Chapter 16

Gene Regulation in Eukaryotes

Sections to study

16.1 Overview of eukaryotic gene regulation

16.2 Control of transcription initiation through promoters and enhancers

16.3 Epigenetics: Control of transcription initiation through DNA methylation

16.4 Regulation after transcription

16.1 Overview of eukaryotic gene regulation

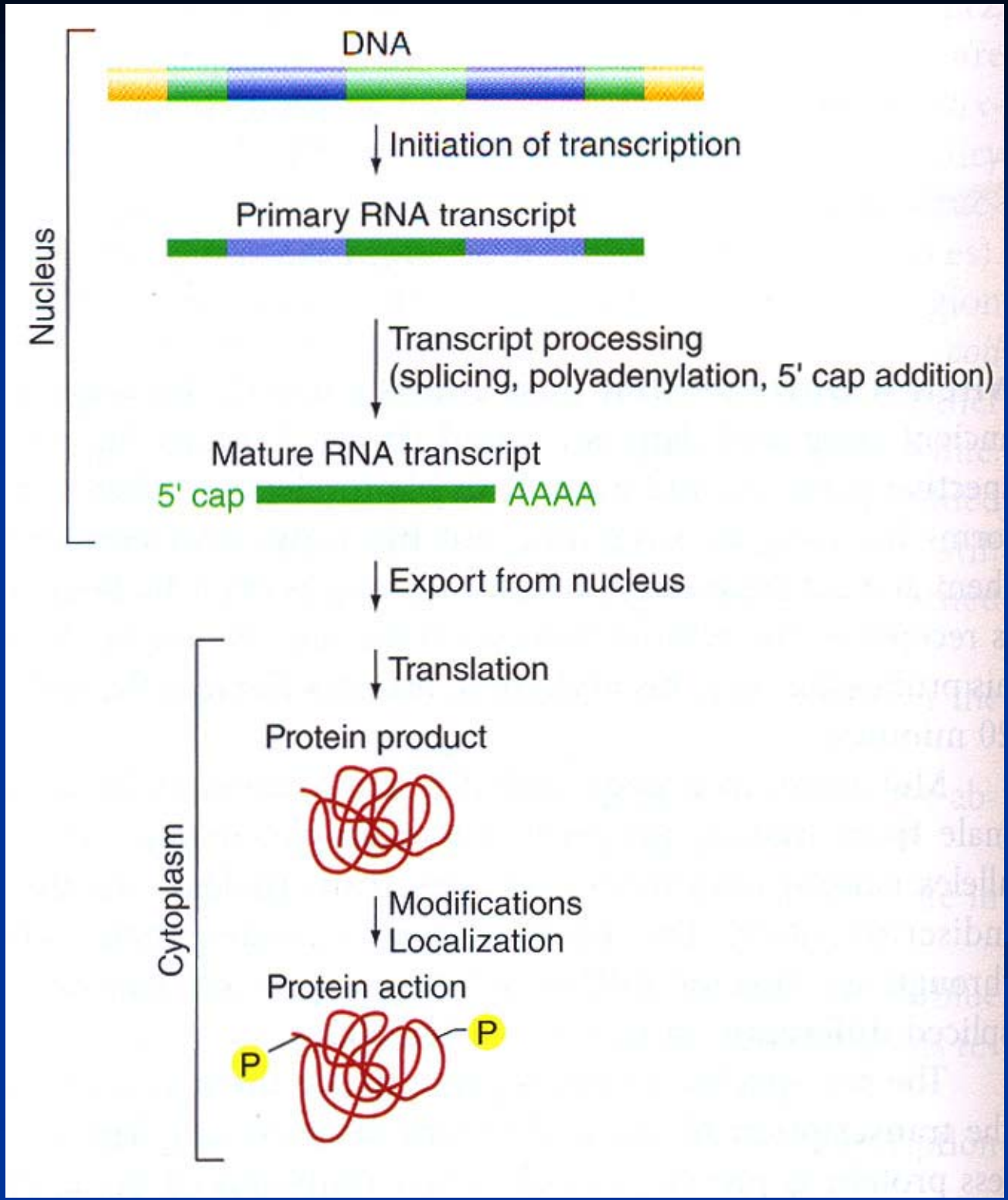


Fig. 16.1

Differences of gene regulation in eukaryotes compared with prokaryotes

- Eukaryotic genomes carry far more DNA, challenging for proteins to locate binding sequences.
- Chromatin structure makes DNA unavailable to transcription machinery.
- Additional RNA processing events occur.
- Transcription and translation are spatially separated.
- Gene regulation needs to control cellular differentiation into hundreds of specialized cell types.

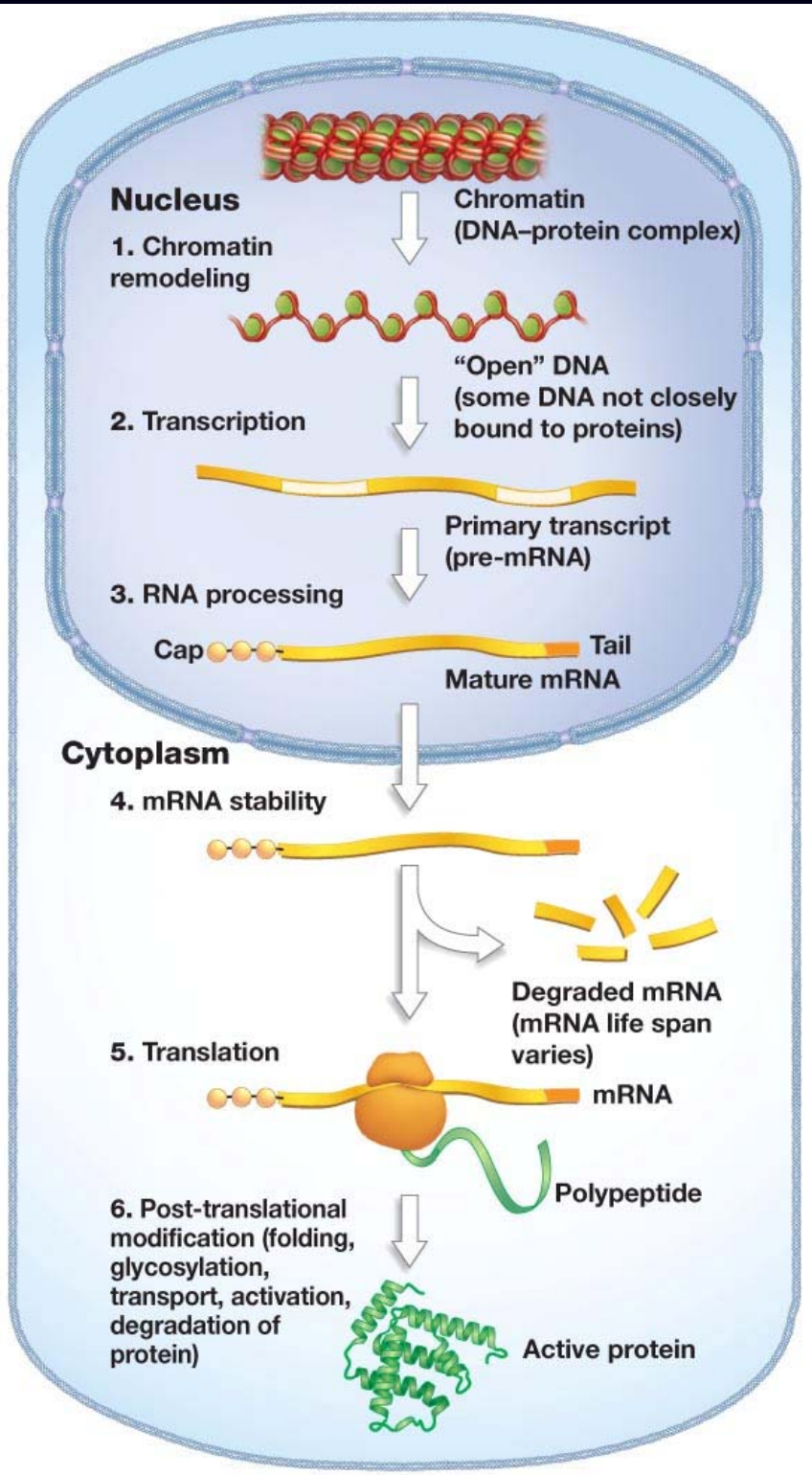
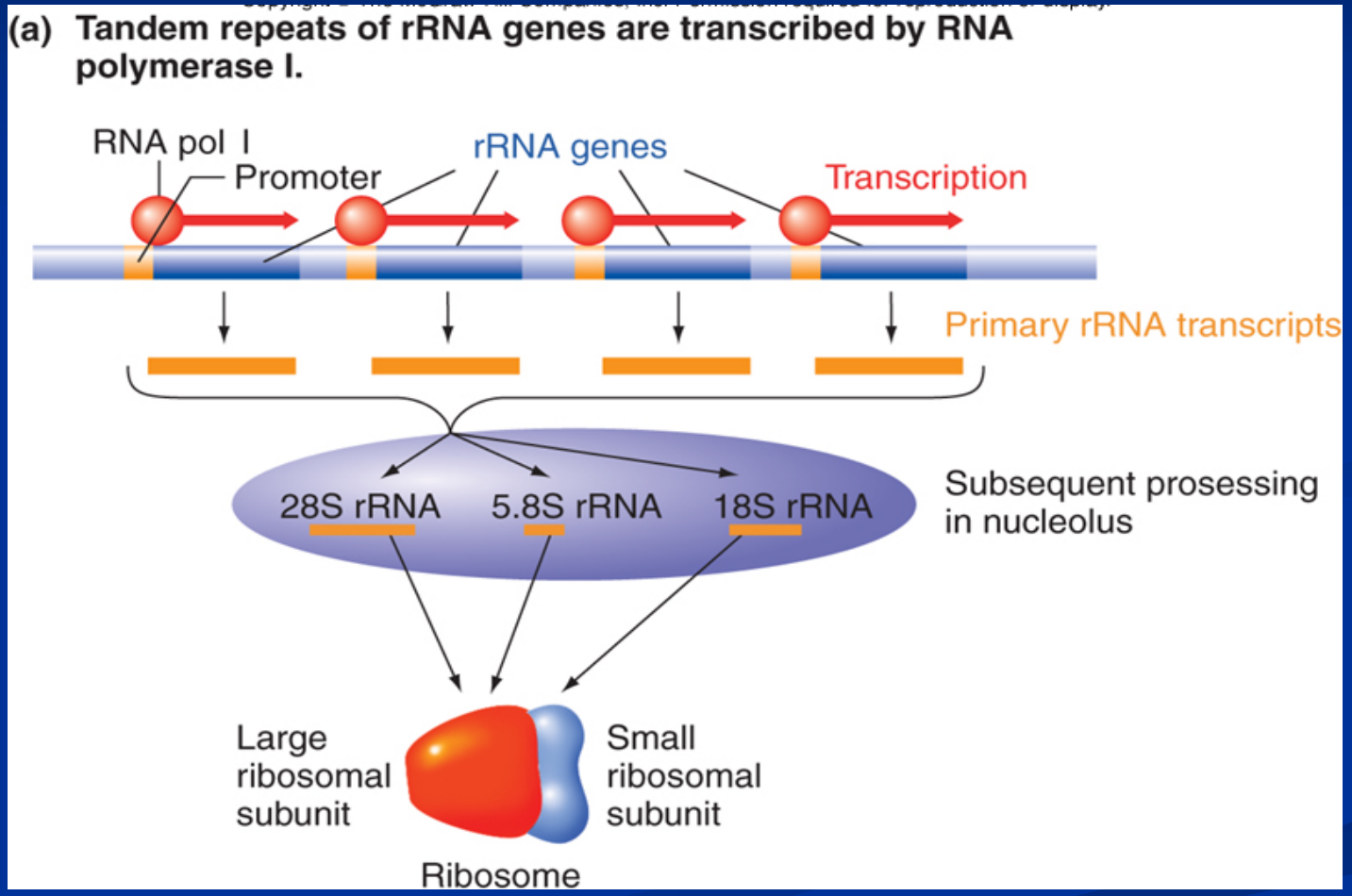


TABLE 18.1 Key Regulatory Differences Between Eukaryotes and Prokaryotes

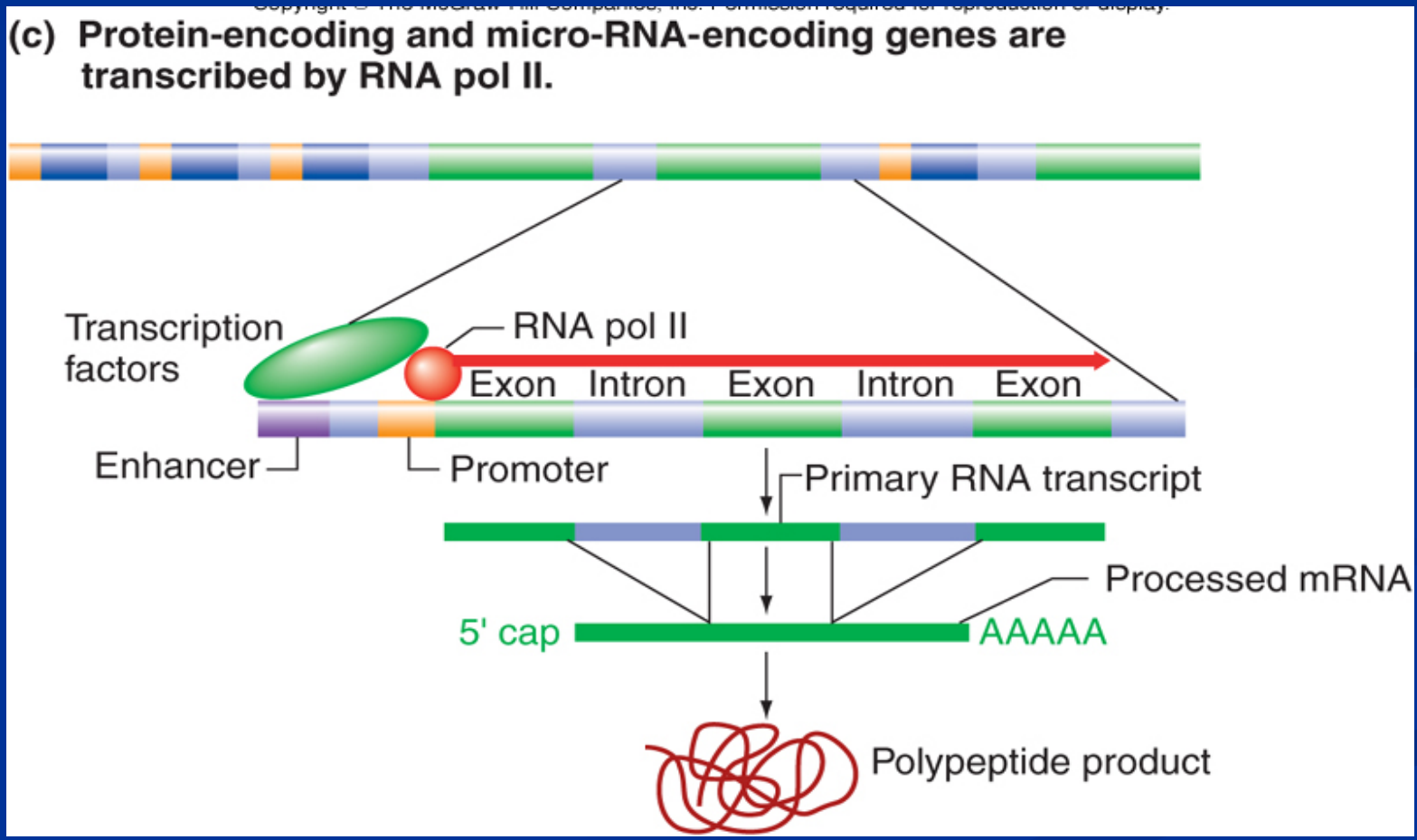
Characteristic	Prokaryote	Eukaryote
Control of transcription through specific DNA-binding proteins	Yes	Yes
Reutilization of same DNA-binding motifs by different DNA-binding proteins	Yes	Yes
Activator proteins	Yes	Yes
Repressor proteins	Yes	Yes
Specificity of binding to DNA by regulatory protein	Specific	Highly specific
Affinity of binding	Strong	Very strong
Role played by chromatin structure	No	Yes
Coordinate control achieved with operons	Yes	Rare
Differential splicing	No	Yes
Attenuation	Yes	No
mRNA processing	No	Yes
Differential polyadenylation	No	Yes
Differential transport of RNA from nucleus to cytoplasm	No	Yes
RNA interference carried out by micro-RNAs	No	Yes

In eukaryotes, three RNA polymerases transcribe different sets of genes.

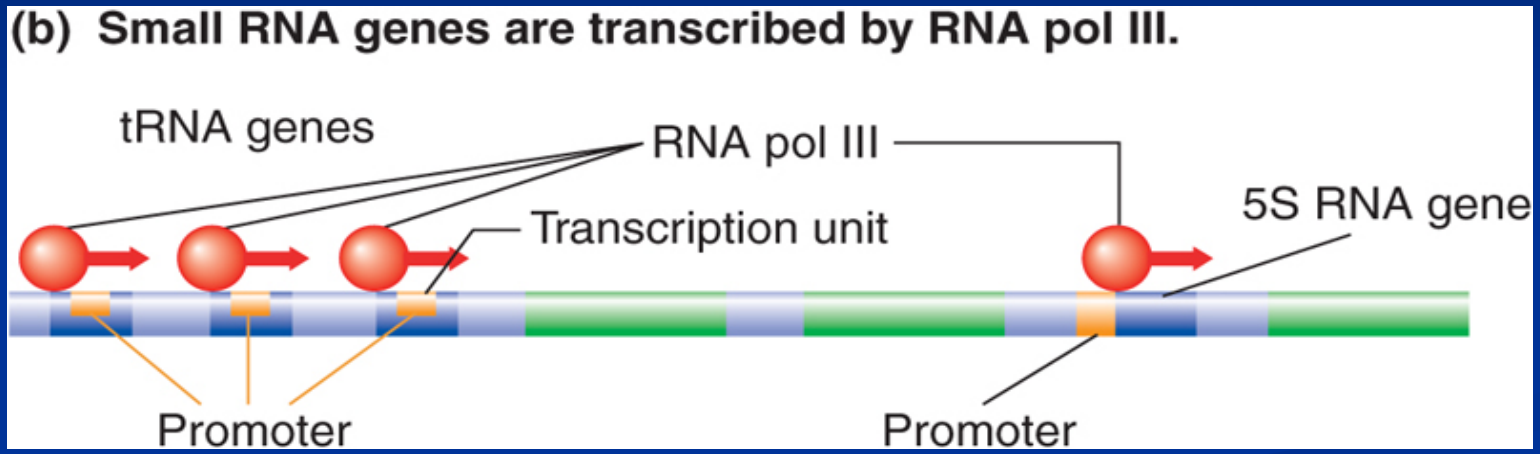
- **RNA polymerase I** transcribes rRNA.
  - rRNAs are made of tandem repeats on one or more chromosomes.
  - RNA polymerase I transcribes one primary rRNA transcript which is further processed to 28S, 5.8S, and 18S rRNA.



- **RNA polymerase II transcribes all protein- and micro-RNA-encoding genes.**
  - **The *cis*-acting regulatory regions of RNA pol. II-transcribed genes contain promoters and enhancers.**



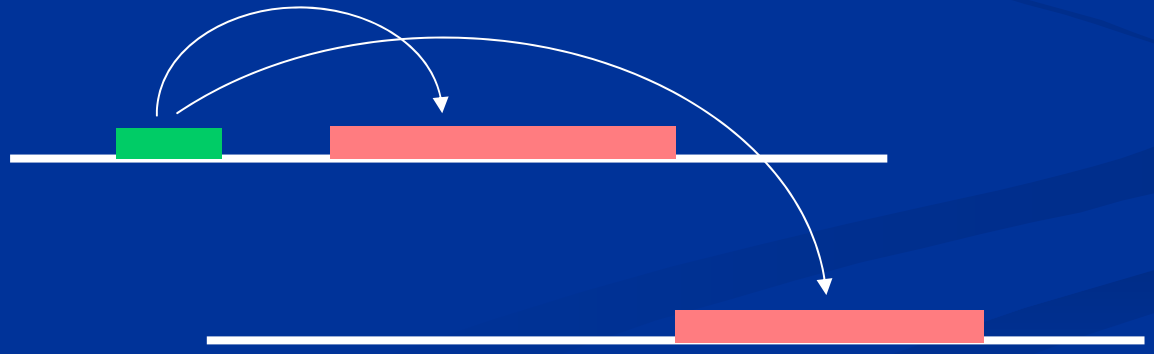
- **RNA polymerase III transcribes tRNAs and other small RNAs (5S rRNA, snRNAs).**



16.2 Control of transcription initiation through promoters and enhancers

Proteins act in *trans*, but DNA sites act only in *cis*.

