第6章 基因表达调控

(Controlling of the Gene Expression)

(Source:Pique,Michael and Peter E.Wright,Dept.(cover photo,science 245 (11 Aug 1989).))

6.1. transcriptional level control

6.2. post-transcriptional level control

6.3.Gene expression control in translation level

6.4. 染色质重建对基因表达的控制

6.5.Programmed Cell Death (PCD) and development

基因表达调控的简介

阐明的科学问题

真核与原核生物如何调控数以千、万计的基因以最为经济、有效的时空模式进行转录，从而实现对环境的适应、细胞的分化, 组织的特化 和个体的发育。

原核生物与真核生物基因表达调控机制具有惊人的相似性

共同的起源与共同的分子基础

- 调控机理上
  - 核酸分子间的互作
  - 核酸与蛋白质分子间的互作
  - 蛋白质分子间的互作
- 调控层次上
  - **transcriptional level**
  - **post—transcriptional level**
  - **translational level**
  - **post—translational level**

基因表达的调控涉及

RNA转录的开/关

数量

选择性加工

蛋白质翻译速率

数量

加工、降解和分泌…

转录水平上的调控是最为经济，灵活，又是最为重要，复杂的调控

- 在复杂的基因组内，确定需要基因转录的起始位点
- 精细调节基因表达的水平，以保证生物体对环境的适应
- cis factor & trans factor 间严格而又灵活的互作
- 保证RNA polymerase 的进行式转录（不中断，准确终止）

遗传信息的概念

C > c

全基因组

10%；结构基因的编码序列

triplet codon（I类遗传信息）

90%；重复，调节序列, Non coding RNA…

基因选择性表达指令

重要的遗传信息（II类遗传信息）

遗传信息表达的方式

- 组成型表达（constitutive expression）

Housekeeping gene

- 诱导型表达

（inducible expression by signaling molecular）

Luxury gene

- 顺、反因子间和反式因之间的互作方式

Epistasis effect

6.1. transcriptional level control

Prok.

operon

stringent response

attenuator

transposon…

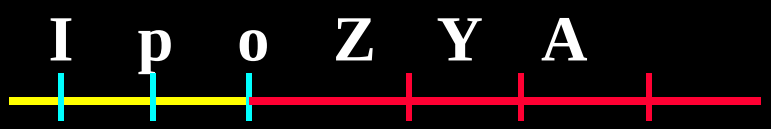
6.1.1. Operon control

(1961 Jacob. & Monod.)

a) operon concept

- 控制某一代谢途径的相关基因,
  紧密连锁地排列在一起, 受同一操纵子控制

Lac operon



- 各结构基因按一定比例协调翻译 (Z : Y : A = 5 : 2 : 1)
- 具有极性突变效应
- P & O基因(cis)紧密连锁 或 彼此重叠
  I 基因(trans) 位点不固定

b) operon control type

Negative & Positive

- I gene → repressor → Negative
- I gene → expressor (apoinducer) 无辅基诱导物 → Positive

i^- or 不加入I基因产物 → operon on

I^+ or 加入I基因产物 → operon off

Neg.

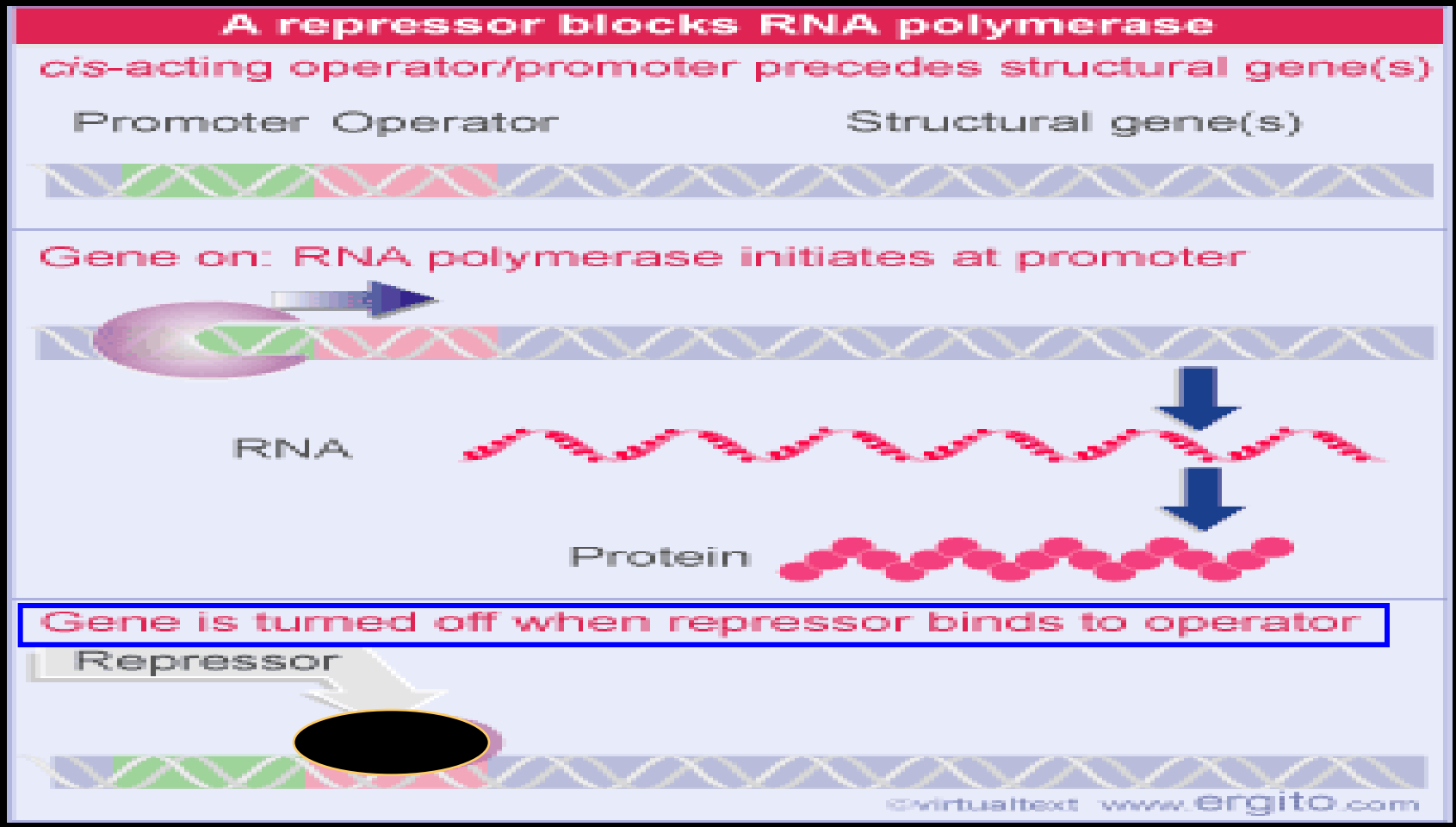
i^- or 不加入I基因产物 → operon off

I^+ or 加入I基因产物 → operon on

Pos.

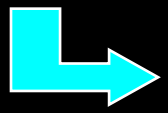
- **Repressor binding on O site**

阻止转录启动



(来源：分子生物学（2007），郑用琏，第239页)

- Expressor binding front p site



激活转录启动

- Operon off  意味转录效率极低

- Negative control 是广泛保险的机制

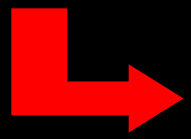
（自然选择使 Prok. 获得选择优势）

Positive control 是灵活，严格，经济的调控机制

c) Model

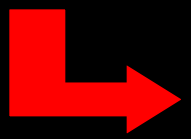
- **Signal molecular be needed for both types**

Add signal mol. → Operon on (inducible operon)



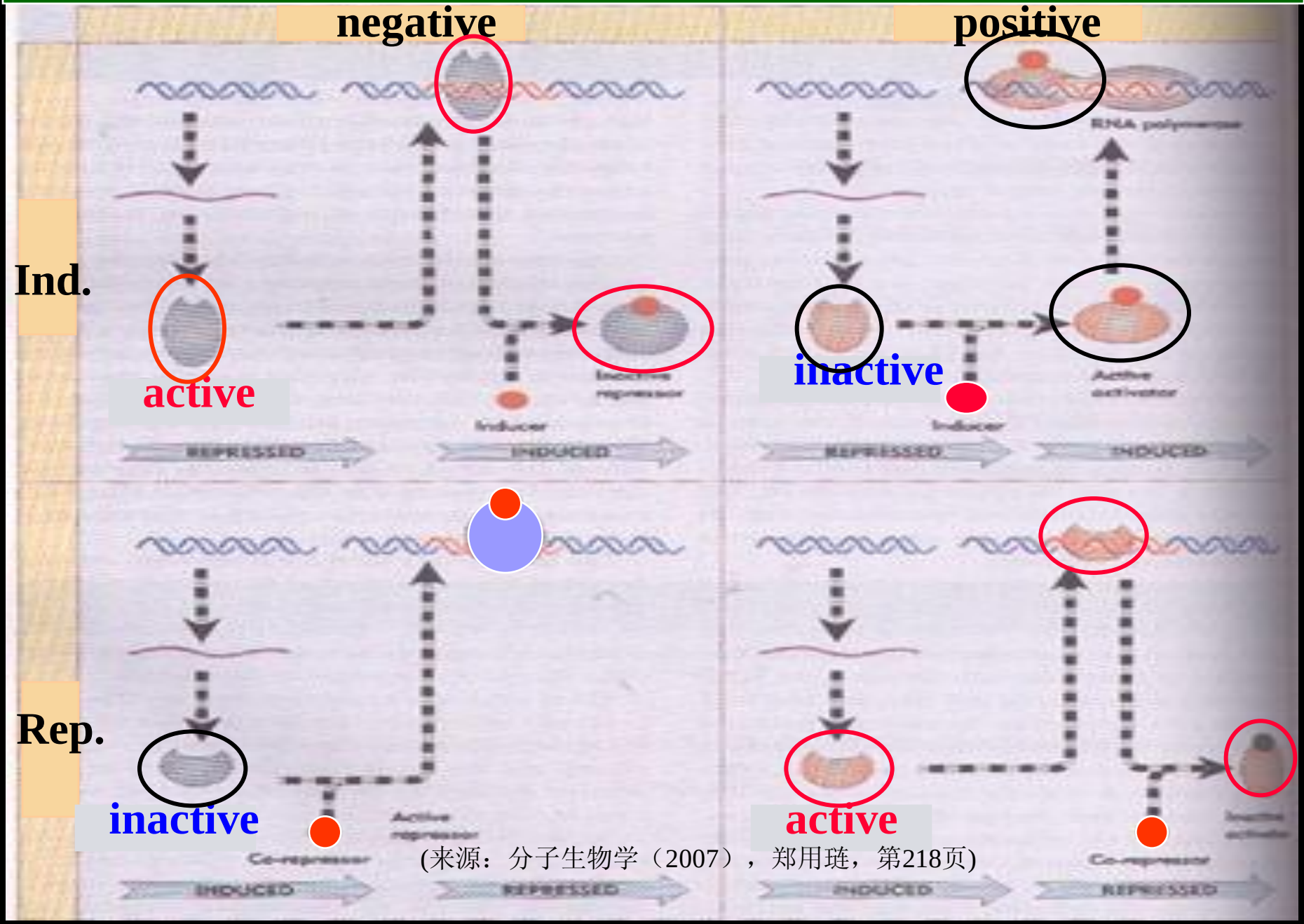
Inducer

Add signal mol. → Operon off (repressible operon)



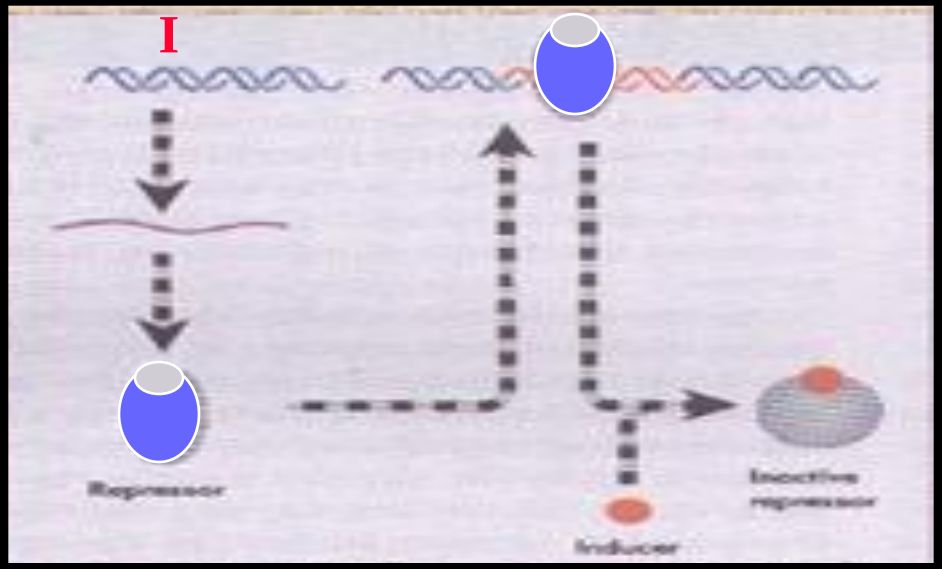
Co-repressor

Operon control model



(来源：分子生物学（2007），郑用琏，第218页)

• **Negative—inducible operon** 例 (分解酶类 lactose operon)



(来源：不详)

I gene → active repressor

38 kd / monomer

↓

tetramer 152 kd

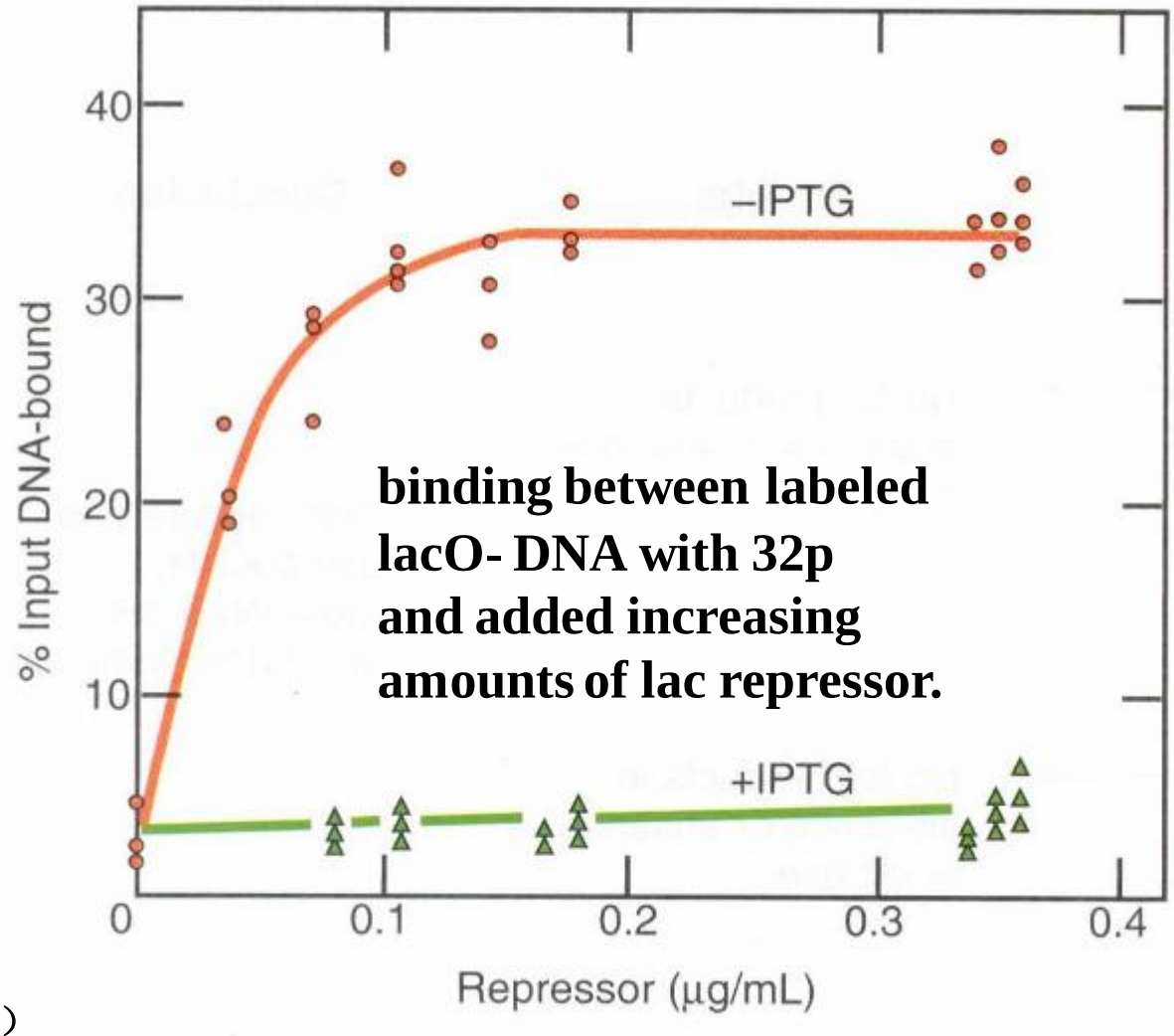
↓

binding on Operator

I^+ ⟶ mut. → i^C (constitutive mut.)

→ i^S (super--reperessible)

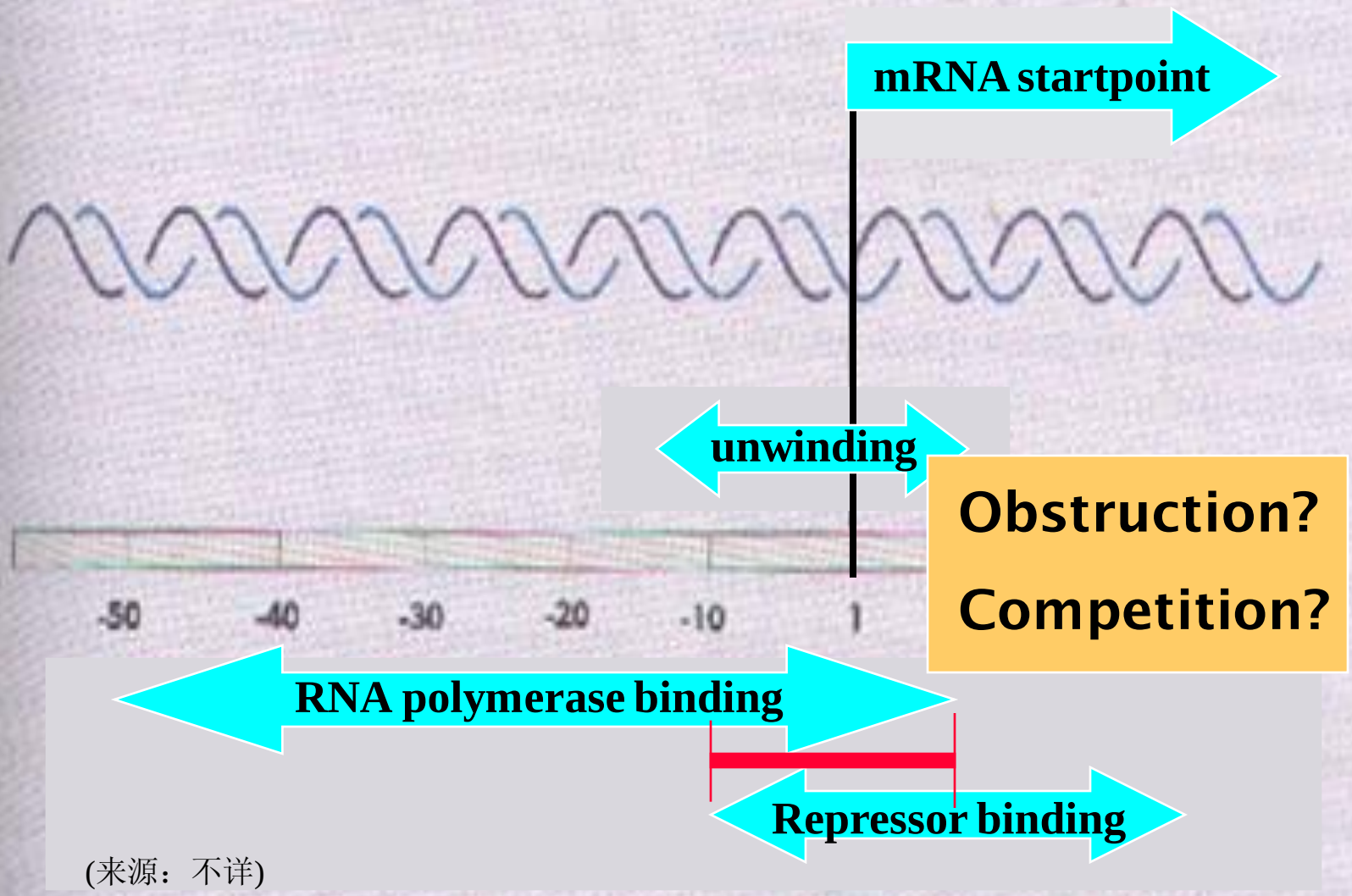
Lac or IPTG lactose analog is inducer of Lac operon



IPTG;异丙基－β －D－硫代半乳糖苷，自己不被分解的强诱导物（安慰诱导物）

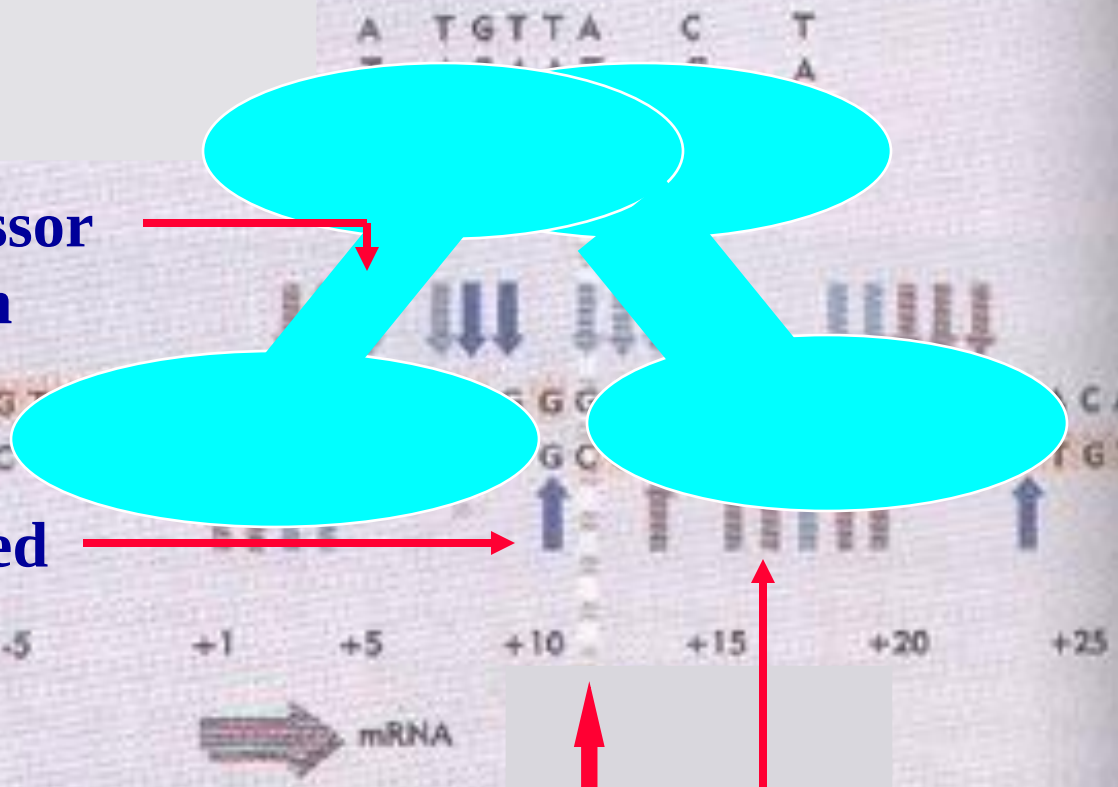
(Source:Cohn. Journal of Molecular Biology, Vol. 34: 366. 1968)

O gene (operator) cis-action factor



(来源：不详)

O gene (operator) cis-action factor



Protected by repressor against methylation

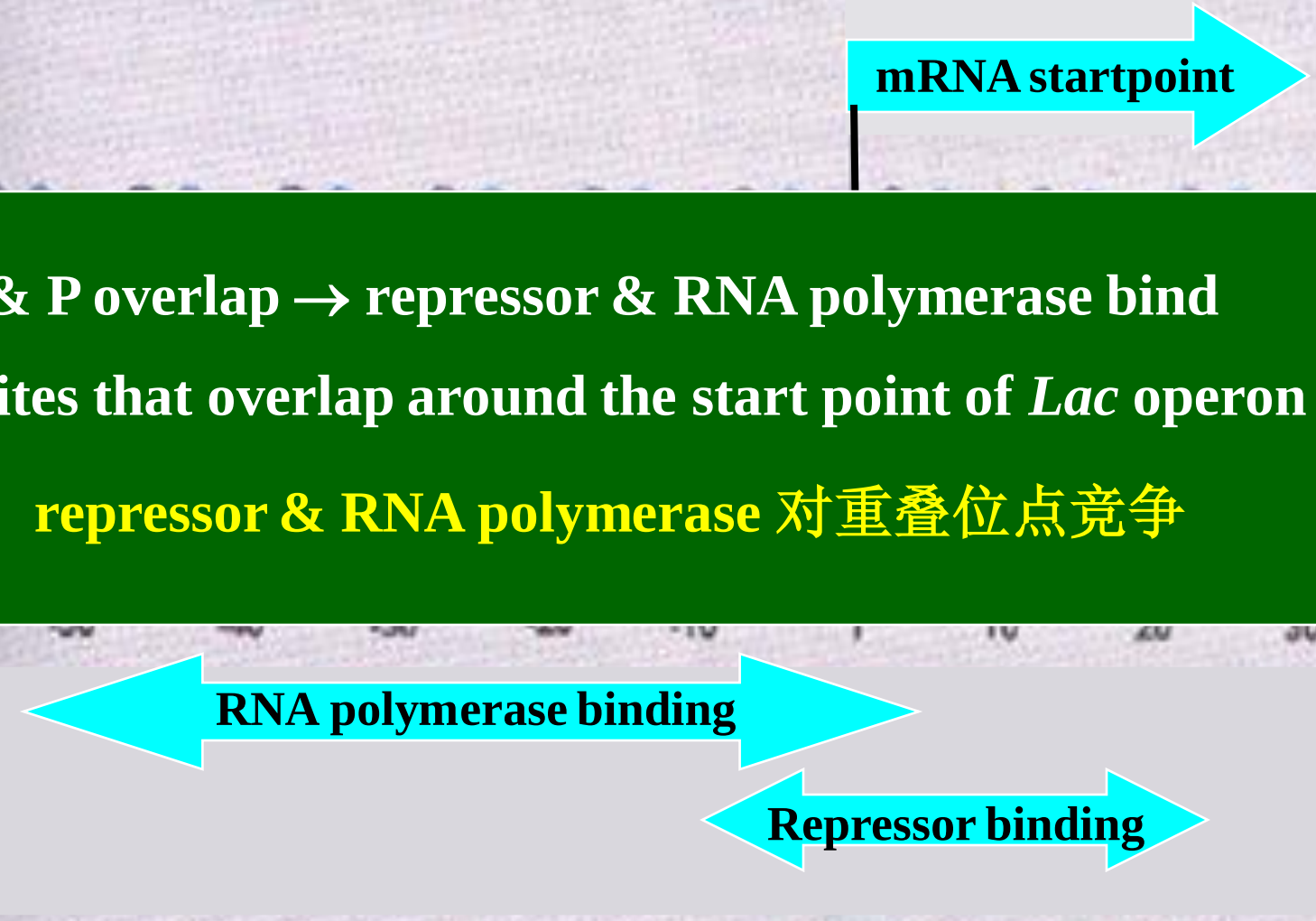
Methylation enhanced by repressor

Symmetrical seq. of 21 bp & G/C as axis

T can be crosslinked to repressor(binding site)

(来源：不详)

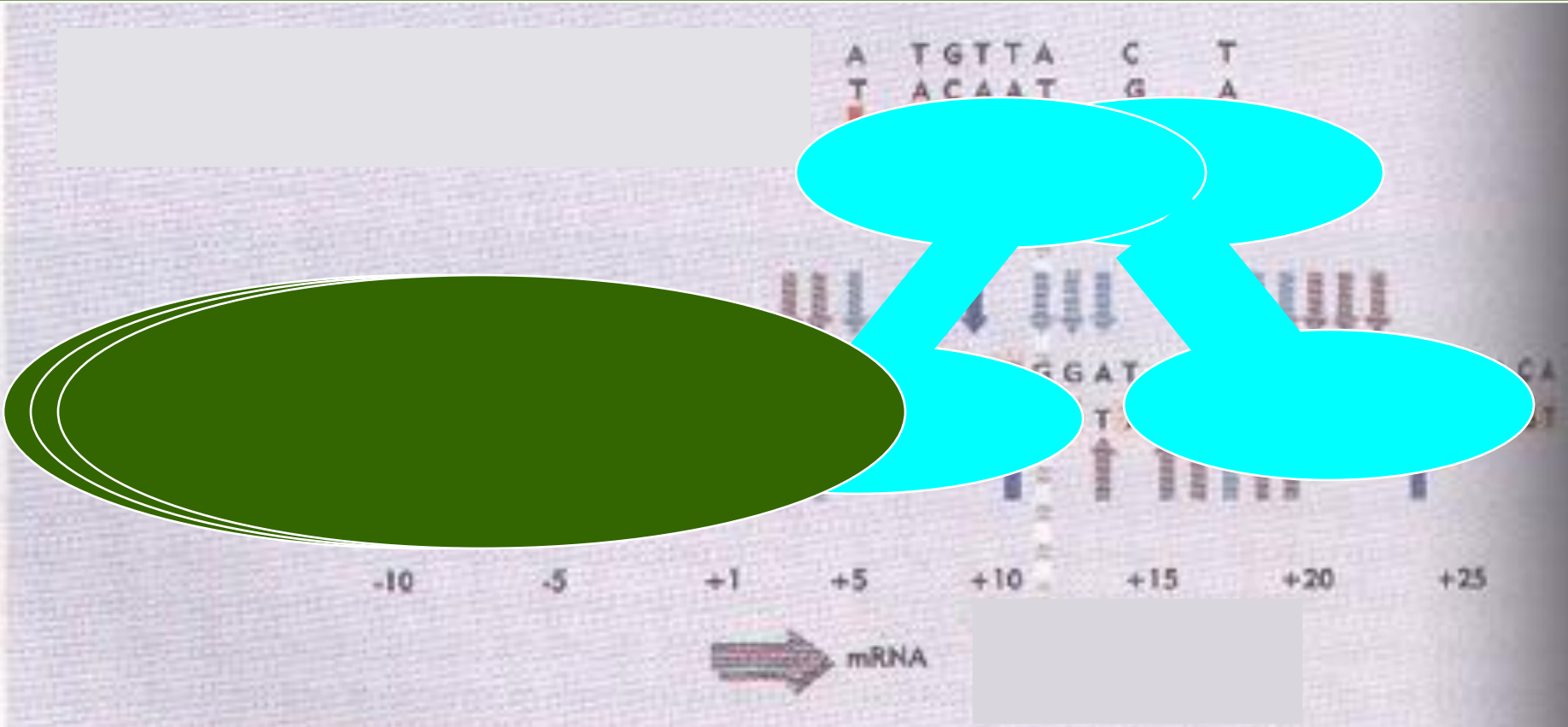
O gene (operator) cis-action factor



O & P overlap → repressor & RNA polymerase bind at sites that overlap around the start point of *Lac* operon

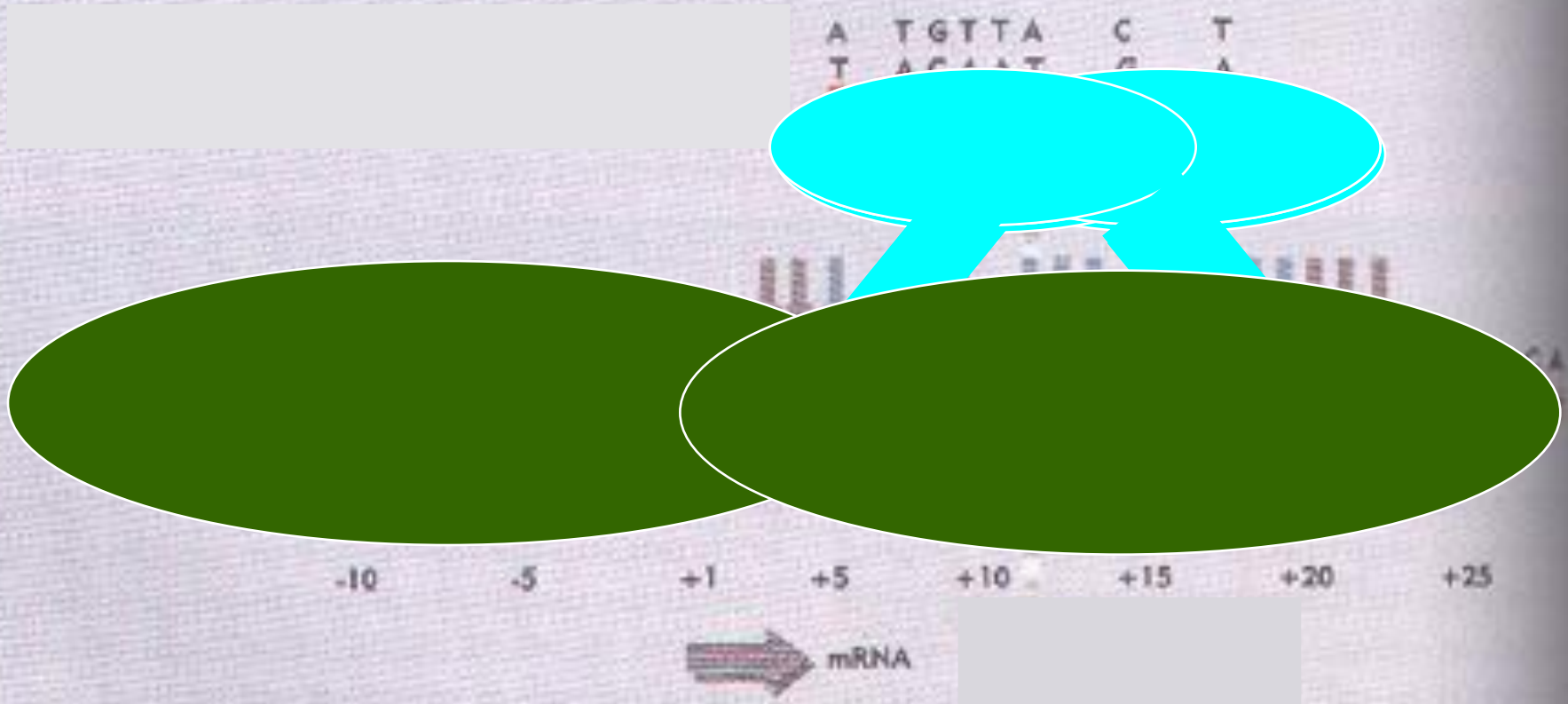
repressor & RNA polymerase 对重叠位点竞争

O gene (operator) cis-action factor



Obstruction

O gene (operator) cis-action factor



O^+ — mut. → O^C occur frequently at left site of axis (?)

---调控机理

repressor tetramer与operator发生特异结合

→ operon off

Inducer (lactose) 与repressor 特异结合

↓

tetramer 变构 → 特异结合力下降1000X

→ operon on

作用于 O 位点上的repressor → 变构 → 脱离O位

作用于游离的repressor

→ 变构 → 失去结合于O位的能力

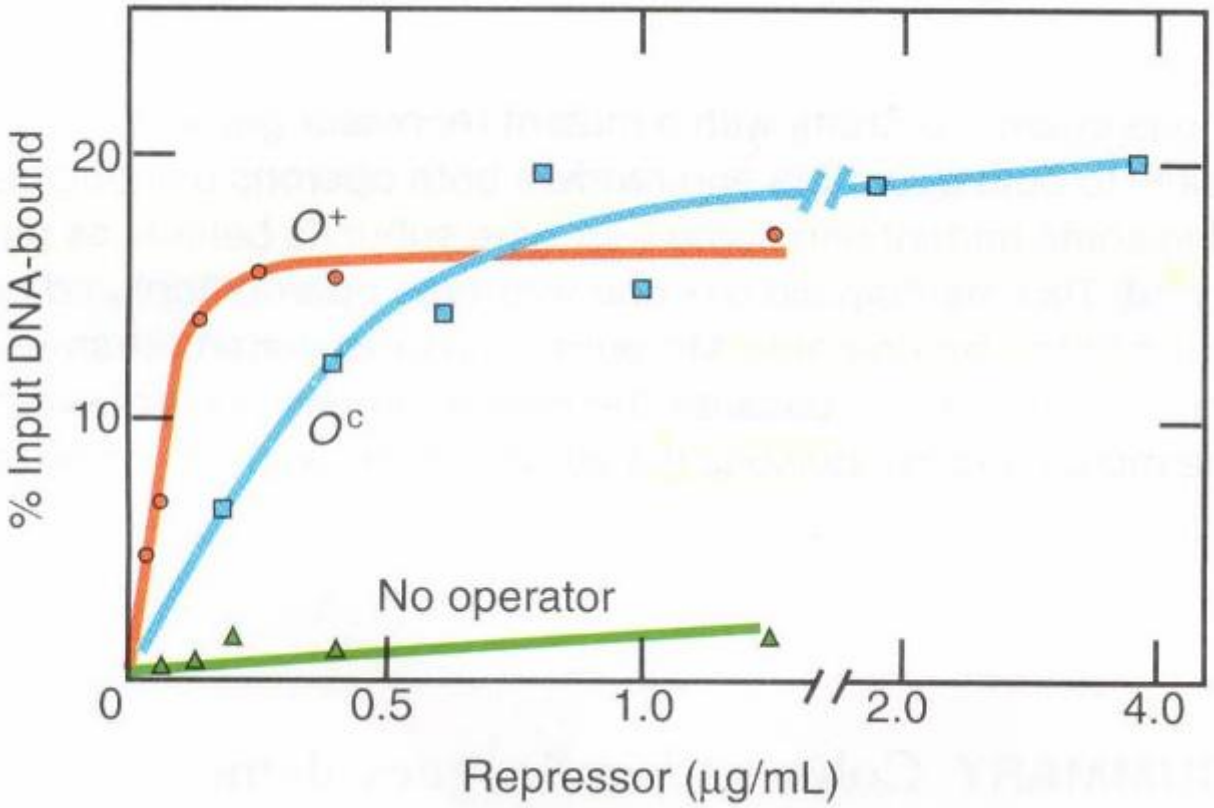
w.t. (I^+ O^+ P^+) 诱导型

add inducer → operon on

no inducer → operon off

O^C mut. (I^+ O^C P^+) constitutive mut. （组成型）

O^C失去与repressor特异结合的能力



wild-type operator (O+)

operator-constitutive mutation (O^c)

control 入Φ80 DNA,

(Source:Molecular Biology(2002),Robert F.Weaver,Page179）

The O^c lac operator binds repressor with lower affinity than does the wild-type operator, O^c required a higher concentration of repressor to achieve full binding.

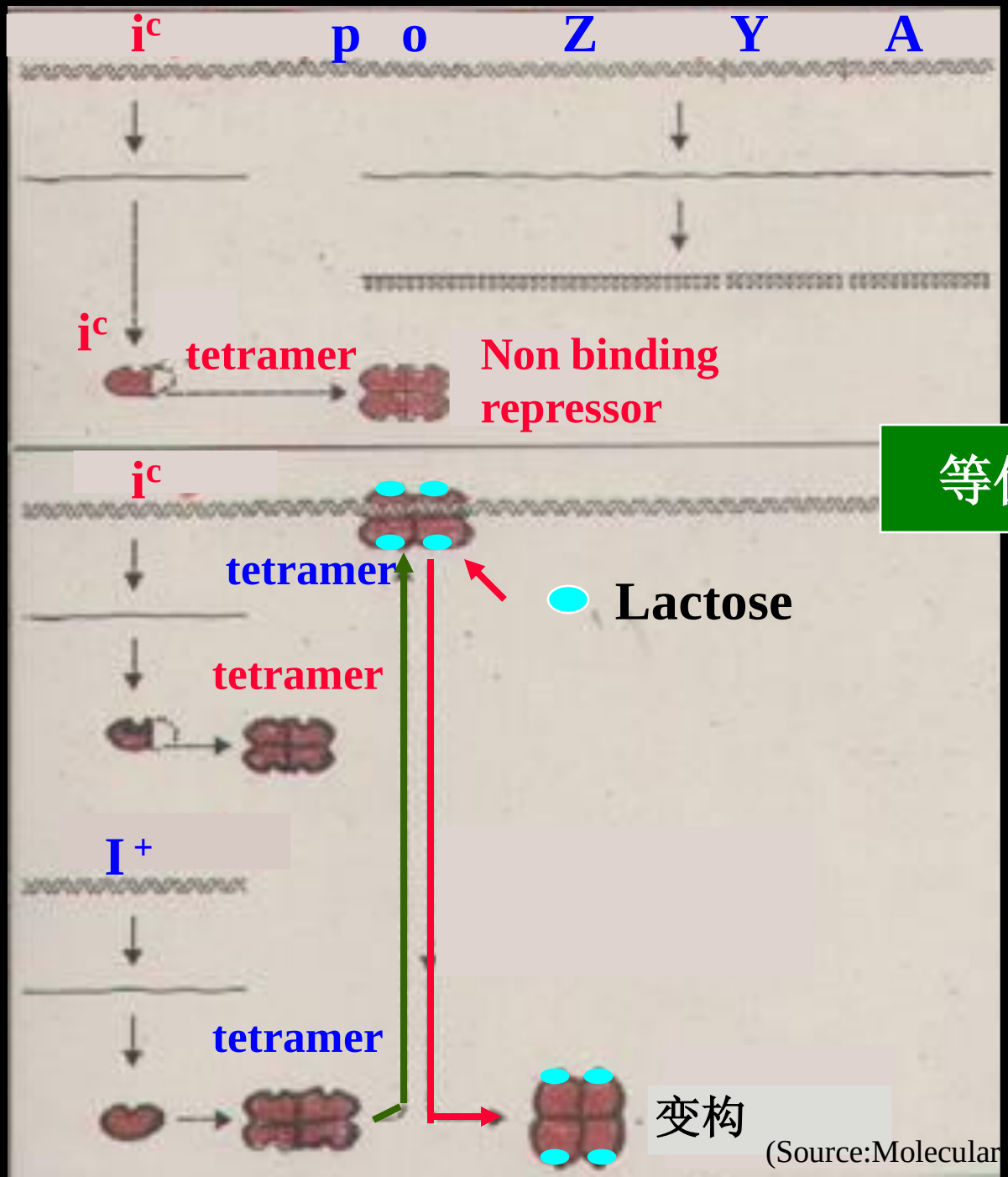
i^C mut. (i^C O^+P^+) constitutive mut. (组成型)

i^C gene产物repressor丧失与O位点结合的能力

i^S mut. (i^S O^+P^+) super-repression mut. (超阻型)

i^S gene 产物repessor 不能与inducer结合

等位基因间的显隐关系



i^C gene产物repressor

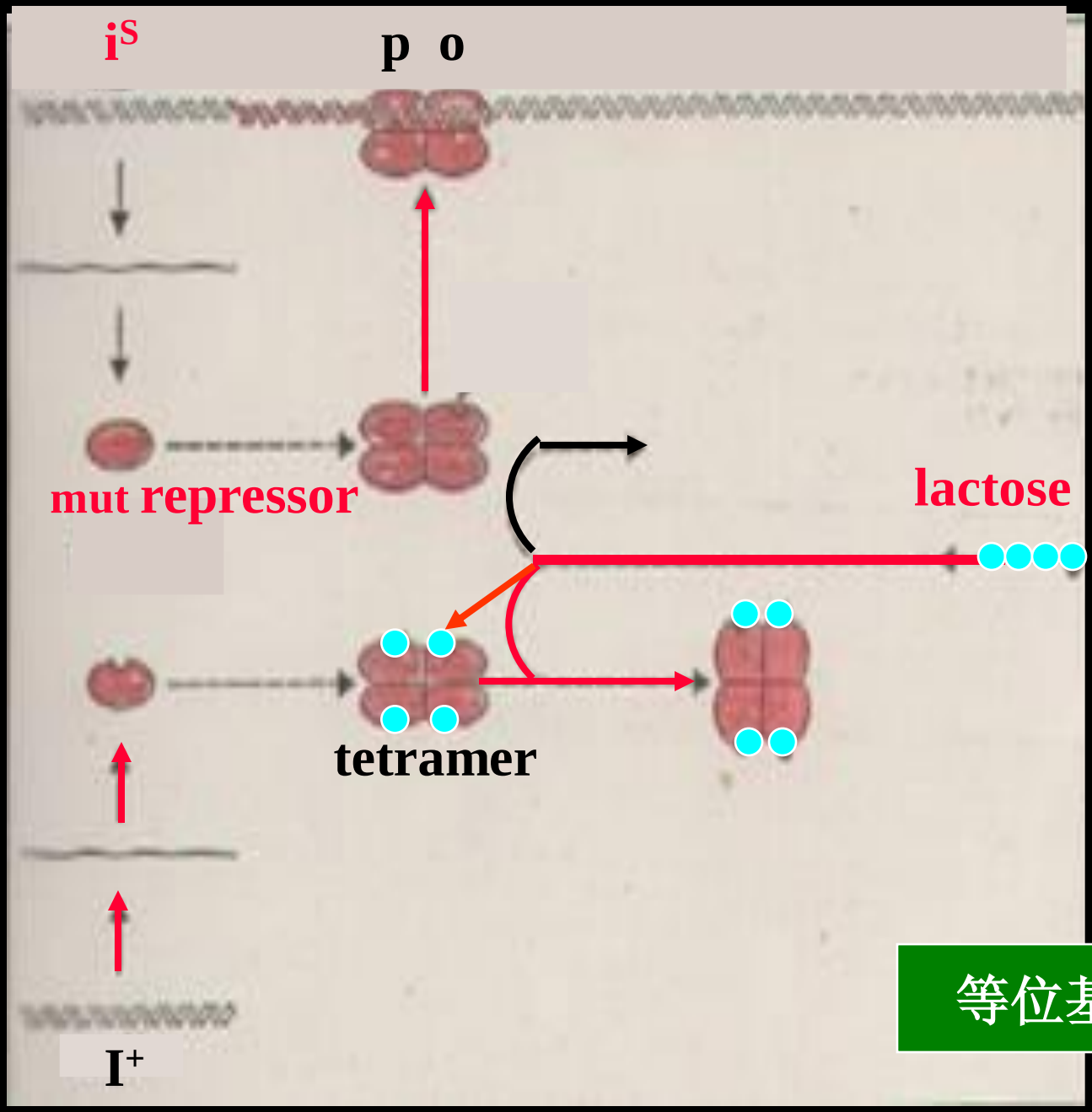
丧失与O位点结合的能力

等位基因间的显隐关系

I^+/I^C

$I^+ > i^C$

(Source:Molecular Biology(2002),Robert F.Weaver,Page177）



i^S gene 产物repessor

不能与 inducer 结合

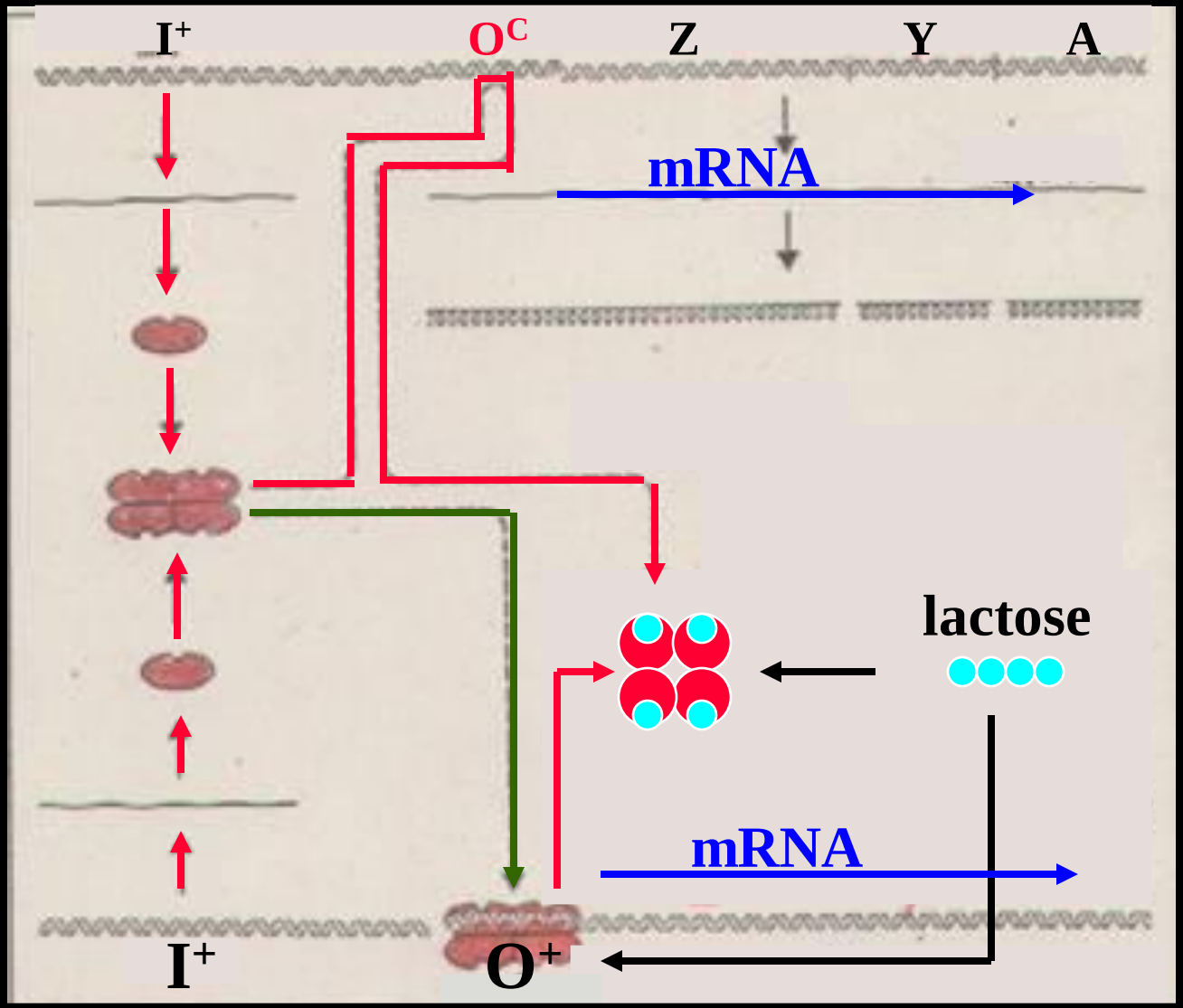
I^S/I^+

$i^S > I^+$

$i^S > i^C$

等位基因间的显隐关系

(Source:Molecular Biology(2002),Robert F.Weaver,Page178）



O^C失去与repressor特异结合的能力

$O^C > O^+$

cis-dominant

等位基因间的显隐关系

cis-dominant

The ability of a site (cis-factor) to control adjacent gene irrespective of the presence in the cell of other alleles of the site.

cis acting factor 对与其紧密连锁基因的控制效应不受其等位基因的影响。

•Positive—inducible operon (多为分解酶类)

I → inactive expressor

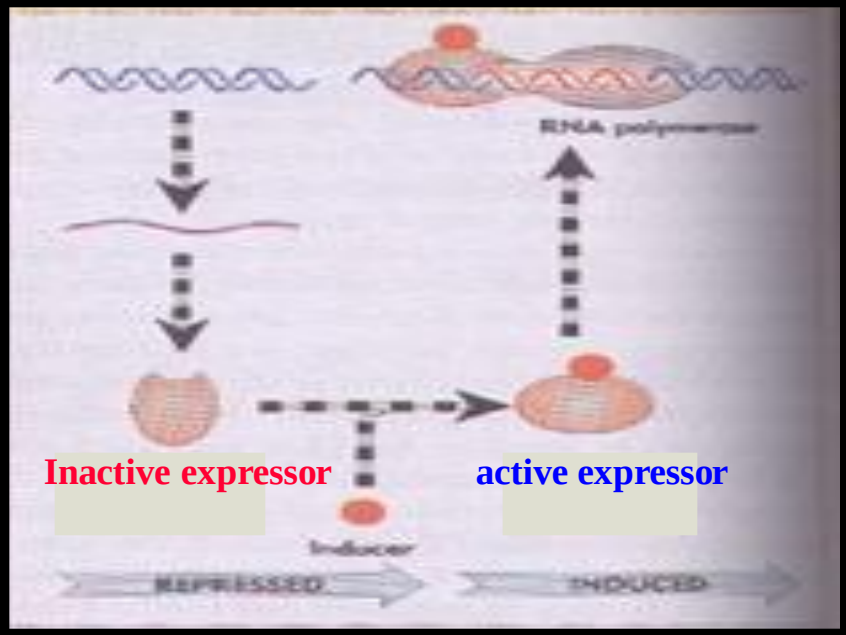
(apoinducer诱导蛋白)

inducer → operon off

active expressor

↓

binding on front P site



(来源：不详)

激活RNA polymerase启动

w.t. ($I^+O^+P^+$) 诱导型

i^S mut. → 超阻突变 (super-repression)

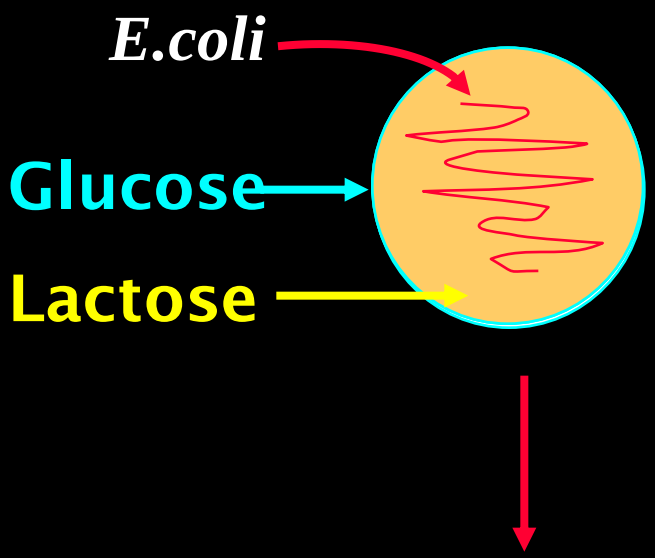
$I^+ > i^S$

↓

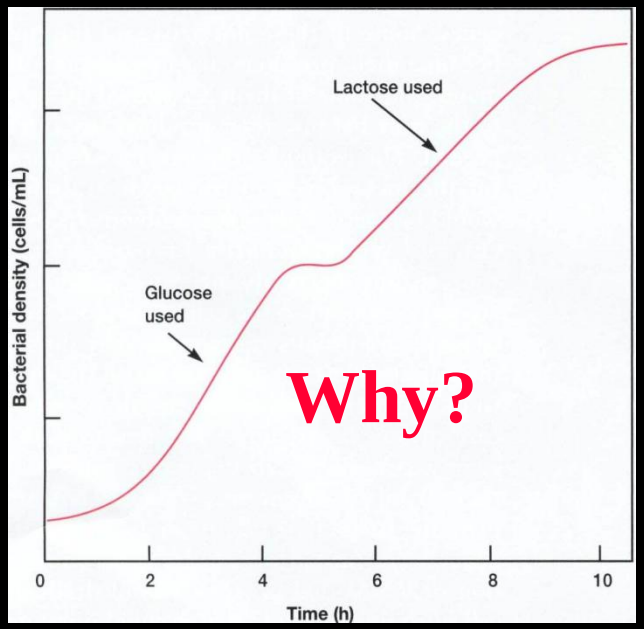
expressor can not be activated by inducer

operon off

e.g. cAMP control (universal controlling system)



(Source:Molecular Biology(2002),Robert F.Weaver,Page173）



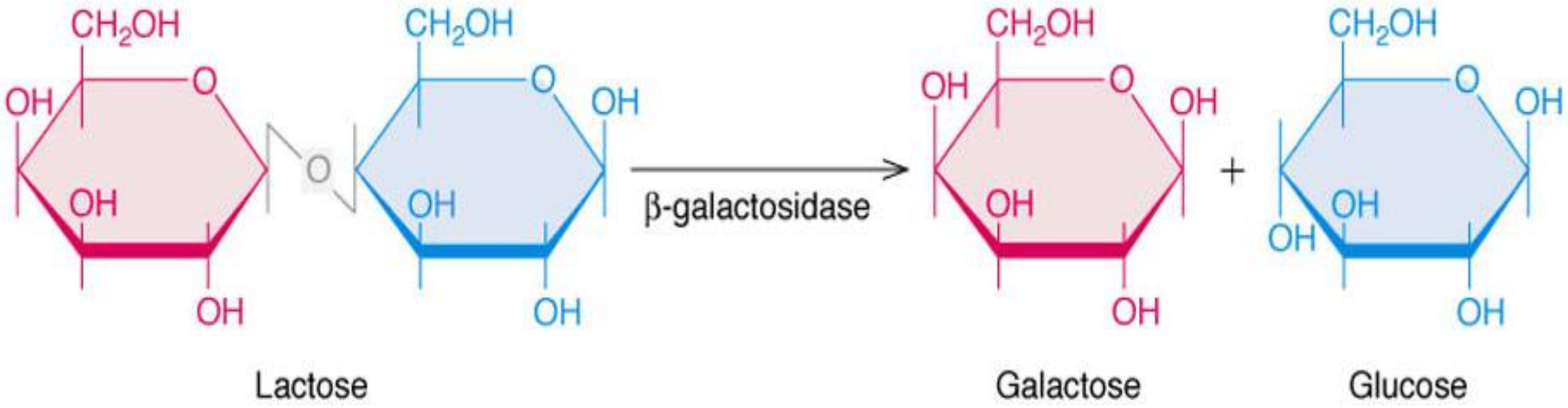
diauxy

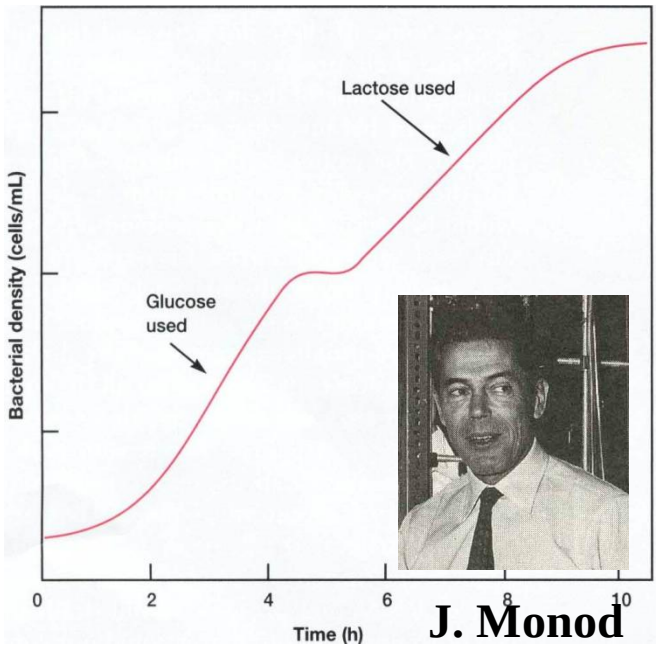
Lac operon open

but no transcripts

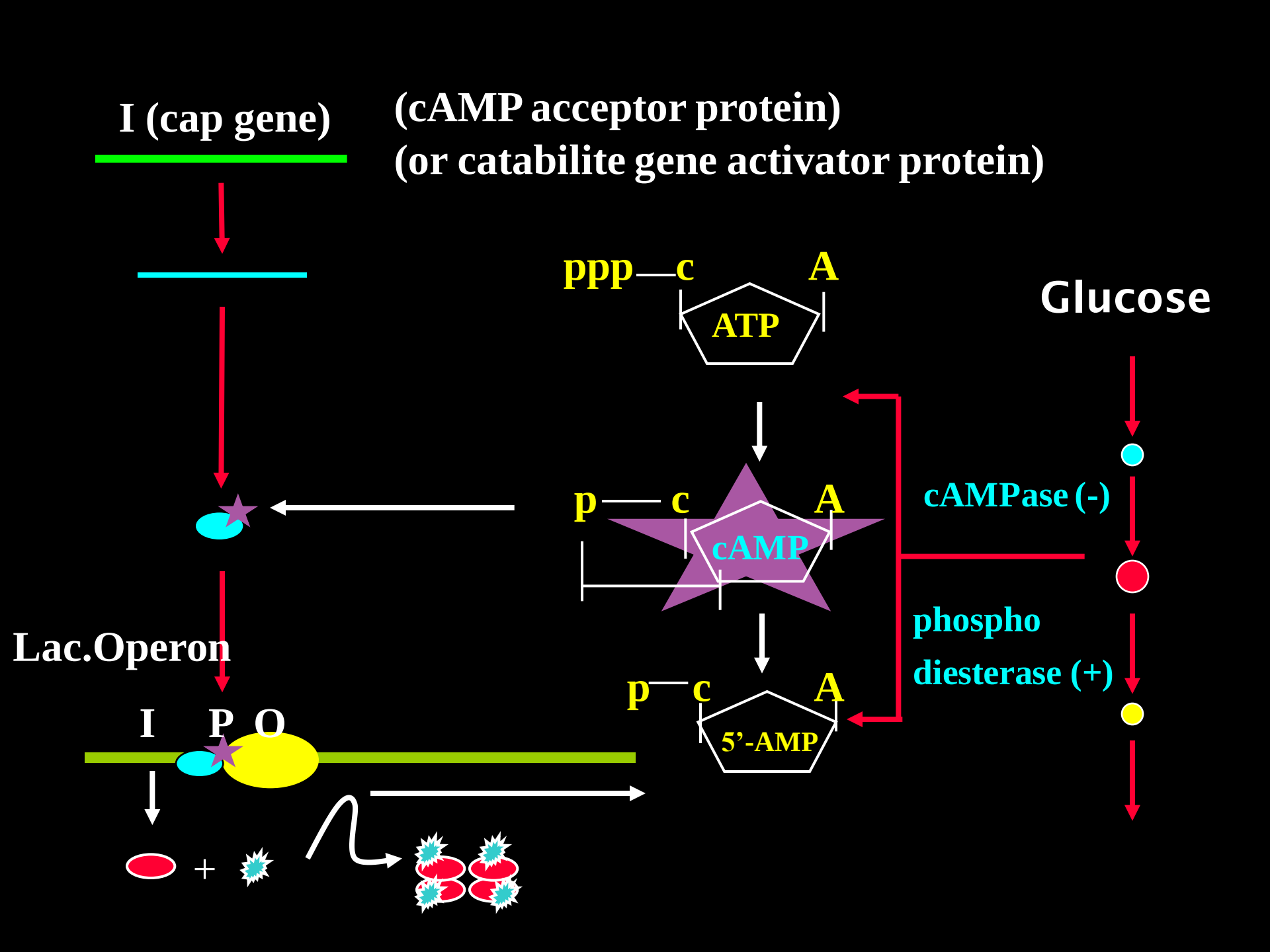
J. Monod

(Source:Molecular Biology(2002),Robert F.Weaver,Page173）



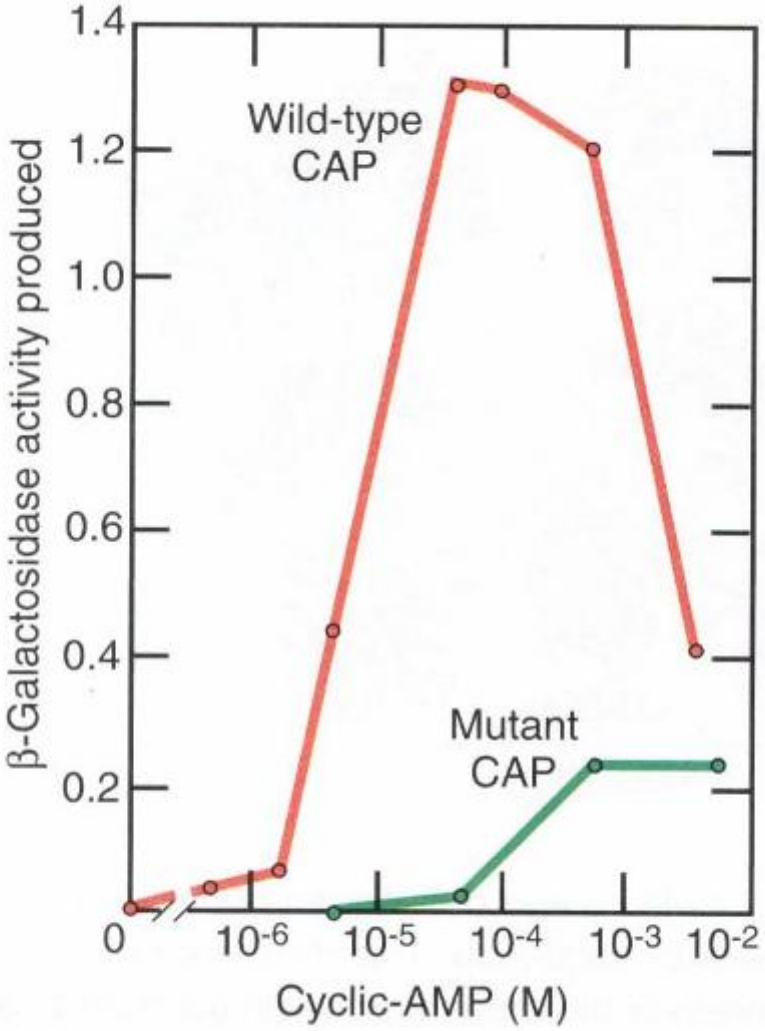


J. Monod

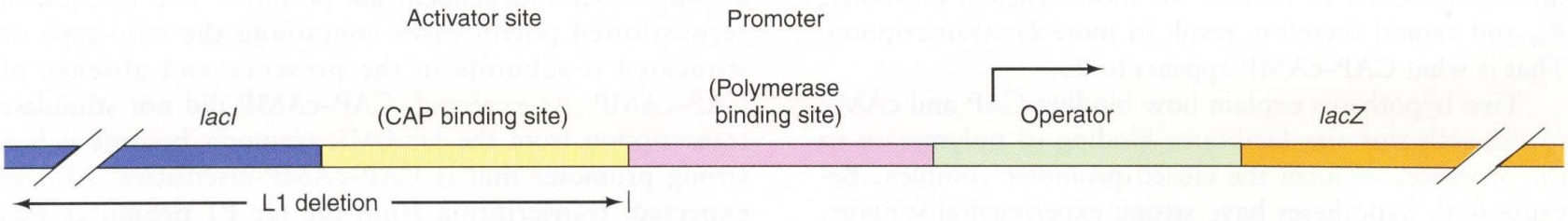

I (cap gene)
(cAMP acceptor protein)
(or catabilite gene activator protein)
ppp—c
A
ATP
Glucose
p—c
A
cAMP
cAMPase (-)
phospho
diesterase (+)
p—c
A
5'-AMP
Lac.Operon
I
P
O
+

