讨论题

1. 过去半个世纪以来，世界上很多国家的人们结婚和生育后代的年龄比他们的父辈和爷爷辈要晚。这种晚婚晚育的趋势对于late-acting的显性致命等位基因可能有什么影响?
2. 人类基因组计划的伟大意义和面临的问题。
3. 对于人的性格，智商等行为，先天和后天哪个更重要? 从行为遗传学和遗传力的角度，介绍经典的双胞胎研究和领养研究实验。讨论基因和环境，哪个对人类行为更重要。
4. 都有哪些机制可以产生遗传的多样性?
5. 生男生女? 在某些动物中，这个问题是由温度决定的。那么温度决定性别适用于人类吗? 如果人类性别由温度决定，我们的生活会是什么样?
6. X染色体失活如何成就三色猫（Calico cat）的毛色?

Lecture 10

遗传密码与蛋白质合成

授课教师：孙亚东

上周内容:

1. Gregor Mendel (1822-1884)-豌豆杂交和遗传学实验

   基因的分离定律和自由组合定律

2. Thomas Morgan (1866-1945)-果蝇

   染色体遗传基因的连锁、交换和伴性遗传（确立了基因学说）

基因以直线形式排列，它决定着一个特定的性状，而且能发生突变并随着染色体同源节段的互换而交换，它不仅是决定性状的功能单位，而且是一个突变单位和交换单位。至此，人们对基因概念的理解更加具体和丰富了。

本周内容:

1. 遗传信息（基因）是有什么物质组成的?
2. 遗传信息（基因）是如何工作的?

亲子鉴定

- 比较的是父母和孩子的什么DNA序列?
- 为什么DNA序列可以作为比较依据?

- 以孟德尔遗传为代表的经典遗传和以分子生物学为基础的现代遗传有什么区别?

经典遗传

- 垂直遗传

  （杂交—从亲代传递到子代）
- 遗传物质在细胞内

  （具体是什么不知道）

分子遗传

- 水平遗传
- 转化现象——细菌细胞之间可以交换遗传物质
- 分离细胞内遗传物质

遗传信息是由什么组成的?

遗传信息特点：多样性，稳定，可复制

DNA，RNA，蛋白质?

研究思路：从豌豆、果蝇等复杂的生命形式的研究，
转移到细菌、病毒等简单生命形式的研究

DNA is the carrier of genetic information

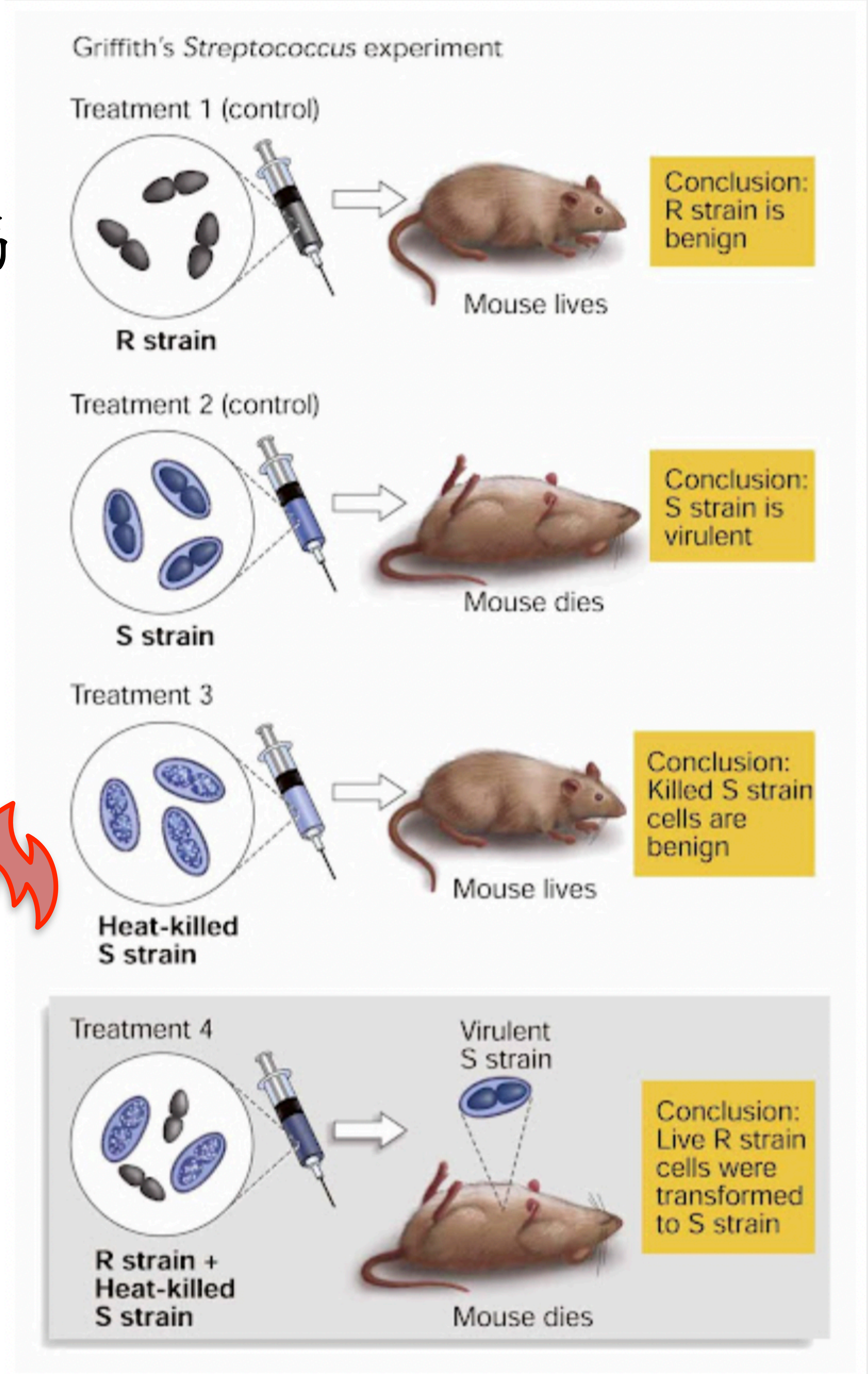
Frederick Griffith

(1877–1941)

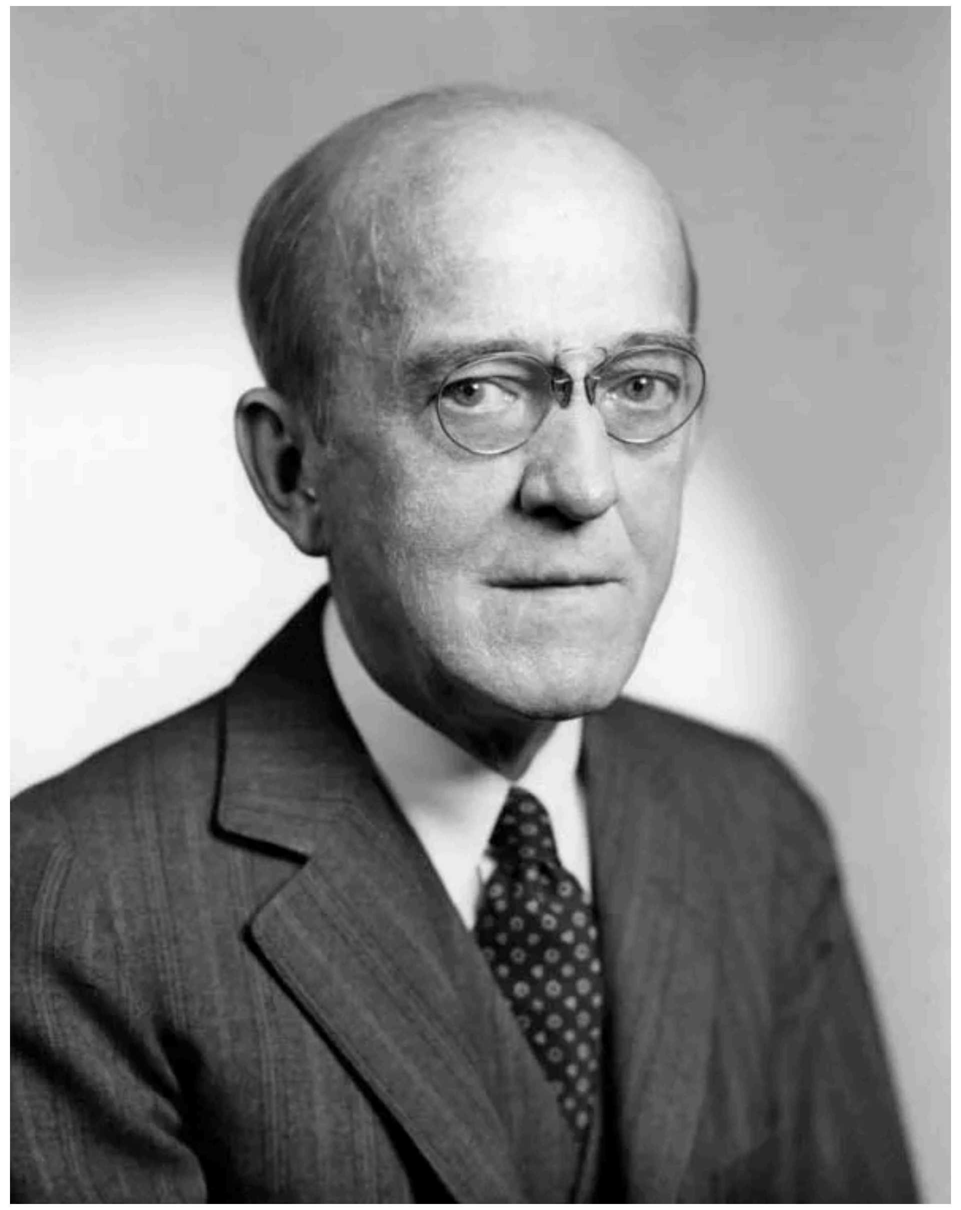
1928年，肺炎球菌

R (rough) —不致病

S (smooth) —致病



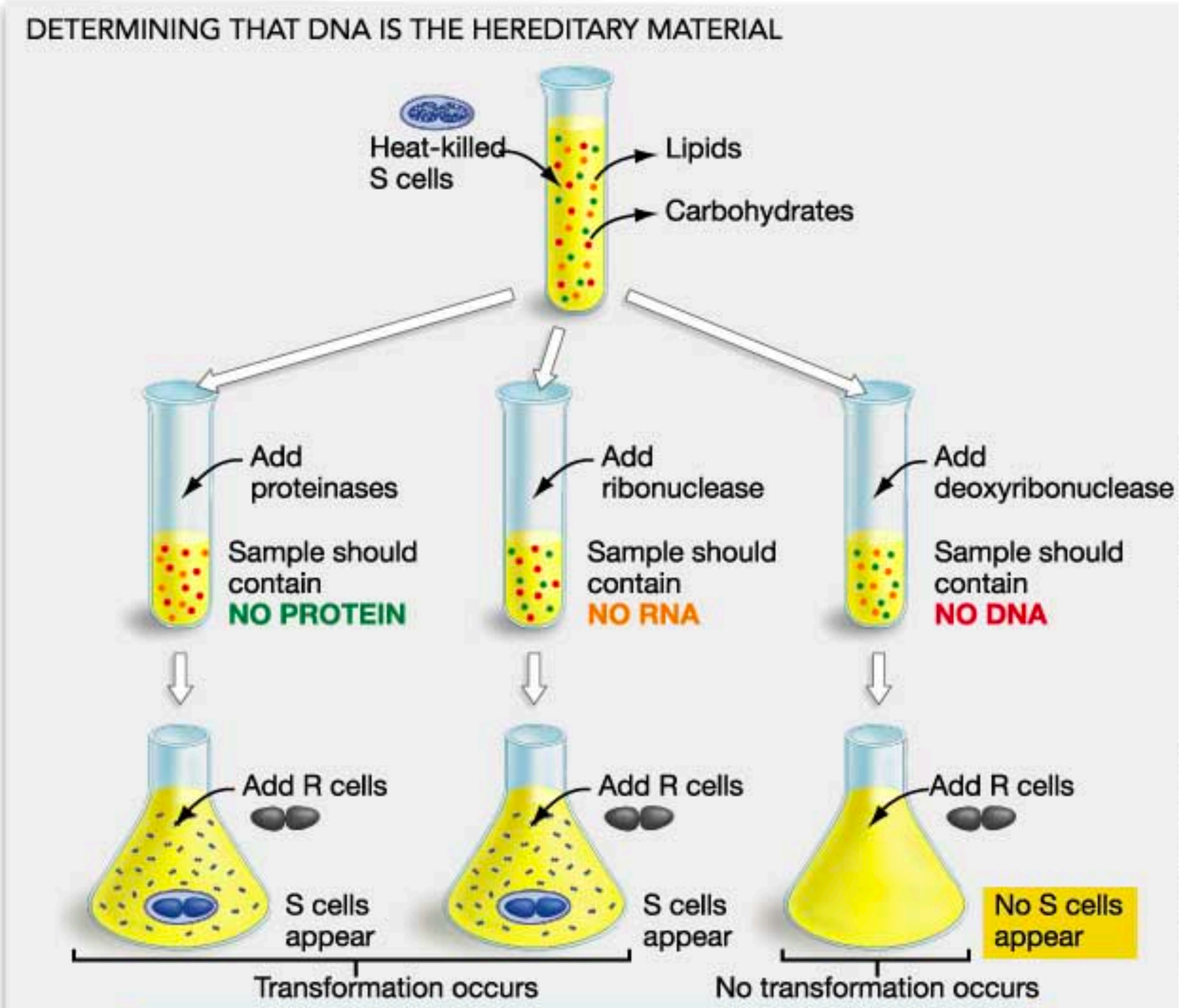
DNA is the carrier of genetic information



Oswald Avery

(1877–1955)

1944年，



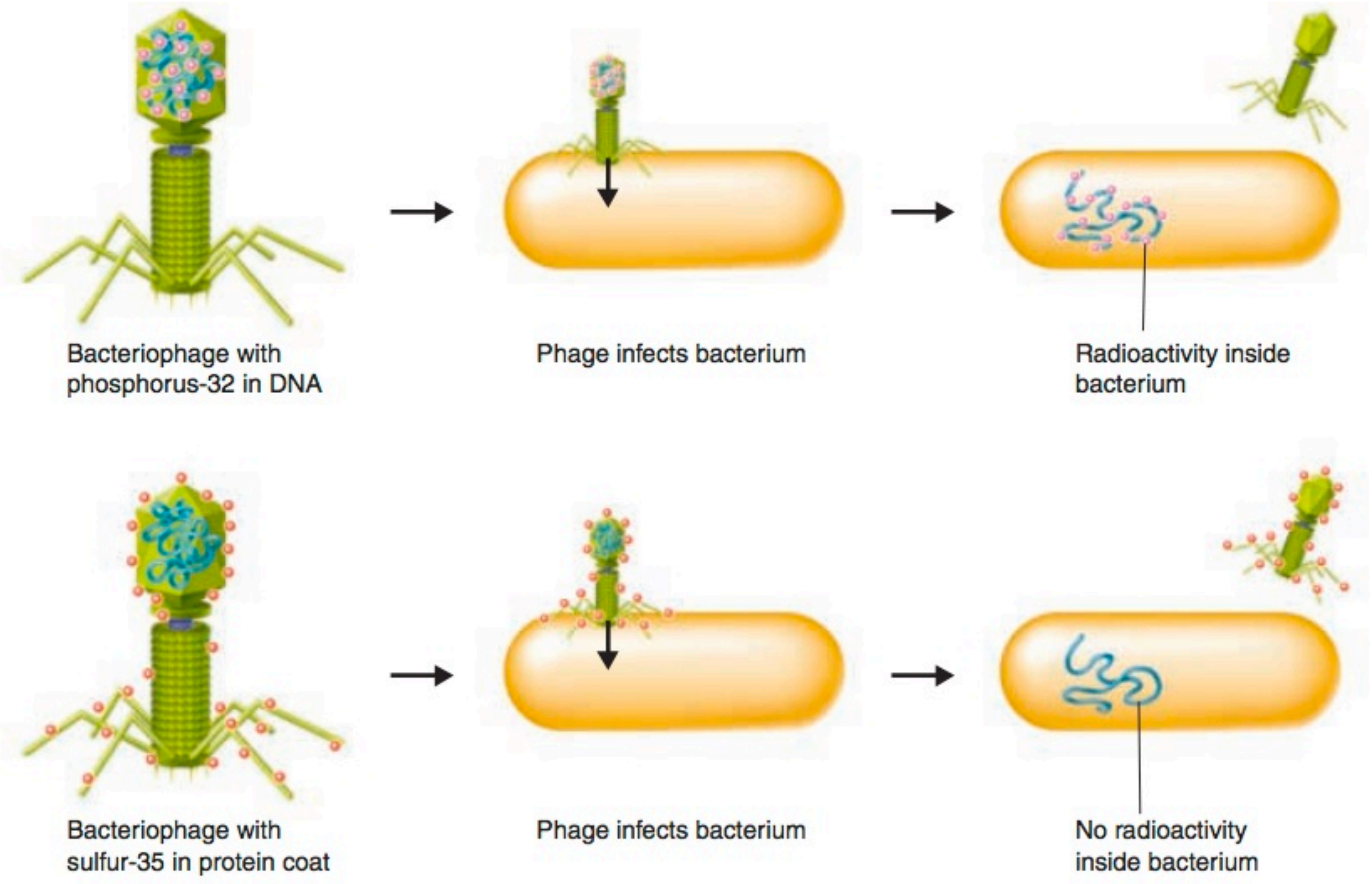
证实了转化因子（即DNA）是遗传物质—被认为是二十世纪最重要的生物学实验！

噬菌体实验-再次证明DNA是遗传物质

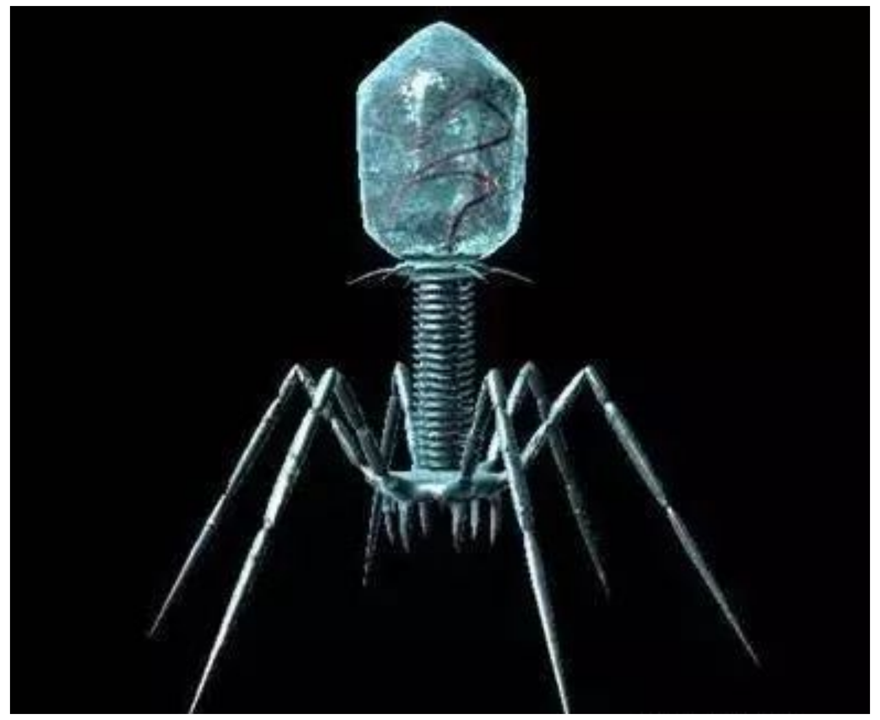
Alfred Hershey
（1908-1997）

Martha Chase
（1927-2003）

1952年，



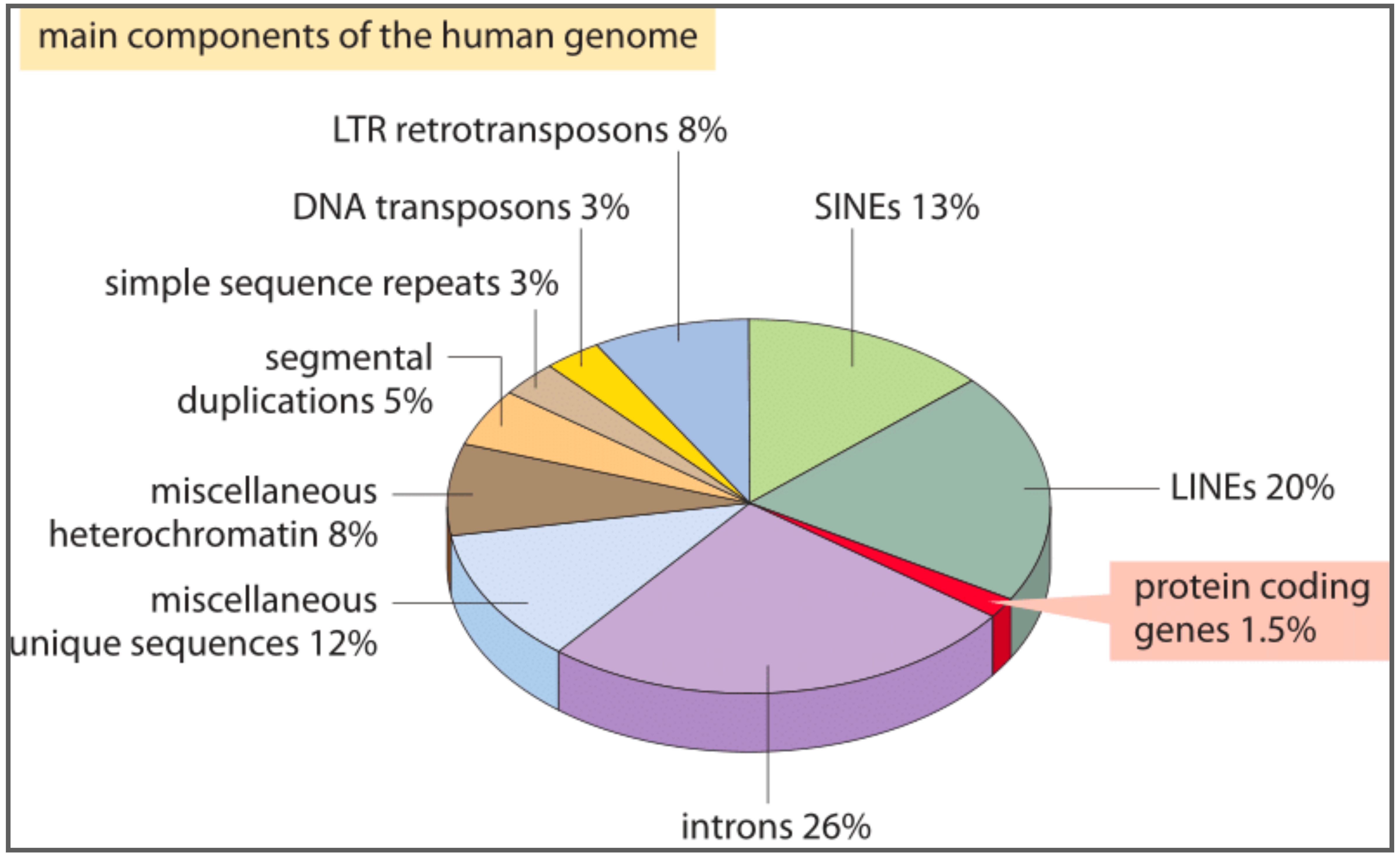
The Hershey-Chase Experiment Alfred Hershey and Martha Chase used different radioactive markers to label the DNA and proteins of bacteriophages. The bacteriophages injected only DNA into the bacteria, not proteins. **From these results, Hershey and Chase concluded that the genetic material of the bacteriophage was DNA.**



遗传信息

DNA包含遗传信息

- 产生什么蛋白质——决定表型(基因)
- 什么条件下产生蛋白质



基因的构成

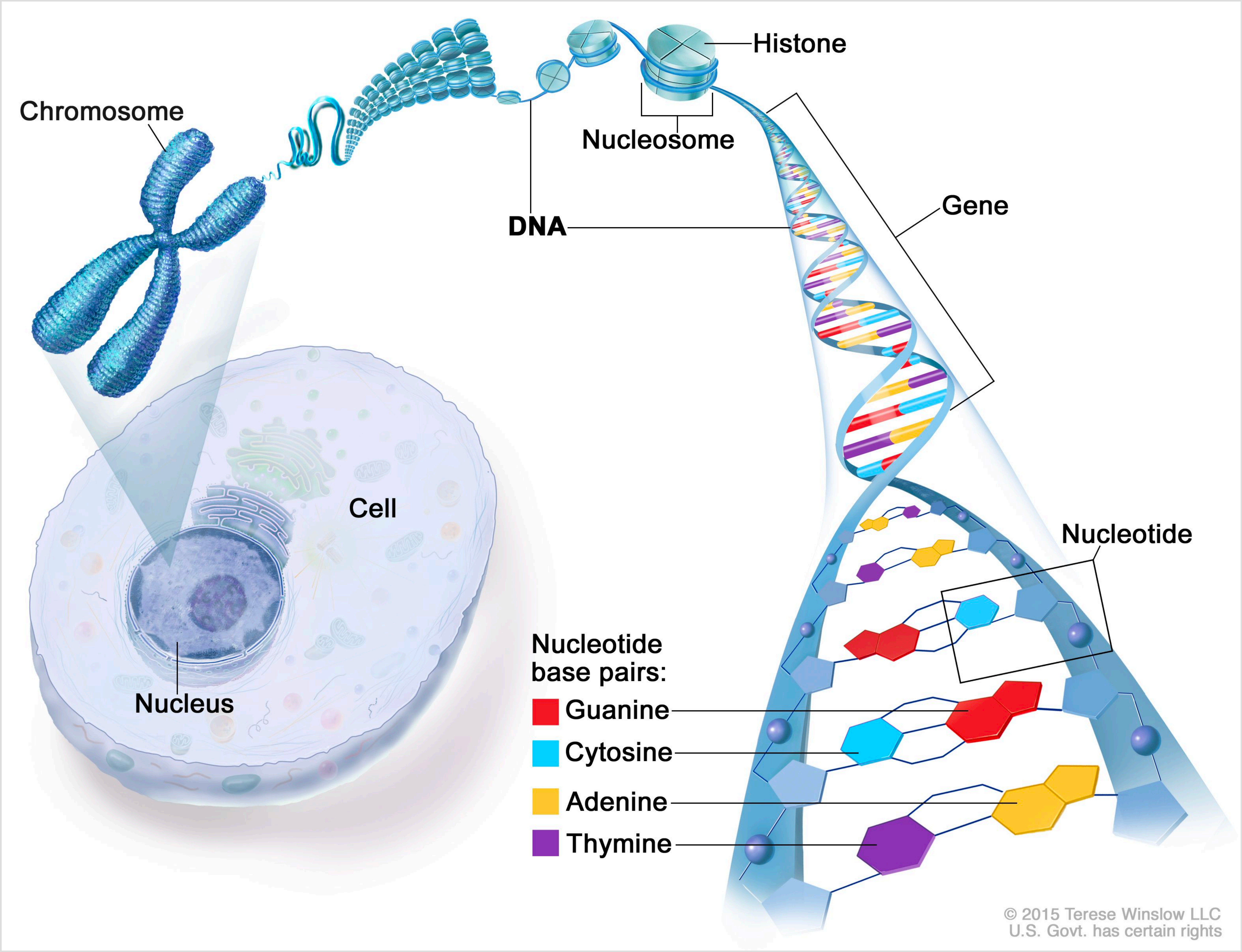
- 编码序列——产生蛋白质的序列
- 启动子序列（promoter）——和转录酶结合的序列
- 调控序列——调控蛋白结合的序列