- ***Cis*-acting elements: Short DNA sequences that constitute the control elements adjacent to genes. They can only influence the expression of adjacent genes on the same DNA molecule.**
- ***Trans*-acting elements: Genes that code for DNA-binding proteins. Their protein products can diffuse through cytoplasm and act at target DNA sites on any DNA molecule in the cell.**



Cis-acting elements of a gene

- **Promoter (启动子): DNA sequences near the beginning of genes that signal RNA polymerase where to begin transcription.**
  - **Promoters contain initiation site and TATA box.**
- **Enhancer (增强子): DNA sequences that augment or repress basal levels of transcription from nearby genes.**
  - **DNA sequences that bind transcription factors.**
  - **Can lie far way from gene.**
  - **Can be reversed.**

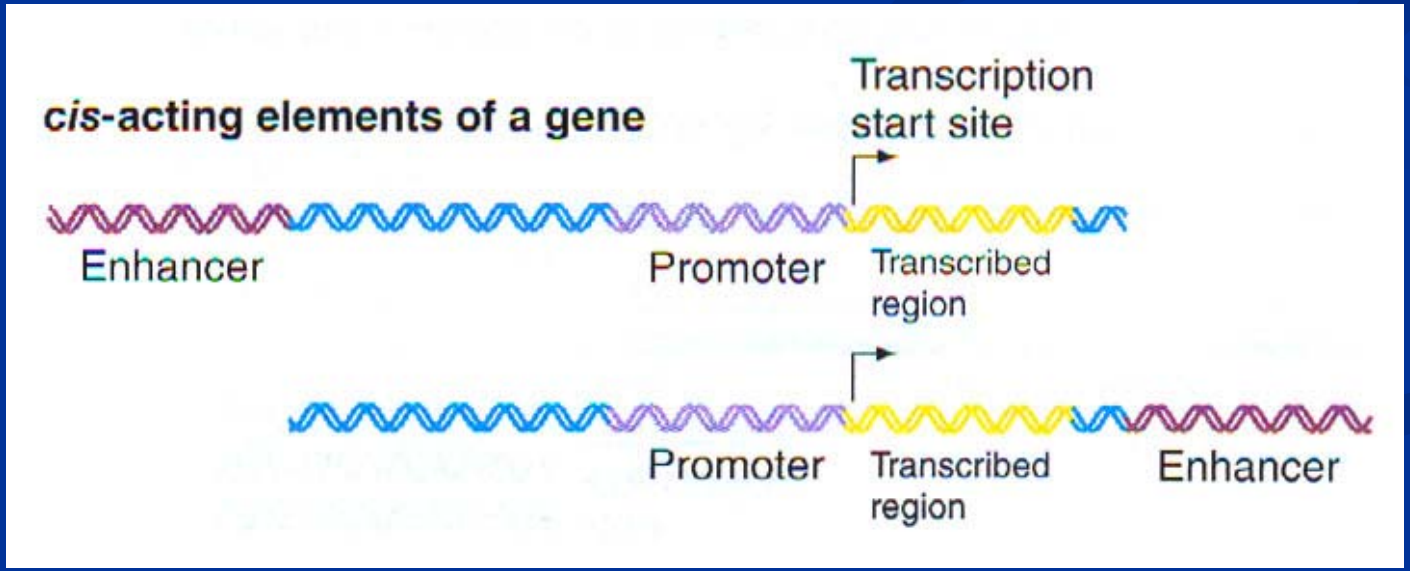


Fig. 16.2

Reporter constructs are a tool for identifying promoters and enhancers

- Promoters and enhancers under investigation are fused to the coding region of reporter genes, such as *lacZ* or GFP.

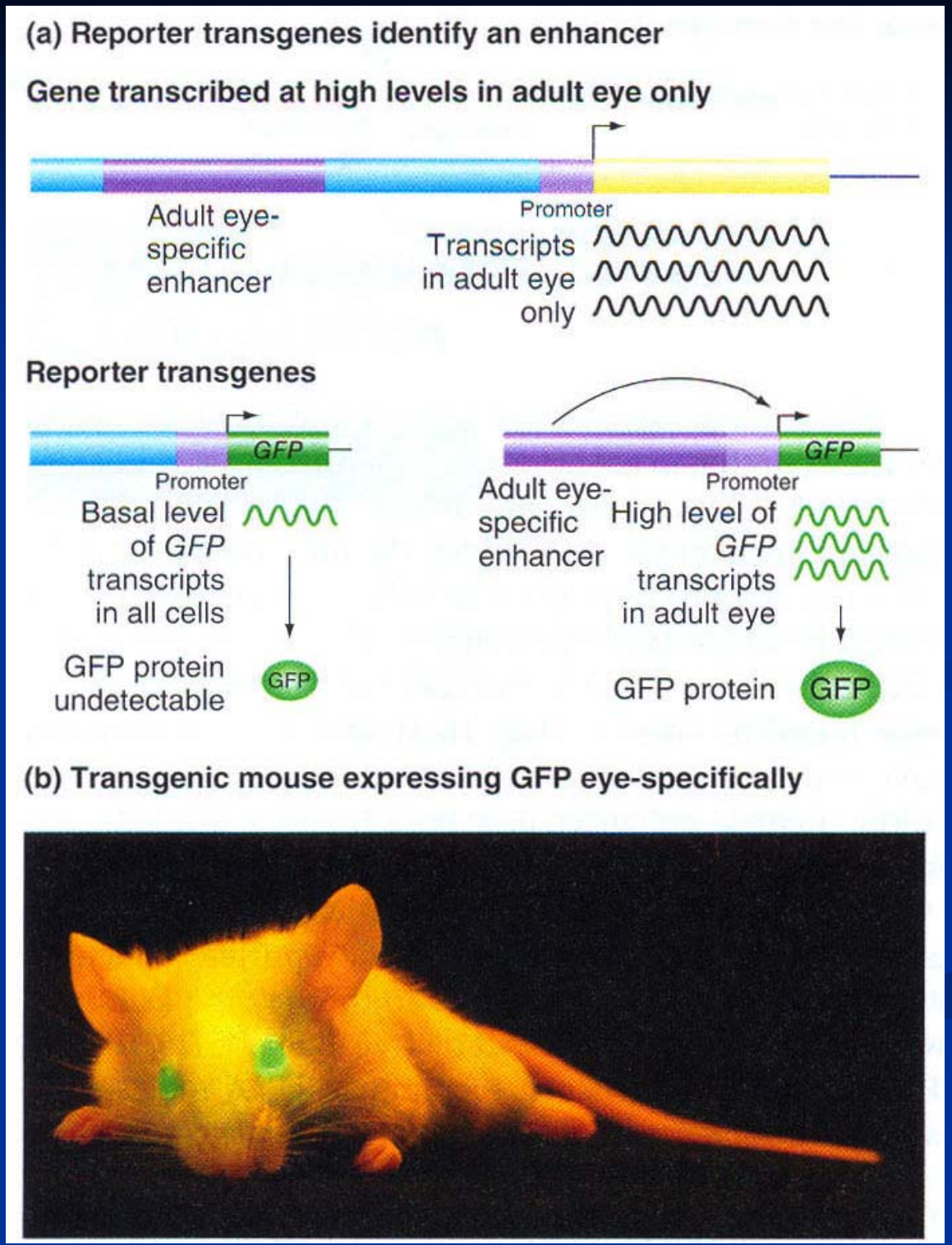
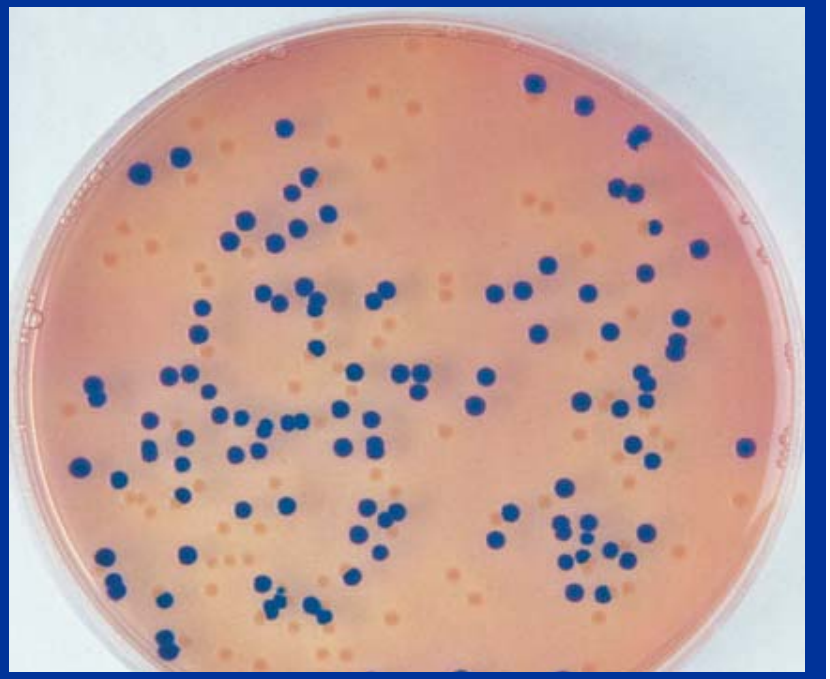


Fig. 16.3

- Reporter constructs can help identify promoters and enhancers.



A

1. Trapping Element

SA Reporter Gene pA

2. Gene

Promoter Exon SD SA Reporter Gene pA SA Exon SD SA Exon pA

3. Transcript

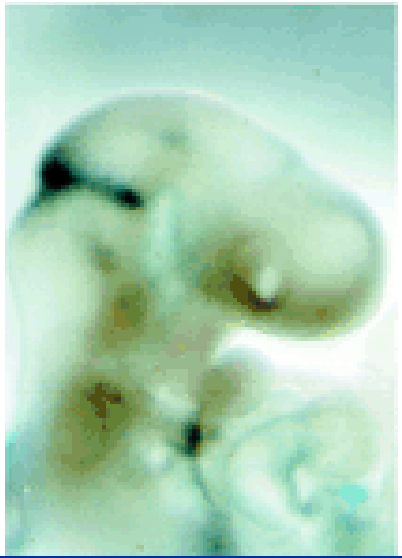
SD SA Reporter Gene AAAAAAAAAA

4. Spliced mRNA

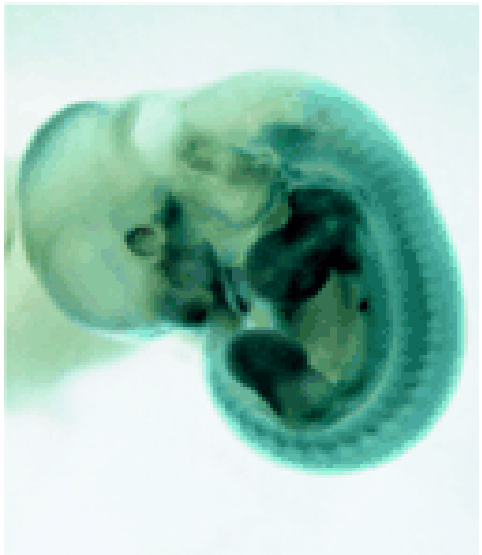
Reporter Gene AAAAAAAAAA

B

E 9.5



E 11.5



Trans-acting elements that control transcription initiation

- **Basal factors**: Proteins that bind to the promoter.
- **Mediator**
- **Transcriptional activators** and **repressors**: Proteins that bind to the enhancers.

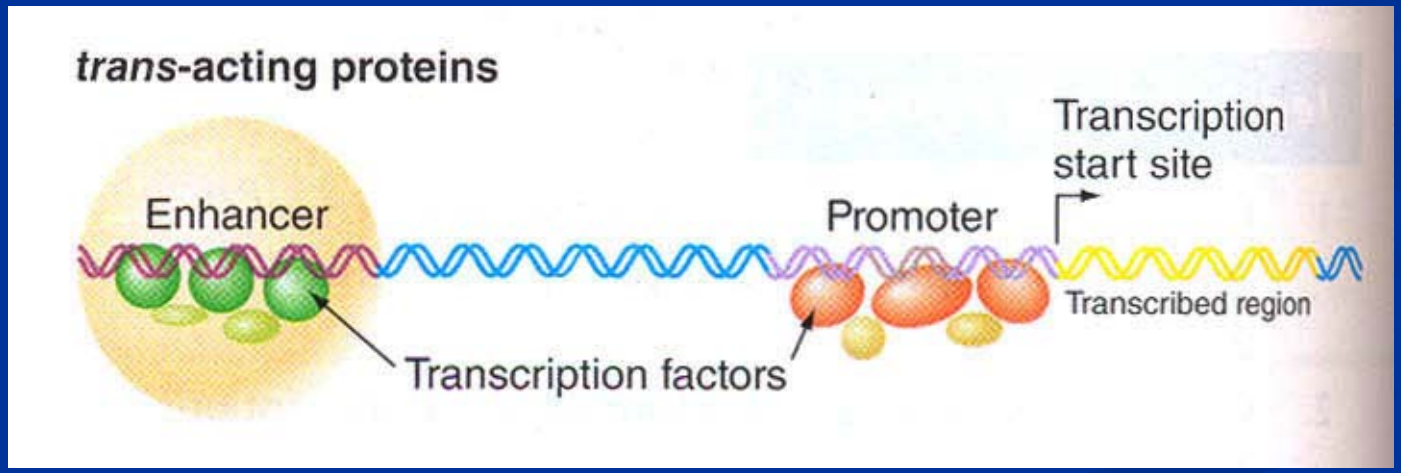


Fig. 16.4

Basal factors

- **Basal factors** bind to the promoter.
  - **TBP** – TATA box-binding protein
  - **TAF** – TBP associated factors
- RNA polymerase II binds to basal factors.

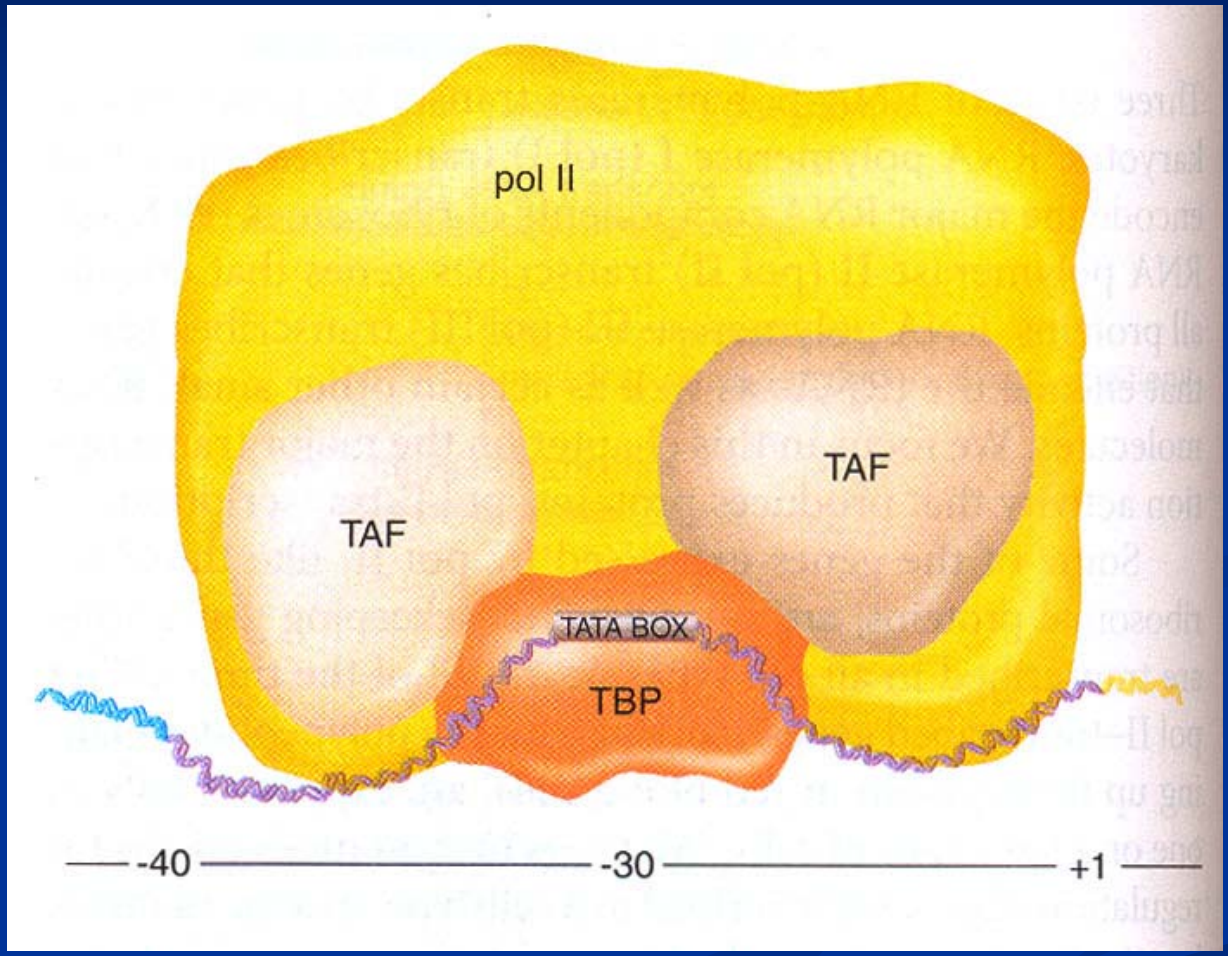
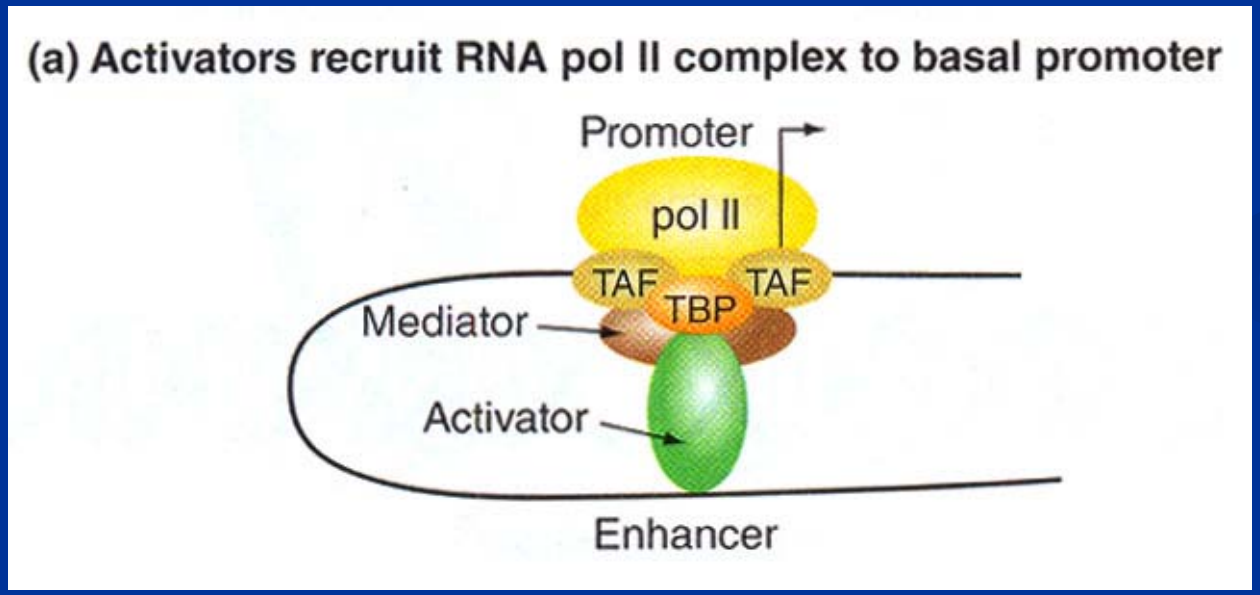


Fig. 16.5

Mediator

- **Mediator**: A protein complex that bridges the RNA pol II complex at the promoter and transcription factors at the enhancer. It is required for the transcription of some eukaryotic genes.
  - A complex that contains more than 20 proteins.



