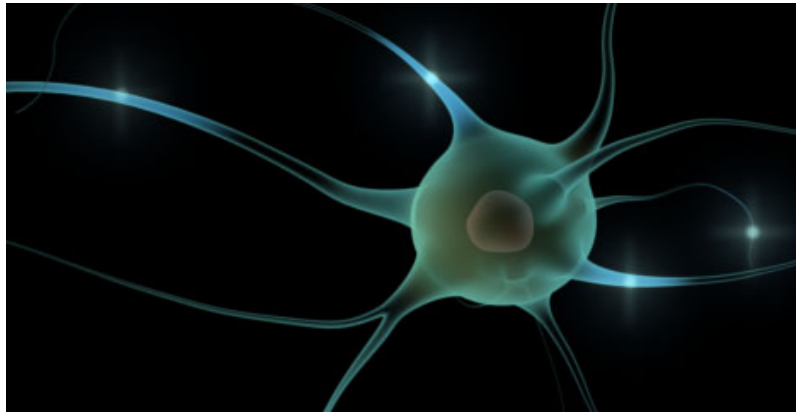
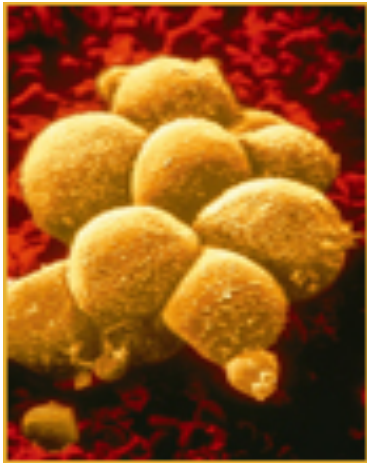


Ciclo celular



Patricia Coltri
coltri@usp.br

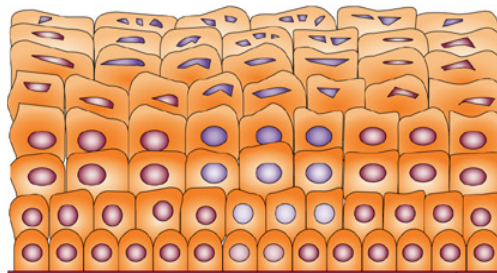
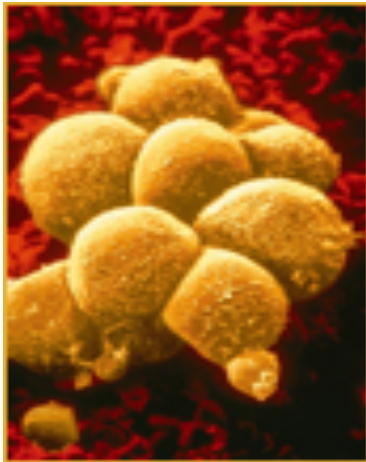
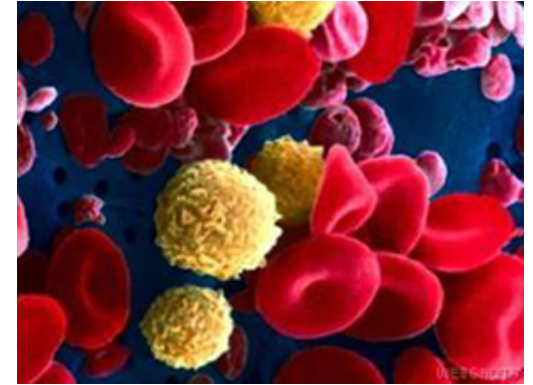
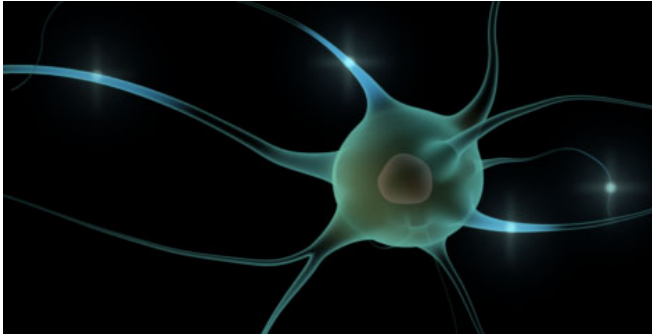
Nesta aula:

- Controle do ciclo celular
- Fatores que interferem no ciclo
- Métodos de estudo

Ciclo celular e proliferação



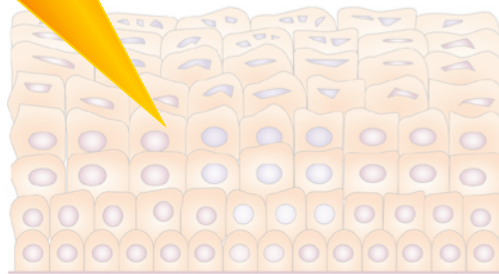
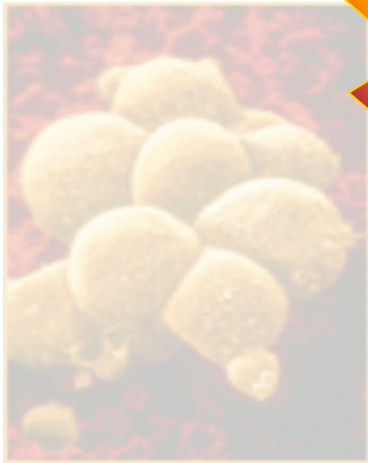
Eventos sequenciais coordenados: geração de células-filhas – geração de organismos



Capacidade proliferativa no mesmo organismo: variável

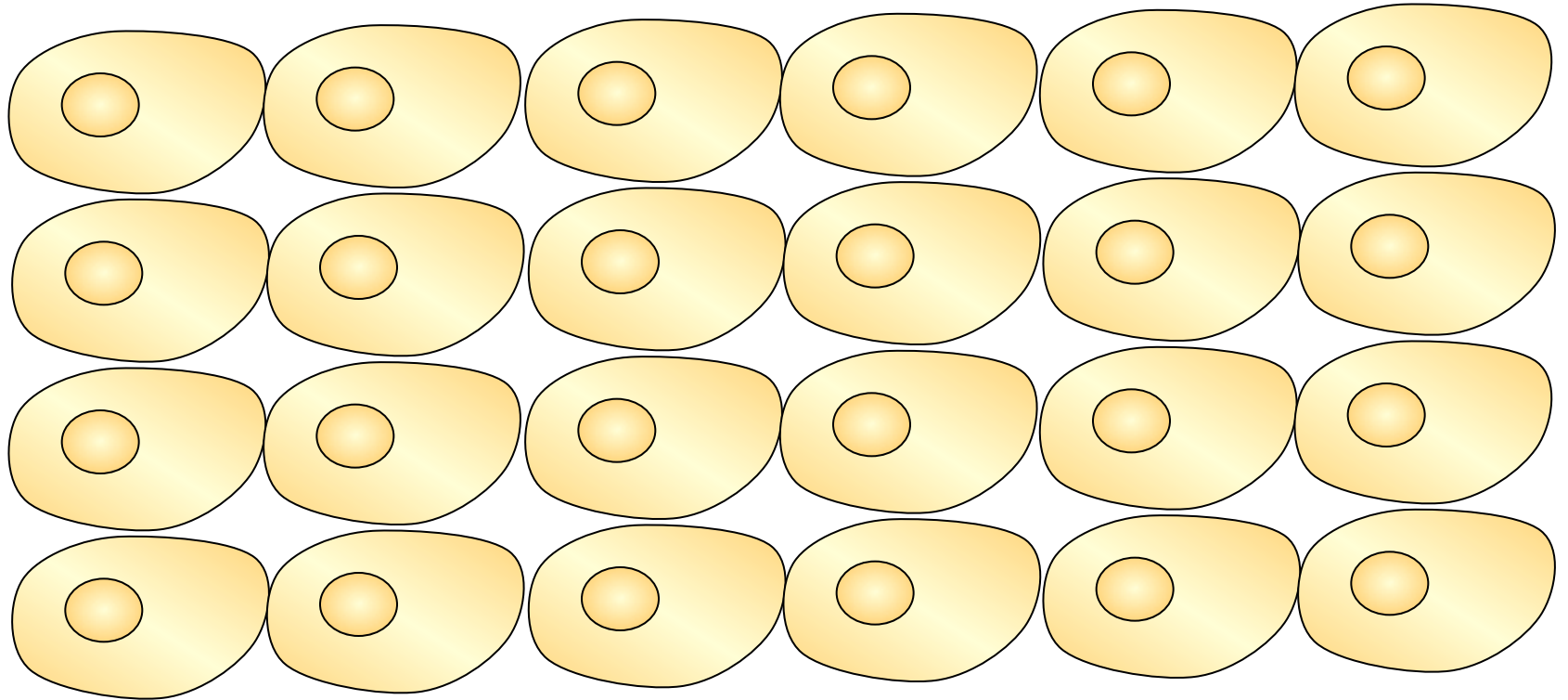


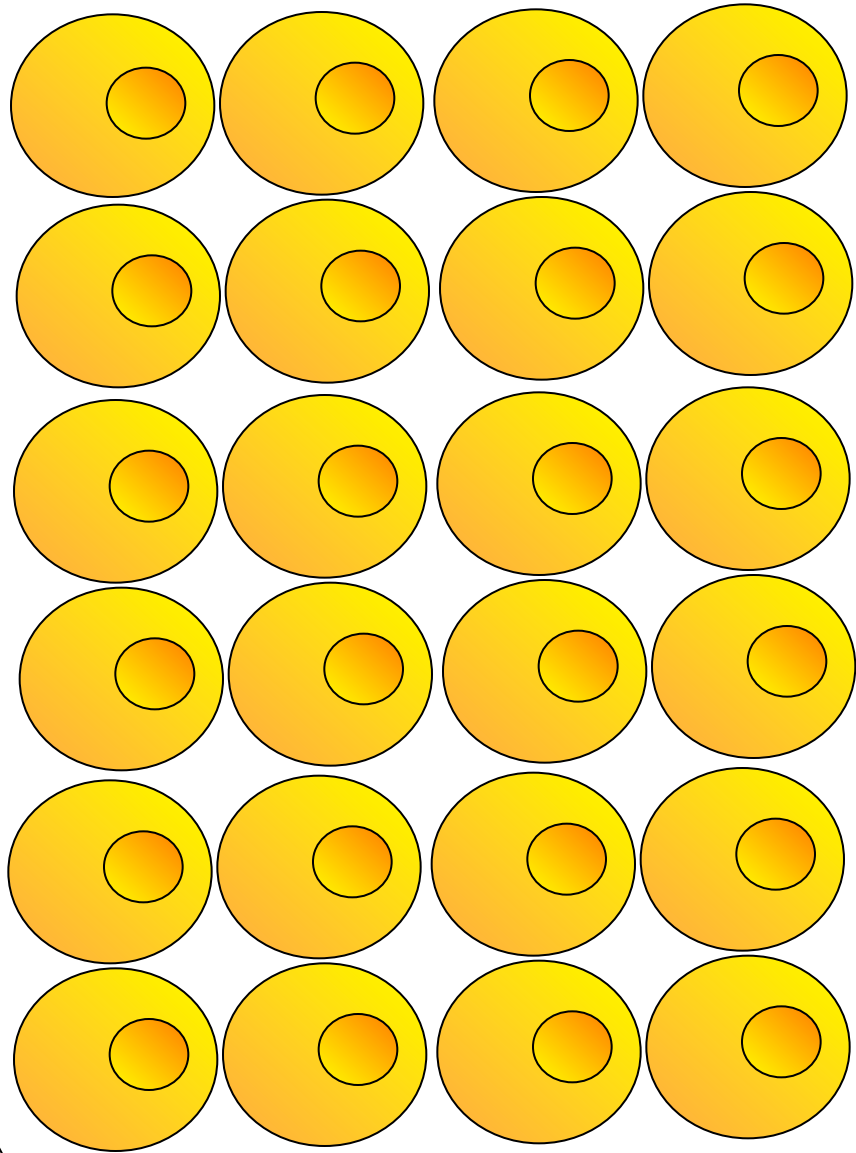
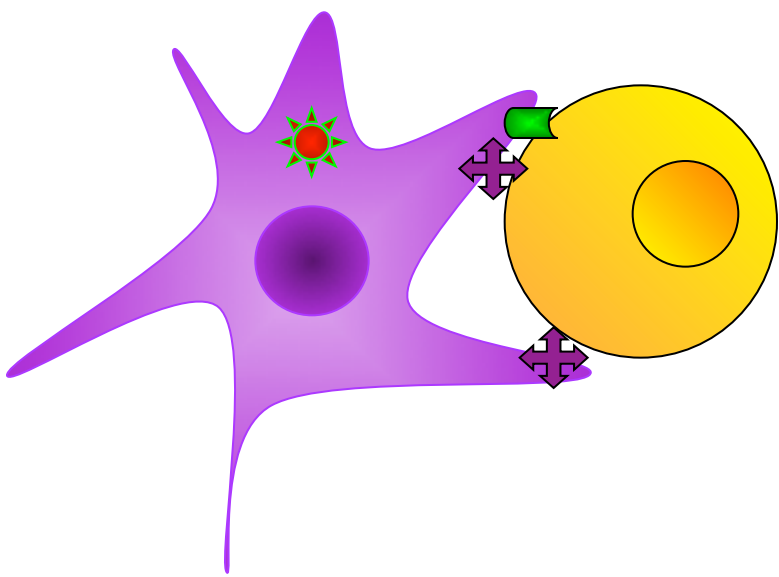
Células podem receber estímulos para iniciar ou parar ciclo celular



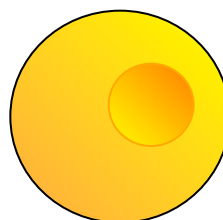
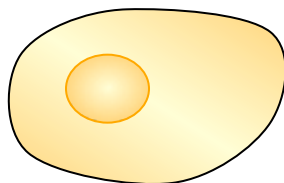
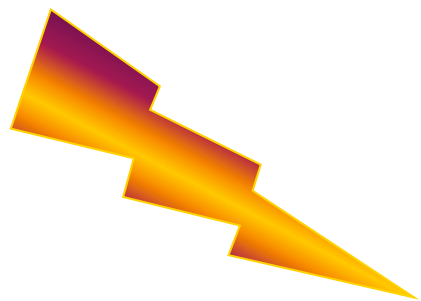
Capacidade proliferativa no mesmo organismo: variável

Regeneração de ferimentos









G0



G1

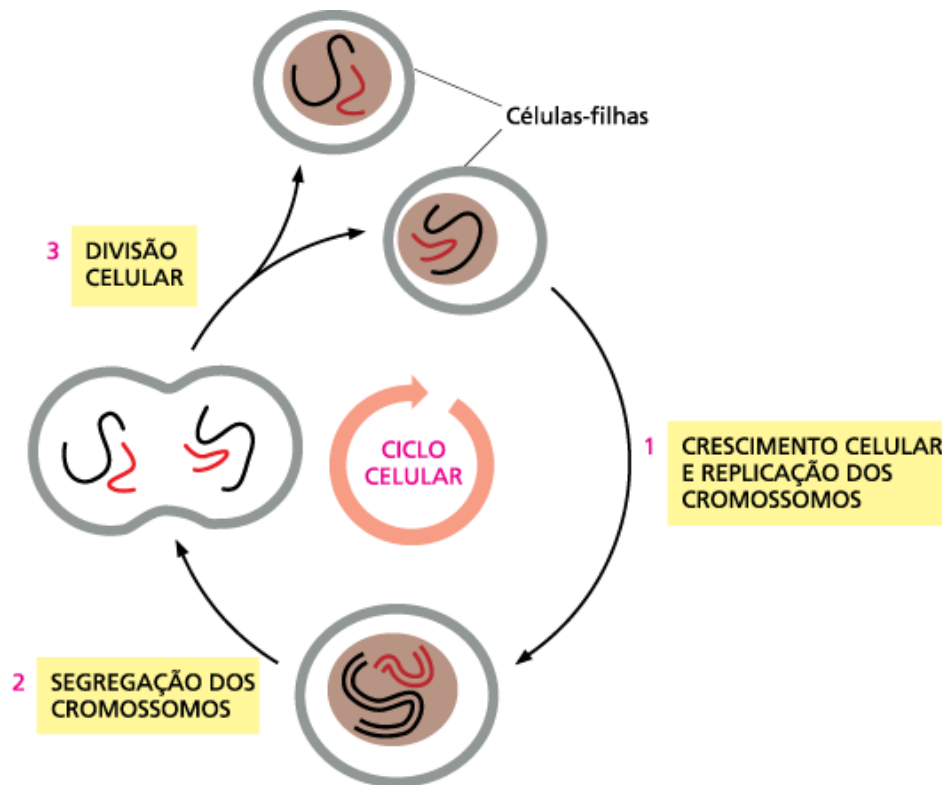
M

S

G2

Ciclo celular

Células se reproduzem com a duplicação de seu conteúdo e divisão em duas



Ciclo celular

TIPO CELULAR	DURAÇÃO DO CICLO CELULAR
Células jovens de embrião de sapo	30 min
Célula de levedura	1,5 – 3 horas
Células epiteliais de intestino	~ 12 horas
Fibroblastos de mamíferos (cultura)	~ 20 horas
Células hepáticas humanas	~ 1 ano

Estímulos: sinais intracelulares e extracelulares



Células podem receber estímulos para iniciar ou parar ciclo celular

Mitógenos: estimulam a divisão celular

Fatores de crescimento: estimulam o crescimento celular (e inibem degradação)

Fatores de sobrevivência: supressão de apoptose

Capacidade proliferativa no mesmo organismo: variável

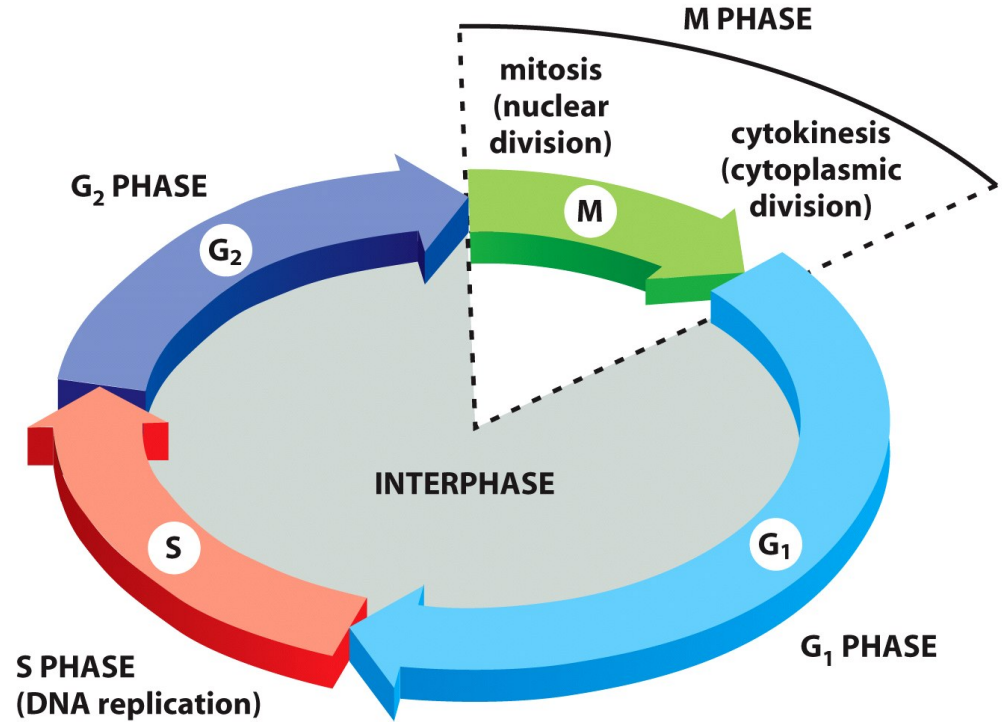
Ciclo celular

Intérfase:

-crescimento da célula e replicação do DNA

Mitose:

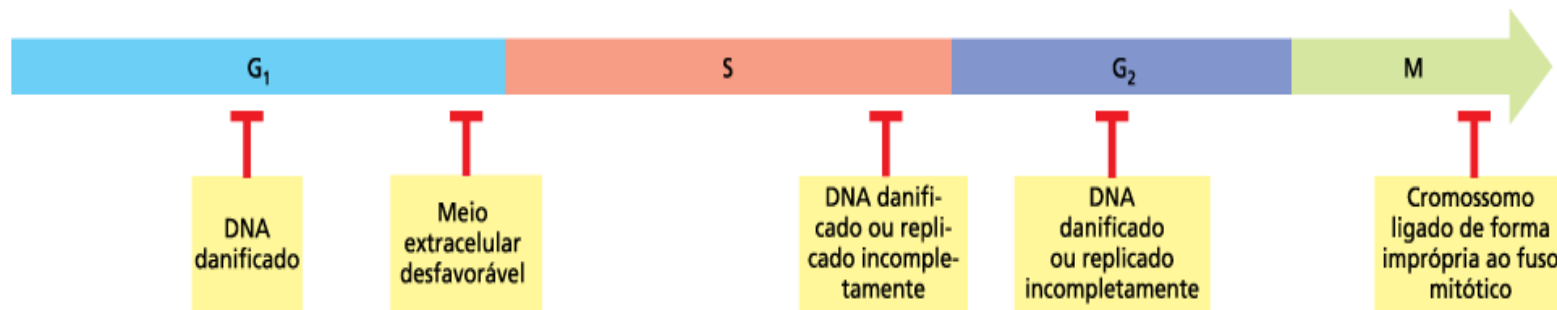
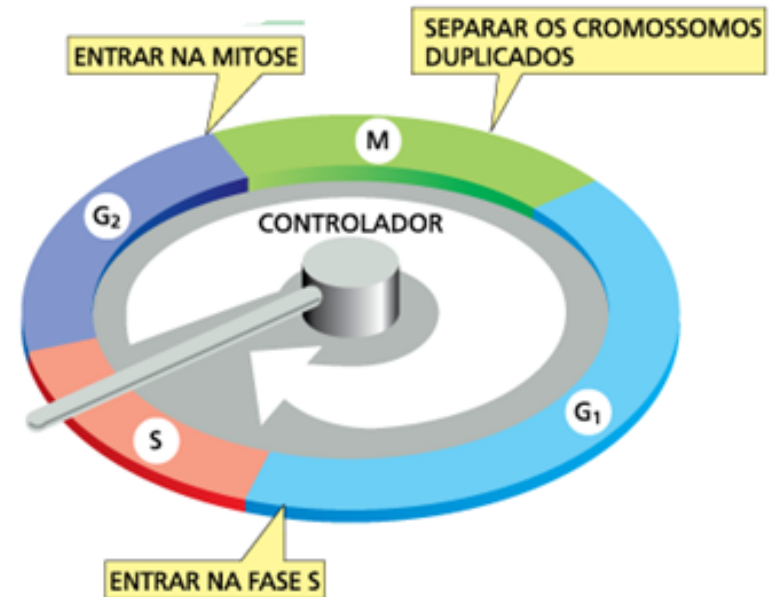
-divisão nuclear (mitose)
-divisão do citoplasma (citocinese)



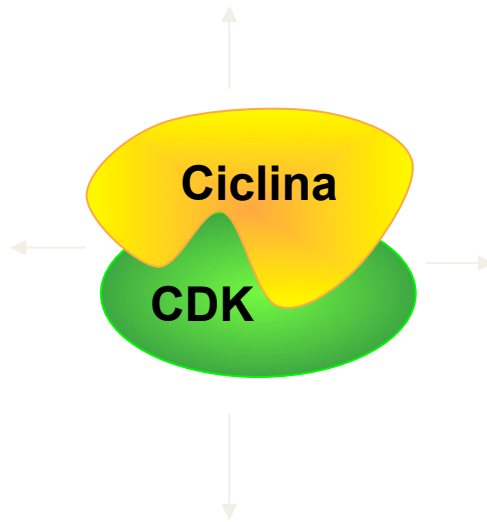
Pontos de verificação

pontos de verificação
são pontos de controle
do ciclo celular

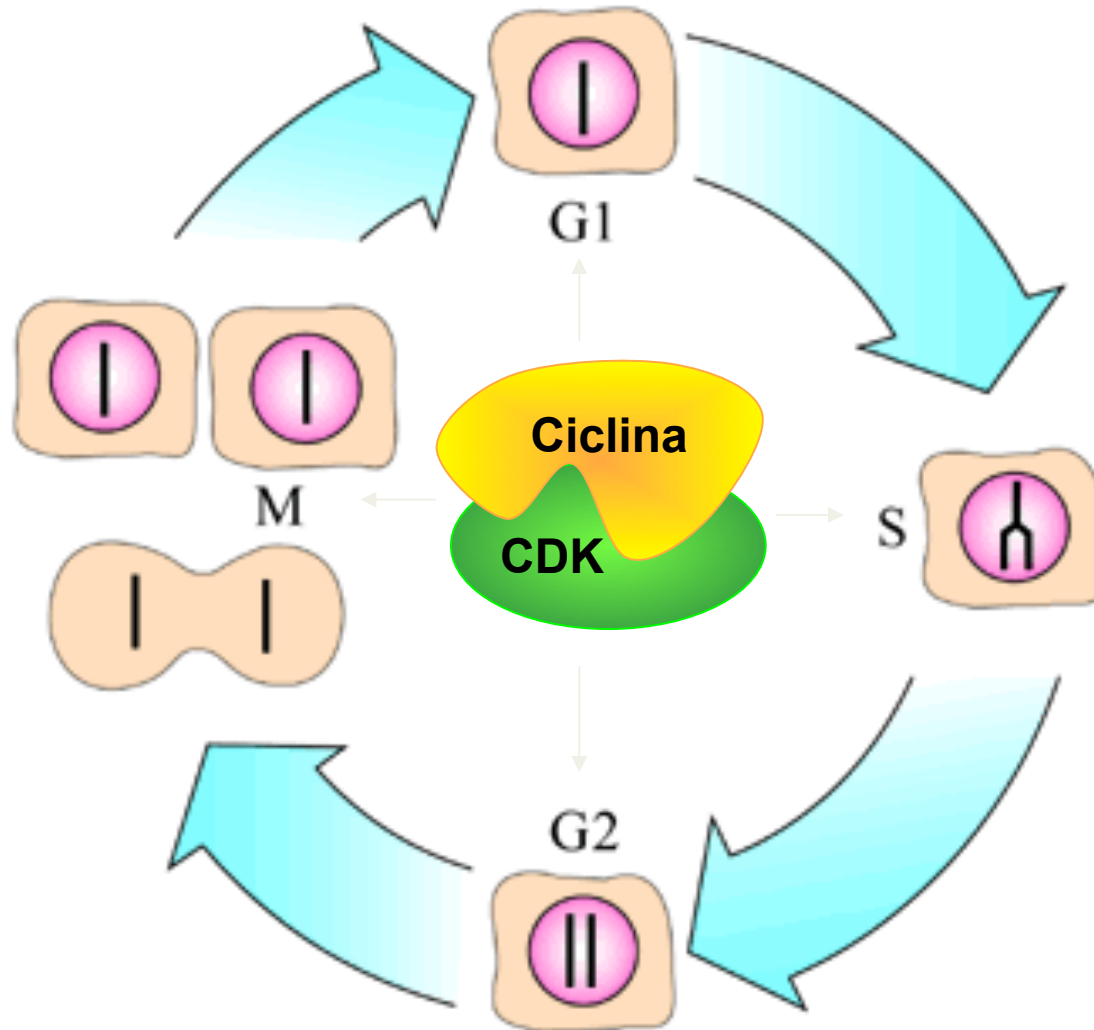
Condições externas e sinais
intracelulares



Controle do ciclo celular

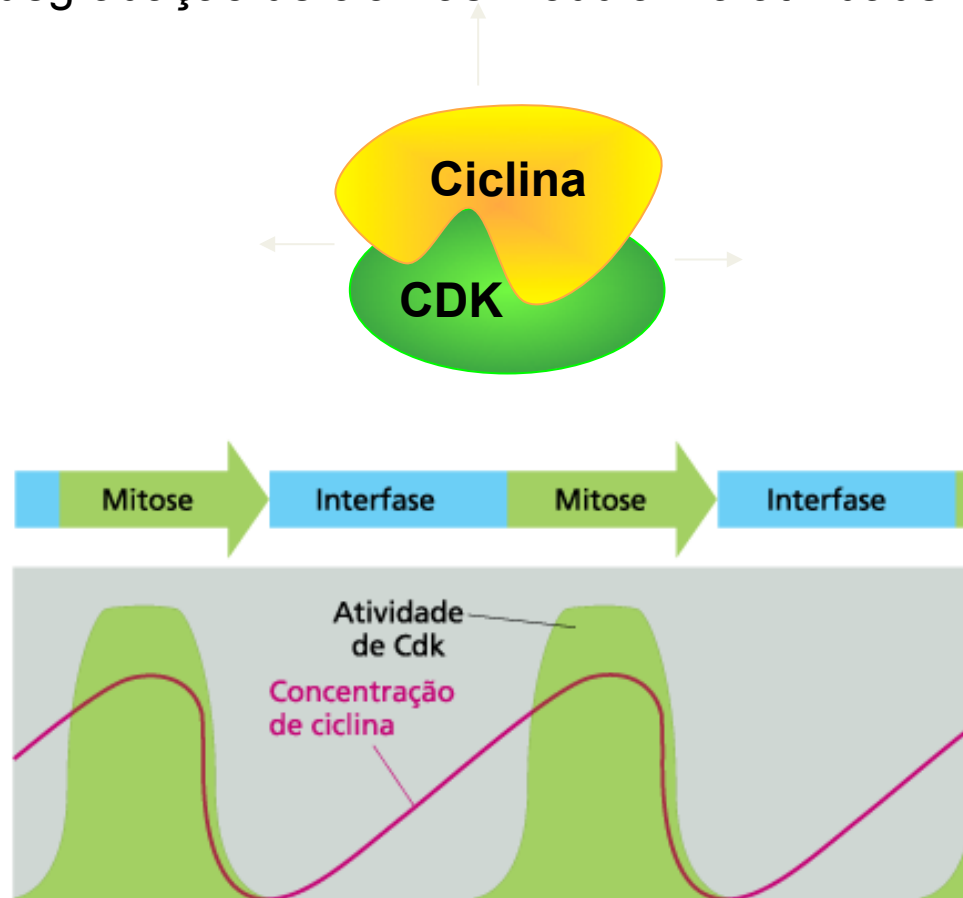


Controle do ciclo celular



Controle do ciclo celular: ciclina-CDK

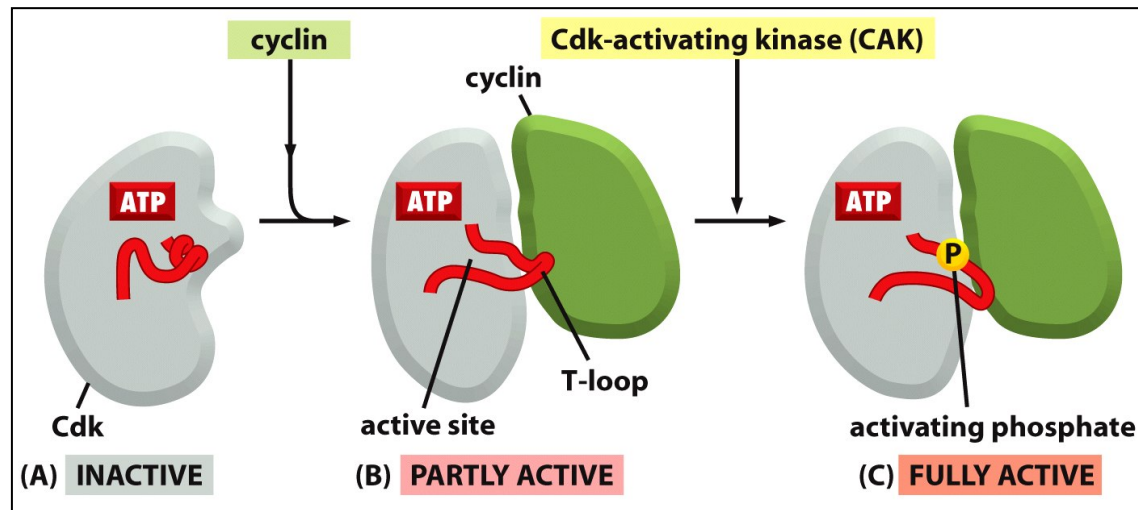
Acúmulo e degradação de ciclinas modulam a atividade das CDKs



Controle do ciclo celular: ciclina-CDK

CDKs: proteínas conservadas; 30-40kDa

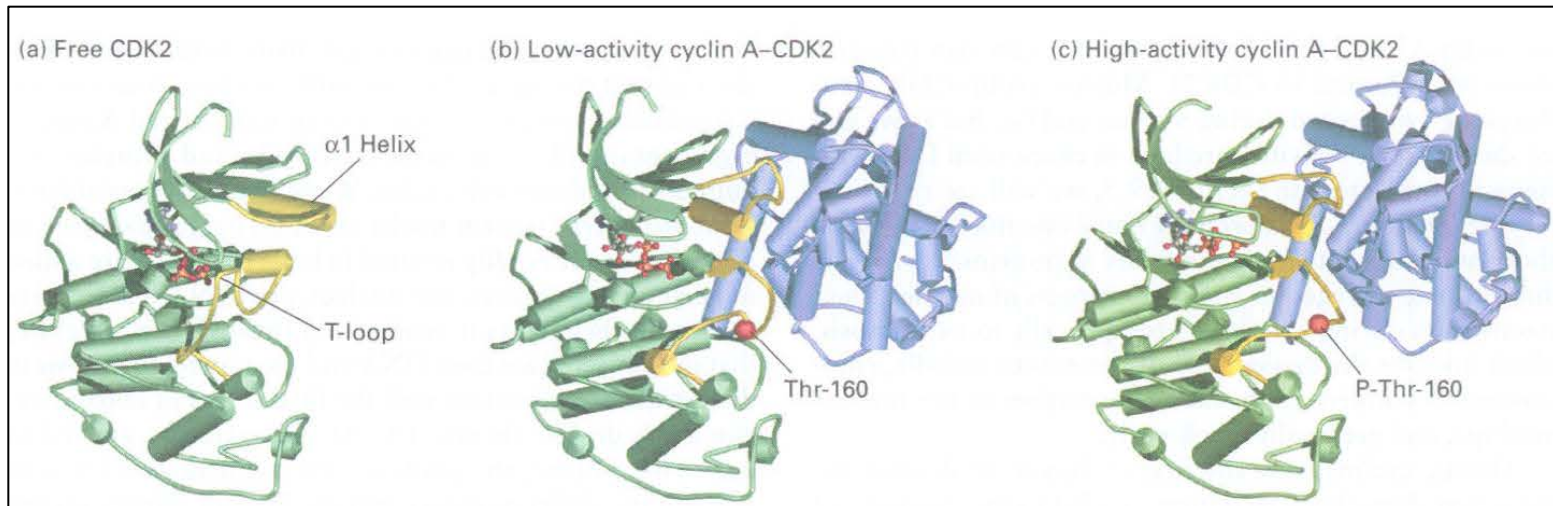
Ativação: 1) ligação a ciclina (altera conformação) e 2) fosforilação sítio ativo



Controle do ciclo celular: ciclina-CDK

CDKs: proteínas conservadas; 30-40kDa

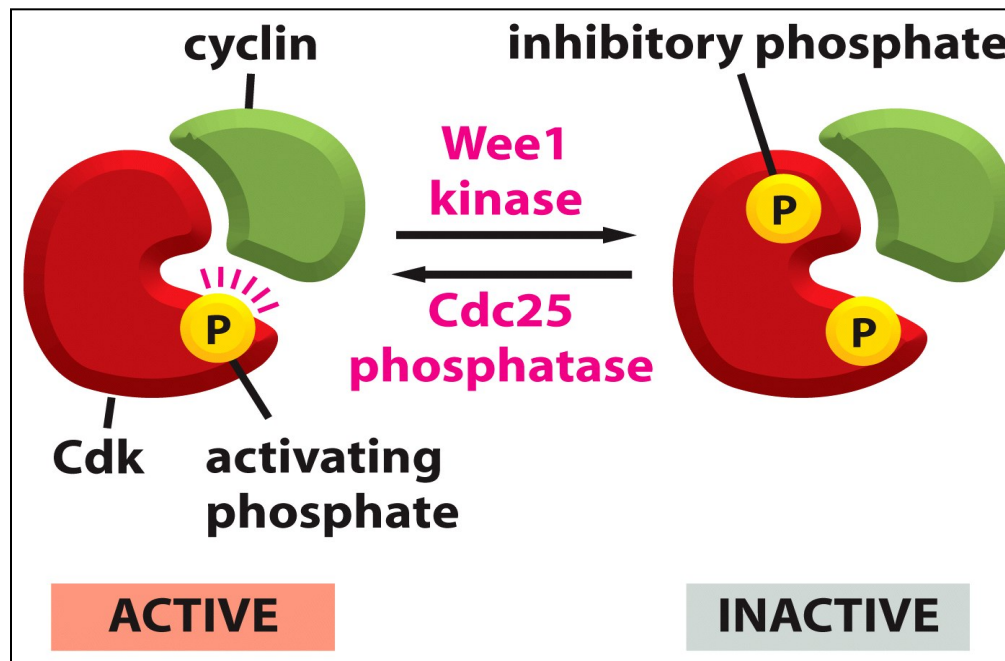
Ativação: 1) ligação a ciclina (altera conformação) e 2) fosforilação sítio ativo



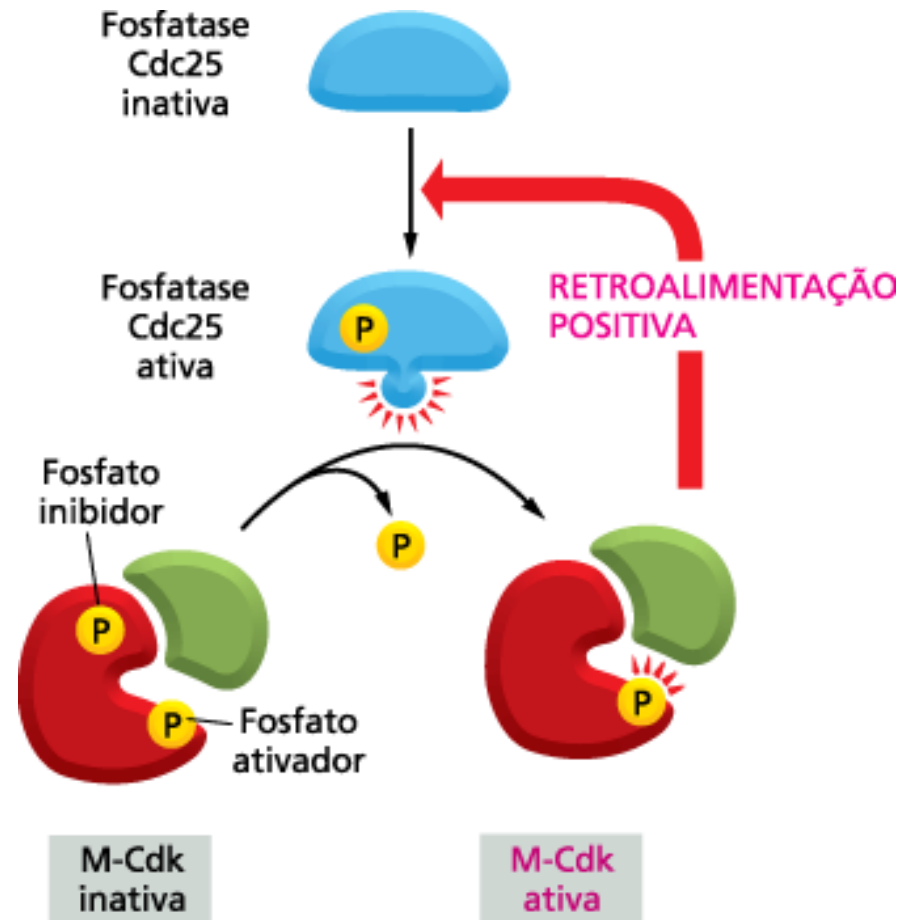
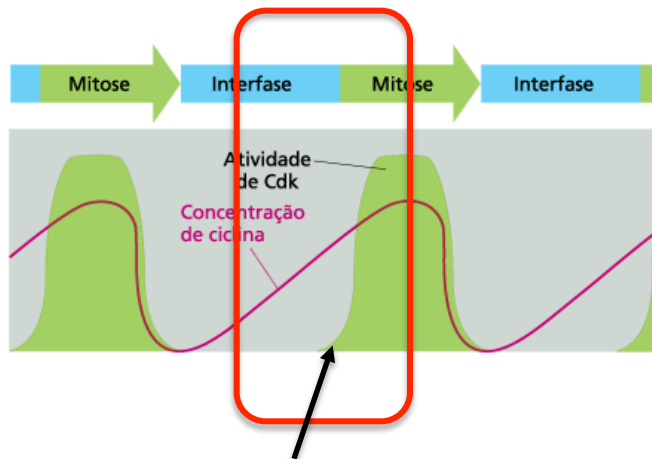
Controle do ciclo celular: ciclina-CDK

