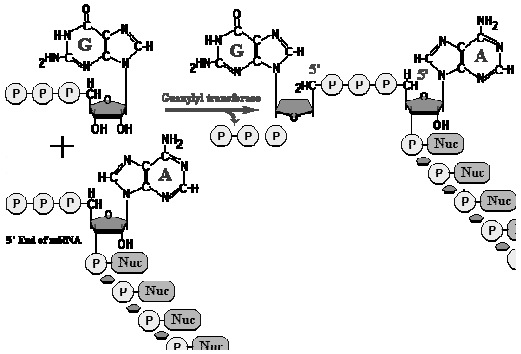
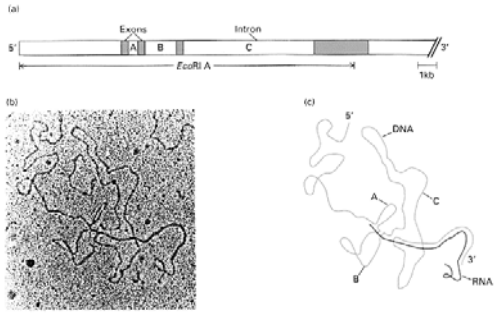
Lecture 4 mRNA splicing and protein synthesis

Another day in the life of a gene.

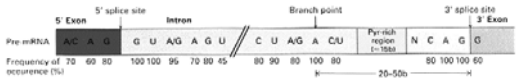
Adding a 5' cap



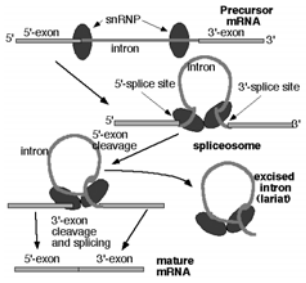
Pre-mRNA has introns



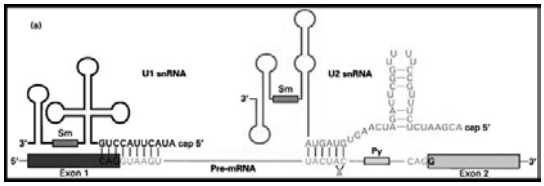
The splicing complex recognizes semiconserved sequences



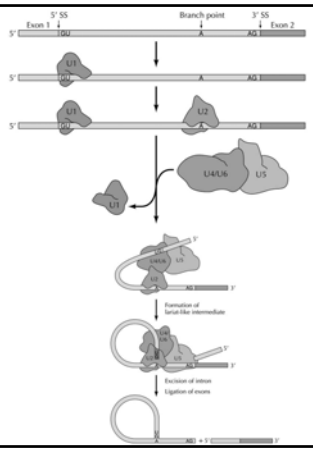
Introns are removed by a process called splicing



Splicing includes multiple proteins and small nuclear RNAs called snRNAs

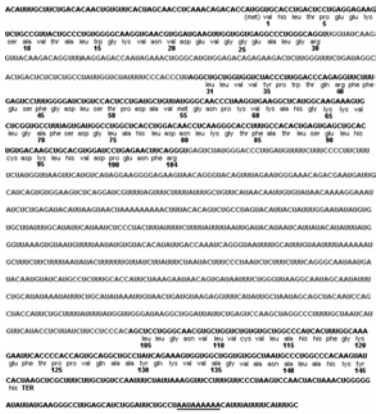


How snRNAs work. Here two snRNAs are shown forming partial hybrids with a pre-mRNA. U1 is forming a hybrid at the junction of the 5' exon and U2 is forming a partial hybrid with a sequence near the 3' exon.



Complexity of genes

- Splicing in some genes seems straightforward such as globin



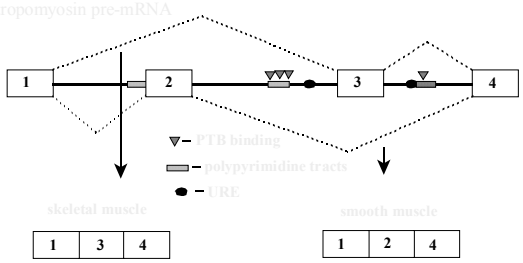
For other genes splicing is much more complex

- Fibrillin is a protein that is part of connective tissue. Mutations in it are associated with Marfan Syndrome (long limbs, crowned teeth elastic joints, heart problems and spinal column deformities. The protein is 3500 aa, and the gene is 110 kb long made up of 65 introns.
- Titin has 175 introns.
- With these large complex genes it is difficult to identify all of the exons and introns.

