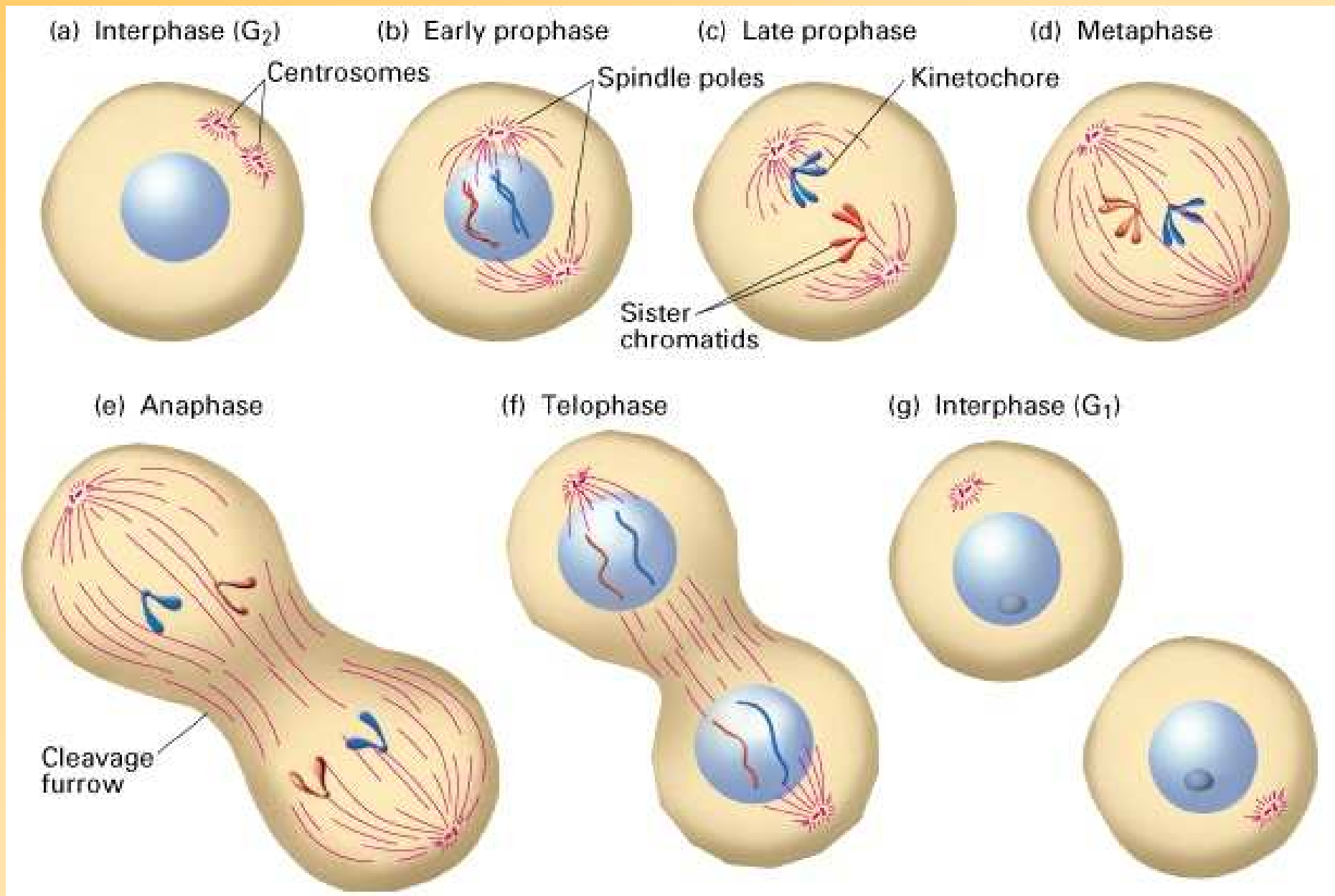
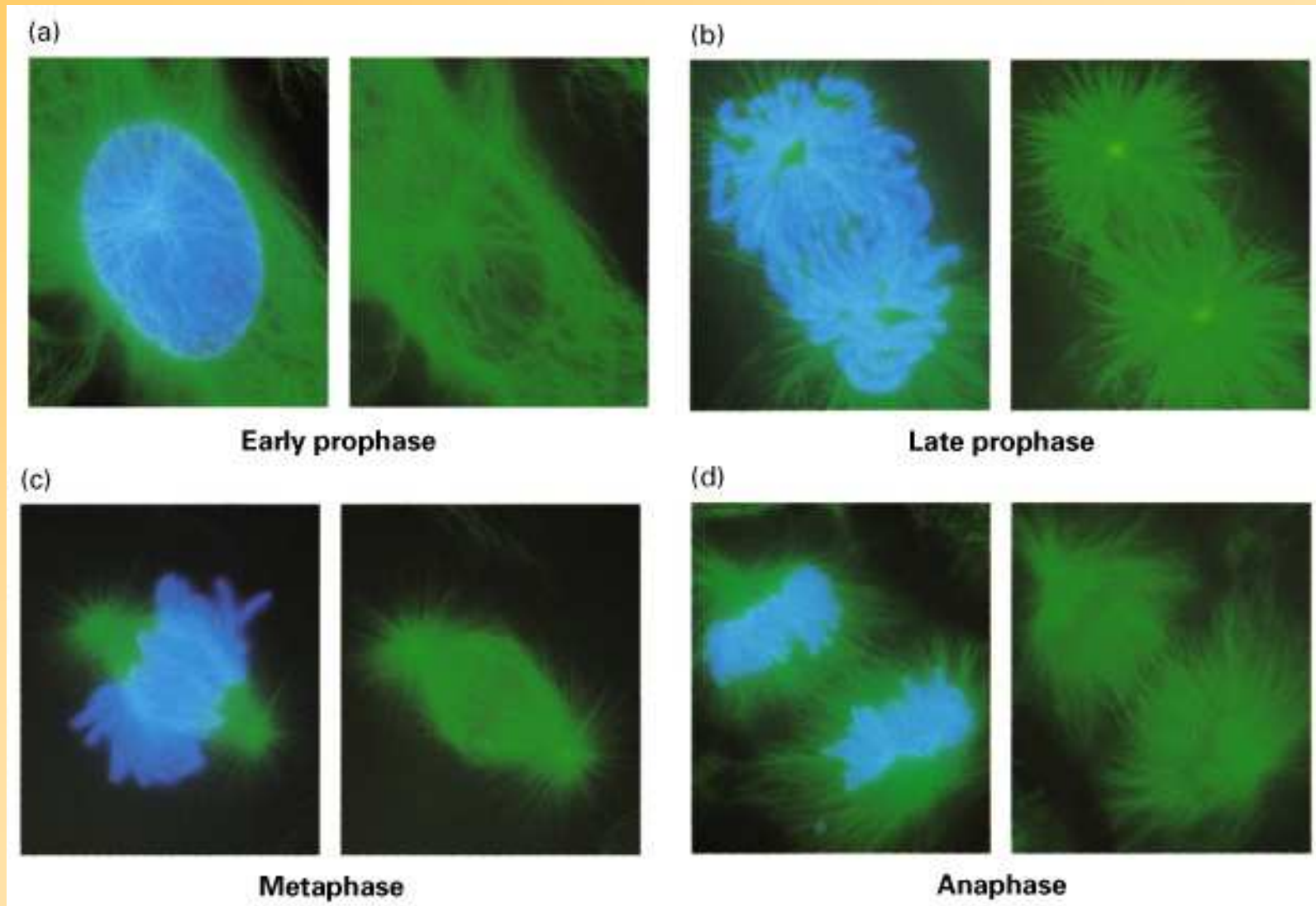


# **Buněčný cyklus - principy regulace buněčného růstu a buněčného dělení**

# Mitóza



# Průběh mitózy v buněčné kultuře fibroblastů



# Buněčný cyklus

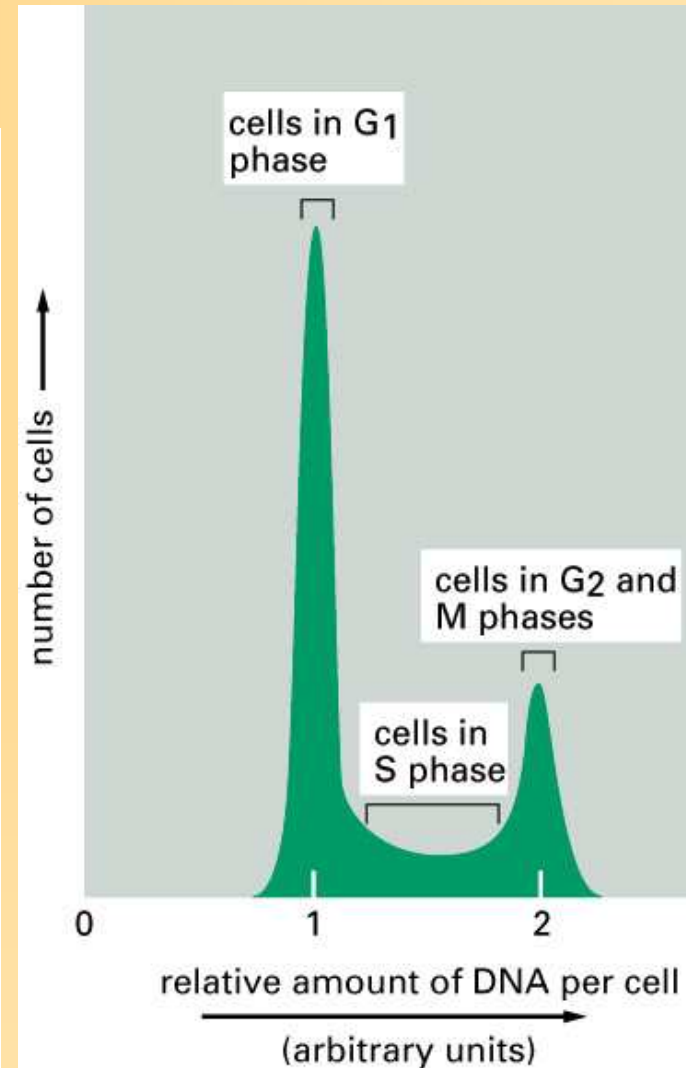
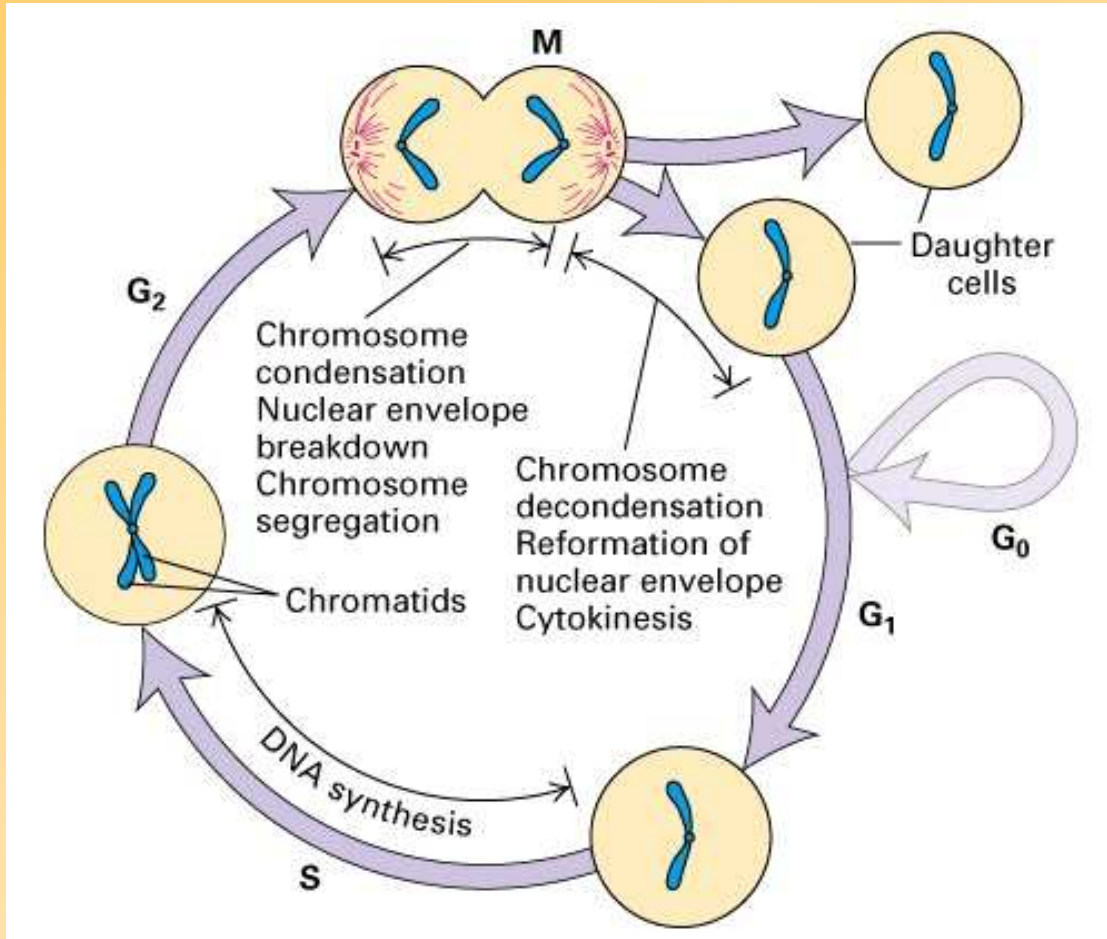
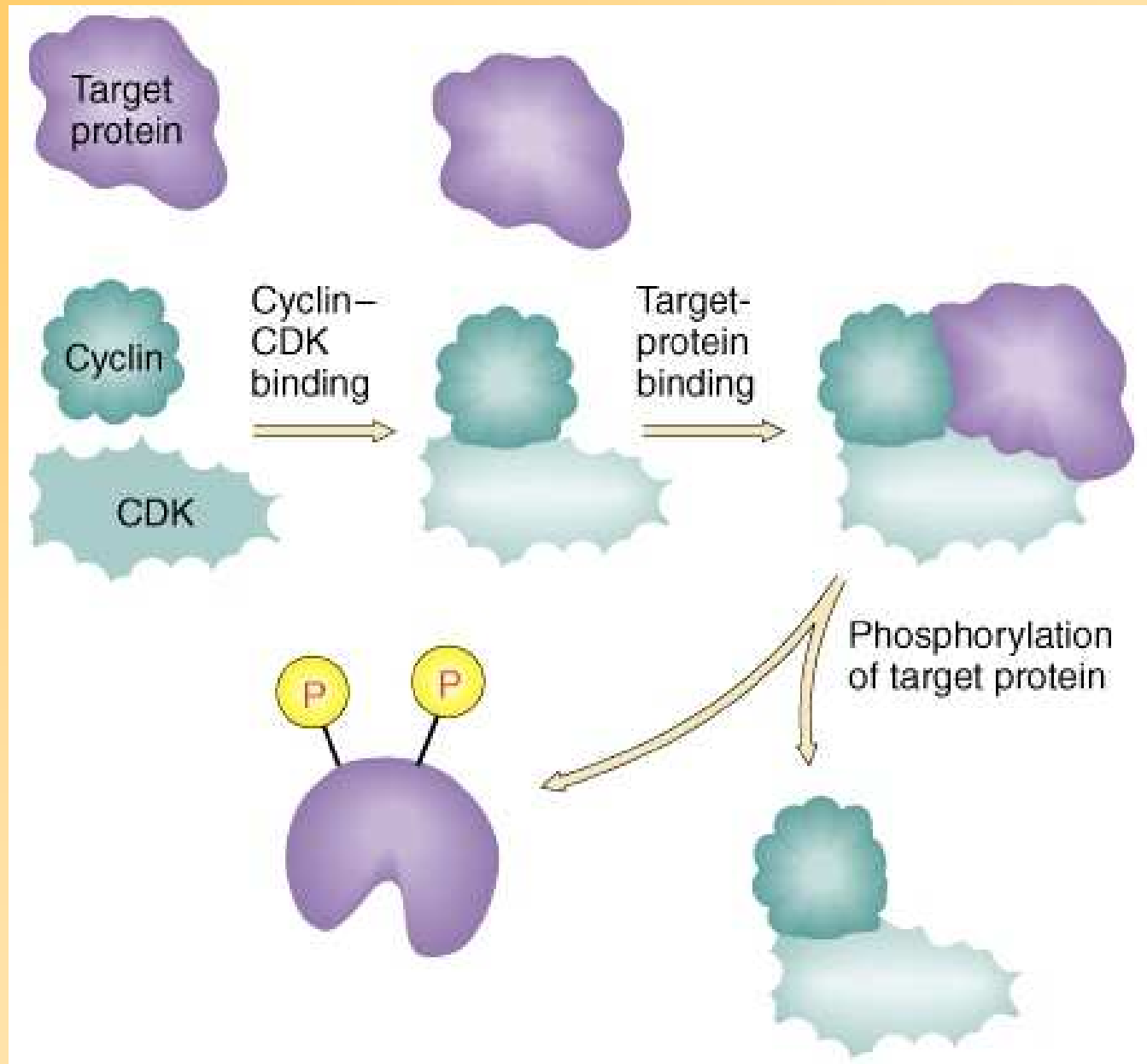


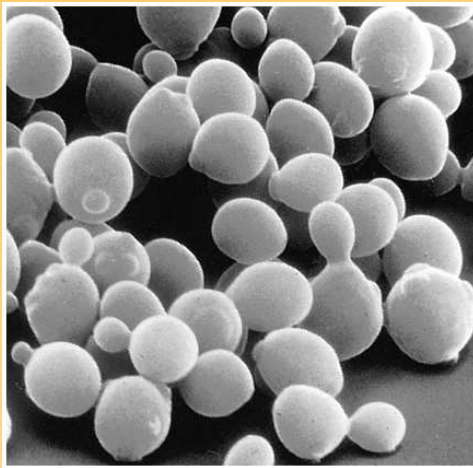
Figure 17-12. Molecular Biology of the Cell, 4th Edition.

# Kinázy závislé na cyklinech kontrolují buněčný cyklus

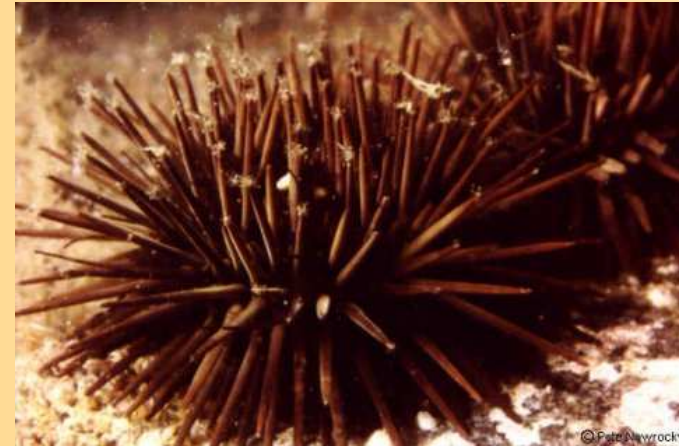


**Kontrola vstupu do mitózy**  
**- výsledek využití různých buněčných modelů**

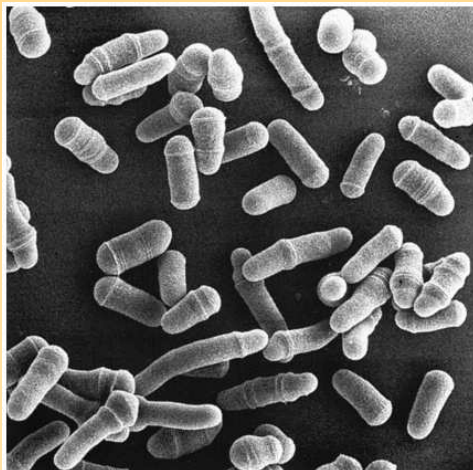
# Experimental Systems Important for Cell Cycle Studies



*Saccharomyces cerevisiae*



*Arbacia punctulata*

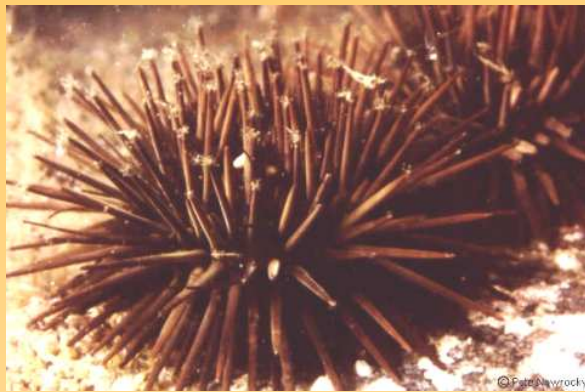


*Schizosaccharomyces pombe*

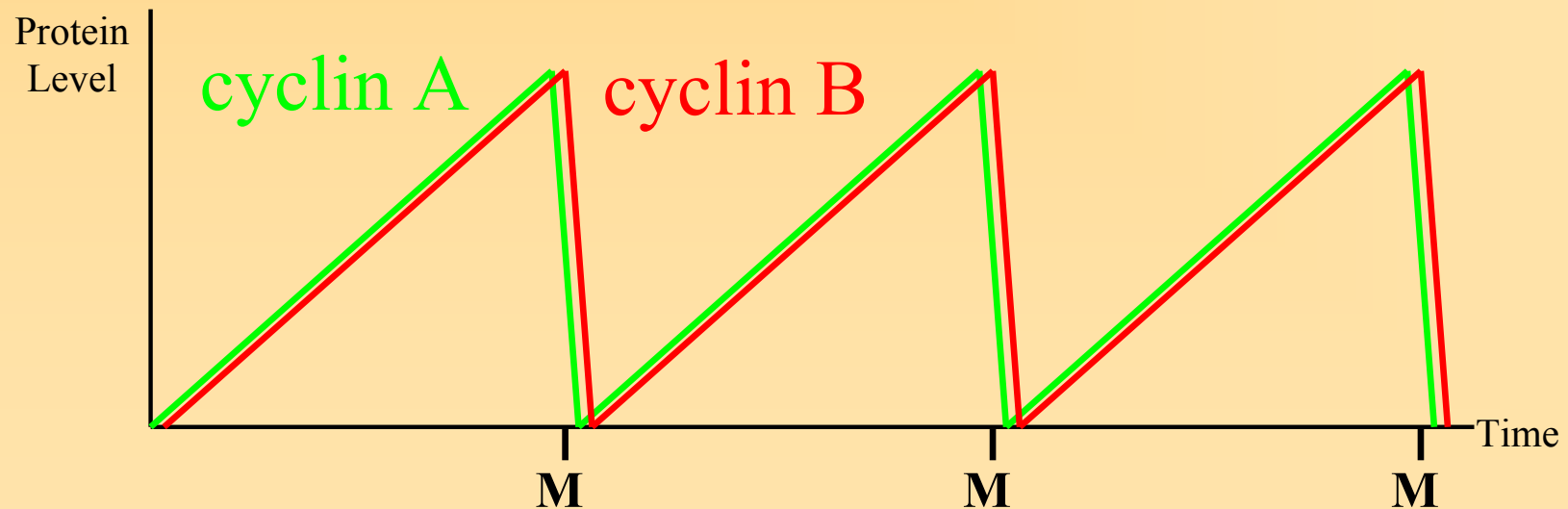
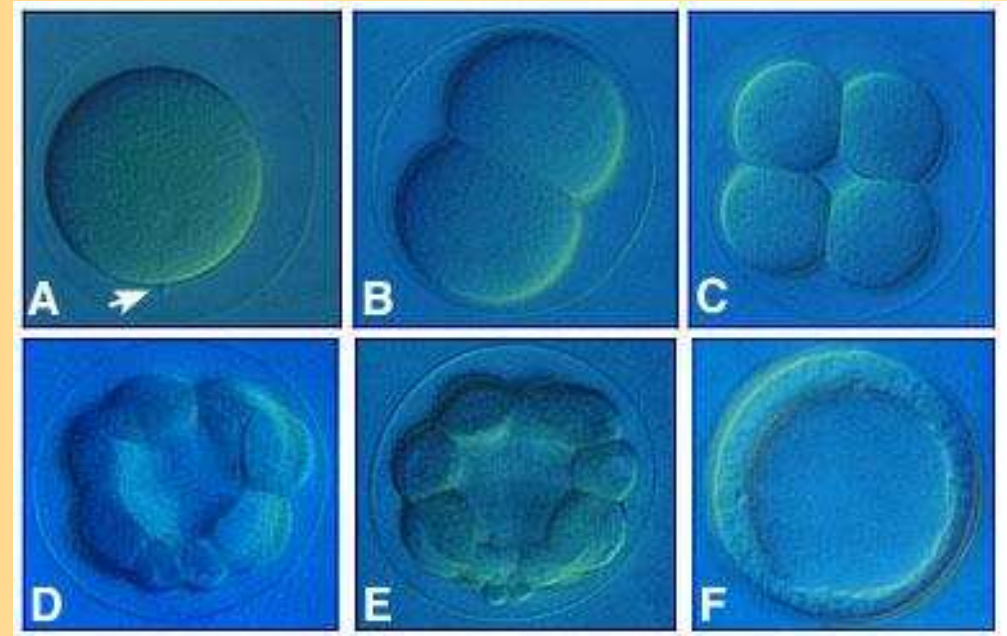


