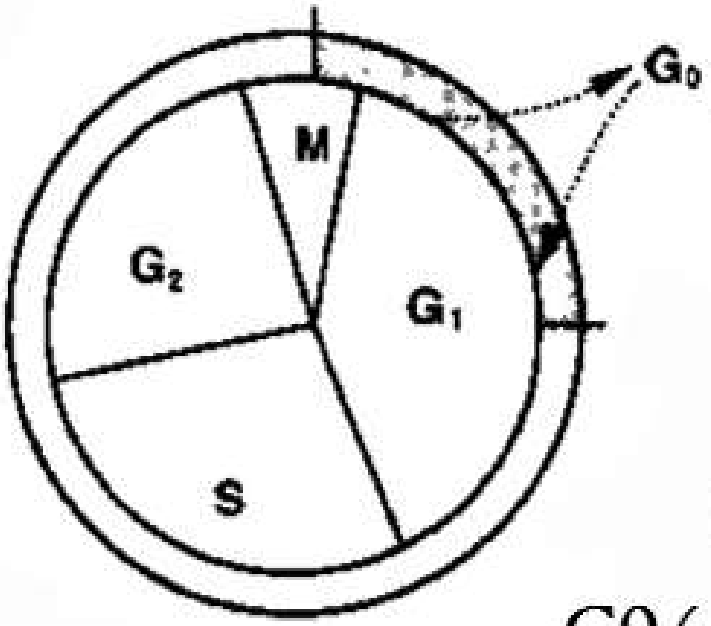
3. Eukaryotic DNA replication

3.1 Cell cycle

- The cell cycle is a cycle process that from one division to the next.
  细胞周期是指从一次分裂到下一次分裂的循环过程。
- The cell cycle involves DNA replication followed by cell division to produce two daughter cells from one parent.



Interphase (间期)

G1 Preparing for replication

S DNA replication

G2 A short gap before mitosis

Mitosis (有丝分裂) M

G0 (quiescence, 沉默期)

- **Eukaryotic DNA replication occurs in S phase.**

3.2 Initiation

3.2.1 Origin

ARSs (autonomously replicating sequences, 自主复制序列)

- **Individual yeast (酵母) replication origins have been cloned into prokaryotic plasmids.**
  - Eukaryotic origins + prokaryotic plasmids → eukaryote
- **Since the origins allow these plasmids to replicate in yeast, they are termed ARSs.**

• Yeast origin structure:

- Minimum size: 11 bp
- Structure: the consensus sequence is [A/T]TTTAT[A/G]TTT[A/T] AT-rich
- The consensus sequence is bound by the origin recognition complex (ORC, 起点识别复合体) which, when activated by CDKs (依赖细胞周期蛋白的激酶), permits opening of the origins for copying.

3.2.2 Only initiate once per cell cycle

- Eukayotic replicons **can only initiate once per cell cycle.**
- **Licensing factor** (许可因子/特许因子)
  - Required for initiation and inactivated after use.
  - Can only enter into the nucleus when the nuclear envelope dissolves at mitosis (有丝分裂), thus preventing premature reinitiation.

复制前细胞核内许可因子有活性

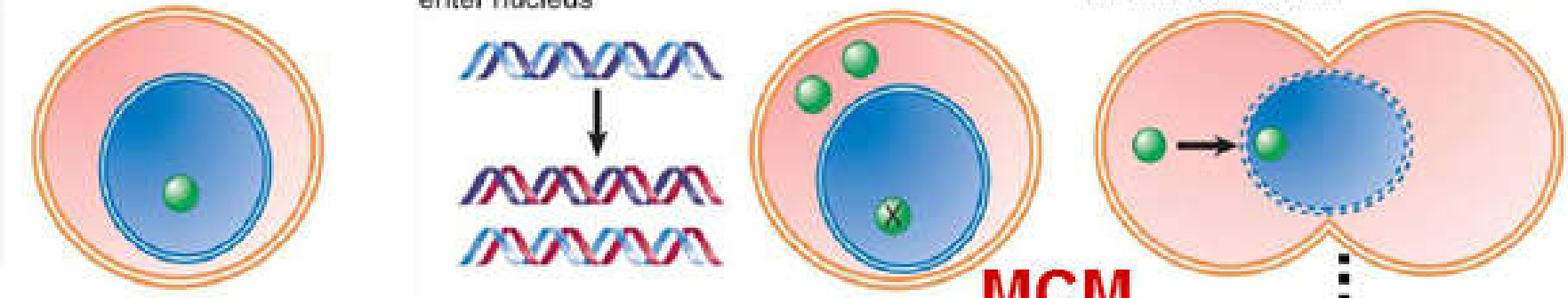
复制后,细胞核内许可因子失活,细胞质内的特许因子不能进入核内

有丝分裂期核膜的溶解使许可因子进入细胞核

Prior to replication, nucleus contains active licensing factor

After replication, licensing factor in nucleus is inactive; licensing factor in cytoplasm cannot enter nucleus

Dissolution of nuclear membrane during mitosis allows licensing factor to associate with nuclear material



