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Lewin, GENES XI

Chapter 26
Operons, prokaryotic gene regulation and yeast GAL4

Lewin's GENES XII (PDF)
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LEWIN'S GENES XI

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Chapter 26
The Operon

26.1 Introduction

- In **inducible regulation**, the gene is regulated by the presence of its substrate (the **inducer**). This is good for catabolic pathways for adapting to new food sources, for instance to use lactose or galactose sugars to grow if there is no glucose.

- In **repressible regulation**, the gene is regulated by the product of its enzyme pathway (the **corepressor**). Genes encoding enzymes in anabolic pathways to make amino acids or nucleotides or other needed products can be transcriptionally repressed when there is enough of the pathway product.

26.1 Introduction

- In **negative regulation of transcription**, a repressor protein binds to an **operator** to prevent a gene from being expressed.
- In **positive regulation**, a transcription factor is required to bind at the promoter in order to enable RNA polymerase to initiate transcription.

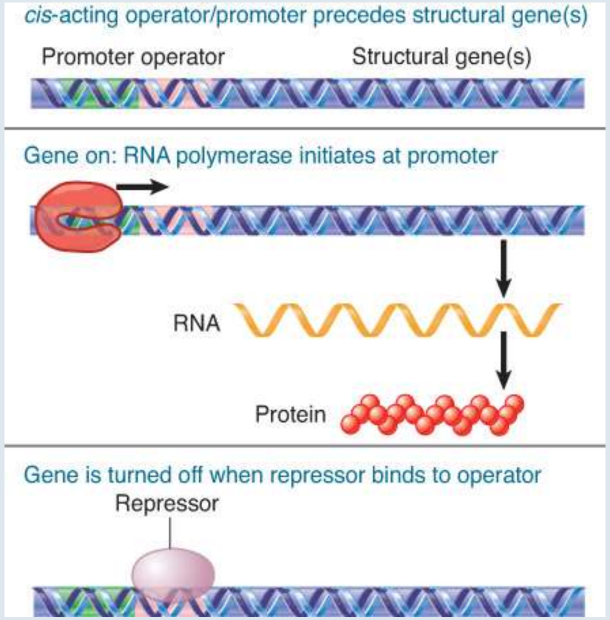


Figure 26.02: In negative control, a trans-acting repressor binds to the cis-acting operator to turn off transcription.

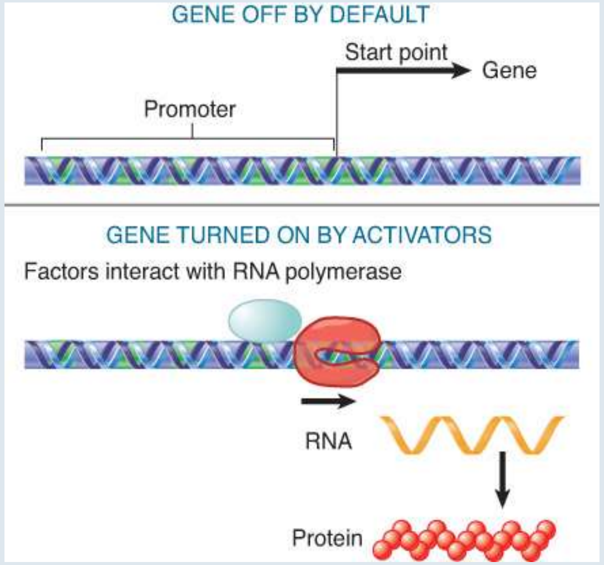


Figure 26.03: In positive control, a trans-acting factor must bind to cis-acting site in order for RNA polymerase to initiate transcription at the promoter.

26.2 Bacterial operons are Structural Gene Clusters that 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**.

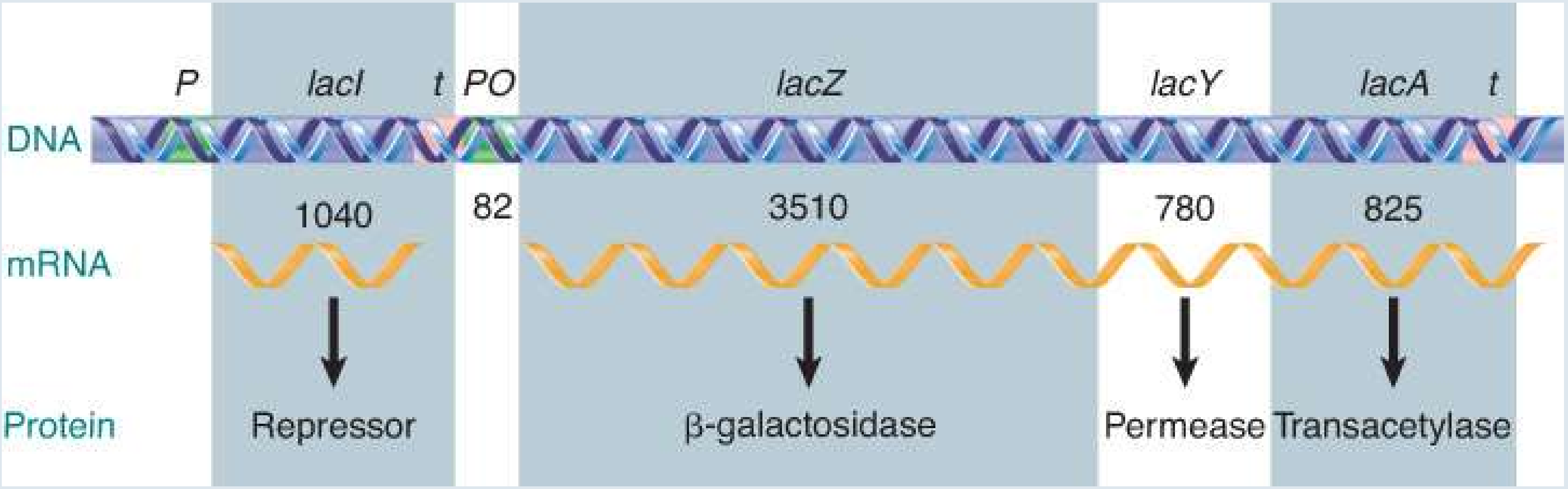


Figure 26.05: The lac operon occupies ~6000 bp of DNA.

26.3 Famous inducible genes and repressors in *E. coli*

The *lac* Operon Is under Negative control by lac repressor

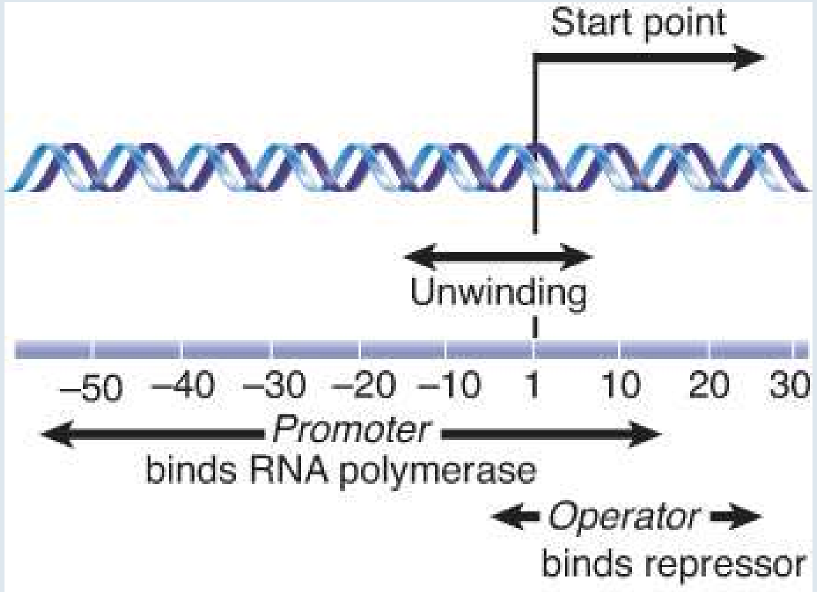


Figure 26.06: lac repressor and RNA polymerase bind at sites that overlap around the transcription startpoint of the lac operon.

- 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 (e.g., Lactose), the *lac* operon is expressed only at a very low (basal) level.

26.3 The *lac* Operon Is Inducible by lactose

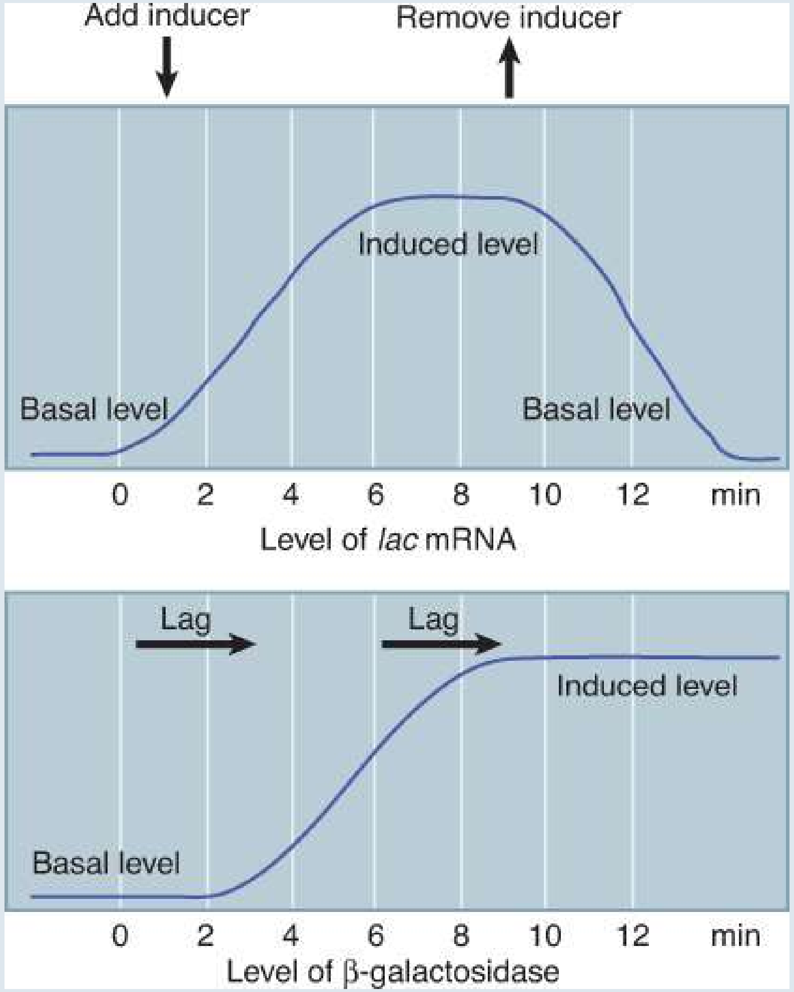
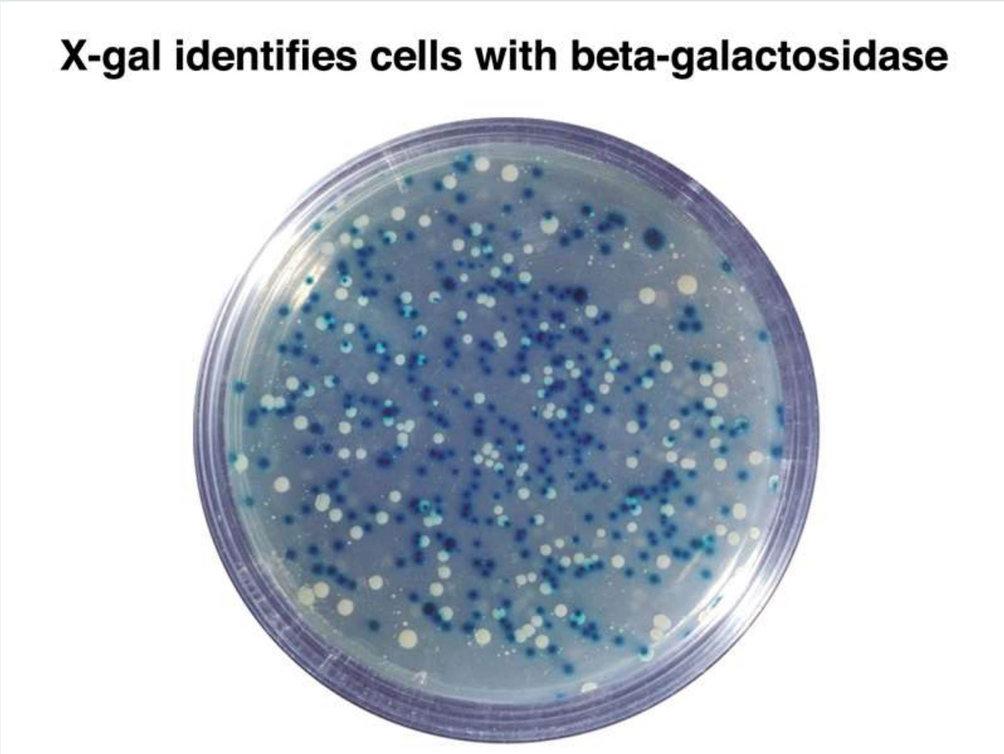
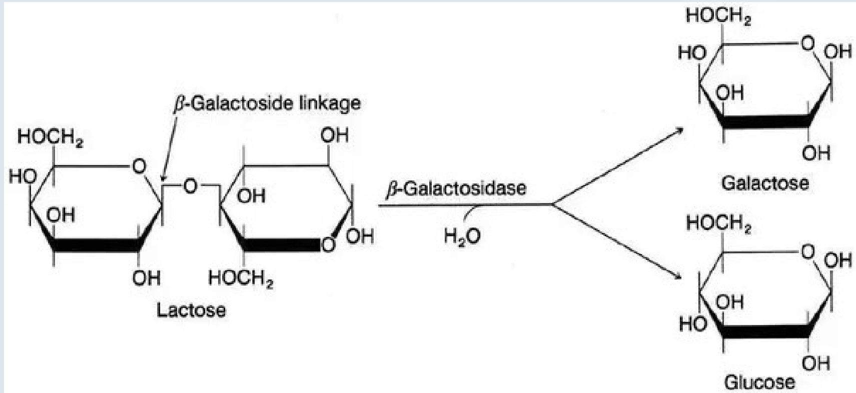
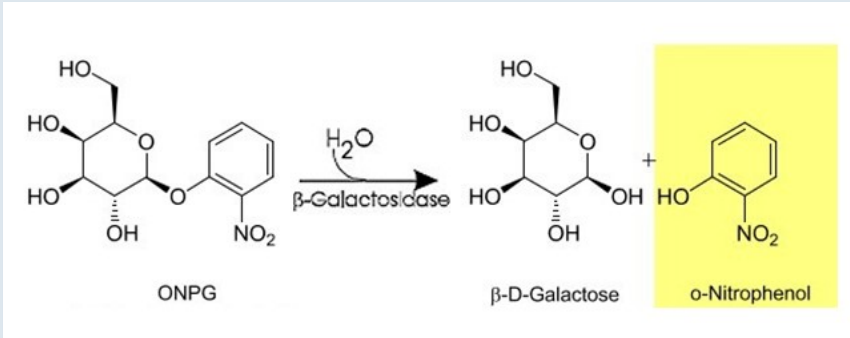


Figure 26.07: Addition of inducer results in rapid induction of lac mRNA, and is followed after a short lag by synthesis of the enzymes.

lacZ (β-galactosidase)) plate tests and liquid culture assays fo beta-gal activity units.







ONPG hydrolysis (right side of figure), by cells gives a soluble yellow product for spectrophotometric measurement of exact *lacZ* (β-galactosidase) activity units

26.3 The *lac* Operon Is under negative transcriptional control by Lac Repressor and is inducible by galactosides

- The Lac repressor protein (LacR) is encoded 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.
- **IPTG** (Isopropyl-thio-galactoside) is a **gratuitous inducer –** a non-hydolysable inducer that resembles the authentic inducer of transcription (Allolactose). It is stable and is not a substrate for the induced enzymes.
- The *lac* mRNA is extremely unstable; as a result, induction can be rapidly reversed.

26.4 *lac* Repressor Is Controlled by a Small-Molecule 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. In experiments we induce with artificial IPTG, not metabolized, a gratuitous inducer.

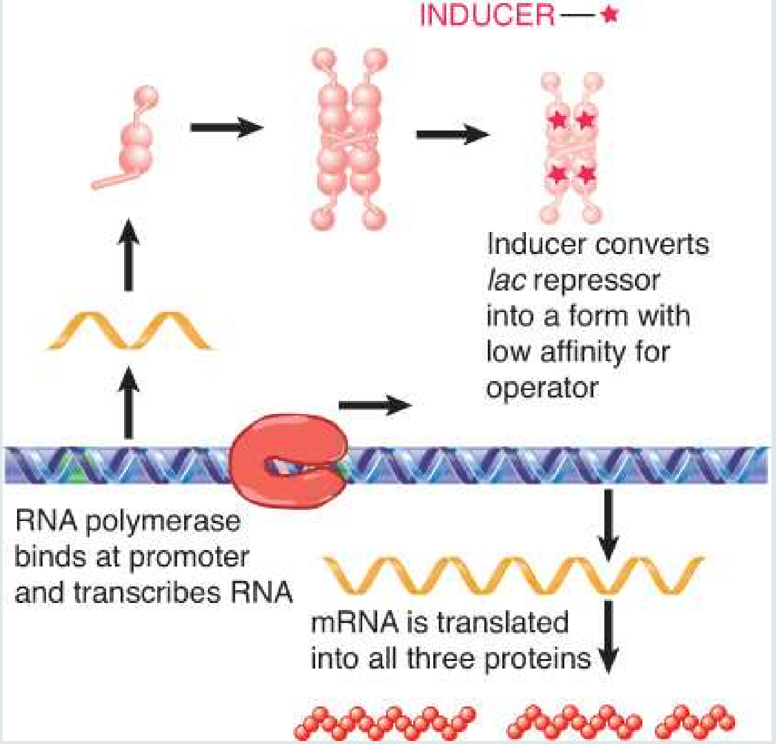


Figure 26.09: Addition of inducer converts repressor to a form with low affinity for the operator. This allows RNA polymerase to initiate transcription.