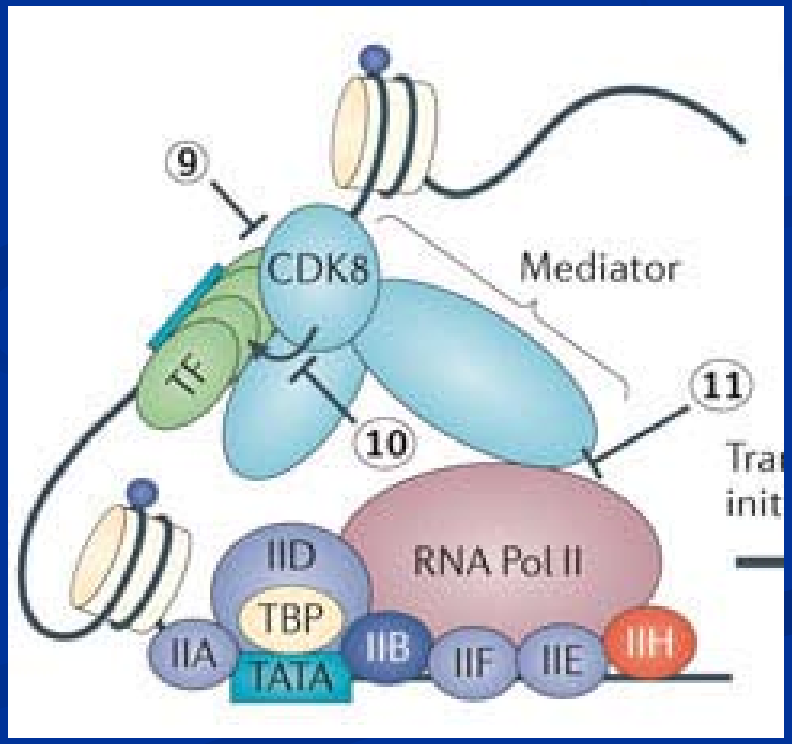
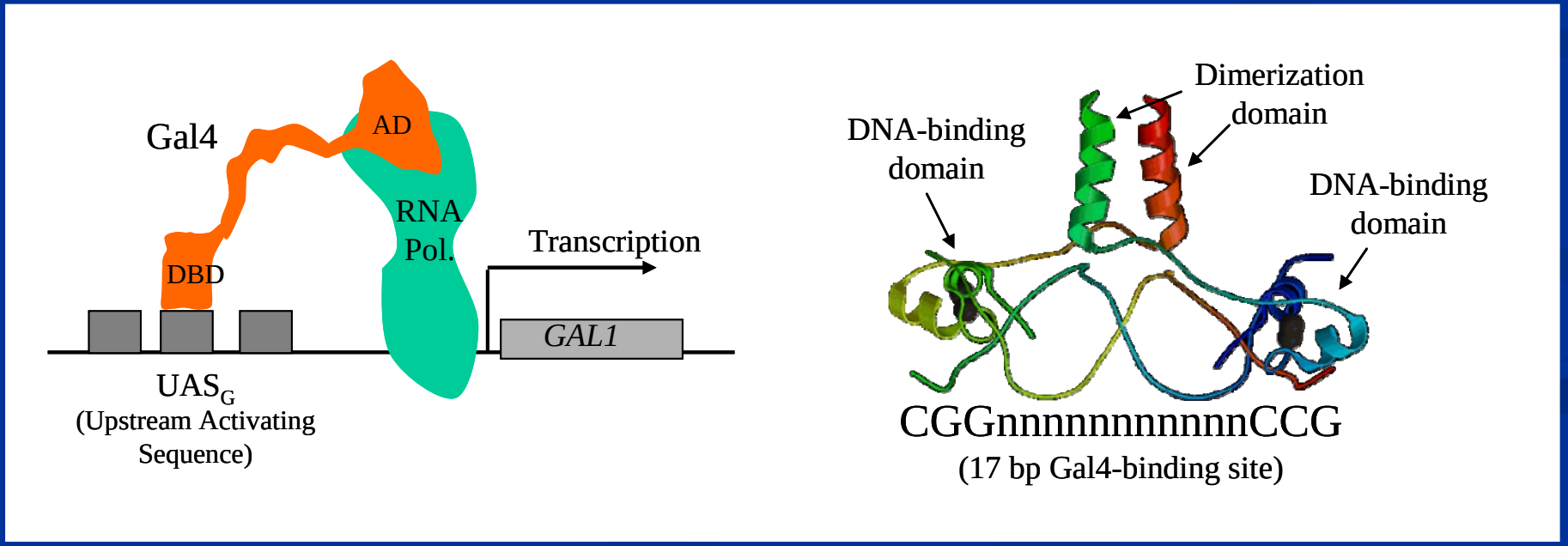


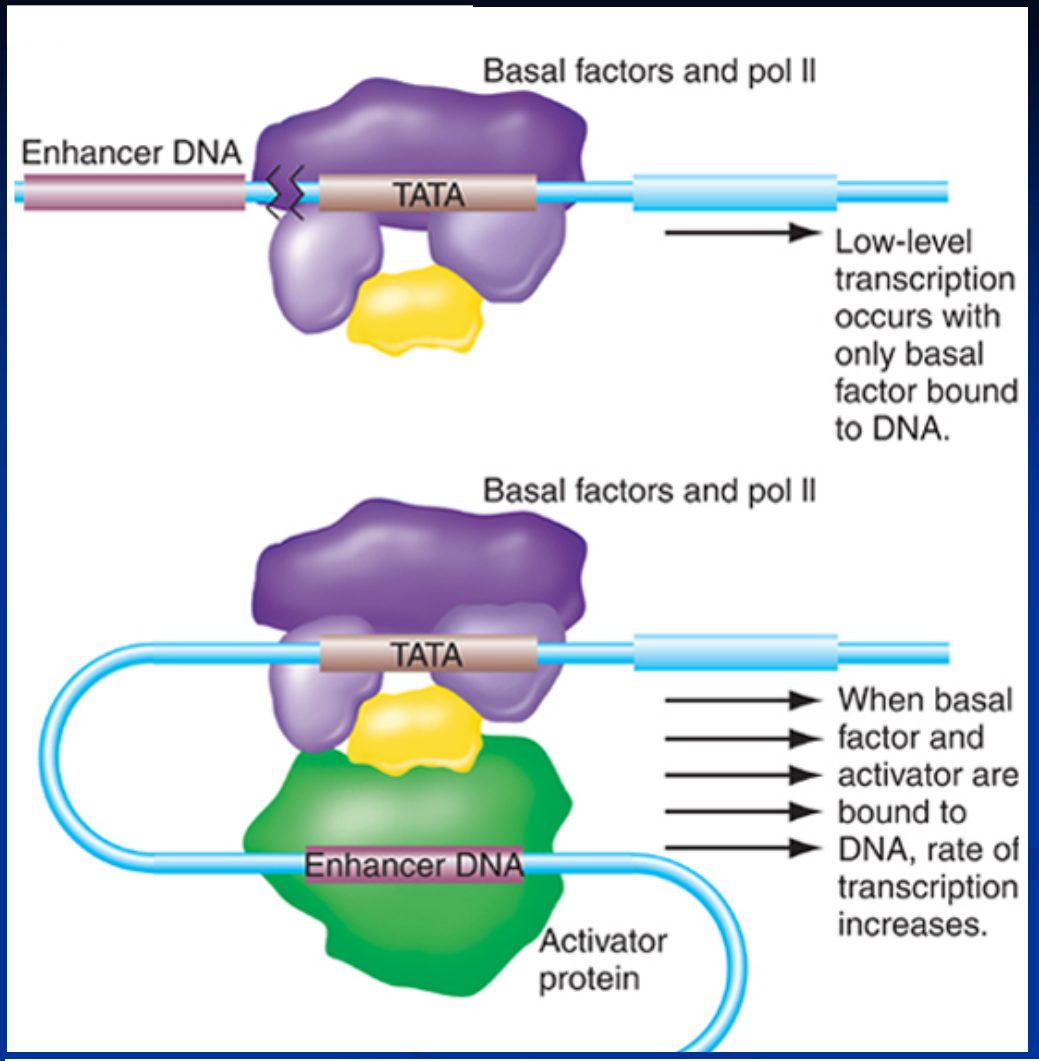
Fig. 16.6a

Activator proteins

- Interact with other proteins to activate and increase transcription above basal levels.
  - Also called **transcription factors.**
- Two structural domains mediate these functions:
  - DNA-binding domain, bind to enhancer DNA in specific ways.
  - Transcription-activating domain



- **Transcriptional activators bind to specific enhancers at specific times to increase transcriptional levels.**



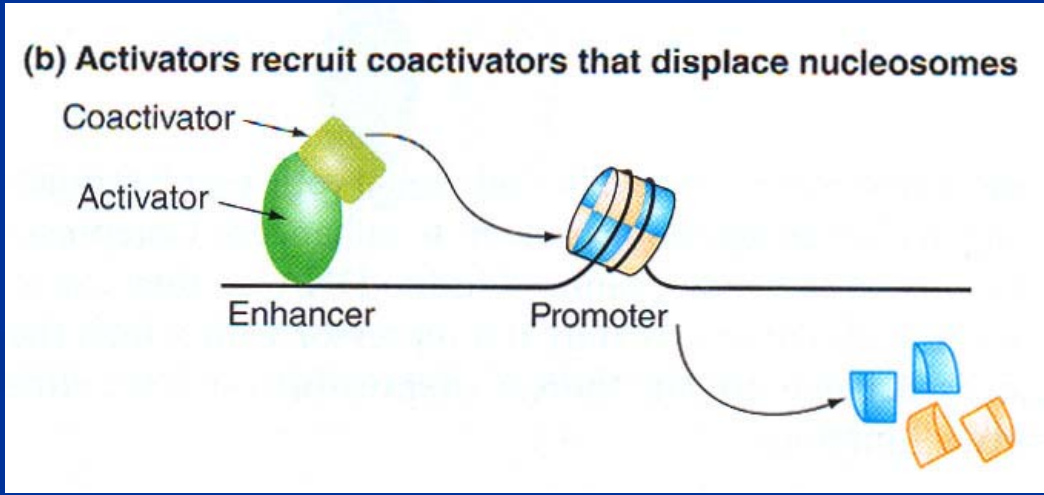
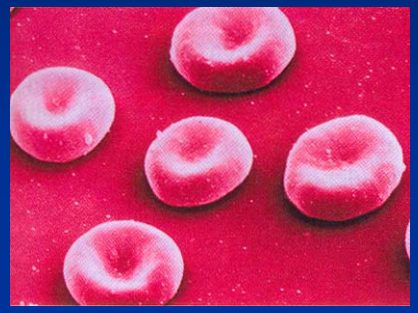


Fig. 16.6

The locus control region (LCR) is a *cis*-acting regulatory sequence that operates sequentially on a cluster of related genes



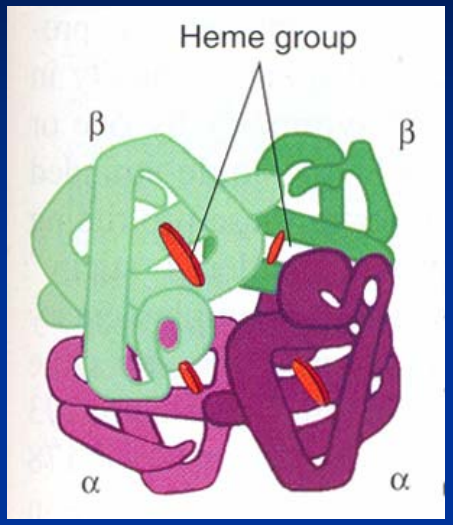
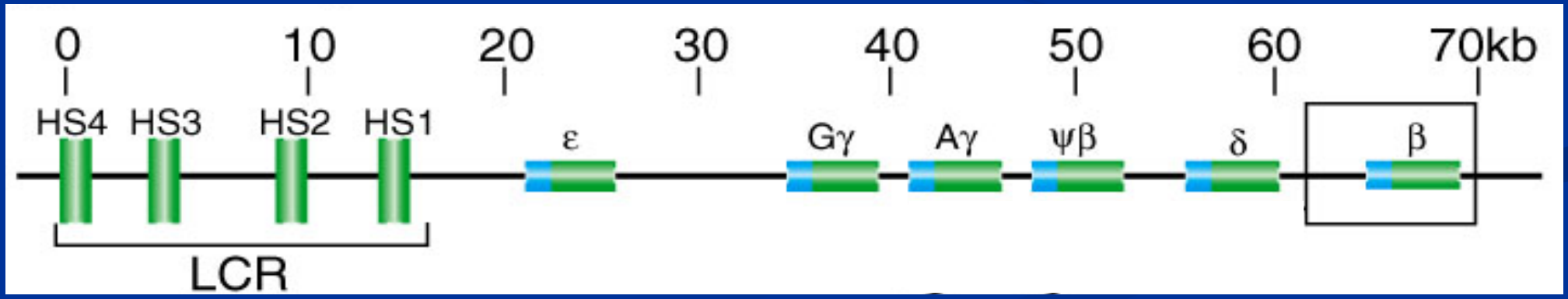
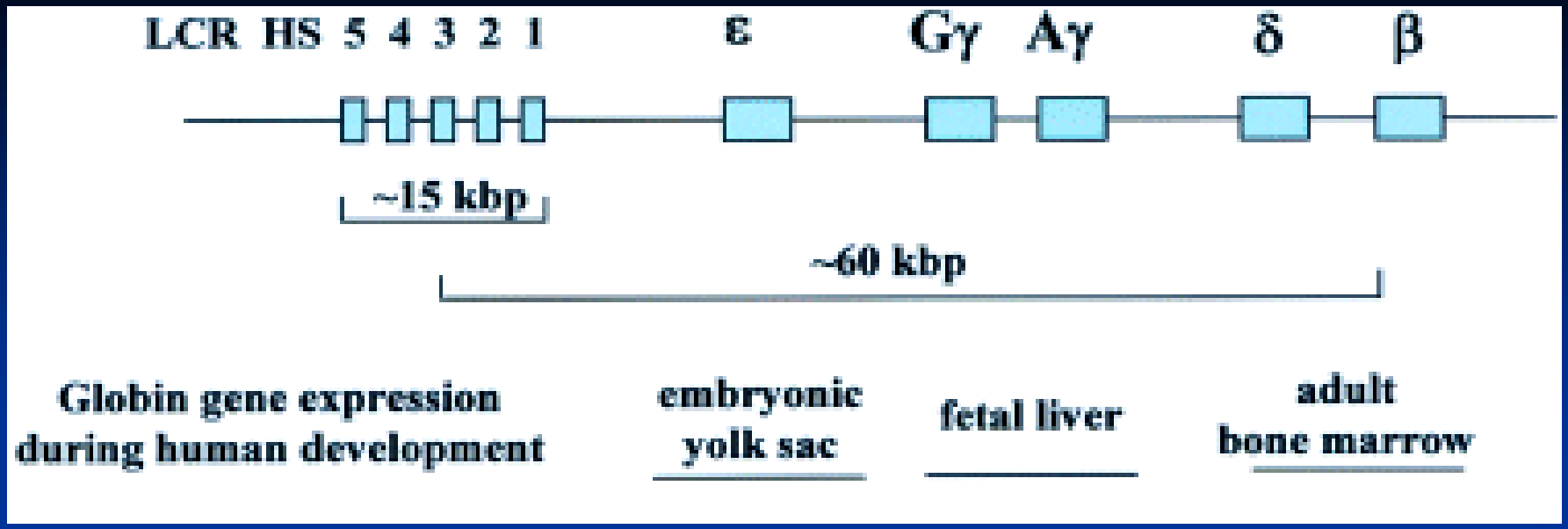


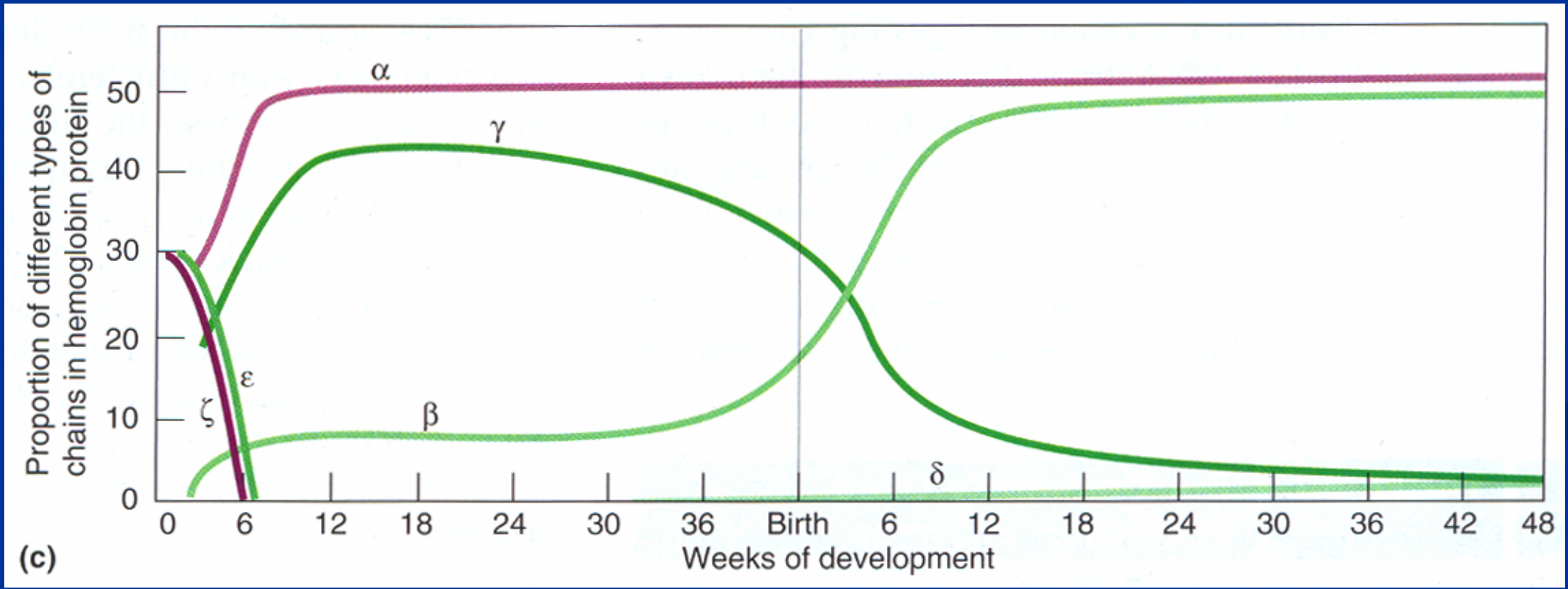
Fig. 9.1a, b

- Human β-globin gene cluster contains five genes that can all be regulated by a distant LCR (locus control region), 50 kb upstream of β-globin gene.