Alternative RNA splicing

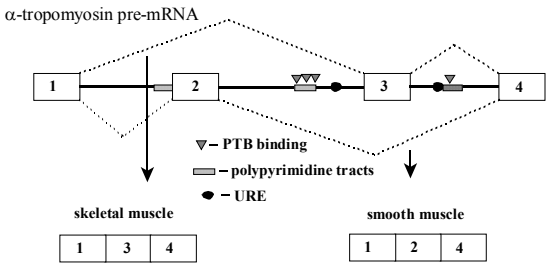
- Shortly after the discovery of splicing came the realization that the exons in some genes were not utilized in the same way in every cell or stage of development. In other words exons could be skipped or added. This means that variations of a protein (called isoforms) can be produced from the same gene.

Alternative splicing of a tropomyosin



There are 3 forms of polypyrimiding tract binding protein (PTB) PTB1, PTB2 and PTB4. Binding of PTB4 to the polypyrimidine suppresses splicing while binding of PTB1 promotes splicing. In smooth muscle exon 3 of a-tropomyosin is not present. Thus, PTB4 is expressed in smooth muscle while PTB1 is not.

Alternative splicing of a tropomyosin



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Gene Expression II

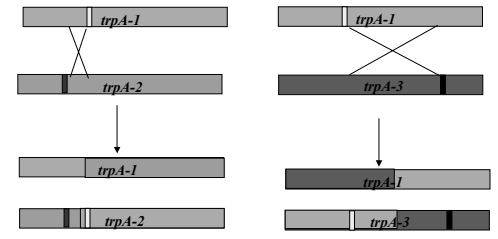
Translation of the mRNA into protein

Show movie

How does DNA function as a code for protein synthesis?

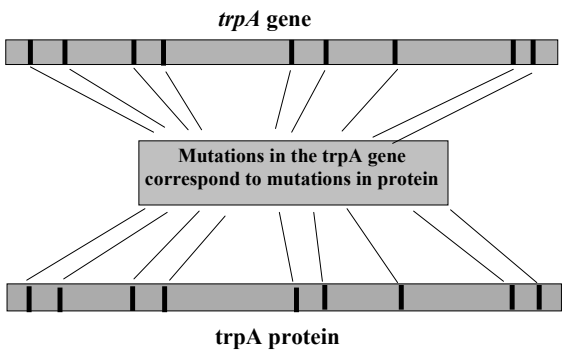
- The experiments of Charles Yanofsky and Syndey Brenner demonstrated that the sequential arrangement of nucleotides along a gene code for a sequential arrangement of amino acids in its encoded protein.
- The code in DNA (and ultimately mRNA is read in triplets).
- The code is degenerate.

Yanofsky precisely mapped the positions of a series of mutations in the *TrpA* gene

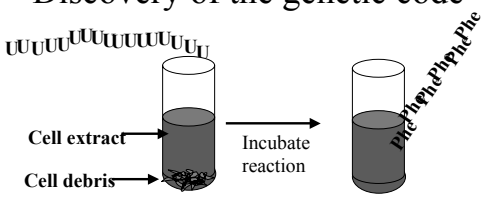


Low recombination frequencies show that theses mutations are closely linked

High recombination frequencies show that these are far apart



Discovery of the genetic code



CCCCCCCCCCCCCCCCCC → Pro-Pro-Pro-Pro-Pro

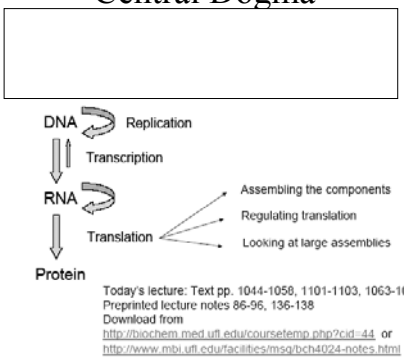
CACACACACACACACACACA → His-Thr-His-Thr-His-Thr

