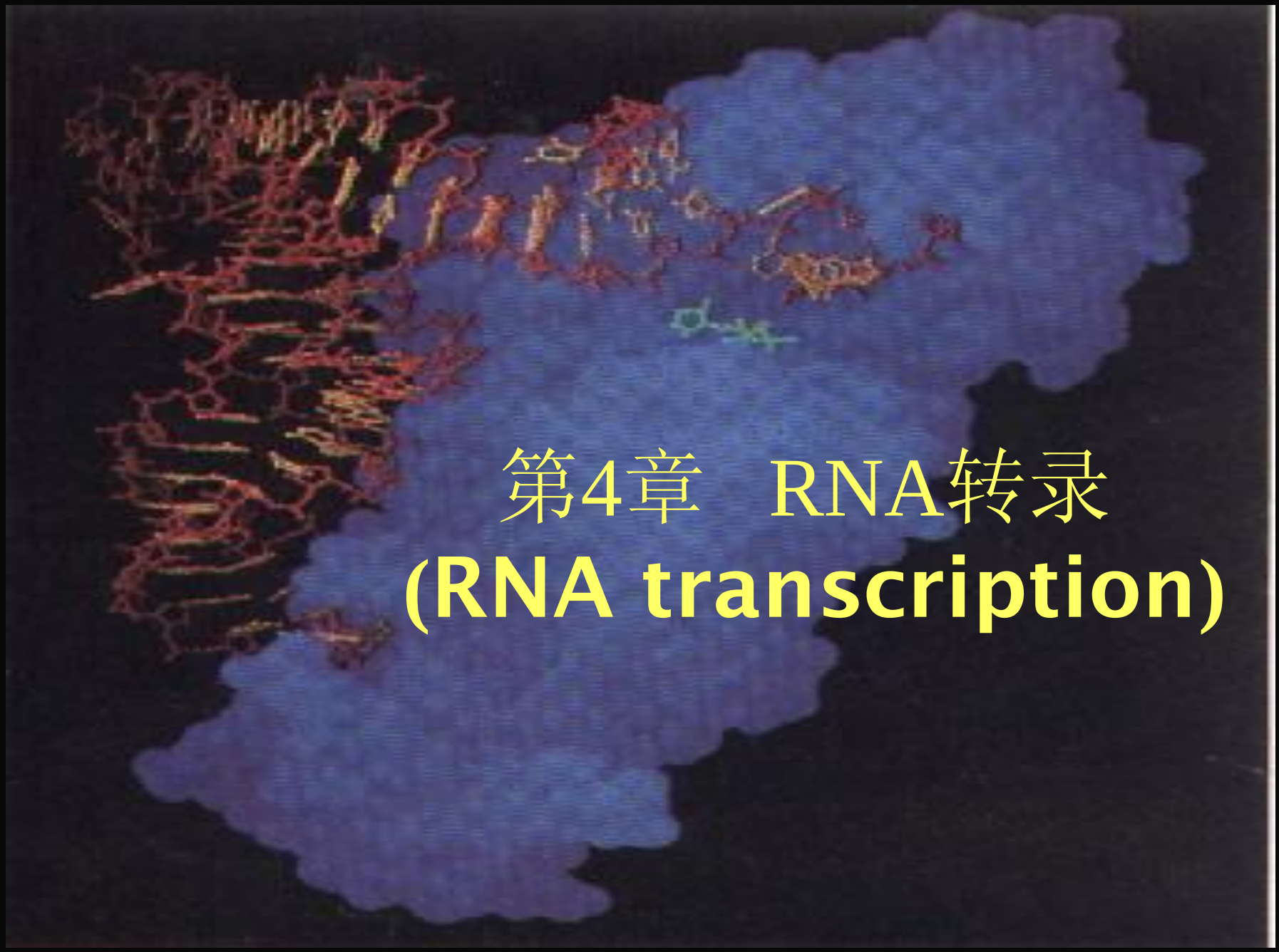


第4章　RNA转录
(RNA transcription)

4.1. RNA Transcription promotion in prokaryotes

4.2. RNA transcription promotion in Eukaryotes

4.3. Transcription termination

4.4. Pre-RNA processing

基 本 概 念

若 干 基 本 概 念

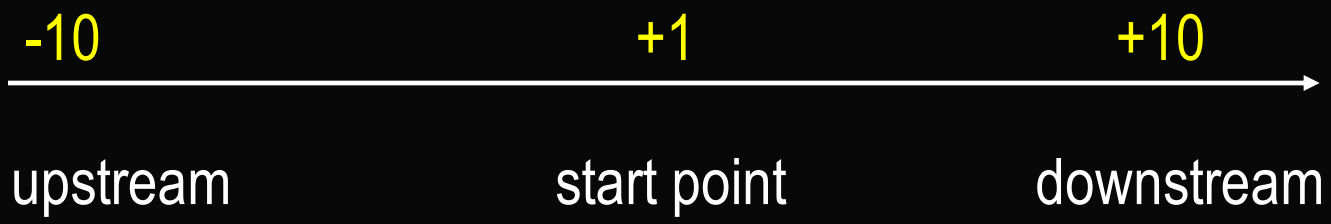
- 基因表达的第一步
- 以D. S. DNA中的一条单链作为转录的模板
- 在依赖DNA的RNA聚合酶的作用下
- 按A═U，C≡G 配对的原则，合成RNA分子
- 模板单链 DNA的极性方向为3’→ 5’, 而非模板单链DNA的极性方向与RNA链相同，均为5’→ 3’.

(书写DNA序列时，仅写非模板序列，可不注明极性方向)

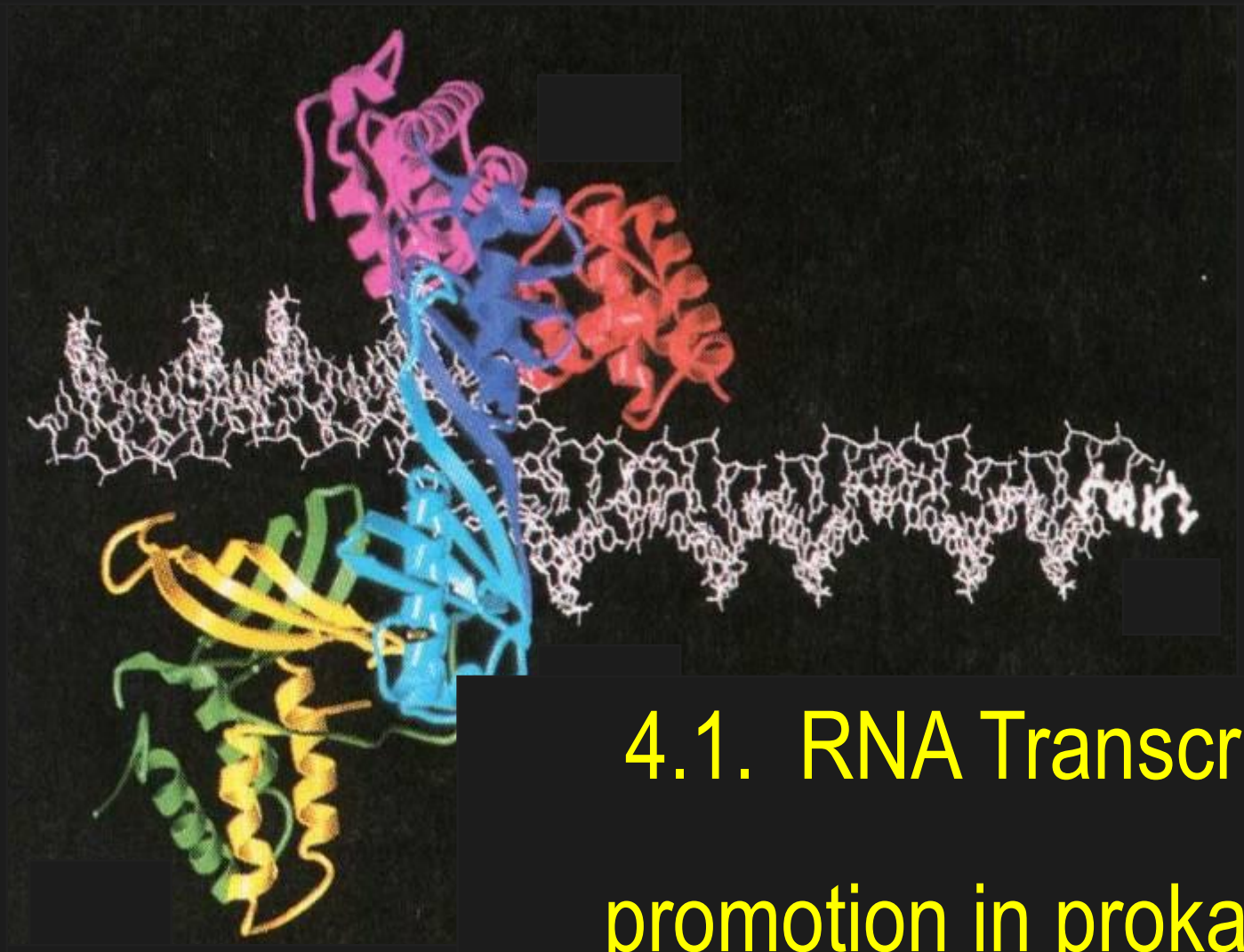
DNA { 5’---ATGAGTA----3’ Non-template (sense strand)
3’----TACTCAT----5’ template (antisense strand)

RNA 5’----AUGAGUA----3’

- 某一基因只以一条单链DNA为模板进行转录（不对称转录）
- RNA的转录包括promotion, elongation, termination 三过程
- 从启动子（promoter）到终止子（terminator）称为转录单位 （transcriptional unit）
- 原核生物中的转录单位多为 polycistron in operon
  真核生物中的转录单位多为monocistron, No operon
- 转录原点记为+1，其上游记为负值，下游记为正值



(来源：不详)



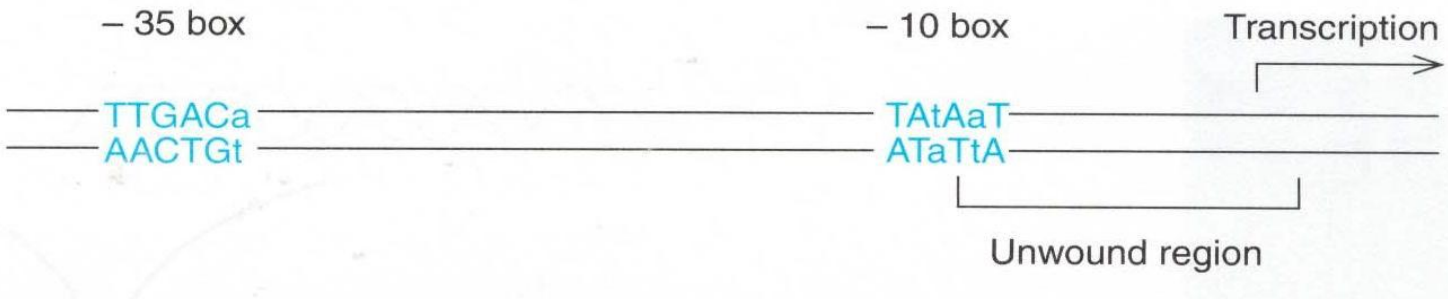
4.1. RNA Transcription

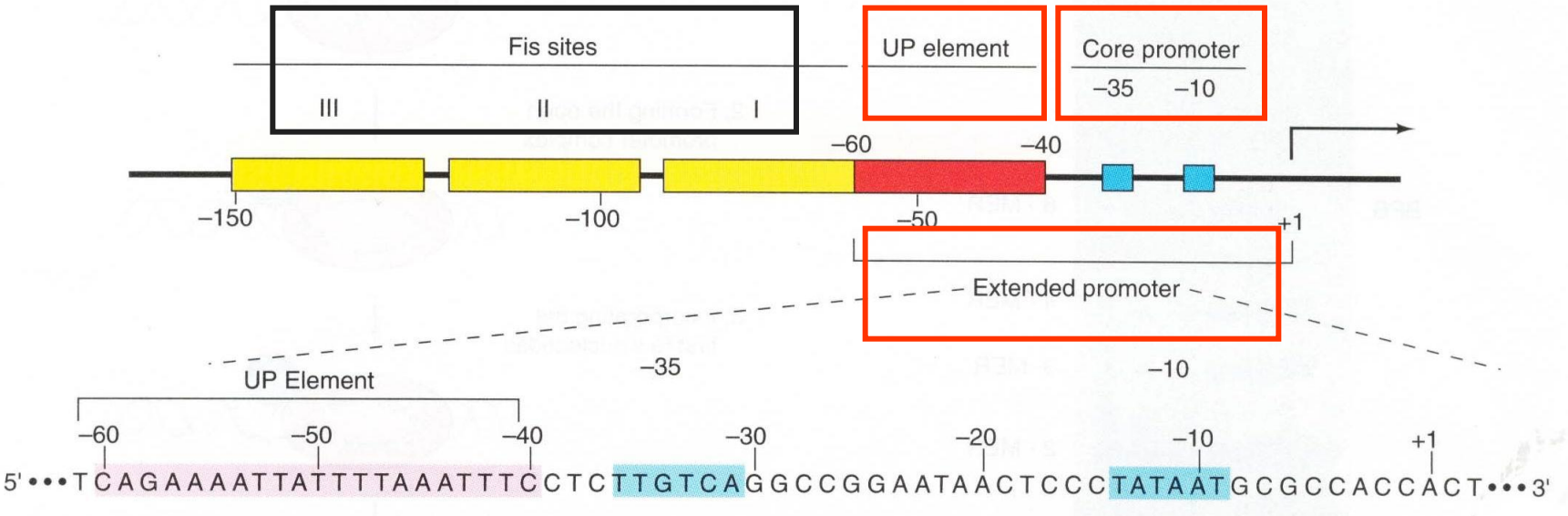
promotion in prokaryotes

(来源：不详)

4.1.1. Promoter 的结构与功能 (Prok. *E.coli*)

a) promoter 由两个重要部分组成



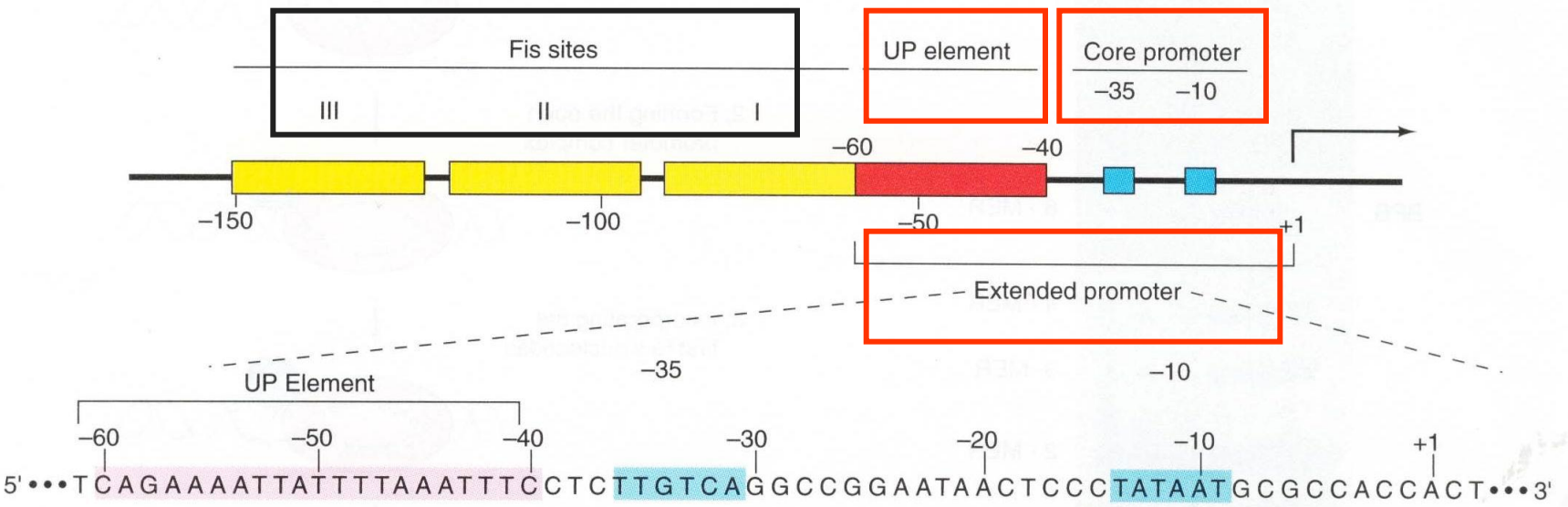


(来源：分子生物学（2007），郑用琏，第30页)

4.1.1. Promoter 的结构与功能 (Prok. *E.coli*)

a) promoter 由两个重要部分组成

- -170 ~ -60 Fis site;
- binding sites for the transcription-activator protein Fis.
- they , do not bind to RNA polymerase , are not classical promoter elements, but instead are members of enhancers.



(来源：分子生物学（2007），郑用琏，第30页)

- -60-70 ~ -40　up element

CAP—cAMP binding site

基因表达调控的正控制位点

CAP;　cAMP Acceptor Protein

(环化AMP受体蛋白)

分解代谢操纵子的激活蛋白

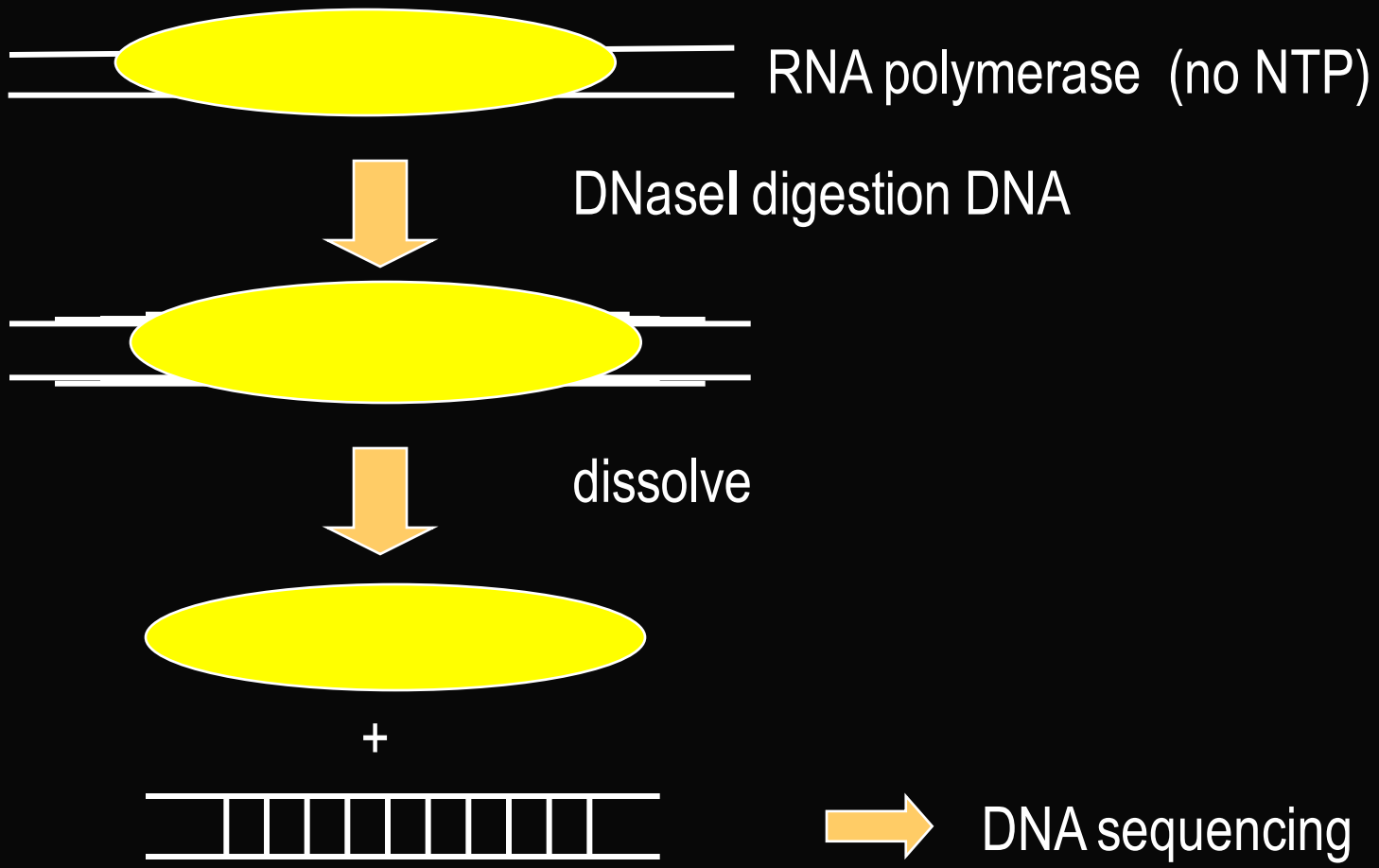
Catabolite gene Activator Protein

(降解物基因激活蛋白)

- -35 ~ -10　core promoter

RNApol. binding site

Promoter region identified by DNAaseI footprinting method



Core promoter region including

Sextama Box ; -35 site RNApol. loosely binding site

RNApol. recognition site (R site)

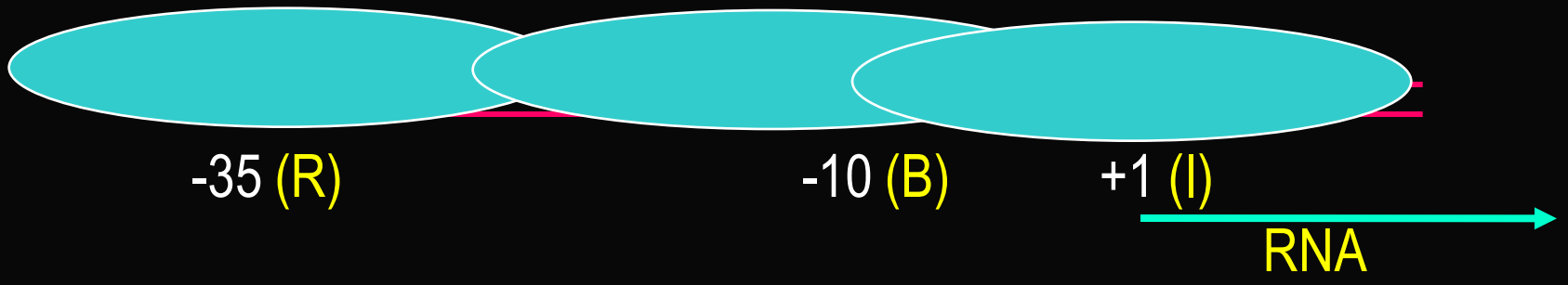
TTGAC (Sextama Box)

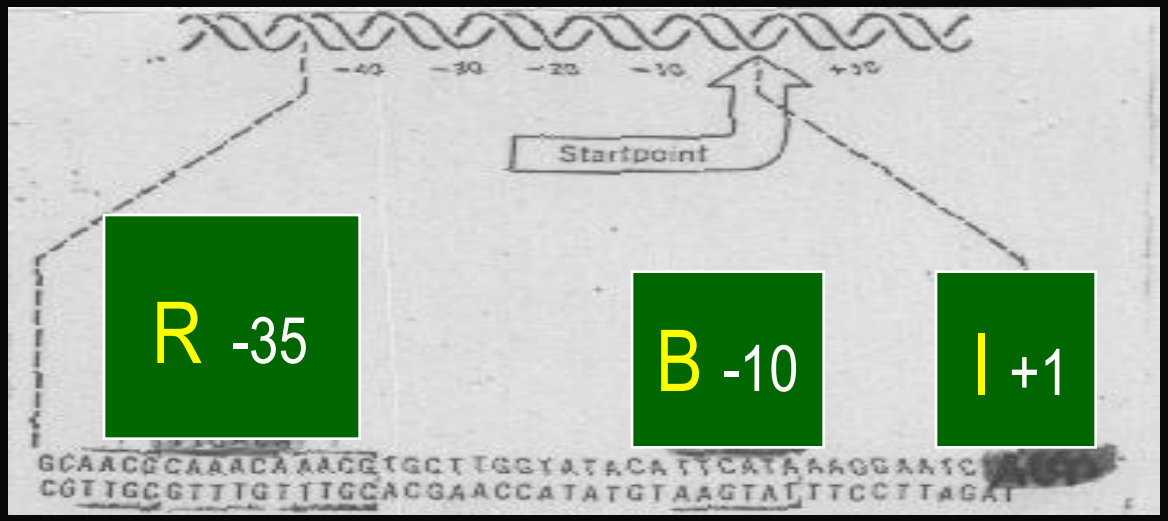
Pribnow Box ; -10 site RNApol. firmly binding site (B site)

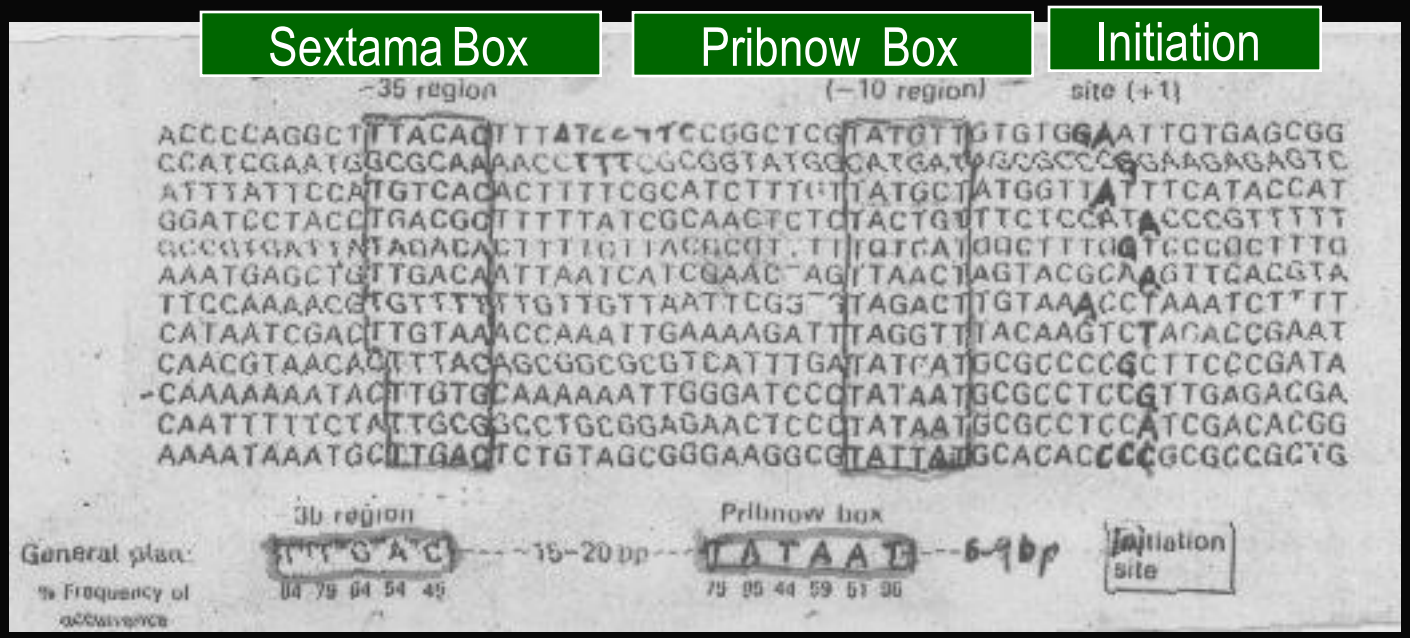
TATAAT (pribnow Box)

Initiation site ; +1 RNA transcriptional startpoint (I site)

A/G

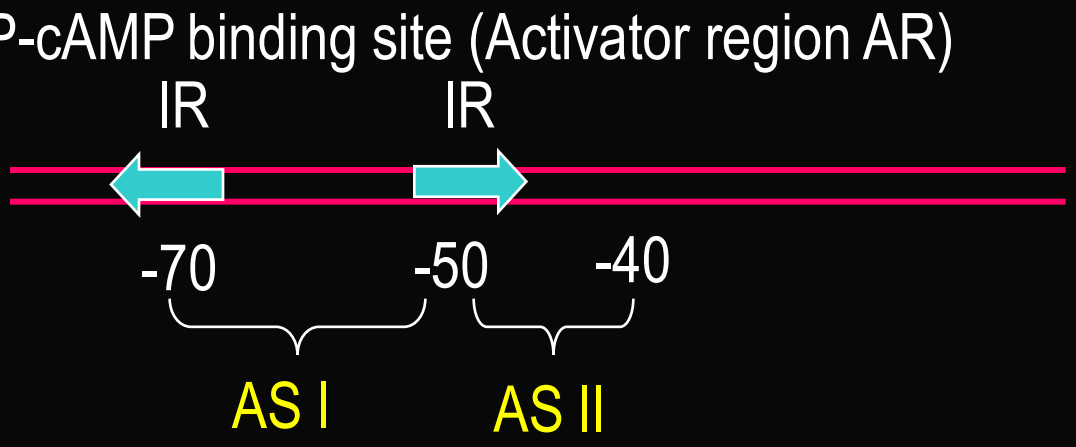






(来源：分子生物学（2007），郑用琏，第123页)

a) Up element



- AS I; IR是CAP-cAMP 的强结合位点（-70 ~ -50）
- AS II; CAP-cAMP 的弱结合位点（-50 ~ -40）
- AS I + CAP-cAMP

cooperative effect → 提高AS II 的结合效率

- AS II + CAP-cAMP → promotion → RNApol. into Sextama Box
  - → into Pribnow Box
  - → starting transcription
- Null AS II → RNApol. into pribnow Box But transcription off

AS II + CAP-cAMP 复合体
促使Sextama Box 附近GC岛区的双螺旋结构稳定性降低，
Pribnow Box 的解链温度降低，利于转录启动

c) Pribnow Box 的突变与遗传效应

R B I

----$T_{82}T_{84}G_{78}A_{65}C_{54}A_{45}$-----$T_{80}A_{95}T_{45}A_{80}A_{50}T_{96}$------c(A/G)t---- (100)

λ-C17 TATAAT

G A

λ-pRE AAGTAT

C C

LRNA-str TAAAAT

C

Bio-p98 TAAATT

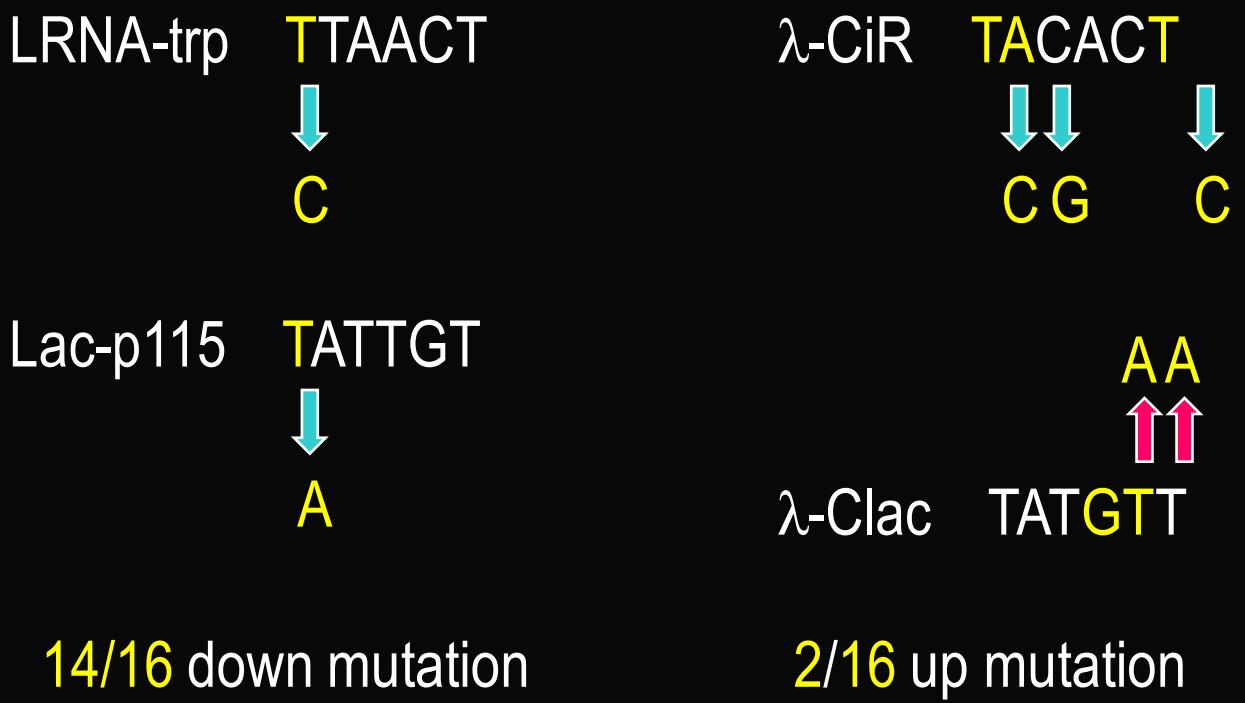
C

gal TATGGT

T

LRNA-tyr TATGAT

C C



- Probnow Box A/T → G/C 双螺旋体稳定性加强，转录率下降
- Pribnow Box 中A/T→T/A，改变了碱基堆积状况

  改变了RNApol.与模板链的结合效率

  转录效率上升（或下降）

- Sextama Box 与Pribnow Box 间距17bp,

  有利于RNApol启动

  间距趋近于17 bp， up mutation

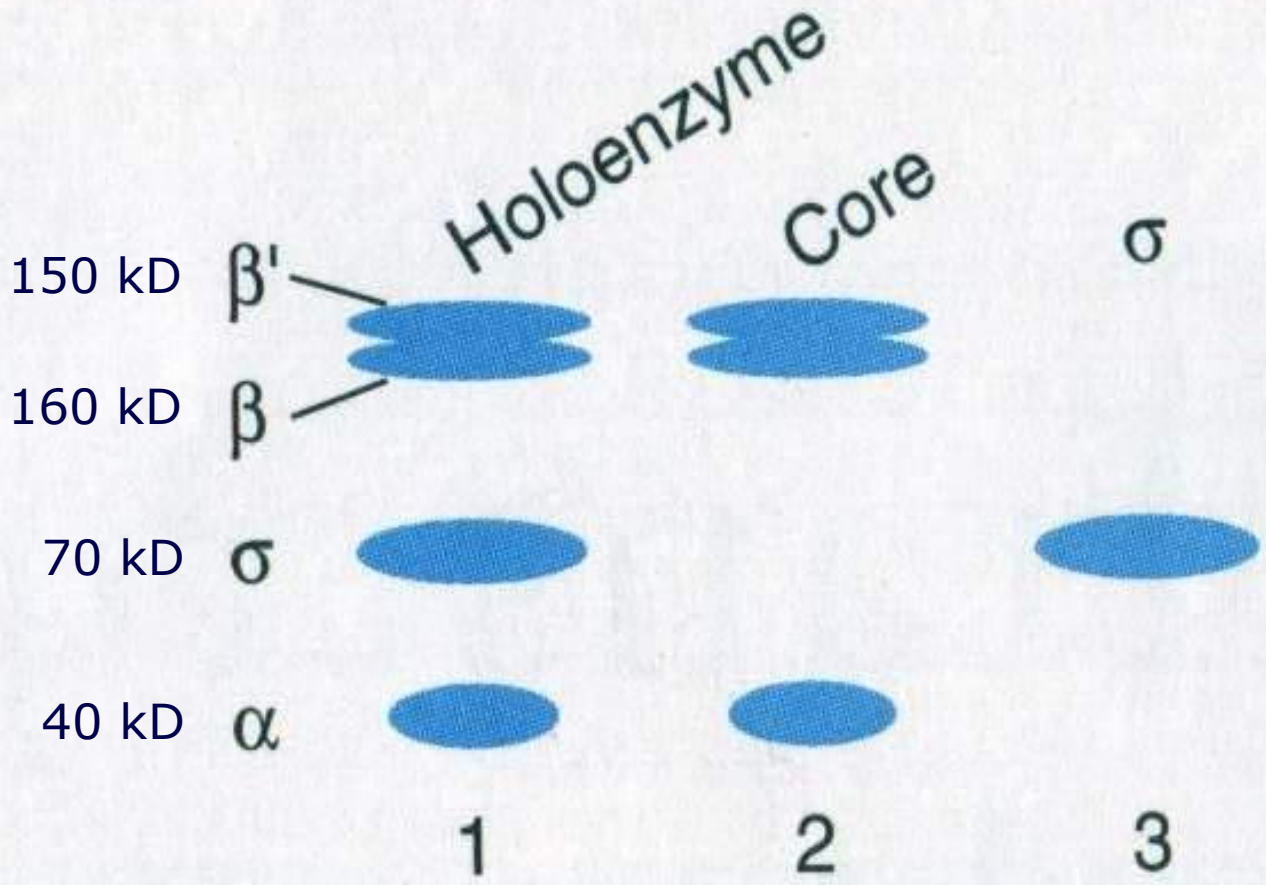
  间距远离于17 bp， down mutation

17bp的间距较17bp的序列对转录更为重要

4.1.2. RNA polymerase in prokaryotes

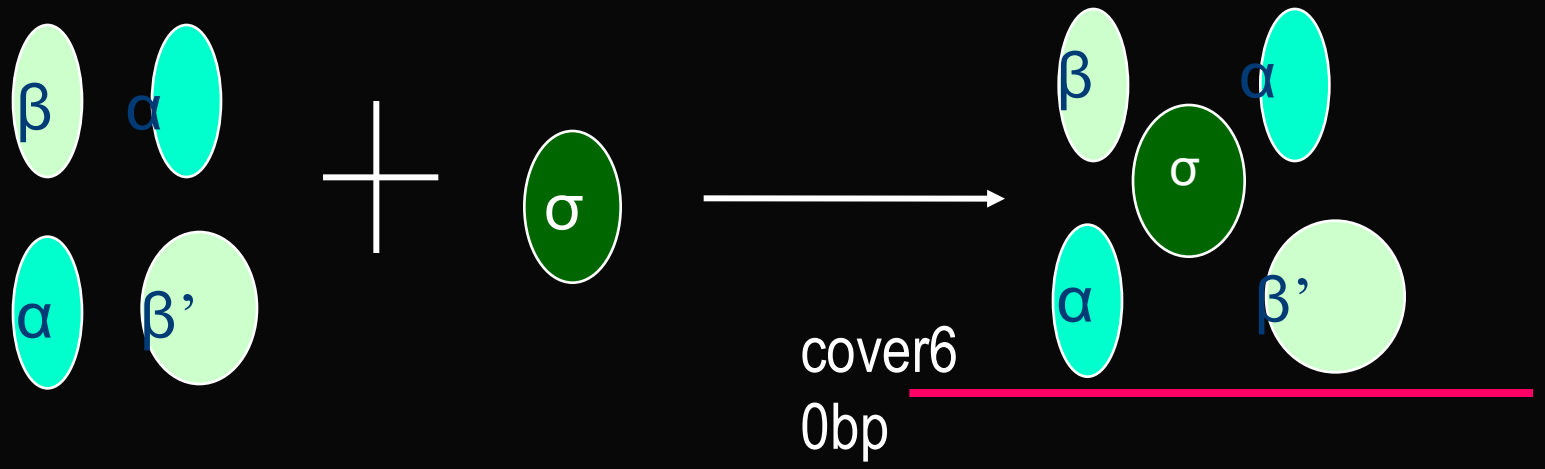
separation of the subunits of E. coli RNA polymerase by SDS-PAGE

Richard Burgess and Andrew Travers (1969)



(来源：分子生物学（2007），郑用琏，第30页)

4.1.2. RNA polymerase in prok.



Core Enzyme

for elongation

依靠静电作用力

非专一性与非特异DNA 结合

半衰期；60’

Holo Enzyme

for initiation

依靠空间结构

专一性与 特异DNA结合

半衰期；数小时

σ 因子；

★ 重复使用(Re-usable)

★ 使 Holo-enzyme识别Sextama Box, 与模板链结合

★ 修饰 RNApol 构型

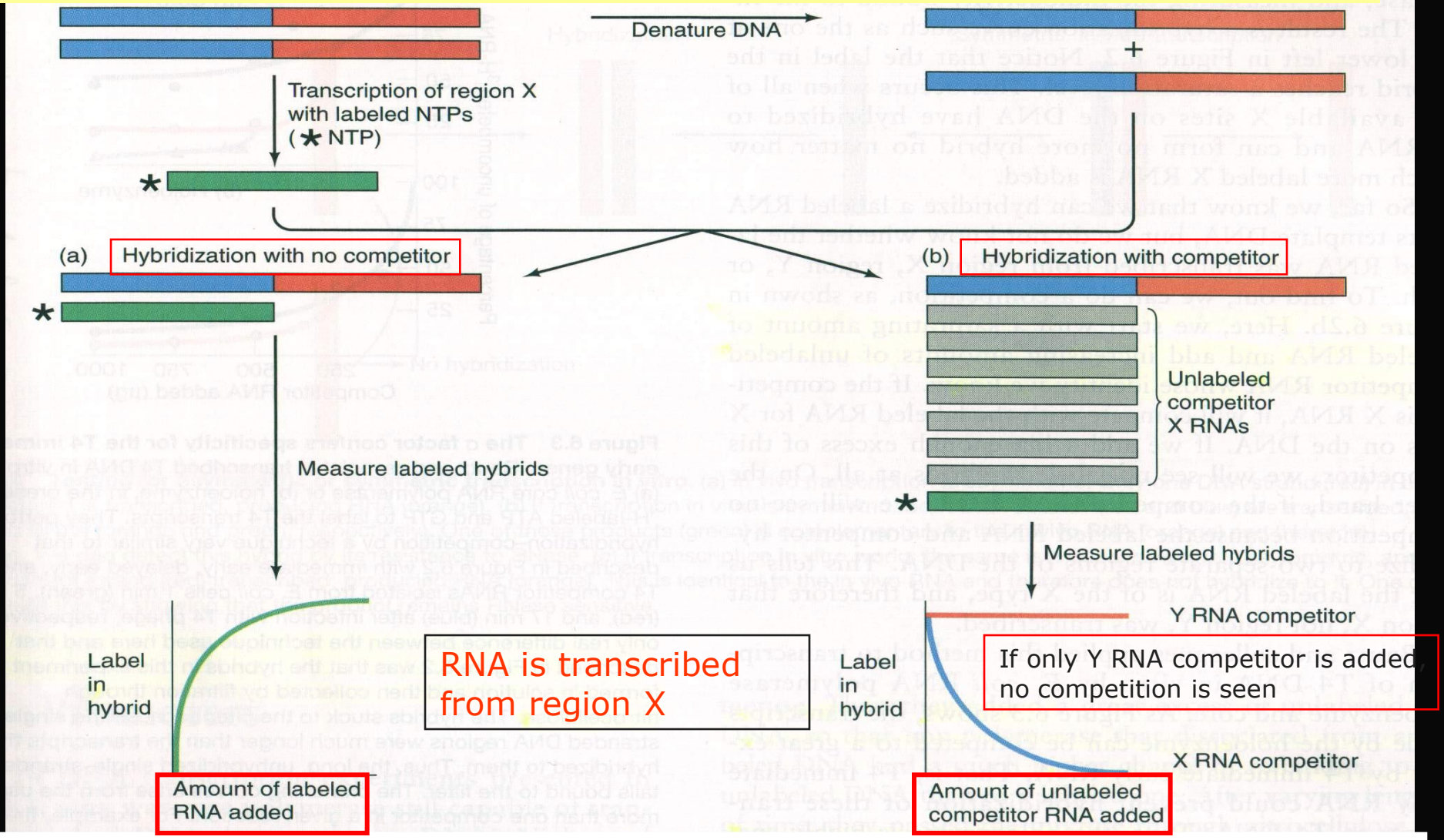
降低全酶与DNA的非专一性结合力

增强全酶与R, B site的专一性结合力

导致RNA链的延伸缓慢

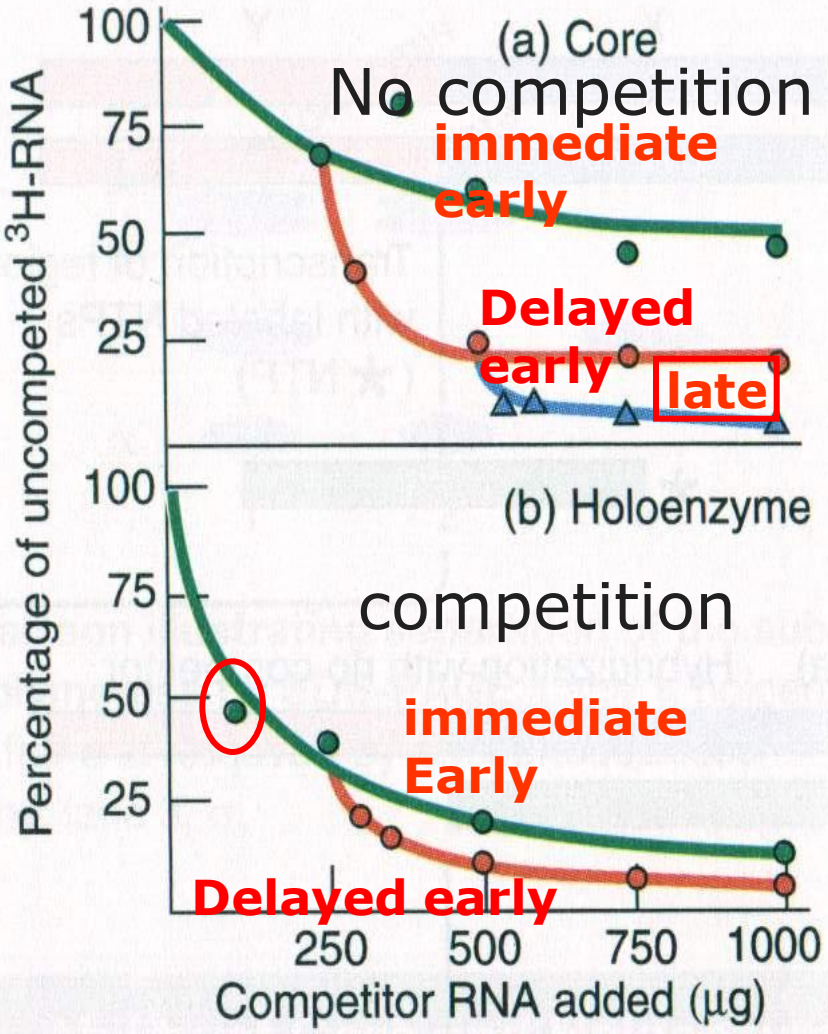
被标记的转录物与相应的RNA-competitor才能表现为“competition”

被标记的转录物特异性越高，表现的“competition”效应越明显



(来源：不详)

T4 competitor RNAs isolated from E. coil cells 1 min (green), 5 min (red), and 17 min (blue) after infection with T4 phage



(来源：不详)

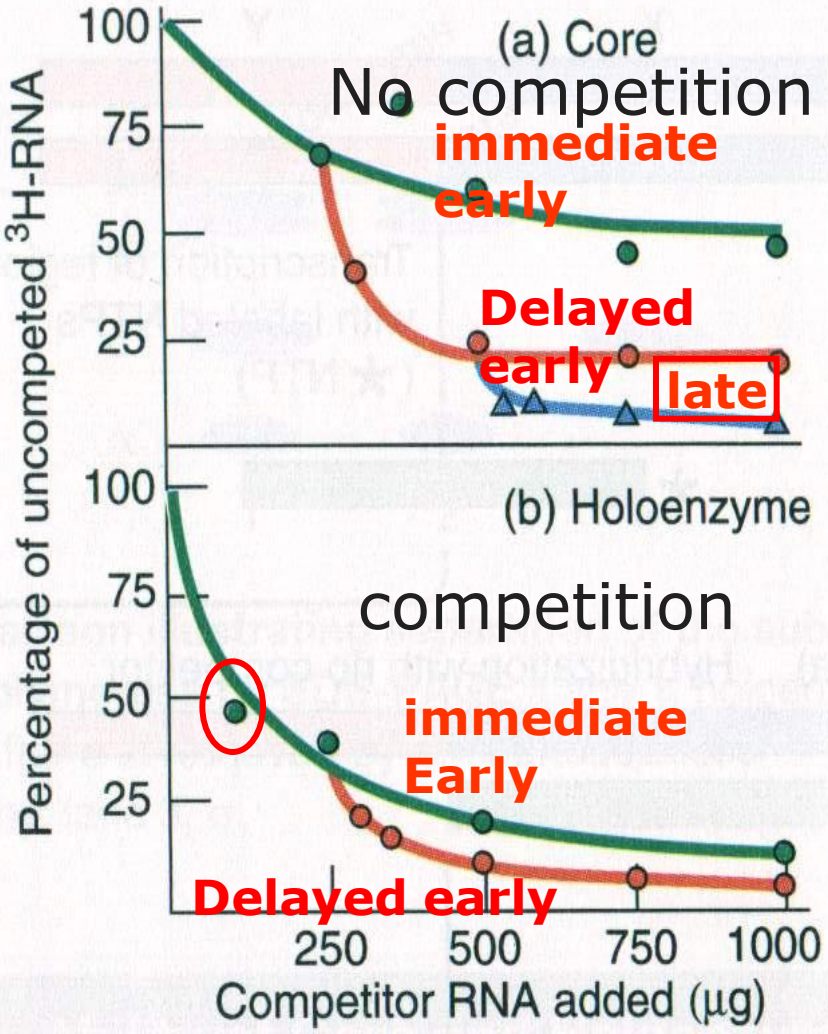
By contrast core enzyme (without σ subunit) has no specificity

Holoenzyme

(with σ subunit)

is highly specific for immediate early and delayed early genes of transcription

T4 competitor RNAs isolated from E. coil cells 1 min (green), 5 min (red), and 17 min (blue) after infection with T4 phage



(来源：不详)

By contrast core enzyme (without σ subunit) has no specificity

Holoenzyme

(with σ subunit)

is highly specific for immediate early and delayed early genes of transcription

H^3-T7 DNA + Holo E/Core E

↓

Excess unlabeled T7 DNA

↓

Holo E/Core E dissociated from DNA-E, then removed

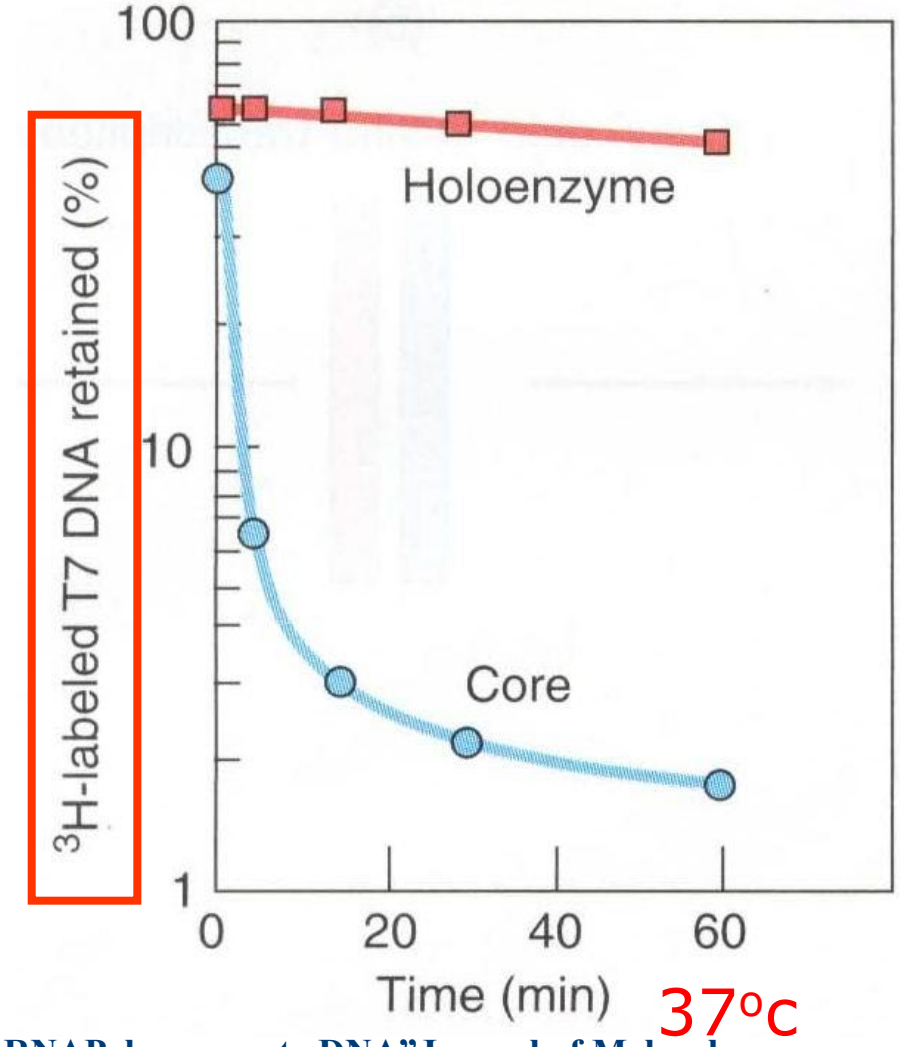
↓

Filtered mixtures through Nitrocellulose

↓

Monitor H^3-T7 DNA/E retained

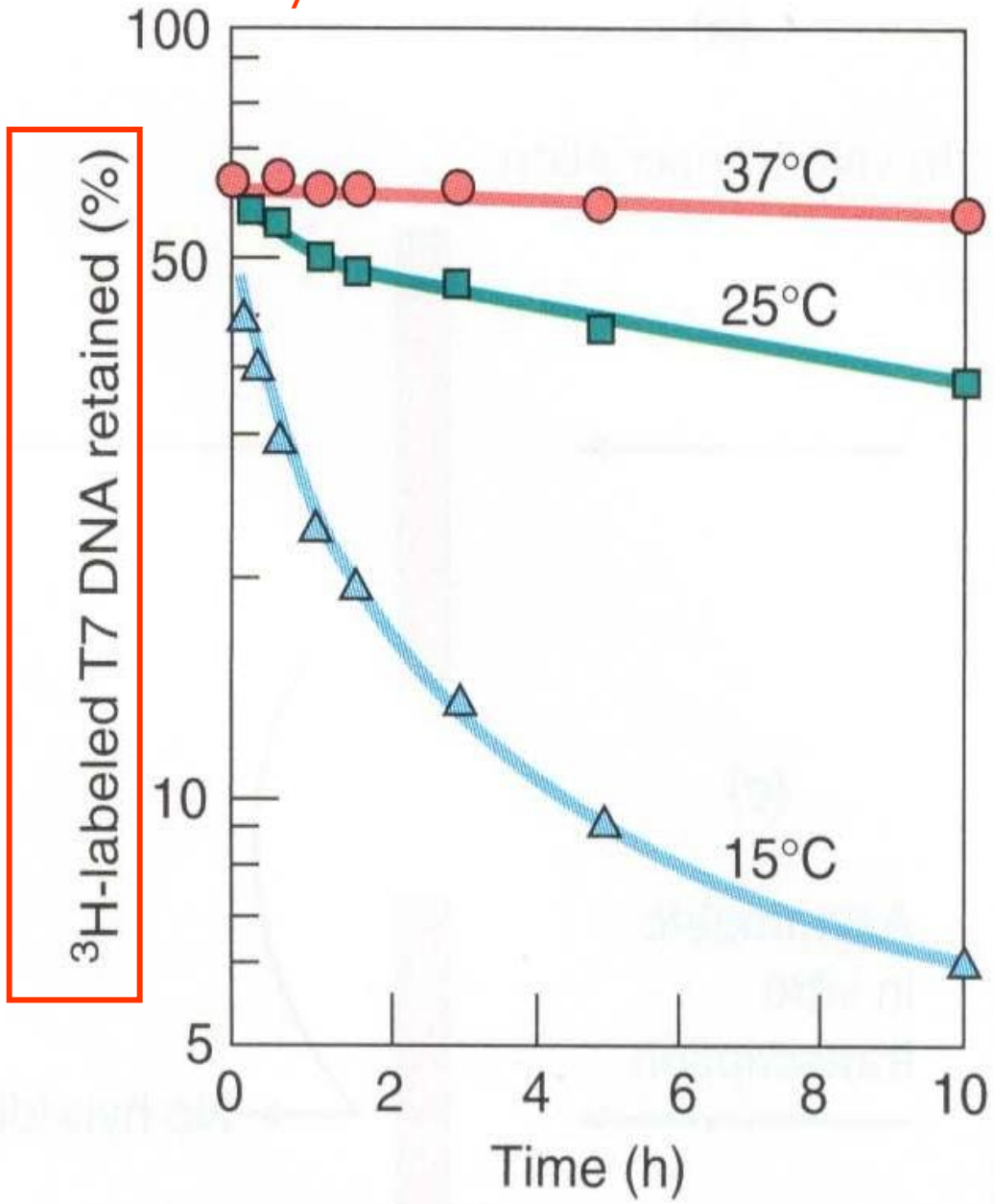
Sigma stimulates tight binding between RNA polymerase and promoter



(source:"Studies of the Binding of Escherichia coli RNAPolymerase to DNA"Journal of Molecular Biology,Vol.70,157-85,1972)

Sigma stimulates tight binding between RNA polymerase and promoter

Holoenzyme and ^{3}H-labeled T7 DNA

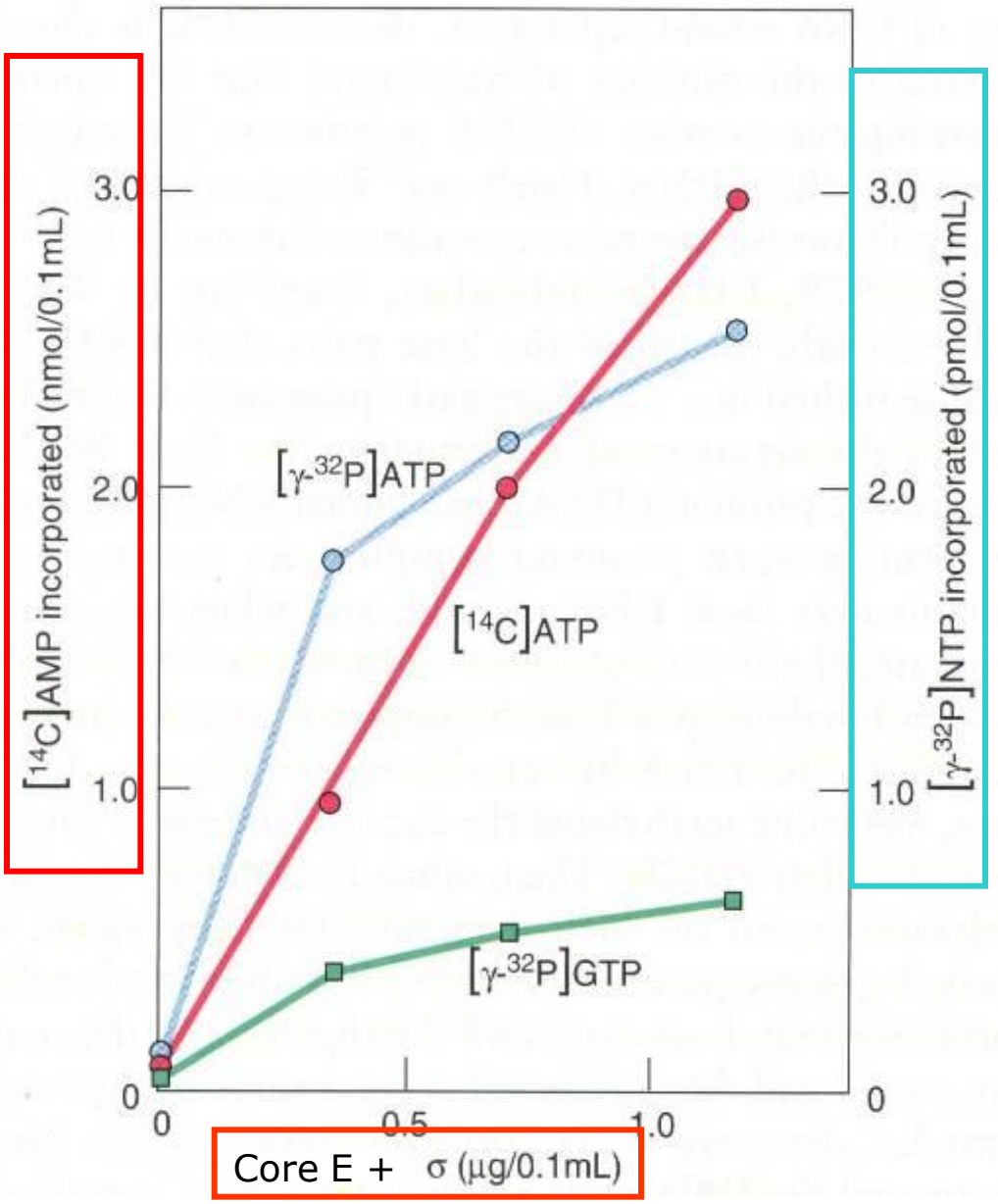


higher temperature favors tighter binding between RNA polymerase holoenzyme and T7 DNA (37°c)

?

