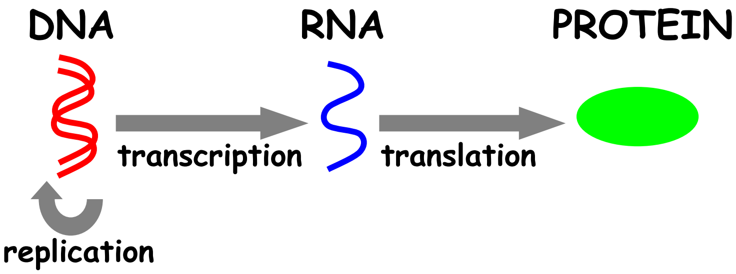
Molecular Biology



Proofreading (p. 961-962)

What happens if the incorrect nucleotide is incorporated during replication?

daughter 5′ G C G A T G (A)
template 3′ C G C T A C G T A A 5′

DNA Polymerase has 3′-->5′ exonuclease activity

5′ G C G A T G (A)
3′ C G C T A C G T A A 5′

Pol pauses, removes ntd.

5′ G C G A T G
3′ C G C T A C G T A A 5′

Pol resumes replication

5′ G C G A T G C A T T 3′
3′ C G C T A C G T A A 5′

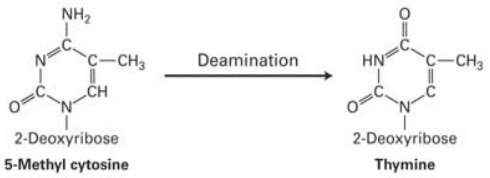
DNA Repair (p.962-970)

TABLE 23-1 Some Human Hereditary Diseases and Cancers Associated with DNA-Repair Defects

Disease	DNA-Repair System Affected	Sensitivity	Cancer Susceptibility	Symptoms
PREVENTION OF POINT MUTATIONS, INSERTIONS, AND DELETIONS				
Hereditary nonpolyposis colorectal cancer	DNA mismatch repair	UV irradiation, chemical mutagens	Colon, ovary	Early development of tumors
Xeroderma pigmentosum	Nucleotide excision repair	UV irradiation, point mutations	Skin carcinomas, melanomas	Skin and eye photosensitivity, keratoses
REPAIR OF DOUBLE-STRAND BREAKS				
Bloom's syndrome	Repair of double-strand breaks by homologous recombination	Mild alkylating agents	Carcinomas, leukemias, lymphomas	Photosensitivity, facial telangiectases, chromosome alterations
Fanconi anemia	Repair of double-strand breaks by homologous recombination	DNA cross-linking agents, reactive oxidant chemicals	Acute myeloid leukemia, squamous-cell carcinomas	Developmental abnormalities including infertility and deformities of the skeleton; anemia
Hereditary breast cancer, BRCA-1 and BRCA-2 deficiency	Repair of double-strand breaks by homologous recombination		Breast and ovarian cancer	Breast and ovarian cancer

SOURCES: Modified from A. Kornberg and T. Baker, 1992, *DNA Replication*, 2d ed., W. H. Freeman and Company, p. 788; J. Hoeijmakers, 2001, *Nature* 411:366; and L. Thompson and D. Schild, 2002, *Mutation Res.* 509:49.