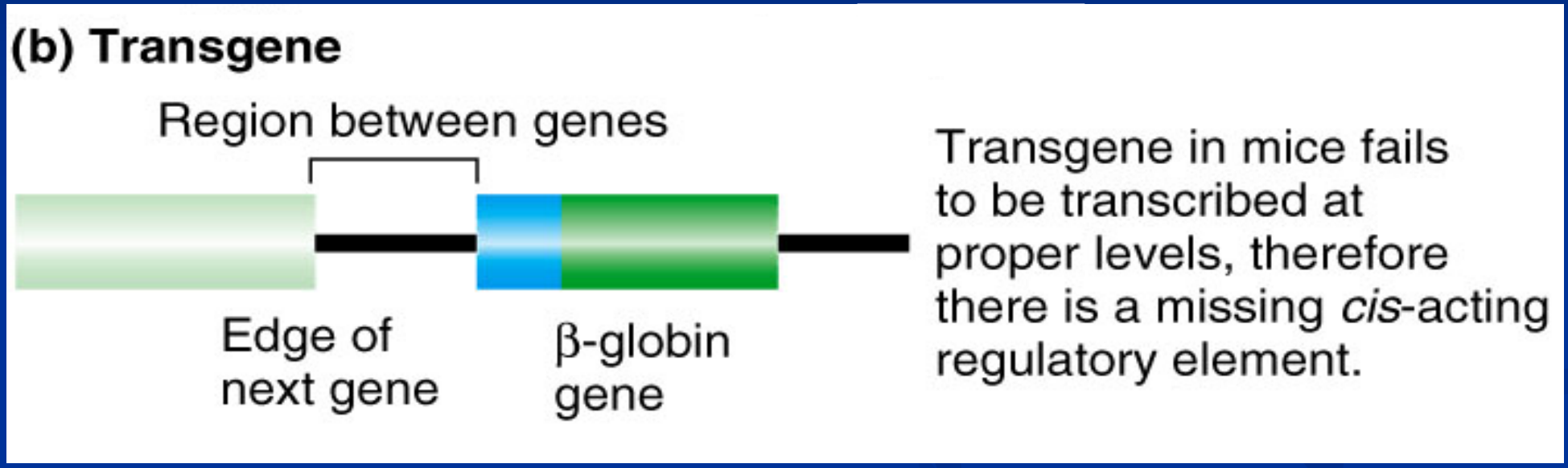


Chr. 11

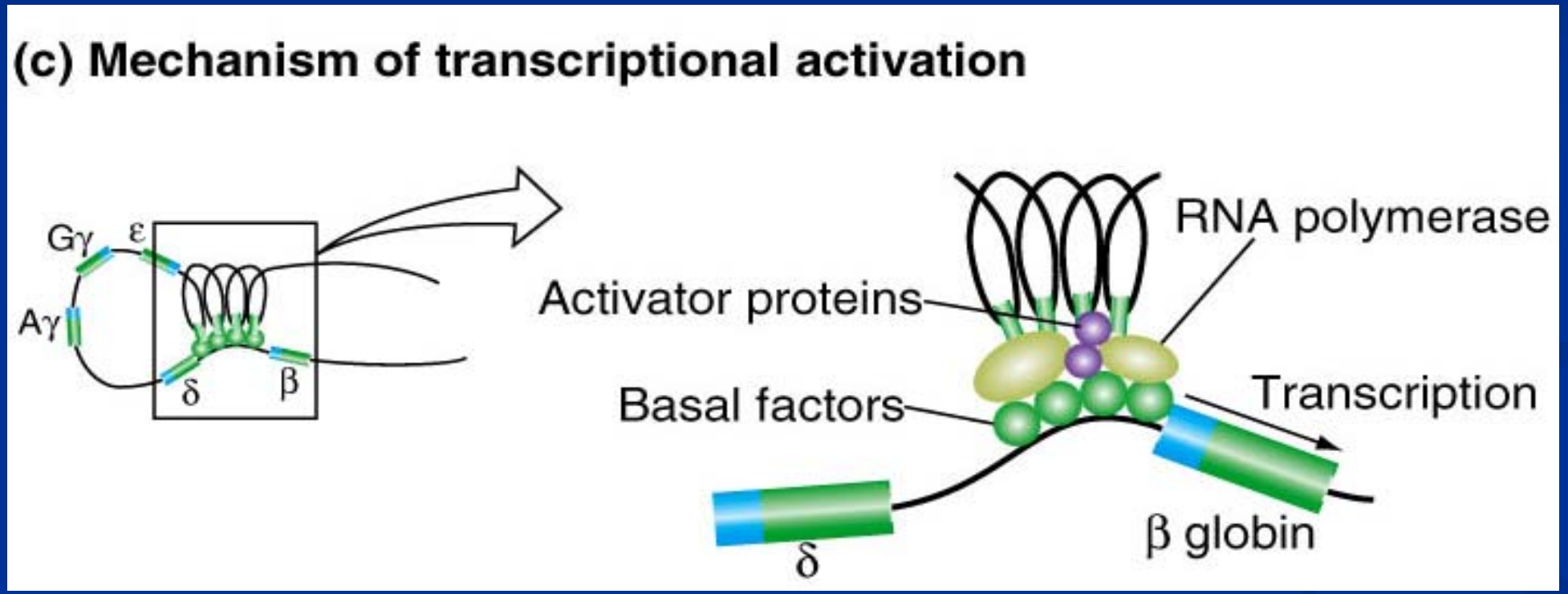

LCR HS 5 4 3 2 1
ε
Gγ
Aγ
δ
β
~15 kbp
~60 kbp
Globin gene expression during human development
embryonic yolk sac
fetal liver
adult bone marrow

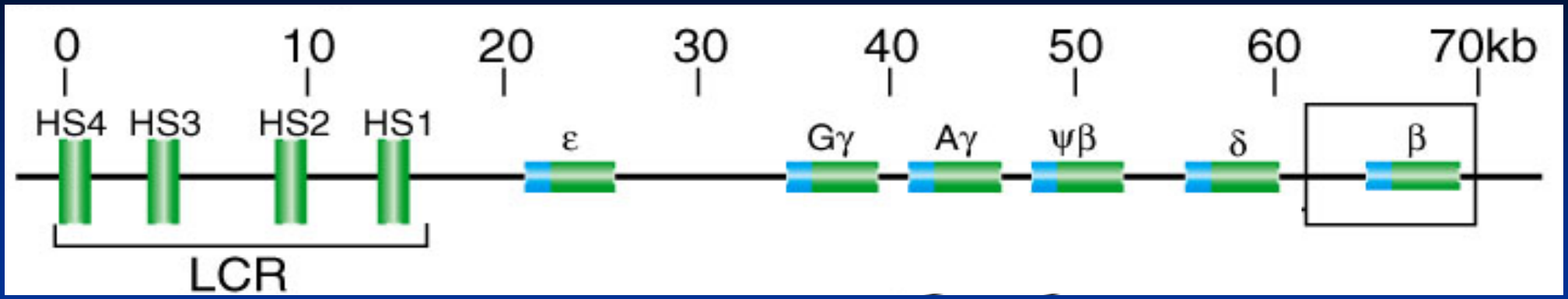

Proportion of different types of chains in hemoglobin protein
50
40
30
20
10
0
α
γ
ε
ζ
β
δ
0
6
12
18
24
30
36
Birth
6
12
18
24
30
36
42
48
Weeks of development
(c)

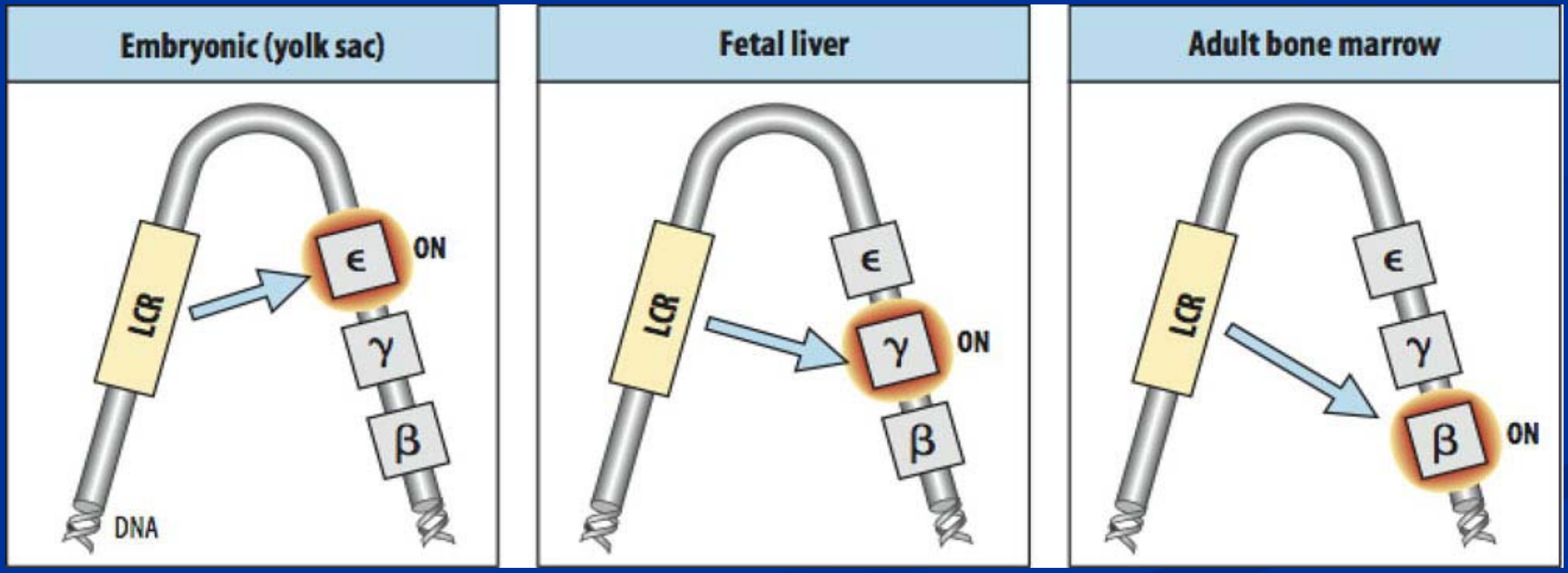
LCR is needed for activation of β-globin gene



LCR could effect activation of distant globin genes through DNA looping

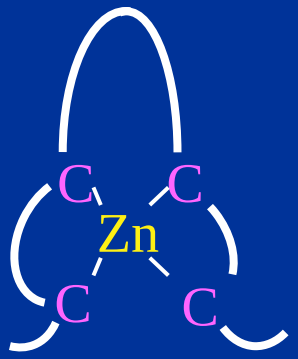



0
10
20
30
40
50
60
70kb
HS4
HS3
HS2
HS1
ε
Gγ
Aγ
ψβ
δ
β
LCR

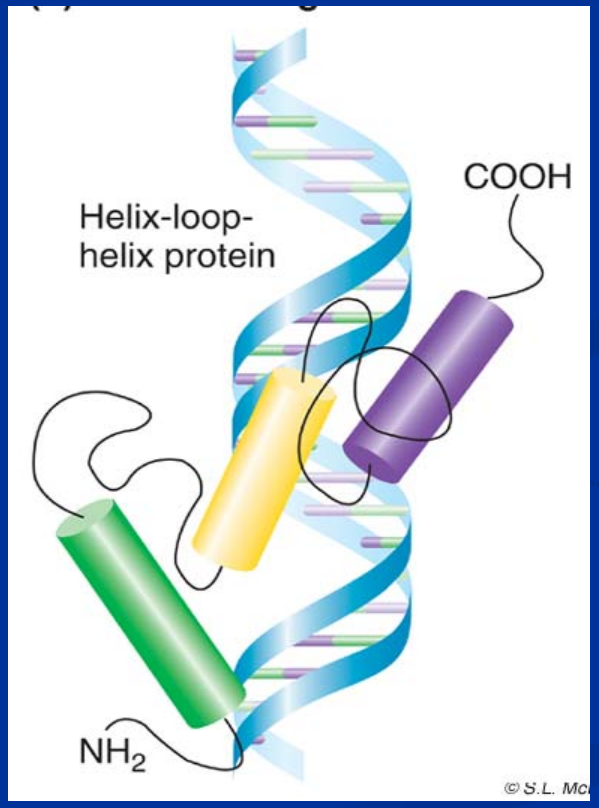

Embryonic (yolk sac)
Fetal liver
Adult bone marrow
LCR
ε
γ
β
ON
DNA

Common DNA-binding motifs of transcription factors

- Helix-loop-helix
- Helix-turn-helix
- Zinc-finger: found mostly in eukaryotes



Zinc finger



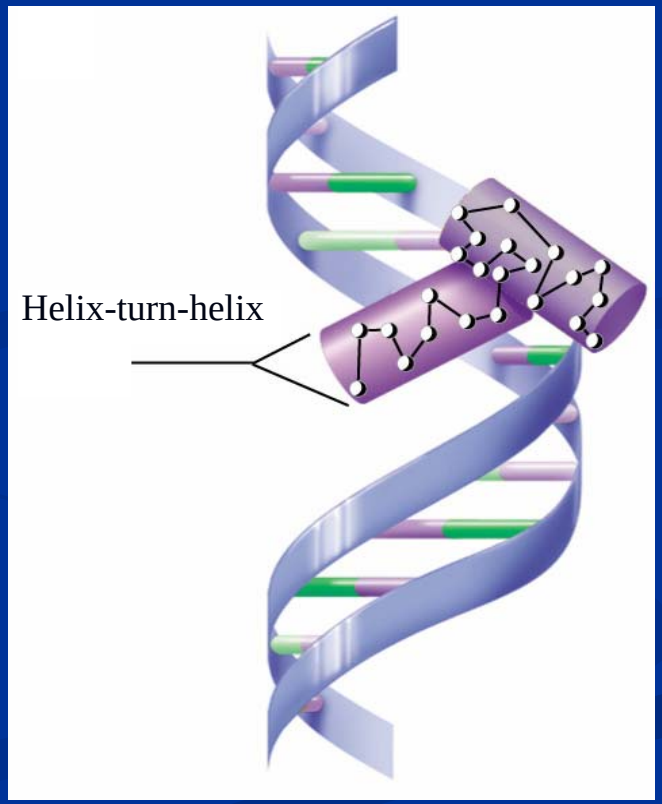


Fig. 16.7

Some activators have a third domain that is responsive to specific signals from the environment

- **Coactivator: Proteins or other molecules that play a role in transcriptional activation without binding directly to DNA.**

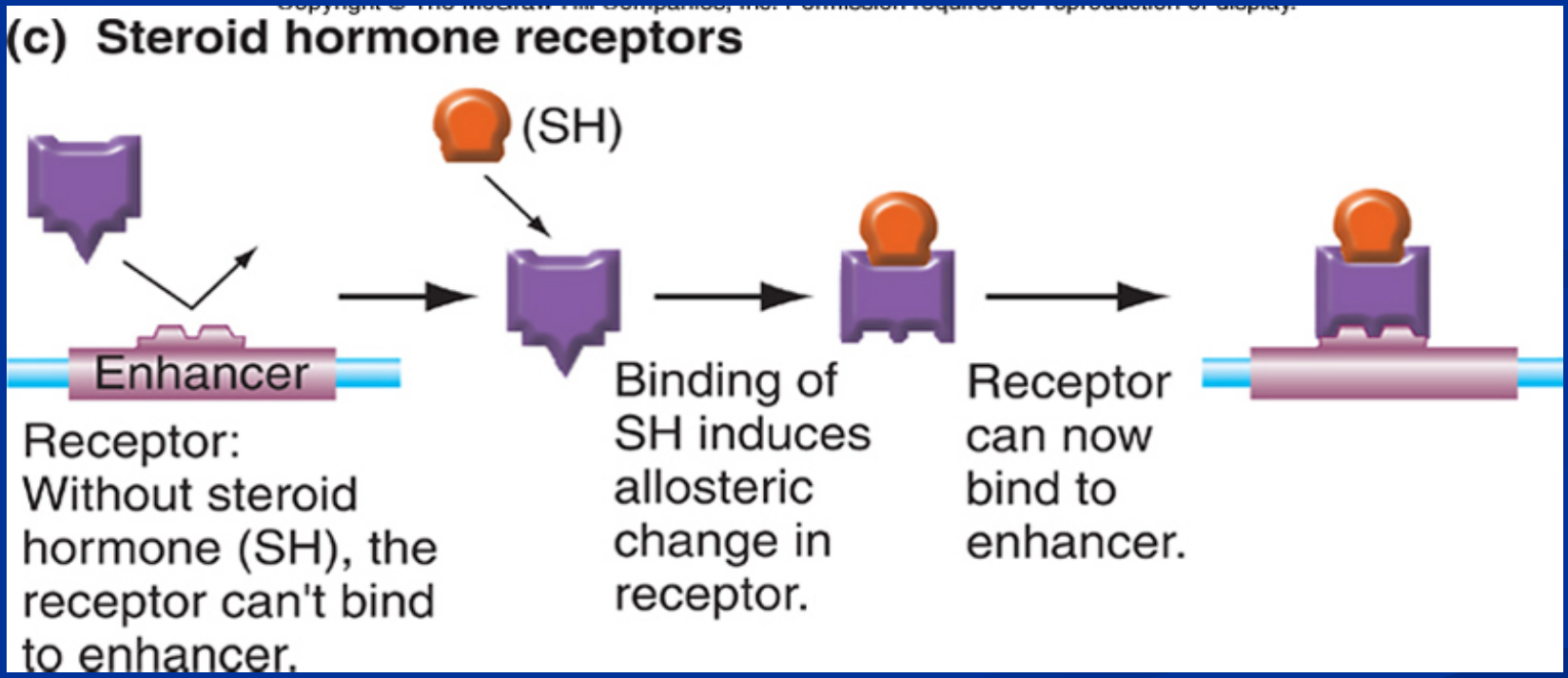


Fig. 16.14

Most transcription regulators are multimeric proteins

- **Homomers** – multimeric proteins composed of identical subunits.
  - Jun-Jun homodimer
- **Heteromers** – multimeric proteins composed of nonidentical subunits
  - Jun-Fos heterodimer

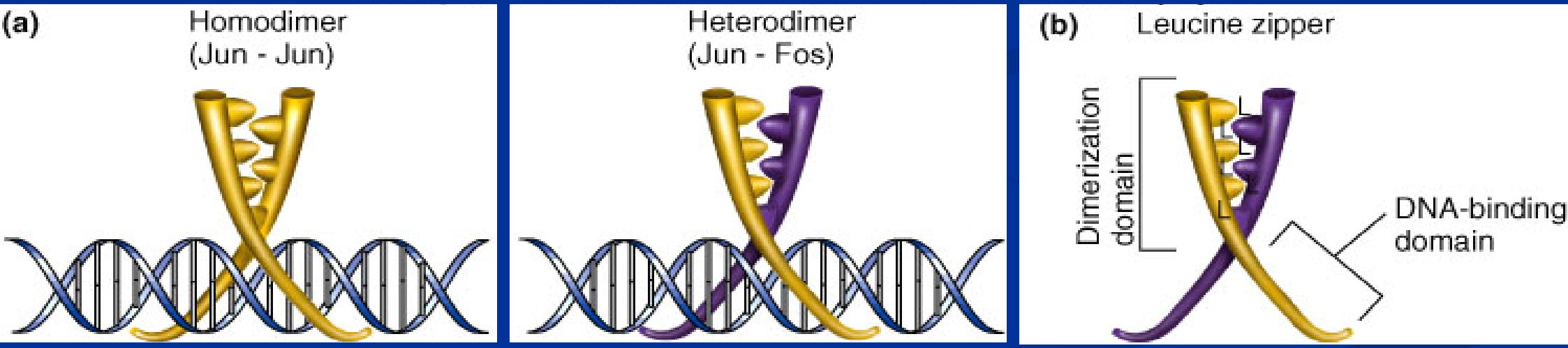


Fig. 16.8

Repressors

- **Repressors**: Transcription factors that bind to specific cis-acting elements and thereby diminish or prevent transcription.
  - Some repressors can interact with RNA pol II basal complex and prevent it from binding the promoter.
  - Other repressors are enzymes such as histone deacetylases (HDACs) or histone methyl transferases (HMTs) that modify histone tail amino acids, resulting in closed chromatin.

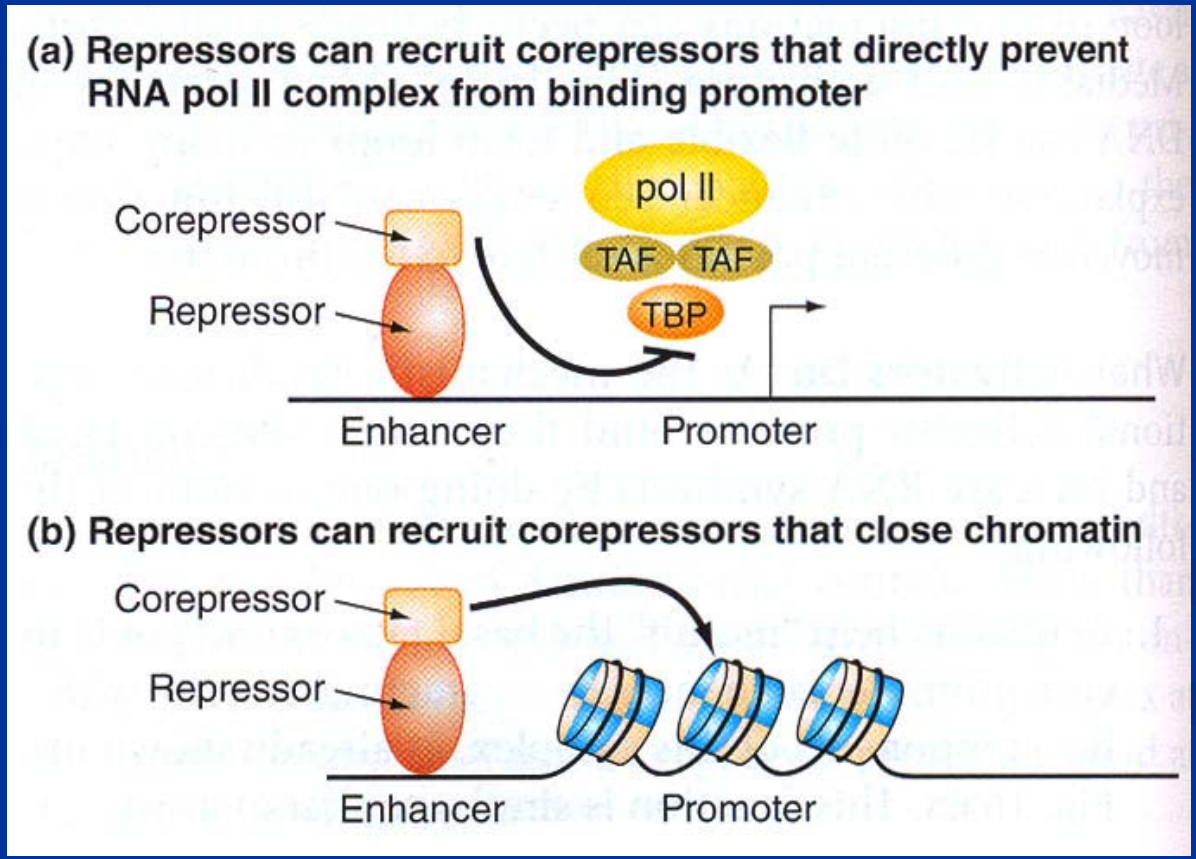


Fig. 16.9

Indirect repression

- **Indirect repressors:** Proteins that interferes with the function of an activator without binding DNA.