CAP-cAMP complex is important for lac operon transcription

But too much cAMP obviously interfered with β-galactosidase synthesis. And cAMP has many effects, some may indirectly inhibit some step in expression of the *LacZ* gene in vitro

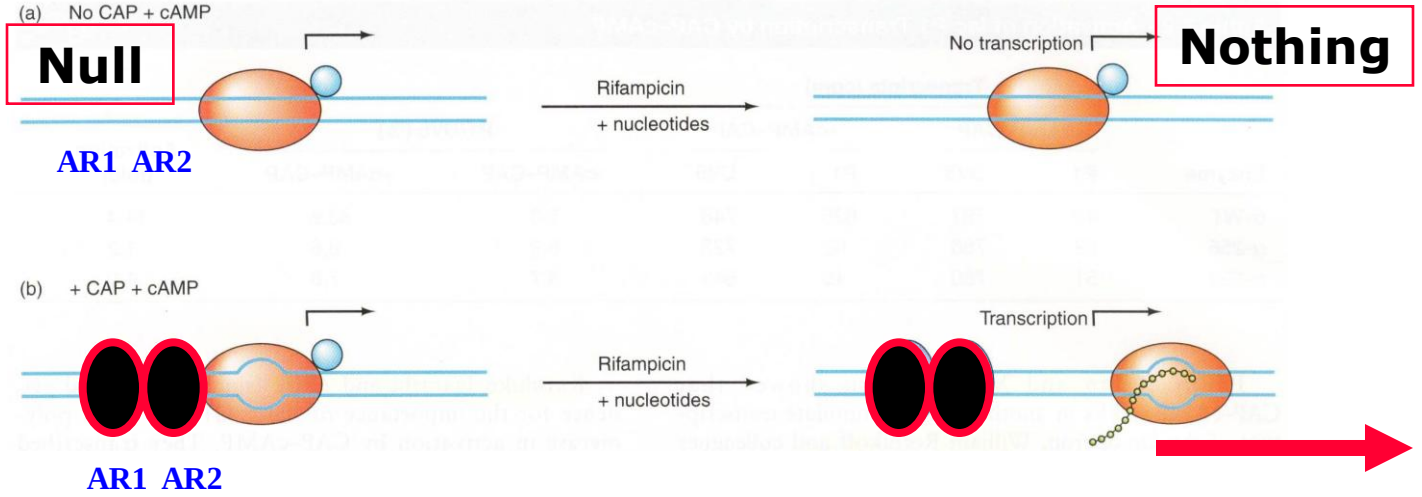


Stimulation of β-galactosidase synthesis by cAMP with wild-type and mutant CAP (Source: Pastan 1970 *P. N. A. S.* 480-487, June 66 (2))



(Source:Molecular Biology(2002),Robert F.Weaver,Page184）

The lac control region.
just upstream of the operator, contains the activator site (or CAP binding site)

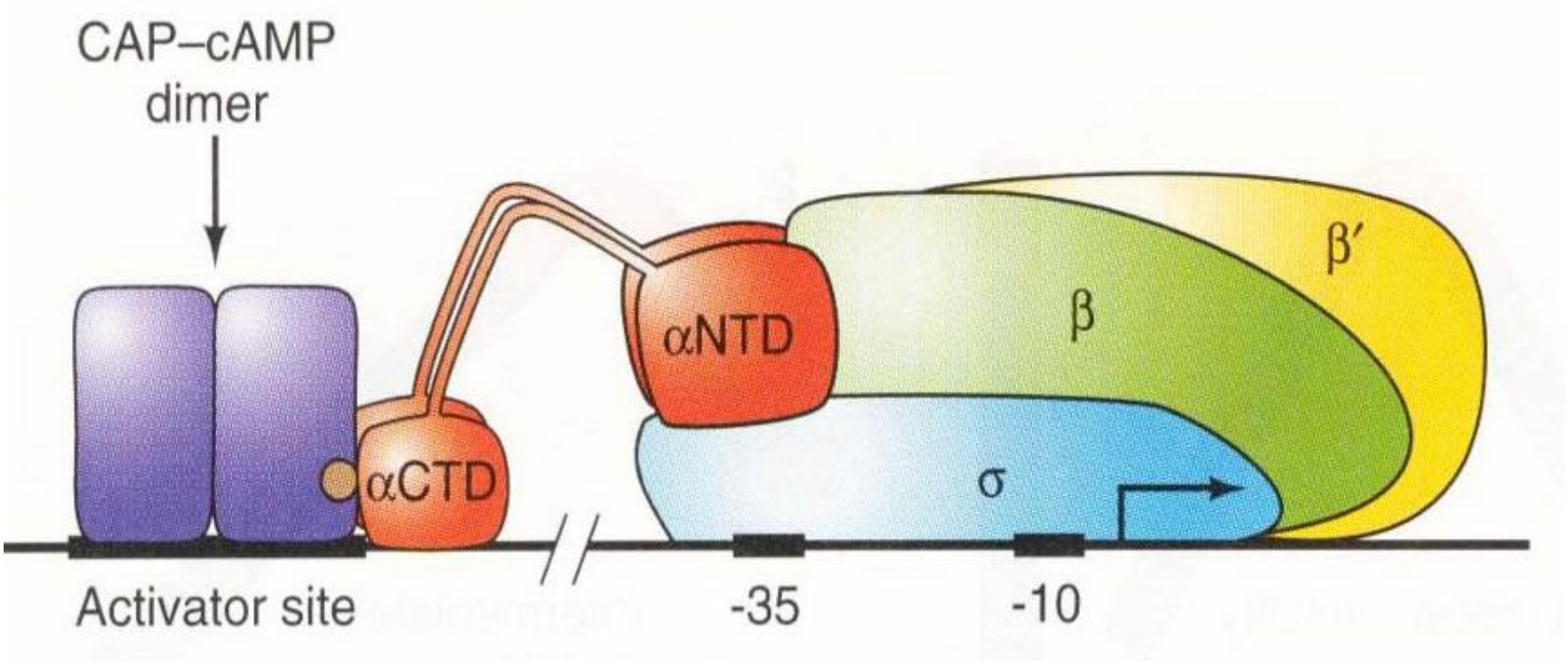


(Source:Molecular Biology(2002),Robert F.Weaver,Page184）

CAP plus cAMP allow formation of an open promoter complex

Hypothesis for CAP-cAMP activation of lac

(Source:**Busby, S. and R.H. Ebright Cell 79:742, 1994**)



The CAP-cAMP dimer binds to its activator site on the DNA, and the α-CTD interacts with a specific site on the CAP protein This strengthens binding between polymerase and promoter.,

cAMP—CAP

具有广泛生理效应的正控制系统

存在于多种基因表达调控体系中

Lac operon expression

lactose

(Negative-inducible)

cAMP

(Positive-inducible)

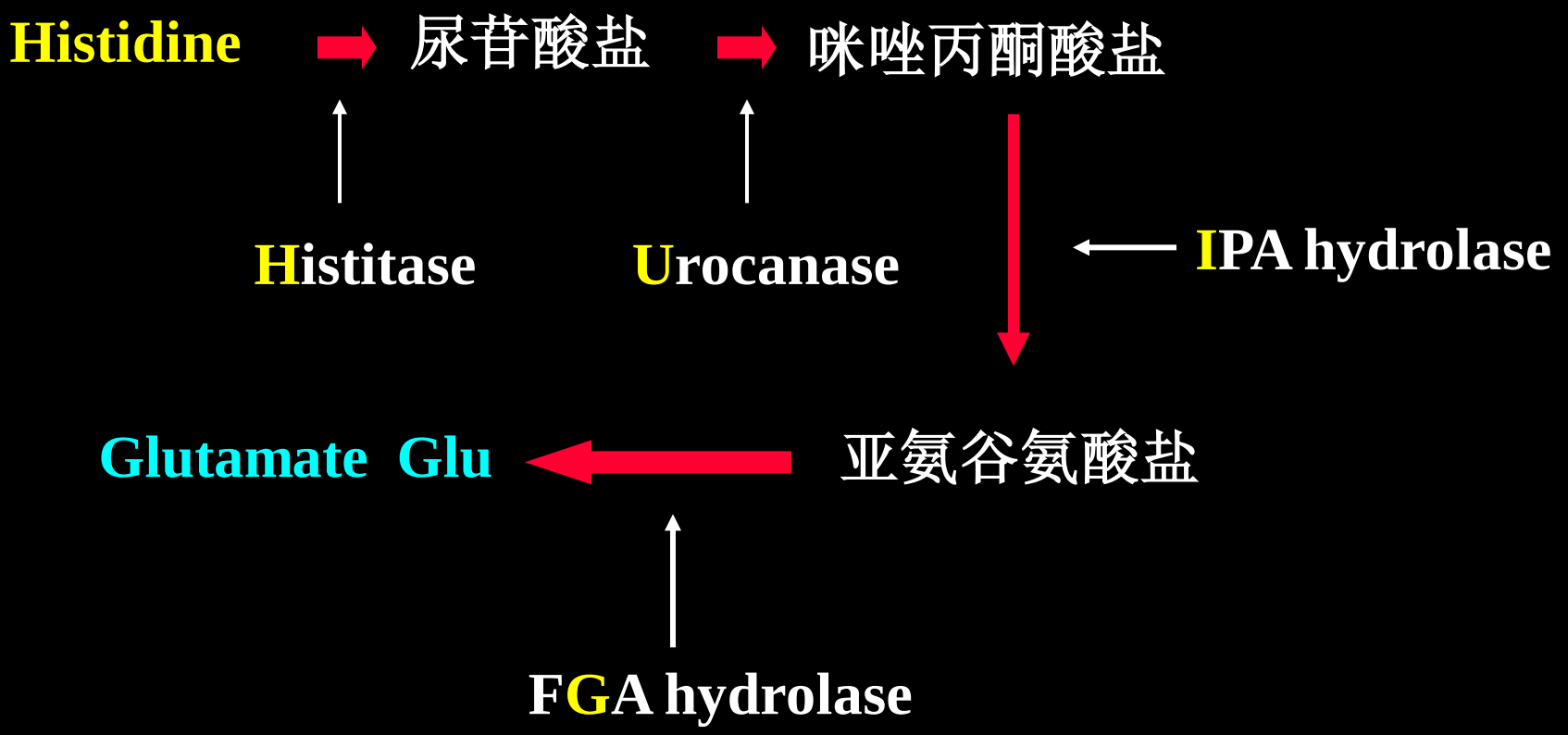
d) 基因表达调控的综合实例

e.g.1 Histidine Utilization operon (Hut)

e.g.2 λ phage 发育阶段选择调控的分子生物学

e.g. 1 Histidine Utilization operon (Hut)

a) Histidine 代谢过程

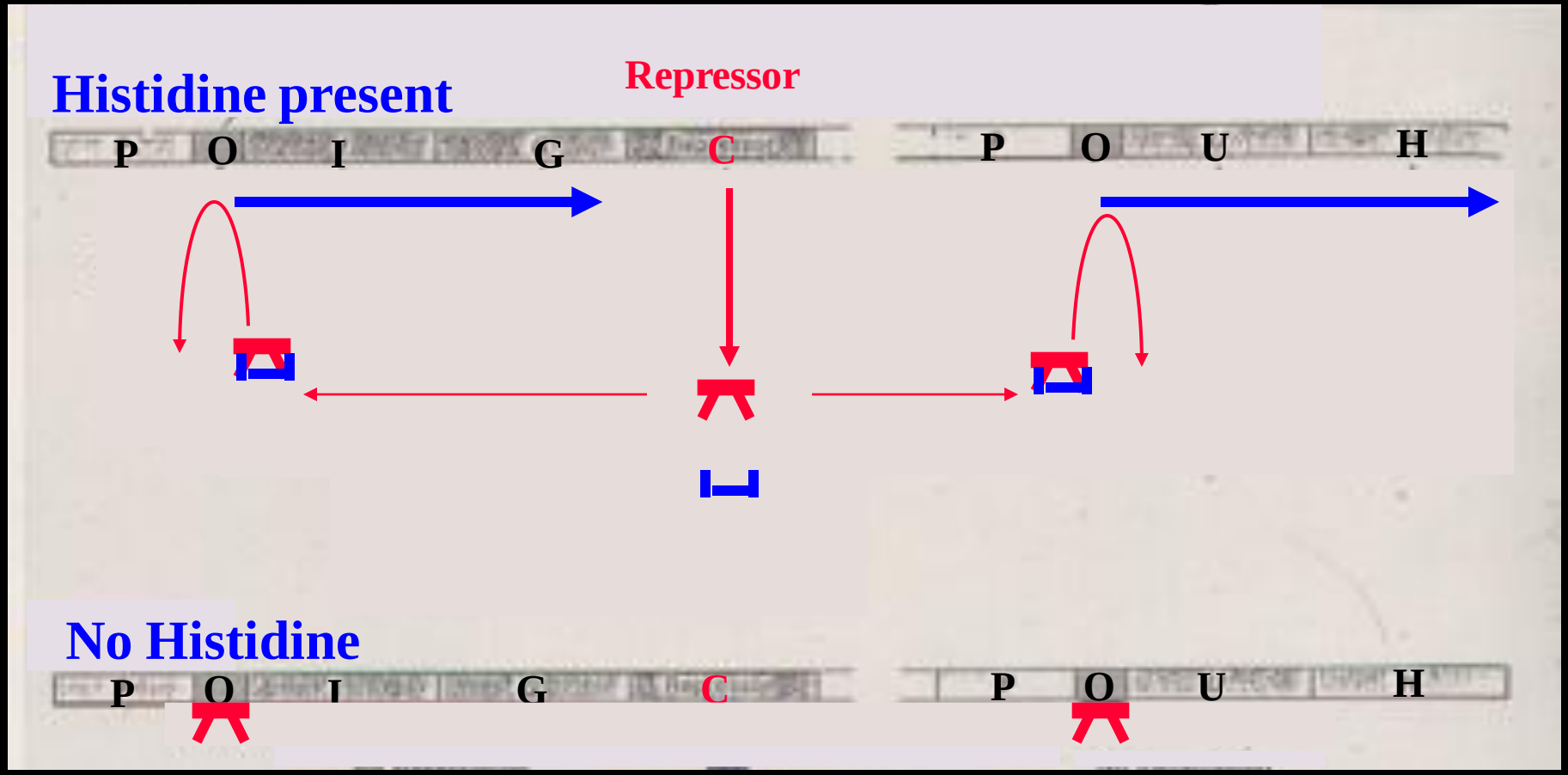


b) operon type & structure

Negative—inducible operon (regulon) by C gene (repressor)

Histidine as inducer

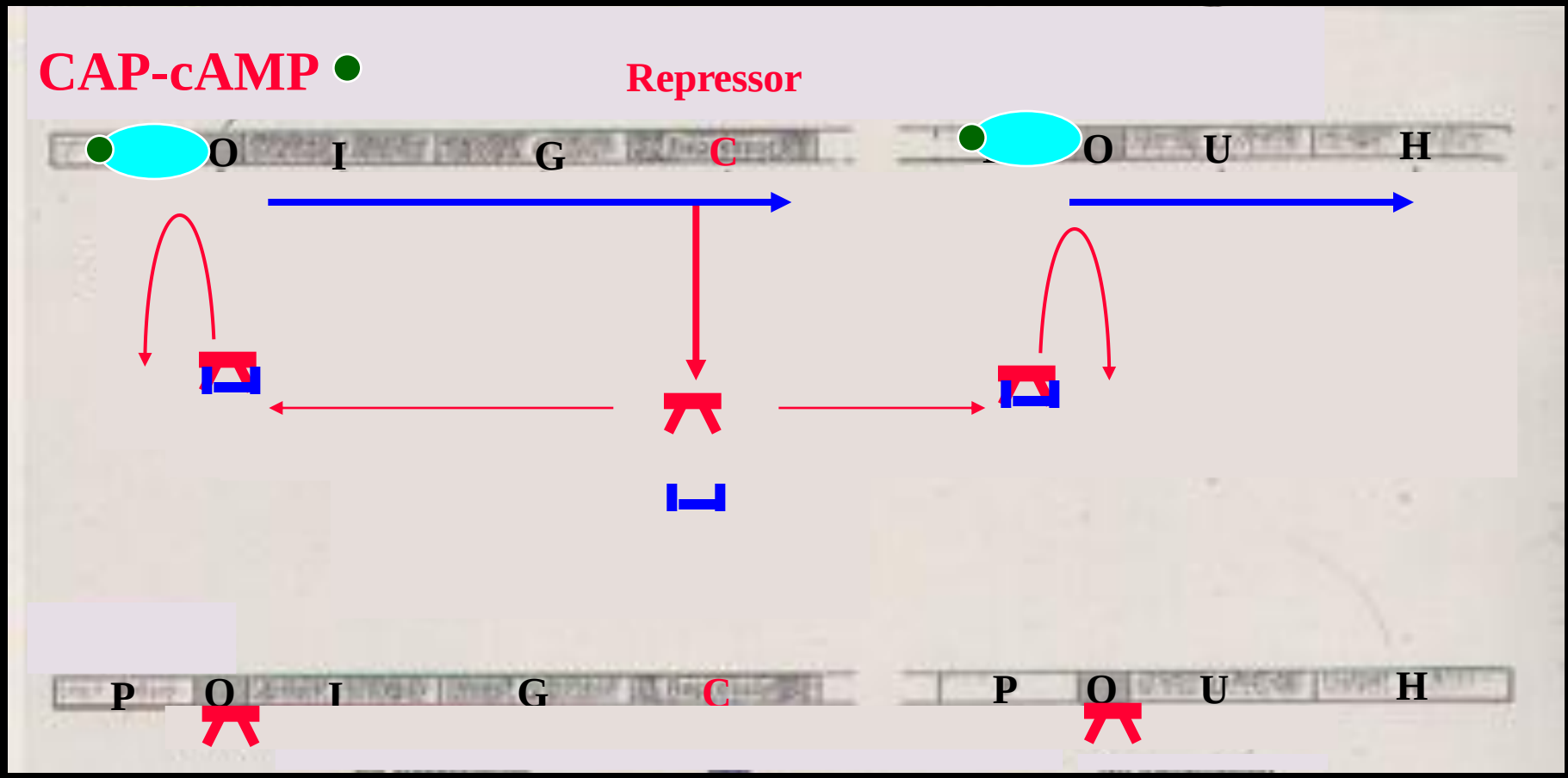
(来源：分子生物学（2007），郑用琏，第226页)



Positive—inducible operon Histidine present

by CAP—cAMP

(来源：分子生物学（2007），郑用琏，第226页)

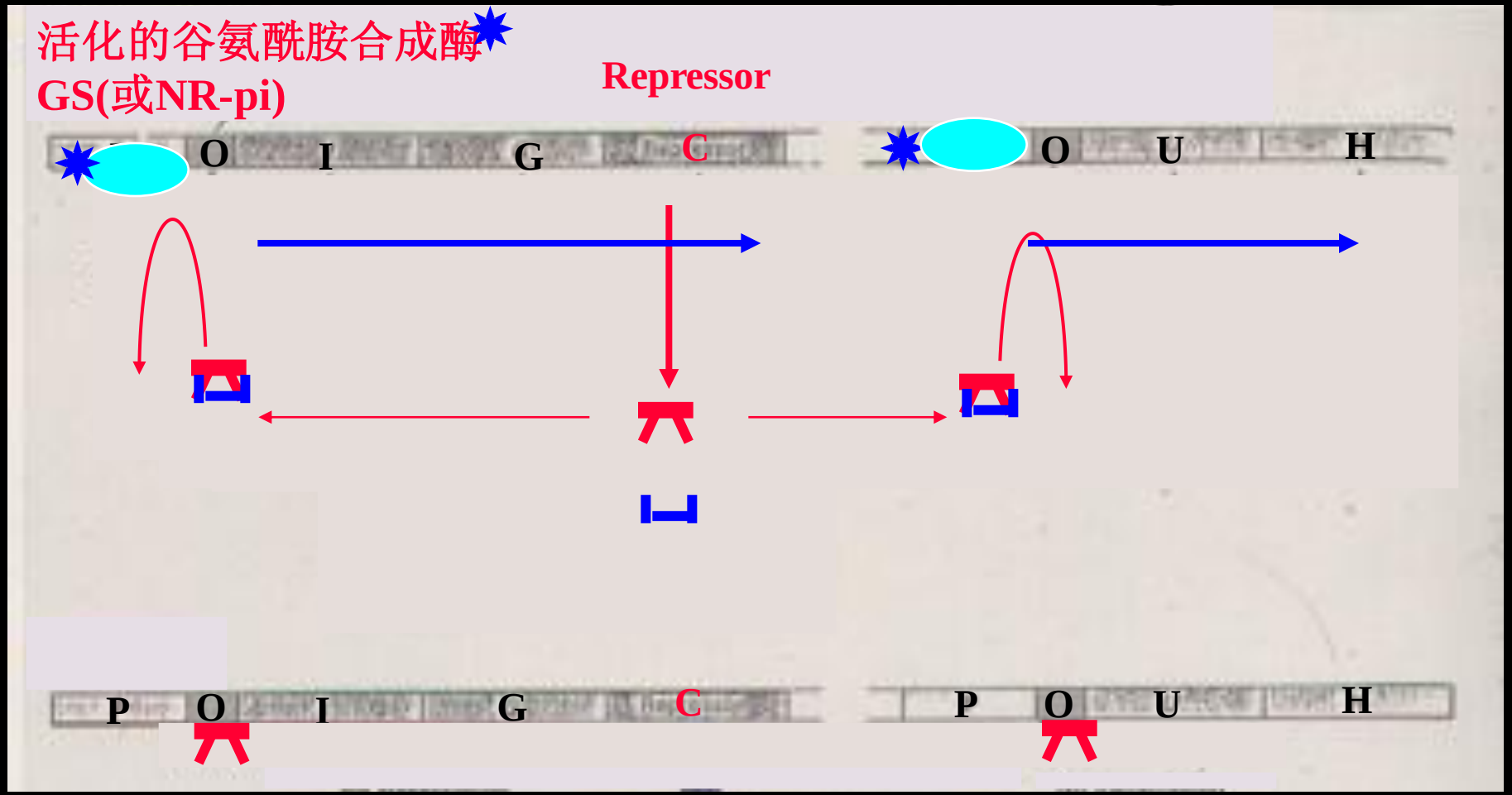


Positive—inducible operon **Histidine present**

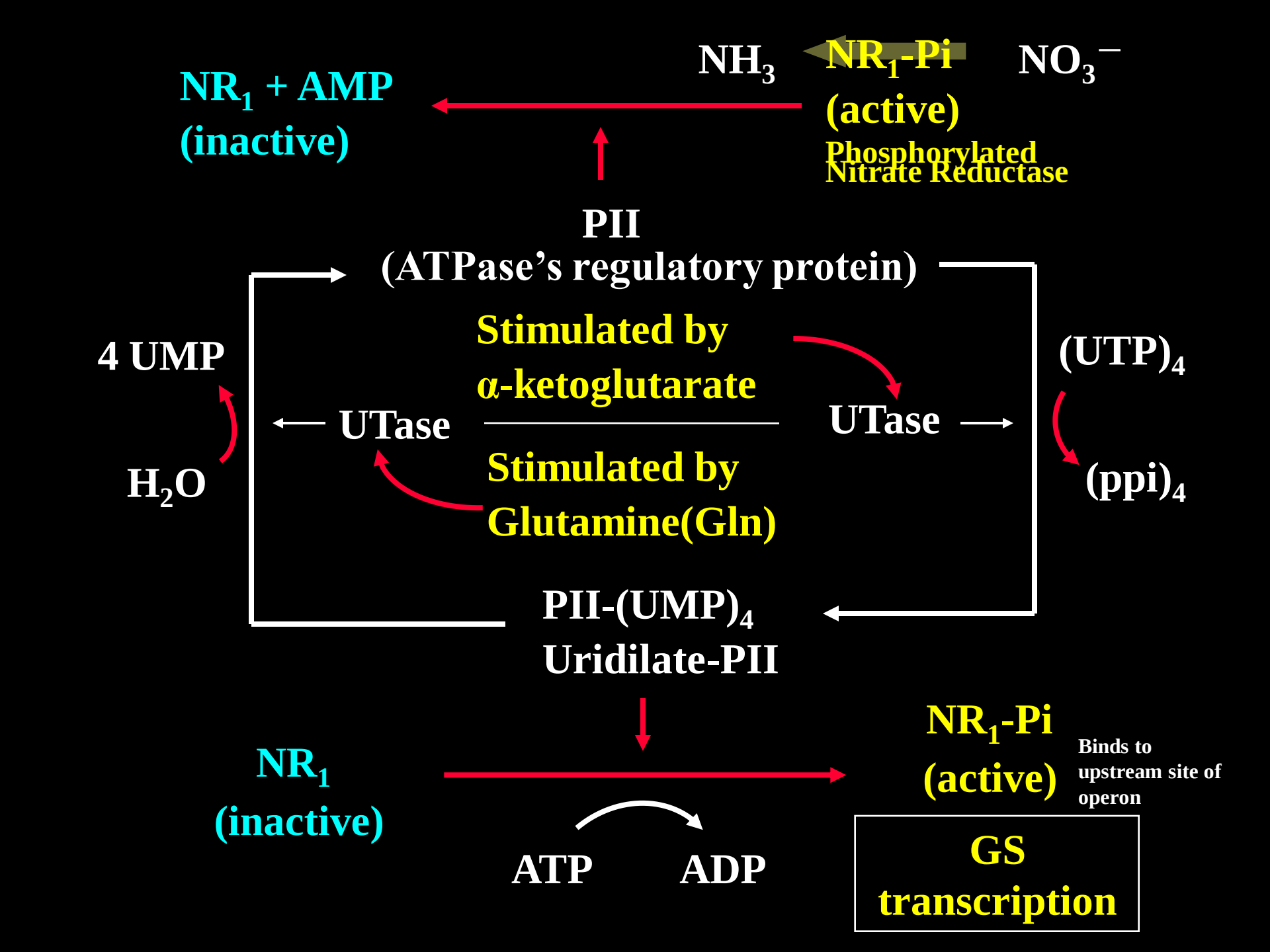
by GS (Glutamine synthetase)

NR-pi (phosphorylated Nitrogen regulator)

(来源：分子生物学（2007），郑用琏，第226页)

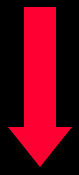


c) GS positive—inducible control model


NH3
NR1-Pi
(active)
Phosphorylated
Nitrate Reductase
NO3−
NR1 + AMP
(inactive)
PII
(ATPase's regulatory protein)
Stimulated by
α-ketoglutarate
4 UMP
H2O
UTase
UTase
(UTP)4
(ppi)4
Stimulated by
Glutamine(Gln)
PII-(UMP)4
Uridilate-PII
NR1
(inactive)
NR1-Pi
(active)
Binds to
upstream site of
operon
ATP
ADP
GS
transcription

NH_3 + α-ketoglutarate

Glu + NH_3 + ATP

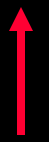


GS

Gln + ADP + Pi

When N starved

α-ketoglutarate



Gln

idling reaction

NR_1-Pi

(GS)

Positive control

Hut Operon

operon type & structure

Negative—inducible operon (regulon)

by C gene (repressor)

Positive—inducible operon

by GS(Glutamine synthetase)

NR-pi (phosphorylated Nitrogen Regulator)

CAP—cAMP

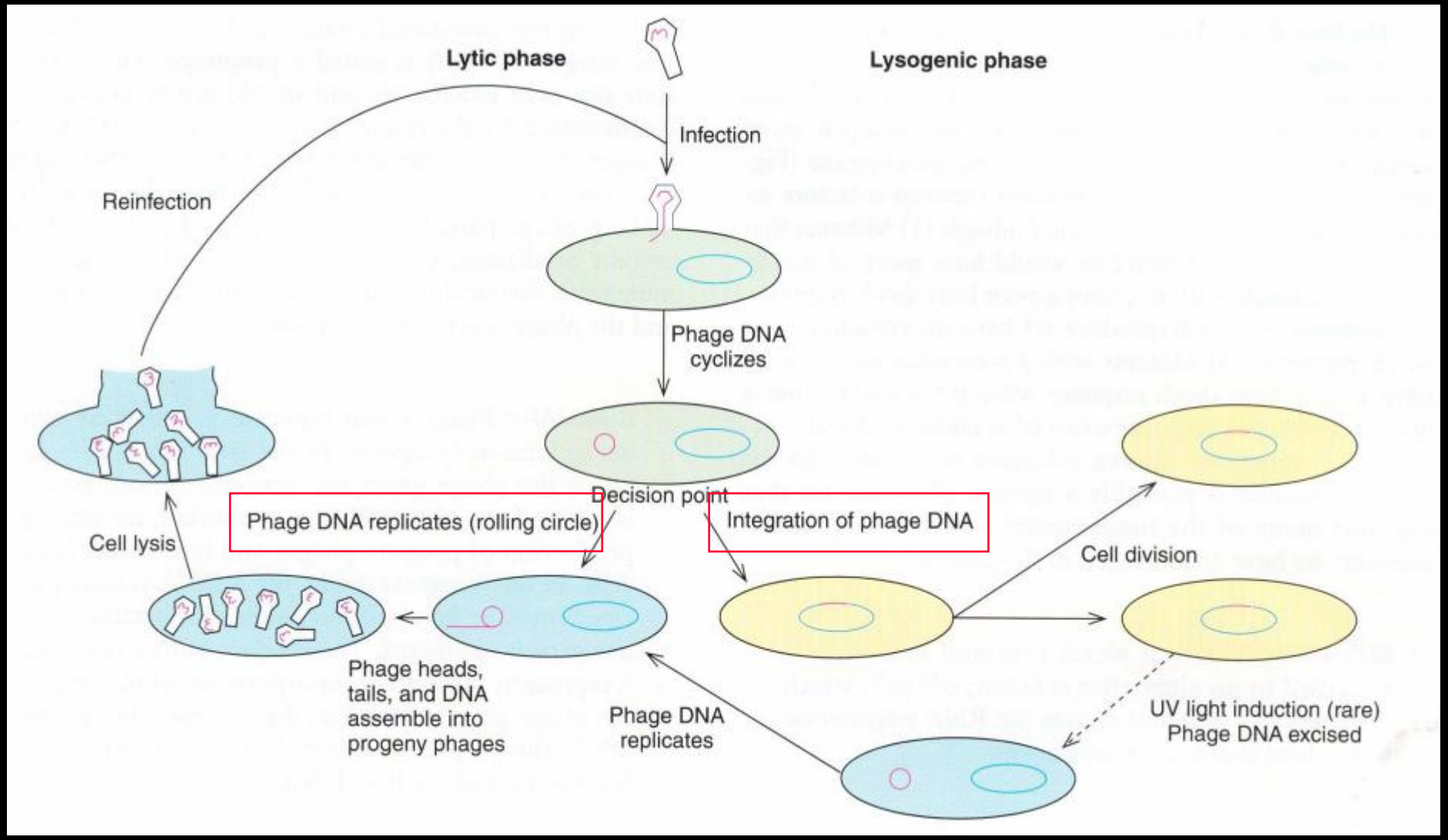
e.g. 2λ phage 发育阶段选择调控的分子生物学

--λ phage 溶原(lysogenic)和裂解(lytic)的发育途径

--λ phage发育途径选择调控区的基因组成

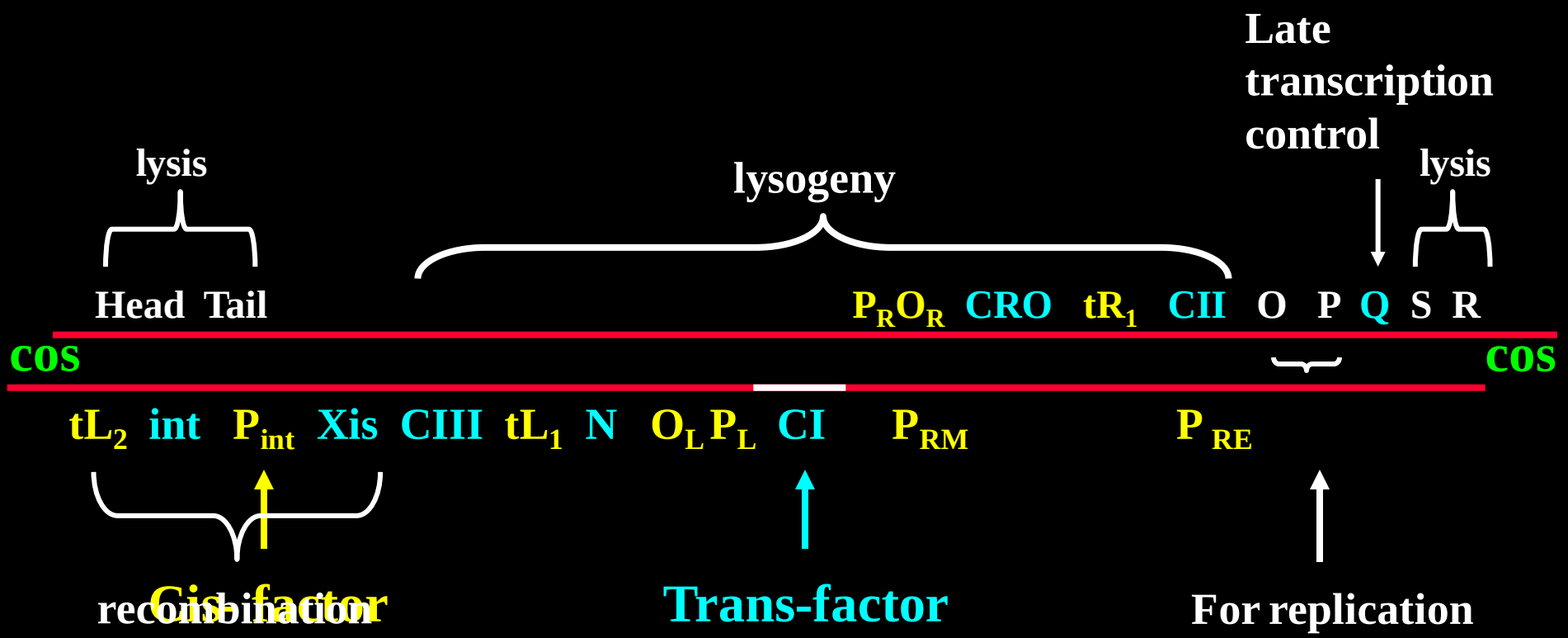
---λ phage发育途径选择调控的分子基础

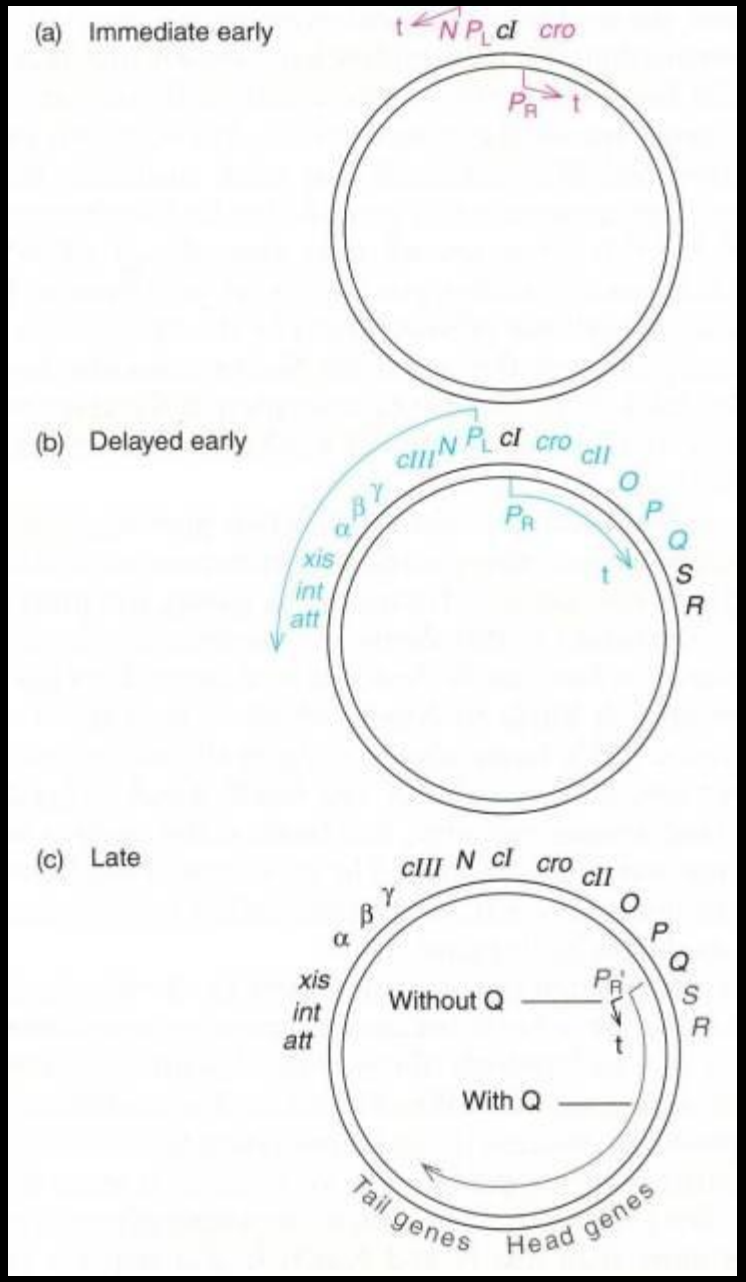
Lytic versus lysogenic infection by phage λ



(来源：分子生物学（2007），郑用琏，第233页)

λ phage发育途径选择调控区的基因组成





λphage developing stage

Immediate early stage;

N & Cro genes transcription

from P_L & P_R

Delayed early stage;

N-p required for anti-termination

at NuT_L & NuT_R

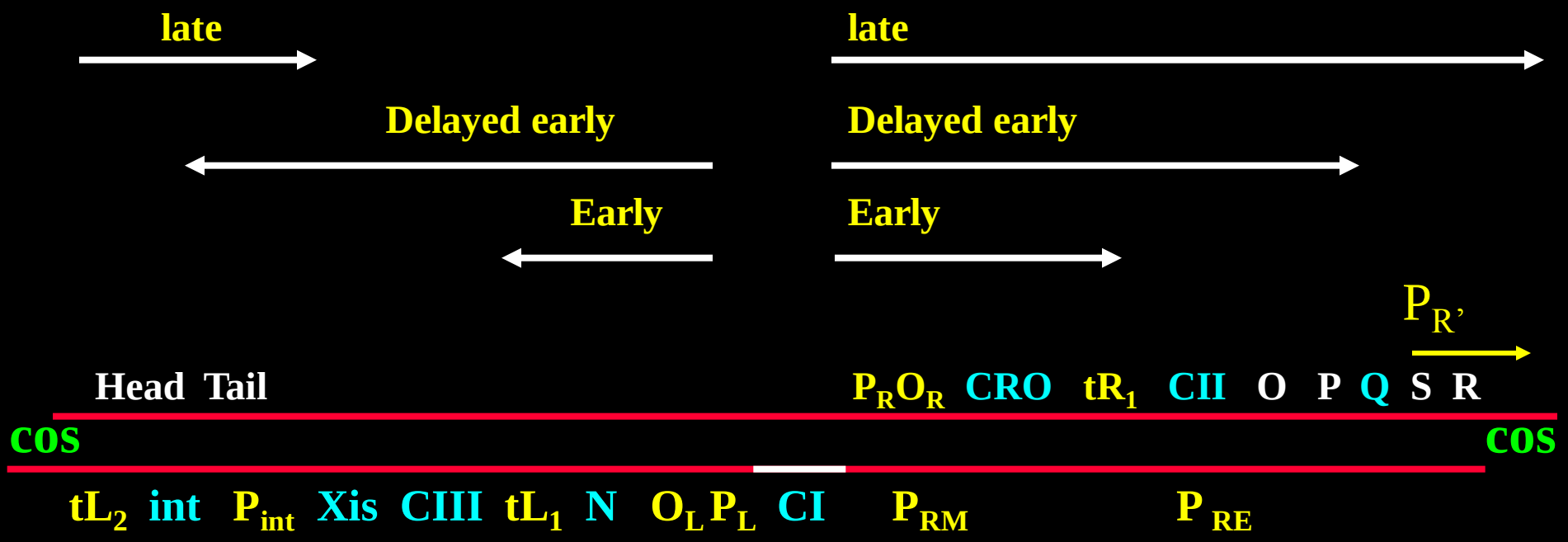
Late stage;

Q-p required

From $P_{R'}$

(来源：分子生物学（2007），郑用琏，第236页)

λ phage发育阶段和调控区



λ phage发育途径选择调控区的基因组成

C gene（与溶原直接相关）

λ → host → lysogenic → turbid colony

C gene mut. → lytic → Clear plaque

CI → cI　可建立溶原，但不易维持

CI-p 26kd

as repressor binding on P_R---O_R, P_L---O_L （负控制）

as expresser for CI self （正控制）

CII → cII 不易建立溶原，一旦建立溶原后，
依靠CI-p可保持溶原

CII-p as expresser

启动P_{RE} → 转录CI gene

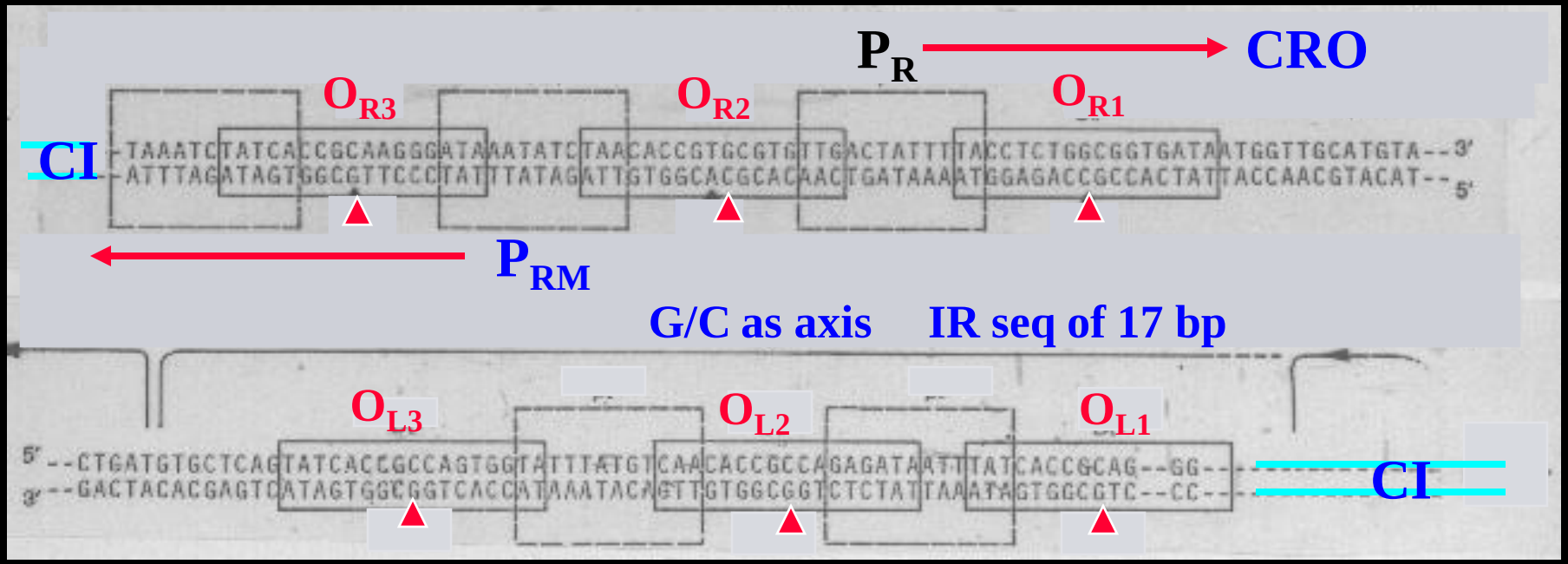
CIII → cIII 参与溶原建立过程

但对溶原化作用不大

CIII-p as expresser

启动P_{RE} → 转录 CI gene

Operator (cis-factor) O_R (O_{R1}, O_{R2}, O_{R3}), O_L (O_{L1}, O_{L2}, O_{L3})



O_{R1}, O_{R2}, O_{R3} 序列的差异，

(来源：分子生物学（2007），郑用琏，第235页)

决定了CI-p, CRO-p与其的结合力

For CI-p (repressor) $O_{R1} > O_{R2} = O_{R3}$

For CRO-p (repressor) $O_{R1} = O_{R2} < O_{R3}$

CI-p/CRO-p的标准浓度　Monomer(200) ⇌ Dimer(50)

结合于O 位点

3 mM 为1标准浓度单位

genotype	O_{R3}	O_{R2} P_R O_{R1}
	CI-p	CI　　CI
O_{R3} O_{R2} O_{R1}	25	2　　1

	O_{R3} P_{RM}	O_{R2}　　O_{R1}
	CRO-p	CRO　　CRO
O_{R3} O_{R2} O_{R1}	1	8　　8

Regulator (trans-factor)

CI-p

acidic protein 236 aa, 26kd

C-end dimerization domain

N-end DNA binding domain

CRO-p (Control of Repressor and Other things)

66aa (3 helix & 3 sheets)

Dimerization domain & DNA bimnding domain

N-p

Anti-termination protein

for delayed early stage

Q-p

Anti-termination protein

for late stage

P_{RE} (Promoter for Repressor Establishment

P_E (Promoter for lysogenic- Establishment

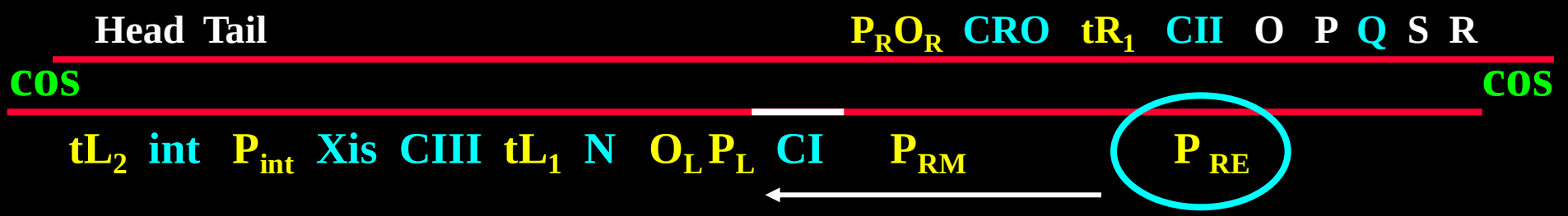
Promoter

Located between CRO---CII

Strong promoter

Positive control site with CII-p,CIII-p

Transcription CI gene & anti-sense CRO RNA



Promoter

P_{RM} (Promoter for Repressor Maintenance)

P_M (Promoter for lysogenic -Maintenance)

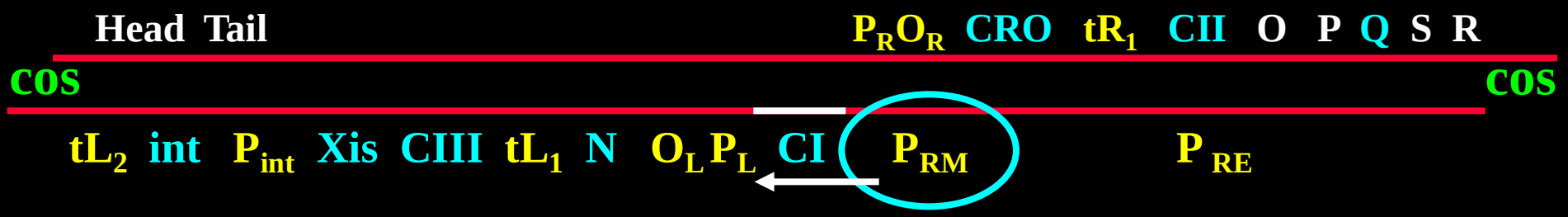
Located between OR2—OR3

Weak promoter (1/7 ~ 1/8 of P_{RE})

Positive control site with CI-p

Negative control site with CI-p & CRO-p

Transcription CI gene



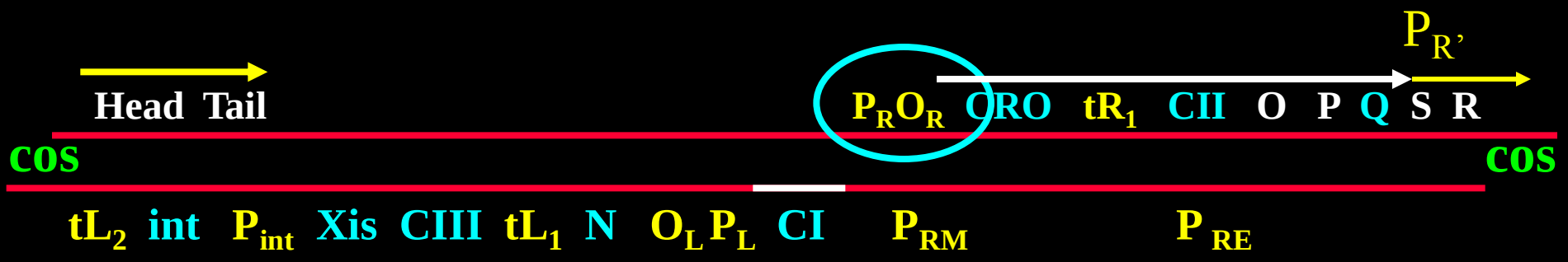
Promoter

P_R (Promoter on Right)

Located between OR1 — OR2

Negative control site with CI-p & CRO-p

Transcription CRO, CII, O, P, Q, S, R, H, T genes



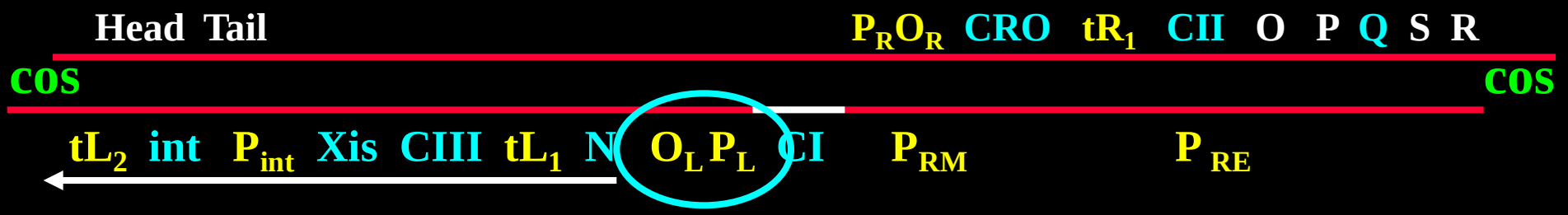
Promoter

P_L (Promoter on left)

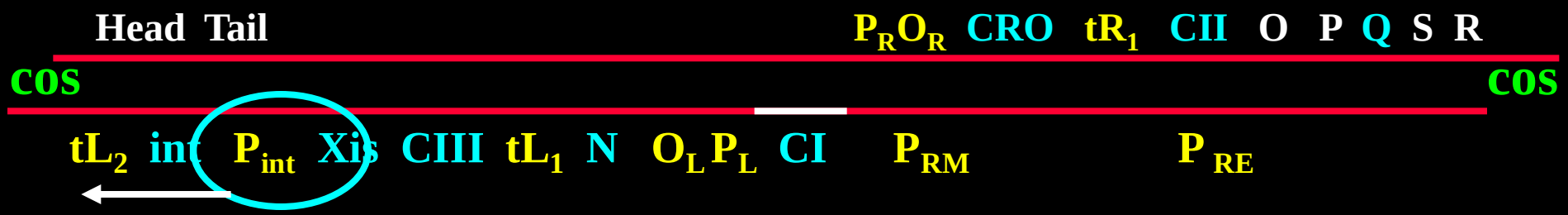
Located between OL1 — OL2

Negative control site with CI-p & Cro-p

Transcription N, CIII genes



Promoter



P_{int} **(Promoter for Integration)**

Located on the downstream of CIII

Positive control with CII-p & CIII-p

Regulation model

Early stage;

