Biology Lecture 1-2: MOLECULAR GENETICS- Transcription, Translation and Control of gene expression

- Describe the essential features of transcription and translation in cells of both prokaryotes (bacteria) and eukaryotes (fungi, plants and animals).
- Understand how point and frame-shift mutations can affect production of functional gene products/proteins.
- Understand the basic processes by which gene expression can be controlled at the level of transcription in prokaryotes and eukaryotes.

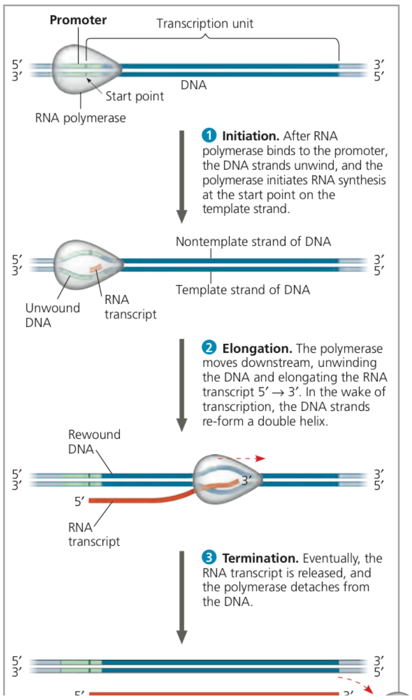
Transcription: the synthesis of RNA using information in the DNA. The two nucleic acids are written in different forms of the same language and the information is just simply 'rewritten' from DNA to RNA.

Messenger RNA (mRNA): carries a genetic message from the DNA to the protein-synthesising machinery of the cell.

Translation: the synthesis of a polypeptide using the information in the mRNA. During this stage, there is a change in language: The cell must translate the nucleotide sequence of an mRNA molecule into the amino acid sequence of a polypeptide. The sites of translation are ribosomes, molecular complexes that facilitate the orderly linking of amino acids into polypeptide chains.

Transcription/Translation in bacteria vs. eukaryotes:

Because bacteria do not have nuclei, their DNA is not separated by nuclear membranes from ribosomes and other protein-synthesising equipment. This lack of compartmentalisation allows translation of an mRNA to begin while its transcription is still in progress. In a eukaryotic cell, by contrast, the nuclear envelope separates transcription from translation in space and time. Transcription occurs in the nucleus, and mRNA is then transported to the cytoplasm, where translation occurs.



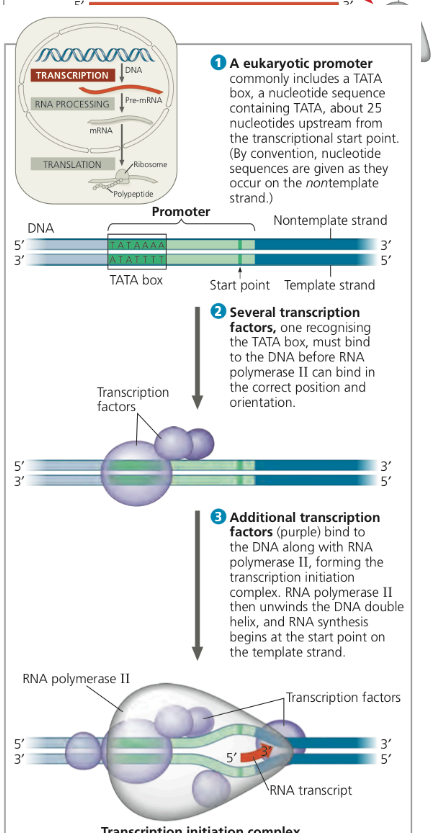
Primary transcript: the initial RNA transcript from any gene, including those specifying RNA that is not translated into protein. Template strand: the strand of DNA that provides the pattern or template for the sequence of nucleotides in an RNA transcript.

* The mRNA is read in the 5'-3' direction.

TRANSCRIPTION

Molecular components of Transcription:

- RNA polymerase: pries the two strands of DNA apart and joins together RNA nucleotides complementary to the DNA template strand. RNA polymerases can assemble a polynucleotide only in its 5'→ 3' direction. Unlike DNA polymerase however, RNA polymerases are able to starts a chain from scratch; they don't need a primer.
- Promoter: the DNA sequence where RNA polymerase attaches and initiates transcription.
- Transcription Unit: The stretch of DNA downstream from the promoter.



