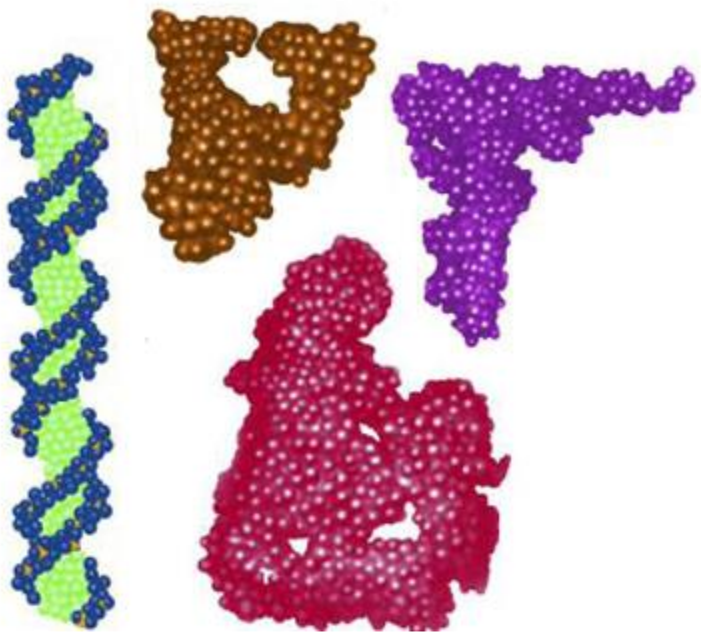
中山大學
SUN YAT-SEN UNIVERSITY



第七章　　核酸

（nucleic acid）

生物化学（一）

生命科学学院　　　　　李莲

lilian-75@163.com

蛋白序列和核酸序列的读法

- LSTTEVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGERVQLAHHFSEPEITLIIFGVMAGTIGTILLISYGIRRLIKKSPSDVKPLPSPDTDVPLSSVEIENPETSDQ

N端 C端

- AGGCTAAGGTCAGACACTGACACTTGCAGTTGTCTTTGGTAGTTTTTTTGCACTAACTTCAGGAACCAGCTCATGATCTCAGGATGTATGGAAAAATAATCTTTGTATTACTATTGTCAGAAATTGTGAGCATATCAGCA

5’端 3’端

为什么核酸从左到右是从5’端到3’端?

Chapter 7
Nucleotides and Nucleic Acids

7.1 ***Brief History of Nucleic Acids***

7.2 ***Nucleotides: the Building Blocks***

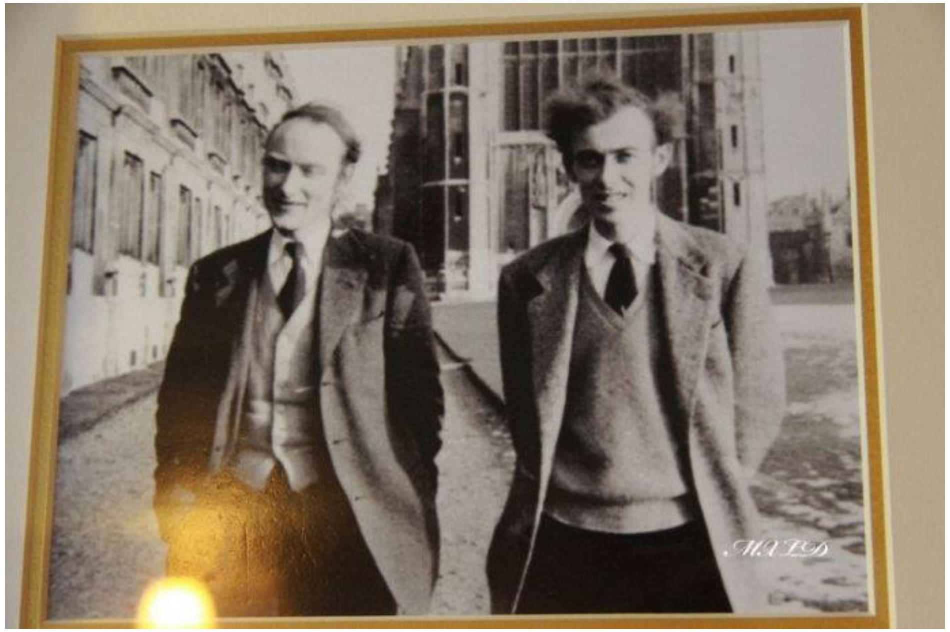
7.3 ***Nucleic Acids: from Structure to Functions***

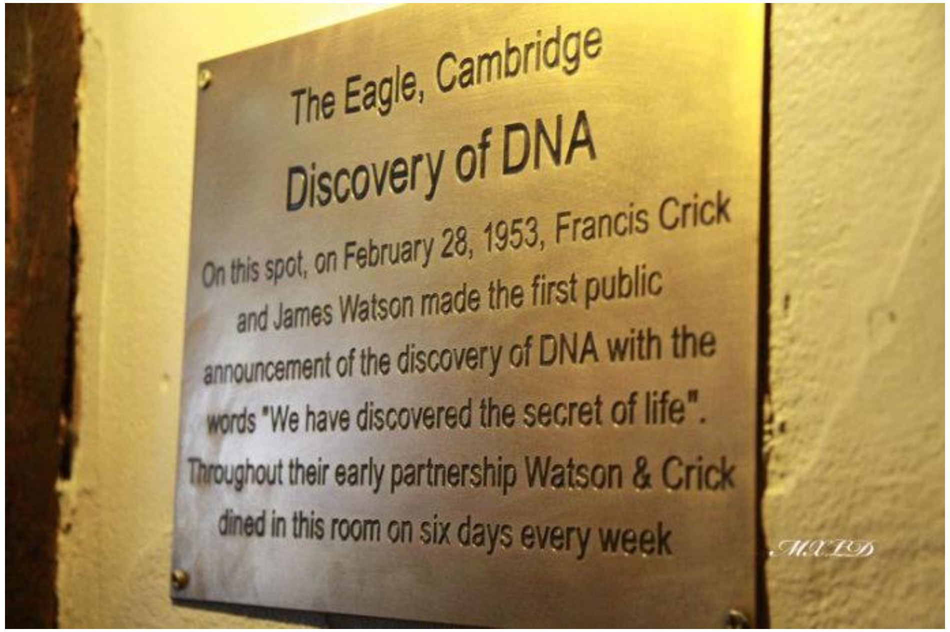
7.4 ***Nucleic Acids-Based Biotechnology***

7.5 ***Other functions of Nucleotides***

7.1 *Brief History of Nucleic Acids*

1953年2月28日午餐时间





Brief History

F. Miescher

Nuclein（核质）

1869

Richard Altmann

Nucleic Acids（核酸）

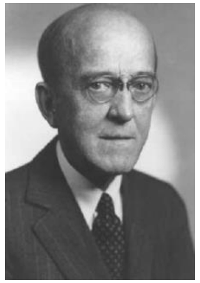
1889

F. Griffith

肺炎链球菌转化实验

1928

O.T. Avery



核酸转化实验

1944

E. Chargaff

碱基组成规律

1947

Herskey 和Chase

噬菌体感染实验

1952

Watson 和Crick

DNA 双螺旋结构

1953

核酸是遗传物质！

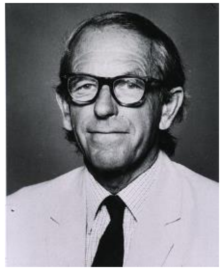
核酸生物技术

1958

Crick

中心法则（Central Dogma）

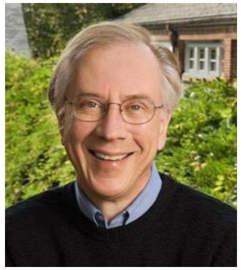
1975



F. Sanger

DNA测序

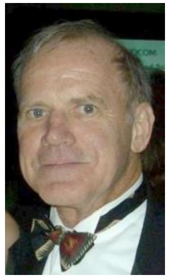
1982



T. Cech等

Ribozyme

1983



Mullis

PCR

1998

Fire and Mello

RNAi

2005

Katalin Karikó、Drew Weissman

核苷碱基修饰

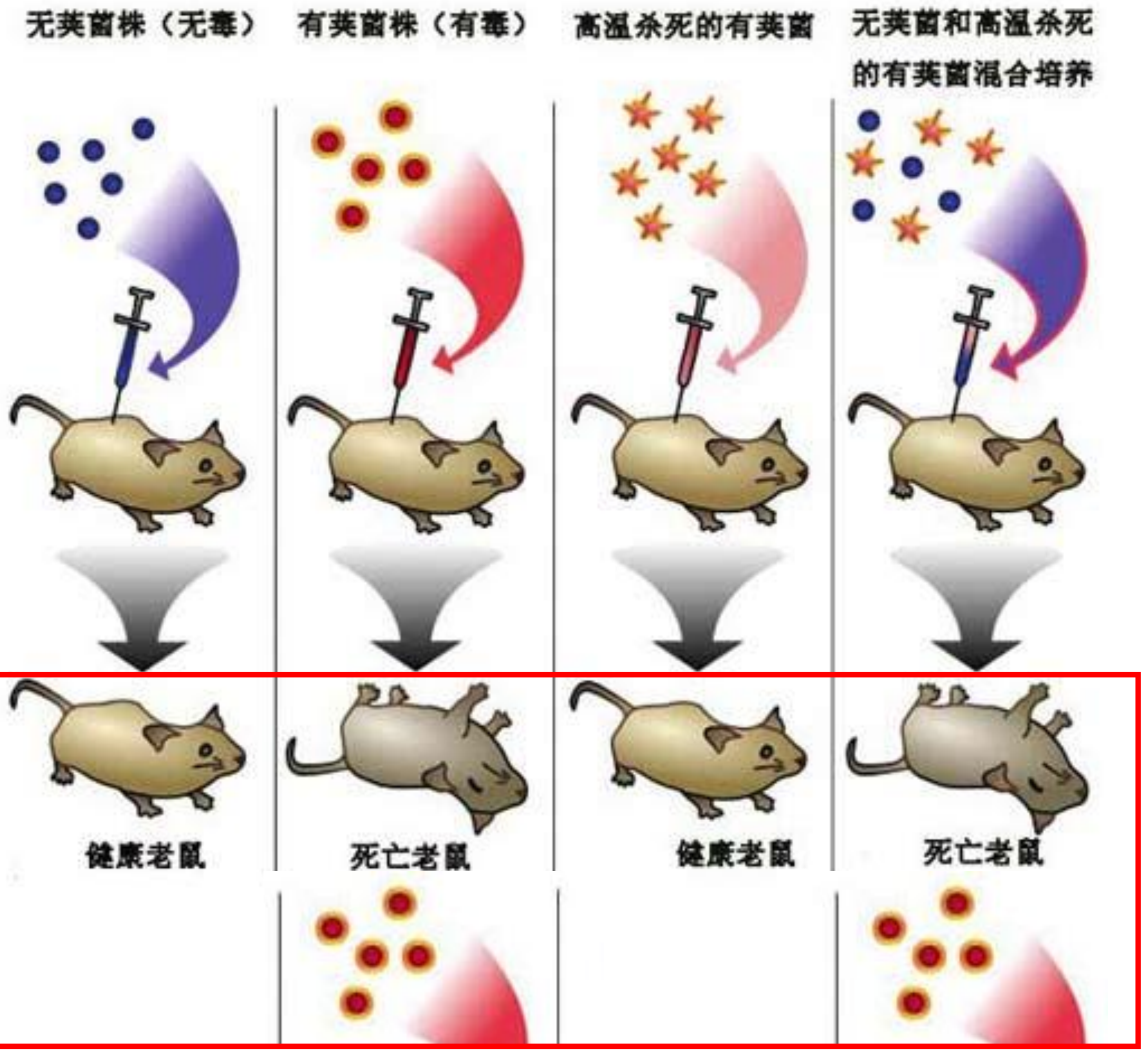
2012

Doudna, Charpentier, Zhang

Genome editing by CRISPR/Cas9

1928, *Griffith's transformation experiment*

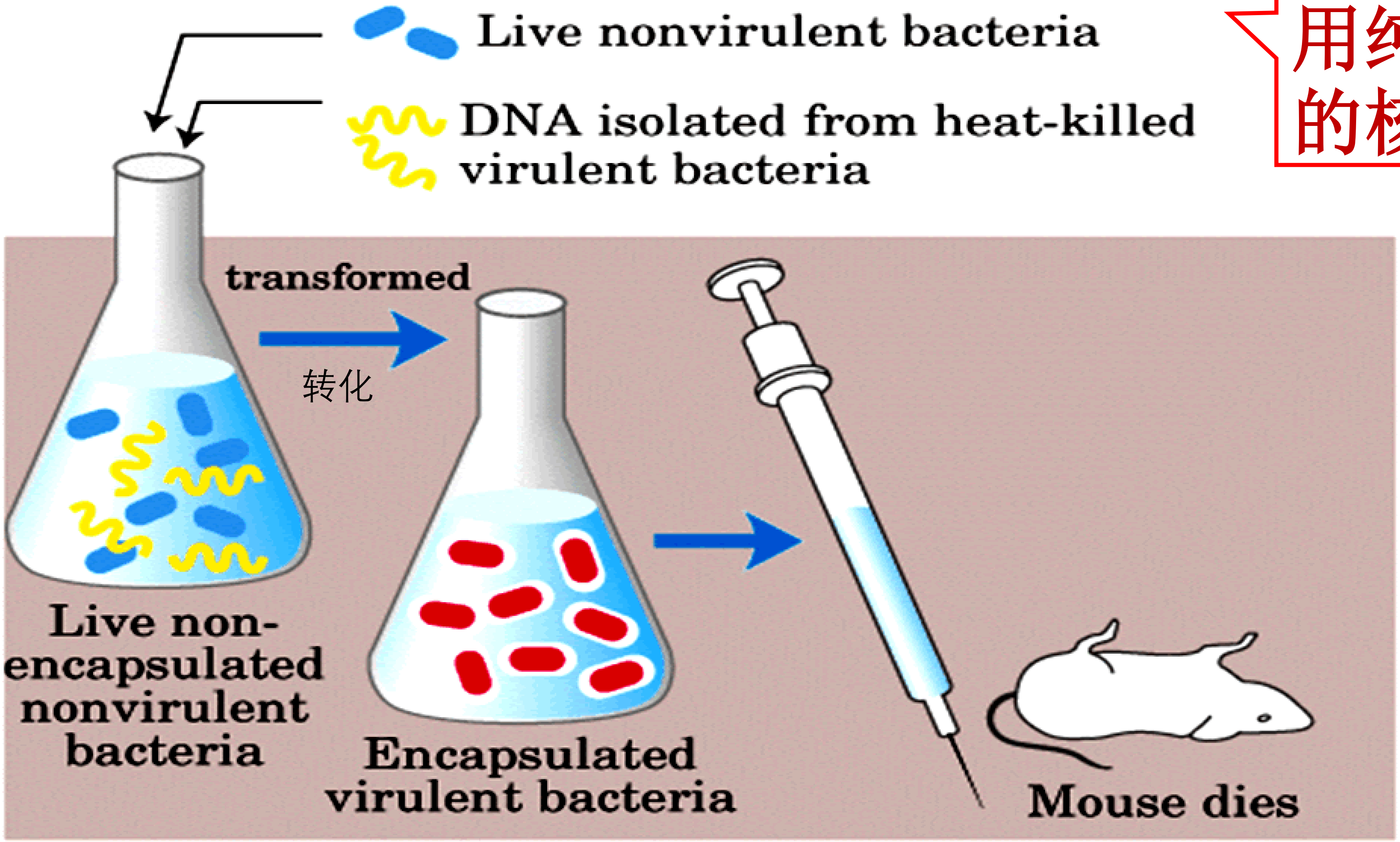
肺炎链球菌转化实验



用蛋白/核酸/糖/脂代替热灭活全菌

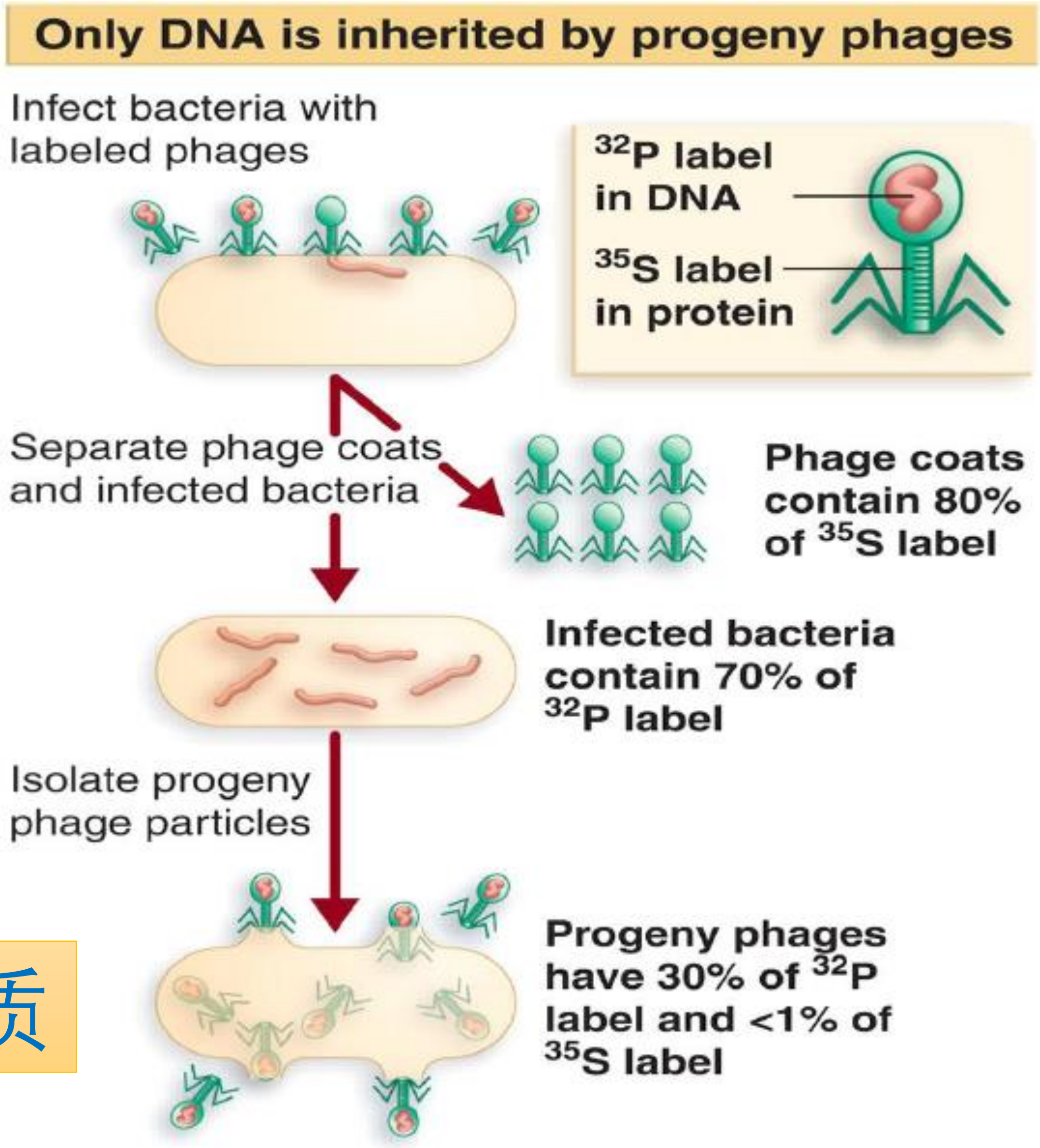
这个实验有什么问题？

1944, Avery-MacLeod-McCarty experiment



用纯化的核酸

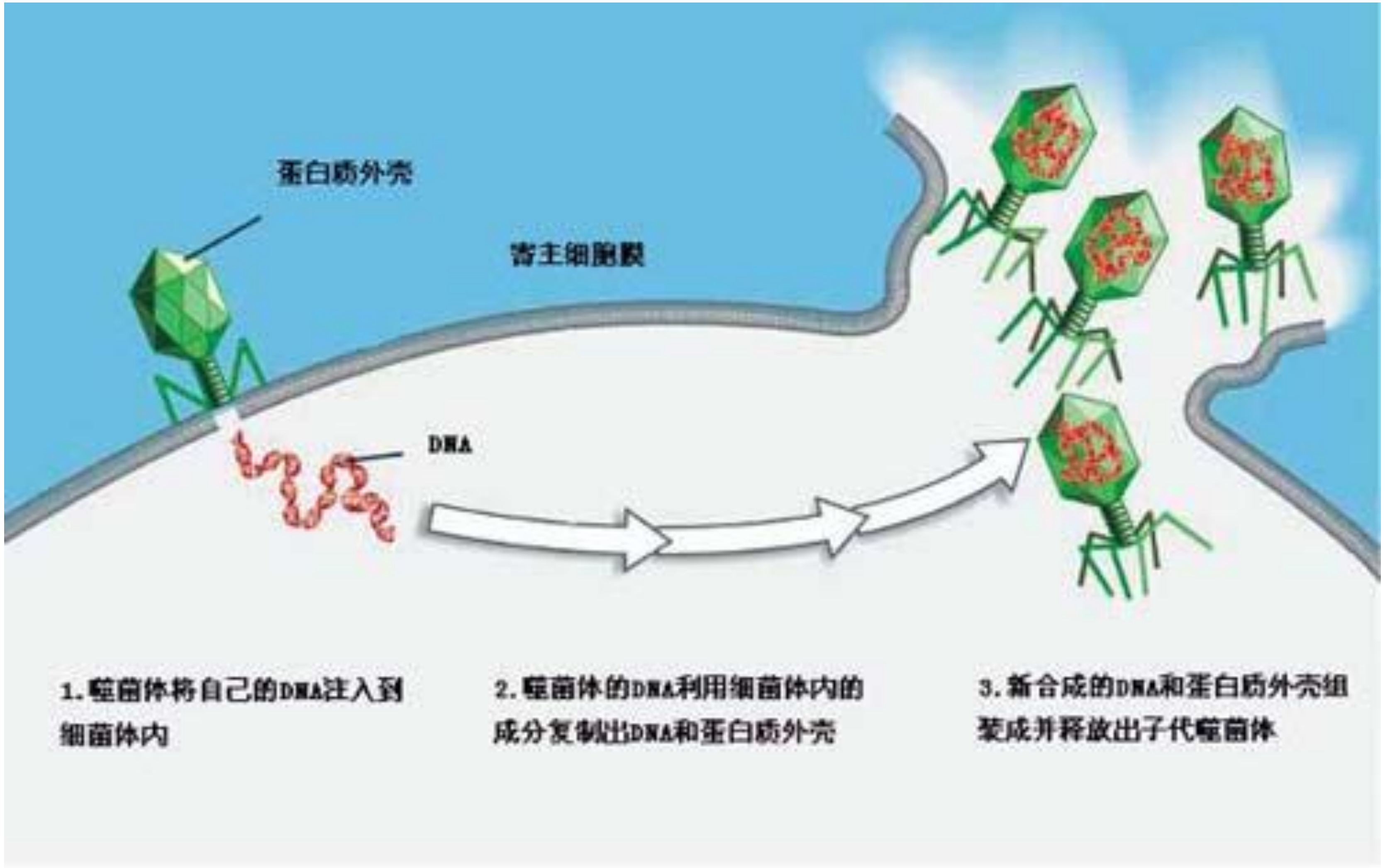
1952, Alfred D. Hershey & Martha Chase experiment



用放射性分别标记核酸和蛋白

核酸是遗传物质

核酸是遗传物质


蛋白质外壳
寄主细胞膜
DNA
1.噬菌体将自己的DNA注入到细菌体内
2.噬菌体的DNA利用细菌体内的成分复制出DNA和蛋白质外壳
3.新合成的DNA和蛋白质外壳组装成并释放出子代噬菌体

Chapter 7
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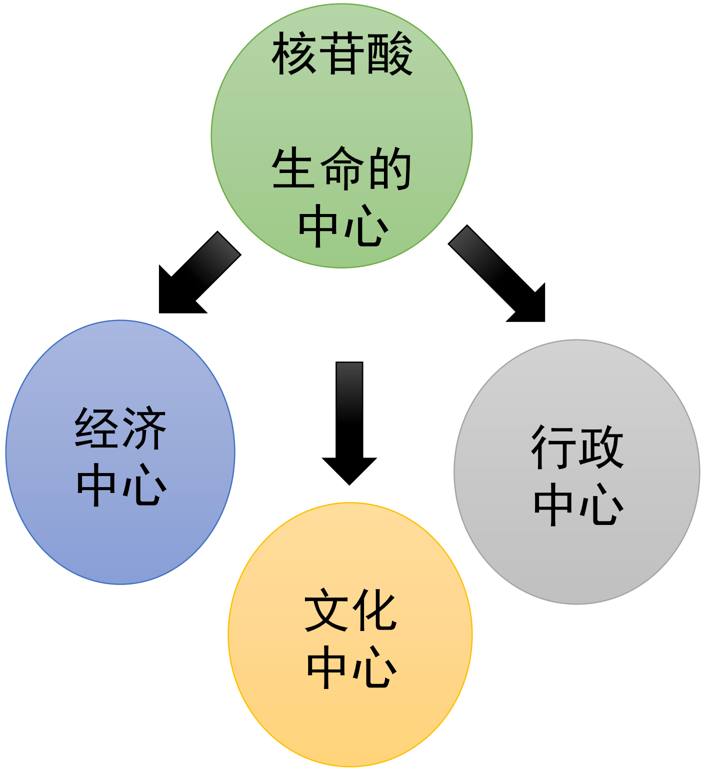
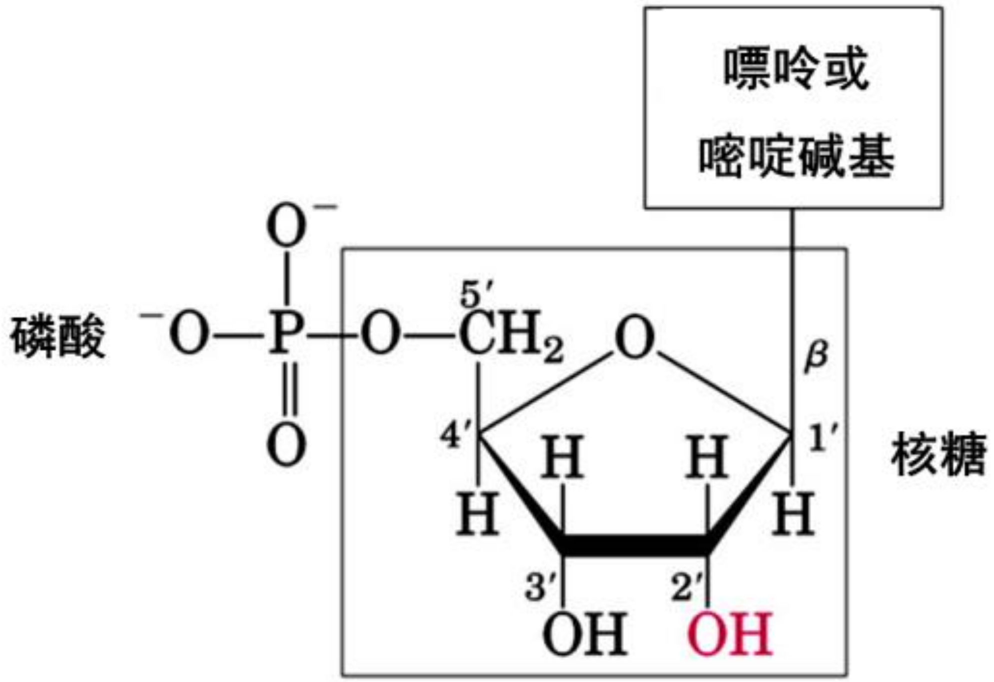
核苷酸的生物功能

- 作为核酸合成的原料
- 体内能量的利用形式
- 参与代谢和生理调节
- 组成辅酶
- 活化中间代谢物，如UDPG



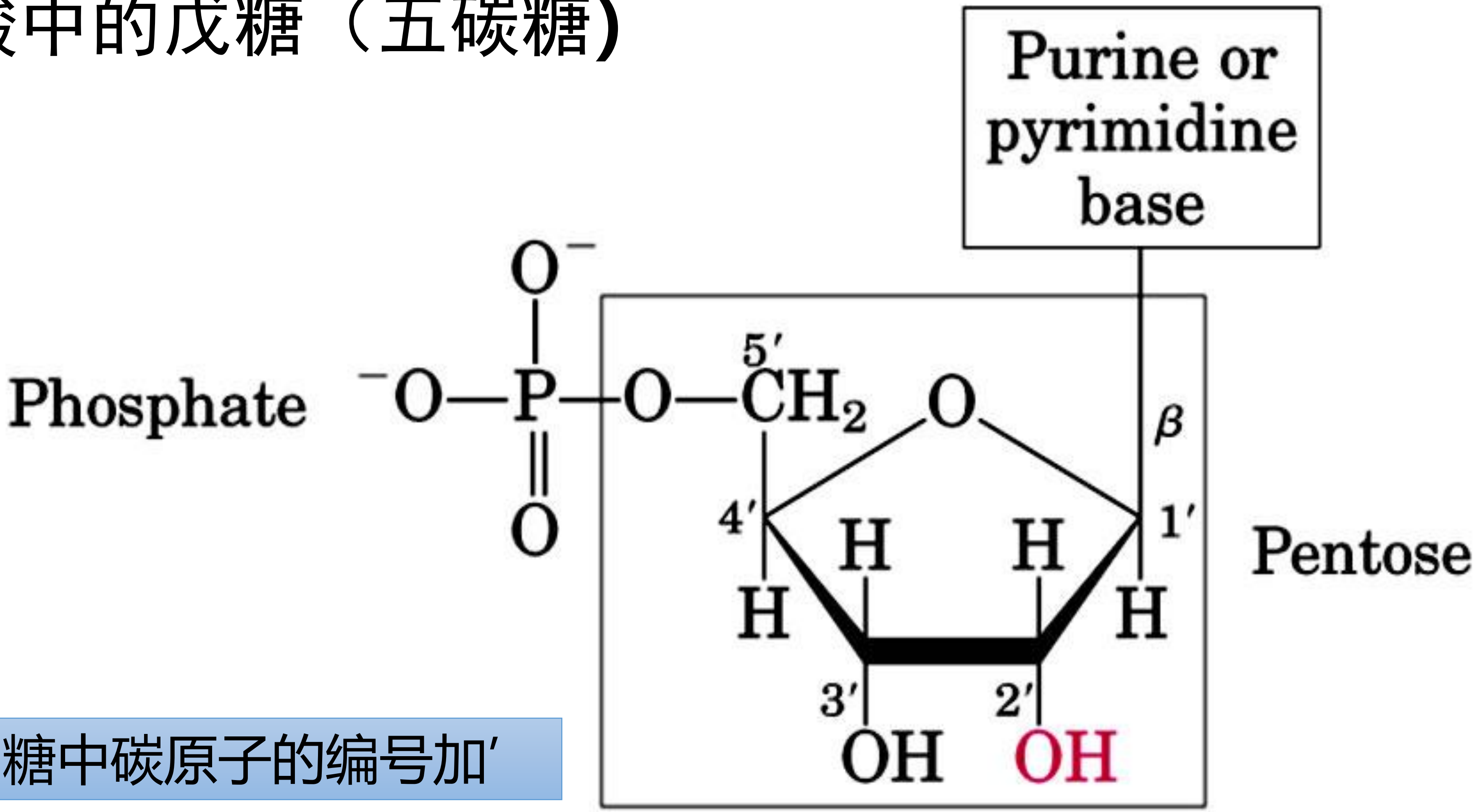
核苷酸的结构



核苷Nucleo*s*ide = 含氮碱基 + 戊糖

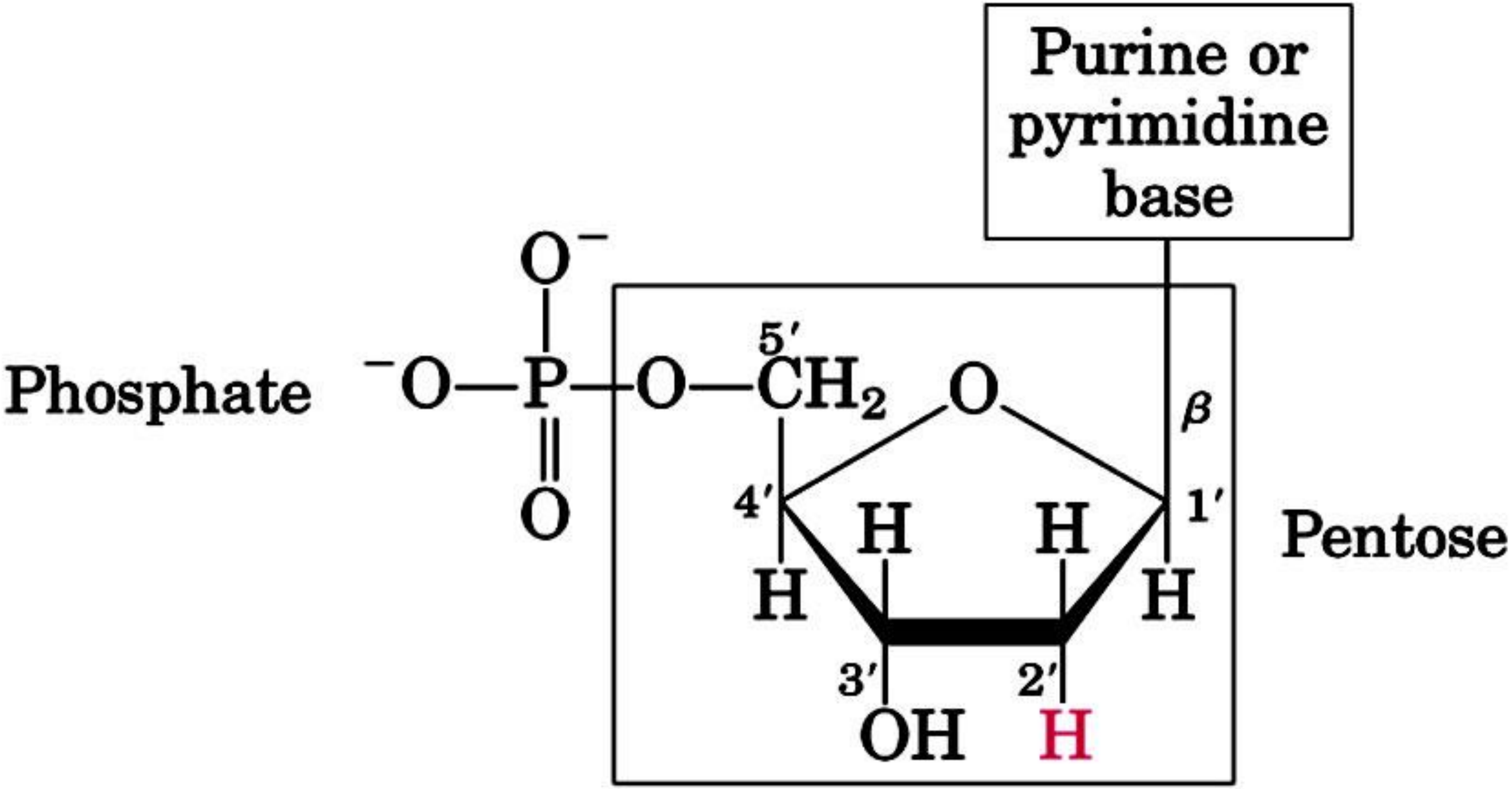
核苷酸Nucleo*t*ide = 核苷 + 磷酸

核苷酸中的戊糖（五碳糖)



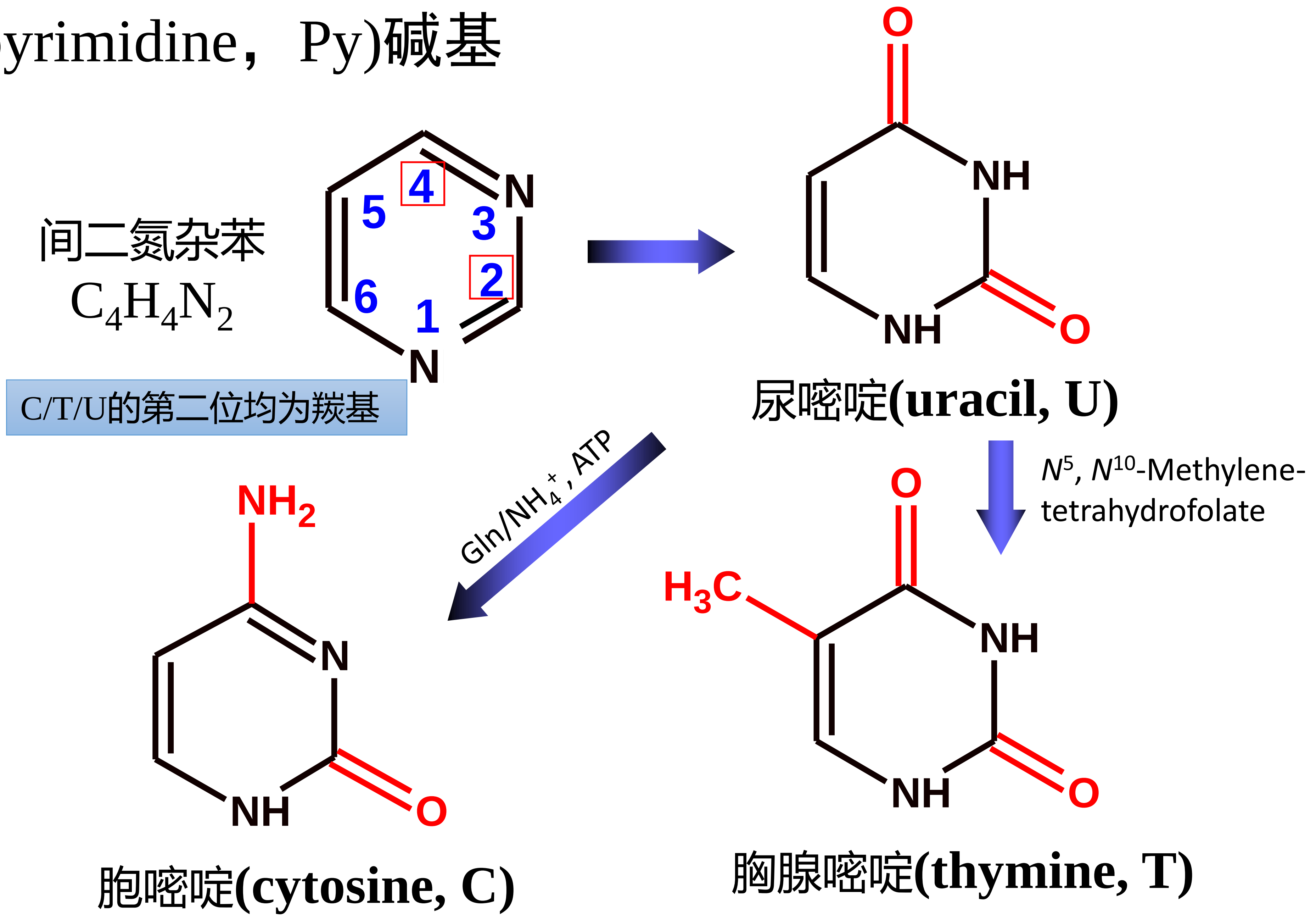
糖中碳原子的编号加′

β*–D–*Ribonucleotide

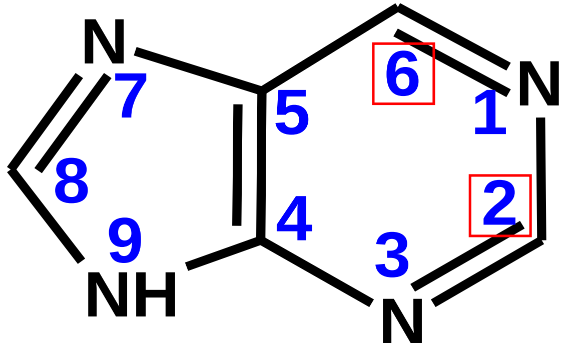


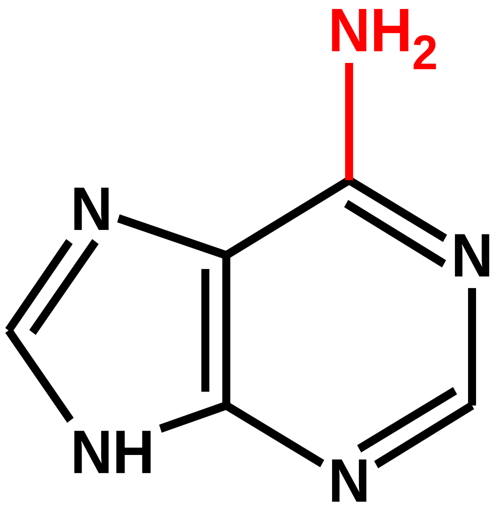
***β*–D–deoxyribonucleotide**

嘧啶(pyrimidine，Py)碱基



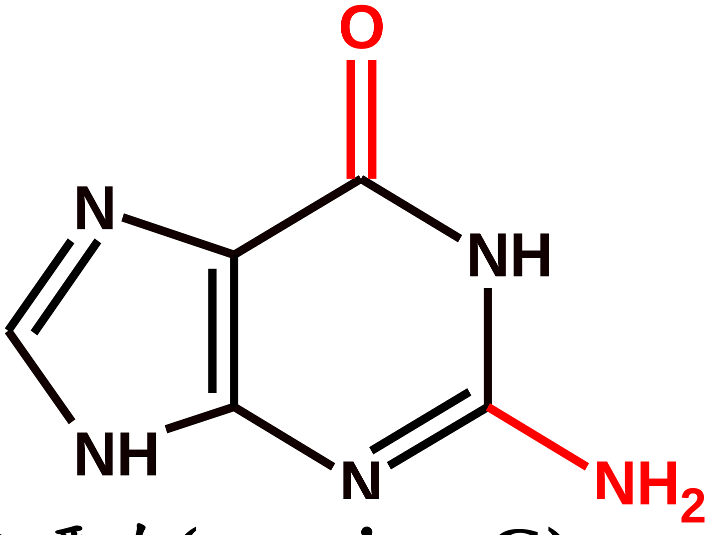
嘌呤(purine，Pu)碱基





腺嘌呤(adenine, A)

