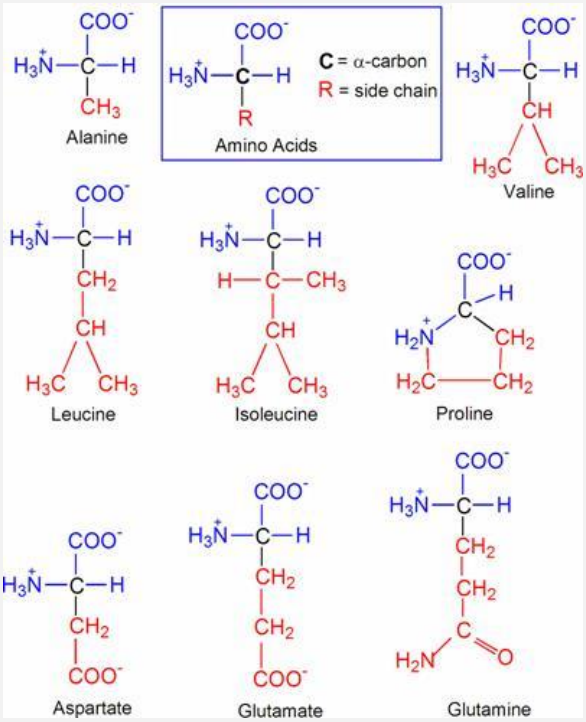
Muscle Protein Synthesis

There is not much to add as an introduction to this content besides a tip on what to focus on. To me, the most practical aspect of protein synthesis is the ATP cost of adding amino acid residues to a lengthening peptide chain. This reality resonates with the observation that exercise training that induces a significant anabolic stimulus is associated with a much greater loss of body fat that just performing long duration endurance exercise training. The biochemical explanation for this is not just in the calories lost in the exercise, but also, and likely to be the most important, the sustained elevation in basal metabolic rate after the exercise to support the high energetic cost of cellular protein synthesis. Such add protein mass then in turn causes a higher sustained basal metabolic rate, allows for increased caloric expenditure during weight supported exercise due to the greater lean mass and the increased physical fitness from the quality training, and as you see, this is a positive feedback cycle that favors fat loss, increased lean body mass, improved physical fitness, and all the added psychological and work productivity benefits that accrue with a more health mind and body.



Amino Acid Biosynthesis

To begin with, of the 20 amino acids, adult humans can produce 11 from intermediates of glycolysis or the TCA cycle, with most derived from the TCA cycle (Table 1).

Table 1. The potential sources of carbon chains for amino acid synthesis.

Source	Amino Acids
Glycolysis	
3-phosphoglycerate	Serine, GLycine, Cysteine
Pyruvate	Alanine
Phosphoenolpyruvate	Tyrosine
TCA Cycle	
α-Ketoglutarate	Glutamate, Glutamine, Proline, Arginine
Oxaloacetate	Aspartate, Asparagine
Essential	Arginine*, Methionine, Threonine, Lysine, Isoleucine, Valine, Leucine, Tryptophan, Phenylalanine, Histidine

* Essential only in children

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Transcription and Translation

First of all, if you are like me, you often have trouble remembering the difference between **transcription** and **translation**. Remember that transcription means that something is transcribed, and to transcribe means to copy. Hence, transcription is the copying of the DNA code to RNA. Translation means something is translated, which means something is interpreted. Thus, translation is the interpretation of the RNA code to produce proteins.

Translation has several steps, and we will go through these one at a time.

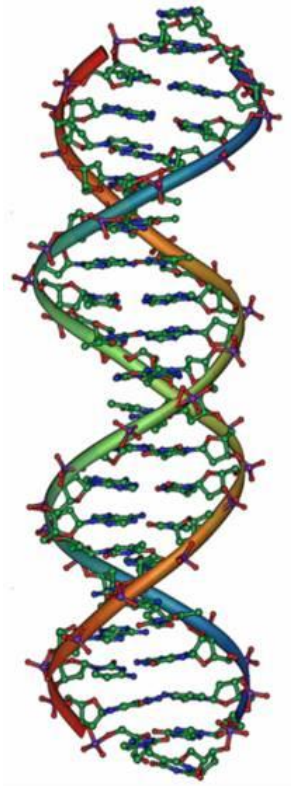

Figure 1. Structure of DNA.

