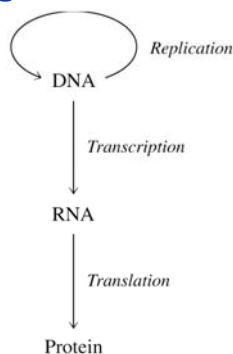


**BCMB 3100 - Chapter 21
Transcription & RNA Processing**

- Definition of gene
- RNA Polymerase
- Gene coding vs template strand
- Promoter
- Transcription in *E. coli*
- Transcription factors
- mRNA processing

- _____ - a DNA sequence that is transcribed (includes genes that do not encode proteins)
- “_____” encode proteins or RNA essential for normal activities of the cell (e.g. enzymes in basic metabolic pathways, tRNAs and rRNAs)

Biological information flow



Four Classes of RNA in living organisms (review)

Messenger RNA (mRNA) - linear “copies” of DNA that encode genetic information. Encode primary structure of protein. ~1-3% of total RNA, relatively unstable (discovered by Jacob & Monod).

Non-coding RNA

Ribosomal RNA (rRNA) - ~80% of total RNA, part of ribosomes (translation machinery)

Transfer RNA (tRNA) - ~15% of total RNA, 73-95 nucleotides long, carry activated amino acids to ribosomes during translation

(Small RNA) - may have catalytic activity and/or associate with proteins to enhance activity, some involved with RNA processing (includes snRNA and microRNA, the latter involved in mRNA degradation, translation inhibition and chromatin remodeling), **(long non-coding RNAs (long ncRNAs))**: functions being determined.

RNA Synthesis (*E.coli*)

Transcription

DNA →→→→→→→→ RNA

RNA Polymerase (450 kd) (1960, Hurwitz; Weiss)

(binds DNA template) $\alpha_2\beta\beta'\omega$ (holoenzyme)
(recognizes promoter & initiates synthesis)

forms phosphodiester bond
binds rNTPs

(see Table 21.2
& Fig. 21.2)

$\alpha_2\beta\beta'\omega$ (core enzyme)

Transcription in *E.coli*

- 1) RNA polymerase searches for initiation sites (~2000 in 4,000,000 bp)
- 2) Unwinds DNA to produce single-stranded template
- 3) Selects correct ribonucleotide and catalyzes the formation of phosphodiester bonds (totally processive)
- 4) Detects termination signals
- 5) Interacts with activators & repressor proteins

RNA Synthesis

- 1) Initiation
 - 2) Elongation
 - 3) Termination
- } RNA polymerase is responsible for these function in RNA synthesis

E. coli RNA polymerase synthesizes all major types of RNA:

mRNA, tRNA, rRNA, small RNA

Requirements for Transcription

DNA template

RNA polymerase

Transcription factors

NTPs (ATP,CTP,GTP,UTP)

Mg⁺⁺

} **Note:** RNA Polymerase does NOT require a primer. RNA chains can be initiated de novo.

Mechanism of elongation is the same as for DNA Polymerase: nucleophilic attack by 3'-OH on α -phosphate of NTP

Fig 21.3

- RNA polymerase reaction

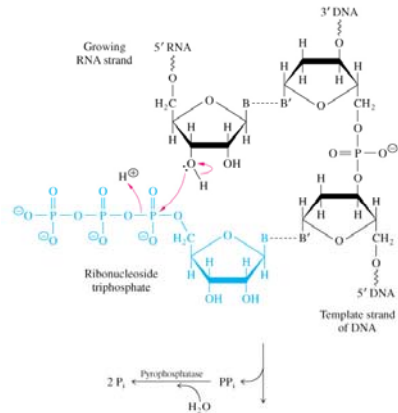


Fig 21.5 Orientation of a gene

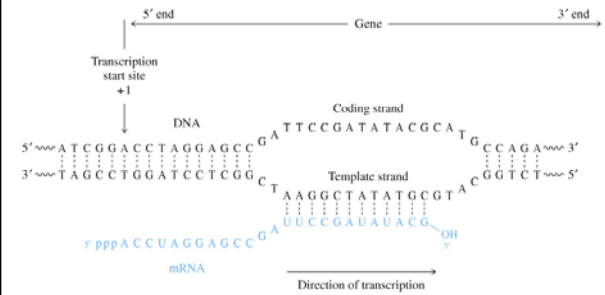
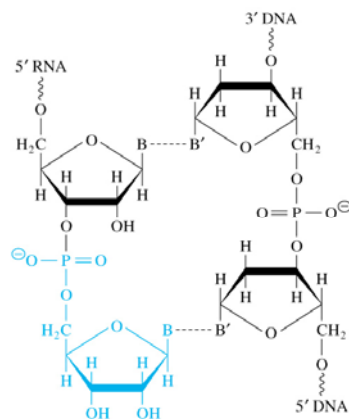


