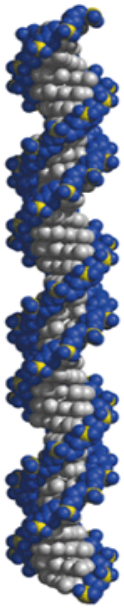
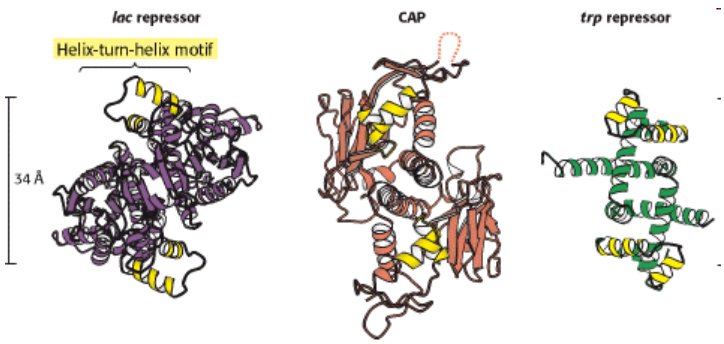
Most operator sequences are short inverted repeats

The *lac* operator

Many DNA binding proteins interact through a helix-turn-helix motif

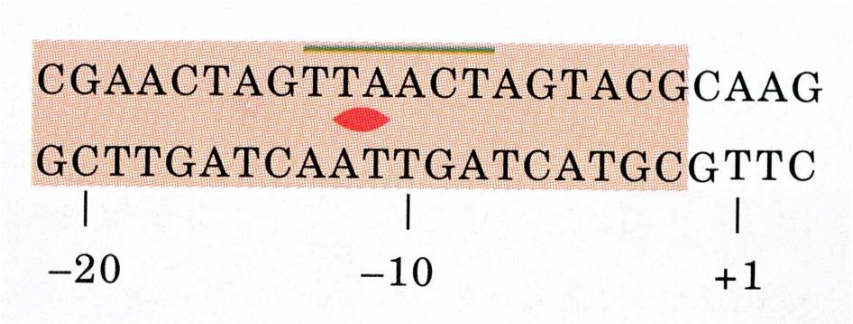




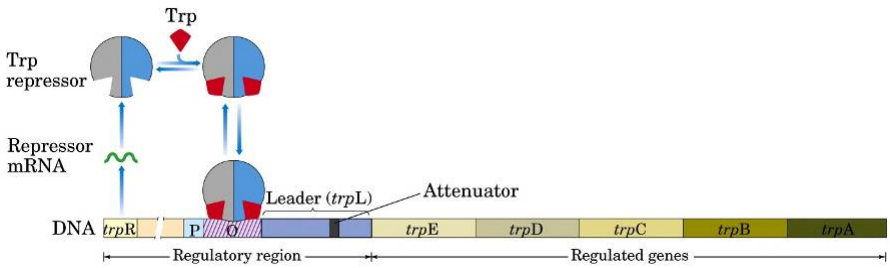
(c)

Helix-Turn-Helix Motif. These structures show three sequence-specific DNA-binding proteins that interact with DNA through a helix-turn-helix motif (highlighted in yellow).
In each case, the helix-turn-helix units within a protein dimer are approximately 3.4 nm apart, corresponding to one full turn of DNA