Mutations arise when DNA repair fails



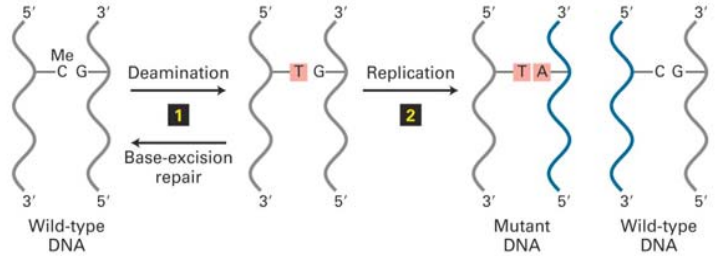


Fig 23-25

DNA Repair Systems in Eukaryotes

1. Base excision - only incorrect ntd. is removed

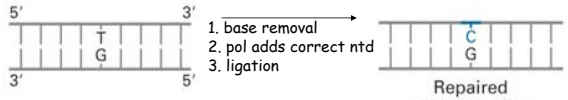


Fig 23-27

2. Mismatch repair

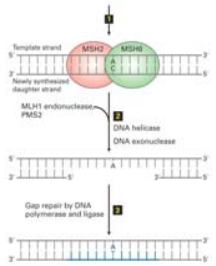


Fig 23-28

3. Nucleotide excision

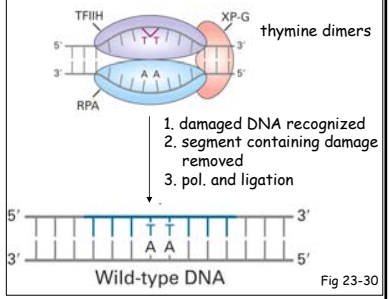


Fig 23-30

What if there is a double-stranded break?

1. Homologous recombination - "error free"
   undamaged sister chromatid serves as template

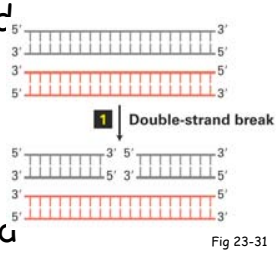
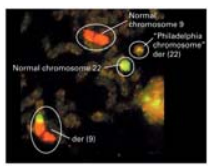


Fig 23-31

2. End-joining - "error prone"
   two separated ends rejoined
   OR
   different broken ends joined-translocations



Replicating DNA in vitro by the Polymerase Chain Reaction (PCR)

Requirements:

1. template DNA
2. primers (DNA oligos ~20nt)
3. dNTPs
4. Polymerase*

*Taq Polymerase

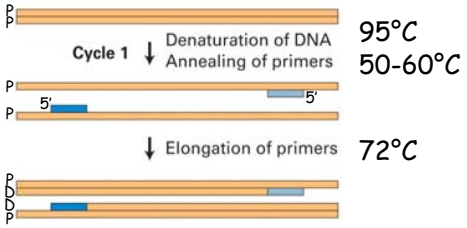


Fig 9-24

Repeat cycle 20X --> 1-million fold amplification

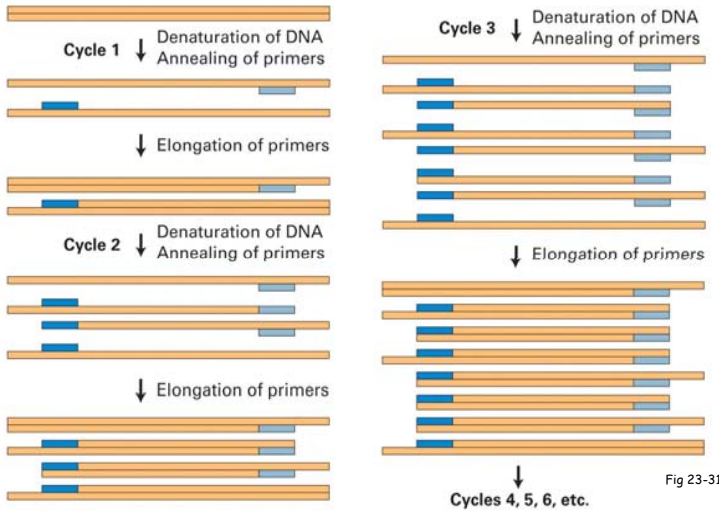
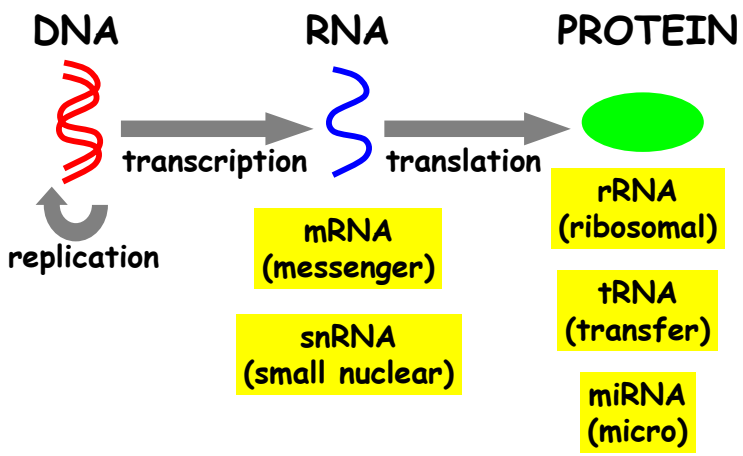


