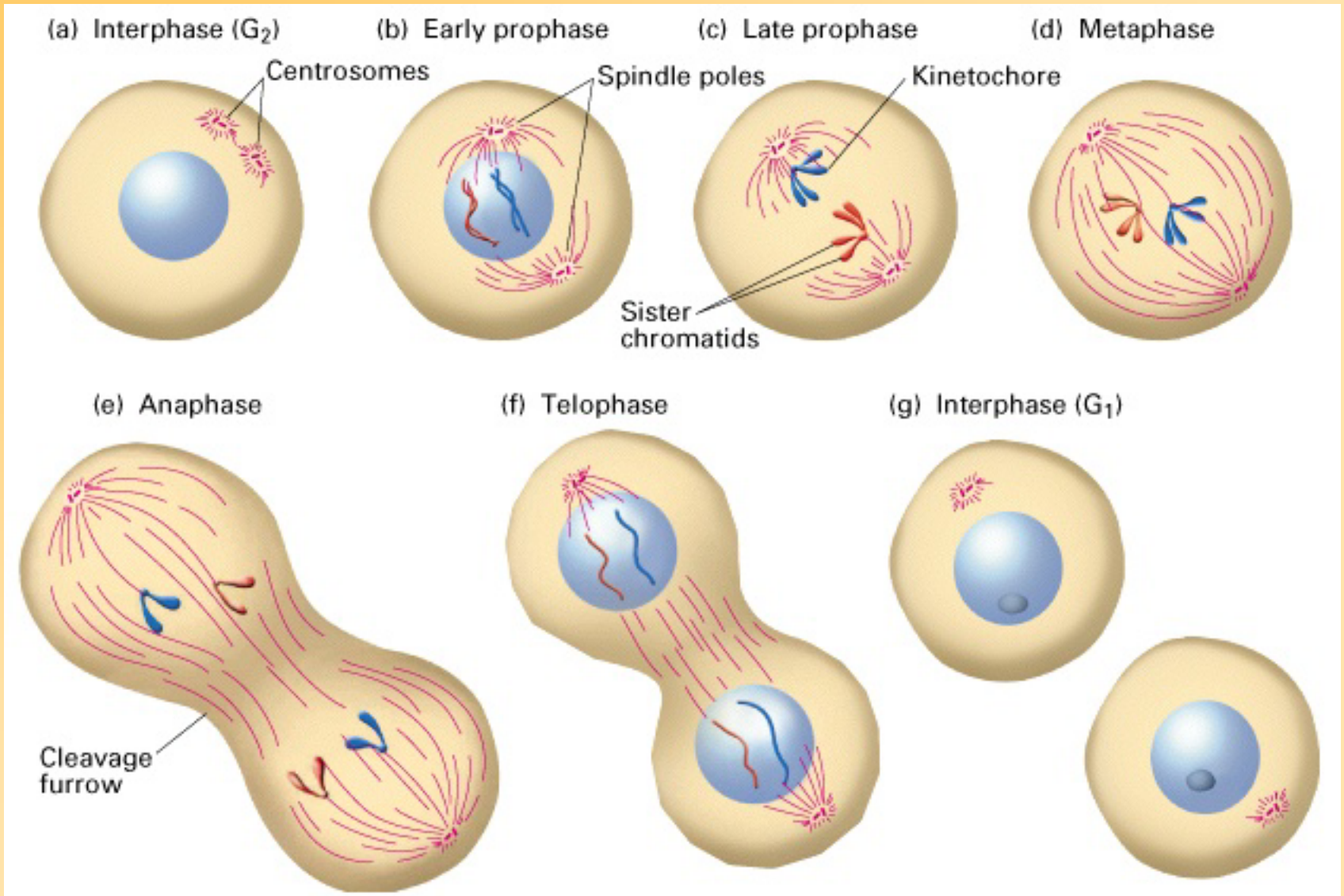


Buněčný cyklus - principy regulace buněčného růstu a buněčného dělení

Mitóza



Buněčný cyklus

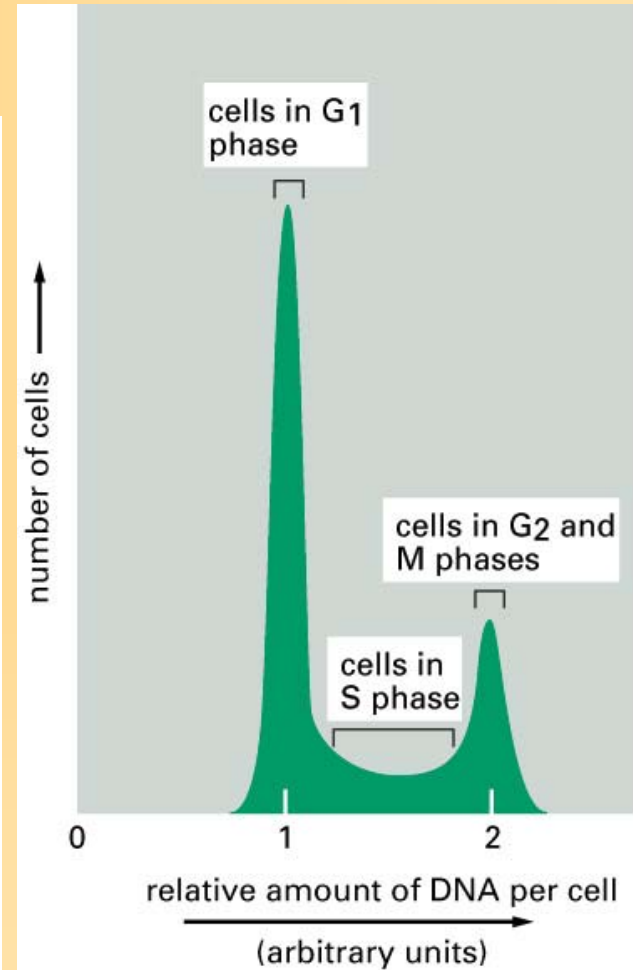
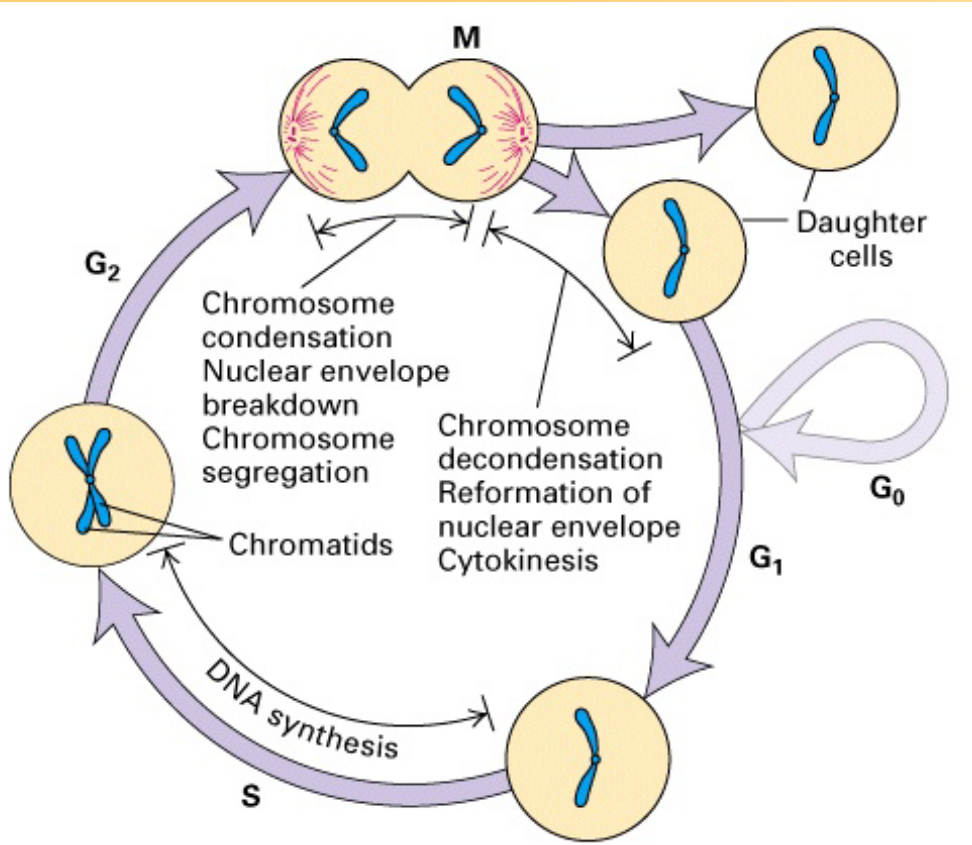
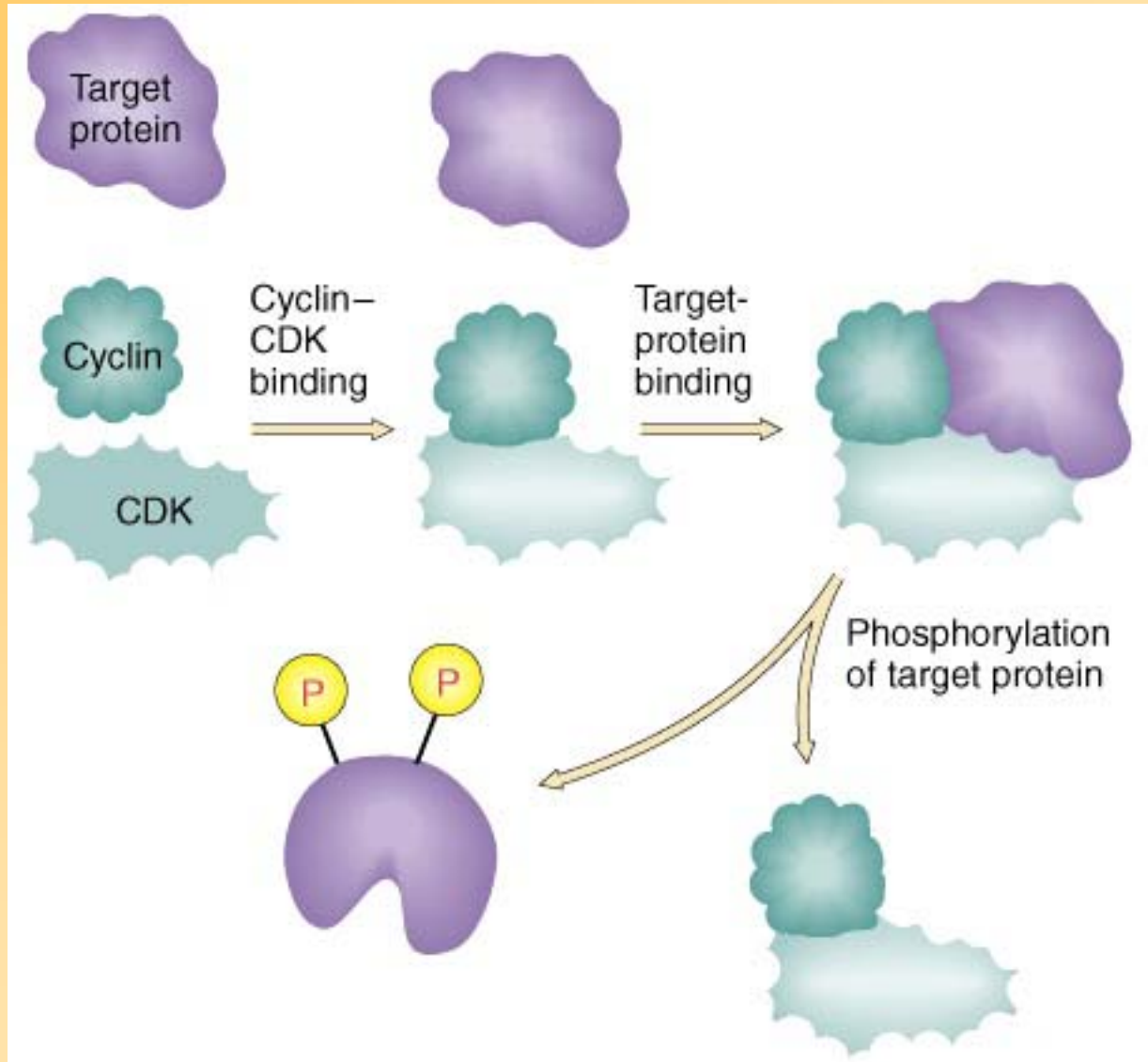


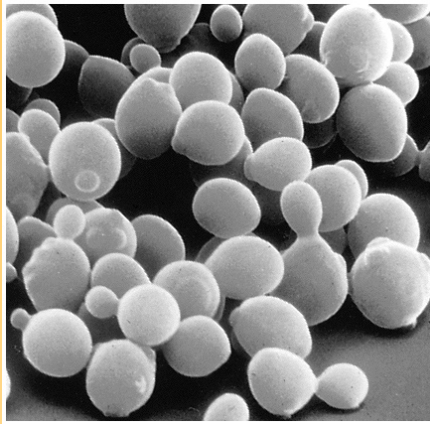
Figure 17-12. Molecular Biology of the Cell, 4th Edition.

Kinázy závislé na cyklinech kontrolují buněčný cyklus

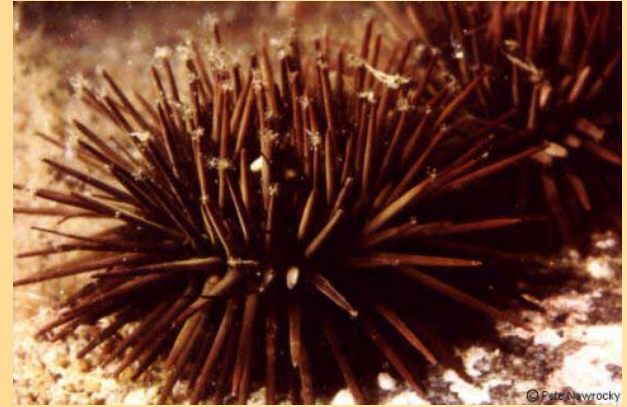


Kontrola vstupu do mitózy
- výsledek využití různých buněčných modelů

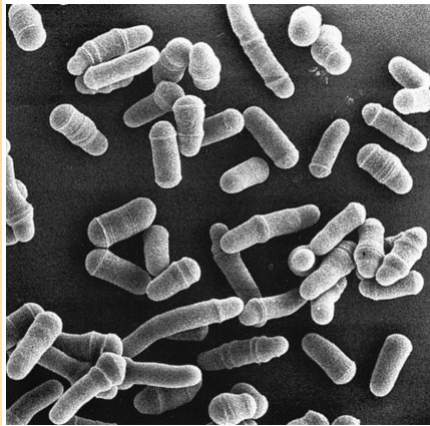
Experimental Systems Important for Cell Cycle Studies



Saccharomyces cerevisiae



Arbacia punctulata

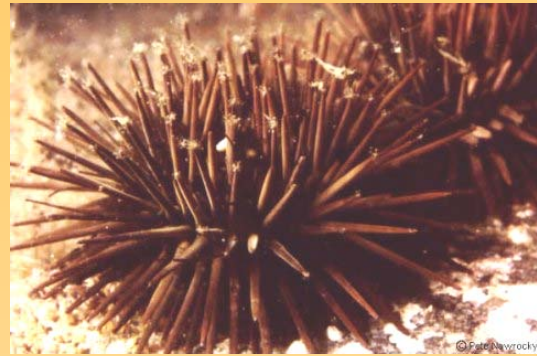


Schizosaccharomyces pombe

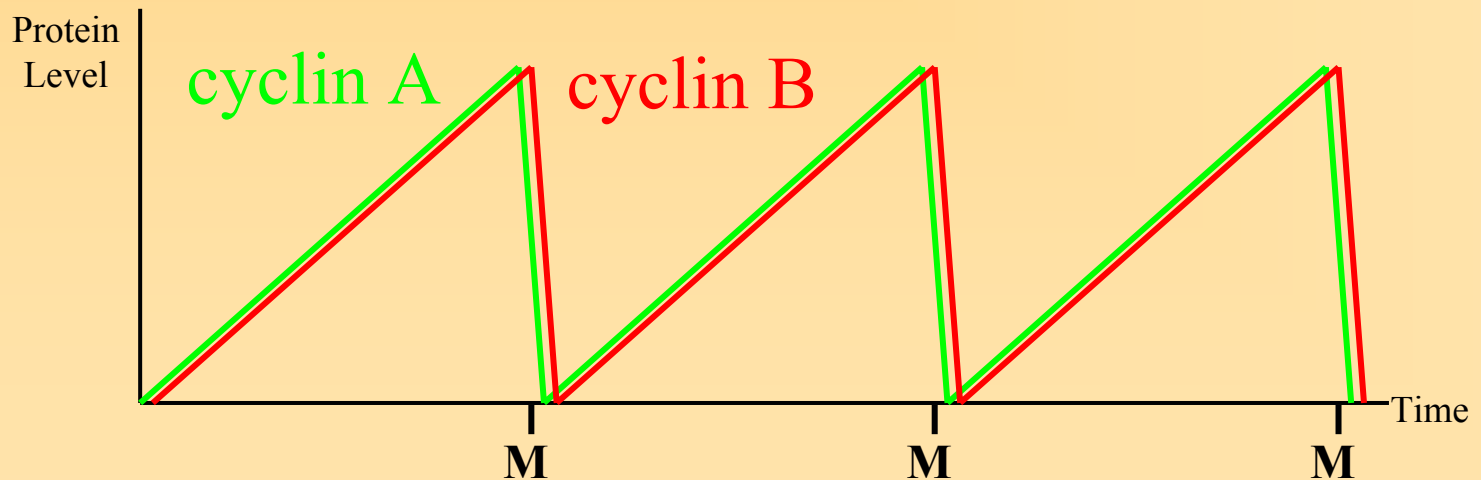
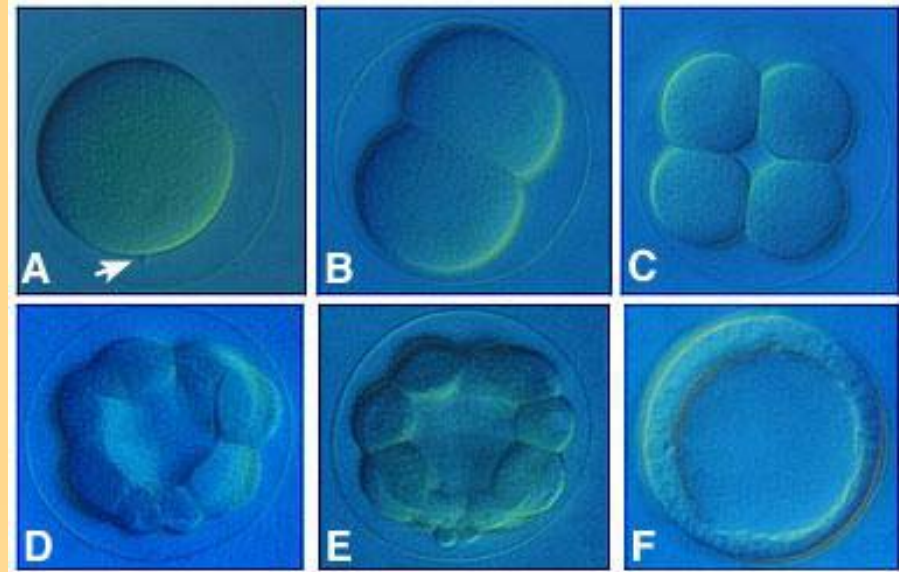


