Regulation of Transcription in Prokaryotes

Trans-acting factors (proteins)

Cis-acting elements (DNA sequences)

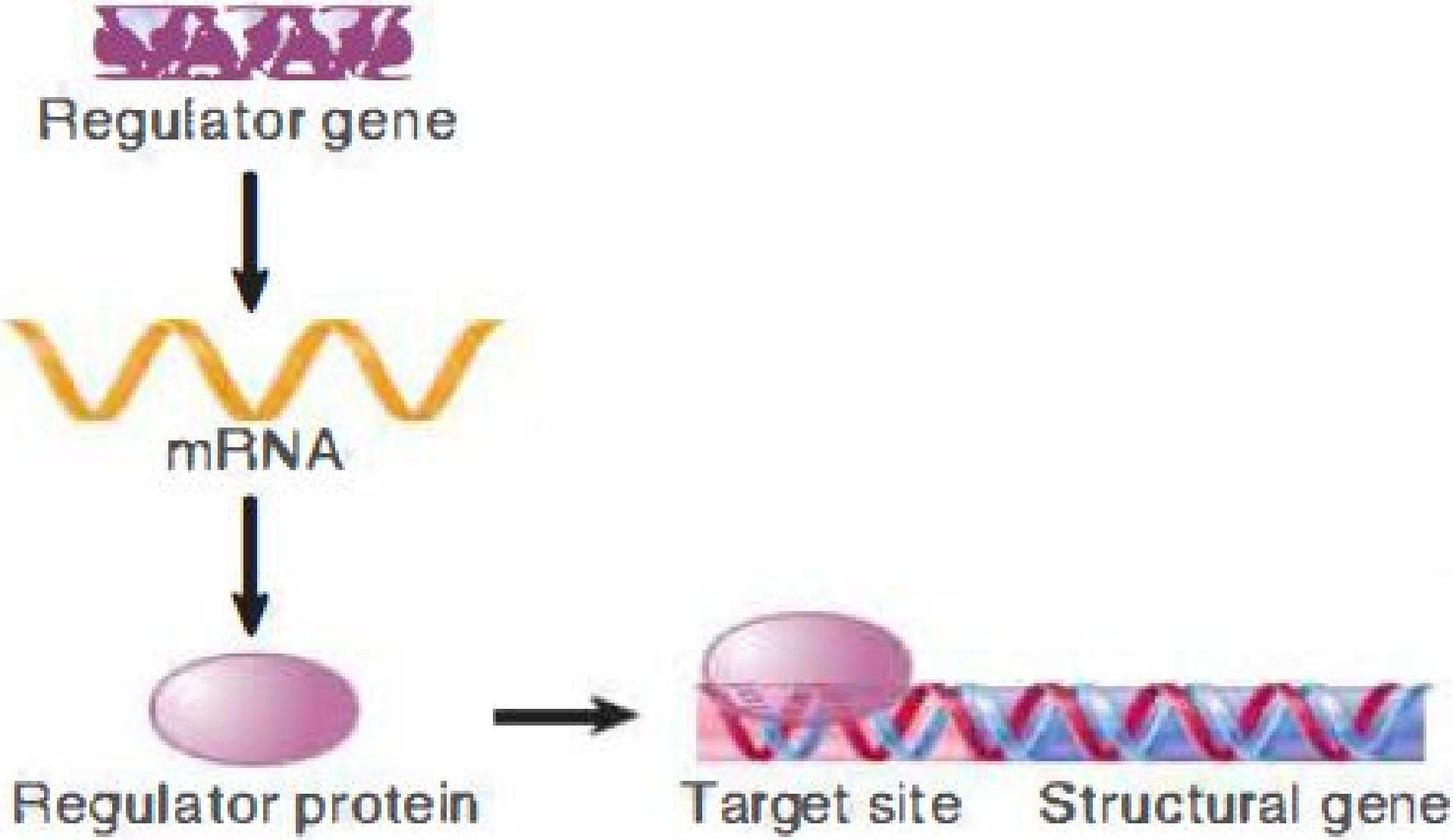


FIGURE 26.1 A regulator gene codes for a protein that acts at a target site on DNA.

Negative control of transcription

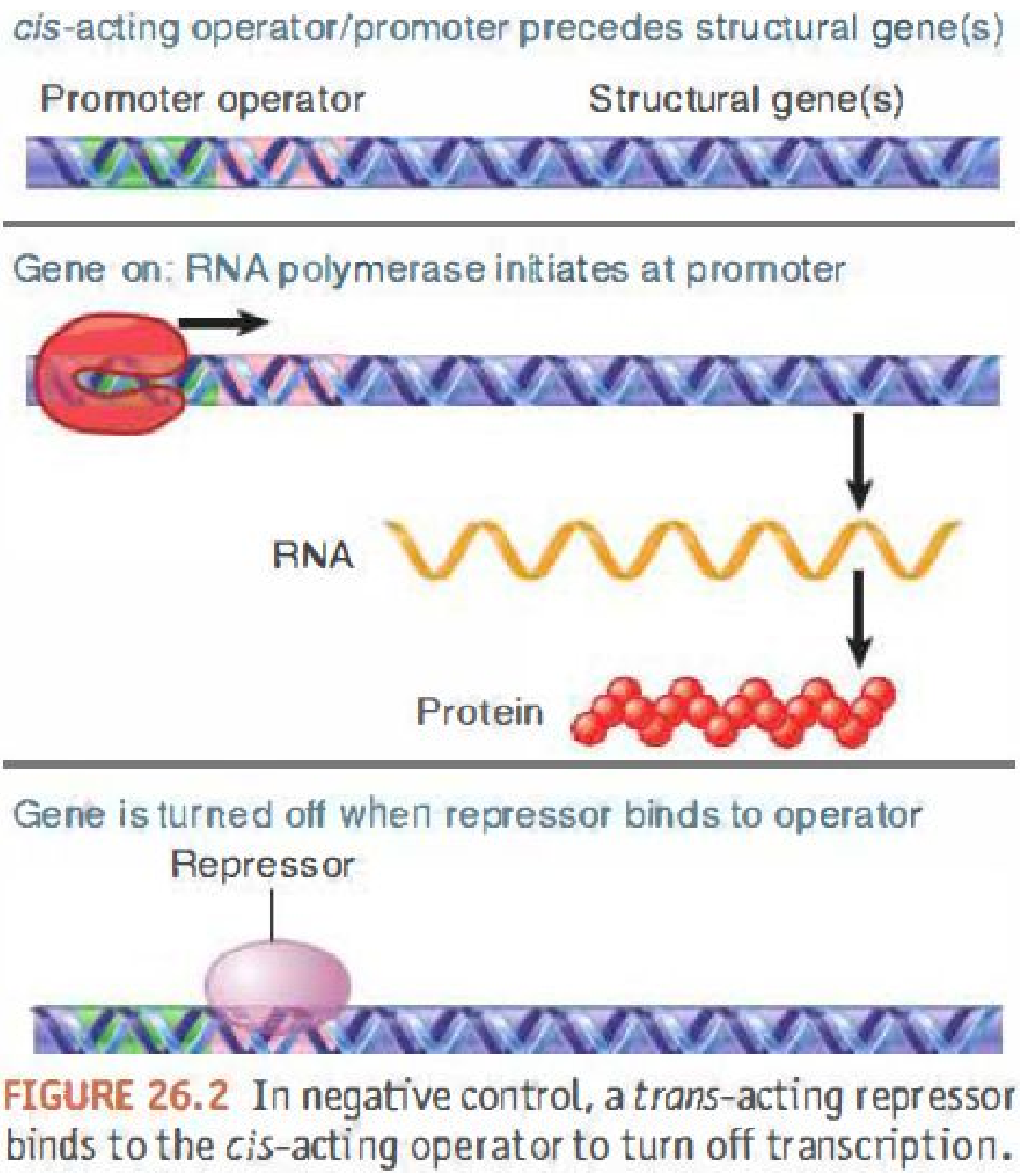


FIGURE 26.2 In negative control, a *trans*-acting repressor binds to the *cis*-acting operator to turn off transcription.

<u>Positive</u> control of transcription

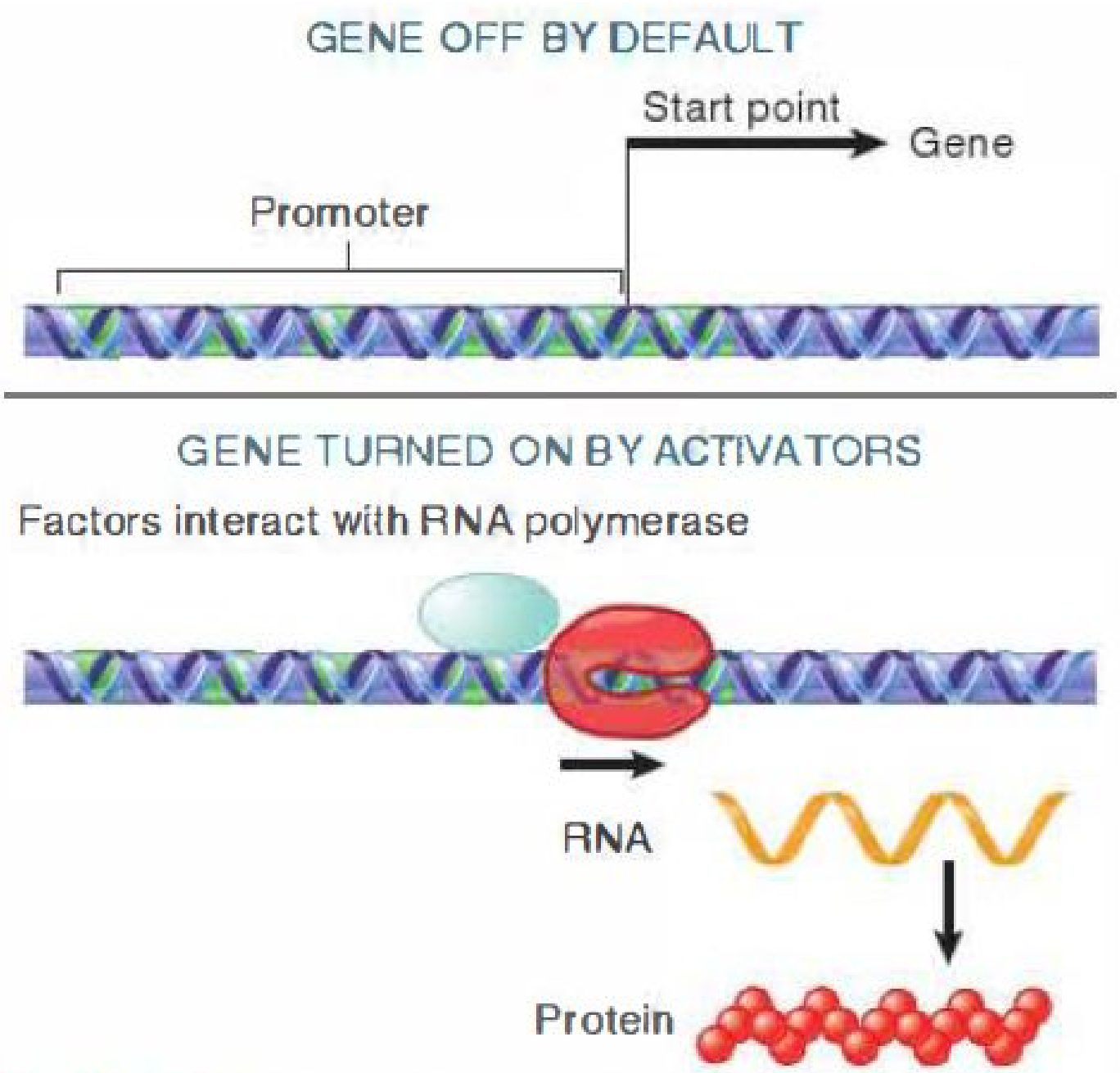


FIGURE 26.3 In positive control, a *trans*-acting factor must bind to the *cis*-acting site in order for RNA polymerase to initiate transcription at the promoter.