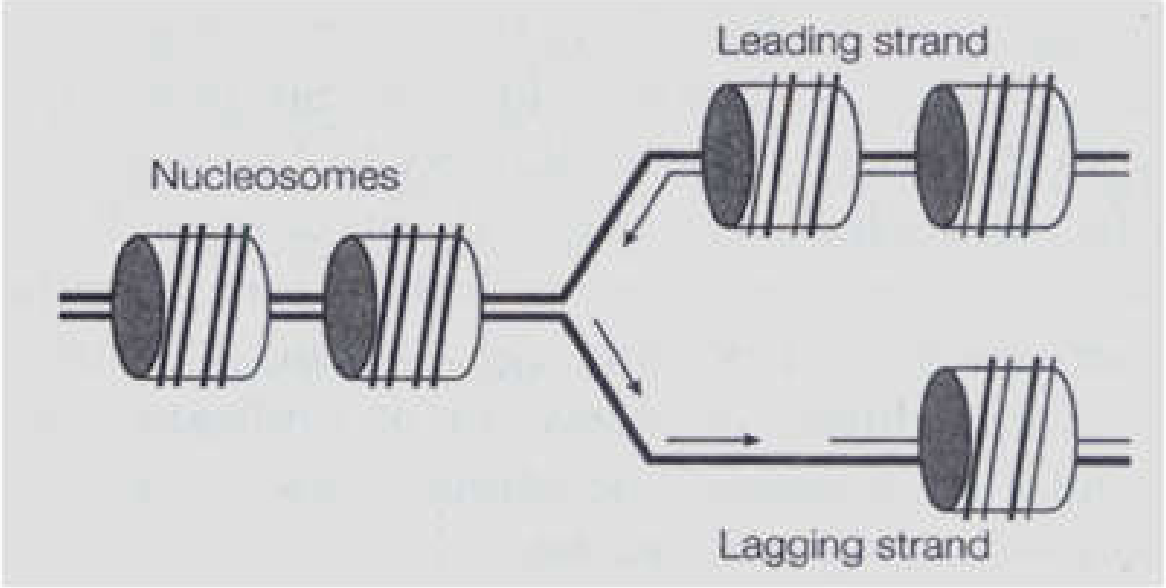
Cell division generates daughter nuclei competent to support replication

3.2.3 Initiation order (顺序)

- The first part is in euchromatin which includes transcriptionally active DNA.
- The second parts are within heterochromatin.
- The last are for centromeric and telomeric DNA.

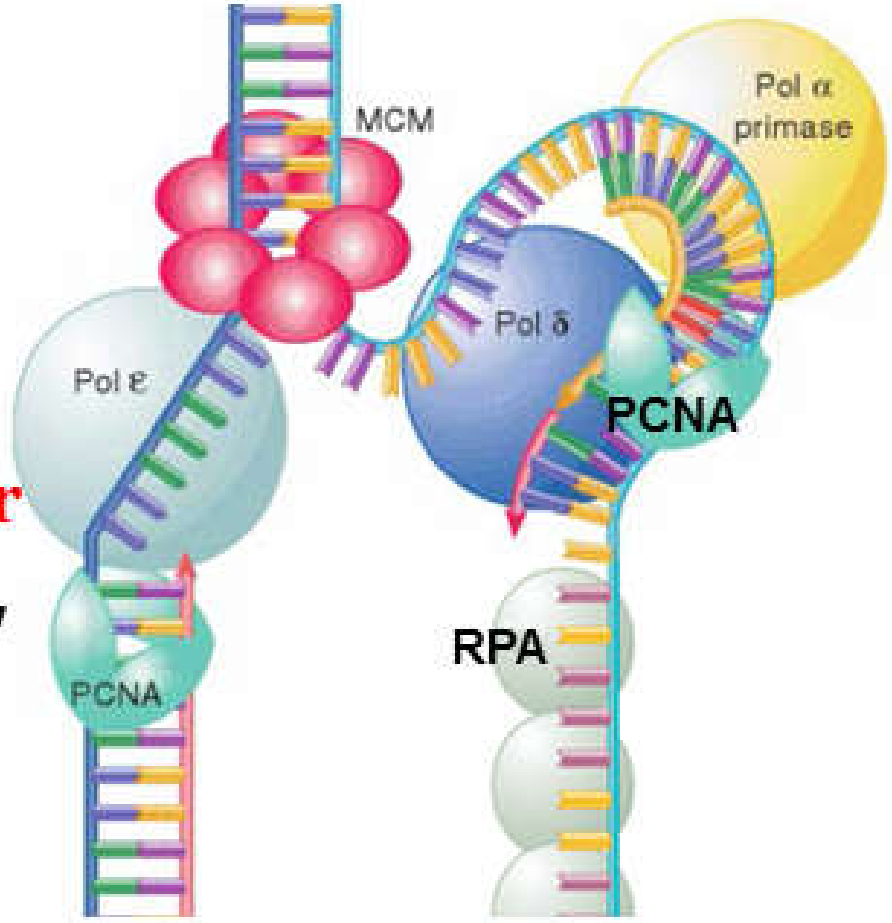
3.2.4 DNAs are unwound from the nucleosomes

- DNA must be unwound from the nucleosomes at the replication forks.
- After the fork passes, new nucleosomes are assembled from a mixture of old and newly synthesized histones.