Xenopus laevis

Cyclin was Discovered in Sea Urchin Embryos



can stimulate to
→
lay lots of eggs

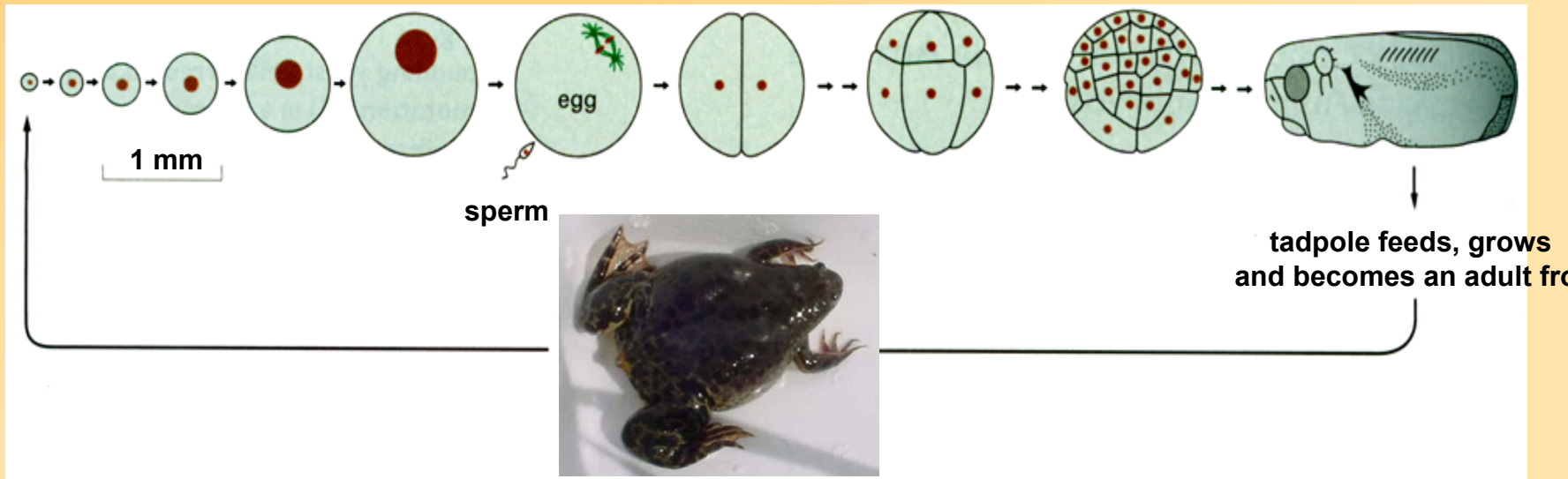


Frog life cycle

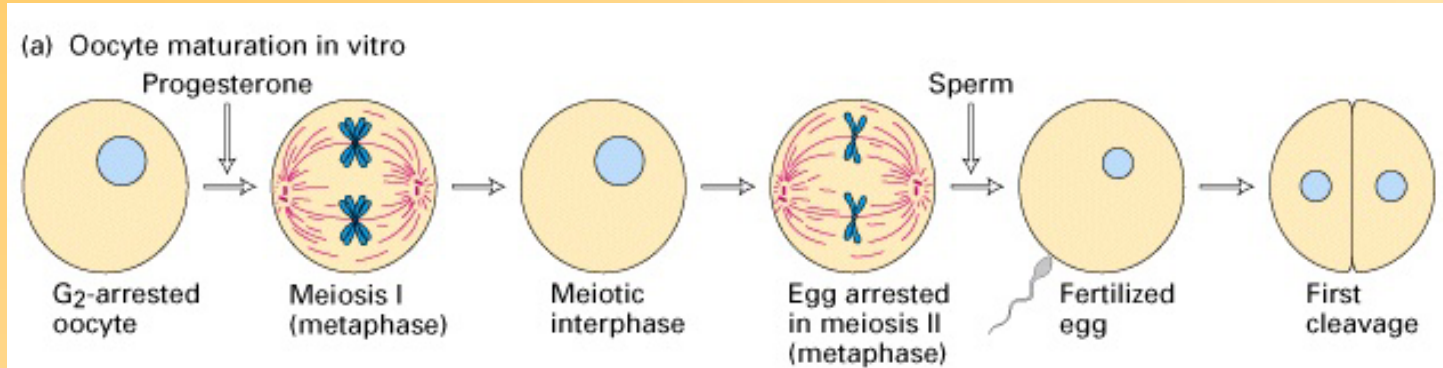
OOCYTE GROWS WITHOUT DIVIDING
(MONTHS)

FERTILIZATION

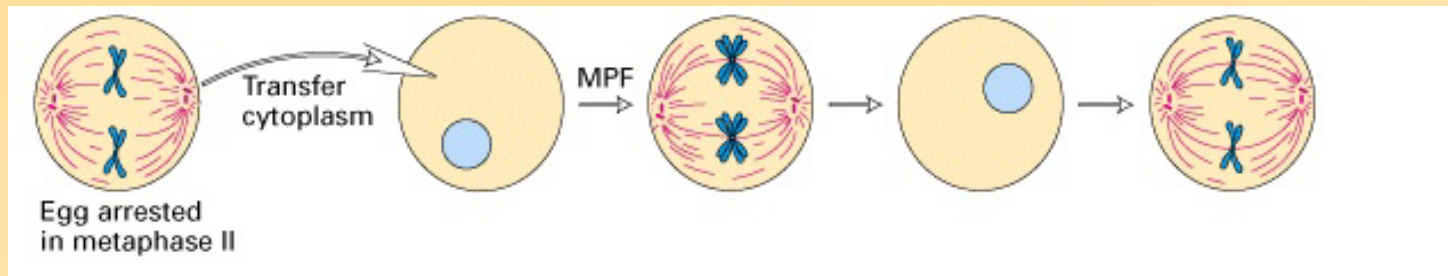
FERTILIZED EGG DIVIDES WITHOUT GROWING
(HOURS)



The Maturation of Frog Eggs

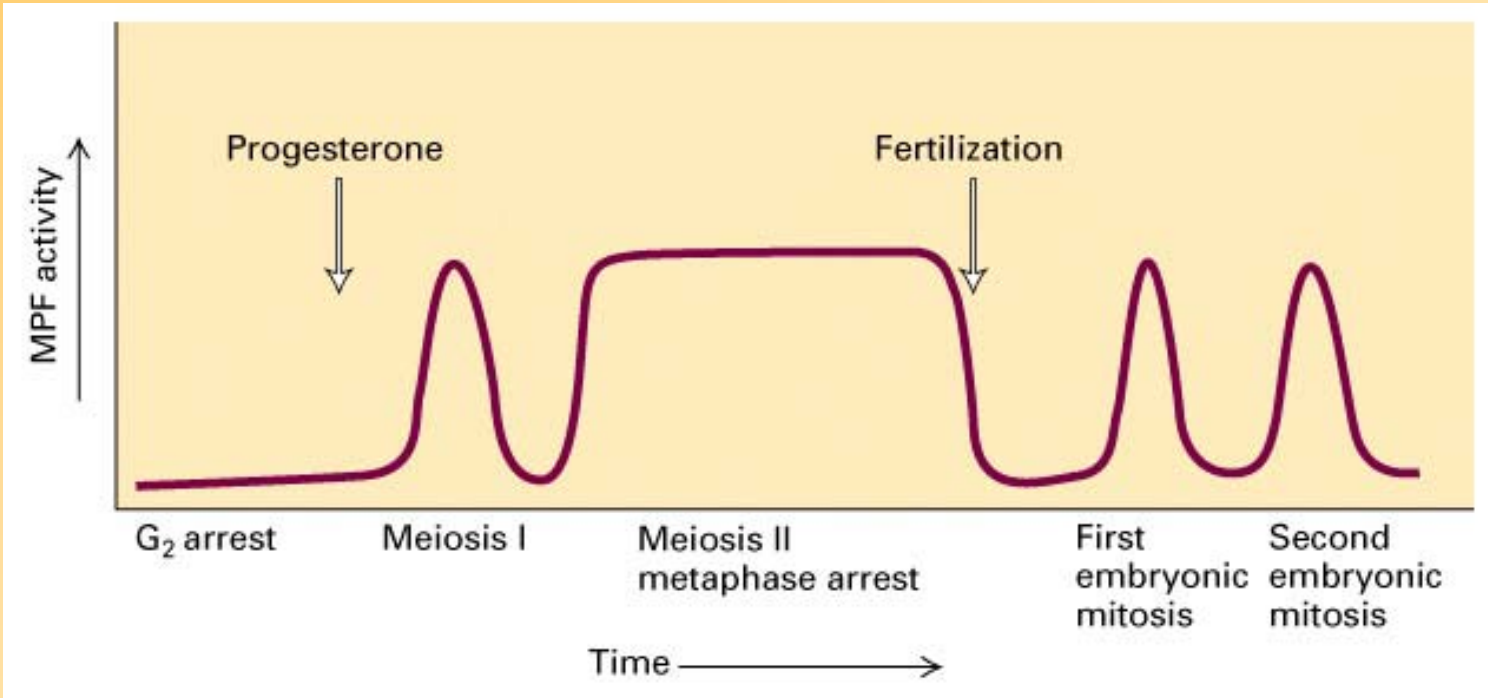


An Assay for Maturation Promoting Factor (MPF)



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)

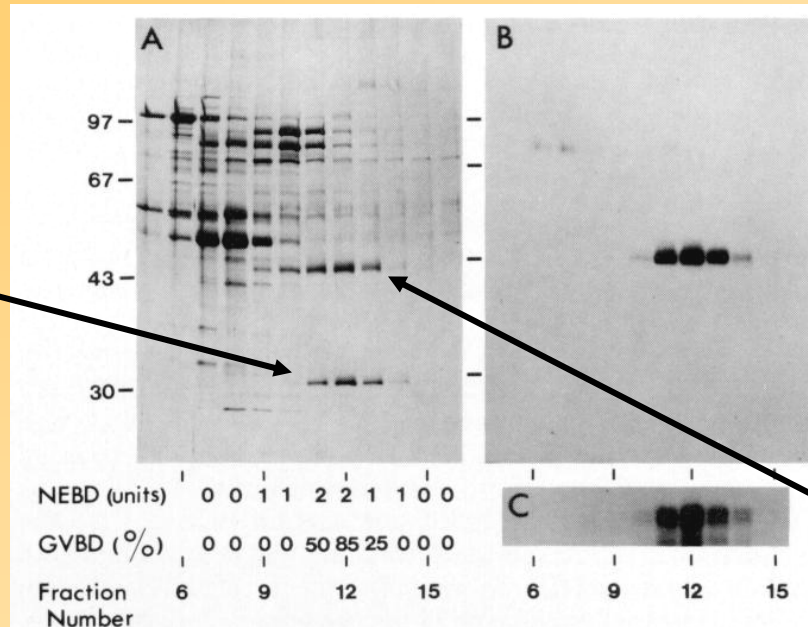
MANFRED J. LOHKA*, **MARIANNE K. HAYES†**, AND **JAMES L. MALLER**

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

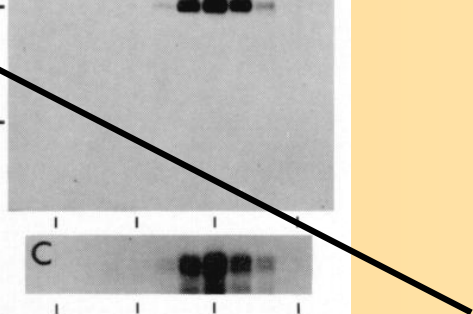
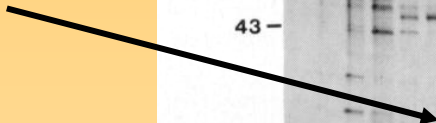
Communicated by Raymond L. Erikson, December 22, 1987 (received for review October 10, 1987)

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*⁺!!
(Cdc28 in *S. cerevisiae*)

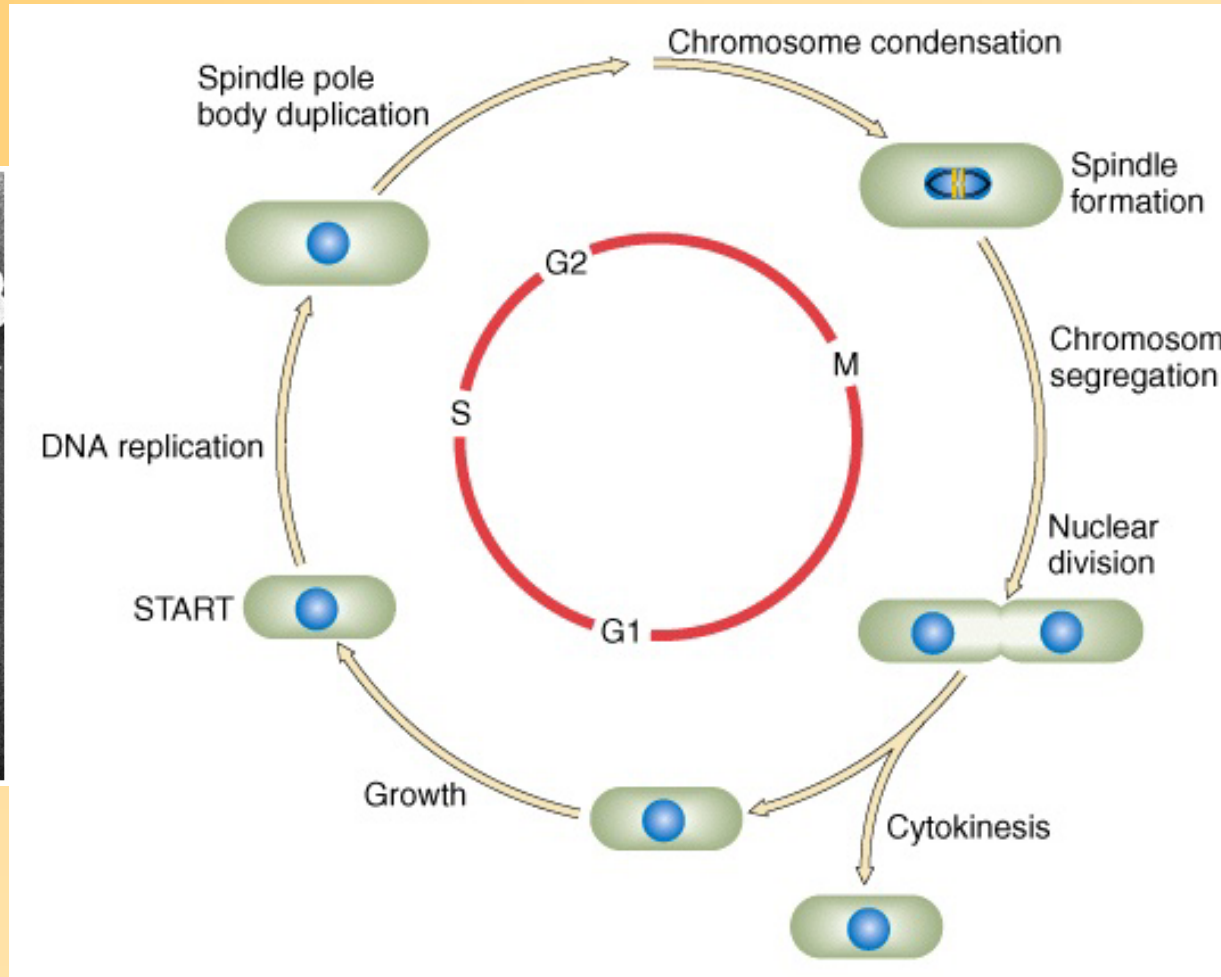
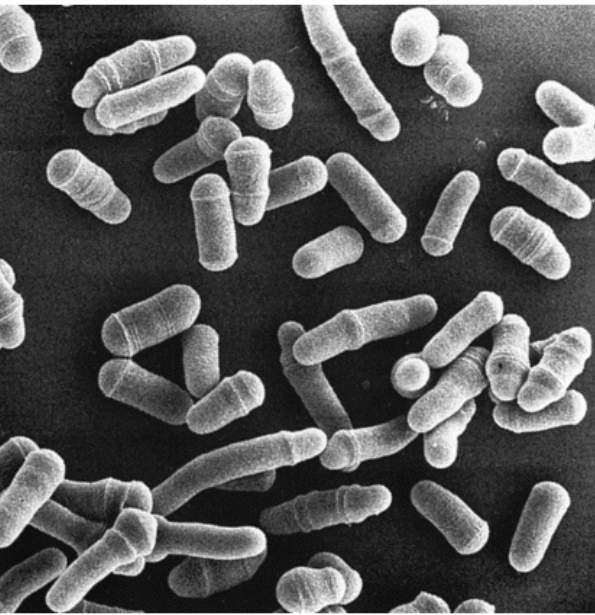


This is cyclin!!

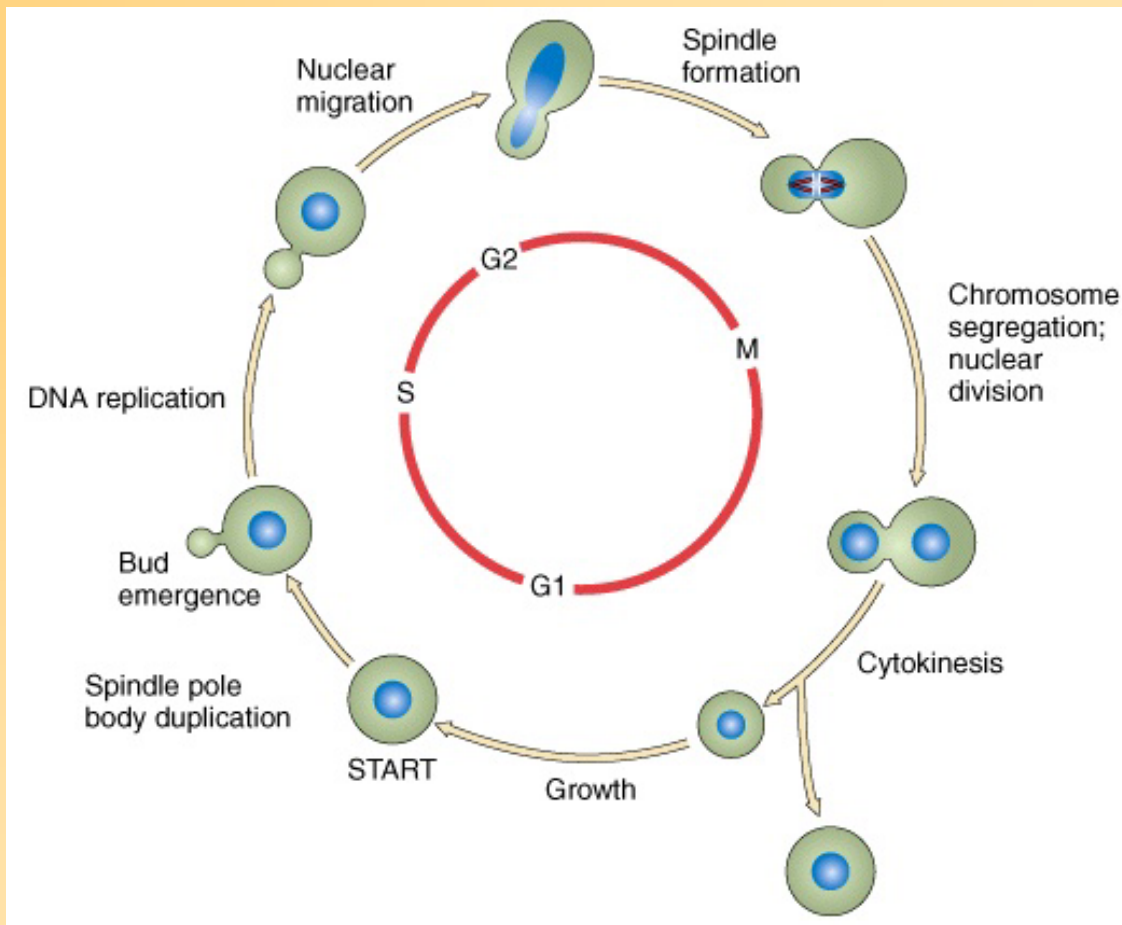
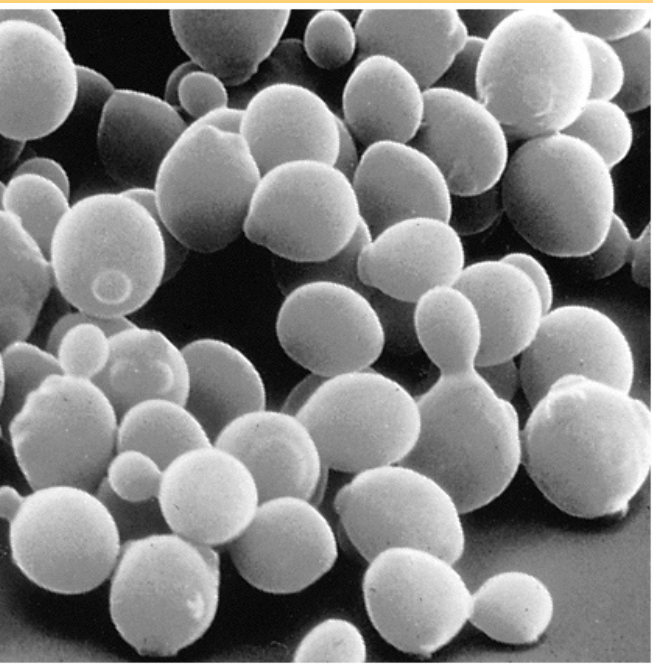
Which = *cdc13*⁺
in *S. pombe*

FIG. 2. Polyacrylamide gel analysis of fractions eluting from the Mono S column. A 45- μ l aliquot of fractions 5-16 was incubated with [γ -³²P]ATP and electrophoresed through a 10% NaDodSO₄/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 μ l. GVBD is expressed as the percentage of oocytes that underwent GVBD during a 2-hr incubation in cycloheximide (0.5 μ 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 ≈ 270 nmol \cdot min⁻¹ \cdot mg⁻¹.