*Xenopus laevis*

# Cyclin was Discovered in Sea Urchin Embryos



can stimulate to  
lay lots of eggs



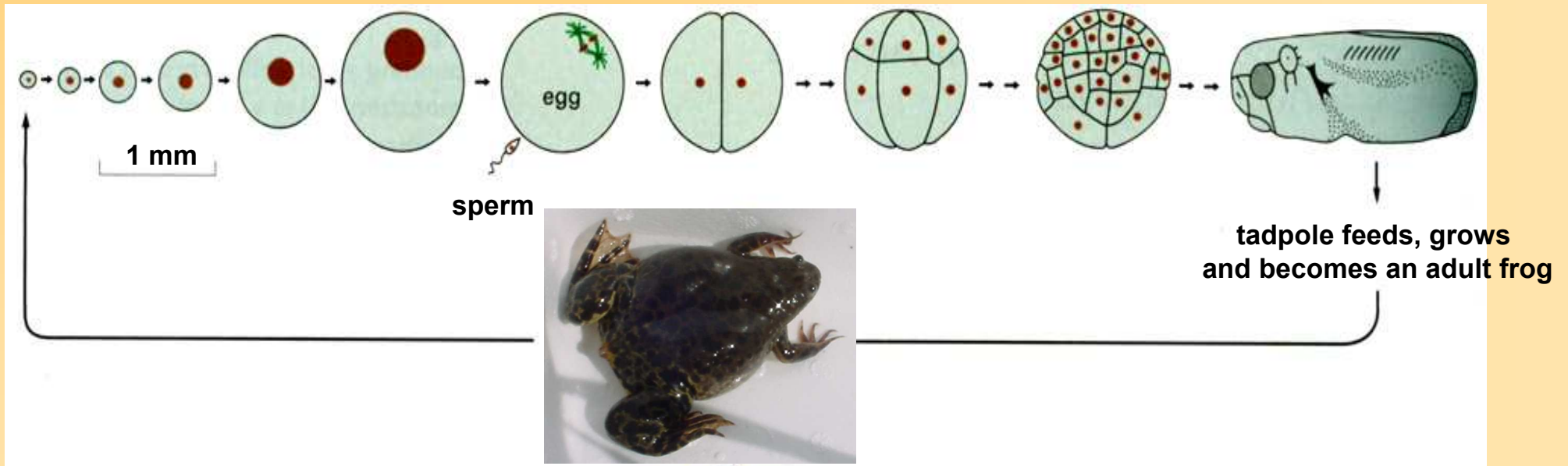


# Frog life cycle

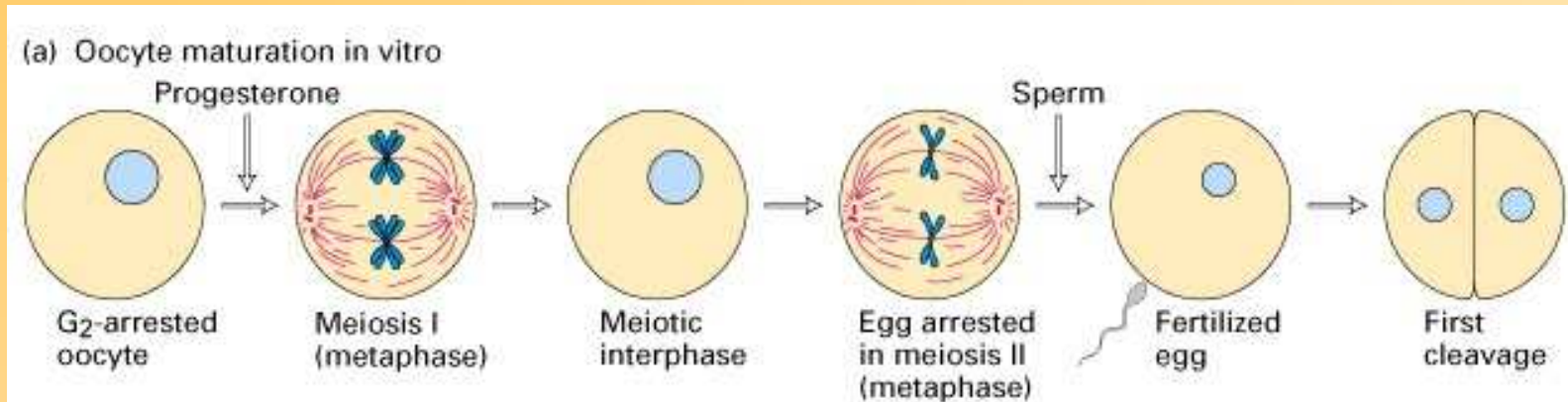
OOCYTE GROWS WITHOUT DIVIDING  
(MONTHS)

FERTILIZATION

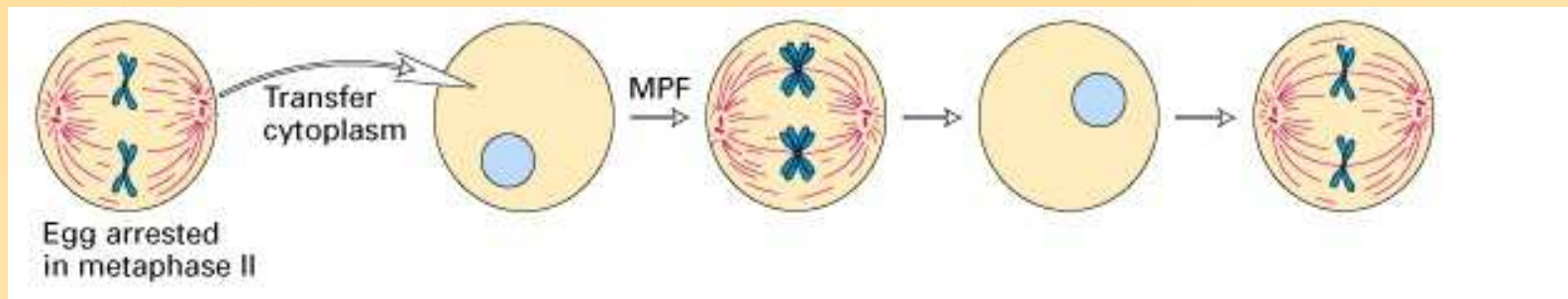
FERTILIZED EGG DIVIDES WITHOUT GROWING  
(HOURS)



# The Maturation of Frog Eggs

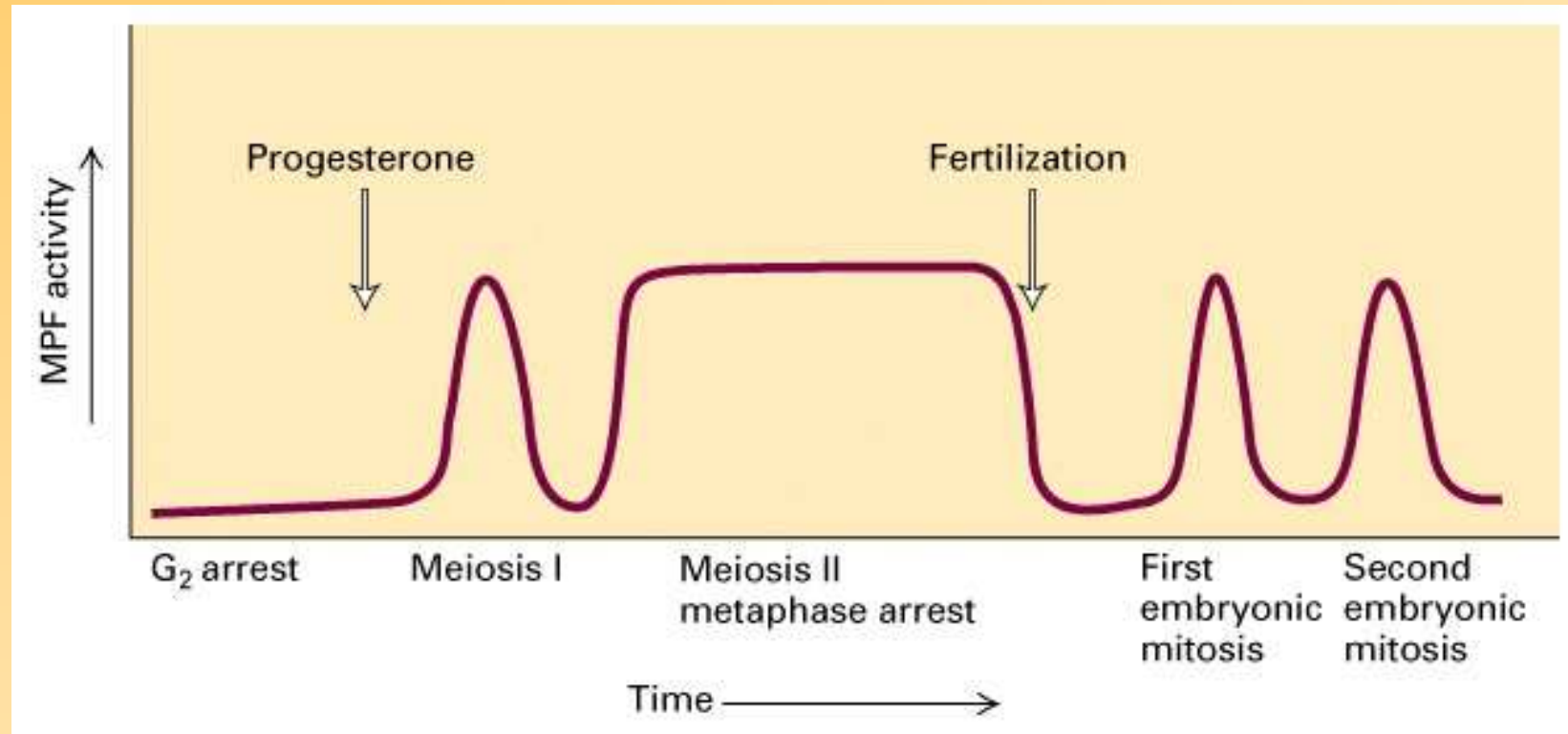


## An Assay for Maturation Promoting Factor (MPF)



Yoshio Masui, 1971

# MPF Activity Peaks Before Each Cell Division



Moreover, MPF has kinase activity

*Proc. Natl. Acad. Sci. USA*  
Vol. 85, pp. 3009–3013, May 1988  
Cell Biology

## **Purification of maturation-promoting factor, an intracellular regulator of early mitotic events**

(cell cycle/mitosis/protein phosphorylation)

MANFRED J. LOHKA\*, MARIANNE K. HAYES†, AND JAMES L. MALLER

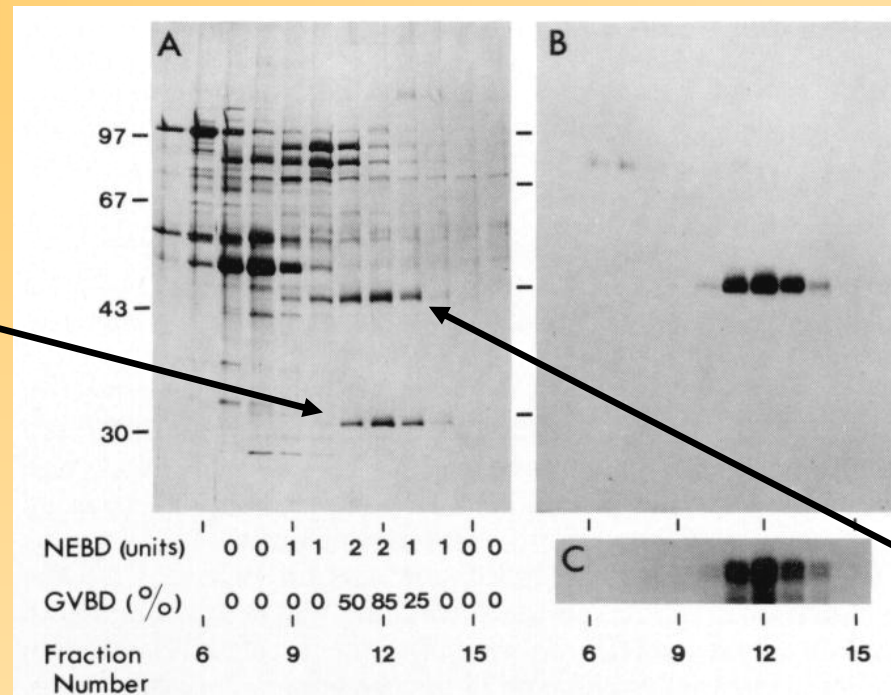
Department of Pharmacology, University of Colorado School of Medicine, Denver, CO 80262

*Communicated by Raymond L. Erikson, December 22, 1987 (received for review October 10, 1987)*

**ABSTRACT** Maturation-promoting factor causes germinal vesicle breakdown when injected into *Xenopus* oocytes and can induce metaphase in a cell-free system. The cell-free assay was used to monitor maturation-promoting factor during its purification from unfertilized *Xenopus* eggs. Ammonium sulfate precipitation and six chromatographic procedures resulted in a preparation purified >3000-fold that could induce germinal vesicle breakdown within 2 hr when injected into cycloheximide-treated oocytes. Proteins of 45 kDa and 32 kDa were correlated with fractions of highest activity in both assays. These fractions contained a protein kinase activity able to phosphorylate the endogenous 45-kDa protein, as well as histone H1, phosphatase inhibitor 1, and casein. The highly purified preparations described here should help to identify the mechanism of action of maturation-promoting factor and to elucidate the role of protein kinases in the induction of metaphase.

## Purification of MPF: The Birth of Cyclin Dependent Kinases

This is *cdc2*<sup>+</sup>!!  
(Cdc28 in  
*S. cerevisiae*)

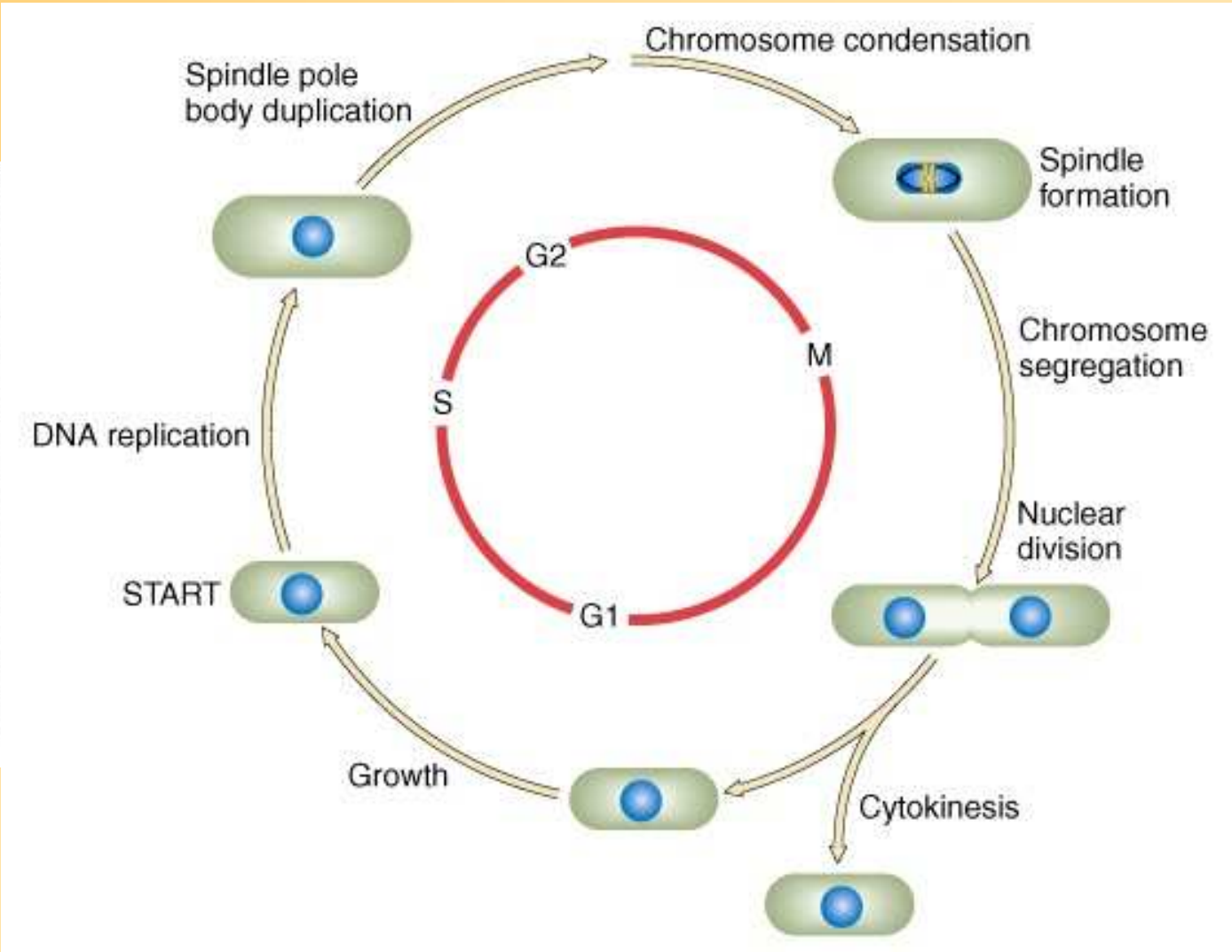
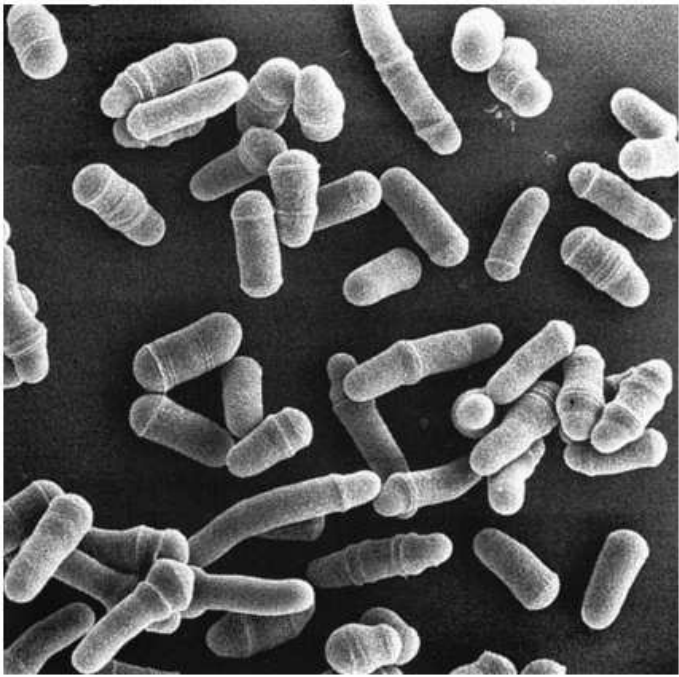


This is cyclin!!

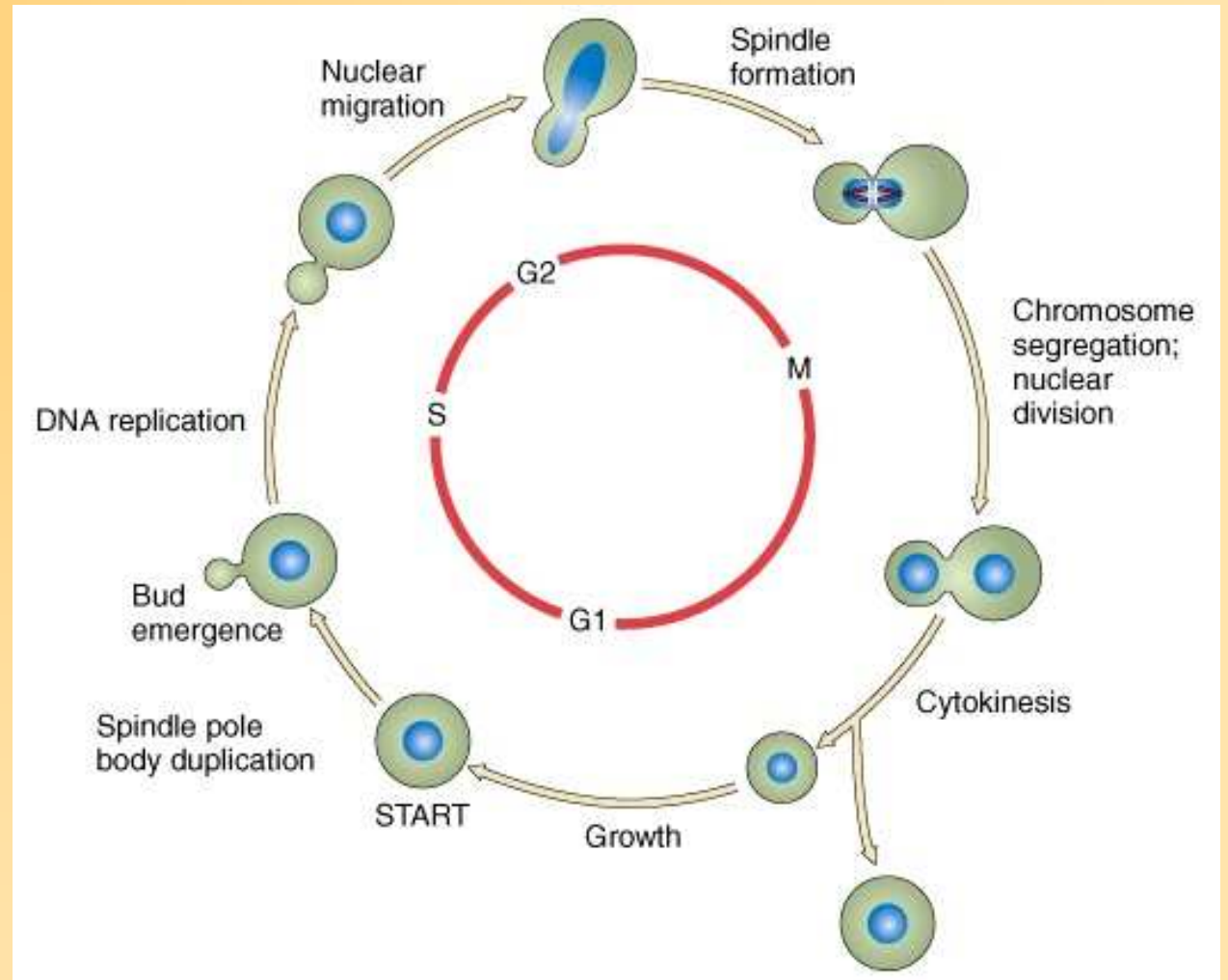
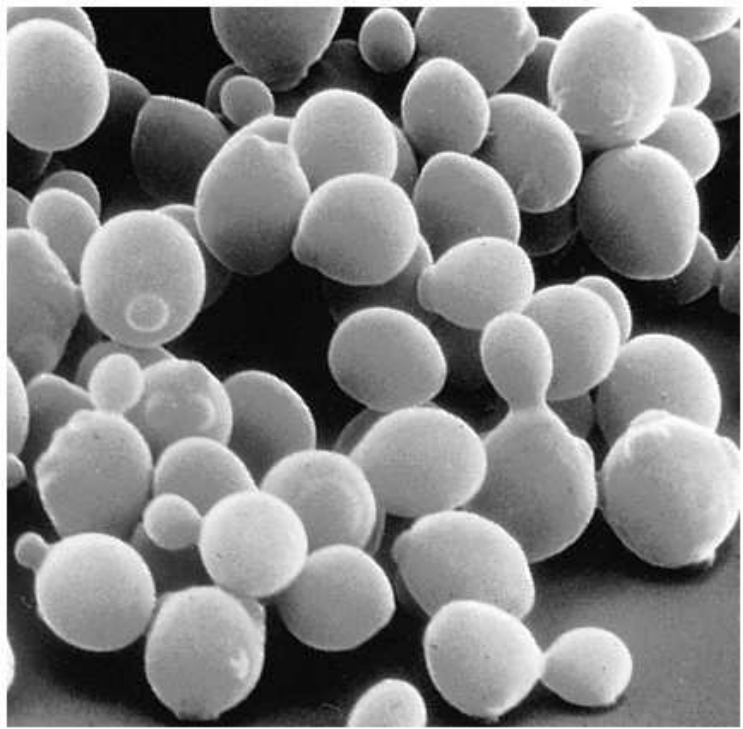
Which = *cdc13*<sup>+</sup>  
in *S. pombe*

FIG. 2. Polyacrylamide gel analysis of fractions eluting from the Mono S column. A 45- $\mu$ l aliquot of fractions 5-16 was incubated with [ $\gamma$ -<sup>32</sup>P]ATP and electrophoresed through a 10% NaDodSO<sub>4</sub>/polyacrylamide gel. (A) Silver-stained polyacrylamide gel of purified MPF. The activity of the fractions in the cell-free assay (NEBD) and in the oocyte microinjection assay (GVBD) is shown below the gel. NEBD is expressed in units/50  $\mu$ l. GVBD is expressed as the percentage of oocytes that underwent GVBD during a 2-hr incubation in cycloheximide (0.5  $\mu$ g/ml). (B) Autoradiograph of the silver-stained gel shown in A. (C) H1 kinase activity of purified MPF. Mono S fractions 6-15 were assayed for H1 kinase activity. The autoradiograph of the region of the gel with histone 1 is shown. Fraction 12 had a specific activity of  $\approx 270$  nmol $\cdot$ min<sup>-1</sup> $\cdot$ mg<sup>-1</sup>.

