

Topic 7: Nucleic acids (HL)

7.1 DNA structure and replication

- U1 Nucleosomes help to supercoil the DNA.
- U2 DNA structure suggested a mechanism for DNA replication.
- U3 DNA polymerases can only add nucleotides to the 3' end of a primer.
- U4 DNA replication is continuous on the leading strand and discontinuous on the lagging strand.
- U5 DNA replication is carried out by a complex system of enzymes.
- U6 Some regions of DNA do not code for proteins but have other important functions.
- A1 Rosalind Franklin's and Maurice Wilkins' investigation of DNA structure by X-ray diffraction.
- A2 Use of nucleotides containing dideoxyribonucleic acid to stop DNA replication in preparation of samples for base sequencing.
- A3 Tandem repeats are used in DNA profiling.
- S1 Analysis of results of the Hershey and Chase experiment providing evidence that DNA is the genetic material.
- S2 Utilization of molecular visualization software to analyse the association between protein and DNA within a nucleosome.

Hershey & Chase experiment

Experiment target: **T2 Bacteriophage** (a kind of virus, containing DNA and a protein coat) & **E. coli bacteria**

How does virus work?

- Virus injects their genetic material into cell
- Non-genetic part of the virus (protein coat) remains outside the cell
- Infected cell produces large amount of viruses based on the genetic material in the cell
- The cell bursts releasing copied viruses

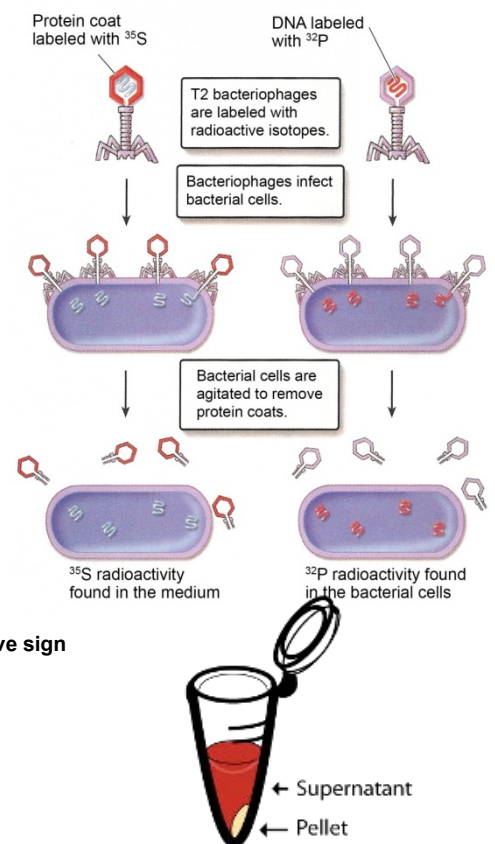
Experimental methodology:

- Amino acid containing radioactive isotopes were used to label the virus: ^{35}S is used for labelling the protein coat, and ^{32}P is used for labelling DNA
- Centrifuge is used for vigorous shaking in order to separate virus and infected bacteria:
Pellet (solid) contains bacteria and **supernatant** (liquid) contains protein coat.

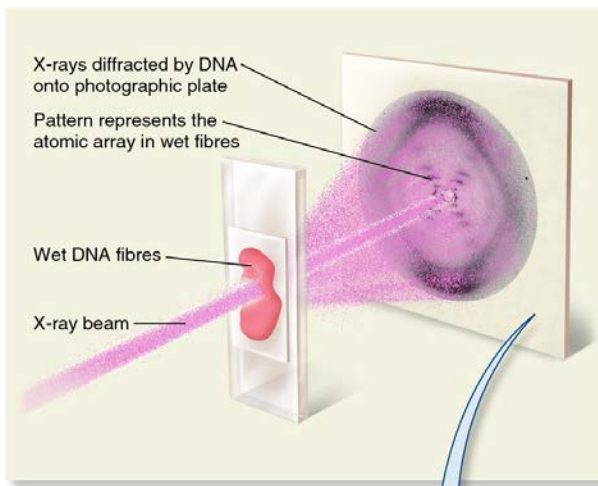
Findings:

- When protein coat is only radioactively labelled, **new viruses made shown no radioactive sign**
- When DNA is only radioactively labelled, **new viruses made shown radioactive sign**

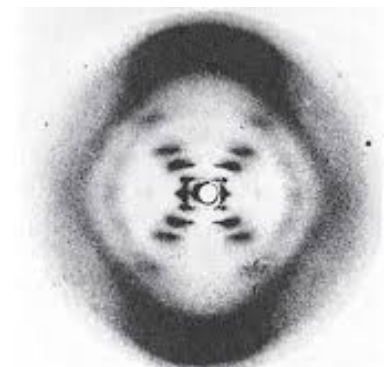
Hypothesis: DNA controls genetic information.



Rosalind Franklin's and Maurice Wilkins' investigation of DNA's structure by X-ray diffraction

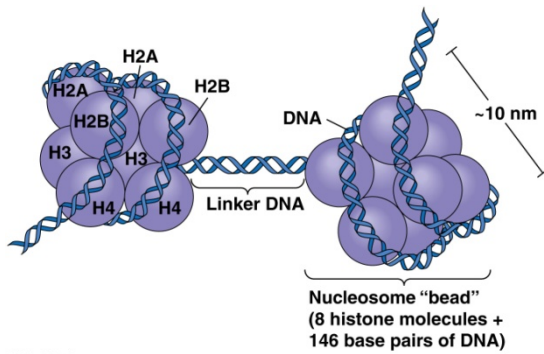


Rosalind Franklin is the first woman to take a photo of DNA using x-ray diffraction. Her investigation leads to the findings that DNA is a **double helix**.