The Operon

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.

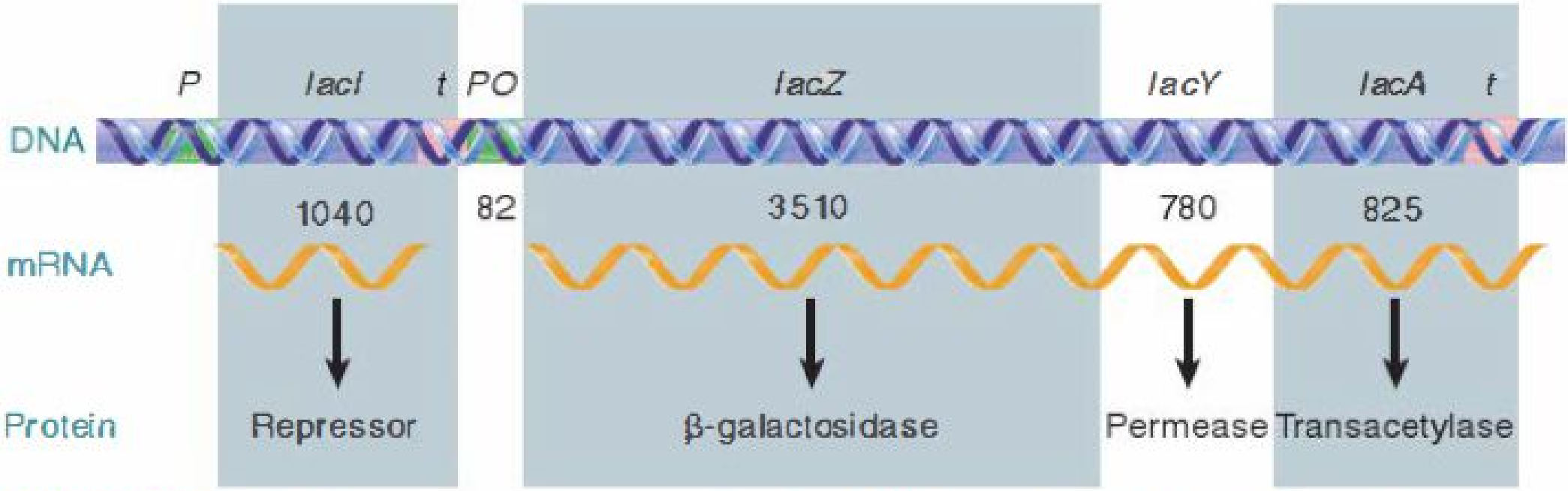
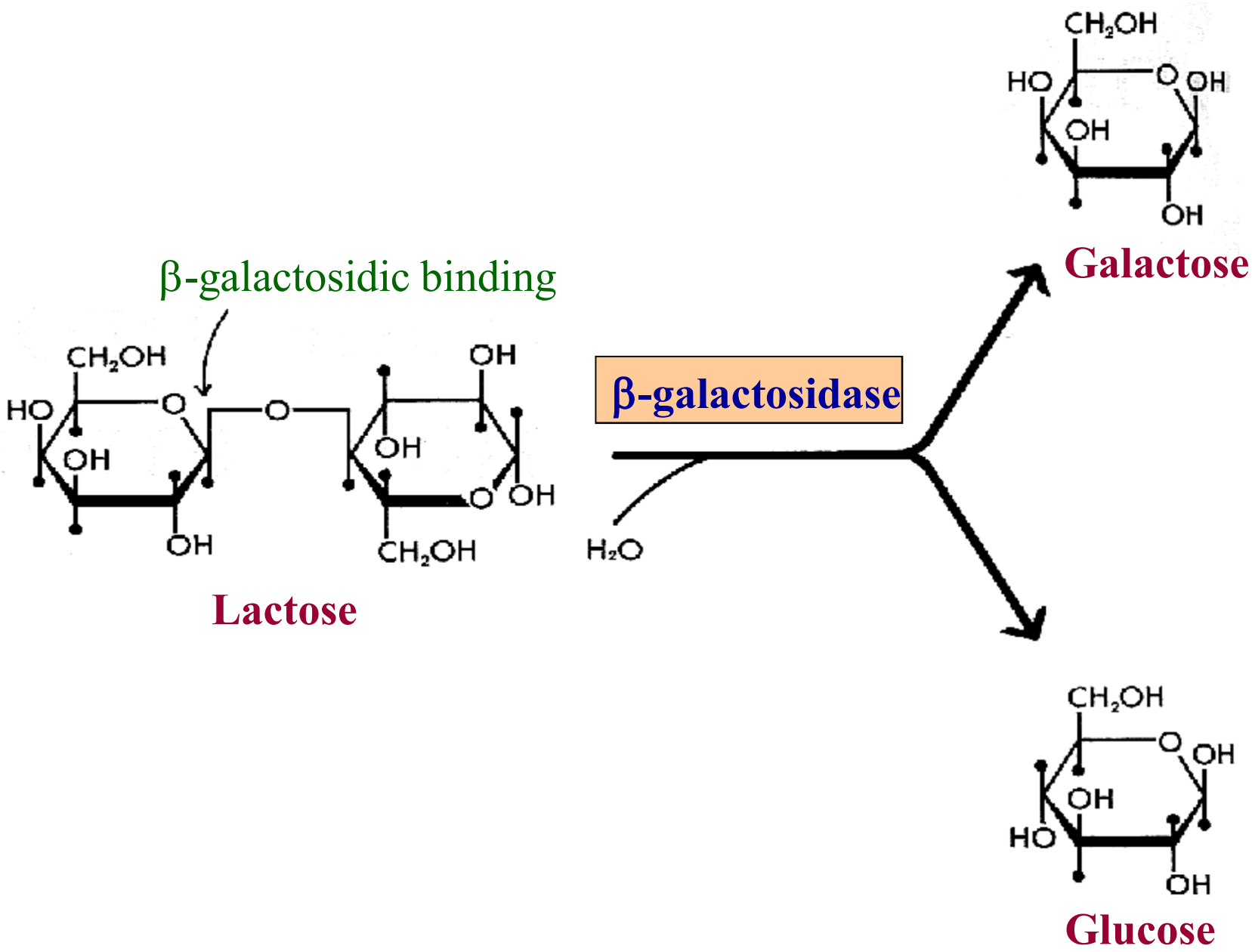


FIGURE 26.5 The *lac* operon occupies ~6000 bp of DNA. At the left the *lacI* gene has its own promoter and terminator. The end of the *lacI* region is adjacent to the *lacZYA* promoter, *P*. Its operator, *O*, occupies the first 26 bp of the transcription unit. The long *lacZ* gene starts at base 39, and is followed by the *lacY* and *lacA* genes and a terminator.


