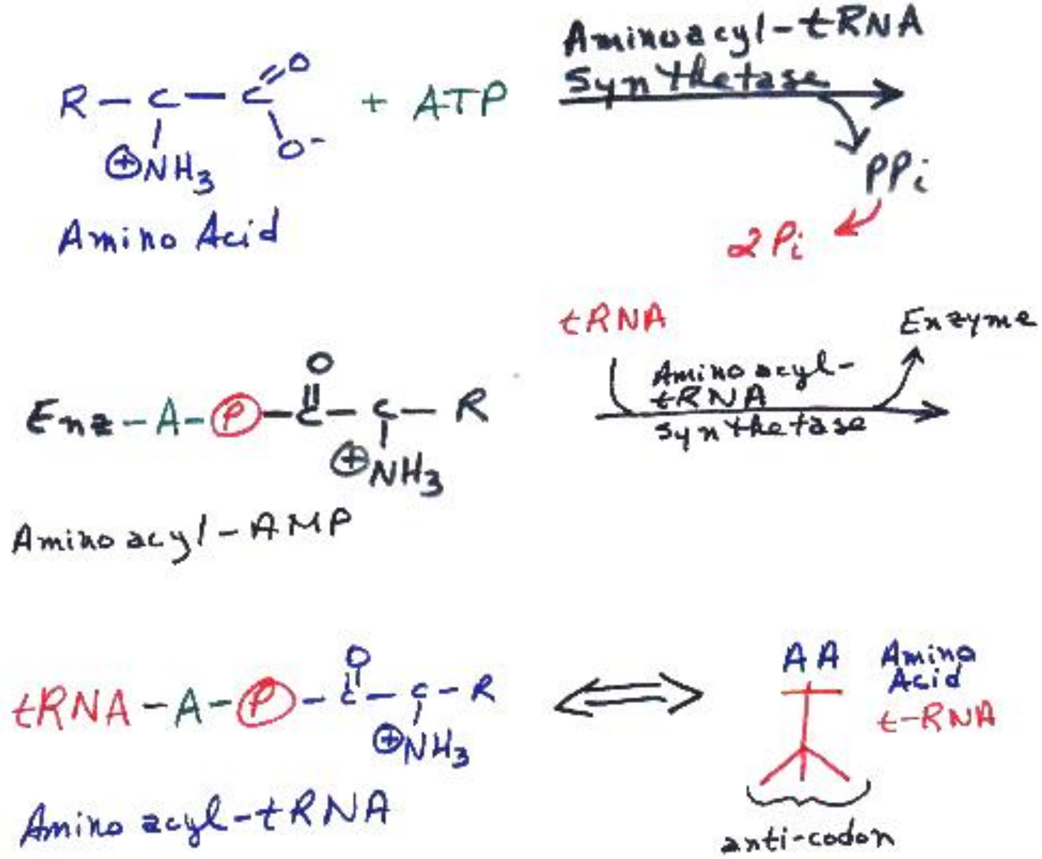
Translation: A Four-Step Process

How DNA, RNA, Enzymes and Ribosomes Work as An Intracellular Team

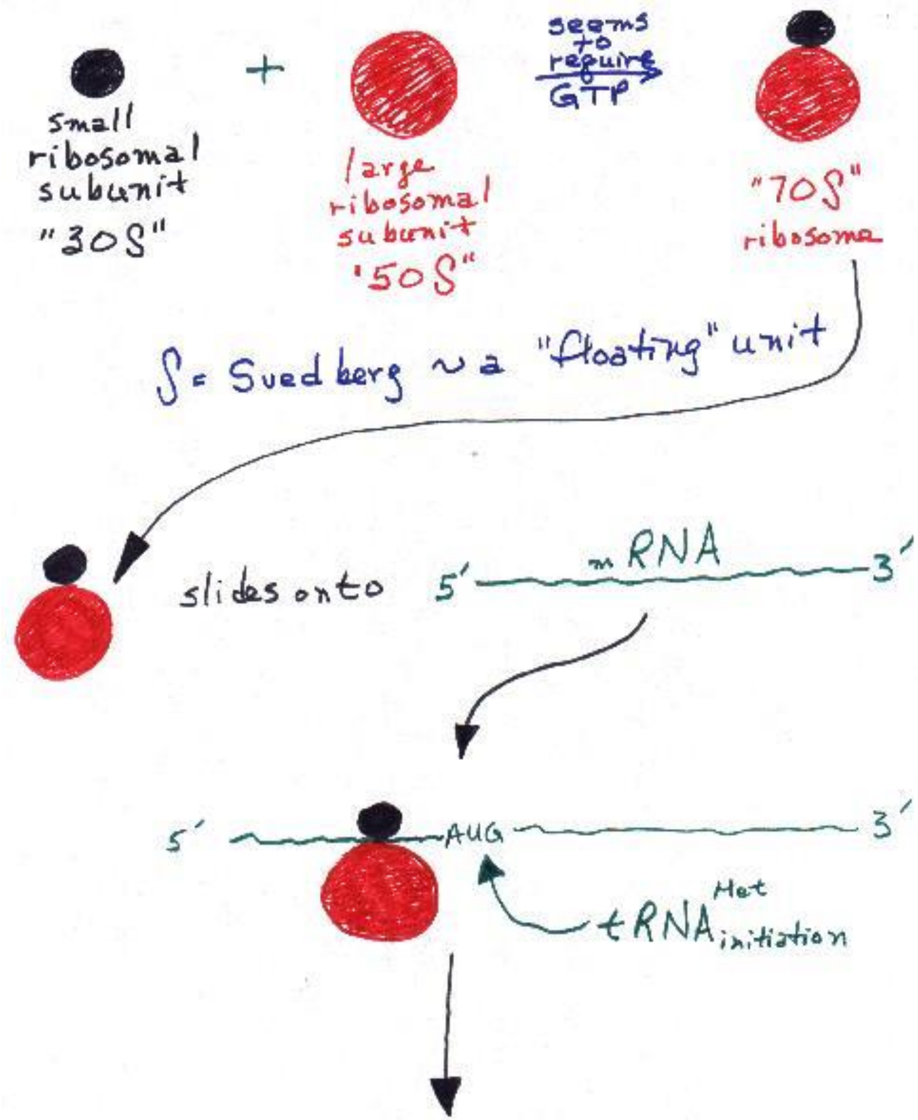
- The four steps, in order, in translation are
  - 1) Activation,
  - 2) Initiation,
  - 3) Elongation and
  - 4) Termination.



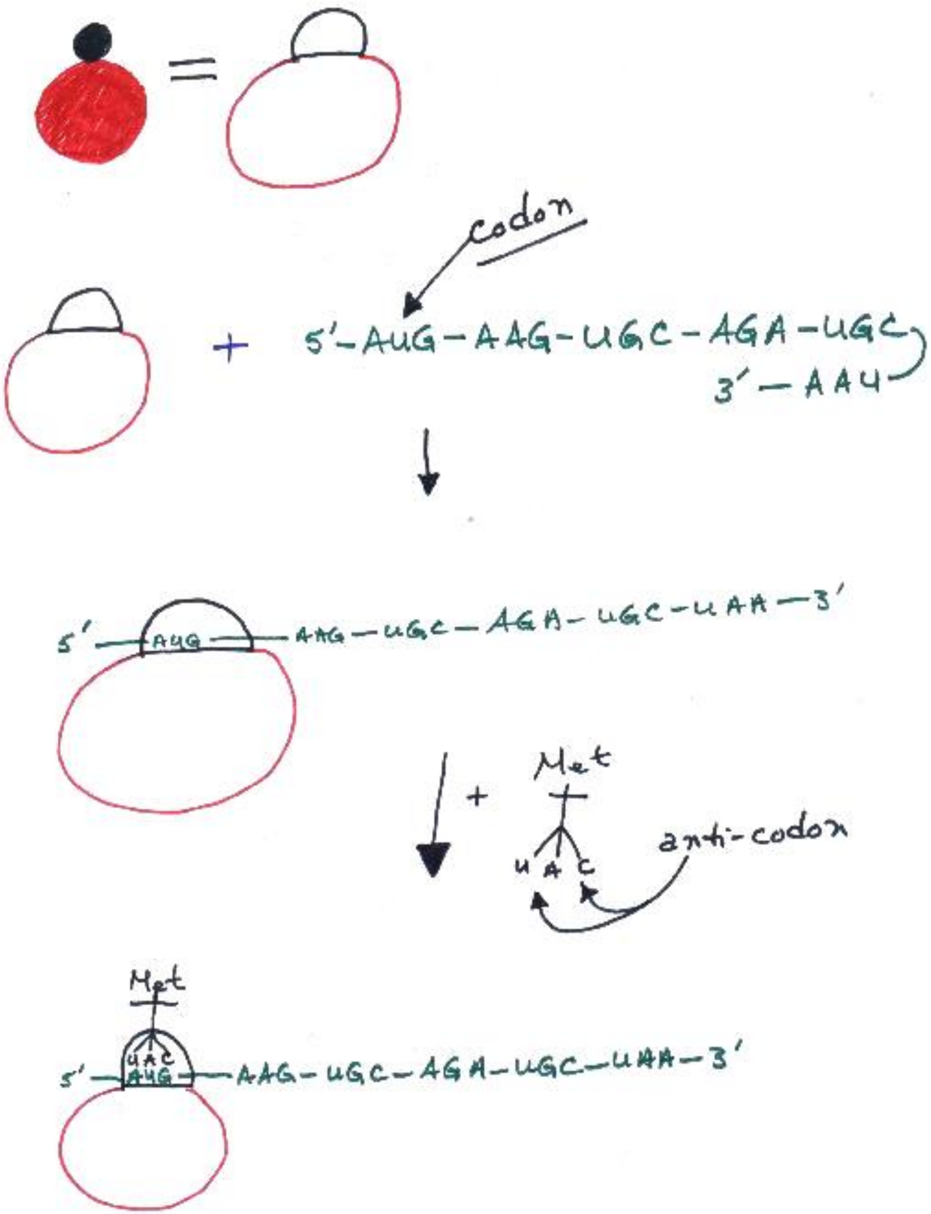
- Activation requires the activation of a tRNA such that it binds to its proper amino acid.
- The amino acid reacts with ATP in the presence of aminoacyl-tRNA synthetase to form the aminoacyl-AMP-aminoacyl-tRNA synthetase derivative/complex and inorganic pyrophosphate (PP_i; the equivalent of 2 phosphates [P_i]).
- This reaction is driven by the hydrolysis of the ATP to the AMP derivative and the PP_i.
- The release of the PP_i is what provides the energy to drive this reaction forward.
- The aminoacyl-AMP derivative then reacts with the appropriate tRNA, releasing the aminoacyl tRNA synthetase and the aminoacyl-tRNA.