Fission yeast: *Schizosaccharomyces pombe*

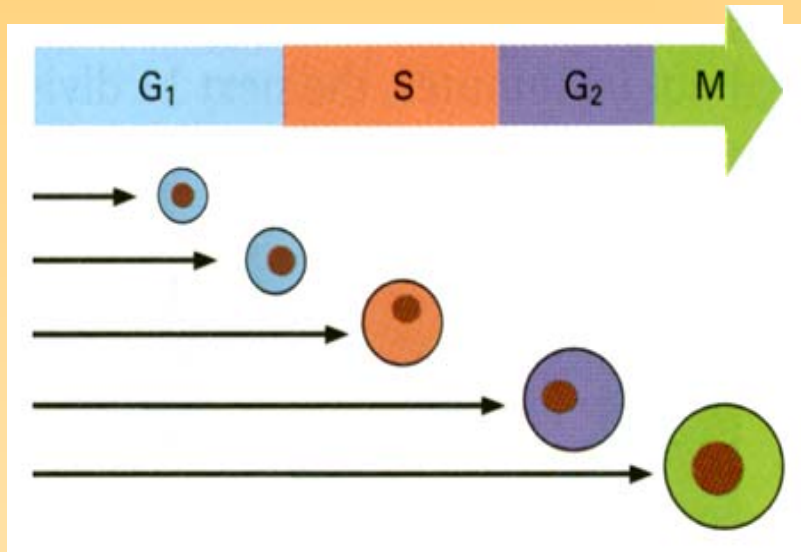


Budding Yeast *Saccharomyces cerevisiae*

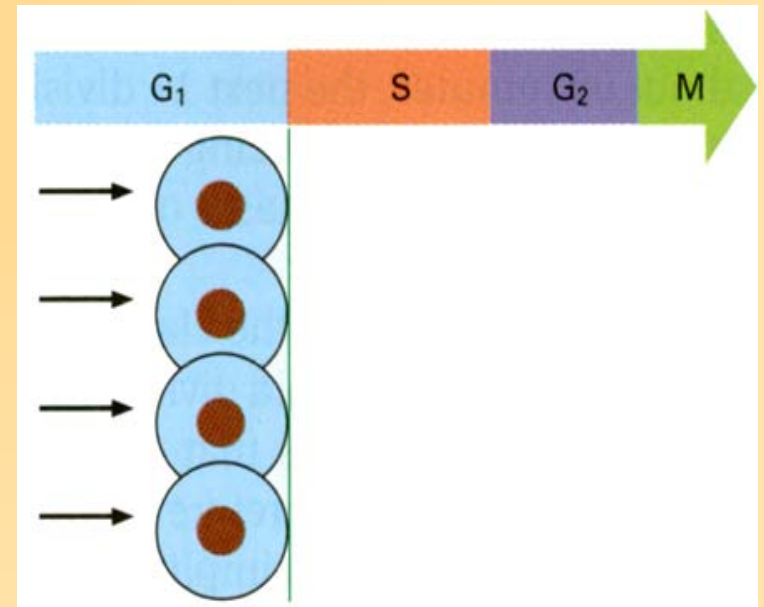


Cdc Mutants Arrest at the Same Cell Cycle Phase

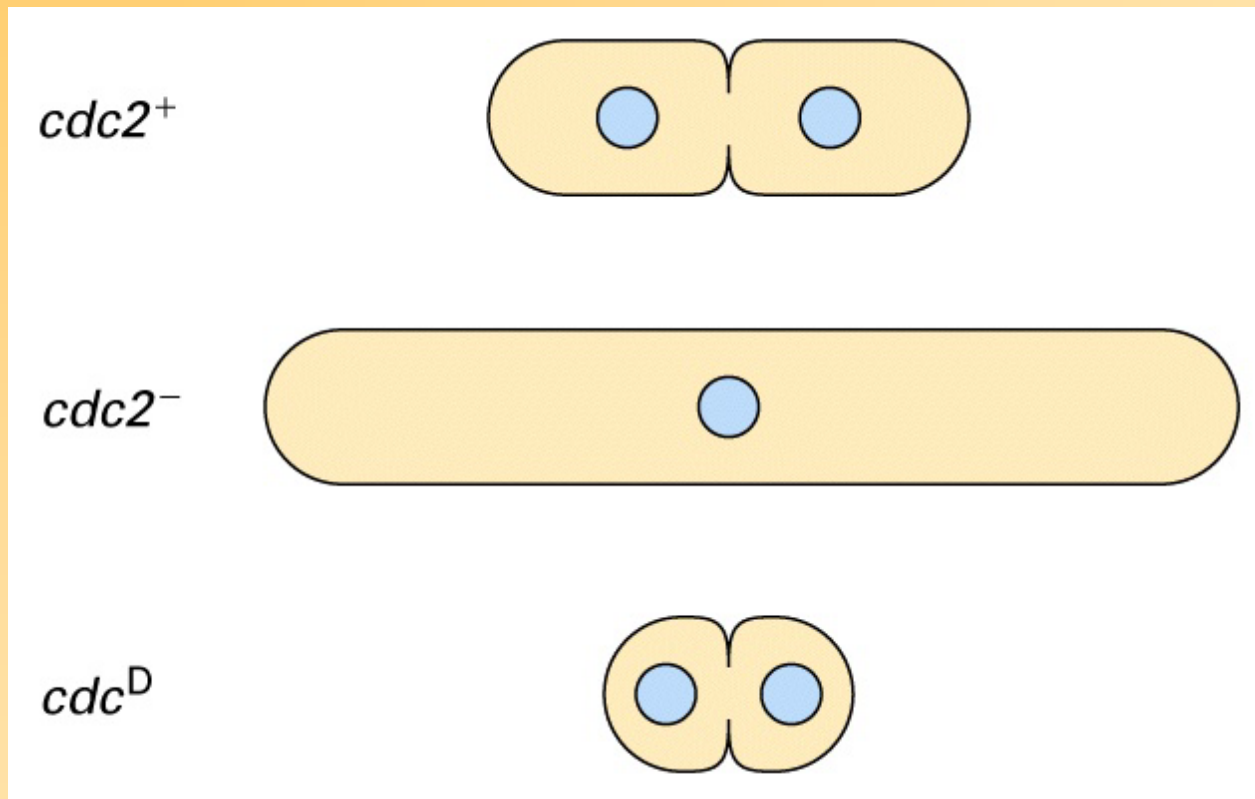
Permissive (low) temperature



Restrictive (high) temperature

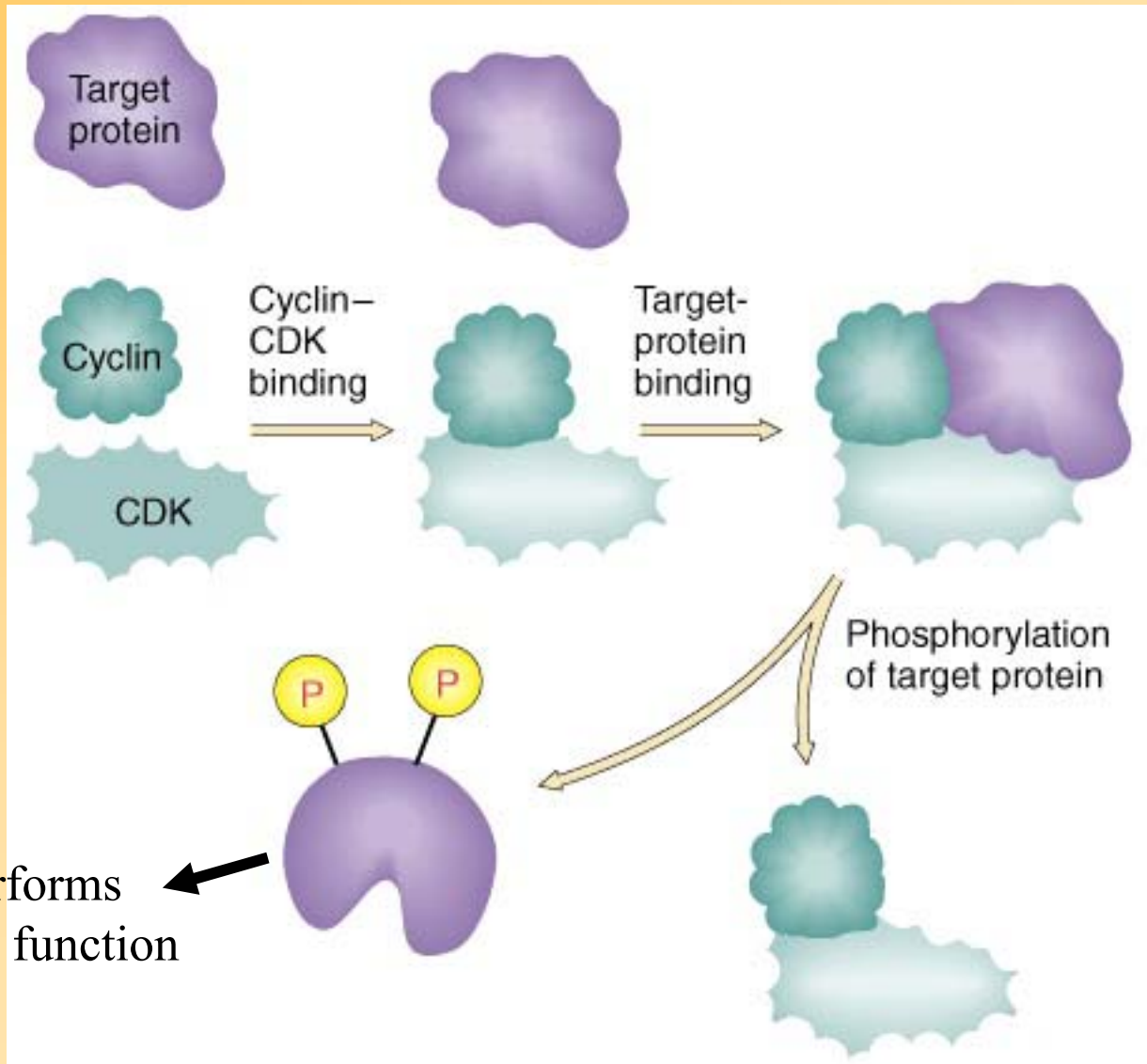


Cdc Genes Encode Proteins Needed for the G2-M Transition: Studies in *S. pombe*



cdc2⁺ encodes a kinase
Moreover = *cdc28* in *S. cerevisiae*!

Phosphorylation of CDK Targets Changes Their Activity



Now performs
a cell cycle function

Jak jsou CDK regulovány?

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

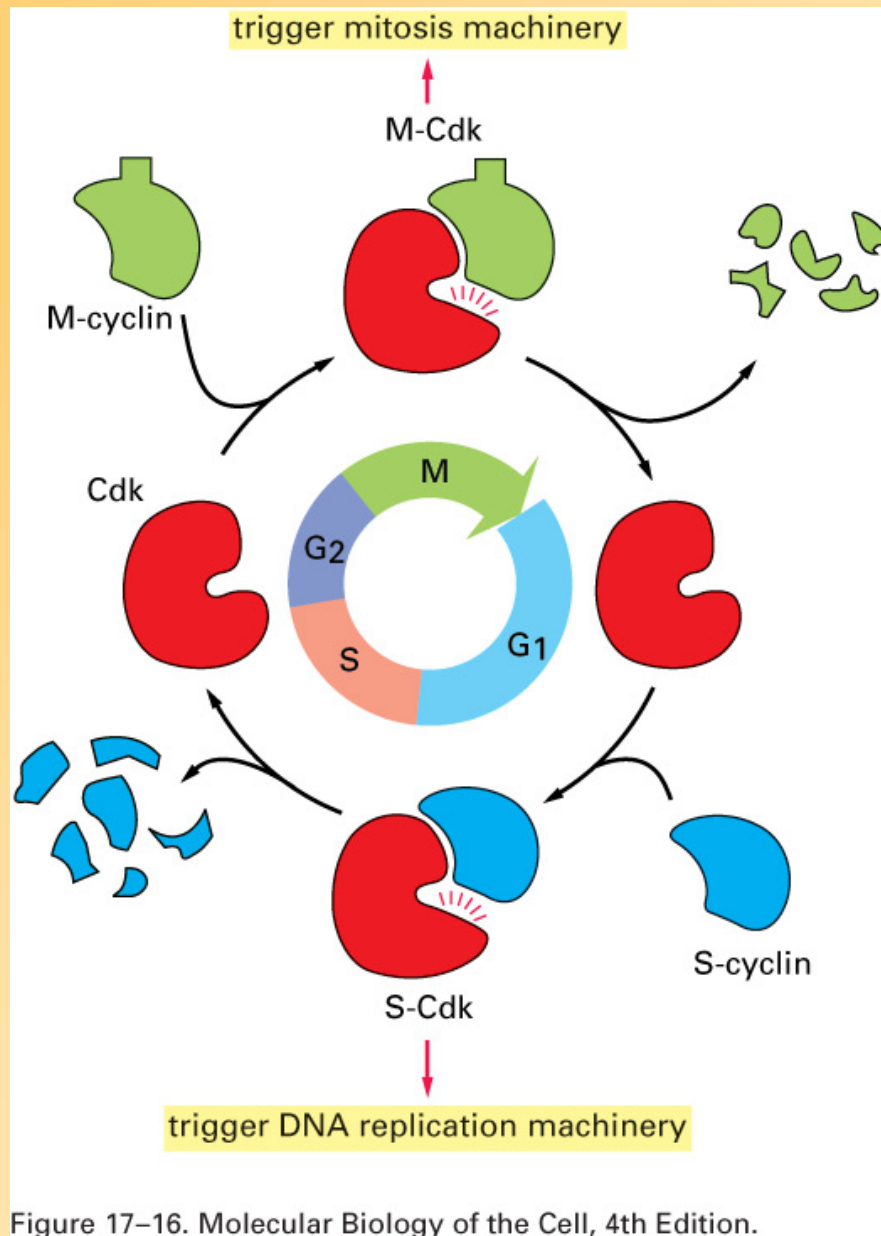
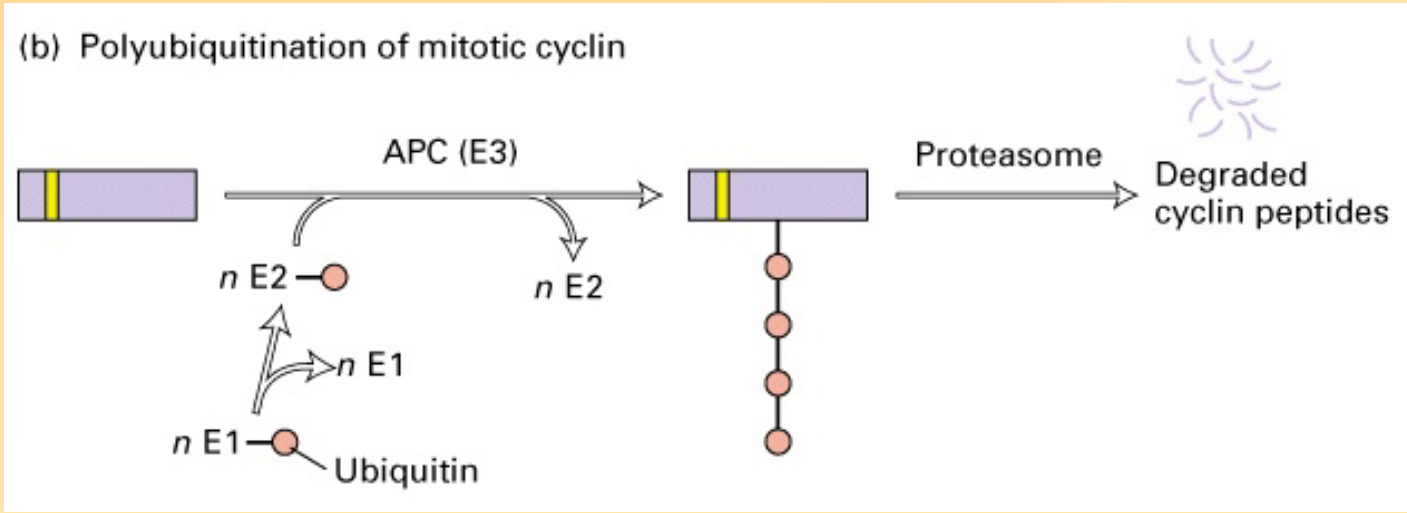
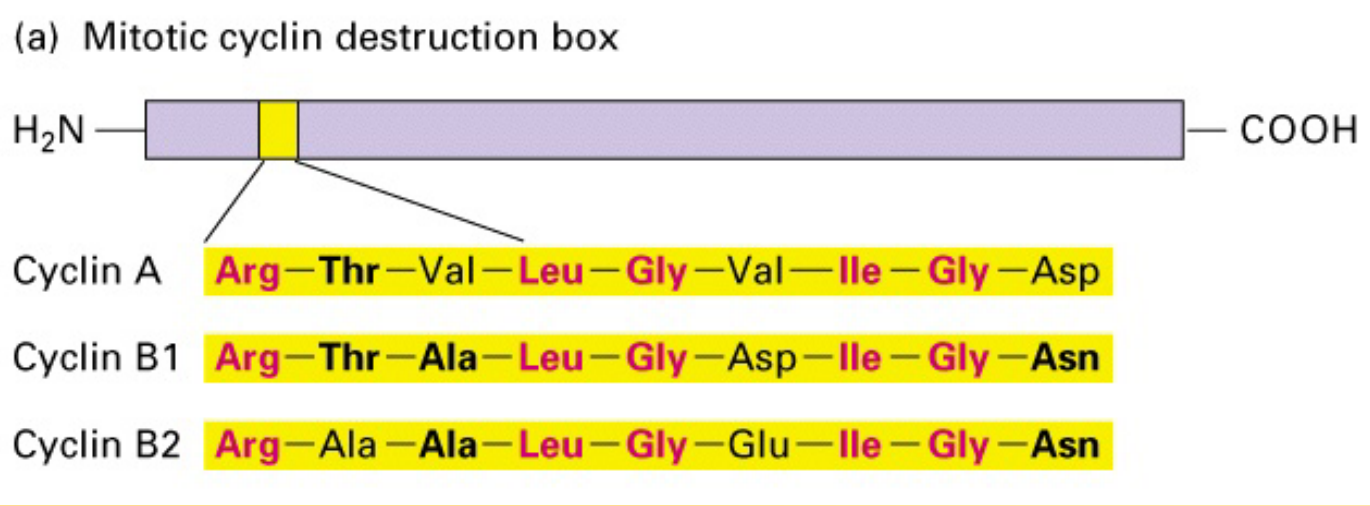


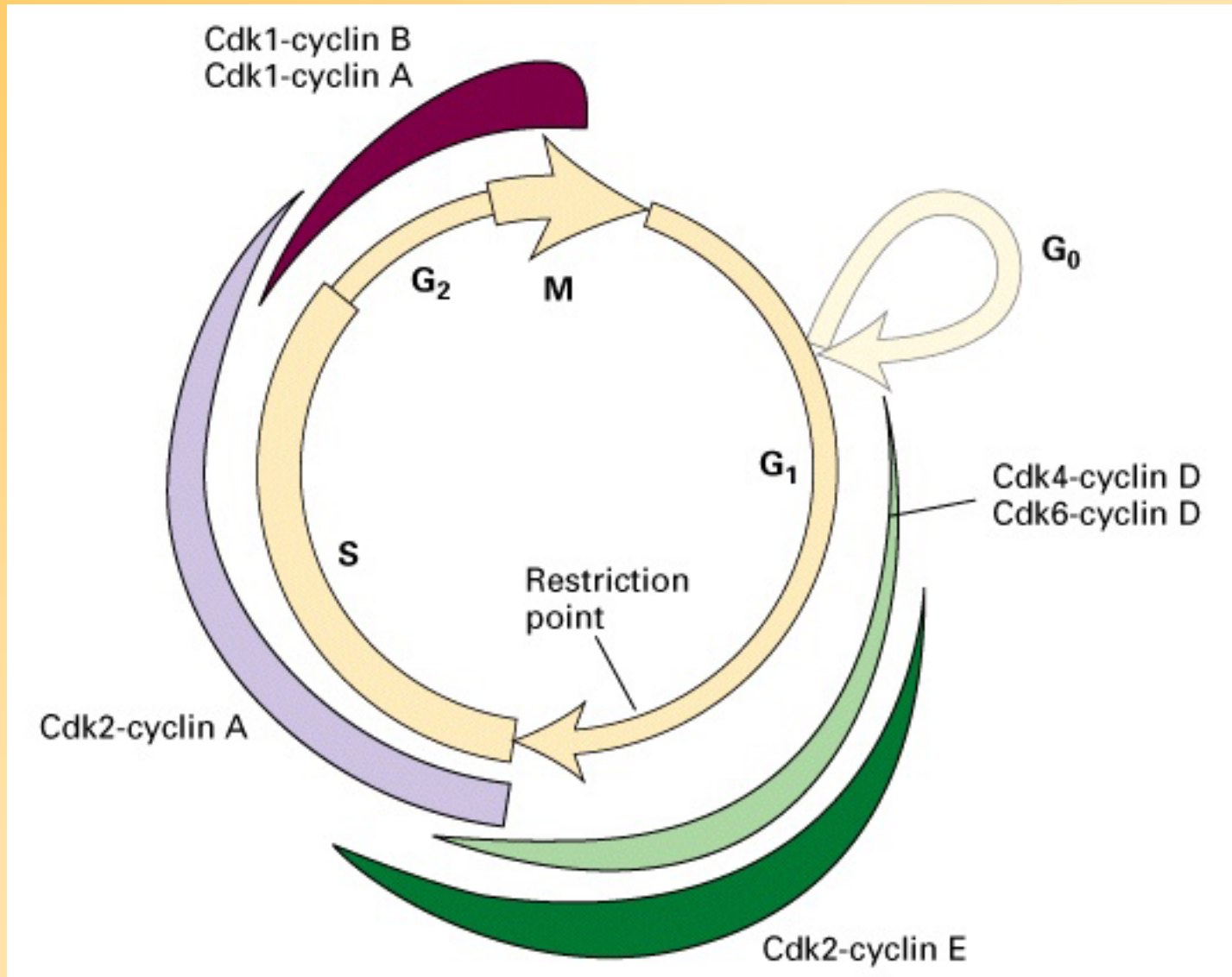
Figure 17-16. Molecular Biology of the Cell, 4th Edition.