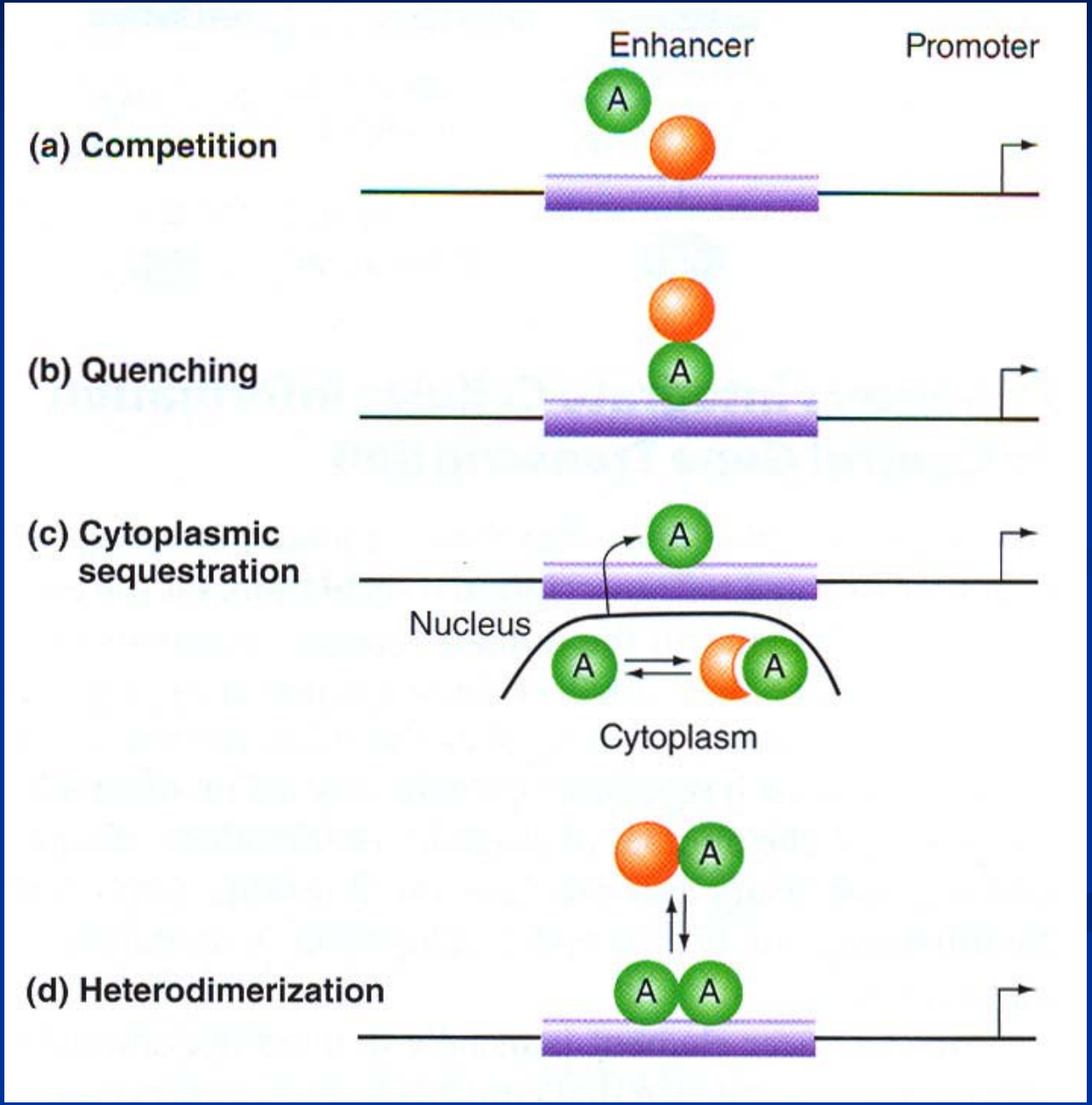


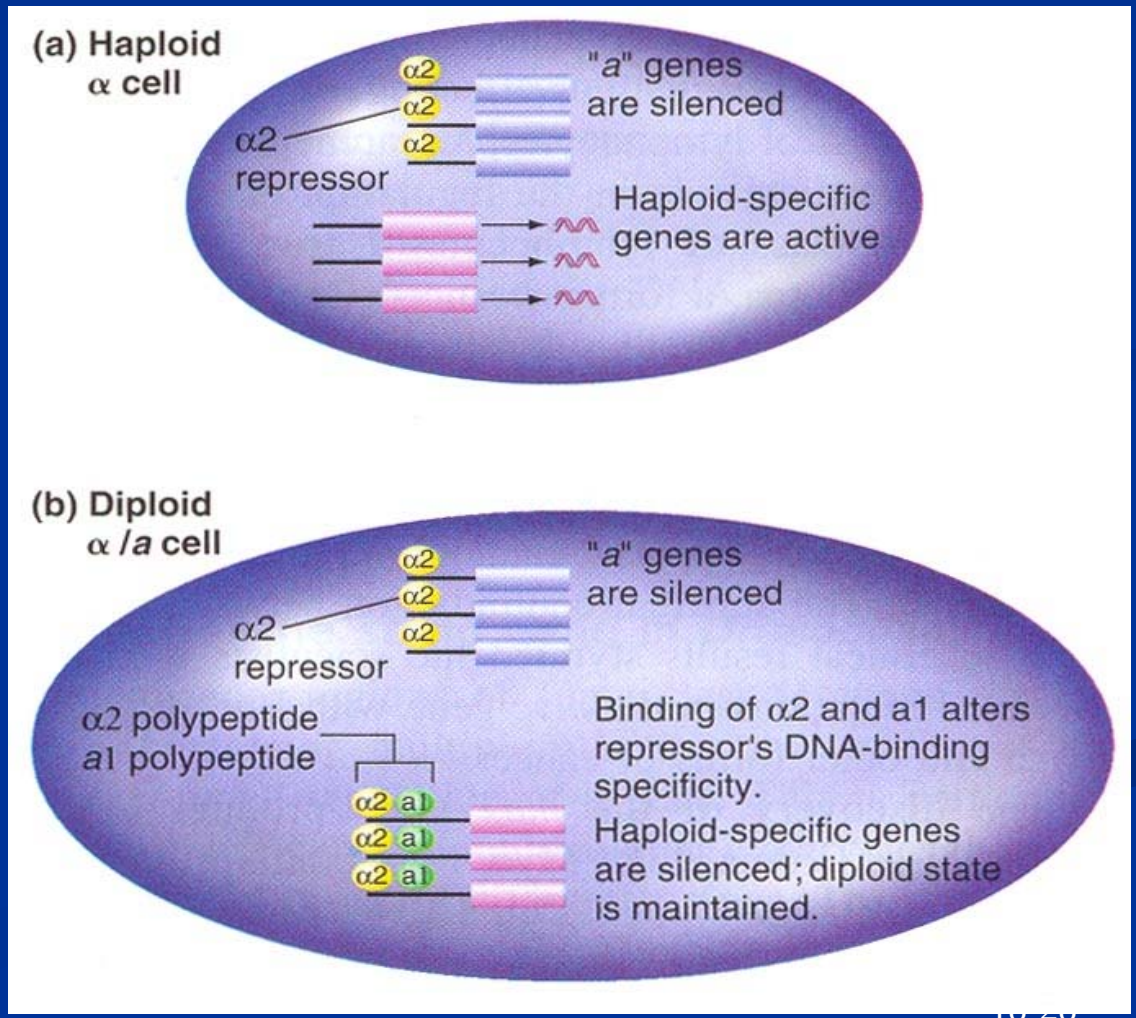
Fig. 16.11

Transcription factors may act as activators or repressors

- Action of transcription factor depends on:
  - Cell type, the presence of interacting proteins.
  - Gene that it is regulating, promoter sequence.

Yeast α2 repressor determines mating type of α cell.

- Haploid – α2 factor silences the set of "a" genes.
- Diploid – α2 factor dimerizes with a1 factor to silence haploid-specific genes.



Insulators organize DNA to control enhancer/ promoter interactions

- **Insulator: A transcriptional regulation element in eukaryotes that stops communication between enhancers on one side of it with promoters on the other side.**

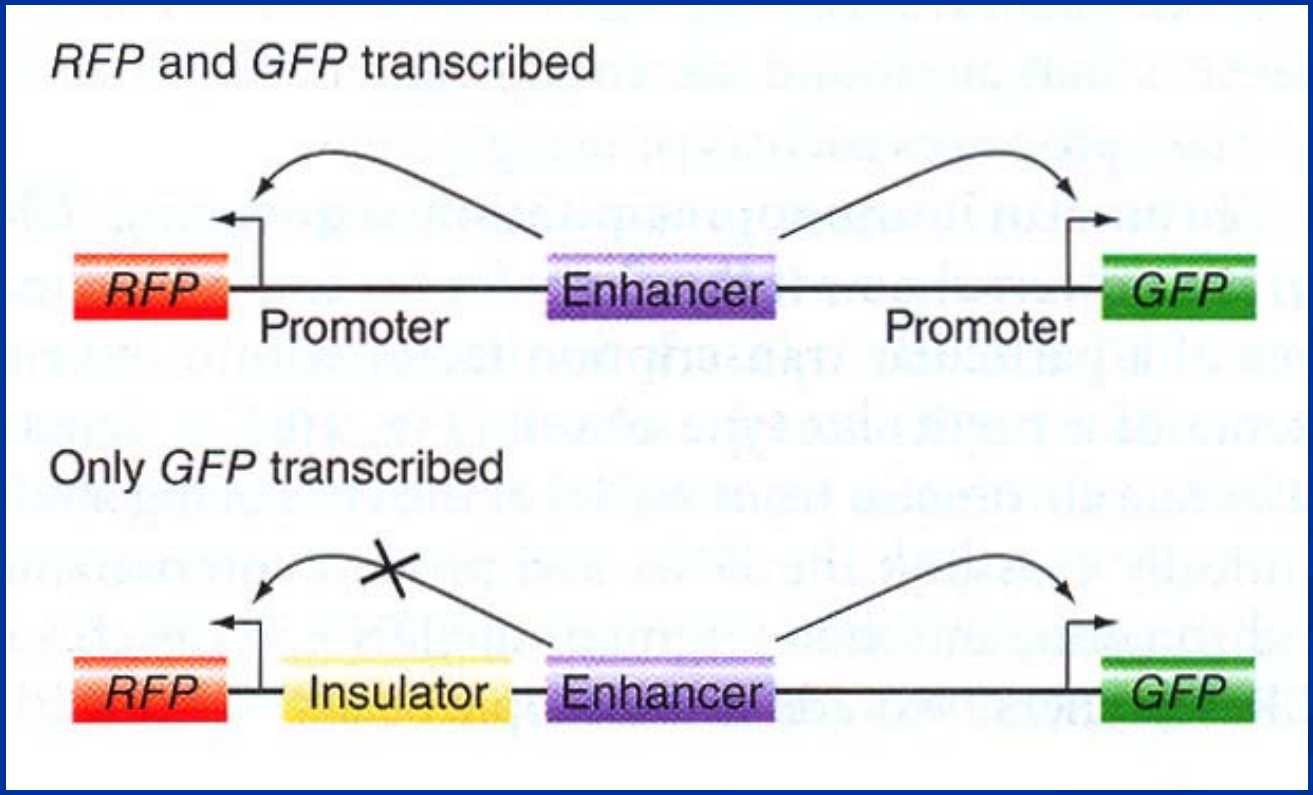


Fig. 16.15

How insulators work?

- Insulators bind a protein called CTCF (CCCTC-binding factor) and organize genomic DNA into loops. A promoter and an enhancer can not interact with each other if an insulator lies between them.
- The functions of some insulators are more complex than simply blocking enhancers.

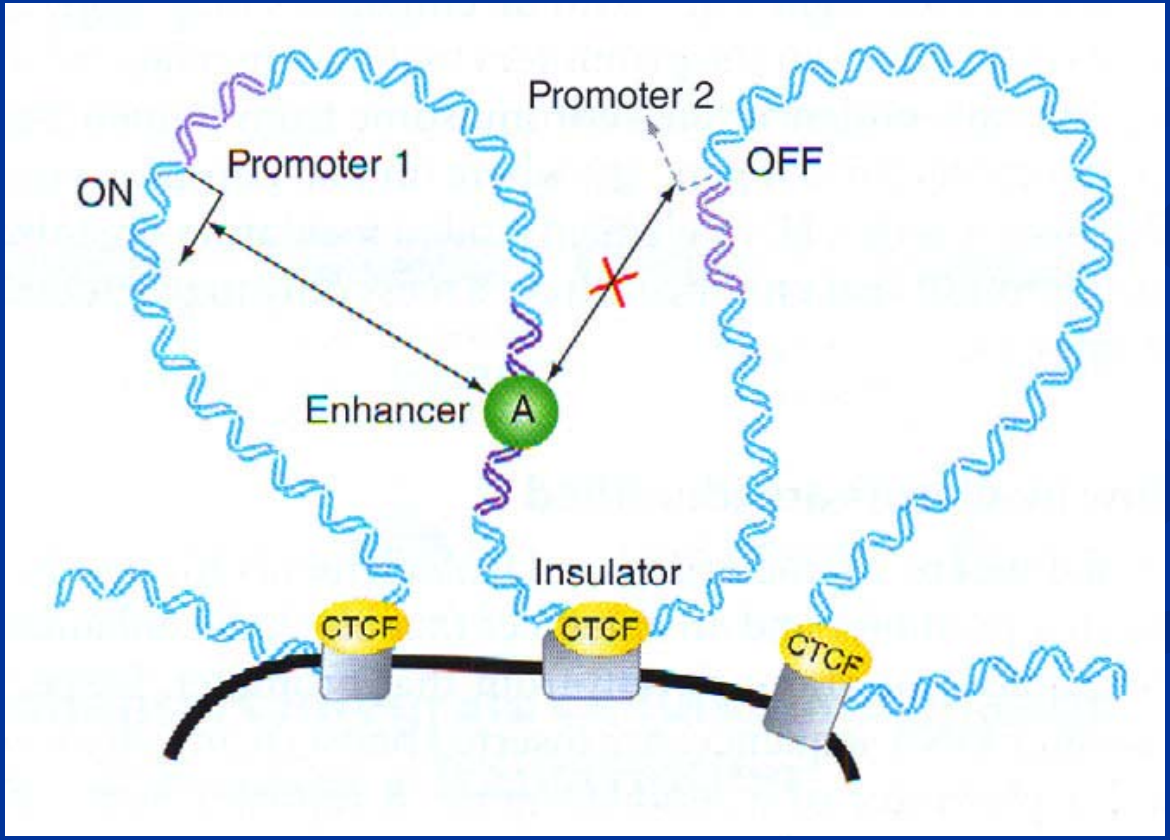
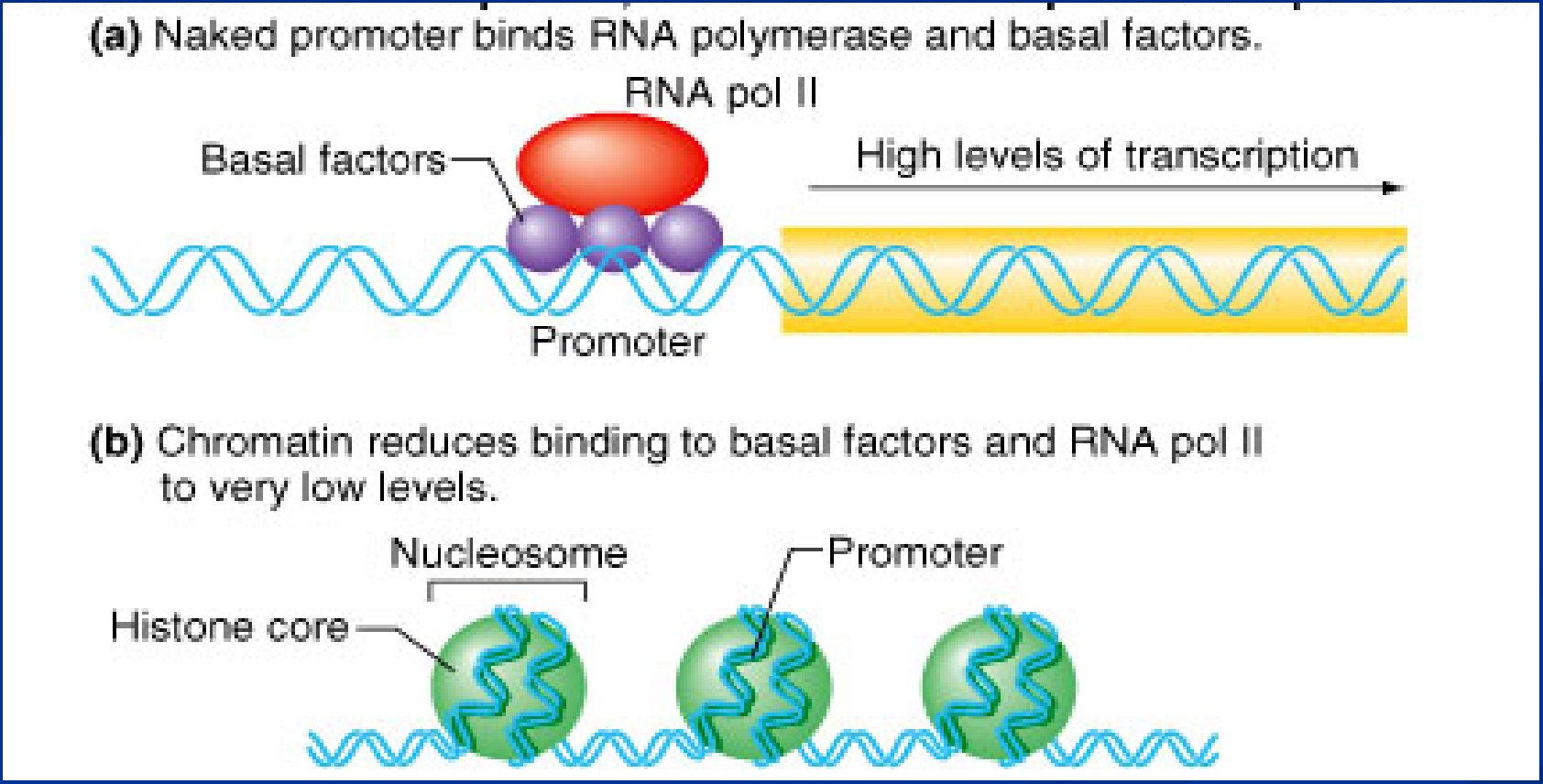


Fig. 16.16

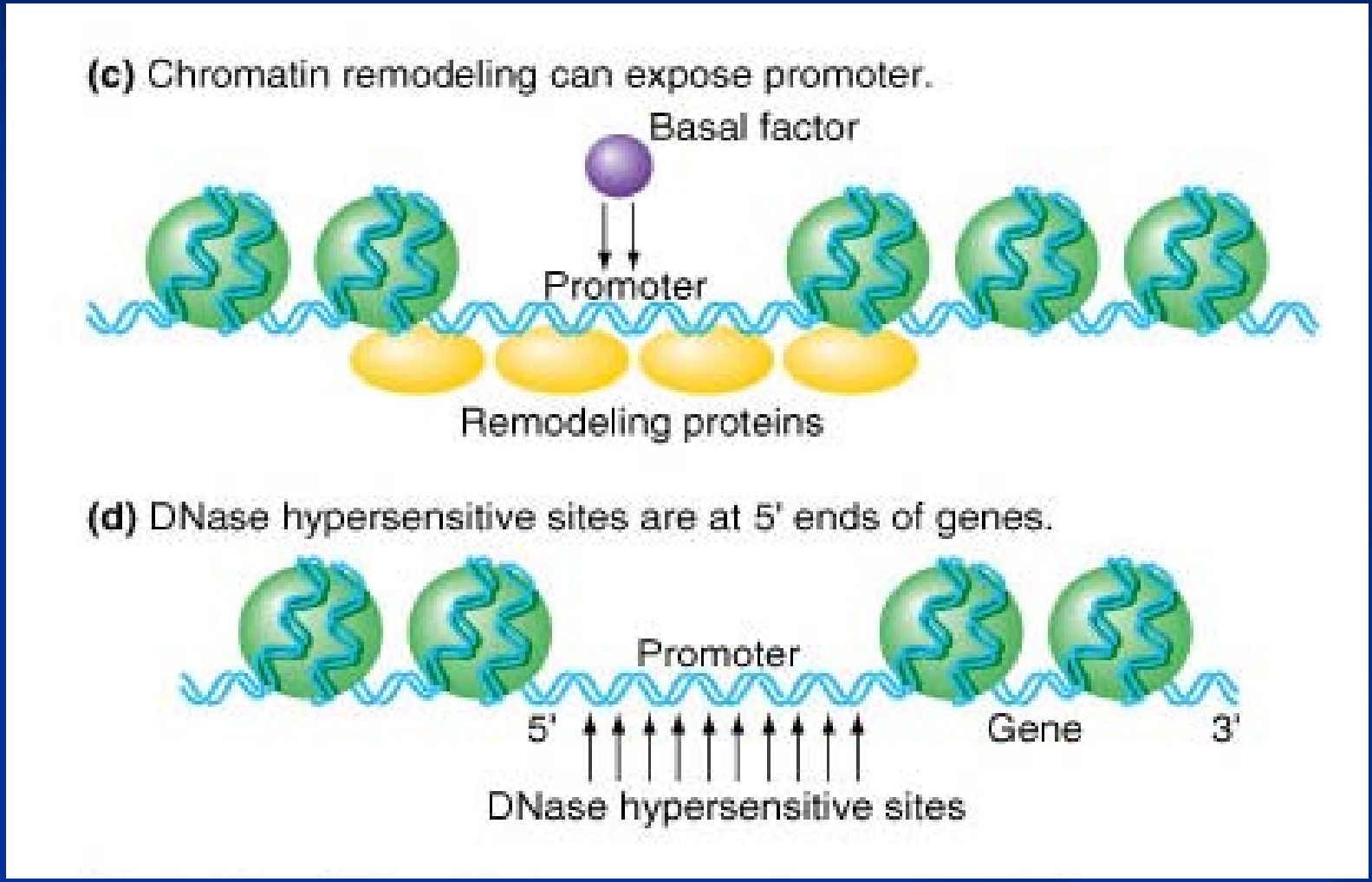
16.3 Epigenetics: Control of transcription initiation through DNA methylation

Chromatin structure reduces basal transcription.



