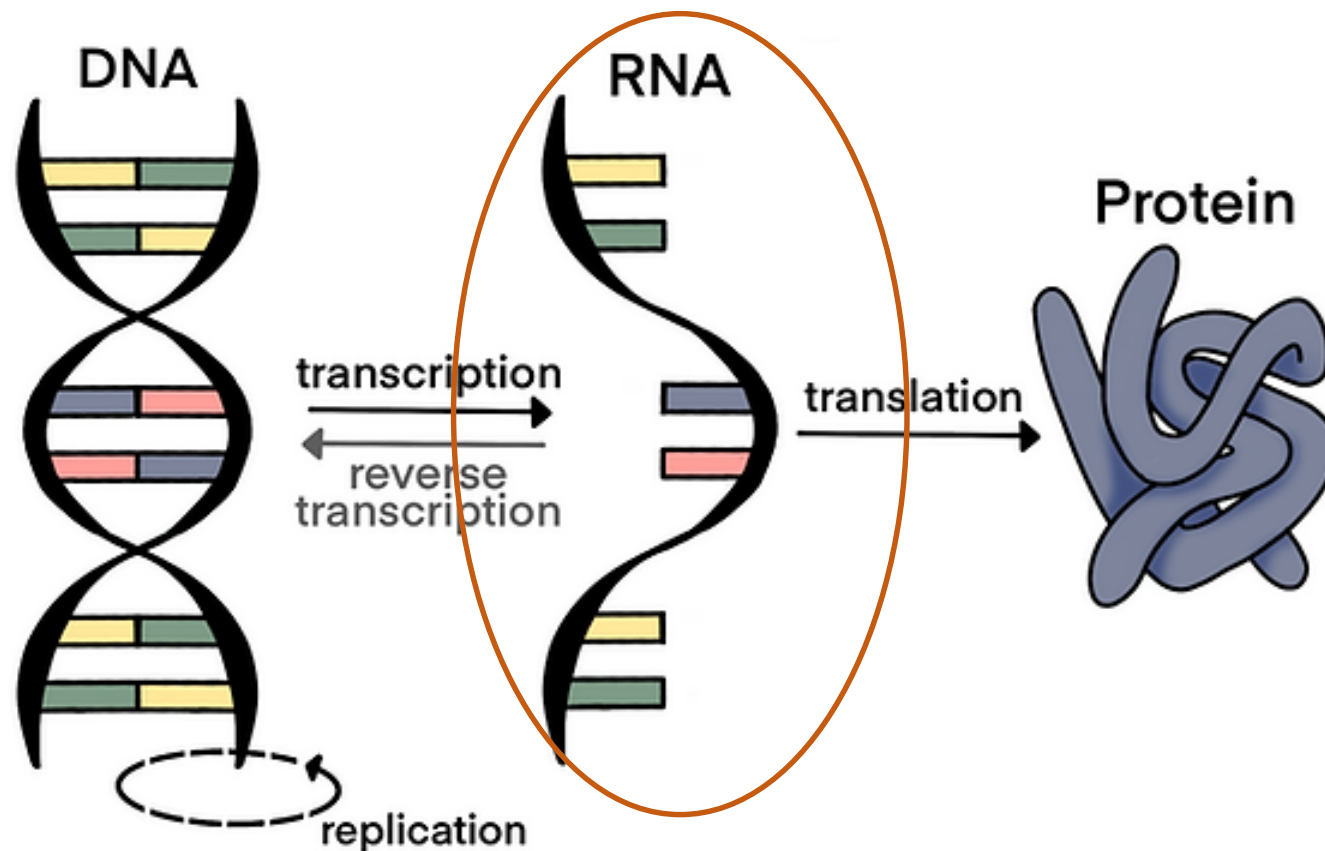
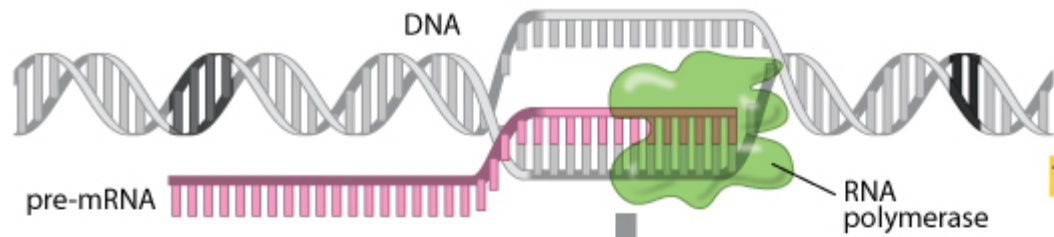


原核生物的转录与调控

Central dogma 中心法则



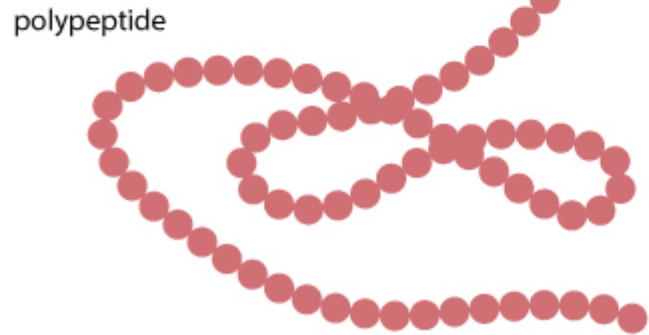
分子生物学的核心是**基因的表达与调控**



Transcription

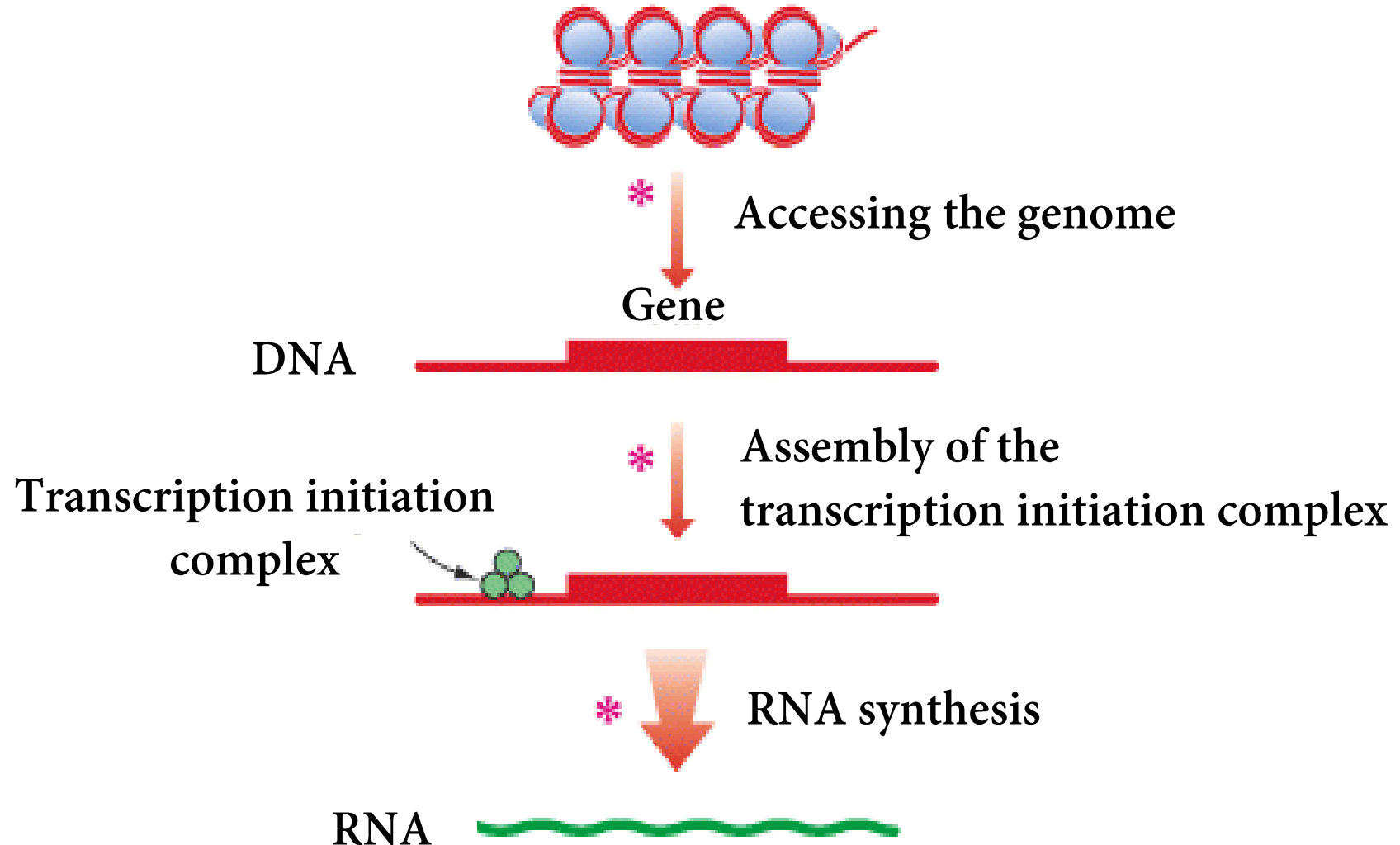


Translation

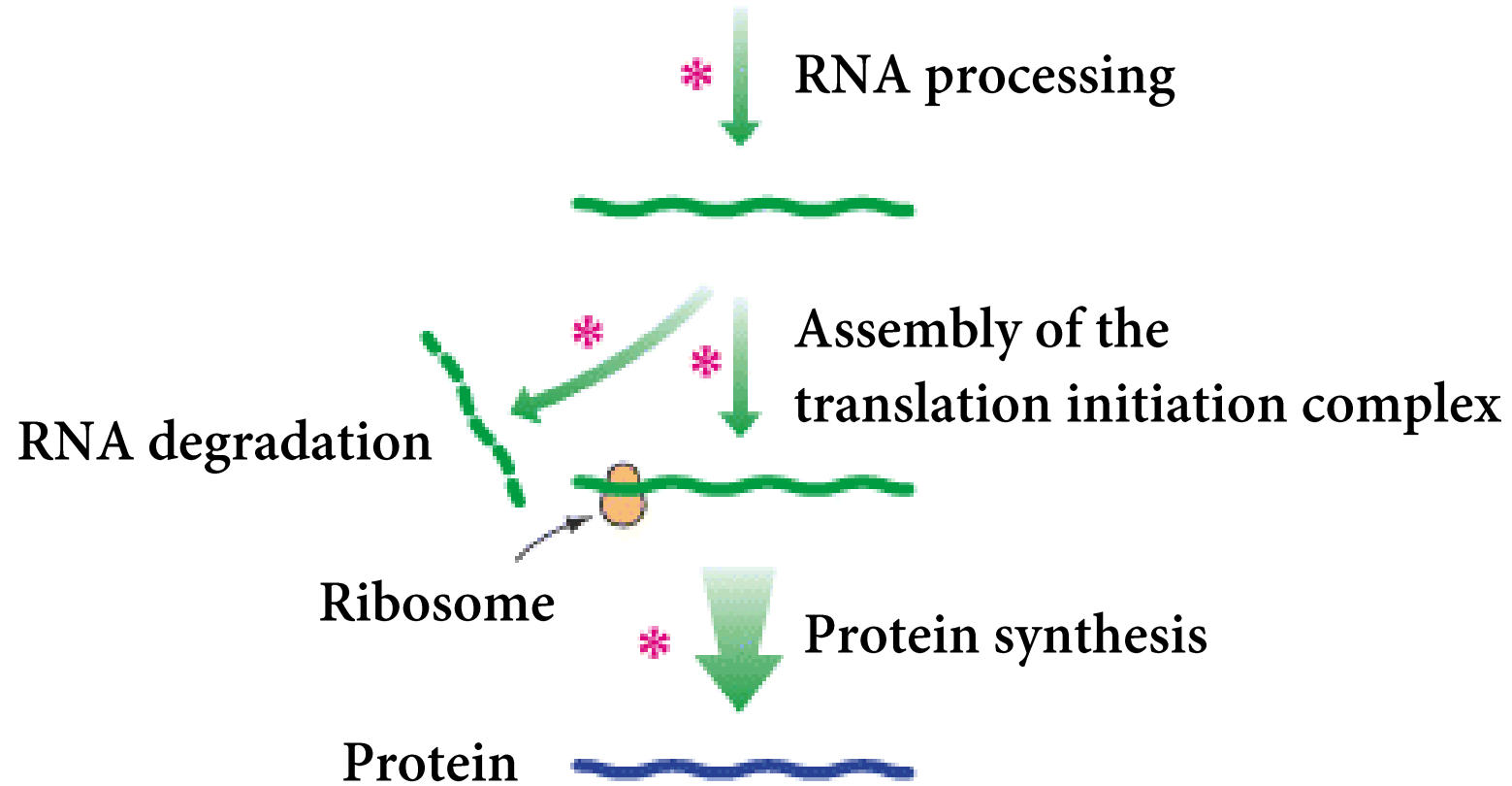


RNA聚合酶如何识别转录起始位点?
RNA聚合酶怎么知道哪里该从DNA上掉下来?
DNA链与RNA链配对?
为什么有的基因表达高有的基因表达低?