CDKs: proteínas conservadas; 30-40kDa

Regulação mais fina: kinase Wee1/ fosfatase Cdc25



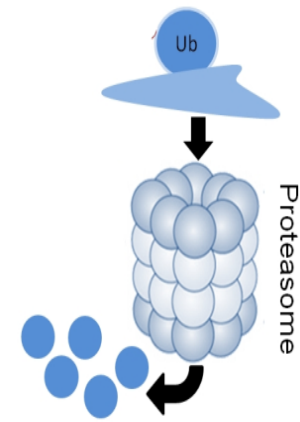
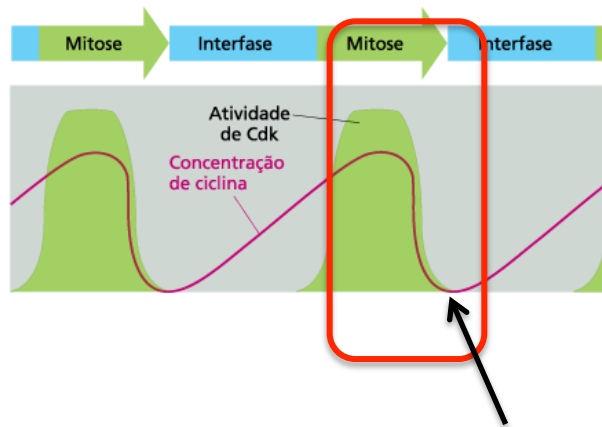
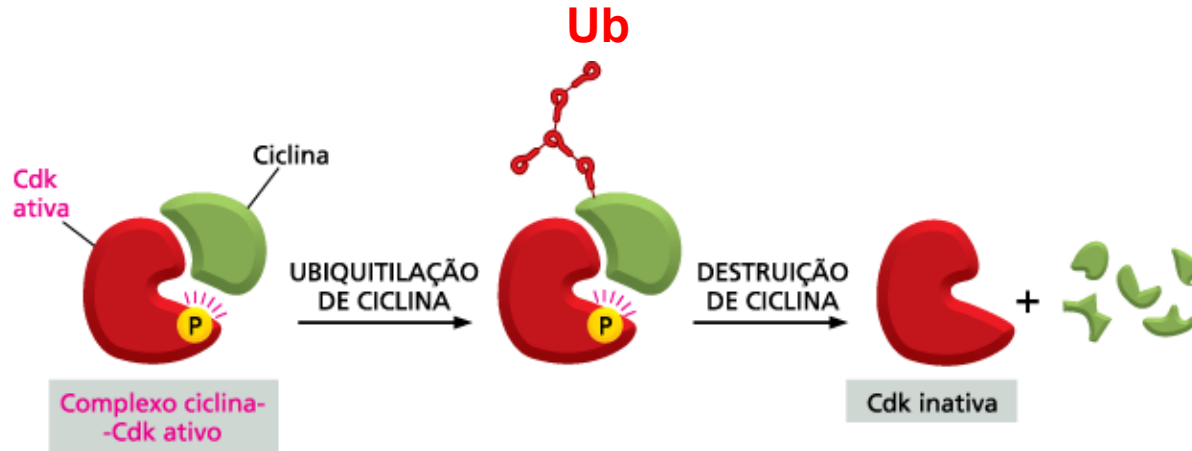
Controle do ciclo celular: ciclina-CDK



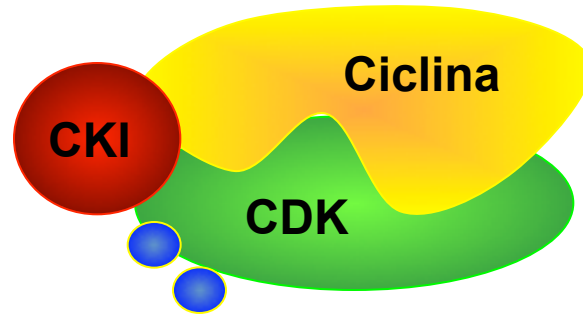


Controle do ciclo celular: ciclina-CDK

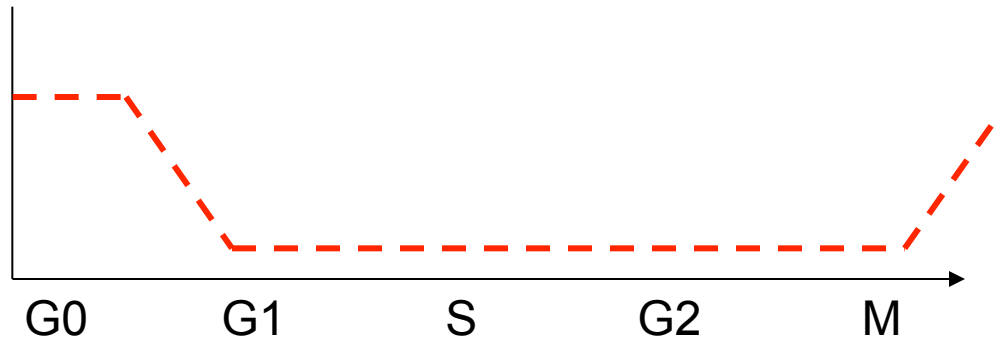
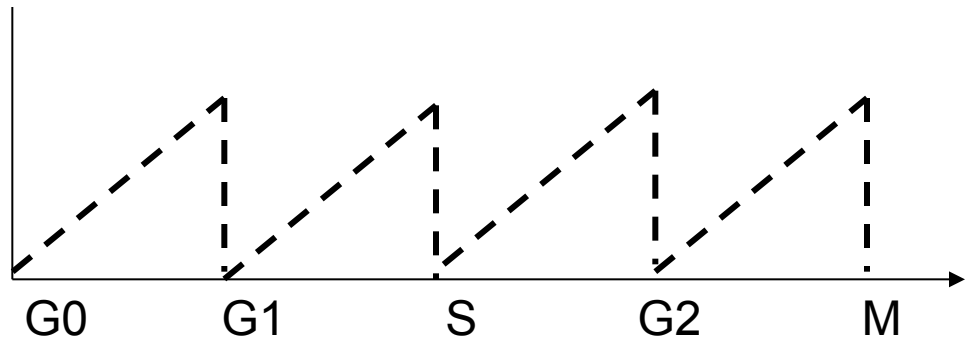
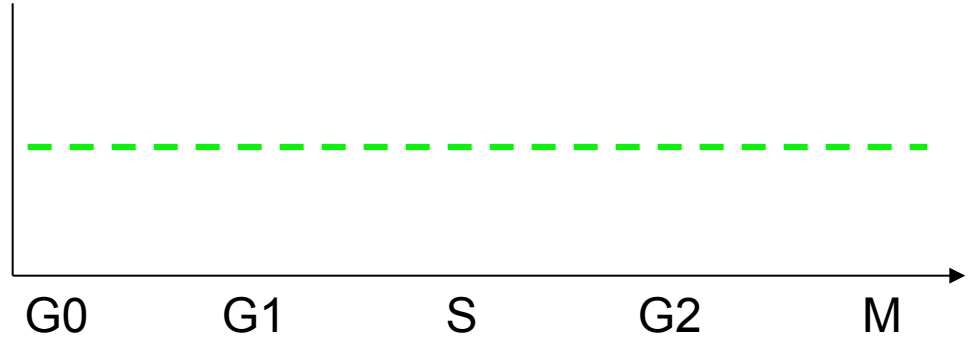
Degradação: proteassoma



Controle do ciclo celular: ciclina-CDK



Interações com proteínas inibitórias: CKIs



CDK, Ciclinas e **CKI**

CDK1 Ciclinas A, B

p27, p21, p57

cip/kip

CDK2 Ciclinas A, E

CDK3 Ciclina E

CDK4 Ciclina D

p15, p16, p18, p19

INK4

CDK5 Ciclina D

CDK6 Ciclina D

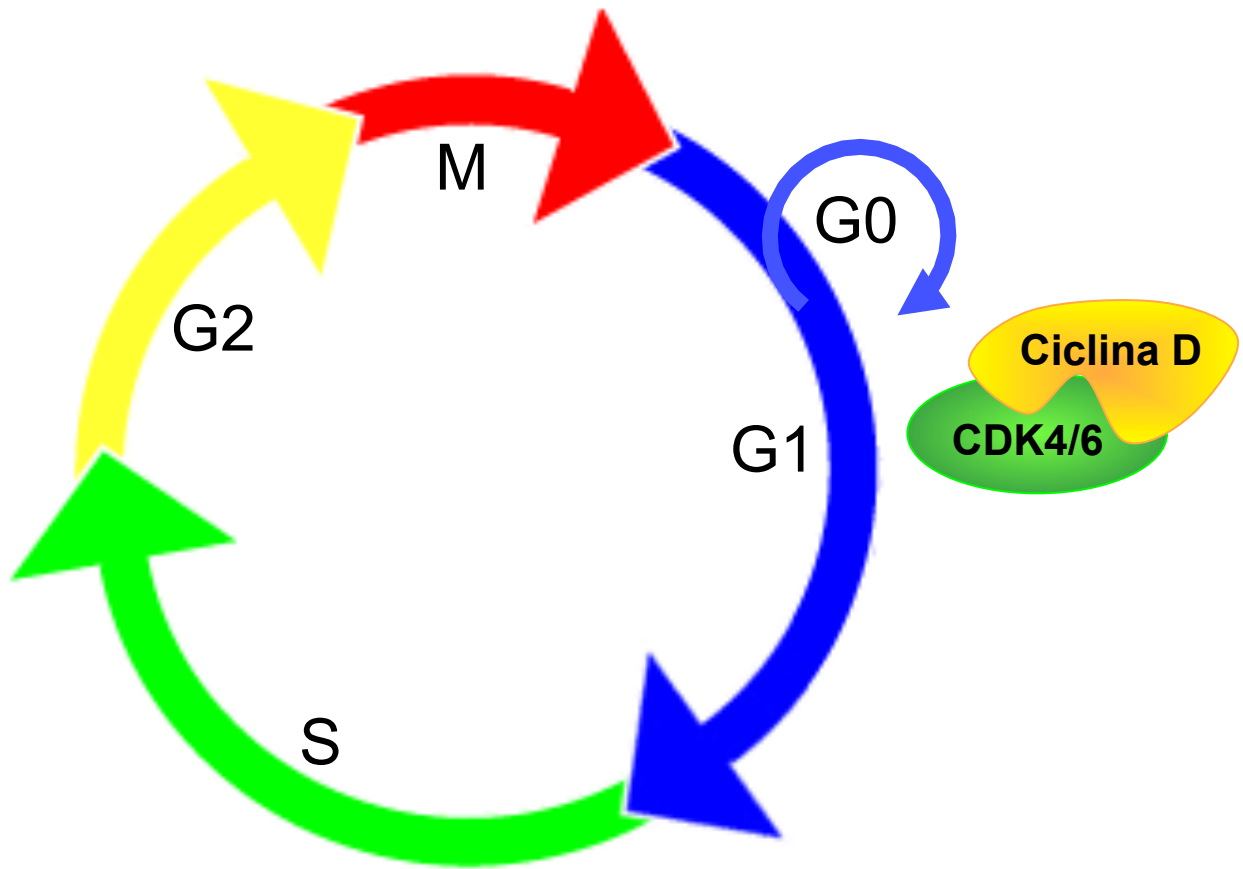
CDK7 Ciclina H

CDK8 Ciclina C

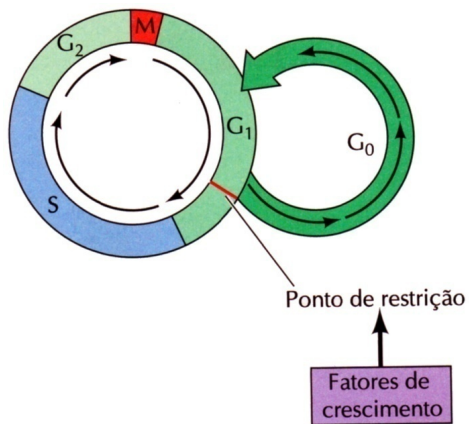
CDK9 Ciclina T

CDK, Ciclinas e fases do ciclo

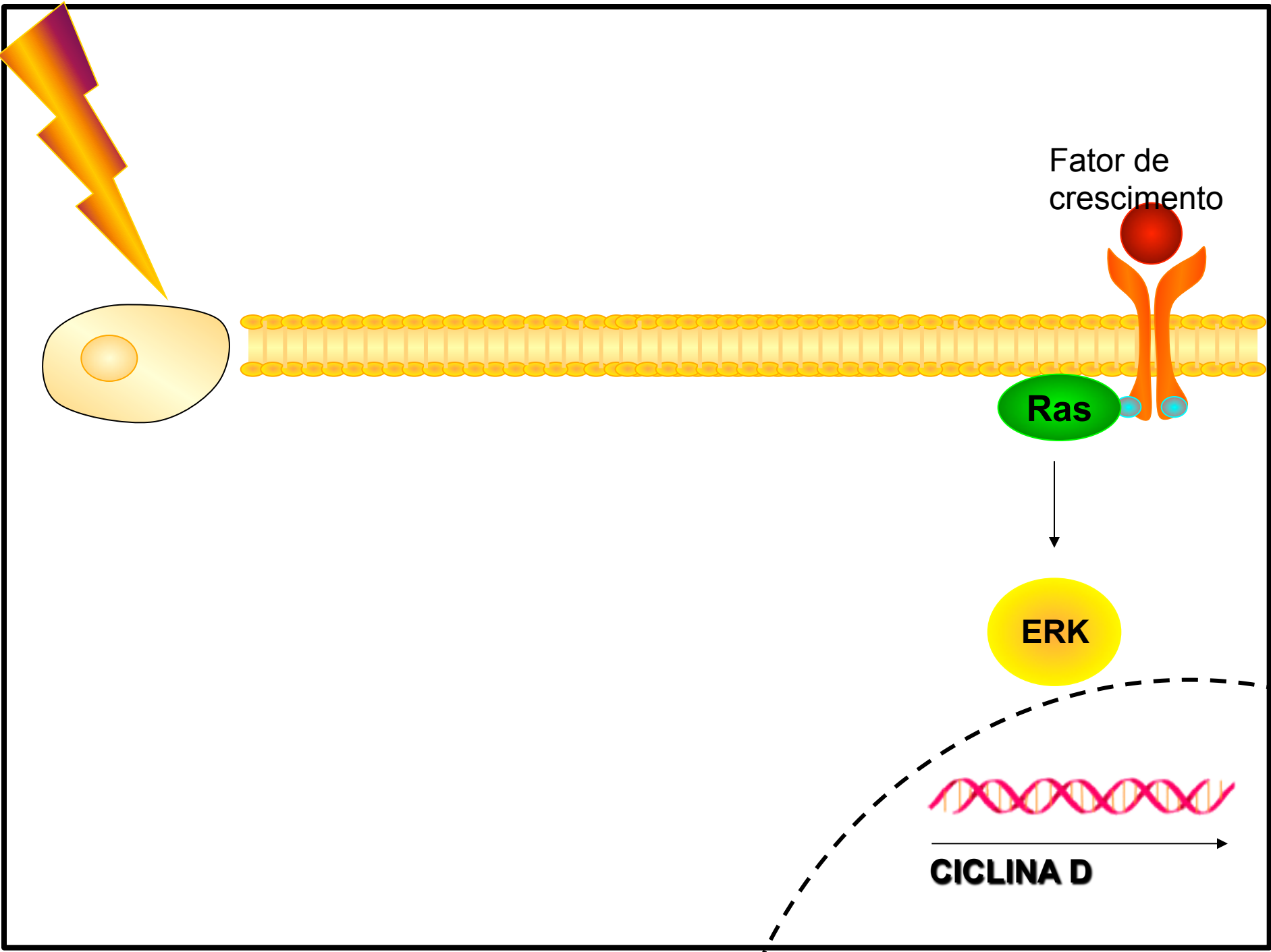
CDK1	Ciclinas A, B	G2/M
CDK2	Ciclinas A, E	G1/S, S
CDK3	Ciclina E	G1/S
CDK4	Ciclina D	G1/S
CDK5	Ciclina D	Diferenciação neuronal
CDK6	Ciclina D	G1/S
CDK7	Ciclina H	CAK
CDK8	Ciclina C	Regulação transcricional
CDK9	Ciclina T	G1/S



Saída para G_0 ?