6-氨基嘌呤

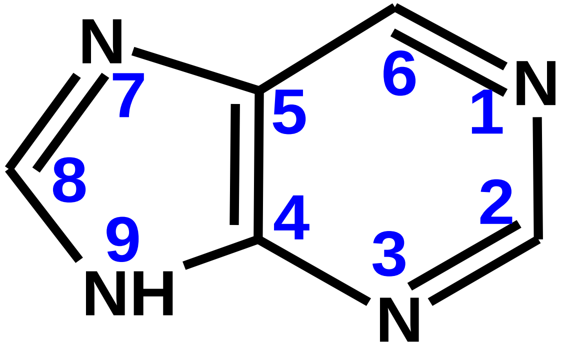


鸟嘌呤(guanine, G)

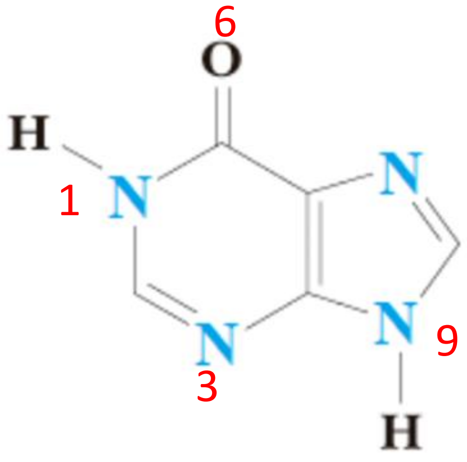
2-氨基-次黄嘌呤

- 大环优先，
- 杂原子编号最低，
- 公用碳原子编号最低，
- O、S、NH、N优先递减，
- 氢原子编号最低。

其他修饰的嘌呤碱基



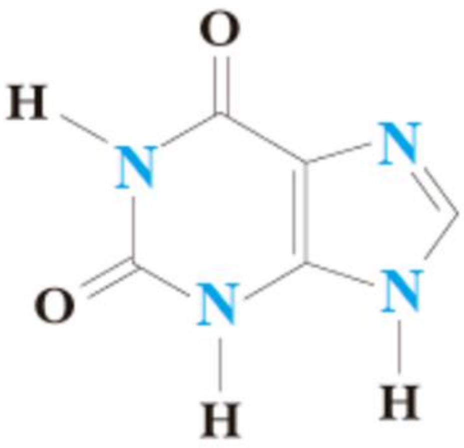
嘌呤环



Hypoxanthine

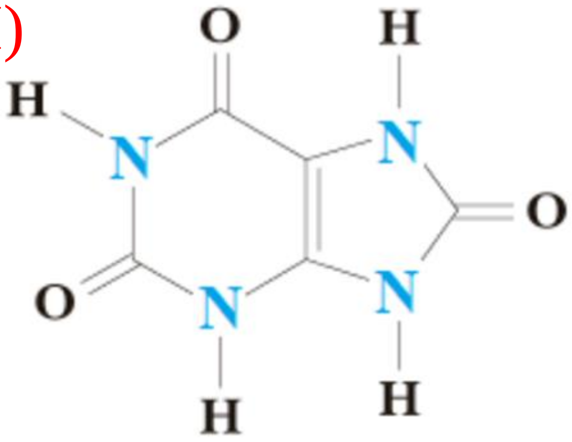
次黄嘌呤

(I)



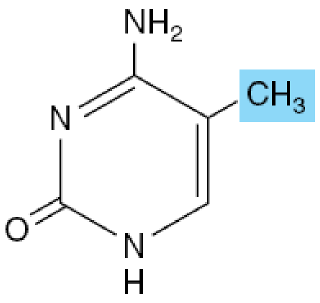
Xanthine

黄嘌呤



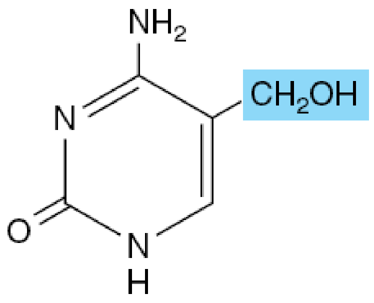
Uric acid 尿酸

uncommon naturally occurring pyrimidines and purines



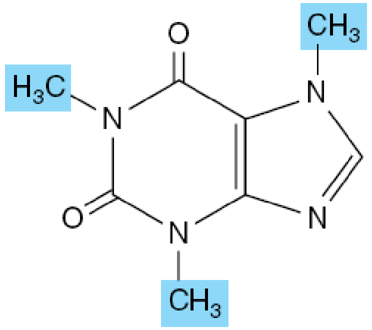
5-Methylcytosine

5-甲基胞嘧啶 (m5C)



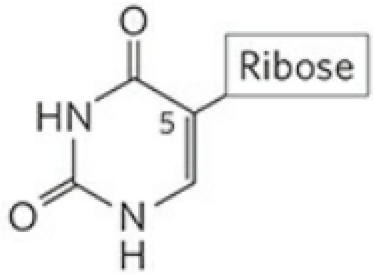
5-Hydroxymethylcytosine

5-羟甲基胞嘧啶



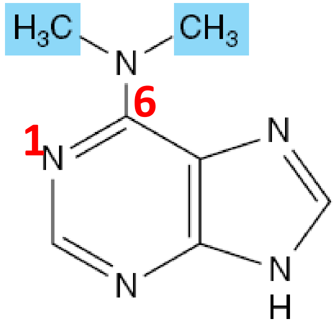
Caffeine

咖啡因



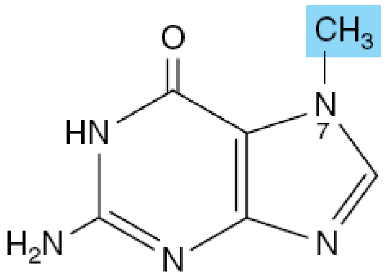
Pseudouridine

假尿嘧啶



Dimethylaminoadenine

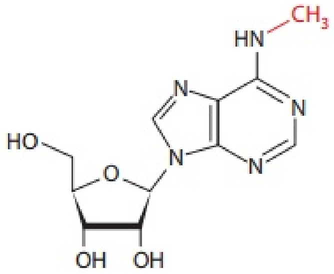
二甲氨基腺嘌呤



7-Methylguanine

7-甲基鸟嘌呤

（mRNA帽子）

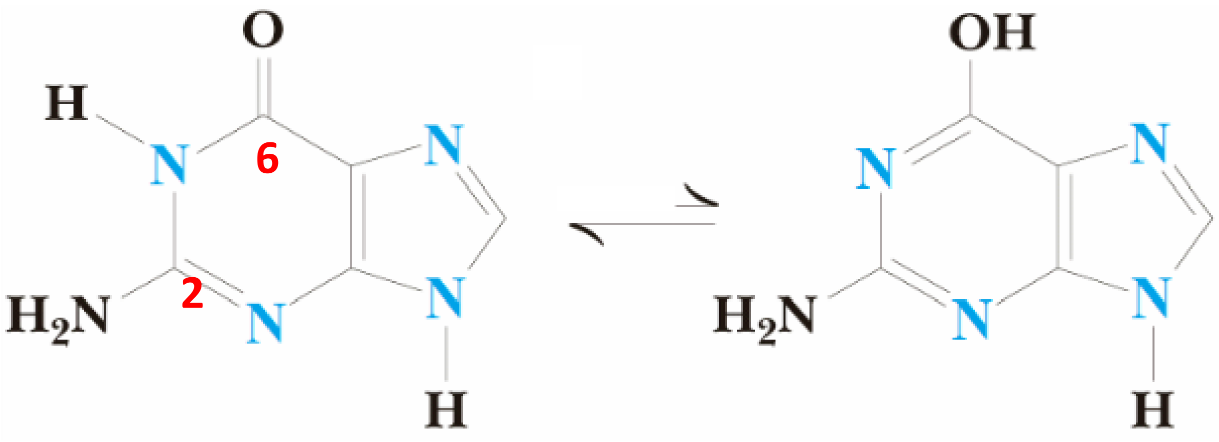


N6-甲基腺嘌呤

（N6-methyladenosine, m6A）

酮-烯醇互变异构（Keto-enol tautomerism）

乌嘌呤(guanine, G)



酮

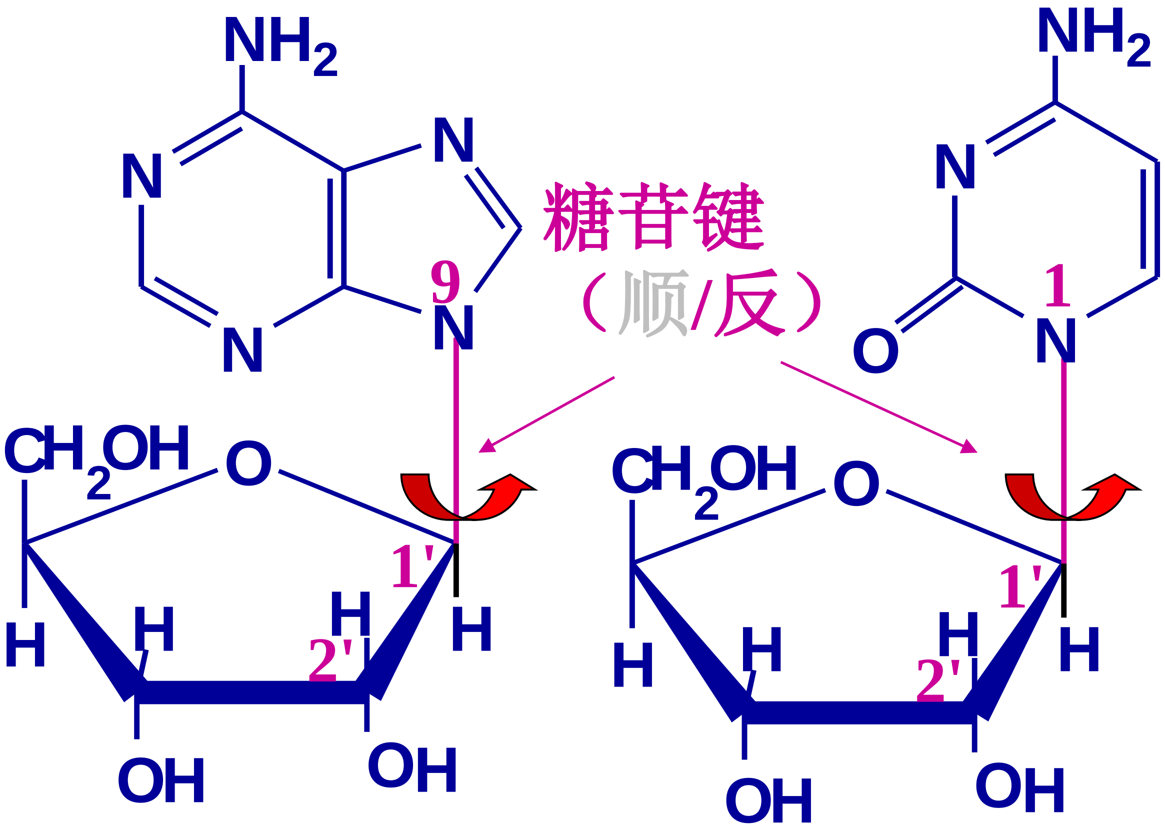
烯醇

2-氨基-次黄嘌呤
（99.99%）

2-氨基-6-羟基嘌呤
（0.01%）

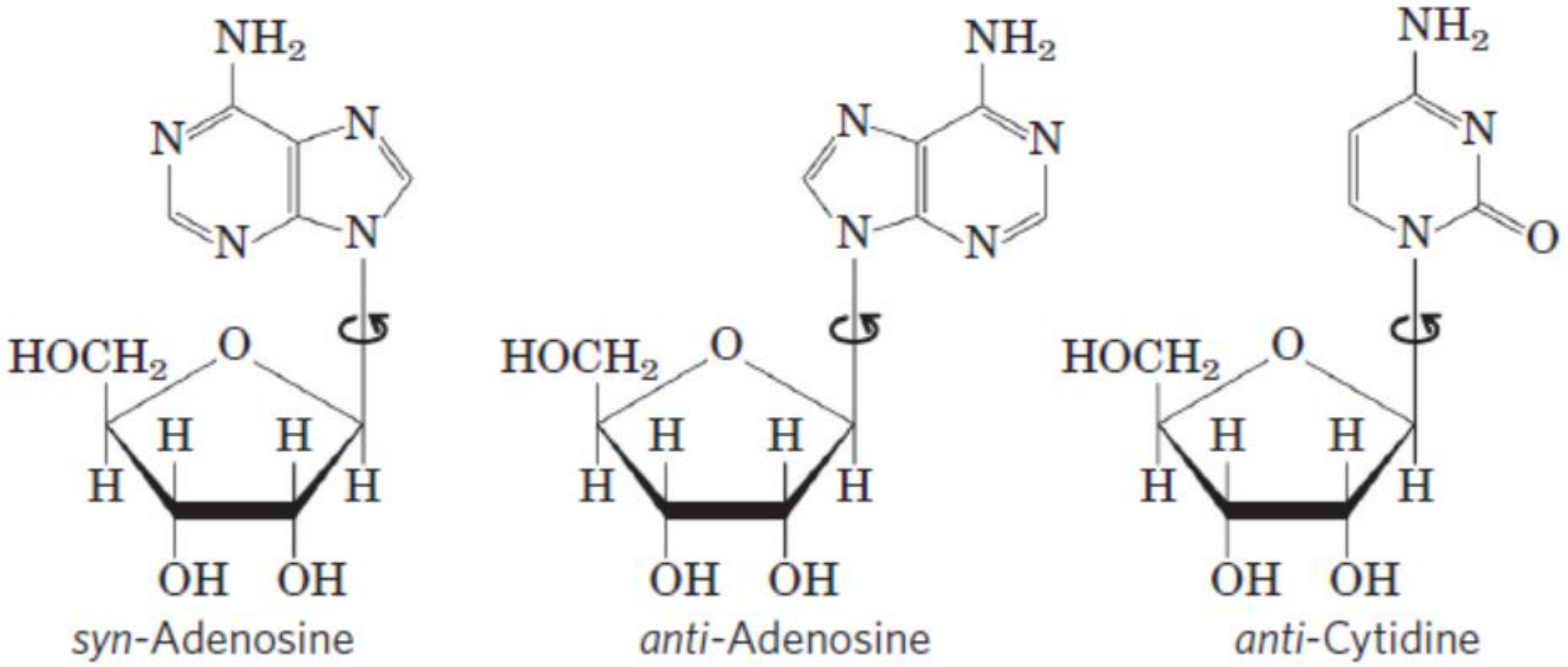
在碱性条件下，促进烯醇式。如果把DNA放在碱性条件下，双螺旋会解离。
羰基只能作为氢的受体，烯醇化后作为H的供体

核糖核苷 （ribonucleoside）



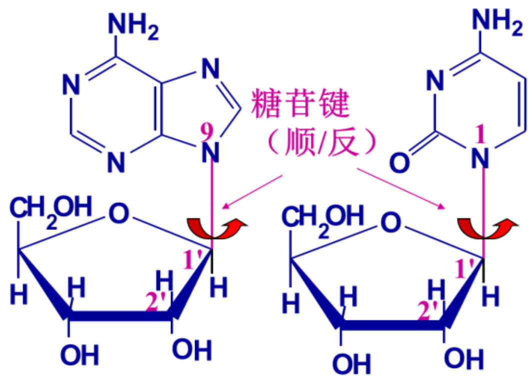
嘌呤N-9或嘧啶N-1与核糖C-1′通过β-N-糖苷键相连形成核糖核苷
(ribonucleoside)。

The *syn*- and *anti*- conformation

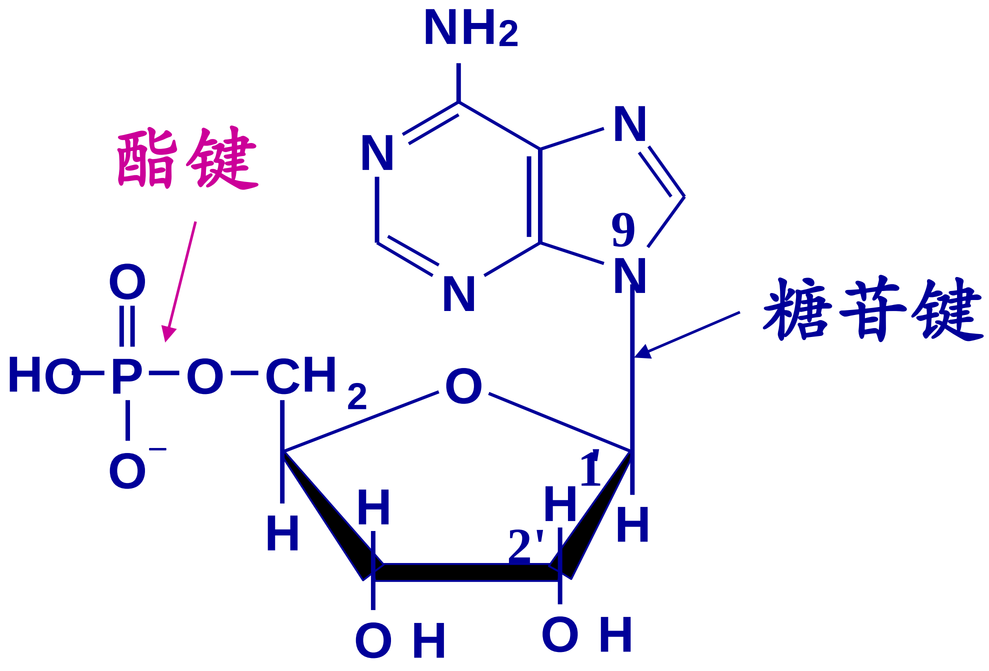


Linkage of a base to a sugar

- N-glycosidic bond
- -idine or –osine, deoxy-
  - Cytosine → Cytidine（胞苷）Deoxycytidine
  - Uracil → Uridine（尿苷）
  - Thymine → Thymidine（胸苷）Deoxythymidine
  - Adenine → Adenosine（腺苷）Deoxyadenosine
  - Guanine → Guanosine（鸟苷）Deoxyguanosine
- *Syn*- or *Anti*- conformation
- Sugars make nucleosides more water-soluble than free nitrogenous bases

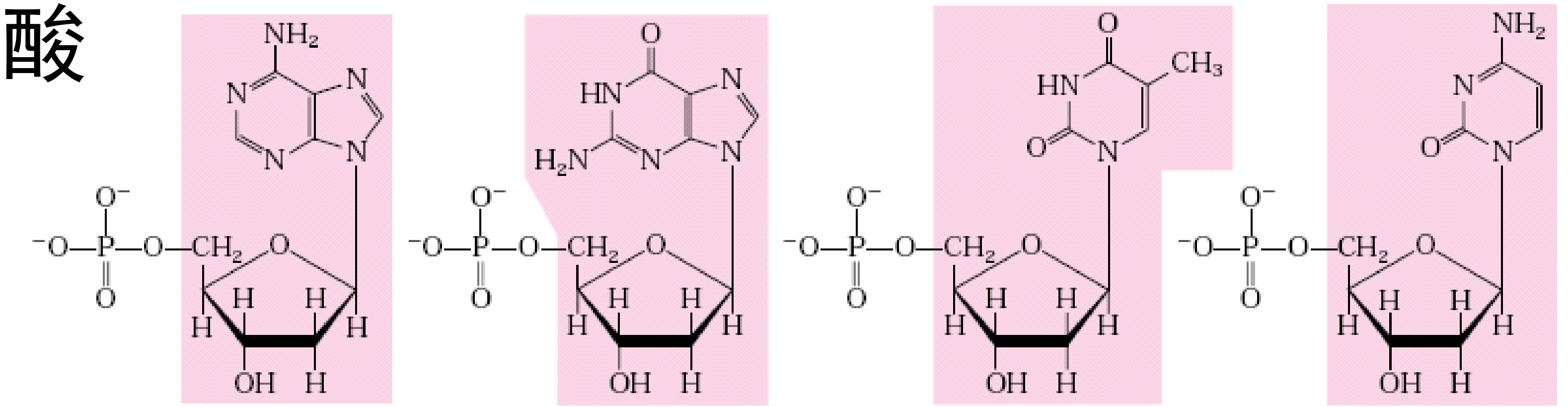


核苷酸(ribonucleotide)



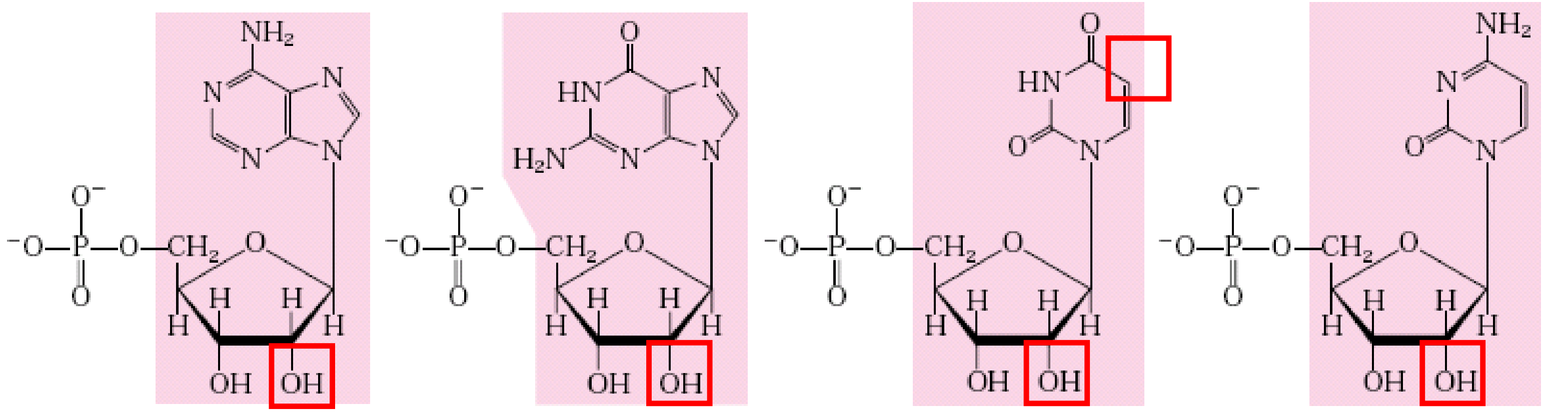
核苷与磷酸通过酯键结合构成核苷酸(ribonucleotide) 。

8种常见的核苷酸



Nucleotide:	Deoxyadenylate (deoxyadenosine 5′-monophosphate)	Deoxyguanylate (deoxyguanosine 5′-monophosphate)	Deoxythymidylate (deoxythymidine 5′-monophosphate)	Deoxycytidylate (deoxycytidine 5′-monophosphate)
Symbols:	A, dA, dAMP	G, dG, dGMP	T, dT, dTMP	C, dC, dCMP
Nucleoside:	Deoxyadenosine	Deoxyguanosine	Deoxythymidine	Deoxycytidine

(a) **Deoxyribonucleotides**



Nucleotide:	Adenylate (adenosine 5′-monophosphate)	Guanylate (guanosine 5′-monophosphate)	Uridylate (uridine 5′-monophosphate)	Cytidylate (cytidine 5′-monophosphate)
Symbols:	A, AMP	G, GMP	U, UMP	C, CMP
Nucleoside:	Adenosine	Guanosine	Uridine	Cytidine

(b) **Ribonucleotides**

- 核苷酸
  - 核苷
    - 戊糖
      - β-D-2′-脱氧核糖
      - β-D-核糖
    - 碱基
      - 嘌呤：A、G
      - 嘧啶：C、U、T
  - 磷酸

单数N，5特殊，嘧啶1，嘌呤9，
嘧啶：2纯氧4氧或N，嘌呤互补。

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7.5 ***Other functions of Nucleotides***

Outline

- **Classification of nucleic acids**

- **Structure of nucleic acids**
  - **Primary**
  - **Secondary**
  - **Tertiary**

- **Function of nucleic acids**

DNA链的方向是5′ → 3′

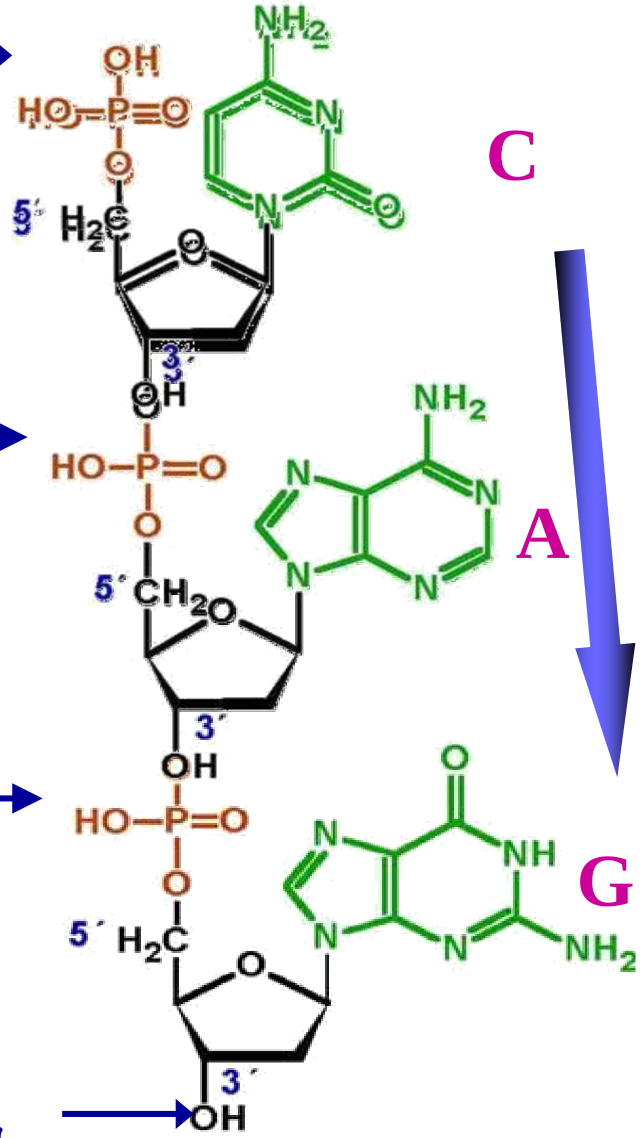
5´-末端 →

磷酸二酯键 →

Phospho-di-ester bond

磷酸二酯键 →

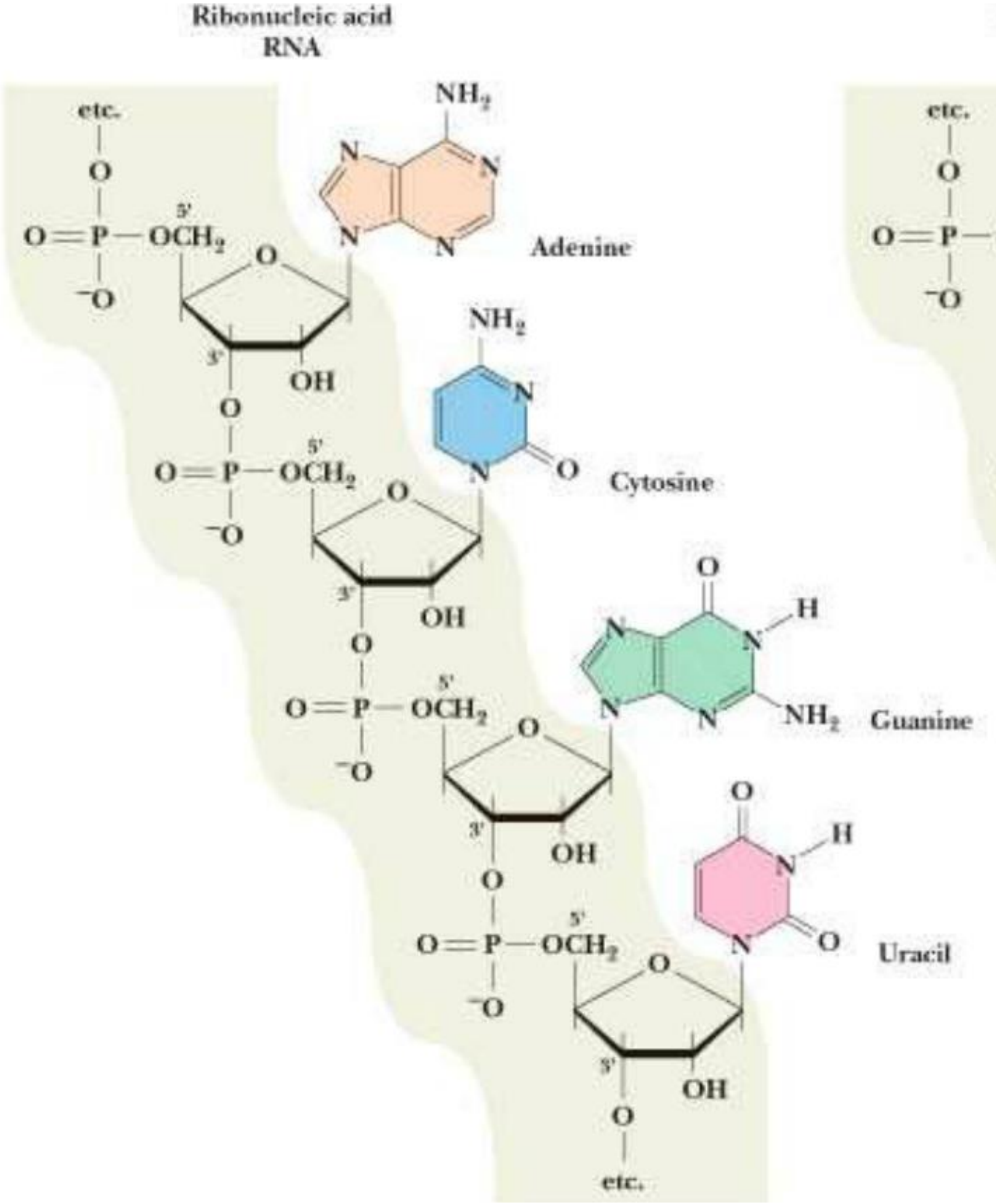
3´-末端 →



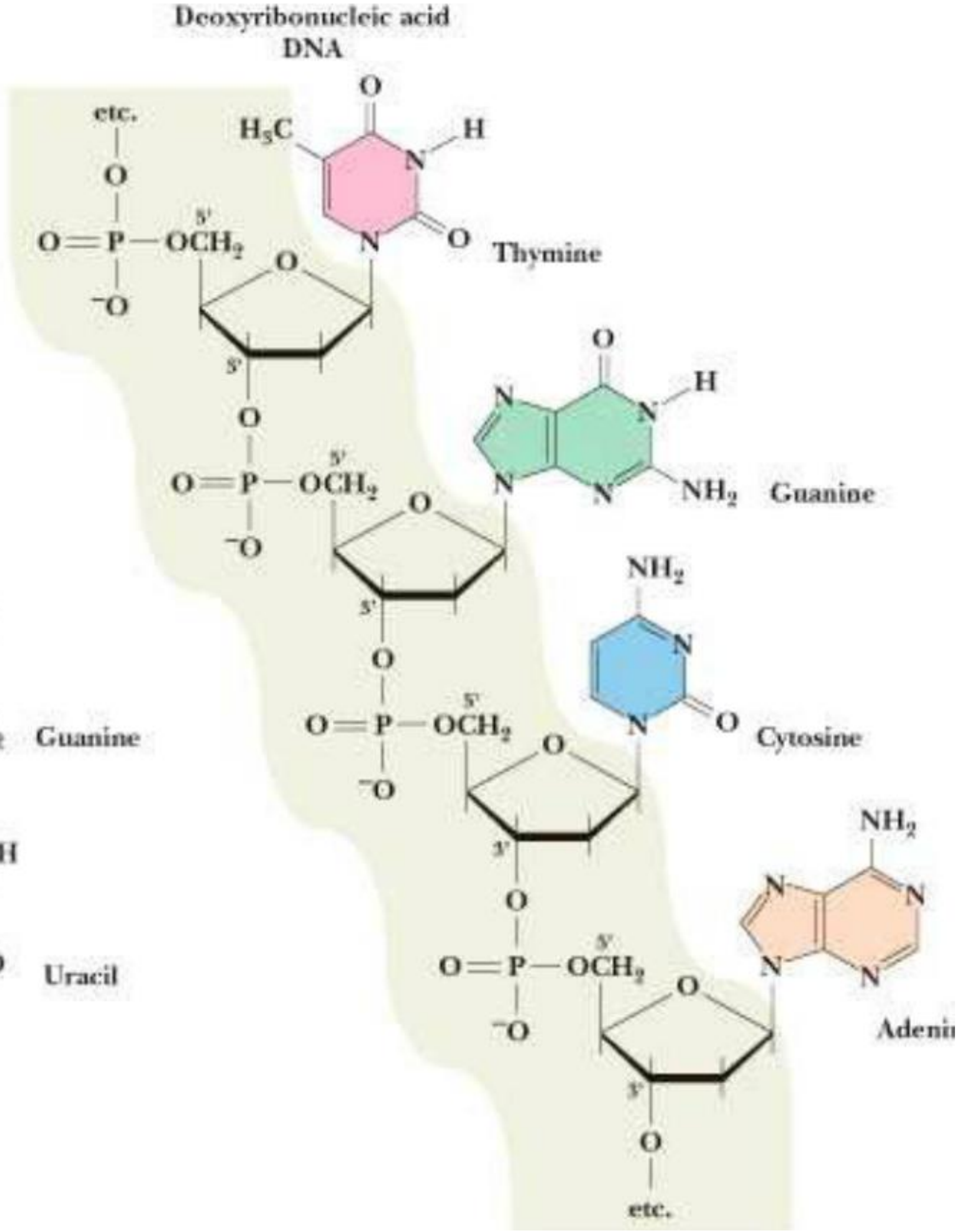
交替的磷酸基团和戊糖构成了核酸的骨架 (backbone)。

1. Phosphodiester bridges
2. Linear or circular
3. 5’ and 3’ end of linear molecule
4. Negative charges

RNA


Ribonucleic acid
RNA
etc.
Adenine
Cytosine
Guanine
Uracil
etc.

DNA


Deoxyribonucleic acid
DNA
etc.
Thymine
Guanine
Cytosine
Adeni
etc.