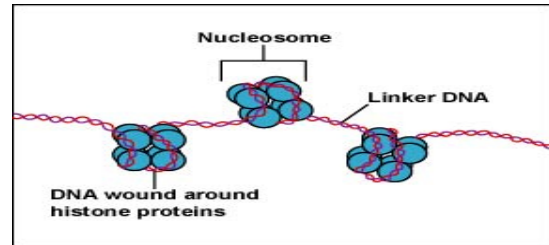


Nucleosome

- Eukaryotic DNA supercoiling is organized by nucleosome
- Nucleosomes are formed by wrapping DNA around **histone protein**
- Supercoiling in general helps regulate transcription because only **certain areas of the DNA are accessible** for the production of mRNA by transcription. This regulates the production of a polypeptide.
- Nucleosomes will be further supercoiled to form chromosome, which is only formed in cell division.

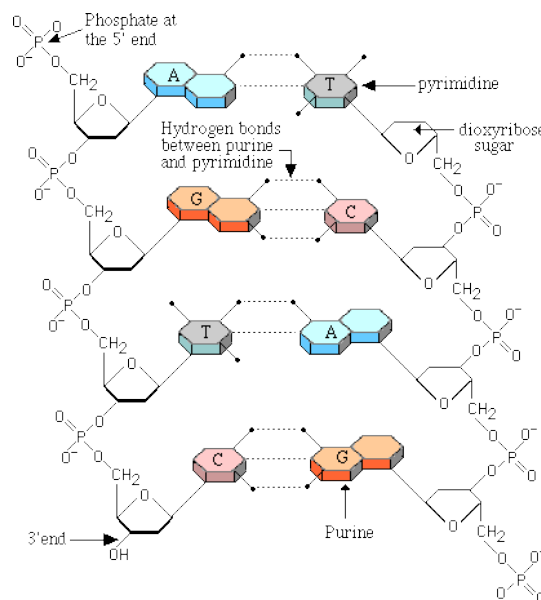


DNA will wrap around the histone protein (as shown as the blue ball in the diagram) first, histones will then stick together to form nucleosome (8 histone protein). Only the DNA connecting two nucleosome (Linker DNA in the diagram) is accessible and can be transcribed into mRNA to produce polypeptides



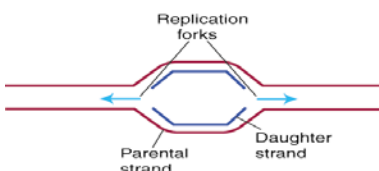
DNA Structure

- DNA is double stranded and shaped like a ladder, with the sides of the ladder made out of repeating phosphate and deoxyribose sugar molecules covalently bonded together.
- Each deoxyribose molecule has a phosphate covalently attached to a 3' carbon and a 5' carbon.
- The phosphate attached to the 5' of one deoxyribose molecule is covalently attached to the 3' of the next deoxyribose molecule forming a long single strand of DNA known as the DNA backbone.
- DNA strands run anti-parallel to each other with one strand running in a 5' to 3' direction and the other strand running 3' to 5' when looking at the strands in the same direction
- The rungs of the ladder contain two nitrogenous bases (one from each strand) that are bonded together by hydrogen bonds.
- Since these two strands are anti-parallel replication occurs in different directions on the DNA strand
- Purines** are two ring nitrogenous bases and **pyrimidines** are single ring nitrogenous bases.
- Adenine and thymine has **two** hydrogen bonds; Guanine and Cytosine has **three** hydrogen bonds.



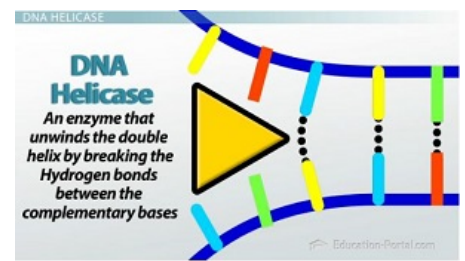
DNA replication

- DNA replication creates two identical strands with each strand consisting of one new and one old strand (**semi-conservative**).
- DNA replication occurs at many different places on the DNA strand called the origins of replication (represented by bubbles along the strand).



Several site "replication bubbles" starts together, so replication process is much faster. Replication starts at the **replication forks**.

- The DNA strand is unwound and separated (hydrogen bonds broken) by an enzyme called **helicase**.
- DNA gyrase is an enzyme that relieves strain on the strand as it is being unwound by the helicase
- Single-stranded binding proteins bind to the open strands and keep the strands apart long enough to prevent the strands from re-annealing before the strands are copied



- **RNA primase** attaches a **RNA primer** to the DNA as starting point and attachment point since DNA polymerases can only add nucleotides to the 3' end of a primer
- **DNA polymerase III** adds free nucleotides found in the nucleus in the **5' to 3' direction** in the direction of the replication fork.
- This strand is called the **“leading strand”** because replication is **continuous** (keeps replicating until it meets another origin of replication).
- Because DNA polymerase III can only add nucleotides to a free 3' end (5'to 3' direction) the other strand is replicated in the opposite direction.
- This strand is called the **“lagging strand”** because replication is delayed until the helicase opens up available nucleotides, to allow replication “back” in the opposite direction to the leading strand. Replication in this direction is **discontinuous**.
- The lagging strand is therefore made as a series of fragments called **“Okazaki fragments”**
- **DNA polymerase I** replaces RNA primer with DNA
- **DNA ligase** glues the Okazaki fragments together to form a continuous strand

Sequence of replication	Protein required	Protein function
1 st Step	Helicase	Unzips the DNA strands
2 nd Step	Primase	Add an RNA primer on the DNA strand as a starting point of replication
3 rd Step	DNA polymerase III	Binds to RNA primer and begins replication in 5' to 3' direction
In Leading strand (helicase pointing 5' end)	DNA polymerase I	Replace RNA primer with DNA nucleotides
In Lagging strand (helicase pointing 3' end)	DNA polymerase I	Replace RNA primer with DNA nucleotides
	DNA ligase	Glue Okazaki fragments together to make a continuous DNA stand

