Regulation of gene expression in eukaryotes

Major principle: Activation of gene activity

Positive Control of Gene expression

General Chromatin structure

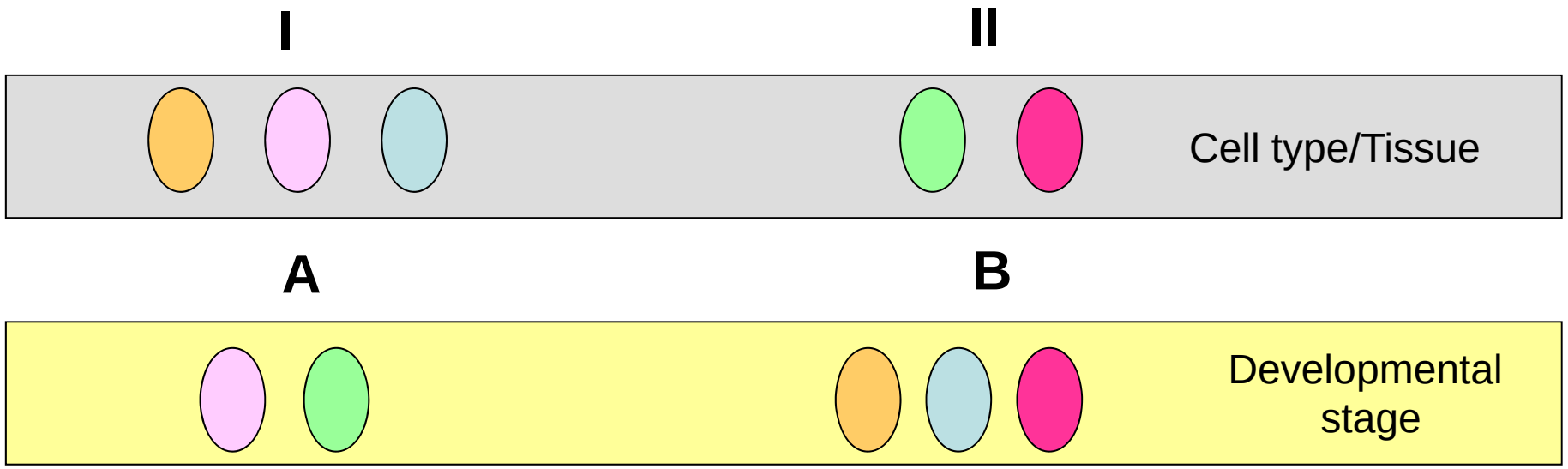
Wide domain regulators

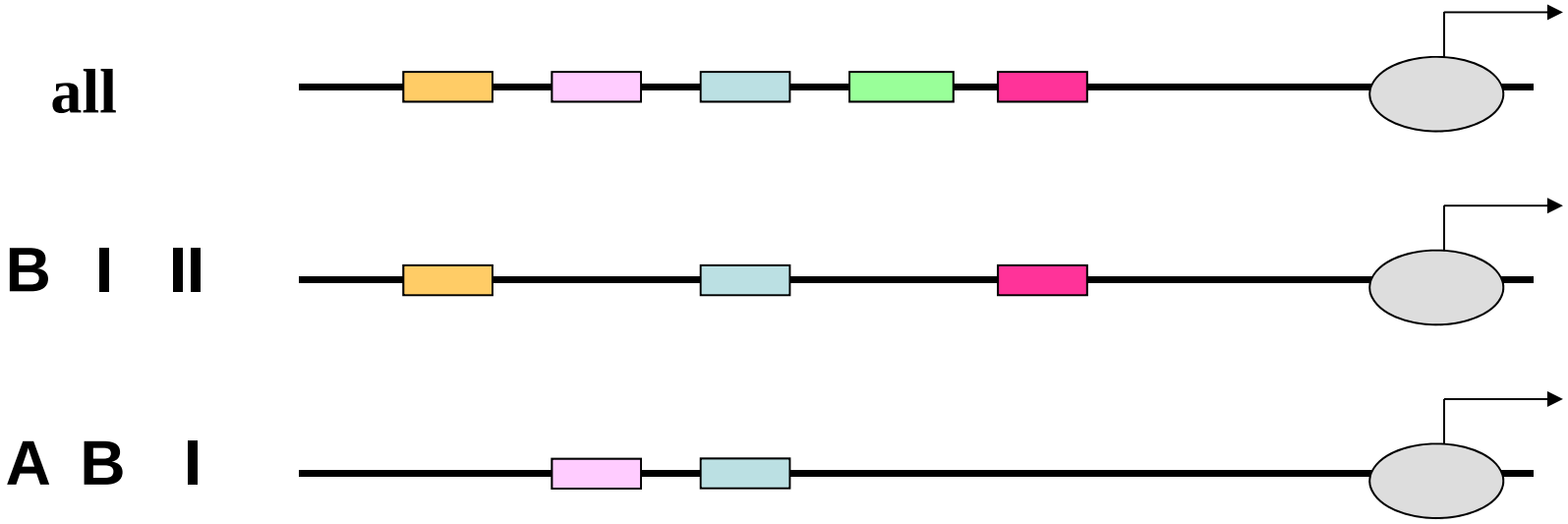
Gene-specific Regulators

Coregulators

Modification of regulators

Combinatorial Principle





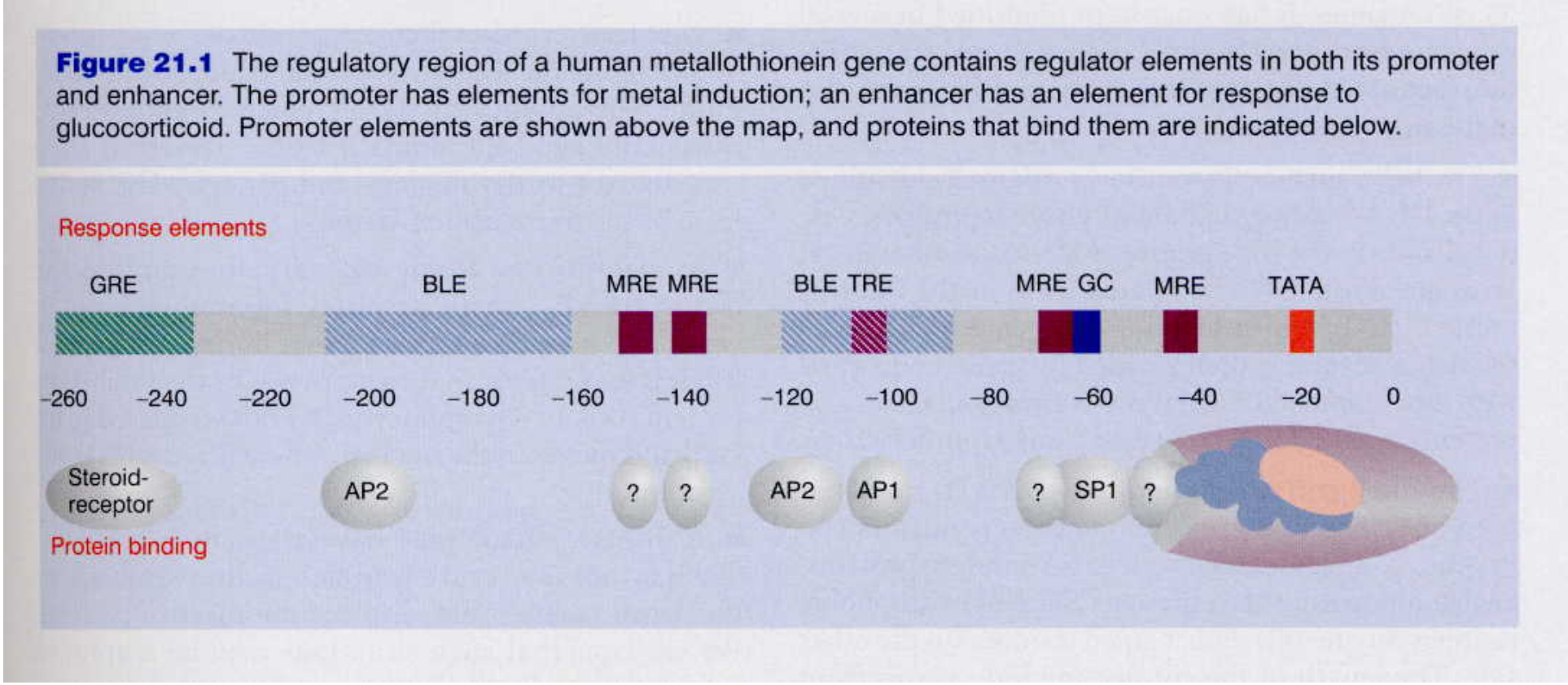


Figure 21.1 The regulatory region of a human metallothionein gene contains regulator elements in both its promoter and enhancer. The promoter has elements for metal induction; an enhancer has an element for response to glucocorticoid. Promoter elements are shown above the map, and proteins that bind them are indicated below.

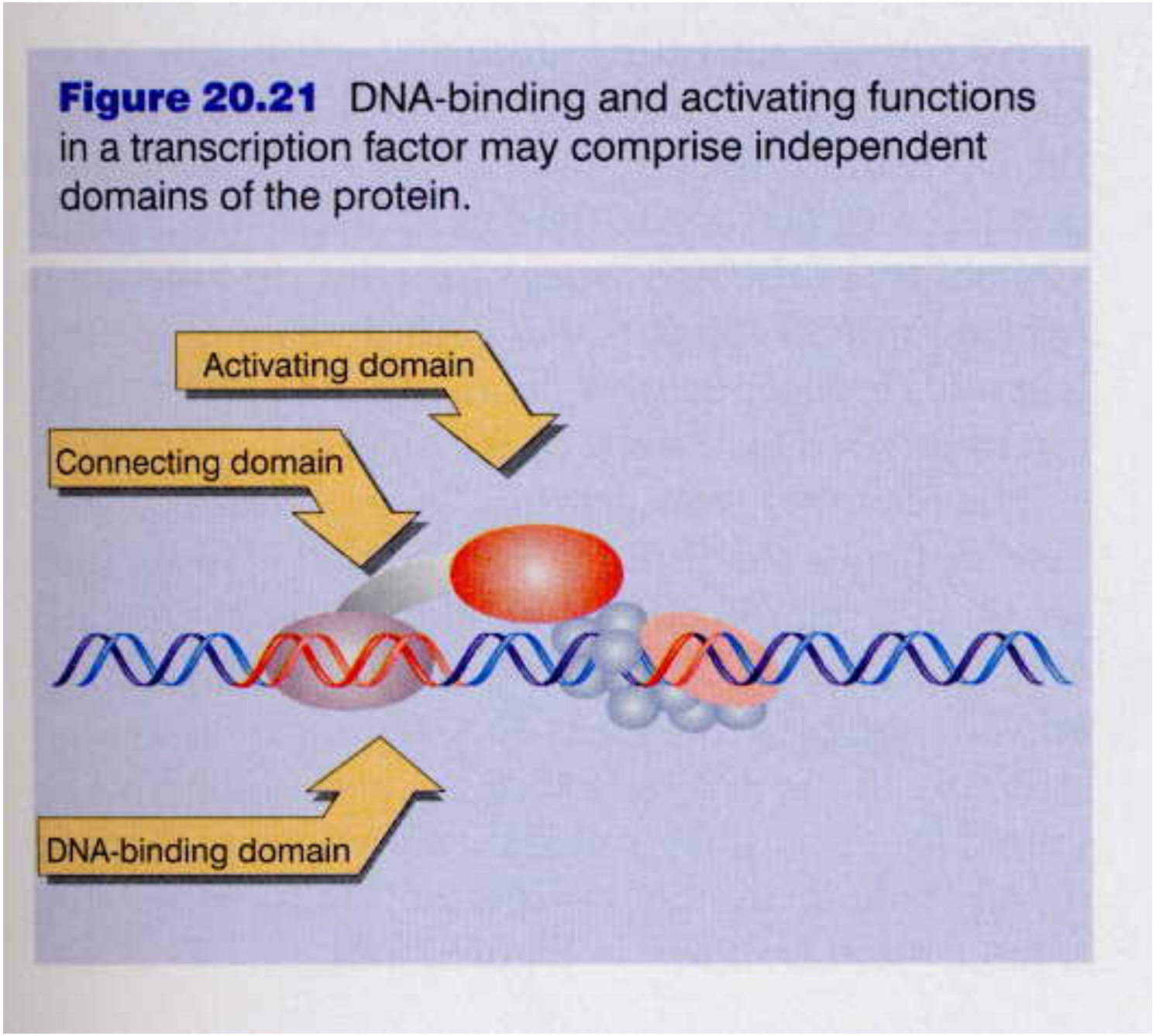
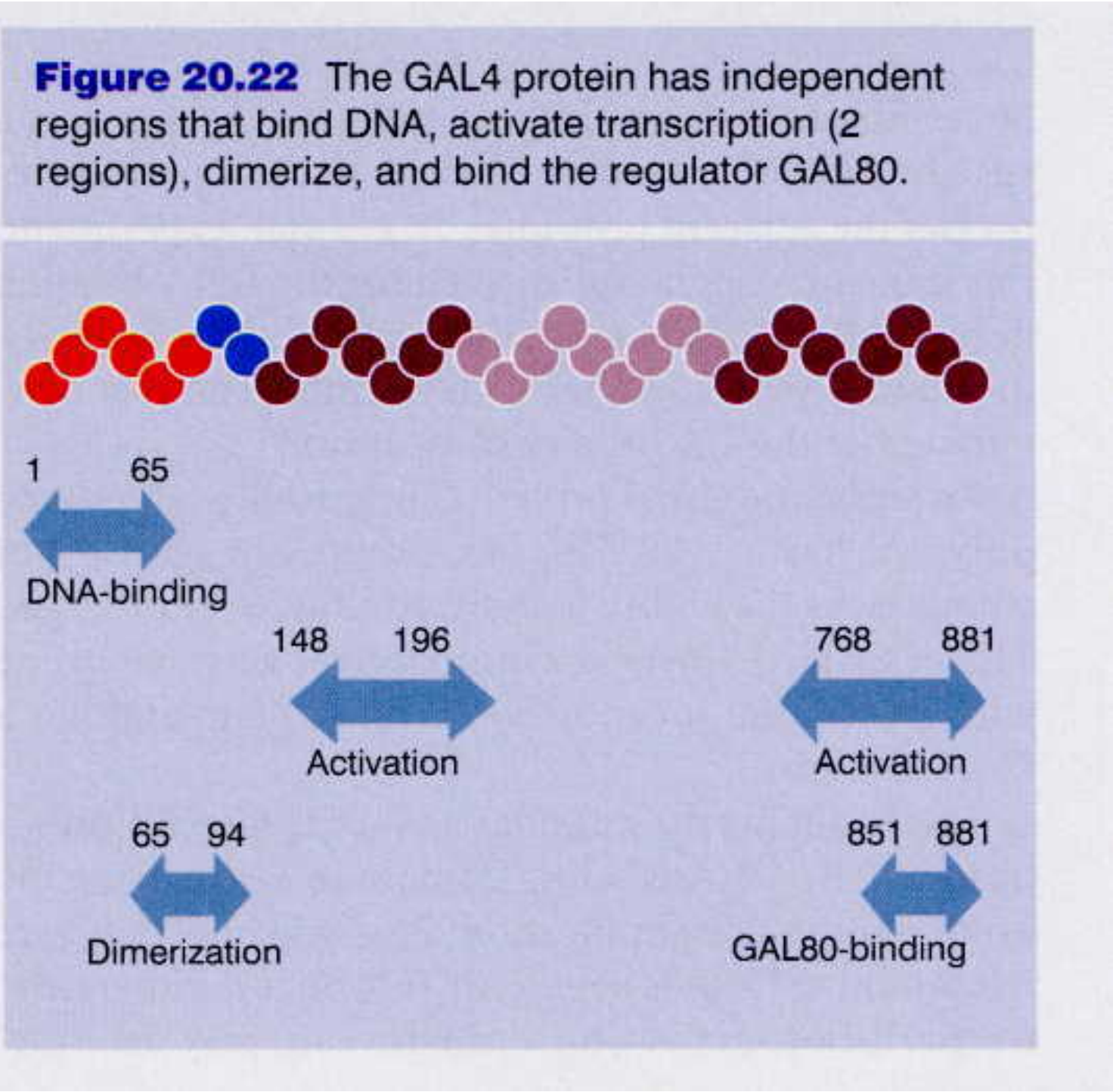


Figure 20.21 DNA-binding and activating functions in a transcription factor may comprise independent domains of the protein.

Figure 20.22 The GAL4 protein has independent regions that bind DNA, activate transcription (2 regions), dimerize, and bind the regulator GAL80.



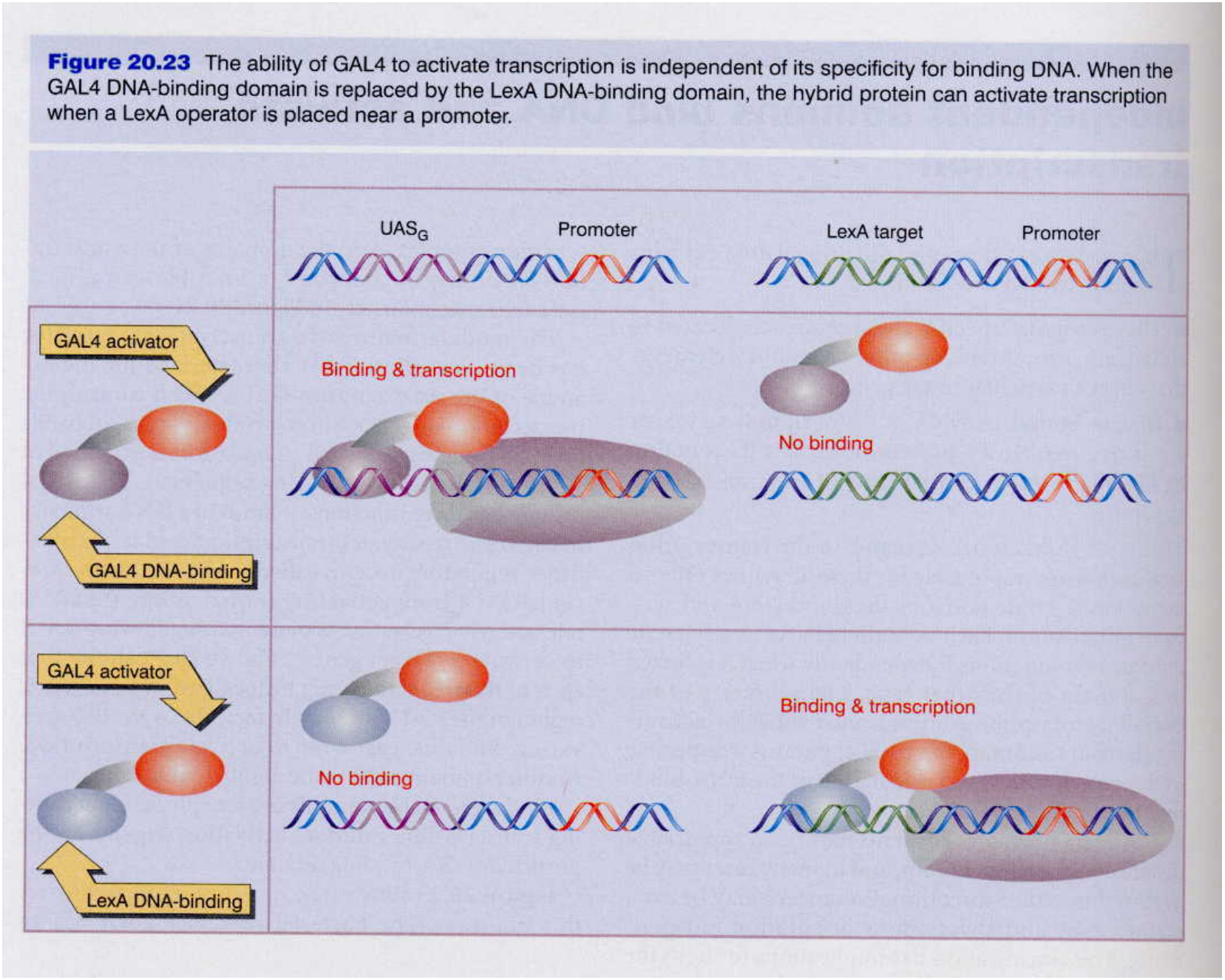


Figure 20.23 The ability of GAL4 to activate transcription is independent of its specificity for binding DNA. When the GAL4 DNA-binding domain is replaced by the LexA DNA-binding domain, the hybrid protein can activate transcription when a LexA operator is placed near a promoter.

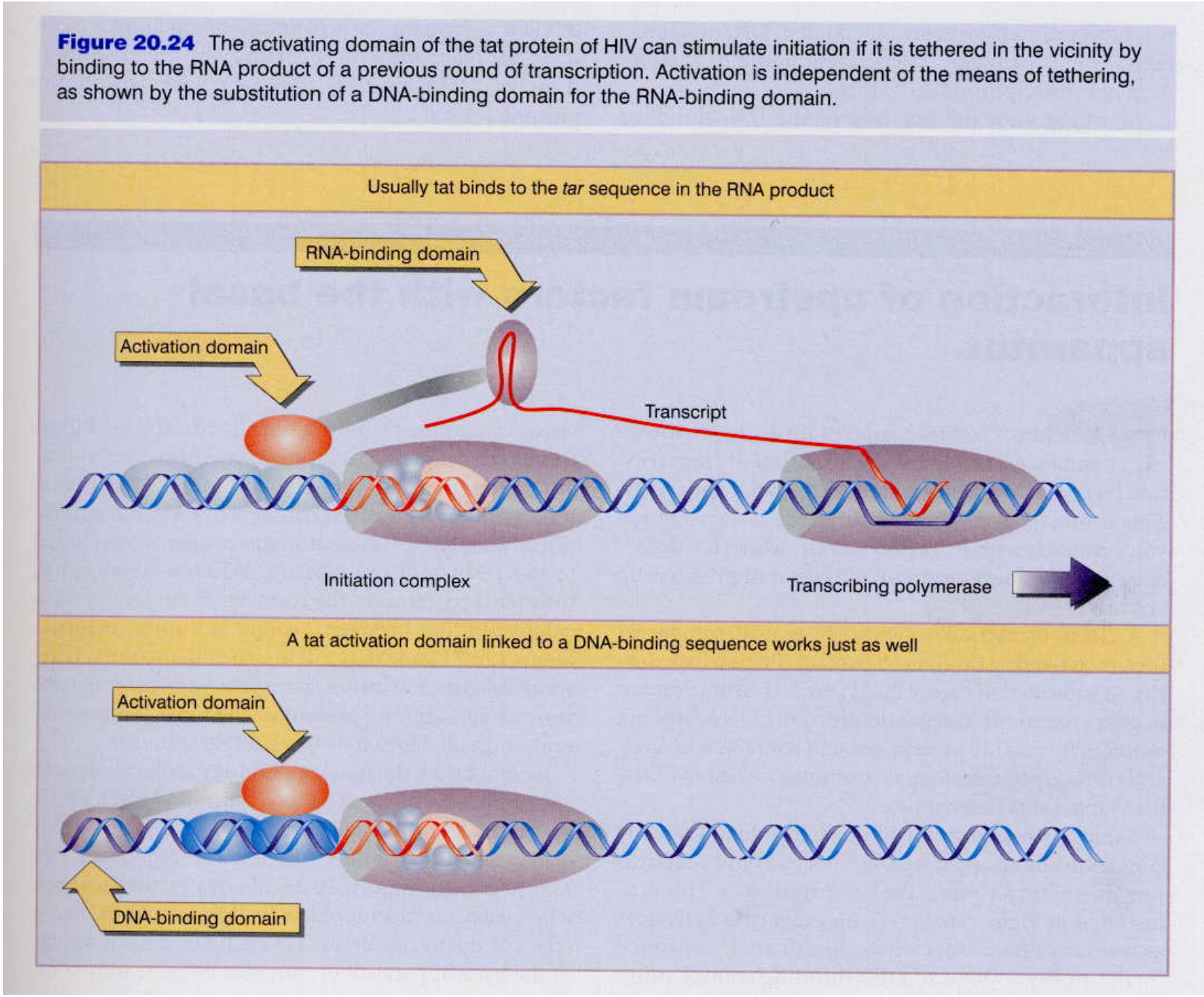


Figure 20.24 The activating domain of the tat protein of HIV can stimulate initiation if it is tethered in the vicinity by binding to the RNA product of a previous round of transcription. Activation is independent of the means of tethering, as shown by the substitution of a DNA-binding domain for the RNA-binding domain.

