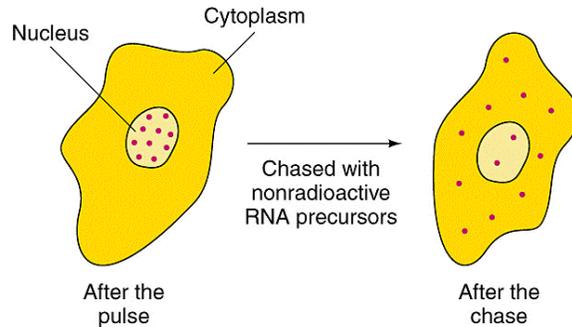


Chapter 13 Lecture Notes: DNA Function

- I. Transcription (General info)
- A. Transcription is the synthesis of RNA using DNA as a template.
- B. Early evidence suggesting an RNA intermediate between DNA and proteins
1. DNA was in the nucleus but proteins were made in the cytoplasm
 2. RNA synthesis in the nucleus was exported to the cytoplasm



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3. T2 infection of *E. coli* results in phage specific RNA being produced
- C. Properties of RNA – Similar to DNA except
1. Contains ribose instead of deoxyribose
 2. Contains uracil instead of thymine
 3. Single stranded instead of double stranded (although there are regions of pairing)
- D. Misc other info
1. Each RNA species is complementary to one strand (template strand) of the DNA double helix.



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2. Upstream vs. downstream: RNA strand has a 5' and 3' end. Upstream refers to “towards the 5' end” and downstream refers to “towards the 3' end”.
3. The region of DNA that contains sequences that are the signals for transcribing a gene are termed promoters.
4. +1 refers to the basepair where transcription starts; -x refers to x basepairs 5' to the start site

II. Factors required for transcription

A. Prokaryotic

1. RNA polymerase (enzyme that catalyzes the synthesis of RNA from a DNA template).

a) Core enzyme = 3 different types of subunits (2 α ; 1 β ; 1 β')

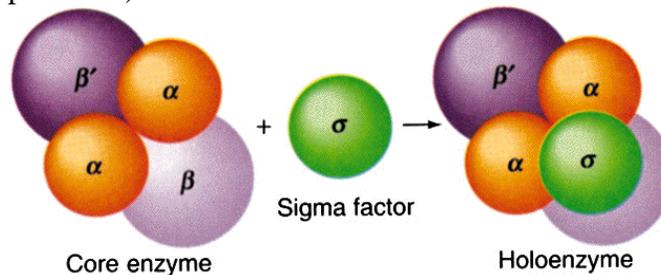
(1) β - binds incoming nucleotides

(2) β' - binds DNA

(3) α - helps with enzyme assembly; interacts with other transcriptional activator proteins; recent work demonstrated that α also interacts with some DNA sequences

b) Holoenzyme = core + σ factor (recognizes the promoter)

c) σ factors - Initially, people thought that there was only one σ factor that functioned to direct RNAP to the promoters of genes. Later, different classes of σ factors were found. Each σ factor directs RNAP to a different type of promoter (differentiated by a specific DNA sequence in the promoter).



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2. Accessory transcription activator proteins

a) Can bind to specific DNA sequences and help RNA polymerase initiate transcription via protein-protein interactions or by altering the structure of the DNA.

b) Transcription of some promoters requires an accessory transcriptional activator; at other promoters, the activators just increase the rate of transcription but are not absolutely required.

3. Template DNA containing gene or genes to be transcribed

4. Promoter - The regulatory element that determine when a gene “turned on” (transcribed) or “turned off”. The promoter DNA is located upstream of the gene and contains a sequence which σ factor of RNAP and other transcription factors bind. Different classes of promoters have different DNA sequences. Deviations from the consensus sequence decrease the level of transcription.

Promoter	Function	-35 sequence	17 bp spacer	-10 sequence
Sigma 70-dependent	Housekeeping	TTGACA		TATAAT
Sigma 32-dependent	Heat shock stress response	TCTCNCCCTGAA		CCCCATNTA
Sigma 28-dependent	Flagella synthesis	CTAAA		CCGATAT
Sigma S-dependent	Stationary phase survival	?		?
Sigma 54-dependent	Nitrogen utilization; pilin	CTGGNA (-24)		TTGCA (-12)

5. Weak promoters (ones that have poor sigma recognition sequences) have additional sequences to which transcriptional activators can bind.