# Fission yeast: *Schizosaccharomyces pombe*

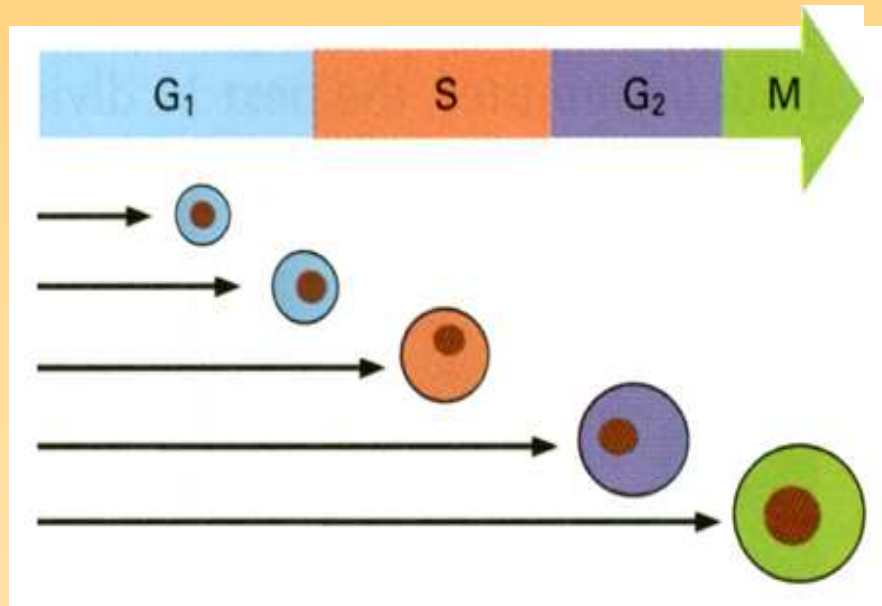


# Budding Yeast *Saccharomyces cerevisiae*

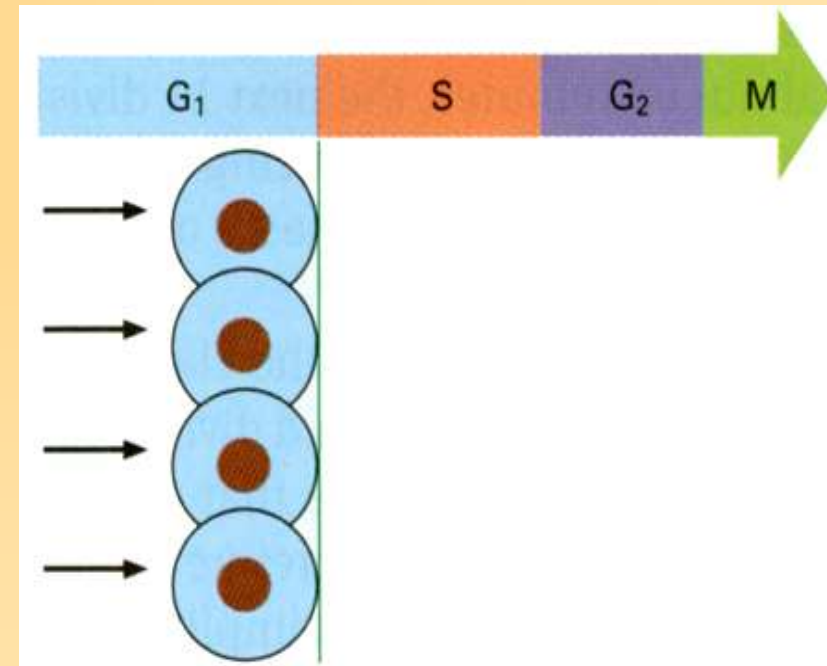


# *Cdc* Mutants Arrest at the Same Cell Cycle Phase

Permissive (low) temperature

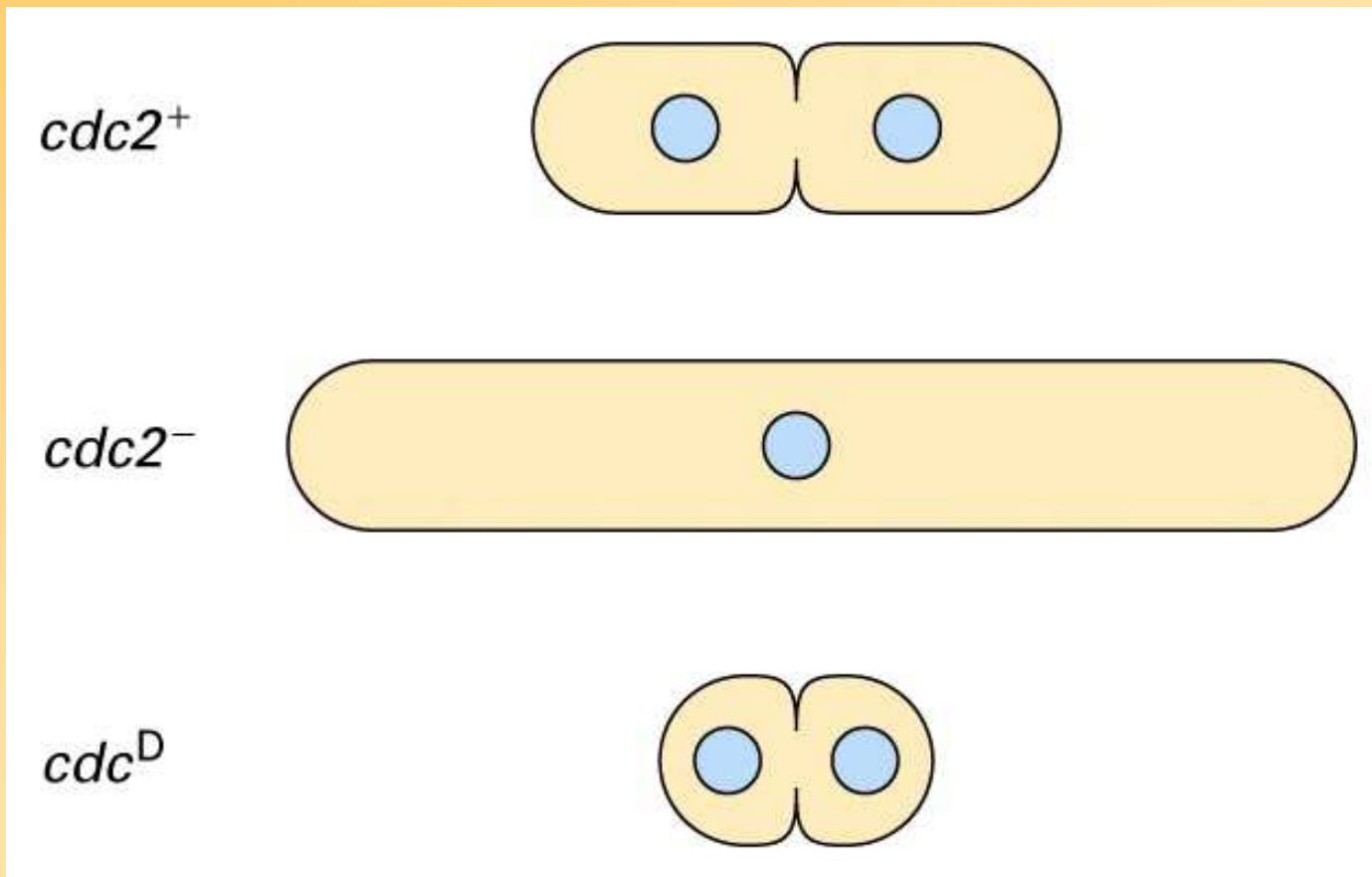


Restrictive (high) temperature



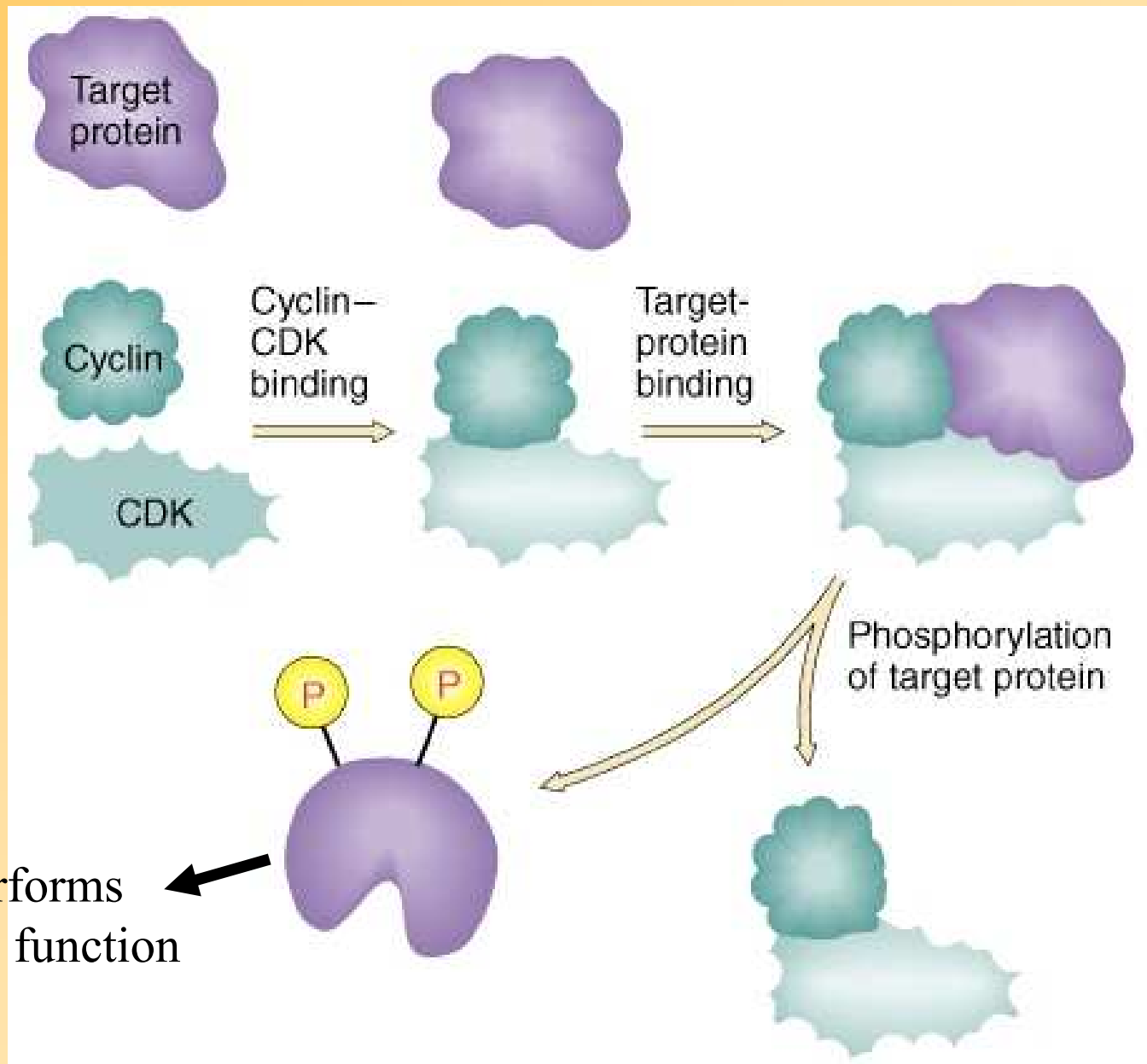


*Cdc* Genes Encode Proteins Needed for the G2-M Transition:  
Studies in *S. pombe*



*cdc2*<sup>+</sup> encodes a kinase  
Moreover = *cdc28* in *S. cerevisiae*!

# Phosphorylation of CDK Targets Changes Their Activity



# Jak jsou CDK regulovány?

1. prostřednictvím syntézy a odbourávání cyklinů
2. fosforylací
3. pomocí CDK inhibitory proteins (CKIs)

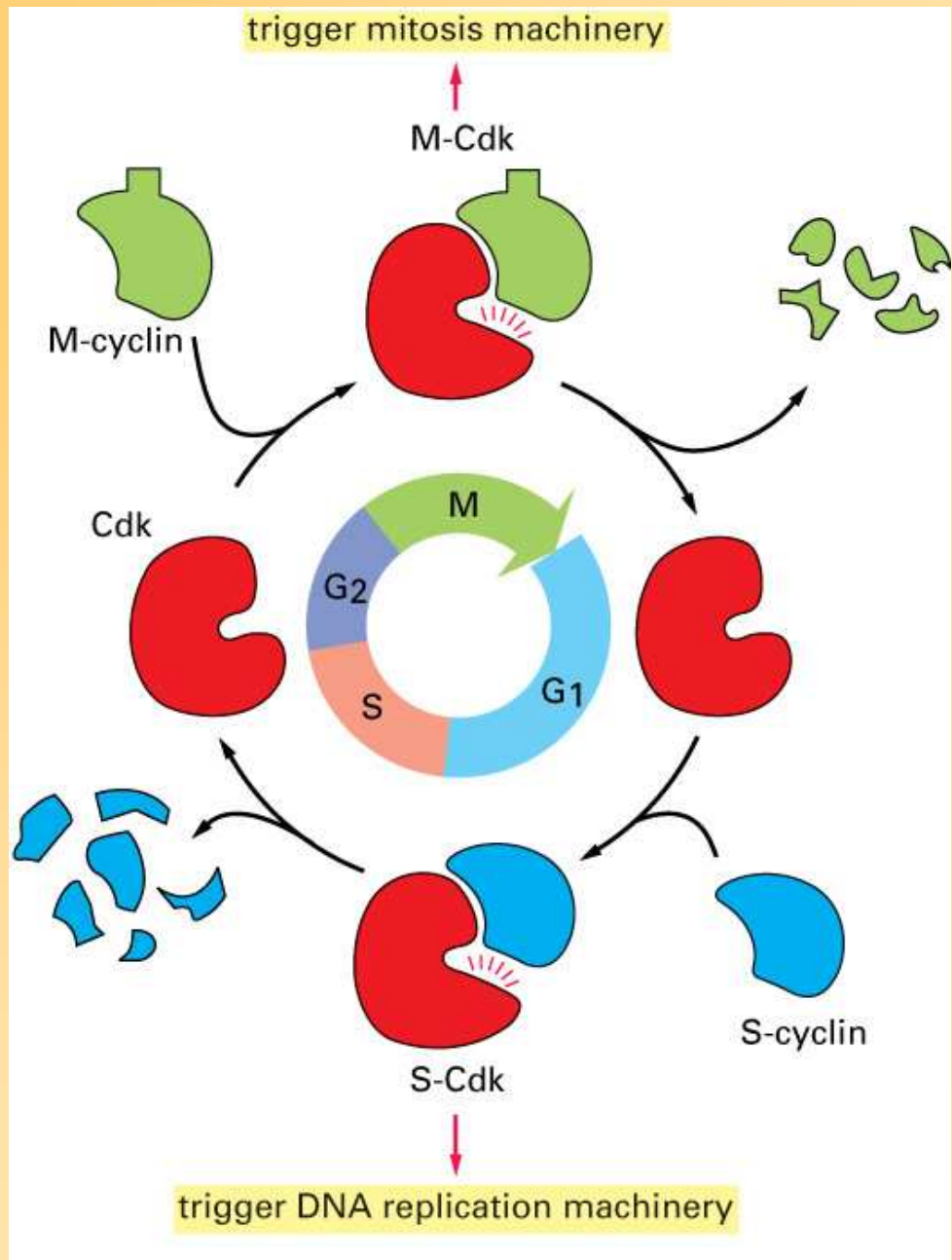


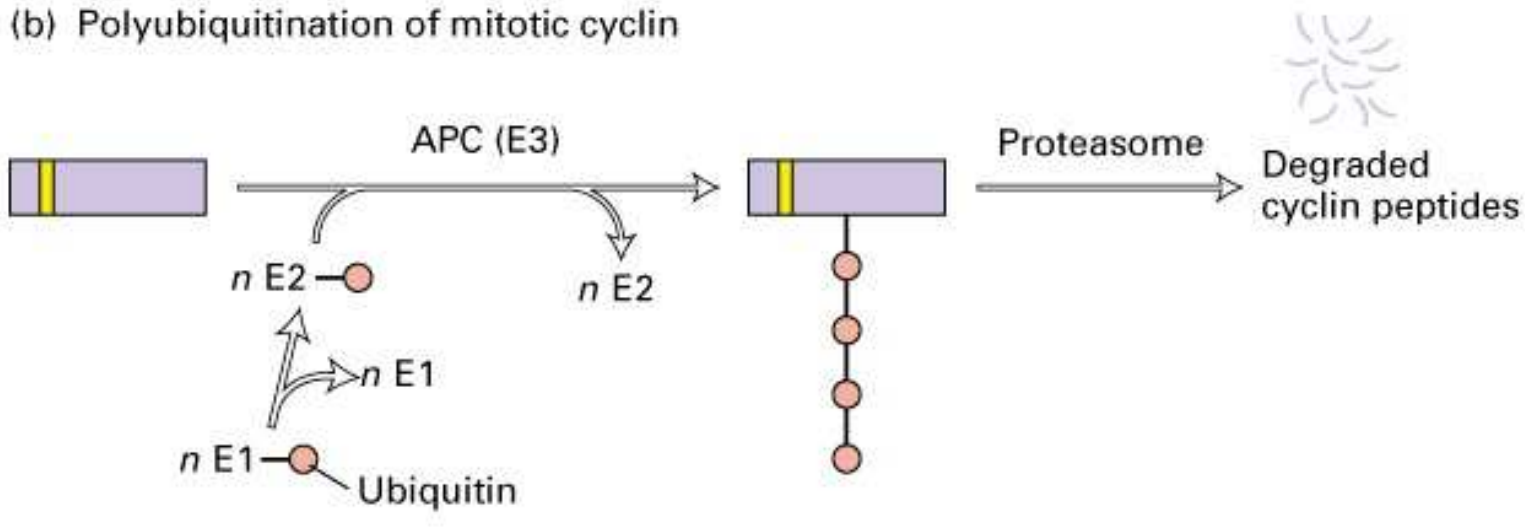
Figure 17-16. Molecular Biology of the Cell, 4th Edition.

