CELL CYCLE

FOR ZOOLOGY HONOURS

SEMESTER II, CCIVH

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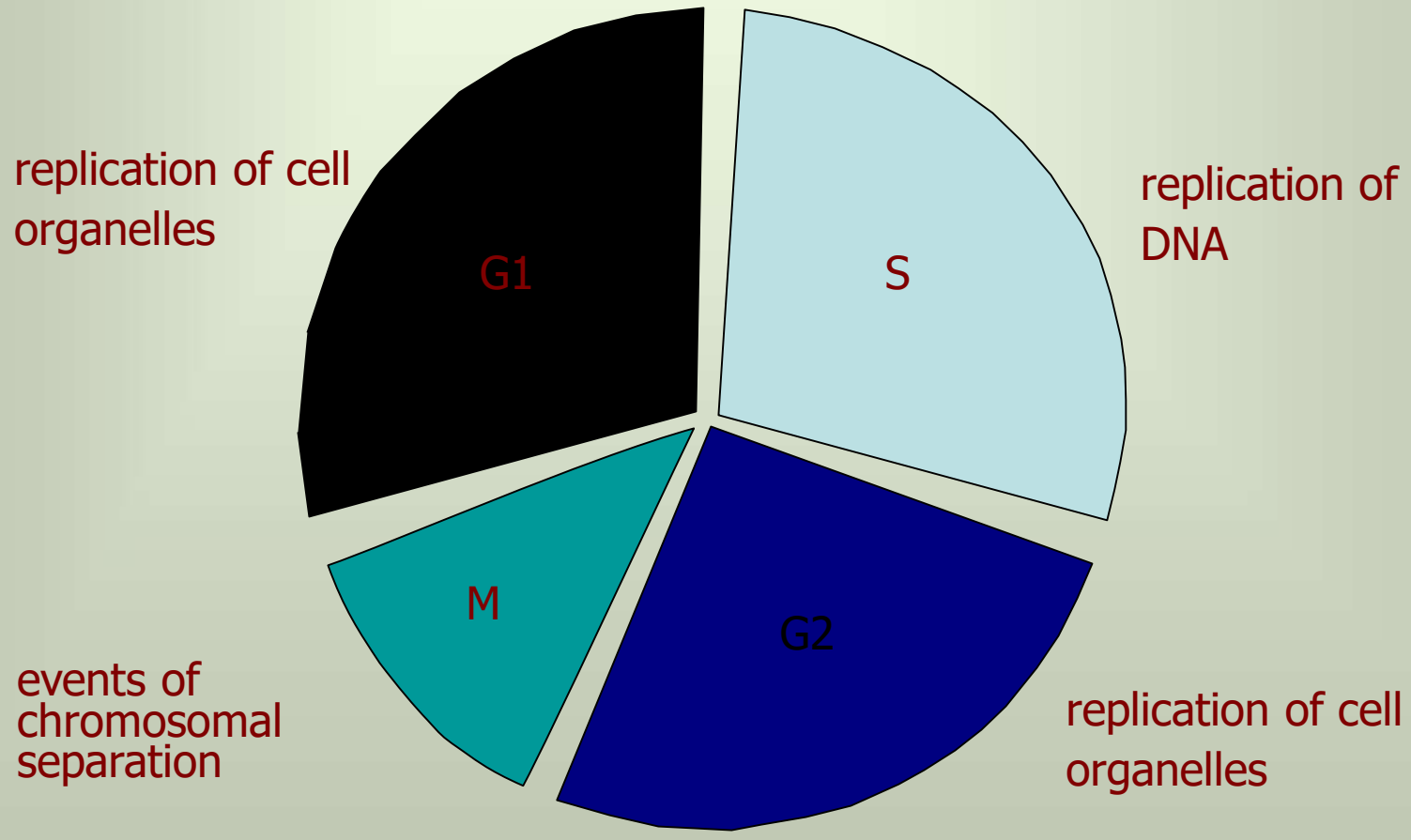
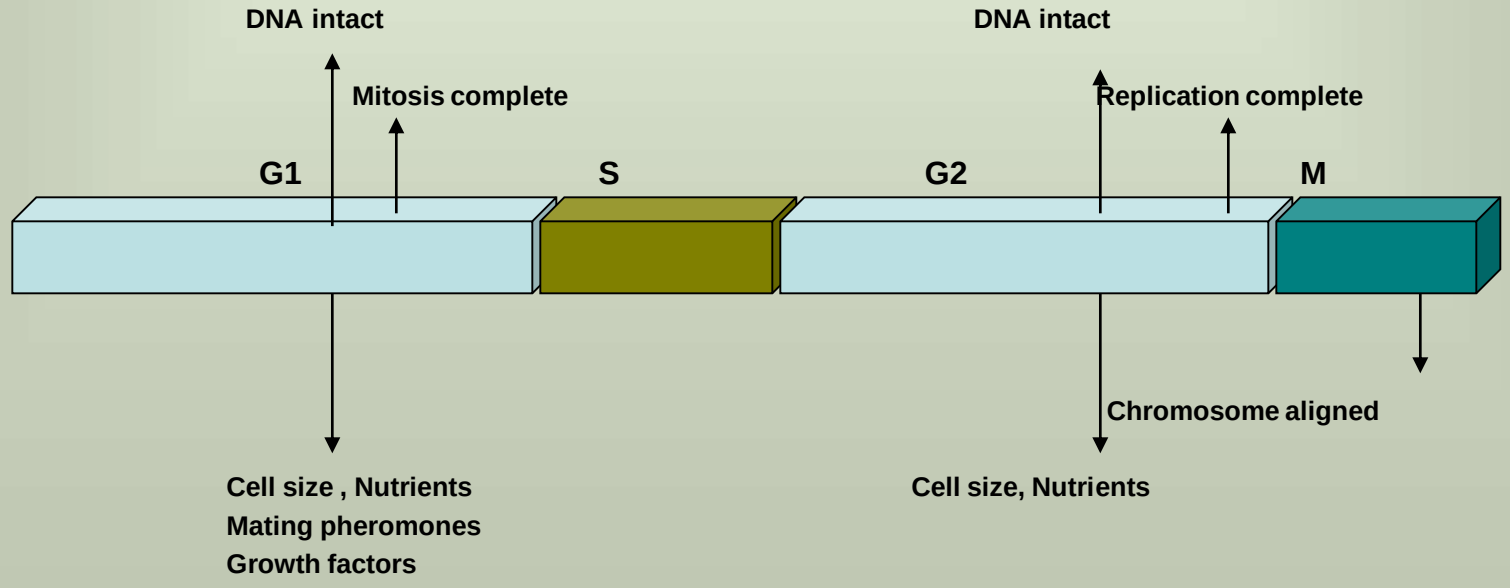
Cell cycle is the sequence of events between two successive cell division

Eukaryotic cell cycle is divided into four discrete phases

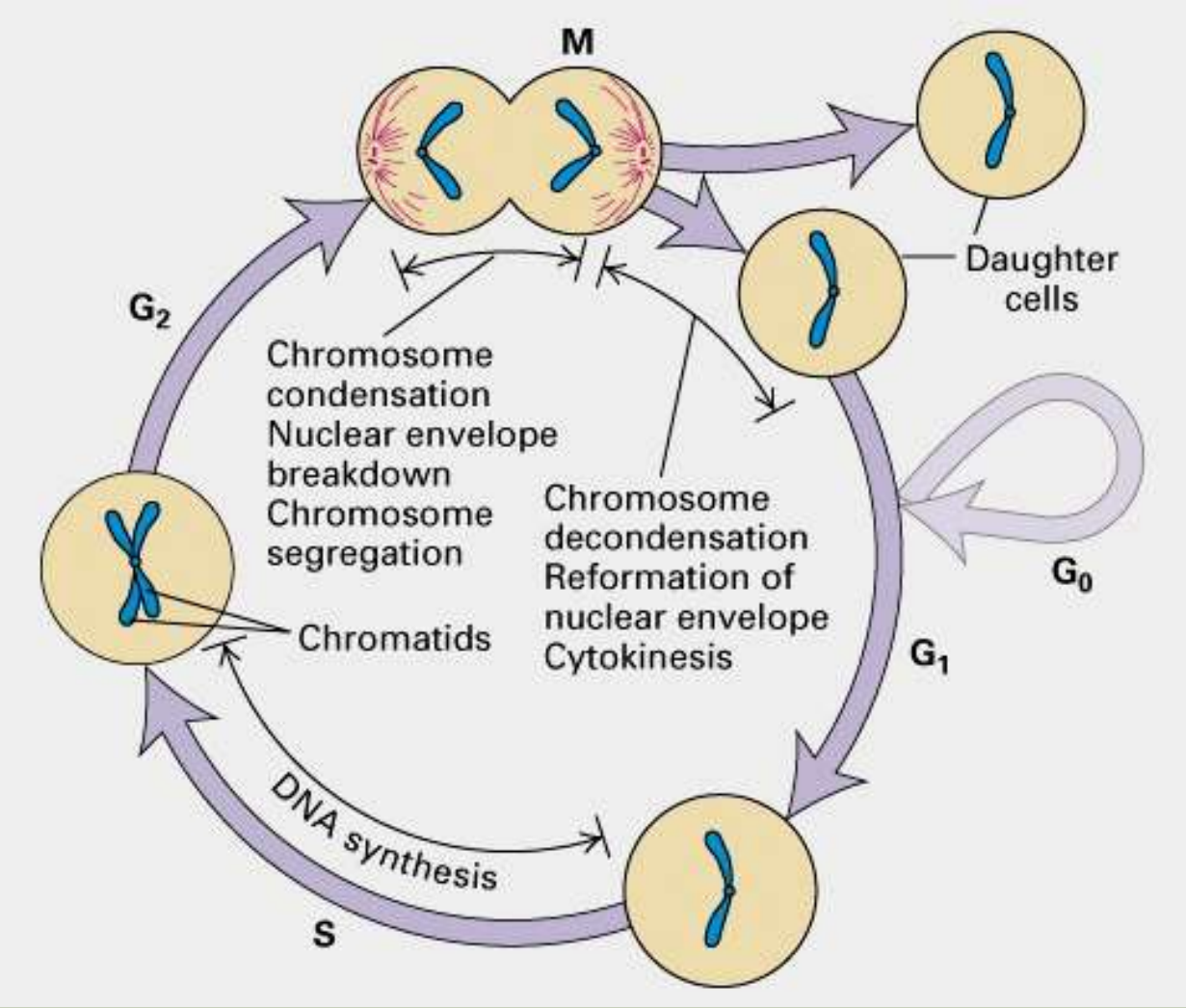


CELL CYCLE CHECK POINTS

- There are several checkpoints in the cell cycle which are clustered in two major groups
  - Those occurring at G1 and regulate entry into the S phase
  - Those occurring at G2 and regulate entry into M phase



The durations of G1 and G2 are variable, even within an organism or cell type. The durations of S and M are usually quite consistent.



