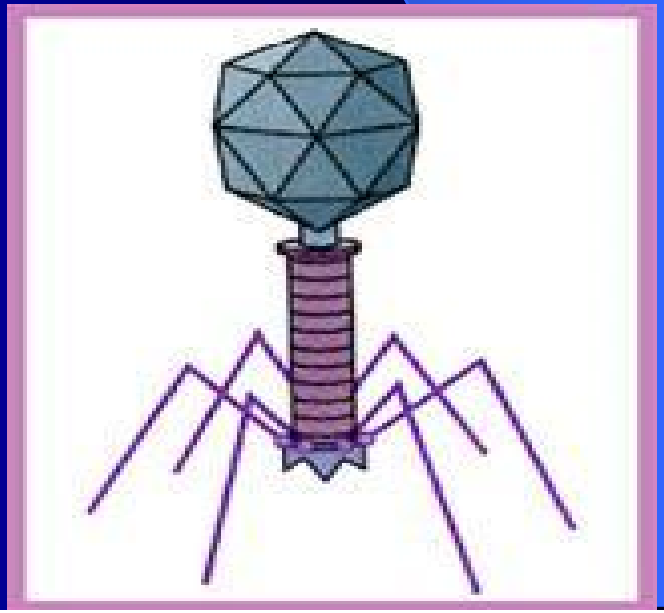
噬菌体的分子生物学

- λ噬菌体的表达调控几乎包括所有原核生物的调控方式：
- 启动子、终止子、诱导、阻遏
- 反义RNA 翻译水平调控
- 抗终止—时序调控
- 溶原/溶菌选择的结构基础



λ phage

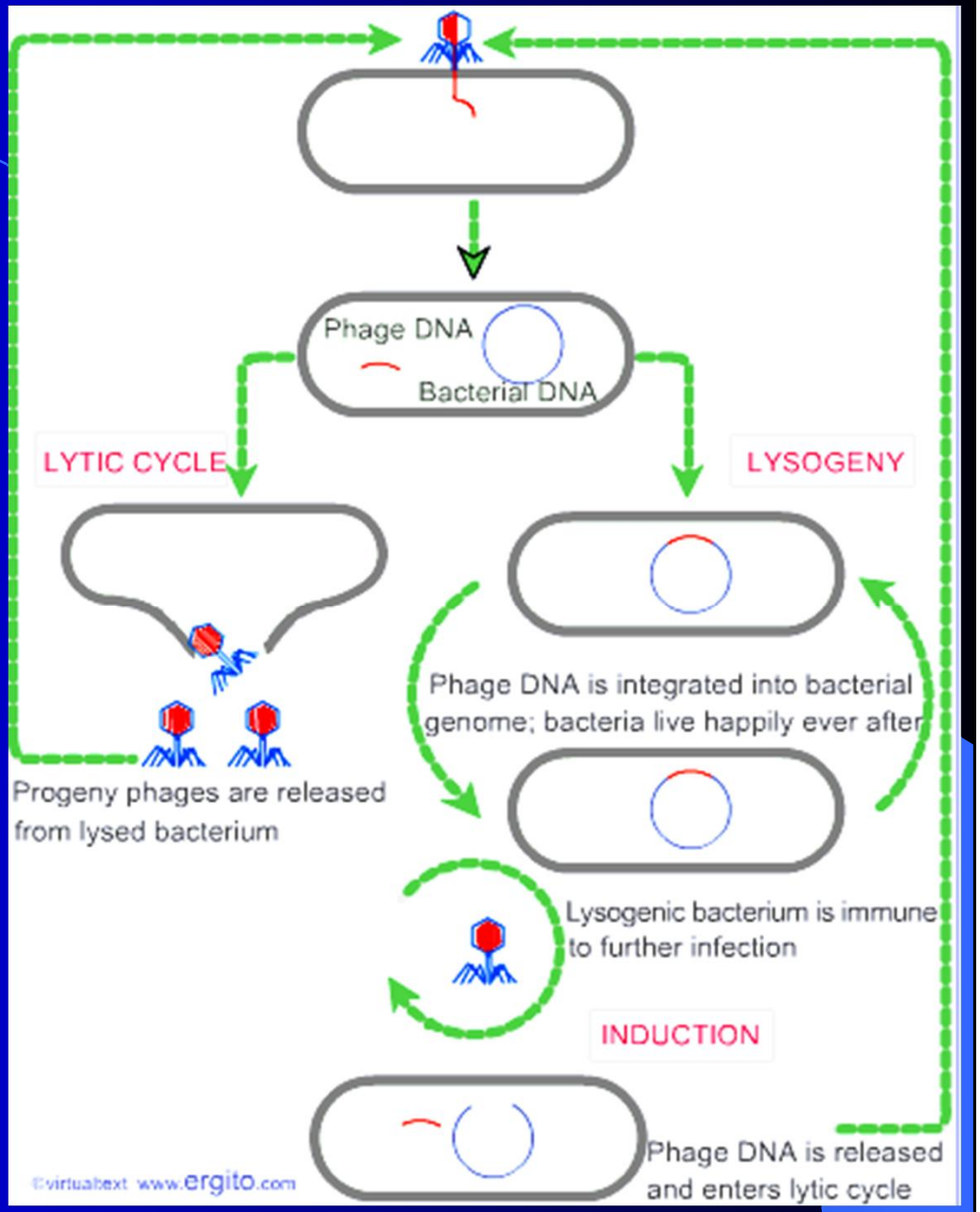
- 溶原/溶菌选择
- 免疫原性：

  同种噬菌体感染

  后进入溶原途径
- 环境因素诱导：

  紫外线等因素

  进入裂解状态

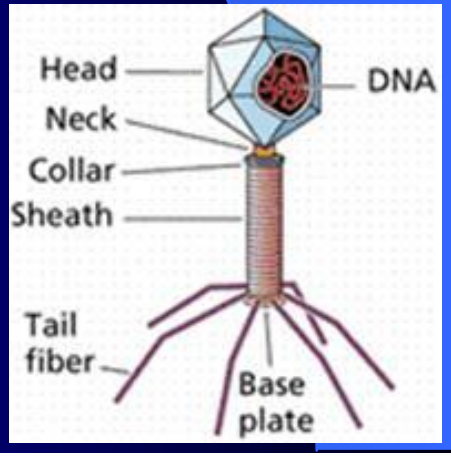


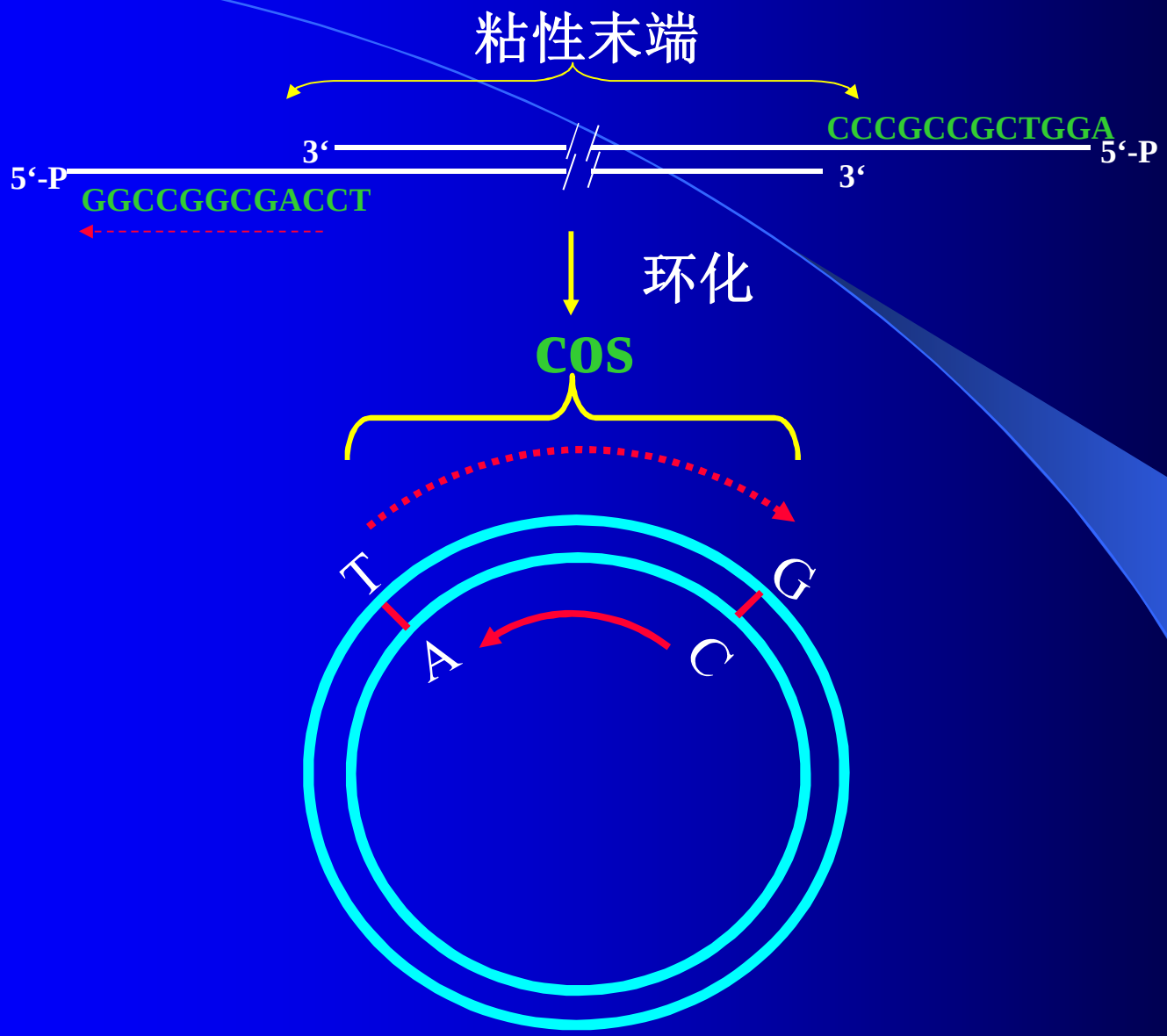
溶原、溶菌过程示意图

λ噬菌体简介

- 双链线状DNA分子，48502bp
- 两条单链5’端有突出的12个碱基，回文对称，富含GC—COS位点
- 从λ噬菌体体内提取的DNA，EcoRI酶切 6 片段

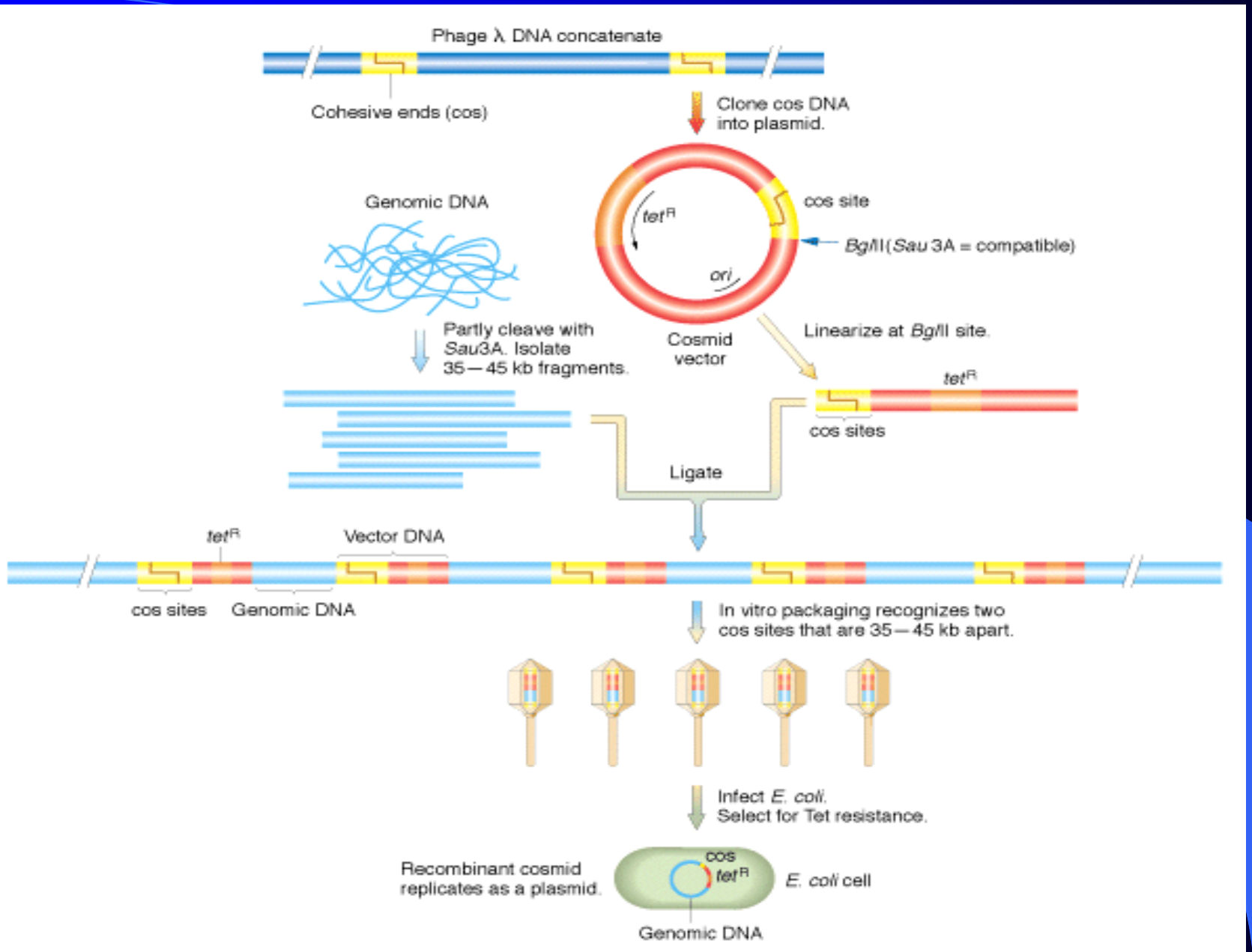
  退火后酶切 5 片段
- 黏性末端切平，则失去感染活性





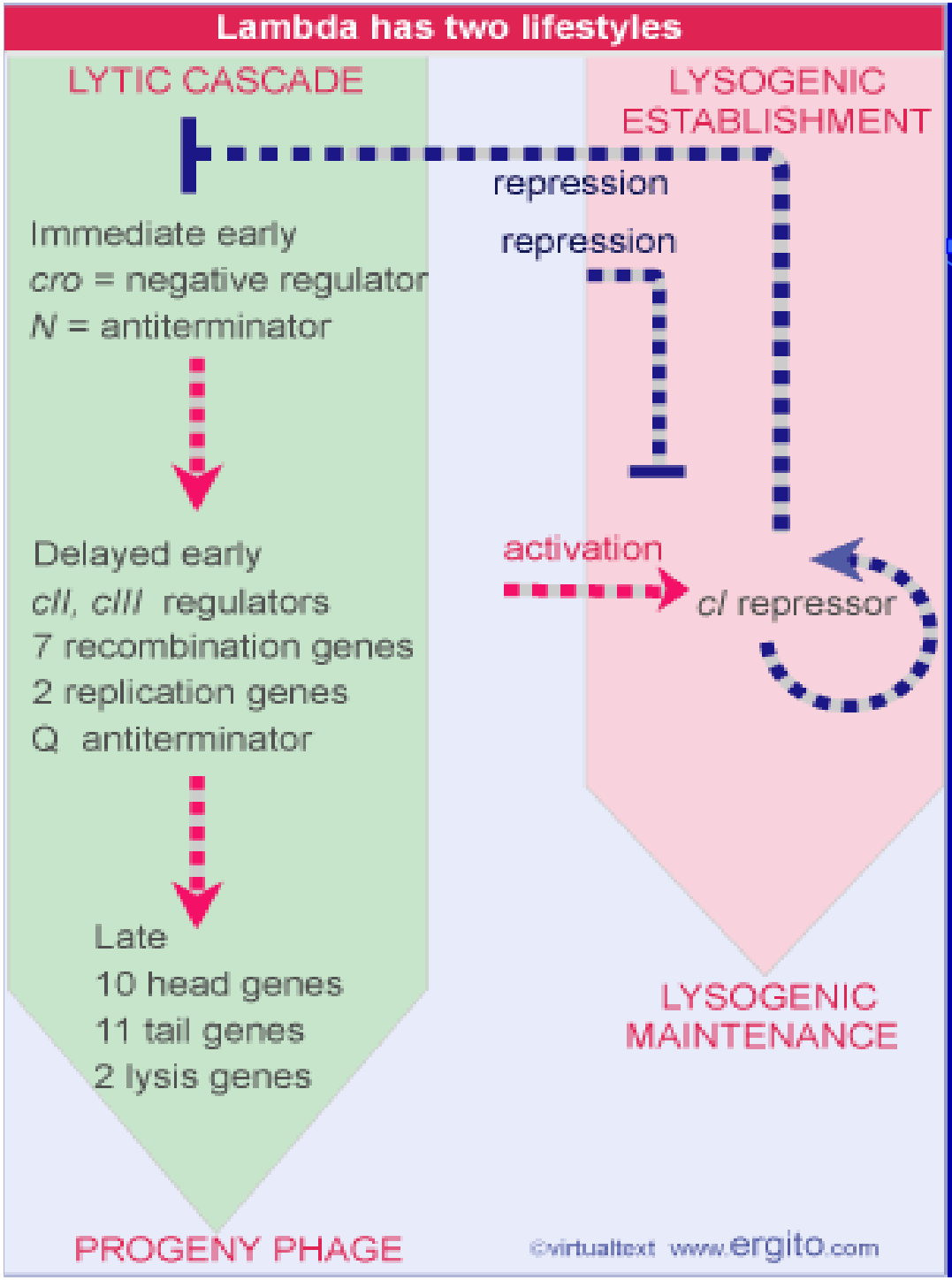
λ 噬菌体黏性末端的环化

λ噬菌体包装过程示意图



科斯质粒(cosmid:cos site-carrying plasmid)

