

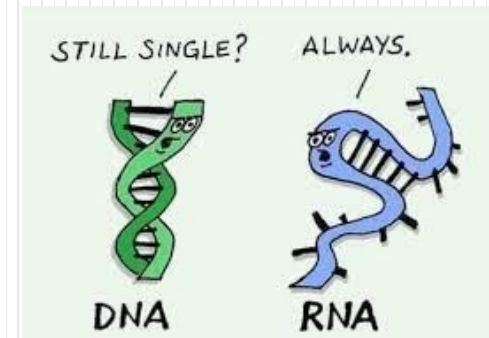
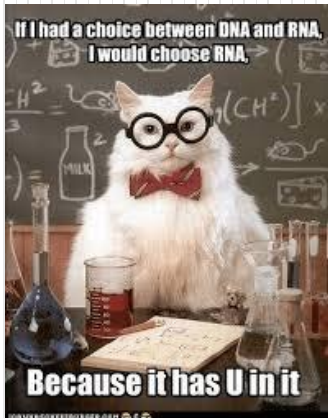
Transcription and translation

Lecture 14

SLE254 Genetics and Genomics

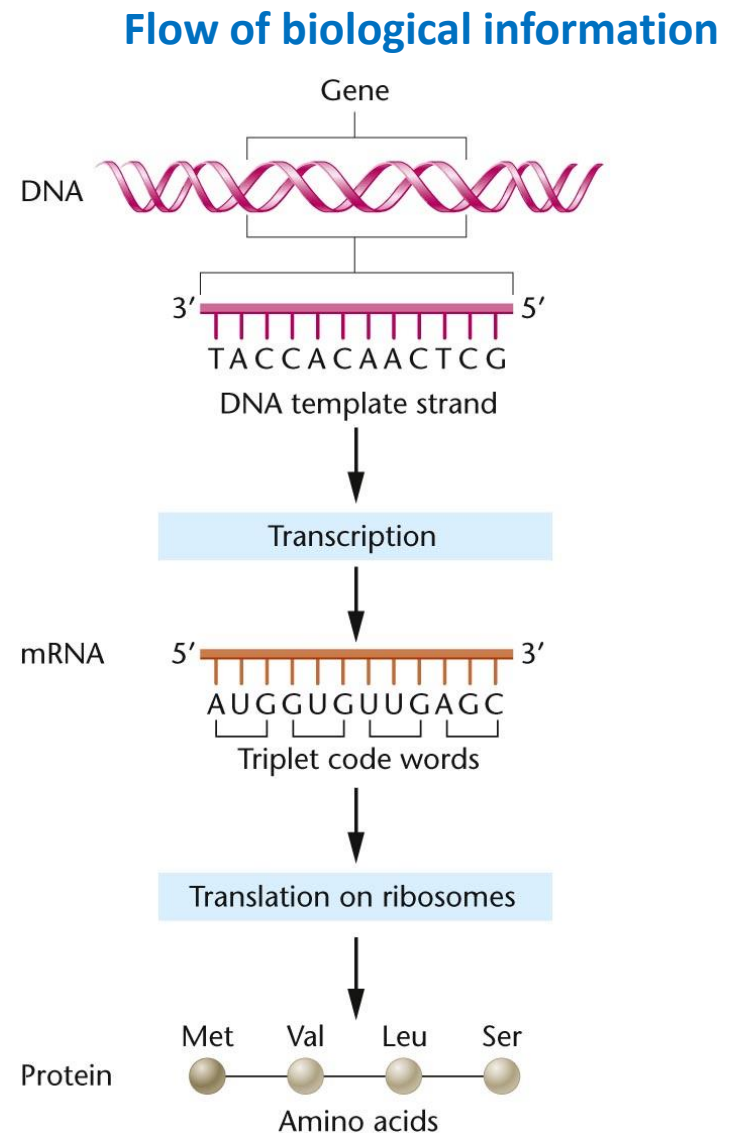
Concepts of Genetics (12th ed)

Chapters 13 & 14 pp321-377



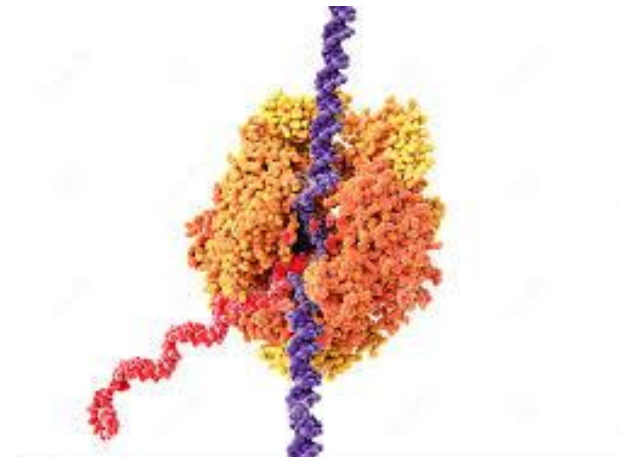
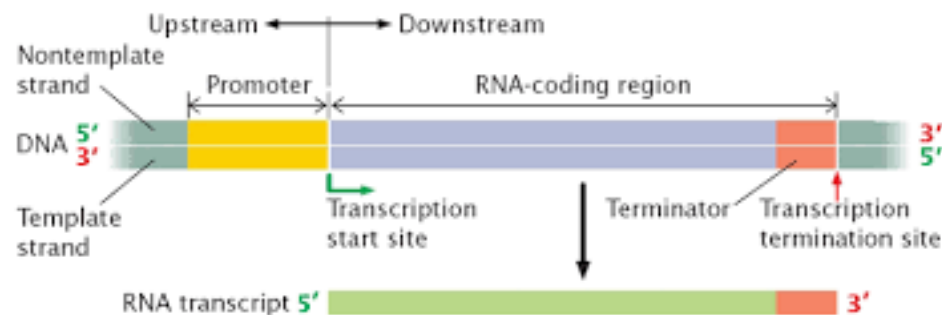
Genes are expressed to create proteins

- Creating a protein from a gene is known as **gene expression**
- Gene expression involves a two step process
 1. The DNA sequence must be copied (**transcribed**) into mRNA
 2. The mRNA must be converted (**translated**) into protein



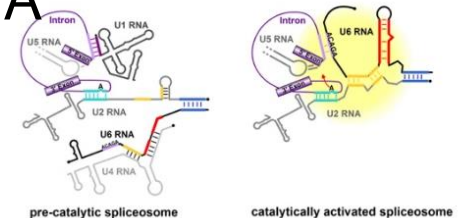
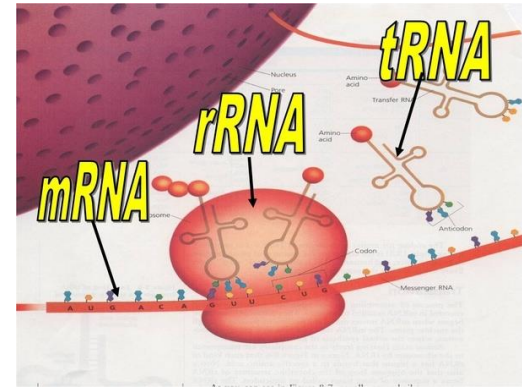
Transcription

- Transcription begins at particular DNA sequences known as **promoter sequences**
- Initiation of transcription is controlled by binding of the enzyme **RNA polymerase** and other proteins to the promoter
- The RNA polymerase copies the DNA sequence into RNA



Types of RNA

- **mRNA** – messenger RNA
 - Translated into protein
- **rRNA** – ribosomal RNA
 - Forms part of a ribosome which is needed for translation of mRNA
- **tRNA** – transfer RNA
 - Acts as a linker between mRNA and amino acids
- **snRNA** – small nuclear
 - Structural and catalytic roles in spliceosomes, the protein/RNA complexes that splice mRNA



Template

- The **template strand** is the DNA strand from which the RNA is actually transcribed
 - It is also termed the **antisense** strand
- The **coding strand** is the strand whose base sequence specifies the amino acid sequence of the encoded protein
 - It is also called the **sense** strand



**Coding strand sequence = mRNA sequence
(except for Uracil replacing thymine)**

Template

5'----- G C A G T A C A T G T C----- 3'

3'----- C G T C A T G T A C A G----- 5'

coding strand = Sense

template strand = Antisense

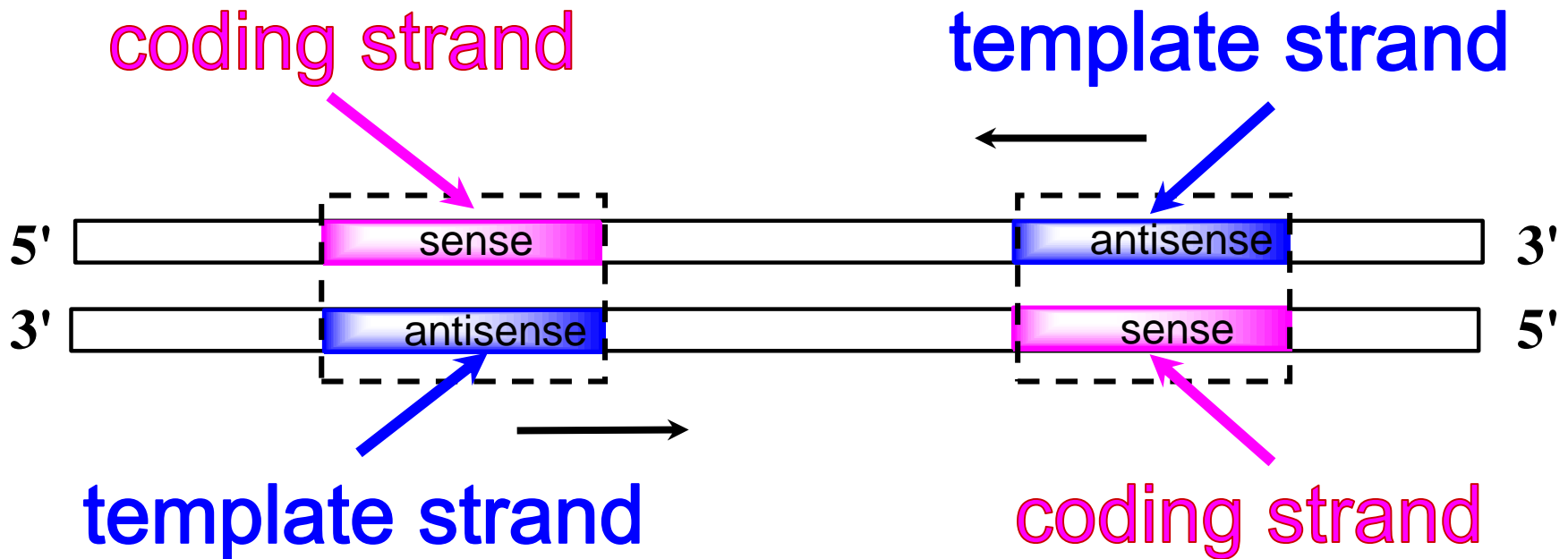
transcription

5'----- G C A G U A C A U G U C----- 3'