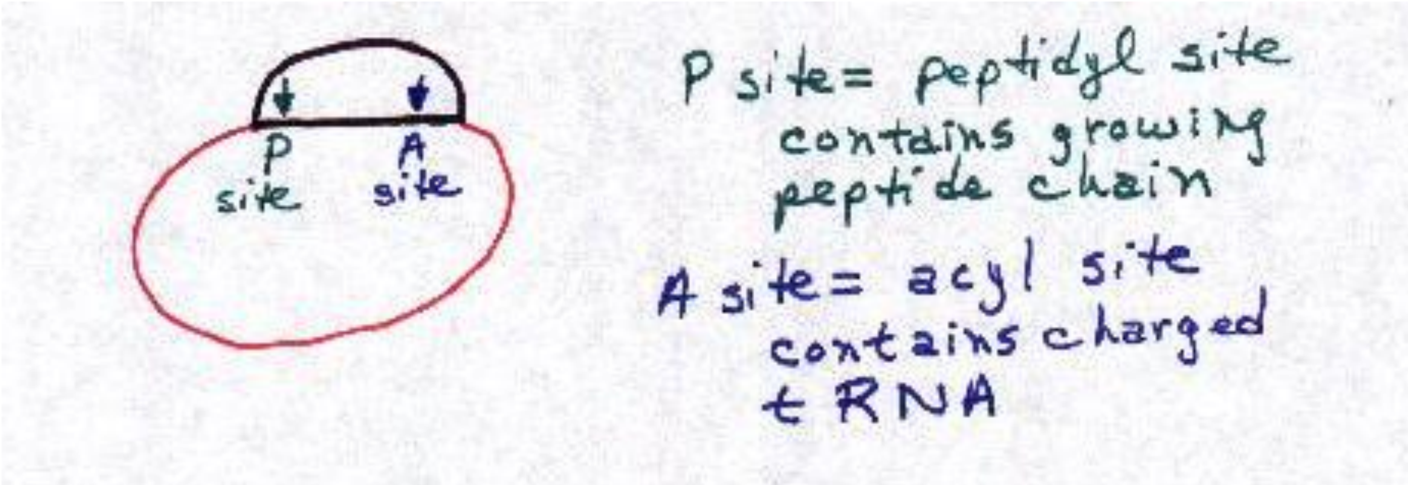
The second step in translation is Initiation

- A small ribosomal (30S) subunit binds with a large ribosomal (50S) subunit following the hydrolysis of GTP to form the 70S ribosome.
- The "S" is the Svedberg Unit which is a unit that measures the floating ability of the particle.



- At this point, translation "goes". The beauty of this mechanism is that only a unique protein is translated from this specific mRNA sequence.