45kb



时序调控（溶菌）

早早期基因:

启动子和宿主基因类似

- Cro 负调控因子
- N 抗终止子

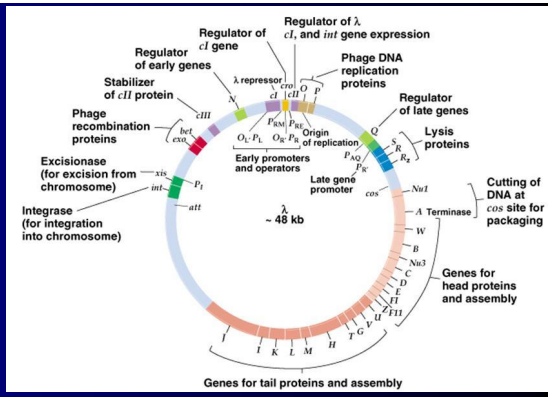
晚早期基因:

- C II 和C III
- 7个重组基因
- 2个复制基因
- Q 抗终止子

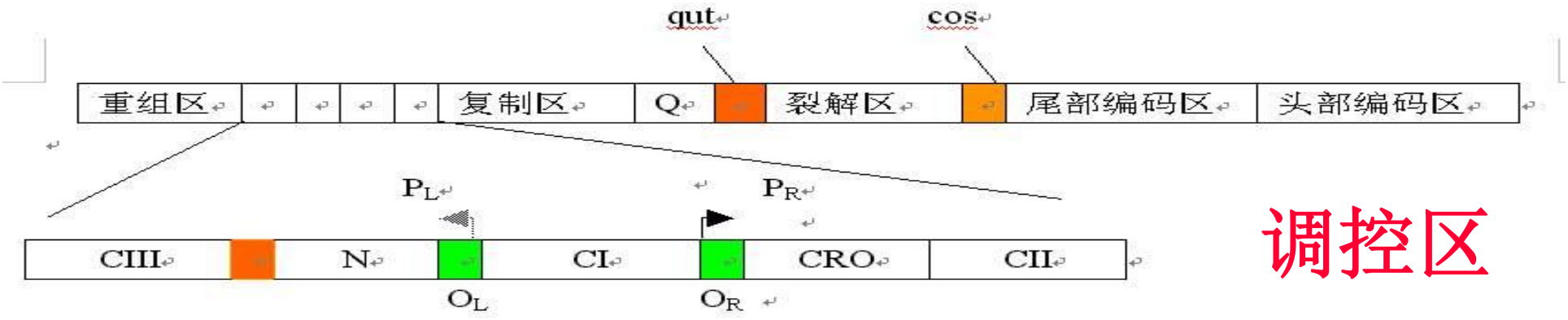
晚期基因:

- 10个头部基因
- 11个尾部基因
- 2个裂解基因

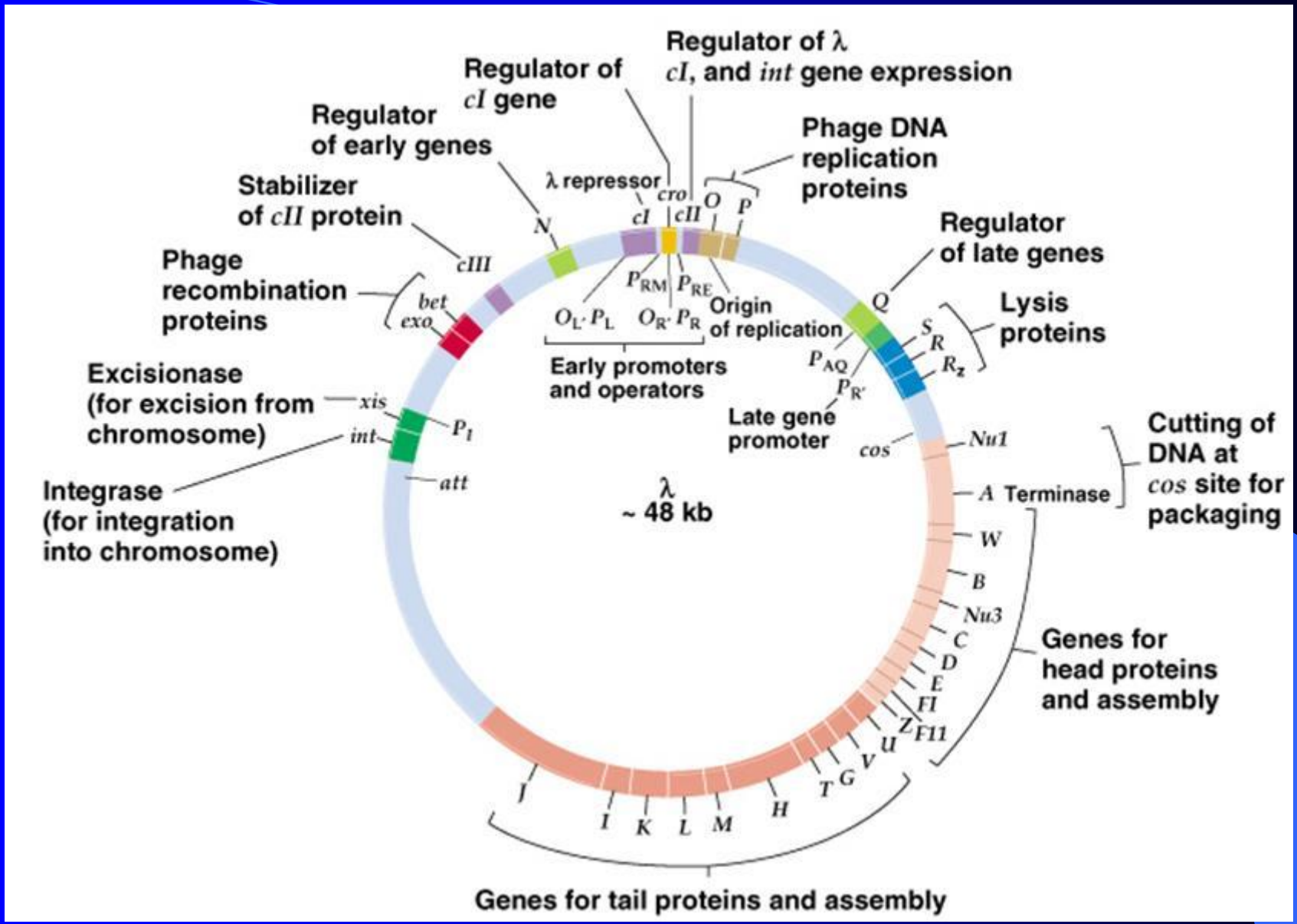
λ噬菌体基因组特征



- 重组区、调控区、复制区、结构基因区
- 与调控有关的基因:C_{II}与C_{III}之间
- N个启动子 每一个转录单位包括功能上并不直接相关的基因
- 转录调控:起始和终止，溶原/溶菌
- 双链结构：结构基因不对称转录

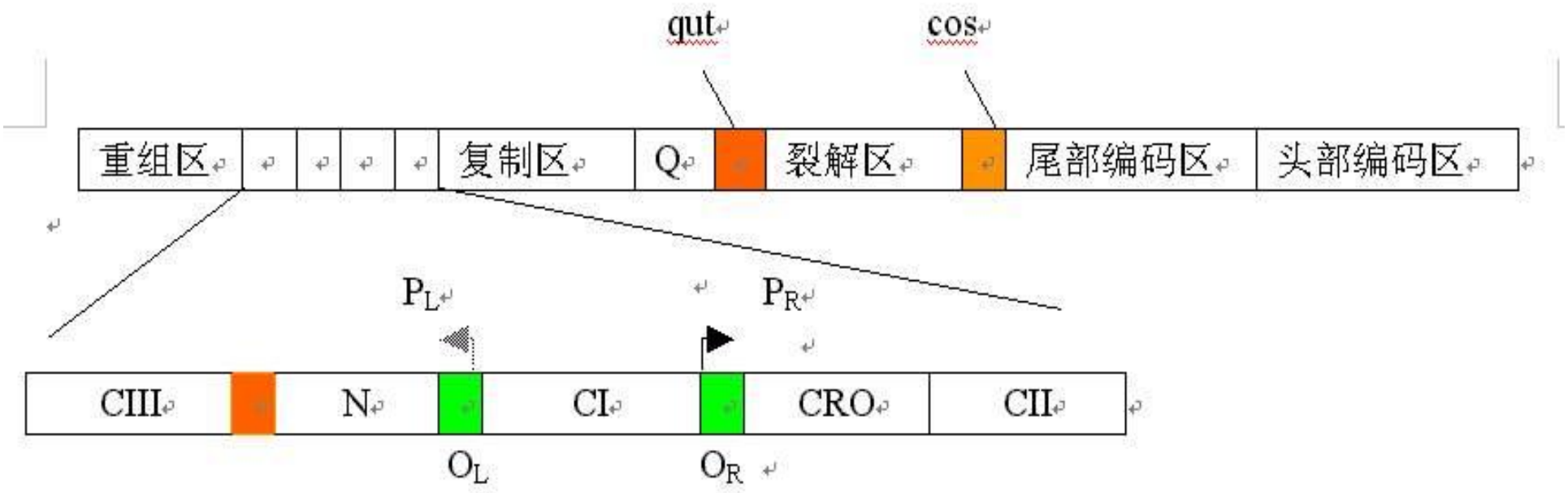


调控区



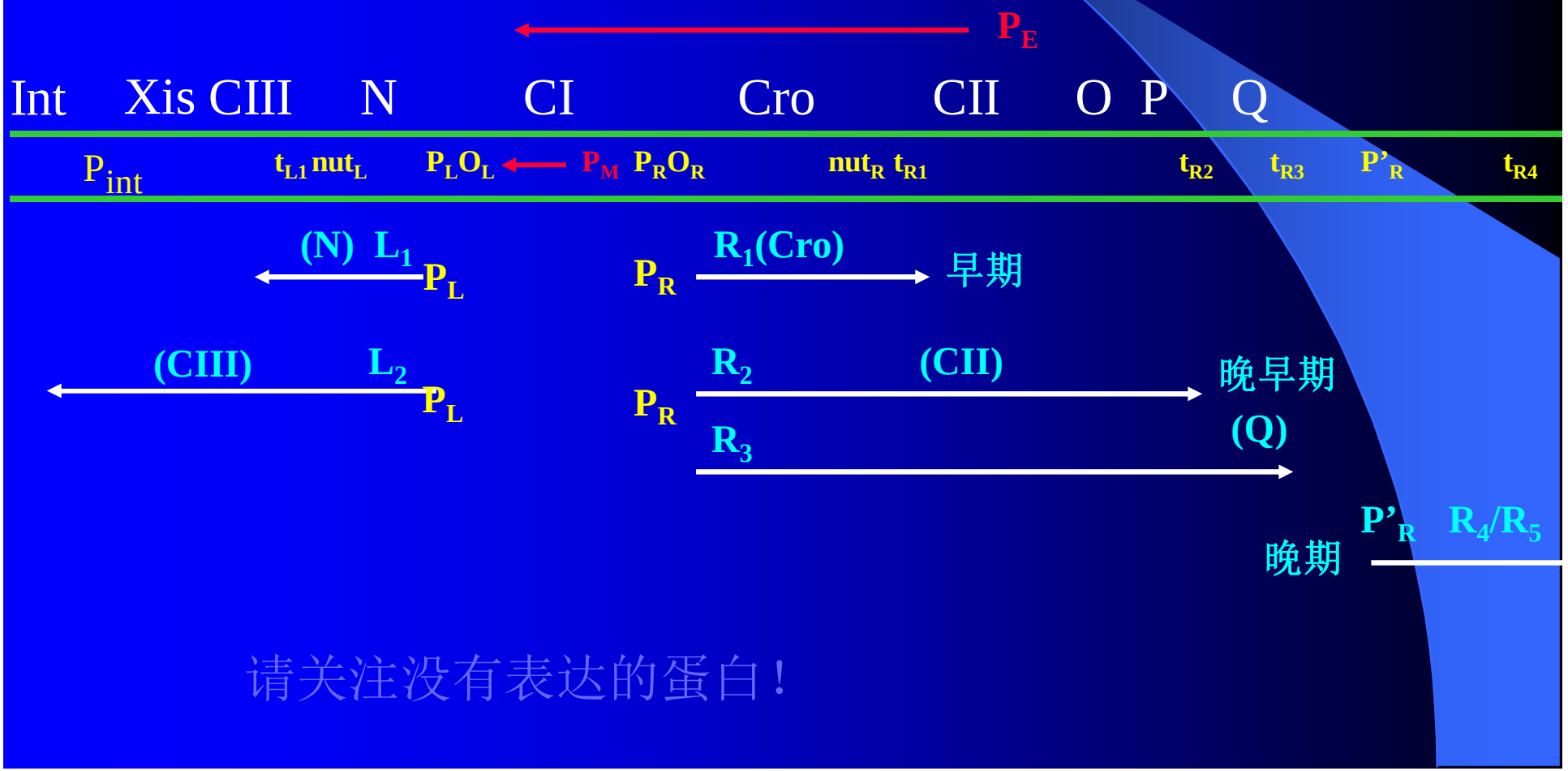
λ噬菌体基因结构示意图

与调控有关的基因



- P_L：λ基因左侧区域转录启动子
- P_R：λ基因右侧区域转录启动子
- O_L：非编码区（约50bp），位于CI和N基因之间
- O_R：非编码区(约50bp)，位于CI和Cro之间