1. <u>Modification of mRNAs</u>

The initial RNA molecule that is transcribed is called **messenger RNA (mRNA)**. It has the prefix "messenger" as it functions to transfer genetic code information (codon sequences) from **DNA** (Figure 1) within the nucleus to the cytosol in preparation for **protein synthesis**. Now, the processes involved from transcription to the start of translation are multiple and complex, and we will not cover all this information for this text. Just remember that the mRNA that is transcribed is edited after transcription in many ways prior to the start of protein synthesis. For example, **intron** sections are removed, **exon** segments are spliced together, beginning and end base sequences are removed and replaced with a "cap" (7-methylguanosine) at the 5' end and 20 to 250 adenylate residues ("**polyA tail**") are added to the 3' end (Figure 2). It is believed that the 5' cap facilitates binding of the mRNA to the **ribosome** to initiate translation. The polyA tail may protect the RNA from enzymatic degradation. Additional post-transcription editing of mRNA also occurs, but currently such editing is not well understood.

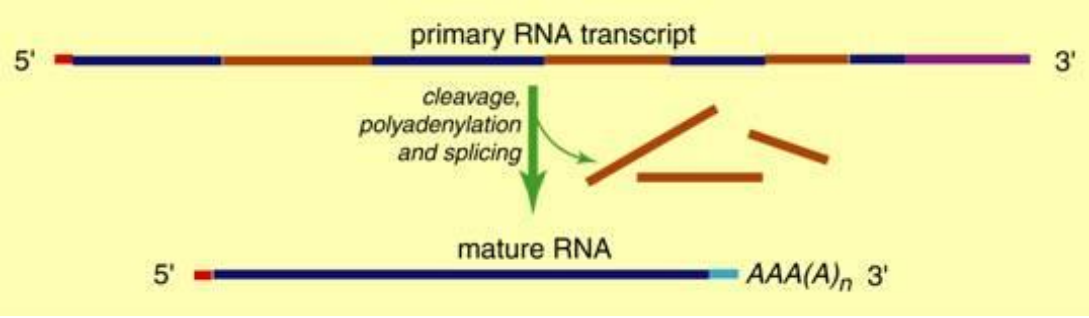

Figure 2. Schematic showing the post-transcriptional editing of mRNA, along with the additional of the 5' cap and 3' polyA sequence.

The end result of transcription is a single strand of mRNA. mRNAs can form complementary double stranded structures with other mRNA or DNA strands, with

the base-pairing rules of DNA still applying. However, the secondary structures of RNA are more complex and variable than for the organized structure of DNA. mRNA strands can also code for more than one protein or polypeptide chain.

2. Production of Transfer RNA (tRNA) and Activation of Amino Acids

To complete the translation of the mRNA codons to amino acids, added RNA is needed to read each codon and specifically attach the required amino acid. Thus, there are specific **transfer RNA (tRNA)** molecules for specific amino acid codons, which of course means that there must be more than 20 different tRNAs. In fact, research tells us that there are more than 32 tRNAs in eukaryotic cells. Each tRNA is a small single stranded RNA molecule that folds into a precise 3 dimmensional structure (Figure 3). Located at the end of the **anti-codon arm** are three base sequences that comprise the **anti-codon** and complement the mRNA, thereby copying the original codon from DNA.

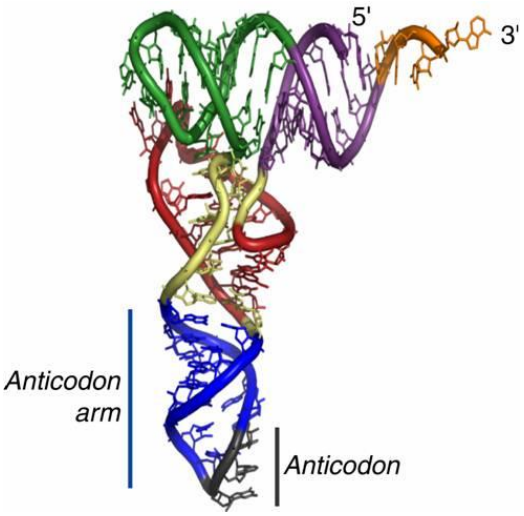

Figure 3. The general structure of tRNAs. Note the consistent 3' and 5' end sequences.