RNApol → $P_R O_R$

→ transcription CRO-p

↓

Stop at T_{R1}

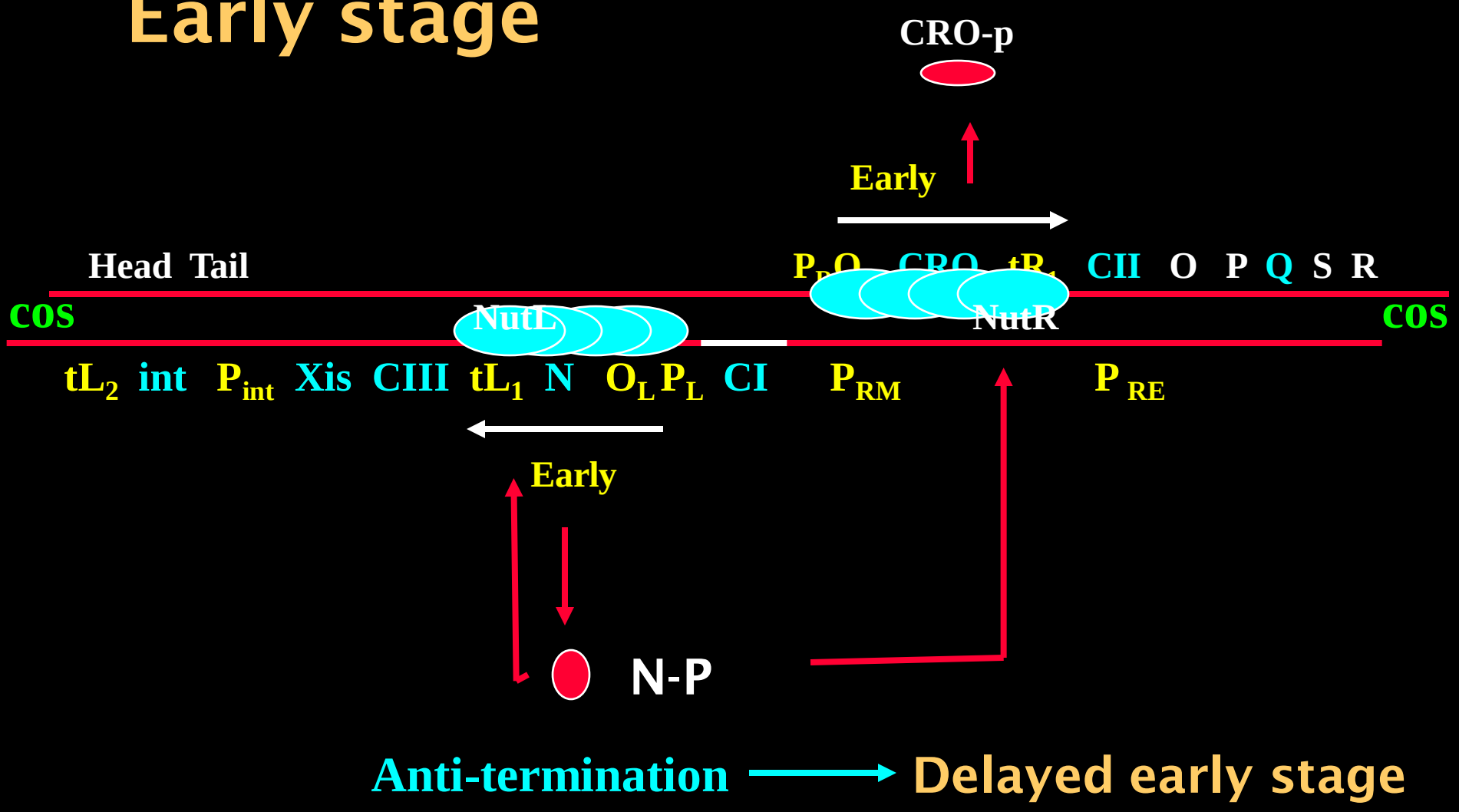
RNApol → $P_L O_L$

→ transcription N-p

↓

Stop at T_{L1}

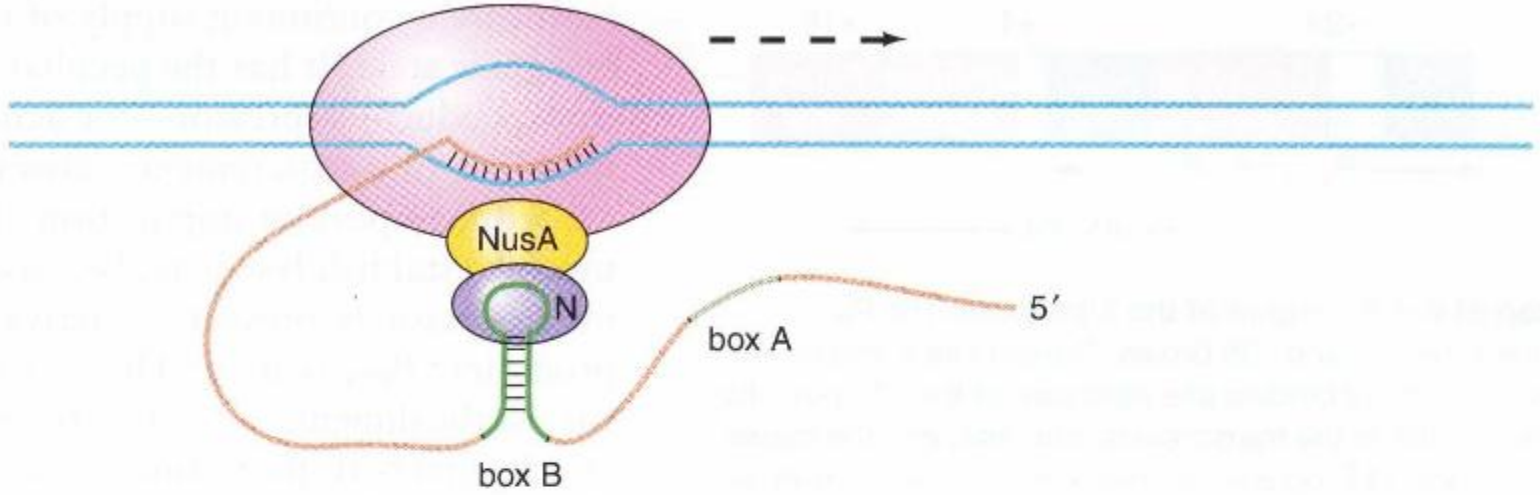
Early stage



Anti-termination → Delayed early stage

(a) Weak, nonprocessive complex

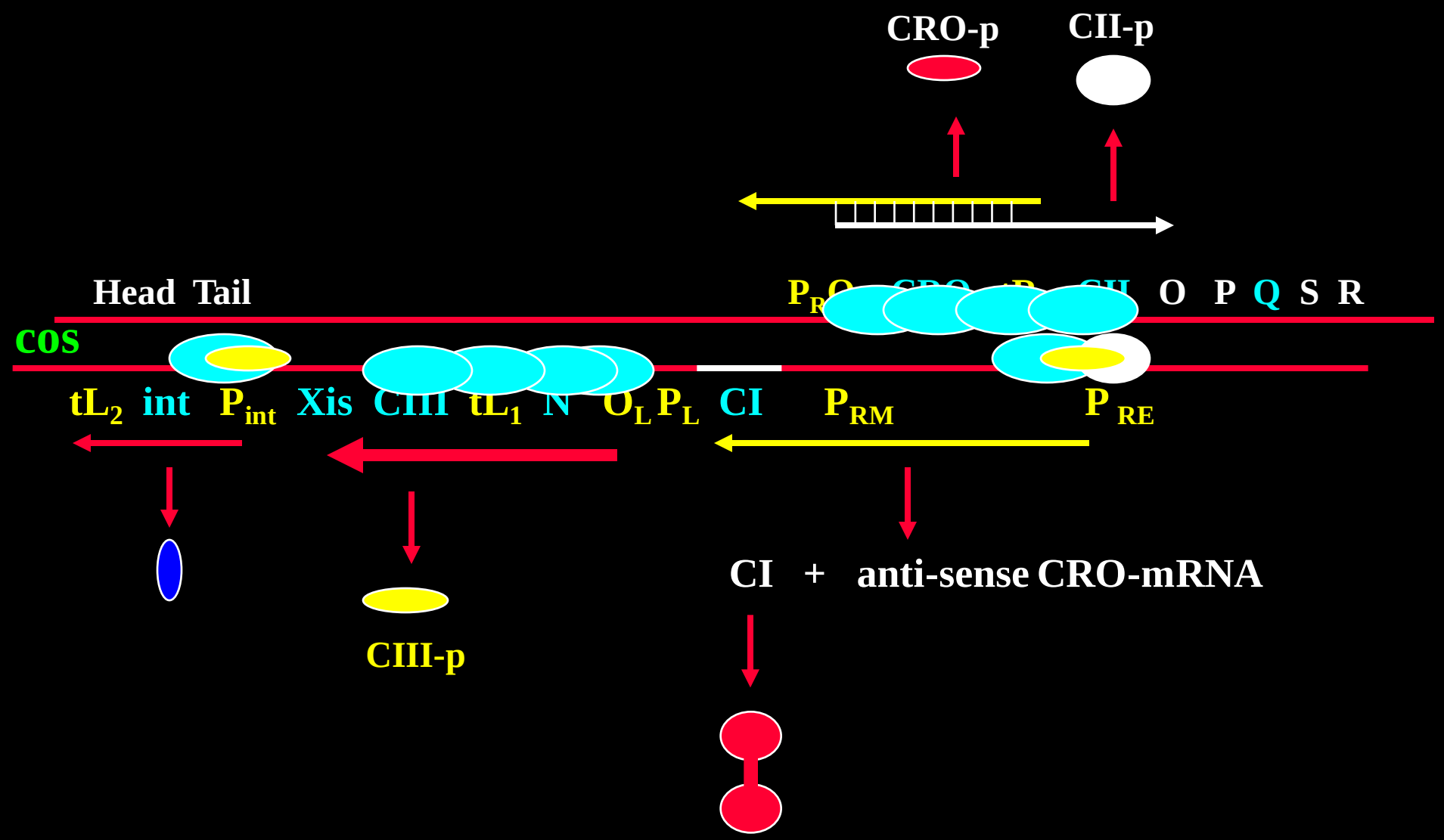
(来源：分子生物学（2007），郑用琏，第237页)



(a) NusA binds to polymerase, and N binds to both NusA and box B of the nut site region, creating a loop in the growing RNA. The antitermination is caused by inhibiting terminator hairpin formation

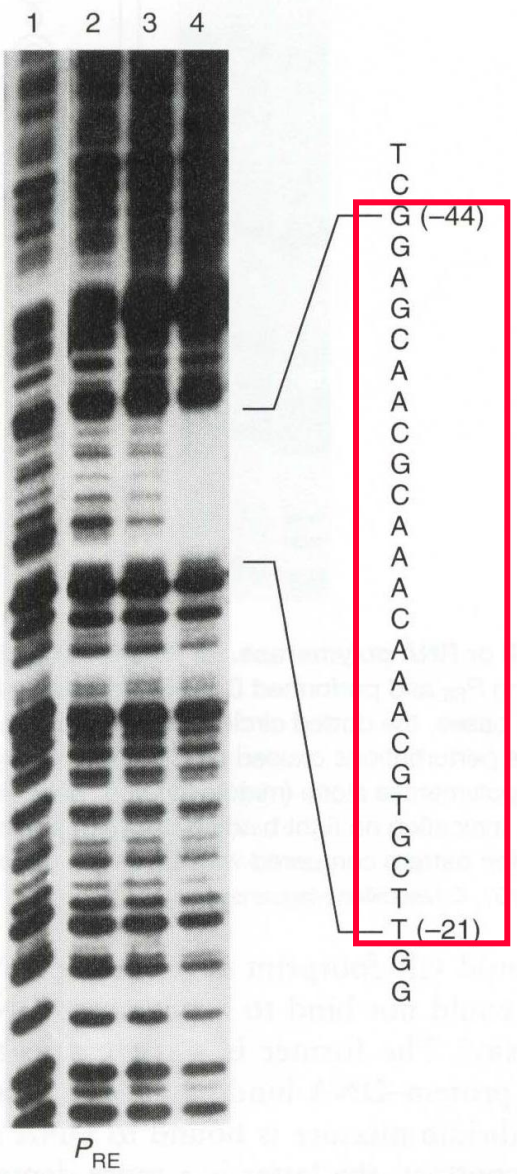
box B

Delayed early stage



interaction between CII and two early λ promoters

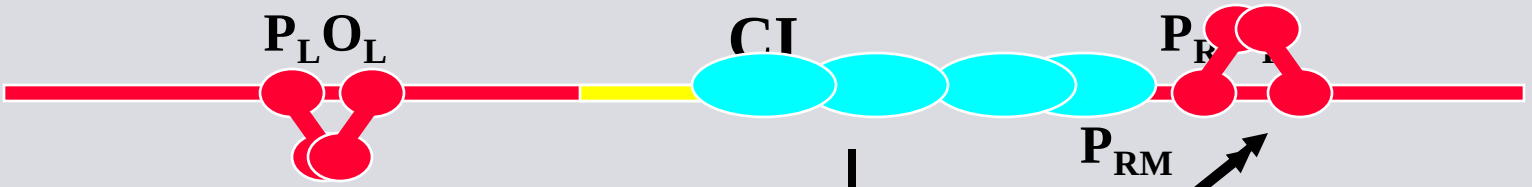
(Source:Ptashne. Nature 304 (1983) p. 705)



1) none CII
2) 10 pmol of CII
3) 18 pmol of CII
4) 90 pmol of CII

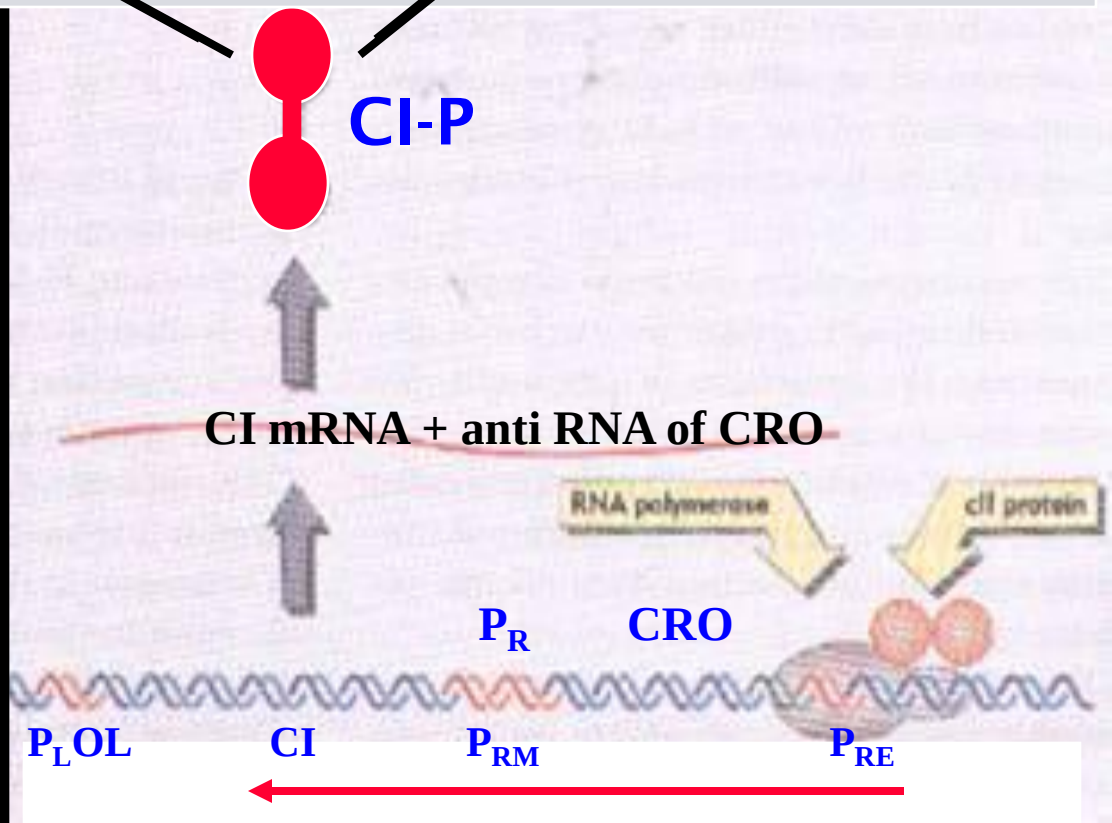
The CII footprint in promoter includes the -35 box (positive control site)

(来源：不详)



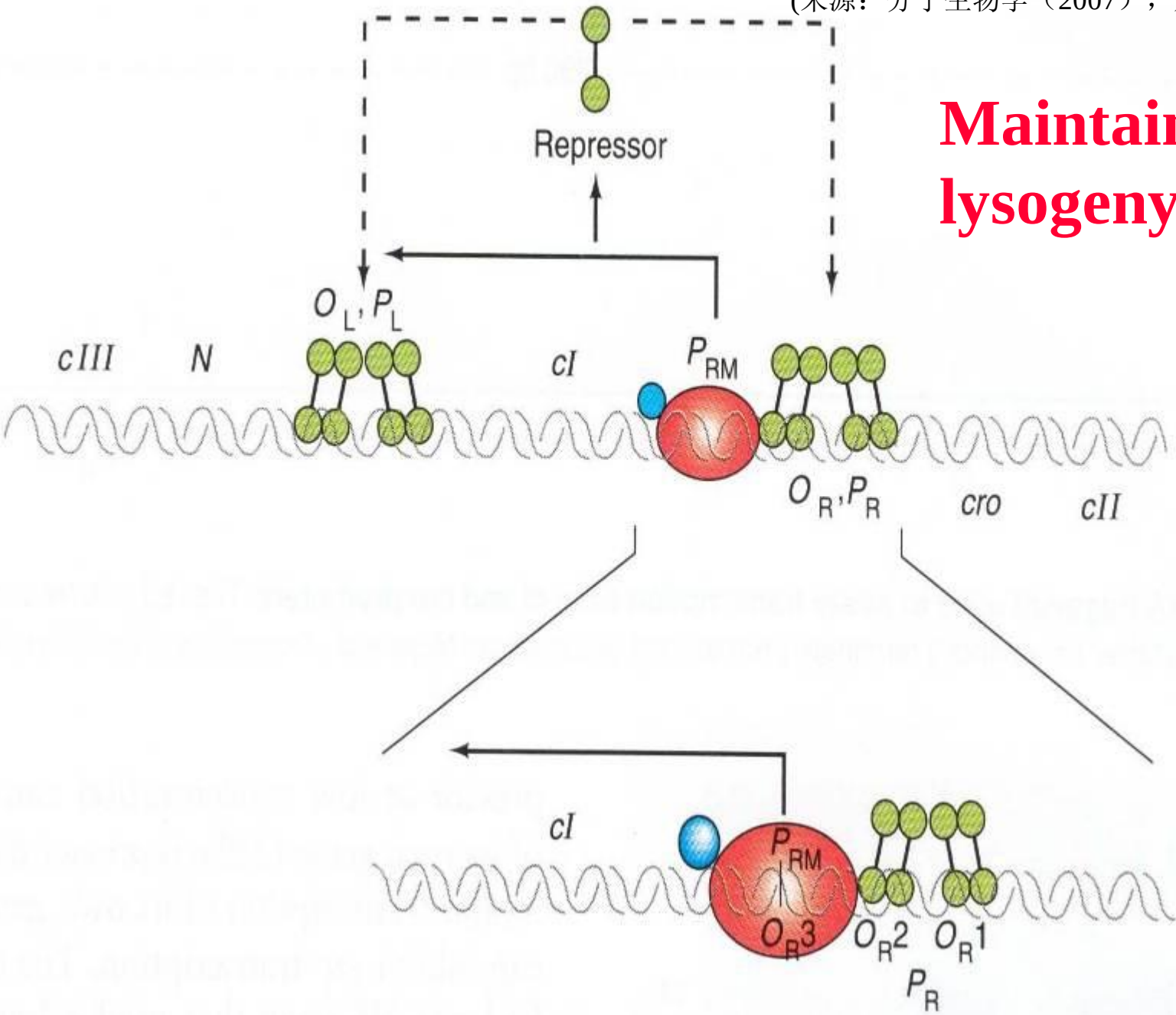
CI-P

Establishes Lysogenic Maintenance

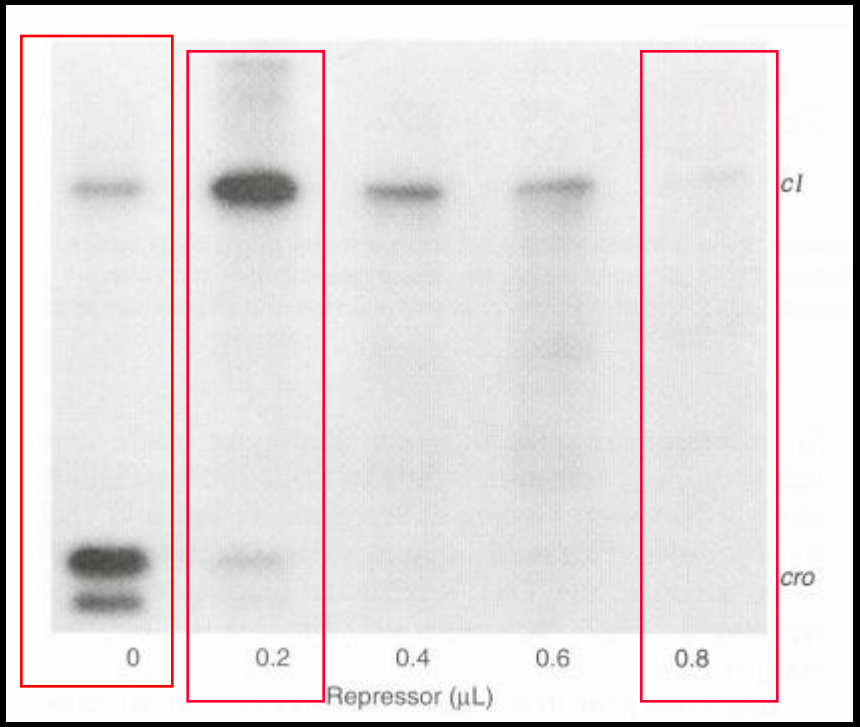


(来源：分子生物学（2007），郑用琏，第239页)

Maintaining lysogeny



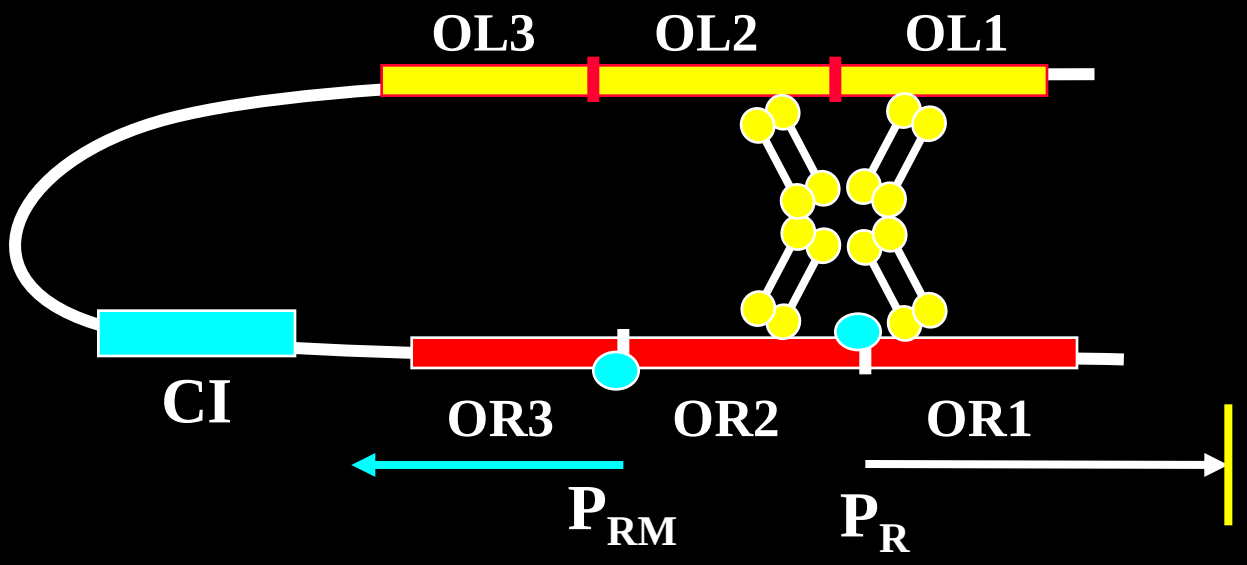
(来源：分子生物学（2007），郑用琏，第236页)

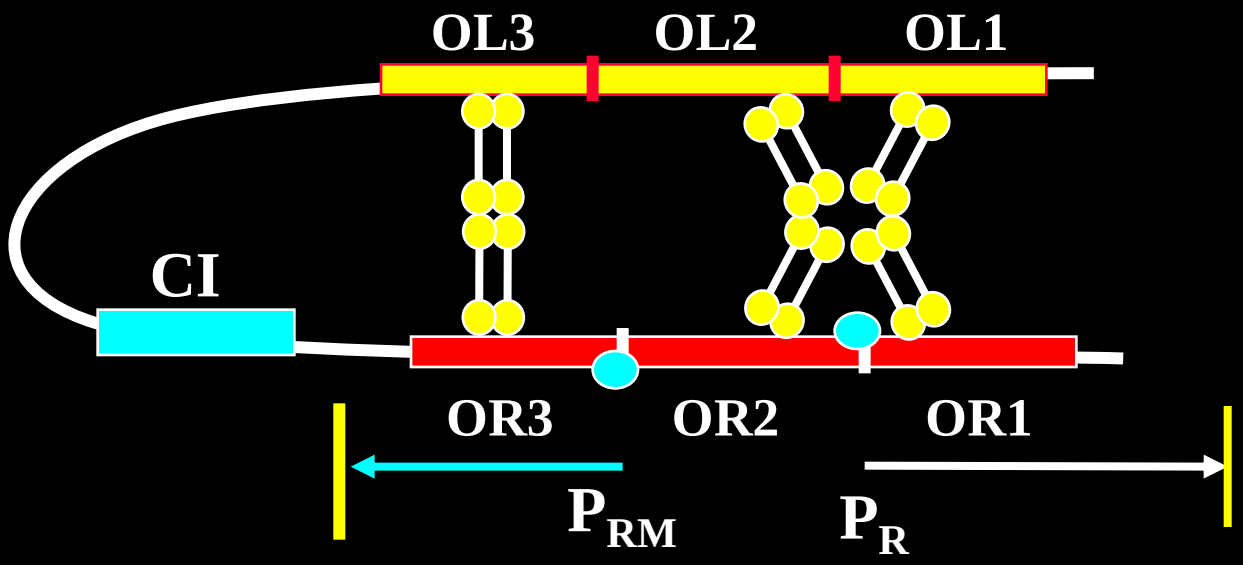


Increasing concentrations of repressor CI.
check the cl and cro transcripts,

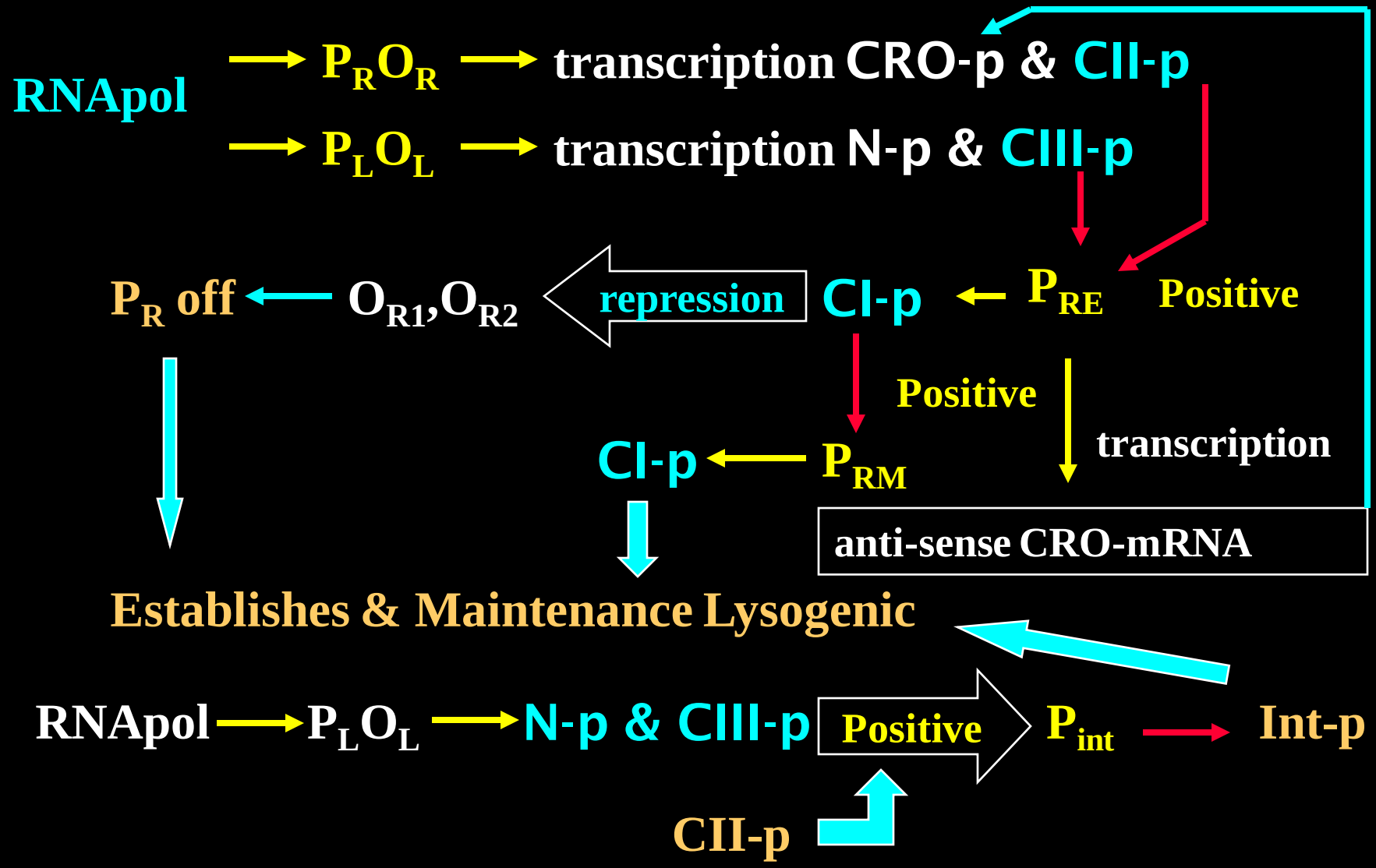
The repressor clearly inhibited cro transcription at low concentration,
but inhibited cl transcription at high concentration.

(Ptashne Meyer et al. Repressor turns off transcription of its own gene. P/VAS 72 (Dec 1975)

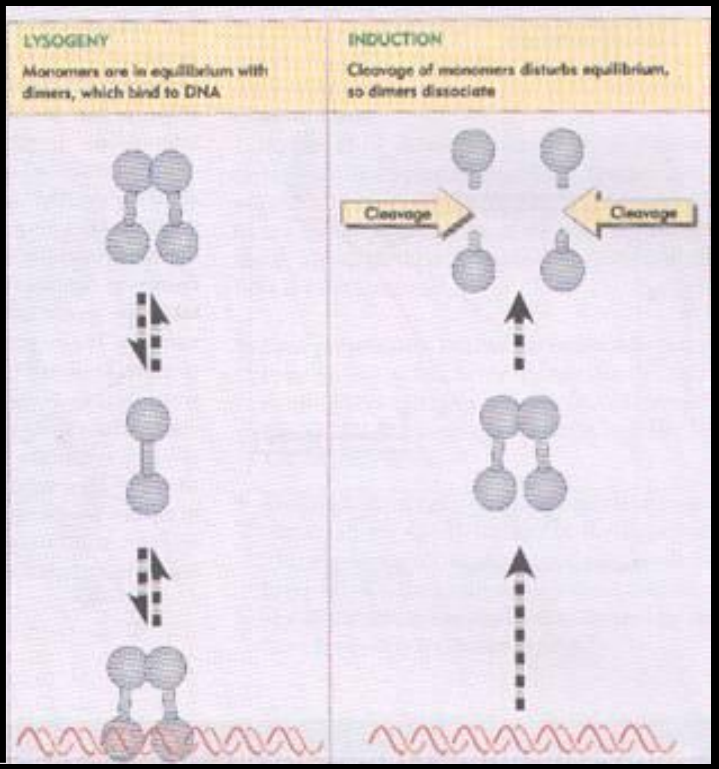

OL3
OL2
OL1
CI
OR3
OR2
OR1
P_{RM}
P_R

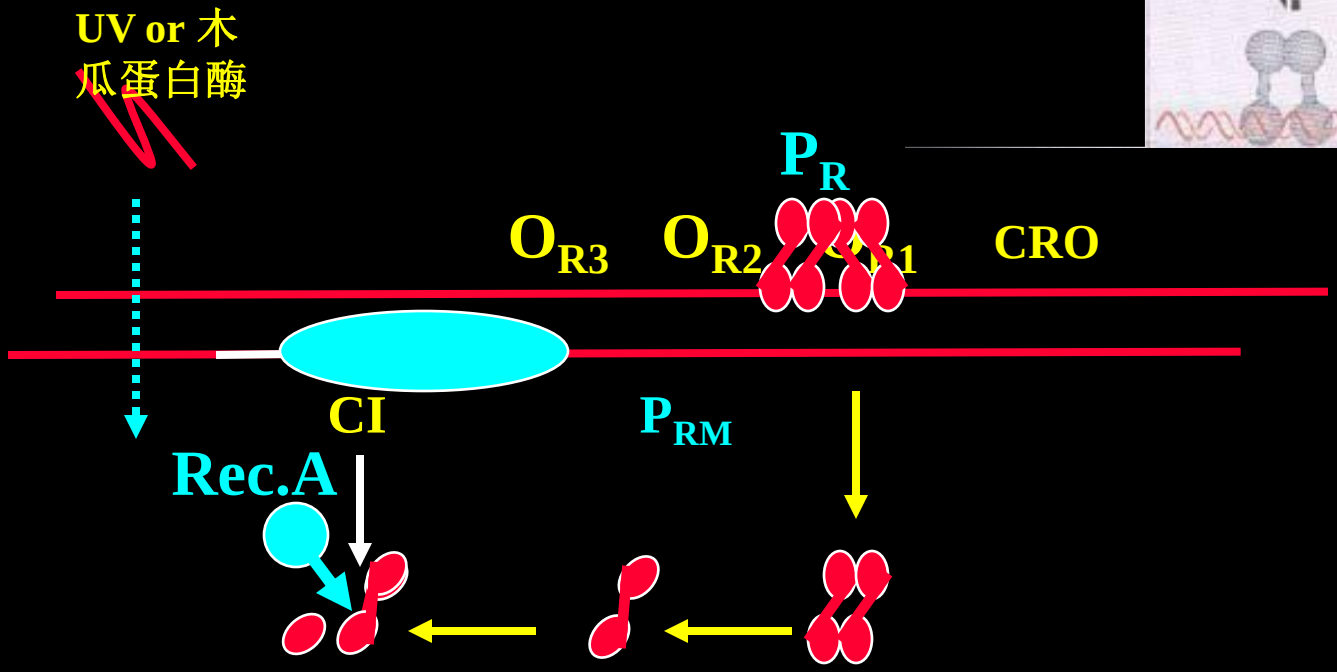

OL3
OL2
OL1
CI
OR3
OR2
OR1
P_{RM}
P_R

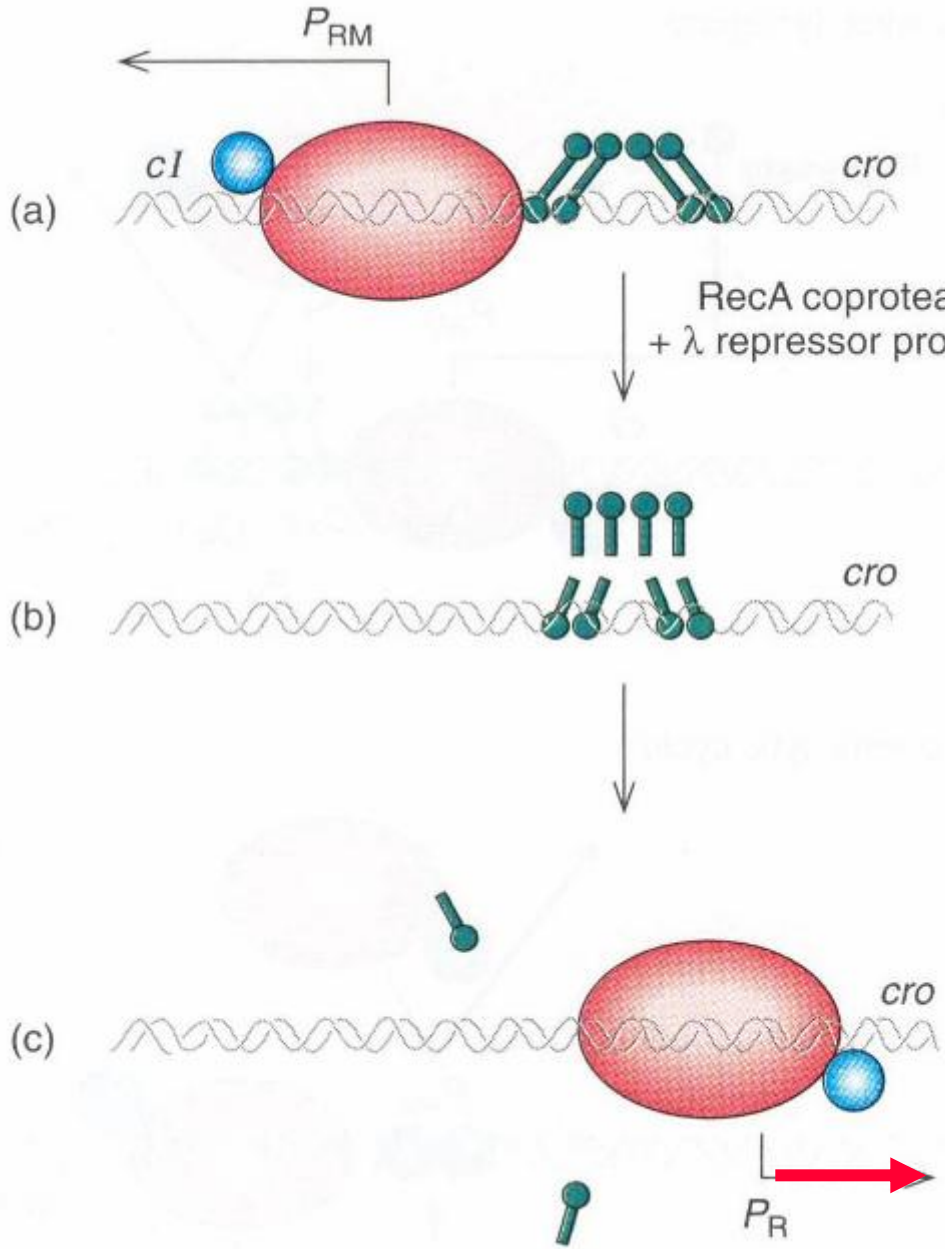
Delayed early stage



Delayed early stage





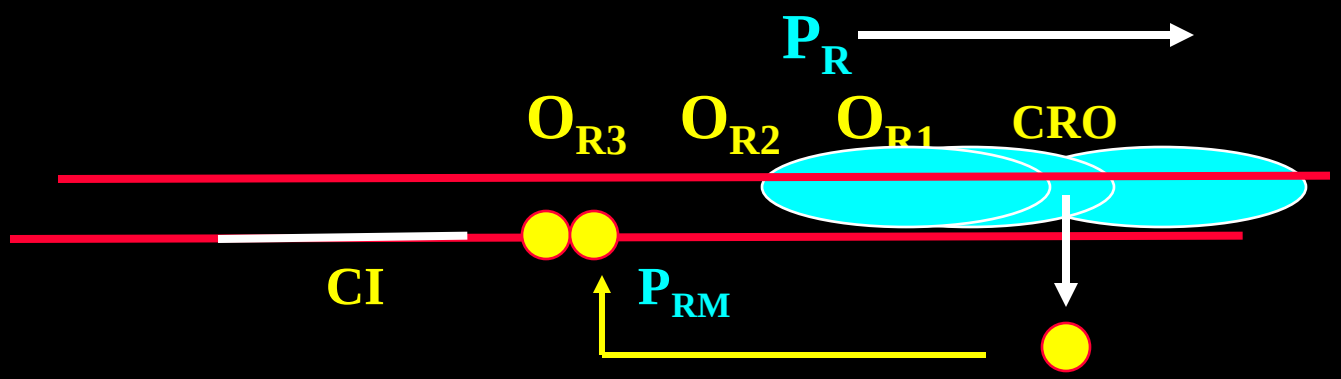


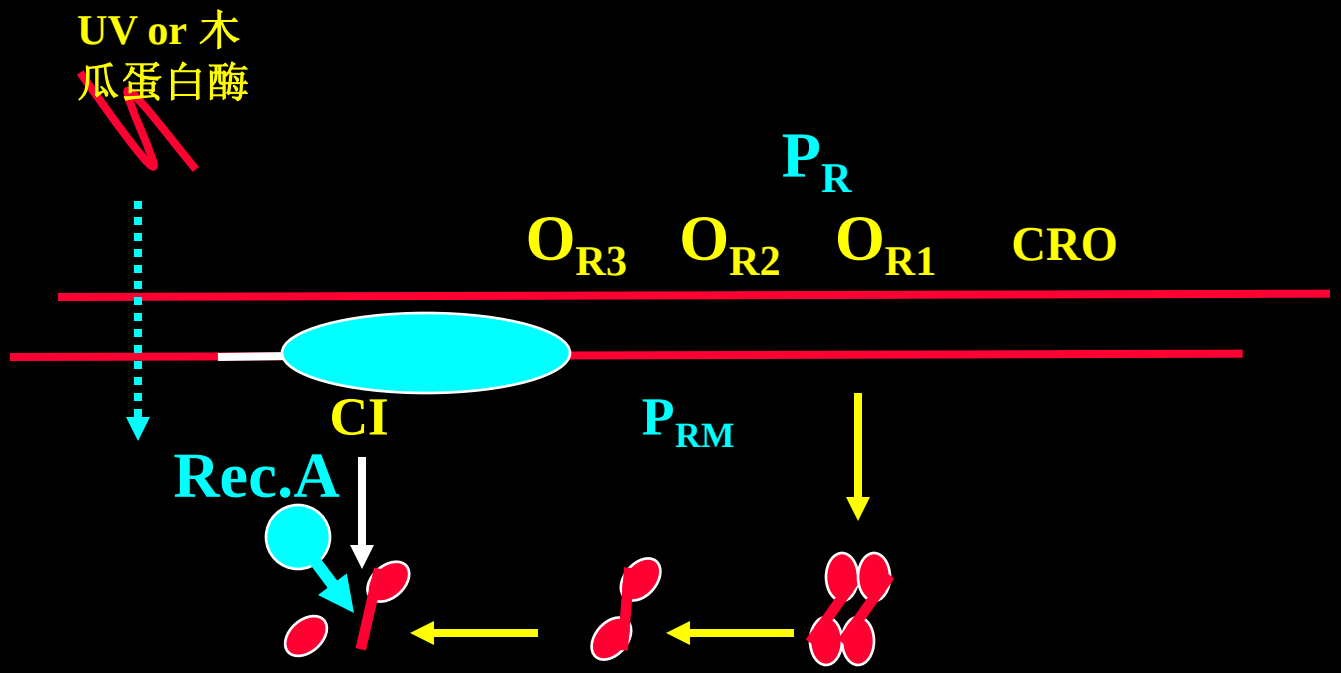
CI-P is bound to OR (and OL) and *cI* is being actively transcribed from the P_{RM} promoter.

The RecA co-protease unmasks a protease activity in the repressor, so it can cleave itself.

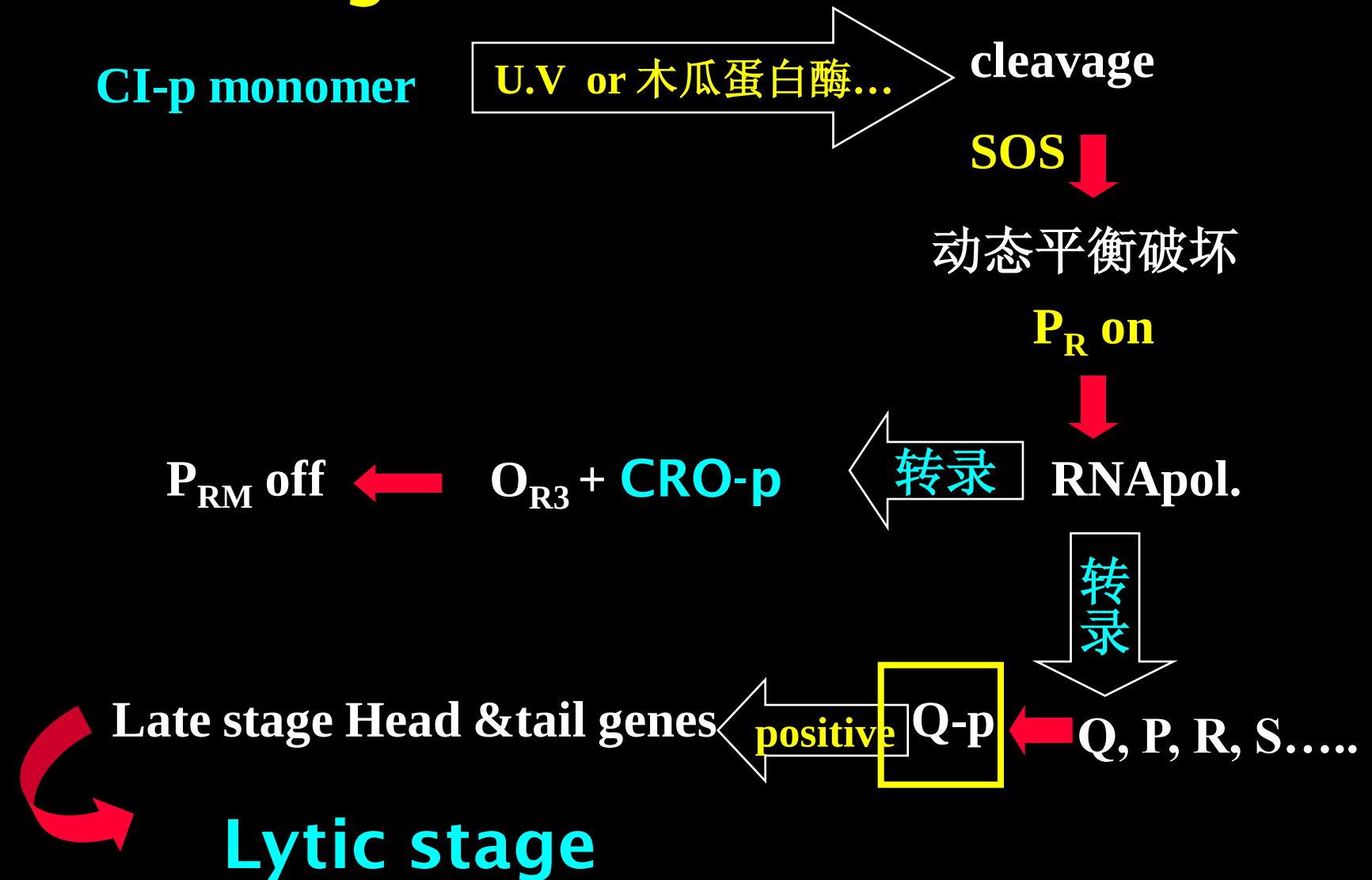
The severed repressor falls off the operator, allowing polymerase to bind to P_R and transcribe cro. Lysogeny is broken.

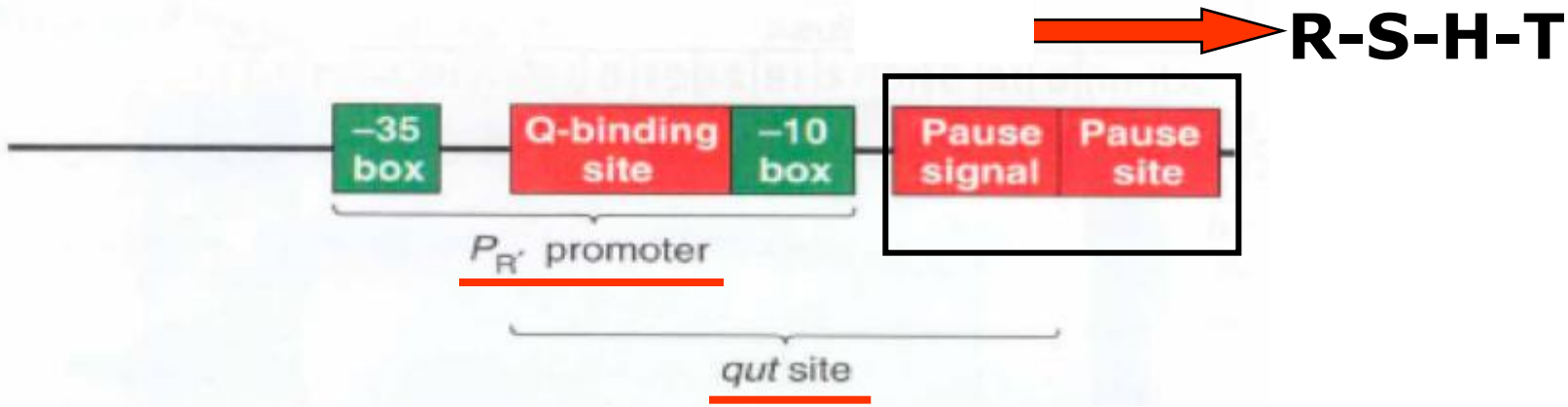
Late stage


P_R
O_{R3} O_{R2} O_{R1} CRO
CI
P_{RM}


UV or 木瓜蛋白酶
P_R
O_{R3} O_{R2} O_{R1} CRO
CI
P_{RM}
Rec.A

Late stage


CI-p monomer
U.V or 木瓜蛋白酶…
cleavage
SOS
动态平衡破坏
P_R on
RNApol.
转录
O_{R3} + CRO-p
P_{RM} off
转录
Q, P, R, S…..
positive
Q-p
Late stage Head &tail genes
Lytic stage



Q-p binding to *qut* site （overlapping 16-17bp with $P_{R'}$）

-Q: RNApol到达Pause site后暂停数分钟， 越过暂停位点，只转录到终止子后便放弃晚期基因的转录。

+Q: Q识别暂停复合体并与qut位点结合，然后Q结合到RNA聚合酶上，忽略终止子，继续转录晚期基因。

Late stage

《老鼠逃离即将沉没的船》

SOS响应溶源菌遭受DNA损伤的信号
帮助λ通过诱导裂解远离不利环境

Late stage

---other controlling system for Lytic way selection

The purpose of λ phage infection E.coli

——Lysis the host of E.coli

自然选择形成；--- λ ➡ host ➡ RNApol. binding P_RO_R

early stage ⬅ CRO-p

---CRO-p binding OR3 ➡ off P_{RM}

具有选择lytic 趋势

如果*cI*先表达则建立溶源态，如果cro胜出则被侵染的细菌会裂解。

如果*cI*基因产生足够多的阻抑物，那么这些蛋白会结合到O_R和O_L上阻止早期基因的进一步转录

如果产生了足够的CRO蛋白，它会阻止cI基因的转录从而阻碍溶源态的建立。

什么因素决定是cI还是cro在竞争中胜出呢？

CII的浓度

CII的浓度越高，就倾向于形成溶源态

控制着CII的浓度

细胞内蛋白酶的浓度（HFL）

培养营养环境因素

为什么λ噬菌体又要选择溶原途径作为自己生活周期的一种方式呢？

选择溶原途径又具有什么生物学意义呢？

• ***HFL*** **gene control of host**

(high frequency lysogenesis)

HFL **gene** → **mut.** ***hfl***

↓

HFL-p → **degradation** CII-p → **lytic**

When [C] starved → ***HFL*** **gene off (筹集粮草)**

↓

degradation CII-p

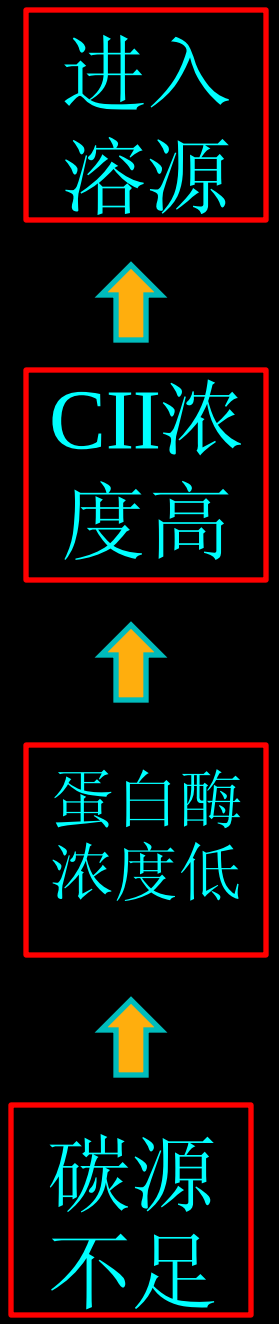
→ **Positive.** → **Lysogenic**

CII的浓度越高，就倾向于形成溶源态

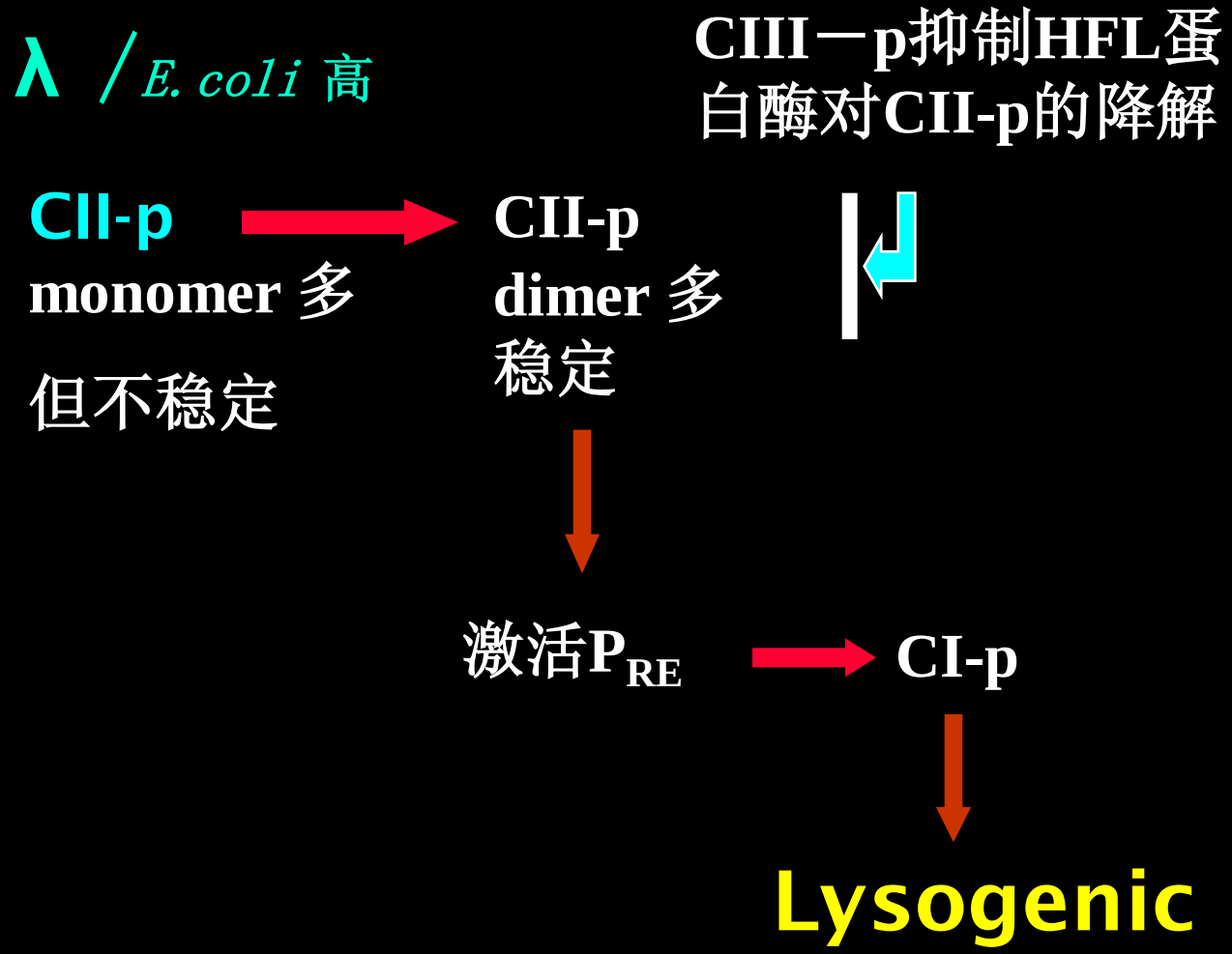
控制着CII的浓度

细胞内蛋白酶的浓度（HFL）

培养营养环境因素



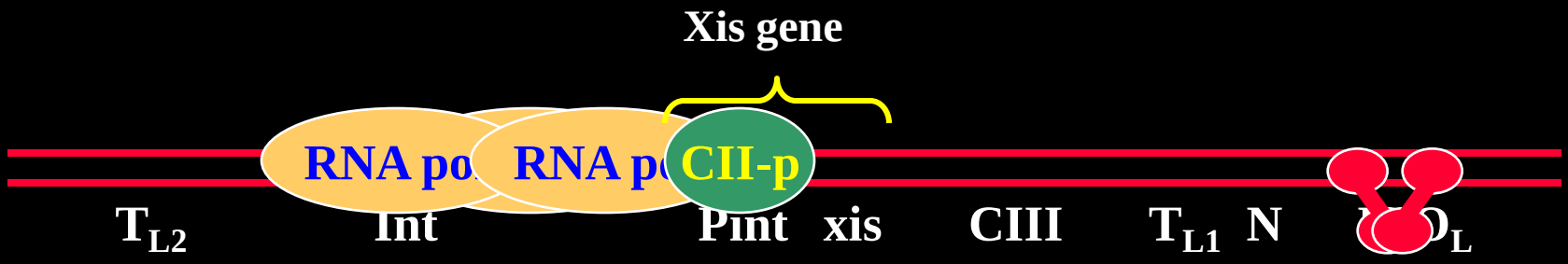
• **MOI ⩾10 (Multiplicity of infection)**

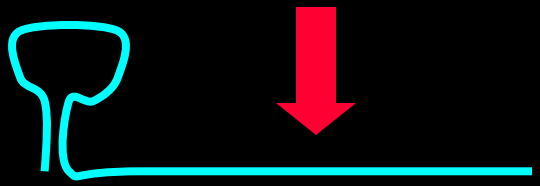


- ***Int* gene and retro-regulation of *sib* site**

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

When lysogenic way be selected



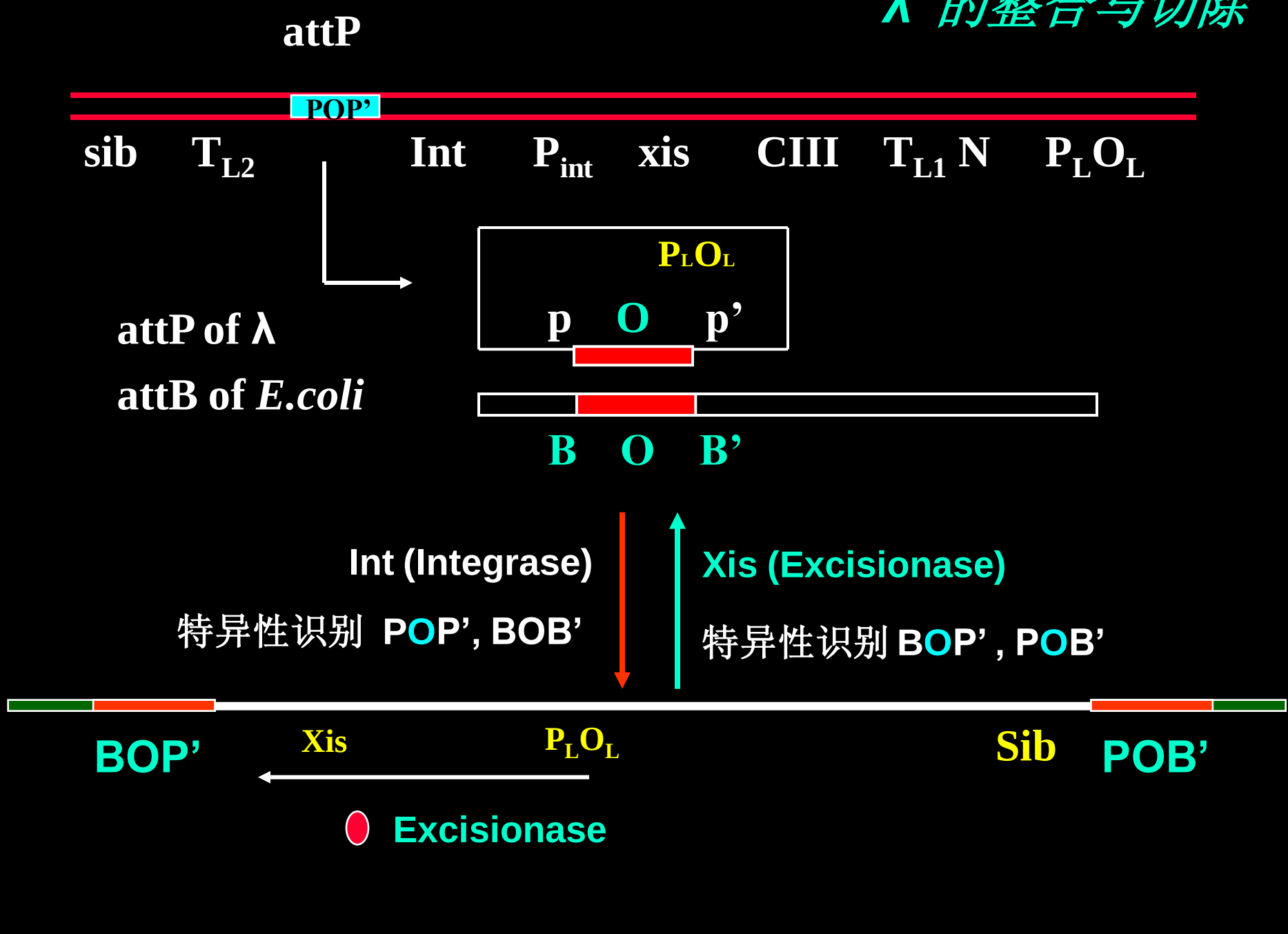


Int mRNA

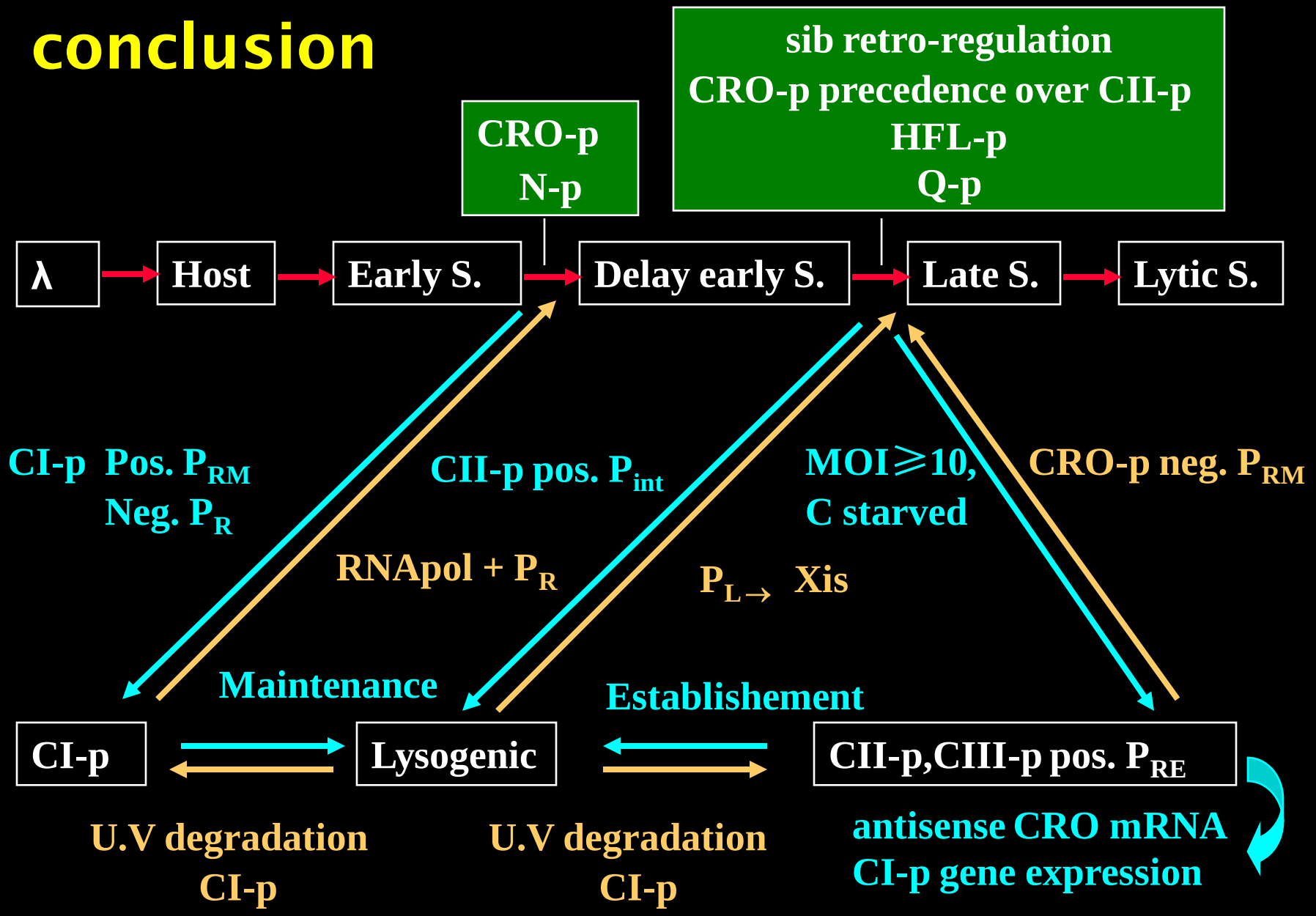
No XIS-p (Pint located in the region of xis)

Integratase → lysogenic way

λ 的整合与切除


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

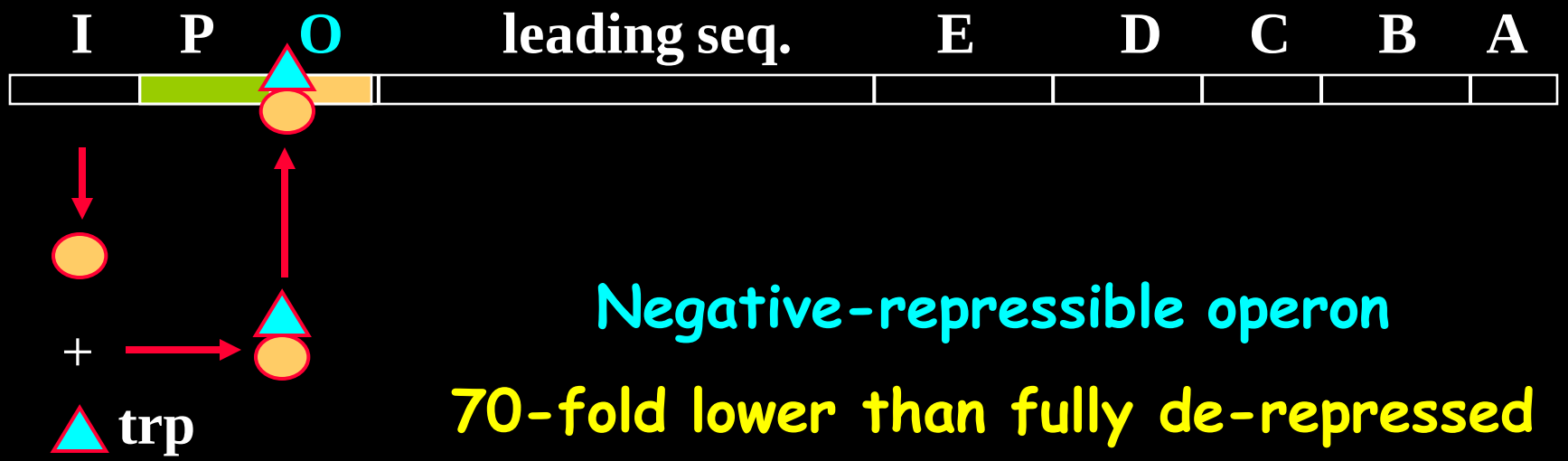
conclusion


sib retro-regulation
CRO-p precedence over CII-p
HFL-p
Q-p
CRO-p
N-p
λ
Host
Early S.
Delay early S.
Late S.
Lytic S.
CI-p Pos. P_{RM}
Neg. P_R
CII-p pos. P_{int}
RNApol + P_R
MOI⩾10,
C starved
$P_{L\rightarrow}$ Xis
CRO-p neg. P_{RM}
Maintenance
Establishement
CI-p
Lysogenic
CII-p,CIII-p pos. P_{RE}
U.V degradation
CI-p
U.V degradation
CI-p
antisense CRO mRNA
CI-p gene expression

6.1.2. Attenuator control

a) discovery

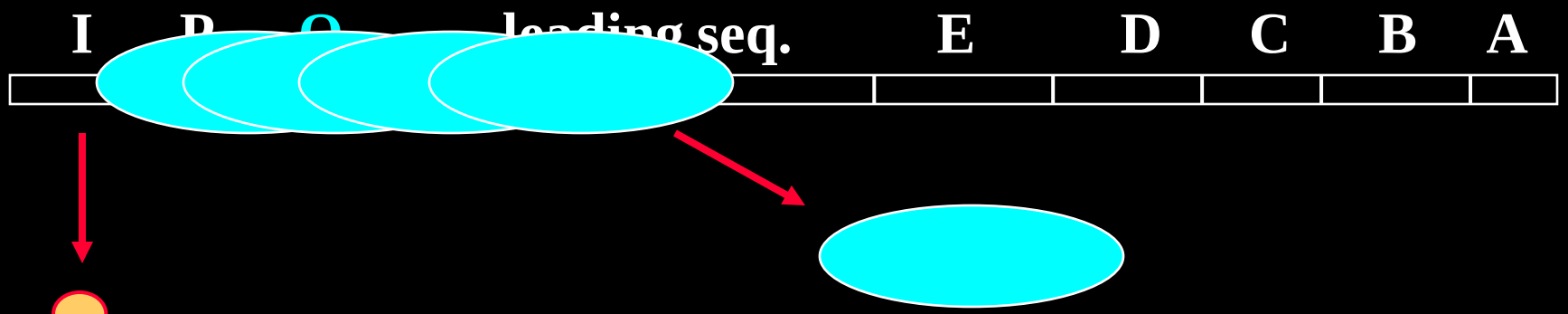
- *E.coli* trp synthetase operon (C. Yanofsky *stanford Univ.*)



Negative-repressible operon

70-fold lower than fully de-repressed

- 1968. Imamato Lab.