The *trp* operator is a palindromic DNA sequence

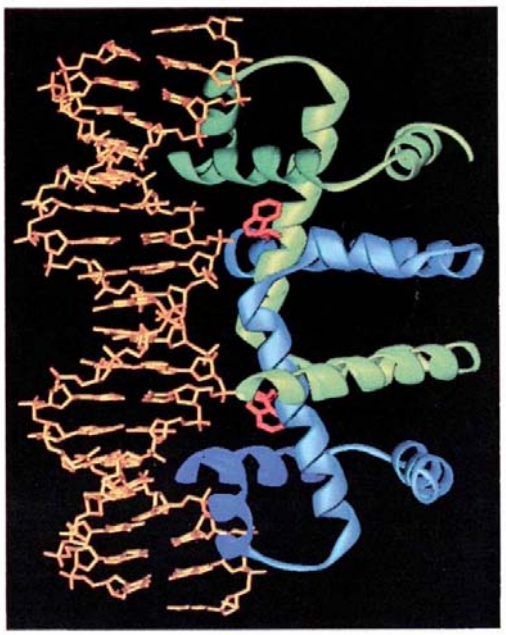


The *trp* operon is negatively regulated by the Trp repressor



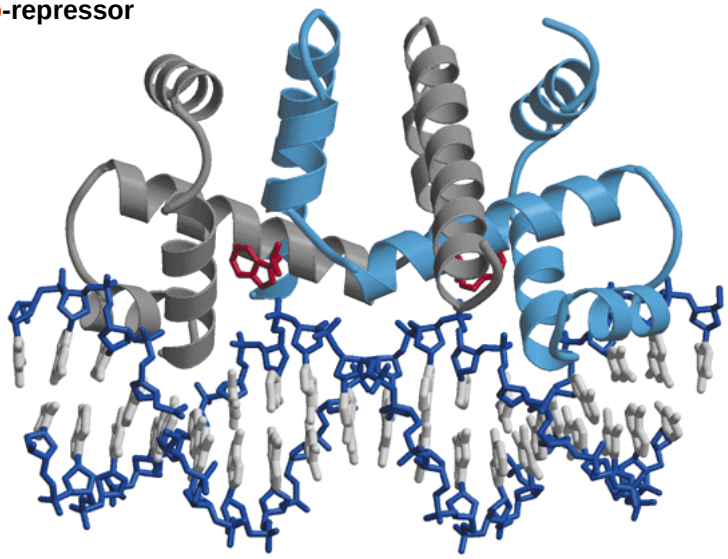
Trp repressor binds its target DNA sequence only when it itself is bound by its corepressor, tryptophan.