λ噬菌体基因的时序表达



请关注没有表达的蛋白！

N-p

Anti-termination protein

for delayed early stage

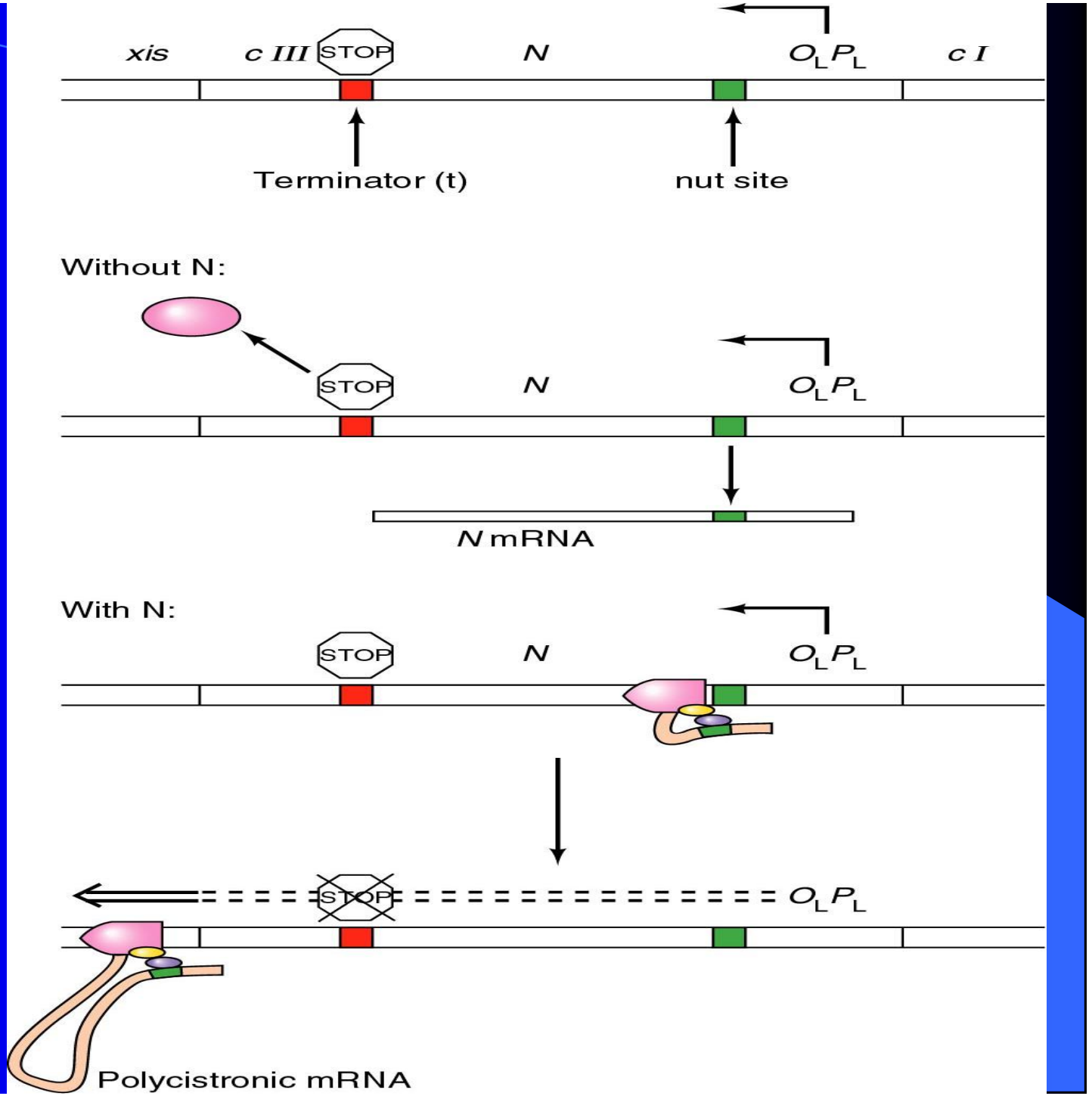
Q-p

Anti-termination protein

for late stage

N

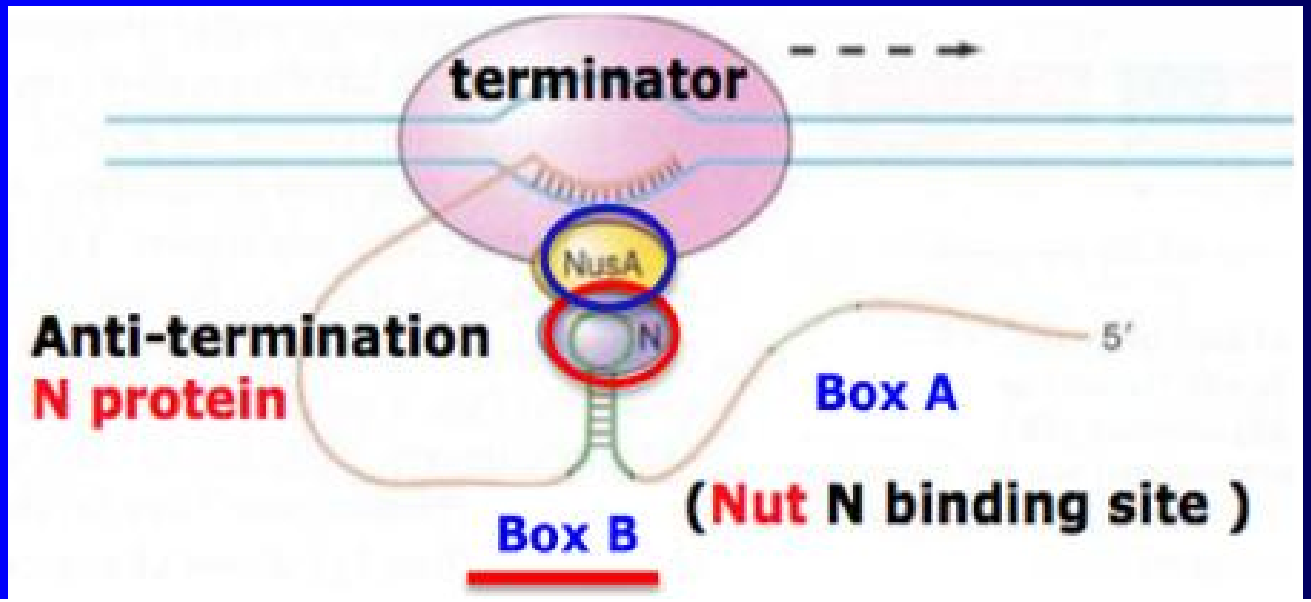
NusA


xis
c III
STOP
N
$O_L P_L$
c I
Terminator (t)
nut site
Without N:
STOP
N
$O_L P_L$
N mRNA
With N:
STOP
N
$O_L P_L$
STOP
$O_L P_L$
Polycistronic mRNA

NusA 蛋白

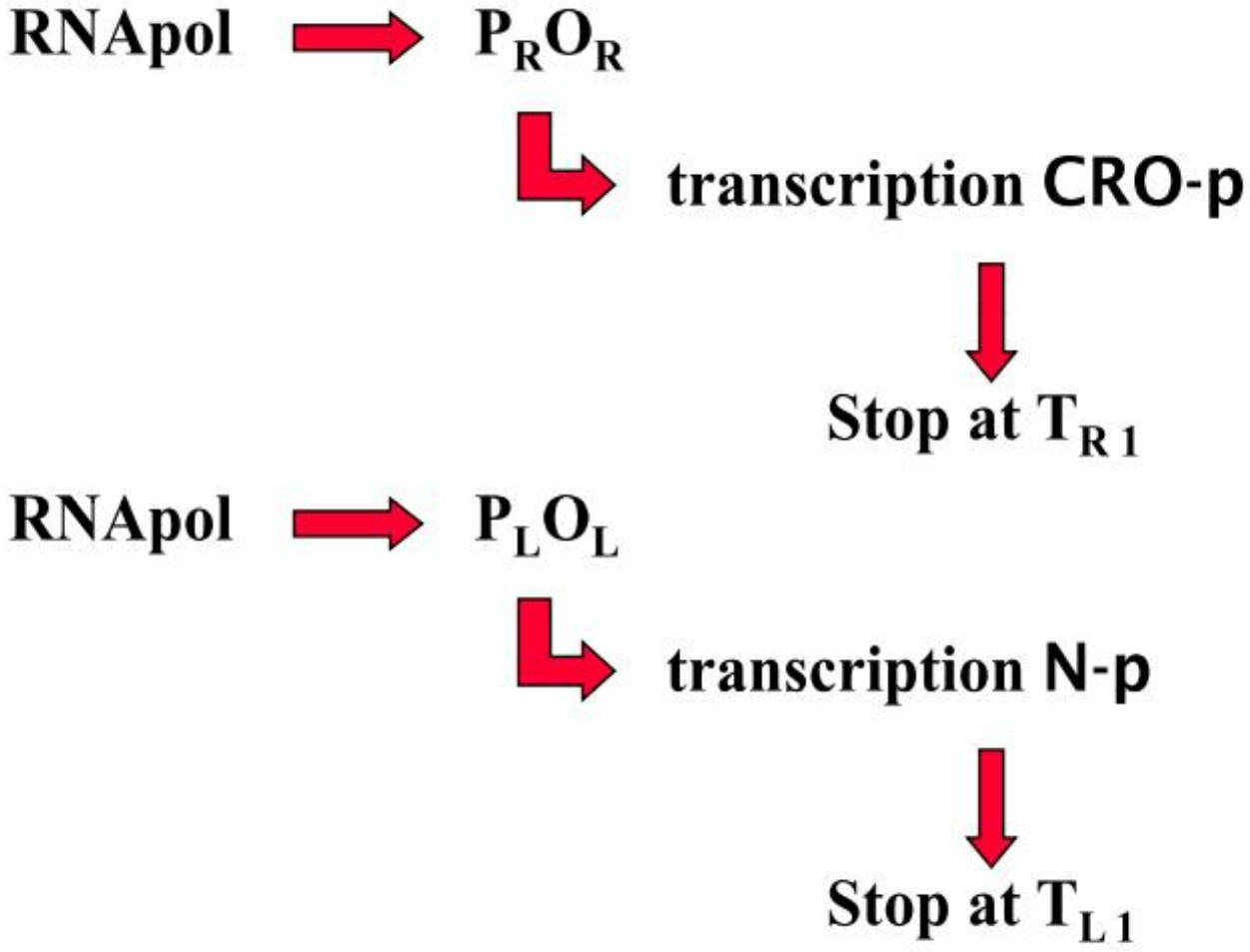
- antitermination N protein utilization substance
- 转录起始→ σ因子脱落 → NusA 与 核心酶结合

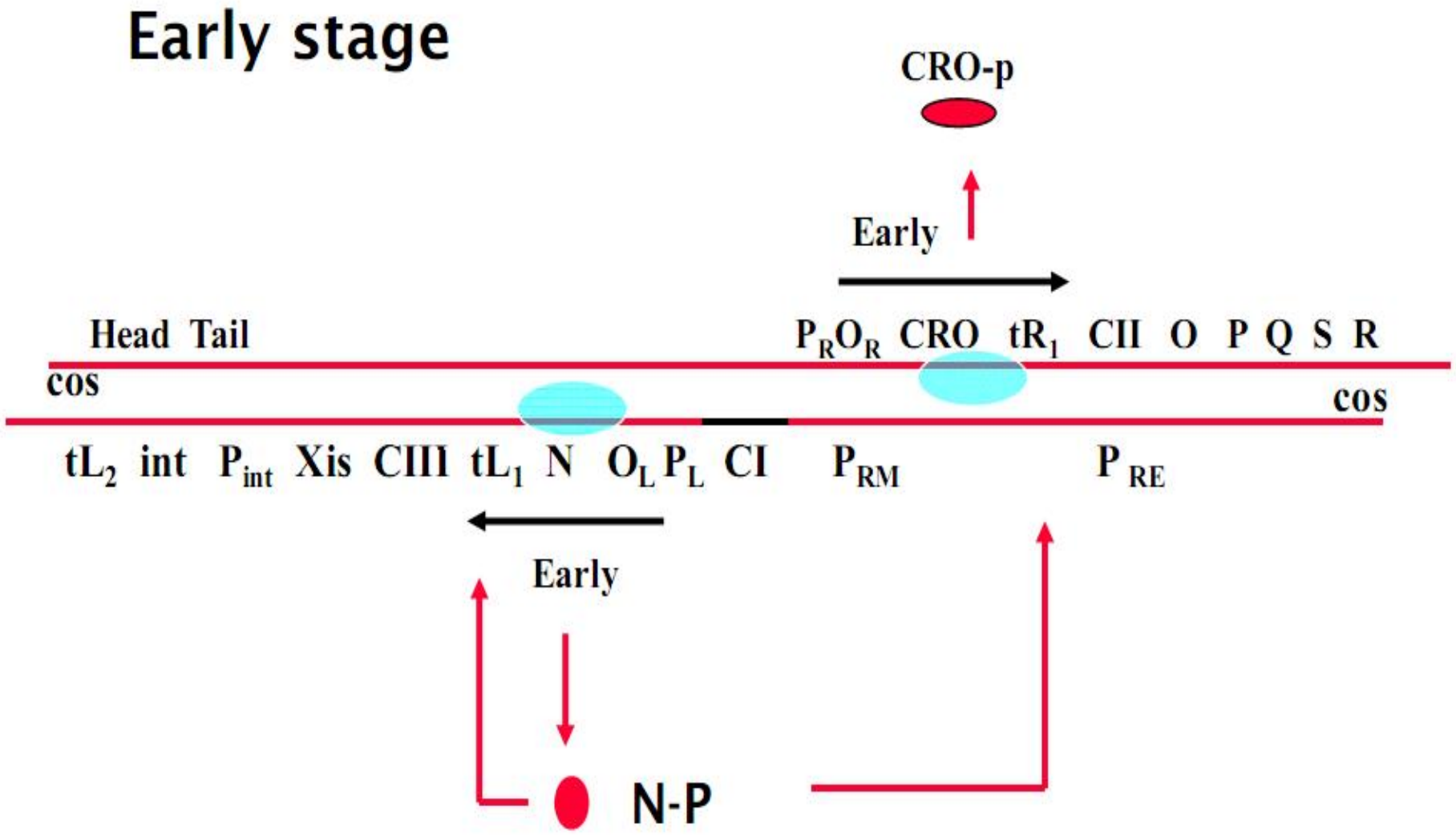
  迫使RNApol 在 terminator停留
- 与ρ一起协同作用使RNA转录停止



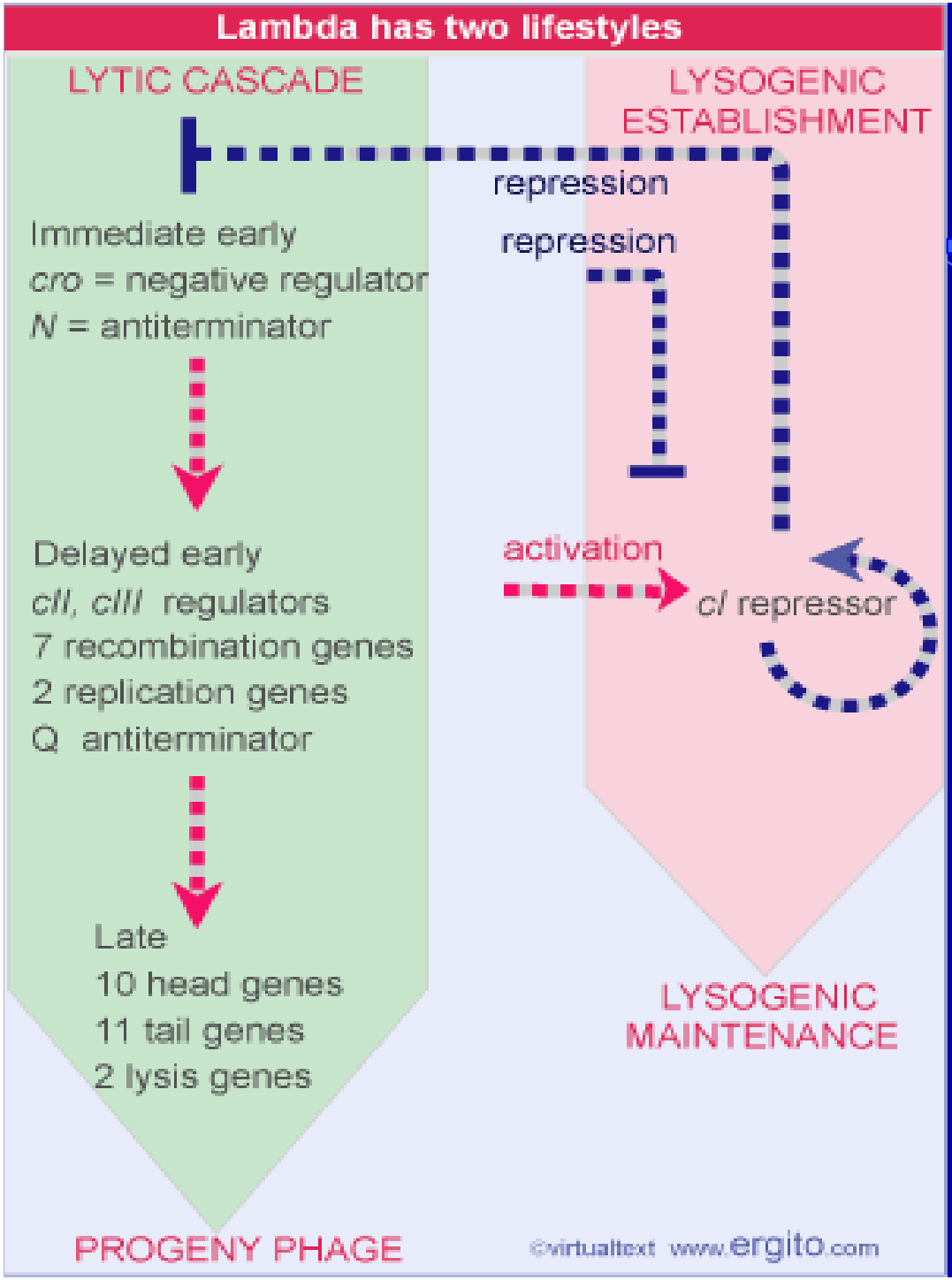
Regulation model

Early stage;