β-galactosidic binding
β-galactosidase
Lactose
Galactose
Glucose
H₂O

Lac Repressor Tetramer

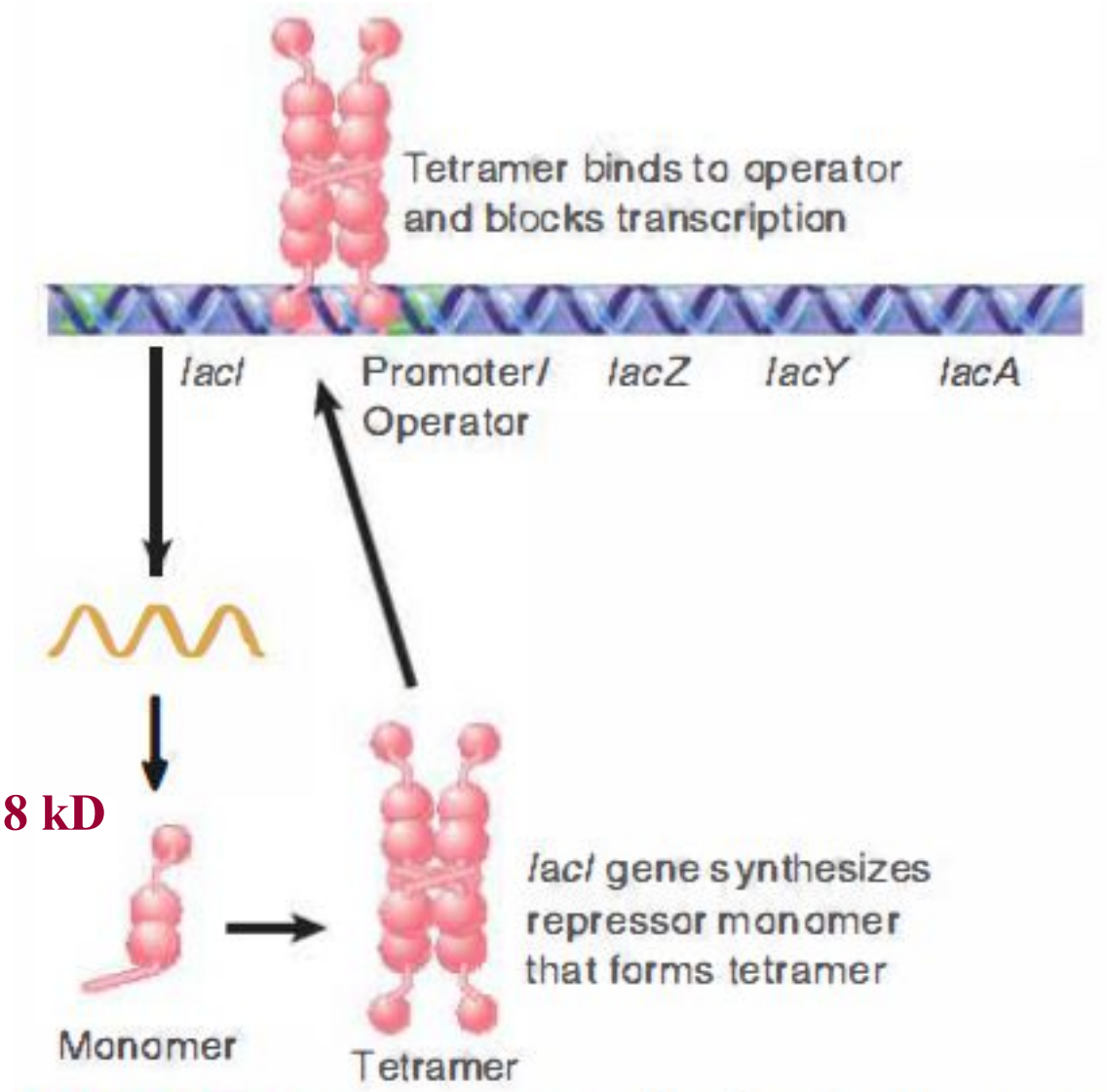
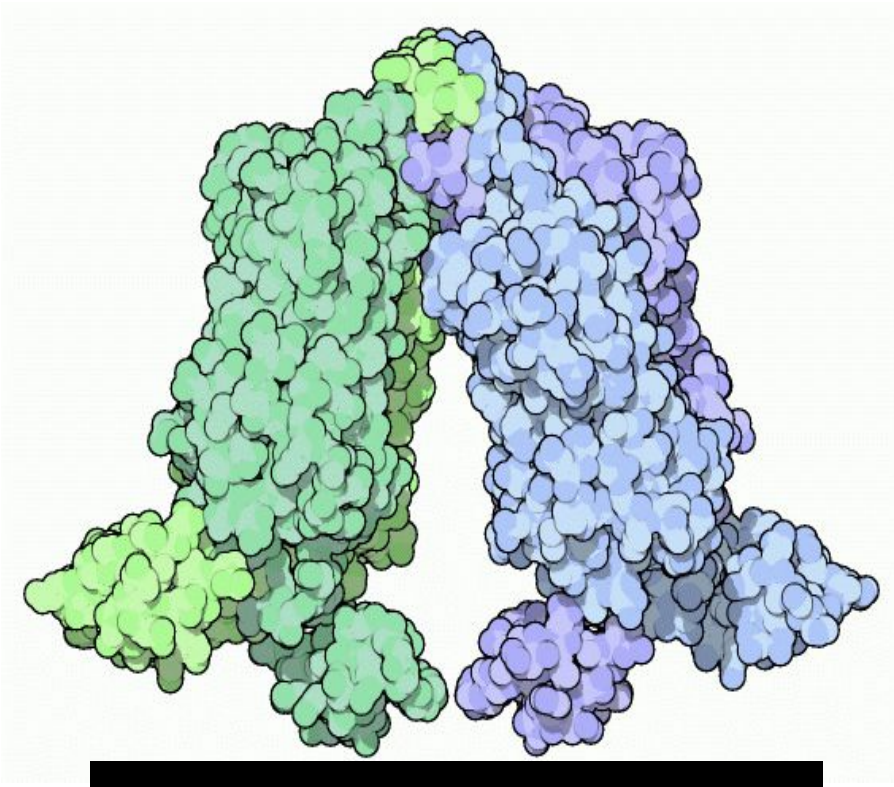
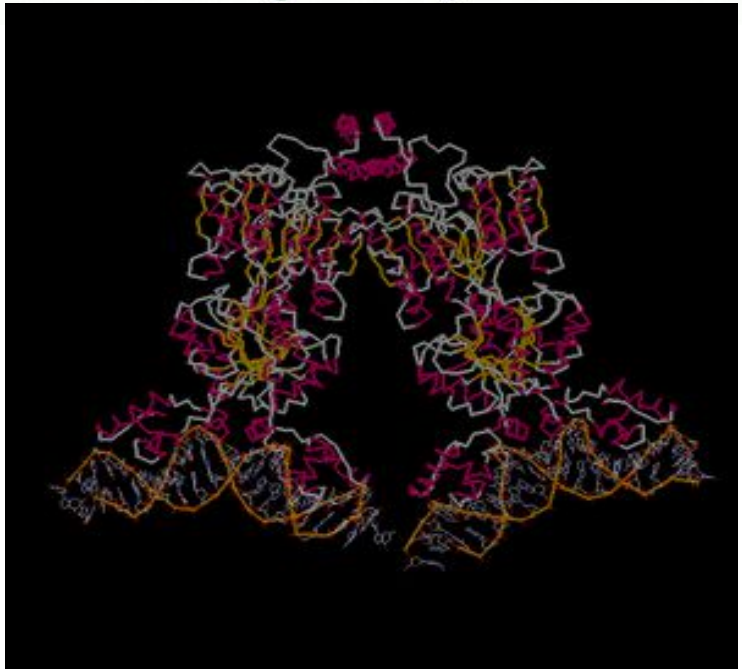
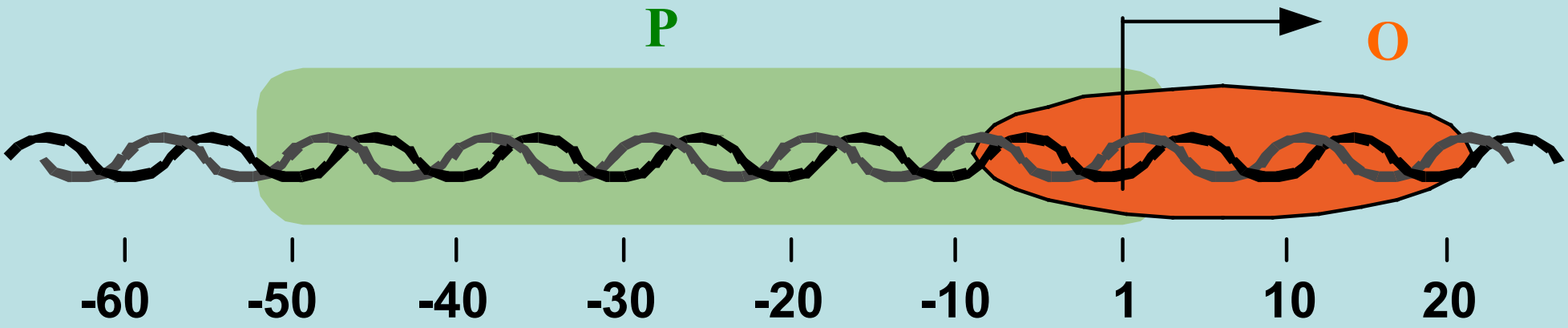


FIGURE 26.8 *lac* repressor maintains the *lac* operon in the inactive condition by binding to the operator. The shape of the repressor is represented as a series of connected domains as revealed by its crystal structure.





Lac repressor and RNA polymerase bind at sites that overlap around the transcription start point of the lac operon.



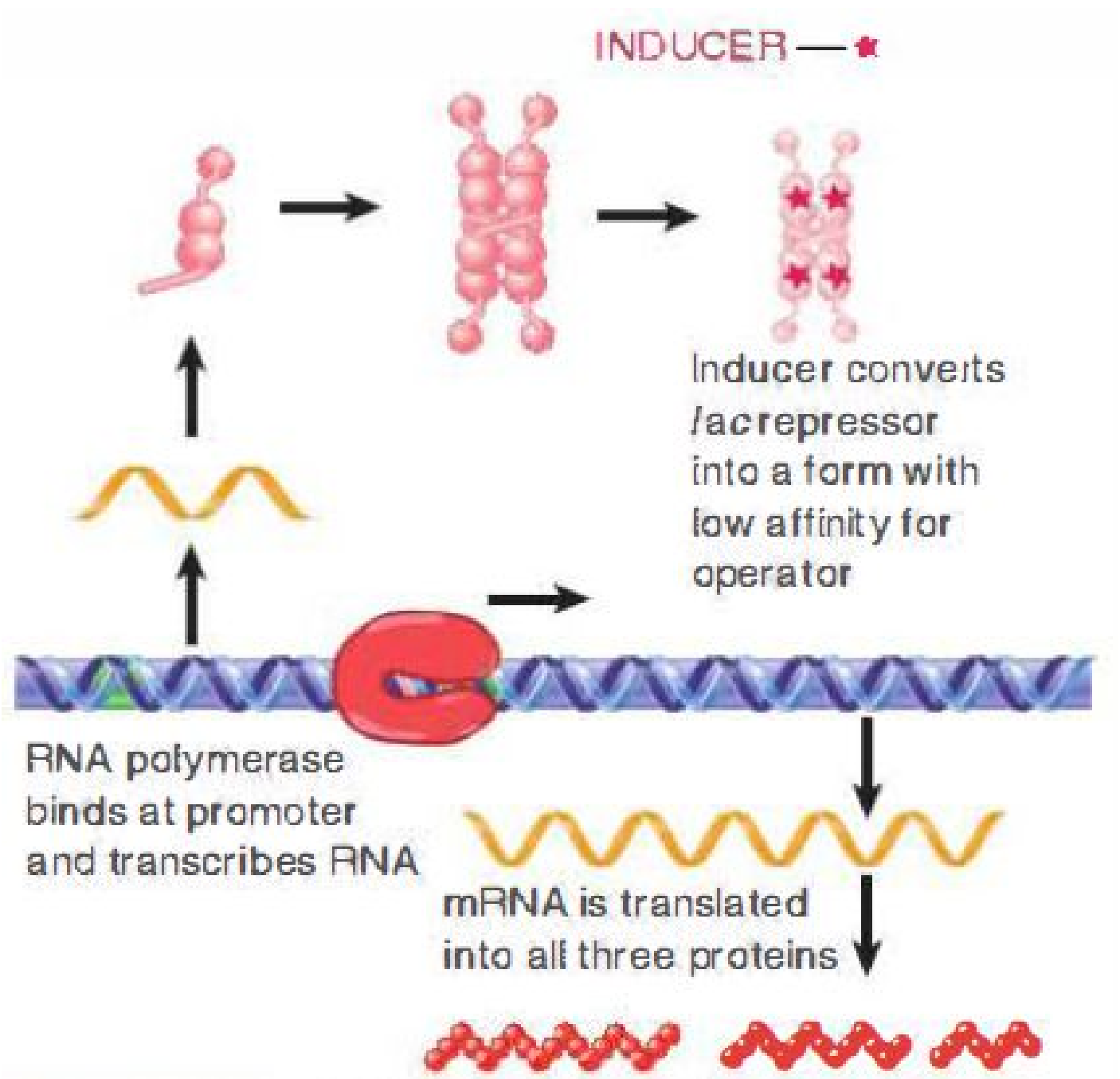


FIGURE 26.9 Addition of inducer converts repressor to a form with low affinity for the operator. This allows RNA polymerase to initiate transcription.

DNA	Repressor	Repressor + inducer
Operator	2×10^{13}	2×10^{10}
Other DNA	2×10^{6}	2×10^{6}
Specificity	10^{7}	10^{4}
Operators bound	96%	3%
Operon is:	repressed	induced

FIGURE 26.23 *lac* repressor binds strongly and specifically to its operator, but it is released by inducer. All equilibrium constants are in M^{-1}.

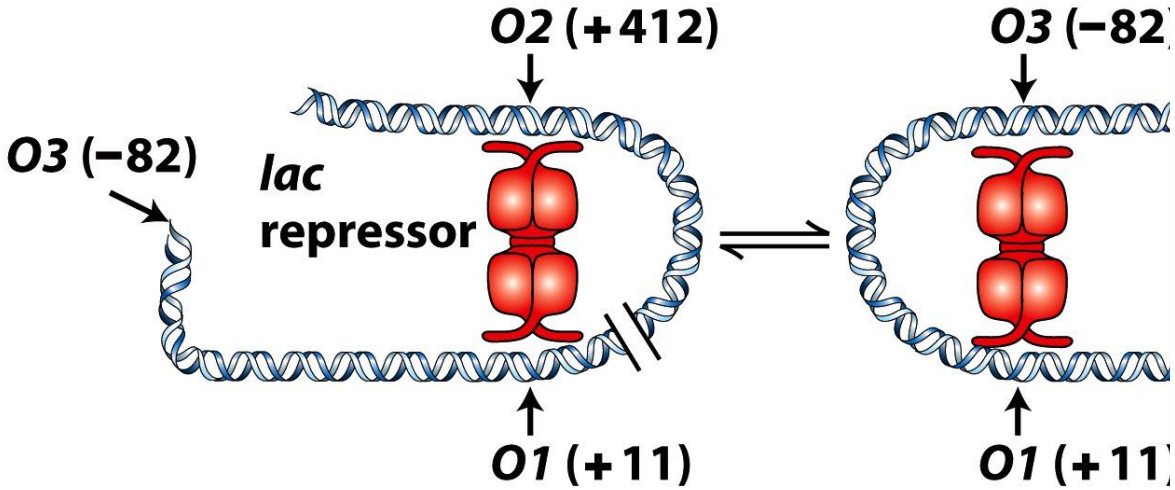


Figure 7-3
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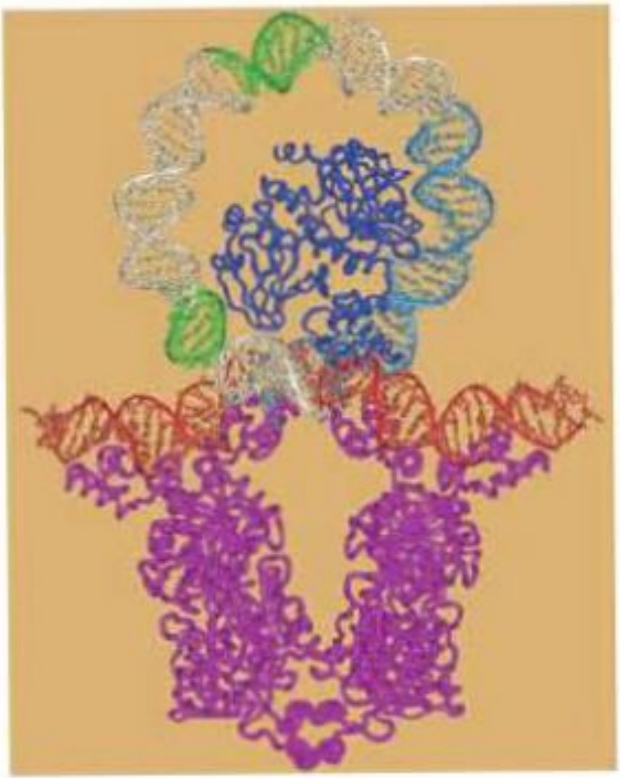