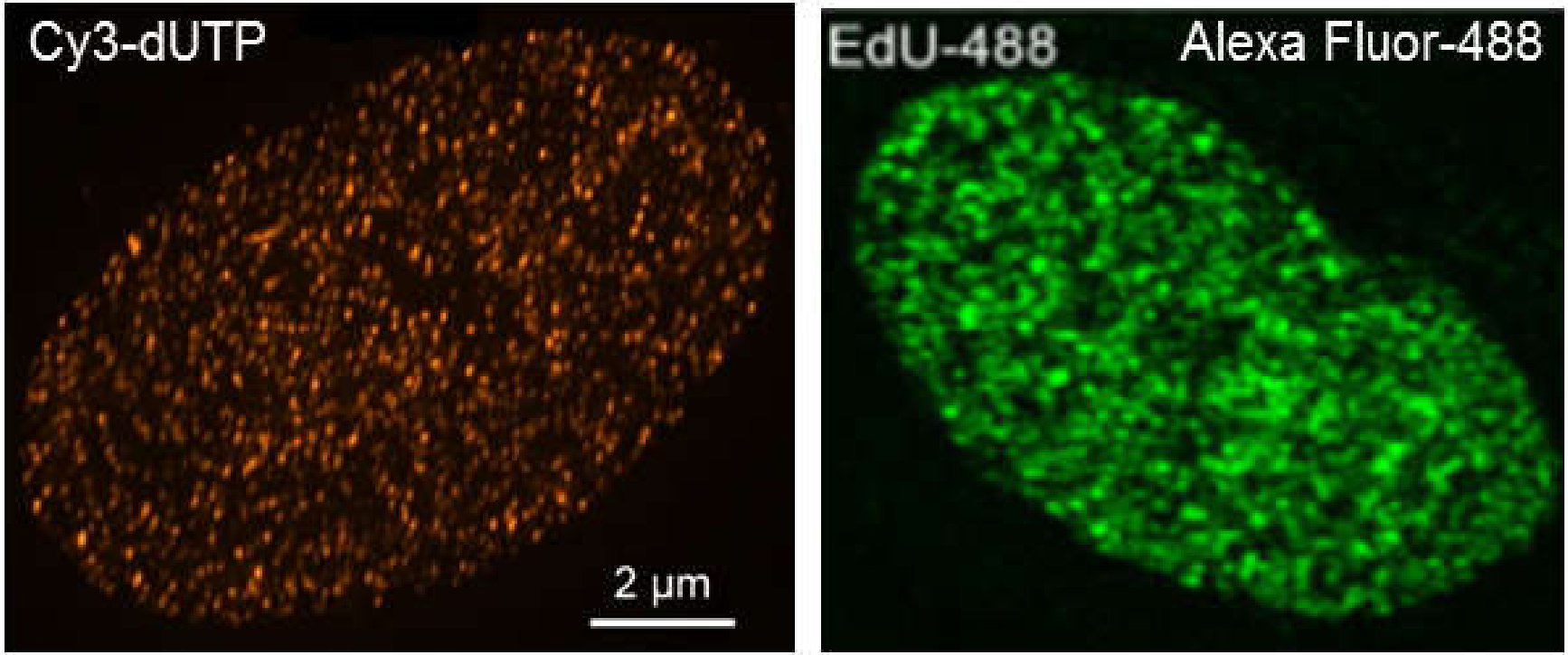
3.3 Elongation

- Three different DNA polymerases are in the eukaryotic replication fork.
- MCM (helicase)
- Proliferating cell nuclear antigen (增殖细胞核抗原, PCNA) endows the complex with high processivity (进行性).



- FEN1/RNase H endonuclease removes primers

- **Replication factories (复制工厂) - all the replication enzymes, DNA associated with the replication forks in replication**



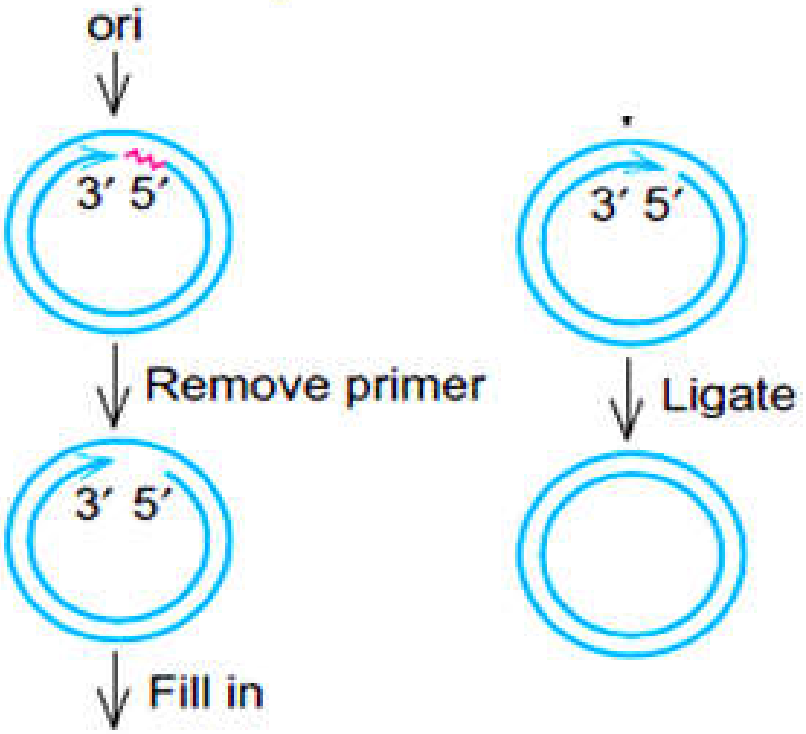
dT analog : dU, BrdU, EdU → Visualizing by fluorescence

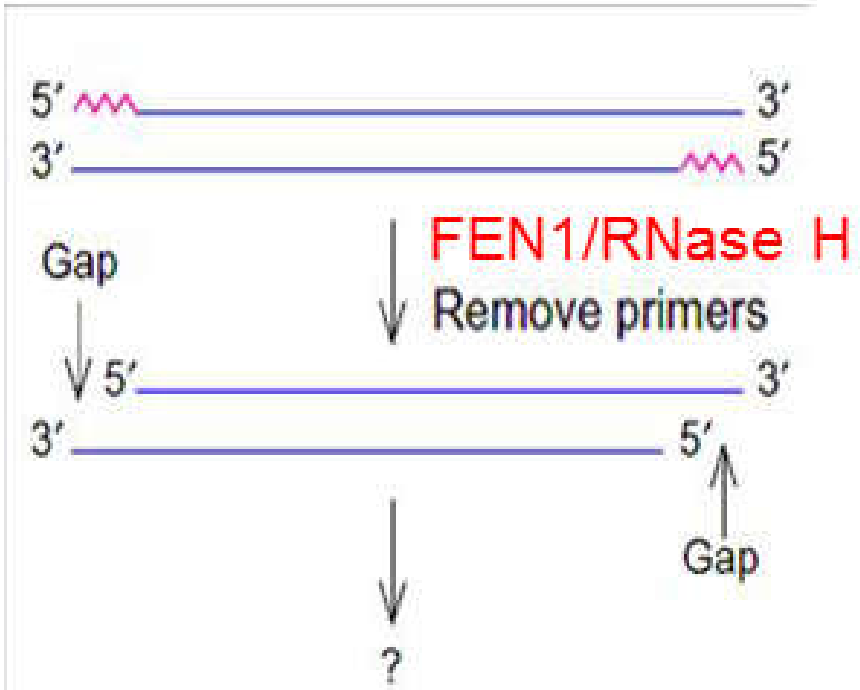
- **Replication factories are immobilized on the nuclear matrix.**
- **Nuclear matrix - a scaffold of insoluble protein fibers which acts as an organizational framework for nuclear processing, including DNA replication, transcription.**