(source:"Studies of the Binding of Escherichia coli RNAPolymerase to DNA"Journal of Molecular Biology,Vol.70,157-85,1972)

σ factor stimulate initiation of transcription



γ-P^{32}ATP

^{14}C-ATP ?

γ-P^{32}GTP ?!

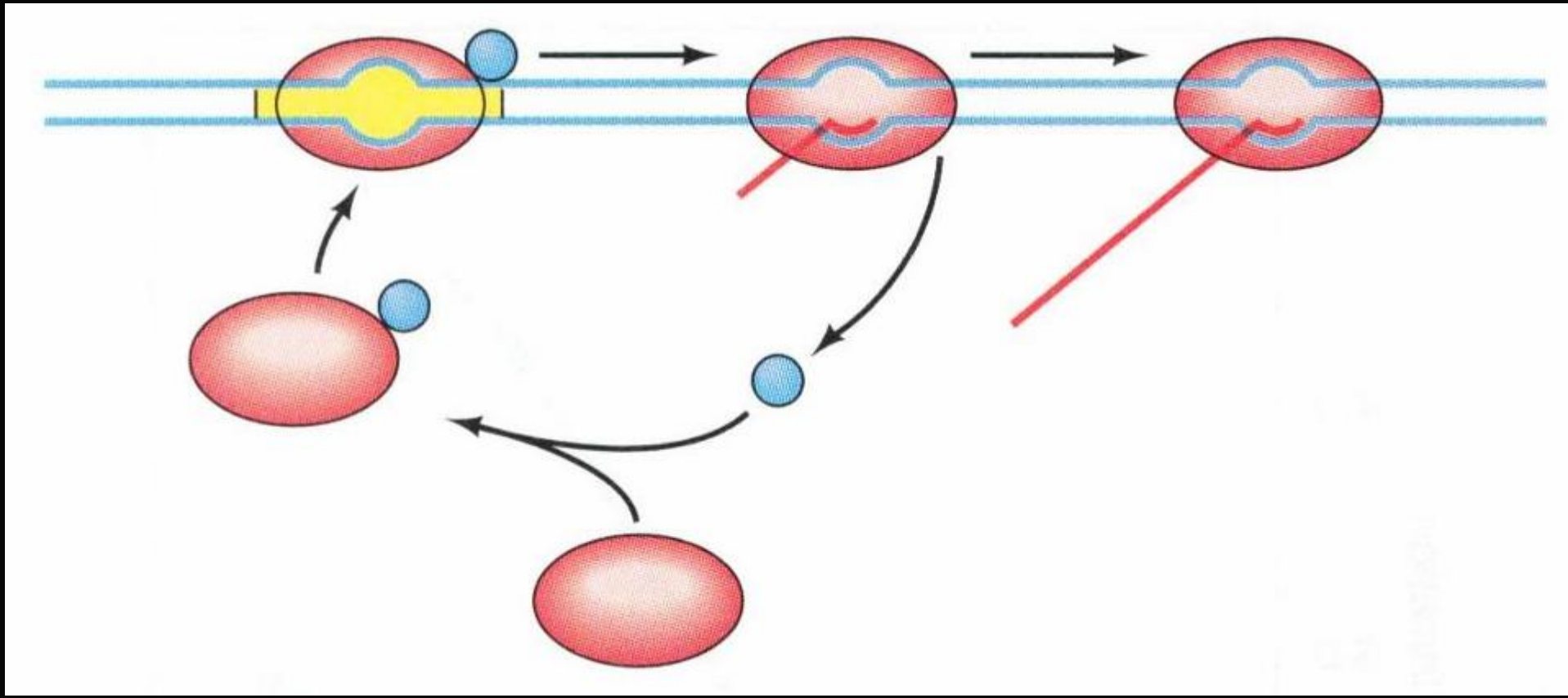
^{14}C-ATP measured bulk RNA synthesis, or elongation

σ could appear to stimulate elongation by stimulation initiation and providing more chains for core E to elongate.

σ factor stimulate initiation of transcription

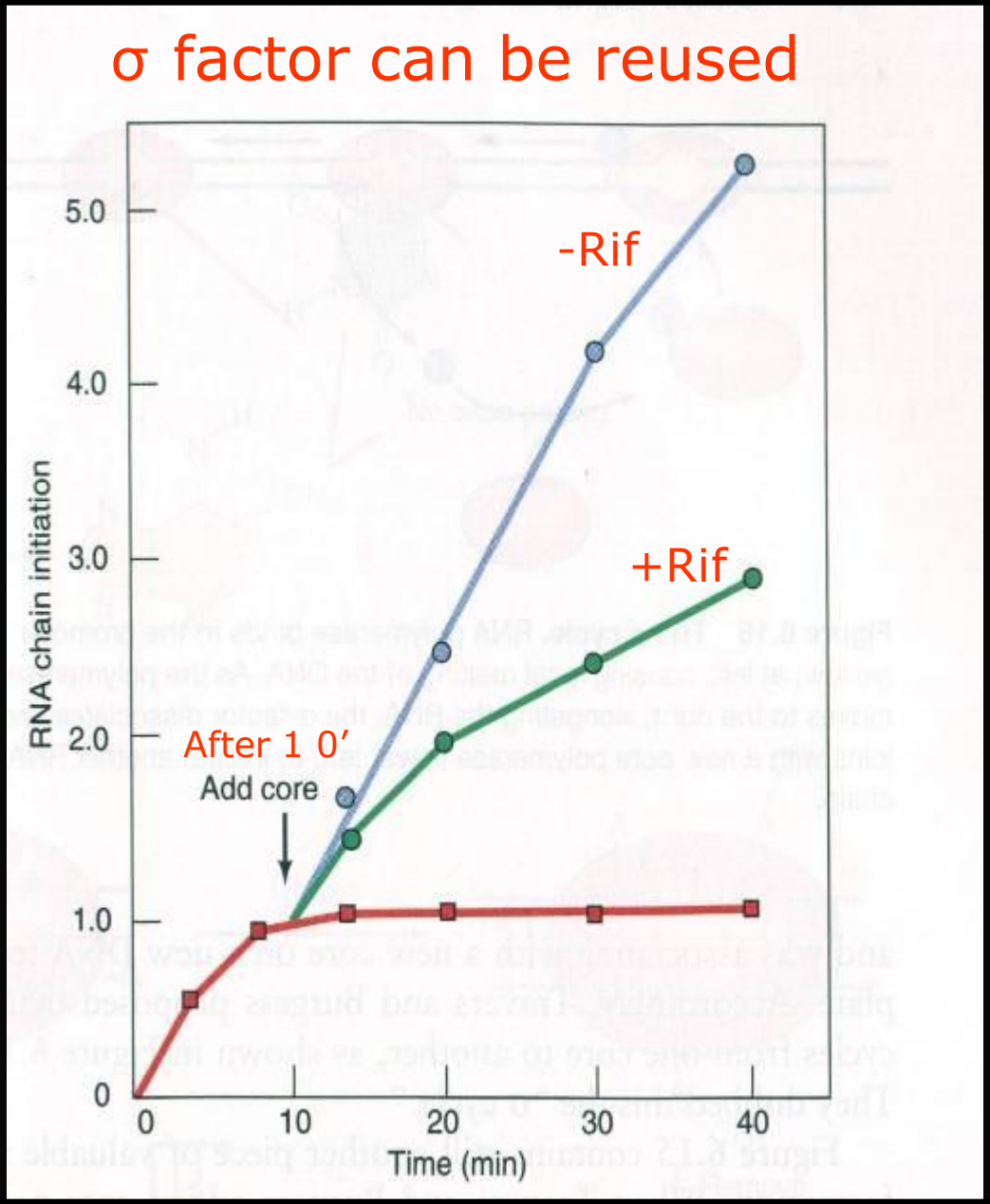
(Source:"Cycle re-use of the RNA polymerase sigma factor".Nature 222:537-40,1969)

σ factor can be reused



(来源：分子生物学（2007），郑用琏，第130页)

σ factor can be reused



initiation of RNA chains measured by [γ-32p]ATP / [γ-32p]GTP incorporation

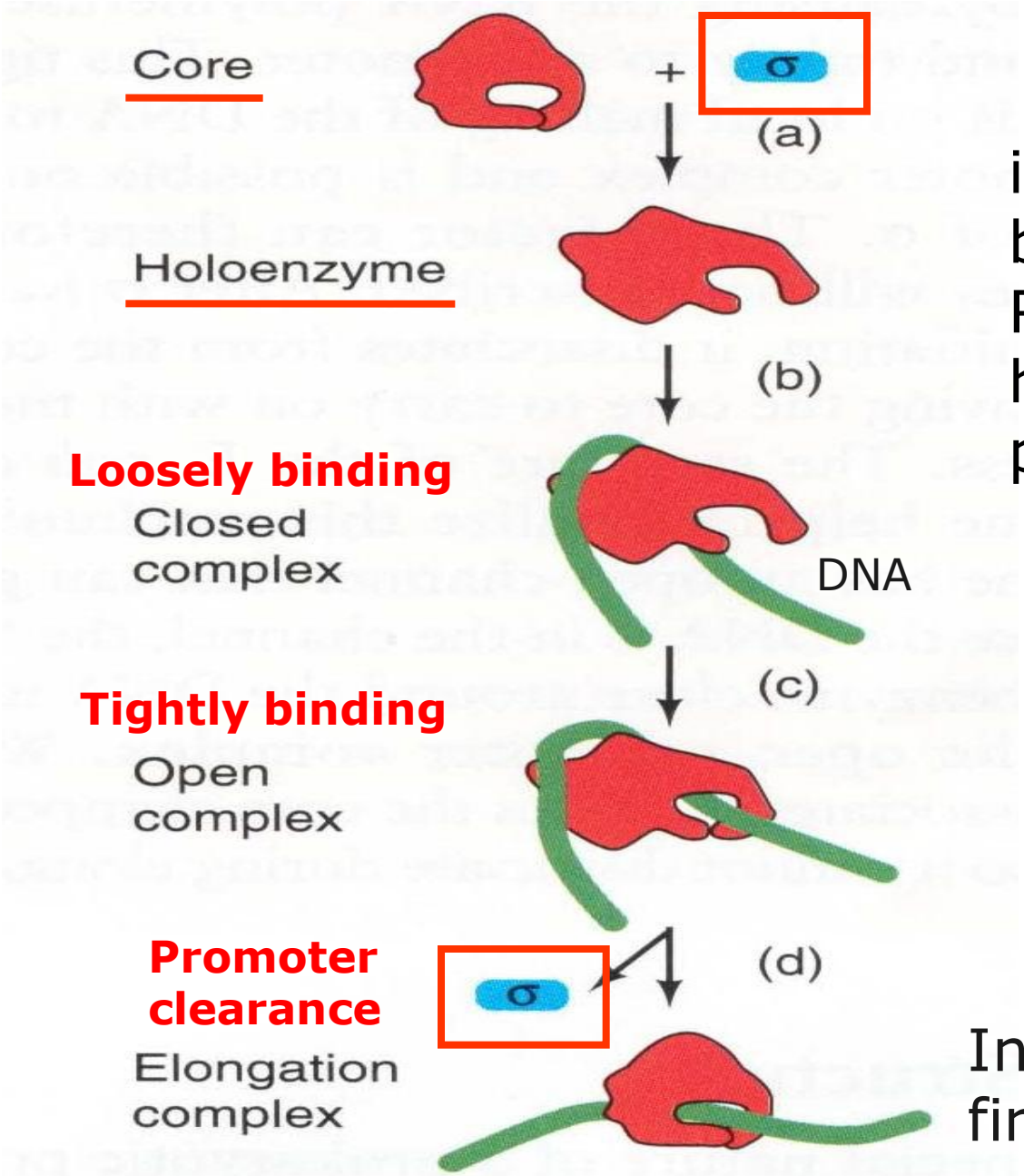
added new, rif^R core-E

↓

restart RNA synthesis

new core E associated with σ that had been released from original holoE

(Source:"Cycle re-use of the RNA polymerase sigma factor".Nature 222:537-40,1969)



Model of the interaction between *E. coil* RNA polymerase holoenzyme and a promoter.

Incorporating the first few Nt

(来源：不详)

α亚基；

★ 核心酶的组建亚基 α+α

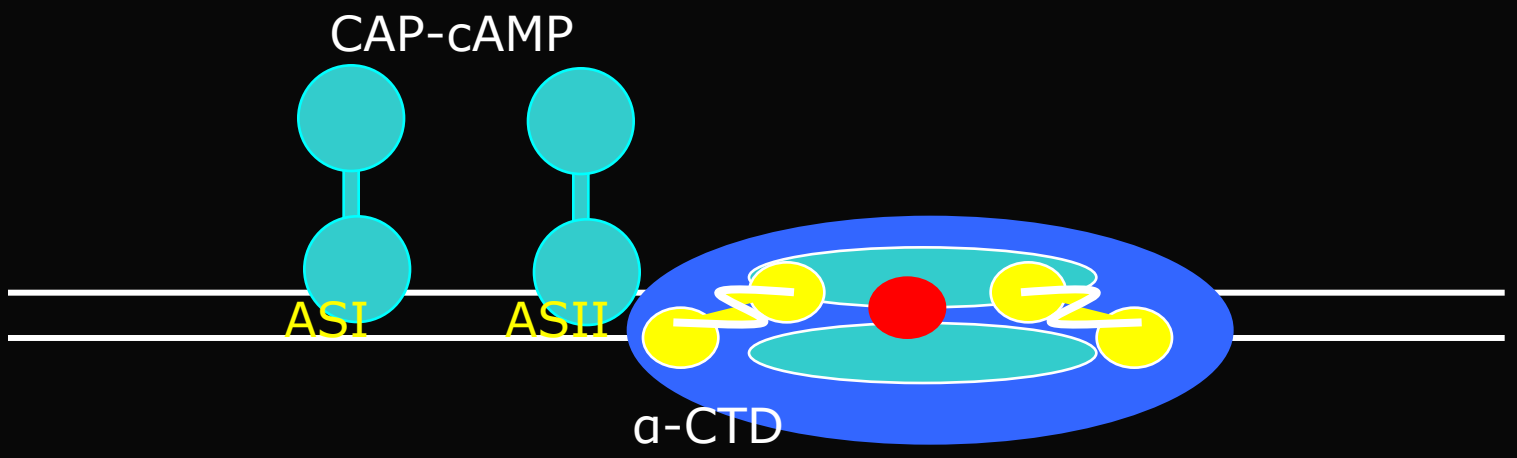
↳ 2α+β → +β’

★ 位于前端的α亚基使双链解链为单链

★ 位于尾端的α亚基使单链新聚合为双链

★ 促使RNApol与DNA 模板链上游元件结合

（CAP-cAMP与AS1、AS2结合）， α亚基CTD (carboxyl terminal donmain)延伸至AS2，与CAP-cAMP结合



Up element + CAP-cAMP ＋ RNA Polymerase复合体

CAP-cAMP 与RNA Polymerase的α-CTD结合，激活转录

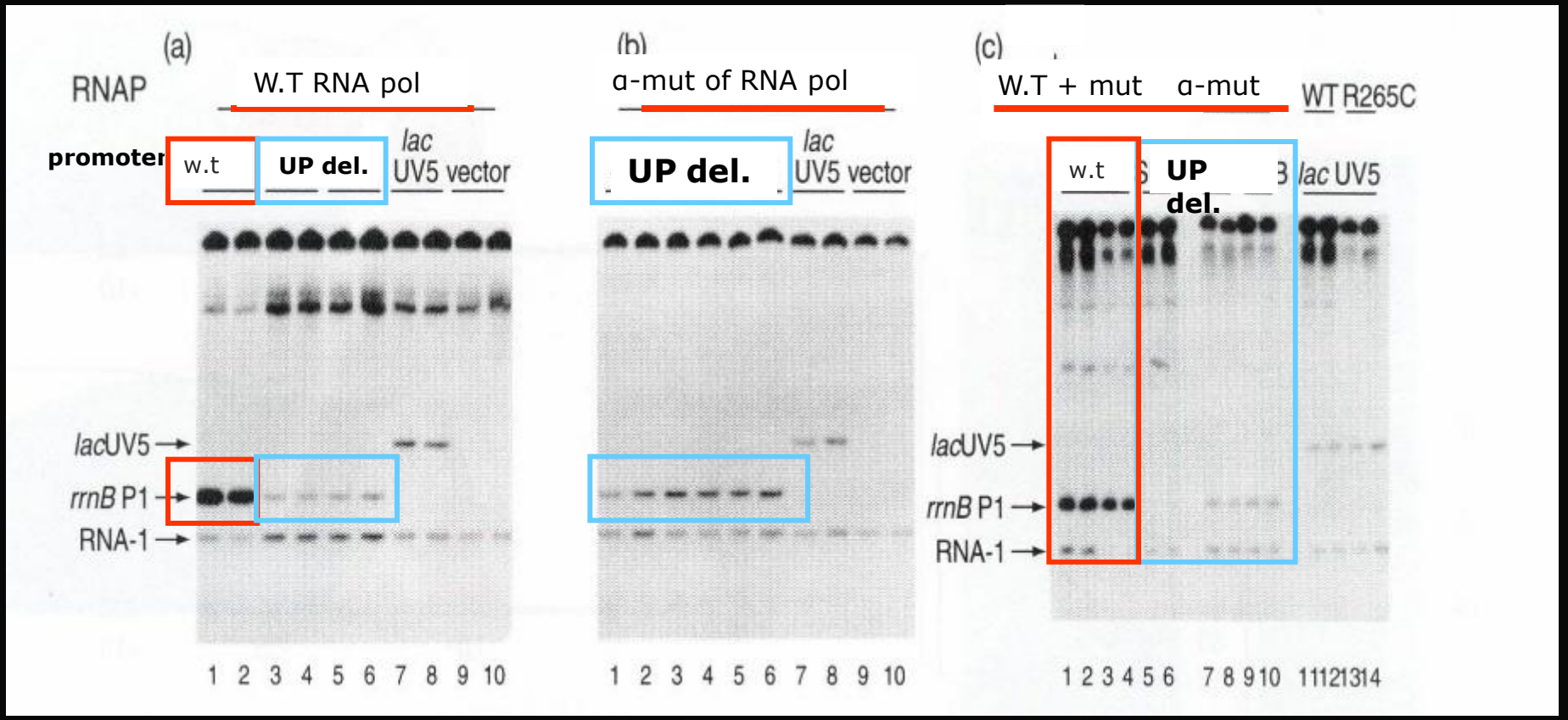
Importance of the α in UP element recognition

Richard Gourse (Science 262 (26 Nov 1993)

- w.t. promoter
  very strong rrnB P1 promoter region (-88 to +1),
  mut. promoter
  (SUB: an irrelevant seq. instead of UP from -59 to -41)
  (-41: lacked the UP upstream of -41 and vector seq instead)

transcribed with three different RNA polymerases(RNA-P),
(1) wild-type polymerase with a normal α-subunit;
(2) α-235, α-subunit was missing 94 aa from C-terminus;
(3) R265C, α-subunit contained at position 265.
Cysteine (C) in place of the normal Arginine (R)

- included a labeled nucleotide to label the RNA
- gel electrophoresis
- performed autoradiography to visualize the RNA products



Importance of the α subunit in UP element recognition

Source:Ross et al.,A third recognition element in bacterial promoters :Dna binding by the alpha subunit of RNA polymerase. Science 262(26 Nov 1993) f.2,p.1408

β亚基；

★ 促进RNA pol + NTP → RNA elongation

★ 完成 NMP之间的磷酸脂键的连接

★ Editing

★ 与 Rho(ρ)因子竞争RNA 3'-end

★ 构成Holo Enzyme后，β因子含有 两个位点

I site (Rif ^{S}) ; 专一性地结合ATP or GTP

要求高浓度的ATP or GTP

E site (Rif ^{R}) ; 对 NTP 非专一性结合

β’亚基；

★ 强碱性亚基

★ 促使RNA polymerase与非模板链（sense strand）结合

★ 受K酶抑制

Holo Enzyme 含有五个功能位点

- ★ sense strand DNA binding point(β’)
- ★ DNA/RNA hybrid site(β)
- ★ D. S.DNA unwinding point(α)
- ★ S. S.DNA rewinding point(α)
- ★ σ factor point

原核生物RNApol (Core) 的结构与功能

Enzyme Movement

DNA coding strand (β')

Rewinding point (α)

Unwinding point (α)

Core Enzyme 使 DNA 形成10-17bp的解链区

DNA template strand

RNA binding site

RNA/DNA hybrid (β)

(来源：分子生物学（2007），郑用琏，第131页)

原核生物RNApol (Core) 的结构与功能

Enzyme Movement

I site (Rif ^{S}) ; 专一性地结合ATP or GTP

E site (Rif ^{R}) ; 非专一性结合 NTP

I E

RNA binding site

RNA/DNA hybrid (β)

(来源：分子生物学（2007），郑用琏，第131页)

locate the determination
of Rifampin resistance

Purification of the individual subunits of *E.coli* RNA polymerase in Urea electrophoresis buffer

(Alfred heil & Ziling 1970)

Separation and reconstitution of RNA polymerase

Source:Heil,A.andZillig,W.Reconstitution of bacterial DNA-dependent RNA-polymerase from isolated subunits as a tool for the elucidation of the role of the subunits in transcription.FEBS Letters 11(Dec 1970)p.166,f.1.)

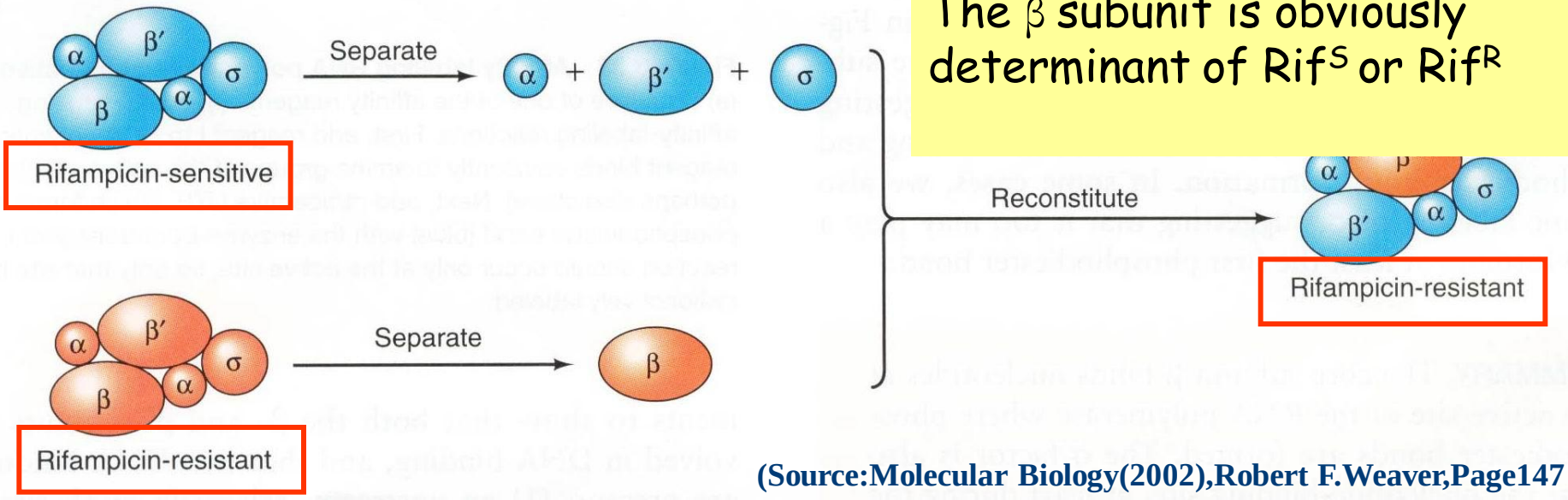
Reconstitution of RNApol.

the α+α+ δ+ β′ from Rif ^{R} E.coli + β from Rif ^{S} E.coli

The reconstituted RNA polymerase was Rif ^{S} , regardless of the origin of the other subunits

the α+α+ δ+ β′ from Rif ^{S} E.coli + β from Rif ^{R} E.coli

The reconstituted RNA polymerase was Rif ^{R} , regardless of the origin of the other subunits



The β subunit is obviously determinant of Rif^{S} or Rif^{R}

(Source:Molecular Biology(2002),Robert F.Weaver,Page147)

Rifampcin对RNApol 的抑制表现

- Rif 与ATP / GTP对I site的竞争

  Rif紧密与β亚基结合　→ 阻止 ATP/GTP对I site 的填充

- 当I, E site被pppXpYpZ(3Nts)填充，

  Rif失去抑制效应

Rifampcin是RNA合成起始的抑制剂

NusA protein ;

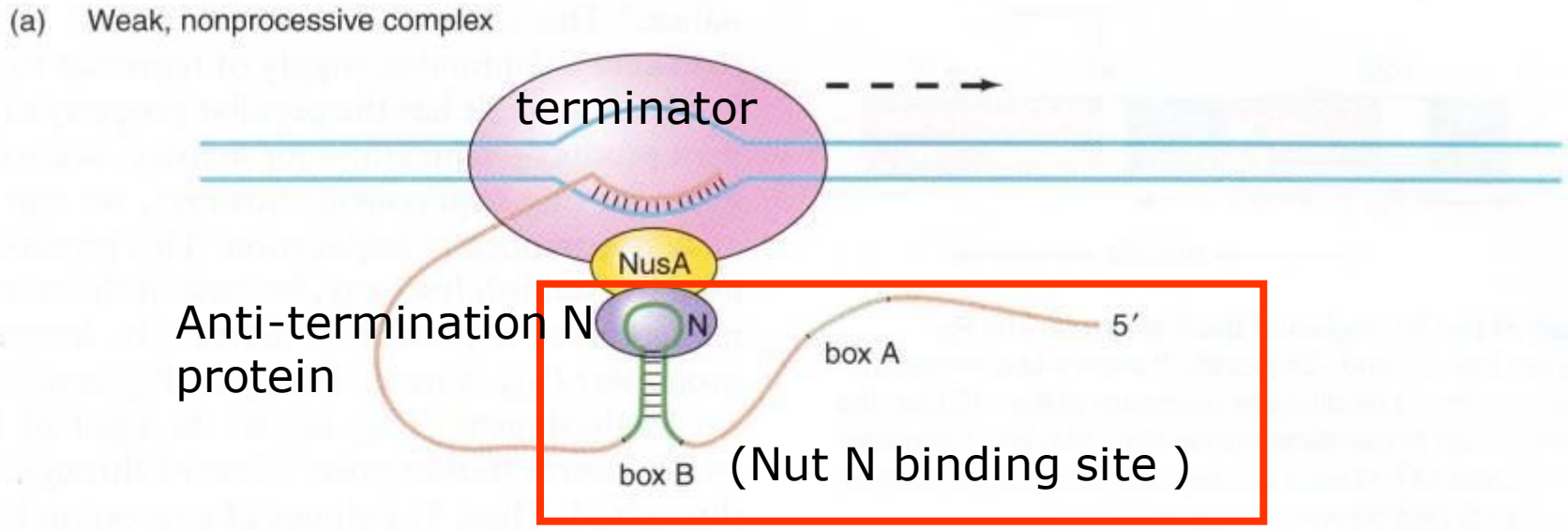
(antitermination N protein utilization substance)

★ 69 Kd, acid protein

★ RNApol-attaching factor

★ After initiation → substituting σ → NusA + core E

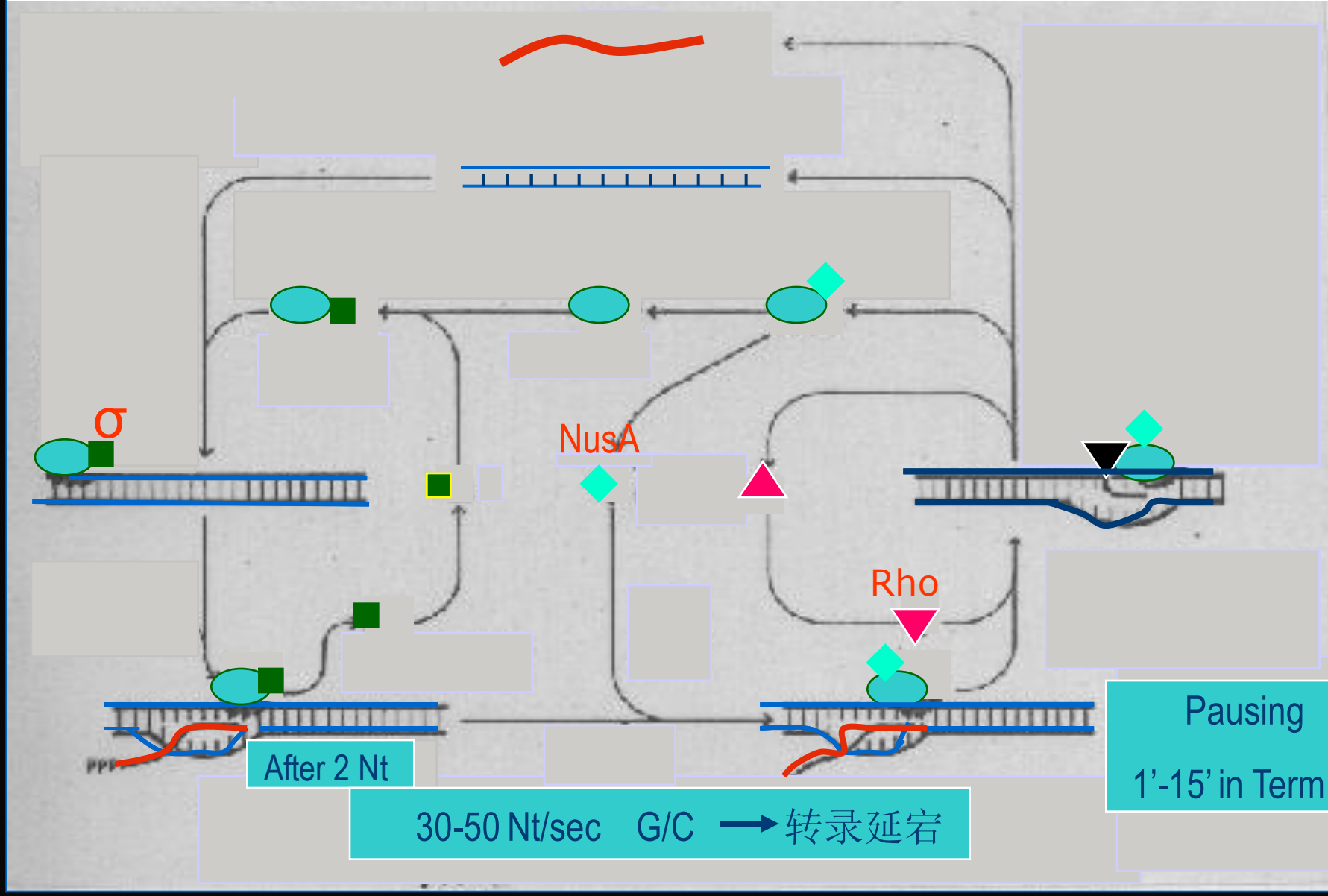
★ Impel RNApol. pausing in terminator for 1’-15’ and waiting for Rho factor to stop transcription of RNA



(Source:Molecular Biology(2002),Robert F.Weaver,Page213）

NusA binds to polymerase, and N binds to both NusA and box B of the nut site region of the transcript, creating a loop in the growing RNA. This complex is relatively weak and can cause antitermination only at terminators near the nut site (These conditions exist only in vitro.).

原核生物转录的起始与延伸过程



(来源：分子生物学（2007），郑用琏，第133页)

Promoter与σ factor 间的结合专效性

- 决定了 strong promoter & weak promoter

★ 启动子中-35, -10 区序列的差异影响与 RNApol 中 σ 因子的结合能力

★ 不同启动子的-35，-10区序列间存在较为保守的标准序列(标准启动子)

σ factor is responsible for recognition of consensus sequence of promoter and only required for initiation.

- Transcriptional regulation by alternative factor

in *E.coli* for stress condition

----- When 37℃； genes expressed in *E.coli* by RNApol with σ^{70}

----- When ＞ 37℃;

More then 17 proteins are expressed in *E.coli* through transcription by RNApol using an alternative σ^{32} , which have own specific promoter consensus sequence

***E.coli*的热激响应是由一种可转变的σ-因子σ^{32} (σ^{H})所控制，σ^{32} (σ^{H}) 取代σ^{70}(σ^{A})来介导RNA聚合酶与热激蛋白基因的启动子结合。**

Results and Discussion

Molecular Biology: gene & interaction

Interaction:

Cis & Trans: Protein & DNA (DNA pol & DNA, RNA pol & promoter), more to come, and more to find

Trans & Trans? Cis & Cis?

Case study:

Understand the concept, principle, and conclusion

Get familiar with the way of thinking and investigation

Develop the ability to identify, dissect, and solve problem

Platform:

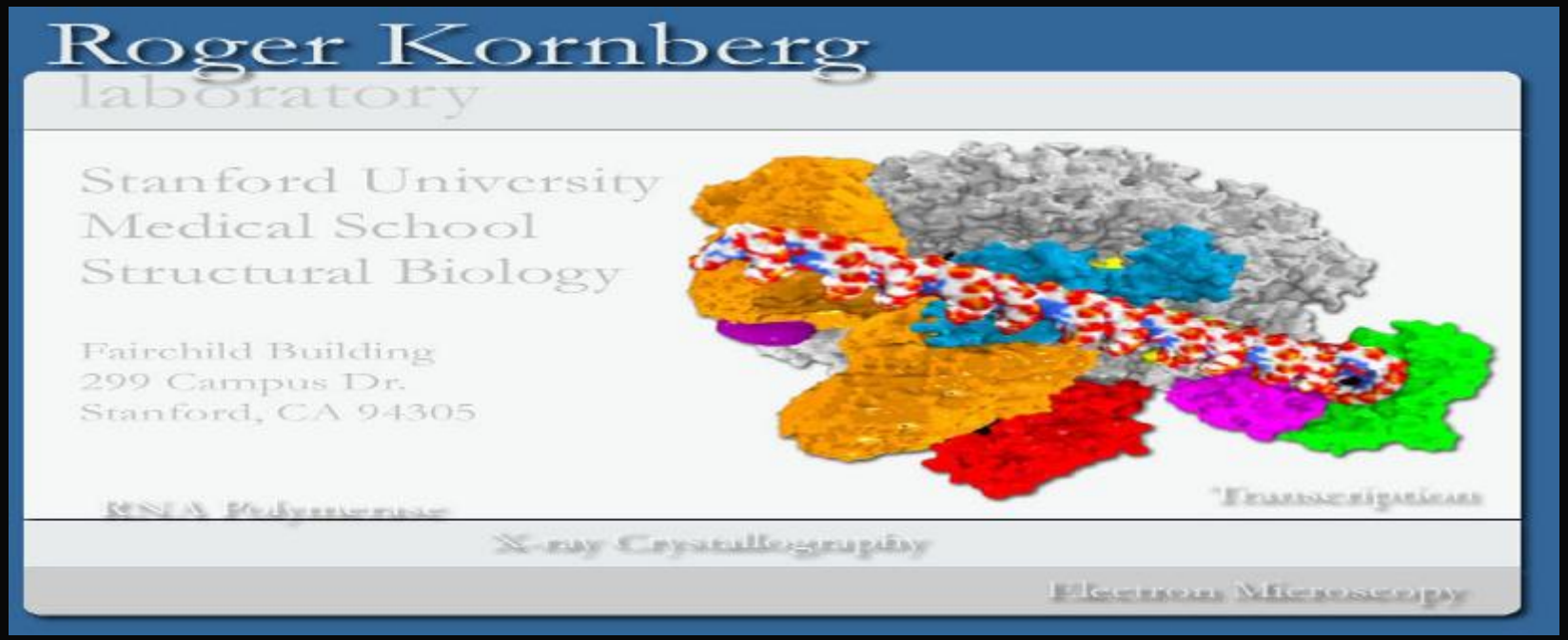
Mind training and curiosity development

Enjoyment: the beauty of nature & the sparks of human wisdom

4.2. RNA transcription promotion in Eukaryotes

2006年度诺贝尔化学奖授予美国科学家罗杰.科恩伯格，以表彰他对真核转录的分子基础所作的研究。

2007.10.26晚病逝，享年89岁



(来源：不详)

4.2.1. Biological state before transcriptional starting

外界信息的感知与传递

- 细胞膜 → 细胞核

细胞信号传递

- 染色体状态的调整

  核小体 包装的解体

  H3 protein outer

  $DNase^S$

- Transcriptional factor (TF, trans factor)

  RNApol

  Cis-factor

} complex

4.2.2. 与转录启动相关的cis-factors

- 基本水平转录的 cis-factor

(promoter or basic factor)

Core promoter (Cap site, TATA box 必需因子)

UPE (Upstream Promoter Element) (CAAT box, GC box)

For housekeeping gene (constitutive expression)

- 特异诱导高效表达的cis-factor

Enhancer

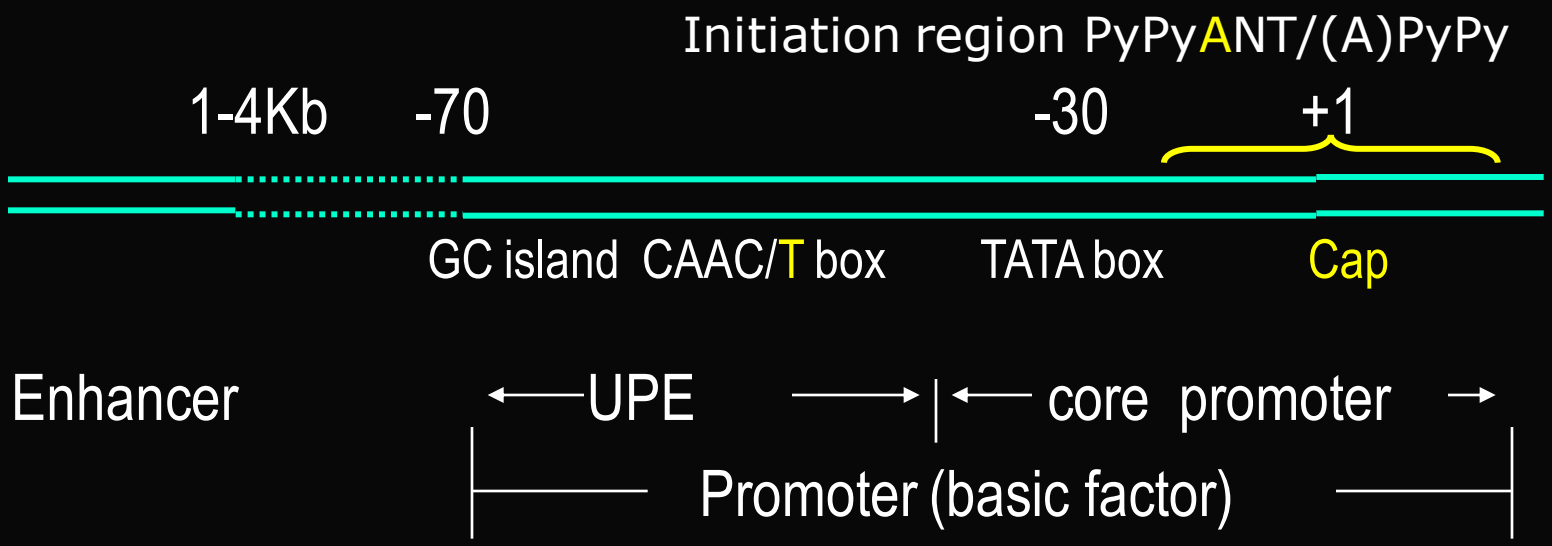
For luxury gene (inducible expression)

4.2.2.1. Promoter for basic transcription

(class II recognized by RNA polymerase II)

- Cap site ; initiation point (+1)

  Capping m^7GpppA/G------70 ±---AUG---(in mRNA)



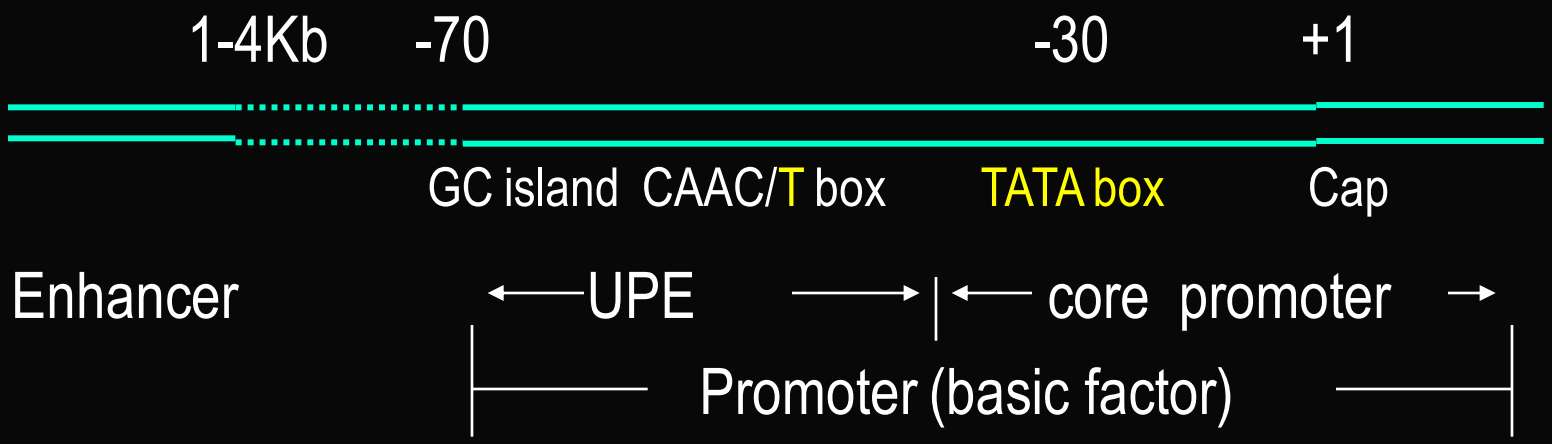
Promoter for basic transcription

- TATA box / Hogness box / Goldberg-Hogness box (-30)

  Rich AT and rich GC flanked

  rich GC------T A T A A (T) A A (T)------rich GC

  82 97 93 85 63 (37) 83 50 (37)



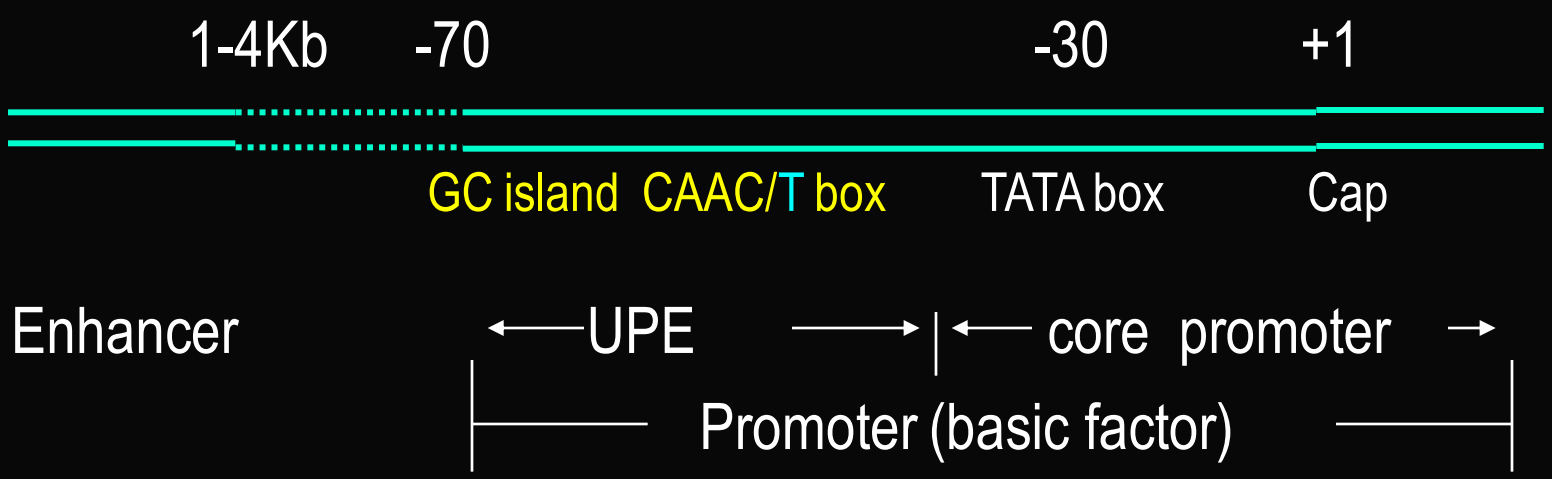
Promoter for basic transcription

- GC island (C no methylated) and CAAT box (CAT box UPE) (-70)

  GCGC-----GGC(T)CAATCT----

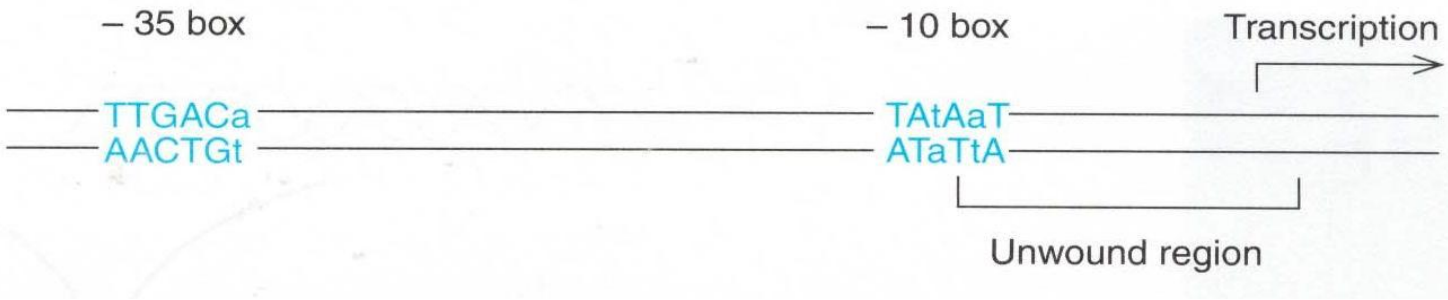
  Response to effect of transcription

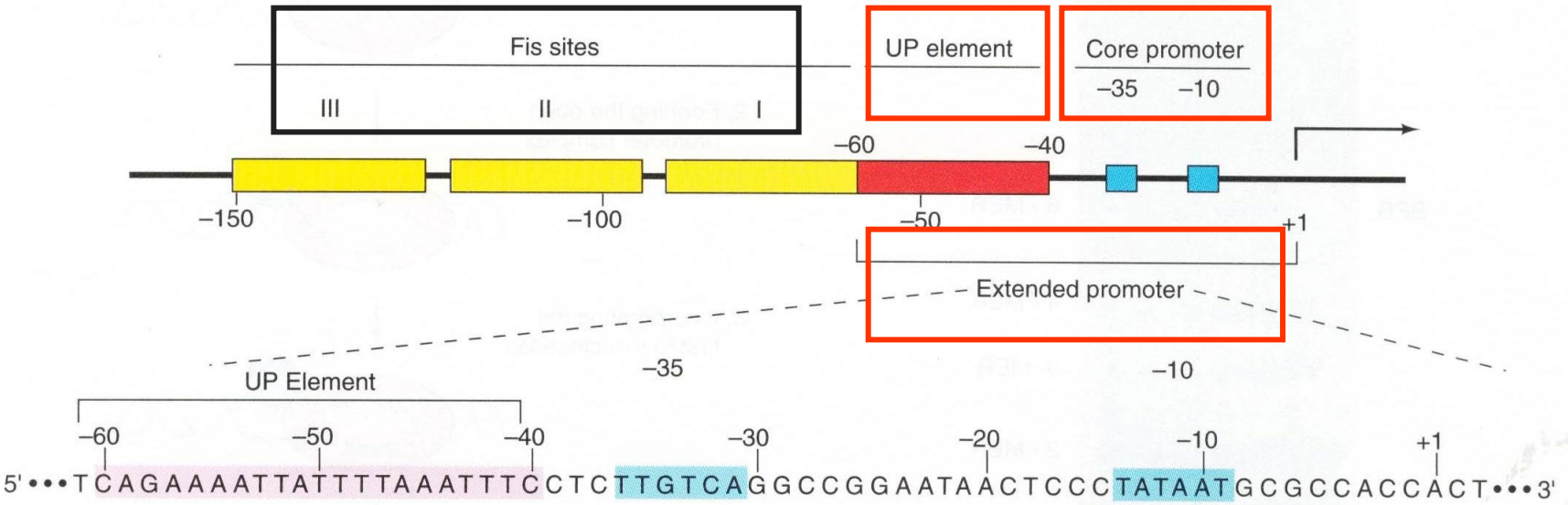
  Range 30 bp ±



注意与Prok. *E.coli* Promoter 结构与功能的比较

a) promoter 由两个重要部分组成

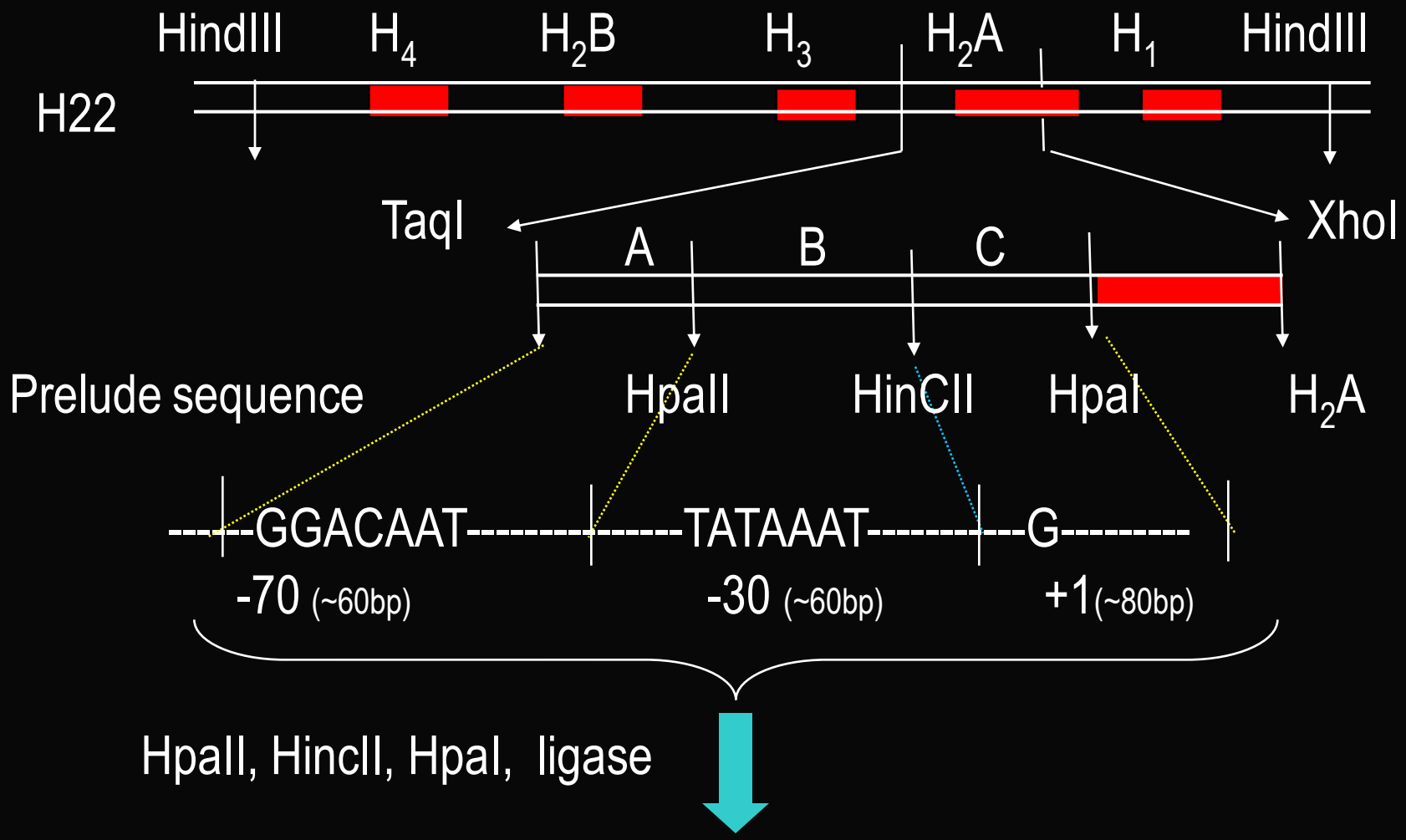




Source:Ross et al.,A third recognition element in bacterial promoters :Dna binding by the alpha subunit of RNA polymerase. Science 262(26 Nov 1993) f.2,p.1408

First identifying of Promoter's function in Euk.