Fig 21.3 (cont)

E. coli transcription rate: 30-85 nucleotides/sec

Error rate = 10^{-6}

RNA Polymerase has no exonuclease activity



Transcription is initiated at promoter sites on the DNA template

Fig 21.6 Promoter sequences for housekeeping genes from 10 bacteriophage and bacterial genes (coding strand)

Gene	Consensus sequence	Transcription start site
<i>λP₂</i>	TTGACT	TTGCA
<i>λP₁</i>	TTGACA	ATCAGCAGGAC
<i>trp</i>	TTGACA	AGTTCACGTAA
<i>lac</i>	TTTACA	ATTGGAGCGG
<i>lacU5</i>	TTTACA	ATTGGAGCGG
<i>araBAD</i>	TTGACT	TTCTCCAT
<i>rnaA1</i>	TTGACA	ATGACACGGAA
<i>rnaA2</i>	TTGACA	ATGACACGGAA
<i>galP1</i>	TTGACA	ATGACACGGAA
<i>galP2</i>	TTGACA	ATGACACGGAA

Strong promoters correspond to consensus sequence (once in 2 sec)

Weak promoters have substitutions (~ once in 10 minutes)

_____ subunit of RNA polymerase is responsible for specific initiation of transcription

σ recognizes promoter sequences

σ_{70} recognizes promoters of house keeping genes

In eukaryotes, transcription factors are required for formation of transcription complex

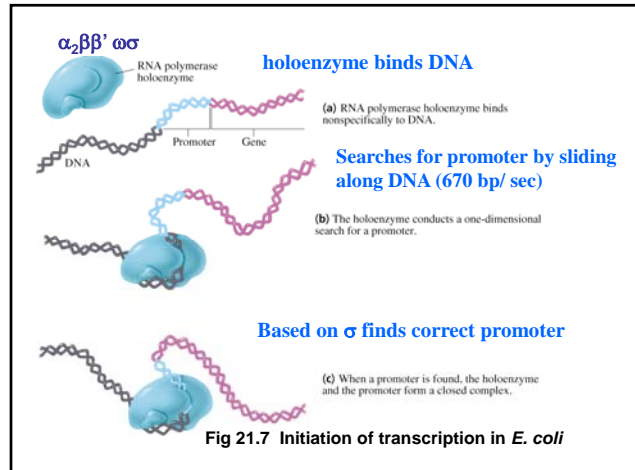


Table 21.3

TABLE 21.3 *E. coli* σ subunits

Subunit	Gene	Genes transcribed	-35	Consensus	-10
σ^{70}	<i>rpoD</i>	Many	TTGACA	TATAAT	
σ^{54}	<i>rpoN</i>	Nitrogen metabolism	None	CTGGCACNNNNNTTGCA ^a	
σ^{38}	<i>rpoS</i>	Stationary phase	?	TATAAT	
σ^{28}	<i>flaI</i>	Flagellar synthesis and chemotaxis	TAAA	GCCGATAA	
σ^{32}	<i>rpoH</i>	Heat shock	CTTGAA	CCCATNTA ^a	
σ^{phage}	gene 55	Bacteriophage T4	None	TATAATA	

^aN represents any nucleotide.