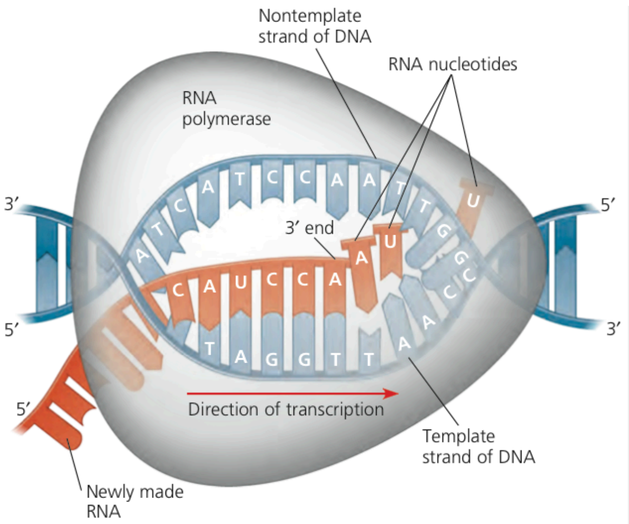
Synthesis of an RNA Transcript:

There are three stages of transcription: initiation, elongation and termination of the RNA chain.

1. **Initiation**
    - RNA polymerase binds to a promoter that determines where transcription starts and which of the two strands of the DNA helix is used as the template.
    - In eukaryotes, a collection of proteins called transcription

factors mediate the binding of RNA polymerase and the initiation of transcription. Only after transcription factors are attached to the promoter does RNA polymerase II bind to it. The complex of transcription factors and RNA polymerase II bound to the promoter is called a transcription initiation complex.

- Once the appropriate transcription factors are firmly attached to the promoter DNA and the polymerase is bound in the correct orientation, the enzyme unwinds the two DNA strands and begins transcribing the template strand at the start point.



2. Elongation

- As RNA polymerase moves along the DNA, it untwists the double helix, exposing DNA nucleotides for pairing with RNA nucleotides.
- The enzyme adds nucleotides to the 3′ end of the growing RNA molecule as it continues along the double helix.
- The new RNA molecule peels away from its DNA template and the DNA double helix re-forms.

3. Termination

- Termination differs between bacteria and eukaryotes. In bacteria, transcription proceeds through a terminator sequence in the DNA. The transcribed terminator (an RNA sequence) functions as the termination signal, causing the polymerase to stitch from the DNA and release the transcript, which requires no further modification before translation.
- In eukaryotes, RNA polymerase II transcribes a sequence on the DNA called the polyadenylation signal sequence, which specifies a polyadenylation signal in the pre-mRNA. The pre-mRNA then undergoes processing, the topic of the next section. Al- though that cleavage marks the end of the mRNA, the RNA polymerase II continues to transcribe. Since the new 59 end isn’t protected by a cap, however, enzymes degrade the RNA from the 59 end. The polymerase continues transcribing, pursued by the enzymes, until they catch up to the polymerase and it dissociates from the DNA.

Q. What enables RNA polymerase to start transcribing a gene at the right place on the DNA in a bacterial cell? In a eukaryotic cell?

Modifying RNA after transcription:

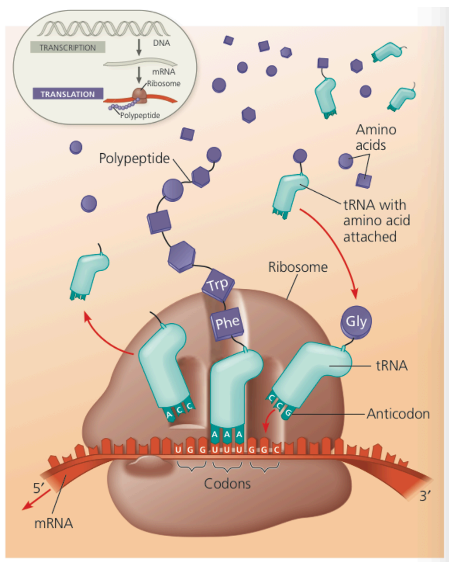
- 5′ end receives a 5′ cap and a poly-A tail is added to the 3′ end (termed pre-RNA). These features facilitate the export of mature mRNA from the nucleus, protect the mRNA from degradation by hydrolytic enzymes and help ribosomes attach to the 5′ end of the mRNA once the mRNA reaches the cytoplasm.
- RNA splicing: non coding regions (introns) are cut out of the RNA molecule (by small RNAs called spliceosomes) whilst exons are maintained and expressed.

Alternate RNA splicing: Alternative splicing is a regulated process during gene expression that results in a single gene coding for multiple proteins. In this process, particular exons of a gene may be included within or excluded from the final, processed messenger RNA (mRNA) produced from that gene.