指导基因是否能够产生蛋白

DNA和基因

基因：部分DNA；

编码蛋白质



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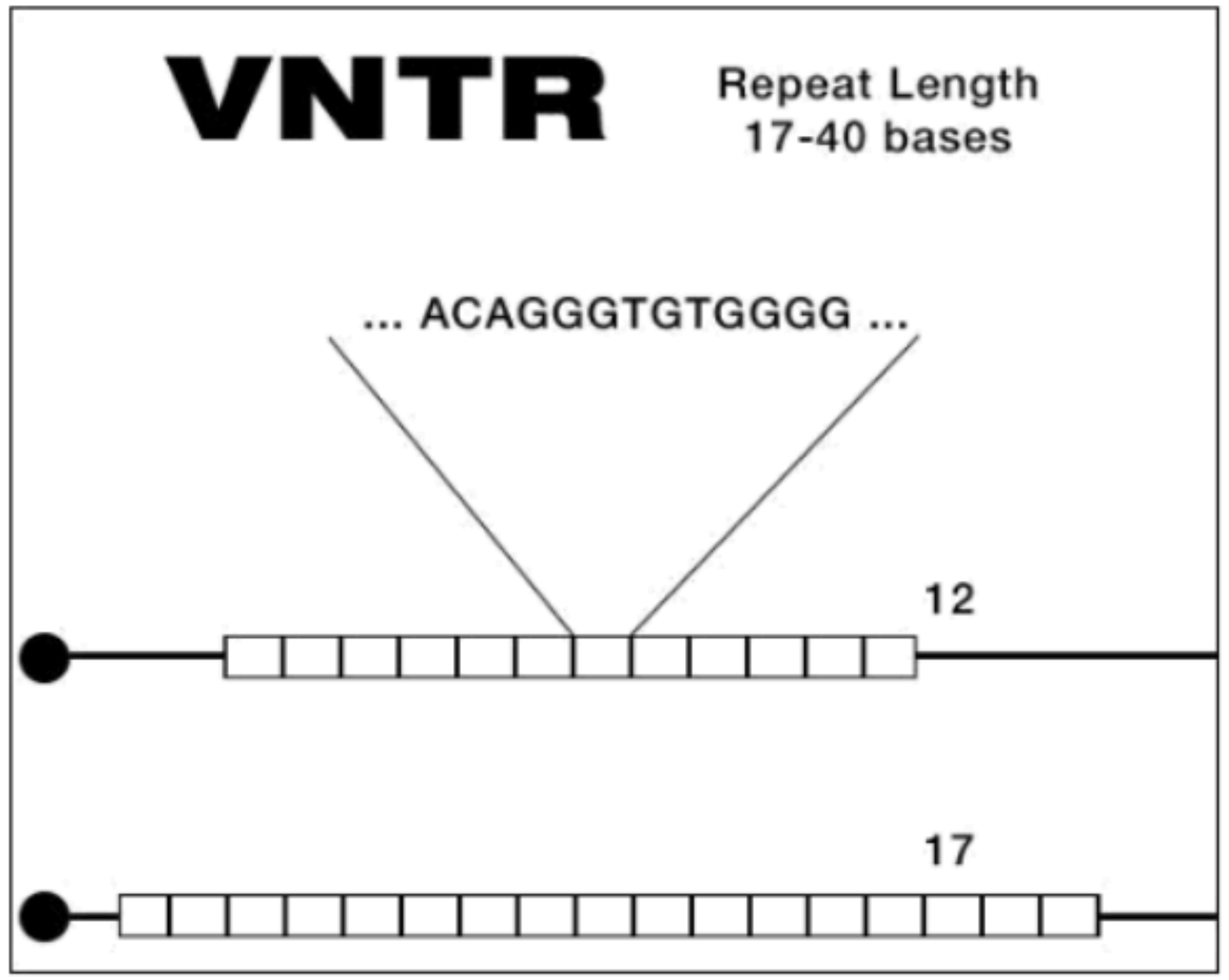
亲子鉴定 DNA指纹鉴定

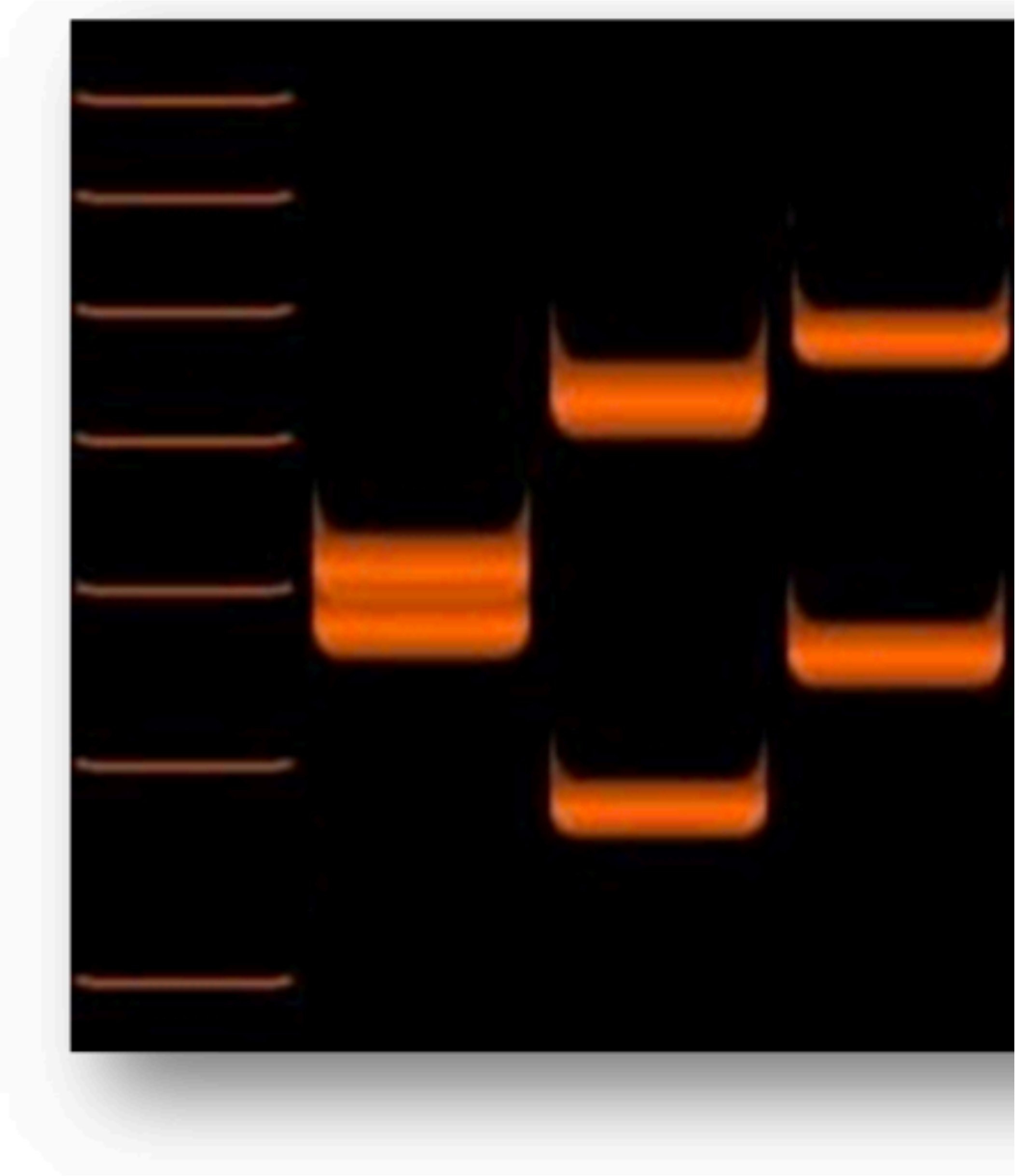
- 检测： 可变的串联重复序列（Variable Number of Tamdem Repeats）
- 这种序列特点：

  -短，连续的重复序列

  -每个个体中的拷贝数不同

  -也叫做遗传标记





DNA指纹鉴定

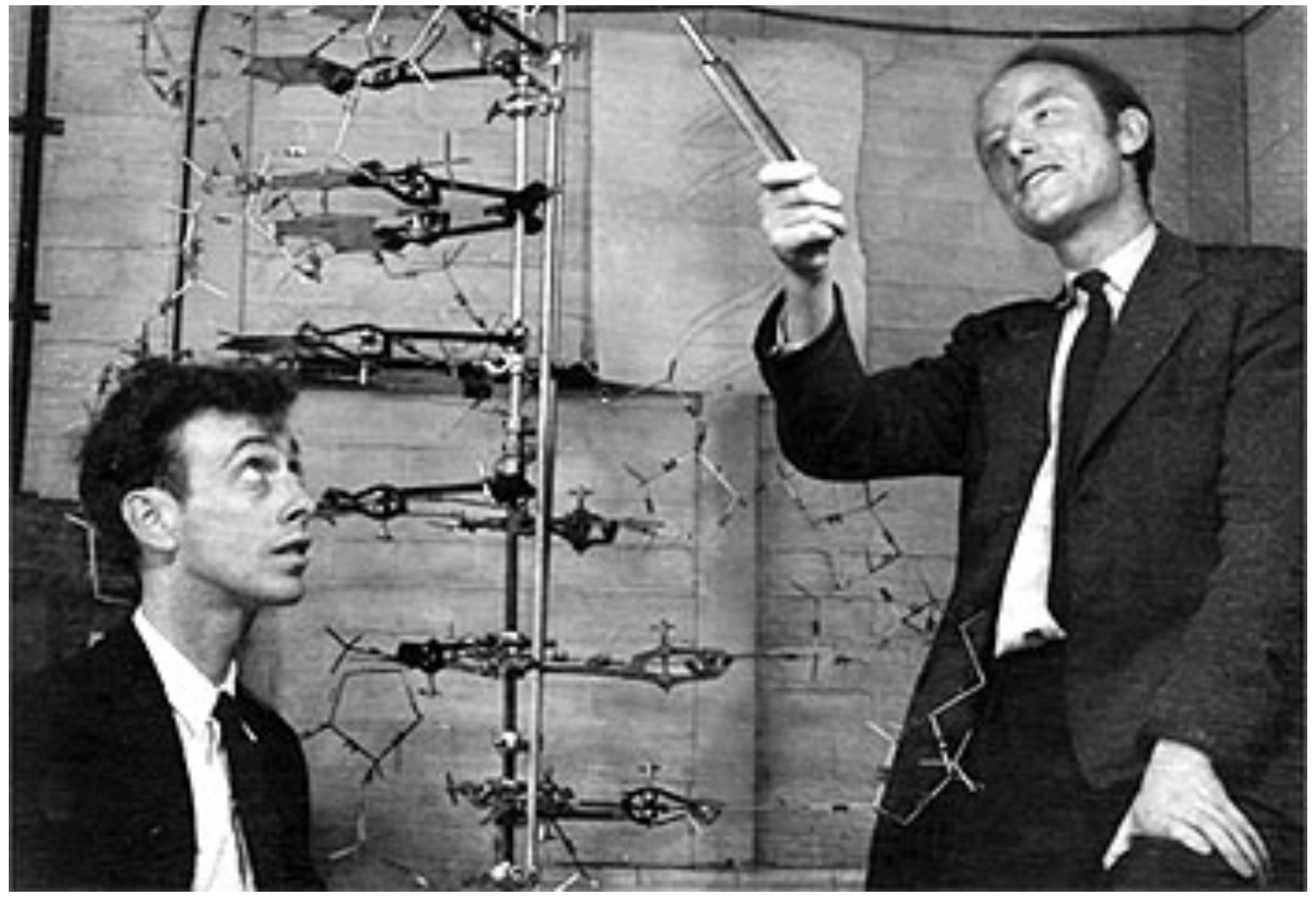
- 遗传位点的检测

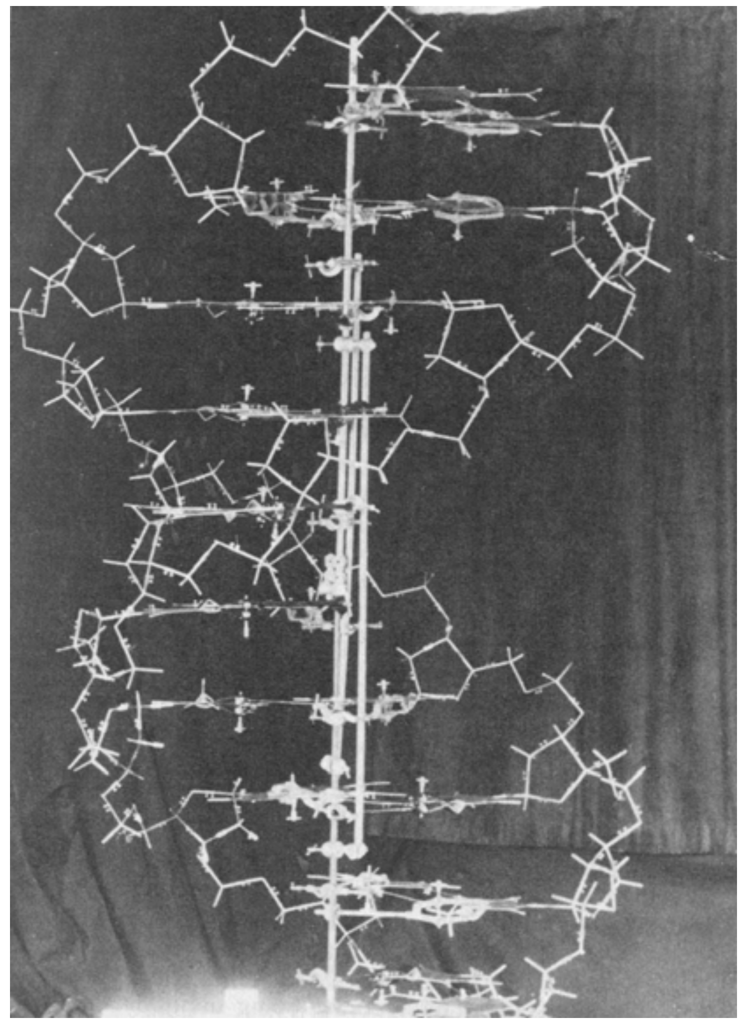
  作十几到几十个DNA位点做检测

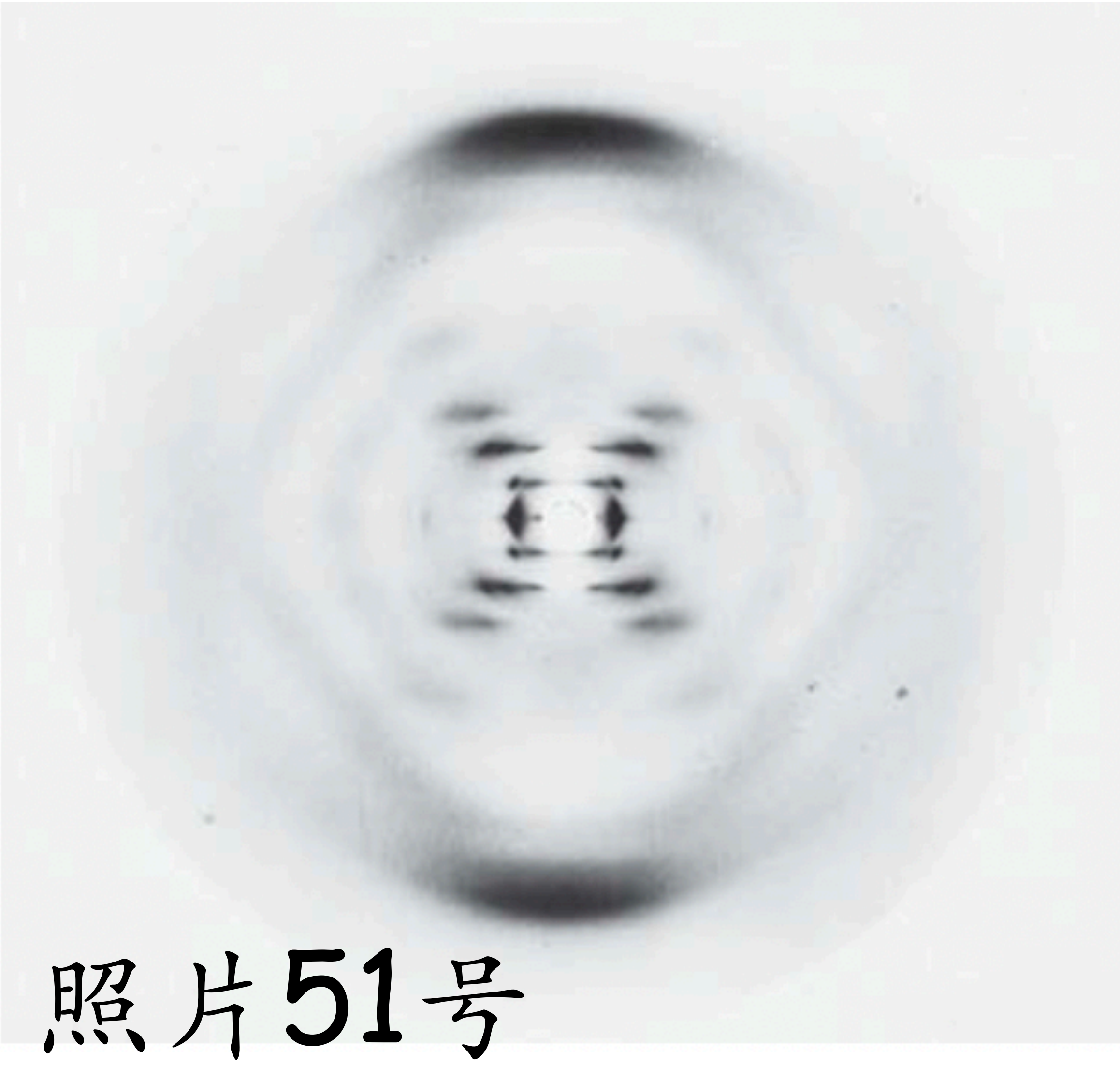
DNA结构的发现

Milestone in structural biology

In 1953 (by Watson, Crick, Maurice Wilkins and Rosalind Franklin)







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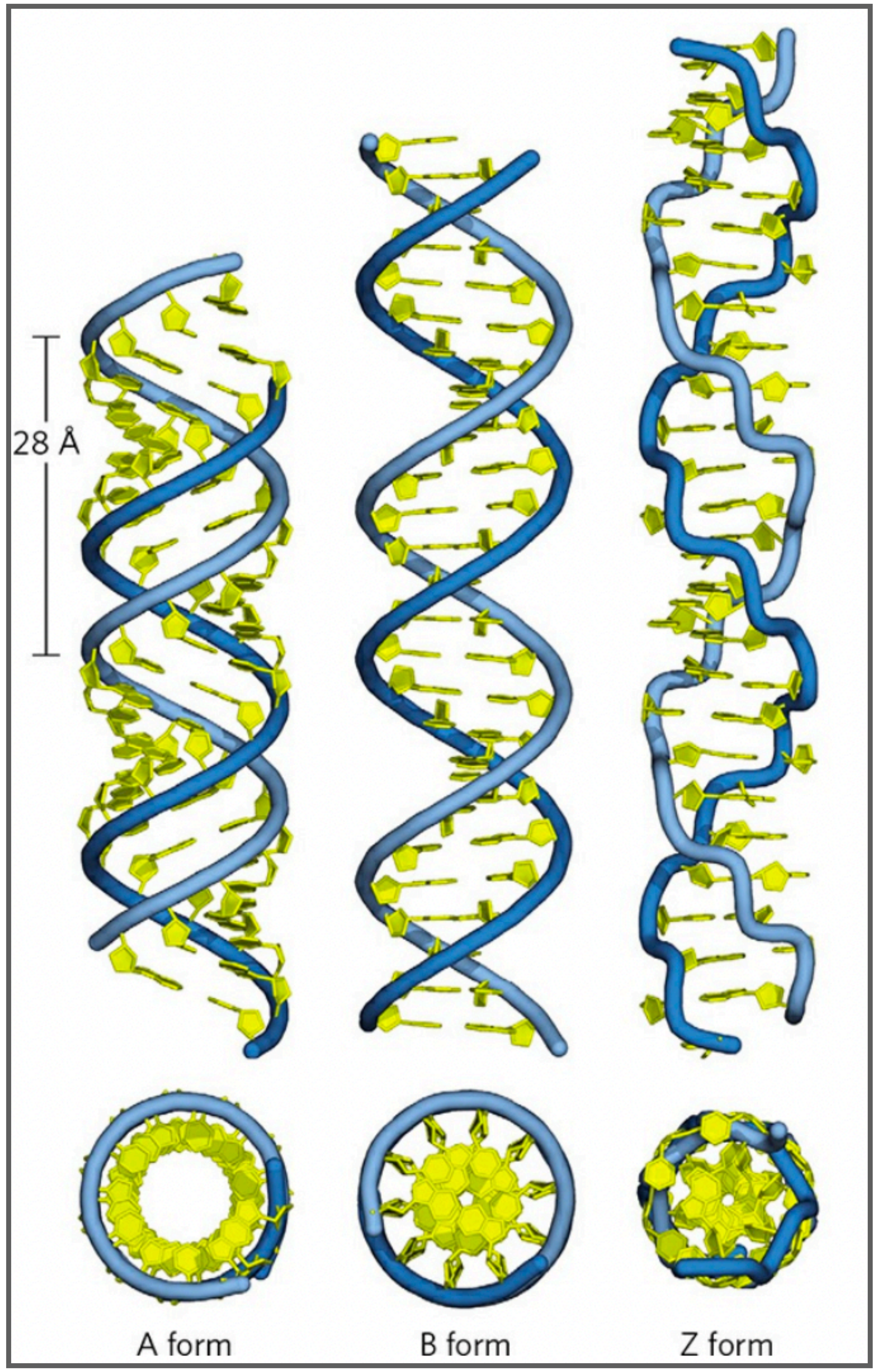
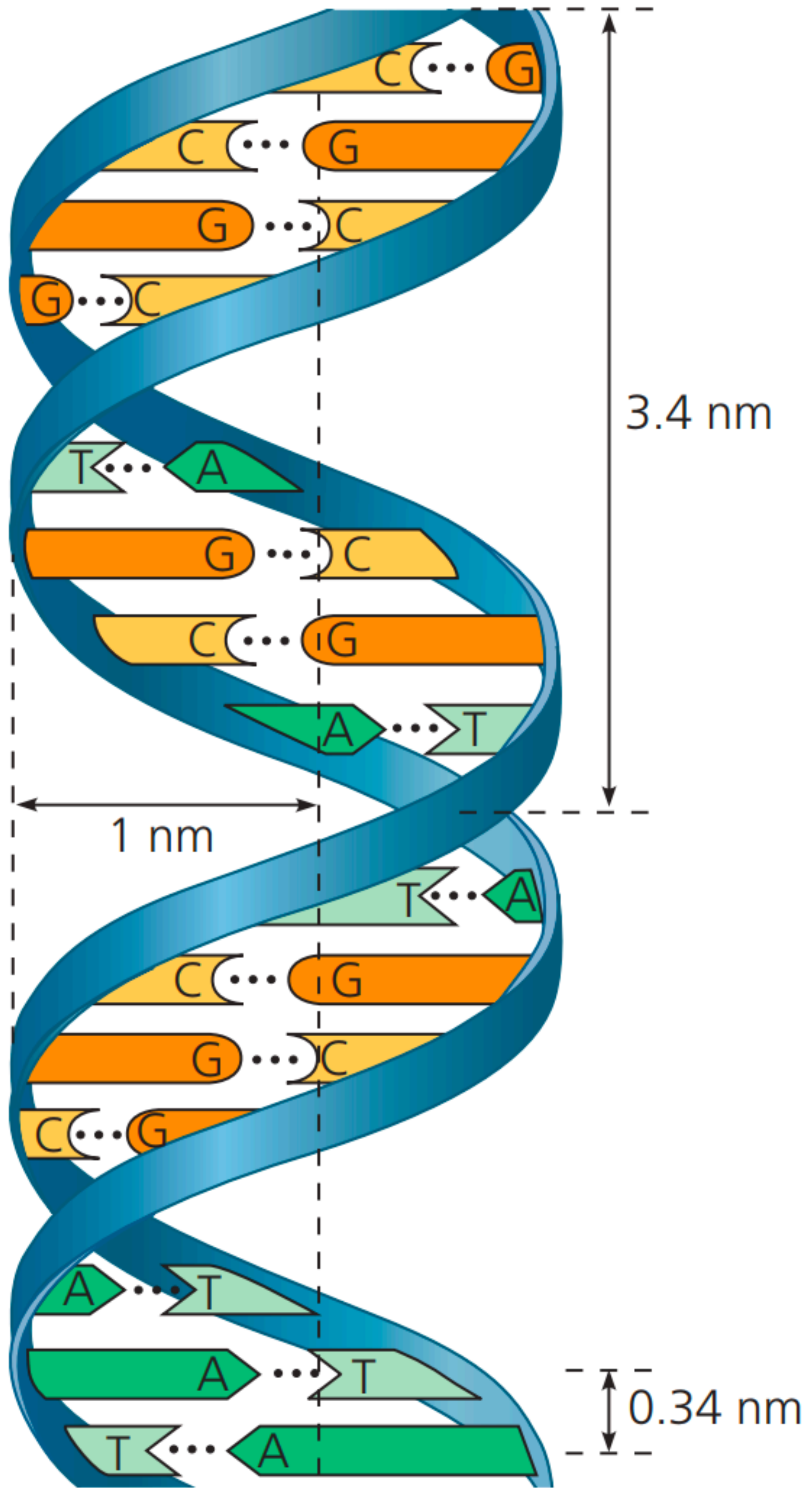
Courtesy of Maurice Wilkins, King's College, London.

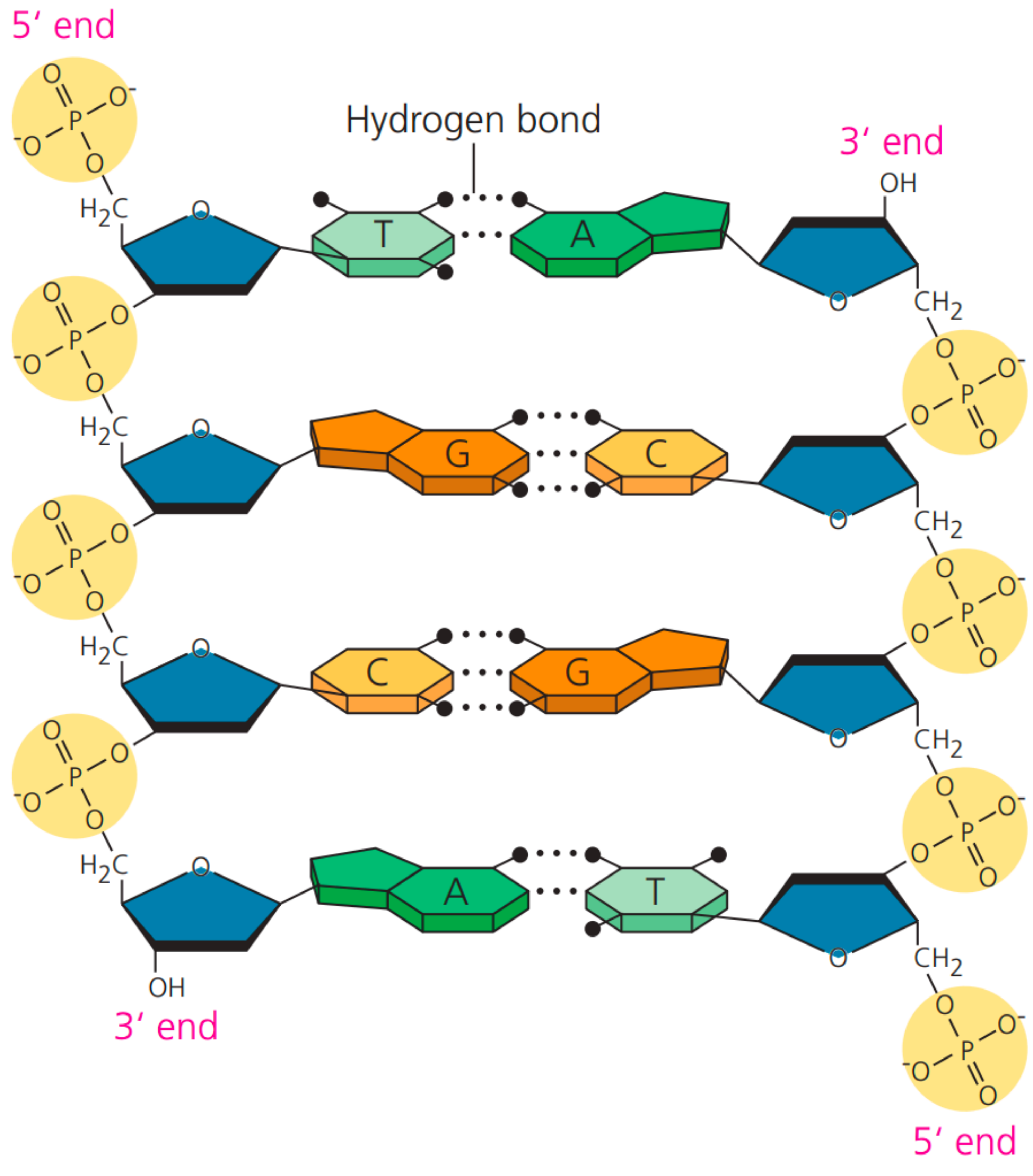
(1920–1958)