N蛋白、Cro蛋白的功能



时序调控

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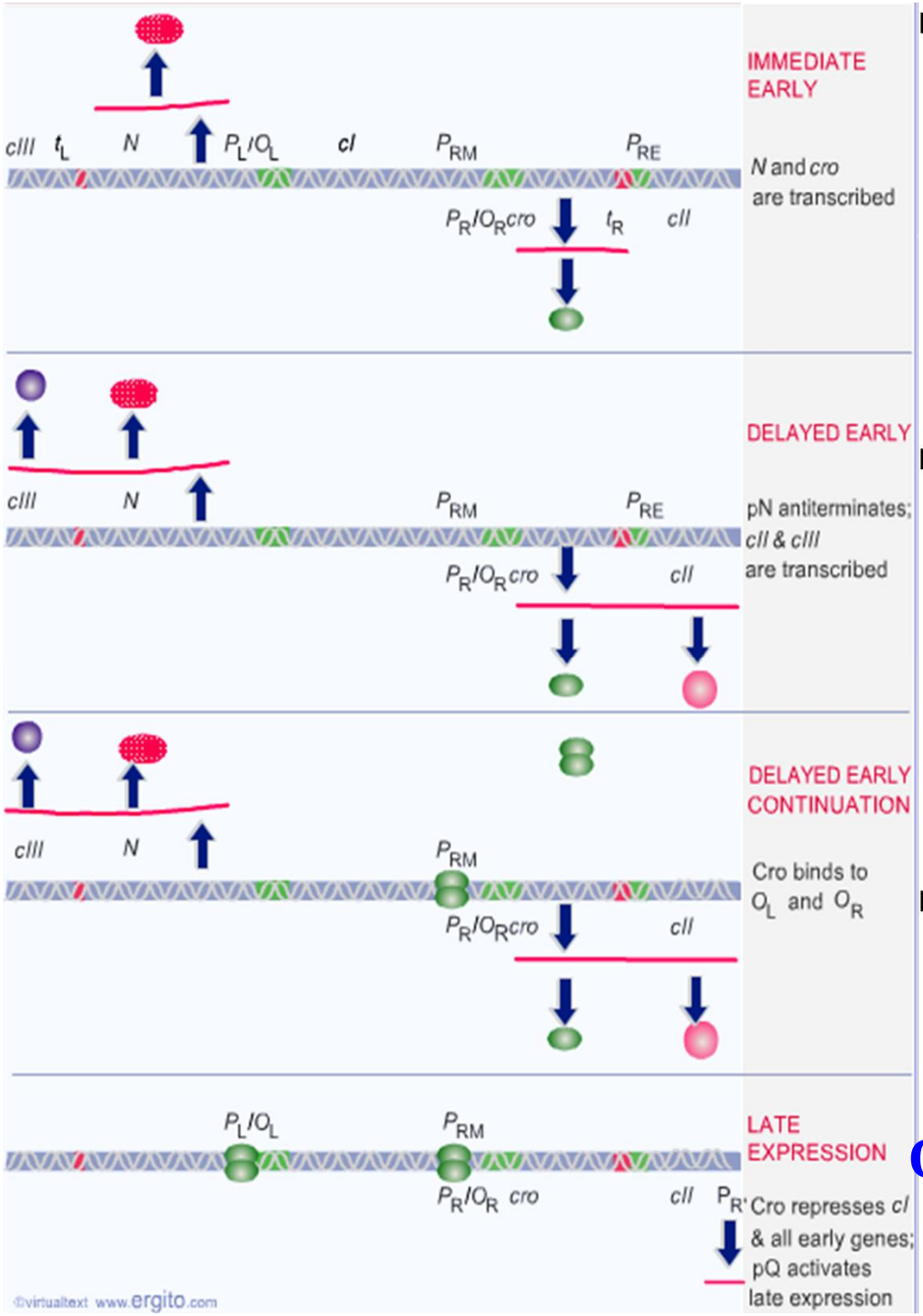
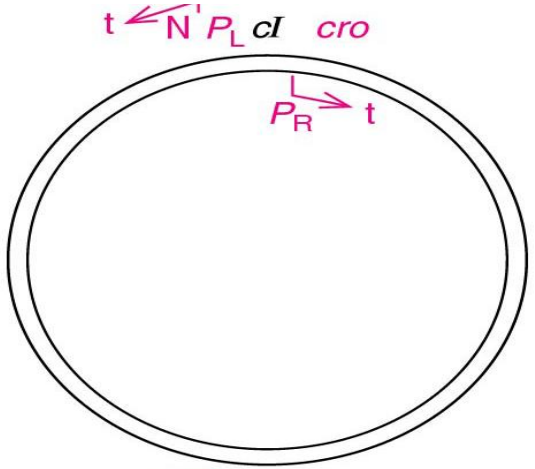
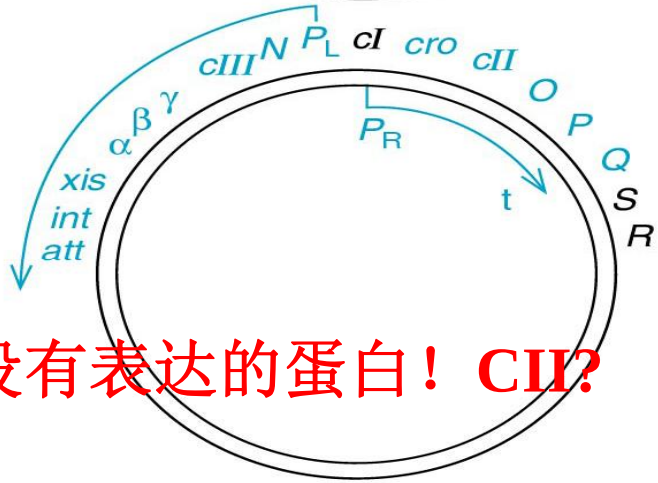
- 10个头部基因
- 11个尾部基因
- 2个裂解基因



Immediate early

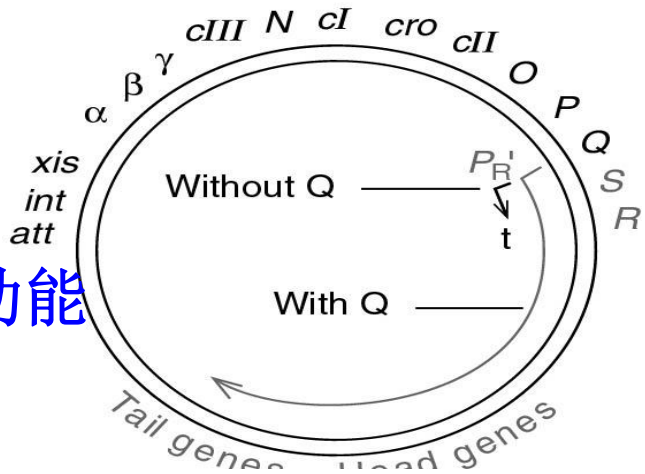


Delayed early

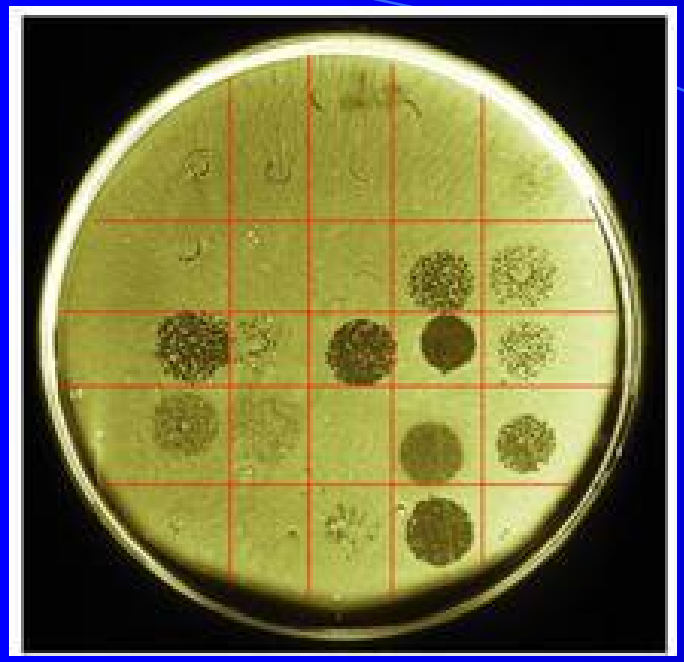


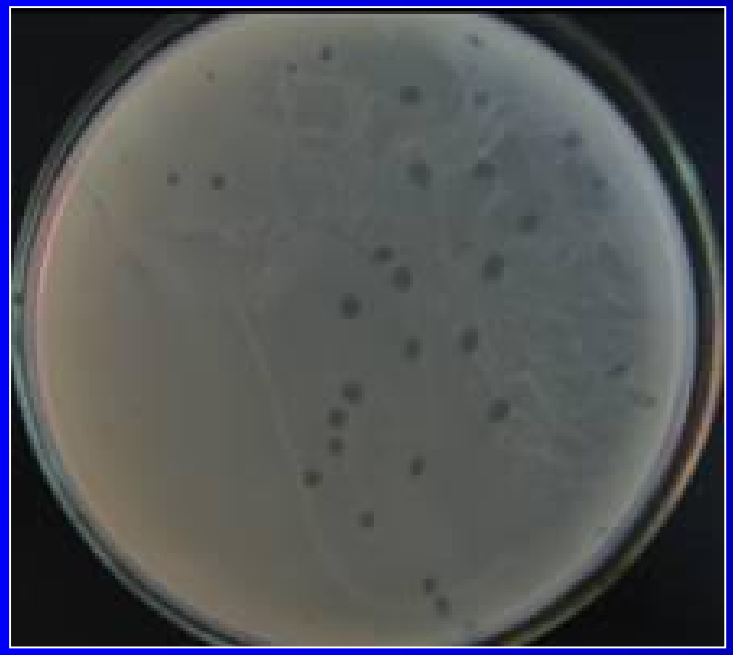
请关注没有表达的蛋白！CII?

Late

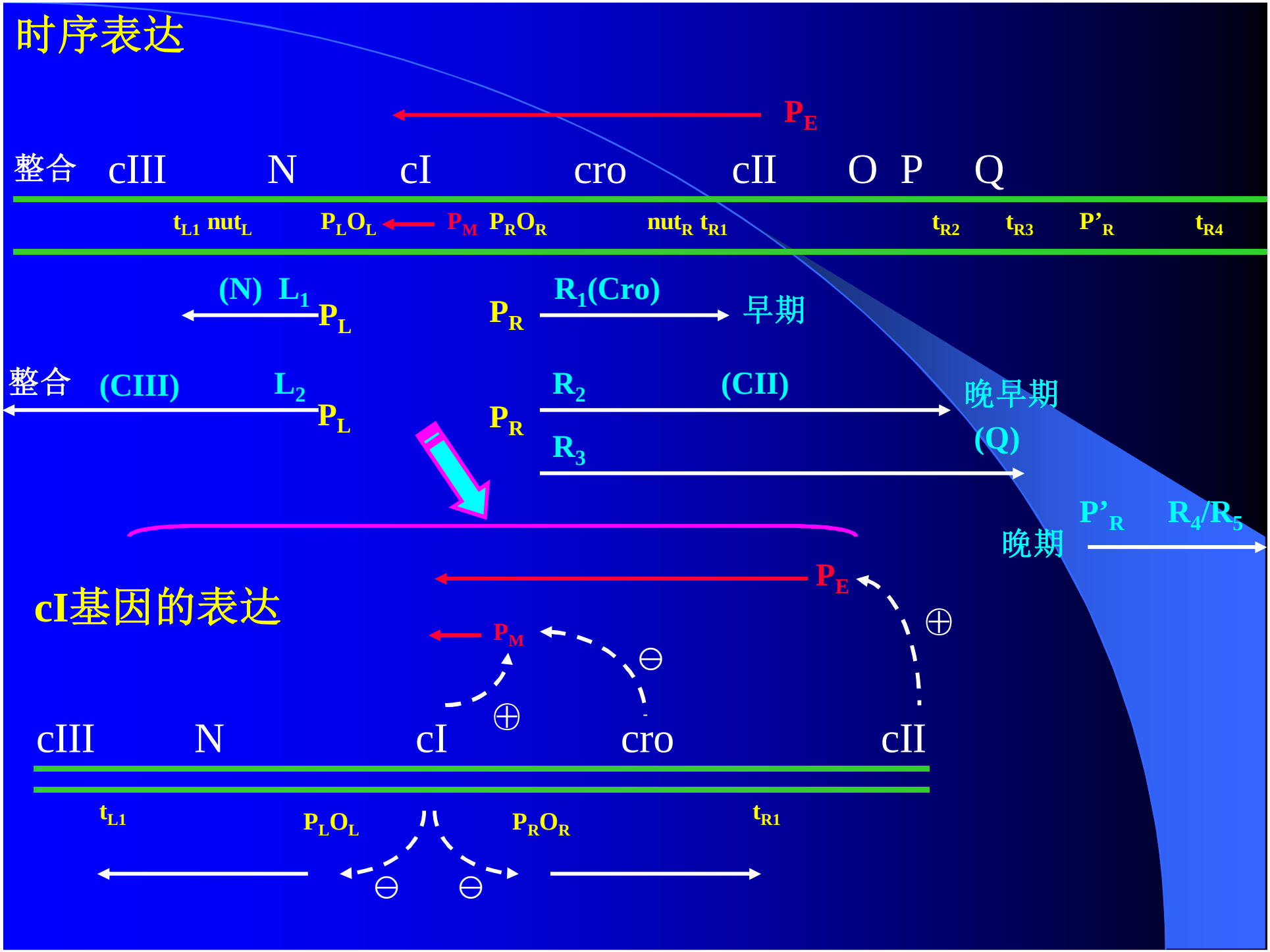


Q蛋白的功能





CI蛋白的功能？


时序表达
P_E
整合 cIII N cI cro cII O P Q
t_{L1} nut_L P_LO_L P_M P_RO_R nut_R t_{R1} t_{R2} t_{R3} P'_R t_{R4}
(N) L_1 P_L
P_R R_1(Cro) 早期
整合 (CIII) L_2 P_L
P_R R_2 (CII) 晚早期
(Q)
R_3
P'_R R_4/R_5
晚期
cI基因的表达
P_E
P_M
cIII N cI cro cII
t_{L1} P_LO_L P_RO_R t_{R1}