FIGURE 26.22 When a repressor tetramer binds to two operators, the stretch of DNA between them is forced into a tight loop. (The blue structure in the center of the looped DNA represents CRP, which is another regulator protein that binds in this region.) Reproduced from M. Lewis et al., *Science* 271 (1996): 1247–1254 [http://www.sciencemag.org]. Reprinted with permission from AAAS. Photo courtesy of Ponzy Lu, University of Pennsylvania.

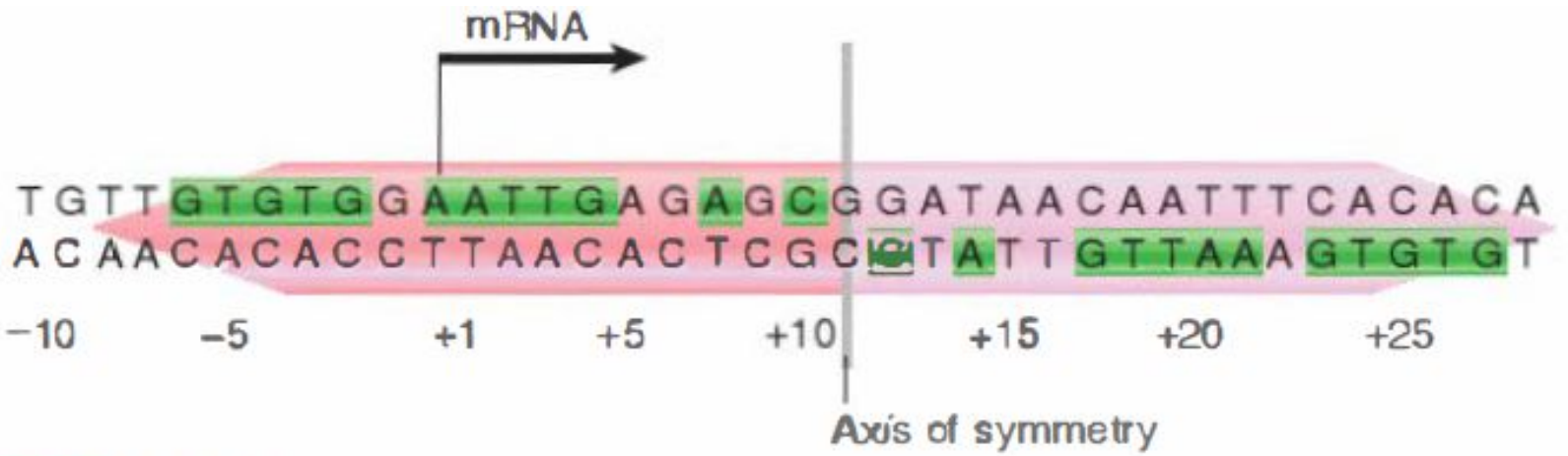


FIGURE 26.17 The *lac* operator has a symmetrical sequence. The sequence is numbered relative to the start point for transcription at +1. The pink arrows to the left and to the right identify the two dyad repeats. The green blocks indicate the positions of identity.

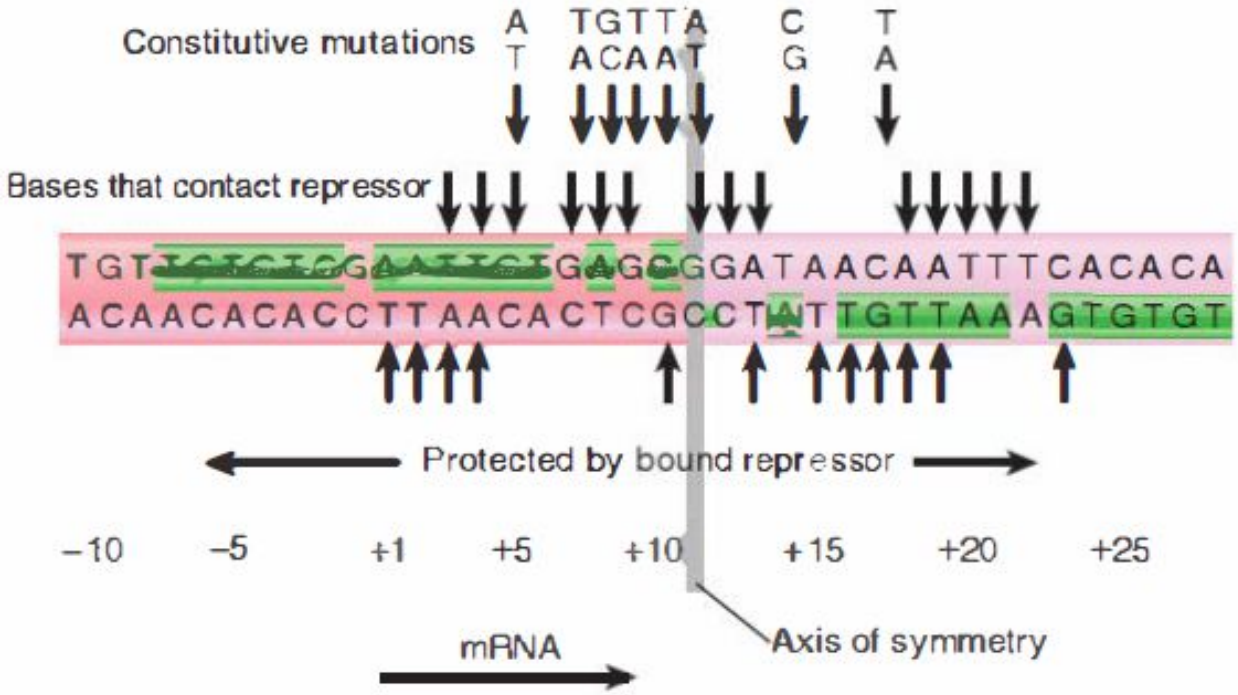


FIGURE 26.19 Bases that contact the repressor can be identified by chemical crosslinking or by experiments to see whether modifications prevent binding. They identify positions on both strands of DNA extending from +1 to +23. Constitutive mutations occur at 8 positions in the operator between +5 and +17.

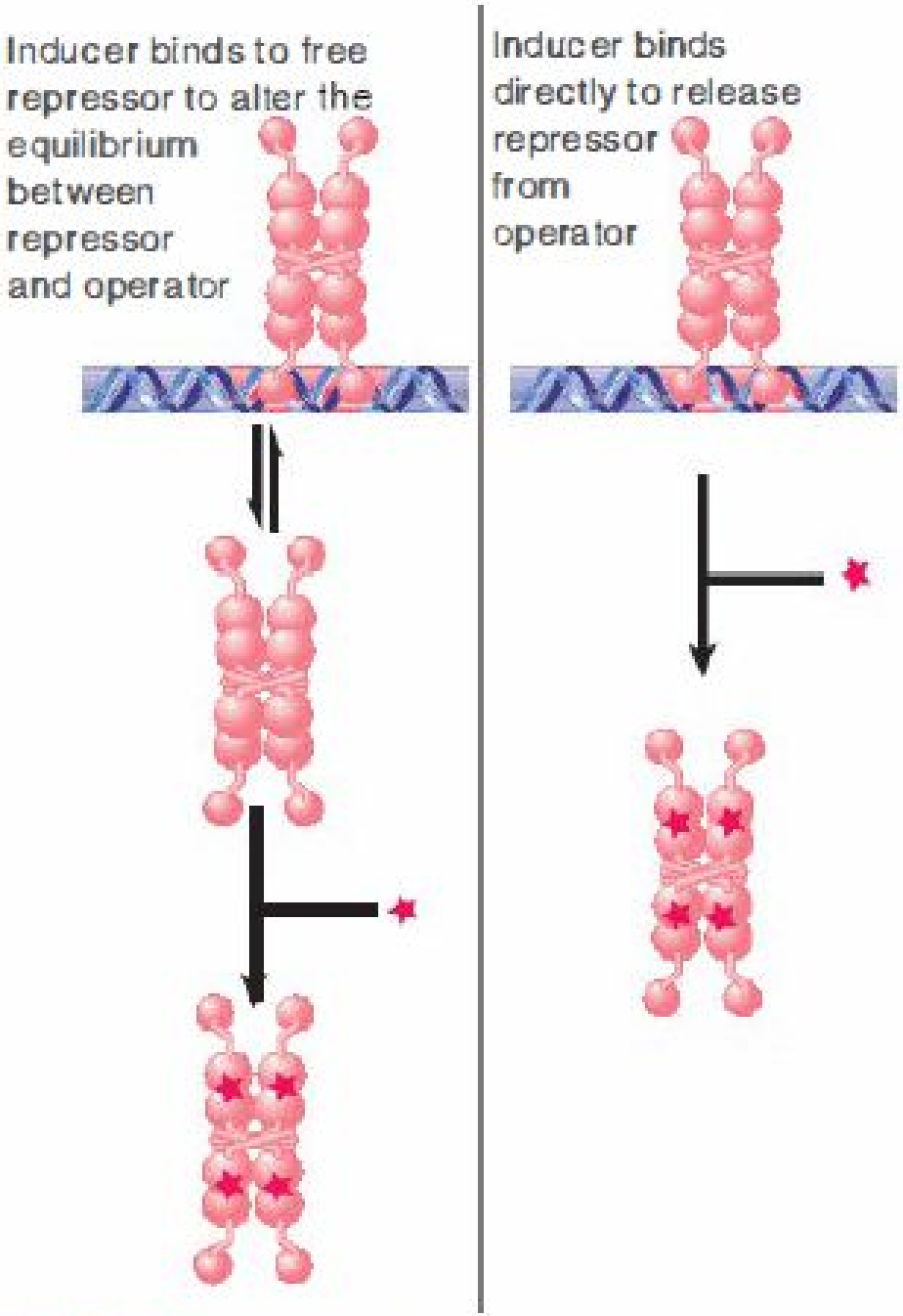


FIGURE 26.20 Does the inducer bind to the free repressor to upset an equilibrium (left) or directly to repressor bound at the operator (right)?