6. NTPs, Mg^{2+}

B. Eukaryotic

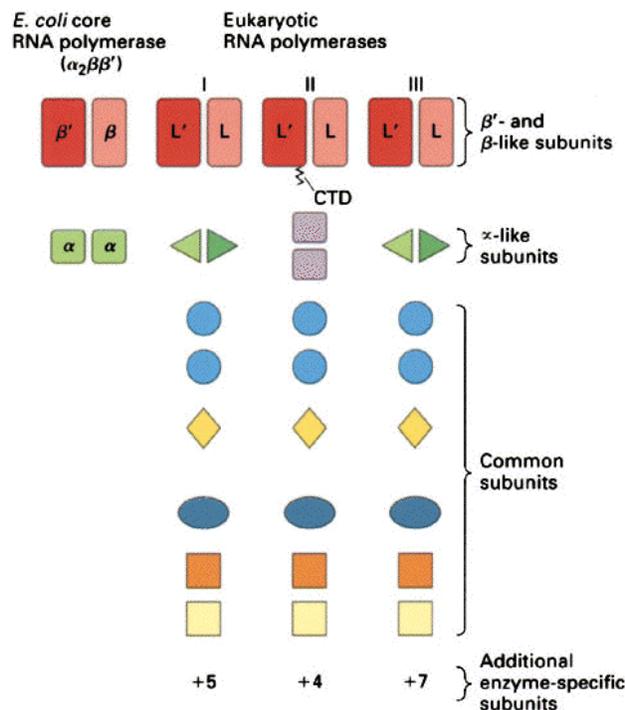
1. RNA polymerases – Much more complex than prokaryotic RNAP (numerous additional factors required, multiple polymerases)

a) RNAP I – synthesizes ribosomal RNA

b) RNAP II – synthesizes messenger RNA

c) RNAP III – synthesizes transfer RNA and 1 type of rRNA

2. Eukaryotic RNAPs have subunits that are homologous to α , β , and β' of prokaryotic RNAP; however, eukaryotic RNAP also contain many additional subunits.



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3. Template DNA containing the gene to be transcribed

4. Eukaryotic promoters – contain some combination of the following

a) contain a TATA rich region located -25 to -30 from the start of transcription

b) Upstream from the TATA region is a variably located sequence containing the sequence CCAAT (frequently at -75)

c) GC box

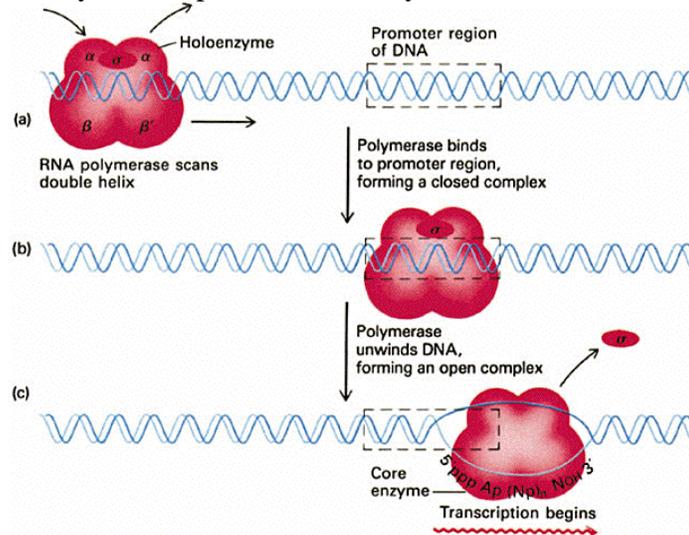
d) Some promoters have other sequences located either upstream or downstream that maximize the level of transcription called enhancers

5. NTPs, Mg^{2+}

III. Prokaryotic transcription

A. Initiation

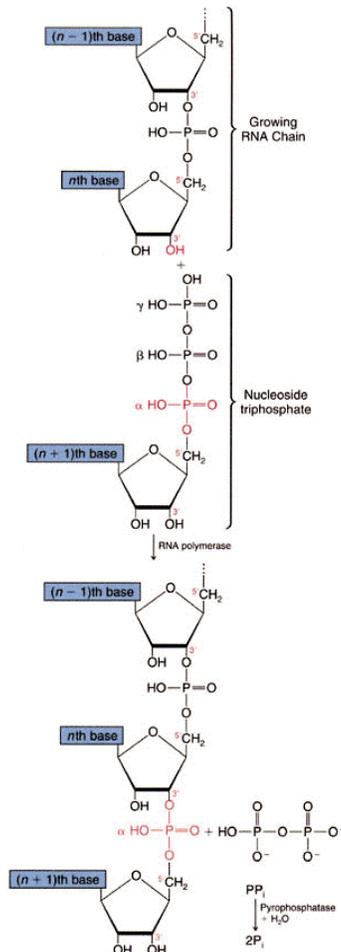
1. RNAP scans the DNA looking for promoters.
2. σ factor of RNAP binds the corresponding σ factor recognition sequence in the promoter.
3. Recent evidence suggests that at some promoters, the α subunit may bind to AT rich regions upstream of the sigma binding sites.
4. RNAP is bound covering approx. 60 basepairs. The DNA is still a double helix (closed complex).
5. RNAP unwinds the DNA resulting in open complex formation.
6. First nucleotides are added to start RNA chain. Transcriptional initiation has occurred!
7. Accessory transcription factors may aid in all of the above listed steps.