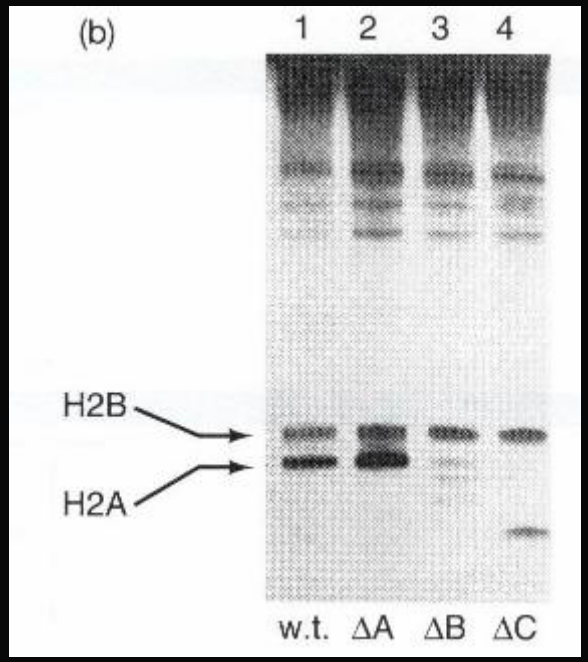
Grosschedl & Max Bernsteil (1980) Sea Urchin *in vivo*



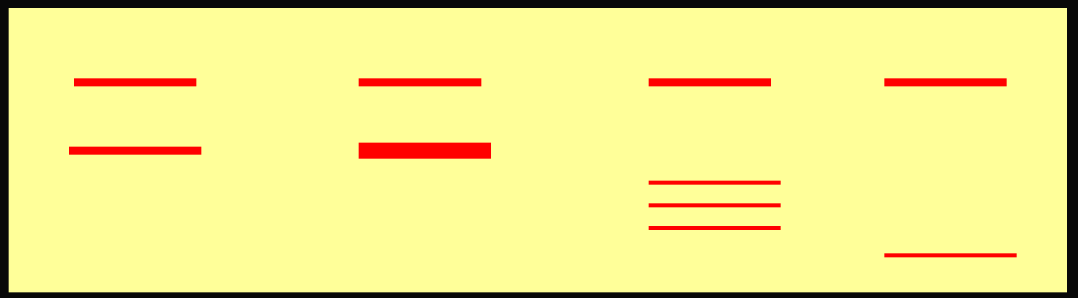
△A(△B, △C) H_2A fragment of H22 of *Sea Urchin*

Xenopus leavis (*frog*) oocytes

GTP$^{\alpha 32}$, purifying RNA & electrophoresis

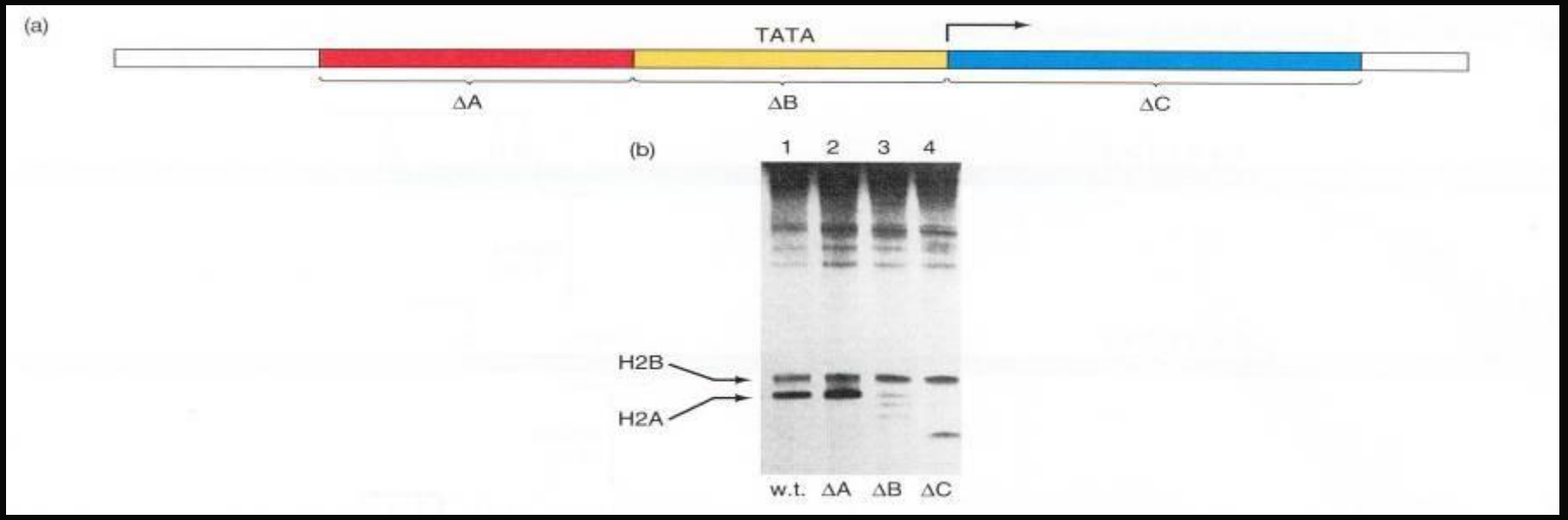


W.t H22 △A H_2A △B H_2A △C H_2A



1.0 1.9 0.22 0.24 H2A/H2B

Source:Grosschedl and Bimstiel PNAS 77 (Mar 1980) p. 1434

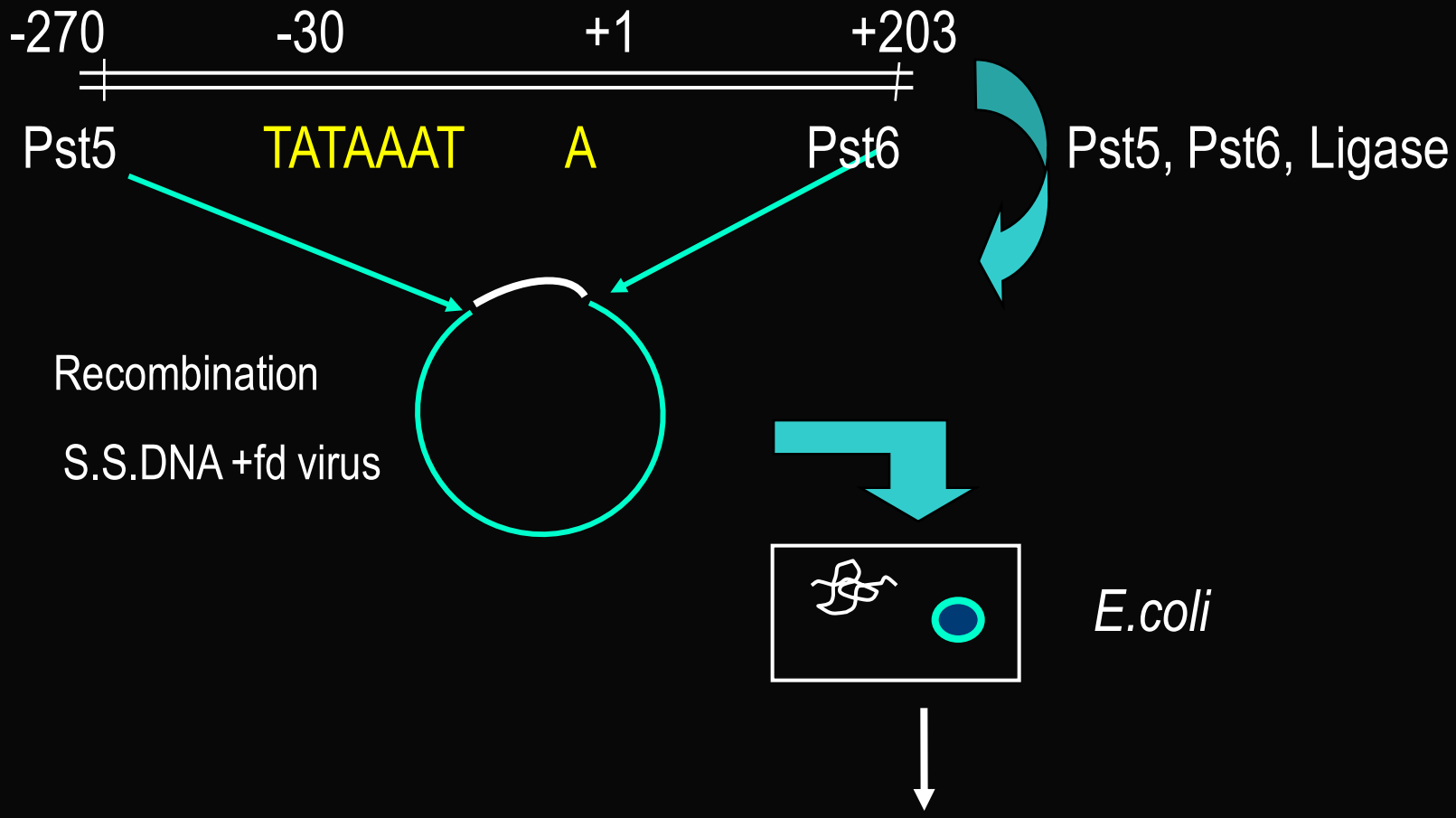


(来源：分子生物学（2007），郑用琏，第128页)

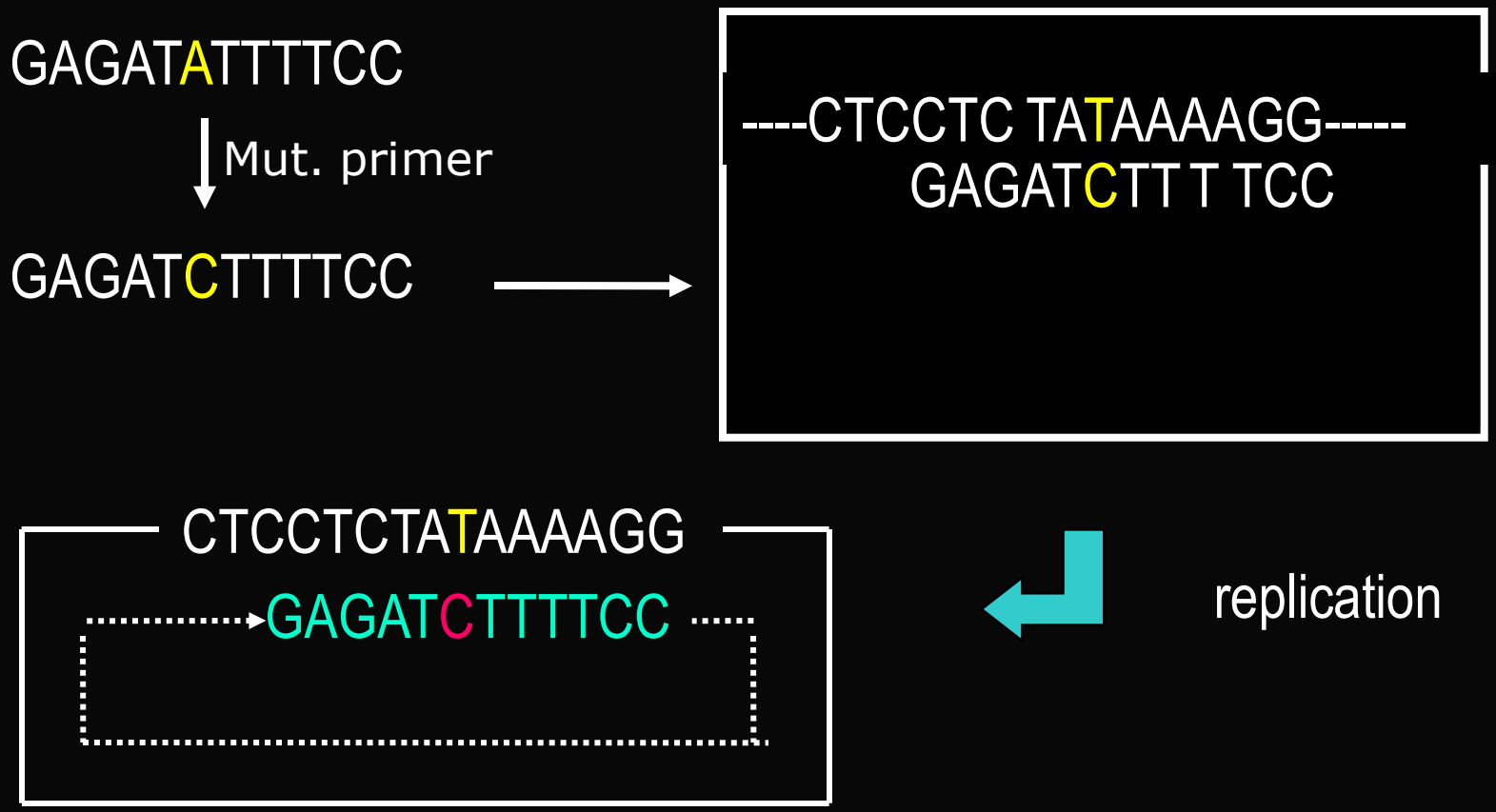
- Deletion A actually stimulated transcription
- Deletion B (including the TATA box) caused a decrease in amount of transcription and the appearance of new transcripts that result from heterogeneous start sites
- Deletion C had little effect on the amount of transcription, but shifted the start site downstream, so a shorter RNA appeared.

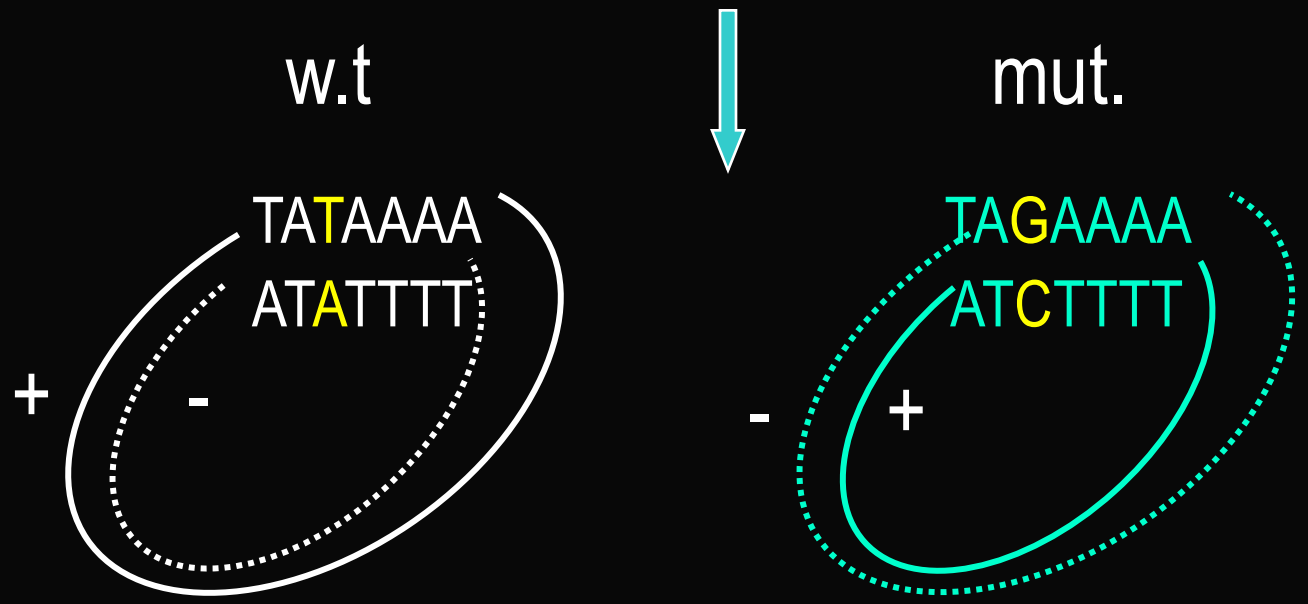
TATA box function

Hen ovalbumin gene expression (Chambon)



Adding a primer (but, A mut. to C in synthesized s. s. DNA which pairing to TATA box) into *E.coli*





transcription ovalbumin RNA

In vitro 100% 50% ?

conclusion

- 30bp from TATA box to I site is required
- Promoter functions

CAAT box (modulator) controlling transcriptional efficiency

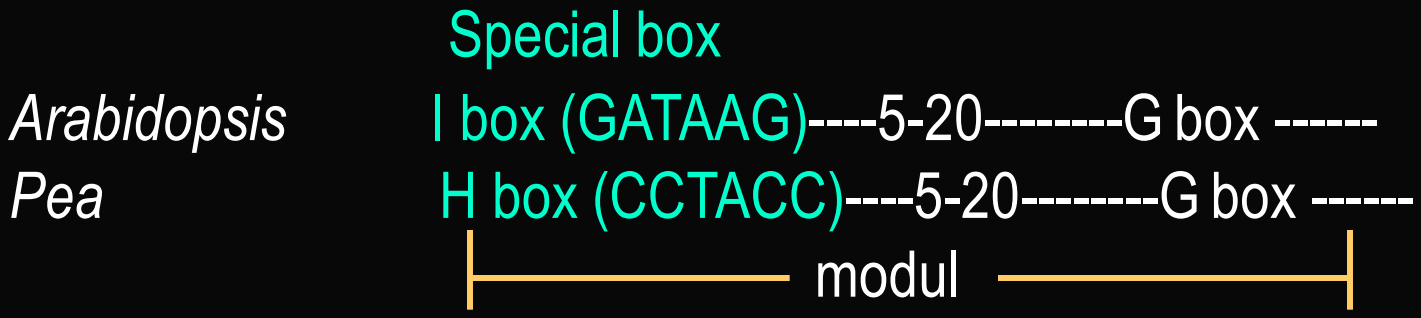
(GC island !!)

TATA box (selector) controlling starting point and efficiency

I site (initiator) determine initiation point and capping of mRNA

4.2.2.2. Cis-factor for inducible expression

- Inducible expressed by environment **Hotspot**



HSE (Heat Shock Element)
GRE (Glucocoricoid Response Element) 糖皮质激素应激元件
MRE (Metal Response Element)
TSE (Tissue Special Element)

Ig ATGGAAAT + Oct-2 factor →B 细胞表达
GH ATGAATAT + Pit-1 factor → 脑下垂体表达

Enhancer

Chambon discovered the first enhancer in the 5'-flanking region of the SV40 early gene.

★ Enhancer与Promoter的比较

Enhancer	Promoter
Enhance expression	Basic expression
Position not be fixed	isolated region
Bi-directional element	Mono-directional element
No for special gene	only for special gene

SV40 早期基因转录单位 S (*Chambon*)

(Source:Molecular Biology(2002),Robert F.Weaver,Page274）

72bp region deletion →
- transcription go down
- into upstream of β-globin gene

↓

Transcription up 200X

Behavior
- Cell & tissue specialty of Enhancer effect
- Distance effect (over 2 kb)
- Orientation-independent & position-independent
- Polarity effect

★ Enhancer 的结构与功能

----Enhancer 由两个以上的增强子成分(Enhancer Element)组成

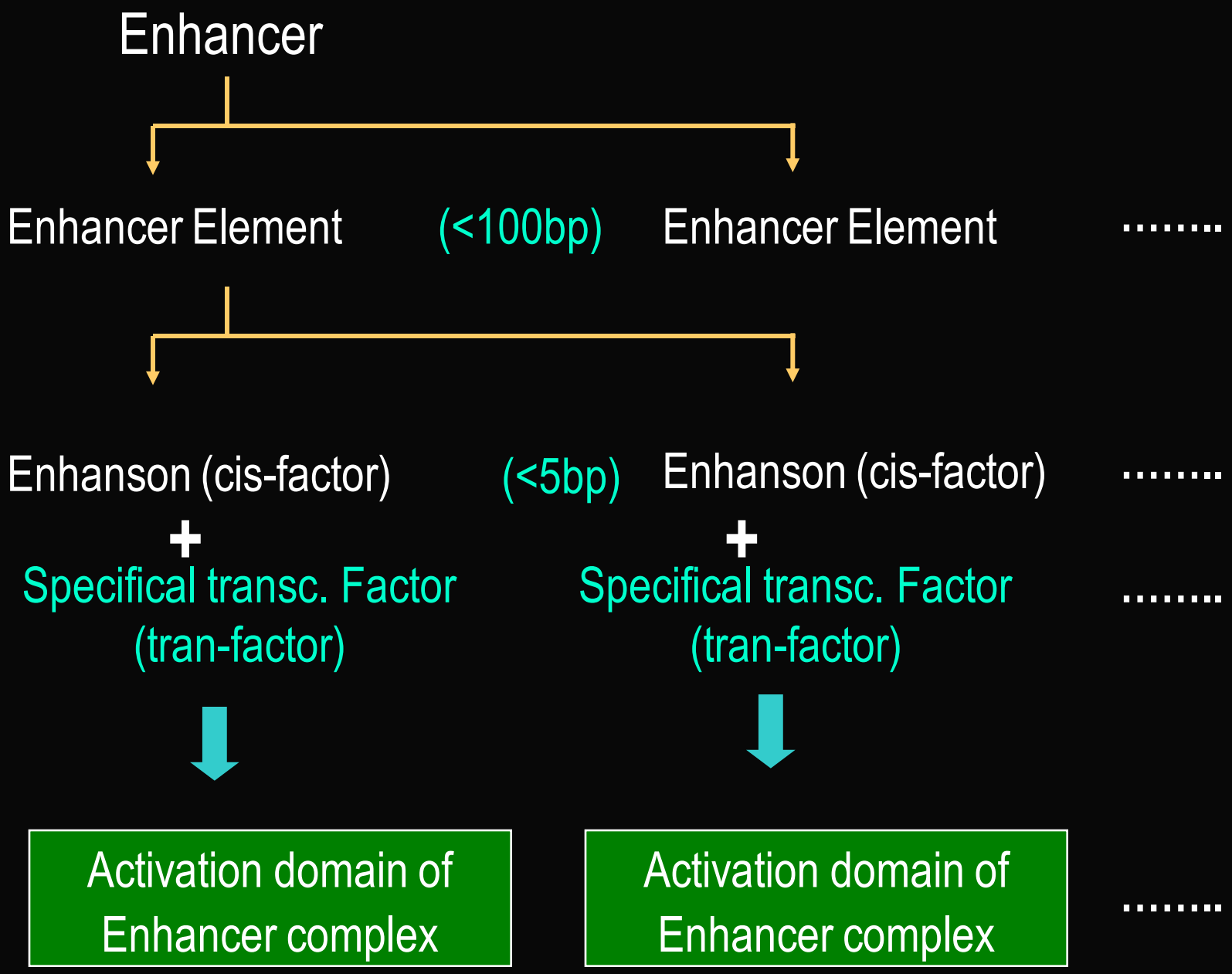
----Enhancer Element 必需由两个紧密相连,具有间距效应的增强子元 (Enhanson）组成

----各个Enhanson(cis-factor)与激活蛋白(trans-factor)结合

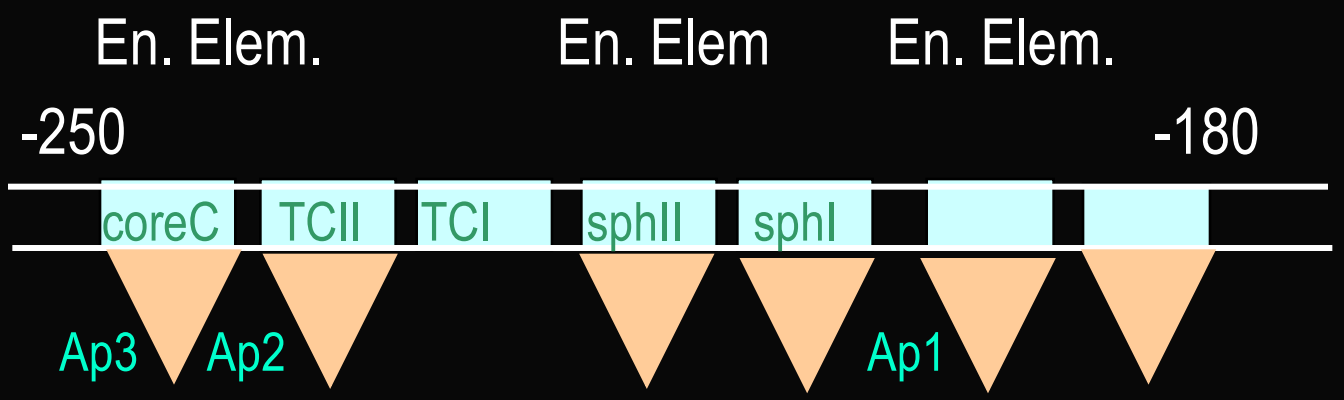
促进转录的复合体 + UPE + core promoter

基本转录复合体

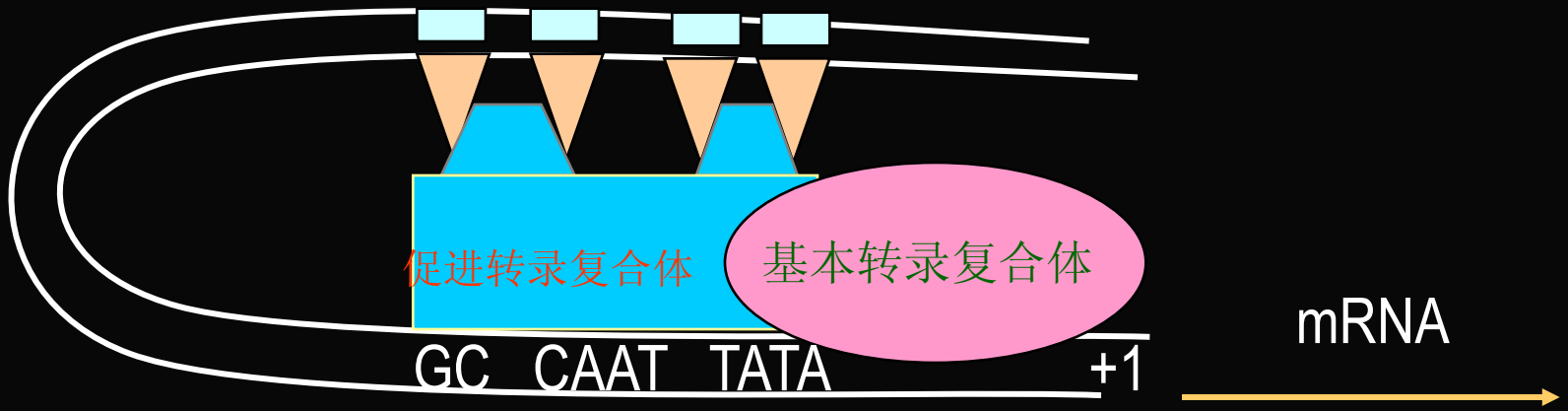
增强特异性转录

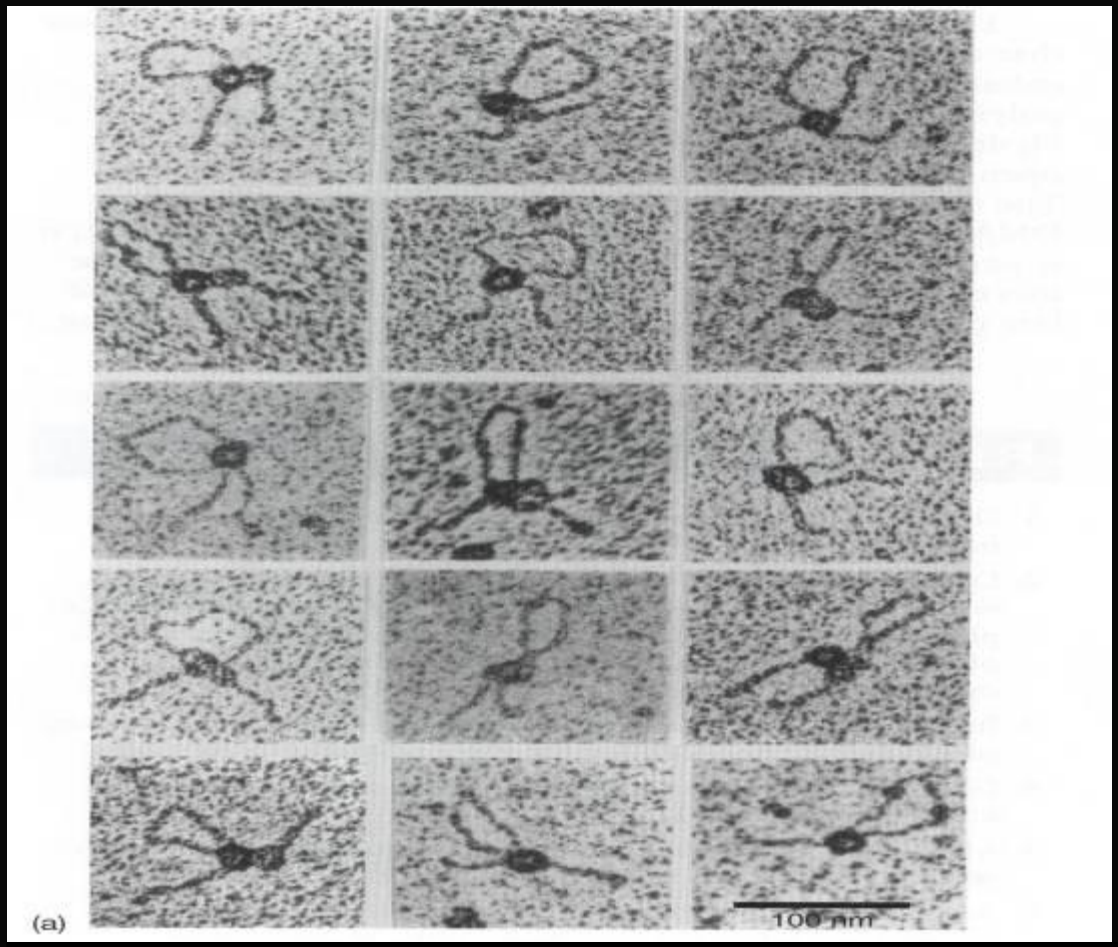

Enhancer
Enhancer Element
(<100bp)
Enhancer Element
.....
Enhanson (cis-factor)
(<5bp)
Enhanson (cis-factor)
.....
+
+
Specifical transc. Factor
(tran-factor)
Specifical transc. Factor
(tran-factor)
.....
Activation domain of
Enhancer complex
Activation domain of
Enhancer complex
.....

e.g. SV40 Enhancer (-179~ -250)



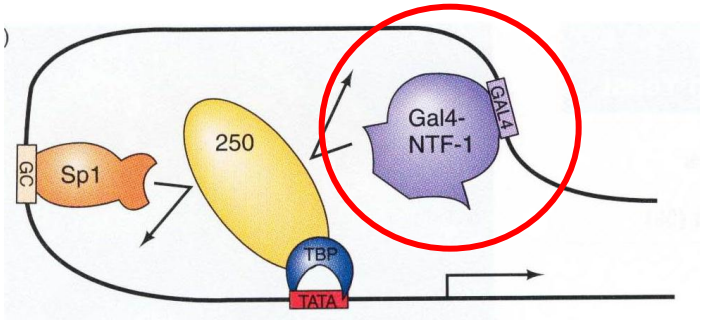
远距离控制，无方向性

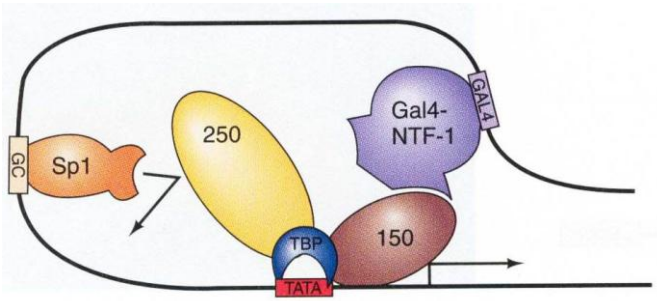


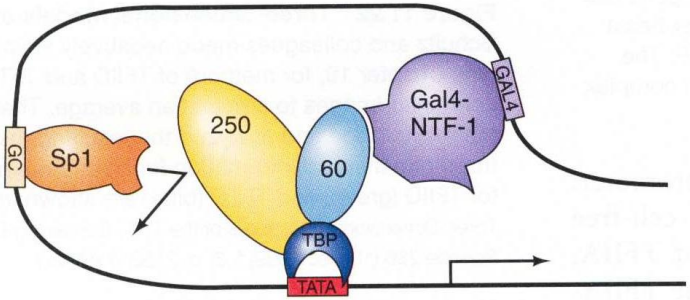


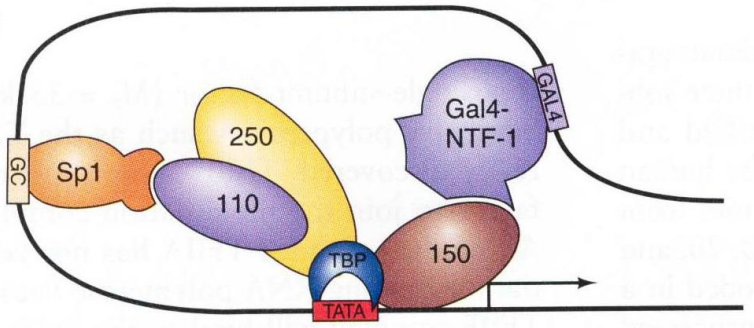
Looping of promoter-enhancer region

Source:Kustu, Echols 1990 PNAS 87 (July)

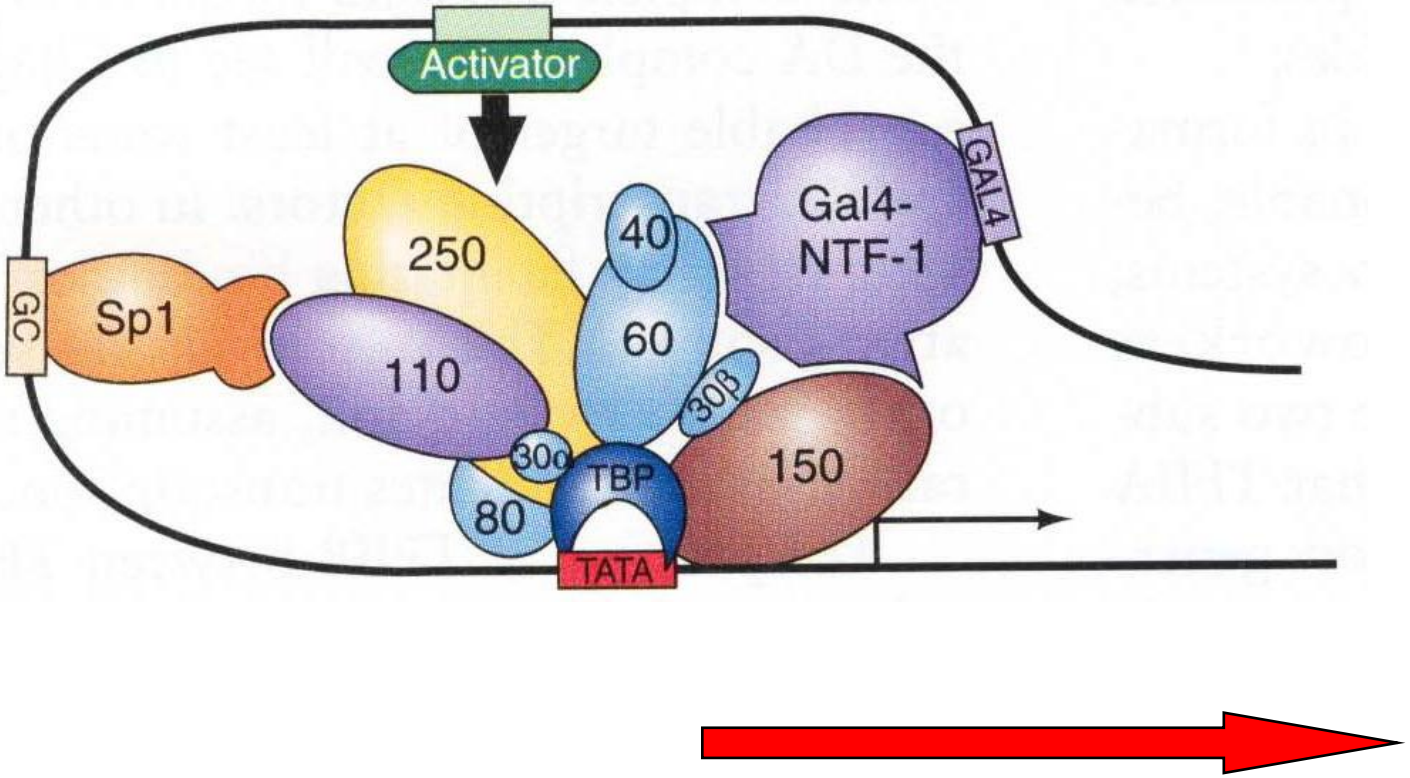








Source:Chen J.L,Assembly of recmbinant TFIID reveals differential coactivator requirements for distinct transcriptional activators.Cell 79:101,1994



促进转录的复合体 + UPE + core promoter

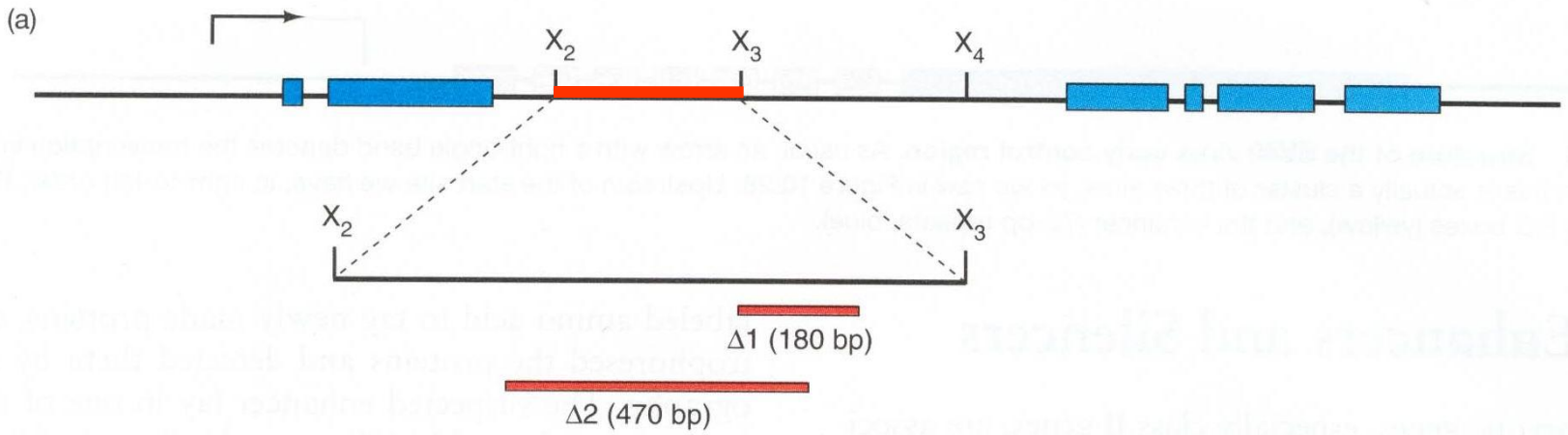
增强特异性转录

Source:Chen J.L,Assembly of recmbinant TFIID reveals differential coactivator requirements for distinct transcriptional activators.Cell 79:101,1994

---Enhancer complex与近启动子的transcriptional complex构型契合，而不与RNA polymerase 结合

--- 特化细胞内，具全部特异转录因子（trans-factor）才表现增强效应即增强子的组织特异性

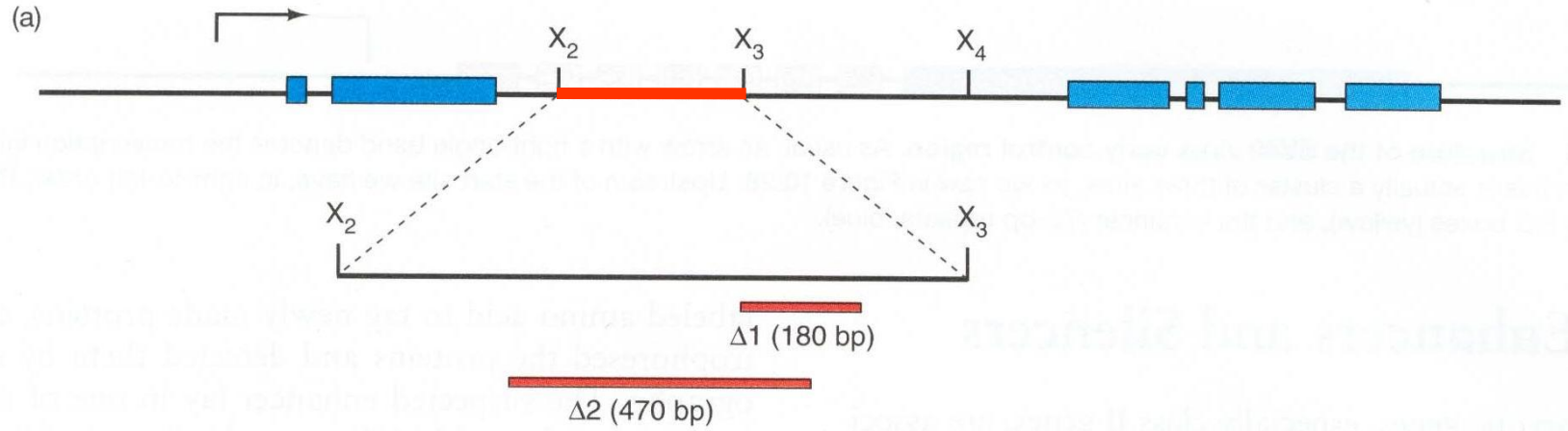
--- 增强子的复杂结构，以正控制方式防止基因表达的紊乱

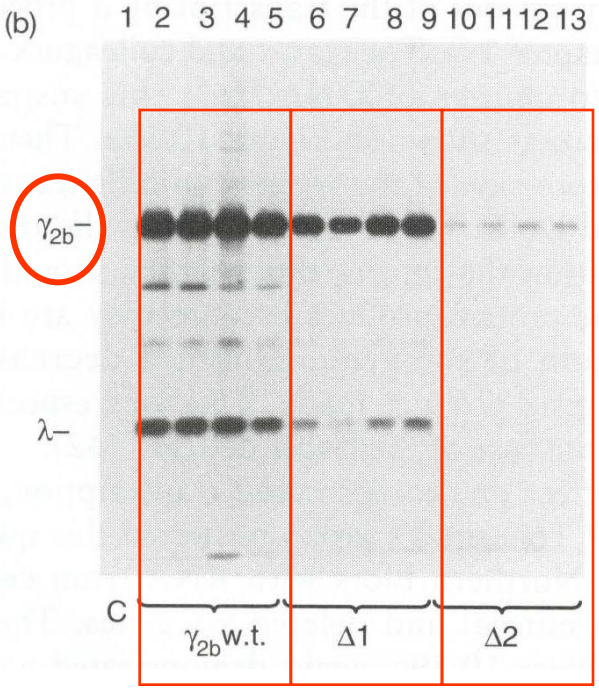


1983 Susunmu Tonegawa found an enhancer within X2-x3 of γ2b gene

- γ2b; a gene that encodes the larger subunit of a particular mouse antibody

Source: 1983 Cell 33 (July) p. 719

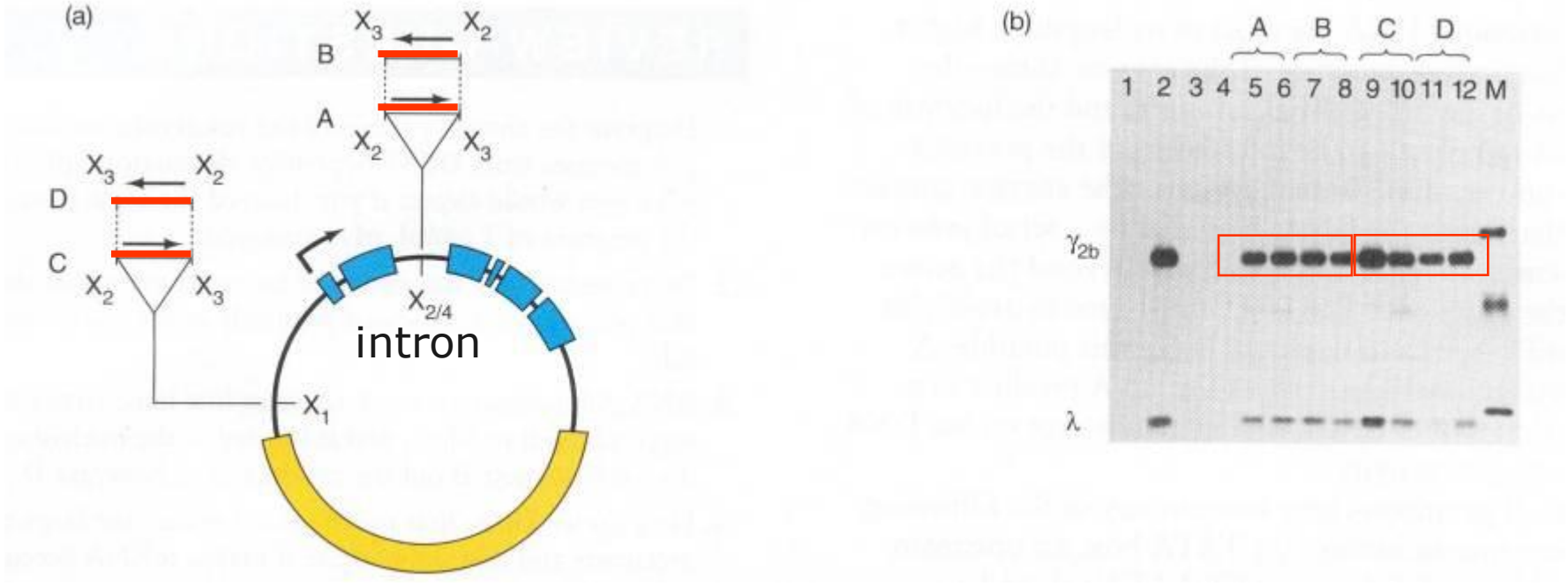




- results: The deletions within the intron should have no effect on the protein product because ….
- Why the Δ1(180bp X3), Δ2 **(**470bp X2-X3)caused a decrease in the amount of gene product made ?

Source:Gillies 1983 Cell 33 (July) p. 719

(Source:Molecular Biology(2002),Robert F.Weaver,Page276）



The enhancing element in the γ2b gene is orientation- and position-independent.

Silencer

can act at a distance (at least 1 kb away)

to modulate transcription

somehow cause the chromatin to coil up

into a condensed

Inaccessible

Inactive form

thereby preventing transcription of neighboring genes

Silencer

沉默子: 一种通过一段延伸的DNA区域影响染色质结构, 从而调节临近基因转录关闭的DNA 元件

Ogourne S. and Antalis T.M. 1998. Transcriptional control and the role of silencers in transcriptional regulation in eukaryotes . *Biochem. J*. 331:1-14

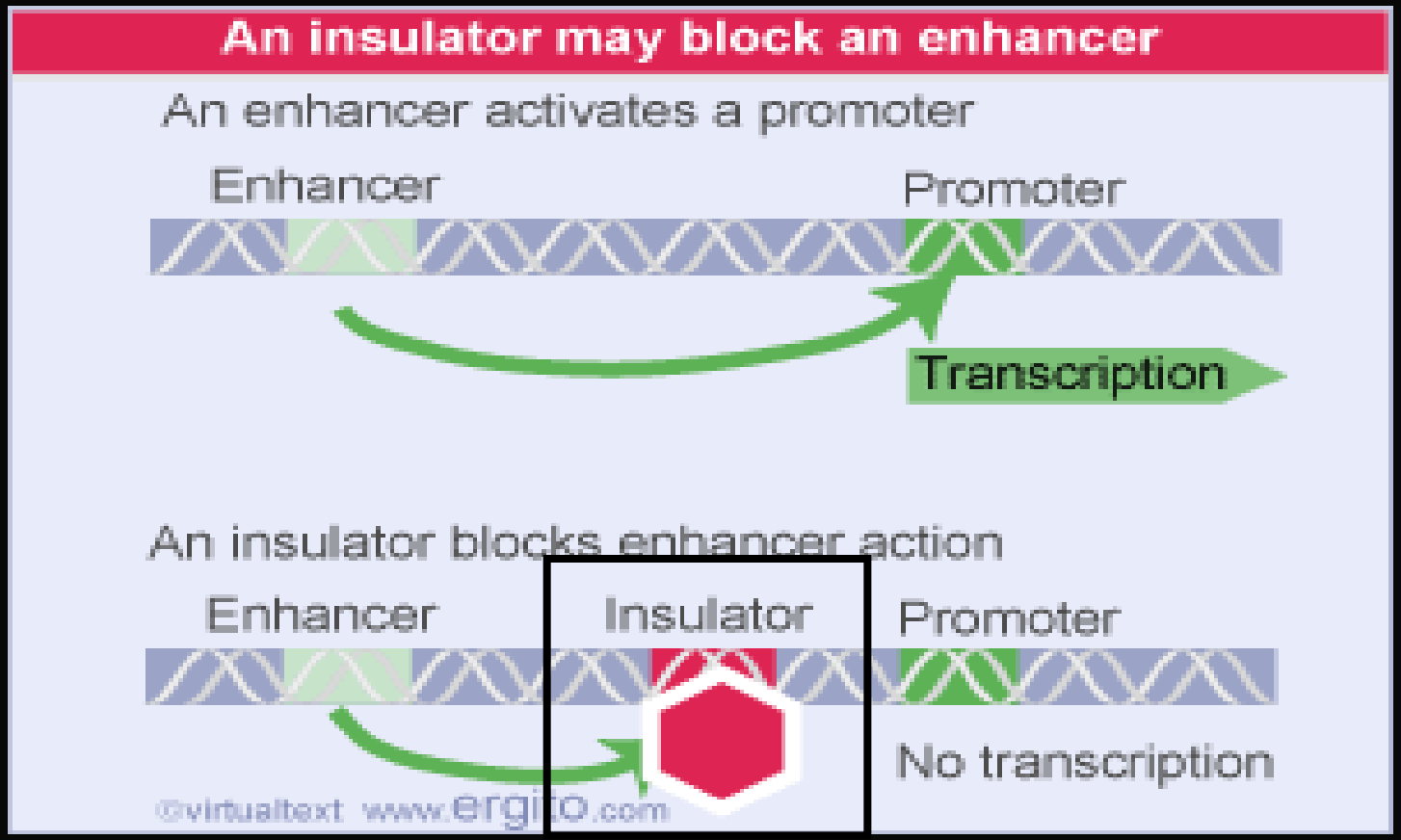
Insulator: (绝缘子)

Elements that prevent the passage of activating or inactivating effects

能够阻断激活或失活效应通过的元件

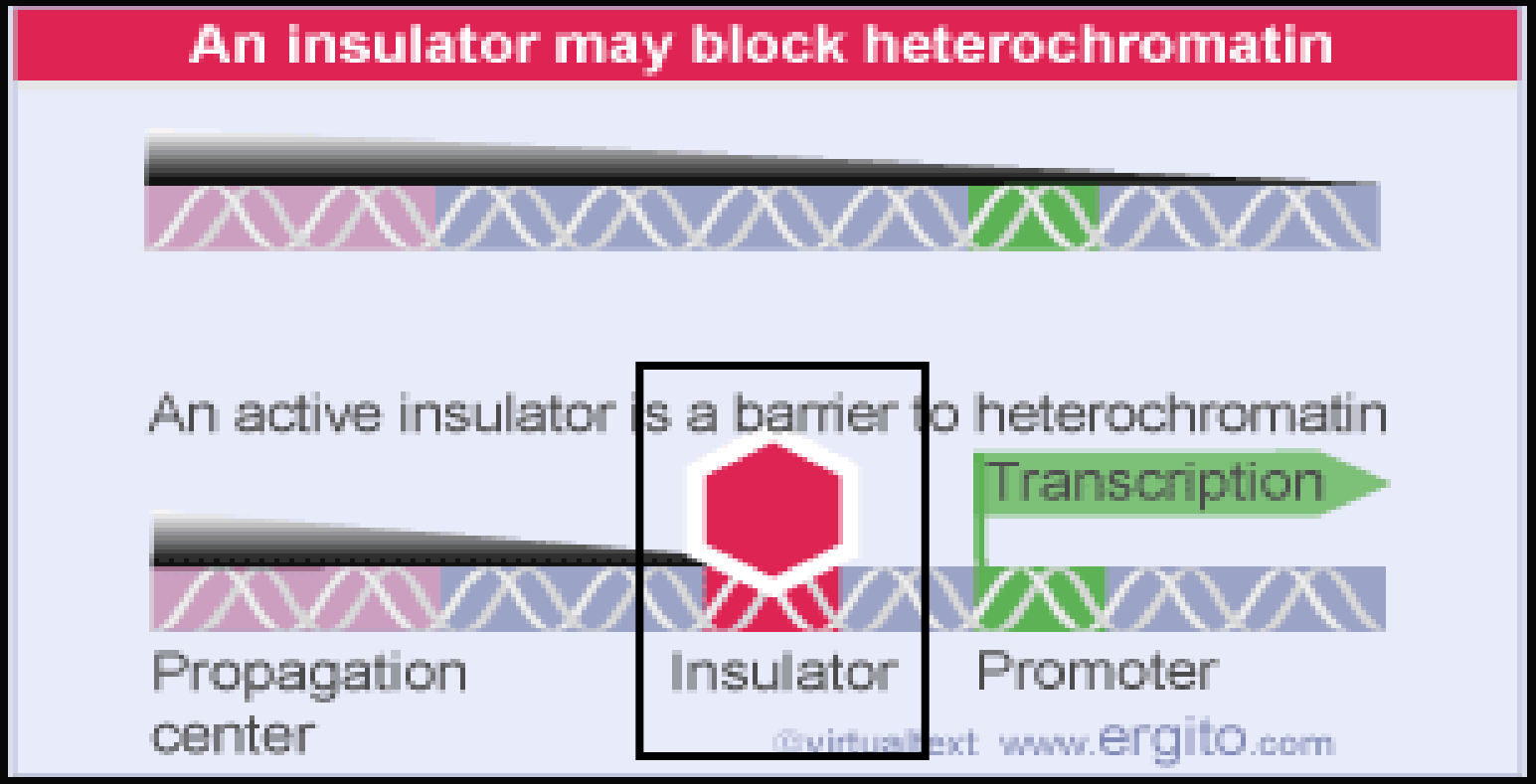
具有两种功能:

- When an insulator is placed btw enhancer & promoter , it prevents the enhancer from activating the promoter



(来源：分子生物学（2007），郑用琏，第146页)

• When an insulator is placed btw active gene & heterochromatin , it provides a barrier that protects the gene against the inactivating effect that spreads from the heterochromatin



(来源：分子生物学（2007），郑用琏，第146页)

4.2.3. 与基本转录相关的T.F.

General transcription factors

真核生物中RNApol的作用必须有其他相关转录蛋白在启动子处的先期结合才能启动转录

Eukaryotic RNA polymerases are incapable of binding by themselves to their respective promoters.

Instead, they rely on proteins called transcription factors to show them the way. Such factors are grouped into

Two classes:

- general transcription factors
- gene-specific transcription factors.

Two classes:
general transcription factors (GTF)
gene-specific transcription factors (STF)

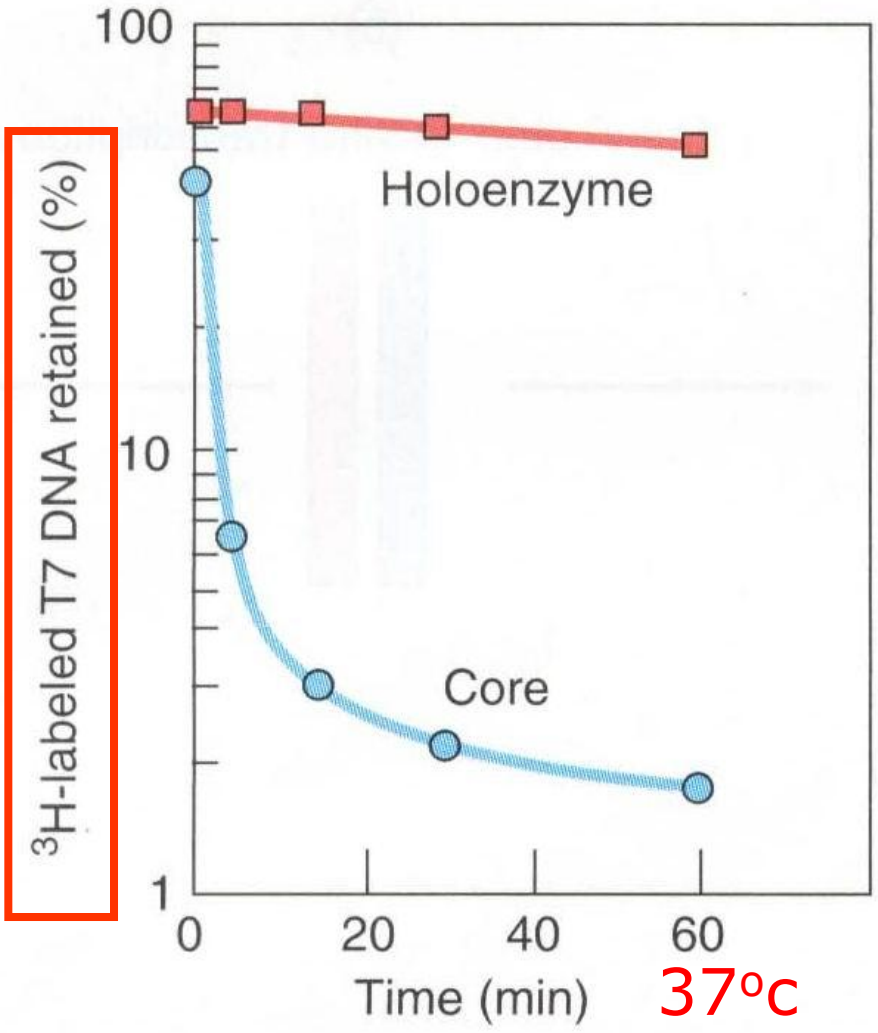
- The general transcription factors

GTF can attract the RNA polymerases to their respective promoters, but only to a weak extent. Therefore, these factors can stimulate only a basal level of transcription.

H^3-T7 DNA + Holo E/Core E

Sigma stimulates tight binding between RNA polymerase and promoter

Monitor H^3-T7 DNA/E retained



Source:D.C.and Chamberlin,M.J.,Journal of Molecular Biology ,Vol.70,157-86,1972

Sigma stimulates tight binding between RNA polymerase and promoter

与基本转录相关的T.F.

General transcription factors (GTF)

真核生物中RNApol的作用必须有其他相关转录蛋白在启动子处的先期结合才能启动转录

4.2.3.1 General T.F. for basic transcription

- TF (I, II, III) Transcriptional Factor
- TBP (TATA box Binding Protein)
- TAF (TBP Associate Factor)
- RAP (RNApol. Associate protein)

TIC (Transcriptional Initiate Complex)

4.2.3.2. RNA polymerase in Eukaryotes

RNApol. I, II, III

⟶ Including L, L' subunit & 7-12 small subunits

L' with 78%±homologous

between 3 RNApol. I, II, III

L'~ β'of prokaryote RNApol. & L ~ β

(功能相似)

进化渊源

RNApol II (B);

---For pre-mRNA transcription

---Located in nucleoplasm

---L' maximum subunit 240 kd & have specific COOH-end

named CTD

(Carboxyl Terminal Domain) only in RNApol II

---CTD-end;

7aa repeats & high frequency phosphorylation

Tyr—Ser^{P}—Pro—Thr^{P}—Ser^{P}—Pro—Ser^{P}

Yeast 26X
Drosophila 44X
Rat & Human 52X

} repeat unit of 7 aa / in CTD

---依CTD中，Ser,Thr 磷酸化与否，将L' 分为3个Subforms

II_O (240kd)

高度磷酸化

CTD over-phosphorylated

II_A (220kd)

基本结合型

Initially binds to promoter

II_B (180kd)

蛋白酶水解

Non-physiological form

使RNApol易于离开Promoter → 转录延伸

提高转录效率10X II_A

---RNApol II + >20TFIIs

TIC → 转录启动

(Transcriptional Initiation Complex)

TFIID; A sort of protein complex (TBP & > 8 TAF)

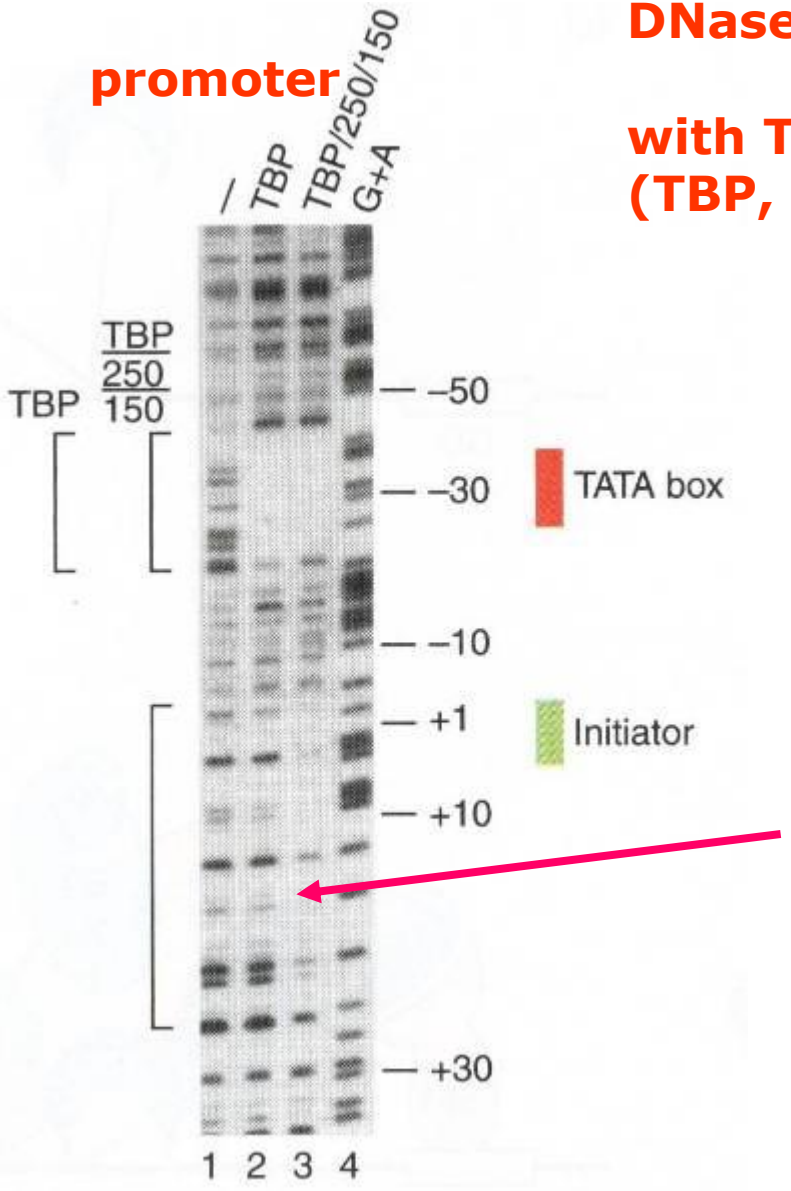
(TBP); needed for RNApol I, II, III

Very high conserved C-end domain of 180 aa

Binds with DNA in minor groove & wilds it

Determination initiation starting site

Control UPE effect for basic transcription



DNase I footprinting the hsp70

with TBP and the ternary complex (TBP, TAFII250, and TAFII150)

1, no protein
2, TBP
3, ternary complex
4, marker.