Different experimental systems have contributed distinct kinds of information to our understanding of the cell division cycle

The progress of the cell cycle is controlled at specific checkpoints

- Mammalian cells enabled to identify the subdivision of interphase into G1, S, and G2
- Yeast cells have provided insight into the pathways that control the decision to move from one stage to the next
- Embryos (amphibian and echinoderm) have provided synchronized cells for biochemical studies and the identification of important control proteins

Intrinsic and extrinsic information provide cues to regulatory proteins of cell cycle r that co-ordinates cell growth & cell division

Regulatory proteins ensures that preceding stage of the cell cycle is completed before the next stage commences

During transition from one phase of cell cycle to another, there is sudden bursts of kinase activity, which activates the regulatory proteins

Checkpoint regulatory proteins inhibit specific kinase activity when the internal or external cues are unsuitable

Checkpoints: Quality Control of the Cell Cycle

- **G1 checkpoint:** This regulatory mechanism senses
  - **DNA damage** before the cell enters S phase
  - Feels the environmental cues like **nutrition** and **growth factors**

- **S phase check point:** This regulatory mechanism senses
  - **DNA damages** during S phase,
  - monitor the presence of the **Okazaki fragments** on the lagging strand during DNA replication. The cell cycle will no proceed further unless Okazaki fragments are cleared.

Checkpoints: Quality Control of the Cell Cycle

- **G2 checkpoint :** This regulatory mechanism senses
  - **DNA damage** after DNA replication
  - Completion of DNA replication
  - **Repair of DNA damage**

- **Spindle checkpoints**
  - detect any failure of spindle fibers to attach to **kinetochores** and arrest the cell in **metaphase** (M checkpoint)
  - detect improper alignment of the spindle itself and block cytokinesis
  - trigger apoptosis if the damage is irreparable

Discovery of Cell Cycle Promoting Factors

Nuclei fusion experiments provided evidence of M-phase promoting factors

Fusion	Result	Conclusion
S x G1	Both nuclei replicate	S phase nucleus contains an promoting factor
S x G2	S-phase nuclei completes replication, G2-phase nucleus waits for S-phase nucleus to complete replication and then both cells enter the M phase	G2 nucleus cannot respond to S-phase activator (a re-replication block,) S-phase activator is also an inhibitor of mitosis
M x G1, M or G2	Interphase nucleus enters precocious mitosis (regardless of state of chromosome replication)	M nucleus contains an M-phase promoting factor
G1 x G2	Neither nucleus undergoes replication or mitosis	Both S-phase and M-phase activators are present transiently

Nuclear transplantation studies with Xenopus eggs provided some interesting results

- interphase nuclei formed spindles when injected into eggs arrested at metaphase of meiosis I
- cytoplasm from meiosis-I metaphase cells induce meiosis in oocytes arrested in G2
- The substance responsible was termed **maturation promoting factor (MPF)**
- **MPF** can induce mitosis in somatic cells and is identical to **M-phase promoting factor**

CONTROL OF THE CELL CYCLE
Cyclins & CDKs

- **Cyclin-dependent kinases regulates the cell cycle activity**
- **(Cdks) levels in the cell remain fairly stable, but each must bind the appropriate cyclin for activation. In vertebrates the CDKs are**
  - **a G1 Cdk (Cdk4) / CDC28 (yeast)**
  - **a S-phase Cdk ((Cdk2 or p34/Cdc2)**
  - **a M-phase Cdk (Cdk1) cdc2 (yeast)**

- **Cyclins: levels in the cell rise and fall with the stages of the cell cycle**
  - **a G1 cyclin (cyclin D)**
  - **S-phase cyclins (cyclins E and A)**
  - **mitotic cyclins (cyclins B and A)**

CONTROL OF THE CELL CYCLE
Cyclins & CDKs

- **The anaphase-promoting complex (APC).**

  (The APC is also called the cyclosome, and the complex is often designated as the APC/C.)

The APC/C

- **triggers the events leading to destruction of the cohesins thus allowing the sister chromatids to separate;**
- **degrades the mitotic cyclin B.**

Cyclin-Dependent Protein Kinases (CDKs)

- **CDKs** were discovered in ***S. cerevisiae*** cdc mutants.
- There exists a family of structurally and functionally related CDKs in all eukaryotes
- The activities of the CDKs are governed by another group of proteins known as **cyclins**
- Phosphorylation reactions by CDKs are reversed by the action of phosphatases
- CDKs themselves are also regulated by phosphorylation of specific rsidues like Tyrosine and Threonine

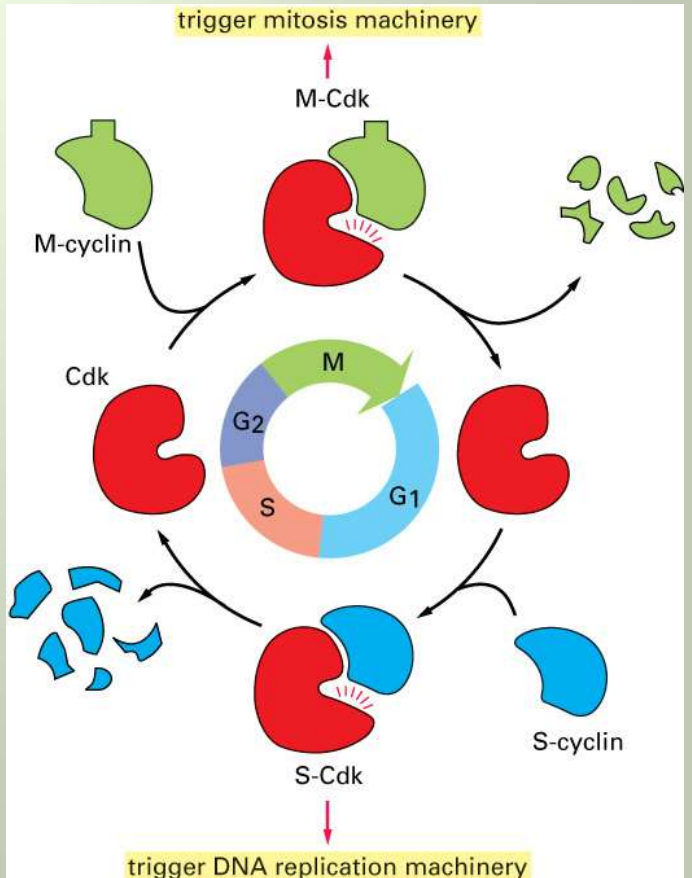


Figure 17–16. Molecular Biology of the Cell, 4th Edition.

