Major Shifts in Bacterial Transcription

- Bacteria control the transcription of a very limited number of genes at a time through the use of operons
- More radical shifts in gene expression require more fundamental changes in the transcription machinery
- Three major mechanisms:
  - σ-factor switching
  - RNA polymerase switching
  - antitermination

8.1 Sigma Factor Switching

- Phage infection of bacterium subverts host transcription machinery
- In process, establishes a time-dependent, or temporal, program of transcription
  - First early phage genes are transcribed
  - This is followed by the later genes
  - Late in the infectious cycle there is no longer transcription of the host genes, only phage genes
- Change in the genes that are transcribed is caused by a change in transcription machinery, in RNA polymerase itself

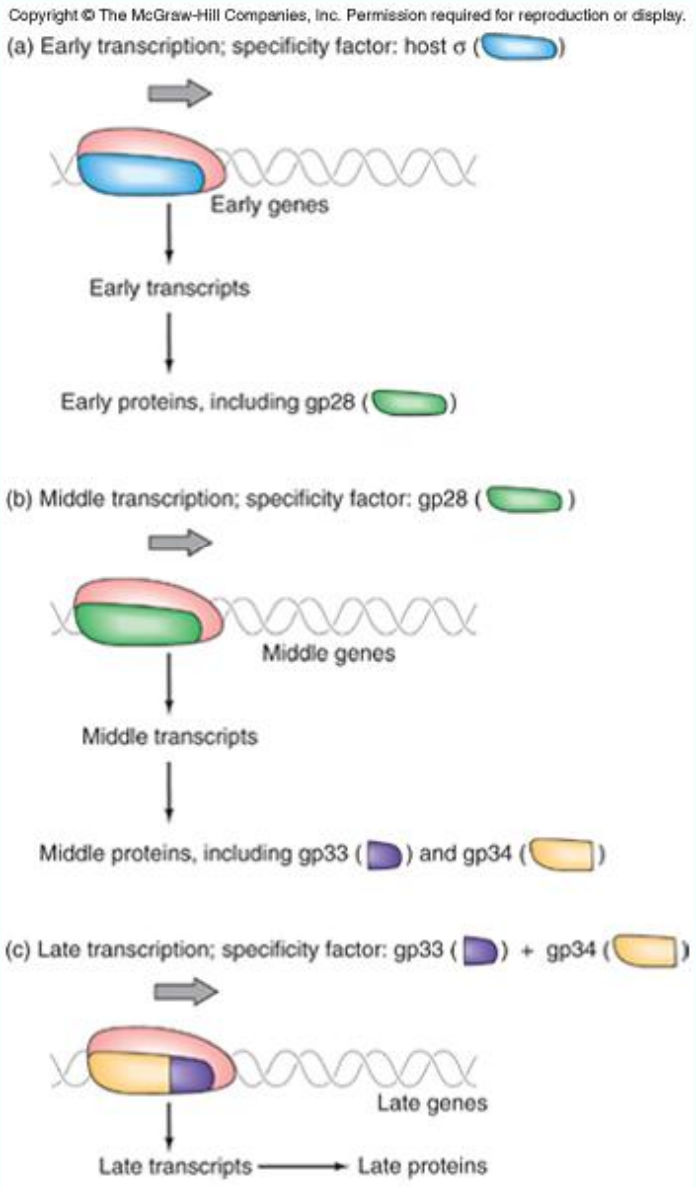
Phage Infection

- σ is the key factor in determining specificity of T4 DNA transcription
- To shift the transcription process σ is a likely candidate
- Study of the process done in *B. subtilis* and its phage, SPO1
- Like T4, SPO1 has a large genome
- SPO1 has a temporal program of transcription

Temporal Control of Transcription in SPO1

- Temporal transcription program:
  - First 5 minutes: expression of early genes
  - After 5 – 10 minutes: expression of middle genes
  - After 10 minutes to end: late genes expressed

(The Phage does not carry its own RNA polymerase)



Bacillus subtilis RNA polymerase

The *B. subtilis holoenzyme closely* resembles the *E. coli enzyme. Its core consists of two* large (β and β'), two small (α), and one very small (ω) polypeptides; its primary σ-factor has a molecular mass of 43,000 kD, somewhat smaller than *E. coli's primary* σ (70,000 kD).

In addition, the polymerase includes a δ-subunit with a molecular mass of about 20,000 kD. This subunit helps to prevent binding to non-promoter regions, a function performed by the *E. coli* σ-*factor but* not by the smaller *B. subtilis* σ *-factor.*

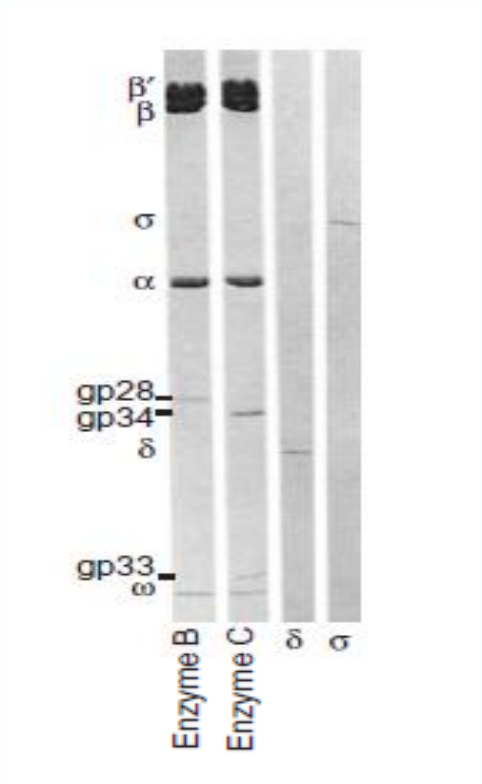


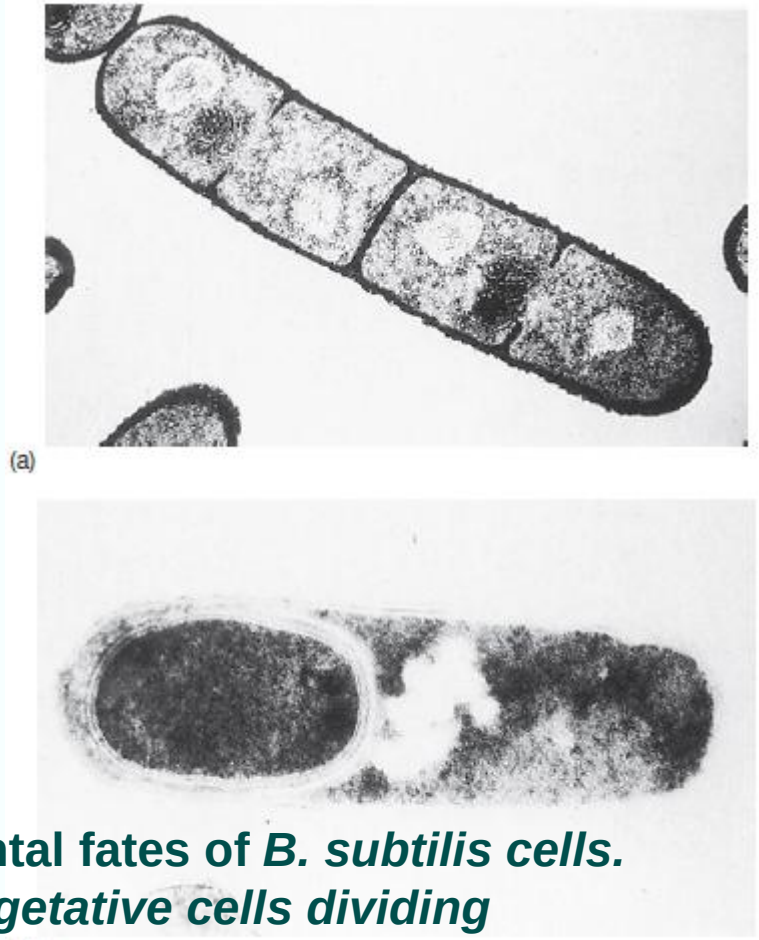
Figure 8.2 Subunit compositions of RNA polymerases in SPO1 phage-infected *B. subtilis cells. Polymerases were separated by* chromatography and subjected to SDS-PAGE to display their subunits plus. Enzyme B (fi rst lane) contains the core subunits (β', β, α, and ω), as well as gp28. Enzyme C (second lane) contains the core subunits plus gp34 and gp33. The last two lanes contain separated δ- and σ-subunits, respectively.