λ噬菌体Promoter

◆ *P R/PL： Strong promoter*

Negative control site with CI/ CRO

Promoter for Repressor-Establishment

◆ *P E： Promoter for lysogenic-Establishment*

Weak promoter： Transcription CI gene （**anti-sense CRO RNA**）

Positive control with CII/CIII

◆ *P M： Promoter for lysogenic –Maintenance*

Weak promoter： Transcription CI gene

翻译效率低 **RNA链长短区别/SD序列**

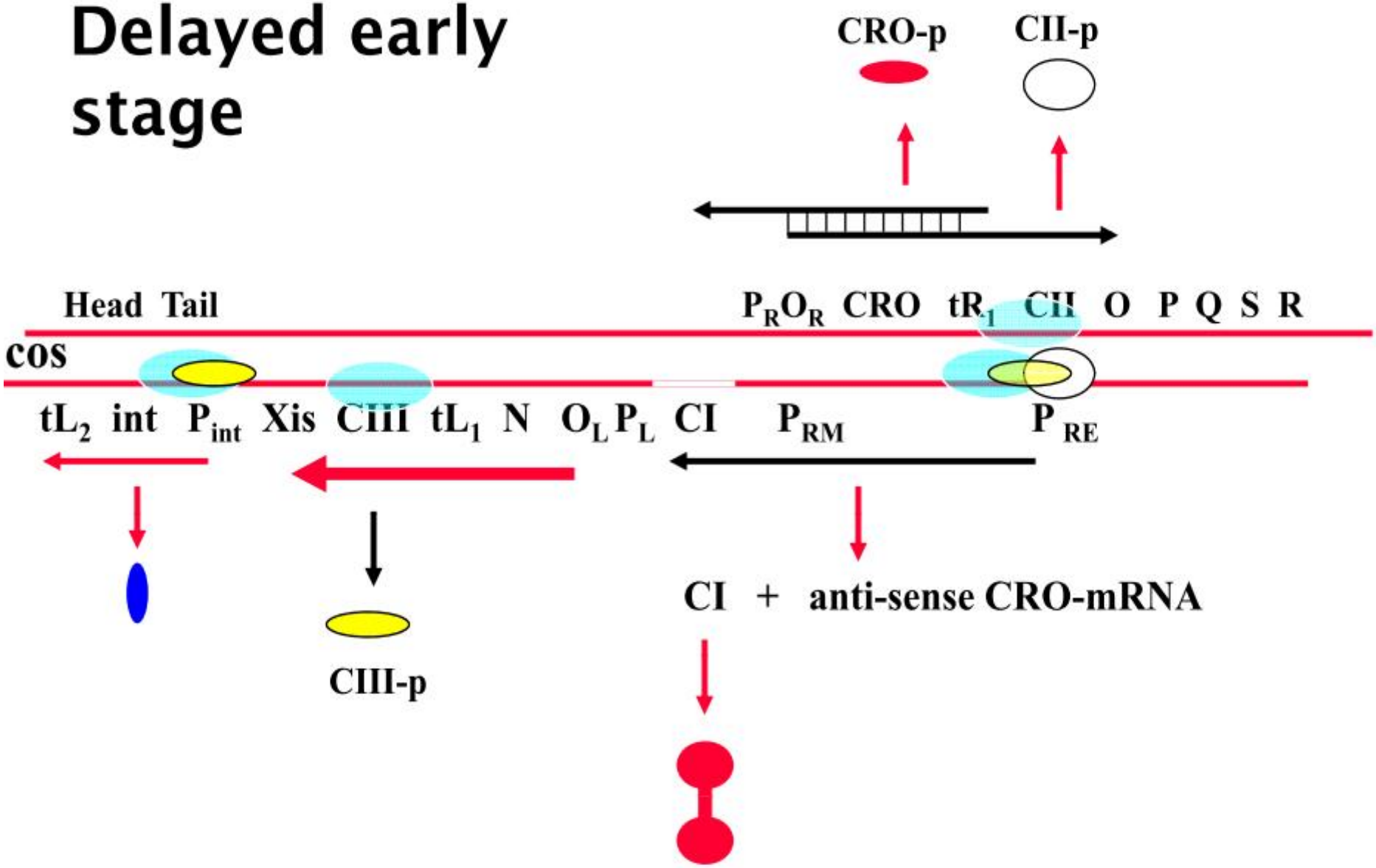
Positive control with ？？

Negative control site with CI/ CRO

P int ： Promoter for Integration

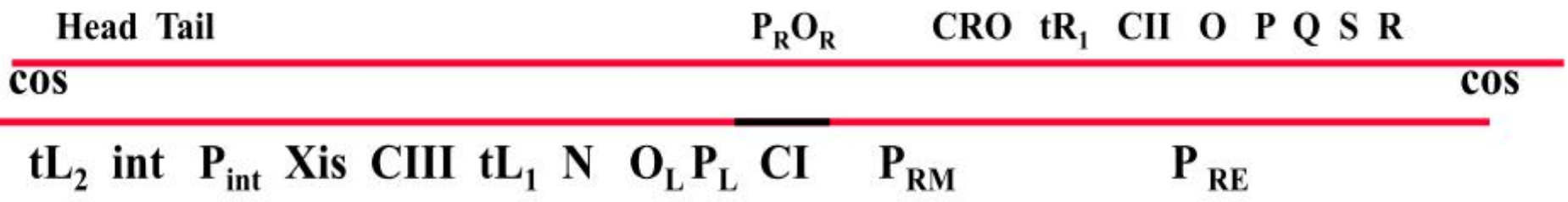
Located on the downstream of CIII, Positive control with CII/CIII

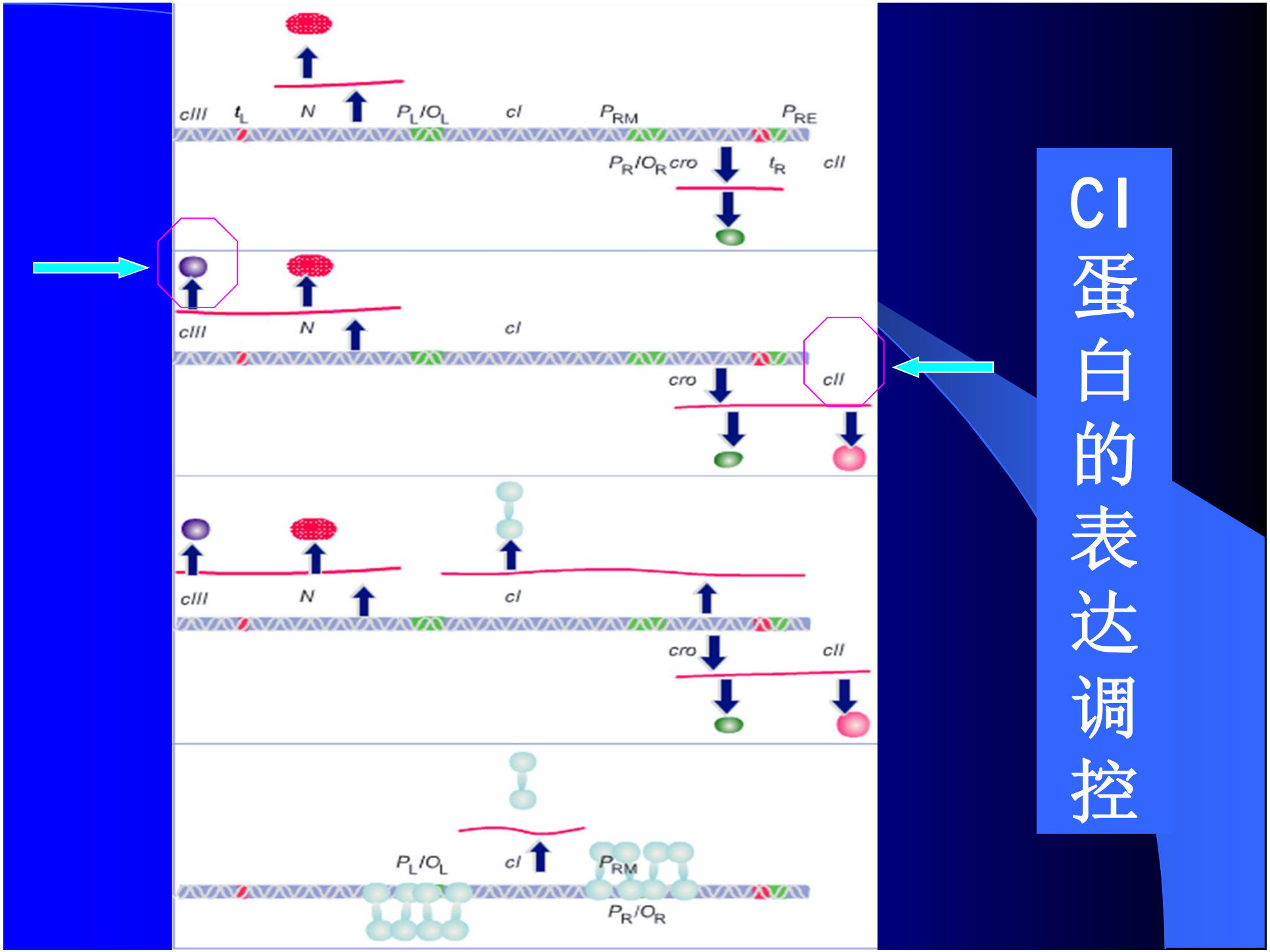
Delayed early stage



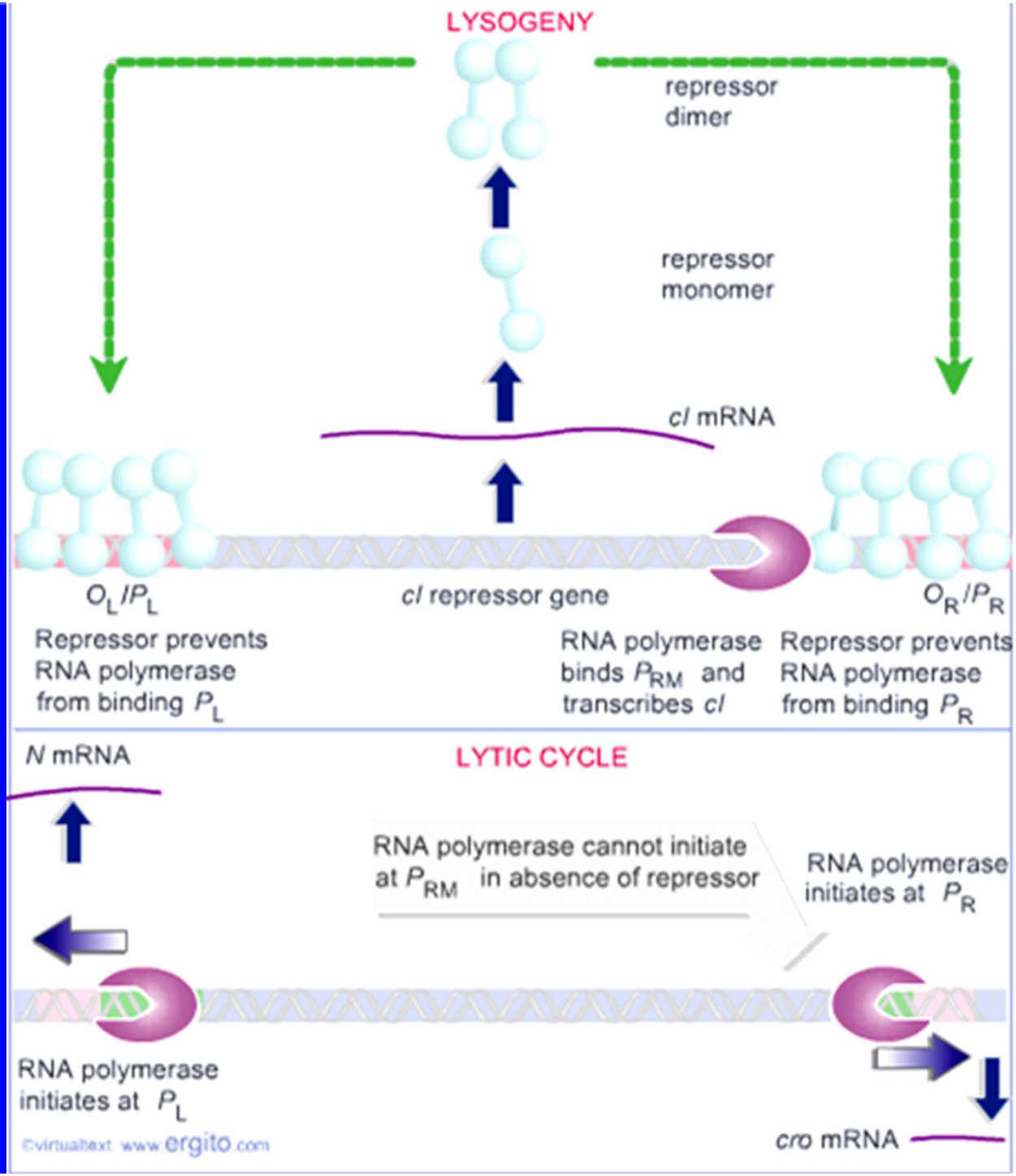
与λ噬菌体溶原选择相关的蛋白

- ？ 通过PE建立溶原，但不能保持溶原
- ？蛋白作为正调启动 PE 转录 CI gene，维持溶原
- CIII 参与溶原建立过程
- N: *Anti-termination protein for delayed early stage*
- Int蛋白




cIII
t_L
N
P_L/O_L
cI
P_{RM}
P_{RE}
P_R/O_R
cro
t_R
cII
cIII
N
cI
cro
cII
cIII
N
cI
cro
cII
P_L/O_L
cI
P_{RM}
P_R/O_R

CI蛋白的表达调控



CI蛋白的功能

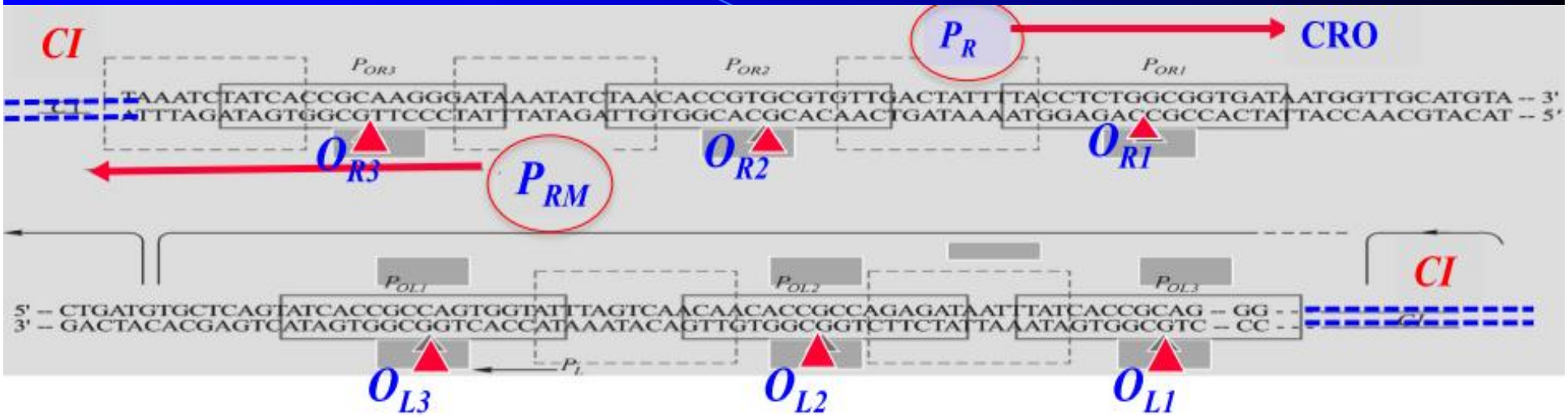
- 阻遏蛋白维持溶原循环过程
- 循环过程一旦被打乱，噬菌体将进入溶菌过程

λ噬菌体溶原状态下表达的蛋白？

决定溶原溶菌选择的分子基础?