Yeast Size and Morphology Through the Cell Cycle

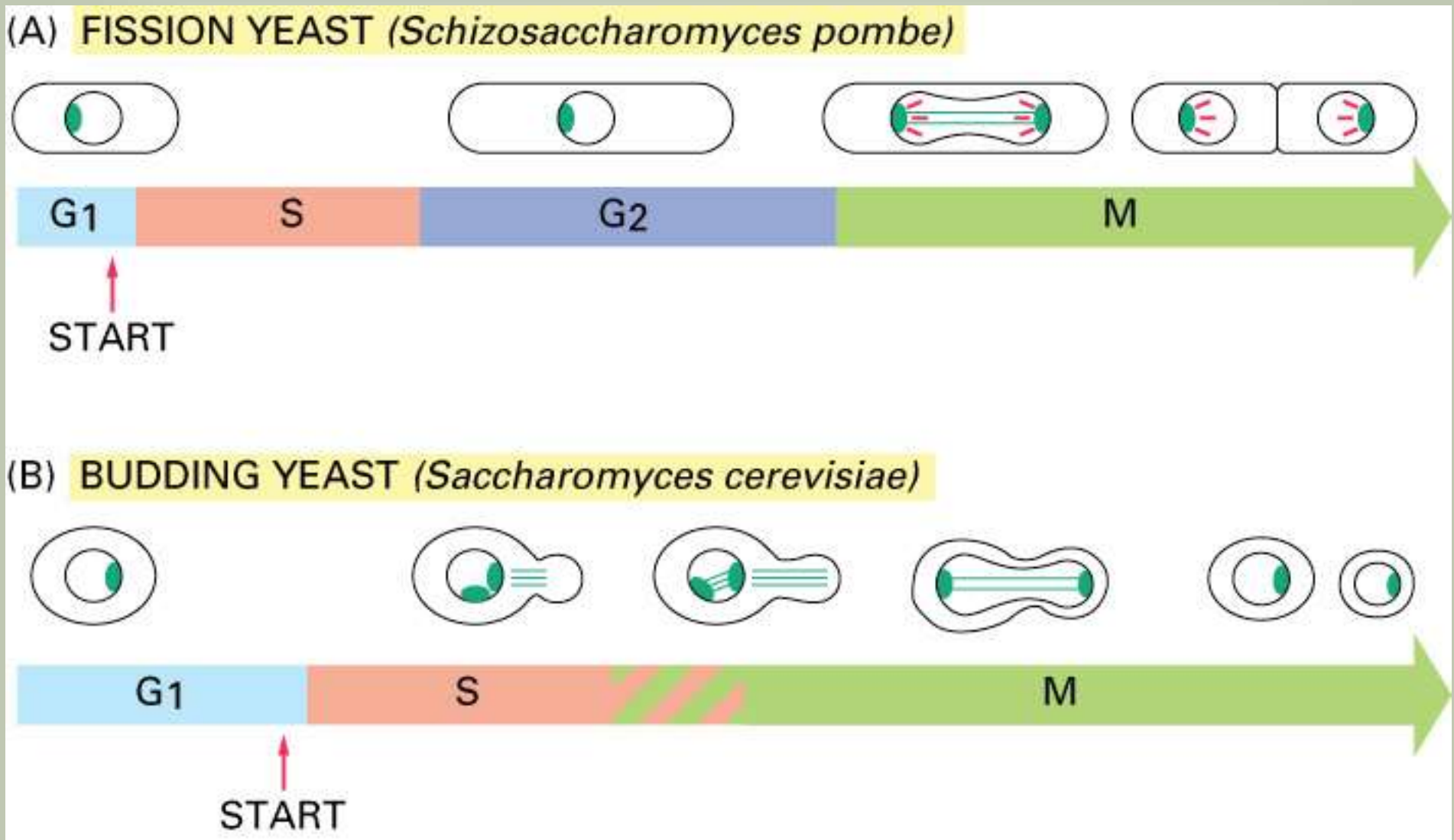
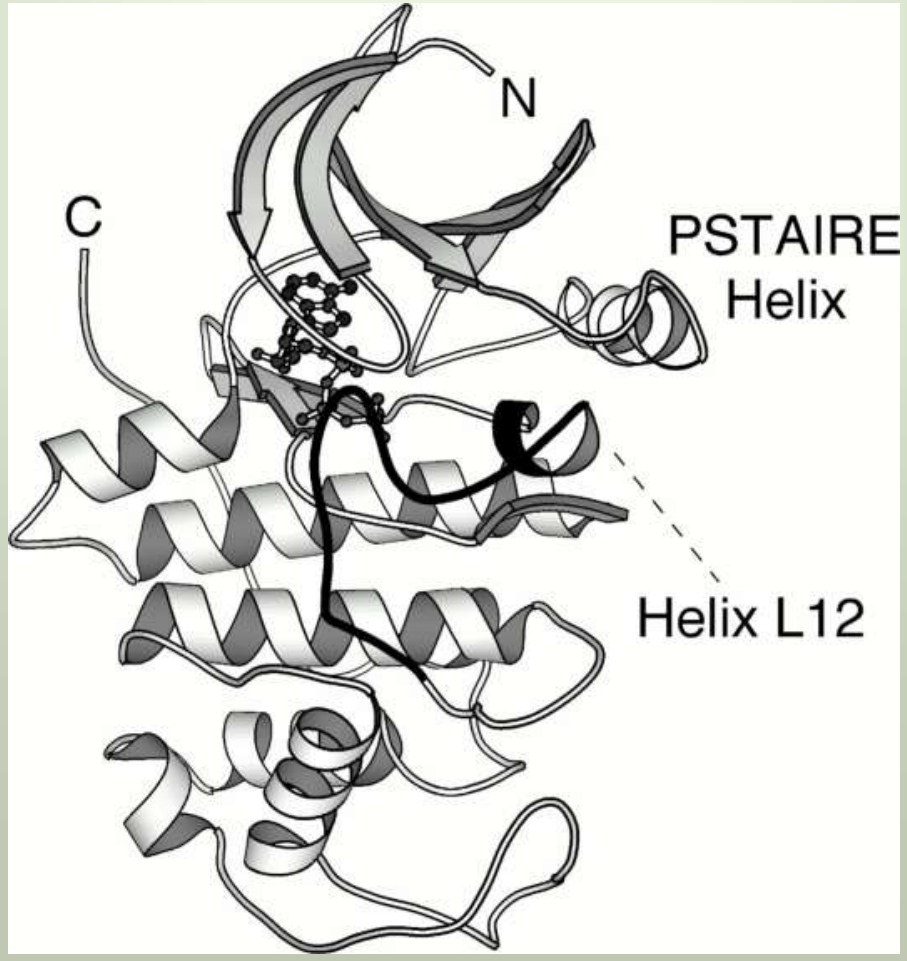


Figure 17–4. Molecular Biology of the Cell, 4th Edition.

Discovery of CDKs

- Cell division cycle (cdc) mutants of ***S. cerevisiae*** gets blocked at **START** because of a defective gene – ***CDC28***
- The product of ***CDC28*** is a 34kD protein kinase and is the principal regulator of the G1-S transition.
- In ***S. pombe***, a similar gene called ***cdc2***, encode a homologous protein kinase (Cdc2) which regulates the transition G1 to S and G2 to M phase.
- Genes encoding similar kinases were subsequently isolated from vertebrates (functions equally well in mutant yeast to produce wild-type cell cycle function
- Significantly, the *Xenopus* homology of CDC28/cdc2 is **p34CDC28/Cdc2** and is a component of **MPF**, which function specifically at the G2-M transition.

Structure of a CDK (Human CDK2)



CYCLINS

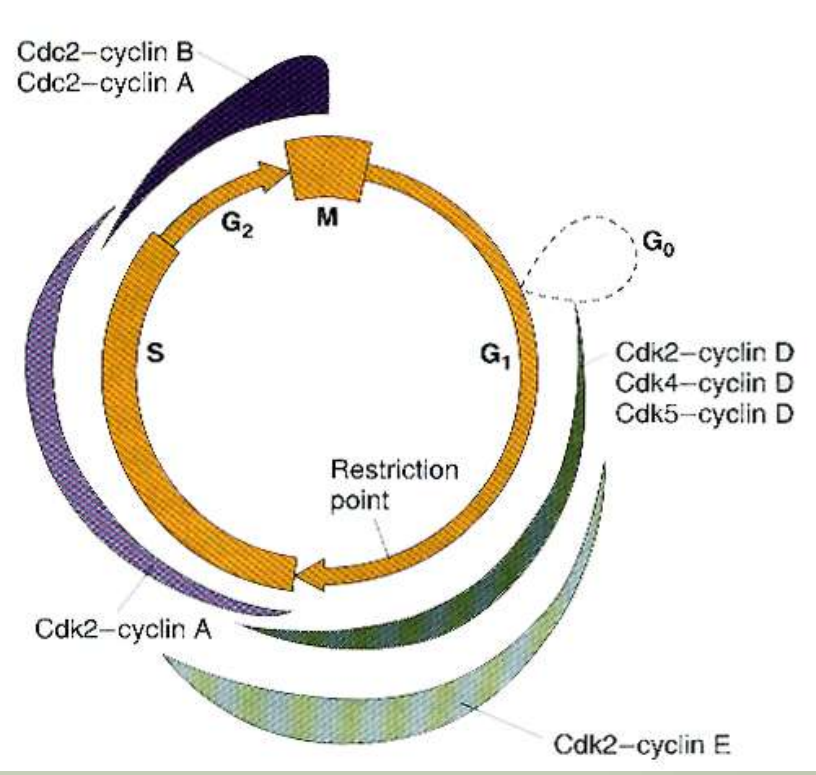
Cyclins are proteins that appear only at specific time during cell division and then are degraded very quickly

Cyclins binds specifically with different CDKs and are responsible to induce kinase activity

