DNA TRANSCRIPTION

Chemical diff between DNA and RNA

- The type of sugar they contain.
- DNA has deoxyribose sugar, while RNA has ribose sugar.
- Deoxyribose has one less oxygen atom than ribose, which is why DNA is called "deoxy" and RNA is called "ribo".

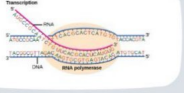
In RNA
base thymine (T) > uracil (U)
(Carries the same genetic information)
RNA = normally single stranded (ss)
DNA = double stranded (ds)

genetic information

- Genetic information is used by being transcribed from DNA into RNA. (transcription)
- Messenger RNA (mRNA) which carries the information from the genes into the rest of the cell.
- DNA must be pulled apart temporarily. Used as a template to synthesize a complementary RNA molecule.

Manufacturing the Message

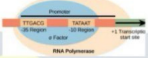
Once sigma has found a promoter [/]
and RNA polymerase has successfully bound to it [/]
then > sigma subunit drops off.
> remaining part of bacterial RNA polymerase makes the mRNA.
The DNA double helix is opened up > single strand of RNA generated using DNA strands as a template.



Beginning of a gene

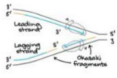
- RNA polymerase > several protein subunits
- Sigma subunit recognises two special sequences; (-10region and -35region)
- The stretch of DNA region = upstream region where RNA polymerase binds = promoter
- Perfect sequences = consensus sequence
  -10: TATAAT | -35:TTGACA

- A few highly expressed genes do have these exact sequences in their promoter.
- Promoter strength depends partly on how closely matched the ideal consensus sequence.



RNA Polymerase

function (binds to the DNA, opens the double helix, manufactures an RNA message (molecule))



- The process begins when RNA polymerase binds to a specific region of DNA called the promoter
- RNA polymerase unwinds the DNA double helix creating a region of non-paired deoxyribonucleotides.
- Transcription is under control of the enzyme RNA polymerase. Transcription of mRNA Complementary to DNA.

Which strand to copy?

- only ONE of the strands of DNA is copied
- The sequence of the RNA is complementary to the template strand of the DNA (synthesised).
- sequence of new RNA molecule = sequence of the DNA

Short segments of the chromosome

- During cell growth, each gene (or small group of related genes) is used to generate a separate RNA copy when, and if it is needed.
- Each of these mRNA molecules carries the information from a short segment of a chromosome.

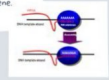
RNAP Know Where to Stop?

yes! by recognizing a specific sequence in DNA called terminator sequence;
-separated by several bases followed by a string of As

- The sequences on the same strand of an RNA molecule can pair up to generate a "stem and loop" or "hairpin" structure
- The string of As > a run of Us (Once the RNA polymerase reaches the stem and loop it stops)

- Lots of possible hairpin structures = cause RNA polymerase slow down or stop briefly (depends on size)
- If there's no string of Us, RNA polymerase will start off again.

- A string of Us paired with a string of As = very weak structure. (RNA & DNA fall apart, RNAP idling)
- Once the DNA and RNA have separated at the terminator structure, RNAP falls off and wanders away to find another gene.



3.0 TRANSCRIPTION

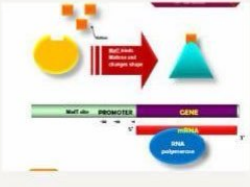
3.7: HOUSEKEEPING GENE

- Genes that are switched on **all the time**
- Most of -10 & -35 are very identical to consensus
- Is recognized by **sigma** subunit of **RNAP** and is switched on automatically in all conditions

- For genes that have poor recognition sequence, **gene activator** will be used to facilitate the recognition process.
- Is activated by regulators (eg: co-activators, co-repressors)

3.8: ACTIVATOR (MALTOSE IN E.COLI)

- MalT(activator) binds and detects maltose, then change its own shape
- Active form (MalT+maltose) is ready to bind to DNA
- The genes intended for using maltose are only expressed when this activator is available



3.9: NEGATIVE REGULATON (LACL PROTEIN)

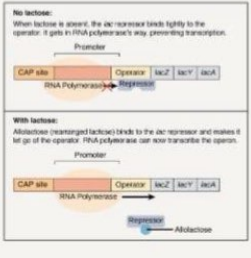
- Better known as **repressors.** Opposite effect
  activators
- Lactose is a sugar found in milk

Absence of lactose

- LacI binds to DNA stretch (operator sequence)
- Causing RNAP **cannot bind** to operator sequence

Presence of lactose

- Lactose binds to LacI protein
- LacI changes shape and falls off DNA
- RNA can bind to operator sequence, genes fo
  using lactose are switched on



CHAPTER 4: DNA TRANSLATION

WHAT ARE PROTEINS?

- Linear chain of monomers that are folded into complex 3D shape (quarternary form)
- amino acid chains are called polypeptide

STRUCTURAL PROTEIN

- Are found making up many subcellular structures
- e.g: flagella with which bacteria swim around
- outer coats of viruses
- fibers inside of muscle cell

TRANSPORT PROTEIN

- Carry molecules from one side to another
- To function, to needs extra components like cofactor or prosthetic group which are not proteins
- **Prosthetic group** - fixed to the protein
- **Cofactors** - free to wander around from protein to protein
- **Apoprotein** - protein without prosthetic group

ENZYMES

- Proteins that carry out chemical reactions
- Enzyme binds to substrate then perform some chemical operations
- Needs an **active site**
- **Active site** - produced by folding up the polypeptide chain correctly

REGULATORY PROTEIN

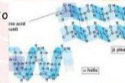
- Vary enormously
- Many can bind both small signal molecules and DNA
- Presence/absence of signal molecule determines whether or not the gene is switched on

TRANSLATION

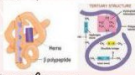
PROTEIN STRUCTURE

- **PRIMARY PROTEIN** — sequence of a chain of amino acids

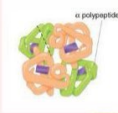
- **SECONDARY PROTEIN** — local folding of the polypeptide chain into helices or sheets.