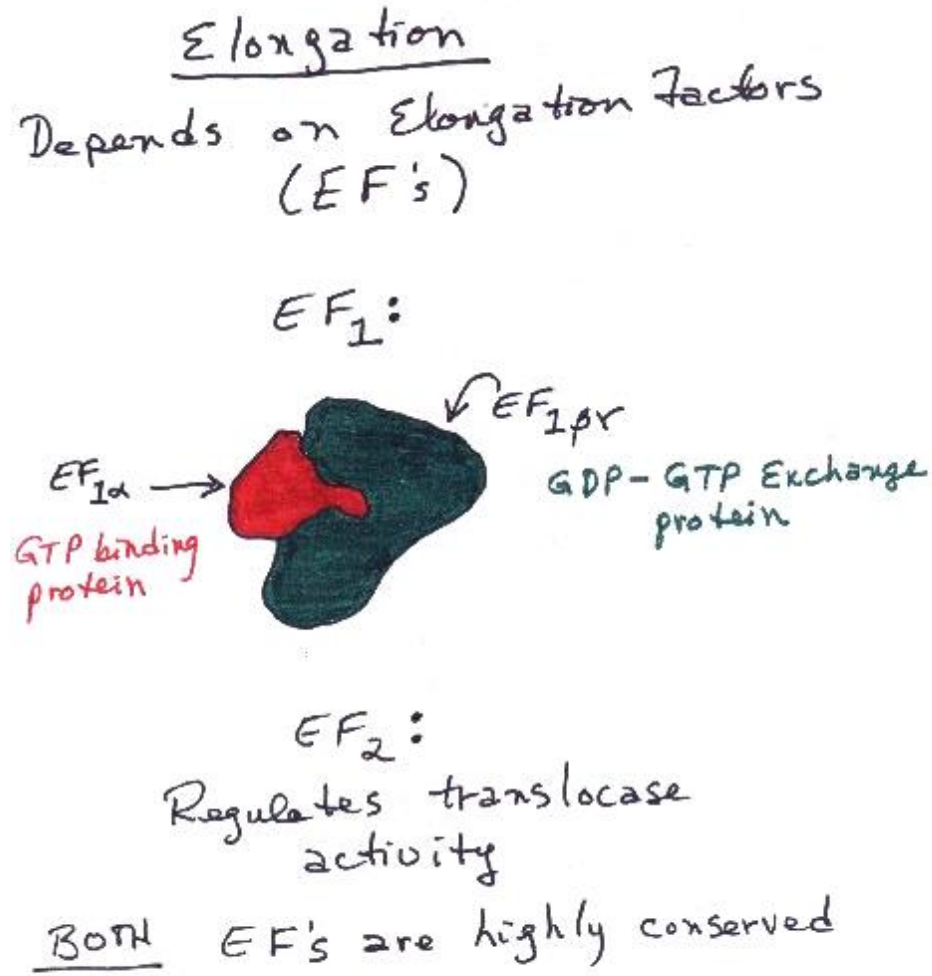




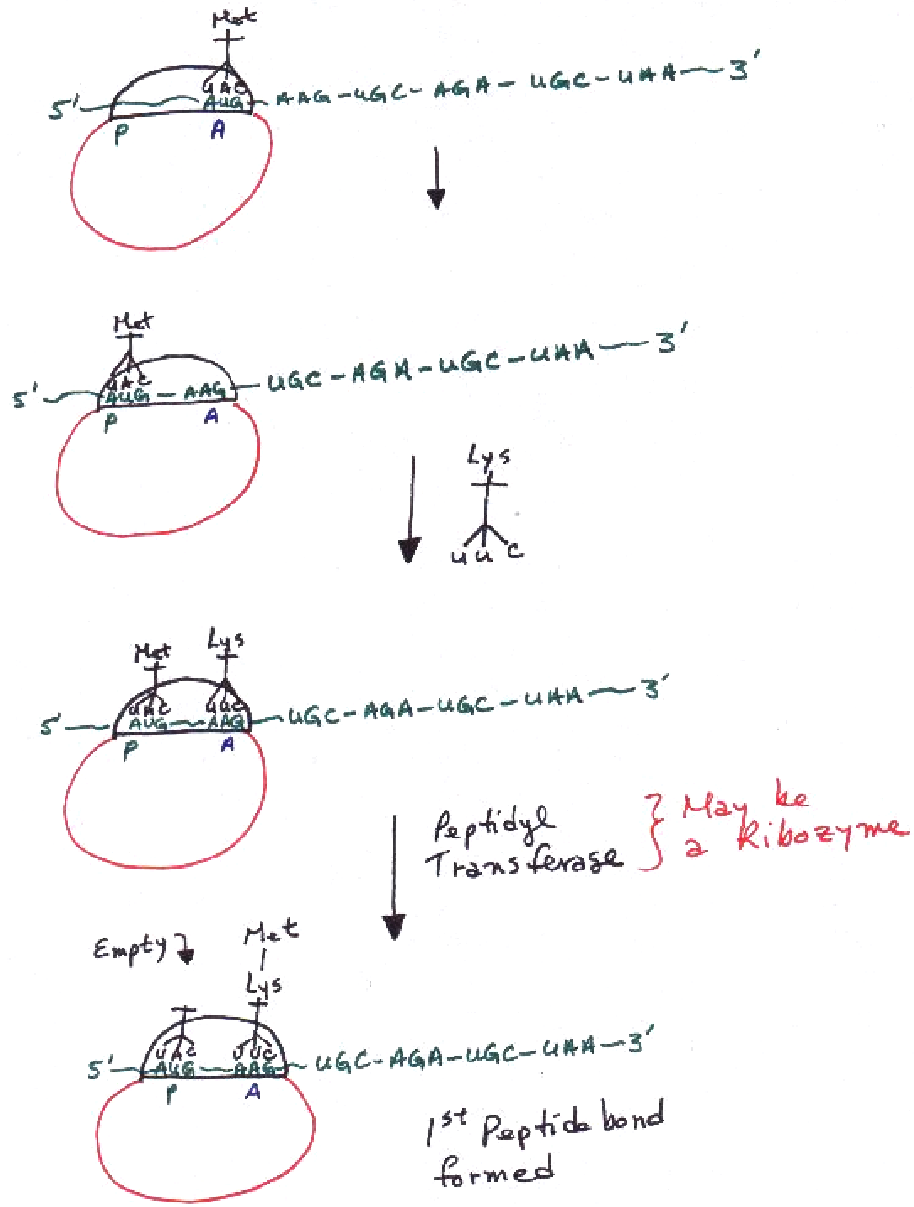
- There are two sites in the 70S ribosome: the "P" site and the "A" site.
- The "P" site is the peptidyl site and contains the growing peptide chain.
- By convention, this site is on the left-hand side of the 70S ribosome.
- The "A" site is the acyl site.
- This latter site contains the charged (activated) tRNA and is drawn on the right side of the 70S ribosome, next slide.

- The $tRNA^{Met}_{Initiation}$ is transported to the A site of the 70S ribosome.