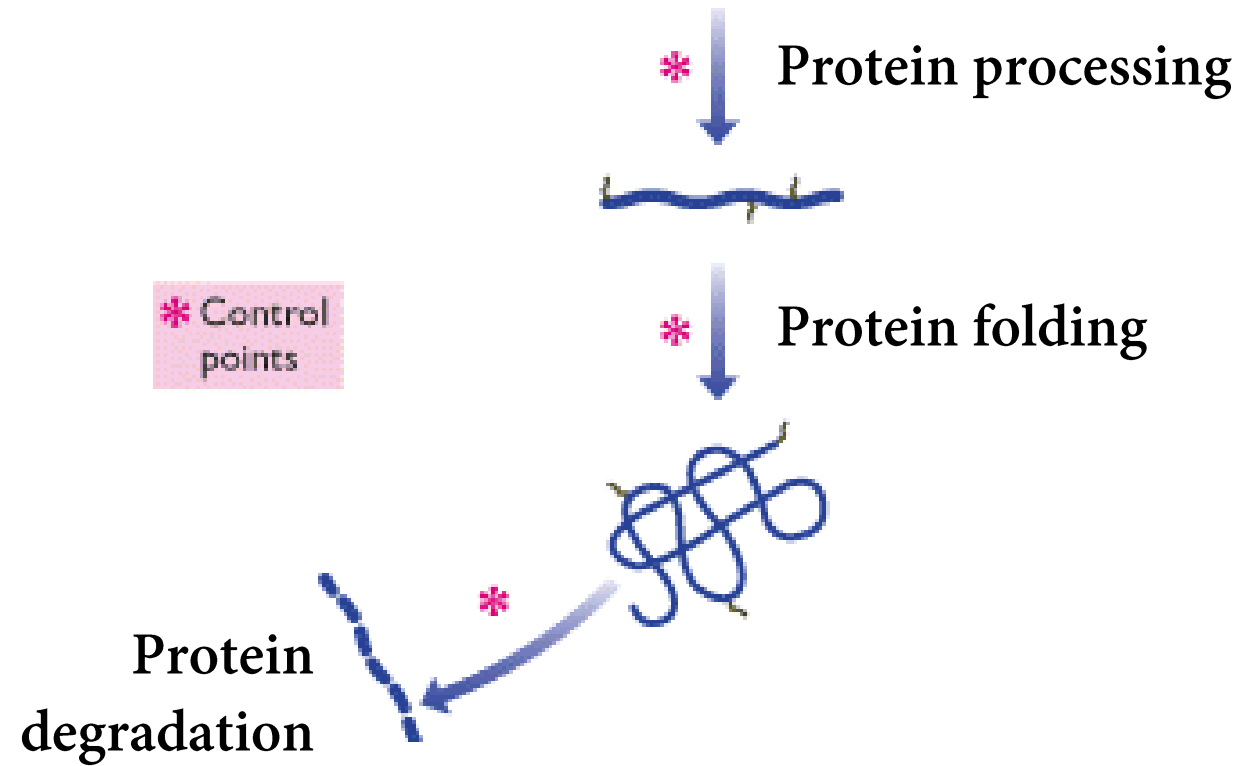
Central dogma - “new”



Central dogma - “new”



Central dogma - “new”



原核生物的转录与调控

1. 原核生物的转录

- 转录的概念、基本过程
- RNA聚合酶

2. 原核生物转录的调控

- 转录水平：乳糖操纵子&色氨酸操纵子
- 转录后调控

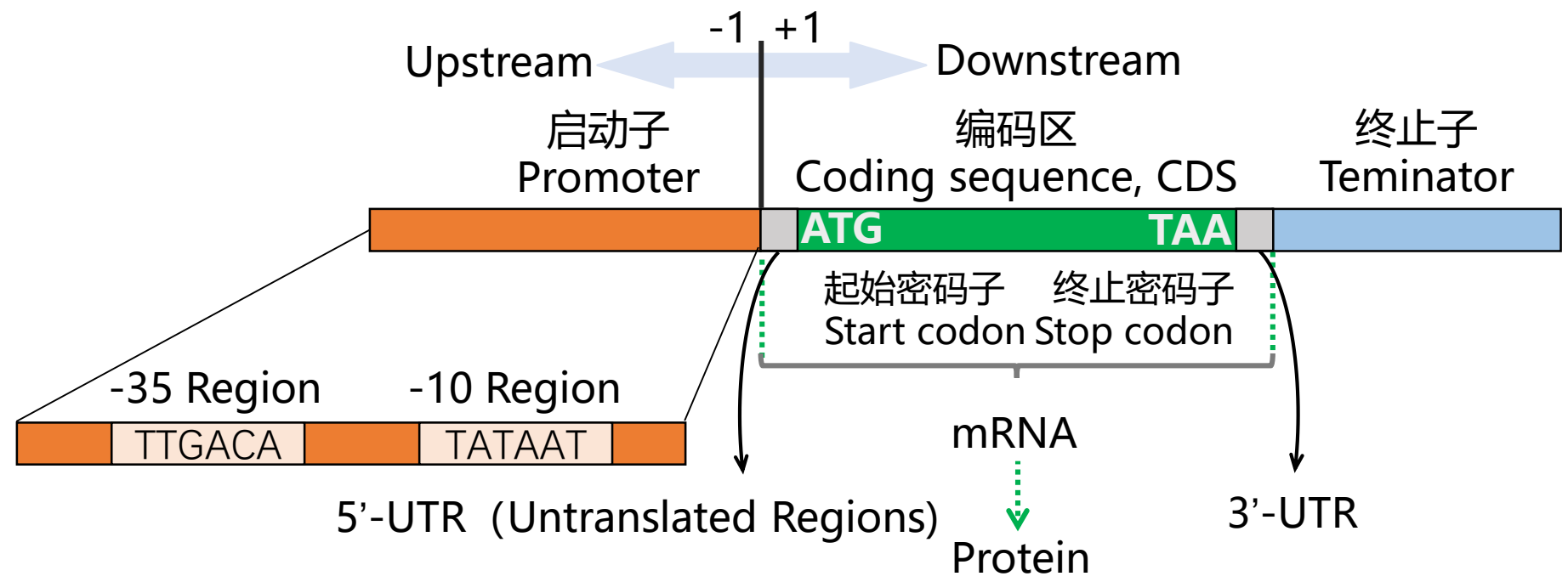
3. 细菌转录的转换模式

- σ 因子的转换
- 特异性RNA聚合酶
- 抗终止子机制

基因

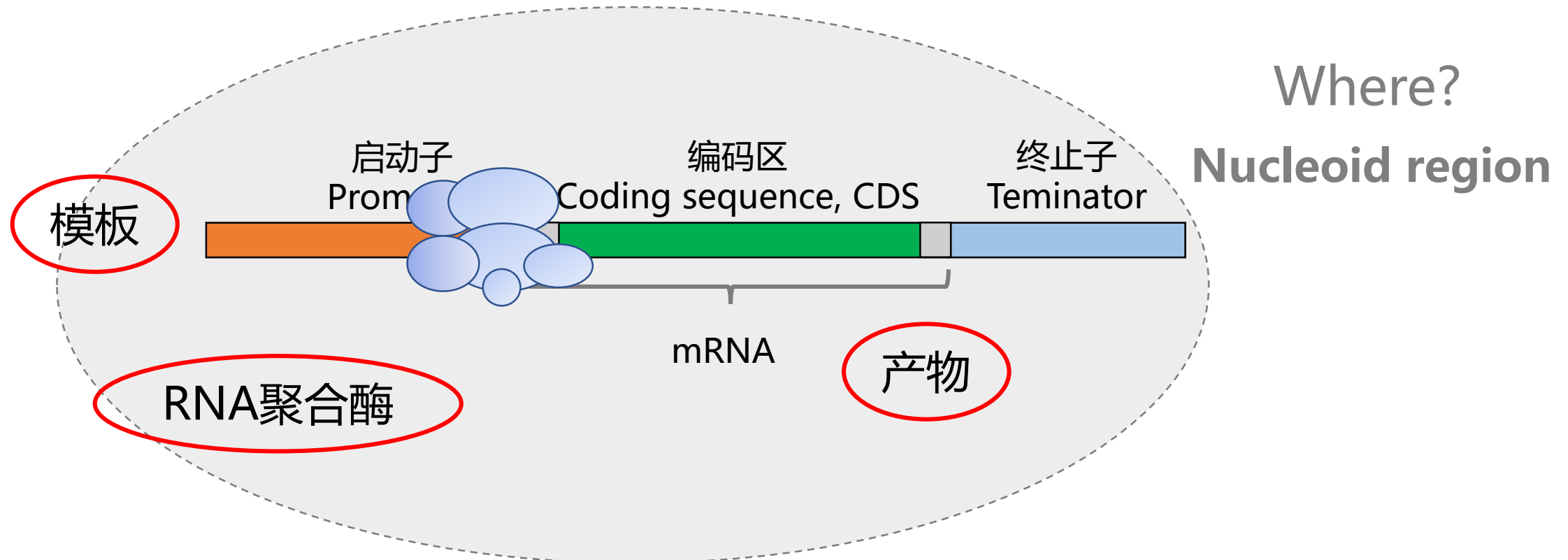
A typical prokaryotic gene

转录起始位点(transcription start site, TSS): 一个基因的5'端转录的第一个碱基



真核生物的mRNA通常只包含一个基因，而原核生物的mRNA可包含多个基因

转录(transcription)



转录：拷贝出一条与DNA链序列完全相同（除了T→U之外）的RNA单链的过程

转录起始 -> 延伸 -> 终止

Contents

I. RNA

II. RNA聚合酶

III. 转录起始

IV. 转录延伸

V. 转录终止

1. 原核生物中一个基本的转录单元的DNA序列组成
2. 原核生物中转录的基本步骤以及各个步骤的参与者
3. 影响转录效率的因素
4. 复制和转录的比较

I. RNA的结构、分类和功能

RNA(ribonucleic acid)的结构

1. RNA是由核糖核苷酸通过磷酸二脂键聚合成的单链线性分子

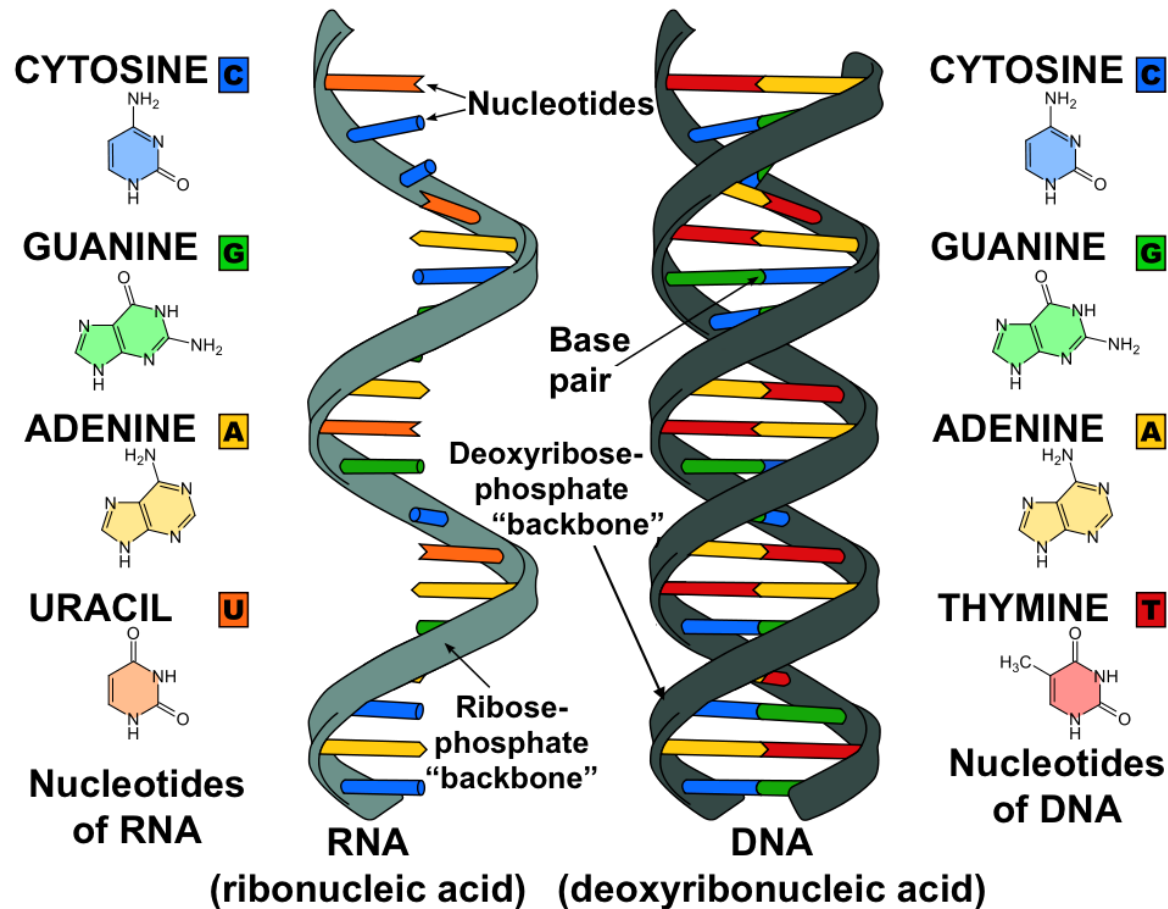
Watson-Crick pair

- A-U (~2 kcal/mol)
- G-C (~3 kcal/mol)