核基质是由不溶性蛋白纤维组成的支架，作为核中各种反应(包括DNA复制和转录)进行时的组织框架行使功能。

3.4 Termination

3.4.1 The problem of 5'-ends of linear chromosomes





- **The ends of linear chromosomes cannot be fully replicated. Thus, genetic information could be lost from the DNA.**

3.4.2 Telomere

- **The ends of eukaryotic chromosomes (telomeres) consist of hundreds of copies of a simple, non-informational repeat sequence (e.g. TTAGGG in humans) with the 3'-end overhanging to the 5'-end.**

3'-AATCCCAATCCCAATCCC-5'

5'-TTAGGGTTAGGG(TTAGGG)nTTAGGG-3'

n=several hundred

- **The exact sequence of the repeat in a telomere is species-specific.**

➢ In *Tetrahymena* (四膜虫), it is TTGGGG.

3.4.3 Telomerase

(1) Telomerase structure

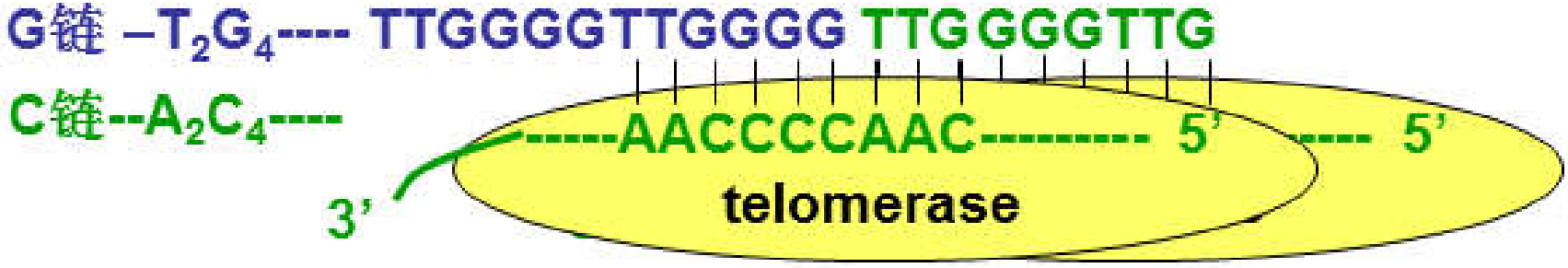
Telomerase contains a short RNA molecule, part of whose sequence is complementary to the telomere repeat. (Reverse transcriptase 反转录酶)

(2) Telomere replication

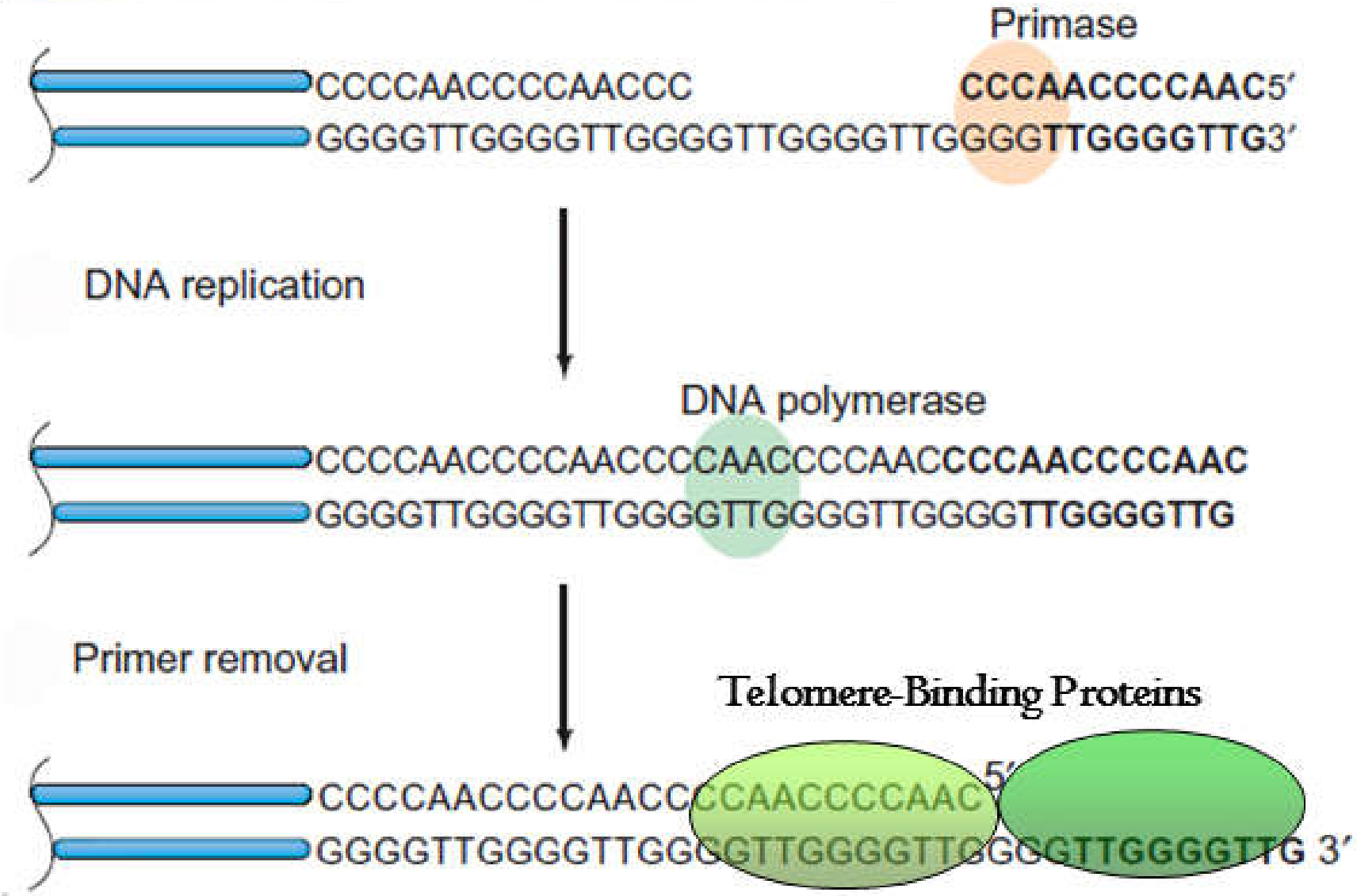
Hybridization (杂交)