- The ribosome slides down the mRNA in such a manner that the $tRNA^{Met}_{Initiation}$ is "shifted" -- presumably by the translocase regulated by $EF_{1\ and\ 2}$ -- into the "P" site, exposing the next codon (AAG; codon for Lys).
- Thus ends initiation and begins elongation.

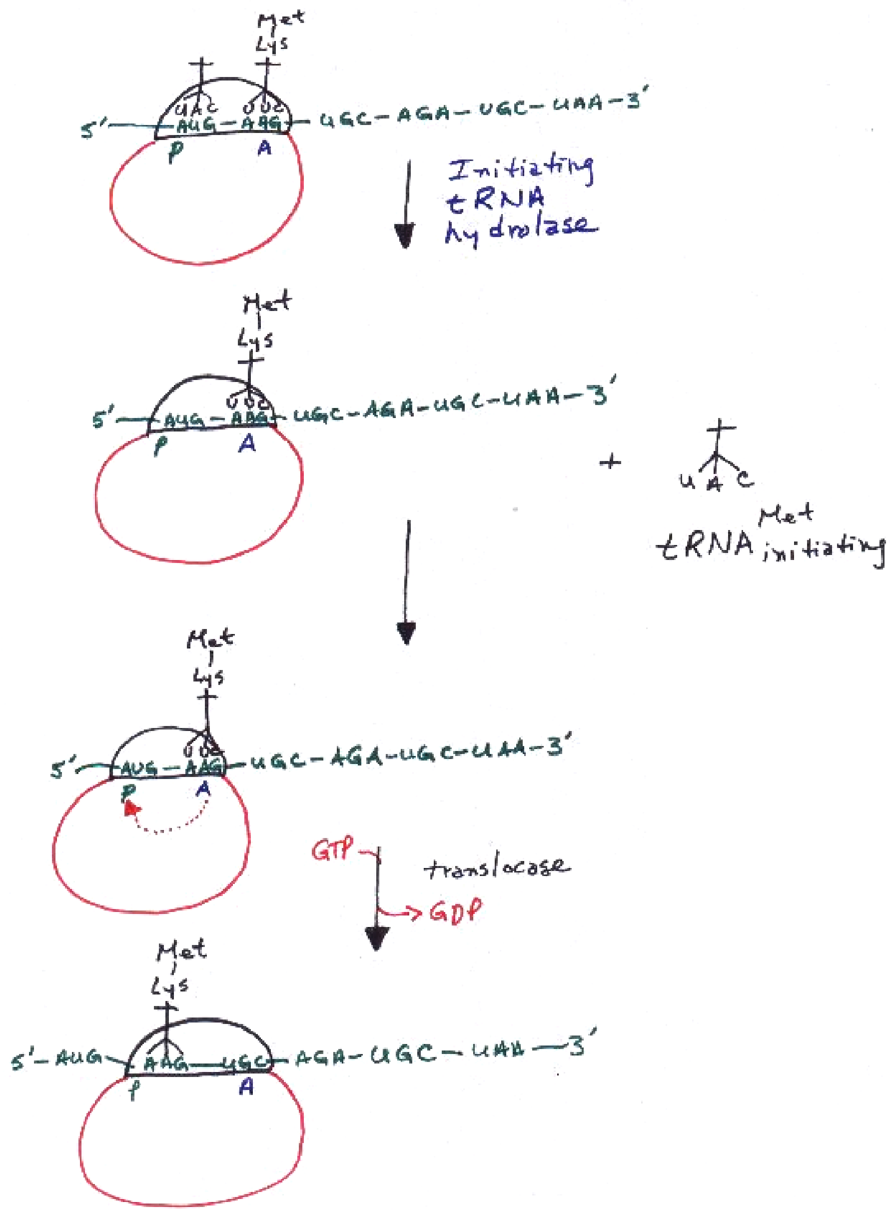
- Elongation depends upon elongation factors (EF's).
- EF_1 consists of $EF_{1\alpha}$ (a GTP binding protein) and $EF_{1\beta\gamma}$ (a GDP-GTP exchange protein), right.
- EF_2 regulates translocase activity (this enzyme is coming up shortly).
- Both EF's are highly conserved, i.e., they are found across nature having closely related structures/sequences.