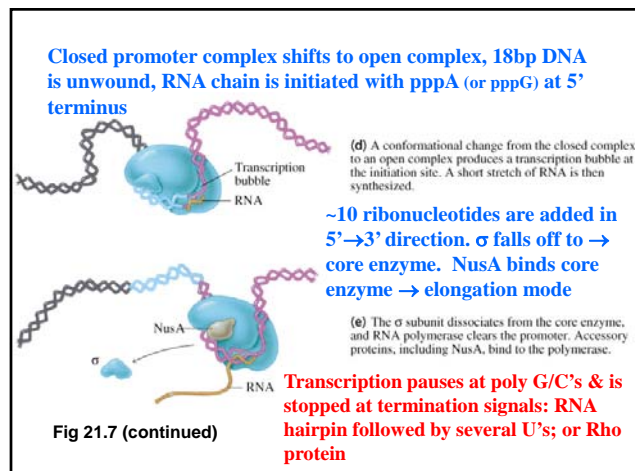
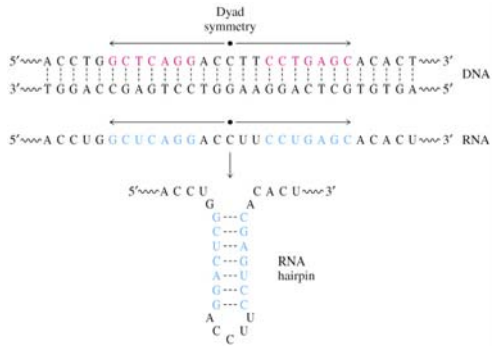


Fig 21.8 Formation of an RNA hairpin



Prokaryote RNA Polymerase synthesizes
mRNA, tRNA and rRNA

Eukaryotes have three RNA Polymerases

RNA Pol I: 18S, 5.8S, 28S **rRNA**

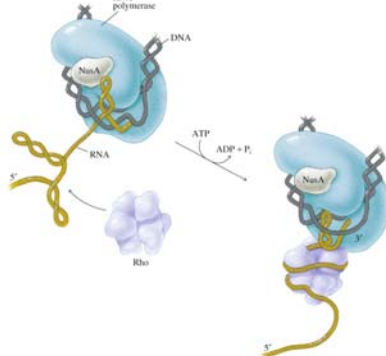
RNA Pol II: **mRNA**

RNA Pol III: **tRNA, 5S rRNA, small RNA**

(see Table 21.4)

Fig 21.9

- Rho-dependent termination of transcription (*E. coli*)
- RNA pol is stalled at pause site
- Rho binds to new RNA, destabilizes RNA-DNA hybrid



Housekeeping genes:

- * encode proteins required for basic metabolism
- * have strong or weak promoters depending upon level of protein required

Regulated (differentially expressed) genes:

- * often regulated at level of transcription

Activators: regulatory proteins that bind DNA & increase rate of transcription of weak promoters.

- * Often interact with RNA polymerase → increase RNA polymerase binding or increase rate of transcription bubble formation (opening) or increase rate of primer formation

activators can be allosterically regulated

Repressors: regulatory proteins that bind DNA and repress transcription

Repressors have many mechanisms for repressing transcription.

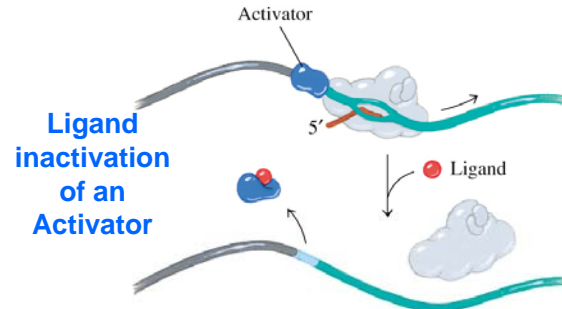
These include:

- * preventing RNA polymerase from binding promoter
- * inhibition of initiation reactions (e.g. transition bubble formation, primer synthesis, promoter clearance)

Repressors are allosterically regulated**

****inducer:** ligands that bind to, and inactivate, repressors

****corepressor:** ligands that bind to, and activate, repressors

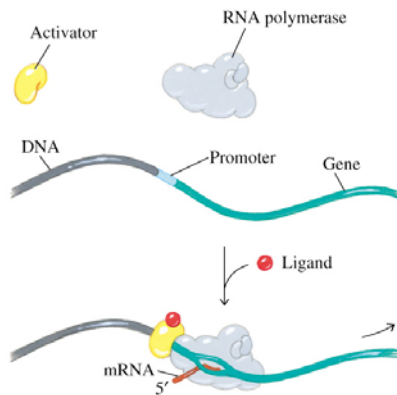


(b) An activator stimulates transcription. In the presence of ligand, the activator is inhibited.