Elongation (延伸)

Translocation (移位)



Filling in the C-rich strand



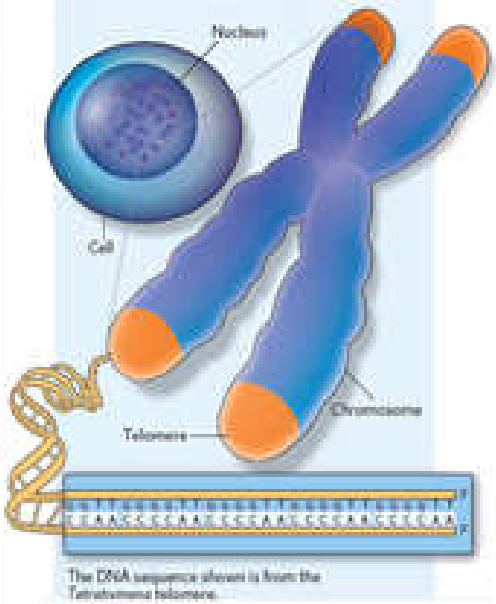
将2009年诺贝尔生理学或医学奖授予美国科学家

卡萝尔·格雷德

伊丽莎白·布莱克本

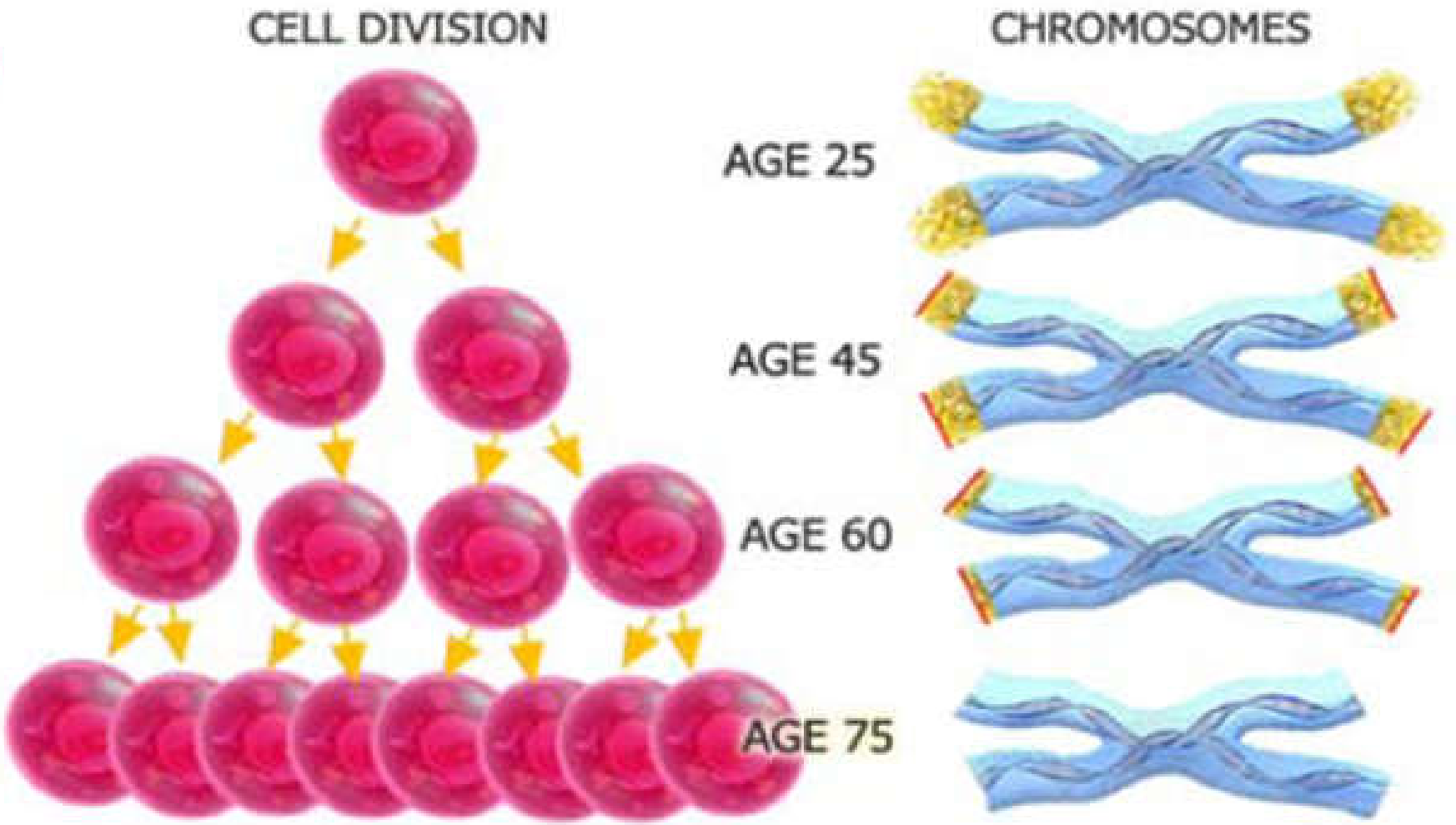
杰克·绍斯塔克

成就："发现端粒和端粒酶是如何保护染色体的"



(3) Special function (biological clock)

- Telomerase activity is repressed in the somatic cells (体细胞) of multicellular organisms, resulting in a gradual shortening of the chromosomes with each generation.