TRANSLATION

Molecular components of Translation:

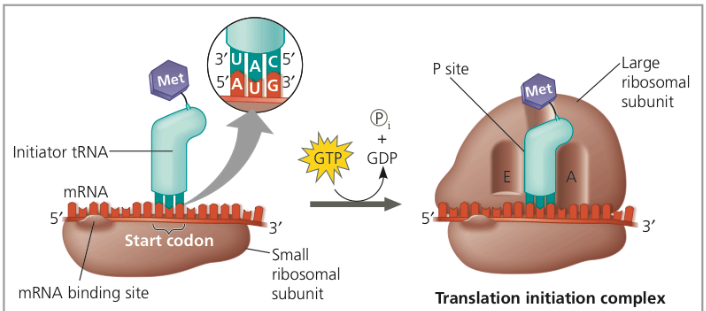
- tRNA translates messages in the form of series of codons along an mRNA molecule. The function of tRNA is to transfer amino acids from the cytoplasmic pool of amino acids to a growing polypeptide in a ribosome.
- Each tRNA molecule translates a given mRNA codon into a certain amino acid. This is possible because tRNA bears a specific amino acid at one end, which at the other end are nucleotide triplets that can base pair with the complementary codon on mRNA.



▲ **Figure 17.14 Translation: the basic concept.** As a molecule of mRNA is moved through a ribosome, codons are translated into amino acids, one by one. The interpreters are tRNA molecules, each type with a specific nucleotide triplet called an anticodon at one end and a corresponding amino acid at the other end. A tRNA adds its amino acid cargo to a growing polypeptide chain when the anticodon hydrogen-bonds to the complementary codon on the mRNA. The figures that follow show some of the details of translation in a bacterial cell.

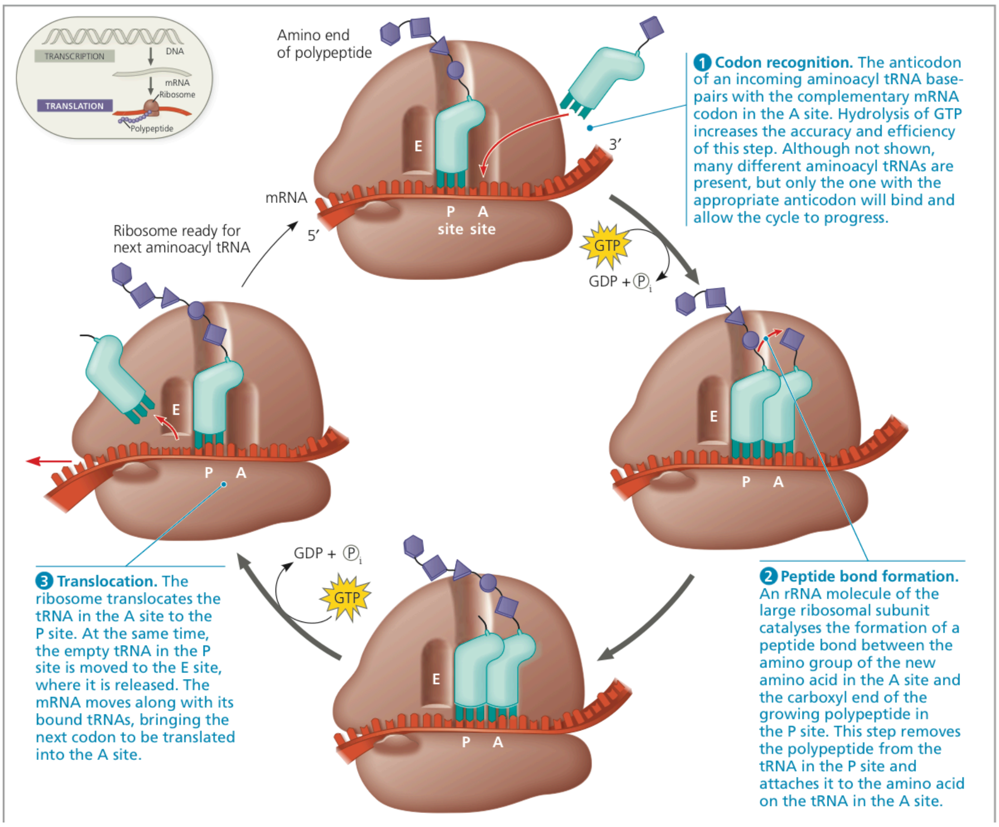
- The anticodon is the part of the nucleotide triplet that base pairs to a specific mRNA codon.
- Like mRNA and other types of cellular RNA, transfer RNA molecules are transcribed from DNA templates. In a eukaryotic cell, tRNA, like mRNA, is made in the nucleus and then travels from the nucleus to the cytoplasm, where it will participate in the process of translation. In both bacterial and eukaryotic cells, each tRNA molecule is used repeatedly, picking up its designated amino acid in the cytosol, depositing this cargo onto a polypeptide chain at the ribosome, and then leaving the ribosome, ready to pick up another of the same amino acid.