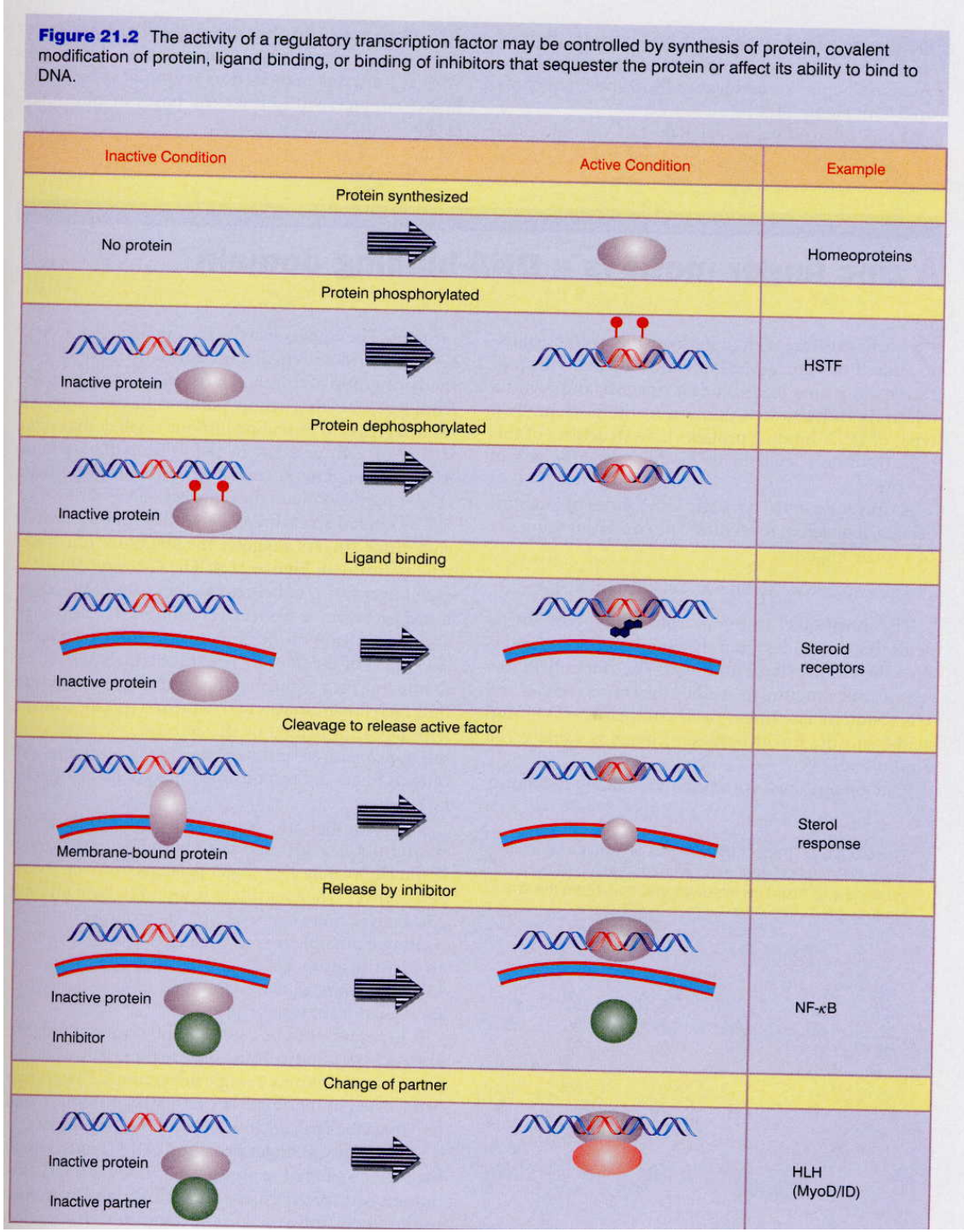


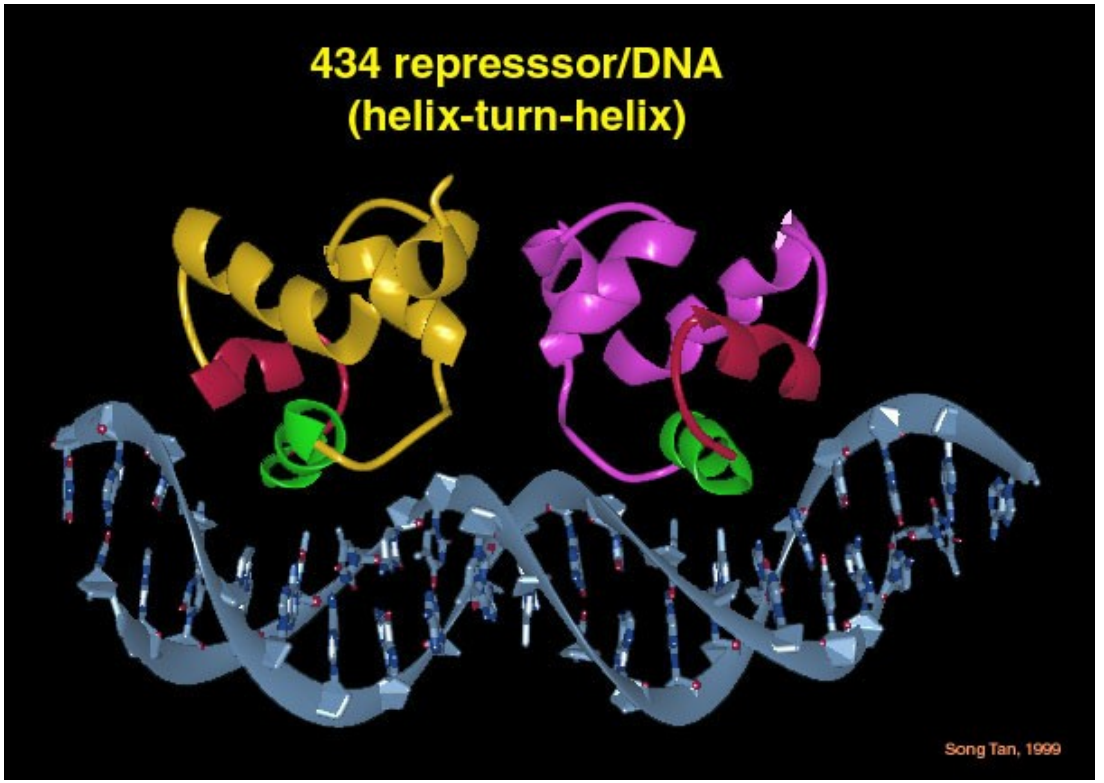
Figure 21.2 The activity of a regulatory transcription factor may be controlled by synthesis of protein, covalent modification of protein, ligand binding, or binding of inhibitors that sequester the protein or affect its ability to bind to DNA.

17.12.13

DNA Binding Proteins - motifs

Helix –Turn – Helix Proteins

The Helix-Turn-Helix motif consists of two a helices and a short extended amino acid chain between them. The more carboxyl-terminal helix can fit into the major groove of DNA. This motif is found in hundreds of DNA-binding proteins, including λ- repressor, tryptophan repressor, catabolite activator protein (CAP), octamer transcription factor 1 (Oct-1) and heat shock factor (HSF),
Source: http://www.web-books.com/MoBio/Free/Ch4F4.htm



Source: http://www.bmb.psu.edu/faculty/tan/lab/gallery/434reprdna.jpg

Siehe auch: http://www.proteopedia.org/wiki/index.php/Helix-turn-helix_motif

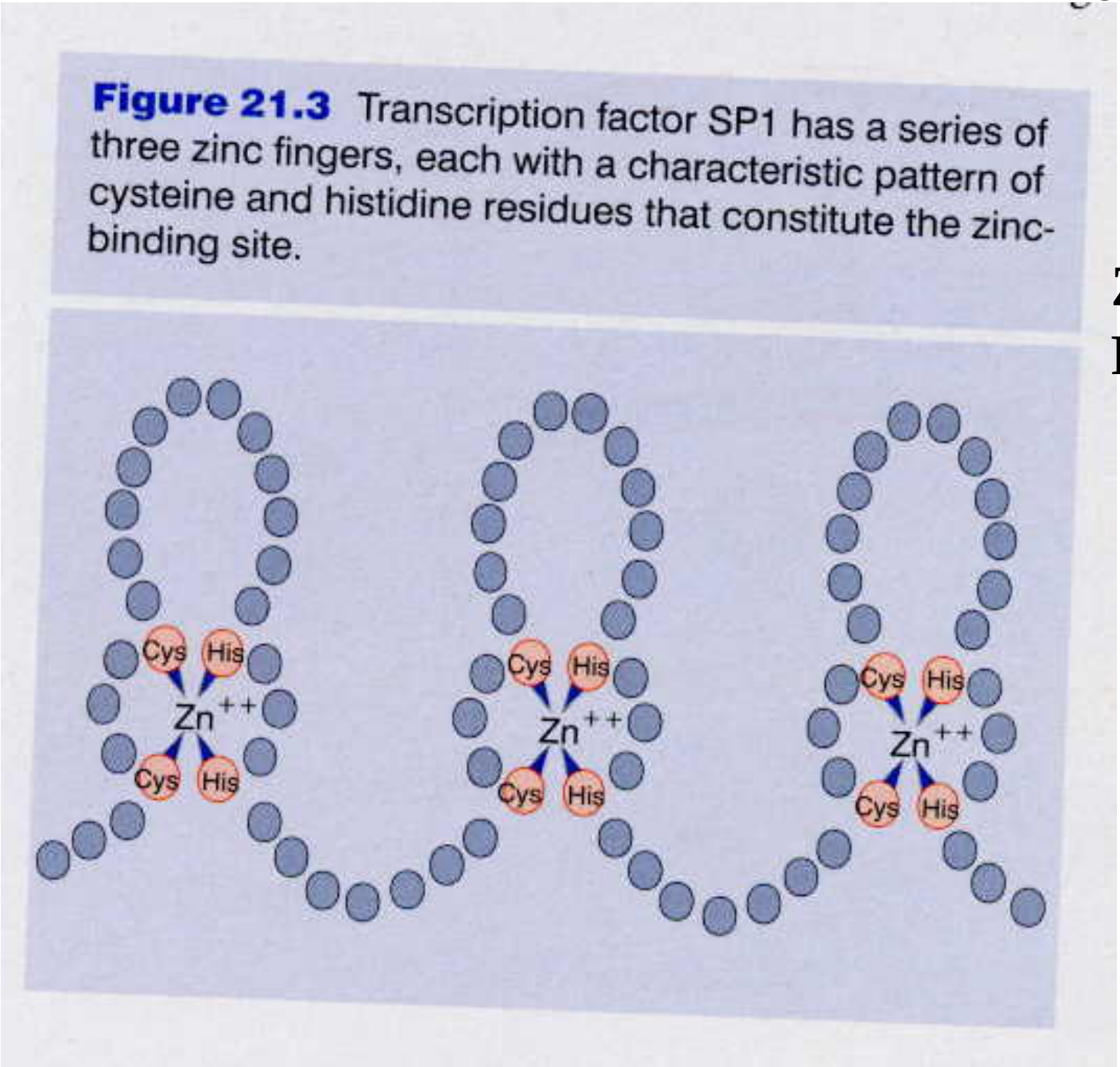


Figure 21.3 Transcription factor SP1 has a series of three zinc fingers, each with a characteristic pattern of cysteine and histidine residues that constitute the zinc-binding site.

Zink-Finger Proteins

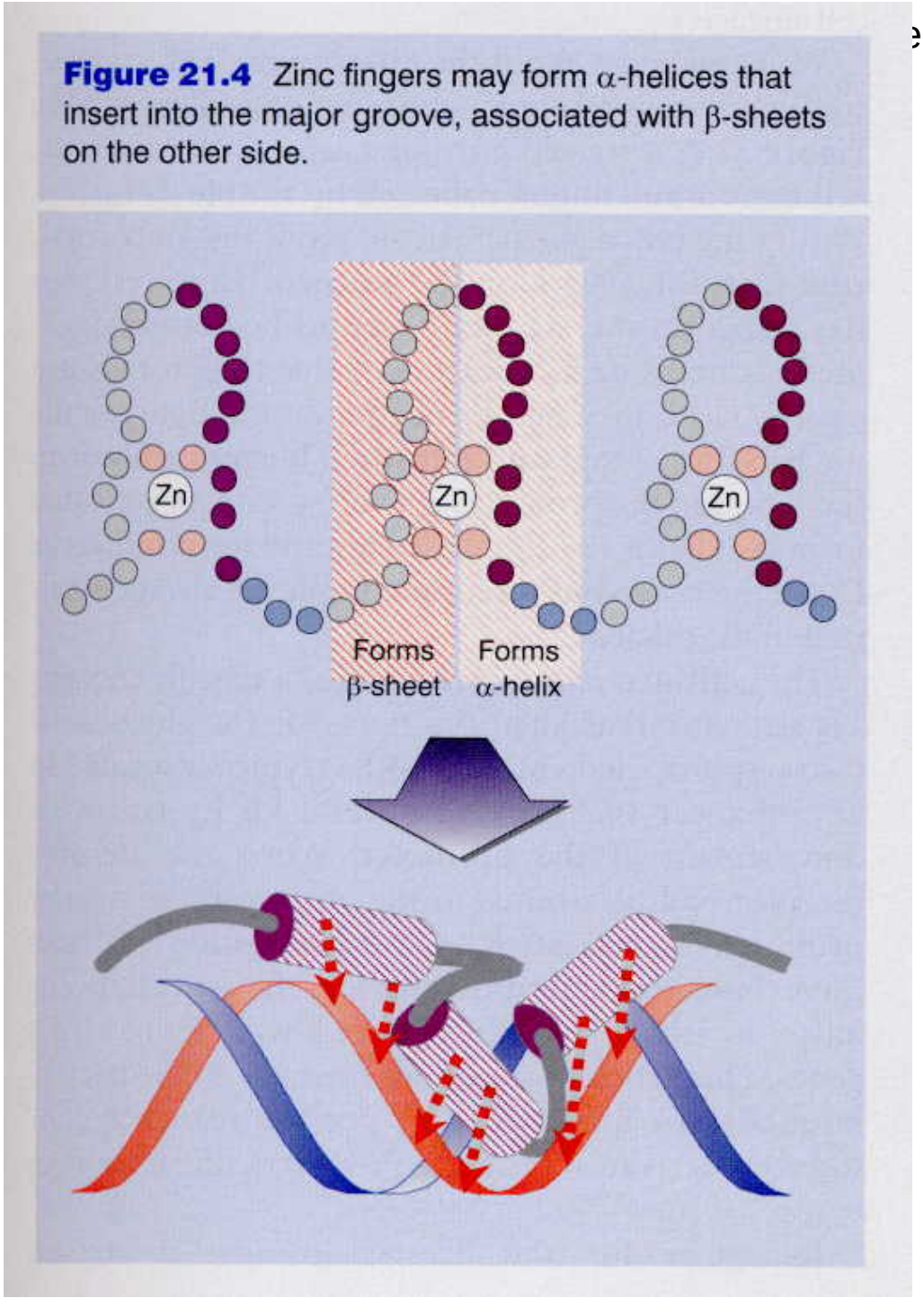


Figure 21.4 Zinc fingers may form α-helices that insert into the major groove, associated with β-sheets on the other side.

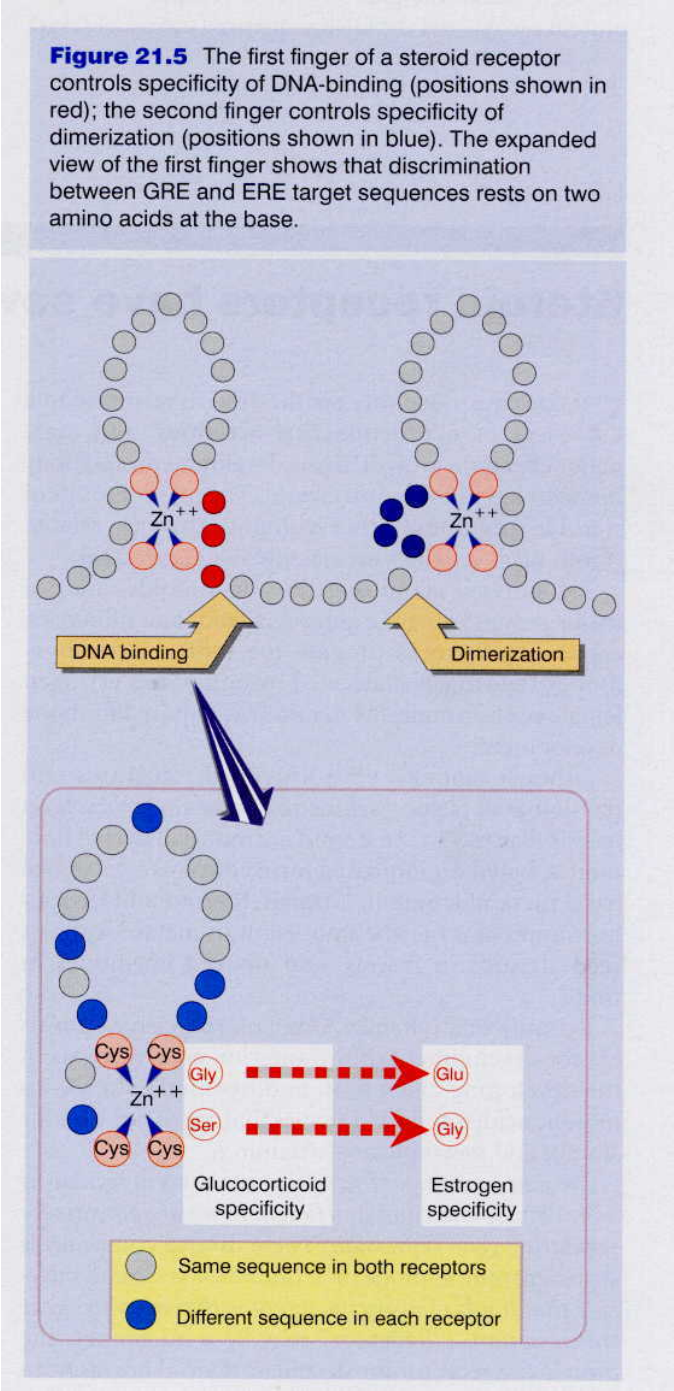


Figure 21.5 The first finger of a steroid receptor controls specificity of DNA-binding (positions shown in red); the second finger controls specificity of dimerization (positions shown in blue). The expanded view of the first finger shows that discrimination between GRE and ERE target sequences rests on two amino acids at the base.

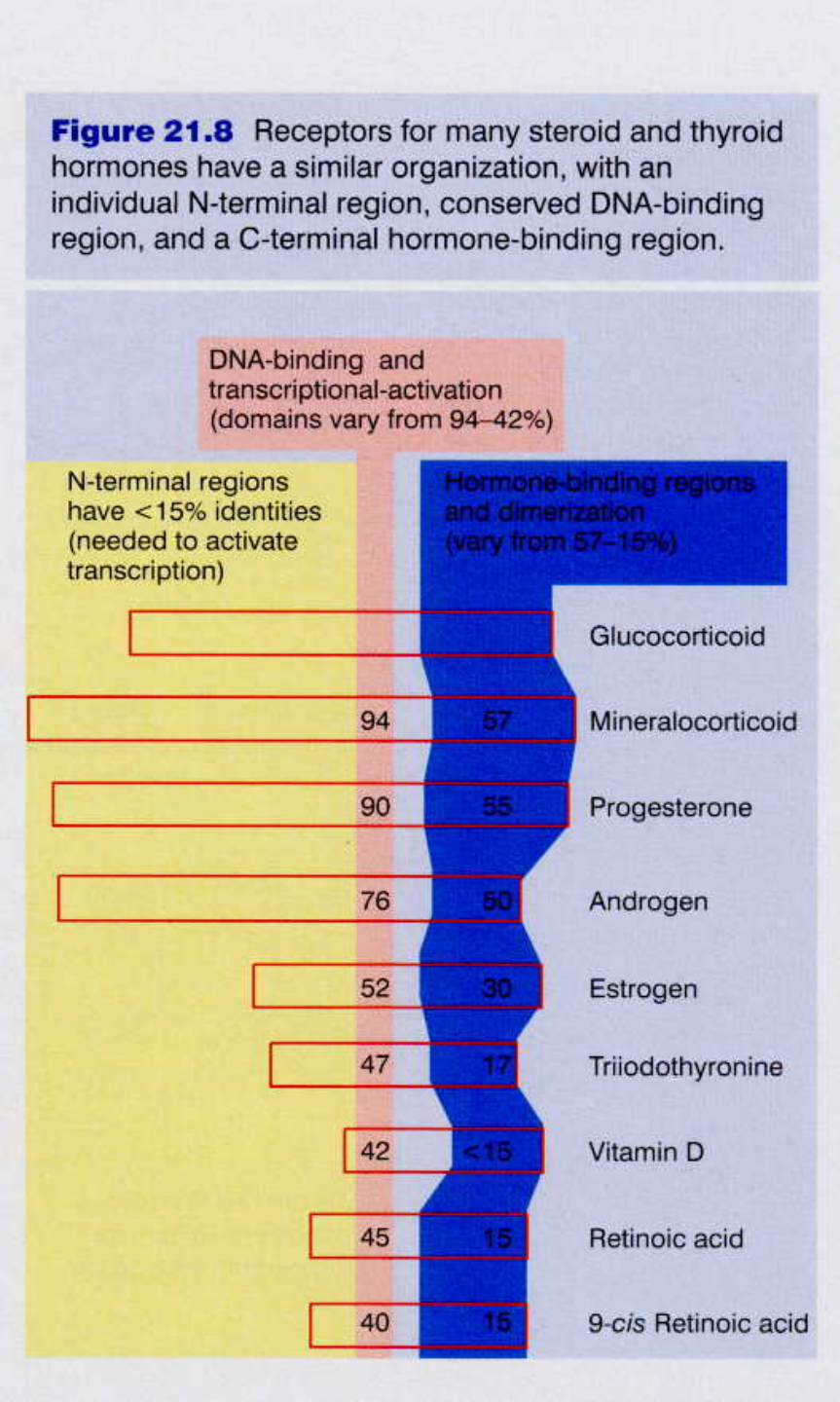


Figure 21.8 Receptors for many steroid and thyroid hormones have a similar organization, with an individual N-terminal region, conserved DNA-binding region, and a C-terminal hormone-binding region.

