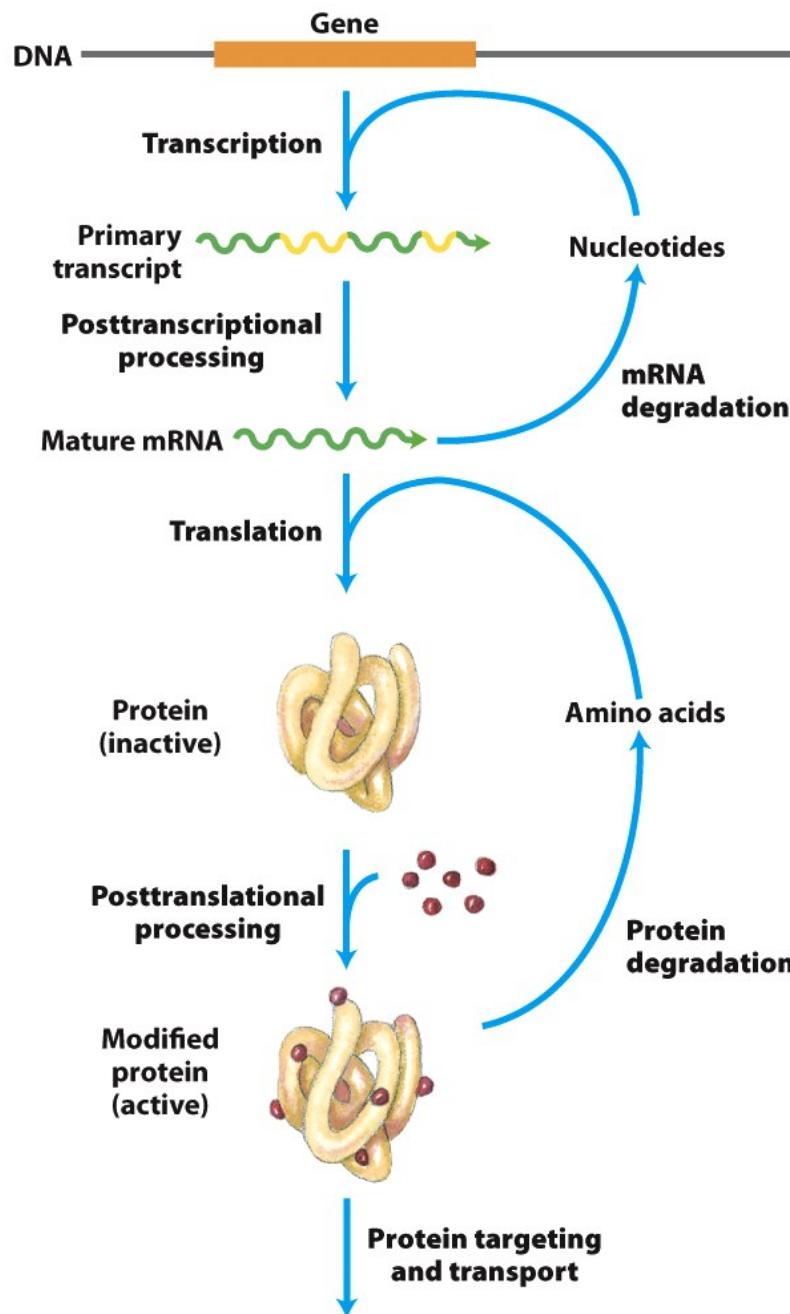


Regulation of Gene Expression

Seven processes that affect the steady-state concentration of a protein



1. Transcription (DNA to RNA)
2. RNA processing (maturation)
3. RNA degradation
4. Translation (RNA to protein)
5. Post-translational modification
6. Protein targeting and transport
7. Protein degradation

Housekeeping genes:

Gene for products that are required at all times.

Constitutive gene expression

Enzymes for metabolic pathways

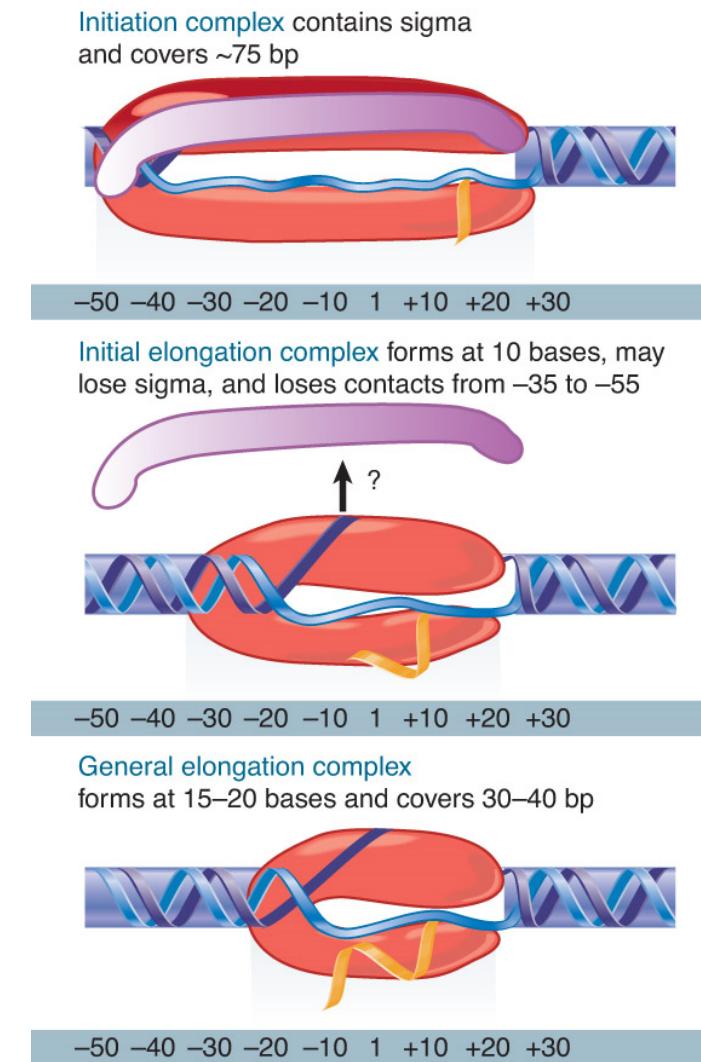
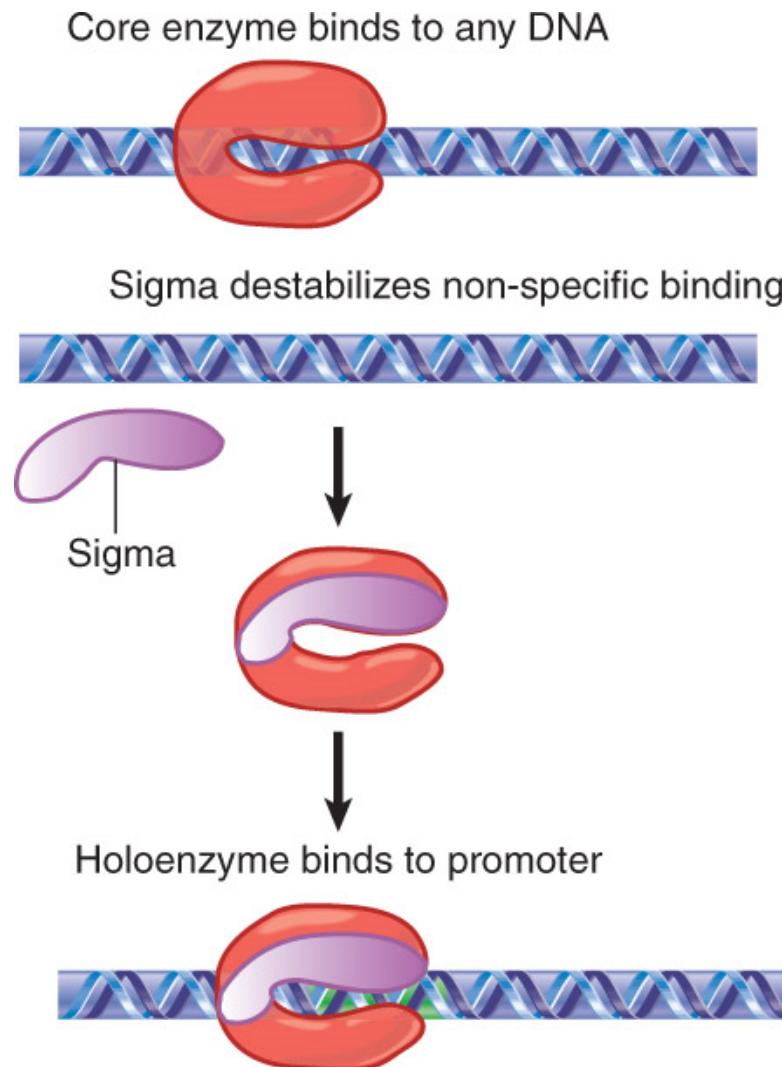
Genes of regulated expression:

Genes for products own cellular levels rise and fall in response to signals

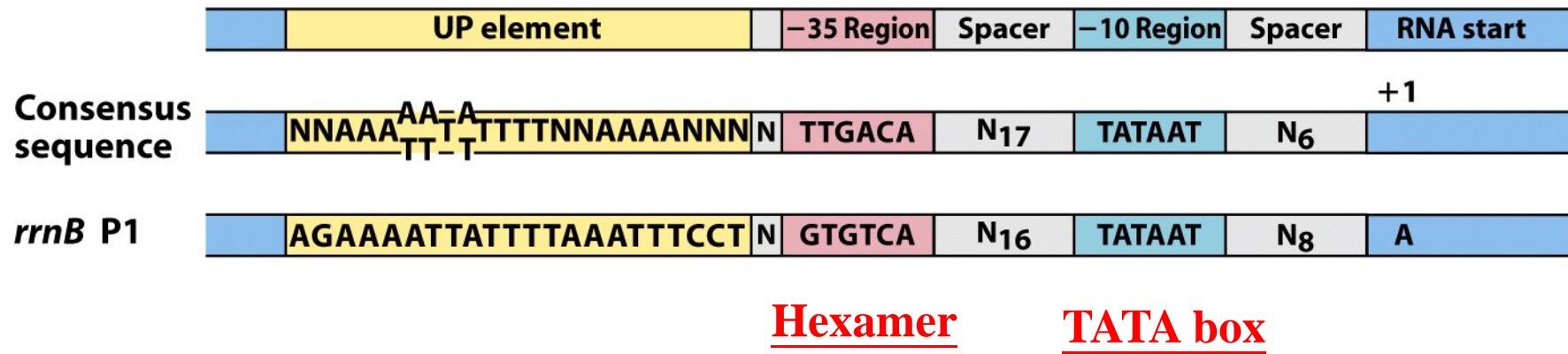
Inducible/ repressible expression

Enzymes for stress responses

RNA polymerase binding to the particular promoter determines the specificity of gene expression



Sigma factor controls binding to DNA by recognizing specific *consensus sequence* in promoter.



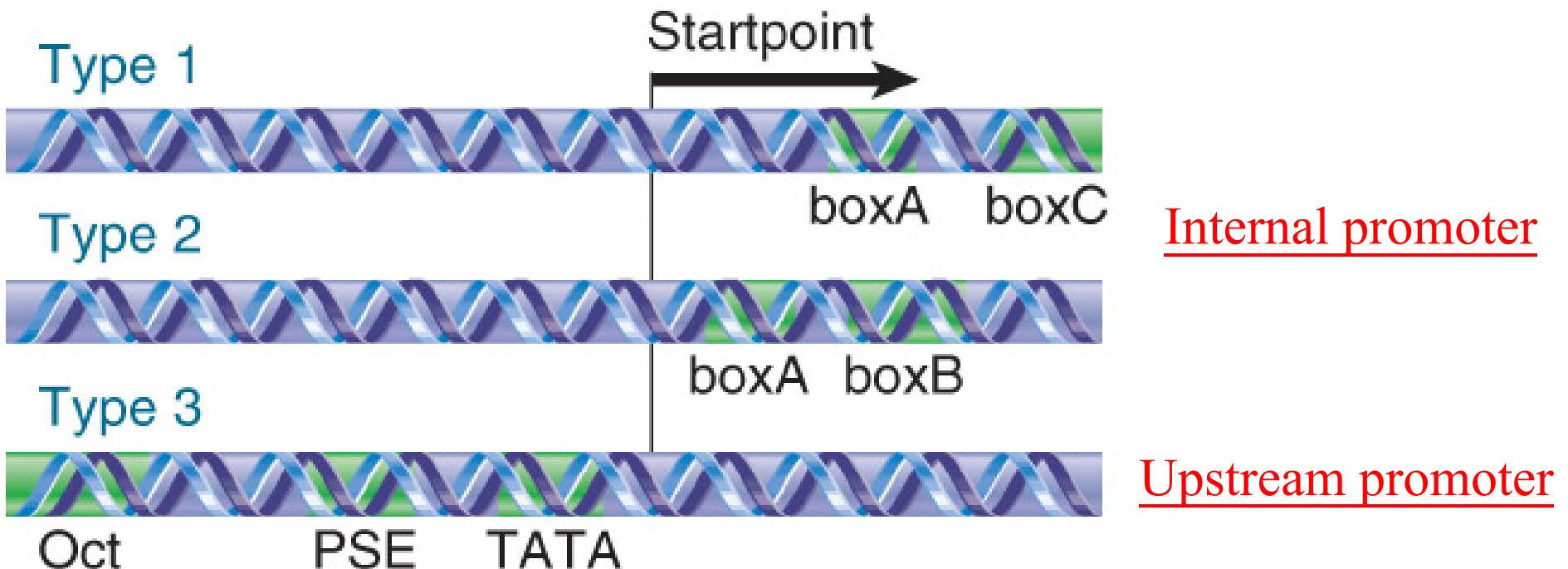
Regulation of housekeeping gene expression:

The binding efficiency of RNA polymerase to promoter sequence

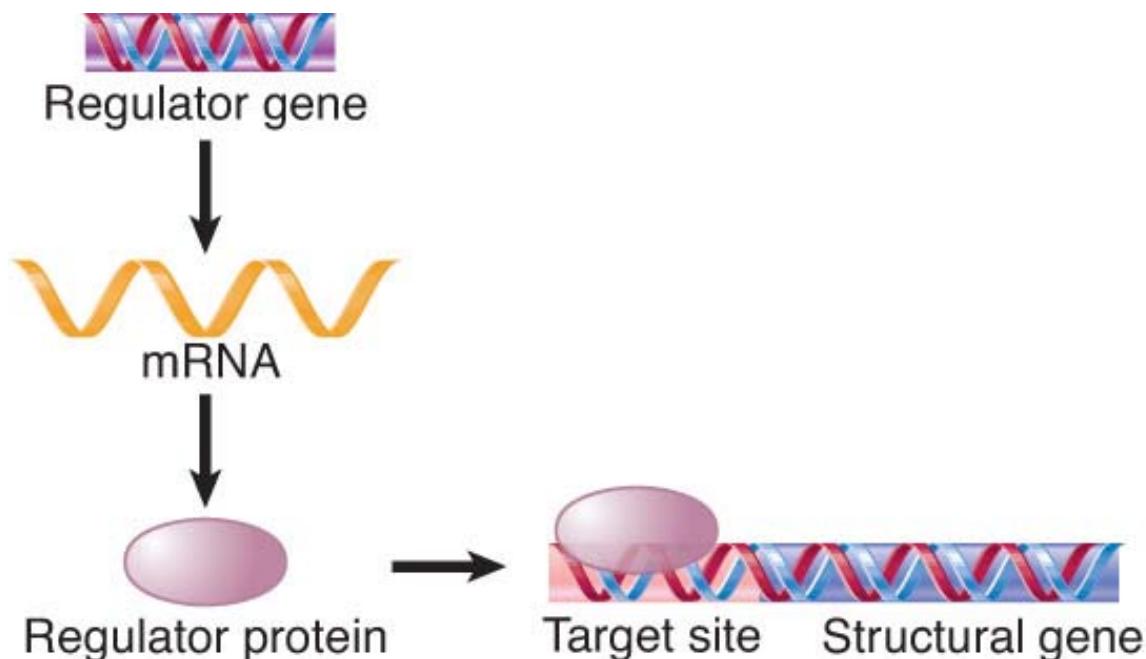
The rate of transcription initiation

The effect of activator and enhancer sequences (UPE)

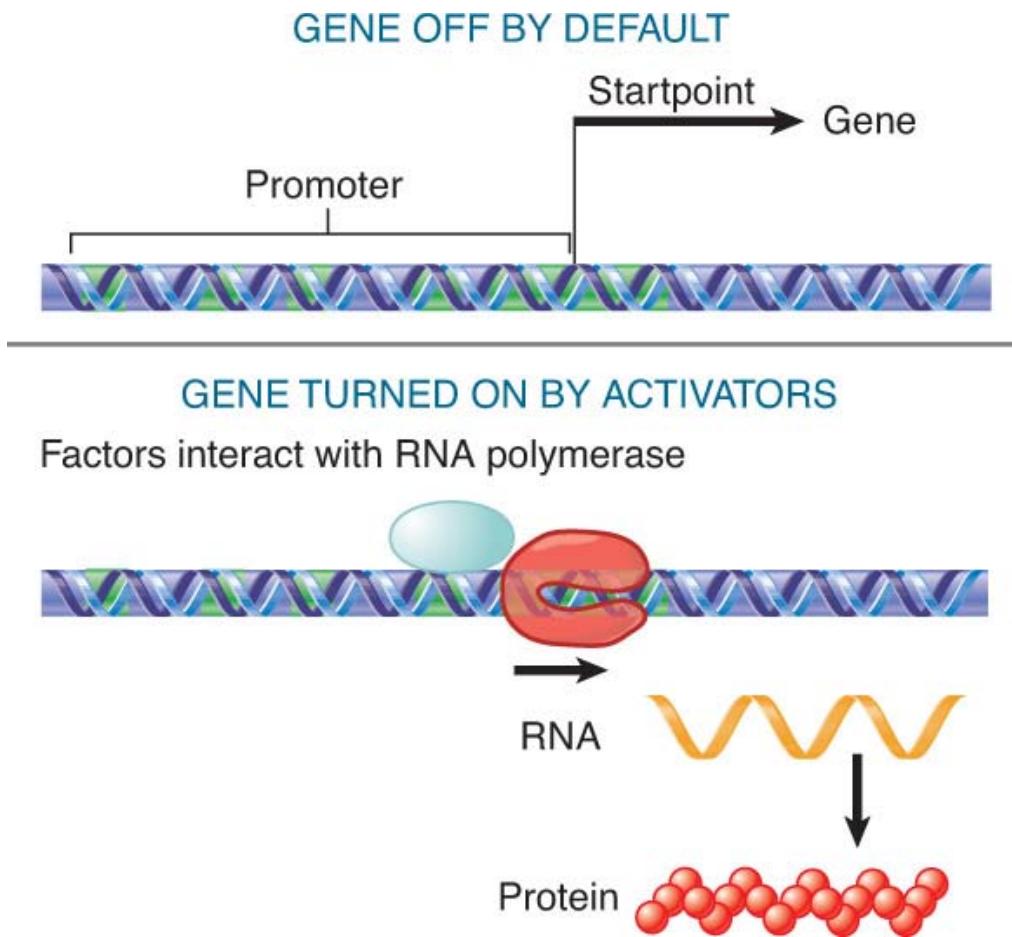
Three types of Pol III promoters:



- **Regulator gene** – A gene that codes for a product (typically protein) that controls the expression of other genes (usually at the level of transcription).
- **Structural gene** – A gene that codes for any RNA or protein product other than a regulator.



Positive control of gene expression



- In **positive regulation**, a transcription factor (*trans-activating factor*) is required to bind at the promoter (*cis-activating site*) in order to enable RNA polymerase to initiate transcription.

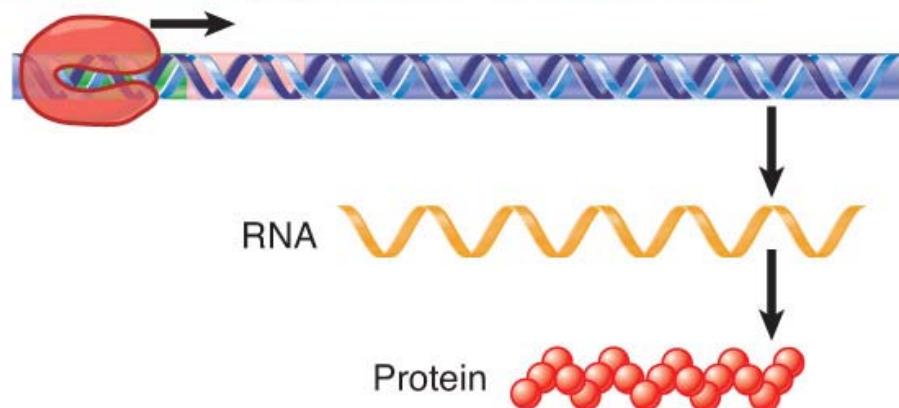
Negative control of gene expression

- In **negative regulation**, a transcription repressor (*trans-activating factor*) binds to the operator or promoter (*cis-activating site*) to turn off transcription.

cis-acting operator/promoter precedes structural gene(s)



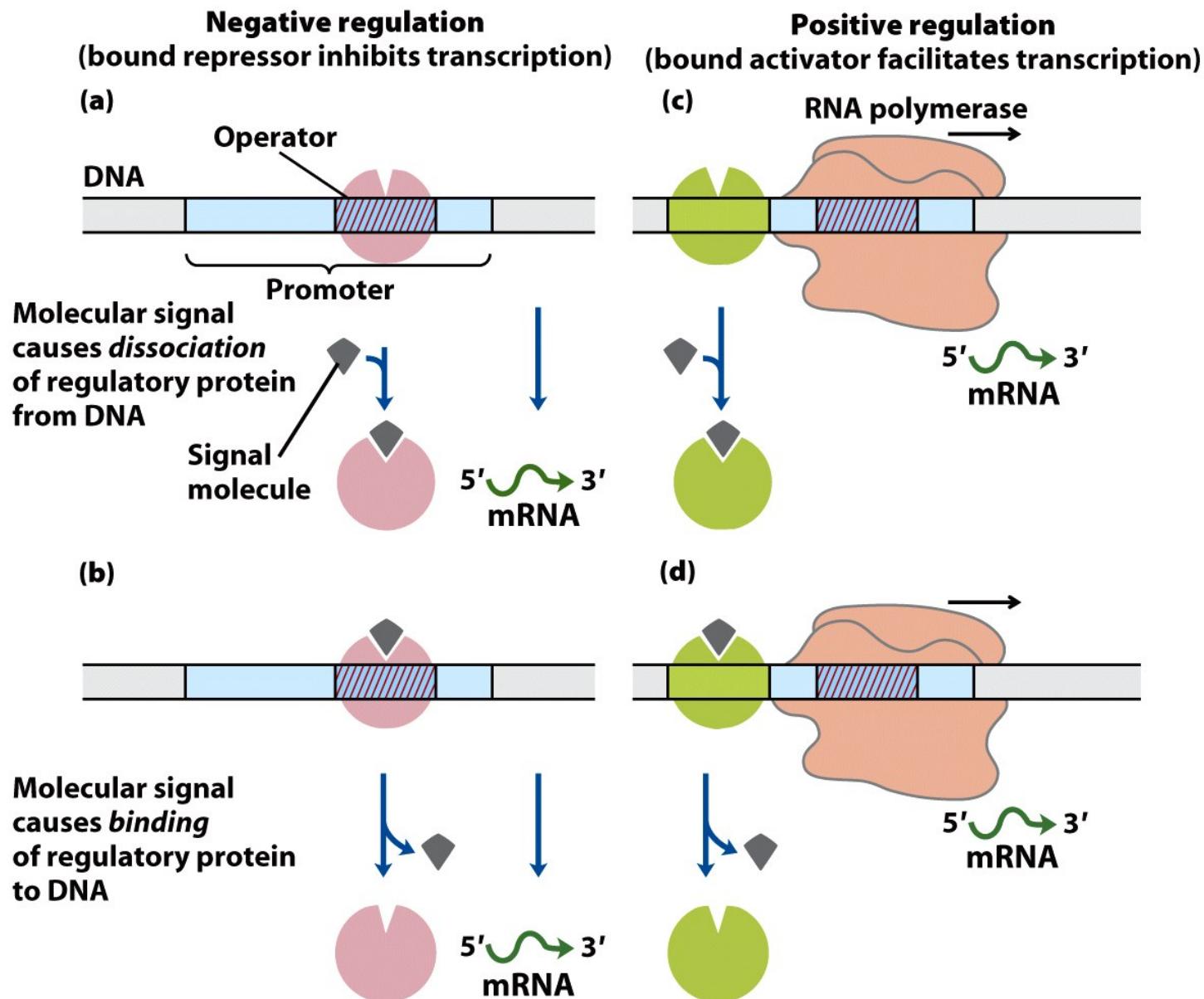
Gene on: RNA polymerase initiates at promoter