trp repressor dimer binds its operator in successive major grooves of the DNA

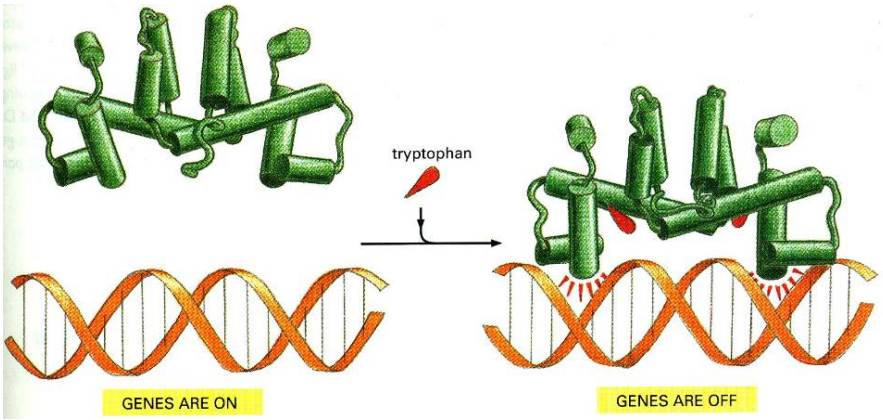


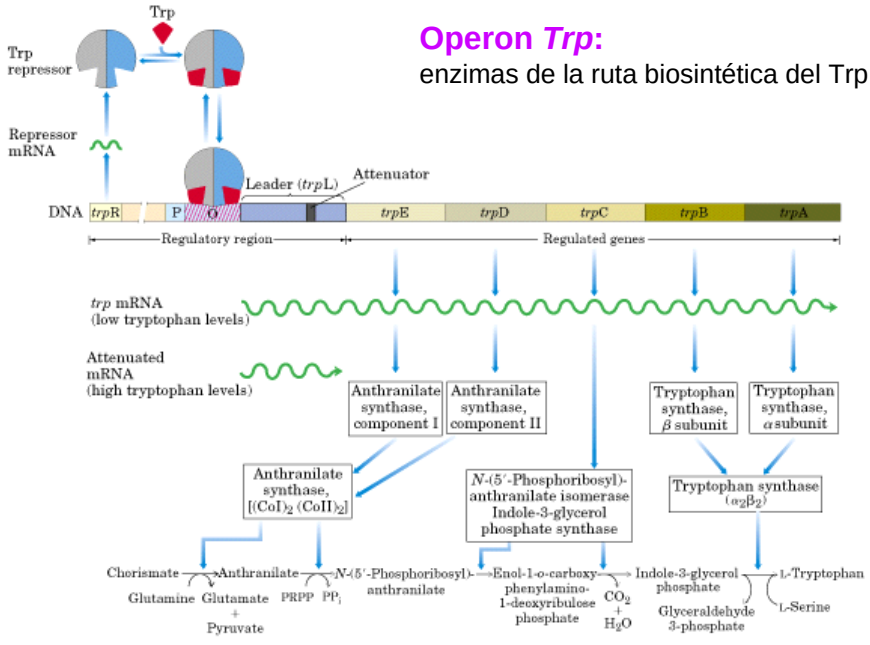
*

Trp-repressor



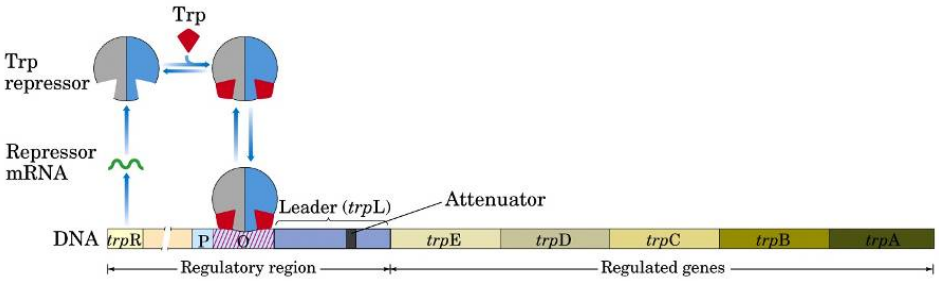
Tryptophan induces a conformational change in Trp Repressor





Operon *Trp*:
enzimas de la ruta biosintética del Trp

El operón *trp* es regulado negativamente por el represor Trp



El represor Trp reconoce el operador sólo cuando está unido a su co-represor, el triptofano.

Rho-independent termination occurs at characteristic sequences in *E. coli* DNA

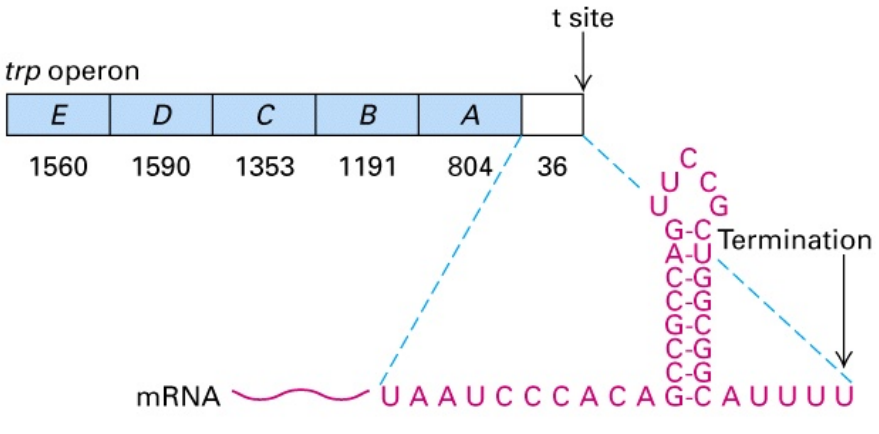


Figure 11-1

Premature termination by attenuation
helps regulate expression of some bacterial operons

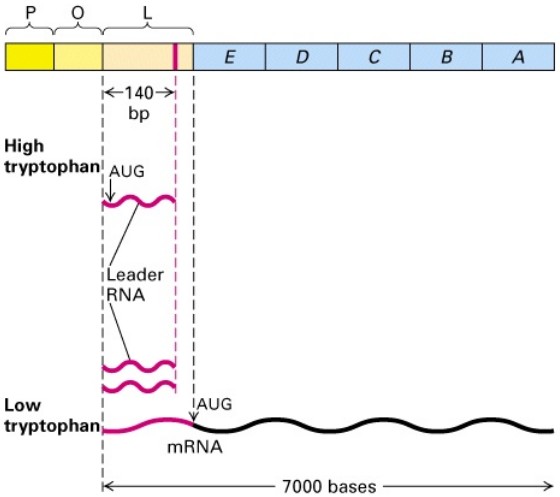
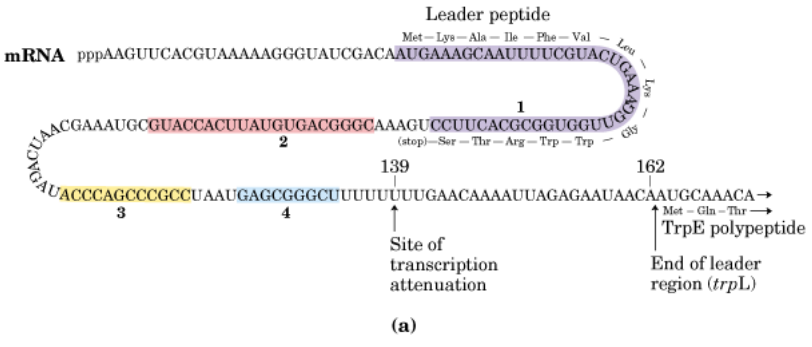
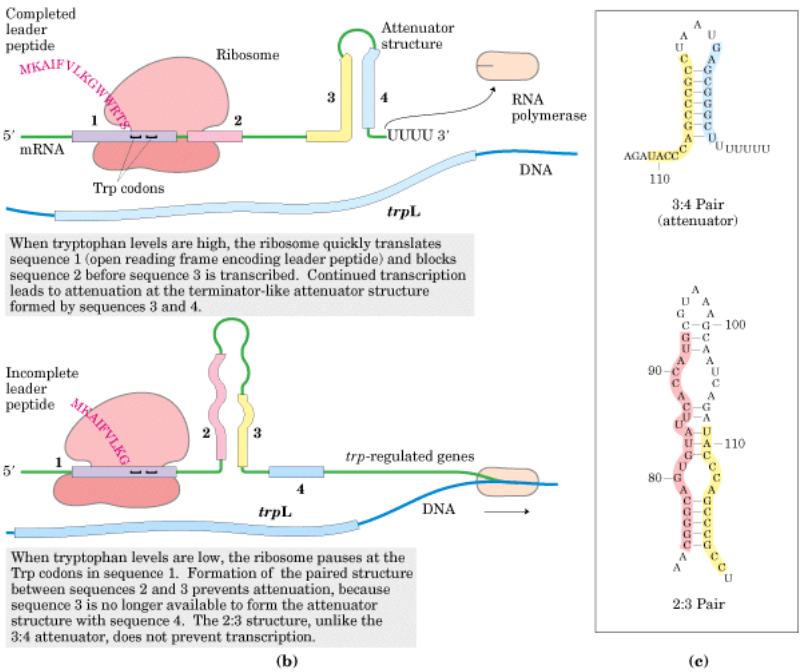