Wobble pair

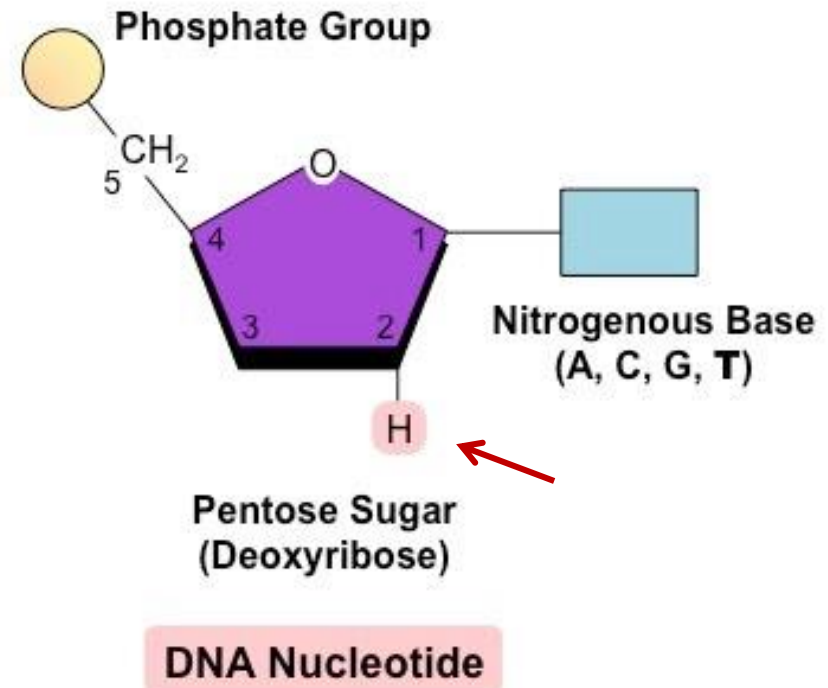
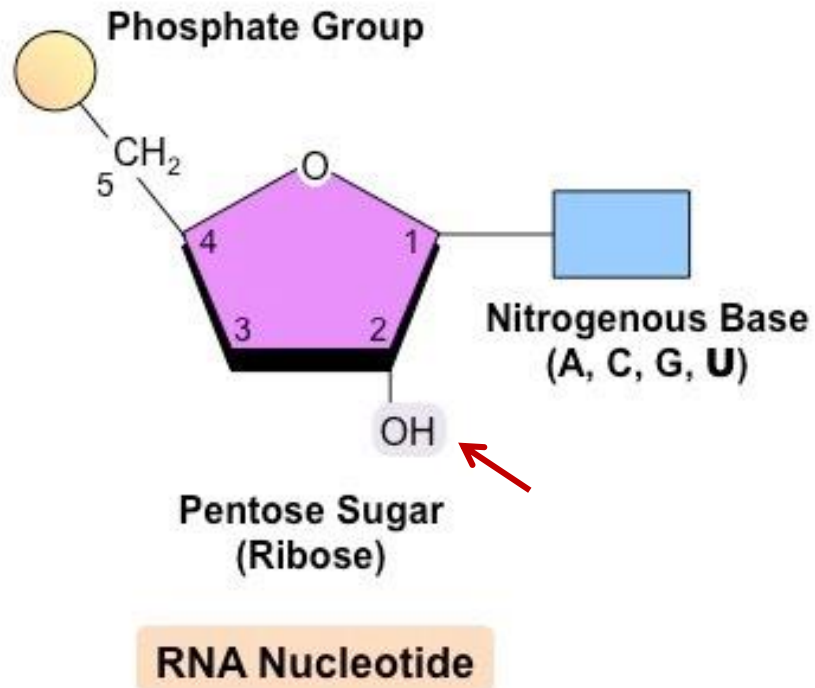
- G-U (~1 kcal/mol)

U->T



I. RNA的结构、分类和功能

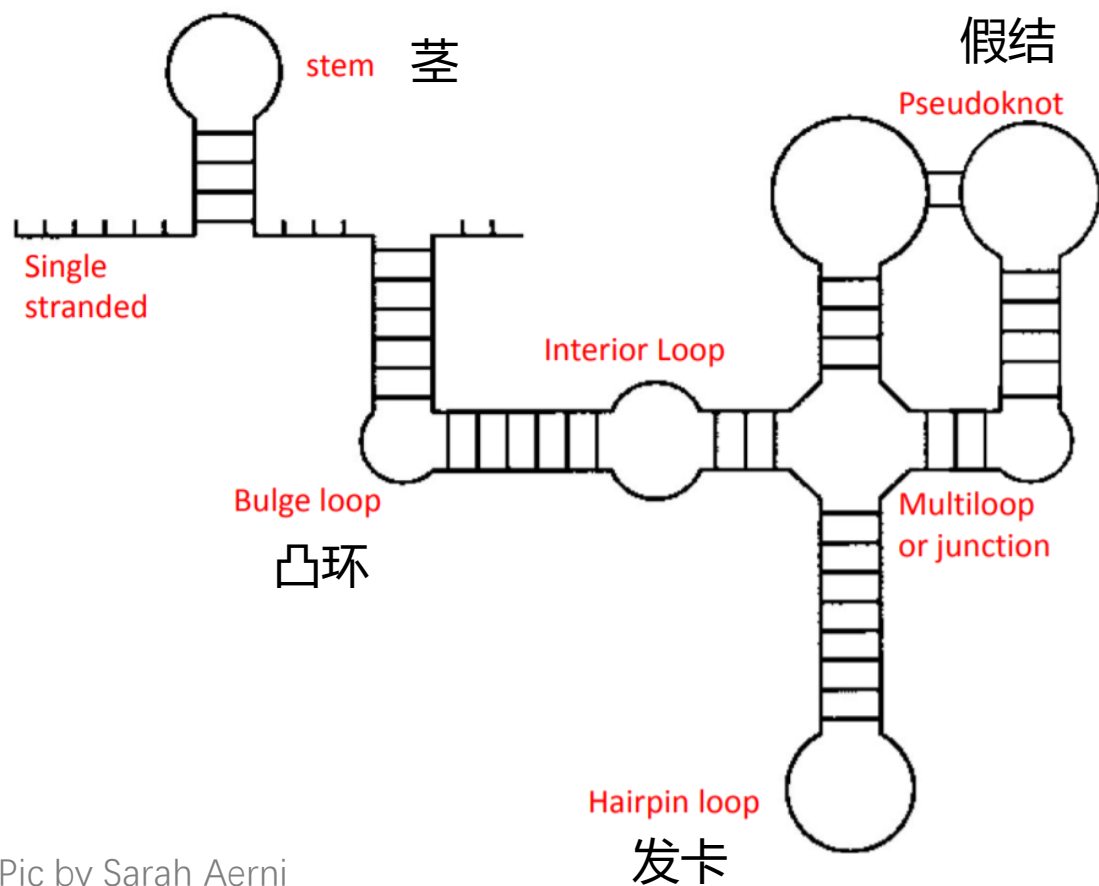
RNA(ribonucleic acid)的结构



I. RNA的结构、分类和功能

RNA的结构

2. RNA链自身折叠形成局部双螺旋，可形成大量的三级结构



I. RNA的结构、分类和功能

RNA的分类

Non coding RNA
(ncRNA)

- messenger RNA (mRNA) ———— 2%
- transfer RNA (tRNA) ———— 16%
- ribosomal RNA (rRNA) ———— 80%
- small interfering RNA (siRNA)
- micro RNA (miRNA)
- small nuclear RNA (snRNA)
- small nucleolar RNA (snoRNA)
- guide RNA (gRNA)
- ...

I. RNA的结构、分类和功能

RNA的功能

1. 作为细胞内蛋白质生物合成的主要参与者
2. 部分RNA可以作为核酶在细胞中催化一些重要的反应
3. 参与基因表达的调控
4. 在某些病毒中，RNA是遗传物质

Contents

I. RNA

II. RNA聚合酶

III. 转录起始

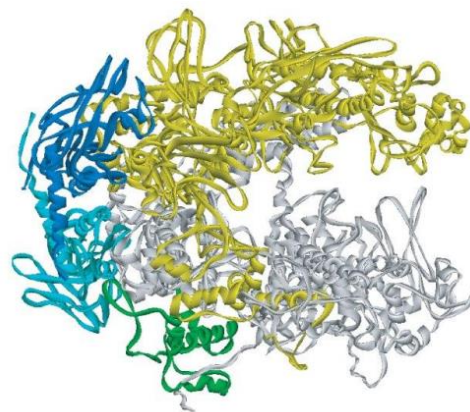
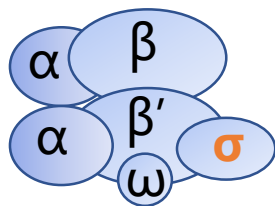
IV. 转录延伸

V. 转录终止

II. RNA聚合酶

RNA聚合酶的结构

- 以双链DNA为模板
- 不需要引物
- 5' -> 3'



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Table 6.1 Ability of Core and Holoenzyme to Transcribe DNAs

DNA Template	Relative Transcription Activity	
	Core	Holoenzyme
T4 (native, intact)	0.5	33.0
Calf thymus (native, nicked)	14.2	32.8

core polymerase
(核心聚合酶)

β' : 160kDa
β: 150kDa
α: 40kDa
ω: 10kDa
σ: 70kDa

holoenzyme
(全酶)

基本的转录装置

指导核心酶转录特异的基因

转录方向



DNA

5' ATCGGCATATATCCAATCGAGATC

3' TAGCCGTATATAGGTTAGCTCTAG

编码链/有义链
Coding strand

模板链/反义链
Template strand



RNA

5' AUCGGCAUAUAUCCAAUCGAGAUC

Contents

I. RNA

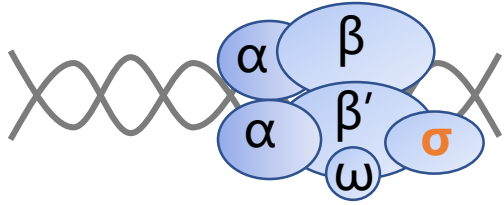
II. RNA聚合酶

III. 转录起始

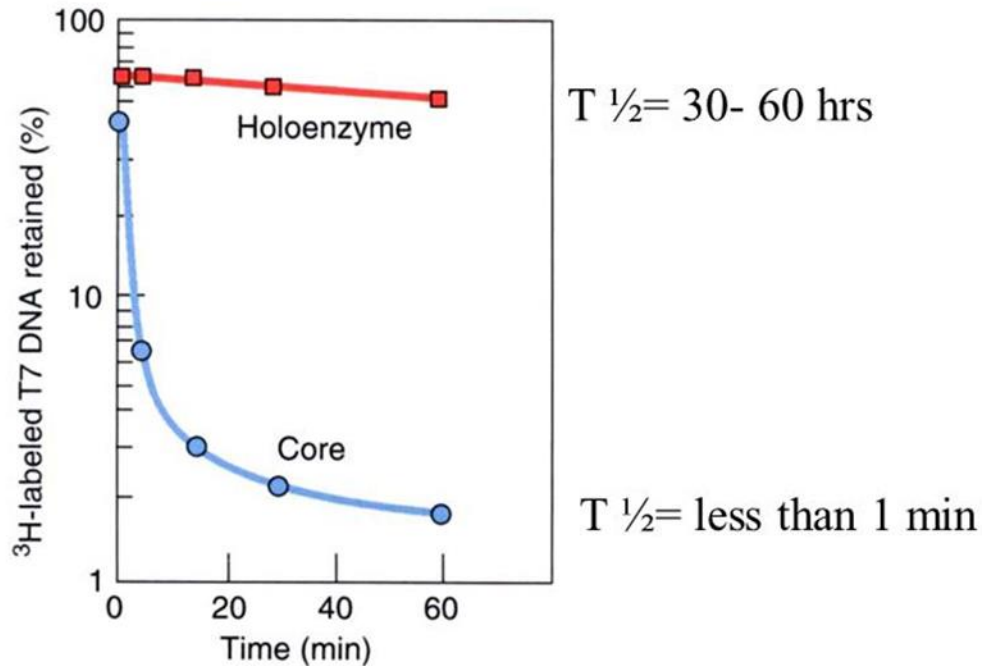
IV. 转录延伸

V. 转录终止

III. 转录起始



启动子: 聚合酶结合位点



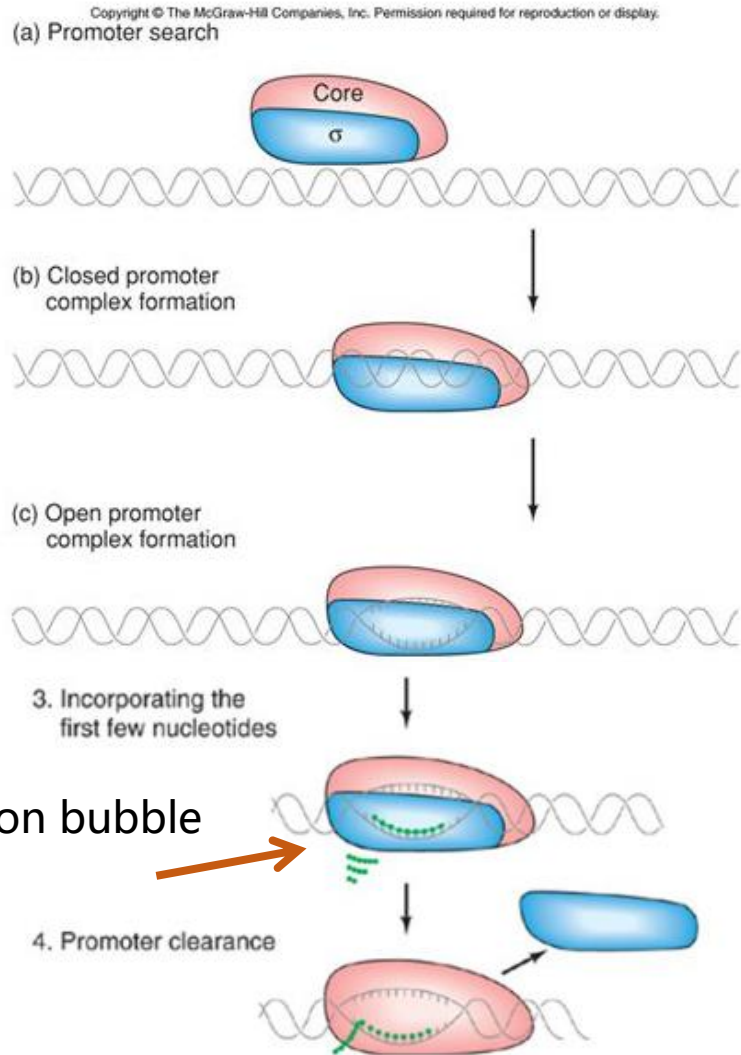
	T7 DNA	core RNA pol
紧密结合位点	8	X
松散结合位点	~1300	√

核心酶 vs 全酶

Figure 6.5 Sigma stimulates tight binding between RNA polymerase and promoter.