Nucleosomes sit atop the promoters of most inactive genes.

Remodeling of chromatin mediates the activation of transcription.



DNA methylation at CpG islands silence gene expression.

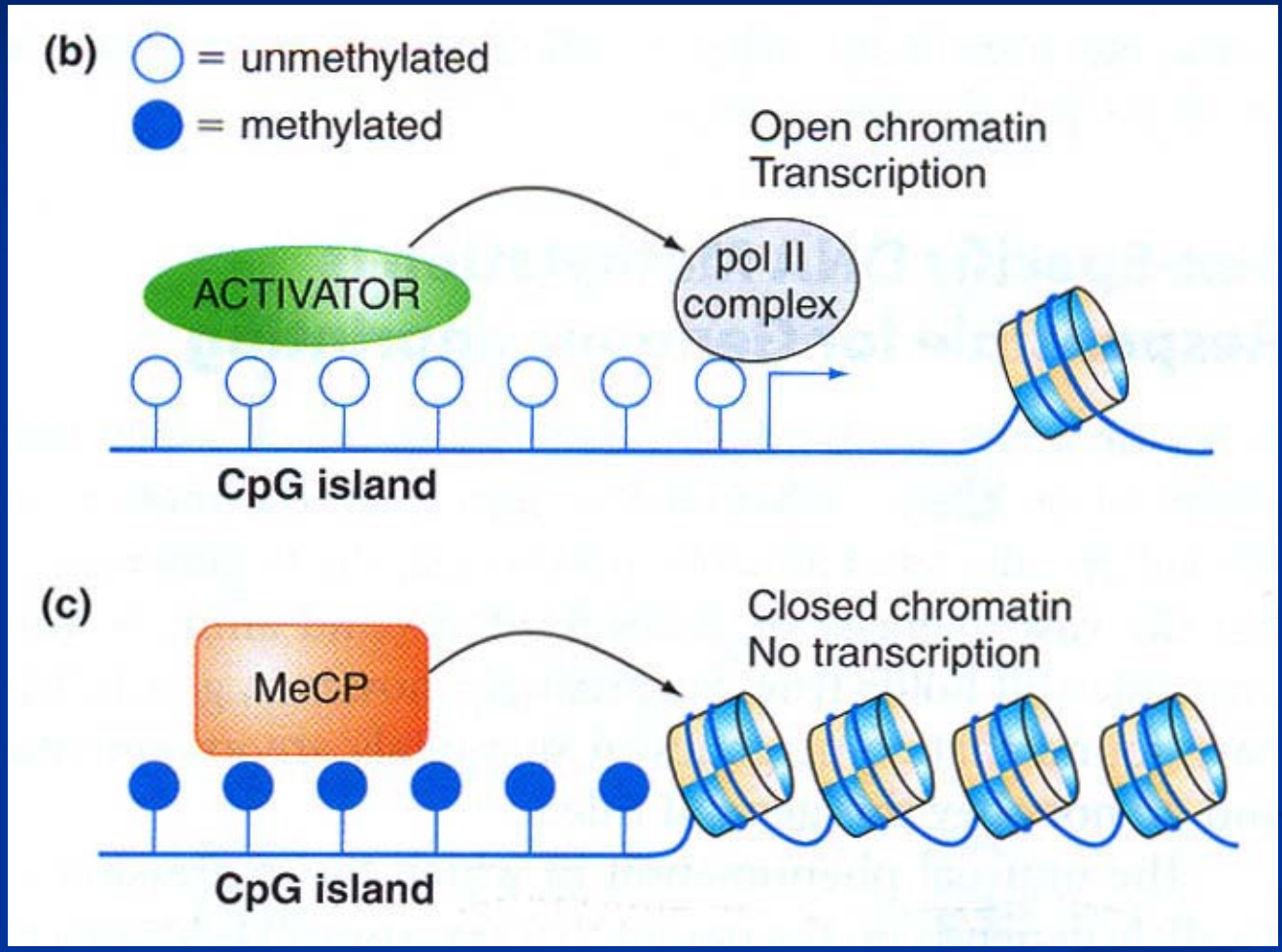
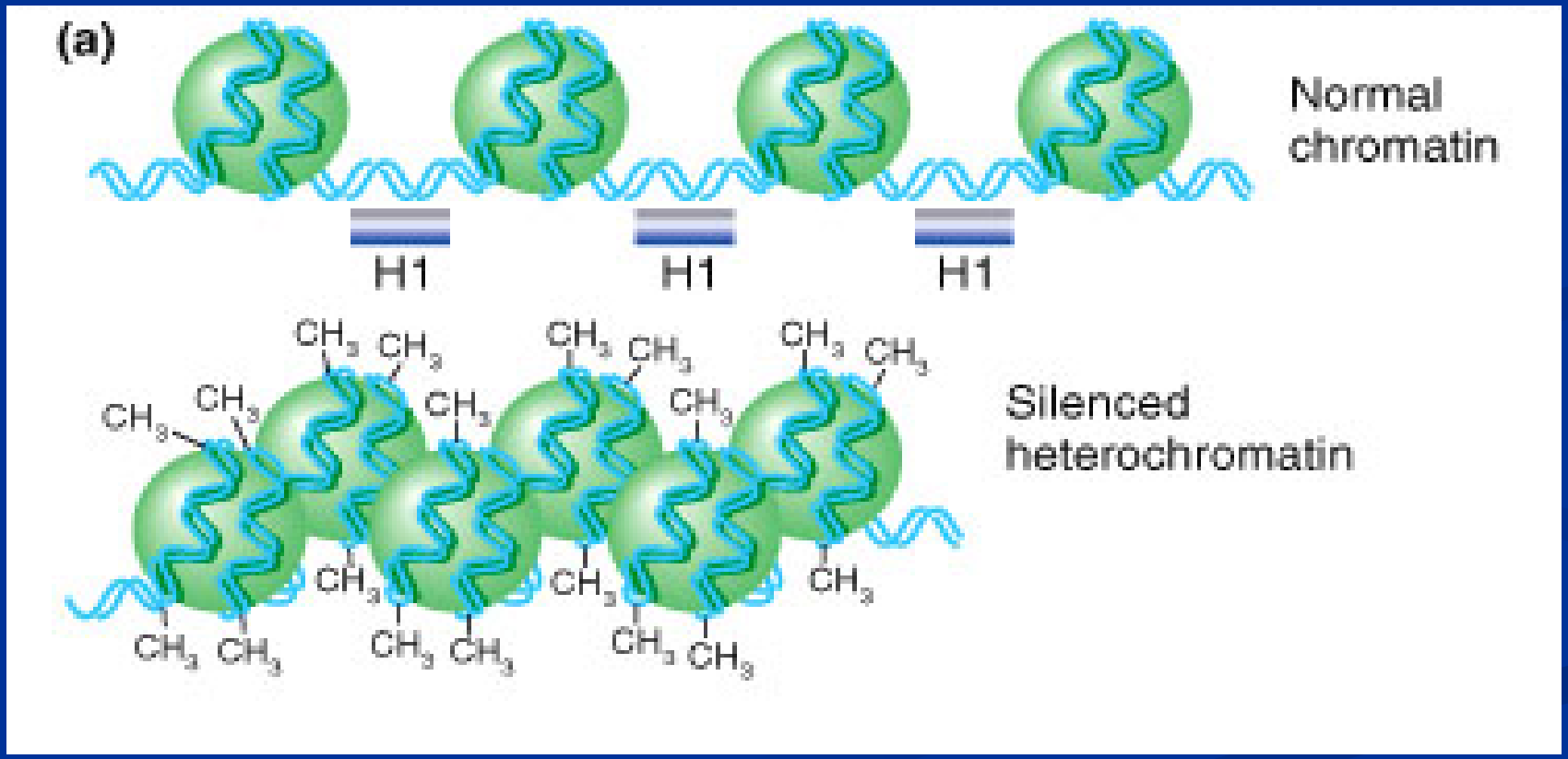


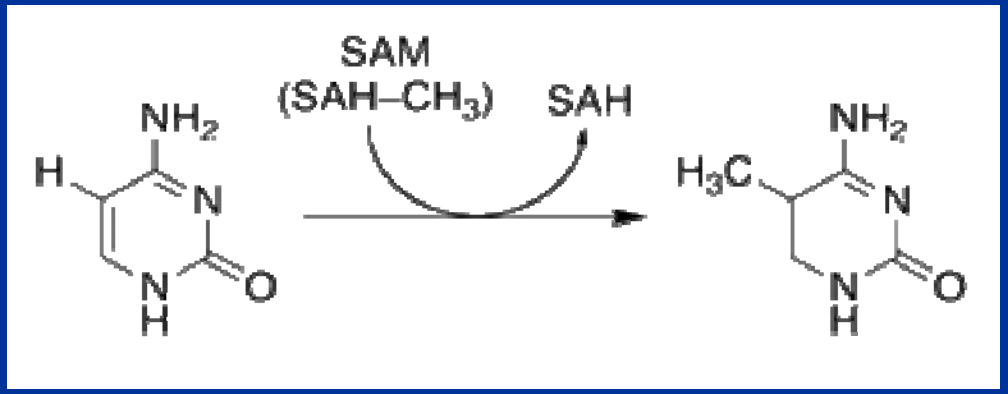
Fig. 16.18b, c

Hypercondensation over chromatin domains causes transcriptional silencing

- Heterochromatic regions on the chromosome are characterized by modified histone H3 and methylated CpG dinucleotide in DNA.



- **DNA methylation is particularly important to the control of the expression of housekeeping genes in vertebrates. It also plays a role in regulating some cell-type-specific genes.**
  - **In the human genome, ~70% of the C residues in CpG dinucleotides are methylated.**



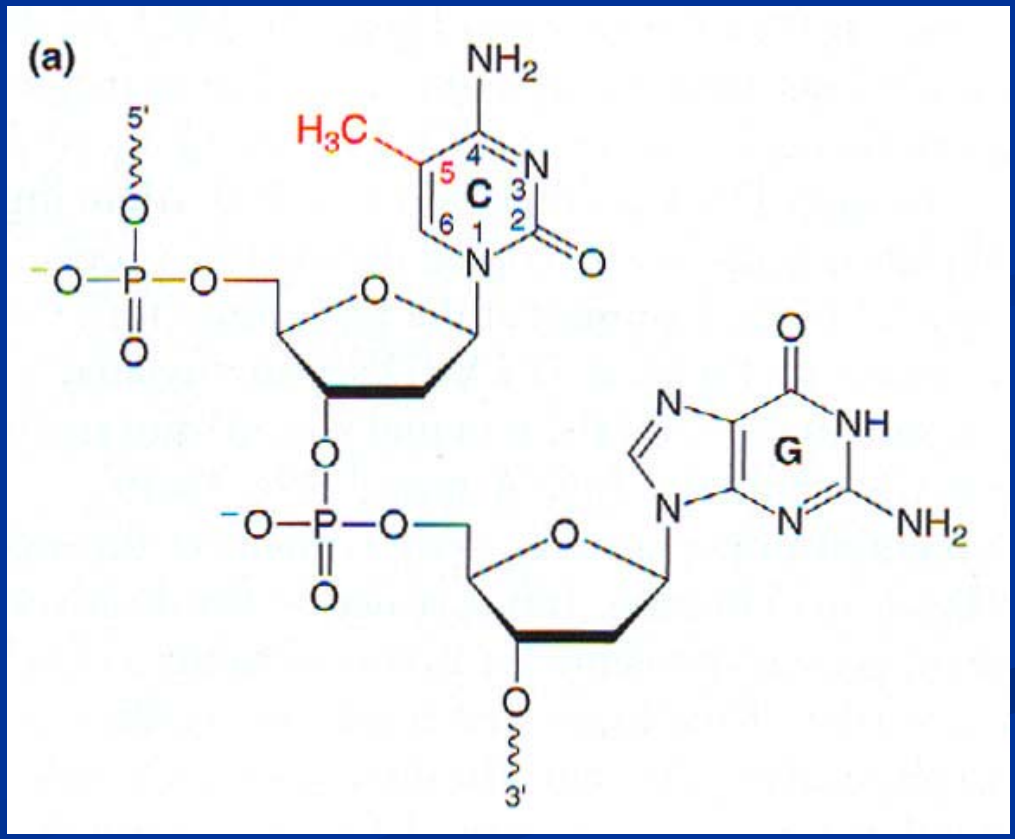


Fig. 16.18a

- **DNA methylation patterns are copied during DNA replication by a special DNA methyl transferase (DNMT) at the replication fork that recognizes hemi-methylated DNA and methylates the newly synthesized DNA strand.**

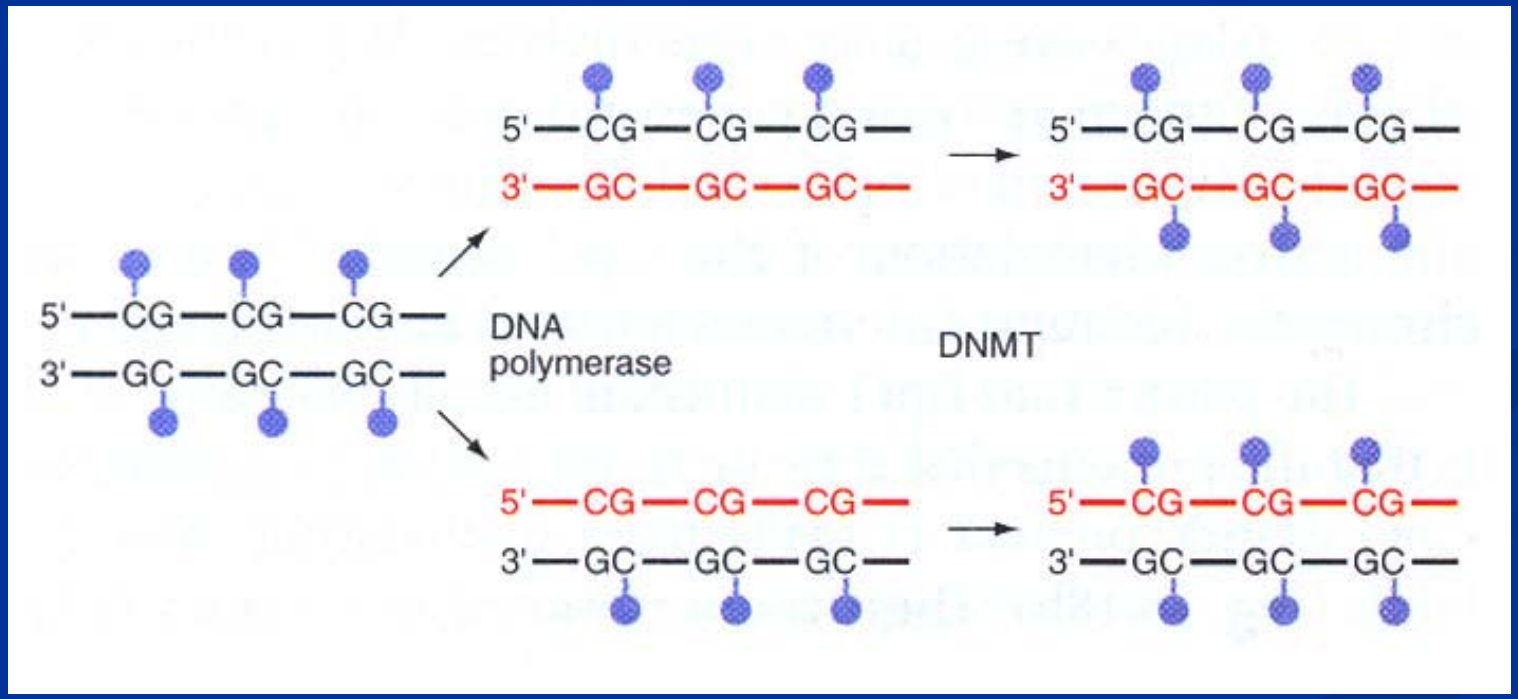
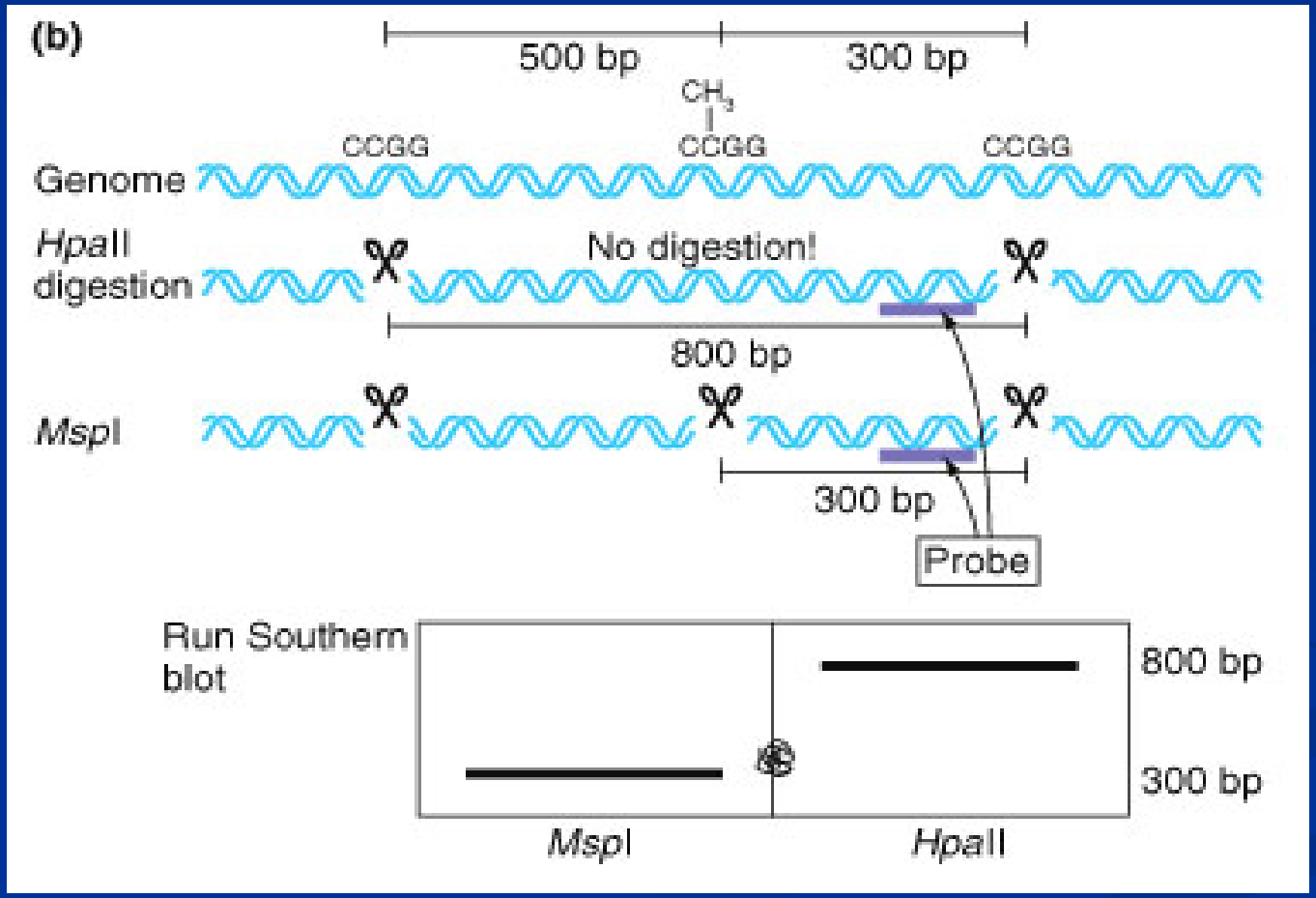