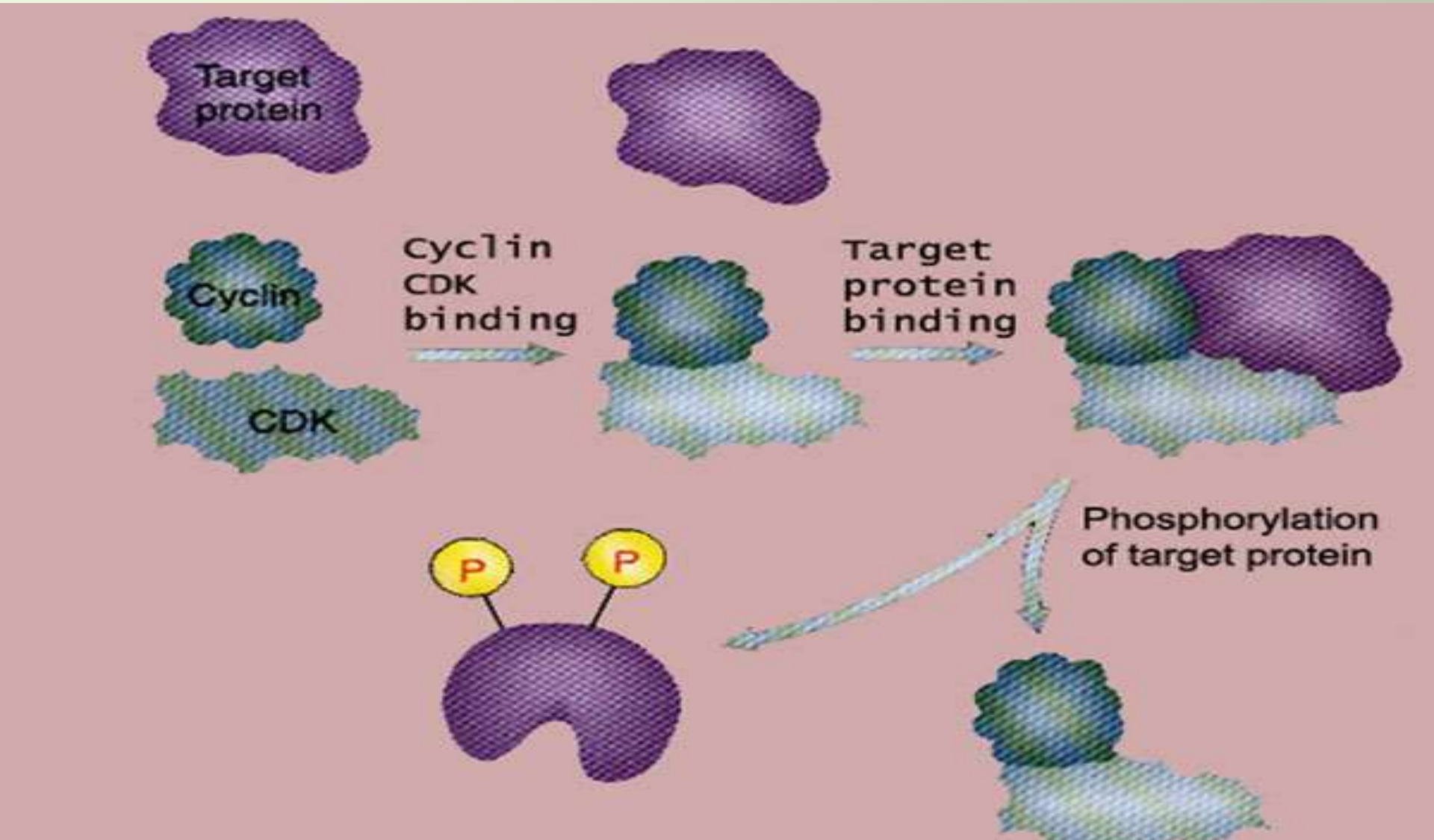
There exists a family of structurally and functionally related cyclins in all eukaryotes

Cyclin-CDK complex activates target proteins by phosphorylation

Cyclin-CDK complex also activates transcription factors for next group of cyclins



Cyclin-CDK mediated phosphorylation of target proteins



Cyclin Diversity

Synthesis of the cyclin and their activity oscillates during the progression of the cell cycle

Presence of diverse cyclins confer substrate specificity to the CDKs.

Change in substrate specificity of CDKs lead to the differential expression of genes

All cyclins carry a conserved motif, the **cyclin box**, which is required for **CDK binding**.

Cyclins which carry the **PEST domain** are inherently unstable undergo **rapid degradation**

The stable cyclins carry a motif called **destruction box** which is required for **ubiquitination**.

Cyclin diversity

Generally three types of cyclins are present in all organisms:-

G1 – cyclins
- regulate G1-S transition
- **CLN1-3** in budding yeasts
- **cig1** and **cig2** in fission yeast
- cyclin **C, D, E, F** in vertebrates

S – phase cyclins
- are required for DNA replication
- **cyclins E and A**

M – phase cyclins
- control the mitosis.
- **CLB1-6** cyclins in *S. cerevisiae*
- **cig13** in *S. pombe*
- **A & B** type cyclins in vertebrates

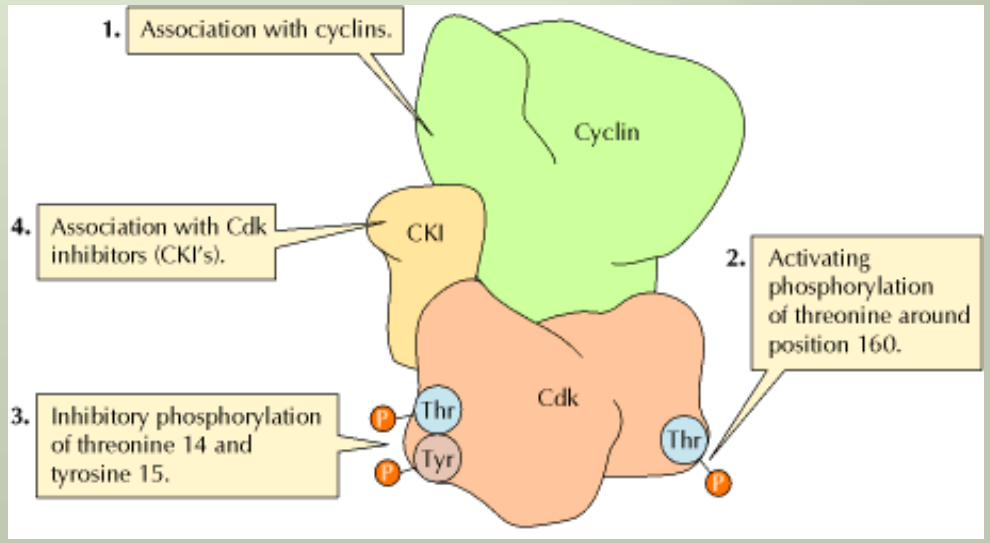
(In vertebrates, the cyclins have been grouped into 8 families - A to H

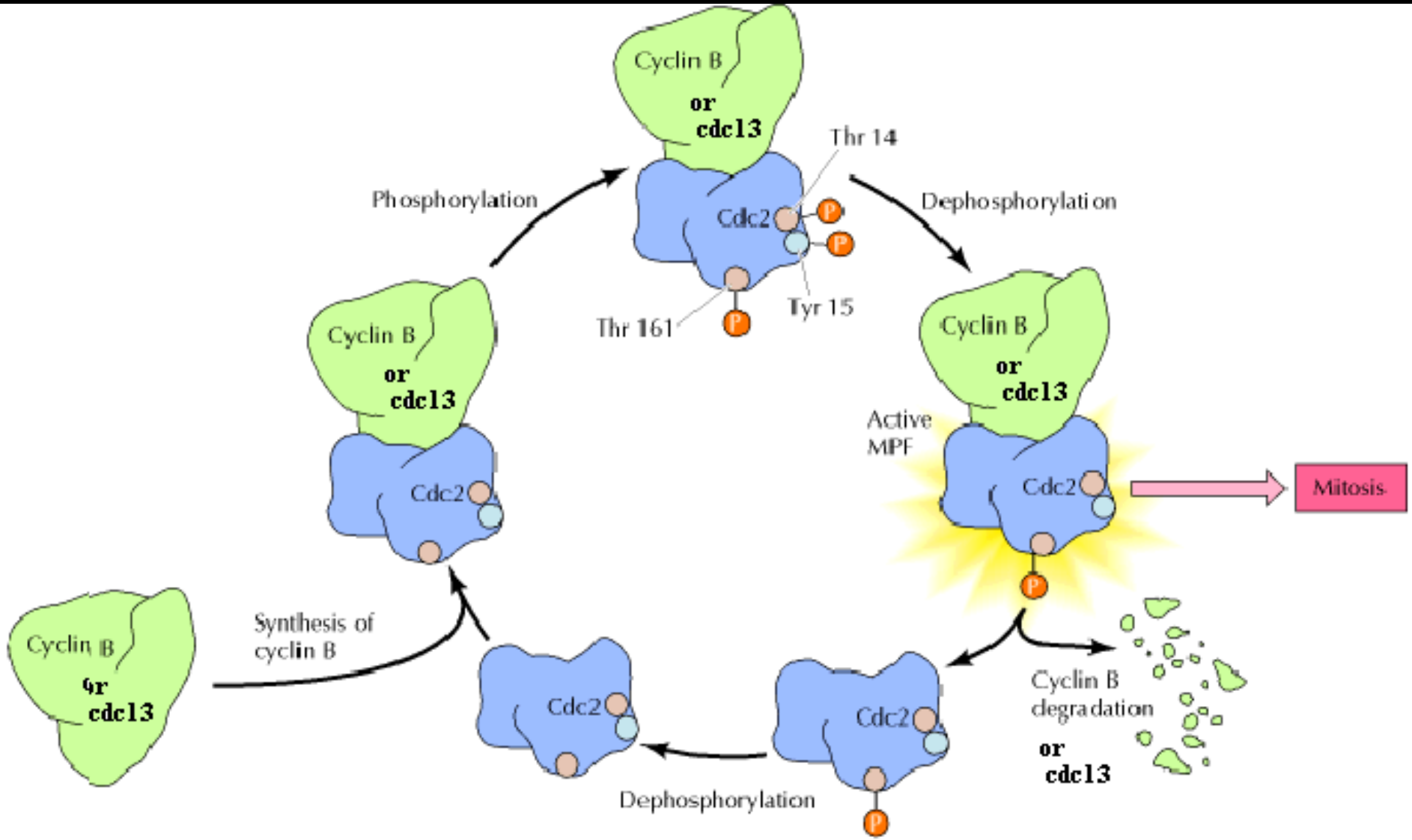
Regulation of Cdks-Cyclin activity

Activity of CDK & its substrate specificity depends on the type of cyclin it binds with