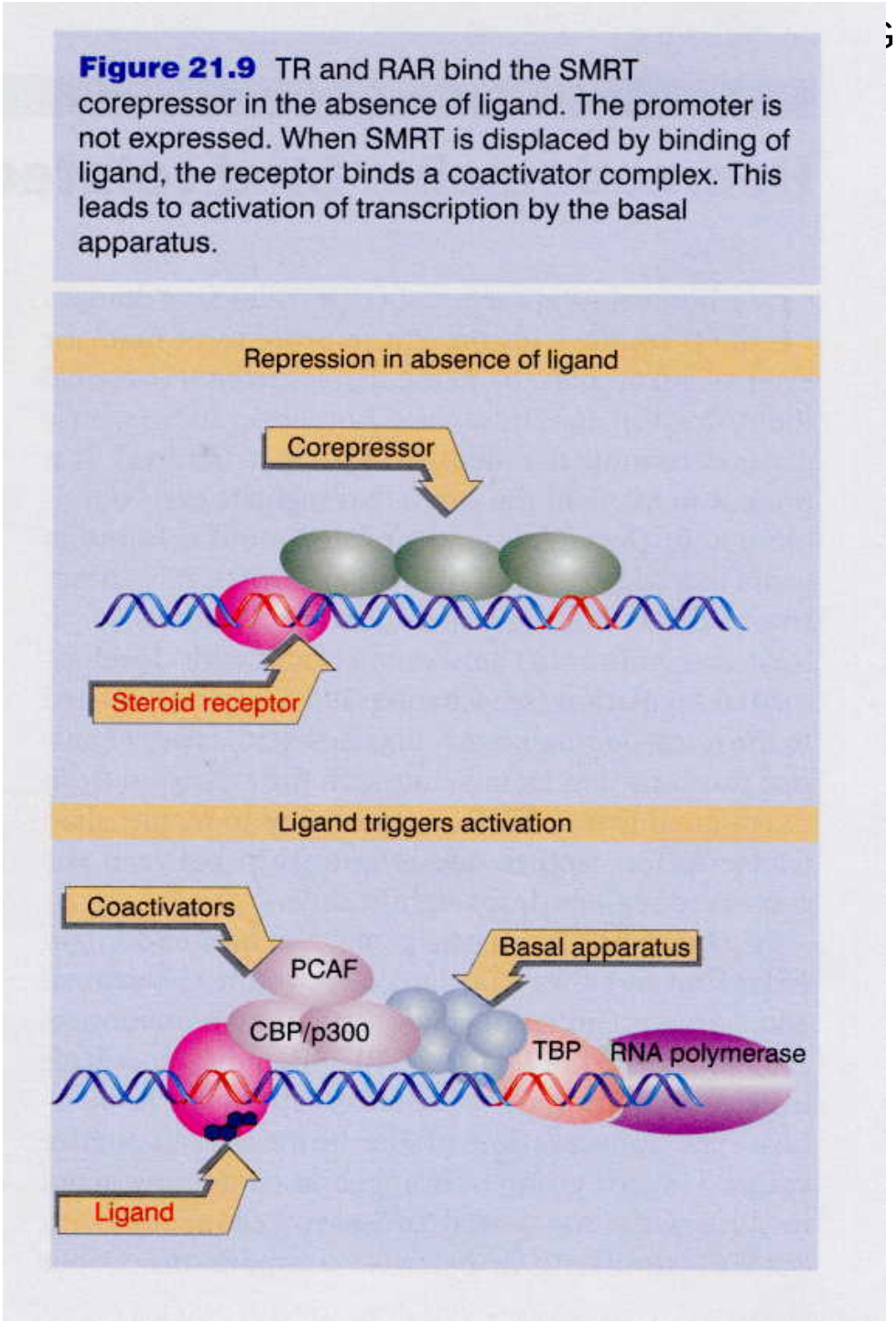


Figure 21.9 TR and RAR bind the SMRT corepressor in the absence of ligand. The promoter is not expressed. When SMRT is displaced by binding of ligand, the receptor binds a coactivator complex. This leads to activation of transcription by the basal apparatus.

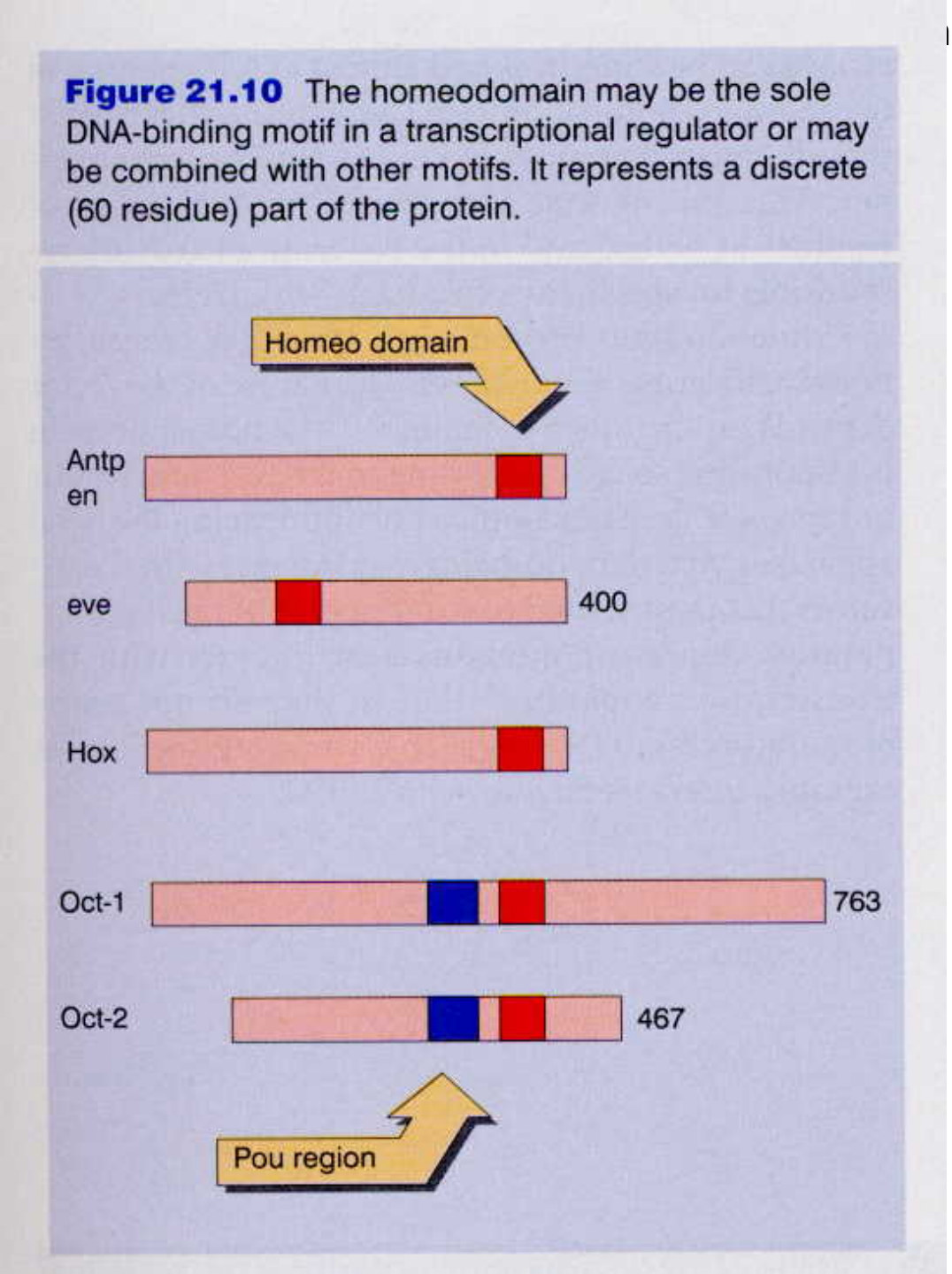


Figure 21.10 The homeodomain may be the sole DNA-binding motif in a transcriptional regulator or may be combined with other motifs. It represents a discrete (60 residue) part of the protein.

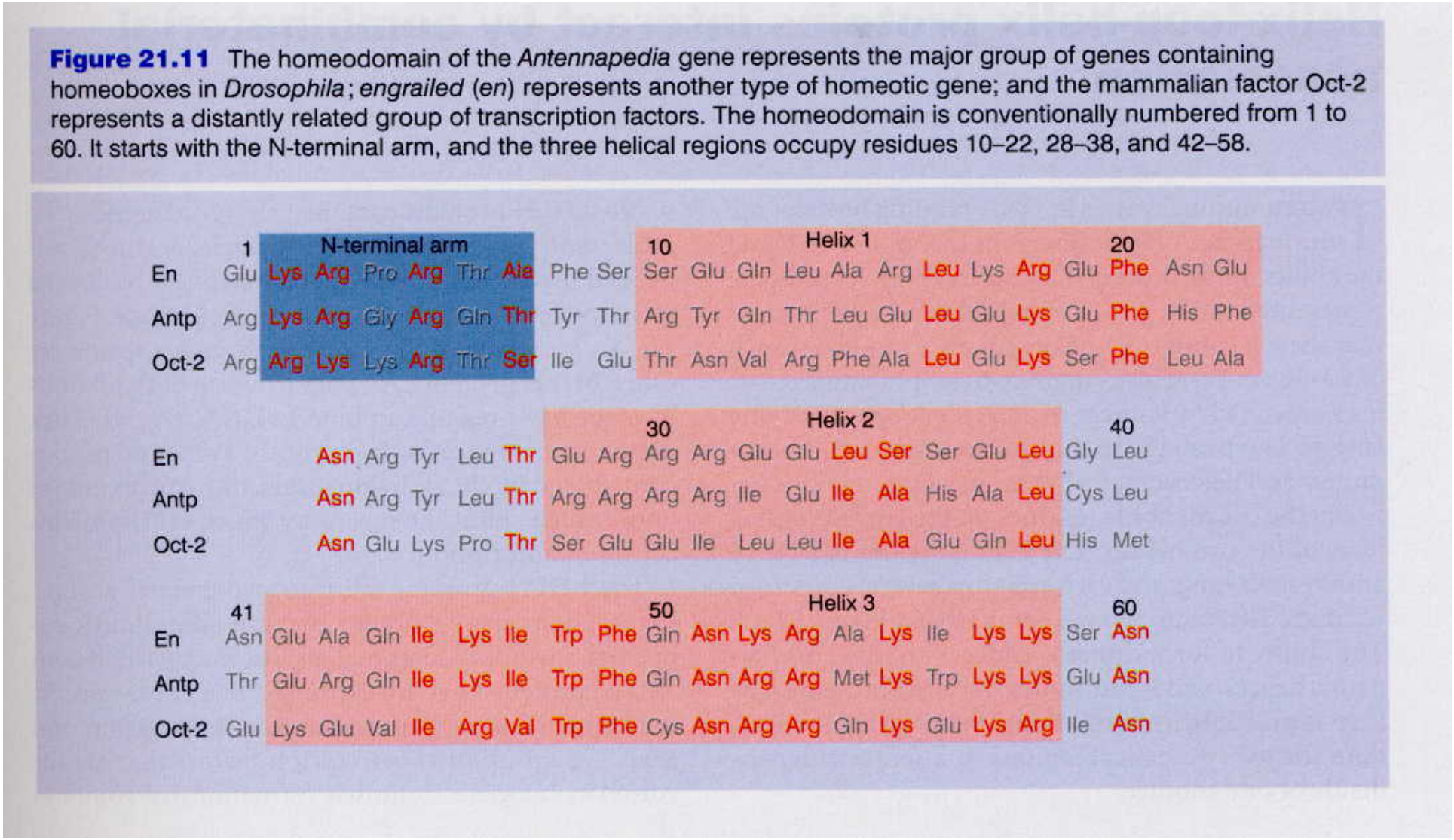
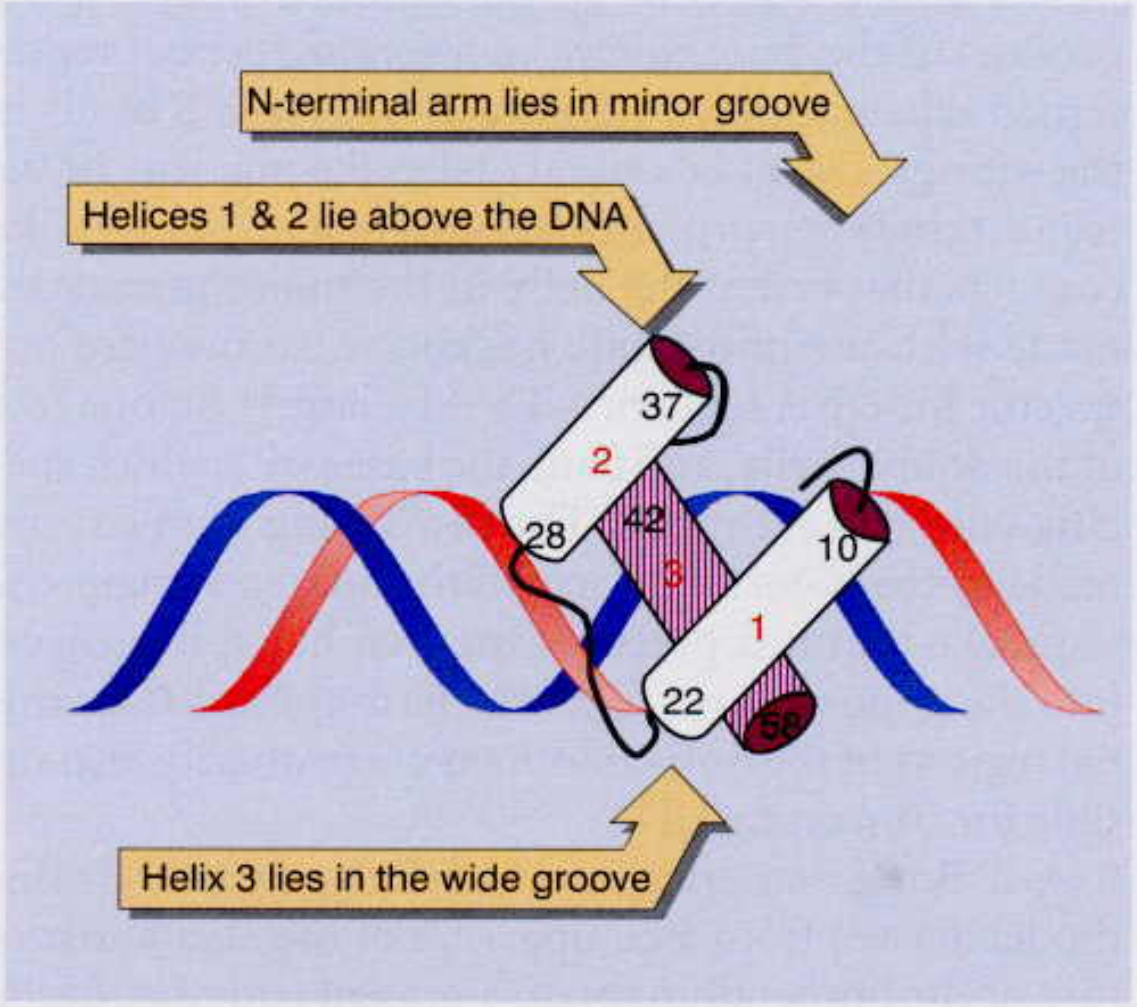


Figure 21.11 The homeodomain of the *Antennapedia* gene represents the major group of genes containing homeoboxes in *Drosophila*; *engrailed* (*en*) represents another type of homeotic gene; and the mammalian factor Oct-2 represents a distantly related group of transcription factors. The homeodomain is conventionally numbered from 1 to 60. It starts with the N-terminal arm, and the three helical regions occupy residues 10–22, 28–38, and 42–58.

Figure 21.12 Helix 3 of the homeodomain binds in the major groove of DNA, with helices 1 and 2 lying outside the double helix. Helix 3 contacts both the phosphate backbone and specific bases. The N-terminal arm lies in the minor groove, and makes additional contacts.



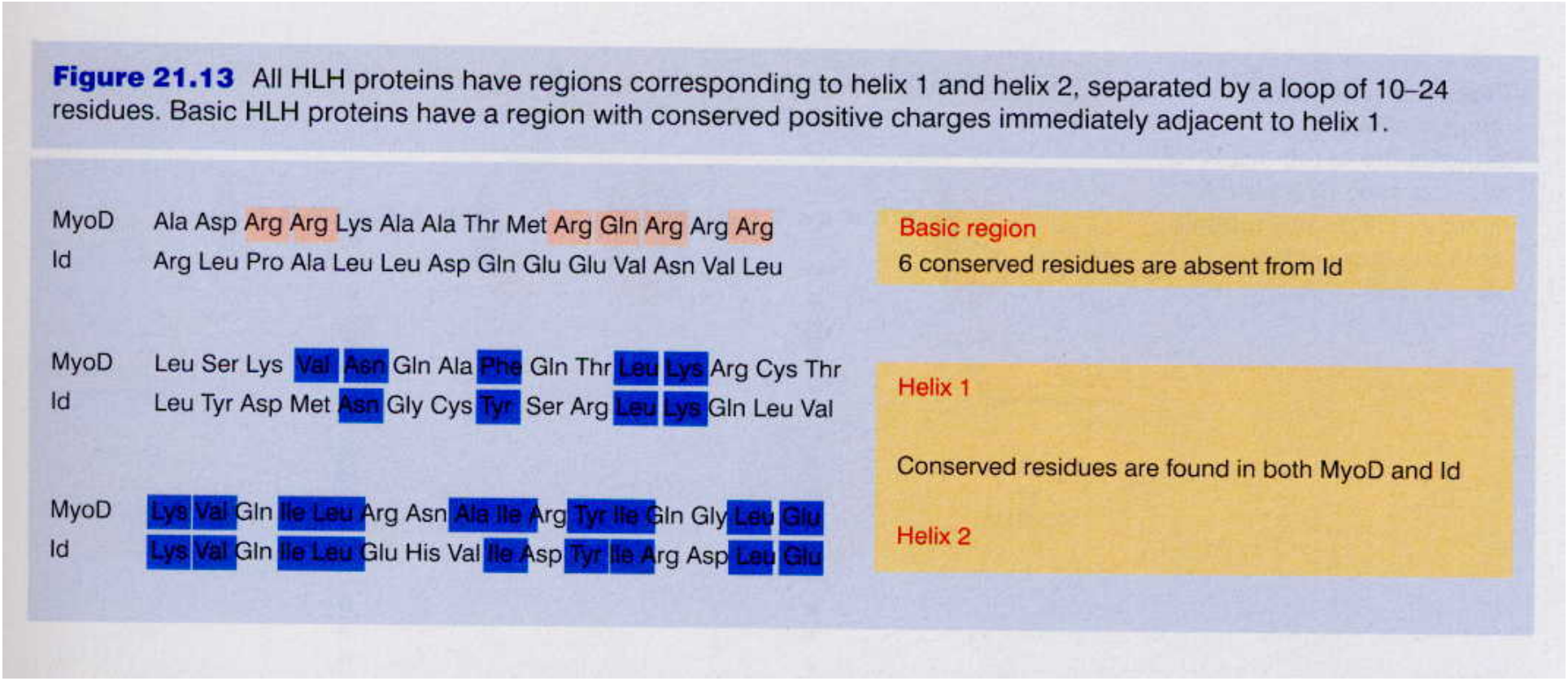


Figure 21.13 All HLH proteins have regions corresponding to helix 1 and helix 2, separated by a loop of 10–24 residues. Basic HLH proteins have a region with conserved positive charges immediately adjacent to helix 1.