# Cyclin Destruction is Controlled by Ubiquitination

(a) Mitotic cyclin destruction box

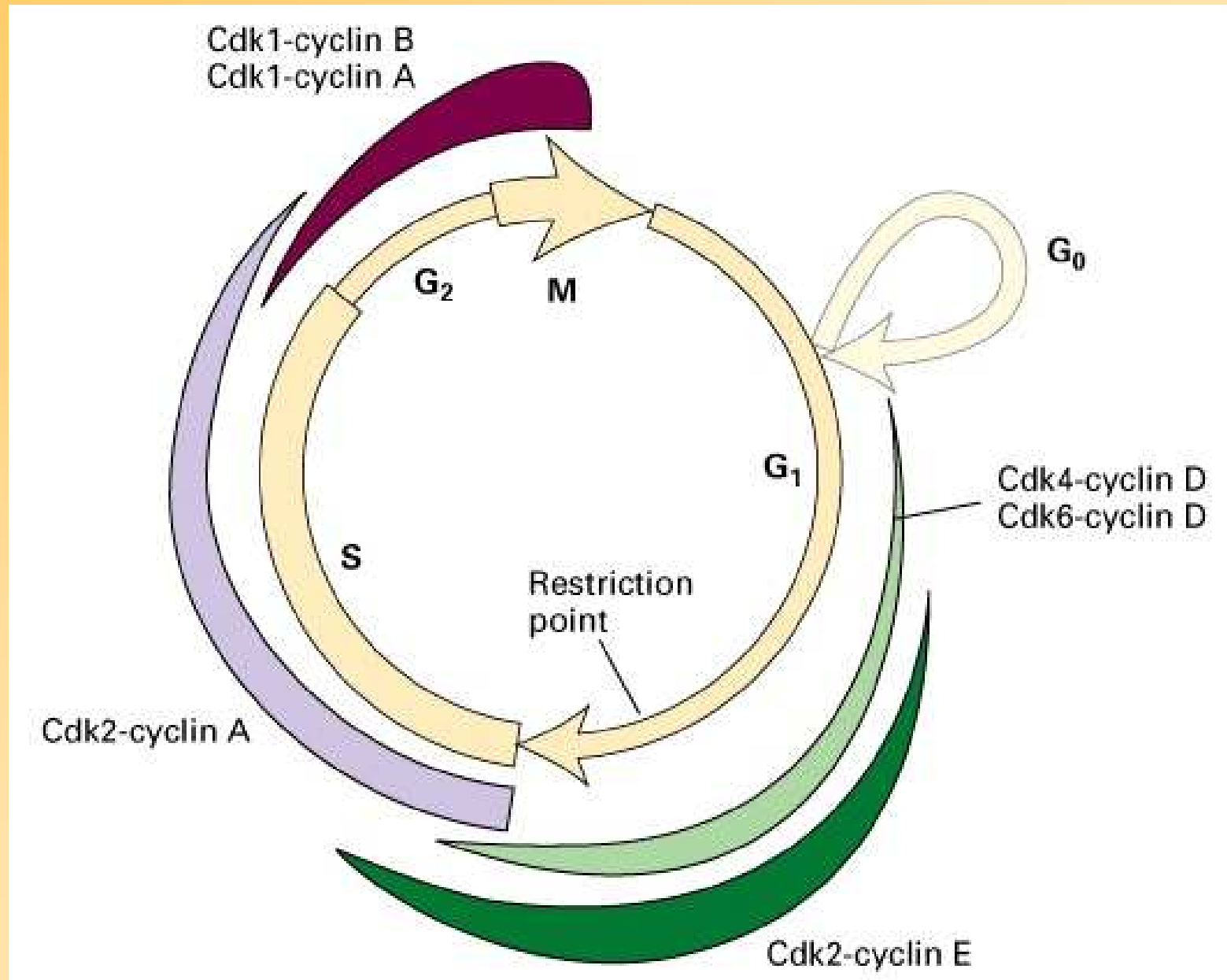


(b) Polyubiquitination of mitotic cyclin



**Představují cykliny jediný způsob regulace CDK?**

# Exprese cyklinů v jednotlivých fázích BC



# CDK jsou regulovány fosforylací

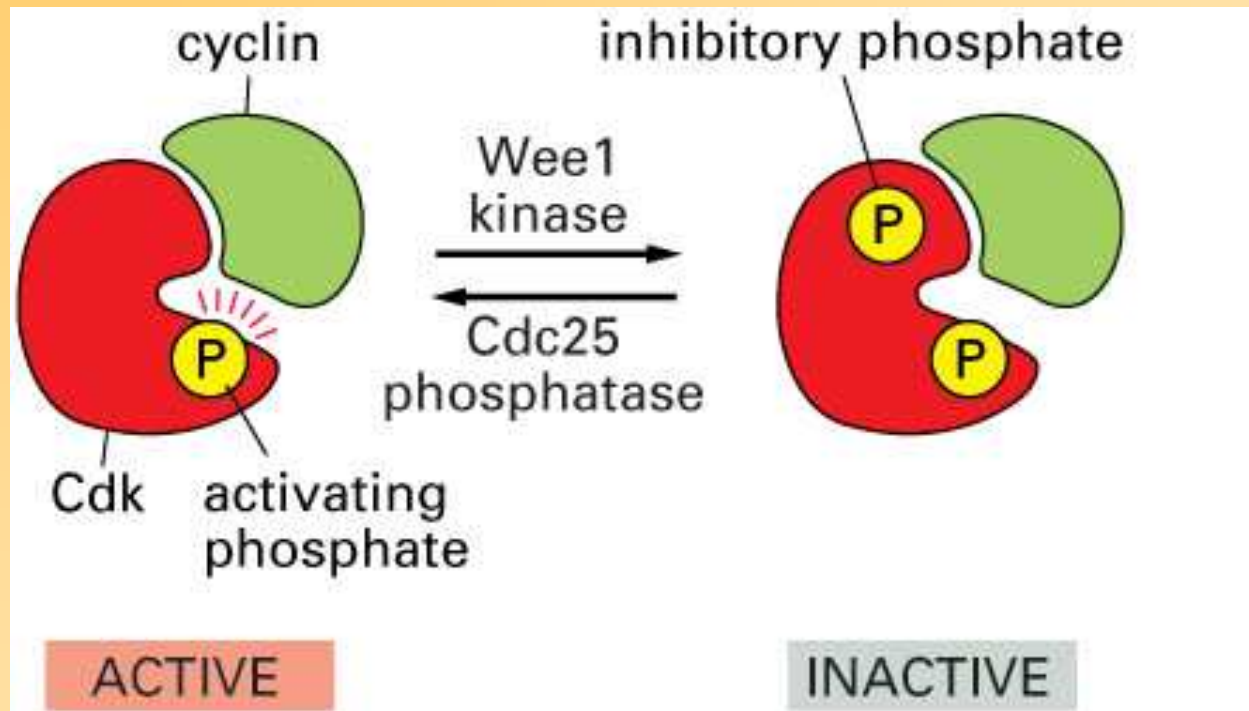
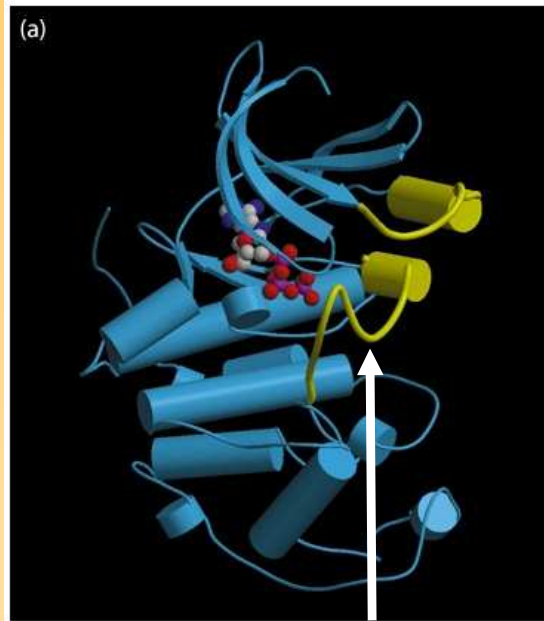


Figure 17-18. Molecular Biology of the Cell, 4th Edition.



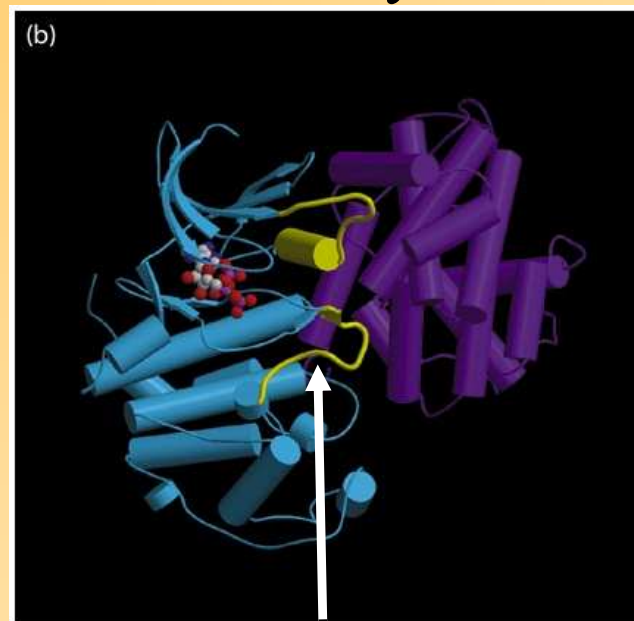
# Conformational Changes Associated with CDK Phosphorylation

Free CDK



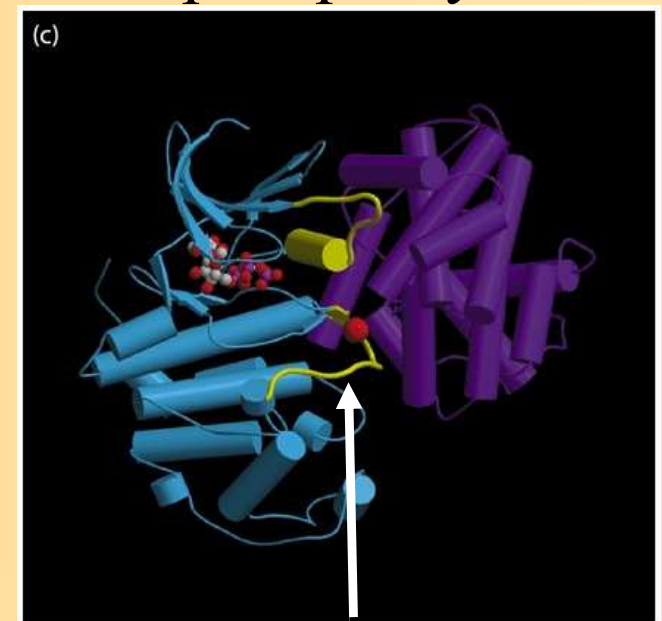
The T-loop blocks substrate access

CDK + Cyclin



Binding of cyclin moves the T-loop

T161 phosphorylation



Phosphorylation moves the T-loop more

# Cyclin Dependent Kinase Inhibitors (CKIs)

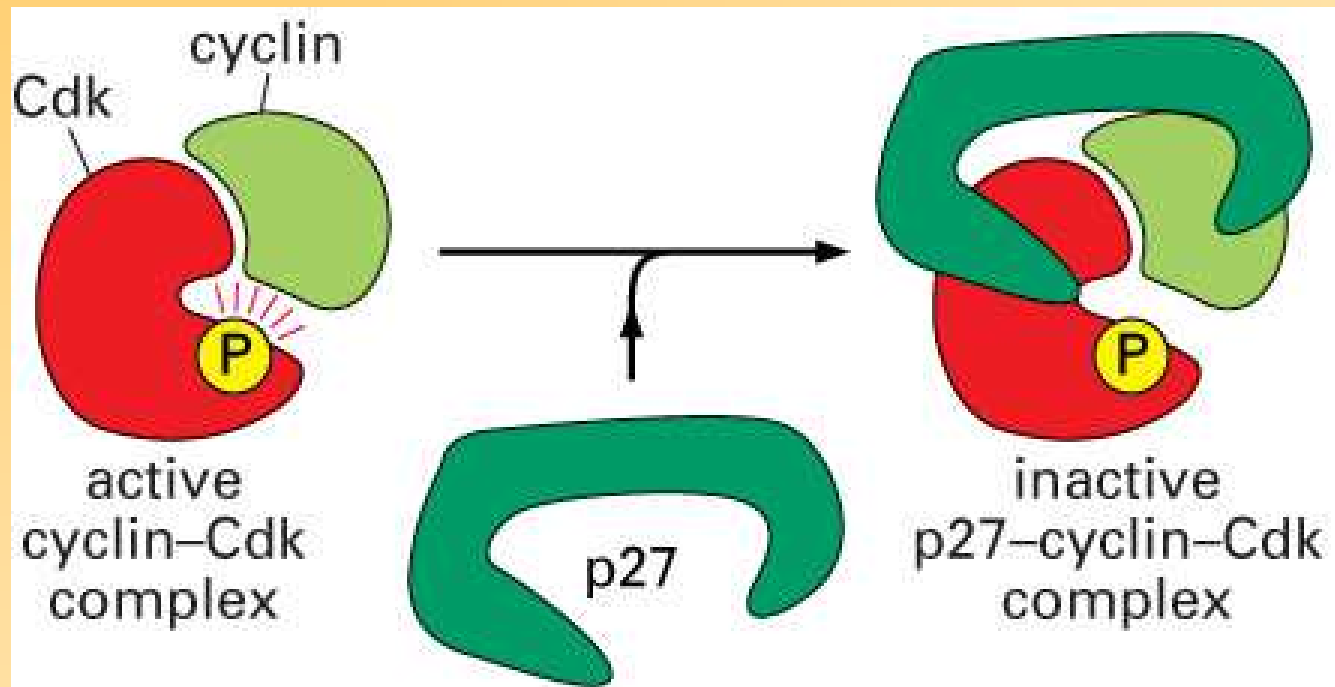
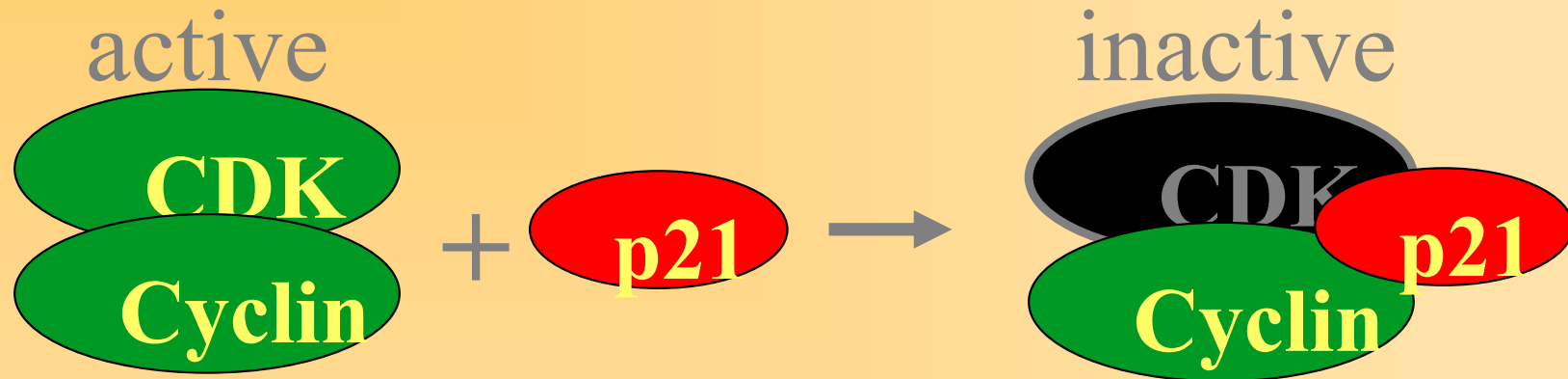


Figure 17-19. Molecular Biology of the Cell, 4th Edition.

# The p21 Family of CDK inhibitors (p21<sup>CIP1/WAF1</sup>, p27<sup>KIP1</sup>, p57<sup>KIP2</sup>)



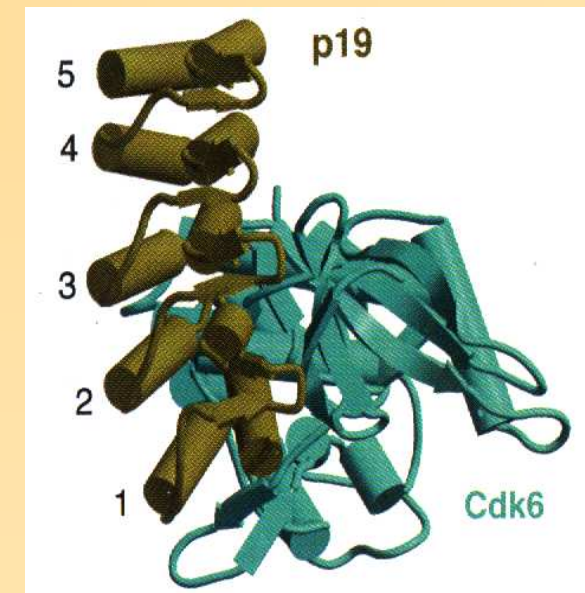
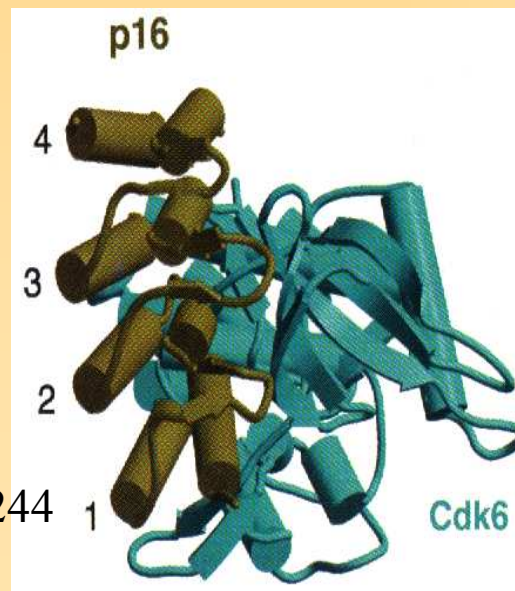
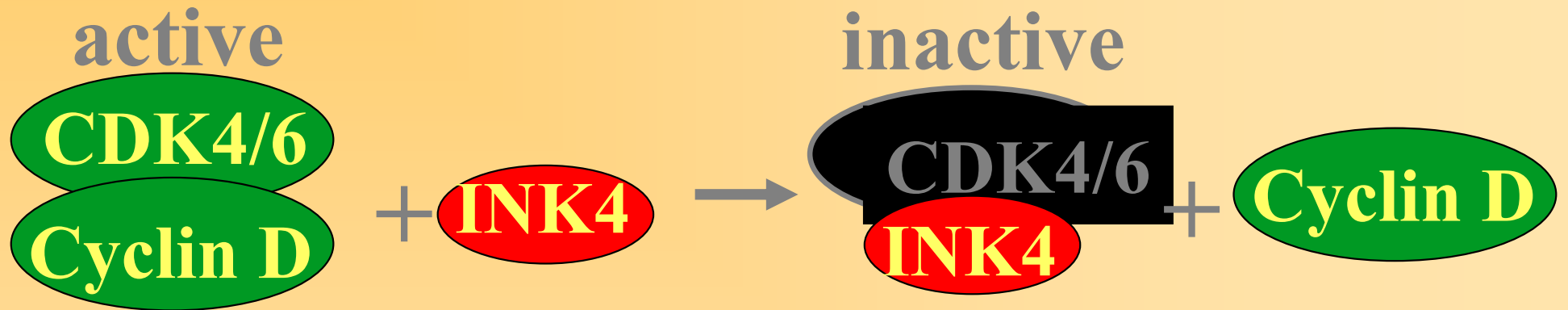
2

Jeffrey et al. (1995) *Nature* 376:313



Russo et al. (1996) *Nature* 382:325

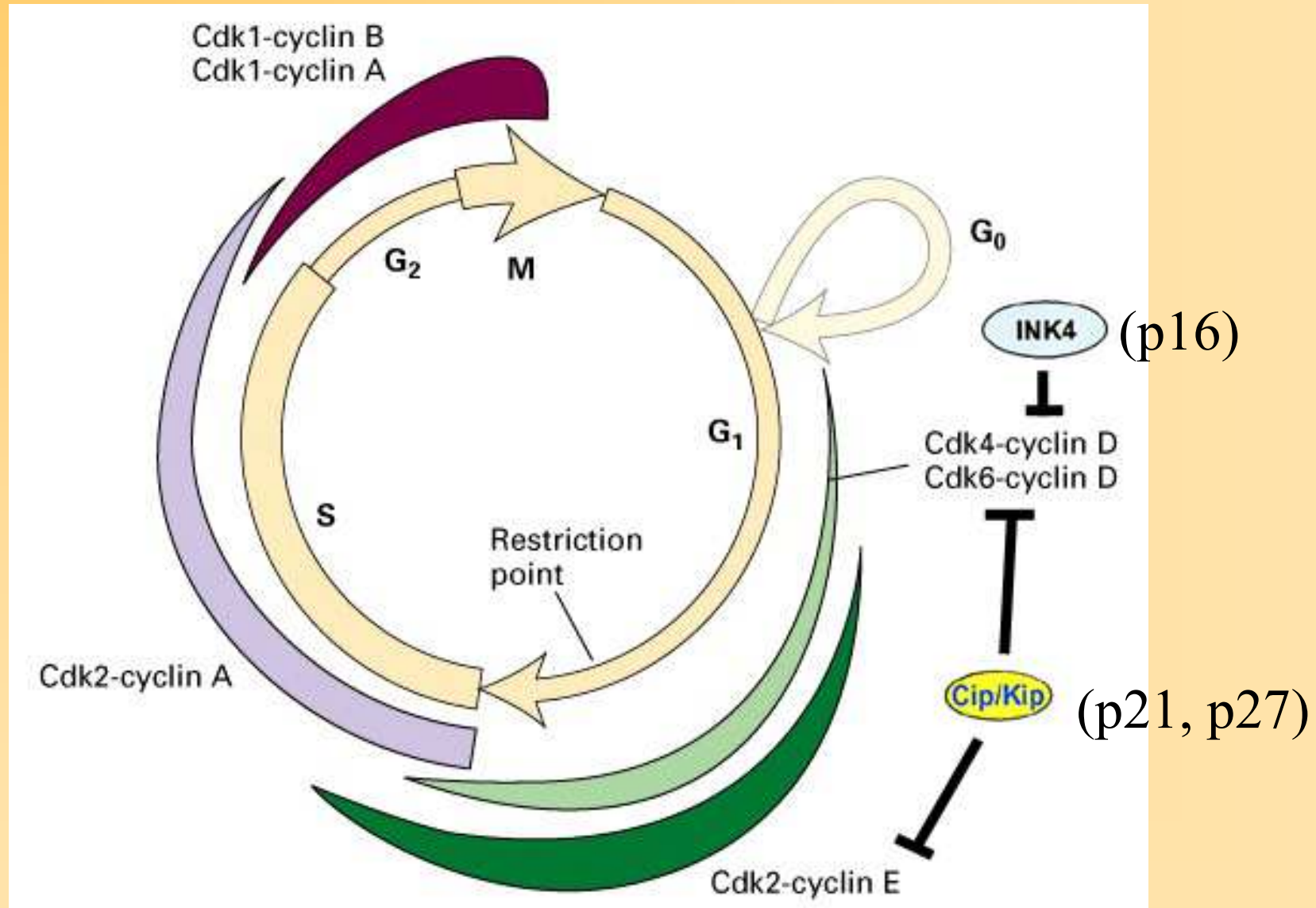
# The INK4 Family of CDK inhibitors (p16<sup>INK4a</sup>, p15<sup>INK4b</sup>, p18<sup>INK4c</sup>, p19<sup>INK4d</sup>)