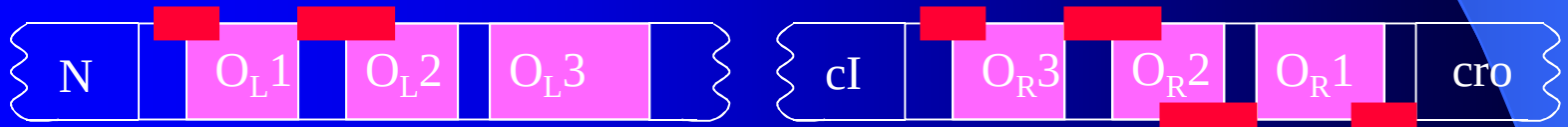
Figure 12.24
Each operator contains three repressor-binding sites, and overlaps with the promoter at which RNA polymerase binds. The orientation of O_L has been reversed from usual to facilitate comparison with O_R.

p /o 结构与启动子活性



左启动子P_L

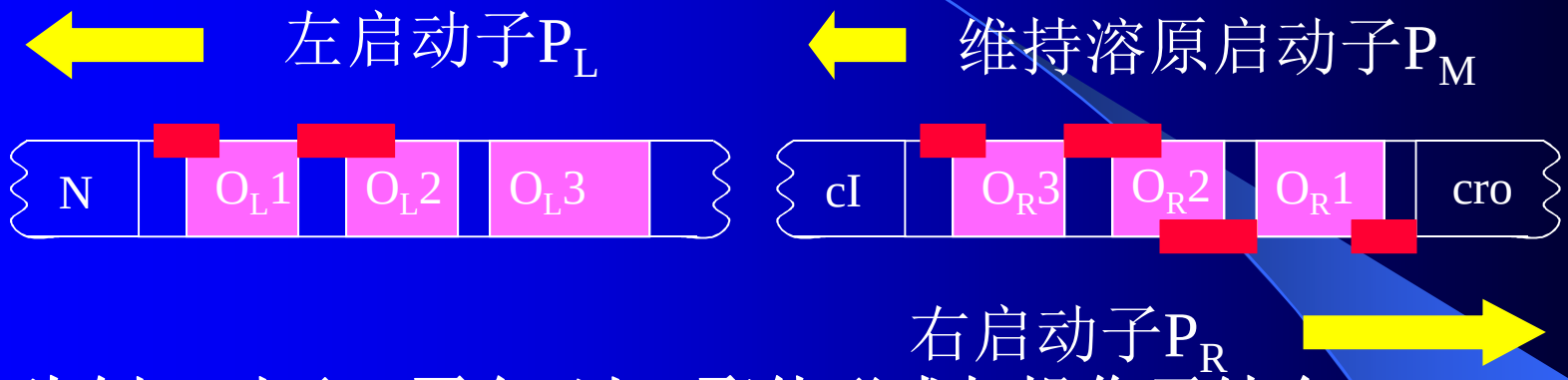
维持溶原启动子P_M



右启动子P_R

promoter /operator 结构互相重叠

O1、O2、O3　三个位点与CI亲和力不同

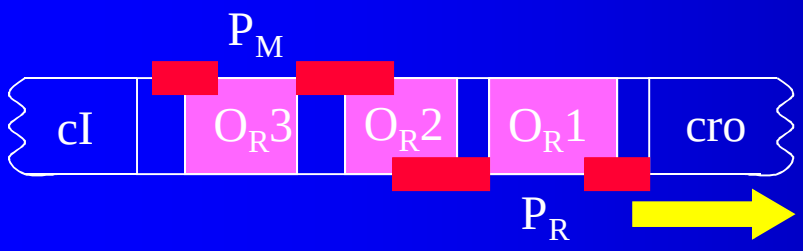


以O_R为例，对于CI蛋白，以二聚体形式与操作子结合：

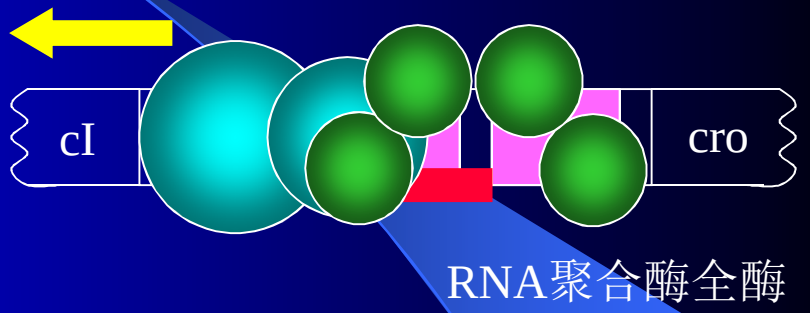
- CI蛋白亲和力：O_R1》O_R2》O_R3
- CI浓度较低时，与O_R1结合，阻止P_R、P_L 转录　（空间环化）
- CI浓度较高时，还与O_R2，促进P_M转录
- CI浓度很高时，进一步与O_R3结合，占据P_M －10区、－35区，阻断P_M进行的CI基因的转录－－－负反馈

CI蛋白与O_R结合对P_R、P_M活性的影响

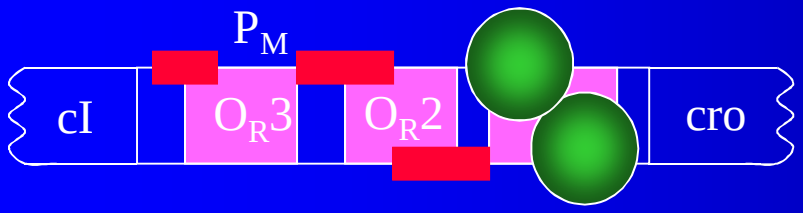
P_R有活性；P_M无活性



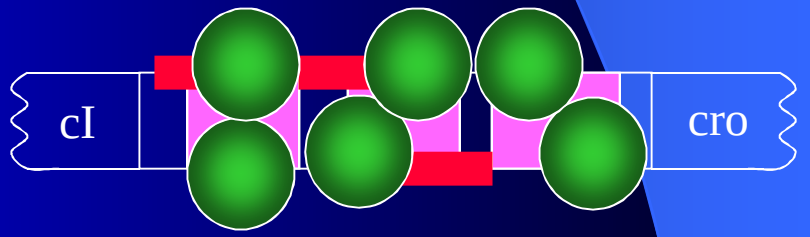
P_M有活性；P_R无活性



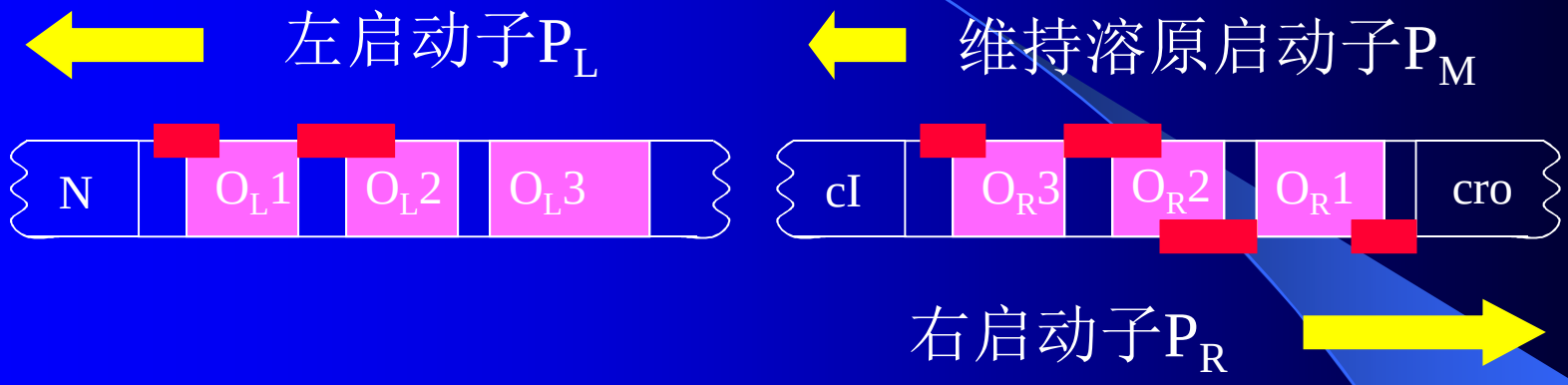
P_R和P_M无活性



P_R和P_M无活性



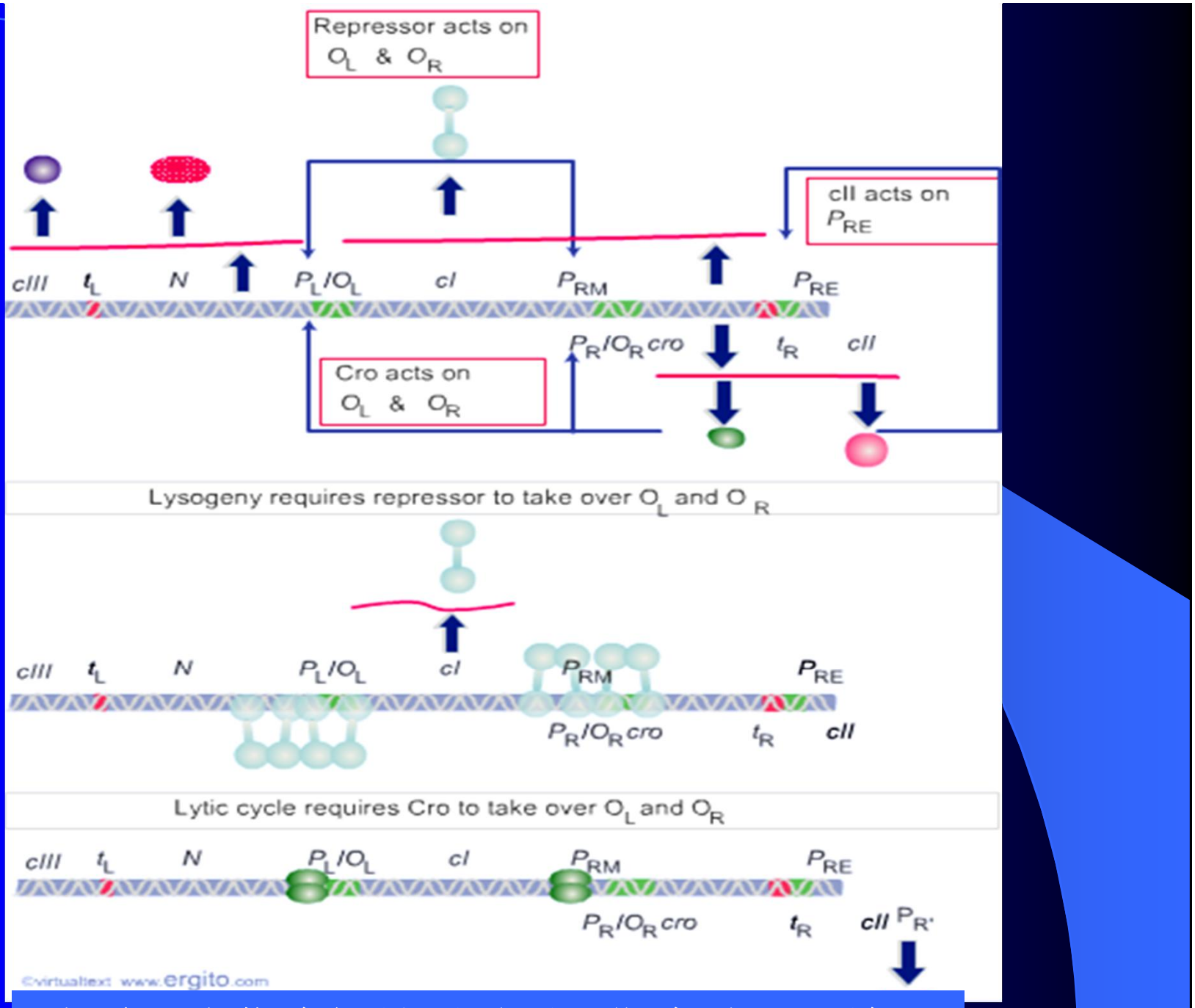
O1、O2、O3　三个位点与Cro亲和力不同



以O_R为例：对于Cro蛋白，也以二聚体形式与操作子结合

- Cro蛋白亲和力：O_R3》O_R2》O_R1
- Cro浓度较低时，与O_R3结合，阻止CI表达，P_R转录
- Cro浓度较高时，还与O_R2 结合，对P_R也有阻遏作用
- Cro浓度很高时，进一步与O_R1结合 ，阻断P_M、P_R转录