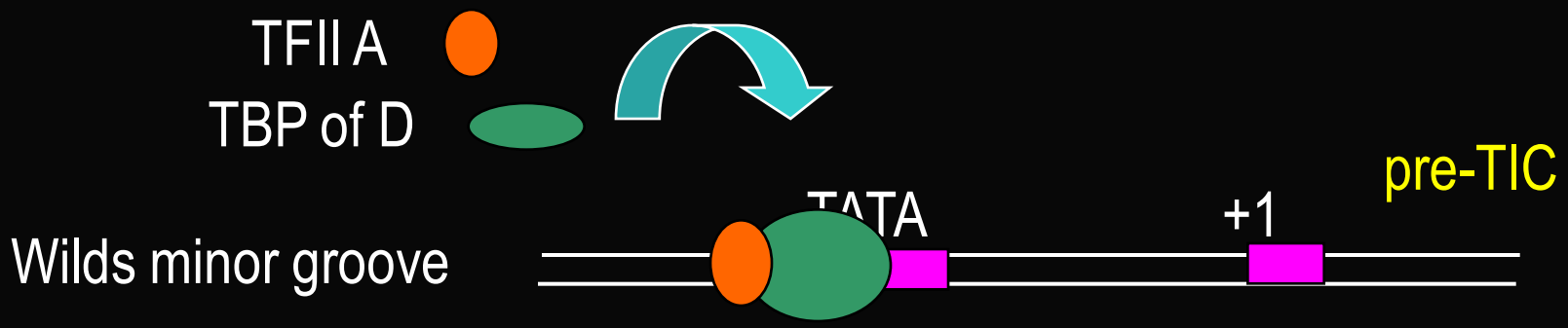
In both lanes 2 and 3, TFIIA was also added to stabilize the DNA-protein complexes, but separate experiments indicated that it did not affect the extent of the footprints

(source:Verrijzer et al Cell 81 (30 June 1995) p. 1117)

TFIIA; Binds to TFII D

Enhances TFII D binding to TATA box

Stabilizing the DNA-TFII D complex



TFIIB; Once TFIID has bound to DNA

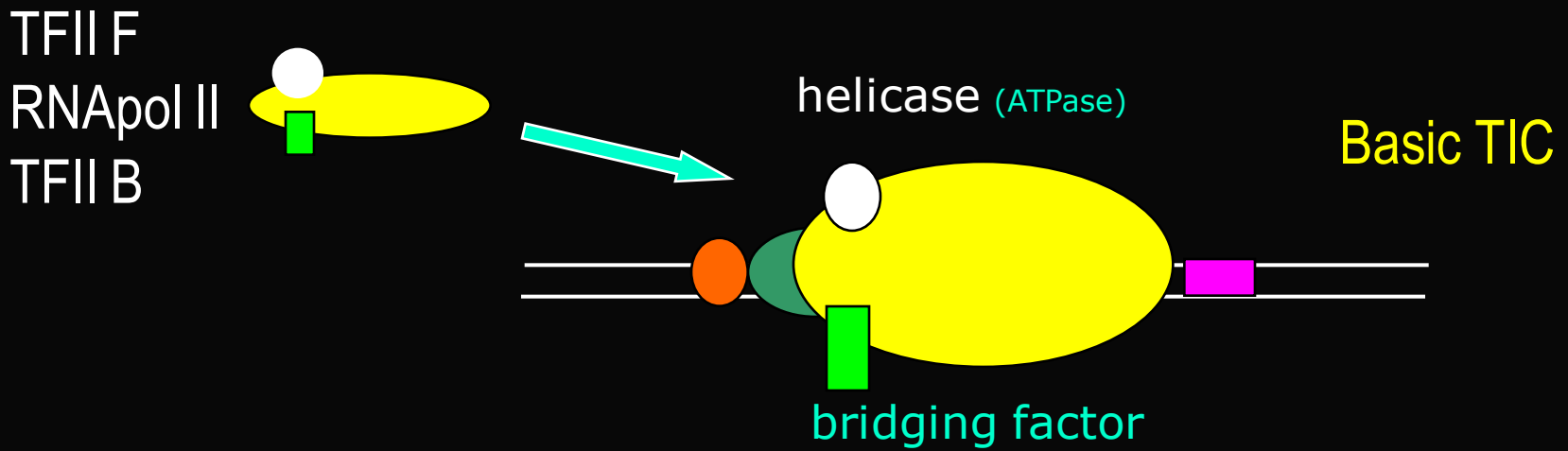
TFIIB binds to TBP of TFIID and RNA pol. II

TFIIB as a bridging factor allowing recruitment of RNApol II to TIC together with RAP30 of TFIIF

TFIIF; Including two subunits of RAP30, RAP74

Binding and recruiting RNApol II to assemble TIC

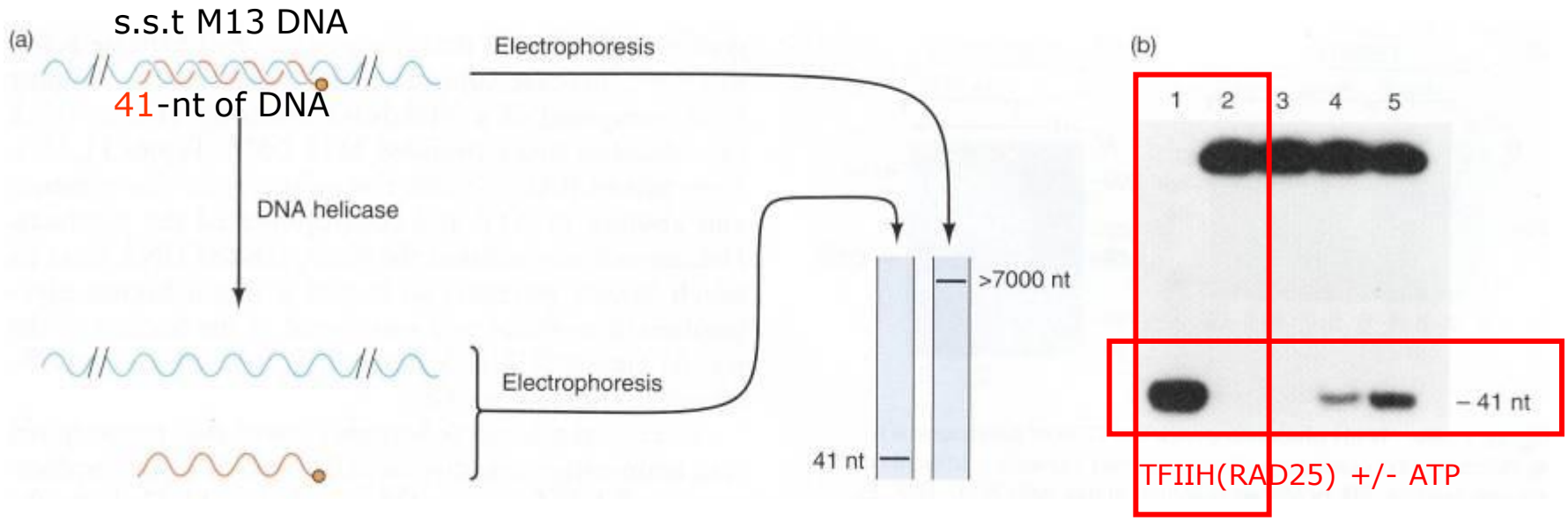
Promoting RNA elongation by its helicase (ATPase)



TFIIH; Large complex made up of > 5 subunits

Helicase activity (ATPase, RAD25…) & kinase activity Phosphorylation of CTD of RNApol II_O

DNA repair



Helicase activity of TFIIH.

(a) The helicase assay. The substrate consisted of a labeled 41-nt piece of DNA hybridized to its complementary region in a much larger, unlabeled, single-stranded M13 phage DNA . DNA helicase unwinds this short helix and releases the
labeled 41 -nt DNA

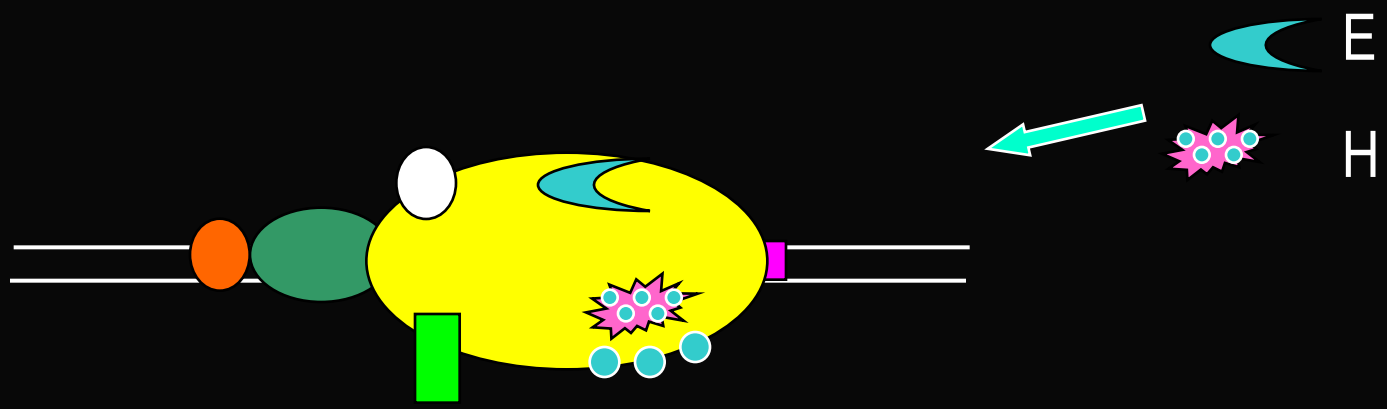
(b) Results of the helicase assay. (1), heat-denatured substrate; (2), no protein; (3), 20 ng of RAD25 with no ATP; (4), 10 ng of RAD25 plus ATP;(5), 20 ng of RAD25 plus ATP.

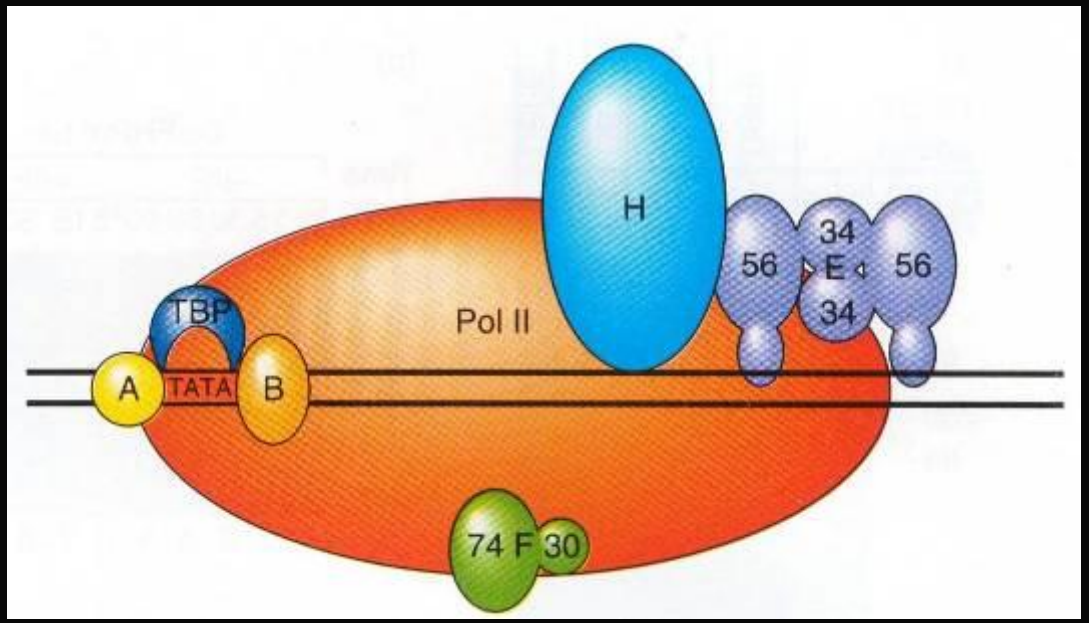
TFIIH(RAD25)

(Source:Gudzer et al. Nature 369 (16 June 1994) p. 579, f. 2c.)

TFIIH; Large complex made up of > 5 subunits
Helicase activity (ATPase, RAD25…) & kinase activity Phosphorylation of CTD of RNApol II_O
DNA repair

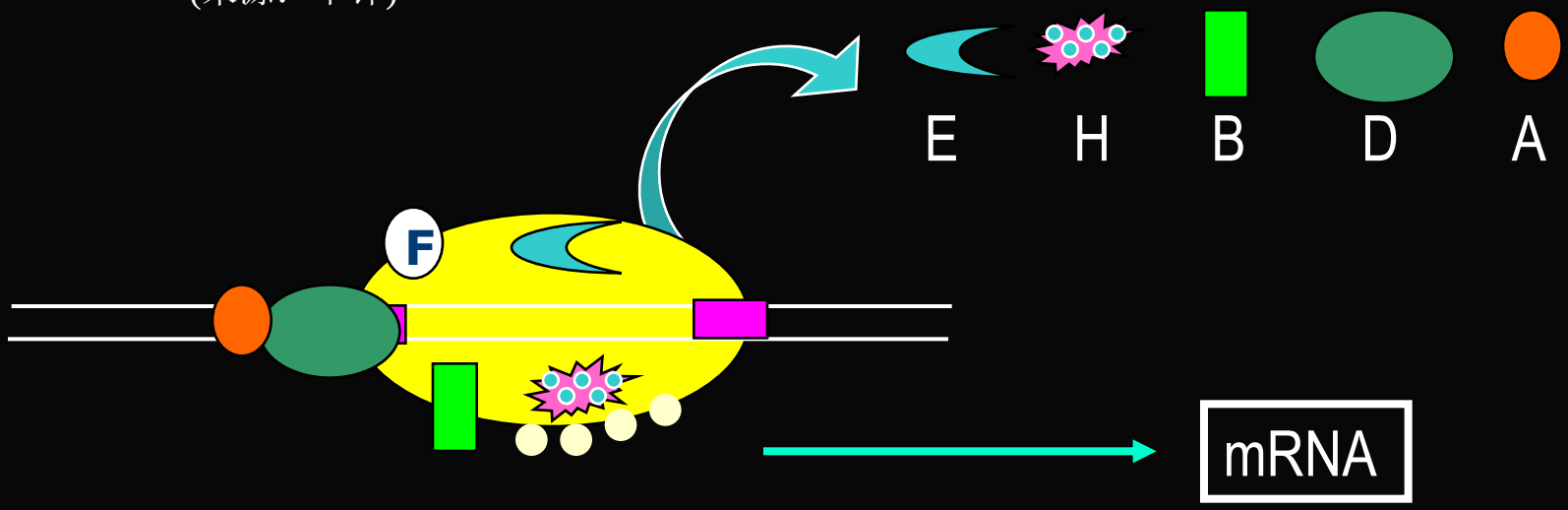
TFIIE; Associate with TFIIH kinase





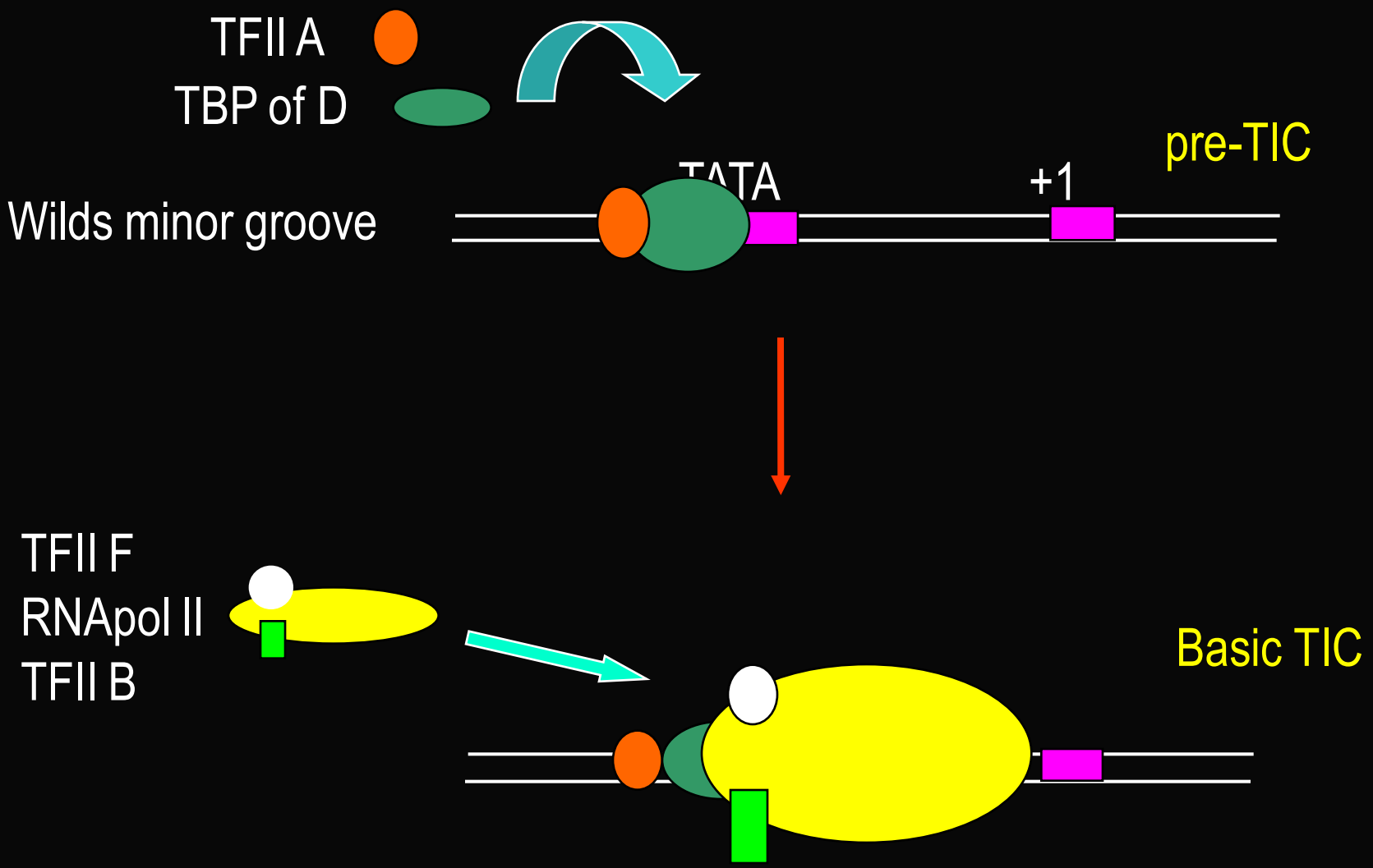
(来源：不详)

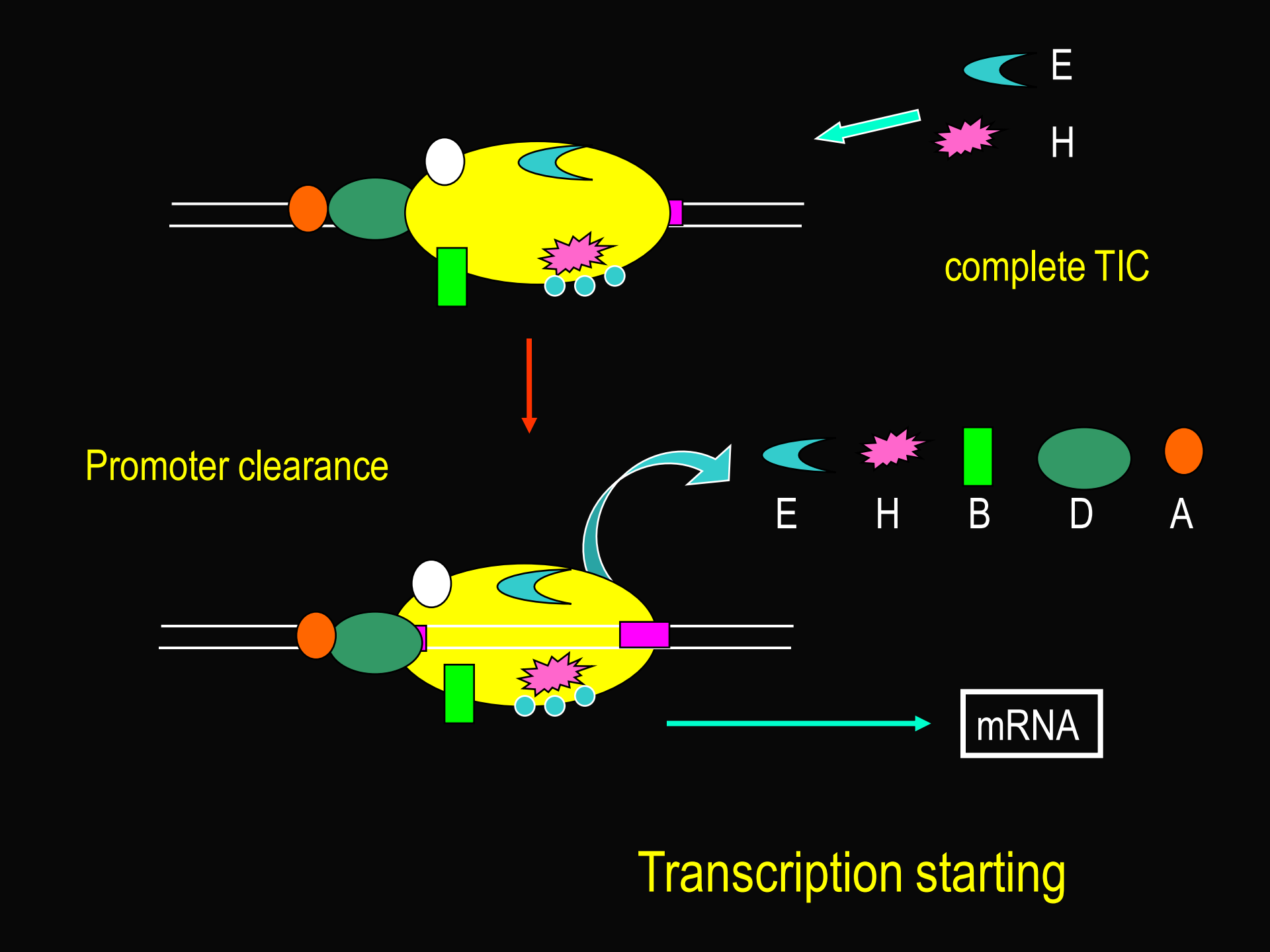
Promoter clearance

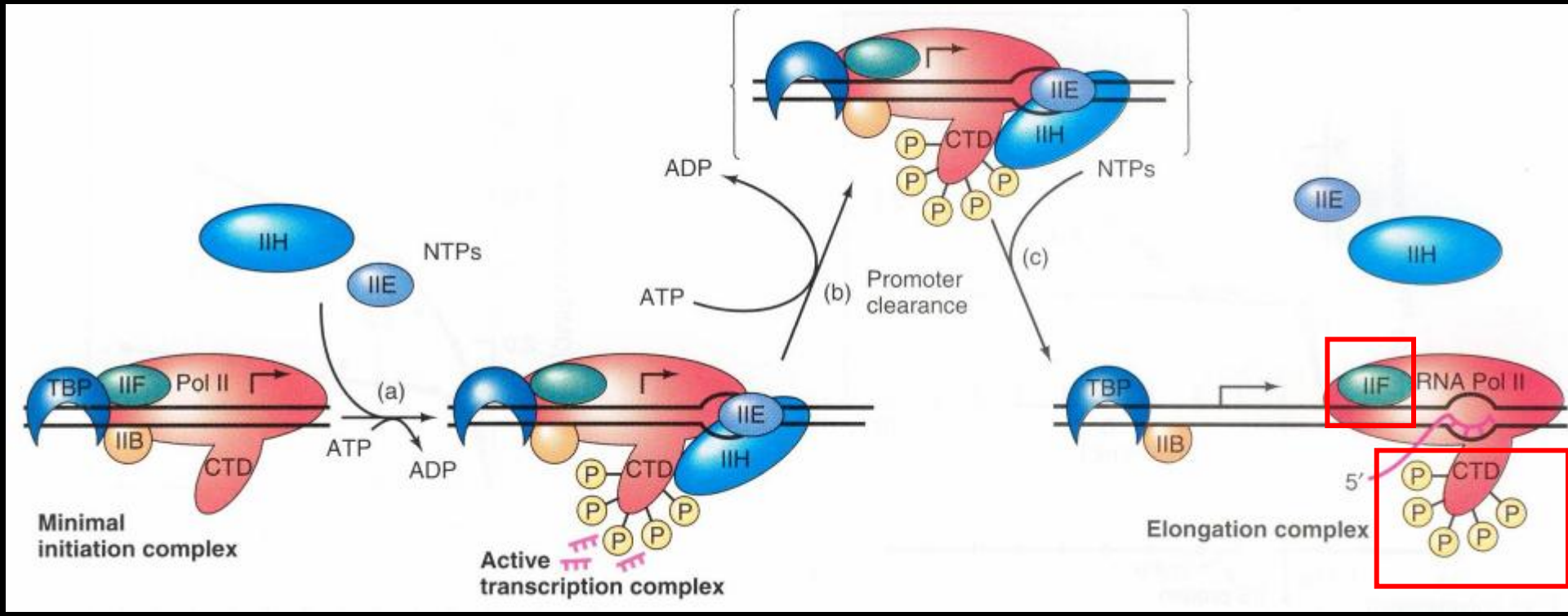


Transcription starting

conclusion


TFII A
TBP of D
pre-TIC
TATA
+1
Wilds minor groove
TFII F
RNApol II
TFII B
Basic TIC


E
H
complete TIC
Promoter clearance
E
H
B
D
A
mRNA
Transcription starting



GTF + TATA box → basal level transcription

Source:J.A.and T.Tjian.1994.Transcription factors IIE and IIH and ATP hyfrolysis direct promoter clearance by RNA polymerase II.Cell 77:145-56.)

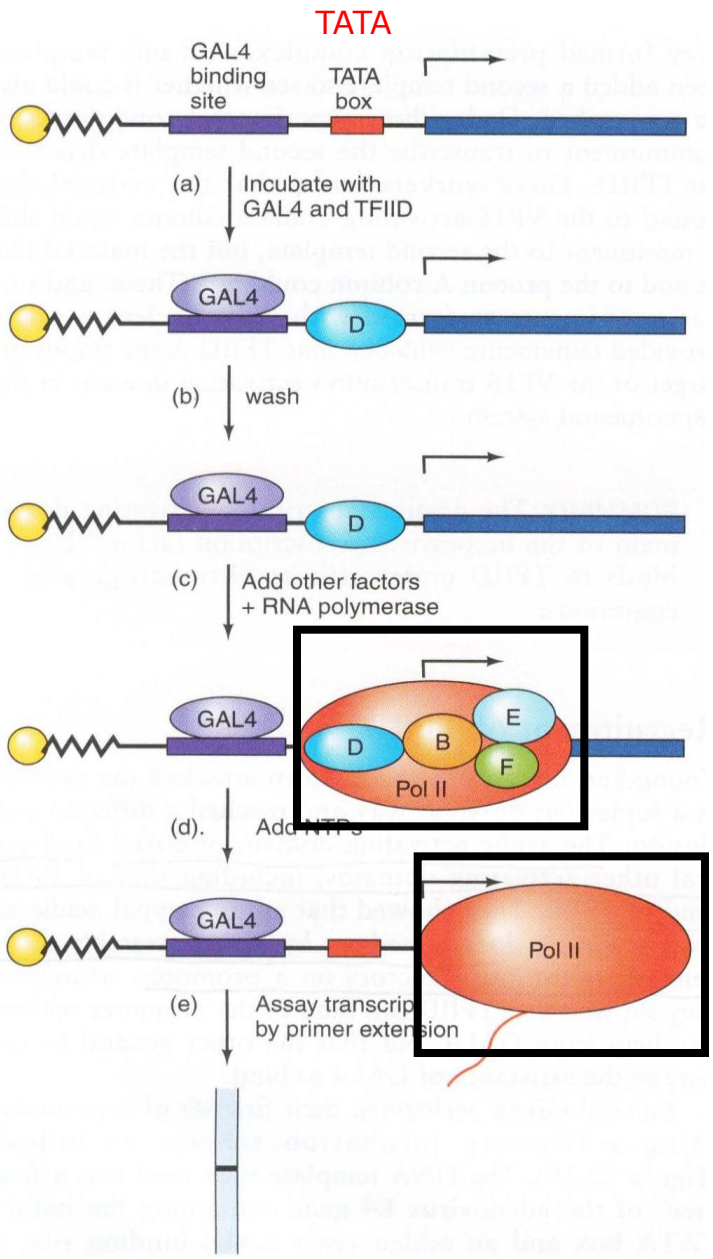
The general T.F. are capable of sponsoring only a basal level transcription.

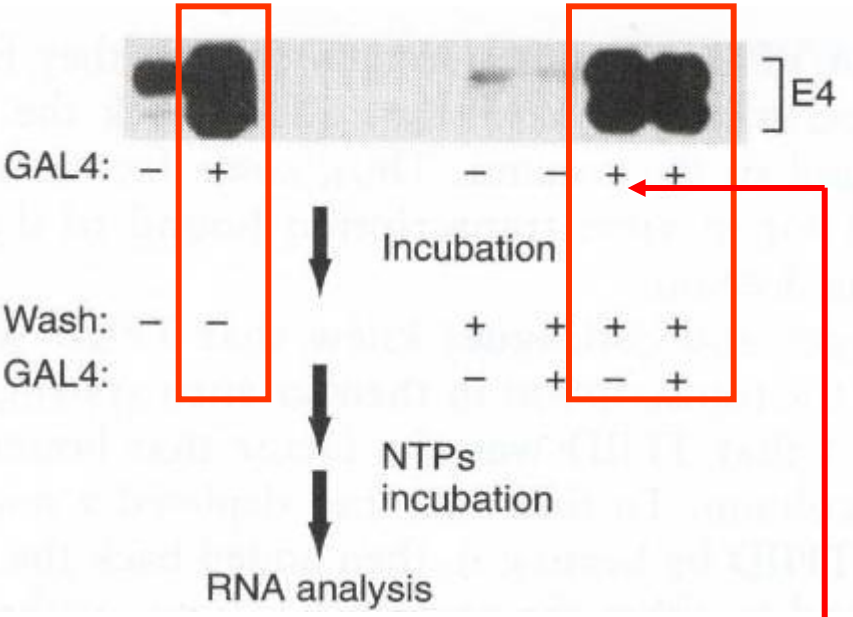
To provide the needed extra boost in transcription, eukaryotic cells have additional, gene-specific transcription factors (activators e.g GAL4).

4.2.3.3. transcription activator

(activator, STF)

for gene- specific inducible transcription





GAL4 stimulates preinitiation complex formation

(来源：分子生物学（2007），郑用琏，第139页)

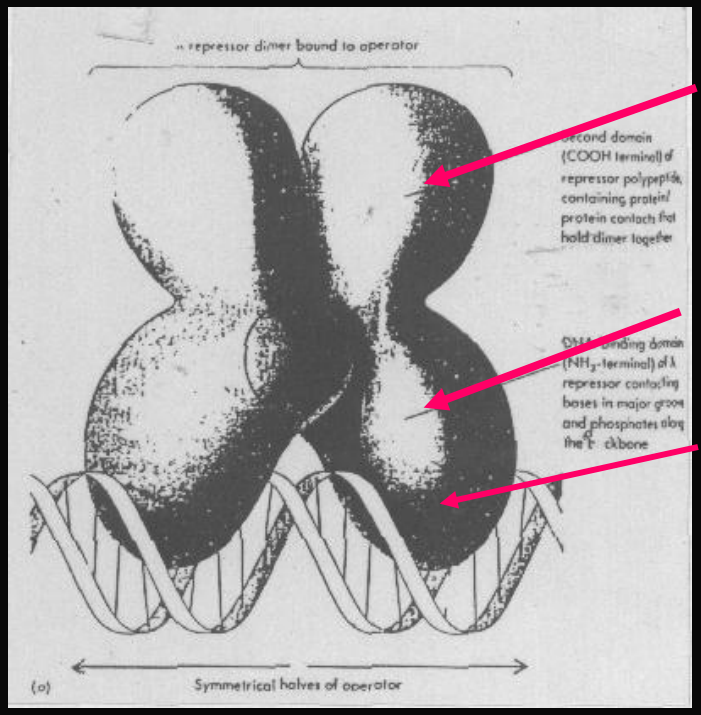
a) character of activator

- 多为变构蛋白，具有2或4个独立的功能结构域（domain）

DNA-binding domain

Transcription regulation domain

activation (or repression) domain



(来源：不详)

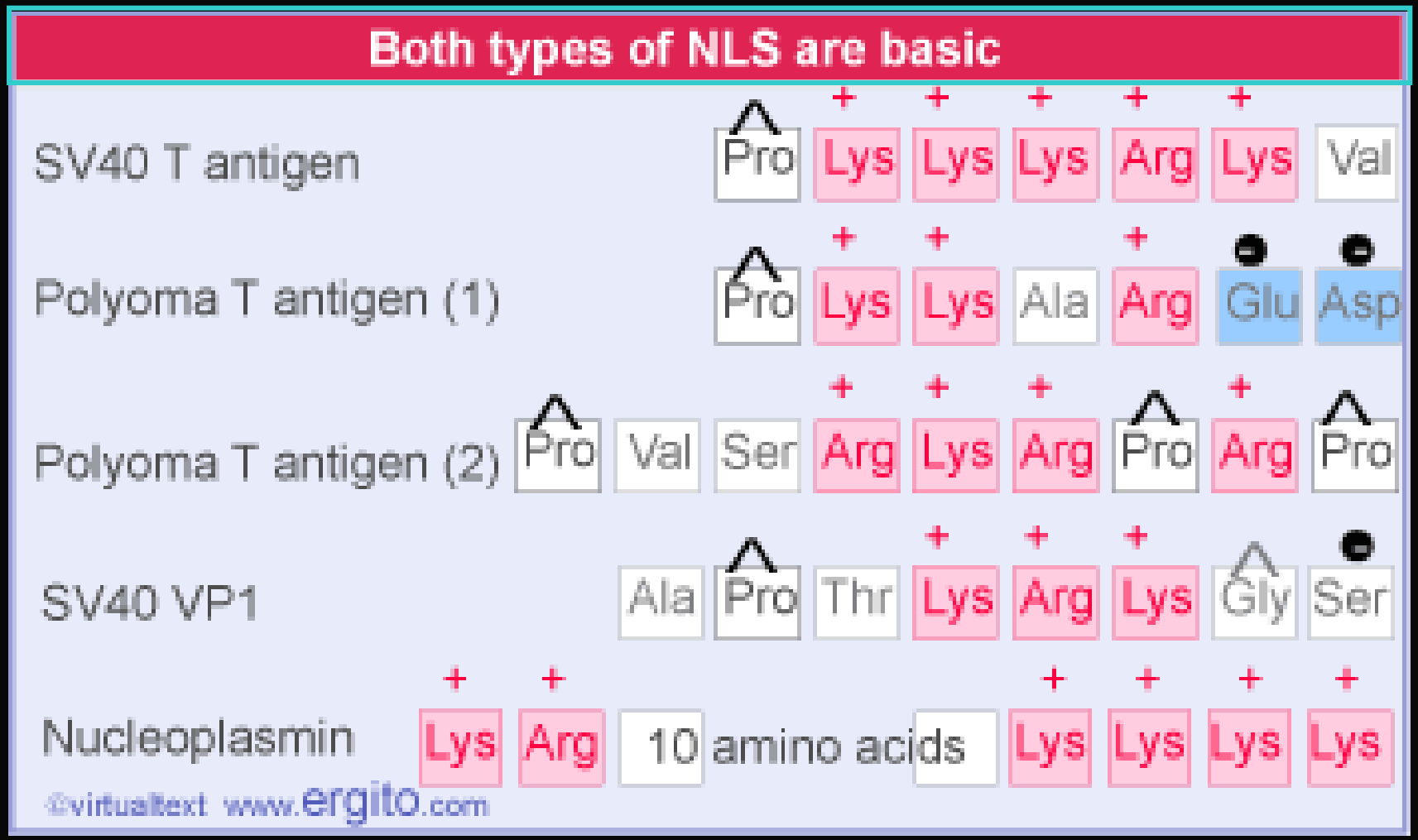
Dimerization Domain (in some dimer factor)

DNA-Binding Domain

Transcription Activation Domain

Nuclear location signal, NLS short peptide rish basic aa Arg, Lys

(or/and N Export Signal, NES)


Both types of NLS are basic
SV40 T antigen
Pro Lys Lys Lys Arg Lys Val
Polyoma T antigen (1)
Pro Lys Lys Ala Arg Glu Asp
Polyoma T antigen (2)
Pro Val Ser Arg Lys Arg Pro Arg Pro
SV40 VP1
Ala Pro Thr Lys Arg Lys Gly Ser
Nucleoplasmin
Lys Arg 10 amino acids Lys Lys Lys Lys
©virtualtext www.ergito.com

(来源：不详)

dimerization domain of activator

(in some transcription factor of dimer / tetremer factor)

Homodimers

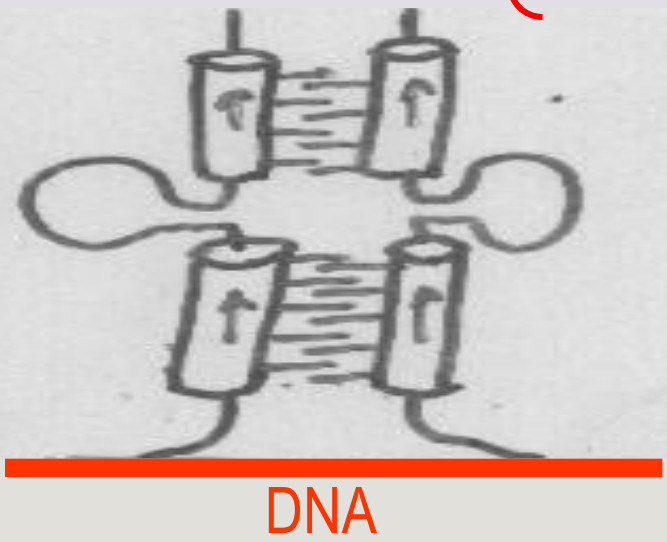
or

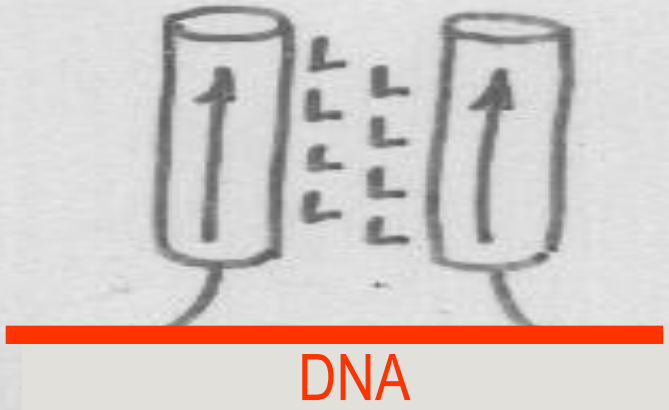
Heterodimers

} held together by dimerization domains

(Including Leu zipper

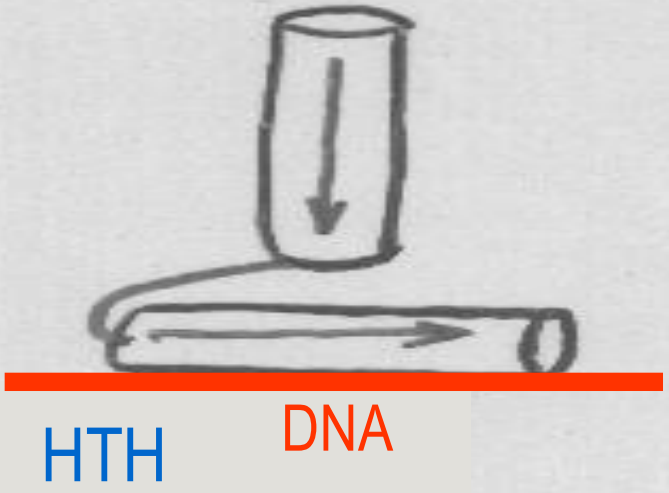
or Helix-loop-Helix)

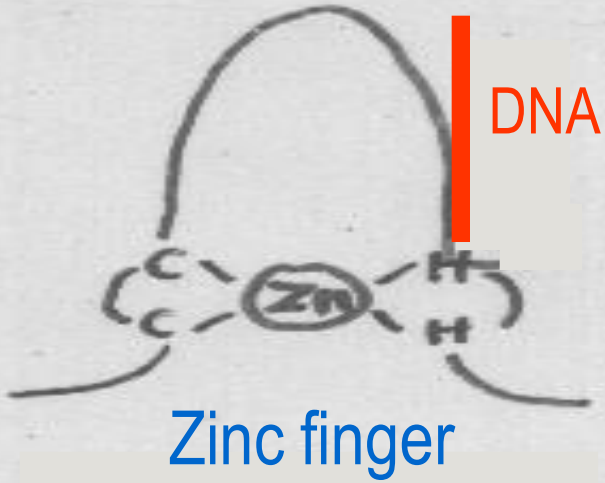




(来源：不详)

DNA binding domain of activator



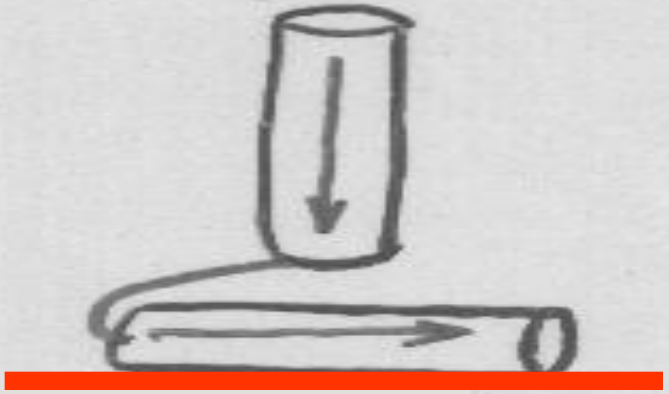


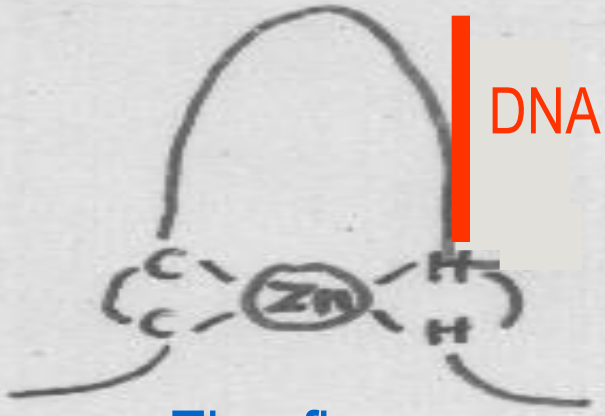
consist of Helix-turn-Helix and encoded by

the homeobox of 60 aa in large activator family

consist of Zinc finger (basic ZIP bZIP)

or basic domain of Helix-loop-Helix (bHLH)





MYB, MADS, WRKY,

AP2/EREBP（APETALA2 / Ethylene—responsive element binding protein），

NAC (NAM, ATAF, CUC2)

ARF (Anaerobic Response Factor)

DNA binding domain

成为转录因子分类的重要标志

(来源：不详)

- Transcription activation domain

(most activator have one or more than one of these domain)

Group 1: acidic domain

Yeast GAL4 typifies this group ,

49 aa domain with 11 acidic aa

Group 2: Glutamine-rich domain

Sp1 have two this domain,

25% Gln, 39 Gln in a span of 143 aa

Group 3: Proline-rich domain

CTF has a domain of 84 aa,

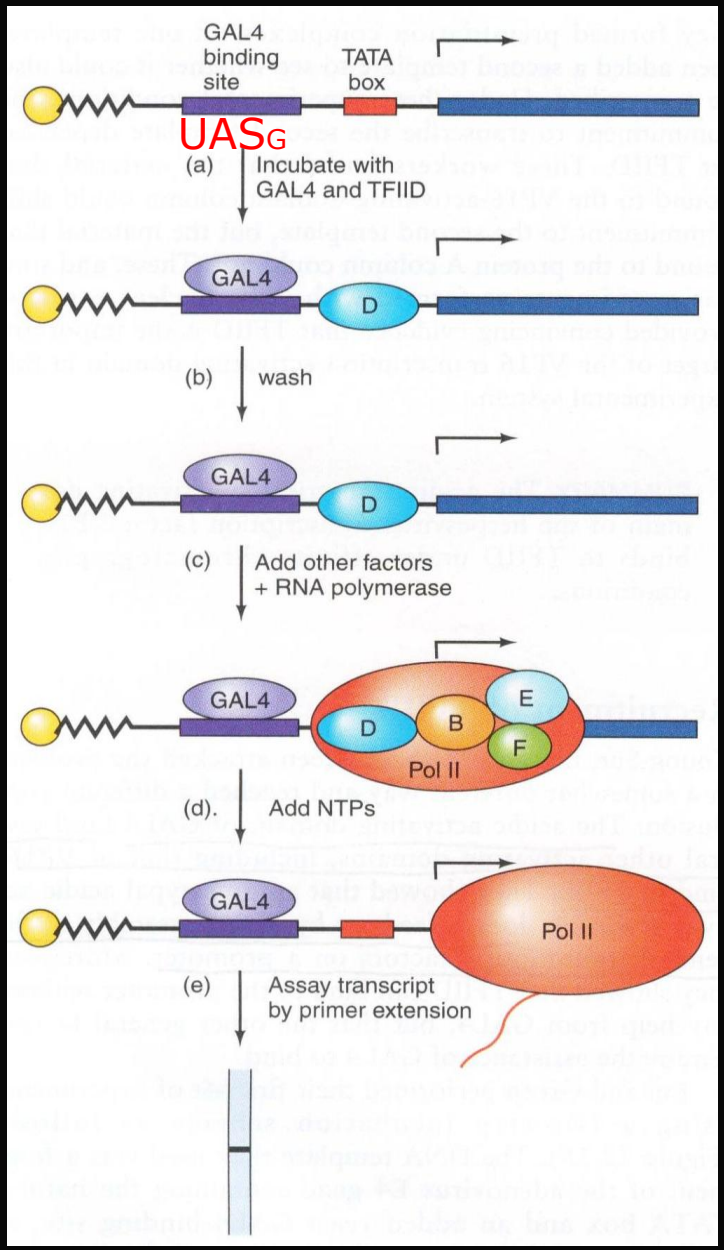
19 of which are Pro

Independence of

DNA-binding domain

and

transcription-activation domain



GAL4 protein : a yeast activator

- a set of genes responsible for metabolism of galactose
- each of GAL4-responsive genes contains a ***GAL4* target site** upstream of transcription start site, called **upstream activating sequence (UAS_G)**
- binding to UAS as Dimer DNA-binding motif is similar to Zinc finger

(来源：分子生物学（2007），郑用琏，第139页)

Roger Brent & Mark Ptashne

chimeric fator

with DNA-binding domain

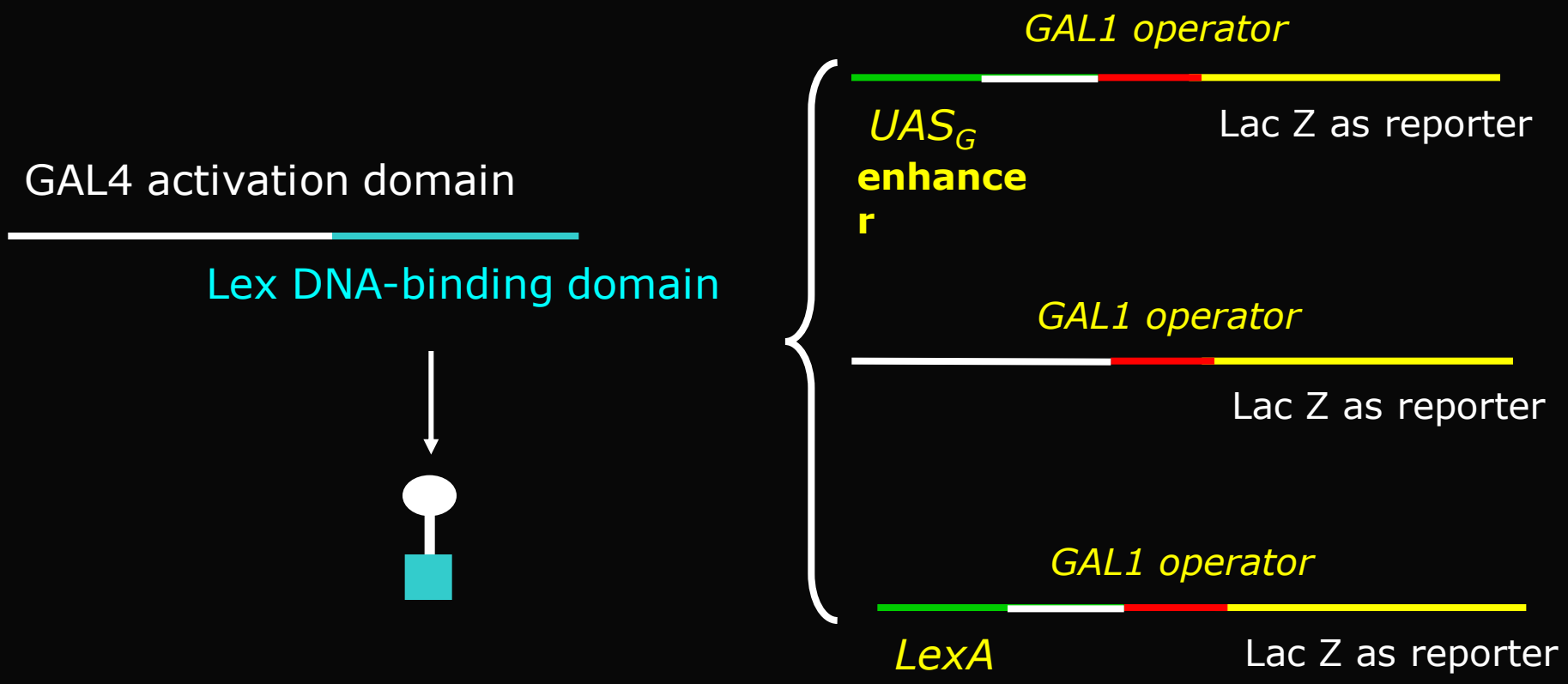
from LexA repressor *(E.coli)*

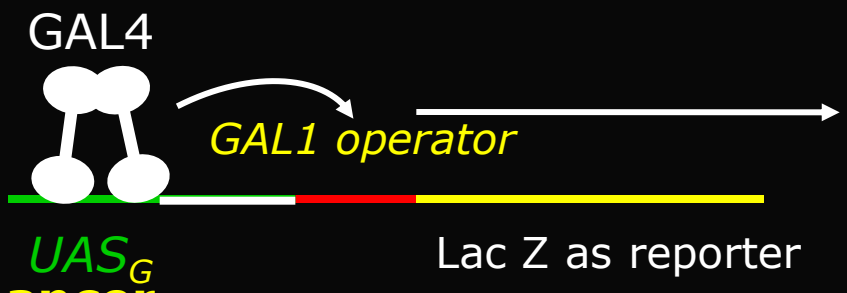
+

Transcription-activation domain

from GAL4 protein

Constructed three set of test recombination Plasmids into yeast(双因子杂交系统)



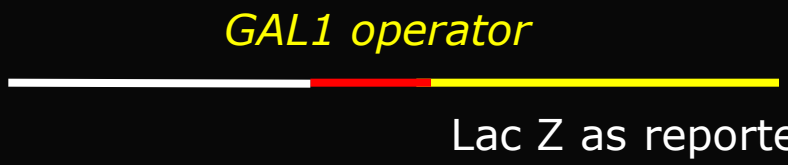


B-galactosidase

1800 units

enhancer

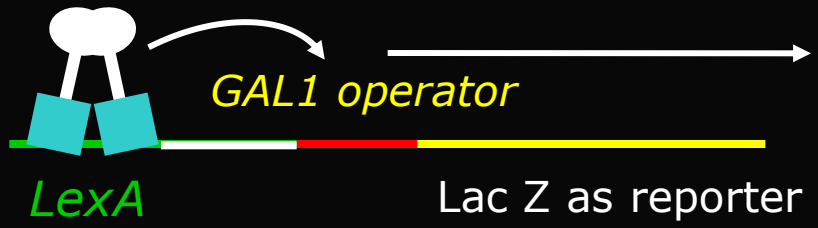
GAL4 + *UAS* ⟶ *enhanciing* ⟶ *GAL1* ⟶ *LacZ*



Nothing

No activator ⟶ no LacZ expressing

GAL4-LexA



520 units

LexA + *LexA op*. ⟶ GAL4 activation ⟶ *GAL1* ⟶ LacZ expressing

Transcription-activating and DNA-binding domain of *GAL4*

can operate quite independently

Function of activation domain

recruitment the RNA polymerase

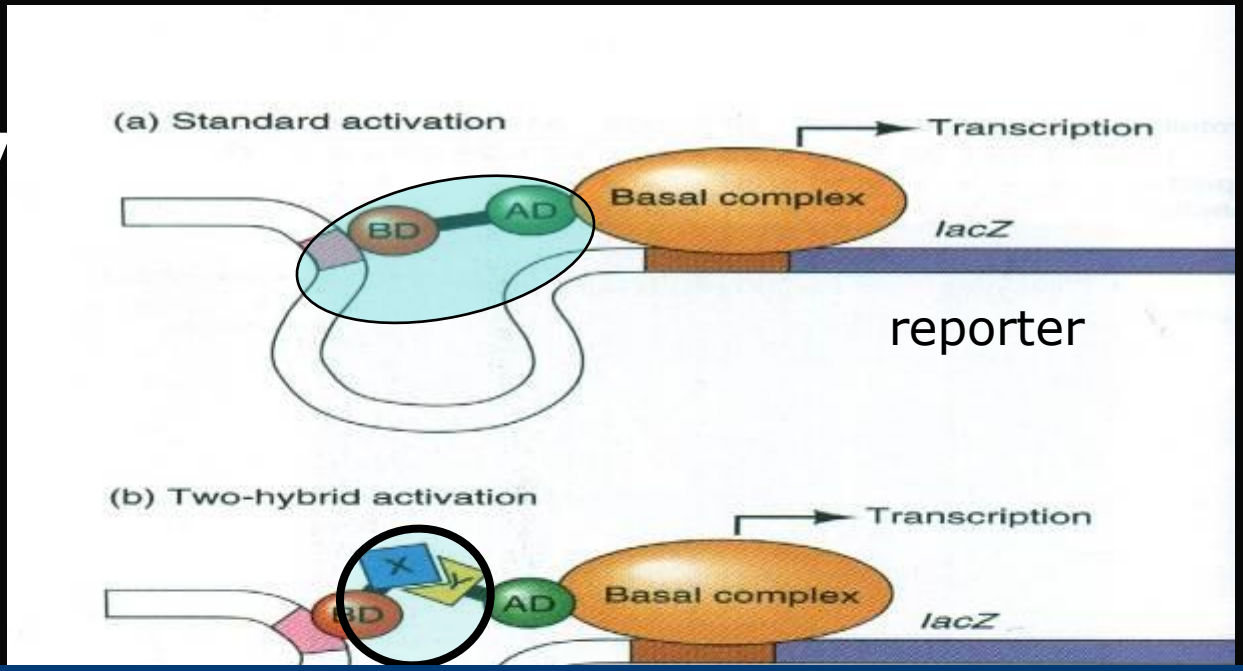
to promoter

Yeast two-hybrid assay

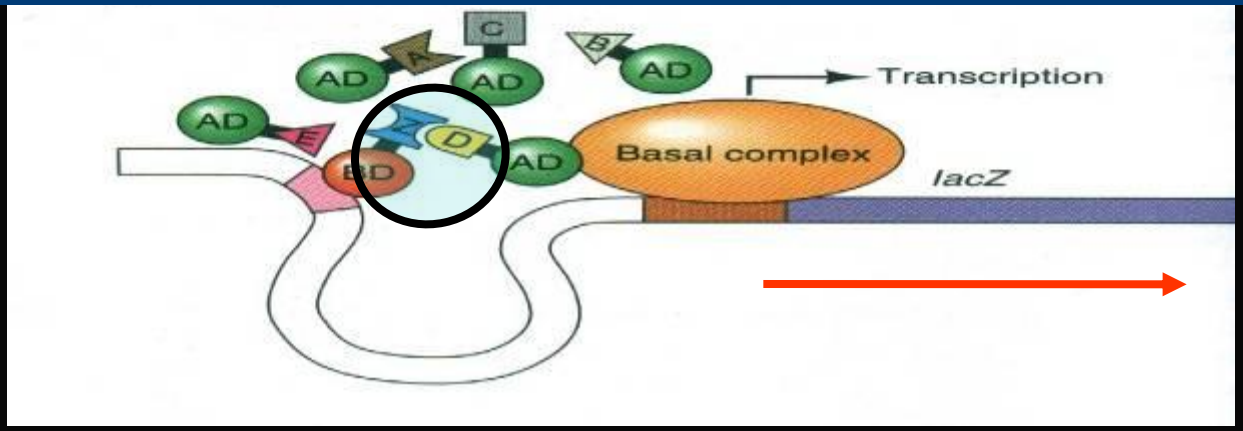
Transcription factor

Xp interaction with Yp

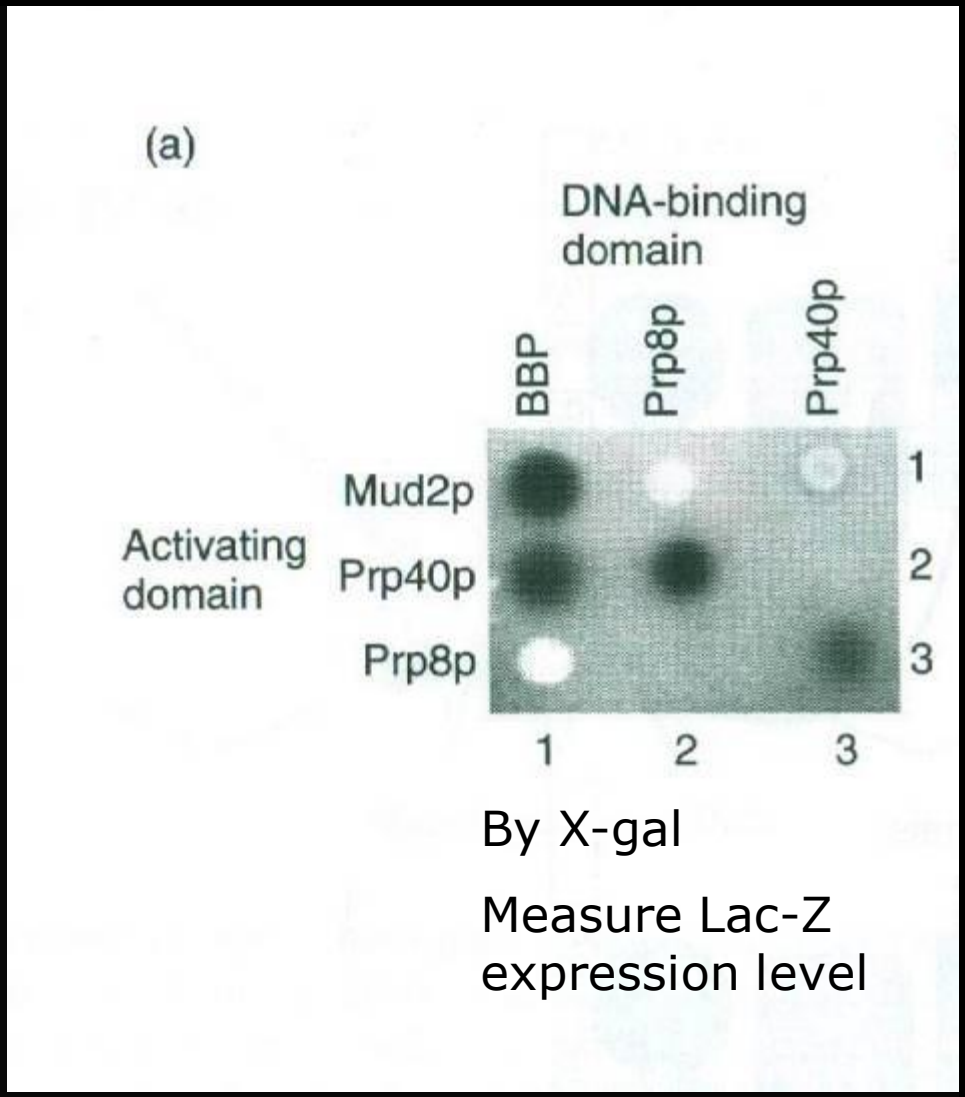
Screen Zp interaction with Dp



将研究的cDNA与AD，BD分别构建两个表达载体转化酵母测验体系（商业化产品）



(Source:Molecular Biology(2002),Robert F.Weaver,Page426）



By X-gal

Measure Lac-Z expression level

BBP + Mud2p

BBP + Prp40p

Prp8p + Prp40p

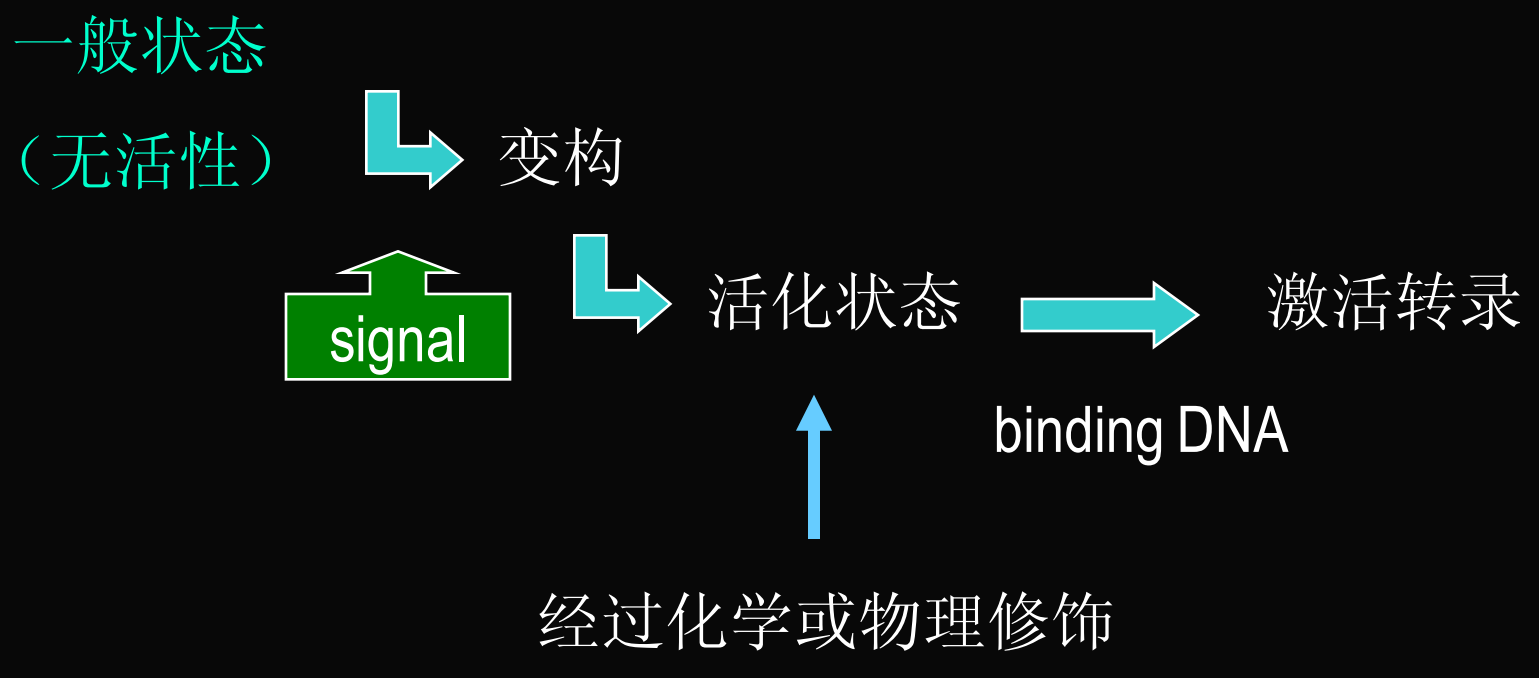
(Source:Abovich and Rosbash Cross-intron bridging interactions in the yeast commitment complex are conserved in mammals