Russo et al. (1998) *Nature* 395:237

Brotherton et al. (1998) *Nature* 395:244

# CKIs Regulate the G1-S Transition



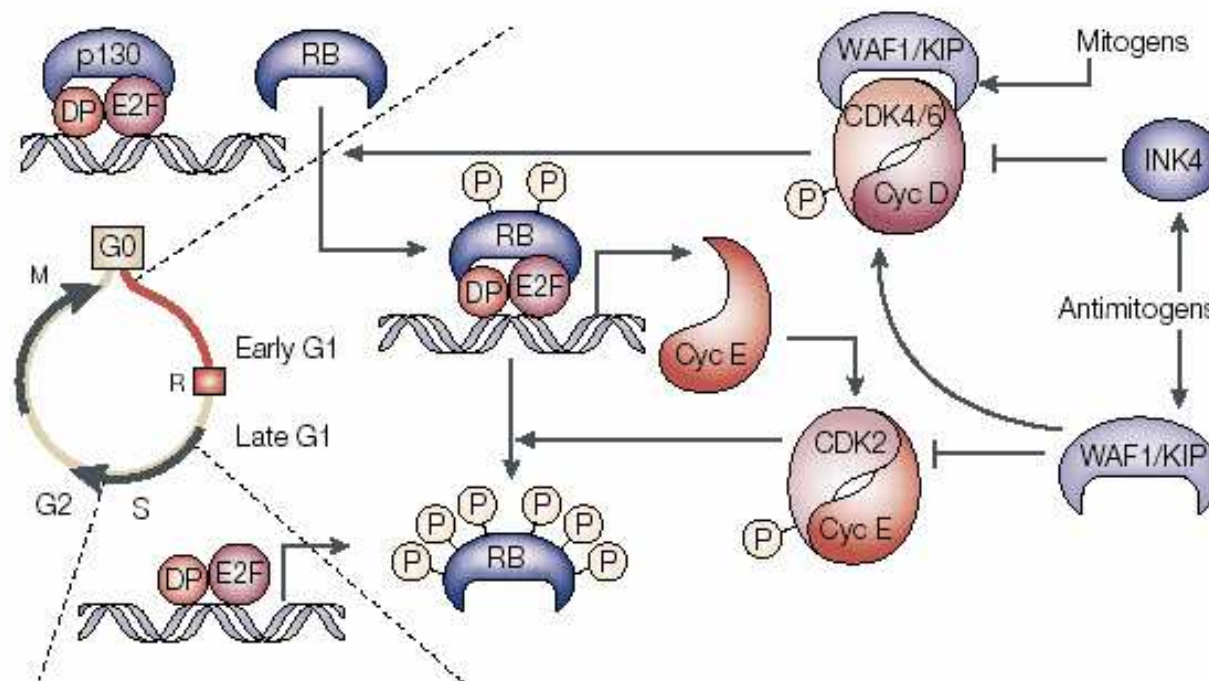
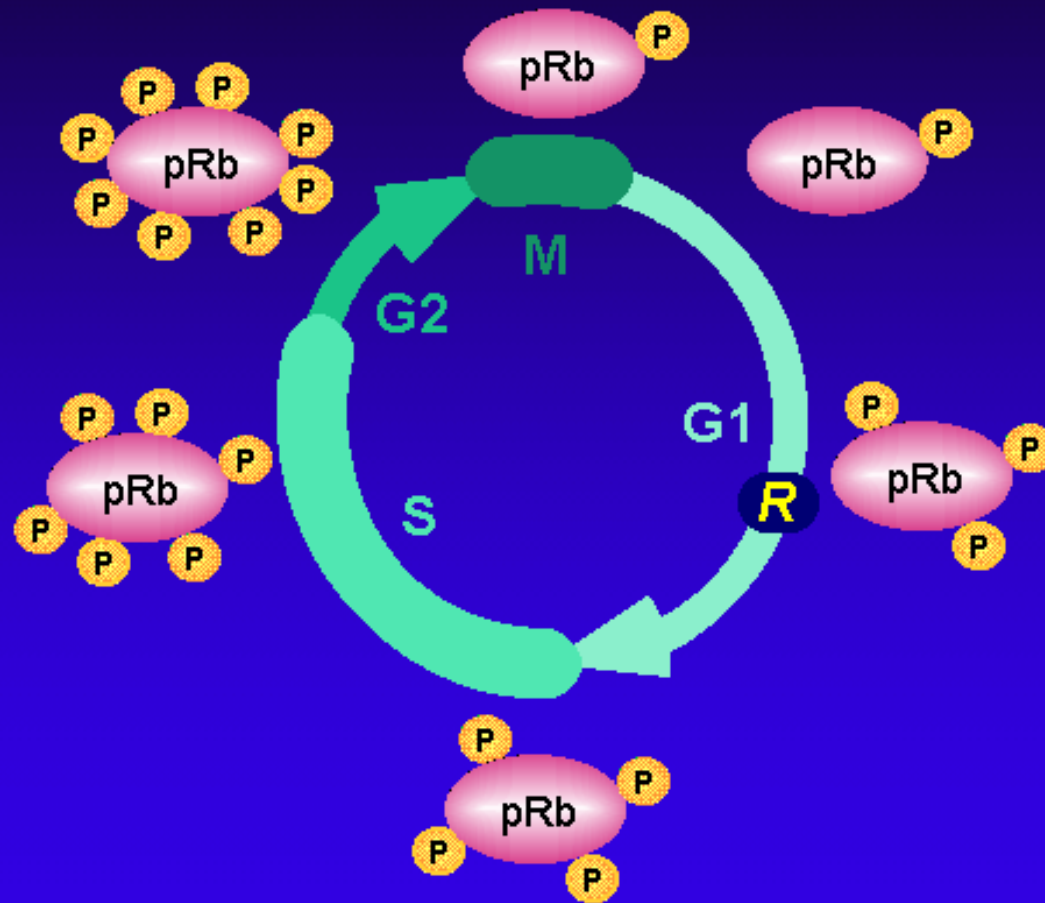
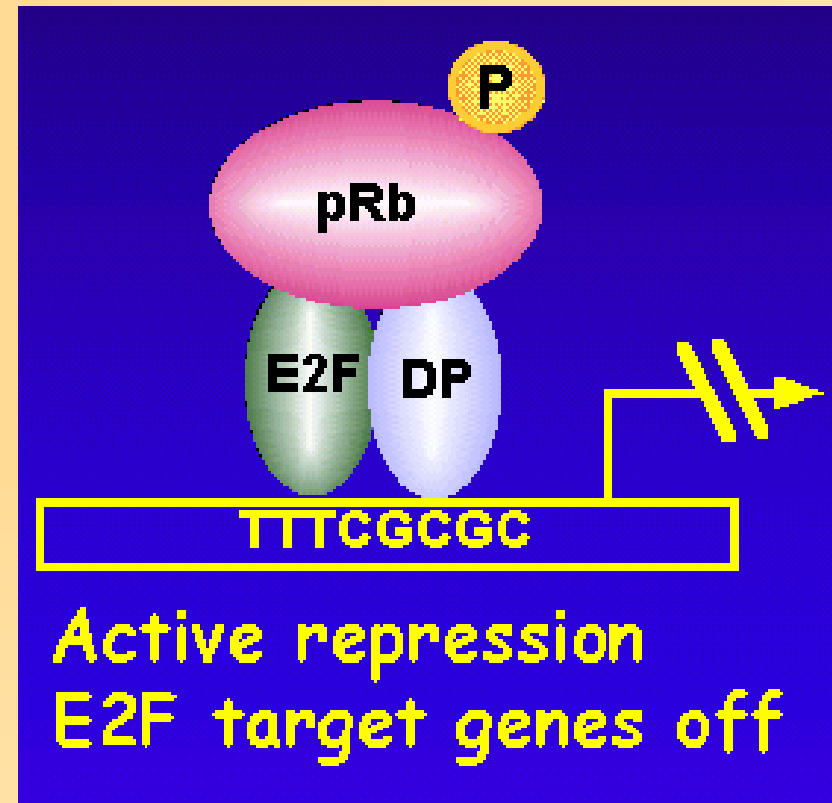
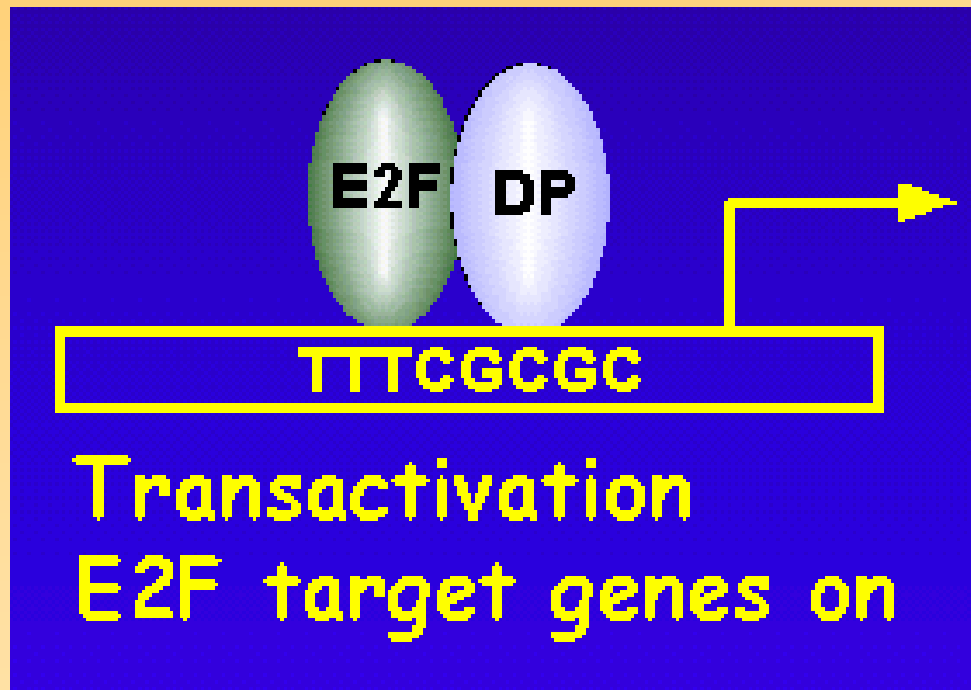


Figure 1 | **Regulation of G1 and the G1/S transition.** In quiescent, G0 cells, E2F-*DP* transcription factors are bound to p130, the principal pocket protein in these cells, which keeps them inactive. In G1, however, RB-E2F-*DP* complexes predominate. Mitogenic signalling results in cyclin D (*Cyc*) synthesis, formation of active CDK4/6-cyclin-D complexes and initial phosphorylation of RB. Partially phosphorylated RB still binds to E2F-*DP*, but the transcription factor is still able to transcribe some genes, such as cyclin E, presumably due to impaired repression. Cyclin E binds to and activates CDK2. It is generally accepted that CDK2-dependent phosphorylation of RB results in its complete inactivation, which allows induction of the E2F-responsive genes that are needed to drive cells through the G1/S transition and to initiate DNA replication. INK4 and WAF1/KIP proteins can inhibit CDK4/6 or CDK2 kinases, respectively, following specific antimitogenic signals. The CDK4/6 complexes can also bind WAF1/KIP inhibitors, while remaining active. This sequesters them from CDK2, which facilitates its full activation. R represents the restriction point that separates the mitogen-dependent early G1 phase from the mitogen-independent late G1 phase.

# Cell cycle regulated phosphorylation of the retinoblastoma protein

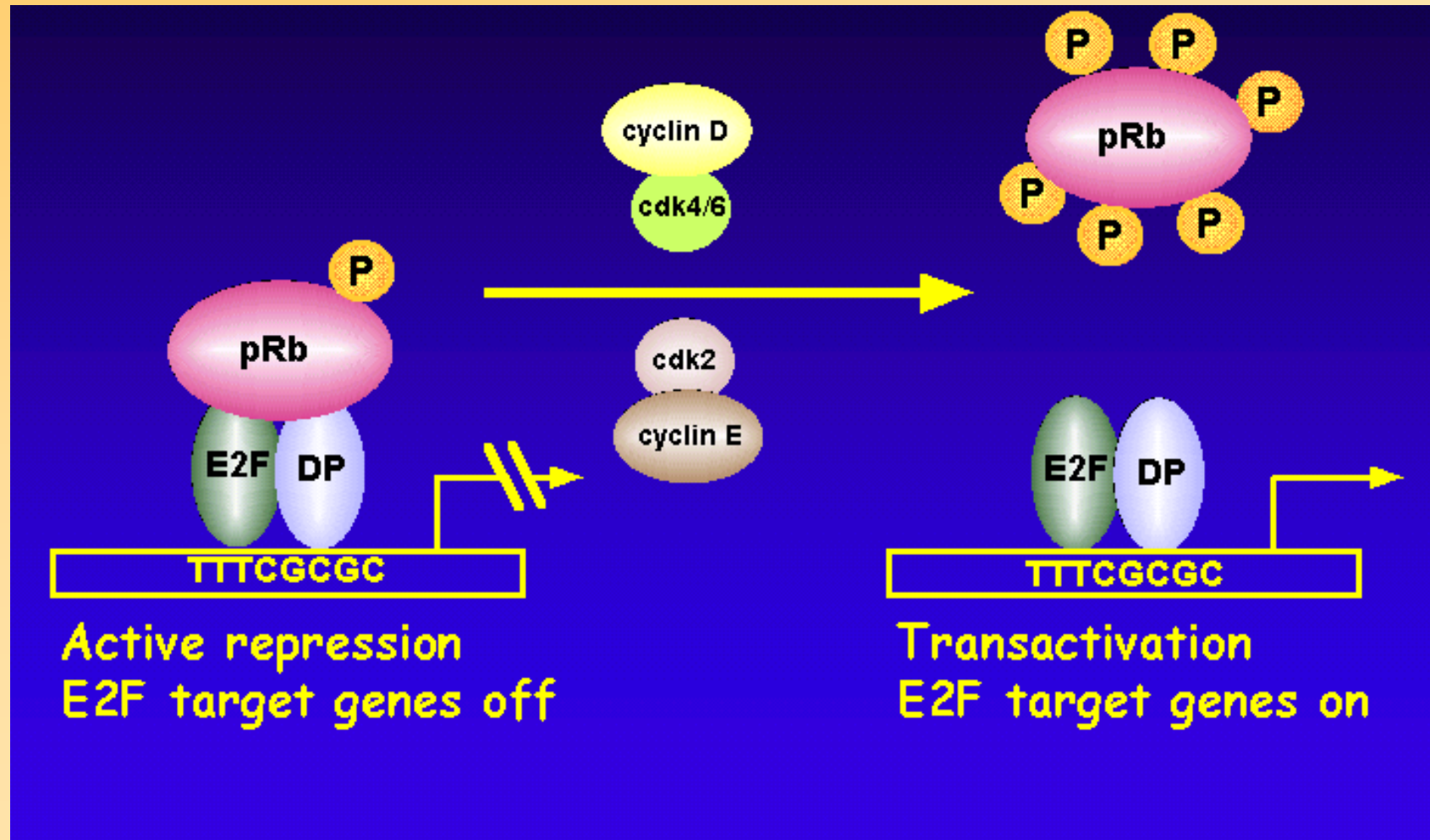


# pRB Binds to the E2F Transcription Factor





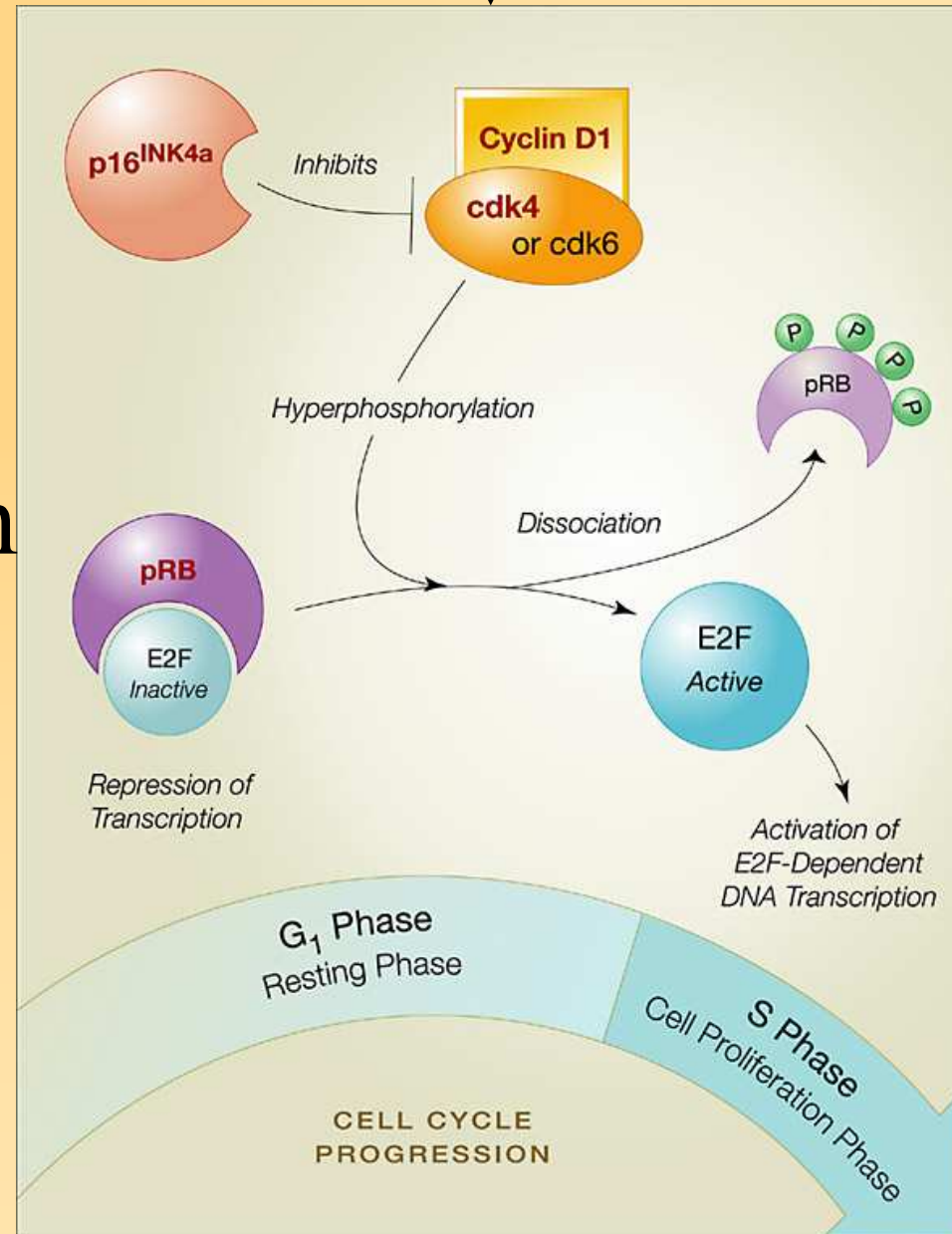
# G1 Cyclin CDKs Phosphorylate pRb



# Proliferation Signals



## p16 Regulates pRB Phosphorylation



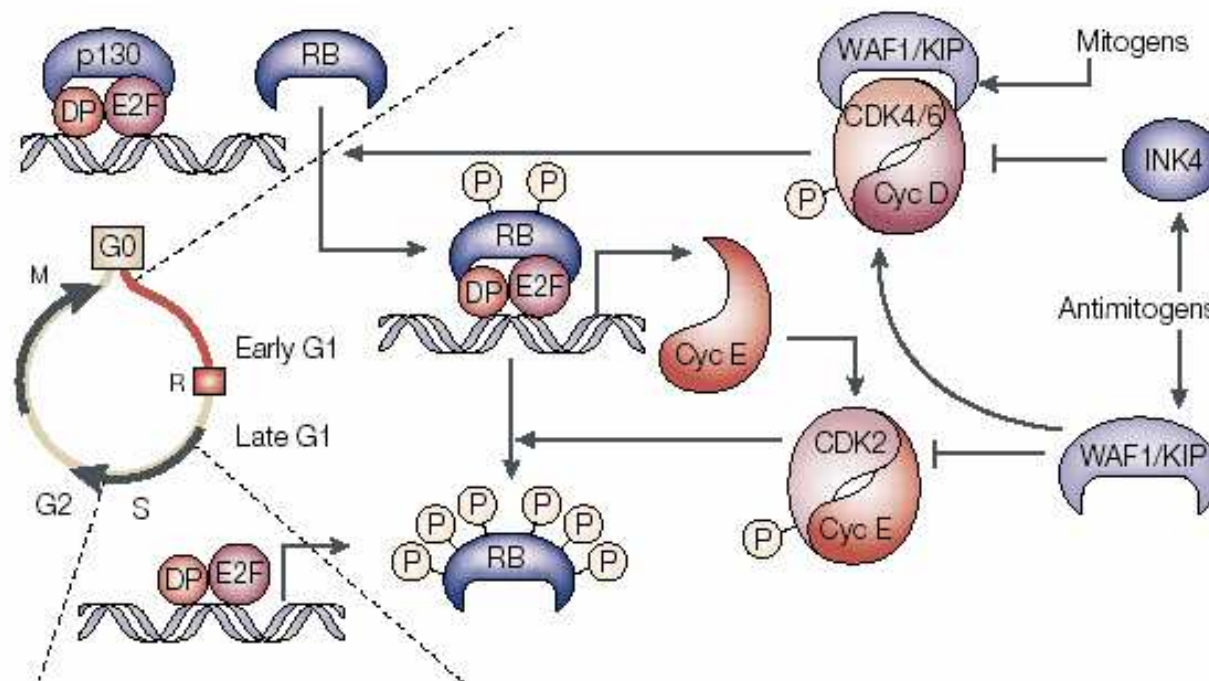


Figure 1 | **Regulation of G1 and the G1/S transition.** In quiescent, G0 cells, E2F-*DP* transcription factors are bound to p130, the principal pocket protein in these cells, which keeps them inactive. In G1, however, RB-E2F-*DP* complexes predominate. Mitogenic signalling results in cyclin D (*Cyc*) synthesis, formation of active CDK4/6-cyclin-D complexes and initial phosphorylation of RB. Partially phosphorylated RB still binds to E2F-*DP*, but the transcription factor is still able to transcribe some genes, such as cyclin E, presumably due to impaired repression. Cyclin E binds to and activates CDK2. It is generally accepted that CDK2-dependent phosphorylation of RB results in its complete inactivation, which allows induction of the E2F-responsive genes that are needed to drive cells through the G1/S transition and to initiate DNA replication. INK4 and WAF1/KIP proteins can inhibit CDK4/6 or CDK2 kinases, respectively, following specific antimitogenic signals. The CDK4/6 complexes can also bind WAF1/KIP inhibitors, while remaining active. This sequesters them from CDK2, which facilitates its full activation. R represents the restriction point that separates the mitogen-dependent early G1 phase from the mitogen-independent late G1 phase.

**Kontrola buněčného cyklu úzce souvisí s:**

▶ **kontrolou buněčného růstu;**

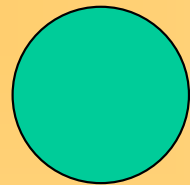
▶ **přítomností růstových faktorů a dalších**

**růstových stimulů a živin;**

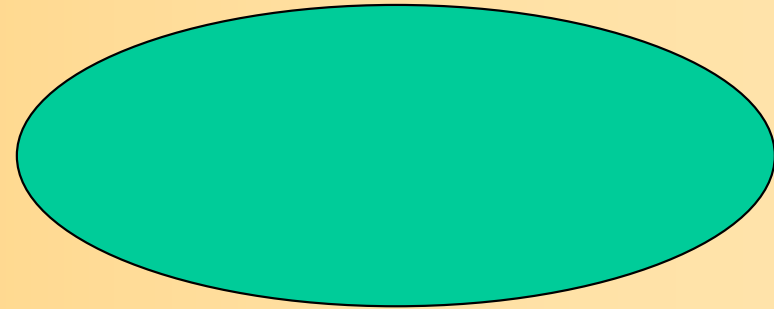
▶ **působením ostatních buněk populace**

**a mezibuněčné hmoty.**

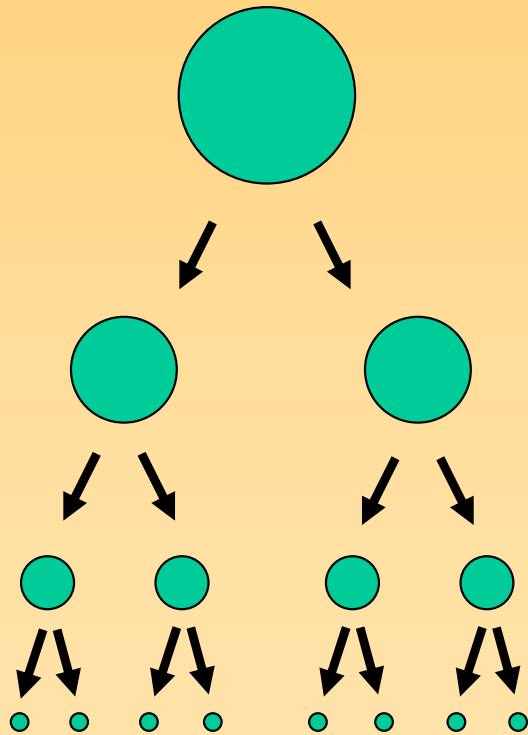
# The Difference Between Growth and Cell Division