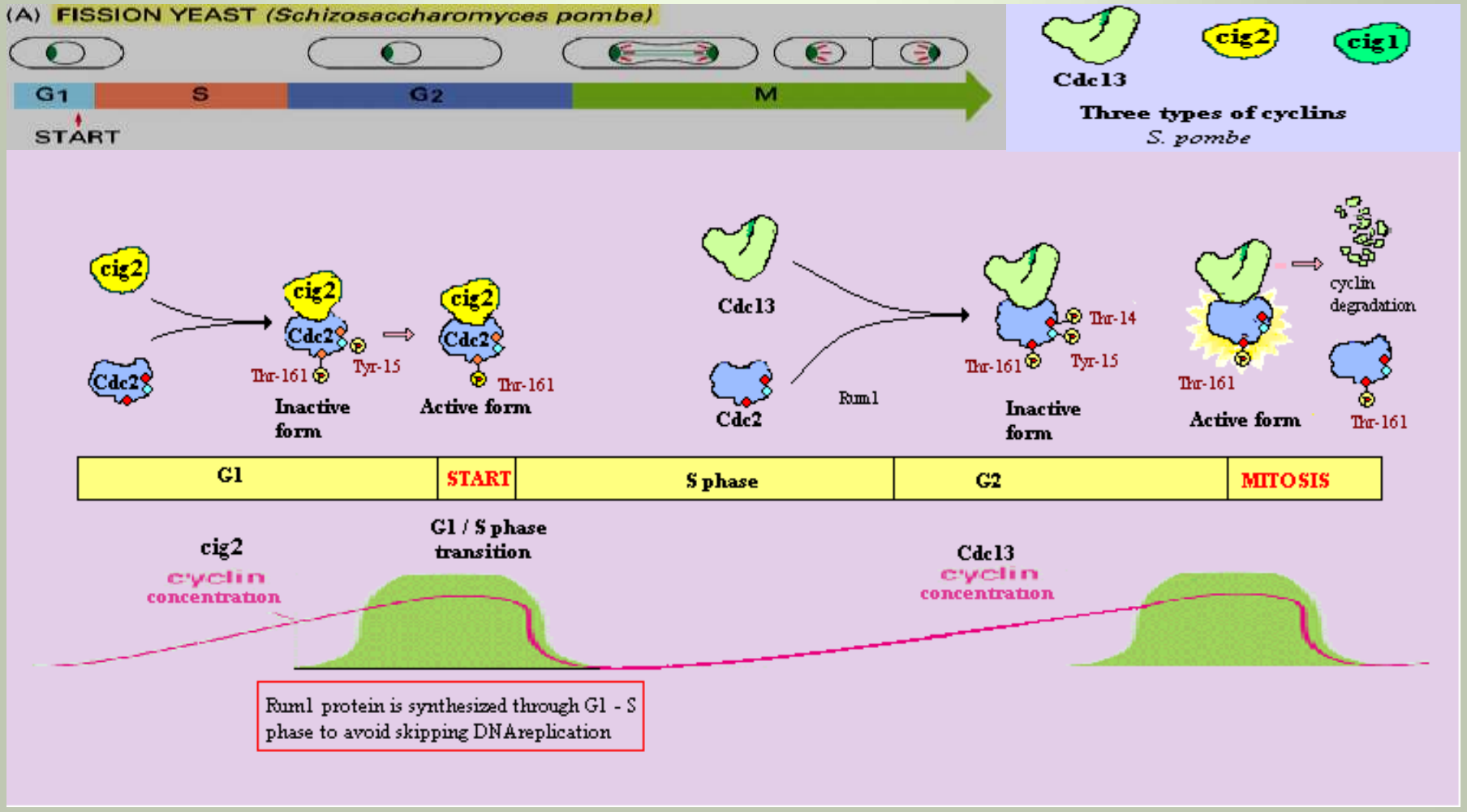
CDK activity also requires phosphorylation of some target amino acid residues in the CDK molecule itself

- CDC28 and Cdc2 kinase in yeast require phosphorylation at Tyr-15 and Thr-161
- Phosphorylation at Thr161 induces the kinase activity
- phosphorylation at Tyr-15 inhibits the kinase activity and dominant over Thr161 phosphorylation




Cyclin B
or
cdc13
Thr 14
Cdc2
P
P
Thr 161
P
Tyr 15
Phosphorylation
Dephosphorylation
Cyclin B
or
cdc13
Cdc2
Active
MPF
Cdc2
P
Mitosis
Cyclin B
degradation
or
cdc13
Cdc2
P
Cdc2
Dephosphorylation
Cyclin B
or
cdc13
Synthesis of
cyclin B
Cyclin B
or
cdc13
Cdc2

CDK regulation in *Schizosaccharomyces pombe*



S. pombe

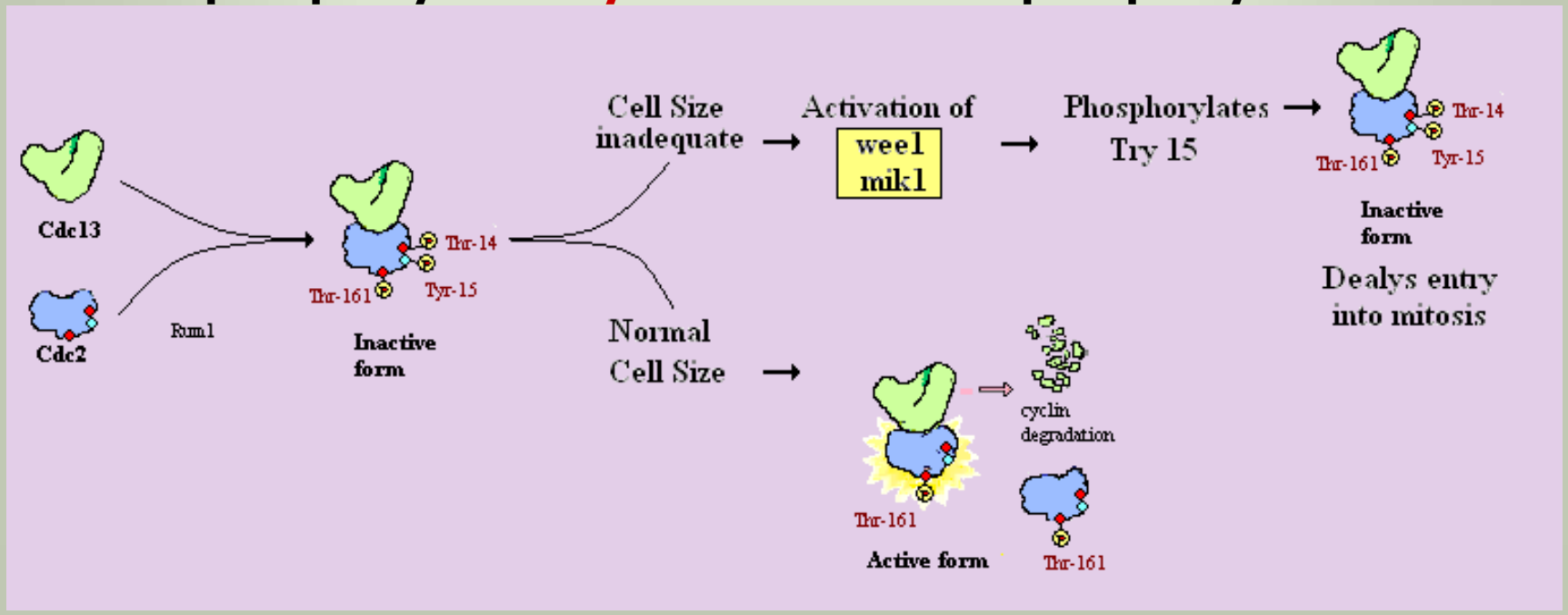
- Mutants lacking cig2, cdc13 and cig1, exhibit complete blockage of the S phase
- However, in mutants lacking both cig1 and cig2, orderly onset of S phase and mitosis can take place suggesting that the orderly activity of **Cdk** appears to depend on the quantitative level of **cdc13**
- Thus, **cdc13** can fulfill three different functions in association with Cdc2:
  - it is essential for the onset of mitosis
  - it prevents re-replication in G2
  - it compensates for cig2 as the major g1 cyclin in mutants lacking cig2.