RNA

Asymmetric transcription

- Both DNA strands can be used as RNA templates
- The transcription direction on different strands is opposite
- This feature is referred to as the **asymmetric transcription**



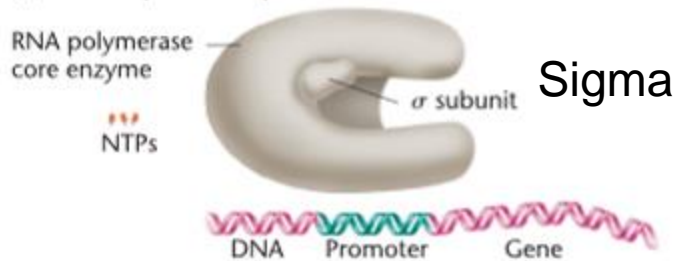
Transcription stages

- The stages of transcription are
 - Initiation
 - Elongation
 - Termination

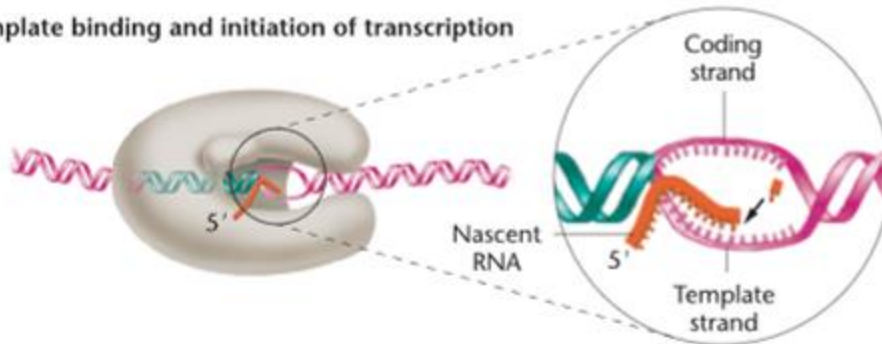
1. Initiation

Prokaryotes

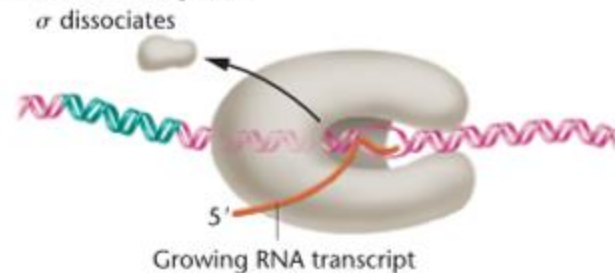
(a) Transcription components



(b) Template binding and initiation of transcription



(c) Chain elongation



Eukaryote

TATA box start of transcription

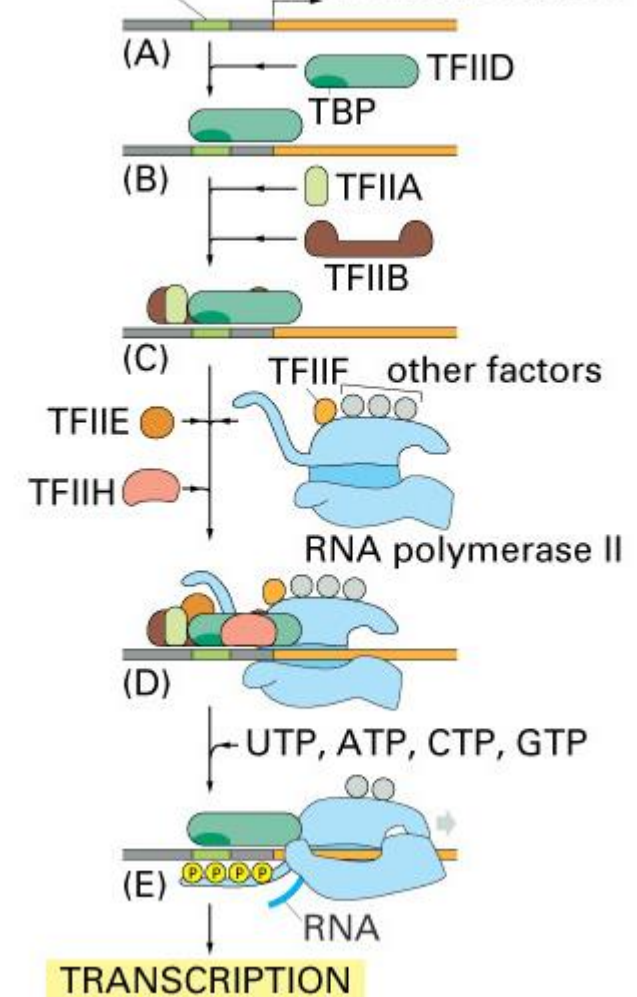


Fig 13.9

RNA polymerase

The enzyme responsible for the RNA synthesis is **DNA-dependent RNA polymerase**

Prokaryotes

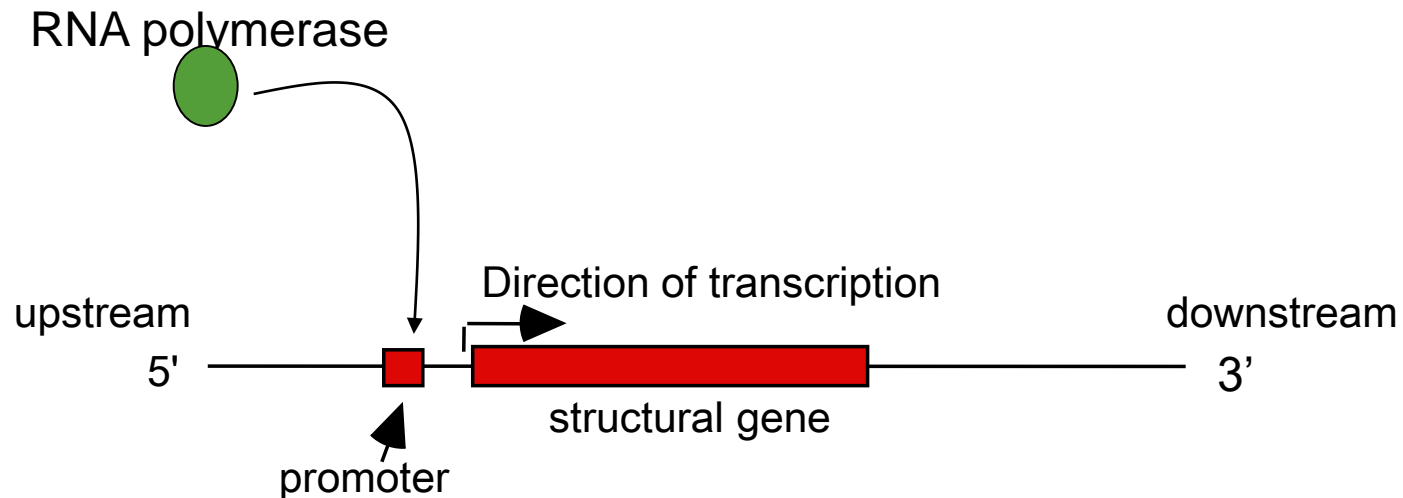
- The prokaryotic RNA polymerase is a multiple-subunit protein of ~480kD

Eukaryotes

- Eukaryotic systems have **three kinds of RNA polymerases**, each of which is a multiple-subunit protein and responsible for transcription of different RNAs

Promoters

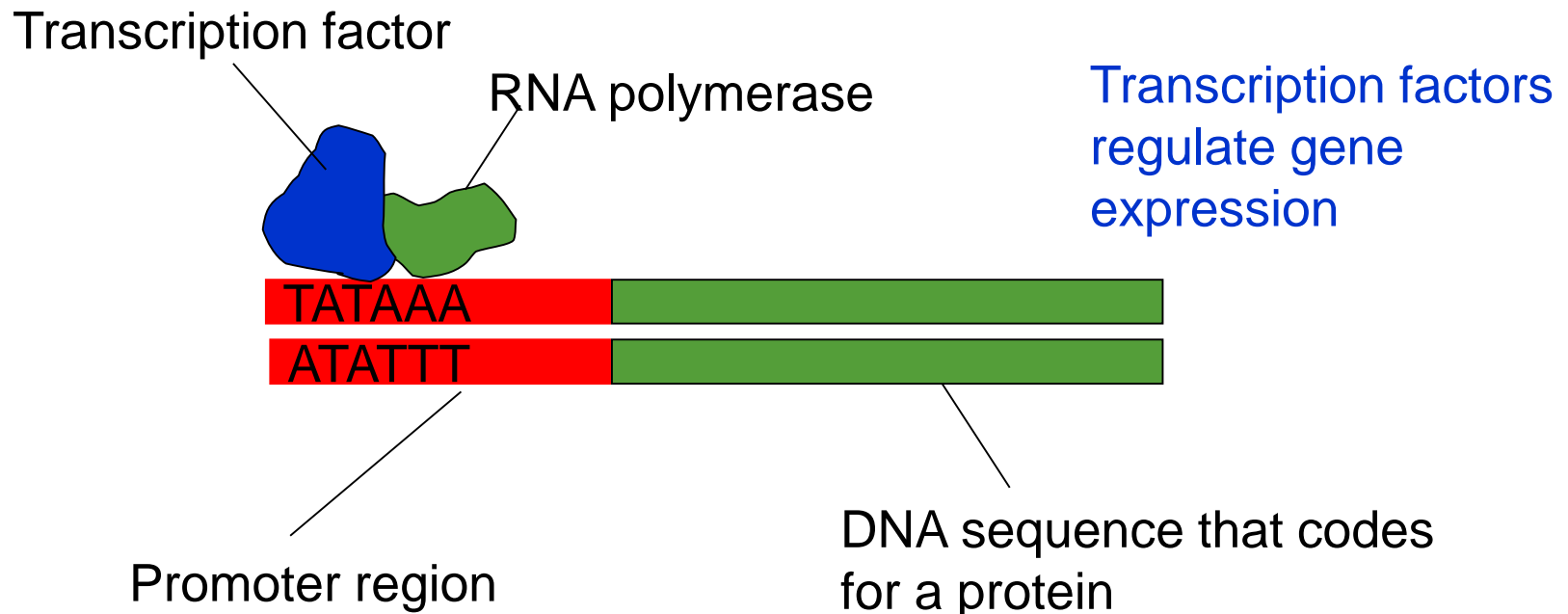
- Promoters direct the binding of RNA polymerases