Figure 11-2





Mechanism of attenuation of *trp*-operon transcription

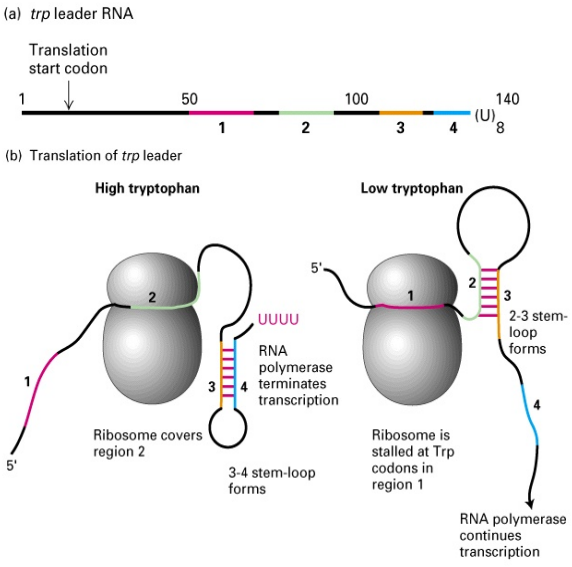
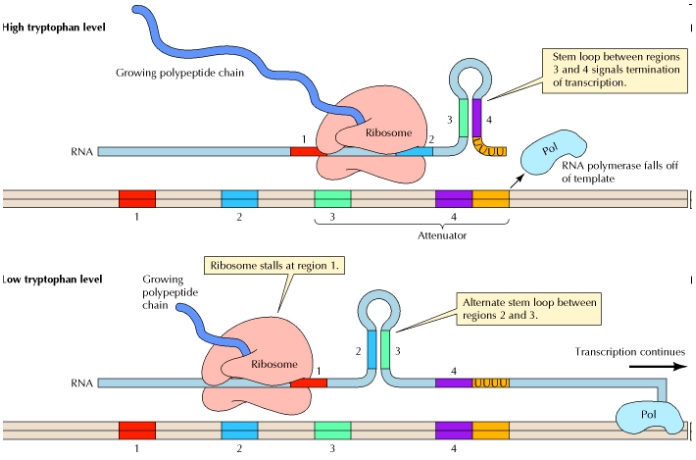