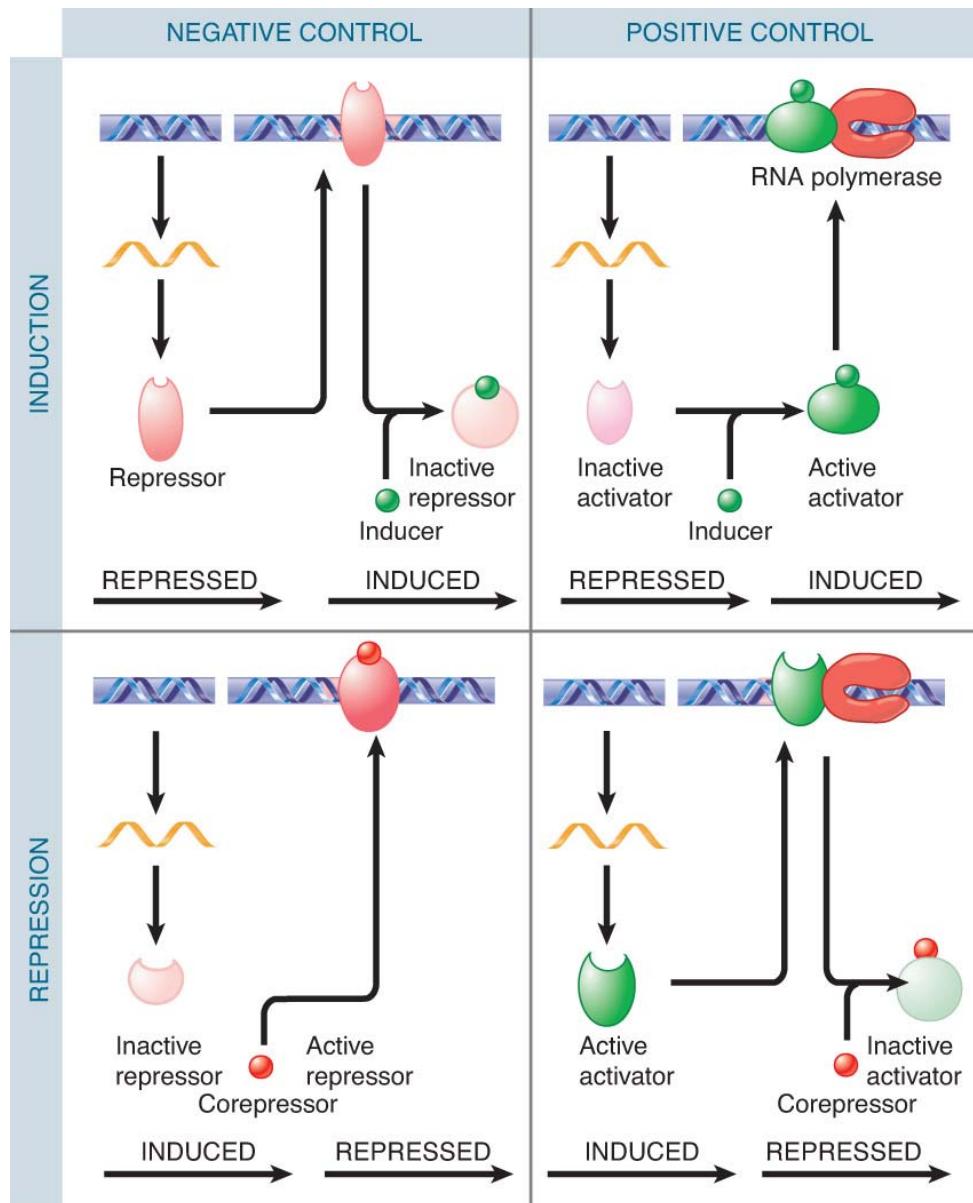
Gene is turned off when repressor binds to operator



Common patterns of regulation of transcription initiation

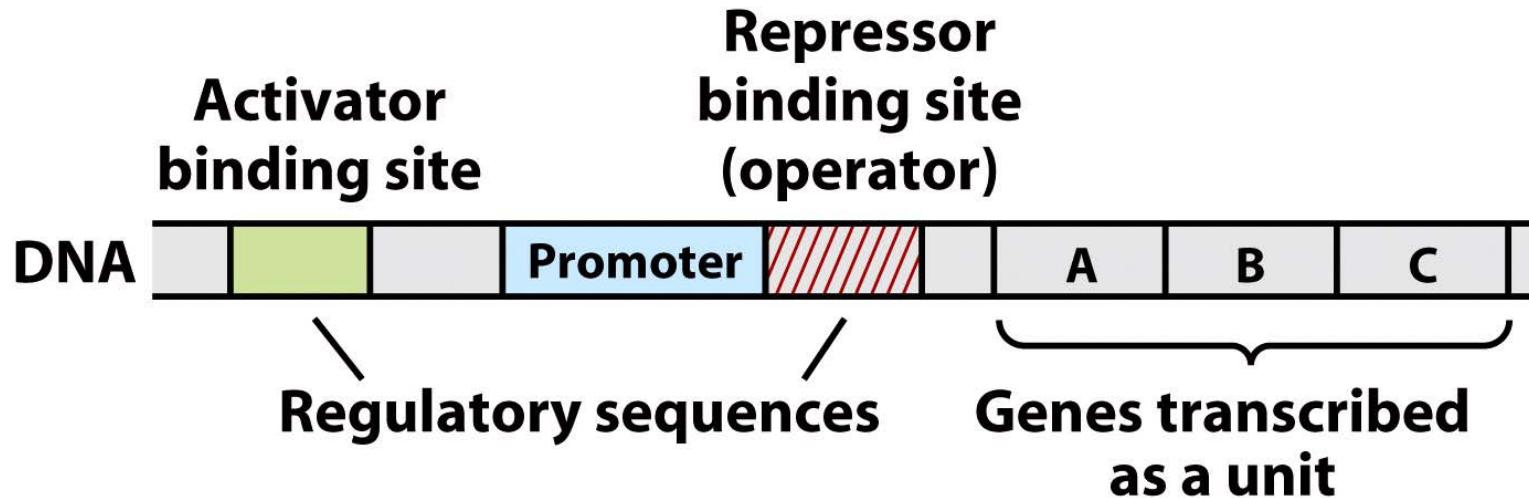


Inducible/ Repressible expressions



- **Negative inducible**
- **Negative repressible**
- **Positive inducible**
- **Positive repressible**

Genes are clustered & Regulated operon

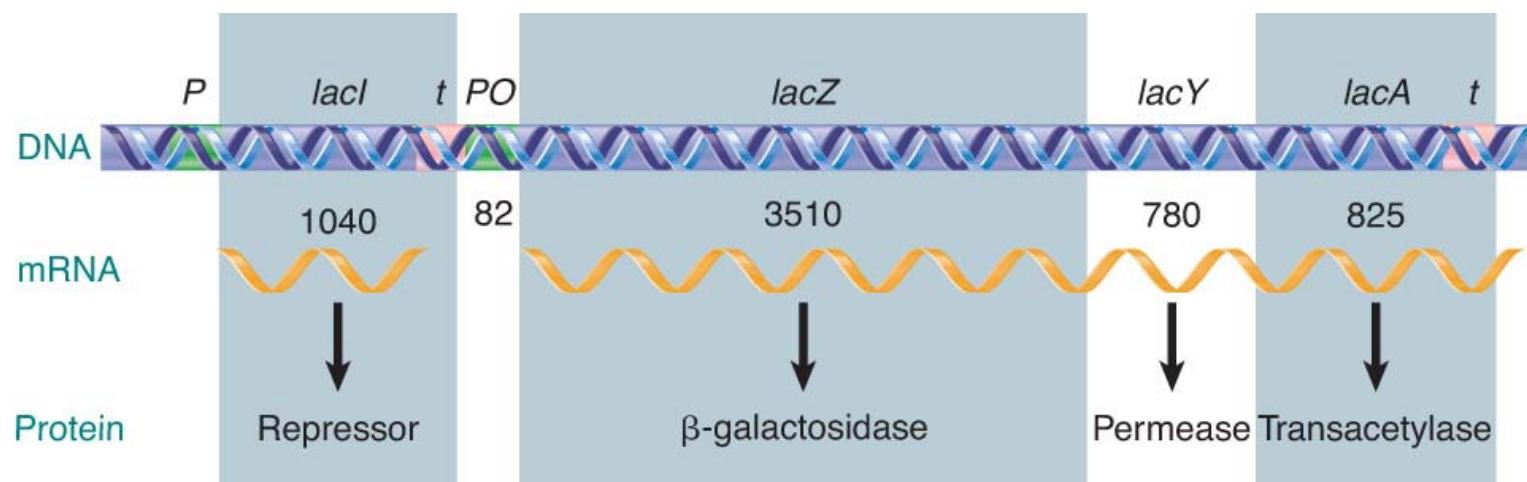


Operon:

- Polycistronic-multiple genes on a transcript-clustered genes
- Single promoter that initiates transcription of the clustered genes
- Additional sequences for activation and repression

Structural Gene Clusters Are Coordinately Controlled

- Genes coding for proteins that function in the same pathway may be located adjacent to one another and controlled as a single unit that is transcribed into a **polycistronic mRNA**.



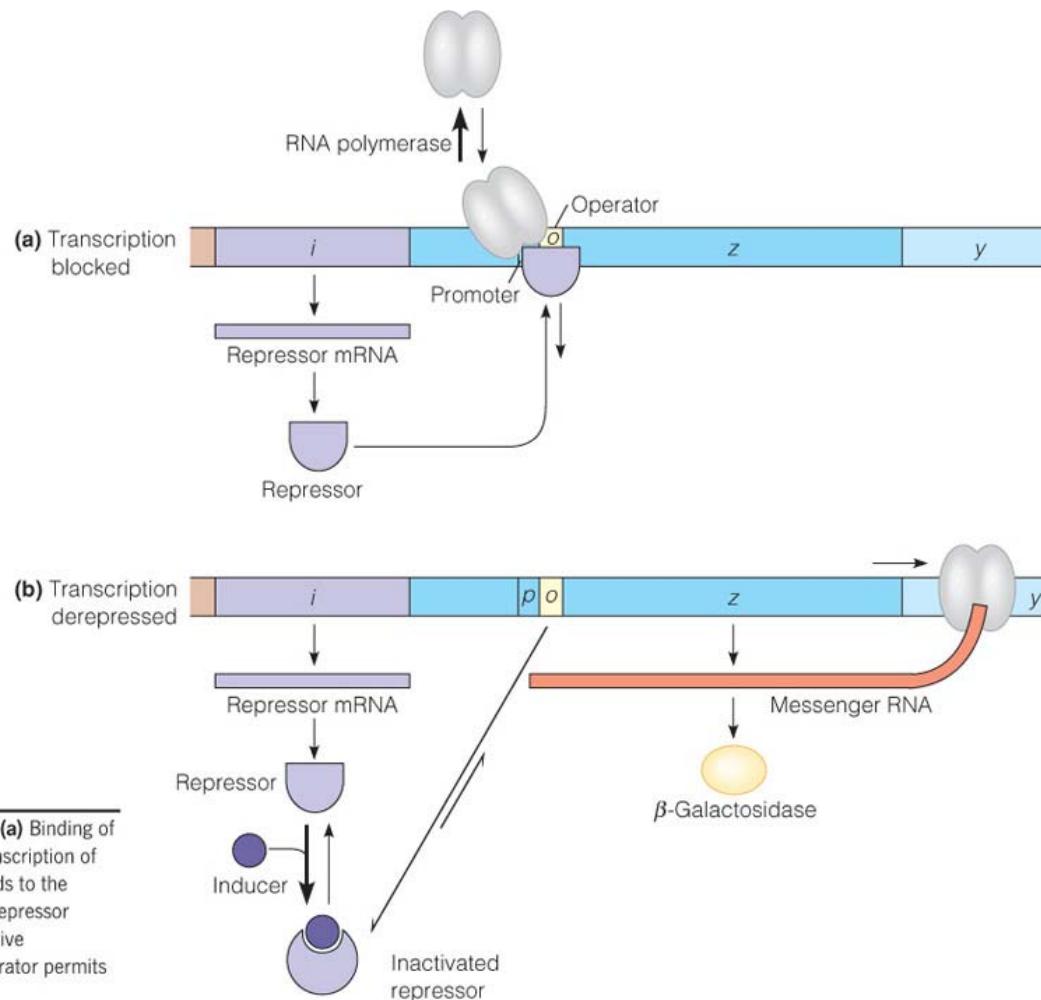
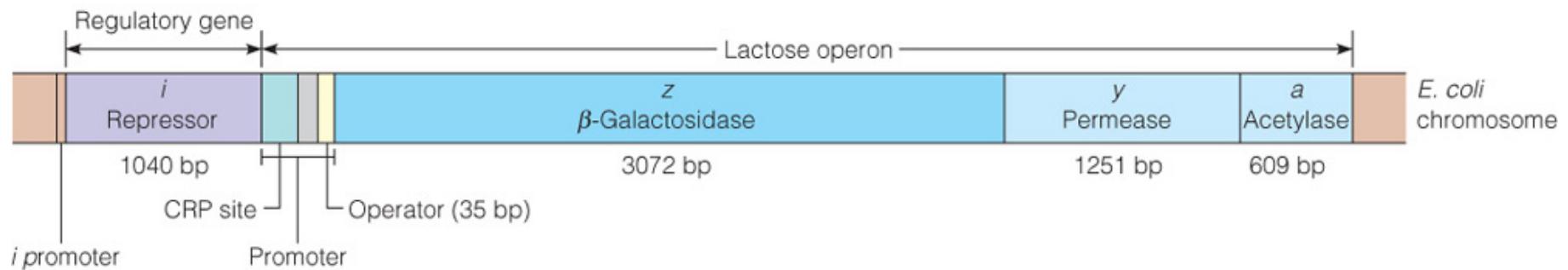
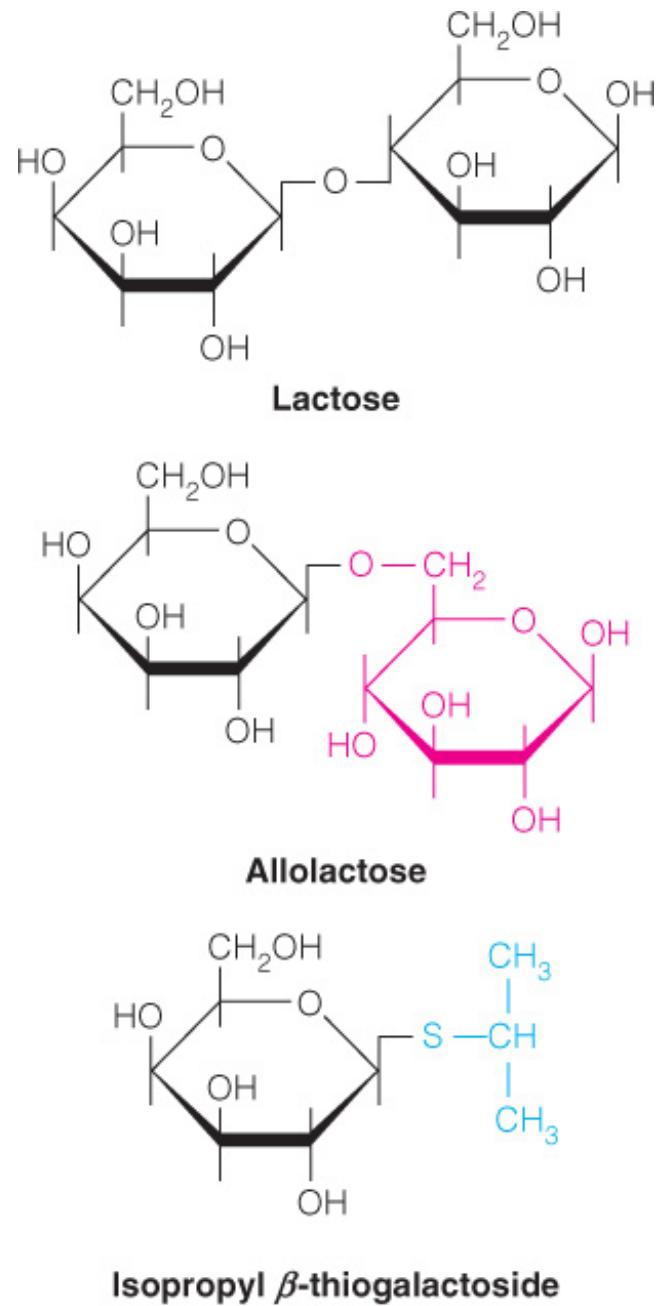
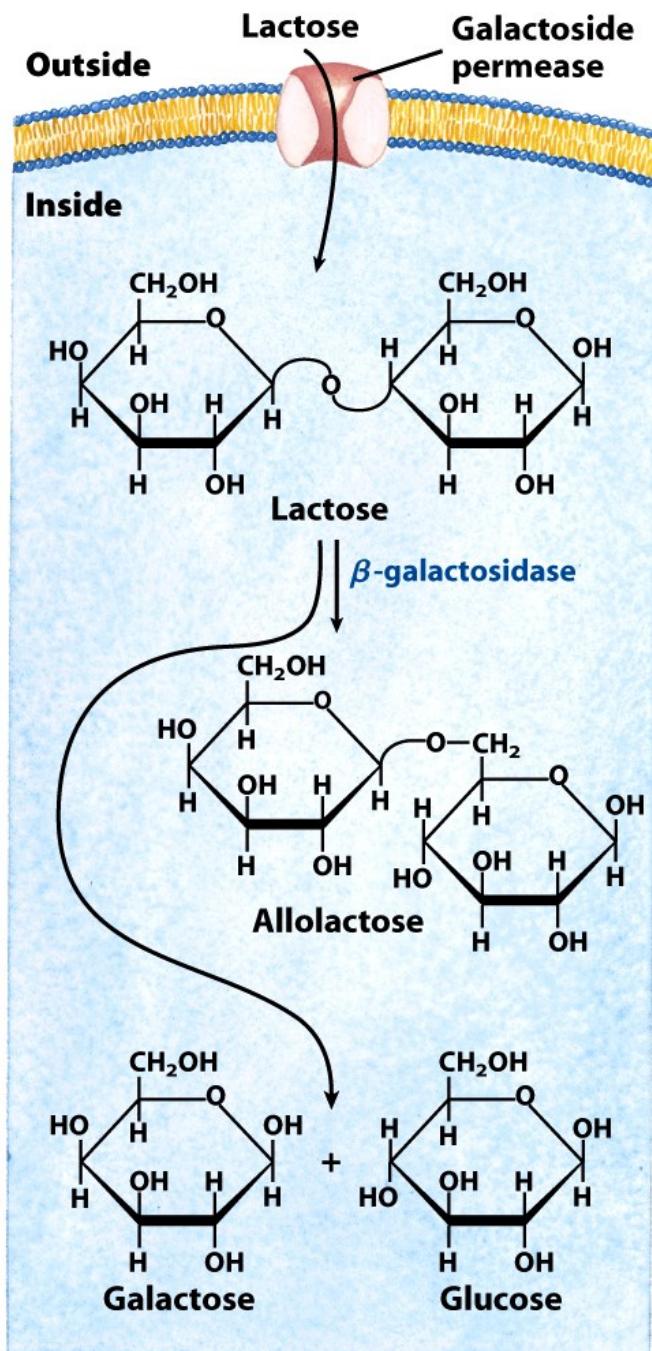
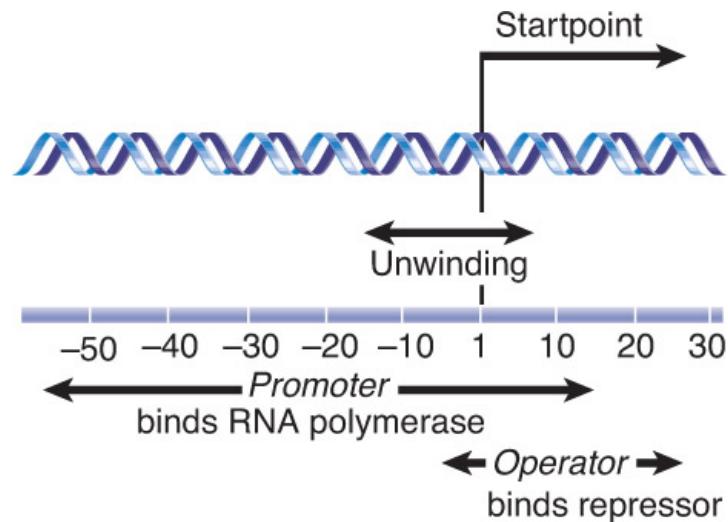


FIGURE 29.2

Configurations of the lactose operon. **(a)** Binding of the repressor to the operator inhibits transcription of the structural genes. **(b)** The inducer binds to the repressor, decreasing the affinity of the repressor for the operator. Dissociation of the inactive repressor-inducer complex from the operator permits transcription of the structural genes.

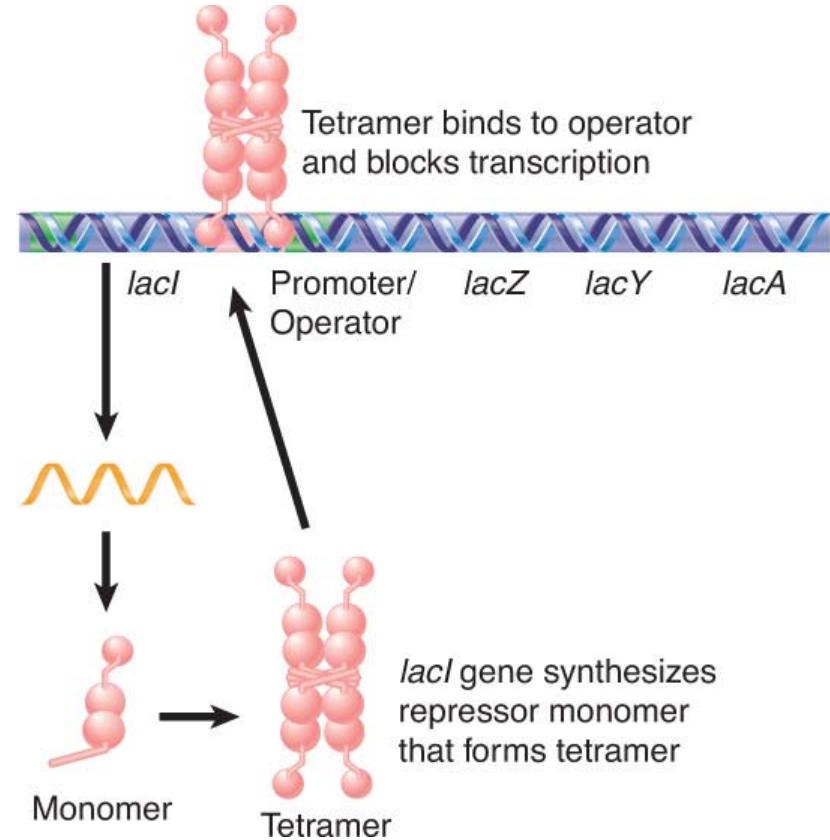
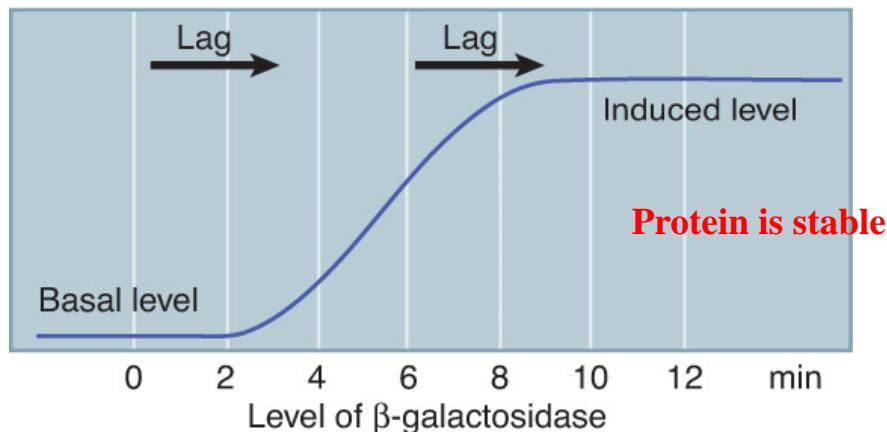
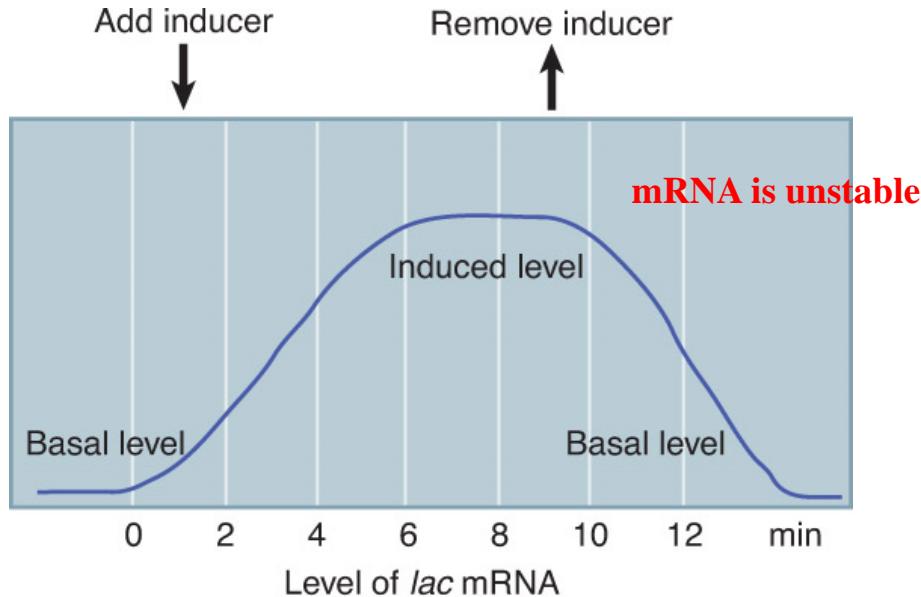