Three Key Questions

- Main differences between RNA and DNA

- **Why DNA is usually double strand, but RNA is usually single strand?**

- **Why DNA uses 2'-deoxyribose, but RNA not?**

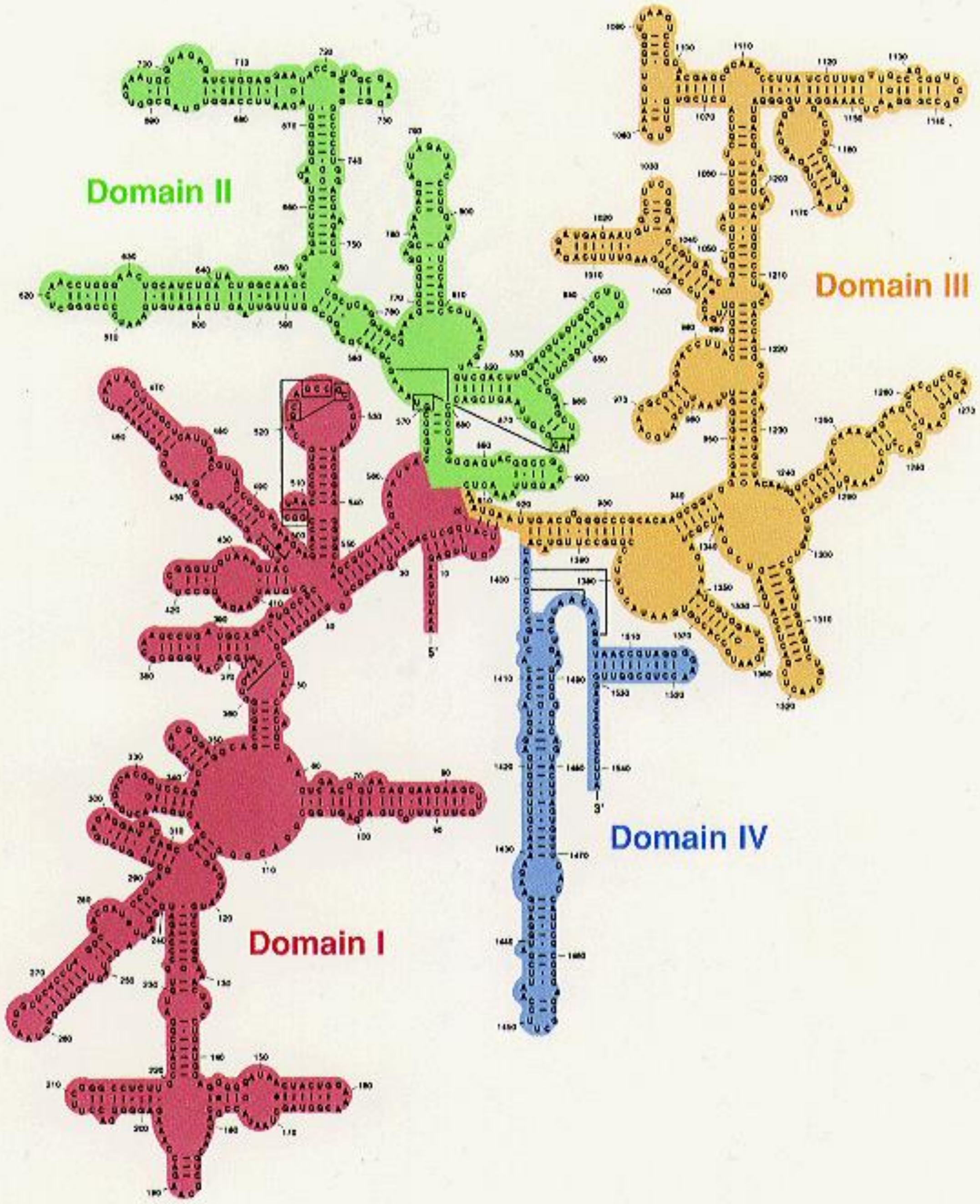
- **Why DNA uses T, but RNA uses U?**

Types and Functions

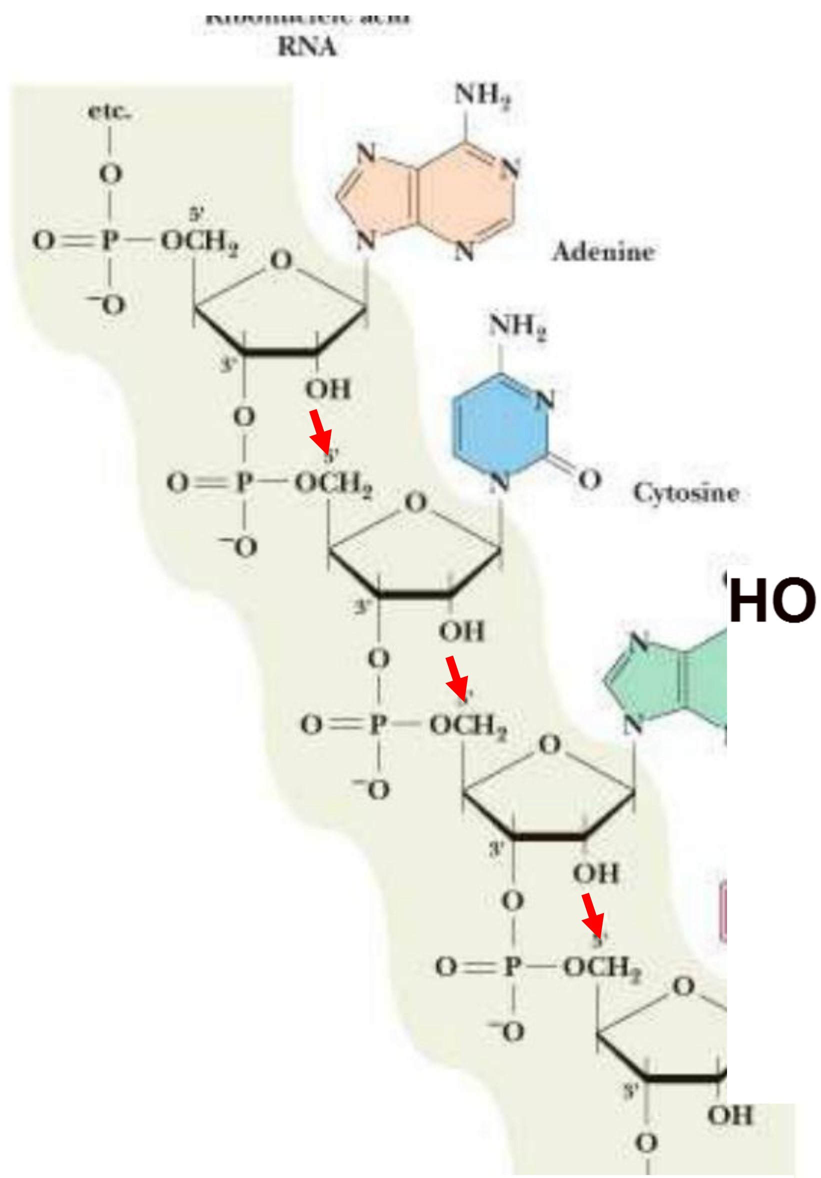
DNA → One world of DNA. One dream of carrying genetic information.

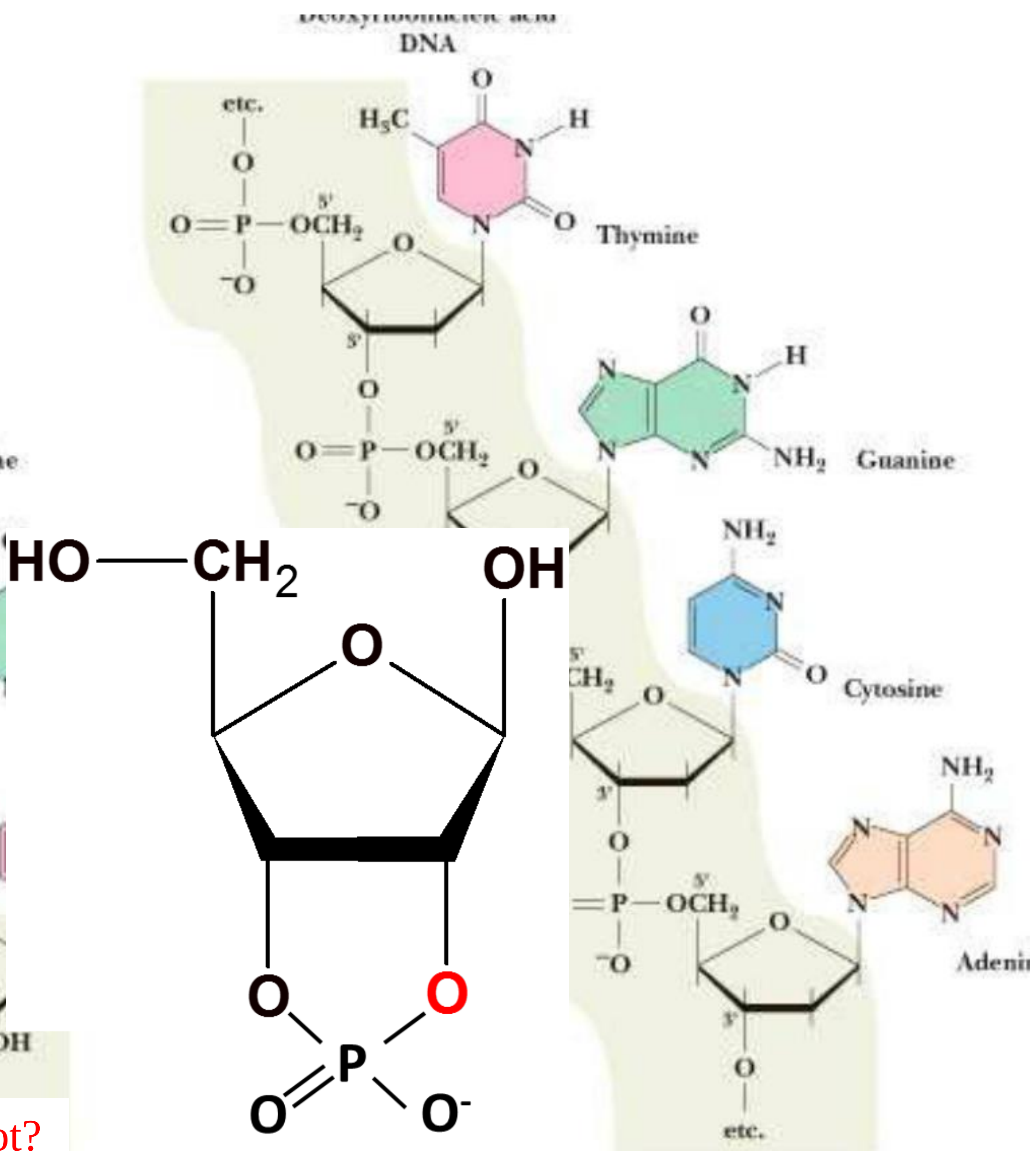
RNA
- Coding-RNA → Messenger RNA (mRNA)
- Noncoding-RNA
  - Ribosomal RNA (rRNA)
  - Transfer RNA (tRNA)
  - Small nuclear RNA (snRNA): mRNA splicing
  - Small nucleolar RNA (snoRNA): rRNA processing
  - Small interfere RNA (siRNA)
  - Guide RNA (gRNA)
  - microRNA、lncRNA
  - Virus RNA……

1. Why DNA is usually double strand, but RNA is usually single strand?


Domain I
Domain II
Domain III
Domain IV
5'
3'

Oxygen atom is capable of nucleophilic（亲核的）reaction





2. Why DNA uses 2’-deoxyribose, but RNA not?

RNA is unstable under at high pH

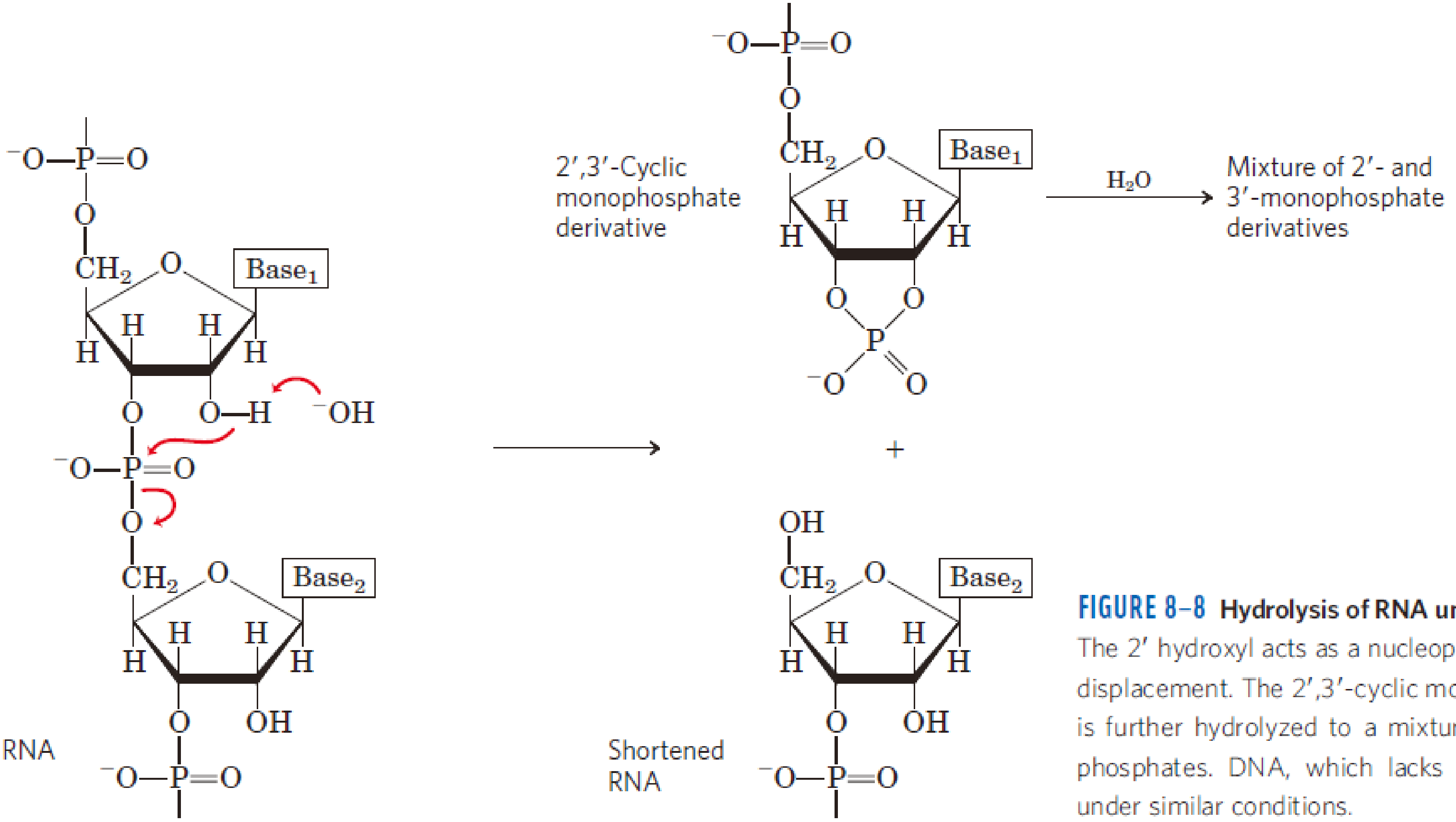
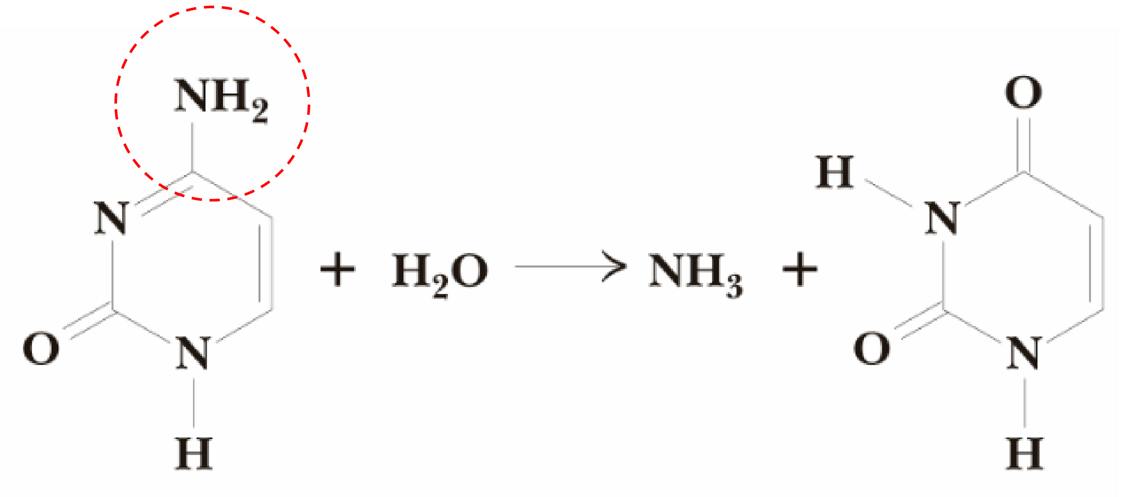


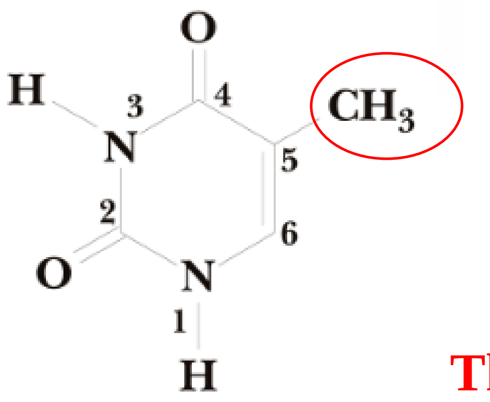
FIGURE 8–8 Hydrolysis of RNA under alkaline conditions. The 2′ hydroxyl acts as a nucleophile in an intramolecular displacement. The 2′,3′-cyclic monophosphate derivative is further hydrolyzed to a mixture of 2′- and 3′-monophosphates. DNA, which lacks 2′ hydroxyls, is stable under similar conditions.

Spontaneous deamination of cytosine can form uracil



Cytosine

Uracil



Thymine

~600 reactions per day in human genome

Uracil-DNA glycosidase (UDG)

尿嘧啶-DNA糖苷酶

5’-AGGTCG-3’
3’-TCCAGC-5’
→
5’-AGGTUG-3’
3’-TCCAGC-5’
→ UDG
5’-AGGTCG-3’
3’-TCCAGC-5’

5’-AGGUCG-3’
3’-UCCAGC-5’
→
5’-AGGUUG-3’
3’-UCCAGC-5’

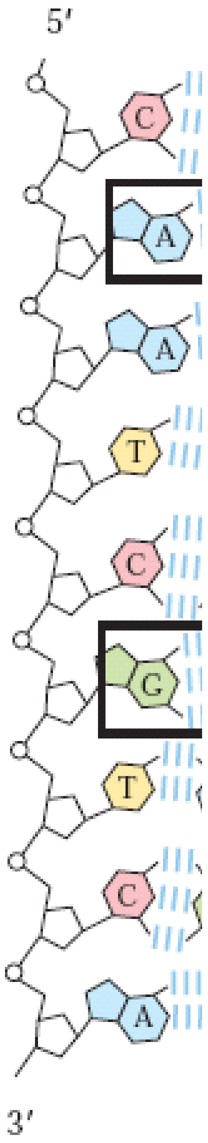
5’-AGGUUG-3’
3’-UCCGGC-5’
→
Which U is True?

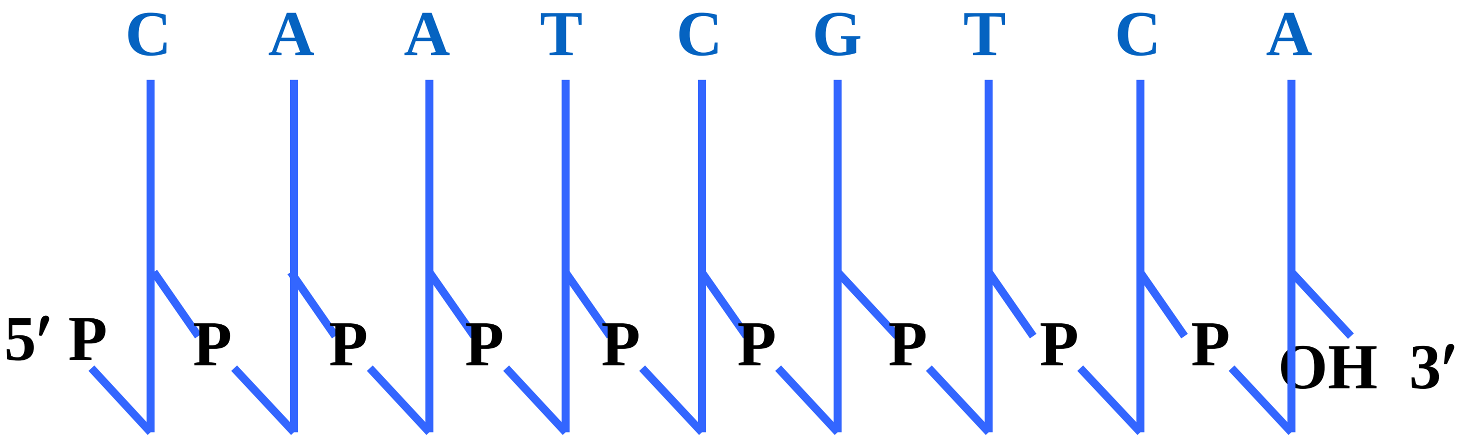
3. Why DNA uses T, but RNA uses U?

Outline

- Classification of nucleic acids
- **Structure of nucleic acids**
  - **Primary**
  - **Secondary**
  - **Tertiary**
- Function of nucleic acids

■ Primary structure：





↓

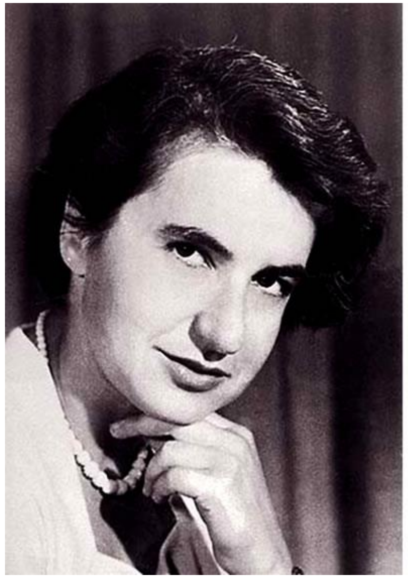
5′ pCpApApTpCpGpTpCpA-OH 3′

↓

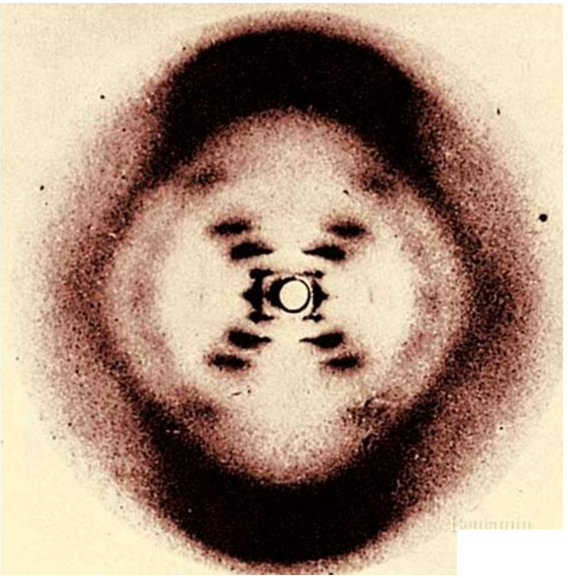
C A A T C G T C A

✦Secondary structure：

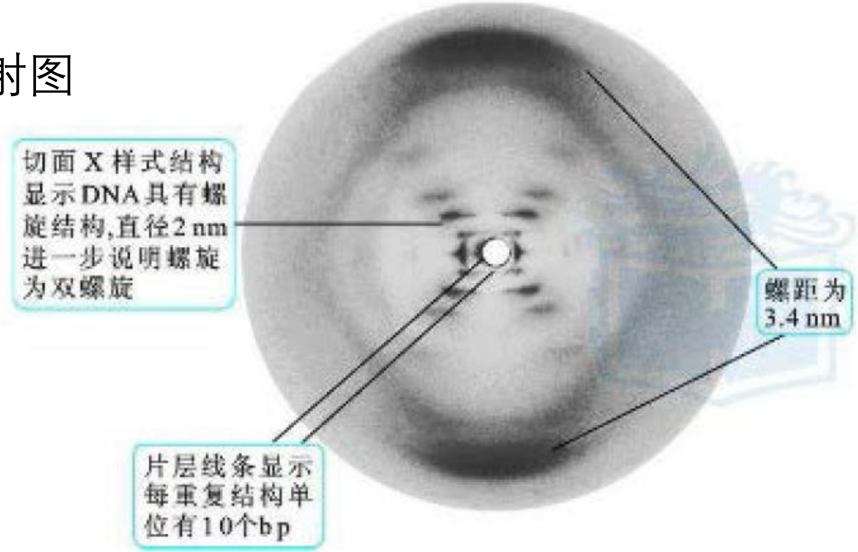
✦*Franklin's X-ray picture for DNA*



Rosalind Elsie Franklin

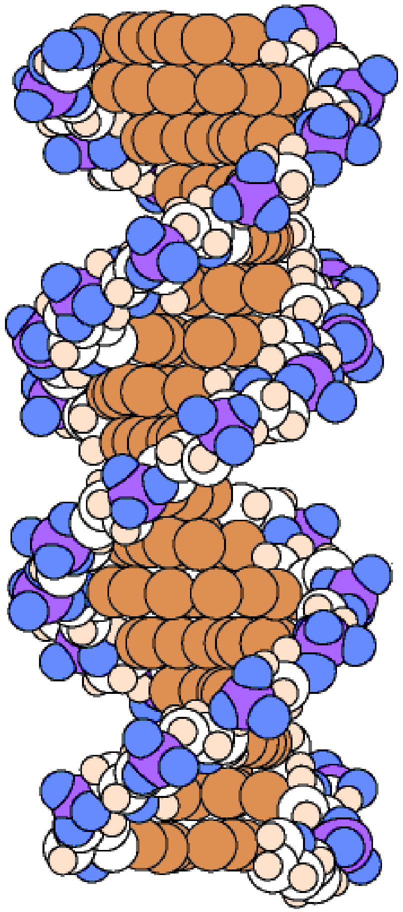


B型DNA X射线衍射图



（1）DNA结构是双螺旋形；
（2）DNA分子半径；
（3）碱基对的间距；
（4）每个完整的螺旋高度。

■ Secondary structure： The Double Helix



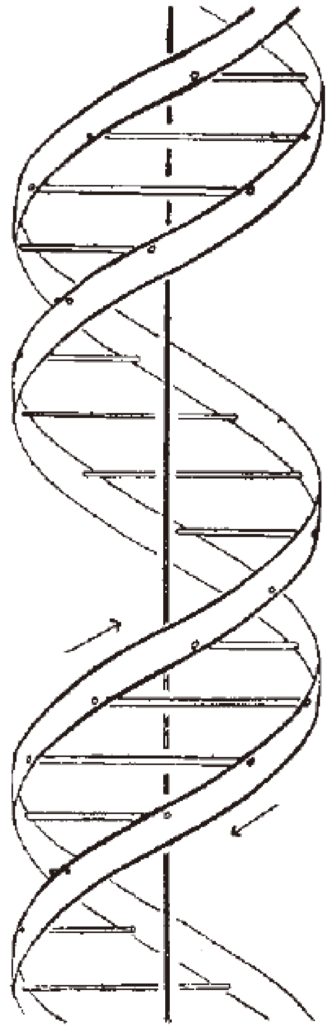
No. 4356 April 25, 1953 NATURE

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

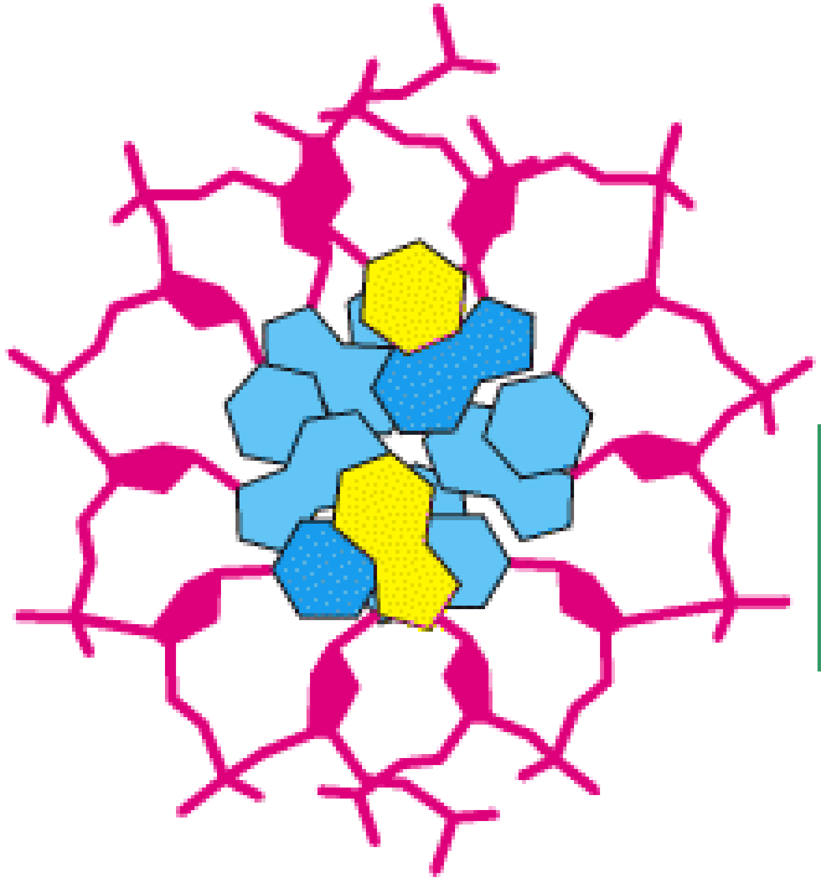
WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

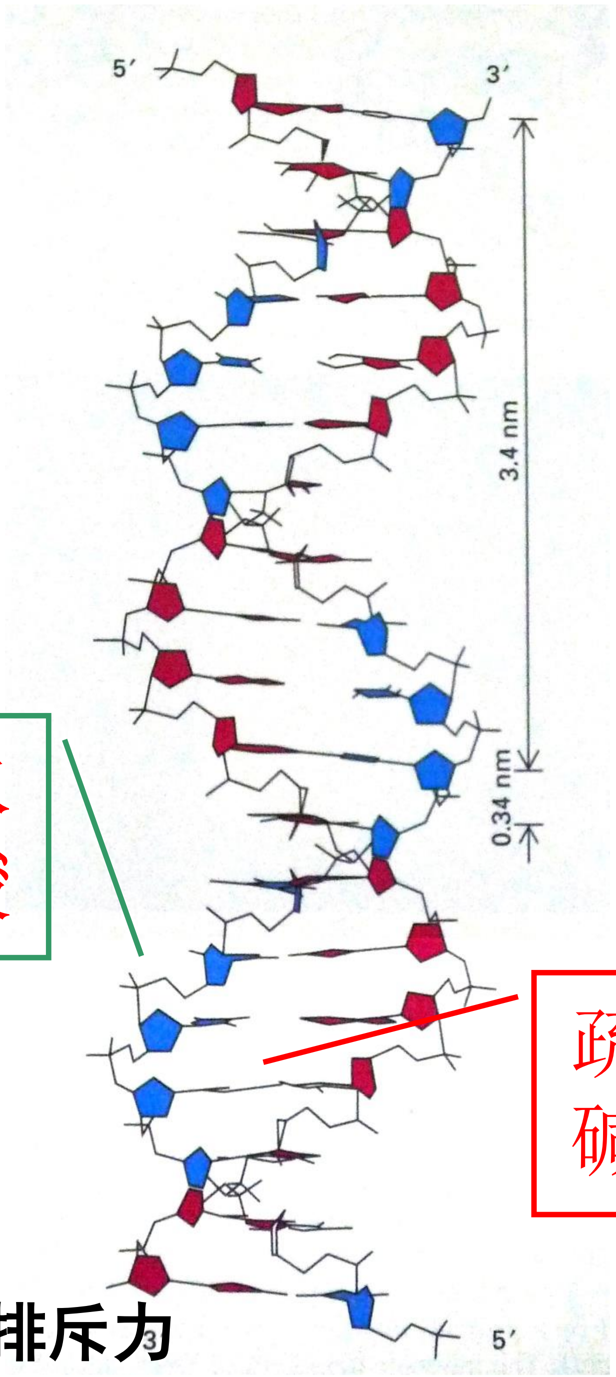


北京中关村的标志
雕塑“生命”