26.4 *lac* Repressor Is Controlled by a Small-Molecule Inducer

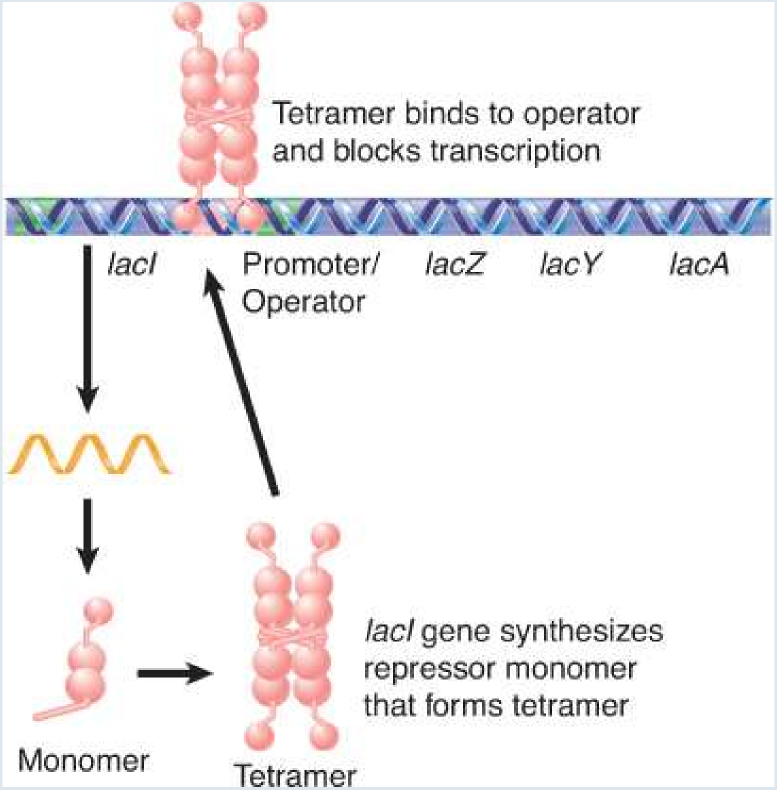


Figure 26.08: lac repressor maintains the lac operon in the inactive condition by binding to the operator.

- 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.

26.5 *cis*-Acting Constitutive Mutations Identify the Operator

- Mutations in the operator cause constitutive expression of all three *lac* structural genes.
- **constitutive expression –**
  A state in which a gene is expressed continuously.
- These mutations are *cis*-acting and affect only those genes on the contiguous stretch of DNA.
- Mutations in the promoter prevent expression of *lacZYA* and are **uninducible** and *cis*-acting.

26.5 *cis*-Acting Constitutive Mutations Identify the Operator

- ***cis*-dominant –** A site or mutation that affects the properties only of its own molecule of DNA, often indicating that a site does not code for a diffusible product. O^C means 'operator constitutive' mutation

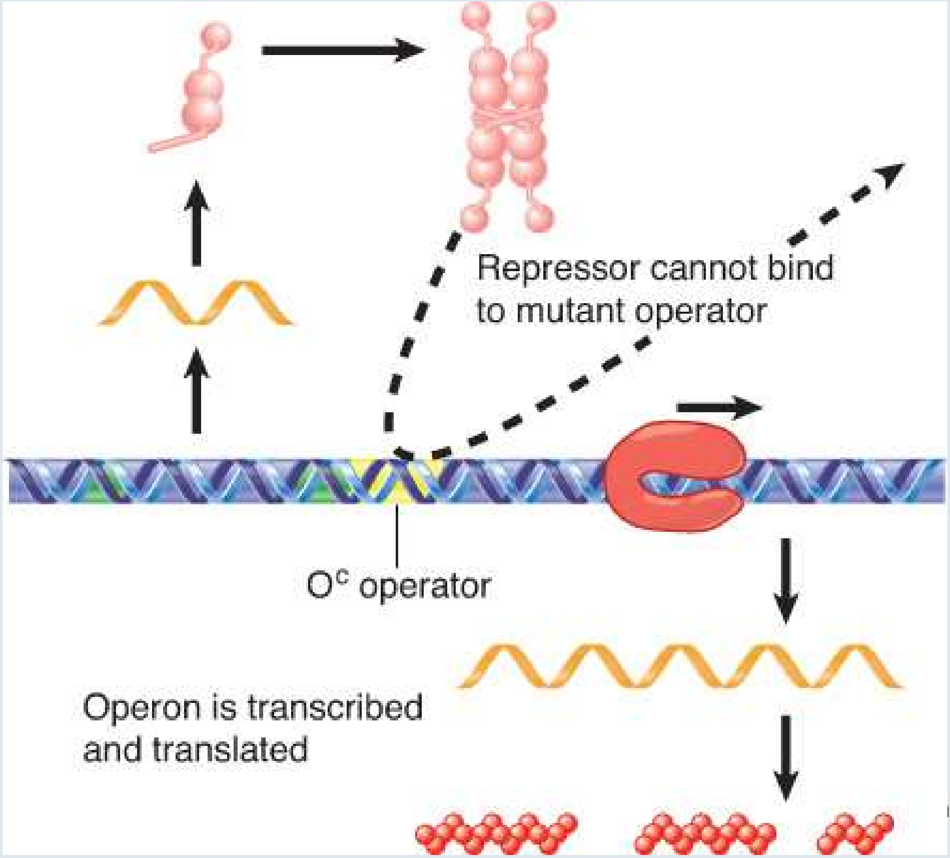


Figure 26.10: Operator mutations are constitutive because the operator is unable to bind repressor protein.

26.6 *trans*-Acting Mutations Identify the Regulator Gene

- Mutations in the *lacI* gene are *trans*-acting and affect expression of all *lacZYA* clusters in the bacterium.
- Mutations that eliminate *lacI* function cause constitutive expression and are recessive (*lacI*$^-$).
- Mutations in the DNA-binding site of the repressor are constitutive because the repressor cannot bind the operator. (also cis-acting)

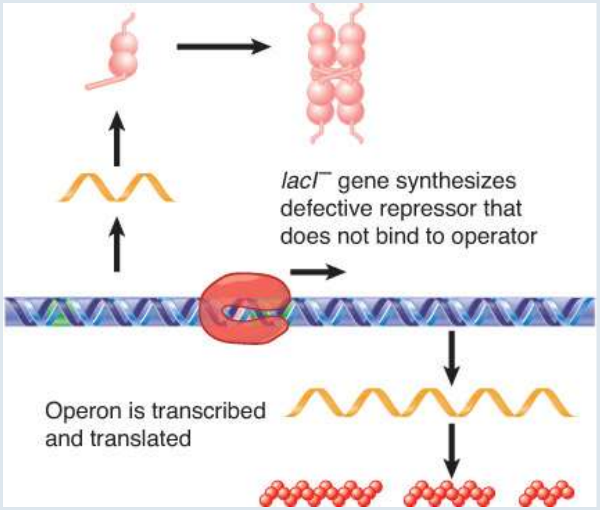


Figure 26.11: Mutations that inactivate the lacI gene cause the operon to be constitutively expressed.

26.6 Other *trans*-Acting Mutations in *lac I*

- Mutations in the inducer-binding site of the repressor prevent it from being inactivated and cause uninducibility. ***lacI^S* Super-repressor mutant**.

- The opposite is an ***lacI^{-d}* uninducible dominant negative** repressor mutant. When mutant and wild-type subunits are present, a single *lacI^{-d}* mutant subunit can inactivate a tetramer whose other subunits are wild-type.

26.6 *trans*-Acting Mutations Identify the Regulator Gene

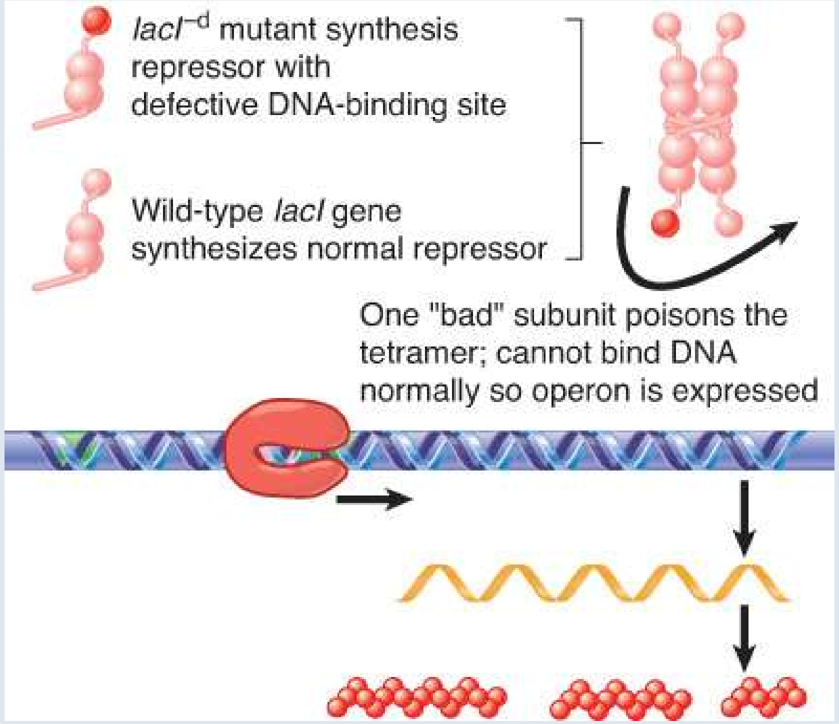


Figure 26.12: A lacI-d mutant gene makes a monomer that has a damaged DNA binding.

- **negative complementation, i.e dominant negative –** This occurs when interallelic complementation allows a mutant subunit to suppress the activity of a wild-type subunit in a multimeric protein.
- *lacI*$^{-d}$ mutations occur in the DNA-binding domain. Their effect is explained by the fact that repressor activity requires all DNA-binding domains in the tetramer to be active.

26.7 *lac* Repressor Is a Tetramer Made of Two Dimers

- A single repressor subunit can be divided into the N-terminal DNA-binding domain, a hinge, and the core of the protein.
- The DNA-binding domain contains two short α-helical regions that bind the major groove of DNA.
- The inducer-binding site and the regions responsible for multimerization are located in the core.

26.7 *lac* Repressor Is a Tetramer Made of Two Dimers

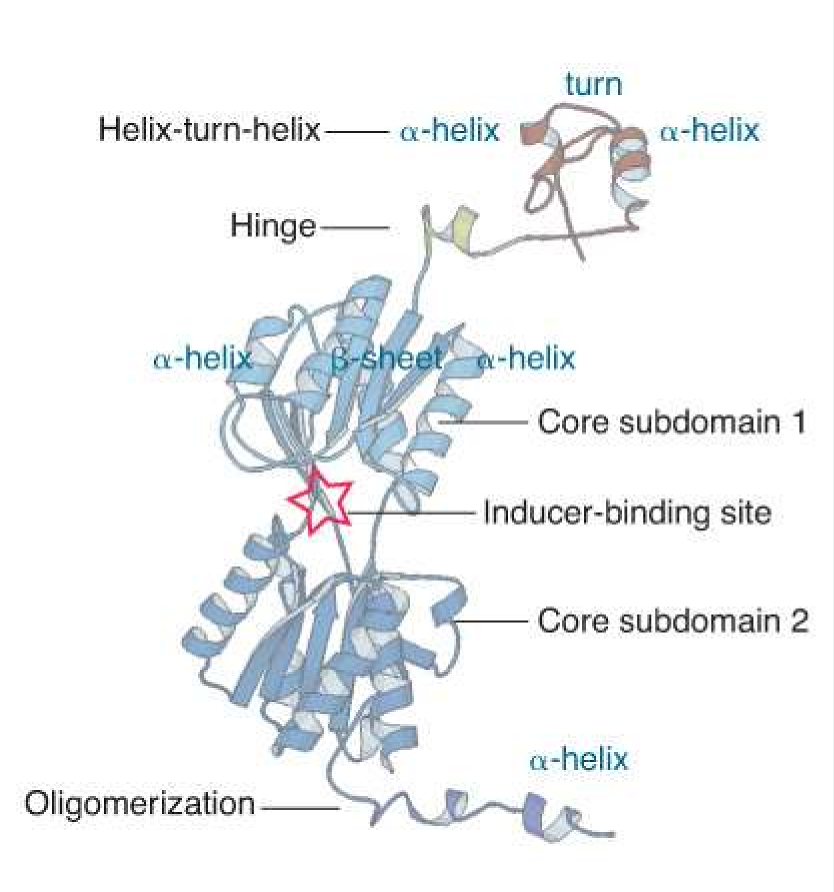


Figure 26.13: The structure of a monomer of Lac repressor identifies several independent domains.

Structure from Protein Data Bank 1LBG. M. Lewis, et al., Science 271 (1996): 1247-1254. Photo courtesy of Hongli Zhan and Kathleen S. Matthews, Rice University.

26.7 *lac* Repressor Is a Tetramer Made of Two Dimers

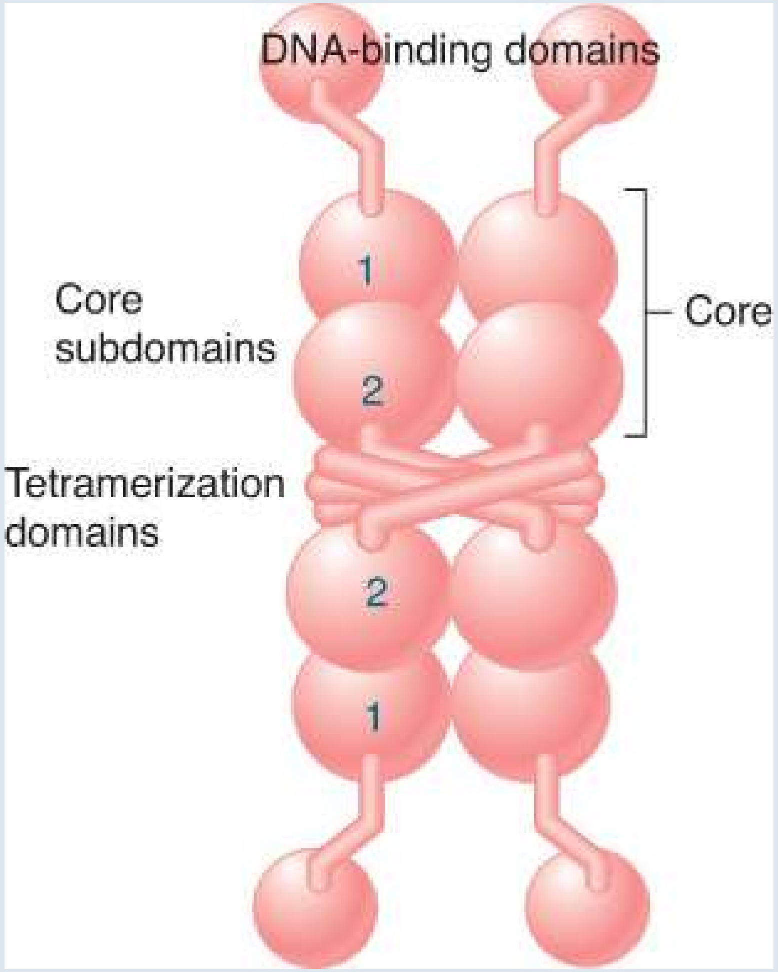


Figure 26.15: The repressor tetramer consists of two dimers.

- Monomers form a dimer by making contacts between core subdomains 1 and 2.
- Dimers form a tetramer by interactions between the tetramerization helices.

26.7 *lac* Repressor Is a Tetramer Made of Two Dimers

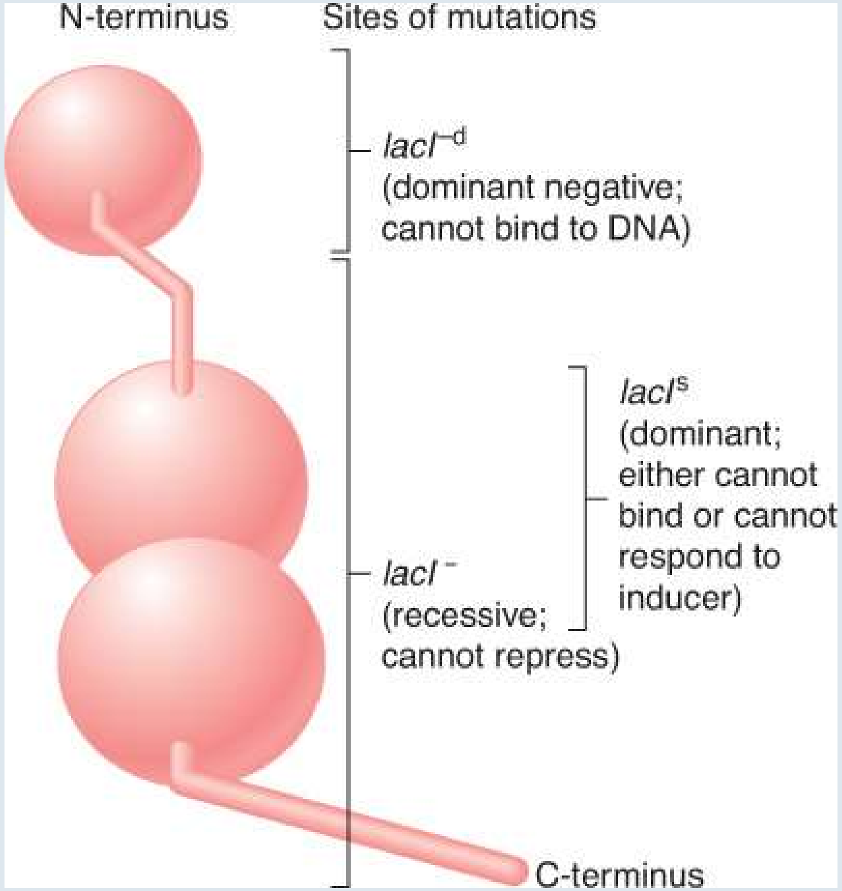


Figure 26.16: The locations of three type of mutations in lactose repressor are mapped on the domain structure of the protein.