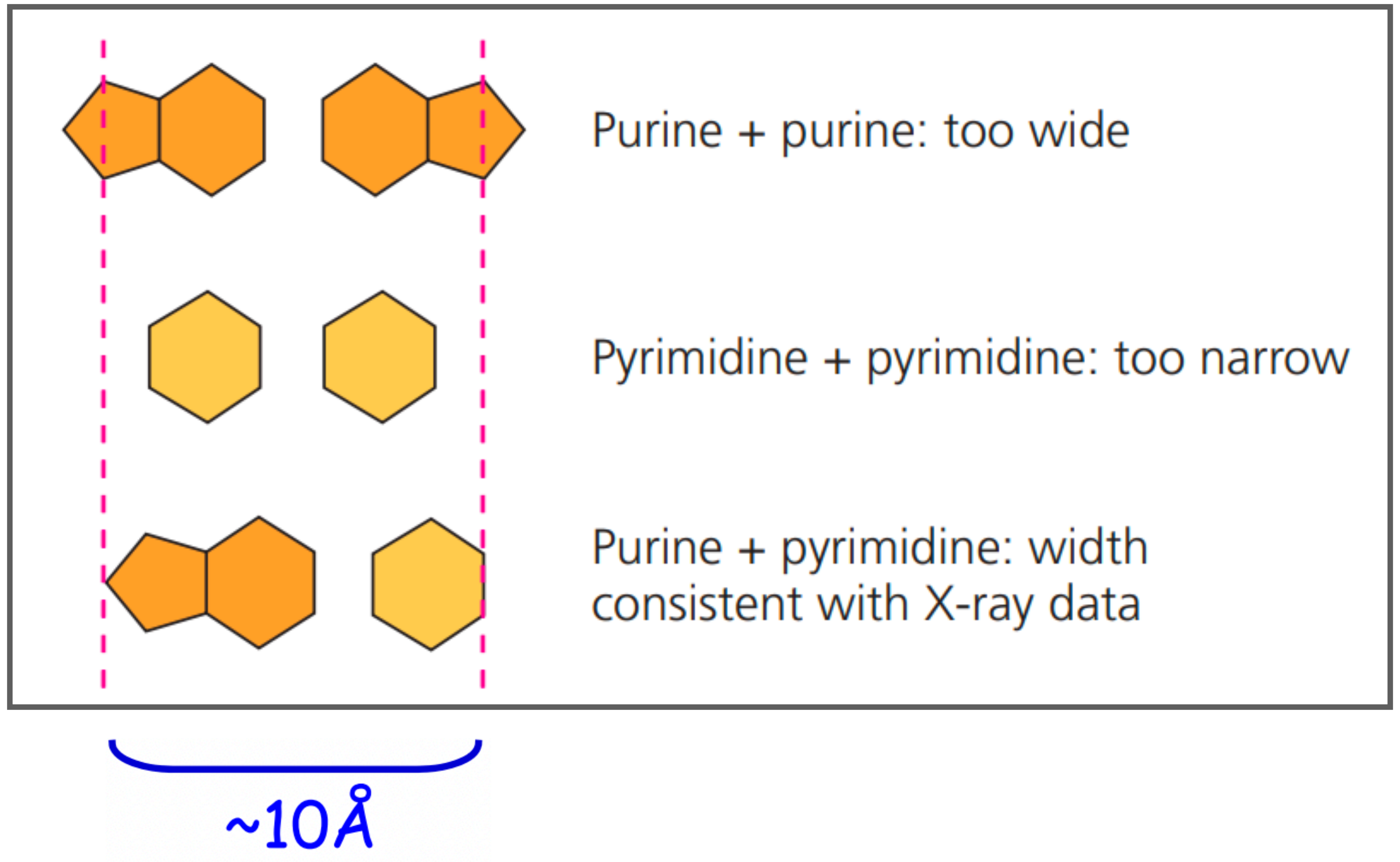
Key features of DNA structure



Double helix



Antiparallel

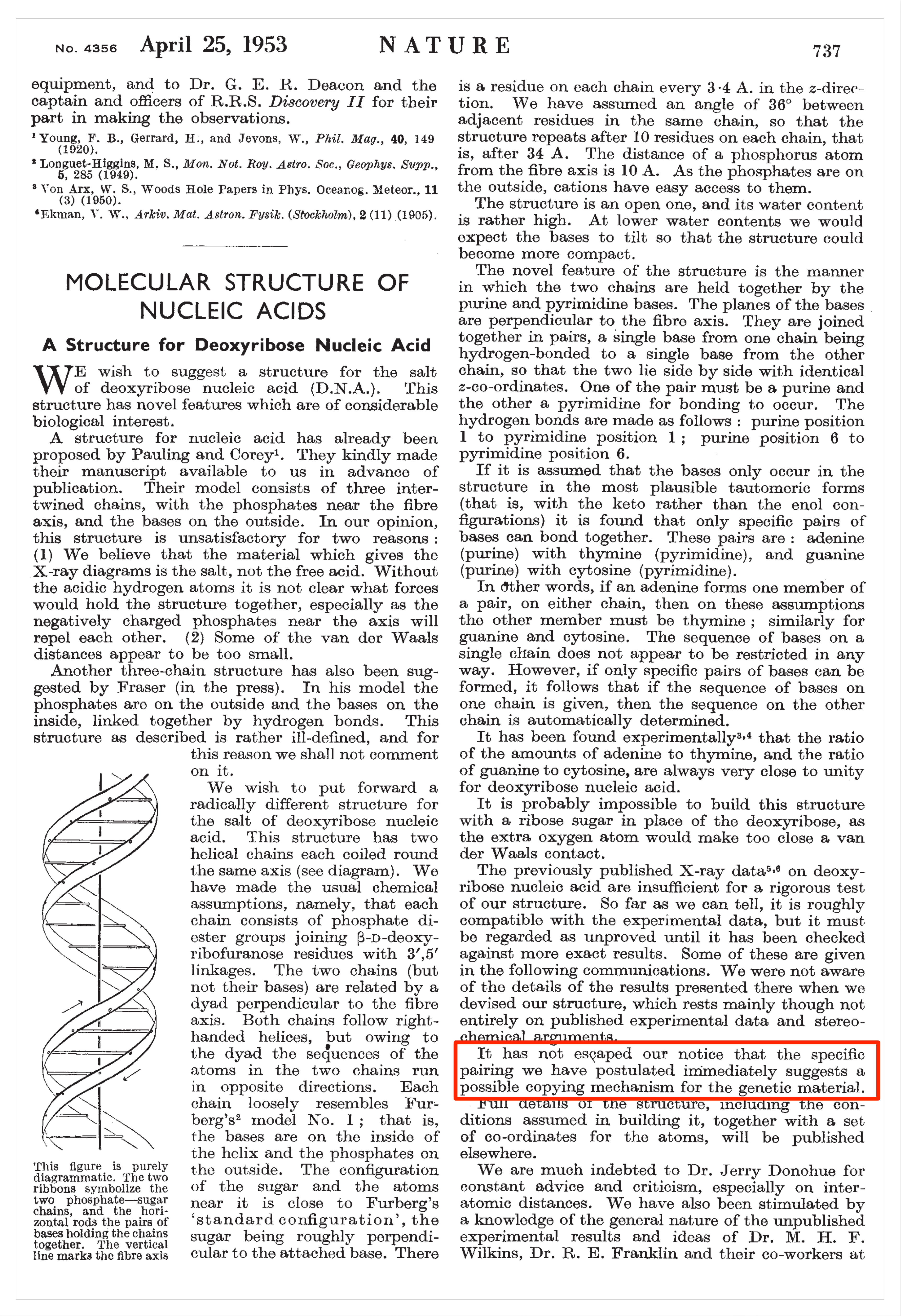


Purine + purine: too wide

Pyrimidine + pyrimidine: too narrow

Purine + pyrimidine: width consistent with X-ray data

Base pairing in DNA



No. 4356 April 25, 1953 NATURE 737

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.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).

[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).

[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.

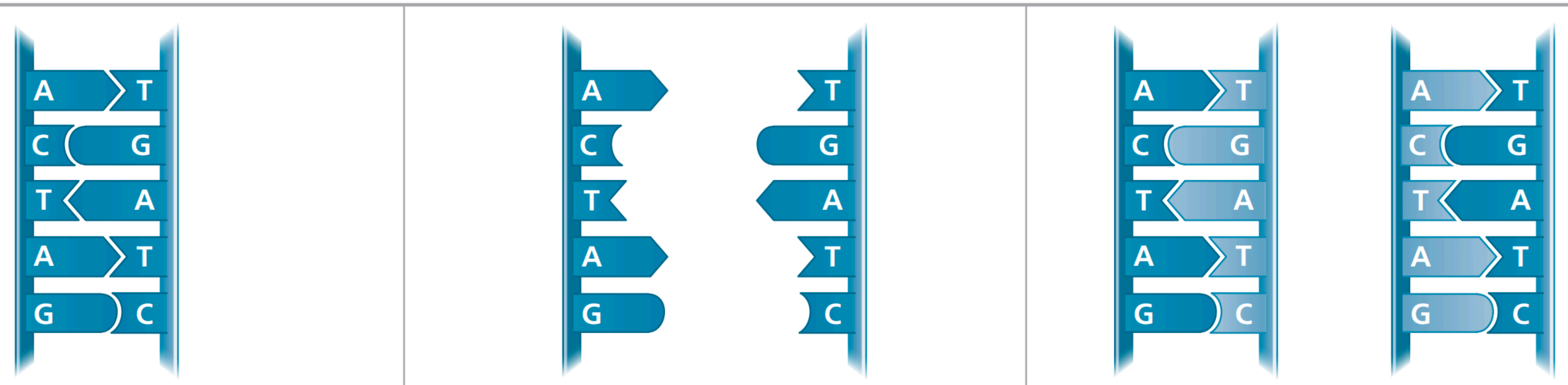
It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

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

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

The Basic Principle: Base Pairing to a Template Strand



(a) The parental molecule has two complementary strands of DNA. Each base is paired by hydrogen bonding with its specific partner, A with T and G with C.

(b) First, the two DNA strands are separated. Each parental strand can now serve as a template for a new, complementary strand.

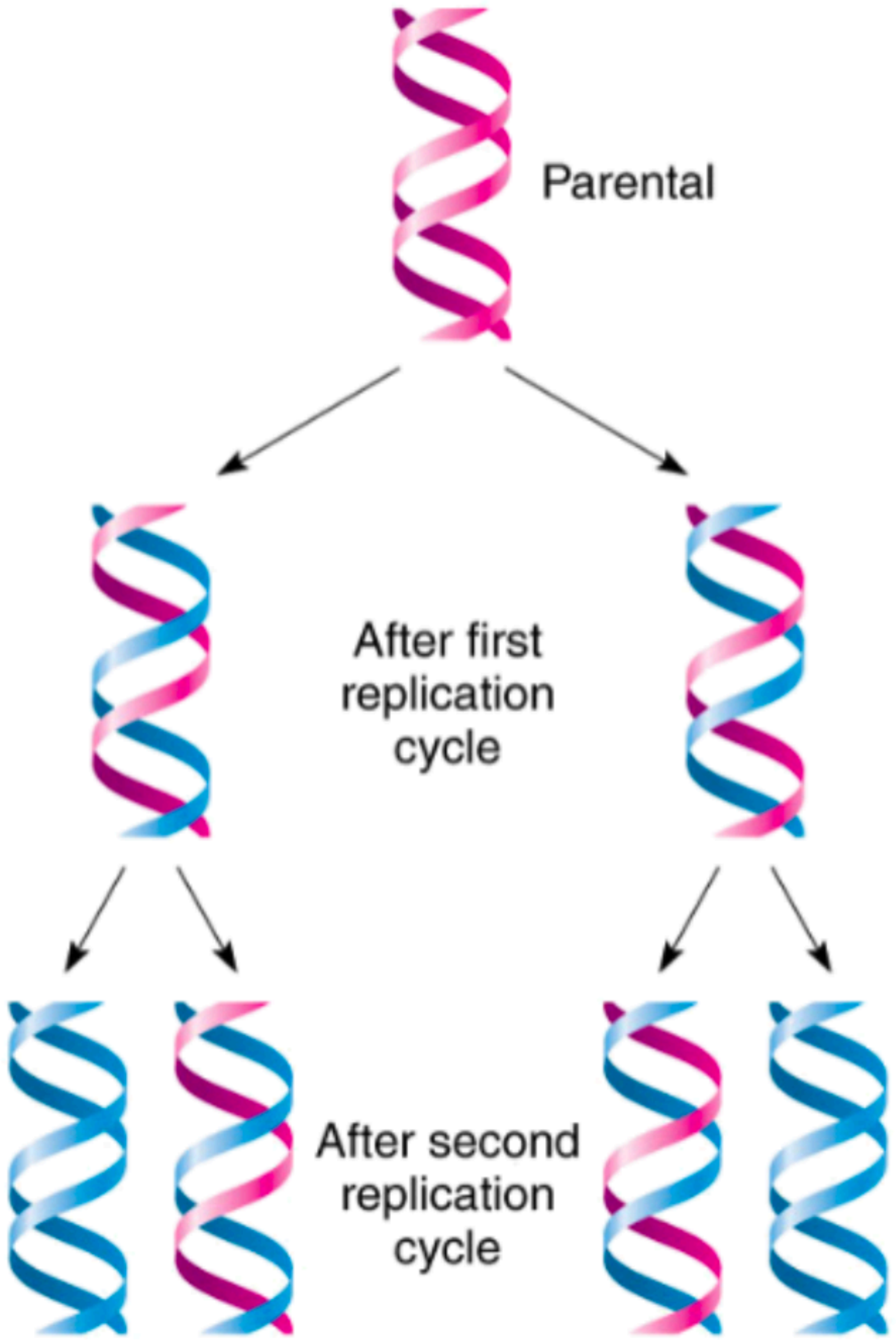
(c) Nucleotides complementary to the parental (dark blue) strand are connected to form the sugar-phosphate backbones of the new "daughter" (light blue) strands.

半保留复制的模式

- 两条双链DNA螺旋先分开成为两条DNA单链
- 这两条DNA单链再各自成为复制模版合成新的DNA链
- 新的DNA双螺旋分子，各自皆包含一条新的DNA单链和一条旧的DNA单链

3种复制模式的提出

半保留复制



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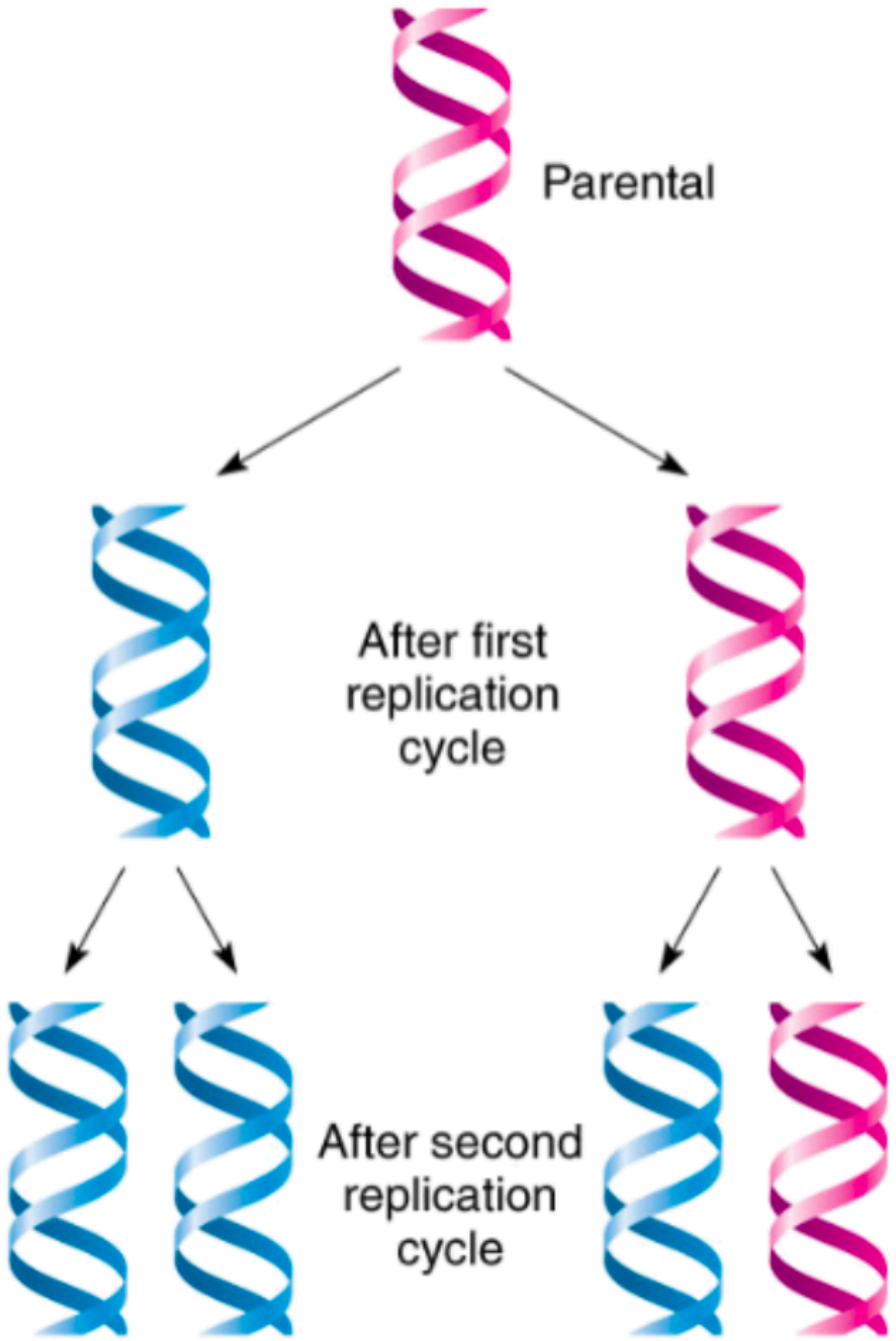

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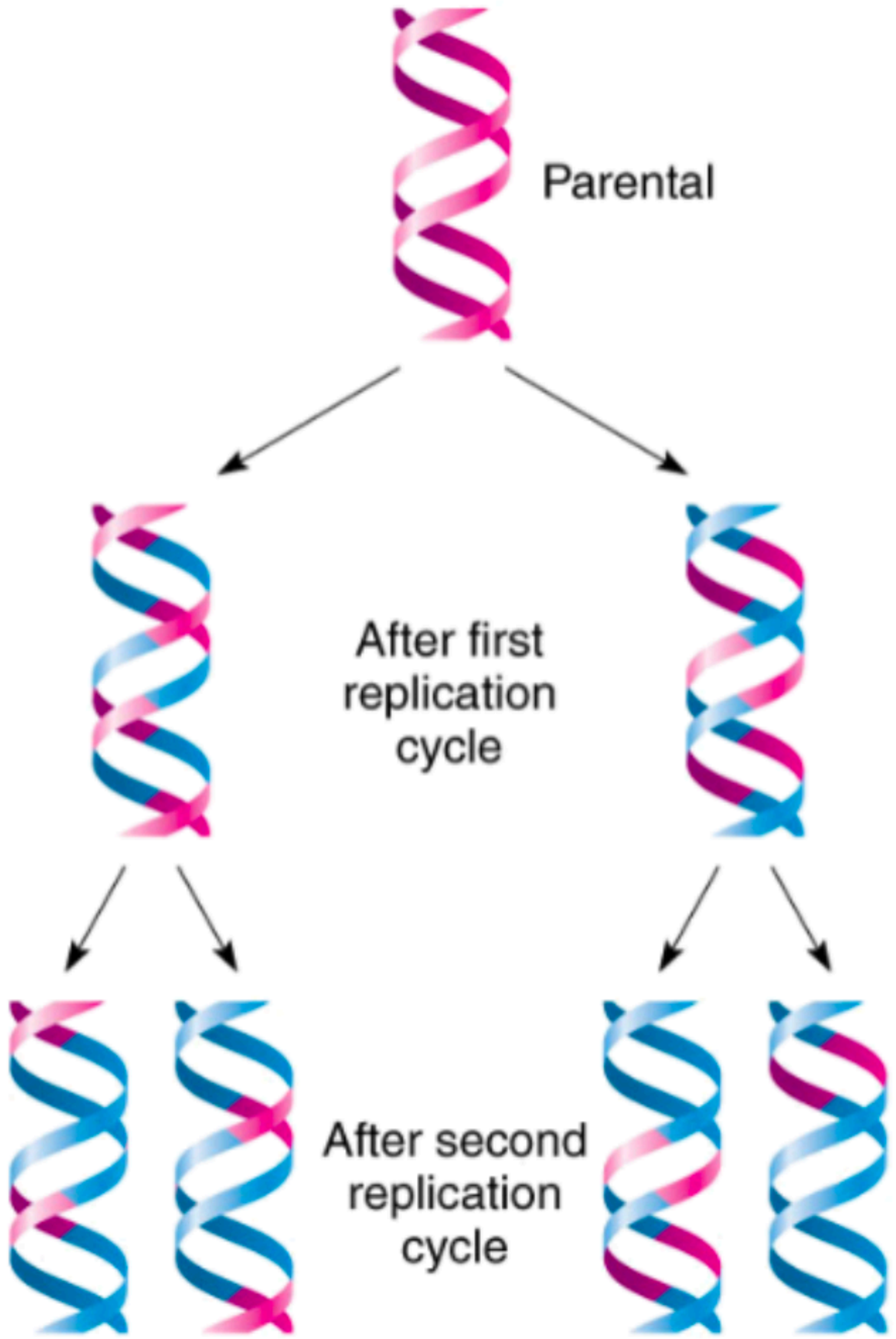
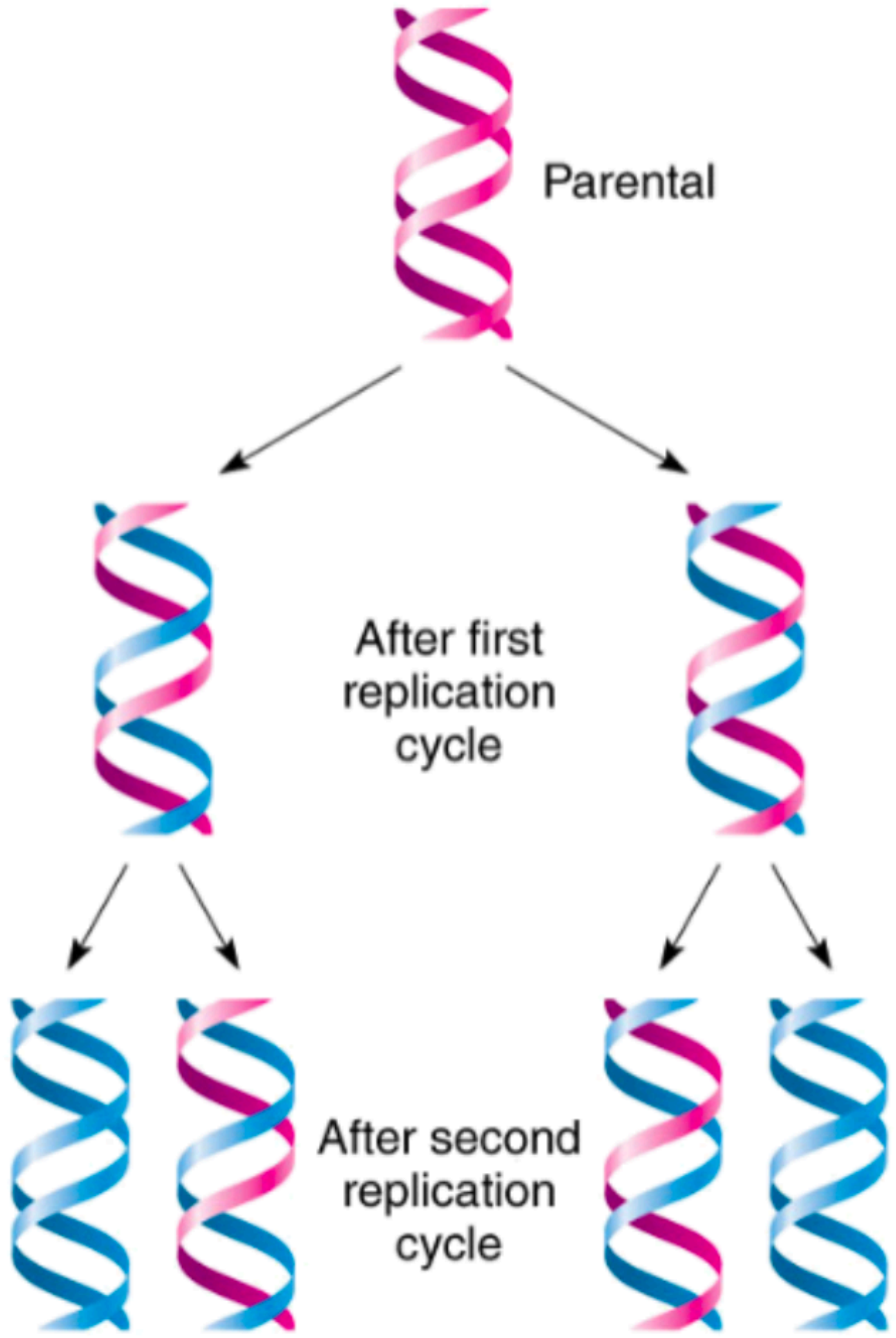


设计这个实验的关键是什么？ 如何区分母链和新合成的链？

半保留复制



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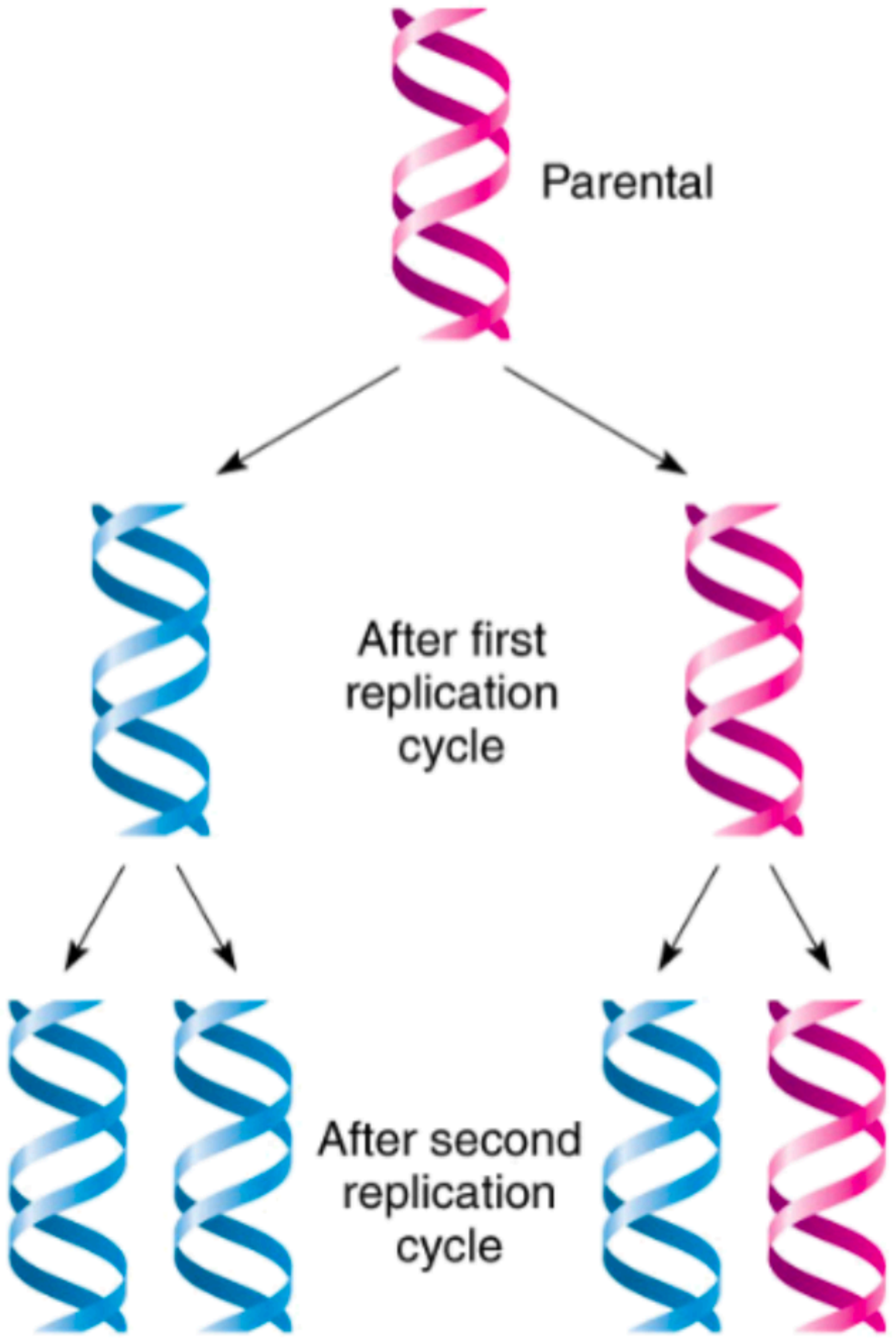

