Molecular Biology

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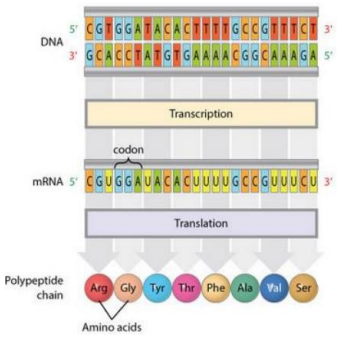
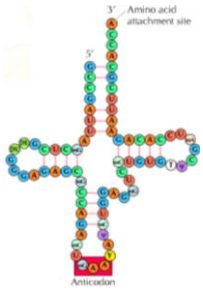
DR.Ahmad Al Qawasmi

ARKAN ACADEMY

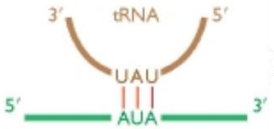
Translation



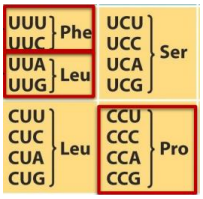
- It is the process of *producing proteins* from the information encoded in the mRNA which requires 3 types of RNA: mRNA, tRNA, rRNA
  - It occurs in the *ribosomes* (protein factory)
- DNA, RNA and proteins are ***Colinear*** meaning that any mutation in the DNA will affect all of them in the same site



- ***tRNA*** *carries amino acids* from the cytosol into the ribosome
  - It is a *short single-stranded RNA* molecules (80 bases long)
  - ***Charged or Activated*** tRNA carries one amino acid at the ***3' end***
  - tRNAs contain stem loop structure, modified and unusual bases such as inosine which is derived from Adenine by deamination
- The genetic information encodes for amino acids in the form of ***3 nucleotide*** codons
  - There are ***64*** possible codons of the genetic code
  - ***61*** codons specify *amino acids*
    - ✓ Each ***codon*** specifies ***only 1 amino acid***
    - ✓ An ***amino acid*** can be encoded by ***more than 1 codon*** (genetic code is ***degenerate***)
  - ***3*** *stop codons* (UAA , UAG, UGA) do not encode any amino acid
  - Mitochondrial mRNA (mtDNA) genetic code is typical of the universal code except for few variants

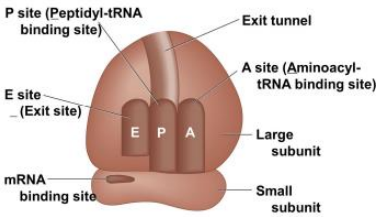


- ***Codon:*** 3 nucleotide sequence on the *mRNA*
- ***Anti-codon:*** 3 nucleotide sequence on the *tRNA*, which is **antiparallel** and complementary to the mRNA codon
- Accurate translation requires two steps:
  - Accurate association of *amino acid to tRNA* via specific interactions determined by the anticodon
  - A correct match between the *tRNA anticodon and the mRNA codon*
    - ✓ ***Wobble base pairing:*** **flexible pairing at the 3rd** base of a codon to the anticodon allowing some tRNAs to bind to more than one codon
    - ✓ An amino acid is usually encoded in many codons sharing the 1st and 2nd nucleotides with some variety of the 3rd nucleotide which acts as a buffer against deleterious mutations



- Ribosomes are the sites of protein synthesis, and they are composed of **proteins** and **rRNAs**
  - They facilitate specific coupling of tRNA anticodons with mRNA codons in protein synthesis
  - The ***RNA*** components are responsible for the ***catalytic function*** of the ribosome
    - ✓ The *peptidyl transferase reaction* of a peptide bond is catalyzed by the rRNA of the large subunit
  - The ***protein*** components ***enhance*** the function of the rRNA molecules
  - Translation occurs from ***N-terminus to C-terminus*** on the polypeptide, and ***5' to 3'*** on mRNA, where the new amino acid is added on the C-terminus