- Different types of mutations occur in different domains of the repressor protein.

26.8 *lac* Repressor Binding to the Operator Is Regulated by an Allosteric Change in Conformation

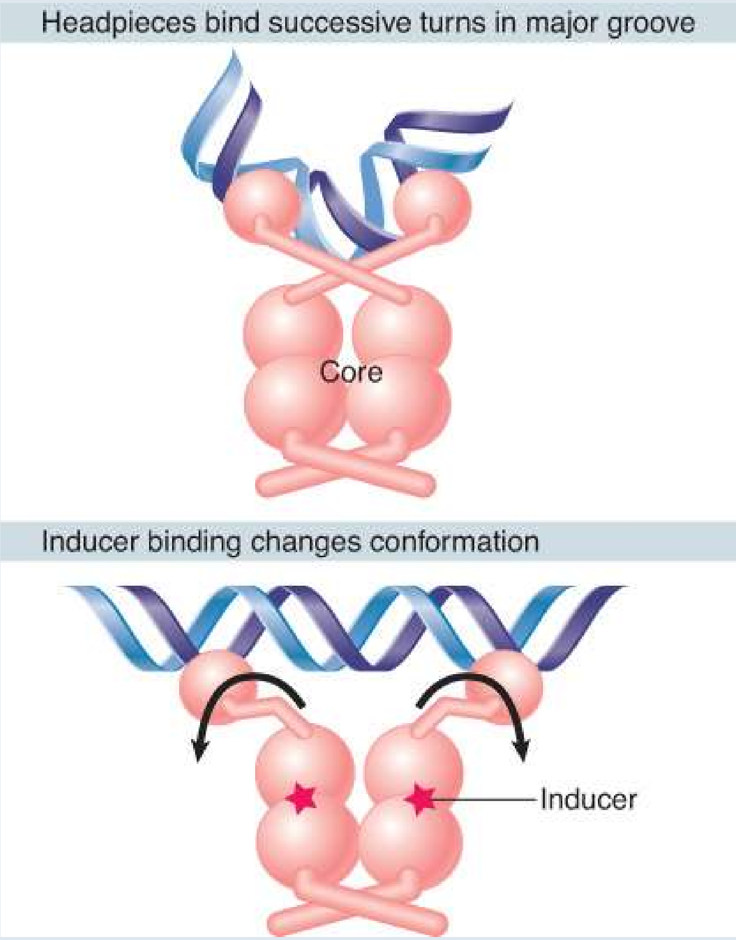


Figure 26.18: The inducer changes the structure of the core.

- Inducer binding causes a change in repressor conformation that reduces its affinity for DNA and releases it from the operator.

26.8 *lac* Repressor Binding to the Operator Is Regulated by an Allosteric Change in Conformation

- *lac* repressor protein binds to the double-stranded DNA sequence of the operator.
- The operator is a **palindromic** sequence of 26 bp.
- Each inverted repeat of the operator binds to the DNA-binding site of one repressor subunit.

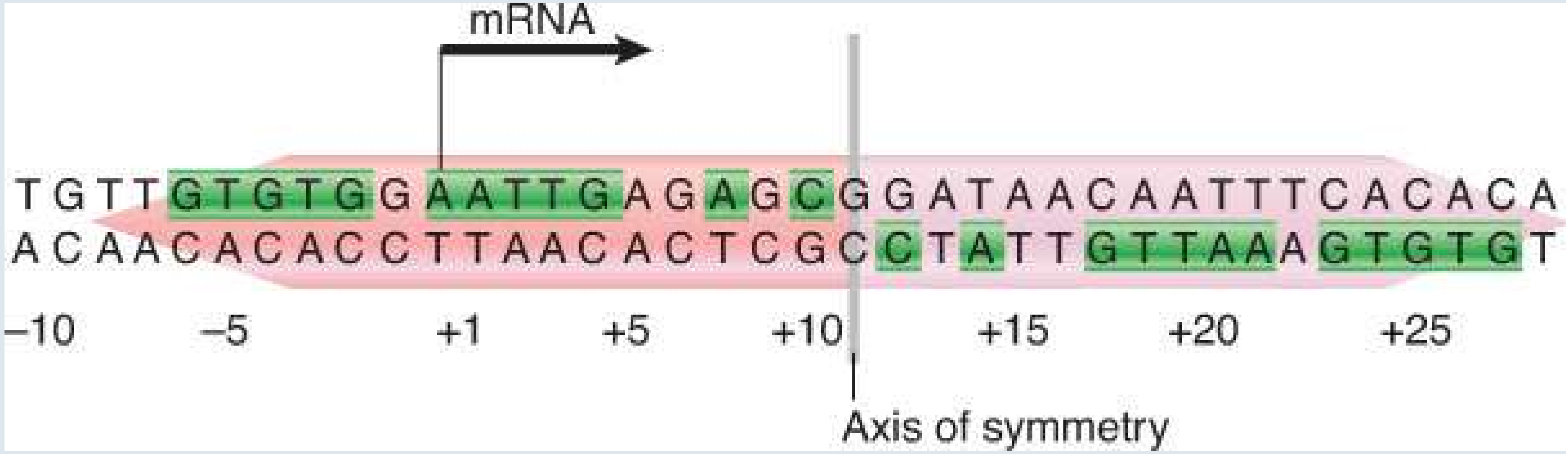


Figure 26.17: The lac operator has a symmetrical sequence.

26.9 *lac* Repressor Binds to Three Operators and Interacts with RNA Polymerase

- Each dimer in a repressor tetramer can bind an operator, so that the tetramer can bind two operators simultaneously.
- Full repression requires the repressor to bind to an additional operator downstream or upstream as well as to the primary operator at the *lacZ* promoter.
- Binding of repressor at the operator stimulates binding of RNA polymerase at the promoter but precludes transcription.

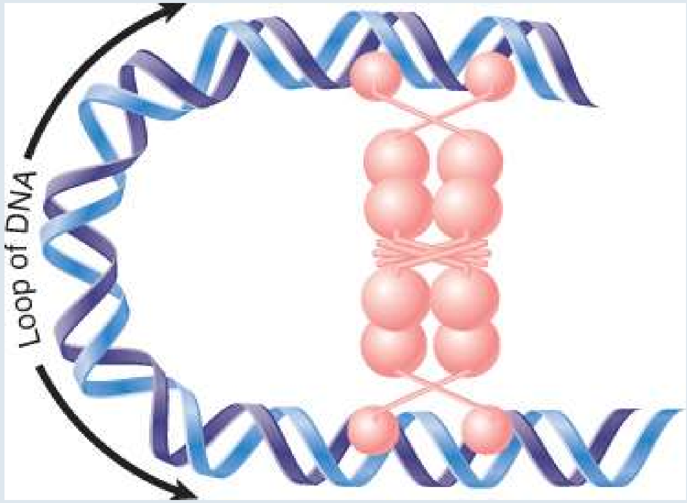
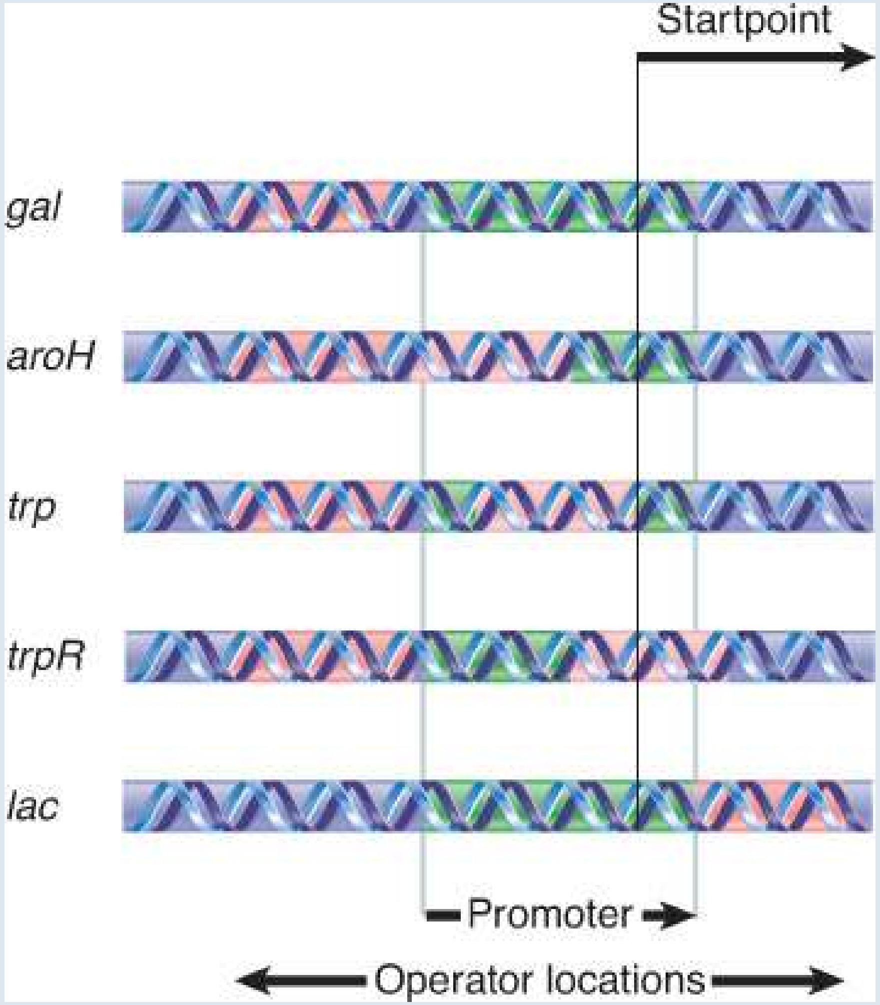


Figure 26.21: If both dimers in a repressor tetramer bind to DNA, the DNA between the two binding sites is held in a loop. Third operator not shown.

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



26.11 **Positive regulation of *lac* transcription**

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.
  - In bacteria this is a positive control system; in eukaryotes, it is completely different.
- **Catabolite repressor protein (CRP)** is an activator protein that binds to a target sequence at a promoter.
- CRP is or was also known as cAMP-dependent, **Catabolite Activator Protein (CAP),** a newer name I find clearer.

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

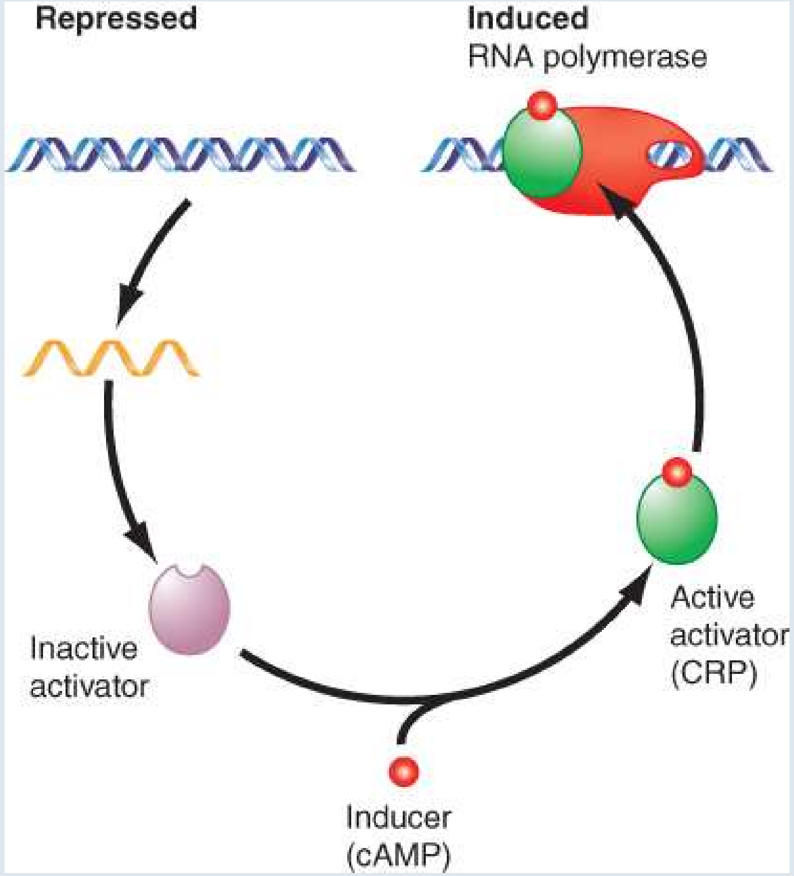


Figure 26.25: cAMP converts an activator protein CRP to a form that binds the promoter and assists RNA polymerase in initiating transcription.

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

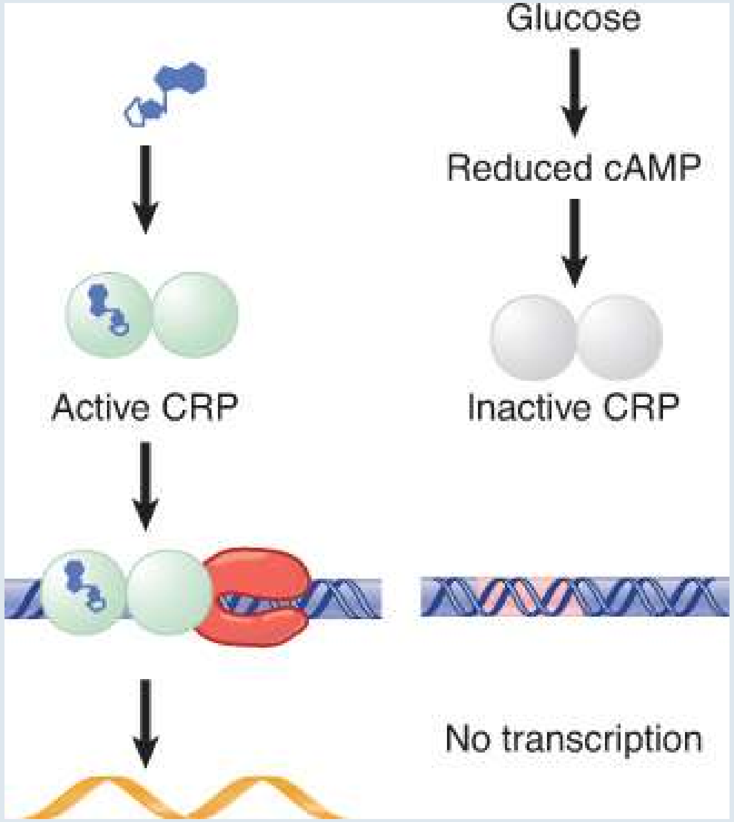


Figure 26.27: By reducing the level of cyclic AMP, glucose inhibits the transcription of operons that require CRP activity.

- A dimer of CRP is activated by a single molecule of **cyclic AMP (cAMP)**. CRP is a major regulator of hundreds of genes for carbon metabolism.
- 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.

26.3 Famous inducible genes and repressors in *E. coli*

The *trp* Operon Is under Negative control by trp repressor and by transcriptional attenuation

26.12 The *trp* Operon Is repressed by *trp* Repressor when tryptophan levels are high

- The *trp* operon is negatively controlled by the level of its product, the amino acid tryptophan (**autoregulation**). This is typical for regulation of synthesis of each amino acid. Tryptophan activates a specific inactive repressor encoded by *trpR*.
- Regulation of nitrogen metabolism also involves a very general positive transcription factor, NtrC, rather like CRP for carbon metabolism. NtrC activates very many nitrogen metabolism genes in response to nitrogen sources like L-glutamine.
- There are about 270 repressors, activators or dual function transcriptional regulator proteins in *E. coli*. Most have sensor domains that respond to levels of key metabolites which also allosterically regulate relevant metabolic enzymes directly. Enzyme allostery controls metabolite flows much more rapidly than transcription regulation.

Transcription attenuation in the leader of the *E. coli trp* operon

26.13 The *trp* Operon Is Also Controlled by Attenuation

- 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× increase in transcription.

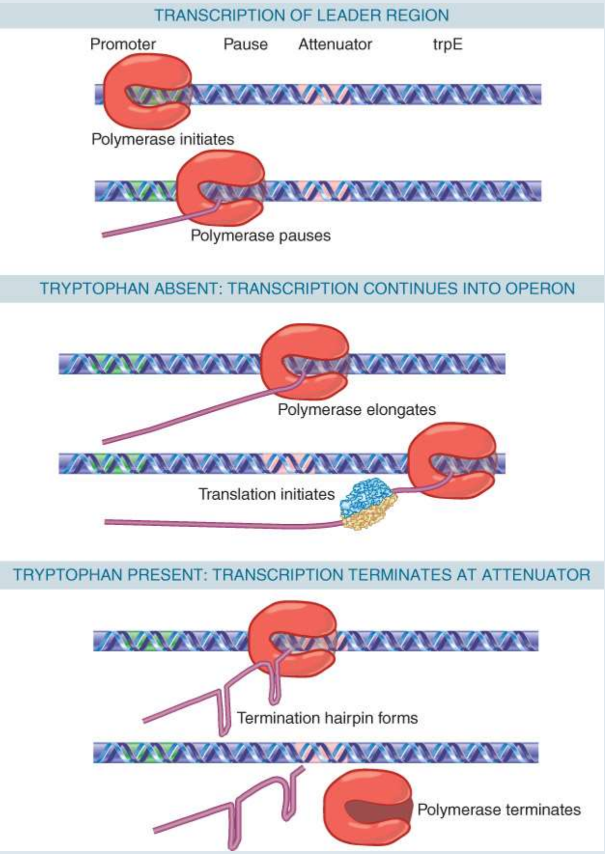


Figure 26.34: An attenuator controls the progression of RNA polymerase into the trp genes.

