Nucleic Acid Metabolism, Introductory Transcription and Translation

The Nickel Tour
Purine Nucleotide Biosynthesis/Anabolism

1. From Asp
2. Folic acid derivative
3. From Gln
4. From Gly
5. From Gly
6. From Cellular respiration
7. From Gly
8. Folic acid derivative
9. From Gln
Purines: Catabolism
Pyrimidines

- Cytosine deaminase is highly elevated in some solid tumor cells
- Inhibition with tetrahydourouridine improves therapy due to reduced drug degradation
More Pyrimidines
Integration of Metabolism

SSDD
Energy Sources from Intermediary Metabolism

- The first system is the phosphagen system. In this system, the source of the energy is ATP. Remember that during muscular contraction, ATP is hydrolyzed to ADP, \( P_i \) and energy. When this happens, there is only enough energy for 5-6 seconds.

- So how do our cells get additional energy?

- Our cells get it via a compound called phosphocreatine (PCr). The concentration of PCr is about 2-3 times greater than the concentration of ATP. When PCr is available, it is hydrolyzed to Cr and \( P_i \) and energy. The \( P_i \) is used to re-phosphorylate ADP to make more ATP.

- This gives us about 15 seconds of maximal contractions and is used for short bursts.
To give you an idea of what is involved in PCr utilization and synthesis, let's examine the PCr shuttle in cardiac and skeletal muscle. This shuttle increases incredibly the movement/transport of high-energy phosphate (ATP) from the matrix of the mitochondrion to the cytosol of the cell.
In the first step (1), an ADP-ATP translocase re-phosphorylates ADP to form ATP in the mitochondrial matrix. This occurs via electron transport/oxidative phosphorylation (the "ETOP" in the graphic). When the ATP is "dumped" into the intermembrane space, it is reacted with creatine via a mitochondrial creatine kinase (6) to form PCr. The PCr is then transported via a creatine-creatine phosphate (C:PCr) transport pore (2) into the cytosol to "dump" into a cytosolic PCr store.
The cytosolic PCr store is derived from an EMP-linked (glycolysis-linked) creatine kinase (CK) (3) and another CK that maintains the equilibrium between C and PCr (or ATP and ADP, if you prefer)(4). The utilization of the PCr from the cytosolic store occurs via an ATP dependent CK that cranks out hugely elevated levels of ATP (5). The ATP is used by processes requiring high energy, e.g., contracting muscle.
Of significance is that there are three isozymes (enzymes with the same function that are found in different tissues and are slightly different, structurally) of CK:

<table>
<thead>
<tr>
<th>CK-BB</th>
<th>CK-MB</th>
<th>CK-MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>= CK-1</td>
<td>= CK-2</td>
<td>= CK-3</td>
</tr>
<tr>
<td>Brain, uterus,</td>
<td>Heart (and skeletal muscle)</td>
<td>Skeletal muscle (and cardiac, too)</td>
</tr>
<tr>
<td>prostate, lung</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Creatine, Urea, TCA and Malate-Aspartate Shuttle Interconnections

1. Creatine: is a nitrogenous organic acid that occurs naturally in vertebrates and helps to supply energy to muscle and nerve cells. In humans and animals, approximately half of stored creatine originates from food (mainly from fresh meat). Ninety-five percent of creatine is later stored in the skeletal muscles.

2. Creatinine: is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). Creatinine is chiefly filtered by the kidney, though a small amount is actively secreted. There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, blood levels rise. Men tend to have higher levels of creatinine because they have more skeletal muscle than women. Vegetarians tend to have lower creatinine levels, because vegetables contain no creatine.

1. Mitochondrial Arginine-glycine: amidinotransferase (E.C. 2.1.4.1)
2. gA Transporter – (from mito to cytosol)
3. Cytosolic S-adenosylmethionine :guanidinoacetate-N-methyltransferase (SAM:gA NMT)
4. Cytosolic Creatine kinase
5. Cytosolic non-enzymatic cyclization
N-acetylglutamate is an obligatory “on switch” for CPS-I.
Note relationships between Urea cycle, TCA, Creatine Synthesis and Asp-Malate Shuttle.
RNA Characteristics with Introductory Transcription and Translation

How DNA, RNA, Enzymes and Ribosomes Work as An Intracellular Team
RNA
Characteristics and Functions
There are Five Kinds of RNA, All of Which are Templated from DNA.