Cell 89(2 may) 1997)

b) 种类与结构特点

activator 的活性调节

受到严格的信号因子的调控



转录因子的结构特点

DNA-binding domain

- Helix(α)-turn-Helix(α) (HTH)
- Cys/Cys, Cys/His Zinc finger

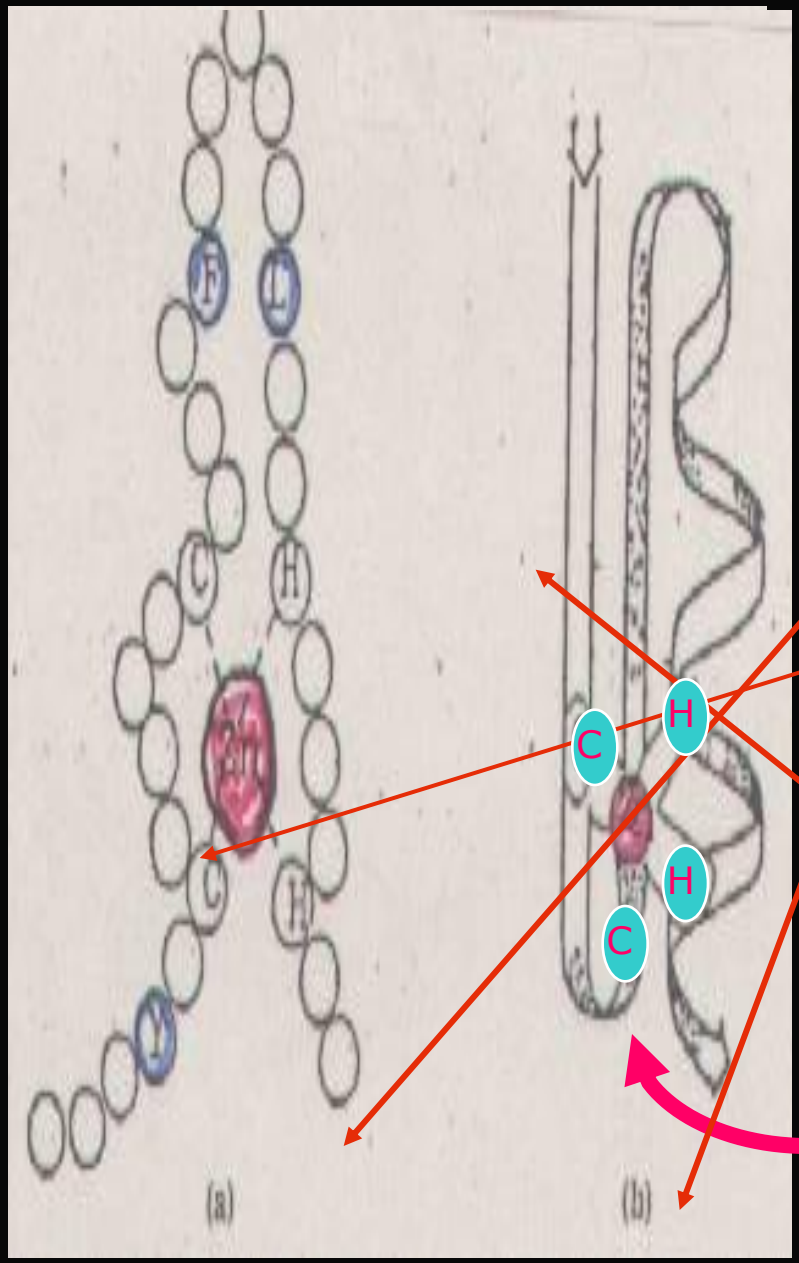
dimerzation domain

- Helix—loop—Helix (HLH or bHLH)
- Leu zipper (bZIP)

Activation domain

- Acidic domain, Gln-rich domain, Pro-rich domain

Zinc finger



Peptide chain

9 direct repeats 30aa / copy

30 aa / copy → one finger

2Cys / 2His or 2Cys / 2Cys types

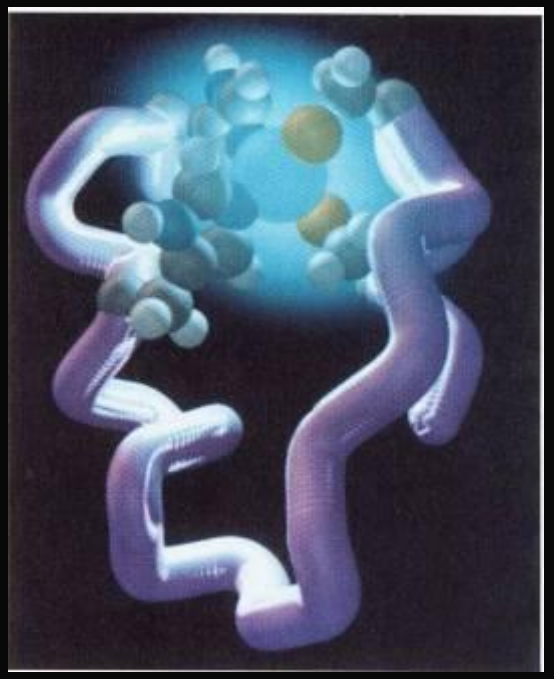
Phe(F) Leu(L) Tyr(Y)
→ core of hydrophobic aa

2Cys (2C) ~ Zn ~ 2His (2C)
to form finger of 1α-helix & 1-βsheet

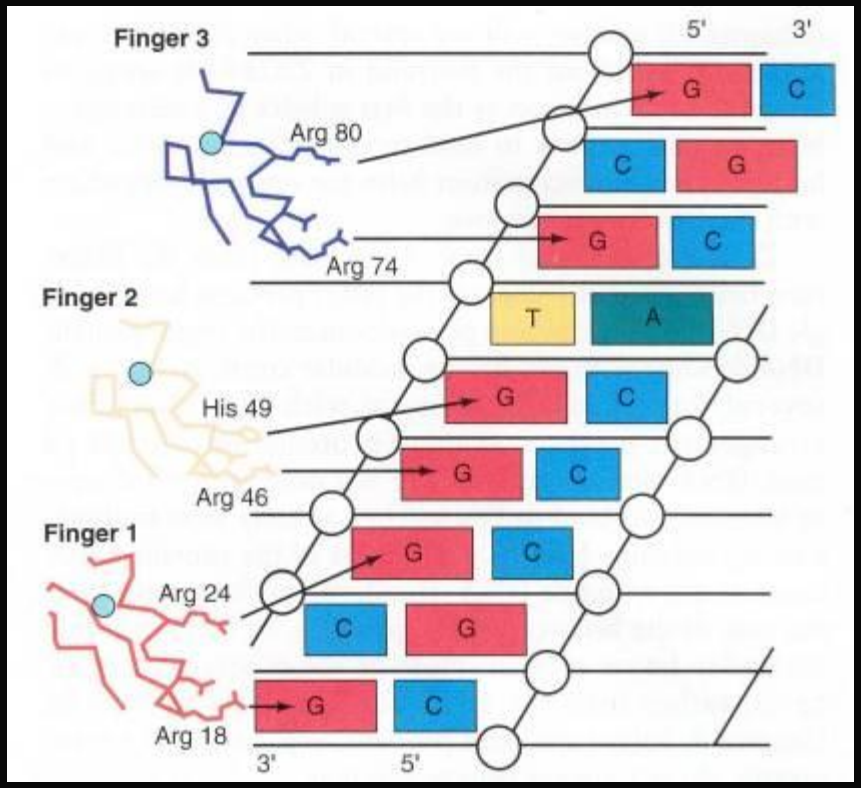
(来源：不详)

Aaron Klug (1985)TFIIIA predict the Zinc finger structure

Carl Pabo obtained the structure of complex between TFIIIA and DNA using X-ray crystallography



Source:cover photo,Science 245(11 Aug 1989)



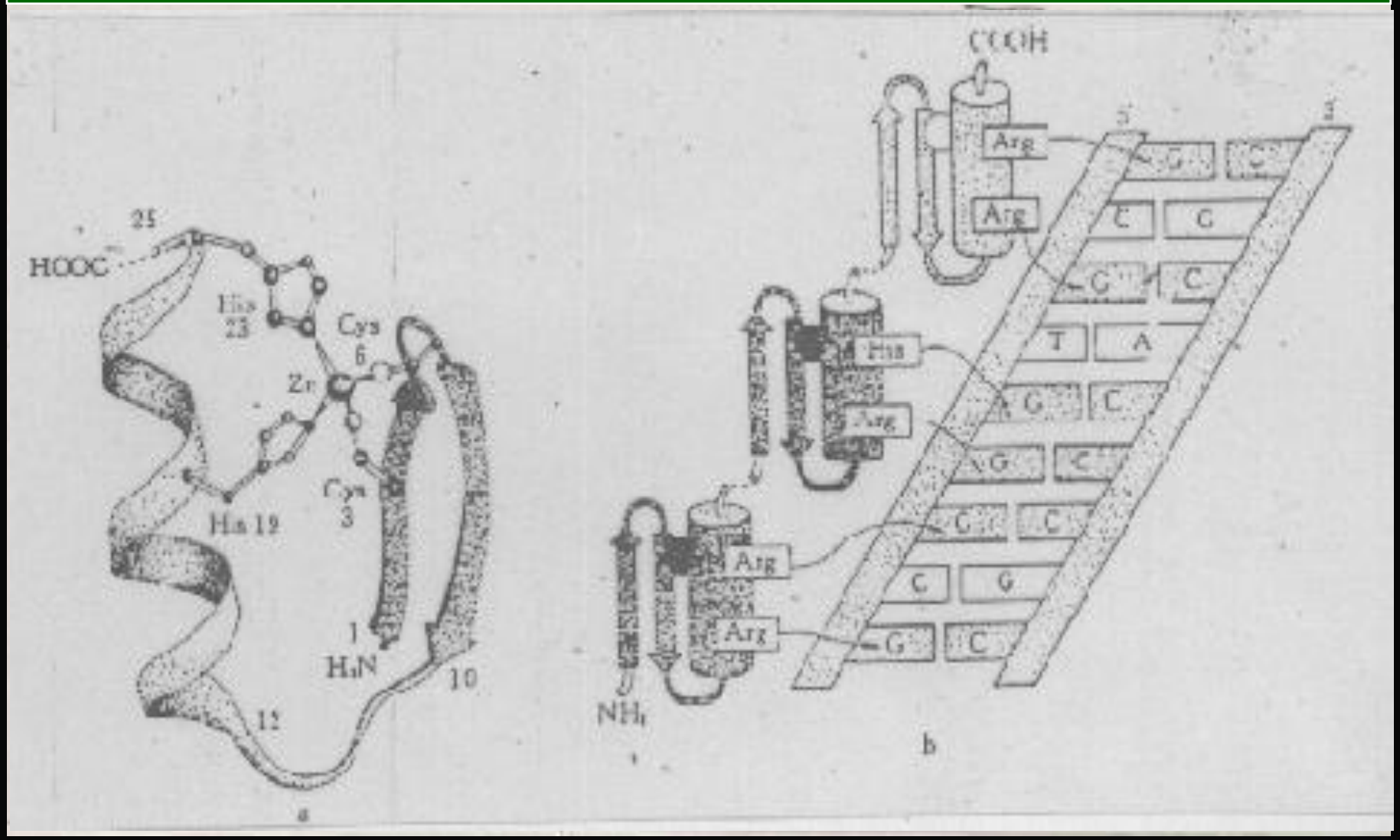
(来源：不详)

TFIIIA three fingers lining up the major groove of DNA which sequence

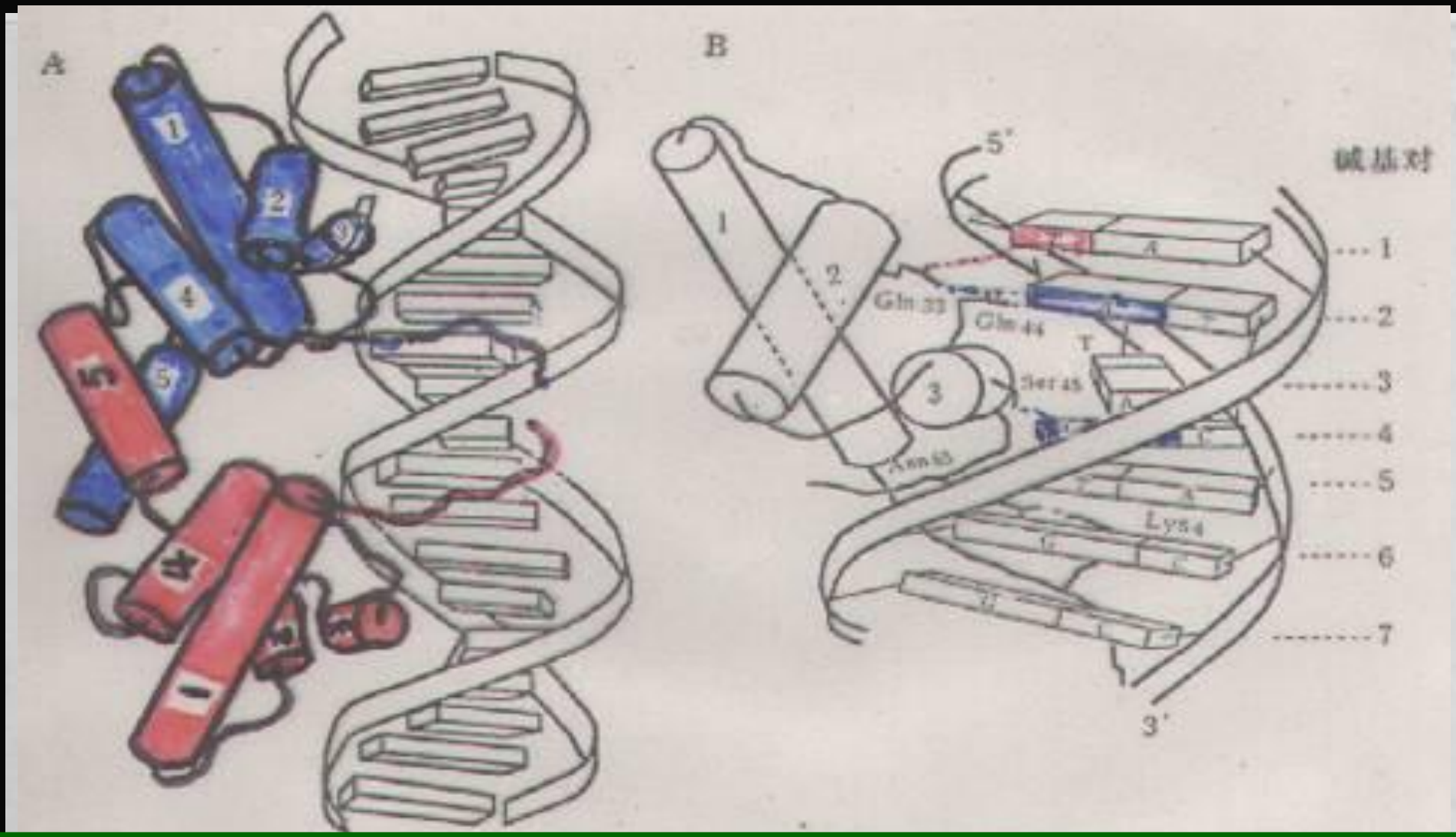
GCGTGGGCG

Fig. III Fig. II Fig. I

Zinc finger binding with cis-factor of DNA



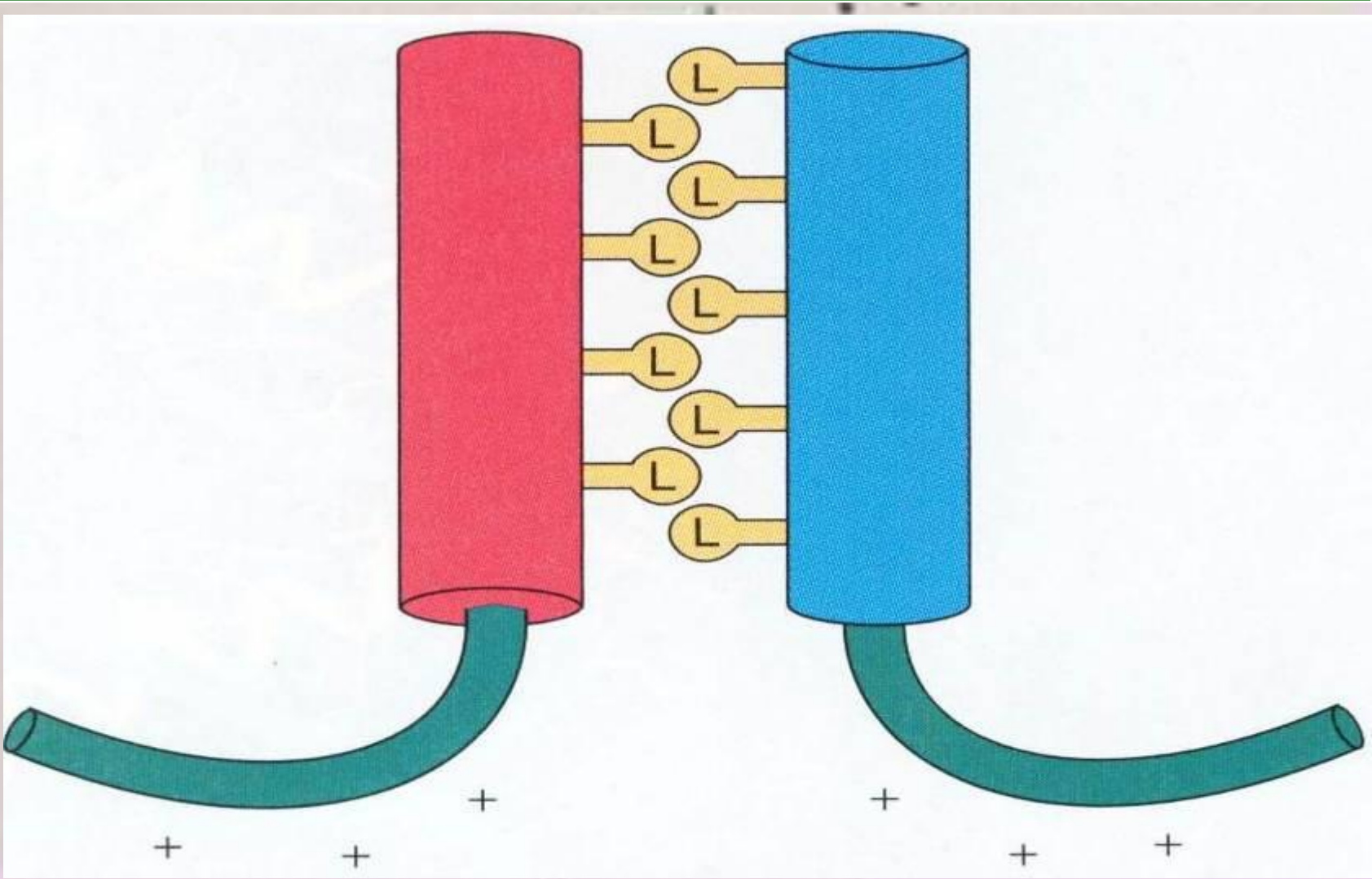
(来源：不详)



Helix-turn-helix domain of homeodomain

(来源：分子生物学（2007），郑用琏，第148页)

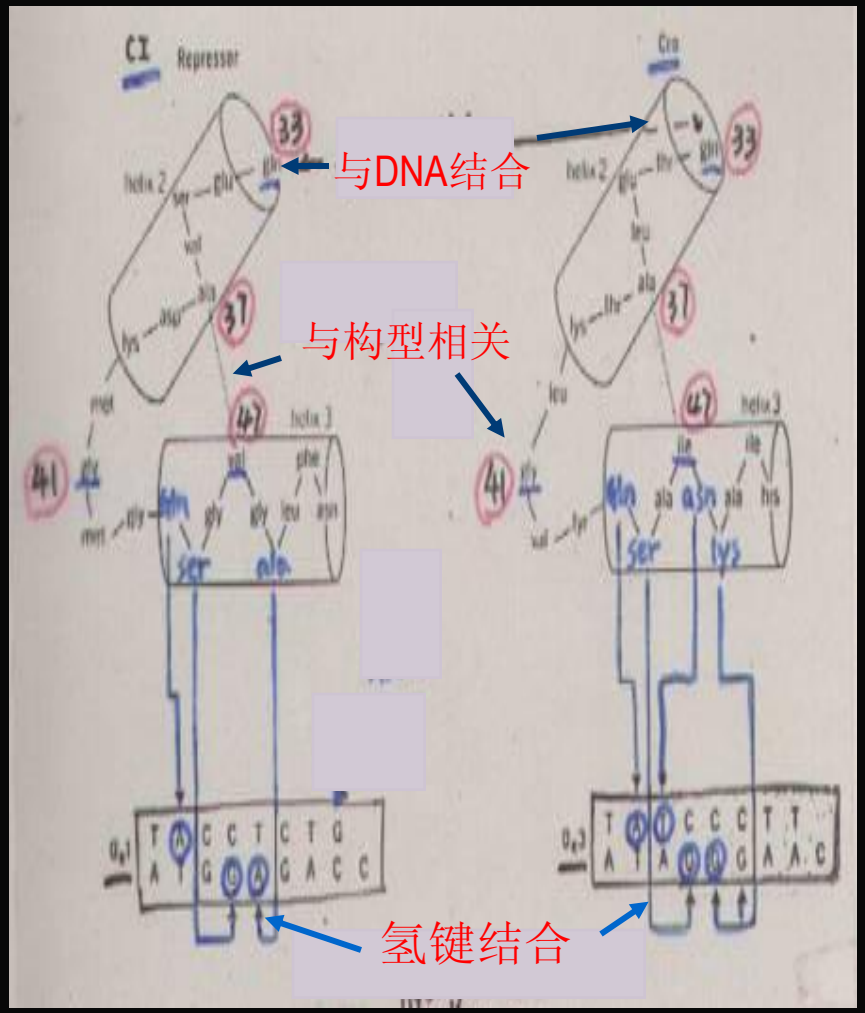
Leu zipper & basic domain of (bZIP)



(来源：分子生物学（2007），郑用琏，第30页)

4.2.3.4. Trans-factor 与cis-actor 结合的 通用原则

- 位点专一性识别，并具特异位点的氢键结合力



(来源：不详)

- Trans因子-- Cis因子间的识别与结合多发生在具有足够信息的DNA的大沟内
- 蛋白质氨基酸与DNA碱基(NH2,O ＝)结合的界面形成大量的氢键连接

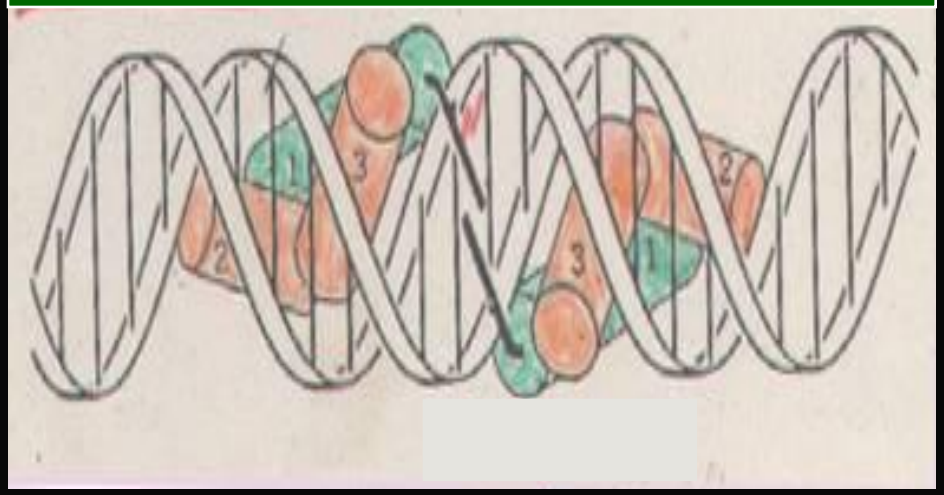
- trans因子与cis因子间的非特异性结合力

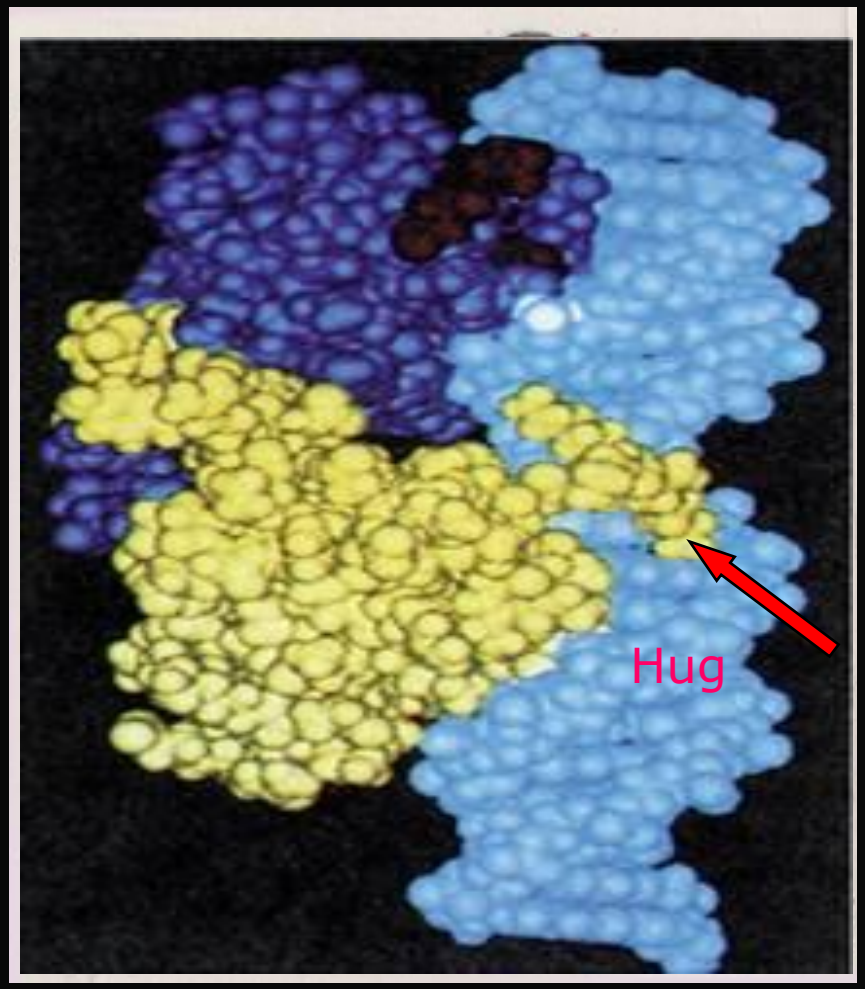
• protein ~ 盐桥 ~ phosphate backbone of DNA

• Hydrophobic focus (△S)

DNA-protein

• N-end arm of helix reach around to other face (to minor groove)



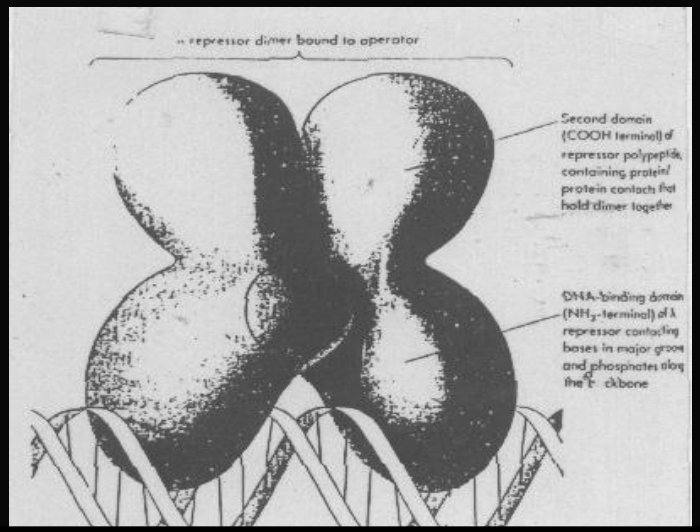


(Source:Hochschild,A.Repressor structure and the mechanism of positive control.Cell 32 (1983)p.322.)

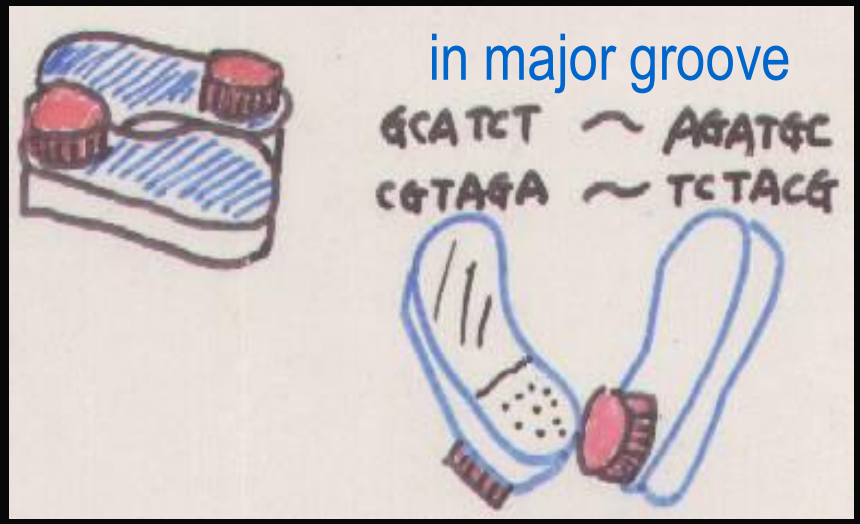
- Dimer or tetramer of trans-F binding in palindromic cis-F

表现一种“二重对称性”

dimer as Right Shoes model binding with palindromic Seq.



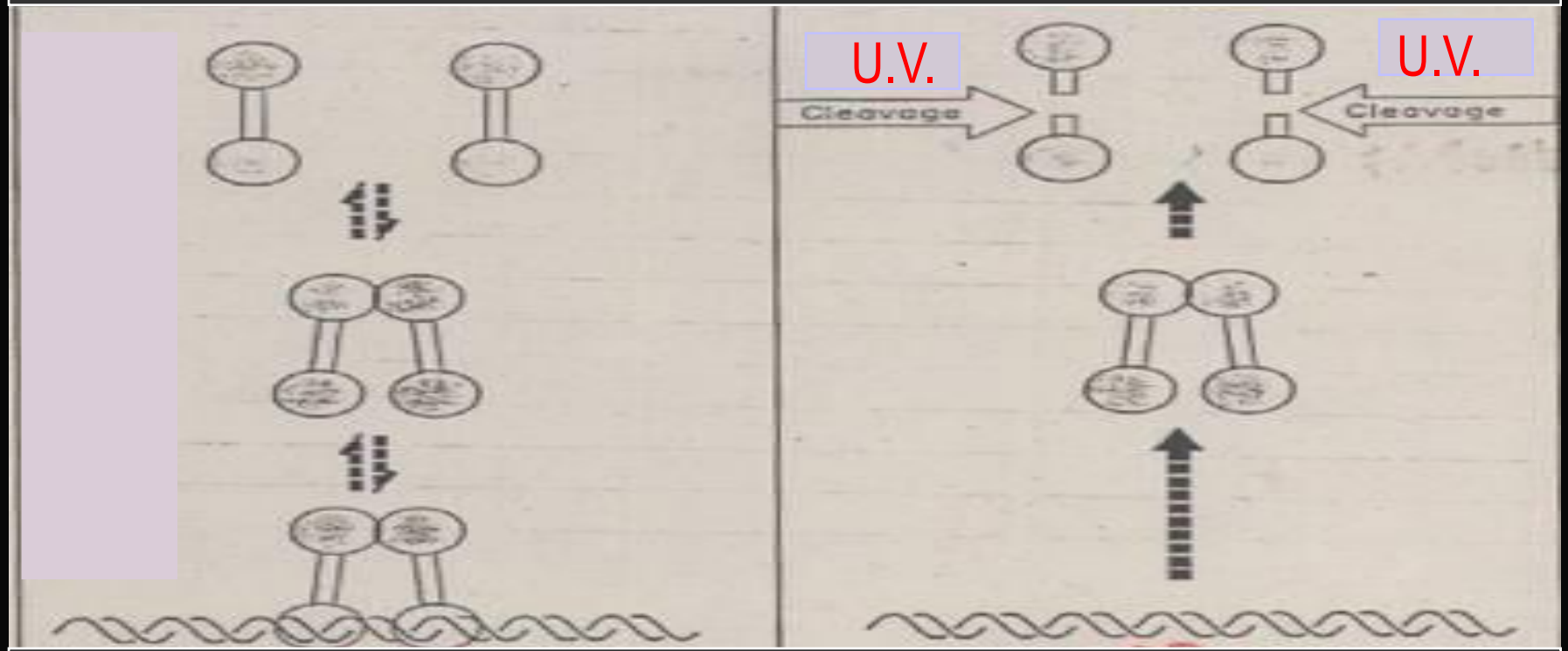
(来源：不详)



● Monomer ⇌ Dimer → cis-factor → gene open

200 ↑ 50 ↑

相对浓度的改变　　准确，灵活地调节基因表达



Cis-factor　　Cis-factor

(来源：不详)

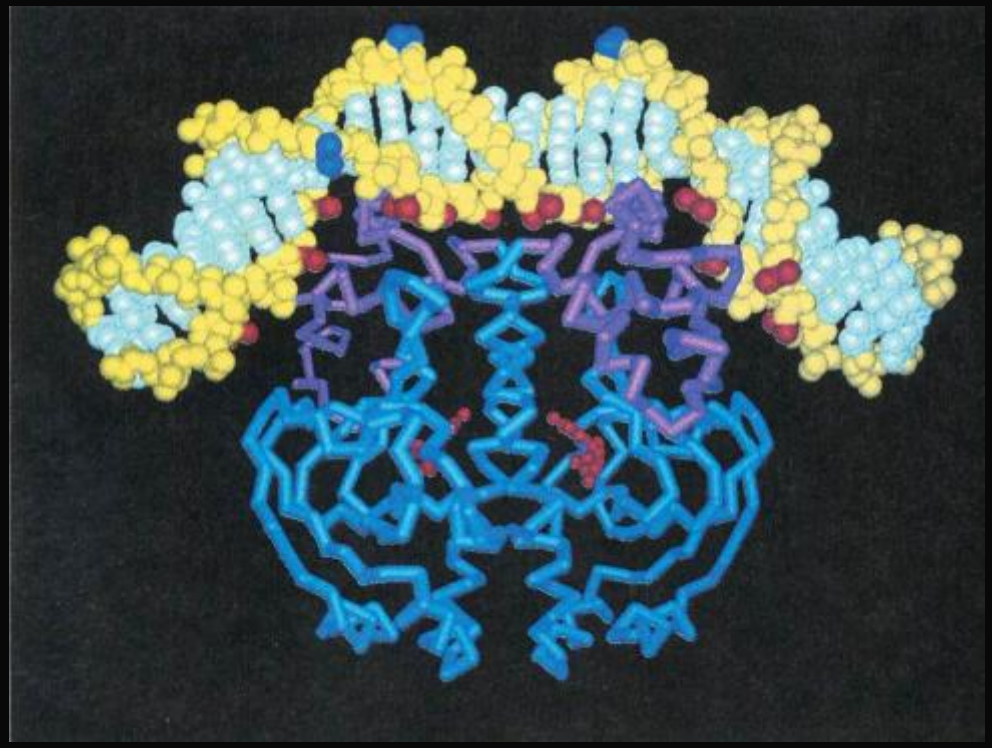
转录因子以三种方式发挥促进转录的功能：

（1）改变DNA构象，使原来包埋在螺旋内部的启动子序列暴露出来，有利于RNA聚合酶与启动子的结合。

（2）影响RNA聚合酶，TF因子与DNA结合形成Basic transcription complex，加强/减弱其特异性结合。

（3）参与其它调节因子之间的interaction & activity

4.3. Transcription termination



4.3.1. 概念

- RNApol leave from terminator
- RNA released from Triplex (RNA, DNA, RNApol)
- different from discontinue / read-through

4.3.2. Terminator 种类

In prokaryotes

基因末端终止子(terminator)

基因内部终止子（attenuator）

Rho-independent terminator / Rho-dependent terminator

In Eukaryotes

Difficult to identify the primary transcript

because pre-mRNA processing

4.3.3. Rho-independent terminator

(simple terminator)

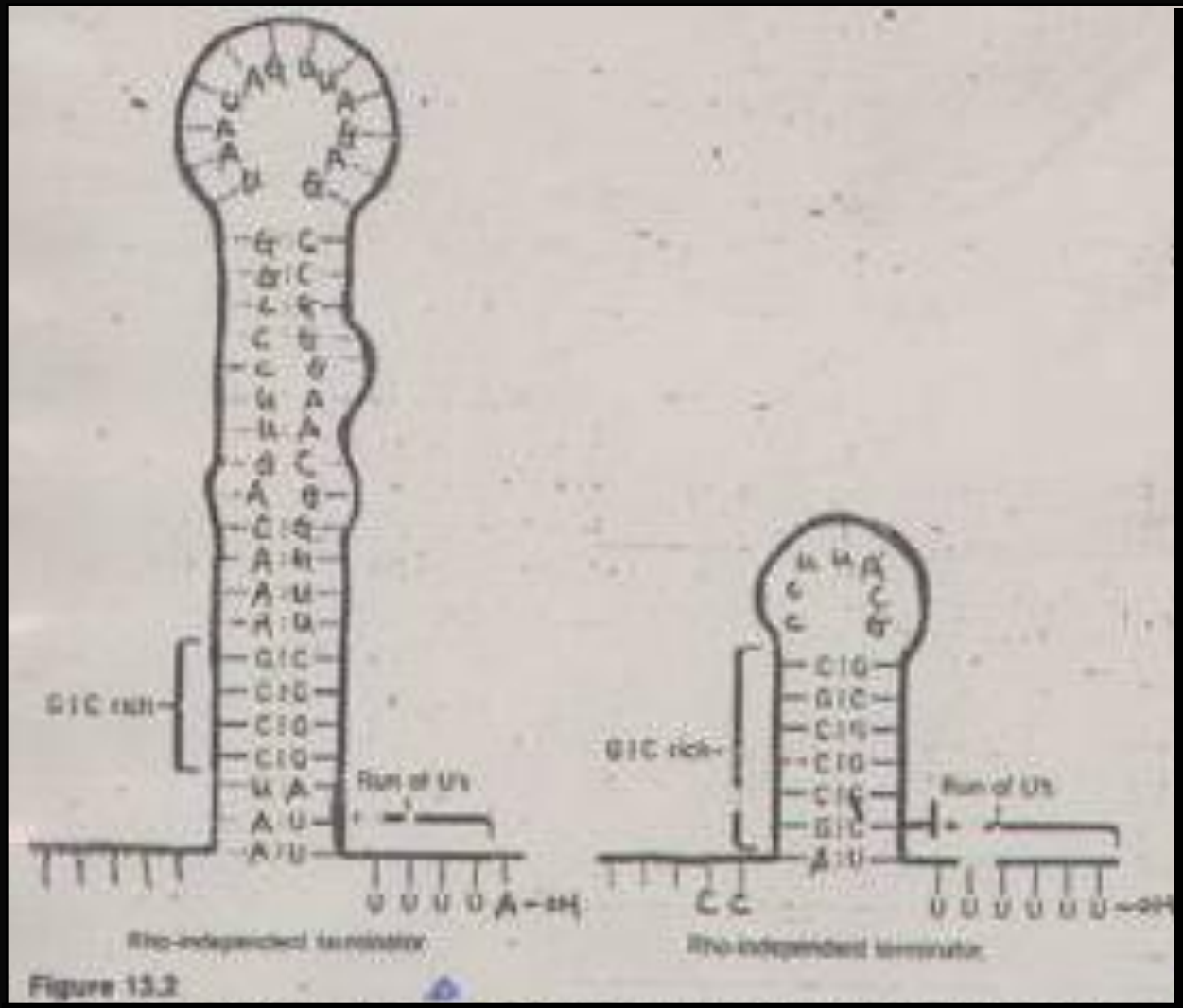


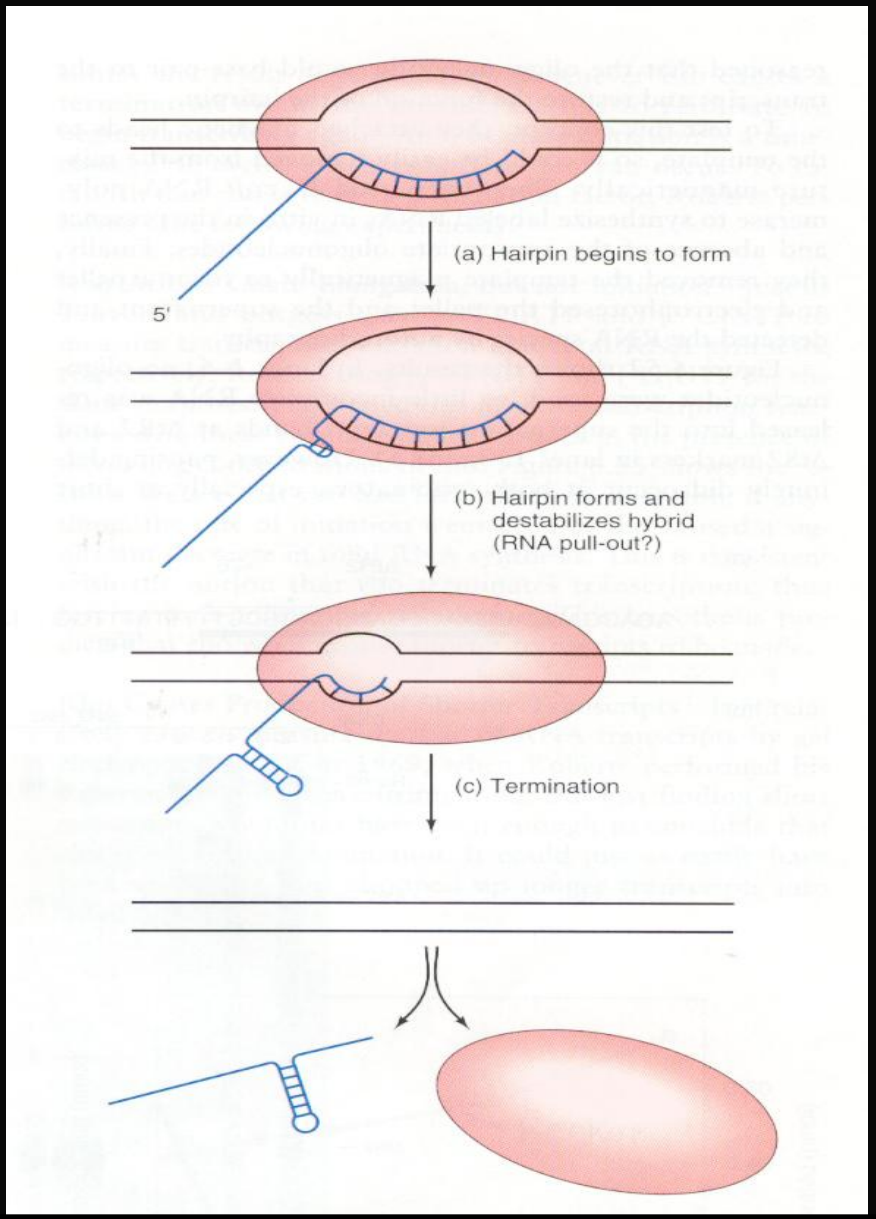
Figure 13.2

(来源：不详)

a) Structure

- Palindromic sequence
- G/C rich region that form hairpins of varying lengths in RNA
- G/C rich hairpin are followed by a run of U residues in RNA

Rho-independent terminator (simple terminator)



(Source:Molecular Biology(2002),Robert F.Weaver,Page164）

b) Function

- G/C rich →

  transcription delay

- NusA-protein →

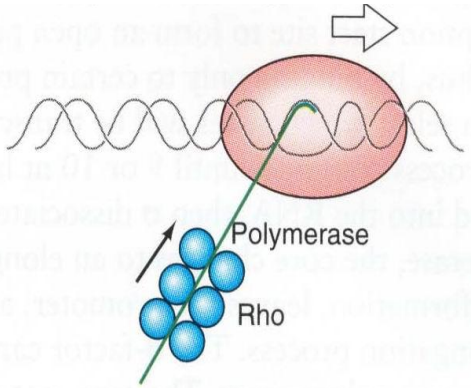
  RNApol pausing

- RNA stem-loop & polyA/U →

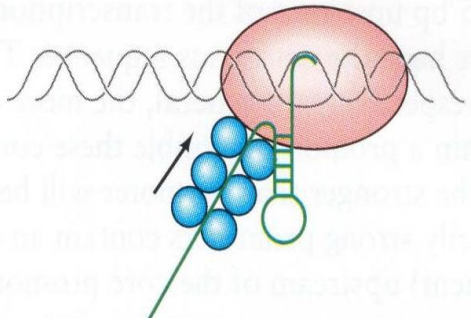
  RNApol leave

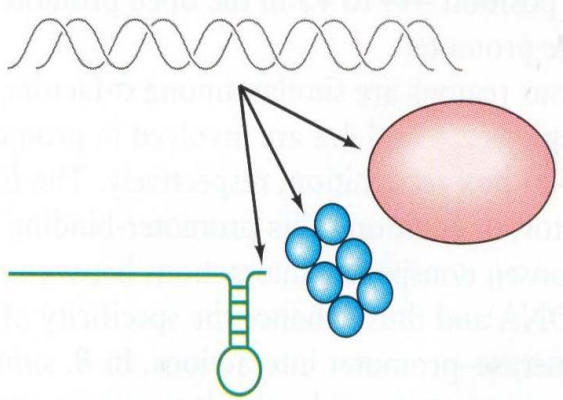
- β’ inhibited by K factor →

  RNApol & DNA separated



4.3.4. Rho-dependent terminator





(Source:Molecular Biology(2002),Robert F.Weaver,Page167）

a) Structure

- Palindromic sequence
- G/C rare in palindrom but form loose hairpin in RNA
- palindromic sequence are not followed by poly A/T in DNA
- Rho factor be needed for termination

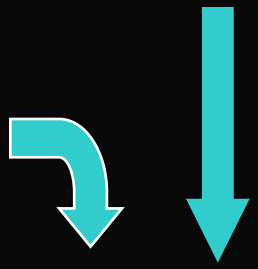
(来源：不详)

b) Function

RNApol + NusA-protein

RNApol pausing 60’’ more or less

K, Rho



or

termination

read-through

c) Rho-factor (ρ)

- Hexamer 55kd neutral helicase-like
- Contest RNA 3'-end with β factor
- ATPase-like, GTPase-like activity need spacer of > 50 Nt
- 转录终止效率取决于紧随回文的下游序列

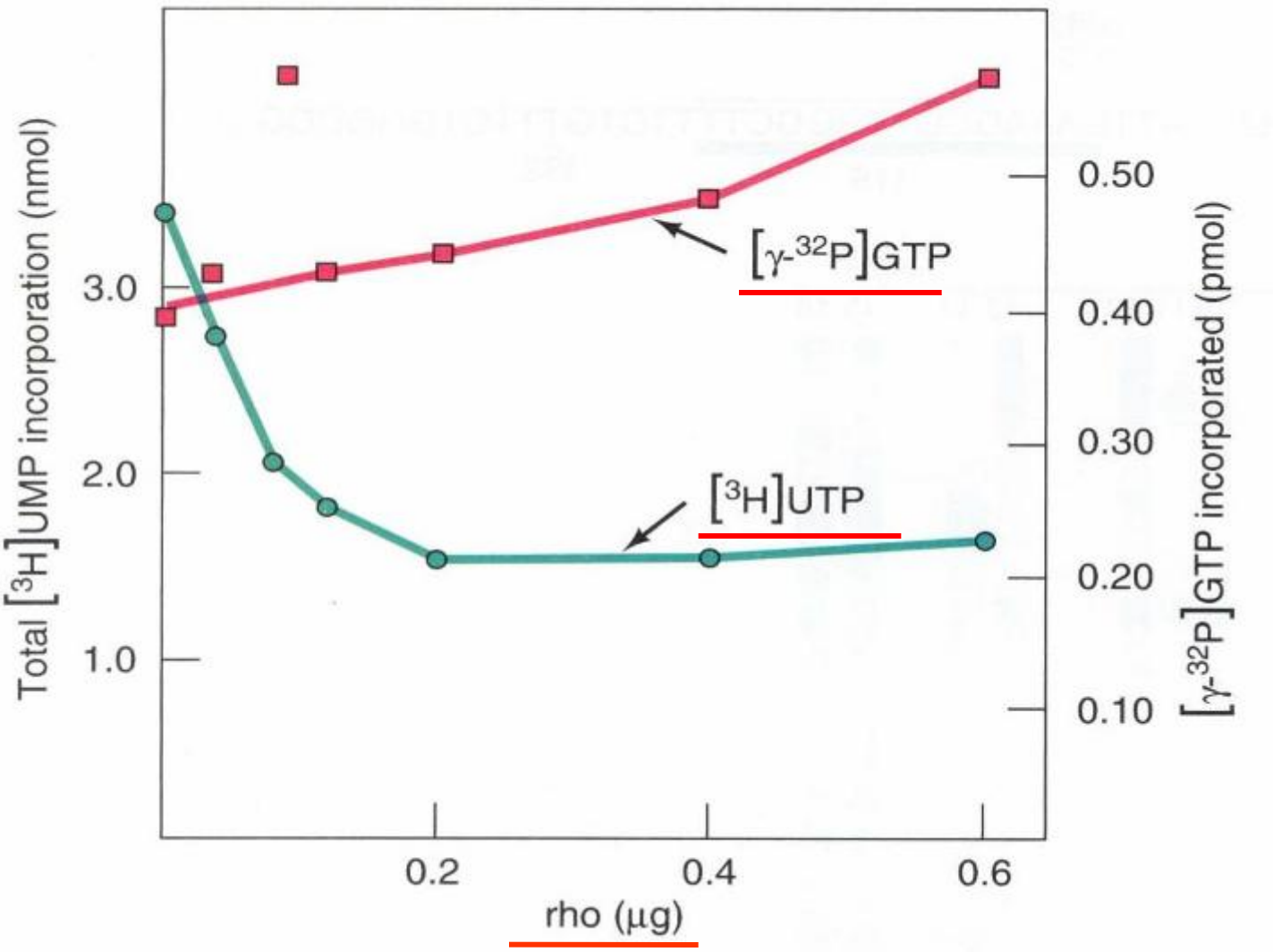
---保证 RNA / DNA 具有一定松弛的结合力

---过低的结合力→ 成为Rho-independent terminator

---过高的结合力→ 引起RNApol read-through

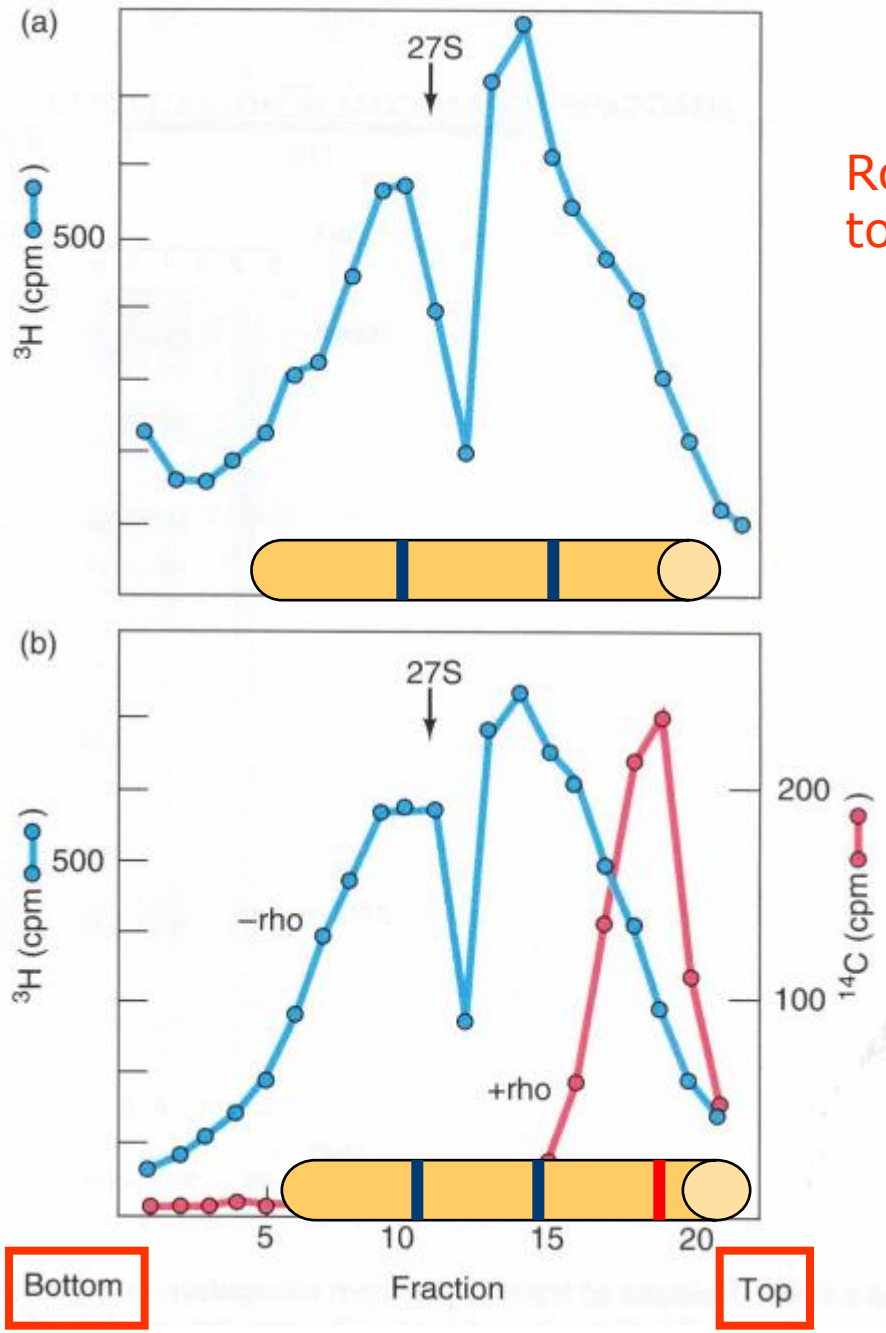
---各依赖Rho 因子的终止子间的效率差异取决于
终止子区DNA / RNA 间结合力的差异

E. coli RNA polymerase to transcribe λ phage DNA



Rho depressed the elongation rate, but not initiation

Source:J.W.Termination factor for RNA synthesis,Nature 224:1168-74,1969.)



Roberts used *E. coil* RNA polymerase to transcribe λ DNA

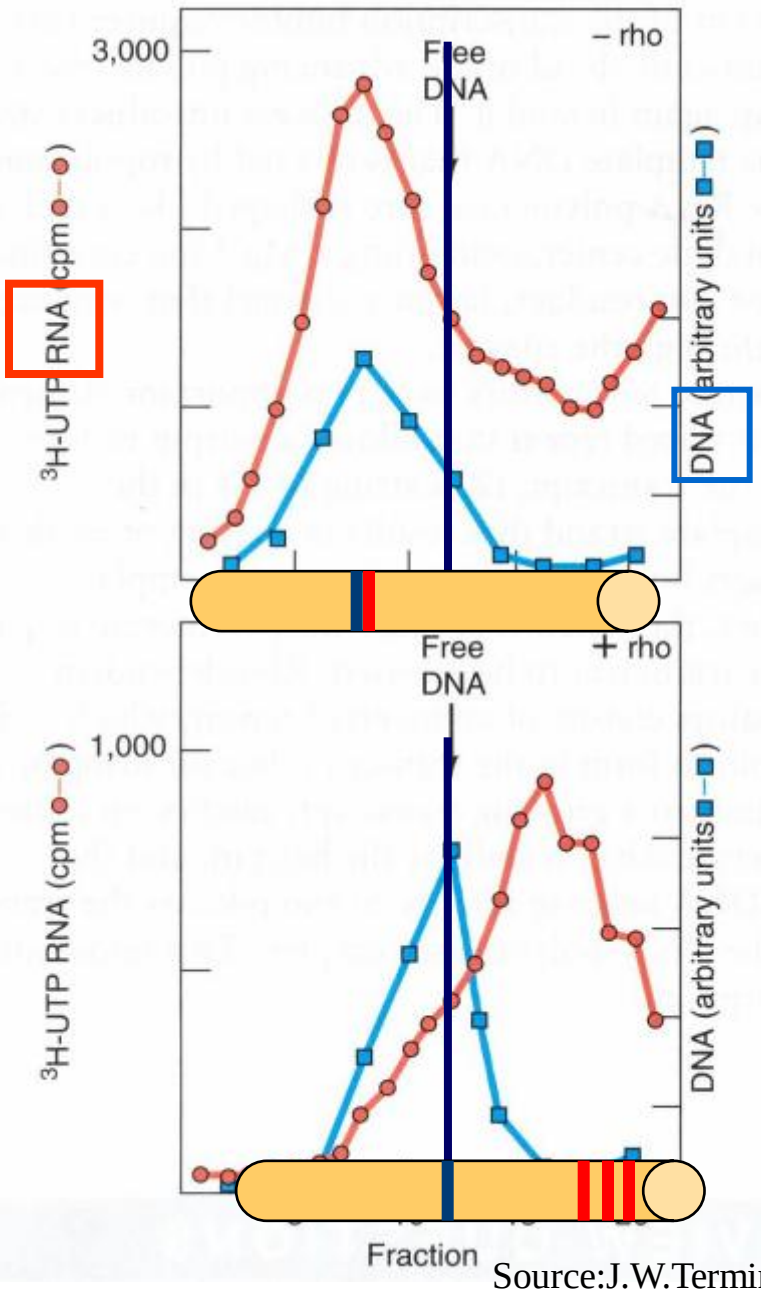
(a) in the absence of Rho. [^{3}H]UTP to label the RNA.

Rho reduces the size of RNA

(b) in the presence of Rho. [^{14}C]ATP to label the RNA, plus the ^{3}H-labeled RNA from panel (a)

Source:J.W.Termination factor for RNA synthesis,Nature 224:1168-74,1969.)