26.13 The *trp* Operon Is Also Controlled by Attenuation

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

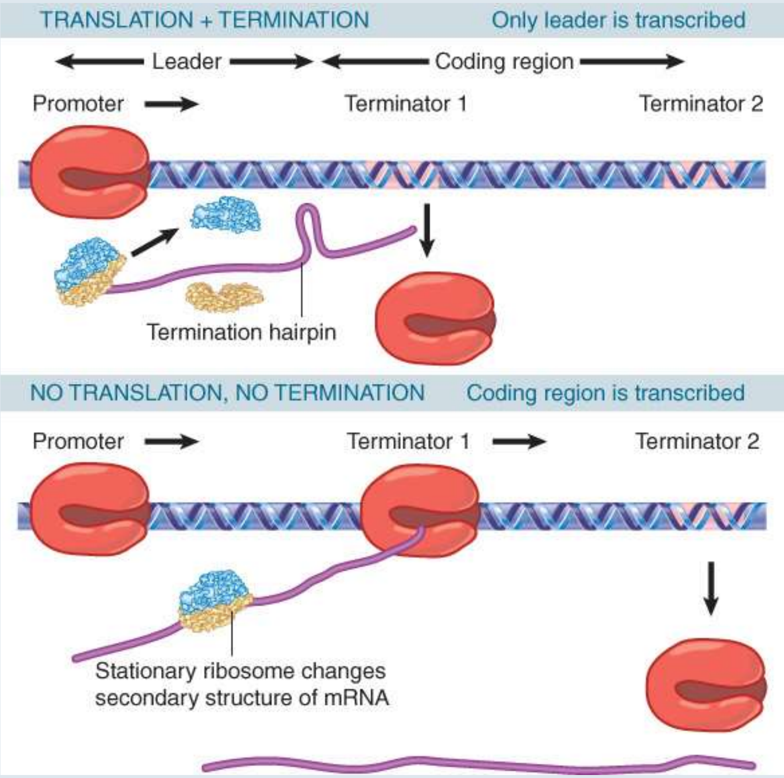


Figure 26.33: Termination can be controlled via changes in RNA secondary structure that are determined by ribosome movement.

26.14 Attenuation Can Be Controlled by Translation

- The leader region of the *trp* operon has a 14-codon open reading frame that includes two codons for tryptophan.
- The structure of RNA at the attenuator depends on whether this reading frame is translated.
- In the presence of Trp-tRNA, the leader is translated to a **leader peptide**, and the attenuator is able to form the hairpin that causes termination.

26.14 Attenuation Can Be Controlled by Translation

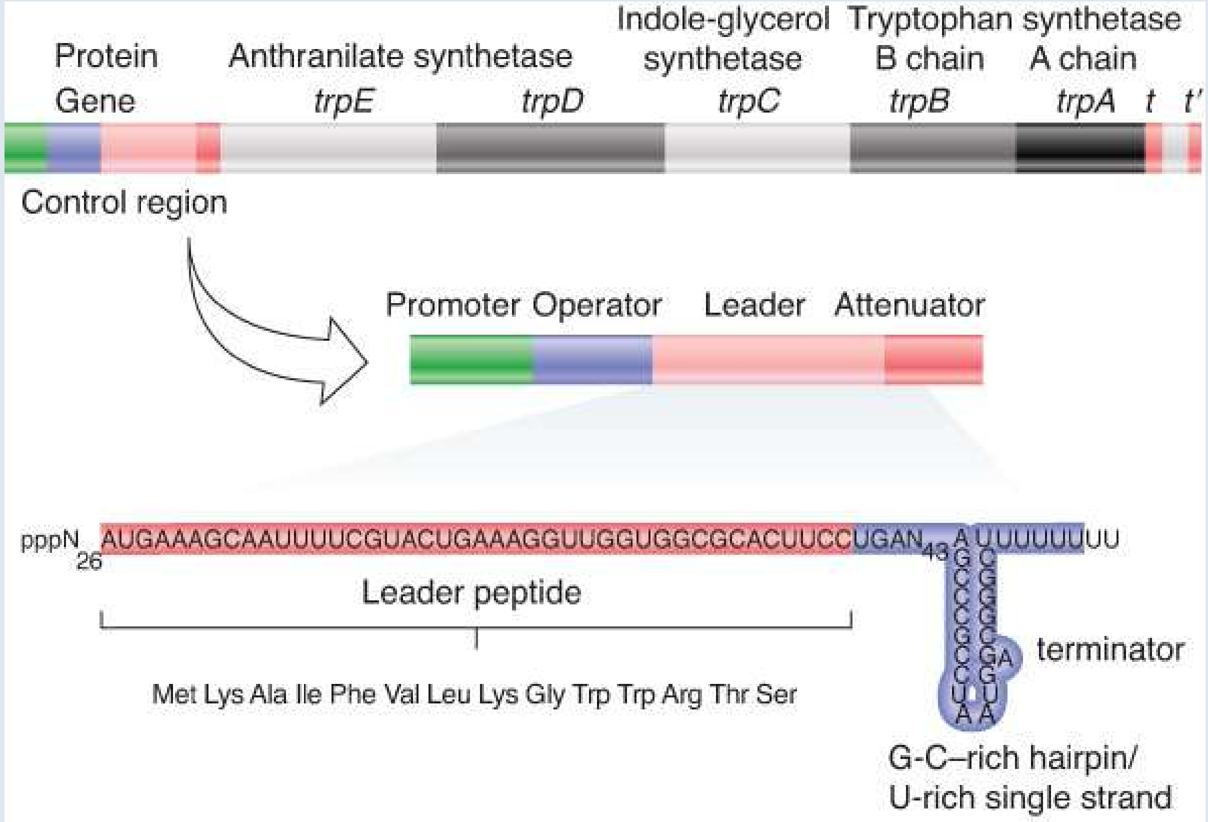


Figure 26.35: The trp operon has a short sequence coding for a leader peptide that is located between the operator and the attenuator.

26.14 Attenuation Can Be Controlled by Translation

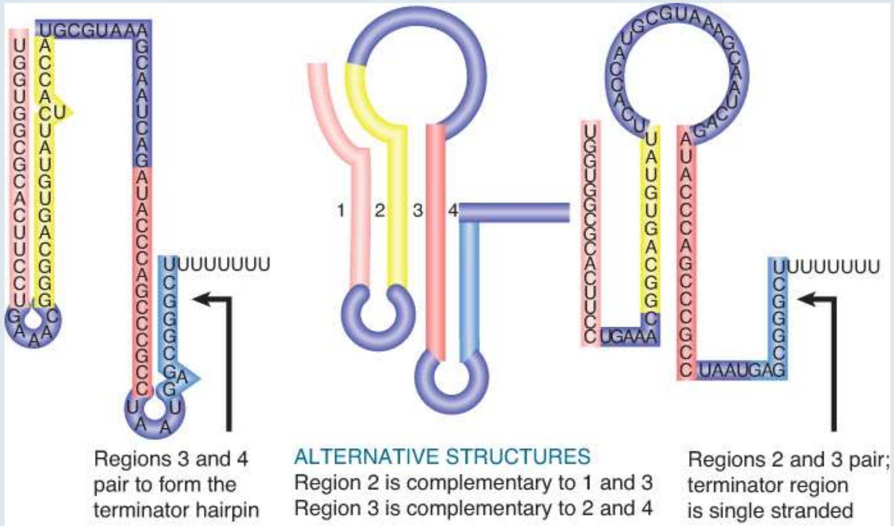


Figure 26.36: The trp leader region can exist in alternative base-paired conformations.

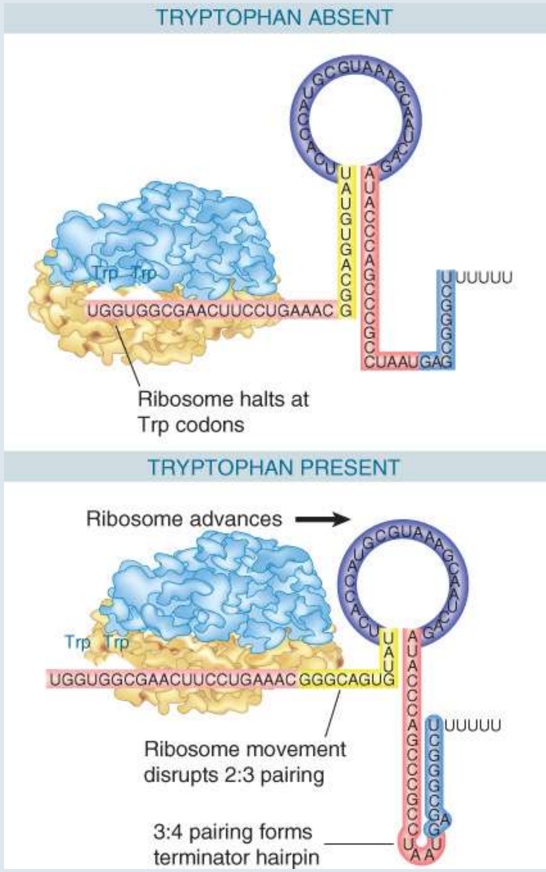


Figure 26.37: The alternatives for RNA polymerase at the attenuator depend on the location of the ribosome.

26.14 Attenuation Can Be Controlled by Translation

- In the absence of Trp-tRNA, the **ribosome stalls** at the tryptophan codons and an alternative secondary structure prevents formation of the hairpin, so that transcription continues.

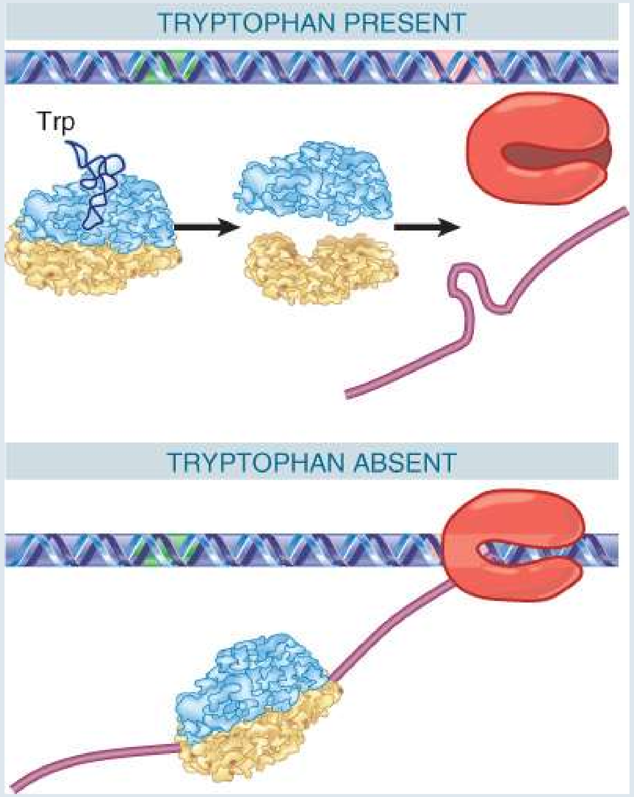


Figure 26.38: In the presence of tryptophan tRNA, ribosomes translate the leader peptide and are released.

27.1 Famous inducible genes and transcriptional repressors in *E. coli.*
Bacteriophage *λ,* the *λ* repressor protein and repressor-operator DNA sequence recognition

- **bacteriophage** (or **phage**) **–** A bacterial virus.
- **lytic infection –** Infection of a bacterium by a phage that ends in the destruction of the bacterium with release of progeny phage.
- **lysis –** The death of bacteria at the end of a phage infective cycle when they burst open to release the progeny of an infecting phage (because phage enzymes disrupt the bacterium's cytoplasmic membrane or cell wall).

27.1 Introduction

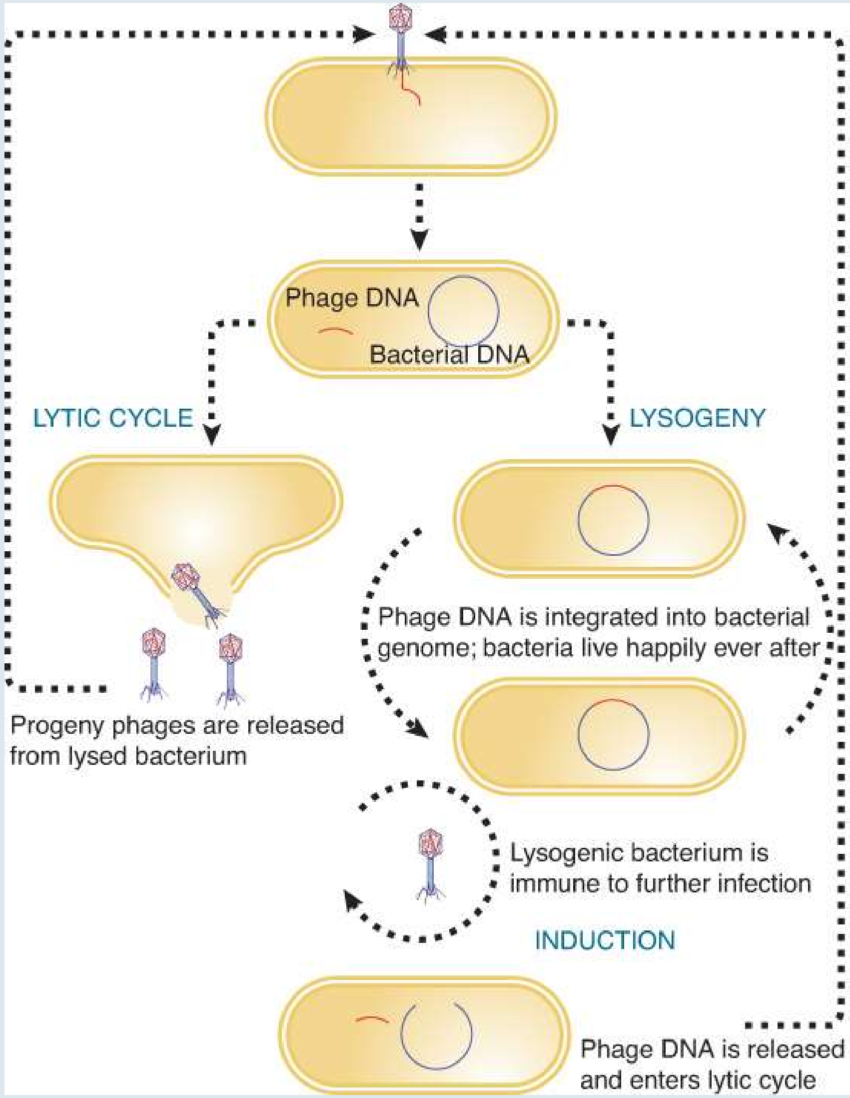
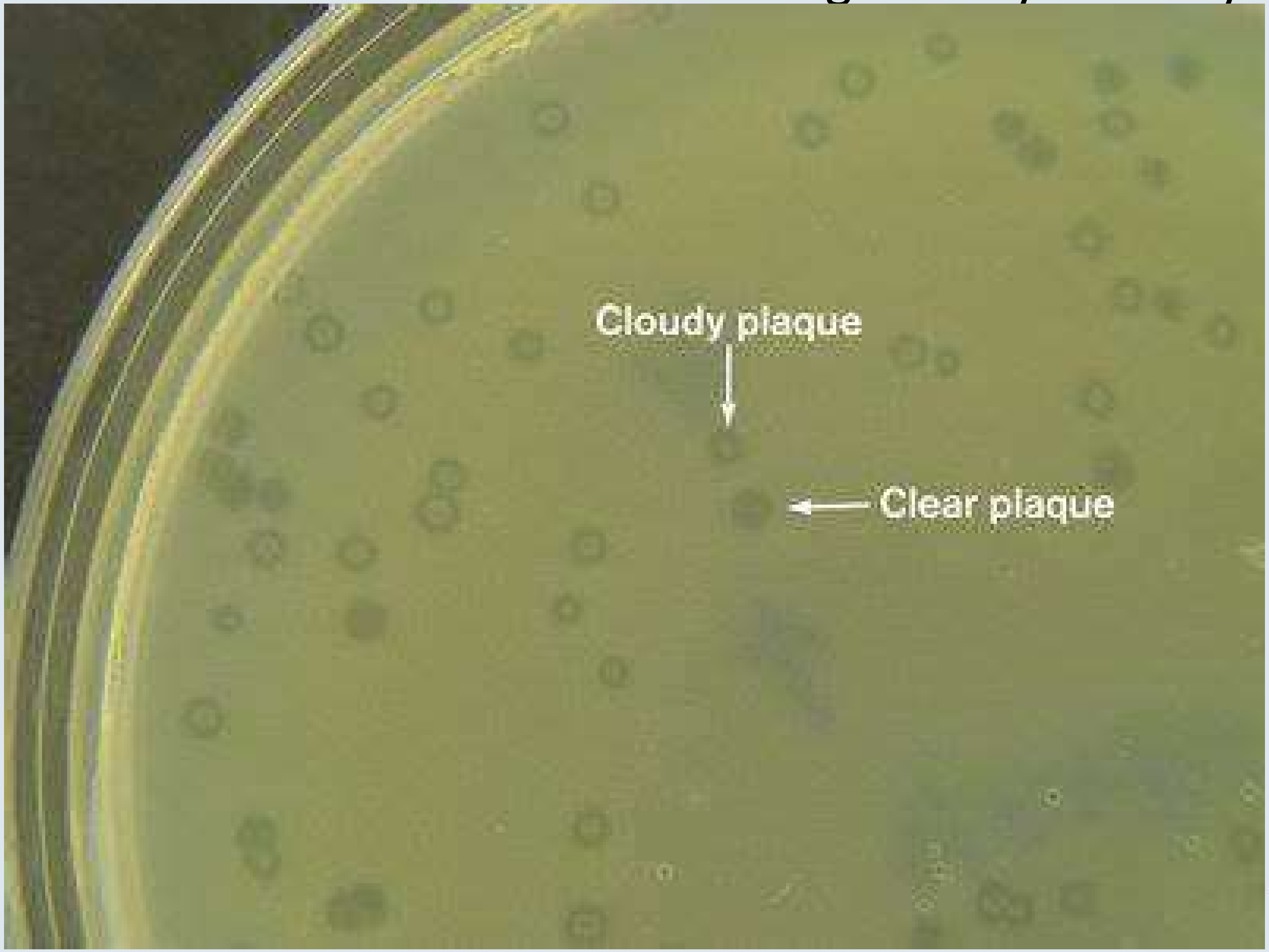


Figure 27.01: Lytic development involves the reproduction of phage particles with destruction of the host bacterium.