Fig 23-31

Types of RNA

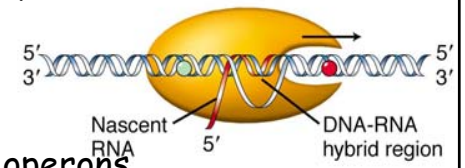


Features of different Eukaryotic RNAs

TYPE	POL	PROCESSED	MATURE SIZE	FUNCTION
mRNA	II	cap, PolyA spliced	variable	translated to protein
rRNA				
tRNA				
snRNA				
miRNA				

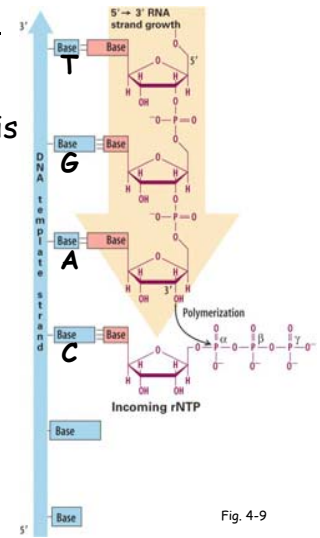
4.2 Transcription of Protein Coding Genes & Formation of Functional mRNA (p.108-115)

1. general transcription
2. initiation
3. elongation
4. termination
5. Prok. mRNAs - operons
6. Euk. mRNAs
7. mRNA processing
8. Northern analysis

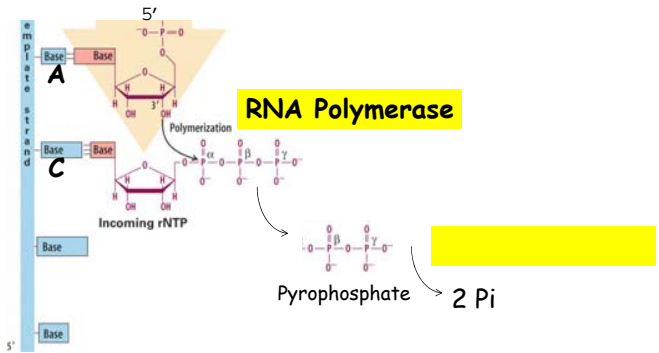


RNA Transcription Requires:

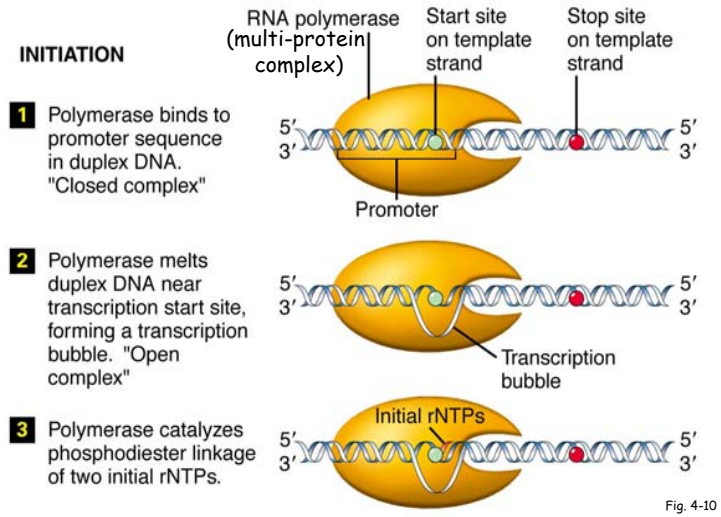
1. Template
2. NTP's for 5′ to 3′ synthesis
3. Proteins