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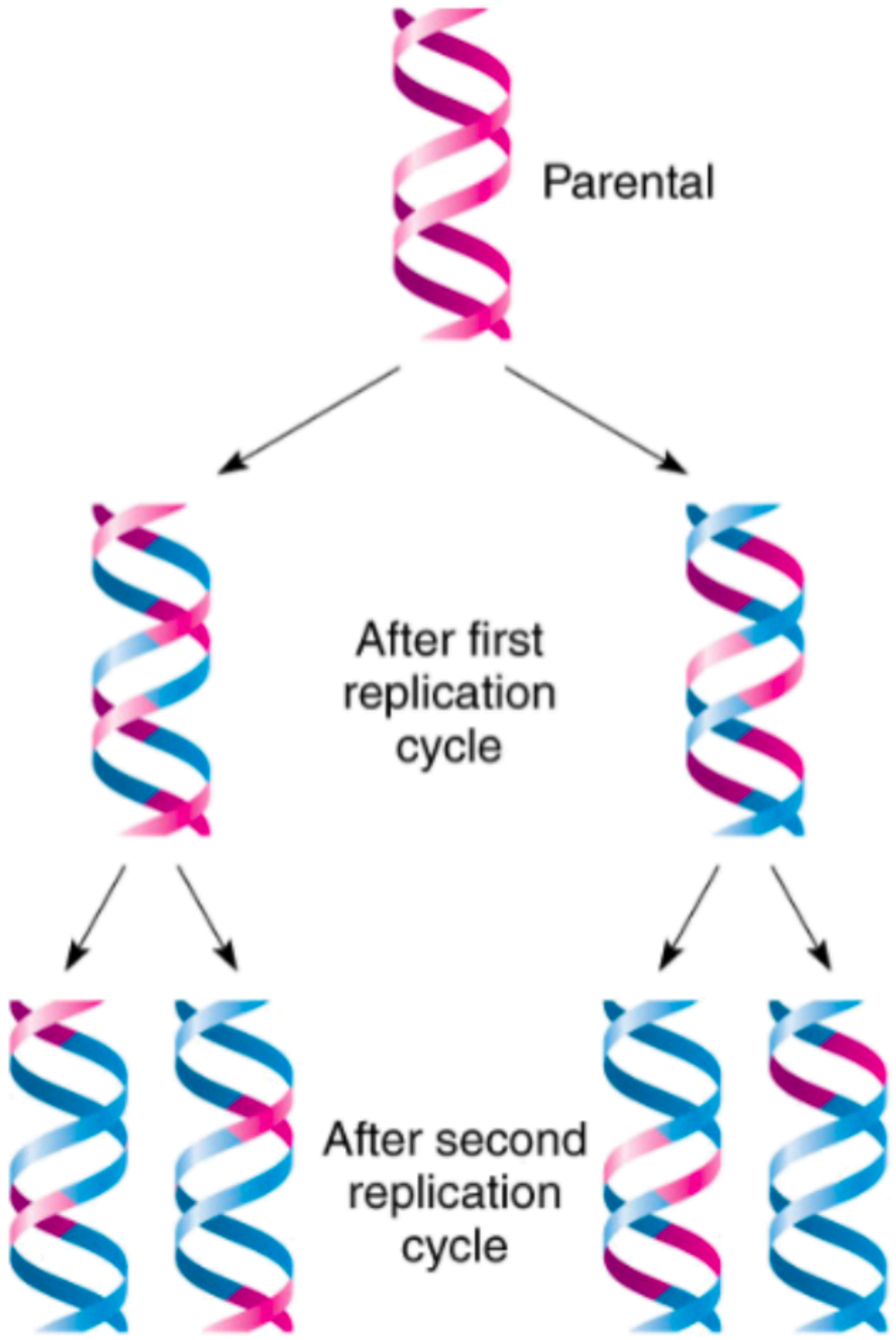
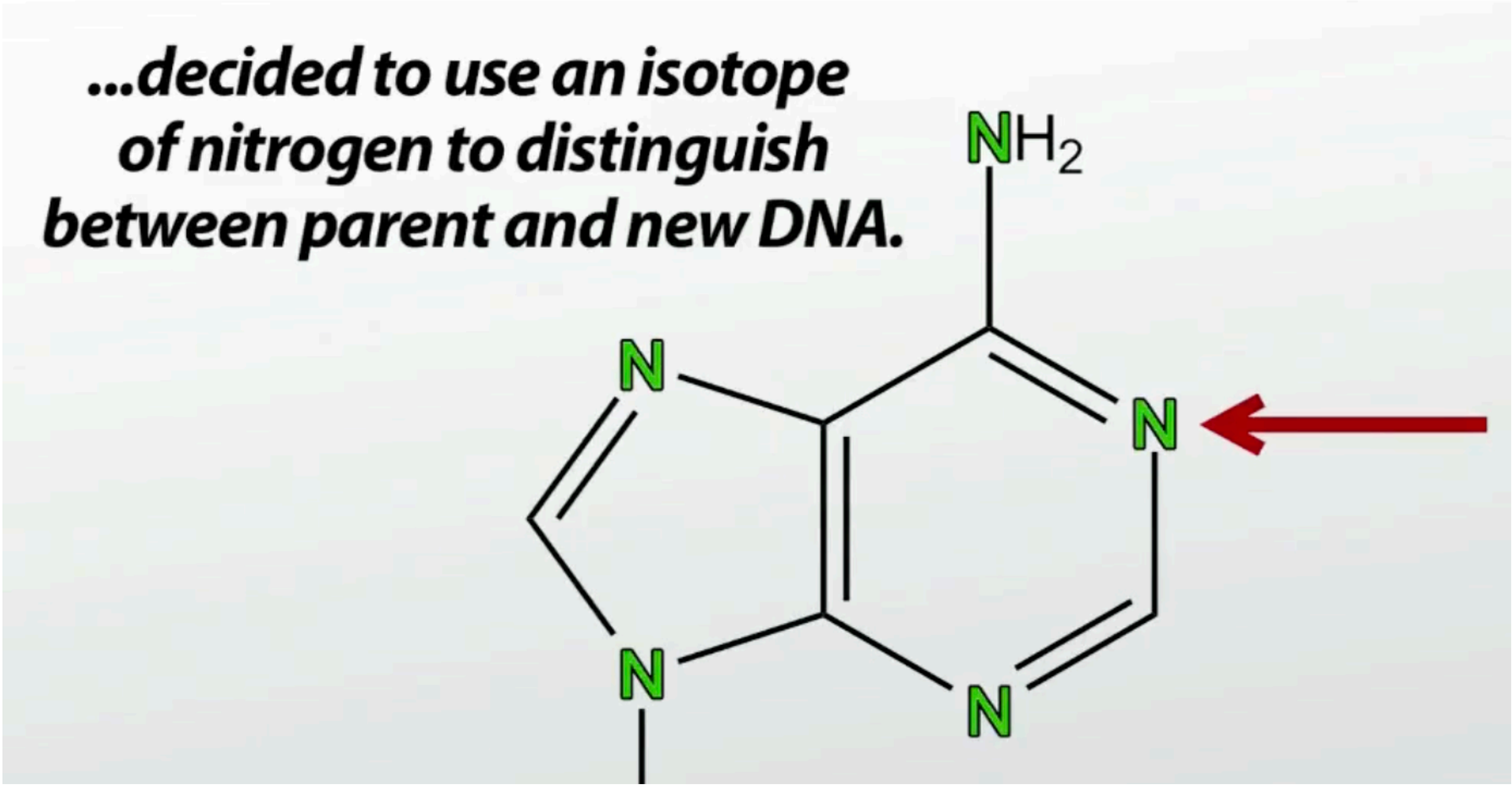
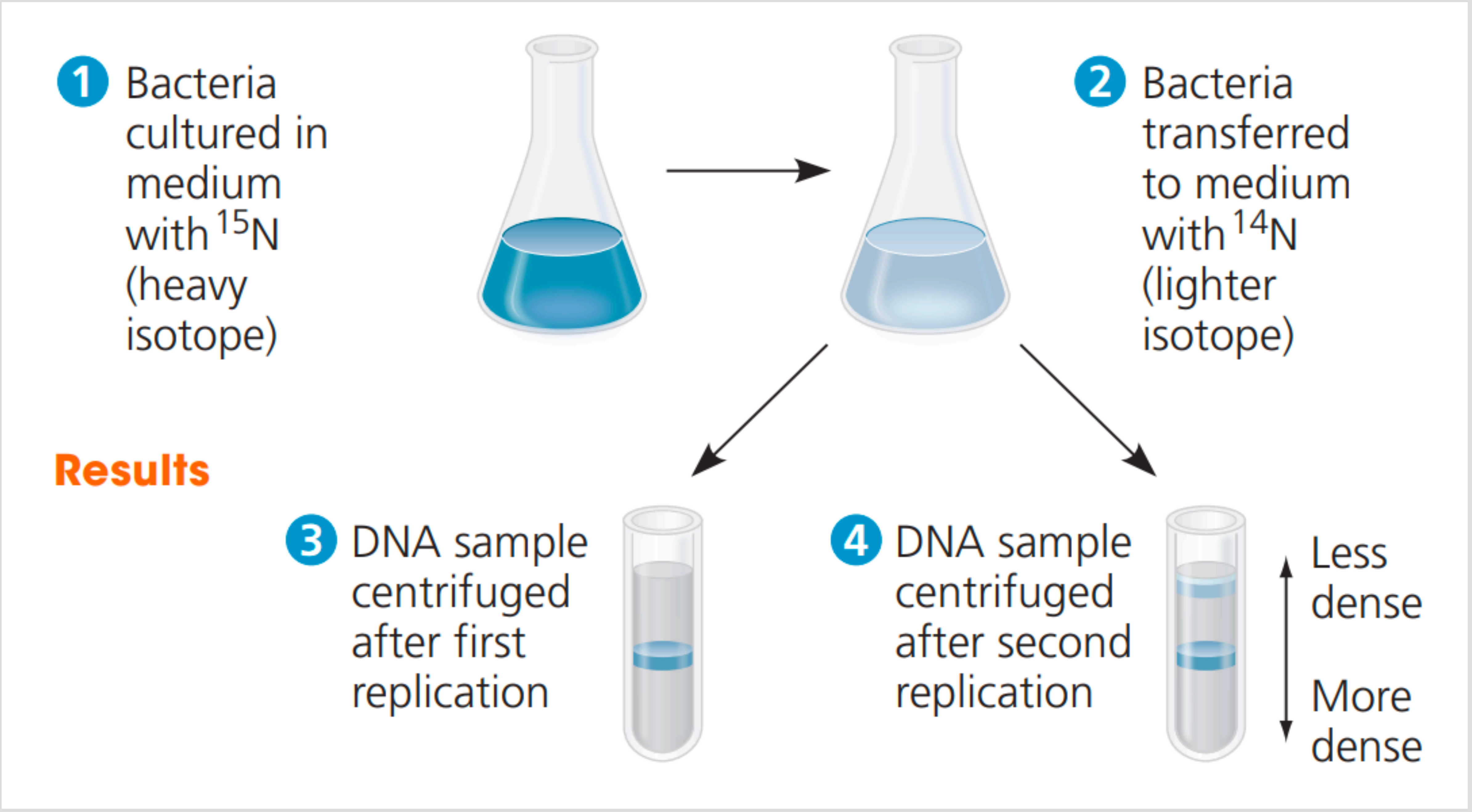


- 区分母链和新链
  - 区别性标记 15N 14N
  - 密度梯度离心



...decided to use an isotope of nitrogen to distinguish between parent and new DNA.

- 如何体现复制过程
  - 活细胞—大肠杆菌
  - 复制轮次



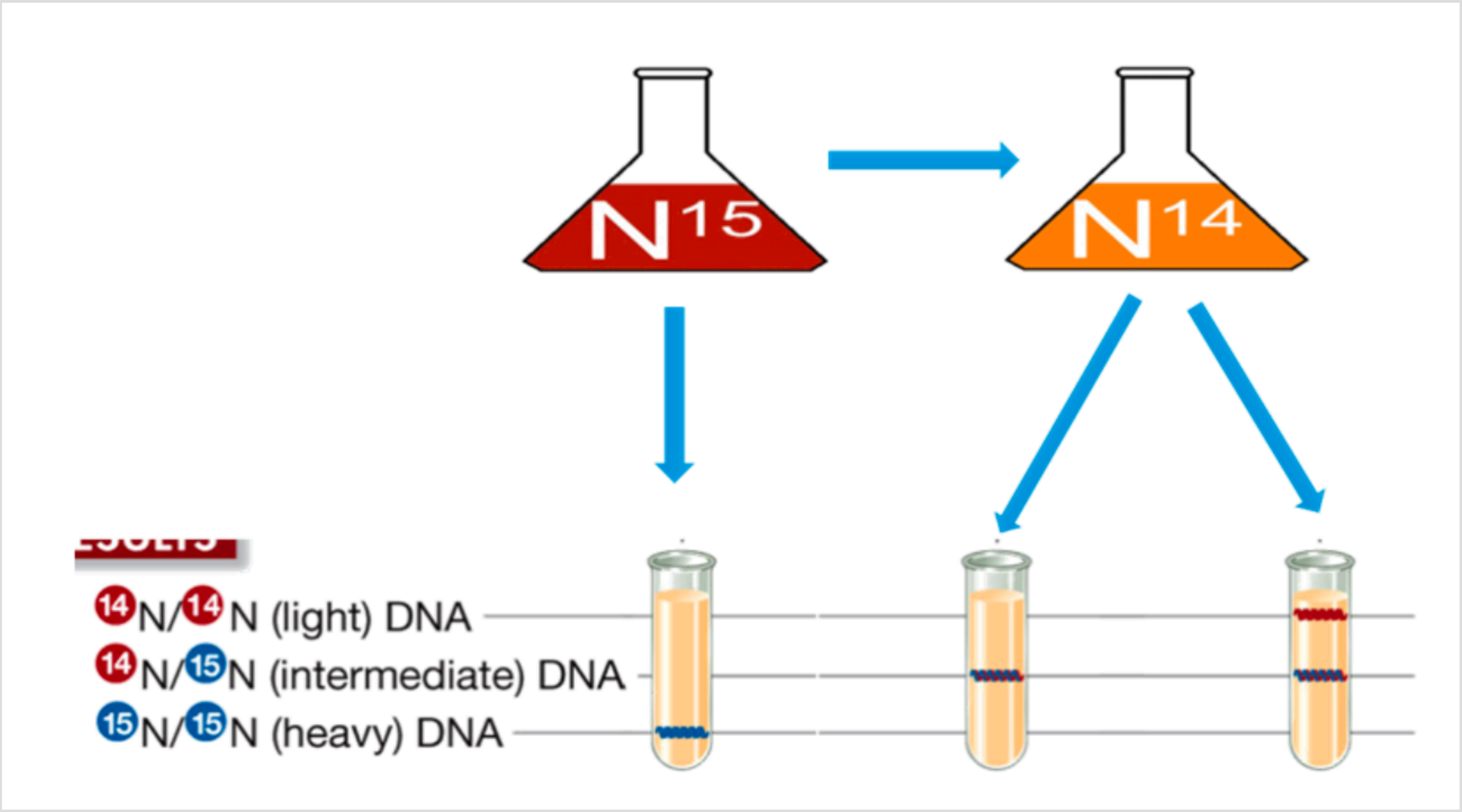
① Bacteria cultured in medium with ^{15}N (heavy isotope)

② Bacteria transferred to medium with ^{14}N (lighter isotope)

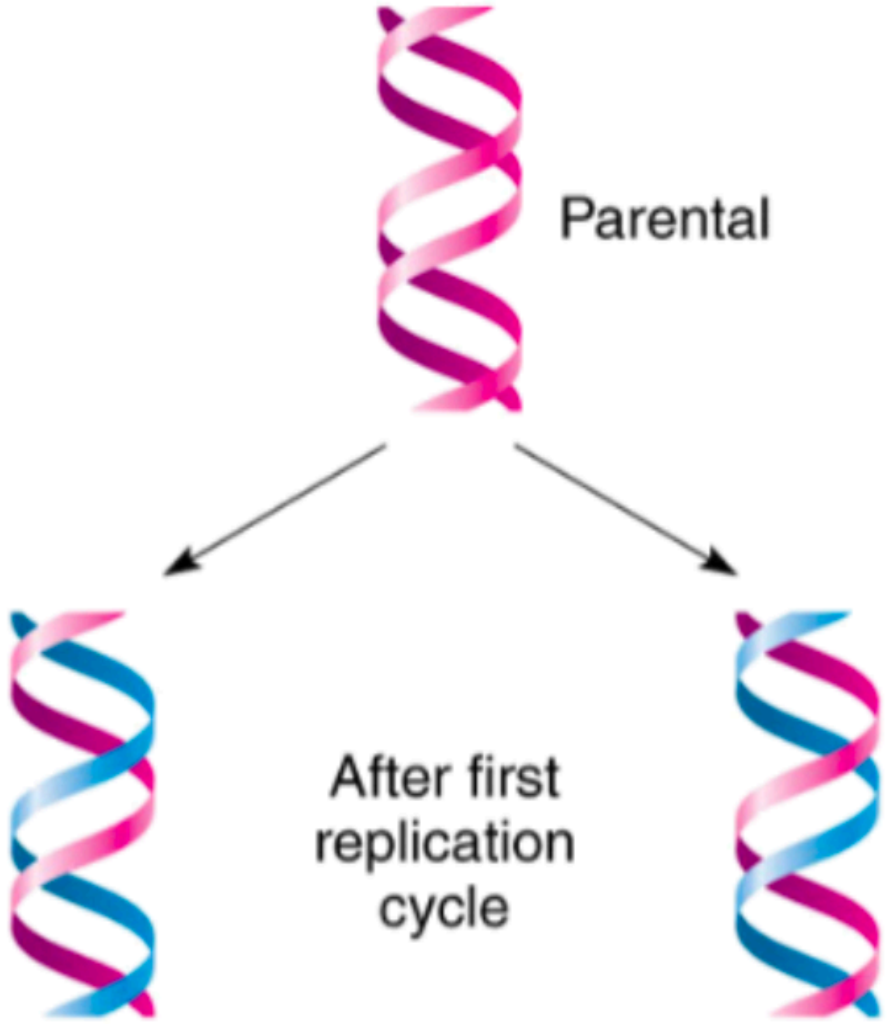
Results

③ DNA sample centrifuged after first replication

④ DNA sample centrifuged after second replication


N^{15}
N^{14}
$^{14}N/^{14}N$ (light) DNA
$^{14}N/^{15}N$ (intermediate) DNA
$^{15}N/^{15}N$ (heavy) DNA

半保留复制



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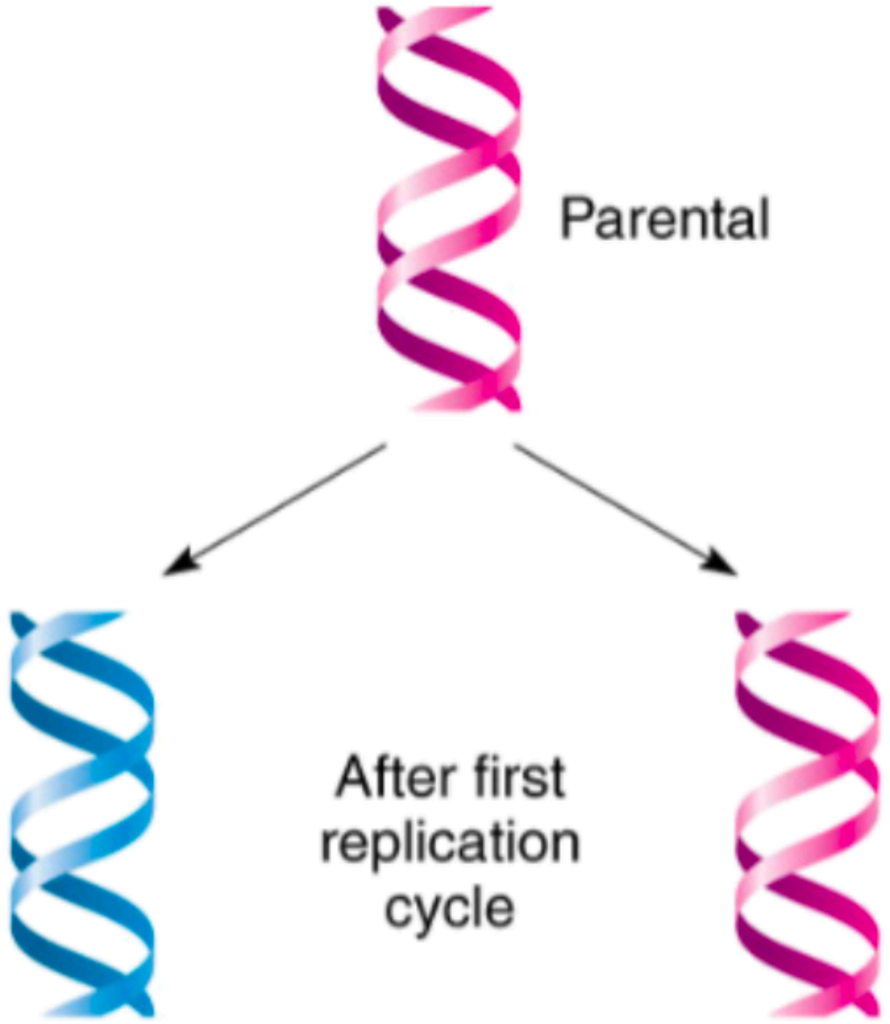

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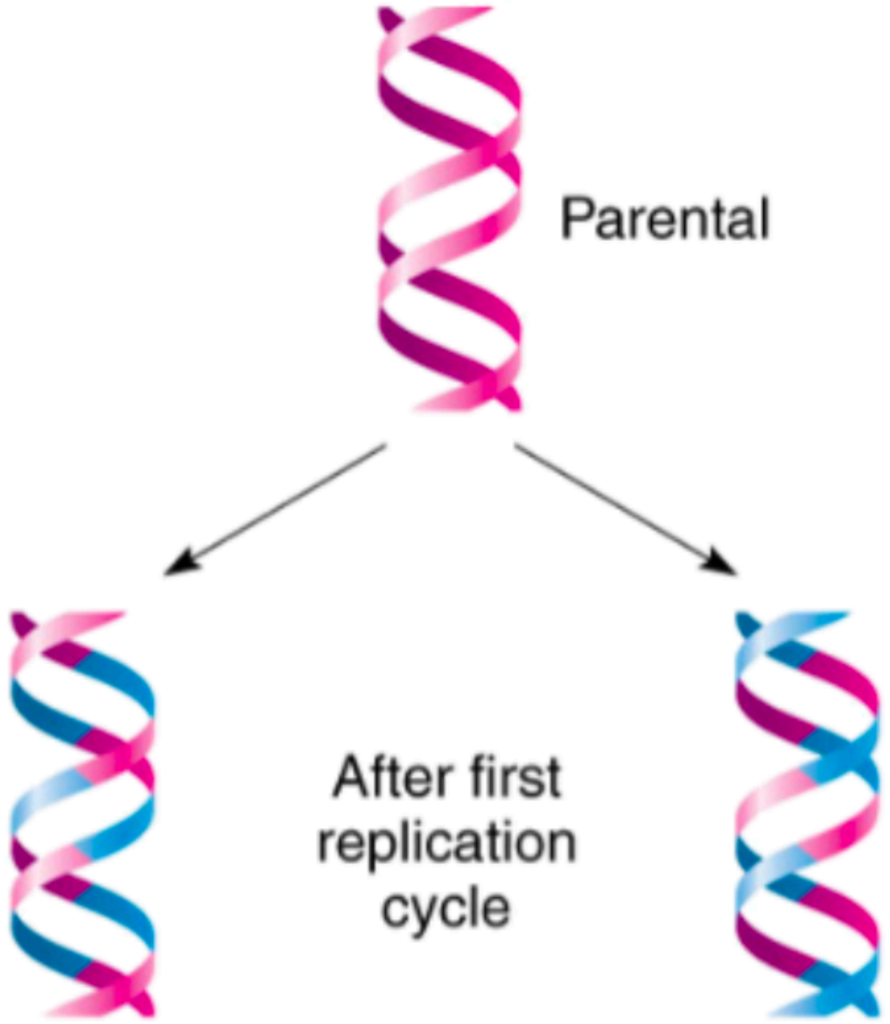
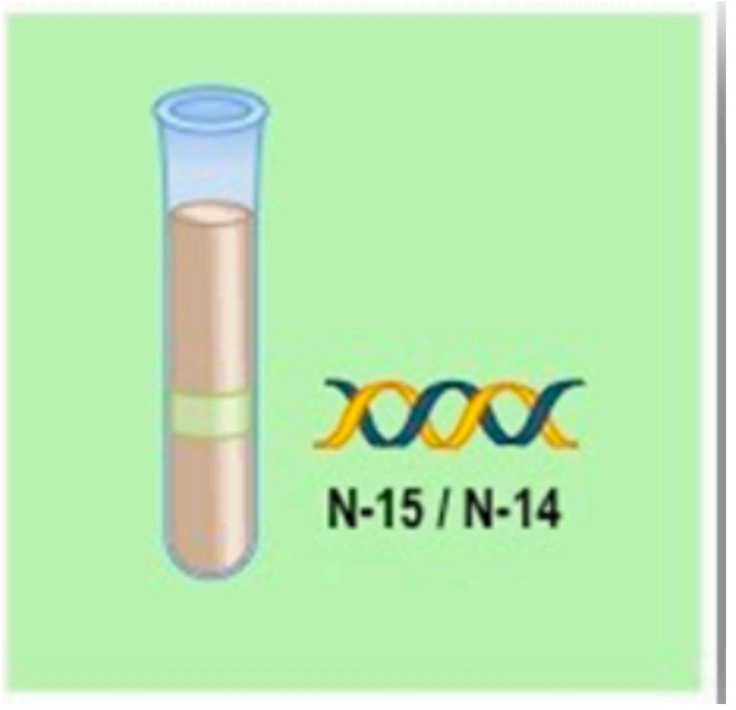
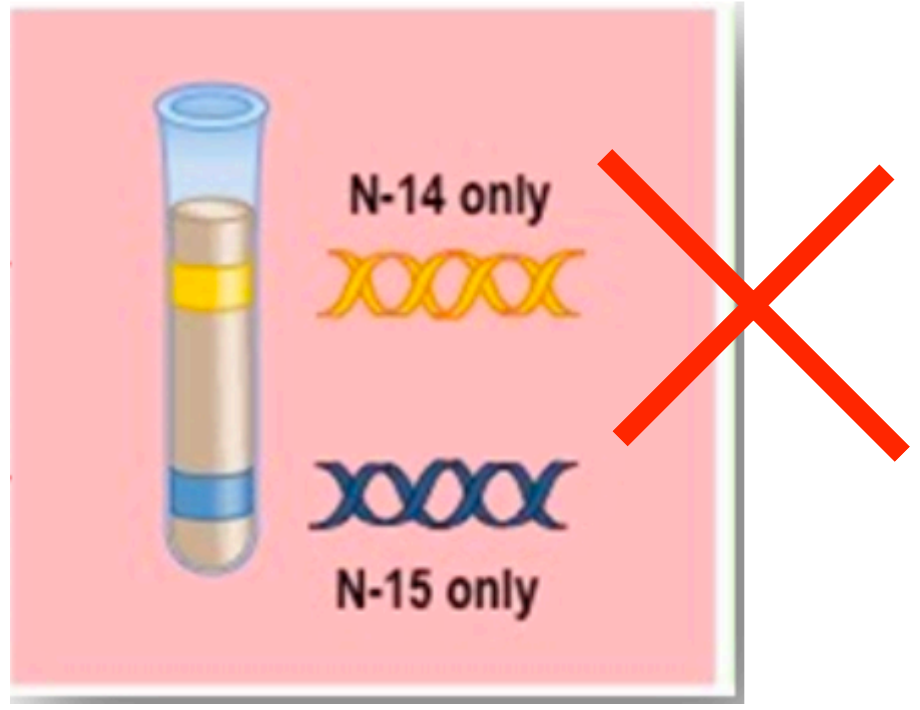
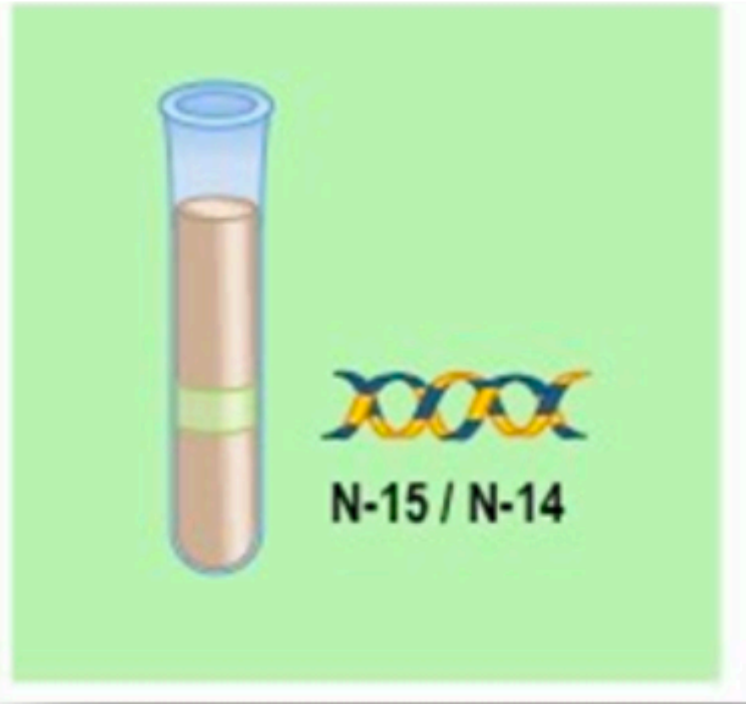