MyoD Ala Asp Arg Arg Lys Ala Ala Thr Met Arg Gln Arg Arg Arg
Id Arg Leu Pro Ala Leu Leu Asp Gln Glu Glu Val Asn Val Leu

Basic region
6 conserved residues are absent from Id

MyoD Leu Ser Lys Val Asn Gln Ala Phe Gln Thr Leu Lys Arg Cys Thr
Id Leu Tyr Asp Met Asn Gly Cys Tyr Ser Arg Leu Lys Gln Leu Val

Helix 1

Conserved residues are found in both MyoD and Id

MyoD Lys Val Gln Ile Leu Arg Asn Ala Ile Arg Tyr Ile Gln Gly Leu Glu
Id Lys Val Gln Ile Leu Glu His Val Ile Asp Tyr Ile Arg Asp Leu Glu

Helix 2

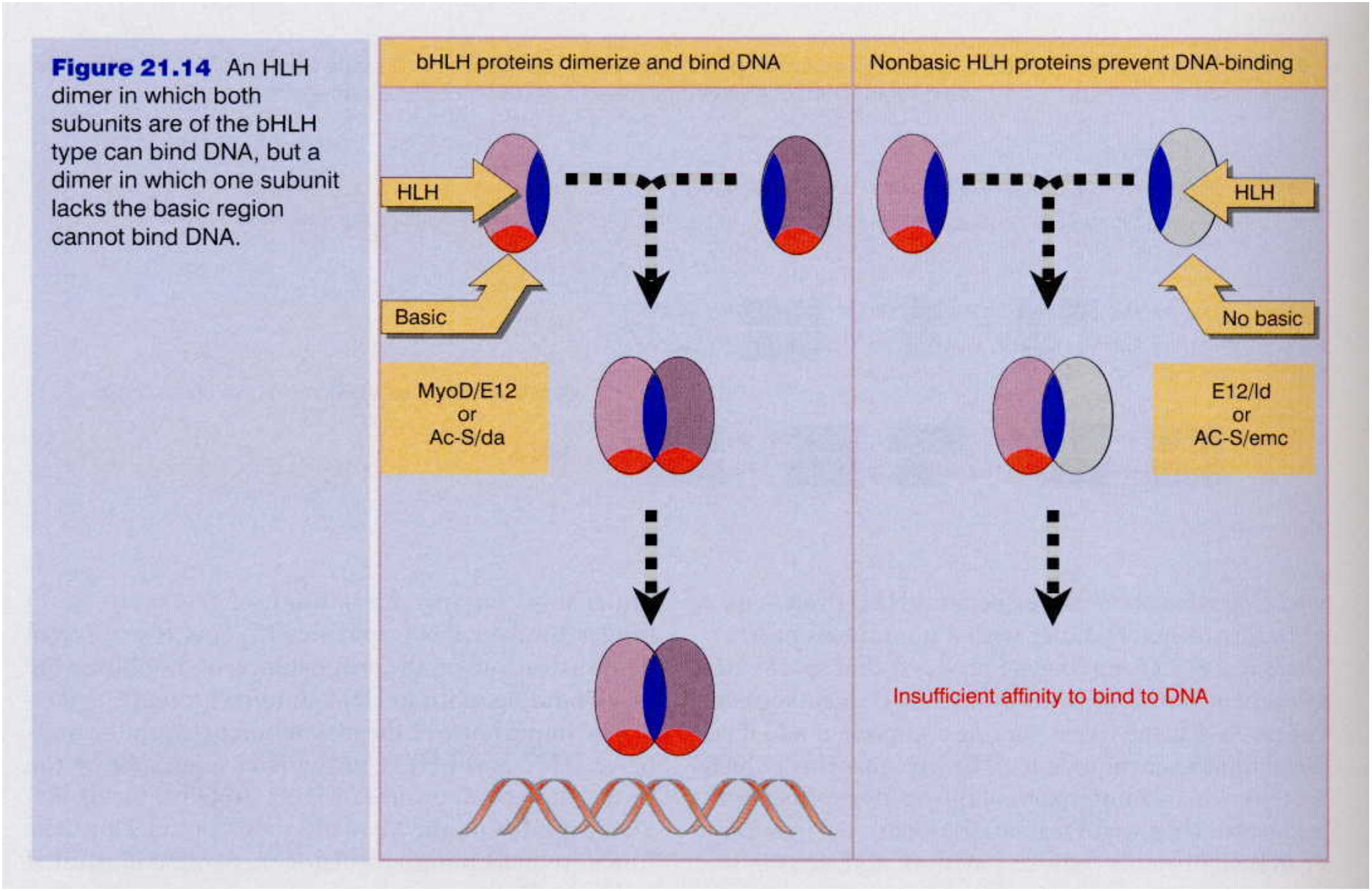
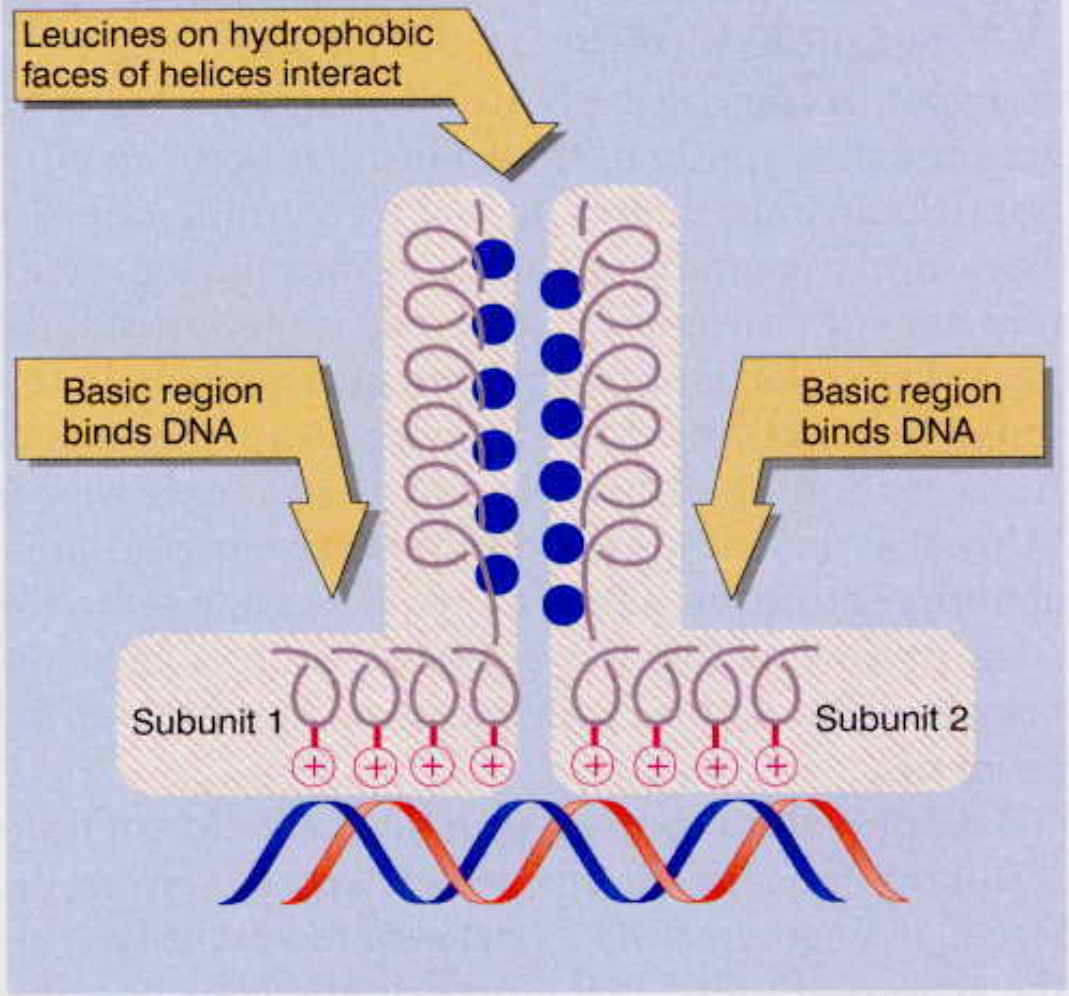


Figure 21.14 An HLH dimer in which both subunits are of the bHLH type can bind DNA, but a dimer in which one subunit lacks the basic region cannot bind DNA.

Figure 21.15 The basic regions of the bZIP motif are held together by the dimerization at the adjacent zipper region when the hydrophobic faces of two leucine zippers interact in parallel orientation.



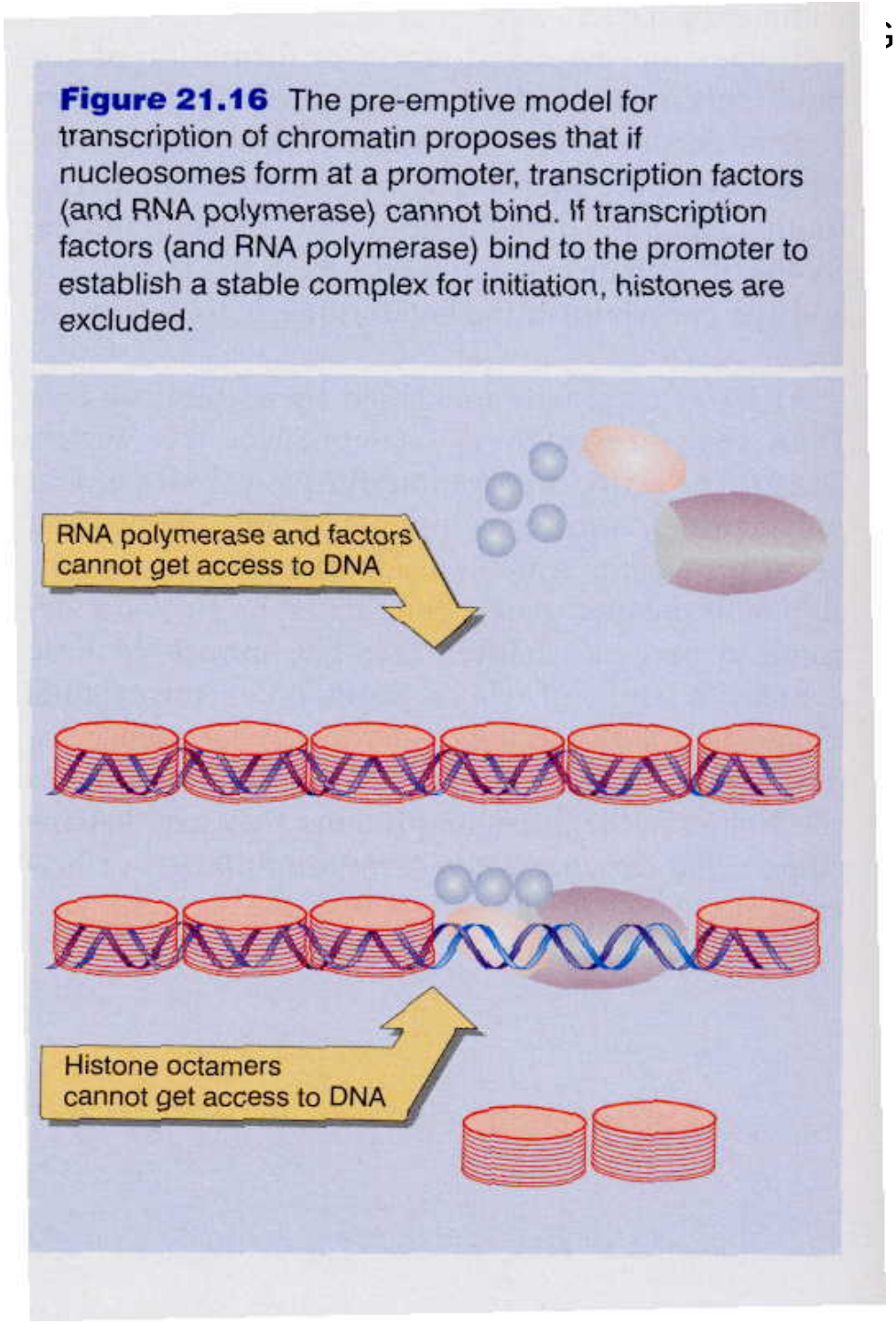


Figure 21.16 The pre-emptive model for transcription of chromatin proposes that if nucleosomes form at a promoter, transcription factors (and RNA polymerase) cannot bind. If transcription factors (and RNA polymerase) bind to the promoter to establish a stable complex for initiation, histones are excluded.

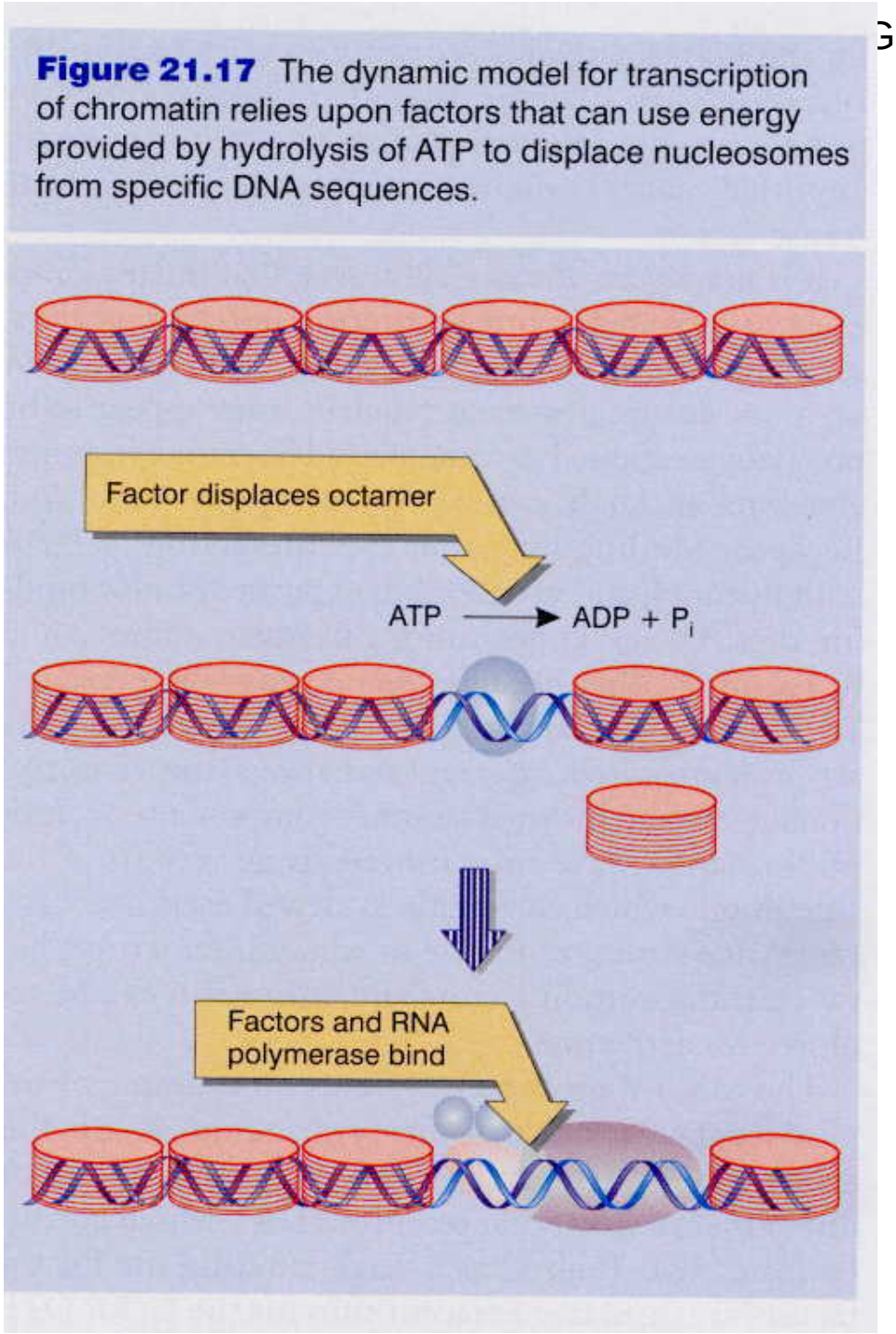


Figure 21.17 The dynamic model for transcription of chromatin relies upon factors that can use energy provided by hydrolysis of ATP to displace nucleosomes from specific DNA sequences.

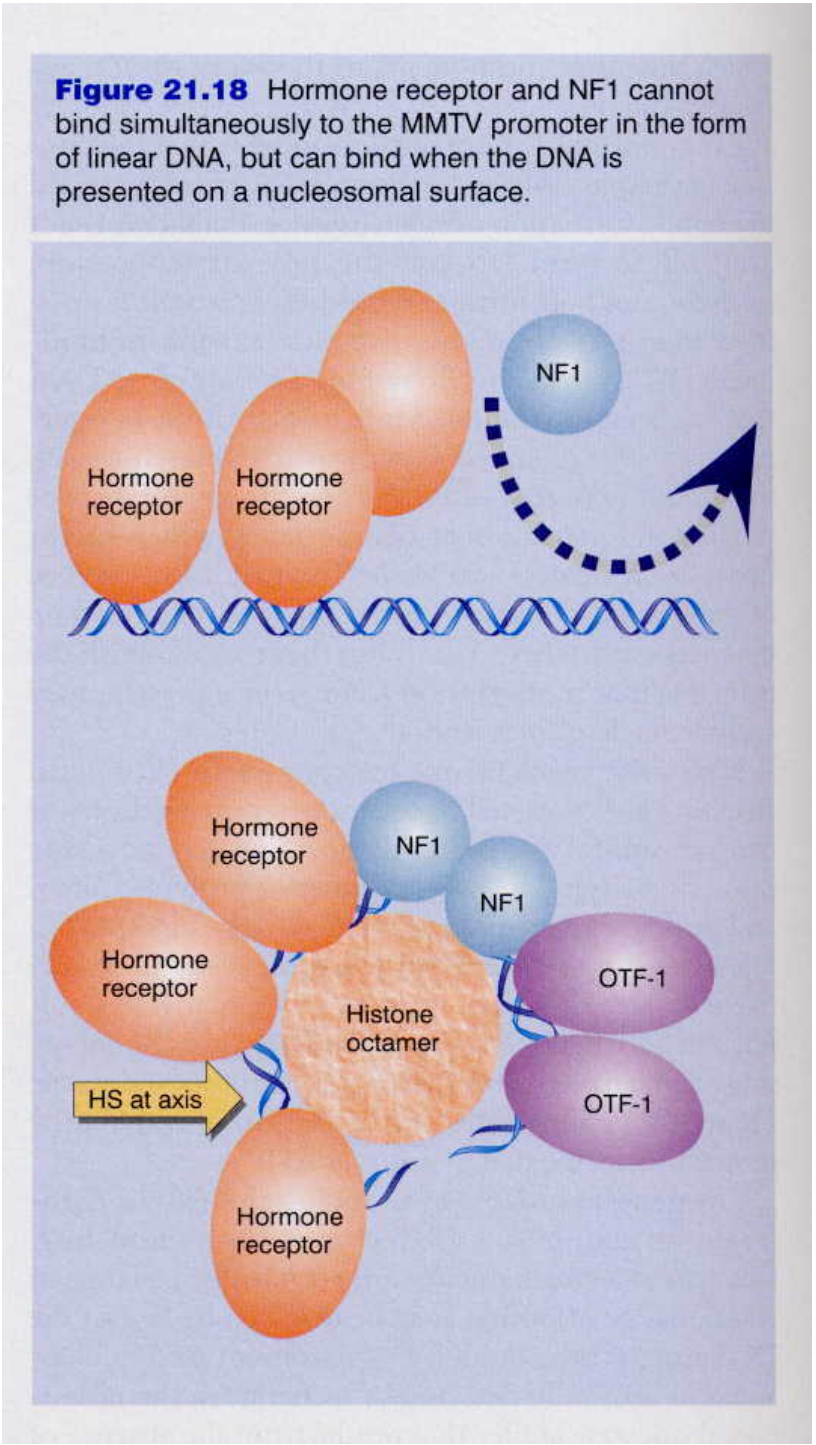


Figure 21.18 Hormone receptor and NF1 cannot bind simultaneously to the MMTV promoter in the form of linear DNA, but can bind when the DNA is presented on a nucleosomal surface.

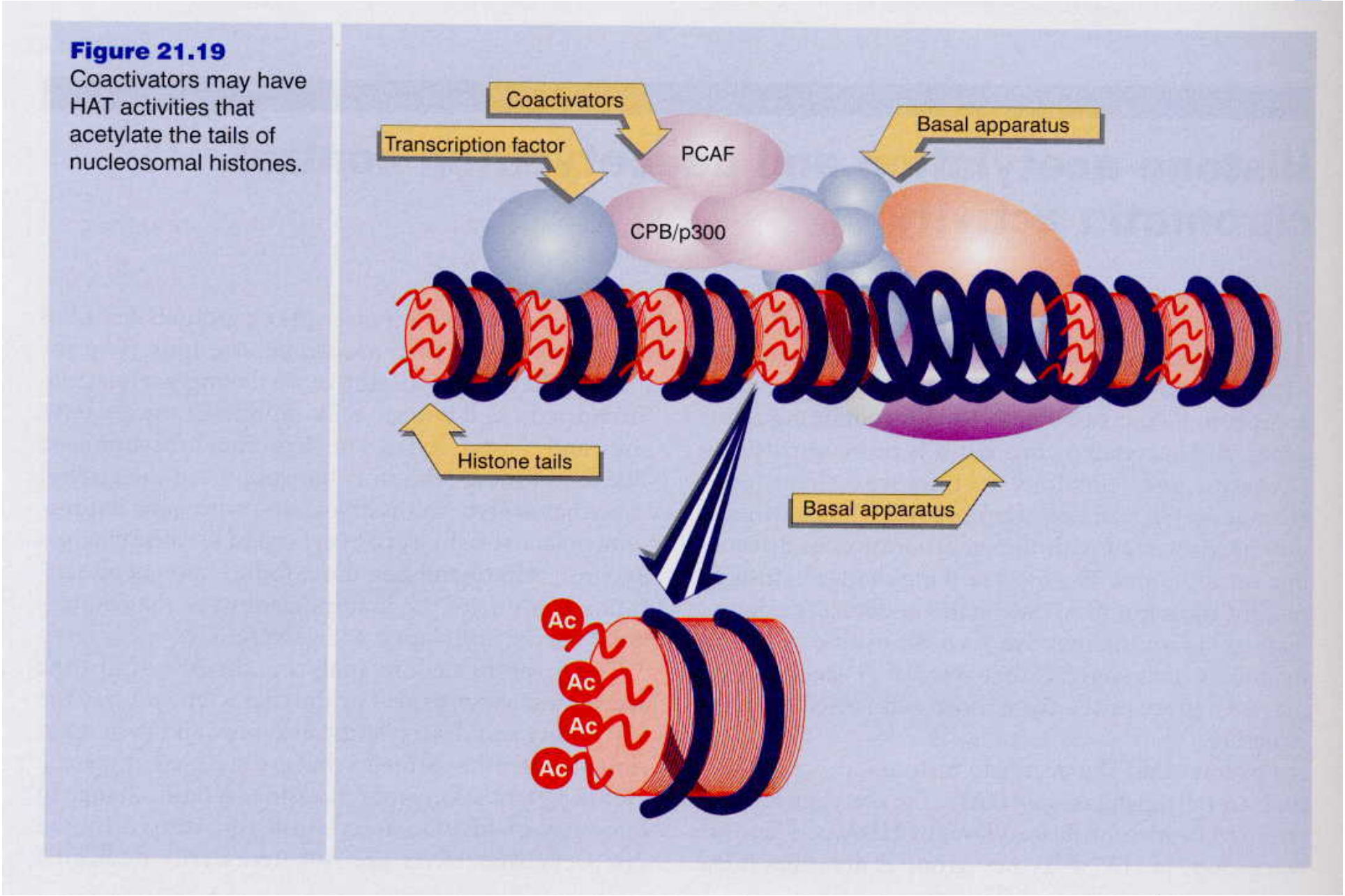


Figure 21.19
Coactivators may have HAT activities that acetylate the tails of nucleosomal histones.

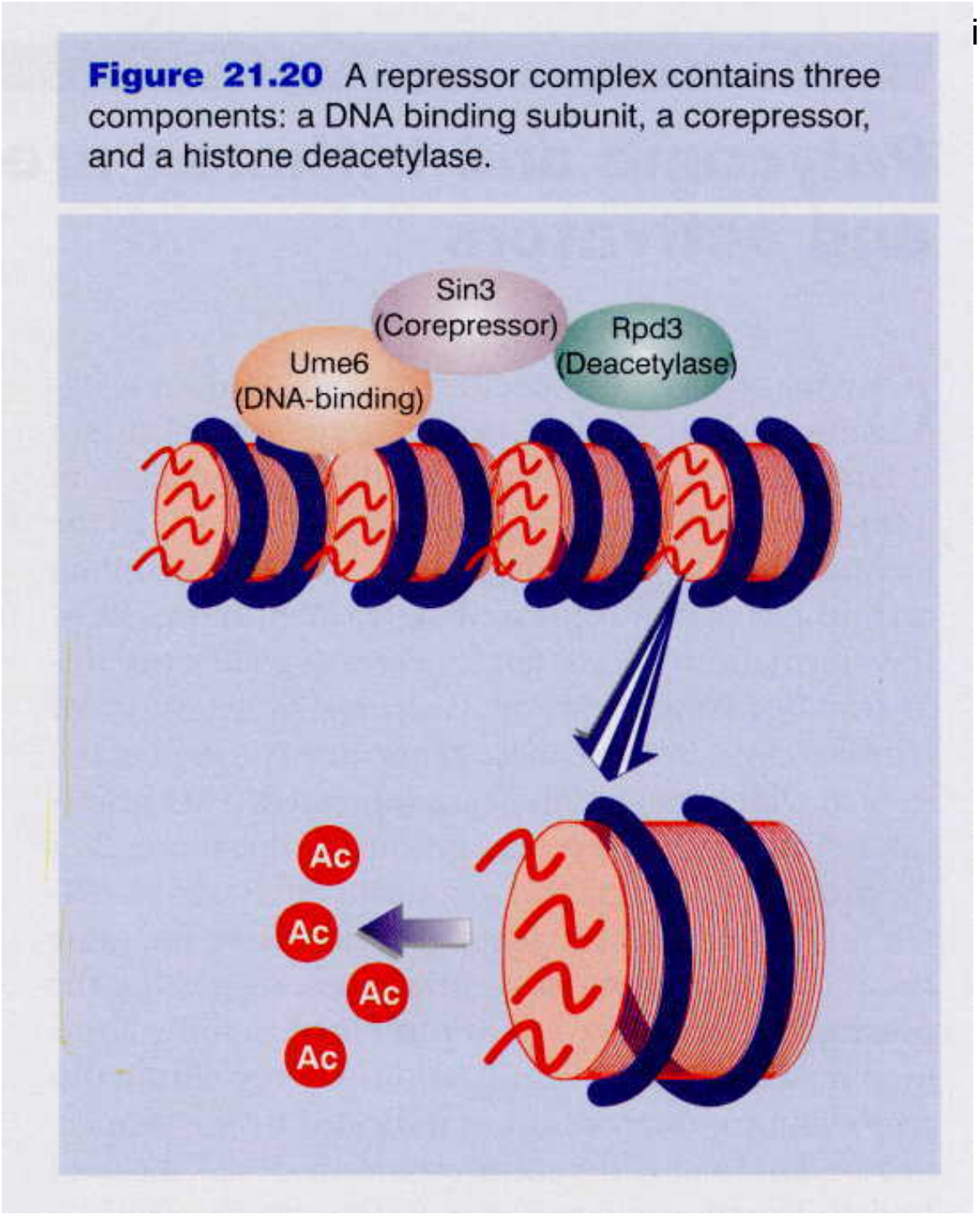


Figure 21.20 A repressor complex contains three components: a DNA binding subunit, a corepressor, and a histone deacetylase.