Cyclin Destruction is Controlled by Ubiquitination



Představují cykliny jediný způsob regulace CDK?

Expresse cyklinů v jednotlivých fázích BC



CDK jsou regulovány fosforylací

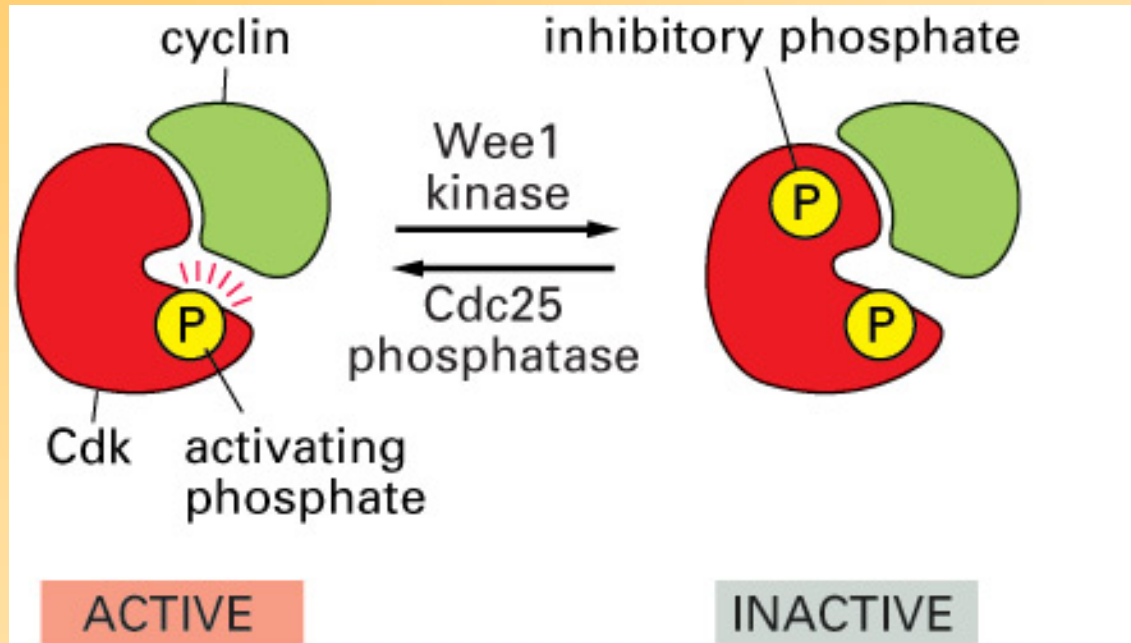
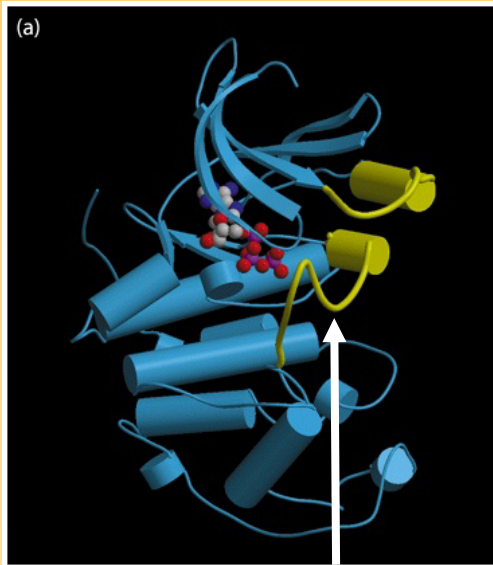


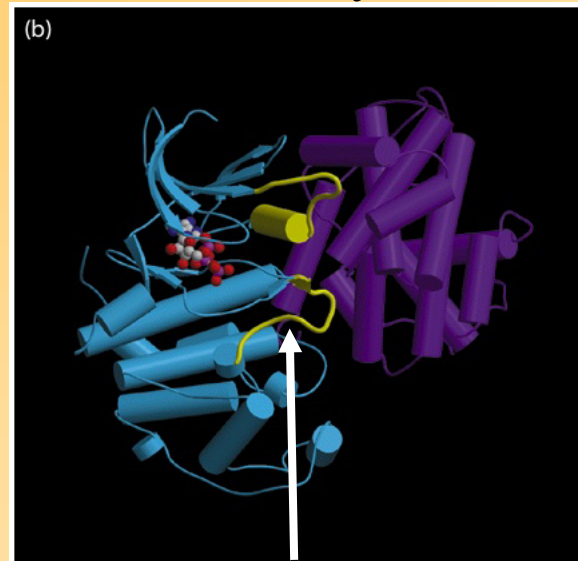
Figure 17–18. Molecular Biology of the Cell, 4th Edition.

Conformational Changes Associated with CDK Phosphorylation

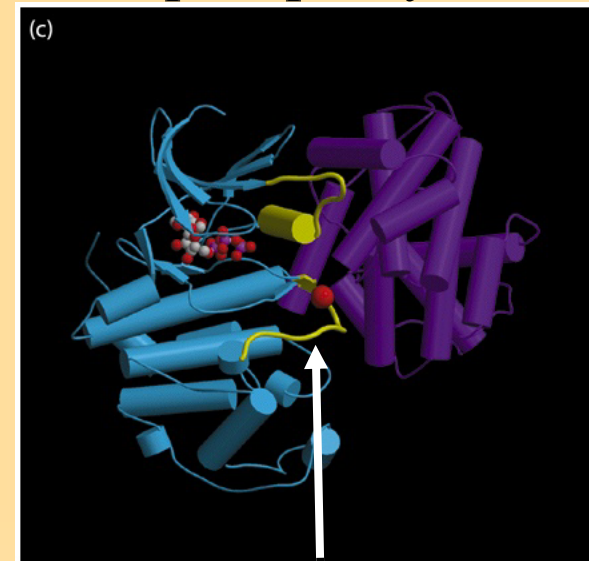
Free CDK



CDK + Cyclin



T161 phosphorylation



The T-loop blocks substrate access

Binding of cyclin moves the T-loop

Phosphorylation moves the T-loop more

Cyclin Dependent Kinase Inhibitors (CKIs)

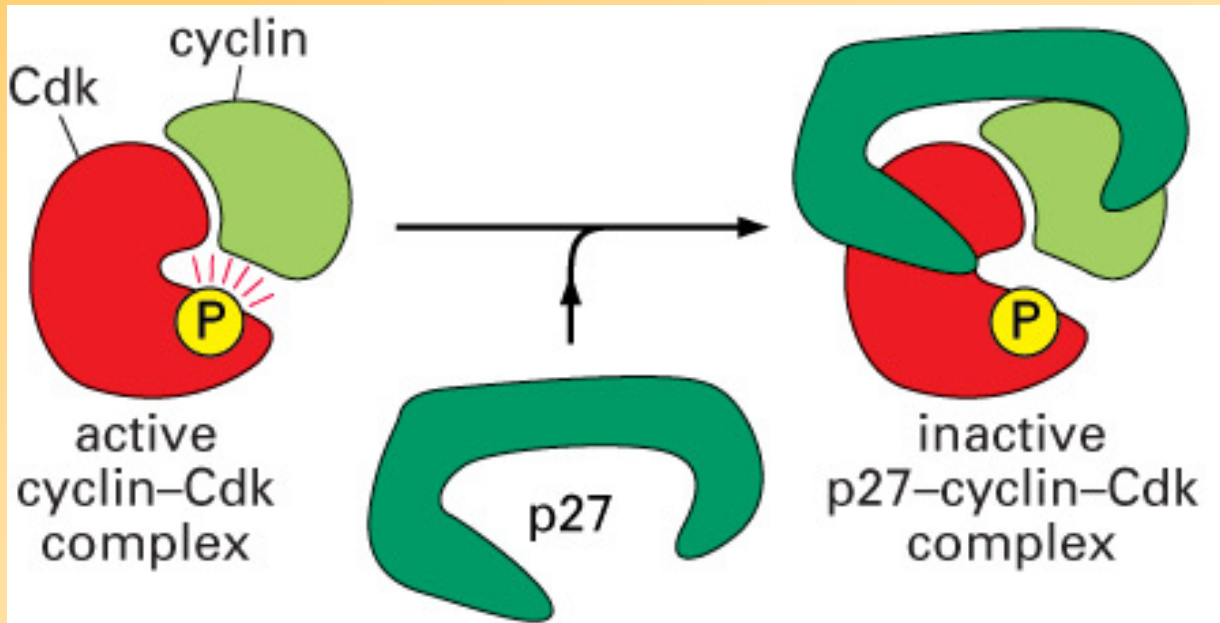
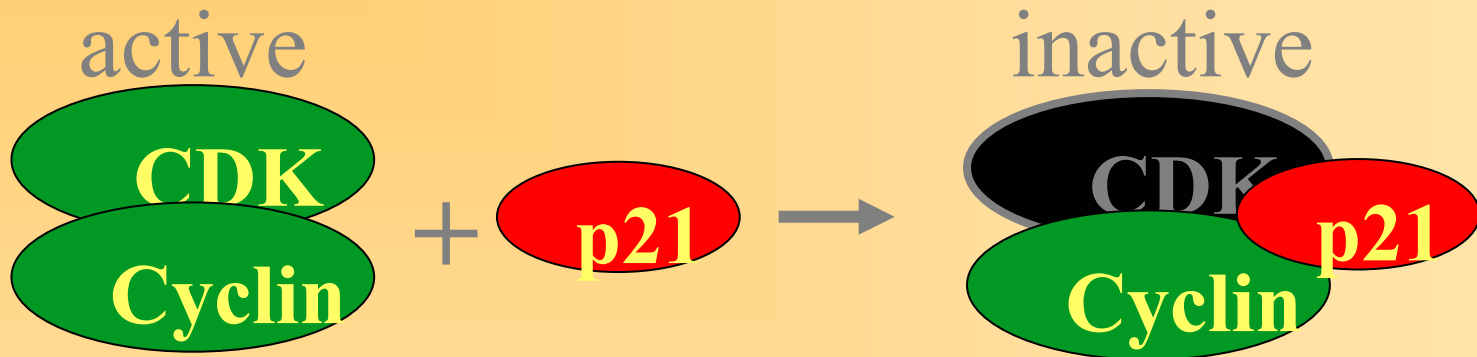


Figure 17-19. Molecular Biology of the Cell, 4th Edition.

The p21 Family of CDK inhibitors (p21^{CIP1/WAF1}, p27^{KIP1}, p57^{KIP2})



2

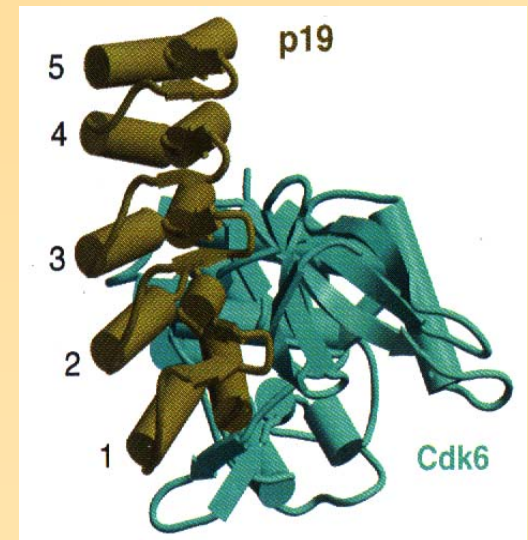
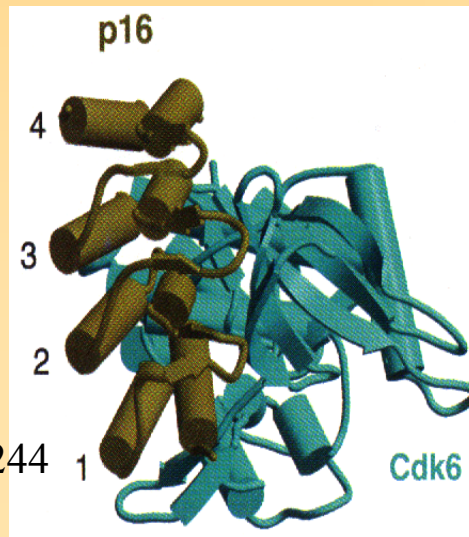
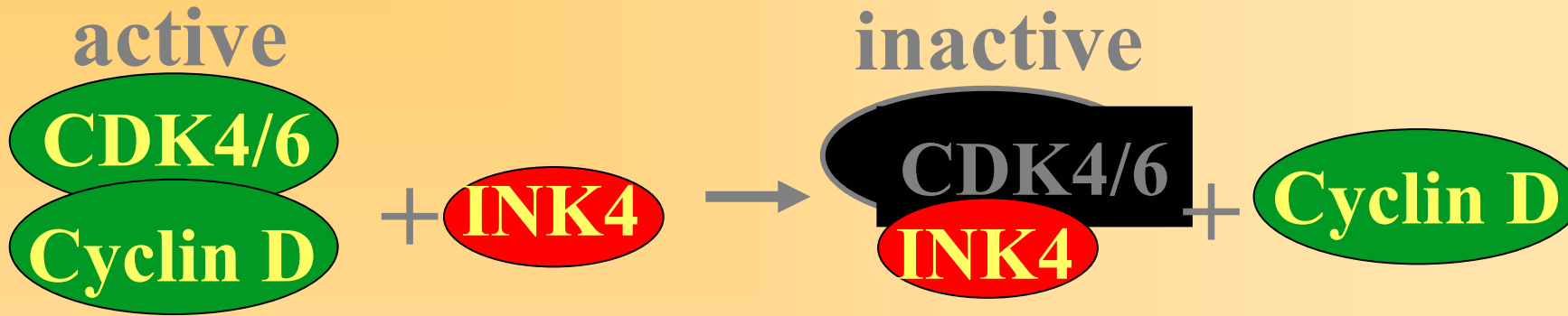
Jeffrey et al. (1995) *Nature* 376:313



A

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

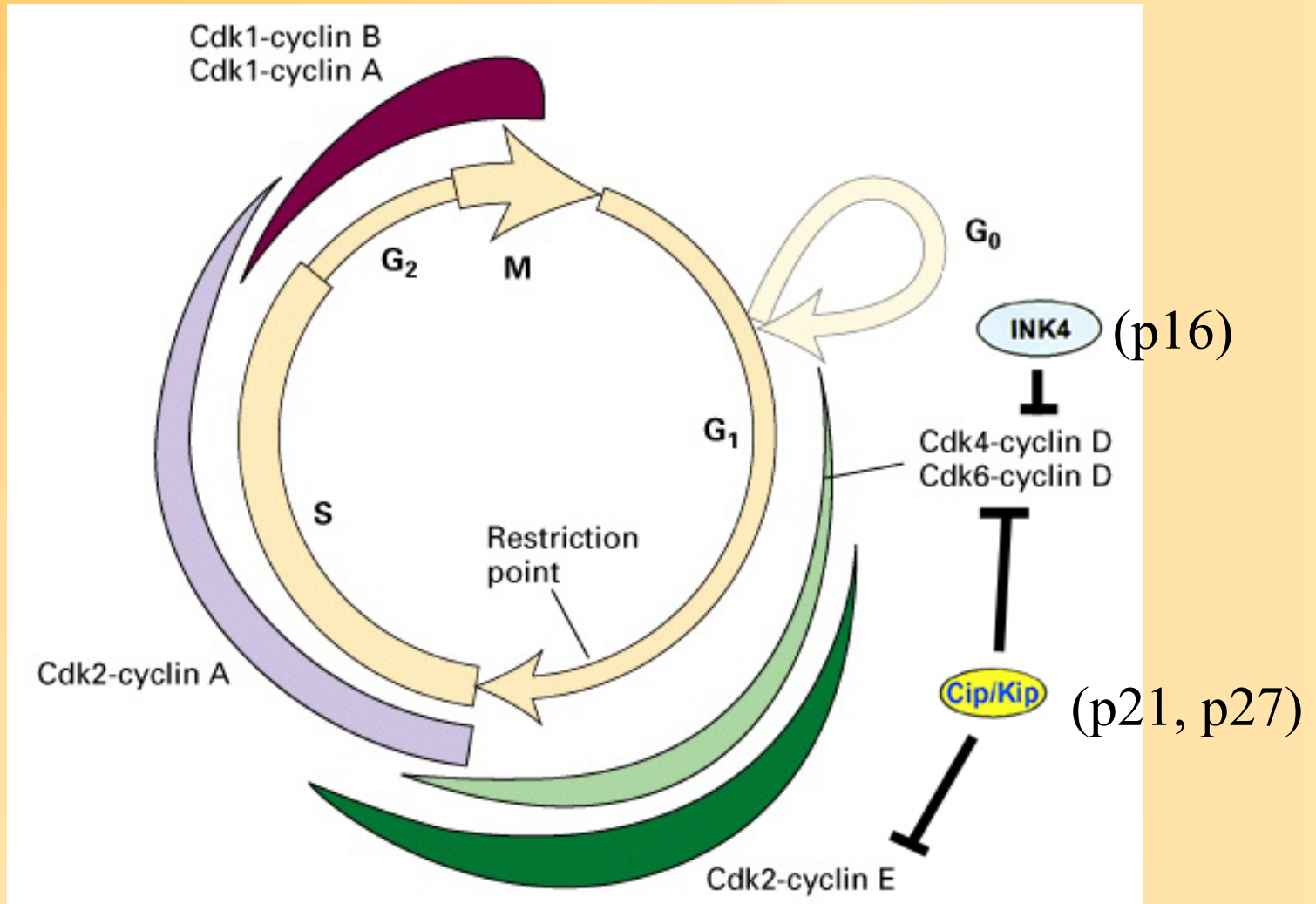
The INK4 Family of CDK inhibitors (p16^{INK4a}, p15^{INK4b}, p18^{INK4c}, p19^{INK4d})