- The first type of RNA is tRNA.
- The "t" stands for "transfer".
- This RNA is the RNA that transfers amino acids to the growing peptide as it is elongating on the ribosome.
- It is single-stranded (SS) and found in the cytosol of the cell.
- tRNA makes up about 15% of all RNA's.
There are at least 20 different kinds of tRNA's as there are at least 20 different amino acids. tRNA has a cloverleaf shape:
1. Bases 1-7 are paired with bases 66-72 to form a double stranded (DS) region in the tRNA that makes it stable/stronger. This region extends through bases 73-76. The whole "arm" is known as the acceptor stem. Note that the 3'-OH is the site of attachment of the amino acid under the direction/catalysis of aminoacyl-tRNA synthetase.

2. Bases 10-13 are paired with bases 22-25 in the DHU loop (on left in graphic). The "H" stands for dihydouridine (DHU).

3. Bases 27-31 are paired with bases 39-43 to form the anticodon loop of the tRNA (bottom of graphic). Bases 34, 35 and 36 make up the triplet that is the anti-codon.

4. Although not DS, bases 44-48 make up the "extra arm" of tRNA.

5. Bases 49-53 pair up with bases 61-65 in the T\(\psi\)C loop. \(\psi\) is pronounced "sigh" and looks like a three-pronged pitchfork; it represents the presence of pseudo-uridine in this loop.

6. The arms or stems of the loops seem to be primarily GU pairs, a rather odd combination, as we typically think about G and C pairing and A and U pairing.

7. The amino acid acceptor end ALWAYS ends with XCCA, where X is any nucleotide, so that A is always attached to the binding/transferable amino acid.
That folds to a more compact "L" shape:
• The region of the tRNA that bonds with the mRNA is called the anti-codon.

• Typically, the tRNA's are identified by which amino acid they transfer, e.g., alanyl tRNA would be represented as \( \text{tRNA}^{\text{Ala}} \).

• As with ALL RNA's, the concentration of adenine is not equal to the concentration of uracil (\([A][U]\)), nor are the concentrations of guanine and cytosine equal (\([G][C]\)).

• This is due to the fact that RNA is single stranded (SS).
• The second type of RNA is "rRNA" or ribosomal RNA.
• It, too is SS.
• It is found in the ribosomes in the cell.
• rRNA makes up about 80% of the RNA's in the cell.
• It is the most stable of the RNA's and is synthesized only when the cell needs more ribosomes.
• The code for the rRNA is found in nuclear DNA.
Third Type of RNA

- Messenger RNA (mRNA) is also SS.
- It is synthesized in the nucleus, is sent to the cytosol of the cell and binds with ribosomes.
- It makes up less than 5% of the RNA's as it doesn't "survive" long enough to make up much of the RNA's.
- It has a half-life \( (t_{1/2}) \) of 4-24 hours.
- mRNA is around only long enough to drive the synthesis of its specific protein, then it is recycled.
- mRNA is synthesized from a single gene unlike t and rRNA.
- mRNA may range from 70 nucleotides in length up to 20000 nucleotides in length.
- The 3' terminus carries a poly-A "tail" that consists of 20-200 adenosine residues that are added after mRNA is synthesized.
Fourth Kind of RNA

• The mitochondrion makes some of its own RNA, as well, called mitoRNA.
• It is SS and found in, believe it or not, the mitochondrion.
• mitoRNA may be t OR rRNA-type.
• It is utilized in the synthesis of mitochondrial protein.
• Remember, though, that the mitochondrion requires nuclear-coded proteins to function, as well.
• The fifth type of RNA is called small nuclear RNA or snRNA (called "snurps").
• It is found in the nucleus of the cell.
• Some snurps are involved in/with RNA processing.
• It consists of and interacts with ribonucleoprotein.
• They are typically named with a "U" followed by a number., e.g., "1", then completed with RNA: U1RNA, U2RNA, U3RNA, ad nauseum.
Transcription and Translation Rapid Overview

1. DNA in nucleus acts as a template for mRNA synthesis (transcription)

2. mRNA leaves nucleus & goes to cytoplasm, where it complexes to ribosomes

3. tRNA carries AA to mRNA

4. tRNA couples briefly E in RNA (translation)

5. tRNA moves off to pick up more AA

6. Stop sequence read & peptide snipped free

7. Ribosomes move along mRNA, adding AA to poly peptide chain
Transcription, i.e., RNA synthesis

- During transcription, only ONE strand of the DNA is actively read and transcribed. The strand to be transcribed contains a promoter (recognition site) that is recognized by RNA Pol. This enzyme causes the DS DNA to separate and it adds on the first of a sequence of nucleotide triphosphates (NTP’s) to initiate transcription.