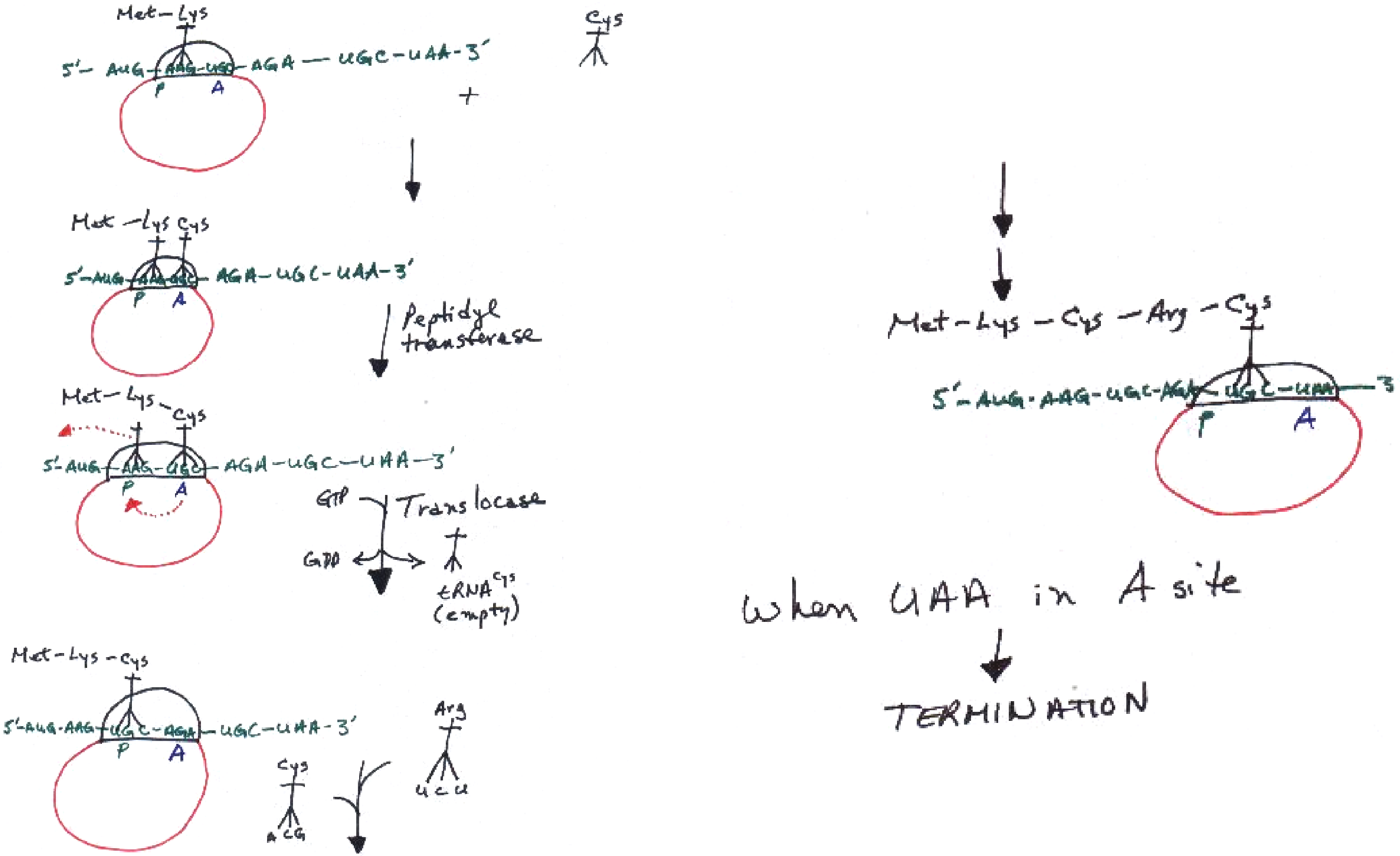


- Lysinyl-tRNA is then transported to the ribosome-mRNA complex.
- Once the latter tRNA is bound, peptidyl transferase (in all likelihood a ribozyme, i.e., RNA acting as an enzyme) tweaks the Met from its tRNA and forms the first peptide bond between the Met and the Lys.
- Lys remains bound to its tRNA in the "A" site of the 70S ribosome.

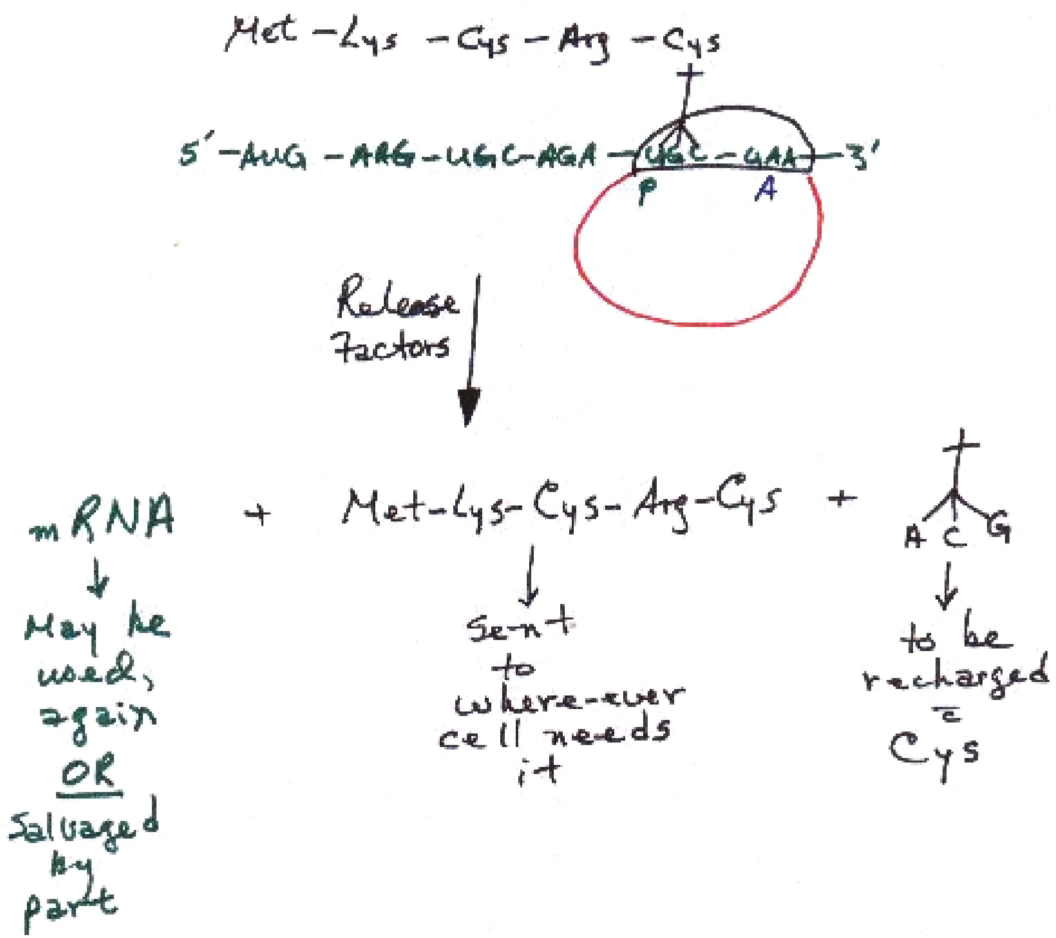


- Elongation continues and this slide demonstrates how the initiating tRNA ($tRNA^{Met}_{Initiation}$) is removed from the tRNA-mRNA-ribosome complex: by an initiating tRNA hydrolase.
- Translocase then drives the 70S ribosome one triplet towards the 3' end of the mRNA, placing the dipeptidyl-tRNA in the P site and making the A site available for the next tRNA.
- Translocase requires GTP for this reaction, i.e., it is energy requiring.





