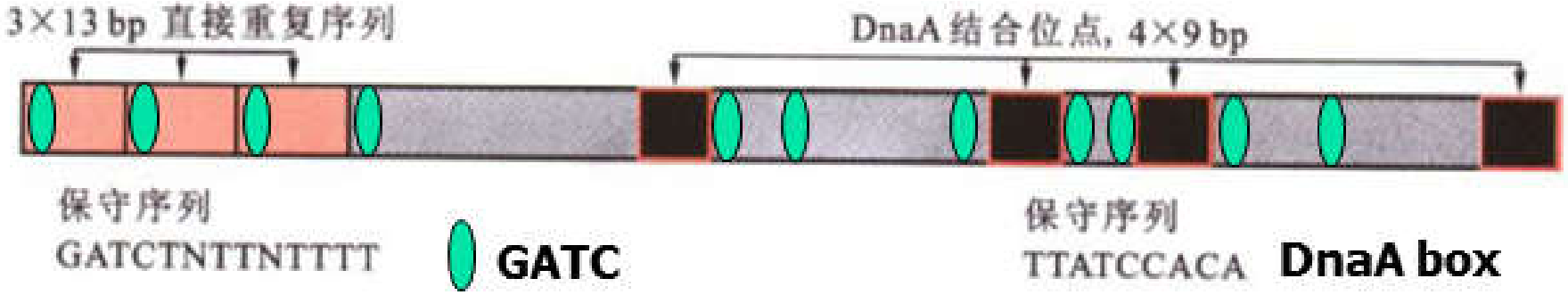
4. Regulation of DNA replication

4.1 Regulation of bacterial DNA replication

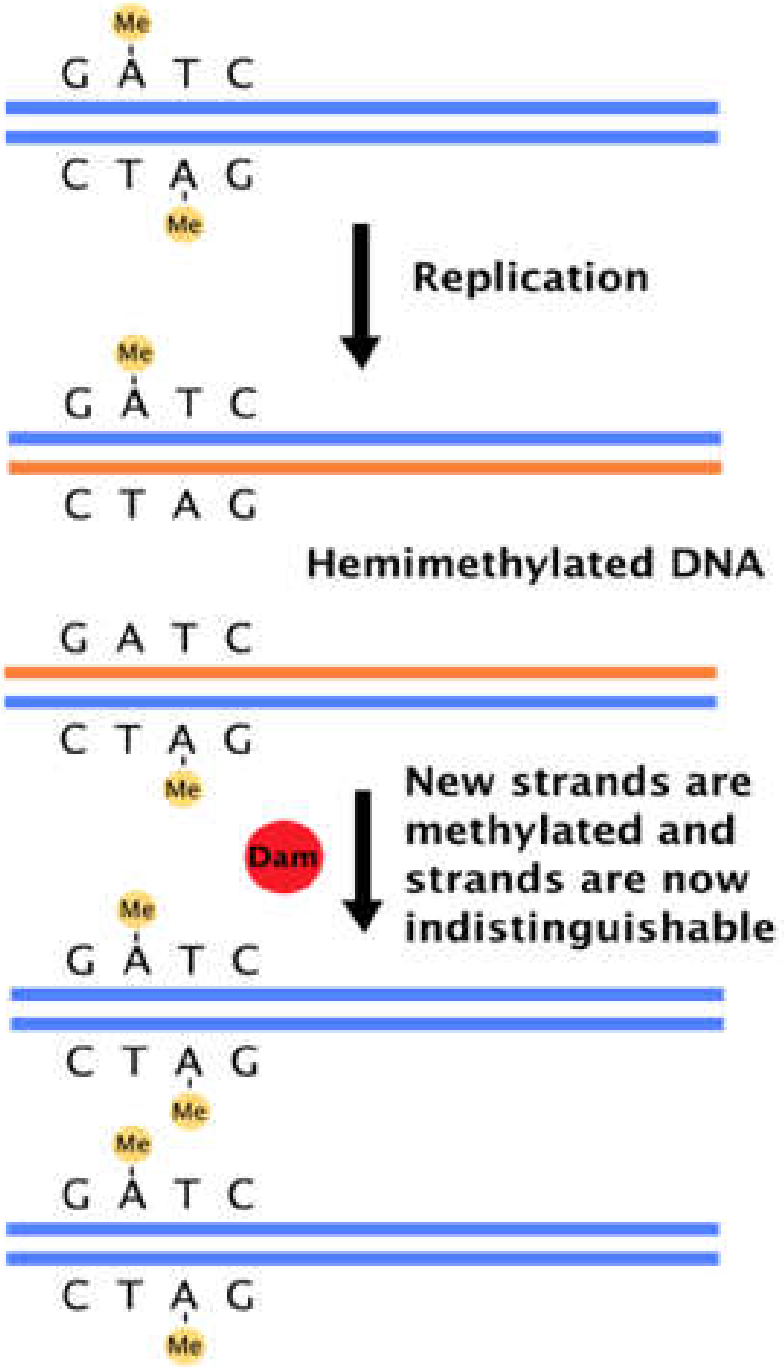
Regulation of DNA replication in bacteria occurs mainly in the initial stage (起始阶段).