Transcription Switching

- This switching is directed by a set of phage-encoded σ factors that associate with the host core RNA polymerase
- These σ factors change the host polymerase specificity of promoter recognition from early to middle to late
  - The host σ factor is specific for the phage early genes
  - Phage gp28 protein switches the specificity to the middle genes
  - Phage gp33 and gp34 proteins switch the specificity to late genes

Sporulation

- During infection, phage SPO1 changes specificity of host RNA polymerase
- Same type of mechanism applies to changes in gene expression during sporulation
- Bacteria can exist indefinitely in vegetative state if nutrients are available
- Under starvation conditions, *B. subtilis* forms endospores - tough, dormant bodies that can survive for years until favorable conditions return



Two developmental fates of *B. subtilis cells.*
(a) B. subtilis vegetative cells dividing
(b) sporulation, with an endospore developing at the left end, and the mother cell at the right and surrounding the endospore.

Sporulation

- During sporulation, a whole new set of genes is turned on, and many vegetative genes are turned off
- The switch occurs largely at the level of transcription
- Several new σ-factors displace the vegetative σ-factor from the polymerase core and direct the transcription of sporulation genes
- Each σ-factor has its own preferred promoter sequence

More than one new σ-factor is involved in sporulation. In fact, several participate: σ^F, σ^E, σ^H, σ^C, and σ^K each play a role, in addition to the vegetative σ^A.
Each σ-factor recognizes a different class of promoter.

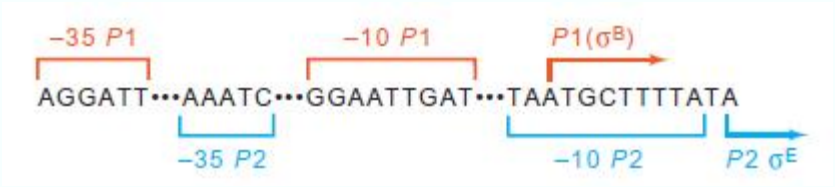
For example, the vegetative σA recognizes promoters that are very similar to the promoters recognized by the major *E. coli* σ-factor, with a -10 box that looks something like TATAAT and a -35 box having the consensus sequence TTGACA.

By contrast, the sporulation-specific factors recognize quite different sequences. The σ^F-factor appears first in the sporulation process, in the forespore. It activates transcription of about 16 genes, including the genes that encode the other sporulation-specific σ-factors. In particular, it activates
spoIIR, which in turn activates the gene encoding σE in the mother cell.

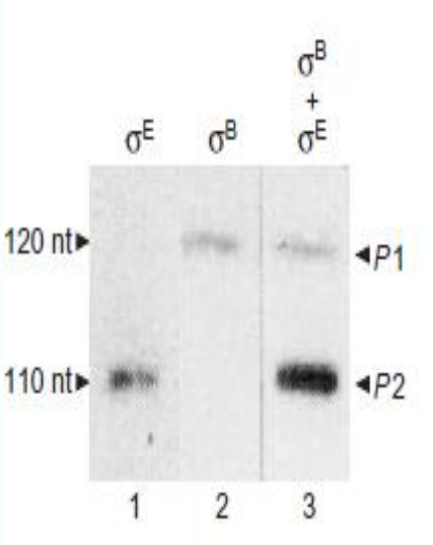
Together, σ^F and σ^E put the forespore and mother cell, respectively, on an irreversible path to sporulation.

Genes With Multiple Promoters

- Some sporulation genes must be expressed during 2 or more phases of sporulation when different σ-factors predominate
- Genes transcribed under different conditions are equipped with two different promoters
  - Each promoter is recognized by one of two different σ-factors
  - This ensures their expression no matter which factor is present
  - Allows for differential control under different conditions



Overlapping promoters in *B. subtilis spoVG. P1* denotes the upstream promoter, recognized by σ^B; the start of transcription and -10 and -35 boxes for this promoter are indicated in red above the sequence. ***P2*** *denotes the downstream promoter,* recognized by σ^E, the start of transcription and -10 and -35 boxes for this promoter are indicated in blue below the sequence.



Specificities of σ^B and σ^E. Losick and colleagues purifi ed the s-factors σ^B and σ^E by gel electrophoresis and tested them with core polymerase using a run-off transcription assay. Lane 1, containing σ^E, caused initiation selectively at the downstream promoter (*P2). Lane 2, containing* σ^B*, caused initiation selectively at* the upstream promoter (*P1). Lane 3, containing both σ-factors, caused* initiation at both promoters.

Bacterial Heat Shock

- The heat shock response is a defense by cells to minimize damage in response to increased temperatures
- Molecular chaperones are proteins that bind to proteins partially unfolded by heating and help them to fold properly again
- Genes encoding proteins that help cells survive heat are called heat shock genes

Almost immediately after *E. coli cells are heated from* their normal growth temperature (37ºC) to a higher temperature (42ºC), normal transcription ceases, or at least decreases, and the synthesis of 17 new, heat shock transcripts begins.
These transcripts encode the **molecular chaperones and proteases** that help the cell survive heat shock.
This shift in transcription requires the product of the *rpoH gene, which* encodes a σ-factor with a molecular mass of 32 kD. Hence this factor is called σ^{32}, but it is also known as σ^{H}, where the H stands for heat shock.

Other σ-Switches

- In *E.coli* the heat shock response is controlled by an alternative σ-factor, σ^{32} or σ^{H} (the H stands for heat shock)
  - Directs RNA polymerase to the heat shock gene promoters
  - Accumulation of σ^{H} with high temperature is due to:
    - Stabilization of σ^{H}
    - Enhanced translation of the mRNA encoding σ^{H}

- **Responses to low nitrogen and starvation stress also depend on genes recognized by other σ-factors**

During nitrogen starvation, another σ-factor (σ^{54}, or σ^{N}) directs transcription of genes that encode proteins responsible for nitrogen metabolism. In addition, although gram-negative bacteria such as *E. coli do not sporulate,* they do become relatively resistant to stresses, such as extreme pH or starvation.
The genes that confer stress resistance are switched on in stationary phase (non-proliferating) *E. coli cells by an RNA polymerase bearing the* alternative σ-factor, σ^{S} or σ^{38}.

These are all examples of a fundamental coping mechanism: Bacteria tend to deal with changes in their environment with global changes in transcription mediated by shifts in σ-factors.

Anti-σ Factors

- These proteins do not compete with σ factor for binding to a core polymerase, they bind directly to σ and inhibit its function
- One example is the product of the *E.coli* ***rsd*** **gene** *(regulator of sigma D)* that regulates the activity of the major vegetative σ, σ^{70} (σ^{D}), the product of the *rpoD* gene (regulator of sigma D)

As long as *E. coli cells are growing rapidly, most genes* are transcribed by E σ^{70}, and no *rsd product,* ***Rsd, is made.***
However, when cells are stressed by
such insults as loss of nutrients, high osmolarity, or high temperature, they stop growing and enter the stationary phase. At this point, a new set of stress genes is activated by the new s-factor, σ^{S}, which accounts for about one third of the total amount of RNA polymerase in the cell.