- Promoters are 'upstream' of the main part of the gene (structural gene) and are **not** themselves transcribed.
- RNA polymerase binds to the promoter.**

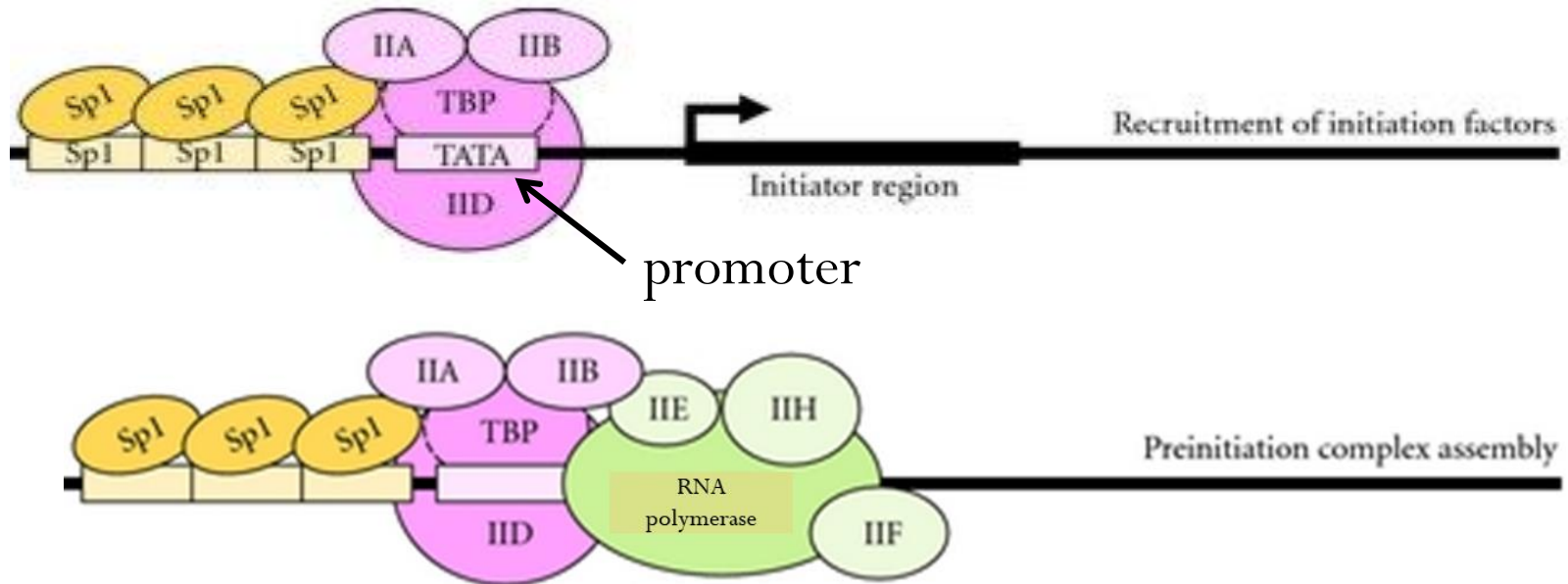
Promoters and transcription factors

- Promoters contain conserved sequences *such as* the **TATA box** and regulatory sequences
- These sequences are recognised by proteins called **transcription factors** that bind and **recruit RNA polymerase**

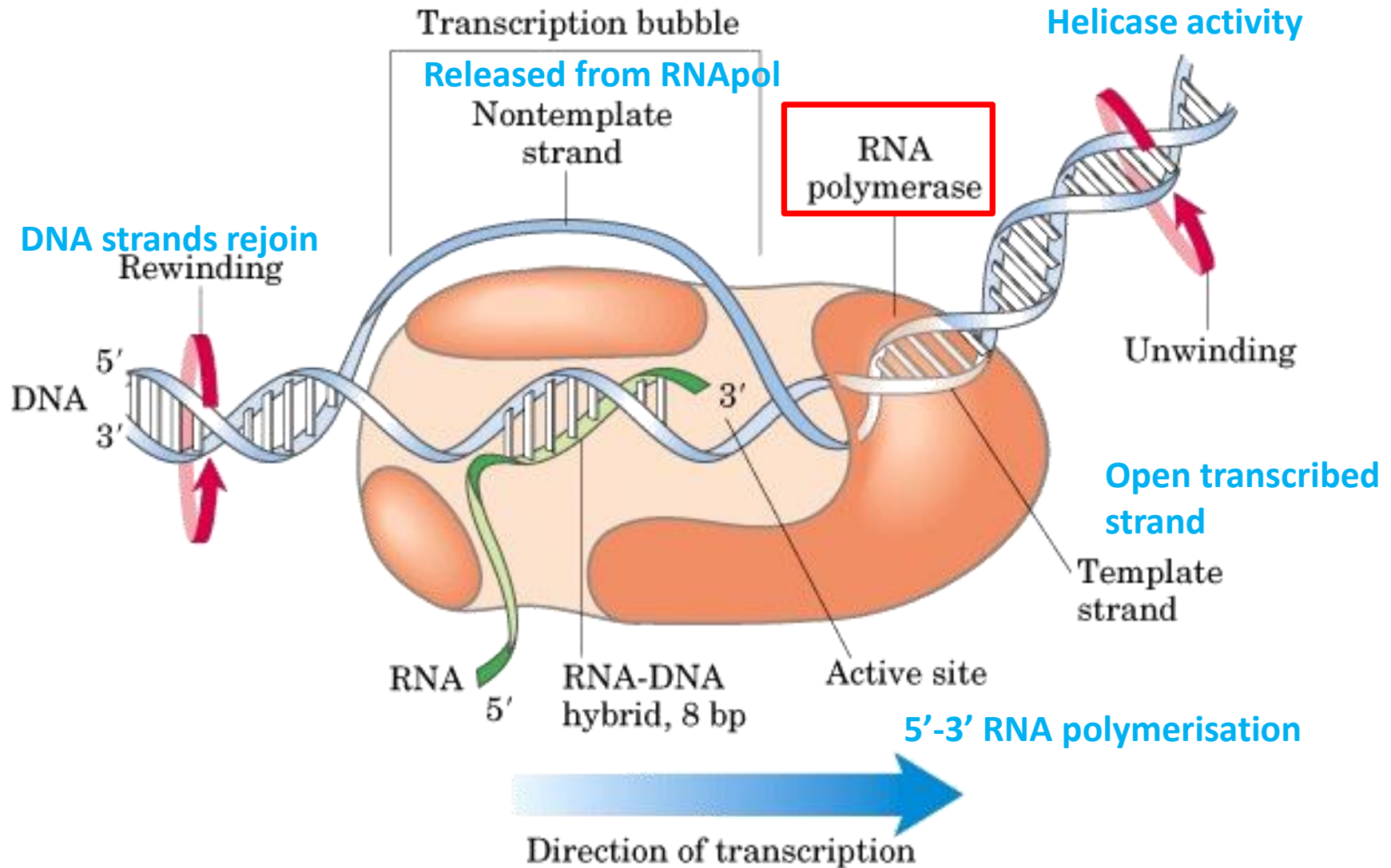


Transcription factors are more complex in Eukaryotes

- Transcription factors (e.g. SP1, TFIID, TBP) regulate gene expression via:
 - enhancing gene transcription
 - facilitating and stabilizing polymerase binding to promoter region
 - helping to create the transcription bubble



Transcription bubble

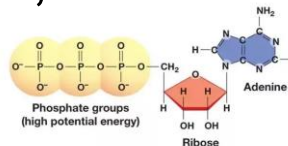




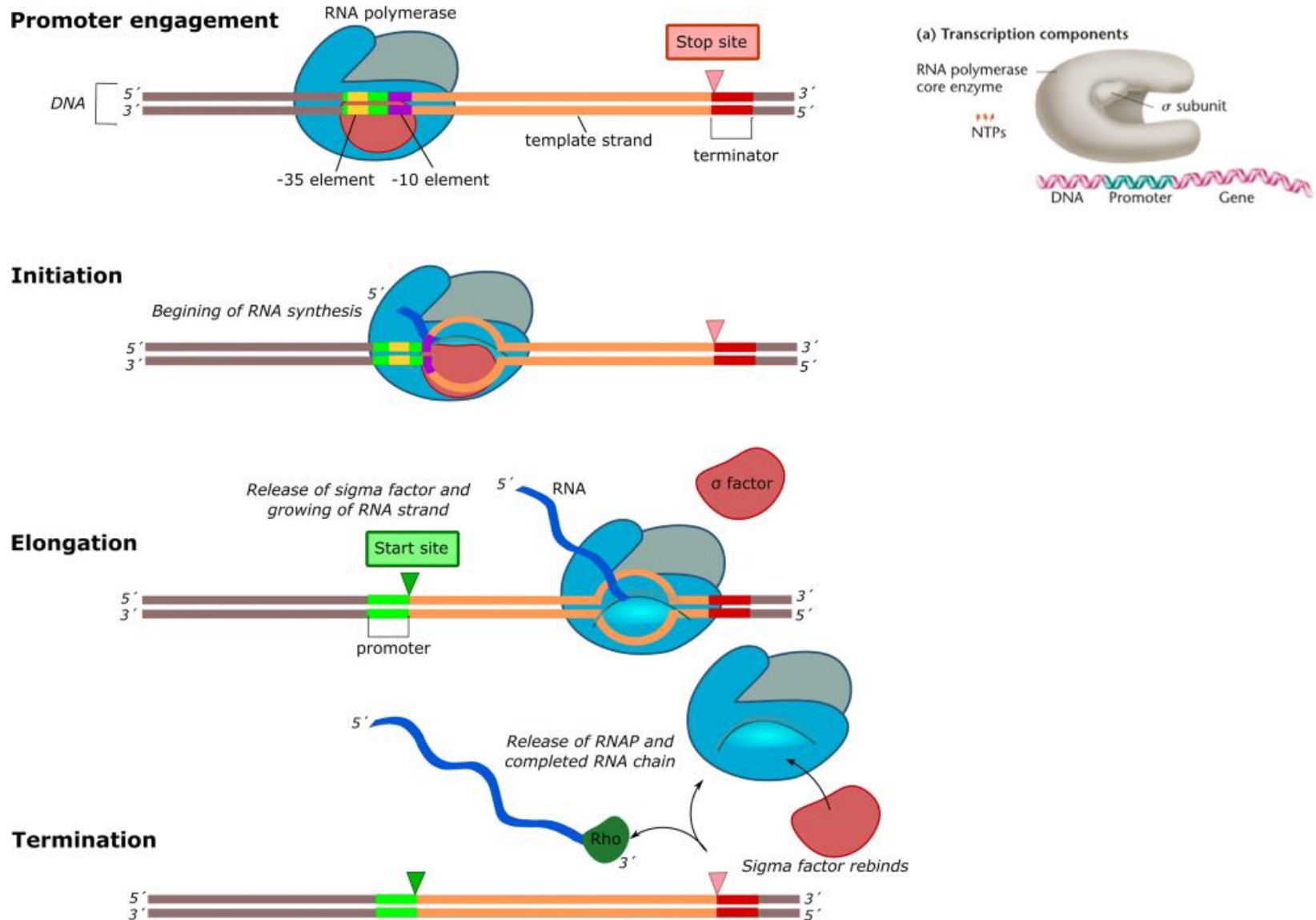
www.dnalc.org

Promoter clearance: prokaryotes

- After initiation, the RNA polymerase **must clear the promoter**
- **Abortive initiation**
 - Tendency for RNA polymerase to release the RNA transcript and produce truncated transcripts
- **The σ factor** (sigma) is a protein needed for transcription initiation (prokaryotes)
- **σ factor** needs to be released, causing the conformational change of the core enzyme, allowing elongation to occur
 - This, like most of the remainder of transcription, is an **energy-dependent process**, consuming **adenosine triphosphate (ATP)**