Fig. 21.14

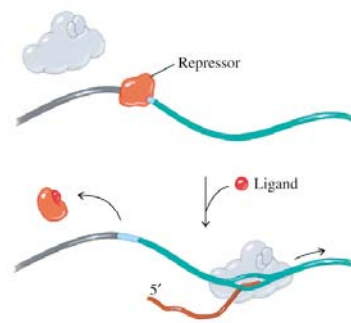
Ligand activation of an Activator

- Strategies for regulating transcription initiation by regulatory proteins

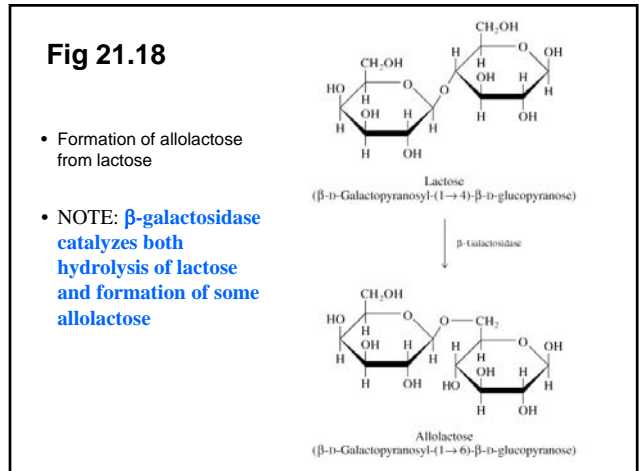
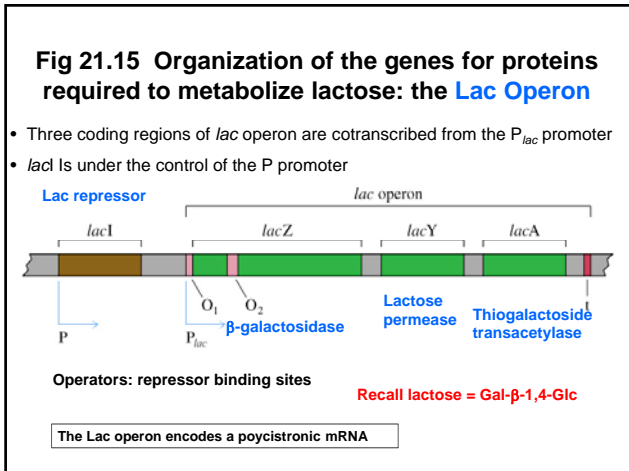
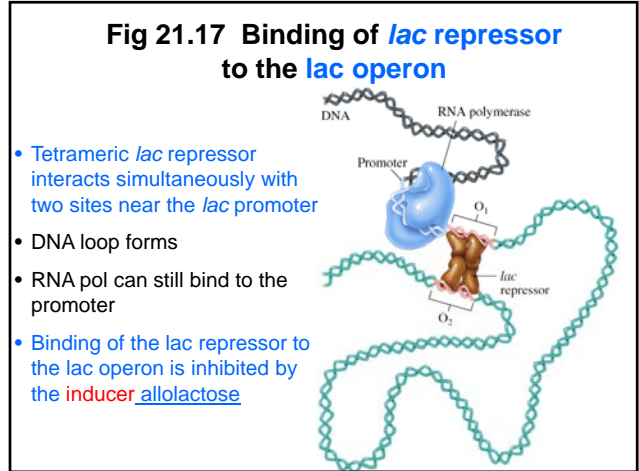
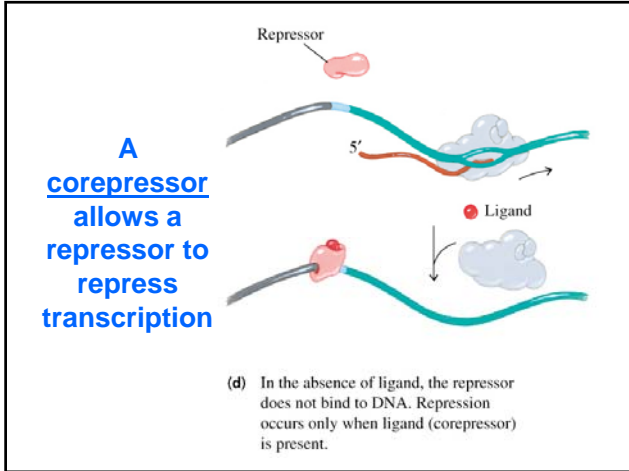


(a) An activator with bound ligand stimulates transcription.