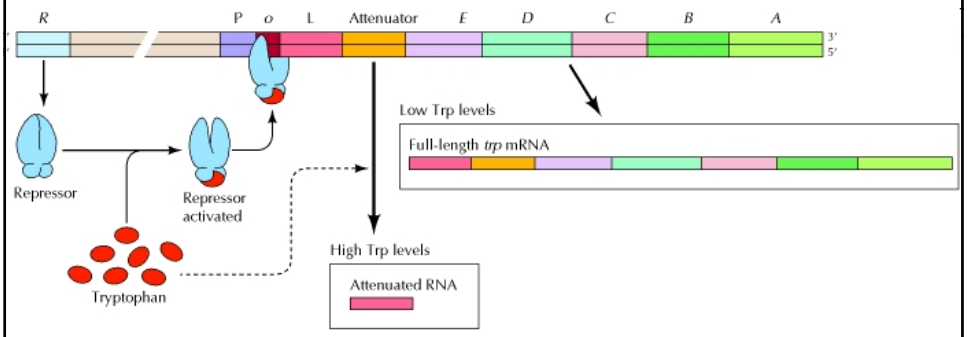
Figure 11-3

Mechanism of attenuation of *trp*-operon transcription



The *trp* mRNA is translated while still being synthesized. In the presence of high levels of tryptophan, the ribosomes proceed along the message slightly behind the site of transcription. Under these conditions, the mRNA regions designated 3 and 4 hybridize to form a stem-loop structure that signals the termination of transcription. In the presence of low levels of tryptophan, however, the ribosomes stall at region 1 of the mRNA, which contains two adjacent codons for tryptophan. In this case, since region 2 is not bound to a ribosome, it is free to form an alternative stem-loop structure by hybridizing to region 3. This hybridization prevents formation of the 3–4 stem loop, and transcription is able to continue past the attenuator sequence.

Regulation of *trp*-operon transcription



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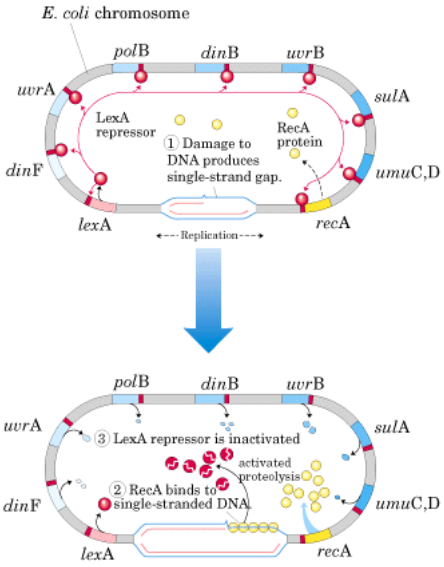
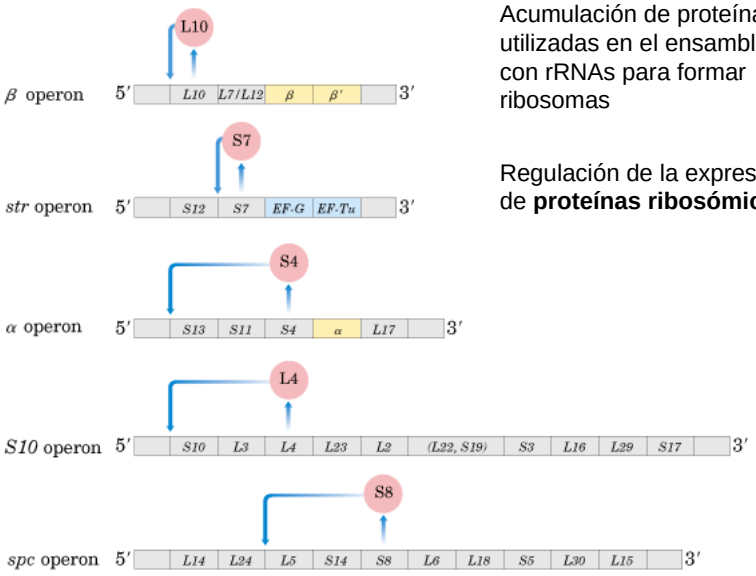
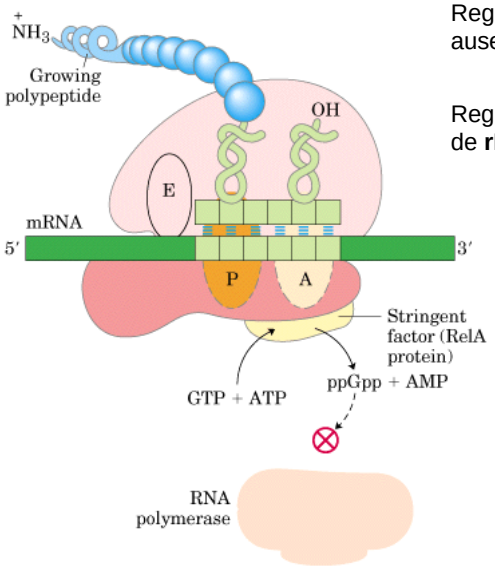