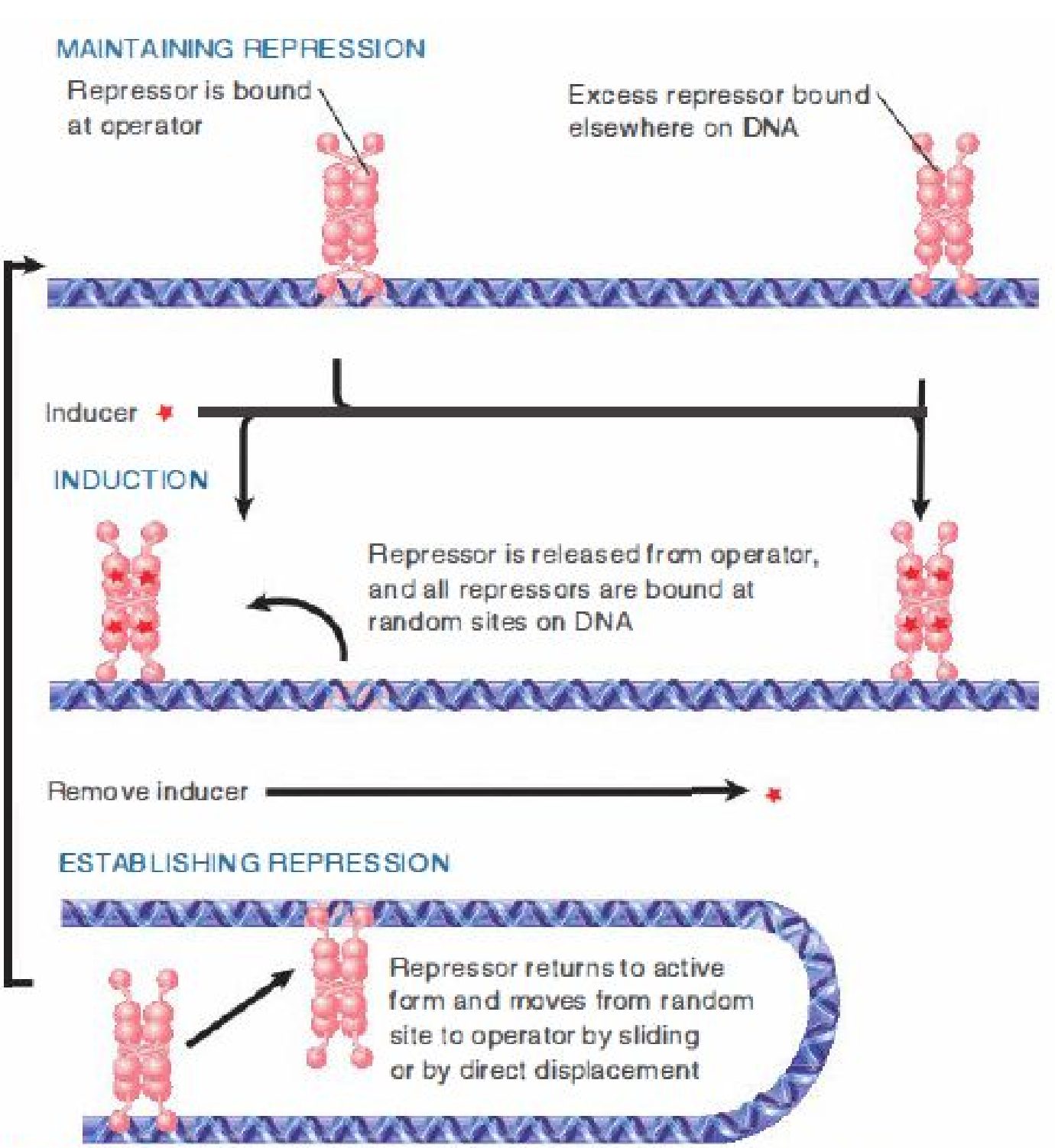


FIGURE 26.24 Virtually all the repressor in the cell is bound to DNA.

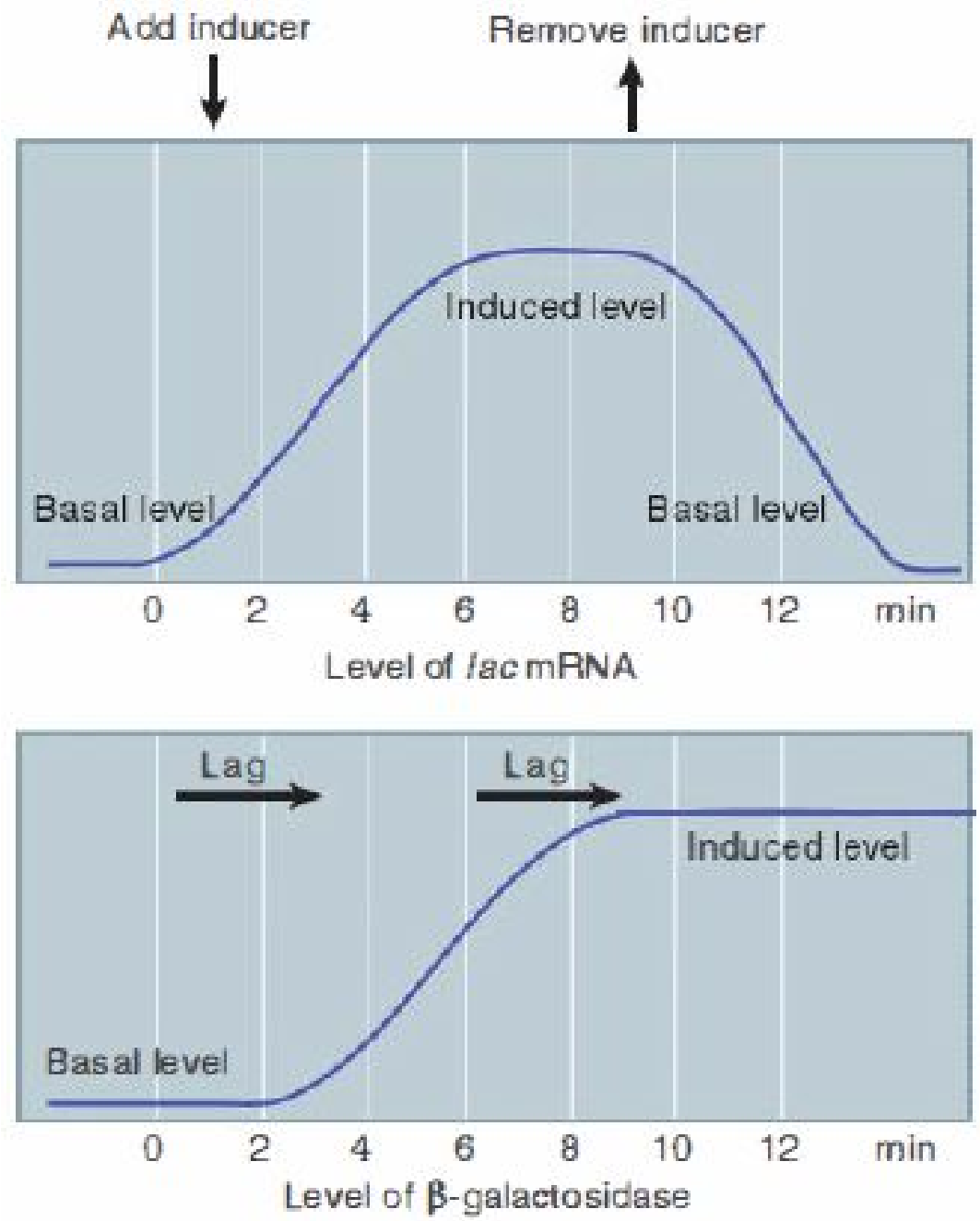
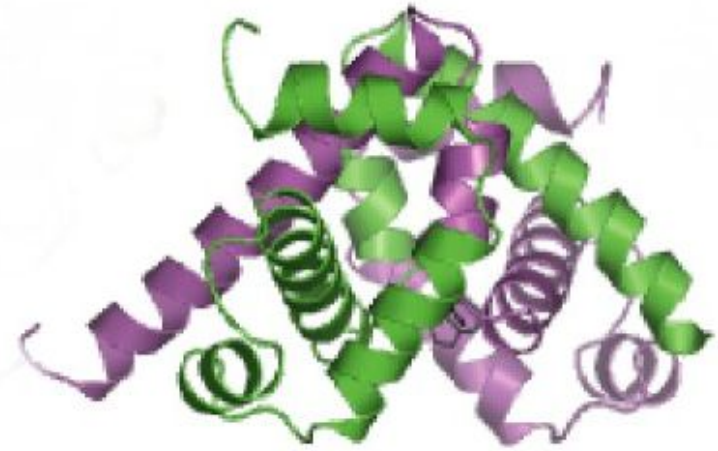


FIGURE 26.7 Addition of inducer results in rapid induction of *lac* mRNA and is followed after a short lag by synthesis of the enzymes; removal of inducer is followed by rapid cessation of synthesis.

Trp (tryptophan) repressor



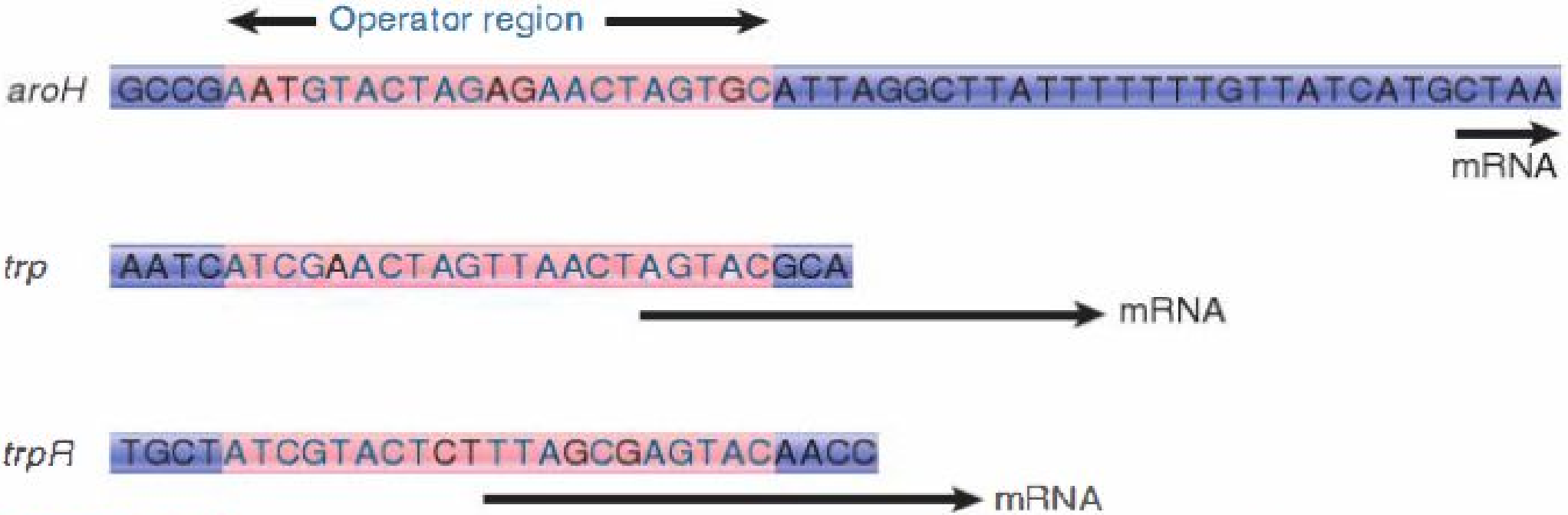


FIGURE 26.31 The *trp* repressor recognizes operators at three loci. Conserved bases are shown in red. The location of the start point and mRNA varies, as indicated by the black arrows.