11.12.12

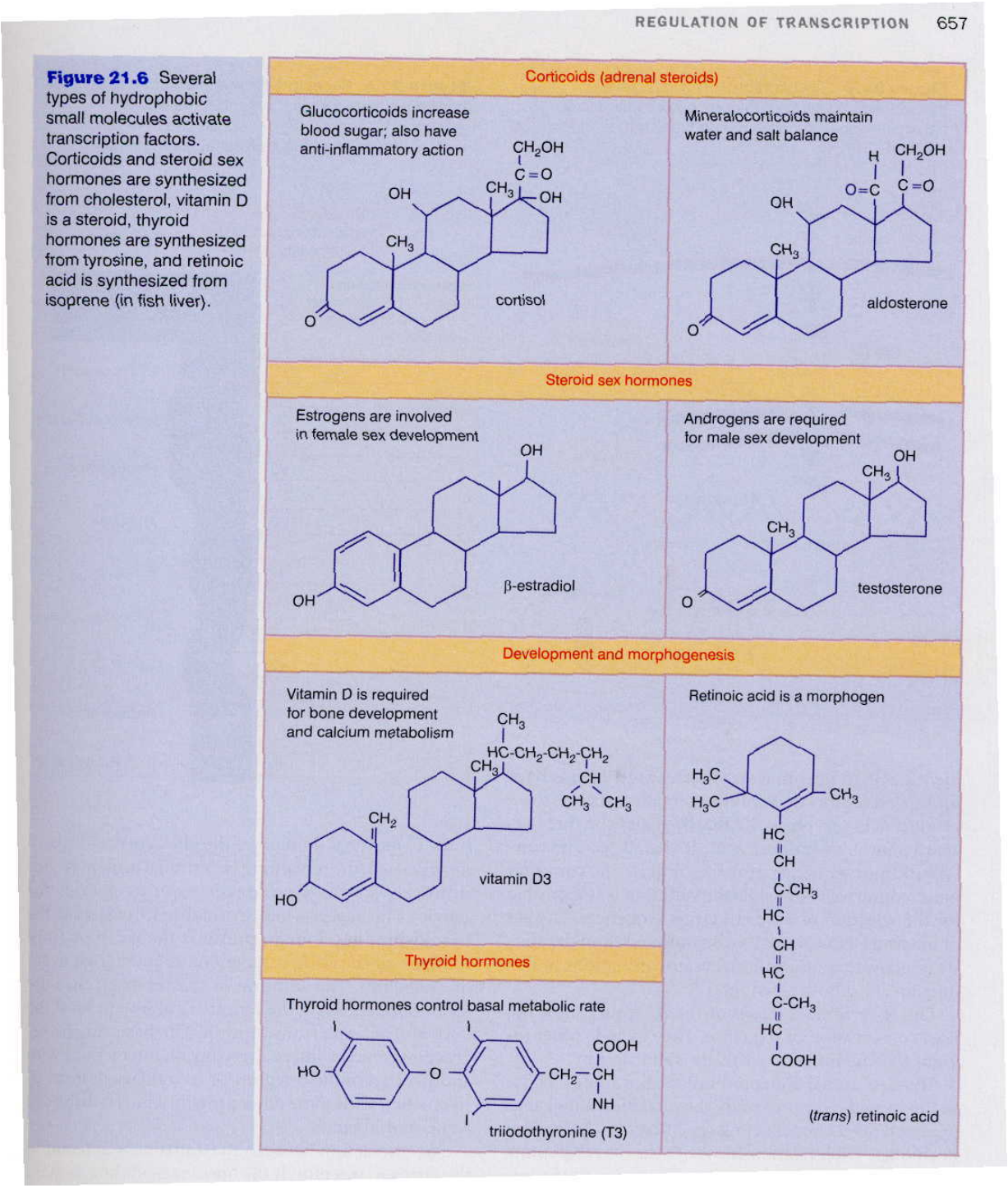


Figure 21.6 Several types of hydrophobic small molecules activate transcription factors. Corticoids and steroid sex hormones are synthesized from cholesterol, vitamin D is a steroid, thyroid hormones are synthesized from tyrosine, and retinoic acid is synthesized from isoprene (in fish liver).

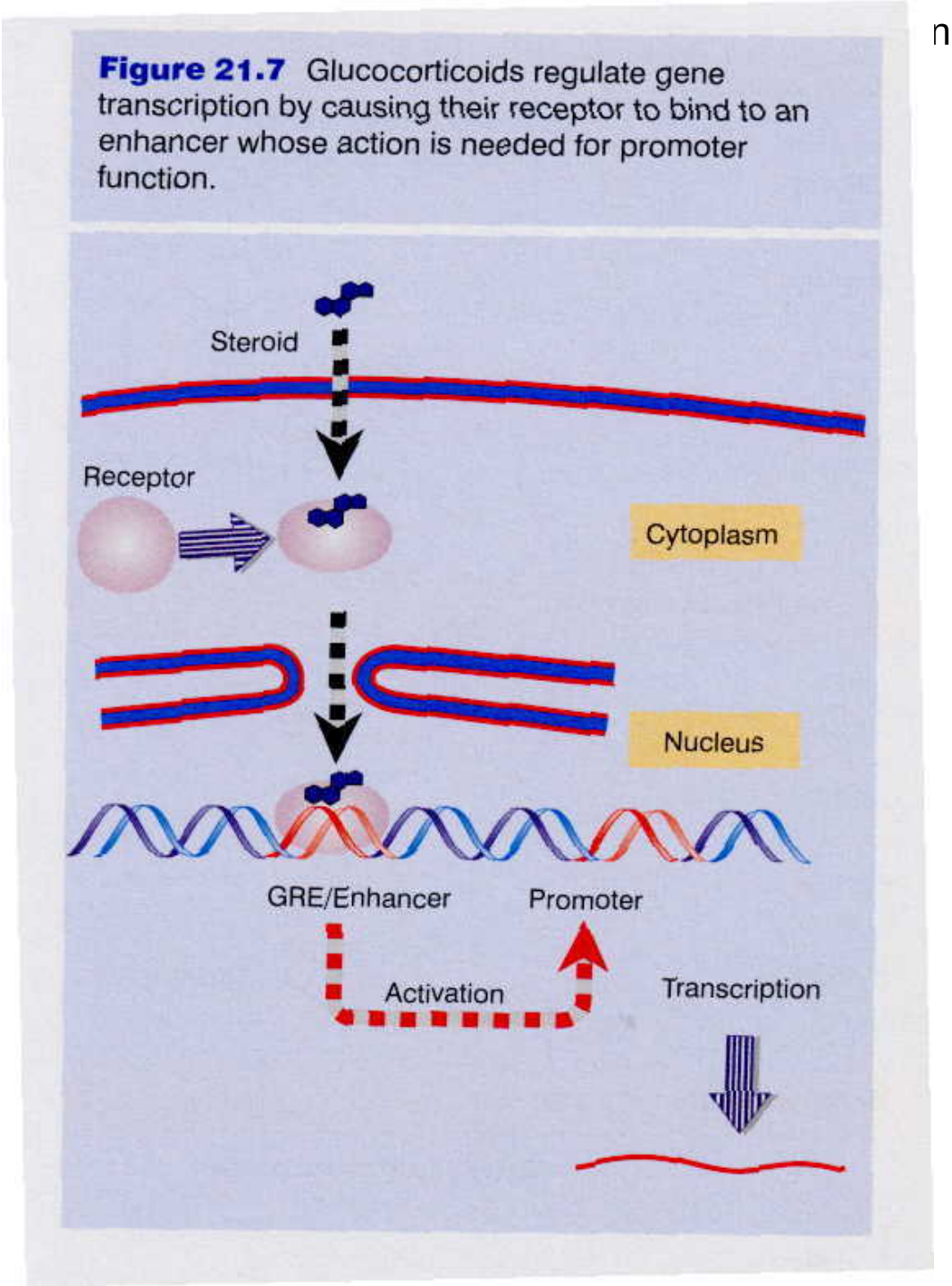


Figure 21.7 Glucocorticoids regulate gene transcription by causing their receptor to bind to an enhancer whose action is needed for promoter function.

Signal transduction

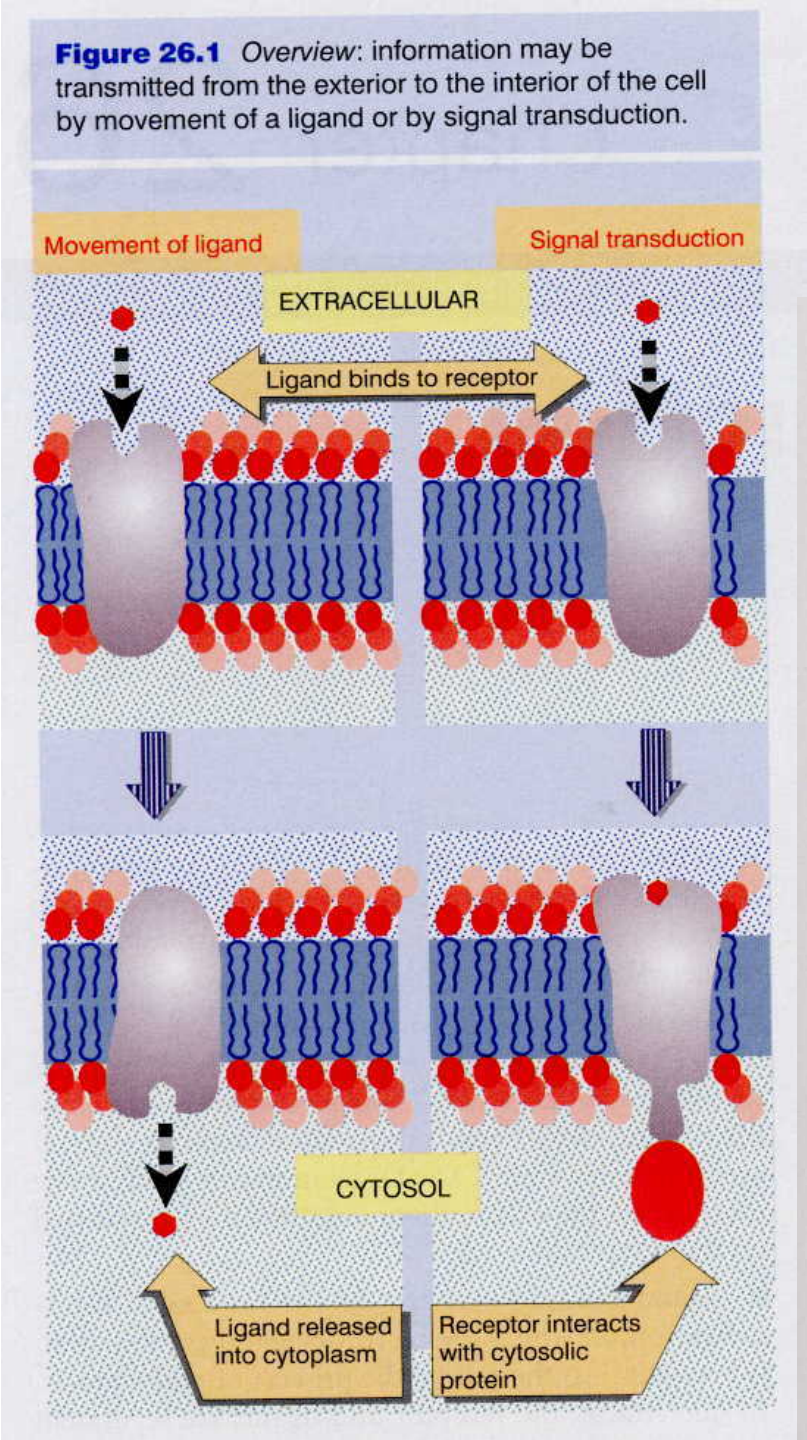


Figure 26.1 *Overview*: information may be transmitted from the exterior to the interior of the cell by movement of a ligand or by signal transduction.

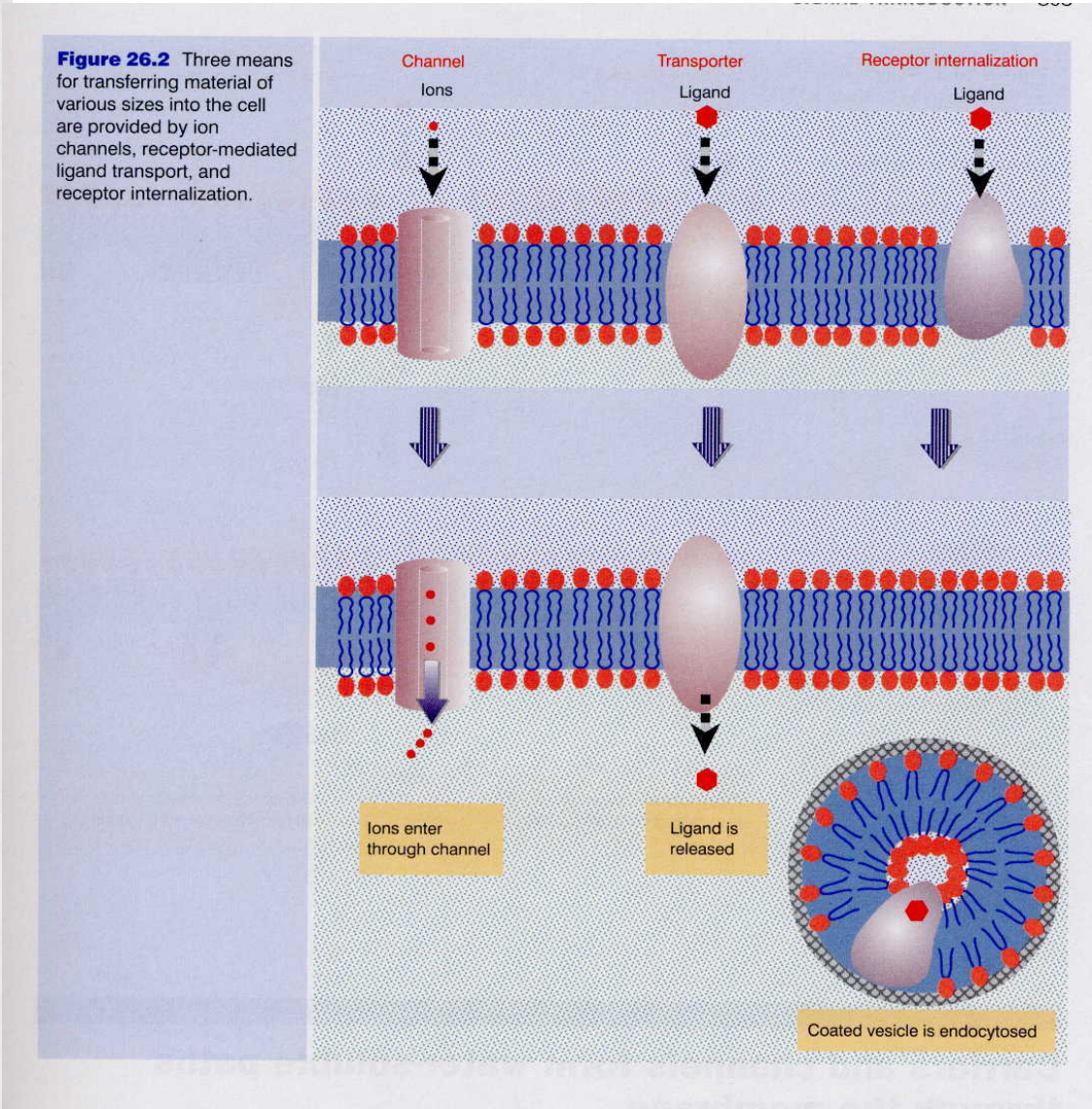


Figure 26.2 Three means for transferring material of various sizes into the cell are provided by ion channels, receptor-mediated ligand transport, and receptor internalization.

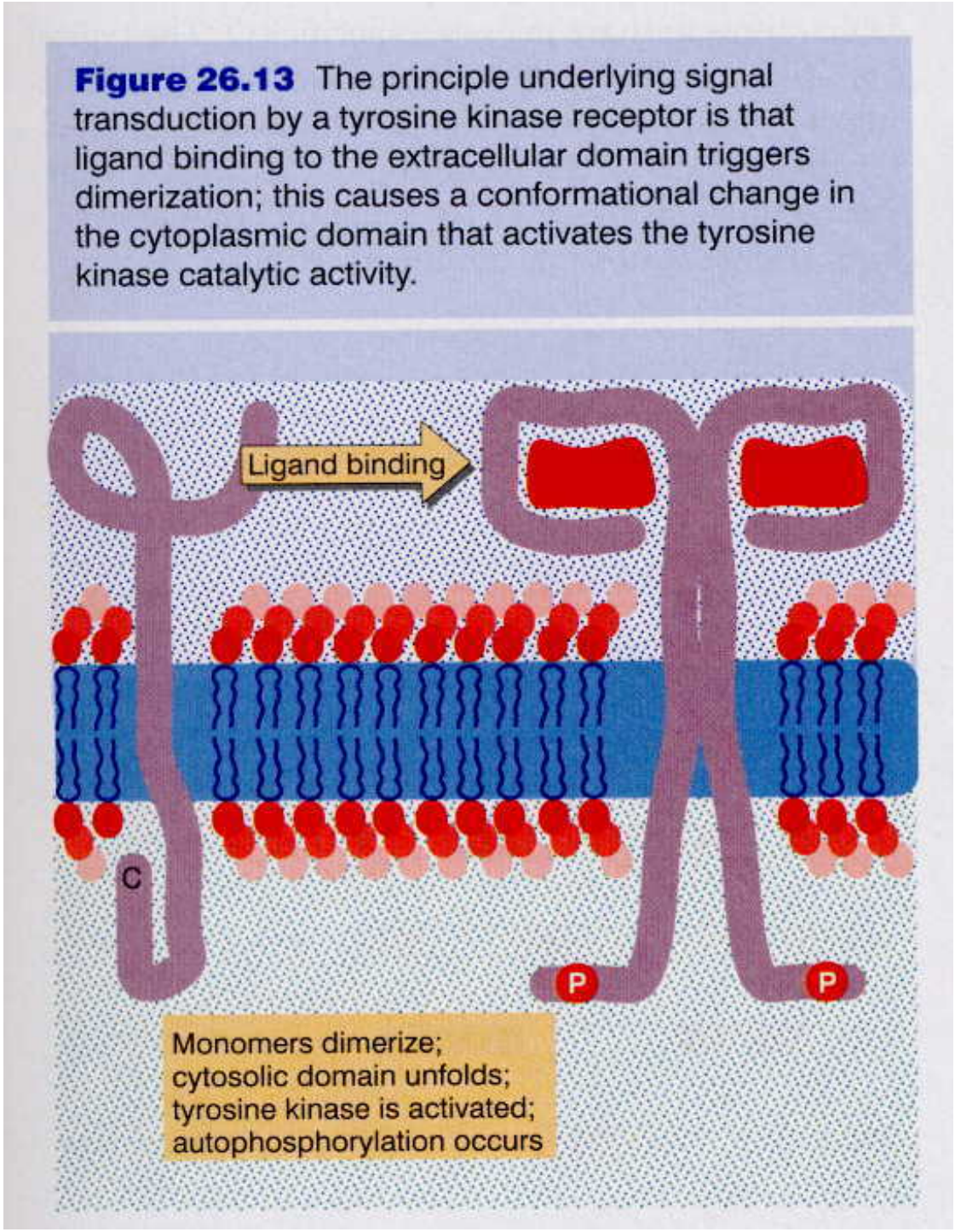


Figure 26.13 The principle underlying signal transduction by a tyrosine kinase receptor is that ligand binding to the extracellular domain triggers dimerization; this causes a conformational change in the cytoplasmic domain that activates the tyrosine kinase catalytic activity.

Figure 26.12 Effectors for receptor tyrosine kinases include phospholipases and kinases that act on lipids to generate second messengers.