Acumulación de proteínas no utilizadas en el ensamblaje con rRNAs para formar ribosomas

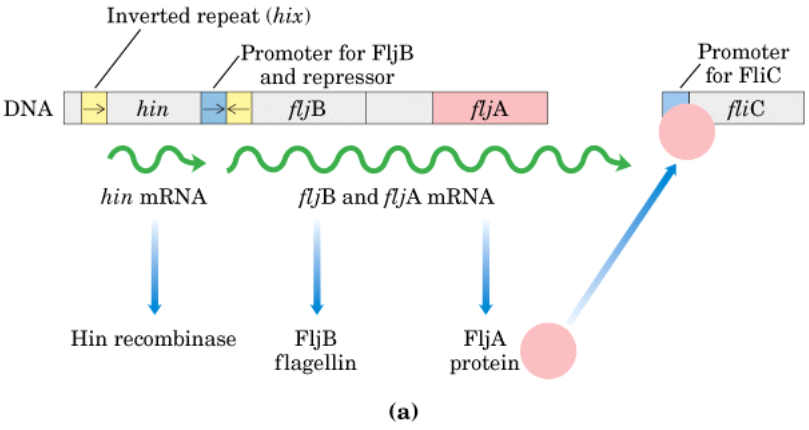
Regulación de la expresión de **proteínas ribosómicas**



Regulación general en ausencia de nutrientes

Regulación de la expresión de **rRNAs y otros**

Regulación por recombinación



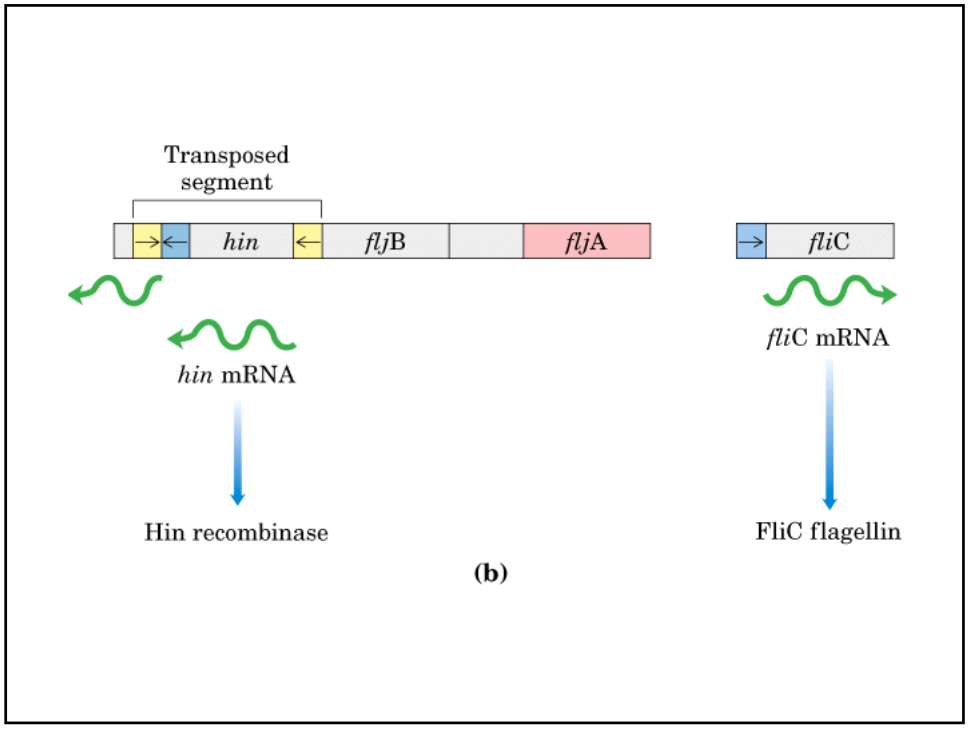


table 28–1

Examples of Gene Regulation by Recombination

System	Recombinase/ recombination site	Type of recombination	Function
Phase variation (*Salmonella*)	Hin/*hix*	Site-specific	Alternative expression of two flagellin genes allows evasion of host immune response.
Host range (bacteriophage μ)	Gin/*gix*	Site-specific	Alternative expression of two sets of tail fiber genes affects host range.
Mating type switch (yeast)	HO endonuclease, RAD52 protein, other proteins/*MAT*	Nonreciprocal gene conversion*	Alternative expression of two mating types of yeast, a and α, creates cells of different mating types that can mate and undergo meiosis.
Antigenic variation (trypanosomes)†	Varies	Nonreciprocal gene conversion*	Successive expression of different genes encoding the variable surface glycoproteins (VSGs) allows evasion of host immune response.