Por exemplo neurônios

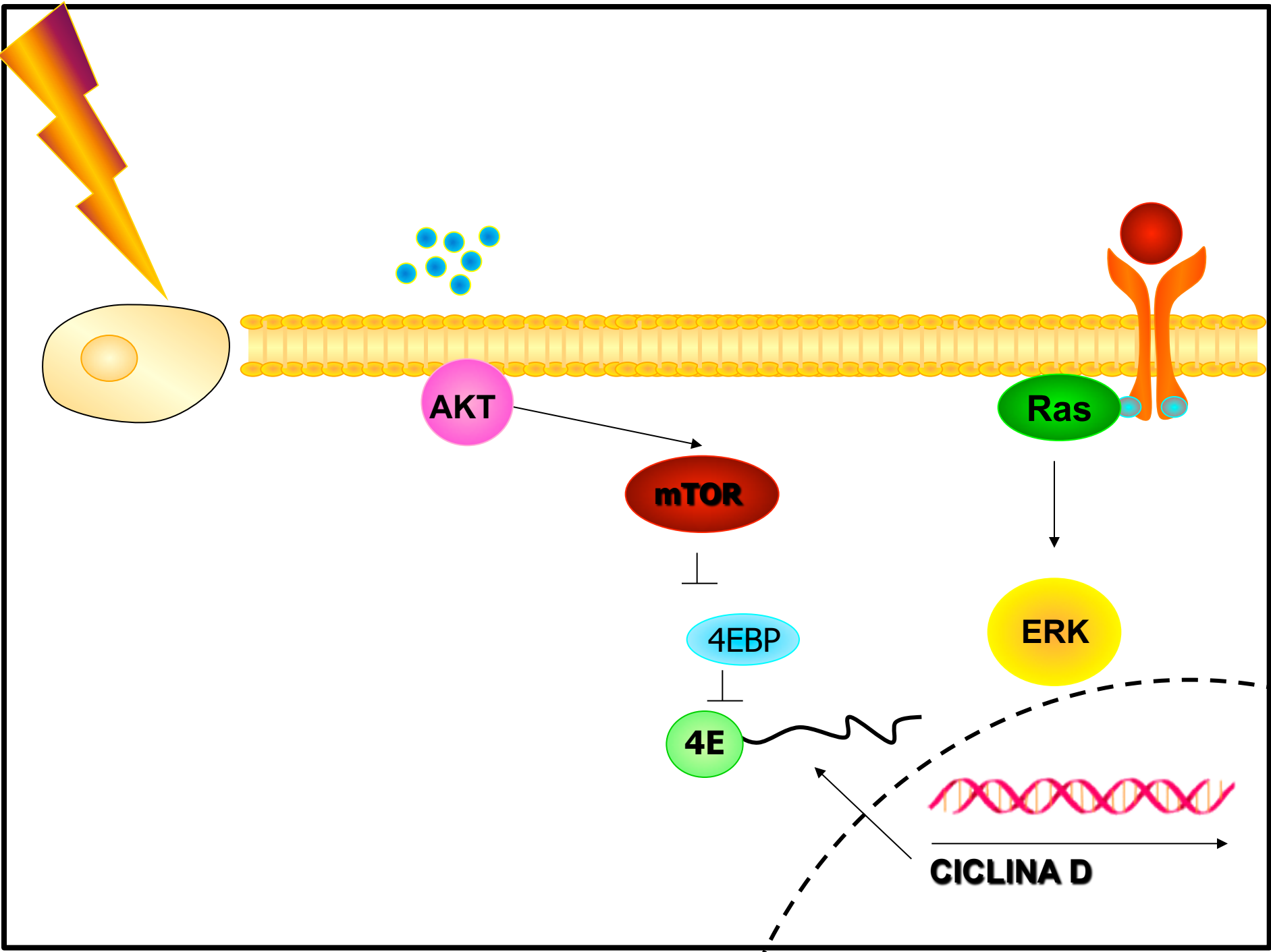


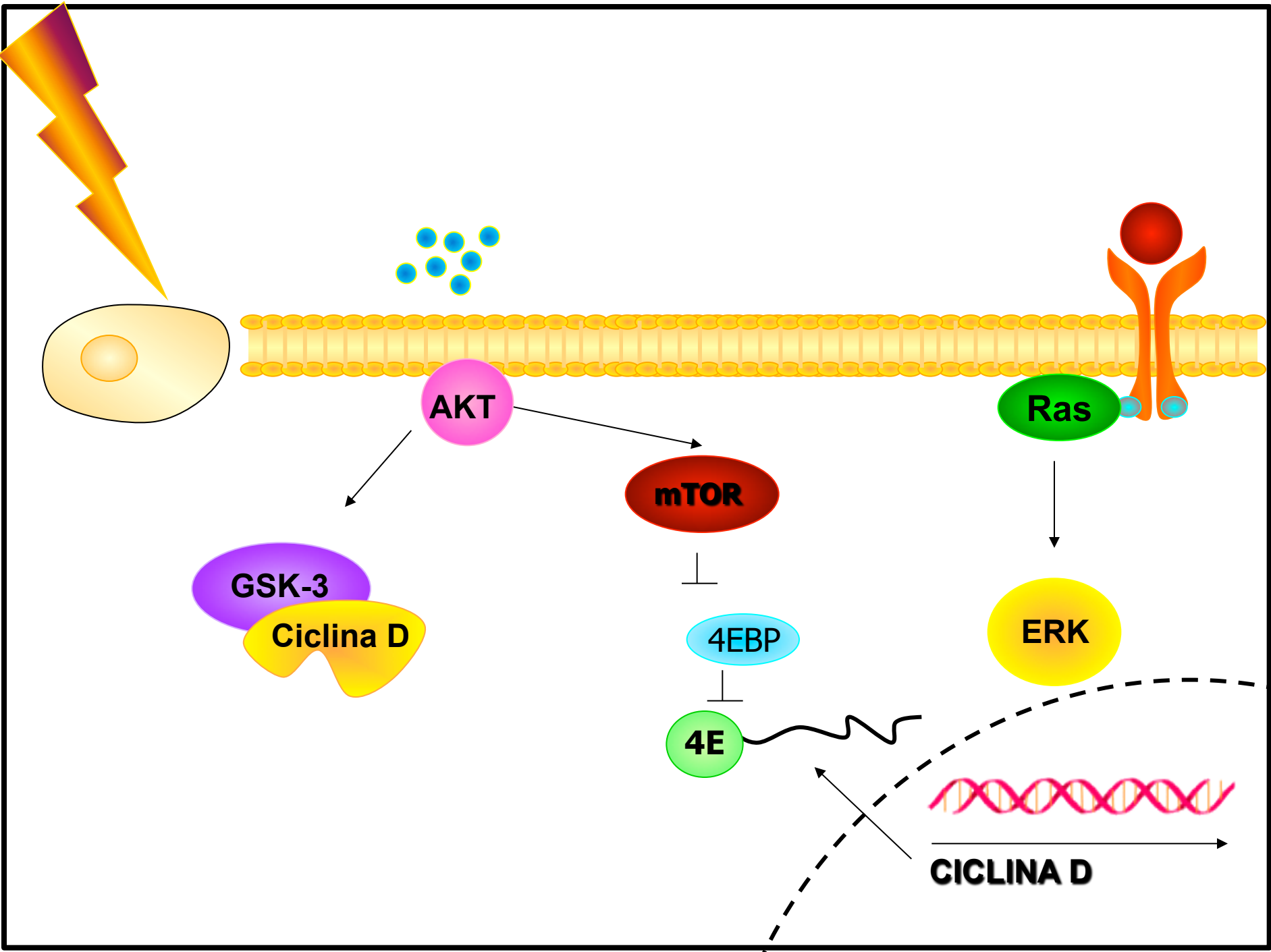
Fator de crescimento

Ras

ERK

CICLINA D





AKT

mTOR

GSK-3

Ciclina D

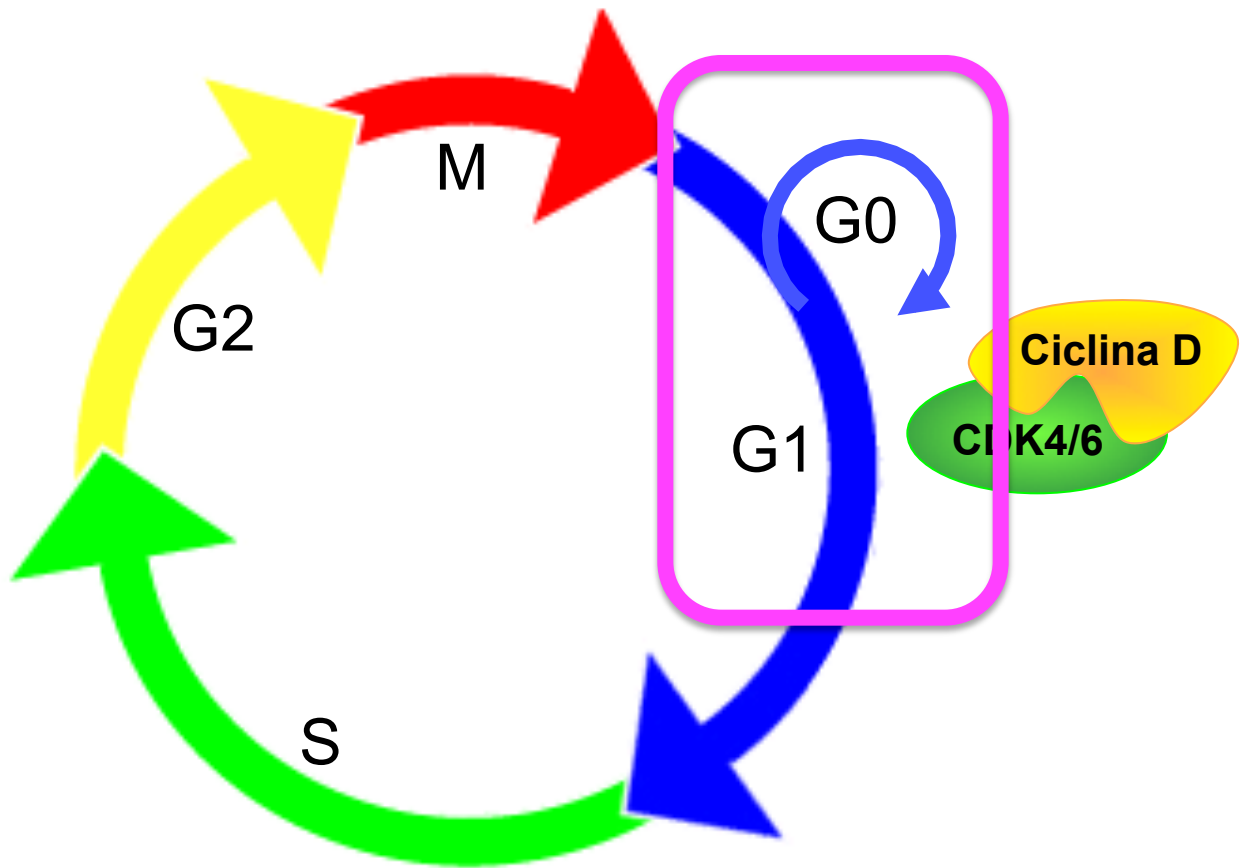
4EBP

4E

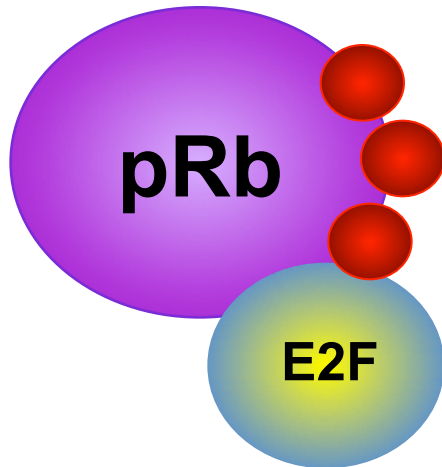
Ras

ERK

CICLINA D

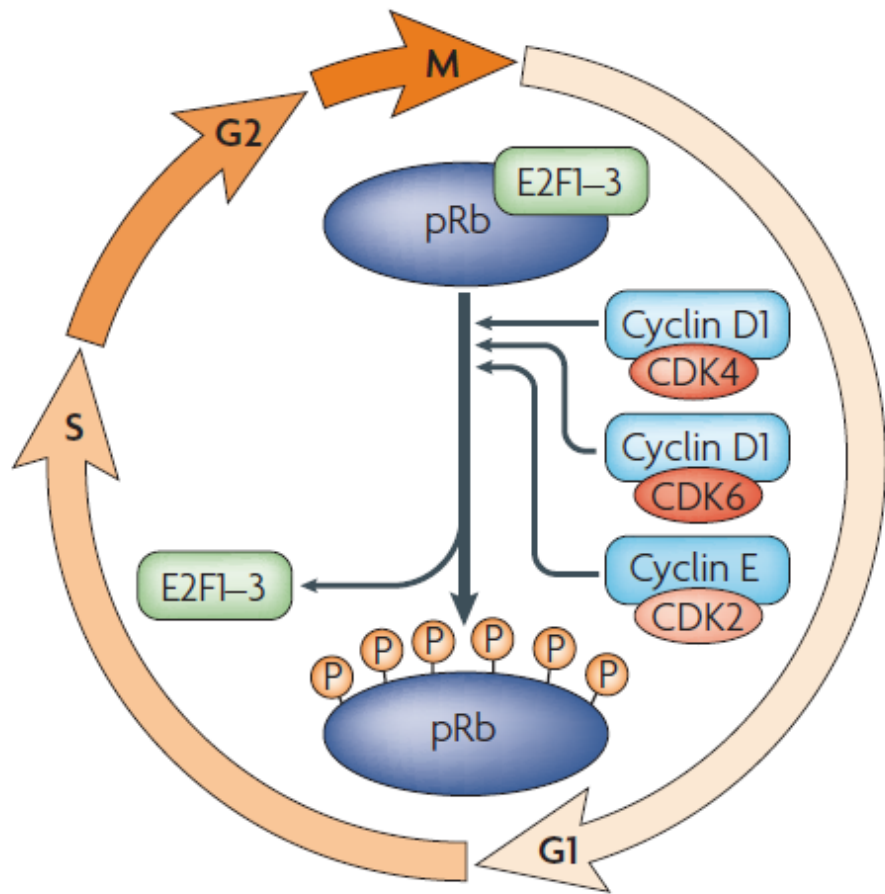


Período de resposta a estímulos extracelulares



- Ciclinas E e A
- CDK1
- c-myc
- E2F

- DNA polimerase α
- Timidina quinase
- Timidilato sintetase
- Hidrofolato redutase

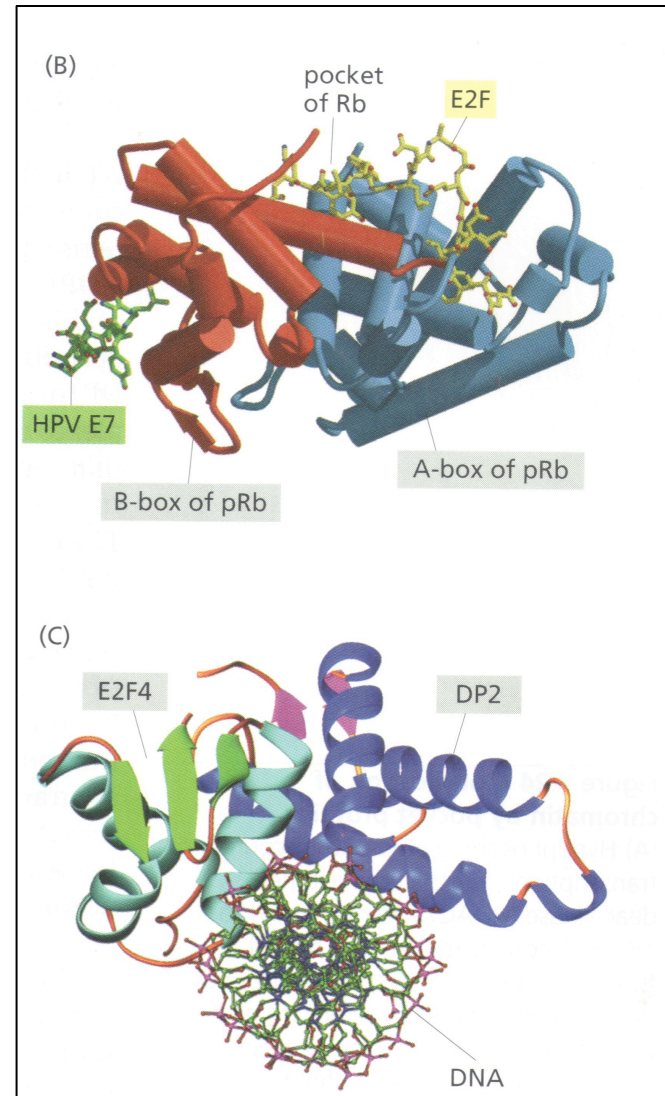


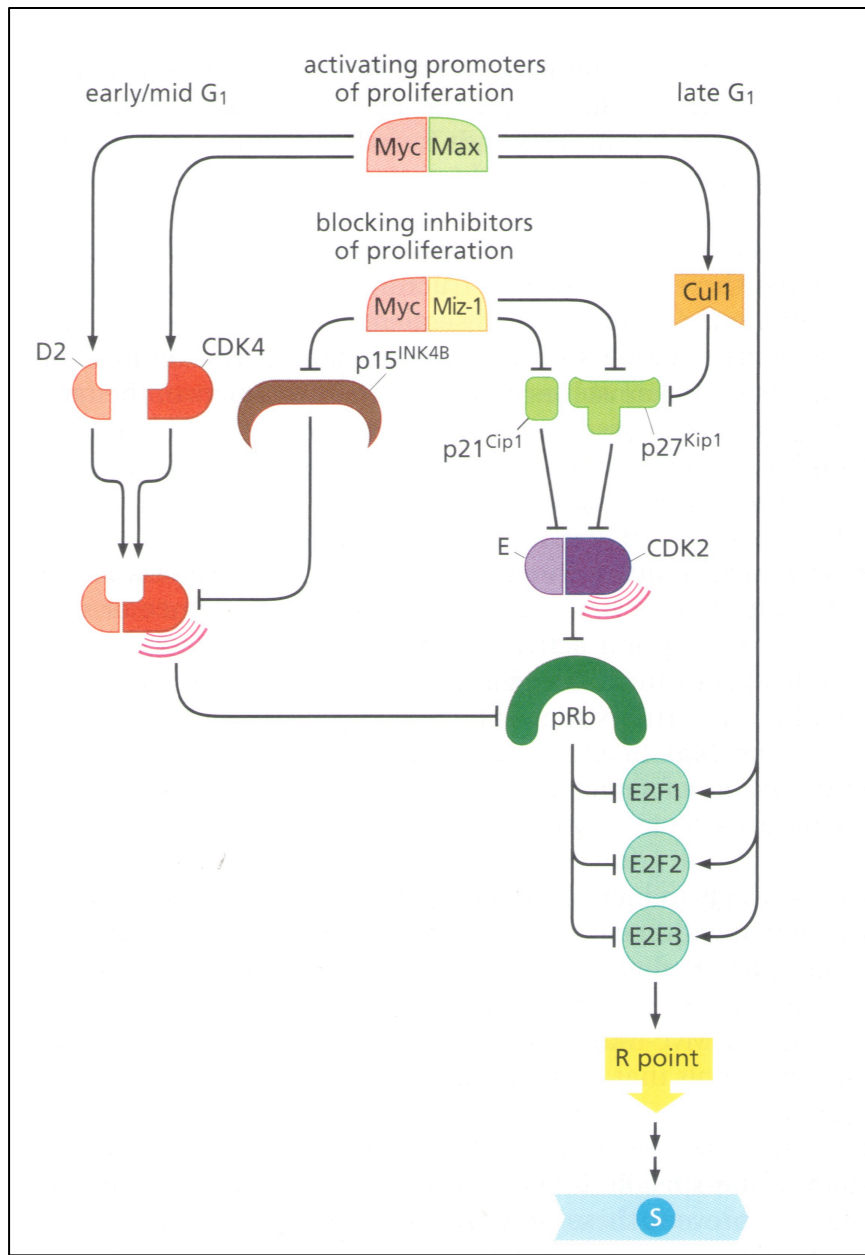
Proteína pRB

Reguladora do ciclo

Ligação com E2Fs pode ser reduzida na presença de algumas proteínas virais → oncoproteínas (HPV)

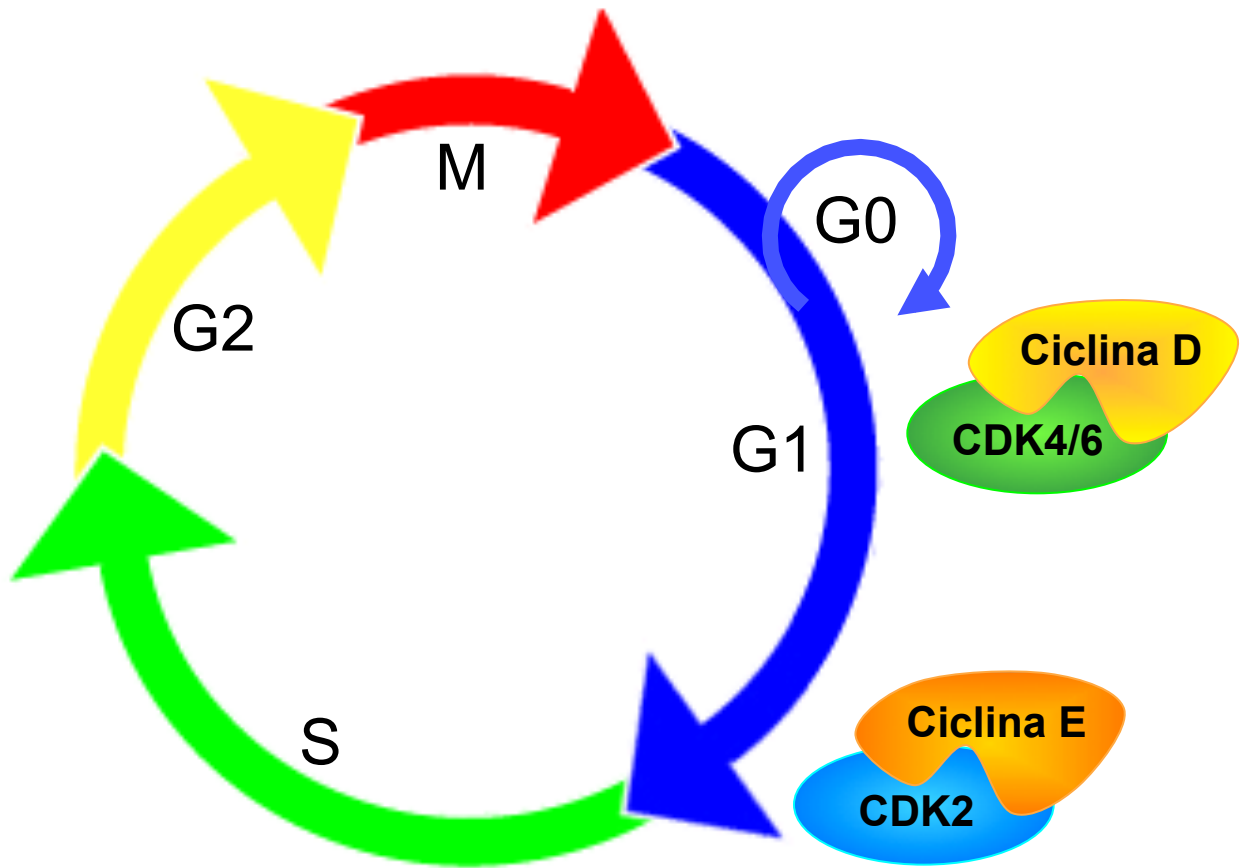
E2F4 ligando ao DNA





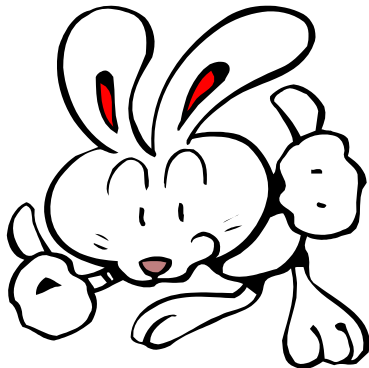
Myc

induz proliferação celular



Ponto de verificação G1/S

- ❖ O tamanho da célula é adequado ?
- ❖ Existe energia suficiente para continuar ?
- ❖ O estímulo para a proliferação continua ?
- ❖ A maquinaria de replicação está presente ?
- ❖ O DNA está íntegro para ser copiado ?



