Lecture 38: DNA Transcription (mRNA), tRNA & Ribosomes

Promoter: DNA sequence that RNA polymerase binds to, initiating mRNA synthesis.

Lac Operator – on/off switch for protein expression

Transcriptional termination signal: Causes RNA polymerase to stop and leave DNA template so that only the gene of interest is transcribed into mRNA.

RNA polymerase (Bacterial)
- Holoenzyme: \( \sigma + \alpha\beta\beta' \)
- Core polymerase = \( \alpha\beta\beta' \) – sufficient for elongation.
- Binds to promoter (P) sequence in a base specific manner via \( \sigma \) subunit.
- Induction of different sigma subunits allows rapid switch of transcription for a large number of genes – e.g. in response to heat shock.
- Uses DNA as a template, making a copy of the template (lower) strand. The upper strand is often called the coding strand, but not all RNAs code for proteins.
- Generates an RNA copy of the DNA template.
- NTPs are polymerized in the 5’→3’ direction. Hydrolysis of P-P drives reaction (ΔG<0, indirect coupling).
- Does not require a primer (makes its own).
- No error checking, the product (mRNA) is ‘disposable’.

Steps in the Production of mRNA:
1. Template binding: Holoenzyme (R) binds only to promoter sites (P), reversibly.
2. "Open complex" formation: A irreversible, committed step, DNA is melted (from -9 to +2).
3. Chain initiation: When the RNA chain is about 10 nucleotides long, σ-subunit dissociates, leaving core enzyme to elongate the RNA processively (i.e. without dissociating from the DNA template).
4. Chain elongation: RNA chain growth is from 5' to 3', and elongation is rapid: about 50 nucleotides/sec.
5. Chain termination: Termination occurs at specific DNA sequences, causing release of mRNA.

Termination in E. Coli:
A. Rho independent termination. 3' end of RNA forms a hairpin structure, causing RNA polymerase to release DNA.
B. Rho dependent termination. Recognition sequence poorly defined, has high cytidine content. Rho uses ATP to track down the RNA 5'→3' until it reaches RNA polymerase, where it causes the release of the polymerase from the DNA.

Inducible Expression of Recombinant Proteins utilizing the Lactose Operon:
- Lac operator – DNA sequence that binds the lac repressor protein, utilized as an on/off switch in the control of recombinant proteins.
- Lac repressor – protein that binds to the lac operator, released from DNA by IPTG
- Lac I gene – chromosomal, produces the lac repressor protein

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TGAACATTTGCTCGGCGCTGATTTGCTGTTGCTGACCGGATAAACATCAGACACAGCGTTATG
-35  -10  --lac operator--
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1. The constitutive expression of high levels of almost any protein is toxic to the bacteria. The protein itself may be toxic, or the simple competition for cellular resources can lead to poor growth of the bacterial host, and in some cases, cell death. Therefore, it is necessary to regulate the production of high levels of recombinant protein.

2. In the Lactose operon production of proteins involved in the metabolism of lactose are controlled by the binding of the lac repressor (a protein that is the product of the lacI gene, produced from the bacterial chromosome) to a region of the DNA near the promoter region of the genes that encode the proteins for lactose metabolism. This segment of DNA is called the lac operator. Although, this system usually controls the expression of enzymes required for metabolism of lactose we can use it to control expression of the HIV mRNA (or any other gene) by simply placing the appropriate DNA segments in the correct location in our expression vector.

3. The lac repressor binds to the DNA when lactose is absent and blocks transcription of the DNA. When lactose is present, it binds to the lac repressor, causing an allosteric change that releases it from the DNA. Since lactose would be rapidly degraded by the bacteria, a non-hydrolyzable analog, isopropyl-thio-galactoside (IPTG), is used instead.