This means that about two-thirds of the σ present in the cell is still σ^{70}; nevertheless, expression of genes transcribed by Eσ^{70} has fallen by over 10-fold. These observations suggest that something else besides relative availability of σ-factors is influencing gene expression, and that extra factor appears to be Rsd, which is made as cells enter stationary phase, then binds to σ^{70} and prevents its association with the core polymerase.

anti anti-σ factors

- Some of these anti-σ factors are even controlled by anti anti-σ factors that bind to the complexes between a σ and and anti-σ factor and release the anti-σ factor

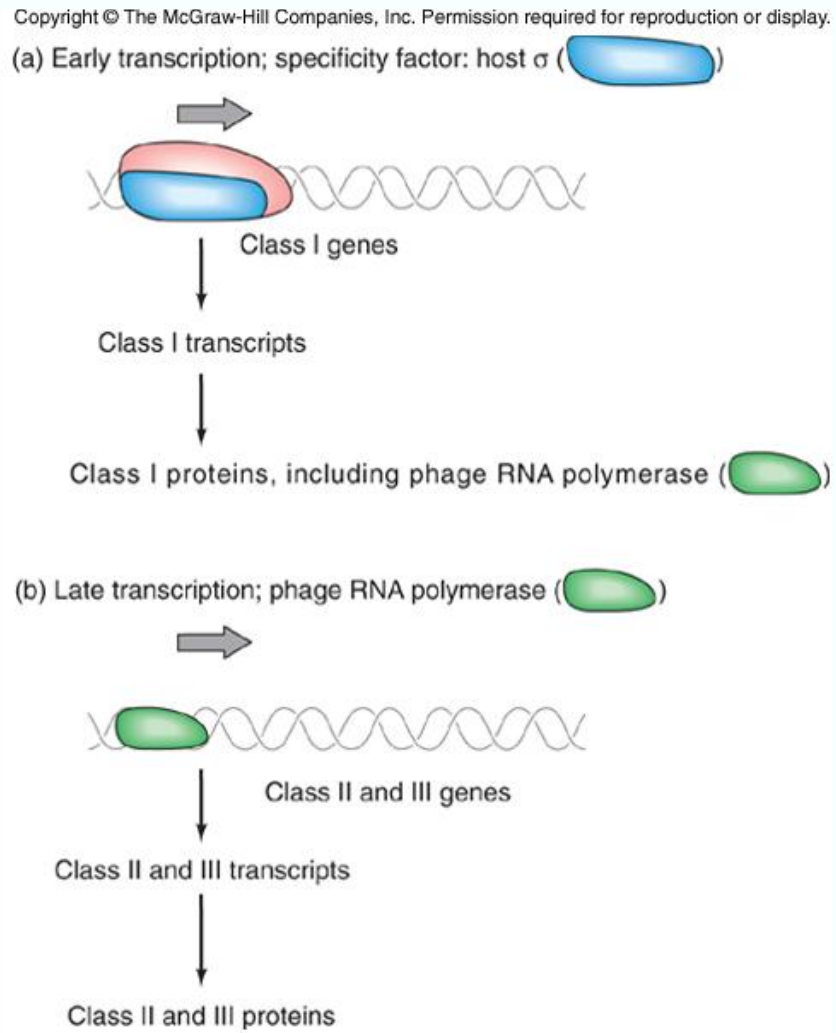
In sporulating *B. subtilis, for example, the antiσ*-factor SpoIIAB binds to and inhibits the activities of two *σ*-factors required at the onset of sporulation, σ^F and σ^G.
But another protein, SpoIIAA, binds to complexes of SpoIIAB plus σ^F or σ^G and releases the *σ*-factors, thus counteracting the effect of the anti-*σ*-factor.
Amazingly enough, the anti-*σ*-factor SpoIIAB can also act as an **anti-anti-anti-** ***σ*** **factor** by phosphorylating and inactivating SpoIIAA.

8.2 The RNA Polymerase Encoded in Phage T7

- Phage like T7 has a small genome and many fewer genes than SPO1
- These phage have 3 phases of transcription: classes I, II, and III
- Of the 5 class I genes, gene 1 is necessary for class II and class III gene expression
  - If gene 1 is mutated, only class 1 genes are transcribed
  - Gene 1 codes for a phage-specific RNA polymerase that transcribes the T7 phage class Ii and III genes specifically

Temporal Control of Transcription

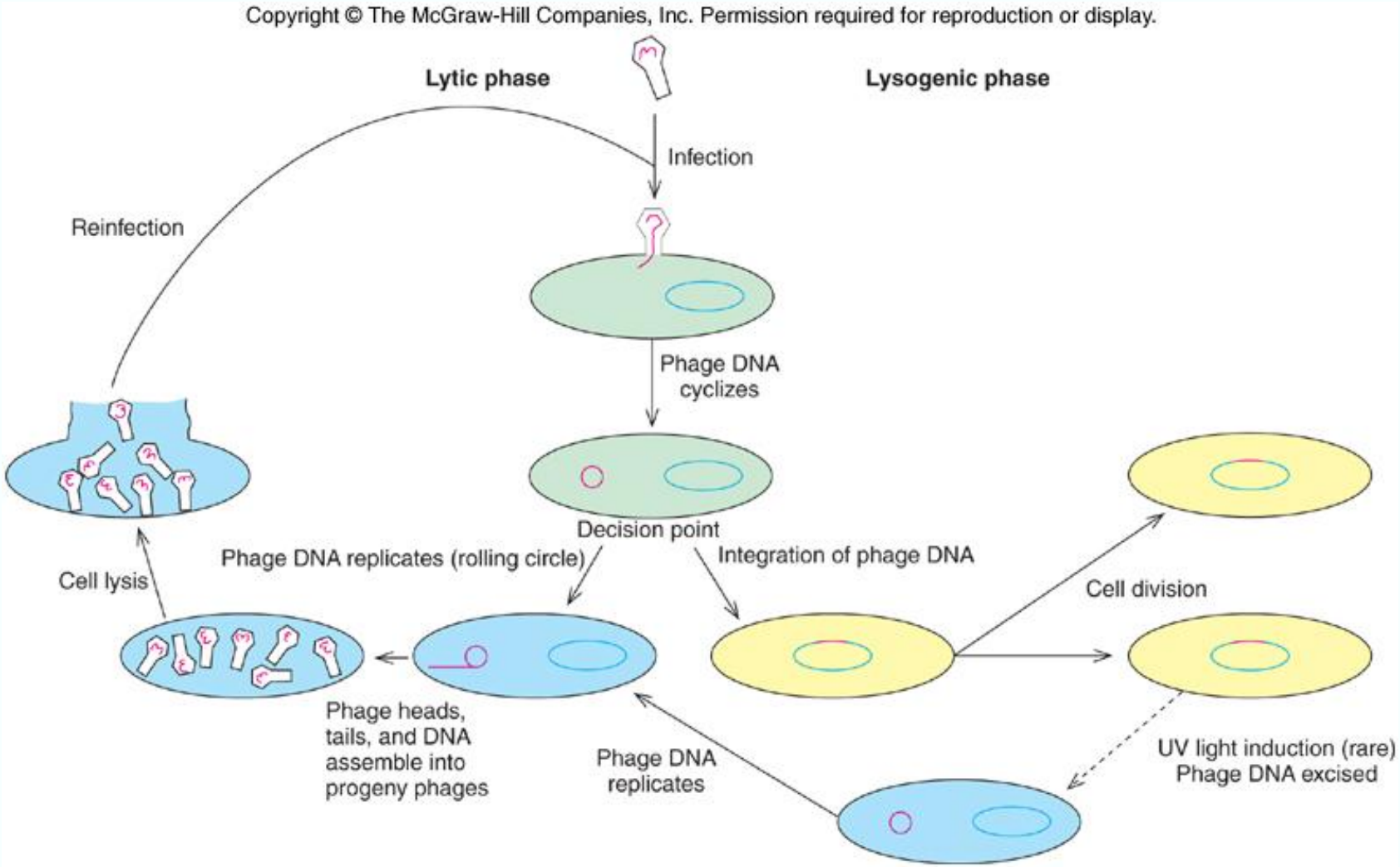
- Host polymerase transcribes the class I genes
- Gene 1 of class I genes is the phage polymerase
- The phage polymerase then transcribes the class II and III genes