Rho releases RNA from DNA template



(a) RNA made in the absence of rho sedimented together with the template in a complex that was larger than free DNA

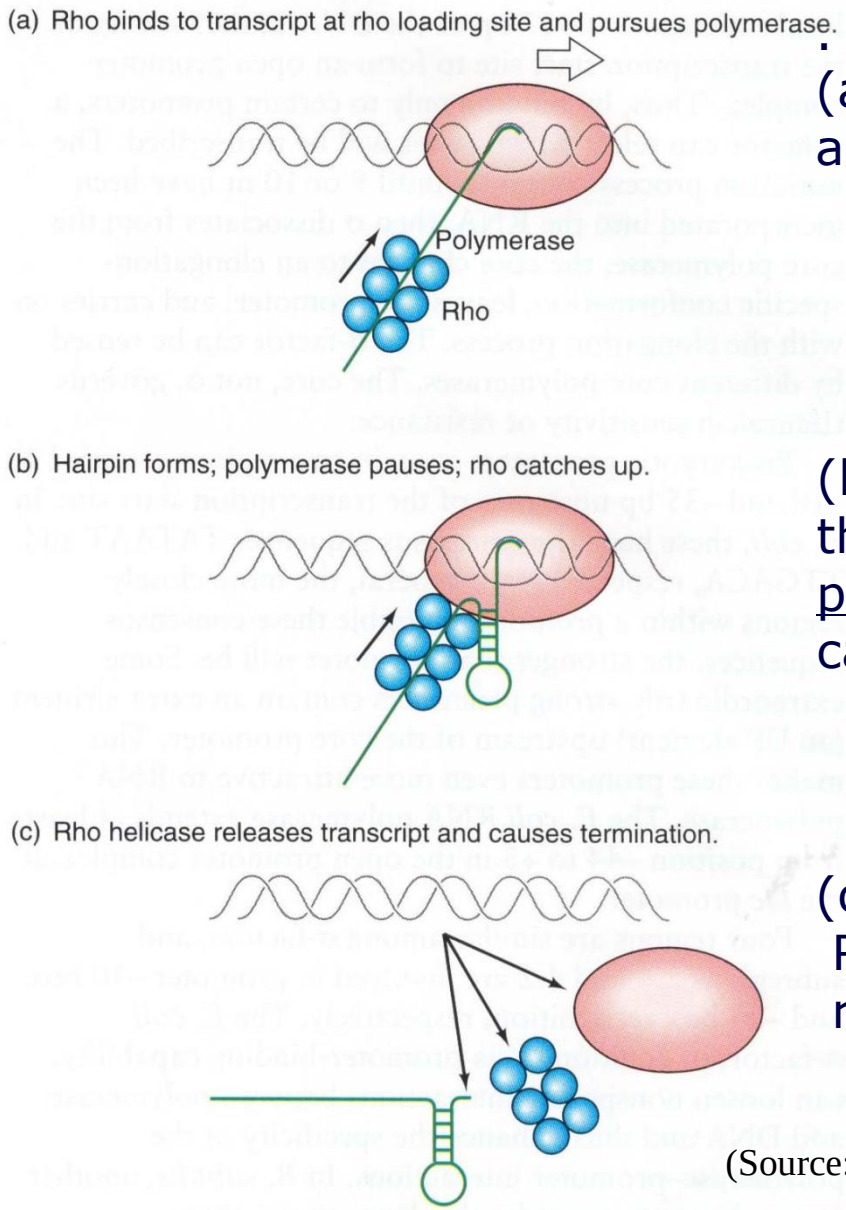
indicating that RNA had not been released from their association with the DNA.

(b) RNA made in the presence of rho sedimented independently of DNA at a position corresponding to relatively small molecules

indicating that rho seems to release RNA transcripts from the DNA template

Source:J.W.Termination factor for RNA synthesis,Nature 224:1168-74,1969.)

model of rho-dependent termination

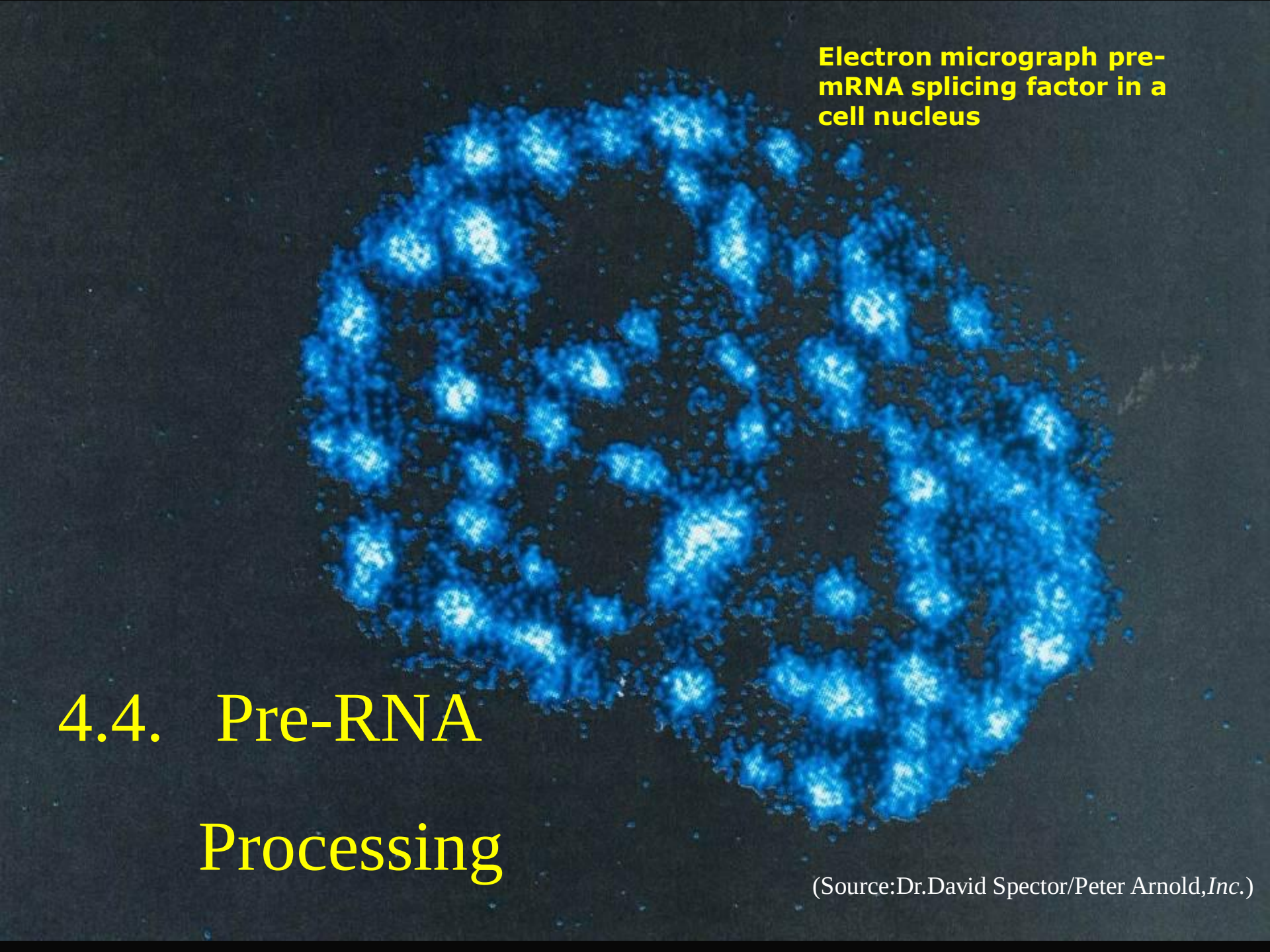


(a) Rho binds to the transcript and pursues the polymerase.

(b) When the hairpin forms in the transcript, the polymerase pauses, giving Rho a chance to catch up.

(c) Rho helicase unwinds the RNA-DNA hybrid and releases the transcript.

(Source:Molecular Biology(2002),Robert F.Weaver,Page167）



Electron micrograph pre-mRNA splicing factor in a cell nucleus

4.4. Pre-RNA Processing

(Source:Dr.David Spector/Peter Arnold,*Inc.*)

4.4.1. 概念;

pre-RNA → capping, tailing, splicing, methylation, editing → mature RNA

生物学意义;

- interrupted gene → remove introns → as template
  (interrupted RNA) (stop codon) (protein translation)
- prevent pre-RNA from digested by RNase
- something new……

4.4.2. pre-RNA capping

- Cytoplasm Polyhedron Virus (CPV)

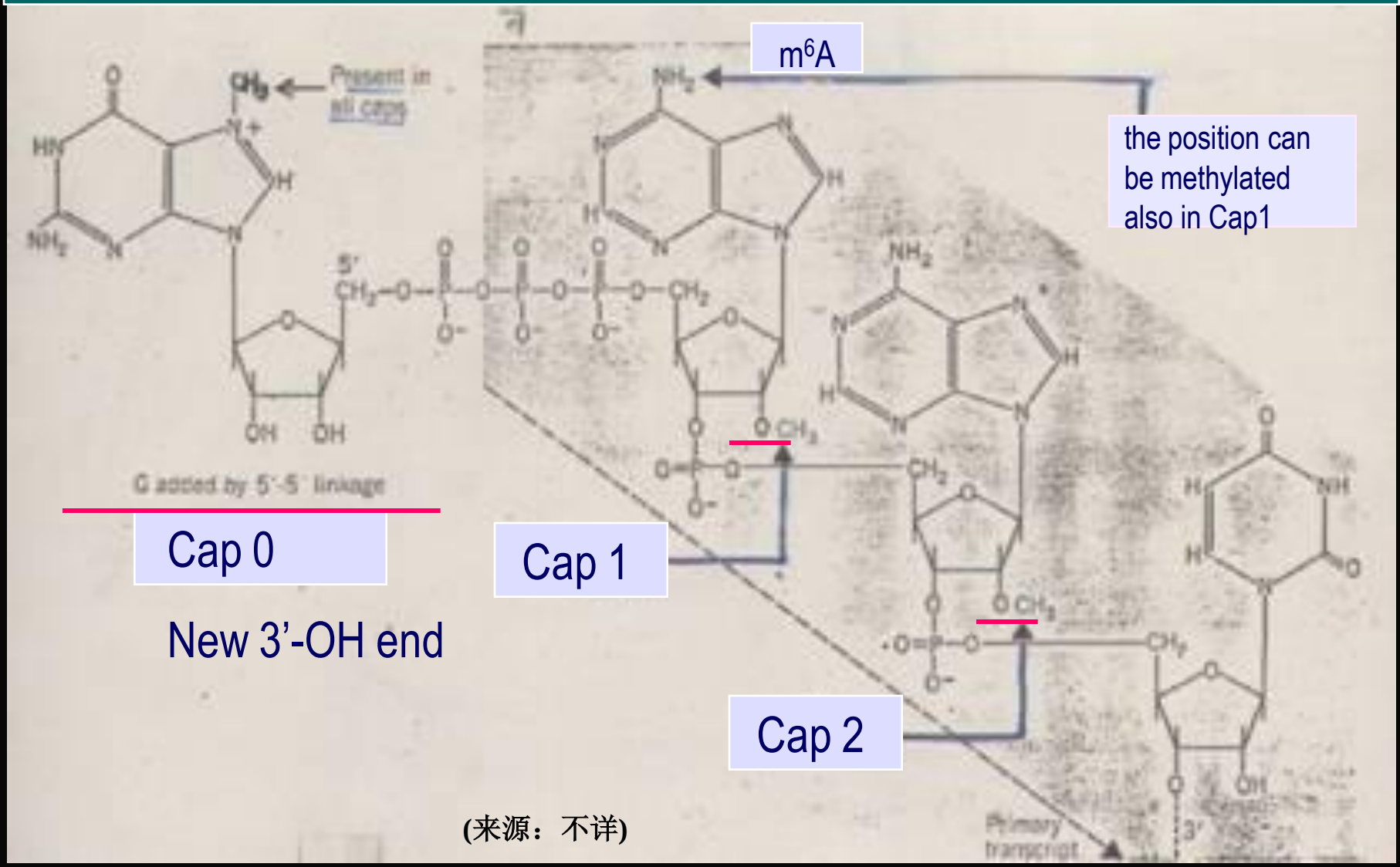
Jan Furuich (1974)

Cap-0 m^7GpppXpYp------- (共有)

Cap-1 m^7GpppXmpYp-------

Cap-2 m^7GpppXmpYmp-------

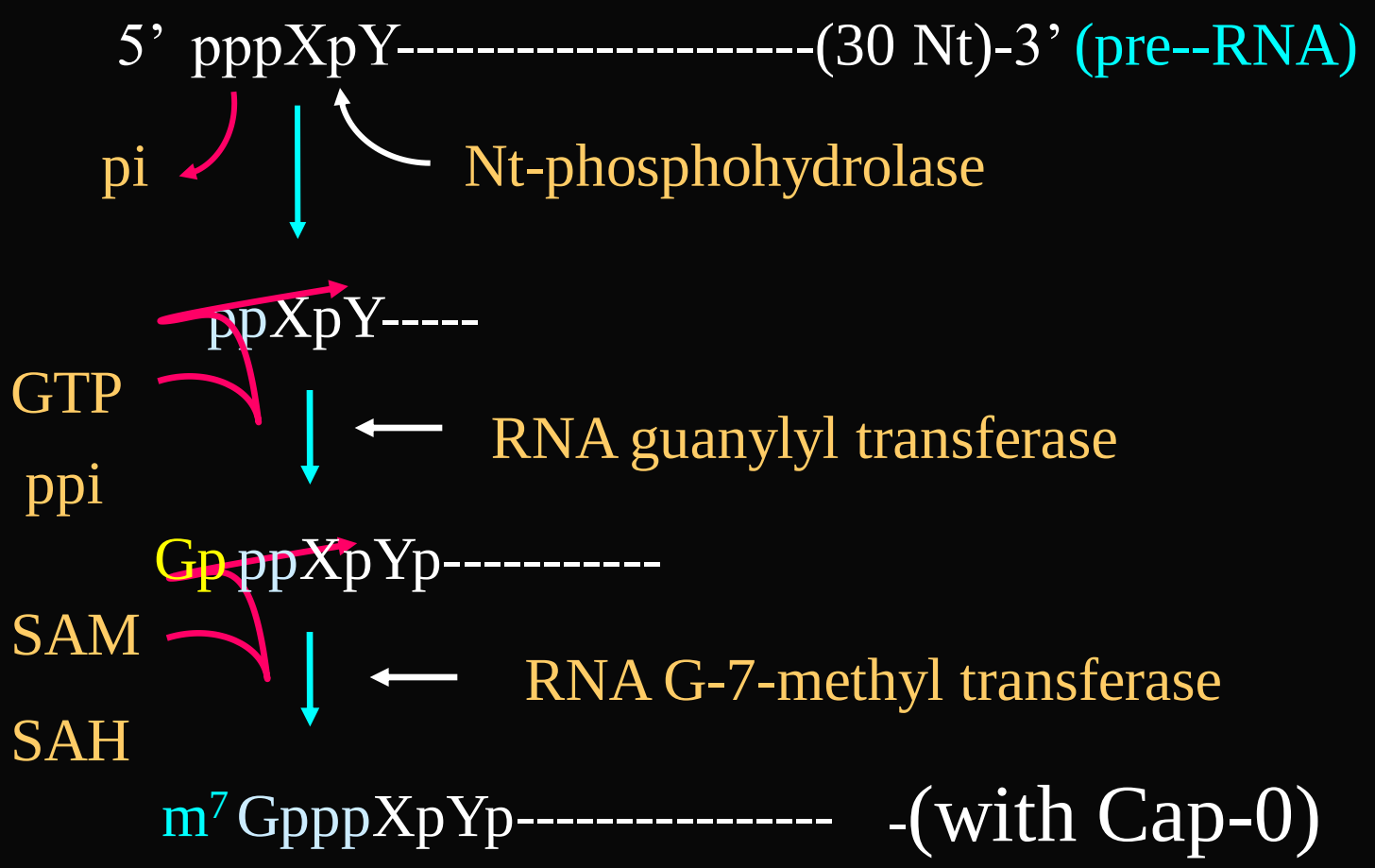
Types & structure of CAP in Eukayotes

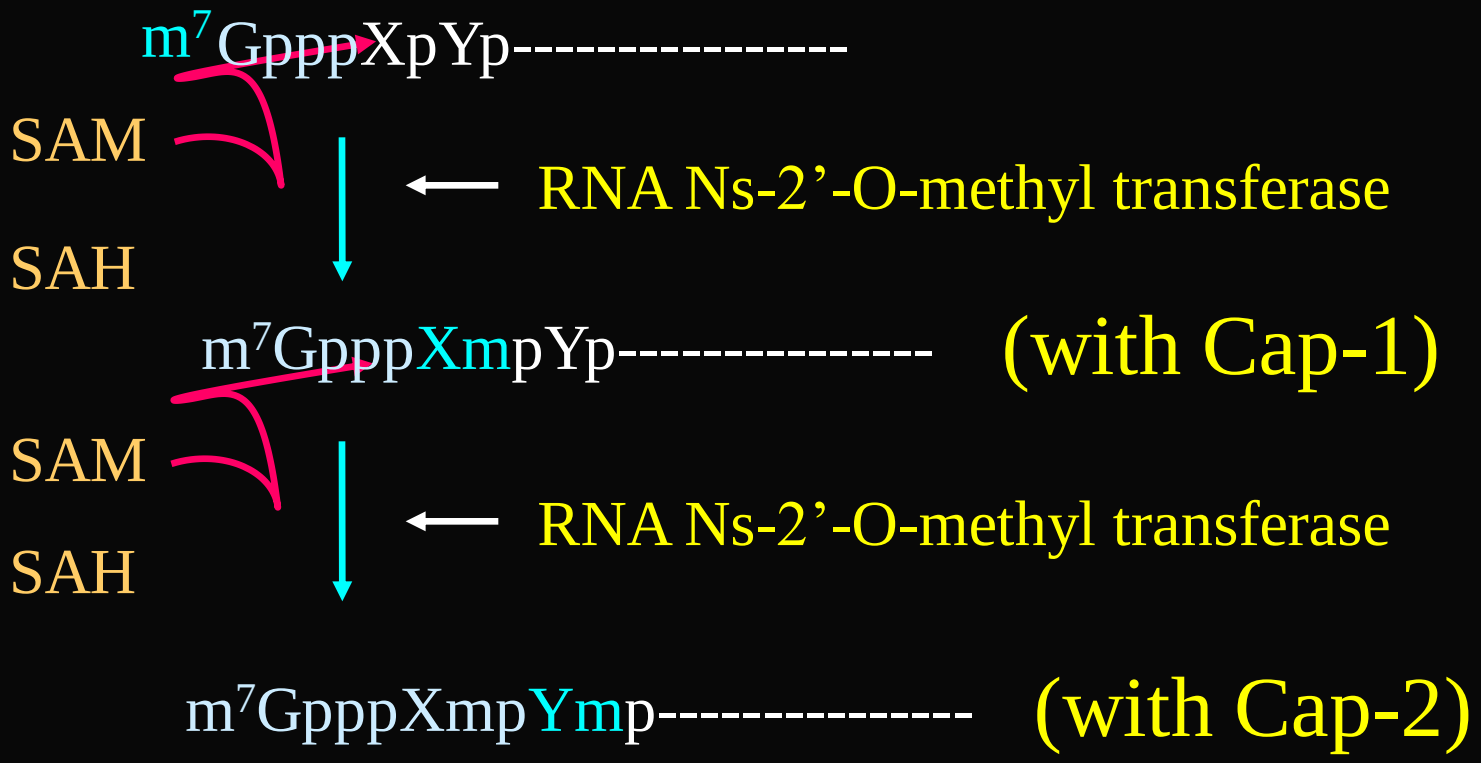


(来源：不详)

mRNA capping proceeding

- After 30 ±Nt tramscripted, pre-RNA capping start

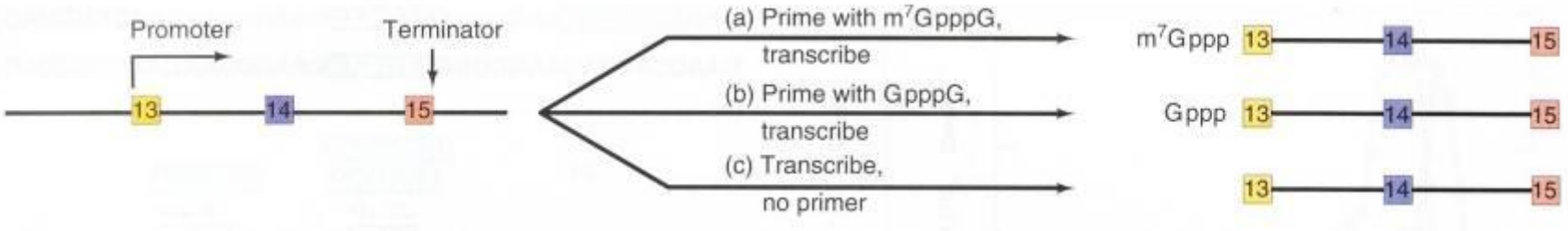


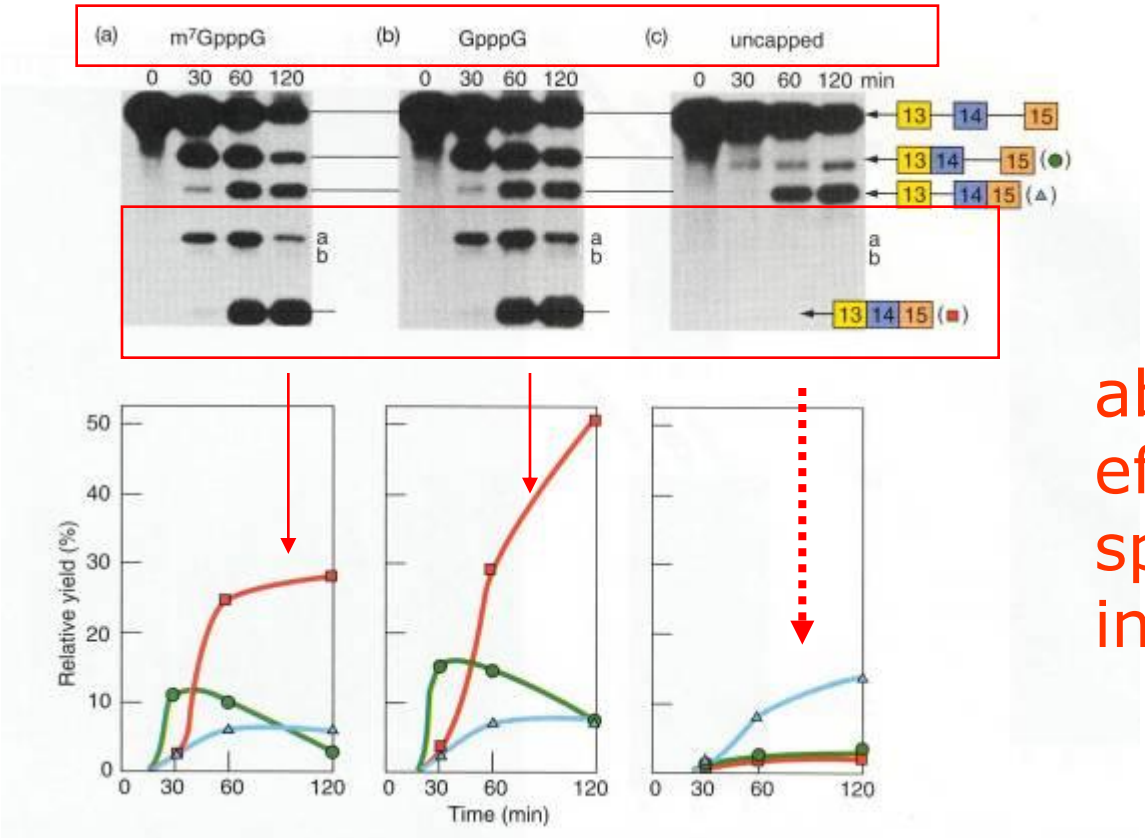


SAM; S - Adenosyl - L - methionine

SAH; S - Adenosyl - L - homocysteine

Production of capped and uncapped splicing substrates





absence of cap effectively inhibits splicing of the first intron.

Source:M.,H.Sakamoto,Preferential excision of the 5' proximal intron from mRNA precursors with two introns USA 84(1987)P.5189,F3.

4.4.3. pre-RNA tailing

- 概念

A poly(A) tail (50-200±) be added at -20 Nt ± tailing signal (AAUAAA) from 3'-end of Pre-RNA

Rabbit α- globin mRNA

5'-------CUUUGAAUAAA----------poly(A) 3'

-20

Rabbit β- globin mRNA

5'-------UGGCUAAUAAA----------poly(A) 3'

-20

Man α- globin mRNA

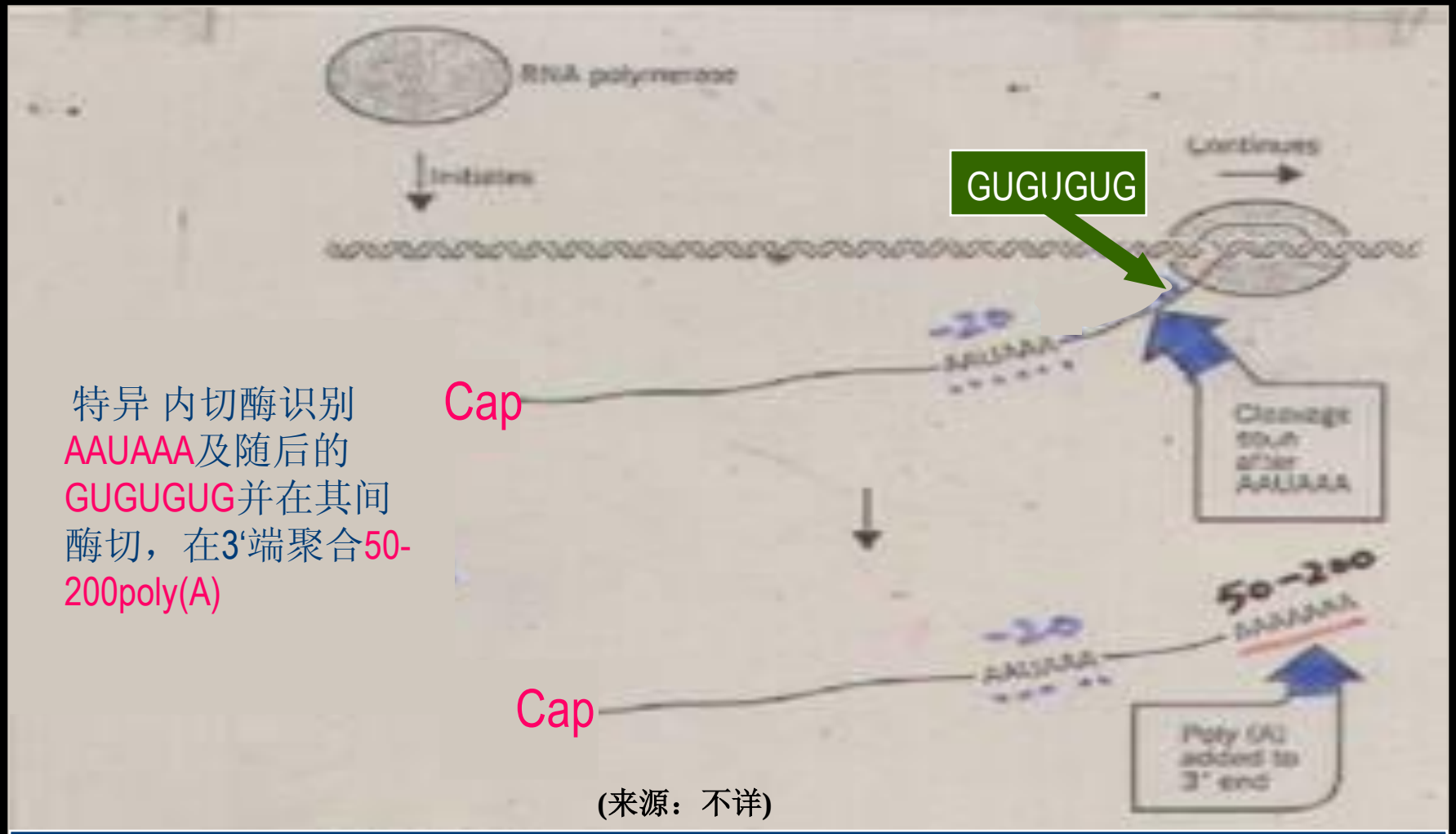
5'-------CUUUGAAUAAA----------poly(A) 3'

-20

Man β- globin mRNA

5'-------UGCCUAAUAAA----------poly(A) 3'

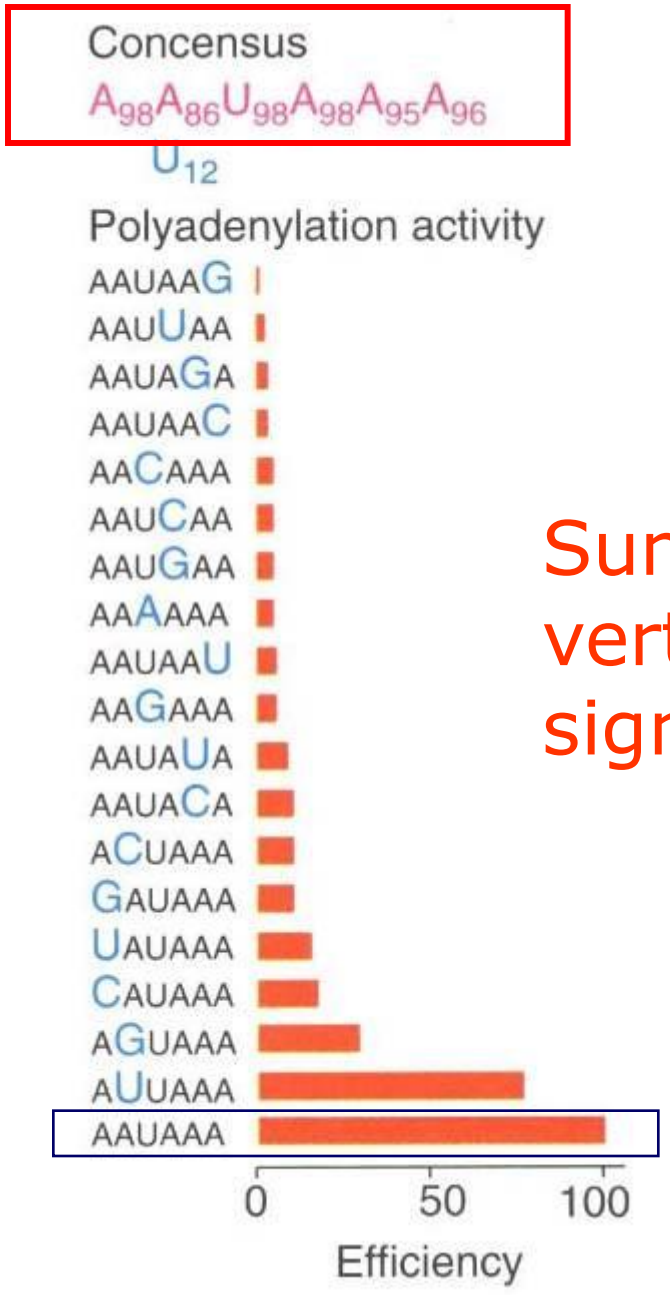
-20



特异 内切酶识别AAUAAA及随后的GUGUGUG并在其间酶切，在3'端聚合50-200poly(A)

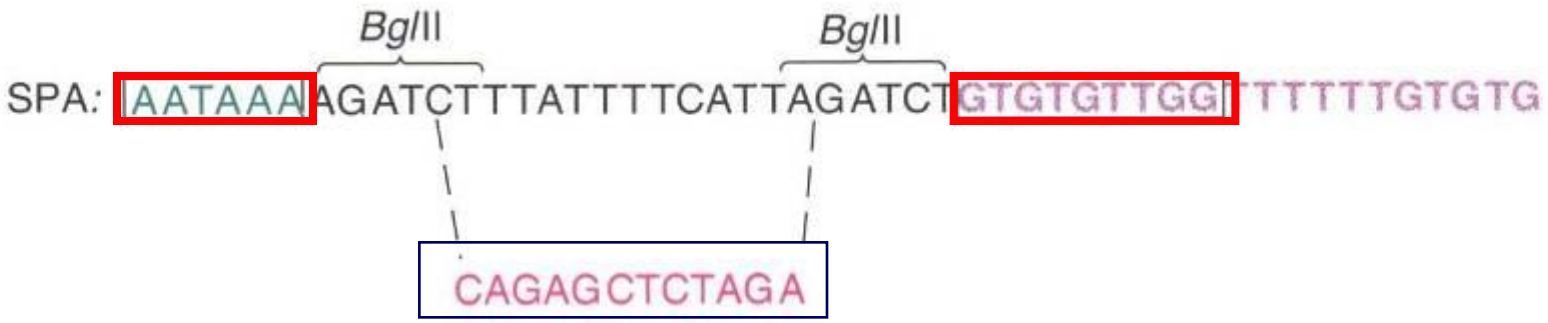
(来源：不详)

Proceeding of pre-RNA tailing Recognizing site



Summary of data on 369 vertebrate polyadenylation signals.

(Source:M. Wickens, "How the messenger got its tail: addition of poly(A) in the nucleus," Biochemical Sciences,1990 vol. 15)



Structure of the synthetic polyadenylation site (SPA)

unrelated sequence could be inserted in place of the natural sequence between the AATAAA and GT/T motifs and Nt substitution made

no difference in efficiency of polyadenylation.

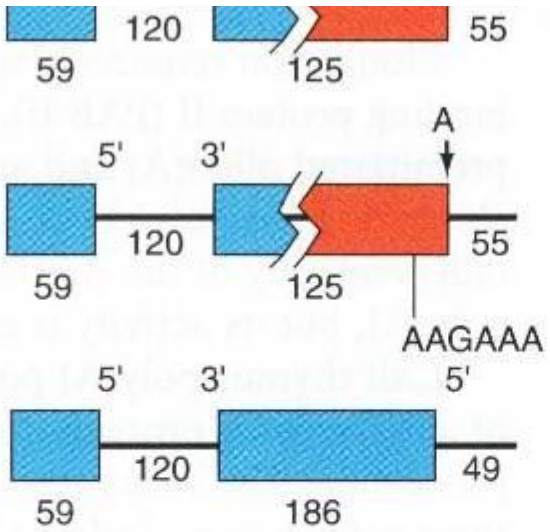
(Source:N. Levitt "Definition of an efficient synthetic poly(A) site," Genes & Development 3:1019-1025, 1989)

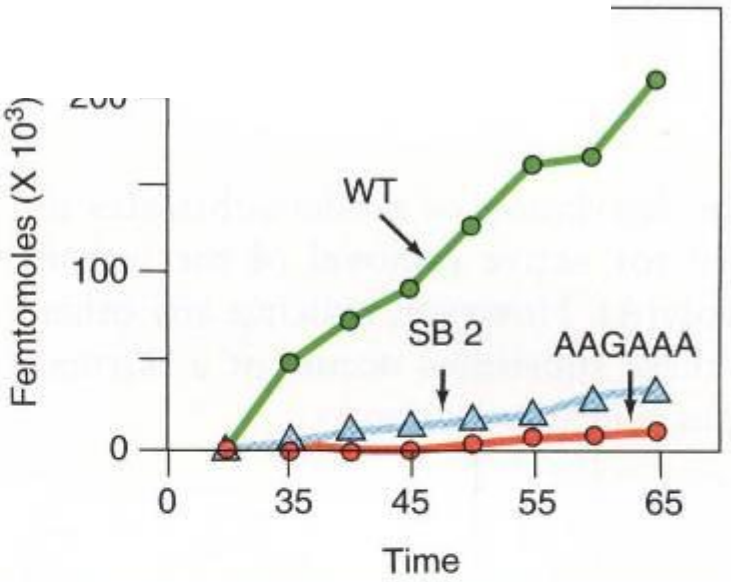
Polyadenylation of model substrates is required for active removal of the intron closest to the poly(A).

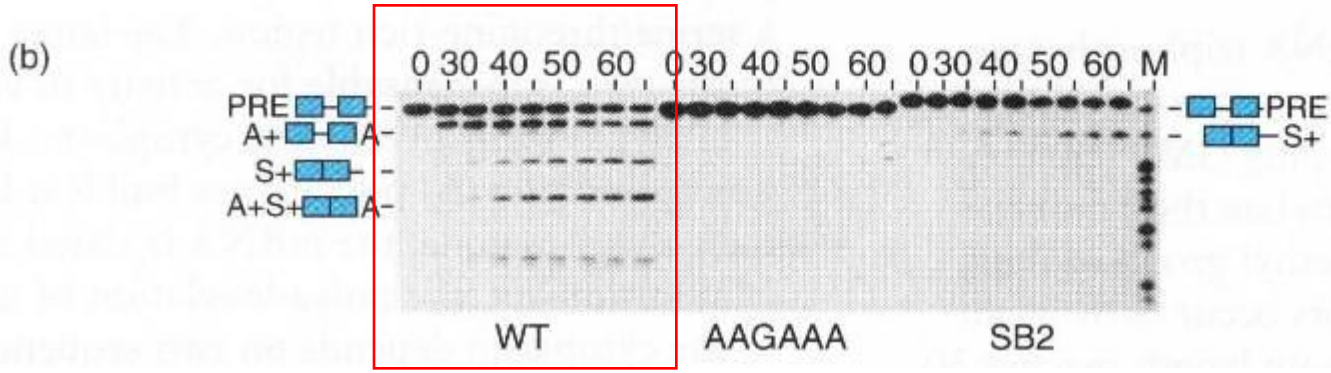
AAU/ WT
polyadenylated

mutation AAGAAA
Un-polyadenylated

No AAUAAA SB 2
Un-polyadenylated







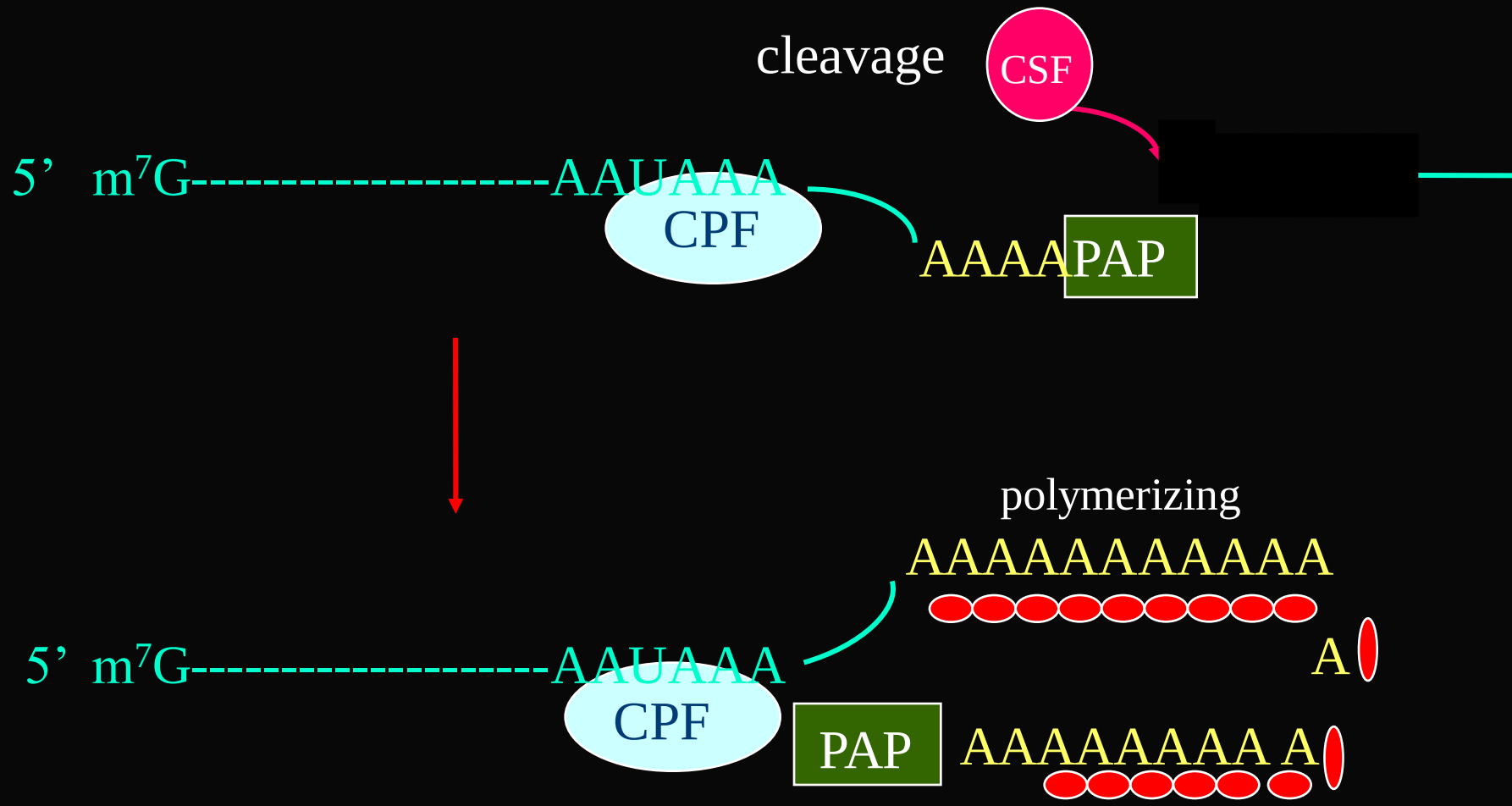
(来源：分子生物学（2007），郑用琏，第162页)

- 过程；

CPF (cleavage and poly(A) specific factor)

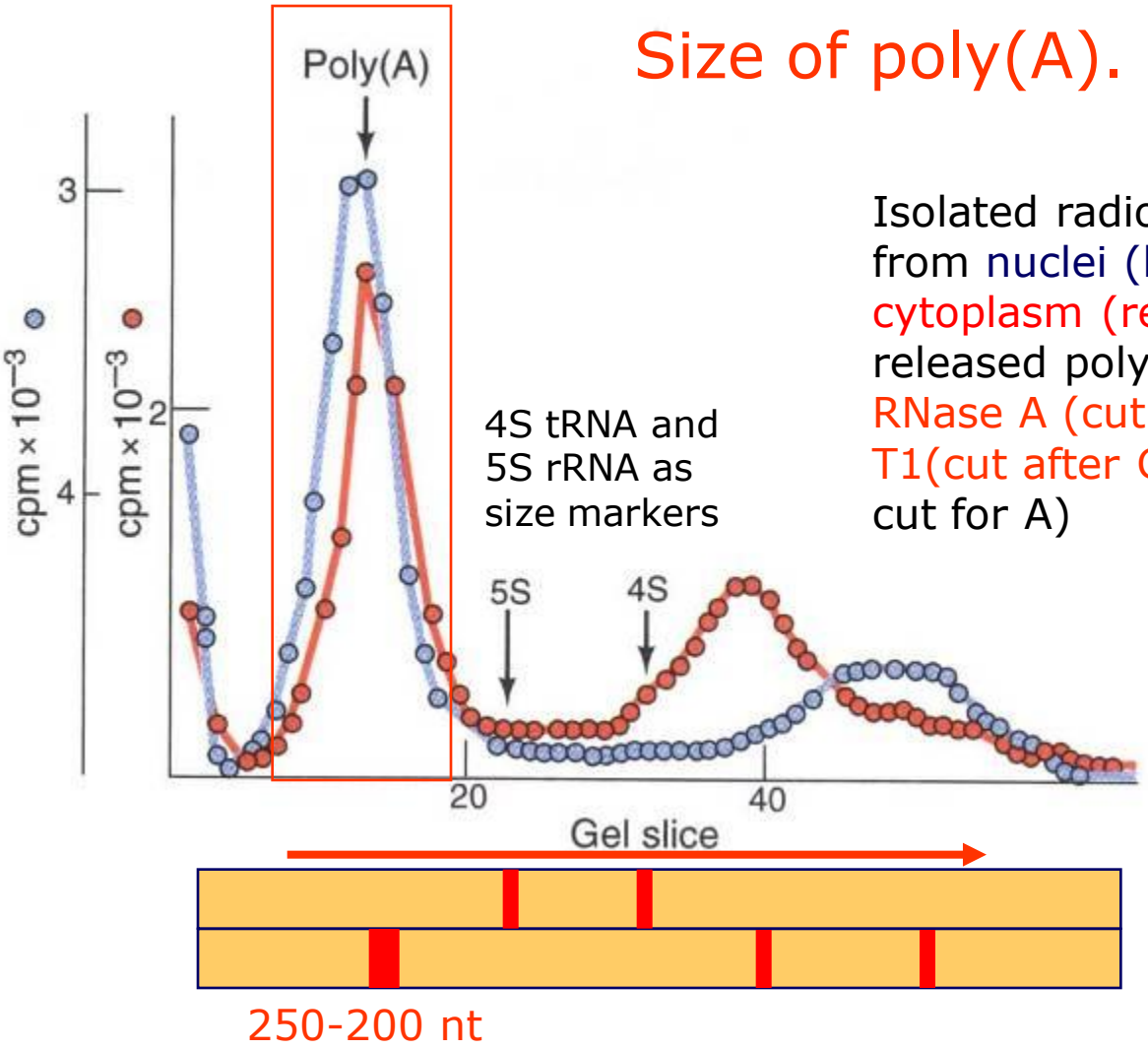
PAP (poly(A) polymerase)

CSF (cleavage stimulation factor)
(stabilize CPF-RNA-PAP complex)



PABP II (poly(A) Binding Protein)
Promotion poly(A) elongation

Size of poly(A).

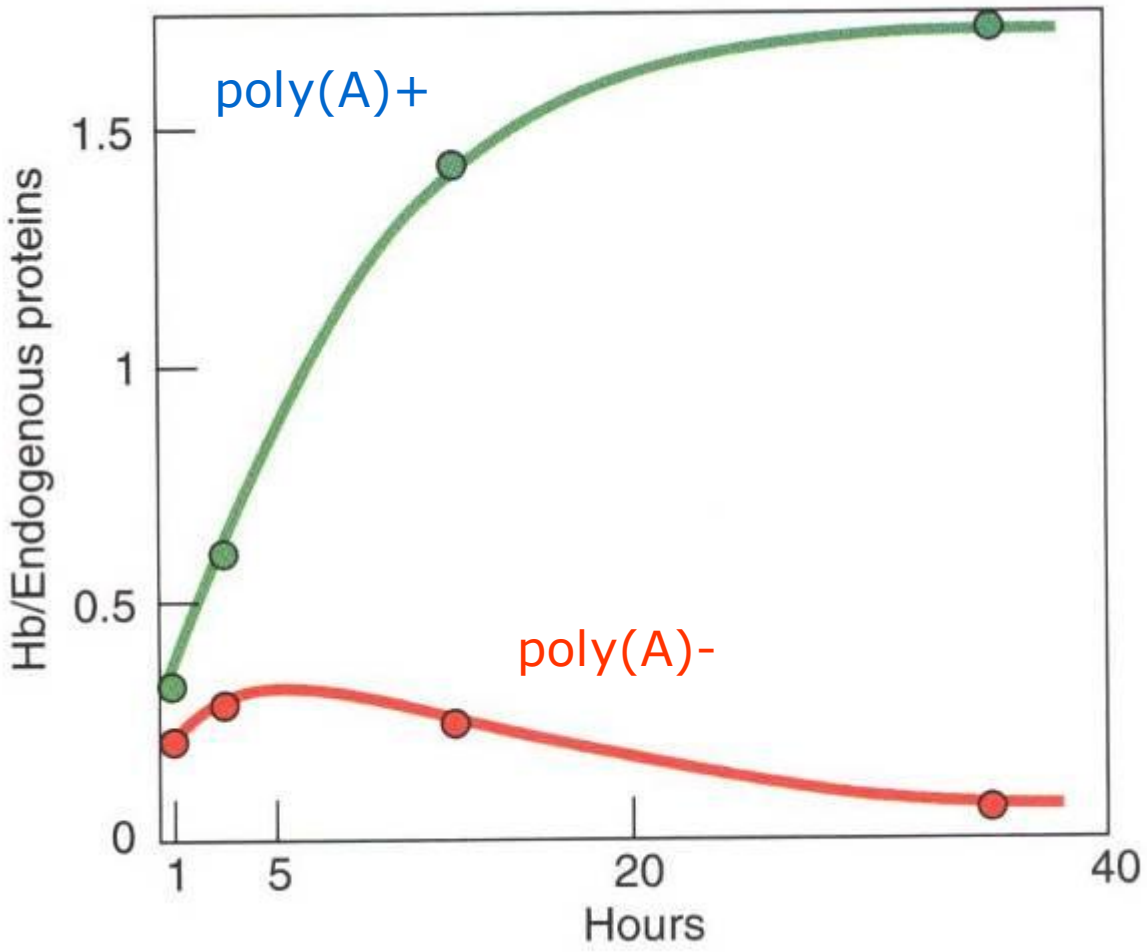


4S tRNA and 5S rRNA as size markers

Isolated radioactively labeled hnRNA from nuclei (blue), and mRNA from cytoplasm (red) of HeLa cells, then released poly(A) from these RNAs by RNase A (cut after U/C)and RNase T1(cut after G) treatment.(No RNase cut for A)

Both poly(A)s electrophoresed more slowly than the 5S marker, corresponding to molecules about 200-250 nt long.

(Source:Sheiness and Darnell Nature New Biology 241:267, 1973)

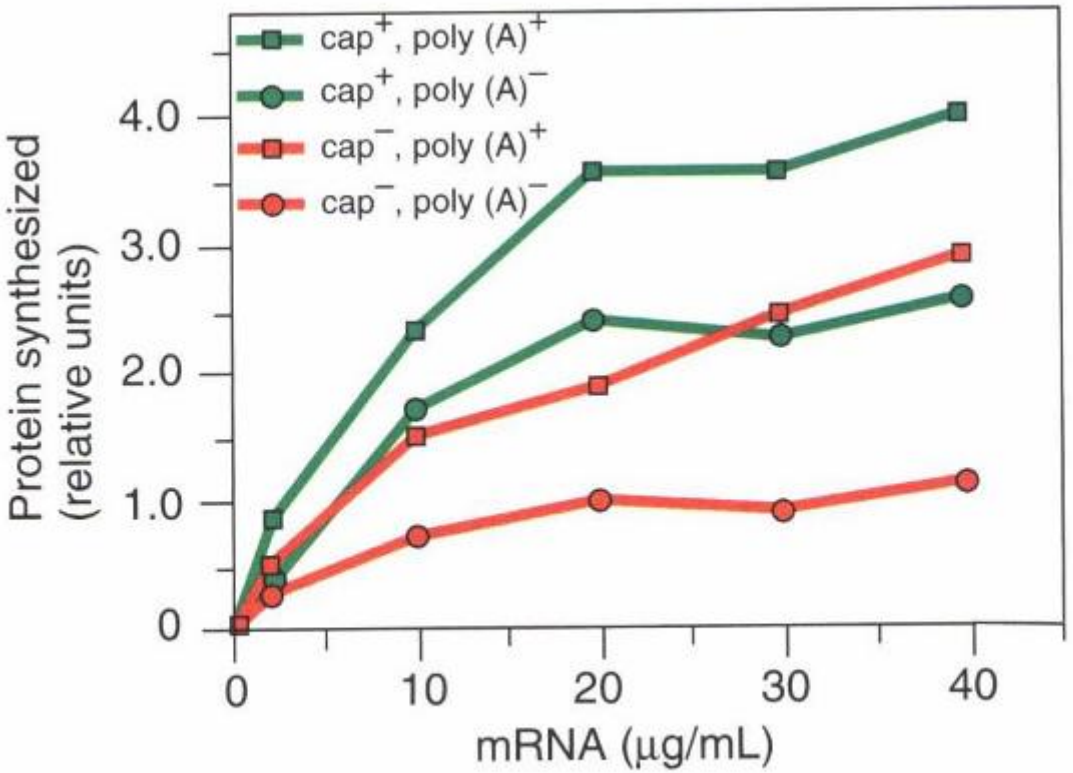


Effect of poly(A) on translation of globin mRNA in oocytes

The poly(A)+ mRNA remained active much longer than the poly(A)- mRNA

(Source:G. Huez et al PNAS 71 (8):3143-3146, August t 974.)

线粒体/叶绿体中Poly(A)导致mRNA的降解?!

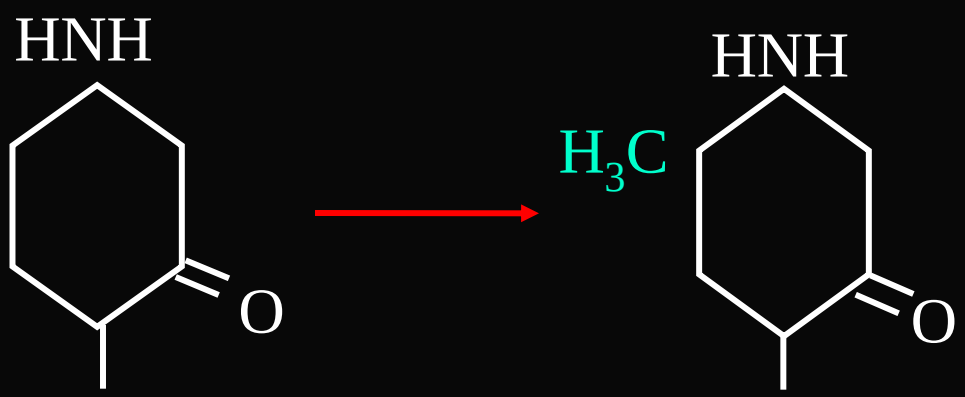


Effect of polyadenylation on translatability and stability of mRNAs.

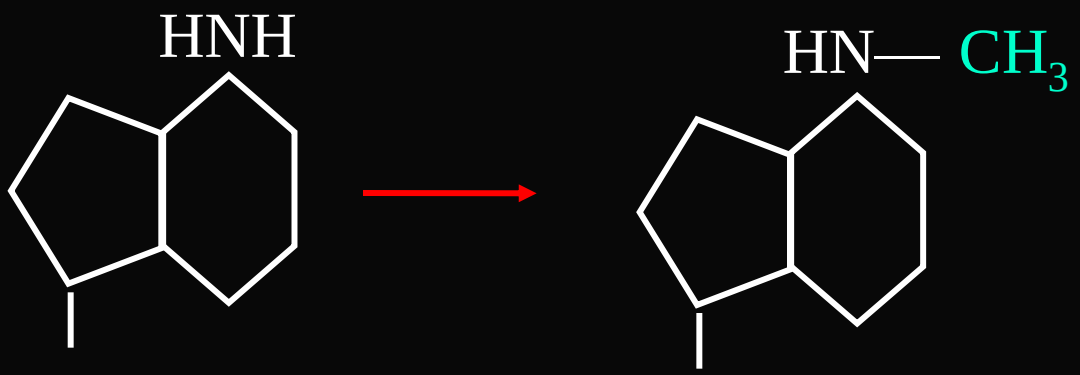
(Source:Munore,D.mRNA poly(A) tail ,a 3' enhancer of a translational initiation.,Molecular and Cellular Biology 10:3445,1990)

4.4.4. RNA internal methylation

m^5C



m^6A



常见tRNA中的修饰核苷酸

Xo^5U	(5-羟基尿苷)
$Cmnm^5U$	(5-羧甲基氨甲基尿苷)
mCm^5U	（5-甲氧基羰甲基尿苷）
Xm^5s^2U	（5-甲基-2硫代尿苷）
K^2C	（2-赖氨酸胞苷）
Com^5U	（5(2)-羟羧甲基尿苷）
I	（Inosine次黄嘌呤）
m^7G	(7-甲基尿苷)
m^5C	（5-甲基胞苷）
m^6A	（6-甲基腺苷）
s^2C	（2-硫代胞苷）
ψ	(假尿苷）
Um	(2’-O-甲基尿苷)
Q	（Queuosine）

4.4.5. pre-RNA 剪接的分子基础

4.4.5.1 剪接装置与底物

a) 核内RNA (nRNA / hnRNA)

- RNApol I → 18s, 5.8s, 28s rRNA (nucleolus)

  RNApol II → mRNA, U1, U2, U4, U5-SnRNA (nucleoplasm)

  RNApol III → tRNA, 5s rRNA, U6-SnRNA (nucleolus & nucleoplasm)

- pre-mRNA $\bar{X}$ ≈7 kb ±; Max.≈20 kb;

  4-10x mature RNA $\bar{X}$≈ 8 Introns

- SnRNA ≤ 250 Nt; 5’-end with Cap-2

  Rich U (named SnRNAU1—U6)

b) RNProt. (核内仅次于组蛋白的重要蛋白)

Ribose nuclear proteosome (RNP)

pre-RNA + SnRNA + > 8 proteins

核糖核蛋白体

c) Intron

--- junction sequence of intron with exon

高度保守，成为剪接过程重要的识别序列

人类许多重要疾病的病因

junction seq. mut. → 异常剪接 → 病症

例：地中海贫血症

1/4 Junction seq. mut. of Globin gene

→ 干扰mRNA成熟

---Introns be classified

into I, II, III by junction seq.

Intron I, II, III 的剪接方式不同

合理有效折叠，形成特定Domain，激活核酶活性

RNA剪接的共性规律

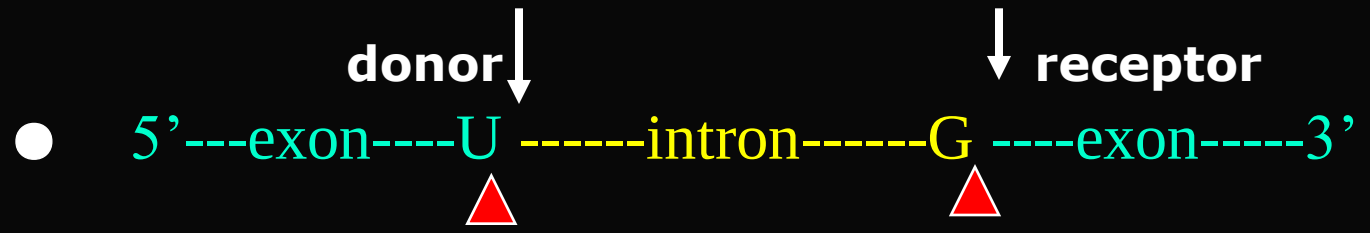
选择供点受点，转移磷酸酯键，剪切连接同步

4.4.5.2

RNA splicing models

Group I splicing model (*tetrahymera* 35s rRNA model)

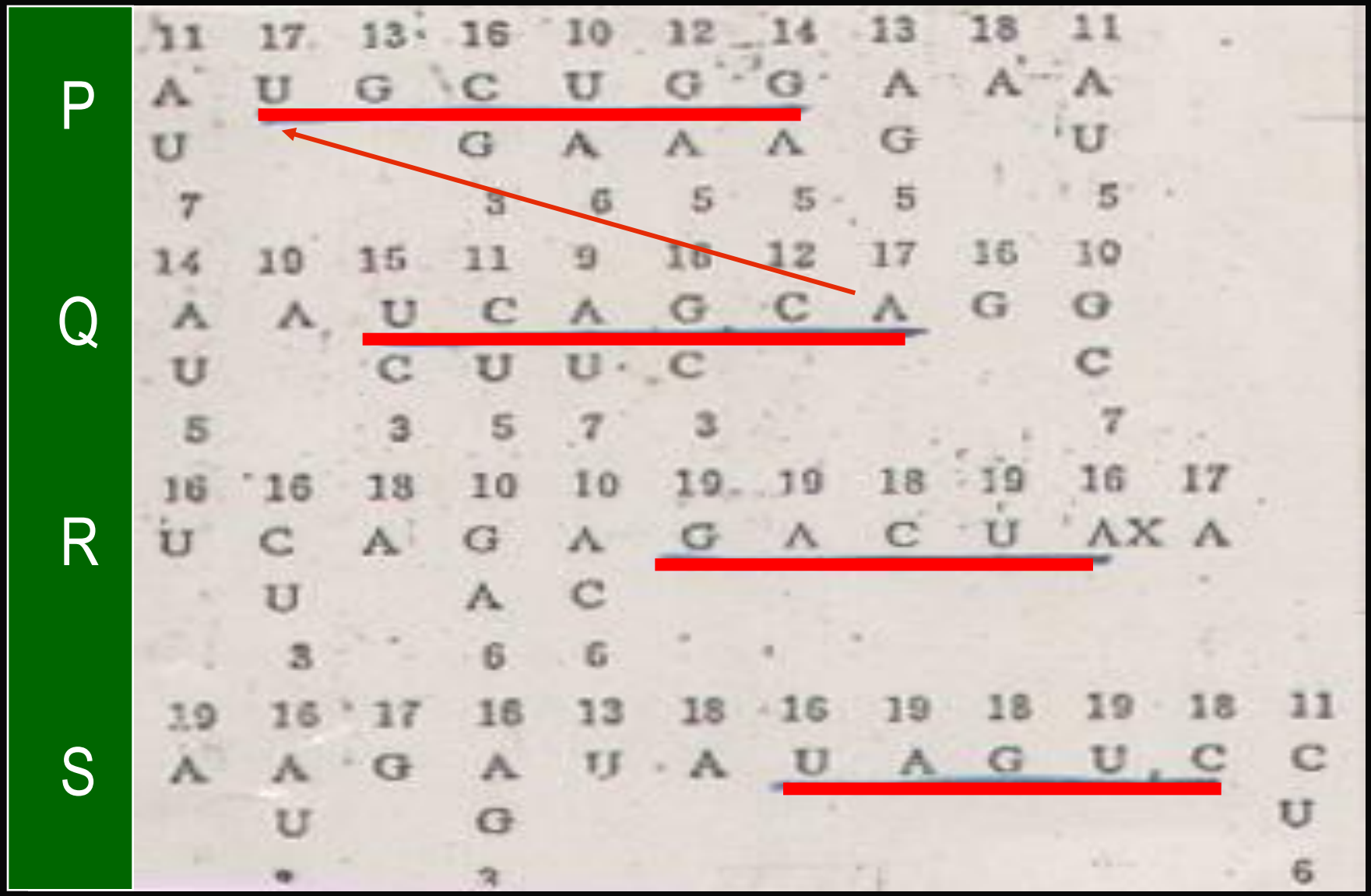
Junction sequence.