The *lac* Operon Is Negative Inducible



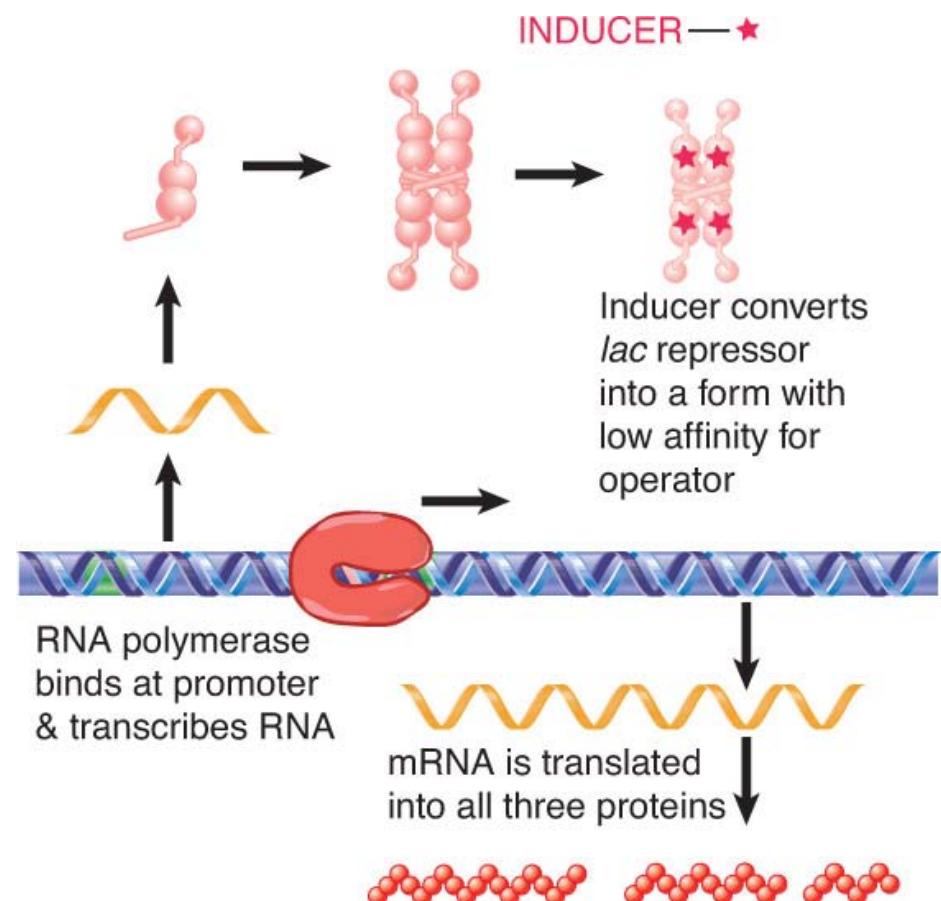
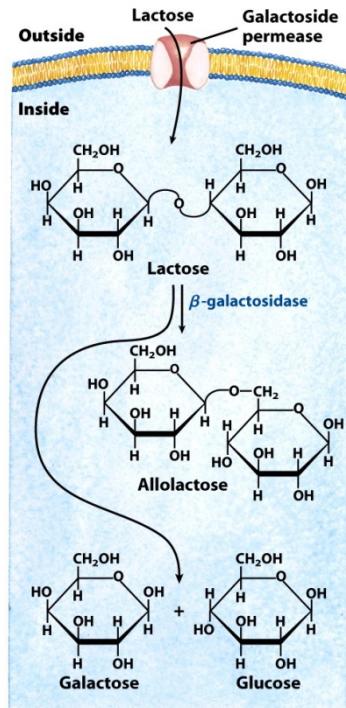
- Transcription of the *lacZYA* operon is controlled by a repressor protein (the *lac* repressor) that binds to an operator that overlaps the promoter at the start of the cluster.
- In the absence of β -galactosides, the *lac* operon is expressed only at a very low (basal) level.

lac expression responds to inducer



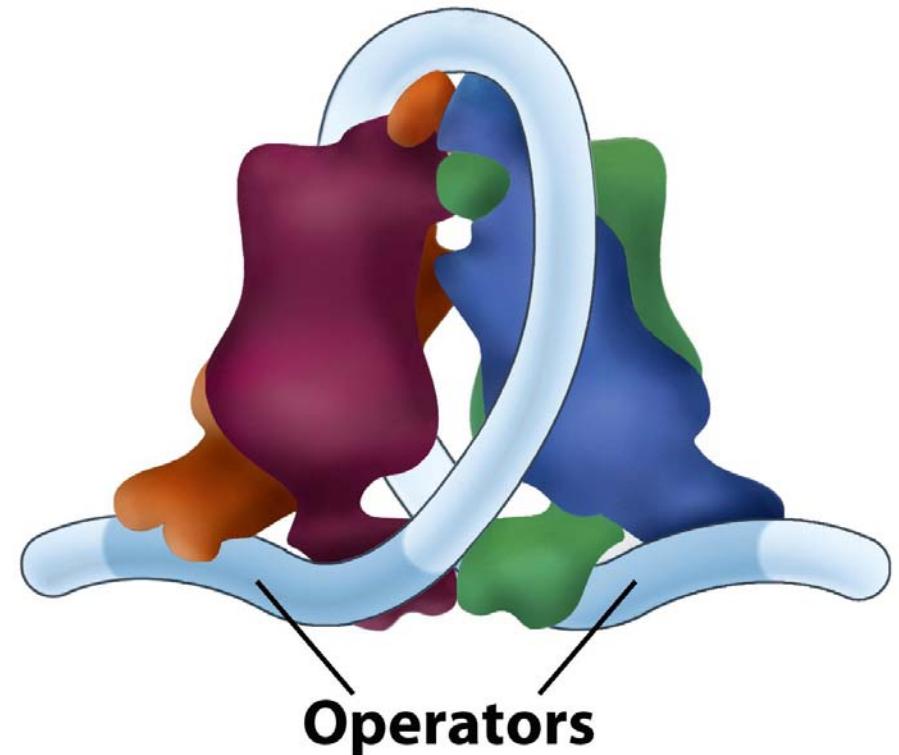
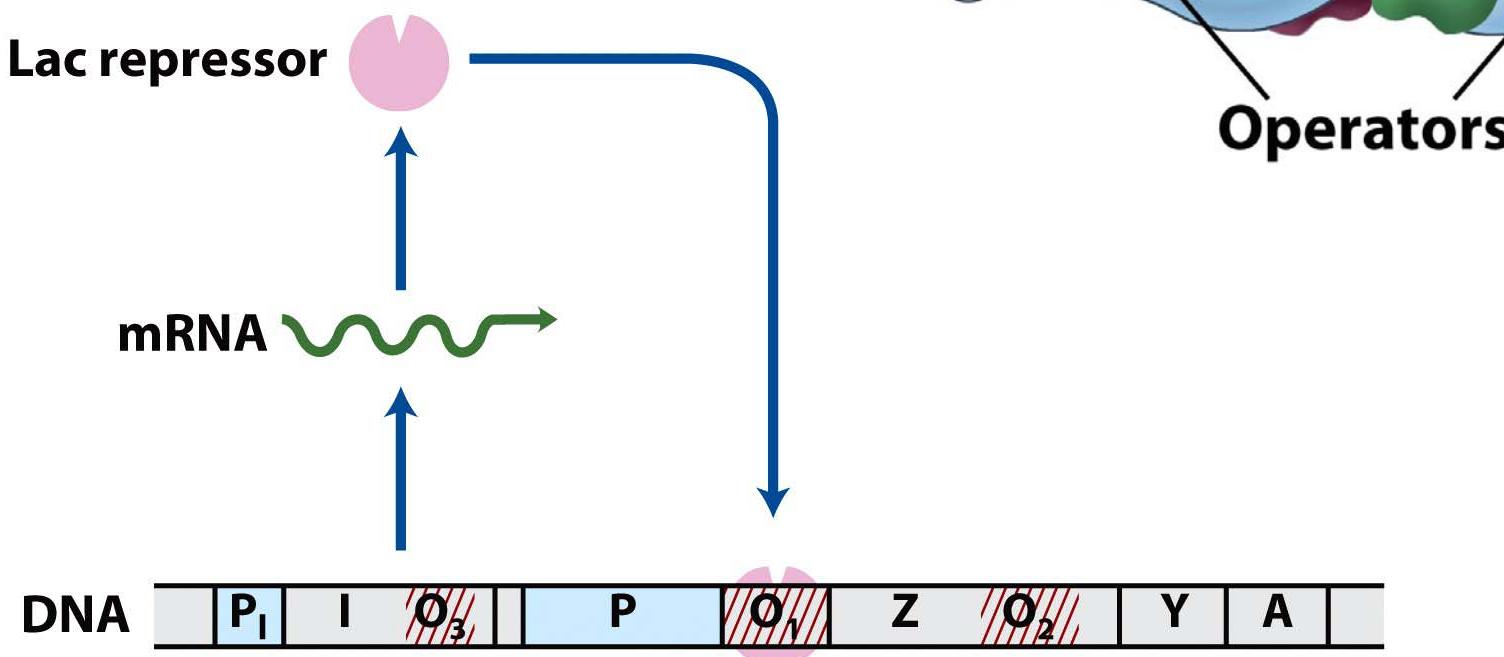
- The repressor protein is a tetramer of identical subunits coded by the *lacI* gene.
- **β-galactoside sugars**, the substrates of the *lac* operon, are its inducer.
- Addition of specific β-galactosides induces transcription of all three genes of the *lac* operon.
- The *lac* mRNA is extremely unstable; as a result, induction can be rapidly reversed.

- An inducer functions by converting the repressor protein into a form with lower operator affinity
- Repressor has two binding sites, one for the operator DNA and another for the inducer
- Repressor is inactivated by an allosteric interaction in which binding of inducer at its site changes the properties of the DNA-binding site (**allosteric control**)
- The true inducer is **allolactose**, not the actual substrate of β -galactosidase



The *lac* operon:

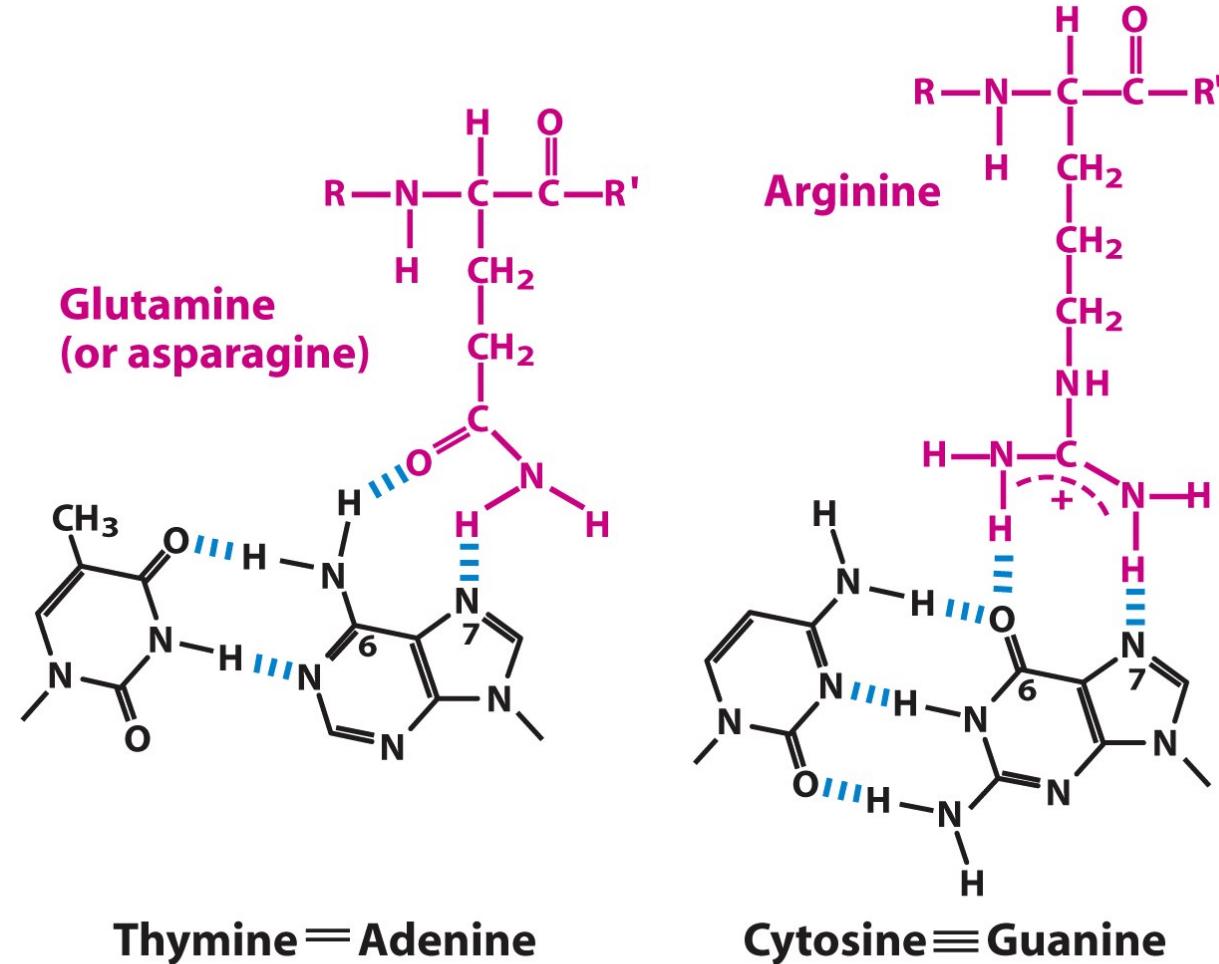
Three operators: O_1 , O_2 , and O_3



The protein binds to DNA sequence:

- Hydrogen bonding (NH₃⁺ & COOH groups)

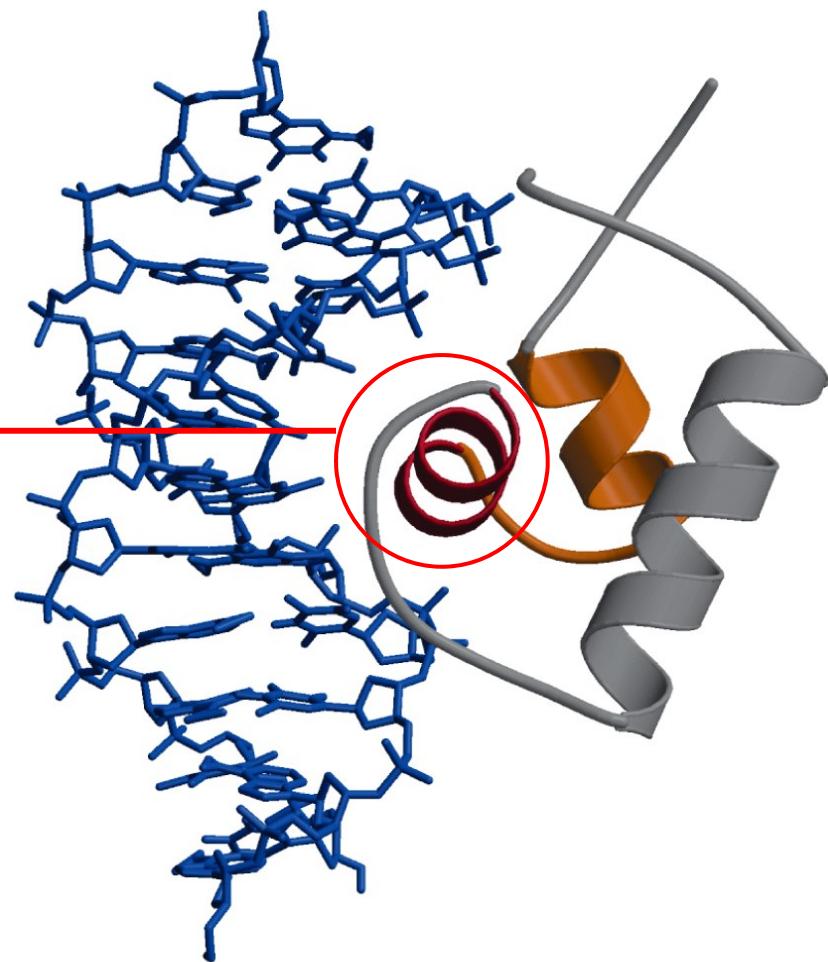
- Asn, Gln, Glu, Lys, and Arg



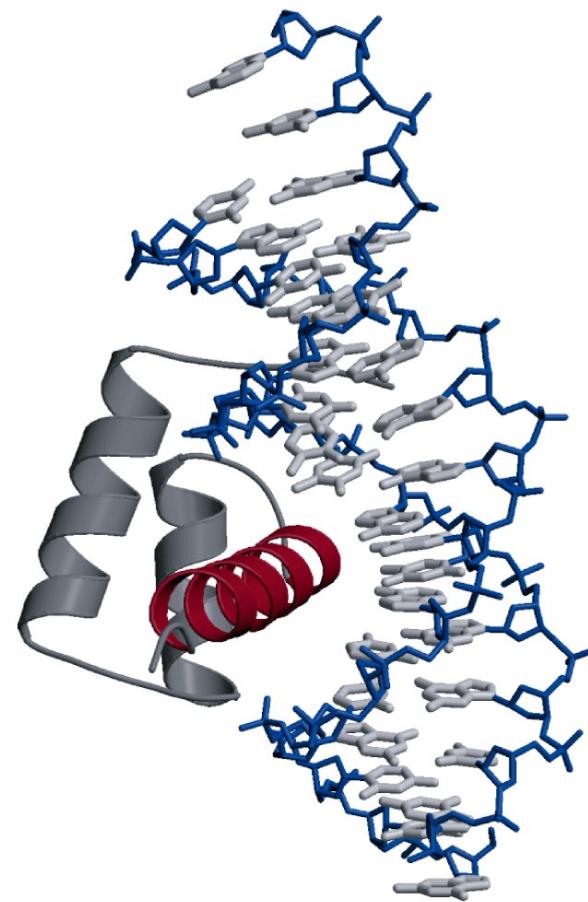
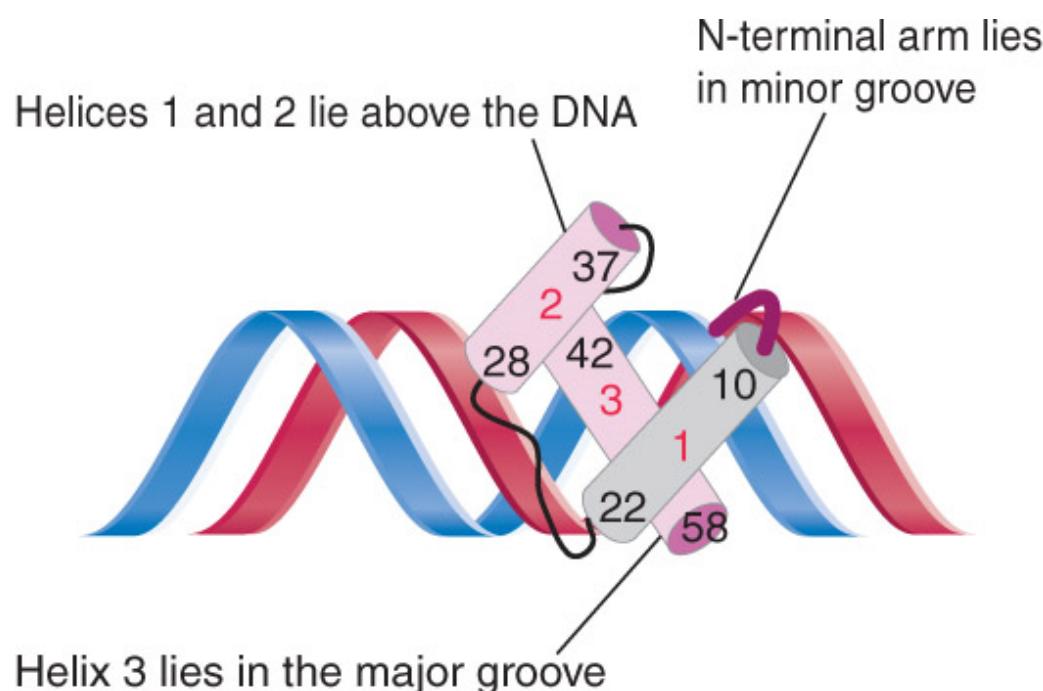
Regulatory domains bind to DNA:

- Helix-turn-helix: 20 amino acids in two short α -helical segments
- 7 to 9 amino acids in each α -helix

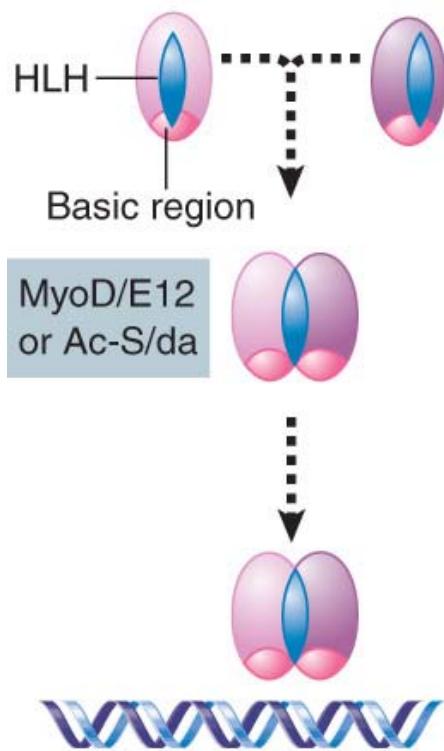
DNA recognition helix ←



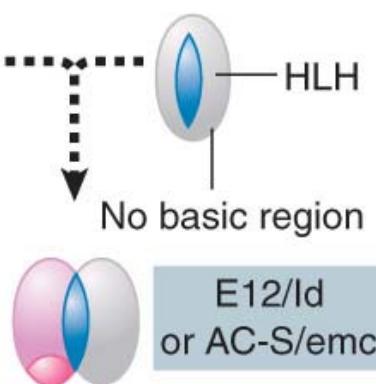
- **helix-turn-helix** – The motif that describes an arrangement of two α -helices that form a site that binds to DNA, one fitting into the major groove of DNA and the other lying across it
- **homeodomain** – A DNA-binding motif (**recognition helix**) that typifies a class of transcription factors



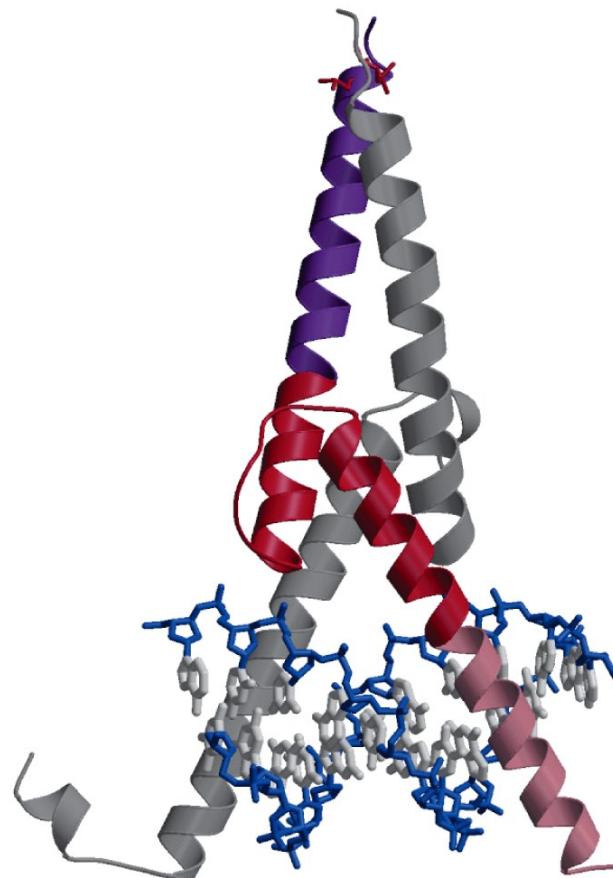
bHLH proteins dimerize and bind DNA



Nonbasic HLH proteins prevent DNA-binding

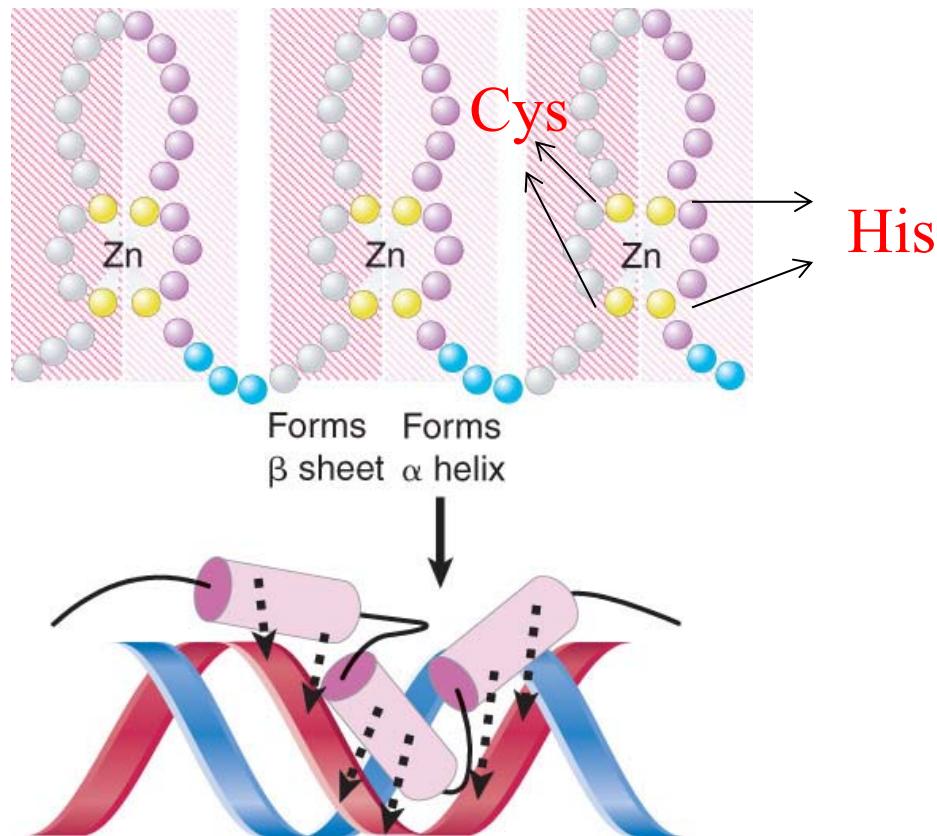


- A basic HLH (bHLH) protein has a basic DNA-binding sequence close to the dimerization motif.