Fig. 16.19

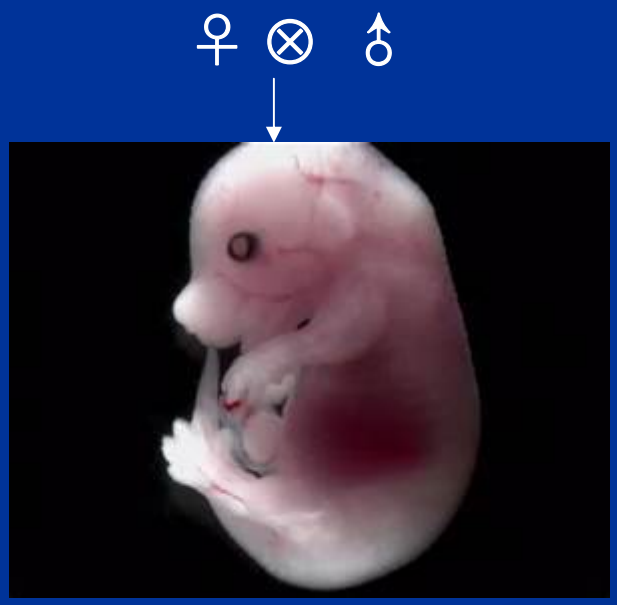
Determine the methylation state of CCGG using restriction enzymes

- ***Msp*I cleaves CCGG and CC*GG; *Hpa*II cleaves CCGG but not CC*GG.**

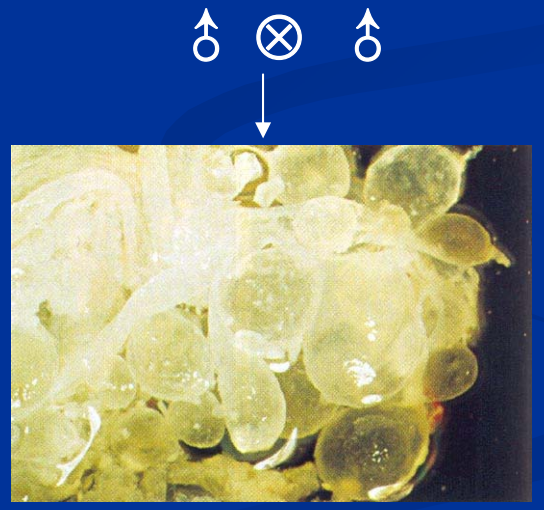


Sex-specific DNA methylation is responsible for genomic imprinting

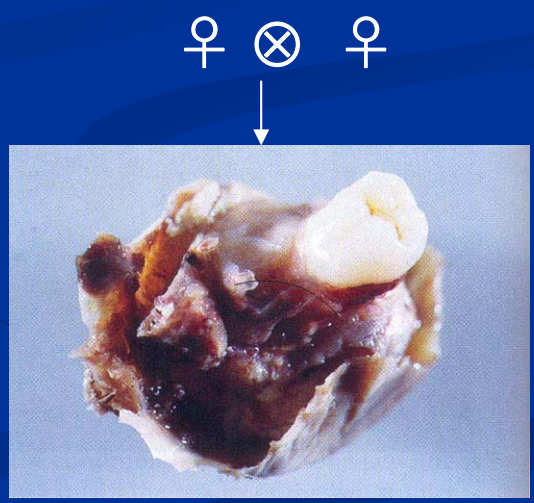
1. In the 1980s, *in vitro* fertilization experiments demonstrated:
   - Mouse embryos with one maternal pronucleus and one paternal pronucleus can develop into normal, viable, and fertile adult mice.
   - Mouse embryos with pronuclei from two females or two males could not develop normally.



Normal embryo

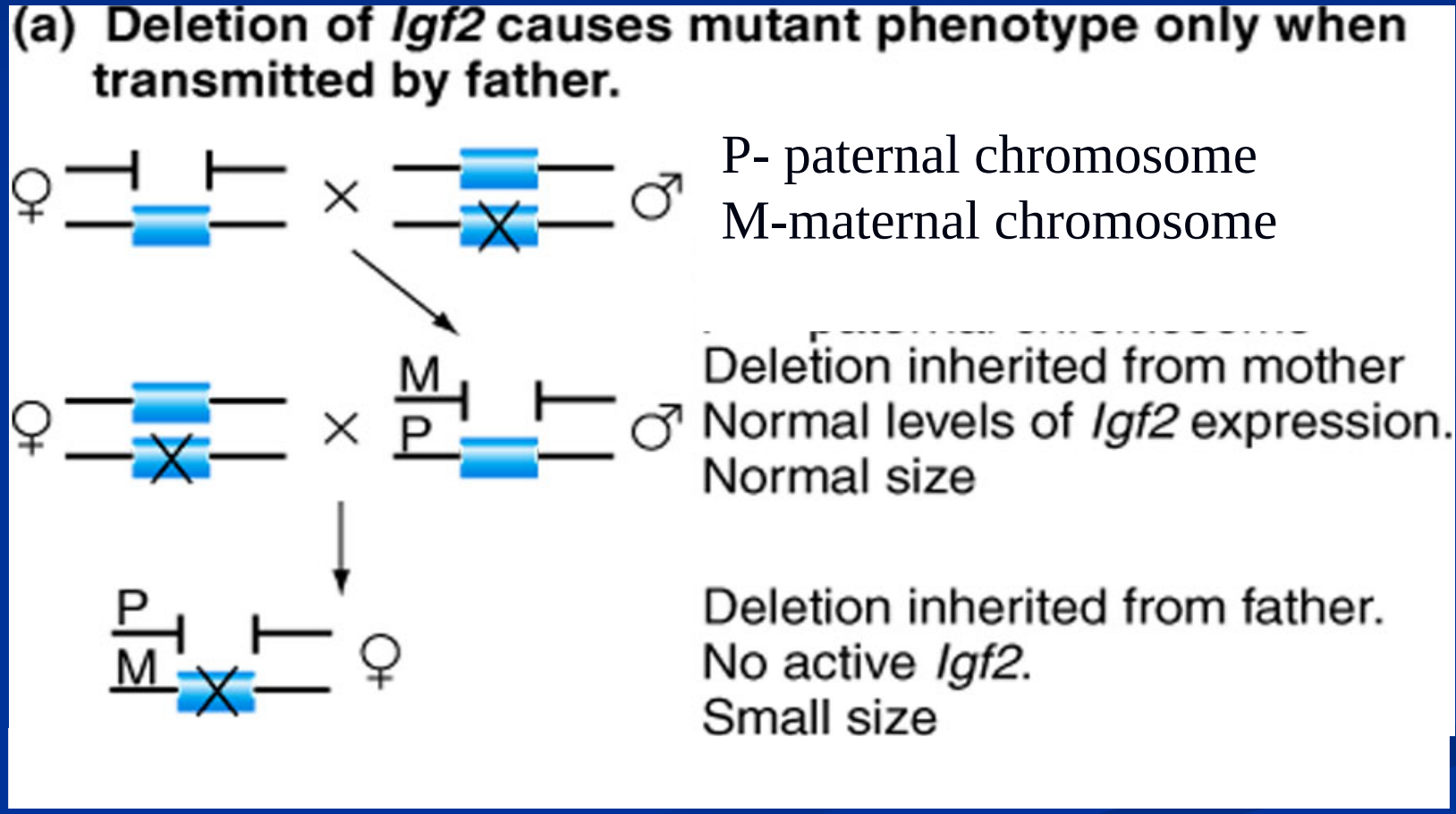


Hydatidiform mole
(葡萄胎)



Teratoma
(畸胎瘤)

2. Mice inheriting *Igf2* deletion from father were weighing 40% below average whereas mice inheriting deletion from mother were normal.



- **Genomic imprinting (基因组印记): The phenomenon in which the expression of an allele depends on the parent that transmits it.**
- **DNA methylation is involved in genomic imprinting..**
  - **Methylation of cytosine in CG dinucleotides within imprinted region.**
  - **Methylated C's silence genes in the region by preventing RNA polymerase and other transcriptional factors from binding to DNA.**

Genomic imprinting and human disease

- For sex-linked genetic traits that are imprinted, the sex of the individual inheriting a mutant allele does not matter. The parental origin of the mutant allele matters.
- Incomplete penetrance may reflect genomic imprinting.

Some individuals inherit the mutation but do not develop into disease.

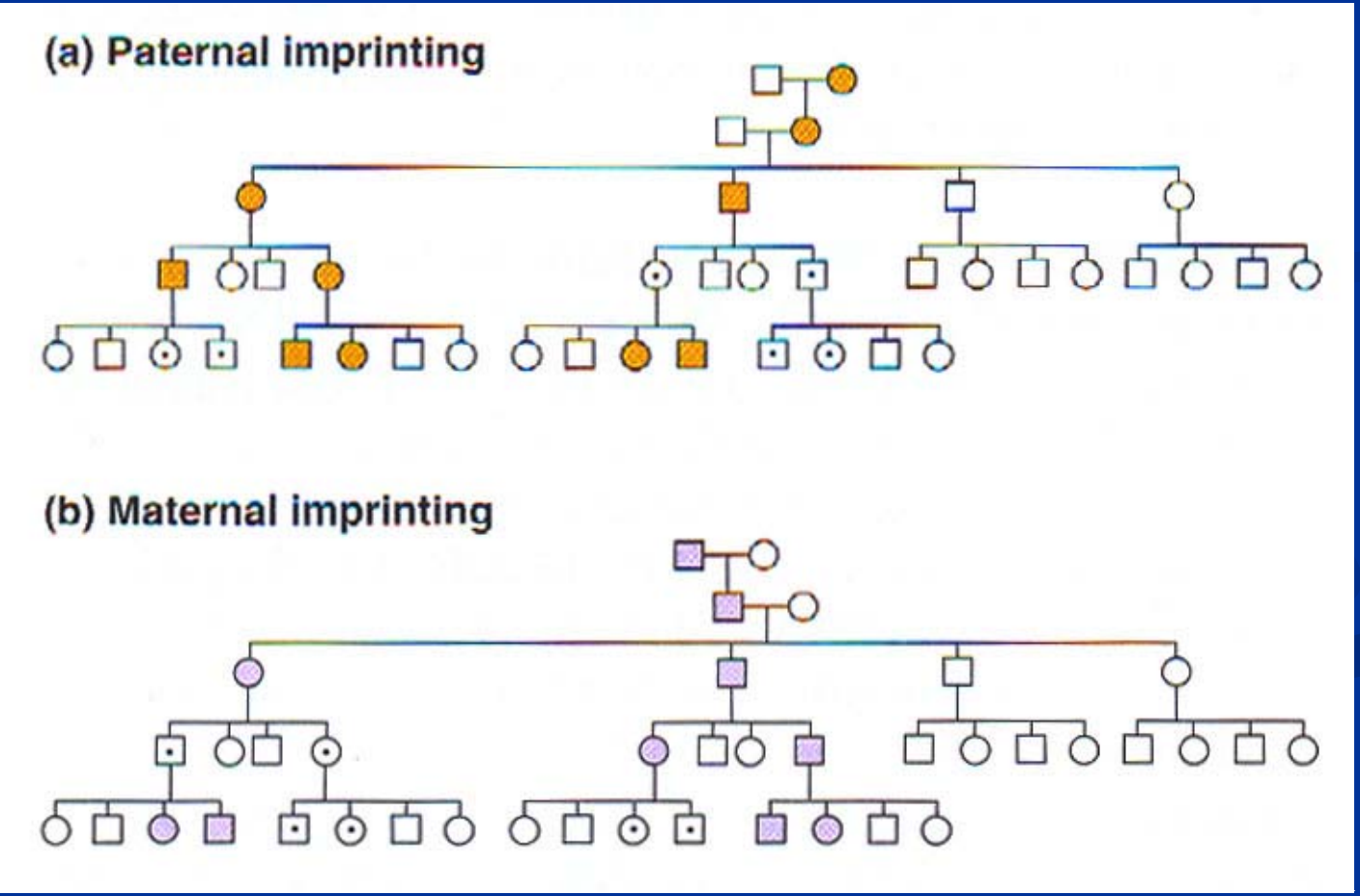


Fig. 16.20

Genomic imprinting is an epigenetic phenomenon

- **Epigenetic (表观遗传的):** A state of gene functionality that is not encoded within the DNA sequence but that is still heritable from one generation to the next.
- Genomic imprinting are reset during meiosis.

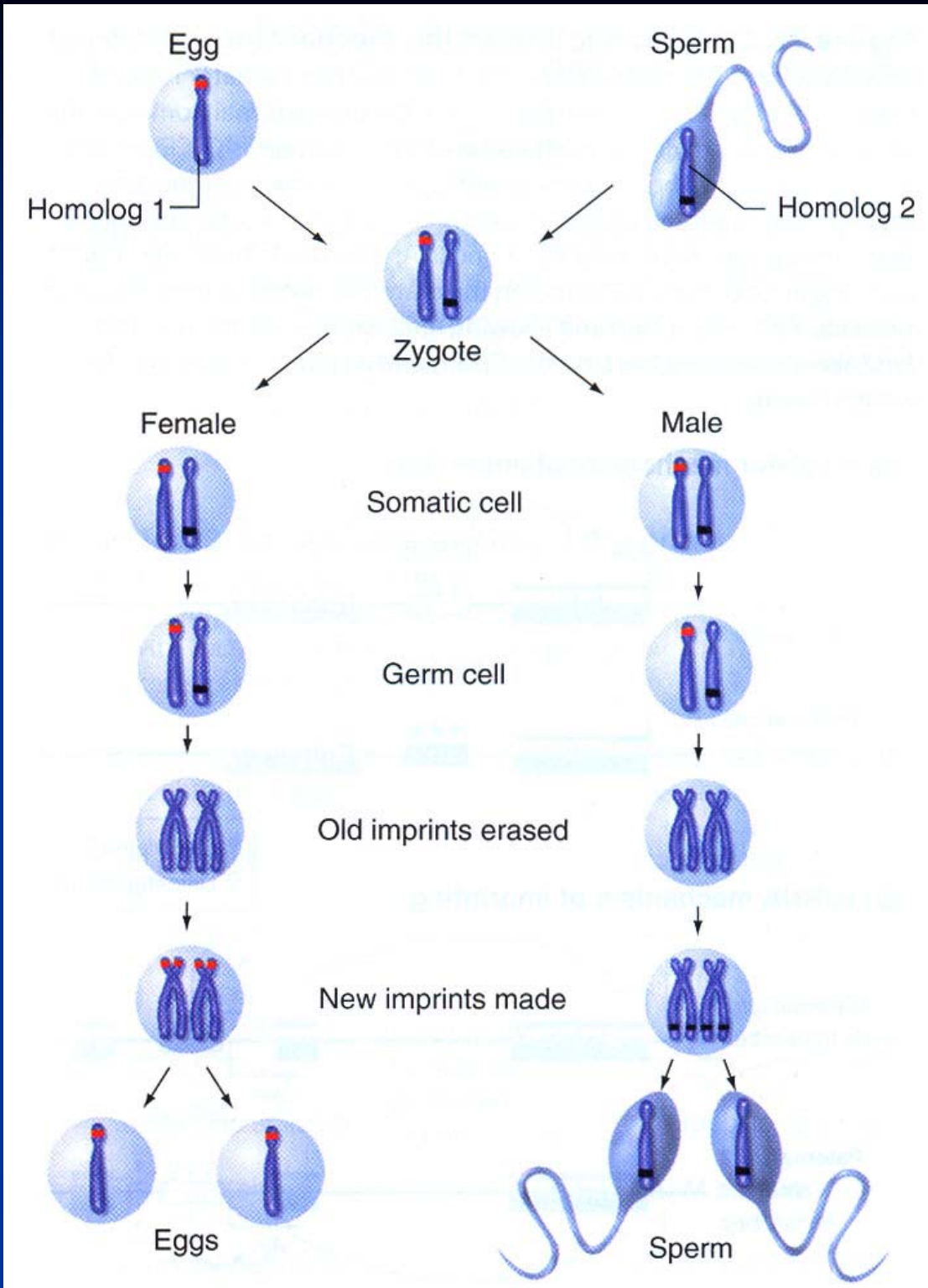


Fig. 16.21

How genomic imprinting works?

- **Insulator mechanism**
  - An **insulator** bound to CTCF blocks the enhancer to interact with *Igf2* promoter.
- **Noncoding RNA (ncRNA) mechanism**
  - In the vicinity of some imprinted genes, the imprinting control region (ICR) encodes an ncRNA whose transcription is controlled by a CpG island. The ncRNA suppresses the expression of the imprinted genes.

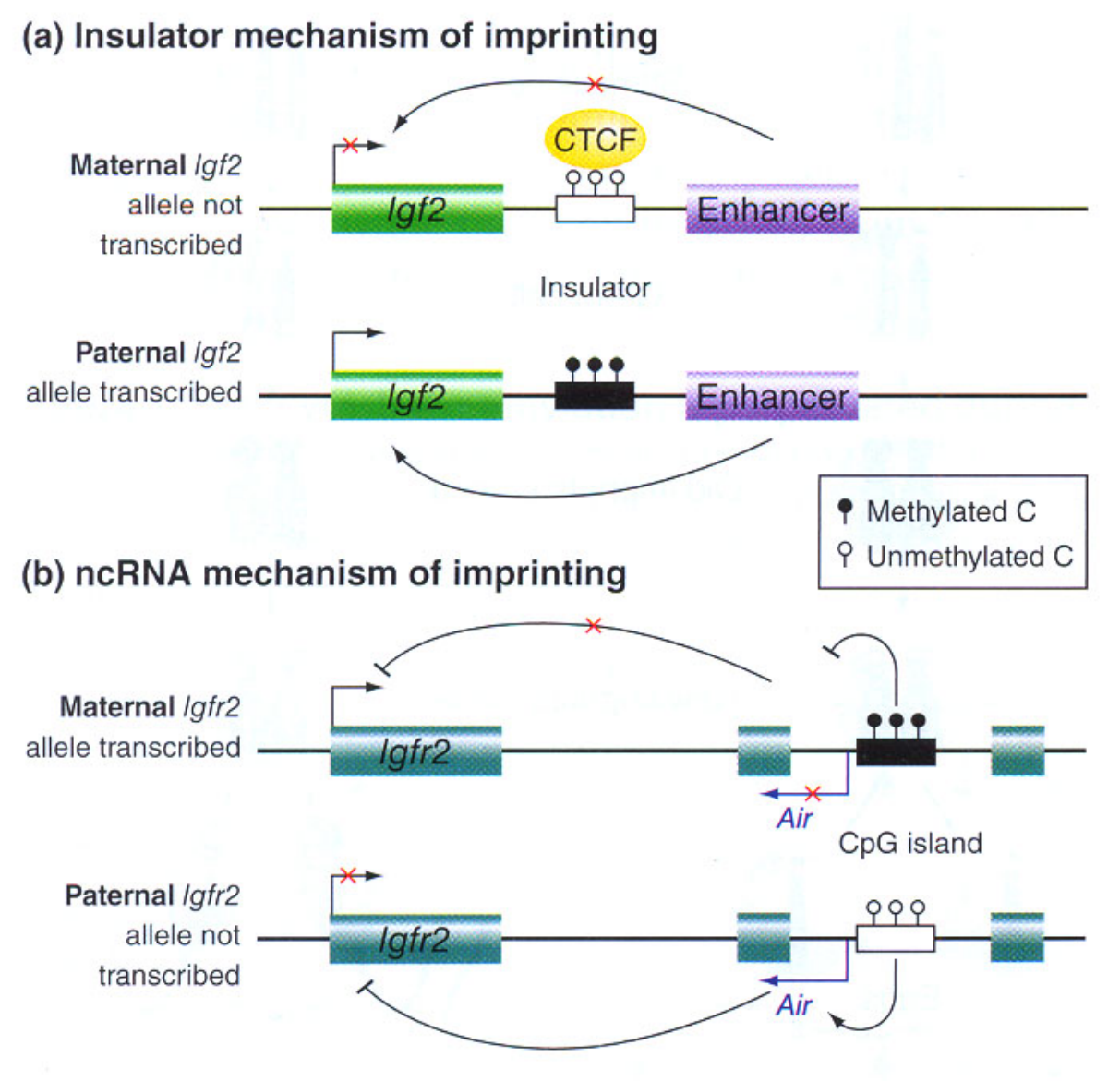


Fig. 16.22

Genomic imprinting is unique to mammals

- ~ 100 of approximately 25,000 genes in the human genome exhibit imprinting.
- Among vertebrate animals, only mammals imprint their genomes.
- Many of the imprinted genes uncovered to date are involved in regulating embryonic growth.
- Most genes imprinted in one mammalian species are also imprinted in other mammal species.

Why imprinting?

The Haig hypothesis

- In 1991, David Haig and colleagues.
- To explain how genomic imprinting get to evolve in mammals.