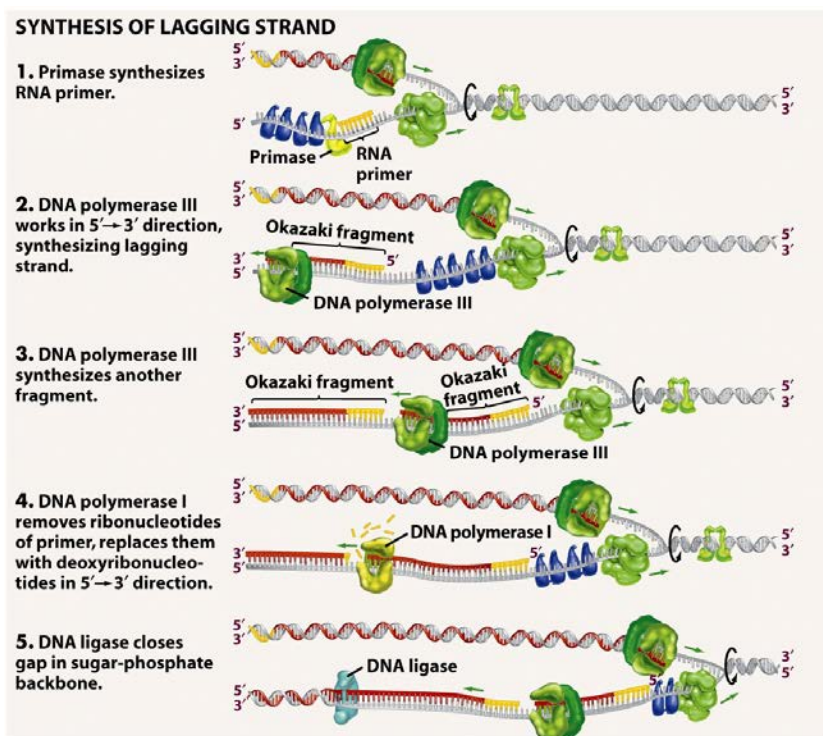
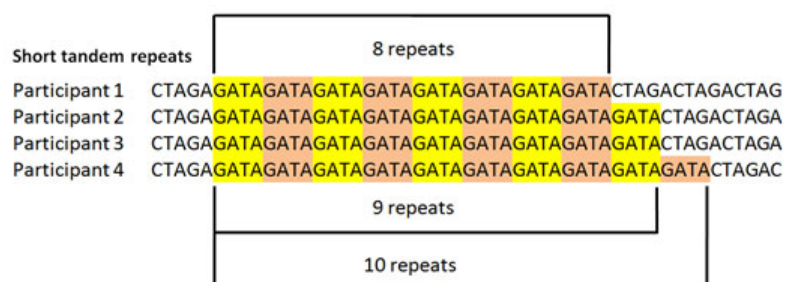


Figure 14-13 Biological Science, 2/e

- Genes contained within DNA called coding sequences, code for polypeptides created during transcription and translation
- The majority of DNA are non-coding sequences that perform other functions such as regulators of gene expression, introns, telomeres.
- **Silencers** are DNA sequences that bind regulatory proteins called repressors that **inhibit transcription**
- **Promoters** are the attachment points for RNA polymerase to transcribe mRNA
- **Enhancers** are DNA sequences that increase the rate of transcription
- **Introns** are the area in DNA for non-coding regions
- **Extrons** are the area in DNA for coding regions
- **mRNA** only copies the information from the exons and ignore what is in the introns. So only exons' information can leave the nucleus
- In DNA there are also many repetitive sequences, especially in eukaryotic DNA, that can make up 5-60% of the genome; specifically, an area of repetitive sequences that occurs on the ends of eukaryotic chromosomes.
- These repetitive sequences called telomeres, protect the DNA during replication. Since enzymes can't replicate all the way to the end of the chromosome, the parts that aren't copied are part of the telomeres. This **prevents the DNA molecule from degradation during replication.**

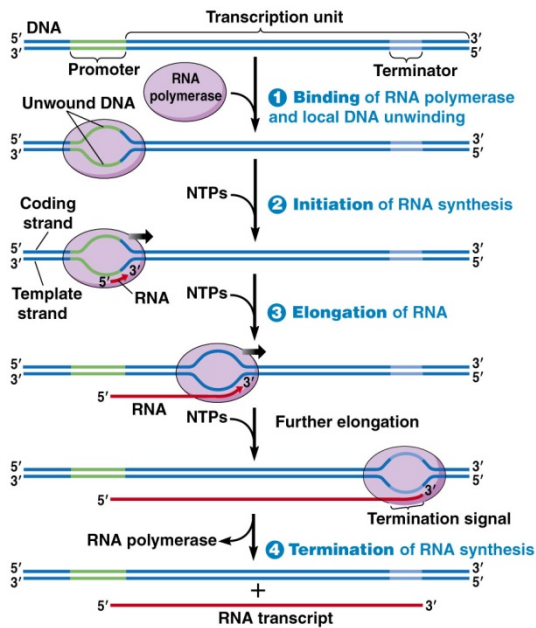


7.2 Transcription and gene expression

- U1 Transcription occurs in a 5' to 3' direction.
- U2 Nucleosomes help to regulate transcription in eukaryotes.
- U3 Eukaryotic cells modify mRNA after transcription.
- U4 Splicing of mRNA increases the number of different proteins an organism can produce.
- U5 Gene expression is regulated by proteins that bind to specific base sequences in DNA.
- U6 The environment of a cell and of an organism has an impact on gene expression.
- A1 The promoter as an example of non-coding DNA with a function.
- S1 Analysis of changes in the DNA methylation patterns.