Gateway and other cloning and directed recombination systems use phage proteins and target sites like the lambda *att* site λ bacteriophage **plaques** on a lawn of *E. coli* are **clear** where all cells were killed by virus infection and cell lysis (- like a coldsore).

Cloudy plaques contain **λ lysogen** cells that are **immune** to further lambda virus infection leading directly to cell lysis



27.8 Lysogeny Is Maintained by the Lambda Repressor Protein

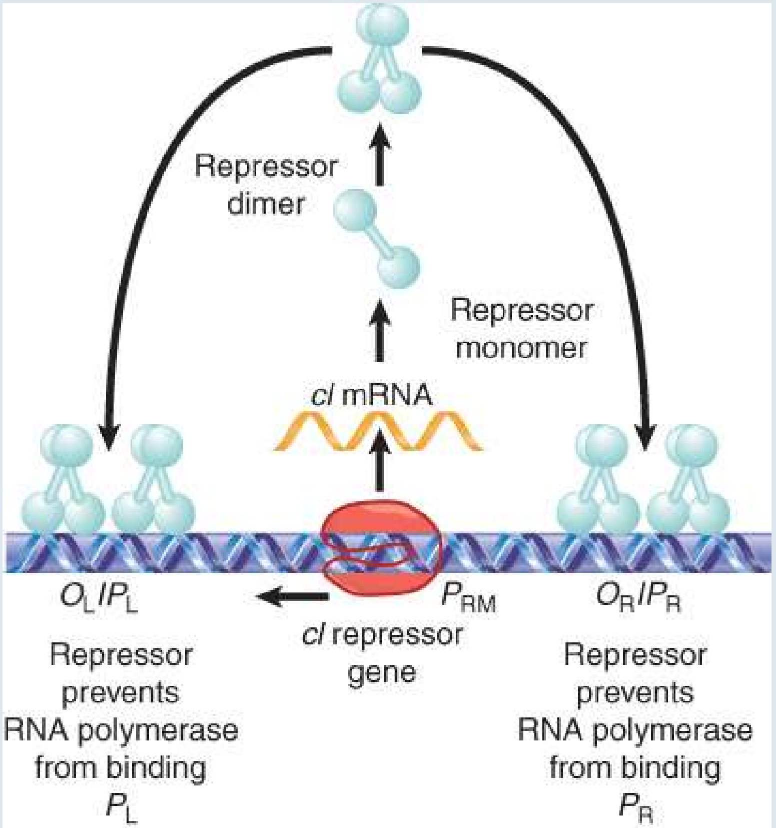


Figure 27.15: Repressor acts at the left operator and right operator to prevent transcription of the immediate early genes (N and cro).

- The lambda repressor, encoded by the *cI* gene, is required to maintain lysogeny.
- The lambda repressor acts at the O_L and O_R operators to block transcription of the immediate early genes.
- The immediate early genes trigger a regulatory cascade; as a result, their repression prevents the lytic cycle from proceeding.

27.9 The Lambda Repressor and Its Operators Define the Immunity Region

- **immunity –** In phages, the ability of a prophage to prevent another phage of the same type from infecting a cell.
- **virulent mutations –** Phage mutants that are unable to establish lysogeny.

27.9 The Lambda Repressor and Its Operators Define the Immunity Region

- Several lambdoid phages have different **immunity regions**.
- A lysogenic phage confers immunity to further infection by any other phage with the same immunity region.

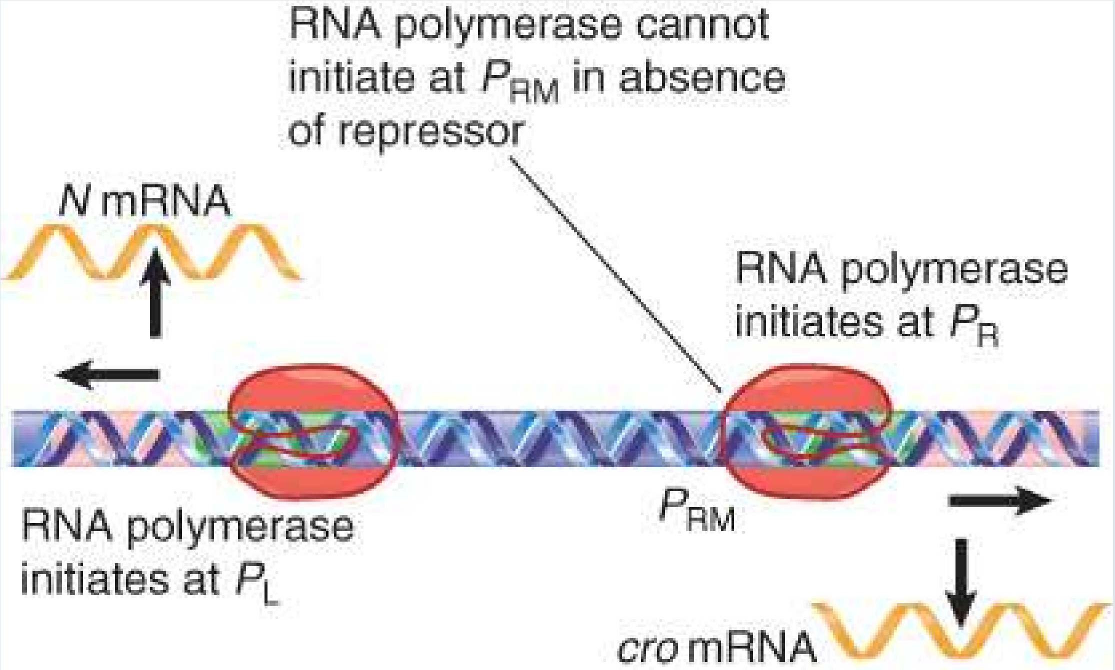


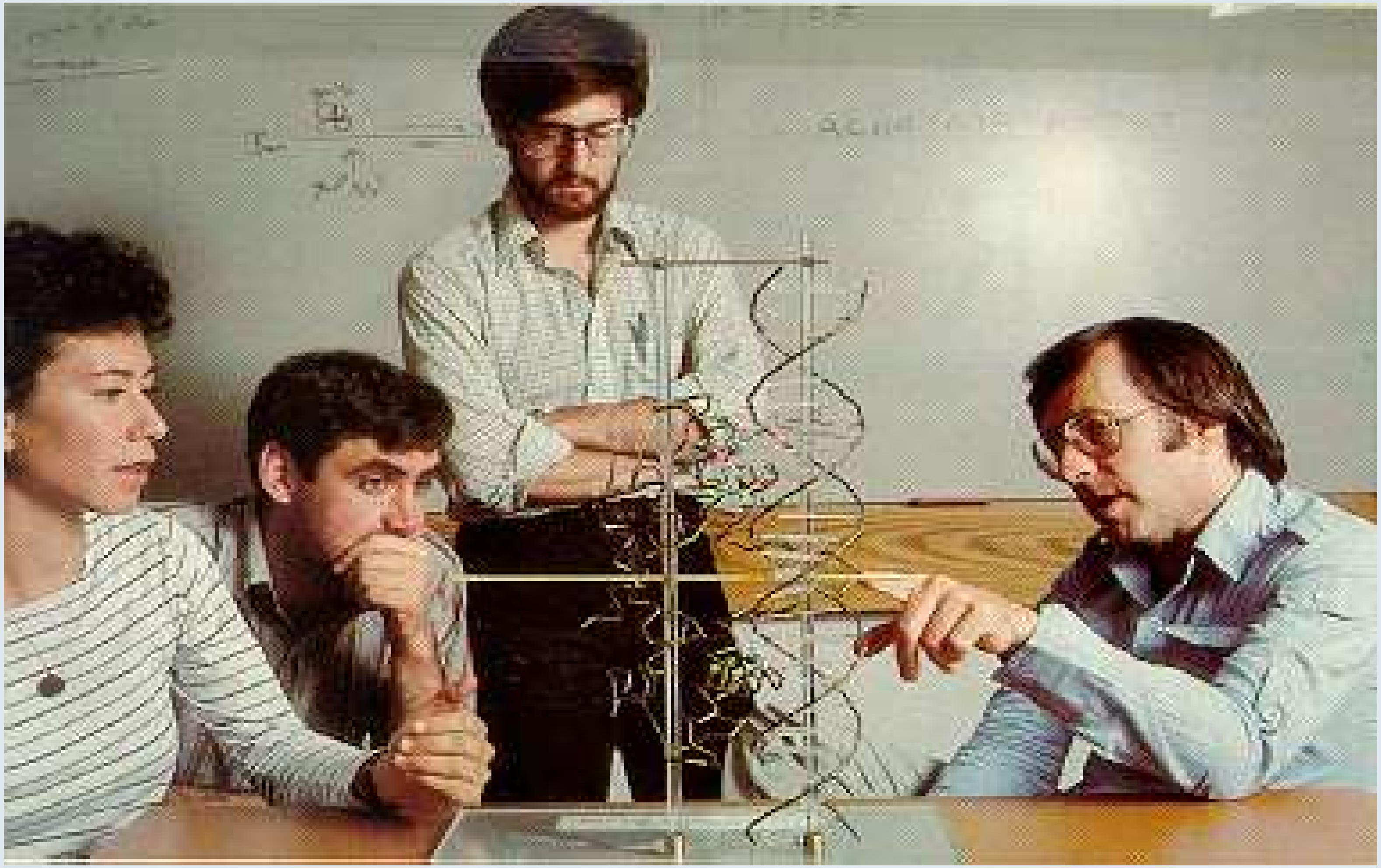
Figure 27.16: In the absence of repressor, RNA polymerase initiates at the left and right promoters.

Need to purify scarce gene regulator proteins drove biotechnology of protein overexpression

- Take *E. coli* cell as a cube 1 micron on each side. A liter is a cube 10 cm on each side. What is the concentration of the lac operator? (Answer. nanomolar; about 10^{-9} M). 10-100 lac repressor molecules per cell and the k_d for operator-binding is 10^{-10} M or lower (tighter).

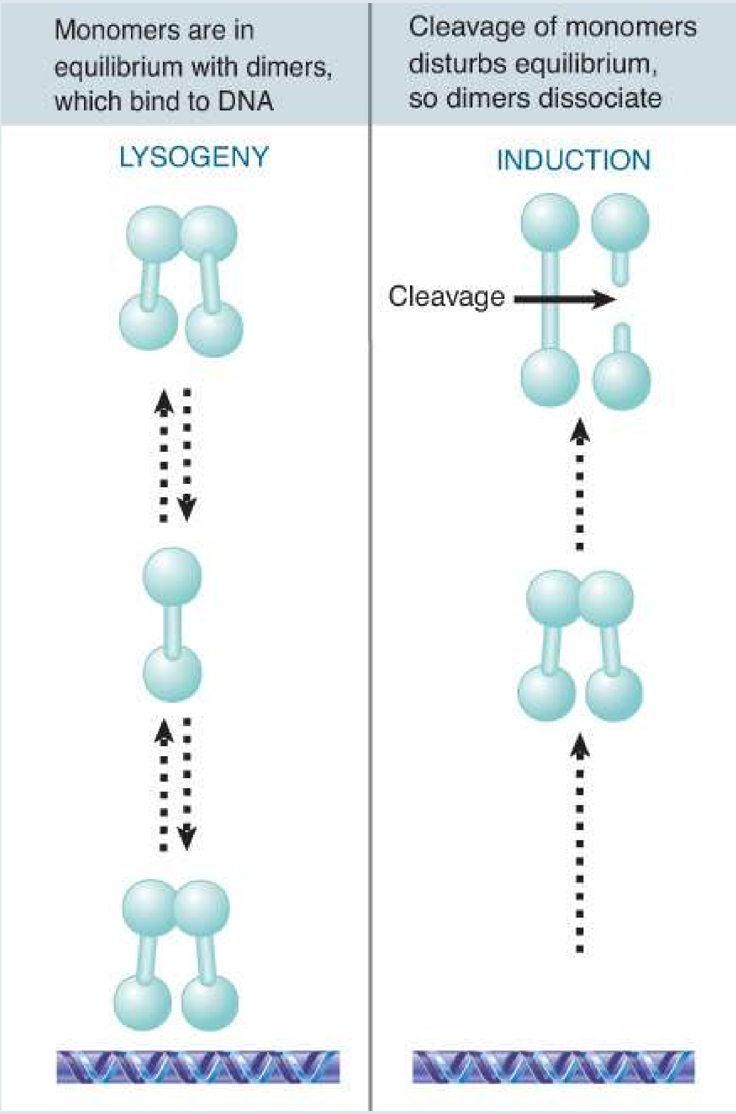
- Lambda repressor was first isolated from an overproducer mutant virus. Later the repressor gene was expressed from *lac* or *tac* promoters. Also, a hybrid ribosome binding site (Shine-Dalgarno sequence), upstream of ATG gave strong translation initiation.

Sequence-specific DNA recognition is the key to differential gene regulation.



Mark Ptashne with graduate students Cynthia Wohlberger, Liam Keegan, Ed Giniger at Harvard, 1982

27.10 The DNA-Binding Form of the Lambda Repressor Is a Dimer



- A repressor monomer has two distinct domains.
- The N-terminal domain contains the DNA-binding site.
- The C-terminal domain dimerizes.
- Binding to the operator requires the dimeric form so that two DNA-binding domains can contact the operator simultaneously.
- Cleavage of the repressor between the two domains reduces the affinity for the operator and induces a lytic cycle.

Figure 27.18: Repressor dimers bind to the operator.

27.11 Lambda Repressor Uses a Helix-Turn-Helix Motif to Bind DNA

- Each DNA-binding region in the repressor contacts a half-site in the DNA.
- The DNA-binding site of the repressor includes two short α-helical regions that fit into the successive turns of the major groove of DNA (**helix-turn-helix**).
- A DNA-binding site is a (partially) palindromic sequence of 17 bp.

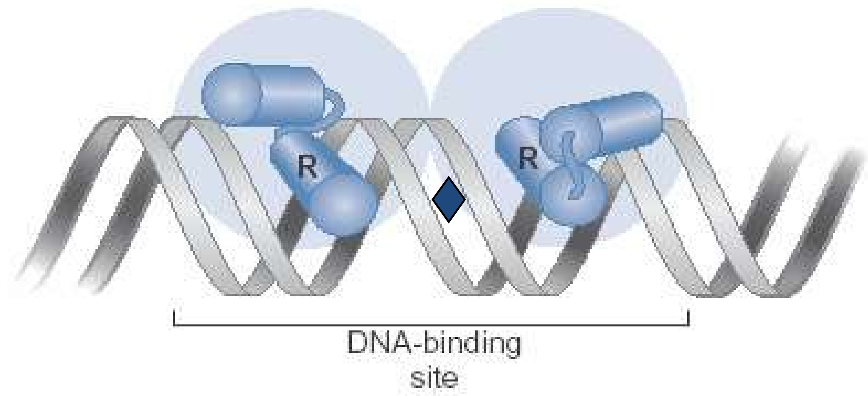
Figure 27.19: The operator is a 17-bp sequence with an axis of symmetry through the central base pair.

Lambda repressor binds as a symmetrical protein dimer to a nearly symmetrical 17 bp DNA sequence.

Symmetric repressor site

5’ T A T C A C C G C C G G T G A T A
A T A G T G G C G G C C A C T A T 5’

FIGURE 16-11 Binding of a protein with a helix-turn-helix domain to DNA. The protein, as is typically the case, binds as a dimer, and the two subunits are indicated by the shaded circles. The helix-turn-helix motif on each monomer is indicated; the "recognition helix" is labeled R.



Recognition helix amino acid side-chains make many sequence-specific contacts to base pairs.