The table of codons

Second letter

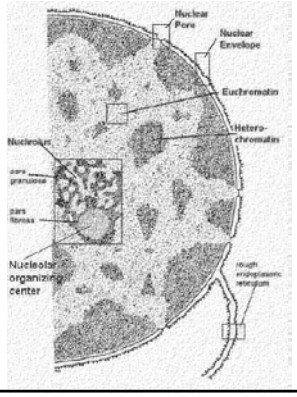
First letter	U	C	A	G	
U	UUU, UUC: Phenylalanine	UCU, UCC, UCA, UCG: Serine	UAU, UAC: Tyrosine	UGU, UGC: Cysteine	U C A G
	UUA, UUG: Leucine		UAA, UAG: Stop codon Stop codon	UGA: Stop codon; UGG: Tryptophan	
C	CUU, CUC, CUA, CUG: Leucine	CCU, CCC, CCA, CCG: Proline	CAU, CAC: Histidine; CAA, CAG: Glutamine	CGU, CGC, CGA, CGG: Arginine	U C A G
A	AUU, AUC, AUA: Isoleucine	ACU, ACC, ACA, ACG: Threonine	AAU, AAC: Asparagine; AAA, AAG: Lysine	AGU, AGC: Serine; AGA, AGG: Arginine	U C A G
	AUG: Methionine; initiation codon				
G	GUU, GUC, GUA, GUG: Valine	GCU, GCC, GCA, GCG: Alanine	GAU, GAC: Aspartic acid; GAA, GAG: Glutamic acid	GGU, GGC, GGA, GGG: Glycine	U C A G

Central Dogma

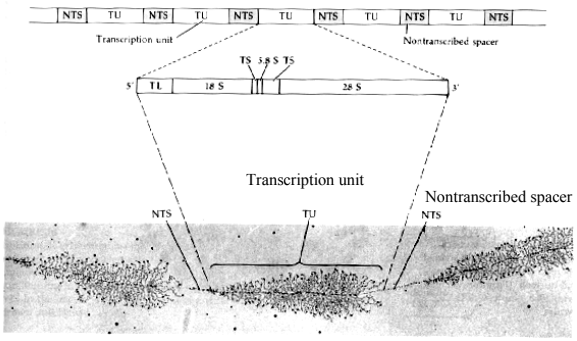


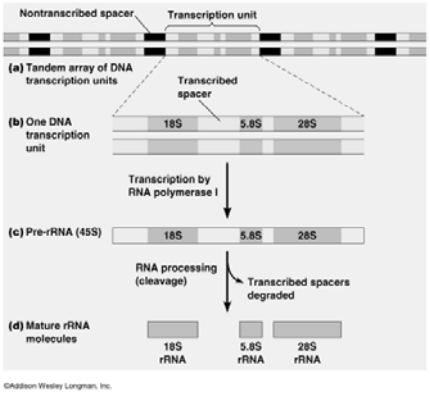
Today's lecture: Text pp. 1044-1058, 1101-1103, 1063-1067
Preprinted lecture notes 86-96, 136-138
Download from
http://biochem.med.ufl.edu/coursetemp.php?cid=44 or
http://www.mbi.ufl.edu/facilities/msg/bch4024-notes.html

Ribosomal RNA synthesis takes place in the nucleolus



Transcription of rRNA

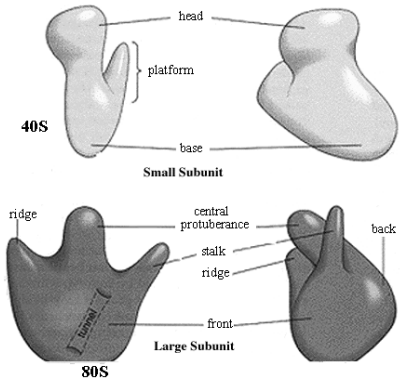




Assembly of ribosomes

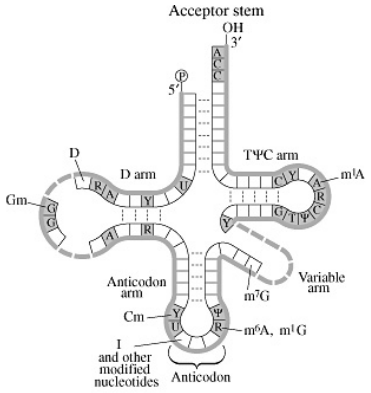
- Ribosomal RNA is transcribed as a 45S precursor RNA, synthesized in the nucleolus by polI from thousands of copies of the gene.
- The 45S precursor (13,000 nt)is processed into 3 smaller RNAs 28S (5000 nt), 18S (2000 nt) and 5.8S (160 nt)
- The 5S subunit is synthesized by polIII from a cluster of 2000 genes located separately from the other ribosomal genes
- Some 80 proteins associate with the rRNAs to make up complete ribosome.
- Small ribosomal subunit (40S) contains 18S rRNA while the large 60S subunit contains the remaining rRNAs

ribosomes



Transfer RNAs (tRNAs)