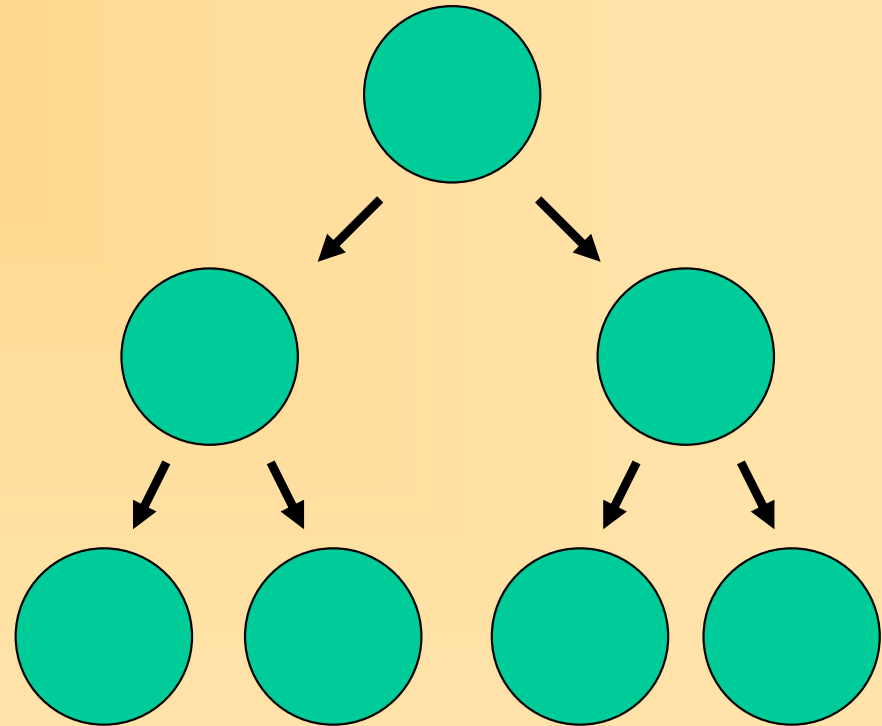
Growth with  
→



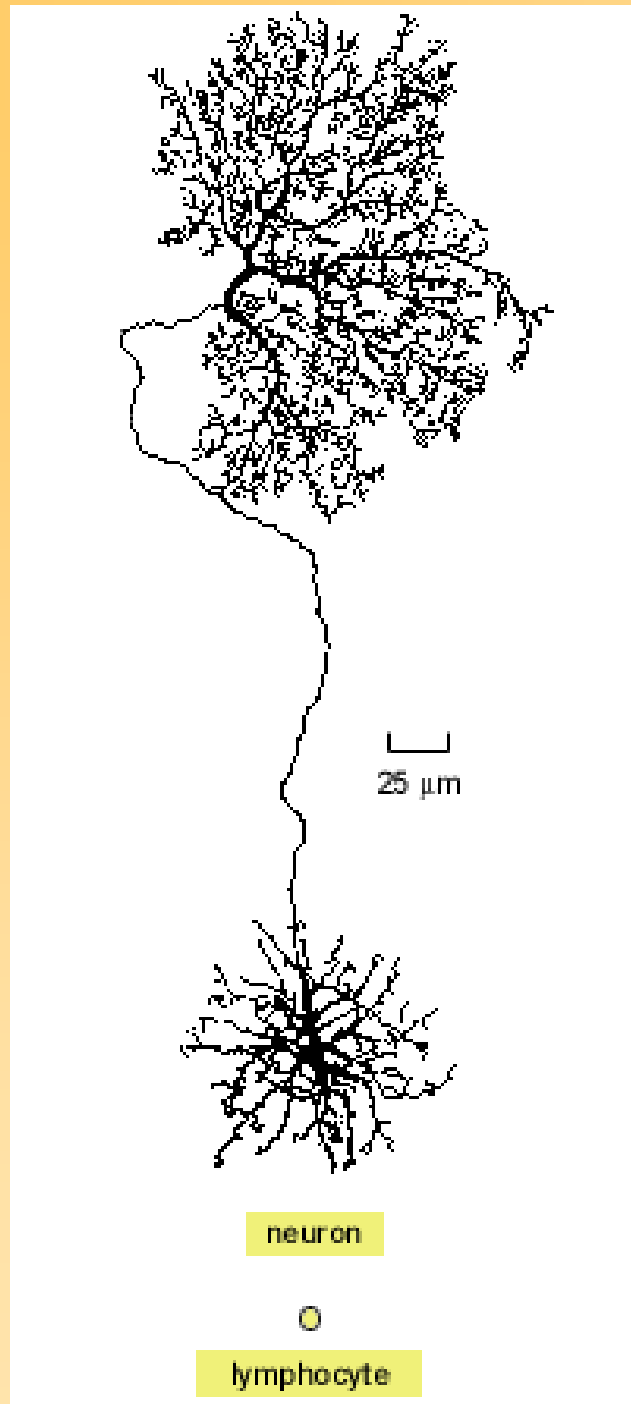
No Cell Division



Cell Division  
No Growth

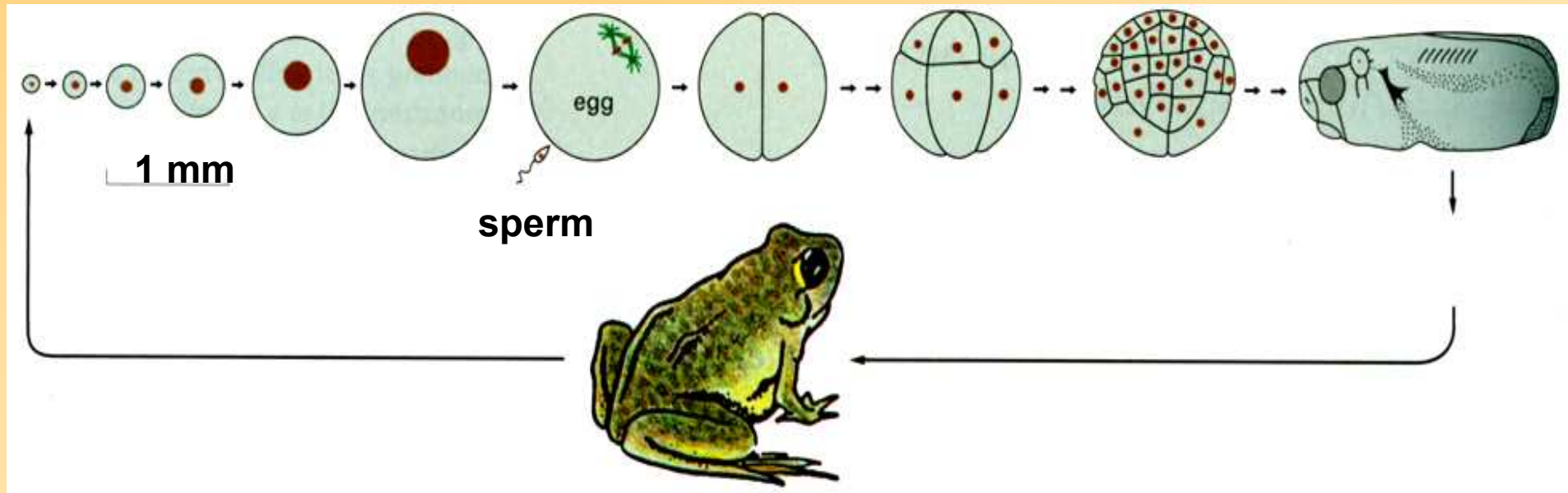


Cell Division + Growth =  
Proliferation!



Growth with  
No Cell Division:  
A Differentiated Neuron

# Cell Division with No Growth: Early Development



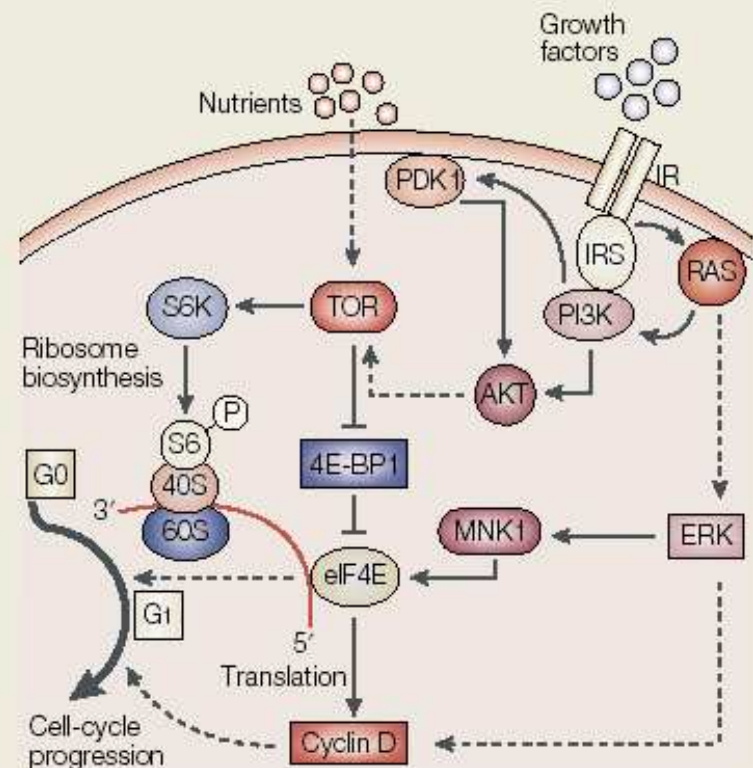
## Box 1 | Cell growth versus cell division

Cell growth (the increase in cell size and protein mass) is a term that has frequently been misused to mean cell proliferation. In fact, both processes are highly coordinated. Only in certain biological systems — such as oocytes, neurons and muscle cells, where cell growth might exist without cell division, and in fertilized eggs, where cell divisions might occur without cell growth — can these processes function in an independent, or even complementary, fashion. In most cells, however, cell division without concurrent cell growth would generate smaller daughter cells, which would affect their viability.

Ribosome biosynthesis is a key process for cell growth. Before entering the cycle, cells need to accumulate sufficient translational machinery, mainly ribosomes, to ensure the rapid processing of transcripts through the cycle. This is accomplished, at least in part, by phosphorylation of the ribosomal S6 protein by S6 kinase (S6K) (see REF 85 for a review). Once the appropriate pool of ribosomes has been achieved, the system is desensitized, either by negative regulators of S6K or by the size of the ribosomal pool (see figure).

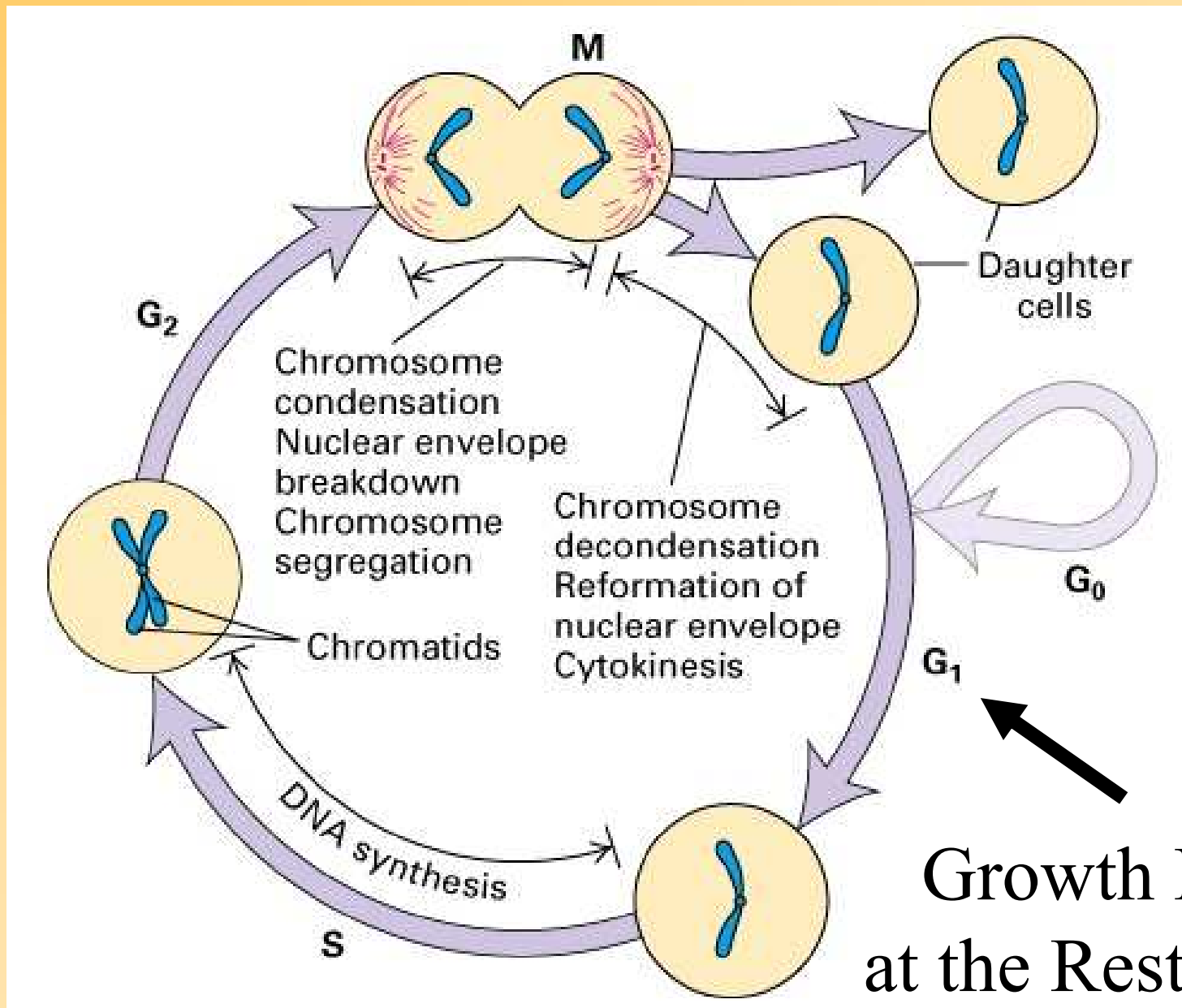
S6K is regulated by mitogenic stimuli mediated through the insulin receptor (IR)/IR substrate (IRS)/phosphatidylinositol-3 kinase (PI3K)/PDK1 pathway. S6K is also regulated directly by TOR, a member of the PI3K-related kinase family<sup>86,87</sup>. TOR is thought to be important in cell growth and amino-acid sensing<sup>87</sup>, but its upstream activators and mechanism of activation are unknown. TOR controls several growth-related readouts, including actin organization, transcription and ribosome biosynthesis.

TOR also affects translation of key regulators of cell proliferation, such as cyclin D and MYC, by phosphorylating 4E-BP1 (a translational inhibitor that is also targeted by AKT/PKB) and causing its dissociation from the initiation factor eIF4E. Mitogen-activated protein kinases such as ERK phosphorylate and activate MNK1, which in turn is able to phosphorylate eIF4E (REFS 88,89). The RAS/ERK cascade is also known to signal to cell-cycle regulators such as cyclin D or KIP1 to induce progression through G1 (REF 12). Several of these proteins, such as PI3K, AKT, MYC and RAS, can be activated as oncogenes, which illustrates the intimate connections between cell growth and cell proliferation.





# Growth Factors Induce Cell Cycle Progression



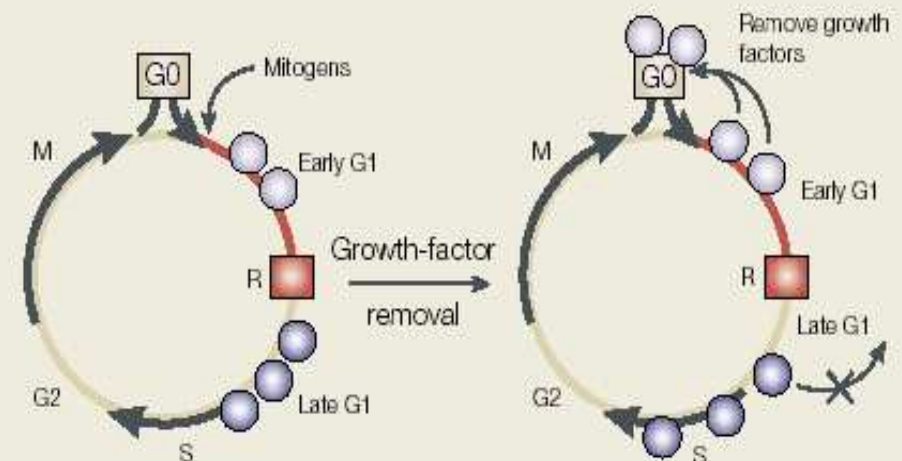
Growth Factors act  
at the Restriction Point

## Box 2 | The Restriction Point

The term 'Restriction Point' was coined in 1974 by Arthur Pardee<sup>90</sup> to define a specific event in G1 after which cultured cells could proliferate independently of mitogenic stimuli. Briefly, cultured mammalian cells that had undergone mitosis within the previous 3 hours could be prevented from progressing through the cell cycle by growth-factor starvation or moderate inhibition of protein synthesis. These cells then re-entered the cell cycle after re-stimulation with growth factors. However, if the cells had undergone cell division more than 4 hours before, they did not respond to mitogen deprivation and advanced through the cell cycle with the same kinetics as unstarved cells. It was postulated that the latter cells had 'passed' the restriction point (R). Today, R is often used to divide the early and late G1 phases.

R does not represent a checkpoint as originally defined in yeast<sup>2,91</sup>. In culture cells, R occurs 3–4 hours after mitosis (see figure). However, entry into S phase is usually initiated 5–13 hours after mitosis. This variability is characteristic of the late G1 phase and accounts for most of the observed differences in the length of the cell cycle. Indeed, the differential kinetics of these two transitions indicates distinct control mechanisms. The molecular events that allow cells to pass R have not been well defined. However, members of the RB family are likely to be important, as ablation of this gene family eliminates R<sup>92,93</sup>.

It has been postulated that loss of regulation of R is critical in cancer. R normally prevents cells from entering the cycle until they have accumulated a certain threshold of mitogen-induced events, so loosening of R control due to mutations in G1 regulators or other, as yet unidentified, genes would allow cells to enter the cycle even in the absence of adequate mitogenic signalling, leading to unscheduled proliferation. Validation of this model will require definition of the molecular players that regulate R.



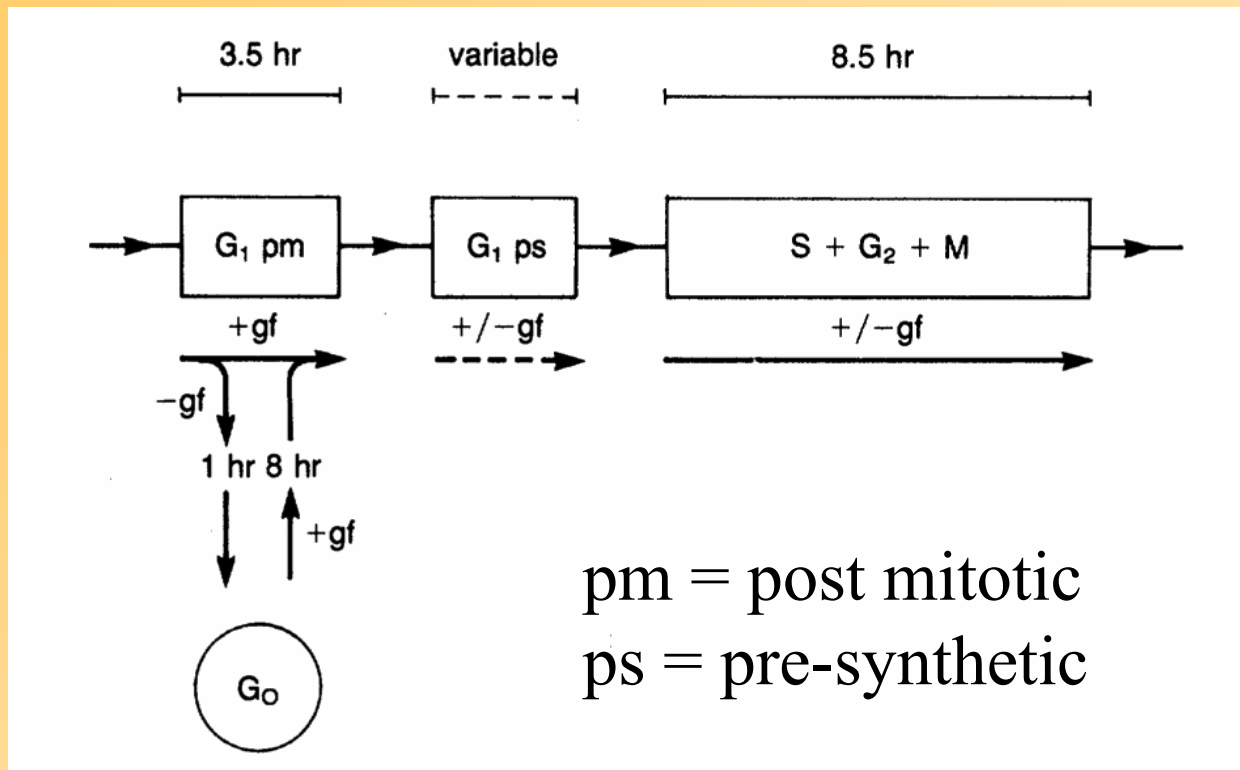
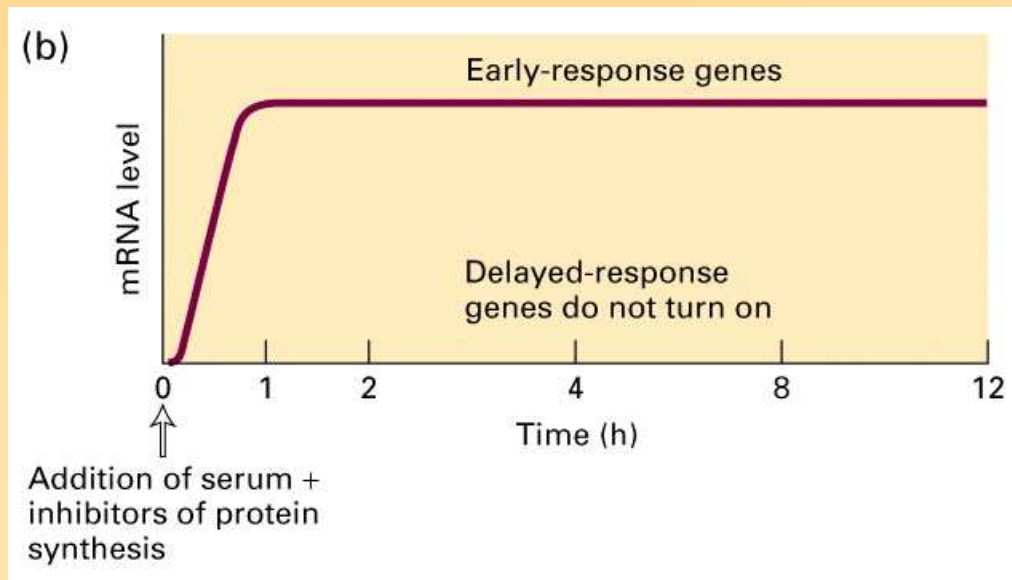
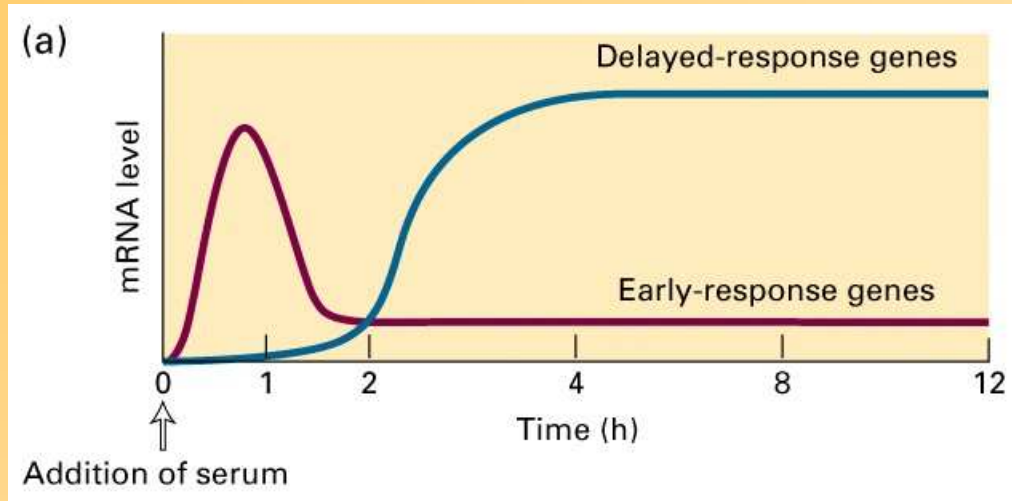
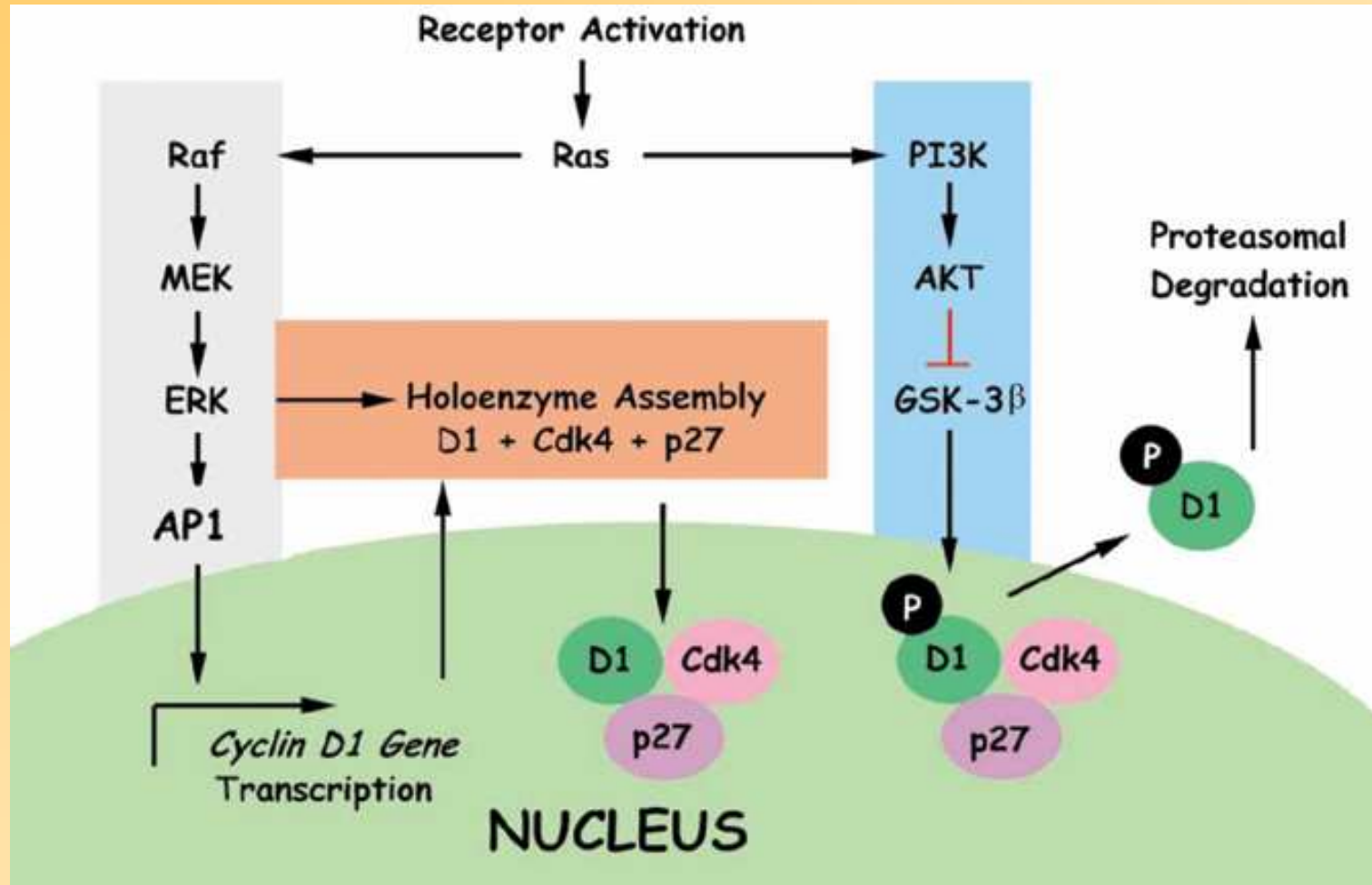