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B. Elongation

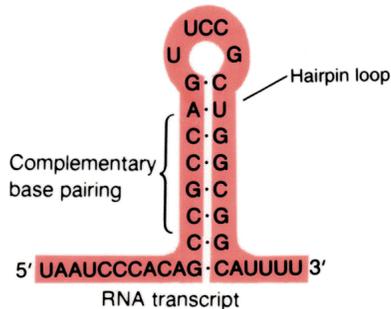
1. Elongation is 5' \rightarrow 3'
2. σ factor is ejected from RNAP after first 2-10 nucleotides are added.
3. Much less is known about this step for transcription than initiation. It was once believed that elongation occurred at a constant rate; however, recent work suggests that RNAP may pause during elongation. In fact, pausing is important in termination (see below).



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C. Termination (2 types)

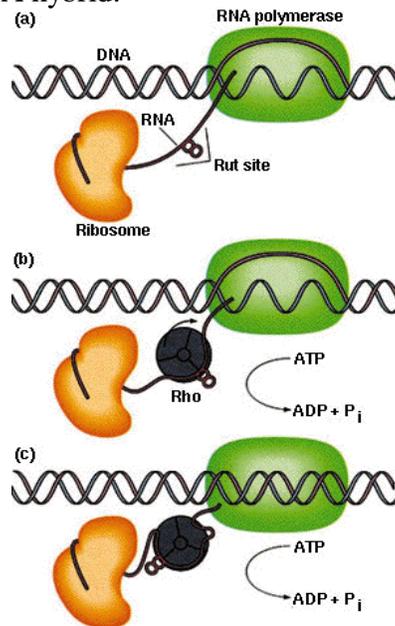
1. Rho independent: A specific sequence at the end of the gene signals termination. The sequence is transcribed into RNA and it is the RNA sequence that is important. This sequence contains numerous Gs and Cs, which forms a hairpin structure, followed by a string of Us.



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The hairpin destabilizes the DNA:RNA hybrid leading to dissociation of the RNA from the DNA.

2. Rho dependent: Rho protein binds to a sequence in the RNA (rut site – not well characterized). Rho moves along the RNA in the 3' direction until it eventually unwinds the DNA:RNA hybrid in the active site, thereby pulling the RNA away from the DNA and RNAP. Rut sites are located 5' to sites in the DNA that cause RNAP to pause. It is thought that this allows Rho to catch up to RNAP and the RNA-DNA hybrid.



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IV. Eukaryotic transcription

A. Initiation and elongation are similar to in prokaryotes; however, there are several important differences.

Table 13-8 Differences in Gene Expression between Prokaryotes and Eukaryotes

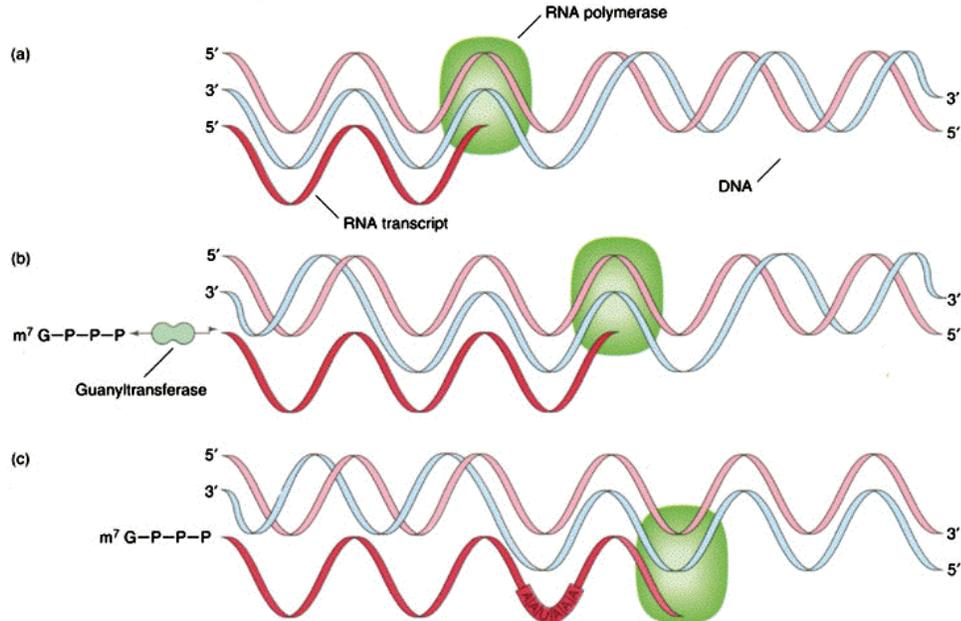
Prokaryotes	Eukaryotes
1. All RNA species are synthesized by a single RNA polymerase.	1. Three different RNA polymerases are responsible for the different classes of RNA molecules.
2. mRNA is translated during transcription.	2. mRNA is processed before transport to the cytoplasm, where it is translated. Caps and tails are added, and internal portions of the transcript are removed.
3. Genes are contiguous segments of DNA that are colinear with the mRNA that is translated into a protein.	3. Genes are often split. They are not contiguous segments of coding sequences; rather, the coding sequences are interrupted by intervening sequences (introns).
4. mRNAs are often polycistronic.	4. mRNAs are monocistronic.