trpR also controls the regulation of <u>its own production</u>, through regulation of the *trpR* gene

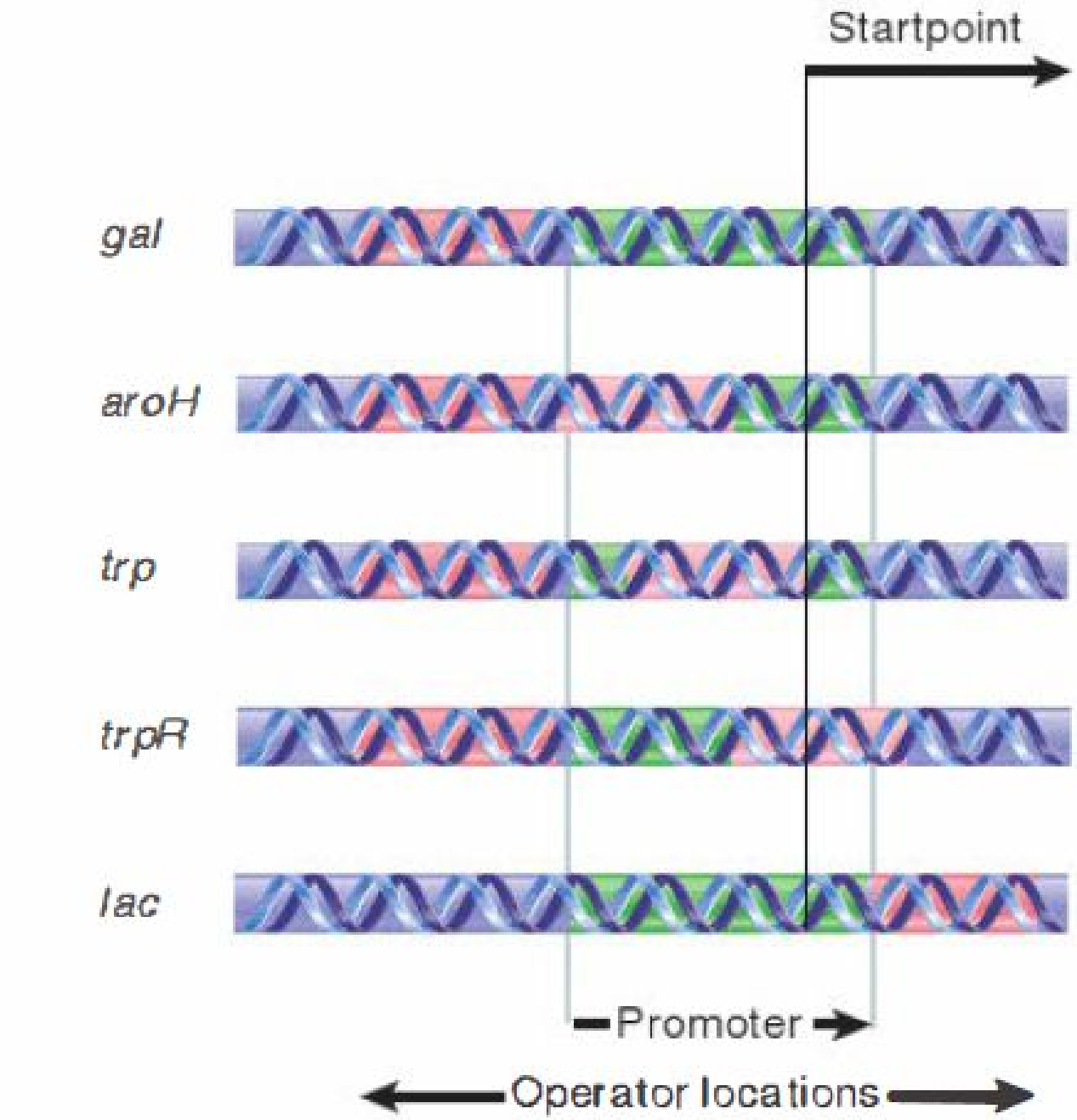
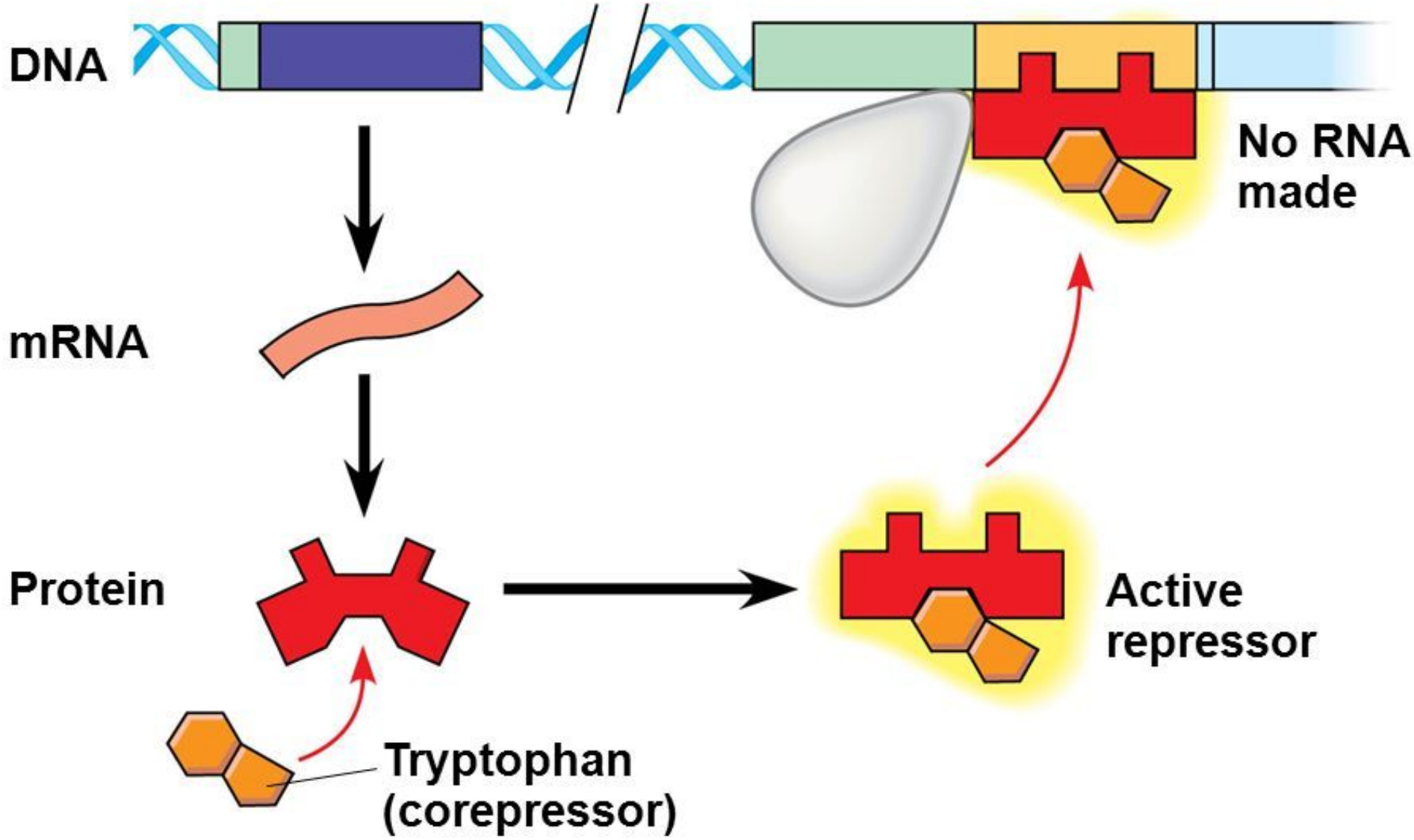


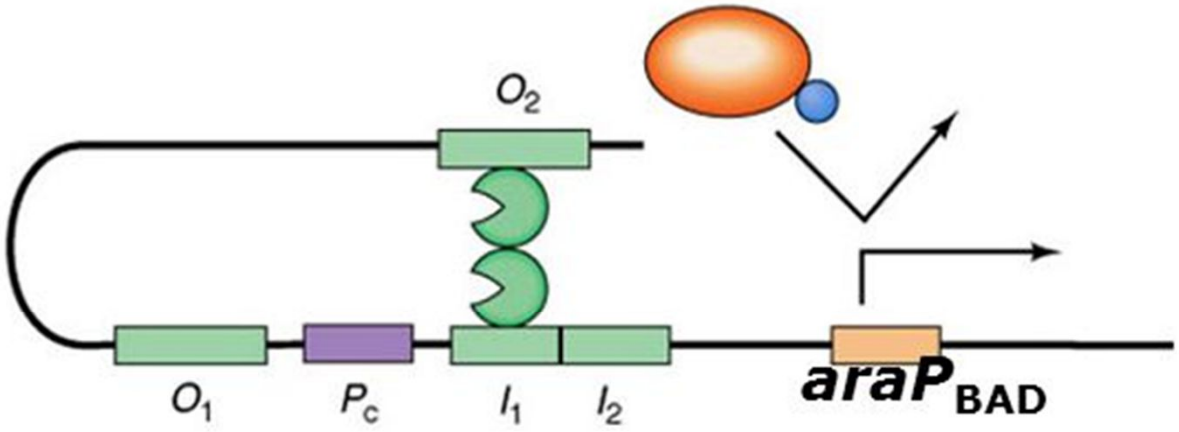
FIGURE 26.32 Operators may lie at various positions relative to the promoter.



(b) Tryptophan present, repressor active, operon off

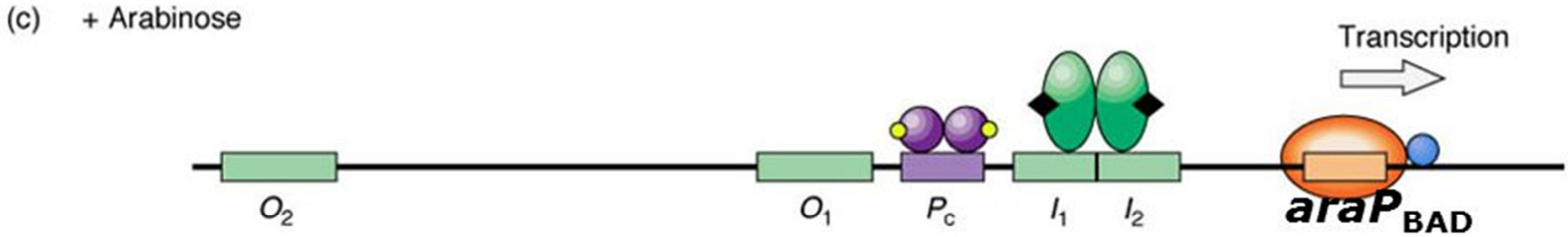
The arabinose operon by E.coli:
an example of negative-positive control

(b) – Arabinose



- When arabinose is absent, the AraC protein acts as a negative regulator.
- AraC acts as a dimer, and causes the DNA to loop. Looping brings the I_1 and O_2 sites in proximity to one another.
- One AraC monomer binds to I_1 and a second monomer binds to O_2.
- Binding of AraC prevents RNA Pol from binding to the P_{BAD} promoter

The arabinose operon by E.coli:
an example of negative-positive control



- When arabinose is present, it binds to AraC and changes AraC conformation
- An arabinose-AraC dimer complex binds preferentially to I1 and I2, and NOT to O2 which causes 'opening' of the loop. This allows RNA Pol to bind to P_{BAD}.
- If glucose levels are low, cAMP-CAP complex binds to P_C.
- Active transcription occurs.