Attachment of an amino acid to a tRNA functions to activate the amino acid, and is catalyzed by an amino acid specific **aminoacyl-tRNA synthetase**. Thus there are 20 different aminoacyl-tRNA synthetase enzymes; one for each amino acid. Such attachment occurs in two steps, where an aminoacyl-AMP intermediate is first formed by the addition of AMP to the carboxyl group of the amino acid (Figure 4). Then the aminoacyl-AMP molecule is attached to the tRNA at the amino acid arm 3' end, with the subsequent release of AMP.

3. Initiation of Protein Synthesis

Remember that the codon AUG occurs at the start of each protein codon sequence, but also codes for the amino acid methionine (Figure 5). This duplication is resolved by the presence of two tRNAs for AUG; one for AUG occurring at the start of a sequence, and one for AUG occurring within a sequence. In bacteria, the tRNA for AUG that codes the start of a code sequence that has methionine attached is then modified by the addition of a formyl group (-HC=O) to the amine end of methionine. This prevents the $tRNA^{fmet}$ from incorrectly being placed within an elongating peptide chain.

The $tRNA^{fmet}$ attaches to a RNA strand associated with a ribosome. Ribosomes are the sub-cellular organelles responsible for directing protein synthesis, and are found both free in the cytosol as well as bound to parts of an intracellular membrane network called the **endoplasmic reticulum**, forming **rough**