- This cycle continues, above, until the stop codon (UAA) is in the A site.
- When UAA is in the A site, this signals for termination to begin.



- Termination, above, is catalyzed by Release Factors that cause the mRNA to be used, again, or salvaged by part, the peptide to be released, modified and sent to where-ever the cell needs it and releases the last tRNA to be re-charged with the appropriate amino acid for future use (in the case of our example, to be re-charged with Cys).

Energy Requirements and Perspective of Translation:

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- 2 ATP's are required to charge each amino acid
- 2 GTP's are required to elongate per elongation step
- 1 calorie = the energy necessary to raise 1 gram of water by 1° C
- 2 ATP's and 2 GTP's give approximately 28,000 calories of energy: this is equivalent to the energy necessary to raise 28 liters of water 1° C.
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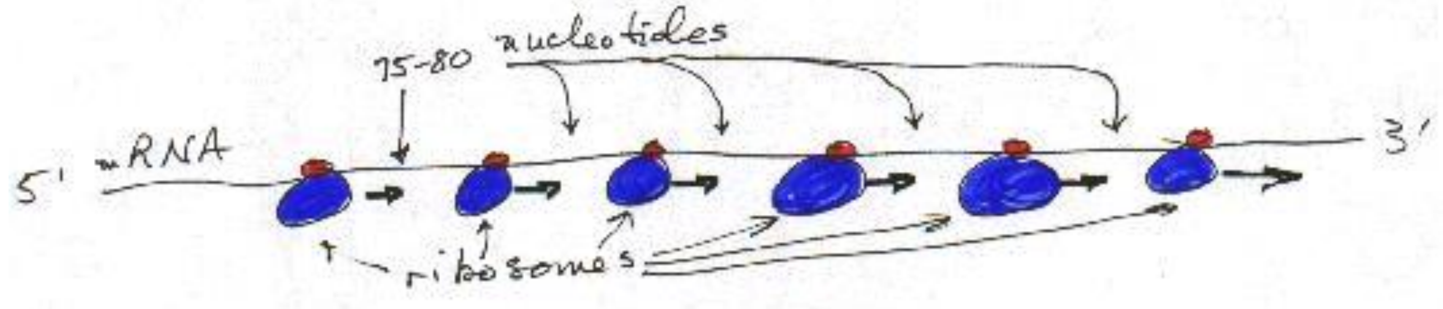
- In short, it takes LOTS of energy to synthesize proteins.
- A portion of that energy has to do with how the proteins are sequentially synthesized: once 25 amino acids (more or less) are linked by peptide bonds during translation, the AUG site is available/exposed for binding by ANOTHER 70S ribosome. This new ribosome initiates ANOTHER round of translation, *ad nauseum*.

- Eventually, the mRNA is literally smothered by ribosomes every 25 or so amino acids, i.e., about every 75-80 nucleotides on the mRNA.
- This smothered mRNA by ribosomes is called a polysome or polyribosome, below.
- This is the general form of the "translation unit in all cells".
- Polysomes increase the rate of translation per unit of time as compared to 1 ribosome on a mRNA strand.



- Once translation is completed, one of at least 4 modifications will occur to the protein[s] (called post-translational modification):
  - 1) glycosylation -- addition of carbohydrate to the protein;
  - 2) phosphorylation -- add a phosphate;
  - 3) proteolytic cleavage -- proteins may be synthesized in an inactive form and require cleavage to become active, e.g., insulin and C-peptide. C-peptide is the portion from pre-insulin that is cleaved to leave active insulin;
  - 4) sub-unit binding -- quaternary structure formations, e.g., the 4 sub-units of hemoglobin binding together, myoglobin subunits binding together, the 3 subunits of arginase binding together.

- Translation is inhibitable, image, right. That very fact makes it of significance to any one going into health care as many micro-organisms are capable of being killed by translation inhibitors such as chloramphenicol (C), tetracycline (T), streptomycin (S), lincomycin (L) and erythromycin (E) to name 5.
- In short,
  - C inhibits/blocks peptidyl transferase,
  - T inhibits binding of charged tRNA to the A site of the ribosome,
  - S blocks proper codon-anticodon binding to cause different peptides to be synthesized,
  - E inhibits the translocase and
  - L blocks peptidyl transferase and blocks tRNA from binding, although not at the same time.