Biogenesis
Translation

Translation

- Process of converting information stored in nucleic acid sequences into proteins
- Sequences of mRNA (messenger RNA) are translated into unique sequence of amino acids in a polypeptide chain (linear order is preserved throughout !)

Translation

- Takes place in the cytoplasm
  - Exception are few proteins coded by mitochondrial and chloroplastic DNA
- Performed on ribosomes

What is necessary?

- Template - mRNA
- tRNAs (transfer RNAs)
  - Linked to amino acids
- Ribosomes
- Many accessory proteins
- Some energy (GTP hydrolysis)

mRNA

- Single stranded molecule of RNA that encodes sequence of the polypeptide
- Transcribed and processed in the nucleus and then exported into cytoplasm
- 5' end has binding sites for translation initiation
- Middle is a coding sequence
- 3' end regulates stability of mRNA

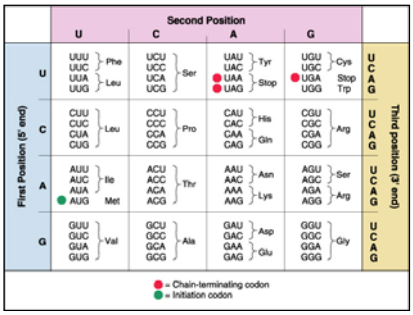
Genetic code

- Codon – specifies the sequence of amino acids
- Initiation (start) codon
  - AUG – methionine
  - Every protein in a cell starts with methionine
- Termination (stop) codons
  - UAA, UGA, UAG

Genetic code

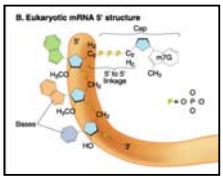
- Universal
- Degenerate - some amino acids are specified by more than one codon
  - 64 possible codons and only 20 amino acids

Genetic code



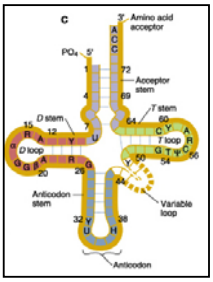
Eukaryotic mRNA

- Codes for one protein
- Is capped on both 5' and 3' ends
- 5' cap - methylated base
  - Protects from nucleases
- 3' poly-A tail
  - 50-200 adenines added post-transcriptionally
  - Protects mRNA from degradation



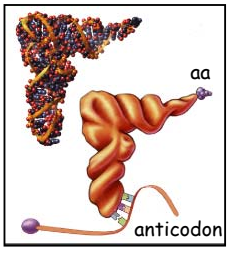
tRNA

- Deliver amino acids to the translational complex
- Serve as adapters between codons in mRNA and amino acid
- 4 stems and 3 loops
  - Anticodon – decoding triplet - localized on the anticodon stem



tRNA

- L-shaped secondary structure
- Anticodon and amino acid are at the opposite arm of the L

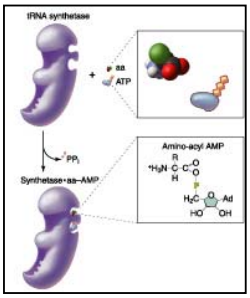


Charging of tRNA

- Linking amino acids to correct t-RNAs
- Catalyzed by aminoacyl-tRNA synthetase (aa-tRNA)
  - Couples an amino acid to its cognate tRNA
  - Fidelity of coupling – 20 different synthetases
- Two steps
  - Activation of amino acid
  - Transfer of amino acid to tRNA

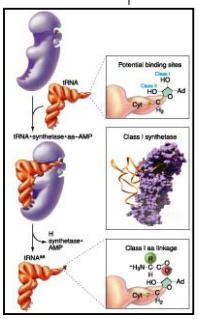
Charging of tRNA

- Activation of amino acid by ATP ⇒ amino acyl AMP
- High energy intermediate



Charging of tRNA

- Transfer of amino acid from amino acyl AMP to 3' end of tRNA (adenine)
- Covalent bond
- High energy bond prepares amino acid for the formation of peptide bond with nascent peptide

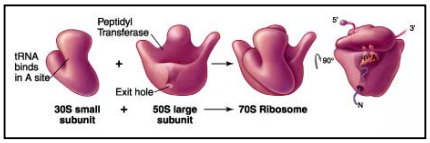


Ribosomes

- RNA-protein complexes (ribonucleoproteins)
- Place of translation (protein synthesis)
- Abundant in cells that synthesize large amounts of protein
- Structurally and functionally similar among species (differ between prokaryotes and eukaryotes)

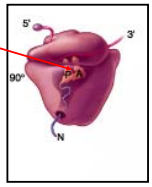
Ribosomes

- Composed of small and large subunit
- Subunits bind together for translation
- Ribosomes self-assemble without additional factors



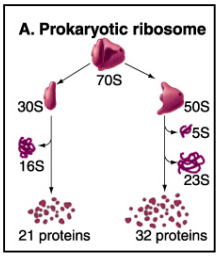
Ribosomes

- Decoding and synthesis takes place in the cavity between subunits
- Ribosomes move along mRNA chain during translation
- New peptide exits through the tunnel in the large subunit



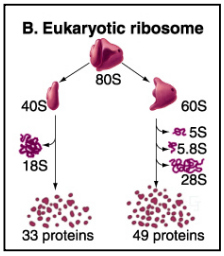
Ribosomes

- Contain rRNA molecules and proteins
- In prokaryotes
  - Large subunit contains rRNA (5S and 23S) and 32 proteins
  - Small subunit contains 16S rRNA and 21 proteins