Effector	Substrate	Products
PLC (phospolipase C) (3 families, PLCα, β, γ)	PIP2 (phosphatidylinositol 4,5-diphosphate)	DAG (diacylglycerol) + IP3 (inositol 1,4,5-triphosphate) DAG activates protein kinase C IP3 mobilizes Ca^{2+}
PLA2 (phospholipase A2)	Phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol)	Arachidonic acid Converted to prostaglandins & leukotrienes
PI3 kinase (phosphatidylinositol-3 kinase)	Phosphatidyl inositol	PI3 (phosphatidyl inositol-3 phosphate)
PI4 kinase (phosphatidylinositol-4 kinase)	Phosphatidyl inositol	PI4 (phosphatidyl inositol-4 phosphate) Converted to PIP2 (phosphatidyl diphosphate)

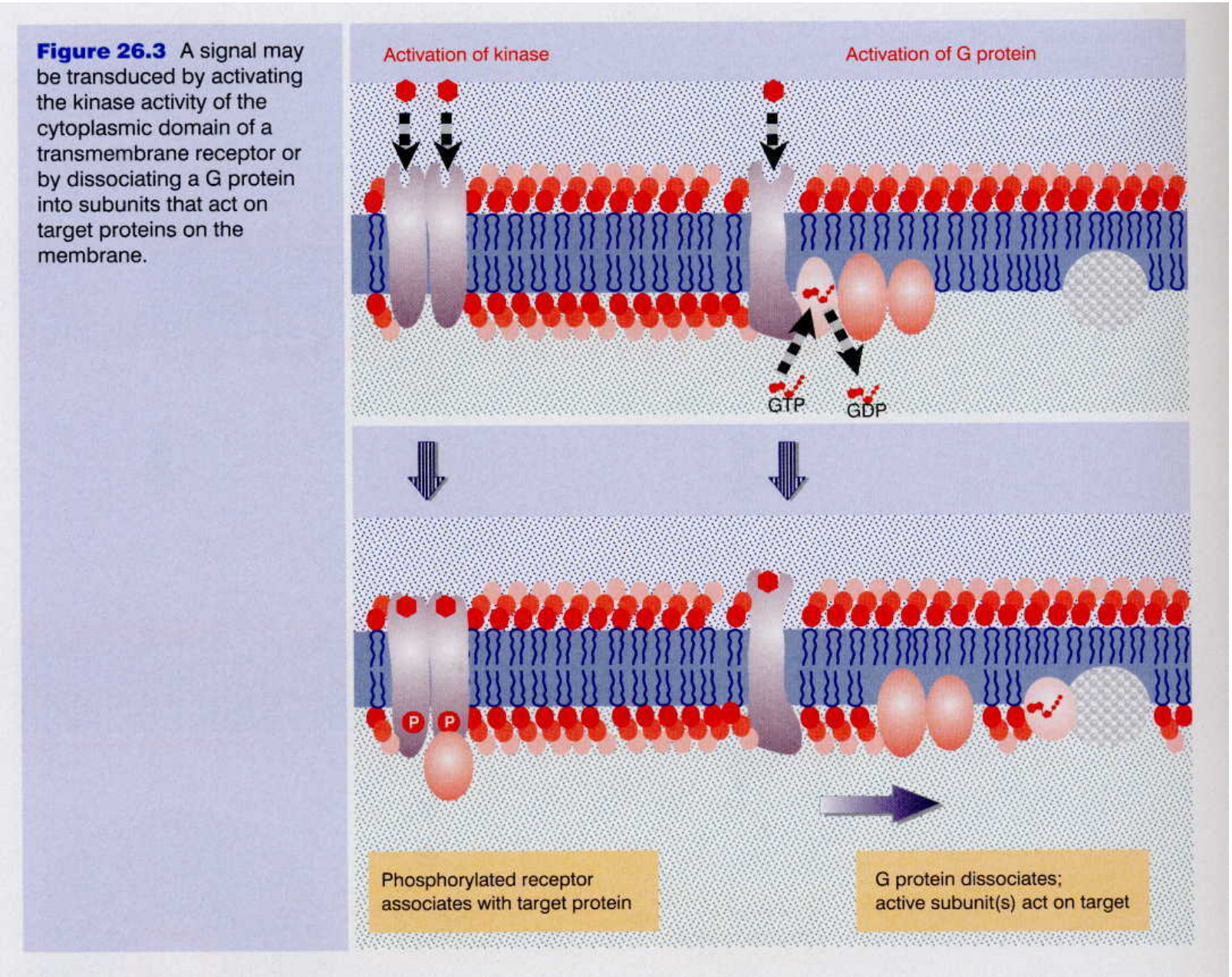


Figure 26.3 A signal may be transduced by activating the kinase activity of the cytoplasmic domain of a transmembrane receptor or by dissociating a G protein into subunits that act on target proteins on the membrane.

H.Schwab

Genetik und Gentechnik

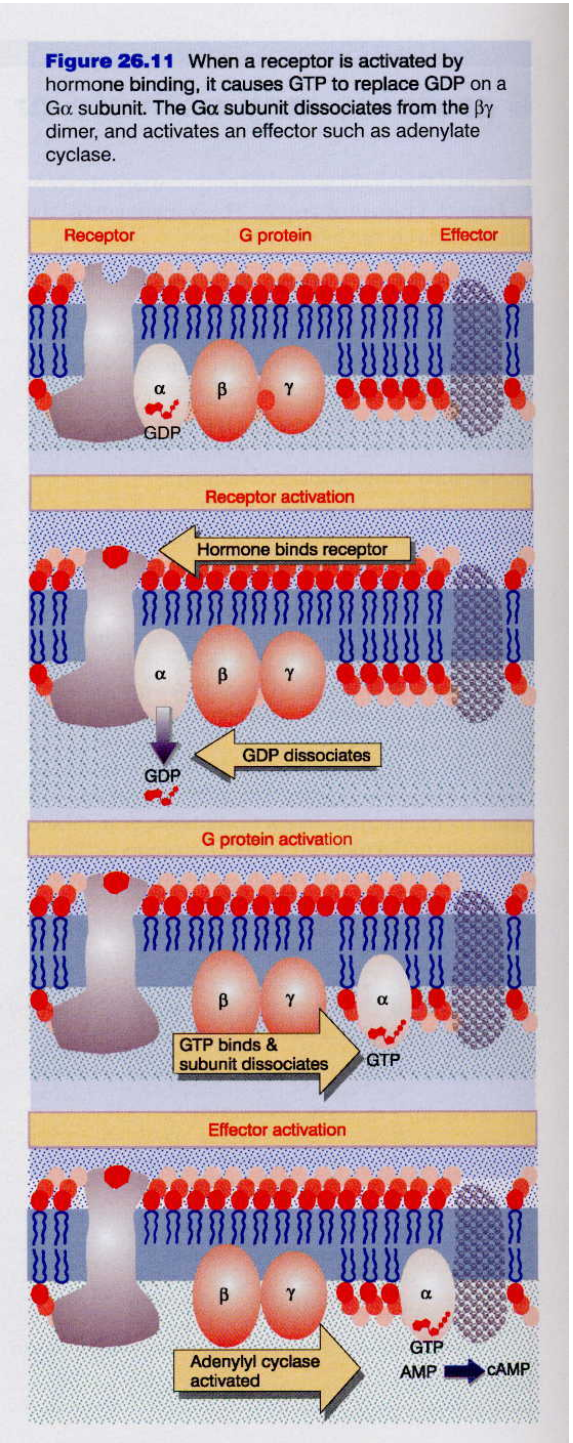


Figure 26.11 When a receptor is activated by hormone binding, it causes GTP to replace GDP on a Gα subunit. The Gα subunit dissociates from the βγ dimer, and activates an effector such as adenylate cyclase.

Figure 26.10 Classes of G proteins are distinguished by their effectors and are activated by a variety of transmembrane receptors.

G protein	Effector function	Second messenger	Example of receptor
s	Stimulates adenylyl cyclase	↑ cAMP	β-adrenergic
olf	Stimulates adenylyl cyclase	↑ cAMP	Odorant
i	Inhibits adenylate cyclase	↓ cAMP	Somatostatin
	Opens K^+ channels	↑ Membrane potential	Somatostatin
o	Closes Ca^{2+} channels	↓ Membrane potential	m2 acetylcholine
t (transducin)	Stimulates cGMP phosphodiesterase	↓ cGMP	Rhodopsin
q	Activates phospholipase Cb	↑ InsP3, DAG	m1 acetylcholine

Locus control region - LCR

Figure 21.22 A globin domain is marked by hypersensitive sites at either end. The group of sites at the 5' side constitutes the LCR and is essential for the function of all genes in the cluster.

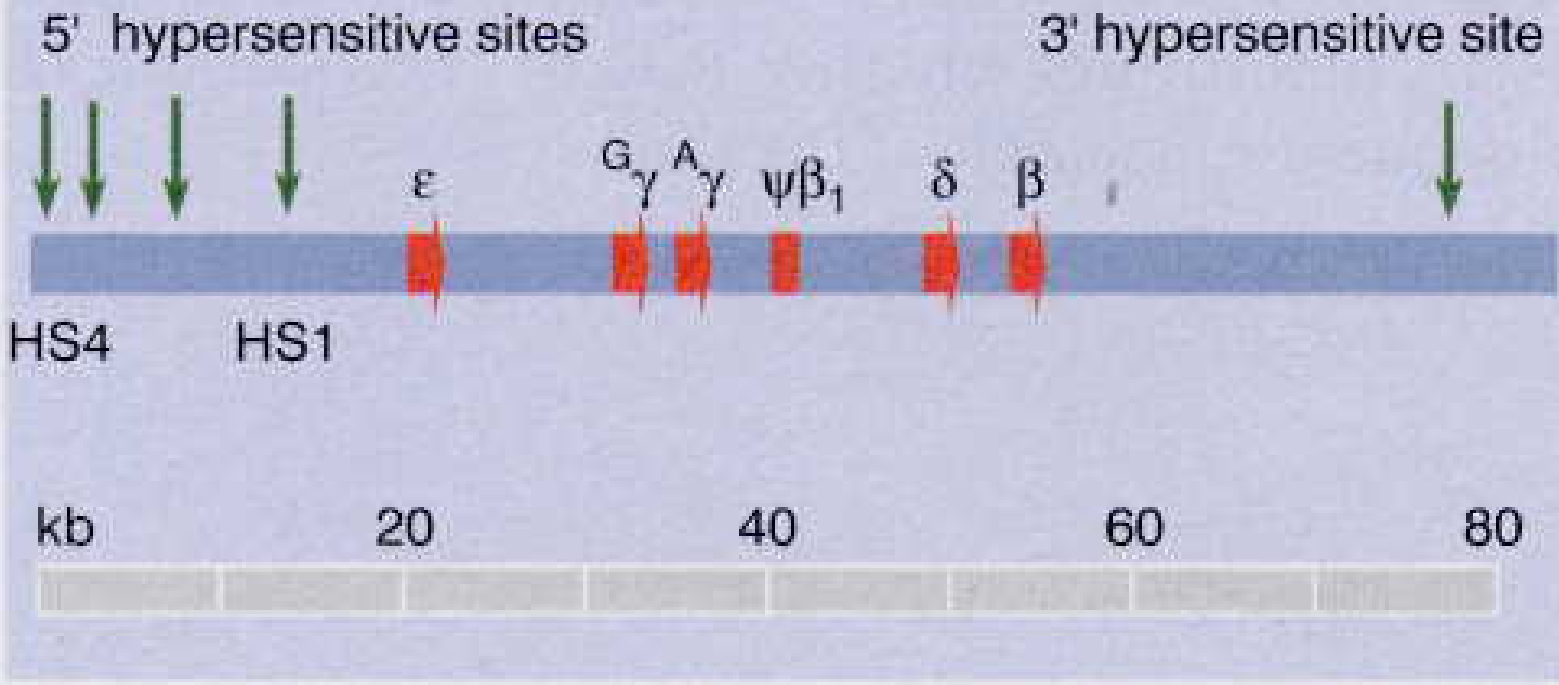


Figure 21.23 Specialized chromatin structures that include hypersensitive sites mark the ends of a domain in the *D. melanogaster* genome and insulate genes between them from the effects of surrounding sequences.

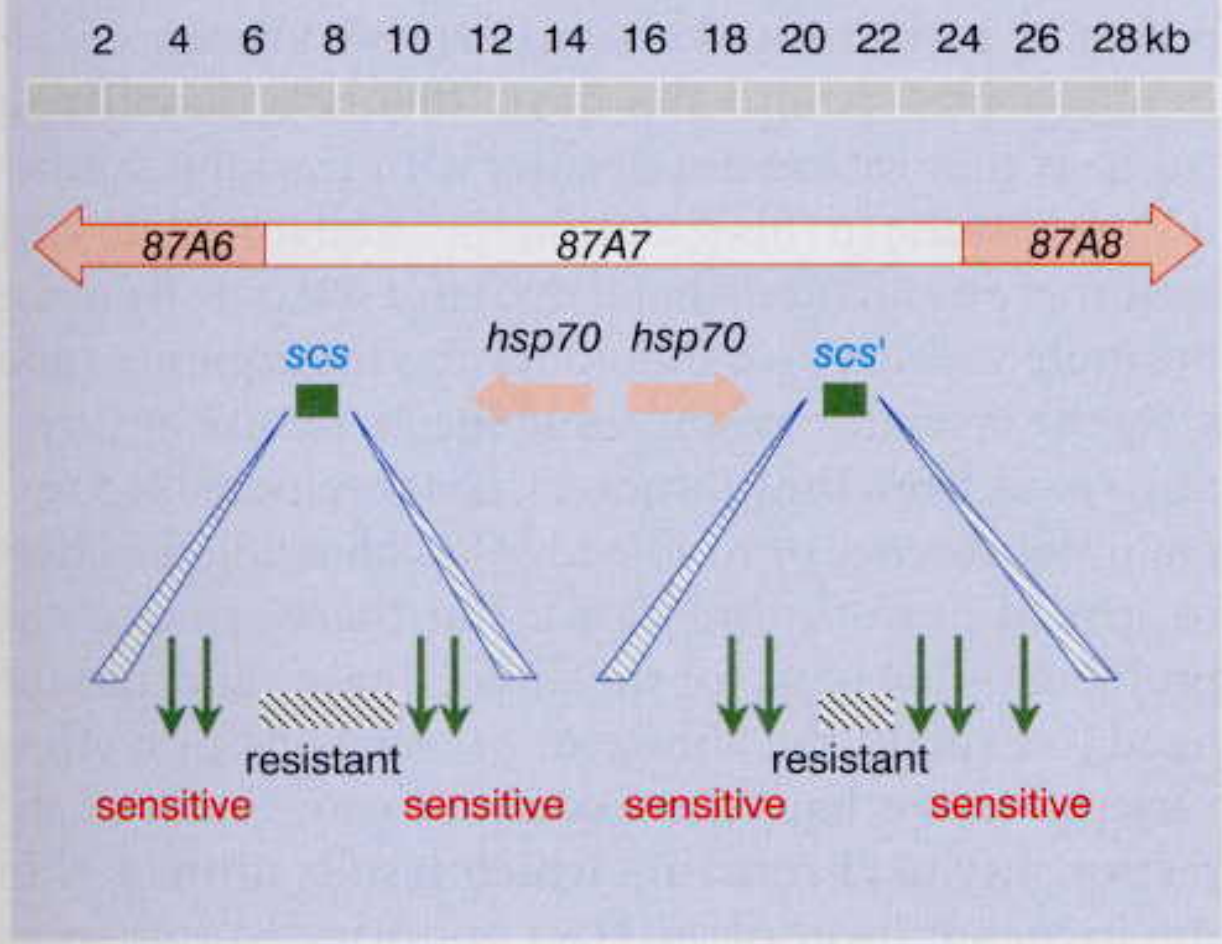
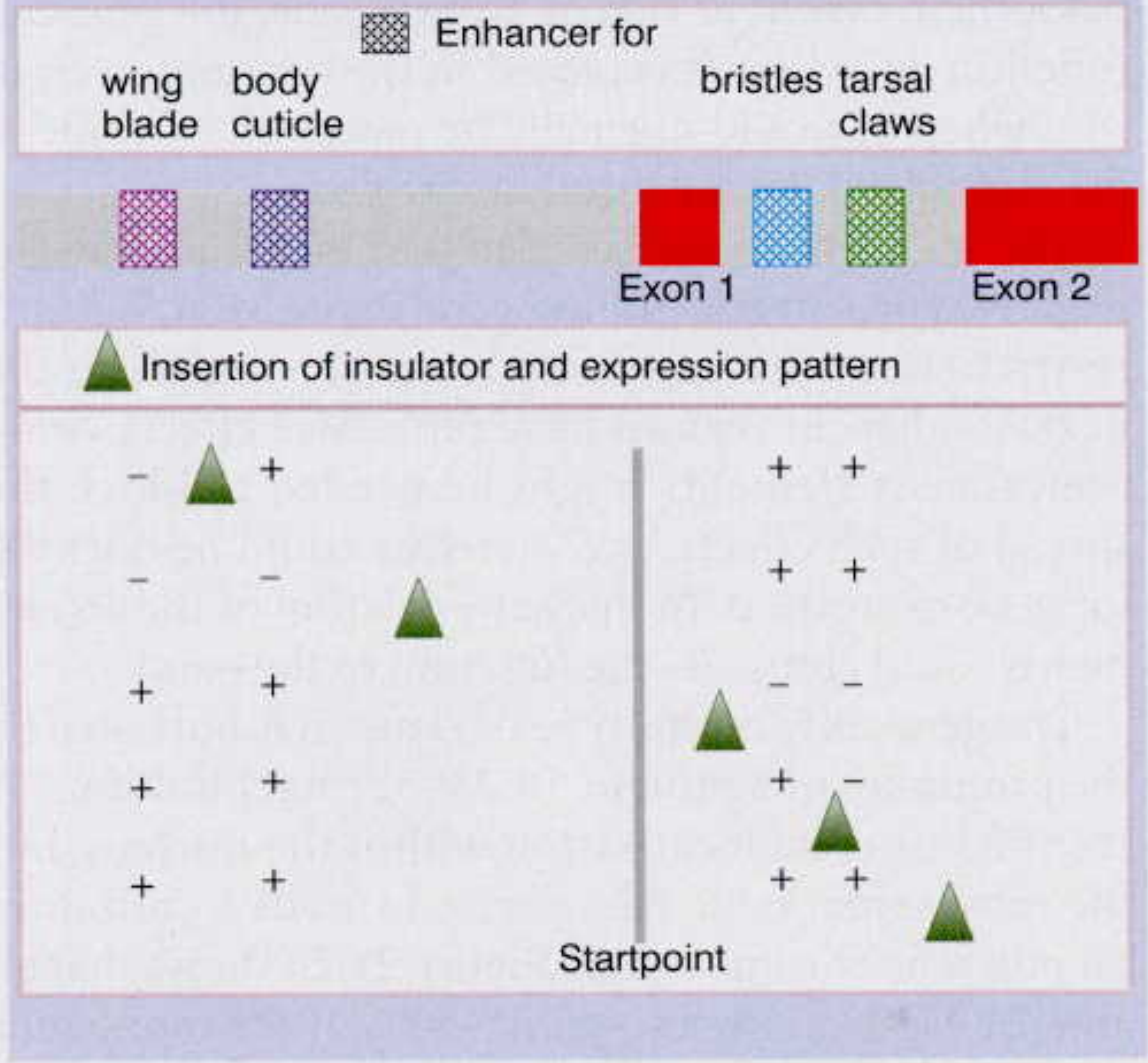
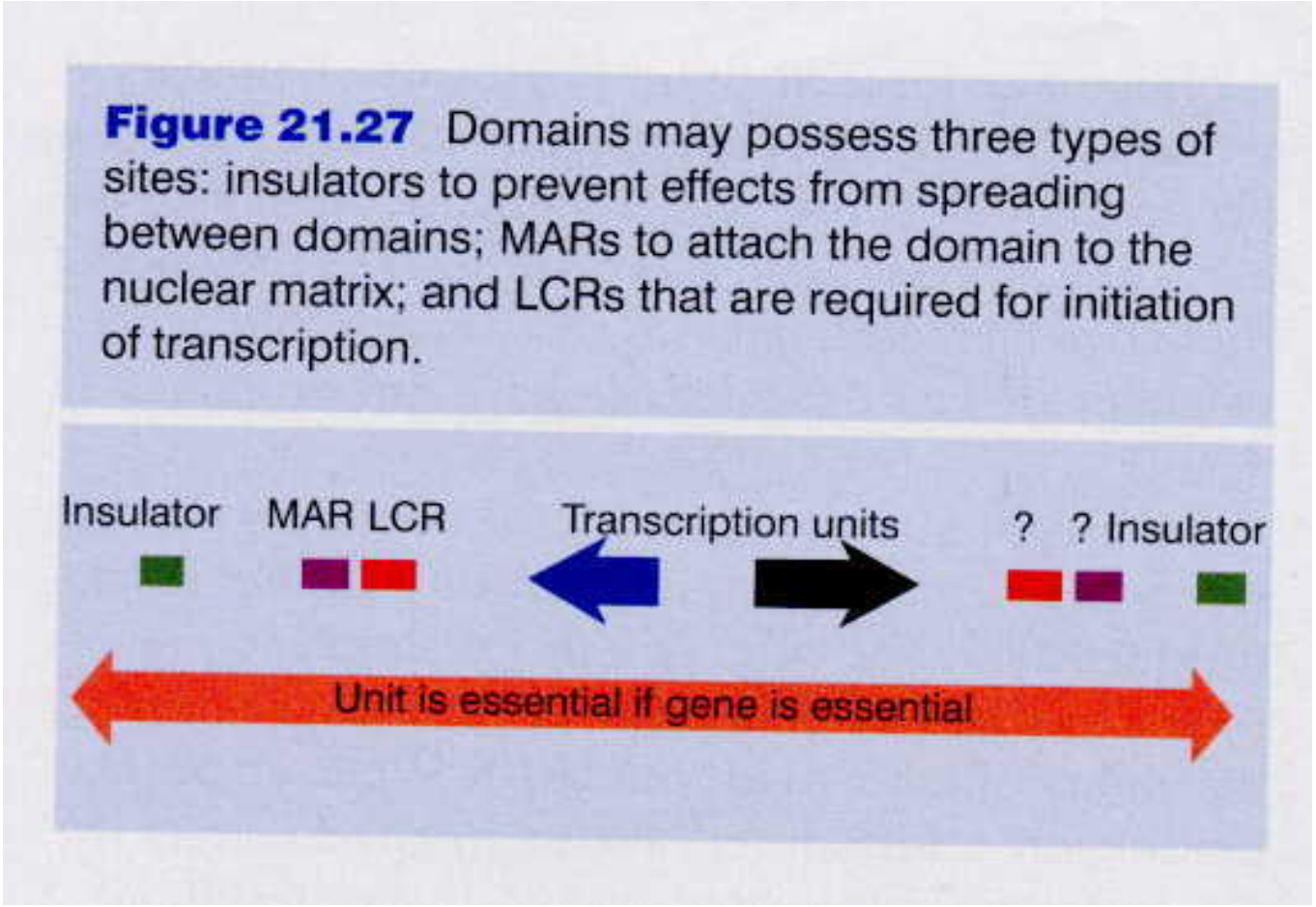


Figure 21.25 The insulator of the *gypsy* transposon blocks the action of an enhancer when it is placed between the enhancer and the promoter.