- tRNAs are small 70-90 nt
- there are about 32 different tRNAs in most organisms
- the tRNAs contain unusual modified nucleotides
- aminoacyl-tRNA synthetases charge tRNAs with amino acids
- tRNAs function to deliver the amino acids to the ribosomes for protein synthesis



Wobble Hypothesis

Wobble Positions in Anticodon and Codon Interactions

C	A	G	U	I
G	U	C	A	C
		U	G	U
				A

Wobble Positions in Codon and Anticodon Interactions

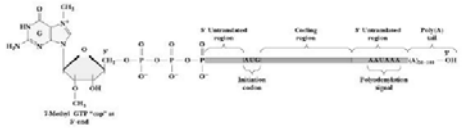
C	A	G	U
G	U	C	A
I	I	U	G
			I

Features of tRNAs

1. exhibit a cloverleaf-like secondary structure.
2. have a 5'-terminal phosphate.
3. have a 7 bp stem that includes the 5'-terminal nucleotide and may contain non-Watson-Crick base pairs, e.g. GU. This portion of the tRNA is called the acceptor since the amino acid is carried by the tRNA while attached to the 3'-terminal OH group.
4. have a D loop and a TpsiC loop.
5. have an anti-codon loop.
6. terminate at the 3'-end with the sequence 5'-CCA-3'.
7. contain 13 invariant positions and 8 semi-variant positions.
8. contain numerous modified nucleotide bases.

Biochemistry 2/e - Garrett & Grisham

Garrett & Grisham: Biochemistry, 2/e
Figure 33.22



Saunders College Publishing

Some general features of protein synthesis

1) mRNA is translated 5' → 3'

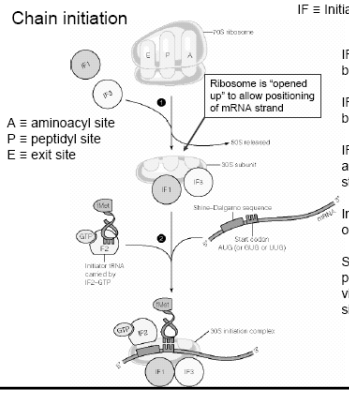
2) Polypeptide chain synthesis is amino (NH_3^+) → carboxy (COO^-)

3) Three phases of the "ribosome cycle"
   a) Initiation: bind initiator tRNA……"AUG"
   b) Elongation: bind aa tRNA and form peptide bonds
   c) Termination

Protein synthesis

Chain initiation

IF ≡ Initiation Factor protein



A ≡ aminoacyl site
P ≡ peptidyl site
E ≡ exit site

IF1 – promotes mRNA binding

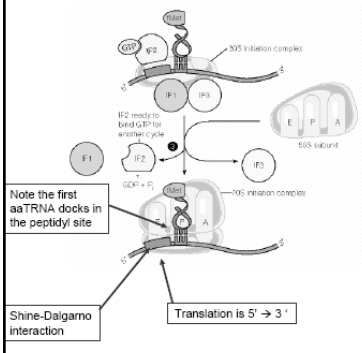
IF2 – initiator tRNA binding; binds GTP

IF3 – Anti-association activity; promotes accurate start & mRNA binding

Initiator tRNA binds at P site on 30S

Shine-Dalgarno sequences position initiation codon in vicinity of decoding site, P site

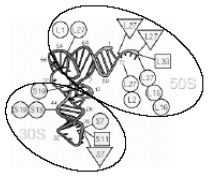
Chain initiation continued



fMet ≡ N-formyl methionine
fMet is unique to prokaryotes
The formyl is removed during elongation and the Met is usually cleaved off later