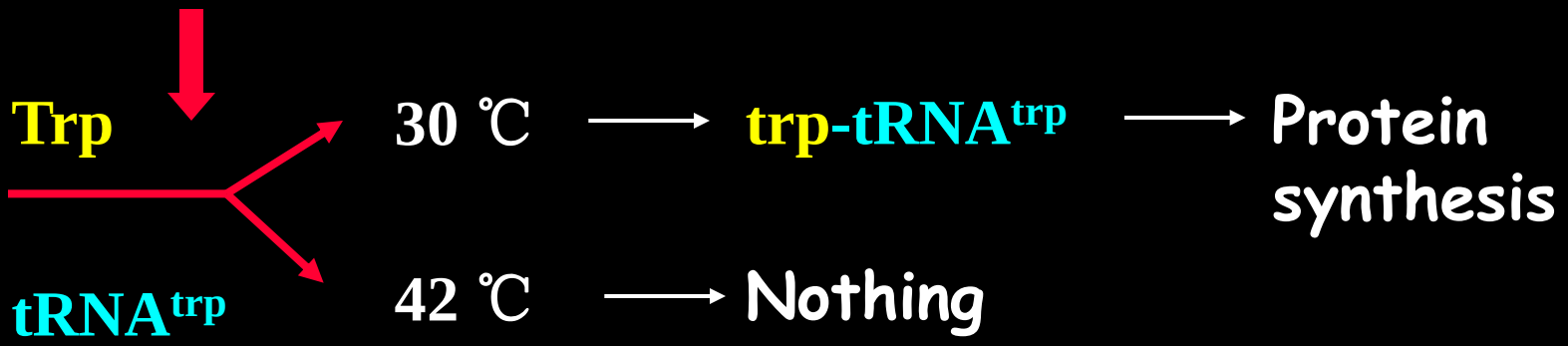
\+ → the complex can not bind with the O site

Low trp

When trp level is low, RNApol moves but falls off in the L.S. and the transcription of the structure genes is blocked.

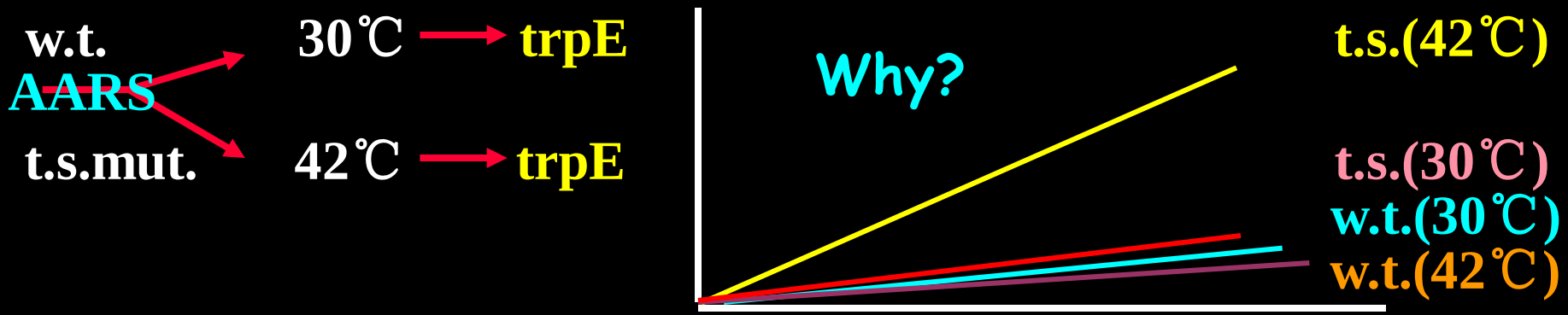
- 1975 Imamato Lab.

E.coli trp-AARS (t.s)



Low trp , operon open

trpE



- t.s.(42℃) AARS inactive

→ no trp-$tRNA^{trp}$ synthesis

RNApol move past L.S

→ transcript of trp synthetase RNA

(E enzyme level is high）

- t.s. (30℃), w.t.(30℃), (42℃) AARS active

→ synthesize trp-$tRNA^{trp}$

RNApol break off at L.S.

→ no transcription of trp synthetase RNA

（ E enzyme level is low）

Conclusion:

---When trp and/or trp-tRNA^{trp} exist in cells,

there is no synthesis of trp

(Fine-tuned control)

--- t.s.(42℃) no AARS and trp-tRNA^{trp},

but take for no trp by error,

（initiate the transcription of trp synthetase RNA)

---trp-tRNA^{trp} is the main factor leading to RNApol. falling off at L.S.

- **Fine-tuned regulation within the leading sequence**

I po L.S. E D C B A

When trp is rich,
B activity detected

genotype	I^+	i^C
Trp Δ ED	0.17	11.8
Trp ΔL.S.ED	1.32	100

?

I^+; operon off low E activity

i^C; operon on high E activity (~70X)

The difference of E activity of Δ ED &ΔL.S.ED (~10X) results from the L. S.

Controls the operon over a 700-fold , range from fully inactive to fully active

0.17 100

- **leading seq with cis-acting domain**

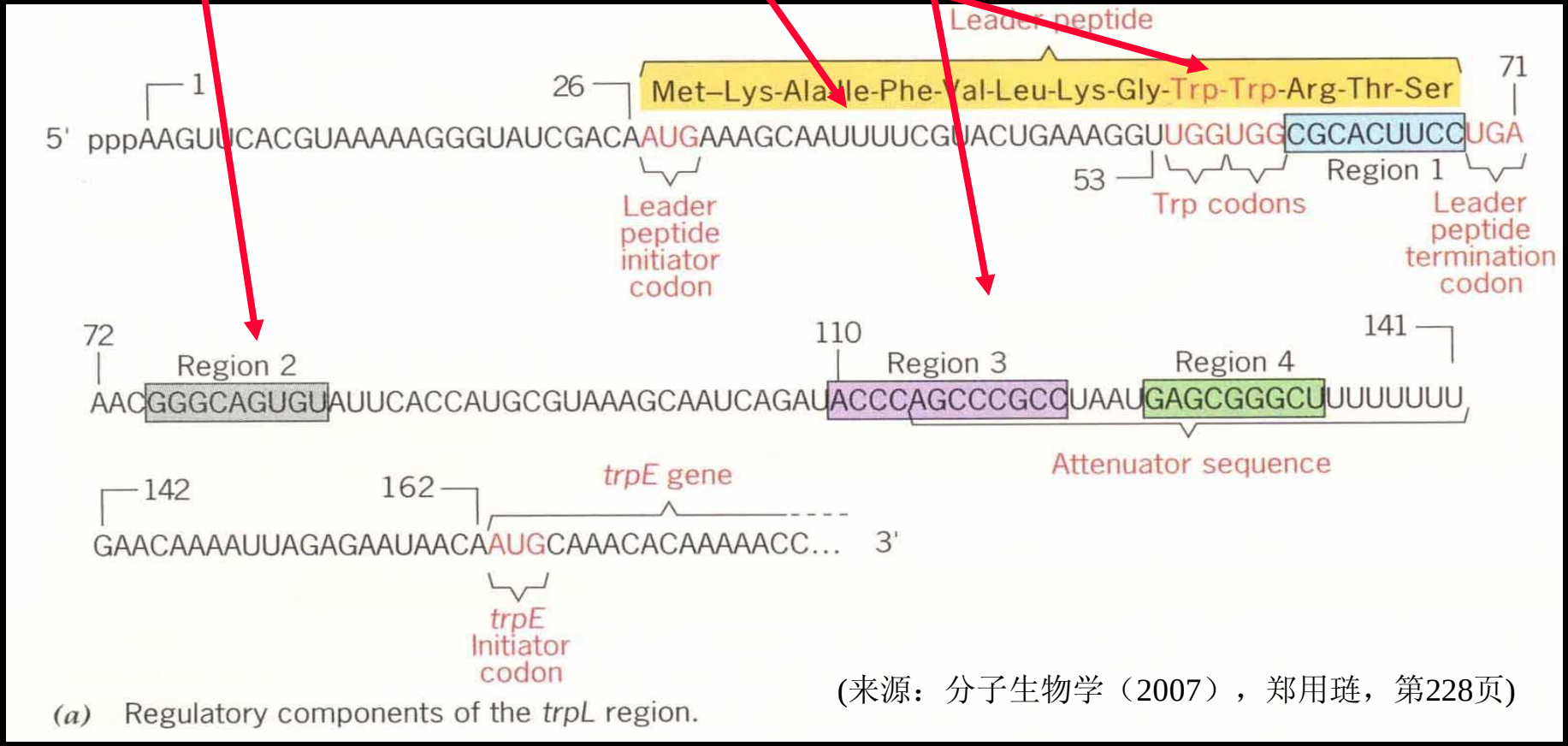
Partial diploid test（trp rich）

Genotype		E activity	A activity
trpi^C	L.S. E D C B A	1.4	14.8
trp $i^C\Delta$ A	L.S. E D C B	1.1	0
trp $i^C\Delta$ E	L.S. D C B A	0	16.5
trp $i^C\Delta$ L-C	B A	0	94
trp $i^C\Delta$ L-C	B A	2.5	102
p- trp $i^C\Delta$ A	p L.S. E D C B		

L.S. without complementary effect.
L.S. is a cis factor with attenuation effect.
Cis-dominant F

b) The primary structure of trp operon RNA

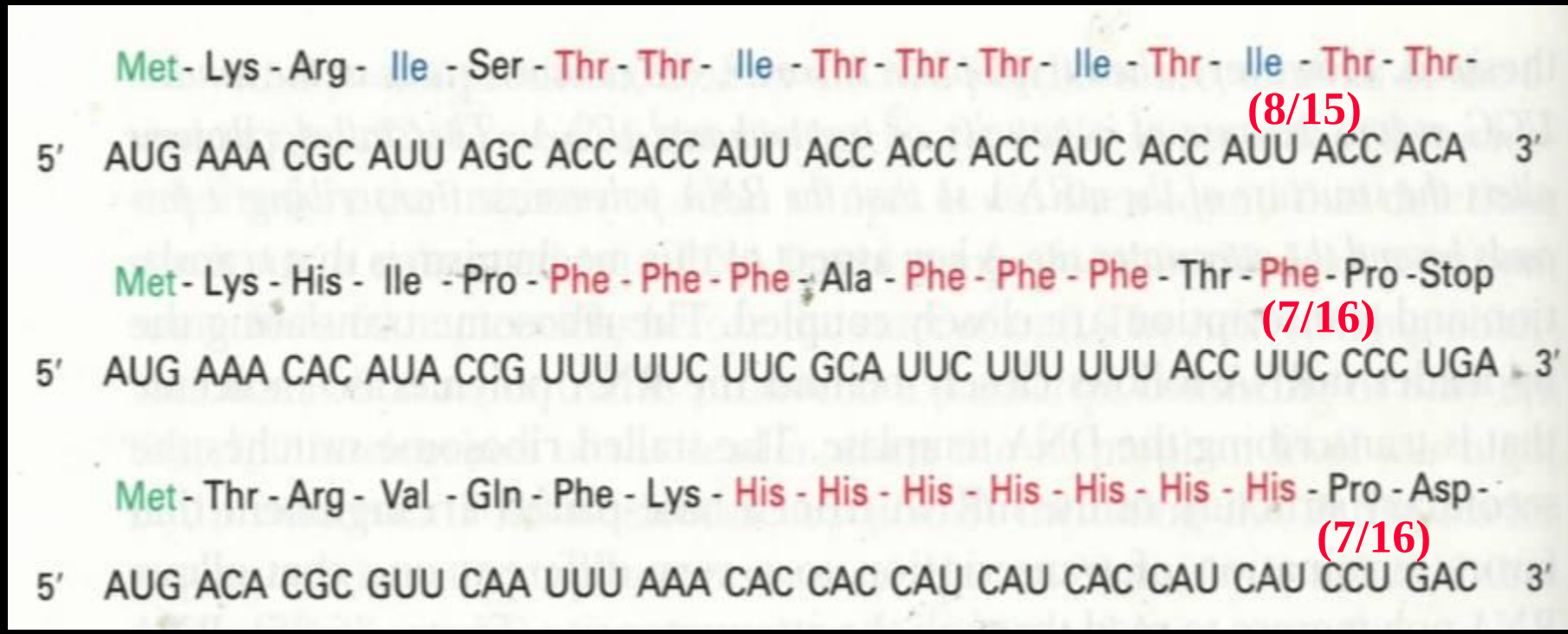
- **60~68—75~83 & 110~121—126~134 (palindromic seq.) with poly (U)**
- **27—68 base → ORF of 14aa**
- **140 base RNA almost binding with protein (translatable seq.)**
- **trp high frequency with 1/7 in 14 aa**



(a) Regulatory components of the *trpL* region.

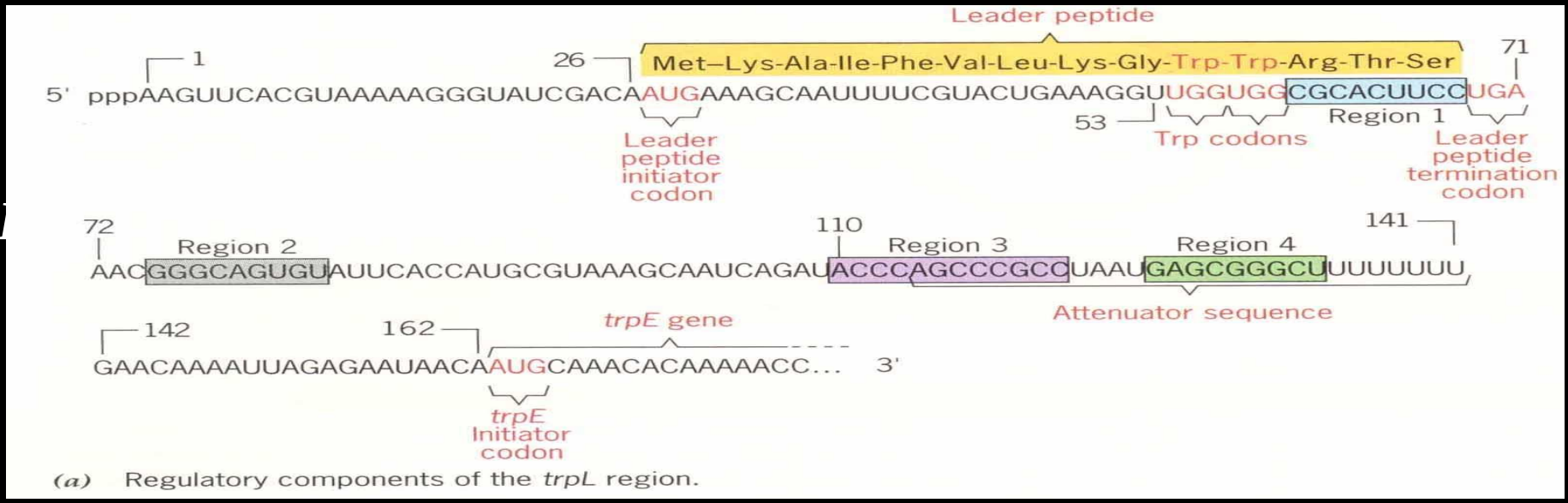
(来源：分子生物学（2007），郑用琏，第228页)

Peptides of L. S. for a few Operons encoding enzymes for amino acid synthesis where transcription attenuation occurs.



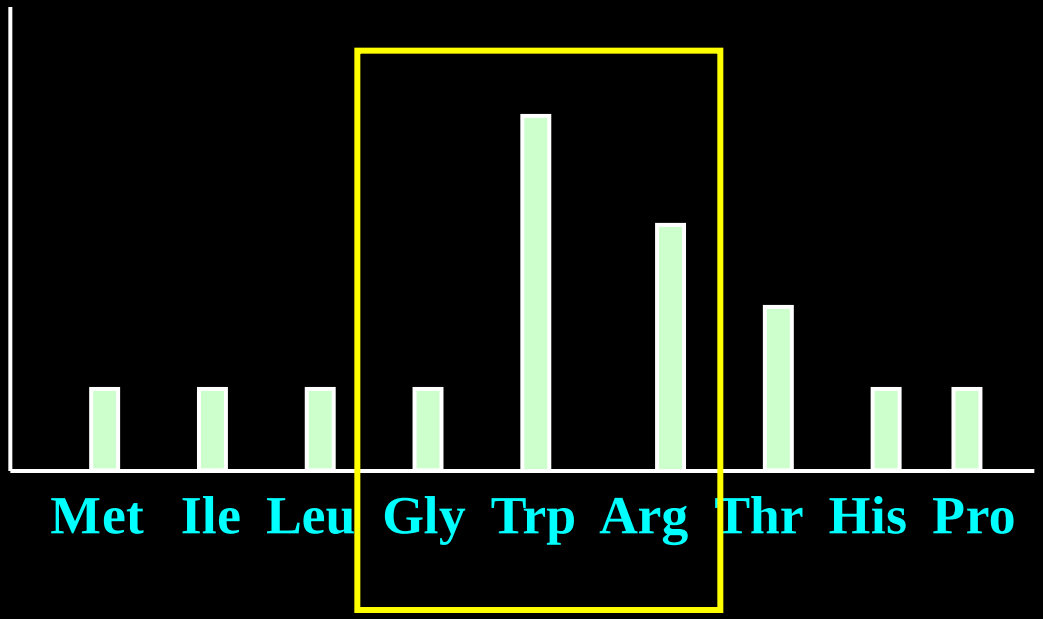
What is meaning ?

(来源：分子生物学（2007），郑用琏，第229页)



(a) Regulatory components of the *trpL* region.

H_3-U mRNA

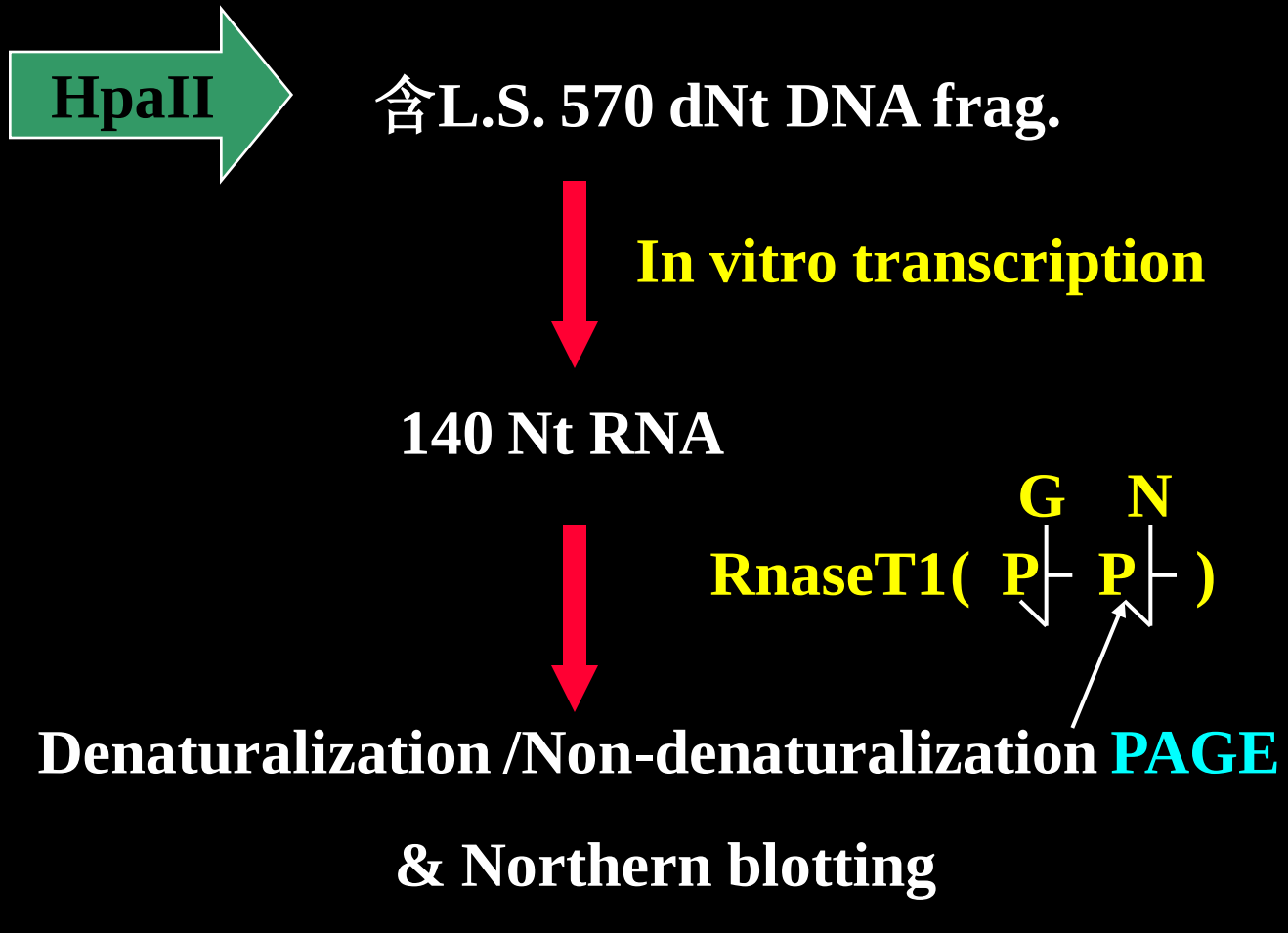


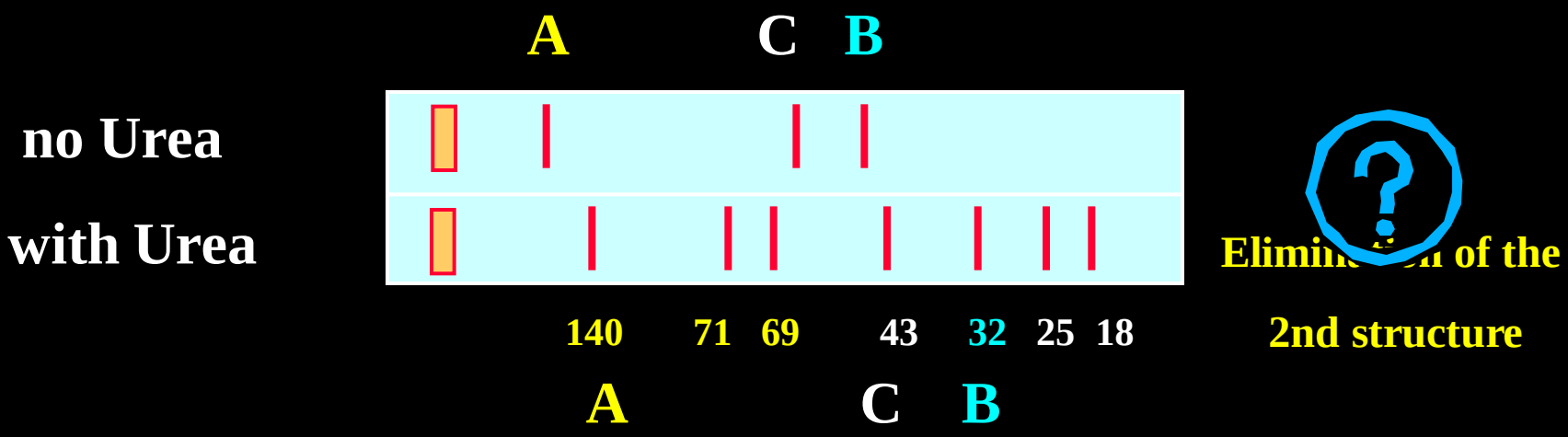
- Trp codon and order play an important role on the expression of the operon
- but Arg is important likewise!

Why?

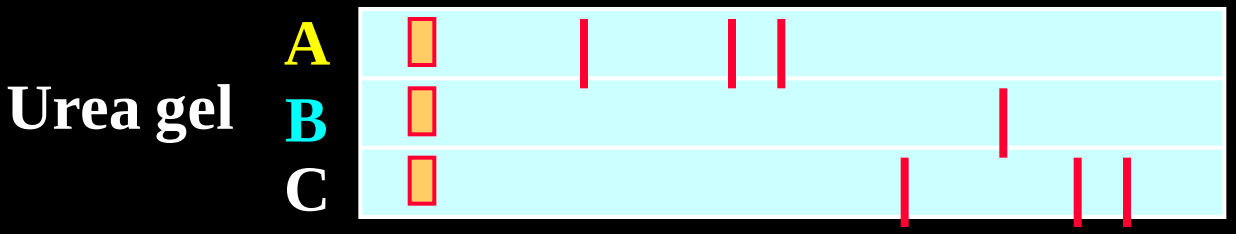
c) The secondary structure of the 140 Nt RNA

- *E.coli* DNA

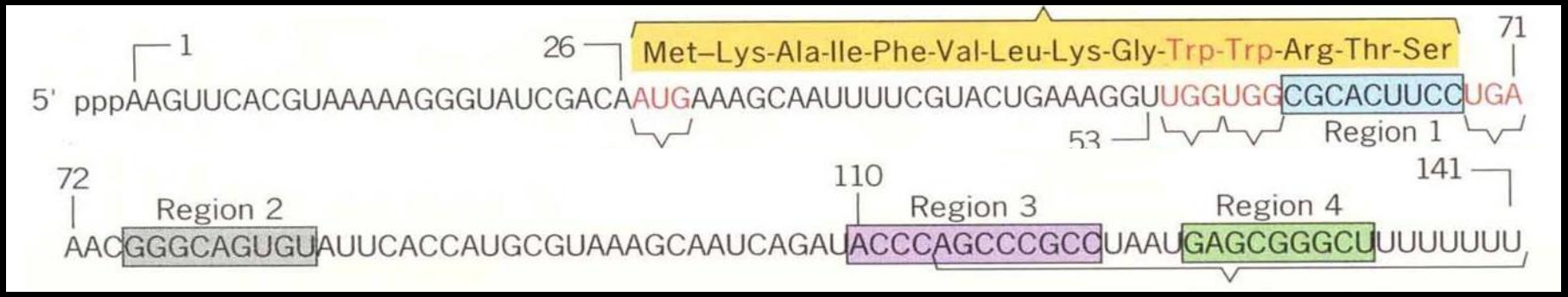




Recycle A, B, C bands



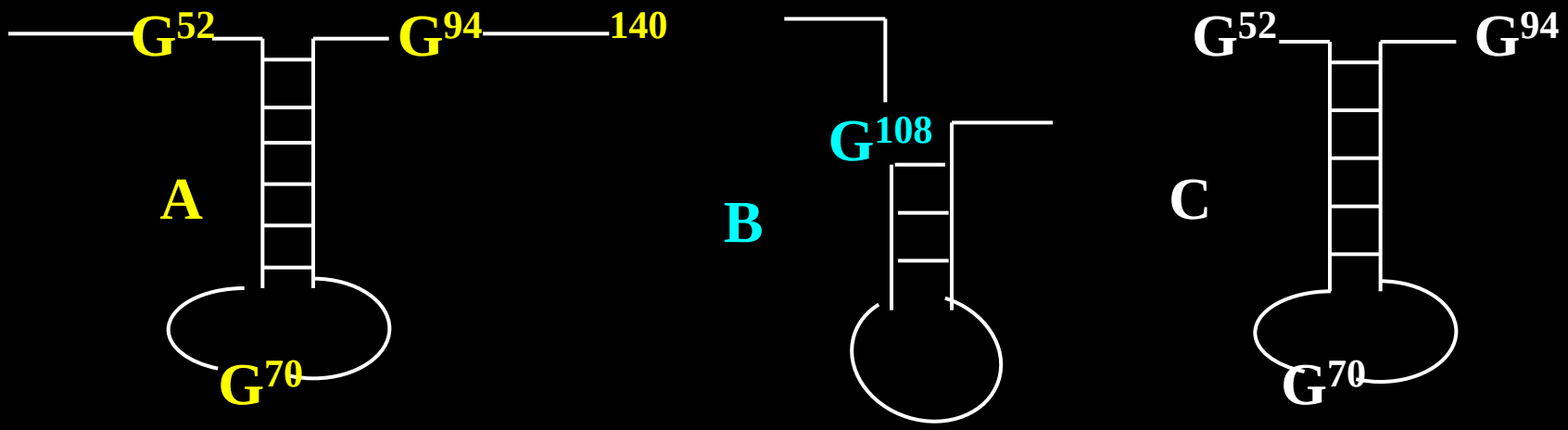
Conclusion:



A band（140Nt）70thNt is G, locates in the single strand

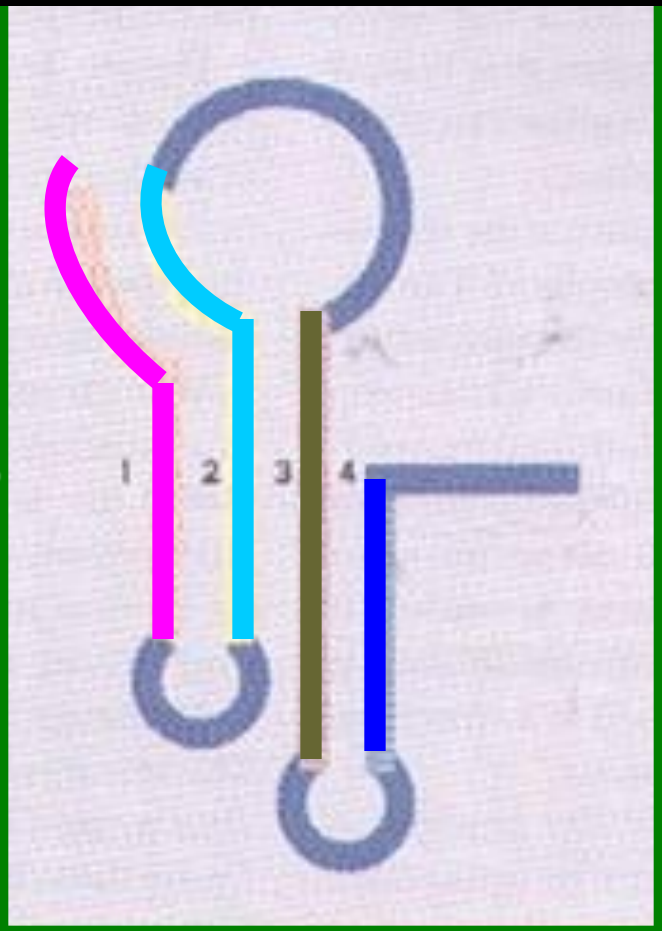
B band（108-140Nt） no G exposes in the single strand

C band（52-94Nt）52th--70th--94th is a fragment of A band's



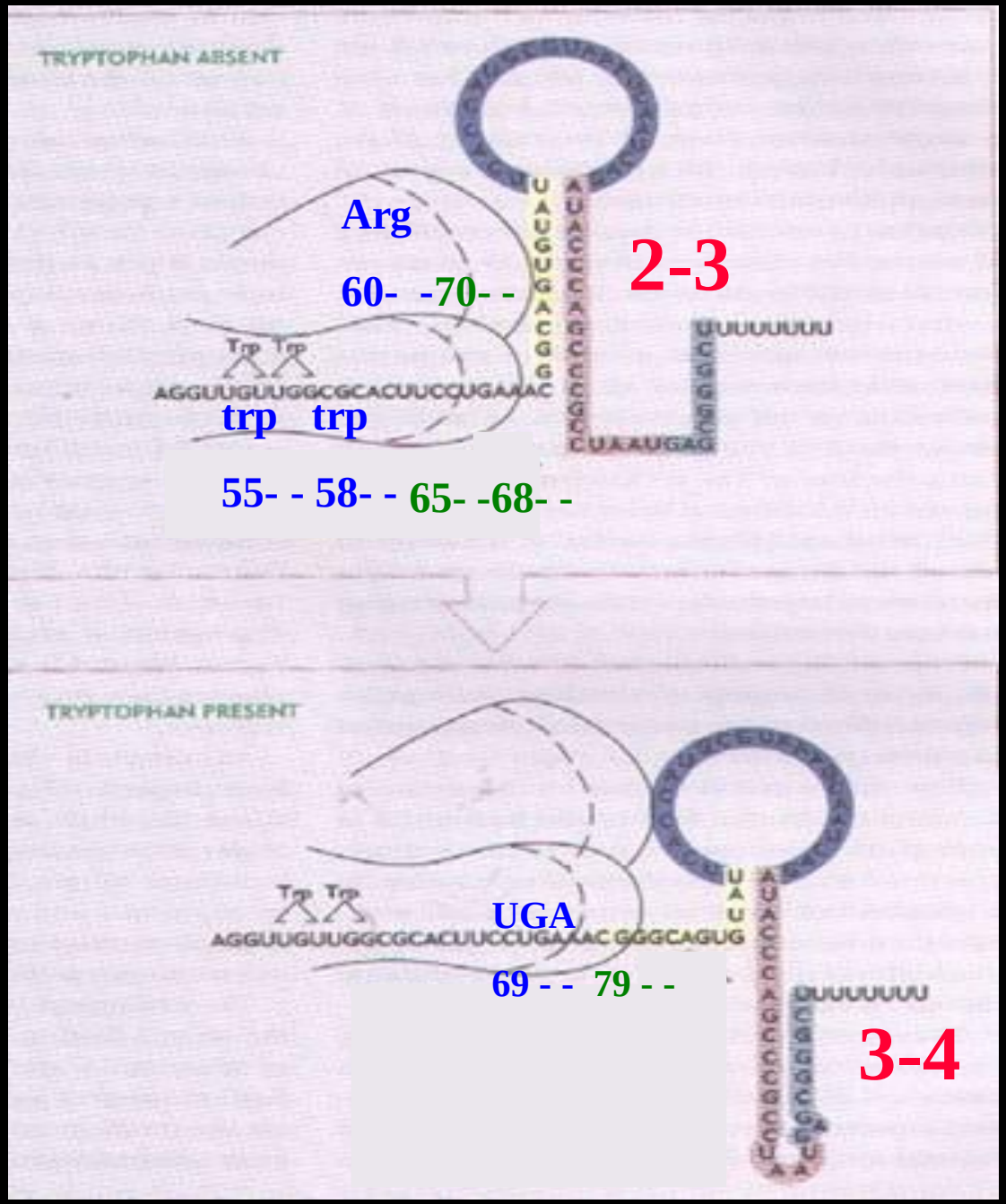
Alternative pairing scheme of 3 forms for three sequence in the trp mRNA leader.

1-2 / 3-4



2 - 3

(来源：分子生物学（2007），郑用琏，第228页)



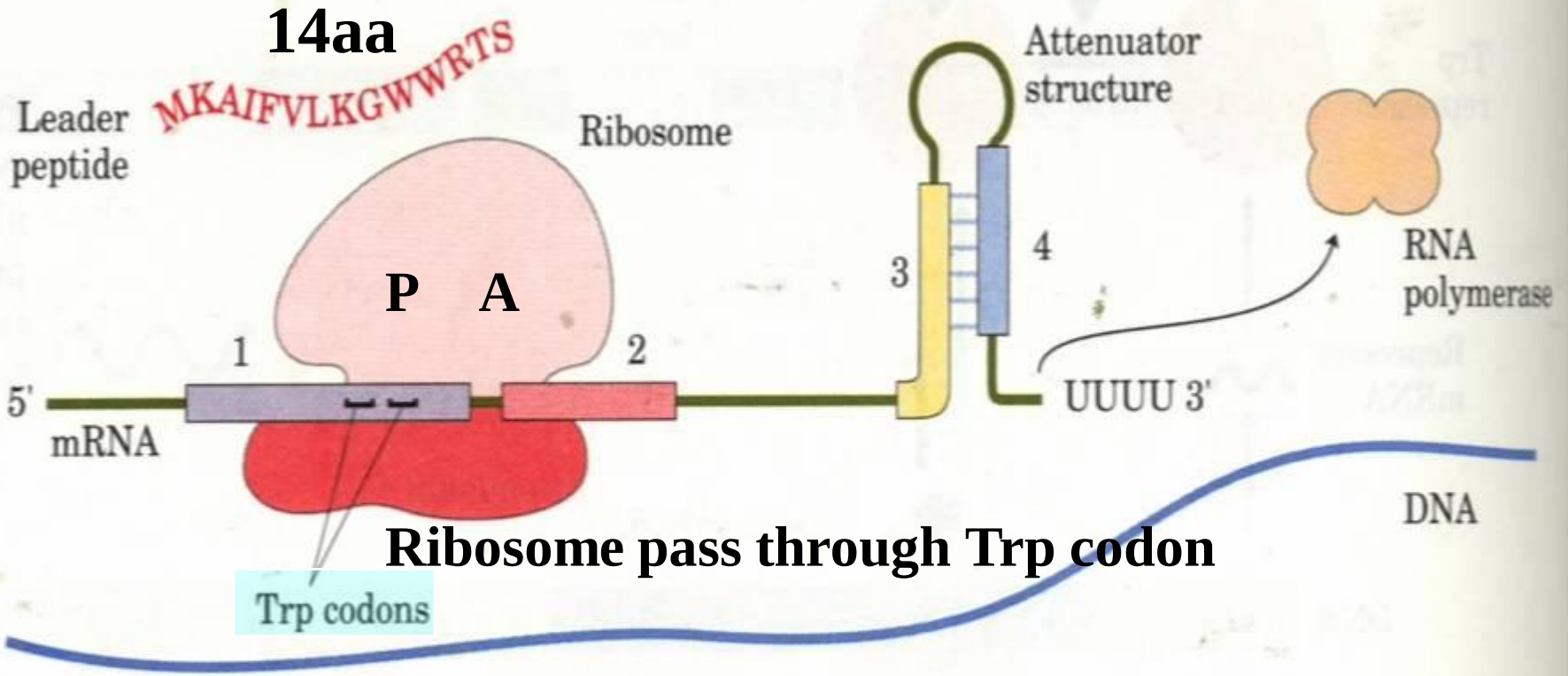
Trp(Arg)

starved

Non-starved

Rho-independent T.

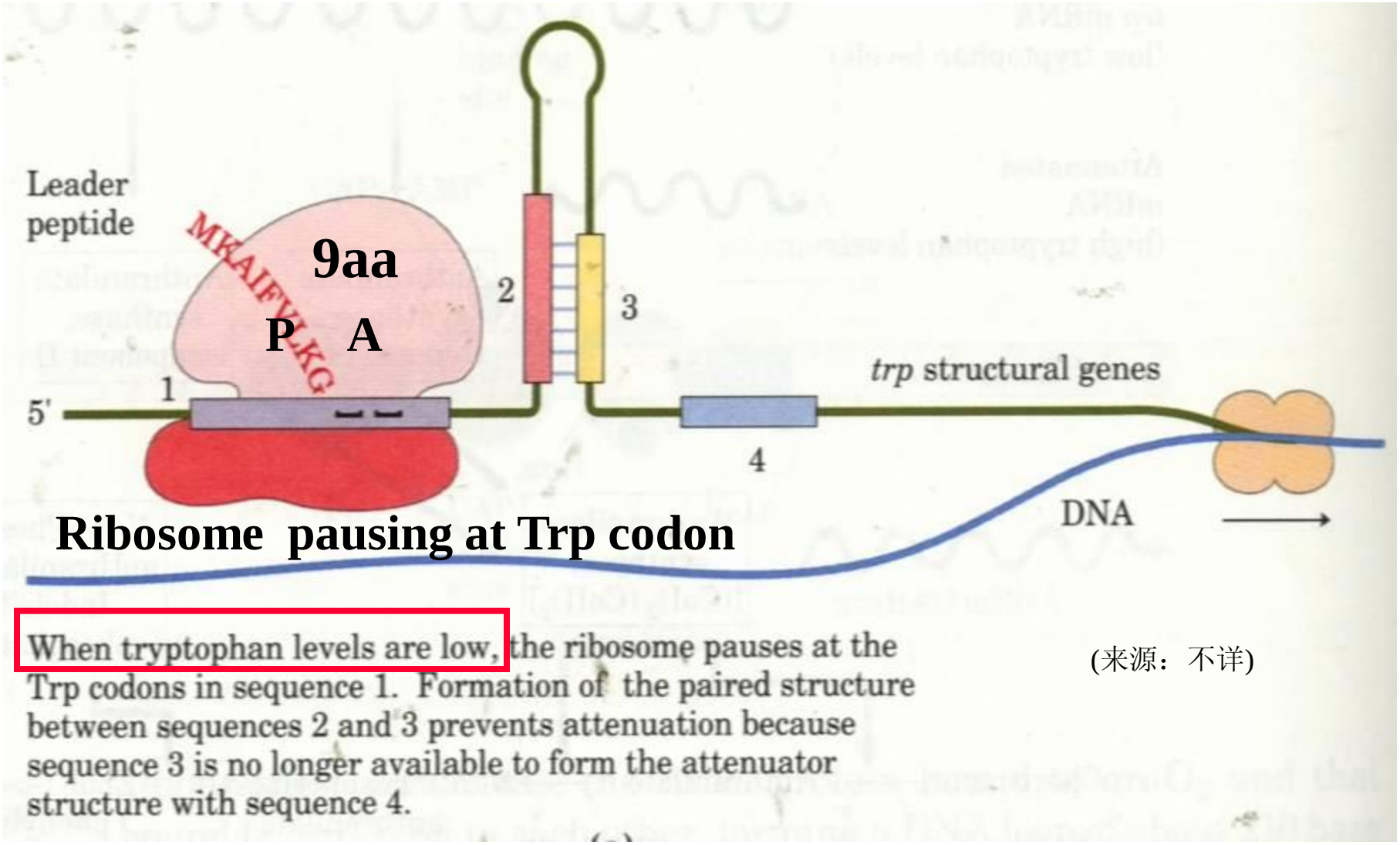
(来源：不详)



Ribosome pass through Trp codon

When tryptophan levels are high, the ribosome quickly translates sequence 1 (open reading frame encoding leader peptide) and blocks sequence 2 before sequence 3 is transcribed. Continued transcription leads to attenuation at the terminator-like structure formed by sequences 3 and 4.

(来源：不详)



Ribosome pausing at Trp codon

When tryptophan levels are low, the ribosome pauses at the Trp codons in sequence 1. Formation of the paired structure between sequences 2 and 3 prevents attenuation because sequence 3 is no longer available to form the attenuator structure with sequence 4.

(来源：不详)

e) The biological significance of Attenuation control

When trp level is low →

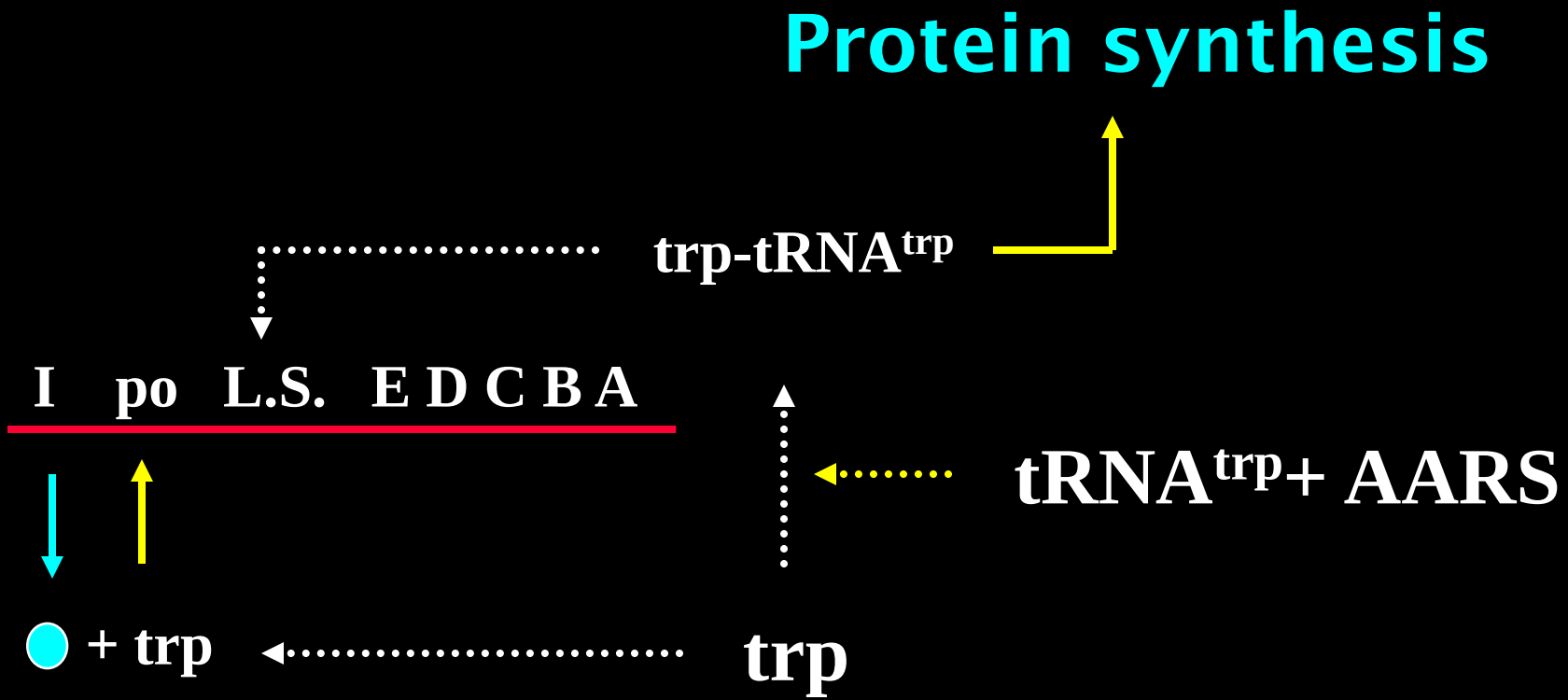
repressor is not enough to close operon

When trp level is high →

little RNApol. Pass through the O site

↓

If anly **trp-tRNA^{trp}** exist in cells, Attenuator will interrupt the RNApol. transcription in the L.S.



Trp synthetase opron

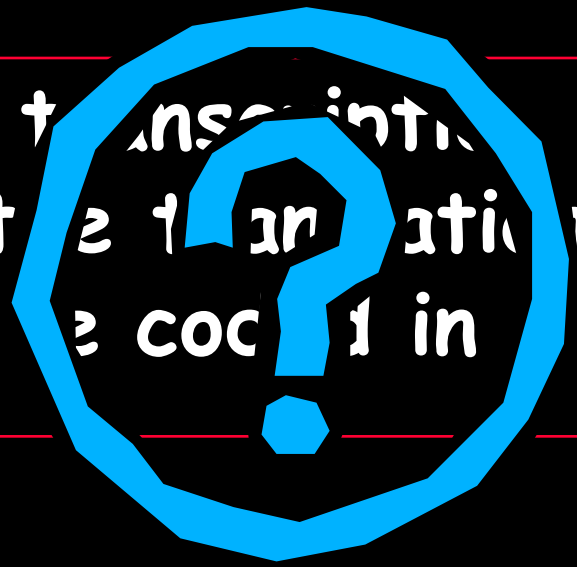
biological significance

The fine-tuned regulation in the Prokaryotes

Enhance the adaptability of prokaryotes to the environment

The mechanism of Attenuation control

Control the transcription of the operon finely via the translation of the short peptide coded in the L.S.



Yanofsky Charles . 1981 . *Nature.* 289;751-758

第6章 基因表达调控

(Controlling of the Gene Expression)

6.2 post-transcriptional level control

(Source:Pique,Michael and Peter E.Wright,Dept.(cover photo,science 245 (11 Aug 1989).))

6.2.1 pre-RNA processing

(for Eukaryots only)

6.2.2 Anti sense RNA and

RNA interference *(RNAi)*

广泛存在于原核生物（*E.coli*）

真核生物（*plant-mammalian*）

基因沉默

- 位置效应（**position effect**）

  整合位点

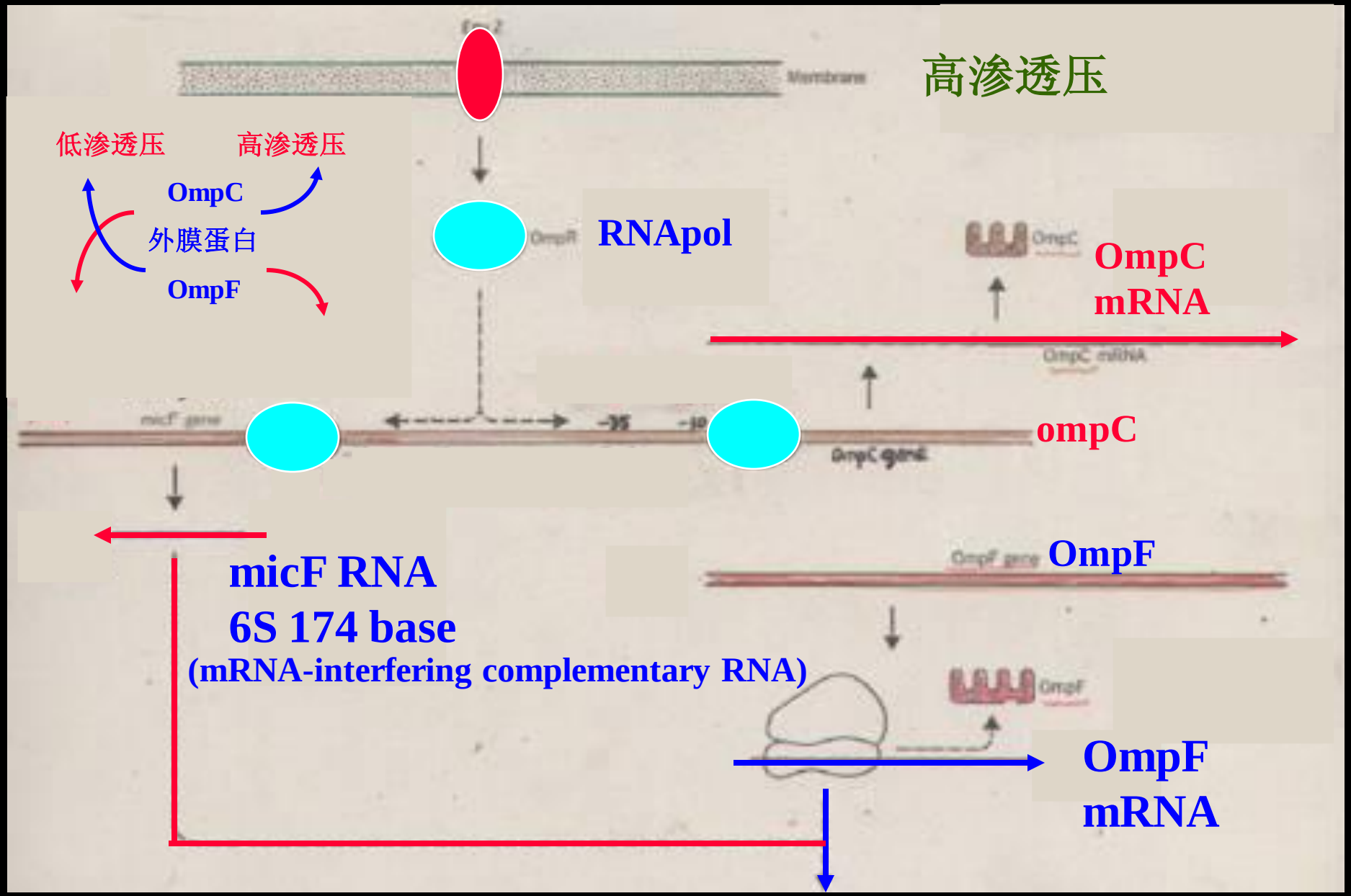
- **转录水平的基因沉默（transcriptional gene silencing, TGS）**

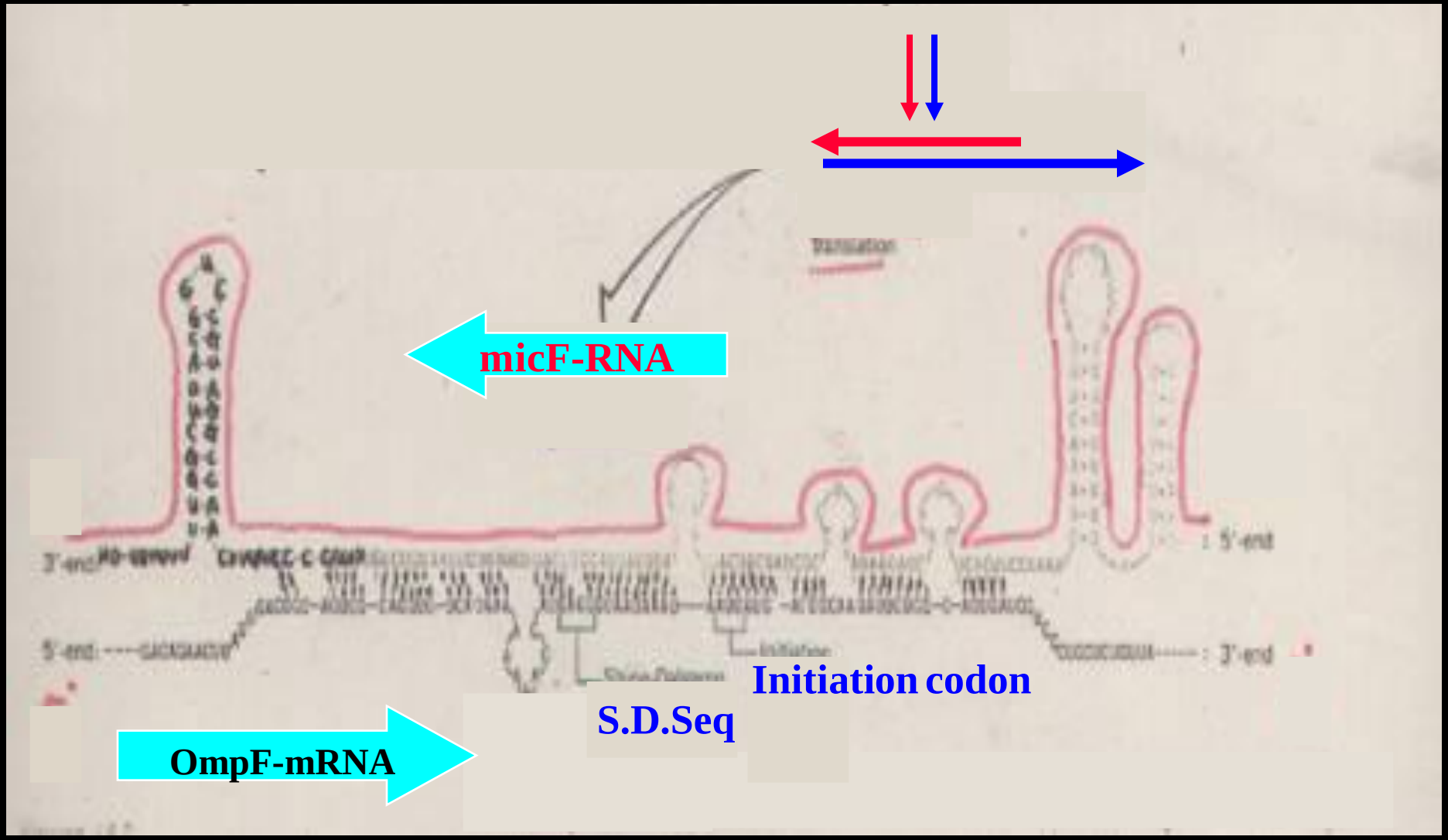
  启动子甲基化或导入基因异染色质化

- **转录后水平的沉默 (post-transcriptional gene silencing, PTGS)**

6.2.2.1 anti-sense RNA control protein translation

(Source:1983. Miruno & Simons)





(来源：不详)

micRNA (anti-sense RNA) binding

5’-end of mRNA(S.D.seq. & AUG.)

遗传特点

---anti-sense gene of CHS (Chalcone synthesis gene)

red flower  **white flower** × **red flower**

dominance /recessive ?

---non complete dominance

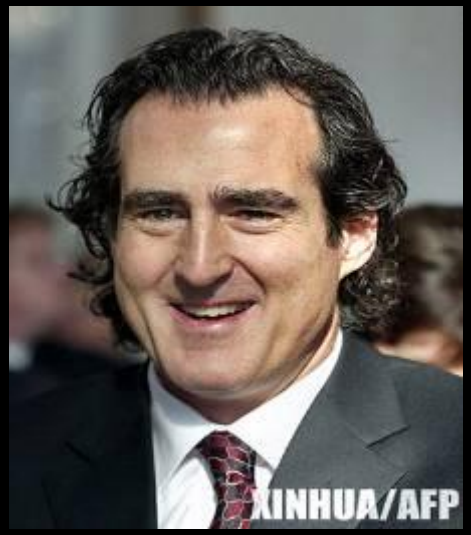
---stable inheritance

---D.S. RNA unstable and rapidly degradated

6.2.2.2RNA interference (RNAi)的发现与证实

Andrew Z. Fire,
斯坦福大学医学院

Craig C. Mello
马萨诸塞州医学院



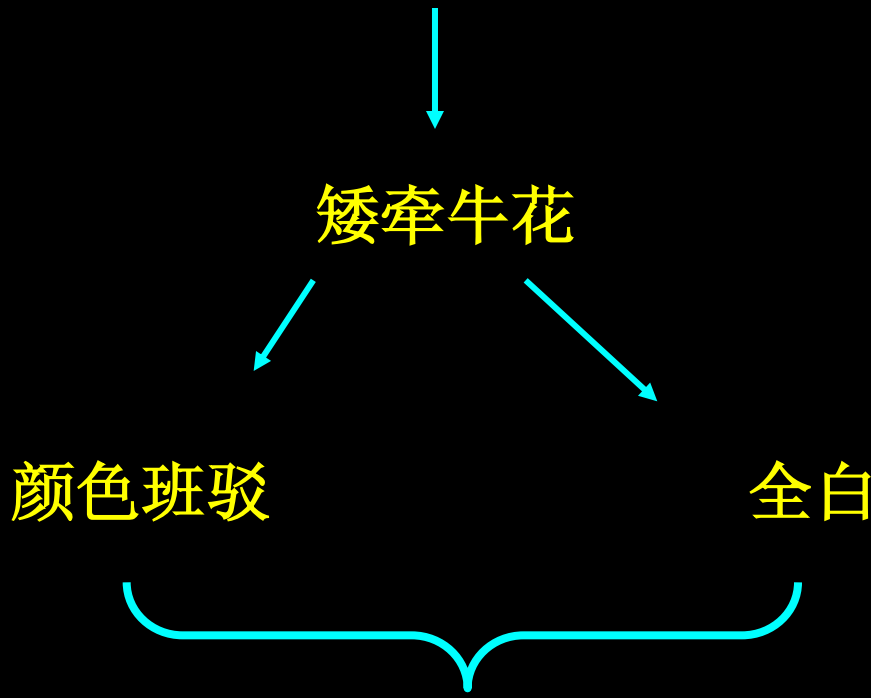
"for their discovery of RNA interference - gene silencing by double-stranded RNA".