4.1.1 Methylation (甲基化) of the bacterial origin

- ***oriC*** **contains eleven GATC that are methylated on adenine (A) on both strands.**



- **Only fully methylated origins can initiate replication.**
- **Replication generates hemimethylated（半甲基化的）DNA, which cannot initiate replication.**
- **There is a 13-minute delay before the GATC repeats are remethylated.**



4.1.2 ATP/ADP

- **DnaA-ATP complex is able to initiate replication.**
- **ADP competes with ATP to bind to DnaA.**

4.1.3 Levels of DnaA

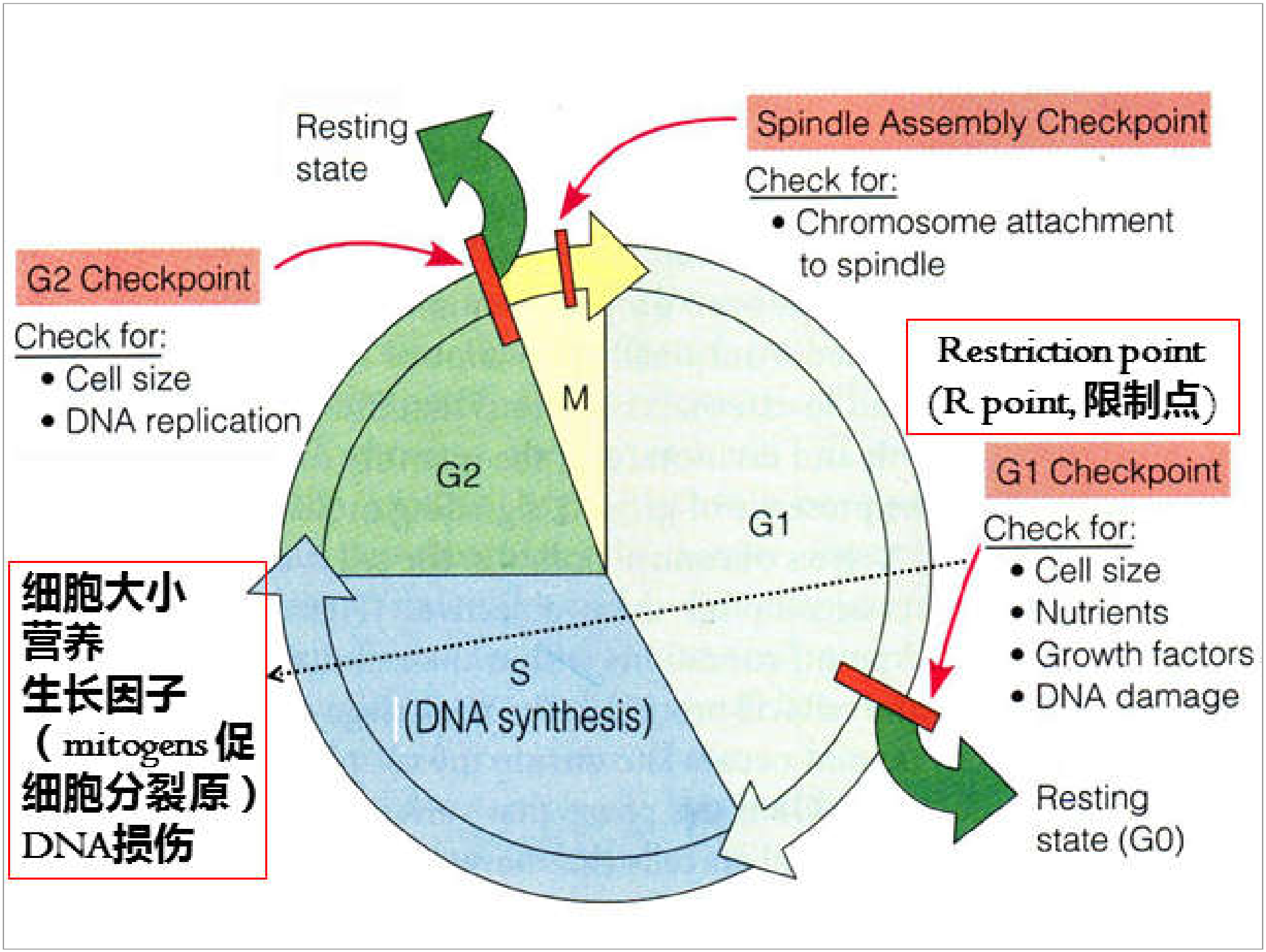
- **Enough DnaA enable initiation of replication.**