Regulation of Cdc2-cdc13 by wee1/milk1 (kinase) and cdc-25 (phosphatase)

Cdc2-cdc13 dimer is regulated by wee1/milk1 (kinase) and cdc-25 (phosphatase)

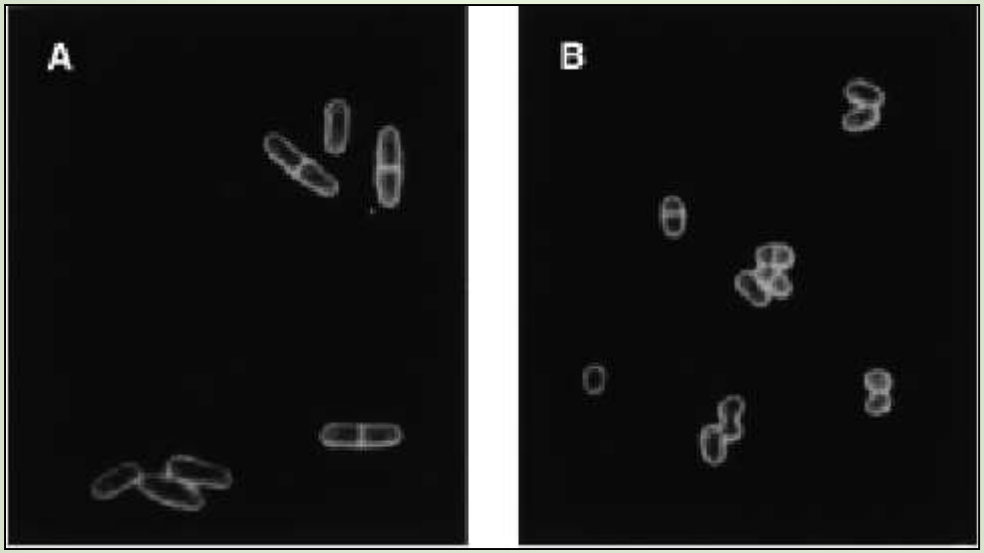
Wee1/milk1 and cdc-25 respond to environmental ques and cell cycle checkpoint signals

Wee1 phosphorylates Try15 while cdc25 dephosphorylates it



CDC mutants with different phenotypes

The first *wee* Mutants Identified a Single Gene *WEE1*



Wee1 acts in G2 to inhibit mitosis
- loss of Wee1 function causes mitosis to begin prematurely

Regulatory Mechanisms

G1/S transition requires Cdc2/cig2 activation but also requires Cdc2/Cdc13 inactivation

Mutation at Cdc13 has several consequences

- fail to enter mitosis
- there is multiple cycle of DNA replication

These observations suggests

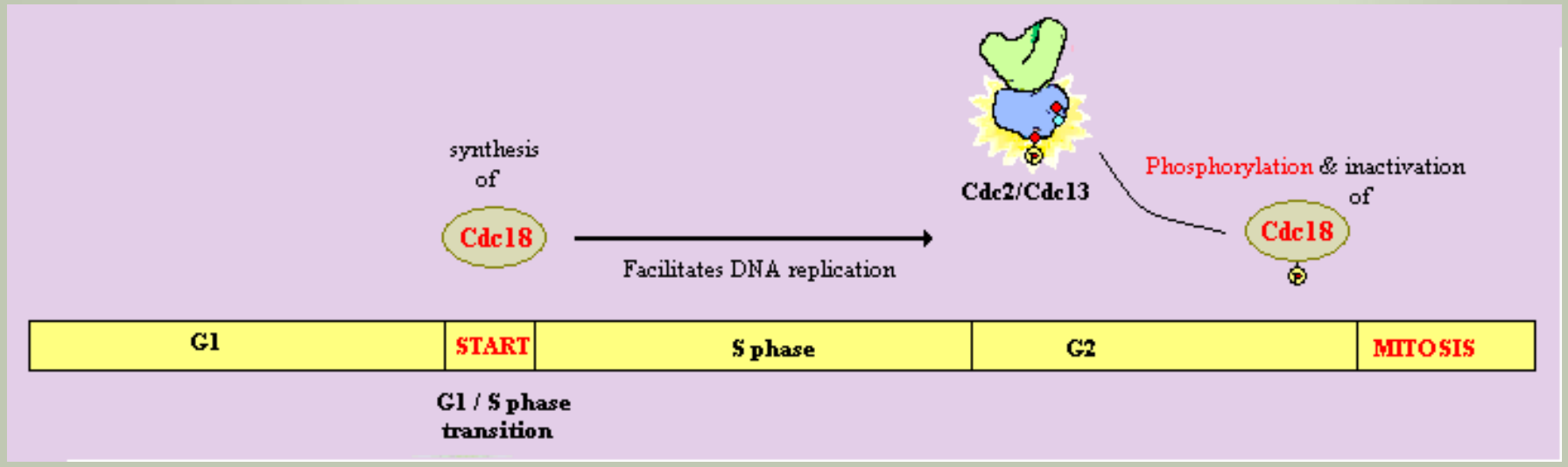
- activated M phase kinases inhibits DNA synthesis
- activated M phase kinases promotes Mitosis

- inactivated M phase kinases allow DNA synthesis to go unchecked
- inactivation prevents another mitosis

This property of Cdc2/Cdc13 thus provides a checkpoint and ensures that DNA replication and cell division occur alternately

Cdc2/Cdc13 mediated control over DNA Replication through **Cdc18** phosphorylation

- The target protein of Cdc2/Cdc13 phoshphorylation is probably **Cdc18** (transcription begins at START)
- **Cdc18** is essential for the entry into the S phase and facilitates DNA replication (over expression causes multiple DNA replication)
- **Cdc18** inactivation by phosphorylation during the entry into the M phase by Cdc2/Cdc13 ensures the prevention of another S phase



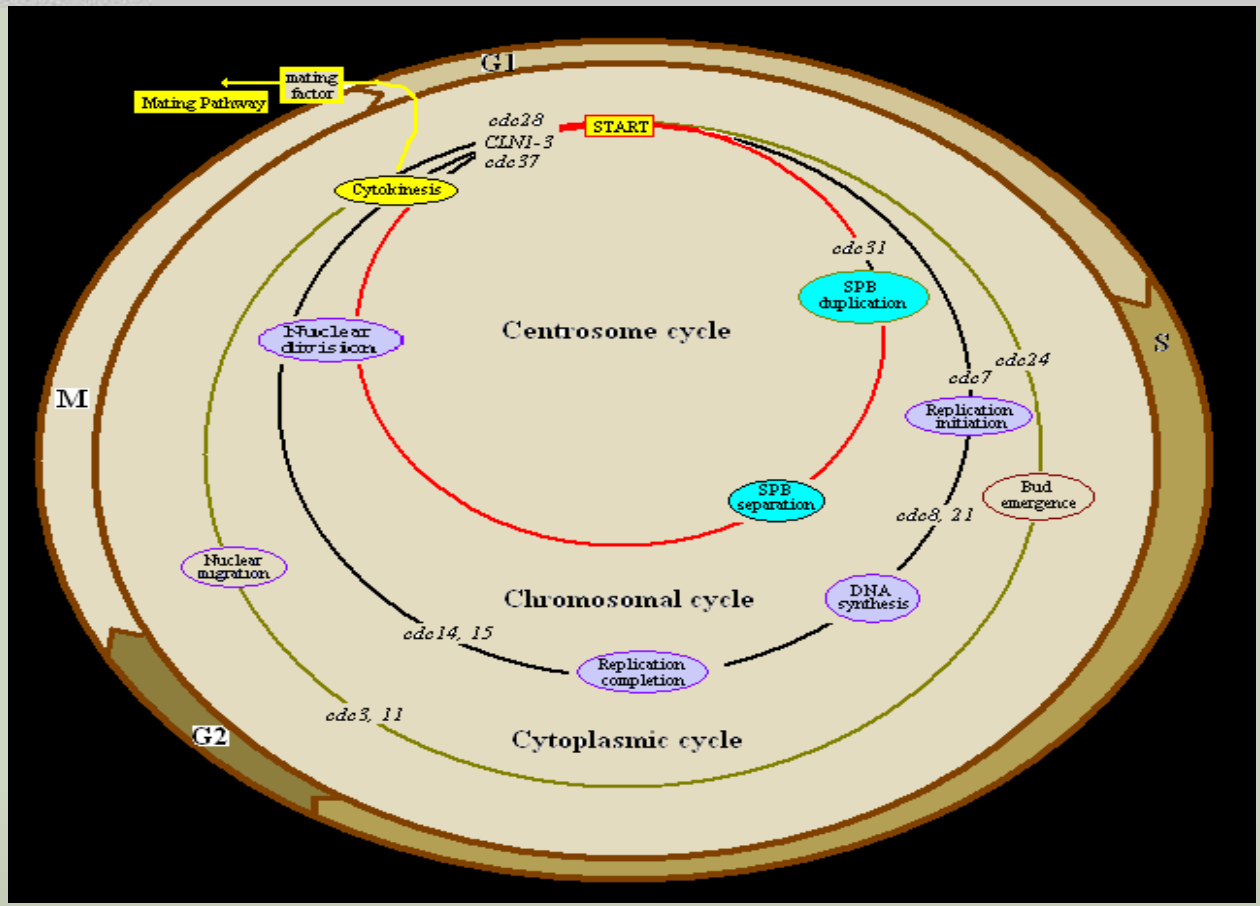
Rum1 mediated regulation of Cdc2/Cdc13

- Rum1 is expressed during G1 and G2 phase
- Over expression of Rum1 causes multiple rounds of DNA replication and cells fail to enter mitosis
- Deletion of Rum1 causes premature entry into M phase

These observations indicate

- **Rum1** inhibits the activity of Cdc2/Cdc13 during the progression of S phase
- Indirectly **Rum1** facilitates the activity of Cdc18 to proceed with DNA replication

CDK regulation in *Saccharomyces cerevisiae*



EVOLUTIONARY SIGNIFICANCES

- All known genes that effect G2/M transition appear to have conserved in evolution
- Counterpart of cdc25 are found in the string gene of *Drosophila malanogaster*
- Analogous proteins are found in amphibian and mammalian cells

Cell Cycle Kinase Inhibitors

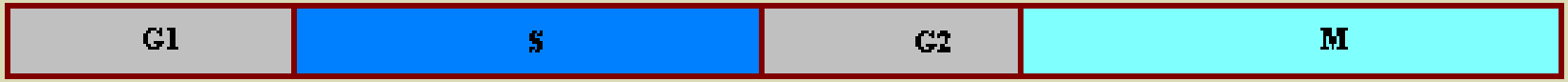
- In S cerevisiae, **CKI** (Cell cycle Kinase Inhibitors) regulate cell cycle through the binding of **CKI-Sic1** to **CDC28-CLB during G1** and maintain the kinase in inactive form
- Sic1 under goes degradation prior to entry into S phase