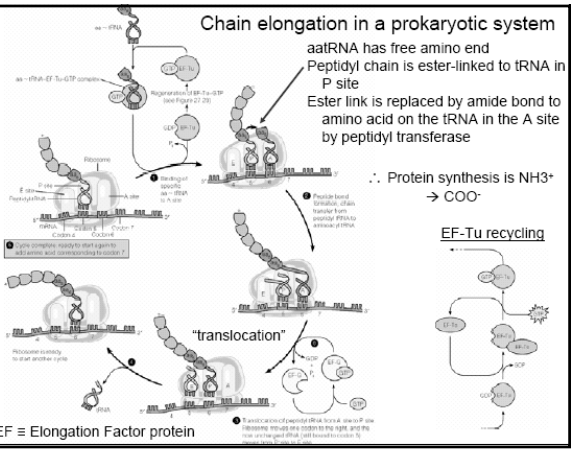
Only $tRNA^{fMet}$ can bind the initiation complex

Chemical cross-linking info:



Triangles ≡ A site
Circles ≡ P site
Squares ≡ E site

Chain elongation in a prokaryotic system

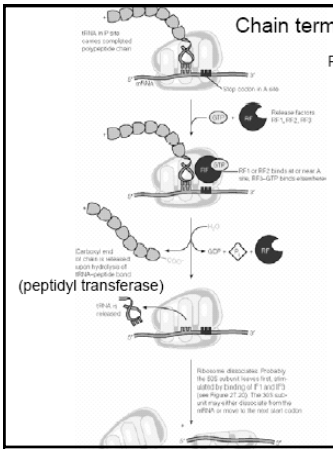


aatRNA has free amino end
Peptidyl chain is ester-linked to tRNA in P site
Ester link is replaced by amide bond to amino acid on the tRNA in the A site by peptidyl transferase

∴ Protein synthesis is $NH3^+$ → COO^-

EF ≡ Elongation Factor protein

Chain termination in a prokaryotic syste



RF ≡ Release Factor protein

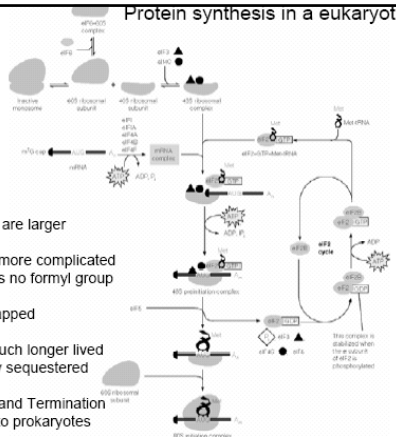
The release factors RF1 or RF2 bind in A site to stop codons UAA (RF1 or RF2), UAG (RF1), or UGA (RF2)

RF3 and GTP bind → release RF1 or RF2

rRNA may be involved in termination as well

Ribosome is recycled

Protein synthesis in a eukaryotic system



Ribosomes are larger

Initiation is more complicated and Met has no formyl group

mRNA is capped

mRNA is much longer lived and actively sequestered

Elongation and Termination are similar to prokaryotes

Comparison of prokaryotic and eukaryotic protein synthesis factors

Prokaryotic factor	Eukaryotic factor	Function
Initiation factors		
IF1		Involved in forming
IF2	eIF2	initiation complex
IF3	eIF3, eIF4C	"
	CBPI	Involved in cap binding
	eIF4A, eIF4B, eIF4F	Search for first AUG
	eIF5	Helps dissociate eIF2, eIF3 eIF4C
	eIF6	Helps dissociate 60S subunit from inactive ribosomes
Elongation factors		
EF-Tu	eEF1α	Delivery of aatRNA to ribosomes
EF-Ts	eEF1βγ	Aids in recycling factor above
EF-G	eEF2	Translocation factor
Release factors		
RF1	eRF	Release of completed
RF2		Polypeptide chain
RF3		"

Protein synthesis in bacteria

mRNA binding to bacterial ribosomes: the Shine – Dalgarno interaction

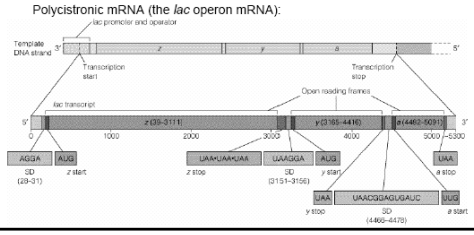
E. coli lac z mRNA 5'...AGGAAACAGCUAUG...3' end

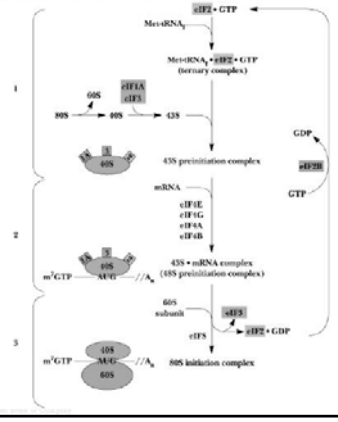
16S rRNA 3' end...AUUCCUCCA...5'

