Hello everybody, congrats on surviving midterms! Hope that everyone was able to have a restful fall break. Keep up your hard work, and enjoy this resource.

Remember: the Tutoring Center offers free individual and group tutoring for this Genetics. Our Group Tutoring sessions will be Thursday from 5:15-6:15 PM at the Sid Rich basement, room 74! All you need to do is walk in. I hope to see you there!

Keywords: Ribosome, Amino Acids, Splicing, Codon, Polypeptides

**Topic of the Week: Translation (15.3)**

Recall: the central dogma of molecular biology follows the flow of information from DNA → RNA → Protein

**Translation:** the synthesis of proteins from an mRNA code

Note: translation happens in the 5’ → 3’ direction along an mRNA sequence; the polypeptide is synthesized in the N(terminal) → C(term) direction

**Prokaryotic:** simultaneous transcription and translation are possible, and use tRNA-*f*-met as initiator tRNA

Initiation: IF-3 binds to small subunit (prevents complexing with large subunit); Shine-Dalgarno sequence on mRNA binds to small subunit at binding site; IF-2 complexes with GTP, which binds to tRNA-*f*-met; IF-1 binds to the small subunit as tRNA finds AUG codon and binding hydrolyzes GTP to GDP+P; all IFs leave; large subunit binds, forming 70s initiation complex.

Elongation: Ribosome has 3 sites*: A, P, and E

1. 70s complex has tRNA-*f*-met occupying P-site
   a. **EF-Tu-GTP** and next charged tRNA form a complex; tRNA enters the A-site
2. **GTP** hydrolyzes to GDP+P, and **EF-Tu** leaves
   a. Peptide bond forms between the C-term of the AA in the P site and the N-term of the AA in the A site; **peptidyl transferase** activity of rRNA near p-site catalyzes this (elongate N → C)
   b. The polypeptide chain transfers from P site to A site
      i. **EF-Ts** converts **EF-Tu-GDP+P** back to **EF-Tu-GTP**.
3. **EF-G** briefly associates with **GTP** to **GDP+P** hydrolysis, causing ribosome to *translocate* down the mRNA (in the 3’ direction)
   a. tRNA with polypeptide chain moves to P-site
   b. tRNA without polypeptide is removed through the exit site

**Termination:** when a terminator (nonsense) codon is read in the A site:
- **RF-1** or 2 binds to terminator codon in the A-site and **RF-3-GTP**.
- Polypeptide chain at P-site is cleaved, and the chain is freed.
- **GTP** to **GDP+P** hydrolysis releases **RFs** and dissociates ribosome-mRNA complex.

**Eukaryotic:** [https://www.youtube.com/watch?v=qlwrhUrvX-k](https://www.youtube.com/watch?v=qlwrhUrvX-k)

**Initiation:** tRNA1 and eIF’s bind to ribosome, then mRNA binds on the small subunit with help of **CBPs**; large subunit complexes and hunts for **AUG**.

**Elongation:** *same process in eukaryotes as in prokaryotes*

**Termination:** *same process in eukaryotes as in prokaryotes*

---

**Highlight #1: pre-mRNA Processing** (14.2)

**Spliceosomal Processing:**
- **Addition of 5’ Cap:** 7-methylguanosine (modified guanine nucleotide) is added in a reverse 5’-5’ bond to the polypeptide strand → prevents degradation and aids recognition of ribosome.
- **3’-Cleavage and Polyadenylation:** a consensus site (**AAUAA**) is recognized -11 to -30 bp from cleavage site; mRNA is cleaved between poly A and poly U consensus and a 50-250bp Adenosine (**poly-A**) tail is added to the 3’ end of the cut → prevents degradation and stabilizes translation.
- **Splicing:** see diagram (right) snRNP = 1 snRNA + proteins → 5 snRNPs make up a spliceosome.

**Alternative Processing:** the same transcribed RNA molecule can be processed in multiple ways; thus, a single mRNA molecule can be *translated* into multiple proteins.
- **Alternate Splicing:** different locations of splices and/or different rearrangement of exons
- **Multiple 3’ Cleavage Sites:** several different cleavage sites on the 3’ end affects the position of polyadenylation.

**Note:** *RNA processing* may occur in Euk’s or Prok’s, but **spliceosomal processing** will only occur in eukaryotes.
Highlight #2: Codons and Wobble (15.2)

**Codons:** a sequence of 3 nucleotides on the mRNA (read 5’→3’) which code for a particular amino acid (left) → number of bases^3 = 4^3 = 64 codons (below right)

**Reading Frame:** the non-overlapping sequence which a ribosome reads from the initiator (AUG) to a terminator

**Sense Codons (61):** more codons than amino acids, so some amino acids are coded by multiple degenerate codons

- **Initiator Codon (AUG):** codes for methionine (Met) in eukaryotes and n-formylmethionine (f-met) in prokaryotes

- **Nonsense Codons (3):** terminator codon (ie end translation) UAA, UAG, UGA.