  因为Q大量表达，　P_R,顺利启动



溶原和溶菌途径是否需要早期启动子开放？

溶原和溶菌的选择

- CI和Cro阻遏

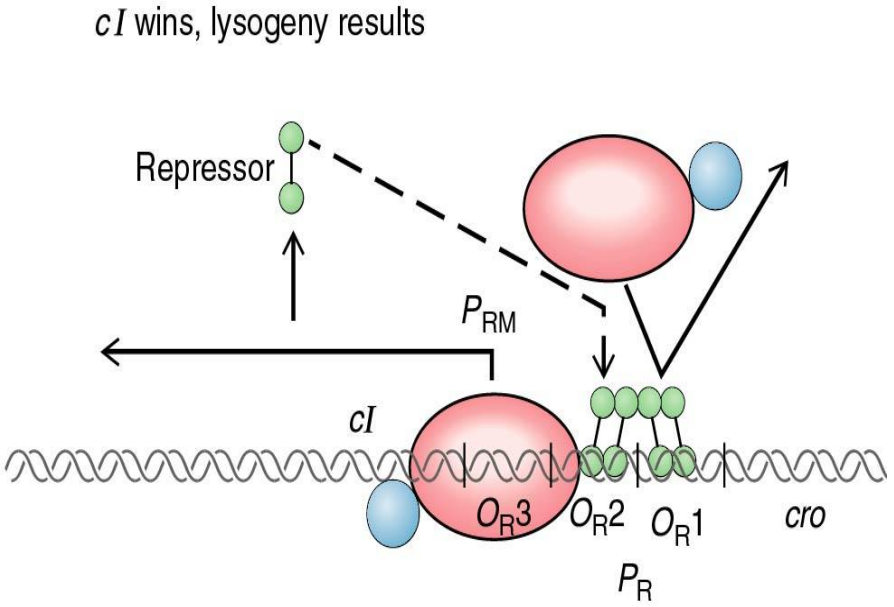
  P_L、P_R目的不同：

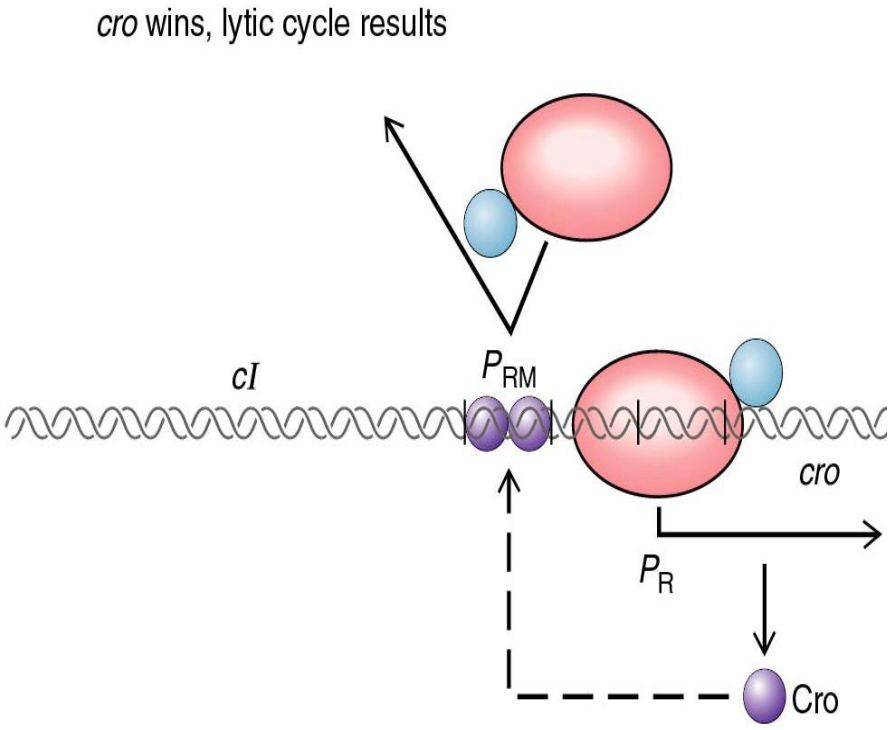
  CI———溶原

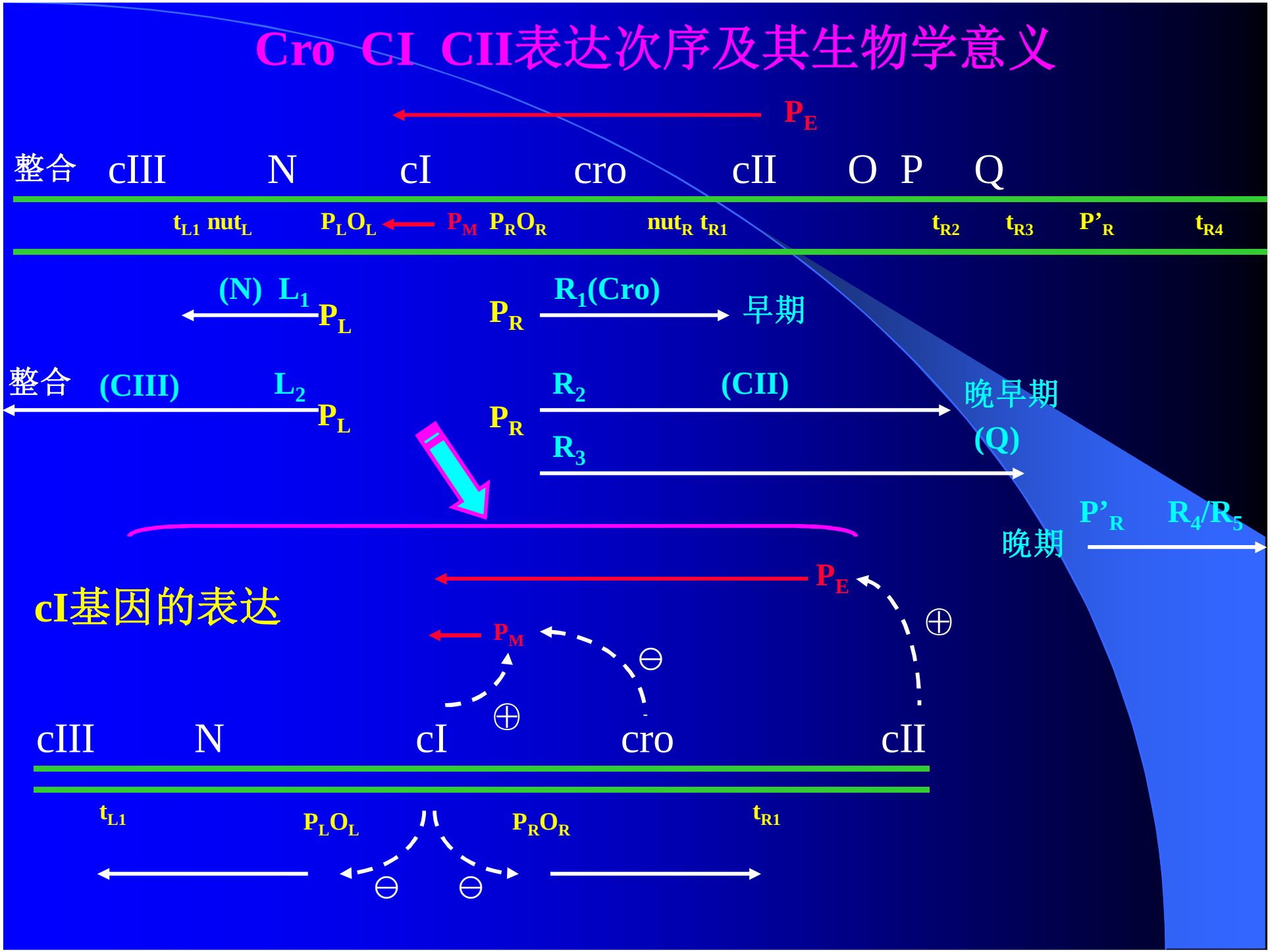
  Cro———溶菌

- 溶原和溶菌的选择：

C？？和Cro竞争Opereator






Cro CI CII表达次序及其生物学意义
P_E
整合 cIII N cI cro cII O P Q
t_{L1} nut_L P_LO_L P_M P_RO_R nut_R t_{R1} t_{R2} t_{R3} P'_R t_{R4}
(N) L_1 P_L
P_R R_1(Cro) 早期
整合 (CIII) L_2 P_L
P_R R_2 (CII) 晚早期
(Q)
R_3
P'_R R_4/R_5
晚期
cI基因的表达
P_E
P_M
cIII N cI cro cII
t_{L1} P_LO_L P_RO_R t_{R1}

思考题

预测表型：

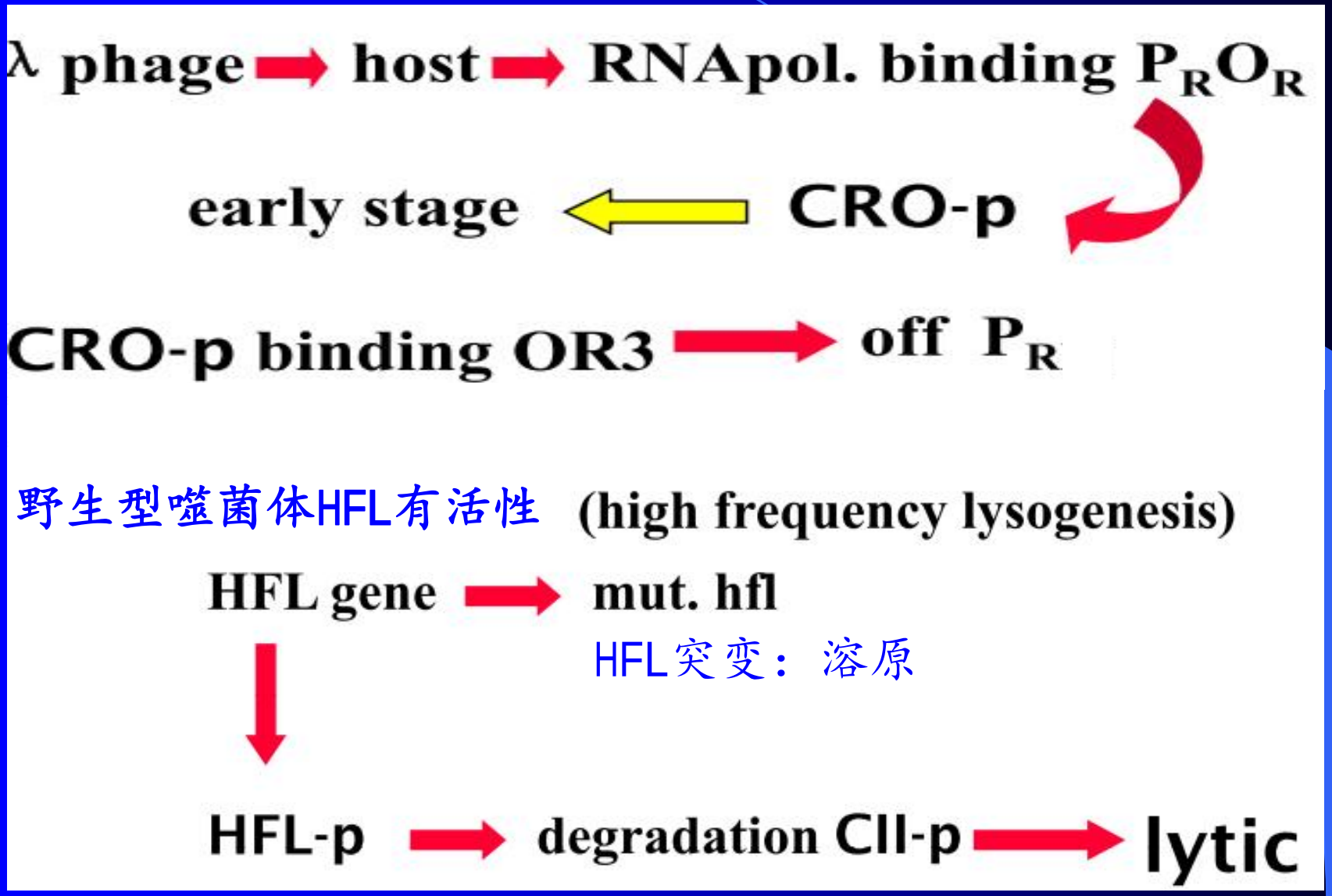
Cro蛋白表达被CI蛋白抑制

CI蛋白突变

CII蛋白突变

N蛋白突变

λ噬菌体感染宿主目的


λ phage → host → RNApol. binding P_RO_R
early stage ← CRO-p
CRO-p binding OR3 → off P_R
野生型噬菌体HFL有活性 (high frequency lysogenesis)
HFL gene → mut. hfl
HFL突变：溶原
HFL-p → degradation CII-p → lytic

