Chapter 17
From Gene to Protein

Key Players in:
- Translation
- mRNA
- tRNA
- ribosome
- amino acids

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tRNA

On this model of a tRNA molecule, identify each of the following:
- 5' and 3' ends
- hydrogen bonds
- unpaired regions
- anticodon loop
- binding site for amino acid
- sequence that pairs with mRNA
The Structure of Transfer RNA

- Single RNA strand (~80 nt)
- Different tRNAs carry different amino acids (on 3' end)
- Anticodon pairs with codon on mRNA

Because of hydrogen bonds, tRNA actually twists and folds into a three-dimensional molecule - tRNA is roughly L-shaped

Two Basic Steps of Translation:
1. A correct match between a tRNA and an amino acid, done by the enzyme aminoacyl-tRNA synthetase (called "tRNA charging")
2. A correct match between the tRNA anticodon and an mRNA codon

Some tRNAs bind more than one codon - why?

Be able to describe the steps of tRNA charging
Ribosomes facilitate specific coupling of tRNA anticodons with mRNA codons in protein synthesis.

- The two ribosomal subunits (large and small) are made of proteins and ribosomal RNA (rRNA).

**Building a Polypeptide**

- The three stages of translation:
  - Initiation
  - Elongation
  - Termination

- All three stages require protein "factors" that aid in the translation process.
Initiation of Translation

Initiation complex assembles:
1. mRNA, small ribosomal subunit, initiation factors, "initiator" tRNA (charged with MET) assemble first
2. Complex scans mRNA for start codon (AUG)
3. Large ribosomal subunit binds to complete complex

Elongation

Elongation factors are involved

Each addition includes:
1. Codon recognition
2. Peptide bond Formation
3. Translocation

Termination

Termination occurs when a stop codon in the mRNA reaches the A site
The A site accepts a protein called a release factor
This reaction releases the polypeptide, and the translation assembly then comes apart
The final product of an expressed gene can be which of the following?

Find all of the correct choices.

a. mRNA
b. tRNA
c. rRNA
d. polypeptide
Completing and Targeting the Functional Protein

Often translation is not sufficient to make a functional protein

• What events happen after translation?

Protein Folding and Post-Translational Modifications

• During and after synthesis, a polypeptide chain spontaneously (or with help) coils and folds into its three-dimensional shape

• Proteins may also require post-translational modifications before doing their job
  – What are some modifications?

• Some polypeptides are activated by enzymes that cleave them – “pro- or pre- or pre-proproteins”

• Other polypeptides come together (interact) to form the subunits of a protein

• Proteins have to be targeted to their proper location

Targeting Polypeptides to Specific Locations

• Two populations of ribosomes are evident in cells: free ribosomes (in the cytosol) and bound ribosomes (attached to the ER)

• Free ribosomes mostly synthesize proteins that function in the cytosol

• Bound ribosomes make proteins of the endomembrane system and proteins that are secreted from the cell

• Ribosomes are identical and can switch from free to bound
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- Polypeptide synthesis begins in the cytosol
- Synthesis finishes in the cytosol unless the polypeptide signals the ribosome to attach to the ER
- Polypeptides destined for the ER or for secretion are marked by a signal peptide
- A signal-recognition particle (SRP) binds to the signal peptide
- The SRP brings the signal peptide and its ribosome to the ER

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**Transcription and Translation**

Of the following, which pertain to transcription, post-transcriptional modification, or translation?

- stop codon
- peptide bond
- P site
- GTP
- amino-acid synthetase
- Poly A tail
- ribosome
- anticodon
- promoter
- terminator sequence
- tRNA
- 5' cap
- Spliceosome
- 5' and 3' UTR
- RNA pol

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Comparing Gene Expression in Bacteria, Archaea, and Eukarya

- While gene expression differs among the domains of life, the concept of a gene is universal.
- Bacteria and eukarya differ in their RNA polymerases, termination of transcription and ribosomes; archaea tend to resemble eukarya in these respects.
- Bacteria can simultaneously transcribe and translate the same gene.
- In eukarya, transcription and translation are separated by the nuclear envelope.
- In archaea, transcription and translation are likely coupled.

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What Is a Gene? Revisiting the Question

- The idea of the gene itself is a unifying concept of life.
- We have considered a gene as:
  - A discrete unit of inheritance (coming soon...)
  - A region of specific nucleotide sequence in a chromosome.
  - A DNA sequence that codes for a specific polypeptide chain.
- In summary, a gene can be defined as a region of DNA that can be expressed to produce a final functional product, either a polypeptide or an RNA molecule.

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The Effect of Mutations on Protein Function

- Mutations are changes in the genetic material of a cell or virus (due to spontaneous mutations during replication or physical/chemical agents).
- Point mutations are changes in just one nucleotide/base pair of a gene.
- The change of a single nucleotide in a DNA template strand can lead to the production of an abnormal protein (but might not).
Example: Sickle Cell Anemia

Wild-type hemoglobin DNA
3’  5’
  1  1  1  1
  1  1  1  1
  1  1  1  1

Mutant hemoglobin DNA
3’  5’
  1  1  1  1
  1  1  1  1
  1  1  1  1

Wild-type mRNA
3’  5’
  1  1  1  1
  1  1  1  1
  1  1  1  1

Mutant mRNA
3’  5’
  1  1  1  1
  1  1  1  1
  1  1  1  1

Types of Point Mutations
Point mutations within a gene can be divided into two general categories

1. Base-pair substitutions
   1. Missense (A instead of T, U instead of A)
   2. Nonsense (T instead of C)
   3. Silent (no change in amino acid sequence)

1. Base-pair insertions or deletions
   1. Frameshift causing extensive missense (1 base-pair deletion)
   2. Frameshift causing immediate nonsense (1 base-pair insertion)
   3. Frameshift causing extensive missense (1 base-pair deletion)
**Substitutions**

- A **base-pair substitution** replaces one nucleotide and its complimentary base with another pair of nucleotides.
- **Silent mutations** have no effect on the amino acid produced by a codon because of redundancy in the genetic code.
- **Missense mutations** still code for an amino acid, but not necessarily the right amino acid.
- **Nonsense mutations** change an amino acid codon into a stop codon, nearly always leading to a nonfunctional protein (early termination).

**Insertions and Deletions**

- **Insertions** and **deletions** are additions or losses of nucleotide pairs in a gene.
- These mutations have a disastrous effect on the resulting protein more often than substitutions do.
- Insertion or deletion of nucleotides may alter the reading frame, producing a **frameshift mutation** (change in reading frame).