*Nonreciprocal gene conversion is a class of recombination events not discussed in Chapter 25. Genetic information is moved from one part of the genome (where it is silent) to another (where it is expressed) in a reaction similar to replicative transposition (see Fig. 25–41).

†Trypanosomes cause African sleeping sickness and other diseases (see Box 22–2). The outer surface of a trypanosome is made up of multiple copies of a single VSG, the major surface antigen. A cell can change surface antigens to more than 100 different forms, precluding an effective defense by the host immune system. Trypanosome infections are chronic and, if untreated, result in death.

Transcription from some promoters is initiated by alternative sigma (σ) factors

TABLE 10-1 Sigma Factors of *E. coli*

Sigma Factor	Promoters Recognized	Promoter Consensus	
		−35 Region	−10 Region
σ^{70}	Most genes	TTGACAT	TATAAT
σ^{32}	Genes induced by heat shock	TCTCNCCCTTGAA	CCCCATNTA
σ^{28}	Genes for motility and chemotaxis	CTAAA	CCGATAT
σ^{38}	Genes for stationary phase and stress response	?	?
		−24 Region	−12 Region
σ^{54}	Genes for nitrogen metabolism and other functions	CTGGNA	TTGCA

SOURCES: C. A. Gross, M. Lonetto, and R. Losick, 1992, in S. L. McKnight and K. R. Yamamoto, eds., *Transcriptional Regulation*, Cold Spring Harbor Laboratory Press; D. N. Arnosti and M. J. Chamberlin, 1989, *Proc. Nat'l. Acad. Sci. USA* **86**:830; R. Hengge-Aronsis, 1996, *Mol. Microbiol.* **21**:887.

La expresión de los genes de un bacteriofago pueden están regulados por:

- Factores sigma alternativos
- RNA polymerasas alternativas
- Anti-terminación

Regulación temporal

Los genes que se expresan en la primera etapa, son transcriptos por la RNAP de la célula huésped (con el factor sigma existente)

Estos genes incluyen algún factor sigma (u otra proteína que cambia las propiedades de la RNAP) o una RNAP codificada por el fago, que permite la transcripción de los genes de la "segunda etapa"

Bacteriophage gene expression regulated by:

- Alternative sigma factors

- The sigma subunit (sigma factor) determines the transcriptional specificity of prokaryotic RNA polymerases. Virally-coded sigma factors often alter the specificity of host RNA polymerase during bacteriophage infections. Alternative sigma factors are also used to alter transcriptional specificity during bacterial sporulation and in other phenomena, such as the bacterial heat-shock response, that we do not have time to explore in this course.
- **Bacteriophage SPO1 infection of *Bacillus subtilis*:** Transcription of the SP01 viral genome can be divided into three time periods, early, middle, and late, based on how much time elapses after infection before the genes in question begin to be transcribed. Early transcription uses host cell sigma factor. A gene coding for an alternative sigma factor, designated gp28, is transcribed during the early period. As it accumulates, gp28 replaces host sigma factor, causing transcription of host genes to stop and transcription of "middle" genes to begin. Middle transcription includes mRNAs for gp33 and gp34. A complex of gp33 + gp34 replaces gp28 as the transcription specificity factor, causing a shift to transcription of late viral genes.
- **Bacterial sporulation:** *Bacillus subtilis* can form highly resistant spores in response to adverse conditions. The sigma factor that is employed during vegetative growth has a molecular weight of 43 kilodaltons. During sporulation, three new sigma factors are made with molecular weights of 29, 30, and 32 kDa. Each of these sigma factors recognizes a set of promoters with different -10 and -35 sequences. As a result of these changes, a very different set of genes is expressed during sporulation.

- Alternative RNA polymerases
- Anti-termination

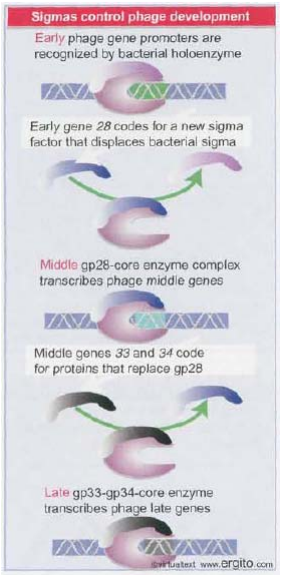
Sigma factors may be organized into cascades

• A cascade of sigma factors is created when one sigma factor is required to transcribe the gene coding for the next sigma factor.

• The early genes of phage SPO1 are transcribed by host RNA polymerase.

