Protein Sequence - coded only by mRNA sequence

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**INITIATION**

Codon Recognition by tRNA Anticodon

       tRNA molecules recognize more than one codon

Translation II

**wobble hypothesis** (Crick) :

       (first two codon bases & last two anticodon) bases form Watson-Crick base pairs

       BUT pairing between (3rd base of codon & first base of anticodon) follows less stringent rules

**Inosine** - appears in several anticodons & can be read by tRNA molecule

       maximizes # codons by allowing **steric freedom** / wobble of 3rd base of codon

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Example. Anticodon for yeast tRNA(Ala) is 5'-IGC-3', which recognizes codons 5'-3' GCU, GCC, GCA

**I on anticodon recognizes G,C,A on codon**

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2 Generalizations:

1.       codons differ in either of first 2 bases must be recognized by different tRNAs

2.       first base of anticodon determines whether tRNA reads 1, 2, 3 kinds of codons

**16S rRNA** - monitors base paring between codon & anticodon

i.e. 16S RNA A1493, A1492, G530 monitors: Anticodon A36 & Codon U1

**16S RNA A1493, A1492** - proofread codon/anticodon pairs

Prokaryotes:

Translation Initiation

       **initiation factors** - **P-loop NTPases** (G protein) which aid assembly of:

1.       30S initiation complex

2.       70S initiation complex by recruiting 50S complex

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Events in Peptide Chain initiation:

1.       **30S Subunit = {IF-3, IF-1}** - prevents premature joining of 50S

2.       add in **N-formyl-Methionine-IF2 + \*GTP\*** on **Shine-Dalgarno Sequence** on mRNA before **AUG**

3.       (2) complex bindsn ear A site

4.       **IF-2** stimulates association of 50S to complex

5.       **IF-2, GDP, P, IF-1, IF-3** leave, leaving **N-formyl-Met-UAC** in **P site**

6.       ready of elongation

**ELONGATION**

**Elongation Factors** - deliver amino acyl tRNAs to ribosome

       EF-Tu, Guanine nucleotide

EF-Tu Binding to Amino Acyl tRNA

       EF-Tu **protects ester linkage** in Amino Acyl tRNA from Hydrolysis

       GTP hydrolysis in EF-Tu occurs only when appropriate base paring occurs with codon-anticodon

       allows free energy of **GTP hydrolysis to contribute fidelity** of protein synthesis

       EF-Tu will **not interact with fMEt-tRNAf**

       **IF2** will **only recognize fMet-tRNAf**

Cycling of EF-Tu-GTP - similar to G protein receptor Cycle

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Translocation Mechanism

1.       EF-G binds 50S

2.       GTP Hydrolysis

3.       Conformational change in EF-G

4.       tRNA & mRNA forced to move through ribosome by distance of 1 codon

5.       **EF-G Translocase** dissociates, leaving ribosome ready to accept next amino acyl tRNA

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Elongation Factors

**G protein** - heterotrimers that bind guanine nucleotides GDP & GTP

**EF-Tu** - G protein that binds aminoacyl-tRNA & delivers to A site

**EF-Ts** - Guanine nucleotide exchange factor (GEF) that replaces GDP on EF-Tu with GTP

**EF-G** - G protein that promotes translocation of mRNA to accept next amino acid

       structure resembles EF-Tu + tRNA

**TERMINATION**

Translation Termination

       if STOP codon i.e. **UAA, UGA, UAG**: Amino Acyl tRNA doesn't bind to A site

 - recognized by **release factors**

**Termination / Release Factor** - contain water for Hydrolysis of polypeptide

1.       **RF1 / RF2** recognize stop codons (UAA & UGA) in A site

& **RF-3** - binds GTP & stimulates RF1 & RF2 binding

2.       stimulates release of completed protein from tRNA in P site

       entire complex dissociated in GTP-dependent fashion

**EUKARYOTIC VS PROKARYOTIC PROTEIN SYNTHESIS**

       difference primarily in translation initiation

       initiator tRNA is methionine & not formyl-Met

       initiator codon always AUG (nearest to the 5' end of mRNA)

       scanning process powered by ATP Hydrolysis

Eukaryotes: Initiation:

Protein Interactions: **Circularize Eukaryotic mRNA**

       Poly A binding protein binds to cap

       enhances ribosomal recycling

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Eukaryotes: Translocation & Termination

1.       EF1 alpha and beta-gamma = EF-Tu and EF-Ts

2.       EF2 - mediates GTP driven translocation (EF-G)

3.       termination carried out by single RF (**eRF1** - recognizes 3 stop codons)

Antibiotics: Interfere with Translation

**Streptomycin** - inhibit initiation & cause prokaryotic mRNA misreading

       highly basic

       interferes with fMet-tRNAf binding , preventing initiation

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**Tetracycline** - binds to 30S subunit & inhibits aminoacyl-tRNA binding

**Chloramphenicol** - inhibits peptidyl transferase activity of 50S ribosomal subunit

**Erythromycin** - binds to 50S subunit & inhibits translocation

Eukaryotes

**Cycloheximide** - inhibites peptidyl transferase activity of 60S ribosomal subunit

Both

**Puromycin** - causes premature chain termination by acting as analog of aminoacyl-tRNA

       analog of terminal amino acyl A part of amino acyl tRNA - dissociated from ribosome

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