DNA Chain



亲水
磷酸

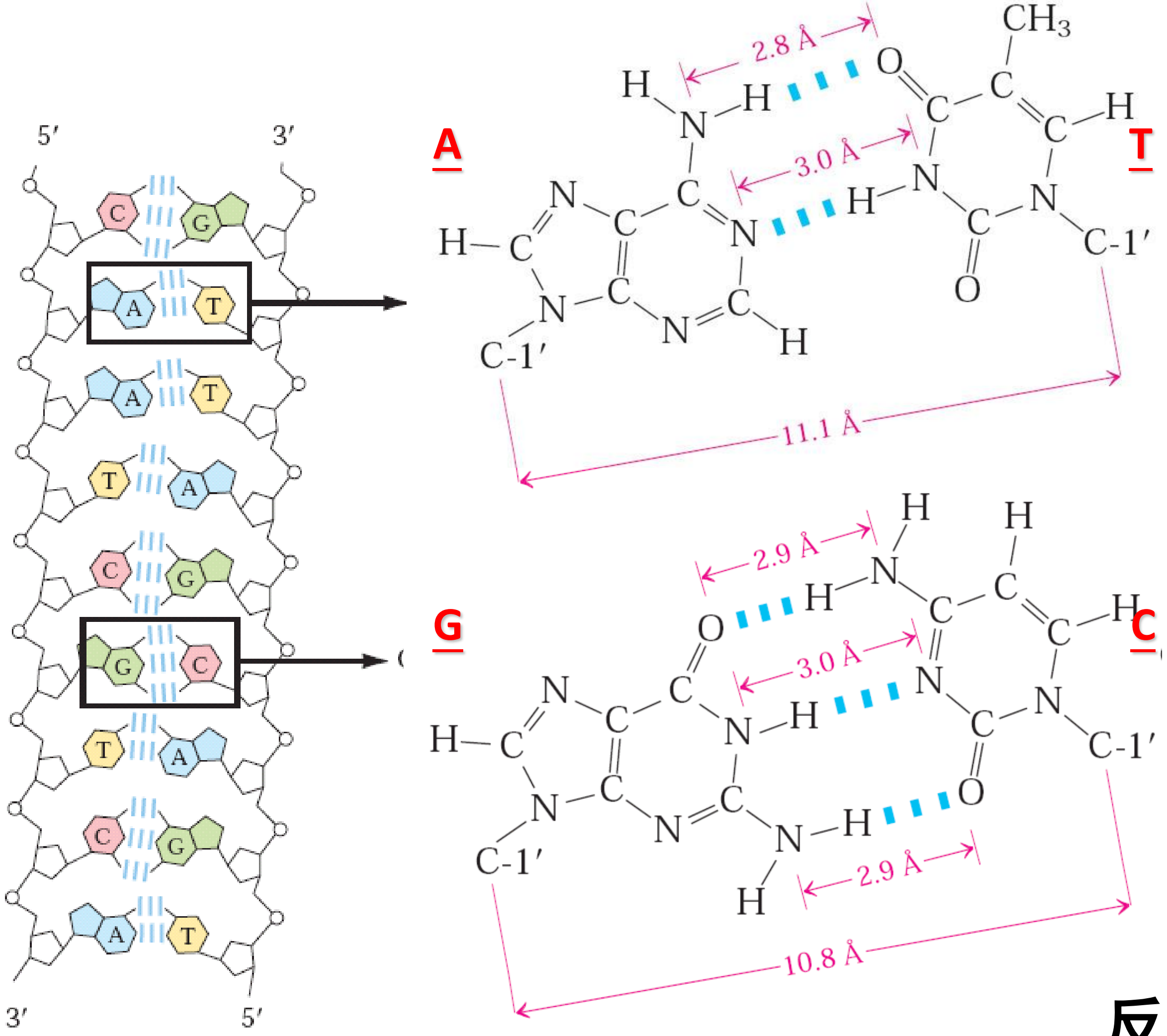


疏水
碱基

氢键、疏水效应和碱基堆积力

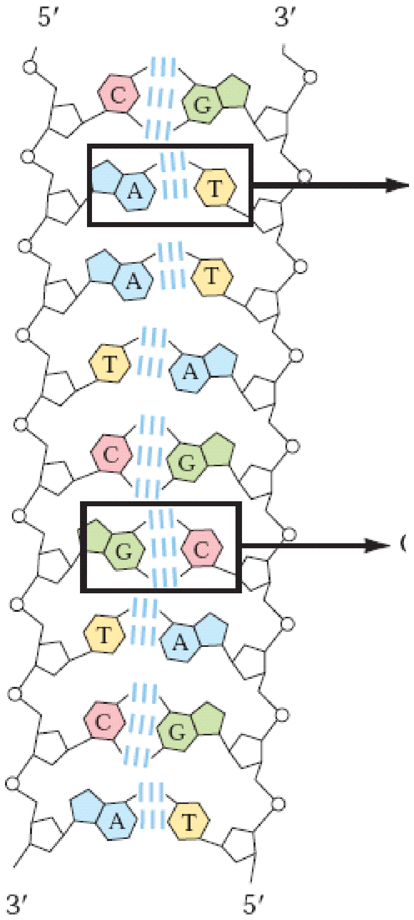
骨架中带负电荷的磷酸基团的静电排斥力

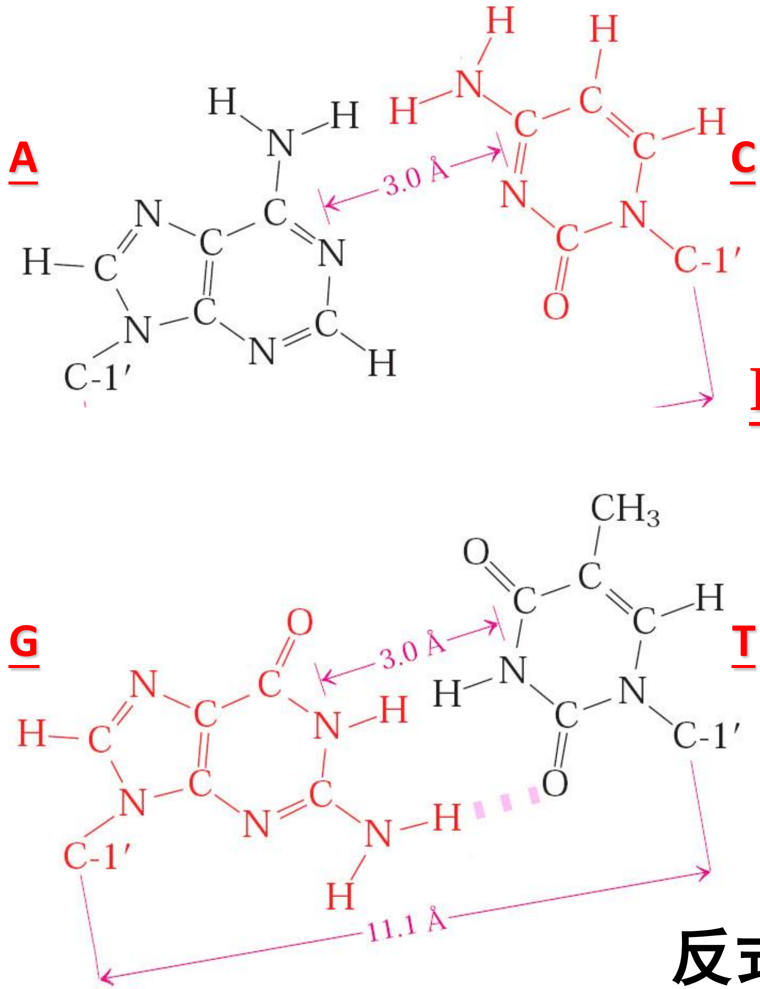
核酸的碱基配对



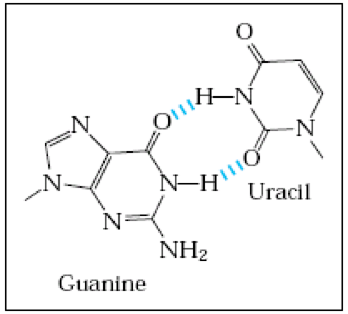
反式构象占优势

核酸的碱基配对



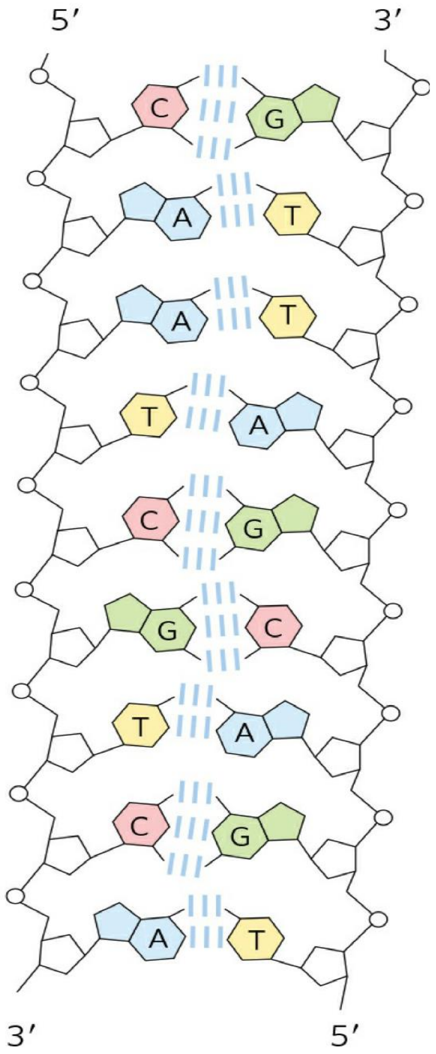


RNA中GU配对

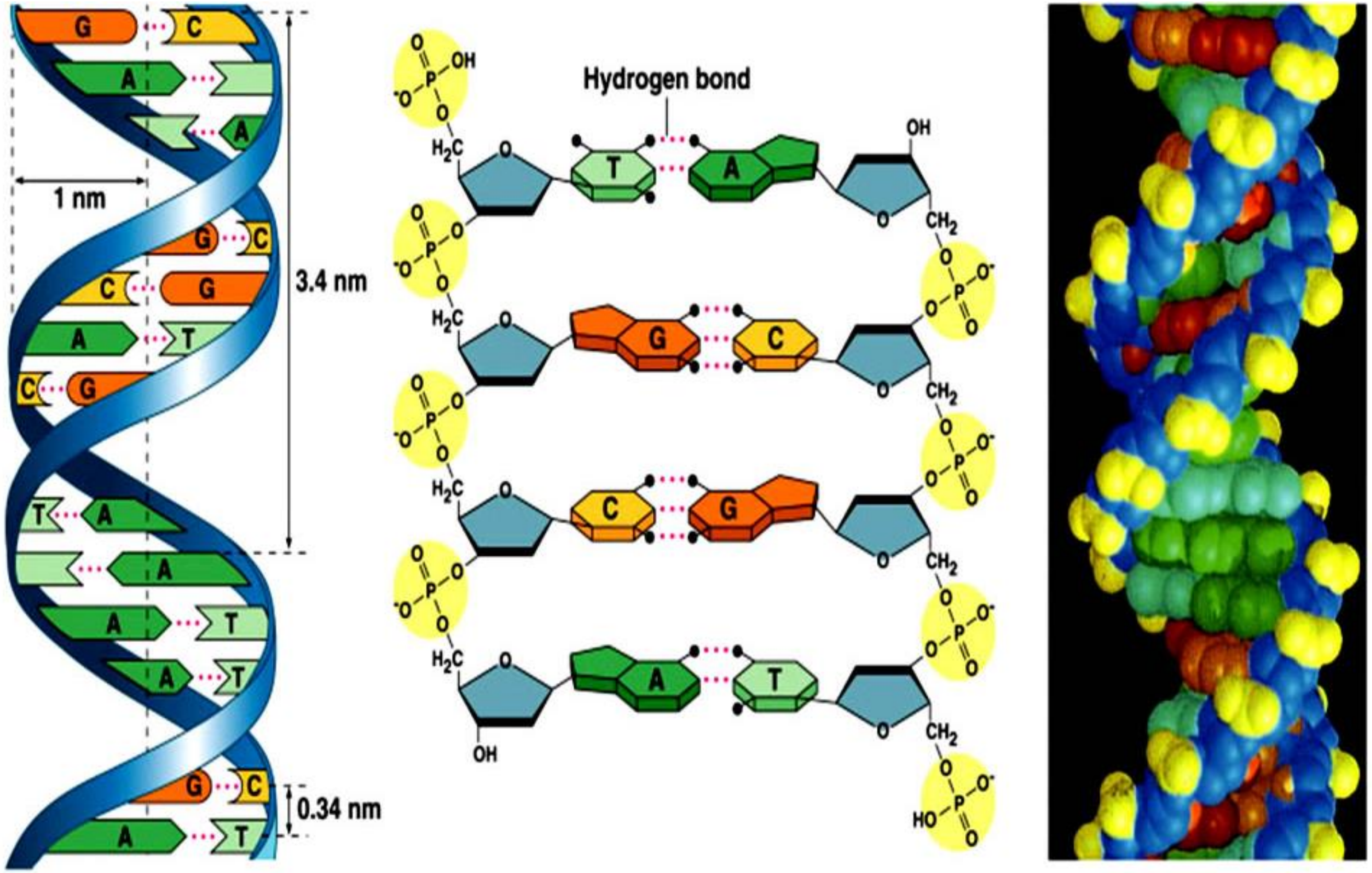


反式构象占优势

DNA Structure: 平行 or 双螺旋？



H键
ππ堆积力
疏水作用

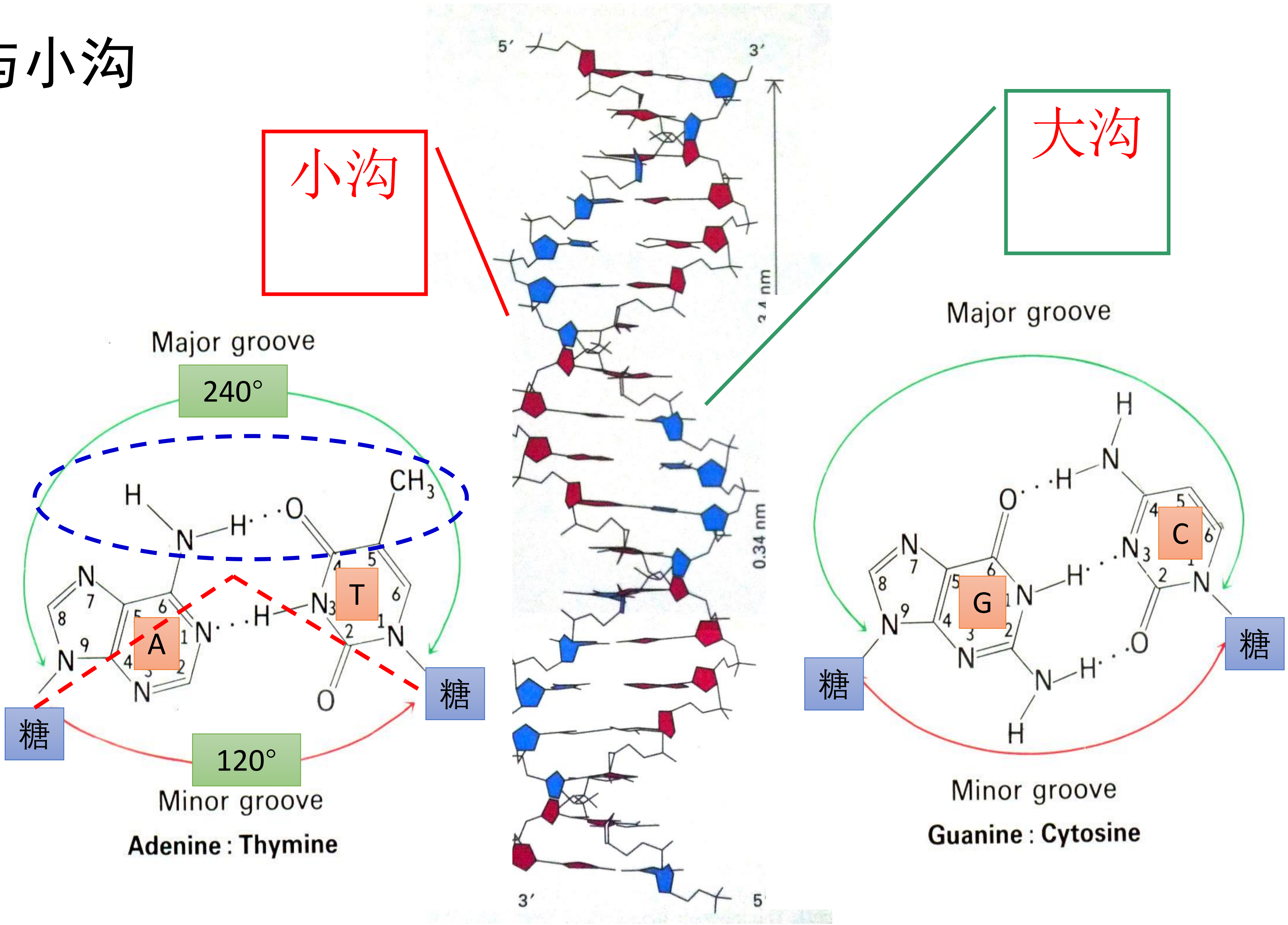


(a) Key features of DNA structure

(b) Partial chemical structure

(c) Space-filling model

大沟与小沟

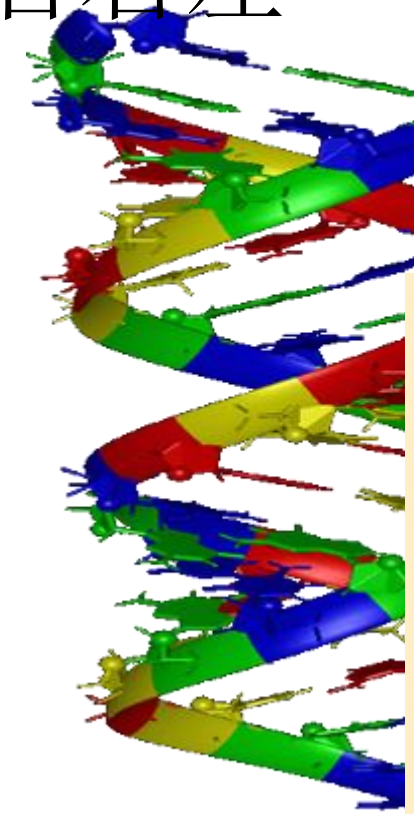


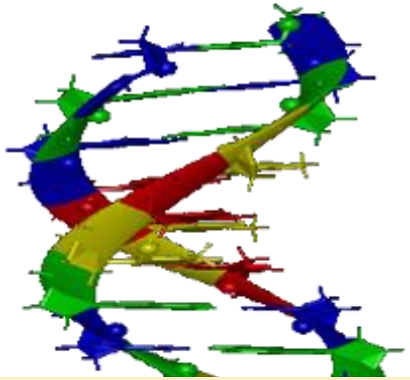
小沟
大沟
5′
3′
3′
5′
3.4 nm
0.34 nm
Major groove
240°
H
N
H
O
CH3
N
N
A
N
T
N
N
N
O
糖
糖
120°
Minor groove
Adenine : Thymine
Major groove
H
N
O
H
C
N3
N
G
N
H
N
N
O
N
H
糖
糖
H
Minor groove
Guanine : Cytosine

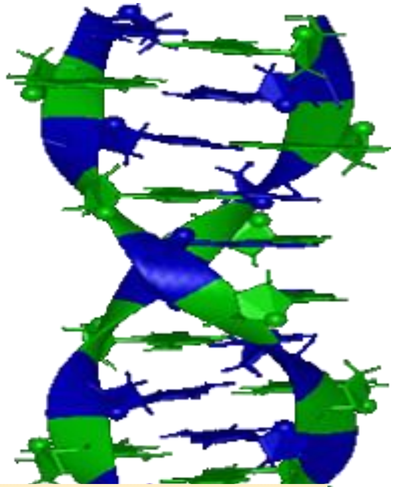
DNA Structure: 不同的构象

右右左
A B Z
右右左

?1 base pairs

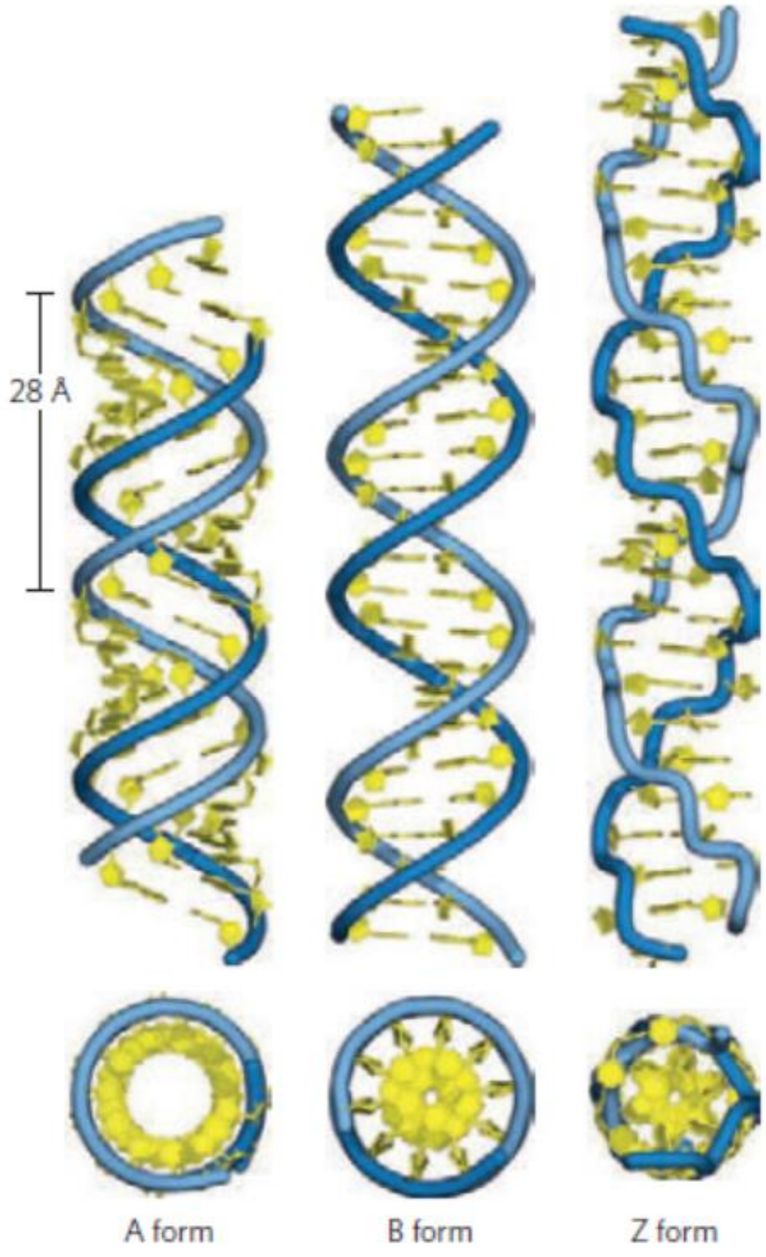






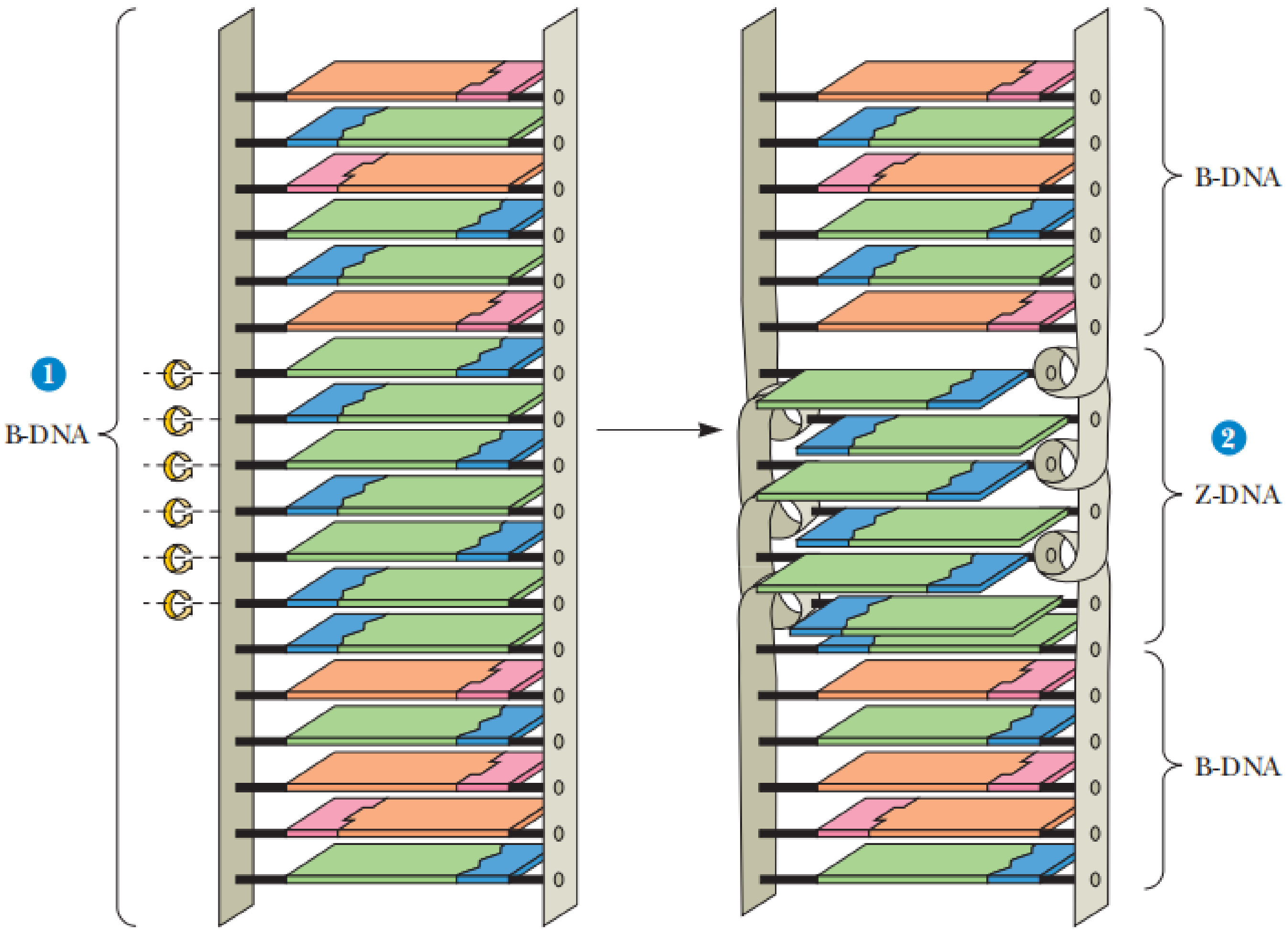
A-DNA

	A form	*B form*	*Z form*
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrimidines; syn for purines

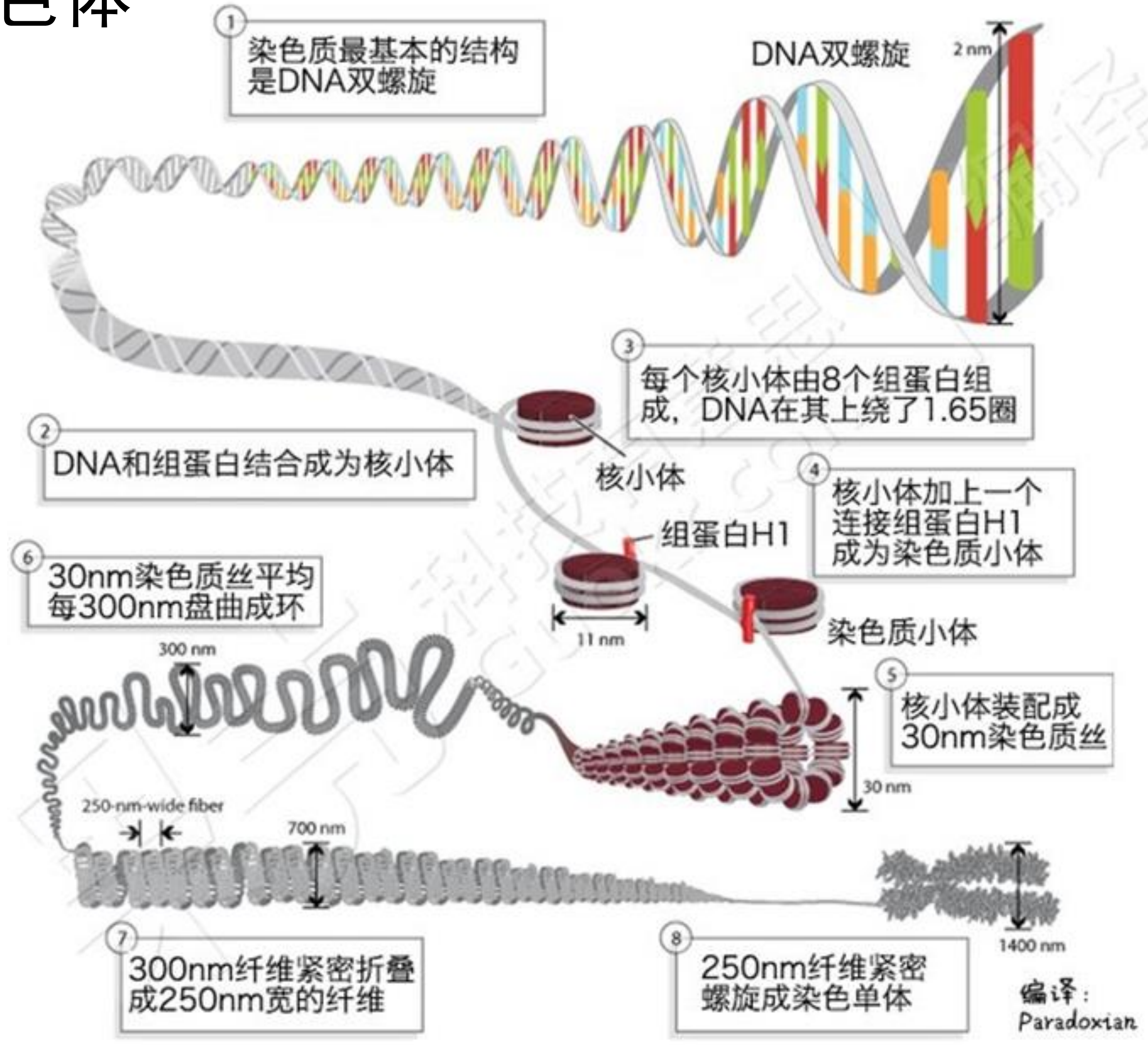


- B-DNA：生理条件下最稳定的构象；
- A-DNA：片段DNA在晶体状态下的构象，
- Z-DNA：生理条件下局部DNA可存在的构象，表示该段DNA可能处于（甲基化）转录抑制状态。

B-型DNA和Z型DNA



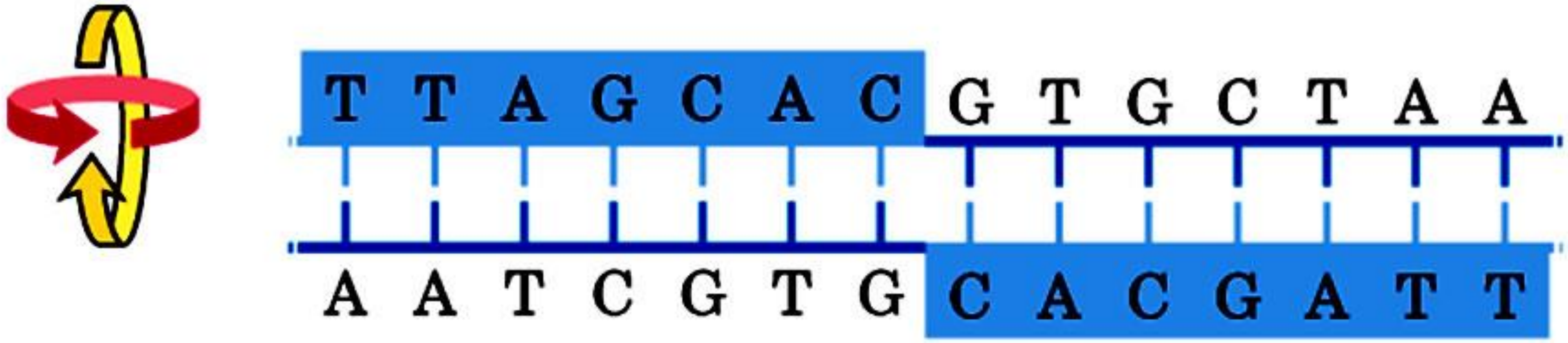
从双螺旋到染色体



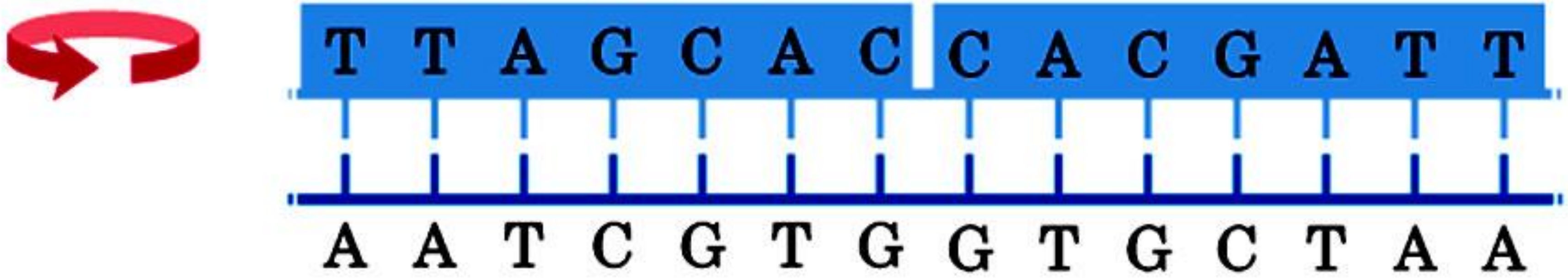
①
染色质最基本的结构
是DNA双螺旋
DNA双螺旋
2 nm
③
每个核小体由8个组蛋白组
成，DNA在其上绕了1.65圈
②
DNA和组蛋白结合成为核小体
核小体
组蛋白H1
④
核小体加上一个
连接组蛋白H1
成为染色质小体
⑥
30nm染色质丝平均
每300nm盘曲成环
11 nm
染色质小体
300 nm
⑤
核小体装配成
30nm染色质丝
30 nm
250-nm-wide fiber
700 nm
1400 nm
⑦
300nm纤维紧密折叠
成250nm宽的纤维
⑧
250nm纤维紧密
螺旋成染色单体
编译：
Paradoxian

DNA Structure: 回文和镜像重复

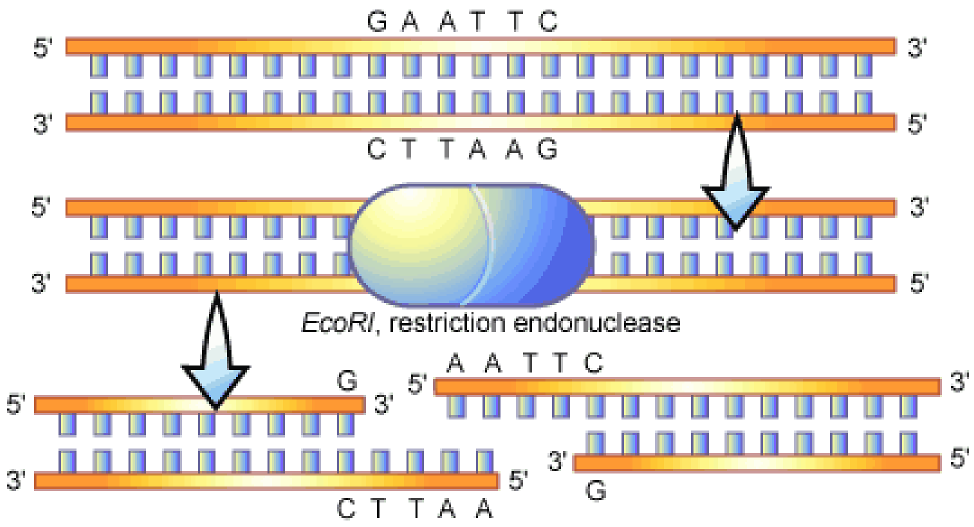
Palindrome 回文：正着读和反着读都是一样。 **rotator、nurses run**



Mirror repeat 反向重复在同一条DNA链



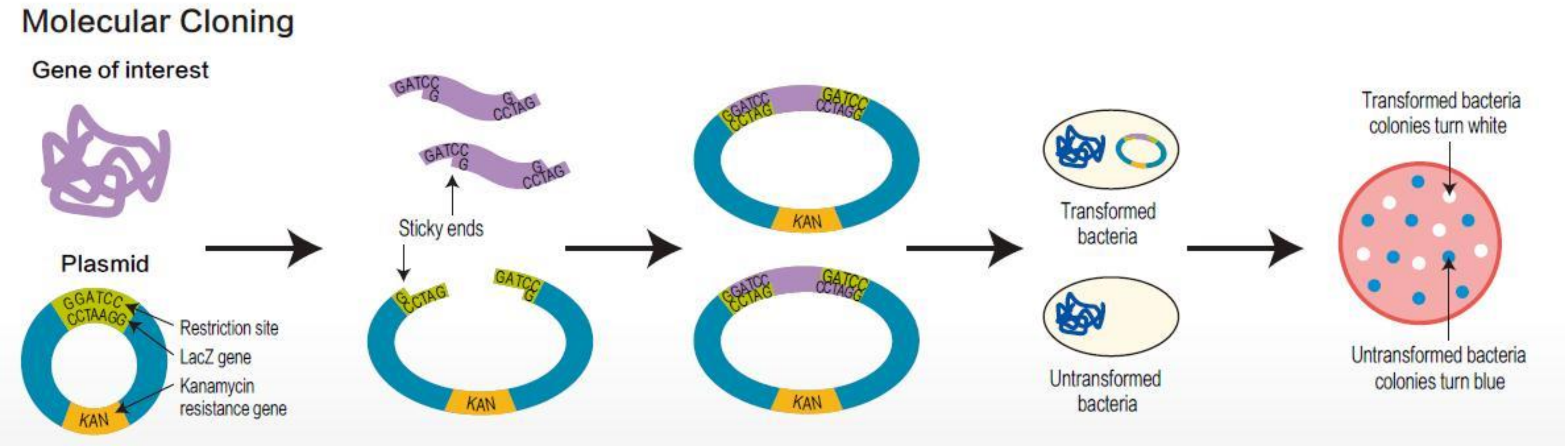
限制性内切酶与分子克隆



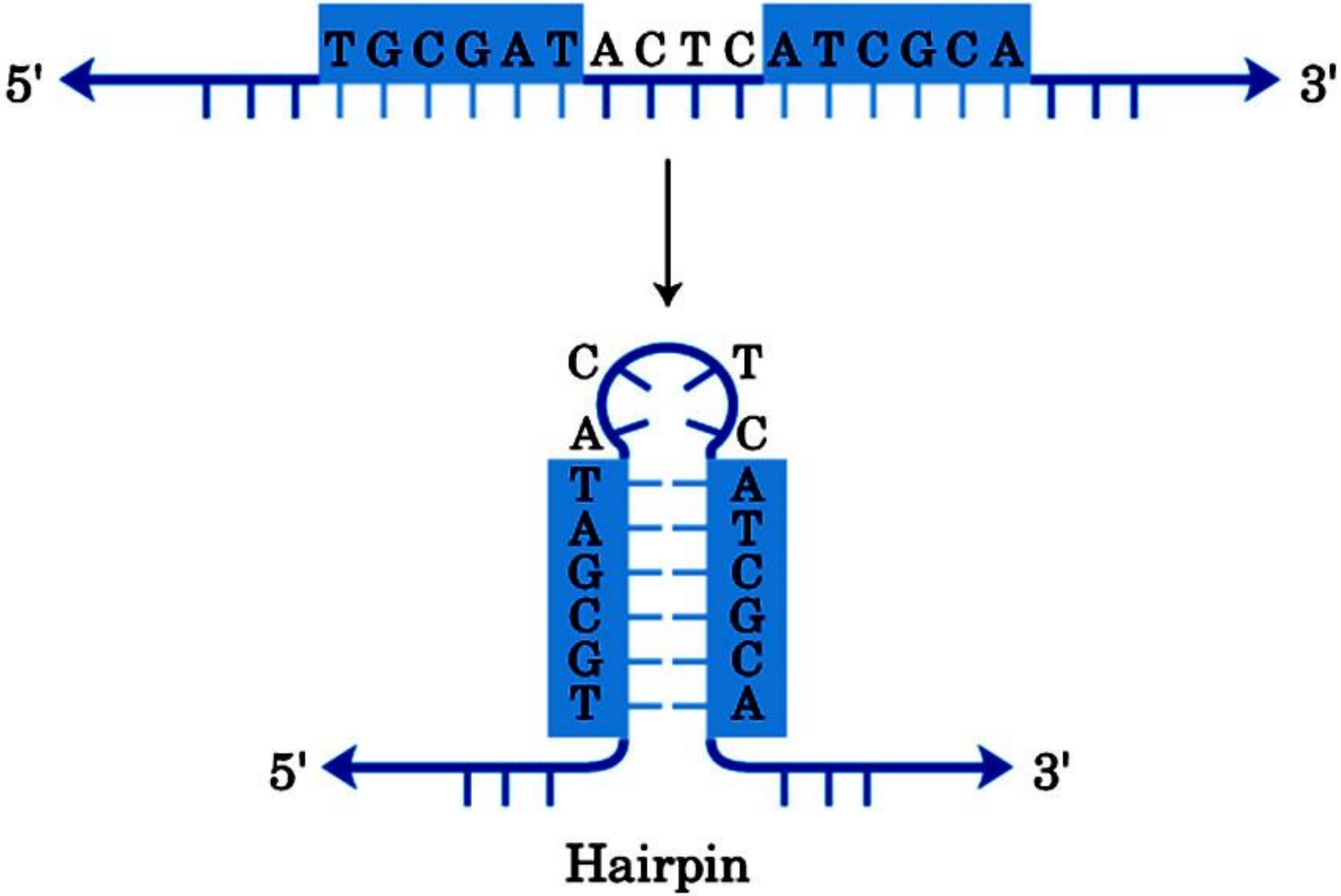
FastDigest BamHI

5' G↓G A T C C 3'

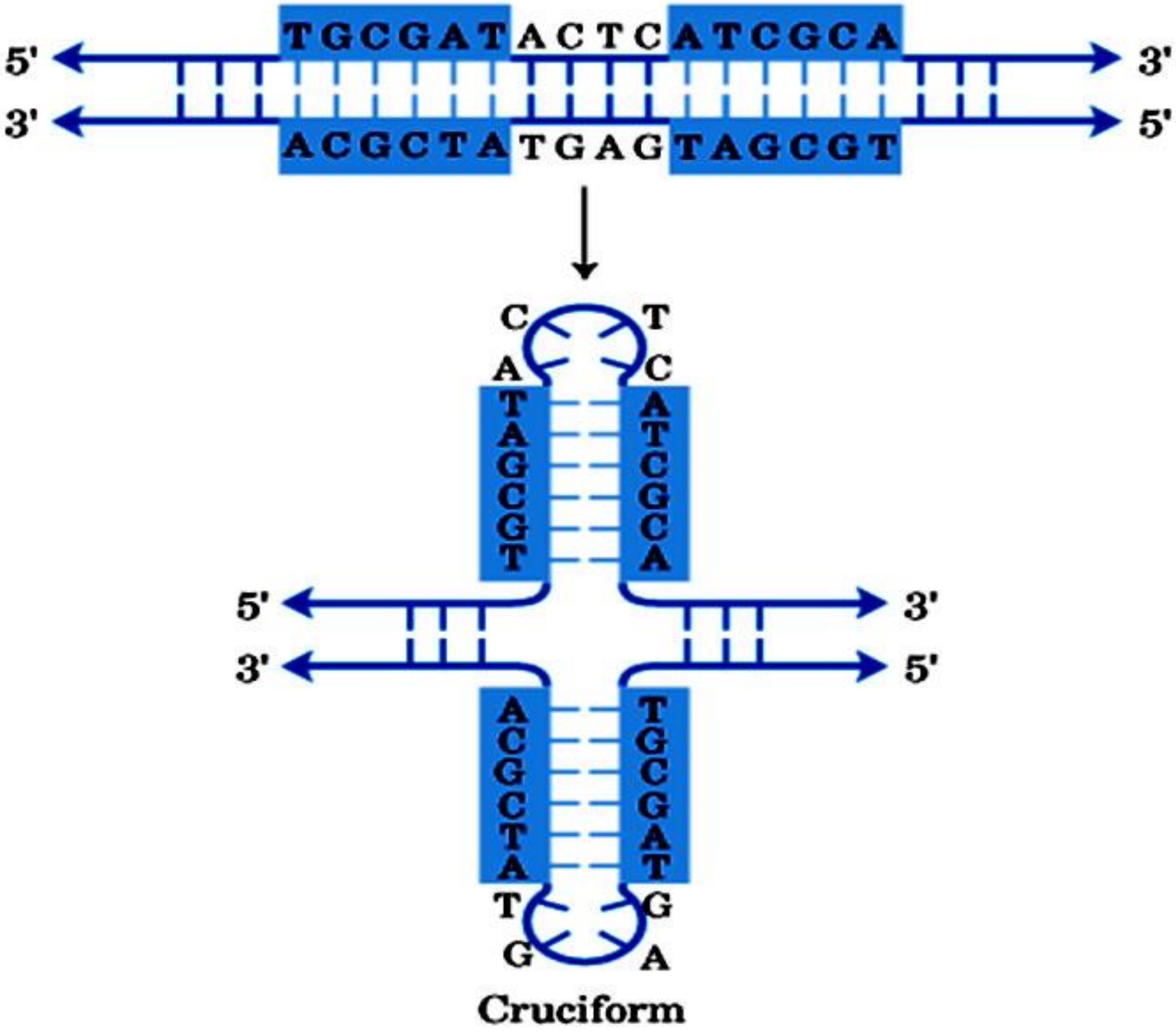
3' C C T A G↑G 5'