FIG. 7. Schematic model of cell cycle in Swiss 3T3 cells. During the first 3.5-hr after mitosis (G<sub>1</sub>pm), the cell makes the decision whether or not to progress through the cell cycle. This decision depends on the presence of growth factors (gf). If the cell senses a lack of growth factors (-gf) in G<sub>1</sub>pm, it will leave the cell cycle within 15–60 min and enter a state of quiescence (G<sub>0</sub>) from which it takes 8 hr to reenter the cycle after the growth factor level in the environment again becomes optimal (+gf) for proliferation. Once the cell has entered G<sub>1</sub>ps, it will eventually initiate DNA synthesis. However, G<sub>1</sub>ps is highly variable in length and in fact responsible for most of the variability in the duration of G<sub>1</sub> and of the whole cell cycle.

# Growth Factors Induce Gene Expression

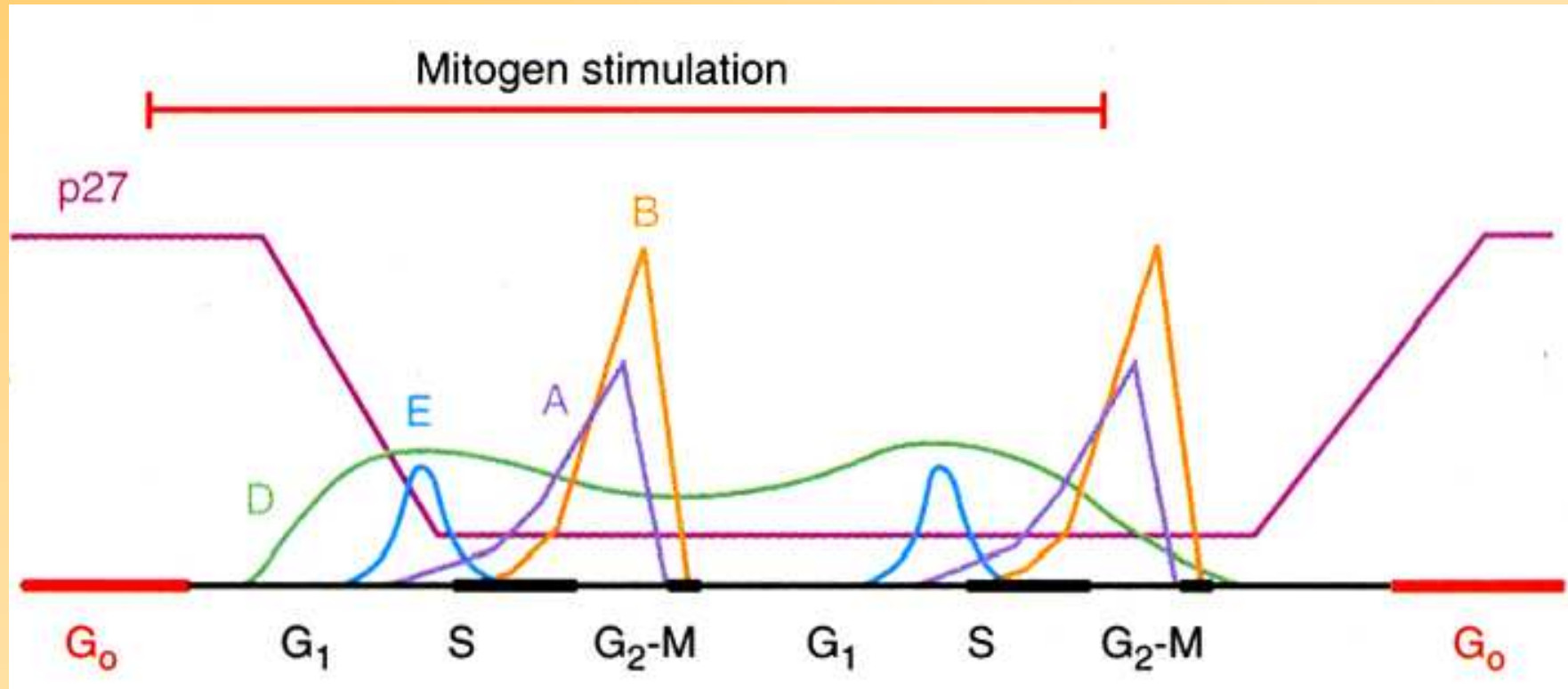


# Growth Factors Induce Cyclin D1 Expression



Sherr and McCormick, Cancer Cell, Vol 2, 103-112 (2002)

# Mitogen Induced Cell Cycle Progression in Cell Culture



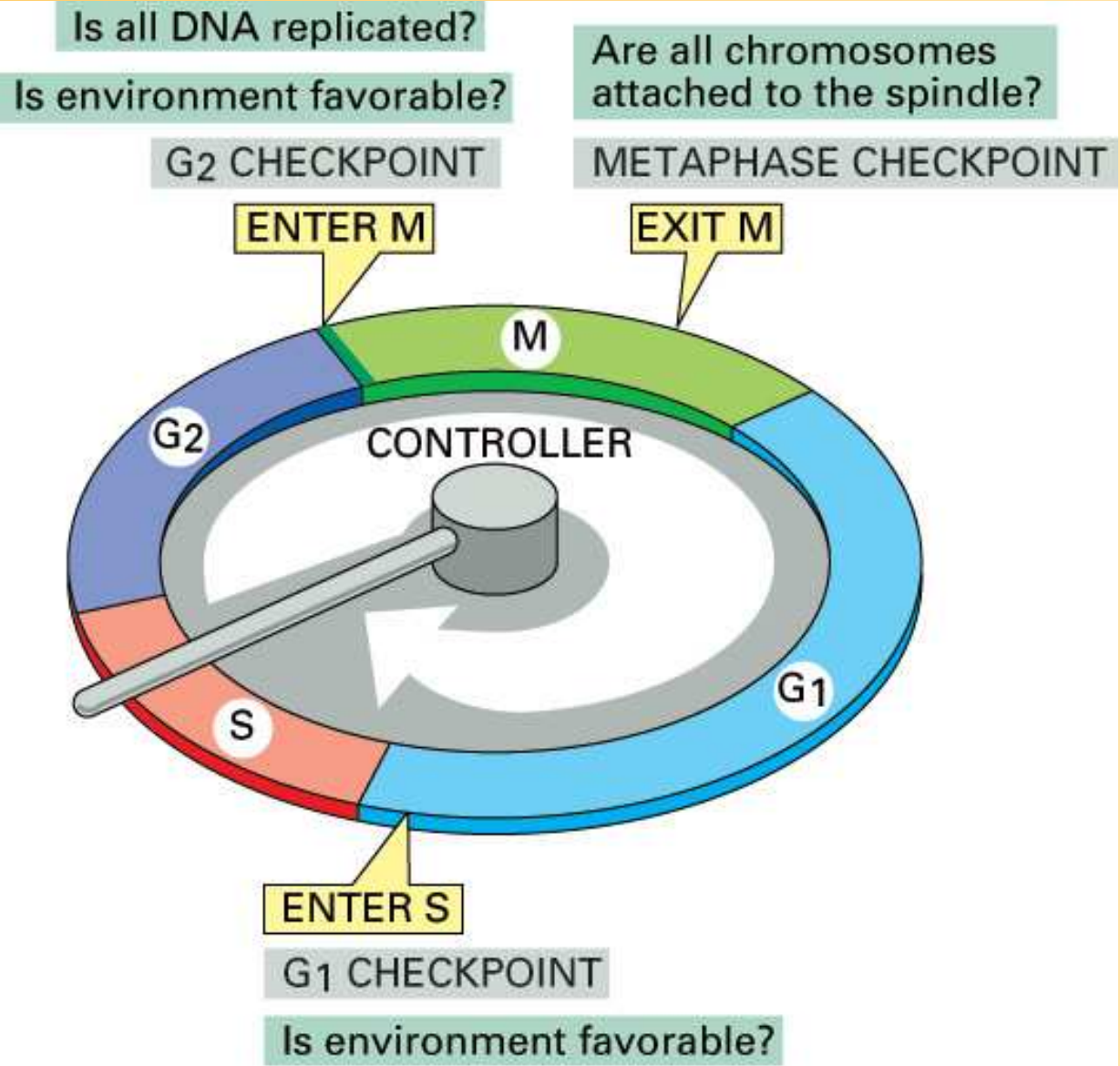


Figure 17-14. Molecular Biology of the Cell, 4th Edition.

# Cell Cycle Checkpoints

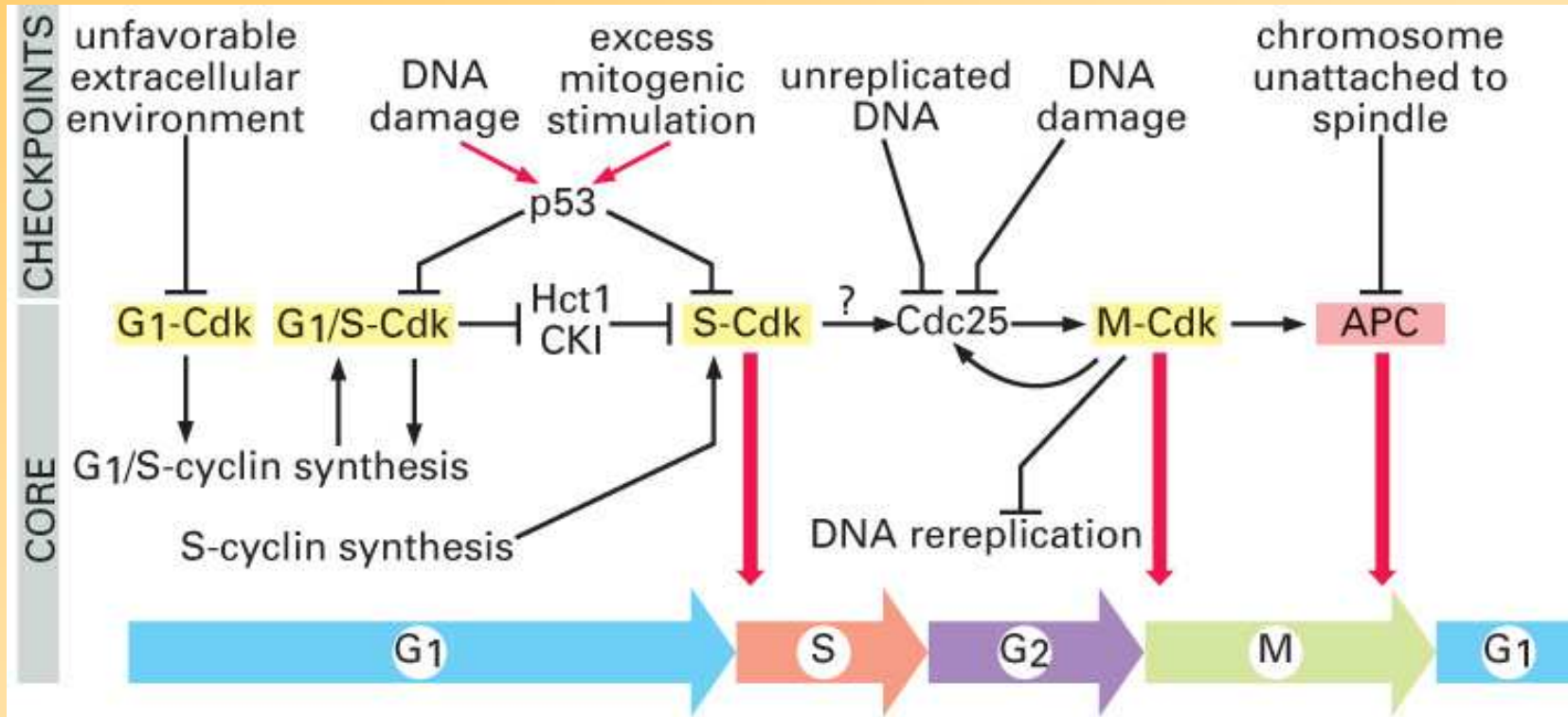
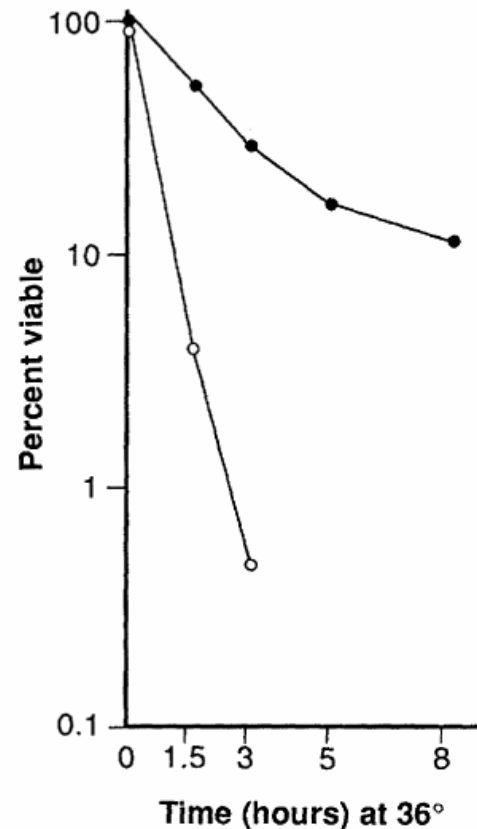


Figure 17-34. Molecular Biology of the Cell, 4th Edition.

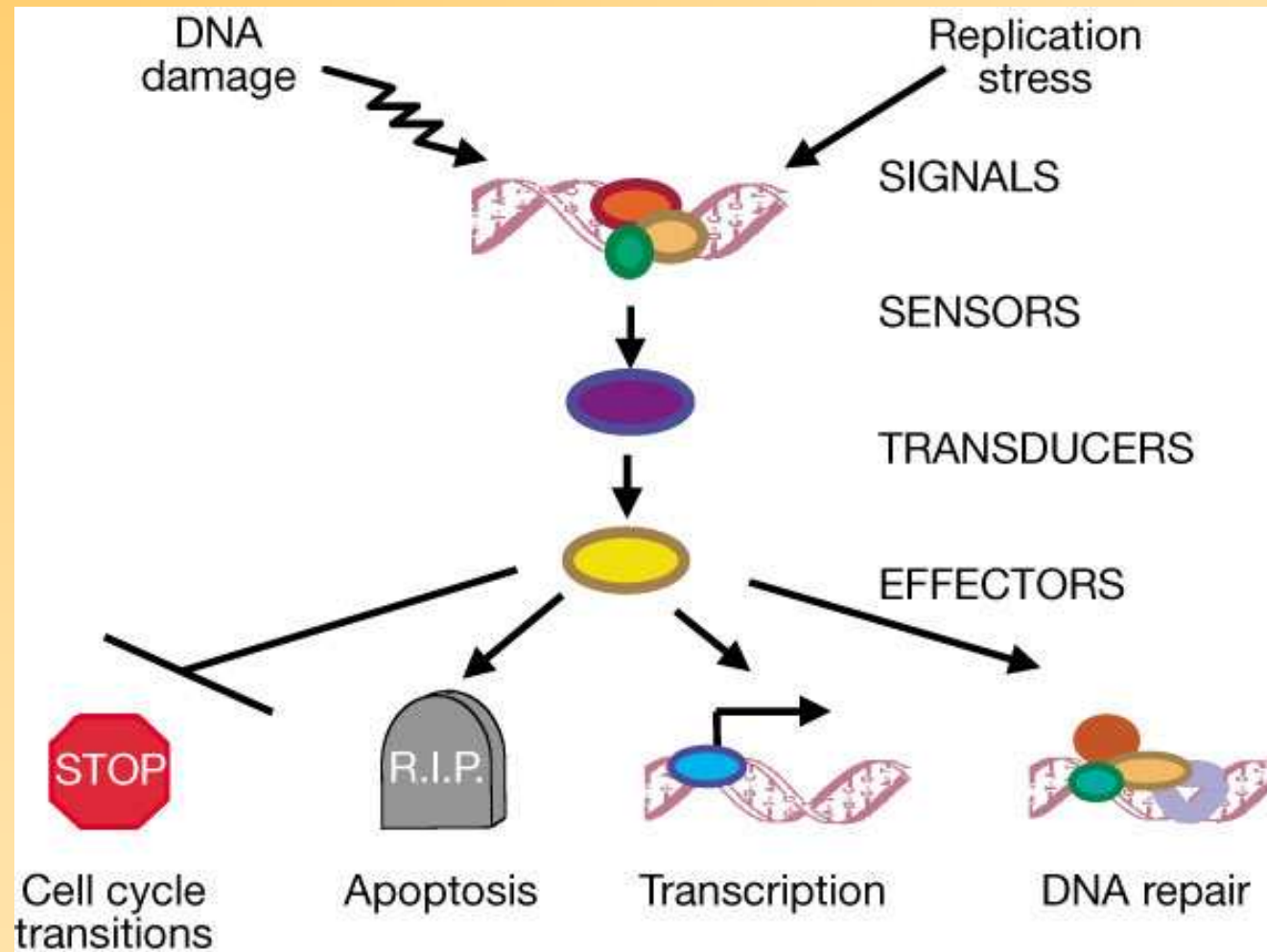


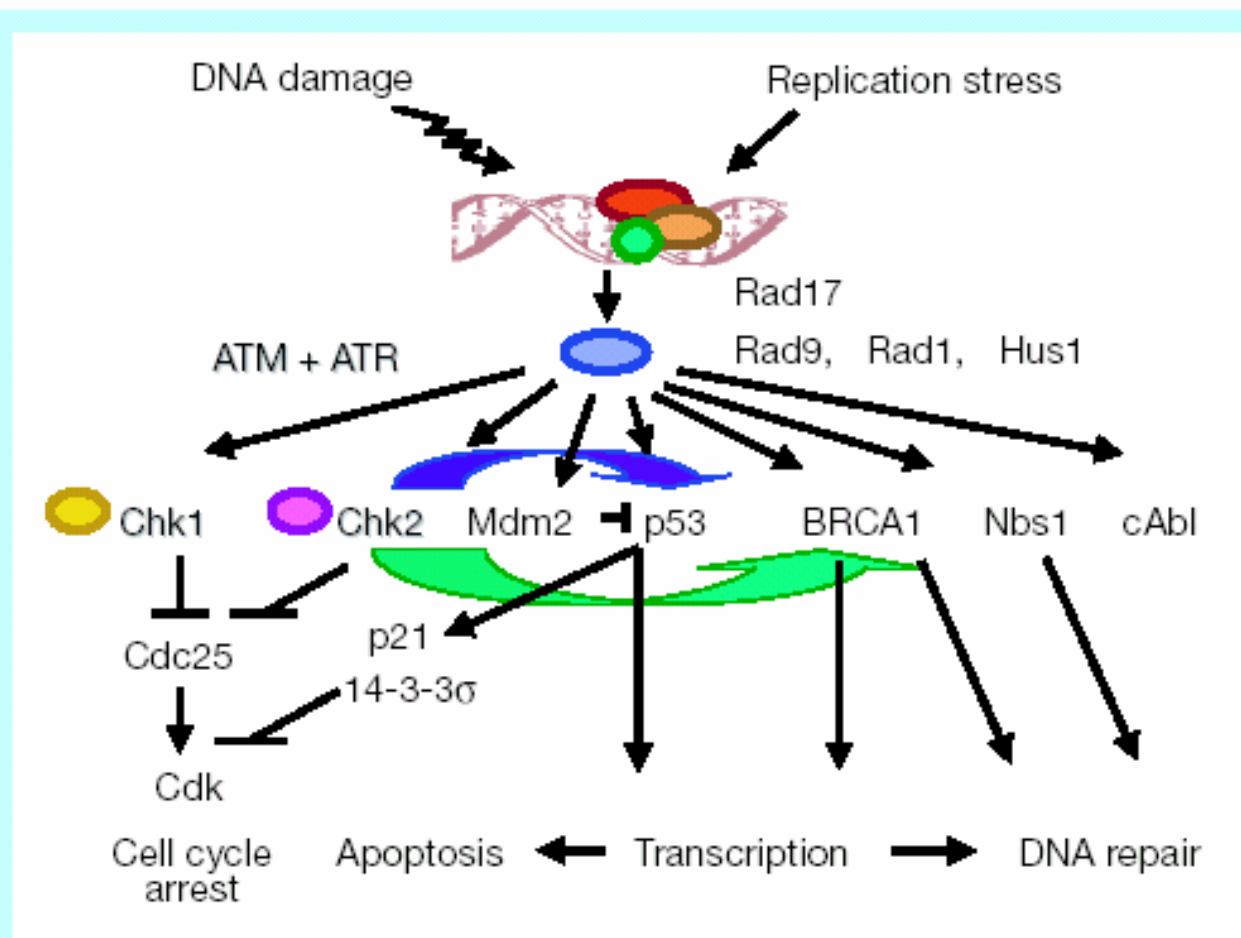
# Cell Cycle Checkpoints Improve Cell Viability



**Fig. 3.** Rapid loss of viability in cells defective both for DNA ligase and for the *RAD9* gene. *cdc9* (●) and *cdc9-rad9* (○) cells growing at 23°C were shifted to the restrictive temperature and viability was determined by plating for viable colonies at the permissive temperature (23°C). The cell viability reported is relative to viability at the time of temperature shift. Results were reproducible in separate experiments and with other congenic strains.