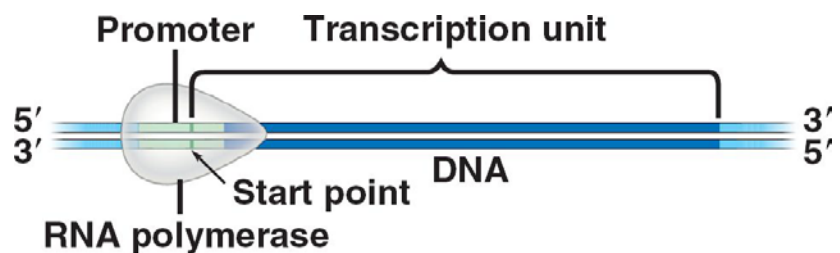
Transcription of RNA

- Transcription occurs in a 5' to 3' direction where the 5' end of the free RNA nucleotide is added to the 3' end of the RNA molecule that is being synthesized.
- Transcription consists of 3 stages called **initiation, elongation and termination**
- Transcription begins when the **RNA polymerase binds to the promoter** with the help of specific binding proteins



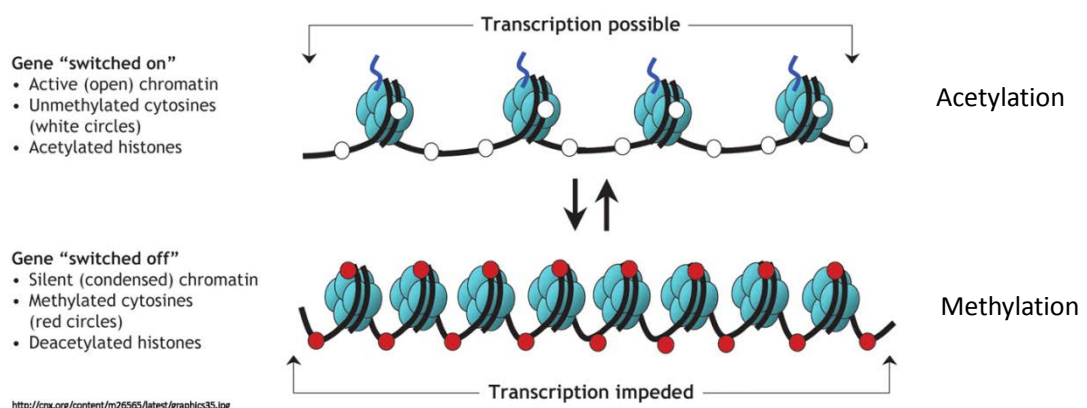
The **promoter region** is a DNA sequence that initiates transcription and is an example of non-coding DNA that plays a role in gene expression. Promoter region is also the **binding site** for RNA polymerase, is the starting point of transcription

Operator allows inhibitor protein to bind to stop the transcription, **Repressor protein** is bind to the operator to prevent RNA polymerase from transcribing genes



Nucleosome regulating gene transcription

- As explained previously in 7.1 eukaryotic DNA wraps around histone proteins and supercoils
- This supercoiling helps regulate transcription because only **certain areas** of the DNA are accessible for the production of mRNA by transcription. This regulates the production of a polypeptide.
- One of the main ways this occurs is through the modification of the histone tails
- Acetylation:** When acetyl groups are added to the positively charged histone tails, they become negative and the DNA repels against them. This **opens up the nucleosome** so the DNA is not as close to the histone anymore and chromatin remodeling can occur. **Acetylation switches on genes.**
- Methylation:** decreases transcription of the gene. It makes histone protein closer and tighter so less gene will be expressed. **Methylation switches off genes.**
- The amount of methylation can vary over an organisms lifetime and can be affected by environmental factors



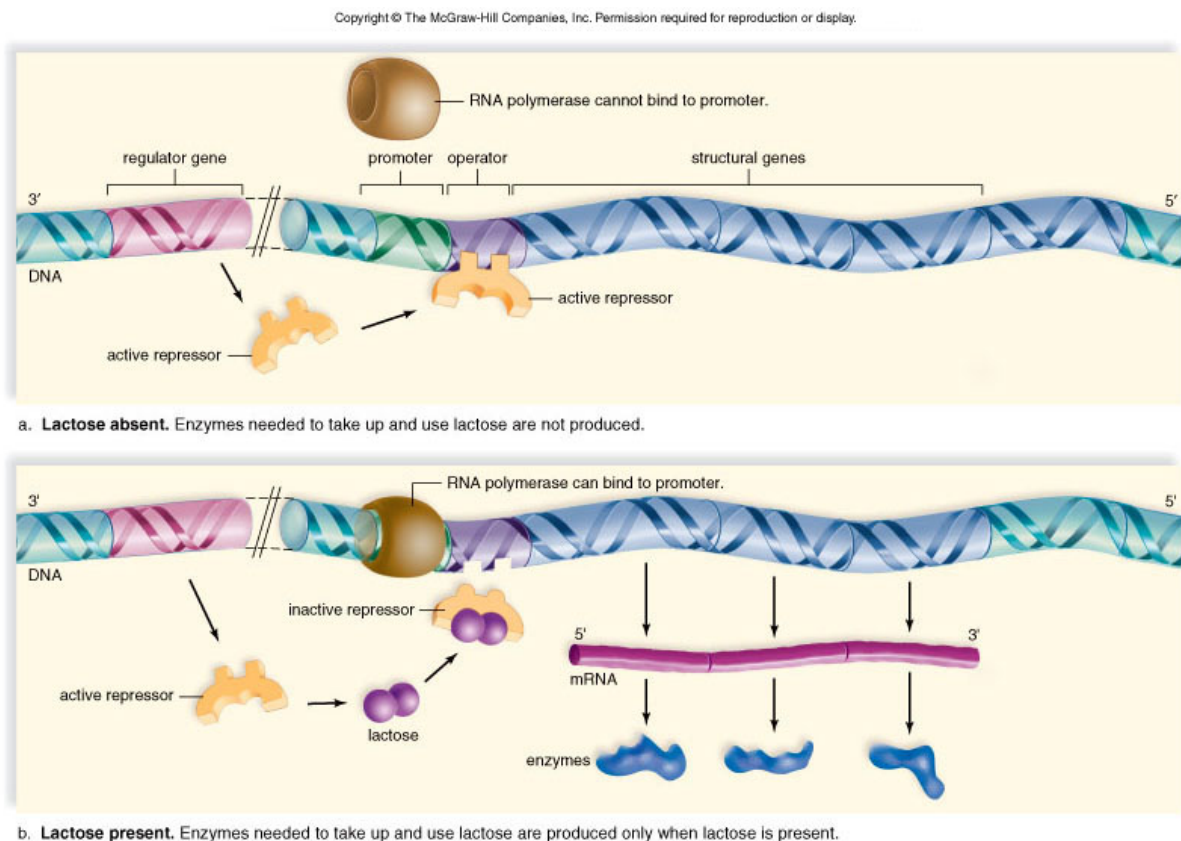
Protein regulation of gene expression

- Gene expression can also be regulated by the environment surrounding the gene that is expressed or repressed
- Specific proteins can regulate how much transcription of a particular gene will occur
- These regulatory proteins are unique to a particular gene
- Regulatory sequences on the DNA that increase the rate of transcription when proteins bind to them are called **enhancers**
- Regulatory sequences on the DNA that decrease the rate of transcription when proteins bind to them are called **silencers**
- Promoter-proximal elements have binding sites closer to the promoter and their binding is necessary to initiate transcription