III. 转录起始

RNA聚合酶 & 启动子



- RNA pol全酶与DNA进行松散结合与再结合，并寻找启动子
- 全酶找到启动子并与之松散结合，形成二元封闭复合物
- 全酶的紧密结合使DNA局部解链，形成二元开放复合物
- RNA聚合酶、DNA与新生RNA形成三元复合物
- σ 因子被释放，核心聚合酶沿模板DNA链移动

III. 转录起始

RNA聚合酶在启动子区

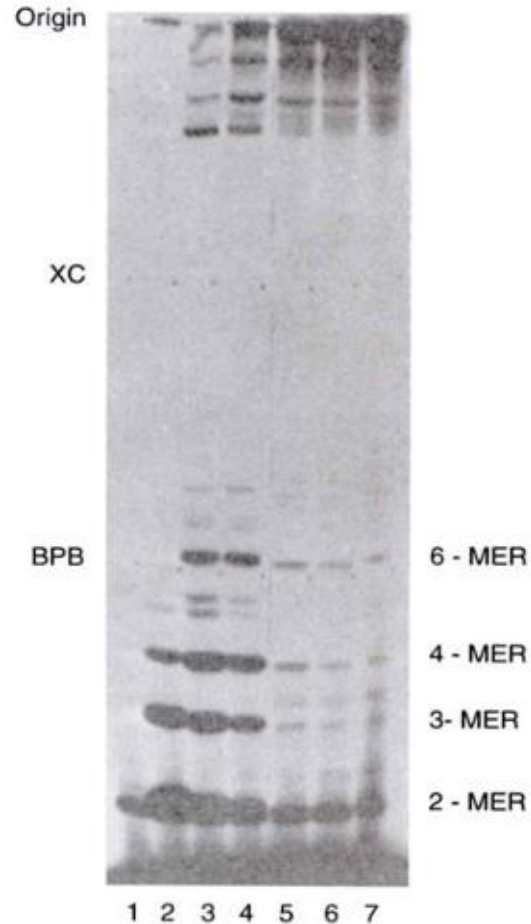
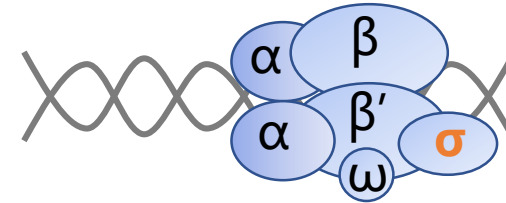


Figure 6.12 Synthesis of short oligonucleotides by RNA polymerase bound to a promoter.

Lane 1: no DNA;
lane 2, ATP only;
lane 3-7: ATP with concentrations of CTP,
GTP, and UTP increasing by two-fold in
each lane



流产式起始

短RNA转录物

2~9个核苷酸

>9个核苷酸

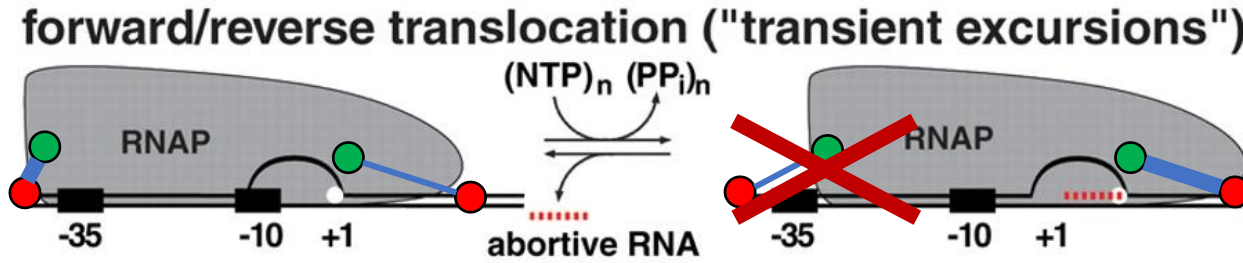
RNA链脱落,
转录终止

离开启动子区,
转录进入延伸阶段

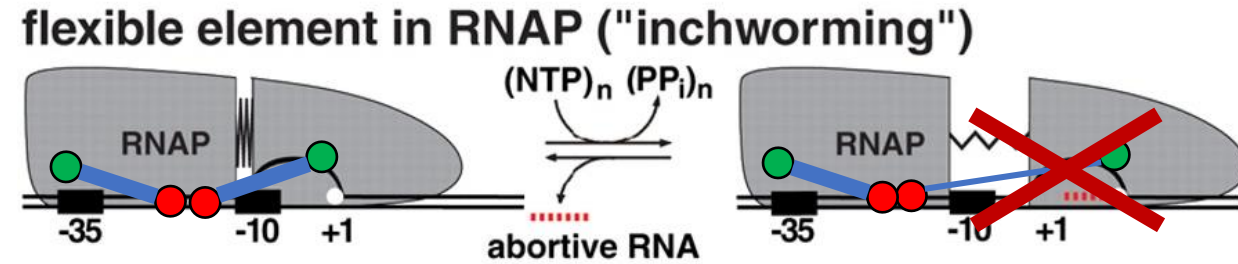
III. 转录起始

启动子清除

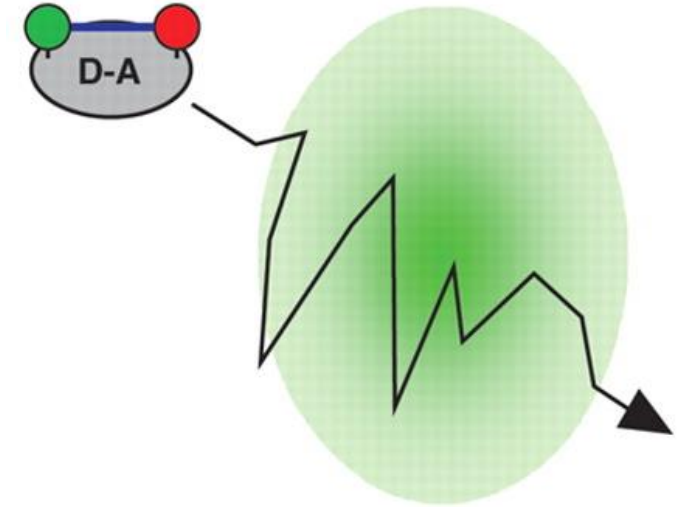
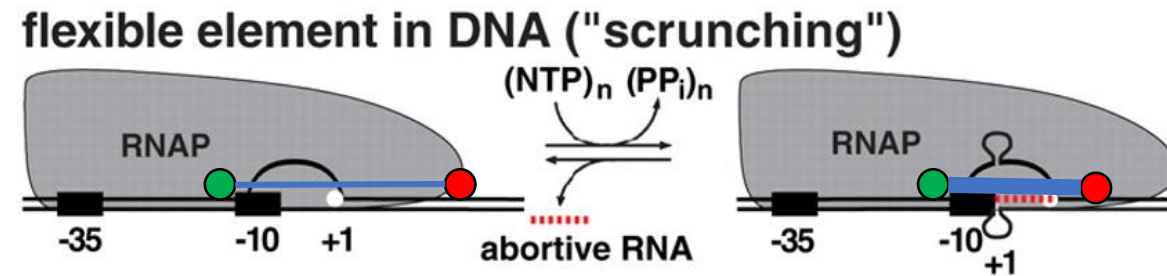
瞬间漂移



缓慢爬行



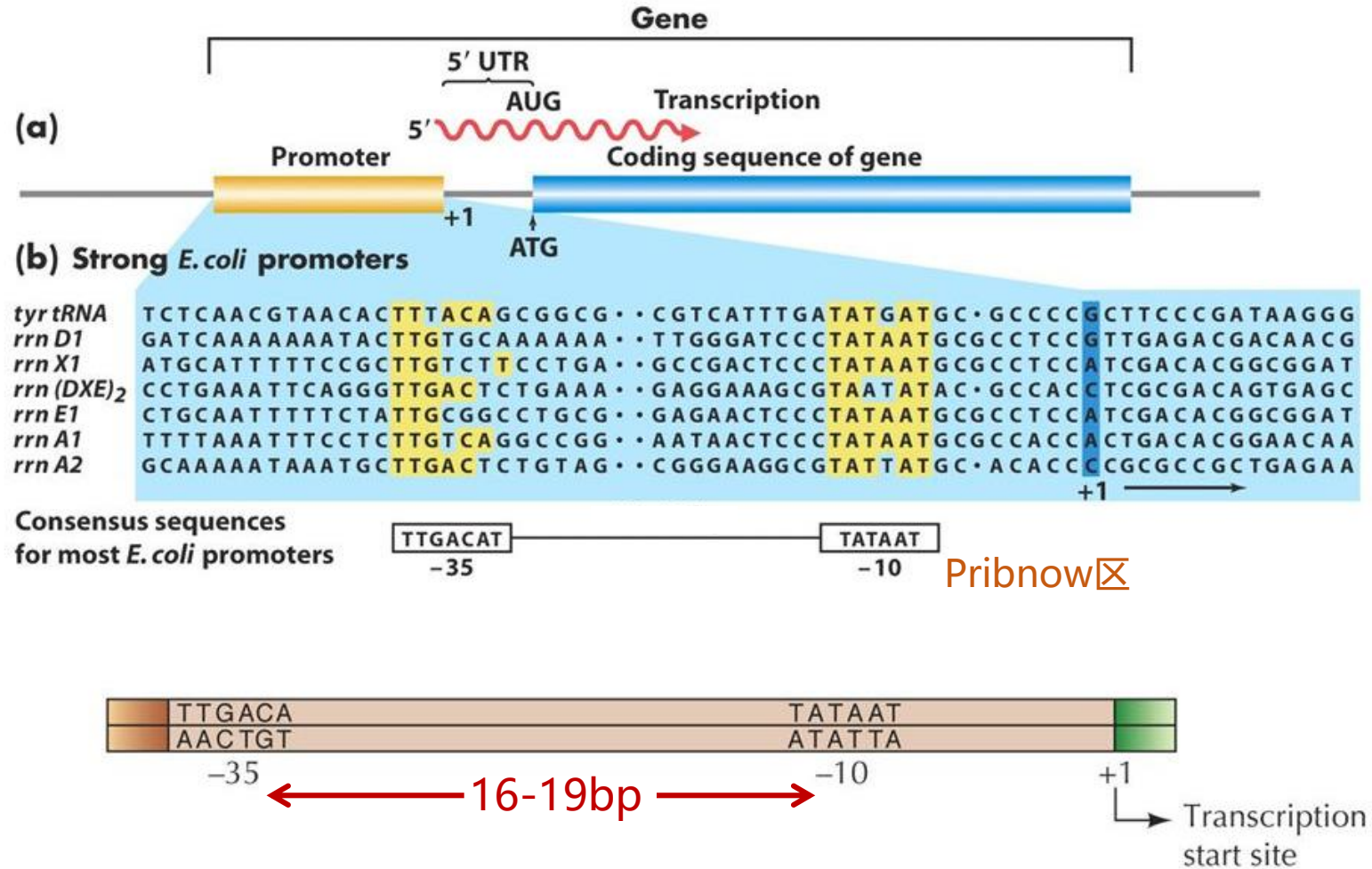
蜷缩



- E. Coli* RNA聚合酶 蜷缩机制:
- 将下游DNA拉入自身内部
 - 无需发生实际移动, 失去启动子上的把手
 - 蜷缩DNA储有足够的能量, 可使聚合酶破坏与启动子的结合