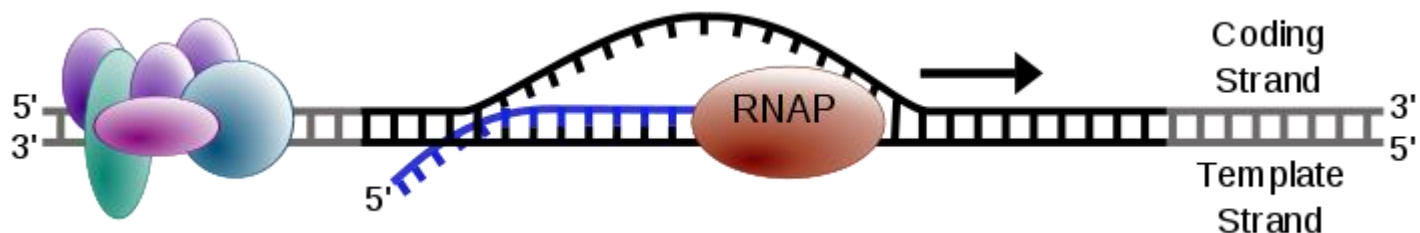


Sigma factor and transcription (prokaryotes)



2. Elongation

- RNA Polymerase unwinds DNA through **helicase** activity
- The RNA polymerase incorporates nucleotides through **complementary base pairing**
 - RNA has uracil instead of thymine, and this binds to adenine on the DNA template
- A strand of RNA is synthesised as the enzyme complex moves along the template DNA
- The separated strands of **DNA come back together** and the **RNA is dissociated**
 - The whole process of elongation is dependent on energy from ATP
 - Long, full length transcripts are produced



3. Termination

- Specific sequences in the DNA signal termination of transcription
- When one of these is encountered by the polymerase, the RNA transcript is released from the DNA
- In prokaryotes, a hairpin loop is formed in the mRNA signalling the end of transcription

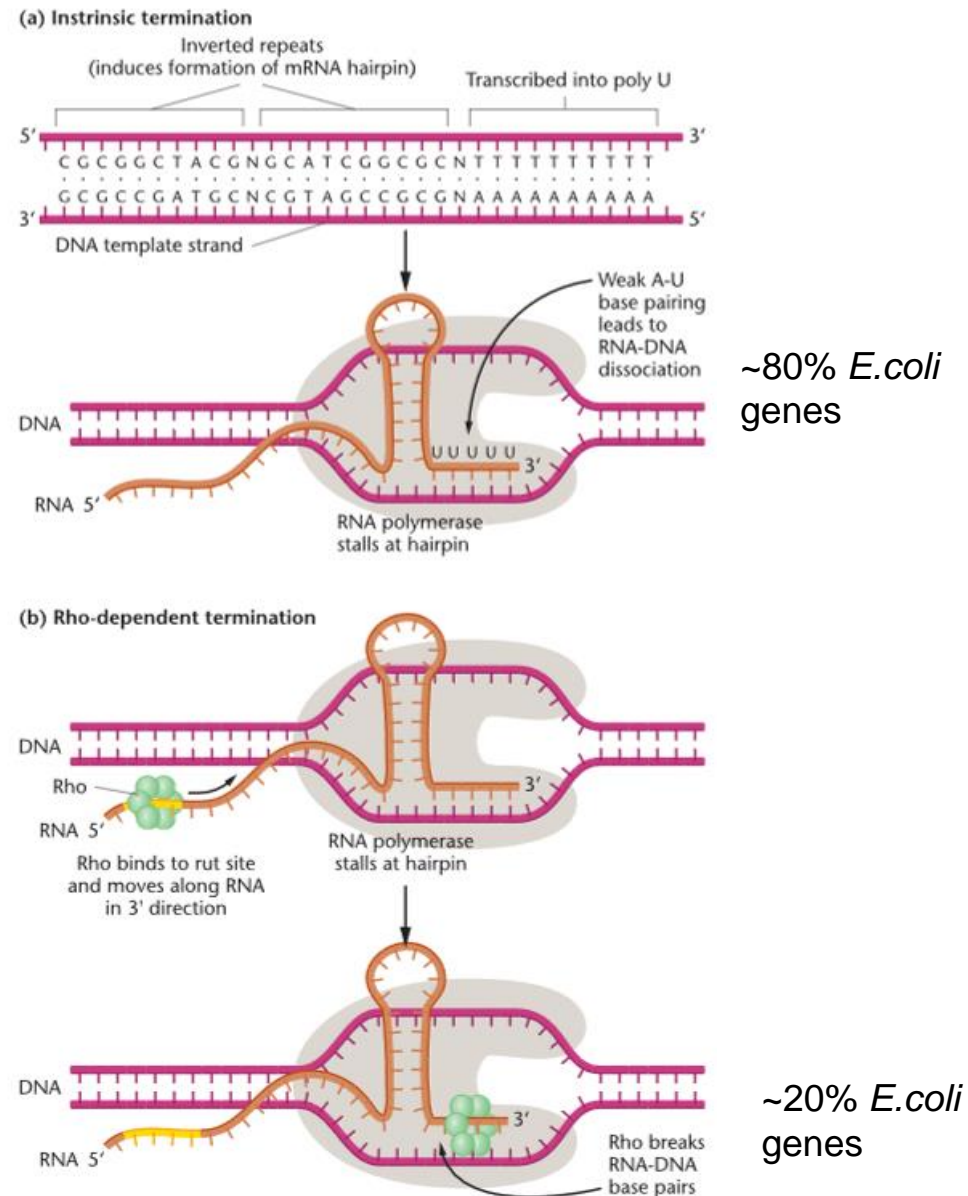
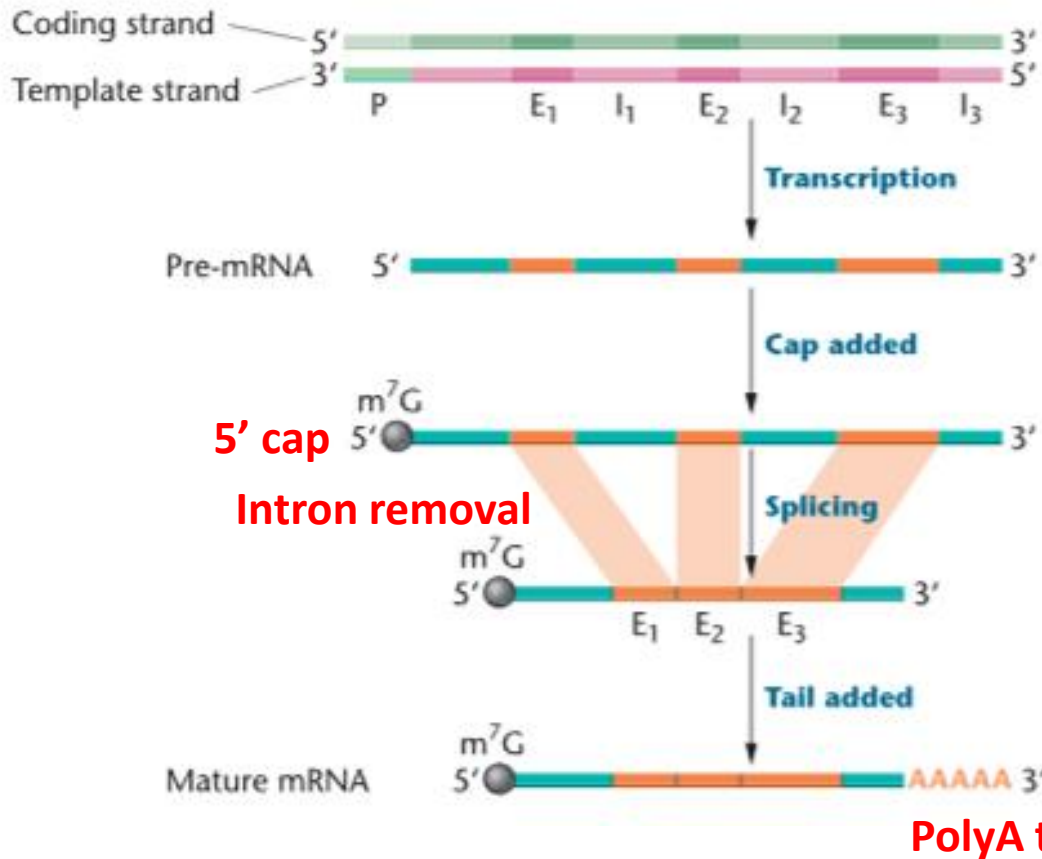