- **TERTIARY PROTEIN** — three-dimensional folding pattern of a protein due to side chain interactions



- **QUARTENARY PROTEIN** — protein consisting of more than one amino acid chain.



RIBOSOME

ibosome binds mRNA and
ranslates it,synthesising a
olypeptide chain.
wo subunits : large (50S)
small(30S)

tRNA

-To read the codon,we need a set of adapter molecules that recognise the codon on the mRNA at one end and carry the corresponding.
these adapters are transfer RNA or tRNA.
-At one end, tRNA has an anticodon consisting of three bases complementary to the three bases of the codon on mRNA.

STRUCTURE OF TRNA

- typical RNA has 4 short base-paired stems &three loops. Cloverleaf structure to show details of base pairing and shows tRNA spreads out.
- anticodon is at the opposite end in anticodon loop. 2 loops of tRNA : ψ (pseudouracil) and D (Dihydrouracil)

READING FRAMES

Bases of mRNA are read in groups of three, starting at 5' end. Always begin with the start codon, A U G.

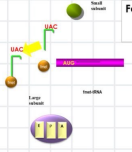
5'GAAAUGUAUGCAUGCCACAAGGAGGCAUCUAAGGA3'

GETTING PROTEIN SYNTHESIS STARTED

- FIRST CODON - AUG (methionine)
- A special tRNA (the initiator tRNA) will be charged with chemically-tagged Met (formyl-Met or fMet) and will bind to the start codon.

INITIATION OF TRANSLATION

- Before protein synthesis starts, the two subunits of the ribosome are floating around separately.
- The 16S rRNA, with the complementary anti S-D sequence, is in the small subunit of the ribosome.
  So FIRST the mRNA binds to a free small subunit.
- NEXT the initiator tRNA (carrying fMet) recognises the AUG start codon.
  You also need three proteins known as initiation factors, that help arrange all the components correctly.
- FINALLY the large subunit arrives, and joins its smaller partner as the initiation factors drop off



Formation of the Initiation Complex

ELONGATION OF A GROWING PROTEIN

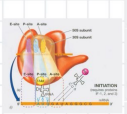
After the large subunit of the ribosome has arrived, the polypeptide chain is made.
The ribosome has three sites for tRNA: the E-site (Exit site), A-site (Acceptor site) and P-site (Peptide site) starting with the fMet initiator tRNA in the P-site.

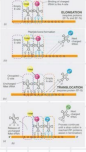
Another tRNA, carrying the exact amino acid, arrives and enters at the A-site. The fMet is cut loose from its tRNA and bonded to amino acid No. 2. So tRNA No. 2 now carries two linked amino acids, the beginning of our growing protein chain.

NEXT another charged tRNA arrives carrying the third amino acid. In order to fit the newcomer into the A-site we must push tRNA No. 2 sideways into the P-site.

THIS IN TURN pushes the free tRNA in the P-site off to the E-site.

As the peptide chain continues to grow, it is constantly cut off from the tRNA holding it (which occupies the P - peptide site) and joined instead to the newest amino acid to be brought by its tRNA into the A-site, hence the name "acceptor" site.





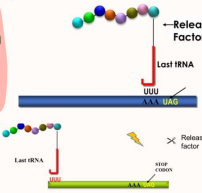
Termination of Protein Synthesis

This is marked by a stop codon (UGA, UAG, or UAA).

As no tRNA exists to read these three codons, the chain can no longer grow. Proteins known as release factors read the stop signal and chop the completed polypeptide chain of the final tRNA.

The ribosome then falls apart into its separate subunits – the termination of polypeptide.

Termination of polypeptide synthesis



Some Proteins Come to a Bad End (Murphy's Law)

- receive a defective mRNA.
- mRNA was never properly finished or whether it was mistakenly snipped short (cause havoc)
- A ribosome that is translating a message into protein expects to come across a stop codon.

tmRNA: because it acts partly like tRNA and partly like mRNA.

Like a tRNA, the tmRNA carries an amino acid (alanine). When it sees a stalled ribosome it binds beside the defective mRNA.

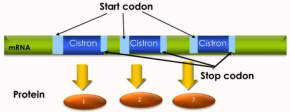
Protein synthesis now continues, first using the alanine carried by tmRNA, and then continuing on to translate the short stretch of message(of about 10 amino acids) that is also part of the tmRNA.

Finally, the tmRNA provides appropriate stop codon so that release factor can disassemble the ribosome and free it for its next assignment.

One mRNA Can Code for Several Proteins

In bacteria, several proteins may be encoded by the same mRNA. As long as each ORF has its own 5-D sequence in front of it, the ribosome will bind and start translating.

ORFs that are translated into proteins are sometimes known as cistrons.



Several Ribosomes Can Read the Same Message at Once

- Once the first ribosome has got moving, another can jump onto the same mRNA and travel along behind.
- In practice, several ribosomes will move along the same mRNA about a hundred bases apart
- This structure is called a polysome(short for polyribosome).

Coupled Translation and Transcription in Bacteria

- When mRNA is transcribed from the original DNA template, its synthesis starts at the 5' end. The mRNA is also read by the ribosome starting at the 5' end.
- this means the ribosome can start translating the message before the synthesis of the mRNA molecule has actually been finished.