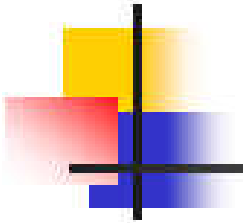
4.2 Regulation of Eukaryotic DNA replication

4.2.1 Cell cycle checkpoints

- Cell cycle progression depends on checkpoints.
- Checkpoints are stages at which the cell cycle may be halted if the circumstances are not right for cell division.

检验点是在环境不适合细胞分裂的情况下可以终止细胞周期的阶段。


Resting state
Spindle Assembly Checkpoint
Check for:
• Chromosome attachment to spindle
G2 Checkpoint
Check for:
• Cell size
• DNA replication
Restriction point
(R point, 限制点)
G1 Checkpoint
Check for:
• Cell size
• Nutrients
• Growth factors
• DNA damage
M
G2
G1
S
(DNA synthesis)
细胞大小
营养
生长因子
（mitogens 促细胞分裂原）
DNA损伤
Resting state (G0)



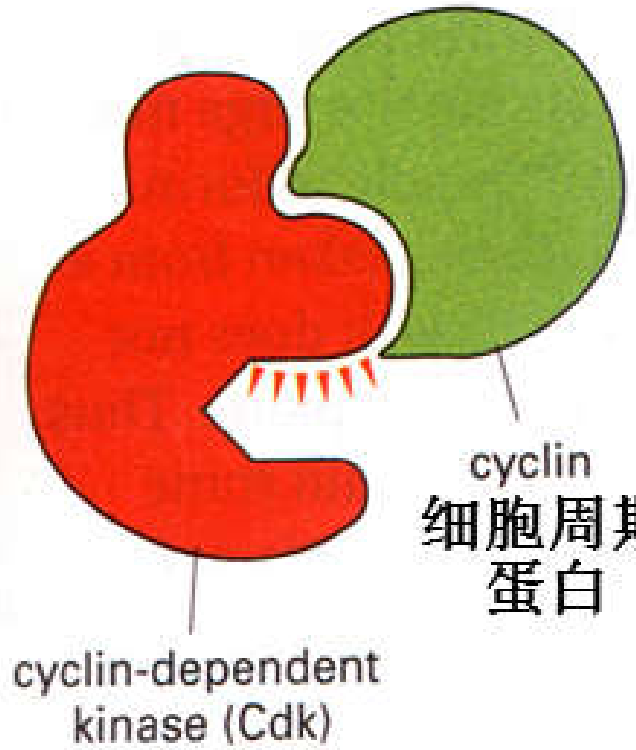
- In animal cells, the G1 phase checkpoint is called the restriction point (R point, 限制点), and in yeast cells it is called the start point.
- At the R point in G1 phase, cells starved of mitogens（促细胞分裂原） withdraw from the cell cycle into the resting G0 phase.