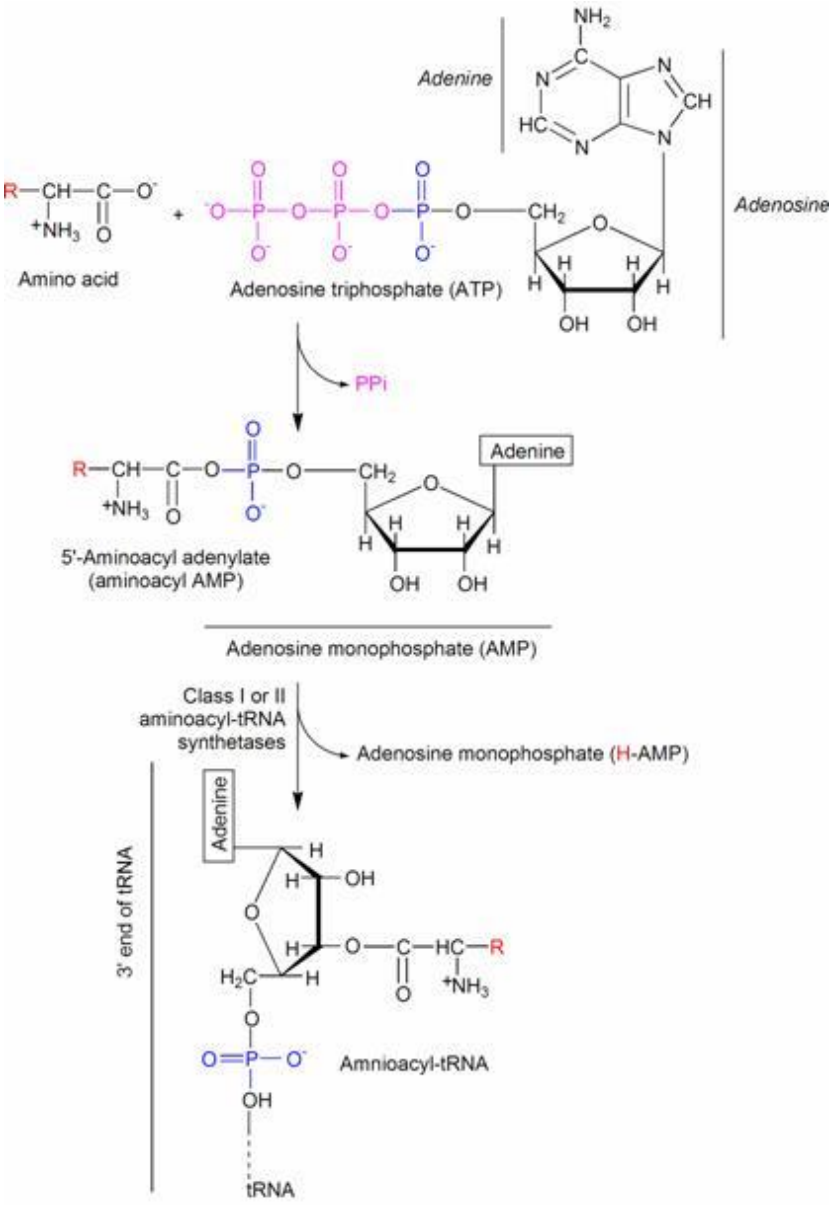

Figure 4. The structural changes that occur during the activation of amino acids to form aminoacyl-tRNAs.

endoplasmic reticulum (Figures 6, 7 and 8). The $tRNA^{fmet}$ attaches to the P-site of the ribosome, and all following aminoacyl-tRNA complexes (($aa\text{-}tRNA^{aa}$), such as $ala\text{-}tRNA^{ala}$ for alanine)) bind to the A site, as explained in the next step of elongation.

4. Elongation of the Polypeptide Chain

When an added $aa\text{-}tRNA^{aa}$ complex binds to the A site, specialized elongation proteins found in the cytosol support a GTP hydrolysis fueled move of the originating formyl-methionine from its initiating tRNA to the $aa\text{-}tRNA^{aa}$ at the A site.

The enzyme activity is provided by **ribozymes** of the ribosome. This movement and free energy release supports the formation of a peptide bond between the amine group of the A-site aminoacyl-tRNA, forming a dipeptidyl-tRNA. The initial $tRNA^{fmet}$ is then released, and the ribosome moves 1 codon further along the mRNA strand, which requires another GTP hydrolysis. The dipeptidyl-tRNA moves to the P-site, and a new codon-specific aminoacyl-tRNA attaches to the A-site. Such actions are repeated along the length of the mRNA strand until the termination codon is reached (Figure 8).

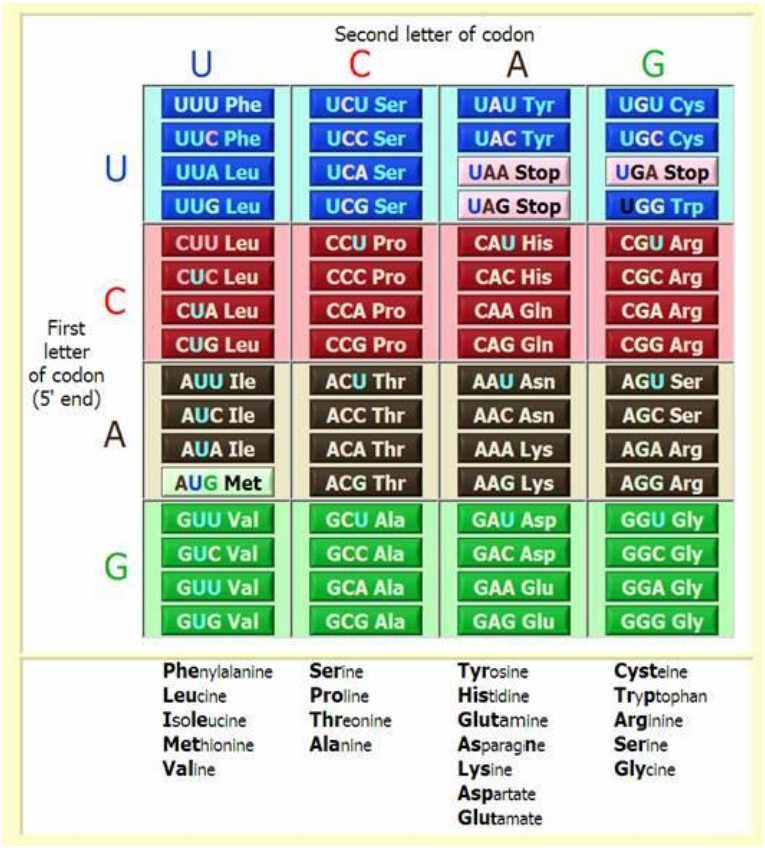

Figure 5. The genetic code from mRNA for amino acids.