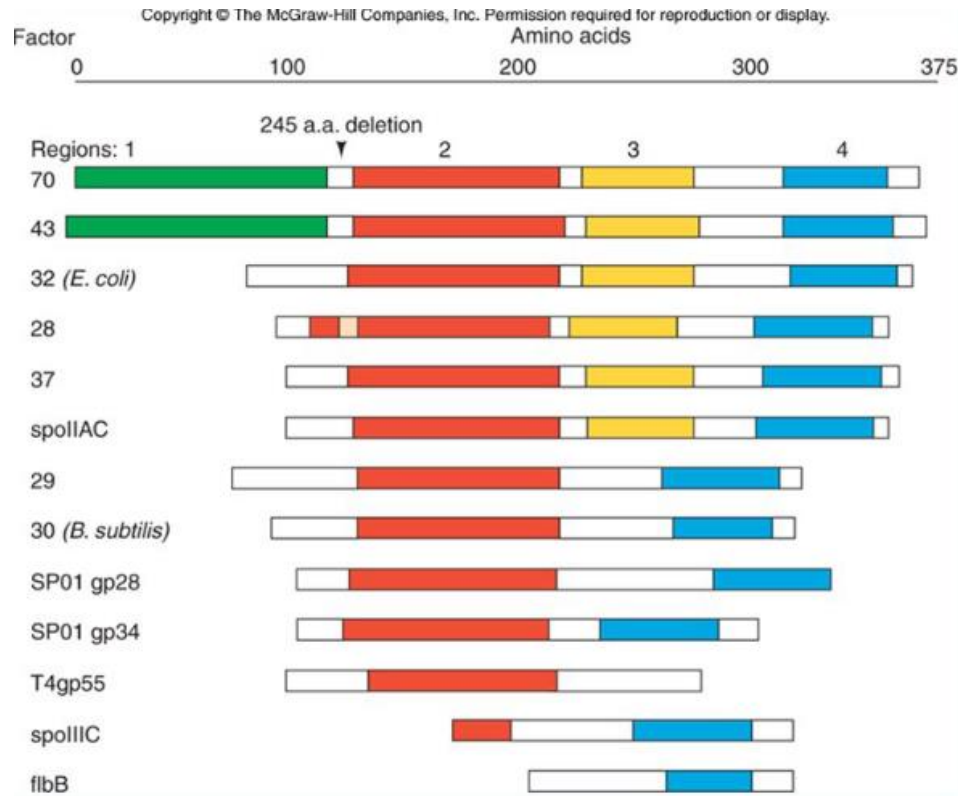
III. 转录起始

启动子: 共有序列(consensus sequence)

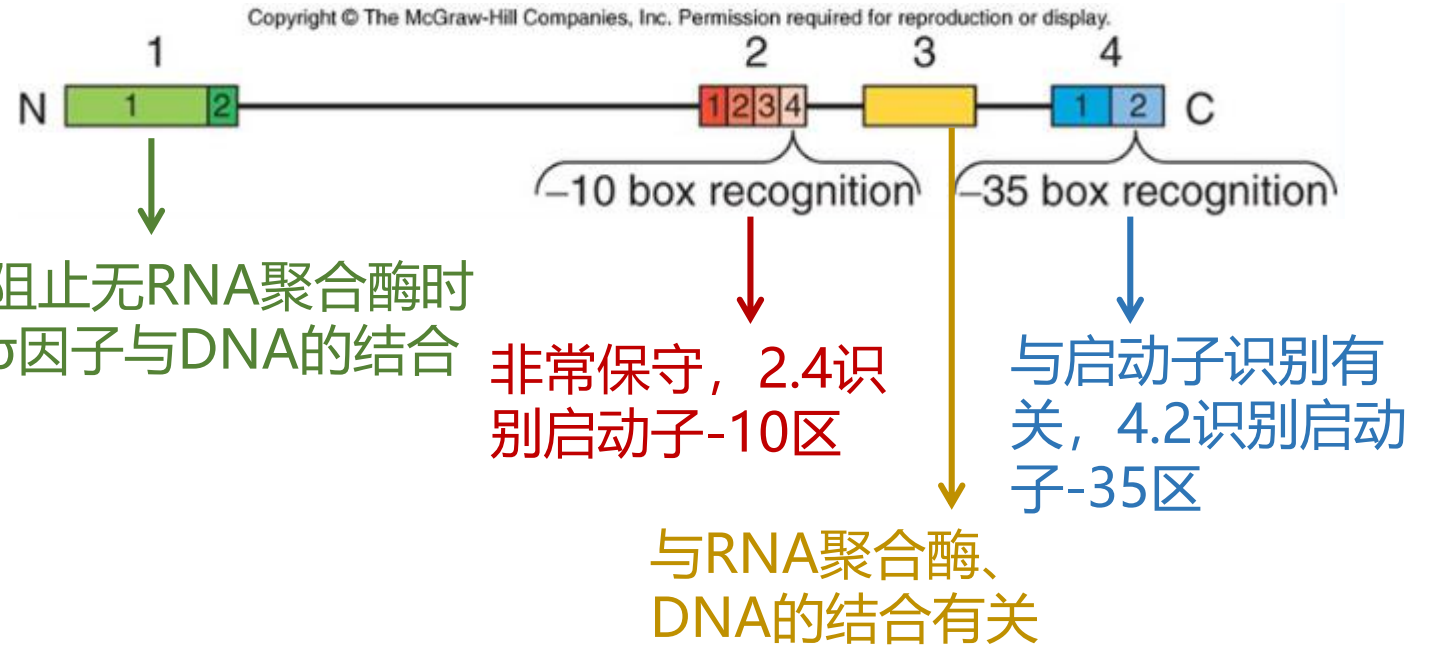


III. 转录起始

细菌 σ 因子结构

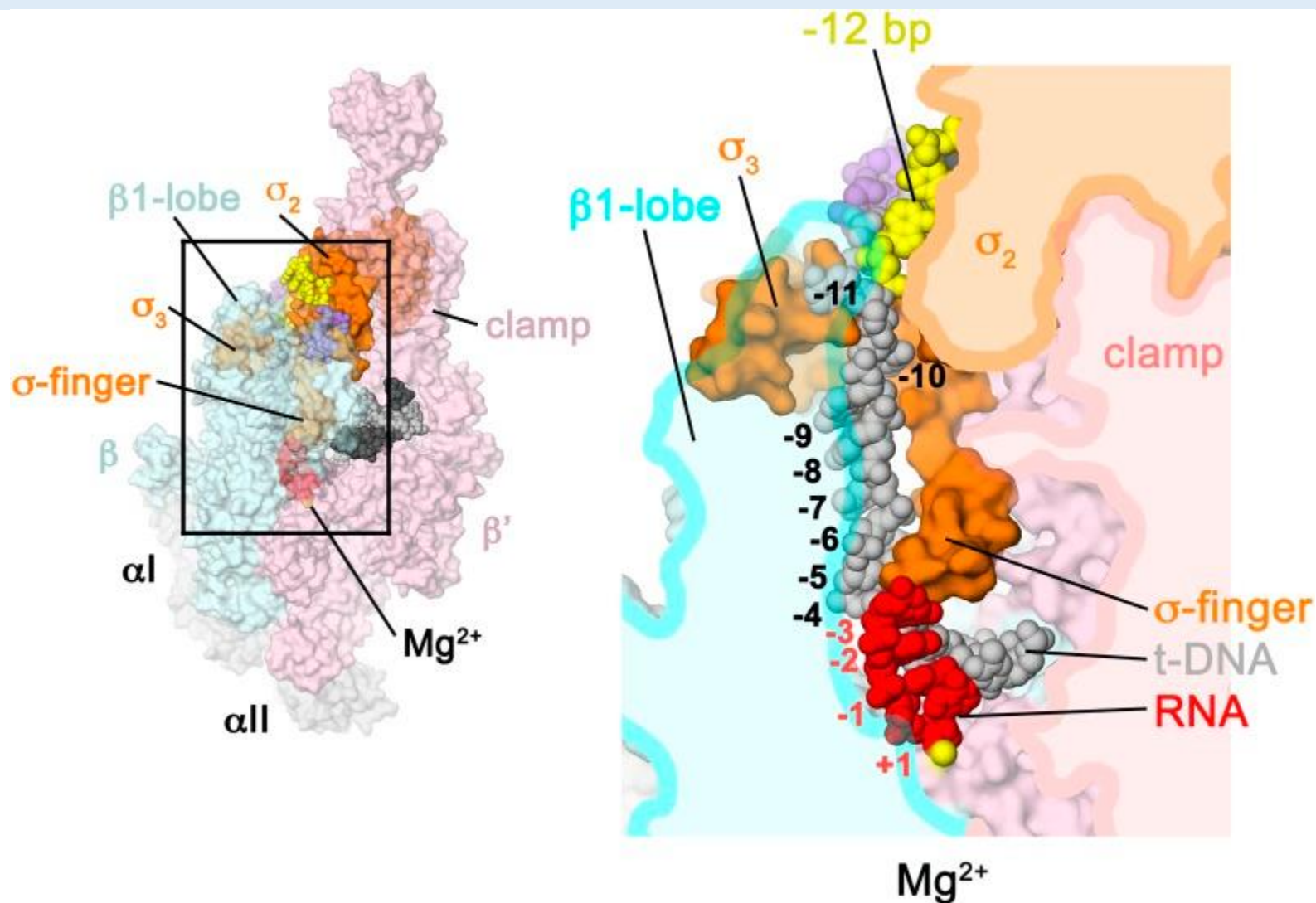


E. coli σ^{70}



III. 转录起始

RNA聚合酶的活性位点



III. 转录起始

启动子: UP element

强启动子

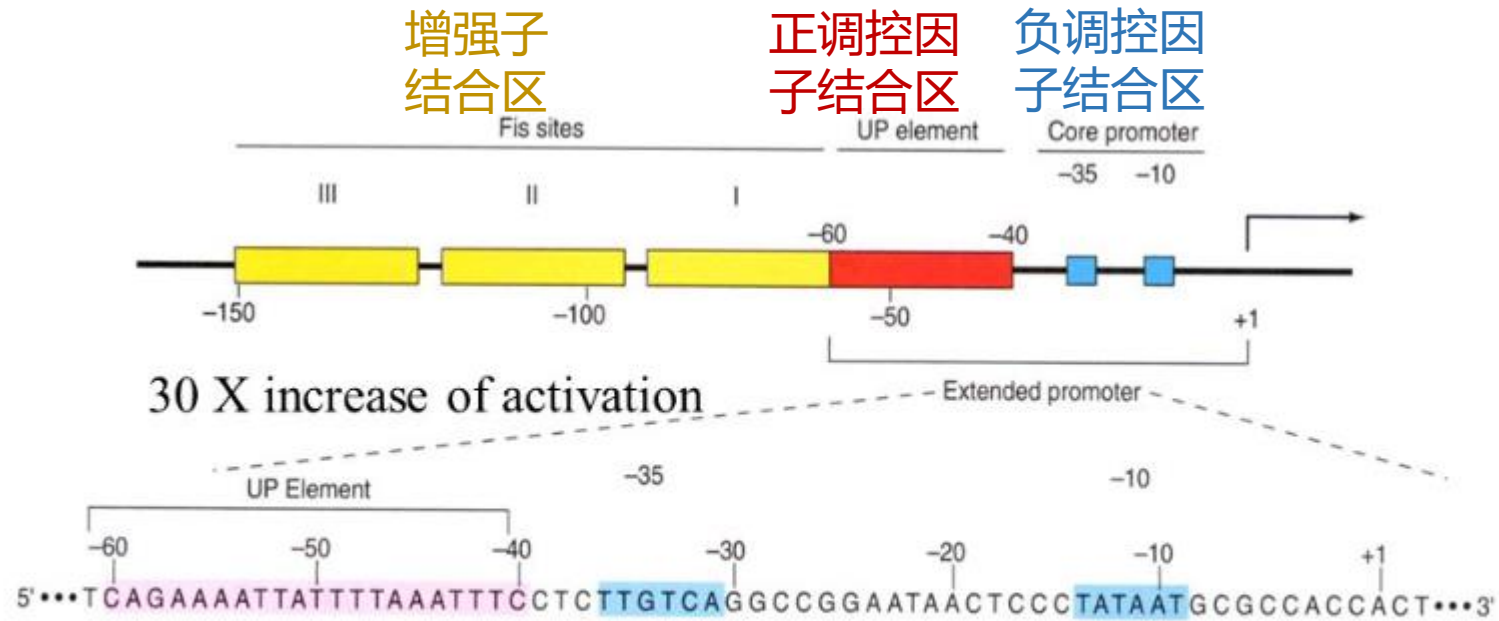
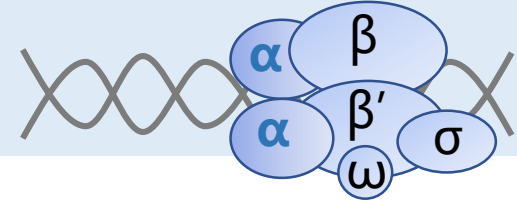


Figure 6.11 The *rrnB* P1 promoter.