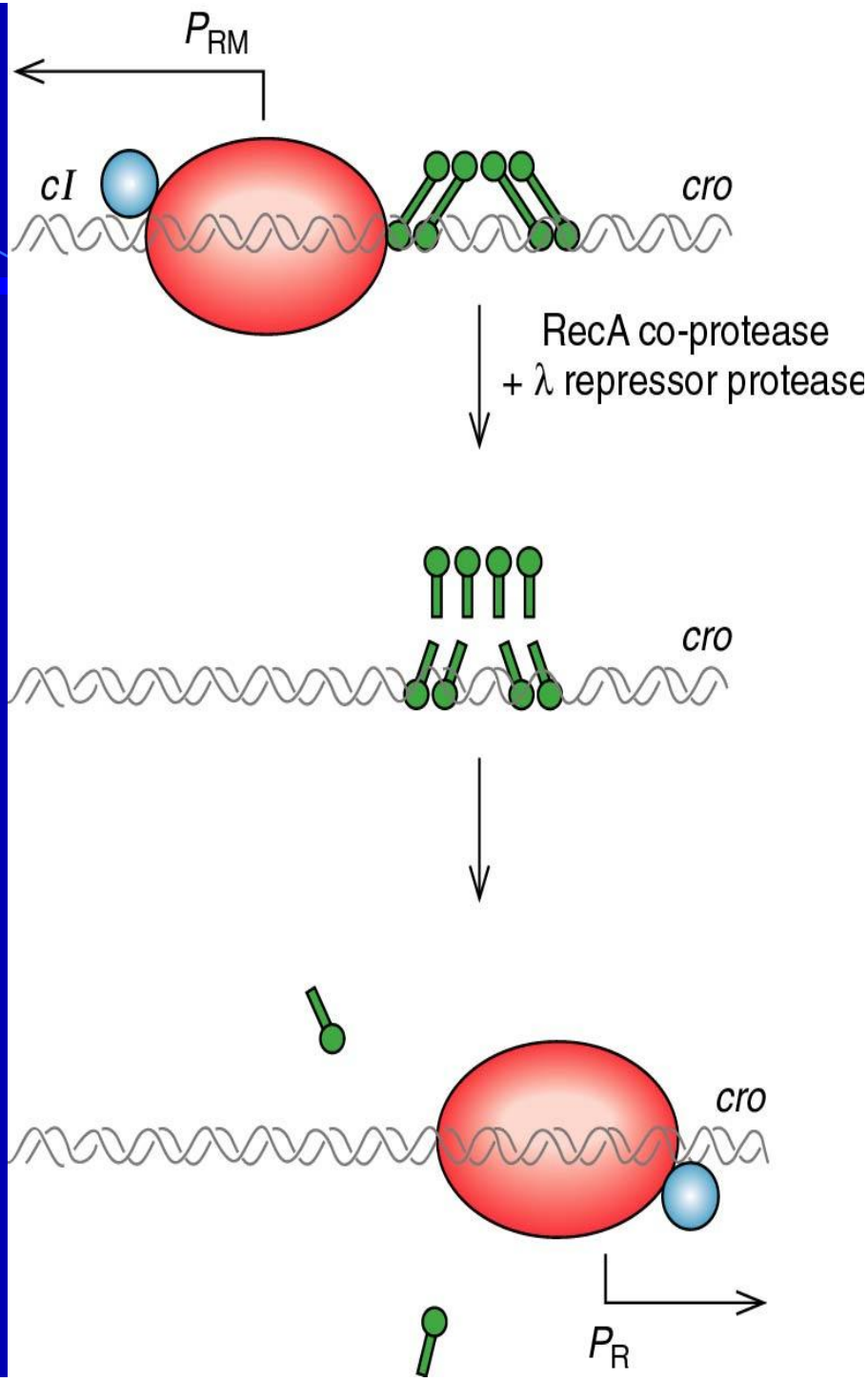
λ噬菌体溶菌建立

- 紫外线或丝裂霉素：
- recA蛋白激活 ⟶

  CI蛋白水解（111—112）

  ⟶ P_R开放

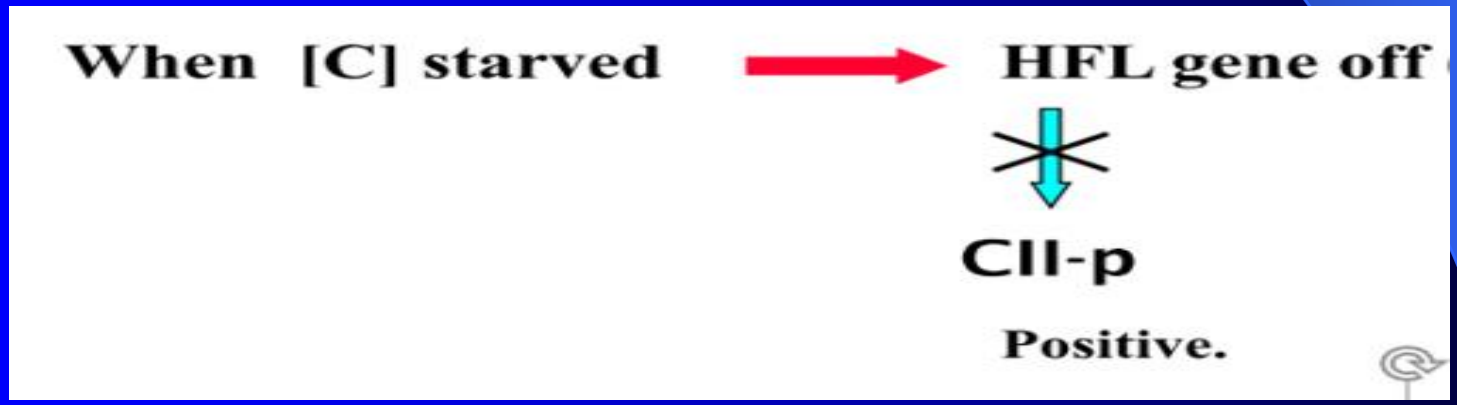
  Cro、Q蛋白大量产生



λ噬菌体溶原化途径的建立

- 营养耗竭：

  cAMP水平上升 ⟶ 宿主hf1蛋白表达下降 ⟶ CII蛋白稳定性提高

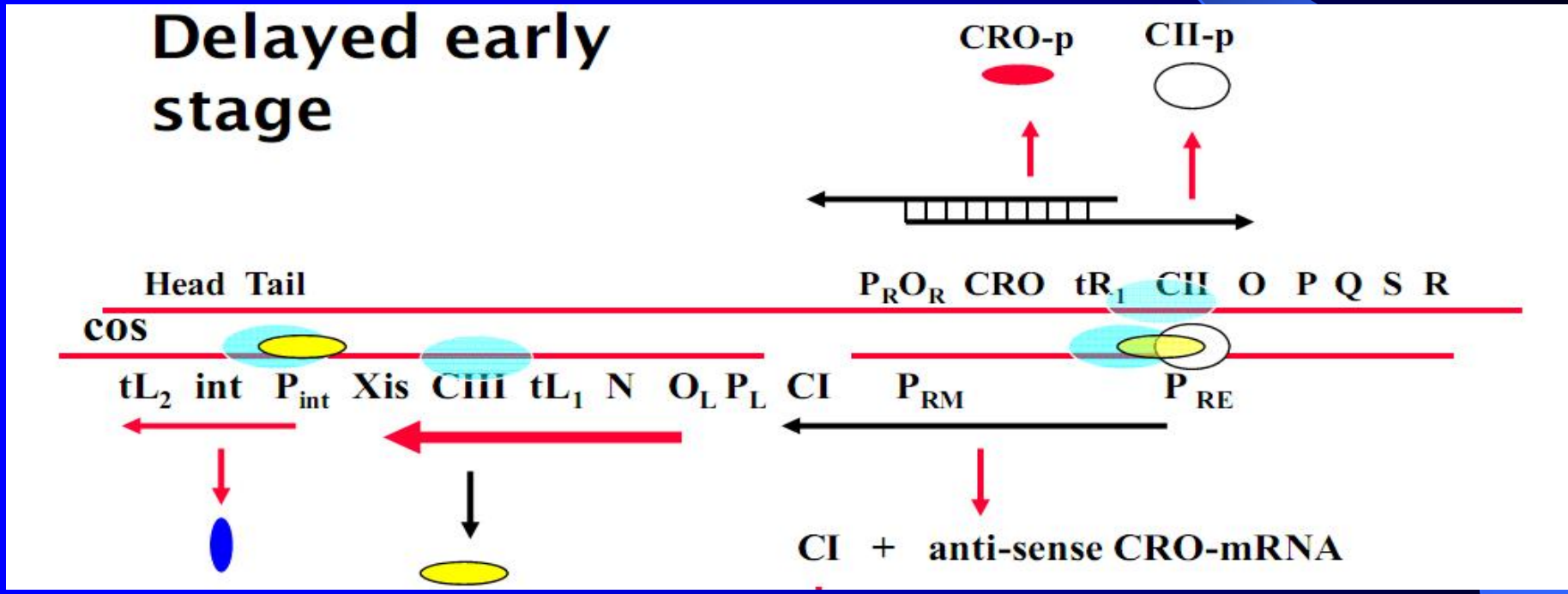


- 多重感染(Multiplicity of infection) MOI ≥10
  意味营养不足 ⟶ CII蛋白稳定性提高

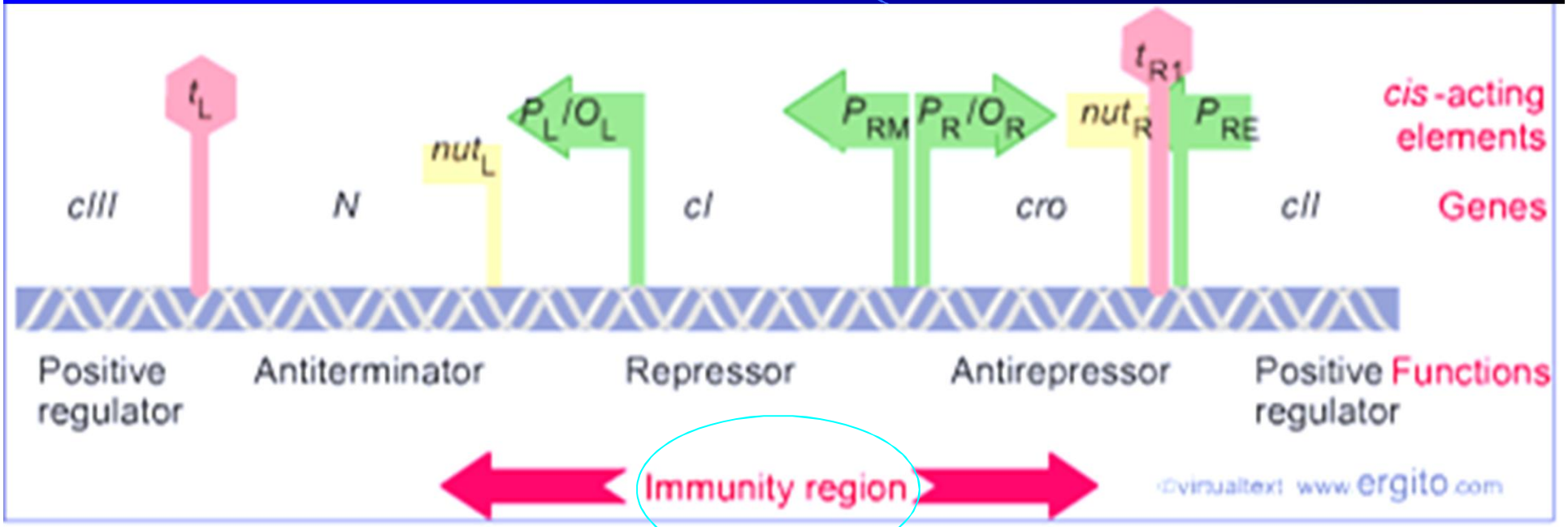
其它

启动子P_E的作用

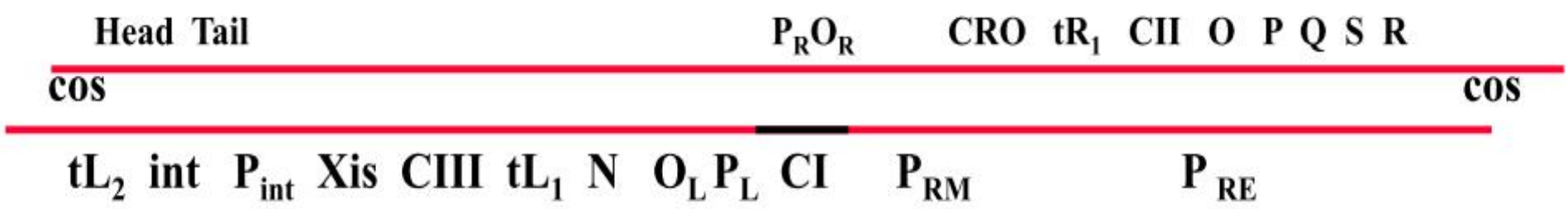
- 表达CI蛋白，翻译效率比P_M高7～8倍
- 使转录以相反的方向经过cromRNA的反义RNA



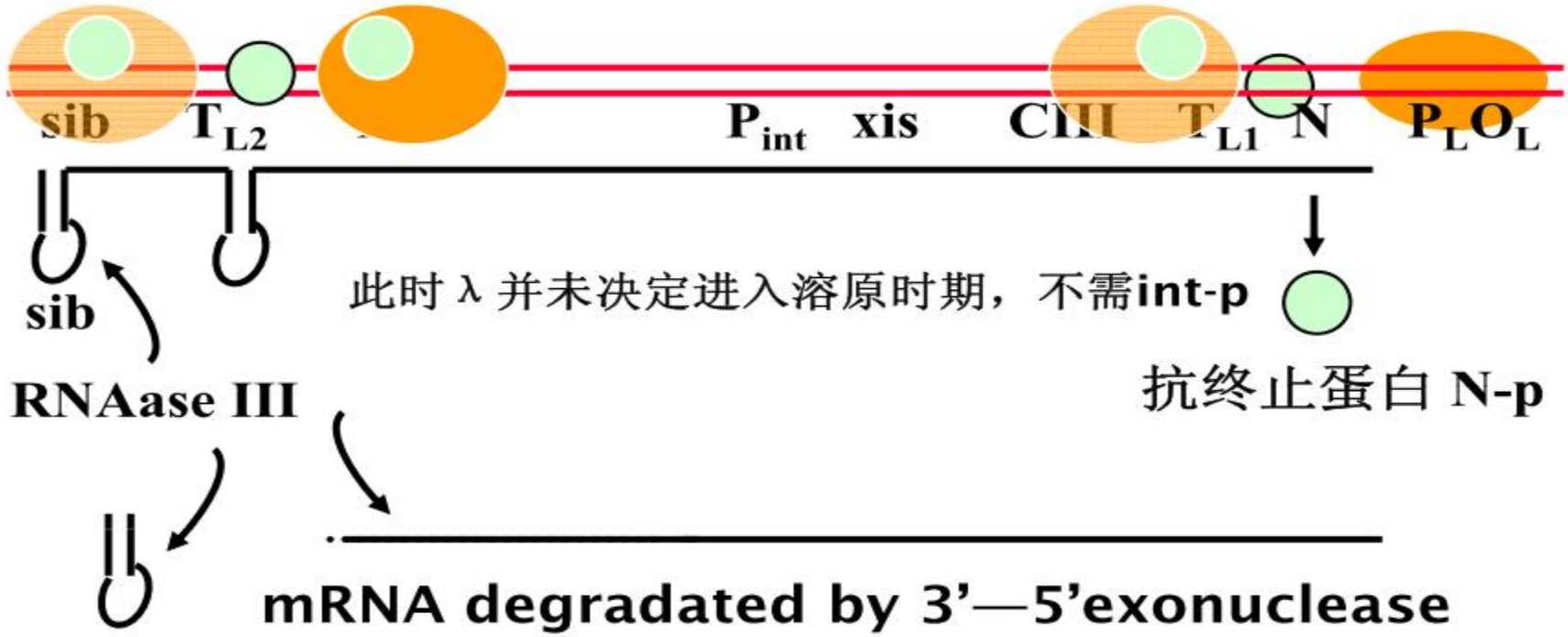
λ噬菌体的调控区



λ噬菌体的免疫区域

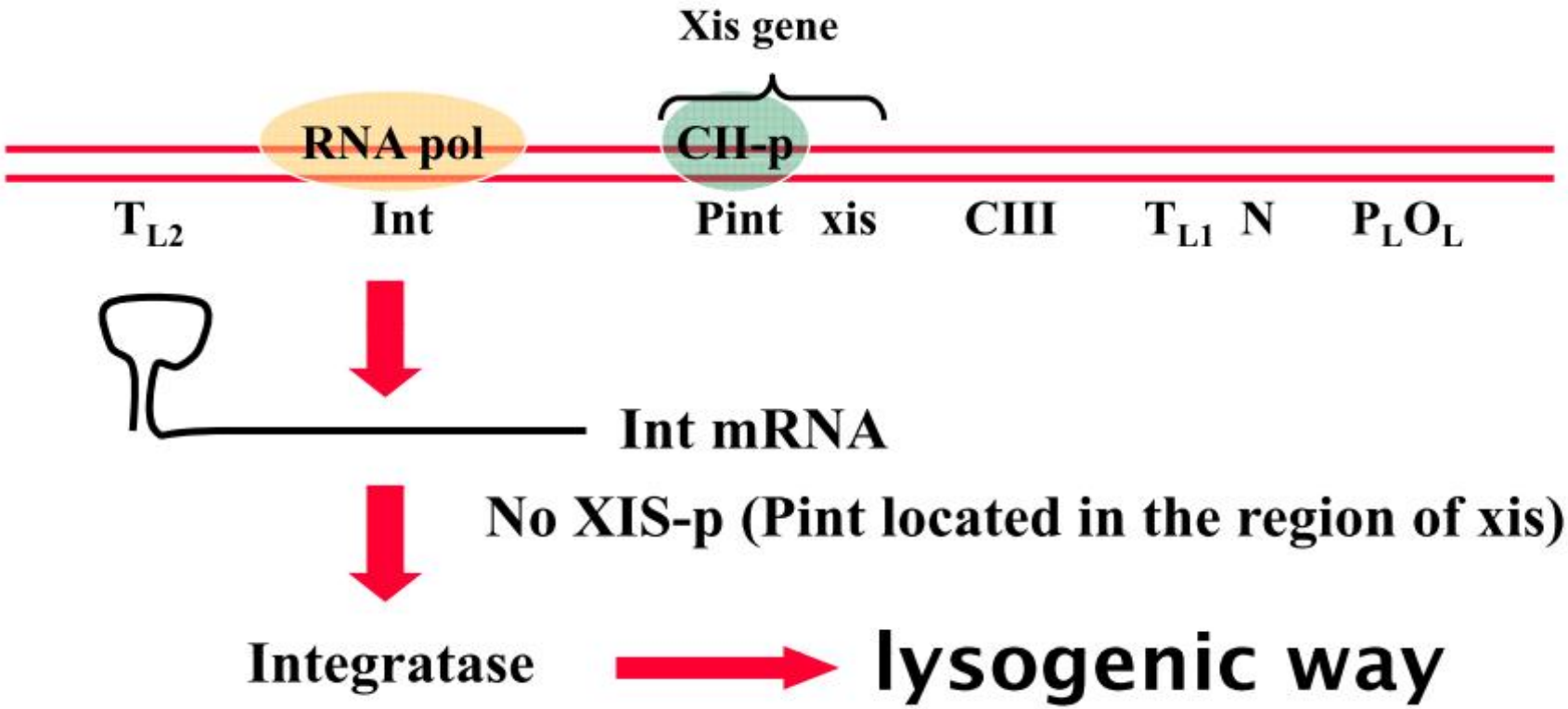


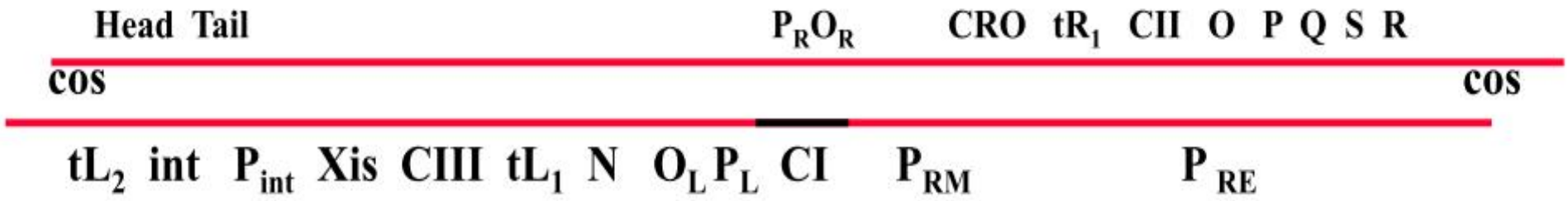
- ***Int*** **gene and retro-regulation of *sib* site**



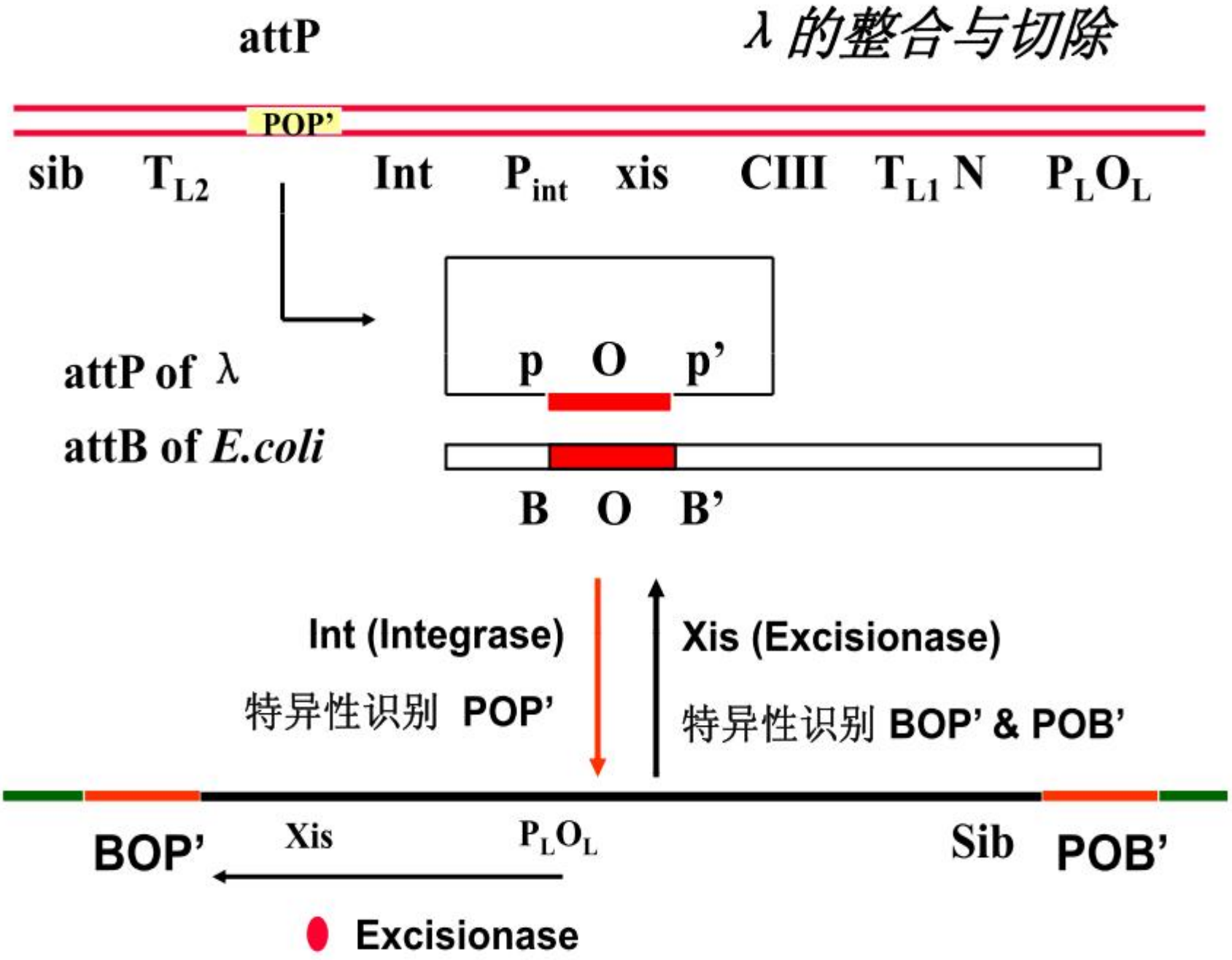
sib site negative control Int gene by sib (retro-regulation)

When lysogenic way be selected





λ 的整合与切除


attP
POP'
sib
T_{L2}
Int
P_{int}
xis
CIII
T_{L1} N
$P_L O_L$
attP of λ
p O p'
attB of *E.coli*
B O B'
Int (Integrase)
特异性识别 POP'
Xis (Excisionase)
特异性识别 BOP' & POB'
BOP'
Xis
$P_L O_L$
Sib
POB'
Excisionase