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

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

CKIs Regulate the G1-S Transition



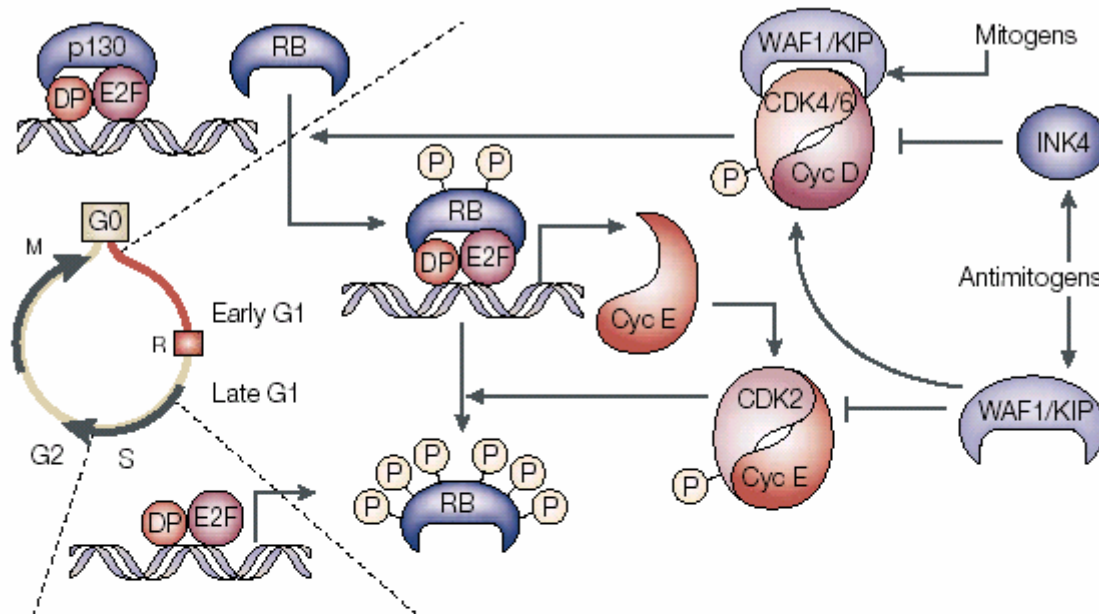
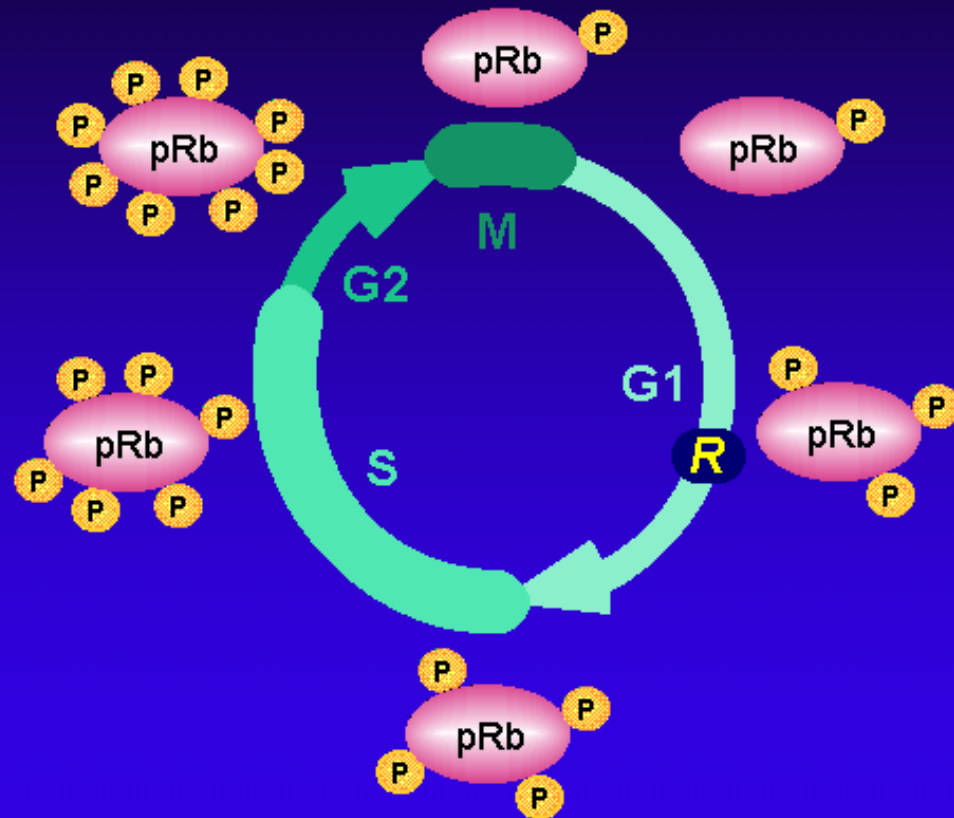
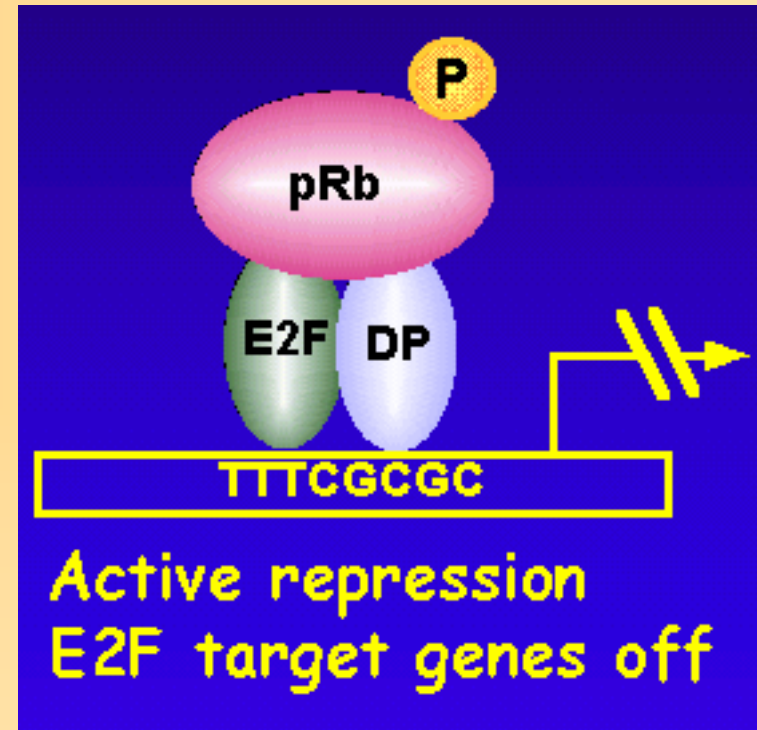
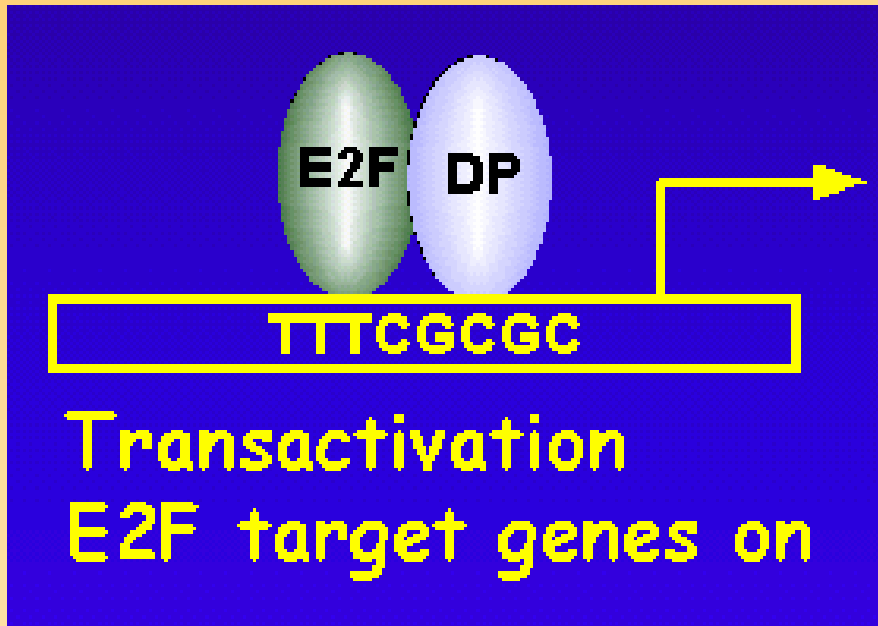


Figure 1 | Regulation of G1 and the G1/S transition. In quiescent, G0 cells, E2F–DP transcription factors are bound to p130, the principal pocket protein in these cells, which keeps them inactive. In G1, however, RB–E2F–DP complexes predominate. Mitogenic signalling results in cyclin D (Cyc) synthesis, 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.

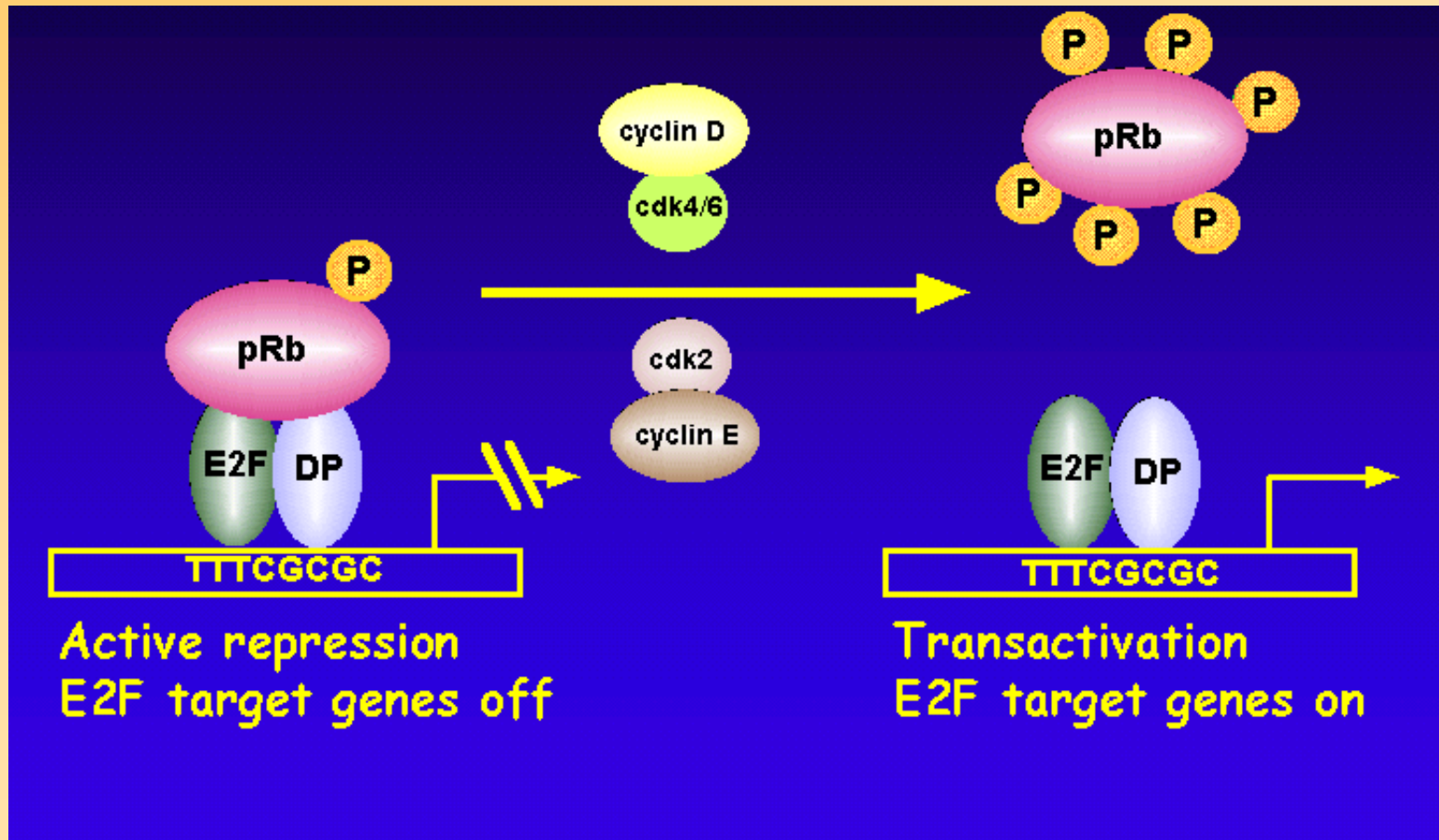
Cell cycle regulated phosphorylation of the retinoblastoma protein



pRb Binds to the E2F Transcription Factor



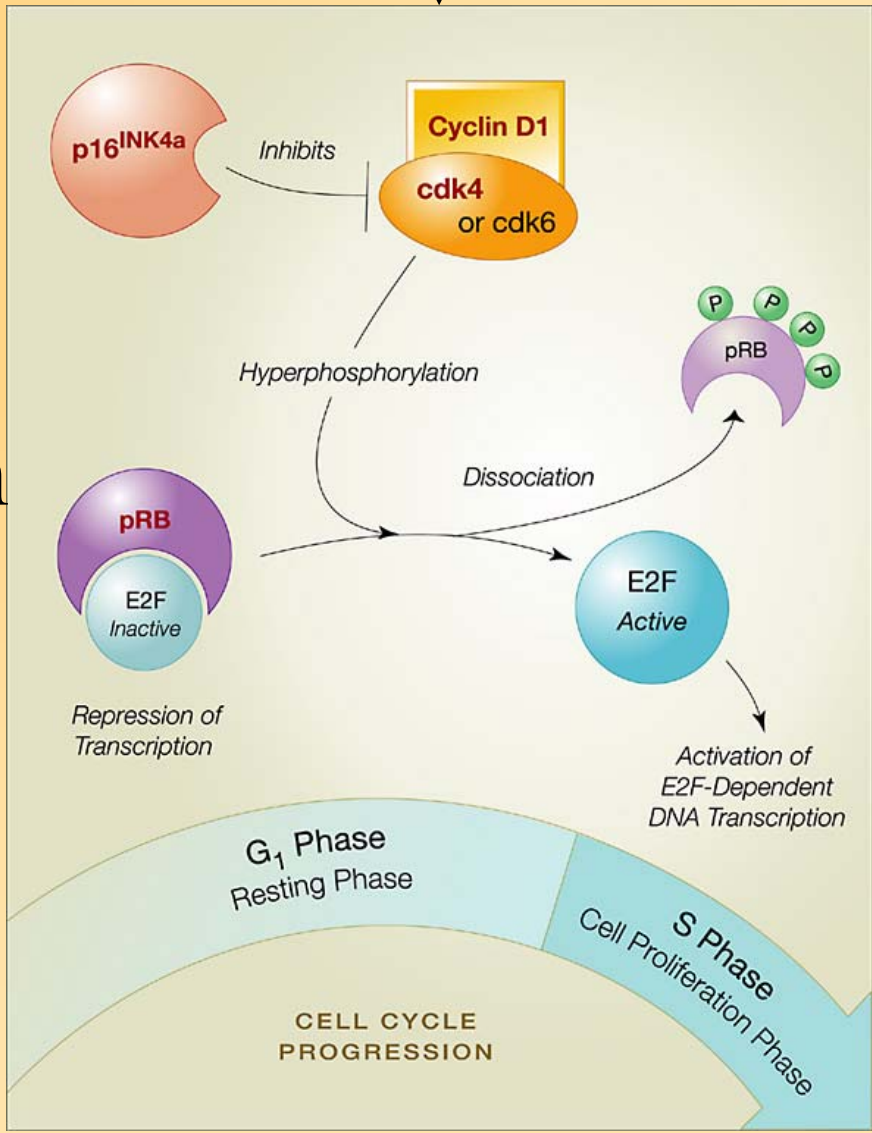
G1 Cyclin CDKs Phosphorylate pRB



Proliferation Signals



p16 Regulates pRB Phosphorylation



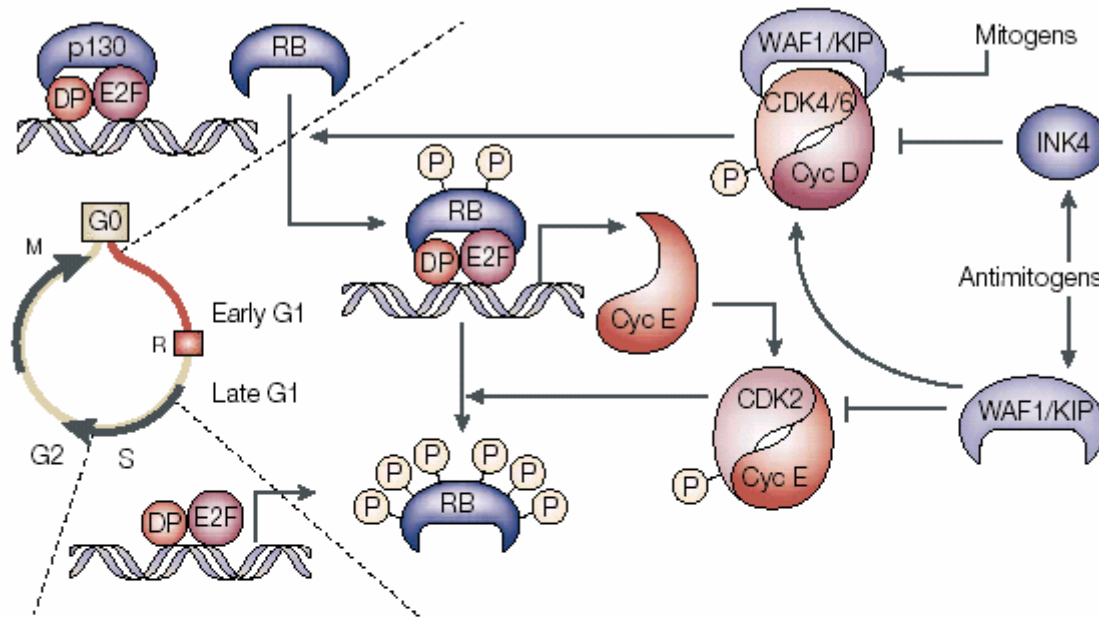


Figure 1 | Regulation of G1 and the G1/S transition. In quiescent, G0 cells, E2F–DP transcription factors are bound to p130, the principal pocket protein in these cells, which keeps them inactive. In G1, however, RB–E2F–DP complexes predominate. Mitogenic signalling results in cyclin D (Cyc) synthesis, 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.

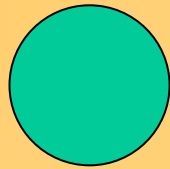
Kontrola buněčného cyklu úzce souvisí s:

▶ **kontrolou buněčného růstu;**

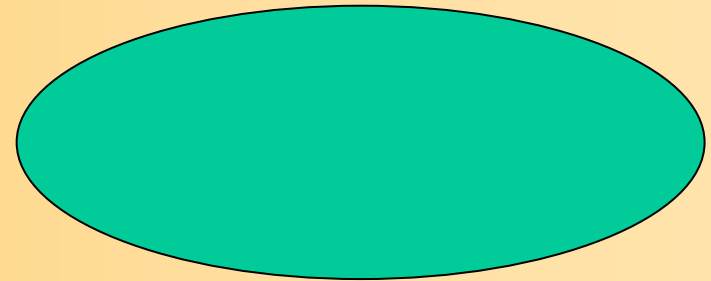
▶ **přítomností růstových faktorů a dalších
růstových stimulů a živin;**

▶ **působením ostatních buněk populace
a mezibuněčné hmoty.**

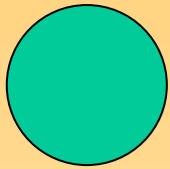
The Difference Between Growth and Cell Division



Growth with

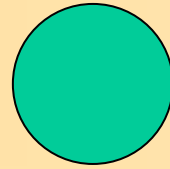
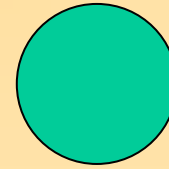
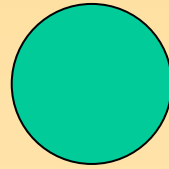
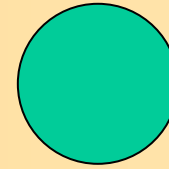
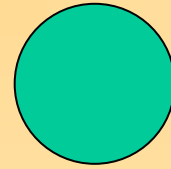
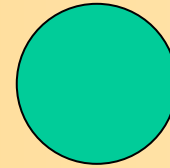


No Cell Division



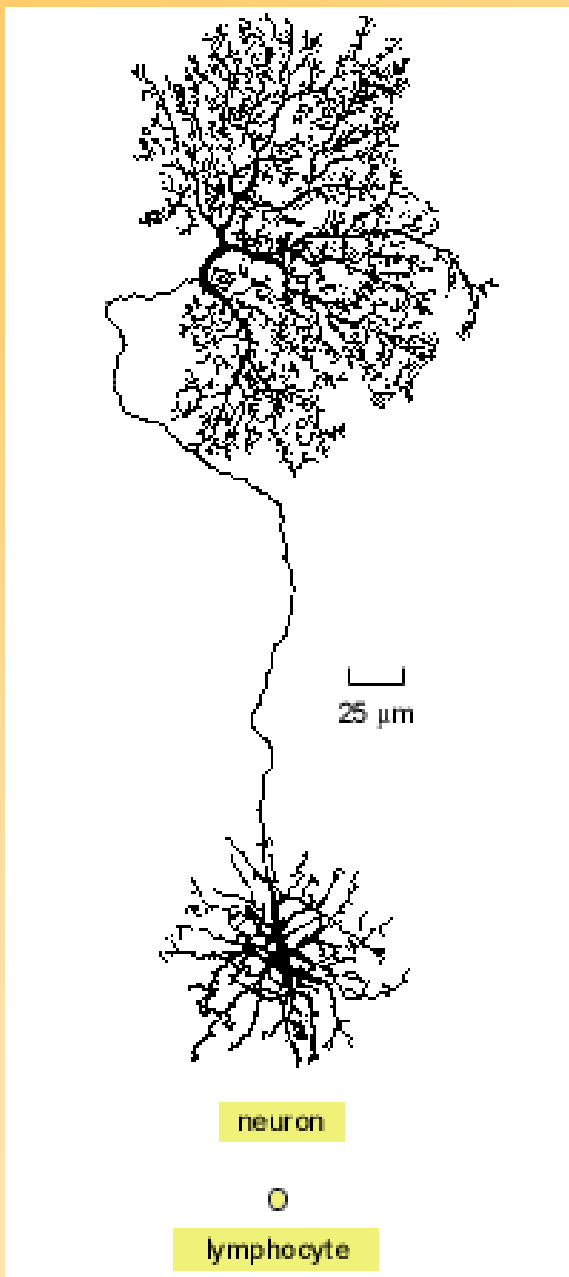
Cell Division

No Growth



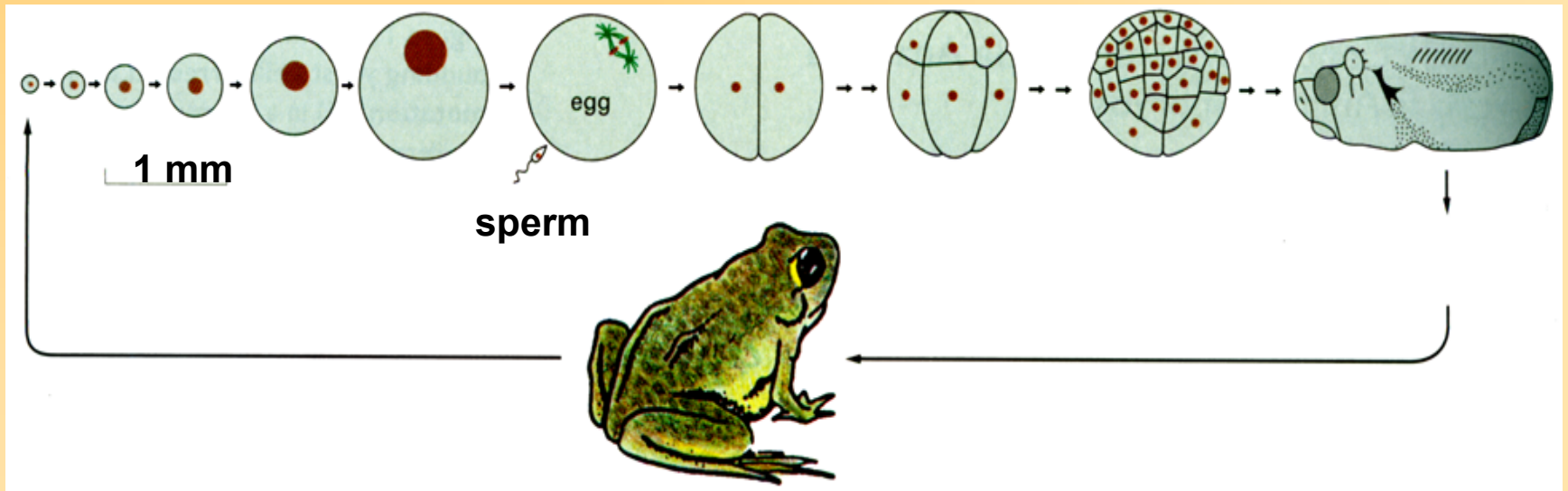
Cell Division + Growth =

Proliferation!