Fig 13.10

Termination in eukaryotes

- The synthesised mRNA must be **processed** before it is exported to the cytoplasm for translation
- Three processing steps for mRNA that happen in the nucleus:
 1. A 7-methylguanosine (MG) cap is added to 5' end
 2. A poly(A) tail is added to 3' end (polyadenylation)
 3. Intron sequences are removed (splicing)

mRNA processing in eukaryotes

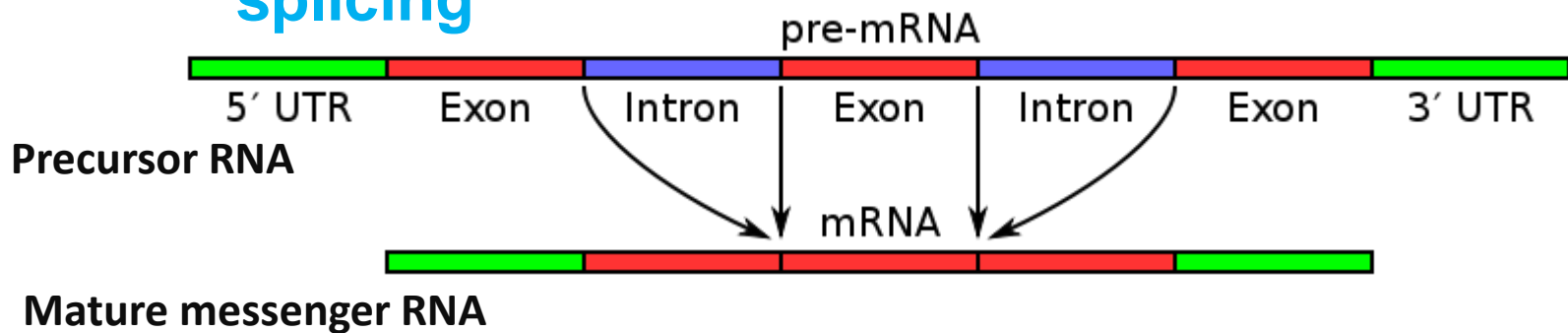


- 1. Caps added to 5'-end of pre-mRNA
- 2. Poly(A) tail added to 3'end
- 3. Introns are spliced out

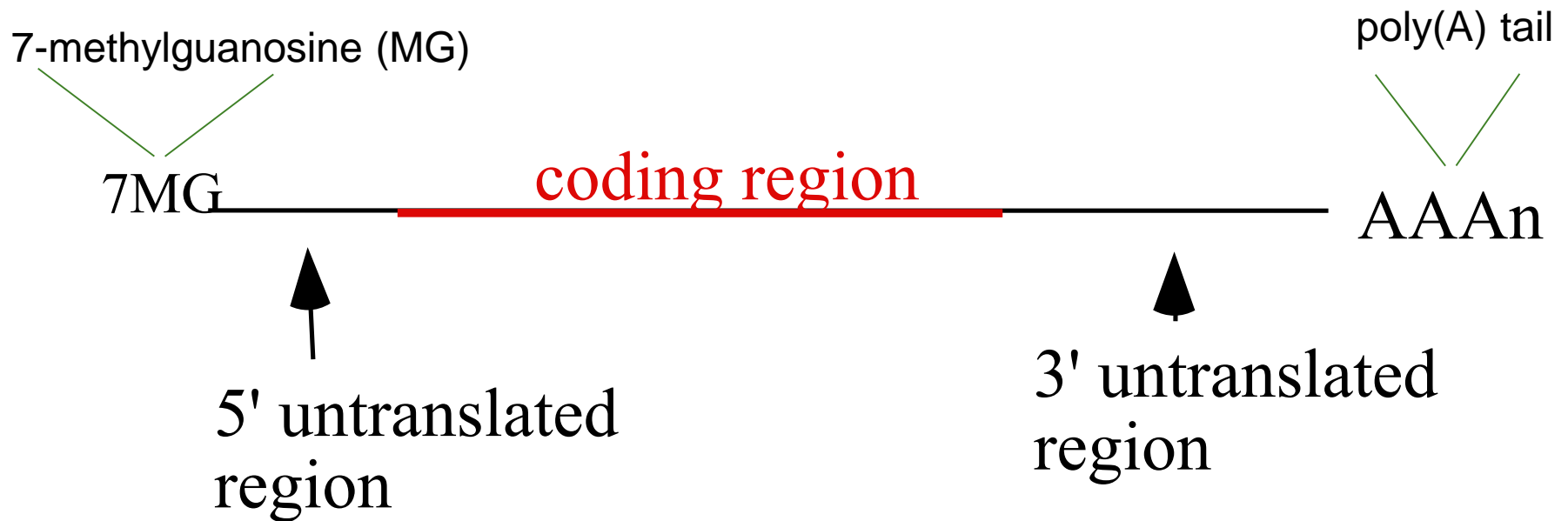
Fig 13.11

Exons and introns

- The coding sequence of most human genes are interrupted by non-coding sequences called **introns**
- The **exons** are the sequences that appear in the mature mRNA
 - Introns are removed in a process known as **intron splicing**



Anatomy of a mature mRNA



Ready for export from the nucleus and translation...

Intron splicing

- Introns are removed from the primary RNA transcript while it is still in the nucleus
- Introns are “spliced out” by RNA/protein hybrids called **spliceosomes**
- The intron sequences are removed, and the remaining ends are re-attached so the final RNA consists of exons only
- **snRNPs**: Small ribonuclear proteins

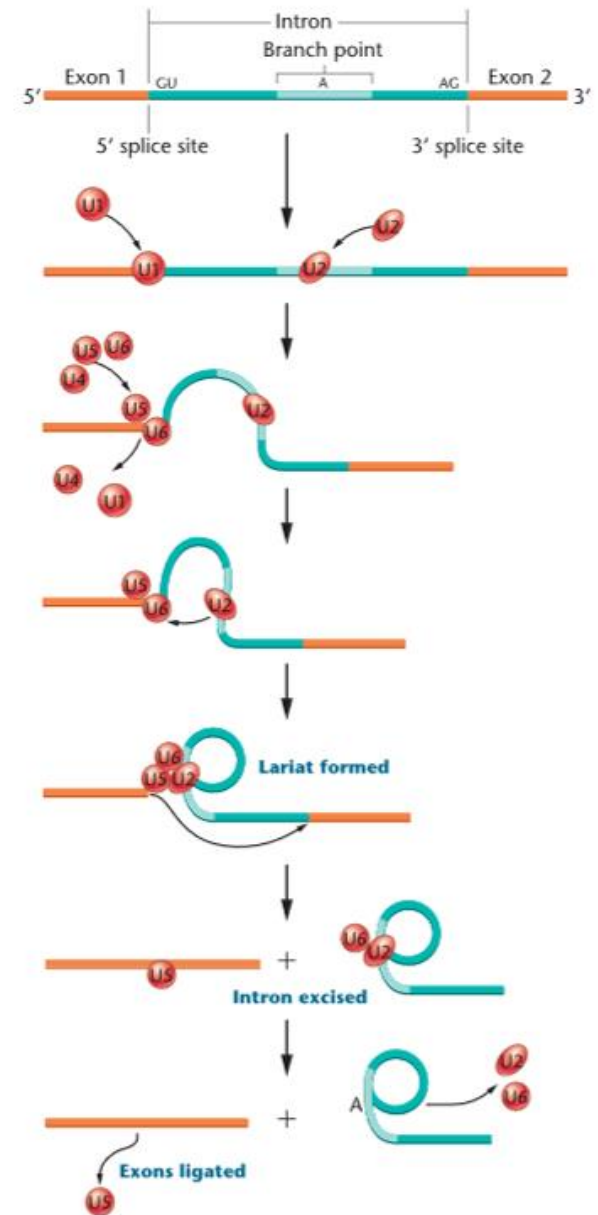
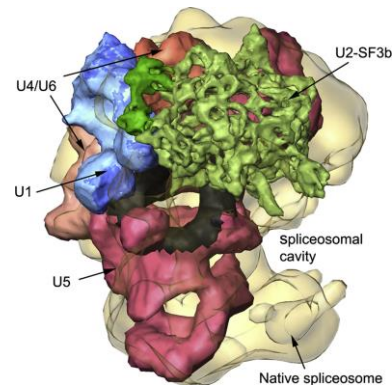


Figure 13.14

Main differences between prokaryotic and eukaryotic transcription

Prokaryotes

- In prokaryotes transcription and translation can occur at the same time (**no nucleus**)

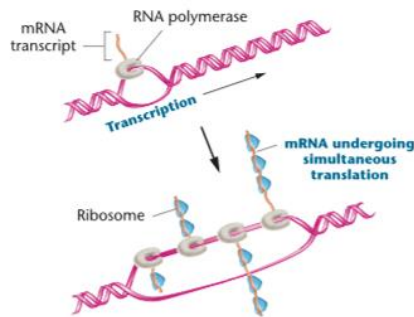


Fig 13.16

- In prokaryotes (e.g. *E.coli*) many genes can be transcribed together into one large mRNA (polycistronic)

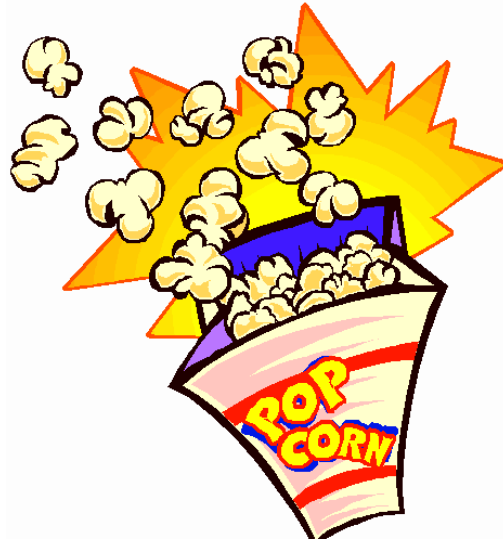
Eukaryotes

- Transcription factors are needed for eukaryotic RNA polymerases – e.g. enhancers and silencers
- Chromatin nucleosomes (histone structures) in eukaryotes need to be uncoiled – called chromatin remodelling
- Eukaryotic genes have introns (intervening sequences) that need to be spiced from pre-mRNA. Usually no introns in prokaryotes