高度保守，转酯反应的攻击位点

- intron 内含有4个序列保守，两两互补的CCS (central core sequence)

形成RNA分子内的二级结构



(来源：不详)

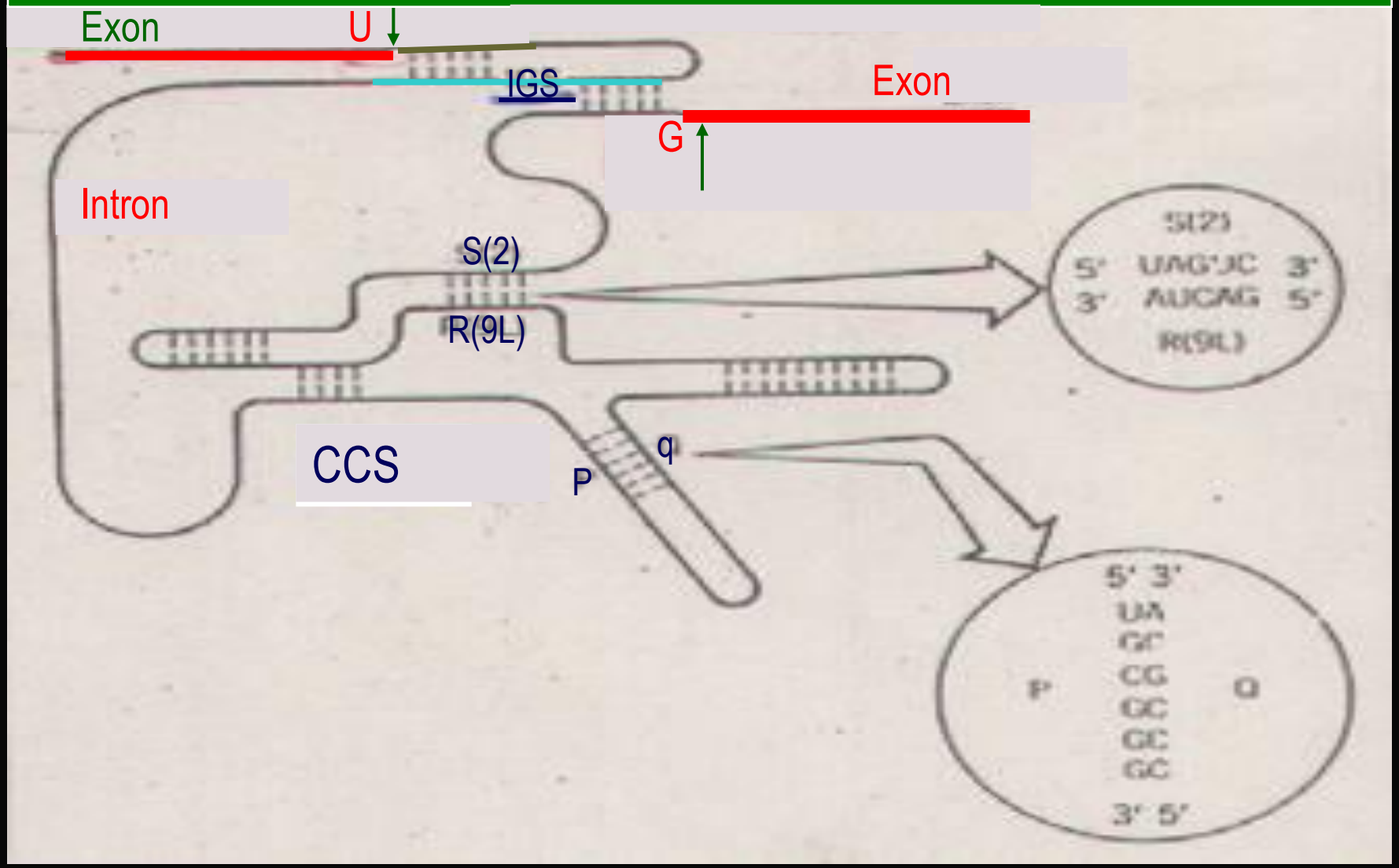
- 靠近5’ junction seq.的intron内，存在一段与5’, 3’ 两个 junction seq. 可发生互补配对，形成二级结构的区域。

Internal guide sequence (IGS)

Splicing model

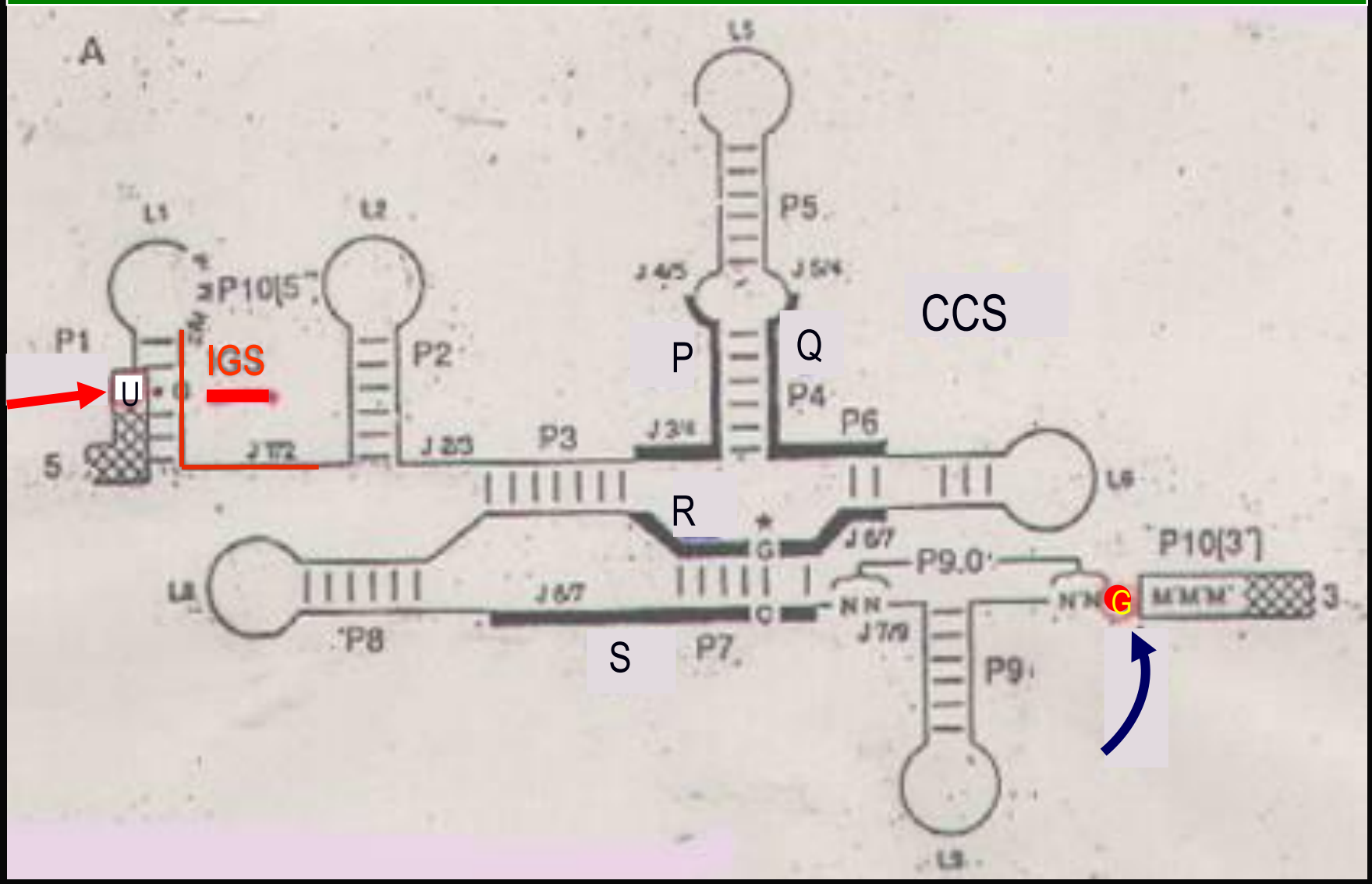
- CCS; P/Q, R/S pairing
- IGS; link two junction seq. → cut intron, ligation exons
- 3 times of trans-esterification between G & U
- RNA auto-splicing as Ribizyme

Splicing mechanism of Intron group I



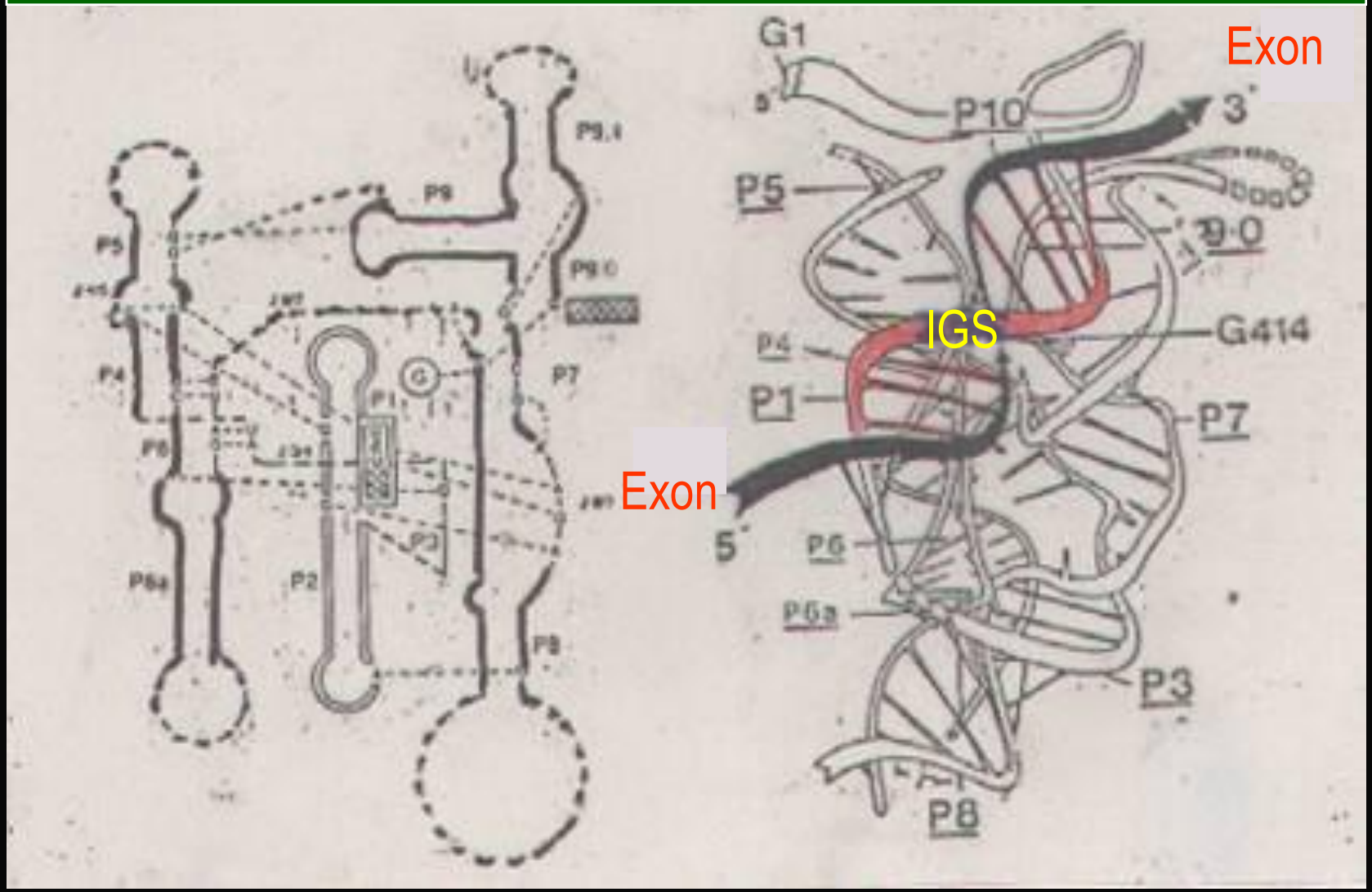
(来源：不详)

Splicing mechanism of Intron group I



(来源：不详)

Splicing mechanism of Intron group I



RNA auto-splicing as Ribizyme

Thomas Cech Univ. of Colorado 1989 Nobel prize

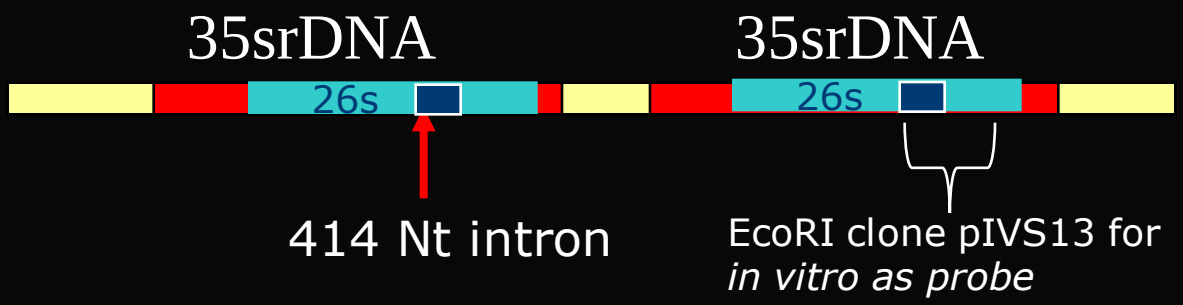
One of the most stunning discovered

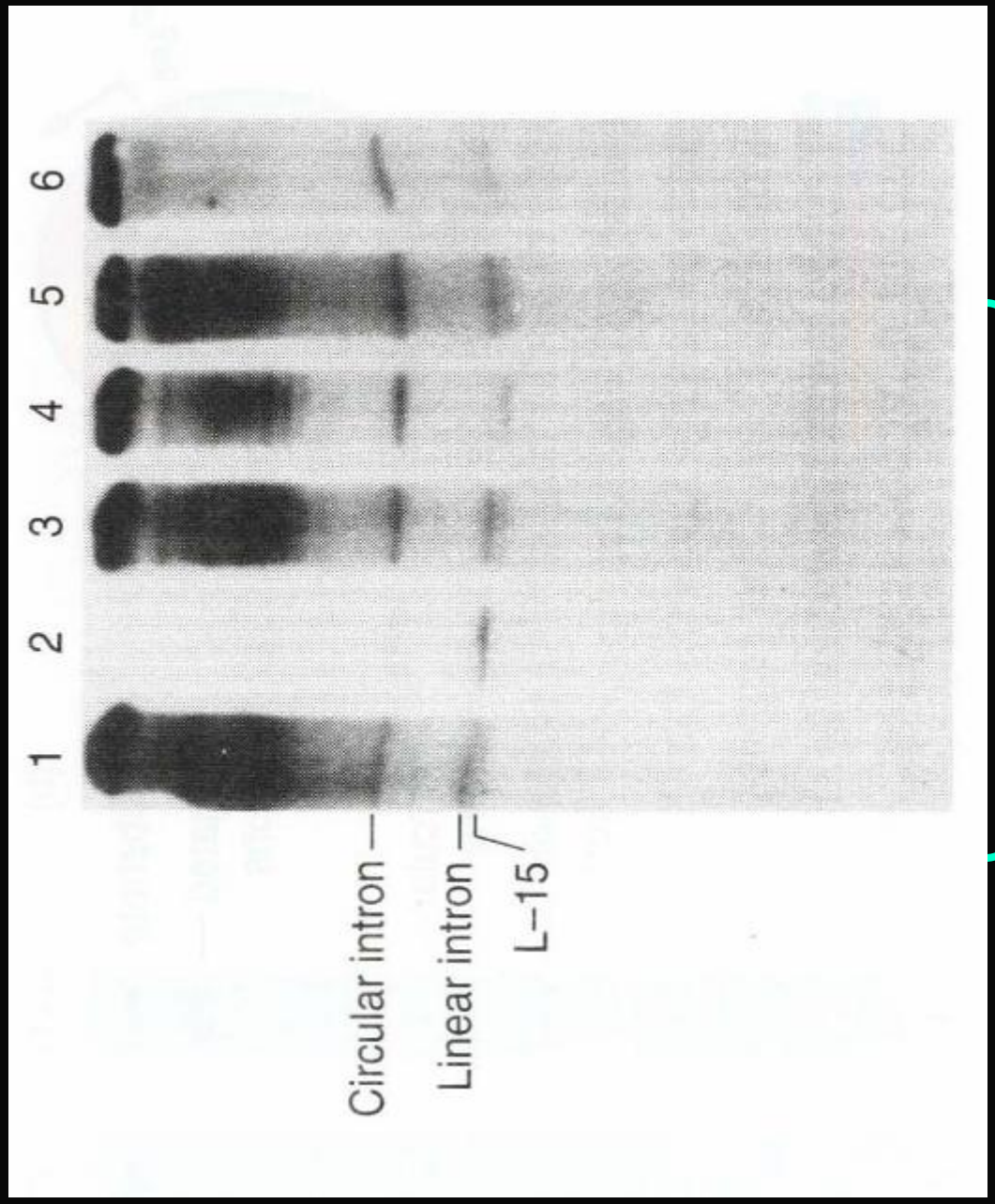
in molecular biology

1981 Thomas Cech ***tetrahymeno thermophila***

染色质结构对基因表达的影响

linear rDNA-dimer including two of 35s rRNA seq.





in vitro

pre-35s rRNA

GTP / GDP/ GMP

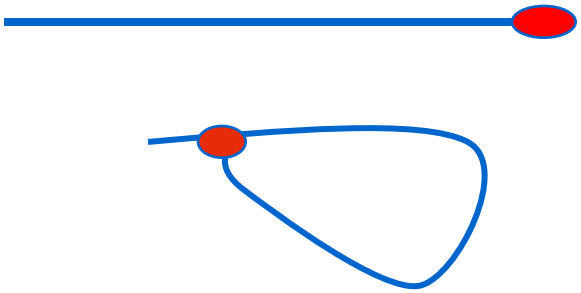
same results

in vitro pre-35s rRNA

重要推测

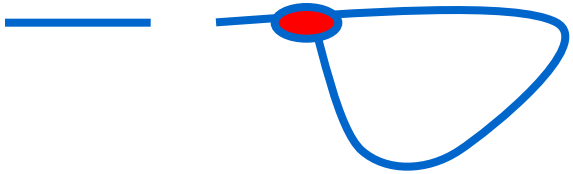
↓ GTP_{α}^{32}

414Nt intron ~ GMP^{32}



↓

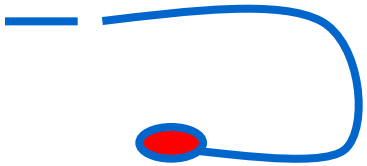
15Nt + 399Nt circle intron



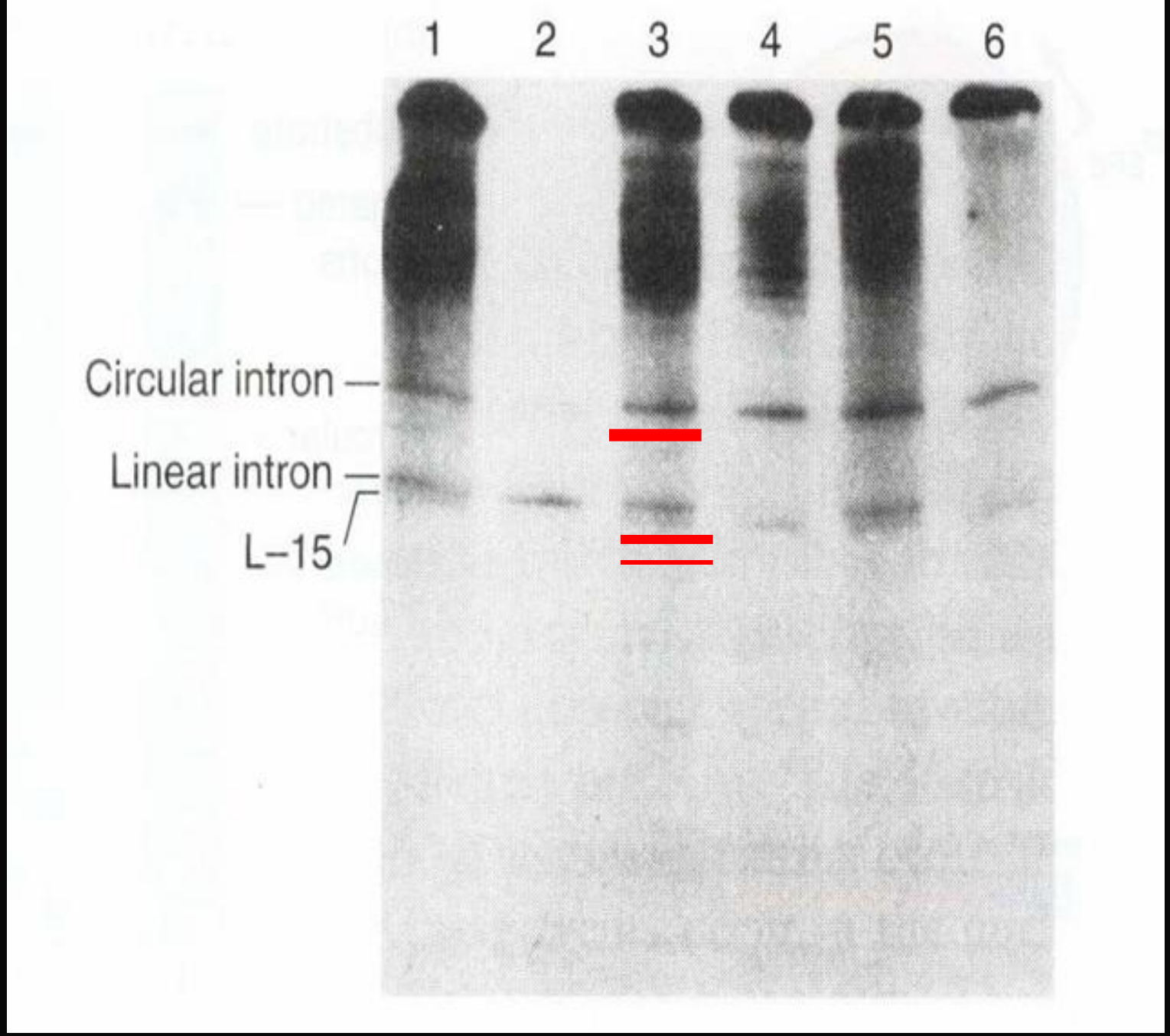
↓

Lose 19 Nt

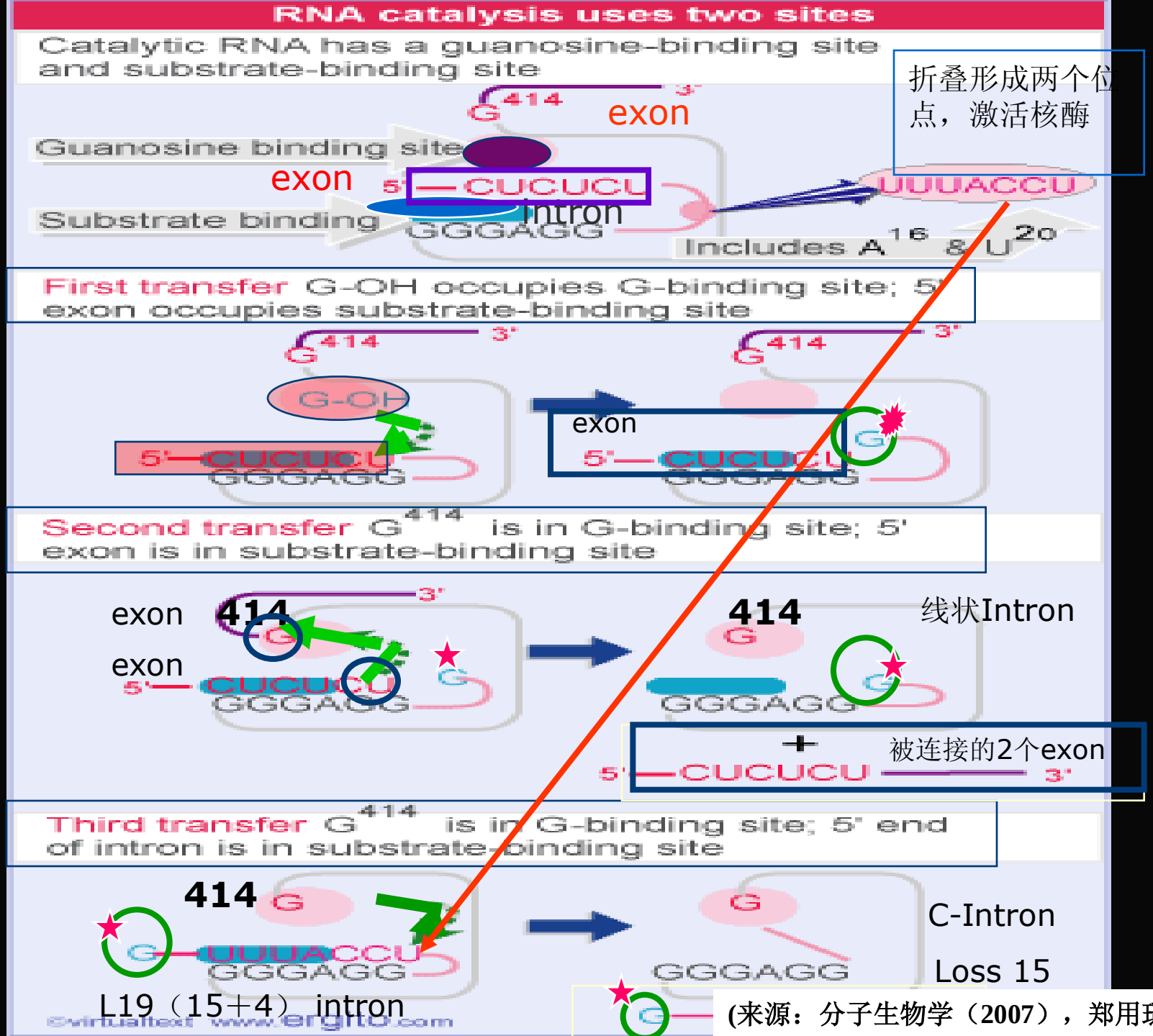
4Nt + 395Nt linear intron



(L19)



(Source:Inane.T.,Intermolecular exon ligation of the rRNA precursor of Tetrahymena:Oligonucleotides can function as 5'-exons.Cell 43(Dec 1985)f.1a,p.432.)



(来源：分子生物学（2007），郑用琏，第30页)

以上结果表明；

- GMP通过转酯（trans-esterification）方式

  → 进入Intron

- Intron 的剪接经过3次转酯反应

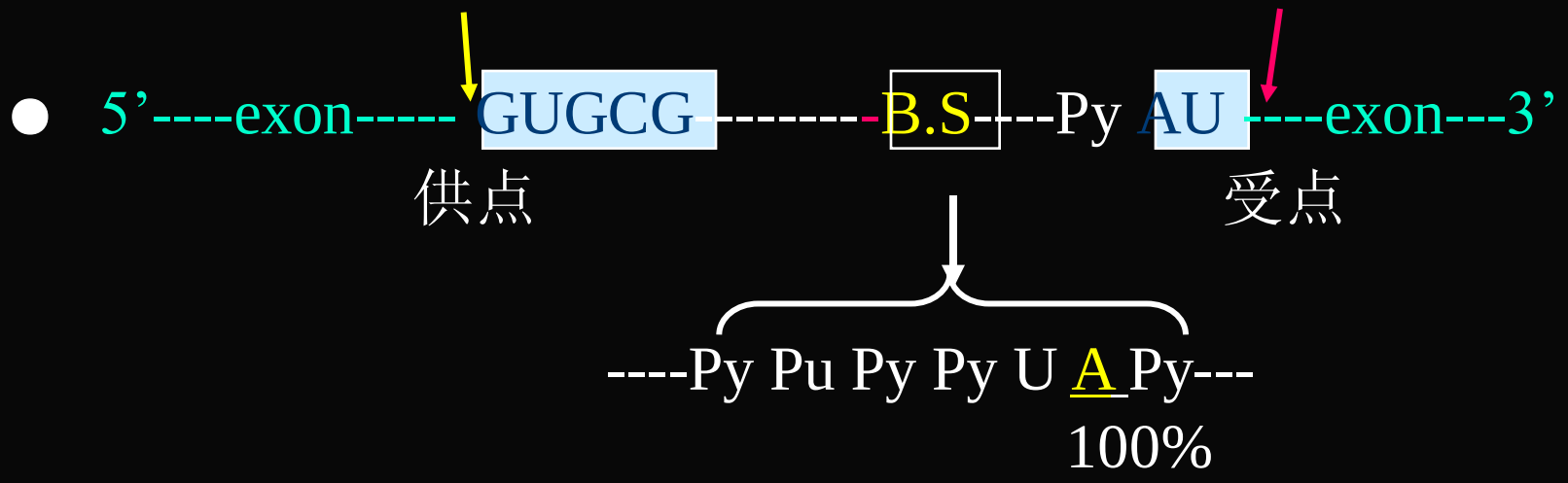
  1^{th} → 将$P^{32}G$插入到RNA中

  2^{ed} → 剪出414Nt的Intran，连接Exon

  3^{rd} → 15＋399Nt（Lose 15）

c) Group II splicing model (Michel 1982)

Junction sequence

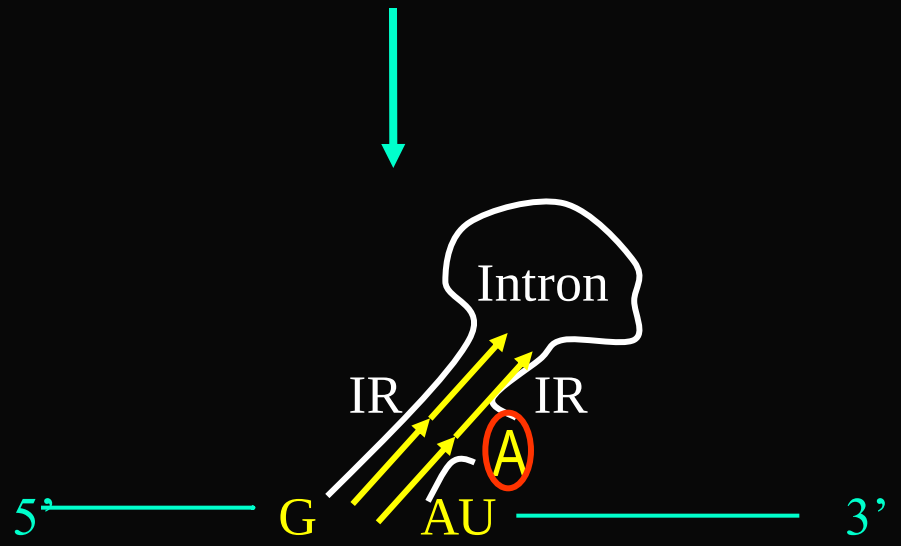


Branch Site of 7Nt

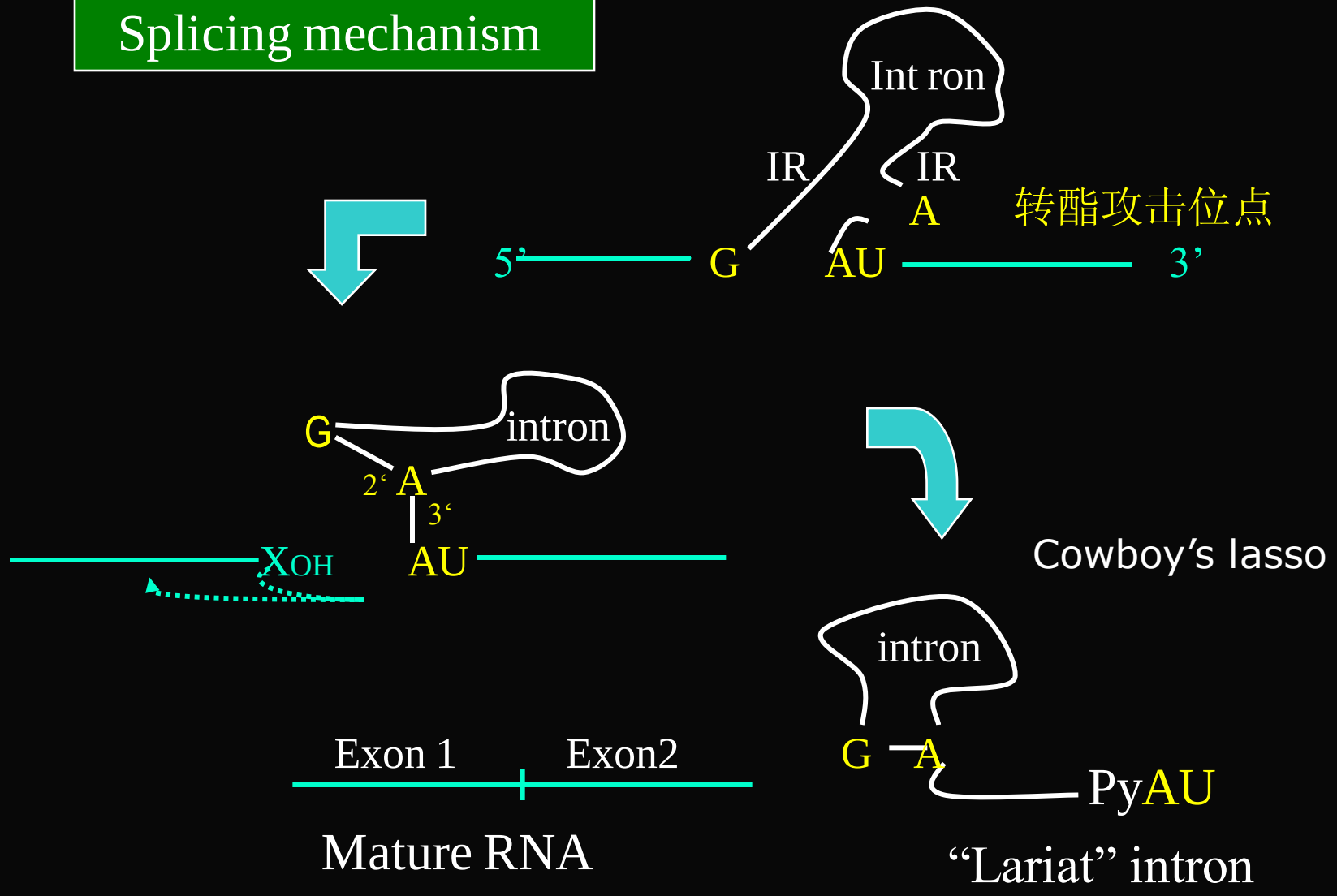
upstream of 3’-junction seq.

- 在Branch Site 的两侧存在一些短的序列，与其上游互成 IR,形成茎环结构(但A不包含在IR序列内, 从而被排除成芽状突起）

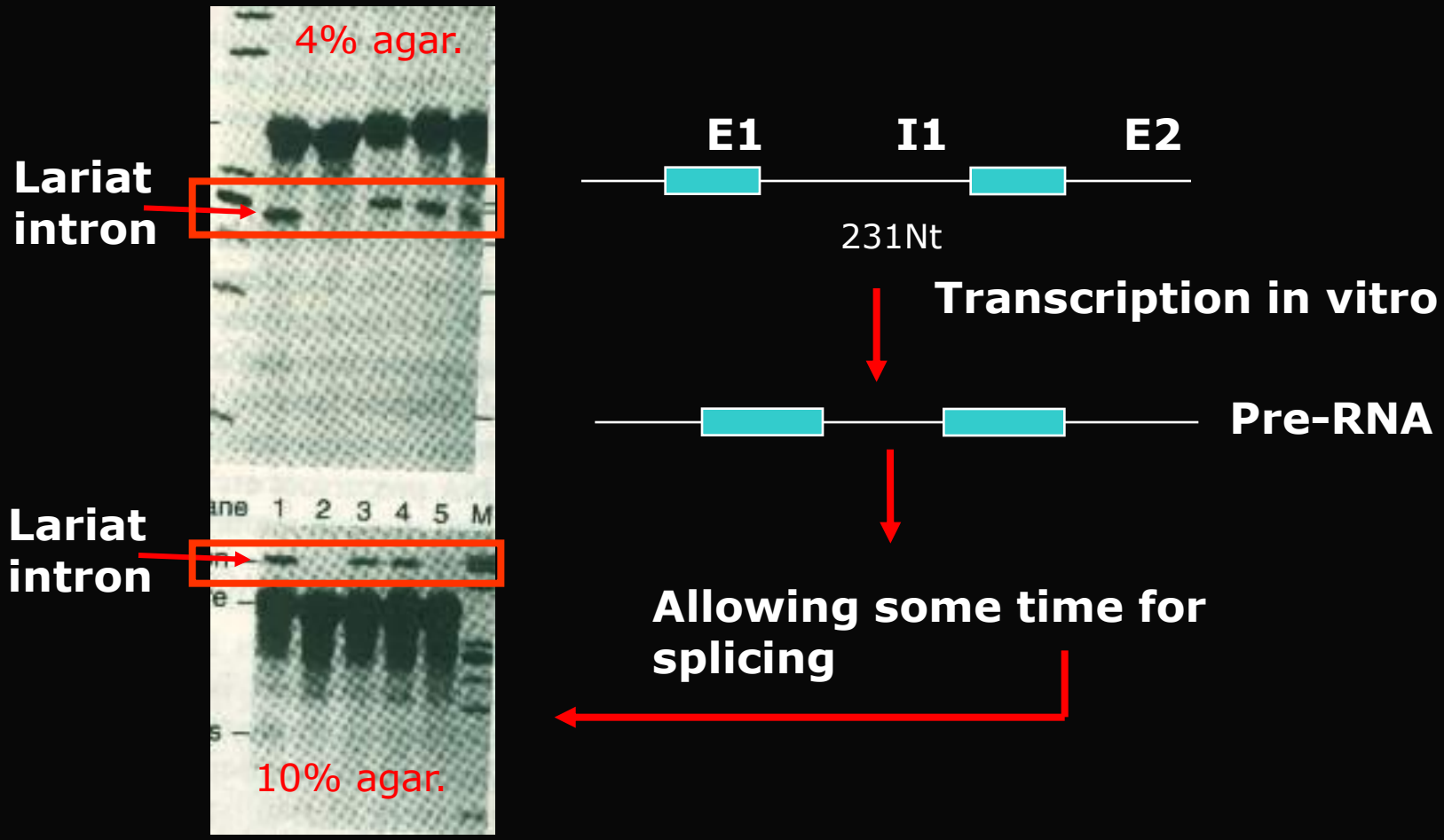
5’--GUGCG----------PyPuPyPyUAPy-----PyAU—3’



Splicing mechanism



Evidence for the Lariat intermediate *1984 Sharp*

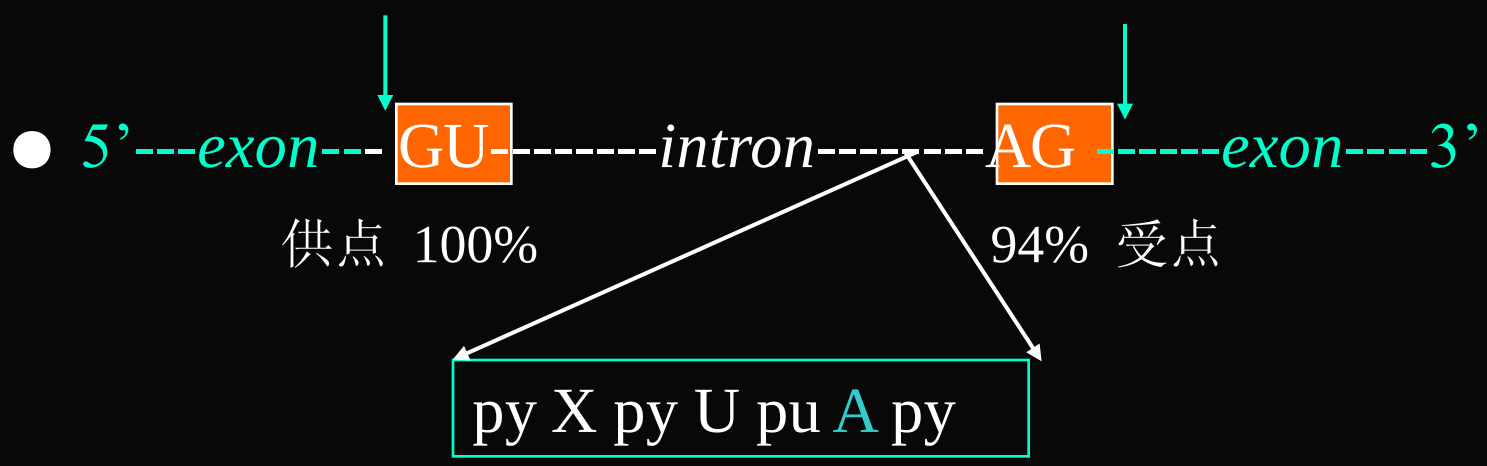


(来源：分子生物学（2007），郑用琏，第166页)

d) Group III splicing model (Chambon)

Junction sequence

Chambon rule



- 7Nt branch site in upstream of 3’ junction seq.

- Intron与 Exon的连接处 存在部分同源的 consensus seq.

Ovalbumin mRNA (6 Intron, 7 Exon)

5’---*E1*---AAUAGGUGA--------*I1*-----UACAGGUUG---*E2*--

---*E2*--CUCAGGUACA-------*I2*-------UCAGGUUG---*E3*--

---*E3*-------CCAGUAA---------*I3*-----UACAGGAA------*E4*--

---*E4*-------AUGGUAA--------*14*-------AAAGGU--------*E5*--

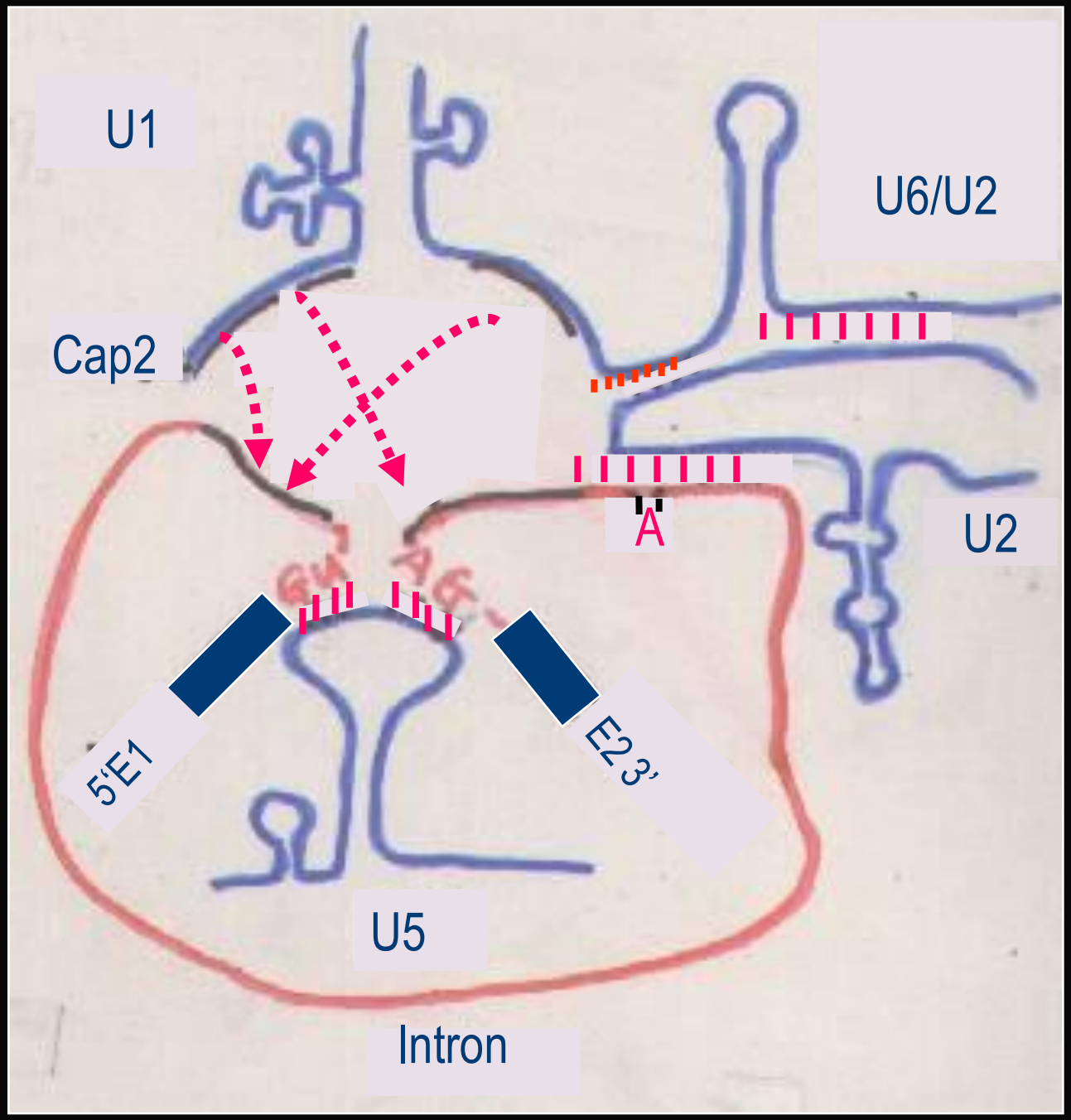
---*E5*-------GAGGUAUAU----*I5*-------CCAGCAA------*E6*--

---*E6*-----GCAGGUAUGG---*I6*-------GCAGCUU-----*E7*---3’

Chambon rule (GU/AG model)

Splicing mechanism (Sharp. P.A. Cell. 1994. 77; p805-815)

- SnRNA (or ScRNA) 与junction seq or Branch site间存在互补区域并参与剪接, 形成 spliceosome。
- Spliceosome 逐级组装， SnRNA 分步替代
- RNProt.在spliceosome中，参与转酯，剪接反应



- SnRNA U1 ~ 5', 3' consensus seq of intron
- SnRNA U2 ~ Branch Site (其中A为转酯攻击位点)
- SnRNA U6 ~ 5' consensus seq. of intron
- SnRNA U6 ~ U4 → SnRNA U6 ~ U2稳定spliceosome
- SnRNA U5 与5'~GU & 3'~AG配对，以连接exons
- Set of SR protein (rich Ser & Arg)

(来源：分子生物学（2007），郑用琏，第166页)

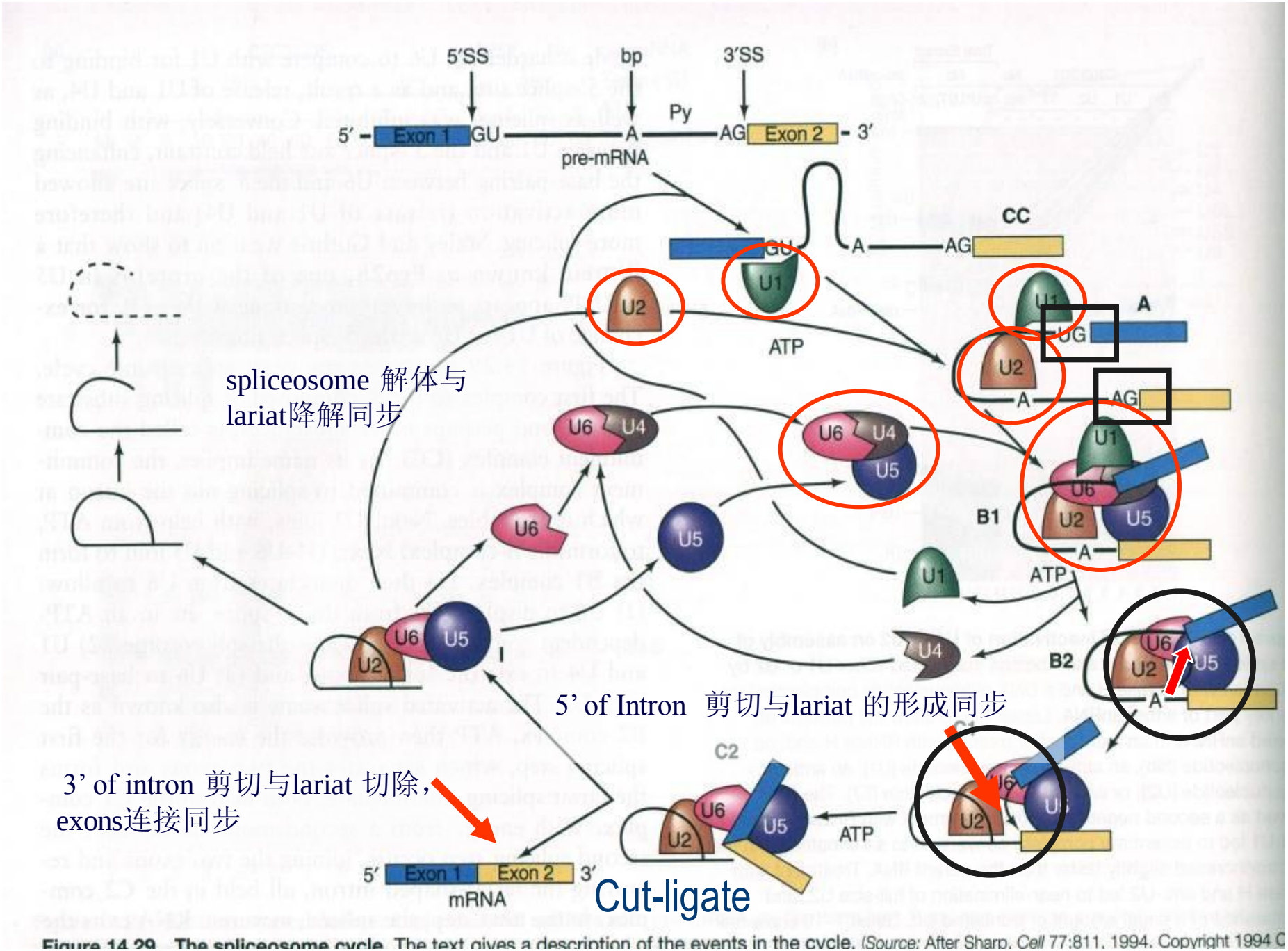
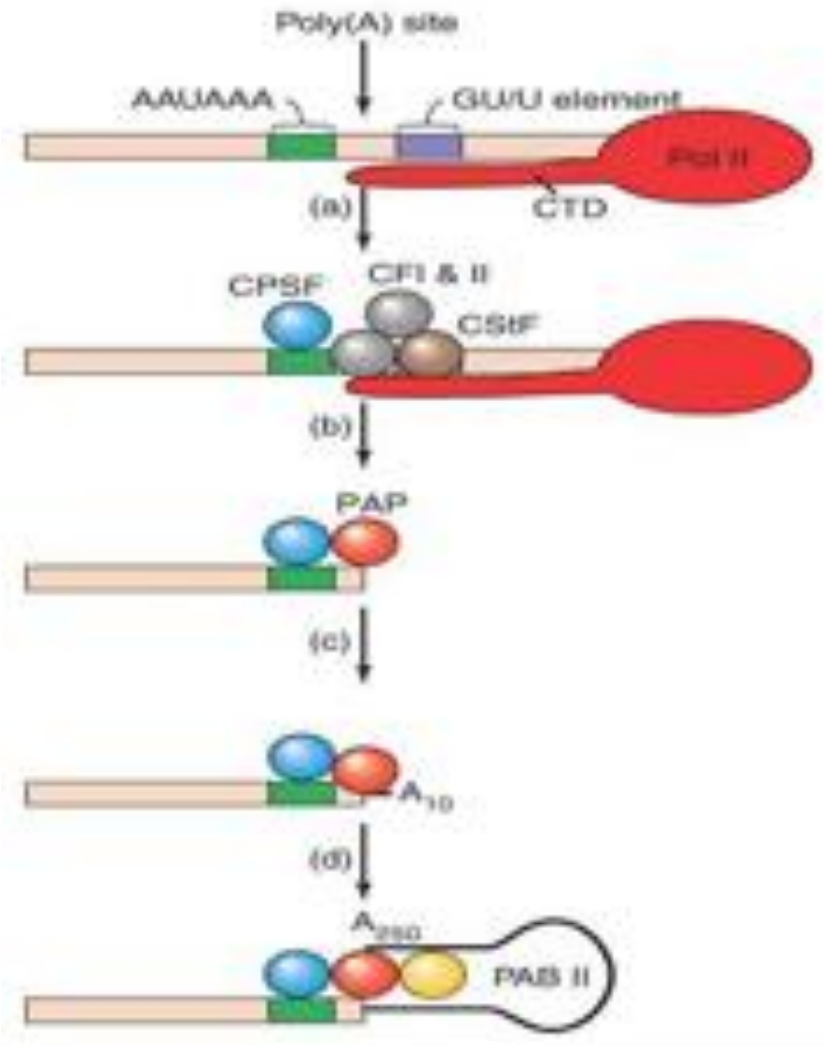


Figure 14.29 The spliceosome cycle. The text gives a description of the events in the cycle. (*Source:* After Sharp, *Cell* 77:811, 1994. Copyright 1994 C



(Source:Molecular Biology(2002),Robert F.Weaver,Page462）

转录与加工几乎同时进行
（转录后的修饰？！）
转录速度愈快
加工效率愈低

所有加工过程的核心元件（unifying element）是:

RNApol Ⅱ -Rpb 1- CTD (carboxyl-terminal domain），

加帽、剪接和多聚腺苷酸化的酶都直接与CTD结合，CTD为这三个加工事件提供了一个平台。

e) 剪接的调控（group III）

- 对供点或受点的选择 ⟶ 剪接的准确与否
- 对受点序列的选择 CAG = UAG > AAG > GAG
- 剪接类型；

组成型剪接 （constitutive splicing）

cutting each intron in one by one

选择型剪接 (alternative splicing)

create isoform protein

>5% gene in mammal

个体发育，细胞分化

Alternative splicing can have profound biological effects

Perhaps the best example is

the Sex-determination

system in Drosophila

- 果蝇性别的发育与性染色体和常染色体的比例相关

XX / AA = 1　　发育成雌性果蝇

XY / AA = 0.5　发育成雄性果蝇

这种调控关系在胚胎发育早期就已确定，并保留记忆于体细胞中

- 3个关键基因参与果蝇的性别决定

sxl (性致死基因)