Growth with No Cell Division: A Differentiated Neuron

Cell Division with No Growth: Early Development

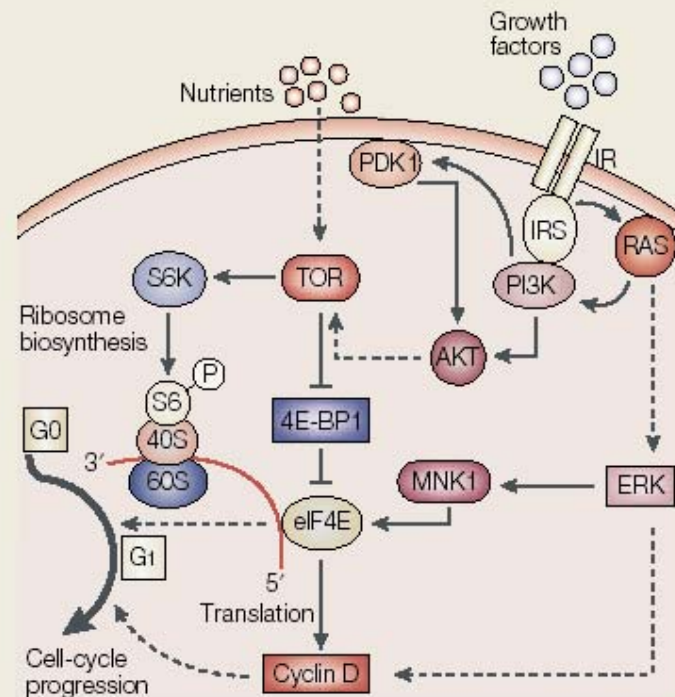


Cell growth (the increase in cell size and protein mass) is a term that has frequently been misused to mean cell proliferation. In fact, both processes are highly coordinated. Only in certain biological systems — such as oocytes, neurons and muscle cells, where cell growth might exist without cell division, 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.

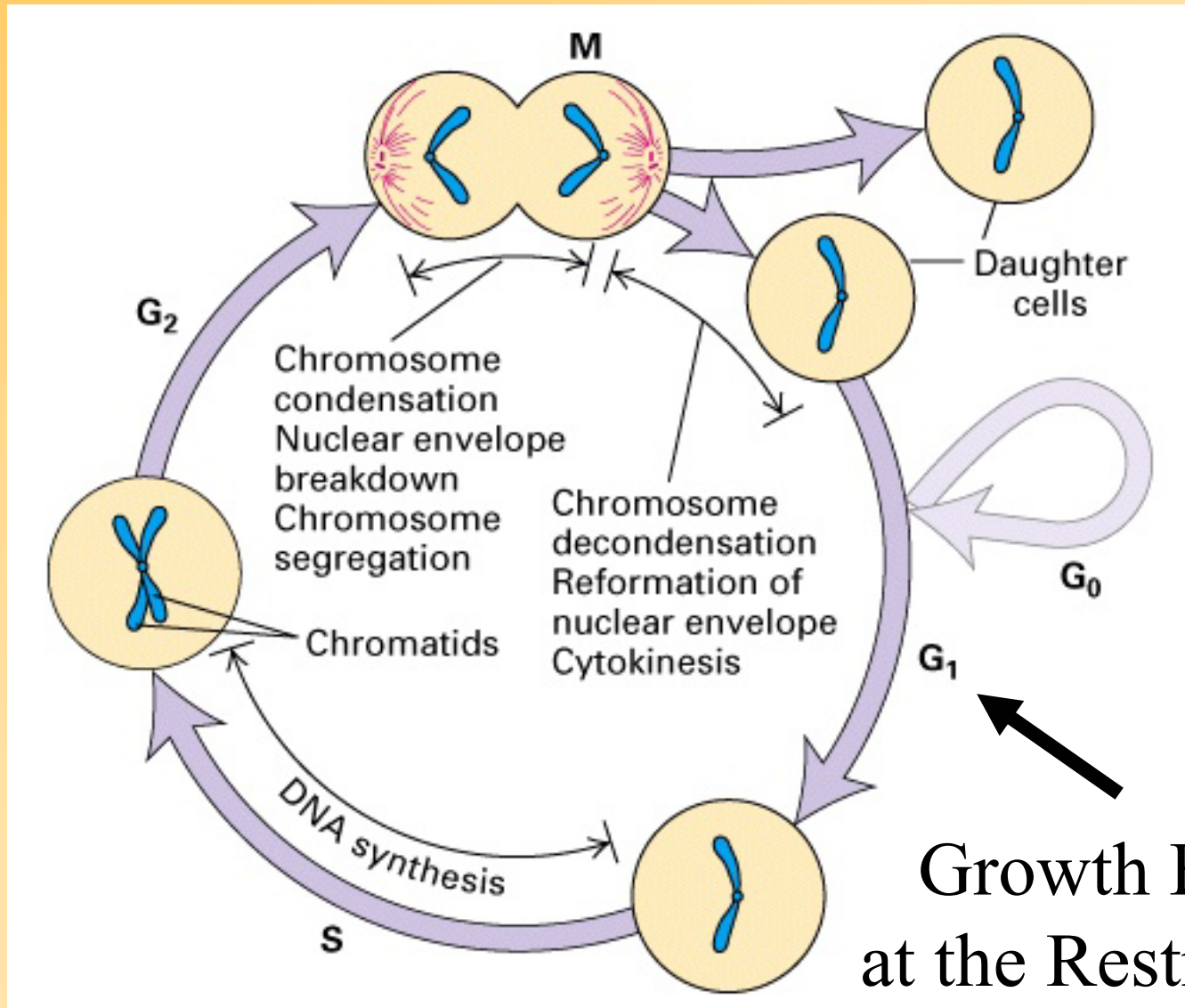
Ribosome biosynthesis is a key process for cell growth. Before entering the cycle, cells need to accumulate sufficient translational machinery, mainly ribosomes, to ensure the rapid processing of transcripts through the cycle. This is accomplished, at least in part, by phosphorylation of the ribosomal 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^{86,87}. TOR is thought to be important in cell growth and amino-acid sensing⁸⁷, 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



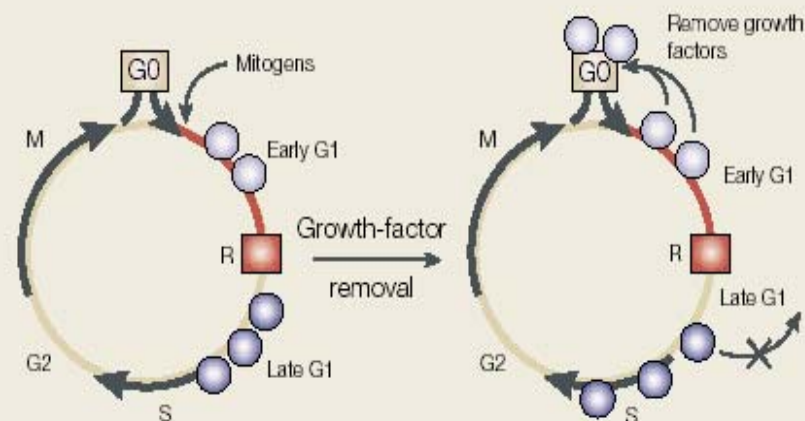
Growth Factors act at the Restriction Point

Box 2 | The Restriction Point

The term 'Restriction Point' was coined in 1974 by Arthur Pardee⁹⁰ 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^{2,91}. 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^{92,93}.

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.



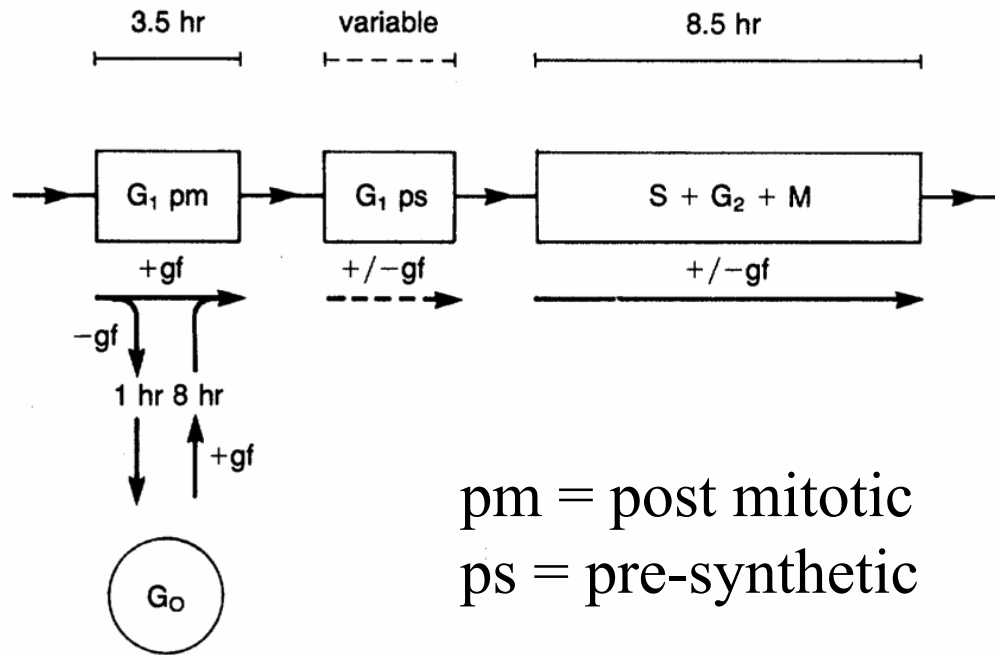
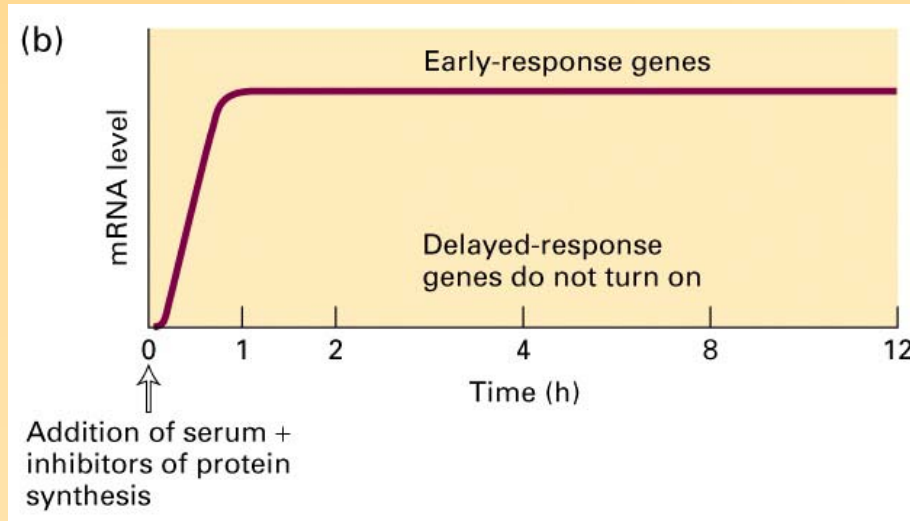
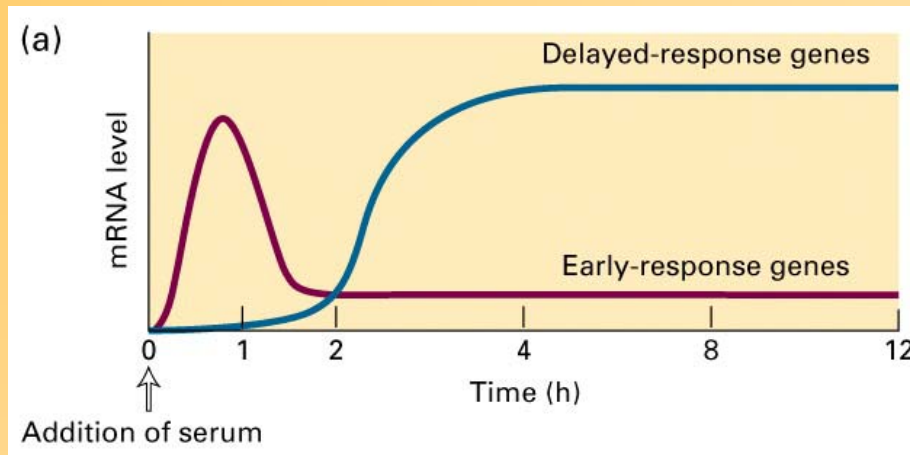
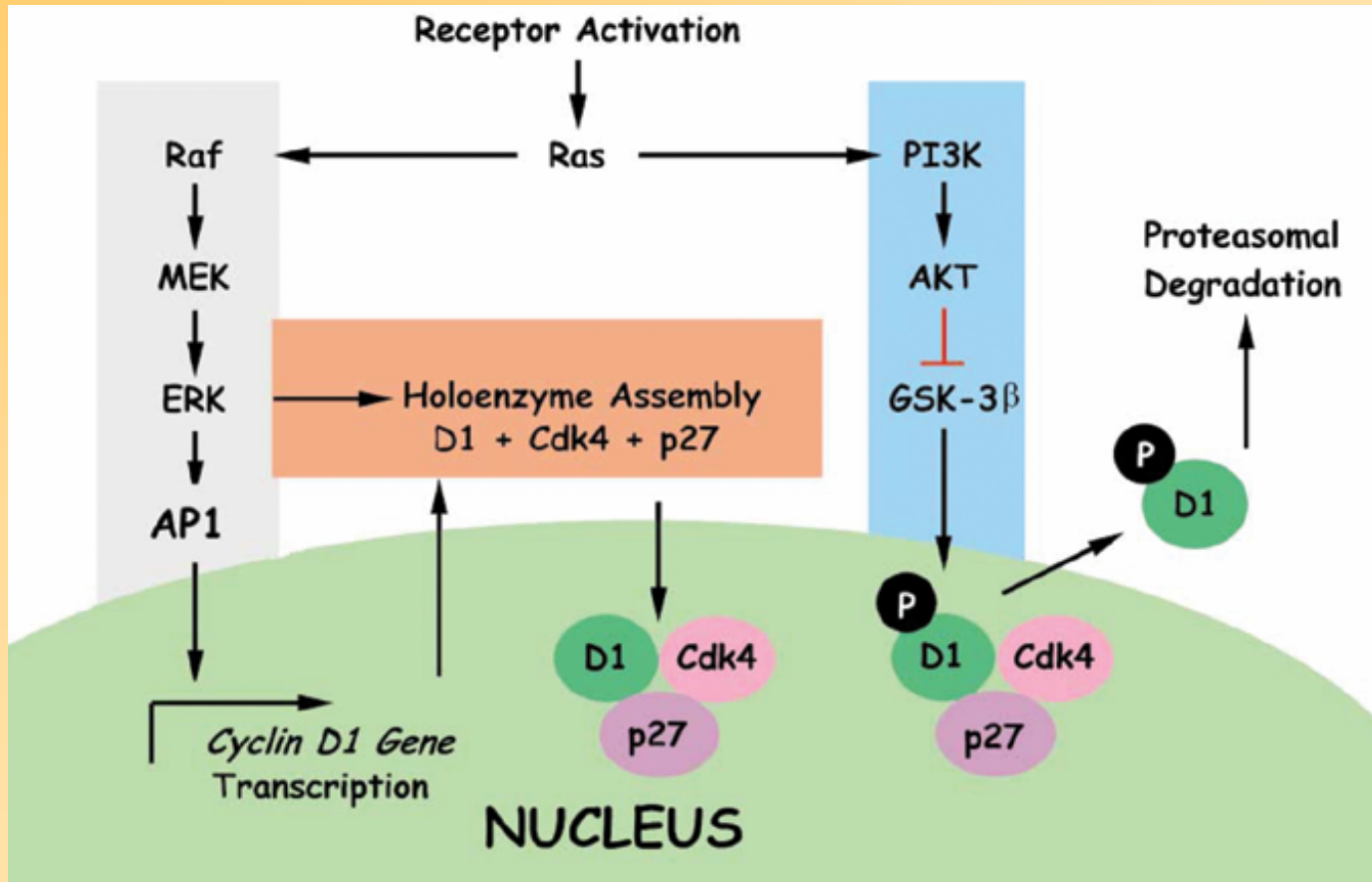


FIG. 7. Schematic model of cell cycle in Swiss 3T3 cells. During the first 3.5-hr after mitosis (G₁pm), the cell makes the decision whether or not to progress through the cell cycle. This decision depends on the presence of growth factors (gf). If the cell senses a lack of growth factors (-gf) in G₁pm, it will leave the cell cycle within 15–60 min and enter a state of quiescence (G₀) 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 G₁ps, it will eventually initiate DNA synthesis. However, G₁ps is highly variable in length and in fact responsible for most of the variability in the duration of G₁ and of the whole cell cycle.