A repressor is inactivated by binding of an inducer



(c) A repressor prevents transcription. Binding of ligand (inducer) to the repressor inactivates the repressor and allows transcription.



Carbon Source	Relative transcription from lac Operon	Reason?
bacteria	Escape synthesis (very low level)	
bacteria	1	
bacteria	50	

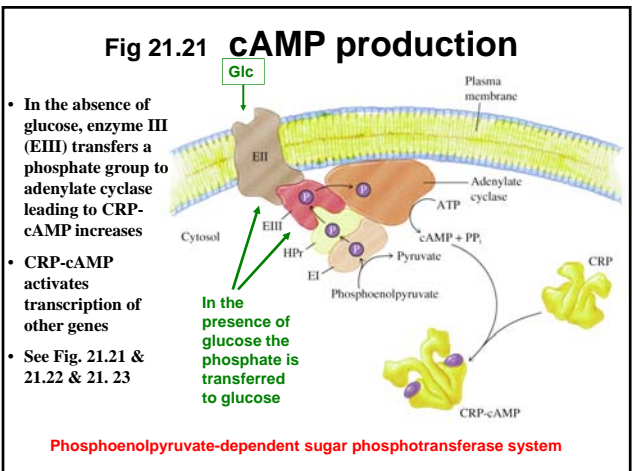
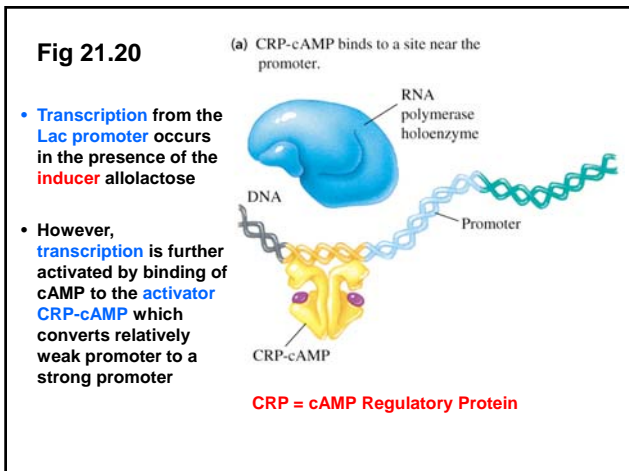
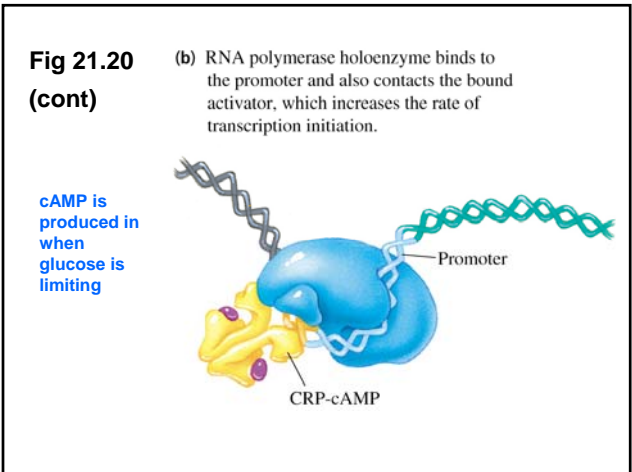
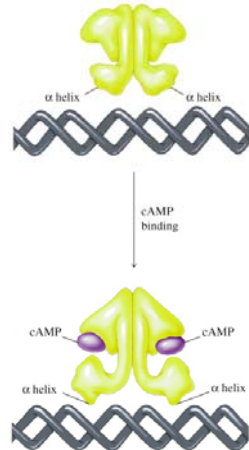


Fig 21.21

- Conformational changes in CRP caused by cAMP binding
- α -Helices of each monomer of the cAMP-CRP dimer fit into major groove of DNA



Many primary transcripts must be further processed to be active. Such transcripts include: tRNA, rRNA and mRNA in eukaryotes

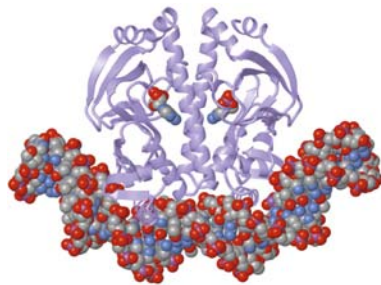
Types of transcript processing

- 1. removal of nucleotides**
- 2. addition of nucleotides**
- 3. covalent modification of nucleotides**

Thus, in some cases the mature transcript includes different bases or modifications NOT encoded by the corresponding gene!!