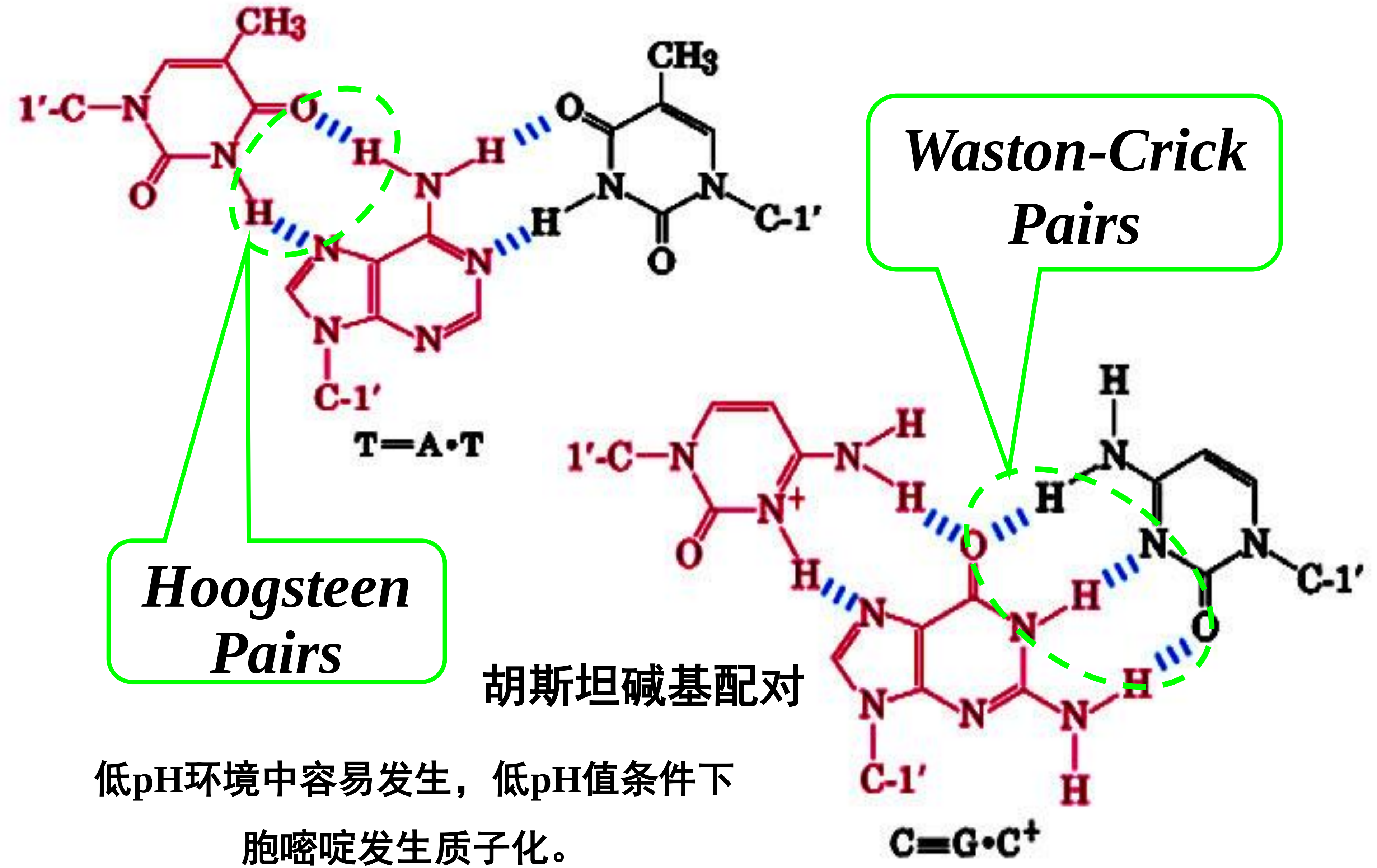
DNA/RNA Structure: 发卡结构（单链）



DNA Structure: “+”字结构（双链）

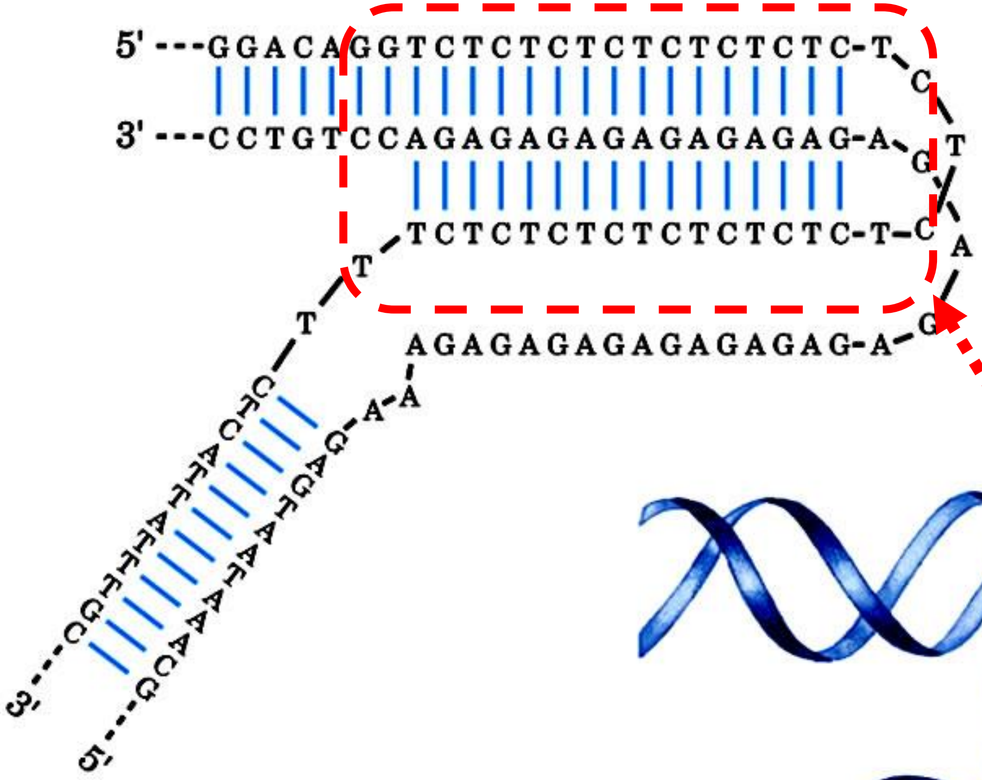


DNA Structure: three DNA strands

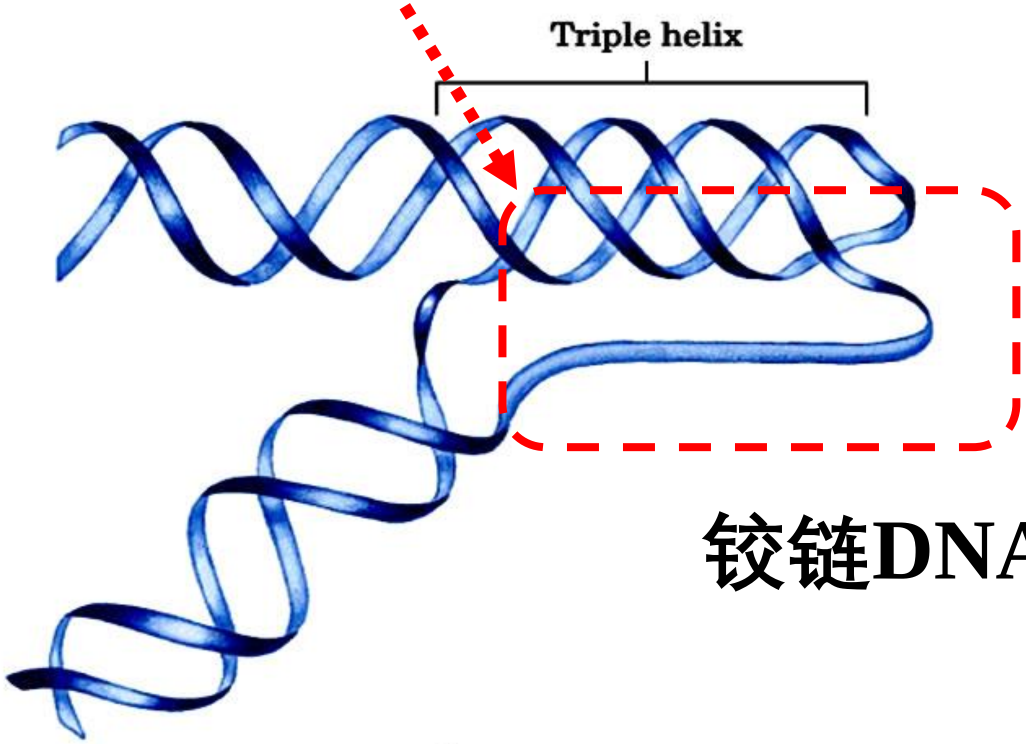


胡斯坦碱基配对

低pH环境中容易发生，低pH值条件下胞嘧啶发生质子化。



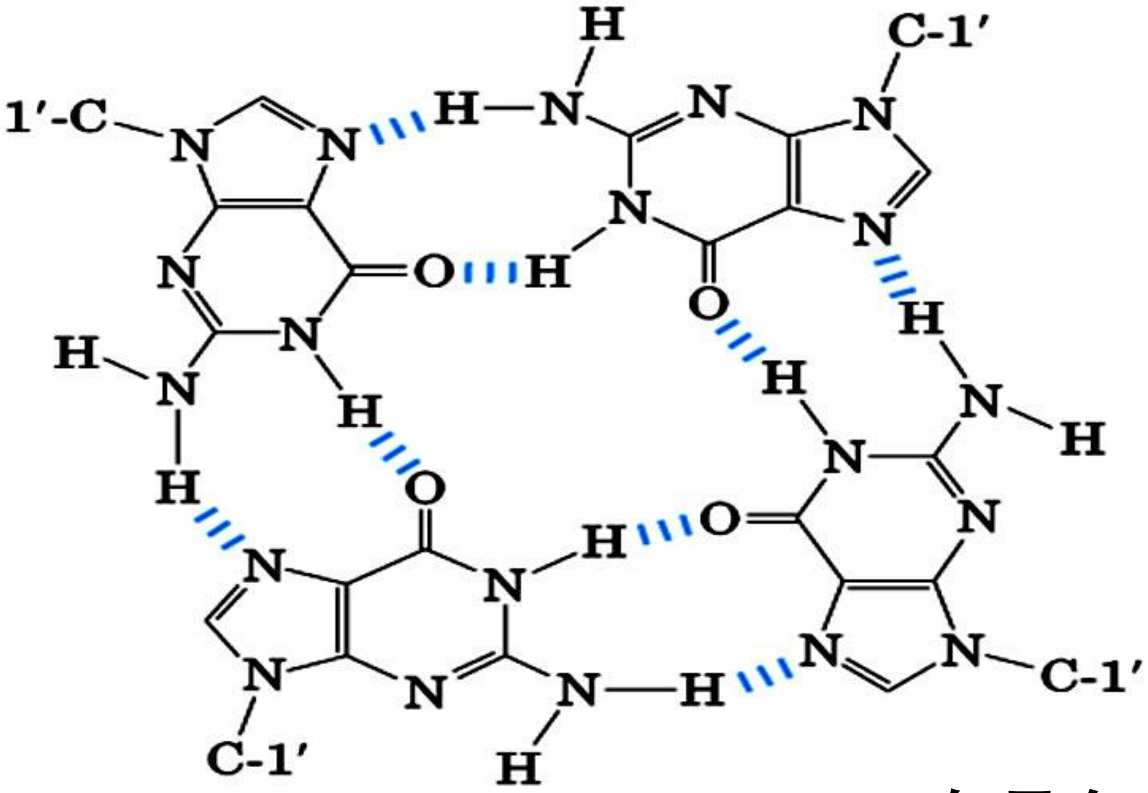
DNA Structure:
H DNA



铰链DNA

多聚嘌呤或多聚嘧啶序列能形成三级螺旋，分布在真核生物表达的调控区，DNA的三聚体能阻断基因的表达，调控细胞代谢。

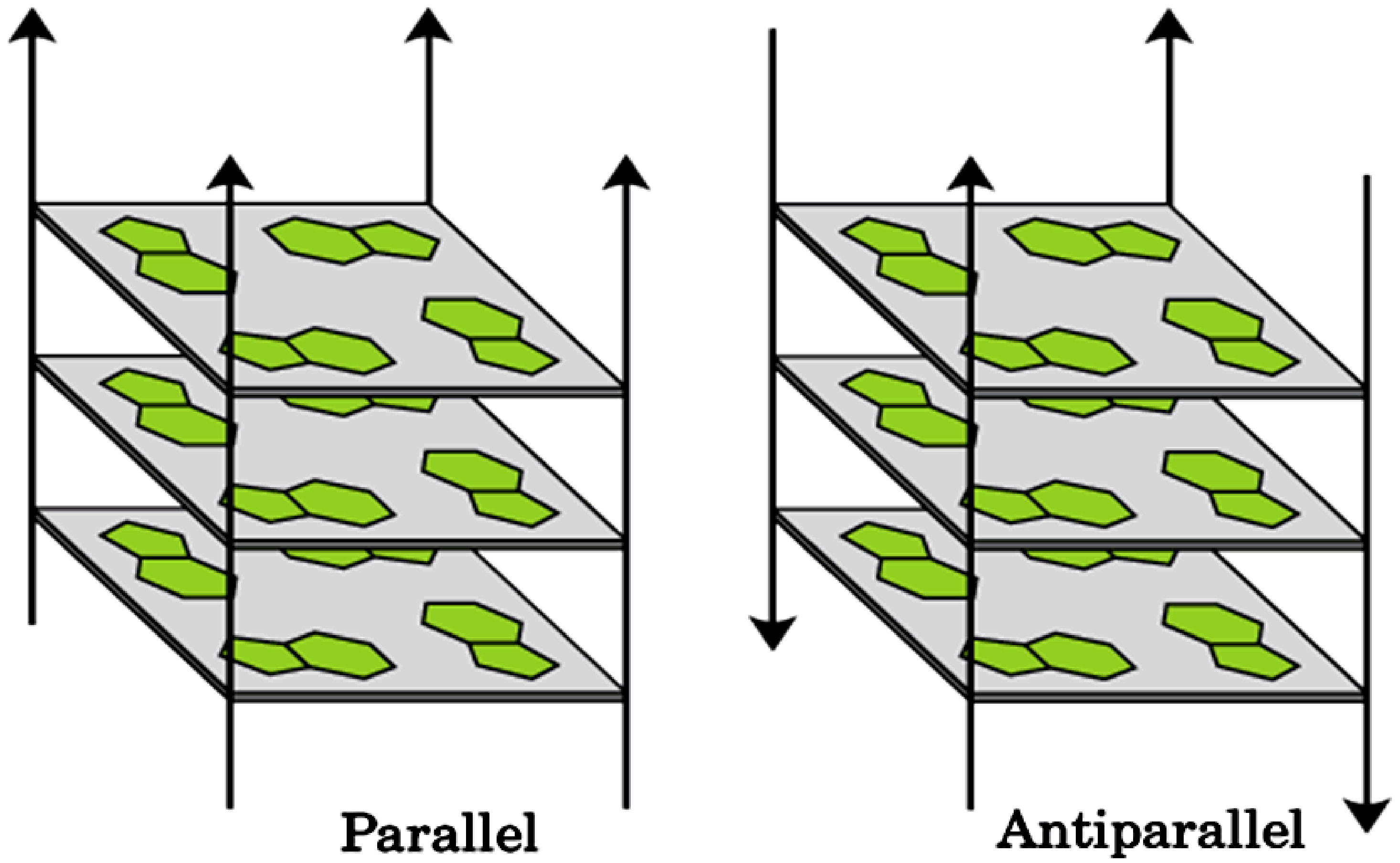
DNA Structure: four DNA strands G四链体



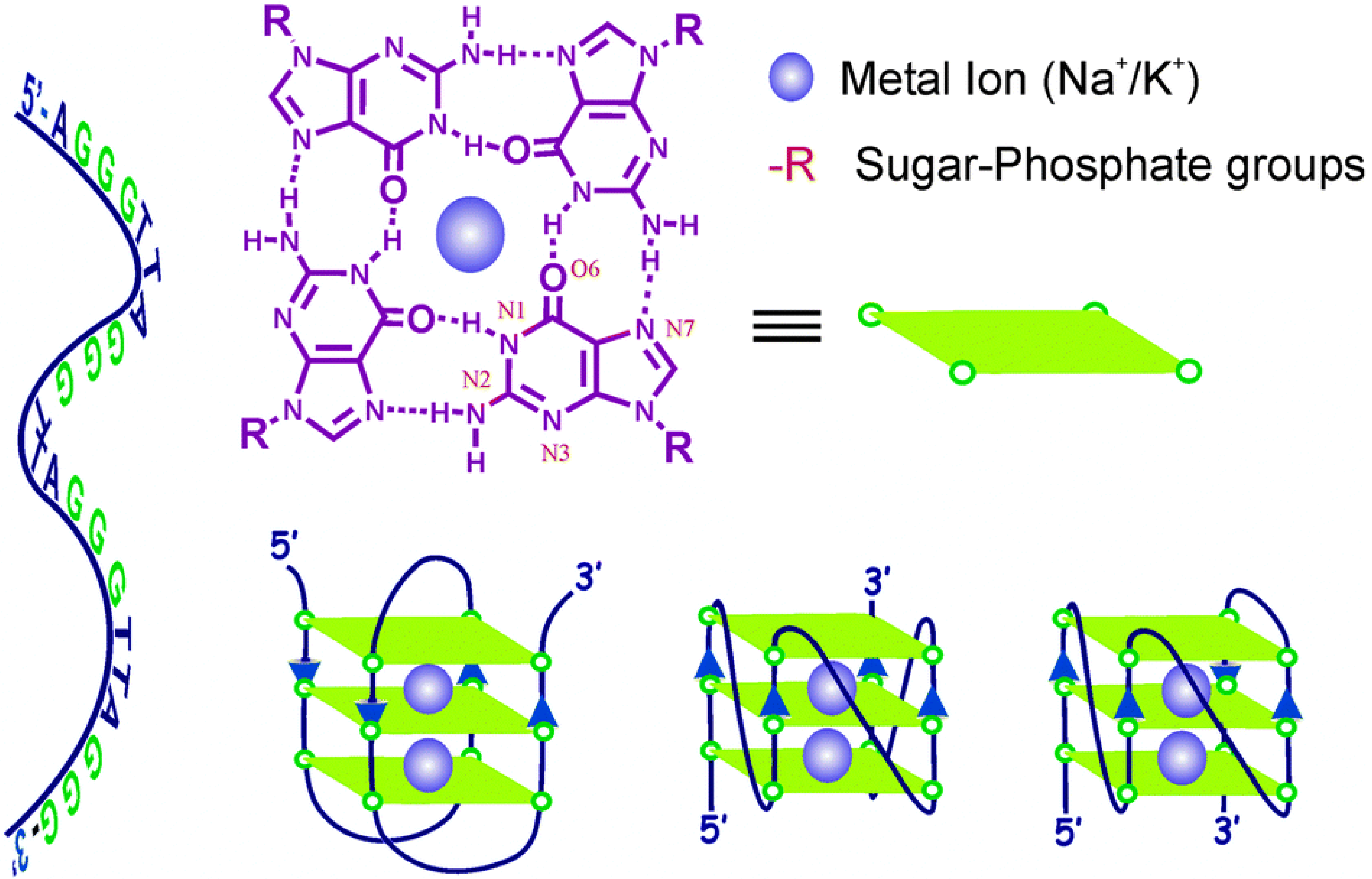
Guanosine tetraplex

鸟嘌呤四聚体

含高的鸟苷酸残基的DNA链易于形成DNA的四聚体。



- *Unusual DNA structures (including triple helix and tetraplex) tend to appear at sites where important events in DNA metabolism (replication, recombination, transcription) are initiated or regulated.*



端粒区

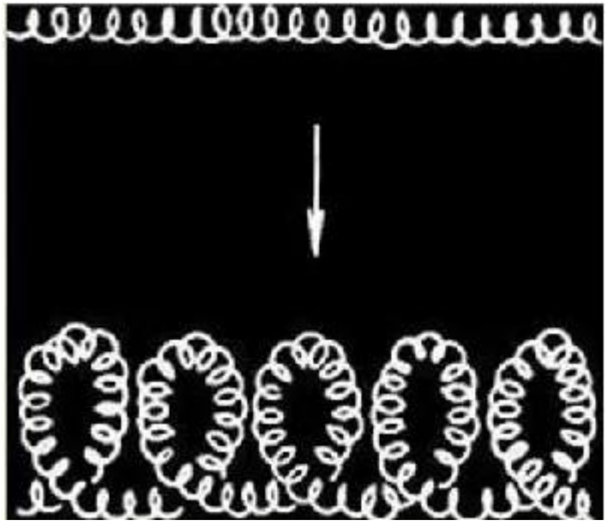
G-rich DNA Sequence

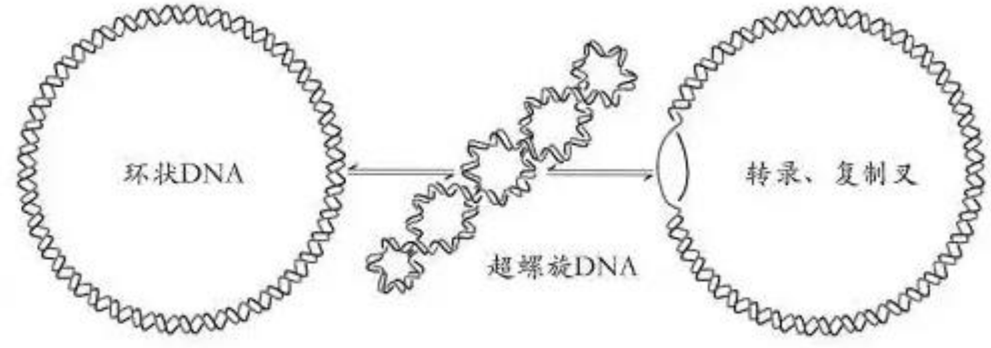
Antiparallel Quadruplex

Parallel Quadruplex

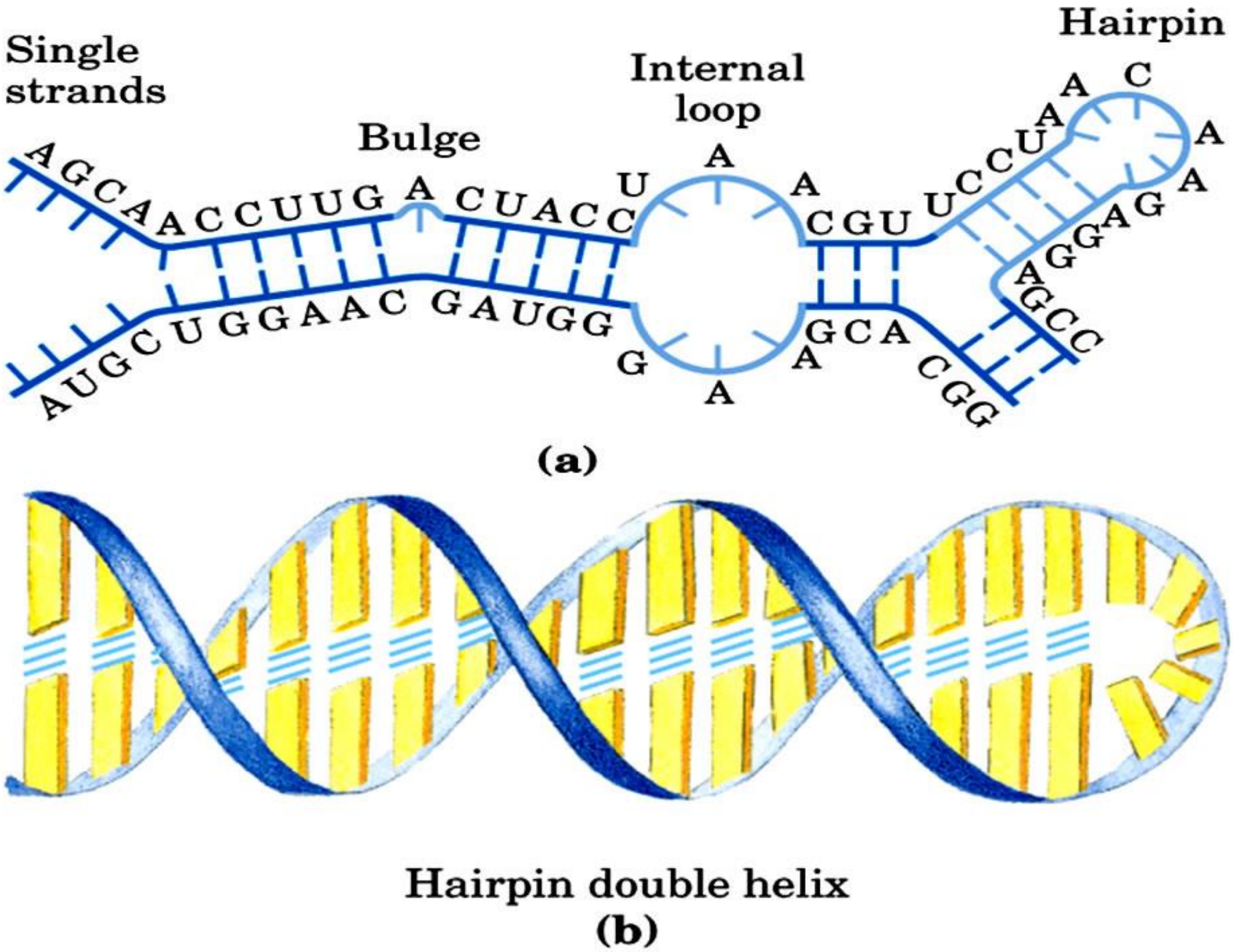
Hybrid Quadruplex

三级结构：DNA的超螺旋

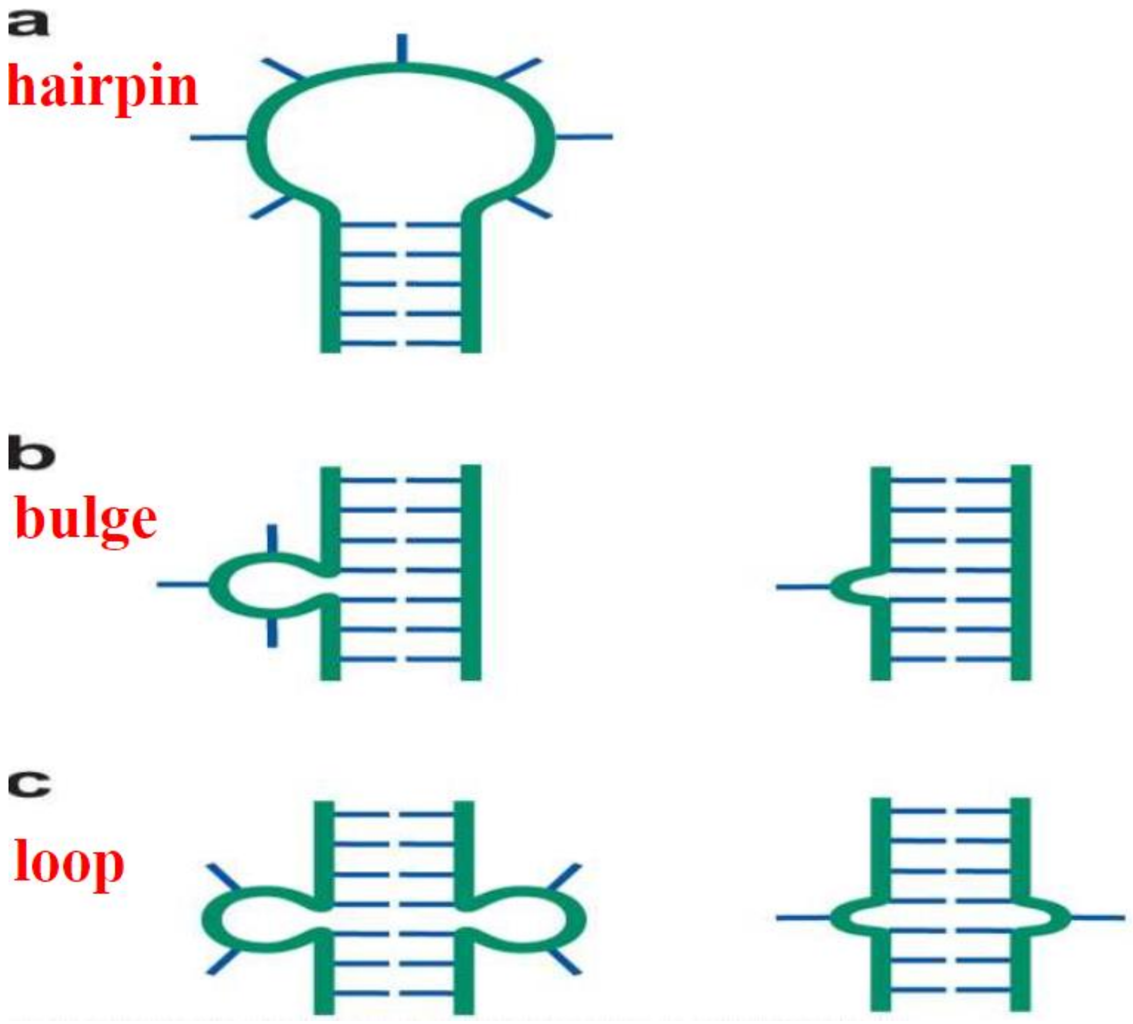




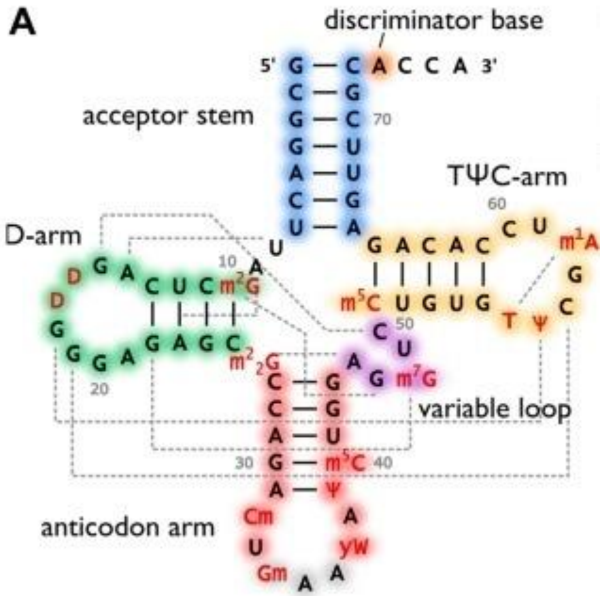
RNA Structure



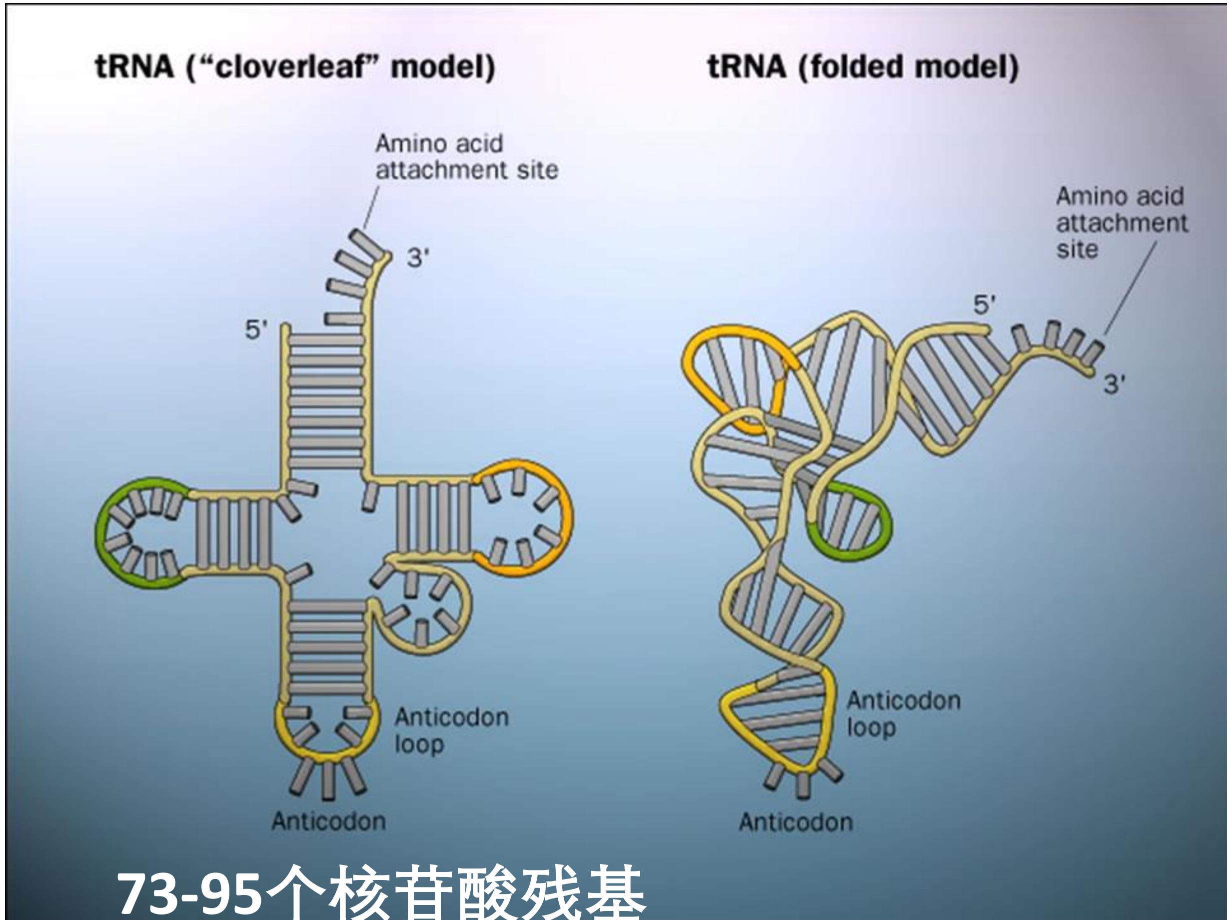
RNA的二级结构——RNA链自身折叠形成类似A-DNA的局部双螺旋



RNA从二级到三级结构



Saccharomyces cerevisiae
cytosolic tRNA^{Phe} (76 nt)



2'羟基能形成氢键以稳定三级结构

核酸的结构小结

类型	DNA	RNA
Primary	**5’-3’**	**5’-3’**
Secondary	**双螺旋，B，A，Z** **发夹/十字** **三螺旋** **四螺旋**	**发夹** **三叶草(tRNA)**
Tertiary	**超螺旋(环形DNA)**	**倒L型(tRNA)**

Chapter 7
Nucleotides and Nucleic Acids

7.1 ***Brief History of Nucleic Acids***

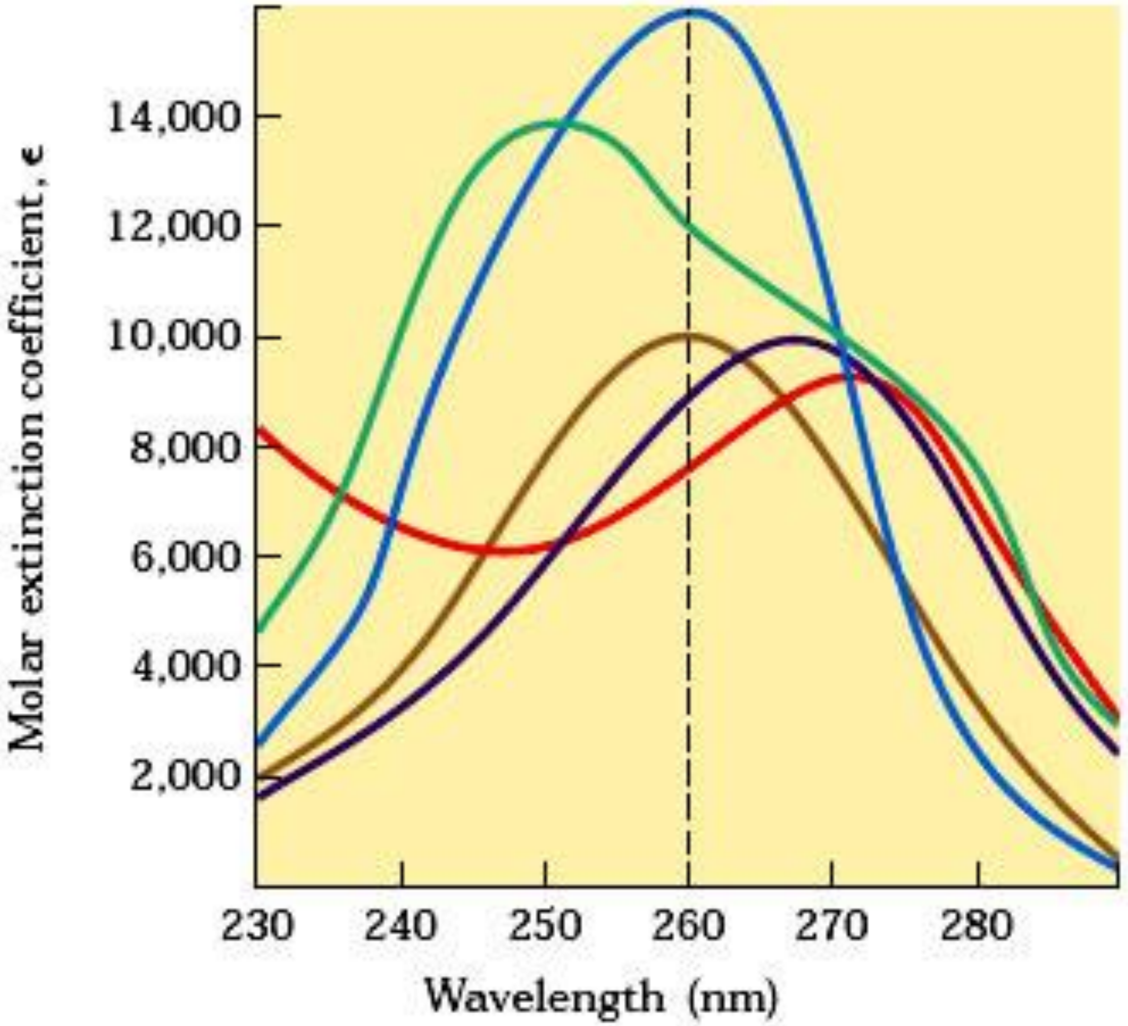
7.2 ***Nucleotides: the Building Blocks***

7.3 ***Nucleic Acids: from Structure to Functions***

7.4 ***Nucleic Acids-Based Biotechnology***

7.5 ***Other functions of Nucleotides***

✦UV absorption



DNA 260/280=1.8
RNA 260/280=2.0

	Molar extinction coefficient at 260 nm, ϵ_{260} ($M^{-1}cm^{-1}$)
AMP	15,400
GMP	11,700
UMP	9,900
dTMP	9,200
CMP	7,500

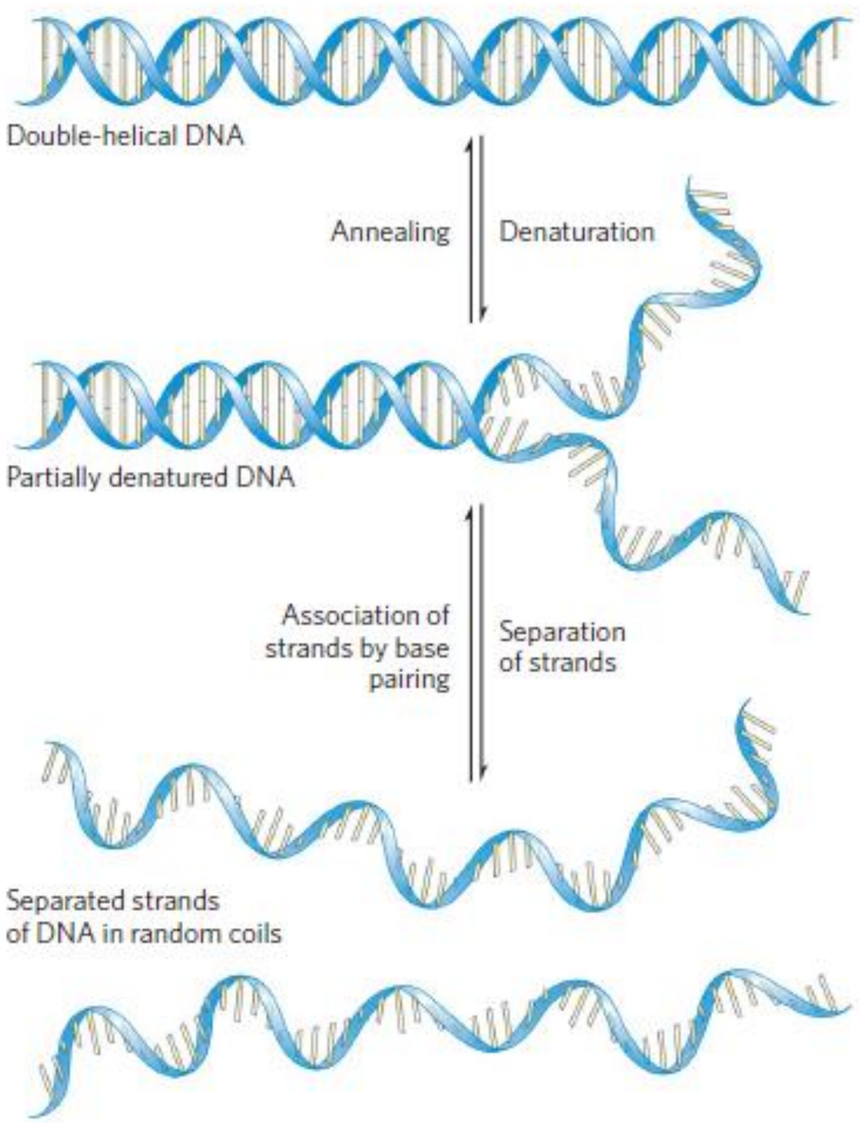
共轭双键

各碱基吸收峰250-270nm，为何只用260?