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B. Termination of transcription in eukaryotes is poorly understood.

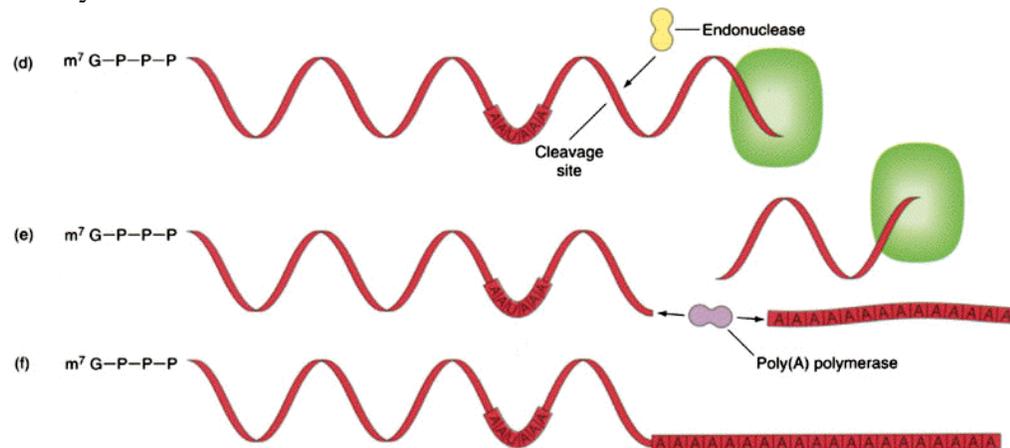
C. RNA processing

1. 5' capping: Occurs early in transcription. Guanosyltransferase adds 5' methylguanosine (Cap) to 5' end of mRNA. The Cap is important for translation initiation and for export from the nucleus.



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2. 3' poly(A) tail: AAUAAA sequence in the RNA signals a cleavage event in the RNA. Poly(A) polymerase then adds 150-200 A residues to the 3' end of the mRNA. The poly(A) tail increases the stability of the mRNA in eukaryotes.

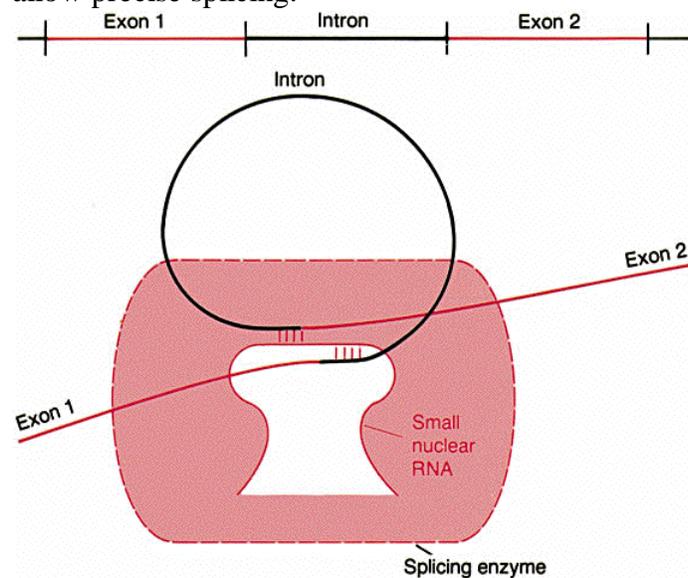


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As a side note, recent evidence has demonstrated that there are poly(A) polymerases in prokaryotes and that some mRNAs have poly(A) tails. Interestingly though, the polyA tail destabilizes the mRNA in prokaryotes.