NP 2006

RNA interference *(RNAi)的发现与证实*

- 1990年， Rich Jorgensen等人发现

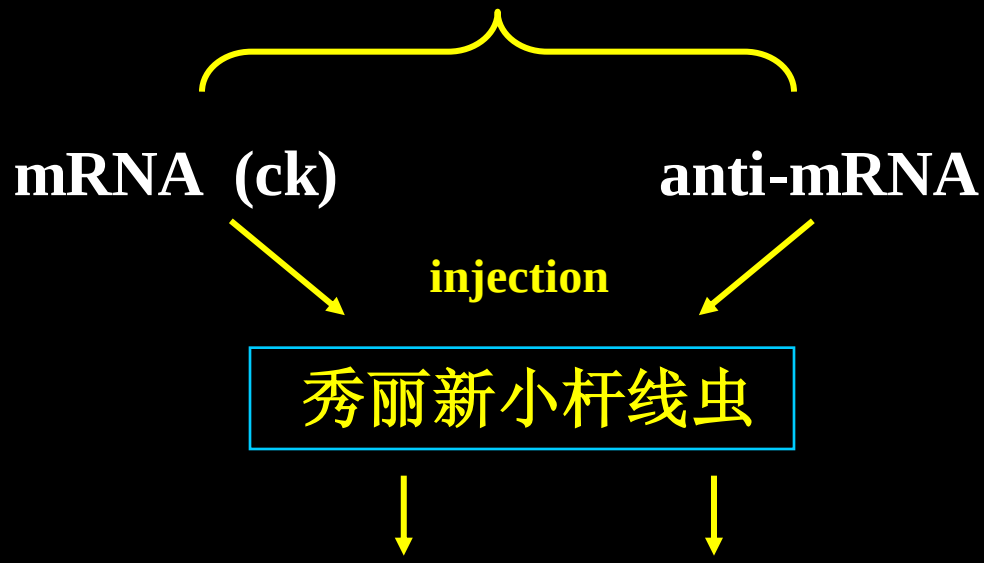
苯基苯乙烯酮合成酶基因



色素的合成不是被增强了，而是被关闭了

Su Guo 1995 康乃尔大学

抑制秀丽新小杆线虫胚胎对称性基因（par-1）



Andrew Fire (1998.2 华盛顿卡耐基研究院)

纯化的 A.S mRNA of mex-3 → *c.elegans* → 极微弱抑制

纯化的 D.S mRNA of mex-3 → *c.elegans* → 特高效抑制

证明； Dr. Su Guo

mRNA of Par-1

mRNA 制备中污染微量D.S RNA

injection ↓ *C.elegans*

↓ 特异性地降解 mRNA of par-1

Interruption expression of par-1

Named RNA interference (RNA 干涉)

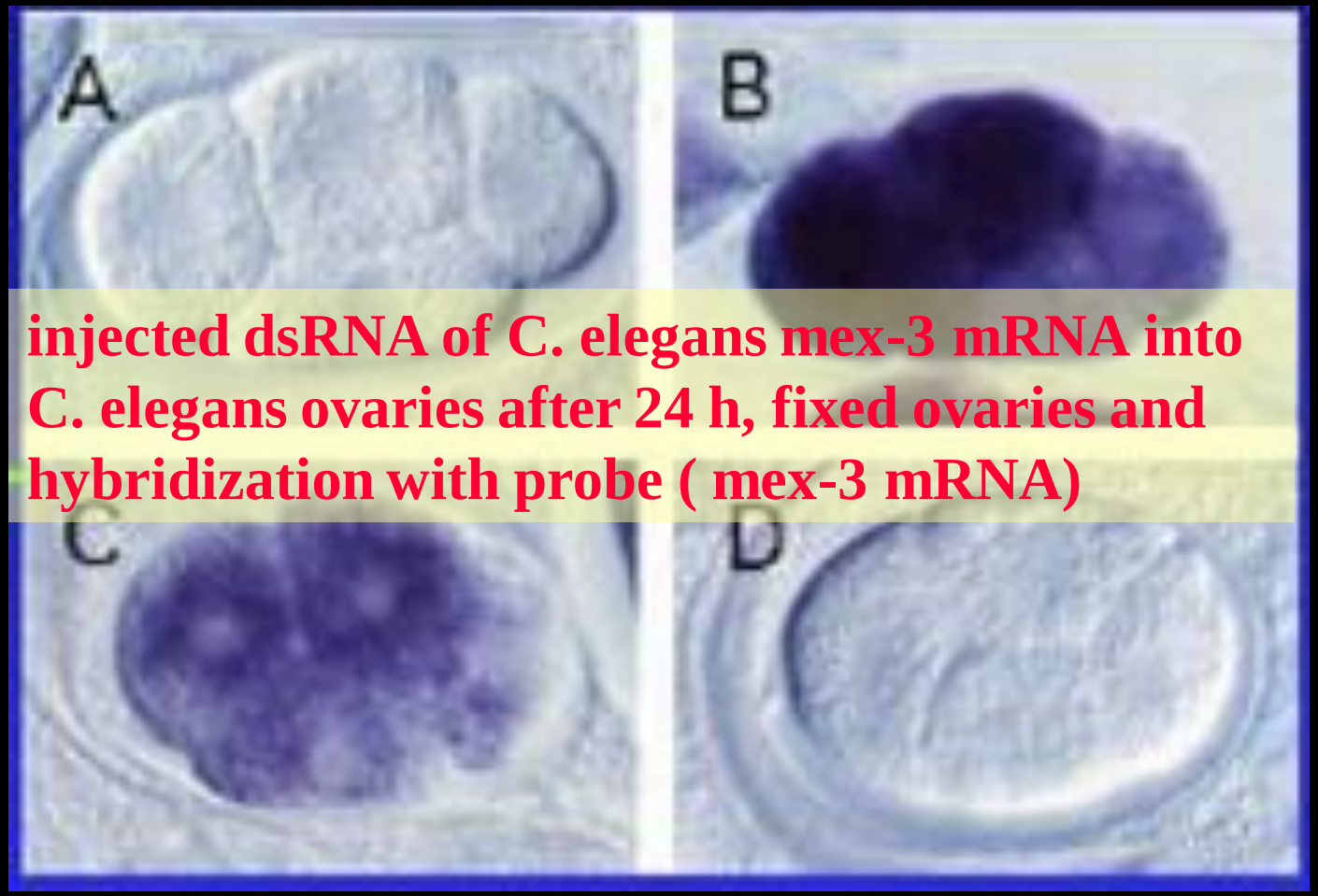
Double-stranded RNA-induced RNA Interference causes destruction of a specific mRNA

No probe

-ck

No injection

+ck



injected dsRNA of C. elegans mex-3 mRNA into C. elegans ovaries after 24 h, fixed ovaries and hybridization with probe (mex-3 mRNA)

Injection antisense RNA

Some mRNA

Injection dsRNA

No detected mRNA

(Source: Fire, Andrew. et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391 (1998) f. 3, p, 809)

- ***Richard Carthew* (1998. 12 匹斯堡大学)**
- ***Clemens* (2000. 6 密歇根大学)**

Drosophila

- ***Zernicka-Goetz* (2000. 2 剑桥大学)**

Rat (mammalian)

- ***Tehurikov* (2000. 8)**

E. coli

真菌、拟南芥、锥虫、水螅、斑马鱼……

注射、浸泡、喂养 → ***C.elegans*** }
电穿孔、基因枪 → ***plant cell*** } **RNA interference**

RNAi 定义

外源或内源性的双链RNA (dsRNA) 进入细胞后引起与其同源的mRNA 特异性降解，抑制相应基因表达，表现出特定基因缺失表型的现象。

RNA干扰抑制基因表达的基本原理

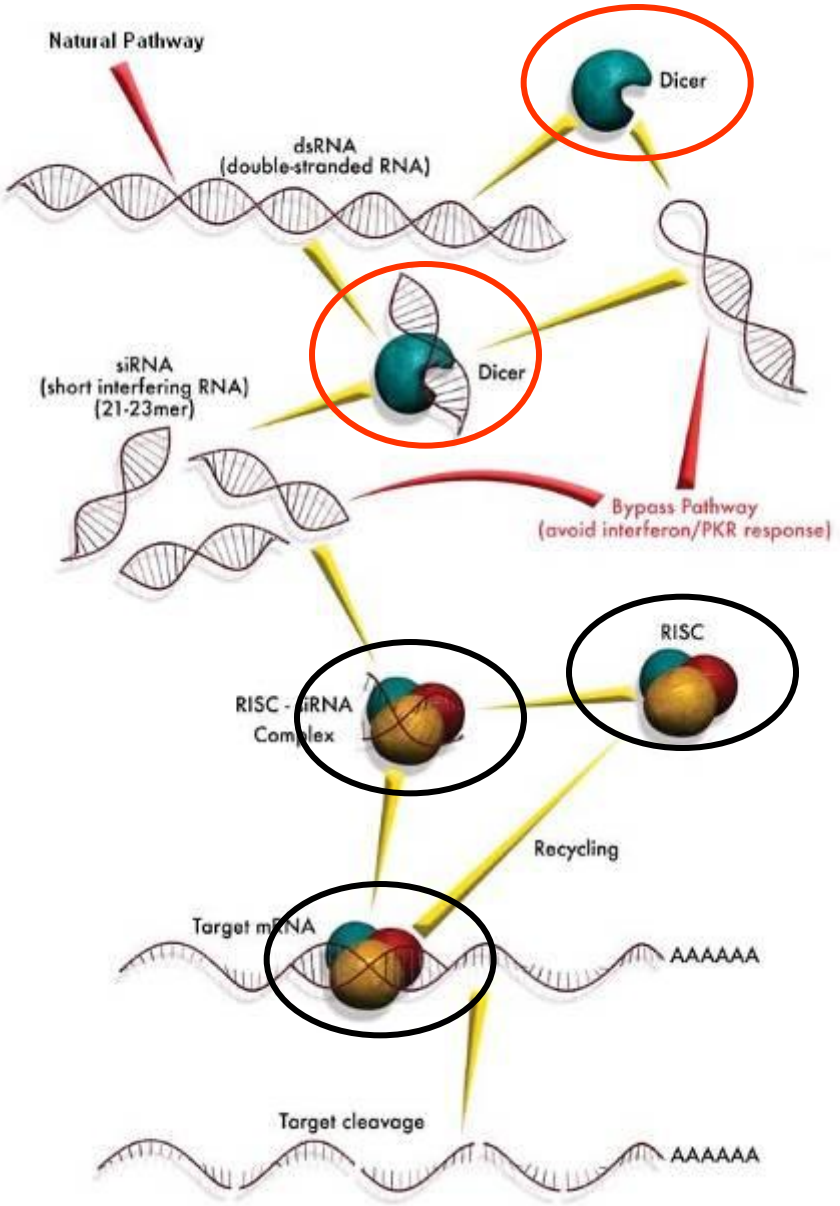
双链RNA (dsRNA) 通过细胞膜进入细胞内, 在Dicer (RNA 酶Ⅲ家族中的一种序列特异性的核酸内切酶) 的作用下, 被切割为21～22bp , 3’端带有2～3nt 末端突出的双链RNA 分子, 这种小的双链的RNA 分子被称为小干扰 RNA (small interference RNA ,siRNA) 。

RNA干扰抑制基因表达的基本原理

siRNA 同相关的酶结合, 形成RNA 介导的沉默复合物 (RISC RNA-induced silencing complex) , RISC在ATP 供能的情况下, 将其 携带的双链 siRNA 变成单链 siRNA 分子, 进而成为有活性的RISC。又称为Slicer

RNA干扰抑制基因表达的基本原理

Slicer 同目标靶mRNA 分子结合, 引发目标RNA 分子的断裂, 进而被核酸酶降解, 导致目的基因的沉默。



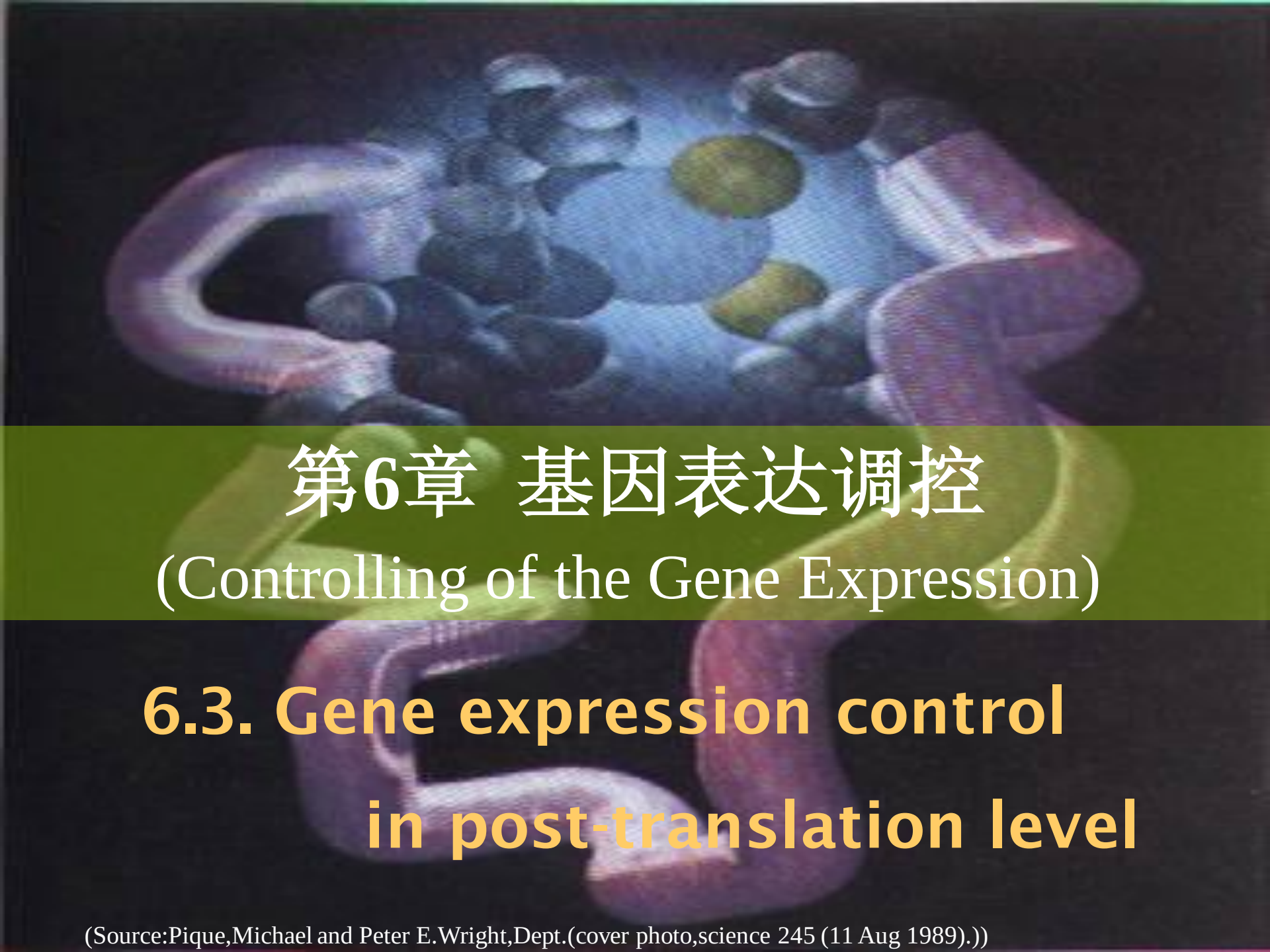
A model for RNAi.

- **begins with dsRNA, which a cellular nuclease cleaves (Dicer) to fragments 21-23 nt long.**

- **Active RISC (Slicer)**

- **the antisense 21-23-nt RNAs can hybridize to sites in the mRNA and dictate cleavage of the mRNA at or near their ends, usually at a U**

(Source:Zamore Cell 101:32, 2000.)



第6章 基因表达调控

(Controlling of the Gene Expression)

6.3. Gene expression control in post-translation level

(Source:Pique,Michael and Peter E.Wright,Dept.(cover photo,science 245 (11 Aug 1989).))

Protein secretion

(targeting)

Protein degradation

Polypeptide chain folding

6.3.1 蛋白质分泌的信号肽假说

（signal hypothesis）

- ***Blobel*** ***in Prok. & Euk.*** **1971. –1975**

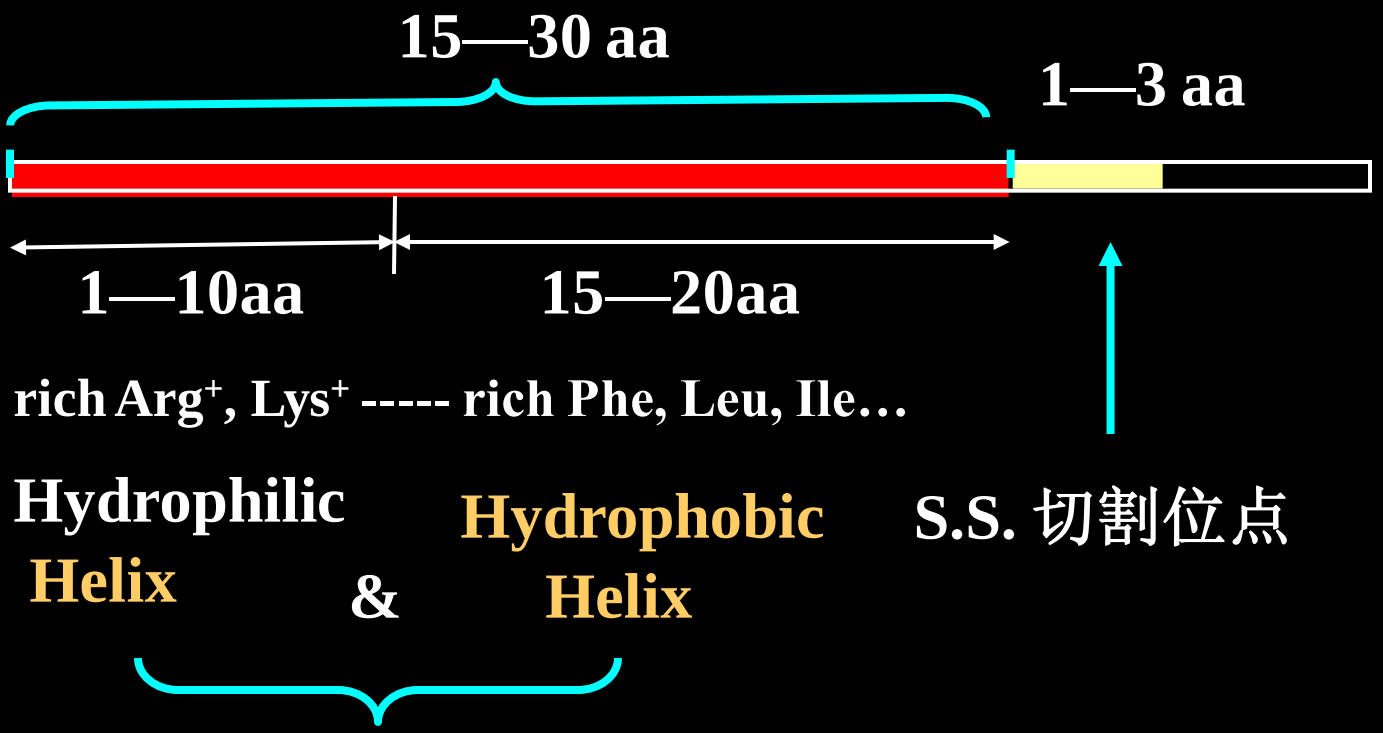
15 — 30 aa leading seq.

in N-end of secretary protein

Leading seq named as signal seq

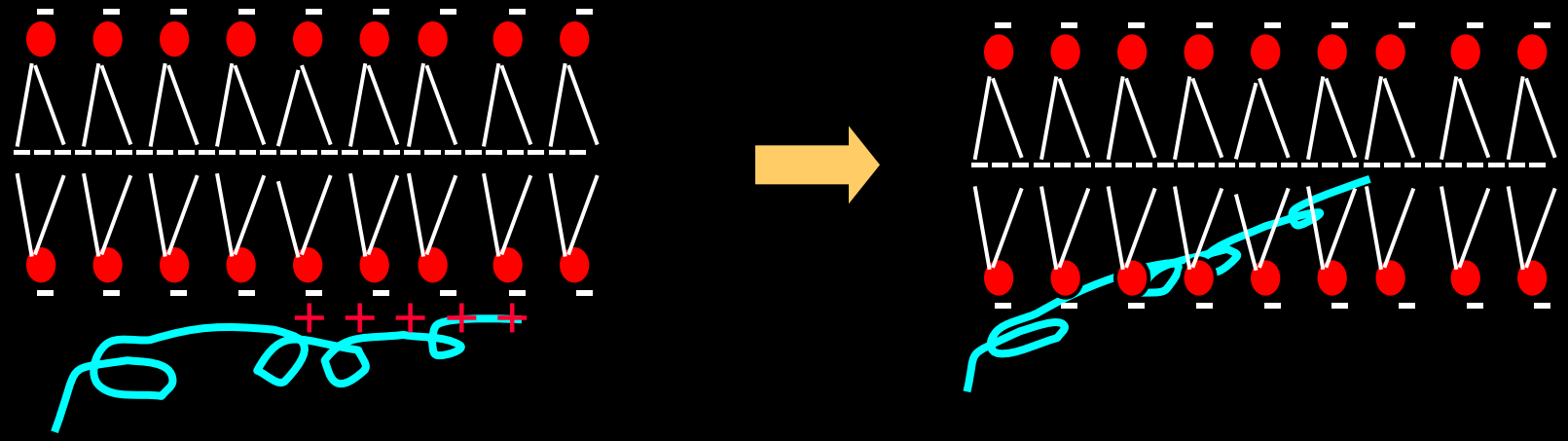
& signal hypothesis

- signal S. 的基本结构



- **signal Seq.引导的穿膜机制**

---signal S. 带正电的区段与带负电的磷脂膜互作,引导蛋白质进入 **inner M.**

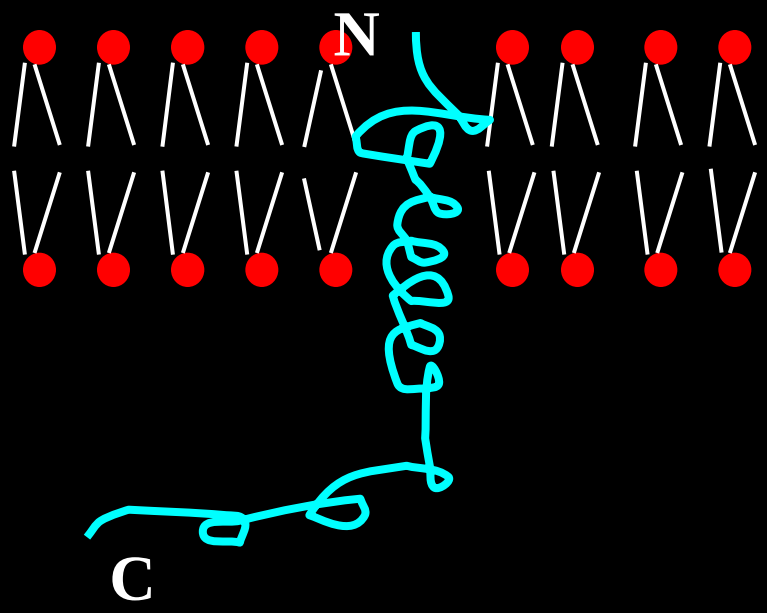


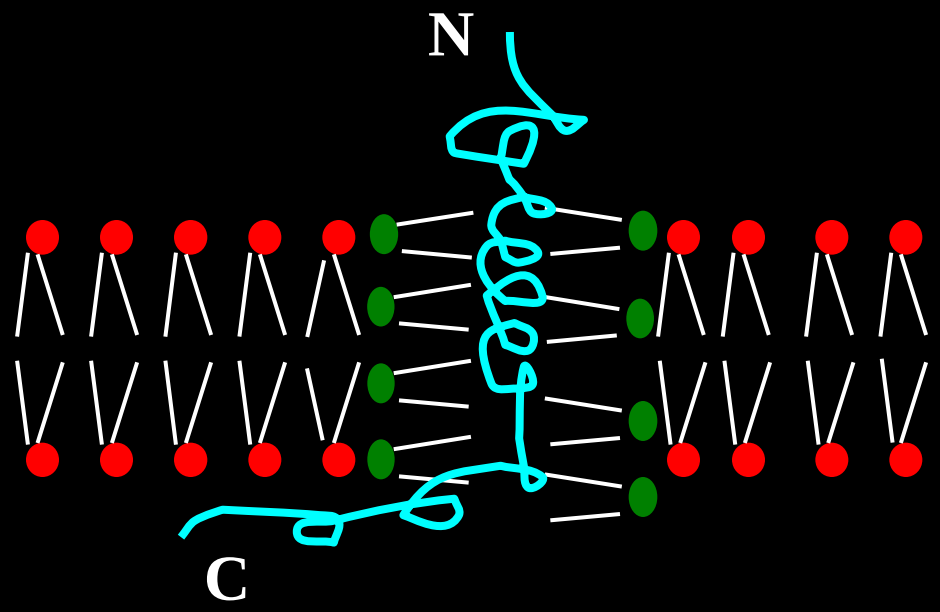
--- 疏水区段嵌入磷脂膜内或形成α helix,

--- 并对磷脂双层膜产生扰动效应，

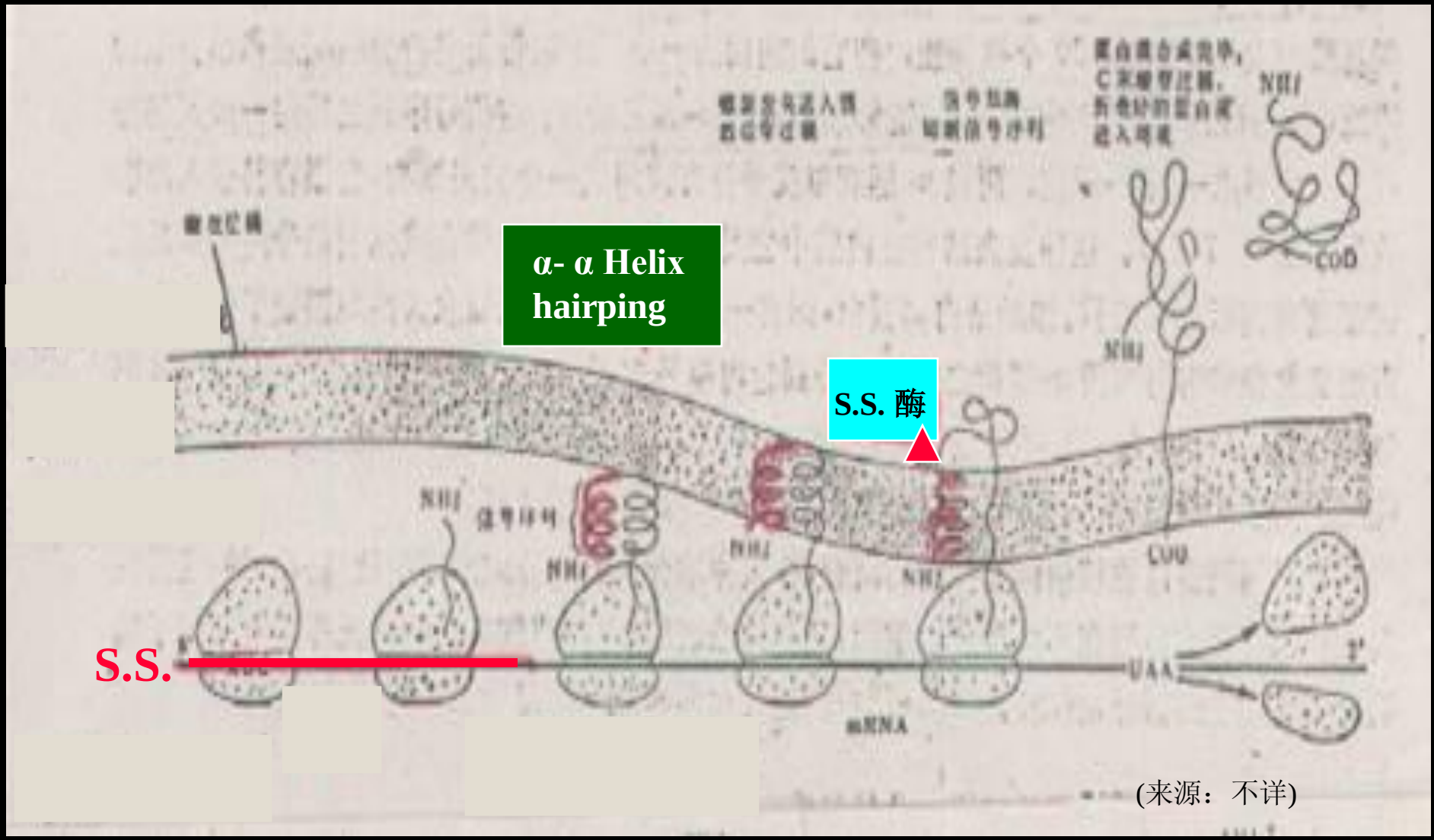
--- 诱发形成非脂双层结构，

以保证Signal S. 所牵引的蛋白质顺利穿膜



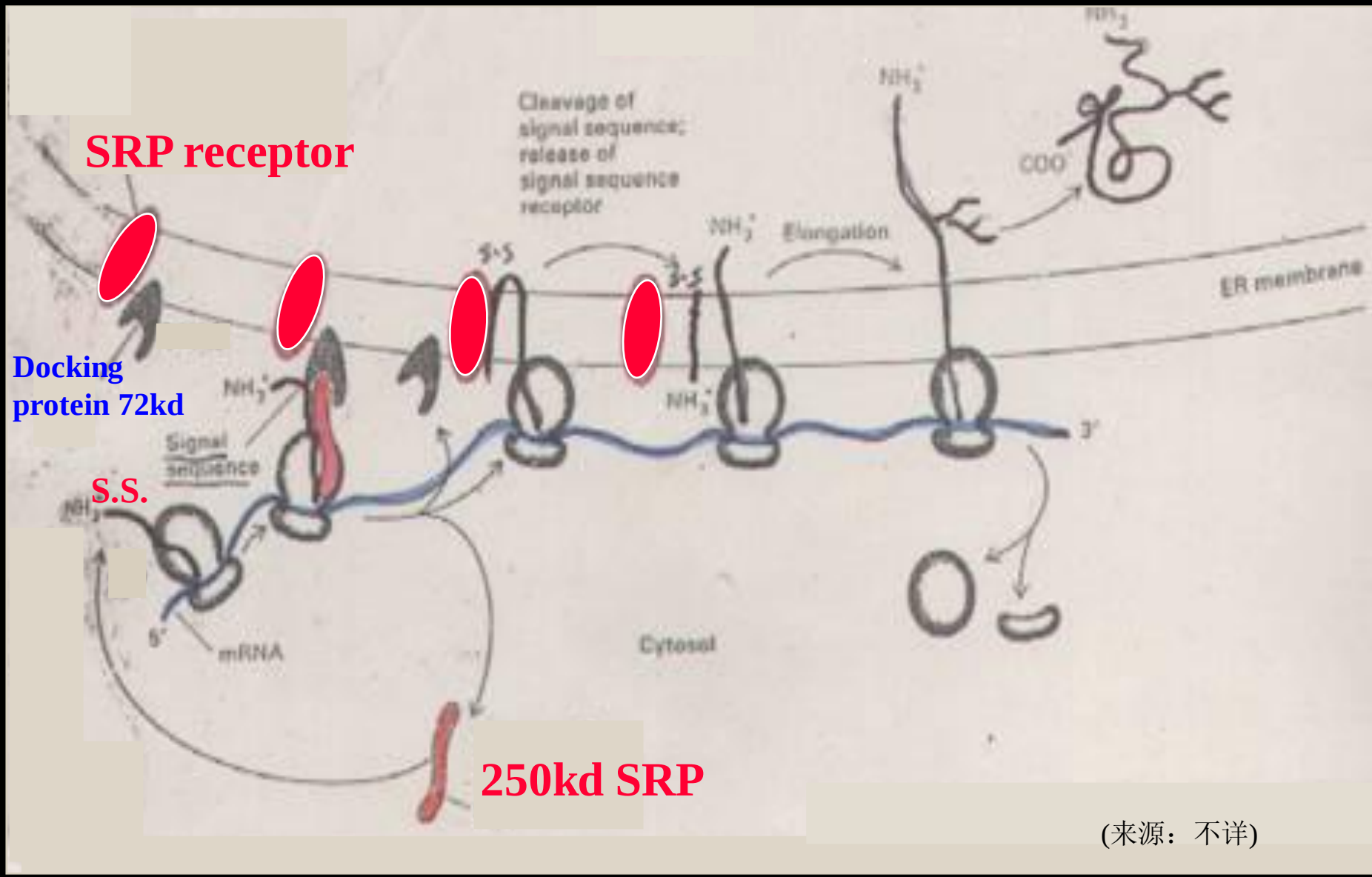


原核生物分泌性蛋白质穿膜的分子模式



(来源：不详)

真核生物分泌性蛋白质穿膜的分子模式



(来源：不详)

6.3.2. protein degradation

a) protein degradation 的调控是生理代谢的需要

各类蛋白的半衰期几乎恒定 e.g. in liver cell of mouse

Ornithine decarboxylase	0.2 hr
RNApol A	0.33 hr
Trp oxygenase	2.5 hrs
Ser dehydrogenase	4.0 hrs
RNA polB	12 hrs

结构蛋白，储藏蛋白 → 半衰期长，结构稳定

催化酶类，代谢酶类 → 半衰期短，不断更新

错译蛋白，失活酶类 → 清除隐患，废物利用

b) 蛋白质降解机制

细胞内高度复杂，精细的调控系统

两个主要的蛋白质降解级链反应

- **PCD过程中的Caspase (trigger protein-splitting enzymes)过程**
- 半衰期短和异常蛋白降解的泛素系统

泛素依赖的蛋白质降解途径（ubiquitin dependent proteolytic pathway）又称Ub途径或泛素/26S蛋白酶体通路是生物体最重要的、有高度选择性的降解细胞内短命调控蛋白的重要调节途径。

泛素/26S蛋白酶体通路的发现

- 1953年 Simpson

  发现生物细胞分解细胞自身的蛋白质是需要消耗能量的（Simpson, 1953）。
- Aron Ciechanover、Avram Hershko、Irwin Rose 在20世纪70年代末至80年代初

  提出了泛素在蛋白质分解中所起的基本作用的假说——“多重步骤泛素化标签假说”

The Nobel Prize in Chemistry 2004

The Nobel Prize in Chemistry 2004

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry for 2004 "for the discovery of ubiquitin-mediated protein degradation" jointly to Aaron Ciechanover, Avram Hershko and Irwin Rose

BACK

Proteins that are marked for hacking into small pieces

For the discovery of Ubiquitin—mediated protein degradation

Irwin Rose
College of Medicine, University of California, Irvine, USA

Avram Hershko
Rappaport Institute, Technion – Israel Institute of Technology Haifa, Israel

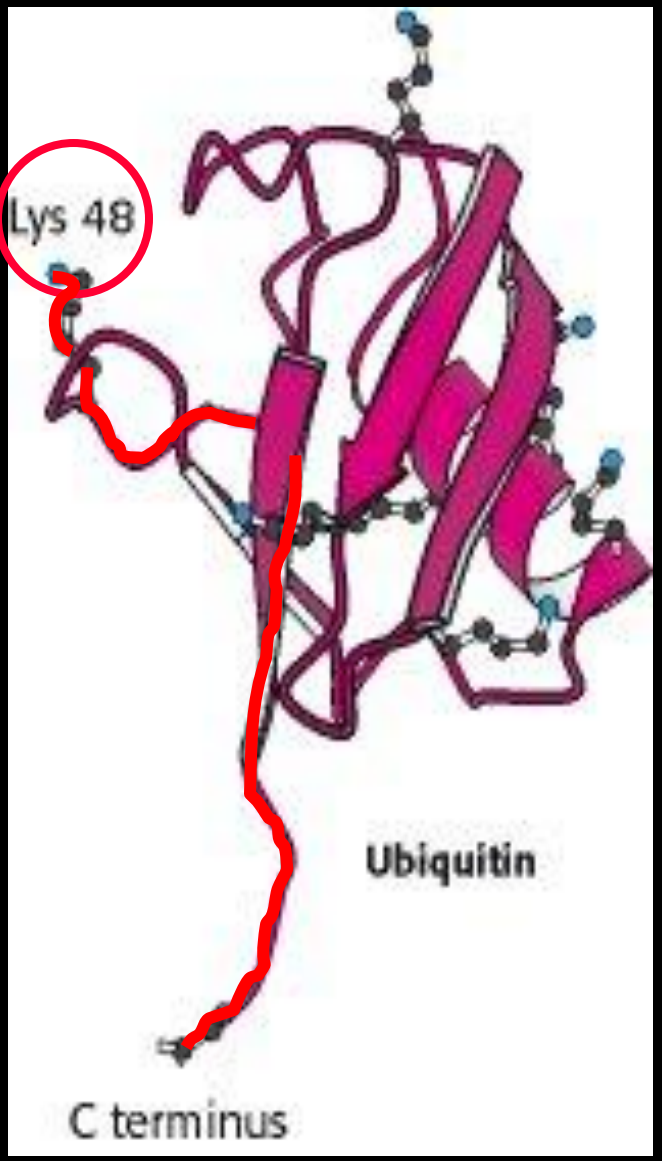
Aaron Ciechanover
Rappaport Institute, Technion – Israel Institute of Technology Haifa, Israel

The discovery was made at the beginning of the 1980s at the Fox Chase Cancer Center in Philadelphia, USA, jointly by the three scientists.

Contents:

Web-adapted Version of the Nobel Poster from the Royal Swedish Academy of Sciences

Ubiquitin (Ub)



- A small (8.5-kD) protein with 76 AA present in all eukaryotic cells

  so named "**Ubiquitin**"

- Highly conserved from yeast to human: differ at only 3 of 76 residues

- Isopeptide bonds: Ub-C-terminus Lys^{48th} -- a protein (**Ubiquitylation**) destined to be degraded

(来源：不详)

Protein ubiquitylation pathway

- Four enzymes participate in the attachment of ubiquitin to each protein

  E1: ubiquitin-activating enzyme

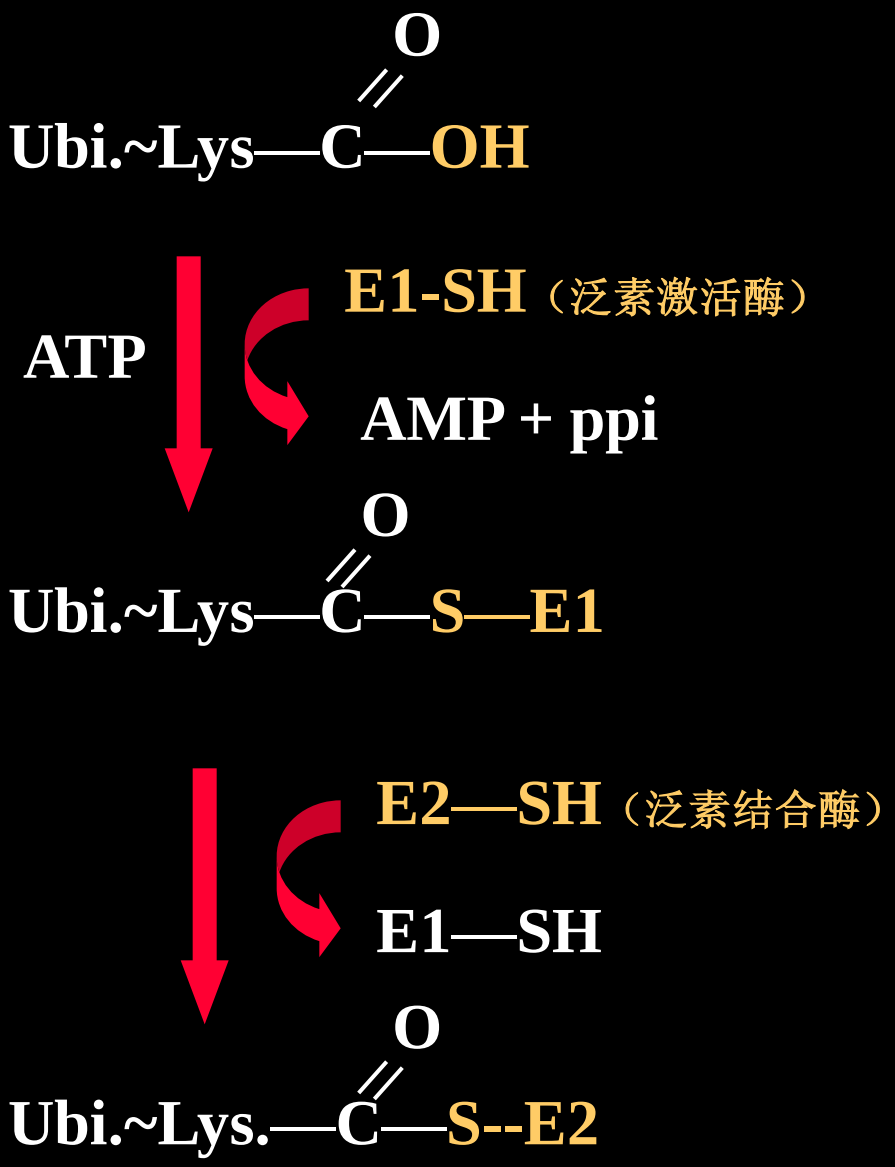
  E2: ubiquitin-conjugating enzyme

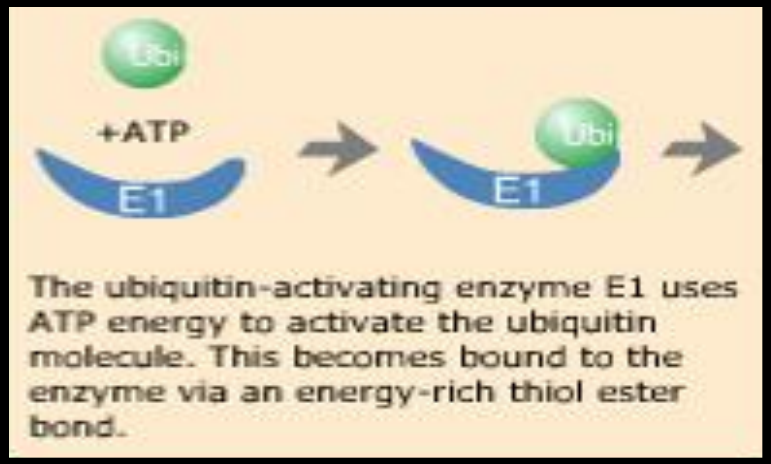
  E3: ubiquitin-protein ligase

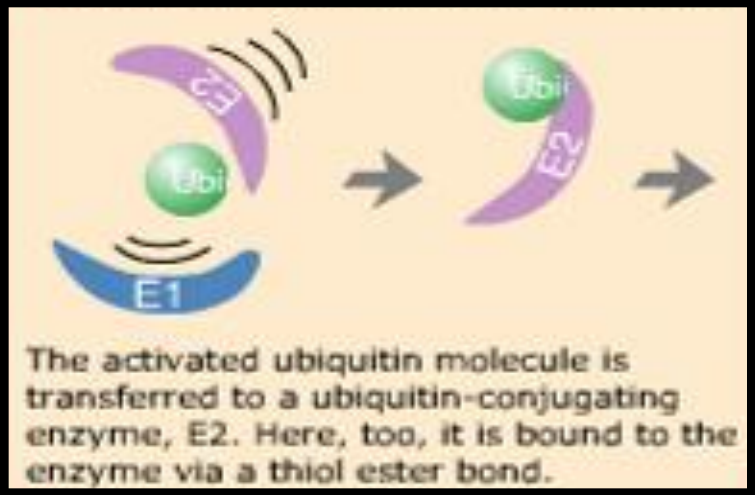
  E4: ubiquitin chain assembly factor

  DUB: de-ubiquitylating enzyme (reversible)

Ubiquitylation







(来源：分子生物学（2007），郑用琏，第274页)

Ubi.~Lys.—C(=O)—S--E2

targeting protein

(E3（泛素蛋白连接酶）对靶蛋白N端aa的识别)

+ Lys- targeting protein (E2-E3 to form a complex that attaches Ubi to a Lys residue on the substrate protein)

Ubi.

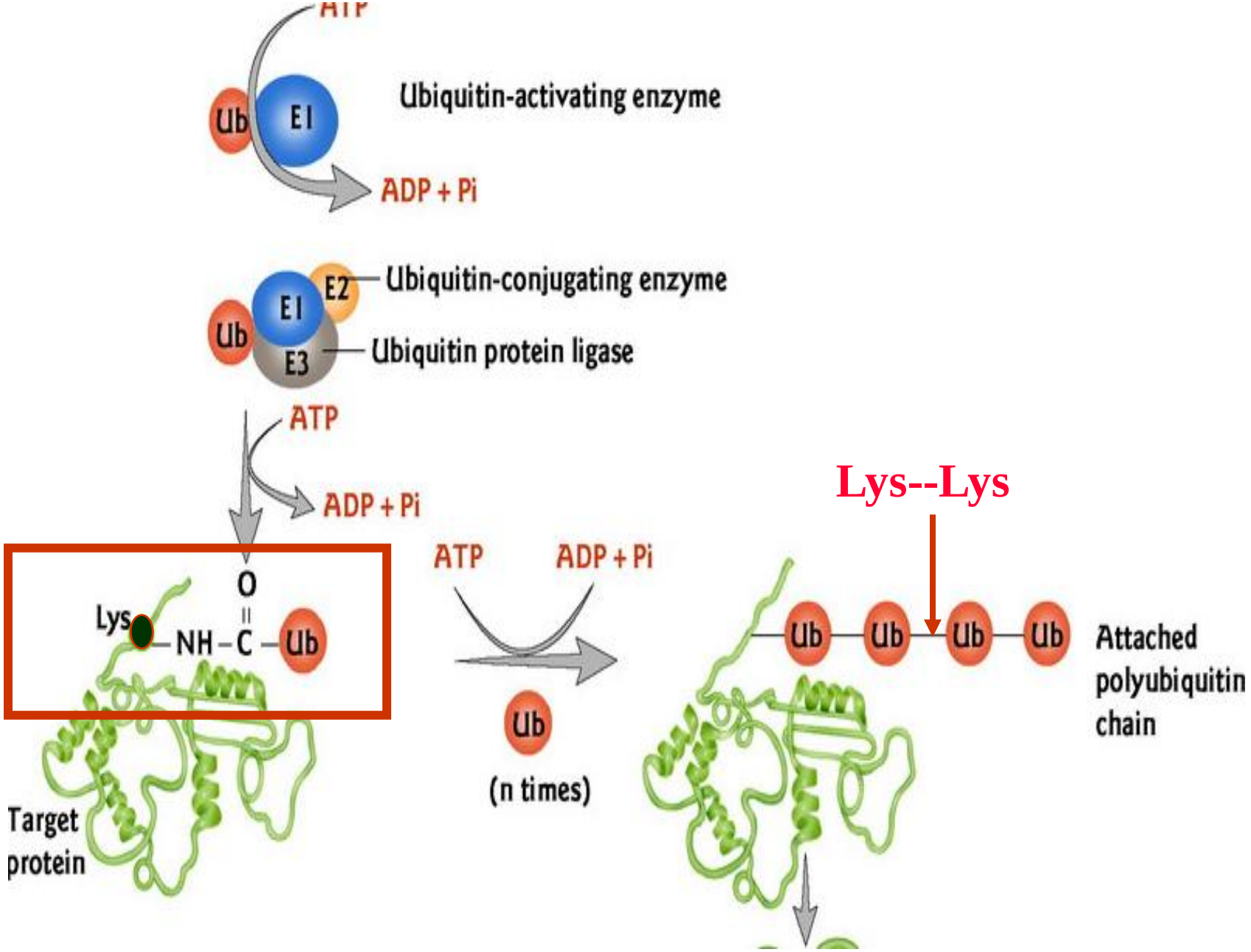
E2

Lys

(poly-Ubiquitylation

and isopeptide bonding)

(来源：分子生物学（2007），郑用琏，第274页)

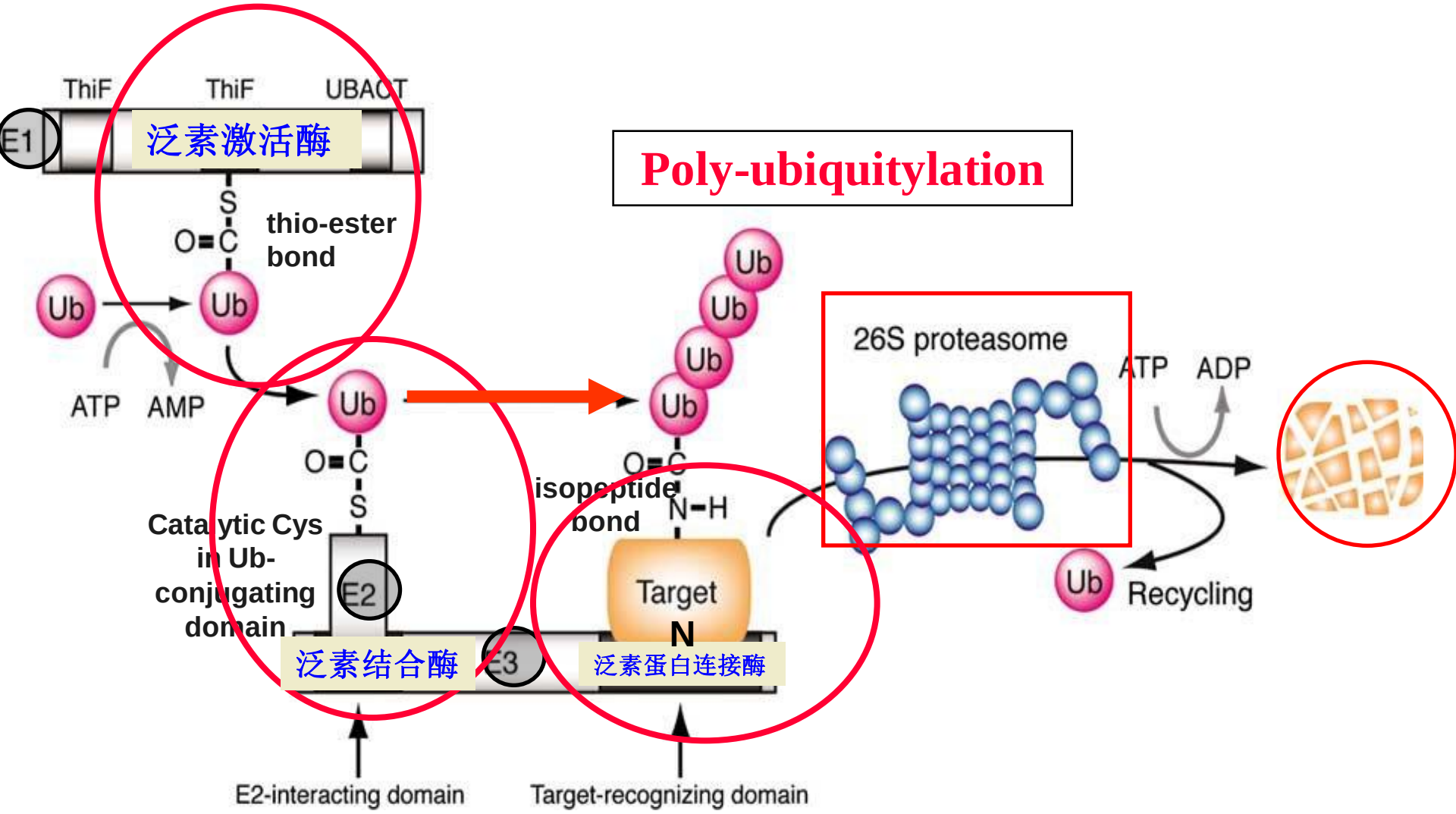


(来源：分子生物学（2007），郑用琏，第274页)

26S蛋白酶体识别

Degradation

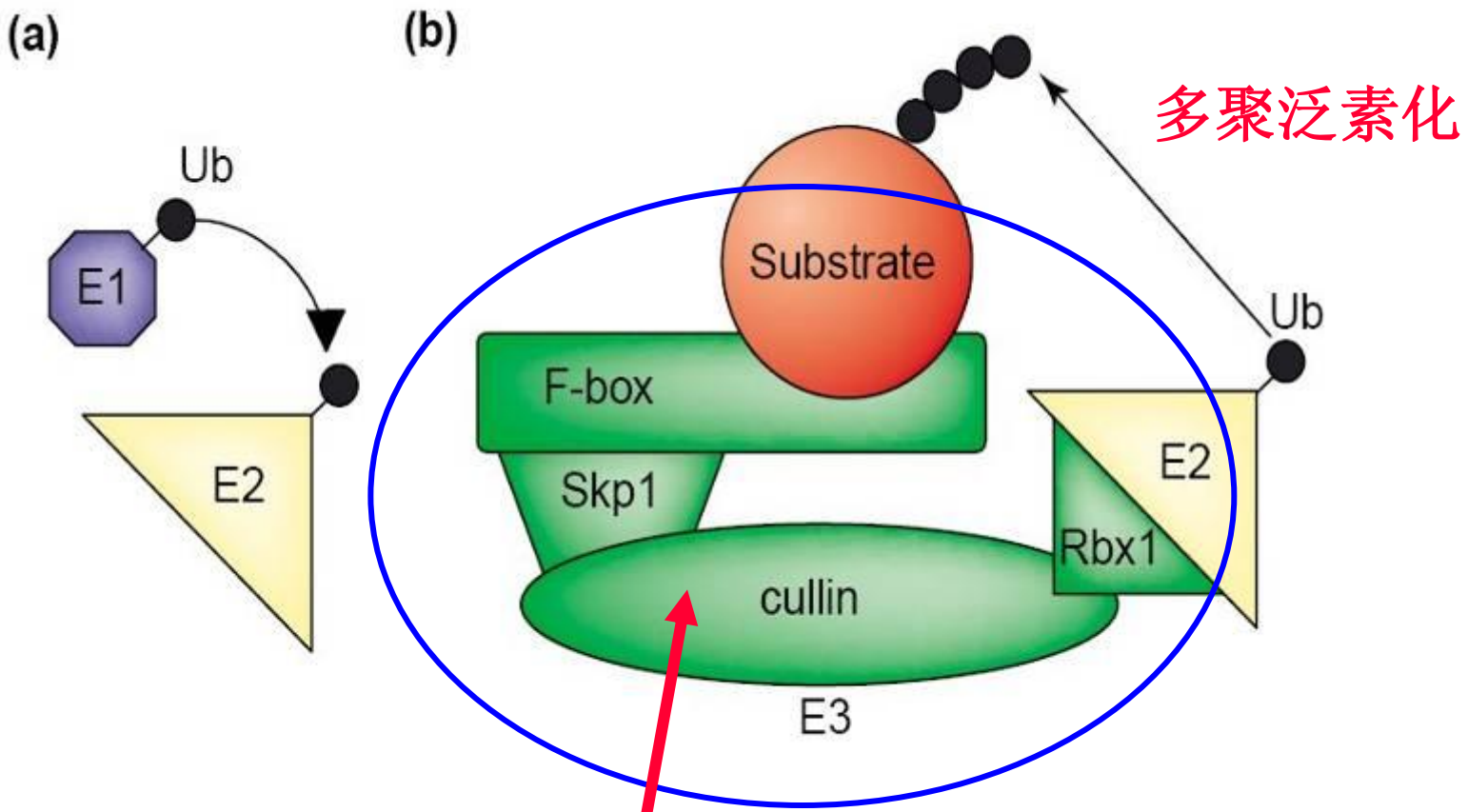
(来源：分子生物学（2007），郑用琏，第274页)



The ubiquitylation pathway and its associated enzymes

From Hatakeyama and Nakayama, *Biochem. Biophys. Res. Commun*, 2003, 302:635-645

某些转录因子的mono-ubiquitylation 被激活

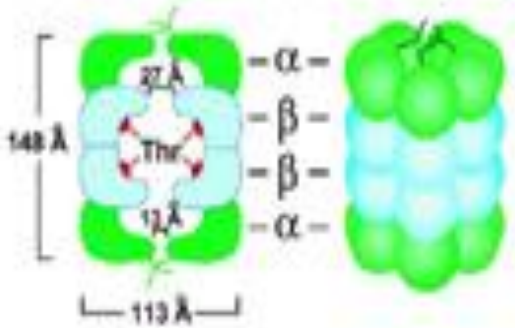


蛋白质在被26S蛋白酶降解前，必须由**E3 ubiquitin ligase enzyme complex (EULEC)**进行多聚泛素化

From Itoh et al., *Trends Plant Sci*, 2003, 8:492-497

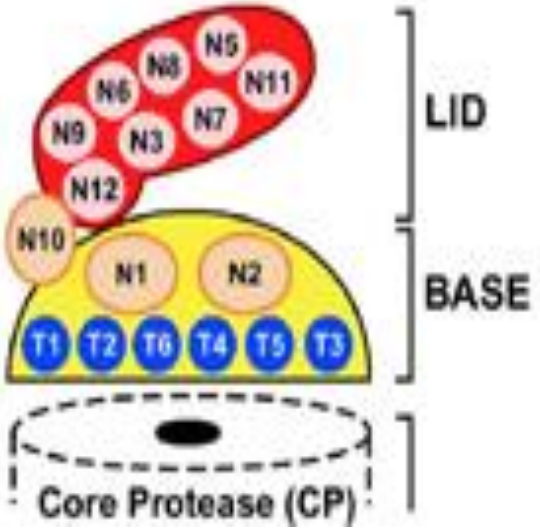
a

20S Core Protease (CP)

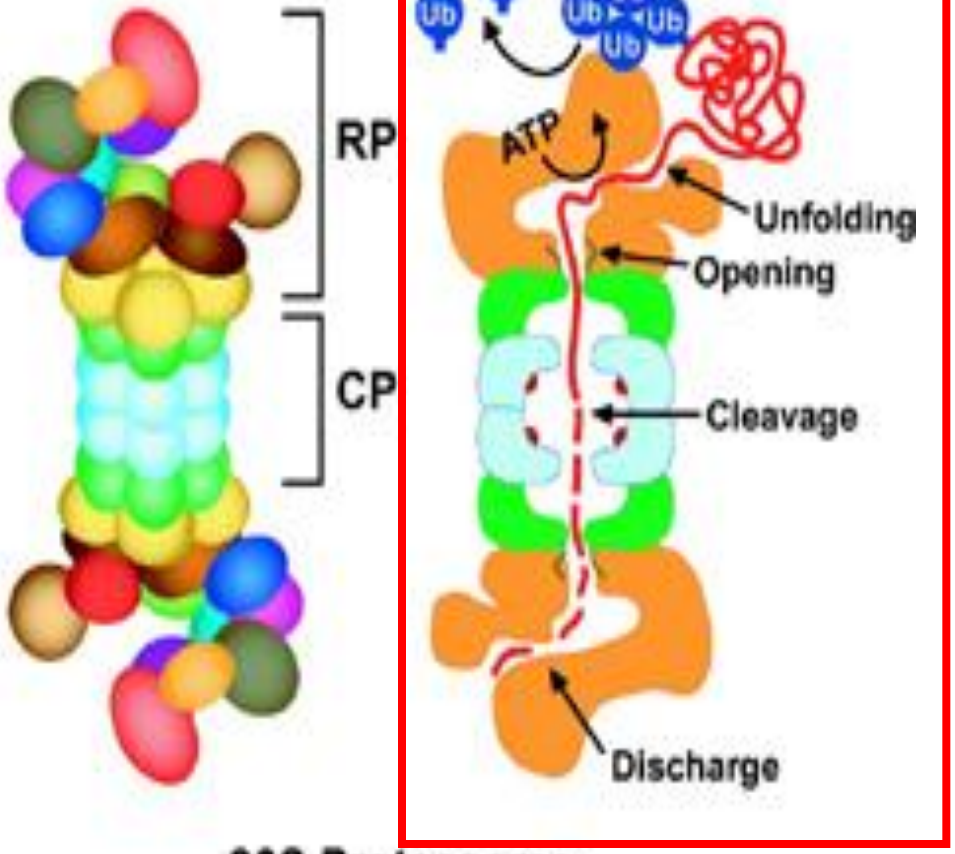


b

19S Regulatory Particle (RP)