Ribosomes

- In eukaryotes
  - Large subunit contains rRNA (5S, 5.8S and 28S) and 49 proteins
  - Small subunit contains 18S rRNA and 33 proteins



Ribosomes

- Cytosolic (free)
- Bound to ER
- Also located in mitochondria and chloroplasts of eukaryotic cells

Free ribosomes

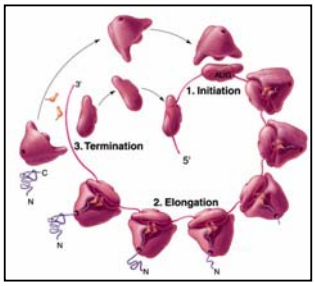
- Found in the cytosol
- May exist as a single ribosome or in groups known as polysomes
- Occur in greater number than bound ribosomes in cells that retain most of their manufactured protein in the cytosol

Bound ribosomes

- Bound to the exterior of the rough endoplasmic reticulum
- Occur in greater number than free ribosomes in cells that secrete their manufactured proteins (e.g., pancreatic cells)

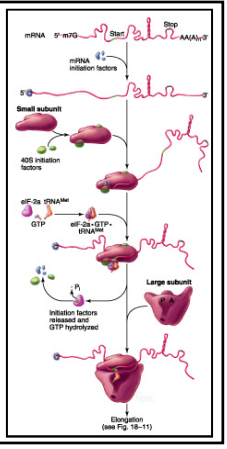
Protein synthesis

- Initiation
- Elongation
- Termination



Initiation

- Attachment of initiator tRNA (Met-tRNA) to start codon on mRNA and assembly of ribosomal subunits

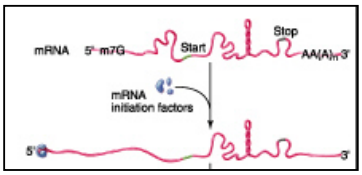


Initiation

- Several initiation factors assist the process
- Strongly regulated process
- Uses energy form ATP or GTP hydrolysis

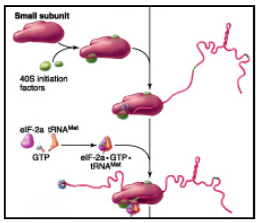
Initiation

- Cap recognition
  - mRNA initiation factors
  - Helicase (ATPase) unwinds mRNA



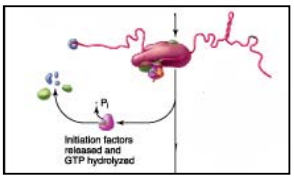
Initiation

- Formation of preinitiation complex
  - Initiator tRNA (Met-tRNA) binds to small ribosomal subunit
  - Initiation factors use GTP hydrolysis



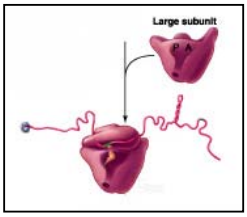
Initiation

- Unwinding and scanning
  - Complex scans and binds to start codon
  - Dissociation of initiation factors



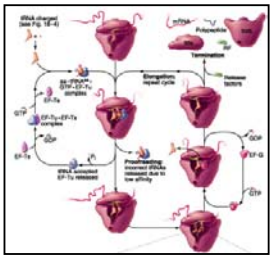
Initiation

- Association with large ribosomal subunit



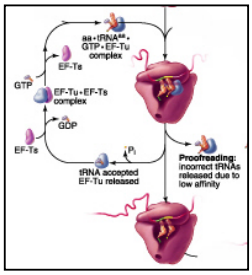
Elongation

- Repetitive cycles of codon directed addition of aa-tRNA
  - Aa-tRNA binding
  - Proofreading
  - Peptidyl transfer
  - Translocation



Elongation

- Aa-tRNA binding
  - Elongation factor eEF-1 delivers amino acid-tRNA to ribosome
  - eEF-1 is bound to GTP



Elongation

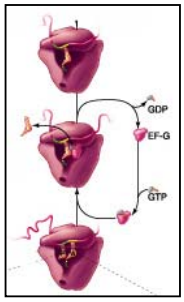
- Proofreading
  - Aa-tRNA is checked against codon
  - GTP hydrolysis and dissociation of eEF-1 from ribosome
  - Correct amino acid-tRNA retained based on codon-anticodon pairing
  - Incorrect amino acid-tRNA escape from the ribosome

Elongation

- Peptidyl transfer
- Large rRNA catalyzes formation of peptide bond
- Precise orientation and stabilization of the transition state

Elongation

- Translocation
- Binding EF-2-GTP
  - Movement of peptidyl-tRNA to P site
  - Sliding of mRNA by 3 bases
  - tRNA moved to the exit
- GTP hydrolysis and release of EF-2



Termination

- Protein factor eTF binds to stop codon and catalyzes hydrolysis of last amino acid-tRNA
- Peptide is released from the ribosome
- Ribosomal subunits dissociate

Polysomes

- In eukaryotes the same molecule of mRNA can be simultaneously translated several times
- Each emerging peptide is synthesized on a separate ribosome
- Many ribosomes on the same “string” of mRNA are called polysomes

What happens after translation?

- A newly synthesized polypeptide chain must undergo post-translational processing to generate the final protein
- Posttranslational modifications include
  - Targeting to the appropriate cell compartment
  - Folding
  - Addition of sugar chains
  - Formation of disulfide bonds

What happens after translation?

- Posttranslational modifications start as soon as the nascent polypeptide emerges from the tunnel in large ribosomal subunit
  - That means these changes happen during translation when the peptide has NOT been finished yet and is still attached to the ribosome (cotranslationally)