Some α 2-thalassemias (anemia due to imbalance of α and β hemoglobin subunits) have been attributed to a defect in polyadenylation. Specifically, there is a mutation in the cleavage site from AAUAAA \rightarrow AAUAAG.

3. Splicing: The primary transcripts often contain intervening sequences (introns) that are removed from the RNA prior to translation by a cleavage reaction catalyzed by snRNPs (small nuclear ribonuclear proteins which contain RNA and protein). Frequently, the splicing site in the intron has a GU at the 5' end and an AG at the 3' end. The snRNP aligns these ends in a lariat formation to allow precise splicing.



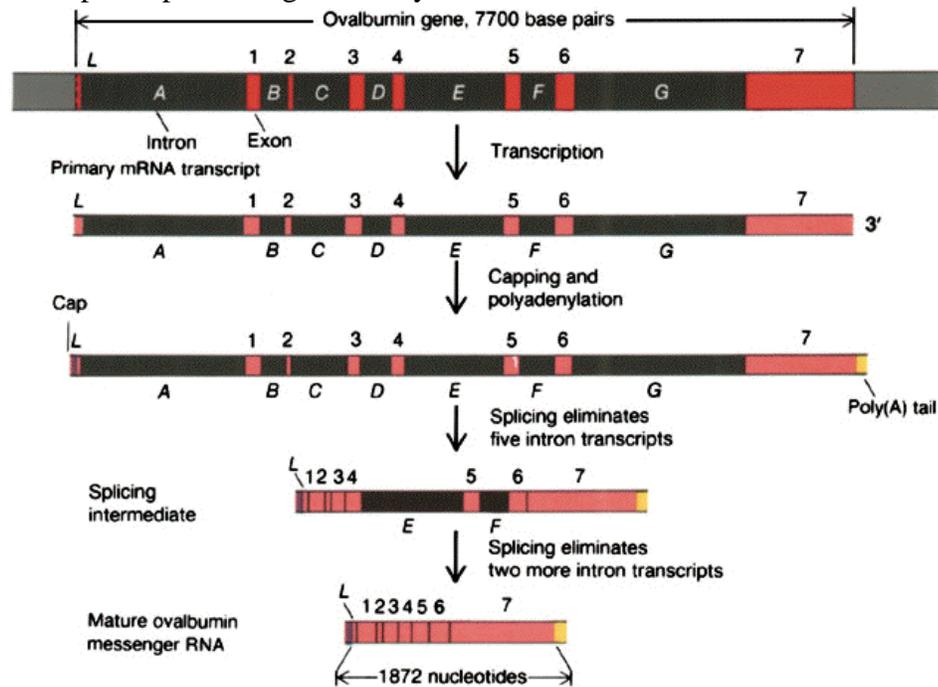
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Complexes containing the snRNP, mRNA, and associated proteins are called spliceosomes.

Splicing is important (1) splicing allows variations of a gene and therefore gene product to be made (2) it has been suggested that exons correspond to functional motifs in proteins and thus the presence of genes that require slicing allows for evolutionary tinkering (3) many viruses have spliced mRNAs and so understanding the process may lead to new therapeutic approaches.

As an interesting aside, people with systemic lupus erythematosus have antibodies directed against snRNP protein subunits. The significance of this is unknown at this time.

Example of processing of eukaryotic mRNA:



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D. RNA export: RNA synthesis and processing occurs in the nucleus. The mature mRNA is then transported through the nuclear pores in the nuclear envelope to the cytoplasm. There is a nuclear complex that is involved in the transport. This complex recognizes the 5' CAP of the mRNA.

V. Translation – General info

A. Translation is the production of a polypeptide (protein) using RNA as a template and tRNA molecules as “adapters” that convert the nucleic acid code to protein code.

B. The nucleotides (letters) of RNA formed codons (words) that specify a particular amino acid.

C. The tRNA contains an anticodon that is complementary to the codon and carries a specific amino acid.

D. Important elements of the genetic code:

1. The code is a triplet code: Each mRNA codon (word) that specifies a particular amino acid in a polypeptide chain consists of three nucleotides (letters). For example, AAG = lysine

2. The code is non-overlapping: The mRNA encoding one protein is read in successive groups of three nucleotides.

3. The code is degenerate: More than one mRNA codon (word) occurs for some amino acids (ie. AAG and AAA are read as both read as lysine)

a) Wobble – certain different codons are recognized by the same tRNAs because the 3rd base in the codon and the 1st base of the anticodon pair via a “loose pairing”. This “loose pairing is according to a set of rules known as the wobble rules.