Translation

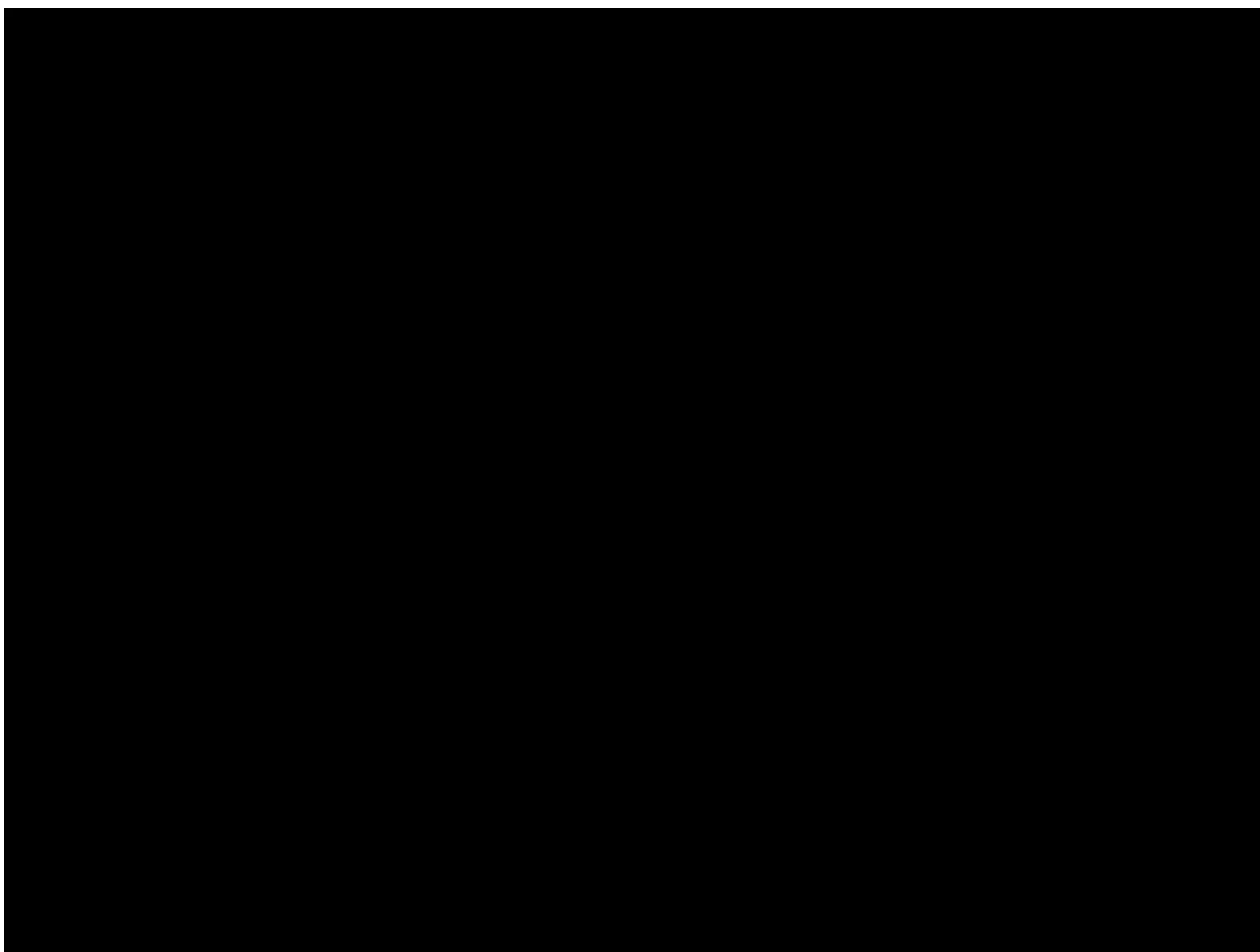
- Translation of mRNA occurs in the cytoplasm on ribosomes
 - Ribosomes consist of **ribosomal RNA (rRNA) and proteins**
- Transfer RNA used as an adapter between an amino acid and the mRNA **codon**
 - A codon is a triplet of bases that specifies an **amino acid**

Movie time

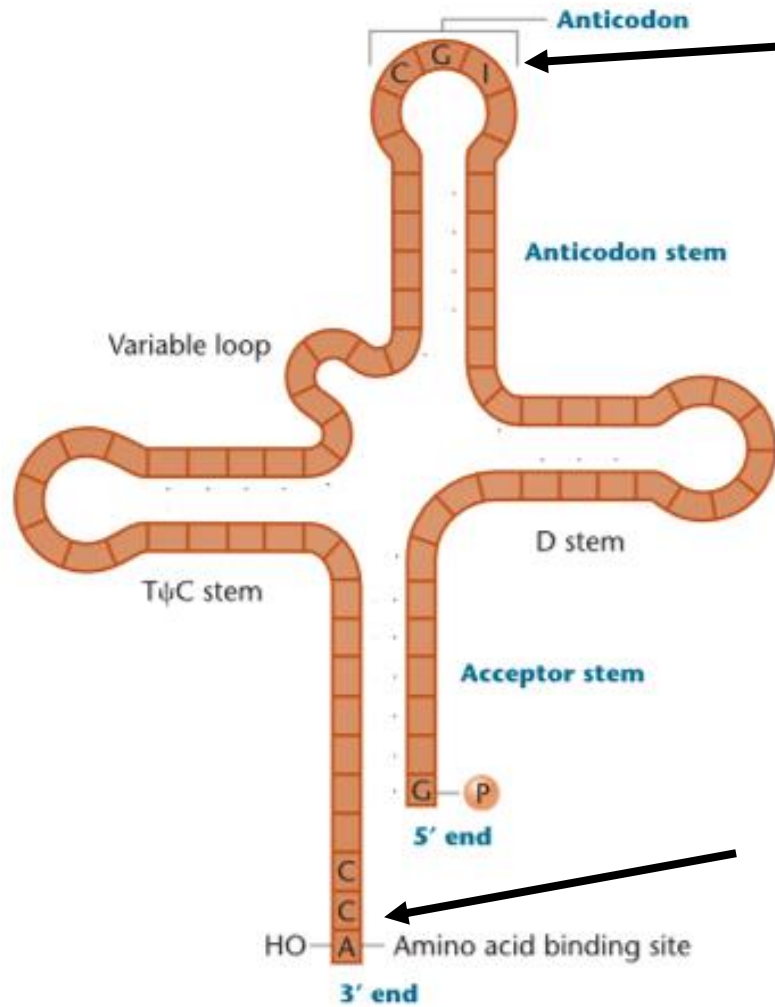


<http://www.youtube.com/watch?v=5bLEDd-PSTQ&feature=relmfu>

3.32min



tRNA molecule



1. Pairs with a complimentary codon sequence in mRNA

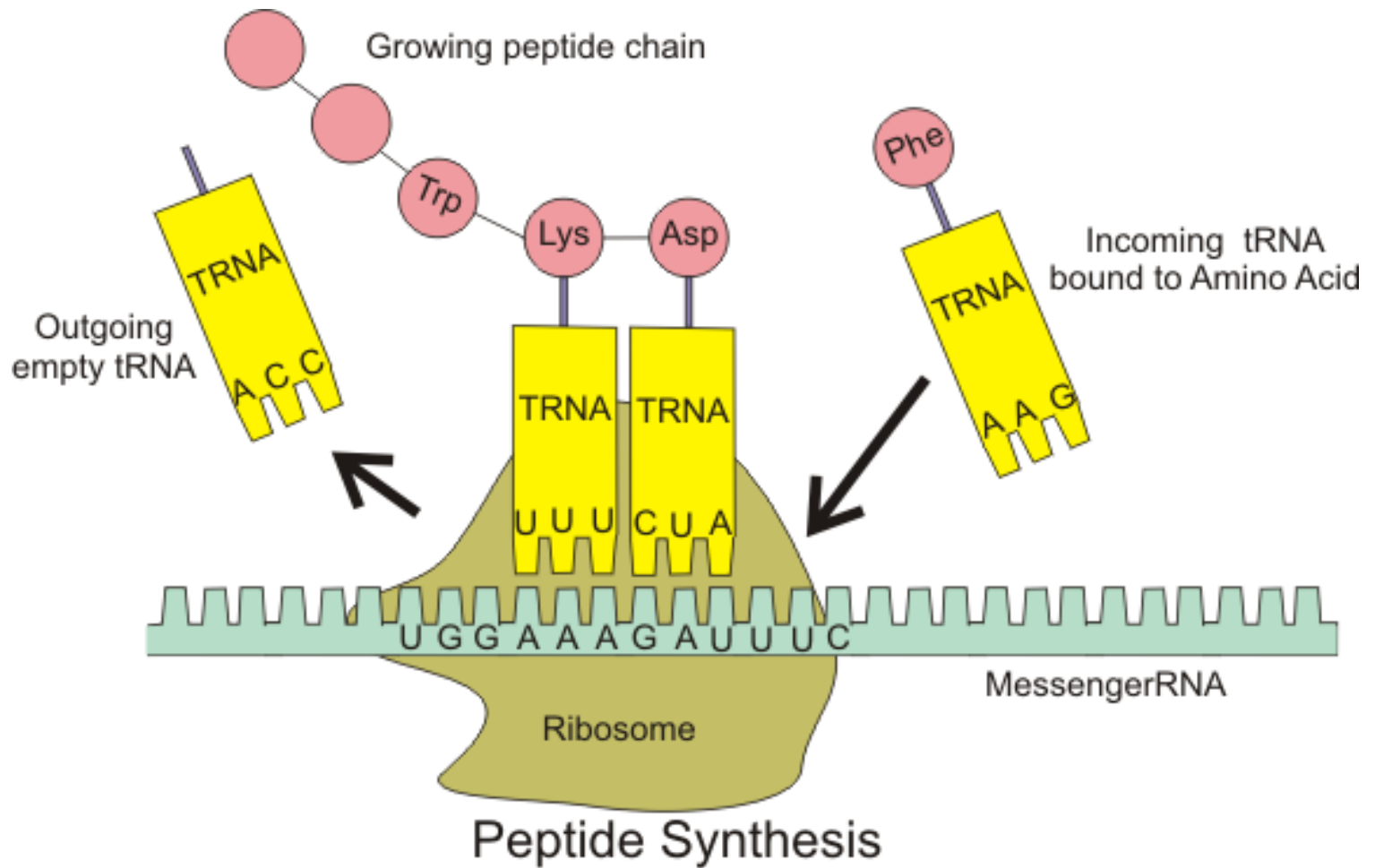
2. Site for attachment of the amino acid specified by the mRNA codon

FIGURE 14.3 Holley's two-dimensional cloverleaf model of tRNA^{Ala}. Hydrogen bonds are designated by dots (. . .).

Amino acids and tRNAs

- There must be a correct match between a tRNA and its amino acid
 - A tRNA that binds to an mRNA codon specifying a particular amino acid must carry only that amino acid
- Each amino acid is joined to the correct tRNA by means of a specific enzyme called **aminoacyl-tRNA synthetase**.
- There are **20 different** aminoacyl-tRNA synthetases – one for each amino acid.