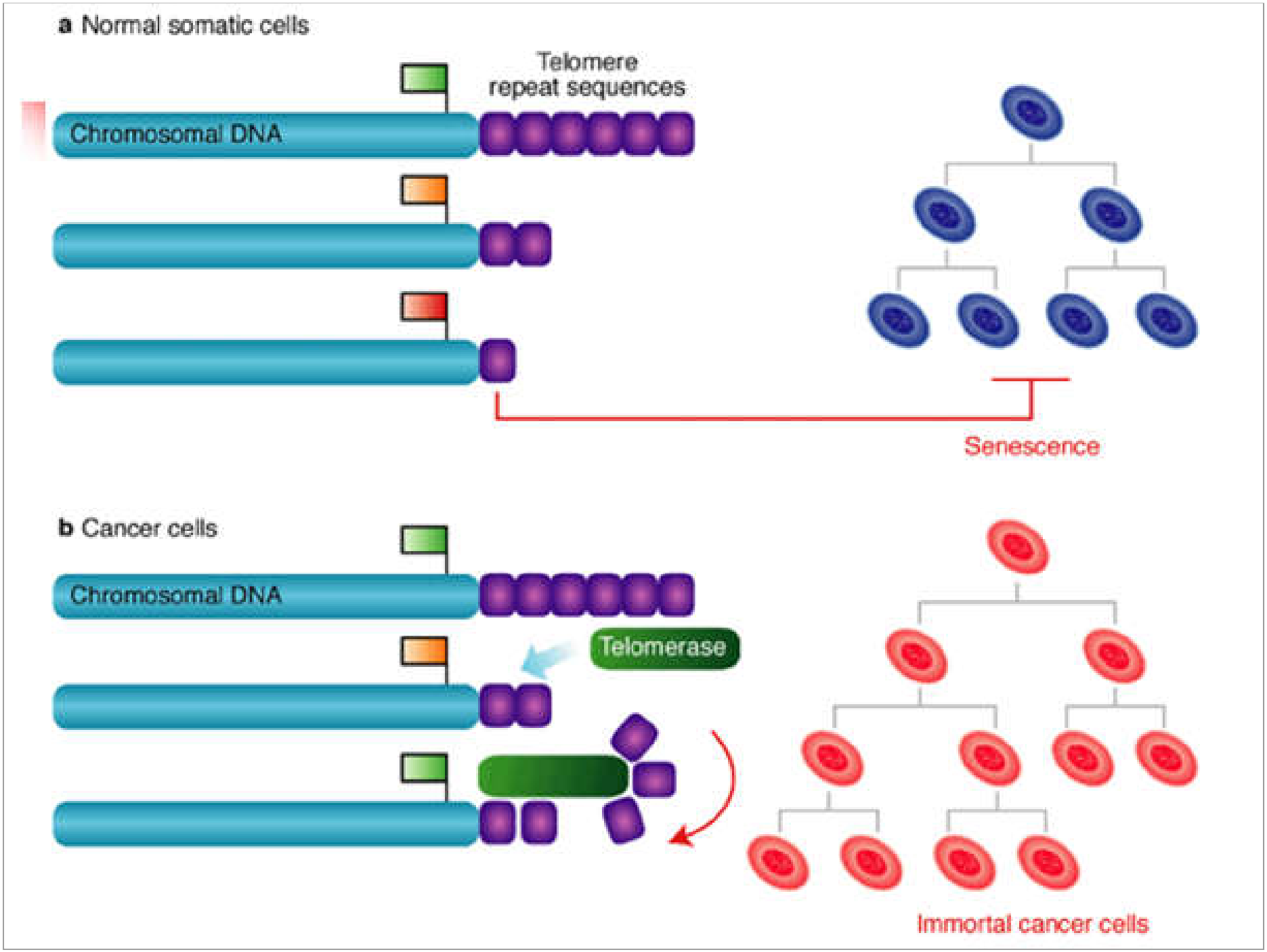


- **As the shortening reaches informational DNA, the cells senesce (衰老) and die.**

- **The length of telomeres has a tight relationship with cell senescence.**

Q: Can we enhance the activity of telomerase to prolong our life?

- **The unlimited proliferative（增殖）capacity of many cancer cells is associated with reactivation of telomerase activity.**


a Normal somatic cells
Telomere
repeat sequences
Chromosomal DNA
Senescence
b Cancer cells
Chromosomal DNA
Telomerase
Immortal cancer cells

Compare DNA replication between prokaryotes and eukaryotes.

Similarities

- Semi-conservative replication
- Semi-discontinuous replication
- RNA priming
- Proofreading

Differences

- Origin
- Terminus
- Replication mode
- Replicon
- Polymerase
- Replication rate
- Size of Okazaki fragments
- Telomeres and telomerases
- Histone

