Temporal control of Transcription in phage SPO-1 infected B. subtilis

Early transcription; specificity factor: host $\sigma$ (blue)

Early genes

Early transcripts

Early proteins, including gp28 (green)

Middle transcription; specificity factor: gp28 (green)

Middle genes

Middle transcripts

Middle proteins, including gp33 (blue) and gp34 (yellow)

Late transcription; specificity factor: gp33 (blue) + gp34 (yellow)

Late genes

Late transcripts

Late proteins
Figure 8.4

T7 RNA Polymerase.

Early transcription; specificity factor: host $\sigma$ (blue circle)

Class I genes

Class I transcripts

Class I proteins, including phage RNA polymerase (green circle)

Late transcription; phage RNA polymerase (green circle)

Class II and III genes

Class II and III transcripts

Class II and III proteins
Figure 8.17

Lambda Life Cycle.

Lytic Phase

Infection

Phage DNA cyclizes

Decision point

Integration of phage DNA

Cell division

Phage DNA replicates

uv light induction (rare)
Phage DNA excised

Lysogenic Phase

Reinfection

Cell lysis

Phage DNA replicates (rolling circle)

Phage heads, tails, and DNA assemble into progeny phages
Lambda

\[ \lambda \text{ genetic map} \]

- Structural
  - 2: Head + Tail
  - 5: Recombination
  - 2: DNA Replication
  - 3: Lysis

- Regulatory
  - 6: Regulatory molecules
    - C, cro: repressors
    - N, Q: anti-terminators
    - CI: promoter activator
    - CIII: promoter co-activator

37 genes
Figure 8.18

Lambda Genome

Head genes → Recombination → Lysogeny

DNA synthesis → Late transcription control → Lysis

Tail genes: att, int, xis, α, β, γ, cIII, N, Cl, cro, cII, O, P, Q, S, R

COS → COS

α, β, γ, xis, int, att → cIII, N, Cl, cro → cII, O, P, Q, S, R, COS
Plaques are turbid
Lytic Life Cycle.

Figure 8.19

Immediate early

Delayed early

Late

Without Q

With Q

Tail genes Head genes
Figure 8.20

(a) N anti-terminates transcription.

Terminator (t)  nut site

(b) Without N:

STOP  N  O_L P_L

N mRNA

(c) With N:

STOP  N  O_L P_L

Polycistronic mRNA
Protein complexes involved in N-mediated anti-termination.

**Figure 8.21**

(a) Weak, nonprocessive complex

(b) Strong, processive complex
Lytic Growth Summary

- $P_L$ and $P_R$ are used by RNAP no cI present
- Cro and N are made
- N anti-terminates tx at $t_L$ and $t_{R1}$
- Polymerase transcribes cIII and recombination genes (L)
- Polymerase transcribes cII and DNA replication genes (R)
- Cro binds to $O_R$ and $O_L$ blocking the $P_{RM}$
- If cII levels are low then lytic growth continues because the $P_{RE}$ is not activated
Lambda

generic map

- att
- L
- R
- (P0)
- (red) (red)
- CIII
- tL
- N
- CII
- cro
- CII
- OP
- Q
- SR
- Rp
- cos

- Head Tail
- TLZ
- Recombination
- Control
- Tail FII ZUVGTHMLKIJ
- Head Nu1 AWBC Nu3 DEFZ

Structural
- 21 Head + Tail
- 5 Recombination
- 2 DNA Replication
- 3 Lysis

Regulatory
- 6 Regulatory molecules
  - Cr, Cro repressors
  - N, O anti-terminators
  - CR promoter activator
  - CIII promoter co-activator

37 genes
Cro vs cI

- Cro and cI are DNA binding proteins that bind to the operator sequences OR and OL
- There are three binding sites for each protein in the operator
- Cro and cI bind with opposite affinities to these three sites
- cI binds first to site 1, then 2 and at high concentrations site 3
- Cro binds to site 3 first, then site 2 followed by site 1
- cI binds to sites adjacent to its own promoter $P_{RM}$ activating its own transcription
- Cro binds to the $P_{RM}$ and prevents transcription from this promoter
The Battle between cro and cl.

(a) cl wins, lysogeny results

(b) cro wins, lytic cycle results

Figure 8.32
Q-mediated anti-termination of the late genes.
Q-”anti-termination”

Q binds to its own promoter through the Q utilization site

Holoenzyme binds to the Q promoter (Pr’) and sigma interacts tightly with the -10 region of this promoter

Q associates with the sigma factor in the 4 domain releasing sigma from the polymerase

Q continues to associate with the polymerase and late genes are transcribed by the Q-containing core enzyme
Paused Complex with Q

Q interacts with a specific site on the DNA known as the *qut* site (Q utilization site) upstream of the transcription site for the late promoter $P_R$.

$\sigma^{70}$ (region 2) interaction with a promoter element (−10 like element) causes a transcriptional pause.

$Q$ interacts with region 4 of $\sigma^{70}$ which is also interacting with a promoter element (−35 like element) which assists in pausing transcription.
Anti-termination Complex with Q

Q is recruited to the RNA polymerase as a subunit and modifies the enzyme into its termination-resistant form as it ravels down the DNA strand.