Fig 21.22 Structure of CRP-cAMP and DNA complex

- Both subunits have a cAMP bound at the allosteric site
- Each subunit has an α -helix in DNA major groove



mRNA Processing

Prokaryote mRNA is NOT further processed. 1^o transcript is directly translated

Eukaryote mRNA IS processed: cleavage, covalent modification, addition of nucleotides & splicing

mRNA processing steps:

5' capping; 3' polyadenylation; splicing

Covalent modification of the ends of the transcript increases RNA stability.

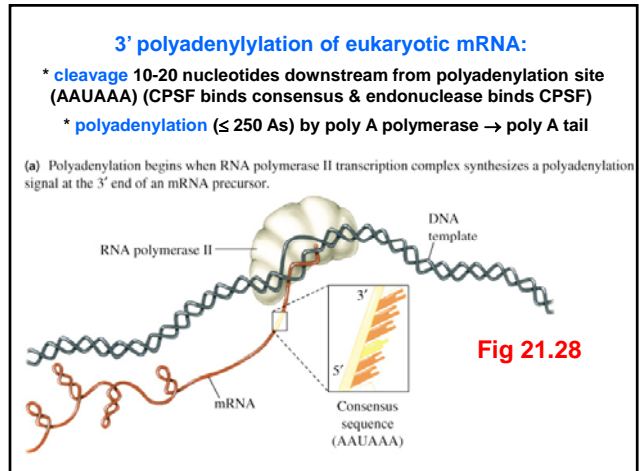
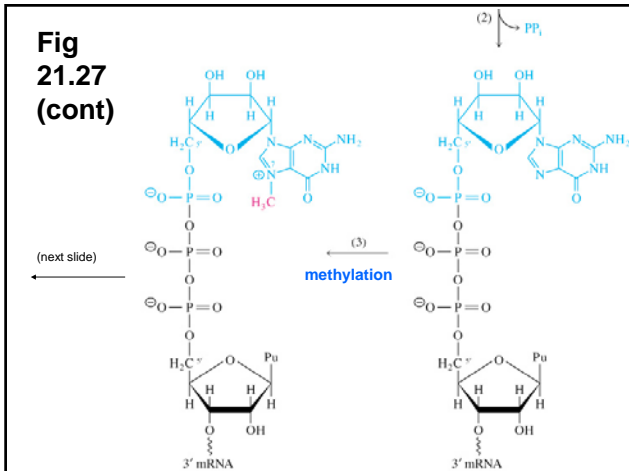
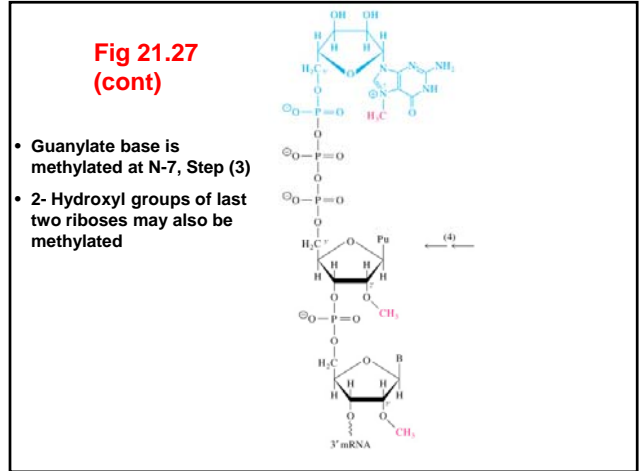
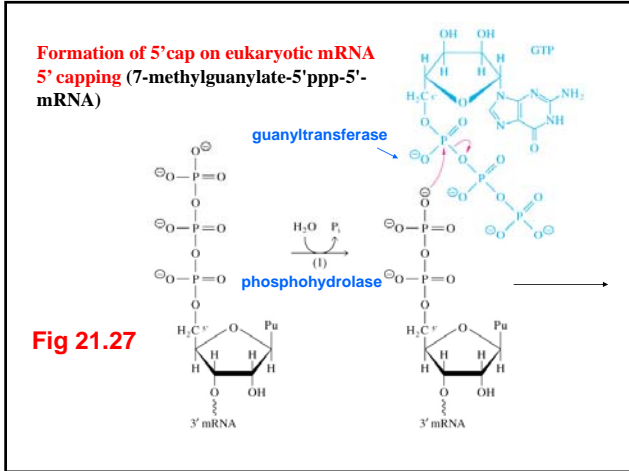