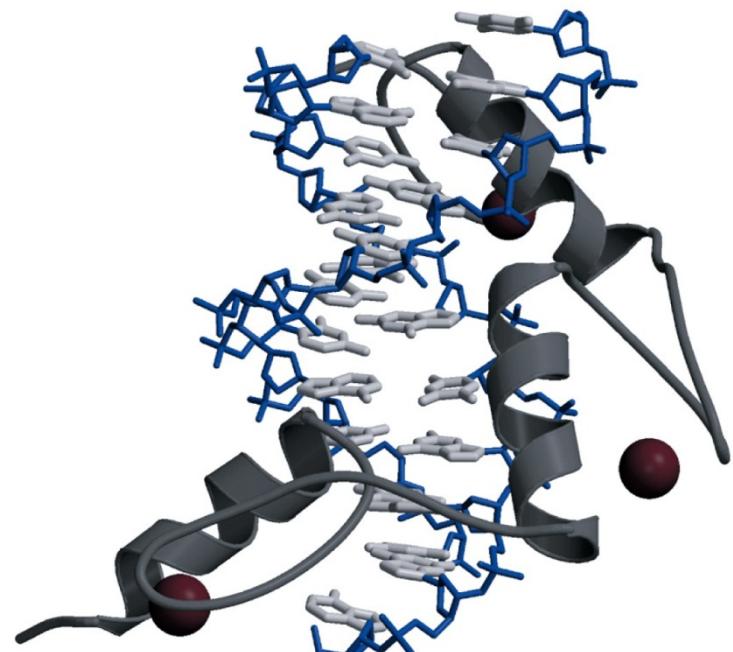


Regulatory domains bind to DNA:

- Zinc finger: 30 amino acid residues form an elongated loop together with Zn²⁺

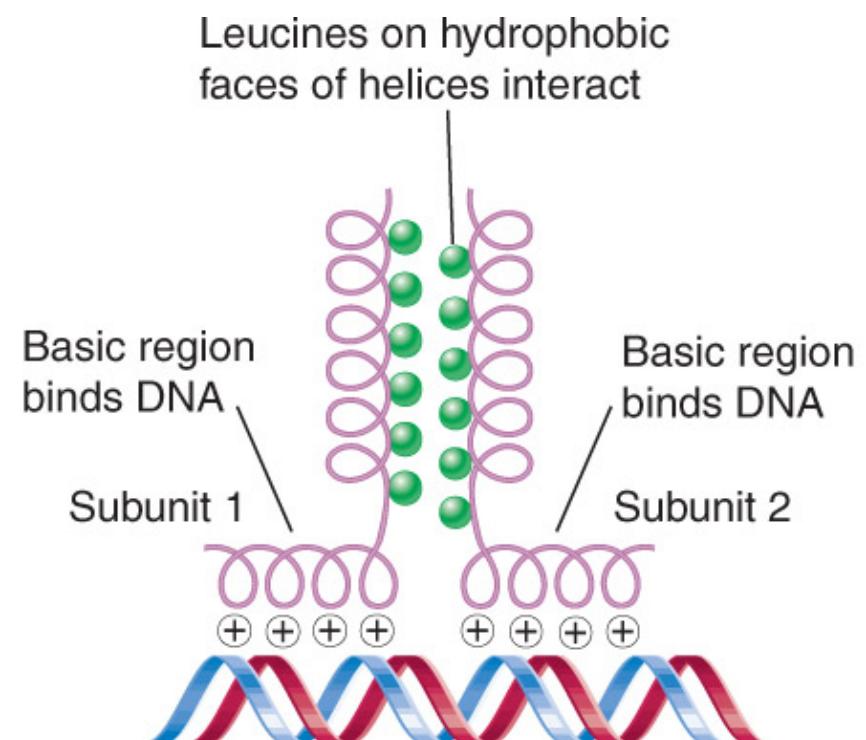
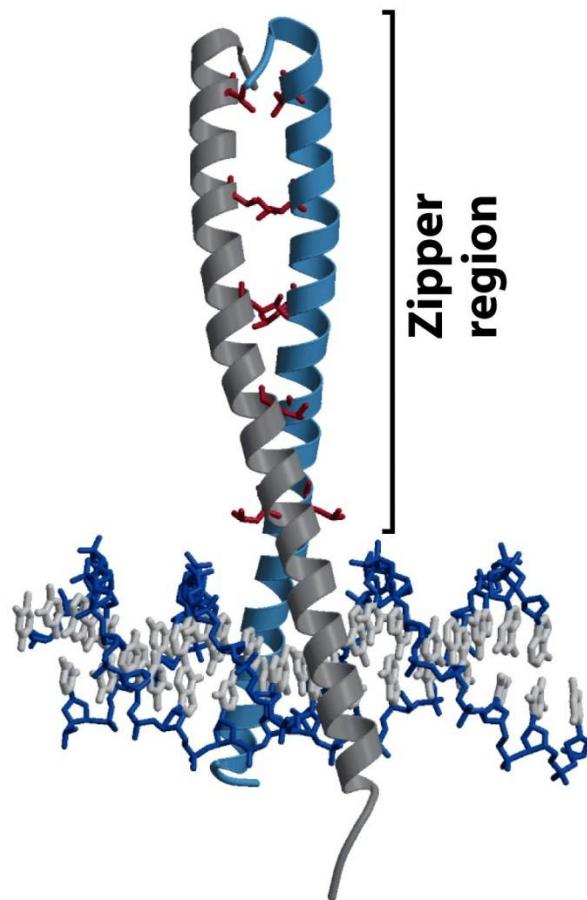


- The consensus sequence:
Cys-X₂₋₄-Cys-X₃-Phe-X₅-Leu-X₂-His-X₃-His

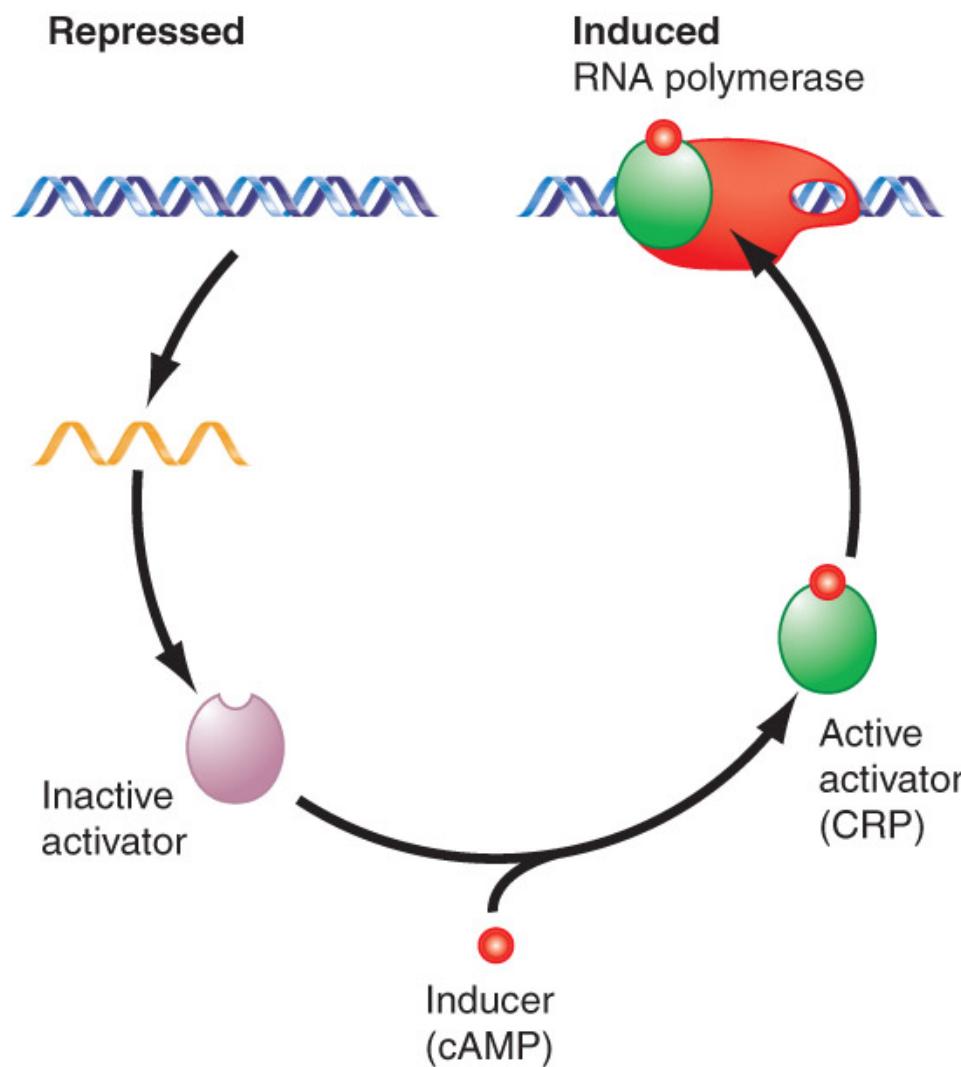


Regulatory domains bind to DNA:

- Leucine zippers: A dimerization motif that is found in a class of transcription factors
- An amphipathic α -helix with a leucine residue in every 7th position

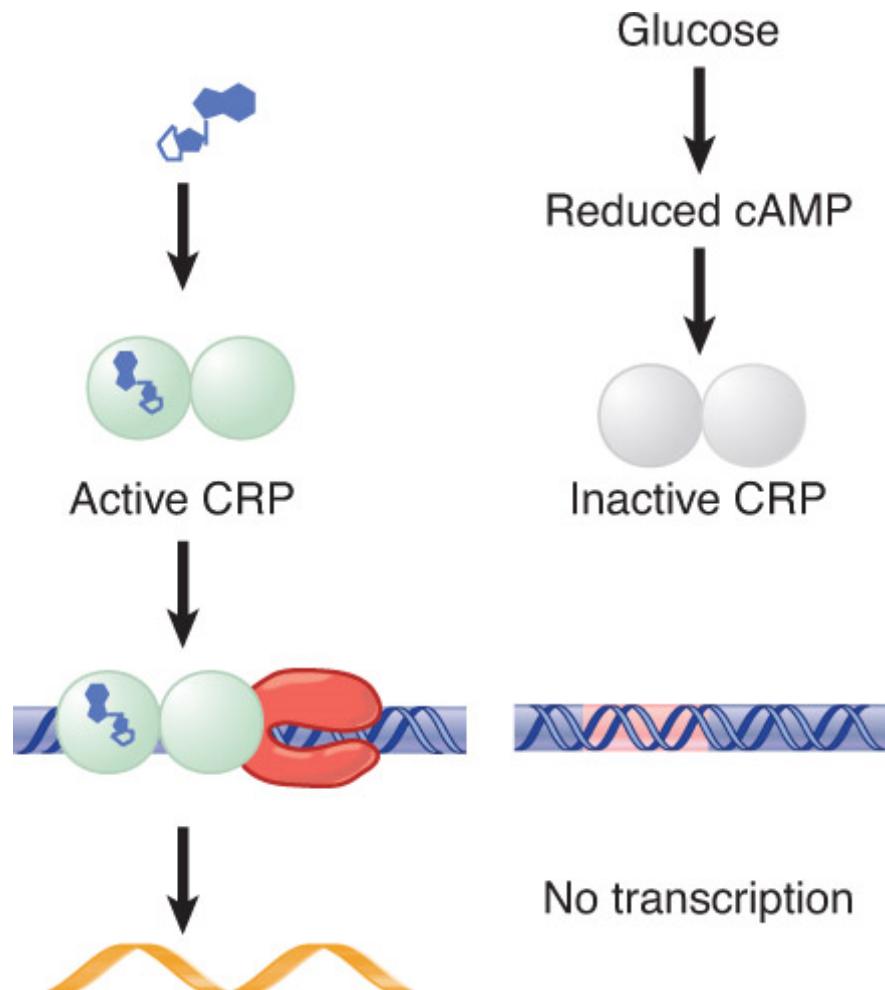
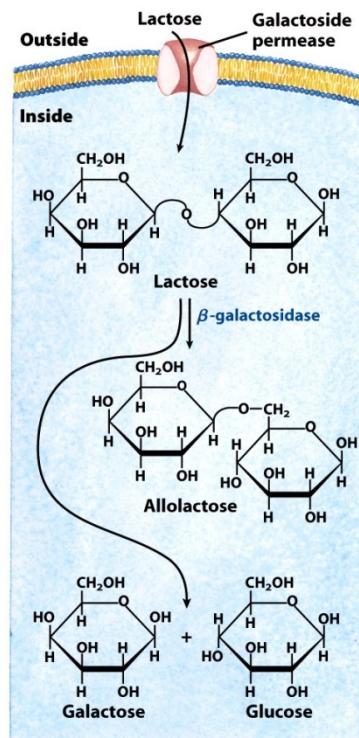


The *lac* Operon Has a Second Layer of Control: Catabolite Repression

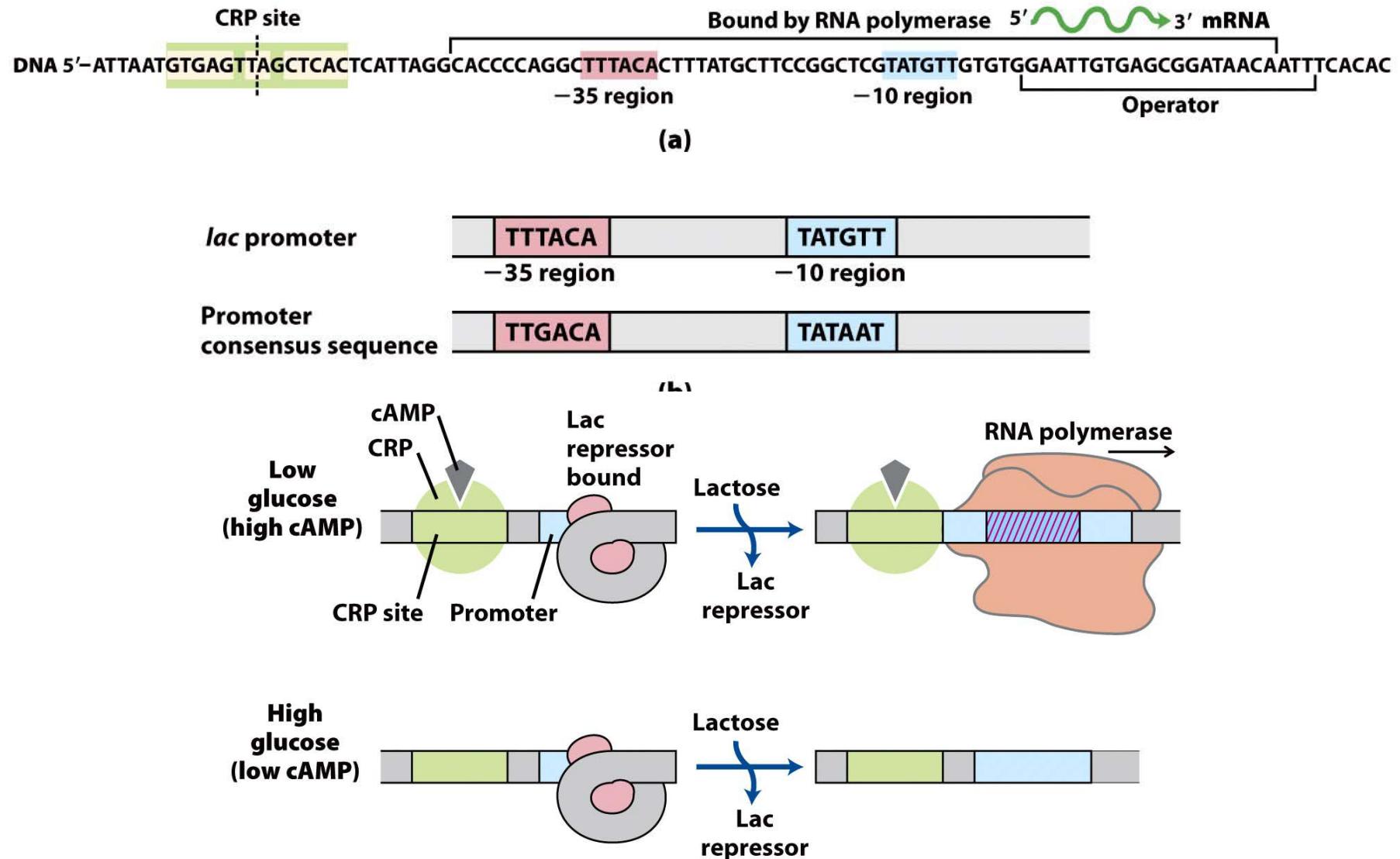


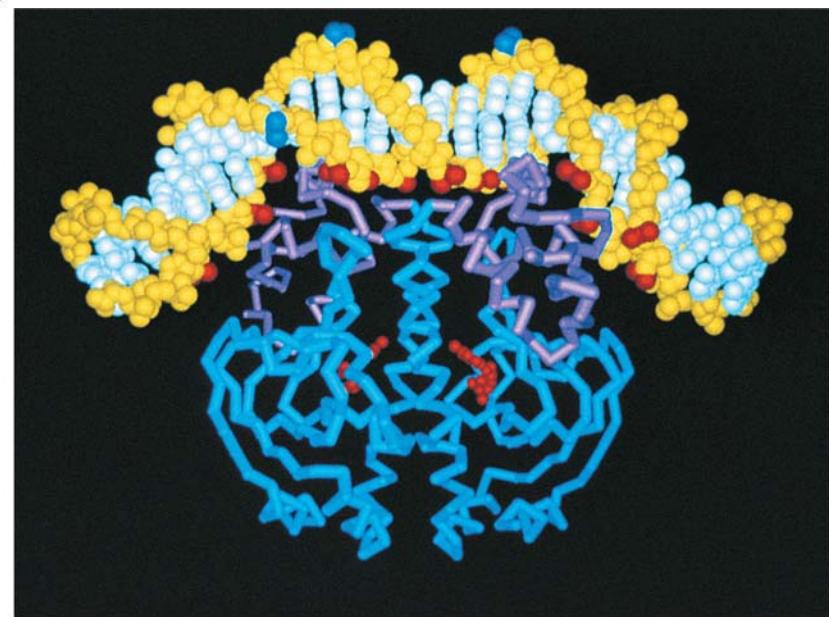
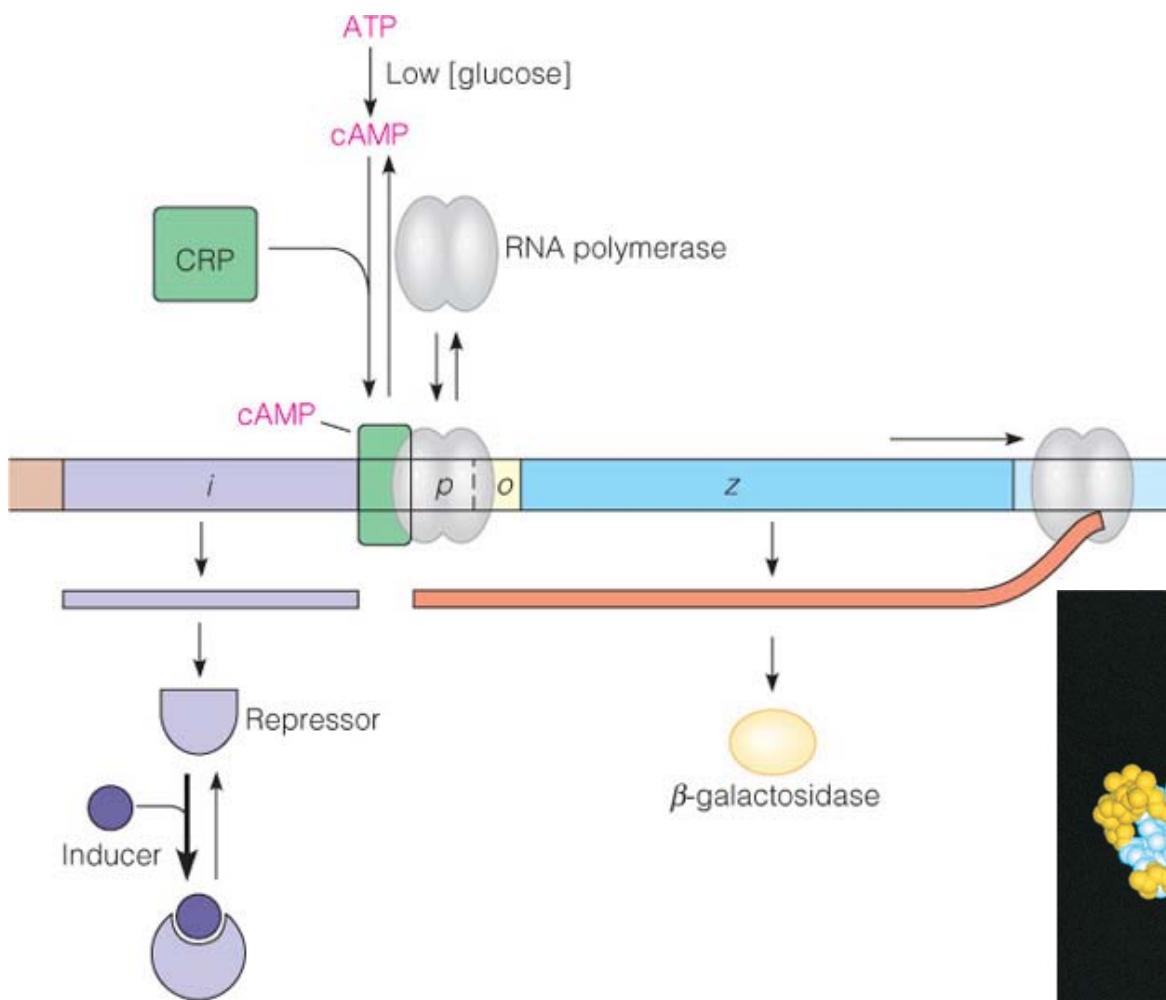
- **Catabolite repression** – The ability of glucose to prevent the expression of a number of genes.
- **Catabolite repressor protein (CRP)** is an activator protein that binds to a target sequence at a promoter.
- A dimer of CRP is activated by a single molecule of **cyclic AMP (cAMP)**.
- cAMP is controlled by the level of glucose in the cell; a low glucose level allows cAMP to be made.
- CRP interacts with the C-terminal domain of the α subunit of RNA polymerase to activate it.