RNA Polymerase Catalyzes Phosphodiester bonds



Transcription Stages - Initiation



Transcription Stages - Elongation

ELONGATION

4 Polymerase advances 3′ → 5′ down template strand, melting duplex DNA and adding rNTPs to growing RNA.

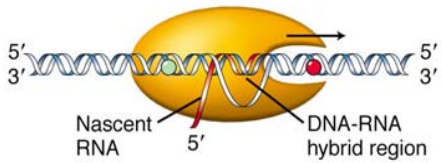


Fig. 4-10

Transcription Stages - Termination

TERMINATION

5 At transcription stop site, polymerase releases completed RNA and dissociates from DNA.

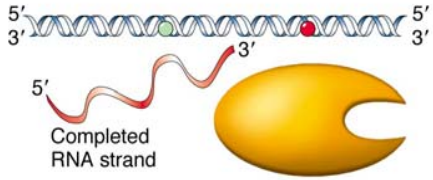
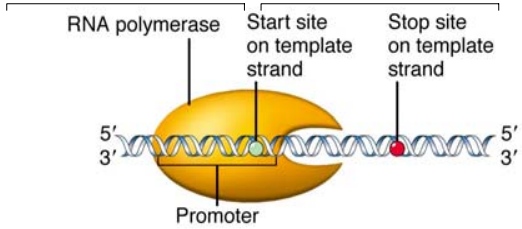


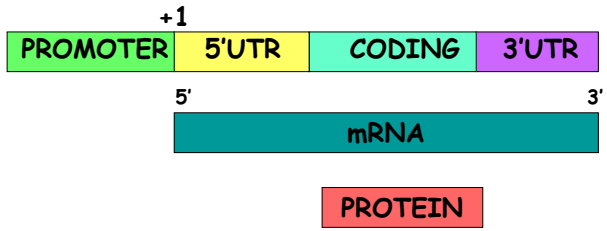
Fig. 4-10

Transcription starts at "+1"

UPSTREAM(-) +1 DOWNSTREAM(+)