5’ T A T C A C C G C C G G T G A T A
A T A G T G G C G G C C A C T A T 5’

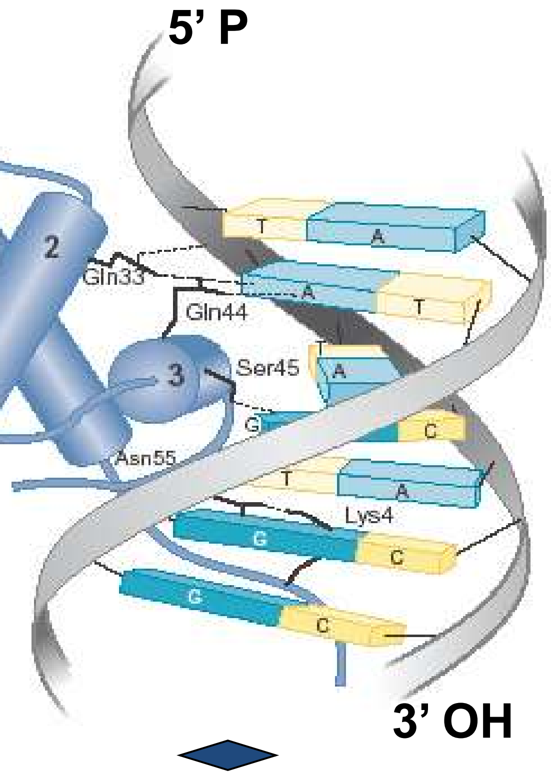
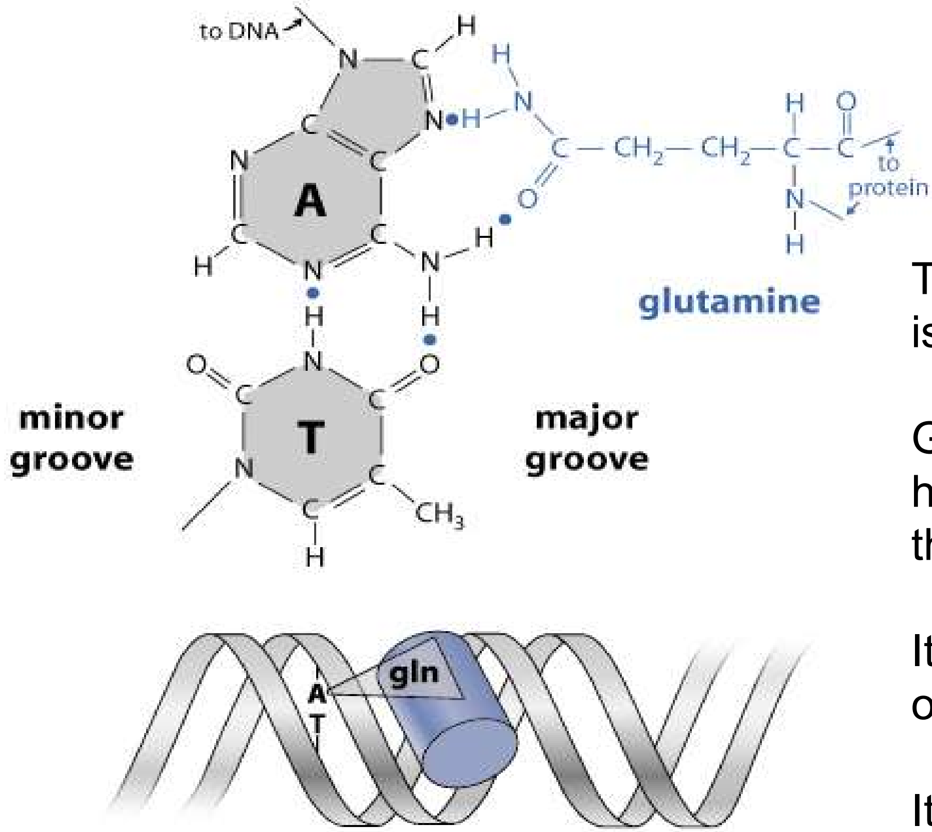


FIGURE 16-12 Hydrogen bonds between λ repressor and base pairs in the major groove of its operator. Diagram of the repressor-operator complex, showing hydrogen bonds (in dotted lines) between amino acid side chains and bases in the consensus half-site. Only the relevant amino acid side chains are shown. In addition to Gln44 and Ser45 in the recognition helix, Asn55 in the loop following the recognition helix also makes contact with a specific base. Furthermore (and unusual to this case, see later in the text) Lys4 in the N-terminal arm of the protein makes a contact in the major groove on the opposite face of the DNA helix. Gln33 contacts the backbone. (Source: Redrawn from Jordan, S. and Pabo, C. *Science* 242: 896, Fig. 3B.)

Example of an amino acid contact that can discriminate between bases in a binding site.



A Genetic Switch, 3rd edition, 2004

Chapter 2, Figure 12

The position of the glutamine is fixed when repressor binds.

Glutamine side chain needs to find the hydrogen donor and acceptor sites in just the right places.

It cannot reach the paired base on the other strand.

It cannot reach the other bases on the same strand.

Only an A base meets the criteria.

Wide major groove means DNA sequence can be recognized by DNA-binding proteins.

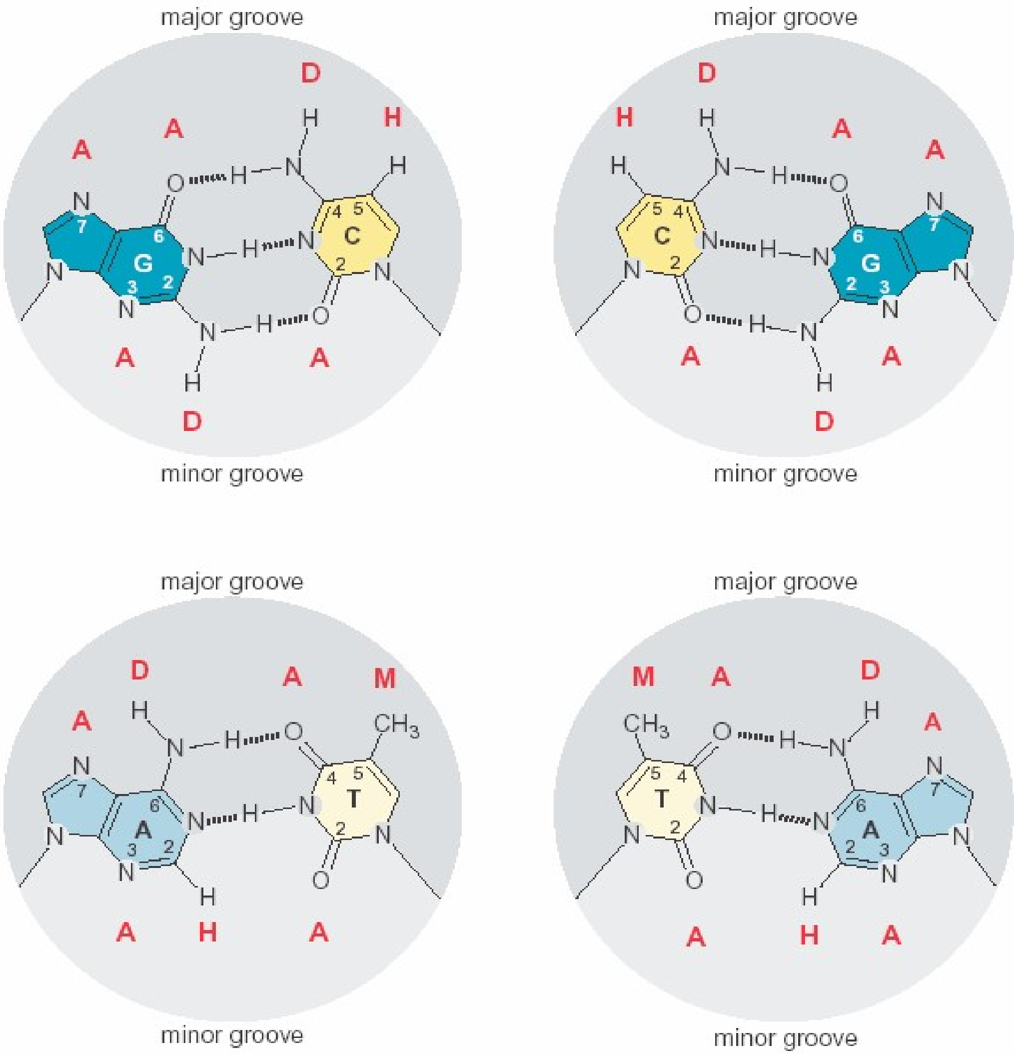


FIGURE 6-10 Chemical groups exposed in the major and minor grooves from the edges of the base pairs. The letters in red identify hydrogen bond acceptors (**A**), hydrogen bond donors (**D**), nonpolar hydrogens (**H**), and methyl groups (**M**).

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com

27.12 Lambda Repressor Dimers Bind Cooperatively to the Operator

- Repressor binding to one operator increases the affinity for binding a second repressor dimer to the adjacent operator.
- The affinity is 10 × greater for O_L1 and O_R1 than other operators, so they are bound first.
- Cooperativity allows repressor to bind the O_L2/O_R2 sites at lower concentrations.

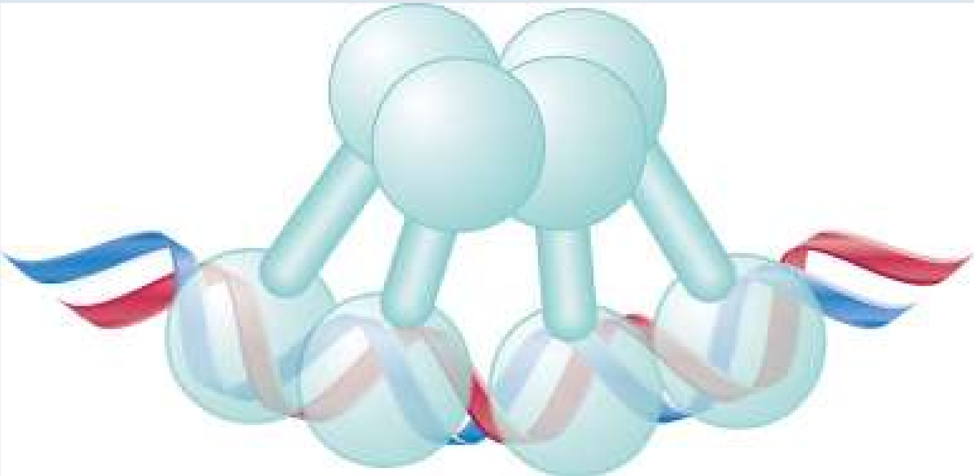


Figure 27.25: When two lambda repressor dimers bind cooperatively, each of the subunits of one dimer contacts a subunit in the other dimer.

27.14 Cooperative Interactions Increase the Sensitivity of Regulation

- Repressor dimers bound at O_L1 and O_L2 interact with dimers bound at O_R1 and O_R2 to form octamers.
- These cooperative interactions increase the sensitivity of regulation.

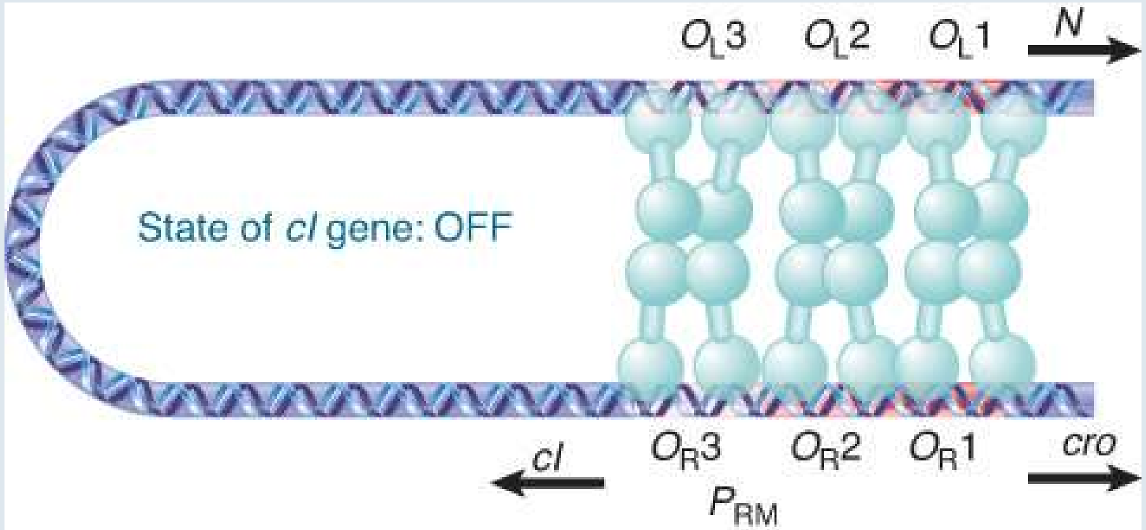


Figure 27.29: Ol3 and Or3 are brought into proximity by formation of the repressor octamer.

27.11 Lambda Repressor Uses a Helix-Turn-Helix Motif to Bind DNA

- The amino acid sequence of the **recognition helix** makes contacts with particular bases in the operator sequence that it recognizes.

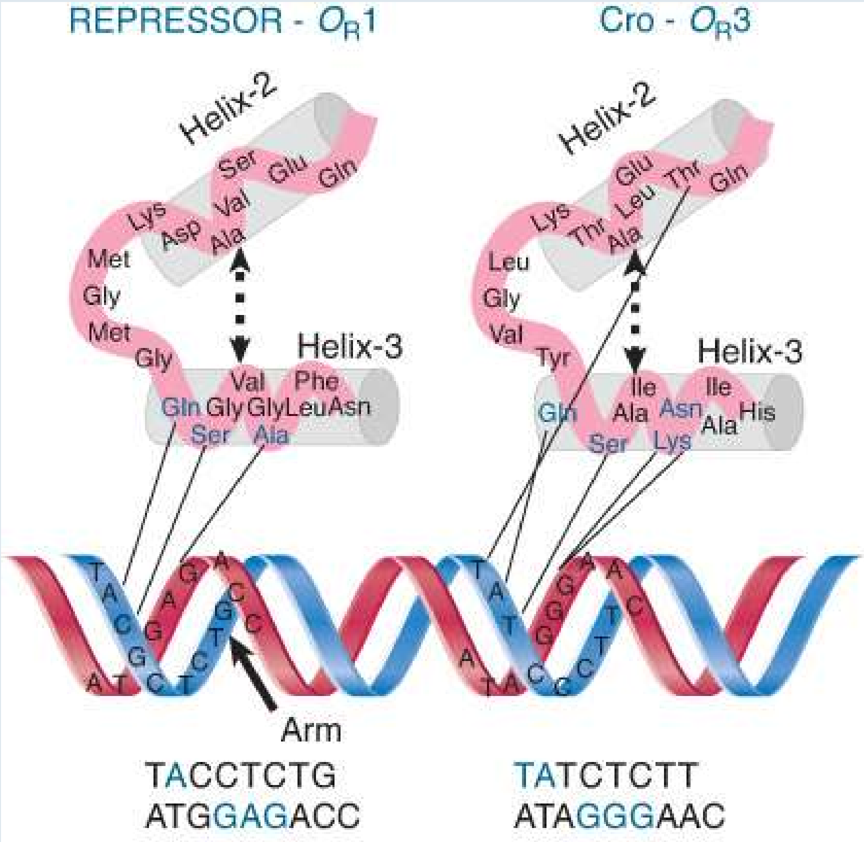


Figure 27.22: Two proteins that use the two-helix arrangement to contact DNA recognize lambda operators with affinities determined by helix-3.

27.13 Lambda Repressor Maintains an Autoregulatory Circuit

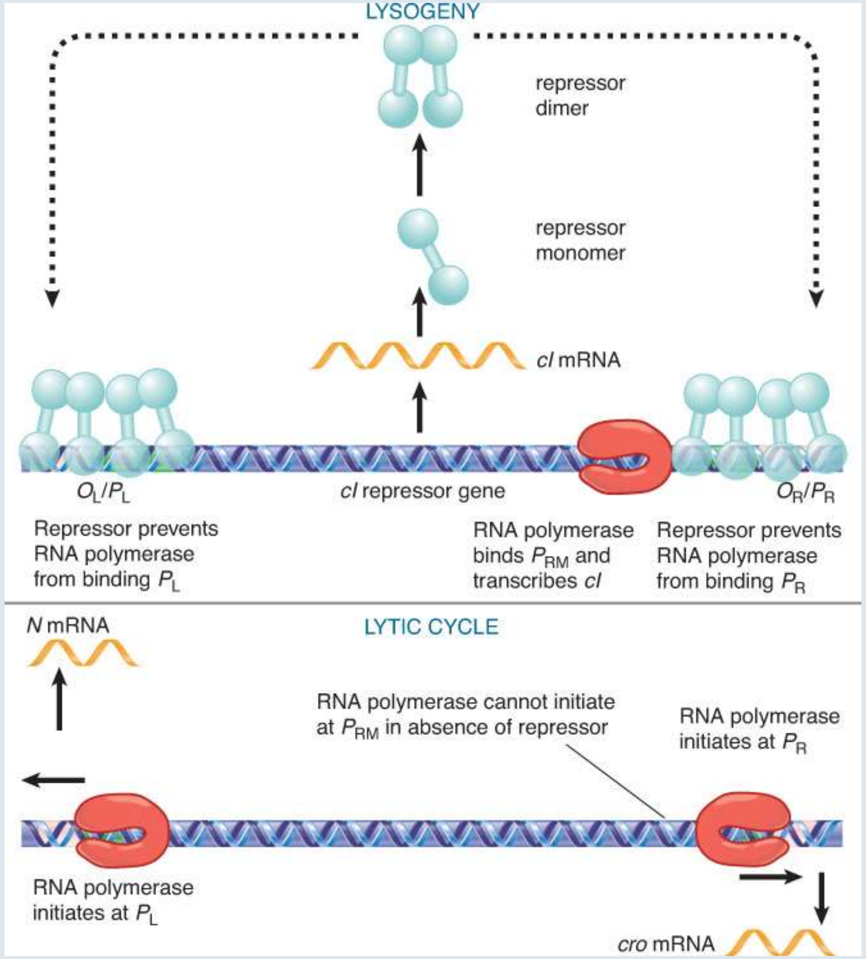


Figure 27.27: Lysogeny is maintained by an autoregulatory circuit.

- Repressor binding at O_R blocks transcription of *cro*, but also is required for transcription of *cI*.
- Repressor binding to the operators therefore simultaneously blocks entry to the lytic cycle and promotes its own synthesis.

27.13 λ Repressor transcriptionally activates *lacI* expression to maintain λ R in lysogenic cells

- The DNA-binding region of repressor at O_R2 contacts RNA polymerase and stabilizes its binding to P_{RM}.
- This is the basis for the autoregulatory **positive control** of repressor maintenance.
- Repressor binding at O_L blocks transcription of gene *N* from P_L.

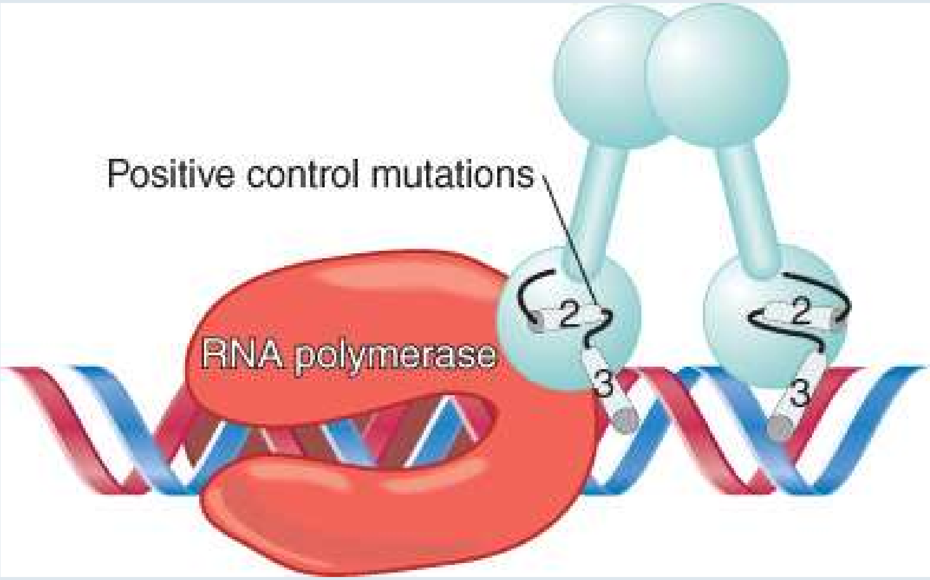


Figure 27.26: Positive control mutations identify a small region at helix-2 that interacts directly with RNA polymerase.