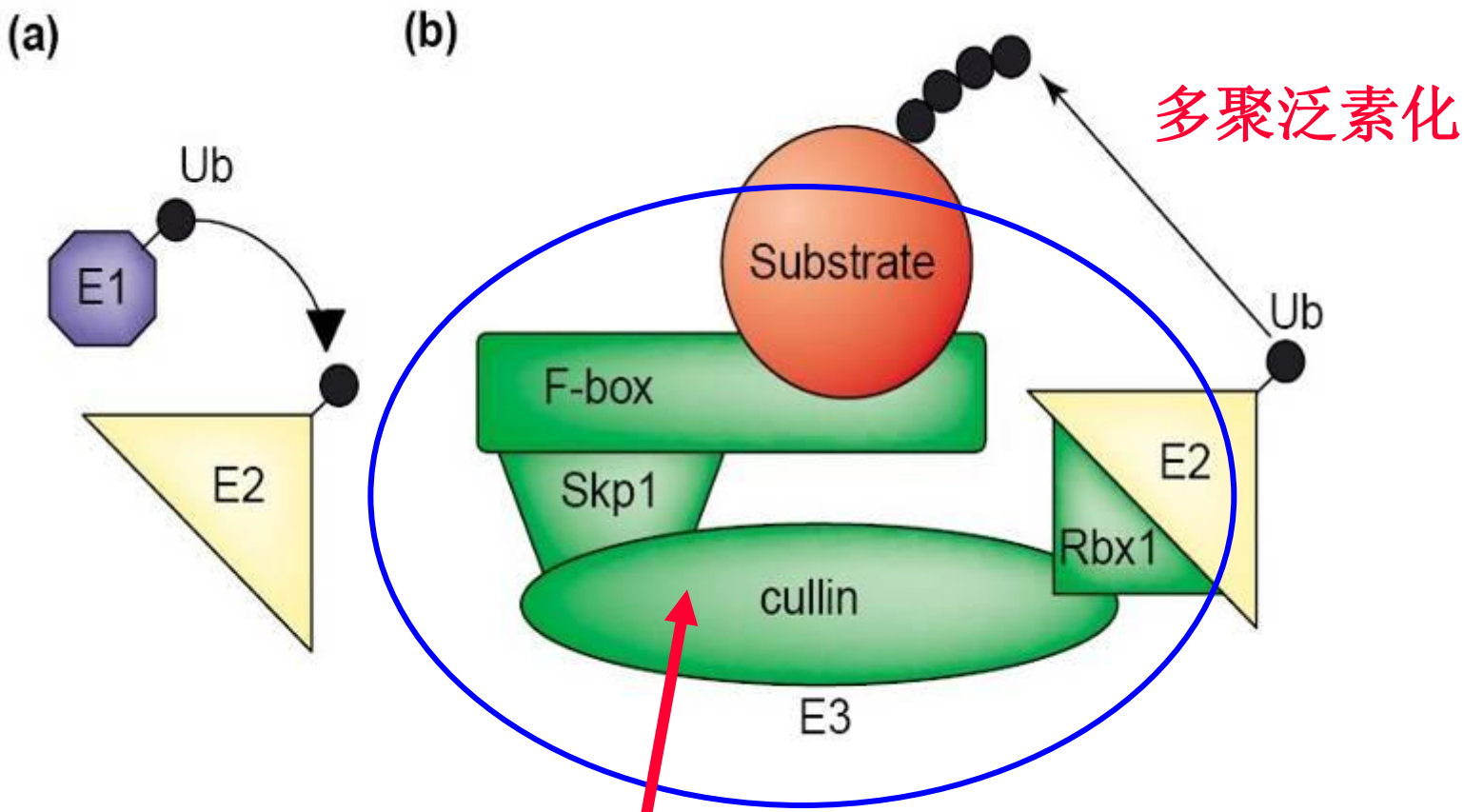


c



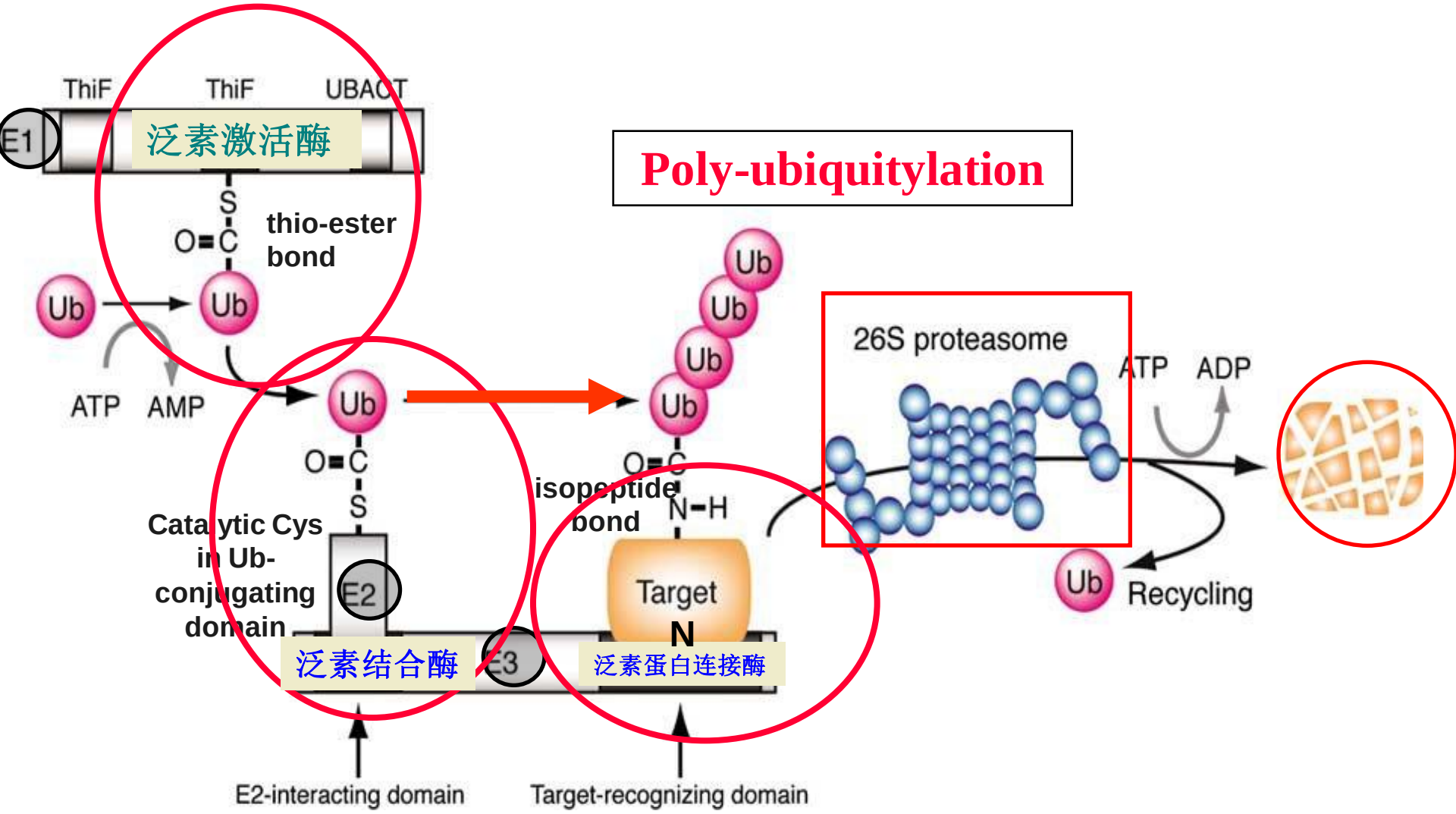
26S Proteasome

某些转录因子的mono-ubiquitylation 被激活



蛋白质在被26S蛋白酶降解前，必须由**E3 ubiquitin ligase enzyme complex (EULEC)**进行多聚泛素化

From Itoh et al., *Trends Plant Sci*, 2003, 8:492-497



The ubiquitylation pathway and its associated enzymes

From Hatakeyama and Nakayama, *Biochem. Biophys. Res. Commun*, 2003, 302:635-645

E3 ubiquitin ligase enzyme

对靶蛋白识别的特异性分为3类

HECT：

Homologous to E6-associated protein Carboxy Terminus

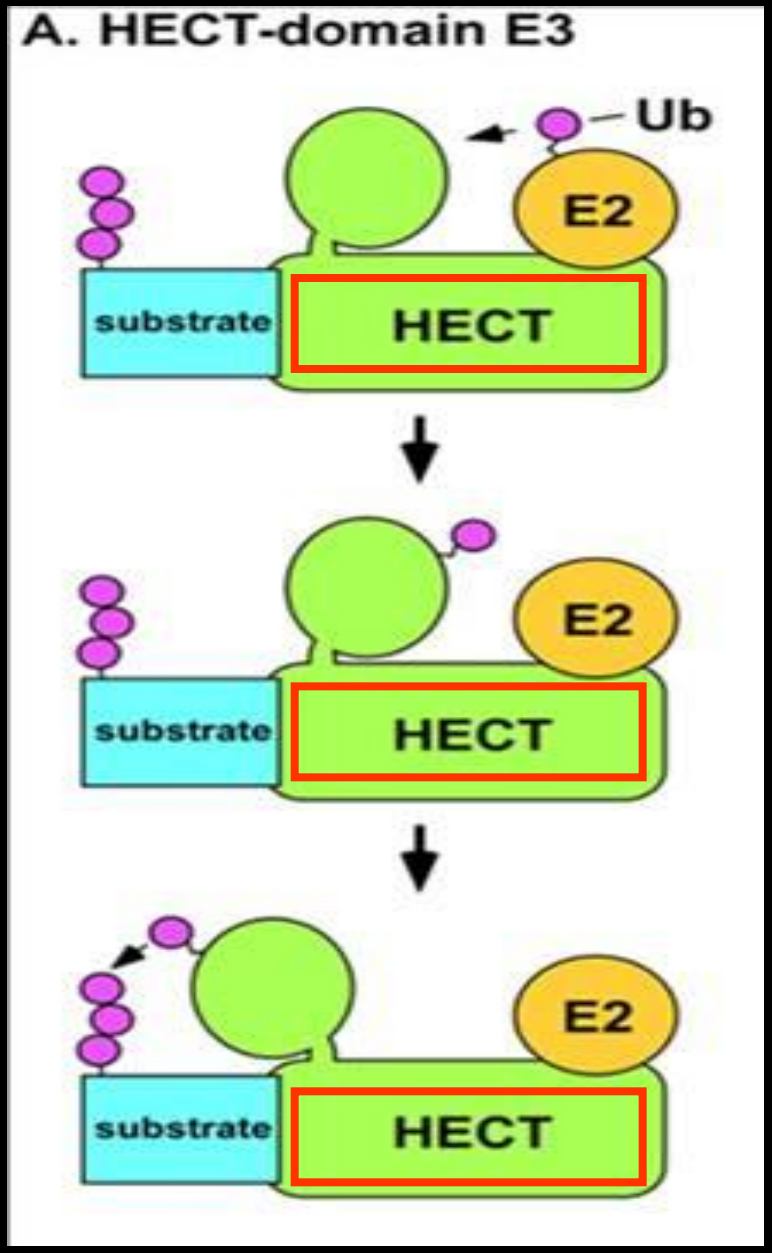
RING-finger； **Really Interesting New Gene-Finger**

U-Box： **RING-Finger modified**

RING-Cullin-E3

共性

- **连接E2，接受Ubi**
- **识别靶蛋白**
- **对靶蛋白完成Poly-Ubi**



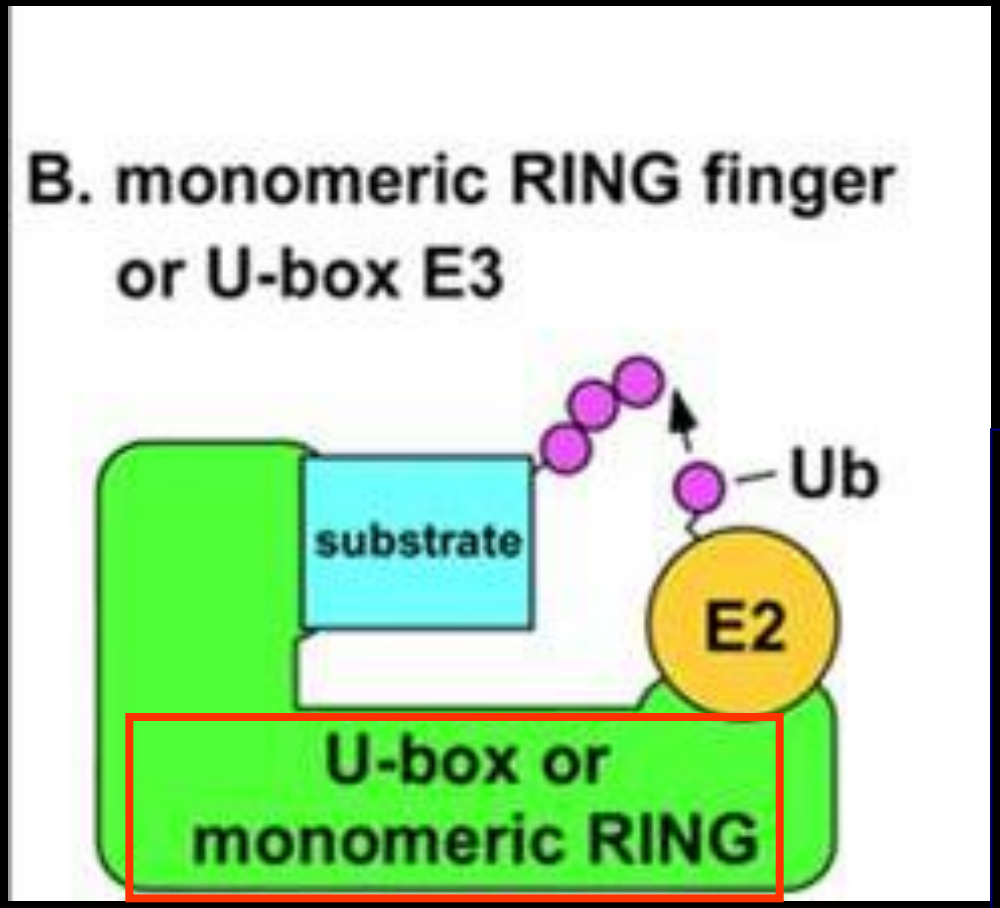
HECT- E3

连接E2

接受Ubi

识别靶蛋白

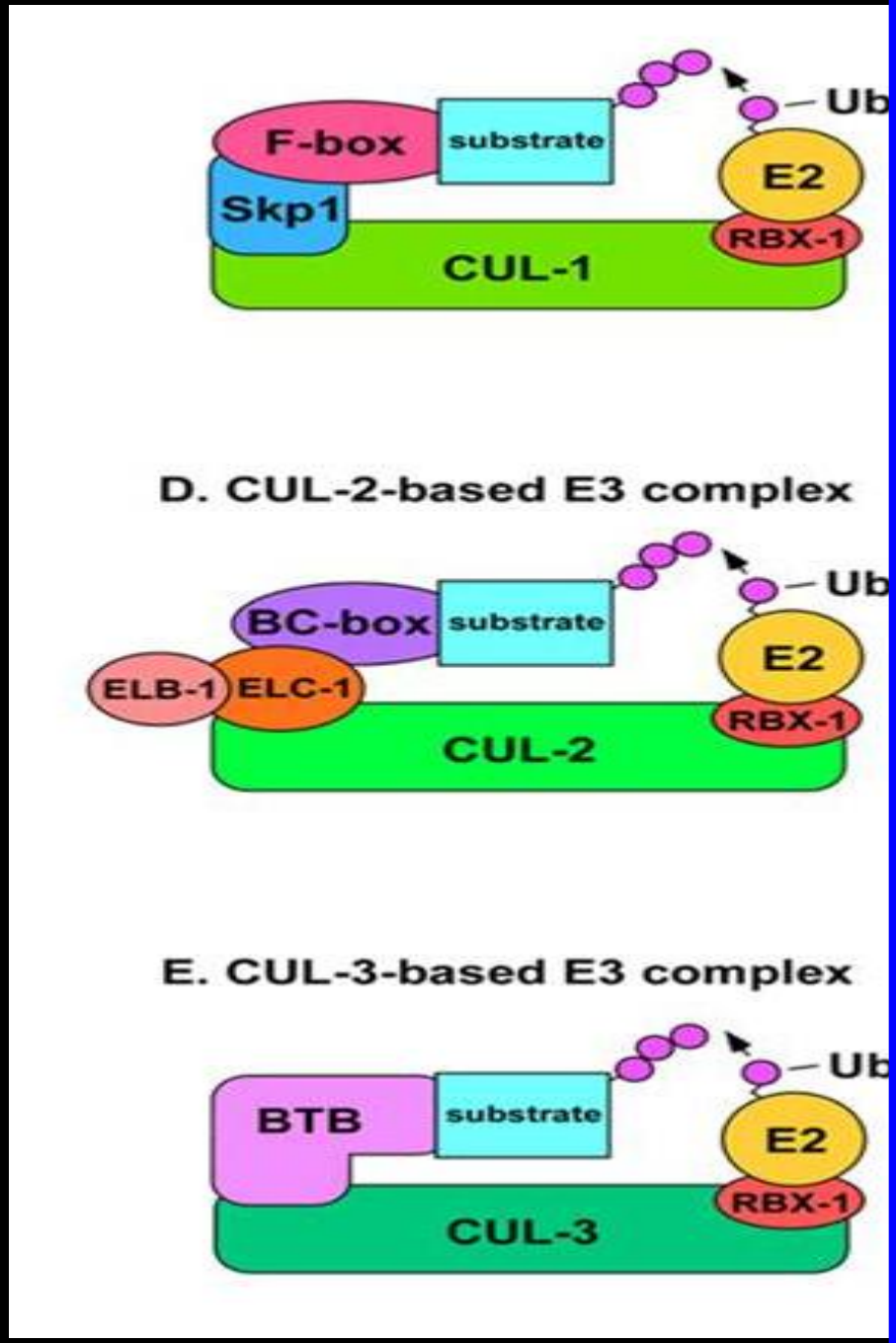
靶蛋白Poly-Ubi



连接E2

识别靶蛋白

靶蛋白Poly-Ubi

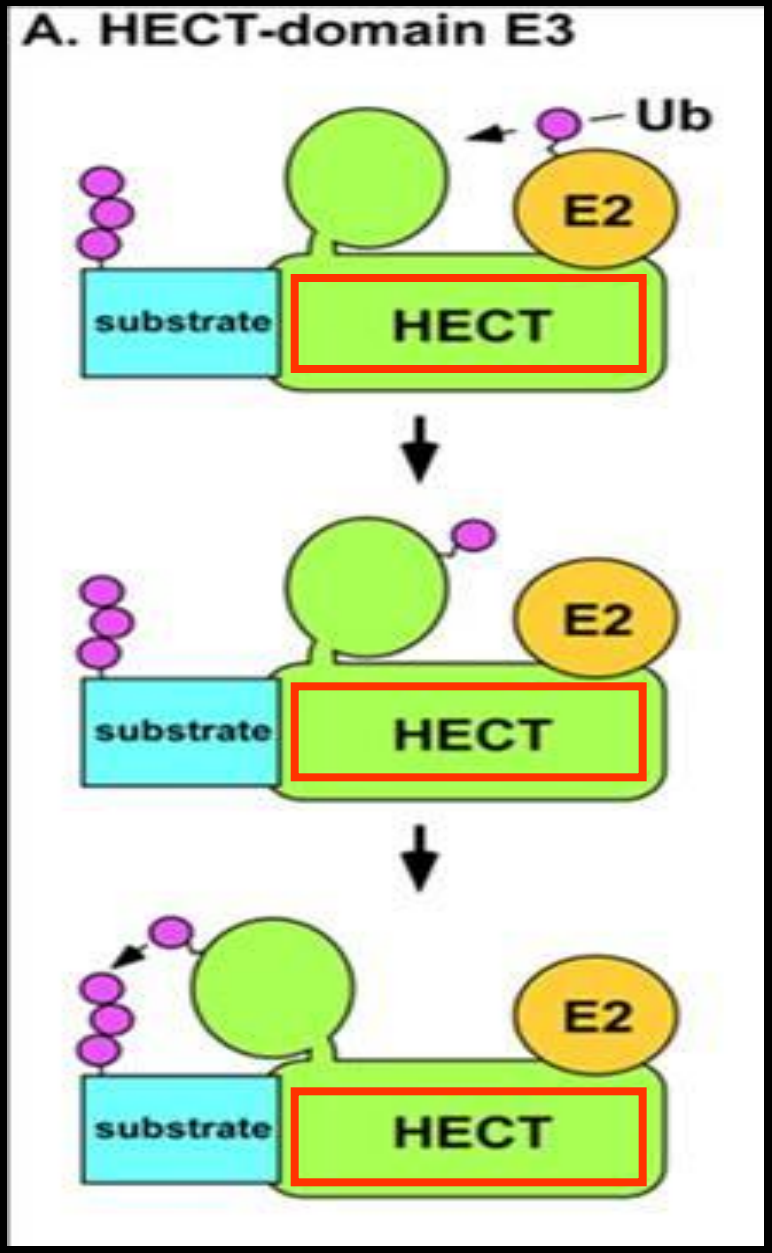


连接E2

识别靶蛋白

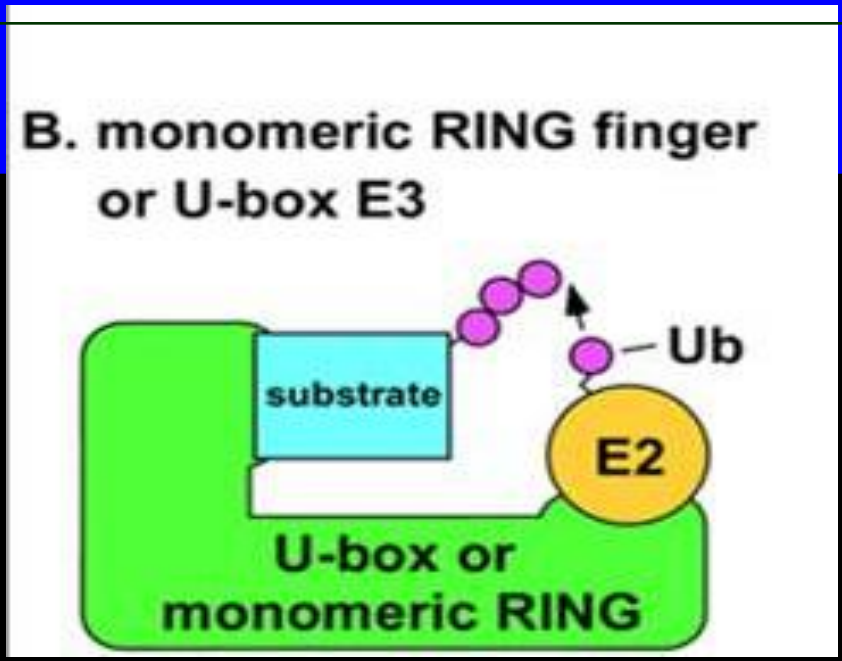
靶蛋白Poly-Ubi

1400种EULEC
识别不同底物
实施降解功能
协调生理活动



HECT-E3 与RING-E3 的主要区别：

- Ubi直接从E2转移到靶蛋白
- Ubi间接通过E3转移到靶蛋白



- 被EULEC识别的N-end aa marker of targeting protein

N-end aa of targeting protein	half—life
• Met, Gly, Ala, Ser, Thr, Val	〉20 hrs (稳定因子)
• Ile, Glu	~ 30’ (不稳定因子)
Gln	~ 10’
Pro	~ 7’
• Leu, Phe, Asn, Lys	~ 3’ (极不稳定因子)
Arg	~ 2’

- 决定与Ubiquitin 发生isopeptide bond 的难易程度
- 决定被 E3 识读结合难易程度
- N 端氨基酸种类对蛋白质寿命的决定性，从原核到真核生物高度一致

泛素/26S蛋白酶体通路与

植物激素应答

植物抗病反应

植物光形态建成

高等植物有性生殖

植物环境胁迫抗性

Molecular Biology

Gibberellin Signaling Pathway

a de-repressible system

moderated by DELLA-p

degradation induced by GA

What is gibberellin (GA)

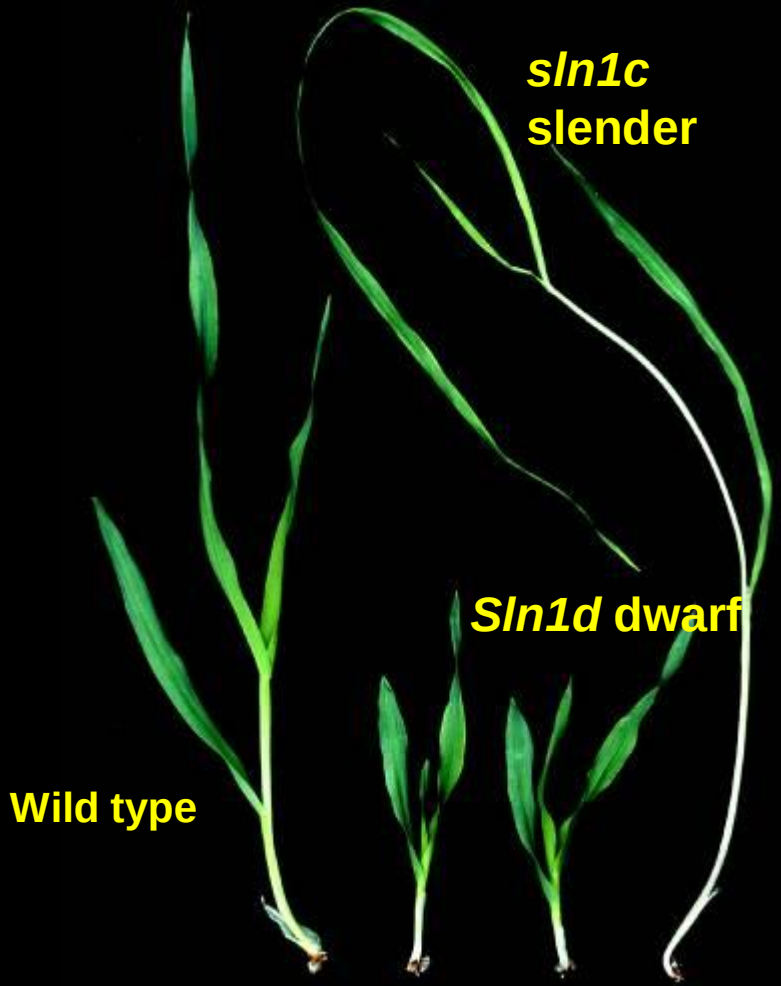
normal infected

Eiichi Kurosawa (1926): Rice foolish seedling (bakanae) disease caused by the fungus *Gibberella fujikuroi*

- Promote stem elongation and leaf expansion

- GA + GA

Dwarf peas



GA-deficient Barley GA mutant

(来源：不详)

6.3.3 Green Revolution

第一次绿色革命；

粮食作物增产20%

Norman E. Borlaug
Director of wheat program (CIMMYT)
1970 Nobel Peace Prize

Green Revolution Gene

Semi-dwarf rice cultivars containing rice ‘G. R’ gene *sd1*

Semi-dwarf maize cultivars containing the ‘G. R’ gene *d8*

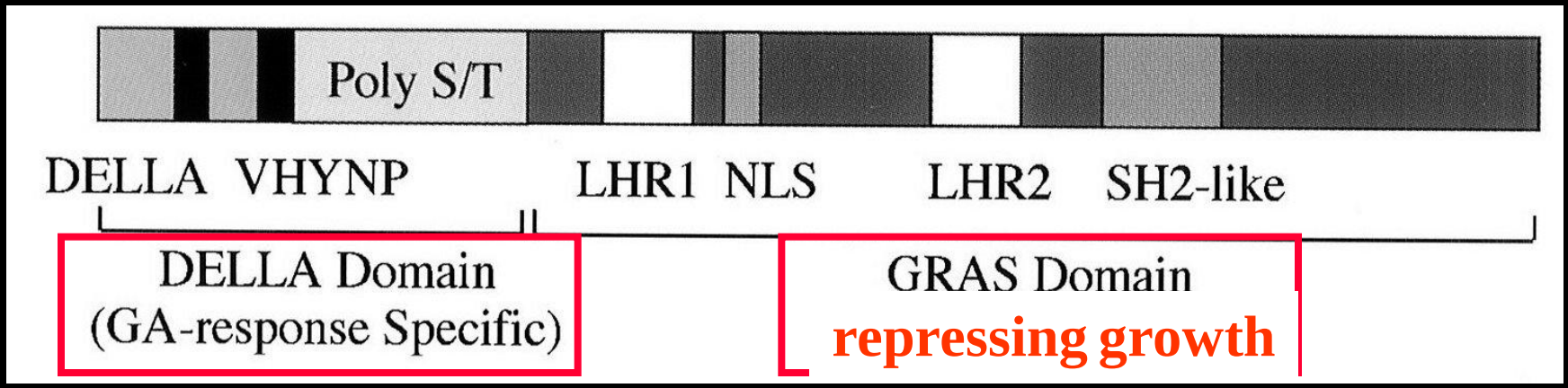
Highly conserved in G.R. gene

Arabidopsis: **(RGA, GAI, RGL1, RGL2, RGL3)**

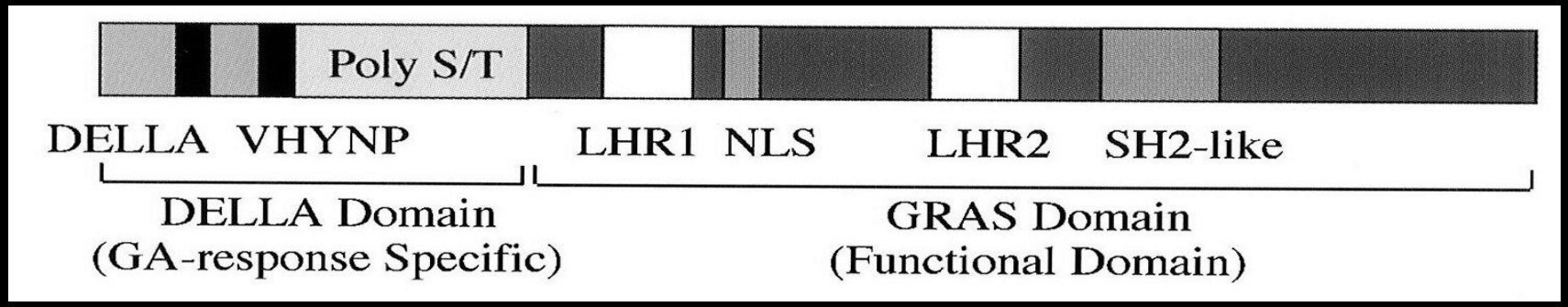
maize : (d8), wheat (Rht)

rice : (SLR1), barley (SLN1)

grape: (VvGAI1), ***Brassica rapa*** **(BrRGA1)**



The DELLA subfamily of GRAS proteins

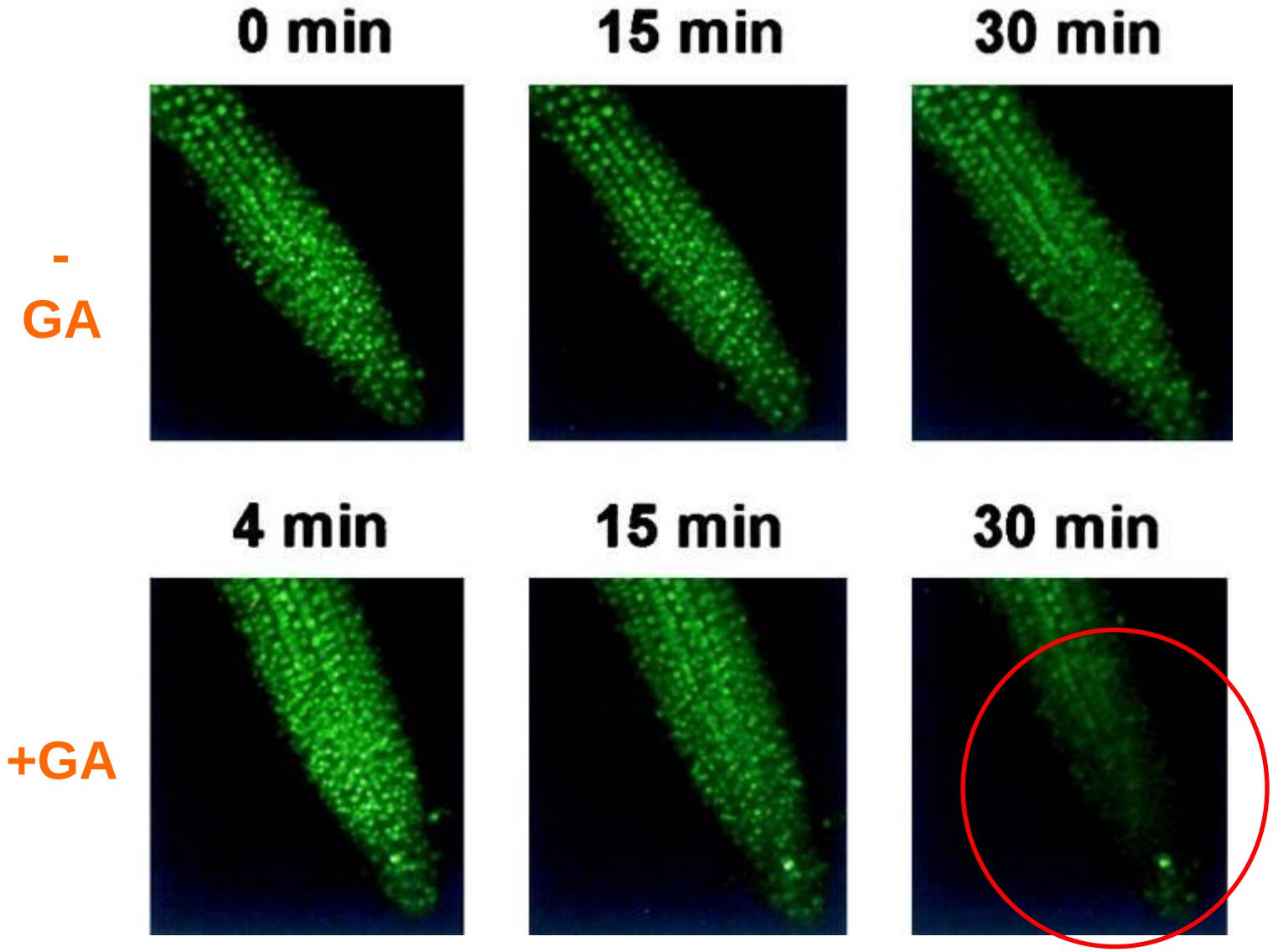


- A conserved and unique N-terminal domain, named DELLA, after a DELLA motif
- Wild type DELLA proteins localize in cell nuclei and disappear by GA treatment
- DELLA domain-mutated are resistant (G.R.gene) to GA-induced degradation (GA-insensitive)

By using immunoblot analysis and GFP fusion protein analysis, it is shown that GA induces rapid degradation of the wild type DELLA proteins,

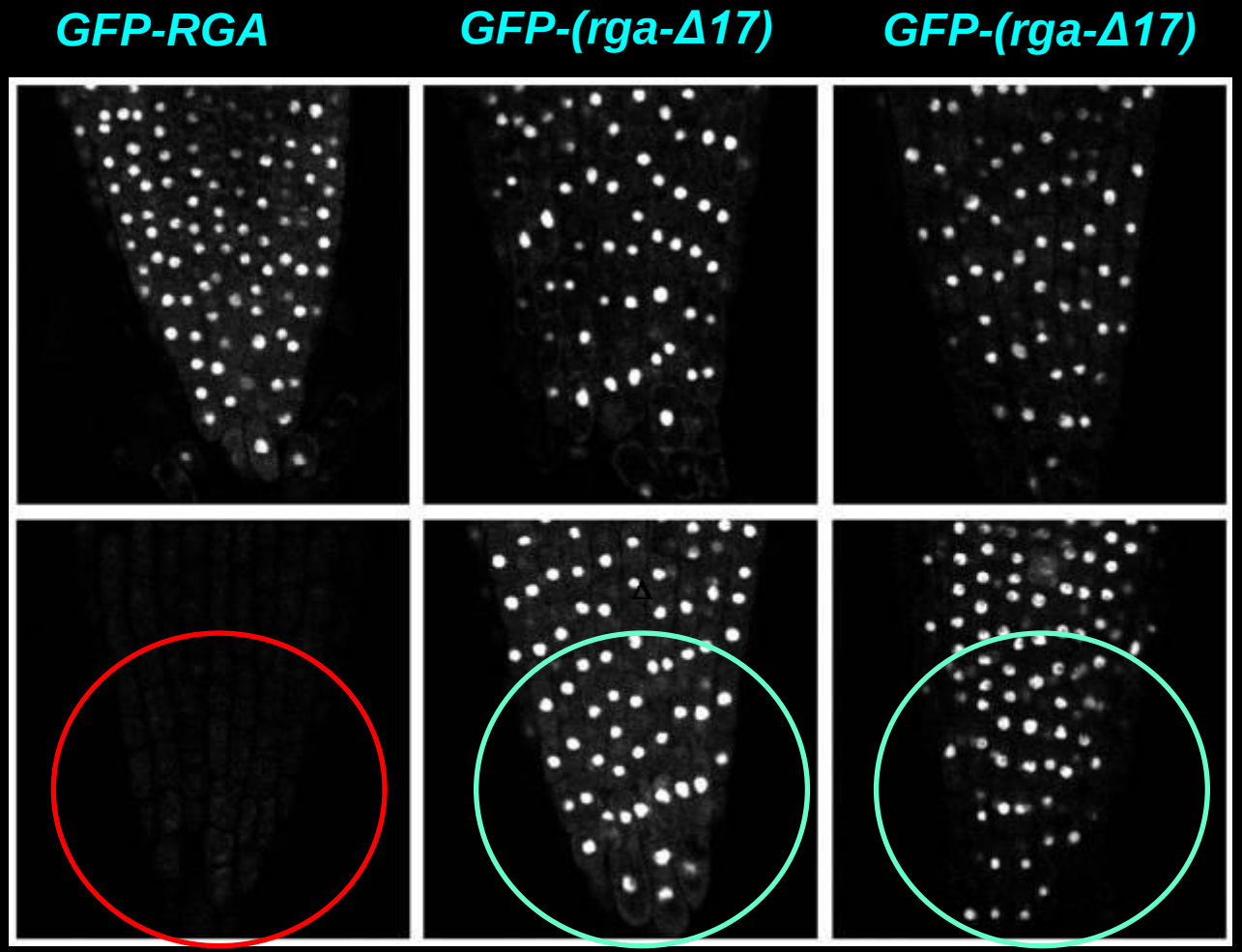
such as GFP-At-RGA

GFP-Os-SLR1



The disappearance of GFP-RGA protein on GA treatment

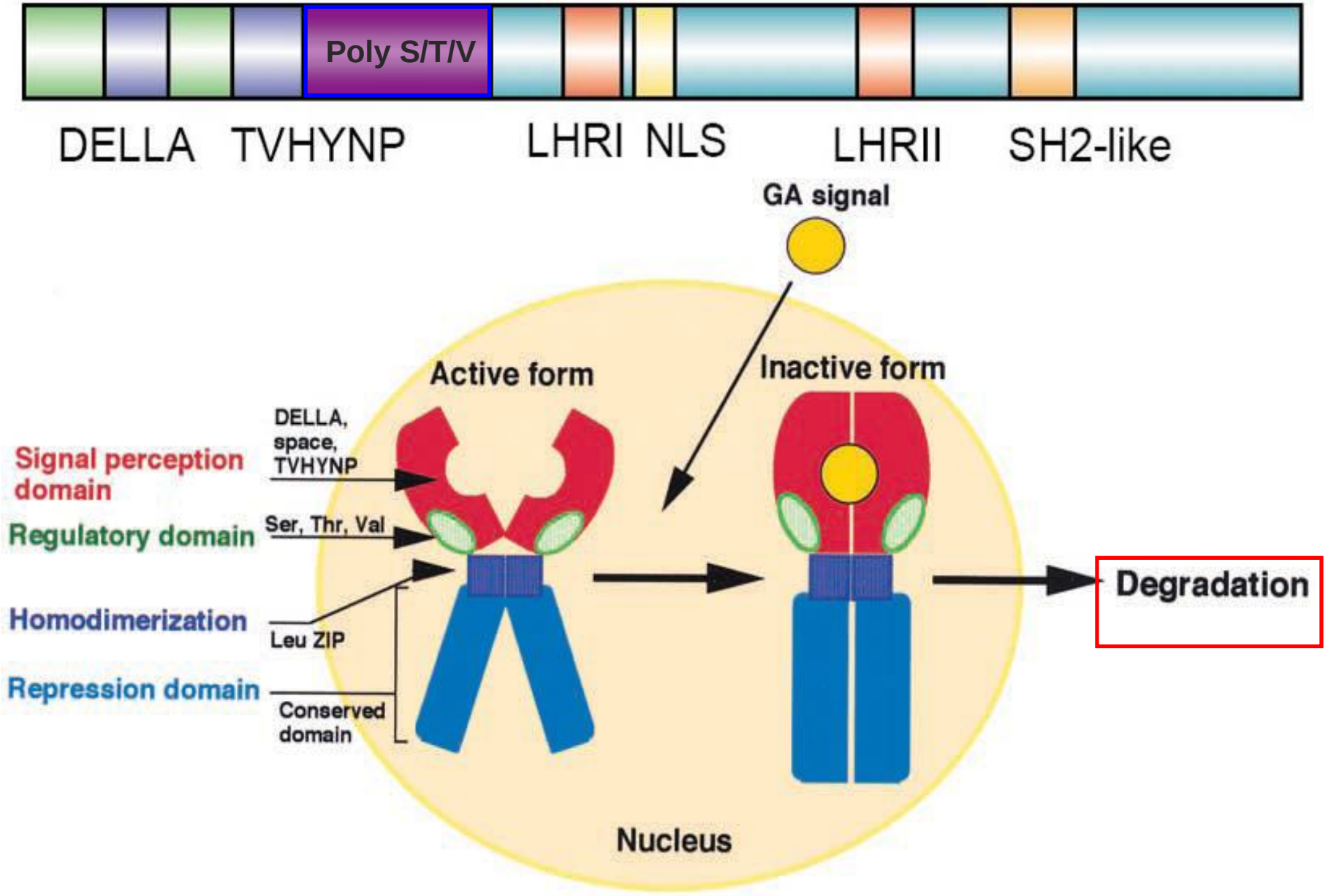
From Silverstone et al., *Plant Cell*, 2001, 13:1555-1566



GFP-(rga-Δ17) proteins are resistant to GA-induced degradation

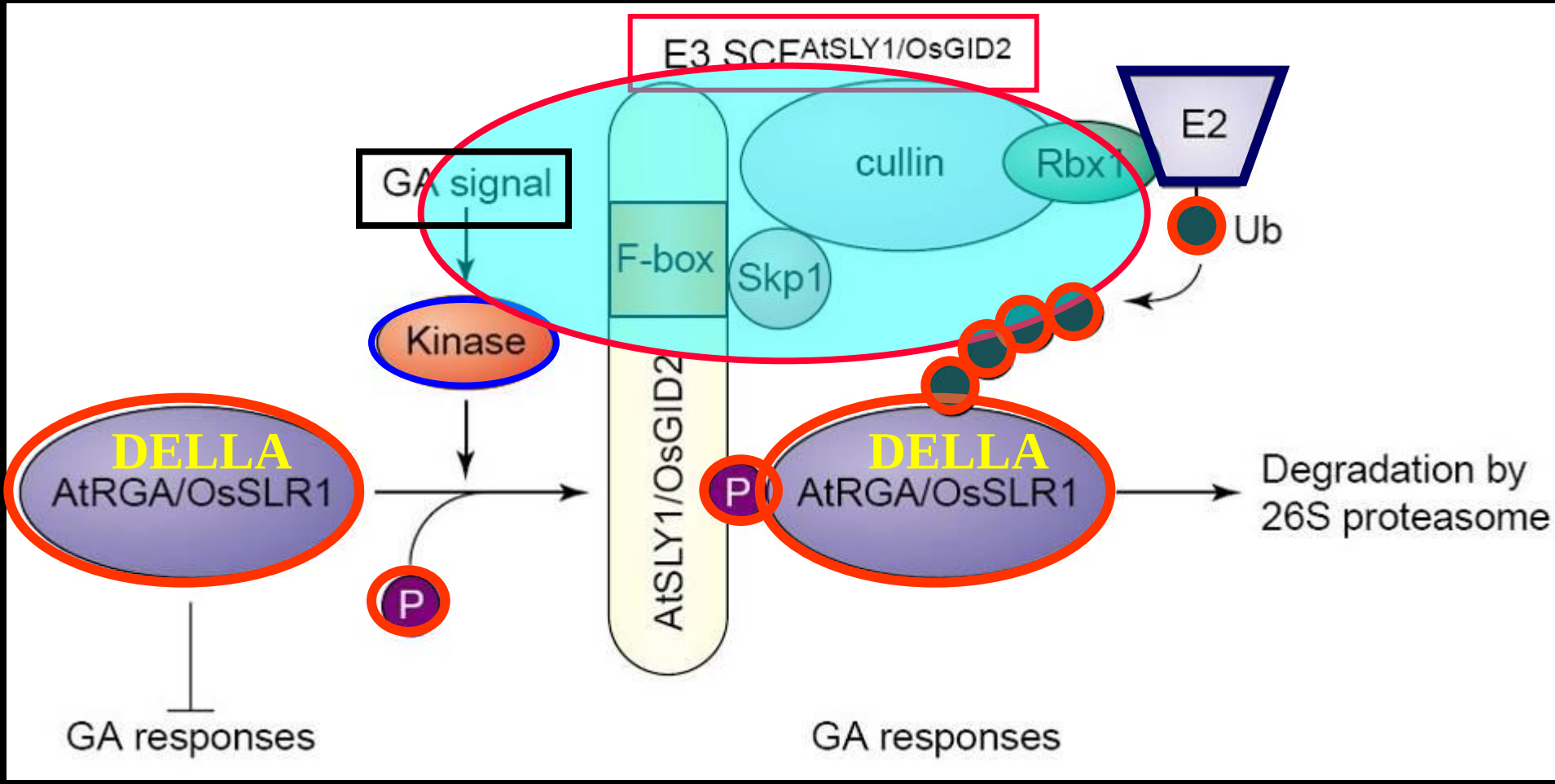
(*rga-Δ17* has the same 17AA deletion in the DELLA region as in *gai-1*)

From Dill et al., *Proc Natl Acad Sci USA*, 2001, 98:14162-14167



Scheme of the functional domains of SLR1 for GA signaling pathway

From Itoh et al., *Plant Cell*, 2002, 14:57-70

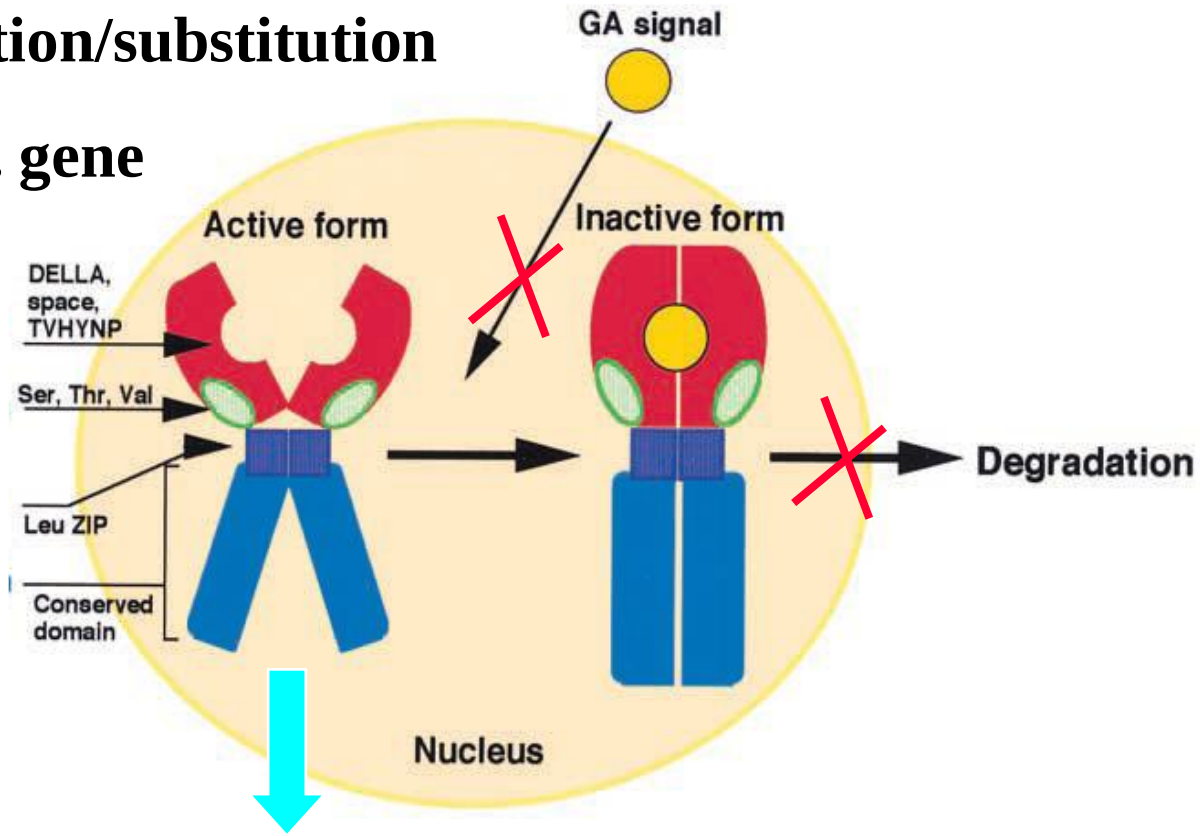


A model of GA induced degradation of DELLA proteins via E3 ubiquitin ligase enzyme complex (EULEC)

From Itoh et al., *Trends Plant Sci*, 2003, 8:492-497

rga-DELLA
deletion/substitution

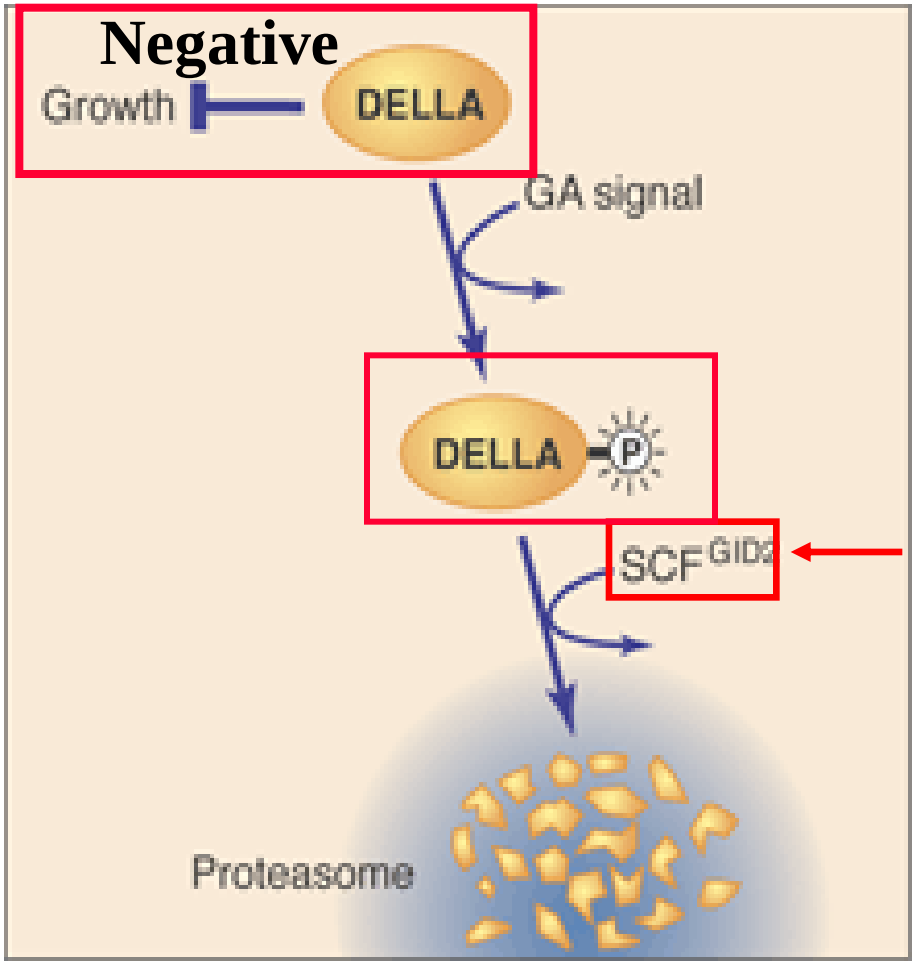
G.R. gene



Repressing stem elongation and leaf expansion

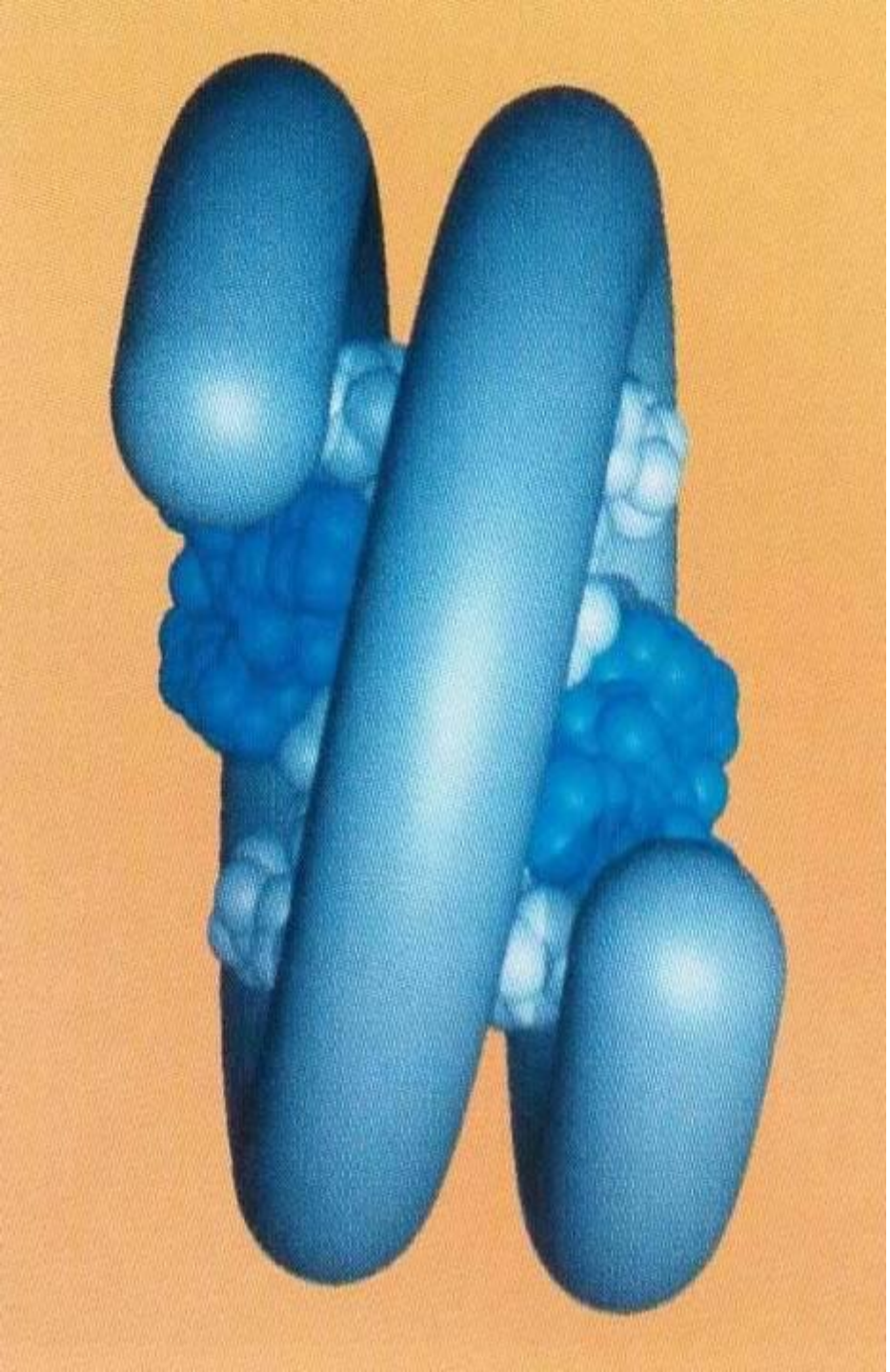
Involvement of the ubiquitin-proteasome pathway in degradation of DELLA proteins

(来源：分子生物学（2007），郑用琏，第275页)



Ubiquitylating enzyme E3 complex

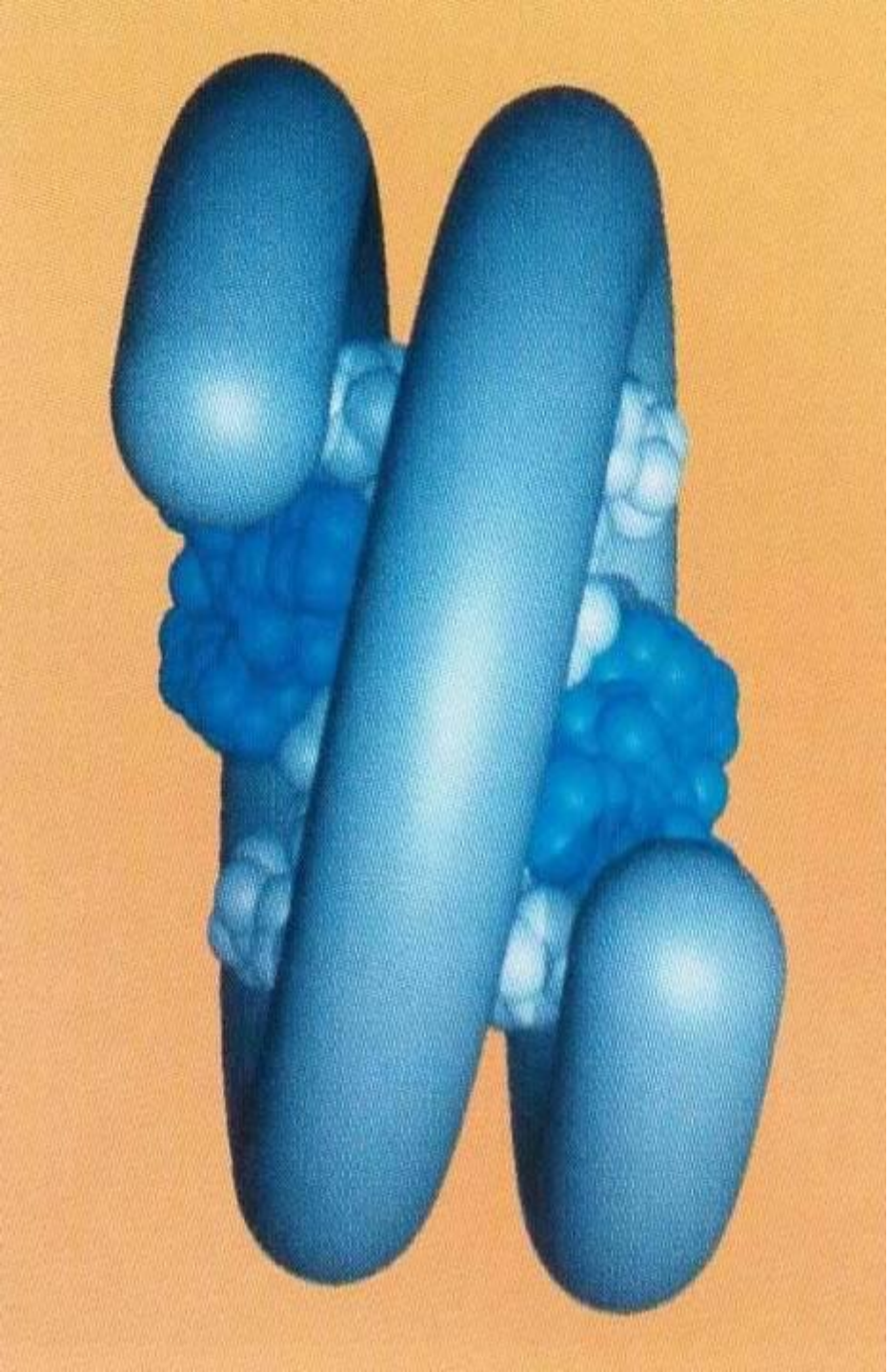
In response to GA, DELLA proteins are rapidly degraded by the ubiquitin-proteasome pathway



表观遗传及其分子机制
Epigenetic and Molecular Mechanism

6.4 染色质重建对基因表达的控制

(Source:Arents,Topography of the histone octamer surface:Repeating structureal motifs utilized in the docking of uncleosomal DNA.USA(Nov 1993))



表观遗传及其分子机制
Epigenetic and Molecular Mechanism

Epi meas

“outside of ”

“in addition to”

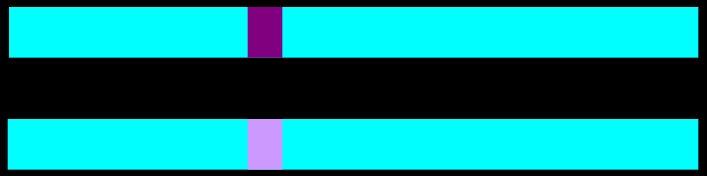
(Source:Arents,Topography of the histone octamer surface:Repeating structureal motifs utilized in the docking of uncleosomal DNA.USA(Nov 1993))

Epigenetic inheritance:

The ability of different states, which may have different phenotype consequences, to be inherited without any change in the DNA sequence.