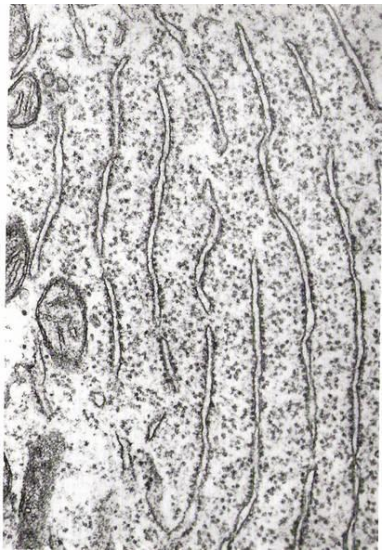

Figure 6. An electron micrograph of the rough endoplasmic reticulum. The dark stained granules are ribosomes.

5. Termination of Protein Synthesis and Release of tRNA

Termination of the elongation of the polypeptide chain is initiated when one of the three possible termination codons (UAA, UAG, UGA) is reached (Figure 5). This process involves the binding of a releasing factor to the termination codon causing the release of the polypeptide chain from the last tRNA, the release of the final tRNA from the ribosome-mRNA complex, and the separation of the ribosome and specialized subunits that support the processes of initiation and elongation. The ribosome is then free to initiate the synthesis of a new polypeptide chain.

6. Three-Dimmensional Folding of the Protein

As the final protein structure is more complex than the primary sequence of amino acids, and

sometimes often requires post-translational modification (Figure 9), the released polypeptide chain is often not yet biologically active. Due to charge distribution characteristics of the primary sequence, folding of the polypeptide chain is a spontaneous process. However, some enzymatic modification is necessary for all amino acids. The methionine start residue is removed, and most proteins have added alteration to both the amine and carboxyl terminal groups. Some protein amino acid functional groups are also chemically modified by phosphorylation, carboxylation, methylation, attachment of carbohydrate side-chains, etc.

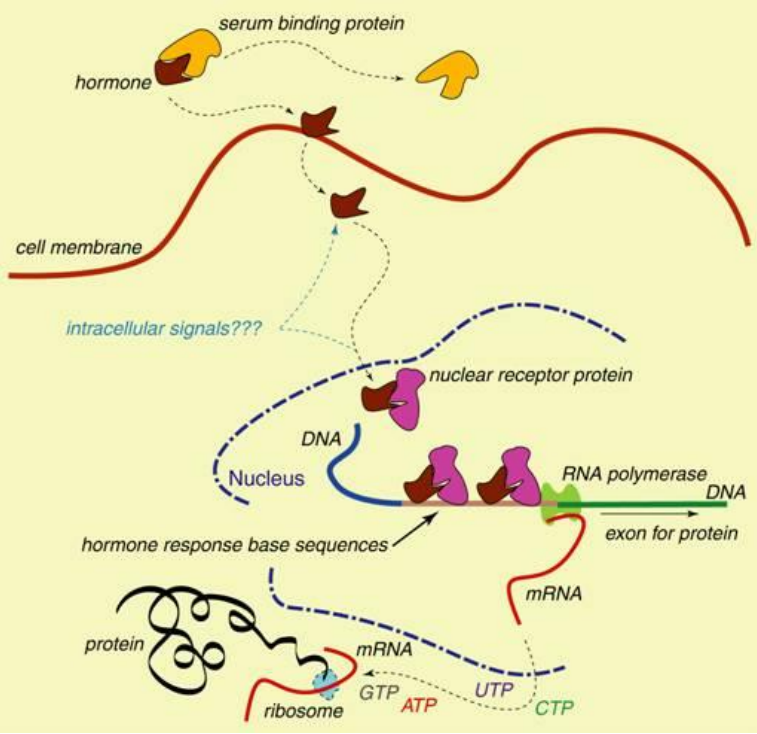

Figure 7. Schematic showing transcription and translation. Note the location for translation (protein synthesis) being the cytosol.