Complementary regions: 3' end of 16S rRNA and 5' untranslated leader of mRNA (Note position of initial codon); AG rich

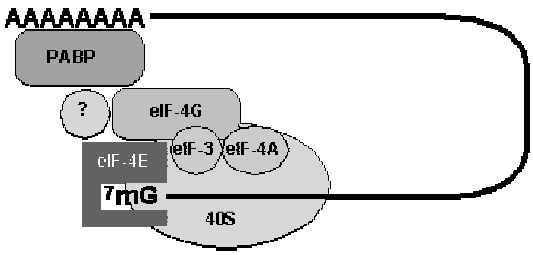
Stronger interactions promote more frequent translation

Polycistronic mRNA (the *lac* operon mRNA):

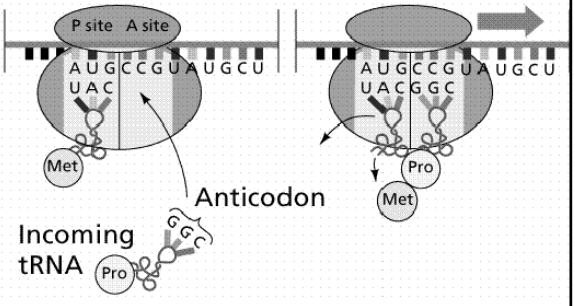


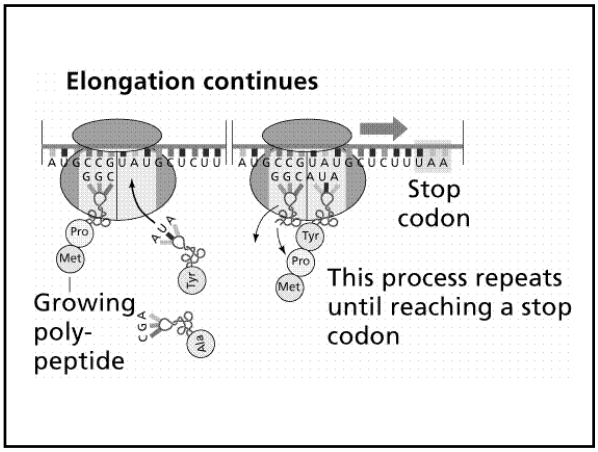


Initiation of translation



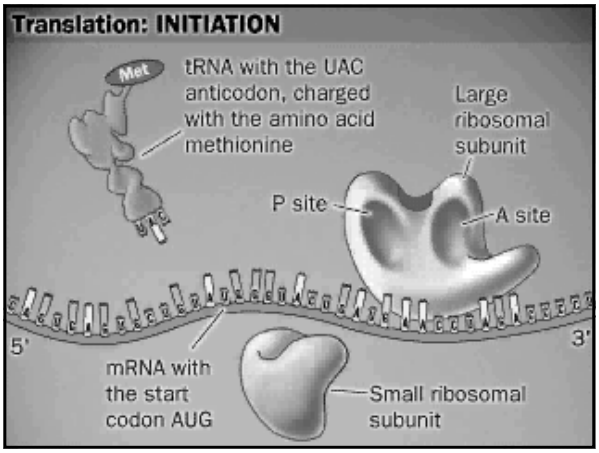
Elongation (translation)