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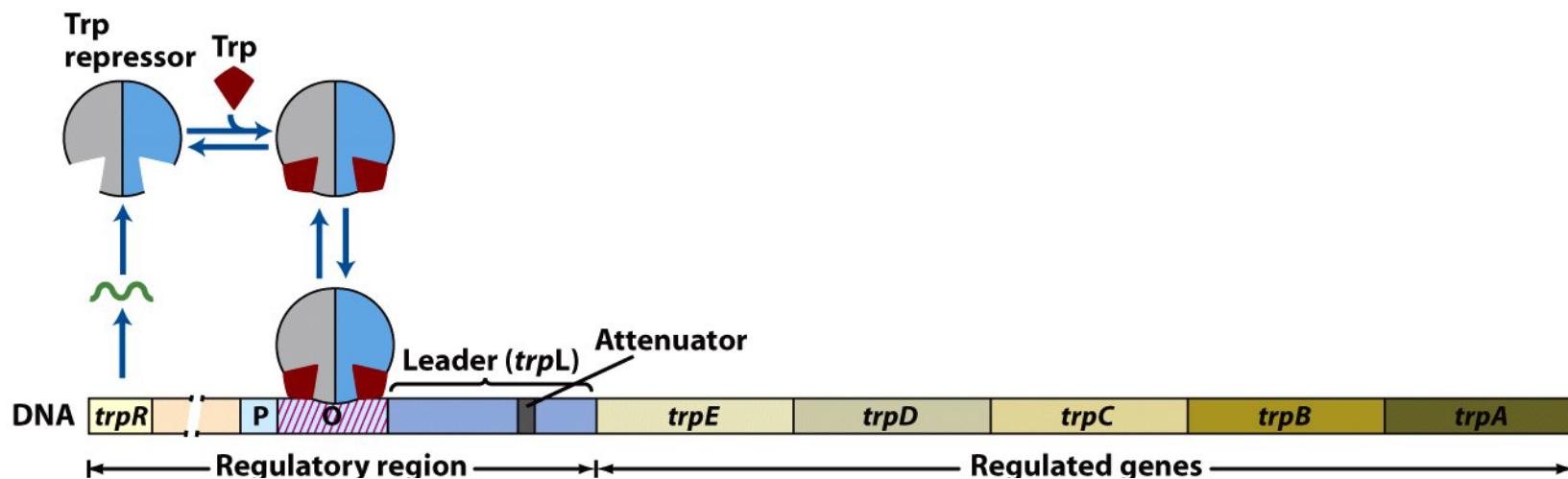
Activation of transcription of the *lac* operon by CRP

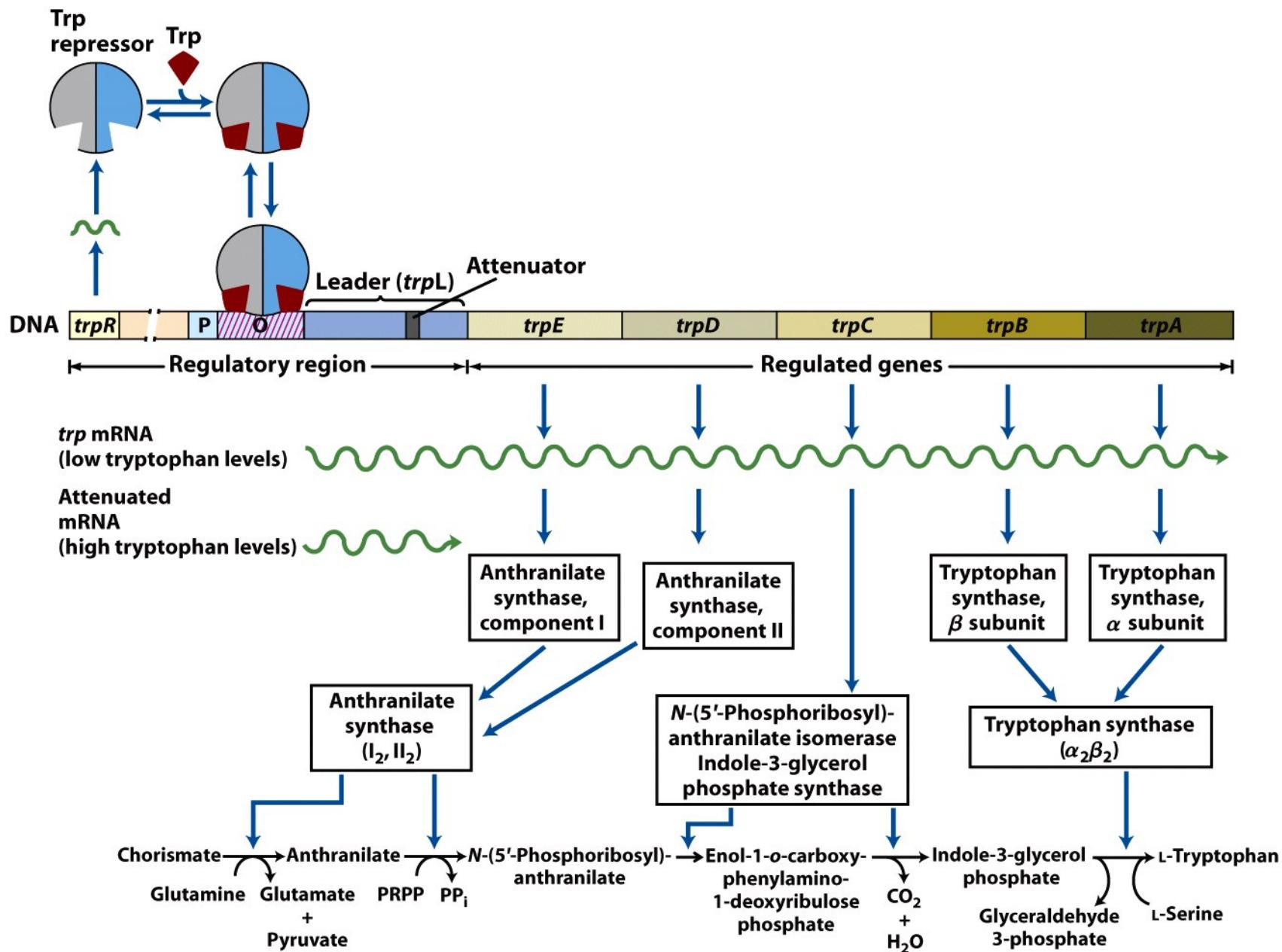




The *trp* operon is a repressible operon with three transcription units

- The *trp* operon is negatively controlled by the level of its product, the amino acid tryptophan (**autoregulation**)
- The amino acid tryptophan activates an inactive repressor encoded by *trpR*
- A repressor (or activator) will act on all loci that have a copy of its target operator sequence





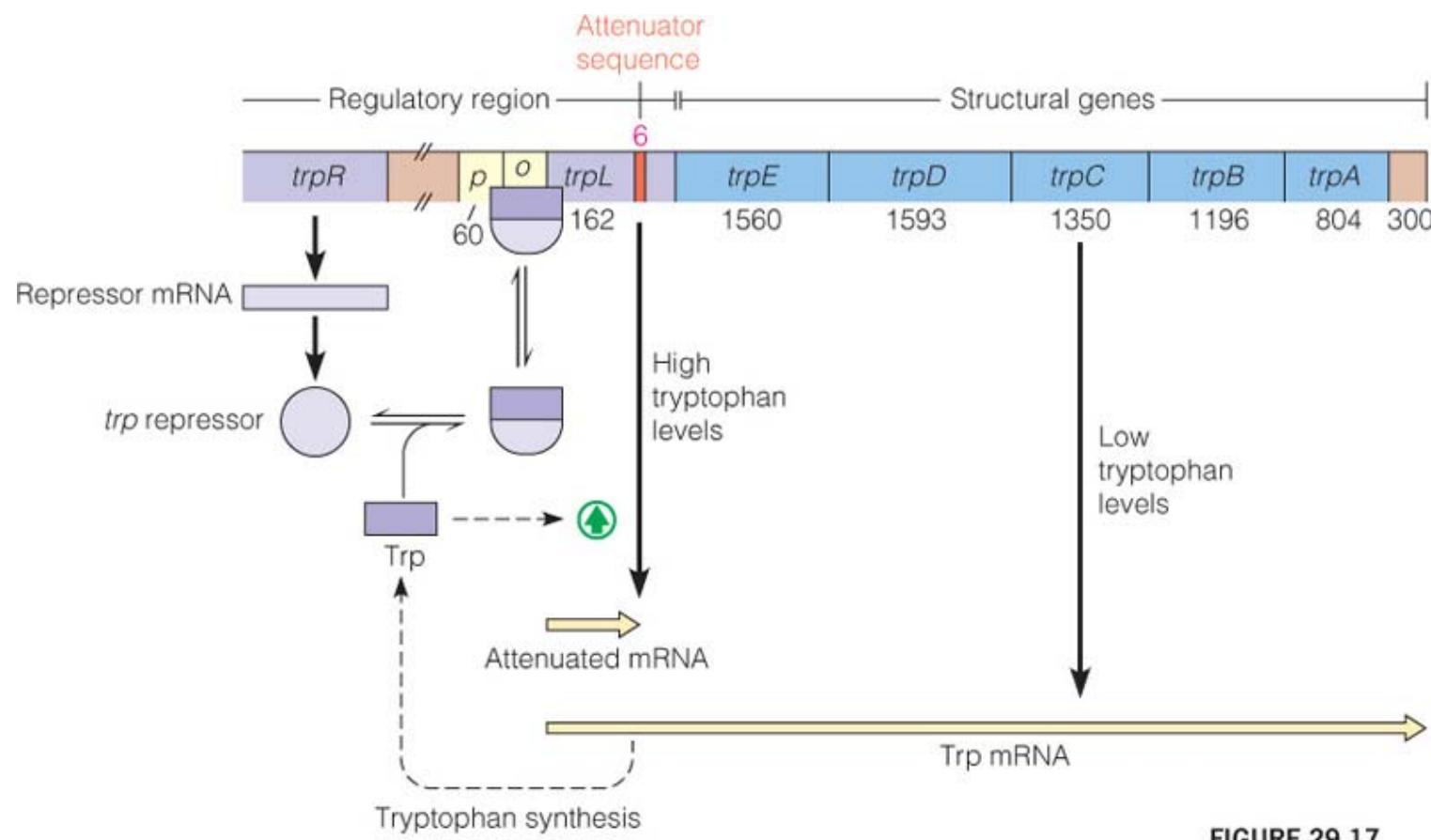
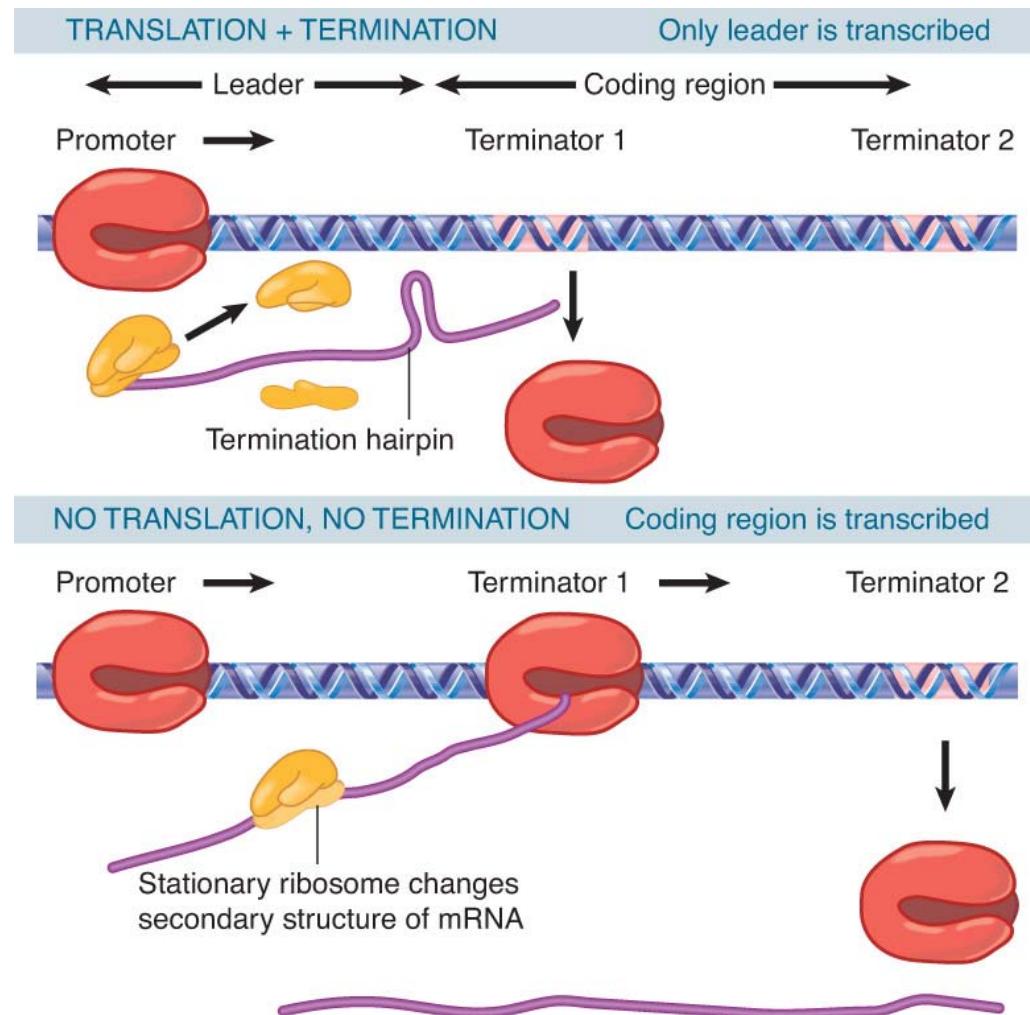


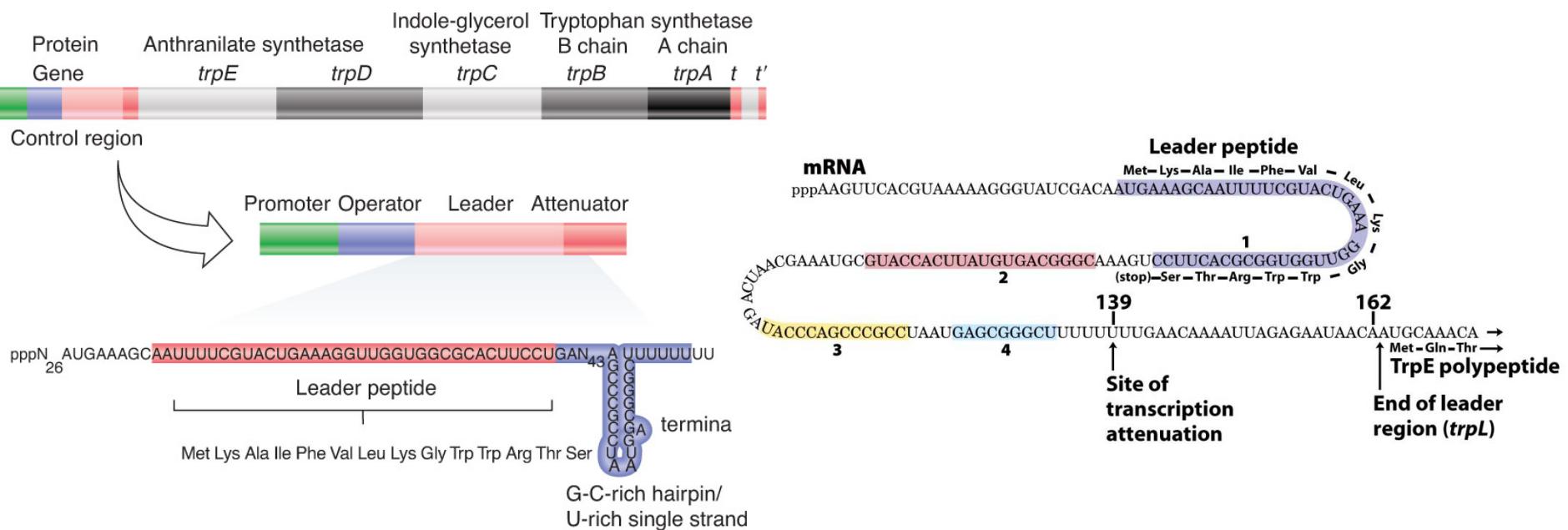
FIGURE 29.17

The *trp* operon. The figure shows regulation by *trp* repressor and by attenuation. *trpA*, the attenuator site, is shown in red.

■ **Attenuation** – The regulation of bacterial operons by controlling termination of transcription at a site located before the first structural gene



- An **attenuator** (intrinsic terminator) is located between the promoter and the first gene of the *trp* cluster
 - The absence of Trp-tRNA suppresses termination and results in a 10 \times increase in transcription



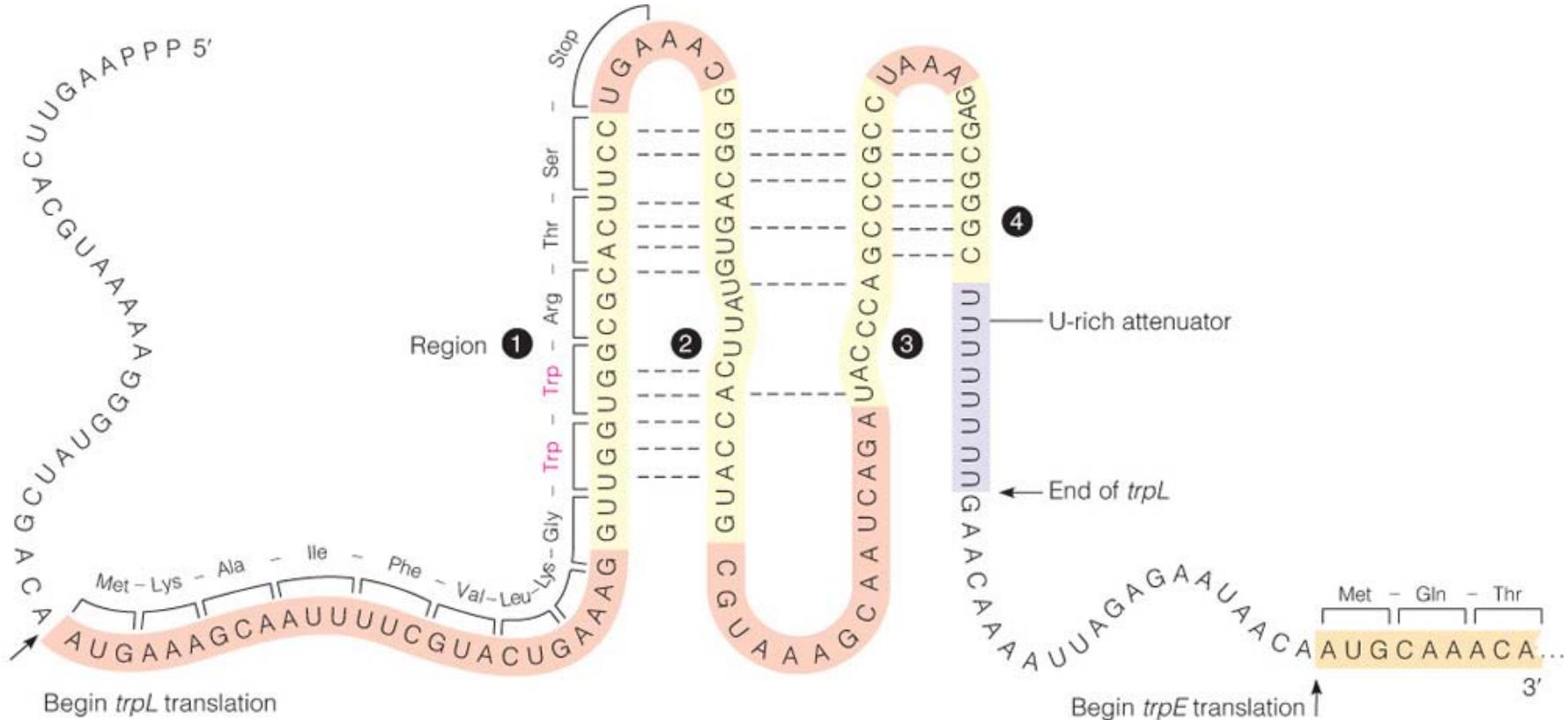
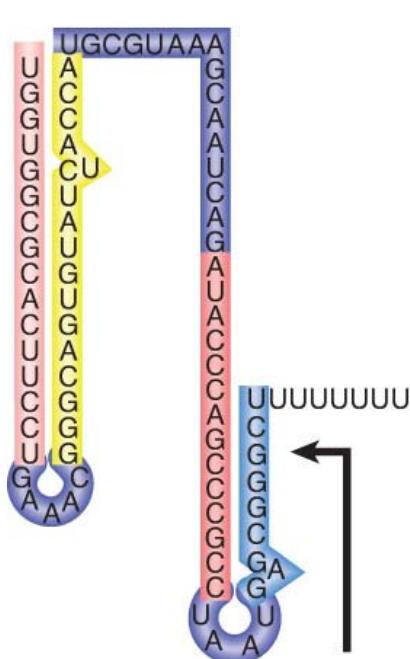


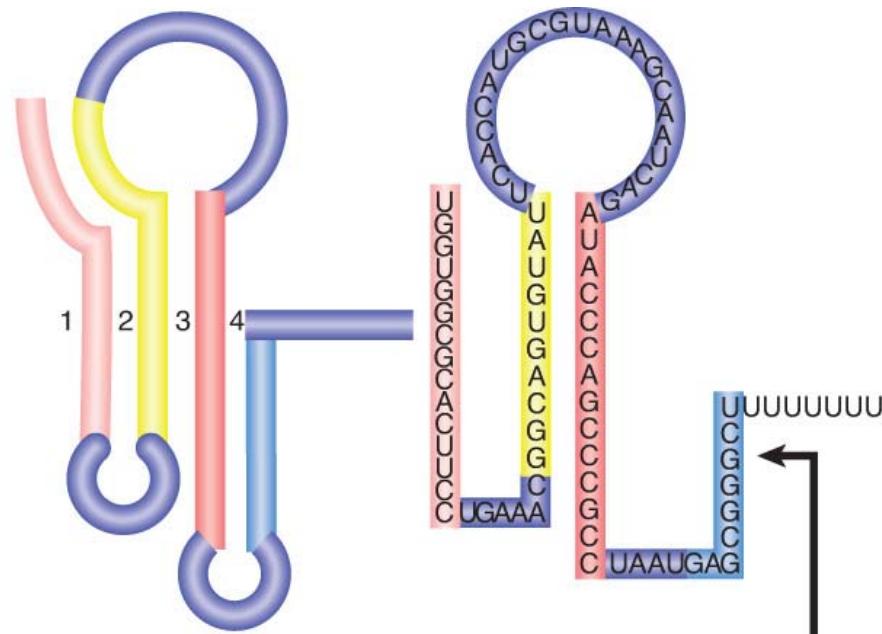
FIGURE 29.18

RNA base sequence of the *trp* leader region. The four internally complementary sequences that participate in attenuation (yellow) are shown, as well as the two *trp* codons (magenta) in region 1 that act as a pause site for RNA polymerase. The translational stop codon after region 1 (see Chapter 28) may serve to prevent needless translation of those few full-length messages that are produced despite attenuation.