在DNA序列没有发生任何改变的情况下，不同表型效应具有可以遗传的能力

Position effect of variegation in Drosophila eyes



Ww w Red eye

（W>w in euchromatin）

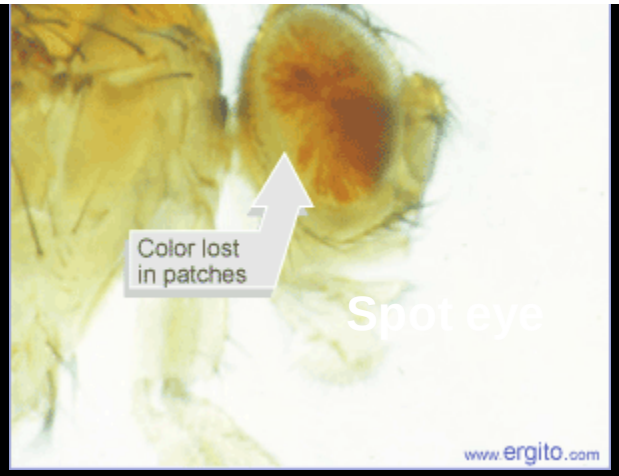
W

Ww white eye

w

（W (near heterochromatin) be silenced

Epigenetic phenomena

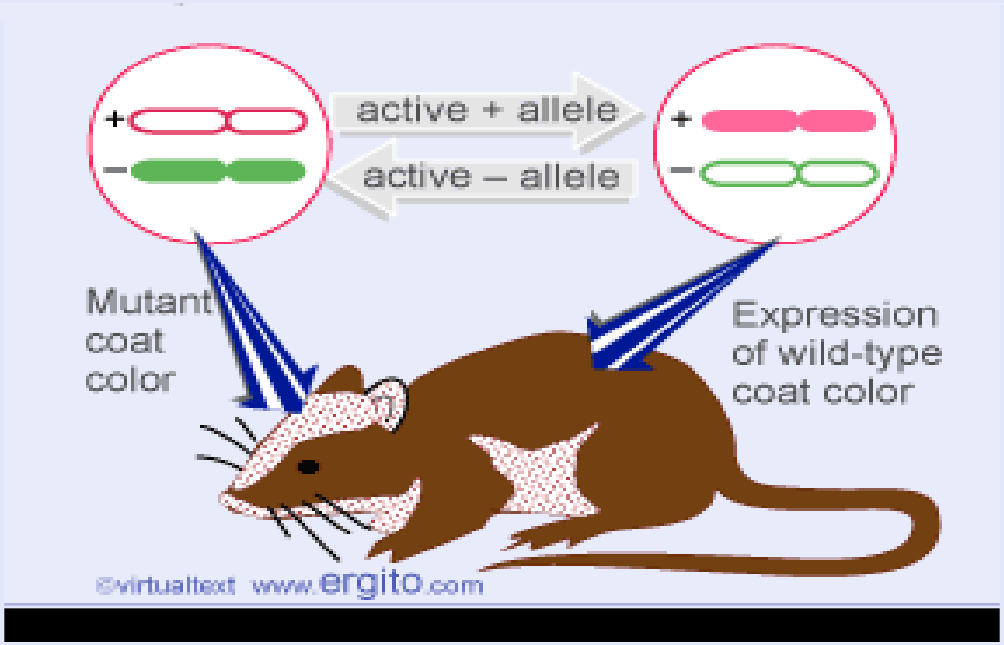


(来源：不详)

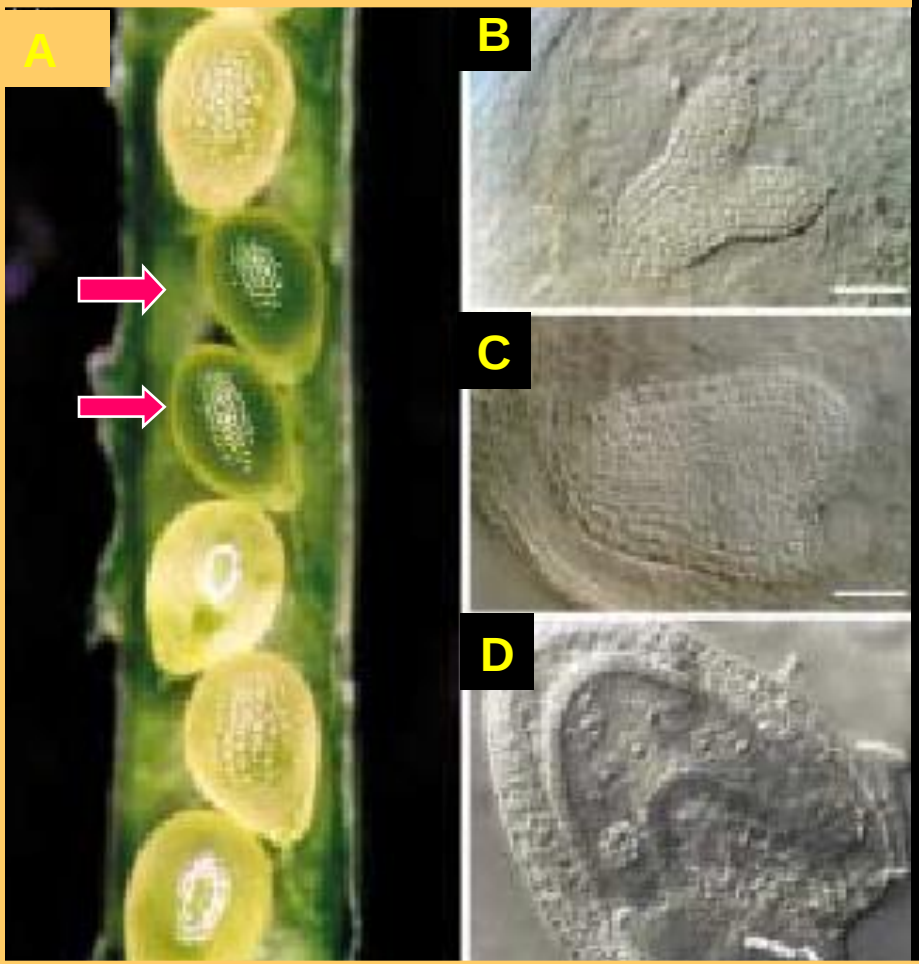
Cellular Memory : Arabidopsis Vernalization

(来源：不详)

One X chromosome is inactived at random

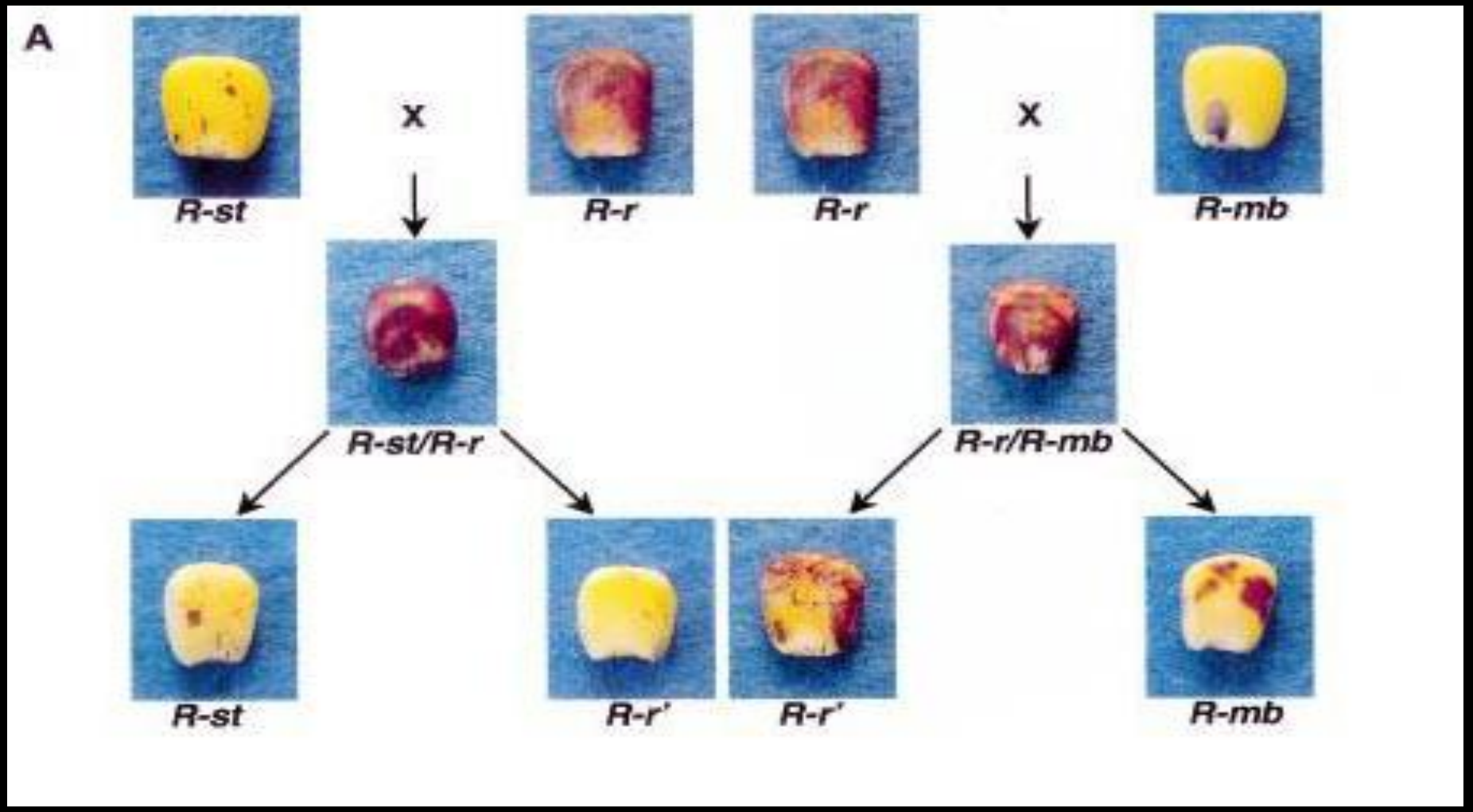


(来源：不详)



parental imprinting

(来源：不详)



Allelic interaction (paramutation)

(来源：不详)

Epigenetic phenomena

- Position-effect variegation
- Inactivation of chromosome X
- Cell-type conversion
- Allelic interaction (paramutation)
- Transgene silencing
- Parental imprinting
- Cell memory: Vernalization
- Heterosis ! ?

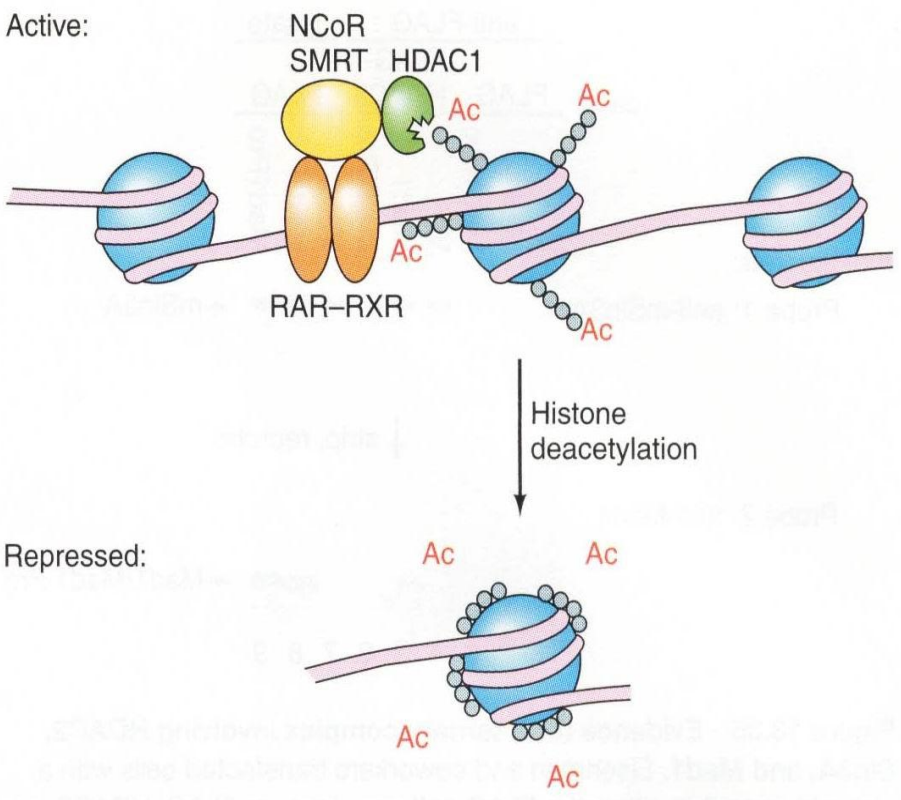
Molecular Mechanism of Epigenetic

包括两个相互作用，彼此渗透，共同影响染色质的结构和基因表达，但又相对独立的方式：

- ATP－dependent Chromatin remodeling
- Chromatin covalent modification

Epigenetic and Molecular Mechanism

染色质重建 Chromatin Remodeling



染色质结构的调整

核小体的松弛

组蛋白的乙酰化

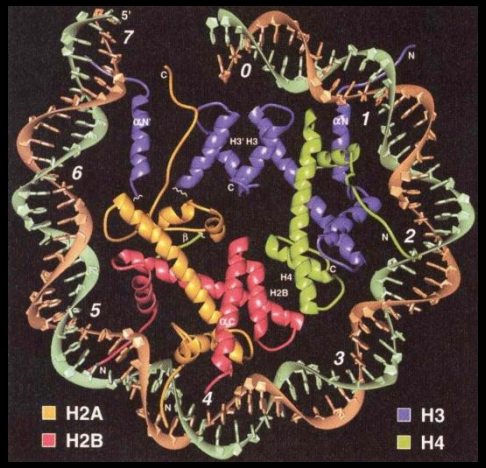
组蛋白的甲基化

沉默子….

(来源：分子生物学（2007），郑用琏，第283页)

Basic unit of chromatin

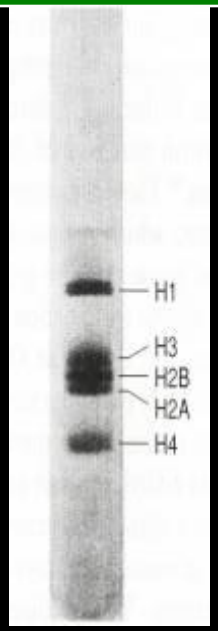
Nucleosome (~ 200bp)



Source:Luger,K.,A,Crystal structure of the nucleosome core particle at 2.8A Resolution.Nature 389(18 Sep 1997)f.2,p.233.)

2 × H_{2A} H_{2B} H_3 H_4 Octamer + core DNA of 146 bp

1.75 cycle

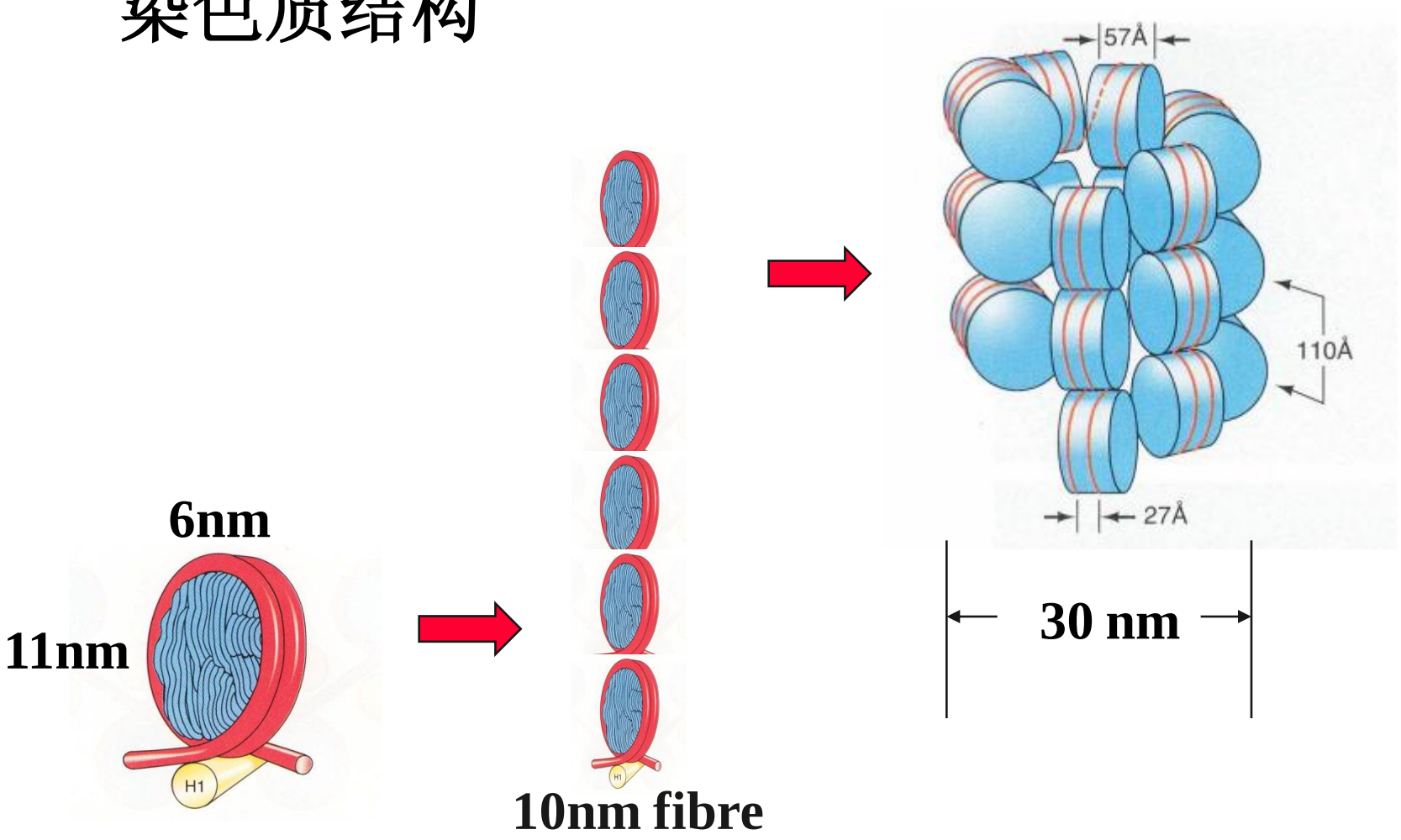


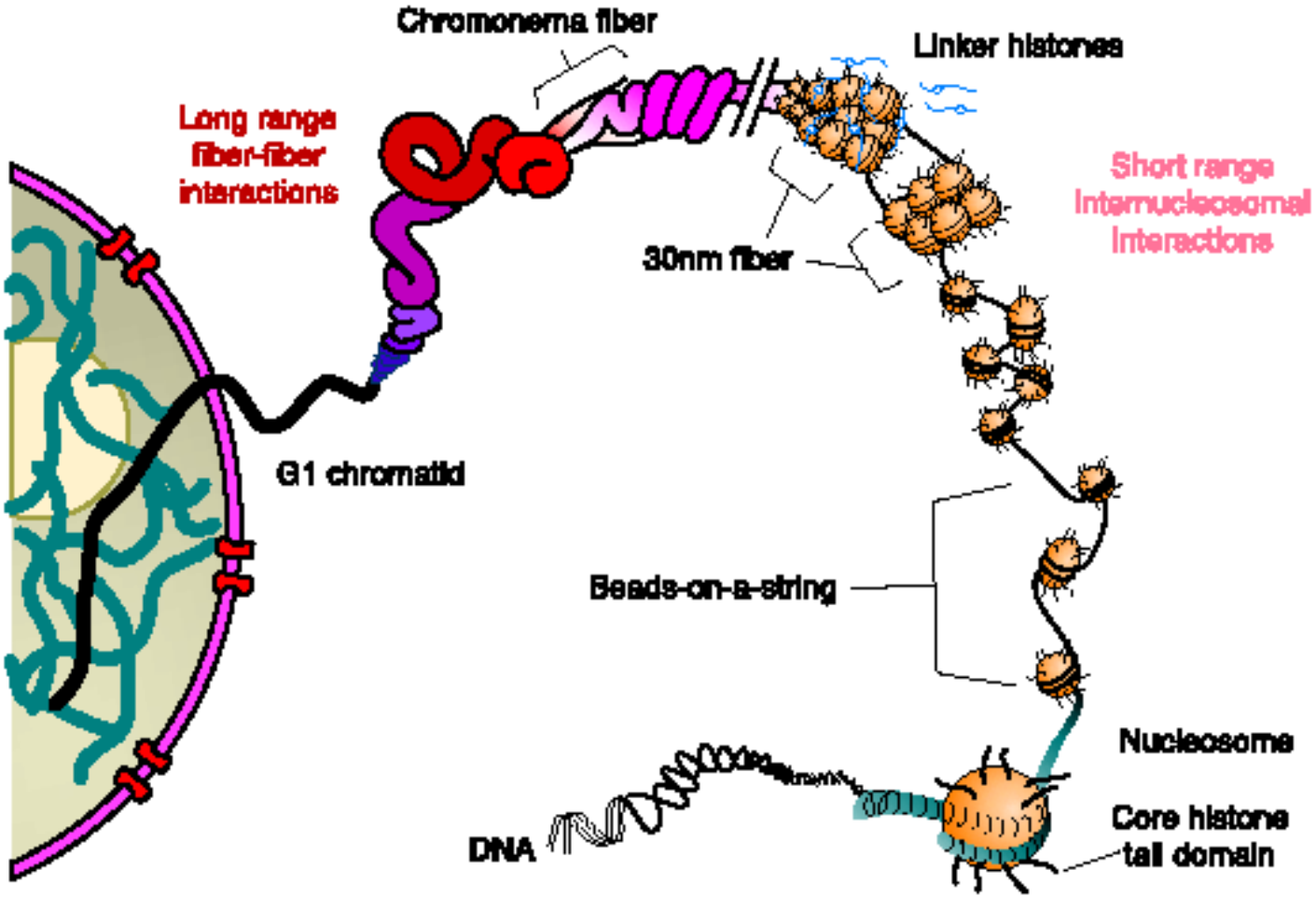
Histone 1 + linker DNA of 20-60bp

(diff. From diff. creature)

(Source: Panyim and Chalkley.Archives of Biochem.&Biophys.130,1969,f,6A,p.343.)

染色质结构





(来源：分子生物学（2007），郑用琏，第280页)

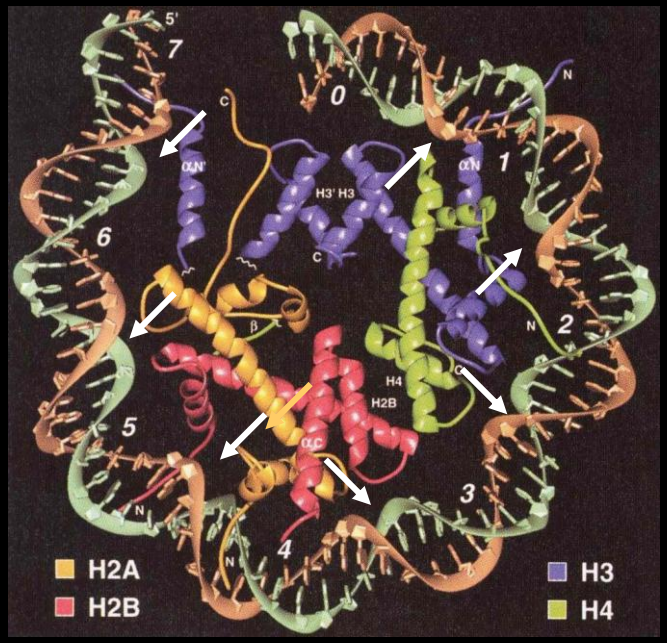
Heterochromatin
Euchromatin

组蛋白中富含 Lys^{+}, $Arg.^{+}$

量大， 进化保守， 与DNA 无专一性结合区

Source:Luger,K.,A,Crystal structure of the nucleosome core particle at 2.8A Resolution.Nature 389(18 Sep 1997)f.2,p.233.)

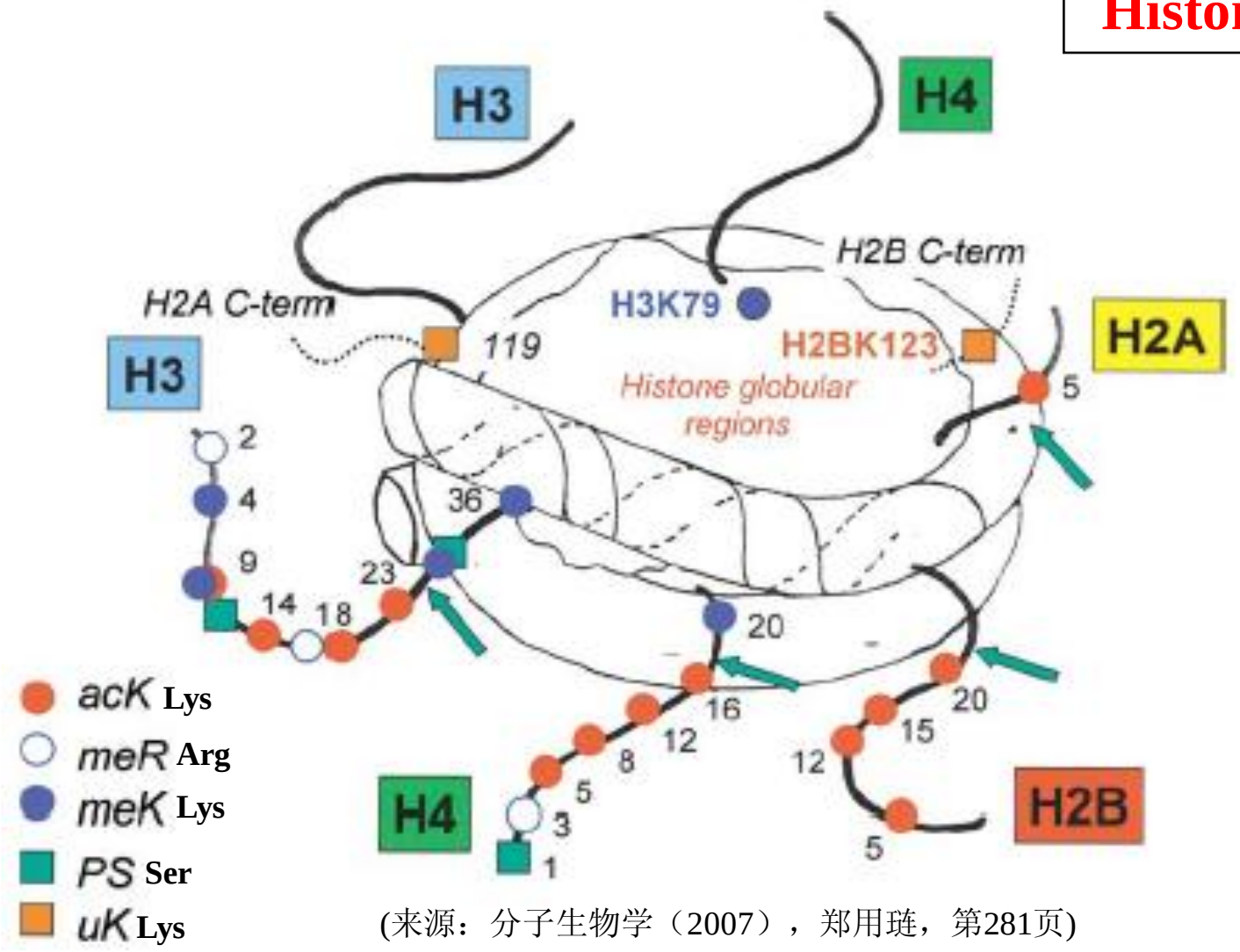
N端外露 Arg^{+}与DNA14个小沟结合



富含Lys^{+}的N端（特别是H4）能保证Histone间的稳定性和与DNA的结合

N端磷酸化、乙酰化等能降低组蛋白正电荷，
使组蛋白间以及与DNA的结合状态发生改变

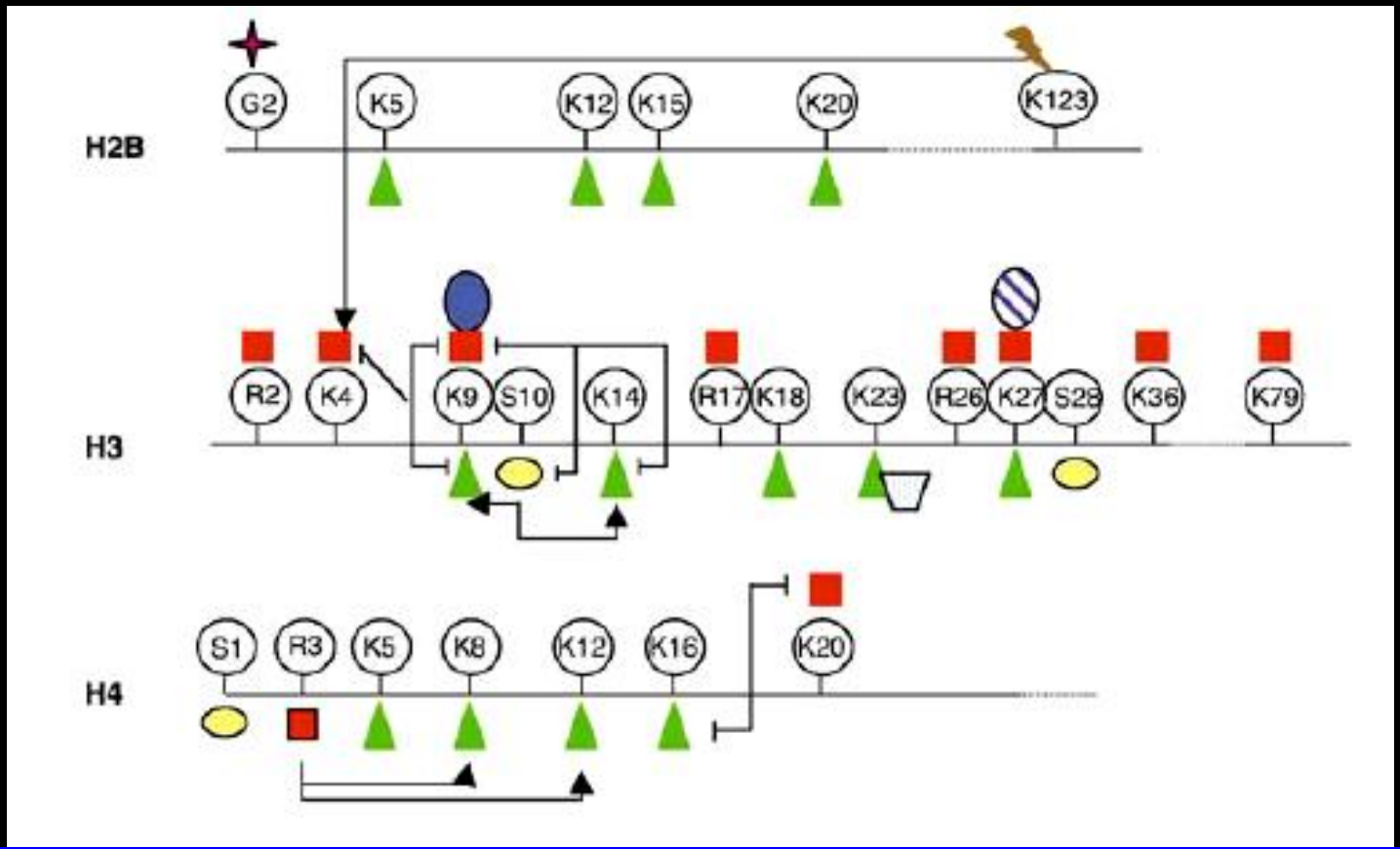
Histone Codon



(来源：分子生物学（2007），郑用琏，第281页)

- **Histone acetyl-transferase (HAT)**
- **Histone deacetylase (HDAC)**
- **Histone methyl-transferase (HMT)**

Histone code



组蛋白一个位点的修饰可以激活或抑制另一个位点的修饰，这种信号的组合可以寓意染色质的类型与性质。这种组合也被称为组蛋白密码

(来源：分子生物学（2007），郑用琏，第281页)

chromatin 状况与基因表达相关

Euchromatin → gene on　　Heterochromatin → gene off

Heterochromatin；

Constitutive Heterochromatin: 组成型异染色质

除DNA复制以外，一直处于高度致密的固缩状态，

DNA从不转录（高度重复序列，着丝点，端粒等）

Facultative Heterochromatin: 特异型异染色质

有时处于异染色质状态，有时为常染色质状态

转录活化区/非转录活化区 Chromatin 的结构差异

in transcriptional activated region

Dnase^{s}

Nucleosome incomplete

No Histone1 in linker DNA

表达非常活跃的rDNA区无核小体结构

凡被Trans-factor结合的区域

均能阻止nucleosome在形成

H_{2A}、H_{2B}、H_3、H_4 acetyleated

降低组蛋白的正电荷

组蛋白解聚 → v-body松弛

Pazin & Kadonaga **1997 Cell. 89;325-328**

从酵母中分离出

组蛋白去乙酰化酶A/B

以及相应的催化组分HDAC1 / RPD3

HDAC1 / RPD3基因突变导致H3/H4超乙酰化

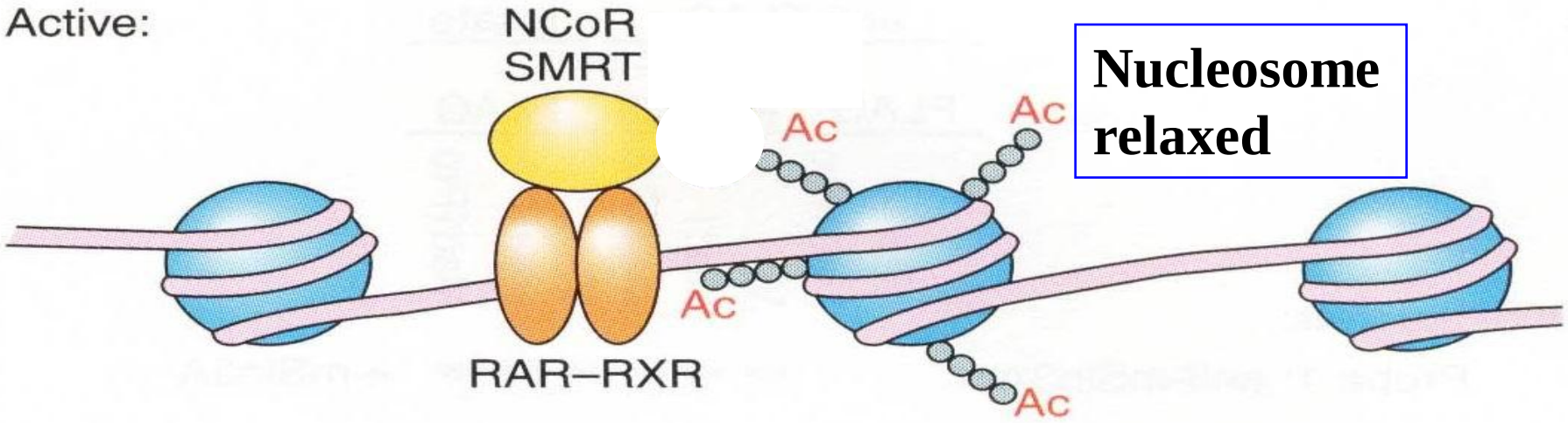
引起基因高效表达

丁酸钠
（去乙酰化酶抑制剂）
→ Hela cell →
80%H3,H4乙酰化
Over acetylation

基因高效表达

Model for

participation of histone deacetylase in transcription repression



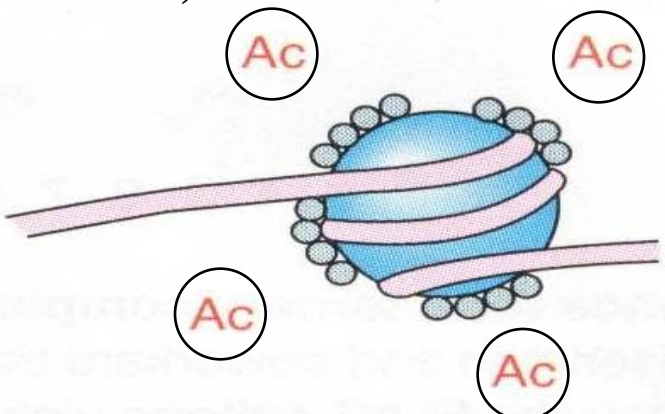
Nucleosome relaxed

histone deacetylase cooperator HDAC1

Histone deacetylation

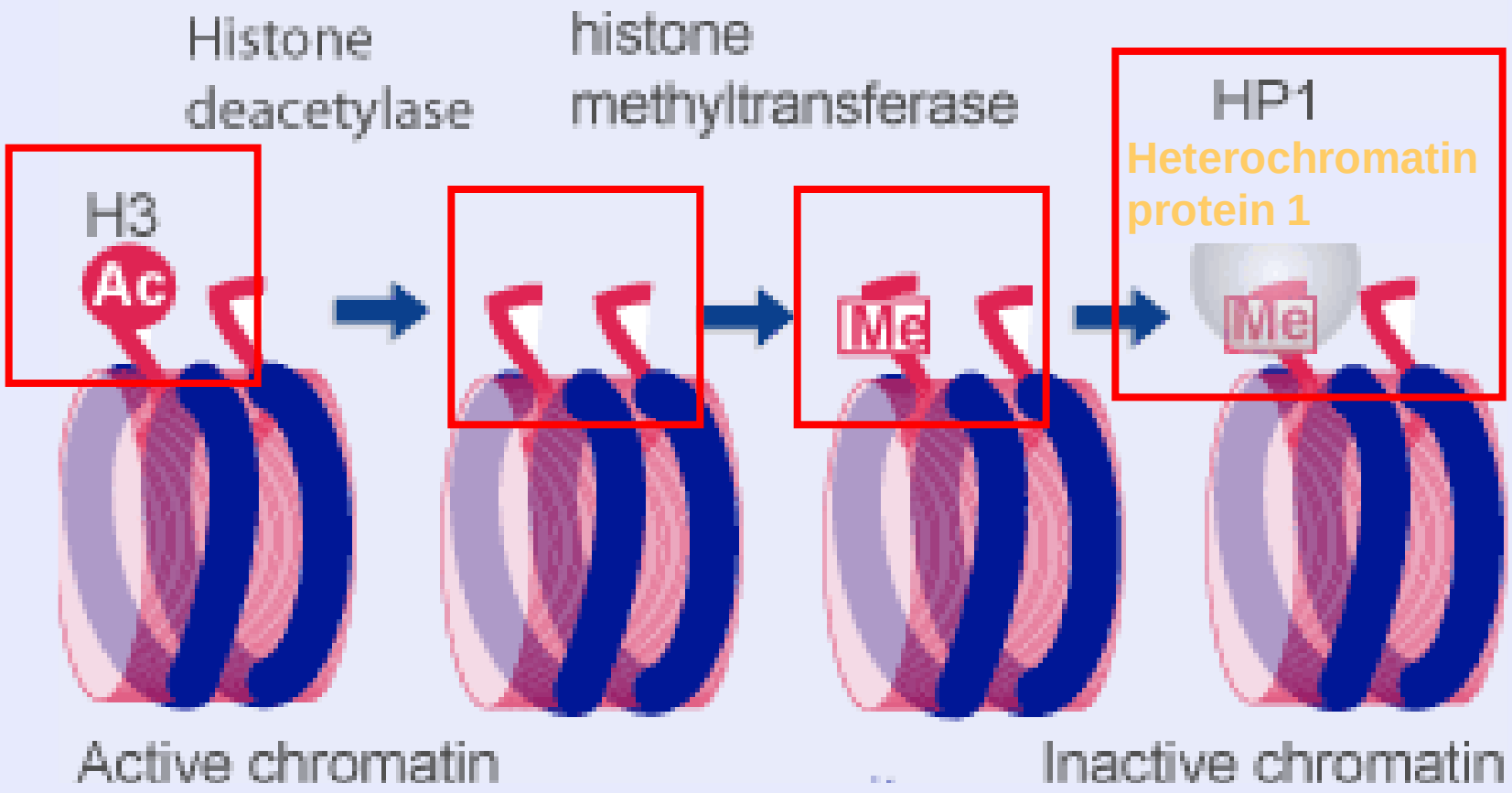
(来源：分子生物学（2007），郑用琏，第283页)

Repressed:



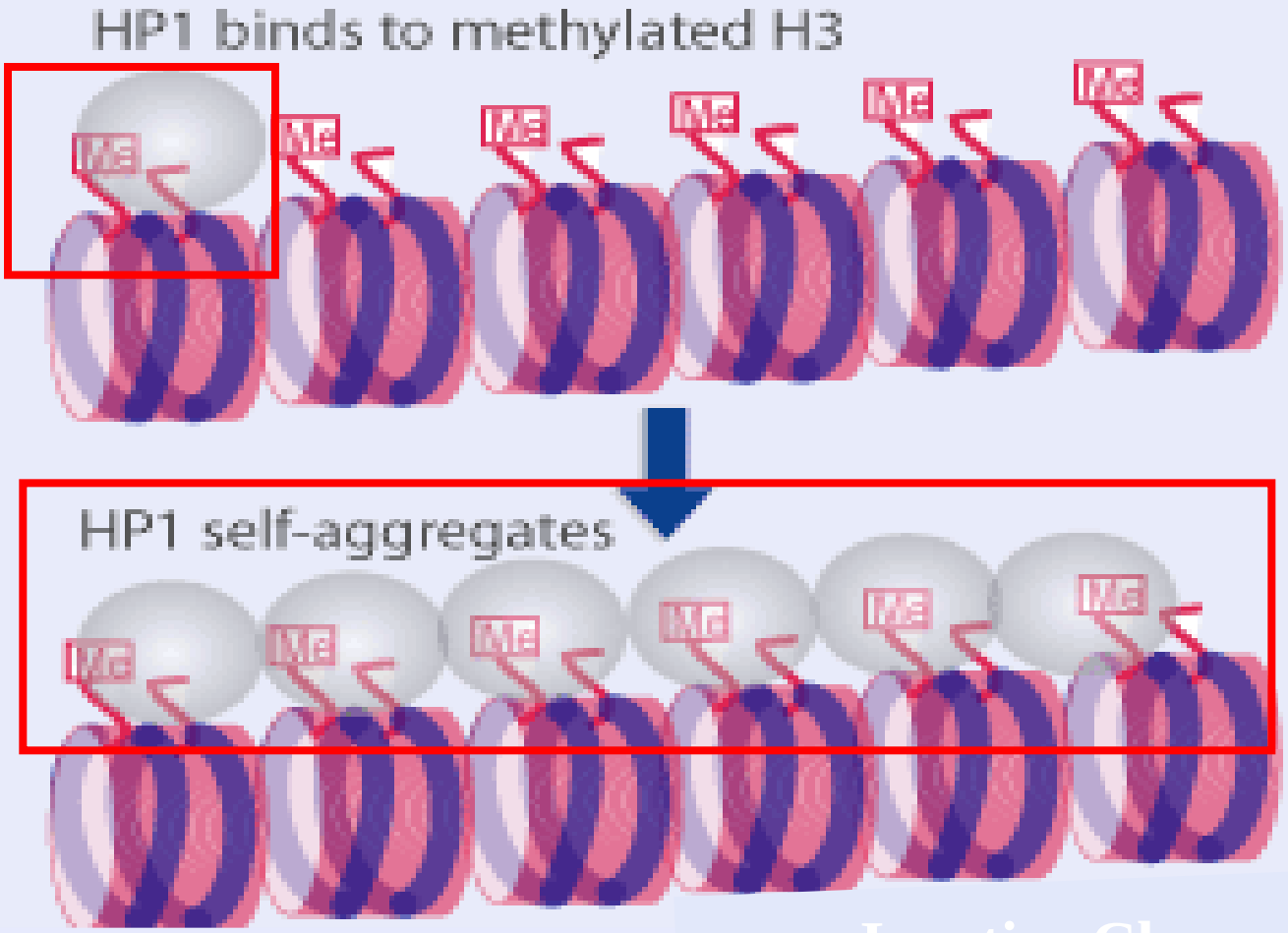
Nucleosome reconstructed

Histone methylation causes inactive chromatin



(来源：不详)

HP1 may propagate heterochromatin



(来源：不详)

Inactive Chromatin

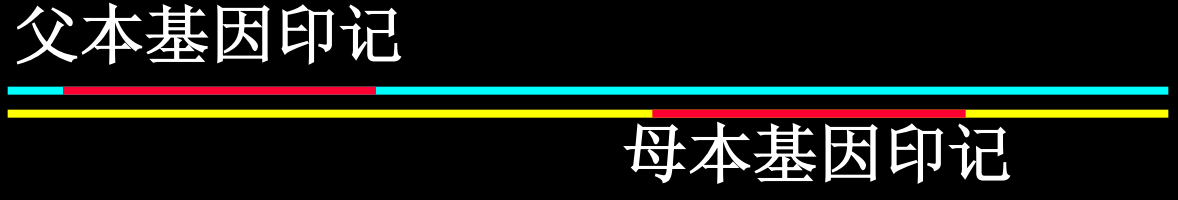
Genome / gene imprint

- **Genome imprinting affects a small subset of genes and results in the expression of those genes from only one of the two parental chromosome**
- **This is caused mainly by differential methylation of the two parental alleles of the imprinting gene and trans-action of small RNA**

*Genome / gene imprint*的特点

- 合子及幼胚期，DNA去甲基化程度高，gene imprint被抑制，表现了细胞的“全能性”

- Genome imprint具有染色体区域性或整体性
- 染色体区域性印记中相邻印记基因具有“相反”的印记作用模式



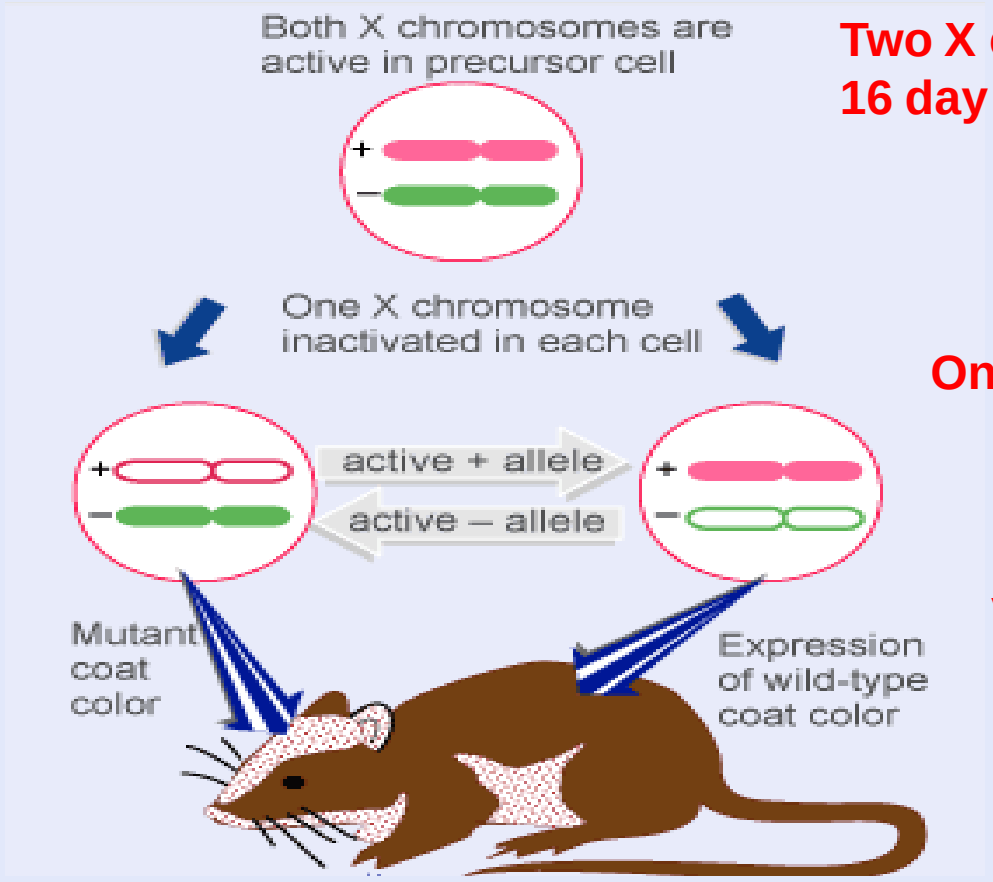
Genome / gene imprint表现的特点

- 印记现象具有组织细胞的特异性

- 基因印记的发生常与该基因的编码区，启动子区及其上下游的CpG序列甲基化相关

- 印记效应会因去甲基化而消失
  (水稻白叶枯病成株抗性？)

One X chromosome is inactived at random



Two X chro. Euchromatin in 16 day precursor cell

One X chro. inactived at random

Variegation

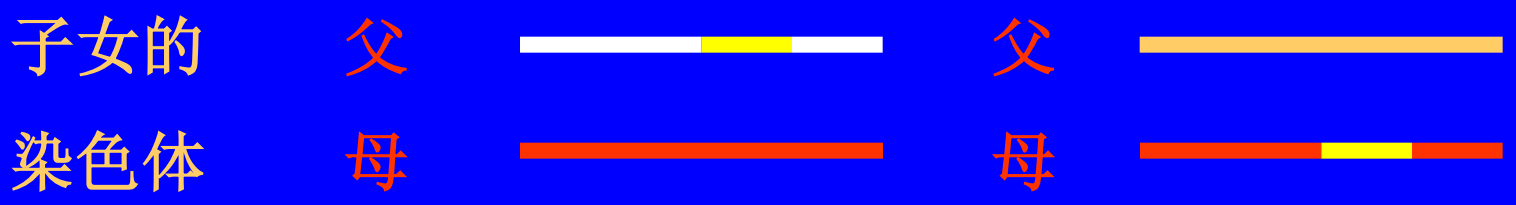
- 被异染色质化的X染色体—常染色质化

  导致雌性哺乳动物宫颈癌，胃癌

- **Prader-willi**症**(PWS)**：肌肉无力、矮小、神经疾病

  Argelmen症（**Ag**）：平衡性差、过分兴奋与发笑

共同的病因：**15q11-q13** 缺失，基因型相同

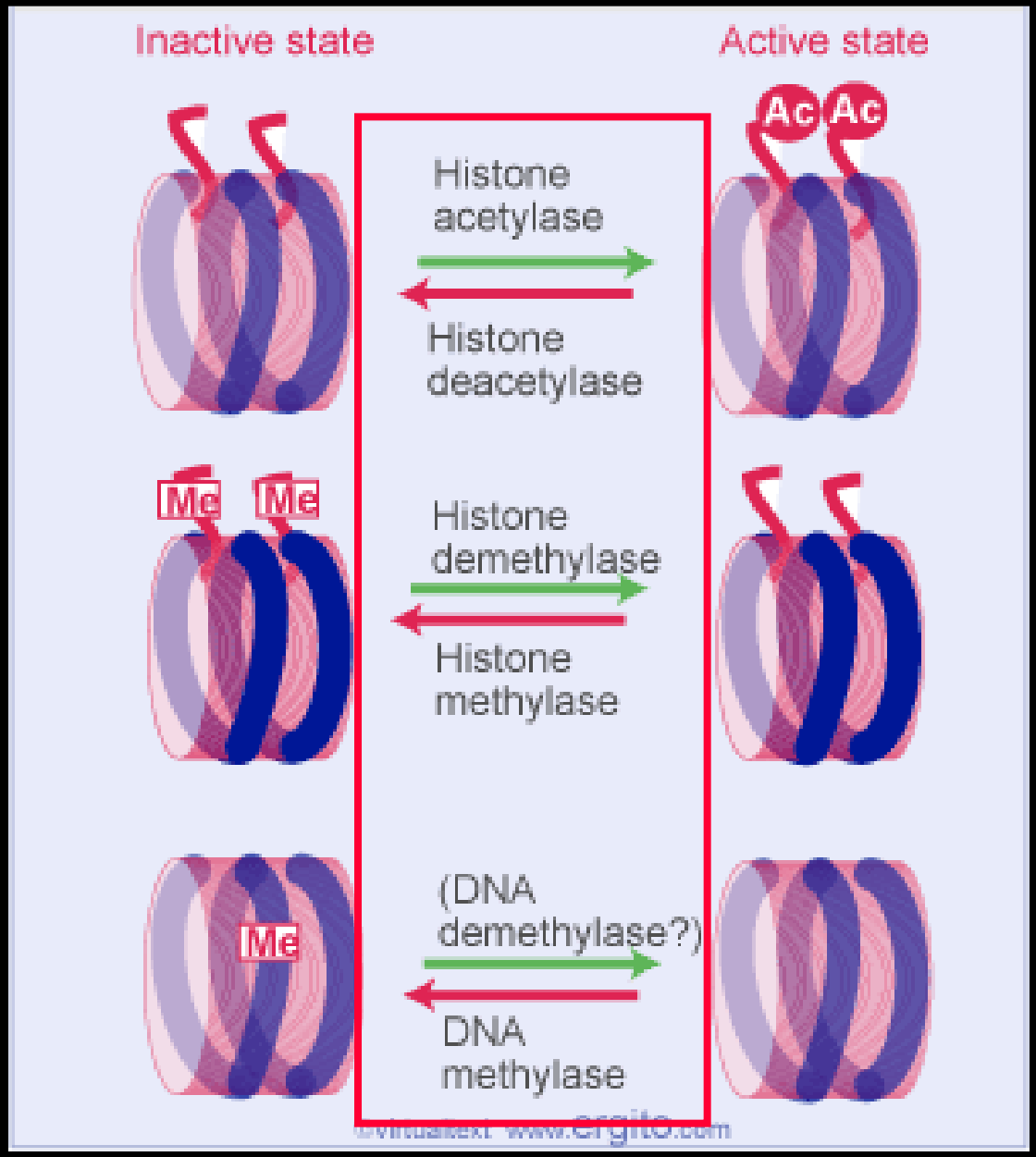


Paternal Dominance of Trans- eQTL Influences Gene Expression Patterns in Maize Hybrids

Ruth A. Swanson-Wagner et al. Science. 1118 (2009);326

We identified ~4000 expression quantitative trait loci (eQTL) that allowed us to identify markers linked to variation in expression. We found that over three-quarters of these eQTL act in trans (78%) and that 86% of these differentially regulate transcript accumulation in a manner consistent with gene expression in the hybrid being regulated exclusively by the paternally transmitted allele.

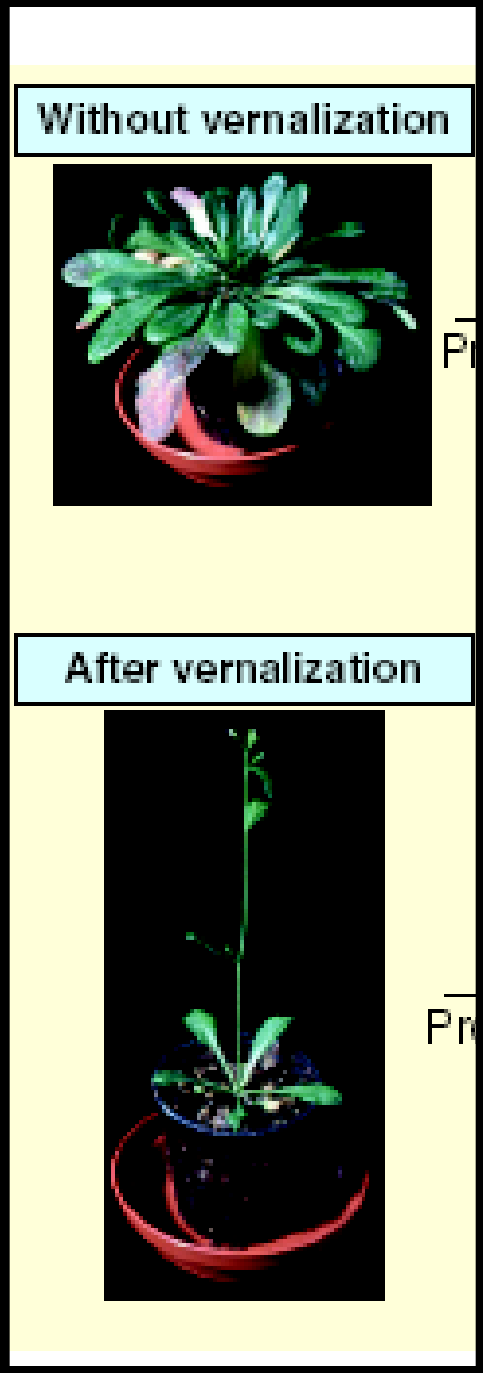
This result suggests that widespread imprinting contributes to the regulation of gene expression in maize hybrids . We hypothesize that at least some paternally dominant trans-eQTL are small RNAs, because small RNAs regulate gene expression in trans.