Fig 21.28 (cont)

CPSF = cleavage & polyadenylation specificity factor

(b) CPSF binds to the consensus sequence and forms a complex containing an RNA endonuclease. The endonuclease catalyzes cleavage of the transcript downstream of the polyadenylation sequence, forming a new 3' end. Poly A polymerase can then bind to the end of the mRNA precursor.

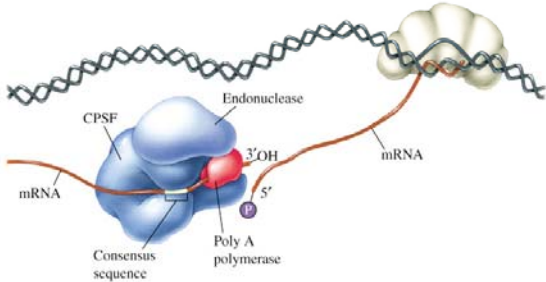


Fig 21.29 Triose phosphate isomerase gene (nine exons and eight introns)

Many eukaryote genes have exons & introns

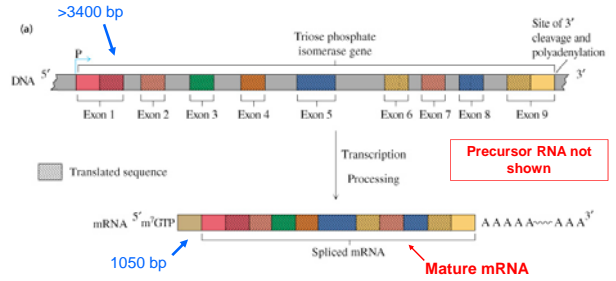


Fig 21.28 (cont)

(c) The endonuclease dissociates and the new 3' end of the RNA is polyadenylated by the activity of poly A polymerase.

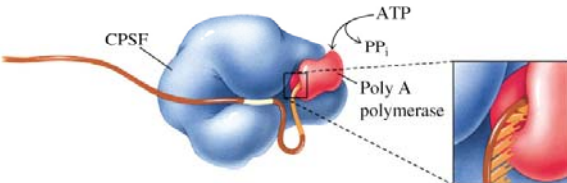
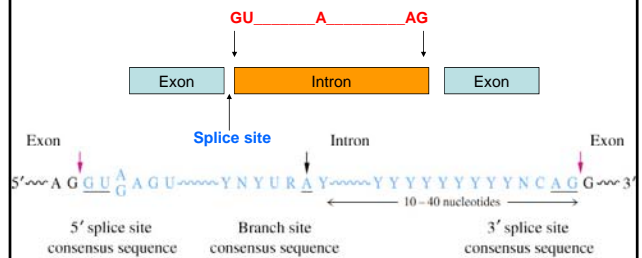


Fig 21.30 Consensus sequences at splice sites in vertebrates



Splicing takes place on _____: complexes of 45 proteins & 5 RNAs called **small nuclear RNA (snRNA)**: U1, U2, U4, U5, U6

snRNA associates with proteins → small nuclear ribonucleoproteins (**snRNPs**).

Fig 21.31 (cont)

(b) The 2'-hydroxyl group is attached to the 5' end of the intron, and the newly created 3'-hydroxyl group of the exon attacks the 3' splice site.