Q binds and displaces σ^70 allowing transcription to continue.
Anti-termination Complex with Q and NusA assistance

Q activity is enhanced by NusA in-vitro
Establishing Lysogeny

- If cII levels are high then lysogenic growth will proceed
- cII activates $P_{RE}$ (repressor establishment)
- cII activates $P_{I}$ (int)
- cII activates $P_{antiQ}$
- Resulting in the synthesis of cI and the turn off of $P_{R}$ eliminating cro synthesis
- cI binds to the $O_{L}$ and $O_{R}$ preventing further $P_{L}$ and $P_{R}$ transcription
- cI activates its own promoter $P_{RM}$ and lysogeny is established
Lambda

\[ \text{\textlambda genetic map} \]

\[
\text{structural} \quad \text{regulatory}
\]

<table>
<thead>
<tr>
<th>Structural</th>
<th>Regulatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 Head + Tail</td>
<td>6 Regulatory molecules</td>
</tr>
<tr>
<td>5 Recombination</td>
<td>( \text{CI}, \text{cro} ) repressors</td>
</tr>
<tr>
<td>2 DNA Replication</td>
<td>( N, Q ) anti-terminators</td>
</tr>
<tr>
<td>3 Lysis</td>
<td>( \text{CI} ) promoter activator</td>
</tr>
<tr>
<td>37 genes</td>
<td>( \text{CIII} ) promoter co-activator</td>
</tr>
</tbody>
</table>
Establishing Lysogeny.

Figure 8.23
cII activates three promoters:

- $P_{\text{int}}$
- $P_{\text{re}}$
- $P_{\text{antiQ}}$
Maintaining lysogeny.

Figure 8.26
The Life-Cycle Decision

- The levels of cII and cIII are critical; they sense the 'health' of the cell.
- Healthy rapidly growing cells have high levels of proteases which degrade cIII and cII.
- cIII tries to block the proteases from cleaving cII.
- Hfl (high frequency lysogen) is a bacterial gene that greatly influences this decision.
- When hfl is absent or mutated, lysogeny is highly favored as this protease cleaves cIII.
- When cells are starved and not dividing, protease levels are low, and cII and cIII levels are stabilized, thus favoring lysogenic growth.
Lambda Integration

- Once cII levels are established cII activates the $P_{\text{Int}}$ promoter allowing the synthesis of int
- $P_{\text{Int}}$ is located in the xis gene so only int is made and integration results
The importance of $P_{\text{int}}$ for integration and lysogeny
Lambda induction

- DNA damage results in the activation of the SOS system in bacteria and the synthesis of recA
- The recA protein causes cI to cleave and dissociate from DNA
- When $O_R$ and $O_L$ no longer have cI bound, $P_R$ and $P_L$ become active and lytic gene expression proceeds
Inducing the Lambda prophage.

(a) $P_{RM}$

(b) RecA co-protease + $\lambda$ repressor protease

(c) $P_R$

Figure 8.33
induction

\[ \lambda \text{ repressor (cl)} \]

\[ \text{cl repressor dimers - prevents RNAP from binding and transcribing at } P_R \]

\[ \text{PRM - Promoter of repressor maintenance (lysogenic genes cl, rexA, rexB)} \]

\[ \text{PR - Promoter of early lytic genes (cro, cII, O and P)} \]

\[ \text{INDUCTION (repressor destroyed)} \]

\[ \text{prevents transcription of } \lambda \]

\[ \text{Cro} \]

\[ P_R \]

\[ \text{RNA Polymerase} \]

\[ \text{strong affinity for } O_R^3 \]

\[ \text{weaker affinity for } O_R^2 \text{ and } O_R^1 \]

\[ \text{weaker affinity for } O_R^3 \]

\[ \text{strong affinity for } O_R^3 \]

\[ \text{cro} \]
Integration and excision of DNA

Integration (int IHF)

Excision (int xis IHF)

*sib protein induces transcription of int, but not xis (lysogeny)

*int and xis equally transcribed upon induction

*cl transcribed during stable lysogenic state

sib out of\( P_L \) reading frame \( \rightarrow \) no hairpin loop
Excision of Lambda DNA

- Integration of lambda DNA into the genome results in the physical separation of the b-region (sib) from int and xis
- Under these circumstances the synthesis of both xis and int occur from PL
- When both int and xis are present excision will occur and the prophage is excised from the genome
Lytic replication

- Lytic replication of \( l \) DNA occurs both when the initial decision was to grow lyticly and after excision from the bacterial chromosome
- \( P_R \) transcription results in the synthesis of the O, P and Q genes
- For the first few replication cycles the \( l \) genome is replicated circle to circle
- Circular DNA cannot be packaged into phage particles
- O protein binds to the ori site internal to the O gene and P binds to O as well as host DNA polymerase
- Rolling circle replication then proceeds resulting in a long concatemer of \( l \) genomes
- The concatemer is cleaved and linear DNA is packaged
Why are plaques turbid?