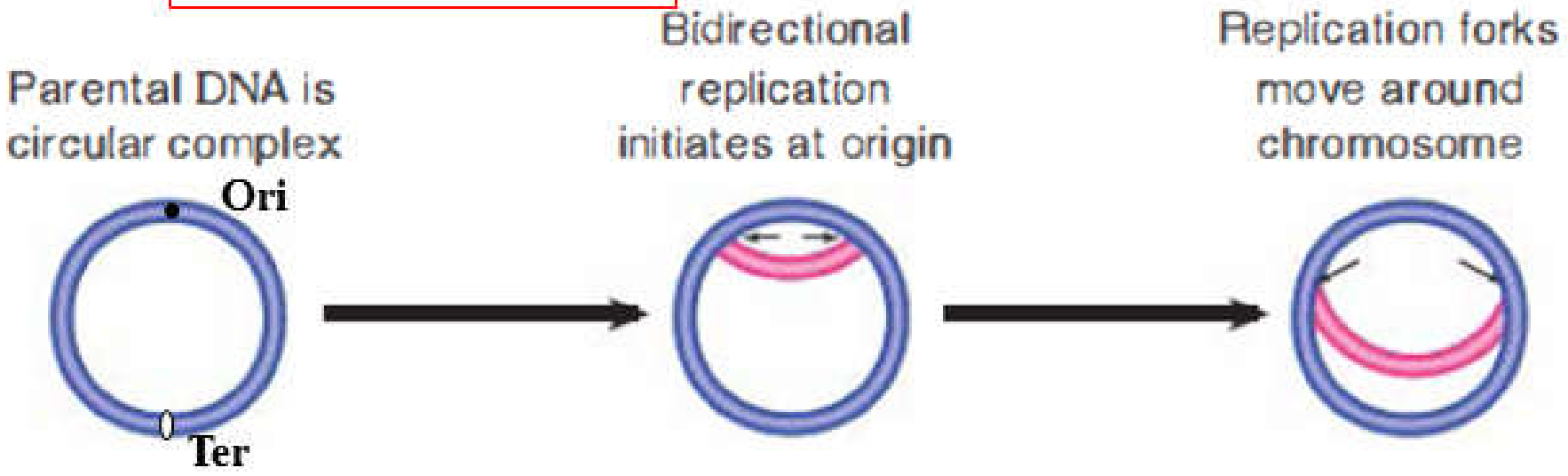
Replication mode 复制方式

(1) θ replication

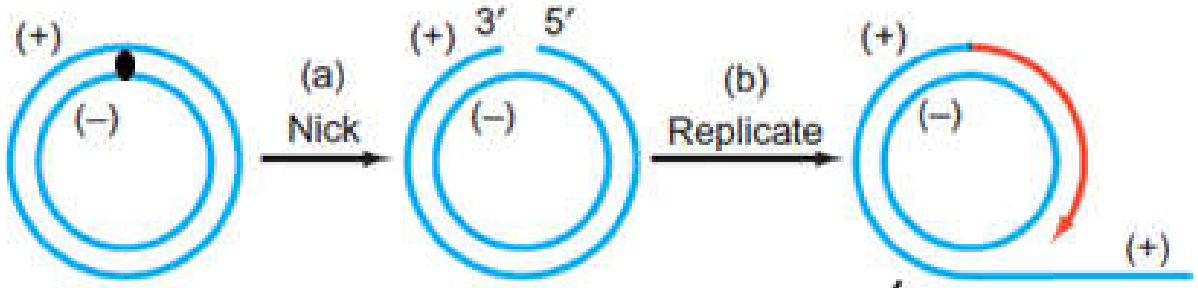
Circular bacterial DNA

Some plasmids

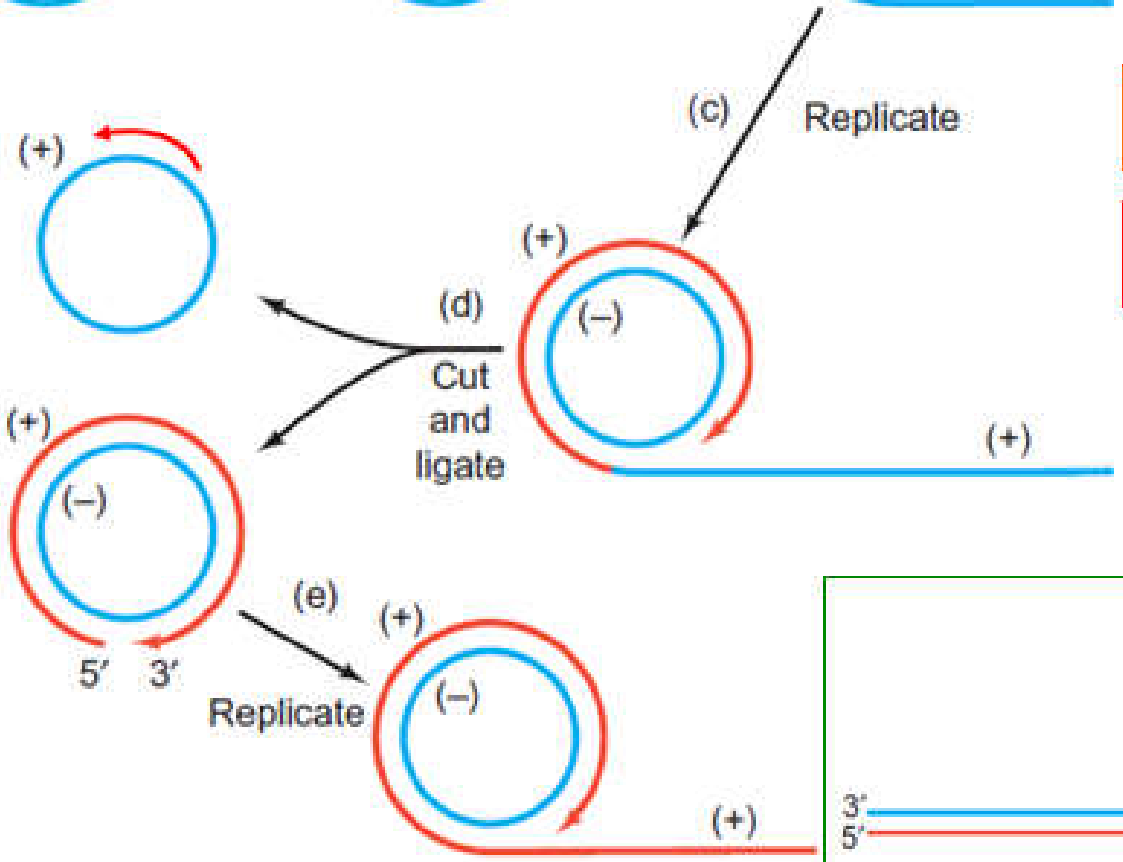
Single origin
Bidirection



(2) Rolling circle replication（滚环复制）



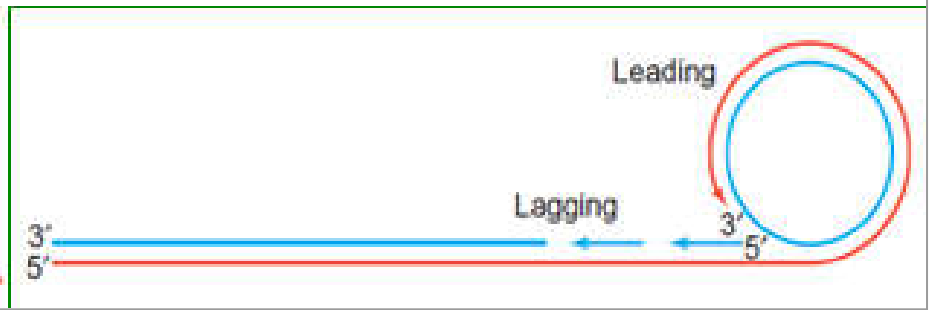