The *trp* leader region can exist in alternative base-paired conformations



Regions 3 & 4
pair to form the
terminator hairpin



ALTERNATIVE STRUCTURES

Region 2 is complementary to 1 & 3
Region 3 is complementary to 2 and 4

Regions 2 & 3 pair;
terminator region
is single-stranded

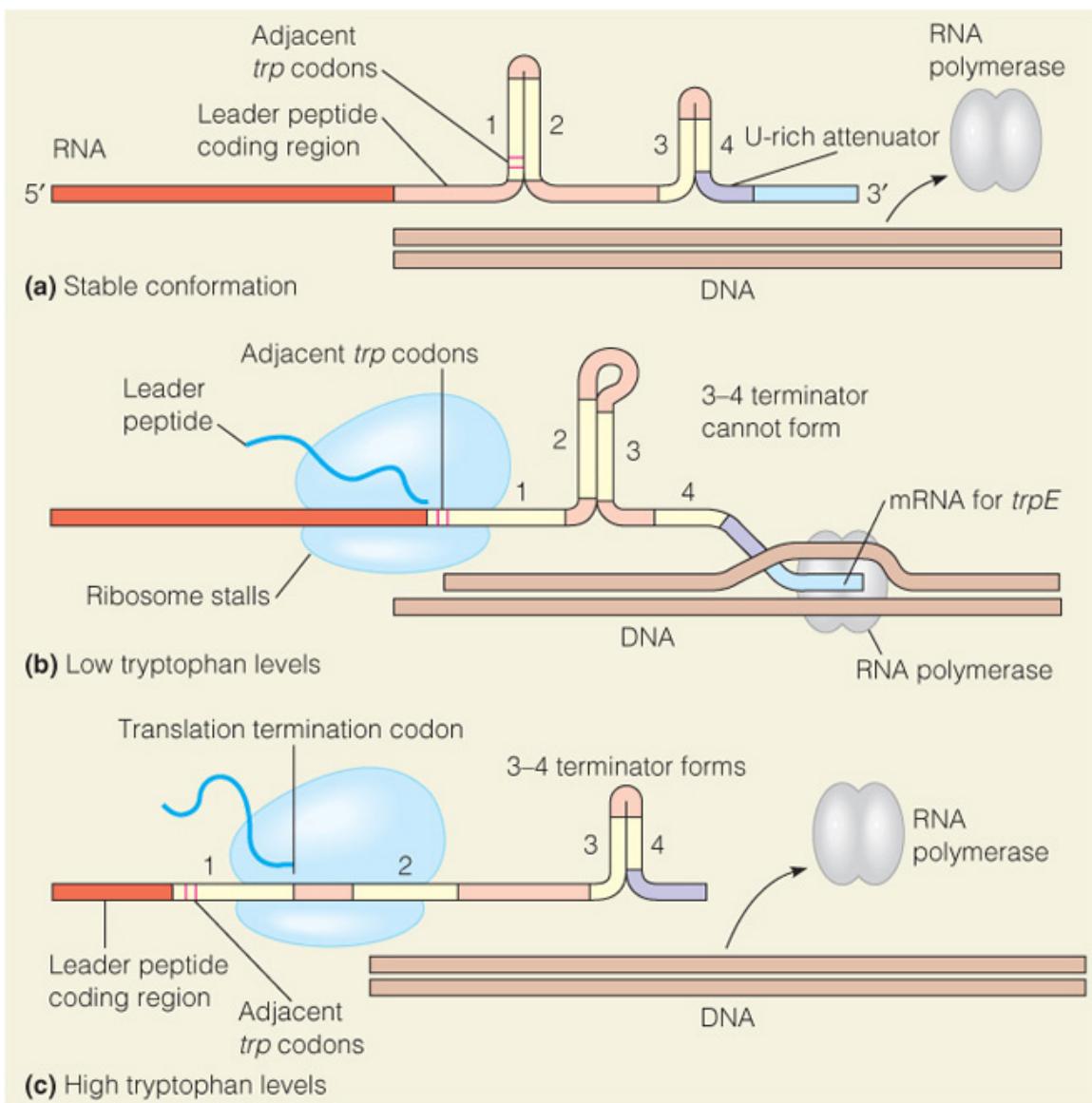
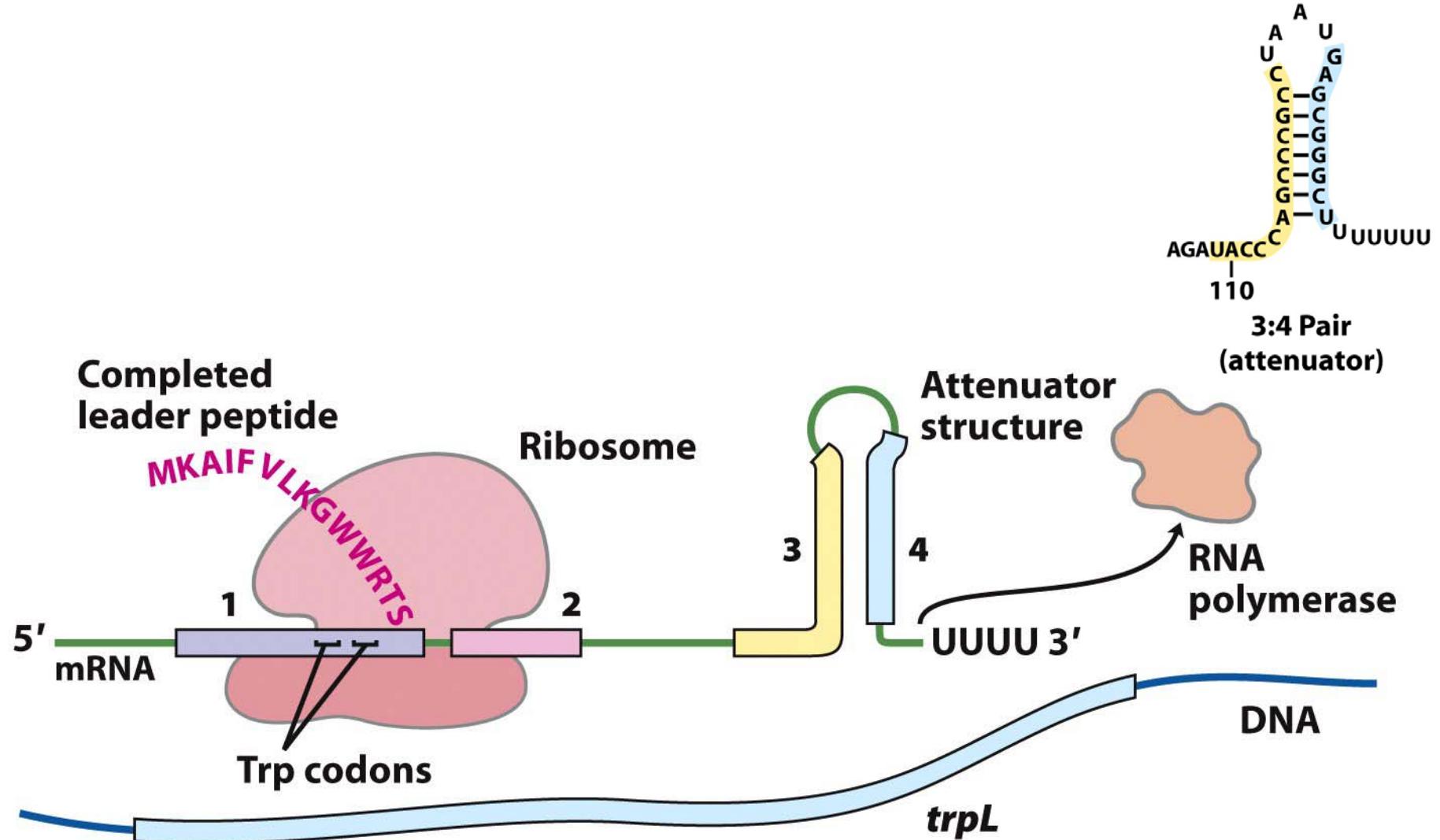


FIGURE 29.19

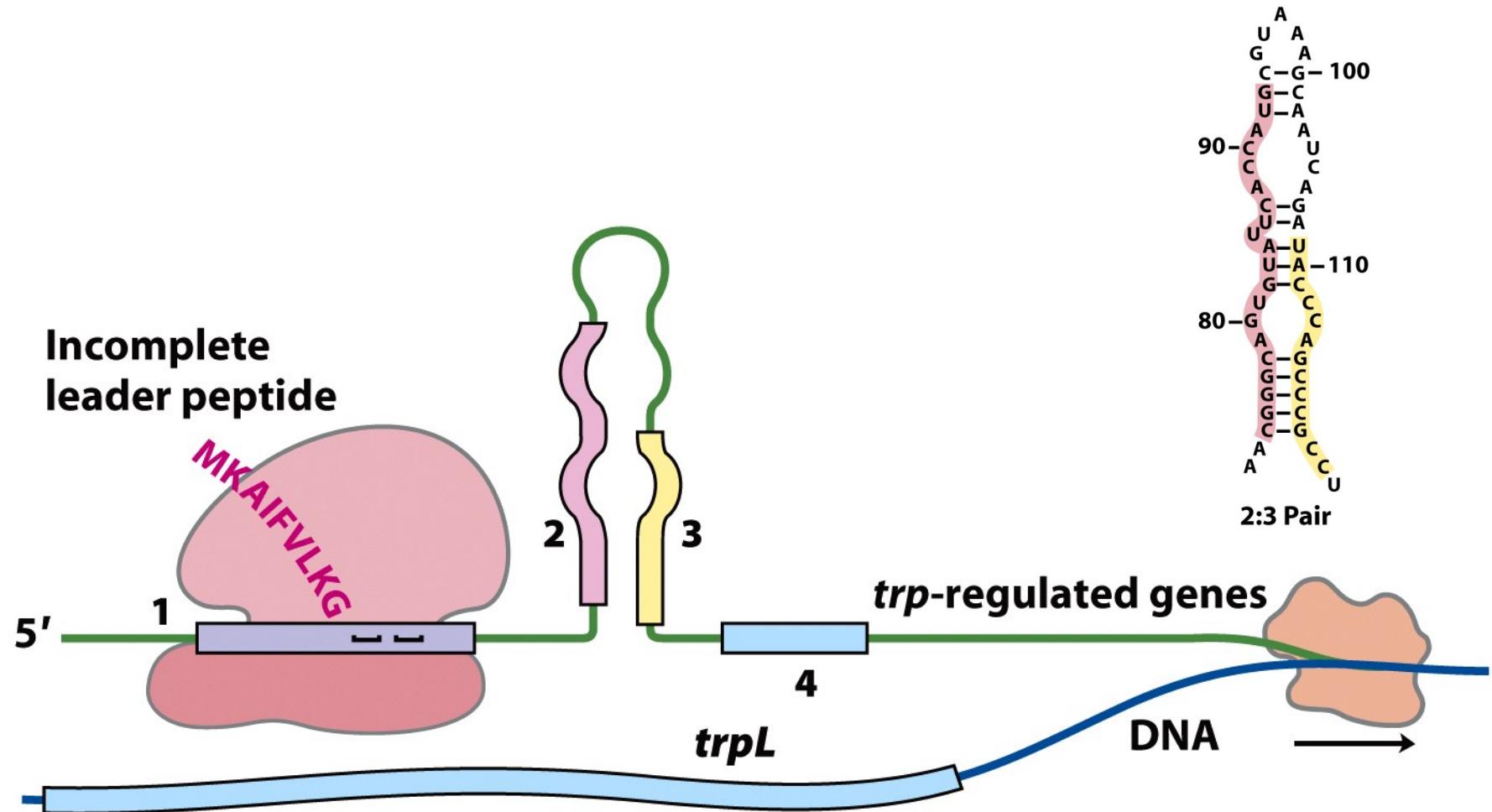
Mechanism of attenuation in the *trp* operon.

(a) Most stable conformation for leader mRNA.

(b) Conformation for leader mRNA at low tryptophan levels. (c) Conformation for leader mRNA at high tryptophan levels.



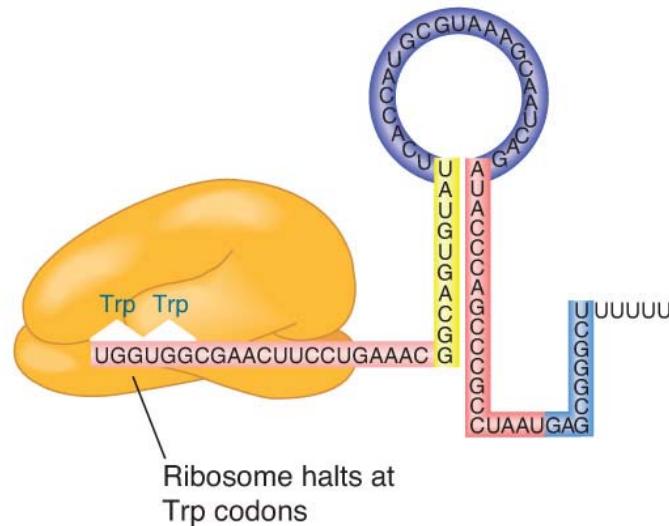
When tryptophan levels are high, the ribosome quickly translates sequence 1 (open reading frame encoding leader peptide) and blocks sequence 2 before sequence 3 is transcribed. Continued transcription leads to attenuation at the terminator-like attenuator structure formed by sequences 3 and 4.



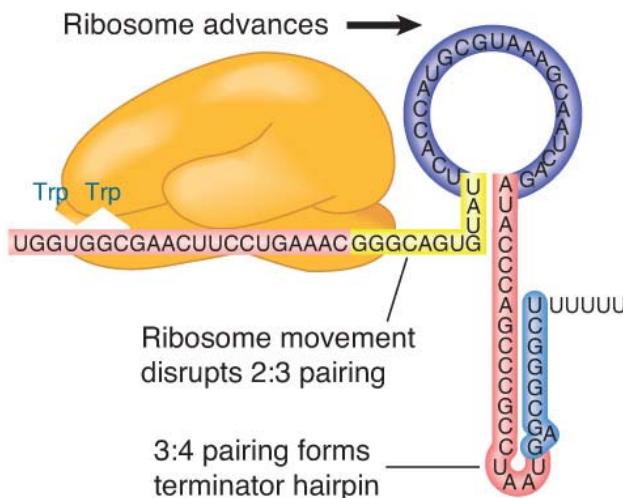
When tryptophan levels are low, the ribosome pauses at the Trp codons in sequence 1. Formation of the paired structure between sequences 2 and 3 prevents attenuation, because sequence 3 is no longer available to form the attenuator structure with sequence 4. The 2:3 structure, unlike the 3:4 attenuator, does not prevent transcription.

Tryptophan controls ribosome position

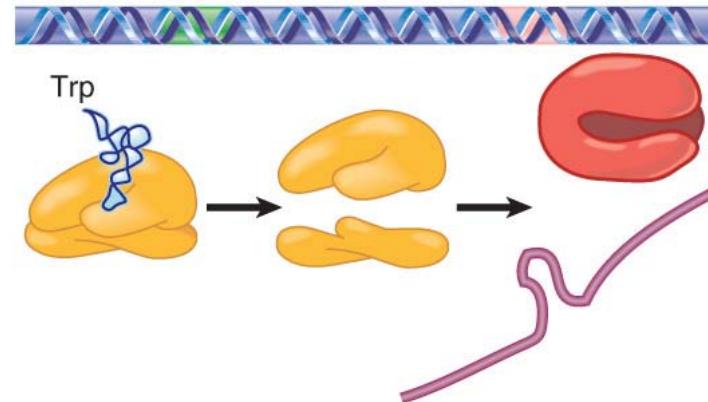
TRYPTOPHAN ABSENT



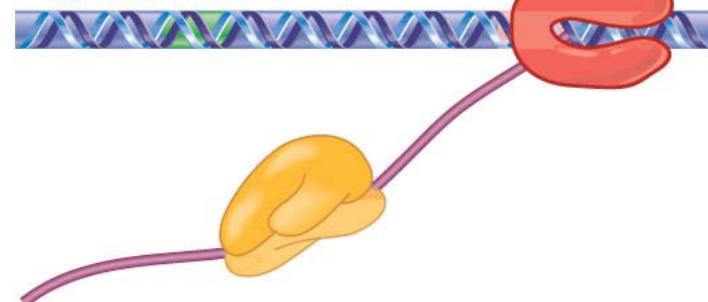
TRYPTOPHAN PRESENT



Tryptophan present



Tryptophan absent



Control of transcription initiation: used for most genes

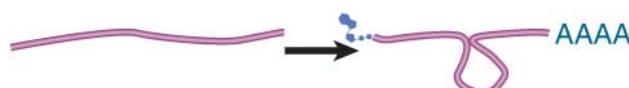
Local structure of the gene is changed



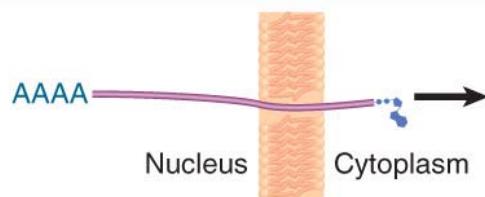
General transcription apparatus binds to promoter



RNA is modified and processed:
can control expression of alternative products from gene



mRNA is exported from nucleus to cytoplasm

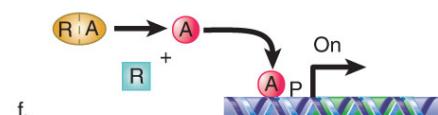
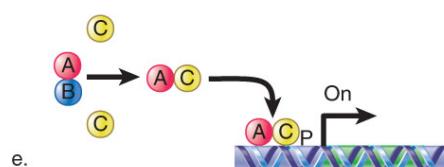
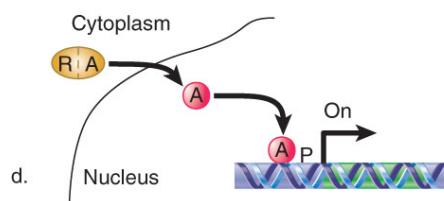
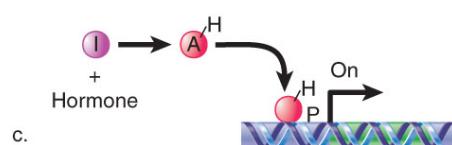
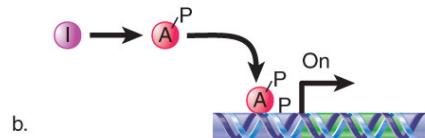
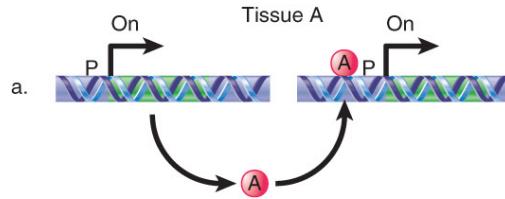


mRNA is translated and degraded



- Eukaryotic gene expression is usually controlled at the level of initiation of transcription by opening the chromatin

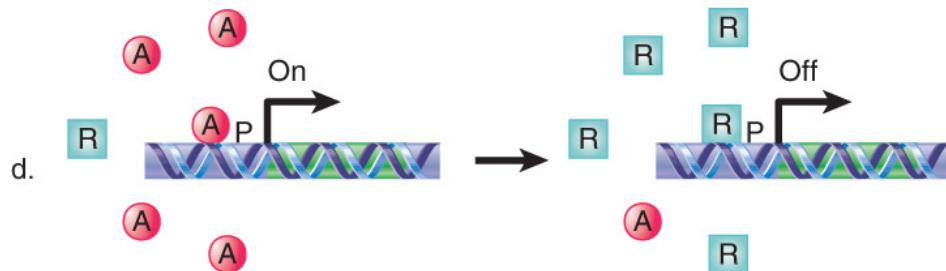
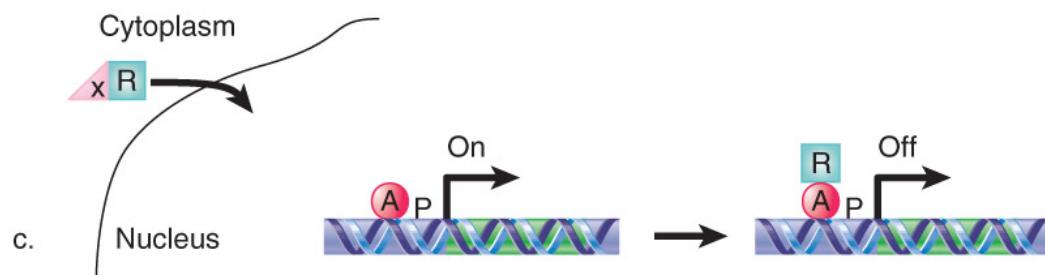
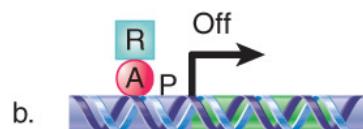
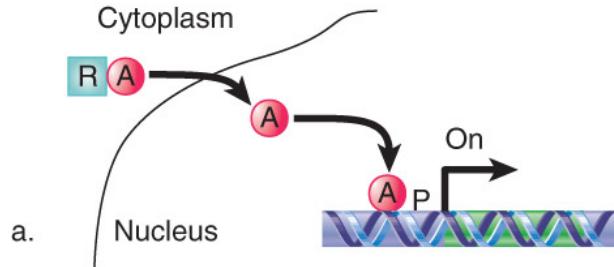
- Activation of gene structure: open chromatin
- Initiation of transcription and elongation
- Processing the transcript
- Transport to the cytoplasm from nucleus
- Translation of mRNA
- Degradation and turnover of mRNA



A Pos TF
I Inactive Pos TF
R Regulator Protein

■ **Activators** – determine the frequency of transcription

- Activators work by making protein–protein contacts with the basal factors
- Activators may work *via* coactivators.
- Activators are regulated in many different ways
- **antirepressor** – A positive regulator that functions in opening chromatin

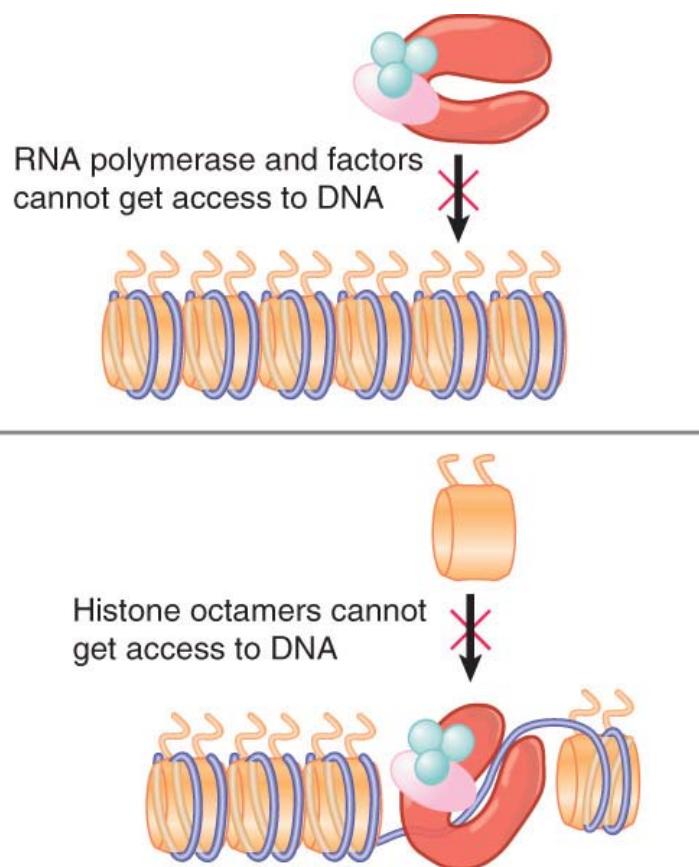


■ **Repressor** – A protein that inhibits expression of a gene.