第一轮复制后预测：







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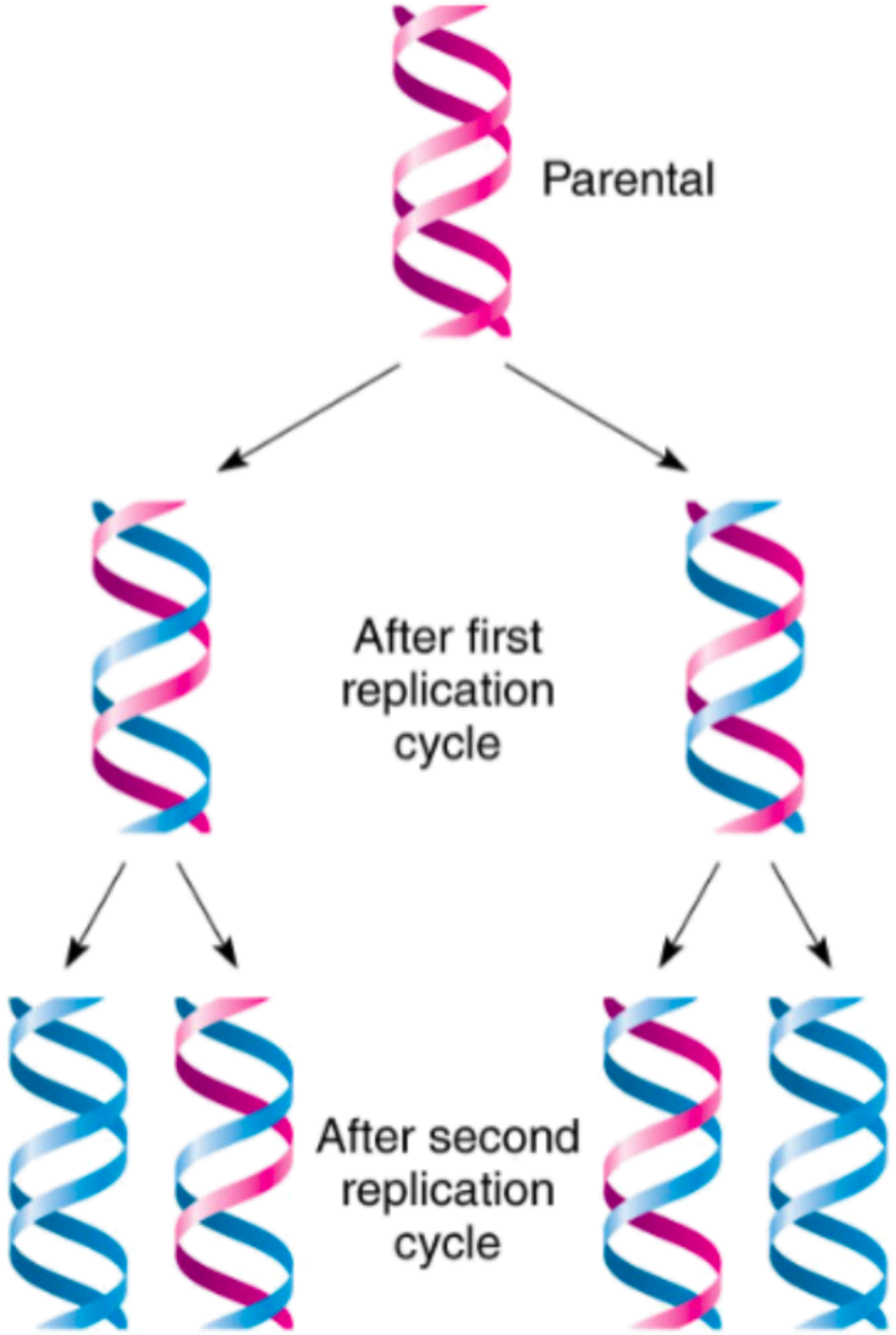
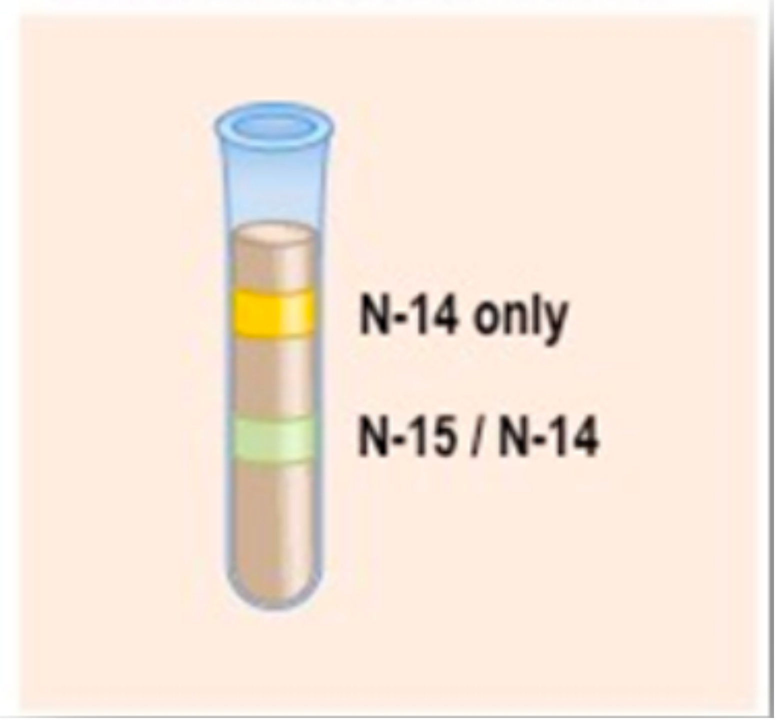
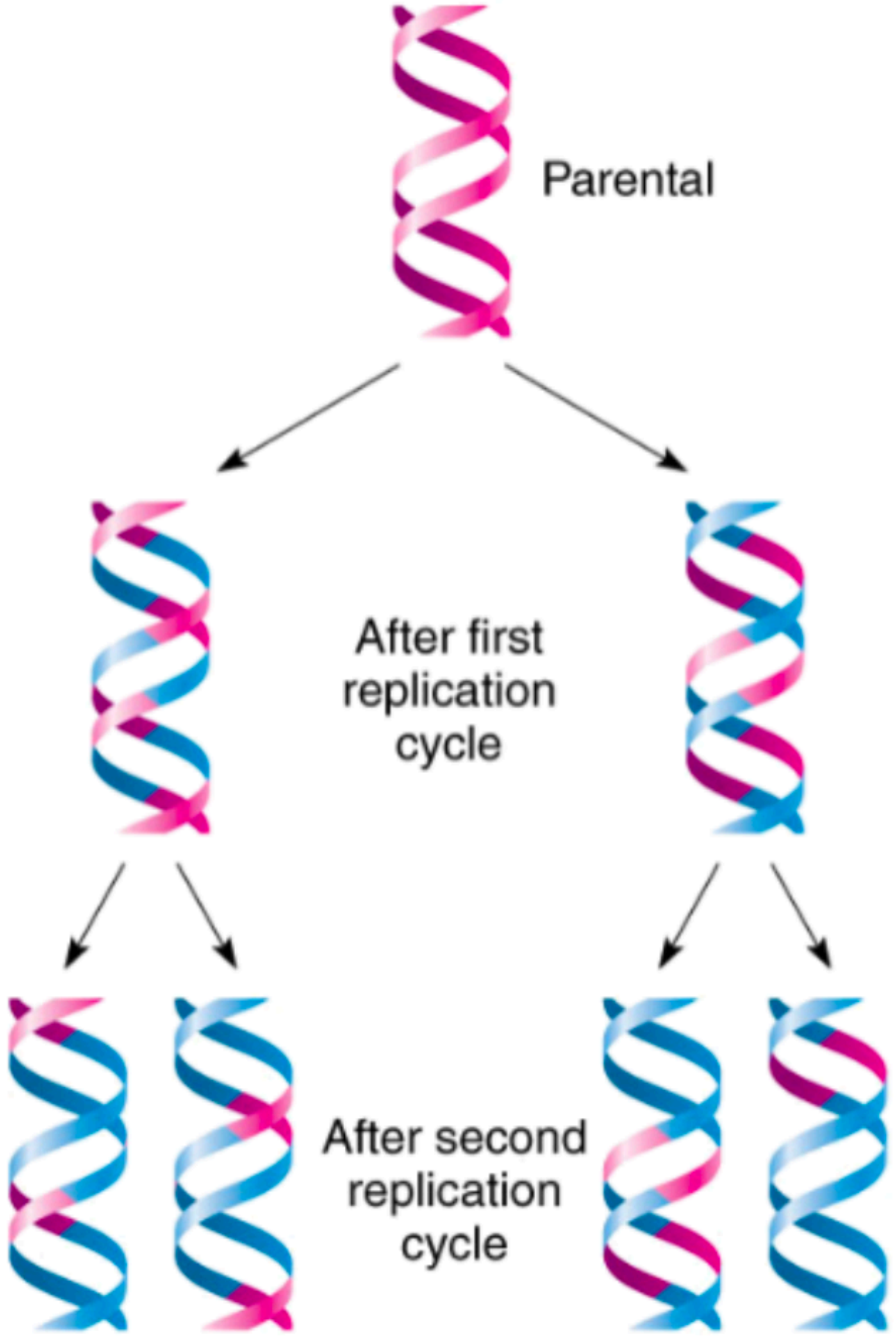


第二轮复制后观察到：

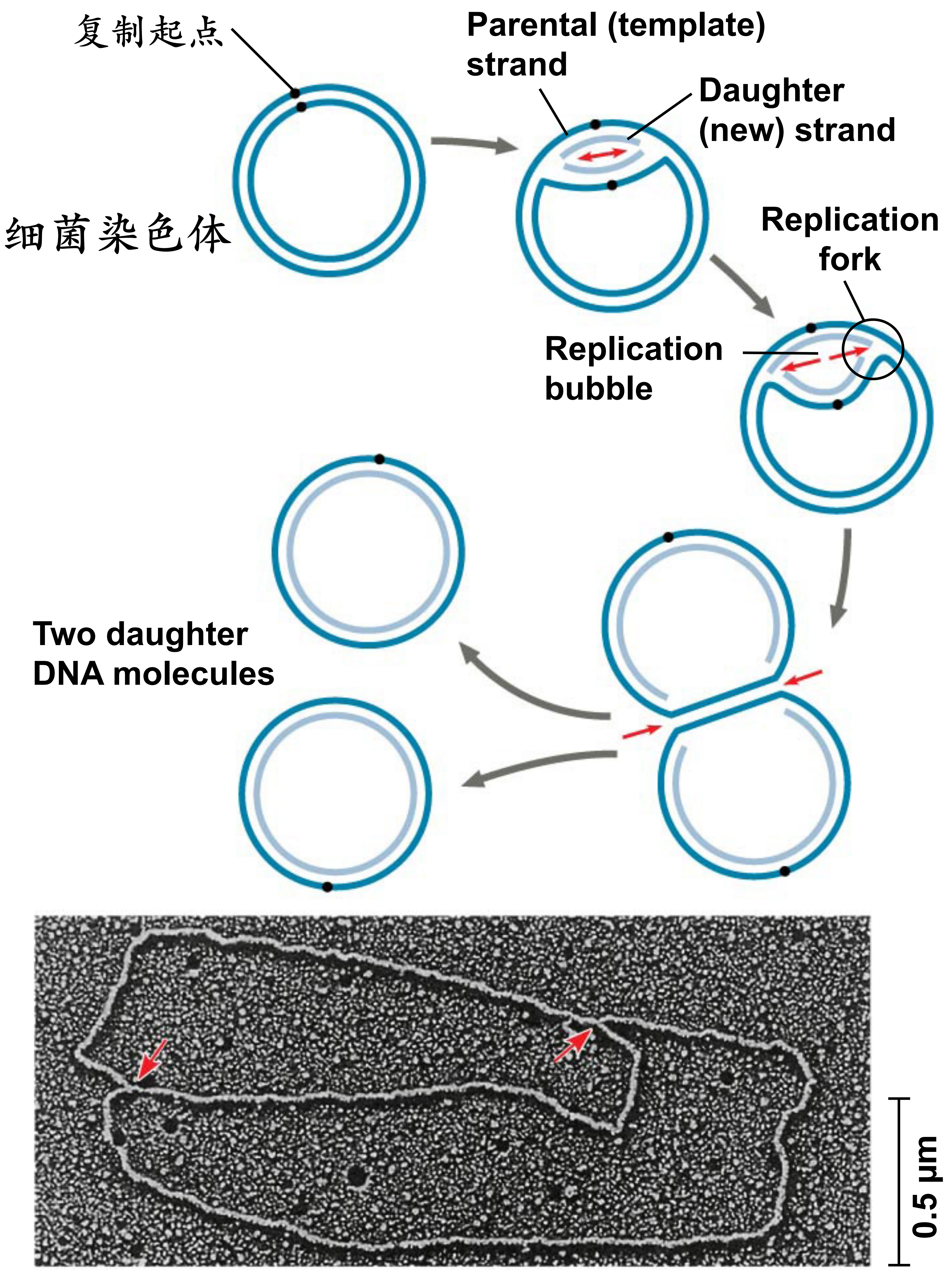


分散式复制

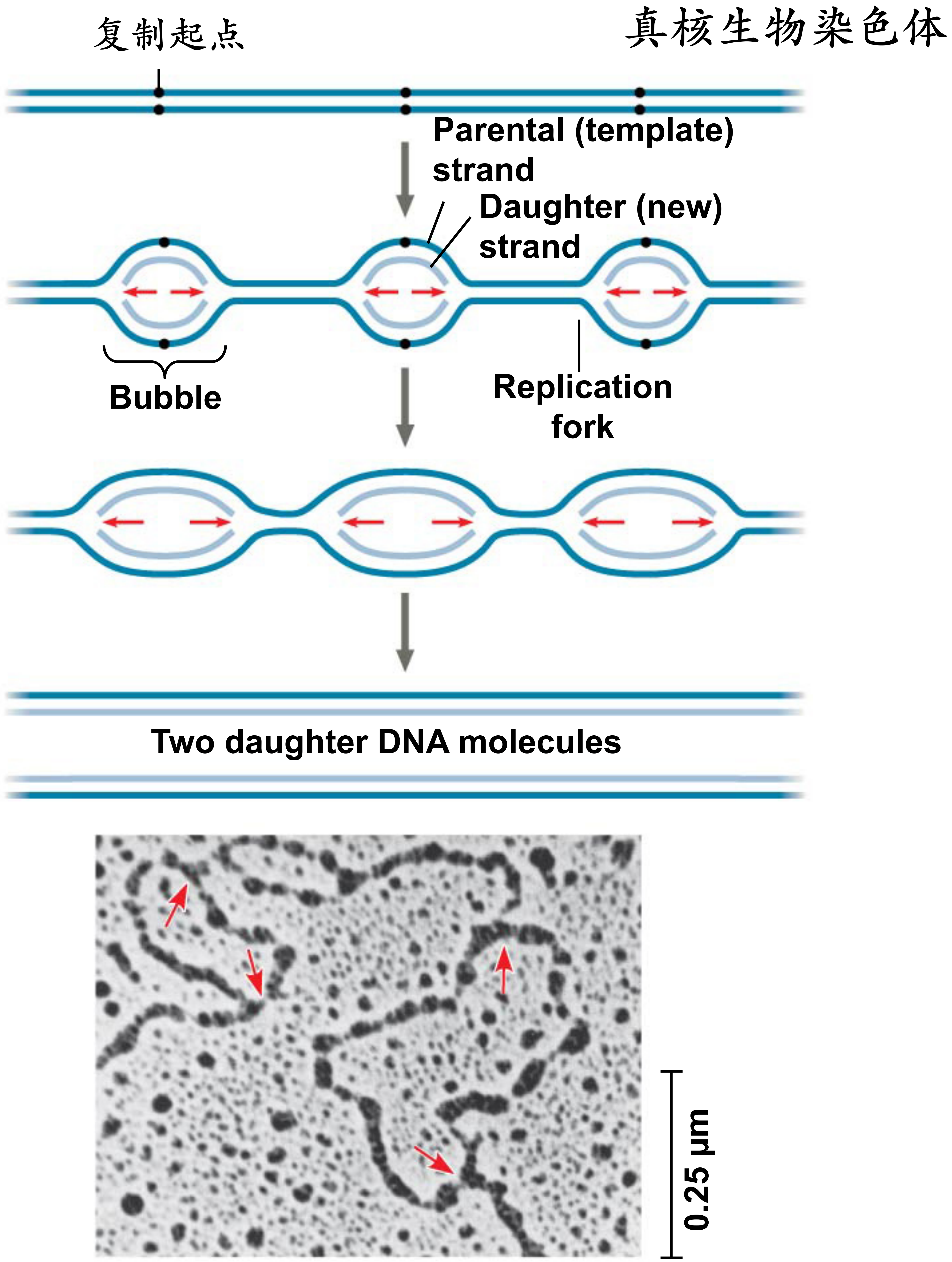


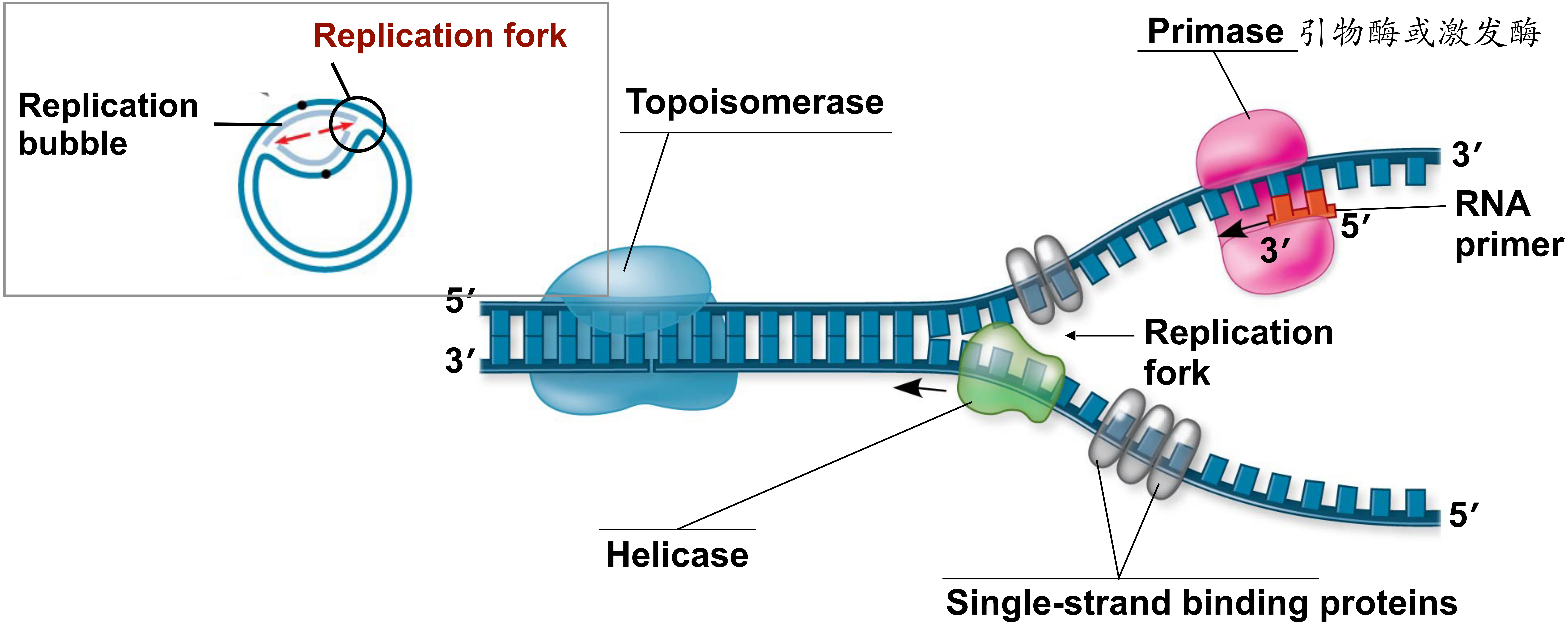
DNA半保留复制保证了所有体细胞携带相同的遗传信息，并可以将遗传信息稳定地传递给下一代

(a) Origin of replication in an *E. coli* cell



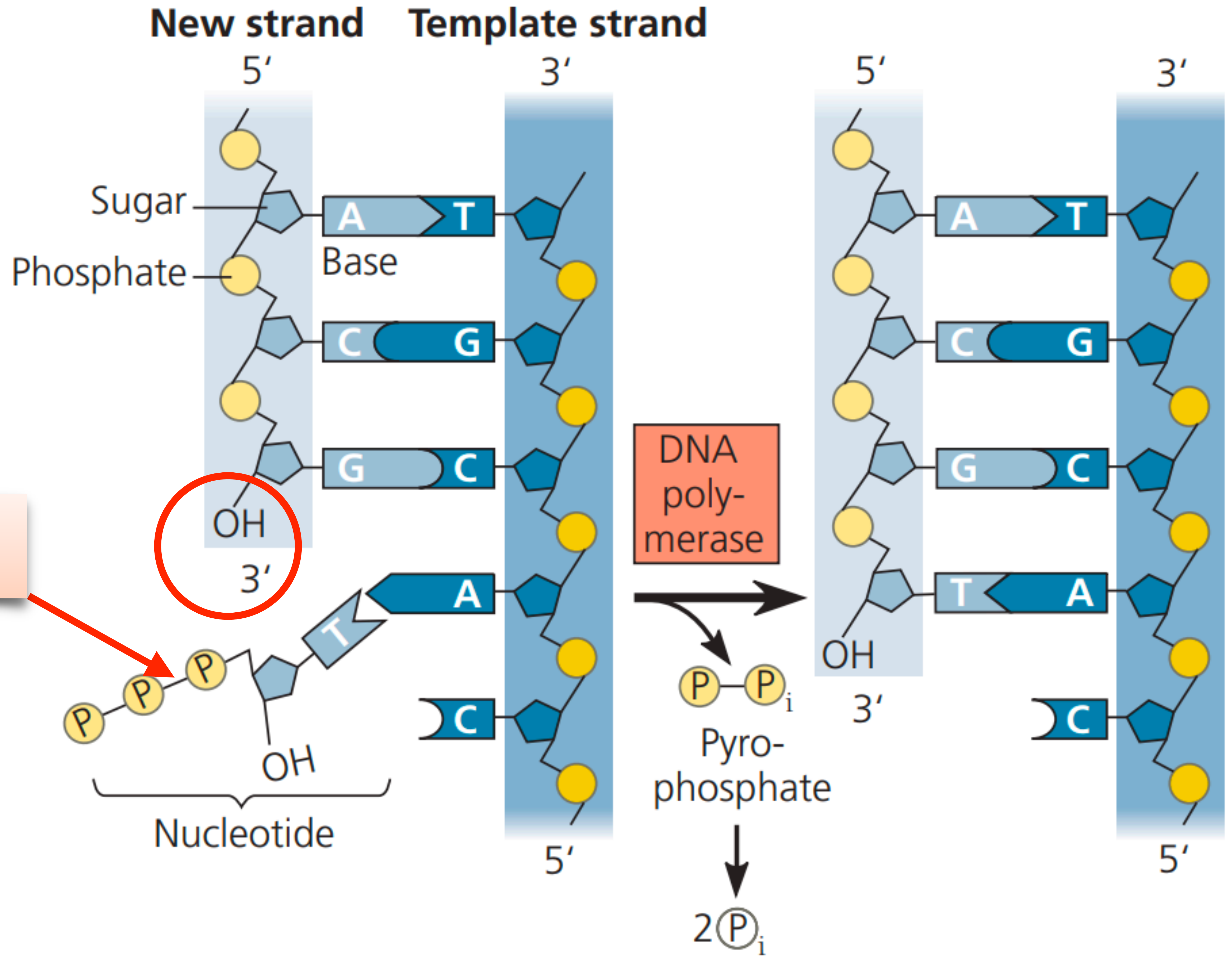
(b) Origins of replication in a eukaryotic cell





- Topoisomerase (拓扑异构酶) corrects "overwinding" ahead of replication forks
- Helicases (解旋酶) are enzymes that untwist the double helix
- Single-strand binding proteins bind to and stabilize single-stranded DNA

- Synthesizing a New DNA Strand



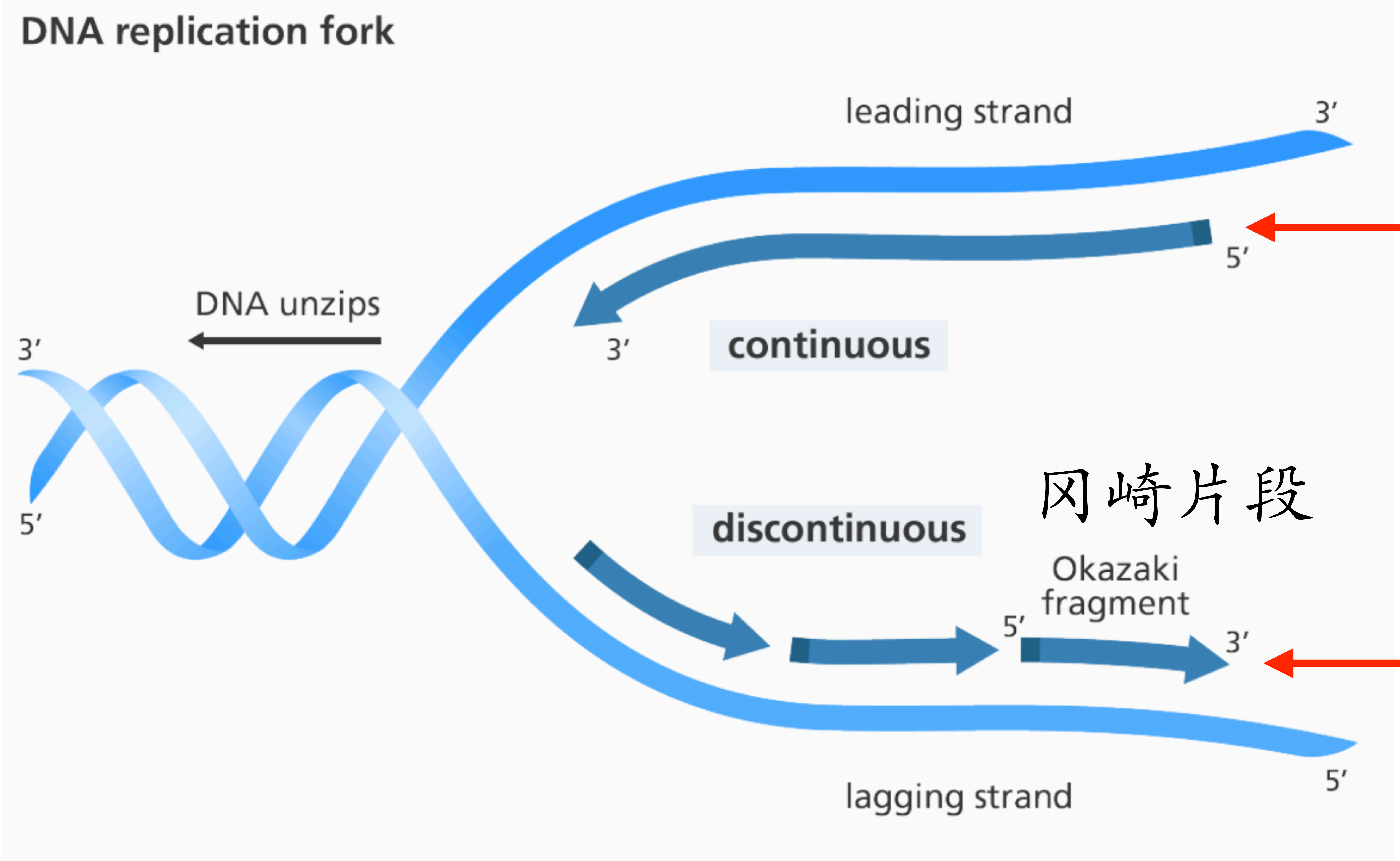
高能磷酸键

A new DNA strand can elongate only in the 5′ — 3′ direction.

How does the antiparallel arrangement of the double helix affect replication?

A new DNA strand can elongate only in the 5′ — 3′ direction.