8.3 Infection of *E. coli* by Phage λ

- Virulent phage replicate and kill their host by lysing or breaking it open
- Temperate phage, such as λ, infect cells but don't necessarily kill
- The temperate phage have 2 paths of reproduction
  - Lytic mode: infection progresses as in a virulent phage
  - Lysogenic mode: phage DNA is integrated into the host genome

Two Paths of Phage Reproduction



Lysogenic Mode

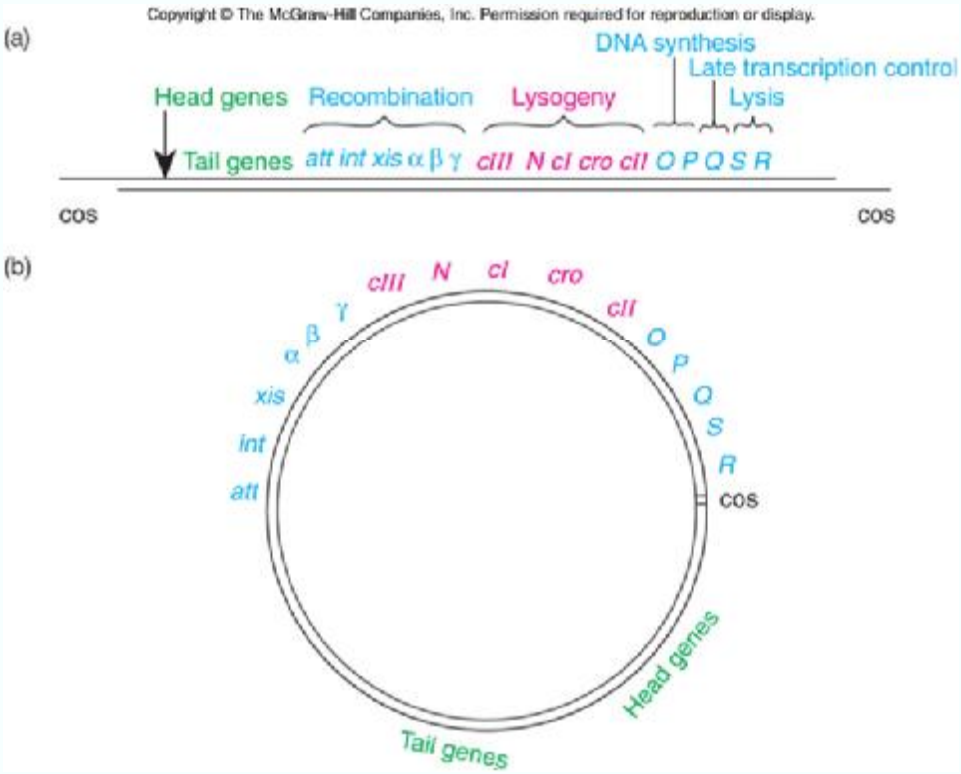
- A 27-kD phage protein (λ repressor, CI) appears and binds to 2 phage operator regions
- CI shuts down transcription of all genes except for *cI*, gene for λ repressor itself
- When lysogeny is established the phage DNA integrates into the bacterial genome
- A bacterium harboring integrated phage DNA is called a lysogen and the integrated DNA is called a prophage
- The phage DNA in the lysogen replicates along with the host DNA

Lytic Reproduction of Phage λ

- Lytic reproduction cycle of phage λ has 3 phases of transcription:
  - Immediate early
  - Delayed early
  - Late
- Genes of these phases are arranged sequentially on the phage DNA

Genetic Map of Phage λ

- DNA exists in linear form in the phage
- After infection of host begins the phage DNA circularizes
- This is possible as the linear form has sticky ends
- Gene transcription is controlled by transcriptional switches



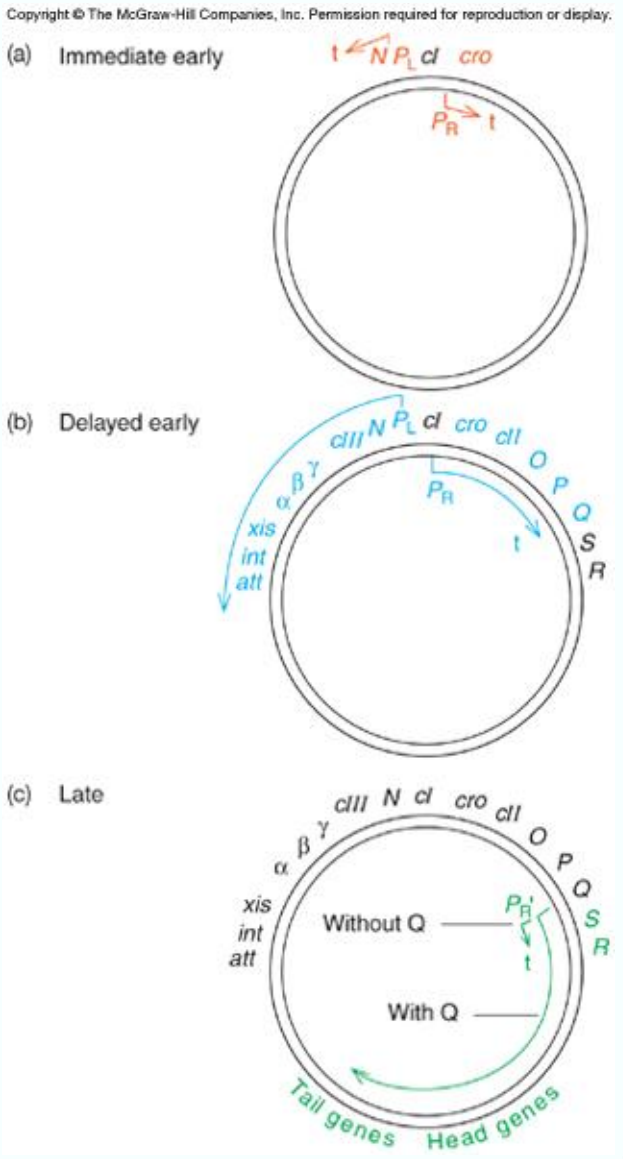
Antitermination

- Antitermination is a type of transcriptional switch used by phage λ
- The host RNA polymerase transcribes the immediate early genes first
- A gene product serves as antiterminator that permits RNA polymerase to ignore terminators at the end of the immediate early genes
- Same promoters are used for both immediate early and delayed early transcription
- Late genes are transcribed when another antiterminator permits transcription of the late genes from the late promoter to continue without premature termination

Antitermination and Transcription

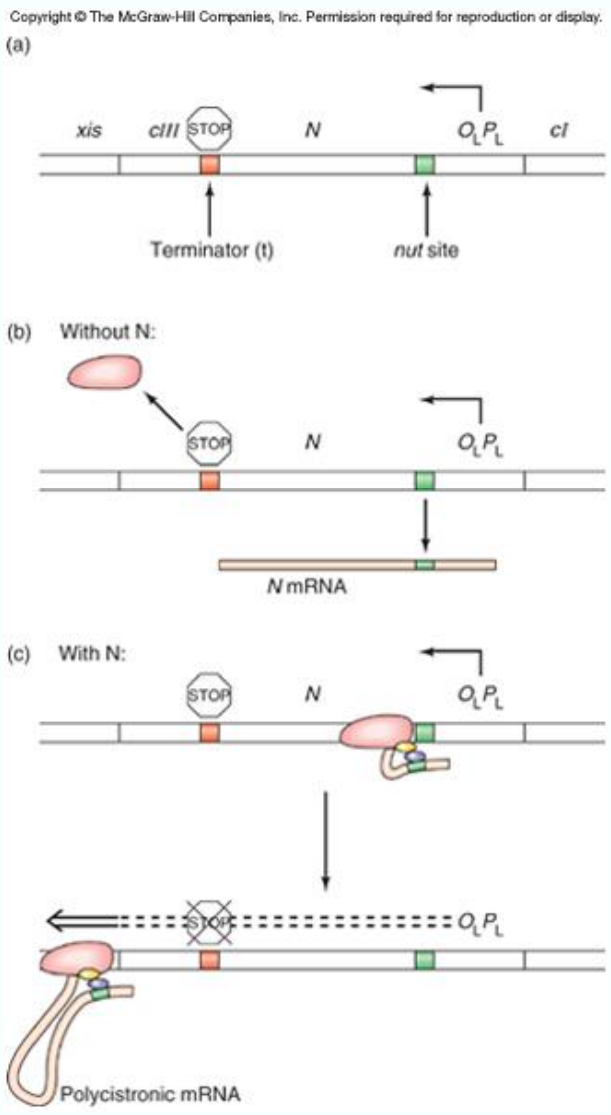
One of 2 immediate early genes is *cro*

- *cro* codes for a repressor of *cI* gene that allows lytic cycle to continue
- Other immediate early gene is *N* coding for N, an antiterminator