Single origin



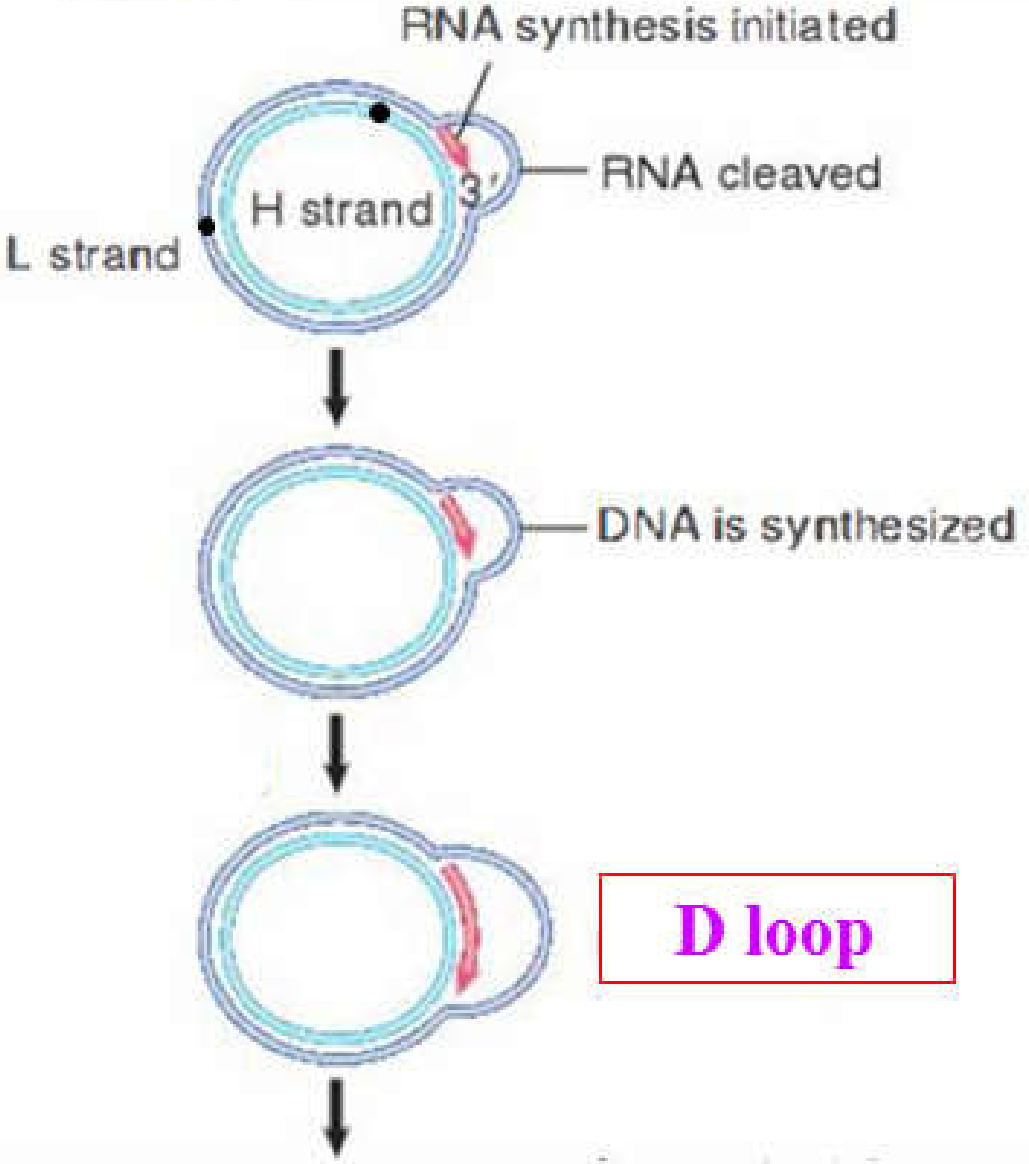
Some phages

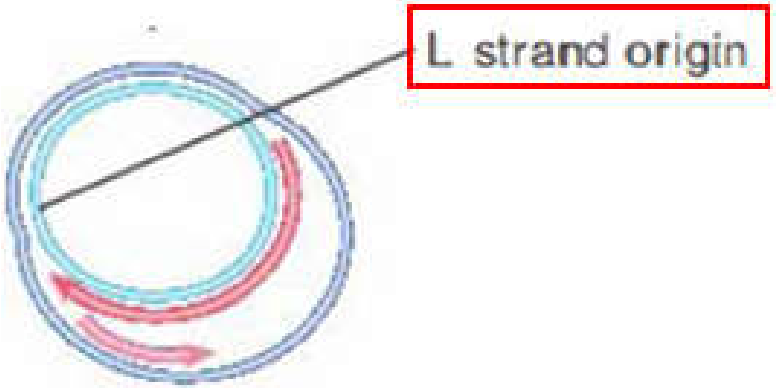
Some plasmids

λDNA



(3) D loop replication





D loop

Mitochondrial DNA
Chloroplast DNA

Two origins

(4) multiple replication bubbles

Eukaryotic chromosome DNA

Mitiple origins
Bidirection