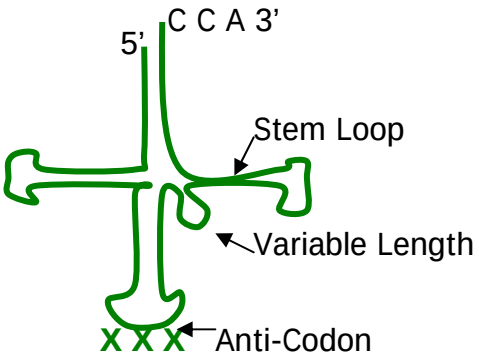
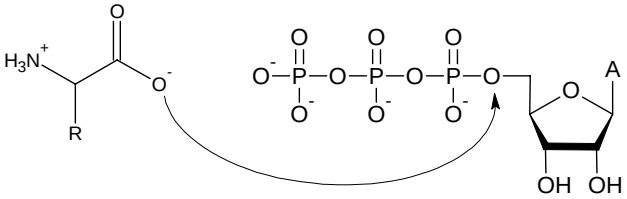
Translation in a Nutshell

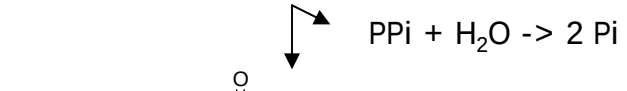
tRNA Charging

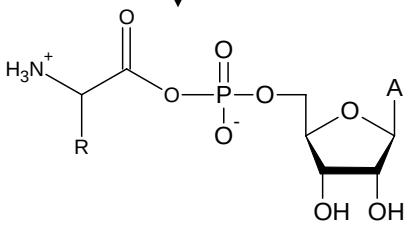


The tRNA shown in the cloverleaf form. In vivo tRNA takes on an "L" form. The major features of the tRNA are labeled for you.

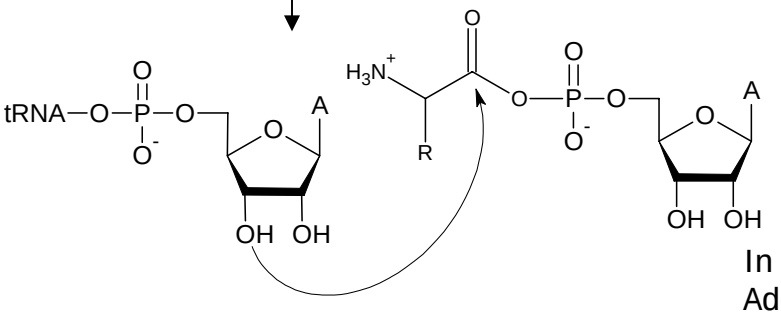


The tRNA is charged by tRNA Synthatase. Synthatases use ATP like in the first step when it activates the Amino Acid.

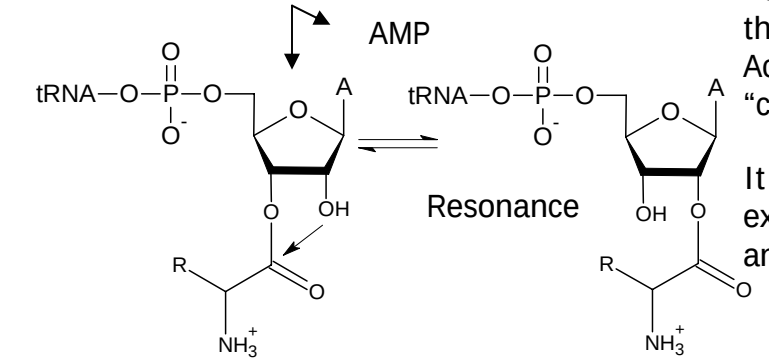




It is important to note that the tRNA Synthatase tests the Amino Acid so that is sure it is adding the correct one. This is done by specific binding pockets and often utilizes the "double sieve" mechanism discussed in class.