DNA Sequence	Binding protein	Function
Enhancers	Activator	Increase greatly the rate of transcribing genes
Silencers	Repressor	Either block or stop the rate of transcribing genes
Promoters	RNA polymerase	Binds to promoter and begins transcription in <u>5' to 3' direction</u>

Example: Lactose production

- In prokaryotic cells such as E.coli **repressor proteins** block the production the enzymes needed to break down lactose in the cell.
- However, when Lactose is present, it will bind to the repressor protein, causing it to fall off, and allowing transcription to occur.
- As transcription occurs, these enzymes are made and lactose is broken down into glucose and galactose. Since there is small amounts of lactose now in the cell, the **repressor** binds again to the operator, blocking transcription from taking place.
- This is an example of **negative feedback**



Environmental impact on gene expression

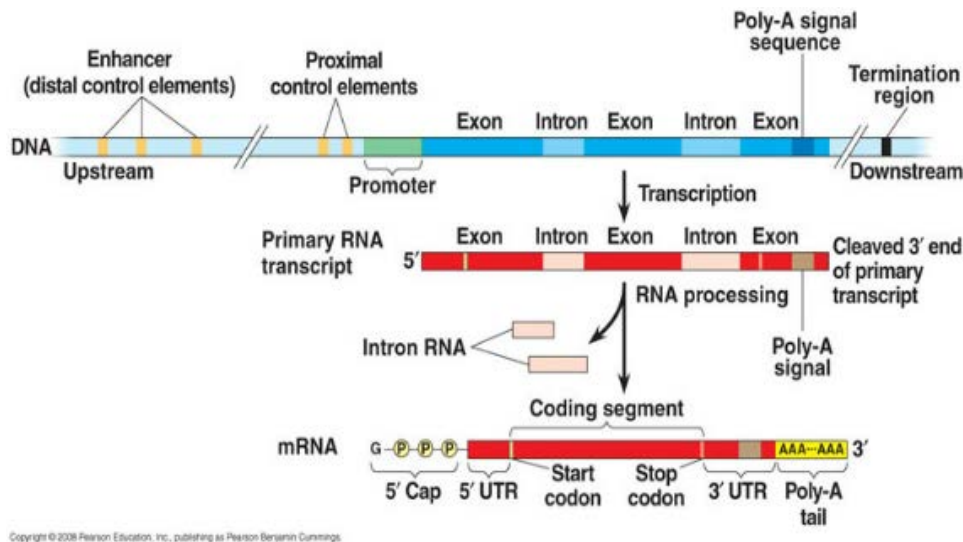
- The external environment in which the organism is located or develops, as well as the organism's internal world, which includes such factors as its **hormones and metabolism** can have an impact on gene expression
- **Temperature and light** are external conditions which can affect gene expression in certain organisms.
- For example, **Himalayan rabbits** carry the **C gene**, which is required for the development of pigments in the fur, skin, and eyes, and whose expression is regulated by temperature (Sturtevant, 1913).
- Specifically, the C gene is inactive above 35°C, and it is maximally active from 15°C to 25°C. This temperature regulation of gene expression produces rabbits with a distinctive coat coloring. In the warm, central part of the rabbit's body, the gene is inactive, and no pigments are produced therefore the fur color is white (picture below). In the rabbit's extremities (i.e., the ears, tip of the nose, and feet), where the temperature is much lower than 35°C, the C gene actively produces pigment, making these parts of the animal black.



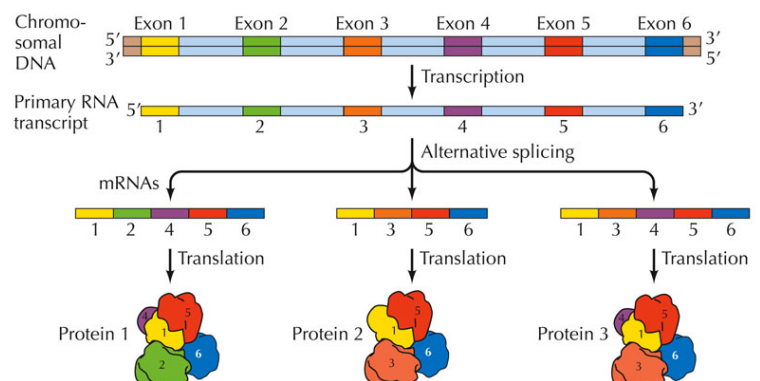
- During embryonic development embryos contain chemicals called **morphogens**, which can affect **gene expression** and thereby affecting the fate of embryonic cells depending on their position within the embryo. **Morphogens regulate the production of transcription factors in a cell**
- An obvious example is how sunlight affects the production of skin pigmentation in humans
- A chemical example, was the use of Thalidomide by pregnant woman for morning sickness. It was thought it was harmless for humans but was not thoroughly tested. The drug was withdrawn too late to prevent severe developmental deformities in approximately 8,000 to 12,000 infants, many of whom were born with stunted limb development. Interestingly, despite the fact that thalidomide is dangerous during embryonic development, the drug continues to be used in certain instances yet today.