- Note that the DNA is the template for the new strand of RNA. In this instance, the bases in RNA will H-bond with the DNA in a complimentary manner, i.e. AU and/or AT (depending on the nucleic acid and orientation) and GC.

- As the polynucleotide progresses, the enzyme elongase extends the polynucleotide chain until termination occurs with the release of RNA (much more on this in the RNA chapter).
• Although gene regulation is complex and not fully understood, there are some initial regulations of transcription about which we do have some knowledge.

• Transcription may be positively regulated
  – 1) hormonally at the level of the DNA; with RNA Pol and with proteins necessary for Pol interaction. This probably does not occur in man;
  – 2) hormonally at the level of the DNA that causes conformational changes of DNA so RNA Pol may bind to it;
  – 3) hormonally where the hormone binds to the transcriptional factor that has to bind to DNA site before RNA Pol may bind;
  – 4) cAMP is even involved: it increases tyrosine aminotransferase, PEPCK and prolactin syntheses.
Conversely, transcription may be negatively regulated

1) hormonally where the hormone acts as an inducer that turns off repressors and/or

2) hormonally where the hormone binds to the DNA to cause a conformational change of chromatin to make the DNA susceptible to RNA Pol.
• Once the mRNA has been synthesized and matured in the nucleus, it is ready for transport through the nuclear envelope into the cytosol to bind to ribosomes.
• tRNA, then, transports the necessary amino acids to the mRNA-ribosomal complex to continue the process of protein synthesis (translation).
• How is it that the two RNA's code for the amino acids?
• The genetic code is based upon triplets, i.e., a set of three nucleotides in sequence that code for a single amino acid.
• Each triplet in mRNA is read from 5' to 3'; this triplet in mRNA is called the codon.
• Each triplet in tRNA is called the anti-codon and is read complimentarily to the codon.
Listed below in the table is an incomplete list of codons for some of the amino acids:

<table>
<thead>
<tr>
<th>Triplet Code (Codon with Amino Acid -- NOT Inclusive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAA = <strong>Stop</strong></td>
</tr>
<tr>
<td>CAC = His</td>
</tr>
<tr>
<td>CUA = Leu</td>
</tr>
<tr>
<td>AAG = Lys</td>
</tr>
<tr>
<td>UAC = Tyr</td>
</tr>
<tr>
<td>CGA = Arg</td>
</tr>
<tr>
<td>AAC = Asn</td>
</tr>
<tr>
<td>AGA = Arg</td>
</tr>
<tr>
<td>UGG = Trp</td>
</tr>
<tr>
<td>GAA = Glu</td>
</tr>
<tr>
<td>AGC = Ser</td>
</tr>
<tr>
<td>UGC = Cys</td>
</tr>
<tr>
<td>GAU = Asp</td>
</tr>
<tr>
<td>GGG = Gly</td>
</tr>
<tr>
<td>ACA = Thr</td>
</tr>
<tr>
<td>CAA = Gln</td>
</tr>
<tr>
<td>AUA = Ile</td>
</tr>
<tr>
<td>GCA = Ala</td>
</tr>
<tr>
<td>CCC = Pro</td>
</tr>
<tr>
<td>UUU = Phe</td>
</tr>
<tr>
<td>AUG = Met <strong>(Start)</strong></td>
</tr>
<tr>
<td>GUG = Val</td>
</tr>
</tbody>
</table>
Note that, in some instances, the difference between amino acids is one (1) nucleotide in the triplet, e.g.,

- mRNA sequence: AUG-CAC-AGA-CCC-UGC-UAA
- amino acid sequence:
  (Start) Met-His-Arg-Pro-Cys-Stop
- If you alter this sequence by placing an "A" after 9 bases, the new sequence is:
  - mRNA sequence: AUG-CAC-AGA-\textcolor{red}{\text{ACC}}-CUG-CUA-A__
  - New amino acid sequence:
    (Start) Met-His-Arg-Thr-Leu-Leu-----
Mutations

- One shift, one base change alters the whole protein after the insertion of the "A".
- A *mutation* is any change that presents in the DNA of a cell.
- Mutations are chance activities and occur spontaneously in all nuclear material.
- Mutations are neither good nor bad (like emotions) and are simply a natural occurrence of cellular activities.
• Mutations can not be predicted, nor can the effects be predicted - caveat: some ARE predictable, i.e., the ones we recognize and have studied extensively.
• Those compounds that interact with DNA and increase the frequency with which bases are altered or which causes the likelihood of mutation are called mutagens.