半不连续复制

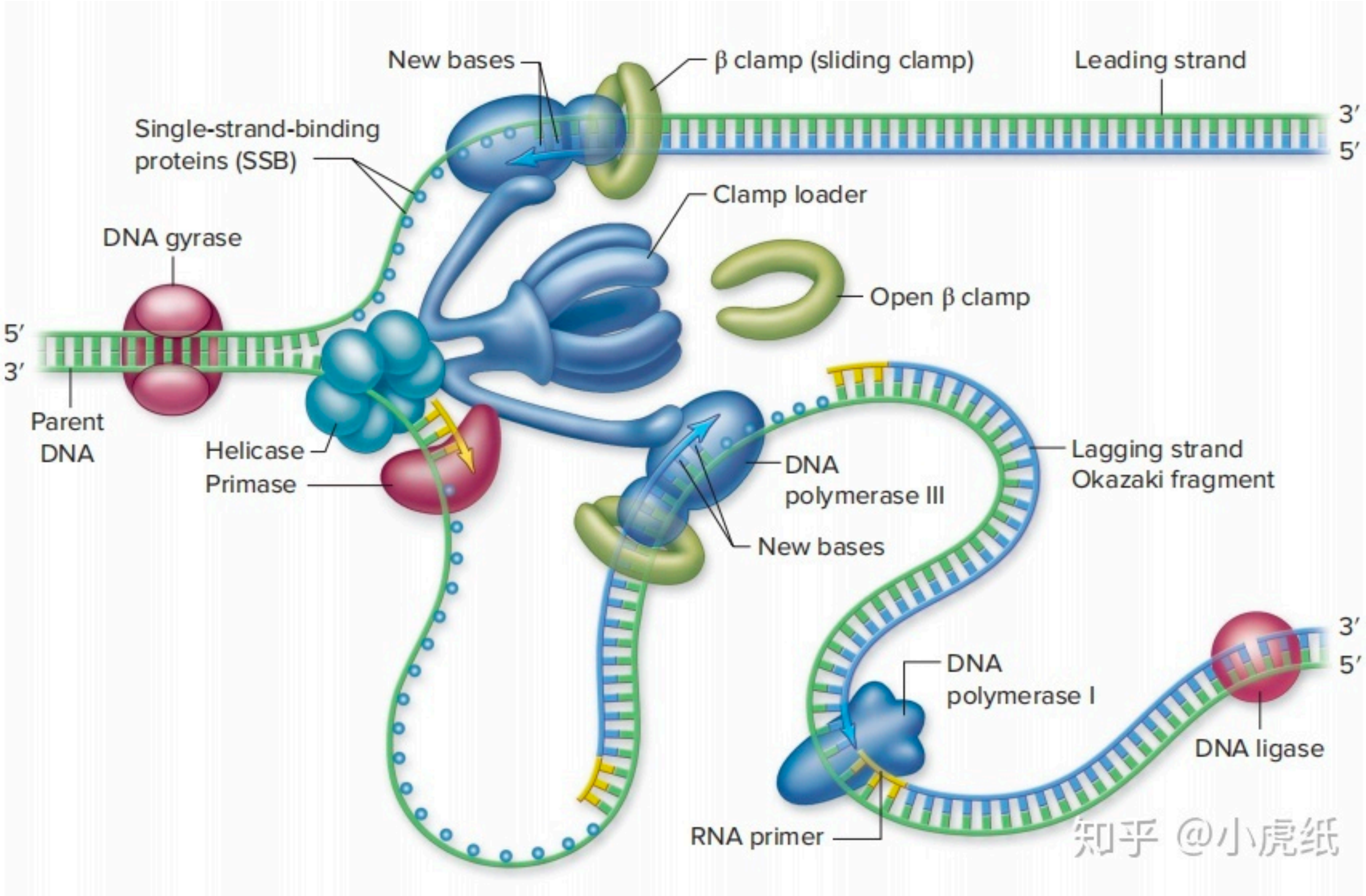


A new DNA strand 顺着合成的方向

冈崎片段

A new DNA strand 逆合成方向

DNA复制过程



- **DNA双螺旋的解链**

-DNA解旋酶在ATP供能下，每分钟旋转3000次解开双螺旋；

-单链DNA结合蛋白马上结合在分开的单链上，以避免产生单链内配对；

-DNA拓扑异构酶来解决由于复制叉的推进而产生超螺旋的问题。

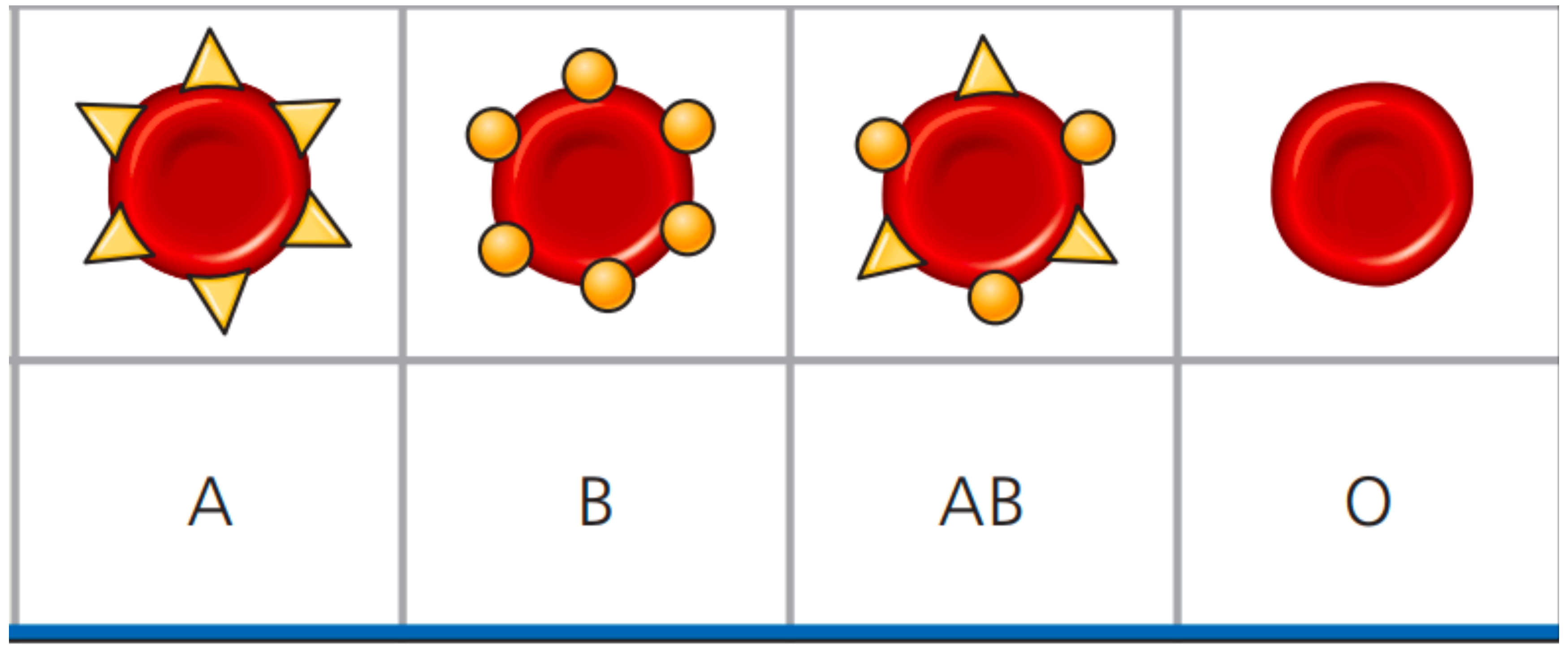
- **DNA合成开始**

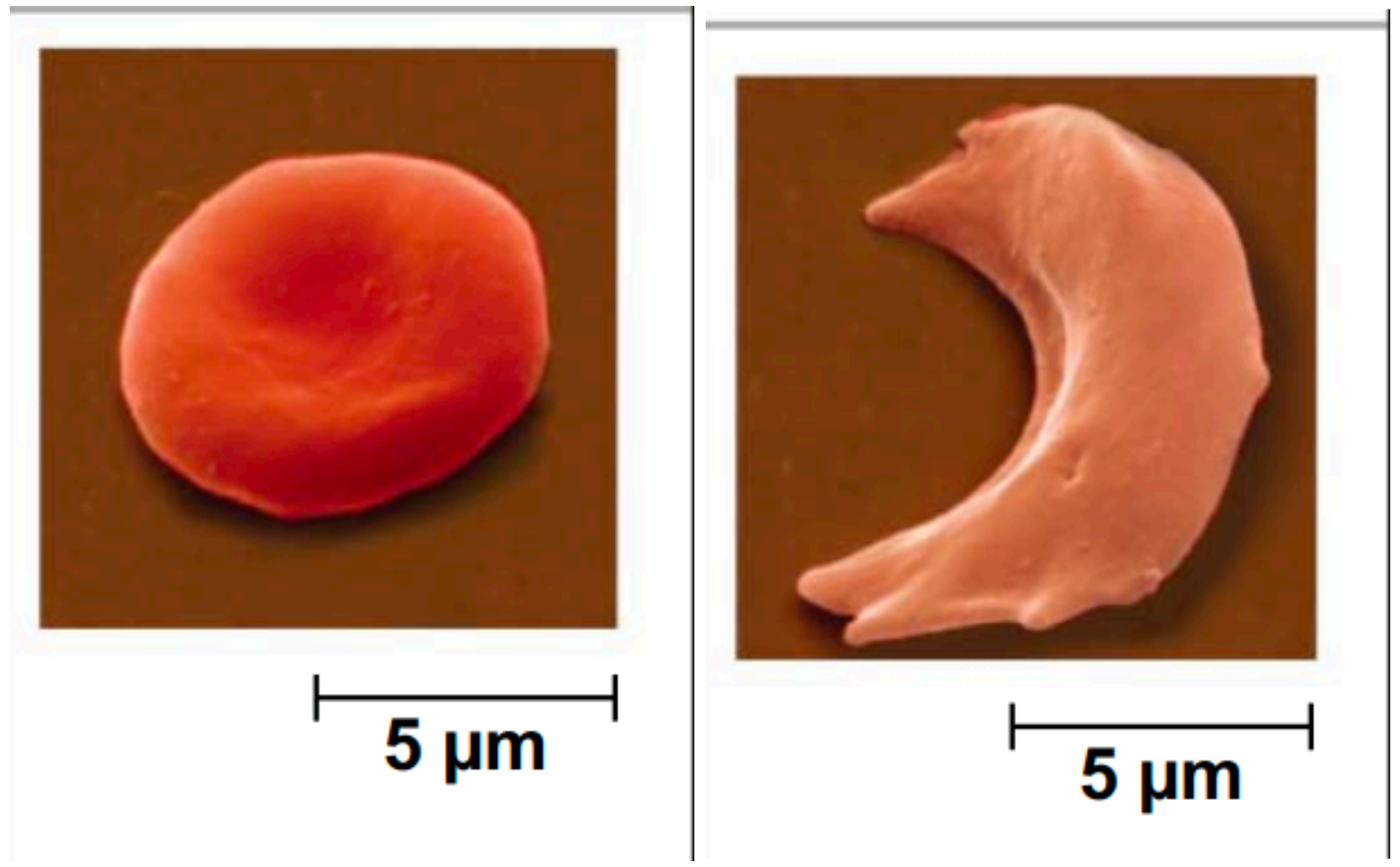
-RNA聚合酶合成一小段RNA引物，DNA聚合酶才开始起作用合成DNA片段。

- 基因复制

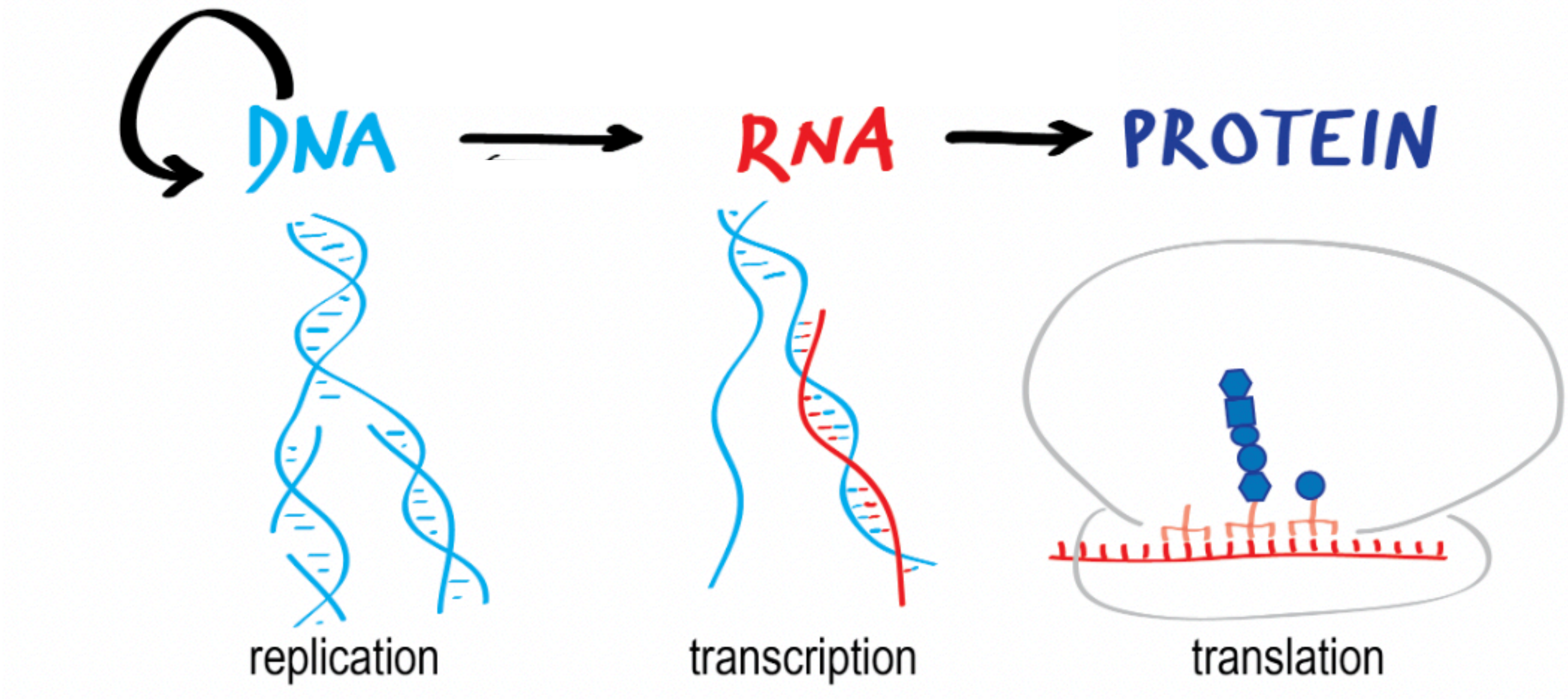
基因是如何决定性状的？

- 基因表达





基因的表达遵从中心法则



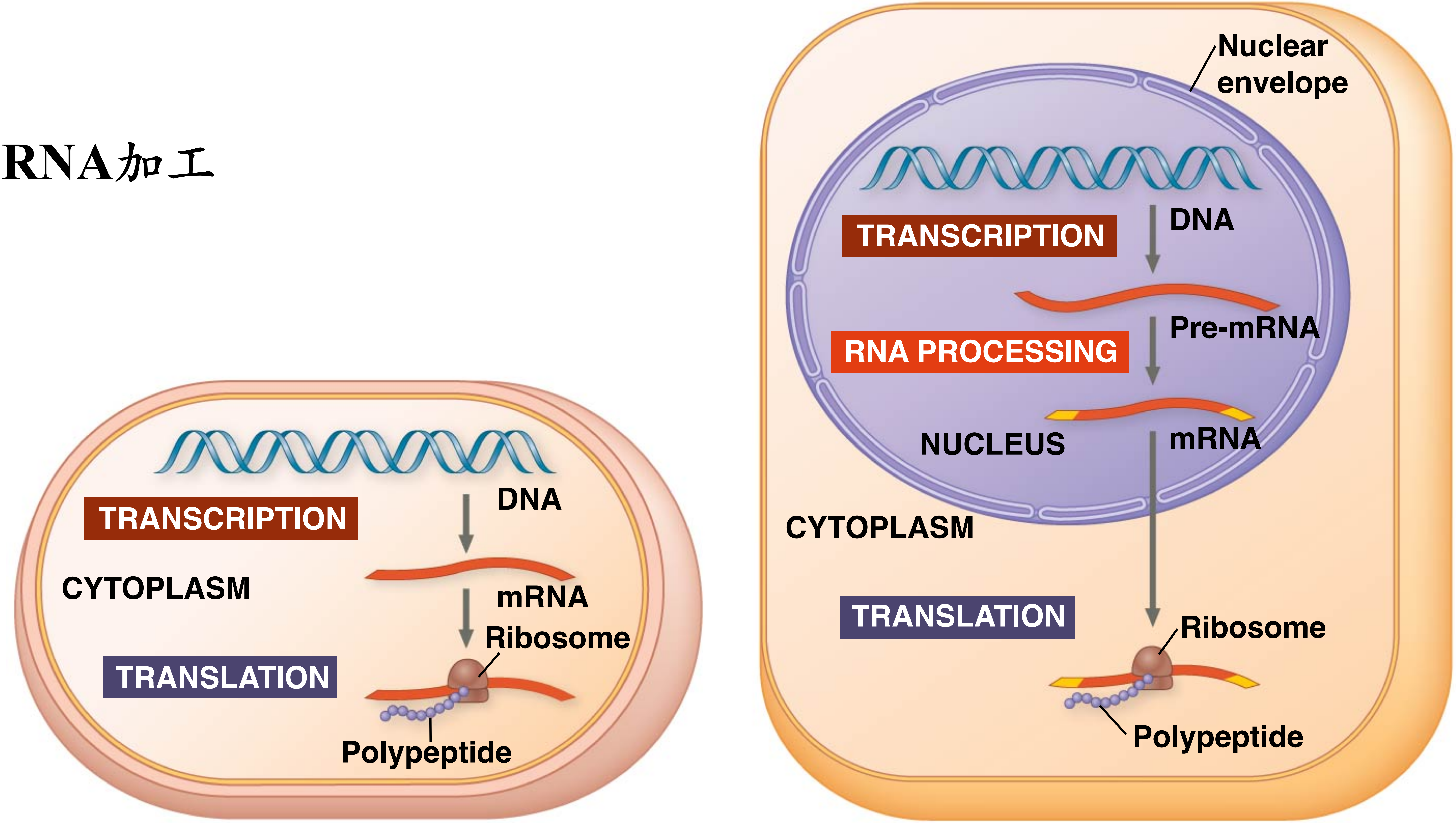
(by Francis Crick in 1957)

- 蛋白质是联系基因型和表型的关键
- 基因表达：从**DNA**到合成蛋白质

Adapted from Vourekas lab website

基因表达的具体步骤

- 转录
- pre-mRNA加工
- 翻译



(a) Bacterial cell

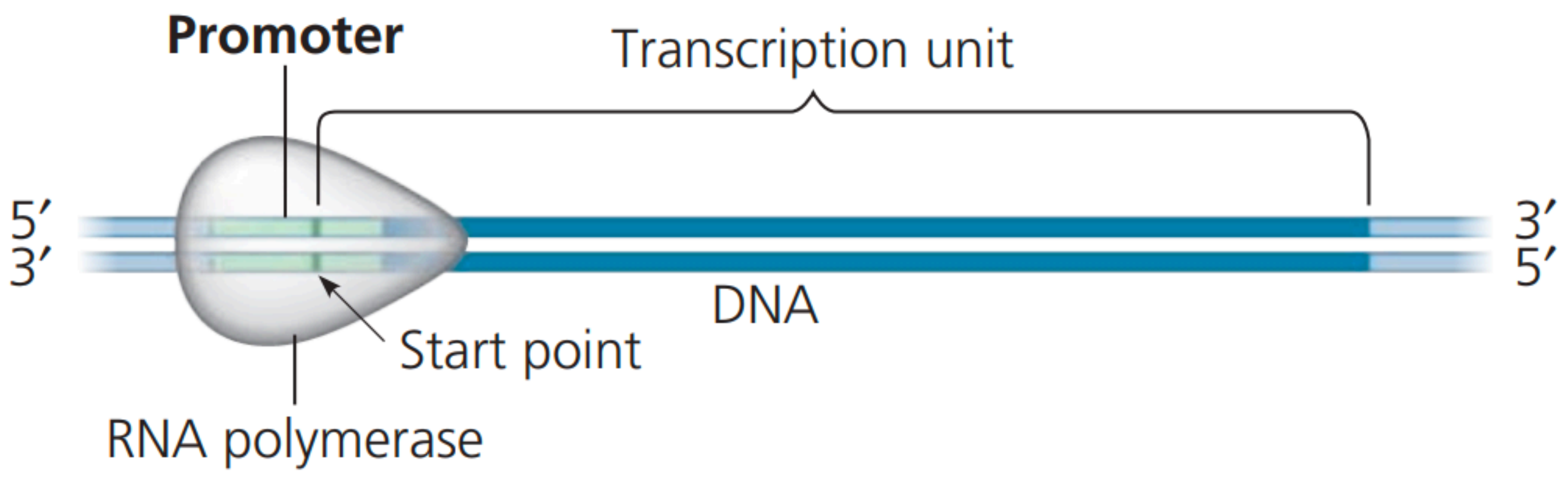
(b) Eukaryotic cell

The first stage of gene expression—Transcription 转录

- 启动子

  一段能使基因进行转录的**DNA**序列

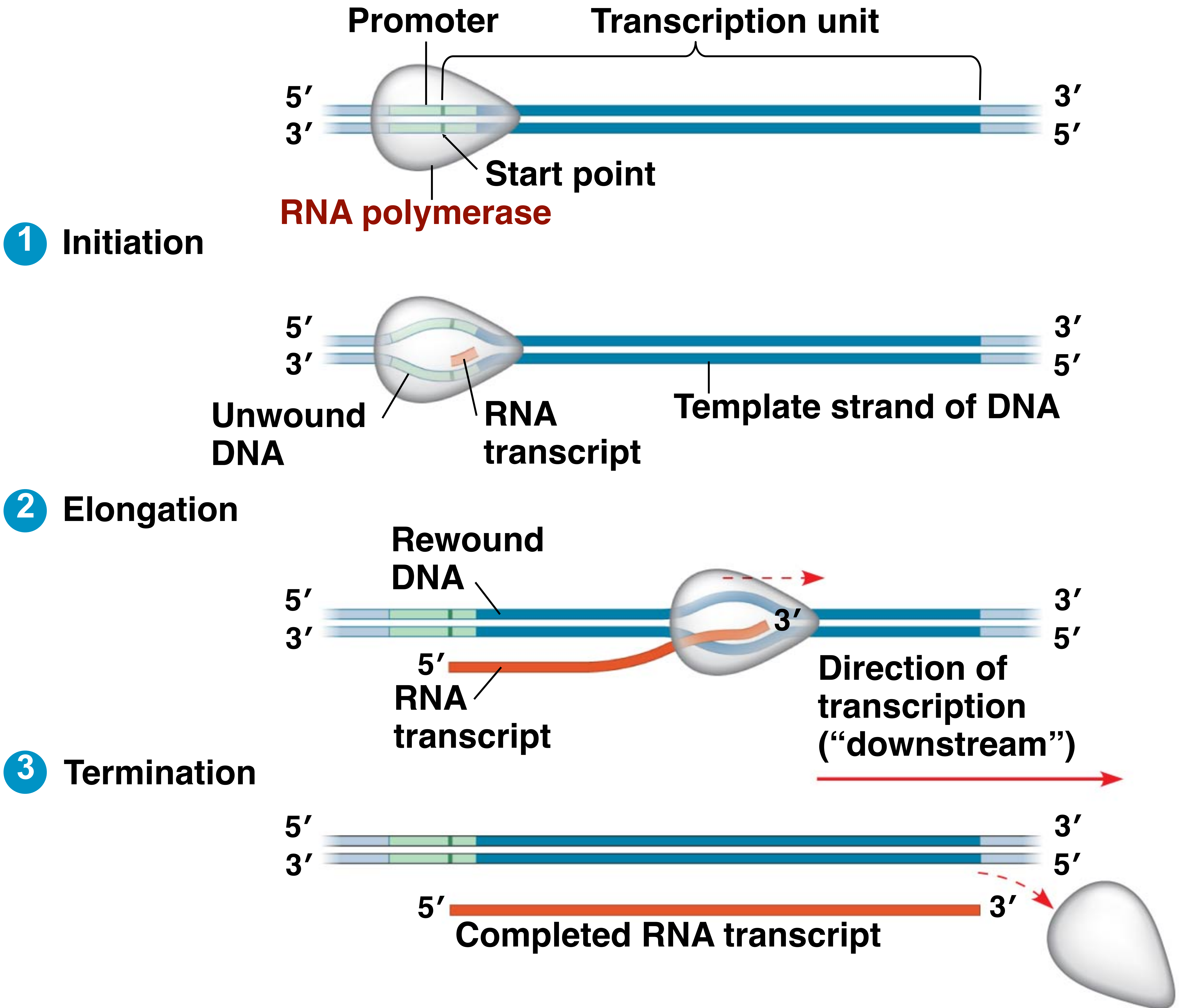
  可被**RNA**聚合酶所识别并和其结合



- 转录：**DNA**指导的合成**RNA**的过程

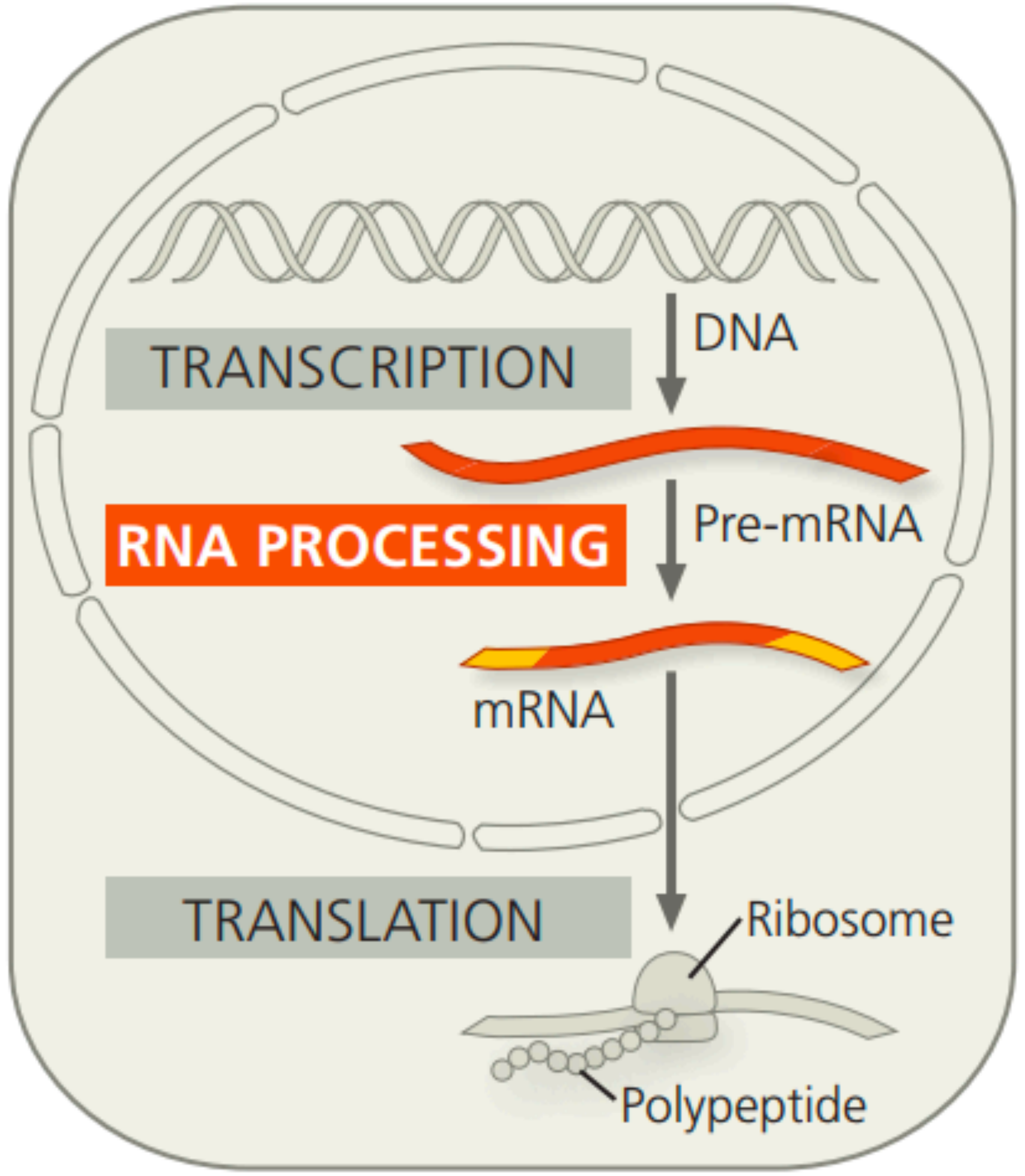
转录

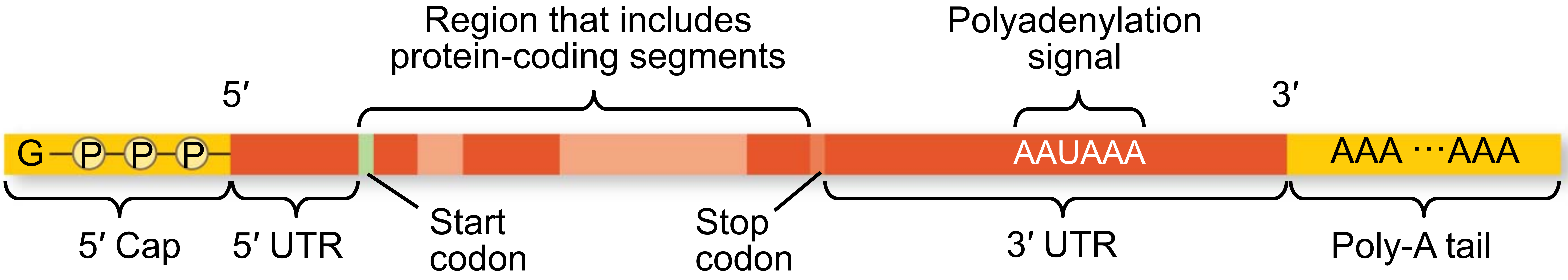
- The RNA is complementary to the DNA template strand
- RNA synthesis follows the same base-pairing rules as DNA, except that uracil substitutes for thymine



pre-mRNA加工

- 在真核生物中，转录后新合成的mRNA是未成熟的，称为前体mRNA (pre-mRNA)。
- 5’端加“帽子” (5’-Cap),
- 3’端加“尾巴” (polyA tail)
- 去除内含子 (Splicing)