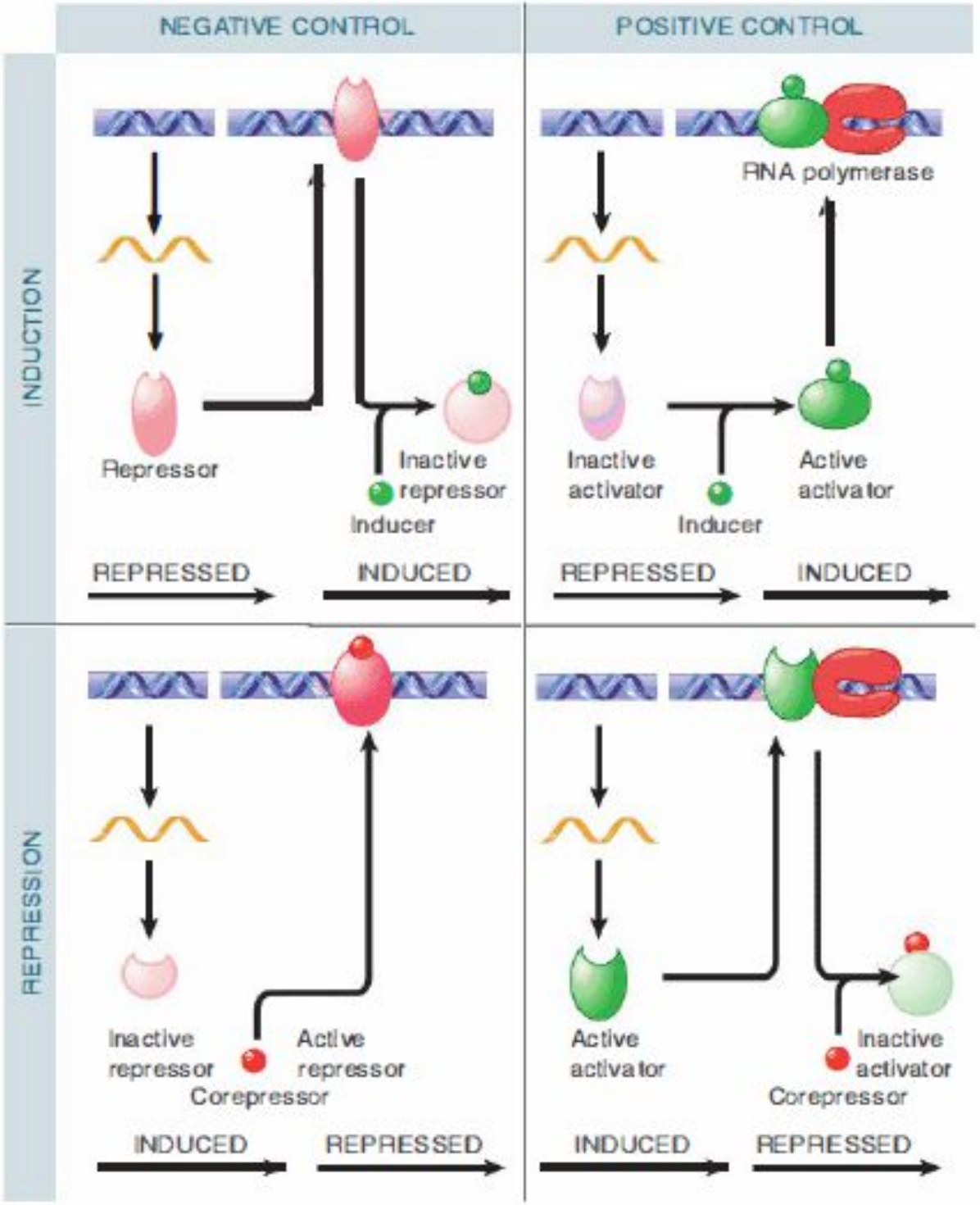
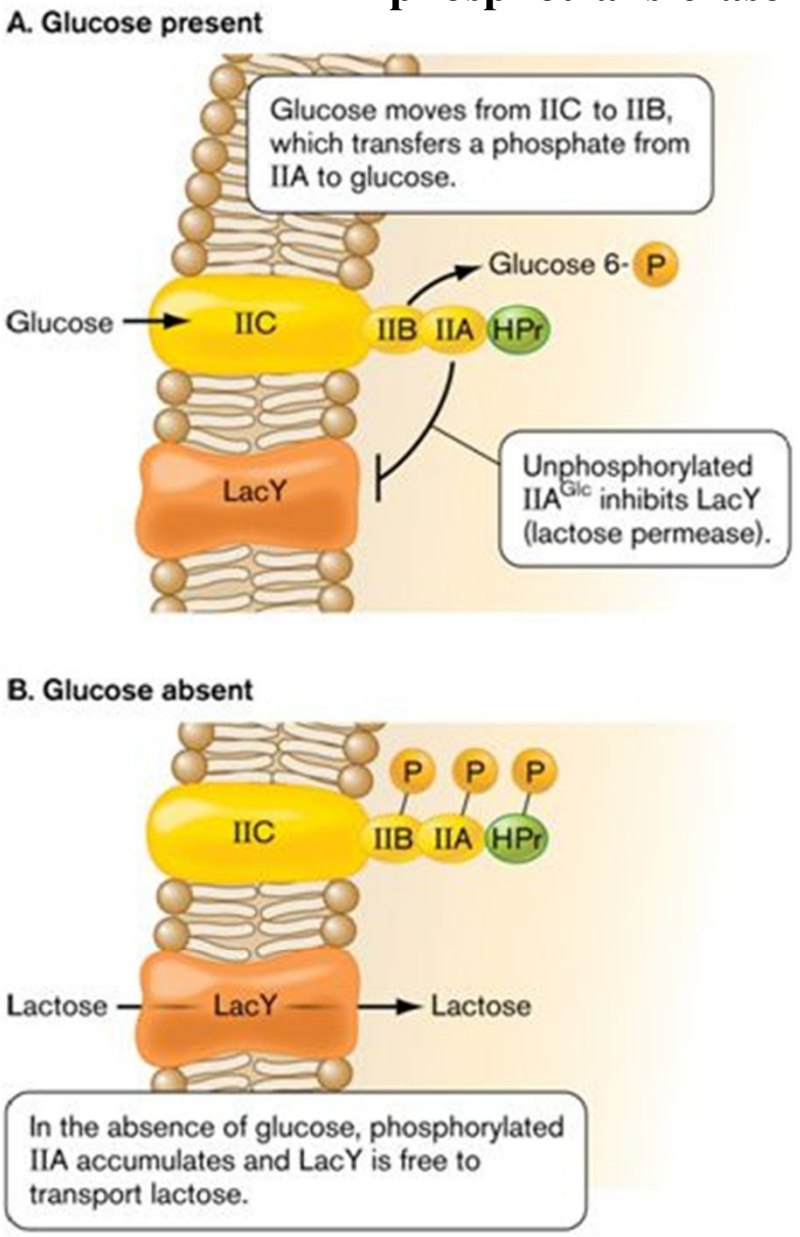


FIGURE 26.4 Regulatory circuits can be designed from all possible combinations of positive and negative control with inducible and repressible control.

Phosphoenolpyruvate:glucose phosphotransferase

- Glucose transport by the phosphotransferase system causes catabolite repression by inhibiting the LacY permease activity.
- - This is termed **inducer exclusion**.



CAP + cAMP
Positive control on Lac operon

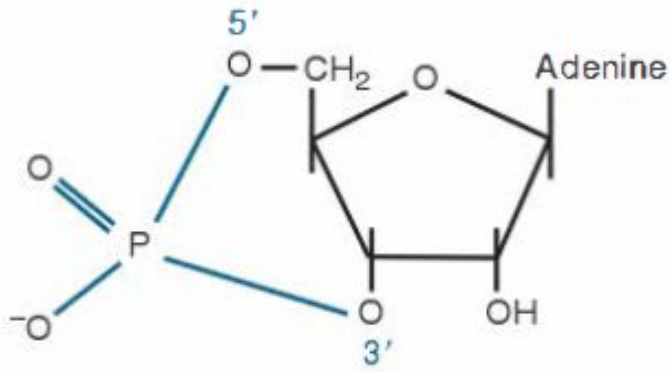


FIGURE 26.26 Cyclic AMP has a single phosphate group connected to both the 3′ and 5′ positions of the sugar ring.

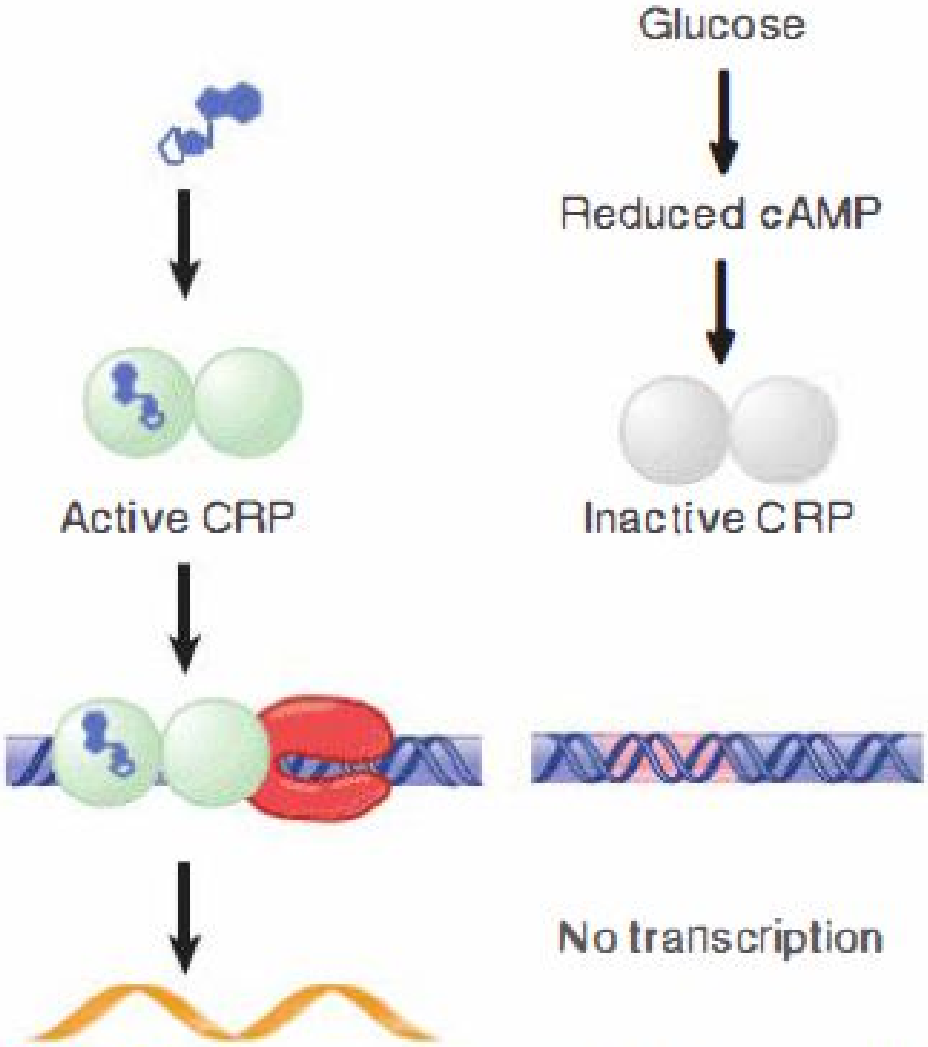


FIGURE 26.27 By reducing the level of cyclic AMP, glucose inhibits the transcription of operons that require CRP activity.