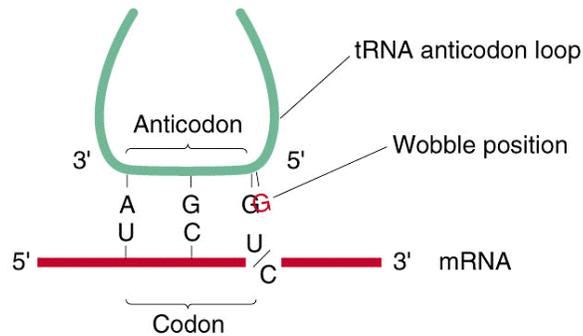


Table 13-5 Codon-Anticodon Pairings Allowed by the Wobble Rules

5' end of anticodon	3' end of codon
G	U or C
C	G only
A	U only
U	A or G
I	U, C, or A

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b) There is more than one tRNA type (therefore more than one anticodon) for some amino acids.

4. The code has start signals (AUG and rarely GUG) and stop signals (UAA, UAG, and UGA). Stop signals are also called nonsense codons because they do not designate an amino acid.
5. The code is commaless.
6. The code is almost universal.
7. The code.....

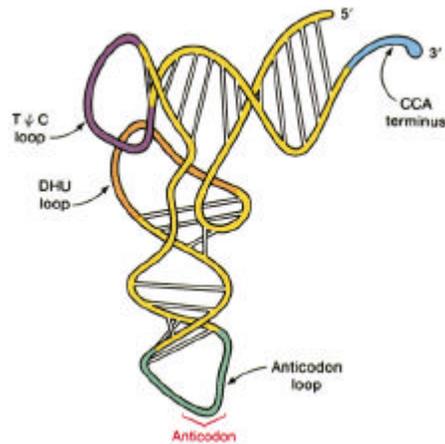
		Second letter				
		U	C	A	G	
U	UUU } Phe	UCU } Ser	UAU } Tyr	UGU } Cys	U	
	UUC } Phe	UCC } Ser	UAC } Tyr	UGC } Cys	C	
	UUA } Leu	UCA } Ser	UAA Stop	UGA Stop	A	
	UUG } Leu	UCG } Ser	UAG Stop	UGG Trp	G	
C	CUU } Leu	CCU } Pro	CAU } His	CGU } Arg	U	
	CUC } Leu	CCC } Pro	CAC } His	CGC } Arg	C	
	CUA } Leu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

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VI. Factors required for translation

A. Prokaryotic

1. mRNA – contains a RBS (ribosome binding site) / also known as a Shine-Delgarno sequence. The RBS is characterized by a core sequence 5' AGGAGU3' located 7+2 nucleotides from the AUG. Deviations from the consensus decrease translation.
2. tRNA – adapter molecules in the information transfer between mRNA and protein which has:
 - a) anticodon which is a 3 nucleotide sequence that is complementary and antiparallel to the mRNA codon
 - b) amino acid attachment site at the 3' end for attachment of the amino acid
 - c) 3-D shape that determines which amino acid will be attached to the amino acid attachment site. Recent studies indicate that the anticodon loop, the D loop, and the aminoacyl stem are all important. The correct attachment of the amino acid to its tRNA is considered the “2nd genetic code” and is still being cracked.