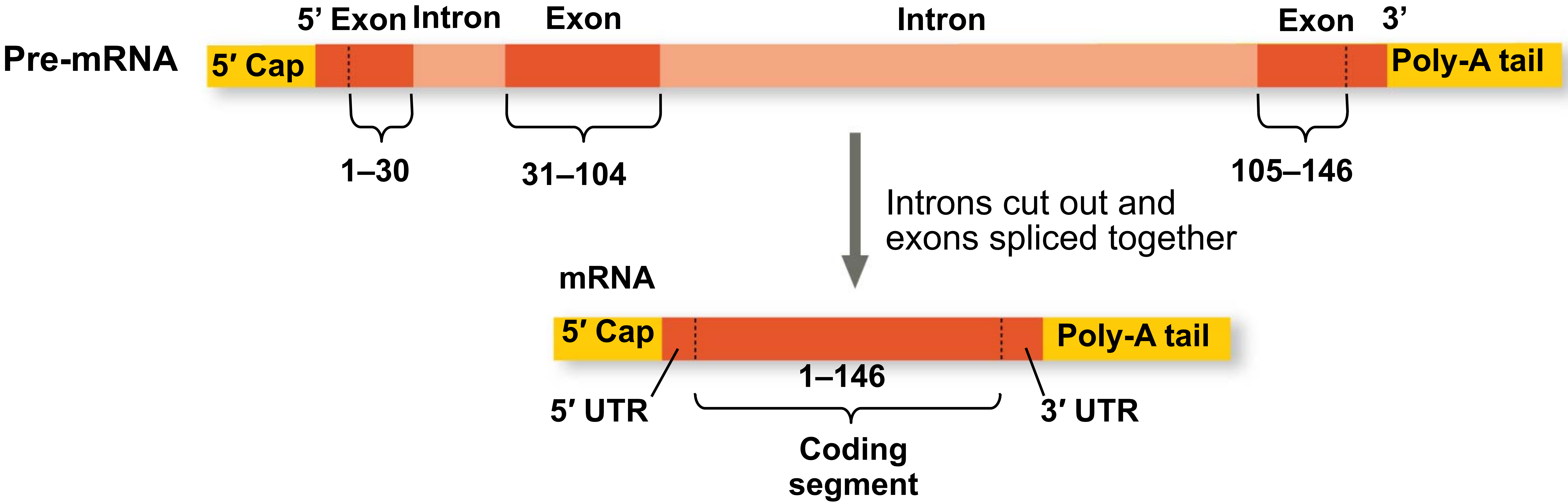


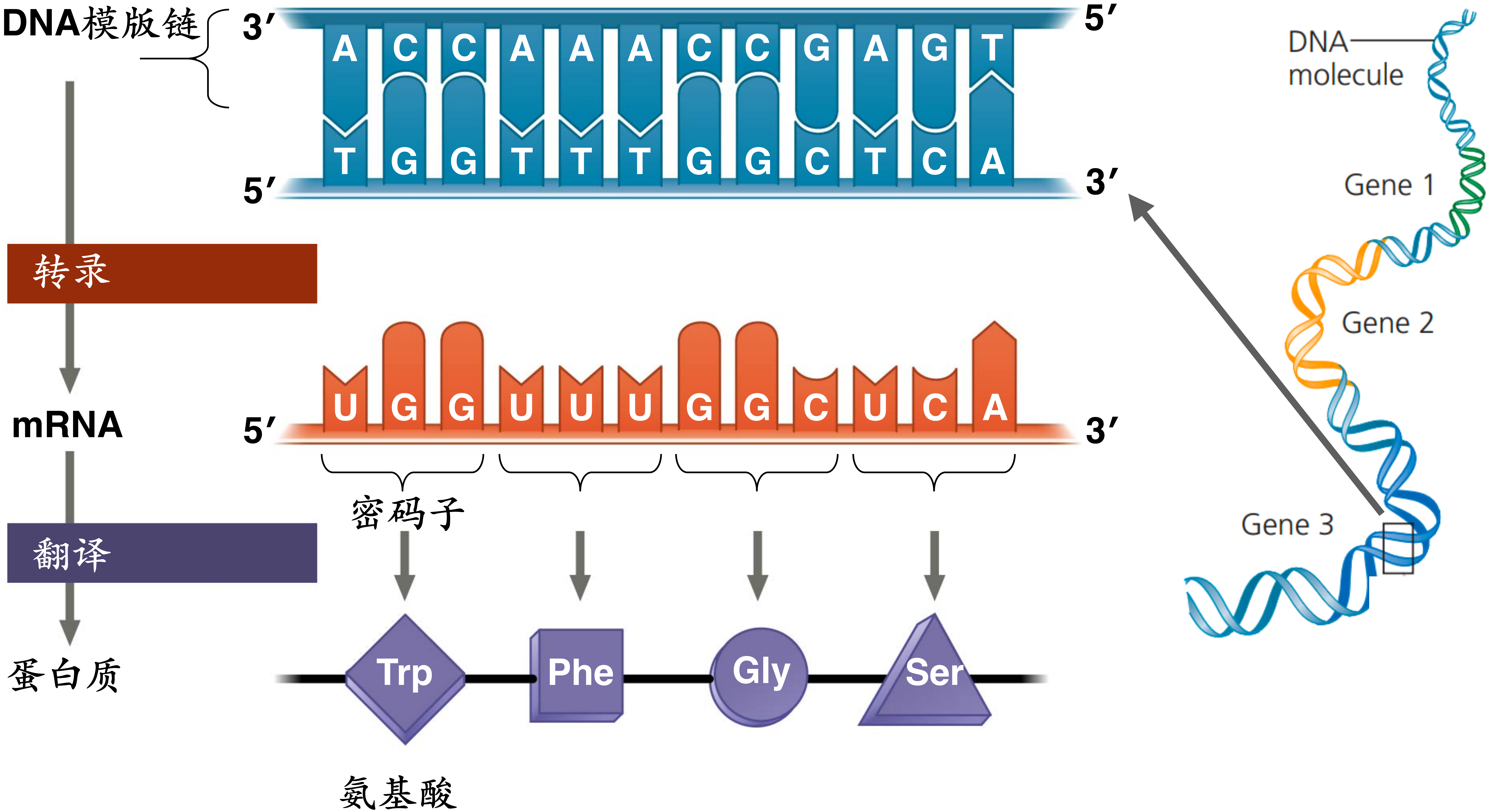


- Splicing 剪接/内含子去除

  内含子 Intron： 非编码区域

  外显子 Exon： 最终被翻译成氨基酸的序列





DNA模板链

转录

mRNA

翻译

蛋白质

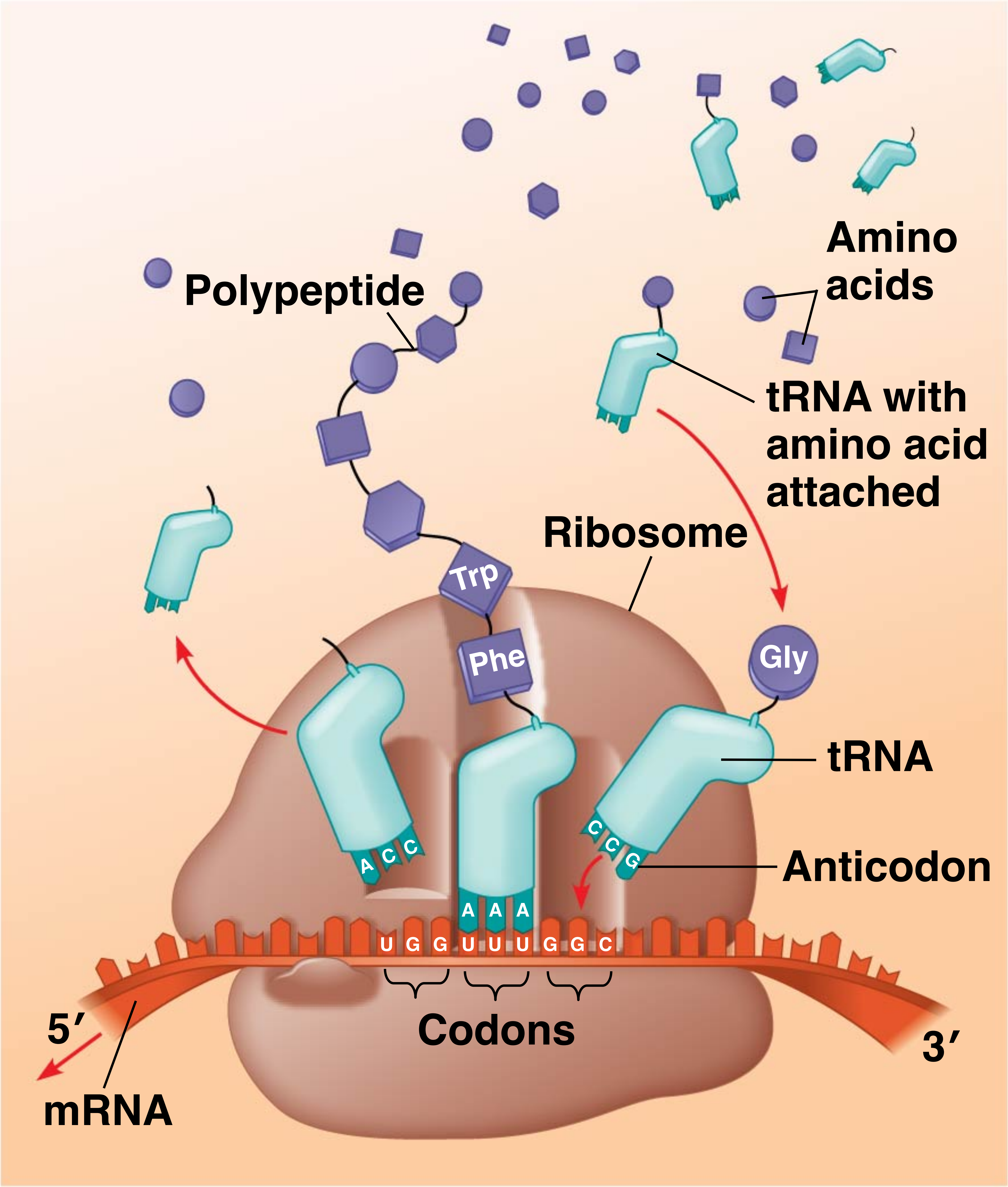
翻译 Translation

从RNA 产生多肽链的过程

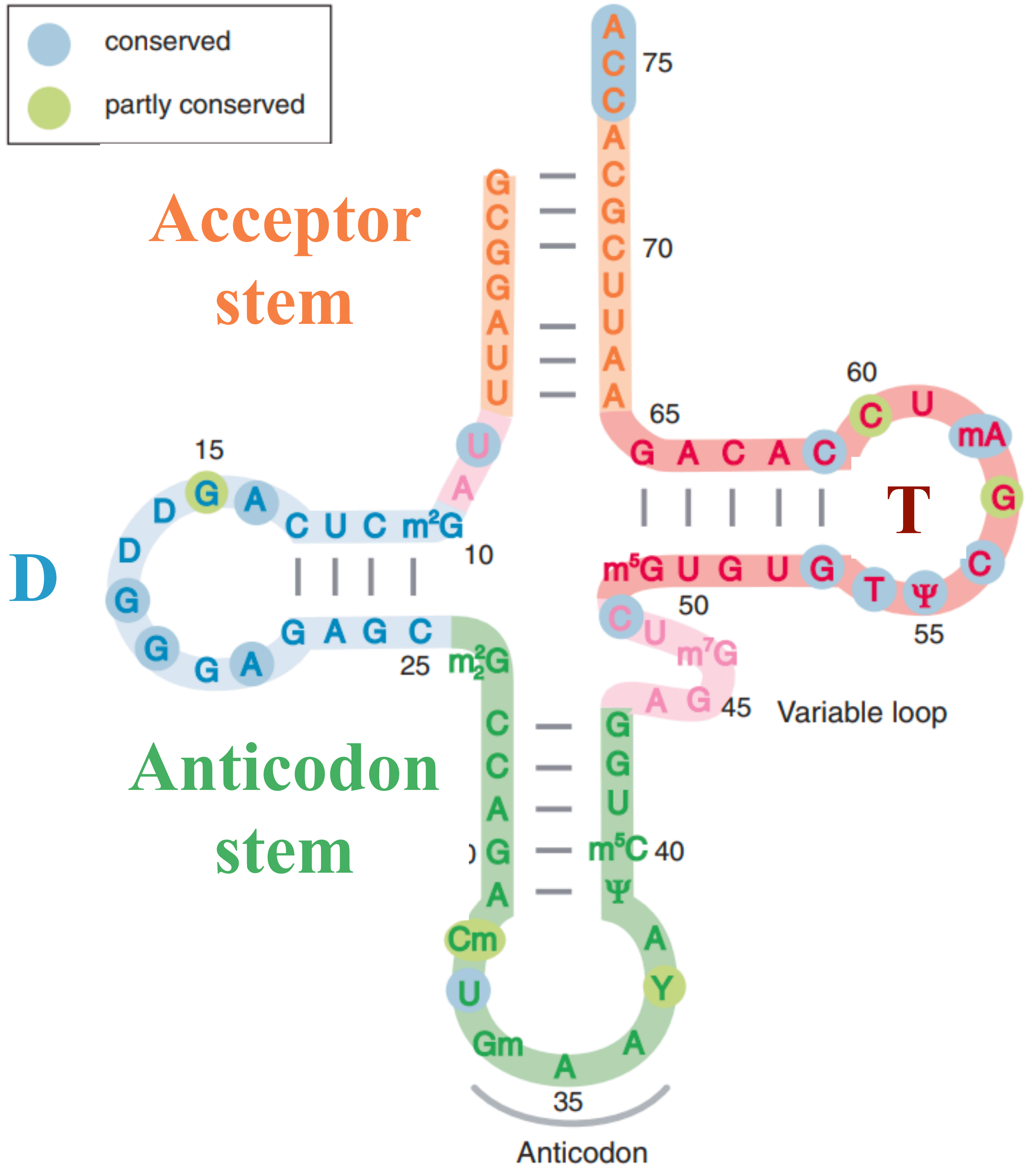
原料：mRNA，tRNA，核糖体，多种氨基酸，多种酶

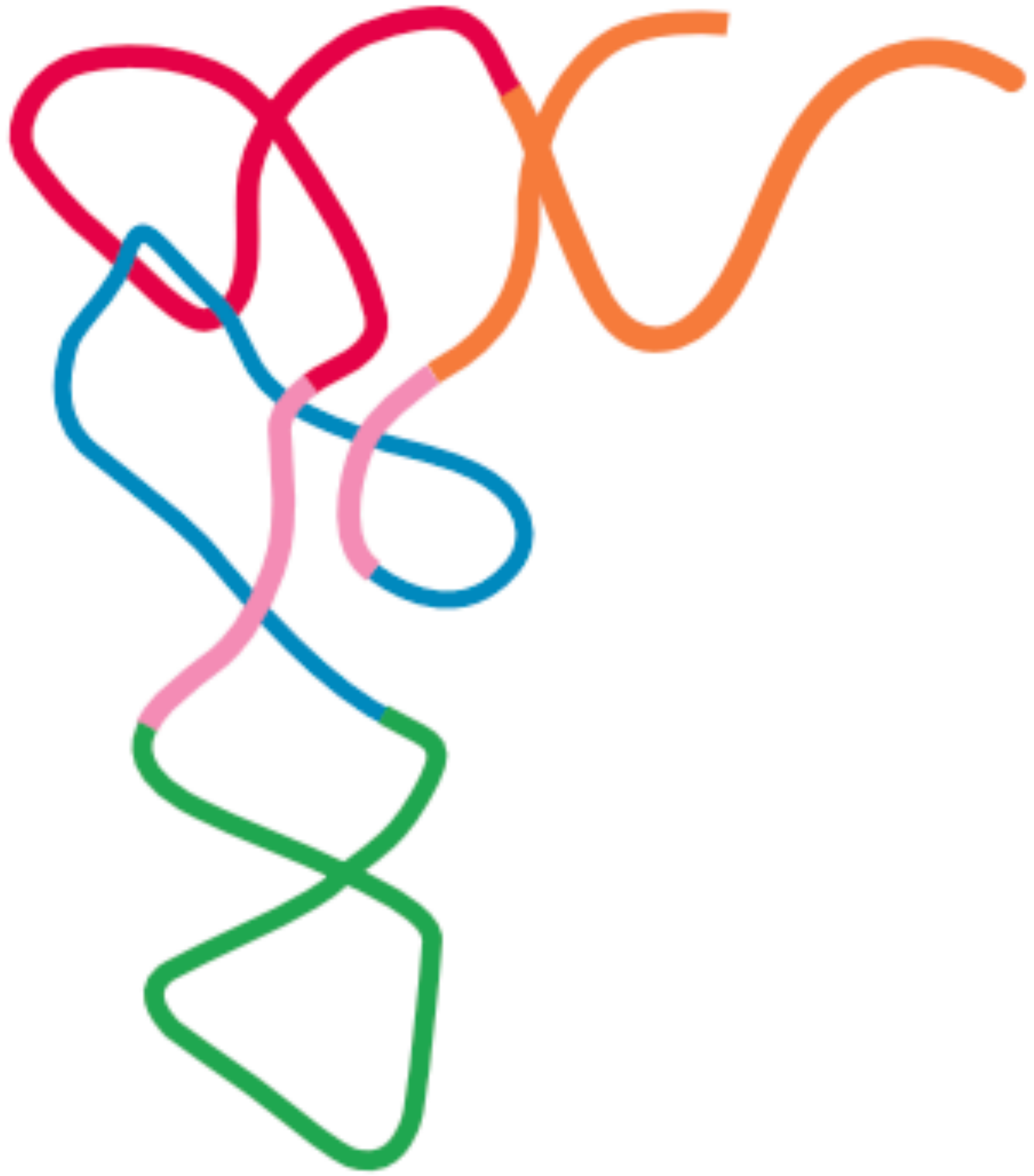
rRNA：核糖体组成成分，占细胞总RNA 80%

过程：起始、多肽链的延长、翻译终止

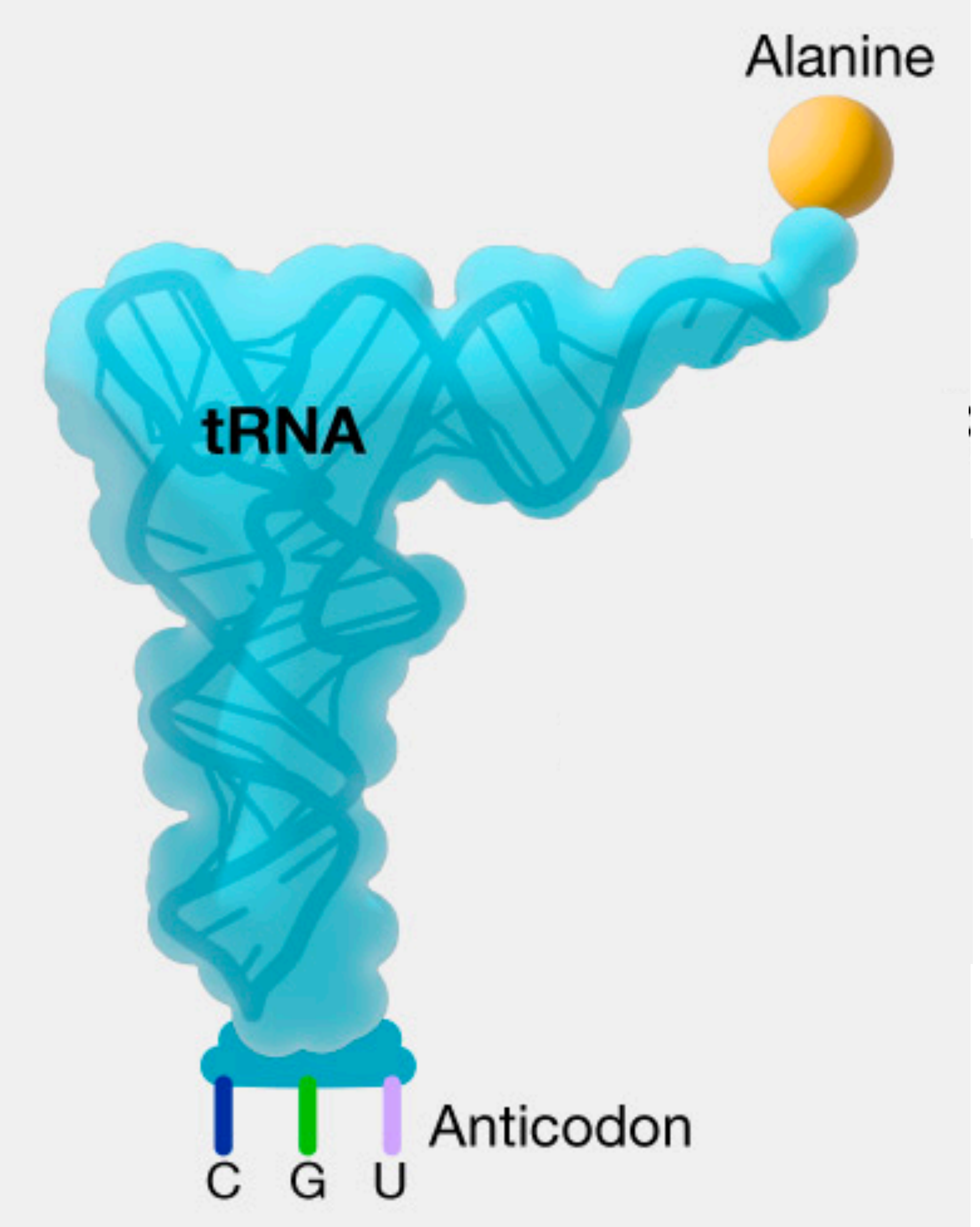


tRNA


conserved
partly conserved
Acceptor stem
D
T
Anticodon stem
Variable loop
Anticodon
G C G G A U U U A
A C C A C G C U U A A
15
10
25
35
40
45
50
55
60
65
70
75
C U C m²G
D G A
D D G G G A
G A G C
m²₂G
C C A G A
Cm U Gm A A Y A Ψ m⁵C U G G
A G m⁷G U C
m⁵G U G U G
G A C A C
C U mA G C Ψ T

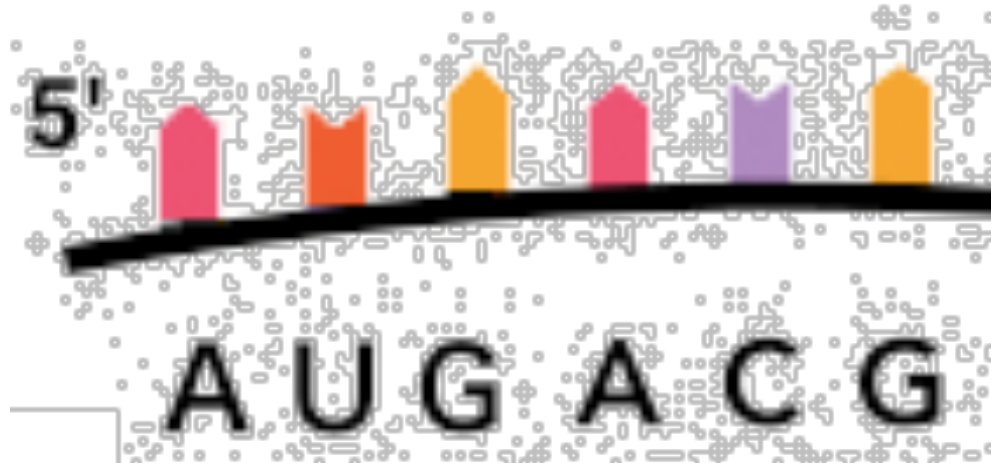


反密码子



- Each type of tRNA molecule can be attached to only one type of amino acid, so each organism has many types of tRNA.

20 amino acids

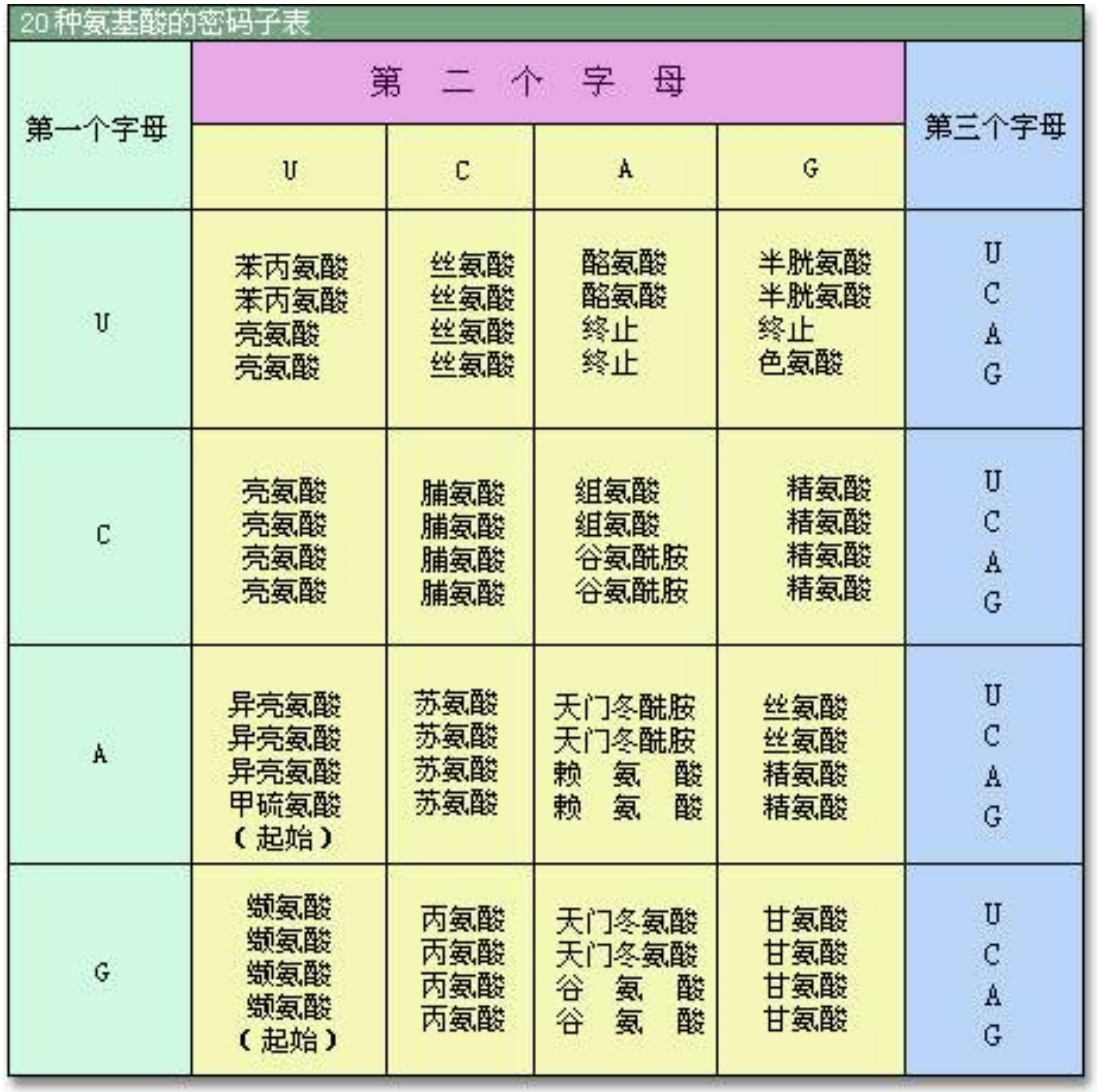


DNA分子碱基只有4种，而蛋白质氨基酸有20种。因此，碱基与氨基酸之间不可能一一对应。

1. 4^1=4种：缺16种氨基酸；
2. 4^2=16种：比现存的20种氨基酸还缺4种；
3. 4^3=64种：由三个碱基一起组成的密码子能够形成64种组合，比20种氨基酸多出44种。