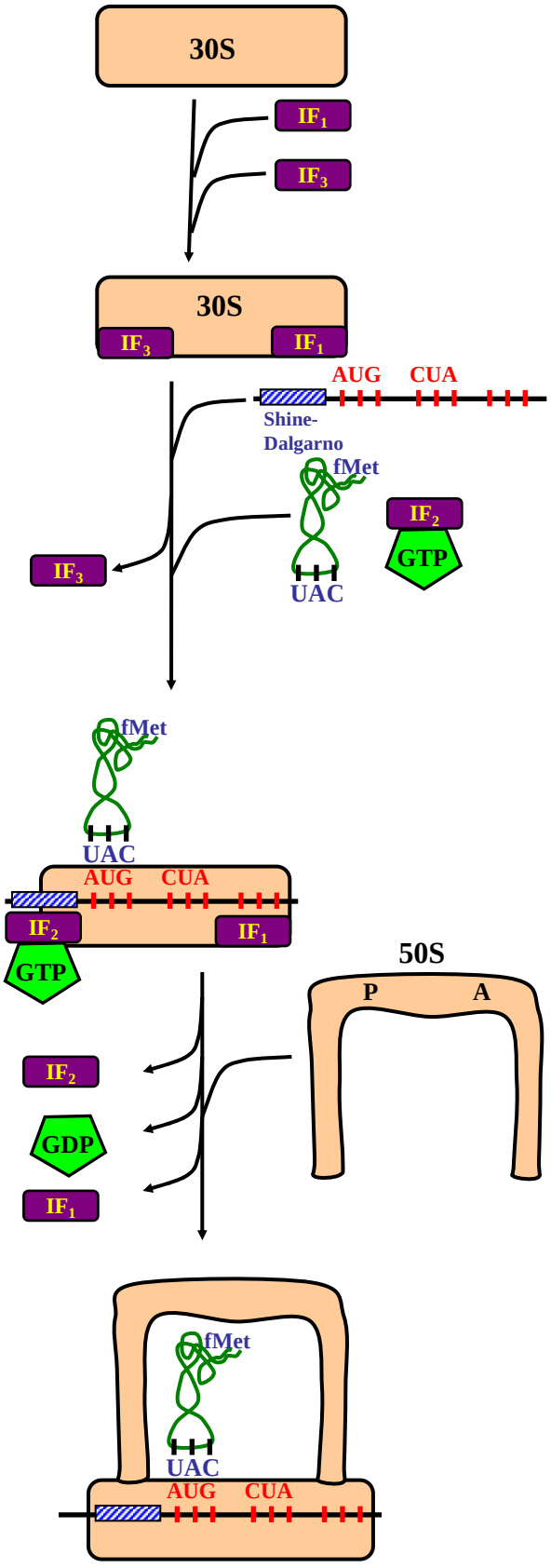
In the next step, the 3' OH of the last Adenine attacks the carboxyl group of the amino acid transferring the Amino Acid to the tRNA. This creates the "charged" tRNA while releasing an AMP.



It is noteworthy that this charged tRNA exhibits resonance between the 2' OH and the 3' OH.

Translation in a Nutshell

Initiation



First the 30S ribosomal subunit (*E. Coli*) is bound by the two initiation factors (IF_1, IF_3). The initiation factors prevent the formation of the 70S ribosome without the presence of mRNA.

Then the 30S complex binds to mRNA at the Shine-Dalgarno Sequence (the Translation "promoter") using GTP-IF_2 and releasing IF_3 along the way. The initiation formylmethionyl-tRNA binds to the AUG.