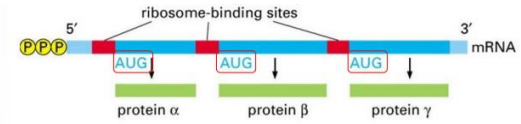
- E. coli contains about 20,000 ribosomes (~25% of the dry weight of the cell)
- Rapidly growing mammalian cells contain about 10 million ribosomes
- Ribosomes consist of 2 subunits (large and small)
- The large subunit has 3 chambers (A, P, E sites)
  - The **P site** holds the tRNA that carries the *growing polypeptide* chain
  - The **A site** holds the tRNA that carries the *next amino acid* to be added to the chain
  - The **E site** is the exit site, where discharged tRNAs *leave* the ribosome



Initiation of Translation

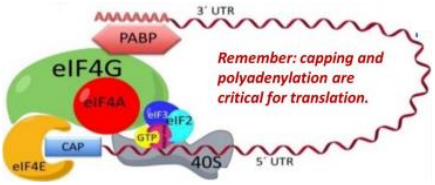
- The first amino acid is added on the site of where **starting codon (AUG)** is read, not from the first codon on the mRNA (codons before AUG are skipped and untranslated)

- **Prokaryotes:** AUG encodes ***N-formyl Methionine***
  - The small ribosomal subunit recognizes the mRNA molecule via *Shine-Dalgarno sequence* upstream the starting codon of and represents a ribosomal-binding site



- **Eukaryotes:** AUG encodes ***Methionine***
  - ***eIF2*** forms a *complex of tRNA and small ribosomal* subunit
    - ✓ eIF2 is activated by ***GTP*** to bind the correct tRNA, after binding GTP hydrolyzed into GDP
    - ✓ Active eIF2/GTP complex must be regenerated by exchanging of the GDP for GTP
  - The small ribosomal subunit recognizes the mRNA molecule via the *5'-cap* (7-mthylguanosine) aided by eIF4

  - Roles of ***eIF4***:
    - ✓ eIF4 form a complex that *links the poly-A tail to the Cap* via poly-A binding protein (***PABP***)
    - ✓ It can *recognize internal ribosome entry site* (*IRES*) exist in mRNAs (when the 5'-cap is not recognized)
    - ✓ Induce the *recruitment of the mRNA* to the tRNA-small ribosomal subunit complex

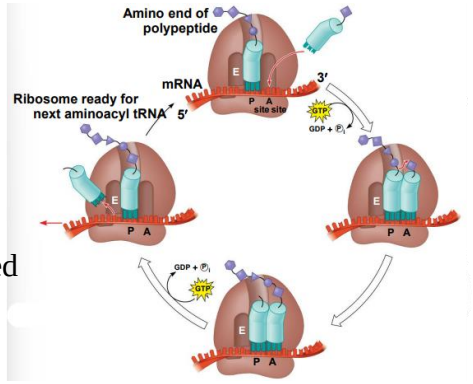


  - tRNA + small subunit form a complex, then mRNA joins, the last one to join is the large subunit

- ***Untranslated regions (UTRs):*** sequences on the mRNA, not translated and do not encode protein
  - *5'-UTRs:* upstream the starting codon
  - *3'-UTRs:* downstream the stop codon

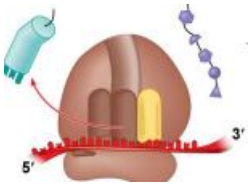
Elongation of Translation

- Involves 3 steps:
  - ***Aminoacyl-tRNA*** binding to the **A site**
    - ✓ *eEF1α* brings next aminoacyl-tRNA to the A chamber
  - ***Peptide bond formation*** between the C-terminus (on P site) and added amino acids (on site A)
  - ***Translocation*** of the charged tRNA **into P site** and the uncharged tRNA **into the E site** to leave the ribosome
    - ✓ *eEF2* is critical in ribosomal translocation

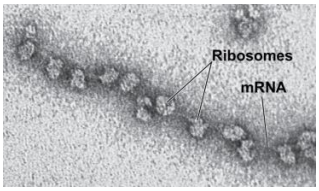


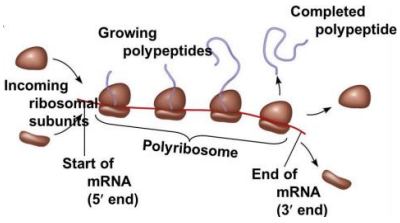
Termination of Translation

- The codons ***UAA***, ***UAG***, and ***UGA*** are the stop signals recognized by a ***releasing factor*** (not tRNA) on the empty A site, which uses 2 GTP for the release of the polypeptide and dissociation of the translation assembly
  - After dissociation, the ribosome can be **reused** in further rounds of translation