Modification of mRNA after transcription

- In eukaryotes, the locations for transcription and translation are separated by the nuclear membrane. This allows for post-transcriptional modification of the mRNA.
- The first product of transcription is pre-mRNA
- As eukaryotic mRNA travels from the nucleus to the ribosomes, non-coding strands of the mRNA called introns are removed to form functional mature mRNA.
- They are removed through **RNA splicing**
- The **exons are spliced together** to form mature mRNA
- Also a **poly A tail** consisting of approximately **100-200 adenine nucleotides** is added to one end of the mRNA and a **5' cap** is added to the other end (these help protect the mature mRNA transcript)



- **Alternative splicing** can also occur with genes that produce multiple proteins, which means that some **exons may also be removed during splicing**, thus producing different polypeptides
- For example, in mammals tropomyosin which is a protein involved in muscle contractions; however, the pre-mRNA is spliced to form 5 different forms of the protein. The mature mRNA that codes for tropomyosin in the smooth muscle of the intestines is missing exon 3 and 10, while the mRNA that codes for tropomyosin in skeletal muscle is missing exon 2.

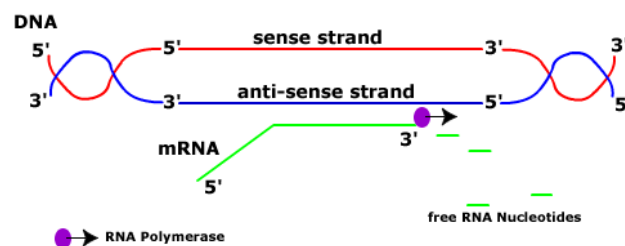


7.3 Translation

U1	Initiation of translation involves assembly of the components that carry out the process.
U2	Synthesis of the polypeptide involves a repeated cycle of events.
U3	Disassembly of the components follows termination of translation.
U4	Free ribosomes synthesize proteins for use primarily within the cell.
U5	Bound ribosomes synthesize proteins primarily for secretion or for use in lysosomes.
U6	Translation can occur immediately after transcription in prokaryotes due to the absence of a nuclear membrane.
U7	The sequence and number of amino acids in the polypeptide is the primary structure.
U8	The secondary structure is the formation of alpha helices and beta pleated sheets stabilized by hydrogen bonding.
U9	The tertiary structure is the further folding of the polypeptide stabilized by interactions between R groups.
U10	The quaternary structure exists in proteins with more than one polypeptide chain.
A1	tRNA-activating enzymes illustrate enzyme -- substrate specificity and the role of phosphorylation.
S1	Identification of polysomes in electron micrographs of prokaryotes and eukaryotes.
S2	The use of molecular visualization software to analyse the structure of eukaryotic ribosomes and a tRNA molecule.

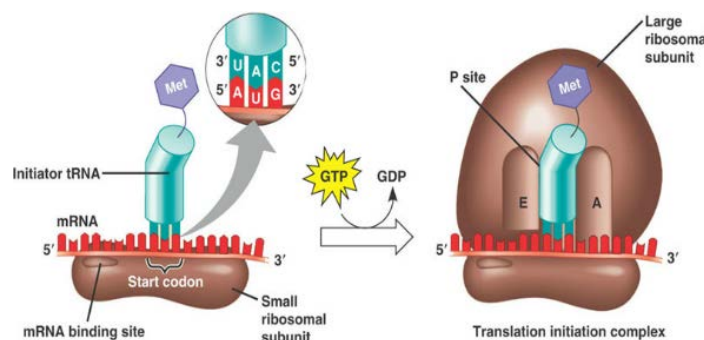
Transcription(revised)

- RNA polymerase binds to a site on DNA (promoter region) at the start of a gene.
- RNA polymerase separates the DNA strands and synthesises a complementary RNA strand.
- RNA copies from the **anti-sense** strand which contains the information on the **sense** strand.
- Once RNA is made, RNA polymerase detaches and DNA double helix reform.



Initiation of translation:

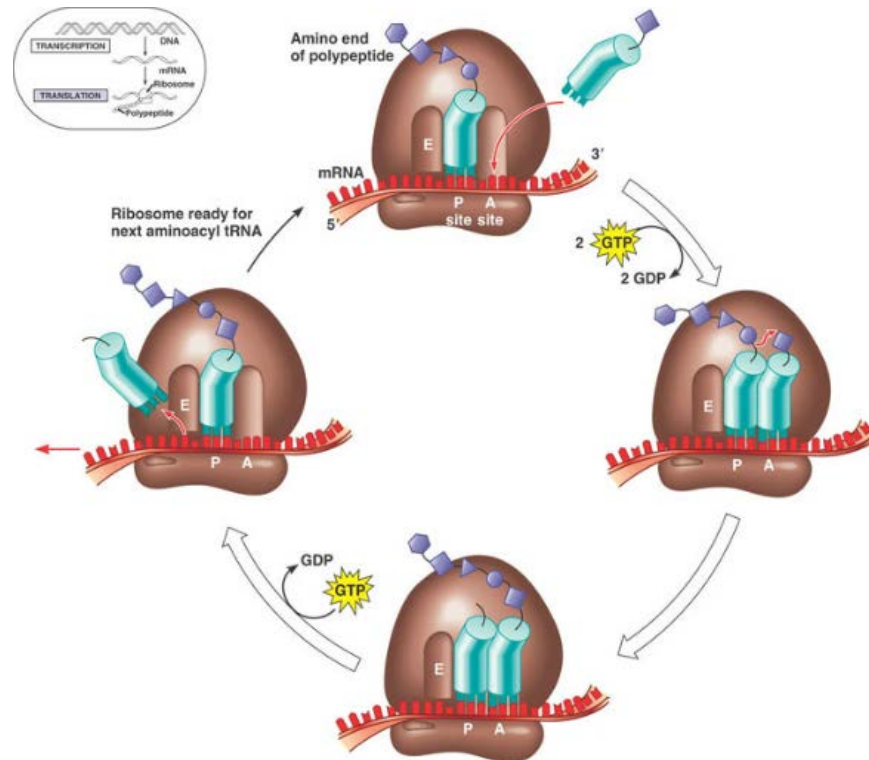
- mRNA binds to the **small (30 s) ribosomal** sub-unit.
- tRNA carrying **Methionine** with the anticodon UAC binds to the codon **AUG (start codon)**.
- This is called the initiation complex.
- The large ribosomal subunit binds to the small ribosome, with the tRNA containing methionine binding at the p-site of large subunit.