In addition to a binding site for mRNA, each ribosome has three binding sites for tRNA, as described in Figure 17.17. The P site (peptidyl-tRNA binding site) holds the tRNA carrying the growing polypeptide chain, while the A site (aminoacyl-tRNA binding site) holds the tRNA carrying the next amino acid to be added to the chain. Discharged tRNAs leave the ribosome from the E site (exit site). The ribosome holds the tRNA and mRNA in close proximity and positions the new amino acid so that it can be added to the carboxyl end of the growing polypeptide. It then catalyses the formation of the peptide bond. As the polypeptide becomes longer, it passes through an exit tunnel in the ribosome's large subunit. When the polypeptide is complete, it is released through the exit tunnel.



1 A small ribosomal subunit binds to a molecule of mRNA. In a bacterial cell, the mRNA binding site on this subunit recognises a specific nucleotide sequence on the mRNA just upstream of the start codon. An initiator tRNA, with the anticodon UAC, base-pairs with the start codon, AUG. This tRNA carries the amino acid methionine (Met).

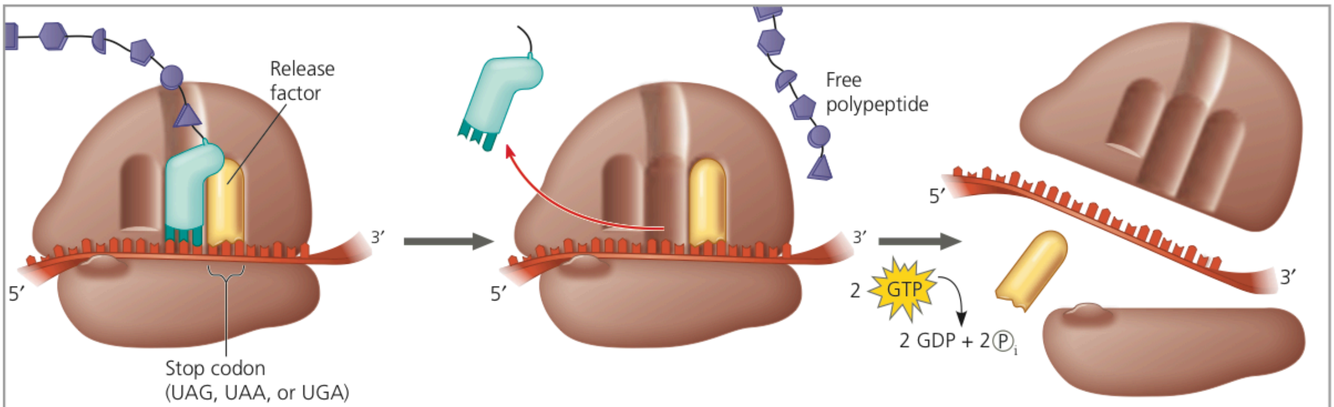
2 The arrival of a large ribosomal subunit completes the initiation complex. Proteins called initiation factors (not shown) are required to bring all the translation components together. Hydrolysis of GTP provides the energy for the assembly. The initiator tRNA is in the P site; the A site is available to the tRNA bearing the next amino acid.



1 **Codon recognition.** The anticodon of an incoming aminoacyl tRNA base-pairs with the complementary mRNA codon in the A site. Hydrolysis of GTP increases the accuracy and efficiency of this step. Although not shown, many different aminoacyl tRNAs are present, but only the one with the appropriate anticodon will bind and allow the cycle to progress.

2 **Peptide bond formation.** An rRNA molecule of the large ribosomal subunit catalyses the formation of a peptide bond between the amino group of the new amino acid in the A site and the carboxyl end of the growing polypeptide in the P site. This step removes the polypeptide from the tRNA in the P site and attaches it to the amino acid on the tRNA in the A site.

3 **Translocation.** The ribosome translocates the tRNA in the A site to the P site. At the same time, the empty tRNA in the P site is moved to the E site, where it is released. The mRNA moves along with its bound tRNAs, bringing the next codon to be translated into the A site.



1 When a ribosome reaches a stop codon on mRNA, the A site of the ribosome accepts a "release factor", a protein shaped like a tRNA, instead of an aminoacyl tRNA.

2 The release factor promotes hydrolysis of the bond between the tRNA in the P site and the last amino acid of the polypeptide, thus freeing the polypeptide from the ribosome.

3 The two ribosomal subunits and the other components of the assembly dissociate.