6.3 Translation: Synthesizing Proteins from mRNA

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tRNA

Transfer RNA (tRNA): an RNA molecule that links the codons on mRNA to the corresponding amino acid for protein synthesis.

Each tRNA has 2 functional regions:

Anticodon loop: sequence of three nucleotides that are complementary to an mRNA codon.

Acceptor Stem: single-stranded region where an amino acid is attached.

tRNA Structure

The anticodon region binds to the correct mRNA sequence. Whereas the acceptor stem required the action of an enzyme to attach the appropriate amino acid to the polypeptide sequence.

Aminoacyl-tRNA synthetase enzymes are responsible for attaching the correct amino acids.



tRNA Synthetase



Each enzyme is specific for one amino acid. When the amino acid binds to the 3' end of the tRNA molecule, ATP is required.

tRNA & Ribosomes

<u>Ribosomes</u>: a cell structure composed of proteins and rRNA that provides the site where protein synthesis occurs.

RIBOSOME SUBUNITS



All prokaryotic and eukaryotic ribosomes are composed of two subunits: large and small subunit.

The large subunit contains 3 sites for binding of the tRNA. It also has a binding site for mRNA.



The small subunit of the ribosome ensures that there is correct pairing between the anticodon and the codon on the mRNA.

Ribosomes



- <u>A site:</u> charge tRNA anticodon binds to the mRNA codon
- <u>P site:</u> tRNA adds the amino acid to the polypeptide chain
- <u>E site:</u> tRNA releases the amino acid and waits to be removed from the ribosome into the cytoplasm

Ribosomes & tRNA

tRNA will move through all three sites on the ribosome and continuously add amino acids to the polypeptide sequence. Remember, there can be three tRNAs in the ribosome because they are highly specialized for one codon.

The third nucleotide of the anticodon on the tRNA is flexible in terms of what it will bind to.



Ribosomes

There are many ribosomes that attach themselves to the RNA at one time. This is known as *'polyribosome'.*



Translation

The process of translation occurs in 3 main steps:

1. <u>Initiation</u>: ribosomal subunits bind to the mRNA and travel to the start codon, whereby a tRNA will bind.

2. <u>Elongation</u>: the ribosome travels across the mRNA strand and bind different tRNA molecules containing the correct anticodon and amino acids. New amino acids are added to the polypeptide strand.

3. <u>Termination</u>: When the stop codon enters the A site of the ribosome and hydrolysis of the peptide bond between tRNA and amino acid occurs. New polypeptide strand is released into the cytoplasm.

Step 1: Initiation (Prokaryotic Cells)

The small ribosomal subunit binds to the start codon AUG of the mRNA sequence. The first tRNA that contains the methionine amino acid also binds to the start codon. (tRNA sequence- UAC)



GTP and other proteins are required to bring in the large ribosomal subunit. The tRNA positions itself in the P site of the ribosome.

The A-site is now available for another tRNA to bind to the next codon.

Step 1: Initiation (Eukaryotic Cells)

The small ribosomal subunit binds to the 5' cap and moves through the mRNA until it reaches the start codon AUG. At this moment, the tRNA will bind along with the large ribosomal subunit.



In both prokaryotic and eukaryotic cell, the first methionine amino acid makes up the N-terminus and the last amino acid makes up the carboxyl end (C-terminus)



Step 3: Termination

The ribosome continues to travel along the mRNA until it reaches the stop codon. The base triplets UAG, UAA and UGA are stop codons and do not code for an amino acid.



A protein (i.e release factor) binds to the A site and helps to release the ribosomal subunits and the tRNA. The last amino acid bound to the tRNA is cut off and polypeptide is released.

Overview of Gene Expression



Checking for Understanding

The triplet of nucleotides in tRNA that is complementary to a triple of nucleotides in RNA is called a(n)

A) codon

B) anticodon

C) ribosome

D) genetic code

E) sequence

Checking for Understanding

How is transcription directly controlled in eukaryotic cells?

- A) through the use of phosphorylation
- B) through the use of operons
- C) transcription factors and activators
- D) through condensed chromatin which allows constant gene activation
- E) through the addition of a 5' cap and a 3' poly-A tail

Homework

Textbook: p. 260 # 13 - 18



Single Gene Mutations

Single-gene mutations may also be categorized according to how they affect the amino acid sequence of a protein.

<u>Silent Mutations:</u>

•Missense Mutation:

•Nonsense Mutations:

1) Silent Mutations

Involves the replacement of one nucleotide with another pair of nucleotides that have no effect on the encoded protein.



Both nucleotide sequences, when transcribed into mRNA they will both code for the same amino acid (i.e glycine)

At times there may be an amino acid change, however the structure of the protein is no compromised.

Missense Mutations

One nucleotide change may cause the wrong amino acid sequence to be inserted into the polypeptide sequence. This amino acid change, may have been an important amino acid that played a vital role in the function of the protein.



If the amino aid has an important function, a mutation in that sequence may cause the protein to be defective or change it role in the cell.

Nonsense Mutations

If there is a change in the nucleotide sequence and it results in a premature stop codon, termination will occur sooner.



The mRNA will be much shorter and may not contain the necessary amino acids to create the correct protein.

Single Gene Mutations

Single gene mutations may also involve the insertion or deletion of a nucleotide.

• <u>Frameshit mutations</u>: result from the insertion or deletion of nucleotides not divisible by three. This causes a change in the reading frame.

wild type	UGU	AAG	AGGA	AUC	UCA	AGC	ս ս ս	AUG	GUC
	Cys	Lys	Arg	lle	Ser	Ser	Phe	Met	Val
mutant	UGU	AAG	AGGA	AUC		AGC	ս ս ս	AUG	d u c
	Cys	Lys	Arg	lle	Stop	Ser	Phe	Met	Val

Chromosome Mutations

There are 4 main types of chromosome mutations that may affect many genes.

<u>1</u>) **Deletion:** a series of nucleotides are deleted from the chromosome

<u>2)</u> Duplication: a series of nucleotides are duplicated on the same chromosome

3) Inversion: a group of nucleotide sequences are inverted in the chromosome

<u>4)</u> Reciprocal Translocation: a group of nucleotides from one chromosome are exchanged with the nucleotides of a different chromosome.

Chromosome Mutations

Causes of Mutations





Formation of a Mutation

Many mutations occur spontaneously during the process of replication. Some mutations may also be due to the DNA transposition.



Certain segments of the DNA will move from one location to another. Segments that can undergo this process are known as 'transposable elements' (i.e. transposons)

Formation of a Mutation

Mutagens:

Physical Mutagen:

Chemical Mutagen:

DNA Repair

If mutations are detrimental and accumulate too quickly, the cell needs to repair the mutation so that certain proteins can be restored.

Specific Repair Mechanisms:

Non-Specific Repair Mechanisms:

Specific Repair

Photorepair is a specific mechanism to repair damage to DNA caused by exposure to UV radiation.

A photolyase enzyme recognizes the damage, binds to the dimer and uses visible light to cleave the dimer.





Non Specific Repair

Excision repair I a non-specific mechanism of DNA repair because it can fix a variety of damage.

Homework

<u>Textbook</u>: p. 266 # 1, 3, 4, 7, 9 & 14