✦*Double-helical DNA & RNA can be denatured*

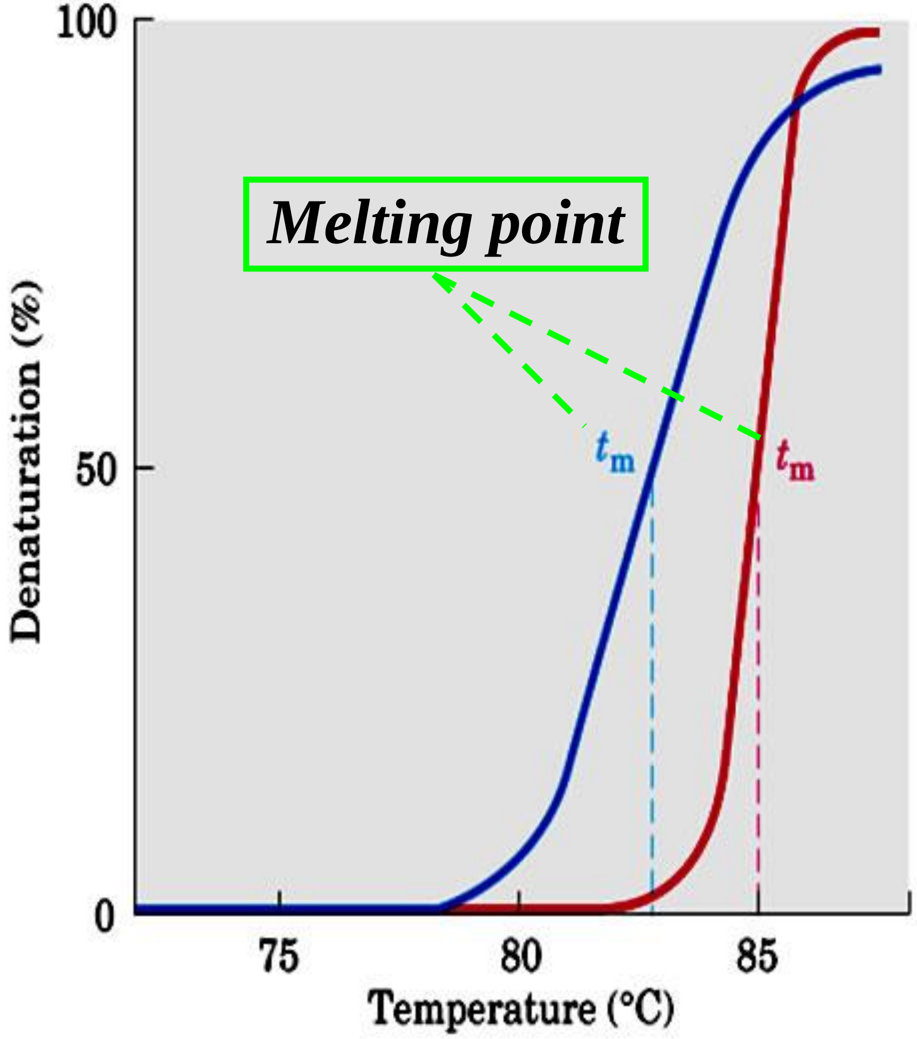
260nm波长，单链DNA的光吸收比双链DNA高12-14%。

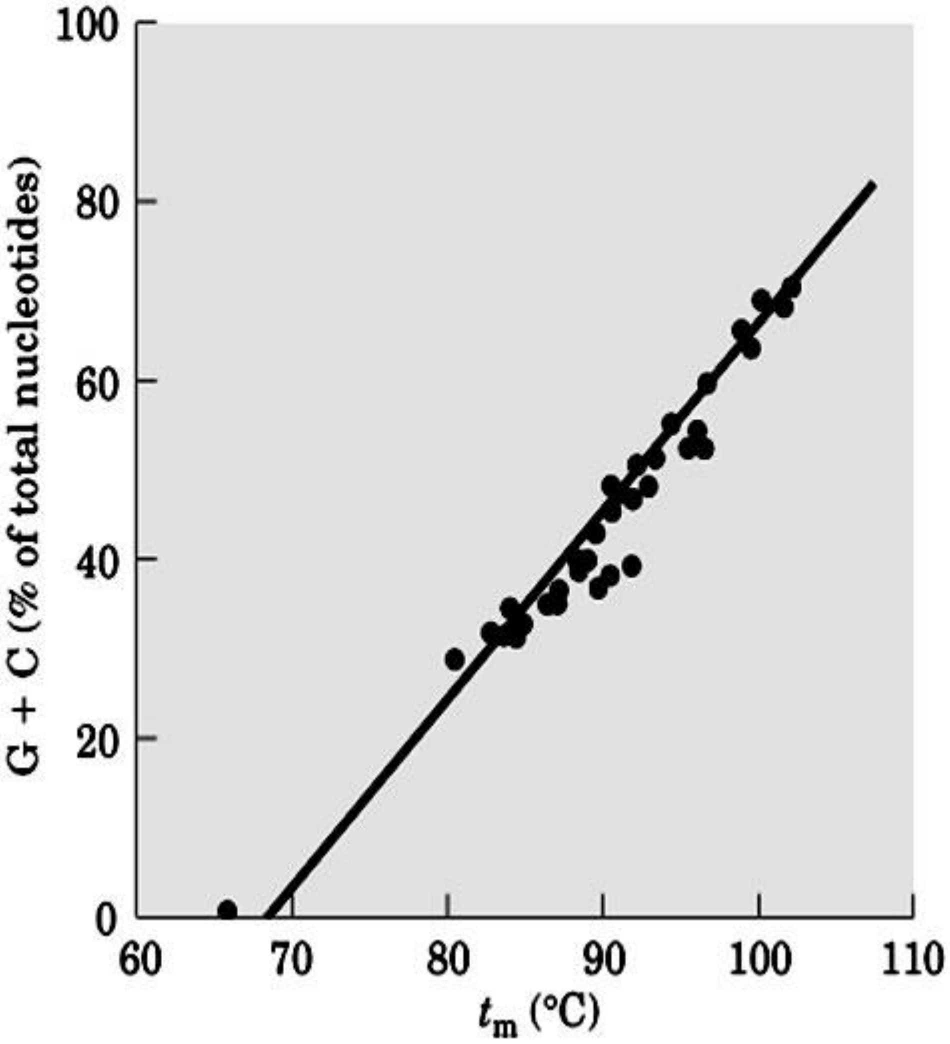
堆积的碱基对互作降低了吸收



增色效应
hyperchromic effect

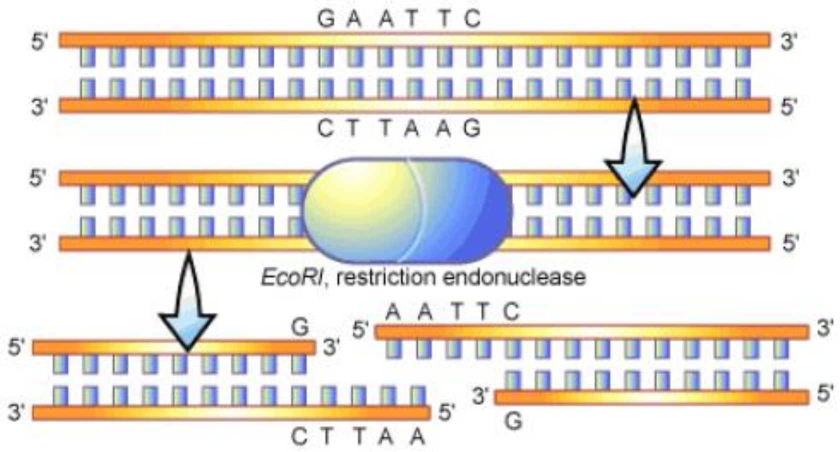
✦Double-helical DNA & RNA can be denatured



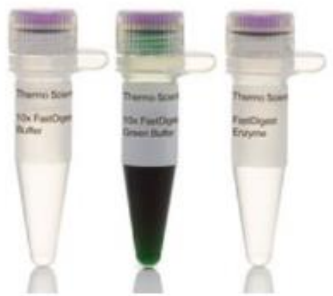


1/2 DNA变成单链时的温度称为熔点Tm。

限制性内切酶与分子克隆

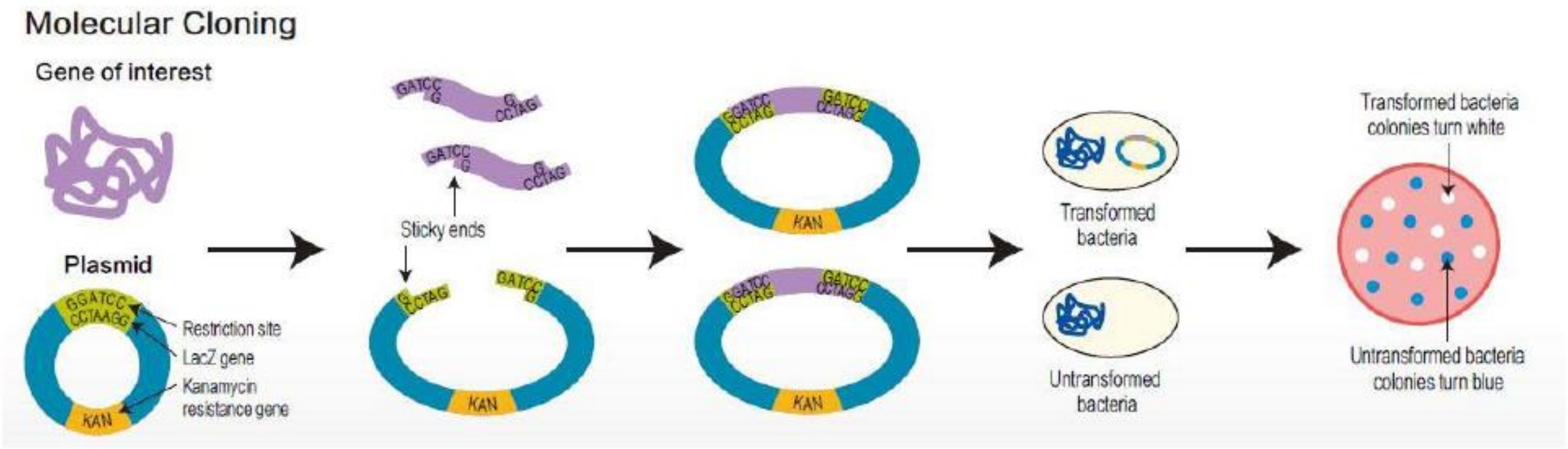


FastDigest BamHI

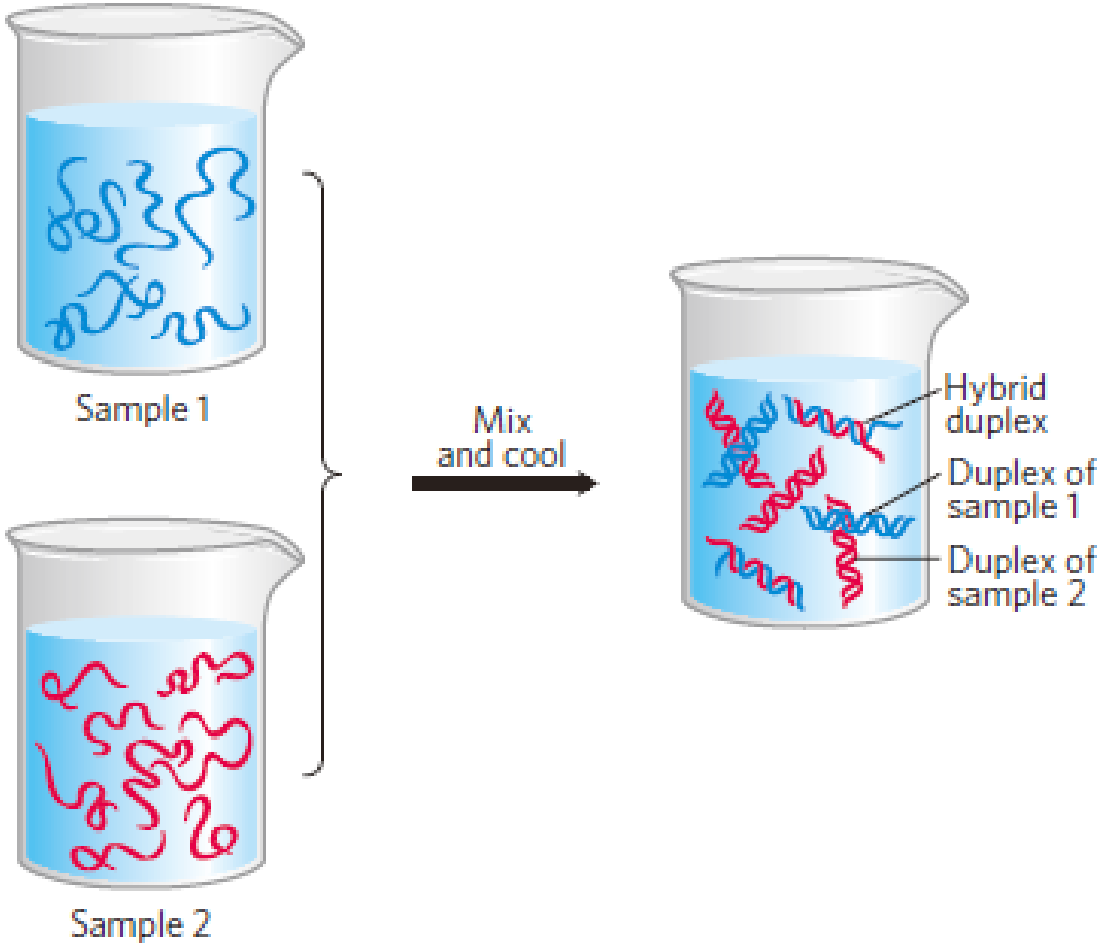


5' G↓G A T C C 3'

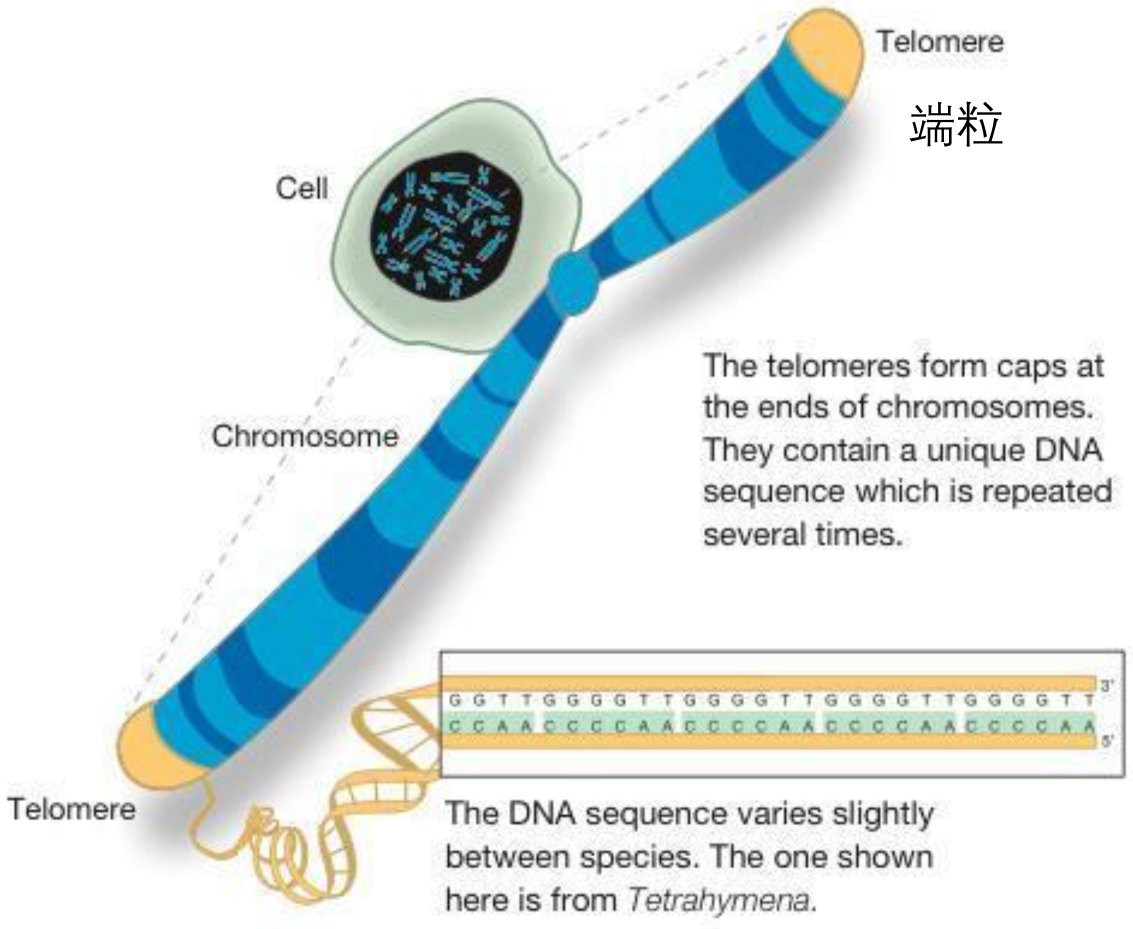
3' C C T A G↑G 5'



✦Nucleic acid hybrid

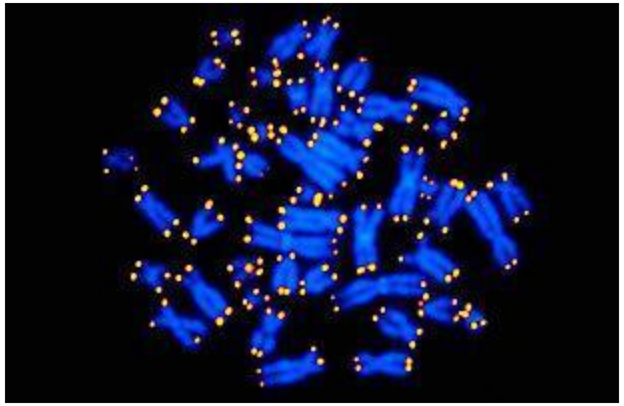


Probe （探针）



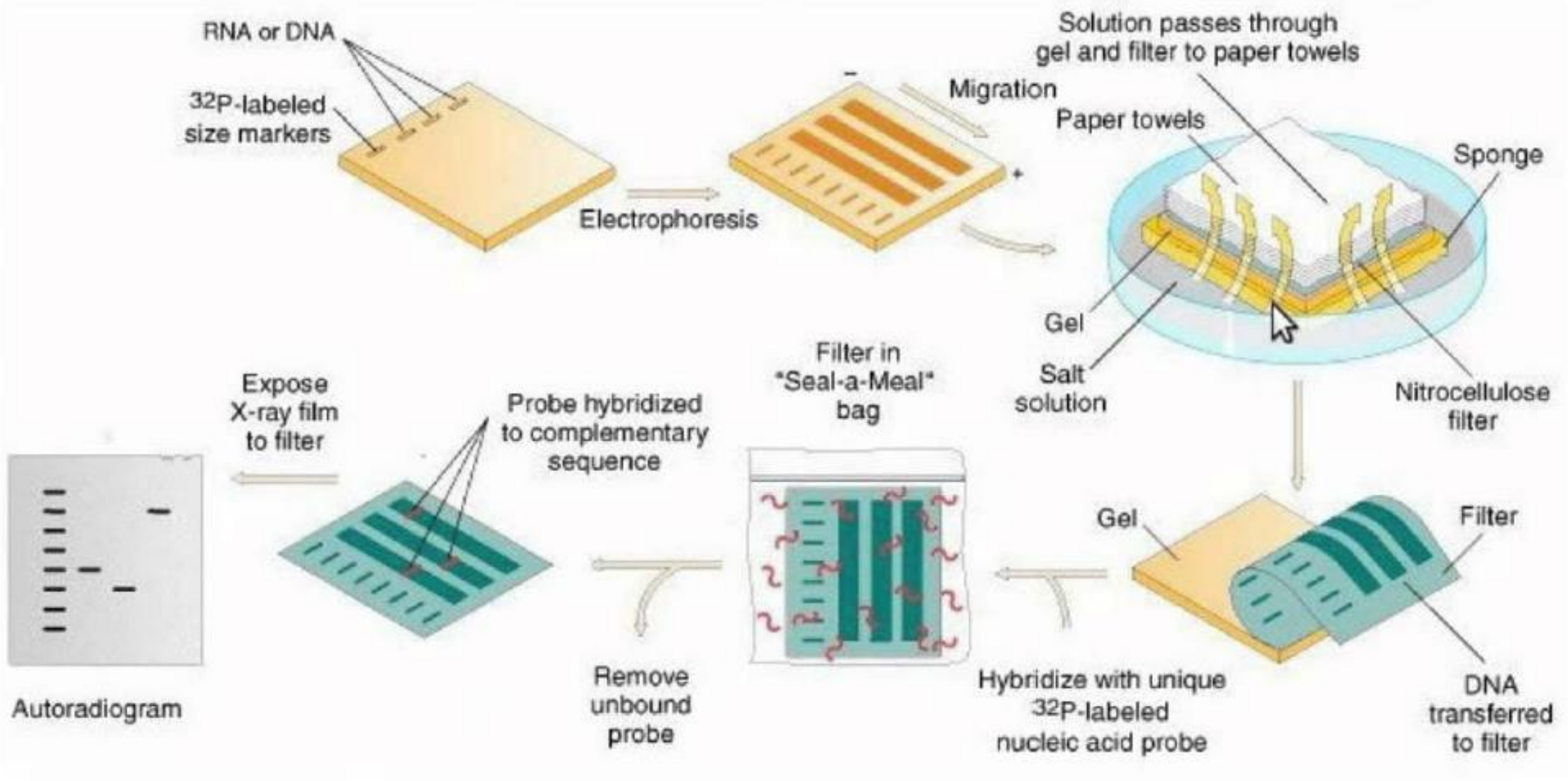
TTAGGGTTAGGGTTAGGGTTAGGG

AATCCCAATCCC

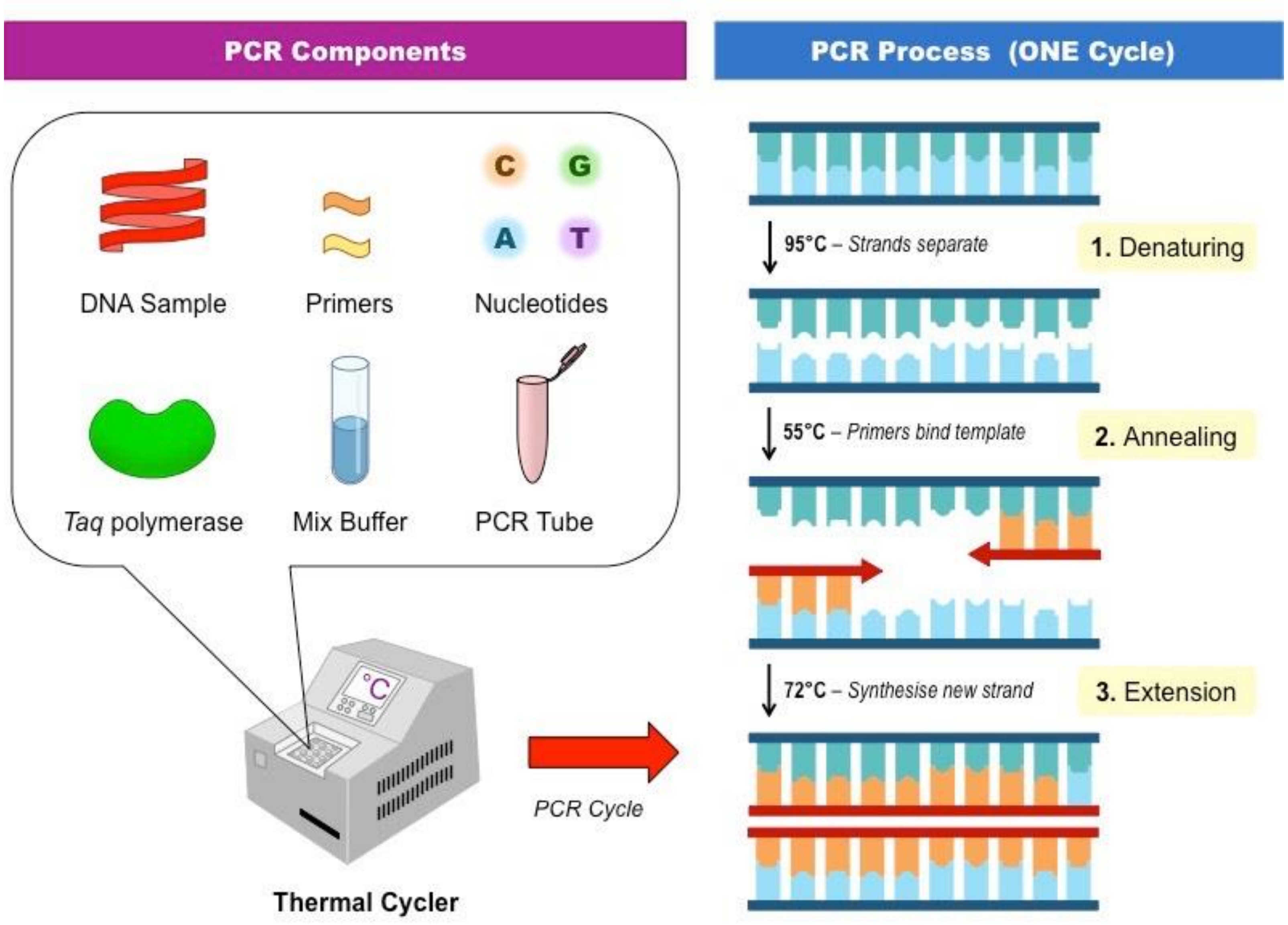


用探针示踪端粒

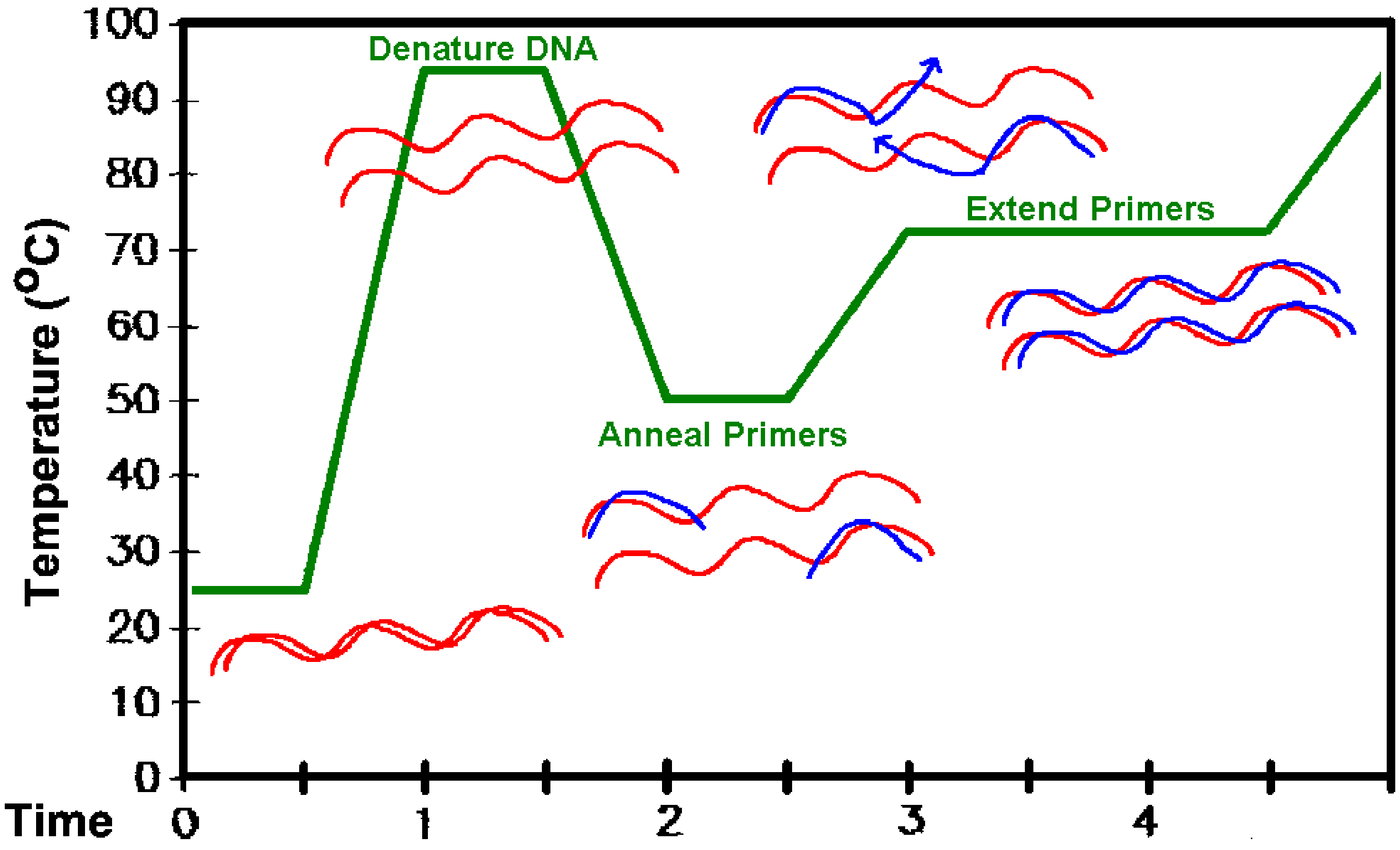
Southern blot（DNA印迹）
Northern blot（RNA印迹）



Polymerase chain reaction (PCR)



One of the PCR cycles



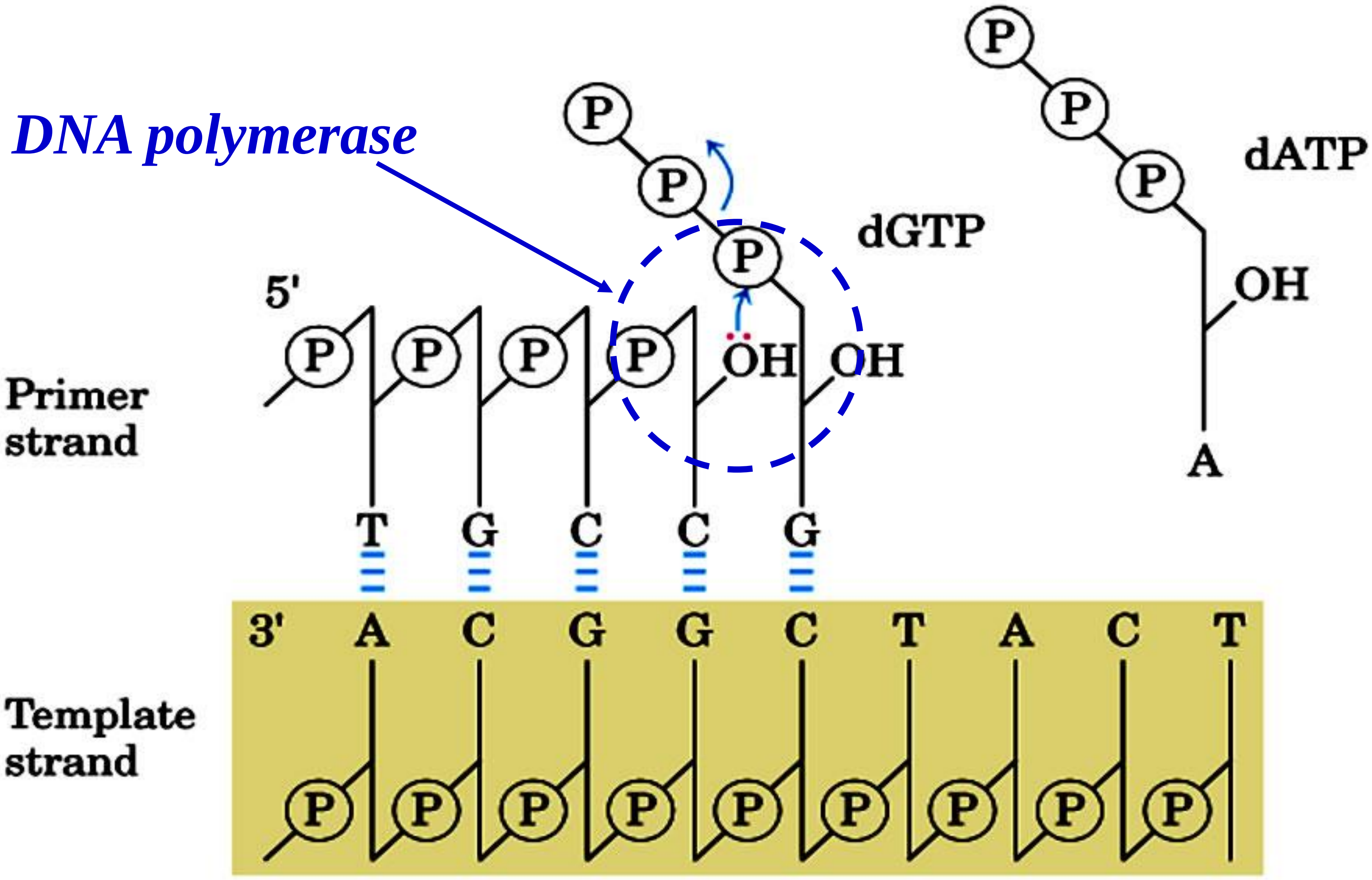
核酸的水解、保存

- 核酸的水解：RNA很怕碱，DNA怕酸，RNA保存在酸性条件下，DNA保存在碱性条件下（pH=8.0）。

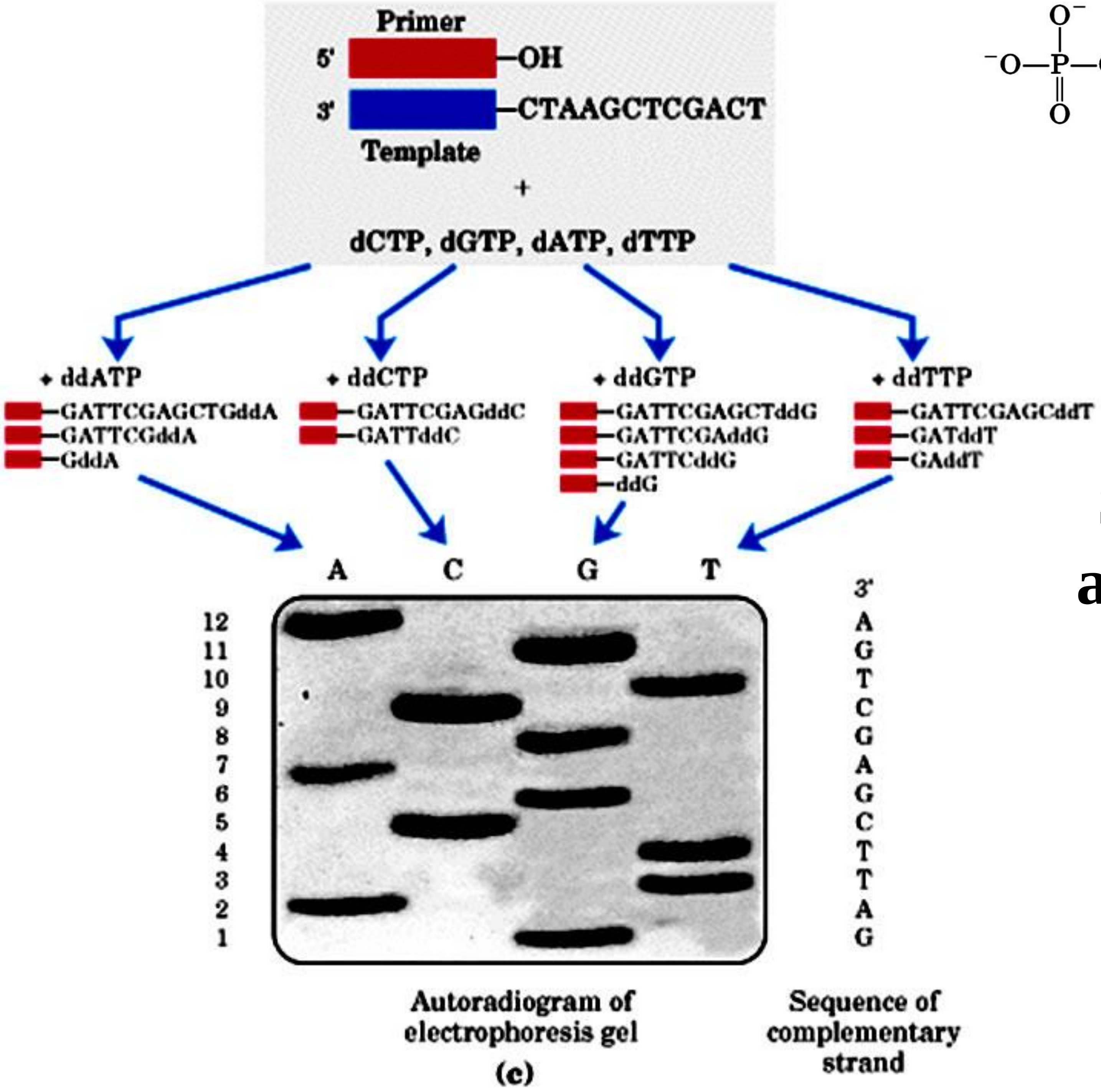
- 在酸性条件下，其嘌呤碱基很容易和核糖分开。N-糖苷键在酸性条件下易发生水解。DNA在酸性条件下会发生脱嘌呤作用。

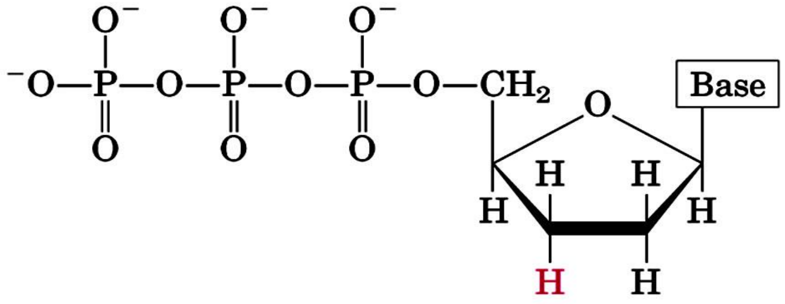
- RNA碱性条件2’-OH反应导致断裂

DNA Sequencing 测序技术



(a)