Elongation of translation

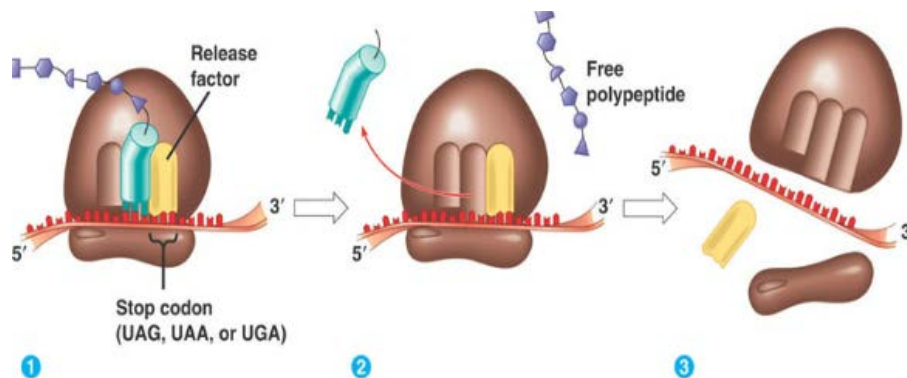
- While the first tRNA is still attached, a second tRNA attaches to the mRNA at the **A site** on the ribosome, carrying the amino acid that corresponds to the mRNA codon.
- The methionine amino acid at the **P site** binds to the amino acid carried by the second tRNA located at the A site.
- The two amino acids are joined together through a **condensation reaction** that creates a **peptide bond between the two amino acids**.
- The ribosome moves along the mRNA one codon shifting the tRNA that was attached to methionine to the **E site**.
- The tRNA is released back into the cytoplasm from the E site, allowing it to pick up another amino acid (methionine) to build another polypeptide.
- Another tRNA moves into the empty A site bringing the next amino acid corresponding to their RNA codon.

- Again, the amino acid is attached to the polypeptide forming a peptide bond, the ribosome slides across one codon and tRNA at the P site moves into the E site releasing it back into the cytoplasm.
- The ribosome continues to move along the mRNA adding amino acids to the polypeptide chain.
- This process continues until a stop codon is reached.



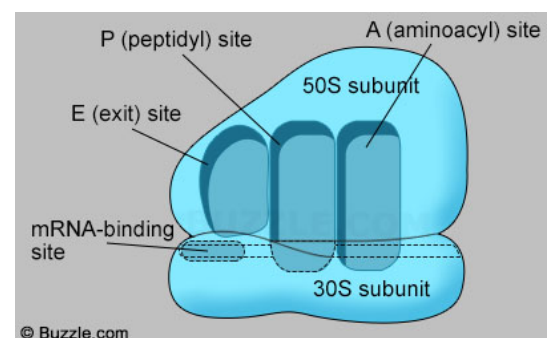
Termination of translation:

- Termination begins when 1 of the 3 stop codons (UAA, UGA, UAG) moves into the A site.
- These tRNA have no attached amino acids.
- When the stop codon is reached the ribosome dissociates and the polypeptide is released.



Ribosome

- Ribosomes are composed with two subunits – large and small subunit
- Large subunit sit on the top and small subunit holds the RNA
- Ribosomes have three sites:
- **A site:** check for the right tRNA matching mRNA, joining to the mRNA (**Activation**)
- **P Site:** form peptide bonds with growing amino acid chain (**Polypeptide**)
- **E site:** empty tRNA exit back to cytoplasm to pick up new amino acid (**Exit**)



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tRNA Structure

- tRNA is a type of RNA molecule that transfers a specific amino acid to a growing polypeptide chain during translation (protein synthesis) at the ribosomes.
- Sections of the tRNA become double stranded through hydrogen bonds formed between base pairs creating **loops**
- A triplet of bases form the **anticodon** which will bind to the corresponding triplet codon on the mRNA strand
- The base sequence of **CCA** at the 3' end forms the **amino acid binding site**

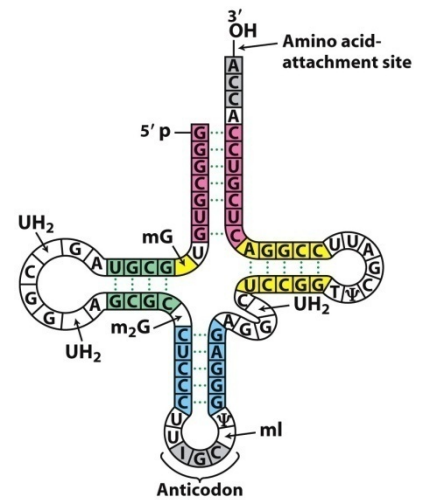
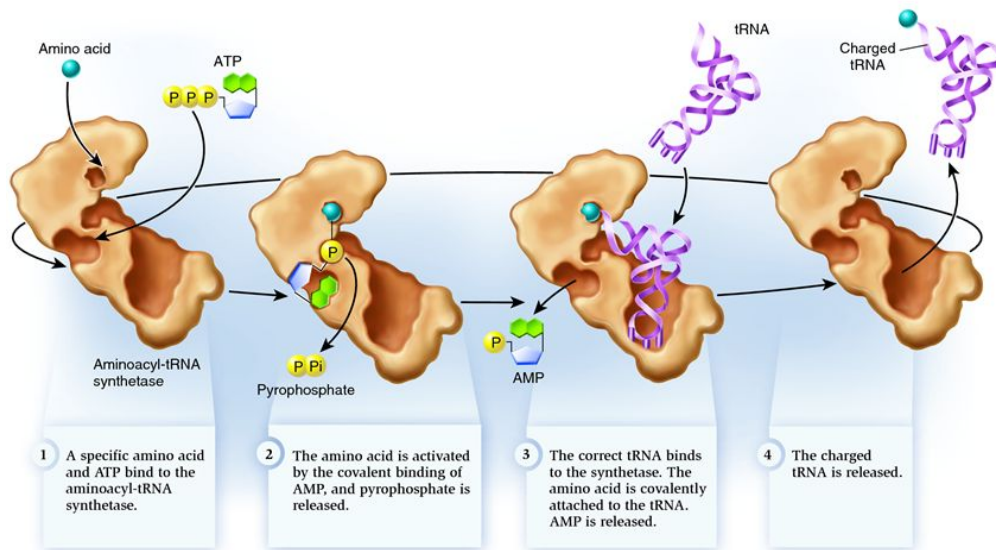