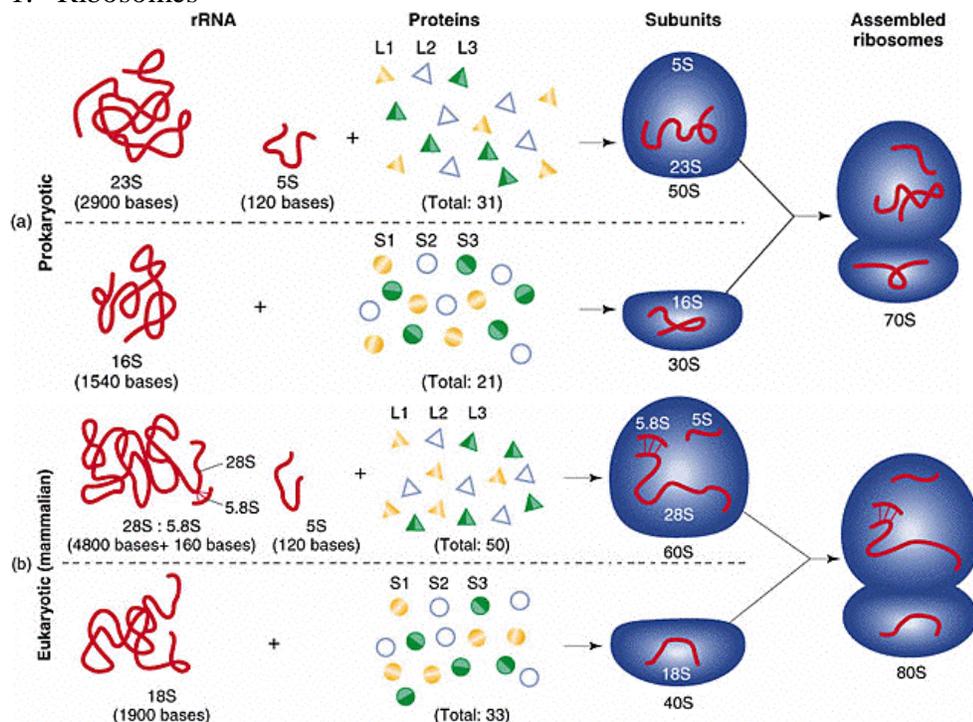
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- d) Isoaccepters = tRNAs with different anticodons but same amino acid.
 - e) Aminoacyl tRNA = tRNA with amino acid attached = charged tRNA.
3. Aminoacyl tRNA synthetase – transfers the amino acid to its proper tRNA; there are 20 of these, each recognizing 1 amino acid and all the tRNAs that to which that amino acid is to be attached
 4. Ribosomes (Note *S* refers to a sedimentation value of the structure in a sucrose gradient)
 - a) Large subunit (50S)– consists of 23S and 5S rRNAs and 31 ribosomal proteins
 - b) Small subunit (30S)- consists of 16S rRNA and 21 ribosomal proteins
 5. Soluble transcription factors
 - a) Initiation factors
 - (1) IF1 – promotes dissociation of ribosomal subunits
 - (2) IF2(•GTP) – required for fMET-tRNA^{met} binding
 - (3) IF3 – required for mRNA binding, finding the AUG
 - b) Elongation factors
 - (1) EF-Tu (•GTP) – binds aminoacid-tRNA to the ribosome
 - (2) EF-Ts – regenerates EF-Tu•GTP
 - (3) EF-G(•GTP) – increases translocation rate
 - c) Termination factors
 - (1) RF1 – recognizes UAA and UAG stop codons
 - (2) RF2 – recognizes UAA and UGA nonsense codons
 - (3) RF3(•GTP) – enhanced RF-1 and –2 binding to ribosome
 6. Amino acids
 7. F-met (N-formyl Met-tRNA)
 8. GTP
 9. ATP (for charging tRNAs)
- B. Eukaryotic (similar to prokaryotes except...)
1. One gene per mRNA (monocistronic)

2. Although the process are similar, the component of eukaryotic and prokaryotic translation can not be mixed
3. ribosomes
 - Interestingly, only two ribosomal proteins and the rRNA which are very highly conserved among prokaryotes and eukaryotes. For euks,
 - a) Large subunit (60S)– consists of 23S, 5.8S, and 5S rRNAs and 50 ribosomal proteins
 - b) Small subunit (40S)- consists of 18S rRNA and 33 ribosomal proteins
4. Soluble translation factors
 - a) Initiation factors
 - (1) eIF1 – promotes dissociation of ribosomal subunits
 - (2) eIF2(GTP) – required for fMET-tRNA^{met} binding
 - (3) eIF3 –
 - (4) eIF4 – important for finding the capped end of the mRNA
 - b) Elongation factors
 - (1) EF (•GTP) – binds AA-tRNA to the ribosome
 - (2) EF₁β – regenerates EF •GTP
 - (3) EF₂(•GTP) – increases translocation rate
 - c) Termination - Several TF (termination factors)
5. No F-met

C. Comparison of Prokaryotic and Eukaryotic factors

1. Ribosomes



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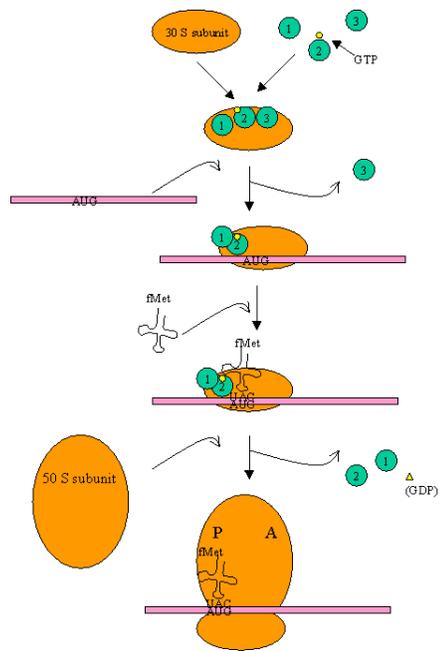
2. Soluble factors

prokaryote	Eukaryote
IF1	eIF1
IF2	eIF2((•GTP)
	eIF-3
IF3	eIF4
EF-Tu	EF (•GTP)
EF-Ts	EF ₁ β
EF-G	EF ₂ (•GTP)
RF-1	TF
RF-2	TF
RF-3	TF