3 types of modification affect chromatin

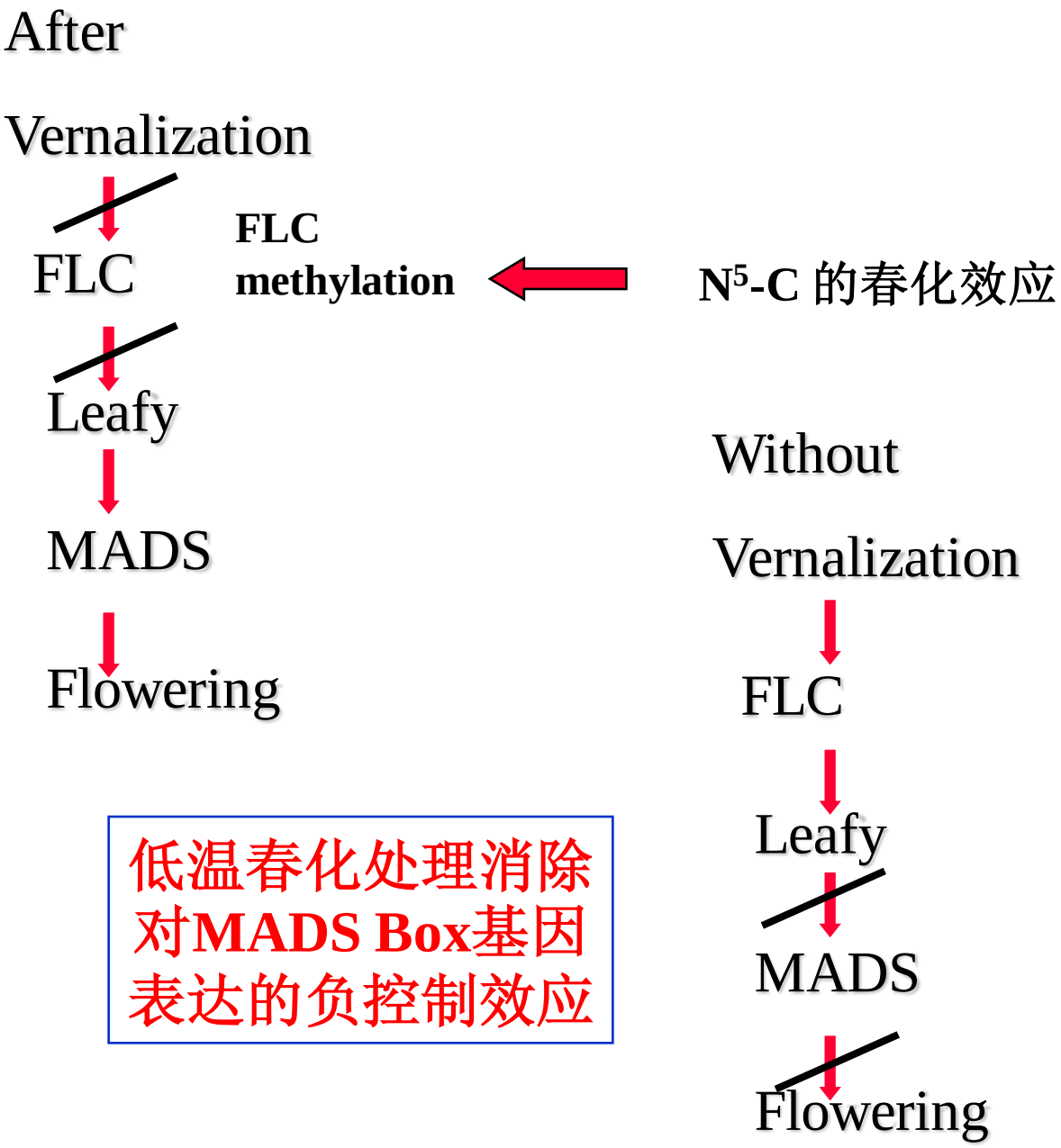
甲基化的CpG序列可招募组蛋白去乙酰化酶，去除组蛋白上的乙酰基团，抑制基因的表达。

（来源：分子生物学（2007），郑用琏，第283页）

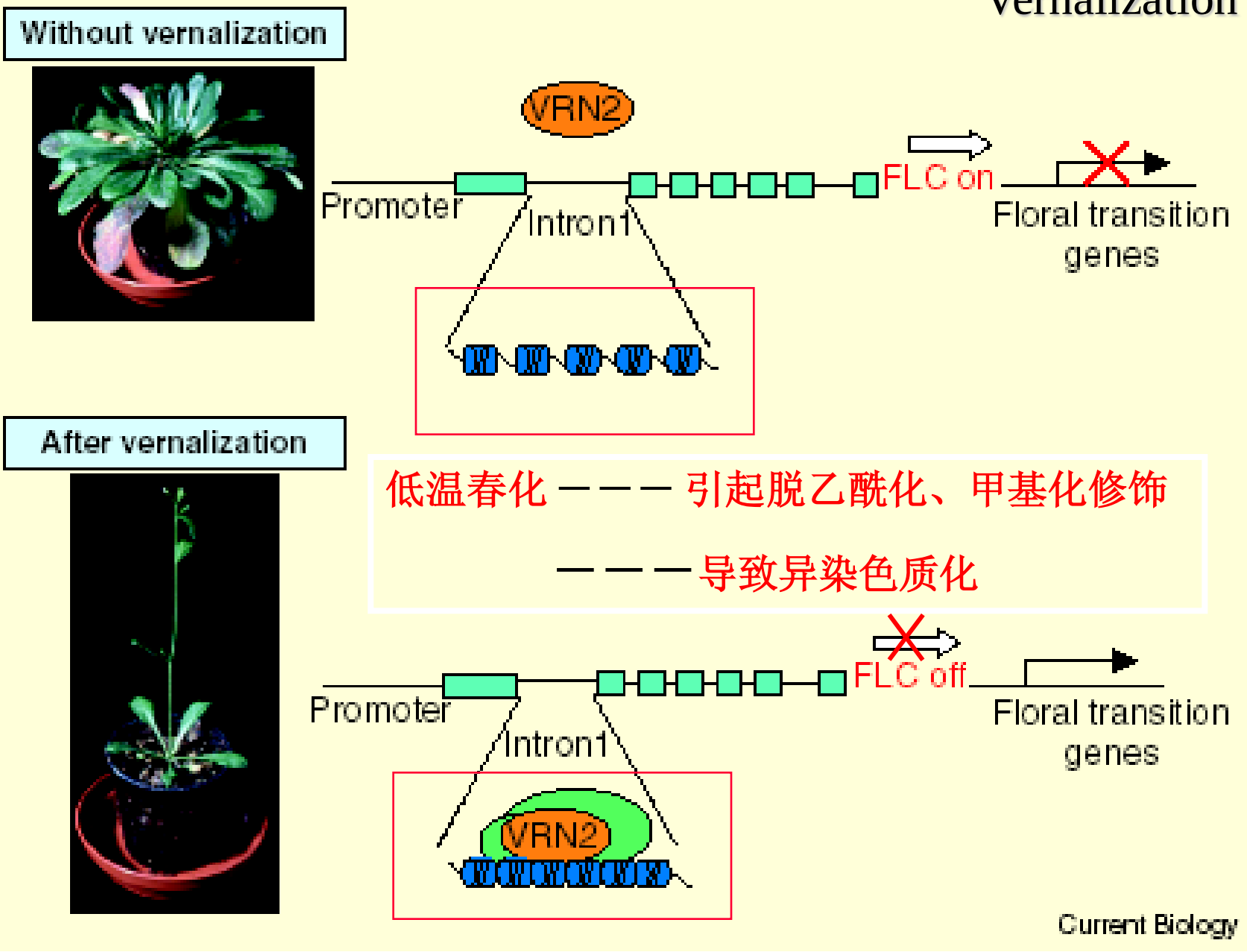


植物低温春化现象的分子机理

（来源：分子生物学（2007），郑用琏，第289页）

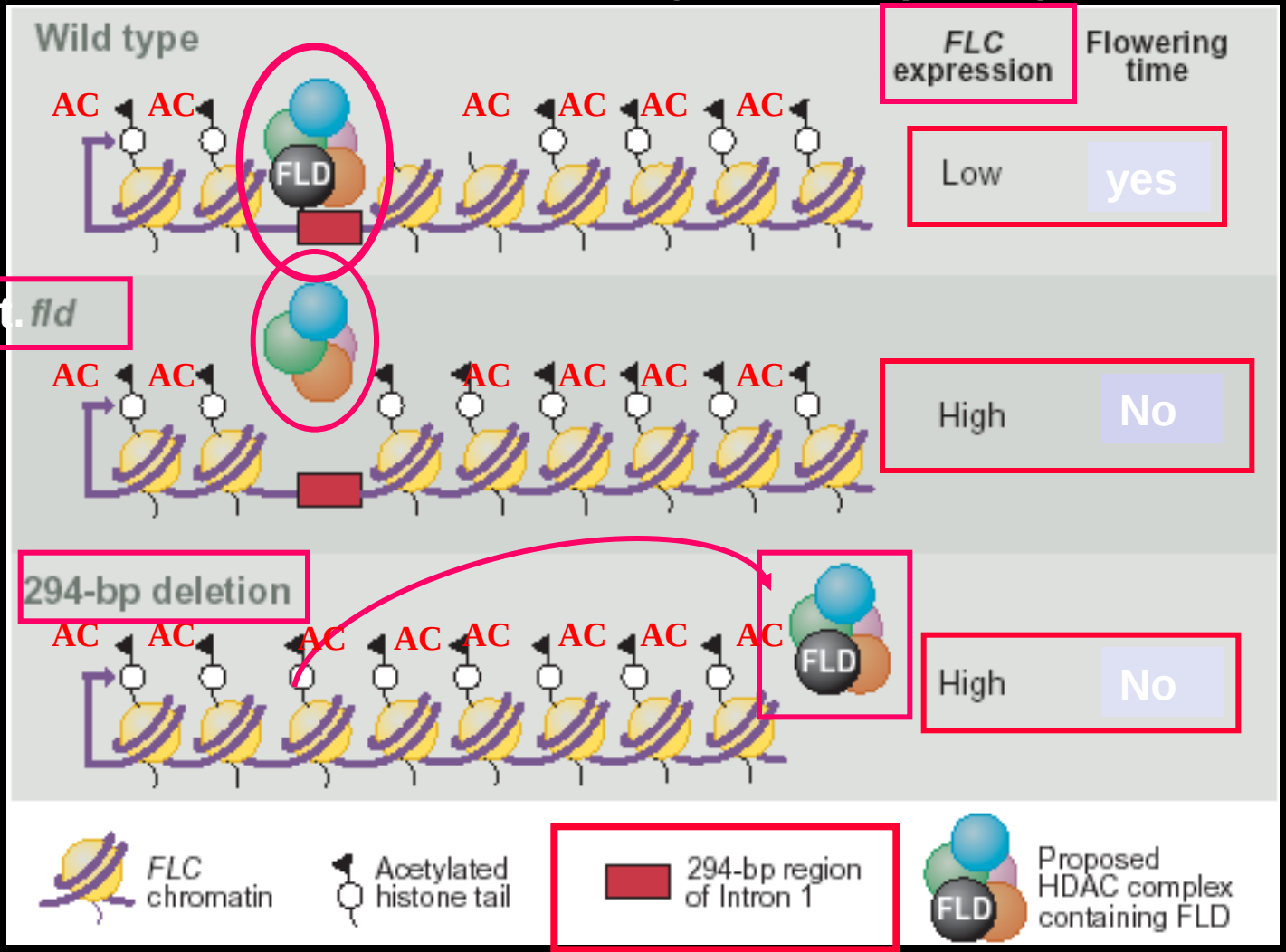

After
Vernalization
FLC
FLC
methylation
N5-C 的春化效应
Leafy
MADS
Flowering
Without
Vernalization
FLC
Leafy
MADS
Flowering
低温春化处理消除
对MADS Box基因
表达的负控制效应

Vernalization



（来源：分子生物学（2007），郑用琏，第289页）

Regulation of the floral repressor FLC by histone deacetylation (FLD)



Chromatin modification & remodeling as the epigenetic basis

- Chromatin structure and remodeling
- DNA methylation
- Histone modification (Ac & Me)
- Relationship between DNA ^{Me} and Histone^{Me}
- Creation of repressive domain
- Heterochromatin formation
- ncRNA

No-coding RNA ncRNA

看家RNA

rRNA，tRNA，UsnRNA，telomere RNA...

调节RNA

microRNA（miRNA），siRNA，

anti-sense RNA...

What is microRNA?

- MicroRNAs are a newly identified class of small single-stranded non-coding RNAs;
- MicroRNAs regulate their target genes via two main mechanisms:

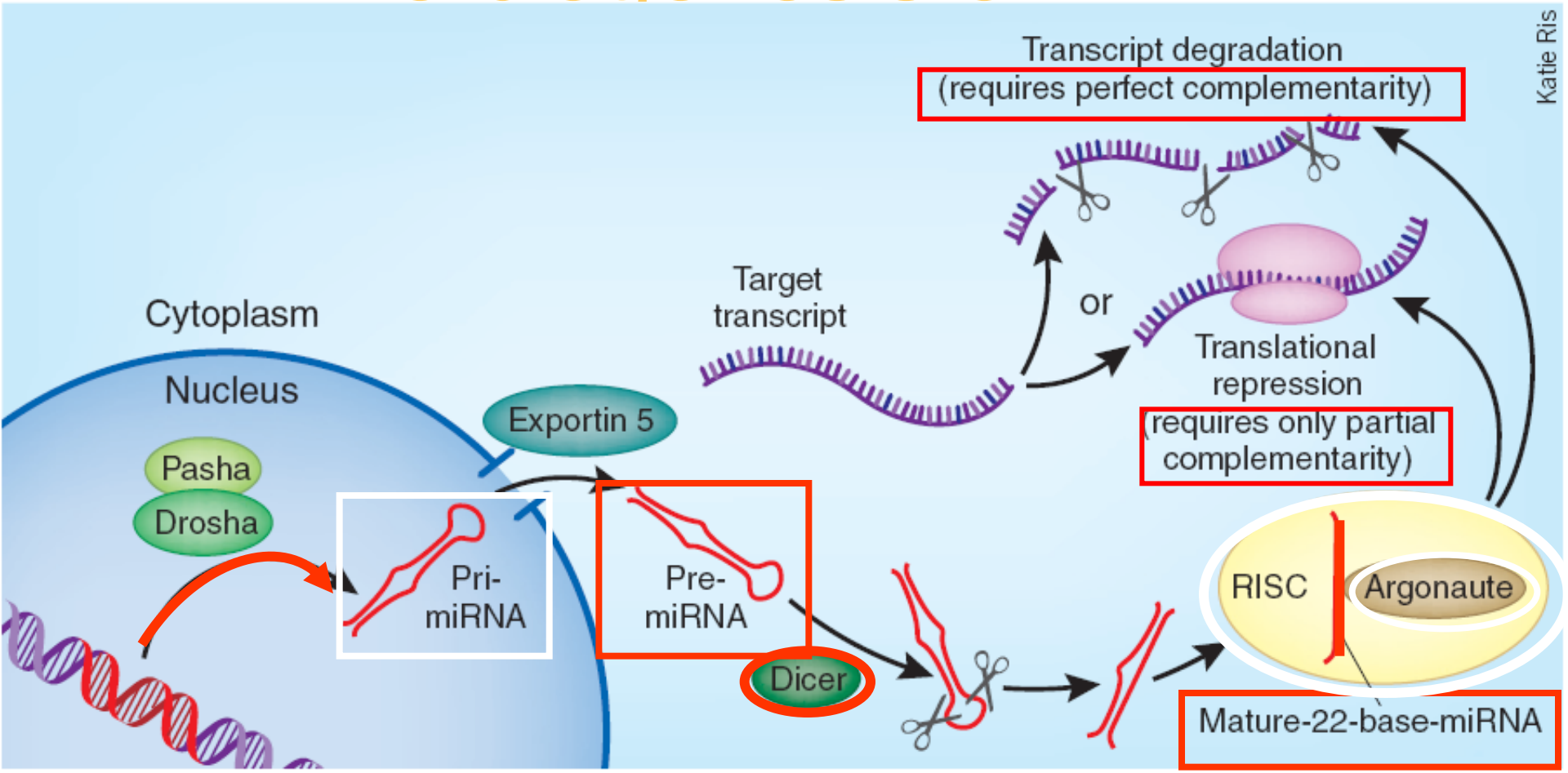
  target mRNA cleavage

  translational repression.

The characters of miRNA

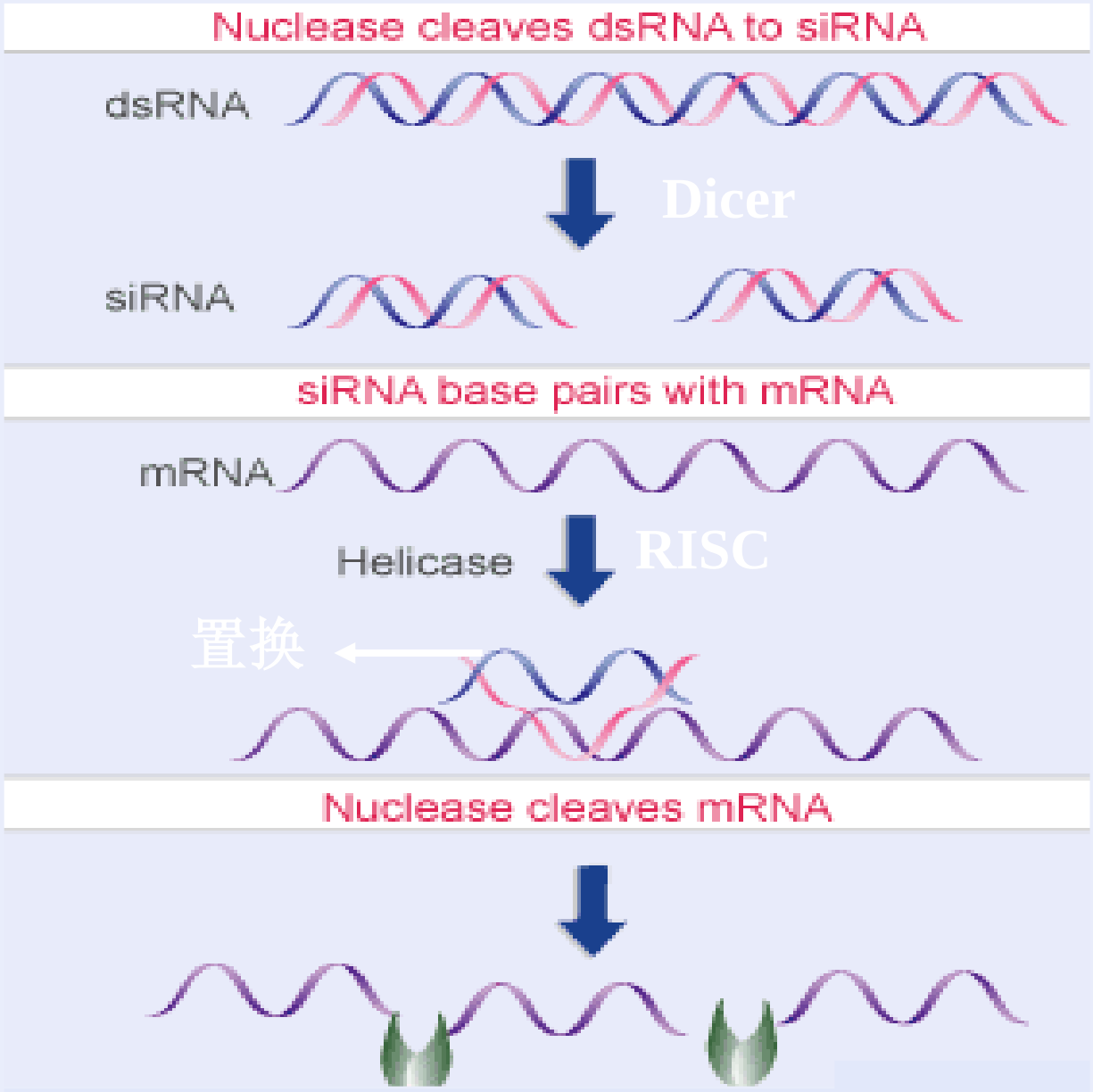
- Length: 18-25nt;
- Single-strand；
- Conserved among species；
- Encoded by internal DNA sequences， which are located in the whole genome, mostly in the interval regions of coding genes,centromeres,or,occasionally in the introns;
- Temporally and tissue-specifically expressed

The biogenesis of miRNA



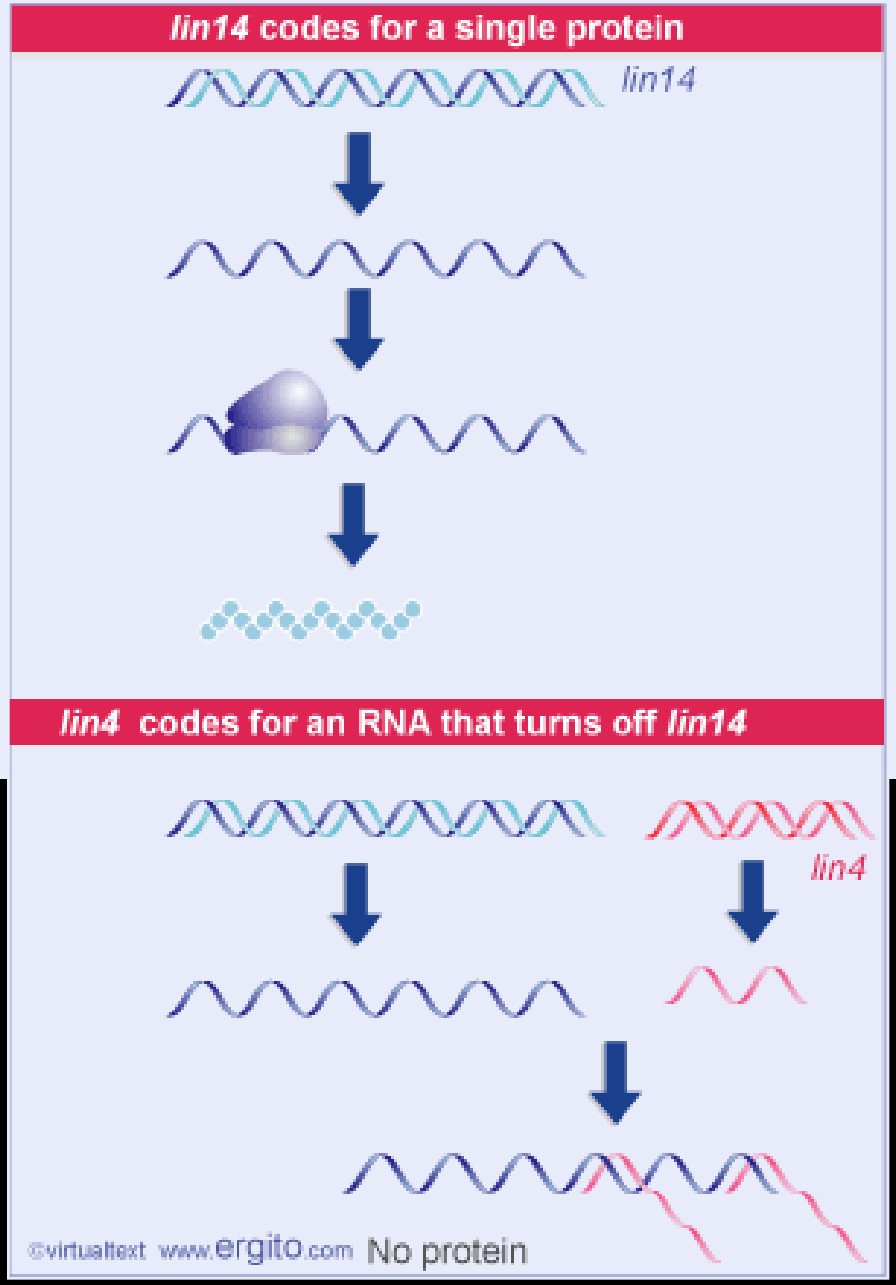
(Source:Review: Geogre S Mack, Nature Biotech., 2007)

siRNA	miRNA
来源于mRNA，病毒RNA，转座子，外源RNA	来源于基因组内的microDNA、剪切的intron...（内源性）
dsRNA由Dicer加工成21Nt±siRNA，在RDRP的作用下可以扩增，siRNA与靶mRNA来源与同一基因	60－100nt，3‘具有2Nt突起茎环结构的pri－pre--miRNA，由Dicer加工成一条双链的21±miRNA，与靶mRNA来自不同的基因
进入RISC体系降解靶mRNA，转录后水平上沉默基因表达，	与mRNA结合，或剪切mRNA，抑制翻译，沉默基因表达(特别是转录因子)。进入RISC体系，在转录后水平上沉默基因表达
与靶基因完全配对，专一性强	与靶基因可不完全配对，因此可调控若干不同的基因
不同内源siRNA具有较大的差异	在近缘物种间具有较高的保守性



RNAi works by generating siRNA

(Source:Molecular Biology(2002),Robert F.Weaver,Page504）

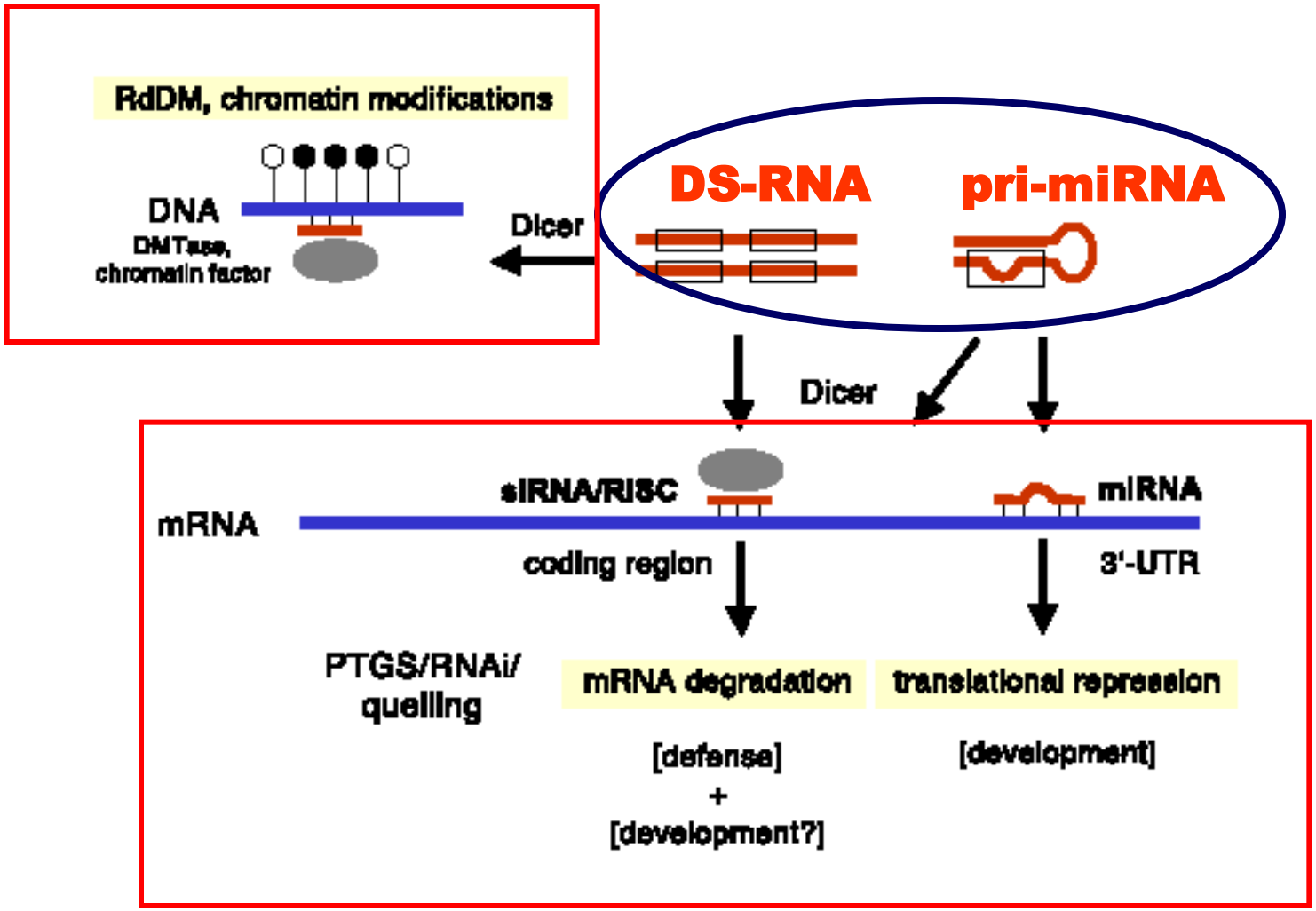


Lin14蛋白调节秀丽新线虫的幼虫发育，但受lin4基因的控制

Lin4 编码22Nt的microRNA, 与Lin14mRNA3‘端非翻译区出现7次重复的10Nt序列互补，抑制Lin14蛋白的翻译

（Source:Molecular Biology(2002),Robert F.Weaver,Page504）

common mechanism for microRNA, RNAi and heterochromatin formation



6.5. Programmed Cell Death (PCD) and development

2002 Nobel Prize

John Sulston ***USA***

Sydney Brenner ***Japan***

田中 耕一

H. Robert Horvitz ***Switzerland***

细胞的“生” → 发育、分裂、繁殖、分化、成熟

细胞的“死” → ….?

--- 高等生物的诞生—发育—成长—死亡

即寿命是生物固有的特征

--- 死亡是生物体不可缺少的生命内容

是生物借以存活的需要

并贯穿寿命的全部周期中

PCD gene 的发现与克隆

人类长寿

癌症发生

提高产量……

--- 细胞死亡的发生及过程是在长期进化中已形成的遗传程序（**PCD**）

这一程序表达的失控

个体发育受阻、病变、畸形、蹼趾、有尾.....

---pcd⁻ → 白细胞不分化，不死亡，不代换

癌症（白血病）

---皮肤, 指(趾)甲(细胞从有生命经PCD向无生命的转化)

pcd⁻ → 无皮，无鳞，无爪 → 失去保护与防御功能

---细胞周期变短, 增殖过快, pcd⁻失控, 细胞死亡速率变低

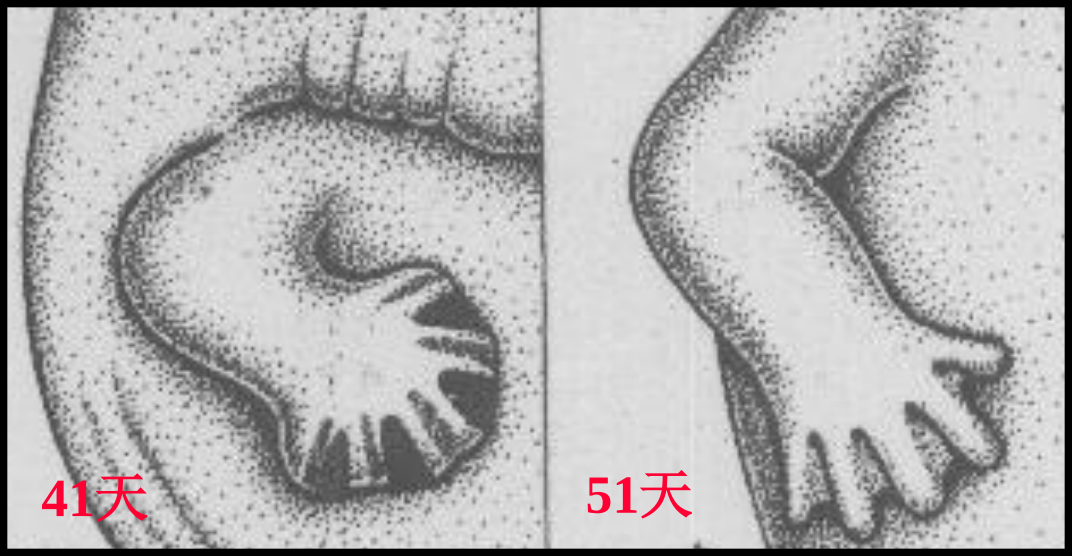
细胞增殖与细胞死亡平衡失调 → 癌症的发生

a) PCD的概念

细胞的死亡；

- necrosis (accidental cell death) 坏死

外因 ➡ 细胞急速死亡，病变死亡



人胚手掌分离过程的PCD (来源：分子生物学（2007），郑用琏，第301页)

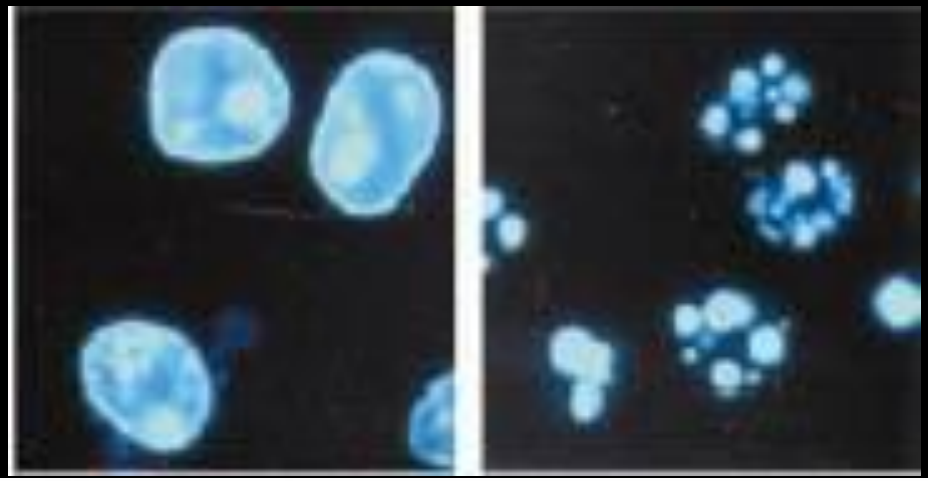
- Programmed Cell Death (PCD)

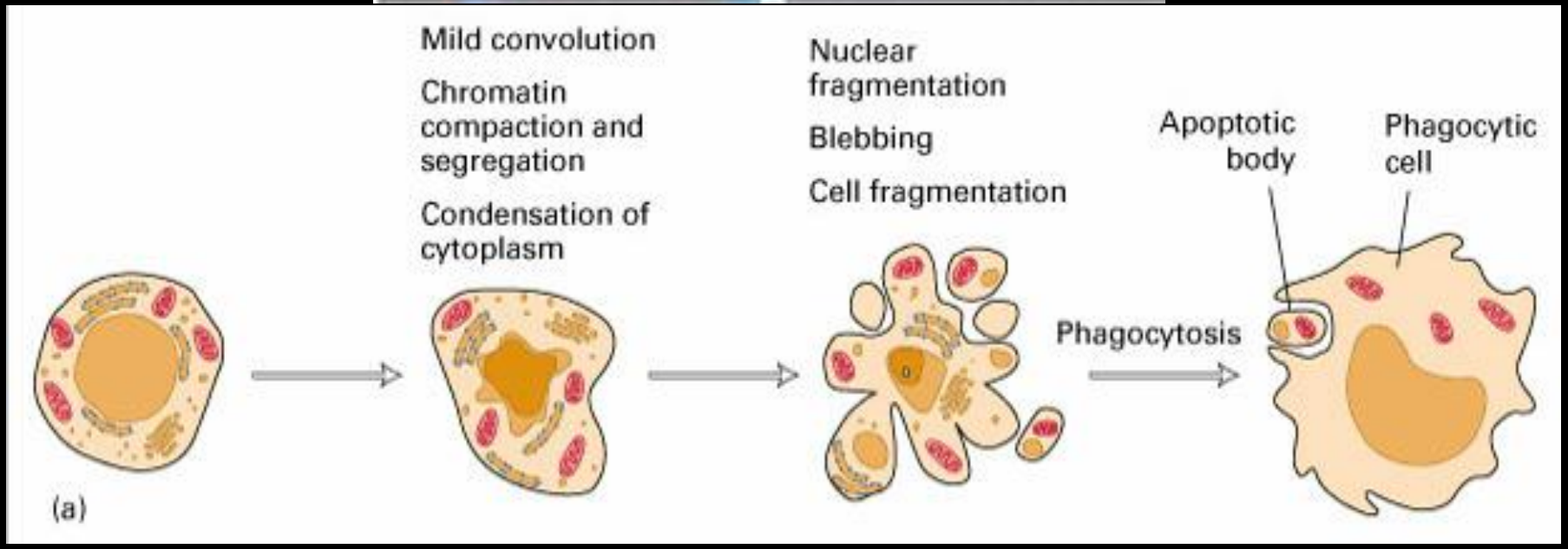
细胞程序性死亡

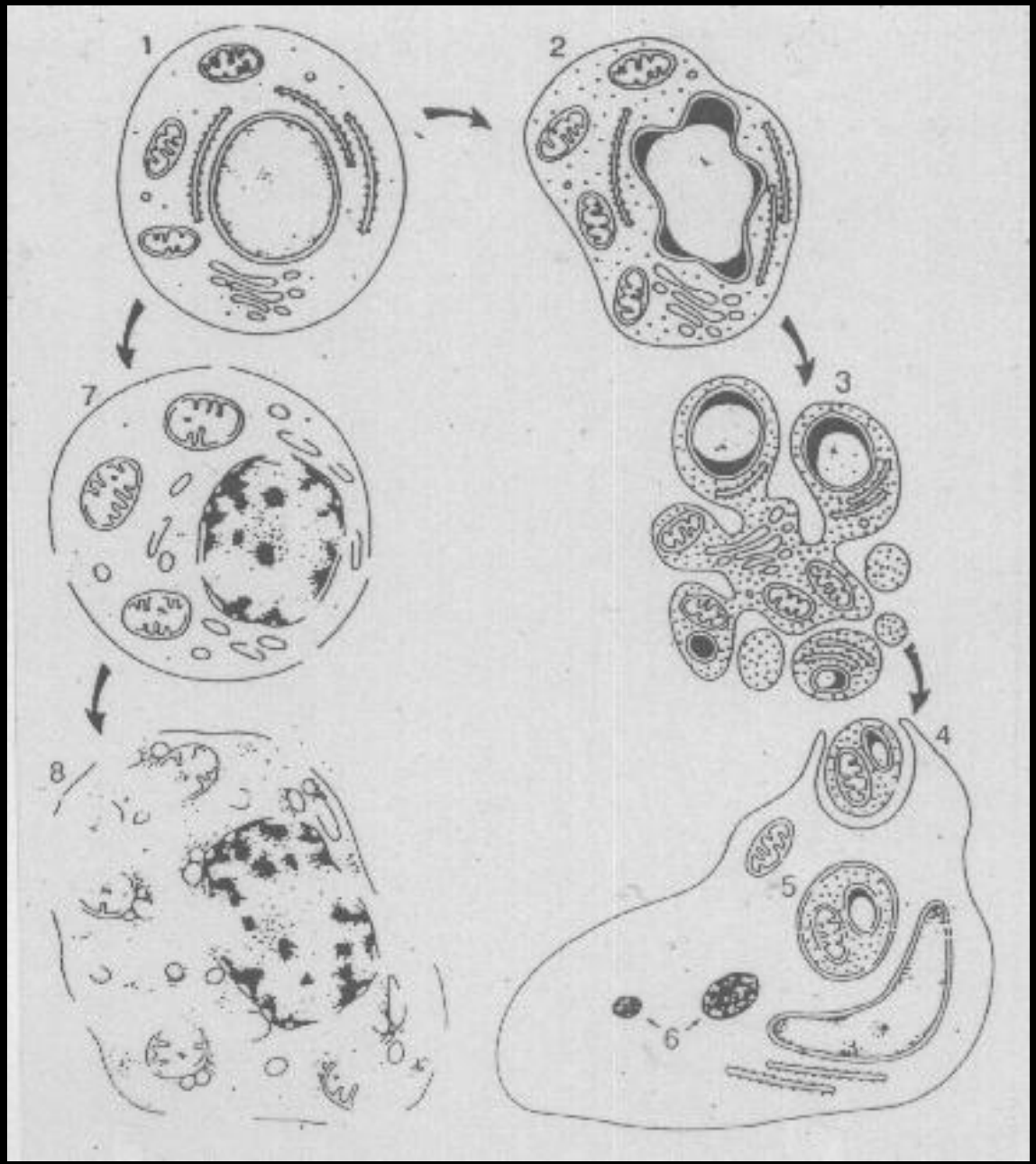
或 Apoptosis 凋亡

生理条件下，在细胞自身遗传程序的控制下，有关基因的正常表达，细胞裂解成apoptotic bodies, 逐渐死亡的现象

Apoptosis




Mild convolution
Chromatin compaction and segregation
Condensation of cytoplasm
Nuclear fragmentation
Blebbing
Cell fragmentation
Apoptotic body
Phagocytic cell
Phagocytosis
(a)



PCD的细胞学特征

细胞变圆变小

与邻近细胞脱离

胞浆浓缩

染色质凝集成月牙状

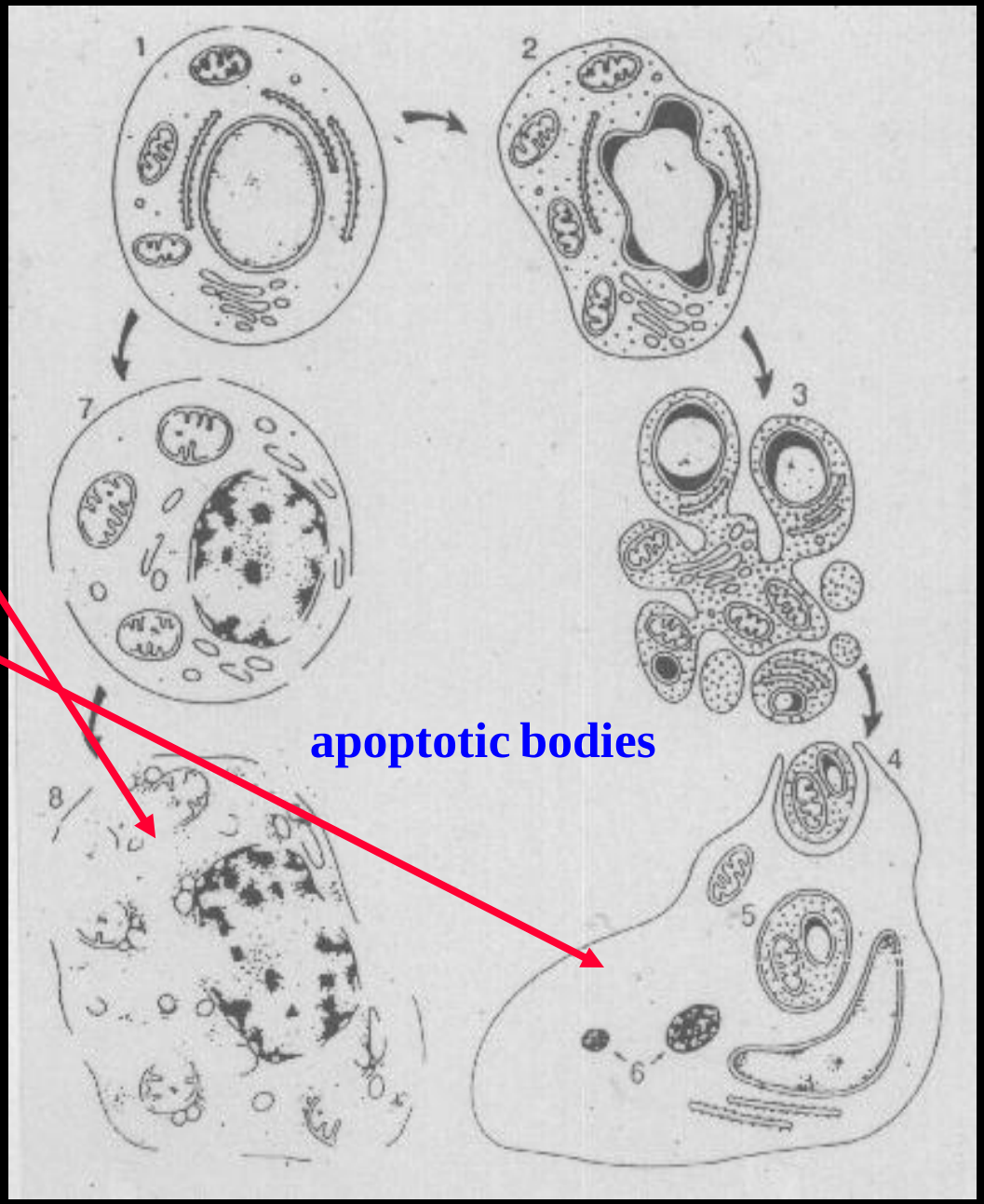
内质网扩张成泡状

并与膜融合

(来源：分子生物学（2007），郑用琏，第302页)

膜内陷，细胞呈沸腾运动，
细胞自行解体成若干有膜包围，
内容物不外溢的凋亡小体
被巨噬细胞吞噬

核内DNA被核酸酶
在核小体单位间降解
电泳检测呈
ladder of 180-200bp

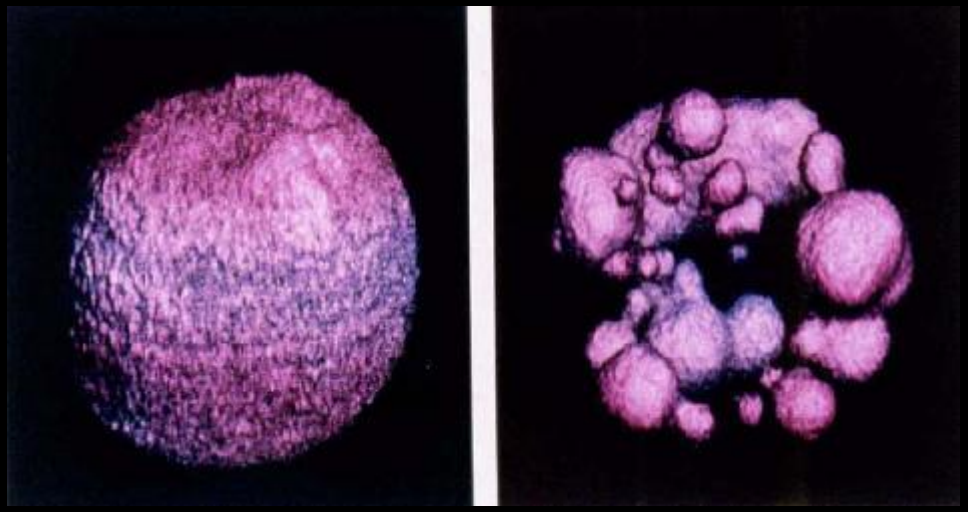


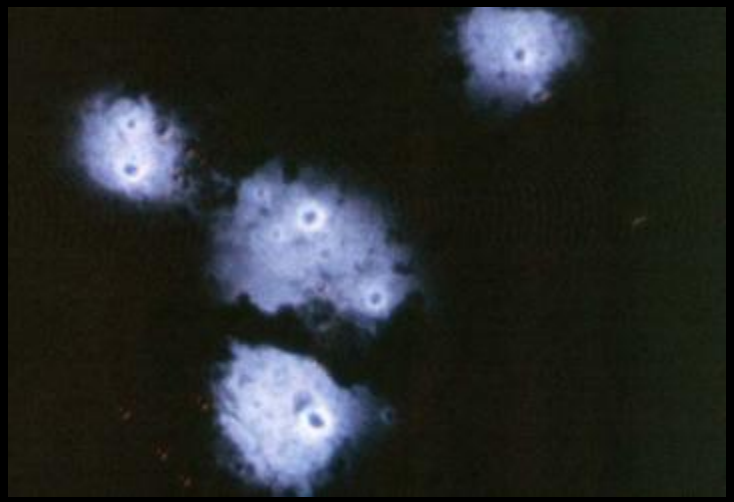
(来源：分子生物学（2007），郑用琏，第302页)

Confocal 3d images of nuclei from apoptotic and necrotic cells

Apoptosis:
"active" cell death;
"cell suicide"

Necrosis:
"accidental" cell death;
"cell murder"





(来源：分子生物学（2007），郑用琏，第301页)

b) 与PCD相关的基因

- 促进PCD的基因

  Ces (cell death specification)细胞凋亡特异基因

  p53抑癌基因

  ICE(interleukin-1β converting enzyme)
  白细胞介素-1β转化酶
  Caspase（半胱氨酸酶家族，蛋白降解途径）….

- 抑制PCD的基因

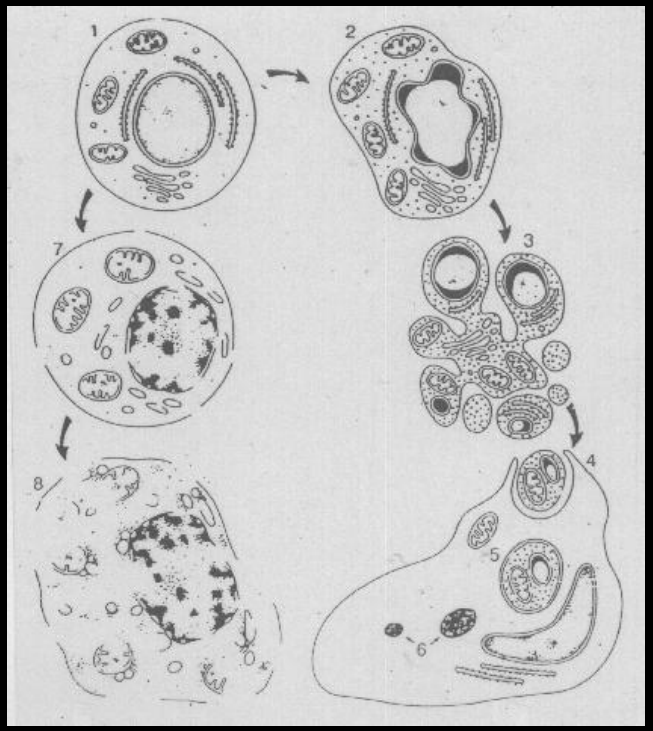
  LAP (inhibitor of apoptosis) 凋亡抑制剂基因

  crm-1(cytokine response modifier A)
  细胞因子应答调节物A基因
  cystatin….

植物的PCD表现与机制

具有与动物细胞相似的

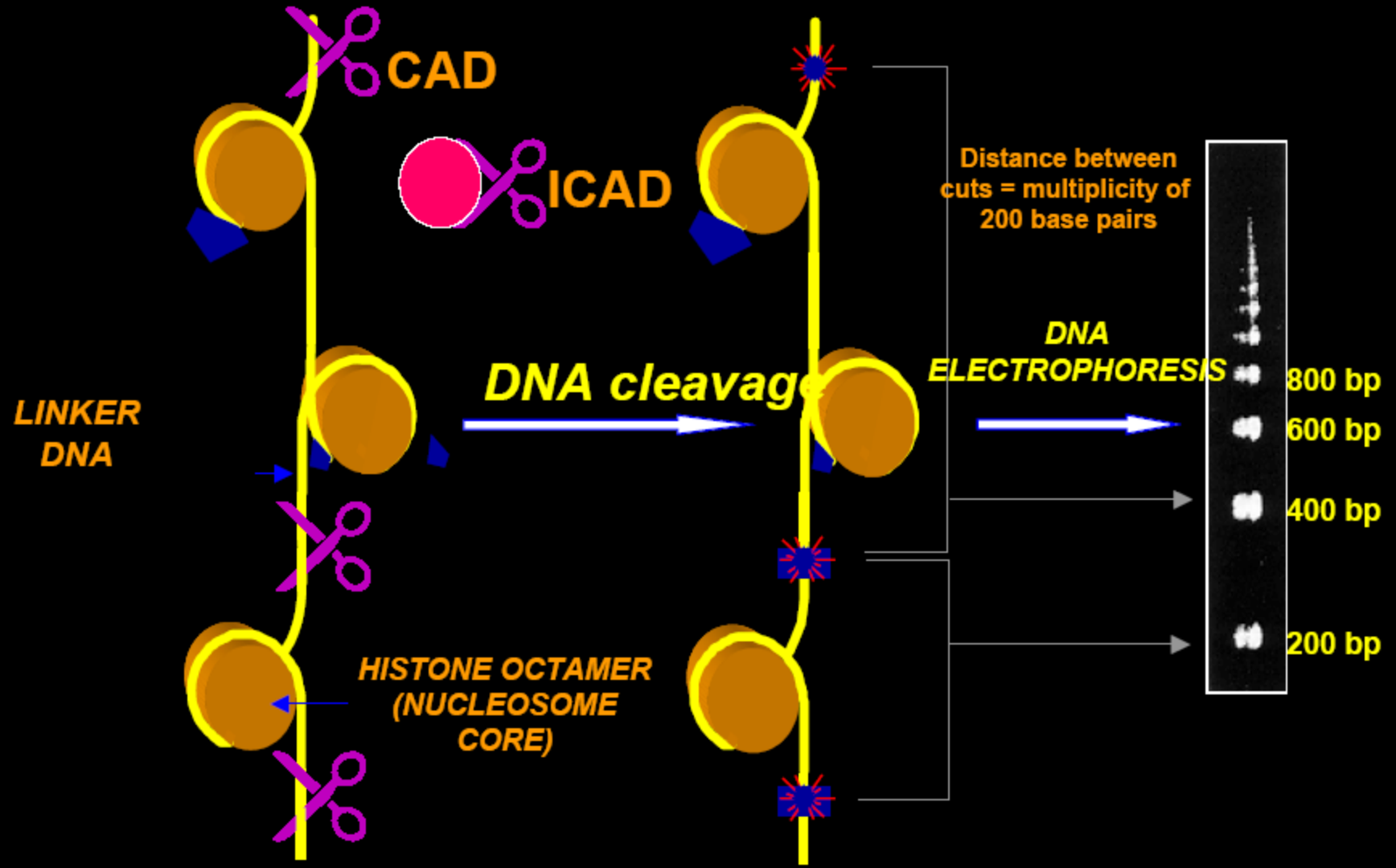
细胞学表型与生化特征



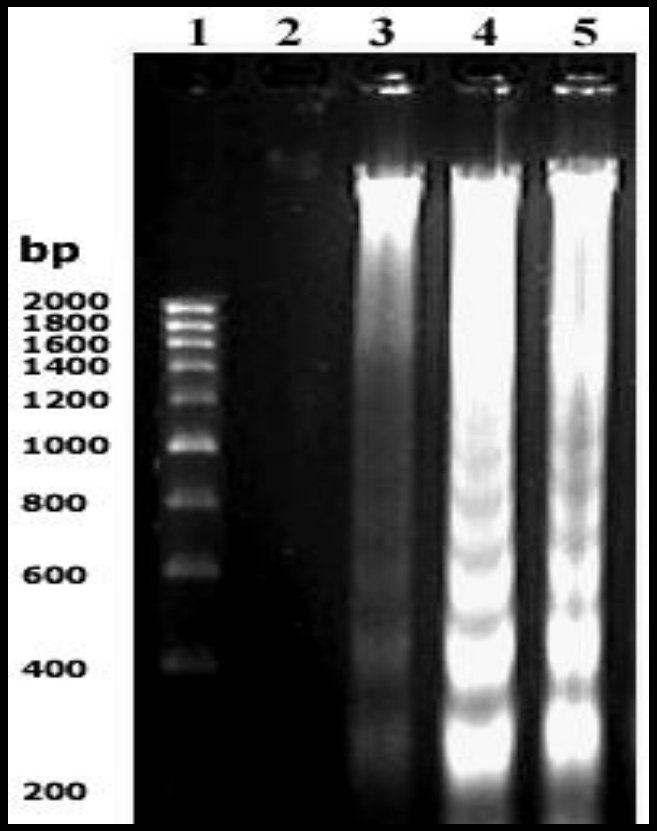
- 花药的发育（CMS？）
- 木质部的分化
- 绒毡层的退化
- 禾本科植物胚乳的发育
- 缺氧条件下诱导的玉米根部通气组织的形成与根冠的形成
- 植物抗病的过敏性反应 → PCD → 枯斑的形成

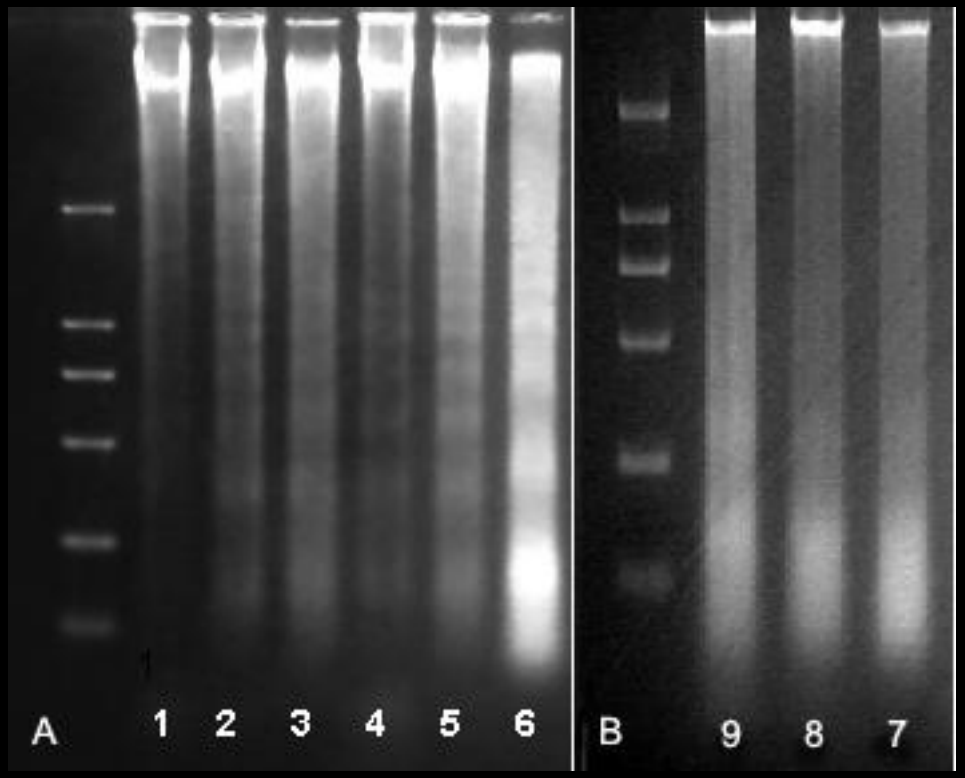
Hypersensitive Response

(来源：分子生物学（2007），郑用琏，第302页)



(来源：分子生物学（2007），郑用琏，第302页)


1
2
3
4
5
bp
2000
1800
1600
1400
1200
1000
800
600
400
200


A
1
2
3
4
5
6
B
9
8
7

植物PCD的相关基因与机制

- 半胱氨酸酶家族（Caspase）

  衰老叶片，果实，花器发育中木质部的形成…Caspase ↑

- 乙烯在PCD中起重要作用

  缺氧条件下，乙烯诱导玉米根部胞内Ca^{++}增加

  → 气生组织的形成

  小麦胚乳形成中，乙烯调节DNA降解

- *acd1,acd2*（*accelerated cell death*）基因

  *Acd*负控制*Arabidopsis HR*诱导的 *PCD*过程

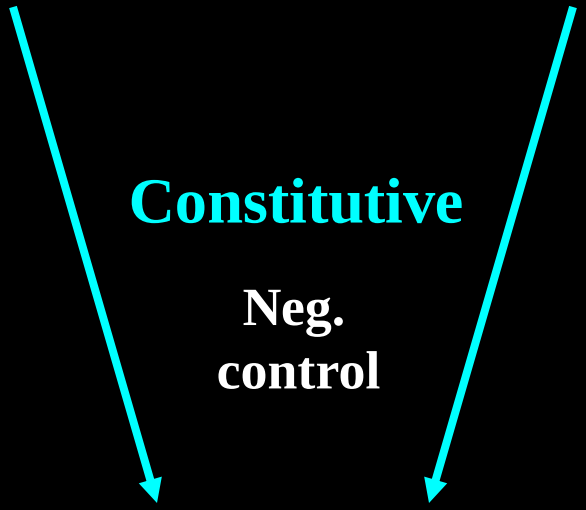
---植物抗病的PCD表现

Acd

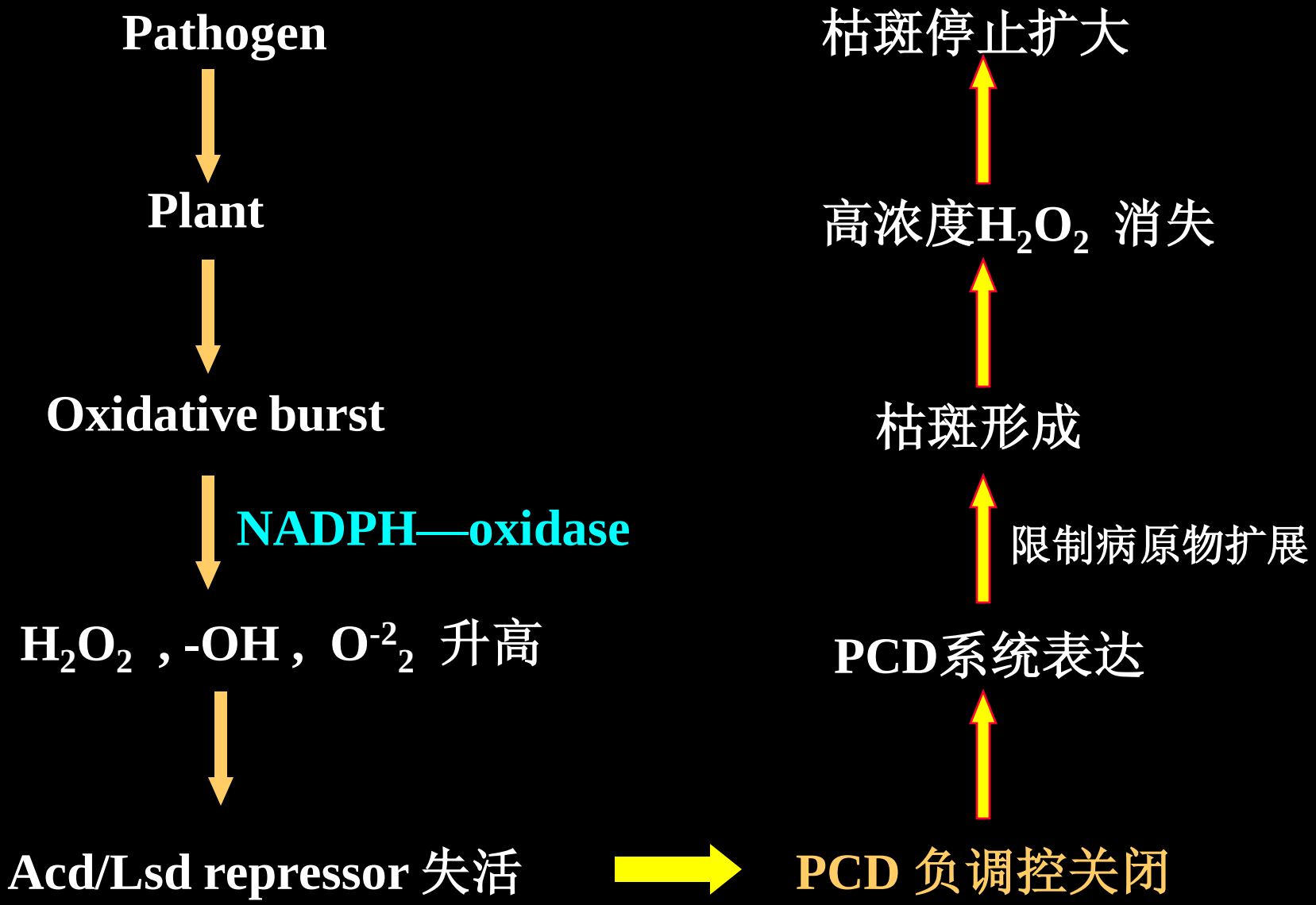
(Accelerates Cell Death)

Lsd

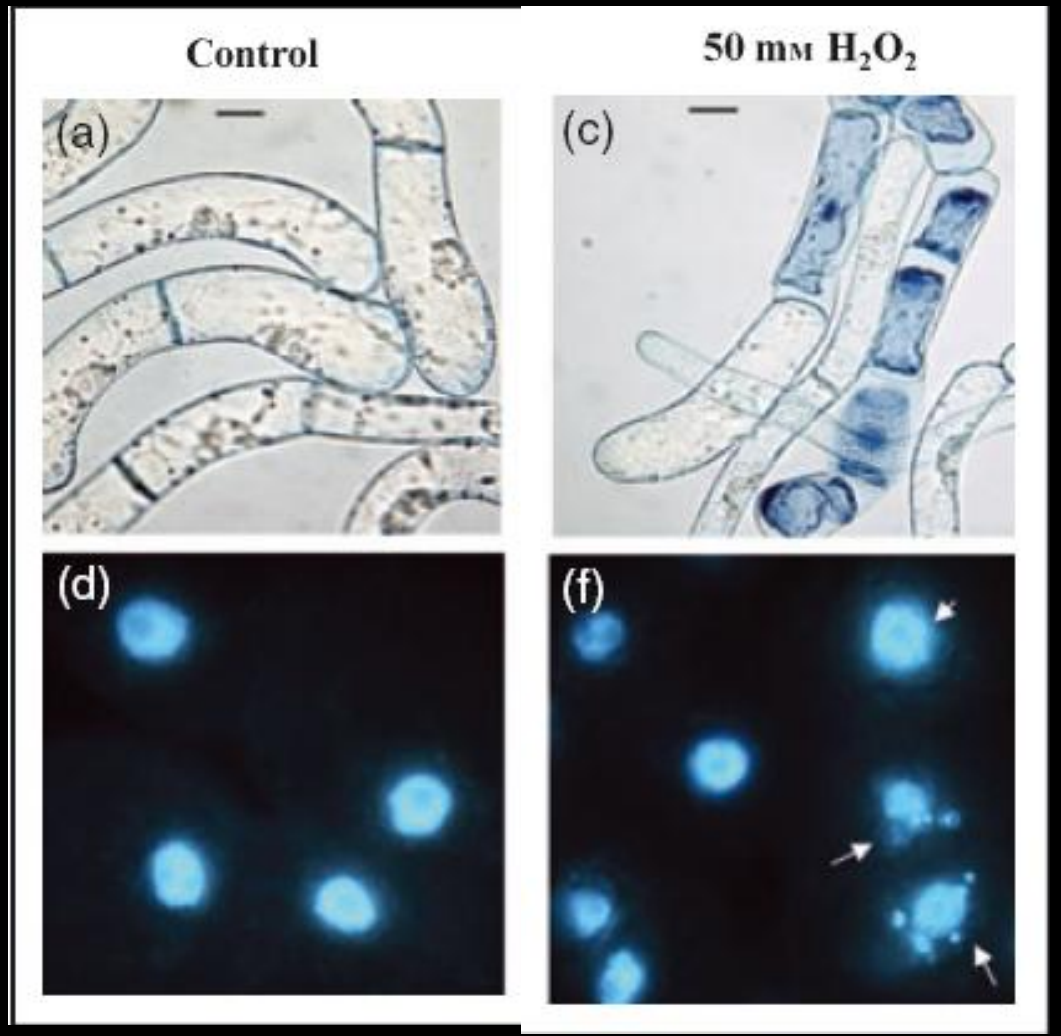
(Lesions Stimulating Disease Resistance)



Programmed Cell Death


Pathogen
Plant
Oxidative burst
NADPH—oxidase
H_2O_2 ，-OH，O^{-2}_2 升高
Acd/Lsd repressor 失活
PCD 负调控关闭
PCD系统表达
限制病原物扩展
枯斑形成
高浓度H_2O_2 消失
枯斑停止扩大

H_2O_2 induced cytoplasm shrinkage, a typical marker of plant PCD.



在发育进程中，PCD何时启动？如何决定某一细胞执行PCD?

机体内是否存在真正杀手基因（killer gene）？

Killer只在PCD细胞中表达！
其产物可使细胞走向死亡！

PCD的信号，传递？

PCD的研究方兴未艾

深入研究，揭示生命奥秘