Animal Cell Cycle Control

- Animal cells uses similar components like the yeast cells to control the cell cycle activity
- Because of the inherent complexity of animal cells, they express a more diversified set of proteins required for cell cycle regulation
- Transition from one stage to another during the progression of the cell cycle is controlled by Kinase activity
- As in yeast cells, the kinases in animal cells have a catalytic subunit and a regulatory unit (cyclins)

Cell Cycle Kinases and their regulatory in Yeast and Animal cells

	Catalytic subunit	Regulatory subunit	Catalytic subunit	Regulatory subunit
S. pombe	Cdc2	Cig2 (Cig1)	Cdc2	Cdc13 (B-like)
S. cervisiae	CD28	CLN1-3	CD28	CLB1-4 (B-like)
Mammals / Frogs	CdK2, 4	cyclins A, D1, D2, D3, E	Cdc2	cyclins A, B1, B2



Cdk2, 4

- Both the Cdks can associate with different types of G1 cyclins
- **Cdk2** is well characterized and show 66% homology with **Cdc2**

Cdc2

- Unlike Yeast cells, animal cells have more than one type of catalytic units (cyclin dependent kinases)
- ~ more than 10 genes are there for Cdc2 are known but which one of them are involved in cell division is not clear

Cdk-cyclin activation and G1/S transition in animal cells

G1 CDKs are called Cdks (Cdks2,4)

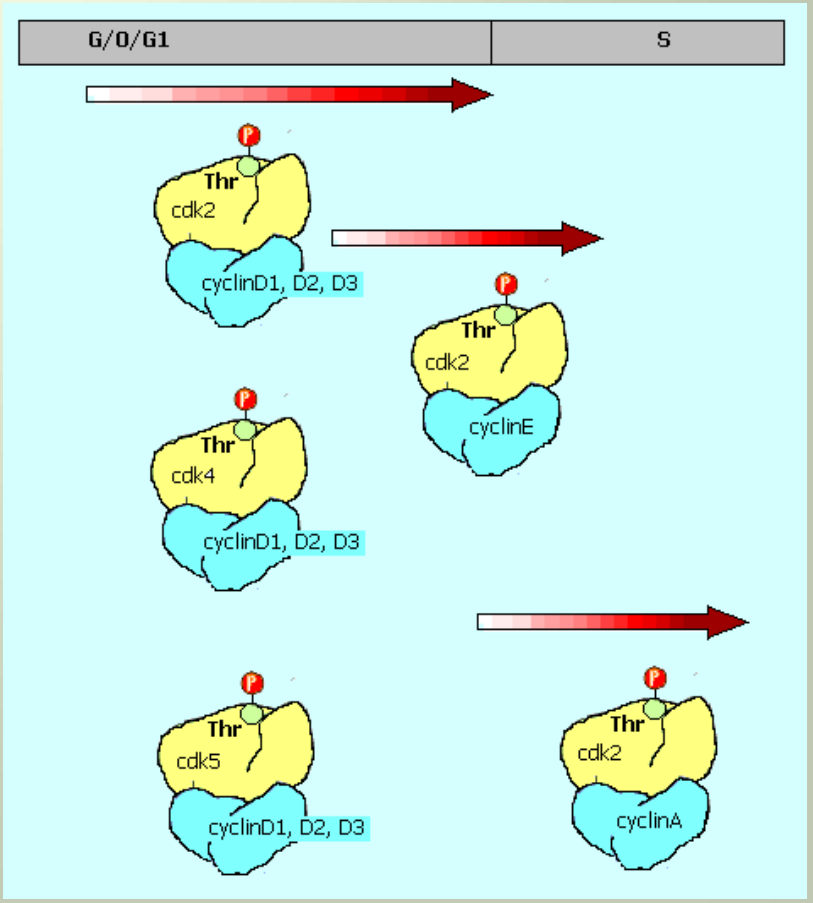
Cdks associate with a variety of G1 cyclins

G1 cyclins includes A, D1, D2, D3 & E

Cdk-cyclin dimer requires the activation of a Thr residue to become activated like Cdc2 in yeast cells

Various Cdk-cyclin dimmers regulate entry into S phase and progression through S phase as shown in the figure

G1 cyclins were identified by their ability to substitute the CLN cyclins in *S. cerevisae*

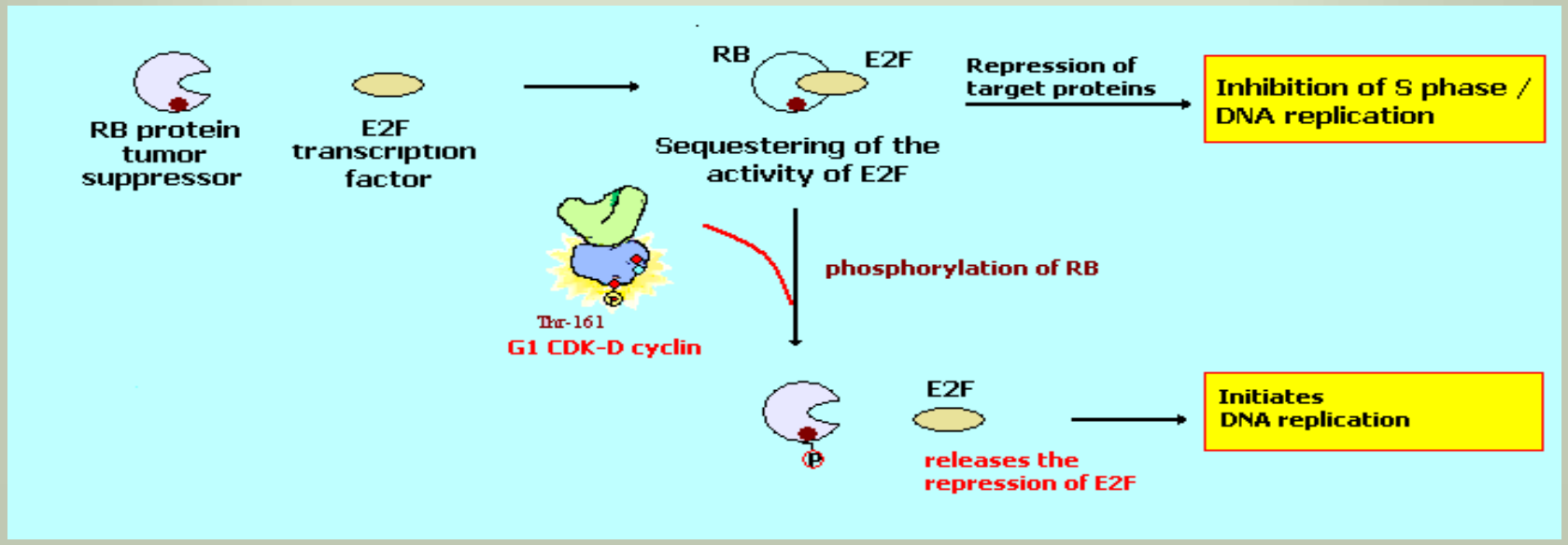


D cyclins

- Growth factors stimulate the synthesis of D cyclins when a cell enters G1 from G0
- The D cyclins have a short half life and their levels decline rapidly when the growth stimulus is withdrawn
- Activity of the D cyclins are sometimes observed in later part of the G1 but not close to G1/S boundary
- The functions of D cyclins may partly be redundant but their ability to associate with different Cdks is not understood
- D cyclins probably trigger the cell in G0 to enter the G1 phase of cell cycle

RB - Substrate for G1 Cdk-cyclin complexes

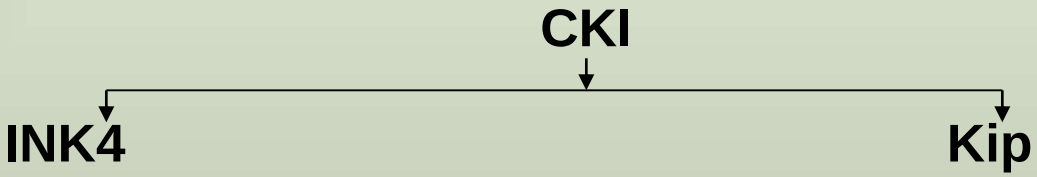
- **RB** is a negative regulator protein of cell cycle
- During the early part of **G1**, unphosphorylated **RB** binds to transcription factors of the **E2F** (transcription factor) family and inhibits transcription of genes necessary for entry into **S-phase**
- Non phosphorylated **RB** forms complexes with **Cdk4,6 D1, 2, 3** (prominent form) and also with **Cdk2 cyclin-E**
- **G1 CDK-D cyclin** complexes phosphorylates **RB** and releases the repression of **E2F**.