• Most mutations impair cell function rather than increase useful cell functions, therefore, mutations are thought of as being harmful.

• Even with incredible minimal exposure to mutagens, there is still a small likelihood that a gene may mutate.

• This is called a spontaneous mutation.

• The frequency of spontaneous mutations vary greatly between genes and organisms.

• When this frequency increases, it is assumed that some mutagen is causing it.
The table, below, summarizes different types of mutations.

<table>
<thead>
<tr>
<th>Type of Mutation</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (&quot;normal&quot;)</td>
<td>DNA is in cell nuclei.</td>
</tr>
<tr>
<td>Point</td>
<td>DNA is in cell nuclei (NO period)</td>
</tr>
<tr>
<td>Insertion</td>
<td>DNA is NOT in cell nuclei.</td>
</tr>
<tr>
<td>Gene duplication</td>
<td>DNA is in IN cell nuclei.</td>
</tr>
<tr>
<td>Gene duplication with point mutation</td>
<td>DNA is in AN cell nuclei.</td>
</tr>
<tr>
<td>Chromosome duplication</td>
<td>DNA is in cell nuclei.</td>
</tr>
<tr>
<td>Translocation</td>
<td>DNA is cell nuclei in.</td>
</tr>
<tr>
<td>Inversion</td>
<td>DNA is in cell ielcun.</td>
</tr>
<tr>
<td>Frameshift*</td>
<td>DNA Ai sin cel Inu clei.</td>
</tr>
</tbody>
</table>

*removal of or insertion of bp sequences that alter the reading of the DNA sequence.
• In terms of mutations of hereditary and somatic forms, when the mutation is passed onto the offspring it is said to be inherited, hereditary, genetic for lower organisms.

• For MAN there are two sort of mutations:
  – hereditary, which occurs ONLY in sex cells: sperm and ova (includes BRCA-1 and BRCA-2); and
  – somatic mutation, which consists of most mutations.

• These occur in all other cells.

• Somatic mutations are NOT hereditary and are NEVER passed onto progeny.

• Hence, there is a genetic and an environmental element involved in cancer.

• It is of significant interest that mutation, aging and cancer are tightly linked by somatic mutations.
The process of using the mRNA to synthesize proteins is called translation and that's the next topic for study.

Translation: A Four-Step Process
• 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 (PPI; the equivalent of 2 phosphates [Pi]).
• This reaction is driven by the hydrolysis of the ATP to the AMP derivative and the PPI.
• The release of the PPI 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.
\[ R - C - C^\circ - \Theta \text{NH}_3 \]

Amino Acid

\[ \text{Enz} - A - \Theta - C - R \]

Amino acyl-AMP

\[ \text{tRNA} - A - \Theta - C - R \]

Amino acyl-tRNA

\[ \text{Aminoacyl-tRNA Synthetase} \]

\[ \text{Amino acyl-tRNA Synthetase} \]

\[ \text{Enzyme} \]

\[ \text{tRNA} \]

\[ \text{Amino Acid} \]

\[ \text{t-RNA} \]

anti-codon
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.
• The $tRNA^{Met}_{\text{Initiation}}$ is transported to the A site of the 70S ribosome.

• The ribosome slides down the mRNA in such a manner that the $tRNA^{Met}_{\text{Initiation}}$ 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).
• EF₁ consists of EF₁α (a GTP binding protein) and EF₁βγ (a GDP-GTP exchange protein).

• EF₂ 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.
• Lysinyll-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.
5'  AAA-UGA-AGA-UGG-UAA-3'

5'  AAA-UGA-AGA-UGA-UAA-3'

5'  AAA-UGA-AGA-UGA-UAA-3'

5'  AAA-UGA-AGA-UGA-UAA-3'

Peptidyl transferase 7 May be a ribozyme

1st peptide bond formed
• Elongation continues and the following slide demonstrates how the initiating tRNA (tRNA$^{\text{Met}_{\text{Initiation}}}$) 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, next two slides, until the stop codon (UAA) is in the A site.
• When UAA is in the A site, this signals for termination to begin.
When UAA in A site

DEPENDENCE
• Termination, next slide, 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).
Met-Lys-Cys-Arg-Cys

5'-AUG-ARG-UGC-AGA-UGC-UAA-3'  

Release Factors

mRNA + Met-Lys-Cys-Arg-Cys +

May be used again or salvaged by part

Sent to wherever cell needs it

to be recharged as Cys
Energy Requirements and Perspective of Translation:

- 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.

- 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.

• 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, next slide. 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.

• Next Slide for Inhibition graphics
• 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.