N Antitermination Function

- Genetic sites surrounding the N gene include:
  - Left promoter, P_L
  - Operator, O_L
  - Transcription terminator
- When N is present:
  - N binds transcript of N utilization site (*nut* site)
  - Interacts with protein complex bound to polymerase
  - Polymerase ignores normal transcription terminator, continues into delayed early genes

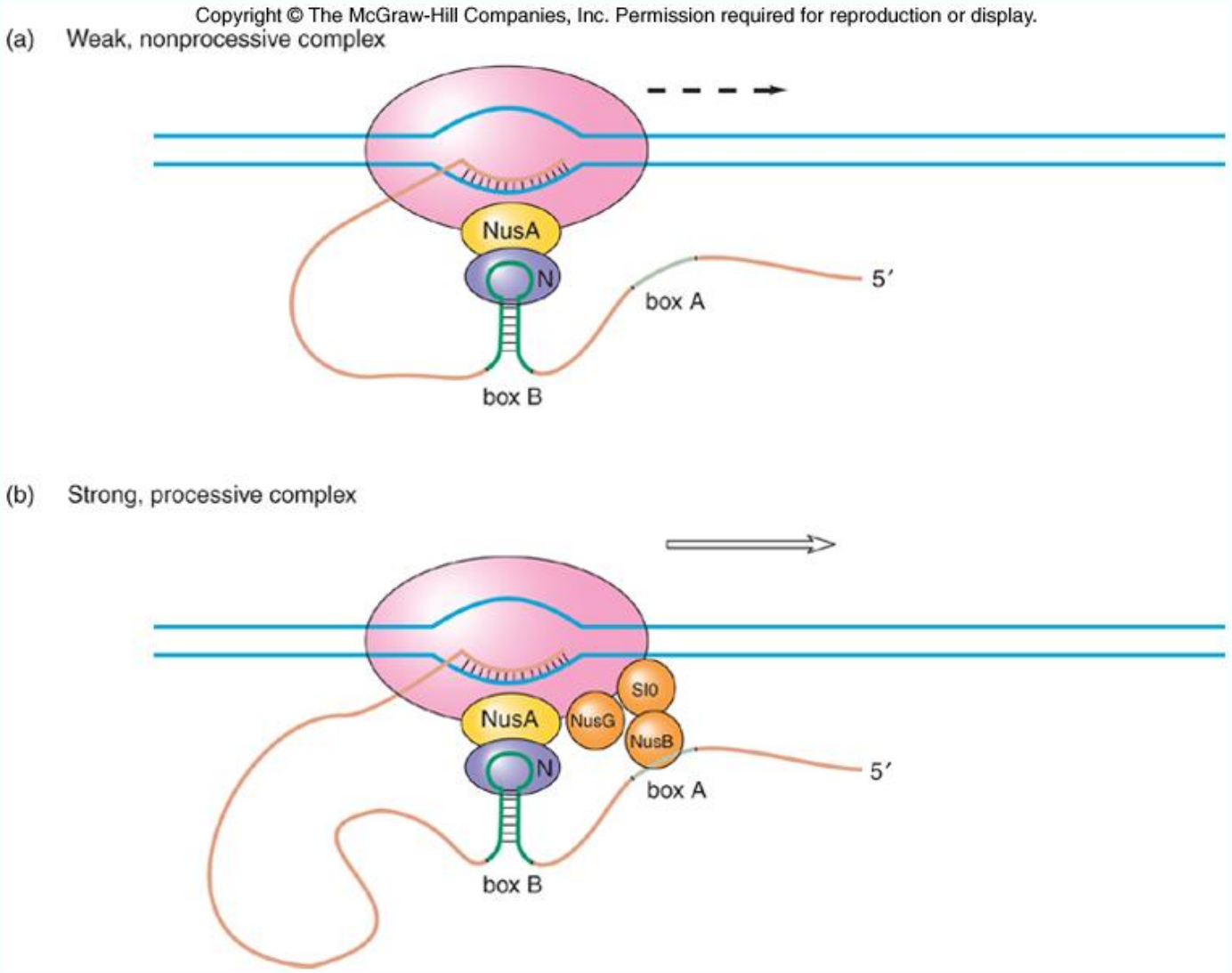


Proteins Involved in N-Directed Antitermination

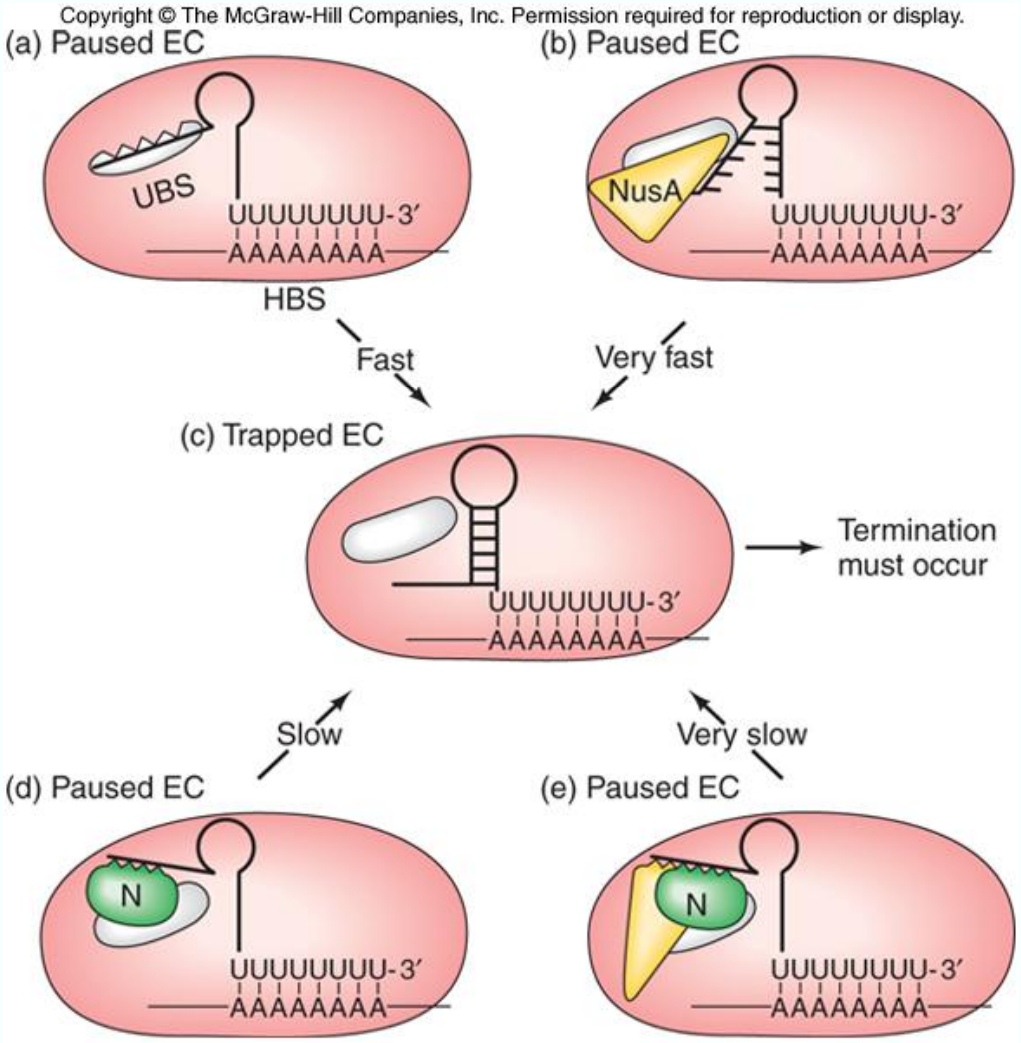
Five proteins collaborate in antitermination at the λ immediate early terminators