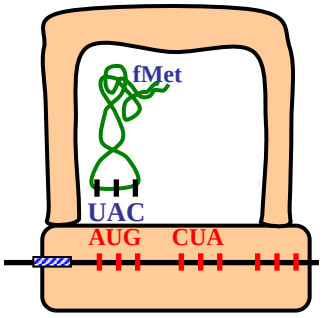
The 50S subunit now binds to the 30S subunit, releasing IF_2 and IF_1 while hydrolyzing the GTP to GDP. Key point: the hydrolysis of GTP to GDP alters the conformation of the protein it is bound to. In this case the IF_2 can no longer bind the 30S subunit and is shed.

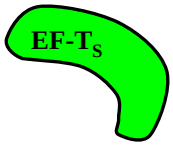
We now have the complete 70S ribosome ready to do its work. As an aside, you should remember you need a high Mg^{2+} concentration to balance the charges. Note that the site where the formylmethionyl-tRNA bound is labeled the "P" site while the next codon over is the "A" site. Think of the "P" site or peptidyl site as the "peptide site" and the "A" site or "amino acyl site" as the "active site."

Translation in a Nutshell

Aminoacyl-tRNA Incorporation (Elongation)

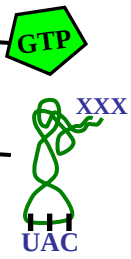
Why use EF-T_u? 1) It protects the Aminoacyl-tRNA from becoming discharged. 2) It also serves to "escort" the charged tRNA into the A site. 3) It gives time for the ribosome to proofread to make sure it chose the correct aminoacyl-tRNA.



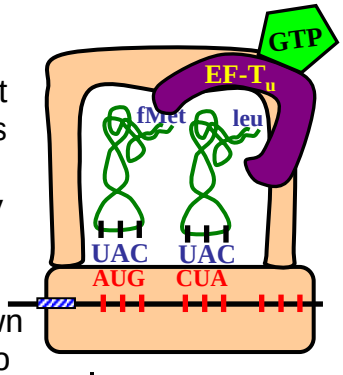


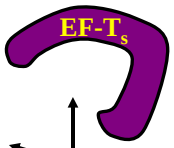
ET-T_s replenishes the GTP and tRNA molecules on EF-T_u

The next charged tRNA is ferried to the ribosome by EF-T_u bound to GTP.

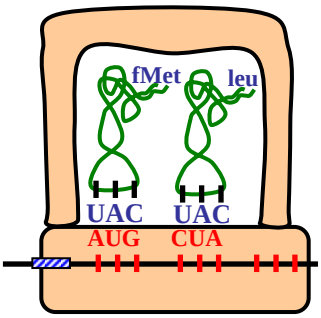


Keep in mind that all charged tRNAs are "sampled" to make sure if they are the correct one. Hence we need to slow down the mechanism to give time for proofreading.





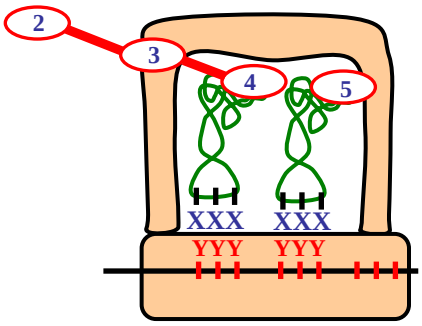
GDP is displaced from ER-T_u by EF-T_s



The codon pairing undergoes "kinetic proofreading" while GTP is hydrolyzed to GDP which alters the shape of the EF-T_u preventing binding. Thus, the GDP-bound EF-T_u is released from the ribosome.