Mechanism of transcription activation of the yeast *GAL* genes; a model for eukaryotic transcriptional gene regulation

Gene numbers increase slower but genome sizes increase faster among the main model organisms.

Bacterial minimum is 1800 genes for independent life and 450 in cell endoparasitic Rickettsias and Mycoplasmas. Craig Venter tries to start a cell like this.

Yeast *S. cervisiae* has 6,000 genes. About 2X basic eukaryotic minimum set 2,700 in *S. bayanus* and or so and less in cell endoparasitic yeast relatives, Microsporidia.

Simpler multicellular animals like *Drosophila* have only about 2.5X as many genes as yeast. Humans and other mammals have less than 2X as many as *Drosophila*.

Sequenced genomes vary from 470 to 30,000 genes

Species	Genomes (Mb)	Genes	Lethal loci
Mycoplasma genitalium	0.58	470	~300
Rickettsia prowazekii	1.11	834	
Haemophilus influenzae	1.83	1,743	
Methanococcus jannaschi	1.66	1,738	
B. subtilis	4.2	4,100	
E. coli	4.6	4,288	1,800
S. cerevisiae	13.5	6,034	1,090
S. pombe	12.5	4,929	
A. thaliana	119	25,498	
O. sativa (rice)	466	~30,000	
D. melanogaster	165	13,601	3,100
C. elegans	97	18,424	
H. sapiens	3,300	~25,000	

4,600 kb

13,500 kb — 3x genome size of *E. coli*

165,000 kb — 10X genome size of yeast

3,000,000 kb — 20X *Drosophila* genome

A classical model of inducible gene expression in a eukaryote.

- ***GAL1*** galactokinase (mutant allele is *gal1*)
- ***GAL2*** galactose permease
- ***GAL7*** galactose uridyl transferase
- ***GAL10*** galactose-glucose epimerase

- ***GAL4*** positive regulator (mutant is *gal4*)
- ***GAL80*** negative regulator (mutant is *gal80*)

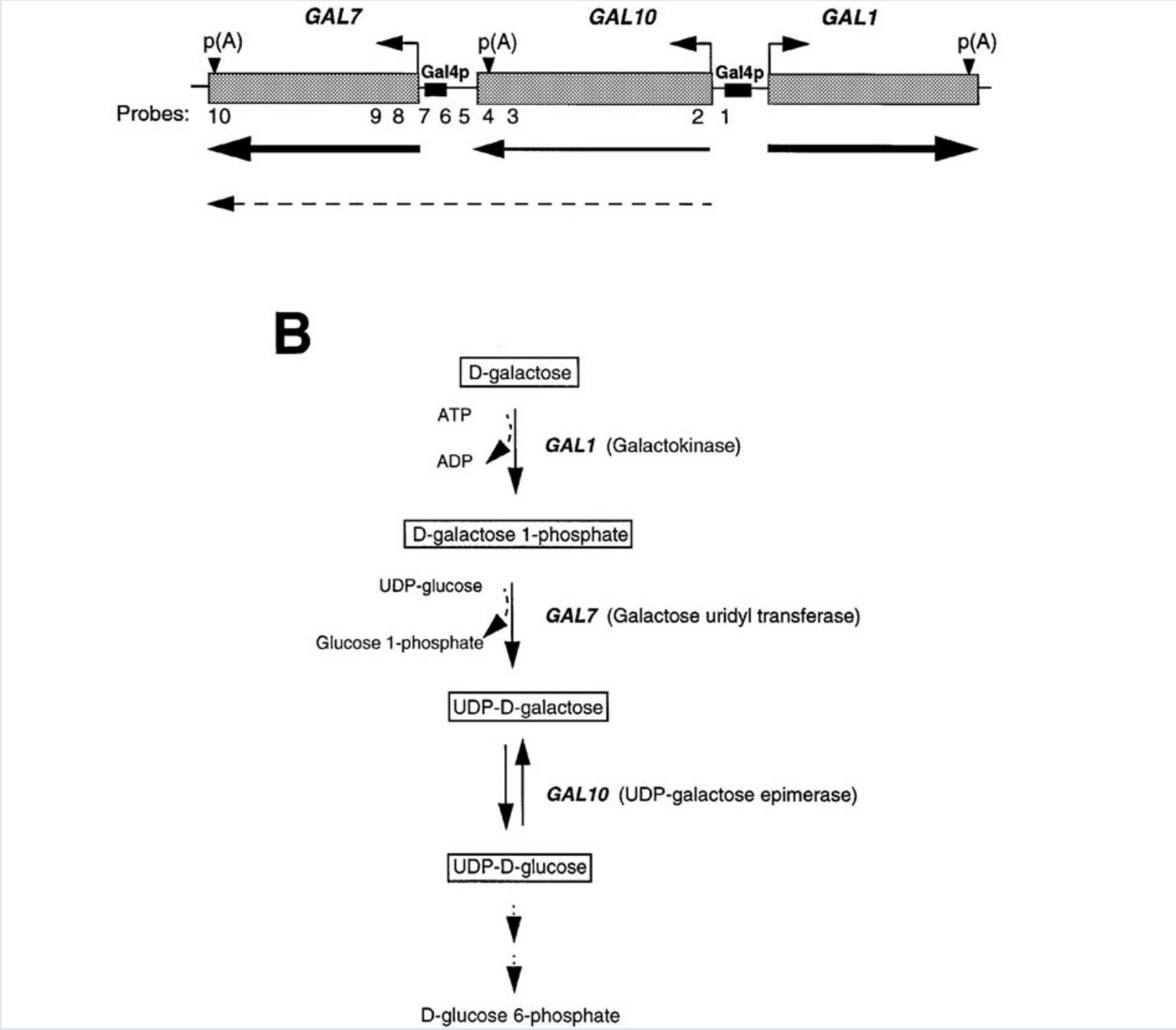
Yeast *GAL* genes are positively regulated at the level of transcription activation.

- A *gal4*, *gal80* double mutant fails to induce expression of galactose enzymes. (gal4 mutation is epistatic to gal80)

- Interpretation is that GAL4 targets the structural genes to activate transcription.

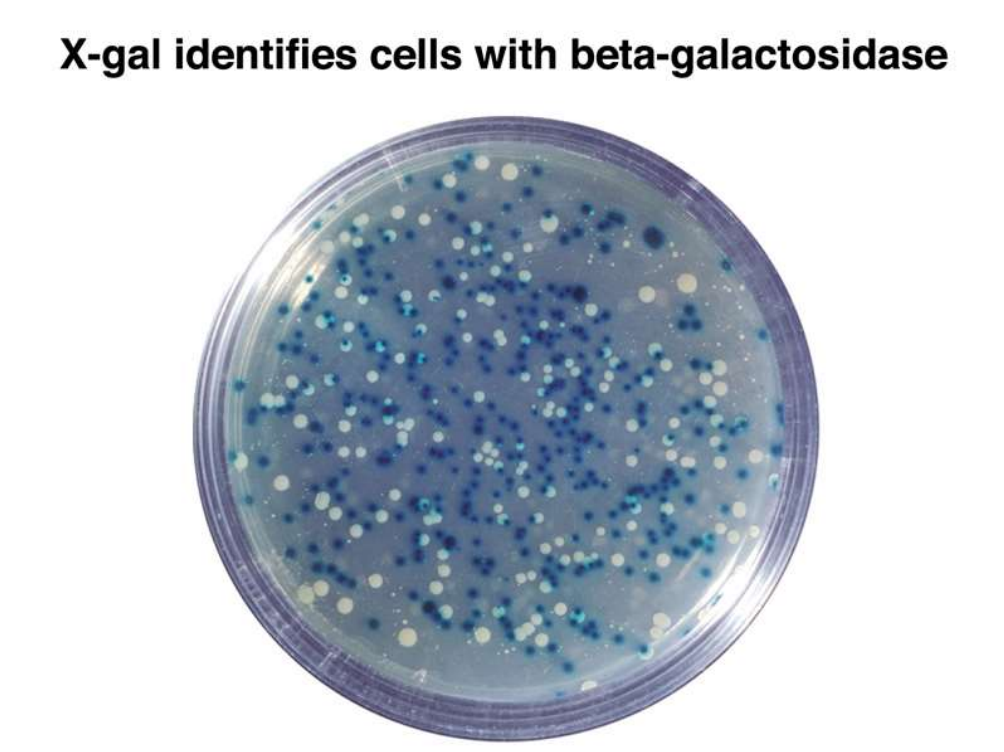
- GAL80 interacts with GAL4 to control its activity.

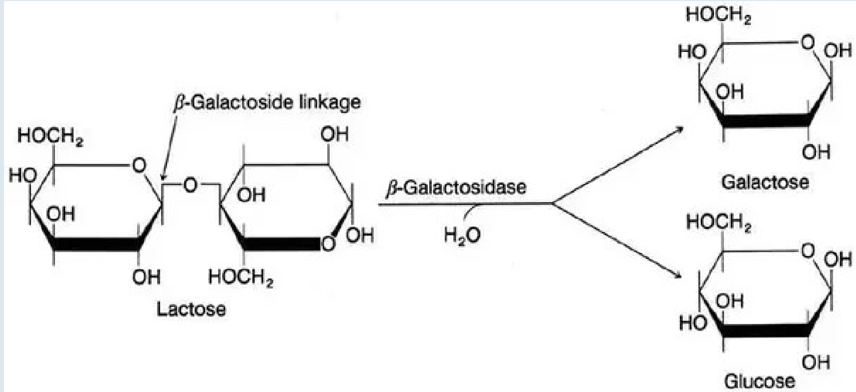
Mechanism of transcription activation of the yeast *GAL* genes.

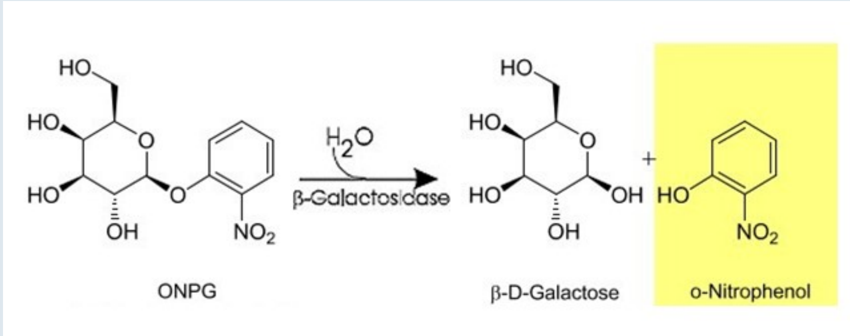


GAL1-lacZ and GAL10-lacZ fusions were used to study yeast *GAL* gene regulation also.

lacZ (β-galactosidase)) plate tests and liquid culture assays for beta-gal activity units.

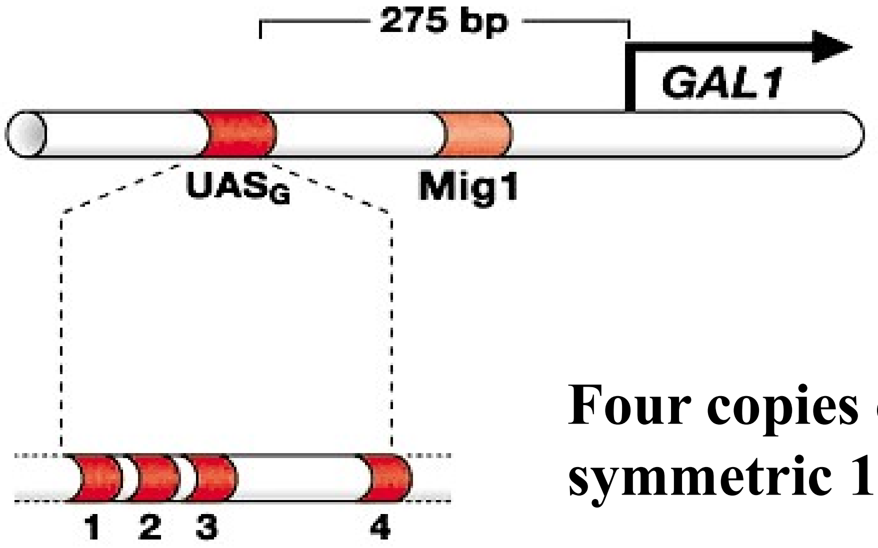






ONPG hydrolysis by cells gives a soluble blue product for spectrophotometric measurement of *lacZ* (β-galactosidase) activity units

Deletion analysis of yeast *GAL1* upstream region defined an Upstream Activating Site (UAS_G).



Four copies of a nearly symmetric 17 bp GAL4 site.

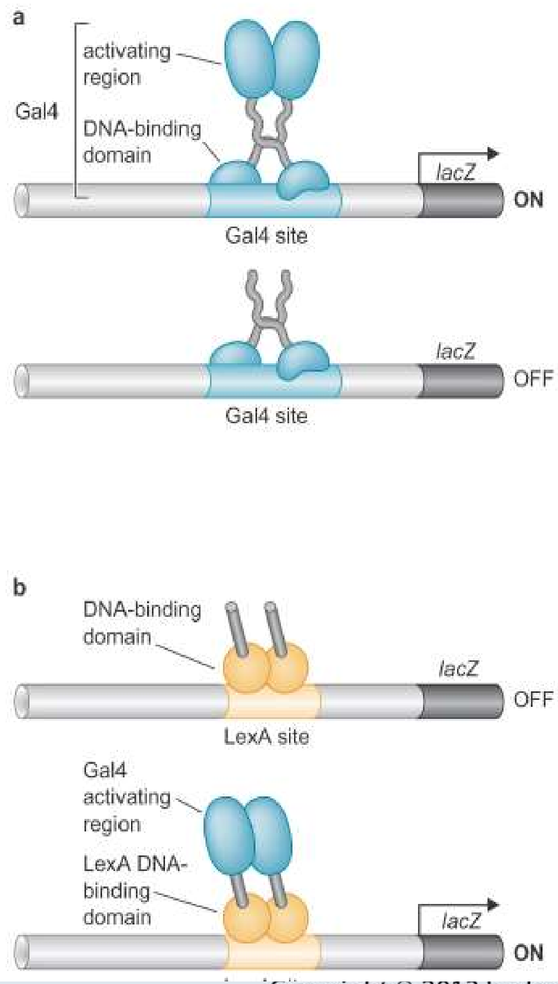
GAL1-lacZ and GAL10-lacZ fusions used for deletion analysis.

UAS_G had many properties of the Enhancer defined in SV40.

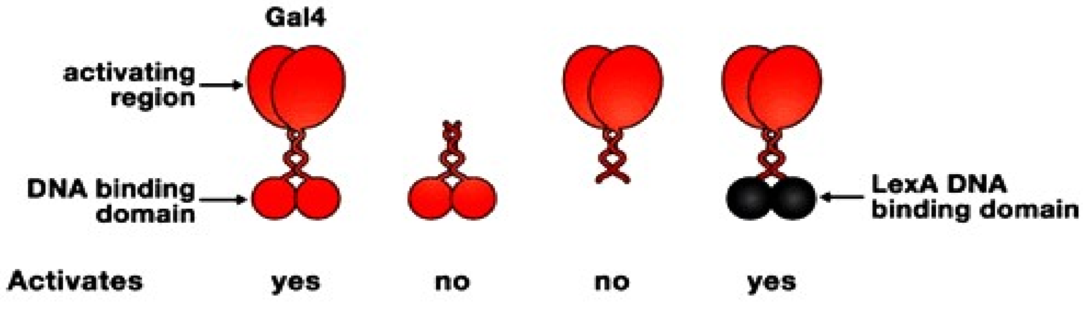
Experiments on the mechanism of transcription activation by GAL4.

Separation of transcription activation from DNA-binding function.

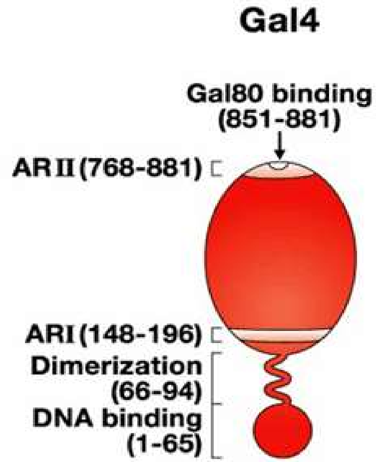
DNA-binding and transcription activation are separable functions.



DNA-binding and transcription activation are separable functions.

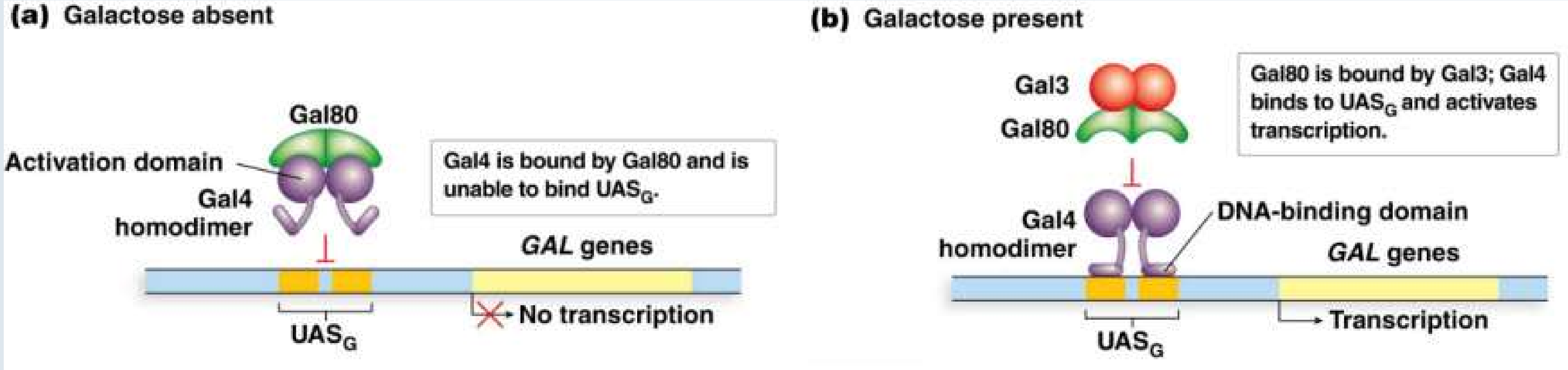


Genes and Signals, © 2002 by Cold Spring Harbor Laboratory Press, Chapter 2, Figure 5.