4. Once the lac repressor leaves the DNA, RNA polymerase can bind, allowing production of mRNA that can be used by the ribosome to produce HIV protease.
RNA molecules involved in protein synthesis:
mRNA – messenger RNA is copy of the DNA that encodes a gene. This was made by RNA polymerase. The mRNA specifies the order of amino acids to be used in making the protein.
tRNA – transfer RNA is the dictionary the converts the codon to a specific amino acid. One part of the tRNA recognizes the codon, the other part contains the aminoacid to add.
rRNA – ribosomal RNA is found in the ribosome and is responsible for most of the function in protein synthesis.

tRNA Structure and Function: There are generally 25-45 different tRNAs/organism. The structure of this single chain RNA molecule is stabilized by W-C H-bonds, non-W-C H-bonds, and phosphate-metal (Mg$^{2+}$) interactions.

- **Acceptor stem**: amino acids are attached to the 3’ terminus of the tRNA
- **Anti-codon arm**: contains the anticodon triplet that translates the codon in mRNA to an amino acid. Watson-Crick H-bonds + other basepairing are used to recognize codons.

"Charging" of tRNA by aminoacyl-tRNA Synthetases (aaRS). These enzymes attach the correct amino acid to the correct tRNA. There are ~25-30 of these enzymes, essentially one for each amino acid. This process is often referred to as "charging" the tRNA. There are separate aminoacyl tRNA synthases for each amino acid/tRNA combination. They add the correct amino acid to the correct tRNA. The aminoacyl tRNA synthase shown above will only bind the tRNA for phe, with the anti-codon 3’-AAG-5’, and will only add Phe to the bound tRNA. No other tRNA will be bound and no other amino acid will be added to that tRNA.
Step 1. Activation of the amino acid (2 ATP equivalents)

Step 2. Transfer of activated AA to tRNA

Editing Charged tRNAs:
Many aminoacyl tRNA synthases have editing capacity, e.g. aa-tRNA, will remove valine that has been incorrectly activated to Val-AMP, preventing the attachment of valine to tRNA.

Only the valine sidechain can fit in the editing site, isoleucine cannot.
Codon-Anticodon Interactions + Wobble pairing.

1. Charged tRNAs are selected by ribosomes solely through codon-anticodon interactions.

2. Degeneracy at the third position of codon-anticodon pairing allows multiple codons/tRNA.

Example: pairing combinations for tRNA^Phe (superscript ‘Phe’ indicates that this tRNA will be attached to Phenylalanine.)

- i) The codon-anticodon pairing is anti-parallel, as are most pairings of nucleic acid strands.
- ii) The anticodon on the tRNA is 5'-GAA-3'.
- iii) The complementary codons in the mRNA are 5'-UUC-3' and 5'-UUU-3'.
- iv) The anticodon GAA can pair with either codon due to degeneracy at the third position (wobble basepair).

In addition to G-U pairing, “wobble” basepairs also involve the base inosine in the anti-codon loop can pair with C, U, or A. Inosine is produced by deamination of adenine:

Ribosome - The ribosomal subunits and their RNA components are named for their sedimentation coefficients, S, which is a measure of how rapidly they move when a centrifugal force is applied. 50S+30S=70S

- 30S - mRNA tRNA interactions.
- 50S - peptide bond formation.
- Exit tunnel - where growing protein chain exits the ribosome, amino term first.

tRNA binding sites:
- A site binds the aminoacyl-tRNA (charged tRNA) containing the next amino acid to be added.
- P site holds the peptidyl-tRNA at the start of each cycle - the growing chain. The peptide shifts to the A site, temporarily, during each addition of an amino acid.
- E site is the exit site for the uncharged tRNA, after peptide bond formation.

<table>
<thead>
<tr>
<th>5' Base</th>
<th>Middle Base</th>
<th>3'</th>
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<tbody>
<tr>
<td>U (=T)</td>
<td>Phe Ser Tyr Cys U</td>
<td></td>
</tr>
<tr>
<td>Phe Ser Tyr Cys C</td>
<td>Leu Ser Term Term A</td>
<td></td>
</tr>
<tr>
<td>Leu Ser Term Trp G</td>
<td></td>
<td></td>
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</tbody>
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50S Subunit
- 31 Proteins
- 23S and 5S rRNA

Responsible for peptide bond formation.

Protein emerges through exit tunnel.

30S Subunit
- 21 proteins
- 16S rRNA - binds to Ribosome Binding Site (SD) on mRNA.

Responsible for mRNA-tRNA pairing.