PROGRESSÃO

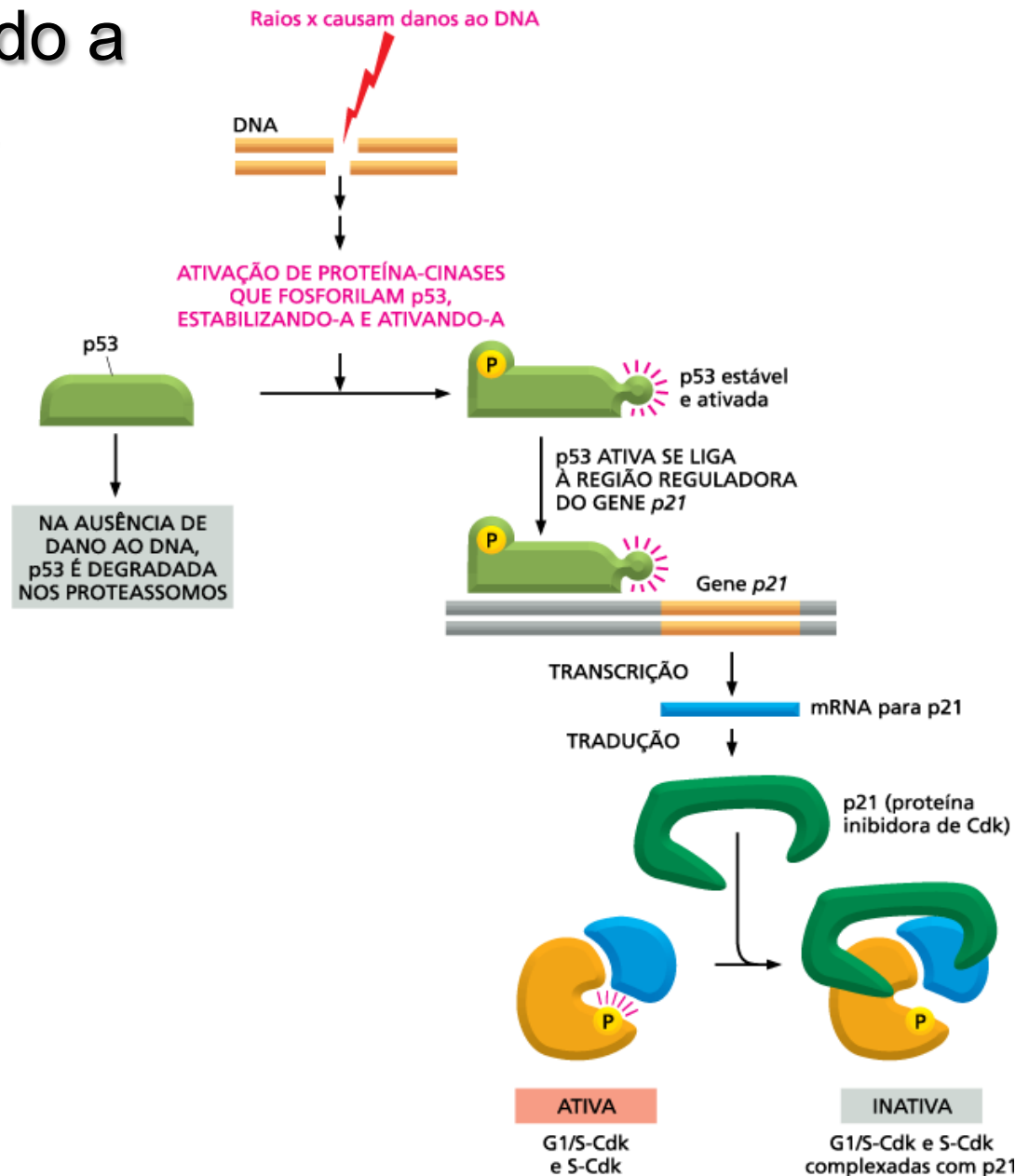
Ponto de verificação G1/S

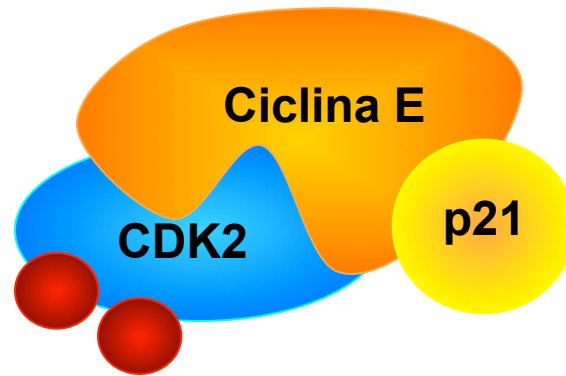
- ❖ O tamanho da célula é adequado ?
- ❖ Existe energia suficiente para continuar ?
- ❖ O estímulo para a proliferação continua ?
- ❖ A maquinaria de replicação está presente ?
- ❖ O DNA está íntegro para ser copiado ?



PARADA OU APOPTOSE

Parada em G1 devido a danos no DNA

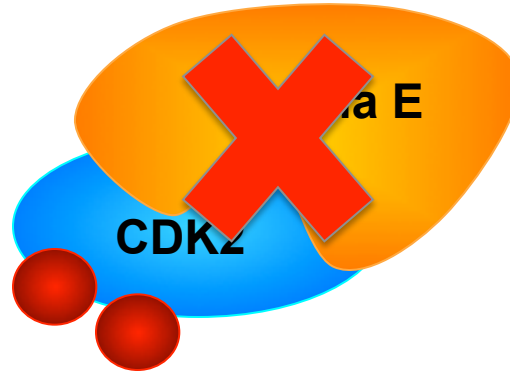


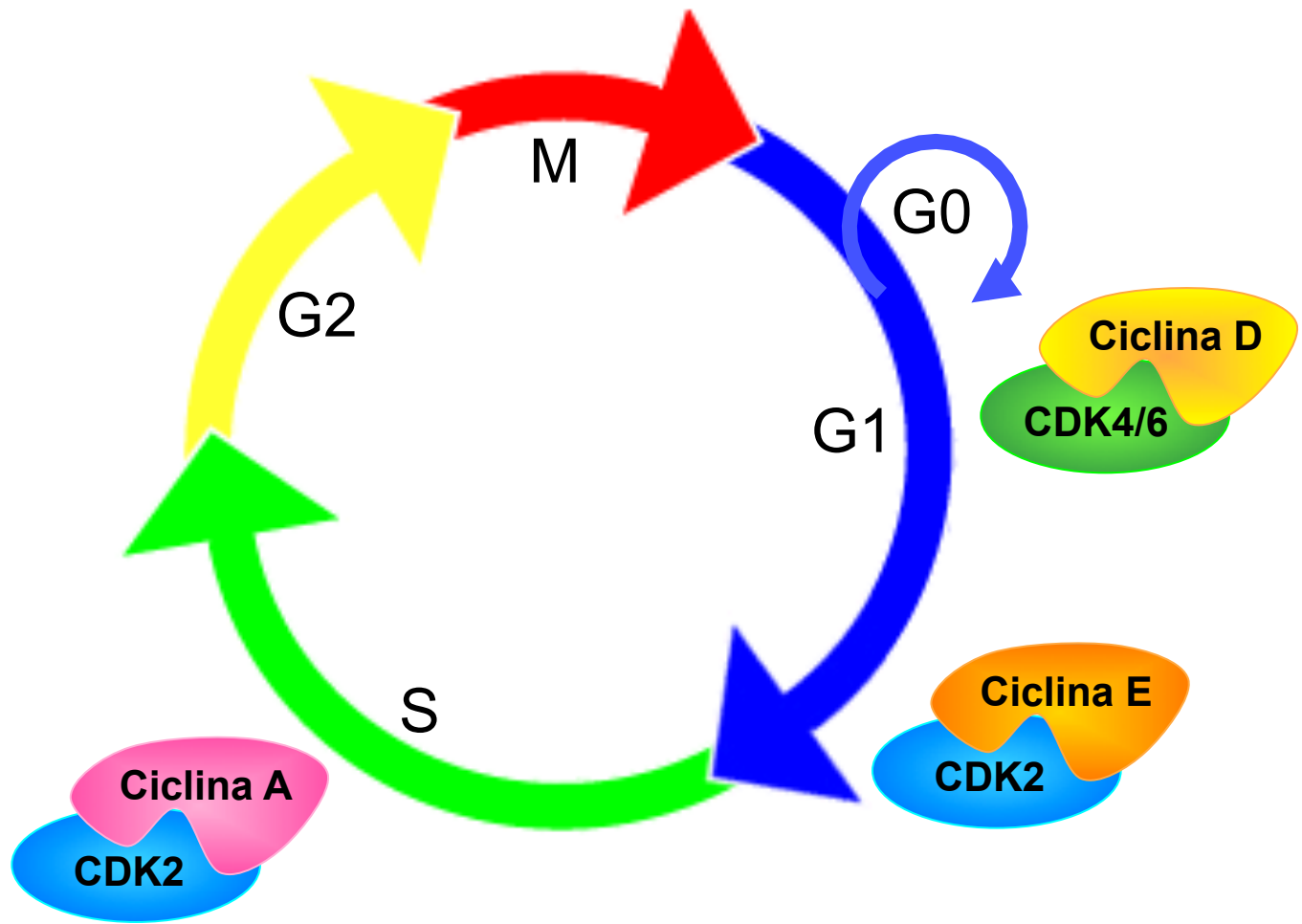


Ciclina E

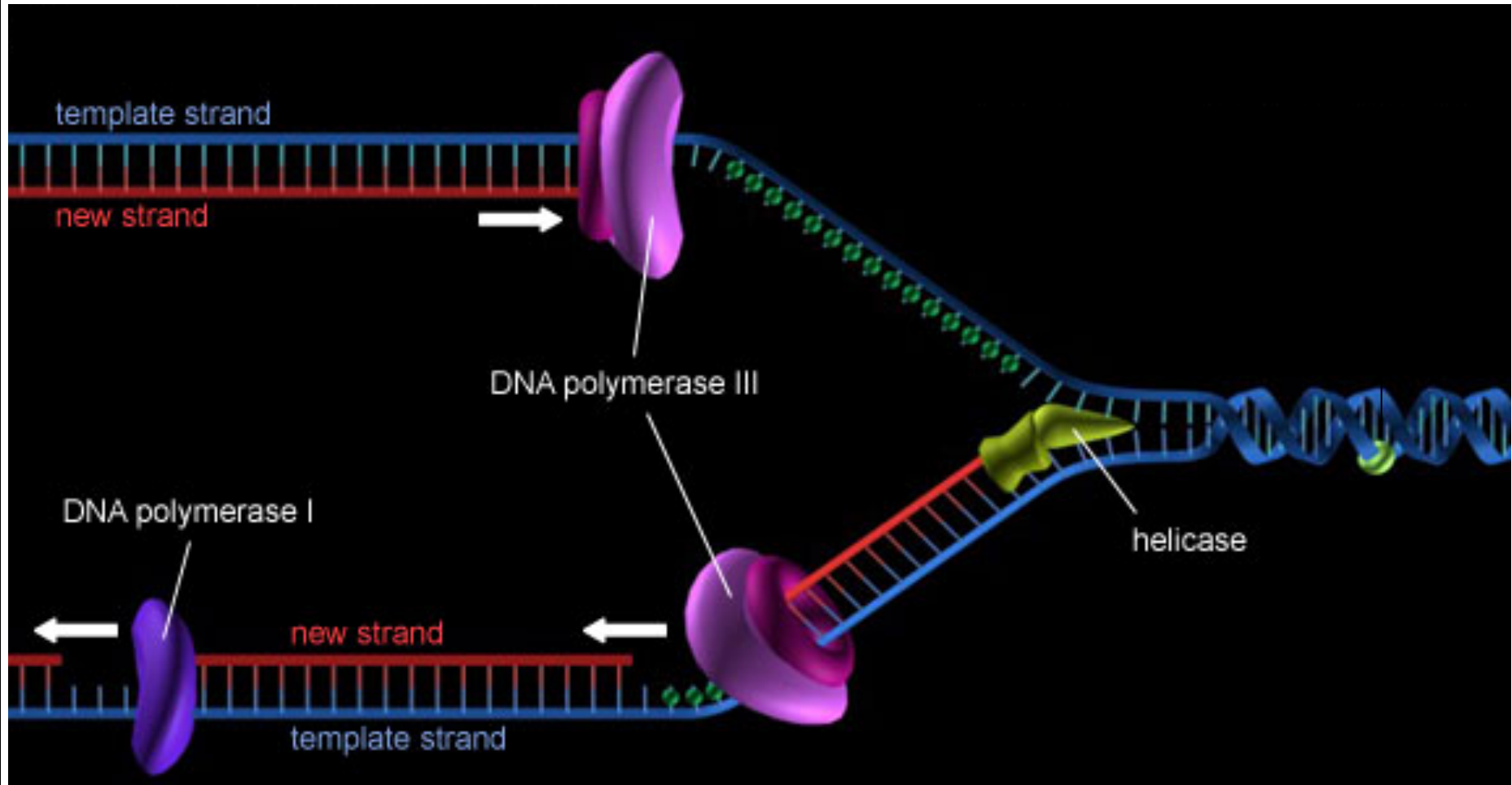
CDK2

p21

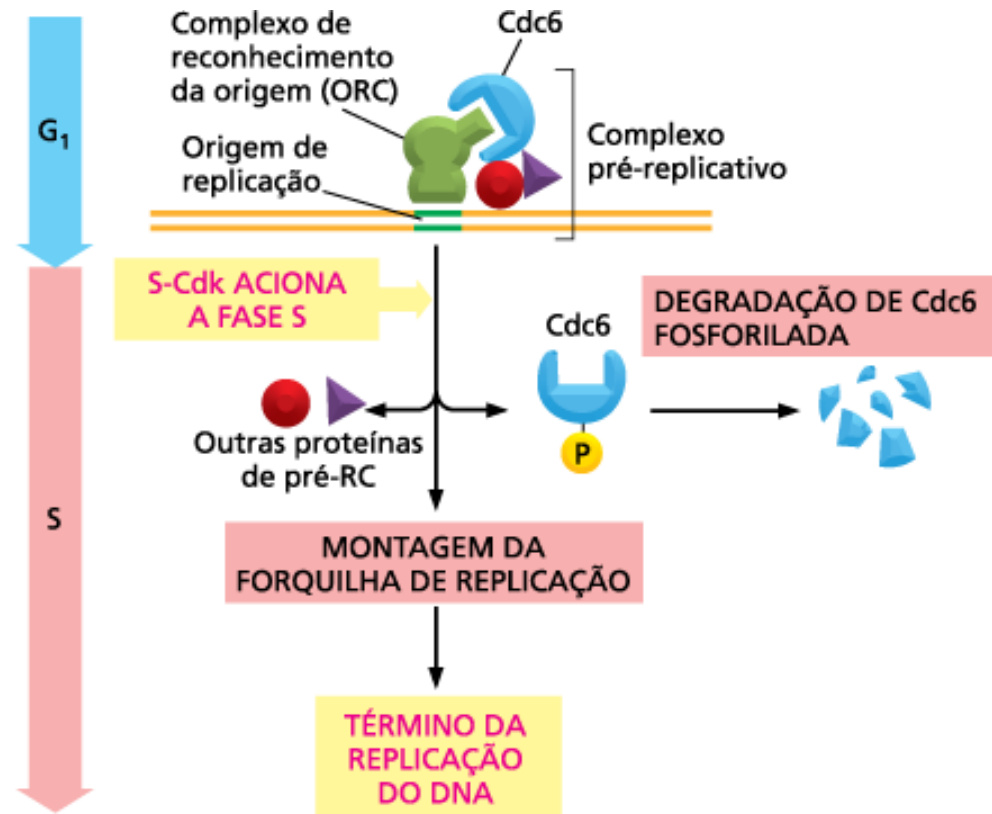




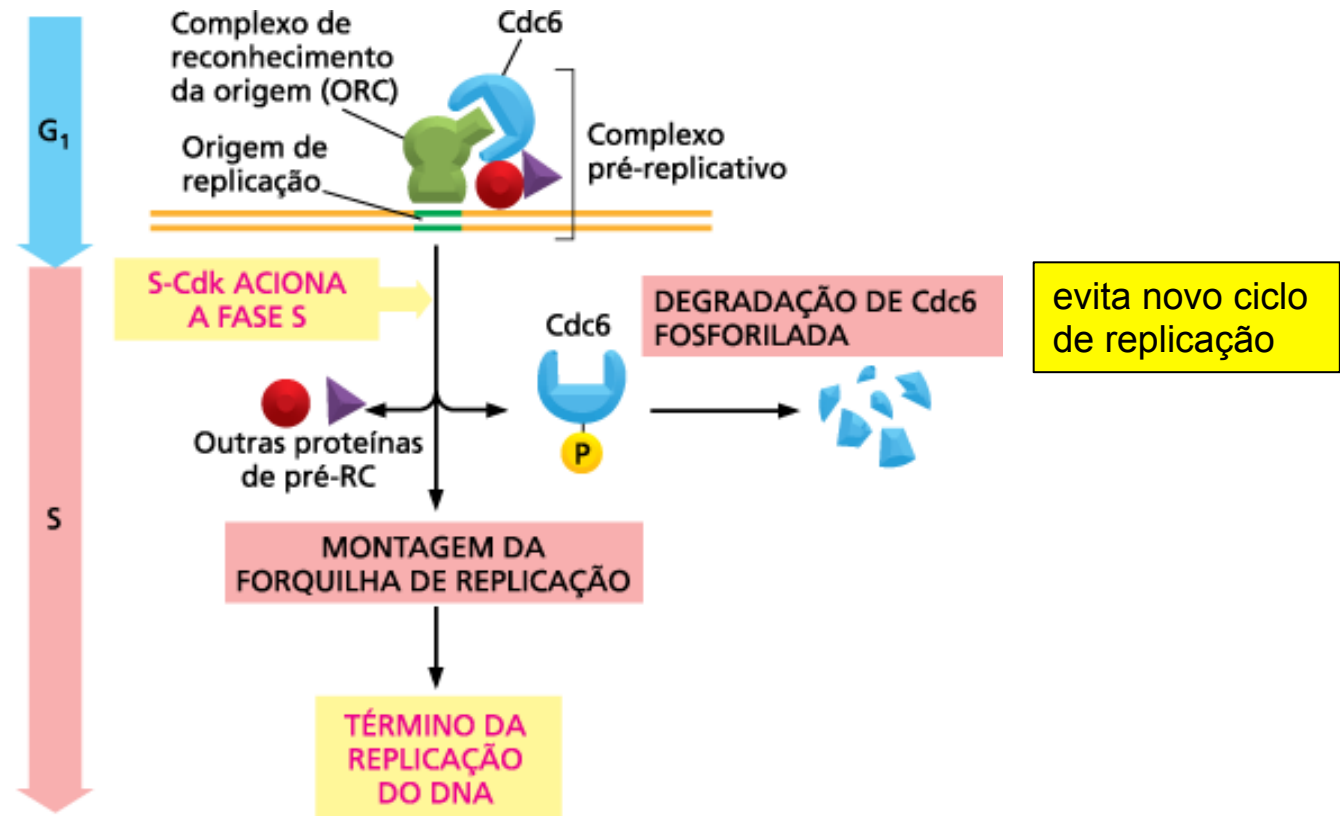
Fase S: replicação do DNA



Controle da replicação do DNA



Controle da replicação do DNA



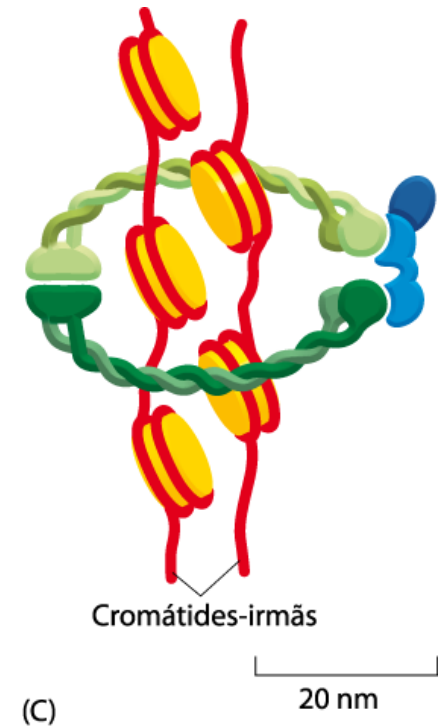
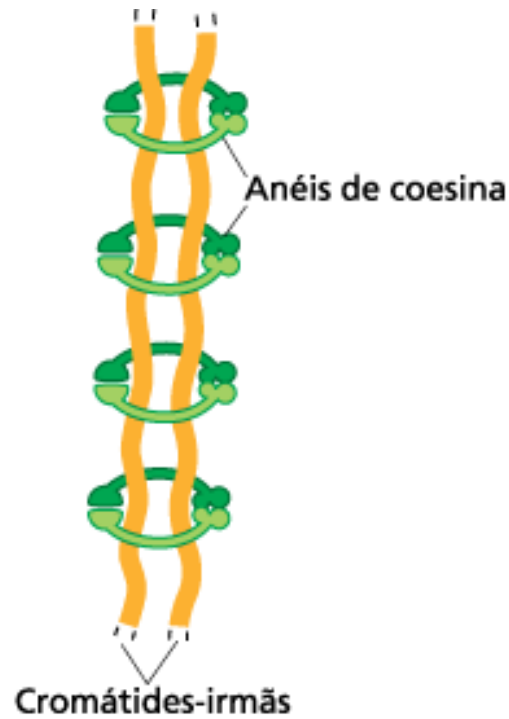
CDK2-ciclina A/ S-CDK:

