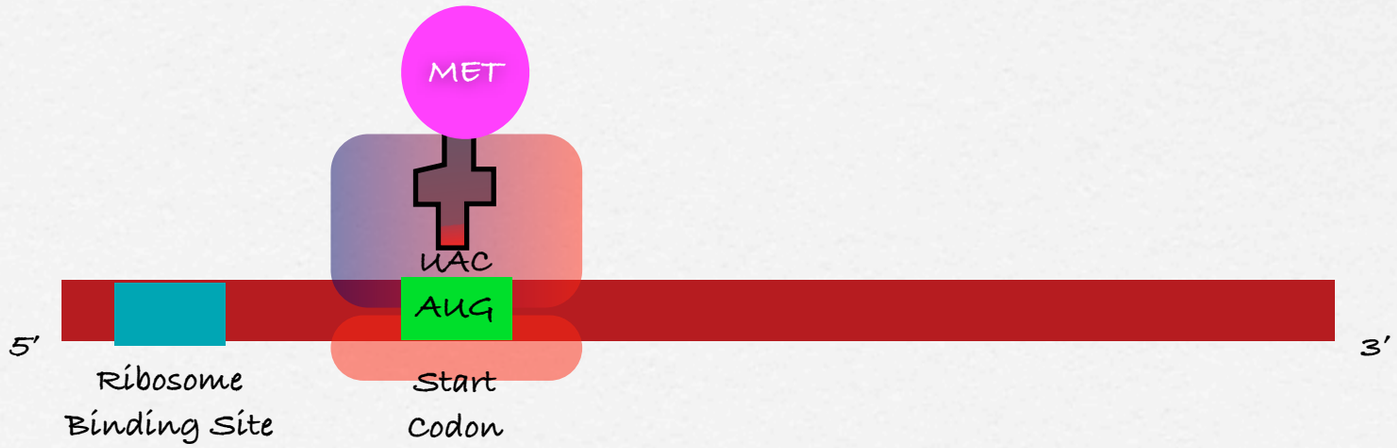
The 50s subunit has three important sites. They are termed the A site, the P site, and the E site. For the purposes of our diagram, the A site is on the right (in red), the P site is in the middle, and the E site is on the left (blue)

Translation I: Initiation (Binding)

- Ribosome binds to specific site on mRNA called Ribosome Binding Site
  - 30s subunit binds first, and the 50s binds second

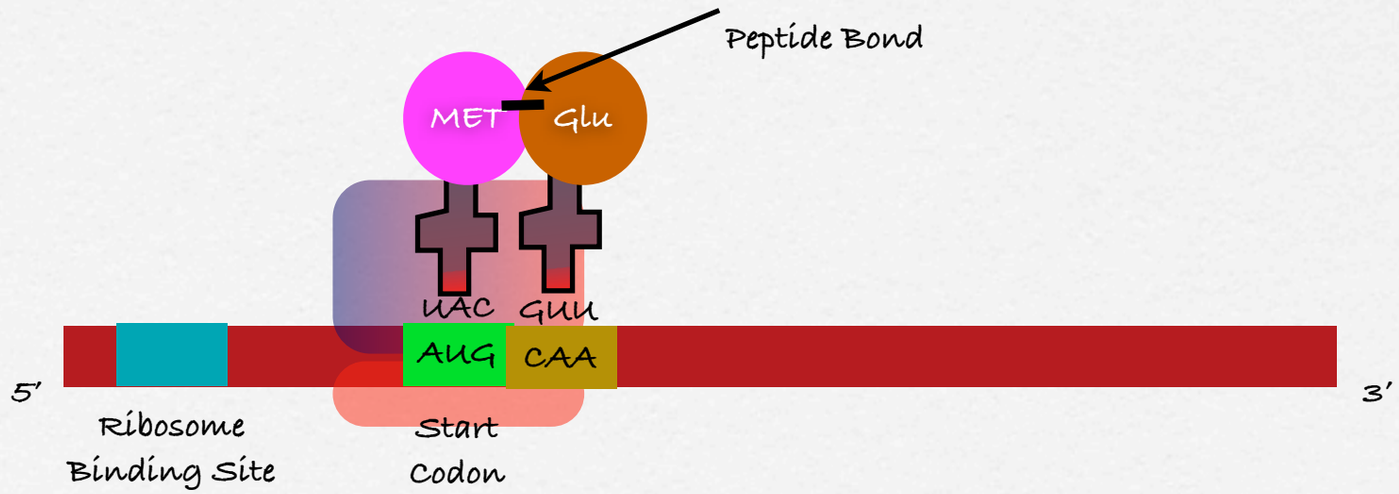
Translation I: Initiation (Start Codon)

- The ribosome moves along the mRNA 5' to 3' until it reaches the start codon
- The start codon is a sequence of three nucleotides, AUG
  - U is uracil, and takes the place of T in RNA
- When this occurs, a tRNA with the matching <u>anticodon</u> binds the E site of the ribosome