- NusA and S10 bind RNA polymerase
- N and NusB bind to the box B and box A regions of the *nut* site
- N and NusB bind to NusA and S10 probably tethering the transcript to the polymerase
- NusA stimulates termination at intrinsic terminator by interfering with binding binding between upstream part of terminator hairpin and core polymerase

Protein Complexes Involved in N-Directed Antitermination



Model for the Function of NusA and N in Intrinsic Termination



Antitermination and Q

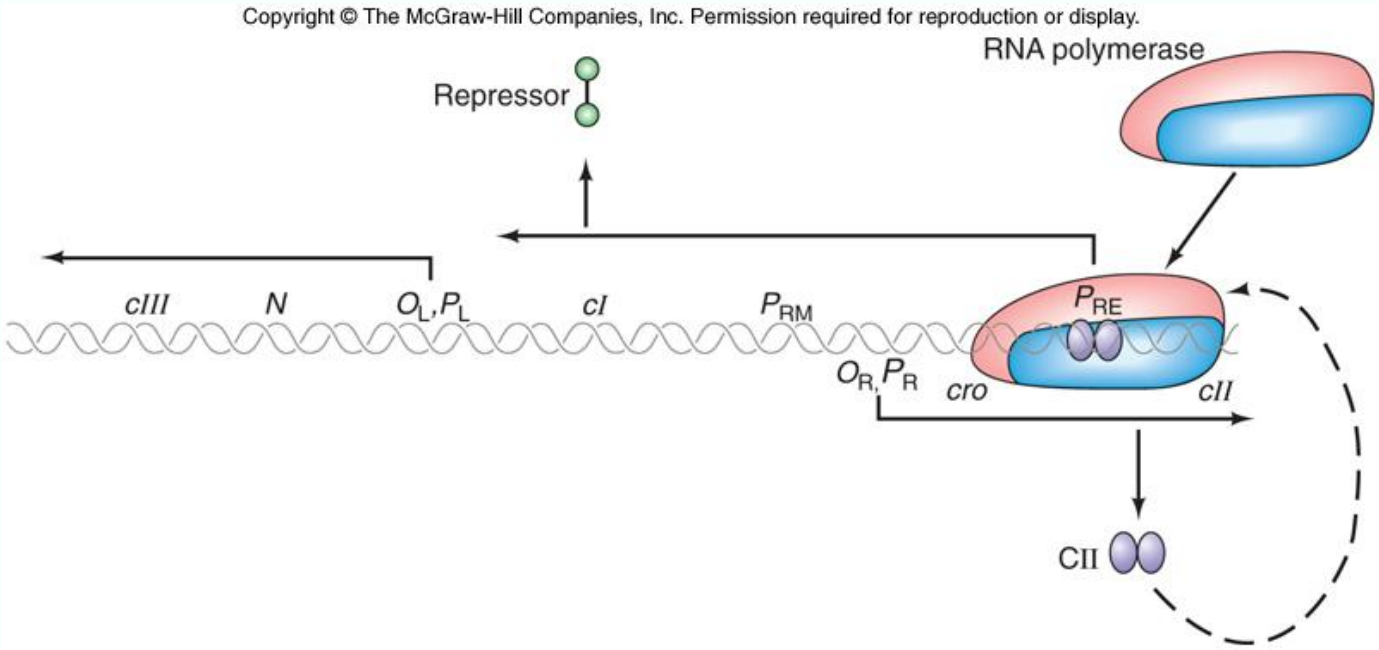
- Antitermination in the λ late region requires Q
- Q binds to the Q-binding region of the qut site as RNA polymerase is stalled just downstream of late promoter
- Binding of Q to the polymerase appears to alter the enzyme so it can ignore the terminator and transcribe the late genes

Establishing Lysogeny

- Phage establish lysogeny by:
  - Causing production of repressor to bind to early operators
  - Preventing further early RNA synthesis
- Delayed early gene products are used
  - Integration into the host genome
  - Products of *c*II and *c*III allow transcription of the *c*I gene and production of λ repressor
- Promoter to establish lysogeny is P_{RE}

Model of Establishing Lysogeny

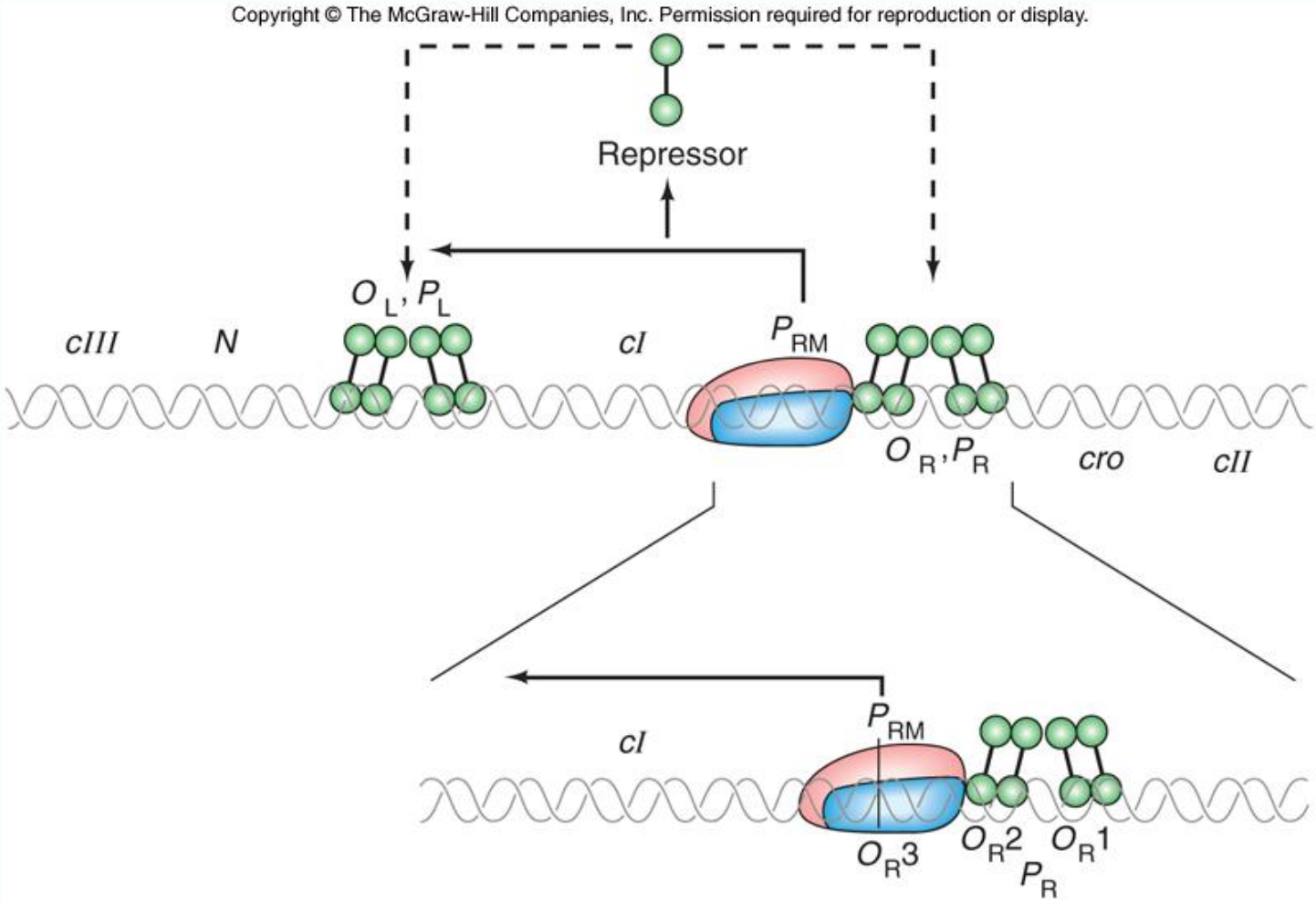
- Delayed early transcription from P_R produces *cII* mRNA translated to CII
- CII allows RNA polymerase to bind to P_{RE} and transcribe the *cI* gene, resulting in repressor