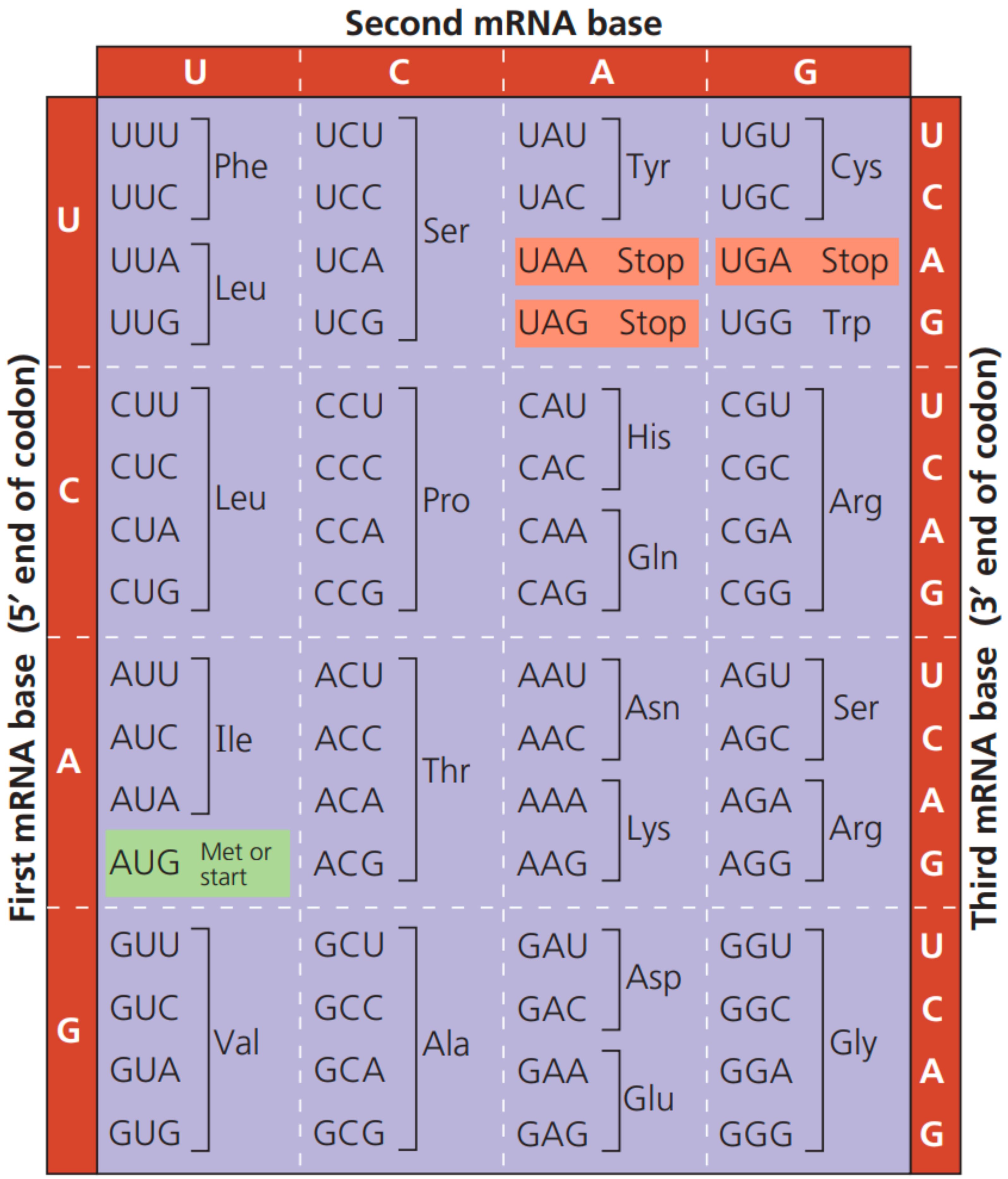
A		A		A
U		U		U
C	X	C	X	C
G		G		G

密码子表



20种氨基酸的密码子表

第一个字母	第二个字母				第三个字母
	U	C	A	G	
U	苯丙氨酸 苯丙氨酸 亮氨酸 亮氨酸	丝氨酸 丝氨酸 丝氨酸 丝氨酸	酪氨酸 酪氨酸 终止 终止	半胱氨酸 半胱氨酸 终止 色氨酸	U C A G
C	亮氨酸 亮氨酸 亮氨酸 亮氨酸	脯氨酸 脯氨酸 脯氨酸 脯氨酸	组氨酸 组氨酸 谷氨酰胺 谷氨酰胺	精氨酸 精氨酸 精氨酸 精氨酸	U C A G
A	异亮氨酸 异亮氨酸 异亮氨酸 甲硫氨酸（起始）	苏氨酸 苏氨酸 苏氨酸 苏氨酸	天门冬酰胺 天门冬酰胺 赖氨酸 赖氨酸	丝氨酸 丝氨酸 精氨酸 精氨酸	U C A G
G	缬氨酸 缬氨酸 缬氨酸 缬氨酸（起始）	丙氨酸 丙氨酸 丙氨酸 丙氨酸	天门冬氨酸 天门冬氨酸 谷氨酸 谷氨酸	甘氨酸 甘氨酸 甘氨酸 甘氨酸	U C A G



First mRNA base (5′ end of codon)	Second mRNA base				Third mRNA base (3′ end of codon)
	U	C	A	G	
U	UUU Phe	UCU	UAU Tyr	UGU Cys	U
	UUC	UCC Ser	UAC	UGC	C
	UUA Leu	UCA	UAA Stop	UGA Stop	A
	UUG	UCG	UAG Stop	UGG Trp	G
C	CUU	CCU	CAU His	CGU	U
	CUC Leu	CCC Pro	CAC	CGC Arg	C
	CUA	CCA	CAA Gln	CGA	A
	CUG	CCG	CAG	CGG	G
A	AUU	ACU	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC	AGC	C
	AUA	ACA	AAA Lys	AGA Arg	A
	AUG Met or start	ACG	AAG	AGG	G
G	GUU	GCU	GAU Asp	GGU	U
	GUC Val	GCC Ala	GAC	GGC Gly	C
	GUA	GCA	GAA Glu	GGA	A
	GUG	GCG	GAG	GGG	G

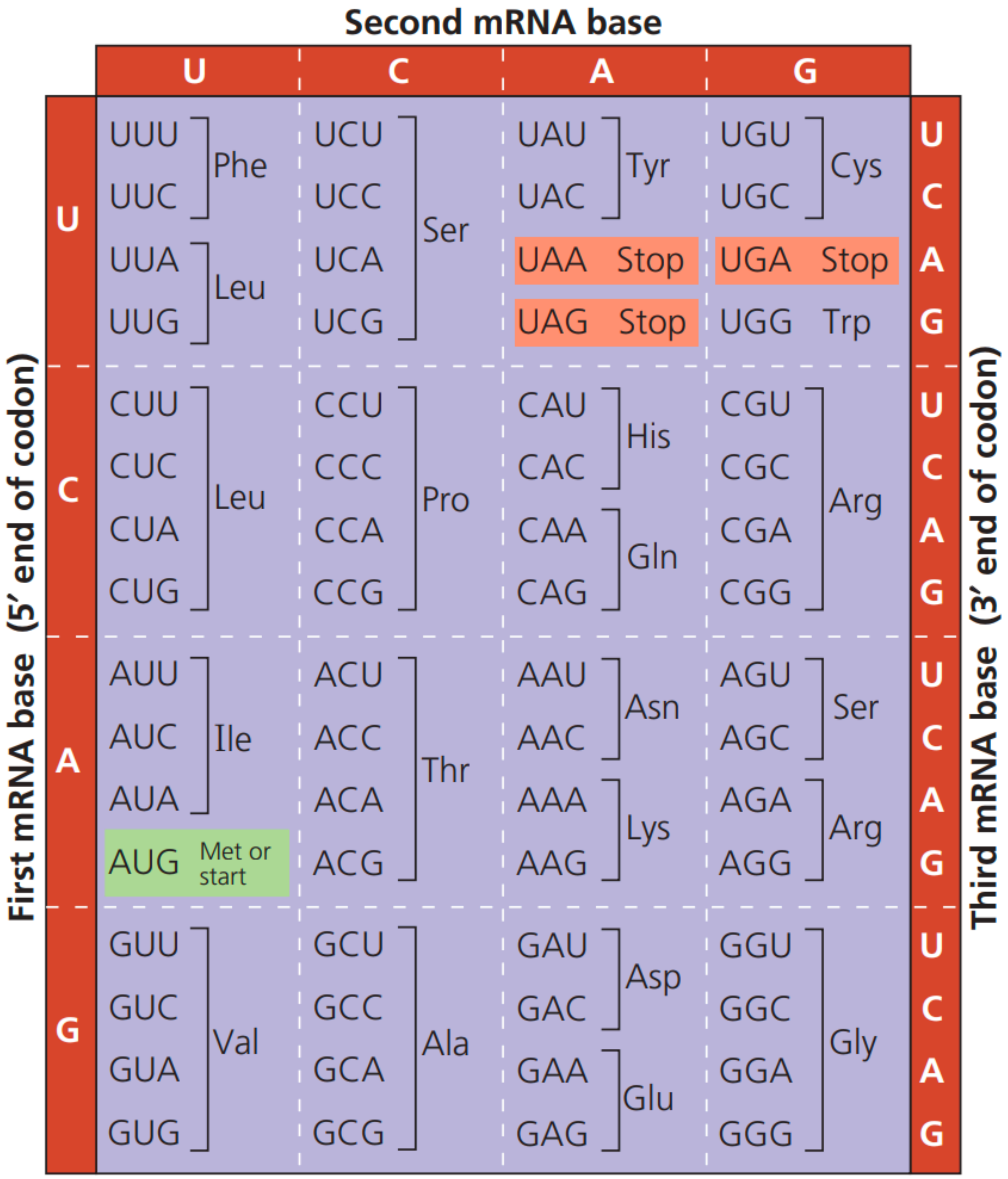
碱基和氨基酸的对应关系

遗传密码的基本特征

- **三联体：** 三个碱基决定一种氨基酸
- **简并性：** 一个氨基酸由二个或二个以上的三联体密码所决定的现象。

简并现象的意义：

同义的密码子越多，生物遗传的稳定性也越大。如：UCU突变为UCC或UCA或UCG，均为丝氨酸。



First mRNA base (5′ end of codon)	Second mRNA base								Third mRNA base (3′ end of codon)
	U		C		A		G		
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met or start	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

遗传密码的基本特征

- **通用性：** 不同的生物密码子基本相同，即共用一套密码子。

在整个生物界中，从病毒到人类，遗传密码通用

4个基本碱基→所有氨基酸

→所有蛋白质

→ 生物种类、生物体性状

<u>The</u> <u>red</u> <u>dog</u> <u>ate</u> <u>the</u> <u>bug</u>

读码框 Reading frame

The red dog ate the bug

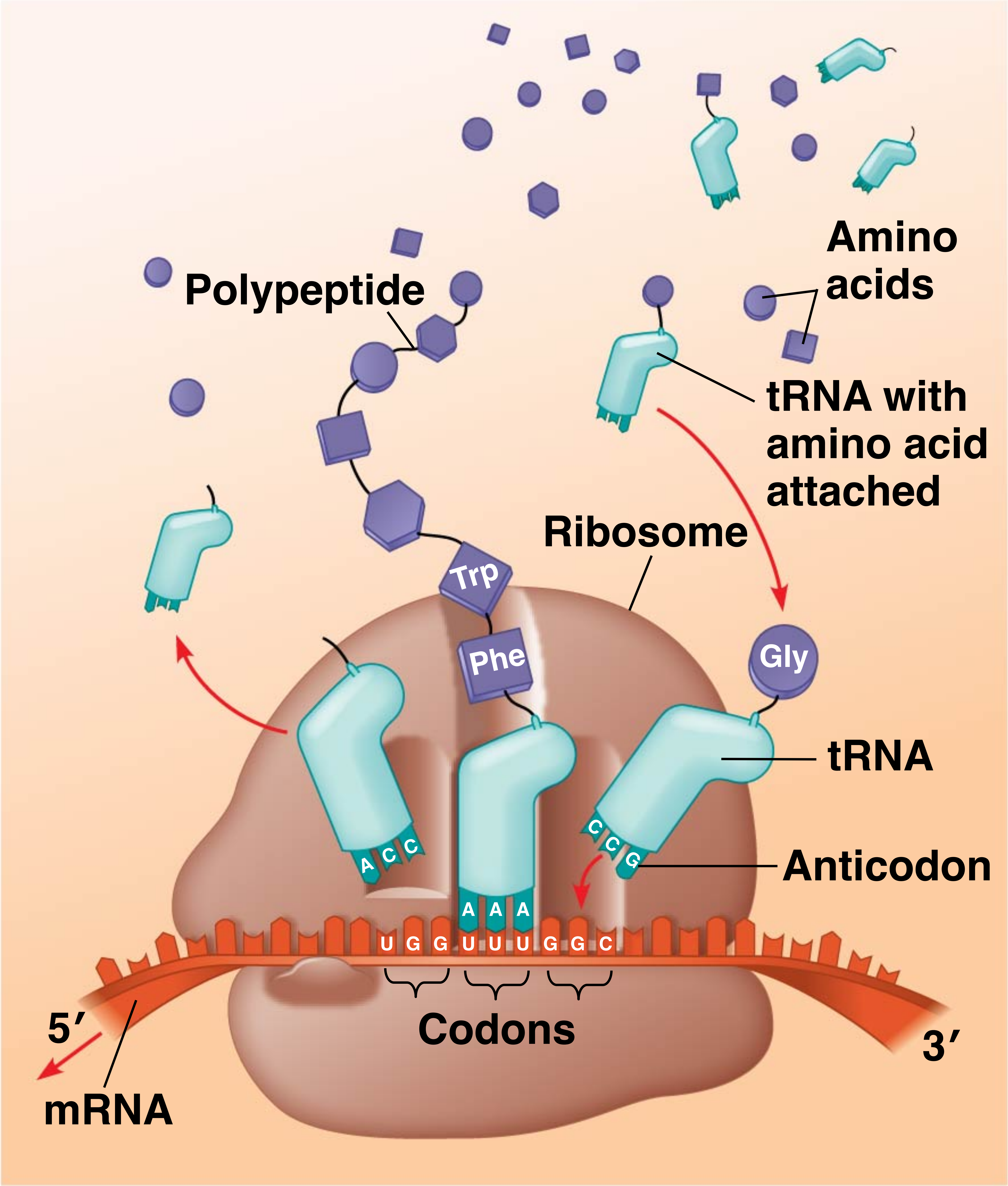
如果中间某个碱基增加或缺失后，阅读就会按新的顺序进行下去，最终形成的多肽链就与原先的完全不一样(称为移码突变)。

her edd oga tet heb ug

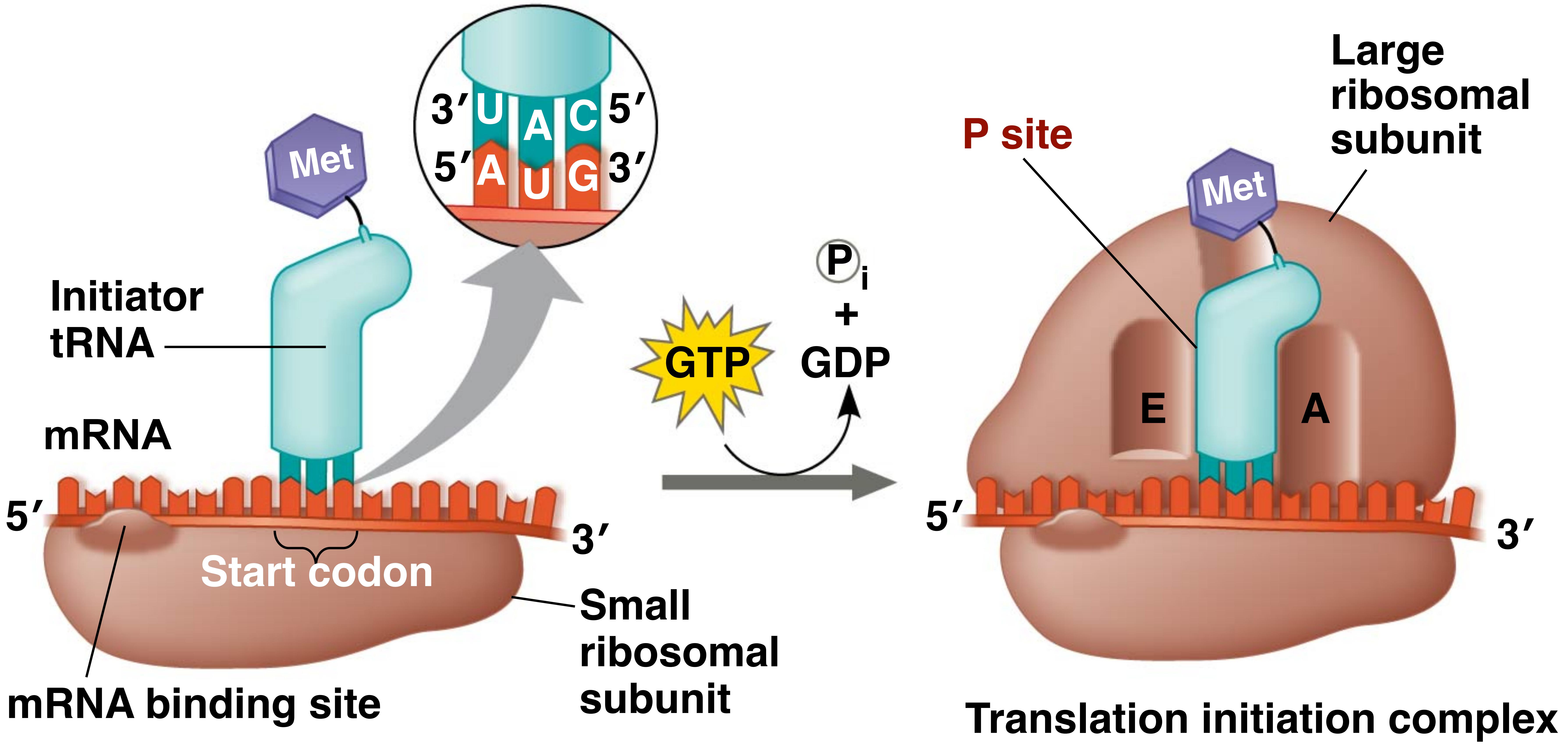
蛋白质翻译涉及两种正确的分子识别

- tRNA必须要和相对应的氨基酸结合
- tRNA的反密码子必须要和mRNA的密码子正确结合

核糖体参与



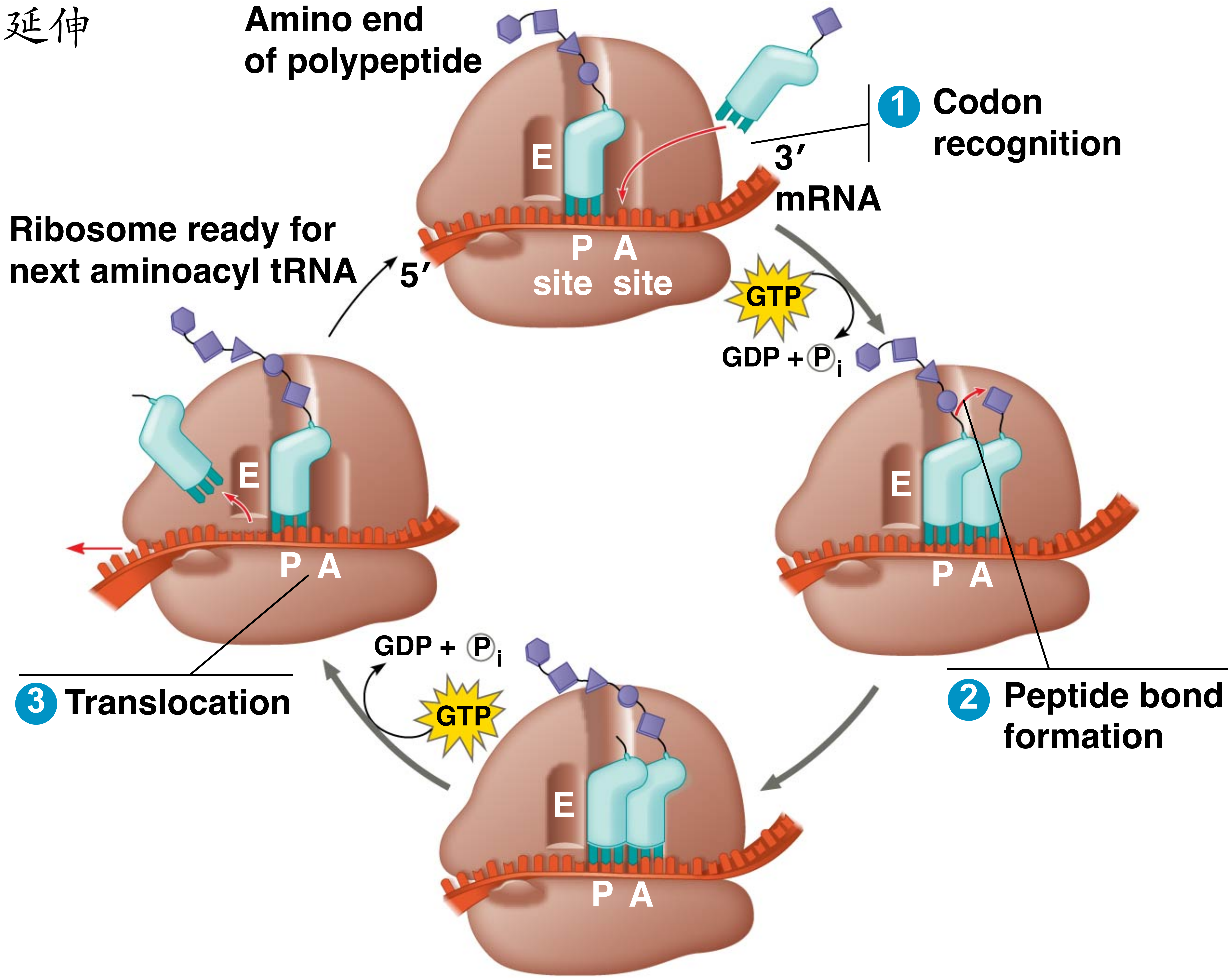
- 蛋白质翻译过程—起始



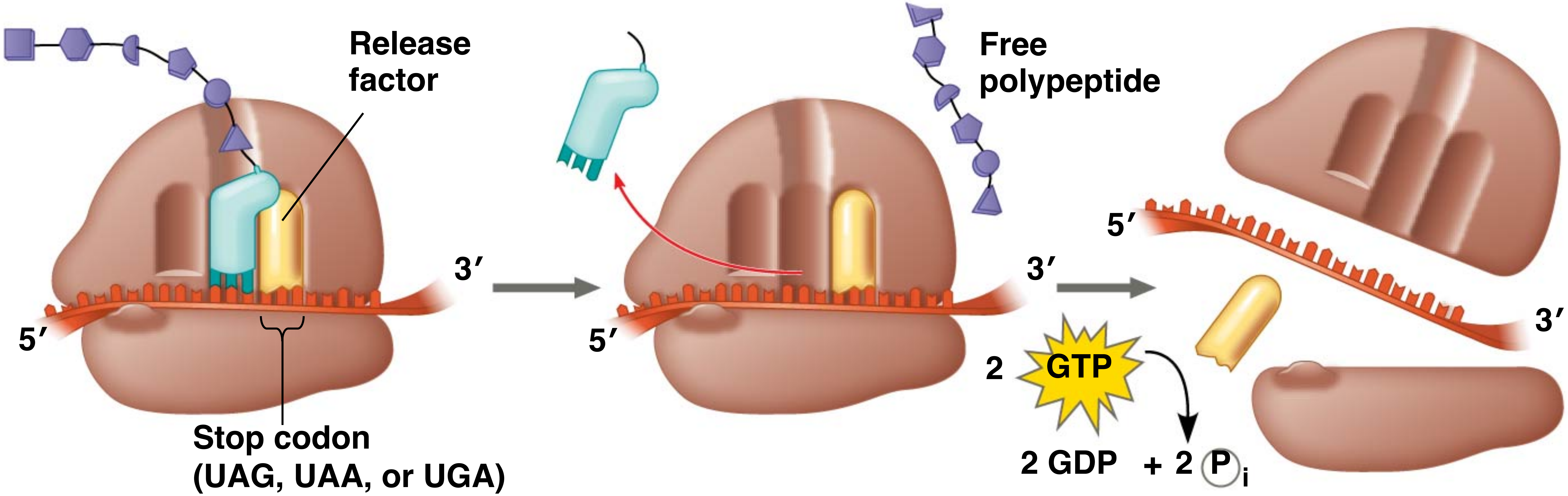
① Small ribosomal subunit binds to mRNA.

② Large ribosomal subunit completes the initiation complex.

- 蛋白质翻译过程—延伸


Amino end
of polypeptide
E
3′
mRNA
5′
P site
A site
① Codon recognition
GTP
GDP + Ⓟi
E
P A
② Peptide bond formation
E
P A
GTP
GDP + Ⓟi
E
P A
③ Translocation
Ribosome ready for
next aminoacyl tRNA

- 蛋白质翻译过程一终止

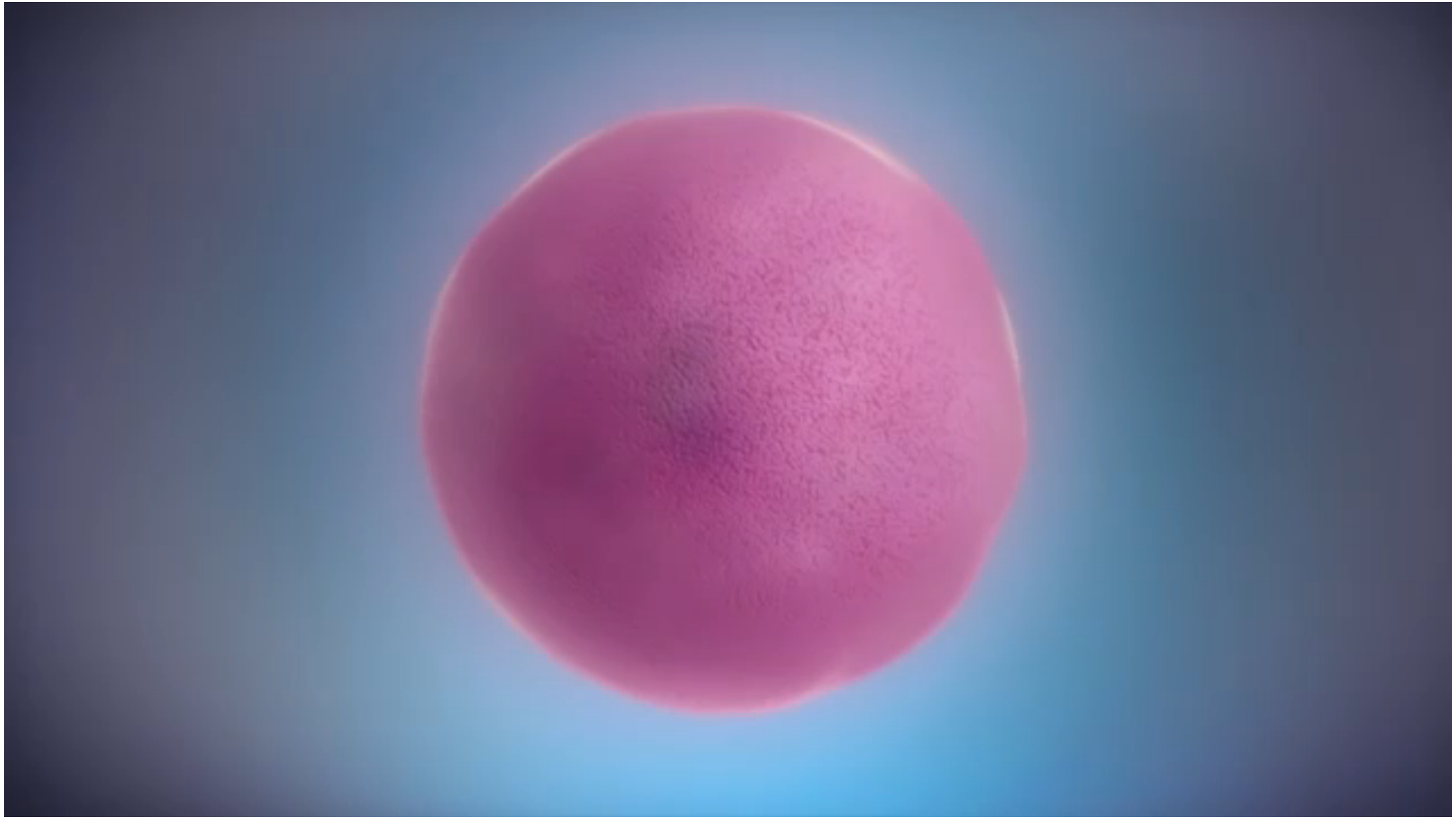


① Ribosome reaches a stop codon on mRNA.

② Release factor promotes hydrolysis.

③ Ribosomal subunits and other components dissociate.

From DNA to Protein



Summary

- 基因复制

  DNA半保留复制及其过程

- 基因表达

  中心法则

  mRNA转录、加工及翻译，密码子的特点