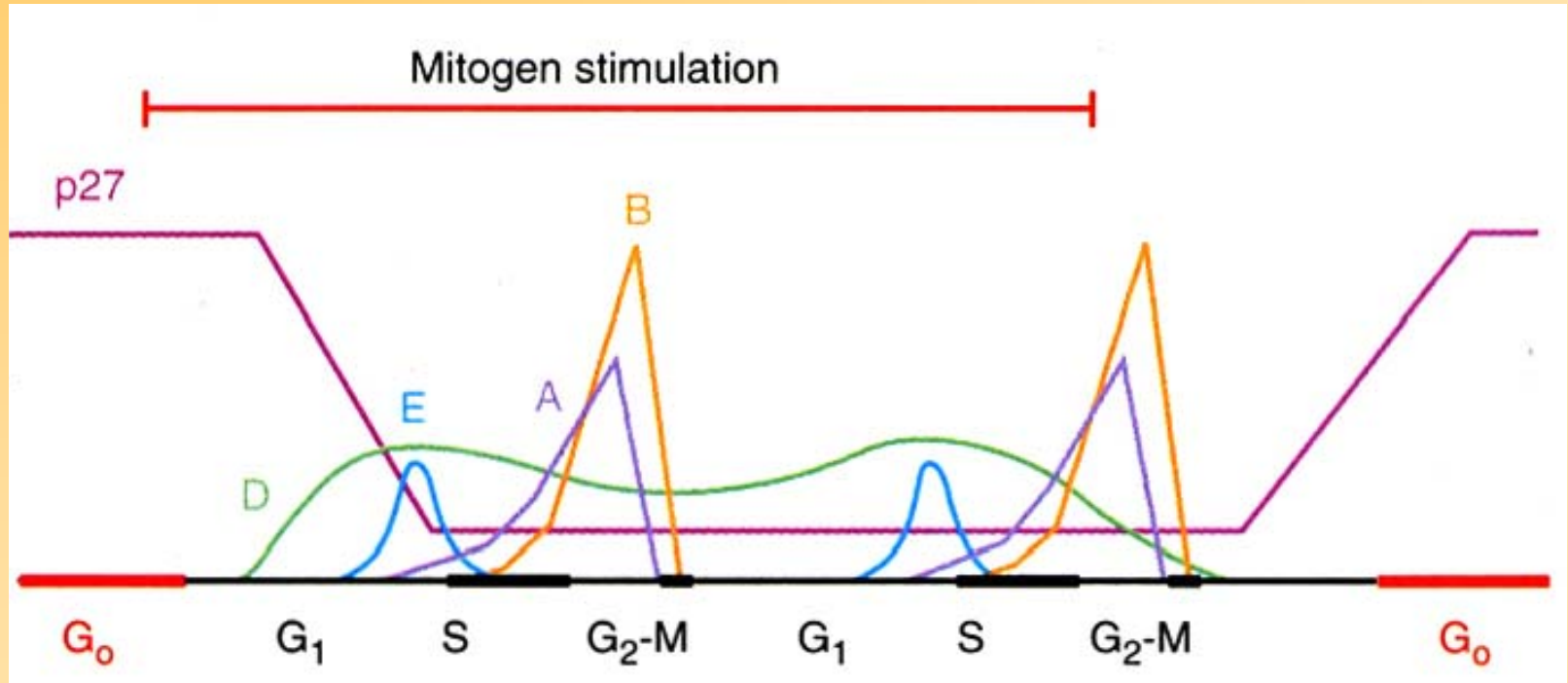
Growth Factors Induce Gene Expression



Growth Factors Induce Cyclin D1 Expression



Mitogen Induced Cell Cycle Progression in Cell Culture



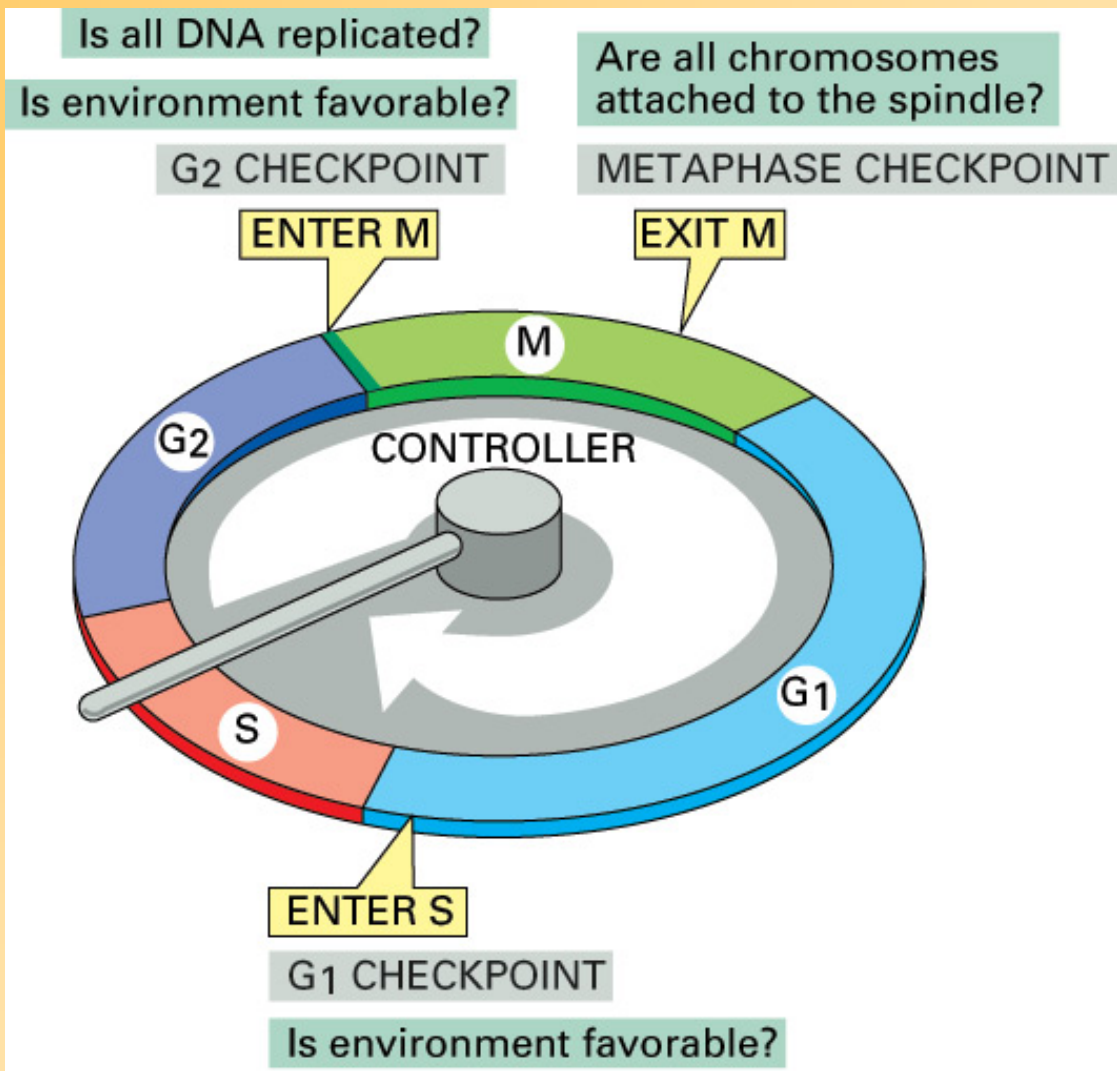


Figure 17-14. Molecular Biology of the Cell, 4th Edition.

Cell Cycle Checkpoints

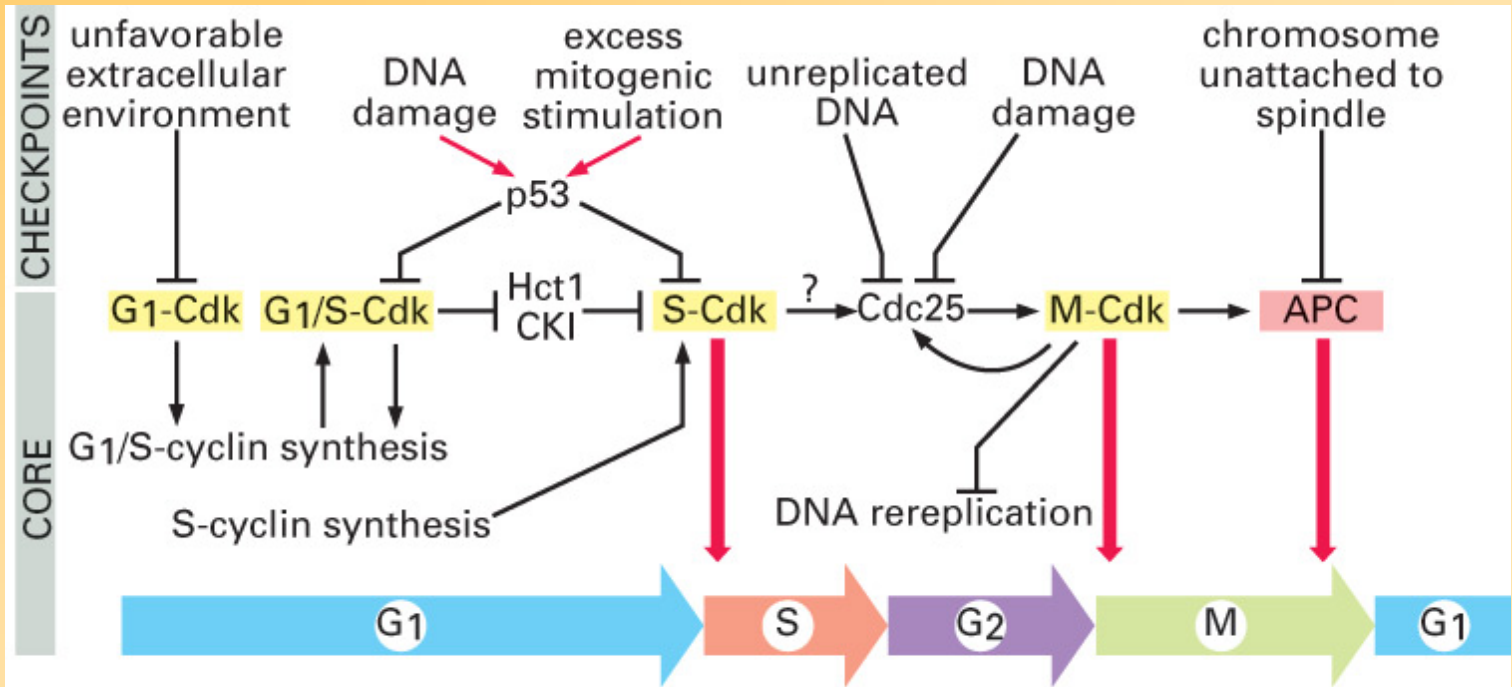


Figure 17-34. Molecular Biology of the Cell, 4th Edition.

Cell Cycle Checkpoints Improve Cell Viability

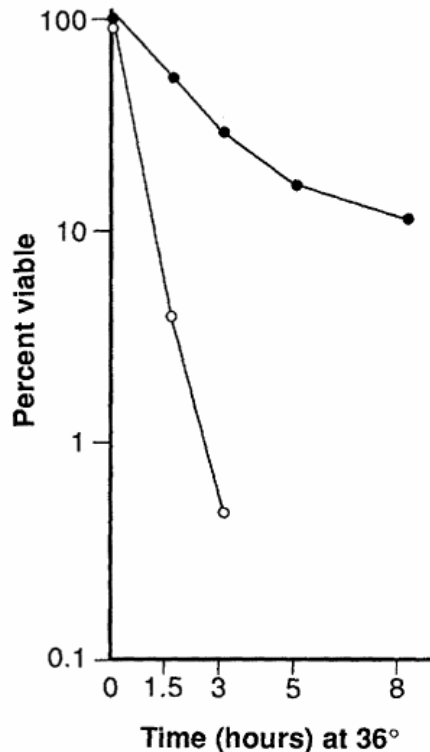
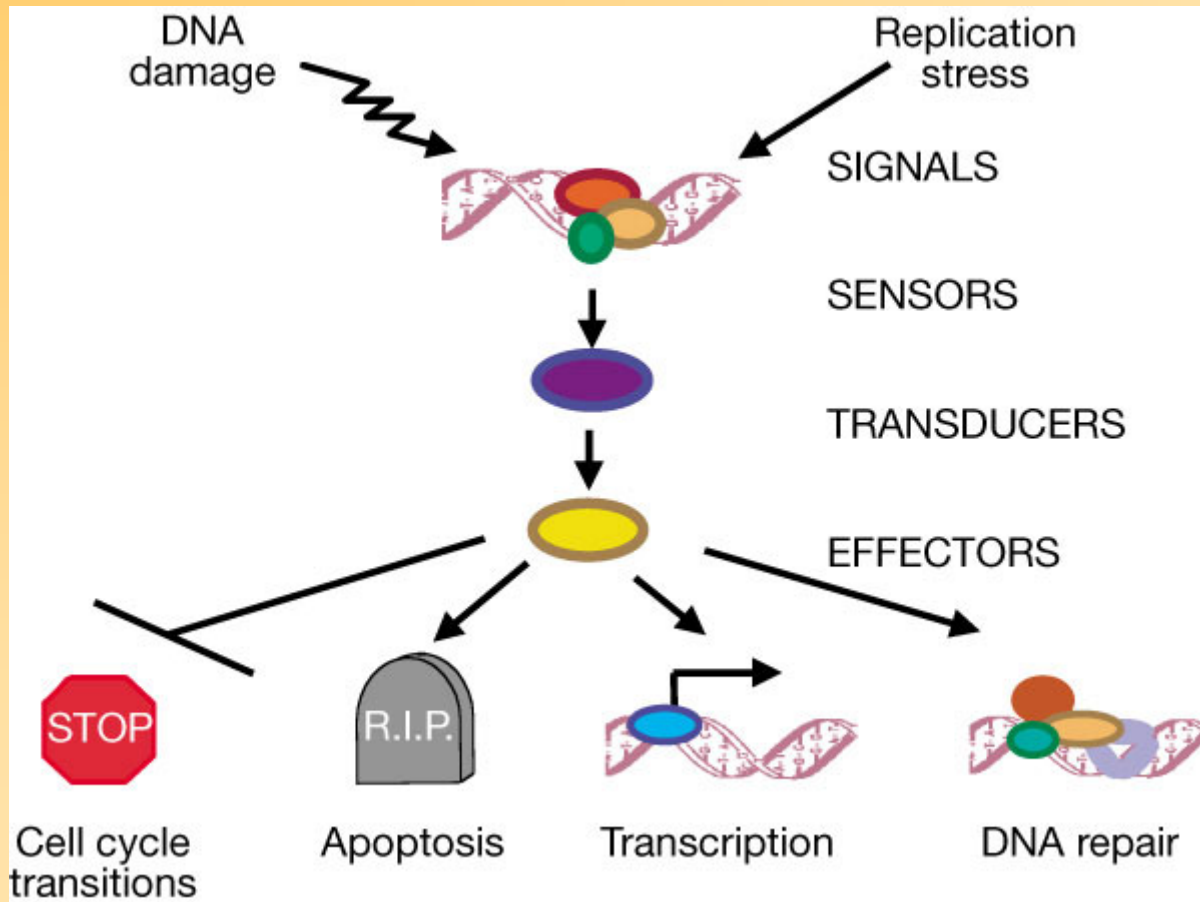


Fig. 3. Rapid loss of viability in cells defective both for DNA ligase and for the *RAD9* gene. *cdc9* (●) and *cdc9-rad9* (○) cells growing at 23°C were shifted to the restrictive temperature and viability was determined by plating for viable colonies at the permissive temperature (23°C). The cell viability 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?



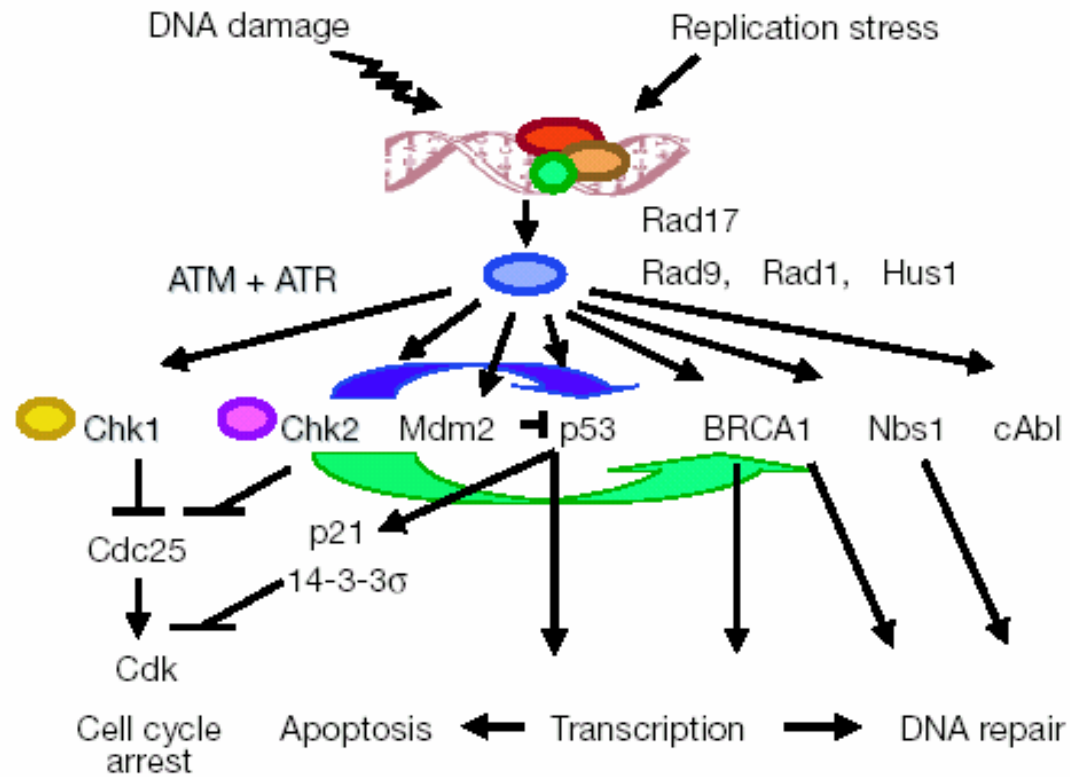
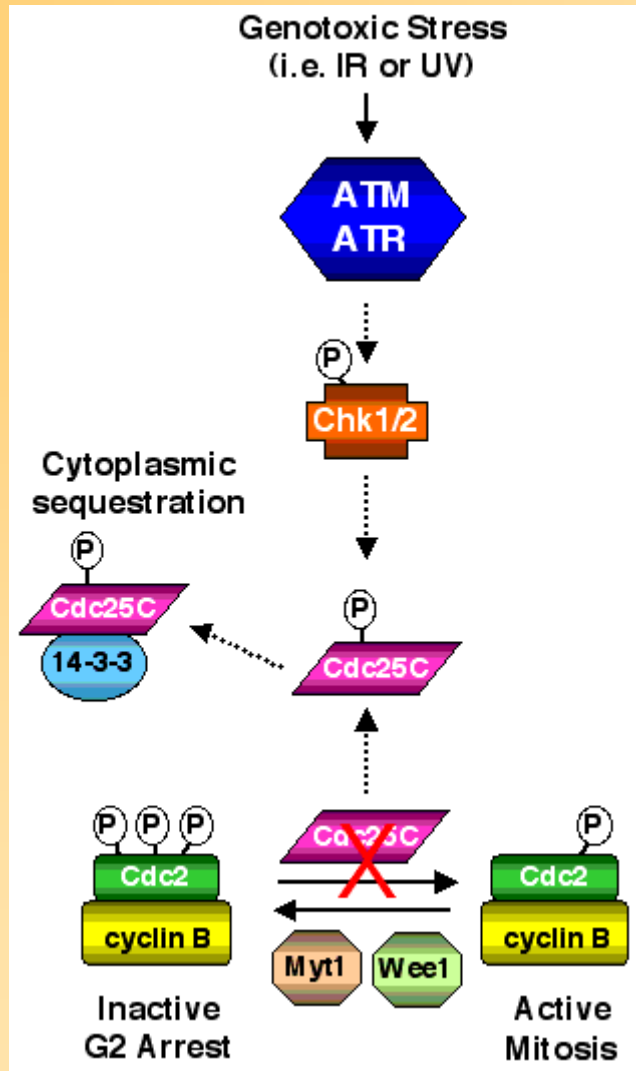


Figure 2 Organization of the mammalian DNA damage response pathway. Arrowheads represent positively acting steps while perpendicular ends represents inhibitory steps. Gene names are shown at the approximate positions where their encoded proteins function in the pathway. Although the general organization 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.