Autoregulation of the *cI* Gene During Lysogeny

- As λ repressor appears, binds as a dimer to λ operators, O_R and O_L results in:
  - Repressor turns off further early transcription
    - Interrupts lytic cycle
    - Turnoff of *cro* very important as product Cro acts to counter repressor activity
  - Stimulates own synthesis by activating P_{RM}

Maintaining Lysogeny

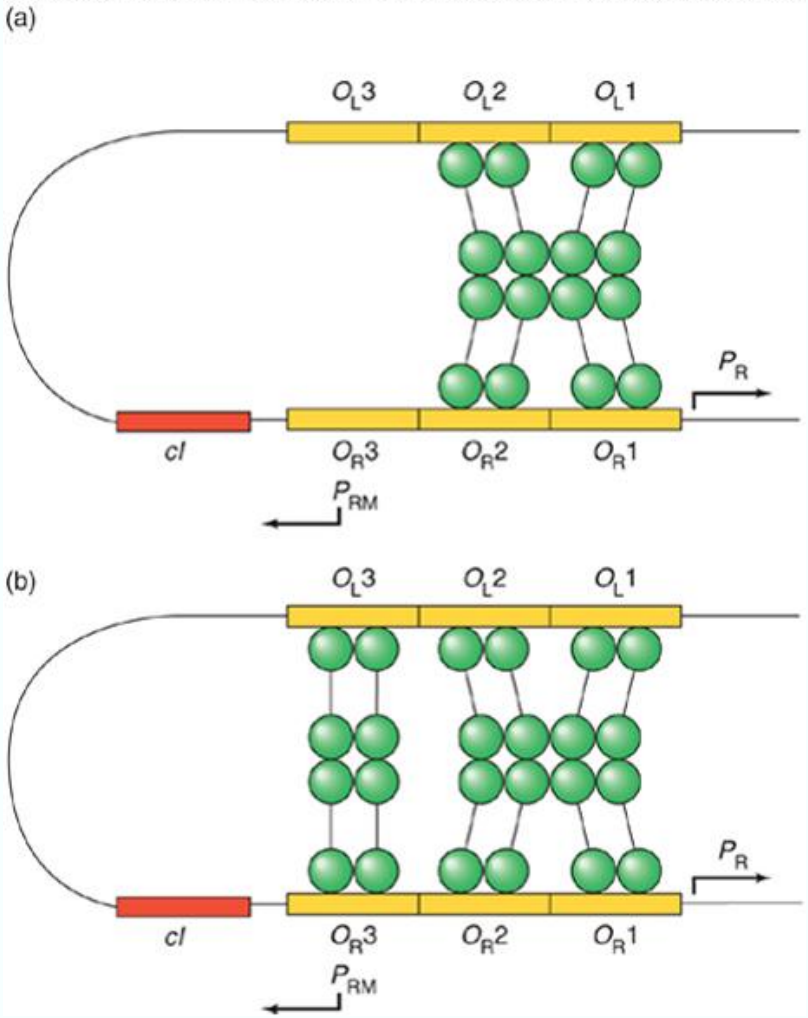


Repressor Protein

Repressor protein

- A dimer of 2 identical subunits
- Each subunit has 2 domains with distinct roles
  - Amino-terminal is the DNA-binding end of molecule
  - Carboxyl-terminal is site of repressor-repressor interaction that makes dimerization and cooperative binding possible

Model of Involvement of O_L in Repression of P_R and P_{RM}



Involvement of O_L in Repression

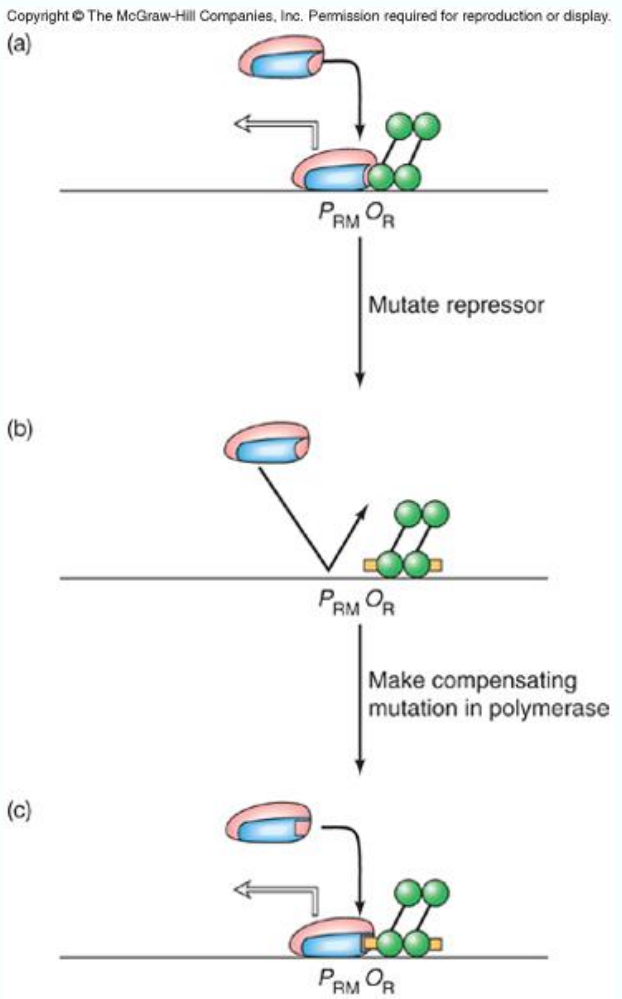
- Repressor binds to O_R1 and O_R2 cooperatively, but leaves O_R3
- RNA polymerase to P_{RM} which overlaps O_R3 in such a way it contacts repressor bound to O_R2
- Protein-protein interaction is required for promoter to work efficiently
- High levels of repressor can repress transcription from P_{RM}
  - Process may involve interaction of repressor dimers bound to O_R1, O_R2, and O_R3
  - Repressor dimers bound to O_L1, O_L2, and O_L3 via DNA looping