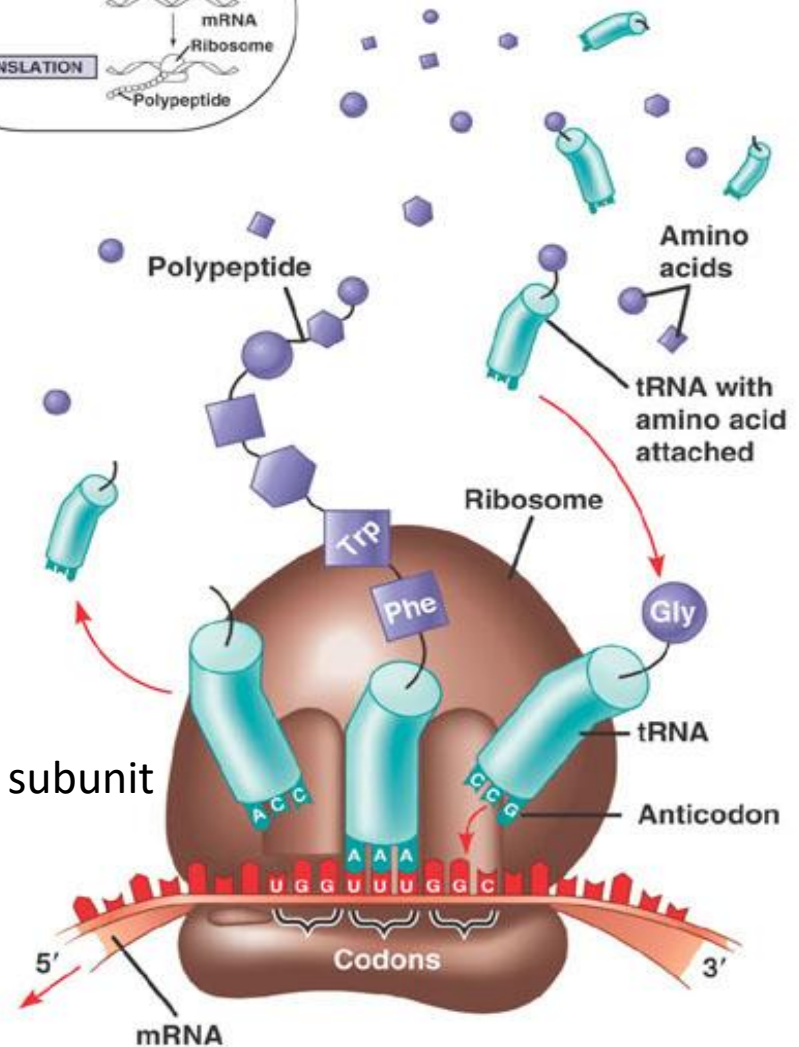
Translation



Translation

- There are **two** ribosomal subunits.
 - The small subunit binds the mRNA
 - The large subunit has binding sites for the tRNAs and also has an enzyme that forms the peptide bond

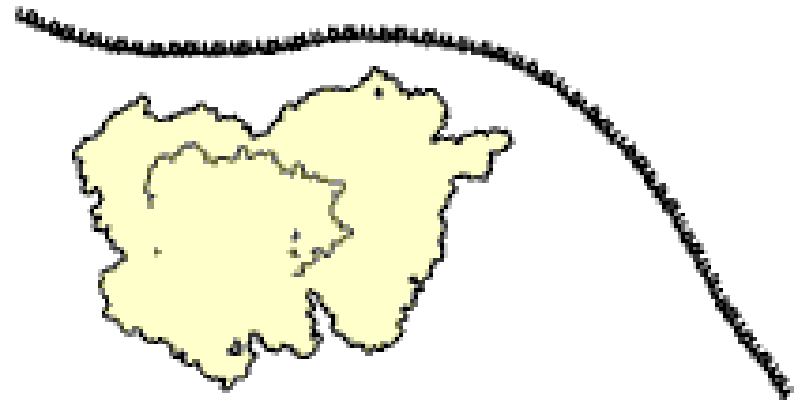
Large rRNA subunit



Small rRNA subunit

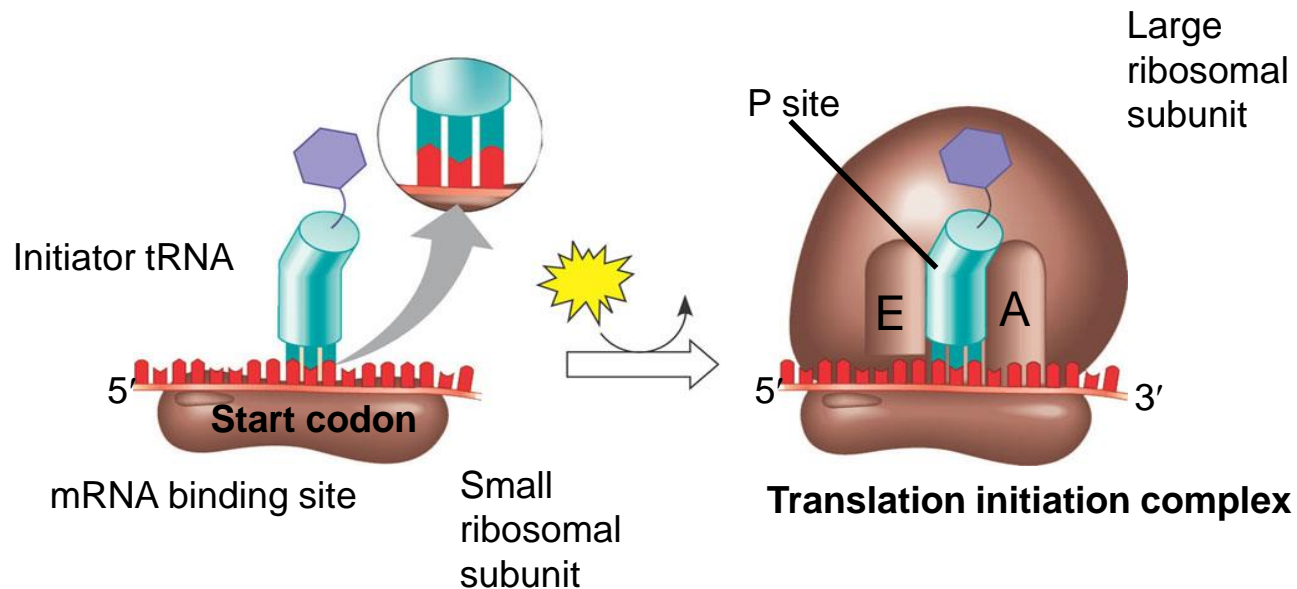
Translation steps

- Translation has three steps
 1. Initiation
 2. Elongation
 3. Termination



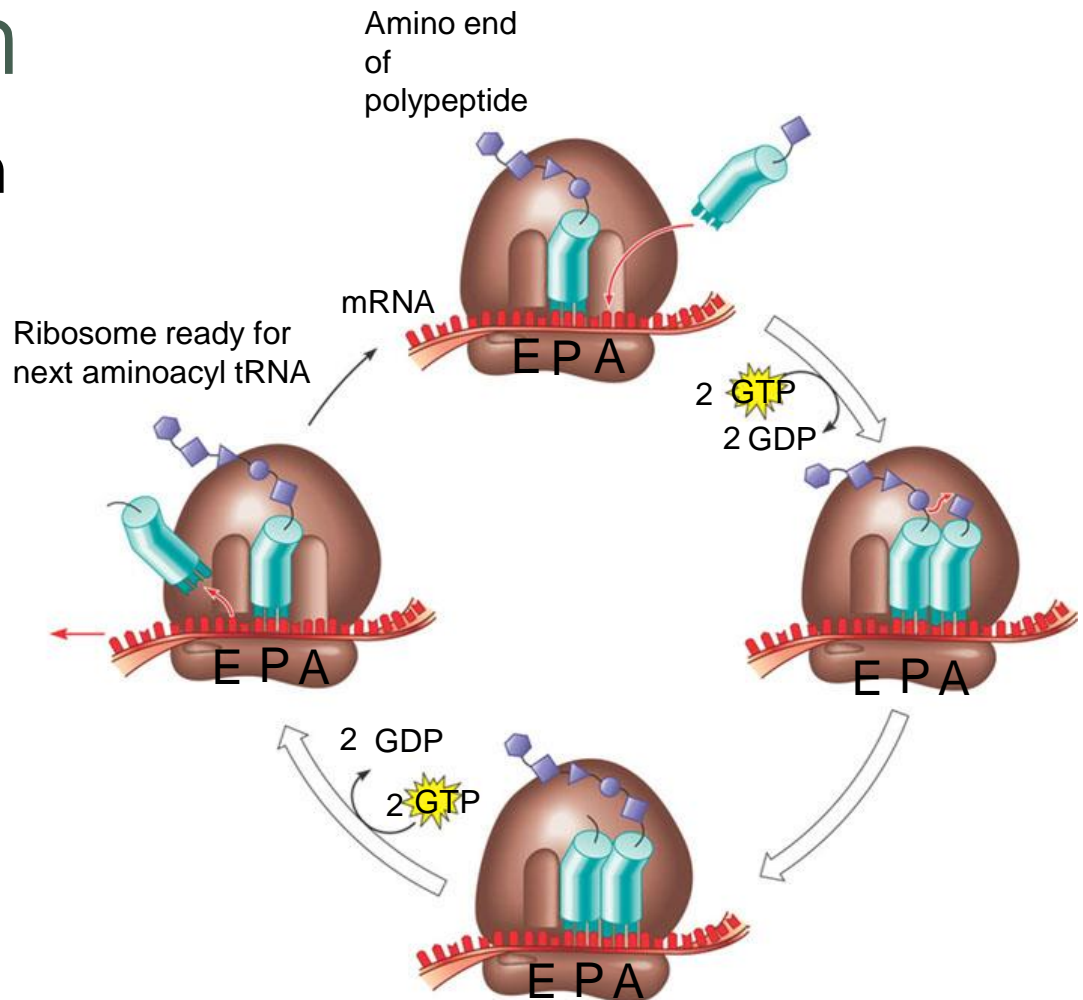
1. Initiation

- The **AUG start codon** is recognized by methionyl-tRNA or Met (Methionine)



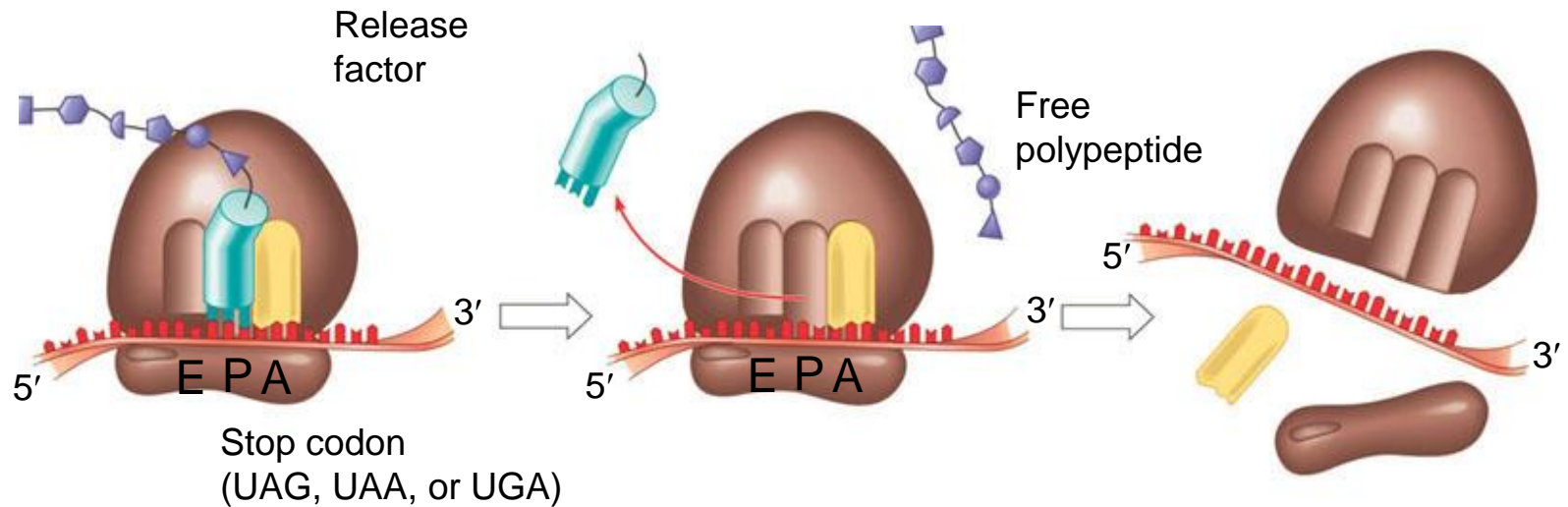
2. Elongation

- Once the start codon has been identified, the ribosome incorporates amino acids into a polypeptide chain
- RNA is decoded by tRNA (transfer RNA) molecules, which each transport specific amino acids to the growing chain



