**Lecture 40: Protein Synthesis (mRNA translation) & Protein Export**

**Given the following mRNA molecule, with the** underlined bases representing the ribosome binding site, identify the correct starting codon.

i) Which AUG is used to start?

 **G**AAUUGUGAGCGGAUAACAAUUUCACAC**AGGAGG**AACAGCUAUGAAAGCUUAAUUUAUG**...**

ii) Given the following tRNAAla molecule. Indicate the anticodon loop and the location of attachment of the amino acid. What amino acid would be attached?

iii) If the codon for alanine is 5’GCT3’, what is the anti-codon?

iv) Label the tRNA sites on the ribosome shown on the right.

**Protein Synthesis - Overview:**

1. The information content of the mRNA is *translated* into a polypeptide chain by the **ribosome**. Three nucleotide bases, or a **codon**, encode each amino acid.

1. mRNA is the template, and synthesis of the polypeptide chain proceeds in the **amino→carboxy** direction as new amino acids are added to the carboxy terminus of the growing peptide chain.

**Step I - Chain Initiation:**

**N-formylmethionine** (fMet) and its charged tRNA function uniquely to initiate chains. The N-formyl group mimics a peptide bond, allowing the first methionine to bind to the site on the ribosome that normally holds the growing peptide.

Steps in formation of **Initiation Complex**:

i) mRNA first associates with the 30S subunit – the ribosome binding site (also called the Shine-Dalgarno sequence) forms Watson-Crick hydrogen bonds to a region at the 3' end of 16S rRNA in the 30S subunit of the ribosome.

ii) fMet-tRNAfMet and the 50S subunit combine to form the 70S initiation complex.

**Step II - Chain Elongation: (**Synthesis of Met-Lys-Ala; mRNA = SD-AUG-AAA-GCU-UAA) RBS = ribosome binding site)

a) fMet-tRNA occupies the P site in the first cycle. Growing protein is found here at the beginning of subsequent cycles.

b) aminoacyl-tRNA-Lys (next AA to be added) is brought to the A site.

c) Peptidyl transfer, or **transpeptidation** occurs - the free amino group of the amino acid in the A-site performs a nucleophilic attack on the carbonyl group of the amino acid (or peptide) attached to the tRNA in the P-site. No energy is required to form the peptide bond.

d) Translocation of the ribosome occurs.

e) The uncharged tRNA leaves the E site.

**Step III - Chain Termination:**

Protein release factor (which is a protein that looks like a tRNA) and a stop codon are required to finish the polypeptide chain. Water hydrolyzes the peptide from the P-site, releasing the complete tri-peptide. The mRNA is released and the ribosome 30s and 50s subunits dissociate.

**B. Export of Recombinant Proteins.**

A specialized *protein* sequence, called the leader peptide, when present as the amino terminal residues of the protein, signals the export of the protein out of the cell. This may reduce the toxicity of the protein and make purification easier since only a small number of native proteins are exported out of the cell.

During the export process, this peptide is cleaved by the **leader** **peptidase** (also known as signal peptidase), producing the mature exported peptide.

The main features of this peptide are:

i) Basic residue at amino terminus

ii) Non-polar segment of ~15 amino acids.

iii) Cleavage site, which is followed by another basic residue (---Ala^Arg---)

A) The complete DNA sequence of expression plasmid, from the promoter to the stop codon is:

-35 -10 mRNA Start RBS Start-----Leader peptide------

**TTGACA**TTTATGCTTCCGGCTCG**TATAAT**GTGTG**G**AAT**TGTGAGCGGATAACAATTTCACACA**GGAGGAACAGCT**ATG**AAACAATCCACAATTGCGCTCGCGCTT

**Promoter Lac operator fMet**LysGlnSerThrIleAlaLeuAlaLeu

-----Leader peptide (cont)----------------|---HIV---Protease-|-----|--His Tag-------|

CTCCCGCTACTATTTACTCCAGTGACCAAAGCGCGC**GAATTC**CCTCAGATC-ttaaatttc**GGATCC**CACCACCACCACCACCACTAA.....

LeuProLeuLeuPheThrProValThrLysAlaArg**GluPhe**ProGlnIle-LeuAsnPheGlySerHisHisHisHisHisHis**Stop**

^ **EcoR1 BamHI**

B) *The peptide, as it comes off of the ribosome will look like:*

**-------------------------Leader Sequence---------------------------------|----HIV protease--| |His Tag..**

**fMet**LysGlnSerThrIleAlaLeuAlaLeuLeuProLeuLeuPheThrProValThrLysAlaArg**GluPhe**ProGlnIle..LeuAsnPhe**GlySer**His6

 **^**

C) After export out of the cell the final product will be as follows (The bold amino acids were added as part of the EcoR1 and BamH1 sites that were required to insert the PCR product into the expression vector.):

 **|----HIV protease----| |----His Tag-------|**

 Arg**GluPhe**ProGlnIle----LeuAsnPhe**GlySer**HisHisHisHisHisHis

**Protein Export Machinery**

1. mRNA binds to the ribosome.

2. When leader peptide emerges from the ribosome it binds to the signal recognition particle (SRP).

3. The ribosome/mRNA/SRP binds to transport machinery(translocon) in the cell membrane.

4. Protein synthesis continues, protein extruded outside the cell.

5. Leader peptidase cleaves off leader peptide.

6. Protein is completely exported out of the cell.

**C. Affinity Purification**:His6-tag binds to immobilized nickel ions on chromatograph beads – allowing one-step purification of HIV protease from culture media via affinity chromatography. Elution with imidazole (competes with His sidechain for Ni+).



 Cell ← Wash → Elute

 lysate

**Summary of Expression Features:**



|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Promoter** | **Lac O** | **RBS** | **Start codon** | **Leader codons** | **HIV P codons** | **His-tag codons** | **Stop codon** | **mRNA term** |
| **mRNA production** | **X** | **X** |  |  |  |  |  |  | **X** |
| **Protein Production** |  |  | **X** | **X** | **X** | **X** | **X** | **X** |  |
| **Protein Export** |  |  |  |  | **X** |  |  |  |  |
| **Protein Purification** |  |  |  |  |  |  | **X** |  |  |

**Comparison of Expression Hosts:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Host** | **Advantages** | **Disadvantages** | **Typical Use** |
| **Bacteria (E. Coli)** | • Easy DNA manipulation• High protein yields | • Lack of post-translational modification (e.g. glycosylation)• Toxic impurities. | • Laboratory research• Diagnostic reagents |
| **Yeast** | • Similar post-translational modification as mammals.• No toxic impurities. | • DNA manipulations more difficult.• Lower protein yield (typically, but not always). | • Laboratory research• Food processing enzymes |
| **Mammalian cells** | • Equivalent protein modification as human proteins.• No toxic impurities | • DNA manipulations more difficult.• Cell culture expensive.• Protein yields can be low. | • Proteins that are used as drugs (e.g. human growth hormone, antibodies.) |

**T7 Expression system** – also widely used.

* T7 Promoter 5’ to coding region is only recognized by T7 RNAP.
* Gene for T7 RNAP is located on chromosome, under control of lac.
* Addition of IPTG induces production of T7 RNAP, followed by production of protein from T7 promoter – only protein that is made is the desired protein.