MAR: Matrix attachment site
LCR: Locus control region
Insulator: prevents influence from surrounding regions

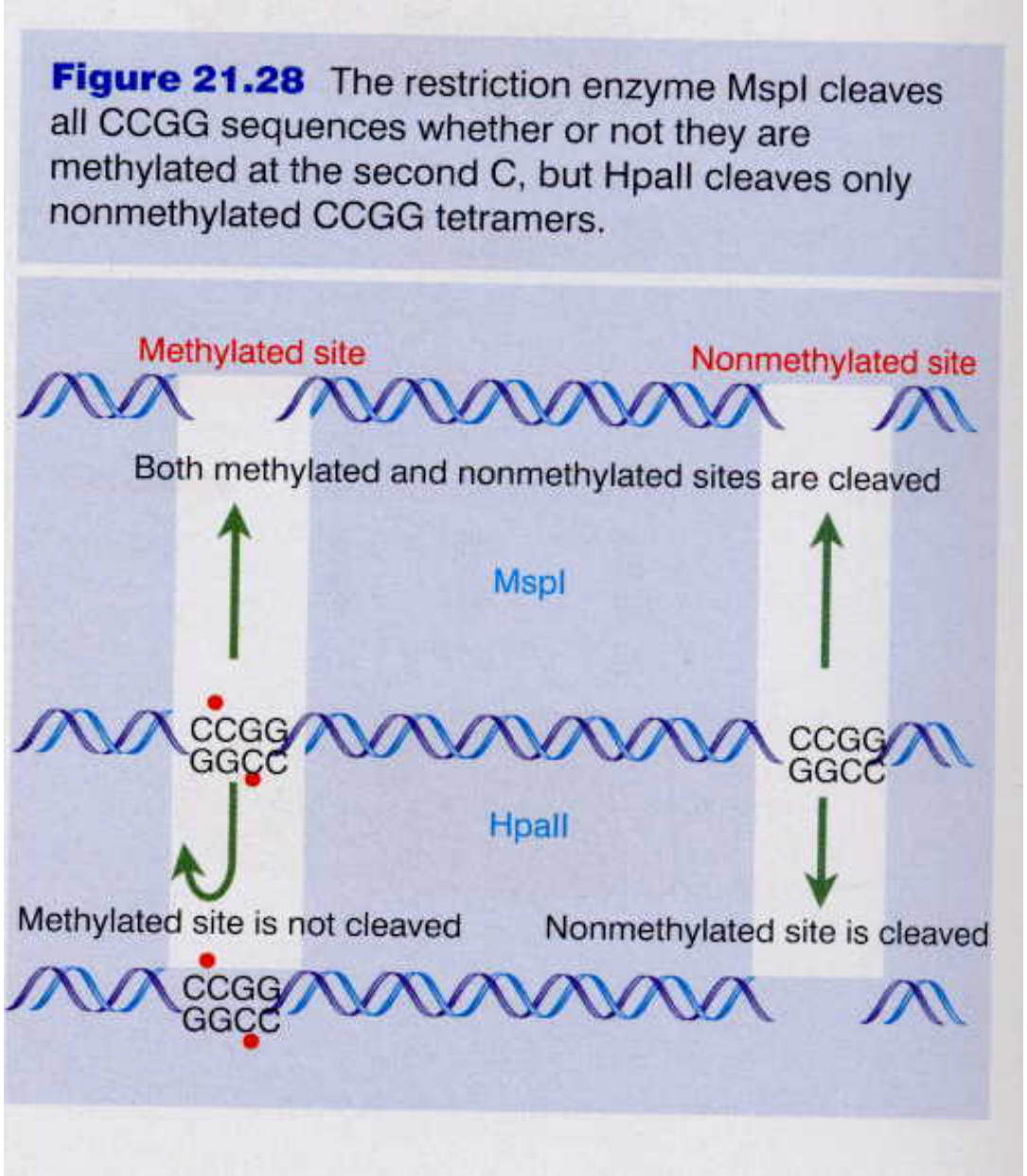


Figure 21.28 The restriction enzyme MspI cleaves all CCGG sequences whether or not they are methylated at the second C, but HpaII cleaves only nonmethylated CCGG tetramers.

Methylation of DNA influences Transcription

Generally:
high methylation – low expression

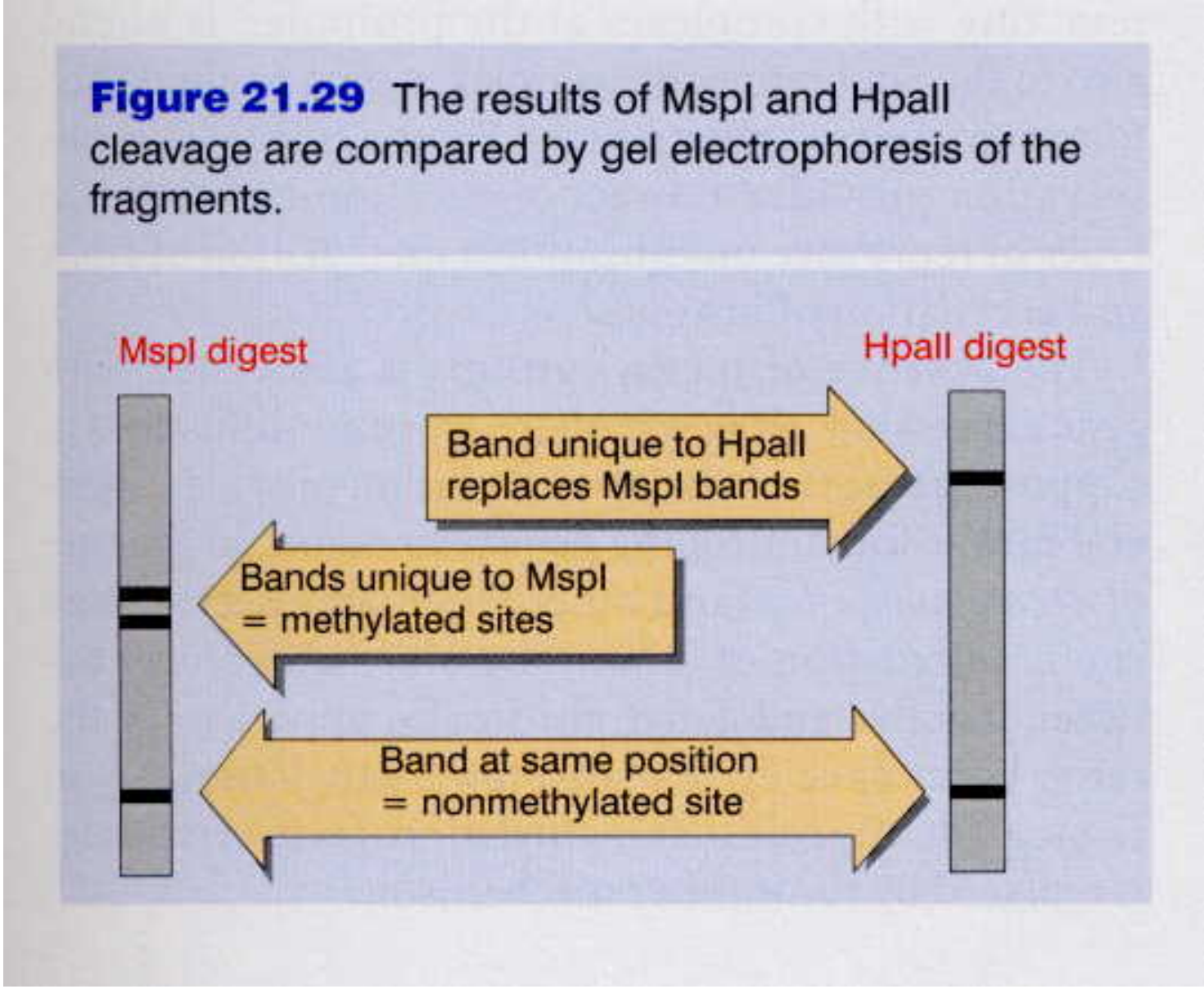


Figure 21.29 The results of MspI and HpaII cleavage are compared by gel electrophoresis of the fragments.

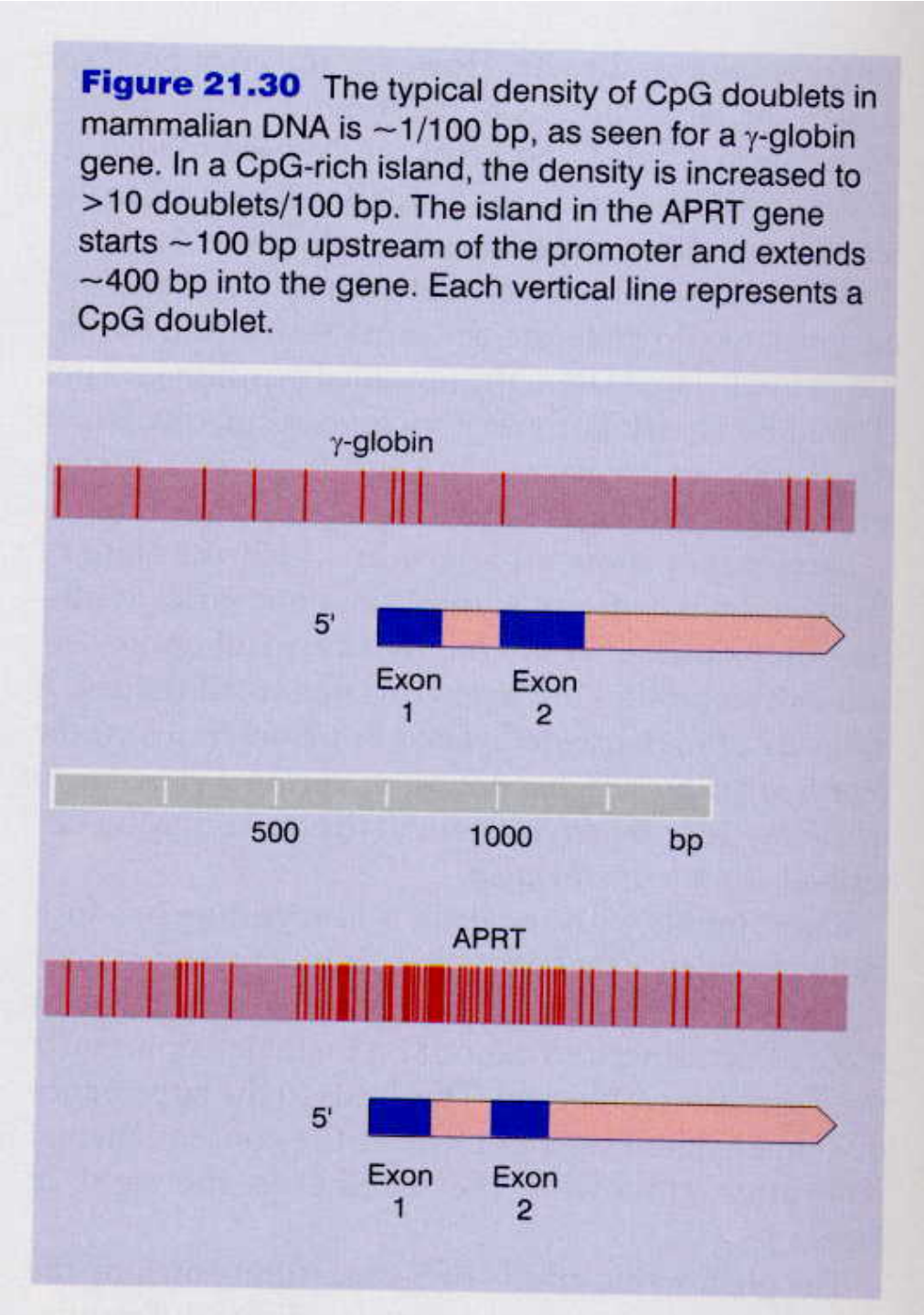


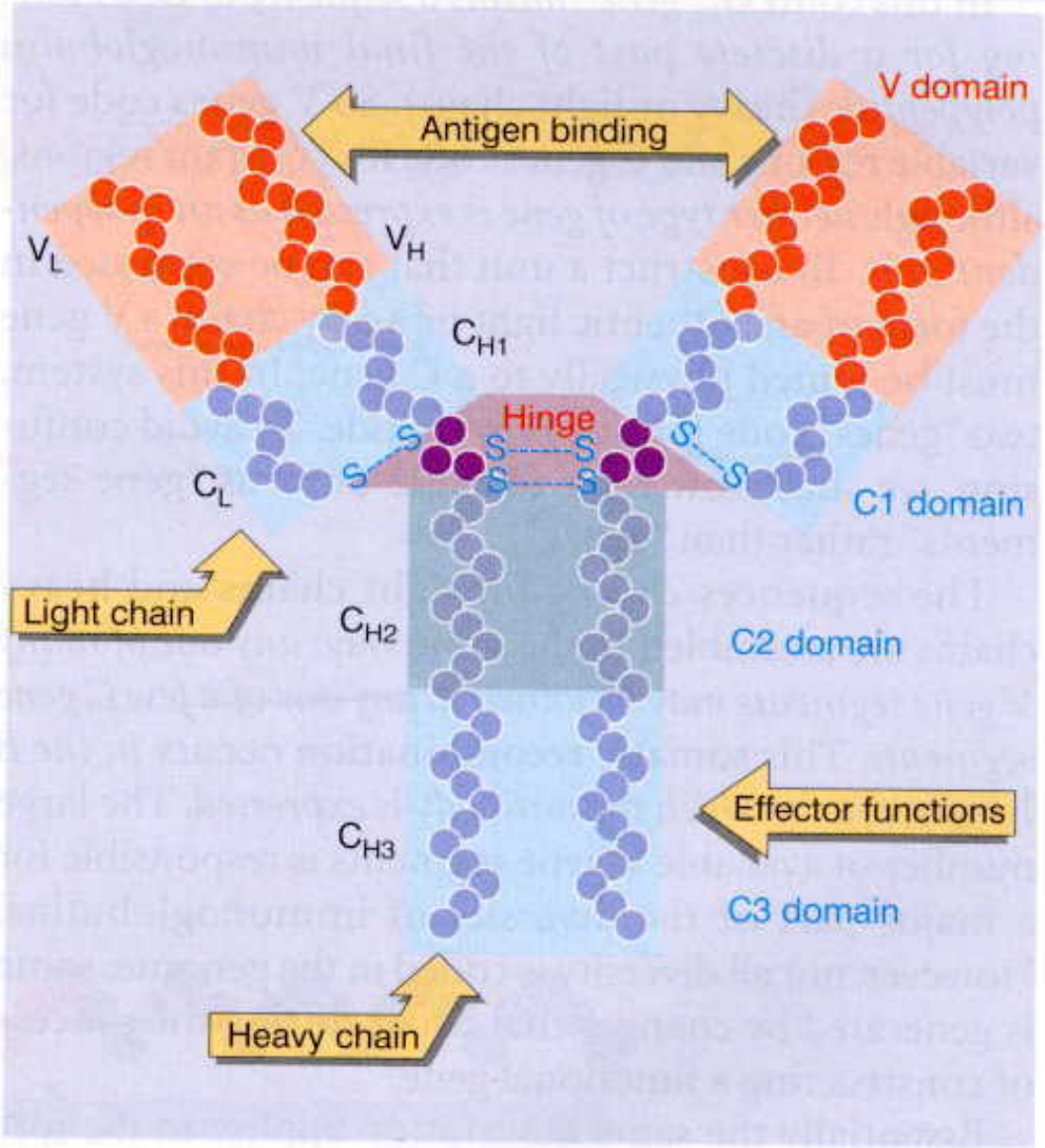
Figure 21.30 The typical density of CpG doublets in mammalian DNA is ~1/100 bp, as seen for a γ-globin gene. In a CpG-rich island, the density is increased to >10 doublets/100 bp. The island in the APRT gene starts ~100 bp upstream of the promoter and extends ~400 bp into the gene. Each vertical line represents a CpG doublet.

Determination of Gene Function by DNA Rearrangements

Figure 24.17 Immunoglobulin type and function is determined by the heavy chain. J is a 'joining protein' in IgM; all other Ig types exist as tetramers.

Type	IgM	IgD	IgG	IgA	IgE
Heavy chain	μ	δ	γ	α	ε
Structure	$(\mu_2L_2)_5J$	δ_2L_2	γ_2L_2	$(\alpha_2L_2)_2J$	ε_2L_2
Proportion	5%	1%	80%	14%	<1%
Effector function	Activates complement	Development of tolerance (?)	Activates complement	Found in secretions	Allergic response

Figure 24.4 Heavy and light chains combine to generate an immunoglobulin with several discrete domains.



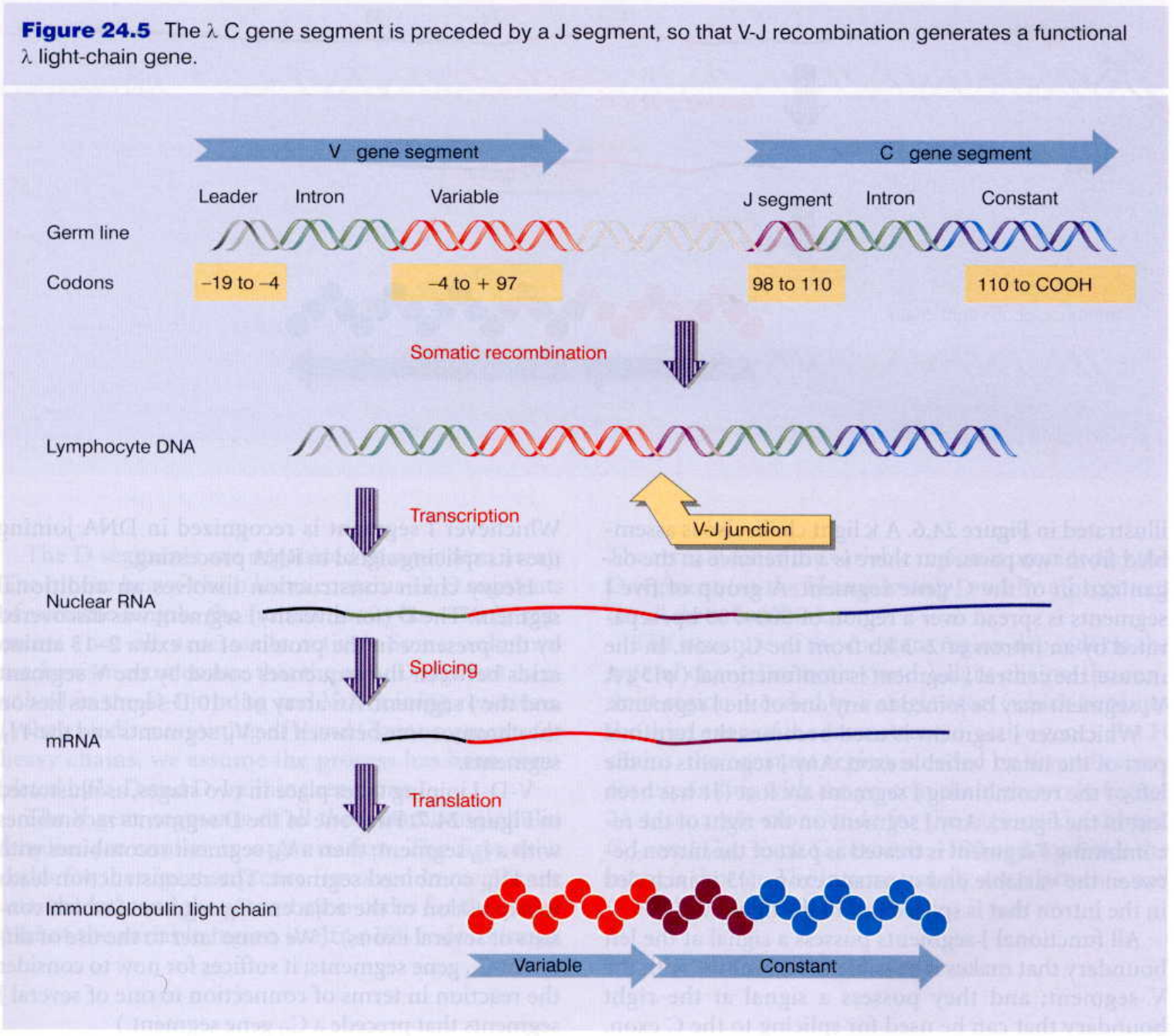


Figure 24.5 The λ C gene segment is preceded by a J segment, so that V-J recombination generates a functional λ light-chain gene.

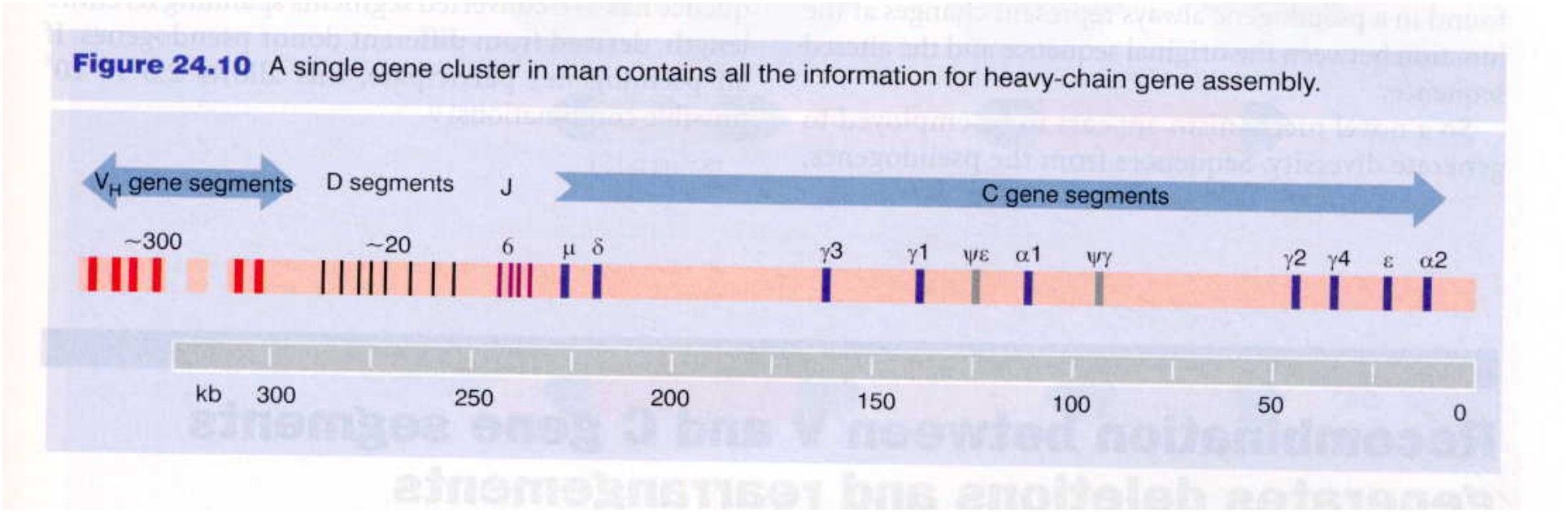


Figure 24.10 A single gene cluster in man contains all the information for heavy-chain gene assembly.