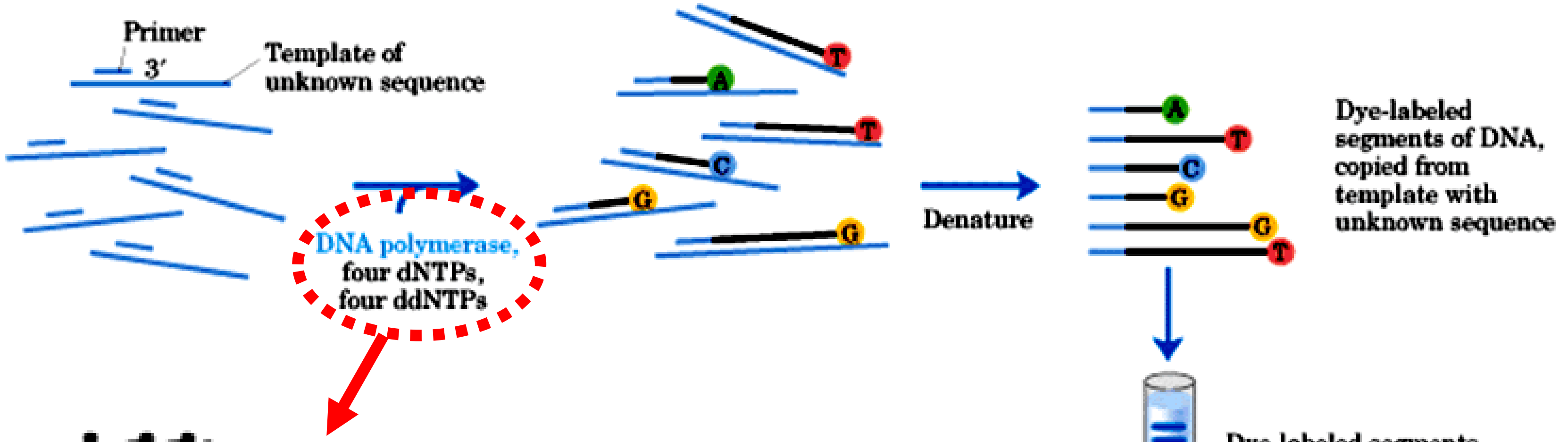


ddNTP analog

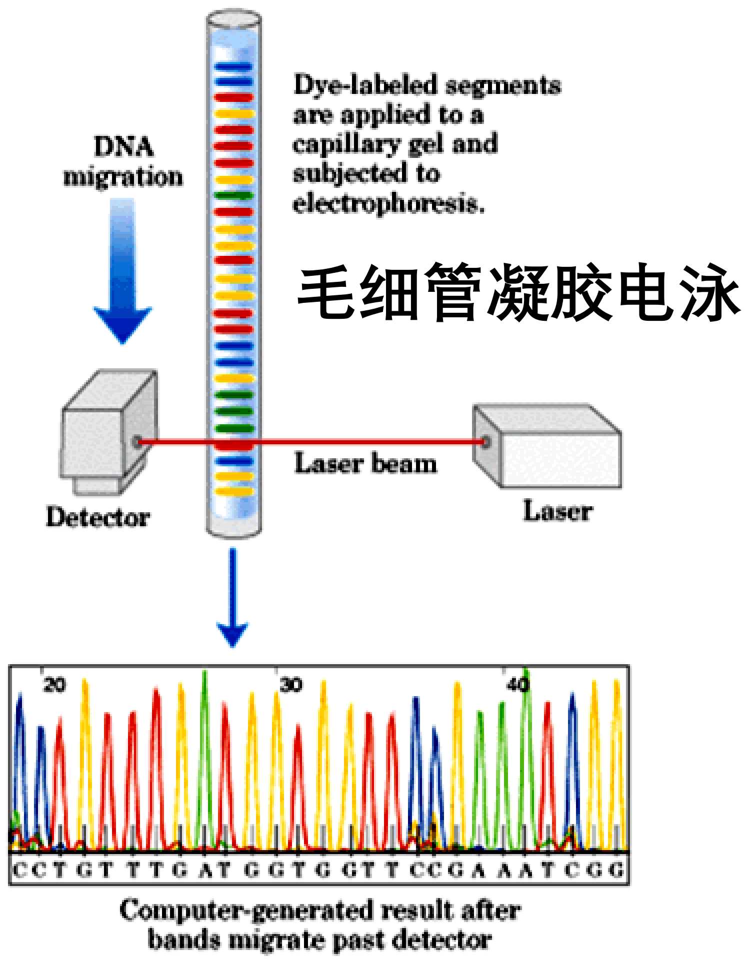
Sanger method is also called **dideoxy method**

(双脱氧终止法)

https://lms.sysu.edu.cn/my/



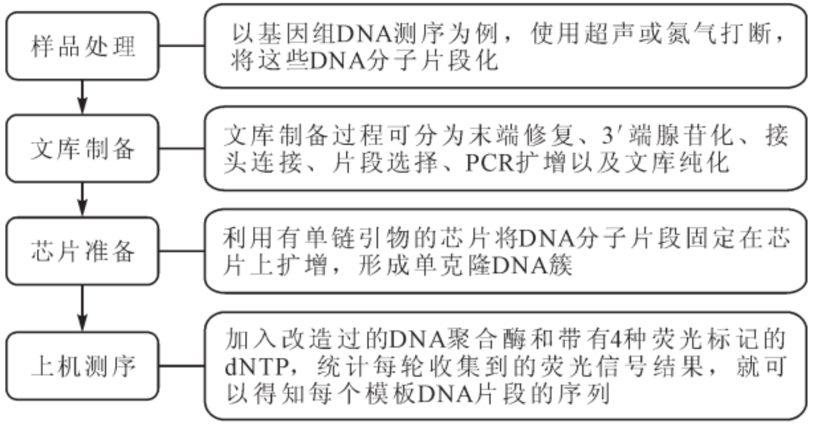
Add:
DNA polymerase
dATP
dGTP
dCTP
dTTP
plus limiting amounts of fluorescently labeled
ddATP
ddGTP
ddCTP
ddTTP

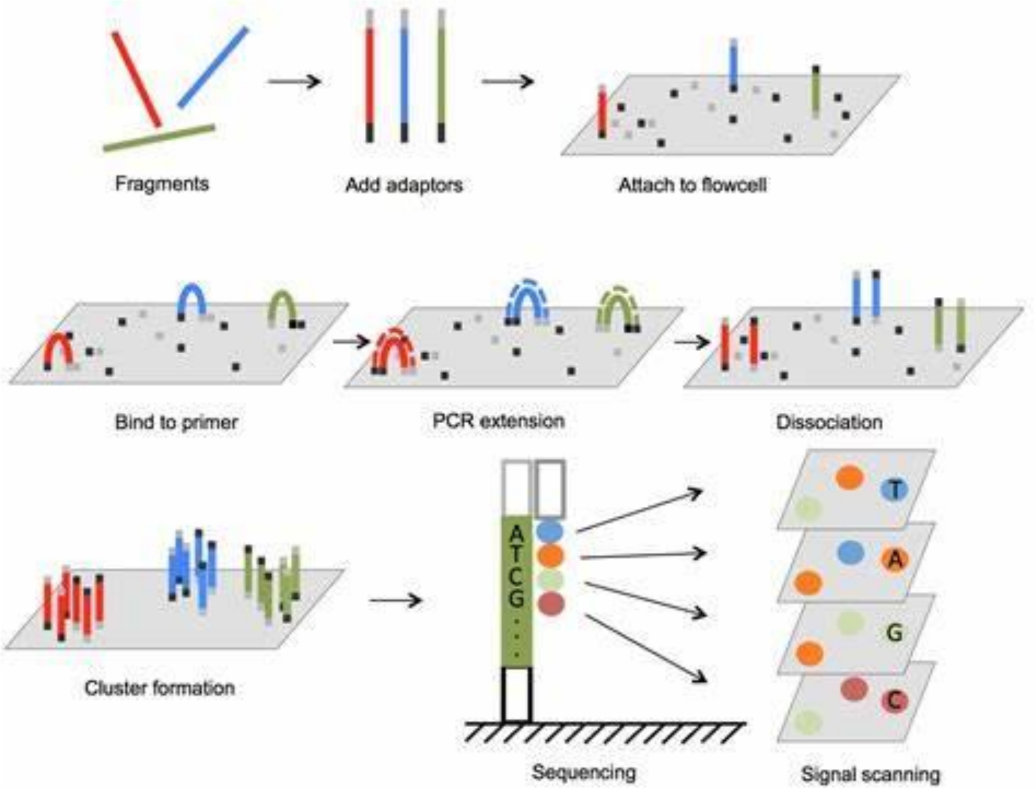


Computer-generated result after bands migrate past detector

荧光标记的策略

二代测序之单碱基延伸测序和结果读取

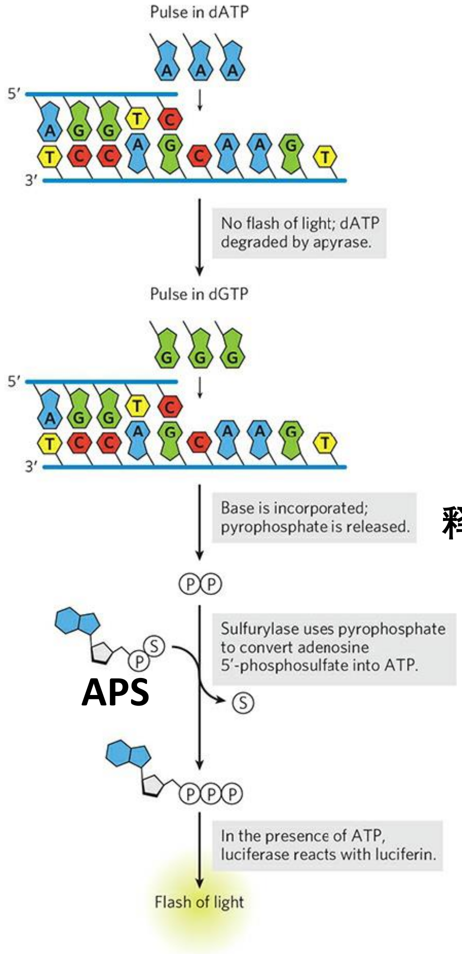




边合成边测序

Next-generation pyrosequencing
焦磷酸测序

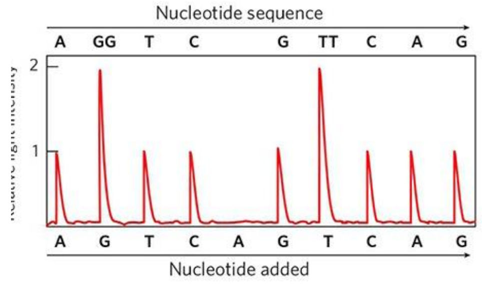
样品处理	利用超声或氮气先将大片段的DNA打断，通过琼脂糖凝胶电泳回收或磁珠纯化来选择500~800 bp的DNA片段
文库制备	包括接头连接和磁珠纯化2步，片段的两端被加入接头，只有两端接头不同种类的才会被磁珠富集
emPCR	将文库与测序磁珠等加入相关物质形成油包水乳浊液体系。每一个液滴，包含1个磁珠和1条单链DNA，经过PCR扩增后，每一个磁珠上将形成密集的DNA簇
反应板准备	反应板上有350万个小孔，每个孔中仅能容纳1个磁珠，将磁珠与测序试剂加入PTP中，使之可用于上机测序
上机测序	4种碱基依次加入反应板，每延伸1个或若干个碱基，就会发出1次光信号，通过记录信号的有无和强度，即可测定DNA序列



释放焦磷酸

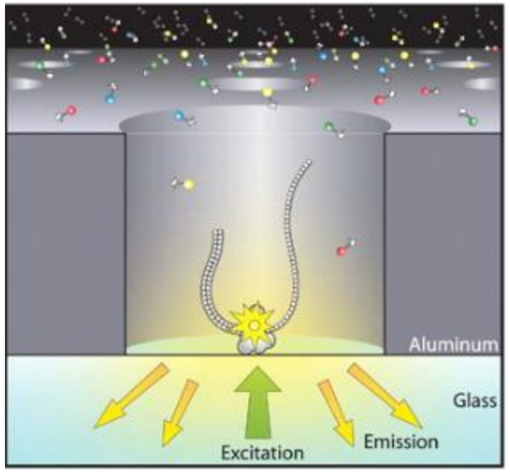
生成ATP

产生荧光素

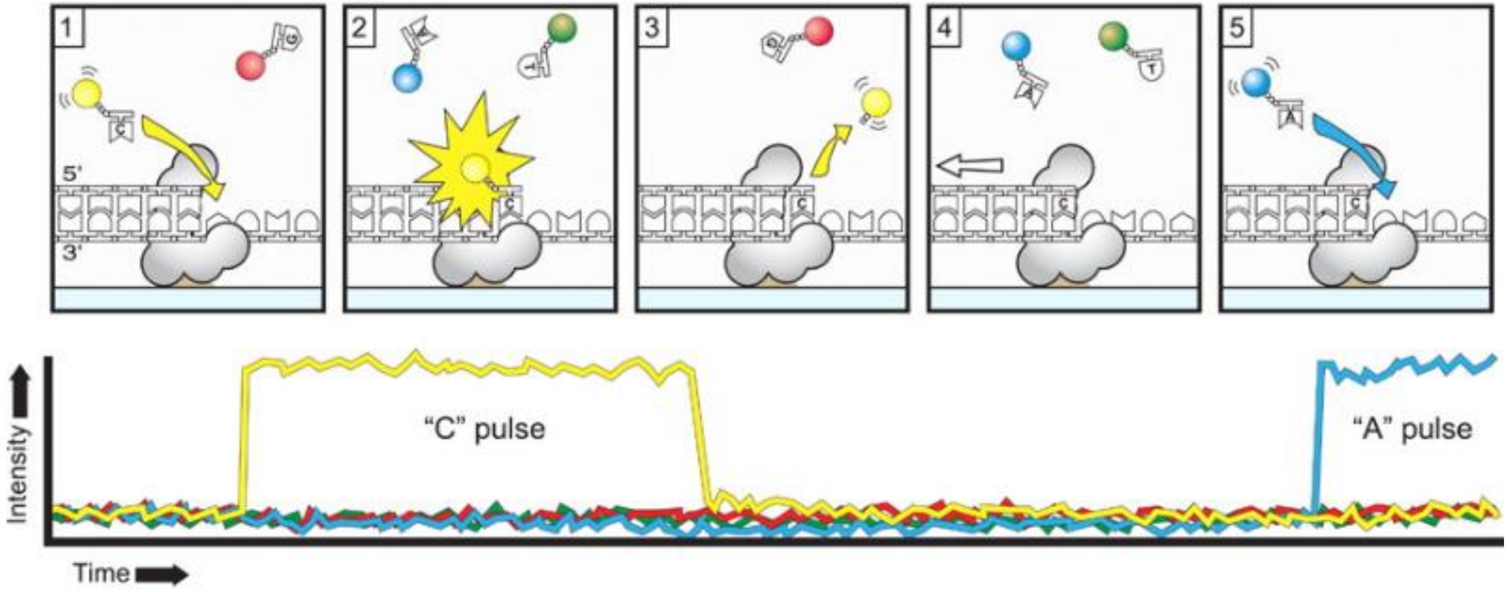


4种酶: DNA 聚合酶(DNA polymerase)、ATP硫酸化酶(ATP sulfurytase)、荧光素酶(luciferase)和三磷酸腺苷双磷酸酶(Apyrase)

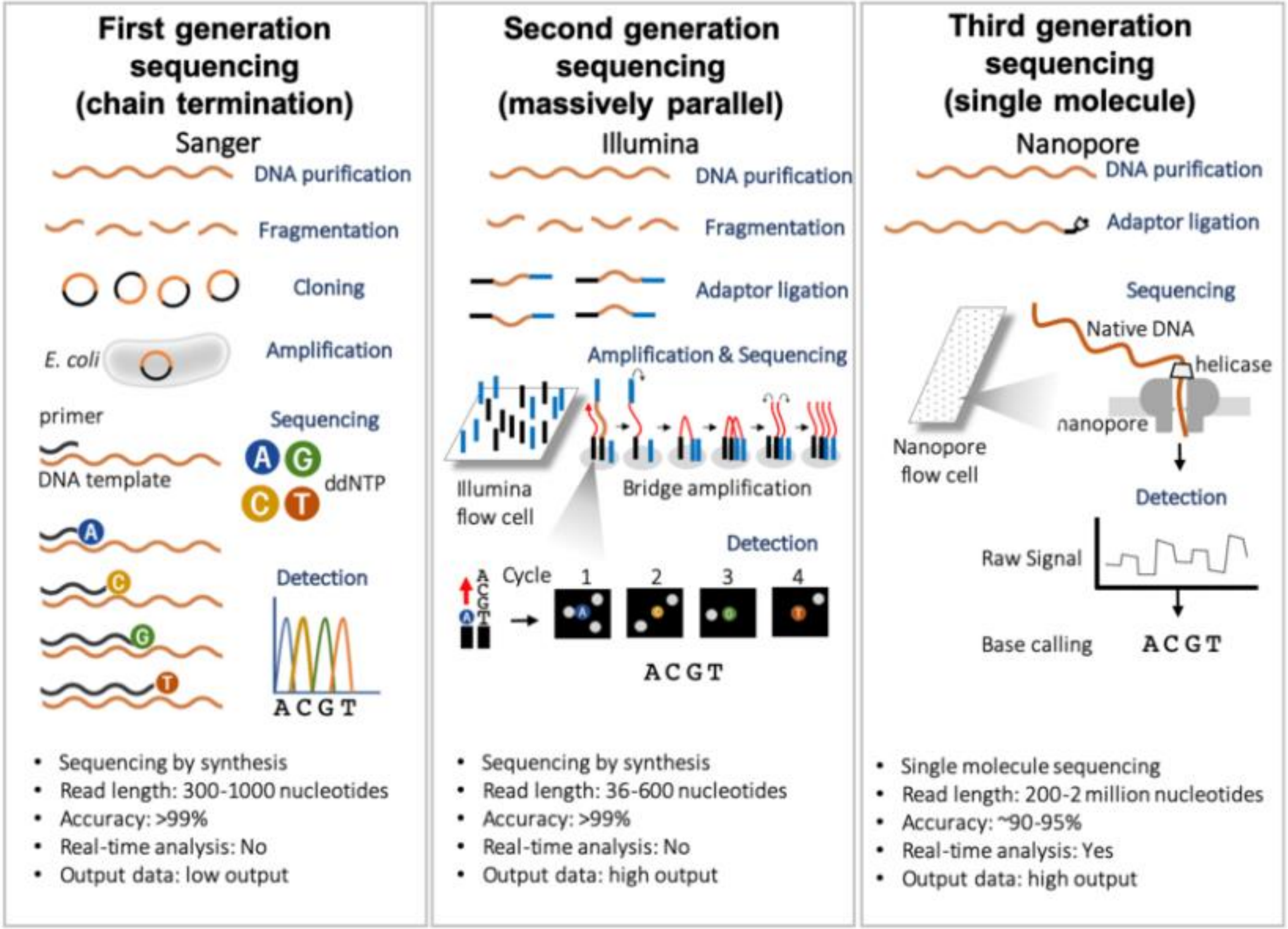
Third generation sequencing 三代测序



1. DNA不需要进行PCR扩增
2. 单分子成像技术的发展



三代测序技术总结


First generation sequencing (chain termination)
Sanger
DNA purification
Fragmentation
Cloning
E. coli
Amplification
primer
DNA template
Sequencing
ddNTP
Detection
ACGT
Second generation sequencing (massively parallel)
Illumina
DNA purification
Fragmentation
Adaptor ligation
Amplification & Sequencing
Illumina flow cell
Bridge amplification
Detection
Cycle
1
2
3
4
ACGT
Third generation sequencing (single molecule)
Nanopore
DNA purification
Adaptor ligation
Sequencing
Native DNA
helicase
nanopore
Nanopore flow cell
Detection
Raw Signal
Base calling
ACGT
Sequencing by synthesis
Read length: 300-1000 nucleotides
Accuracy: >99%
Real-time analysis: No
Output data: low output
Sequencing by synthesis
Read length: 36-600 nucleotides
Accuracy: >99%
Real-time analysis: No
Output data: high output
Single molecule sequencing
Read length: 200-2 million nucleotides
Accuracy: ~90-95%
Real-time analysis: Yes
Output data: high output

Chapter 7
Nucleotides and Nucleic Acids

7.1 ***Brief History of Nucleic Acids***

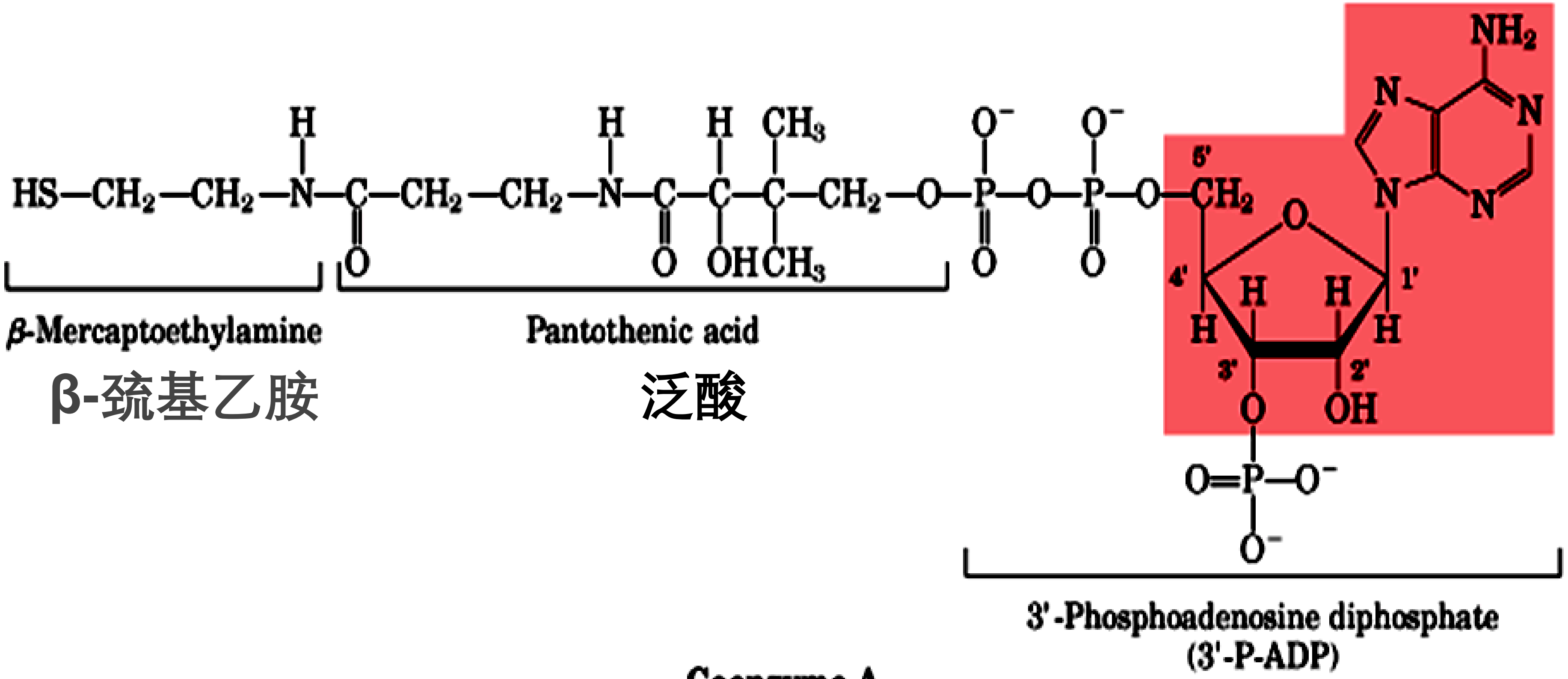
7.2 ***Nucleotides: the Building Blocks***

7.3 ***Nucleic Acids: from Structure to Functions***

7.4 ***Nucleic Acids-Based Biotechnology***

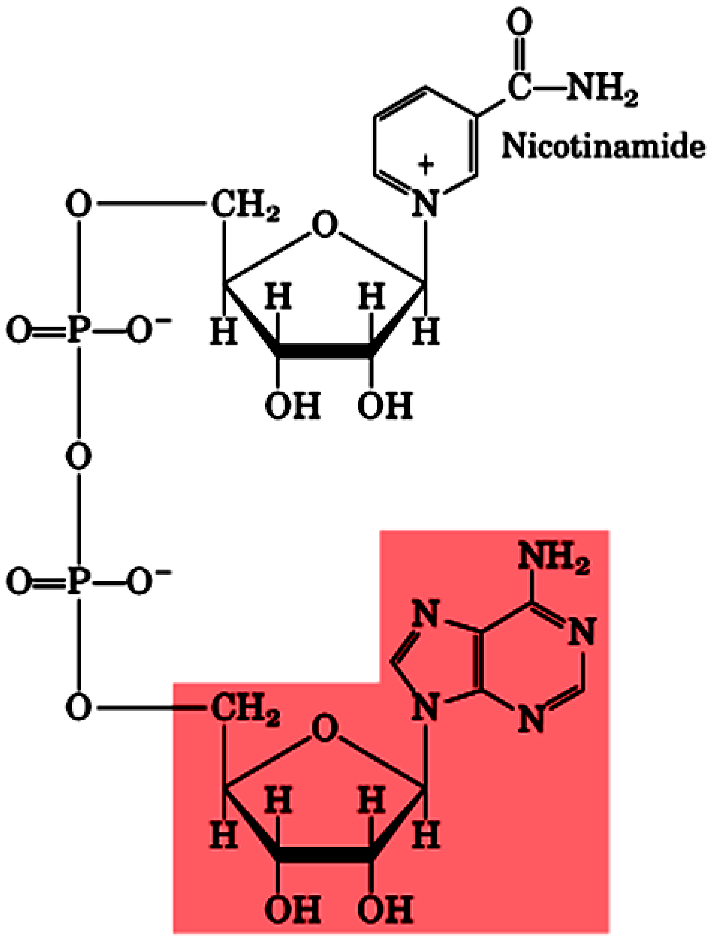
7.5 ***Other functions of Nucleotides***

Adenine nucleotides are components of many enzyme cofactors



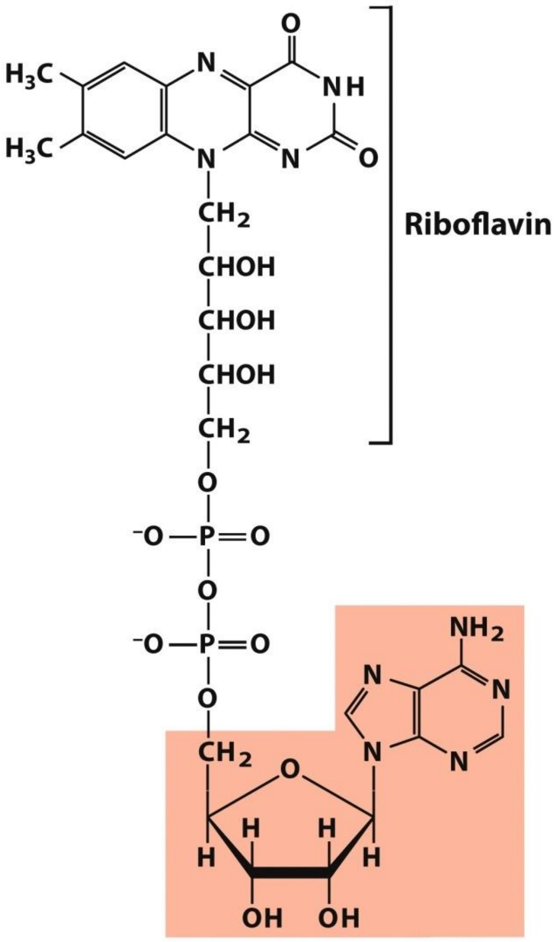
Coenzyme A

转移乙酰基



Nicotinamide adenine dinucleotide (NAD^+)

烟酰胺腺嘌呤二核苷酸

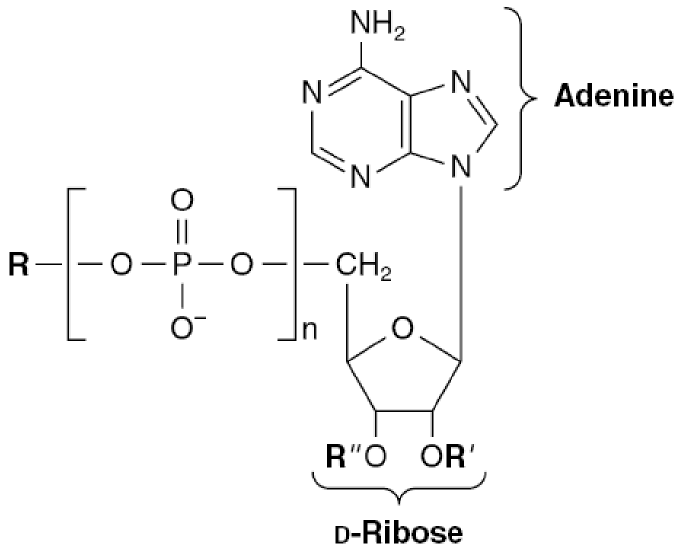


Flavin adenine dinucleotide (FAD)

黄素腺嘌呤二核苷酸

传递氢

Many coenzymes and related compounds are derivatives of adenosine monophosphate

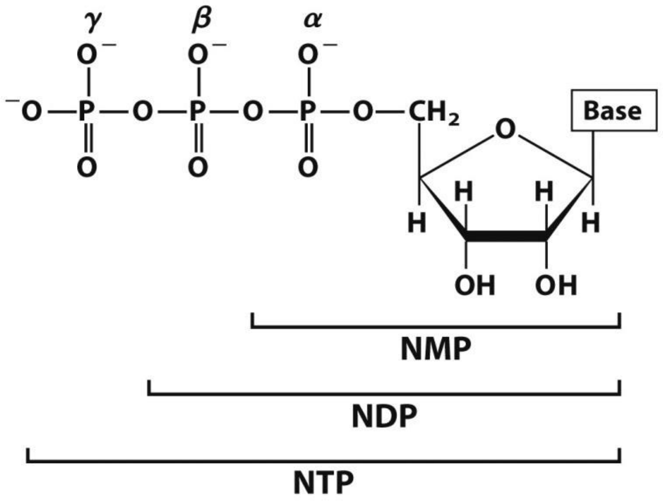


Coenzyme	R	R′	R″	n
Active methionine	Methionine*	H	H	0
Amino acid adenylates	Amino acid	H	H	1
Active sulfate	SO_3^{2-}	H	PO_3^{2-}	1
3′,5′-Cyclic AMP		H	PO_3^{2-}	1
NAD*	†	H	H	2
NADP*	†	PO_3^{2-}	H	2
FAD	†	H	H	2
CoASH	†	H	PO_3^{2-}	2

*Replaces phosphoryl group.
†R is a B vitamin derivative.

Nucleoside phosphates

经济中心
能量货币



Abbreviations of ribonucleoside 5′-phosphates			
Base	Mono-	Di-	Tri-
Adenine	AMP	ADP	ATP
Guanine	GMP	GDP	GTP
Cytosine	CMP	CDP	CTP
Uracil	UMP	UDP	UTP

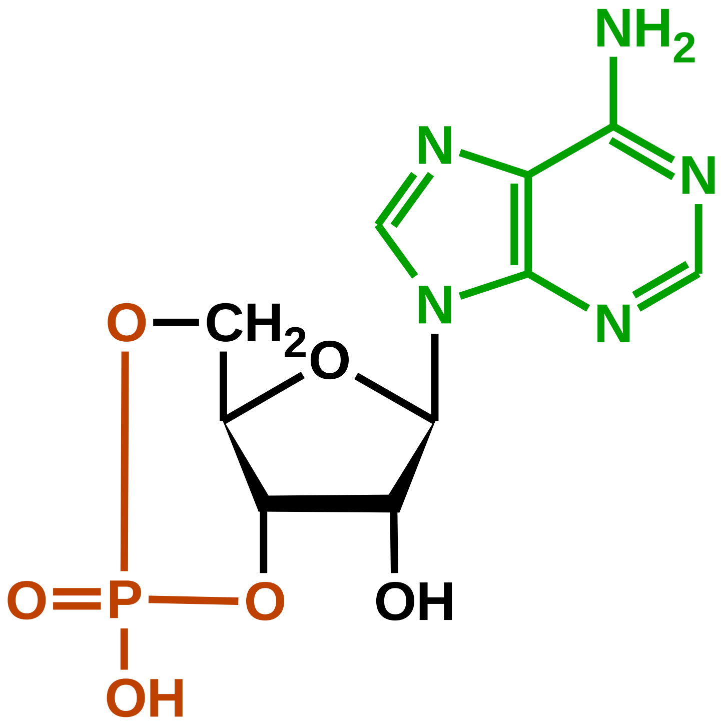
Abbreviations of deoxyribonucleoside 5′-phosphates			
Base	Mono-	Di-	Tri-
Adenine	dAMP	dADP	dATP
Guanine	dGMP	dGDP	dGTP
Cytosine	dCMP	dCDP	dCTP
Thymine	dTMP	dTDP	dTTP

ATP: central role of energy metabolism
GTP: drives protein synthesis
CTP: drives lipid synthesis
UTP: drives carbohydrate metabolism

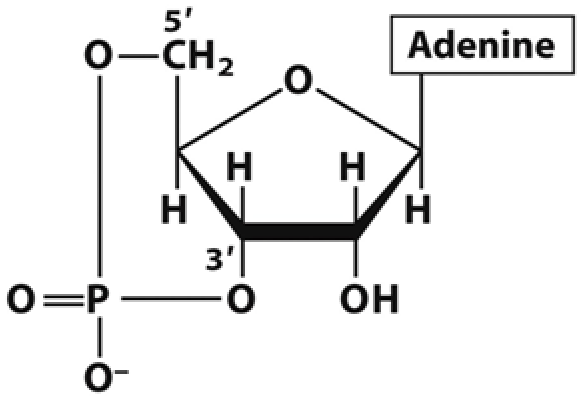
核苷酸衍生物

环化核苷酸：cAMP、cGMP，是细胞信号转导中的第二信使。

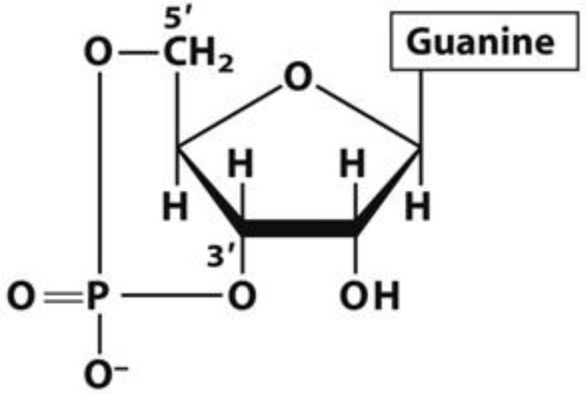
cAMP



行政中心
第二信使



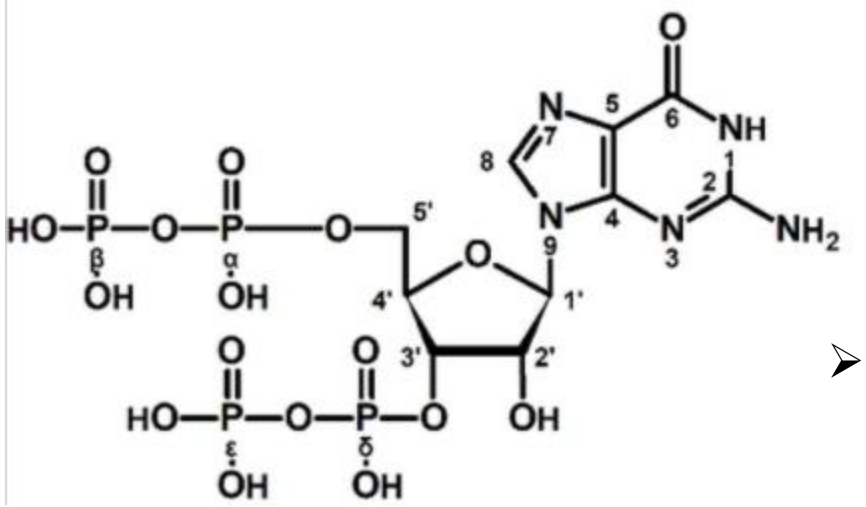
Adenosine 3′,5′-cyclic monophosphate
(cyclic AMP; cAMP)



Guanosine 3′,5′-cyclic monophosphate
(cyclic GMP; cGMP)

cAMP: olfactory sensation, taste sense

cGMP: visual sense



ppGpp: **produced in bacteria in response to a slowdown in protein synthesis during amino acid starvation**

- **ATP and ADP serve as neurotreansmitters in a variety of signaling pathways.**

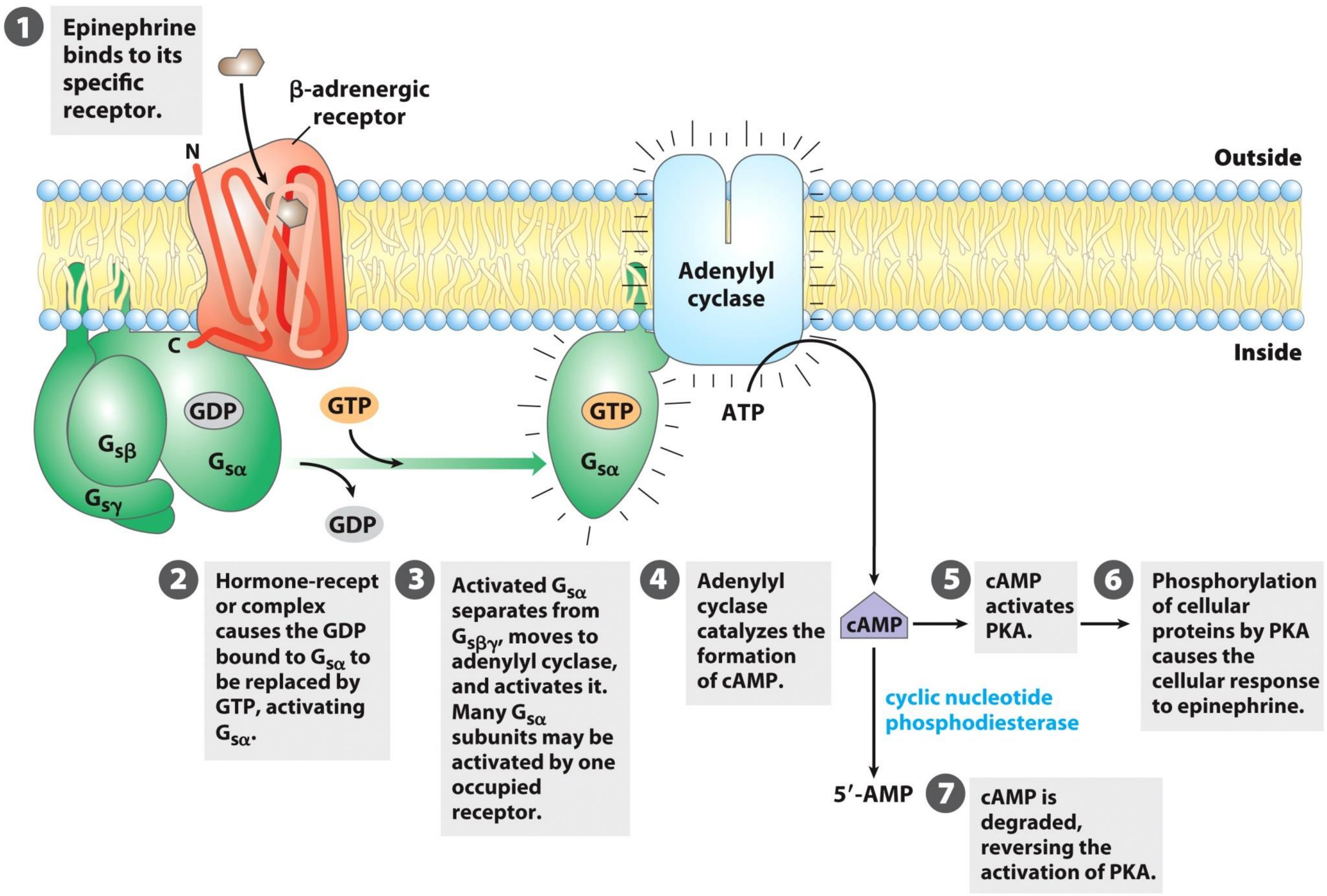
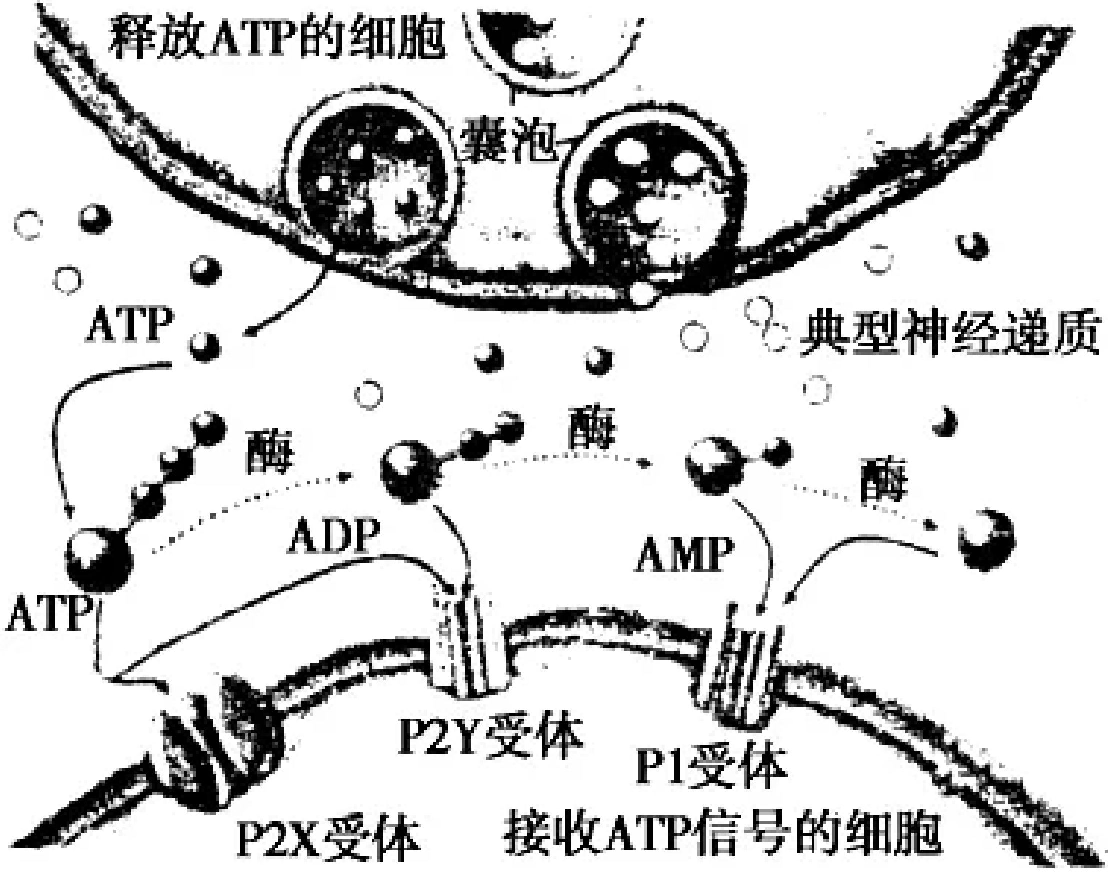
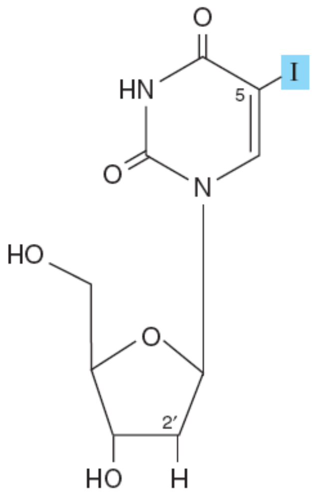

1 Epinephrine binds to its specific receptor.
β-adrenergic receptor
N
C
Outside
Inside
Adenylyl cyclase
GDP
GTP
$G_{s\beta}$
$G_{s\alpha}$
$G_{s\gamma}$
GTP
GDP
GTP
$G_{s\alpha}$
ATP
2 Hormone-recept or complex causes the GDP bound to $G_{s\alpha}$ to be replaced by GTP, activating $G_{s\alpha}$.
3 Activated $G_{s\alpha}$ separates from $G_{s\beta\gamma}$, moves to adenylyl cyclase, and activates it. Many $G_{s\alpha}$ subunits may be activated by one occupied receptor.
4 Adenylyl cyclase catalyzes the formation of cAMP.
cAMP
5 cAMP activates PKA.
6 Phosphorylation of cellular proteins by PKA causes the cellular response to epinephrine.
cyclic nucleotide phosphodiesterase
5′-AMP
7 cAMP is degraded, reversing the activation of PKA.