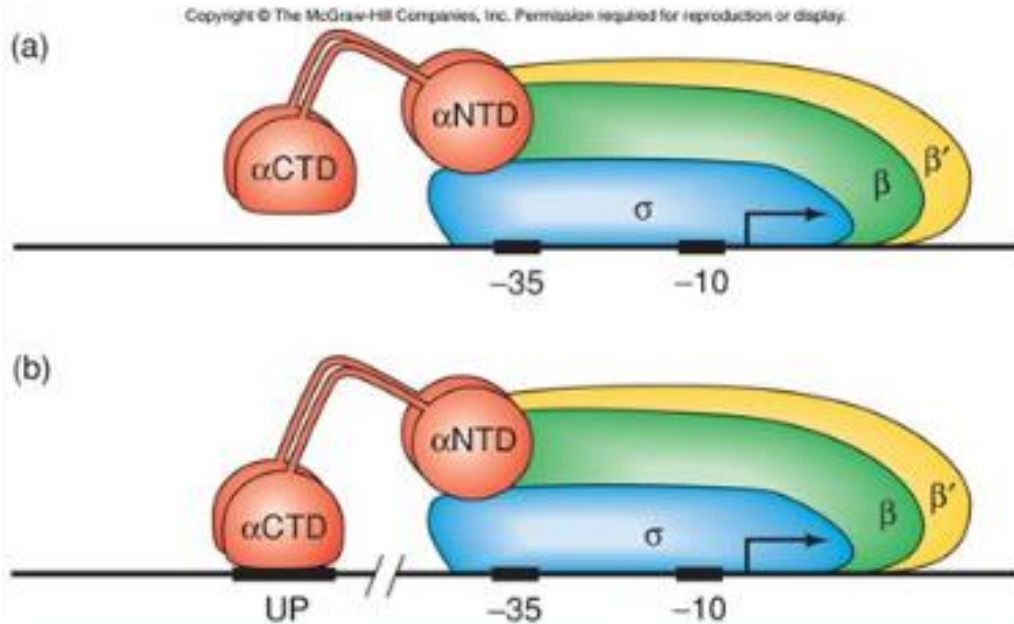
- 仅在RNA聚合酶存在的情况下，UP元件能将*rrnB* P1基因的转录效率提高30倍
- **UP元件是一个启动子元件，被RNA聚合酶自身识别**

III. 转录起始

启动子: UP element



- α 亚基拥有独立折叠的C端结构域 (CTD)，该结构域识别启动子的UP元件并与之结合



在核心启动子中， α 亚基不参与结合

在含有UP元件的启动子中，CTD与UP元件结合

Contents

I. RNA

II. RNA聚合酶

III. 转录起始

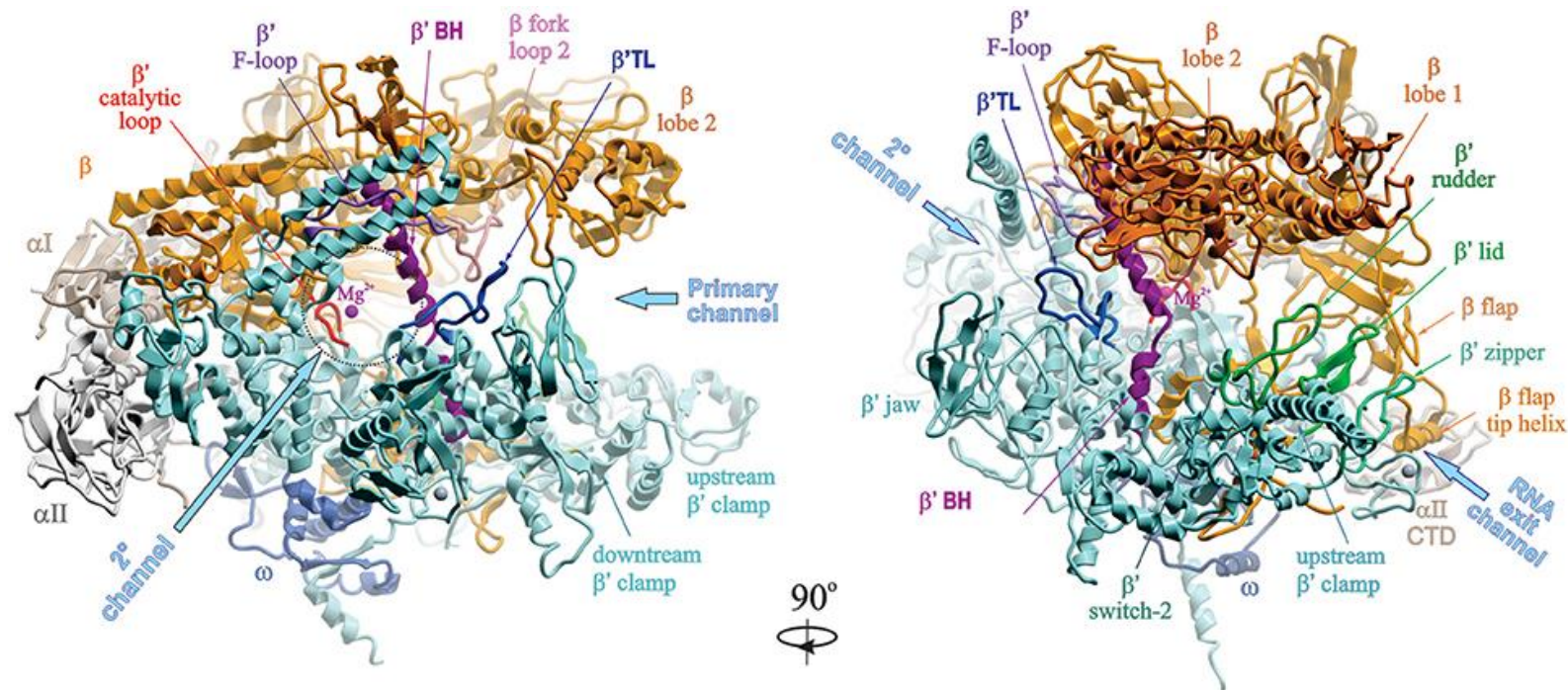
IV. 转录延伸

V. 转录终止

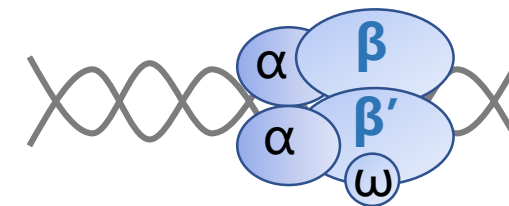
IV. 延伸

核心聚合酶结构

Structure of *T. aquaticus* RNAP core



Primary channel: DNA, RNA-DNA hybrid
Secondary channel: NTP entry
RNA exit channel: mRNA exit



β' & β : 催化中心

IV. 延伸

延伸中的拓扑学问题

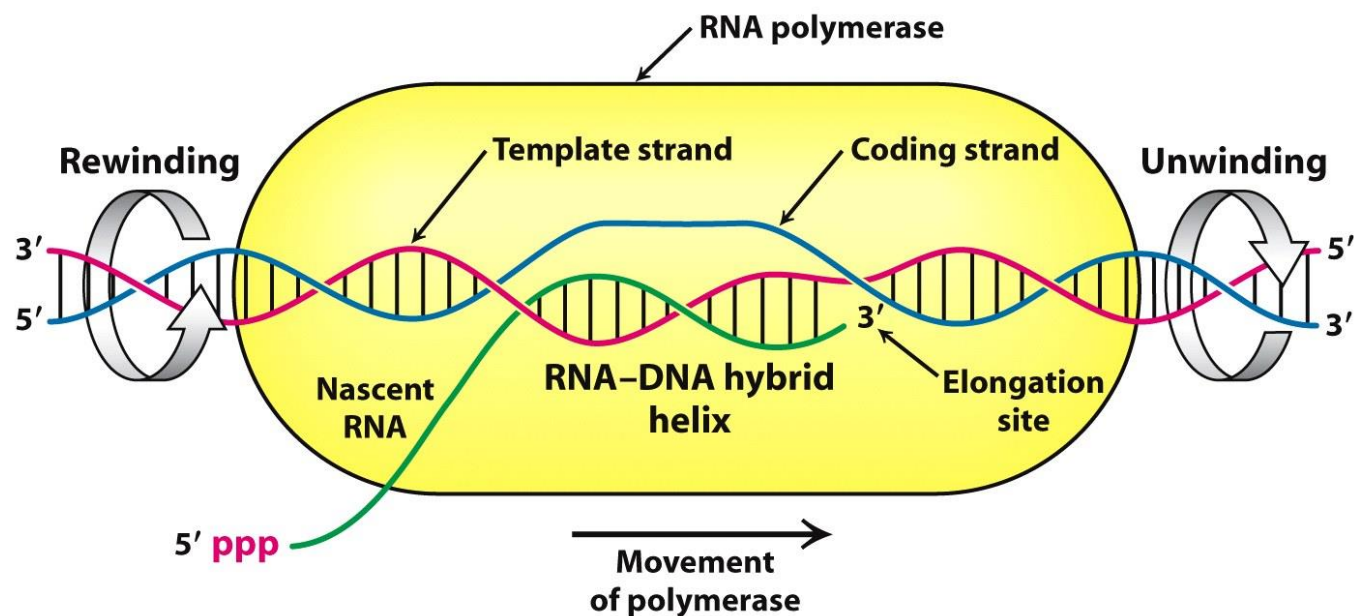


Figure 29.13
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Topoisomerases
拓扑异构酶

- 伴随着RNA聚合酶的移动，DNA模板维持一段短的解链区
- 行进的RNA聚合酶前方的DNA解旋，而其后的DNA又重新聚合，由此在DNA分子内引入的张力通过拓扑异构酶加以释放

Contents

I. RNA

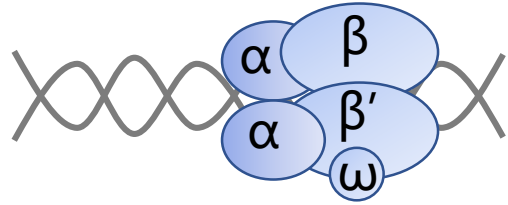
II. RNA聚合酶

III. 转录起始

IV. 转录延伸

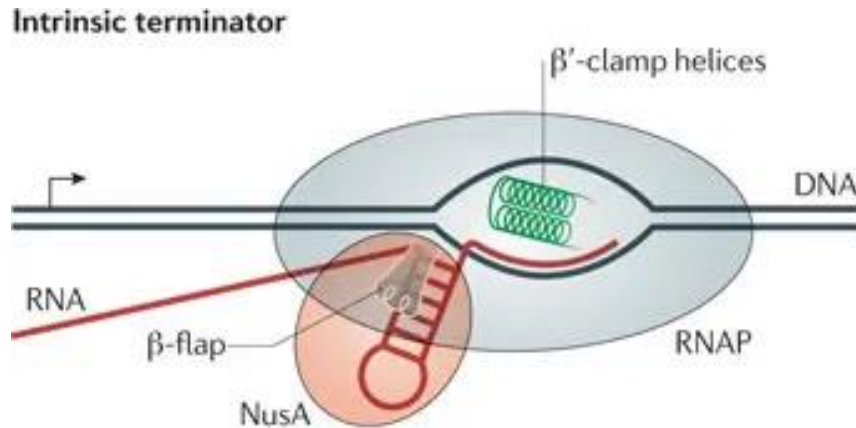
V. 转录终止

V. 转录终止

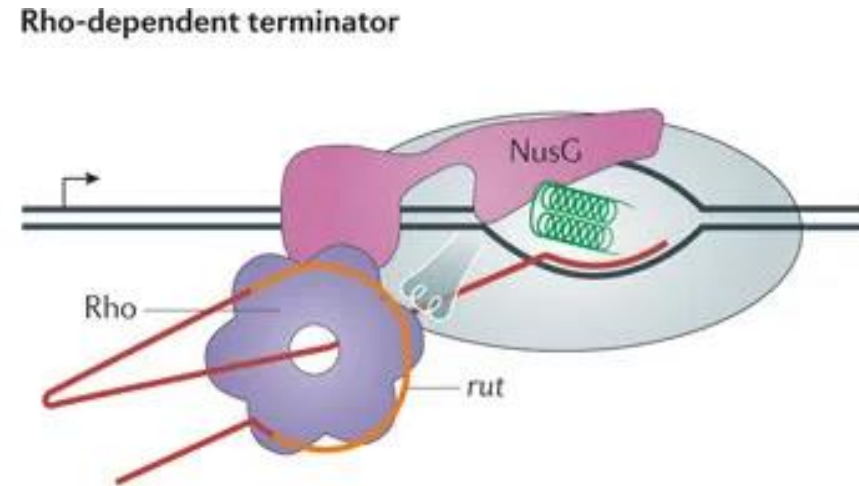


核心酶碰上终止信号，停止加入新的核苷酸，与模板DNA相脱离，释放新生RNA链

内在终止子



依赖于ρ因子的终止子



Nature Reviews | Microbiology

V. 转录终止

内在终止子

反向重复序列 Inverted Repeats:

- 反向互补self-complementary
- 发夹 hairpin

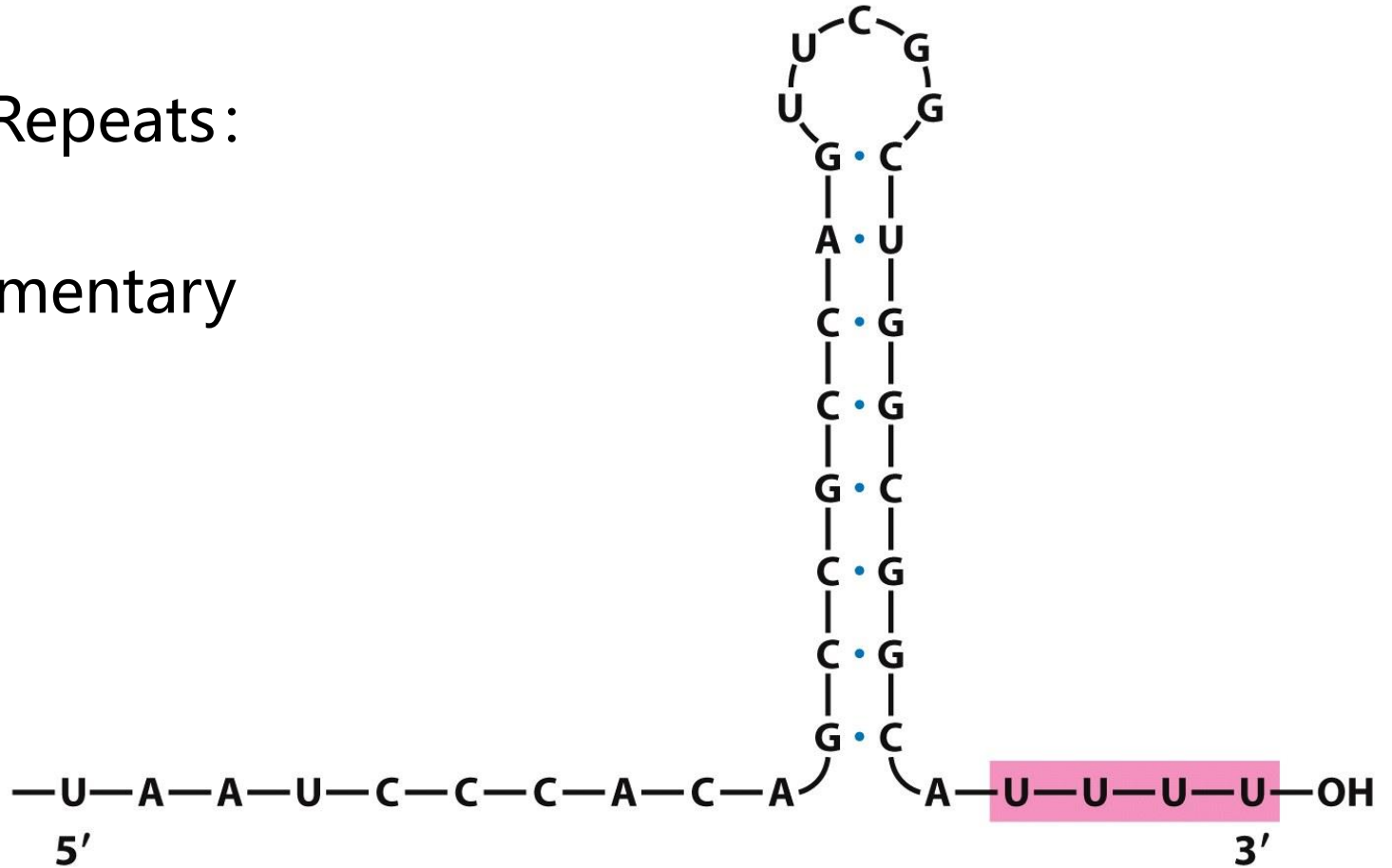
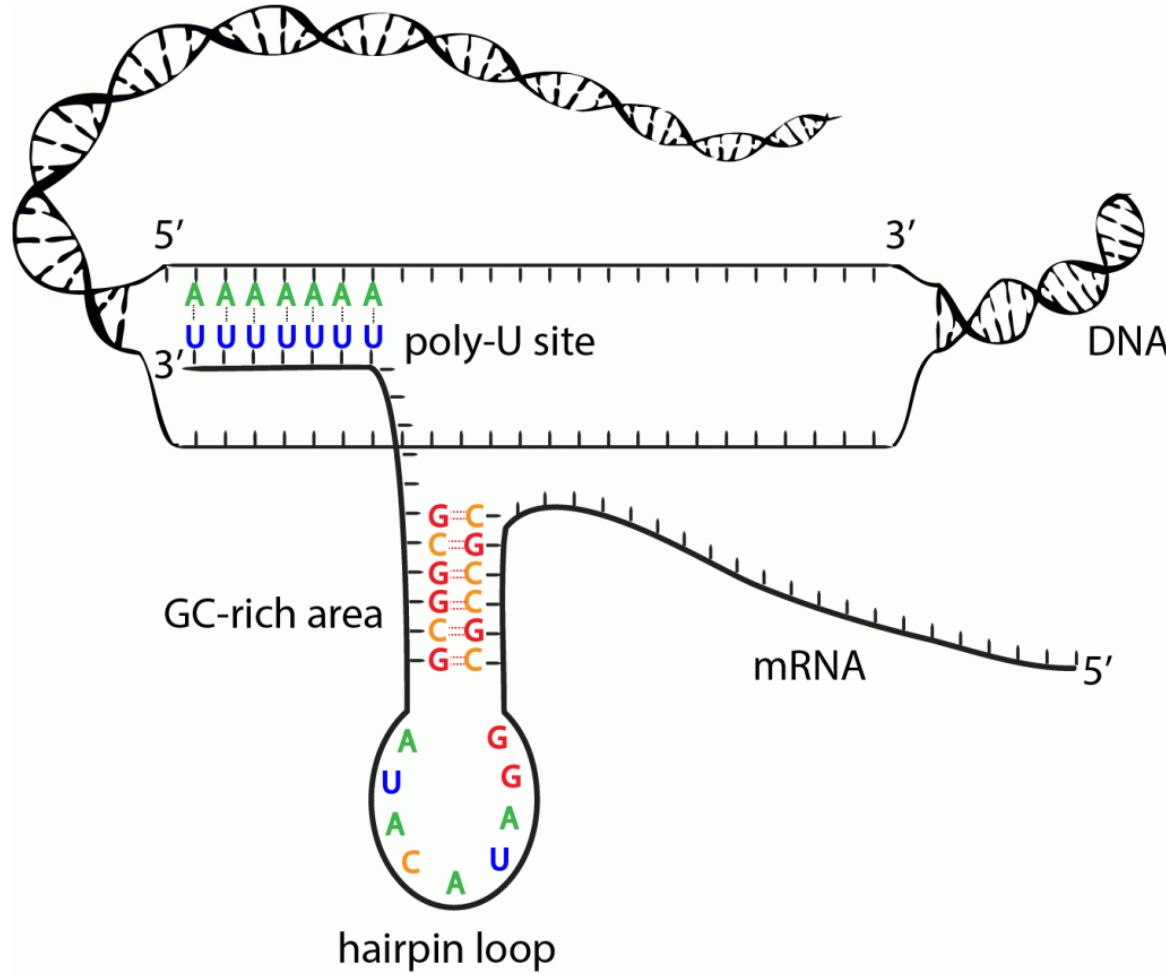


Figure 29.14
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V. 转录终止

内在终止子

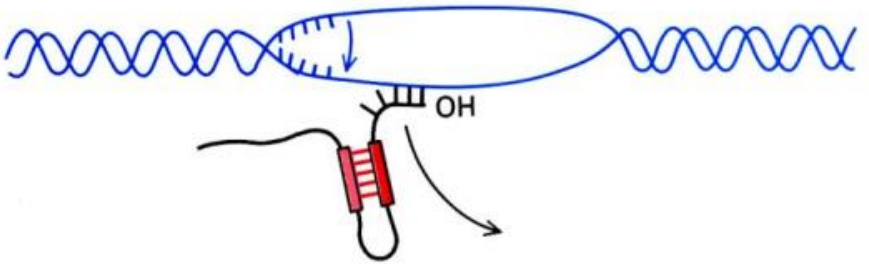
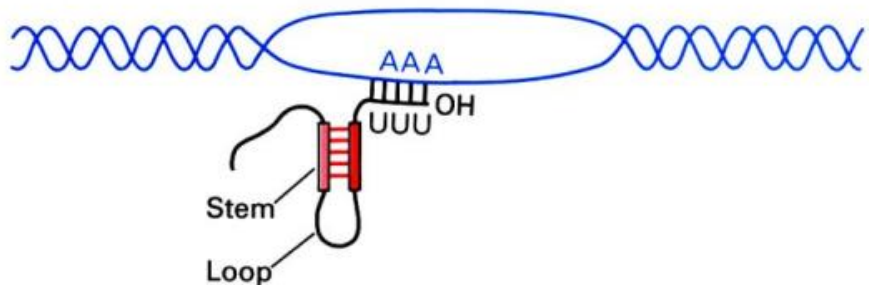
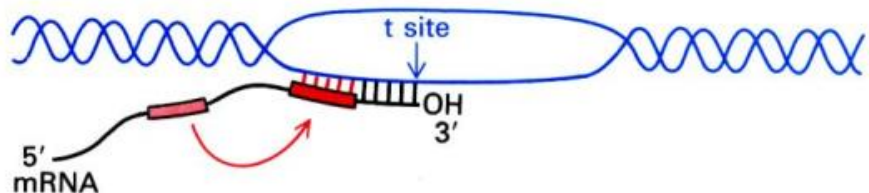


1. 终止位点上游一般存在一个**富含GC碱基**的反向重复序列 (~20个核苷酸)
2. 终止位点前面有一段**4~8个A-T**碱基对序列, 其转录产物的3' 端为寡聚U

V. 转录终止

内在终止子

终止效率与二重对称序列和寡聚U的长短有关



- 在新生RNA中出现发卡结构会导致RNA聚合酶的暂停，破坏RNA-DNA杂合链5'端的正常结构
- 寡聚U的存在使杂合链的3'端部分出现不稳定的rU-rA区域
- 两者共同作用使RNA从三元复合物中解离出来

V. 转录终止

依赖于 ρ 因子的终止子

- 有些终止位点的DNA序列缺乏共性，不能形成强的发夹结构，因而不能诱导转录的自发终止。
- 只有在 ρ 因子存在时，RNA聚合酶才能在DNA模板上准确停止转录。

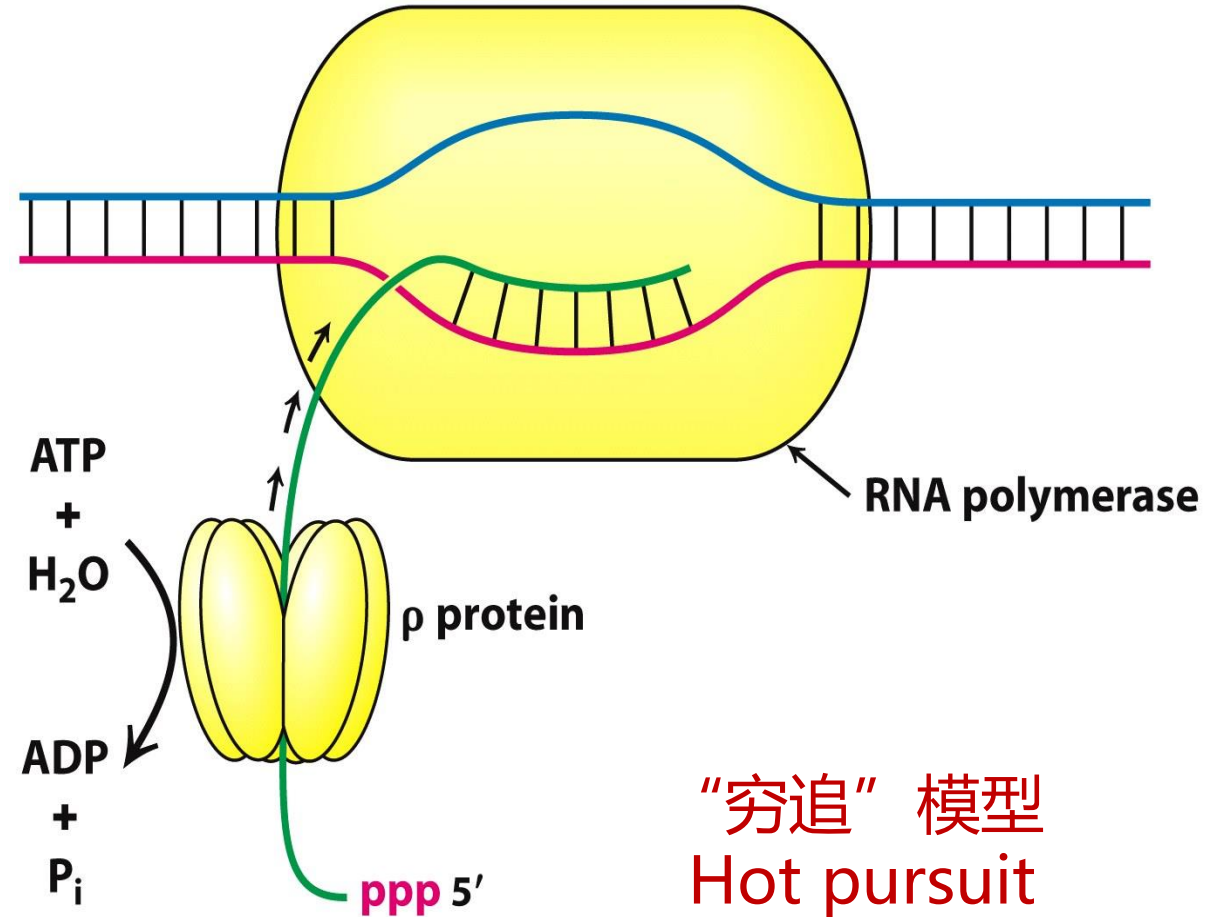
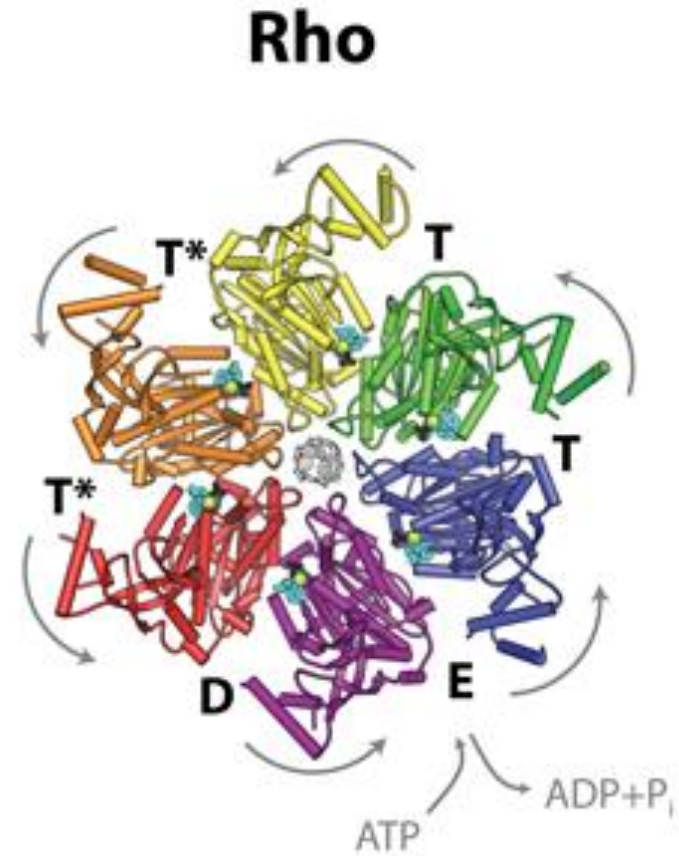