Figure 30.2
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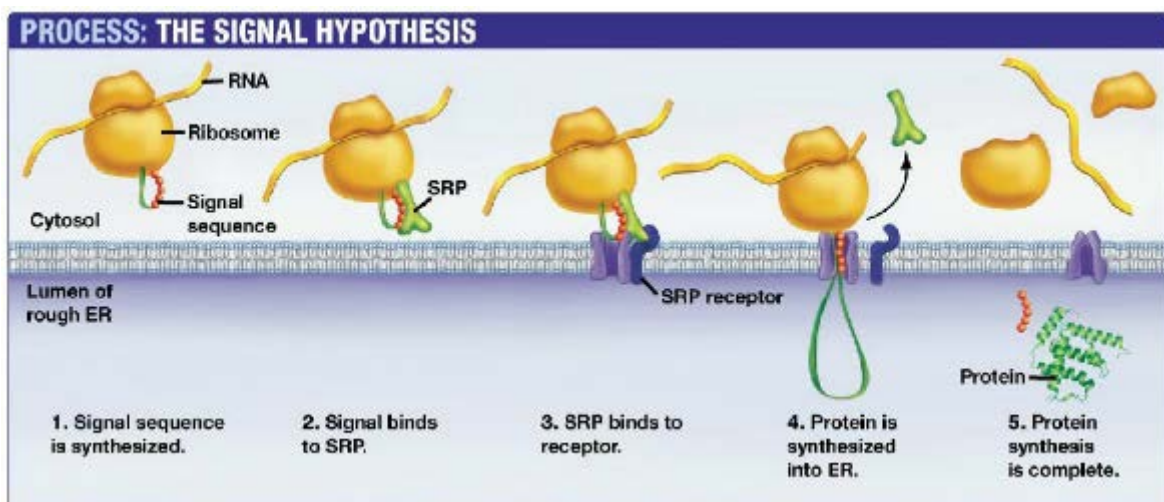
tRNA activating enzyme

- Each tRNA binds with a specific amino acid in the cytoplasm in a reaction catalyzed by a specific tRNA-activating enzyme.
- Each specific amino acid binds covalently to the **3'- terminal nucleotide (CCA)** at the end of the tRNA molecule.
- The binding of the specific amino acid to the tRNA requires energy from ATP.
- Energy between the tRNA and amino acid (**the bond**) will be used in translation to form a peptide bond between amino acids.

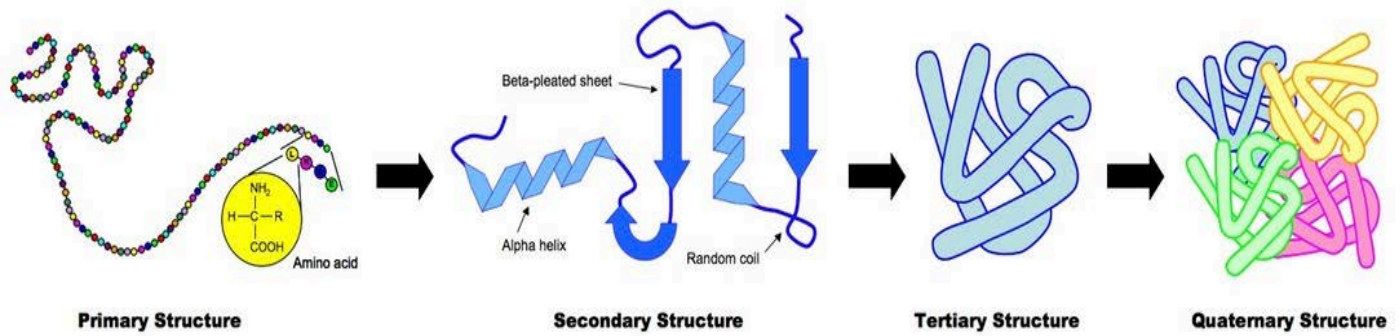


Ribosomes on rough endoplasmic reticulum synthesise protein for secretion

- Ribosomes attached to ER create proteins that are **secreted from the cell** by exocytosis or are used in lysosomes.
- Proteins perform many functions within specific compartments of the cell or in other parts of the body after they are secreted out of the cell
- Proteins that are destined to be used in **lysosomes**, **ER**, **Golgi Apparatus**, the **plasma membrane** or **secreted** by the cell are made by ribosomes bound by the endoplasmic reticulum
- Ribosomes that become bound to the ER are directed here by a signal sequence that is part of that specific polypeptide
- This signal sequence on the polypeptide binds to a signal recognition protein (SRP)
- The **SRP guides the polypeptide and ribosome to the ER** where it binds to an SRP receptor
- Translation can now continue and the polypeptide is deposited into the **lumen of the ER** as it is created for transportation to the correct location



Four levels of protein structure



- **Primary** structure: **basic amino acid chain**
- **Secondary** structure: Held together by **hydrogen bonds** between (non-adjacent) amine (N-H) and carboxylic (C-O) groups, H-bonds provide a level of structural stability. (**alpha helix shape & beta pleated sheet**) (e.g. silk)
- **Tertiary** structure: The polypeptide folds and coils to form a complex **3D shape**. Caused by **interactions between R groups** including **ionic bonds, sulfur bridge, hydrophobic and hydrophilic interaction**. Protein is **globular in nature**.
- **Quaternary** structure: 2 or more polypeptide chains and/or an inorganic compound (**prosthetic group**) (e.g. hemoglobin)