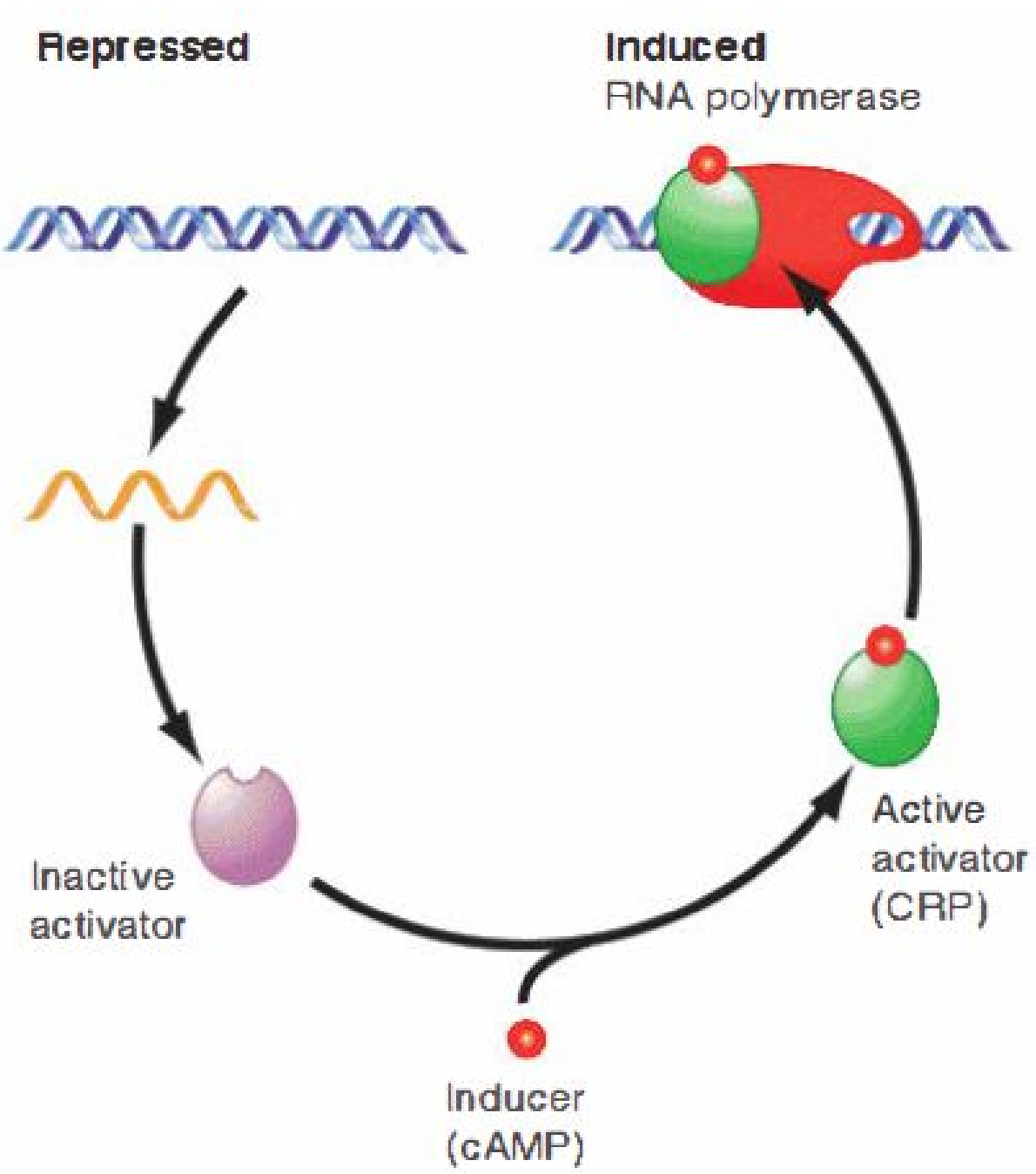


FIGURE 26.25 A small-molecule inducer, cAMP, converts an activator protein CRP to a form that binds the promoter and assists RNA polymerase in initiating transcription.

Catabolite activator protein (CRP gene)

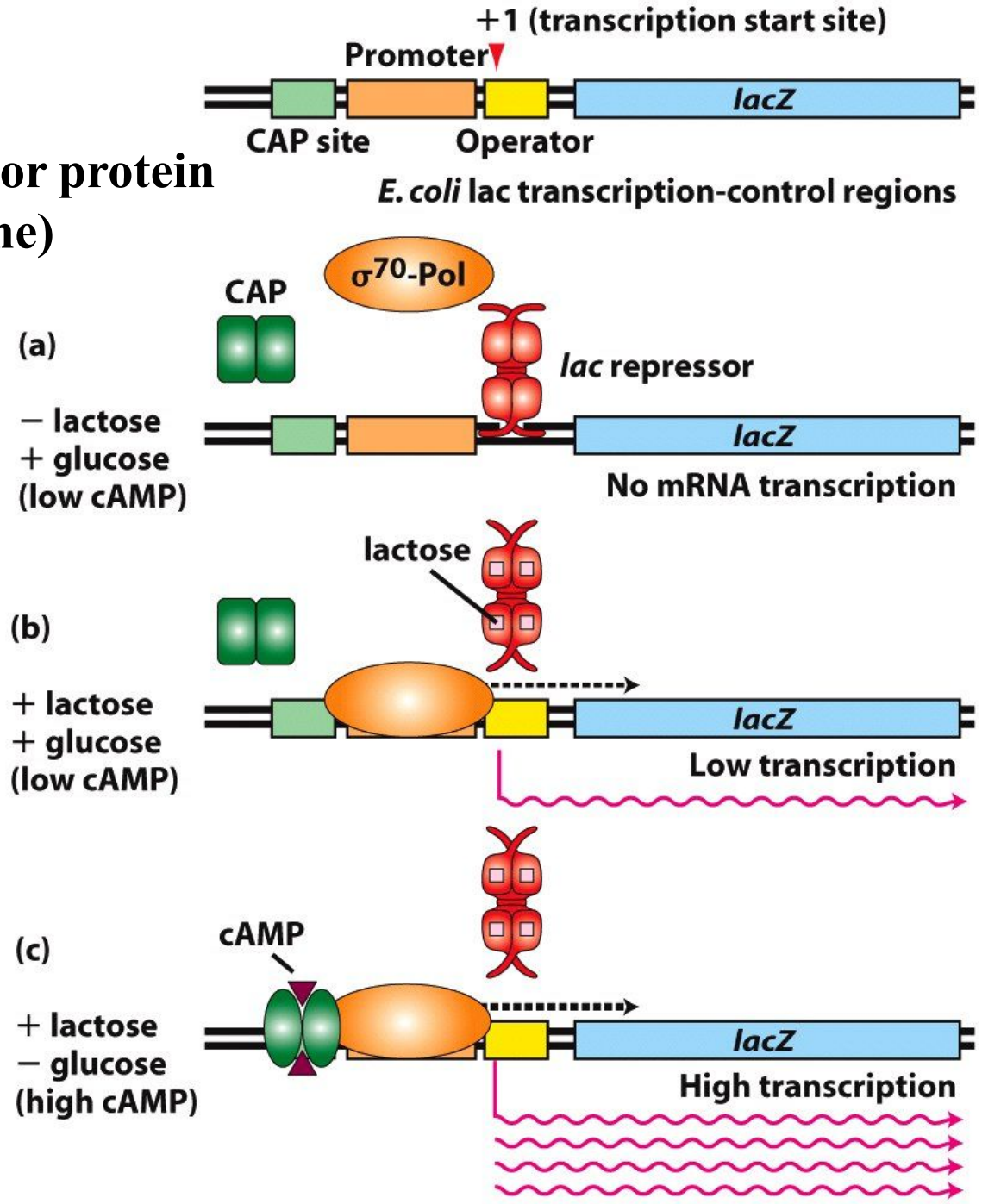


Figure 7-2
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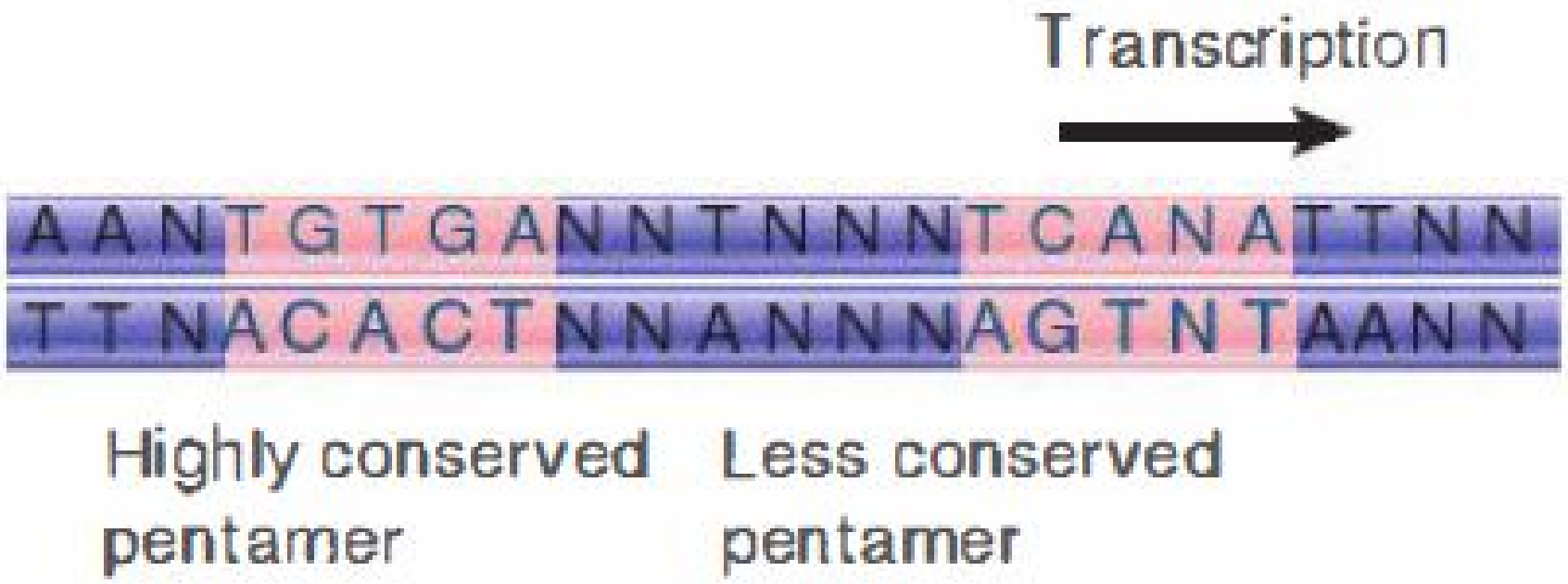

FIGURE 26.28 The consensus sequence for CRP contains the well conserved pentamer TGTGA and (sometimes) an inversion of this sequence (TCANA).

Figure 10.24 The CAP protein can bind at different sites relative to RNA polymerase.

Catabolite activator protein ←→ CRP