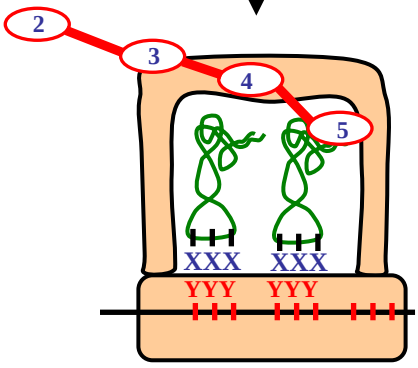
Translation in a Nutshell

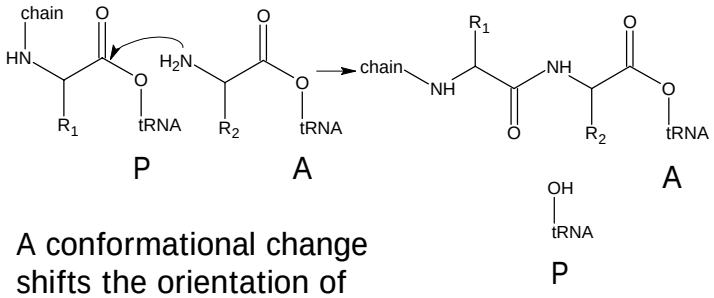
Peptidyl Transfer



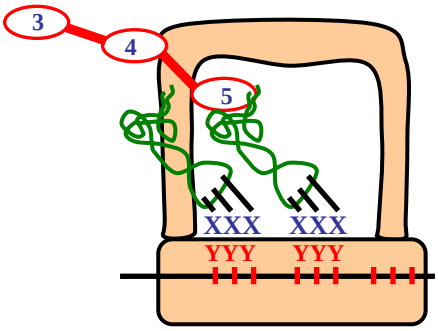
A peptide bond is formed between the amino acids in the P and A sites, resulting in the transfer of the polypeptide chain to the tRNA in the A site.

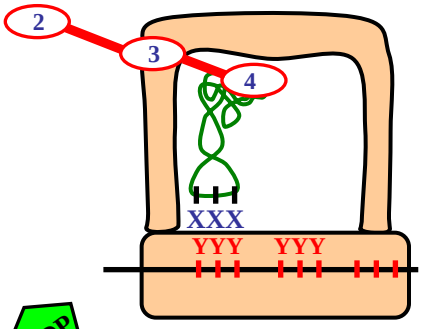
The next aminoacyl-tRNA is incorporated into the empty A site (see above) and the process is repeated until a STOP codon is encountered.





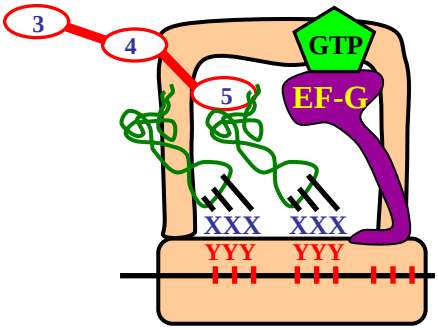
A conformational change shifts the orientation of the tRNAs over but the mRNA does not yet move relative to the ribosome. This is called the **Hybrid State.**

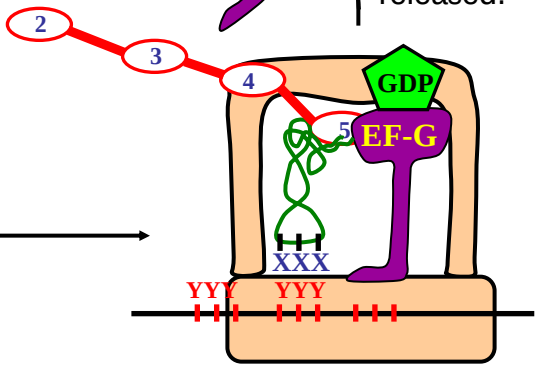




Translocation: GTP-bound EF-G binds to the A site and then uses the energy from hydrolysis of GTP to translocate the ribosome along the mRNA.