Regulation of expression by DNA rearrangements

Yeast mating type switching

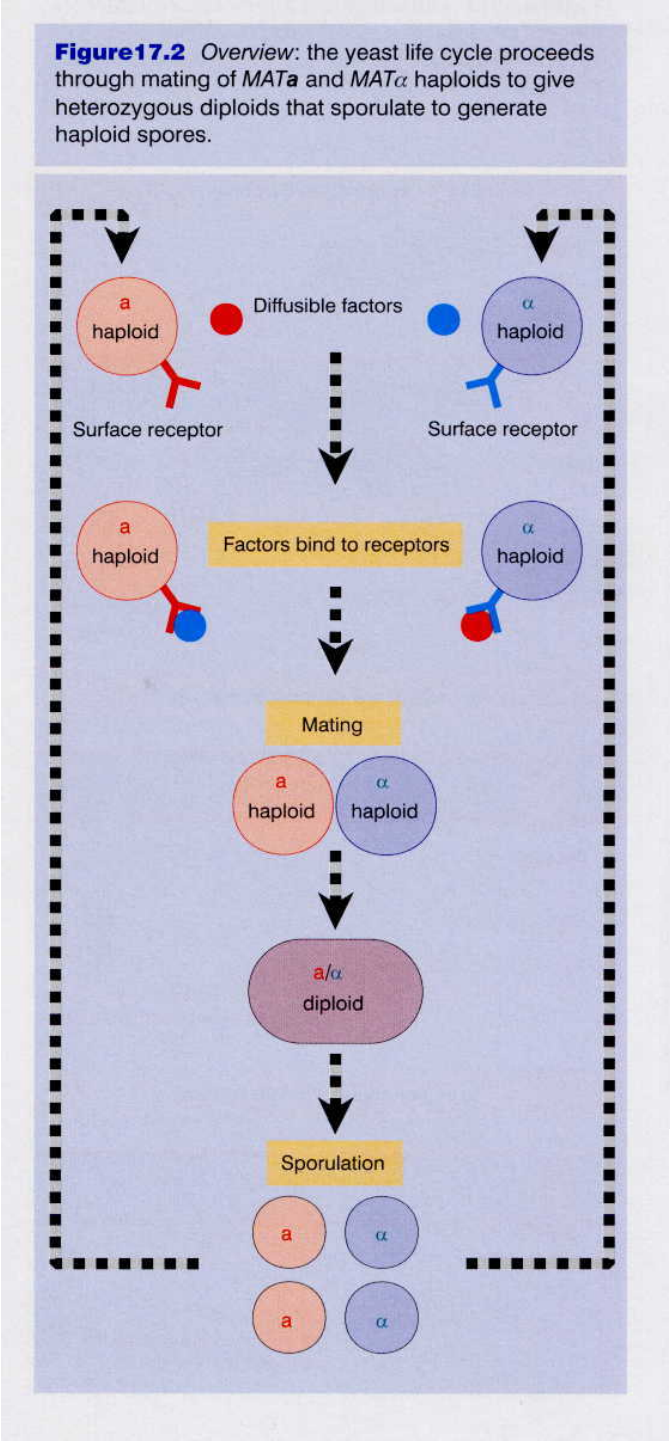


Figure17.2 *Overview*: the yeast life cycle proceeds through mating of *MAT**a*** and *MATα* haploids to give heterozygous diploids that sporulate to generate haploid spores.

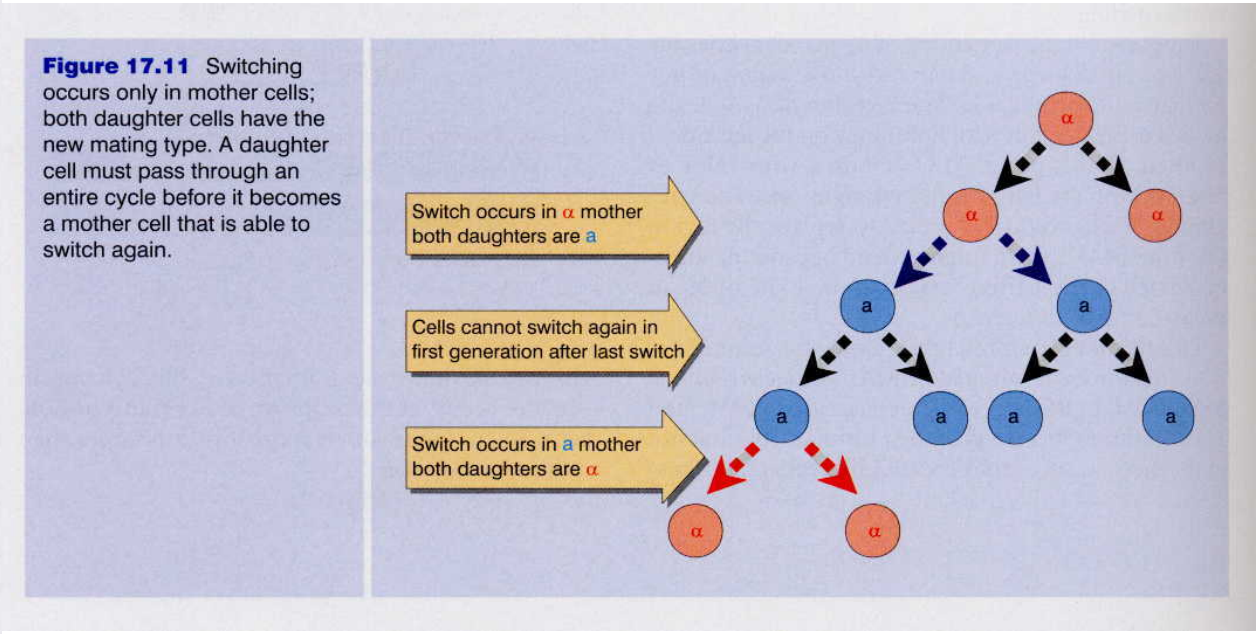


Figure 17.11 Switching occurs only in mother cells; both daughter cells have the new mating type. A daughter cell must pass through an entire cycle before it becomes a mother cell that is able to switch again.

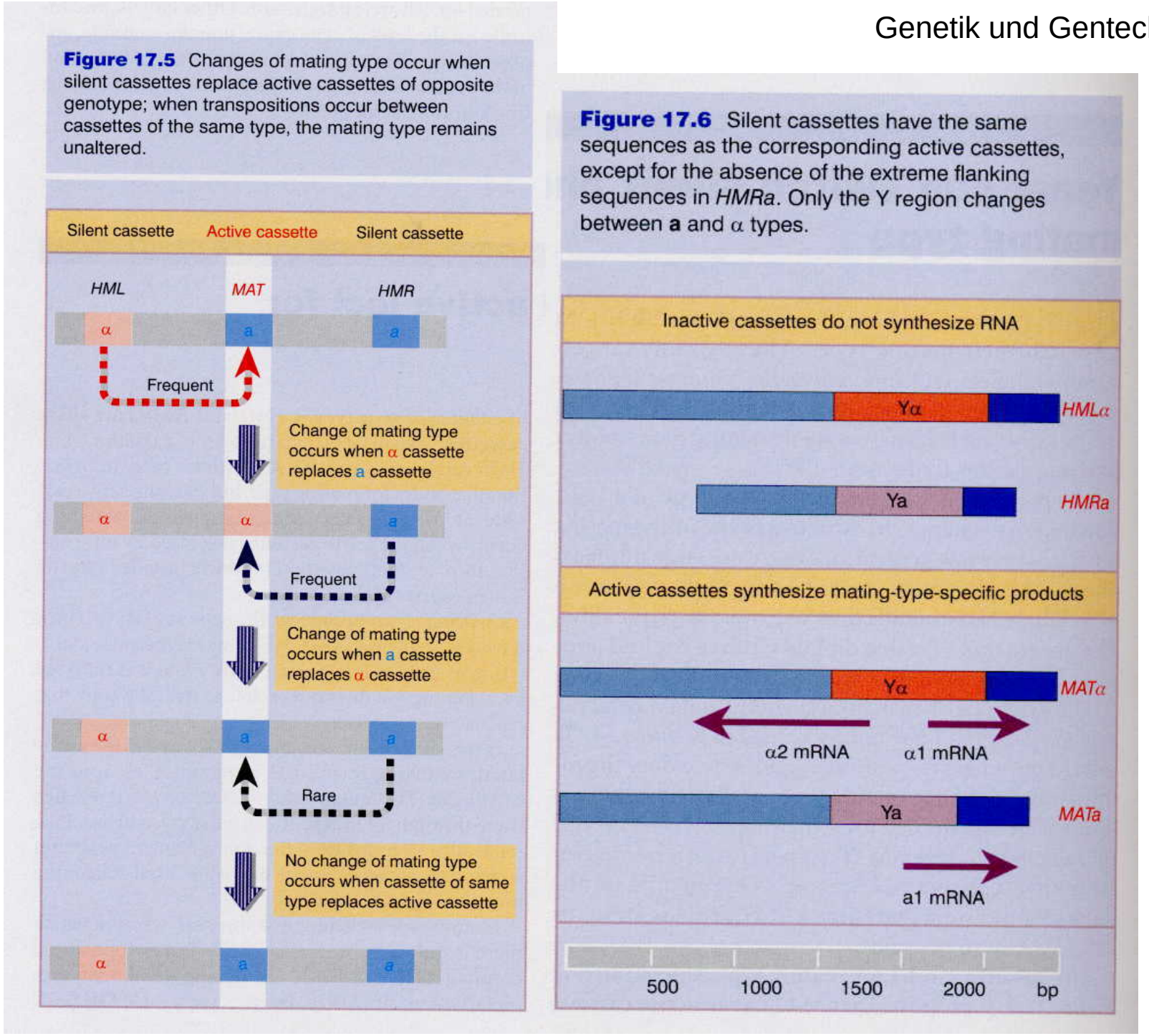


Figure 17.5 Changes of mating type occur when silent cassettes replace active cassettes of opposite genotype; when transpositions occur between cassettes of the same type, the mating type remains unaltered.

Figure 17.6 Silent cassettes have the same sequences as the corresponding active cassettes, except for the absence of the extreme flanking sequences in *HMRa*. Only the Y region changes between **a** and α types.

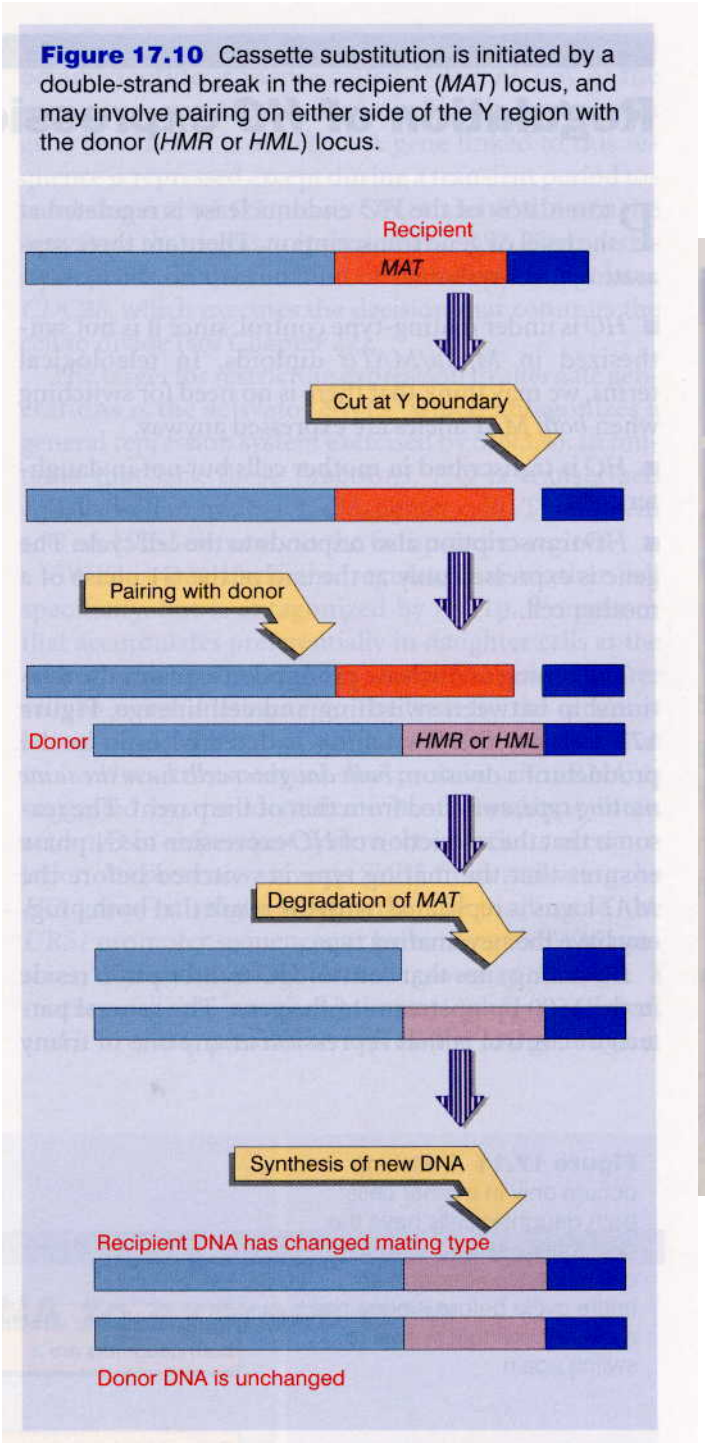


Figure 17.10 Cassette substitution is initiated by a double-strand break in the recipient (*MAT*) locus, and may involve pairing on either side of the Y region with the donor (*HMR* or *HML*) locus.

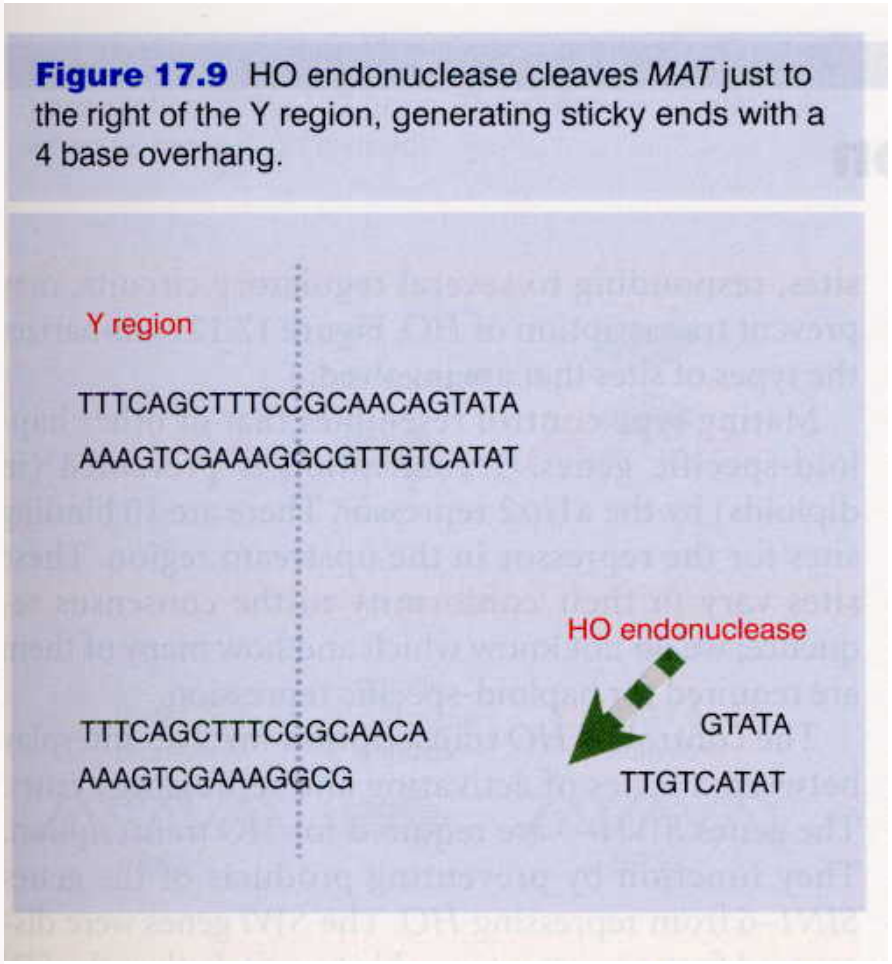


Figure 17.9 HO endonuclease cleaves *MAT* just to the right of the Y region, generating sticky ends with a 4 base overhang.

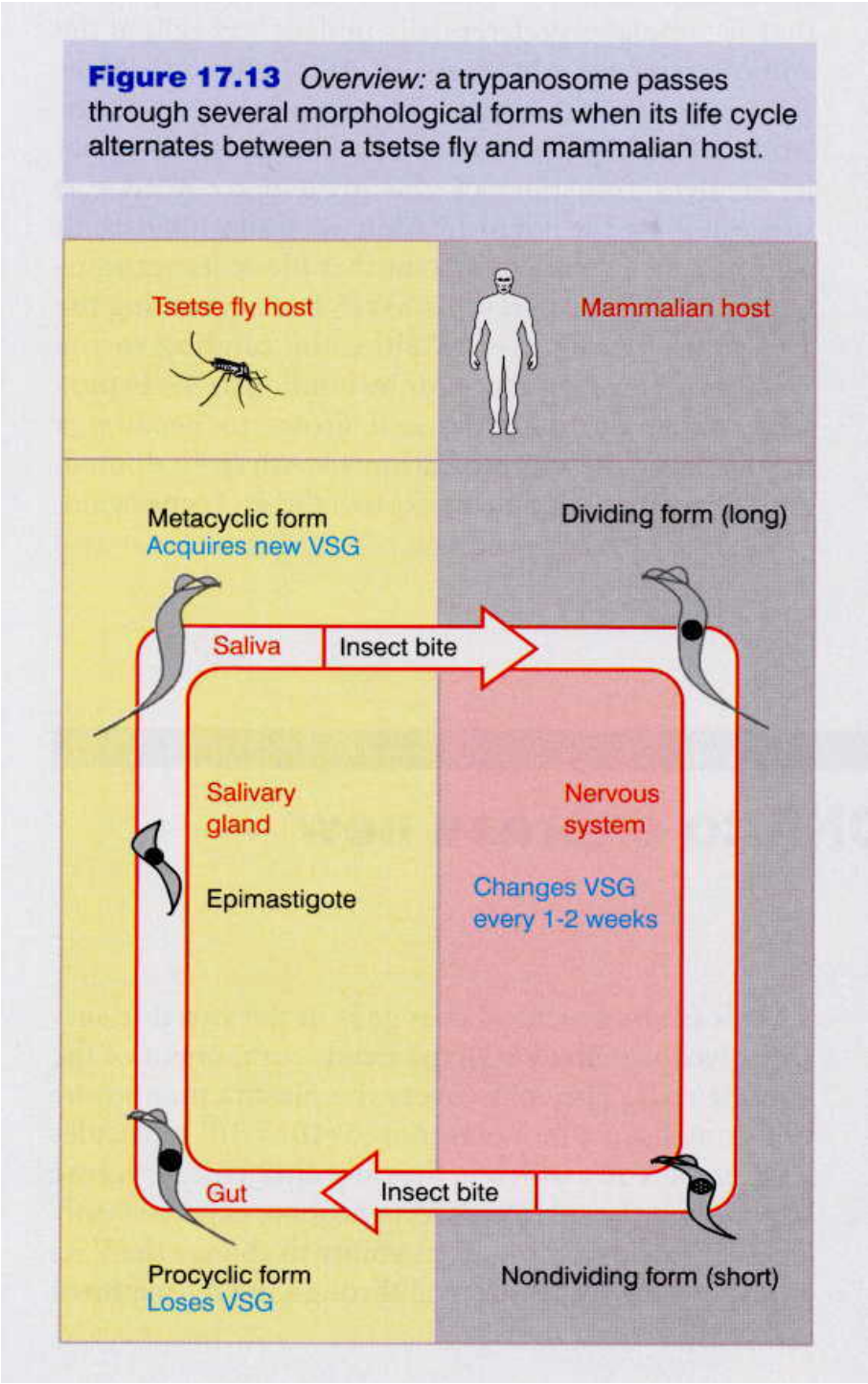


Figure 17.13 *Overview:* a trypanosome passes through several morphological forms when its life cycle alternates between a tsetse fly and mammalian host.

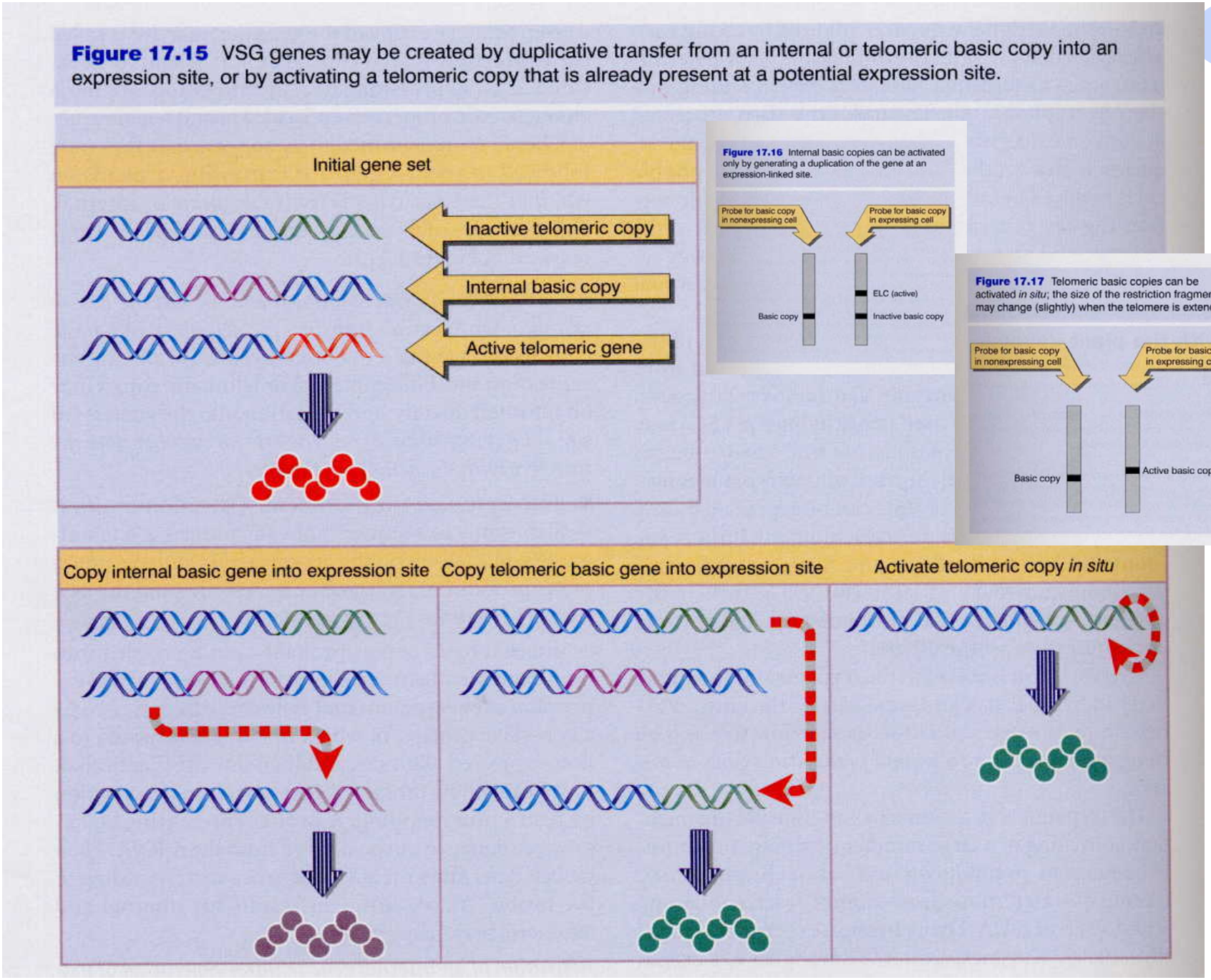


Figure 17.15 VSG genes may be created by duplicative transfer from an internal or telomeric basic copy into an expression site, or by activating a telomeric copy that is already present at a potential expression site.

Figure 17.16 Internal basic copies can be activated only by generating a duplication of the gene at an expression-linked site.

Figure 17.17 Telomeric basic copies can be activated *in situ*; the size of the restriction fragment may change (slightly) when the telomere is extended.

2.12.14