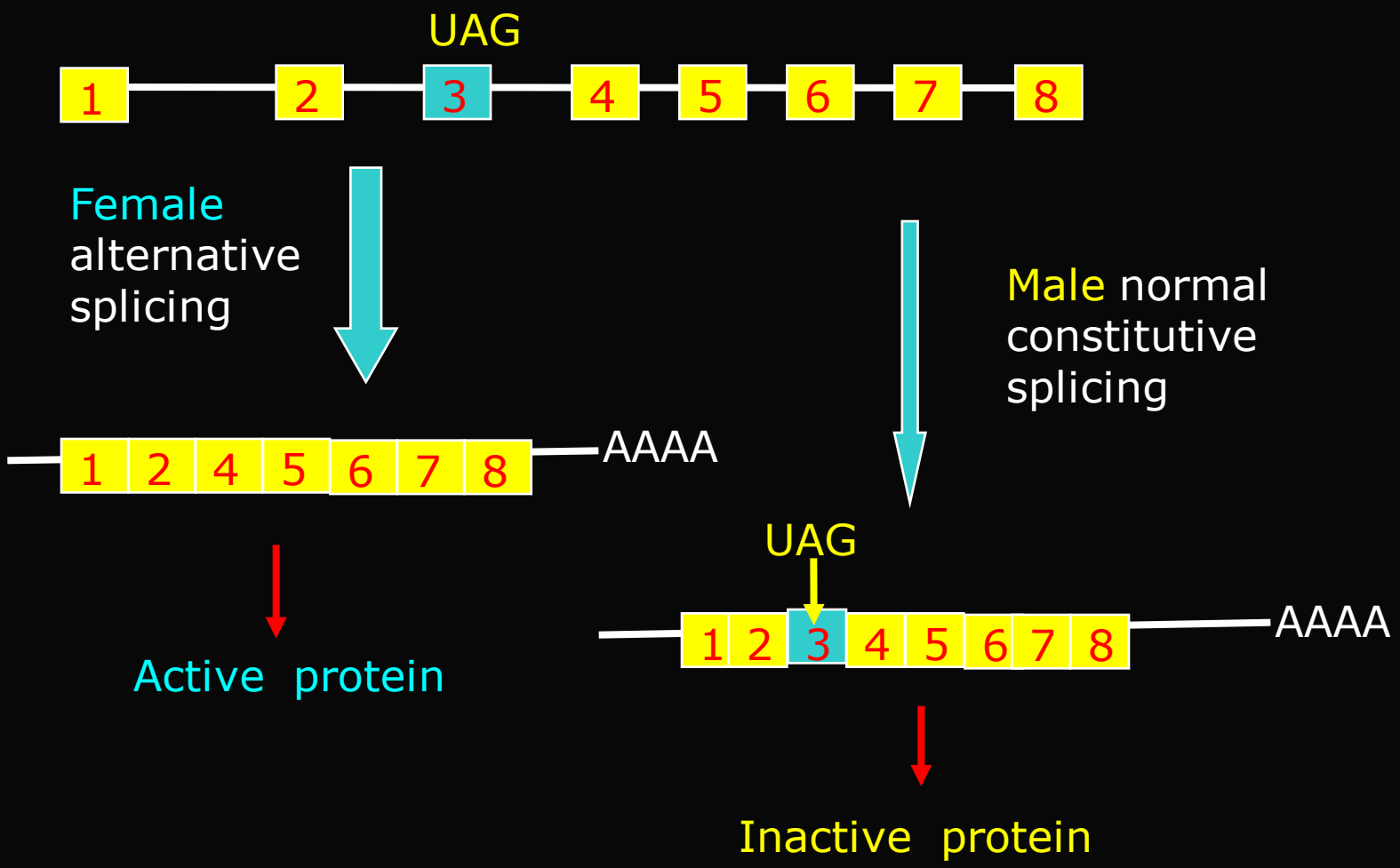
tra (转化基因)

dsx (两性基因)　DSX^{M}-p → ♂

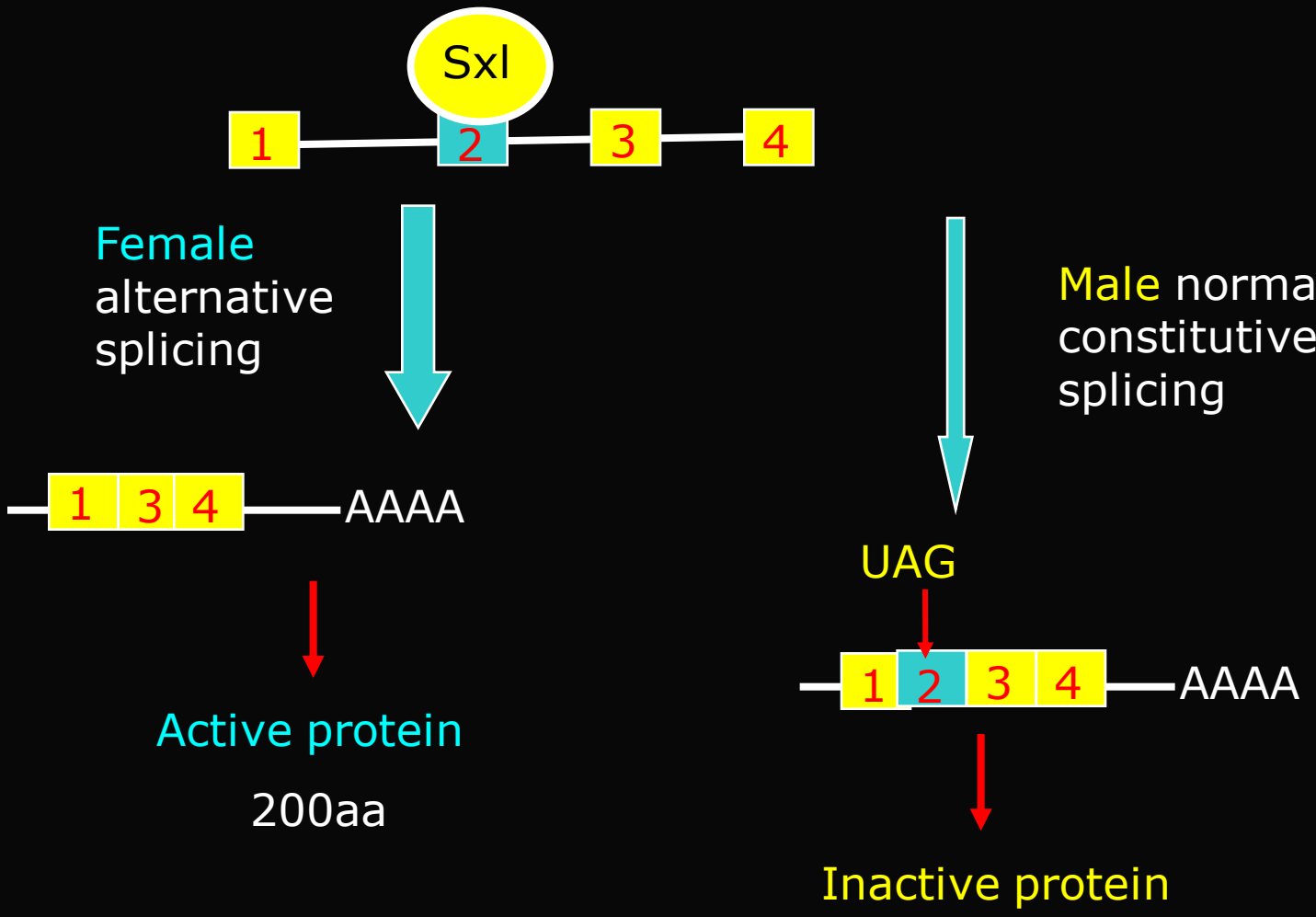
DSX^{F}-p → ♀

参与性别调控的细胞记忆形成依赖选择性剪接的级连调控

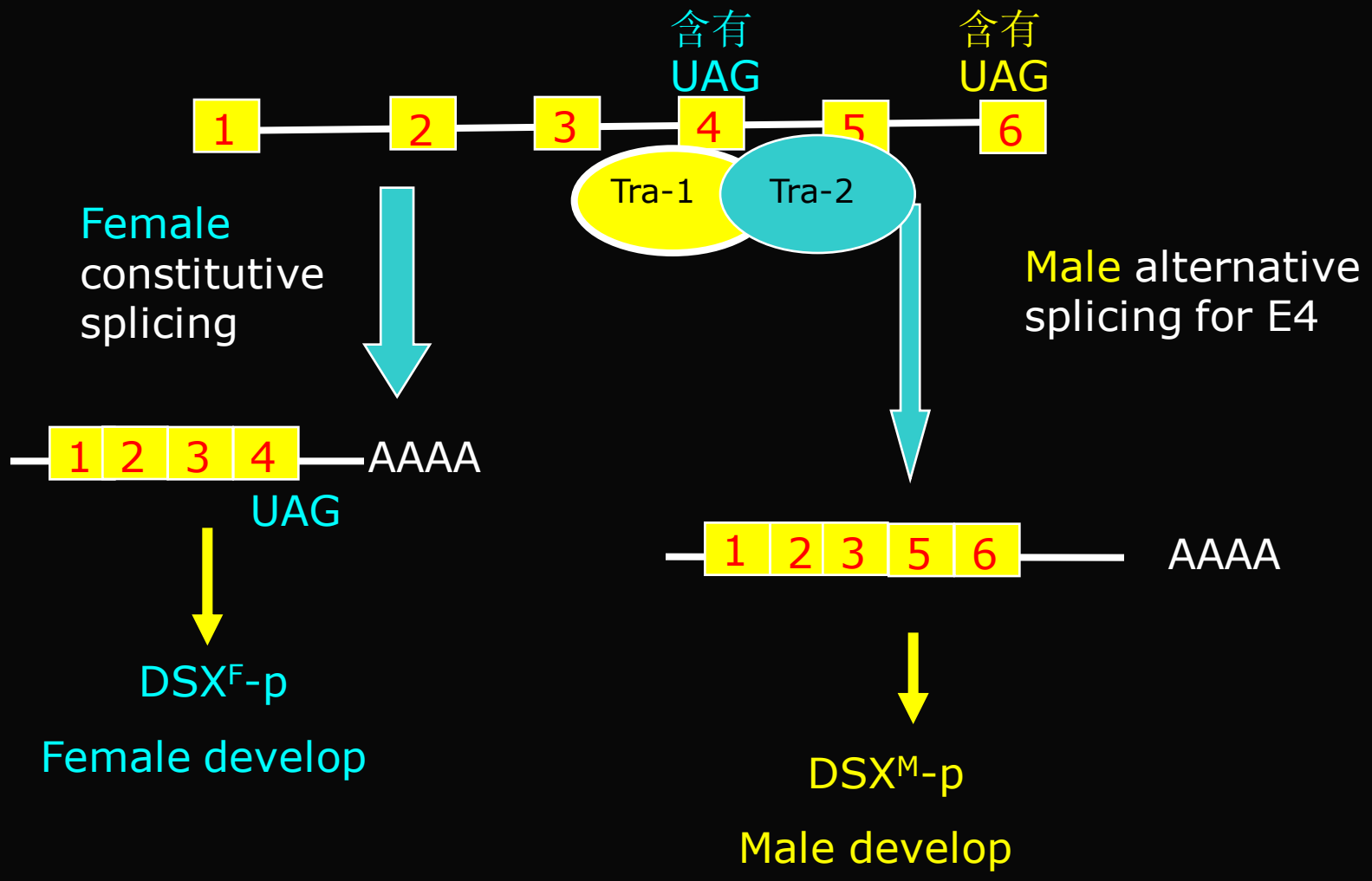
Sxl *Sex lethal* *(8 EXON)*



Tra Transformer (4 EXON)



dsx double sex *(6EXON)*



When X/AA = 0.5 ⇢ ♂

sxl gene　　tra gene

Pre-mRNA
组成型剪接

无活性蛋白　　(带有stop codon 的exon 保留）

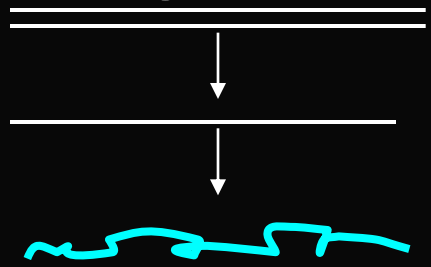
pre-mRNA
择型剪接

选 dsx gene

DSX^{M}-p → 关闭与♀性发育有关的基因

when XX/AA = 1 ⋯⋯▶ ♀

sxl gene （在胚胎向雌性分化的途径中被短暂激活）



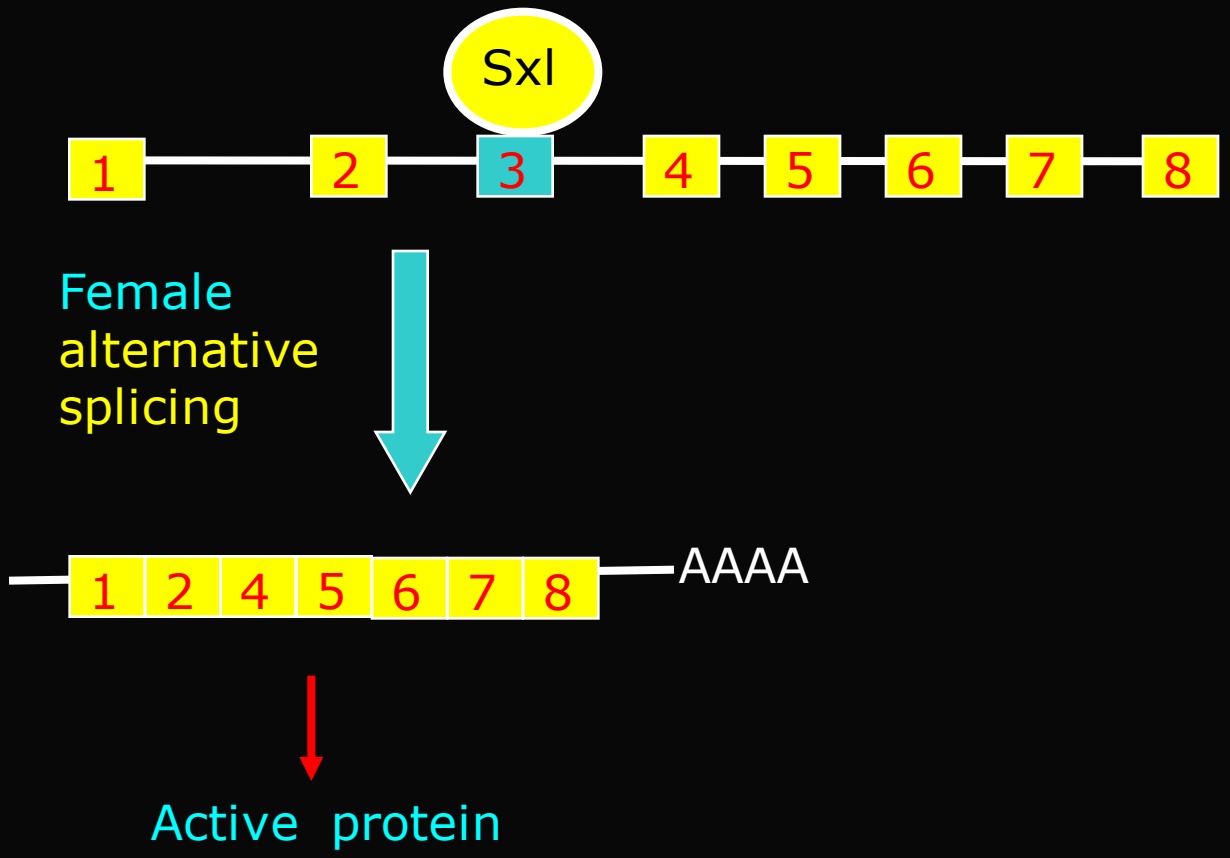
pre-mRNA 选择性剪接，E3 被切除

active Sxl-protein with bi-functions

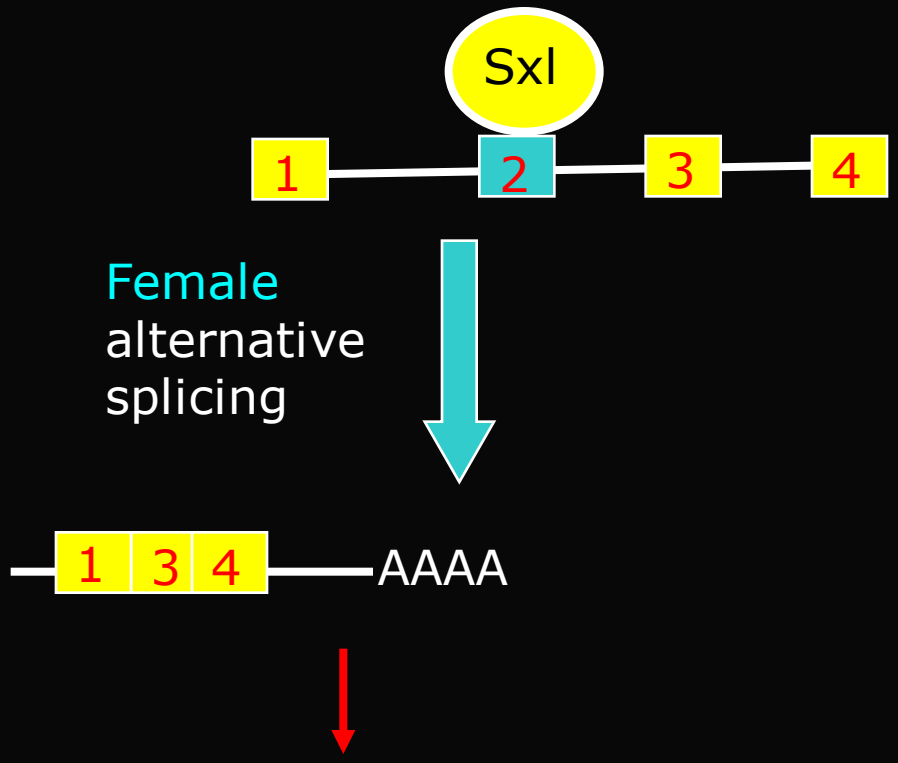
- binding at E3 site of sxl pre-mRNA
  阻止组成性剪接，完成选择性剪接
  持续产生 Sxl-protein (RNP-CS, 具有负控制 阻止组成性剪接)

- binding at Tra pre-mRNA
  阻止组成性剪接，完成选择性剪接
  产生active Tra-protein(RNP-CS）＋ 另一Tra 2-protein
  正控激活dsx pre-mRNA组成性剪接为DSX^F-p, 关闭♂基因

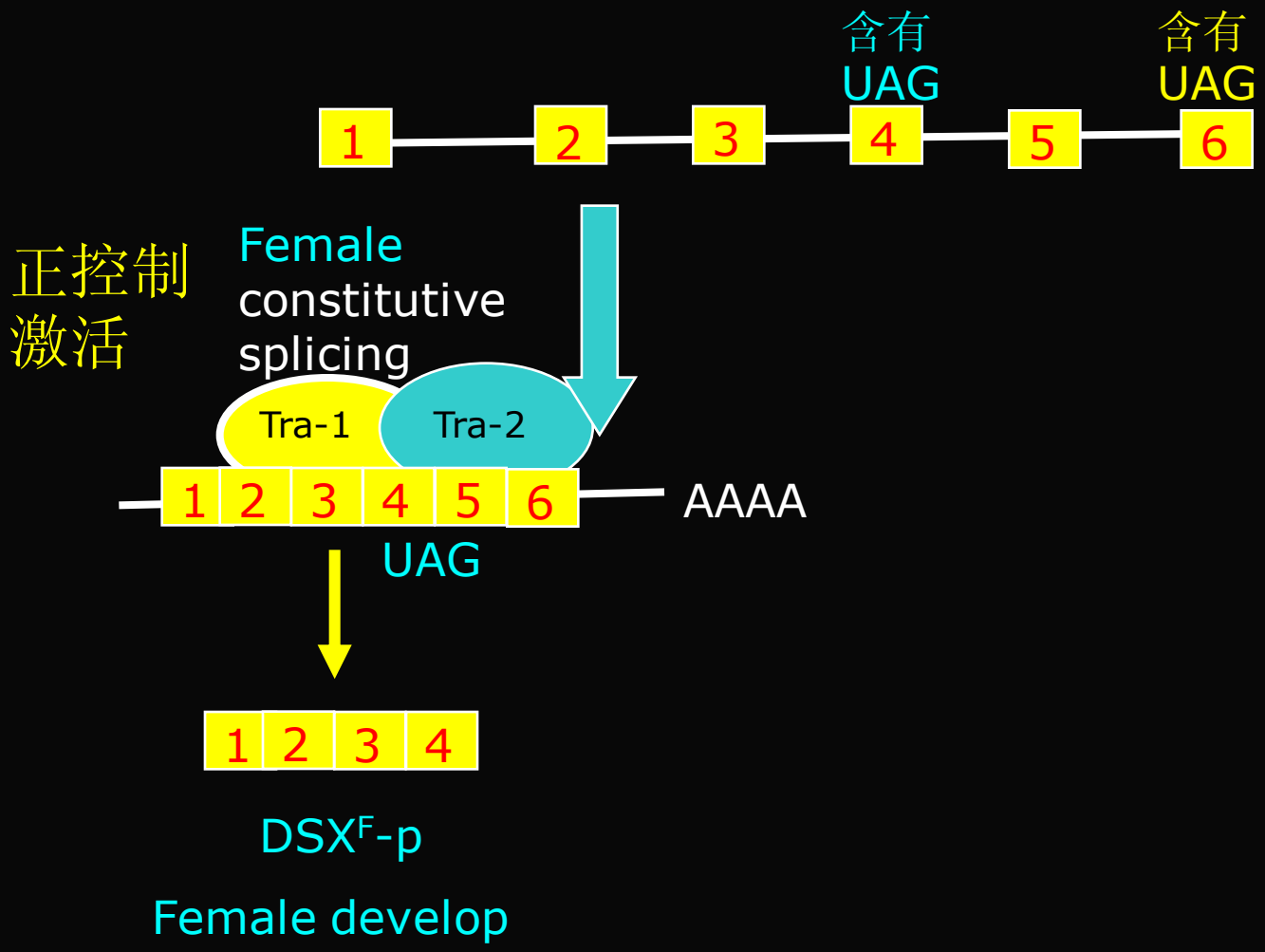
Sxl *Sex lethal* *（8 EXON）*

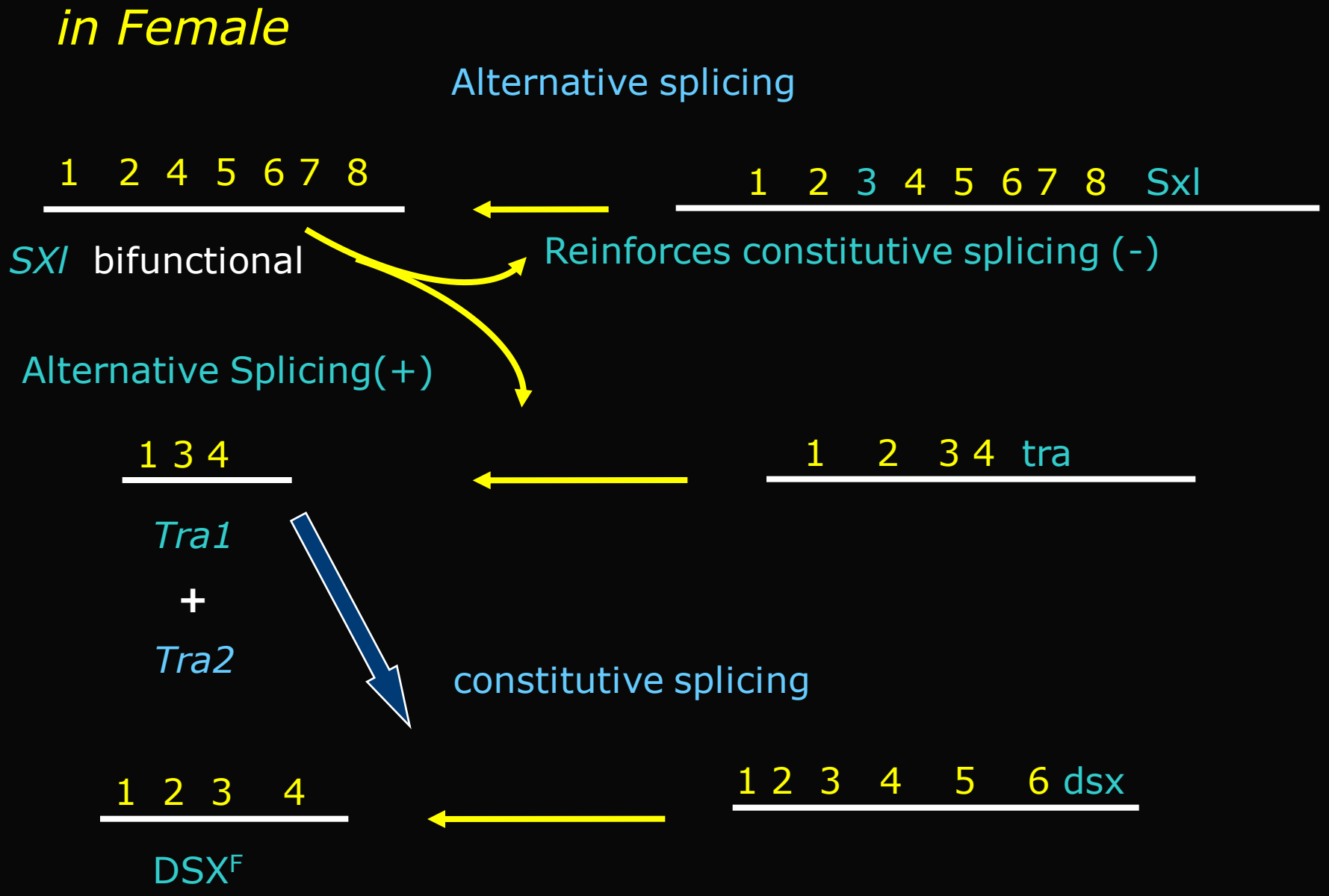


Tra Transformer (4 EXON)



dsx double sex *(6EXON)*




in Female
Alternative splicing
1 2 4 5 6 7 8
1 2 3 4 5 6 7 8 Sxl
SXl bifunctional
Reinforces constitutive splicing (-)
Alternative Splicing(+)
1 3 4
1 2 3 4 tra
Tra1
+
Tra2
constitutive splicing
1 2 3 4
1 2 3 4 5 6 dsx
DSXF

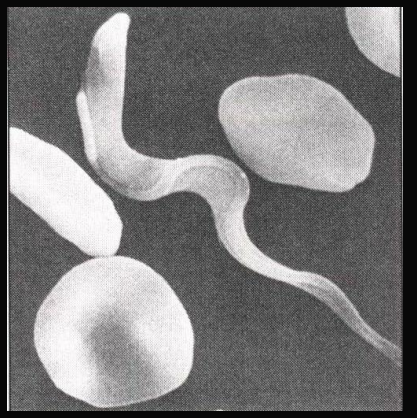
f) 反式剪接（trans-splicing）

cis-splicing	tans-splicing
以一条pre-RNA 为底物	以两条不同来源的pre-RNA 为底物
在spliceosome中完成	在spliceosome中完成
组成型剪接 选择性剪接 对供点或受点的 识别差异	在成熟RNA的leading sequence中 拼接一外来的mini-exon 发生在两条 pre-RNA间的选择 性剪接
lariat intron	Y- intron

反式剪接的发现与证实

锥虫（**trypanosome**）

可变表面糖蛋白（VGS）基因（Van de Ploeg 1982）



(Source:Donelson J.E.,How the trypanosome changes its coat.Scirntific American 45(Feb 1985).)

- matuer mRNA

- 锥虫中几乎所有mRNA的5’-end

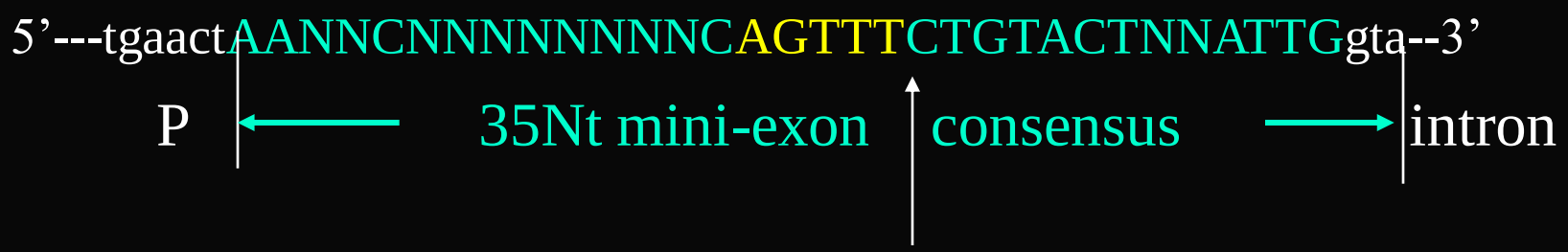
均有相同的 35Nt seq.

(spliced lead or mini-exon)

- 锥虫genome中发现

  包含有35Nt seq.在内的135Nt的重复序列

  （约200个拷贝）



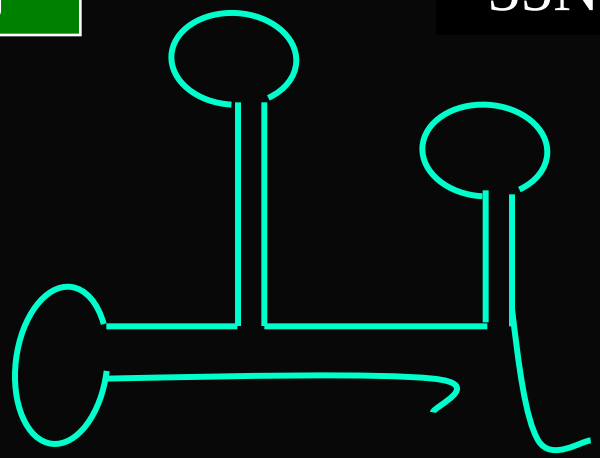
High conservative

- 病毒
  线虫
  植物 (mt, ct)
  人体

  均有发现

trans-splicing 的机制

35Nt SL 3 stem-loop



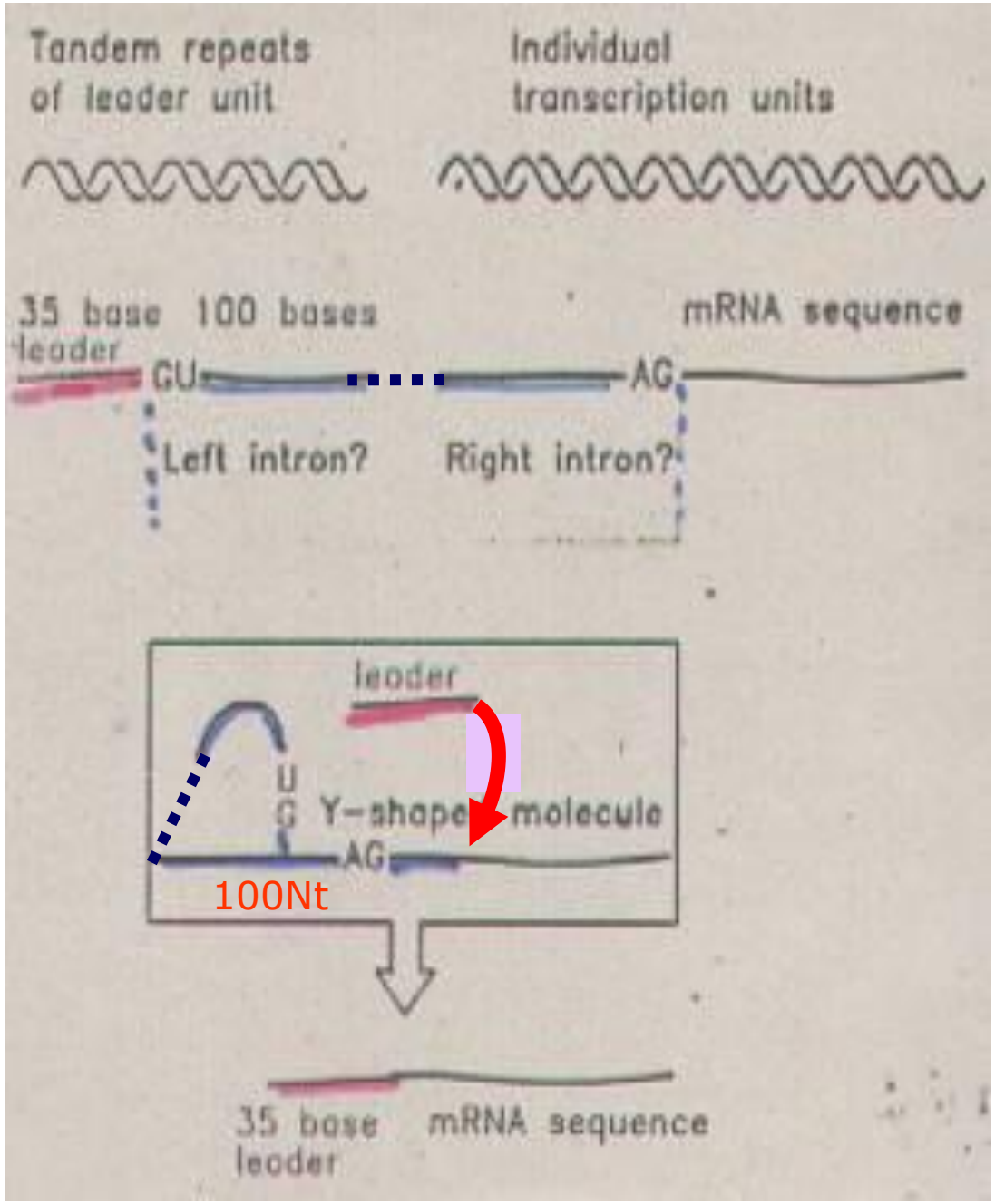
- Splicing Model

--- Group III pre-mRNA中AUG上游（lead seq.）30-70Nt

存在通用方式剪接的受点（5’AG）

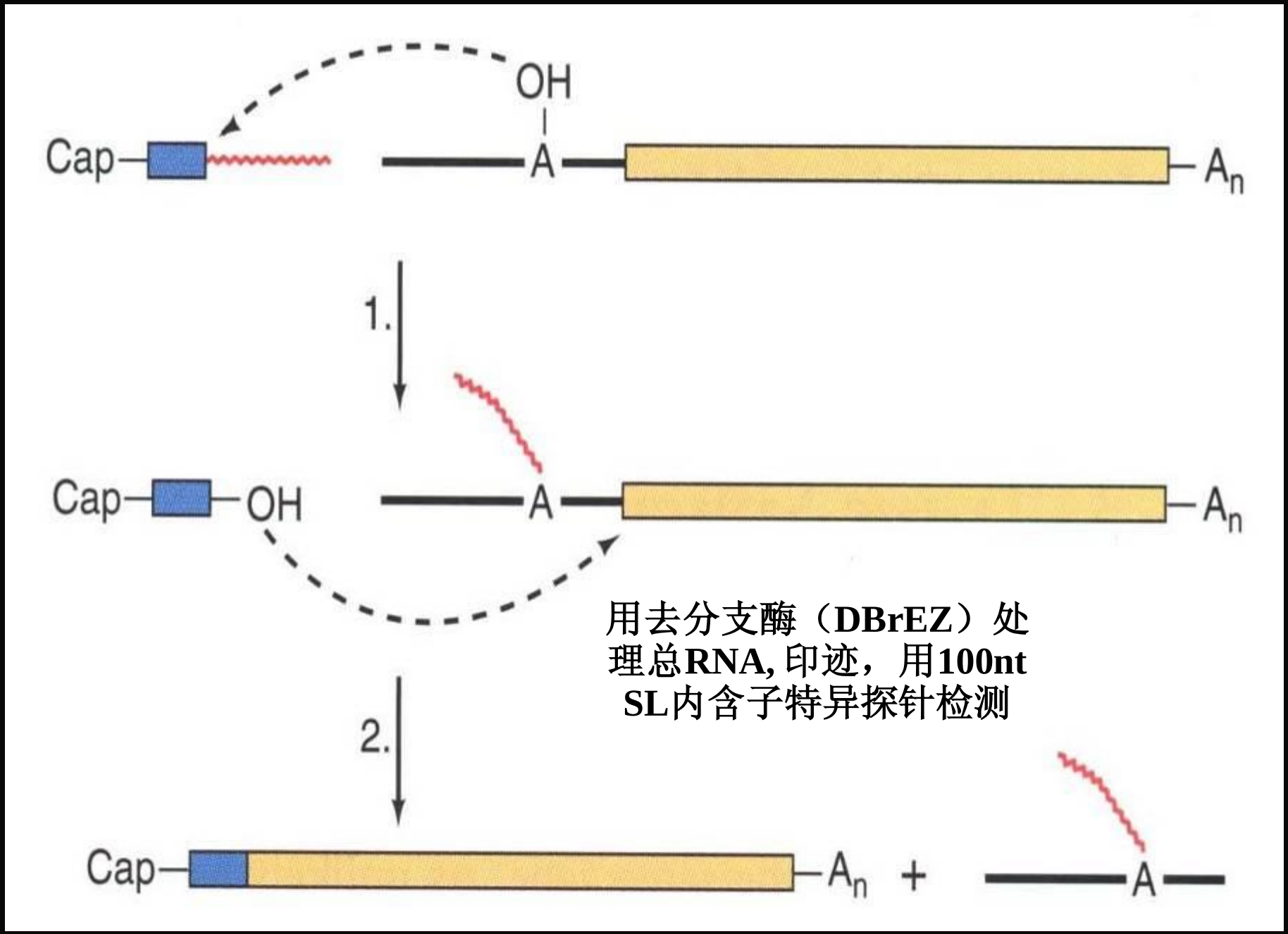
→ 与 SL 中GU供点以2’, 5’磷酸酯键连接

→ 形成Y-intron

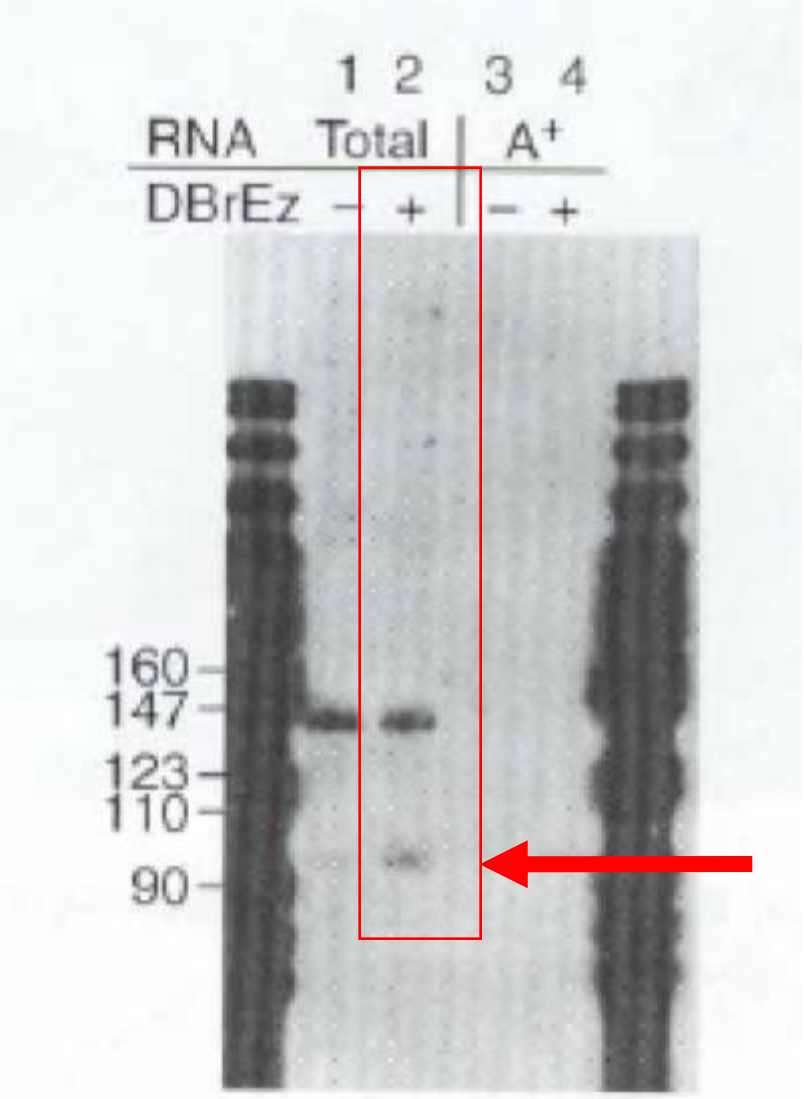


(来源：不详)

用去分支酶（DBrEZ）处理总RNA，印迹，用100nt SL内含子特异探针检测



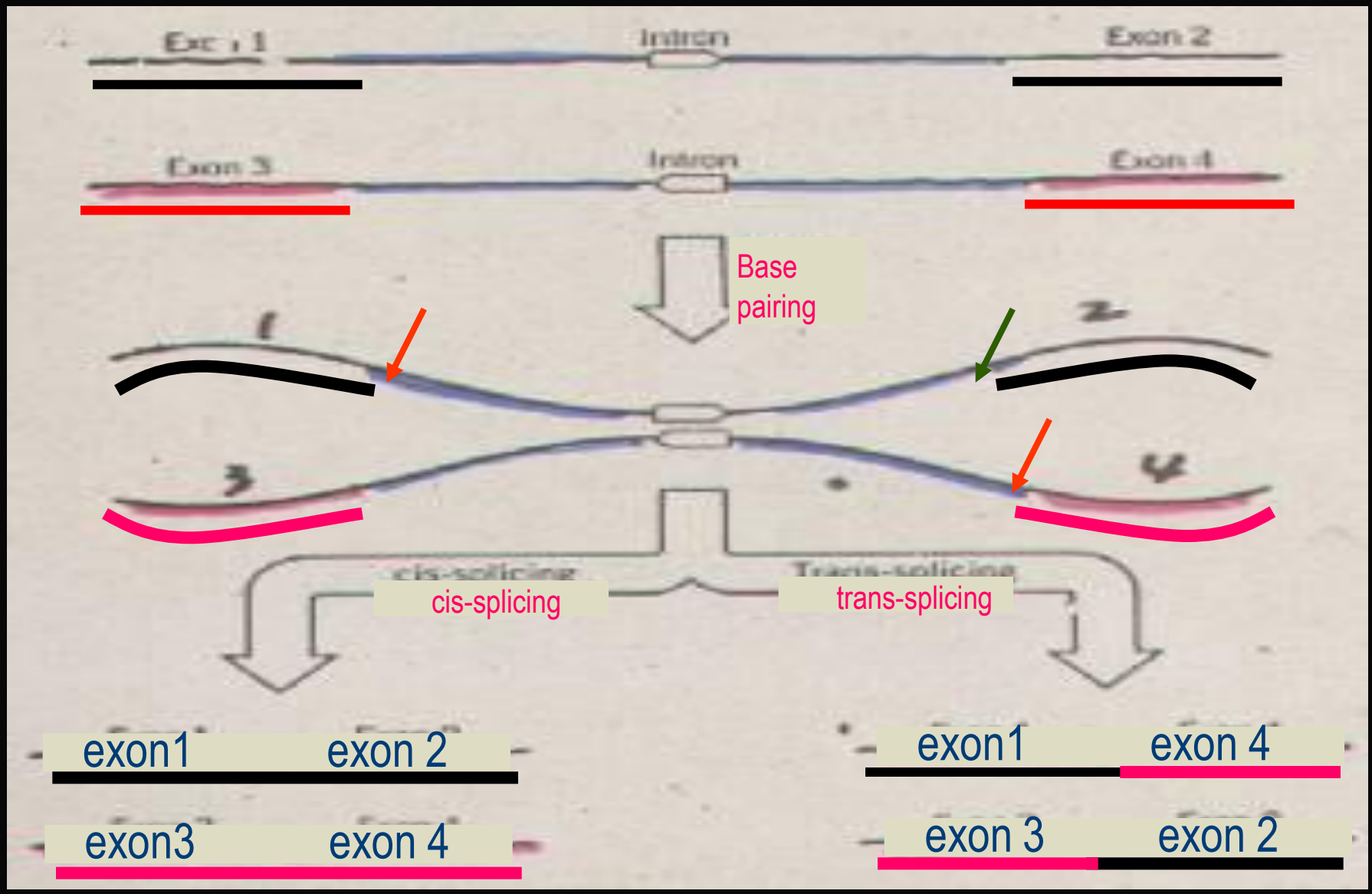
(来源：分子生物学（2007），郑用琏，第169页)



Agabian用^{32}P标记锥虫的RNA，用去分支酶（DBrEZ）处理总RNA, 然后产物经过电泳印迹，用100nt SL内含子特异

Source:Murphy W.J.,Idenfication of a novel Y branch structure as an intermediate in trypanosome mRNA processing.Cell 47(21 Nov 1986).)

--- Complementary introns by base pairing (recombination-like)



(来源：不详)

4.4.6. RNA editing

R. Benne. 1986. 锥虫 线粒体基因组

coxII, coxIII , cob mRNA

U $\rightarrow$ deleted

相继在真核生物(mt)及病毒中发现

---U inserted & deleted in mRNA

---C transition into U in mammal

---多头绒泡菌线粒体中　C, A, U, 的插入

---小麦线粒体 ND3 mRNA中C→U,G→ A;G→U;A→ I

--- some genes edited with high frequency

锥虫 mt coxIII mRNA　　407 U inserted in 145 loci
19 U deleted in 9 loci

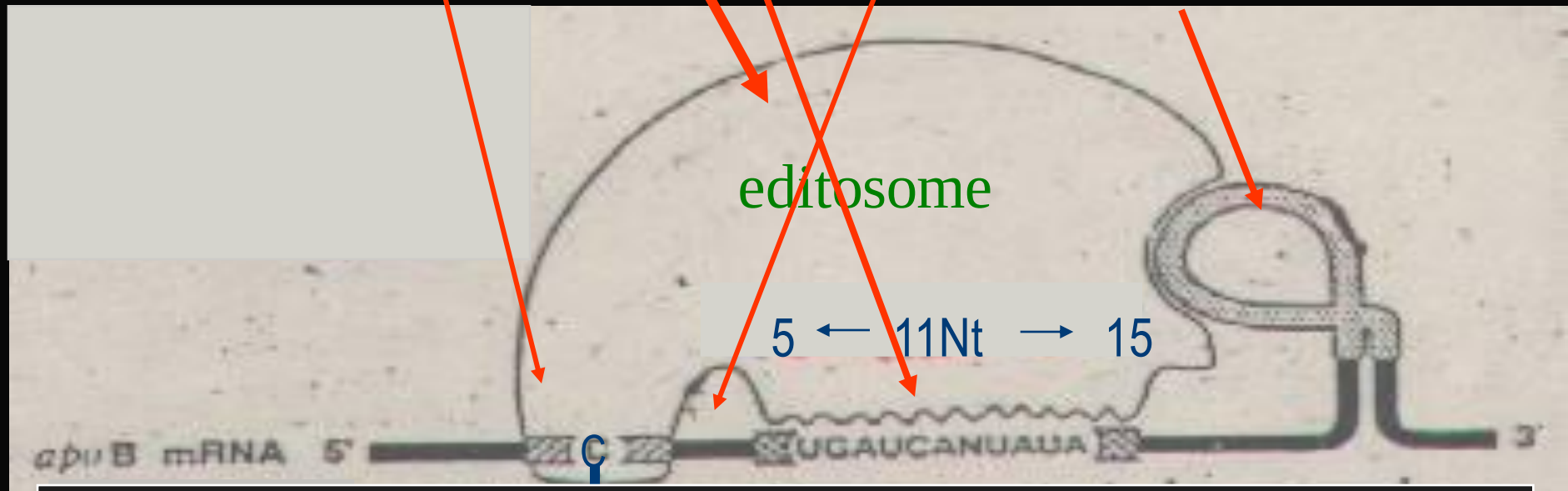
绒泡菌mRNA of ATP synthetaseαsubunit
54 site C inserted

editing 模式：（1）在editosome 中完成

--- C编辑位点上游区特异区　　--- C编辑位点下游5Nt 的间距

--- C编辑位点 下游5-15(11Nt)为非特异区

---非特异区下游为种特异区，与 编辑体发生互作



mooring region (停泊区)

位置与序列并非保守

缺失 → 丧失编辑功能

转移 → 形成新的 editing site

(来源：不详)

editing 模式：

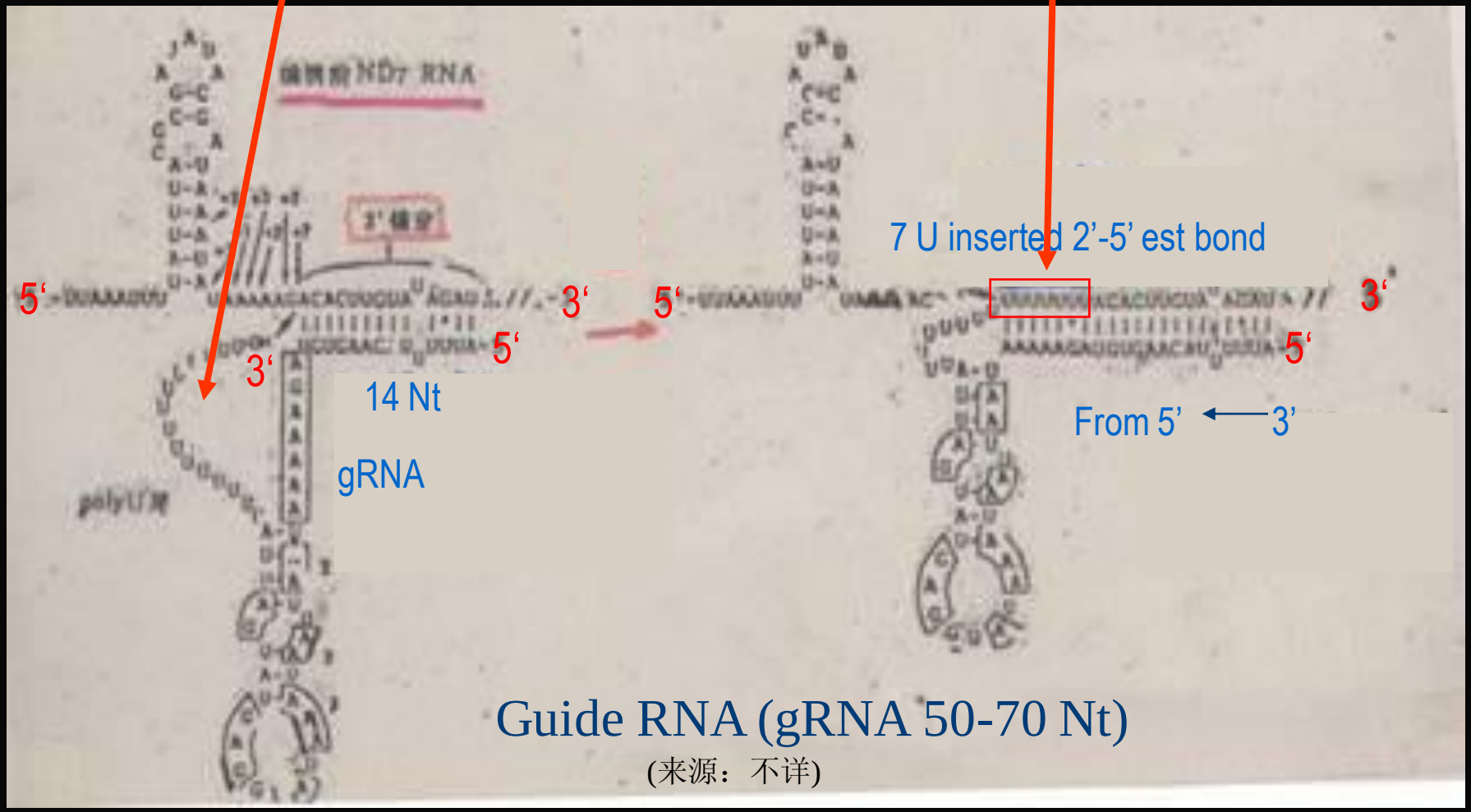
需要回答的重要问题是:

- 用于编辑的尿嘧啶(UMP)的来源?
- mooring region 位置与序列的保守性
- 由gRNA的末端能够通过转酯反应将U转移到前体mRNA中?

或者
由gRNA提供U的掺入的模板：A和G。UMP来自UTP

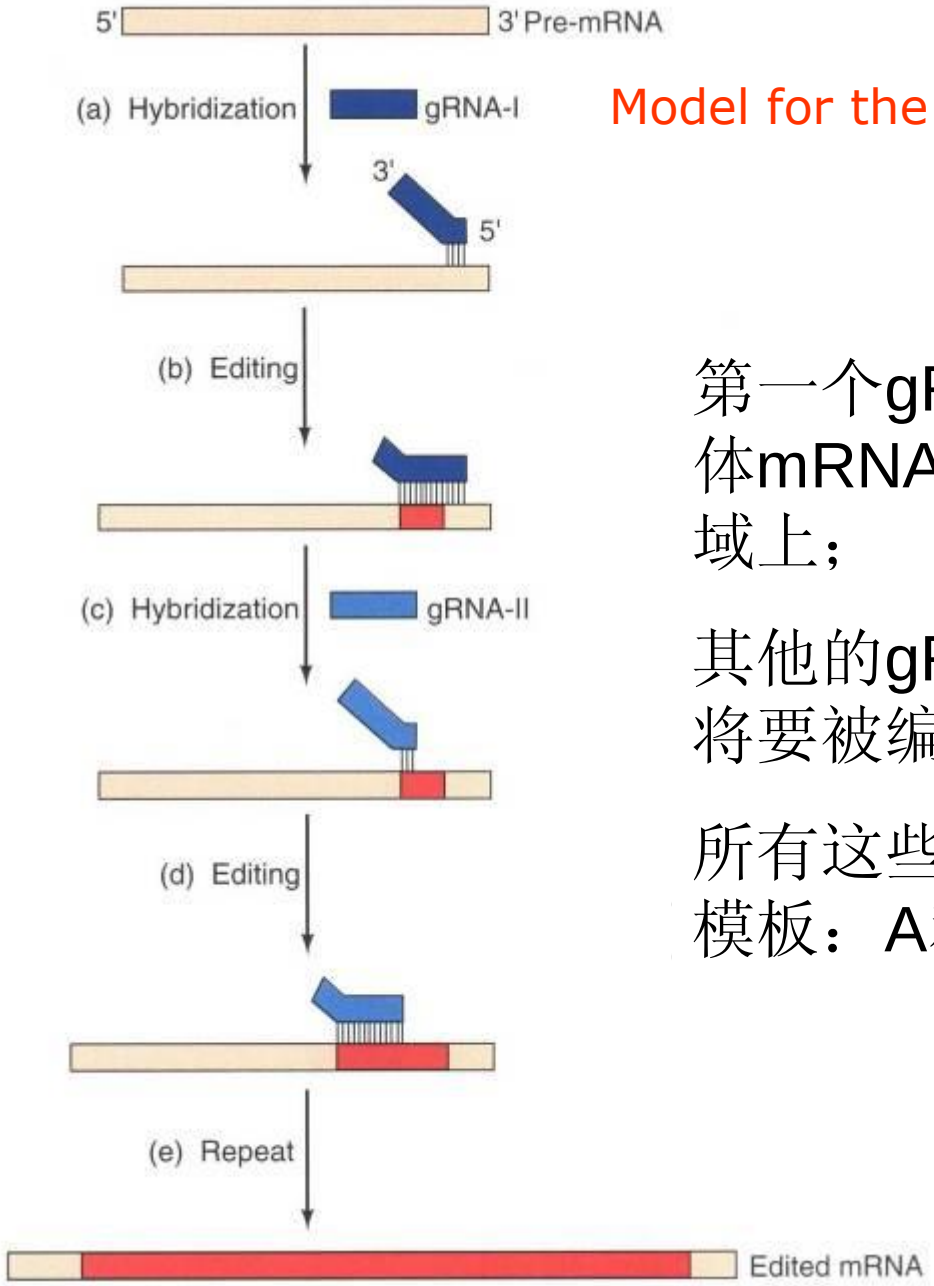
---gRNA as a template & 5-25 polyU at 3’ ---editing (deletion & insertion) from 3’-5’

---editing by trans-esterification ---gRNA have endonuclease & ligase activity



Guide RNA (gRNA 50-70 Nt)

(来源：不详)



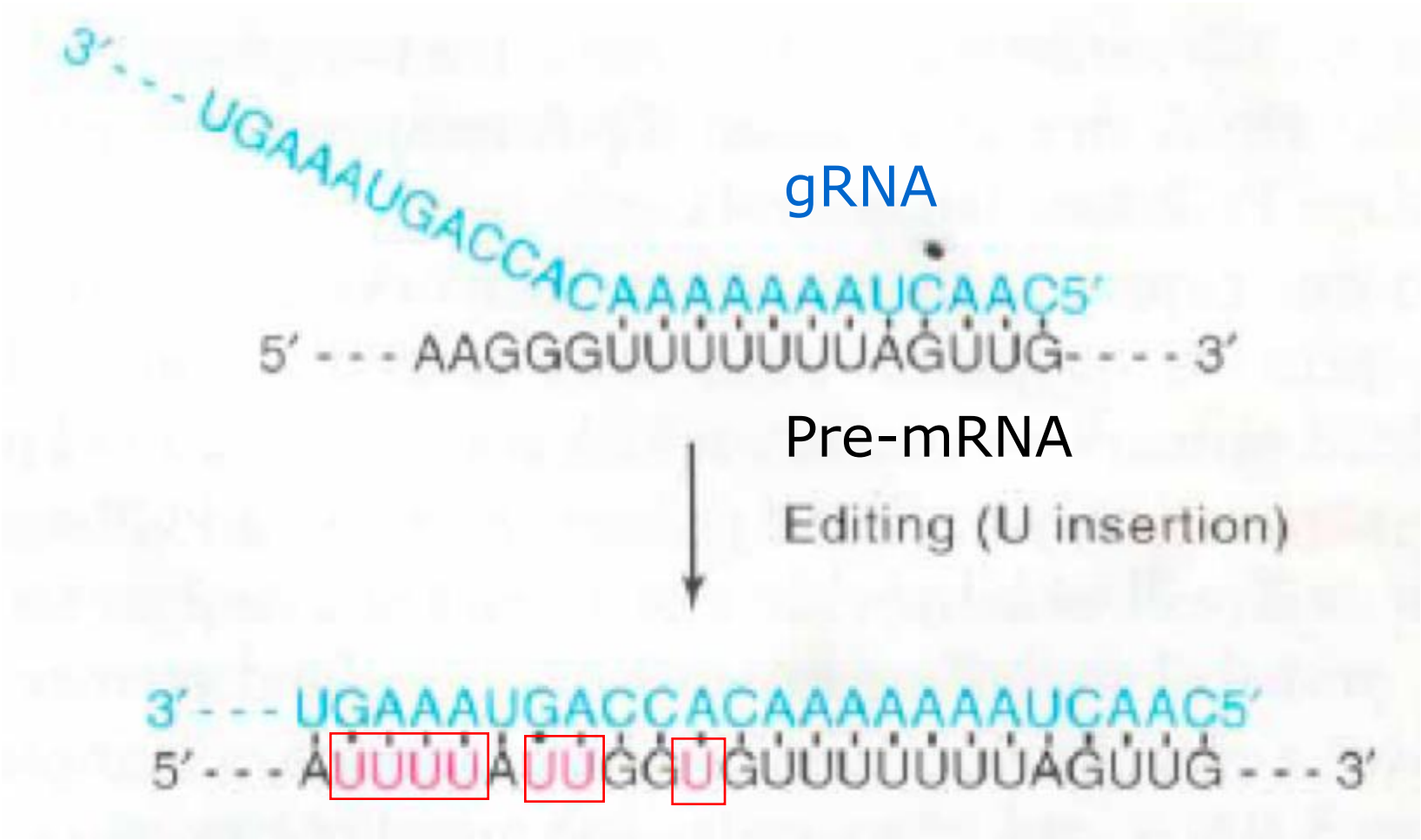
Model for the role of gRNAs in editing

第一个gRNA的5'-端杂交到一个前体mRNA待编辑位点3'-侧未编辑区域上；

其他的gRNA的5'-该区域的5'-区域将要被编辑。

所有这些gRNA提供了U的掺入的模板：A和G。UMP来自UTP

(Source:Molecular Biology(2002),Robert F.Weaver,Page492）



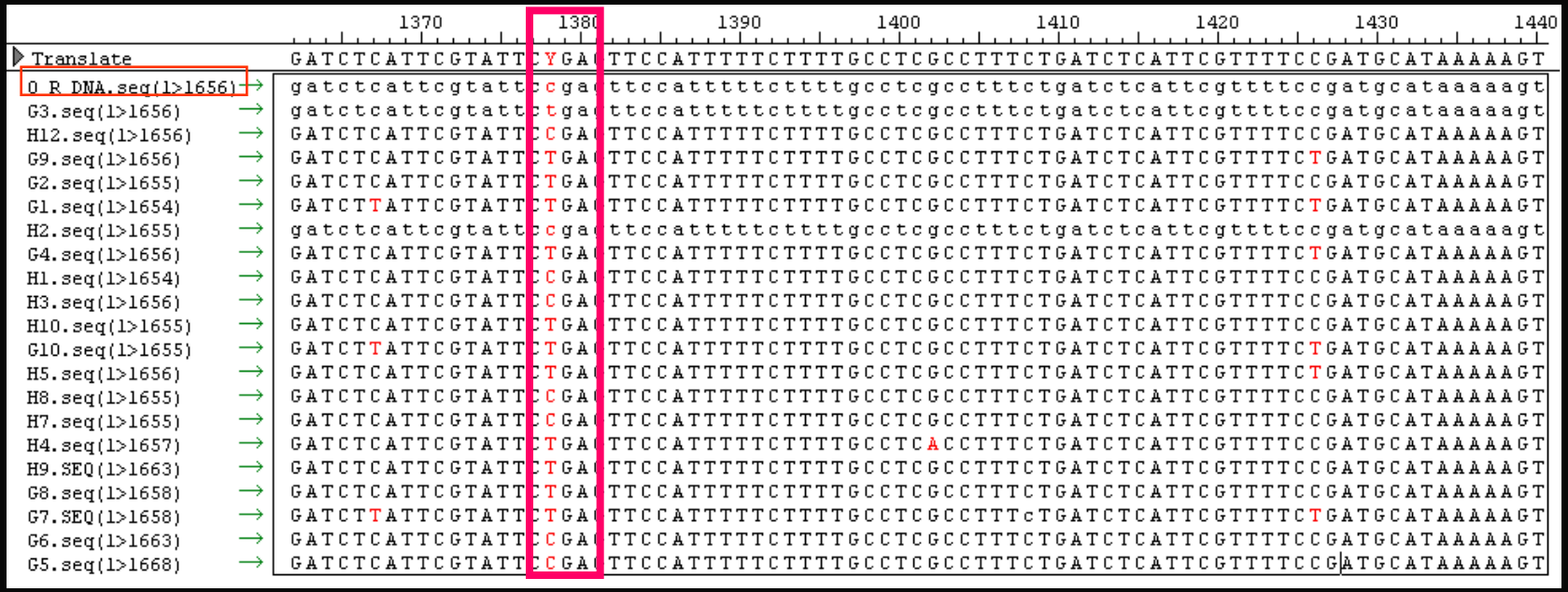
Inserted UMP

(Source:Molecular Biology(2002),Robert F.Weaver,Page492）

Editing sites of orf355-orf77 mRNA

(来源：不详)

41 52 100



Genotype of microspores	41 *orf77* (UCA^{Ser} to UUA^{Leu})	52 *orf77* (CGA^{Arg} to UGA)	100 *orf77* CGA^{Arg} to UGA)
S-Mo17 *rf3*	30%	80%	50%
S-Mo17 *Rf3*	0%	40%	10%

Editing 的生物学意义？ 结论为时过早

Editing 的生物学意义

- 形成/删除AUG, UAA, UAG, UGA…
- 改变codon信息
- 扩大编码的遗传信息量
- mRNA editing 较大程度地改变了DNA的遗传信息，使该基因的DNA序列仅是一串简略意义模糊的序列（abbreviated ）或称为隐秘基因、模糊基因（cryptic gene, cryptogene）
- 中心法则的发展