Figure 12-4a

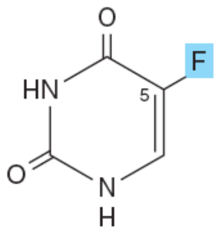
ATP作为信号分子


释放ATP的细胞
囊泡
ATP
典型神经递质
酶
酶
酶
ADP
AMP
ATP
P2Y受体
P1受体
P2X受体
接收ATP信号的细胞

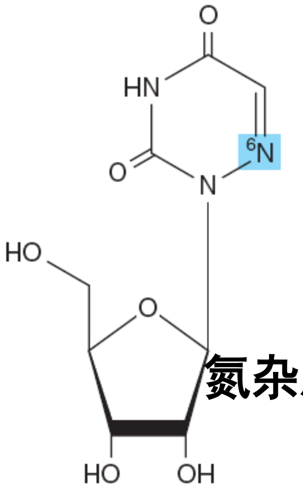
Selected synthetic pyrimidine and purine analogs



5-Iodo-2′-deoxyuridine

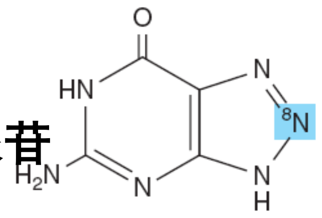


5-Fluorouracil

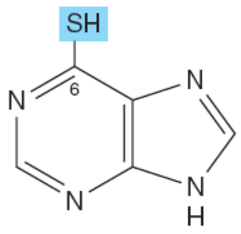


氮杂尿苷

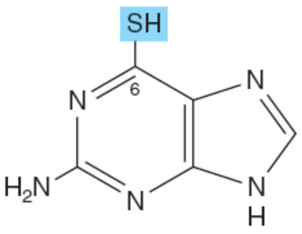
6-Azauridine



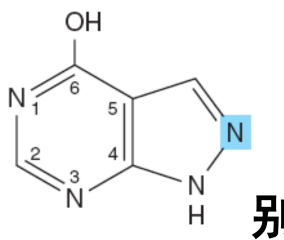
8-Azaguanine



6-Mercaptopurine



6-Thioguanine



别嘌呤醇

Alloburinol

抗肿瘤药物

Summary: Functions of Nucleotides

- **Building blocks of genetic materials**

- **Energy carriers**
  - **ATP: central role of energy metabolism**
  - **GTP: drives protein synthesis**
  - **CTP: drives lipid synthesis**
  - **UTP: drives carbohydrate metabolism**

- **Signaling molecules**
  - **cGMP: visual sense**
  - **cAMP: olfactory sensation, taste sense**

- **Allosteric effectors （酶的变构效应分子）**

核酸分子的大小

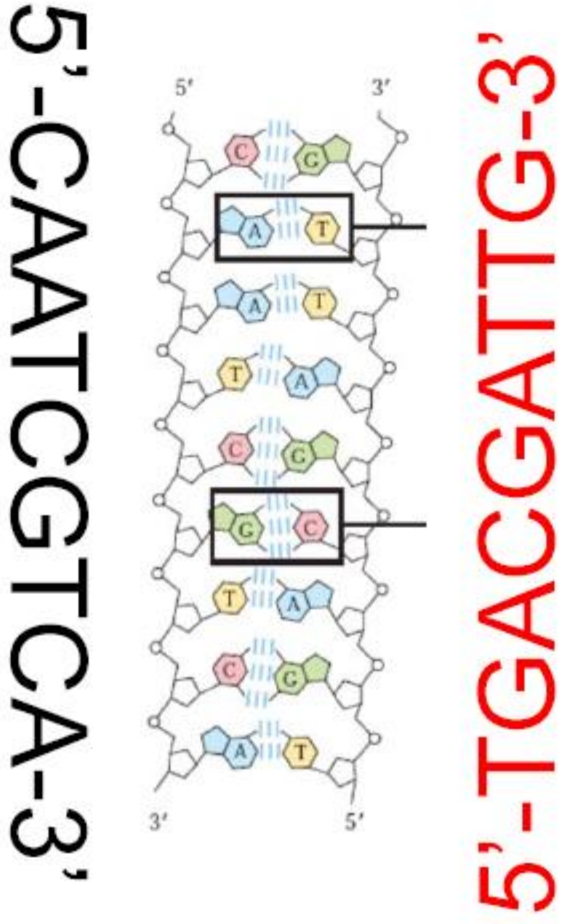
单链常用碱基数目来表示（nt，nucleotides）

双链常用碱基对数目来表示（bp，base-pairs；kb，kilo-base-pairs）

寡核苷酸（oligonucleotide）<50bp

通常DNA的长度可高达千万个碱基对

遗传信息的编码方式（1级结构）



5’-CAATCGTCA-3’

5’-TGACGATTG-3’

5’-CATTCGTCA-3’

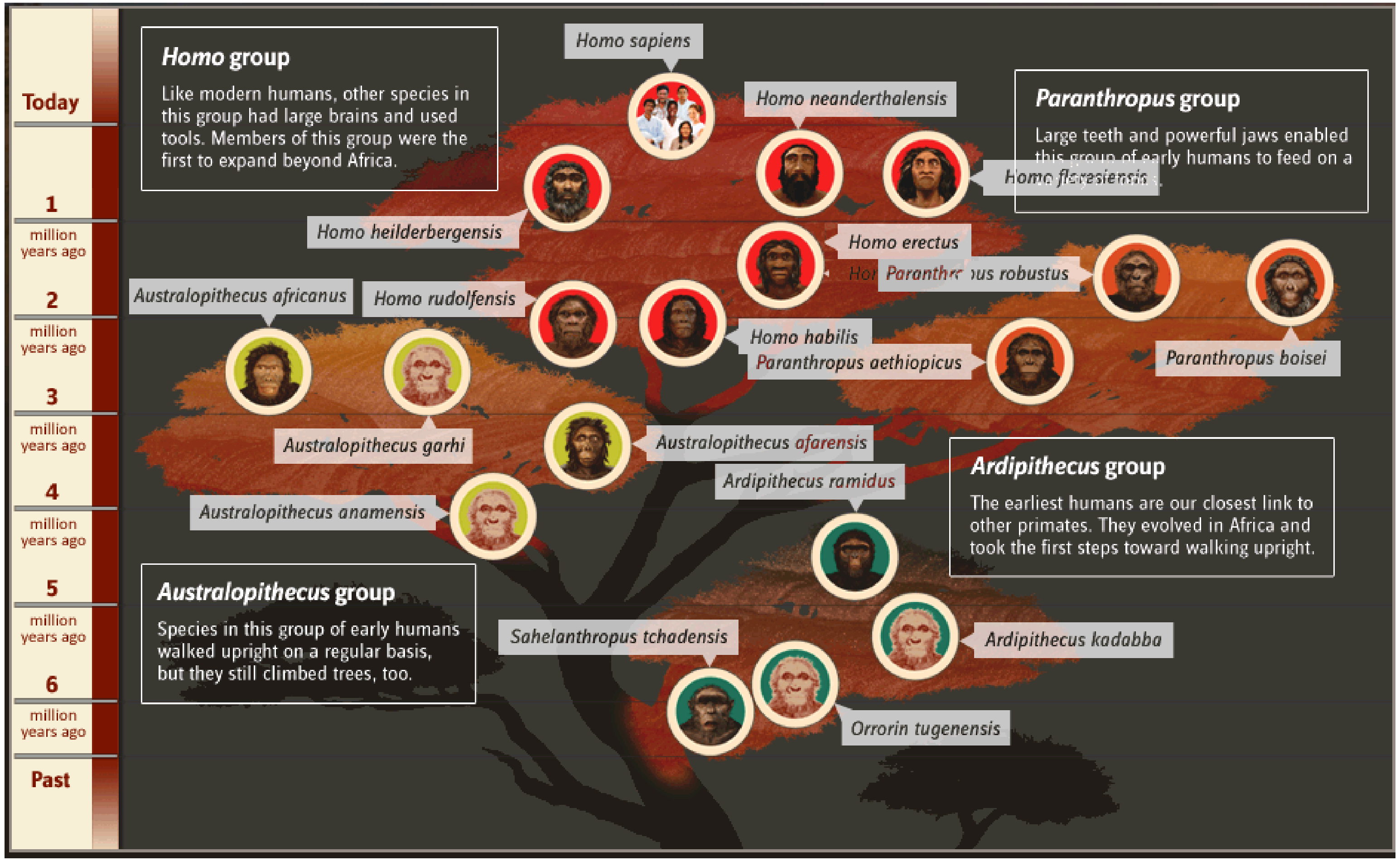
5’-CACTTGTCA-3’

5’-CACTATTCA-3’

遗传信息的差异程度决定亲缘的远近

我们是怎么来的?

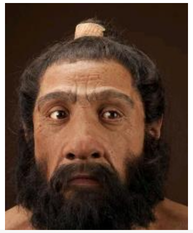
- 人类及灭绝的近亲http://humanorigins.si.edu/

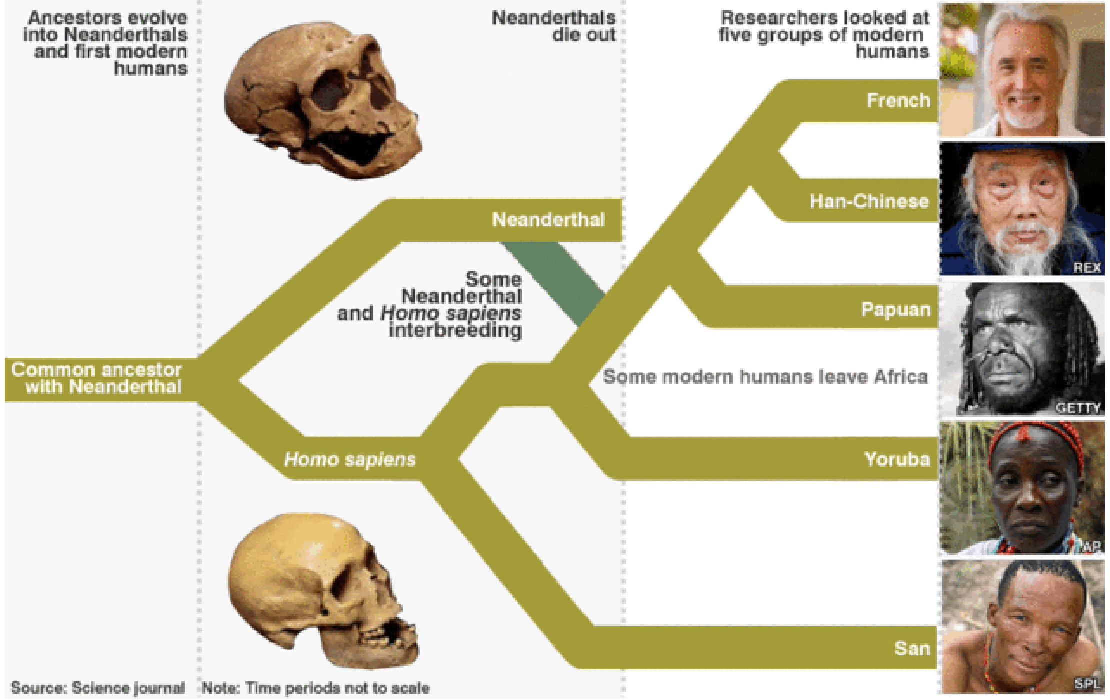


人类的血统

1%的尼安德特人基因

《科学》2010





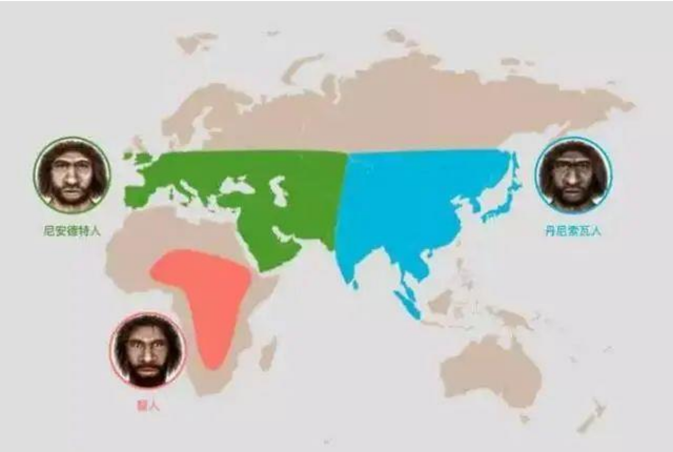
追溯人类的起源

- 走出非洲学说

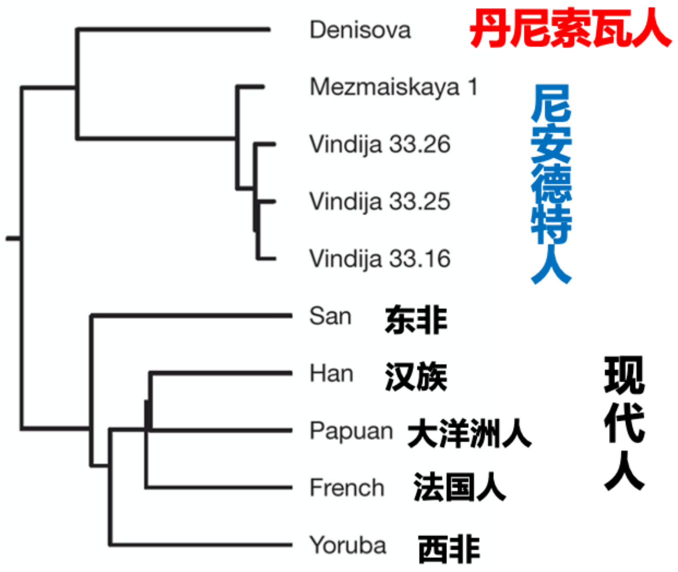
https://genographic.nationalgeographic.

人类的血统

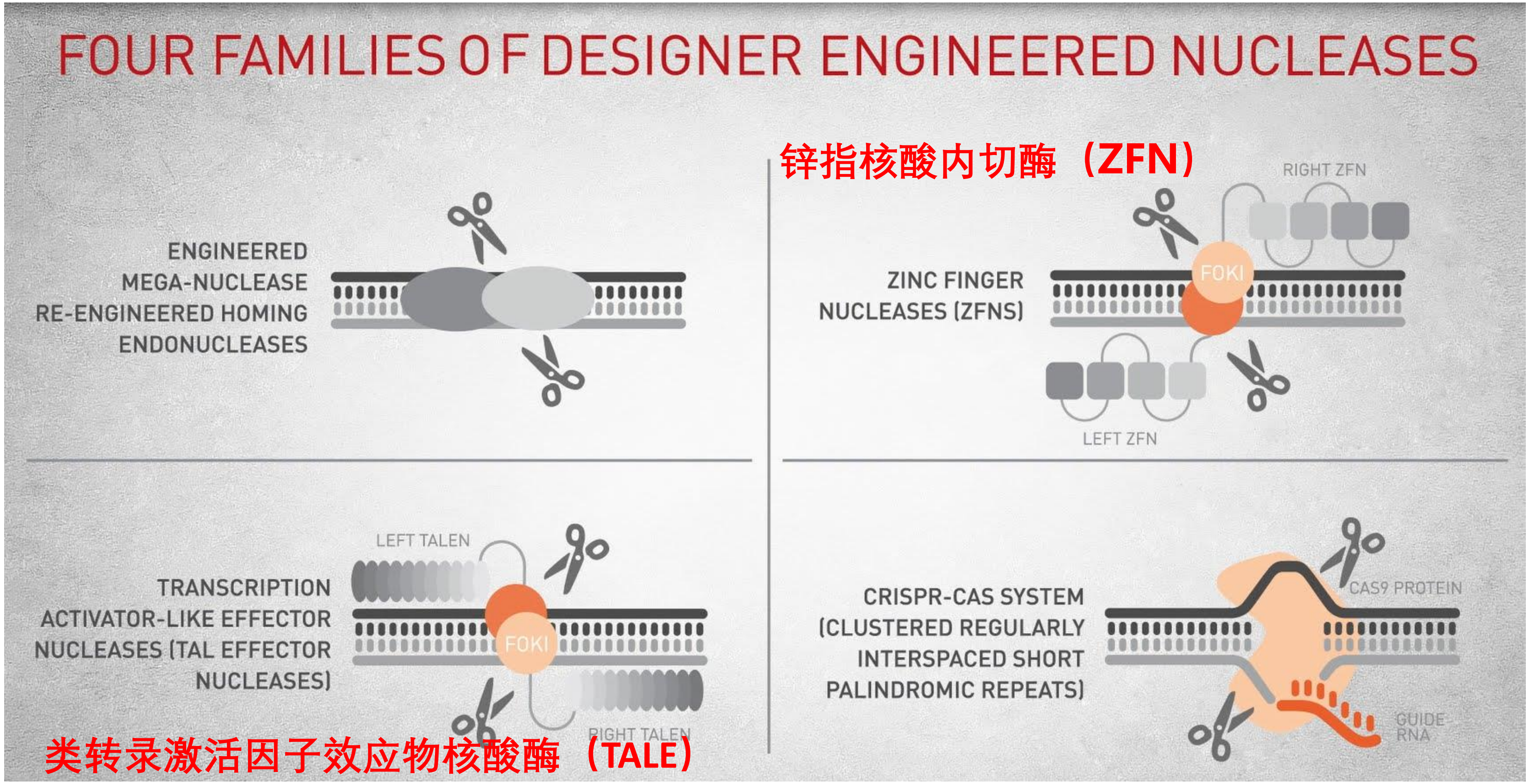
新发现的丹尼索瓦人基因

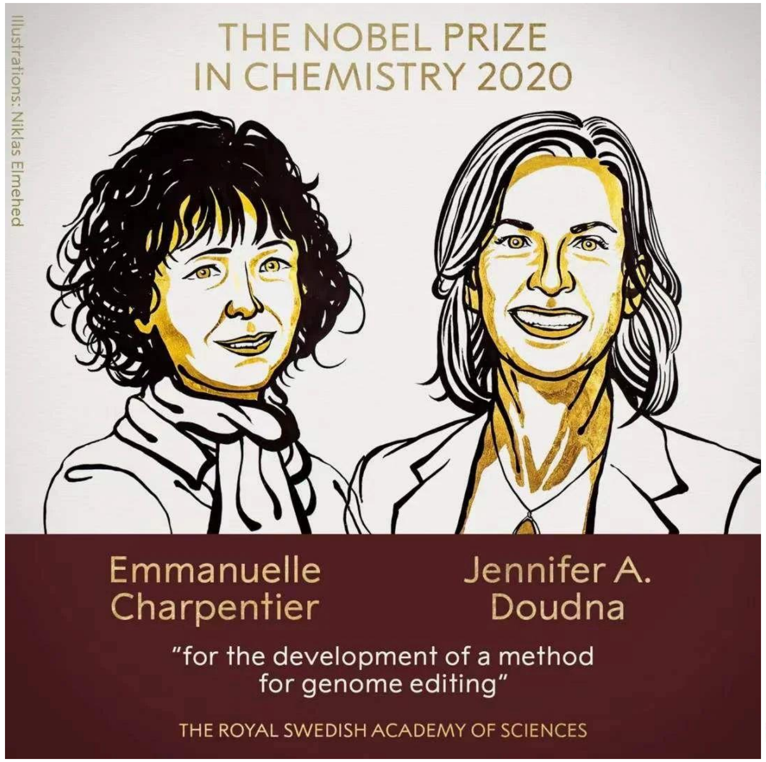


基于核DNA测序的人类谱系分类树



Genome editing technology 基因组编辑技术

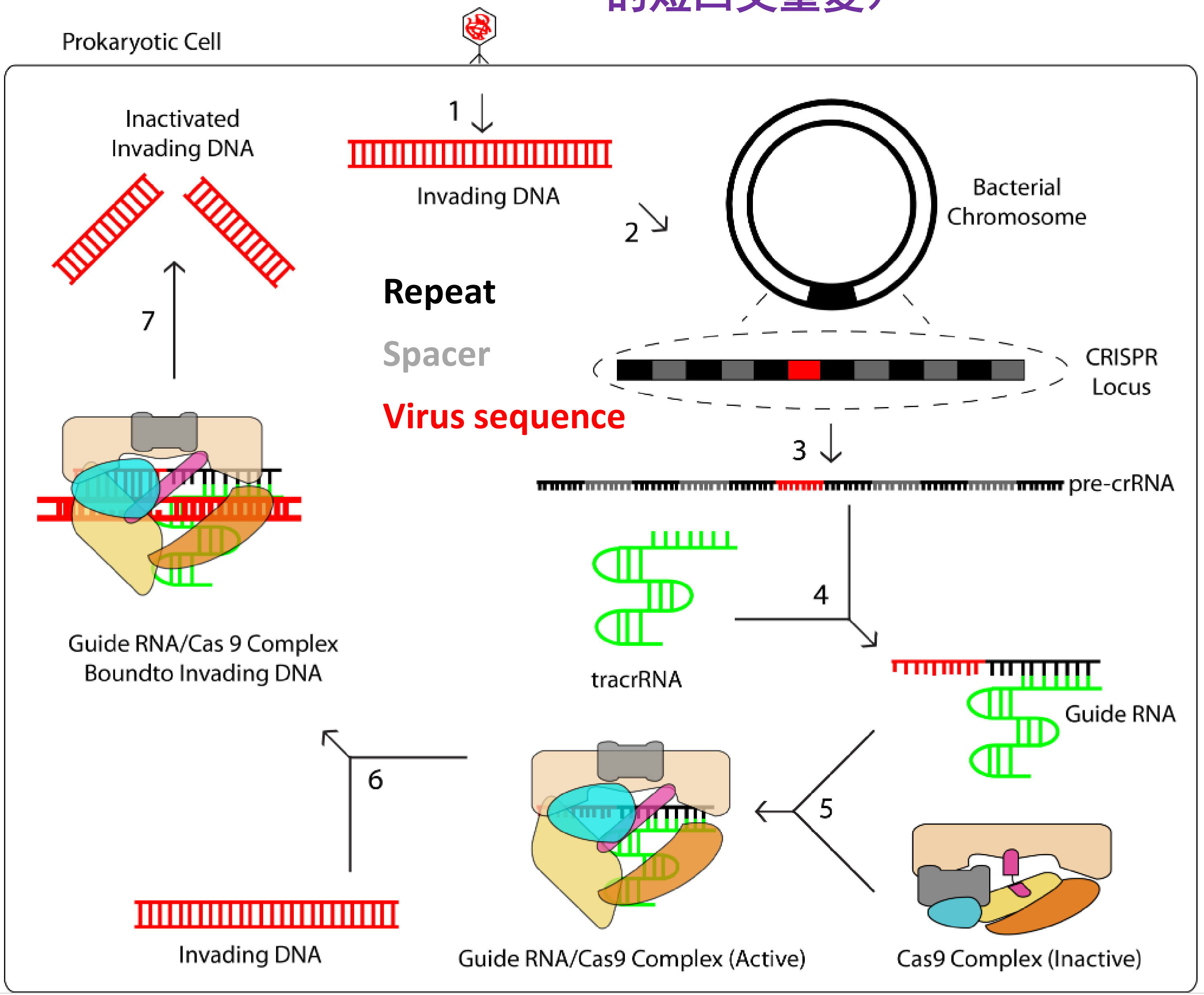




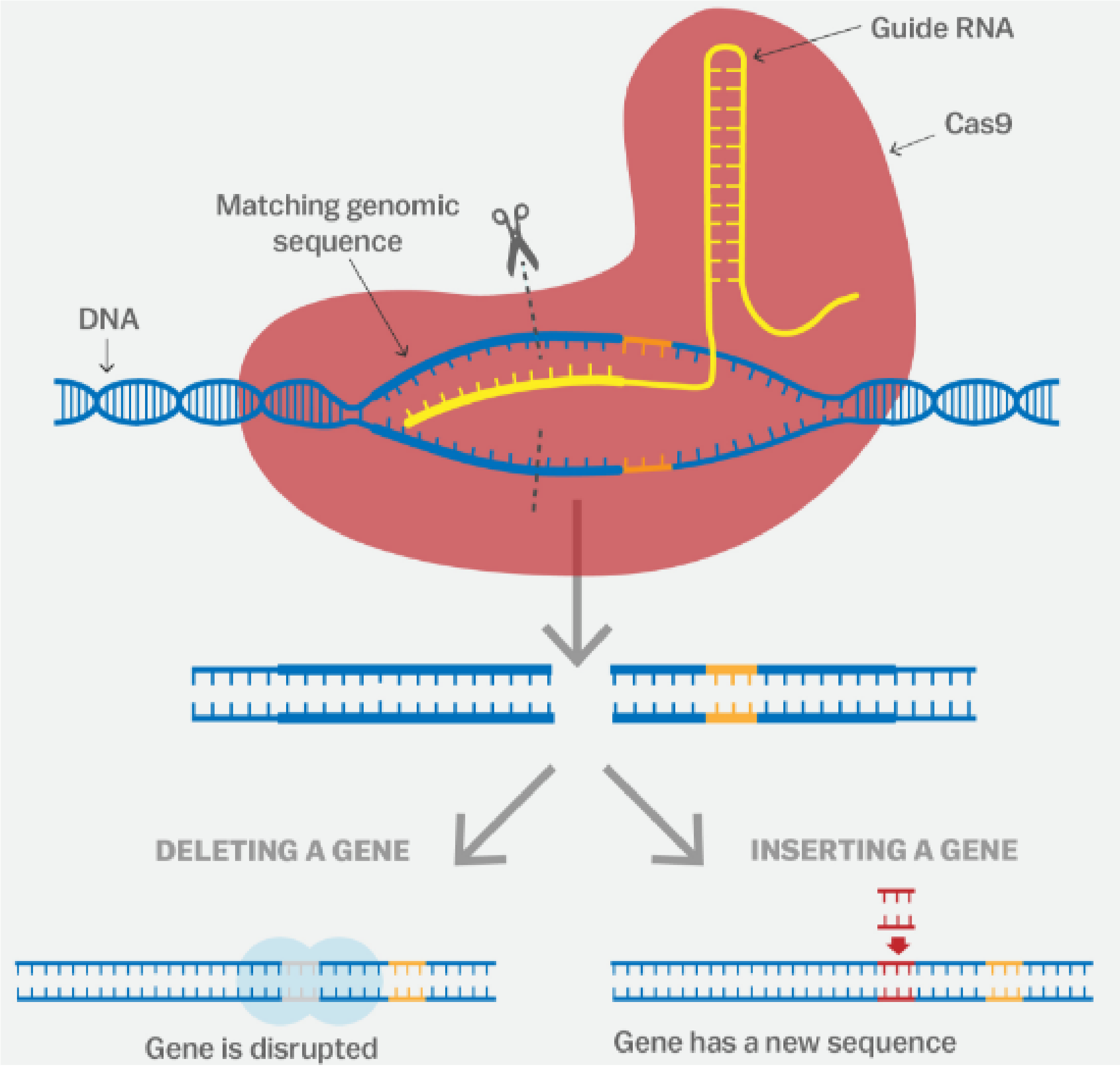
"开发出一种基因组编辑方法" 极其精确地修改动物、植物和微生物的DNA。

CRISPR/Cas9基因剪刀已经彻底改变分子生命科学，为植物育种带来了新的机遇，为新的癌症疗法做出贡献，还有可能使人类治愈遗传疾病的梦想成为现实。

CRISPR（Clustered regularly interspaced short palindromic repeats，有规则间隔的短回文重复）



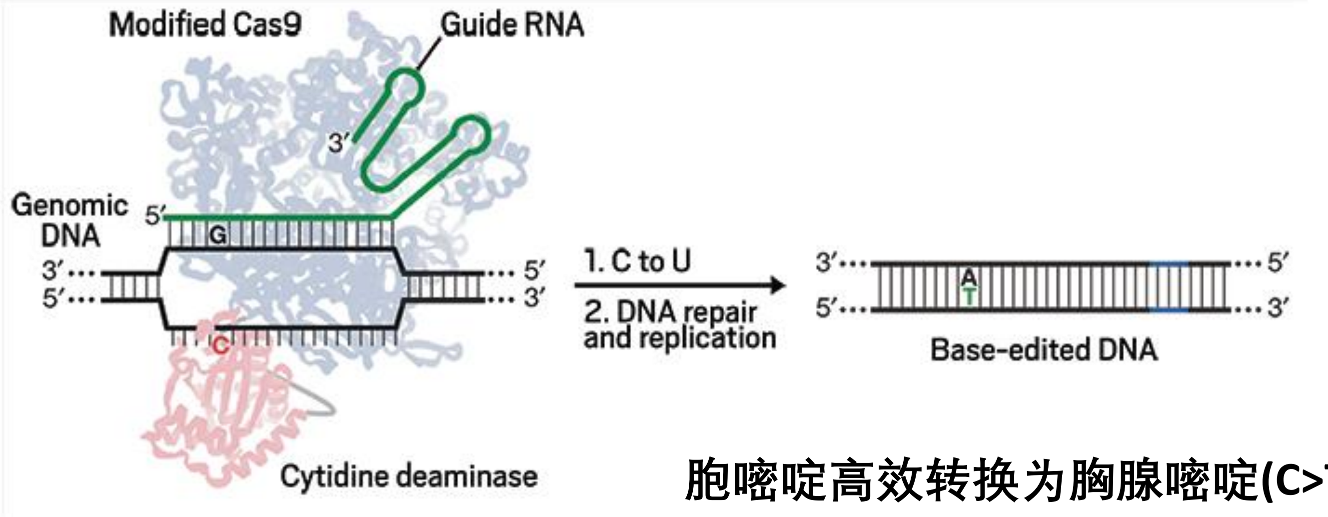
CRISPR-Cas 基因编辑系统



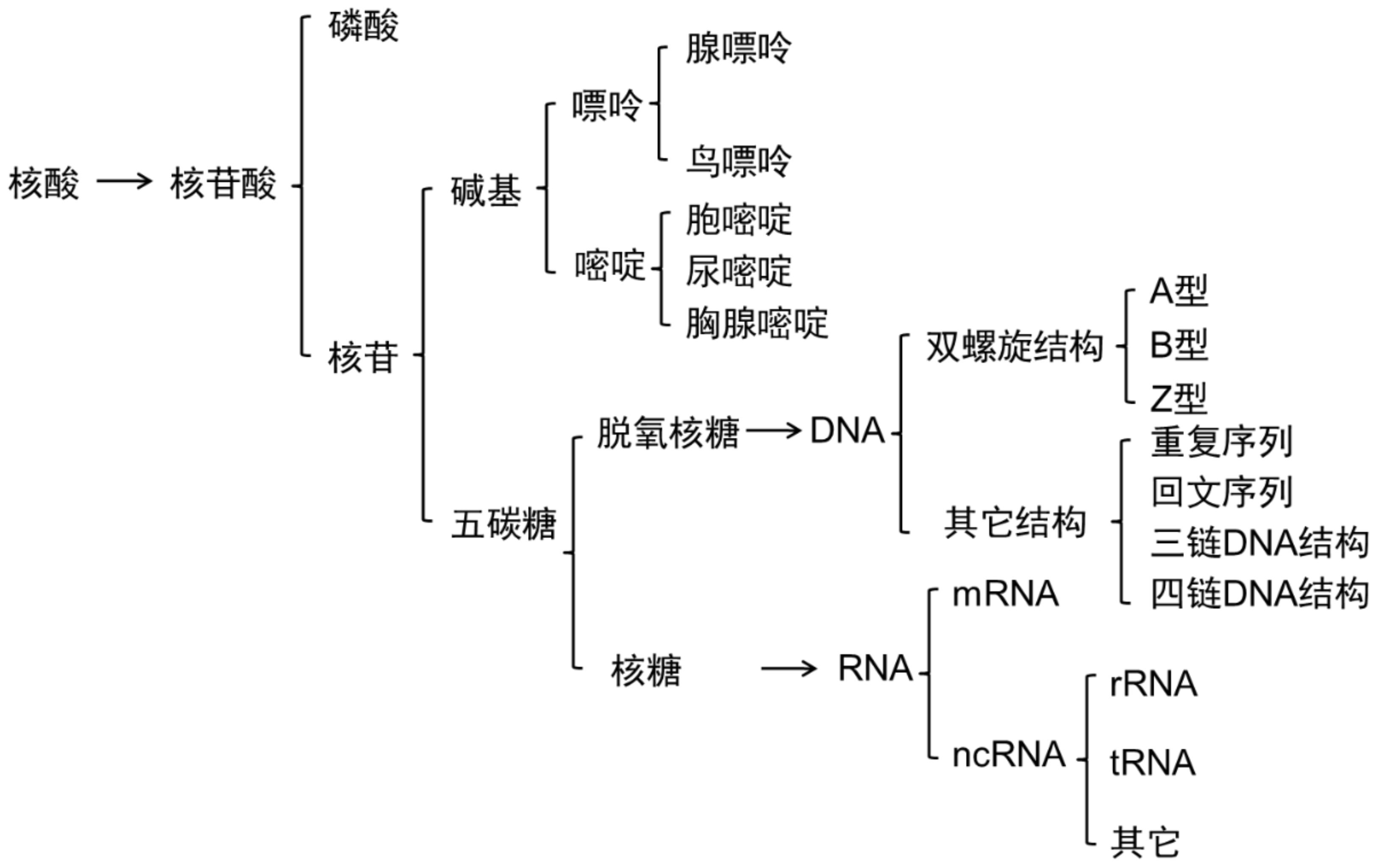
利用一个Cas酶和一段RNA就可以实现基因编辑

Base Editor（BE）单碱基基因编辑系统

胞嘧啶(cytosine, C)　　尿嘧啶(uracil, U)



胞嘧啶高效转换为胸腺嘧啶(C>T)



核酸
核苷酸
磷酸
核苷
碱基
嘌呤
腺嘌呤
鸟嘌呤
嘧啶
胞嘧啶
尿嘧啶
胸腺嘧啶
五碳糖
脱氧核糖
DNA
双螺旋结构
A型
B型
Z型
其它结构
重复序列
回文序列
三链DNA结构
四链DNA结构
核糖
RNA
mRNA
ncRNA
rRNA
tRNA
其它

- 核酸相关技术
  - 核酸测序技术
    - 一代测序技术
    - 二代测序技术
    - 三代测序技术
  - PCR技术
  - 人类基因组计划与进化分析
  - 基因编辑技术

学习目标

- 掌握碱基、核苷和核苷酸的化学结构和理化性质
- 掌握DNA和RNA在结构上和功能上的异同
- 了解核酸的一级结构、二级结构和三级结构的定义
- 了解几种常见的RNA的名称、结构（一级、二级和三级结构）及其功能
- 掌握DNA双螺旋结构的主要内容，A型、B型和Z型双螺旋结构之间的异同
- 掌握核酸的理化性质，特别是DNA的变性、复性及其杂交，知道影响DNA变性和复性的因素
- 了解DNA的生物功能

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

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[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

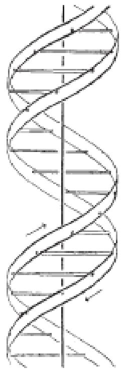
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at