RNA Polymerase/Repressor Interaction

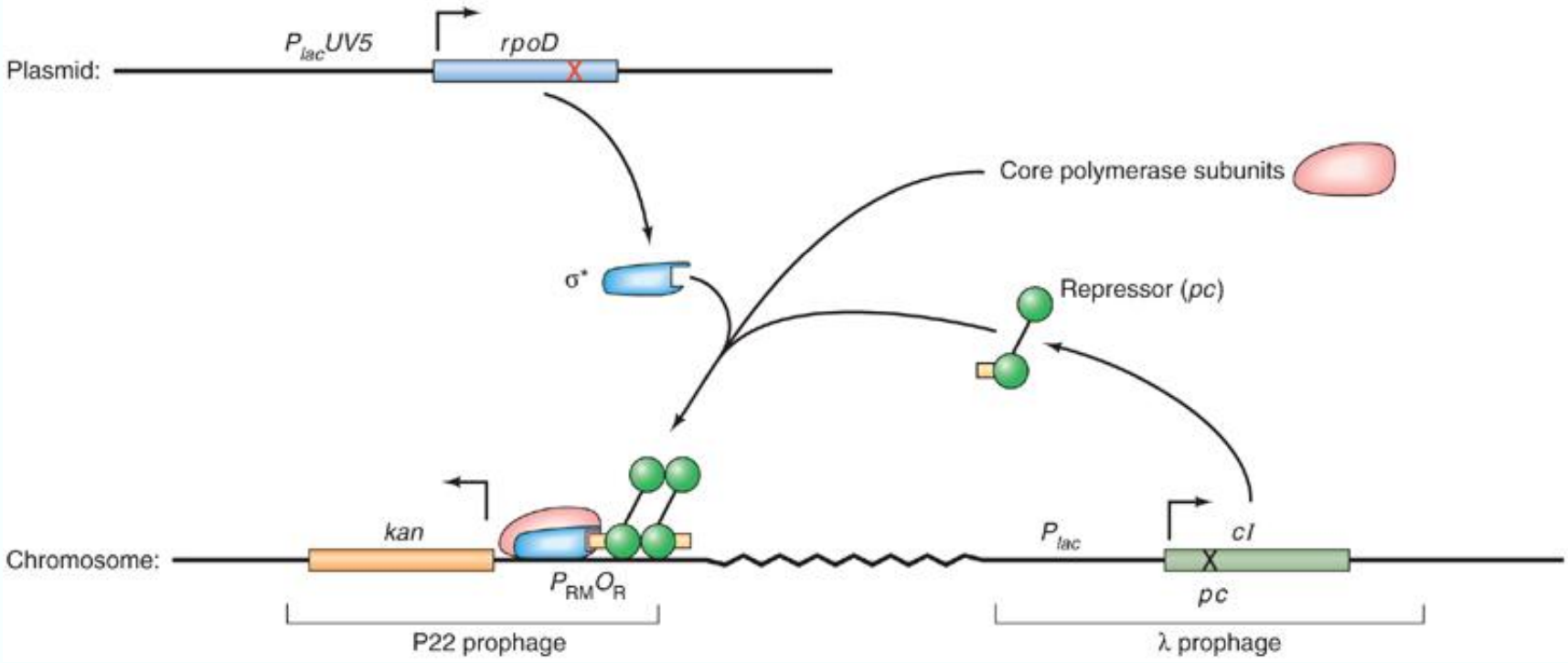
- Intergenic suppressor mutation studies show that crucial interaction between repressor and RNA polymerase involves region 4 of the σ-subunit of the polymerase
- Polypeptide binds near the weak -35 box of P_{RM} placing the σ-region 4 close to the repressor bound to O_R2
- Repressor can interact with σ-factor helping to compensate for weak promoter
- O_R2 serves as an activator site
- Repressor I is an activator of transcription from P_{RM}

Principle of Intergenic Suppression

- Direct interaction between repressor and polymerase is necessary for efficient transcription from P_{RM}
- Mutant with compensating amino acid change in RNA polymerase subunit restores interaction with mutant repressor
- In intergenic suppression, a mutant in one gene suppresses a mutation in another

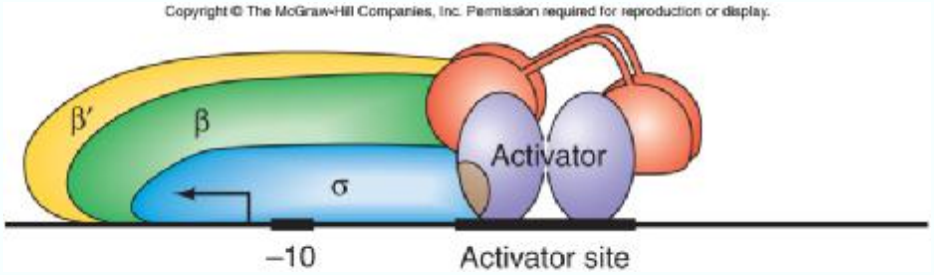


Selection for Intergenic Suppressor



Activation Via Sigma

- Promoters subject to polymerase-repressor activation have weak -35 boxes
- These boxes are poorly recognized by σ
- Activator site overlaps -35 box, places activator in position to interact with region 4

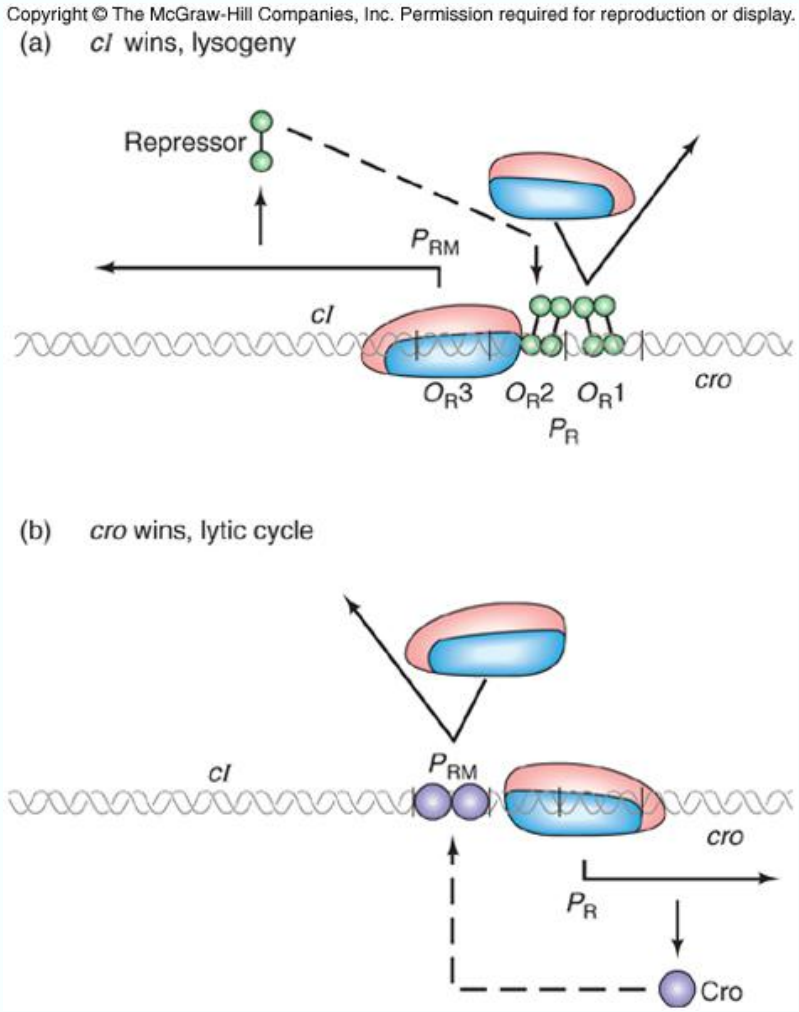


Determining the Fate of a λ Infection

- Balance between lysis or lysogeny is delicate
- Place phage particles on bacterial lawn
  - If lytic infection occurs
    - Progeny spread and infect other cells
    - Circular hole seen in lawn is called plaque
  - Infection 100% lytic gives clear plaque
  - Plaques of λ are usually turbid meaning live lysogen is present
- Some infected cells suffer the lytic cycle, others are lysogenized

Battle Between *cI* and *cro*

- The *cI* gene codes for repressor, blocks O_R1, O_R2, O_L1, and O_L2 so turning off early transcription
- This leads to lysogeny
- The cro gene codes for Cro that blocks O_R3 and O_L3, turning off transcription
- This leads to lytic infection
- Gene product in high concentration first determines cell fate



Lysogen Induction

- When lysogen suffers DNA damage, SOS response is induced
- Initial event is seen in a coprotease activity in RecA protein
- Repressors are caused to cut in half, removing them from λ operators
- Lytic cycle is induced
- Progeny phage can escape potentially lethal damage occurring in host