Figure 29.17
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V. 转录终止

依赖于 ρ 因子的终止子

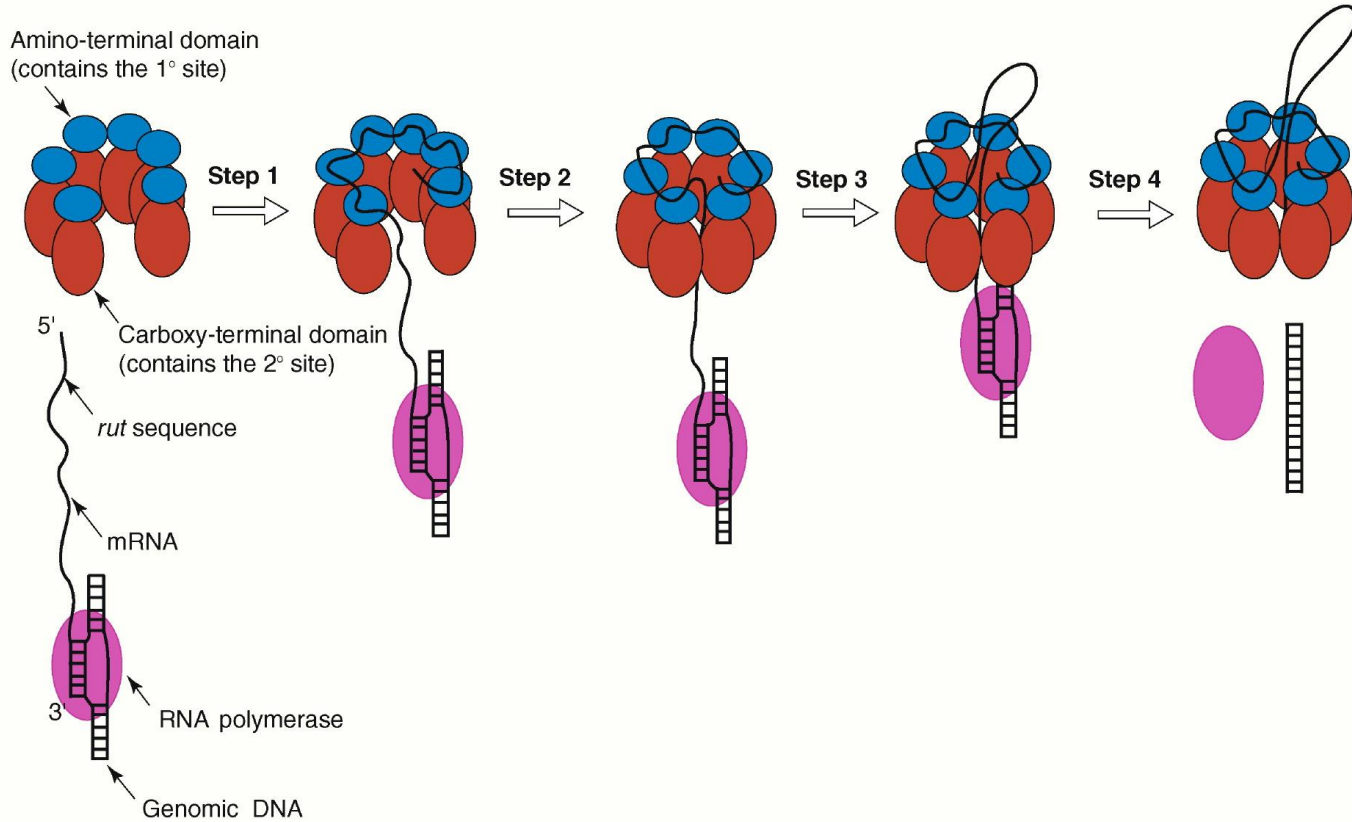
- Rho: 46 KD protein
- 6个相同亚基组成的六聚体
- NTP酶和解螺旋酶活性，能水解各种核苷酸三磷酸
- 转录终止的重要辅助因子



V. 转录终止

依赖于 ρ 因子的终止子

“穷追”模型



1. RNA合成起始后， ρ 因子即附着在新生的RNA链5' 端上(open)
2. ρ 因子继续结合mRNA形成闭环 (closed)
3. 利用ATP水解产生的能量，沿着5' ->3' 方向朝转录泡靠近
4. 当RNA聚合酶移动到终止子而暂停时， ρ 因子到达RNA的3' -OH端追上并取代了RNA聚合酶，使mRNA从模板DNA上释放

Current Biology

Current biology, 2003

rut sequence: ρ 因子在mRNA上的特异结合位点

VI. 复制 VS 转录

复制

DNA \longrightarrow DNA

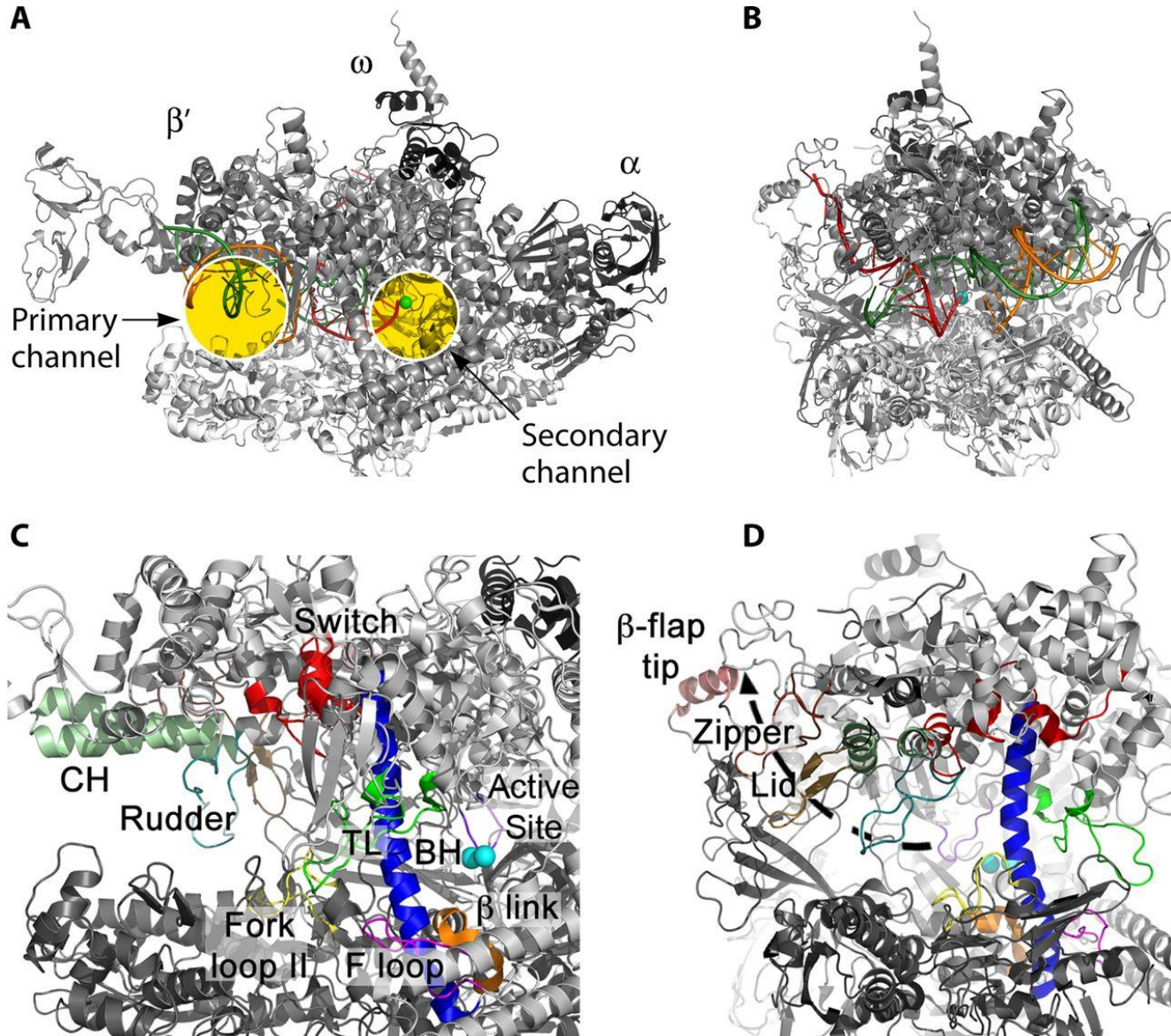
- 模板: DNA
- 产物: 双链DNA
- DNA聚合酶
 - dNTP
 - 5' \rightarrow 3'
 - 需要引物
 - 需要解旋酶
- 合成整个基因组
- 一个细胞周期或细胞分裂一次仅复制一次
- 错误率低

转录

DNA \longrightarrow RNA

- 模板: DNA
- 产物: 单链RNA
- RNA聚合酶
 - NTP
 - 5' \rightarrow 3'
 - 不需要引物
 - 不需要解旋酶
- 合成某部分基因
- 一个转录单元可在一个细胞周期内有多个转录产物
- 错误率高

VI. RNA转录的抑制



RNA聚合酶抑制剂

- primary channel inhibitors
- mobile elements of the primary channel
- secondary-channel inhibitors
- switch region inhibitors
- transcription factors and RNAP binding sites as targets

Classification	Name	Source	Binding site	Comment	References
Inhibitors that disrupt RNAP—DNA/RNA/NTPs interactions	Rifamycins	<i>Amycolatopsis mediterranei</i>	β subunit active site cleft	High resistance spectrum	Campbell et al., 2001; Molodtsov et al., 2013
	Sorangicin	<i>Sorangium cellulosum</i>			Campbell et al., 2005
	GE23077	<i>Actinomadura</i> sp	i/i+1 NTP binding site	Low resistance spectrum but poor membrane permeability	Zhang et al., 2014
	GE-Rif	semi-synthetic	composite of Rif- and GE-binding sites		Zhang et al., 2014
	Microcin	<i>E. coli</i> AY25	2° channel (based on modeling)	High resistance spectrum, active against Gram(–) only	Adelman et al., 2004; Mukhopadhyay et al., 2004
	Myxopyronins	<i>Myxococcus fulvus</i>	β' Switch-2	broad-spectrum antibacterial (Gram+ and Gram–)	Mukhopadhyay et al., 2008; Belogurov et al., 2009; Srivastava et al., 2011
	Corallopyronin	<i>Corallococcus coralloides</i>			
	Ripostatin	<i>Sorangium cellulosum</i>			
	Squaramides	synthetic			Buurman et al., 2012; Molodtsov et al., 2015
Fidaxomicin	<i>Dactylosporangium aurantiacum</i>	β' Switch-2 ($\sigma^{70/S}$ R3.2)*	active against Gram (+)	Artsimovitch et al., 2012	
Lipiarmycin	<i>Actinoplanes deccanensis</i>			Tupin et al., 2010; Morichaud et al., 2016	
Inhibitors that constrain the mobile elements of RNAP active center	Streptolydigin	<i>Streptomyces lydicus</i>	β' BH, β' TL, β fork loop-2 (downstream DNA)*	High resistance spectrum	Temiakov et al., 2005; Tuske et al., 2005; Vassilyev et al., 2007
	Salinamide	marine and soil <i>Streptomyces</i> spp	β' BH hinge region (near 2°-channel), β' F-loop, β -link, (β' TL)*	Poor membrane permeability	Degen et al., 2014
	CBR compounds	chemical compound library	β element between fork loop 2 and β DII, β' F-loop, β' BH hinge region	Low resistance spectrum but tested only in <i>ToI/C</i> strain; cytotoxic	Artsimovitch et al., 2003, 2011; Malinen et al., 2012, 2014; Yuzenkova et al., 2013; Bae et al., 2015c; Feng et al., 2015
	Tagetitoxin	<i>Pseudomonas syringae</i> pv. <i>tagetis</i>	2°-channel; β' catalytic loop and adjacent residues, β' clip; β -link, β active site loop, Mg^{2+} (II) (β' TL)*	Low membrane permeability	Artsimovitch et al., 2011; Malinen et al., 2012; Yuzenkova et al., 2013
Inhibitors that disrupt RNAP- σ^{70} interactions	GKL003	chemical compound library	β' clamp helices (CH)	High affinity ($K_i \sim 6$ nM) but low solubility and permeability	Ma et al., 2013; Yang et al., 2015a
	DSHS00507	drug-like compound library	β' clamp helices (CH)	Effective against Gram-positive bacteria	
	SB series	compound library	undefined	IC ₅₀ : 2~15 μ M but low target specificity	André et al., 2004, 2006

*Indicates possible auxiliary binding site.