**tRNA Charging:** energy requiring 2-step reaction where **aminoacyl-tRNA synthetase** adds an amino acid (AA) to the 5’-CCA-3’ of the tRNA acceptor arm: AA + tRNA + ATP → aminoacyl-tRNA + AMP + 2P

**Wobble:** flexible base pairing between the 3’ position of the codon and the 5’ of the anticodon.

\[
\begin{align*}
5' & \text{C} (\text{anti}) \text{ and } 3' \text{ G (codon)} \\
5' & \text{G and } 3' \text{ U or C} \\
5' & \text{A and } 3' \text{ U} \\
5' & \text{U and } 3' \text{ A or G} \\
5' & \text{I and } 3' \text{ A, U, or C}
\end{align*}
\]

Week 9 Concept Check:

1. A tRNA has an anticodon **5’-I-U-G-3’**. Which of these mRNA could it not bind? (multiple)
   a. 5’-A-A-C-3’
   b. 5’-C-A-U-3’
   c. 5’-C-A-A-3’
   d. 5’-A-A-U-3’

2. What would not be expected to occur in eukaryotic cells?
   a. Polyadenylation of mRNA transcript
   b. Capping mRNA with a 5’-5’ triphosphodiester-bound modified guanine
   c. Looping the 3’-splice site to form a bond to the 2’-OH of an Adenosine
   d. Joining exons and removing the intron lariat within the nucleus

3. The following RNA sequence would be translated to which amino acid sequence?
   5’-AUGAAAAAUGCAUACUGA-3’ (hint: use codon chart from highlight 2)
   a. fMet-Lys-Leu-Gly-Cys-STOP
   b. Met-Lys-Ile-Ala-Tyr-STOP

*All diagrams, tables and figures are the property of Benjamin A. Pierce; Genetics: A Conceptual Approach*
4. A cell is discovered and is kept alive while the researchers study it. The cell is roughly 8μm in diameter - too unambiguous to determine the cell type. If they look at a higher power and see polyribosomes, what type of cell will this be?
   a. Eukaryotic cell
   b. Prokaryotic cell
   c. Plant cell
   d. You can’t tell because polyribosomes are native to many classes of cells

5. A particular drug binds to and inhibits the recognition site of IF-2’s tRNA binding site. What would be the primary effect of this?
   a. Spliceosomes would not cleave the 5’ splice-site of pre-mRNA
   b. The DNA code would not match the translated protein’s codons
   c. The Shine-Dalgarno sequence would not bind to the small subunit
   d. tRNA\textsubscript{i} would not bind to the AUG codon on the small subunit

6. A particular drug binds to and inhibits the recognition site of IF-2’s tRNA binding site. What does it likely do on a broad scale?
   a. Inhibit plant-cell replication
   b. Inhibit viral replication in animal cells
   c. Inhibit bacterial infection without affecting eukaryotic translation
   d. Preventing tumor formation by inhibiting target gene transcription

7. What does the 5’ splice site bind to form the lariat?
   a. Adenosine Residue (2’-OH)
   b. Guanine Residue (2’-OH)
   c. Cytosine Residue (3’-OH)
   d. Adenosine Residue (5’-OH)

8. What is not an immediate result of EF-Tu bound GTP hydrolysis?
   a. EF-Tu dissociates from ribosome
   b. Peptide bond is formed between the A site and P site
   c. Polypeptide chain is transferred to the tRNA in the A site
   d. Ribosome slides in the 3’ direction down the mRNA
THINGS YOU MAY STRUGGLE WITH:

1. Terminology: “IF” = initiation factor; “eIF” = eukaryotic; “EF” = elongation factor; “RF” = release factors; “AA” amino acids; “A [site]” = aminoacyl site; “P” peptidyl site; “E” exit site; “CBP” = cap-binding proteins; “N-term” = N-terminus (-NH$_3$); “C-term” = C-terminus (-COO$^-$)

2. Eukaryotic cells do not have a Shine-Dalgarno sequence; CBPs help complex mRNA with ribosome (bound to tRNA) and the small subunit “scans” until it finds the AUG codon on mRNA

3. Polyribosomes are single mRNA molecules simultaneously translated by multiple ribosomes used by both prokaryotes and eukaryotes to increase the efficiency of translation

4. Hydrolyzing GTP $\rightarrow$ GDP + P$_i$ causes things to leave or dissociate, or gives energy for movement/bonding

5. Release factors don’t have AA’s bound, but do have anticodons.

CONGRATS; You made it to the end of the resource! Again, group tutoring will be every Thursday from 5:15-6:30 PM. I hope to see you there!

Answers:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>A, D</td>
</tr>
<tr>
<td>2.</td>
<td>C</td>
</tr>
<tr>
<td>3.</td>
<td>B</td>
</tr>
<tr>
<td>4.</td>
<td>D</td>
</tr>
<tr>
<td>5.</td>
<td>D</td>
</tr>
<tr>
<td>6.</td>
<td>A</td>
</tr>
<tr>
<td>7.</td>
<td>D</td>
</tr>
<tr>
<td>8.</td>
<td>D</td>
</tr>
</tbody>
</table>

All diagrams, tables and figures are the property of Benjamin A. Pierce; Genetics: A Conceptual Approach