3. Termination

- Translation ends when a stop codon (UAA, UAG, UGA) is reached



Determining the amino acid sequence

- The coding region of the mRNA consists of a sequence of nucleotides that specifies a particular order of amino acids
- Every three consecutive nucleotides in mRNA is a **codon**
- Each codon specifies an amino acid
 - E.g. the sequence AUG CCC contains two codons that specify methionine and proline

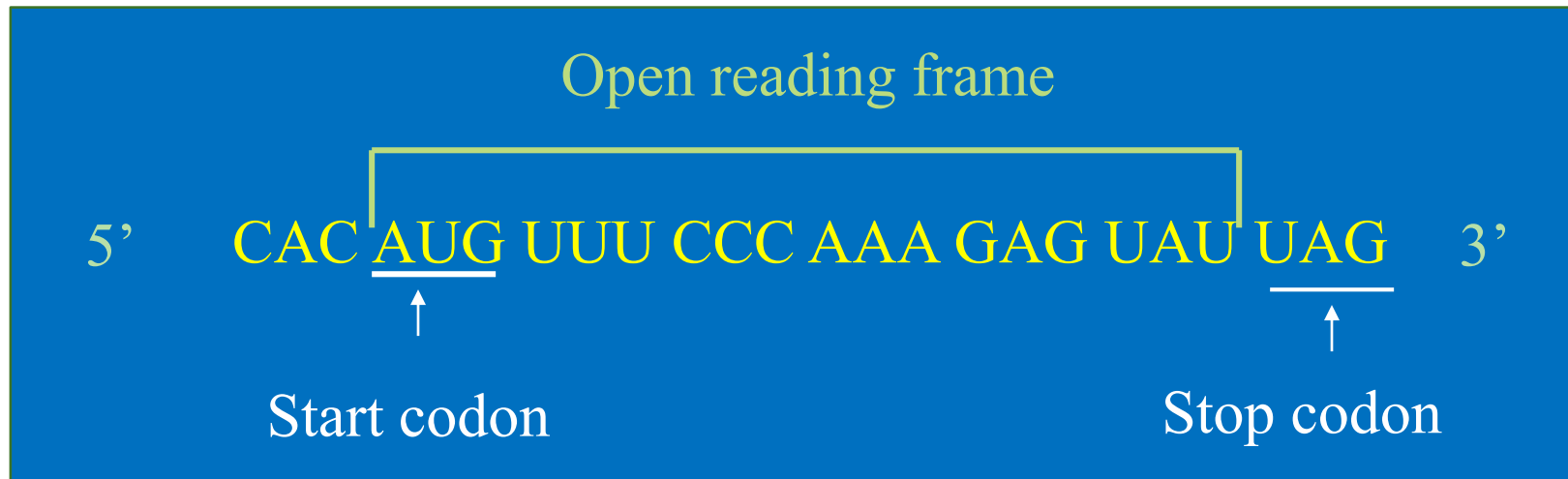
The complete genetic code consists of 64 codons, 61 specify the 20 amino acids and 3 are stop codons

Codons

- **There is more than one codon for most amino acids**
 - 1 met (M) trp (W)
 - 2 phe (F) tyr (Y) his (H) gln (Q) asn (N) lys (K) asp (D) glu (E) cys (C)
 - 3 ile (I)
 - 4 val (V) pro (P) thr (T) ala (A) gly (G)
 - 6 leu (L) arg (R) ser (S)

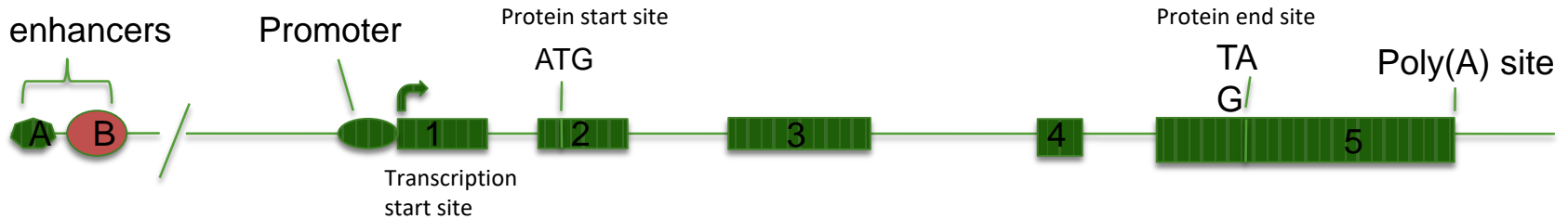
		Second position					
		U	C	A	G		
First position	U	UUU } Phe	UCU } Ser	UAU } Tyr	UGU } Cys	U	Third position
		UUC }	UCC }	UAC }	UGC }	C	
		UUA } Leu	UCA }	UAA Stop	UGA Stop	A	
		UUG }	UCG }	UAG Stop	UGG Trp	G	
	C	CUU }	CCU }	CAU } His	CGU } Arg	U	
		CUC } Leu	CCC } Pro	CAC }	CGC }	C	
		CUA }	CCA }	CAA } Gln	CGA }	A	
		CUG }	CCG }	CAG }	CGG }	G	
	A	AUU }	ACU } Thr	AAU } Asn	AGU } Ser	U	
		AUC } Ile	ACC }	AAC }	AGC }	C	
		AUA }	ACA }	AAA } Lys	AGA } Arg	A	
		AUG Met/start	ACG }	AAG }	AGG }	G	
	G	GUU }	GCU } Ala	GAU } Asp	GGU } Gly	U	
		GUC } Val	GCC }	GAC }	GGC }	C	
		GUA }	GCA }	GAA } Glu	GGA }	A	
		GUG }	GCG }	GAG }	GGG }	G	

Open reading frame



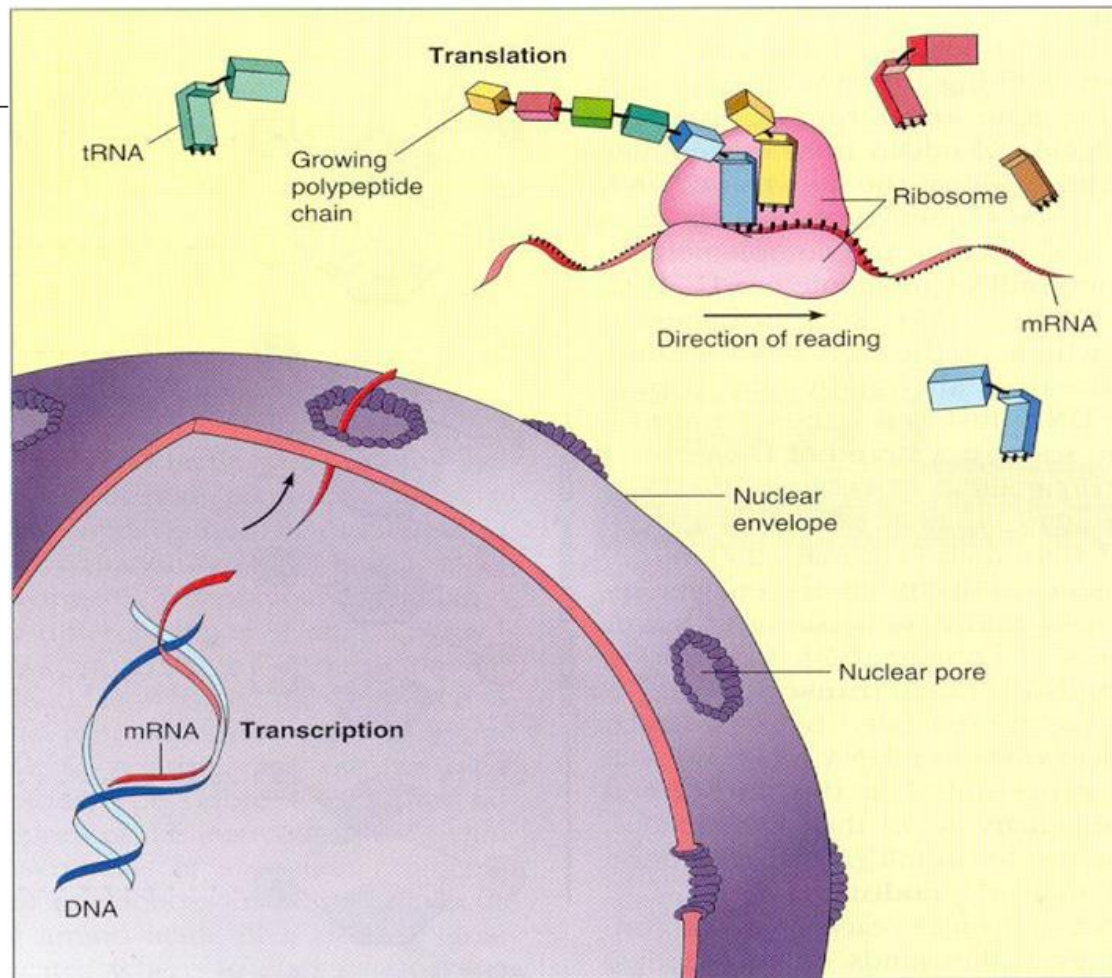
- The **open reading frame** consists of the codons in a gene (or mRNA) that specify the amino acids of the gene product.
- Open reading frame **does not contain the stop codon**

Gene expression problem



1. What effect would mutation of the promoter have on gene expression?
2. If the TAG codon was mutated to TTG what effect would this have?

Translation-Overview



<https://www.youtube.com/watch?v=6YqPLgNjR4Q>

13.17 min