4.2.2 Cyclin-dependent kinase (依赖于细胞周期蛋白的激酶, CDKs)

A major mechanism for control of cell cycle progression is by regulation of protein phosphorylation (磷酸化).

(1) Composition and properties of CDKs

- CDKs are a family of protein kinases (激酶).
- CDKs phosphorylate their substrates on serines and threonines.



CDK=Cdk+cyclin

↑

*cdc*基因表达产物

- **Cyclins are regulatory subunits;**
- **Cdks are catalytic subunits.**
- **The CDKs have no catalytic activity unless they are associated with a cyclin.**
- **Their regulatory function in the cell cycle has been evolutionarily conserved (保守的).**

(2) Three classes of CDK complexes

① G1 phase CDK complexes

- **Prepare the cell for S phase by activating transcription factors that cause expression of enzymes required for DNA synthesis and genes encoding S phase CDK complexes.**
  通过激活DNA合成所需酶表达过程中的转录因子以及激活编码S期CDK复合物的基因表达过程中的转录因子

E2F

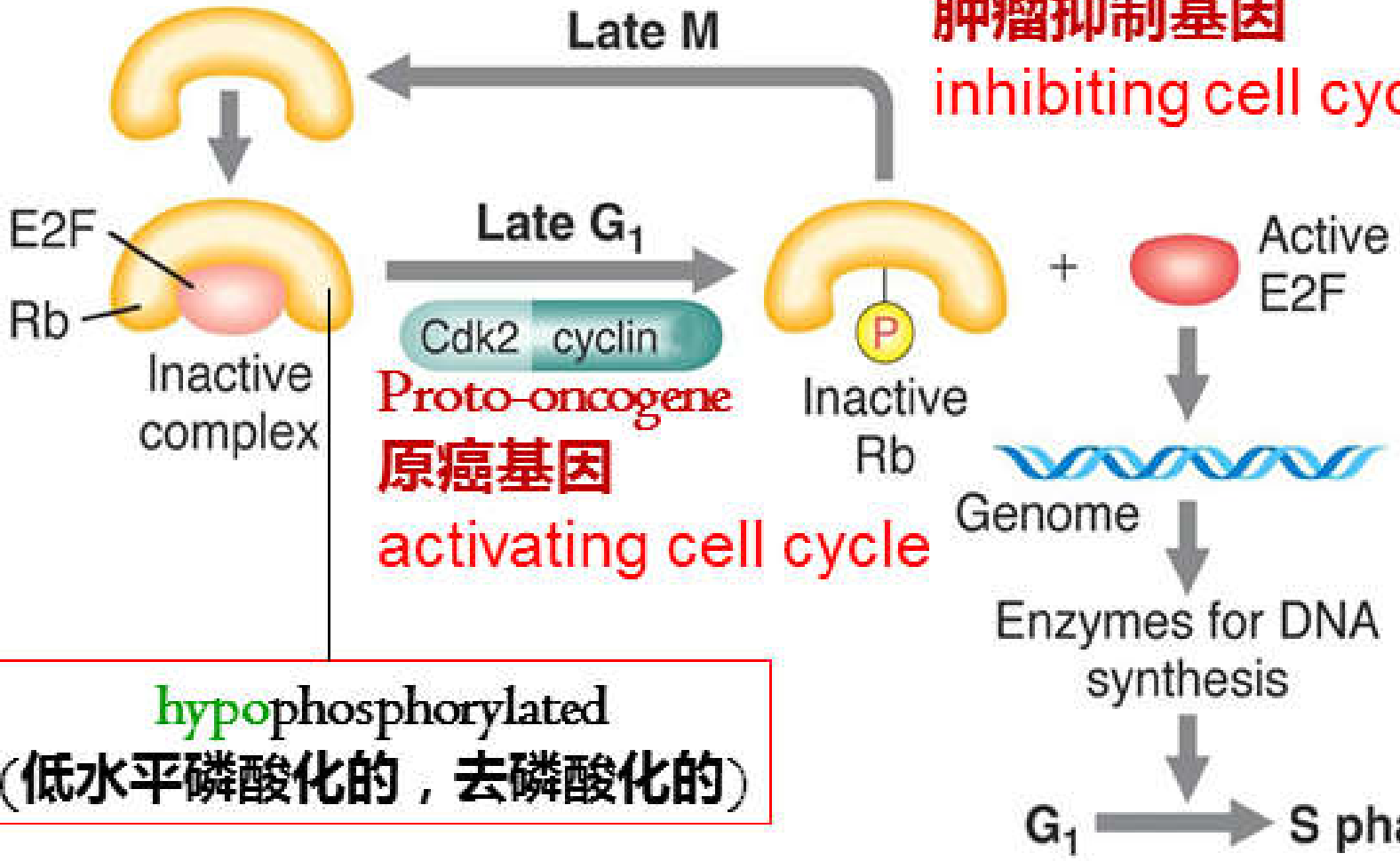
- **Cyclin D, E; Cdk2,4,6.**

The Rb-E2F regulation pathway

- Passage through the key G1 R point critically depends on the activation of a transcription factor, E2F.