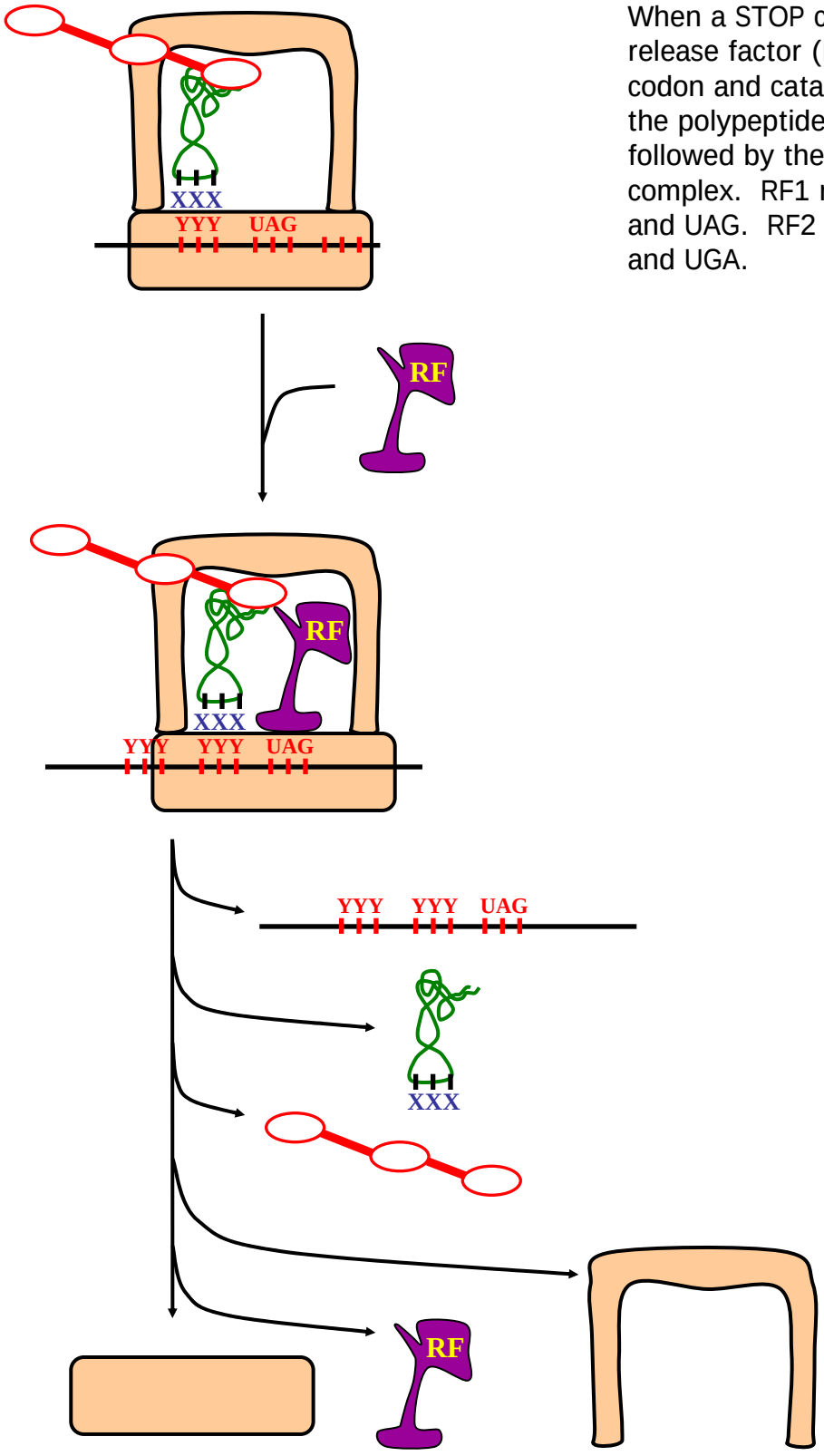
GDP bound EF-G is released.





Translation in a Nutshell

Termination



When a STOP codon is reached, a release factor (RF1 or RF2) binds the codon and catalyzes the hydrolysis of the polypeptide from the tRNA. This is followed by the dissociation of the entire complex. RF1 recognizes codons UAA and UAG. RF2 recognizes codons UAA and UGA.