- dispara replicação do DNA
- impede que a origem seja utilizada novamente

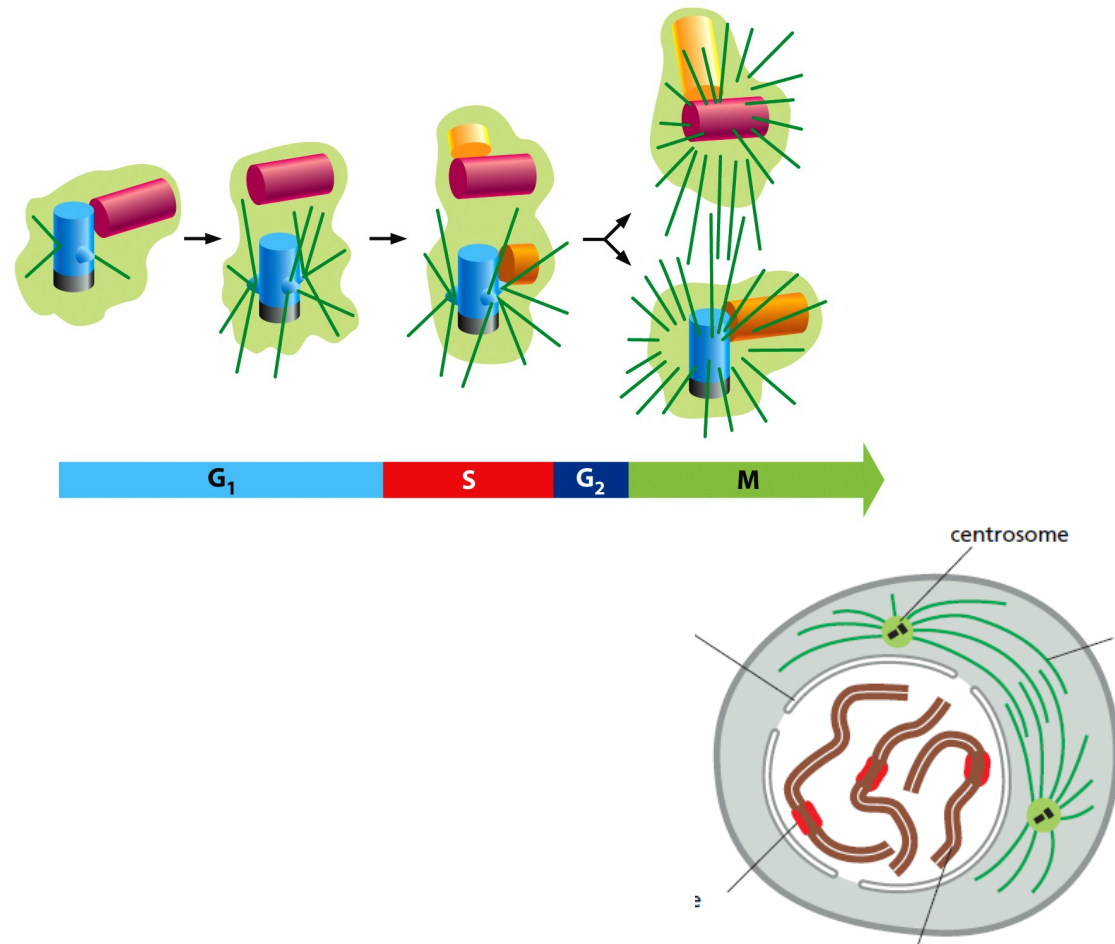
Controle da replicação do DNA

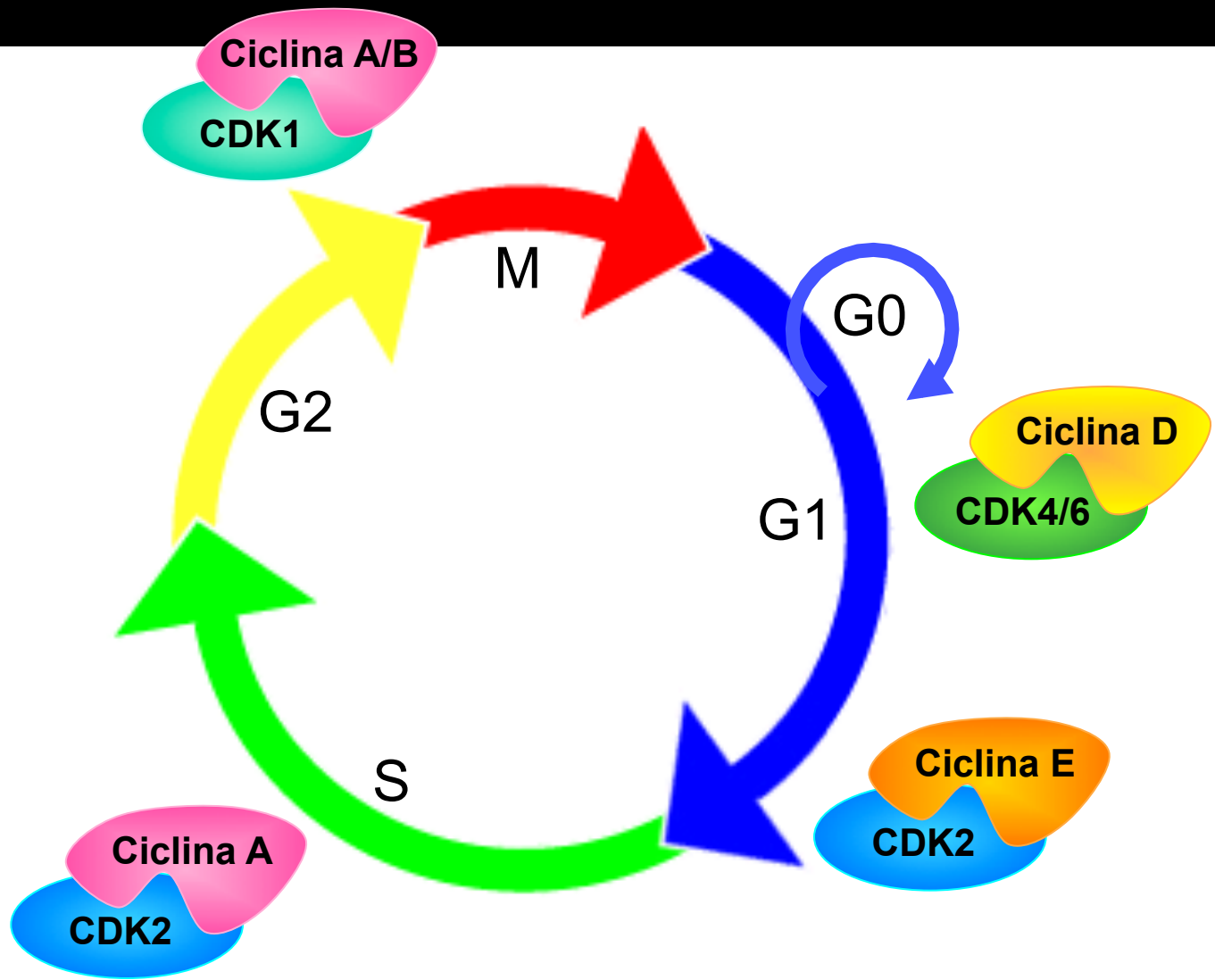
Coesina:

Mantém cromátides-irmãs unidas até o fim da mitose



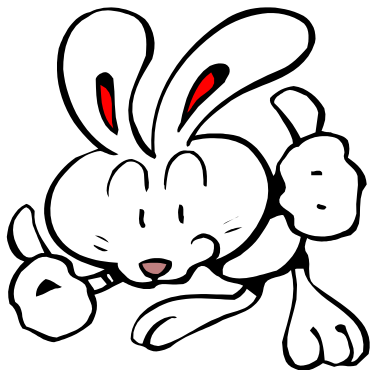
Duplicação dos centrosomos





Ponto de verificação G2/M

- ❖ O tamanho da célula é adequado ?
- ❖ Existe energia suficiente para continuar ?
- ❖ A replicação dos cromossomos foi adequada?
- ❖ Os centrossomos foram duplicados ?



PROGRESSÃO

Ponto de verificação G2/M

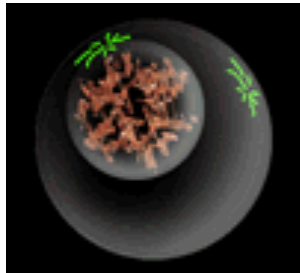
- ❖ O tamanho da célula é adequado ?
- ❖ Existe energia suficiente para continuar ?
- ❖ A replicação dos cromossomos foi adequada?
- ❖ Os centrossomos foram duplicados ?



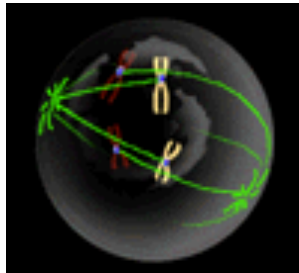
PARADA OU APOPTOSE



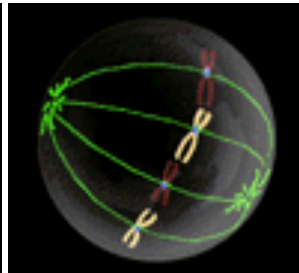
G2



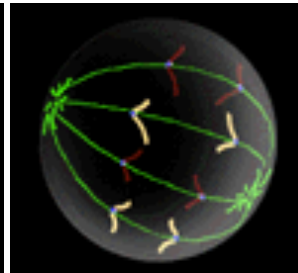
Prófase



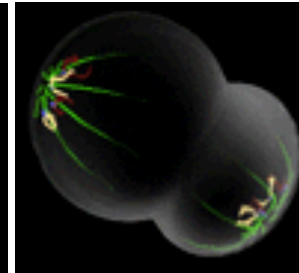
Prometáfase



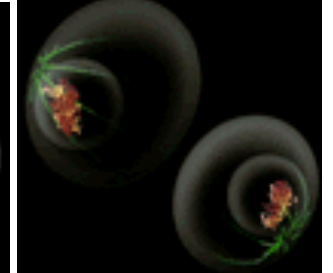
Metáfase



Anáfase



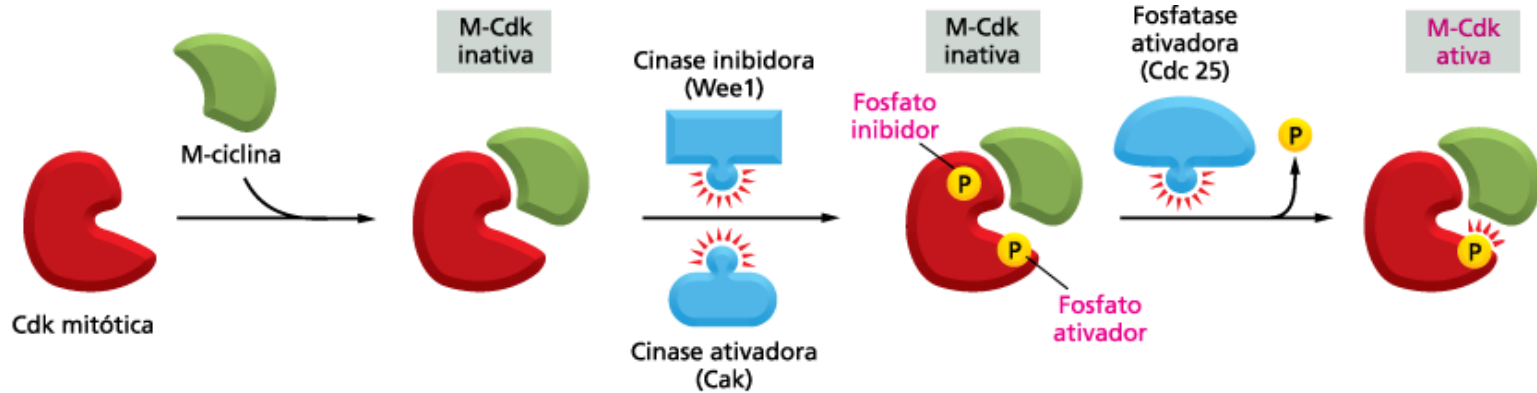
Telófase



Citocinese

MITOSE

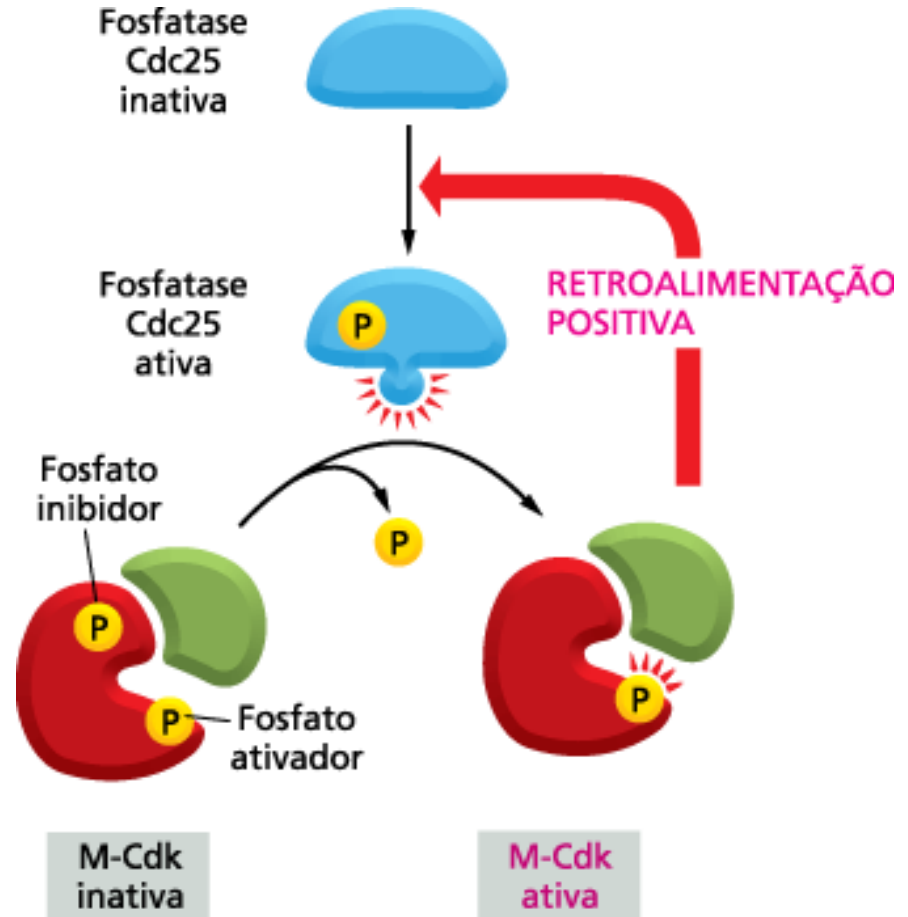
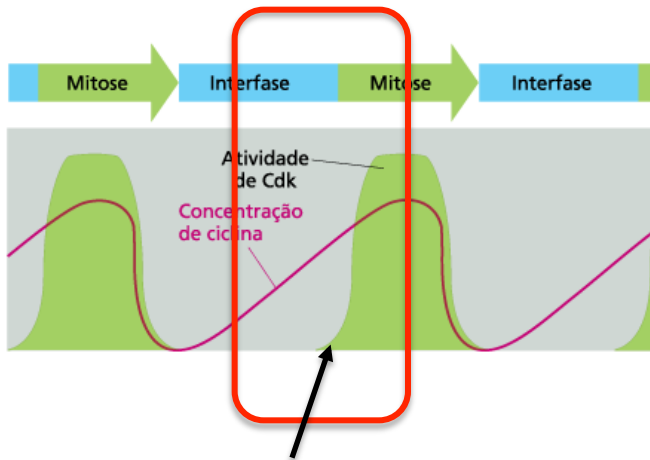
Mitose: ativação de M-CDK



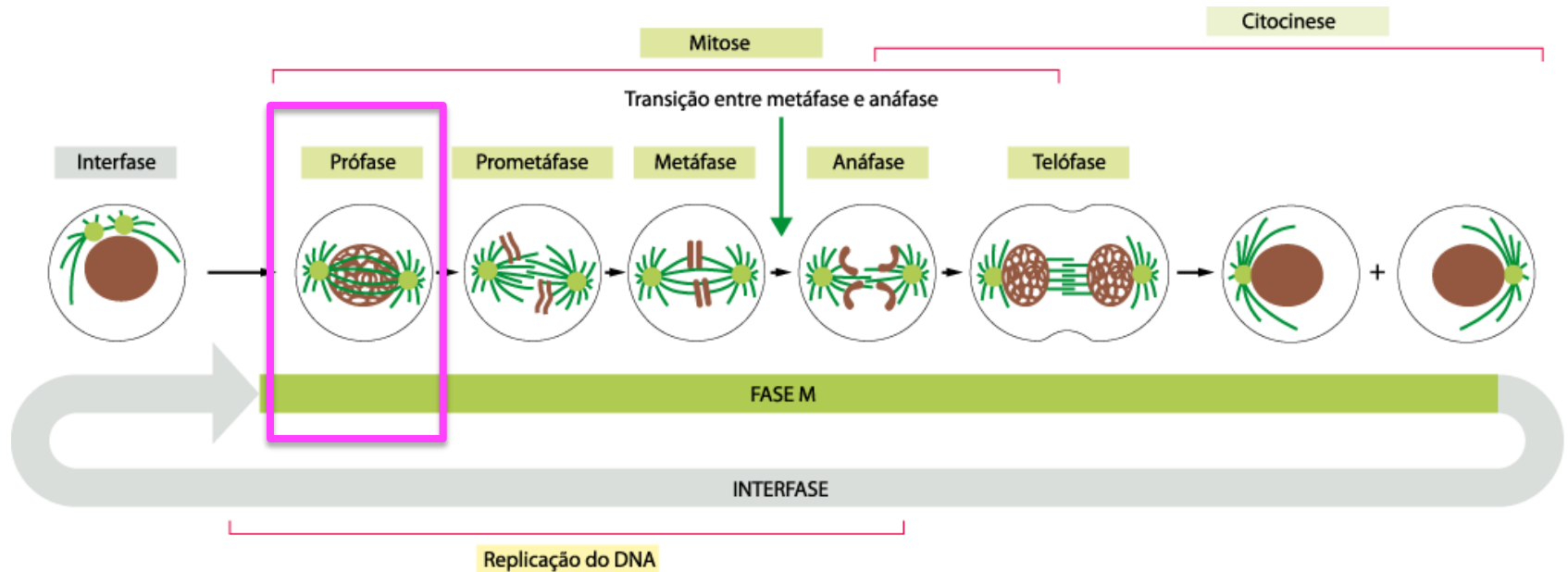
M-Cdk: condensação dos cromossomos e montagem do fuso mitótico

Mitose: ativação de M-CDK

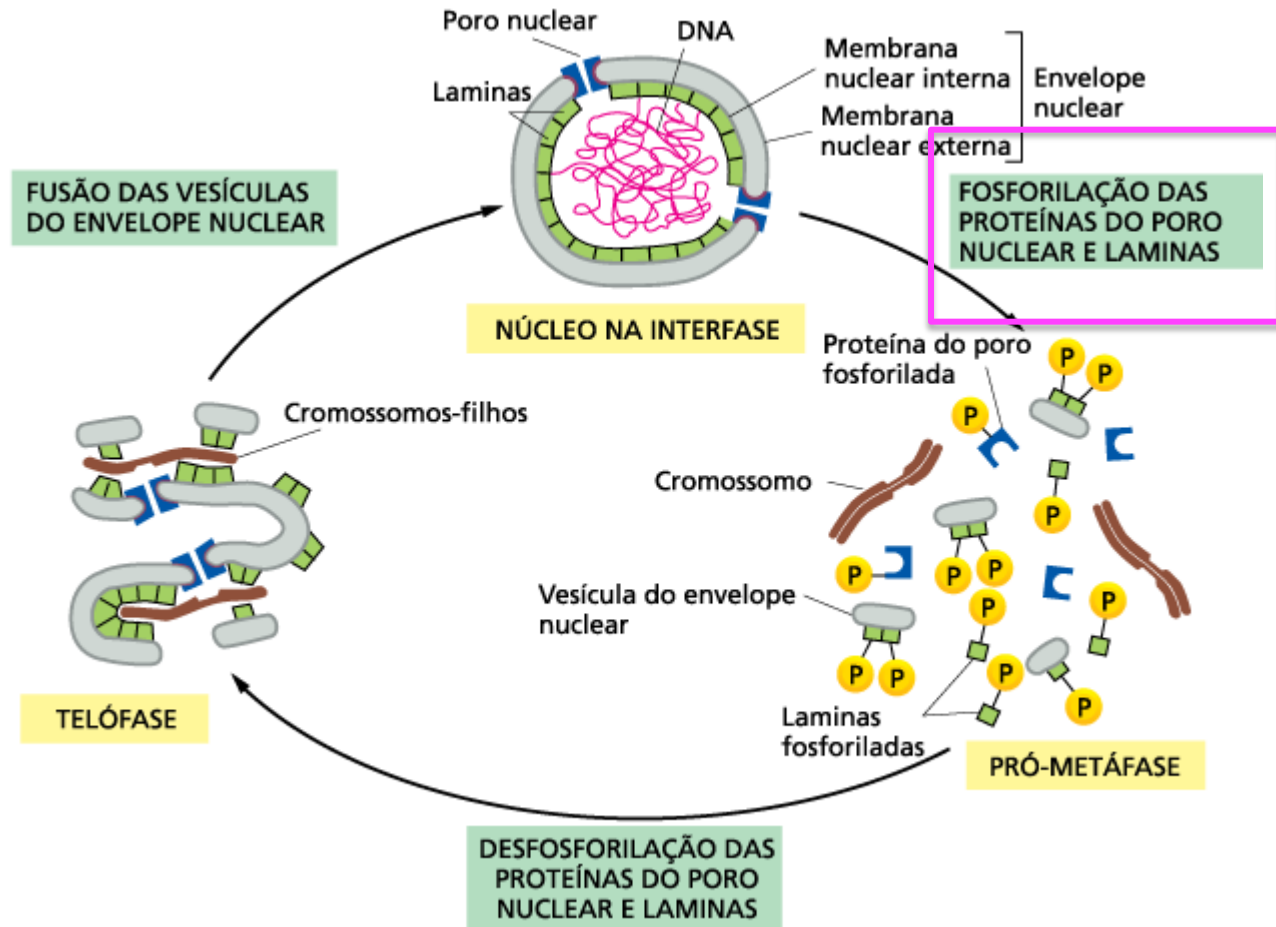
M-Cdk: Retroalimentação positiva de Cdc25