• One of the early genes codes for a sigma factor that causes RNA polymerase to transcribe the middle genes.

• Two of the middle genes code for subunits of a sigma factor that causes RNA polymerase to transcribe the late genes.



Bacteriophage gene expression regulated by:

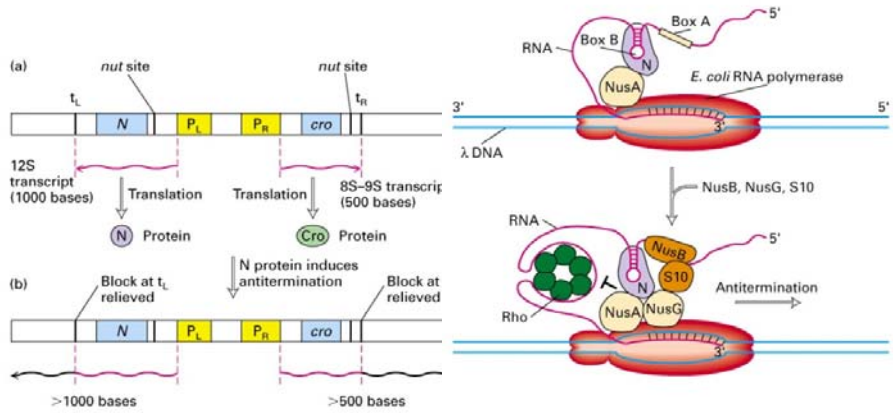
- Alternative sigma factors
- Alternative RNA polymerases
- **Viral-specific RNA polymerase:** Some types of viruses employ a newly synthesized virally-coded RNA polymerase for the transcription of viral genes. As an example, after ***bacteriophage T7*** infects *E. coli*, there is a temporal shift in transcription from early (Class I) genes to later (Class II and III) genes. The Class I genes are transcribed with host RNA polymerase. One of the early gene products is a new RNA polymerase that is highly specific for transcription of Class II and III genes of bacteriophage T7. One of the Class II gene products inactivates the host RNA polymerase, thus completing the switch from host-specific to T7-specific transcription.
- Anti-termination

Bacteriophage gene expression regulated by:

- Alternative sigma factors
- Alternative RNA polymerases
- Anti-termination

- **Antitermination:** The bacteriophage lambda genome can be divided into four operons, designated left, right, late (an extension of right), and repressor. During the initial stages of infection, the early genes in the left and right operons are transcribed with host polymerase for only a short distance before a rho-dependent termination occurs in each operon. One of the early products from the left operon is an ***antiterminator protein*** coded by the ***N gene***. As it accumulates, it blocks the terminations and allows transcription to spread further into the left and right operons (interaction of N with *nut* hairpin-loop structure in the nascent mRNA and host proteins: formation of a more processive complex with RNA polymerase).
- Transcription of the right operon terminates again, somewhat downstream from a gene designated Q. In cells that enter a lytic cycle, the ***Q gene product*** accumulates and functions as another ***antiterminator***, allowing the late operon (actually an extension of the right operon) to transcribe genes needed for completion of the lytic cycle, including those for formation of the phage head and tail. Thus, a series of temporal delays are achieved by forcing further transcription to wait until protein products of earlier transcription events accumulate to levels that are adequate to achieve antitermination. Also, as described below, the second antitermination event fails to occur in lysogeny.

Regulation of the transcripts from the bacteriophage lambda



Antitermination: Default is termination, the factors that bind to the emerging transcript block termination. After shortly after infection, lambda transcribes from the two divergent promoters and transcribes Cro and N. The transcripts terminate at terminators (tL and tR) normally.

The function of the N protein is to inactivate RNA polymerase termination at these sites, allowing for the production of longer transcripts, and hence more genes.

N- binds to the transcript at the nut site (N-Utilization) that lies between the terminator and the promoter. Nut site= Box A and Box B.

Termination is prevented by recruiting elongation factors (NusA,B,G S10).The mechanism is unknown, but it can block both rho-dependent and rho independent termination.

Hypothesis: Terminator sequences and the RNA that is produced inhibits RNA polymerase from passing through the site. Terminator sequences strongly favor termination, but do not assure it. Polymerase can make it through a termination site, but infrequently.

a. Recruiting factors that stimulate elongation may increase the frequency in which polymerase transcribes through the site. By increasing elongation rates, the polymerase may be less sensitive to the effects of the binding of the terminator stem loop to the polymerase ?

b. The polymerase stalls, but the large multisubunit complex that forms prevents the RNA from leaving the active site (being pulled out) allowing more time for elongation to occur?

Or both?