VII. Translation – Mechanism in Prokaryotes

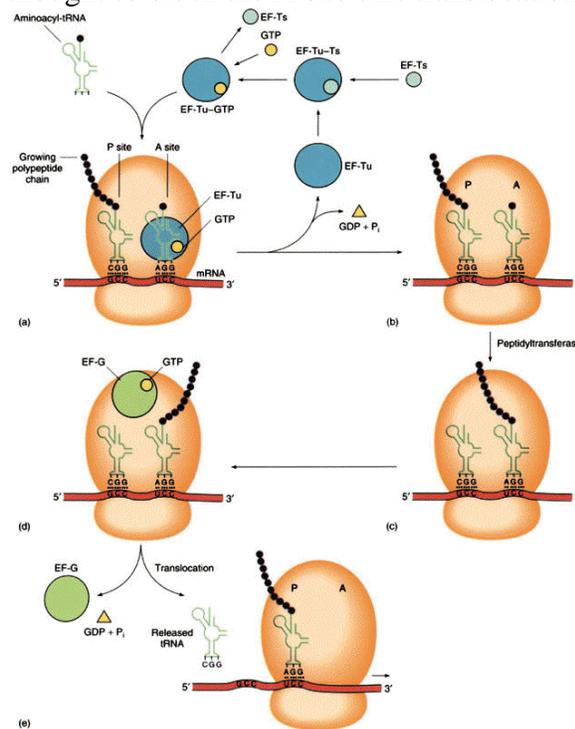
A. Initiation – the purpose of this step is to set the reading frame

1. IF1, IF2•GTP, and IF3 bind to the 30S subunit.
2. Binding of mRNA to the 30S subunit via an interaction between the RBS on the RNA and a complementary sequence at the 3' end of the 16S RNA.
Facilitated by IF3.
3. Release of IF3
4. fMET•tRNA binds to the P site in the 30S subunit with the help of IF-2.
5. GTP hydrolysis and release of IF1 and IF2 drives the attachment of the 50S subunit.



B. Elongation – addition of amino acids to the growing polypeptide chain

1. EF-Tu•GTP-AA-tRNA complex binds to the A site (there is a selection process that goes on at this step whereby if the match between the codon and anticodon is not correct, the complex is released before the next step can occur)
2. GTP hydrolysis
3. Proofreading (if the match between the codon and anticodon is not correct, the complex is released before the next step can occur)
4. EF-Tu release (Note that EF-Tu•GTP is regenerated via the action of EF-Ts)
5. Peptidyl transfer – polypeptide is transferred from the tRNA at the P site to the AA-tRNA complex at the A site. This catalytic activity is thought to involve not only proteins but also the 23S RNA.
6. Translocation – shift of the ribosome one codon towards the 3' end resulting in transfer of the tRNA with the polypeptide chain to the P site (stimulated by EF-G). During translocation the uncharged tRNA in the P site is moved to the E site (for exit) which is thought to block the A site unit translocation is complete.

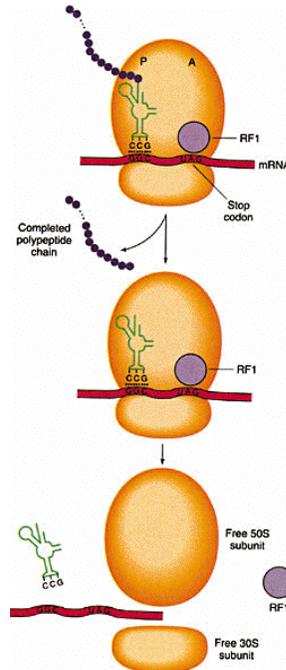


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C. Termination

- a) A termination codon (UAA, UAG, UGA) is presented at the A site
- b) RF1 or RF2 bind to the A site with the help of RF3•GTP
- c) The RF-mRNA-ribosome complex catalyzes peptidyl hydrolysis instead of transfer
- d) The polypeptide is released from the tRNA in the P site.
- e) The GTP associated with RF3 is hydrolyzed causing the release of the 3 RF factors and the tRNA from the ribosome

f) The 30S and 50S subunits dissociate with the aid of IF1 and IF3 and the mRNA is released



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- VIII. Importance in understanding translation in detail.
- A. Translation is a fundamental process to all life.
 - B. Antimicrobial drug design against components of translation machinery that are different between eukaryotes and prokaryotes.
 - C. Antisense DNA designed to bind to the beginning of specific mRNAs to prevent transcription.