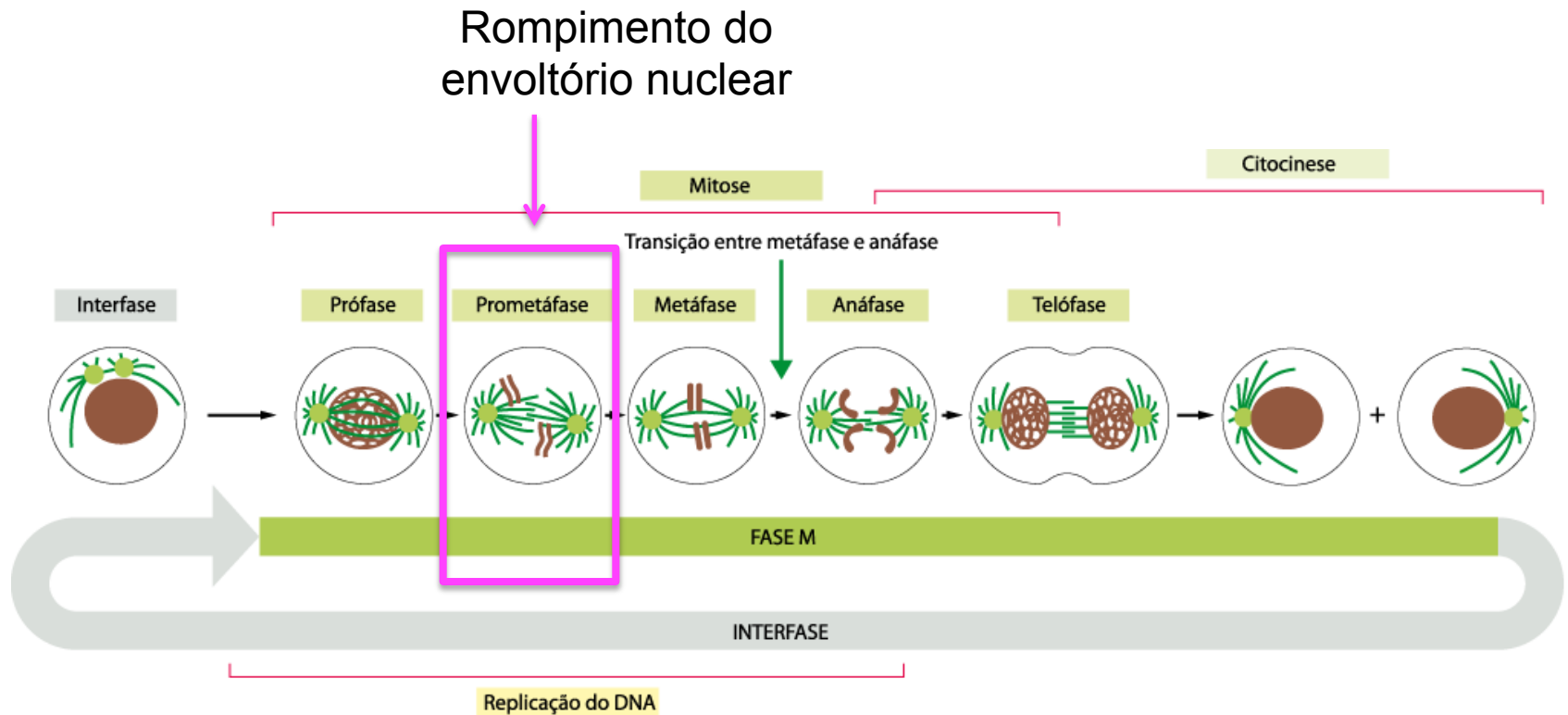
Mitose: ativação de M-CDK



M-CDK: fosforila laminas

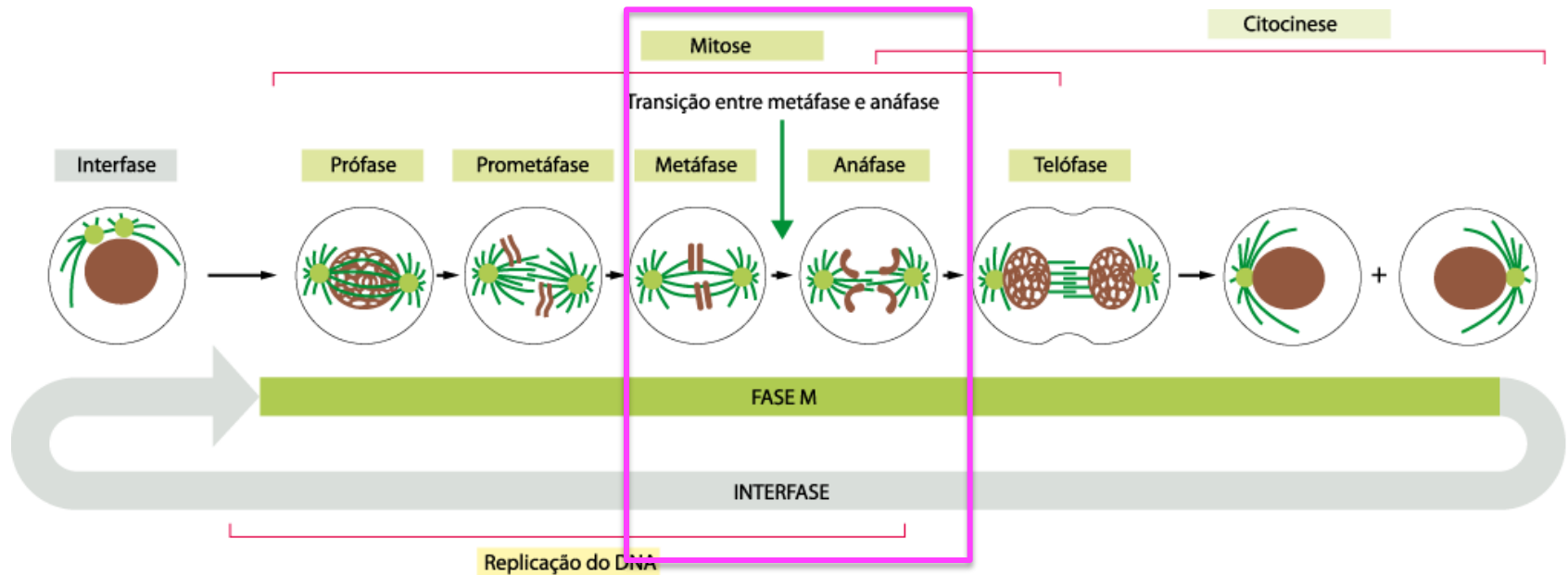


Mitose: ativação de M-CDK



Mitose: ativação de M-CDK

Ponto de verificação M



Todos os cromossomos estão ligados corretamente ao fuso?

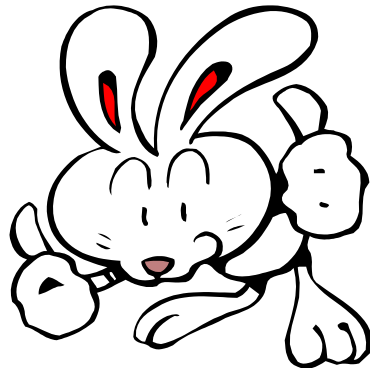
Todos os cromossomos estão ligados corretamente ao fuso?



(A)

SIM

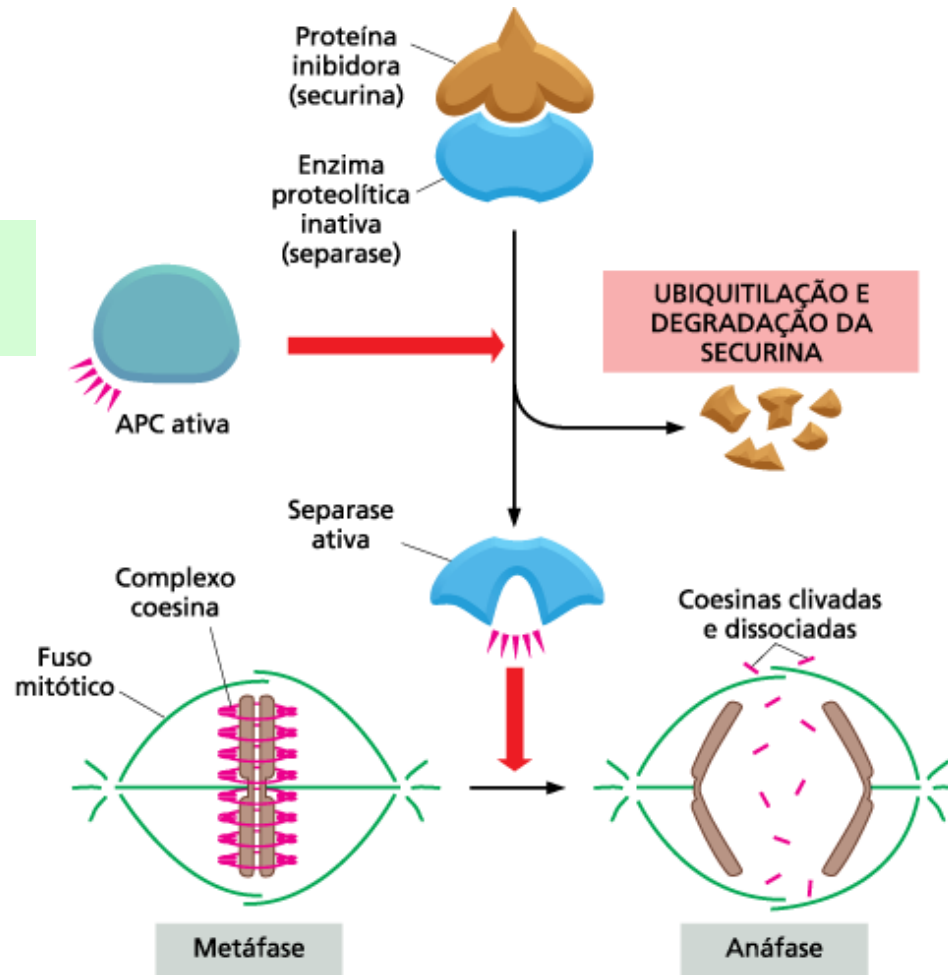
20 μ m



PROGRESSÃO

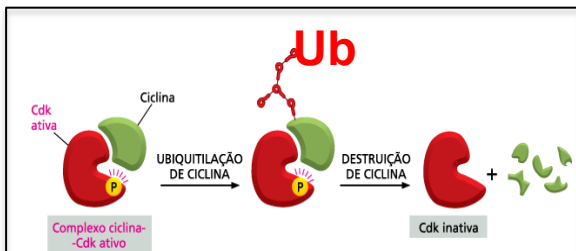
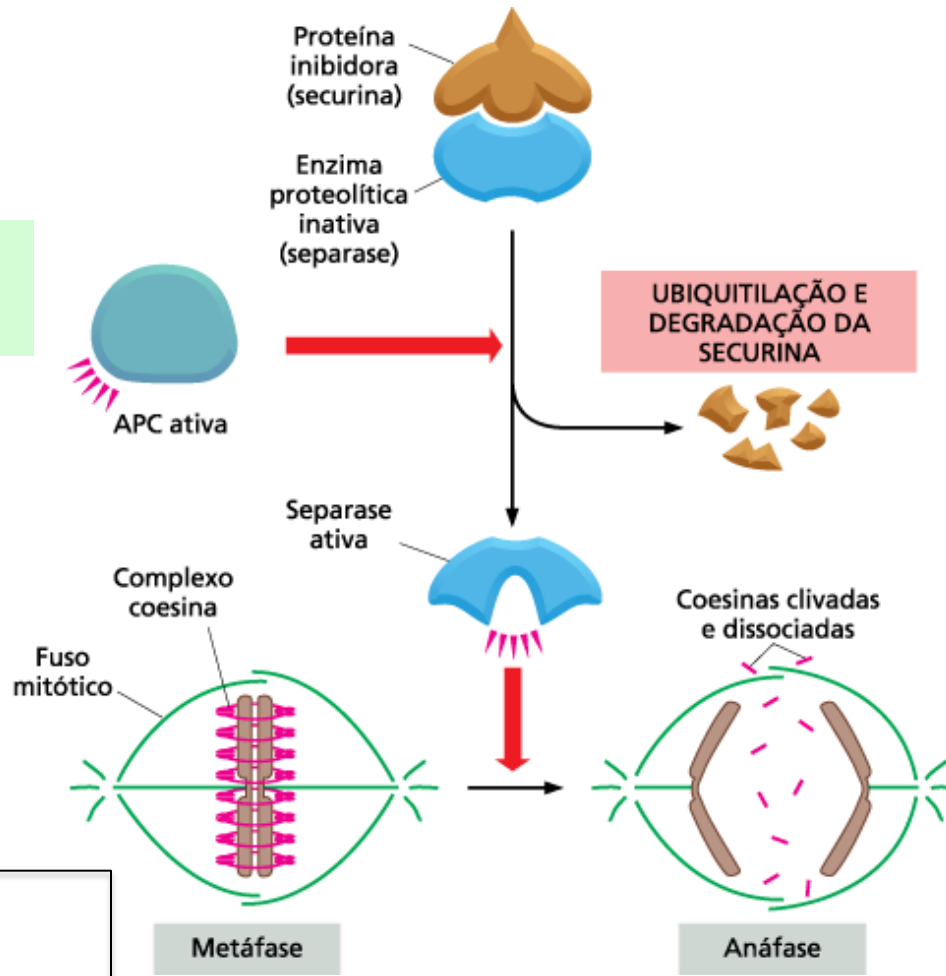
APC: complexo promotor da anáfase

APC/C:
ubiquitina ligase

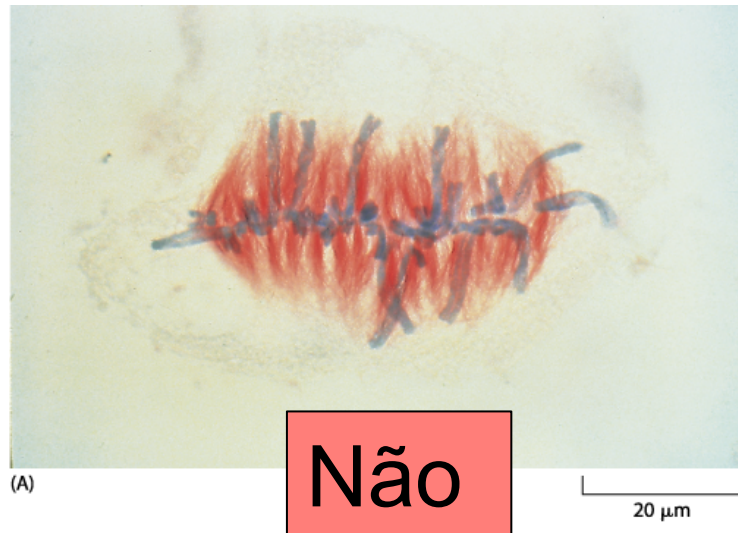


APC: complexo promotor da anáfase

APC/C:
ubiquitina ligase



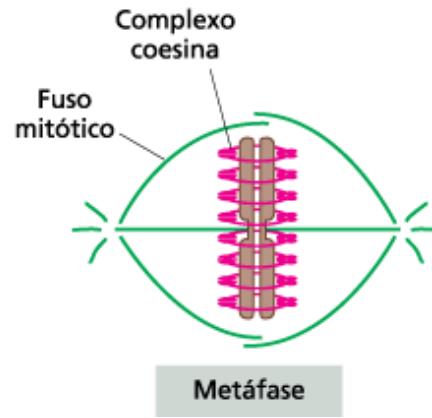
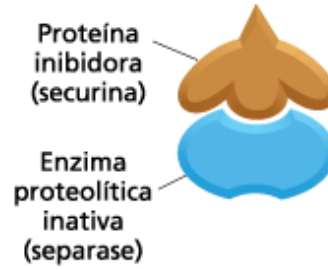
Todos os cromossomos estão ligados corretamente ao fuso?



PARADA OU APOPTOSE

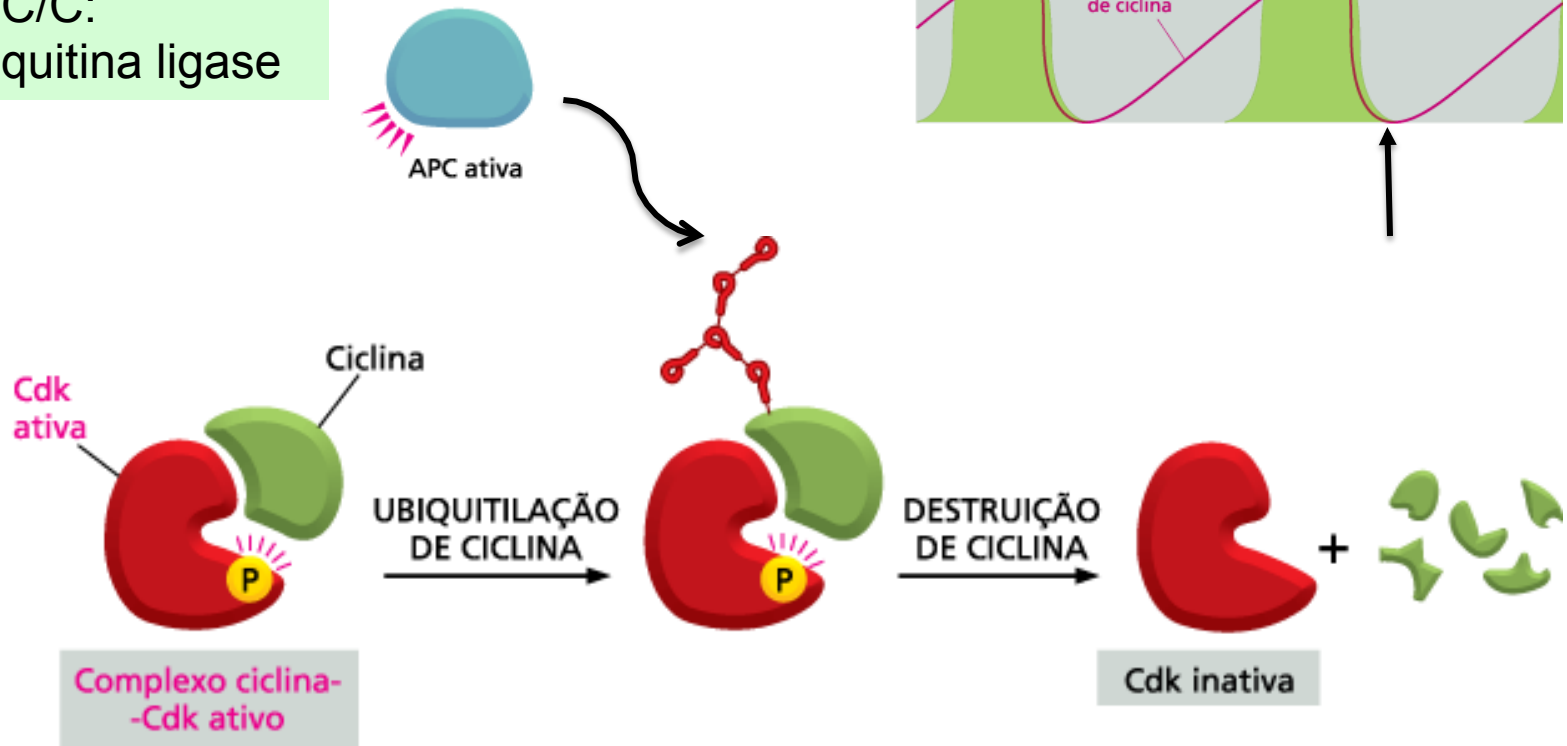
APC: complexo promotor da anáfase

APC/C:
ubiquitina ligase



APC: destruição de M-CDK

APC/C:
ubiquitina ligase



Ciclo Celular e Câncer

(reguladores G1/S)