- E2F stimulates the transcription of genes encoding proteins required for DNA replication and deoxyribonucleotide synthesis as well as for cyclins and Cdks required in later cell cycle phases.

- The activity of E2F is inhibited by the binding of the protein Rb (成视网膜细胞瘤抑制蛋白) and related proteins.

Tumor suppressor gene
肿瘤抑制基因
inhibiting cell cycle



hypophosphorylated
(低水平磷酸化的，去磷酸化的)

② S phase CDK complexes

- **Stimulate the onset of organized DNA synthesis. The machinery ensures that each chromosome is replicated only once.**
  激发有序DNA合成的起始，该机制将确保每一染色体仅复制一次。
- **Cyclin A; Cdk2.**

③ Mitotic CDK complexes (MPF, 促有丝分裂因子)

- **Induce chromosome condensation and ordered chromosome separation into the two daughter cells.**
  诱导染色体凝集并有序地分配进两个子细胞。
- **Cyclin B; Cdk1.**

(3) Regulation of CDKs

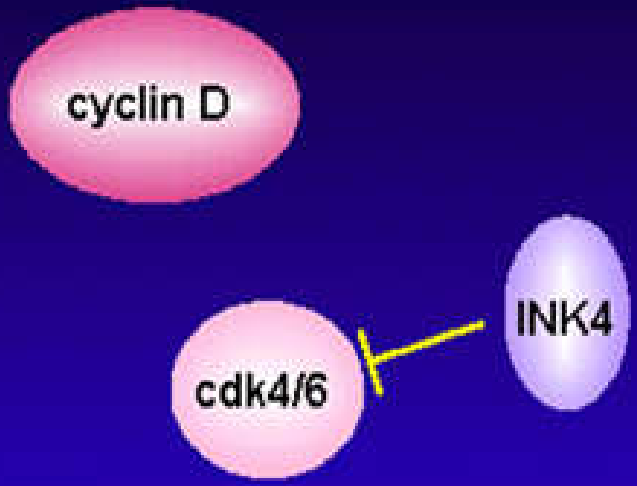
Activity

- Cyclin binding
- Phosphorylation by CDK-activating kinase
- CDK Inhibitors

Number

- Control of the transcription of subunits
- Organized proteolysis (有序的蛋白水解)

Two classes of CDK inhibitors



- **Inhibits CDK2/E and CDK4/D**
  -Blocks G1→S transition
- **Inhibits CDK2/A**
  -Blocks S phase

INK4 family
(Inhibitors of CDK4)

Blocks G1/S transition

e.g. P16,, p15, p18, p19

CIP/KIP family
(CDK-interacting protein)