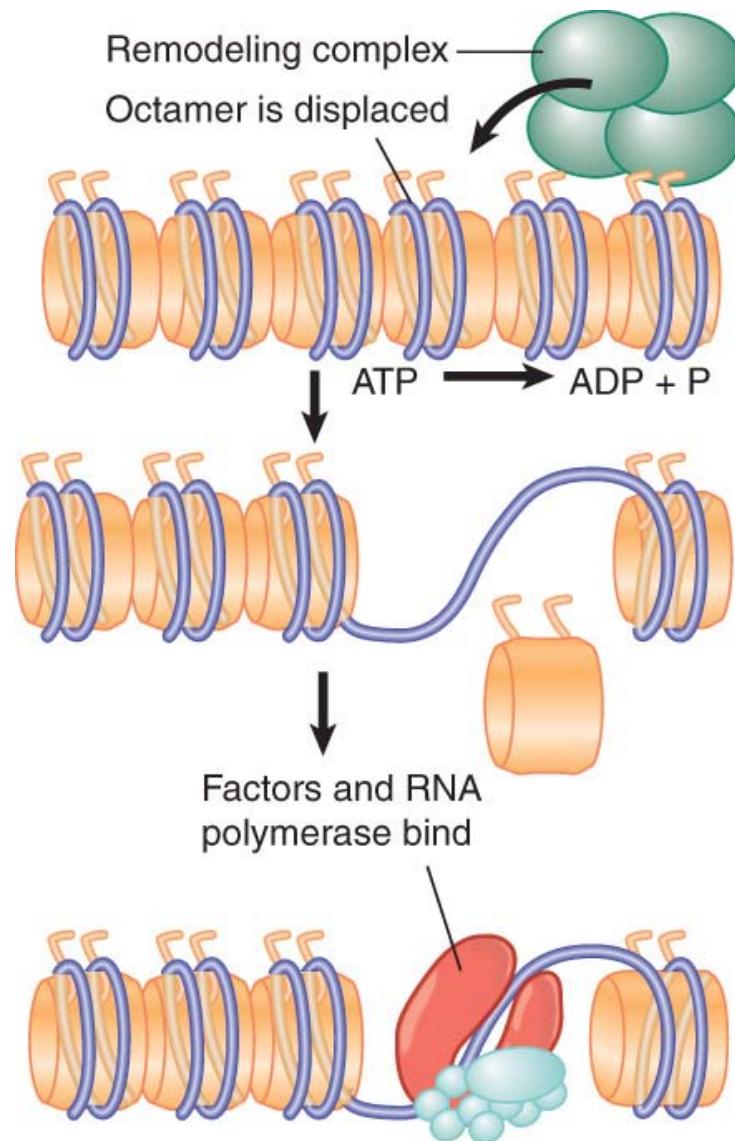
It may act to prevent transcription by binding to an operator site in DNA or to prevent translation by binding to RNA

- Some components of the transcriptional apparatus work by changing chromatin structure
- Repression is achieved by affecting chromatin structure or by binding to and masking activators

Chromatin remodeling is an active process responsible for transcriptional initiation



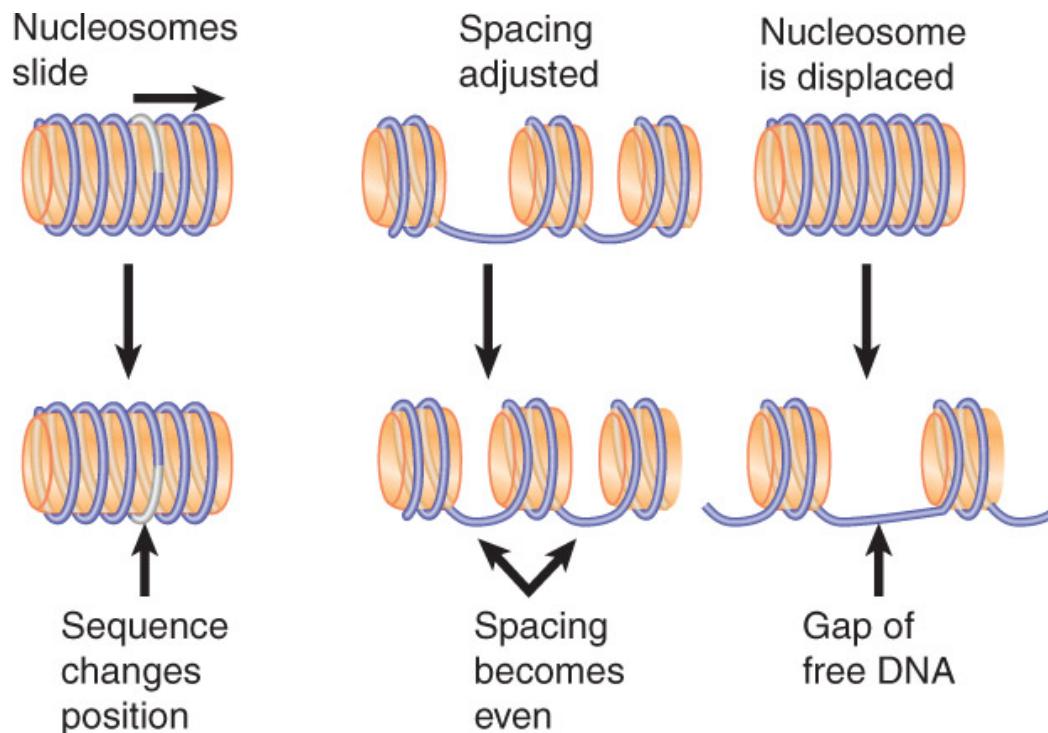
■ **Chromatin remodeling** – The energy-dependent displacement or reorganization of nucleosomes that occurs in conjunction with activation of genes for transcription



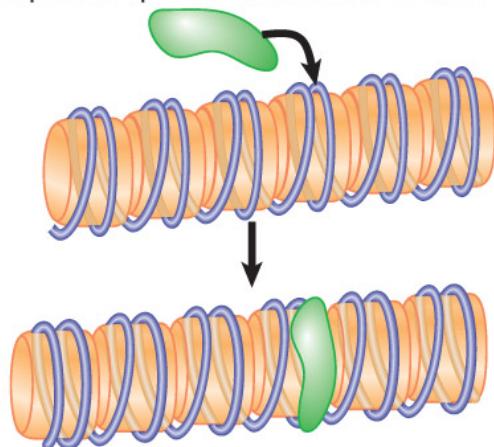
- There are numerous **ATP-dependent chromatin remodeling complexes** that use energy provided by hydrolysis of ATP

Type of Complex	SWI/SNF	ISWI	CHD	INO80/SWRI
Yeast	SWI/SNF RSC	ISW1a, ISWb ISW2	CHDI	INO80 SWRI
Fly	dSWI/SNF (brahma)	NURF CHRAC ACF	JMIZ	Tip60
Human	hSWI/SNF	RSF hACF/WCFR hCHRAC WICH	NuRD	INO80 SRCAP
Frog		WICH CHRAC ACF	Mi-2	

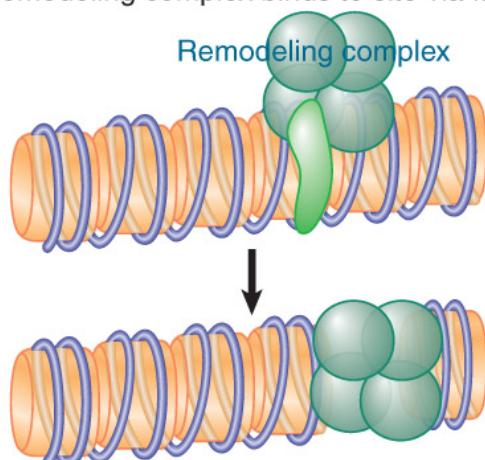
- All remodeling complexes contain a related ATPase catalytic subunit, and are grouped into subfamilies containing more closely related ATPase subunits
- Remodeling complexes can alter, slide, or displace nucleosomes
- Some remodeling complexes can exchange one histone for another in a nucleosome



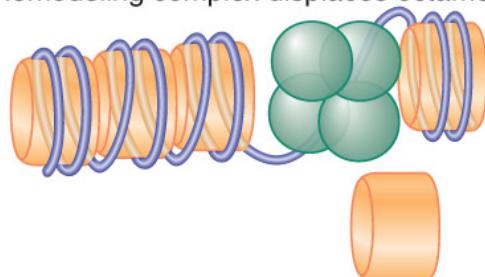
1. Sequence-specific factor binds to DNA



2. Remodeling complex binds to site via factor



3. Remodeling complex displaces octamer

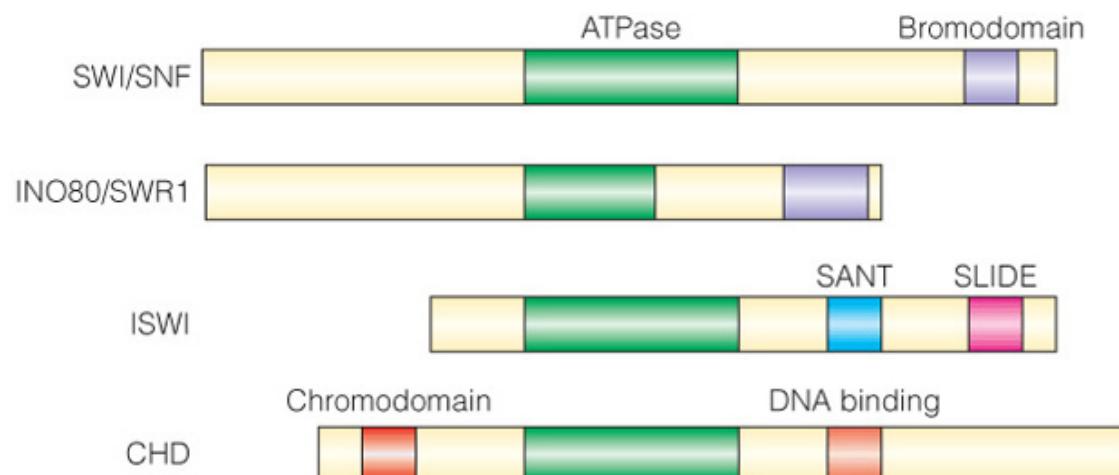


- A remodeling complex does not itself have specificity for any particular target site, but must be recruited by a component of the transcription apparatus.
- Remodeling complexes are recruited to promoters by sequence-specific activators.
- The factor may be released once the remodeling complex has bound.

FIGURE 29.25

Chromatin remodeler families and conserved domains of the ATPase-containing subunit.

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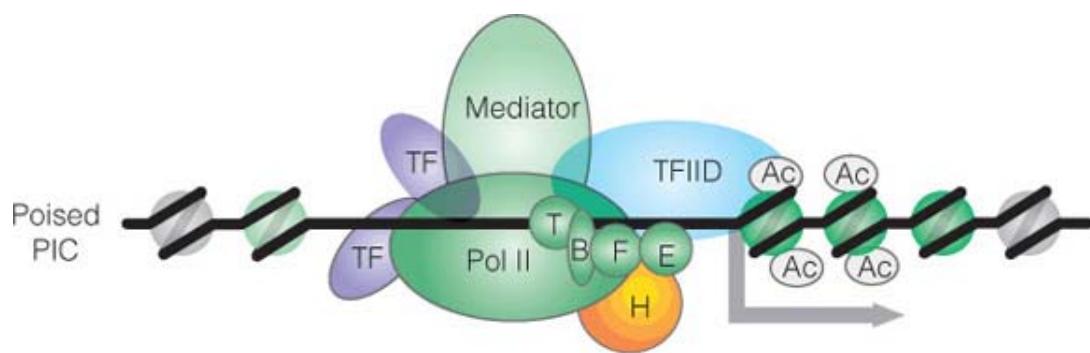


FIGURE 29.26

Schematic view of a transcription preinitiation complex.

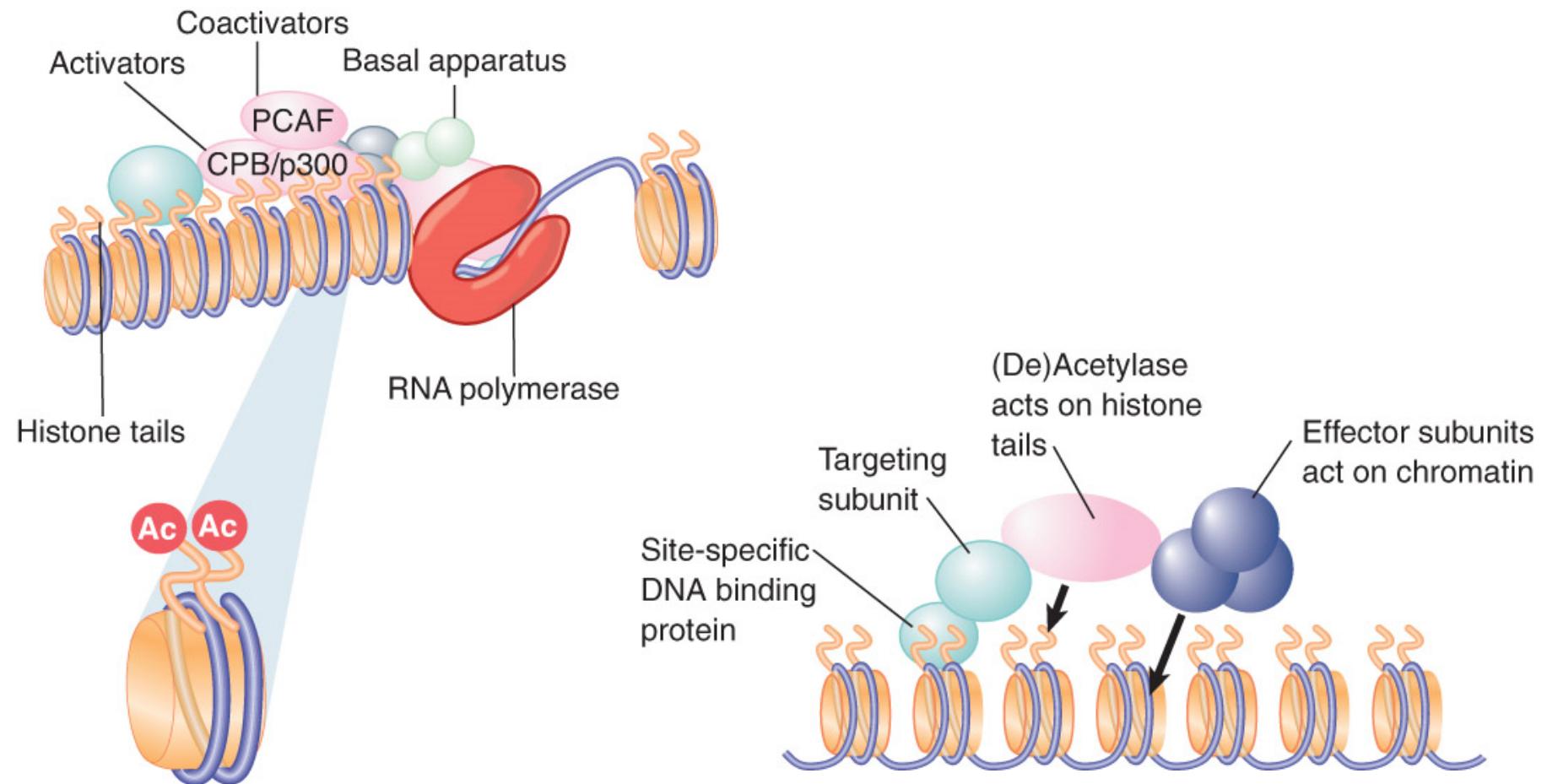
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Histone acetylation is associated with transcription activation

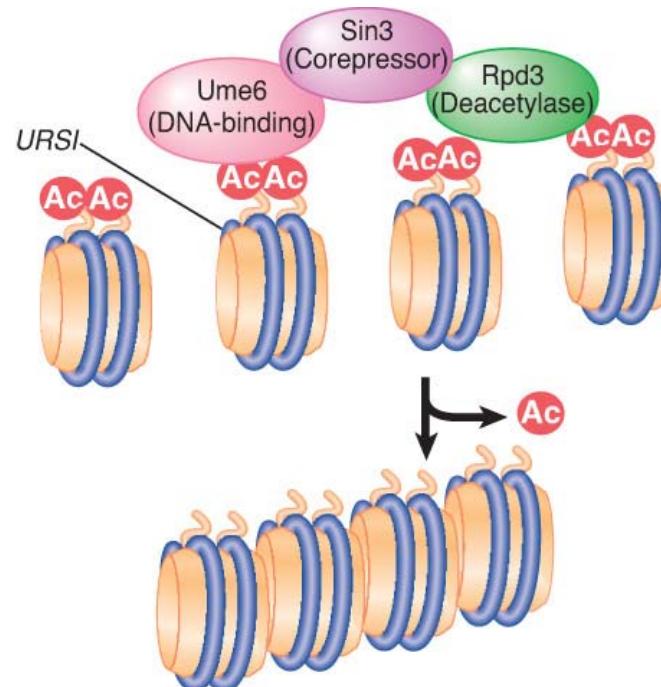
- Newly synthesized histones are **acetylated** at specific sites, then **deacetylated** after incorporation into nucleosomes
- Histone acetylation is associated with activation of gene expression
- **Lysine (K) acetyltransferase (KAT)** – An enzyme (typically present in large complexes) that acetylates lysine residues in histones (or other proteins). Also known as **histone acetyltransferase (HAT)**
- Transcription activators are associated with histone acetylase activities in large complexes

Some coactivators are acetylases



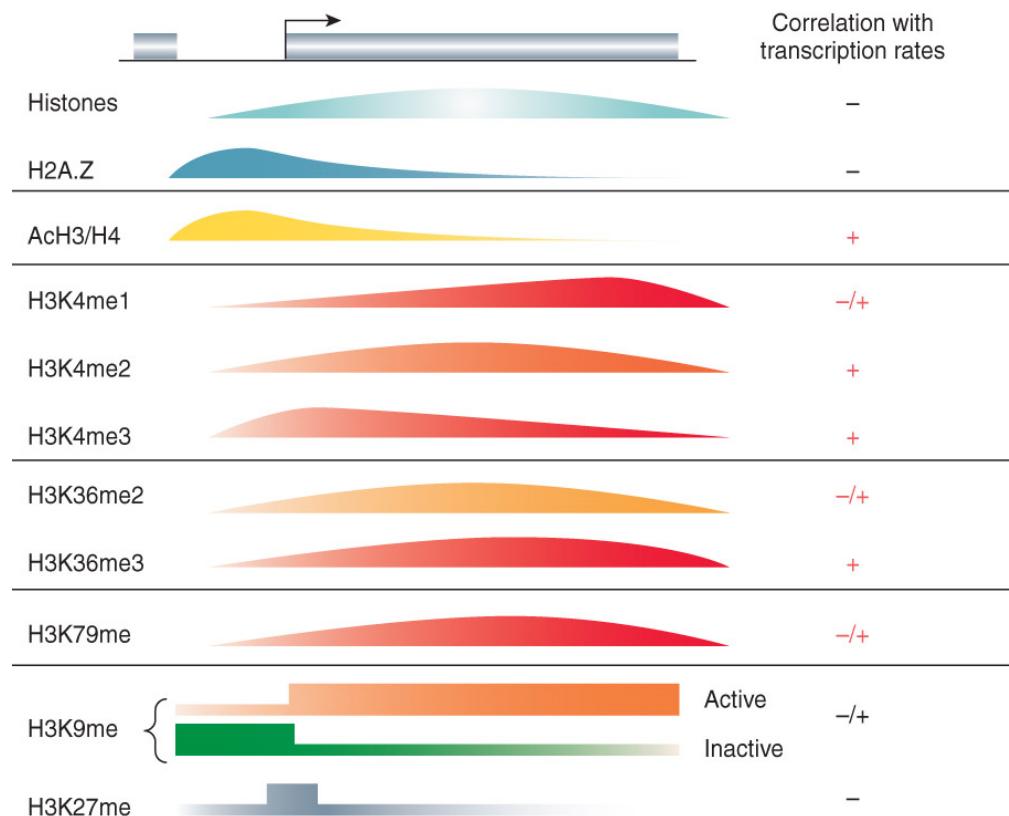
Histone acetylation is associated with transcription activation

- Histone acetyltransferases vary in their target specificity
- Deacetylation is associated with repression of gene activity
- **Histone deacetylase (HDAC)** – Enzyme that removes acetyl groups from histones; may be associated with repressors of transcription
- Deacetylases are present in complexes with repressor activity

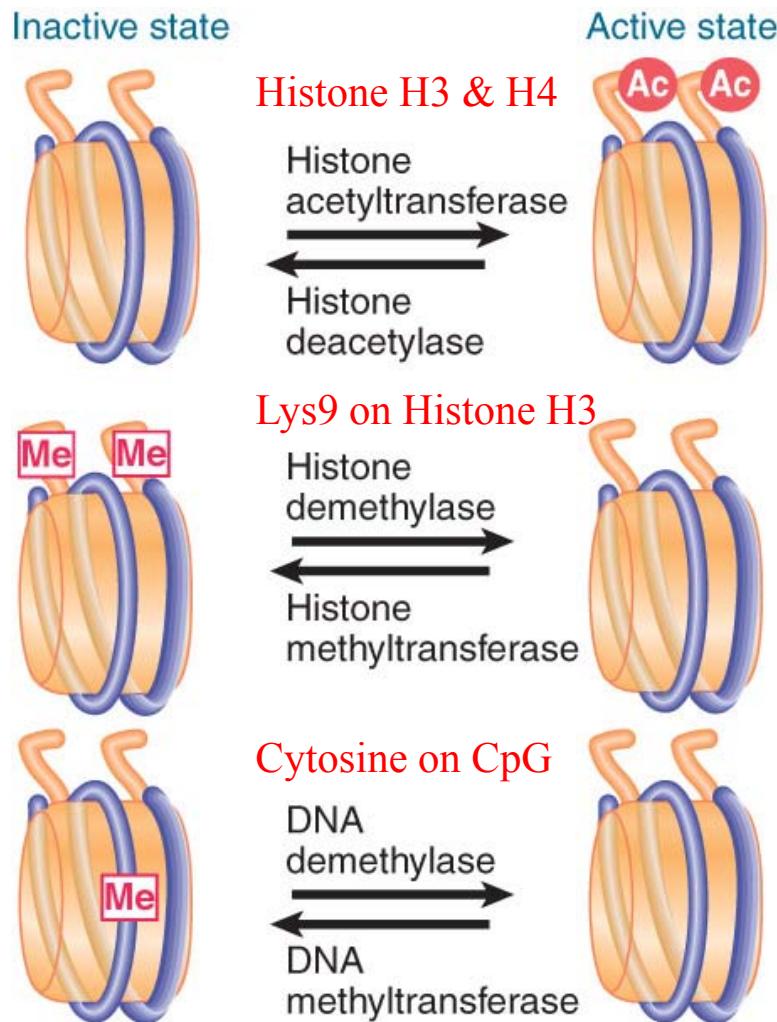


Methylation of histones and DNA is connected

- Methylation of both DNA and specific sites on histones is a feature of inactive chromatin
- The SET domain is part of the catalytic site of protein methyltransferases
- The two types of methylation event are connected.



Promoter Activation Involves Multiple Changes to Chromatin



- Remodeling complexes can facilitate binding of acetyltransferase complexes and vice versa.
- Histone methylation can also recruit chromatin-modifying complexes.
- Different modifications and complexes facilitate transcription elongation.

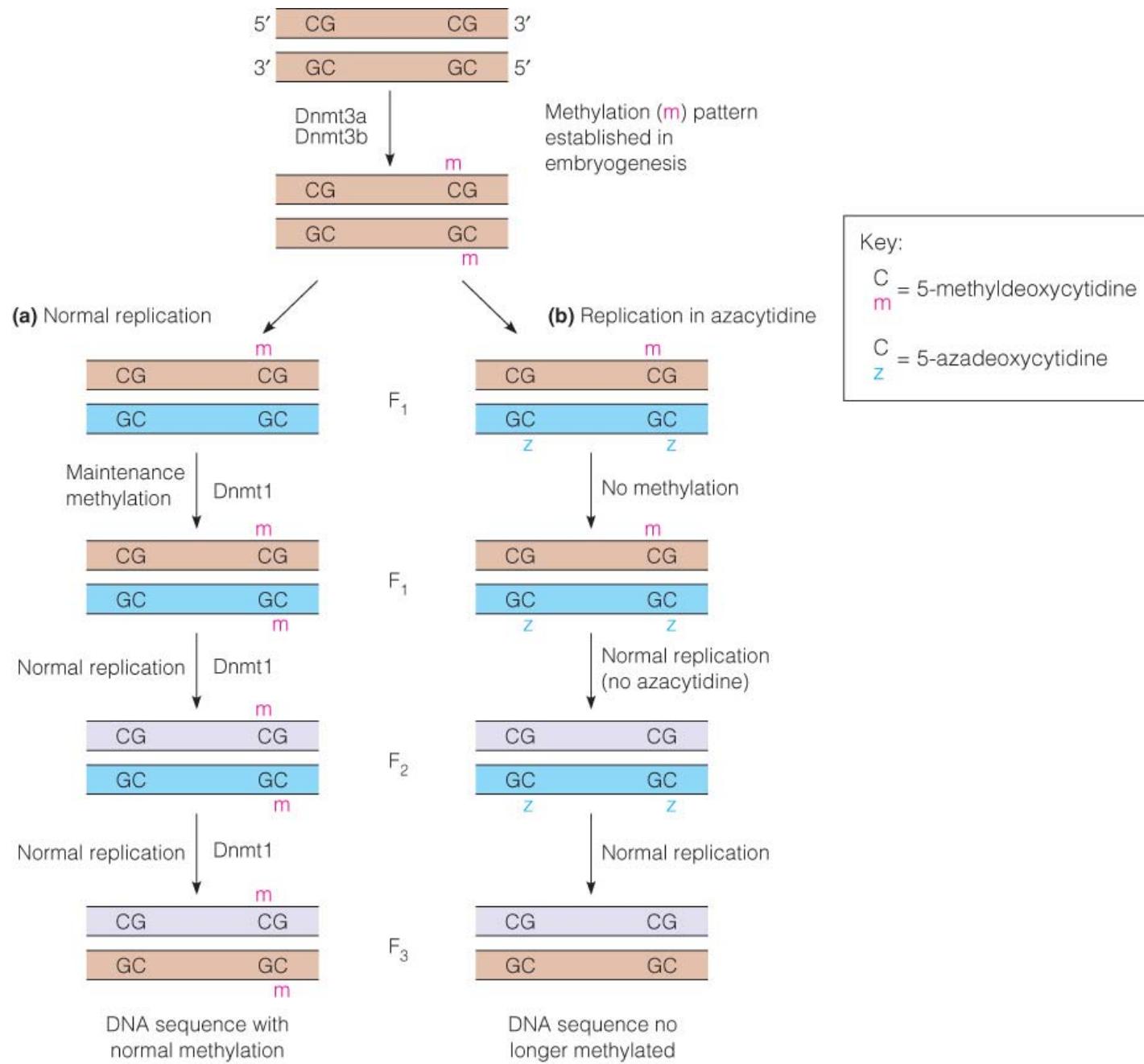
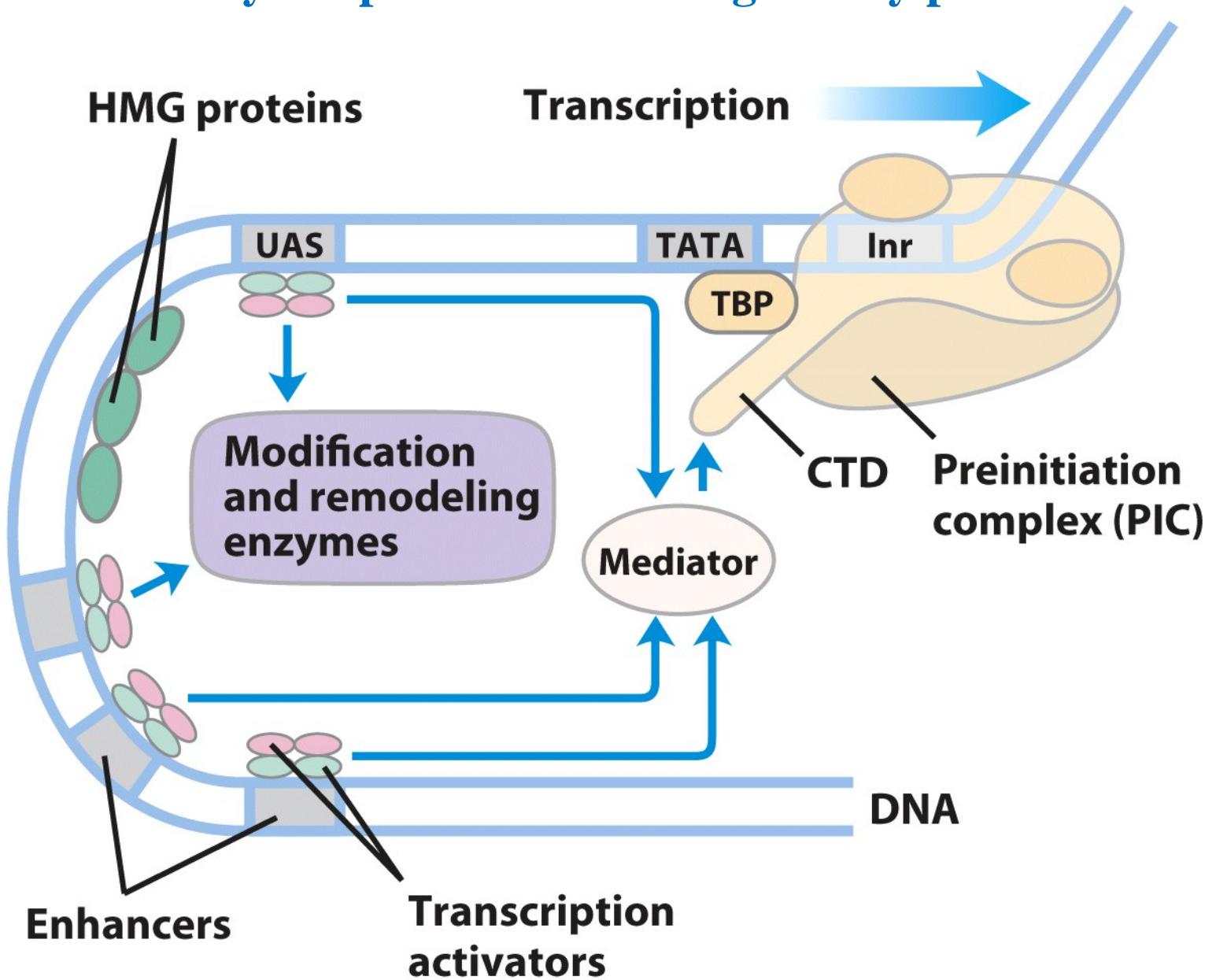