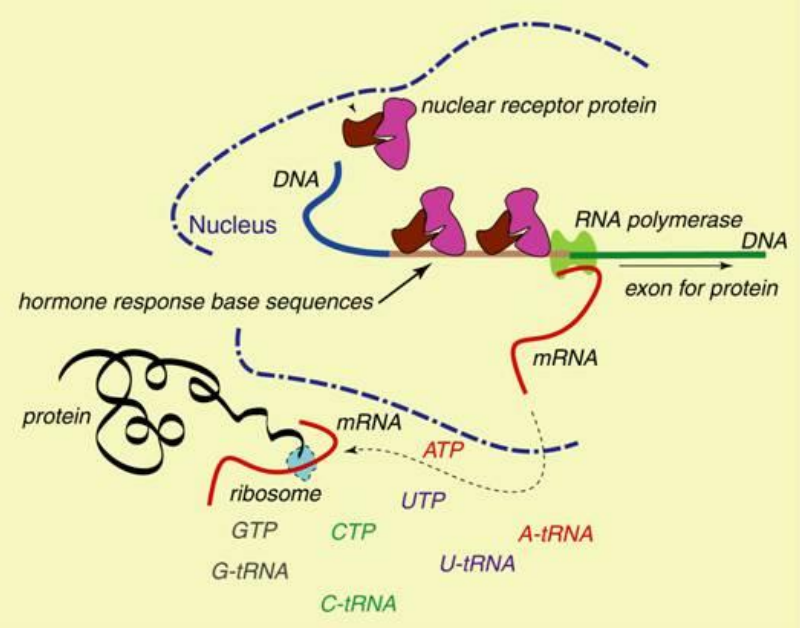

Figure 8. Schematic showing protein synthesis. Note the need for nucleotide specific triphosphates and tRNA molecules.

The energetics of protein synthesis is costly. The cost of each addition of an amino acid is 4 GTP molecules (from activation through peptide bond formation), and as such, protein synthesis does not occur during exercise, but at rest and in recovery from exercise in a nutrient rich condition. For example, a deficiency in a single amino acid will

prevent the synthesis of a given protein. Also, ample supplies of amino acids are needed to support protein synthesis, and adequate fat and carbohydrate is needed to provide the carbon skeletons for non-essential amino acid synthesis and the free energy for ATP and GTP regeneration to fuel the energetics of peptide chain elongation.

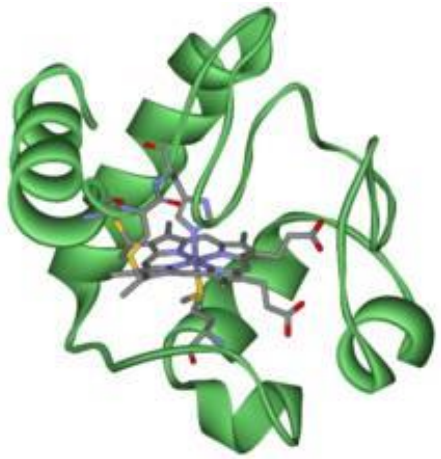

Figure 9. An example of the 3-dimmensional protein structure of cytochrome C.

Glossary Words

transcription is the copying, or more correctly, the transferring of the DNA code to mRNA.

translation is the interpretation of the mRNA code to produce proteins.

messenger RNA (mRNA) is the product of the process of transcription.

DNA is the abbreviation for deoxyribonucleic acid.

protein synthesis is the production of proteins by sequential amino acid addition to a elongating peptide chain.

intron is the section of DNA not involved in the transcription of complement bases in mRNA.

exon is the base segments of DNA that are transcribed to form mRNA.

polyA tail is the 20 to 250 adenylate residues that are added to the 3’ end of a mRNA at the completion of the transcription process.

ribosome is the subcellular organelle located in the cytosol that functions as the structural and regulatory foundation on which protein synthesis occurs.

transfer RNA (tRNA) is the amino acid specific RNA that is responsible for reading each codon and providing the required amino acid for attachment to the growing peptide chain.

anti-codon arm is the structural region of tRNA that contains the anti-codon.

anti-codon is the 3 base sequence region of the anti-codon arm that complements the bases of mRNA for specific amino acids.

aminoacyl-tRNA synthetase is the enzyme that catalyzes the attachment of an amino acid to its specific tRNA.

endoplasmic reticulum is the membranous network within the cytosol that provides structural connections throughout the cell.

rough endoplasmic reticulum is the endoplasmic reticulum that is structurally associated with ribosomes.

ribozymes are enzymes that are associated with each ribosome and facilitate the amino acid transfer reactions of protein synthesis.