e.g. P21,, p27, p57

5. Reverse transcription (反/逆转录)

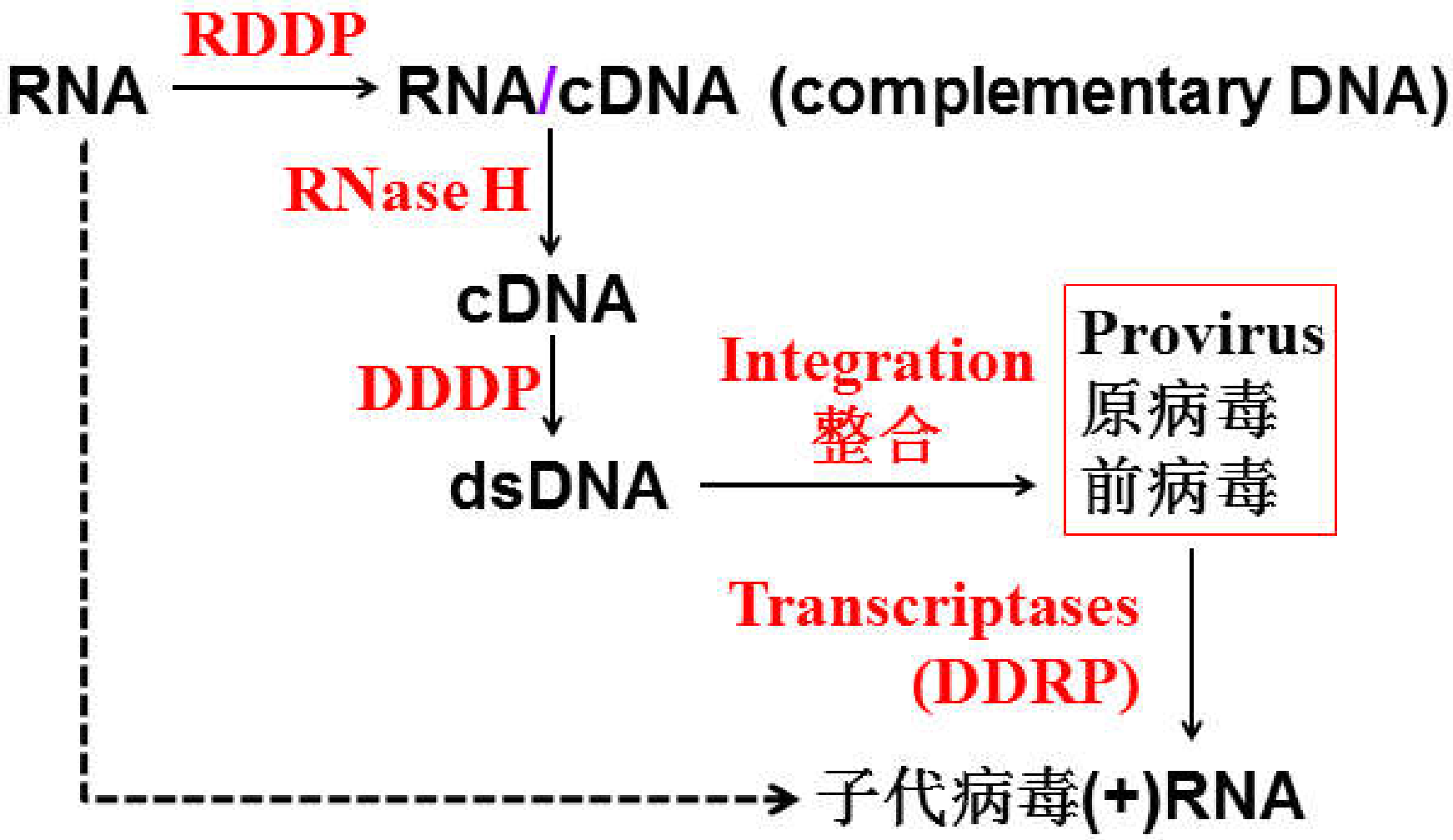
Reverse transcription - the synthesis of DNA from an RNA template. This process is catalyzed by reverse transcriptases (逆转录酶, RDDP).

5.1 Characteristics of RDDP

- **Primer: tRNA**
- **Template: ssRNA**
- **Substrates: dNTP**
- **Has the activity of Ribonuclease H (RNase H)**

RNase H is a nuclease that specifically hydrolyzes the phosphodiester bonds of RNA which is hybridized to DNA.

5.2 Brief process of reverse transcription



Summary

1. Mechanisms of DNA replication
2. Key proteins and their function in DNA replication
3. The process of DNA replication
4. Characteristics of prokaryotic and eukaryotic DNA replication (similarities and differences)
5. Structure and function of telomerase
6. Checkpoints of cell cycle and CDK regulation
7. Characteristics of reverse transcriptase