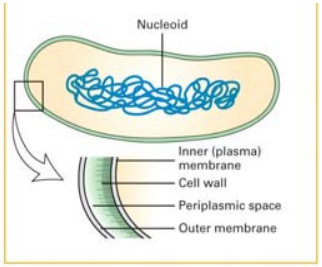


General Gene Structure



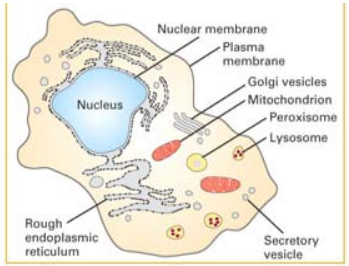
Transcription of Genes

Prokaryotes



- operon - 1 promoter, 1 mRNA, several genes

Eukaryotes



- separate genes
- introns
- pre-mRNAs capped, spliced, PolyA

A Prokaryotic Operon

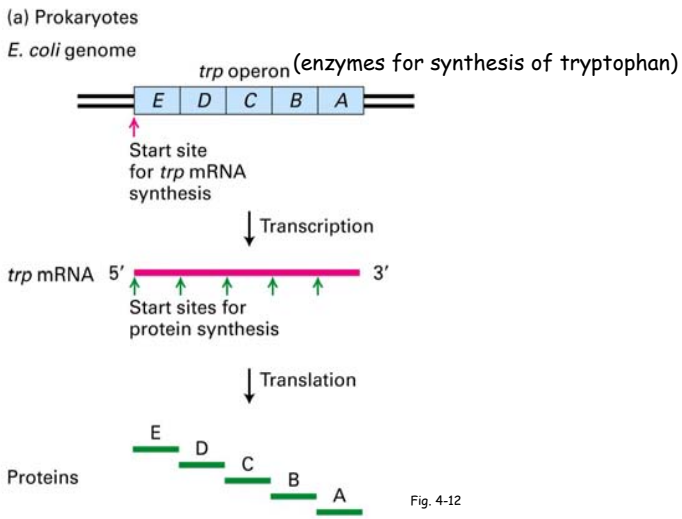


Fig. 4-12

Eukaryotic Gene Expression

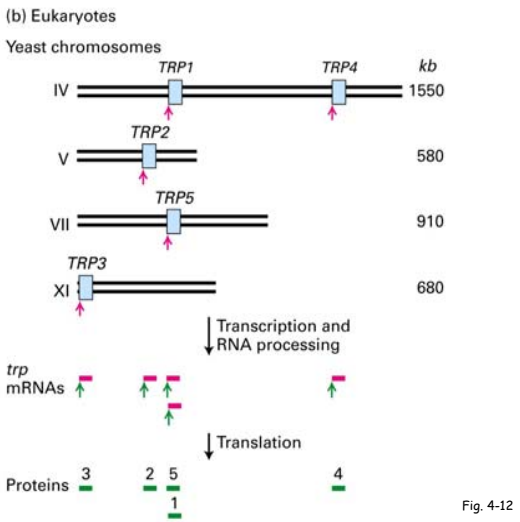


Fig. 4-12

Eukaryotic mRNA Maturation

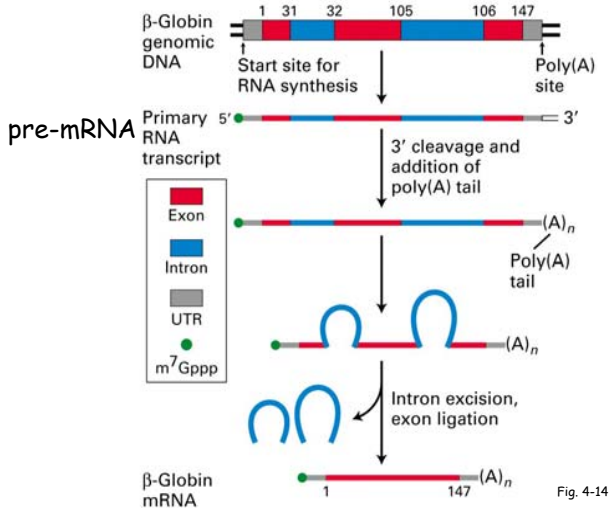


Fig. 4-14

Functions of mRNA processing/modification

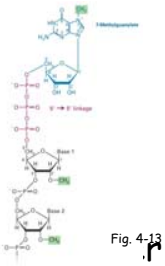


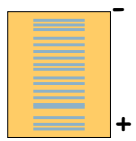
Fig. 4-13

1. 5′ cap: protection
   export signal
   translation
2. splicing: yields mRNA w
   translation code
3. polyA: protection
   export signal
   translation

AAAAAA

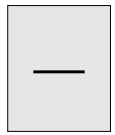
Experimental Detection of RNA

1. isolate total RNA



2. denature and separate RNA by electrophoresis

3. transfer to blot, fix, hybridize



4. detect

Northern Blot of β-globin mRNA

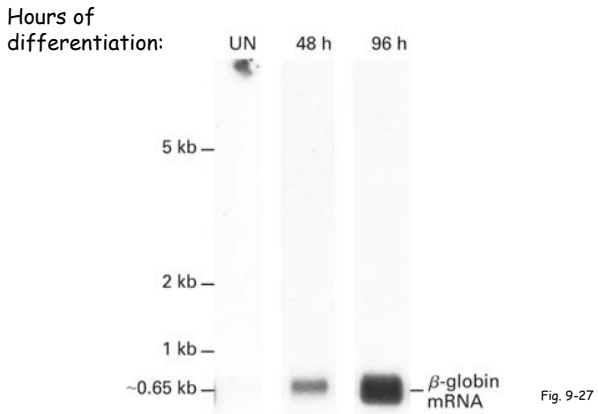


Fig. 9-27

- What can you conclude about activation of the globin gene?
- What predictions can you make about the chromatin structur of the β-globin gene at the three different time points?