- Translation and transcription are *coupled in space and time* in **prokaryotes**
- In **eukaryotes** they are *not coupled* due to the membrane-bound nucleus and RNA processing
- ***Polyribosome (polysome)*** can occur in both eukaryote and prokaryote
- *Many ribosomes* are translating the *same mRNA* molecule simultaneously enabling a cell to make *many copies of a polypeptide very quickly*





Regulation of Translation

- Translation is inhibited by ***kinases*** that *phosphorylate eIF2 and eIF2B* blocking the GTP/GDP exchange
  - *Reticulocytes* (immature erythrocytes) undergoes maturation into mature RBCs when adequate heme is available, which stimulates overall protein (hemoglobin) synthesis
  - If heme supplies are inadequate, protein kinase is activated and phosphorylates eIF2 to inhibit translation and *block hemoglobin synthesis*

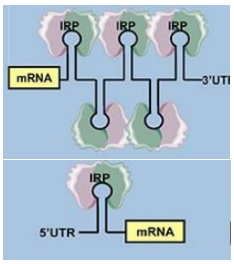
- ***Translational repressors*** can bind to regulatory sequences in the *3′ untranslated region (UTR)* and inhibit translation and bind *eIF4E* (bound to the 5′ cap), blocking formation of normal initiation complex

- Iron is an essential metal for the human body important for Oxygen transport and Enzymatic functions
  - Too much iron can be toxic causing organ failure and Bacterial infection
  - The level of iron is intricately maintained

- ***Liver ferritin protein:*** **stores** 4000 iron atoms when abundant
  - It must be upregulated at *high iron levels*, and down regulated at low iron levels

- ***Transferrin receptor*** mediates iron entry (**uptake**) to peripheral cells via receptor-mediated endocytosis
  - It is upregulated at *low iron levels*, and down regulated at high iron levels

- ***IRE:*** Iron regulatory elements
  - Present *upstream* the coding region in the ferritin mRNA (near the 5' cap)
  - Present *downstream* the coding region in the transferrin receptor mRNA



- ***IRP:*** Iron regulatory protein
  - *At low iron levels*, IRP (1,2) bind the IRE in both mRNAs
    - ✓ In ferritin mRNA, it blocks translation by interfering binding of mRNA to 40S ribosomal subunit *inhibiting synthesis of ferritin* and prevent storage
    - ✓ In transferrin receptor mRNA, it stabilizes mRNA and *enhances synthesis of transferrin receptor* to increase iron uptake
  - *At high iron levels*, IRP1 production is inhibited and IRP2 is degraded
    - ✓ *Enhancing synthesis of ferritin*, increasing iron storage
    - ✓ *Inhibiting synthesis of transferrin receptor*, reducing iron uptake

- ***MicroRNA (miRNA)***
  - It is a short RNA molecule produced by RNA polymerase II
  - Primary micro RNA (pri-miRNA) is single stranded, then processed into ***double stranded*** miRNA
  - Ine of the 2 strands is loaded to a translation repressor protein called ***RISC*** (RNA-induced silencing complex) which binds the *3'-UTR* inducing mRNA degradation or translation inhibition

- ***Short interfering RNA (siRNA)*** are double stranded RNA can be synthetic
  - It can be associated with ***RISC*** by one strand and target mRNA molecules causing them to be cleaved to inhibit translation and block expression
  - It can be used for experimental and therapeutic purposes

- ***Misfolded and unfolded proteins*** are degraded either in:
  - Degradative subcellular organelles like ***lysosomes***
  - ***Macromolecular proteasomes*** when they are *uniquitinylated*

Chaperon are complexes that refolds protein, but if it is unable to be folded it will be degraded

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www.arkan-academy.com

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+962 790408805