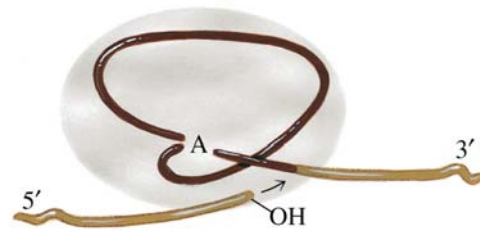


Fig 21.31 Intron removal in mRNA precursors

(a) The spliceosome positions the adenylate residue at the branch site near the 5' splice site. The 2'-hydroxyl group of the adenylate attacks the 5' splice site.

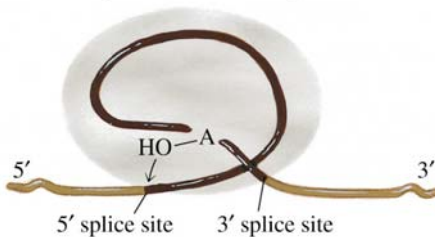
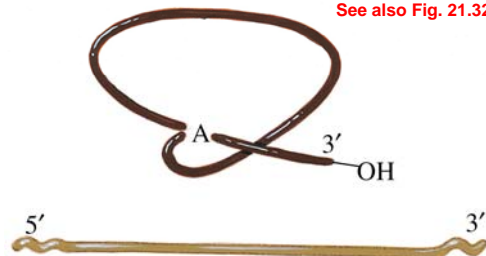


Fig 21.31 (cont)

(c) As a result, the ends of the exons are joined, and the intron, a lariat-shaped molecule, is released.

See also Fig. 21.32



mRNA Processing

Prokaryote mRNA is NOT further processed. 1° transcript is directly translated

Eukaryote mRNA IS processed: cleavage, covalent modification, addition of nucleotides & splicing

mRNA processing steps

1. covalent modification of the ends of the transcript increases RNA stability.

a) **5' end modification:** **capping** (7-methylguanylate-5'ppp-5'-mRNA)

b) **3' end modification :**

* **cleavage** 10-20 nucleotides downstream from polyadenylation site (AAUAAA) (CPSF binds consensus & endonuclease binds CPSF)

* **polyadenylation** (≤ 250 As) by poly A polymerase \rightarrow poly A tail

Extra Information

2. **splicing:** removal of some **internal pieces (introns)** of the 1° transcript and rejoining of the remaining pieces (**exons**).

Junctions between introns and exons = **splice sites**.

Splice sites have consensus 5', 3' and branch sequences required for splicing.

Splicing takes place on **spliceosomes:** complexes of 45 proteins & 5 RNAs called small nuclear RNA (snRNA): U1, U2, U4, U5, U6

snRNA associates with proteins \rightarrow small nuclear ribonucleoproteins (snRNPs).

Fig 21.32 Formation of a spliceosome

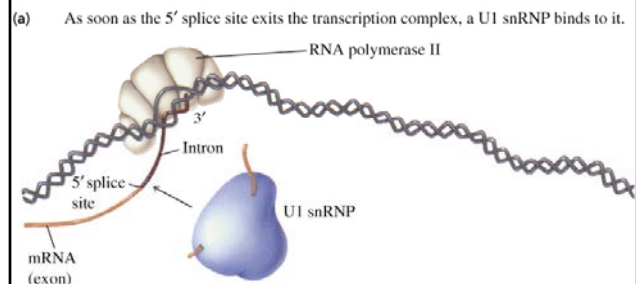


Fig 21.32 (cont)

(b) Next, a U2 snRNP binds to the branch site within the intron.

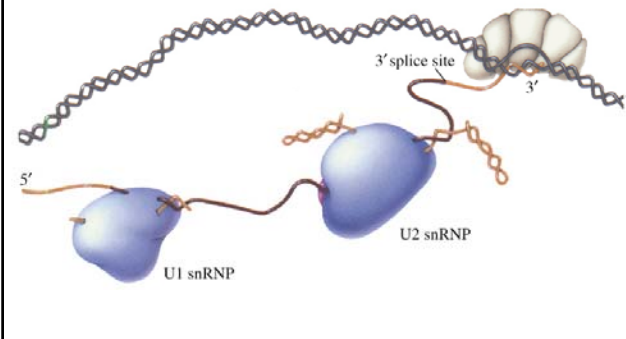


Fig 21.32 (cont)

(c) When the 3' splice site emerges from the transcription complex, a U5 snRNP binds, and the complete spliceosome assembles around a U4/U6 snRNP.