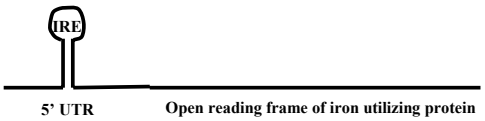
Termination

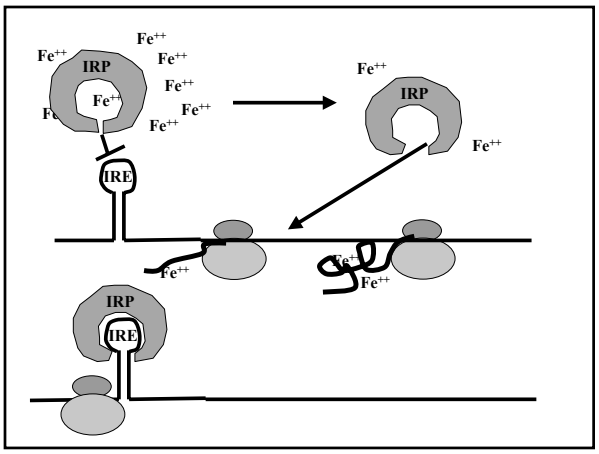
-stop signal on mRNA read by protein release factors causes release of completed polypeptide chain
-RF1 recognizes UAA and UAG
-RF2 recognizes UAA and UGA
-RF3 binds GTP and enhances action of RF1 and RF2
-binding of RF1-RF3-GTP (or RF2-RF3-GTP) to ribosome causes hydrolysis of peptidyl-tRNA
-GTP is then hydrolyzed and the release factors dissociate from ribosome



Regulation of protein synthesis

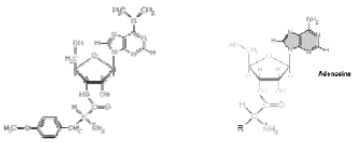
Iron is poorly absorbed and toxicity to cells makes it highly regulated organisms have developed to minimize iron levels. Yet it is essential so at the same time great efforts are made to hang on to it.





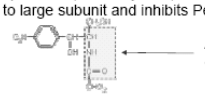
Inhibitors of protein synthesis

1) Puromycin



Causes premature chain termination by entering the A site and transferring to the peptidyl chain

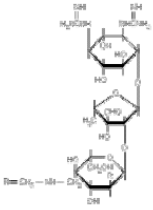
2) Inhibitors of prokaryotic ribosomes
   1) Chloramphenicol (chloromycetin)
      Binds to large subunit and inhibits Peptidyl Transferase activity



Amide link which acts as competitive inhibitor

Side effects include inhibiting mitochondrial ribosomes resulting in "gray baby syndrome" and aplastic anemia in susceptible individuals

2) Streptomycin and other members of the aminoglycoside family of antibiotics



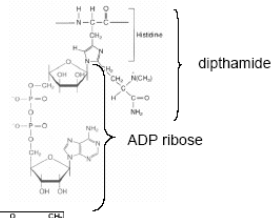
Streptomycin, Gentamycin and Kanamycin inhibit protein synthesis by binding to the rRNA of the small subunit and causing translational errors in reading codons

Side effects include ringing sound in the ears and deafness can occur in susceptible individuals (G → A mutation in small subunit rRNA). Also interferes with mitochondrial protein synthesis.

3) Inhibitors of eukaryotic synthesis

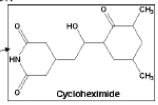
1) Diptheria toxin
Enzyme encoded by a bacteriophage in the bacterium *C. diphtheriae* which uses NAD catalytically to inactivate eEF2 by ADP-ribosylating a modified histidine (diphthamide) in the factor

Catalytic so very potent toxin



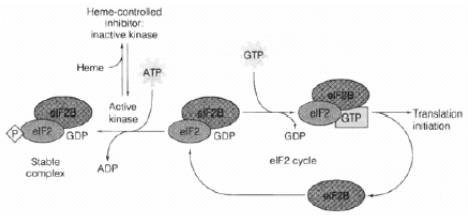
2) Cycloheximide
Inhibits chain elongation

Competitive inhibitor of peptidyl transferase



4) Regulation of translation in erythropoetic cells by heme

In the absence of heme, a heme-controlled kinase becomes active and phosphorylates the α subunit of eIF2. In the phosphorylated state, eIF2 is not able to dissociate from the guanine nucleotide exchange factor IF2B, and the IF2 is no longer available for initiation of protein synthesis.



Immature red cells have no nuclei but reserves of mRNAs for globin synthesis

ANTIBIOTICS INHIBITING TRANSLATION

The bacterial ribosomal structure and the accessory functions differ in many respects from its eukaryotic equivalent. The translation reaction itself can be subdivided into three parts:

1.Formation of the initiation complex, blocked by Streptomycin and Tetracyclins (the latter inhibiting binding of aa-tRNA to the ribosomal A- site at the 30S ribosomal subunit.

2.Introduction of aa-tRNA and synthesis of a peptide bond, inhibited by puromycin (leading to premature termination) and chloramphenicol (probably inhibiting the peptidyltransferase).

3.Translocation of the mRNA relative to the ribosome blocked by erythromycin and fusidic acid (the latter preventing release of EF-G/GDP.