- **Why in mammals?**
  - **Embryos develop inside mother's body. Conflicting interests in transferring nutrients from mother to embryos.**
  - **Polygamous – A female mammal can mate with several males and generate multiple embryos fathered by different males.**

The incentive:

- **From the mother side: Keep embryonic growth under control.**
- **From the father side: The embryos sired by him should grow faster than other embryos even at the expense of the mother.**

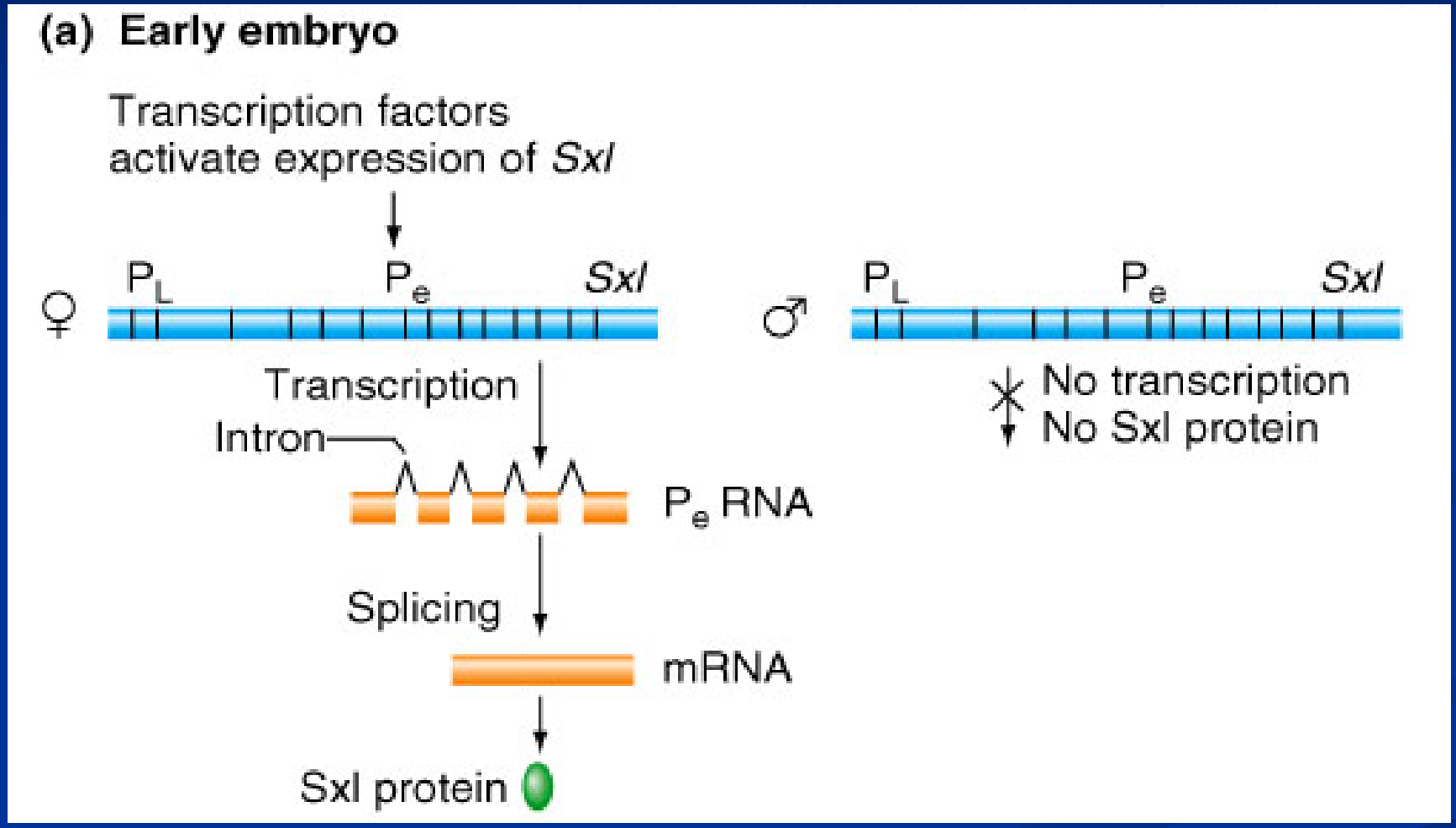
The hypothesis:

- **Maternal genes** that normally enhance fetal growth would be down-regulated.
  - The genes that a fetus receives from its mother are most interested in mom's survival and reproduction in the future.
- **Paternal genes** that normally promote embryonic growth should be active to help a fetus to outgrow its half-sibling fetus.
  - Genes that a fetus receives from its father are most interested in getting more resources from its mother.

16.4 Regulation after transcription

1. Alternative RNA splicing
2. Small RNAs regulate mRNA stability and translation

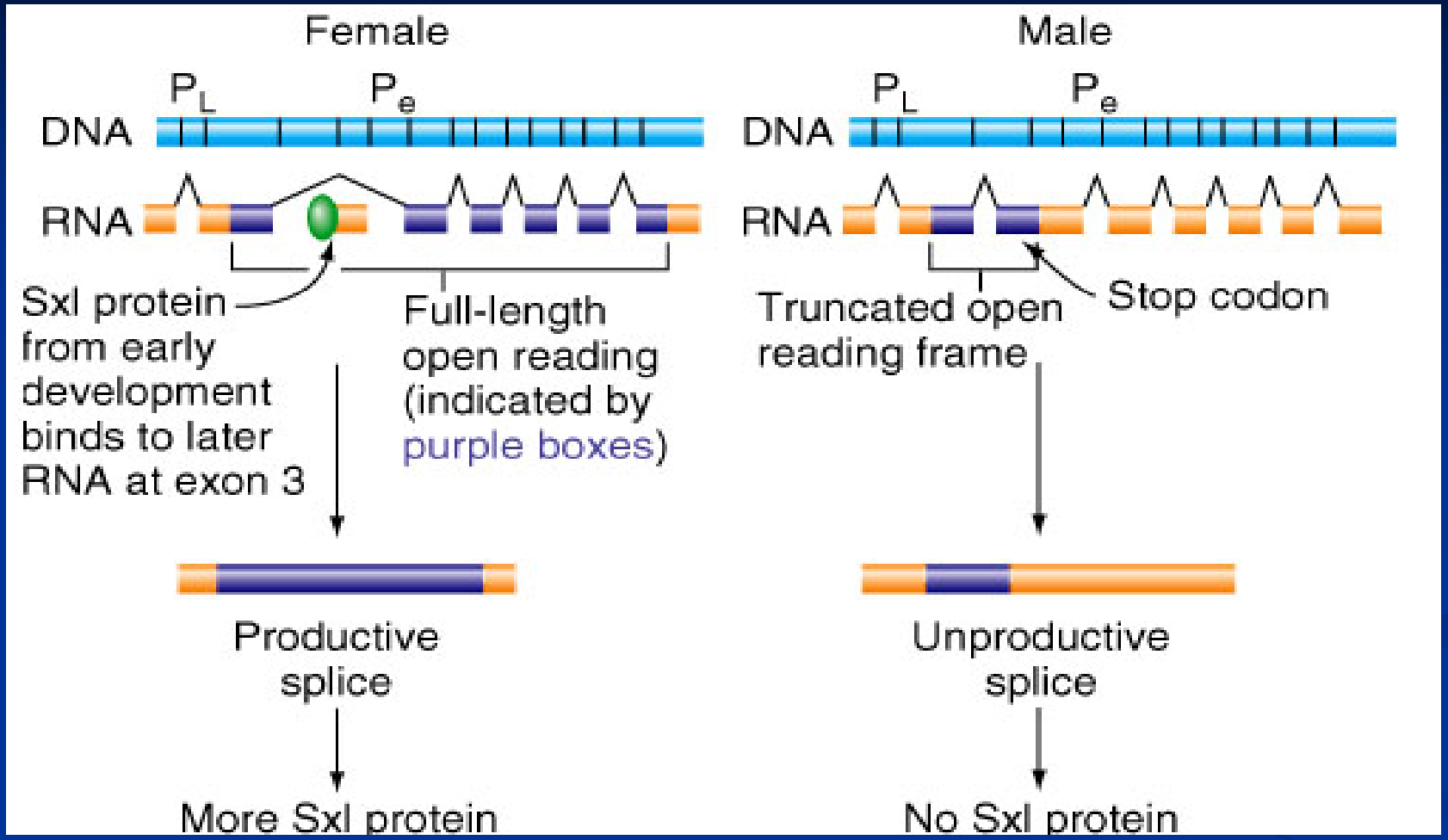
1. Sequence-specific RNA binding proteins can regulate RNA splicing



Sxl protein is essential to the female-specific developmental program.

Fig 16.28

Later in development



Alternative splicing: Production of different mature mRNAs from the same primary transcript by joining different combinations of exons.

Fig 16.28

2. Small RNAs regulate mRNA stability and translation

- 1998-2005 – micro-RNAs identified and characterized in *C. elegans*, 20-23 nucleotides in length.
- Several classes of small regulatory RNAs:
  - Micro-RNAs (miRNAs)
  - Small interfering RNAs (siRNAs)
  - Piwi-interacting RNAs (piRNAs)

TABLE 16.1 Small RNAs in Eukaryotes

	Targets	Effects
miRNAs (micro-RNAs)	• mRNAs	• Block mRNA translation • Destabilize mRNAs
siRNAs (small interfering RNAs)	• mRNAs • Nascent transcripts of chromosomal regions destined to become heterochromatin	• Block translation/Destabilize mRNAs • Recruit histone-modifying enzymes to DNA, resulting in heterochromatin formation
piRNAs (Piwi-interacting RNAs)	• Transposable element transcripts • Transposable element promoters	• Degradation of transposable element mRNA • Facilitate histone modifications that inhibit transposable element transcription

The 2006 Nobel Prize in Physiology or Medicine

For the discovery of RNA interference

Andrew Z. Fire
Stanford U.

Craig C. Mello
U of Massachusetts

RNA interference (RNAi): Trans-acting single-stranded micro-RNAs or small interfering RNAs prevent the expression of specific genes through complementary base pairing.

Micro-RNAs

- ~ 120 miRNAs in plants, ~ 150 in invertebrate animals, ~ 500 in humans.
- Micro-RNAs are products processed from longer transcripts.
  - ¼ from introns of protein coding transcripts
  - ¾ from products of primary transcripts devoid of ORFs

Examples of primary transcripts from micro-RNA-containing genes

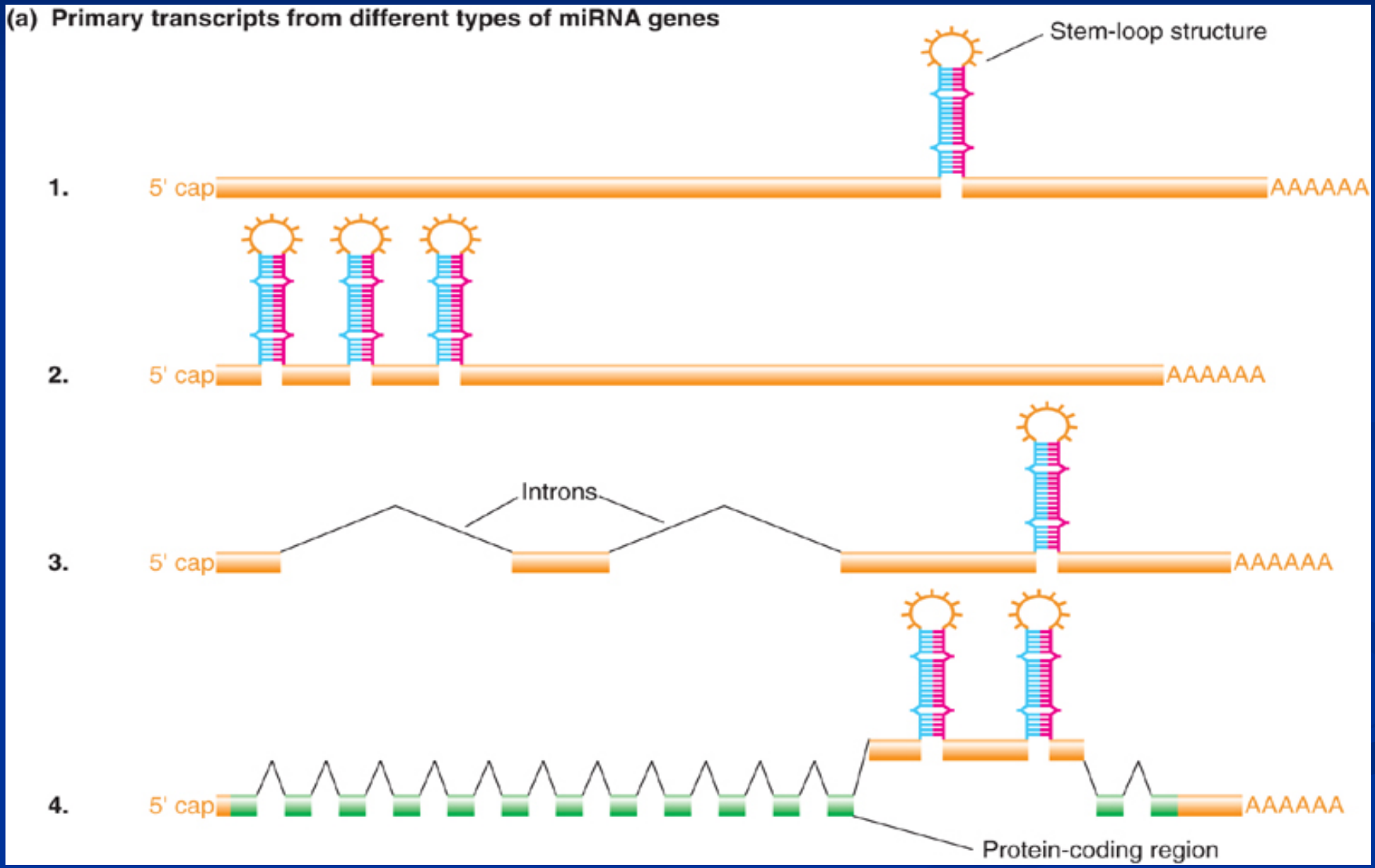
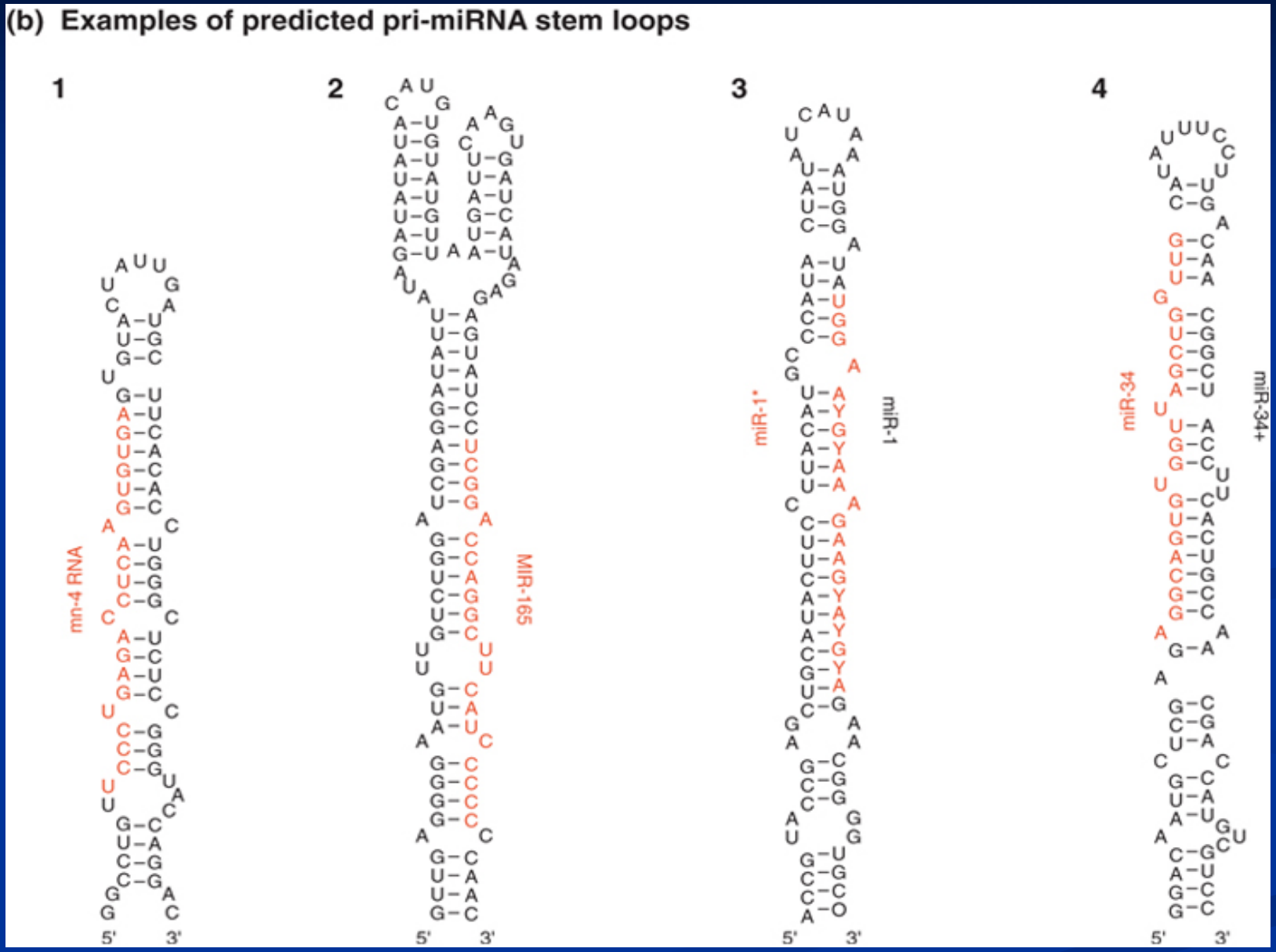


Fig. 16.24


(b) Examples of predicted pri-miRNA stem loops
1
mn-4 RNA
2
MIR-165
3
miR-1*
miR-1
4
miR-34
miR-34+
5'
3'

miRNA processing

- After transcription, pri-miRNAs are recognized by **Drosha** which crops out pre-miRNA stem loops from larger RNA.
- Pre-miRNAs undergo active transport from nucleus to cytoplasm where they are recognized by **Dicer**.
- Dicer reduces the pre-miRNA into a short-lived miRNA*:miRNA duplex which is released and picked up by **RISC**.
- miRNA* strand is degraded, **miRISC** is formed.

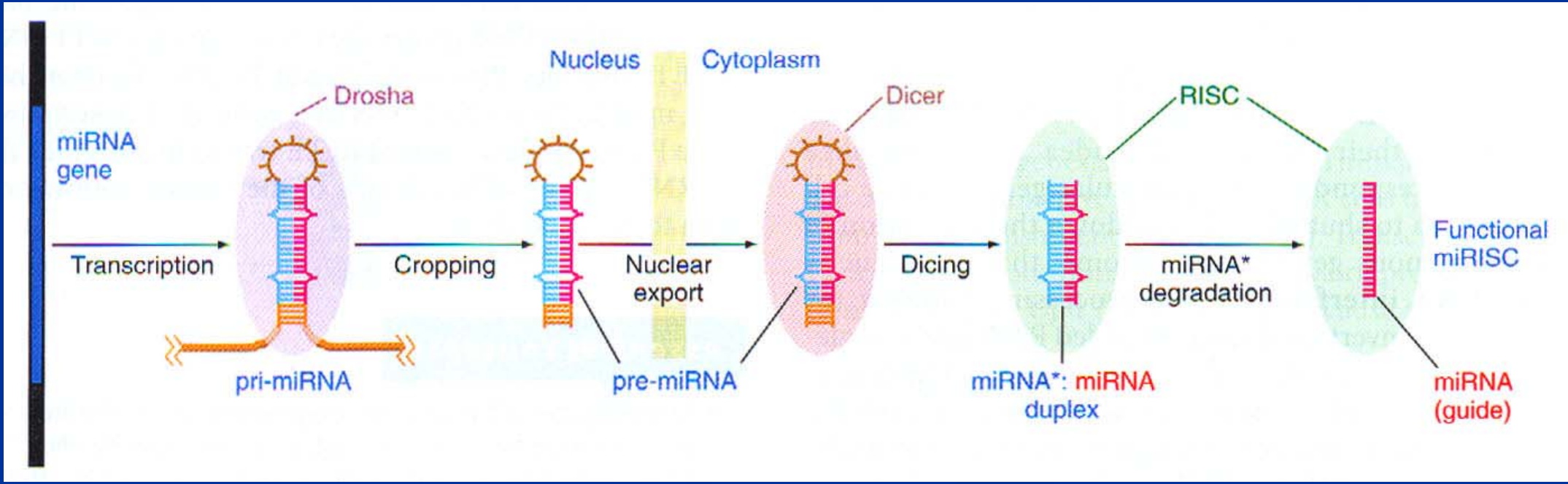


Fig. 16.25

Mechanism of RNA interference

1. **mRNA cleavage**: If miRNA and target mRNA contain perfectly complementary sequences, **miRISC** cleaves the mRNA. RNase rapidly degrades cleavage product.
2. **Translational repression**: If miRNA and its target mRNA have only partial complementarity, cleavage does not occur. **miRISC** remains bound to its target and represses its movement across ribosomes.