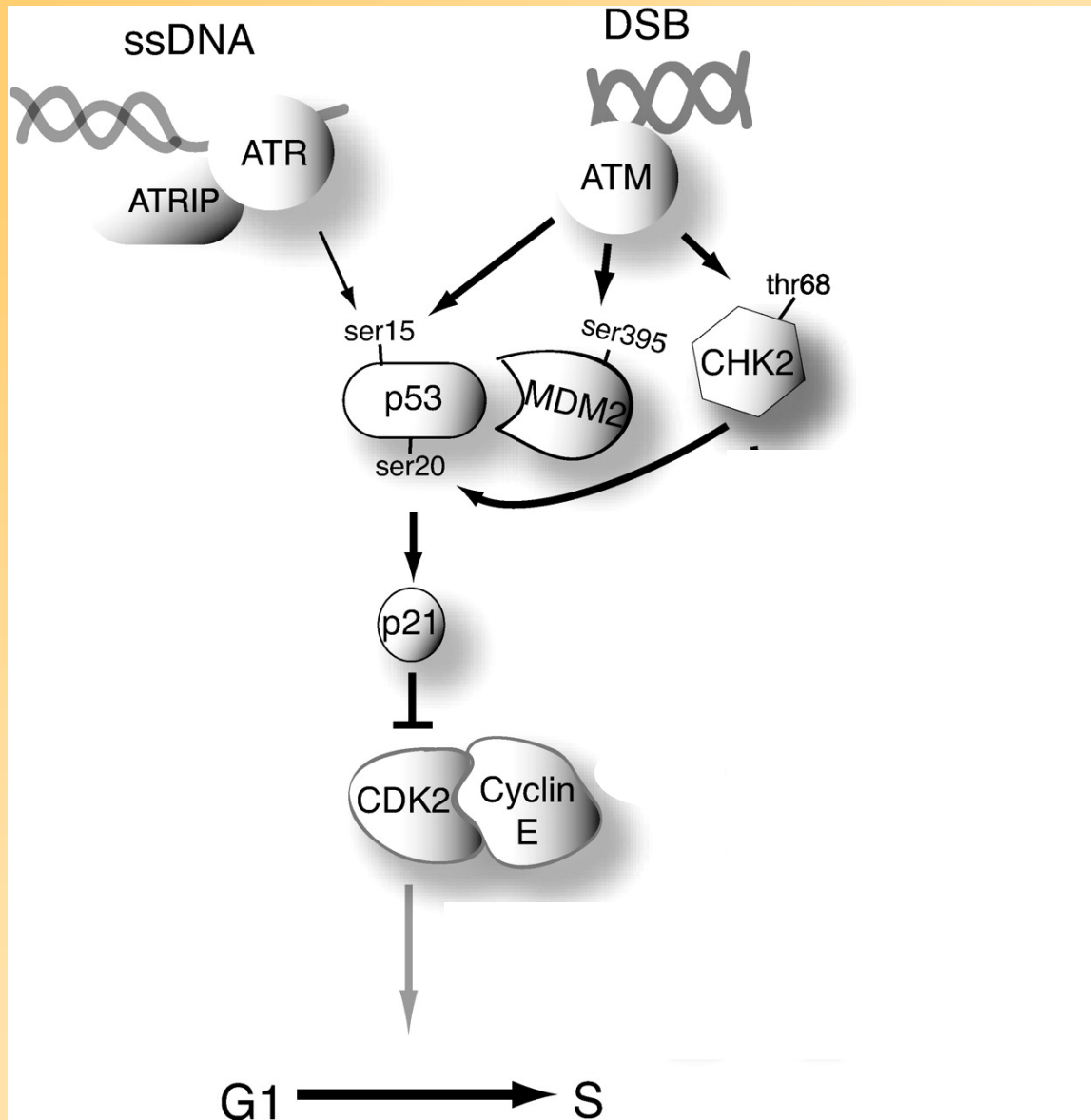


Signal

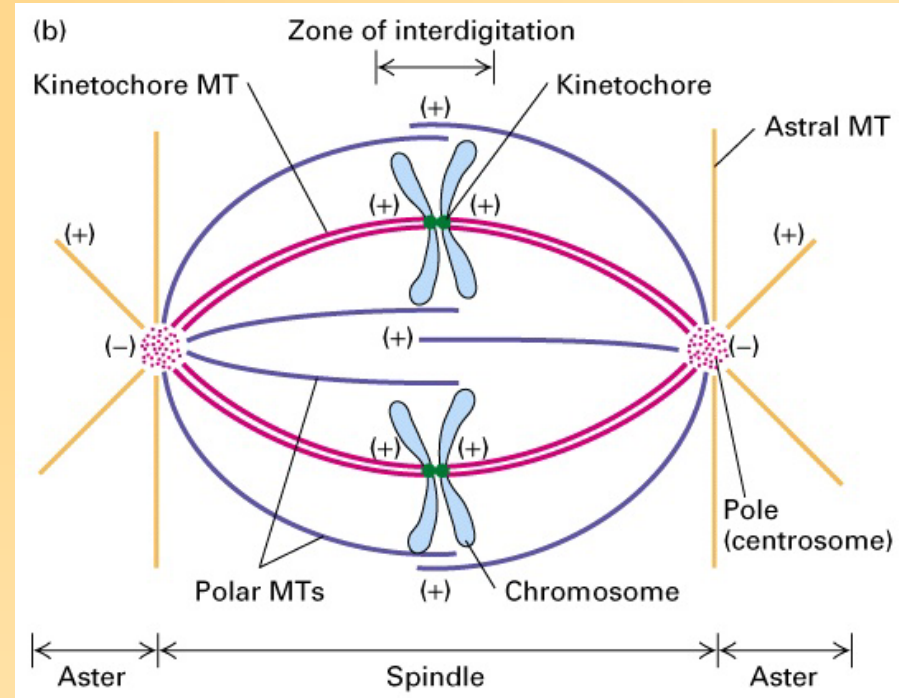
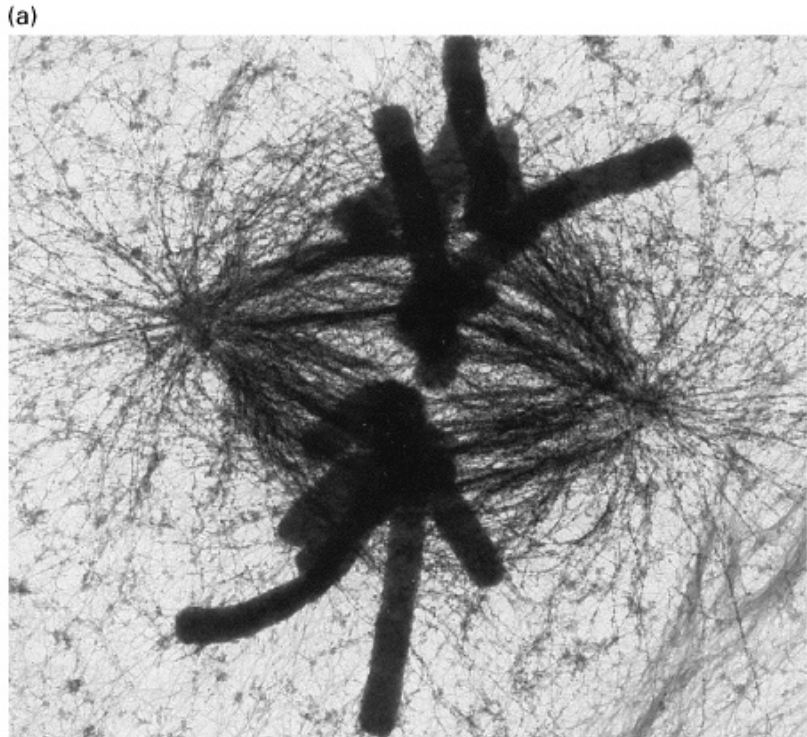
Sensor

Transducer

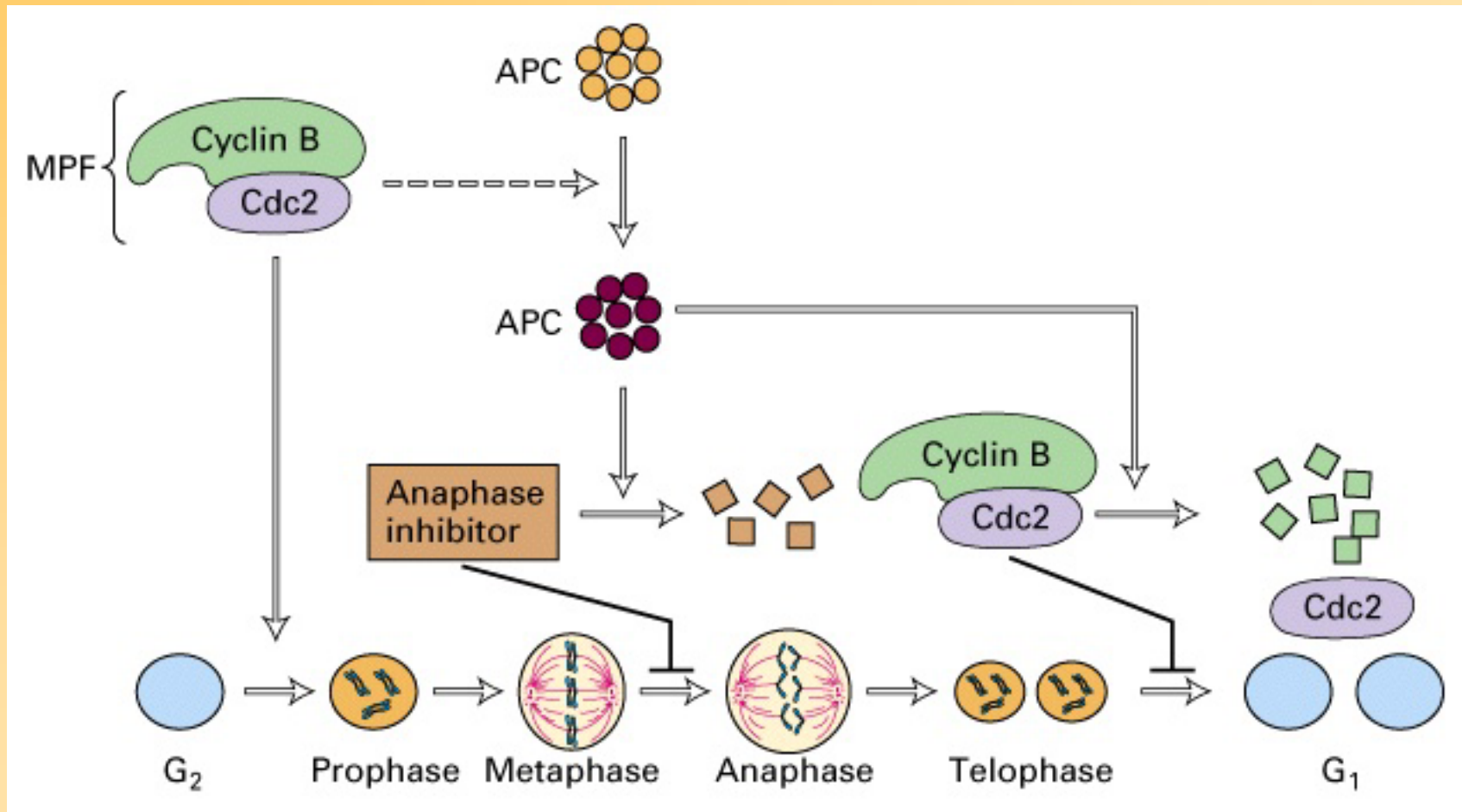
Effector



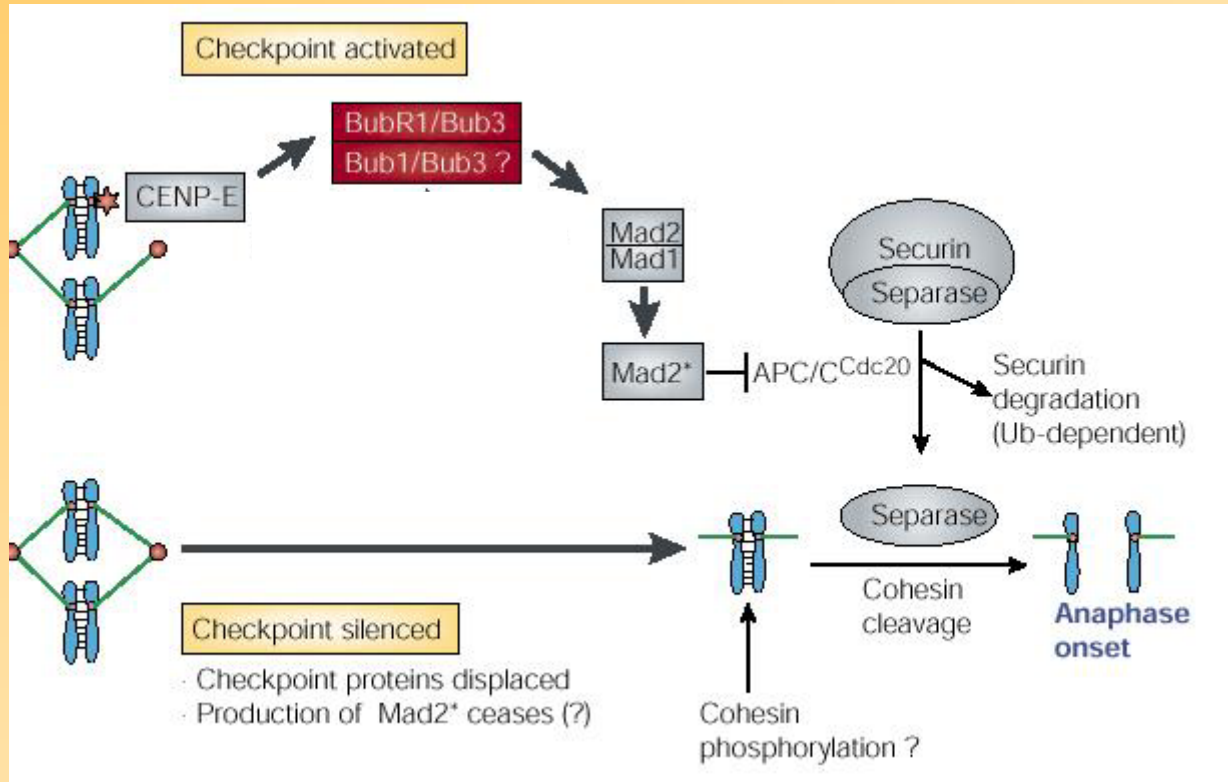
Metaphase in a mammalian cell



The Metaphase to Anaphase Transition

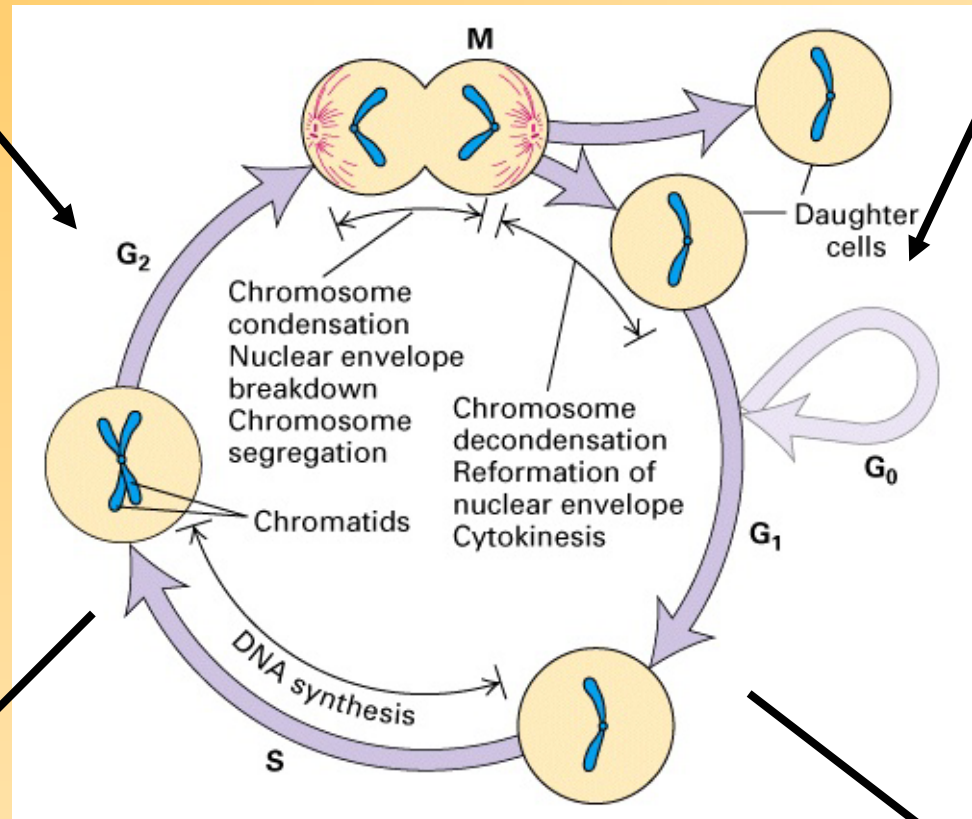


The Spindle Assembly Checkpoint



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

dostatek živin a růstových faktorů
mitogeny



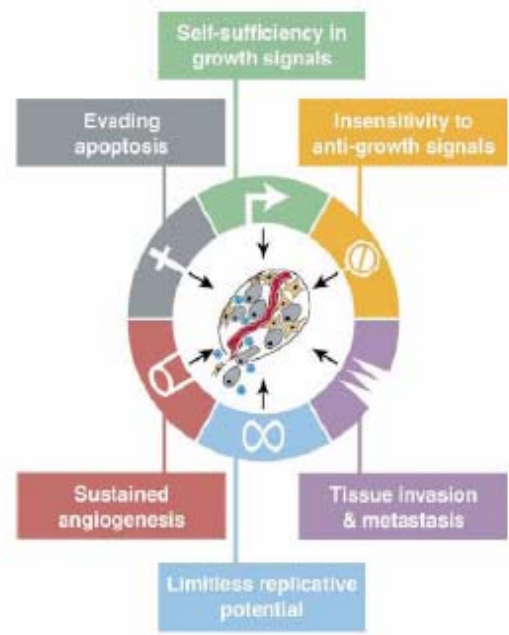
zástava buň.cyklu
(restriction point,
checkpoints)
setrvání v G₀ fázi
apoptóza

proliferace

Hallmarks of Cancer

- Summarized by Hanahan and Weinberg (2000) Cell
- Six changes for cancer – found in most, if not all, cancers

- Self-sufficiency in growth signals
- Insensitivity to growth-inhibitory signals
- Evasion of apoptosis
- Limitless replicative capacity
- Sustained angiogenesis
- Tissue invasion and metastasis



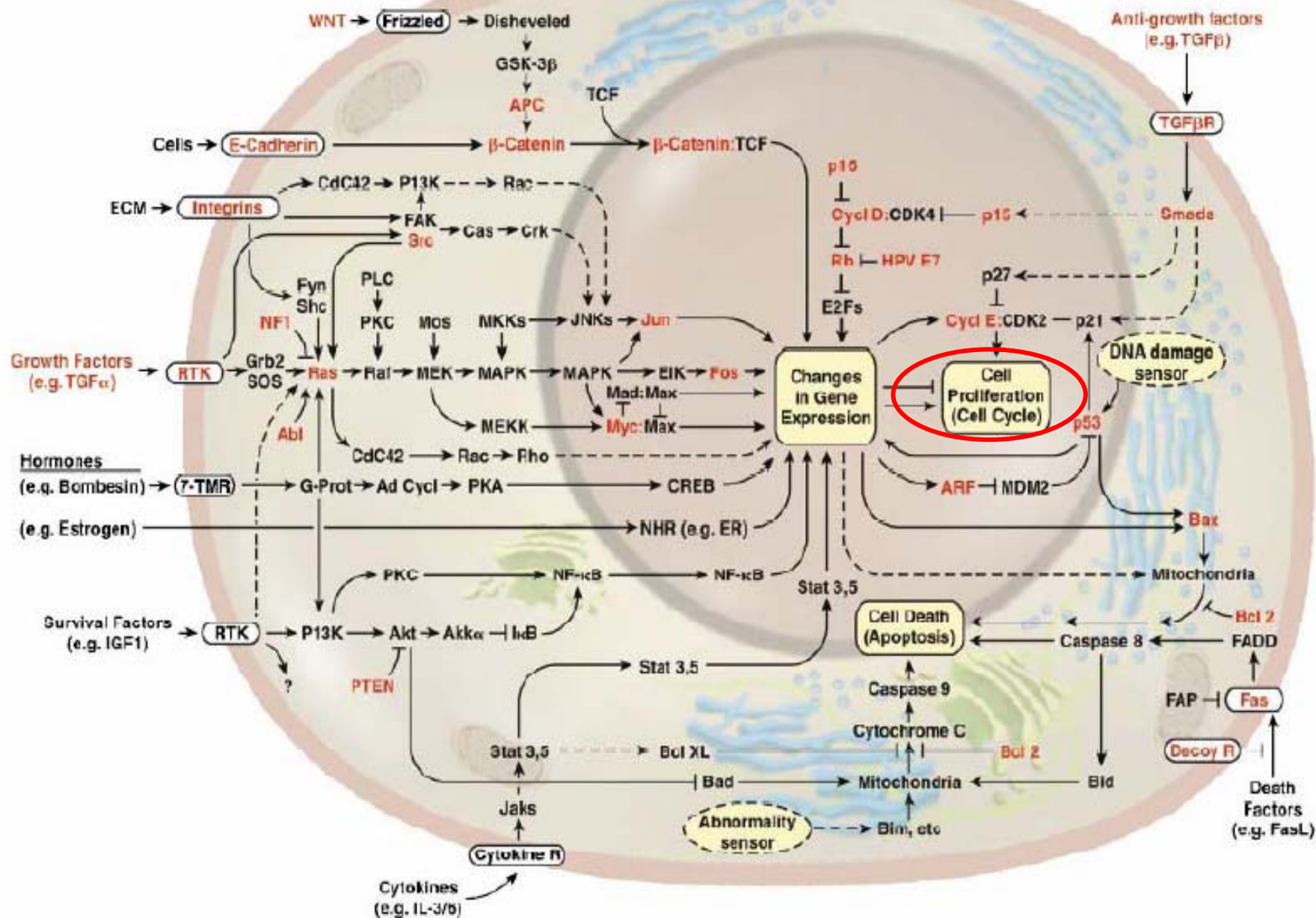


Table 1 Human cancer susceptibility linked to DNA-damage response

Disease	Gene	Number of mutant alleles inherited	Cancer predisposition	Comments
Ataxia-telangiectasia (A-T)	<i>ATM</i>	2	Leukaemia, lymphoma	Most mutations result in null protein phenotype
Nijmegen breakage syndrome (NBS)	<i>NBS1</i>	2	Leukaemia, lymphoma	Fragment of NBS1 protein still expressed in some cell types
A-T-like disorder (ATLD)	<i>Mre11</i>	2	Leukaemia, lymphoma	Hypomorphic mutations in <i>Mre11</i>
Fanconi's anaemia (FA)	<i>FancD2, Brca2</i> (also known as <i>FancD1</i>)	2	Acute myelogenous leukaemias	Other FA genes not directly implicated in checkpoints; <i>Brca2</i> — hypomorphic
Familial breast, ovarian carcinoma syndrome	<i>Brca1, Brca2</i>	1	Breast, ovarian, scattered others	
Li-Fraumeni syndrome	<i>p53, CHEK2</i>	1	Sarcomas, leukaemias, brain tumours, adrenal tumours, others	

This list does not include syndromes resulting from DNA-repair defects, which includes xeroderma pigmentosum, hereditary non-polyposis colon cancers, Bloom's syndrome and other Fanconi's anaemia complementation groups.

Chemo Agents and Cell Cycle Interruption

Antimetabolites: Antimetabolites interrupt the cell cycle by preventing DNA synthesis - the “S” phase.

They interfere with the production of key molecules with the cell – nucleic acids, RNA & DNA.

Examples of Antimetabolites:

Folate Antagonists such as MTX. MTX binds to and inhibits dihydrofolate reductase (DHFR), meaning Folate stays in its inactive form.

Purine Antagonists such as 6-MP (6-Mercaptopurine). 6-MP inhibits purine nucleotide production thus halting DNA synthesis.

Pyrimidine Antagonists such as 5-FU (5-fluorouracil). 5-FU inhibits the production of pyrimidine nucleotides, thus halting DNA synthesis.

Plant Alkaloids

- ❖ Vinca Alkaloids (derived from the *periwinkle* plant) - halt the mitosis phase of the cell cycle by binding to tubulin, thus preventing the assembly of microtubules responsible for chromosome migration. Example: vincristine, vinblastine
- ❖ Paclitaxel or Taxol (isolated compound from the *Pacific Yew* tree) – binds to microtubules and prevents their breakdown. Without the ability to breakdown, microtubules cannot pull chromosomes to opposite ends of the dividing cell.
- ❖ Etoposide (a derivative of the *mandrake* plant) – inhibits topoisomerase II, an enzyme which normally unwinds DNA during replication to relieve tension on the unwinding strand. The DNA which has been nicked by topo II, is unable to unwind and eventually breaks under tension.