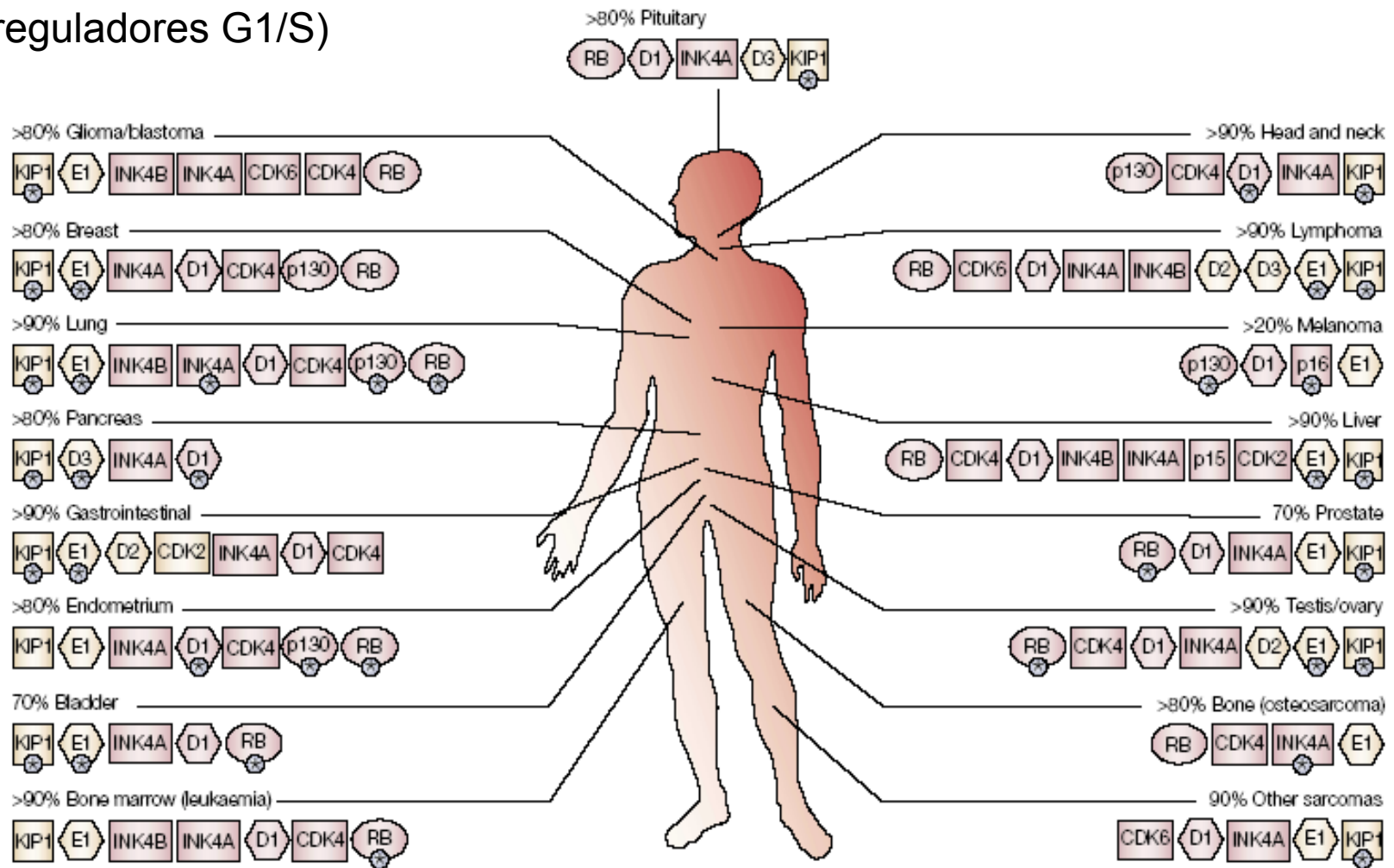


Figure 2 | **Mutation of G1/S regulators in human cancer.** Only alterations that occur in more than 10% of primary tumours have been considered. Numbers represent the percentage of tumours with alterations in any of the listed cell-cycle regulators. The loci in which specific genetic or epigenetic alteration have been defined are in pink. The alterations for which no mechanistic explanation has been provided are in yellow. Alterations relevant for tumour prognosis are indicated by asterisks.

Nesta aula:

- Controle do ciclo celular
- Fatores que interferem no ciclo
- Métodos de estudo

Métodos de estudo do ciclo celular

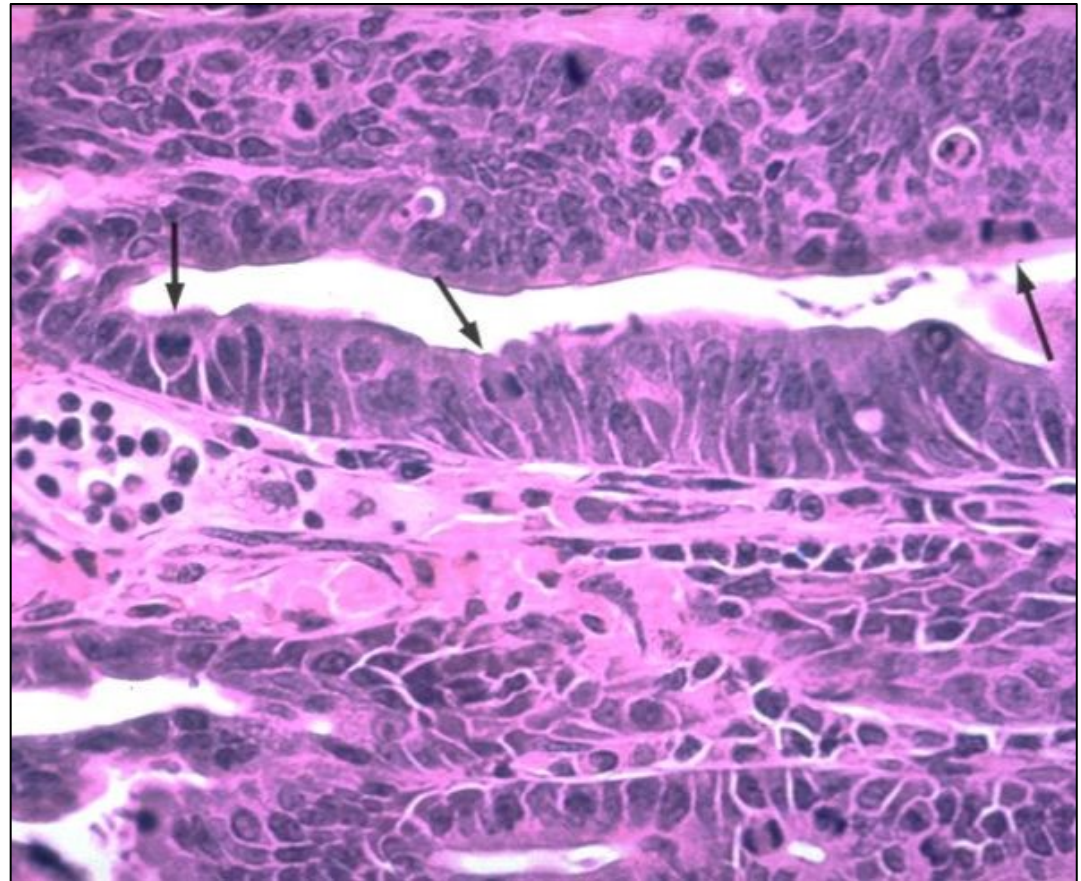
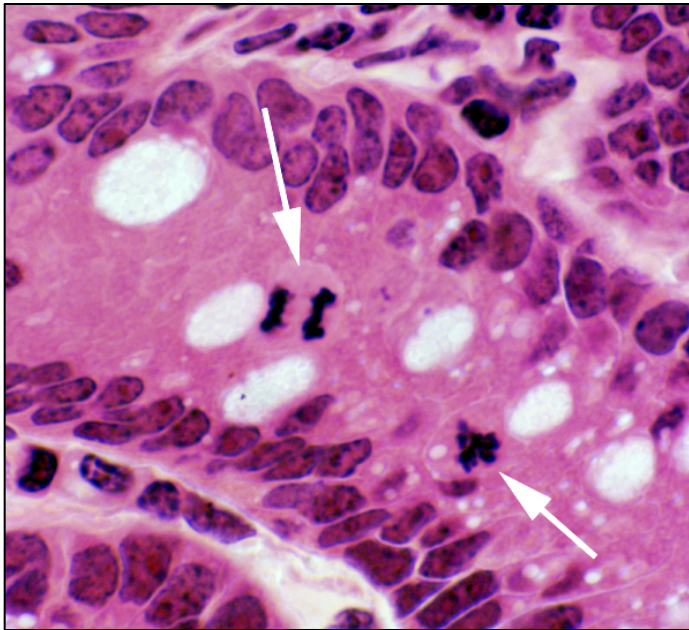
Como determinar a fase do ciclo celular em que uma célula está?

Como estudar uma população de células?



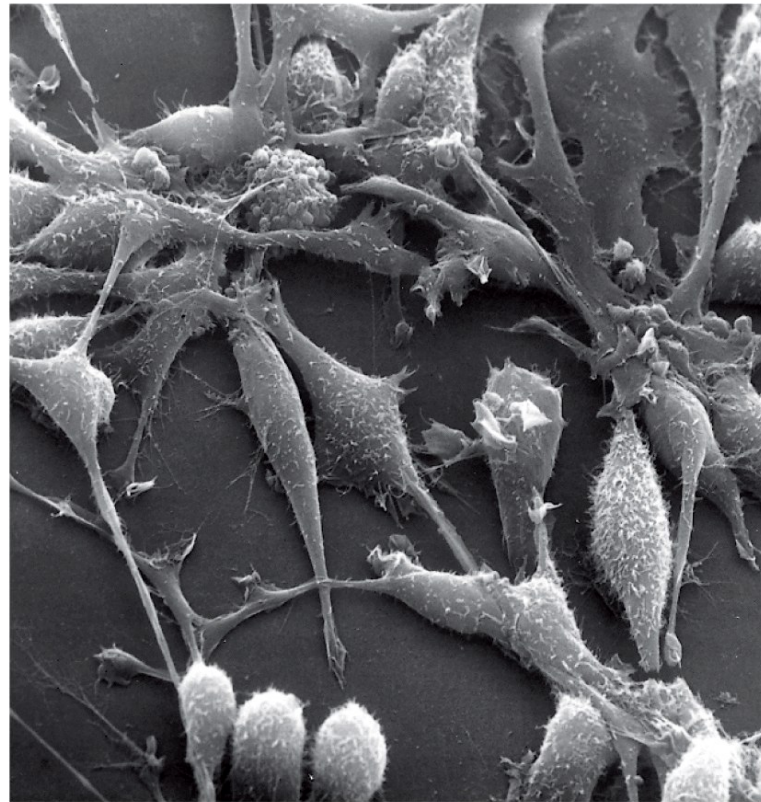
Métodos de estudo do ciclo celular

Microscopia – observação e contagem de células



Métodos de estudo do ciclo celular

Microscopia – observação e contagem de células



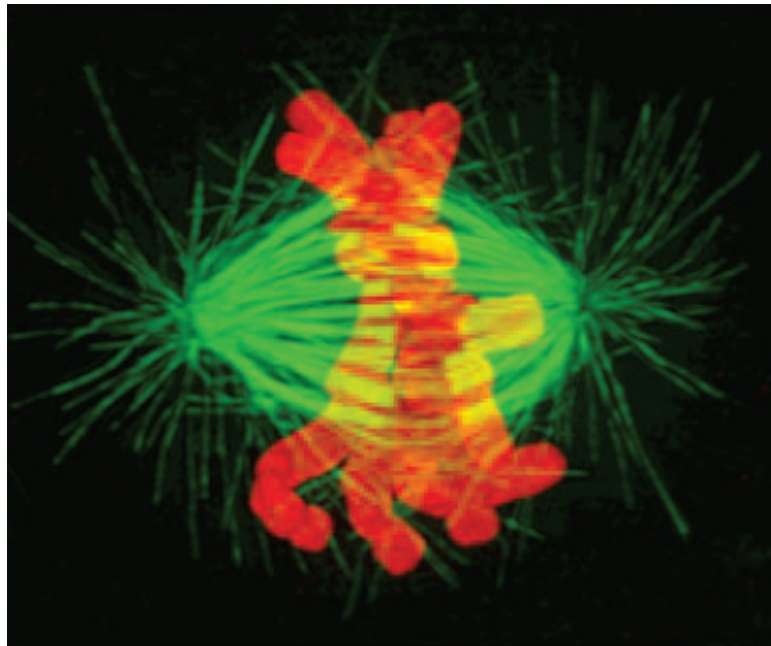
MEV

10 μm

Métodos de estudo do ciclo celular

Microscopia – observação e contagem de células

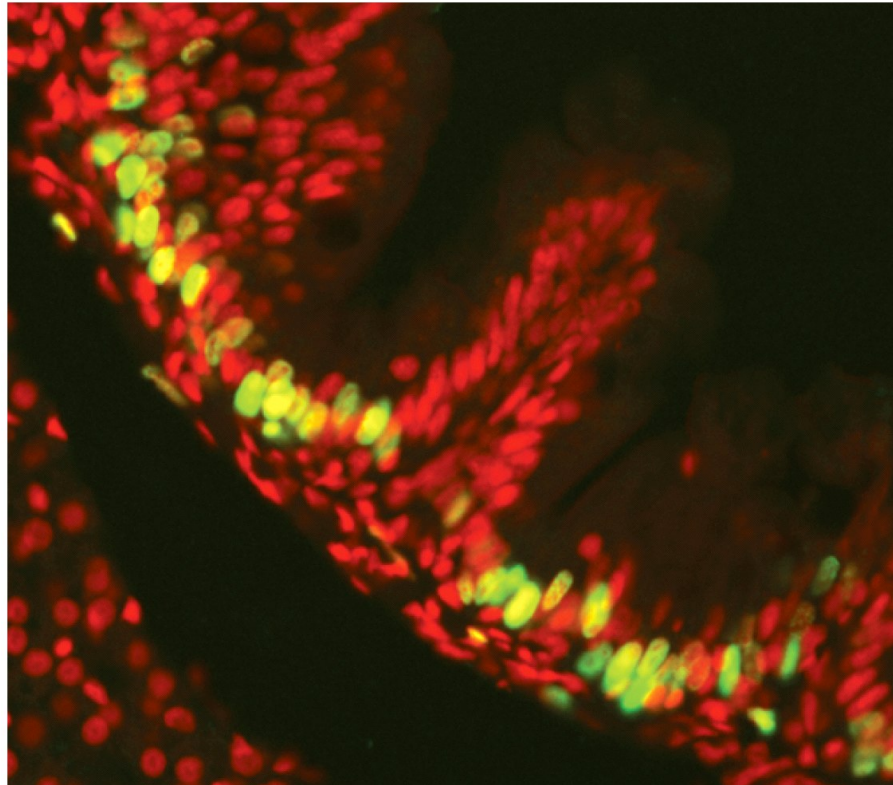
- ✧ Uso de anticorpos que reconhecem citoesqueleto
- ✧ Uso de agentes que marcam DNA (para visualizar mitose)



Métodos de estudo do ciclo celular

Microscopia – observação e contagem de células

- ✧ Uso de agentes que podem incorporar-se ao DNA (fase S) – **BrDU** (deoxi-uridina modificada com bromo)

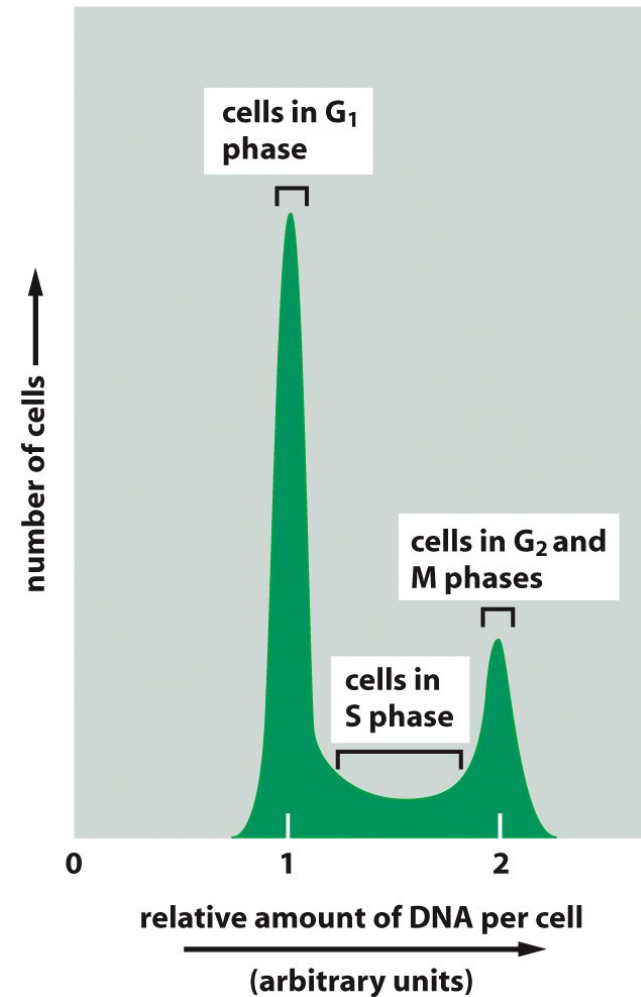


Anti-BrdU: verde
Células: vermelho

Métodos de estudo do ciclo celular

Citometria de fluxo (FACS)

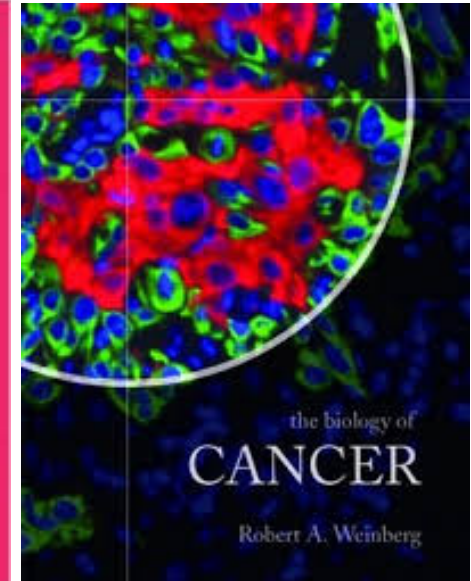
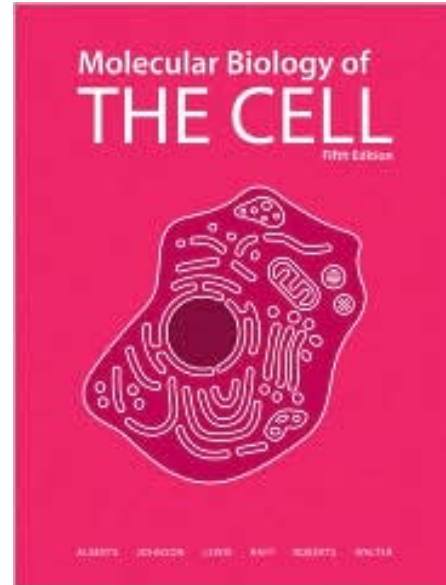
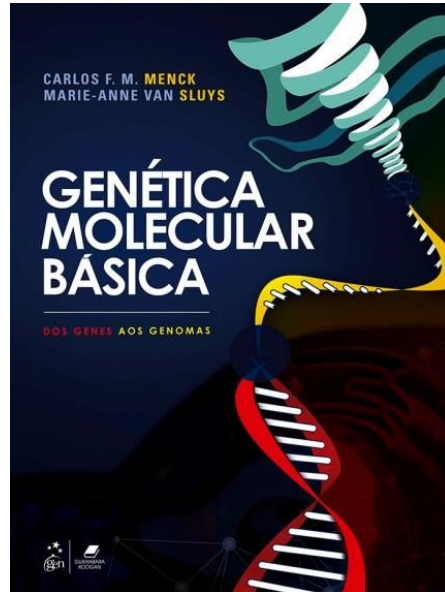
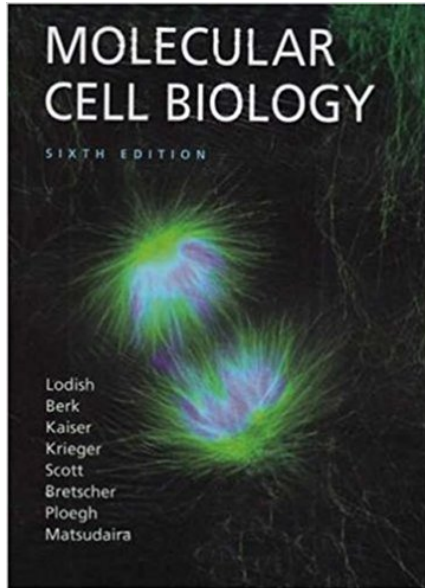
Agente fluorescente ligado ao DNA:
permite inferir a fase do ciclo



Resumo

- Ciclo celular: duplicar o DNA dos cromossomos e as organelas e distribuir esse material igualmente entre as células-filhas
- Pontos de verificação ao longo do ciclo – a passagem de G1 para S compromete a célula com o ciclo
- Controle do ciclo celular: ciclinas-Cdk/ CKI's
- Saída de G_0 : estímulos
- Fosforilação da proteína Rb – fator de transcrição E2F
- Parada em G1 – regulação por p53 e p21
- Métodos de estudo de ciclo celular: marcação com anticorpos/ contagem de células

Para saber mais



Aula:

www.coltri.bio.br/disciplina.html