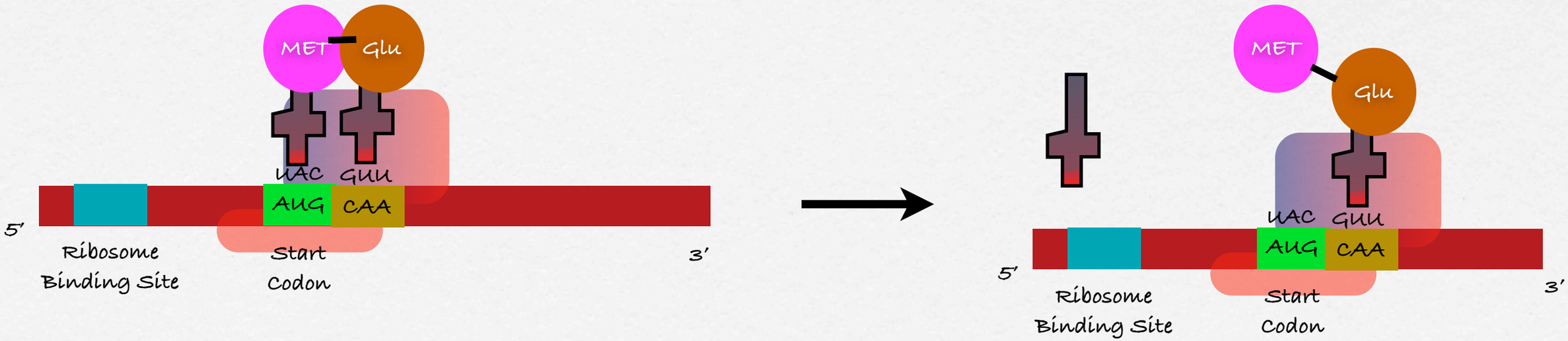
Translation II: Elongation (Peptide Bond)

- Next, another tRNA carrying an amino acid comes into the A site
- This tRNA has an anticodon that matches the codon of the mRNA
- The ribosome catalyzes a reaction, forming a peptide bond between the two amino acids



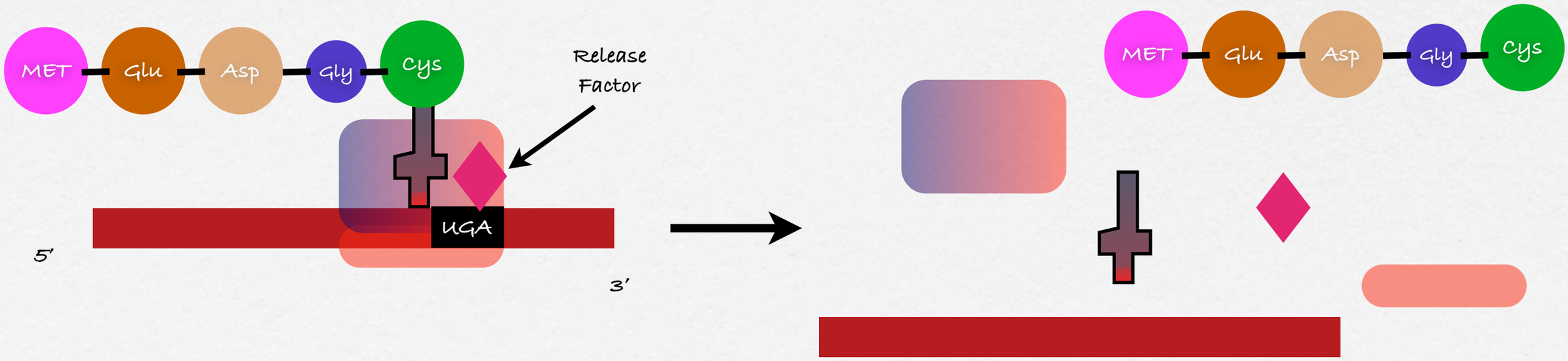
Translation II: Elongation (Ejection)

- The 50s subunit slides over 1 codon (three nucleotides), moving the tRNA in the P site to the E site
- This tRNA is then ejected, but the amino acid stays, bound to the peptide chain
- The 30s subunit slides over next, and elongation continues when the next tRNA binds the next codon in the A site



Translation III: Termination

- Translation is terminated when the ribosome reads the stop codon (UAA, UAG, or UGA)
- Instead of tRNA, a protein called a release factor binds
- When the release factor binds, the ribosome is dissociated and the mRNA and protein are released



Review

- DNA holds the information cells use to make proteins
- Transcription is the process of making an mRNA template from DNA
- Translation, using the ribosome, creates polypeptides (proteins) from mRNA transcripts by matching tRNA anticodons to mRNA codons
- tRNA is the real converter between DNA/RNA language (nucleotides) and protein language (amino acids)

Important Terminology

DNA; Deoxyribonucleic Acid: Double helical storer of genetic information

RNA; Ribonucleic Acid: All components are the same as DNA (sugar, phosphate, base) EXCEPT sugar is ribose, not deoxyribose and the base Thymine in DNA is replaced by Uracil in RNA; RNA is also not usually found in double helical structure, but is often single stranded and/or folded in unusual ways

mRNA: Messanger RNA. A type of RNA that codes for amino acids. mRNA templates are single stranded, and contain a ribosome binding site, start codon, and stop codon.

tRNA: Transfer RNA. Converter between mRNA and amino acids. Each tRNA has an anticodon that matches the a codon on mRNA. Each tRNA also binds a specific amino acid, which means the mRNA codon sequence can be directly translated to an amino acid sequence.

Protein: Main building blocks of biology; proteins are composed of strings of amino acids

Amino Acids: There are twenty different amino acids, which are the building blocks used to make the billions of proteins

Peptide: Strings of amino acids linked by peptide bonds; NOTE: while all proteins are polypeptides, not all peptides are proteins

Enzymes: Enzymes catalyze biological reactions (make them faster). NOTE: Enzymes are a type of protein. Not all proteins are enzymes. Most enzymes end their name in "-ase".

rRNA: ribosomal RNA. RNA that folds into the ribosome.