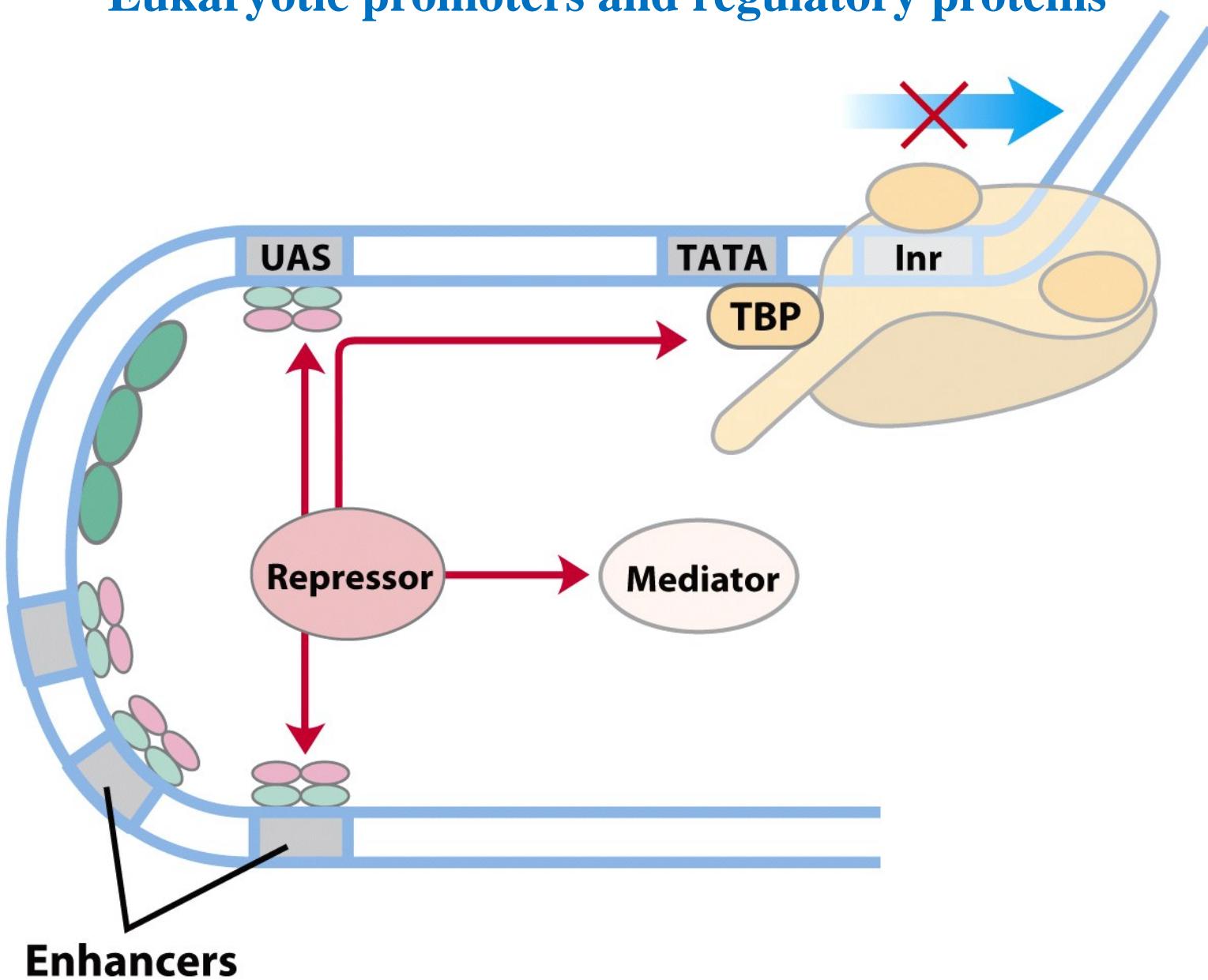
FIGURE 29.29

De novo and maintenance methylation of DNA and the effect of 5-azacytidine upon DNA methylation.

Eukaryotic promoters and regulatory proteins



Eukaryotic promoters and regulatory proteins



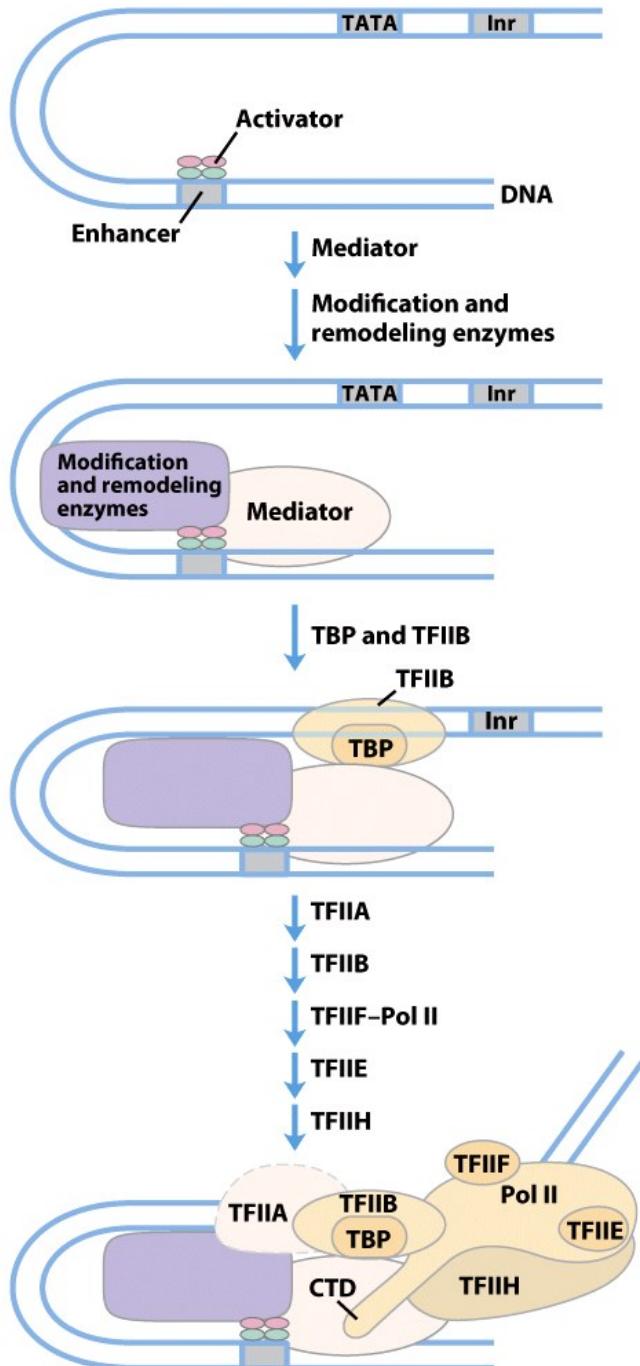


FIGURE 29.35

Blocking of translational initiation by antisense RNA. The transposase gene contains an antisense sequence at its 3' end. When transcribed, this antisense segment can fold back and base-pair to block initiation of translation.

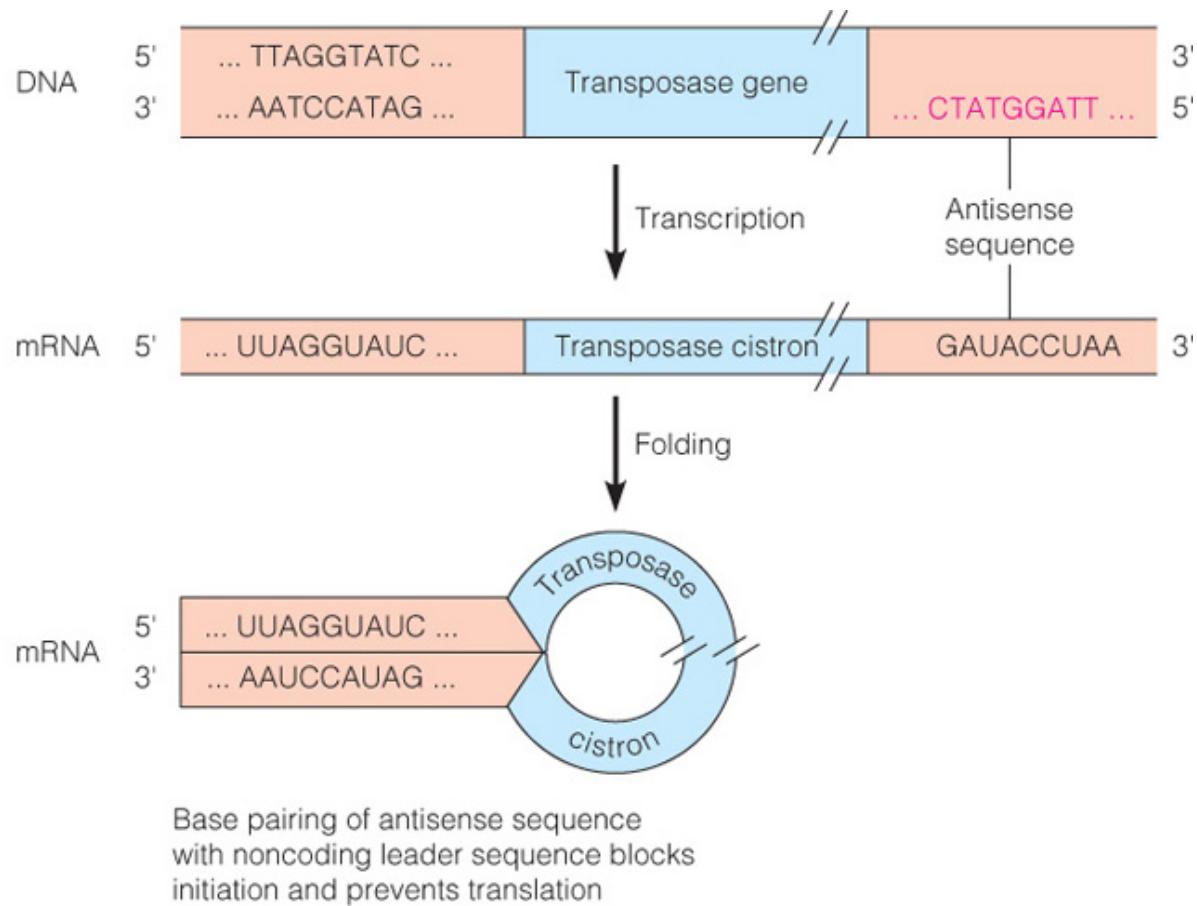


FIGURE 29.36

Inactivation of *ompF* mRNA by pairing with antisense RNA from the *micF* gene. A change in osmolarity stimulates transcription of the *micF* gene. The transcript is largely complementary to a region in *ompF* RNA that includes the translational start site. Hairpin loops within the sequences allow base pairing between complementary regions on the two mRNAs, and in this way both transcripts are prevented from serving as templates for protein synthesis.

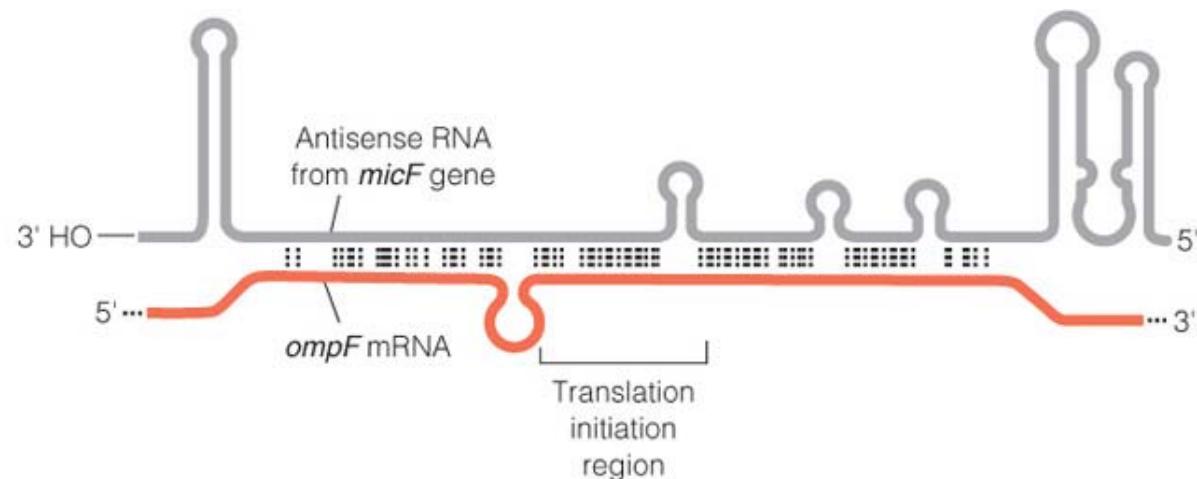


FIGURE 29.37

Regulation of translation in erythropoietic cells by heme levels. If heme levels fall, the heme-controlled kinase becomes active and phosphorylates eIF2 (magenta arrow). This blocks further translation by tying this factor into a stable complex with eIF2B. When heme levels are adequate, the kinase is inhibited, and eIF2 is available for translation initiation.

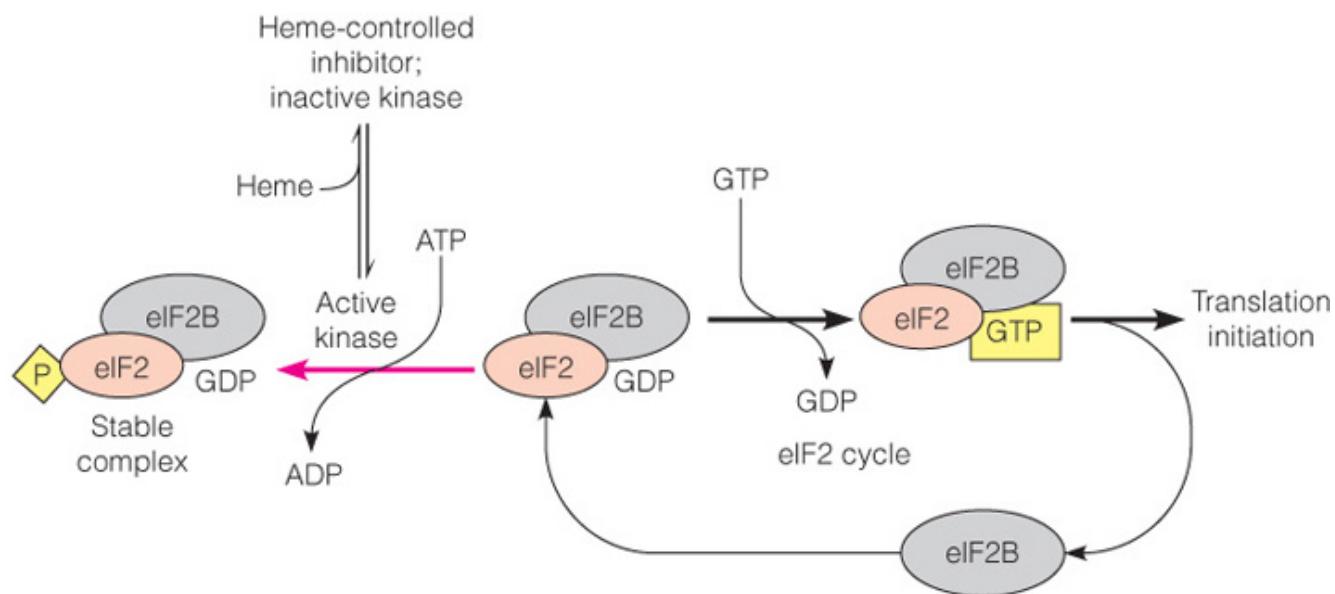
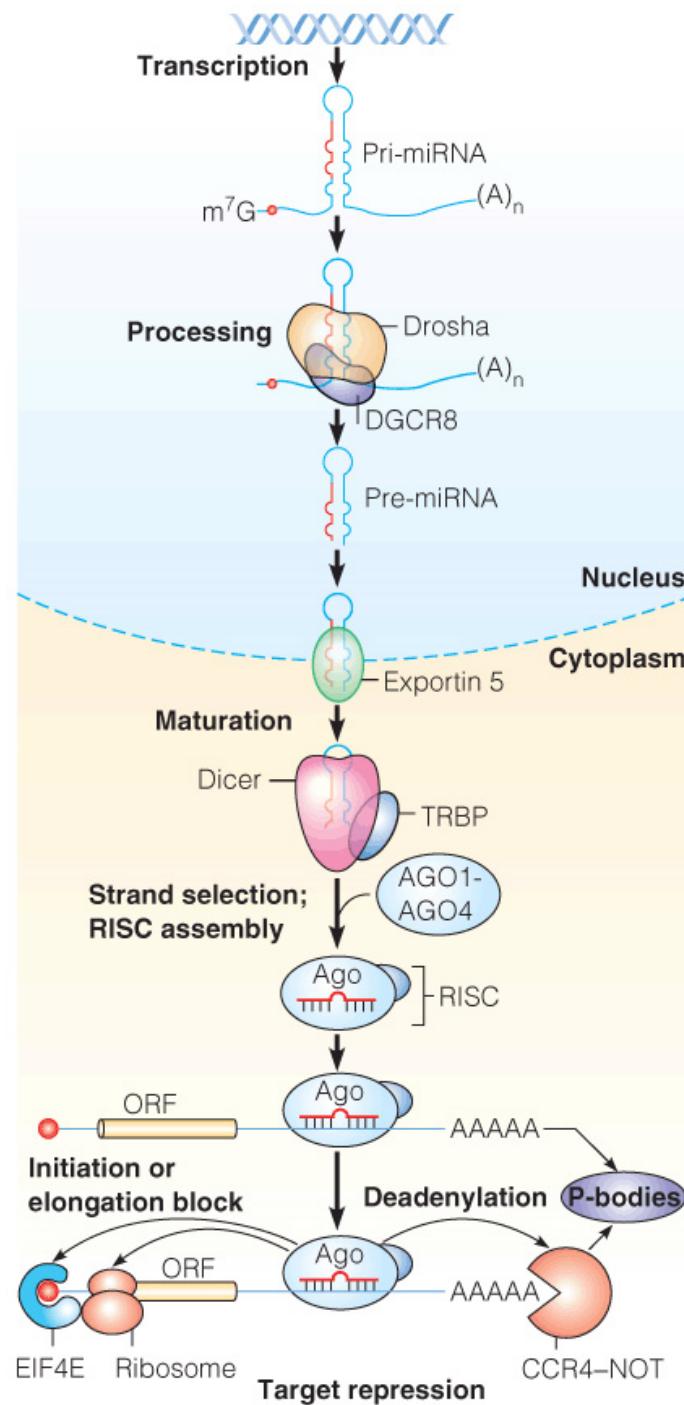


FIGURE 29.38

Biogenesis of miRNA. The mRNA sequence selected for processing can be either in an exon or an intron. For further details see text.

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Riboswitch

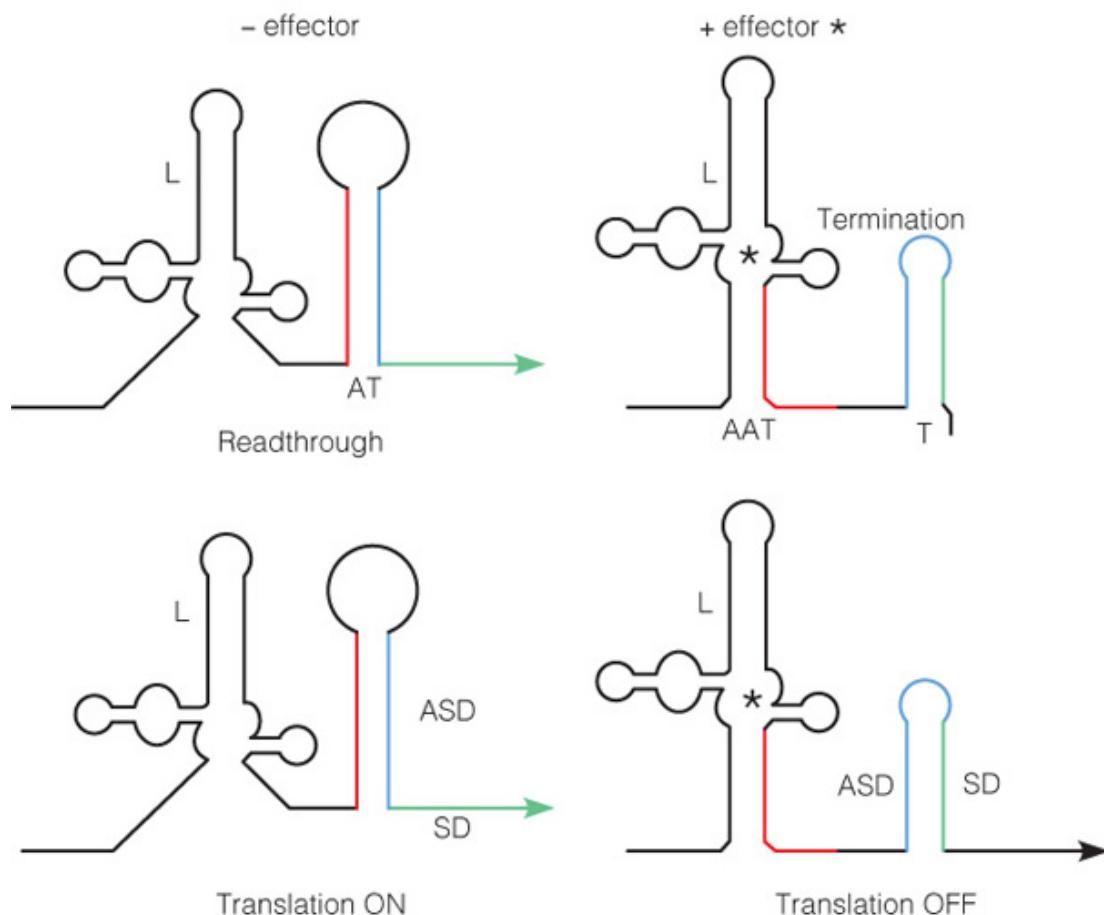


FIGURE 29.40

Mechanisms of action of a riboswitch. In the absence of effector (upper left) the ligand-binding site (L) is unoccupied, and a transcriptional antiterminator (AT) can form (see Figure 29.19, mechanism of attenuation). Alternatively, if the riboswitch functions at the level of translation (lower left), a region containing the Shine–Dalgarno sequence (SD) is not paired with its complement (anti-SD, or ASD), and translation can occur. In the presence of an effector, the mRNA tertiary structure can change to pair the antiterminator sequence with its complement (AAT), allowing a terminator loop (T) to form, leading to transcriptional termination (upper right). Alternatively (lower right), binding the ligand permits the Shine–Dalgarno sequence to pair with its complement (ASD) so that translation cannot initiate.

Genes & Development 22:3383–3390, T. M Henkin,
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