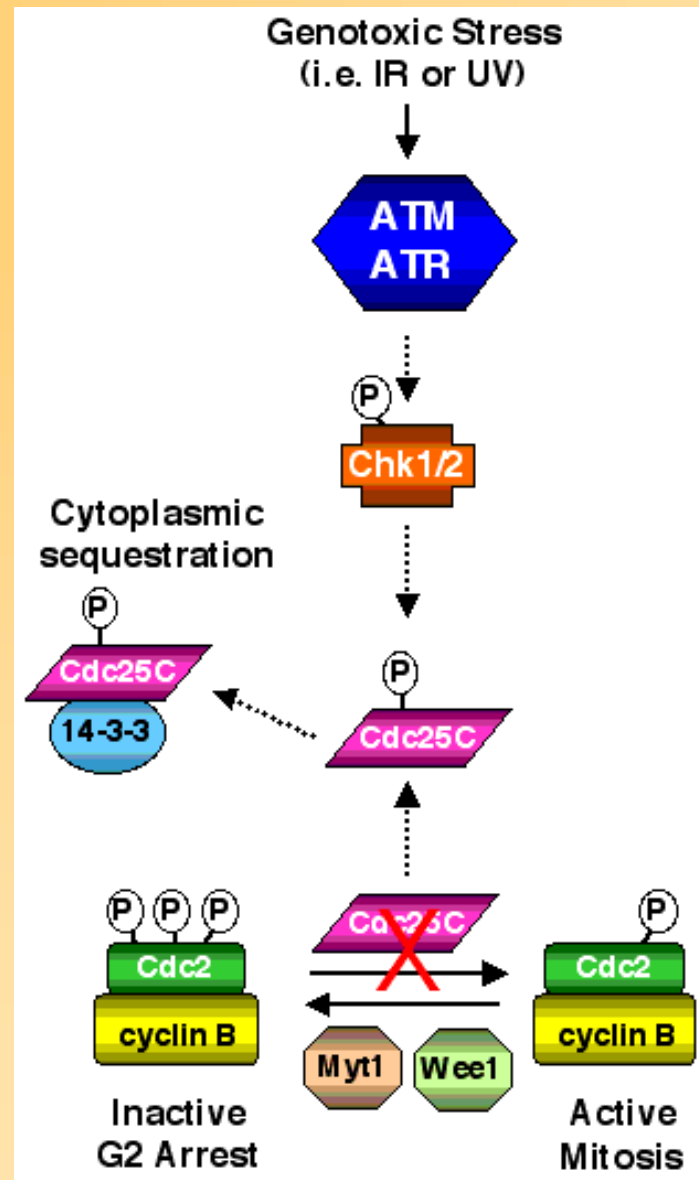
# How do Cell Cycle Checkpoints Work?





**Figure 2** Organization of the mammalian DNA damage response pathway.

Arrowheads represent positively acting steps while perpendicular ends represents inhibitory steps. Gene names are shown at the approximate positions where their encoded proteins function in the pathway. Although the general organization of the pathway is correct, some details are omitted, especially concerning the relationship between the ATR/ATM and Hus1/Rad17/Rad9/Rad1 proteins, which may participate in mutual regulation.

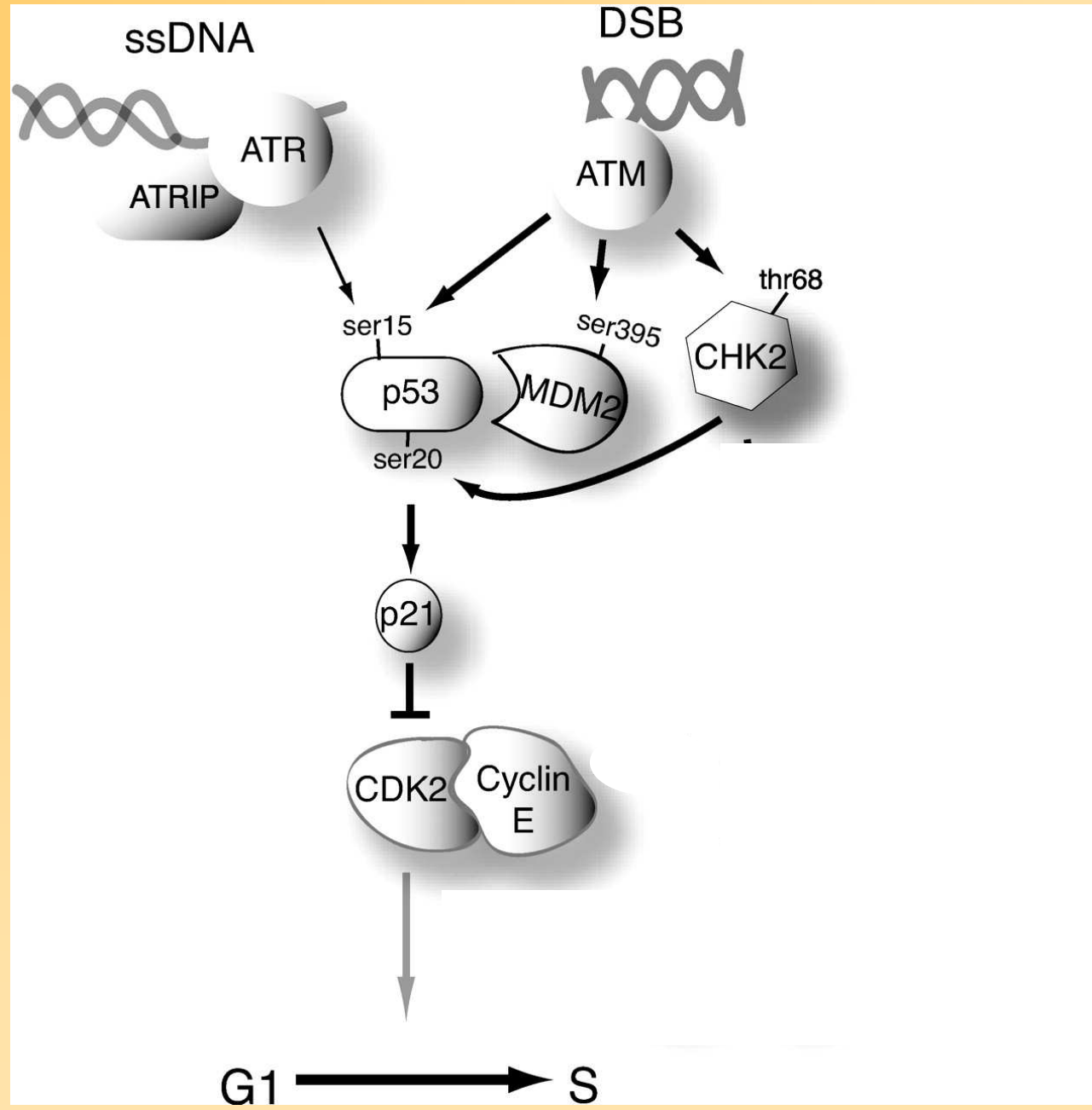


Signal

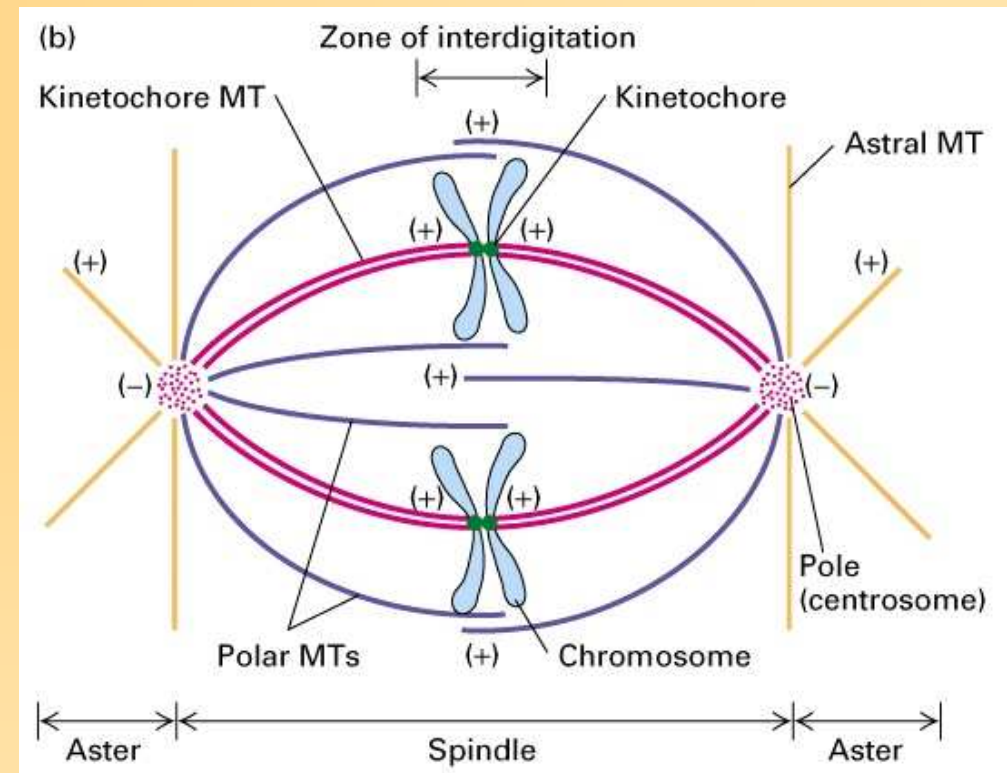
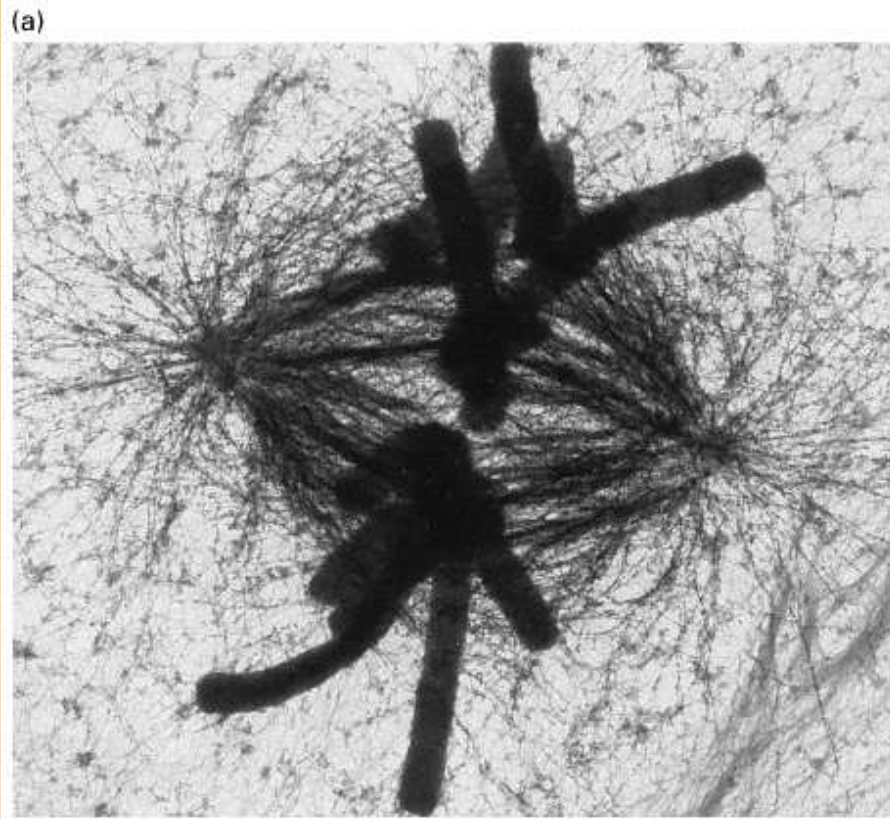
Sensor

Transducer

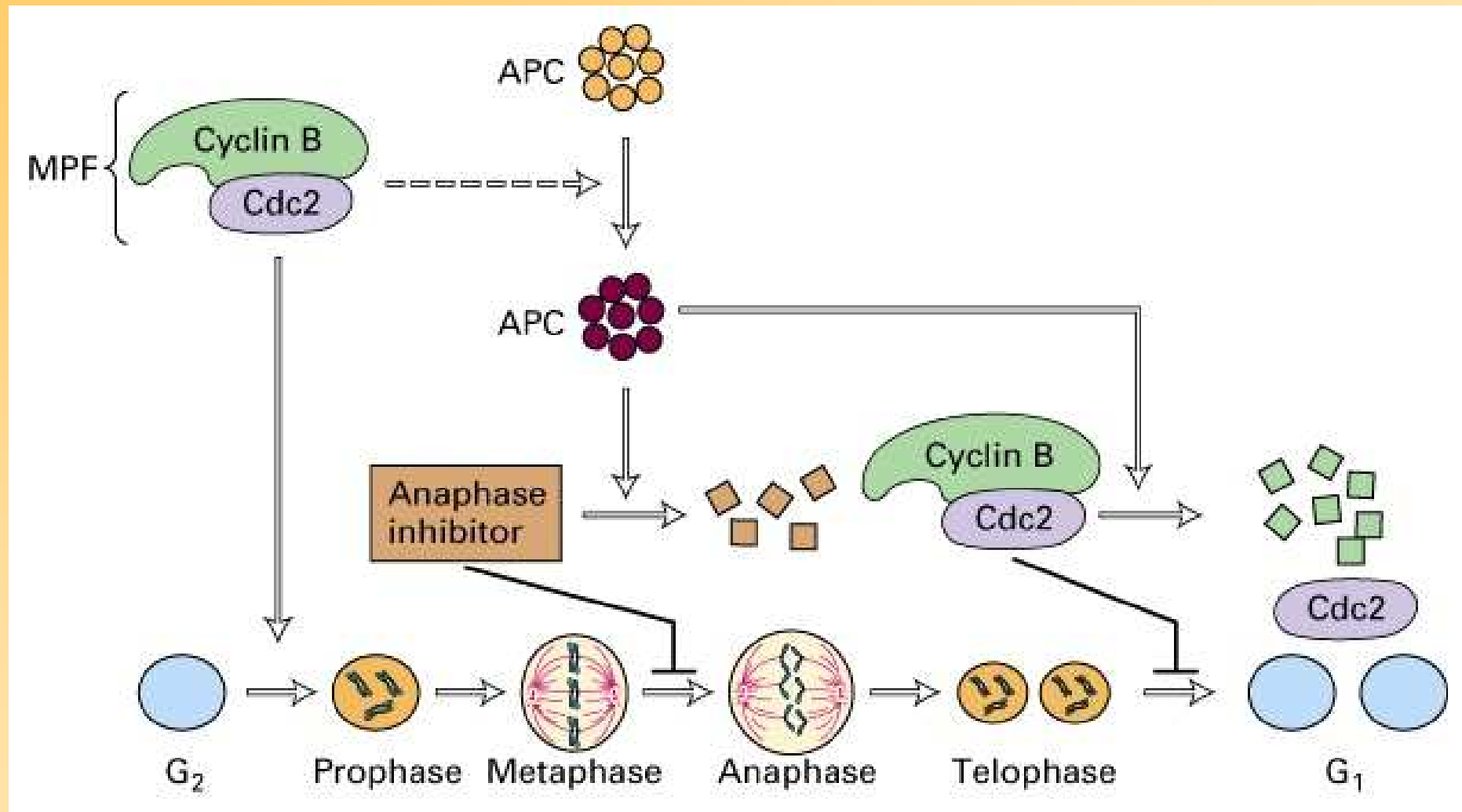
Effector



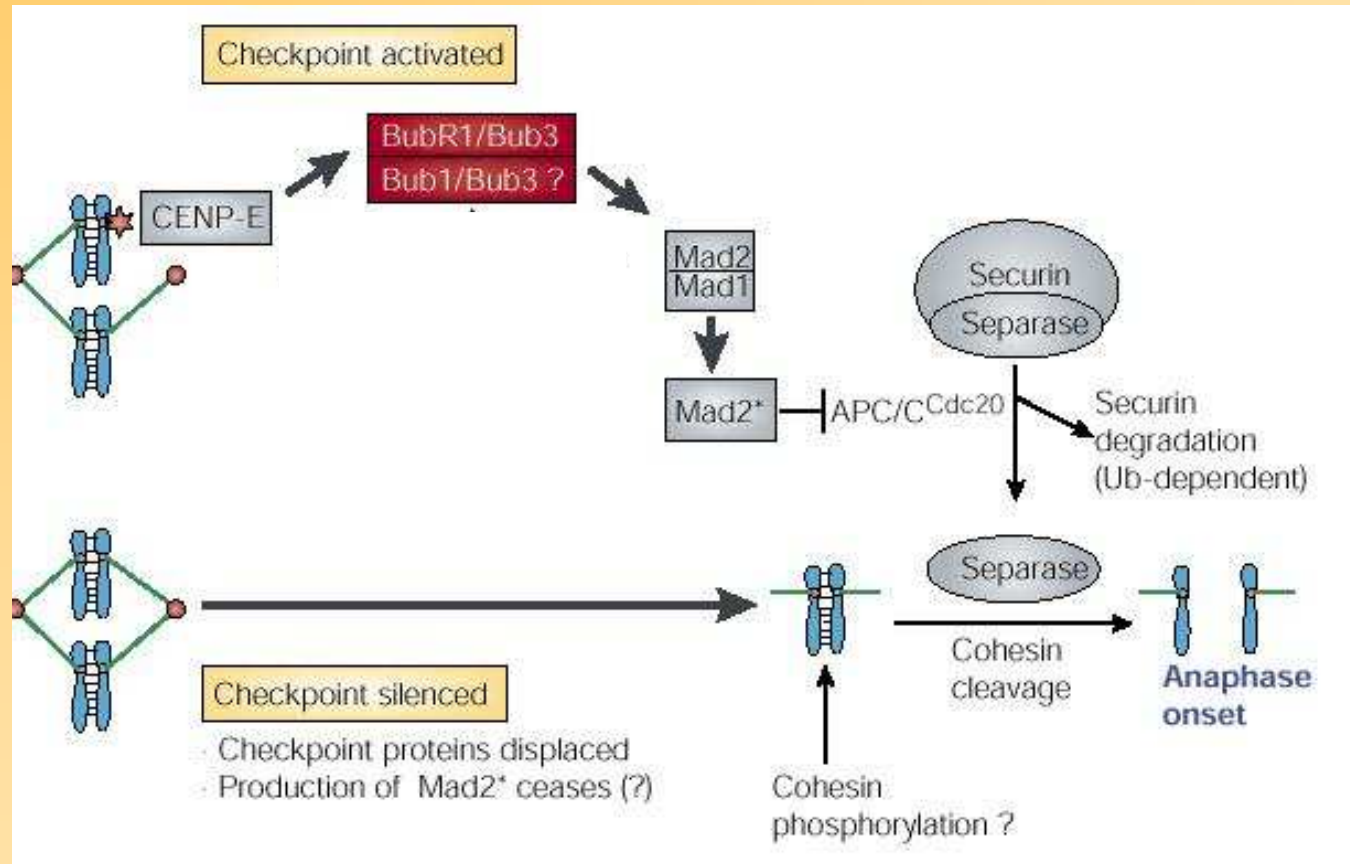
# Metaphase in a mammalian cell



# The Metaphase to Anaphase Transition



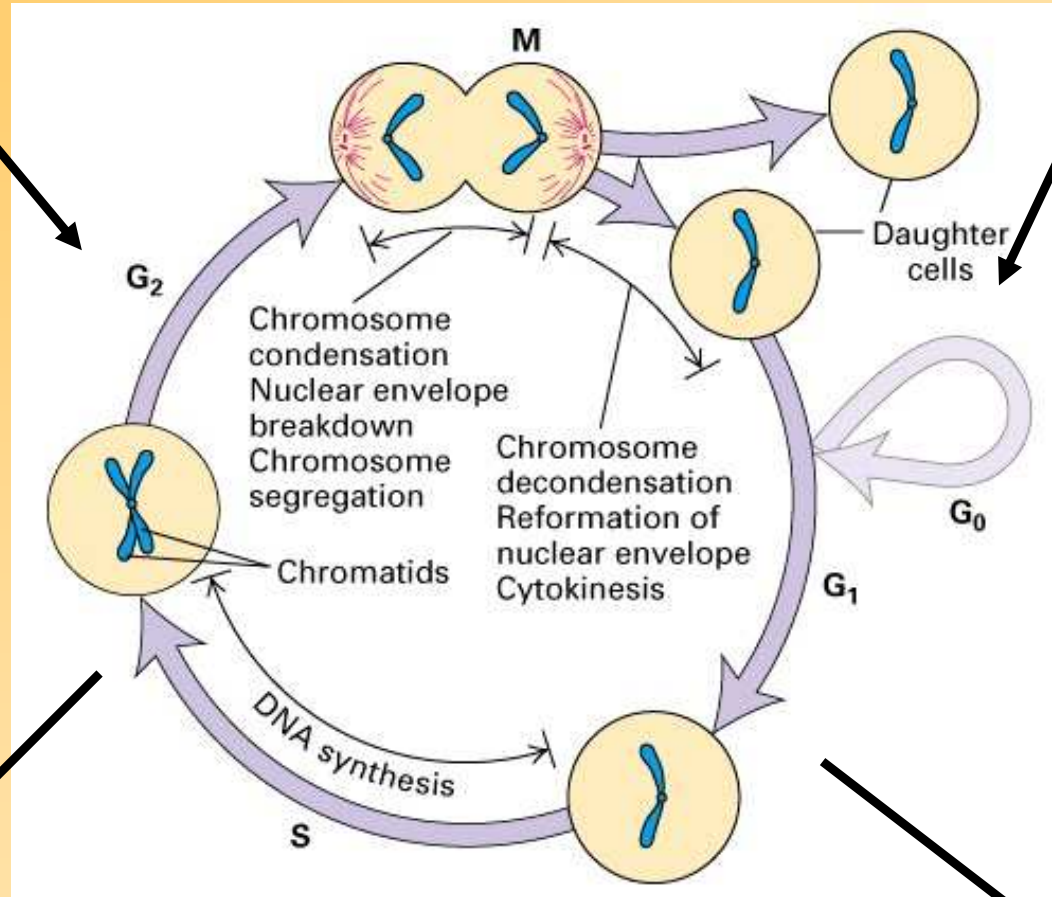
# The Spindle Assembly Checkpoint





poškození DNA  
poruchy buň. cyklu  
poruchy buň. dělení  
nedostatek živin a růstových faktorů  
kontaktní inhibice  
vliv ECM

dostatek živin a růstových faktorů  
mitogeny



zástava buň. cyklu  
(restriction point,  
checkpoints)  
setrvání v G<sub>0</sub> fázi  
apoptóza

proliferace