Genes and Signals, © 2002 by Cold Spring Harbor Laboratory Press, Chapter 2, Figure 4.

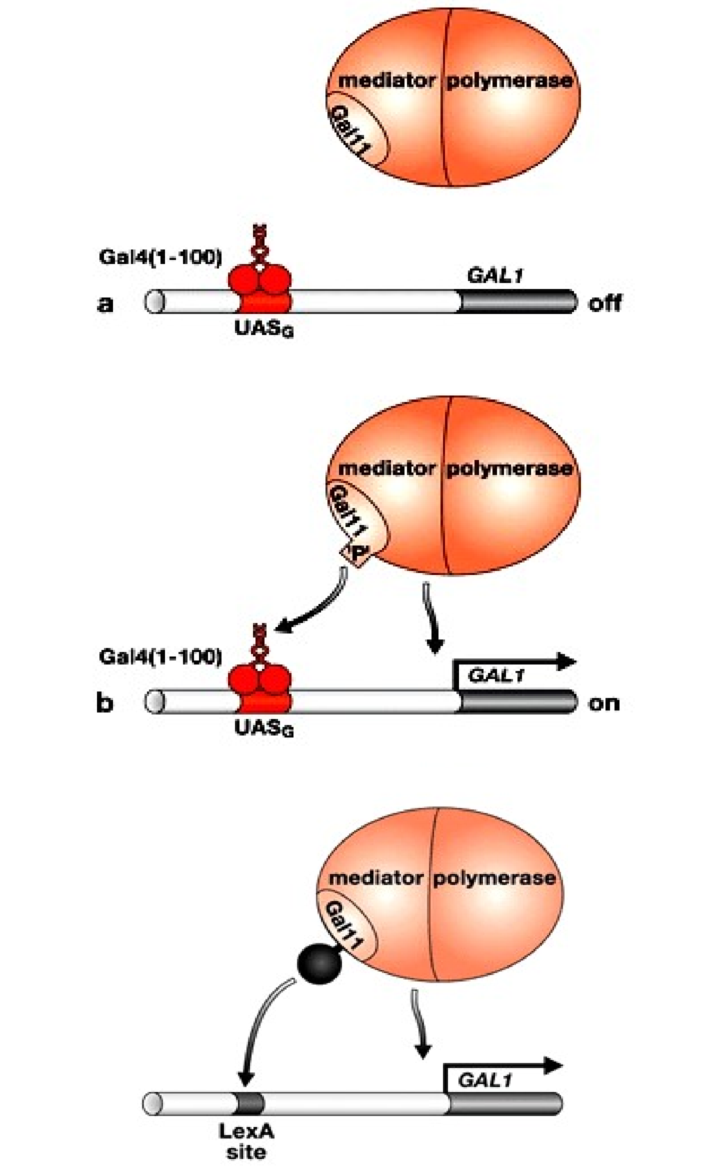
Current understanding of yeast *GAL* gene activation



- GAL1-GAL80 complex DOES bind UAS$_G$ DNA (a is wrong on this).
- GAL3 is the galactose sensor for GAL gene induction
- GAL3 is closely related to GAL1, also binds galactose and ATP
- GAL1 itself acts instead of GAL3 to bind GAL80 in *Kluveromyces lactis*, the whey (skimmed milk) yeast, which hydrolyses lactose and then converts the galactose to glucose

Eukaryotic gene activators recruit RNA polymerase to the promoter.

- Activating regions associate with mediator which in turn associates with RNA polymerase.
- Screen for yeast mutants with increased activation by GAL4(1-100) identified a potentiator mutant in GAL11, a component of Mediator.
- Gal11 when fused to LexA activates transcription. This is an activator bypass experiment.
- Results favour the idea that GAL4 recruits polymerase to the promoter by helping it bind as CAP/CRP does in *E. coli.*



Genes and Signals, © 2002 by Cold Spring Harbor Laboratory Press, Chapter 2, Figure 13.

DNA looping is the simplest explanation for GAL4 action.

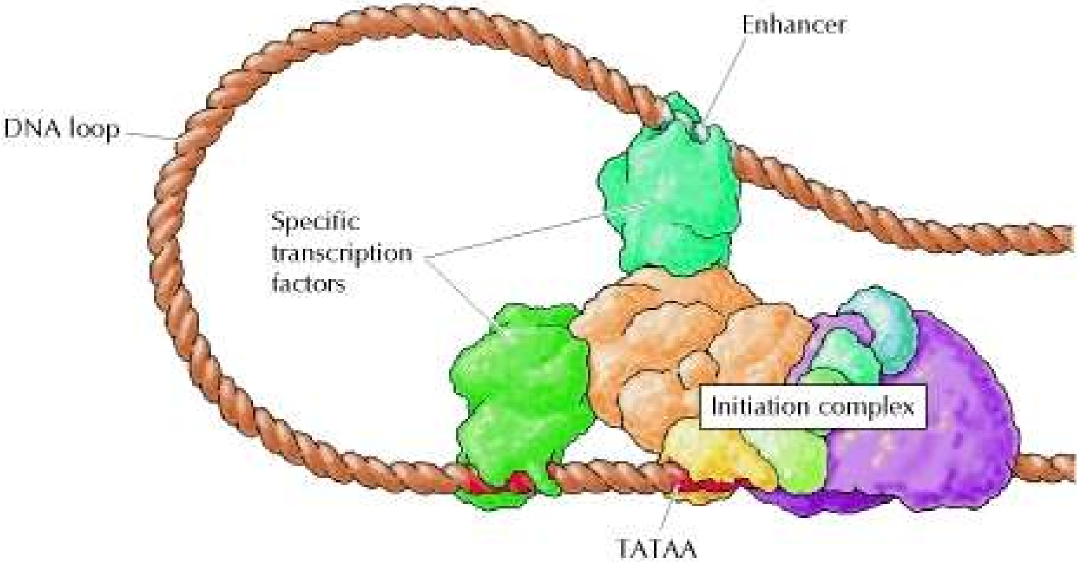
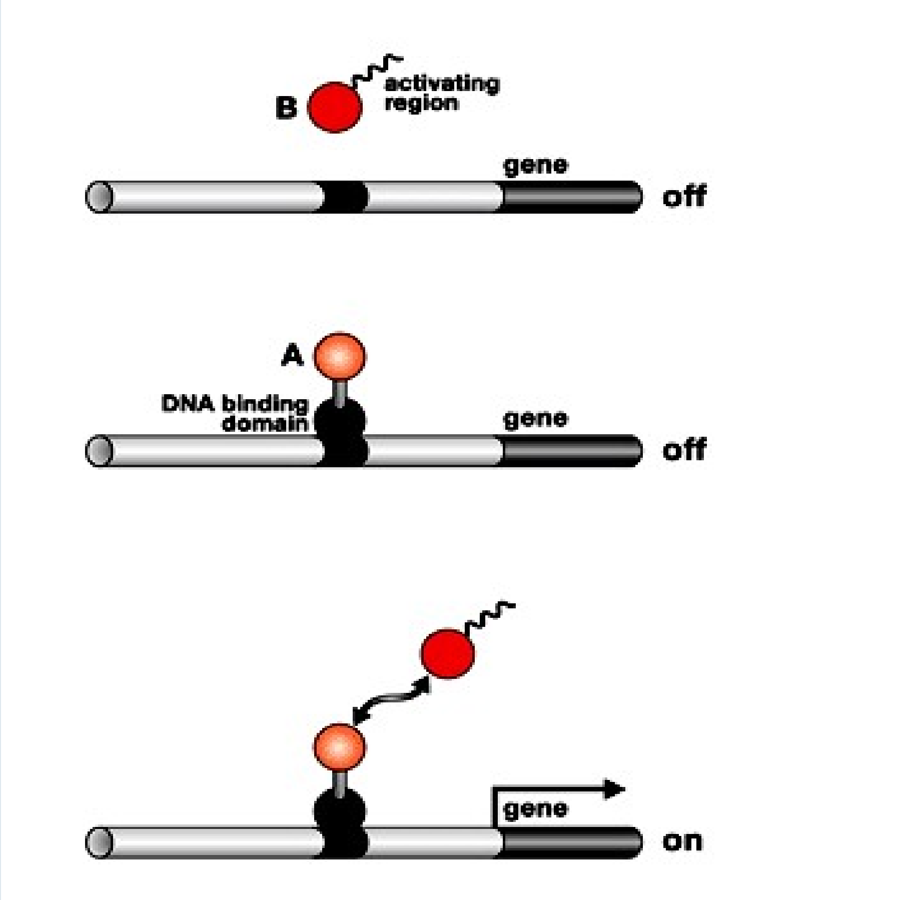


Figure 6.22 DNA looping

Transcription factors bound at distant enhancers are able to interact with general transcription factors at the promoter because the intervening DNA can form loops. There is therefore no fundamental difference between the action of transcription factors bound to DNA just upstream of the promoter and to distant enhancers.

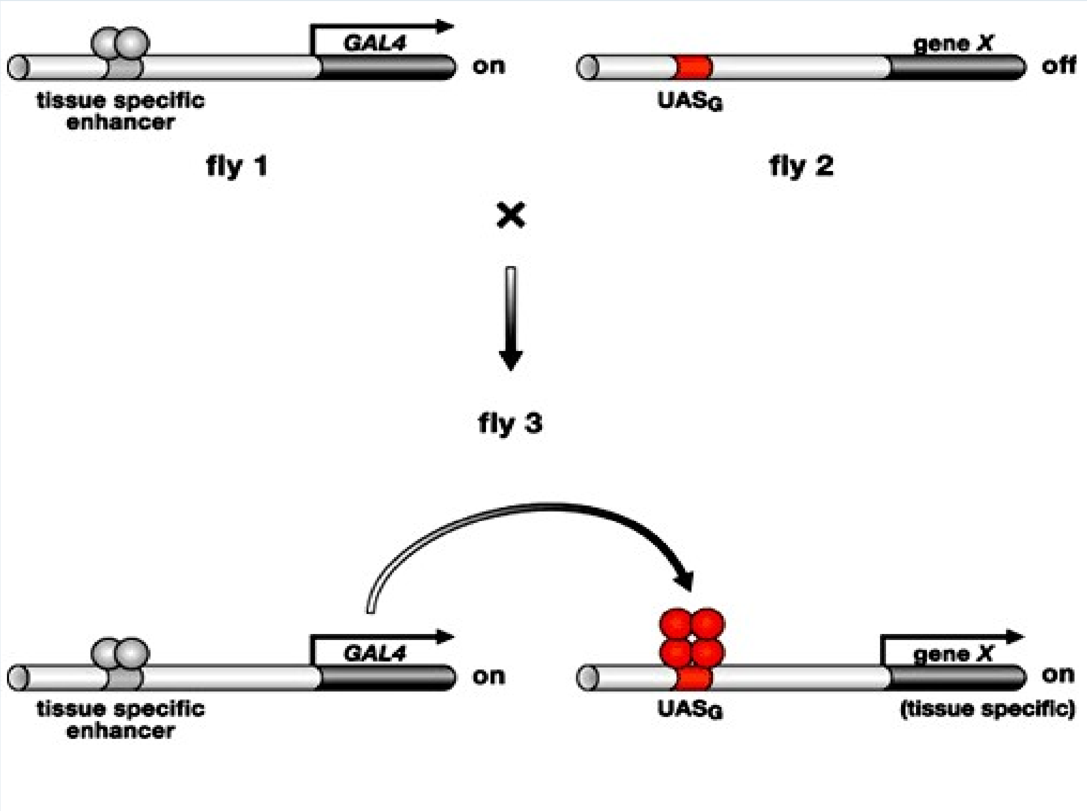
Uses of GAL4 Number 1: The yeast two hybrid system for identification of interacting proteins.

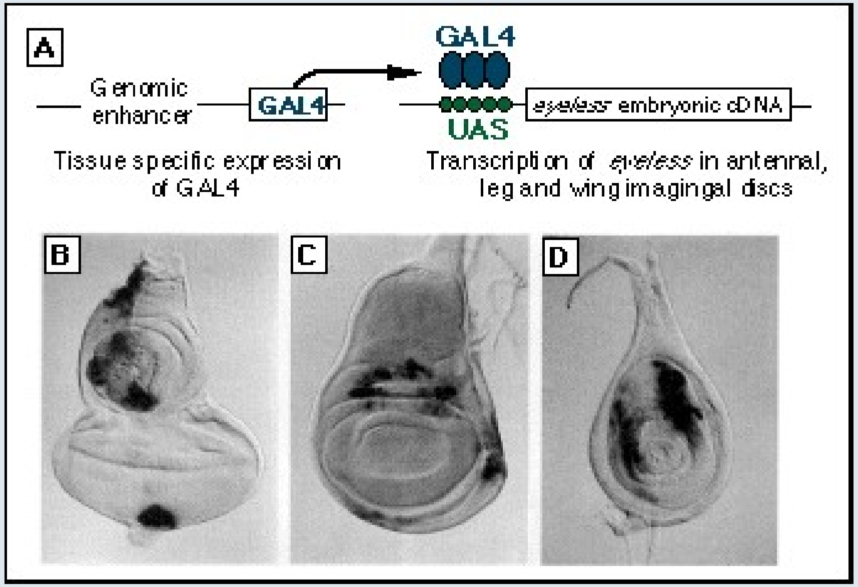


Can be used on a genomic scale to test all proteins against all others. Target protein fused to DNA-binding domain in cells of one mating type mated to a library of activating region fusion proteins.

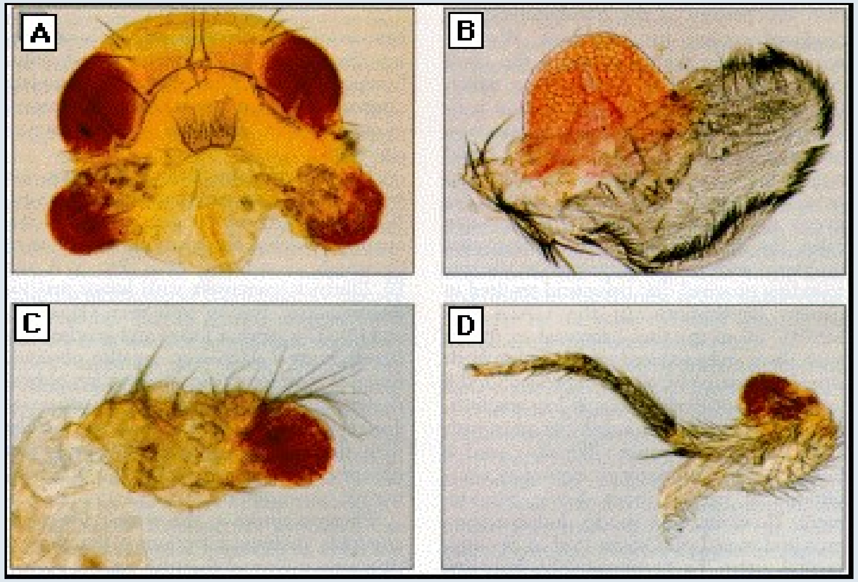
Uses of GAL4 Number 2: GAL4 activates transcription from UAS_G in *Drosophila*.

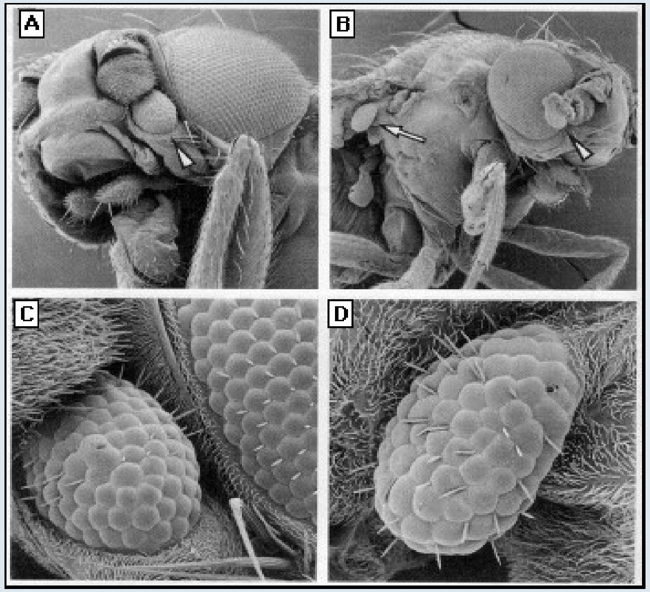
Widely used for targeted protein expression in *Drosophila* (GAL4-UAS two-component system).





Induction of ectopic eyes by GAL4-targeted expression of the *eyeless* gene in *Drosophila*





The GAL4-UAS binary system is used to map brain neurons involved in memories and behaviours and to target gene expression there.

- *rutabaga* mutant flies lack an adenlyl-cyclase required for synaptic plasticity and cannot learn a variety of training tasks.
- A UAS-RUTABAGA construct was expressed under the control of different GAL4 drivers in a *rutabaga* mutant fly to see which brain cells are needed to learn particular tasks.
- Mushroom body cells (somewhat similar to mammalian hippocampus) need RUTABAGA protein at synapses to learn to avoid particular odours and central complex cells need it for visual learning.
- *Drosophila* connectome is all the neuron types and pathways in the whole CNS