Inhibitors of G1 Cdk-cyclin activity

- Certain proteins called **CIK**s (CDK-cyclin Inhibitors) bind to Cdk-cyclin dimmers in quiescent cells and inhibit the phosphorylation **RB,** thus in turn promotes the sequestering of **E2F** factors, and inhibit DNA replication

- CKI proteins are broadly grouped into two classes, each having a family of proteins as noted below :



(Specific inhibitors for Cdk4 & 6)

1. p16INK4A (inhibits cdk-cyclin from phosphorylating RB)
2. p15INK4B
3. p18INK4C
4. p19INK4D

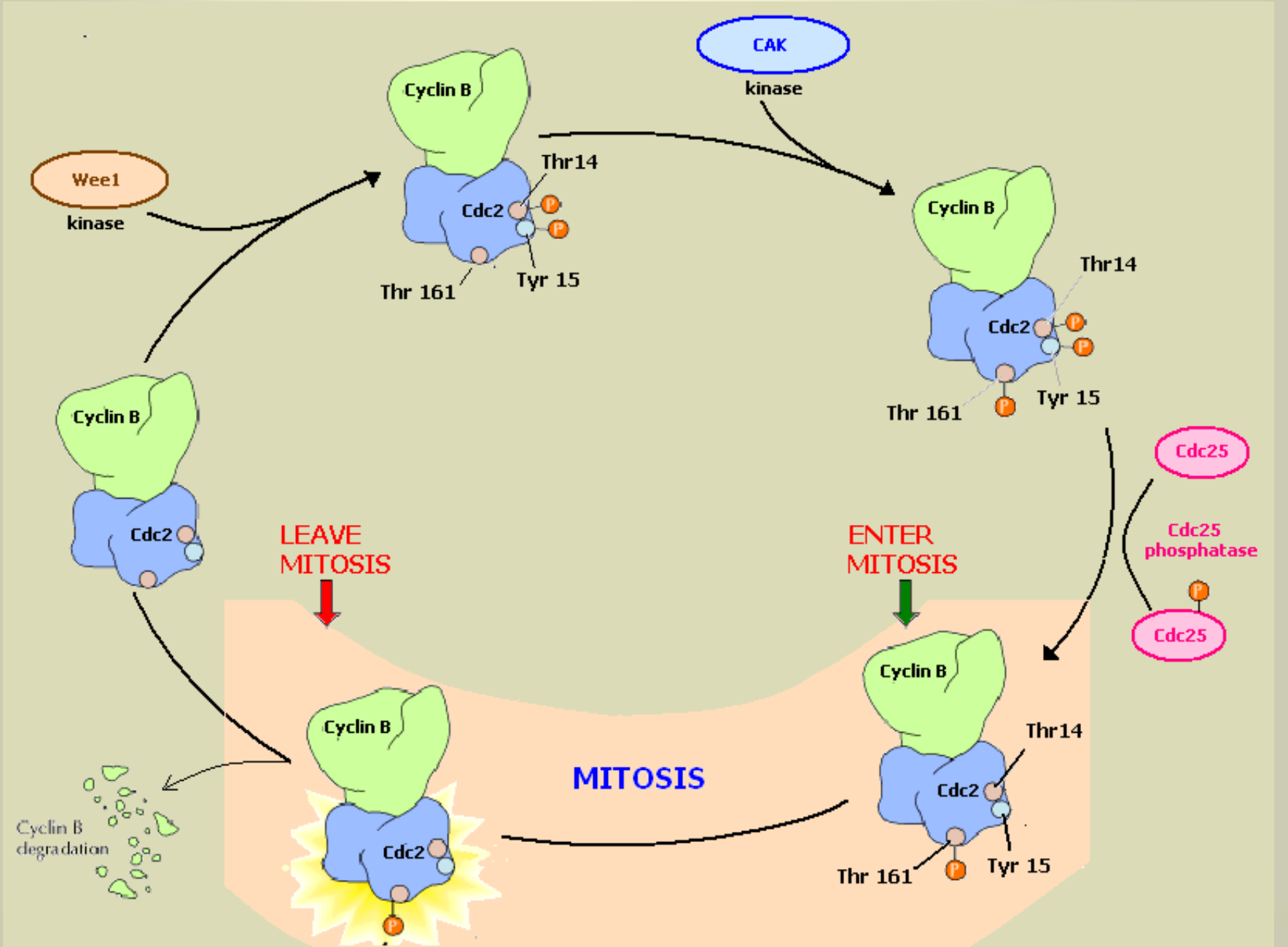
(Inhibit all G1 and S phase Cdk enzymes)

1. p21Cip/WAF1 (universal inhibitor of cdk; G1/S transition)
2. p27Kip1 (blocks progression through S phase)
3. p57 Kip2

Cdc2-cyclinA,B activation and G2/M transition in animal cells

- Higher eukaryotes, the G2/M transition is better understood
- Eukaryotes possess a large number of *Cdc2* genes (~ 10 genes) related to cdc2 homologue.
- It is not clear how many *Cdc2* genes are transcribes and are functional
- Cdc2 has 66% homology with Cdk2
- High levels of Cdc2 is maintain the cell prior to the entry into mitotic phase for activation of key proteins required for the M phase
- Cdc2 associates with Cyclin B during the maturation phase
- CAK (Cdc2 activating kinase) is responsible to phosphorylate Thr-161 and activate Cdc2
- Cyclin B degradation inactivates Cdc2 which is required for the cell to exit the M-phase and reversal of events

Cdc2-cyclinA,B activation and G2/M transition in animal cells



Key substrates of Cdc2-CyclinB

- **Histone H1** - the phosphorylation of this protein may be important for chromosomal condensation to occur

- **p60*src*** - phosphorylation of the mitotic-specific sites of this protein may influence the cytoskeleton and lead to changes in the cell shape and other **DNA binding proteins** that need to be released for chromosomal condensation to occur

- **Lamin** - this is a protein associated with the nuclear envelope

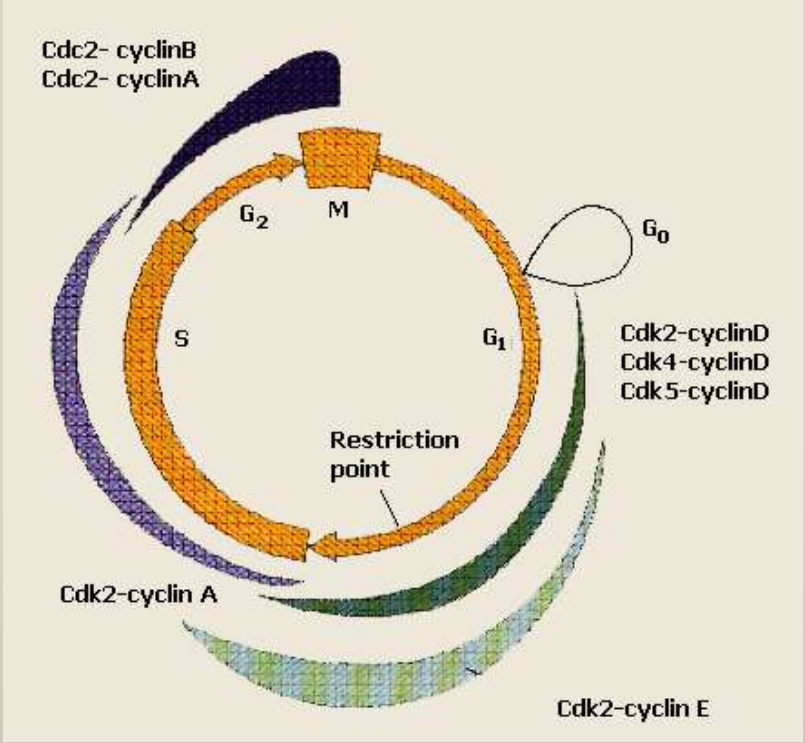
- **Centrosomal protein** - these proteins are associated with centrioles, the organizing center of the cell for microtubules associated with the cytoskeleton

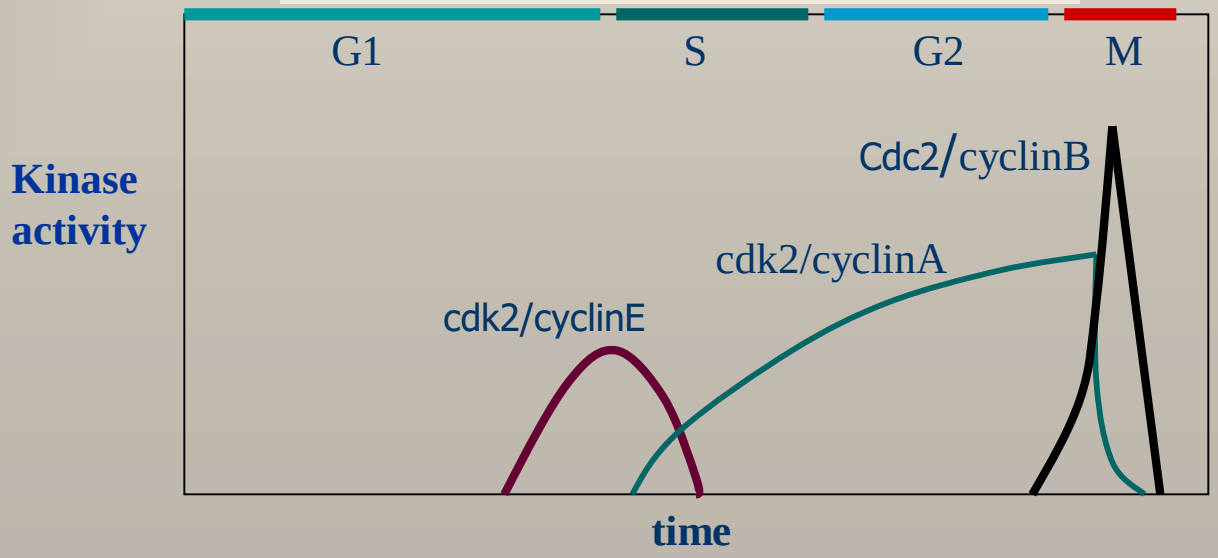
MPF drives the cell through the M phase committal point

MPF activity sets the stage and events necessary for cell division:

- chromosomal condensation
- cytoskeletal reorganization
- nuclear envelope breakdown
- cell shape changes

CDK-Cyclin Activity in Animal Cells



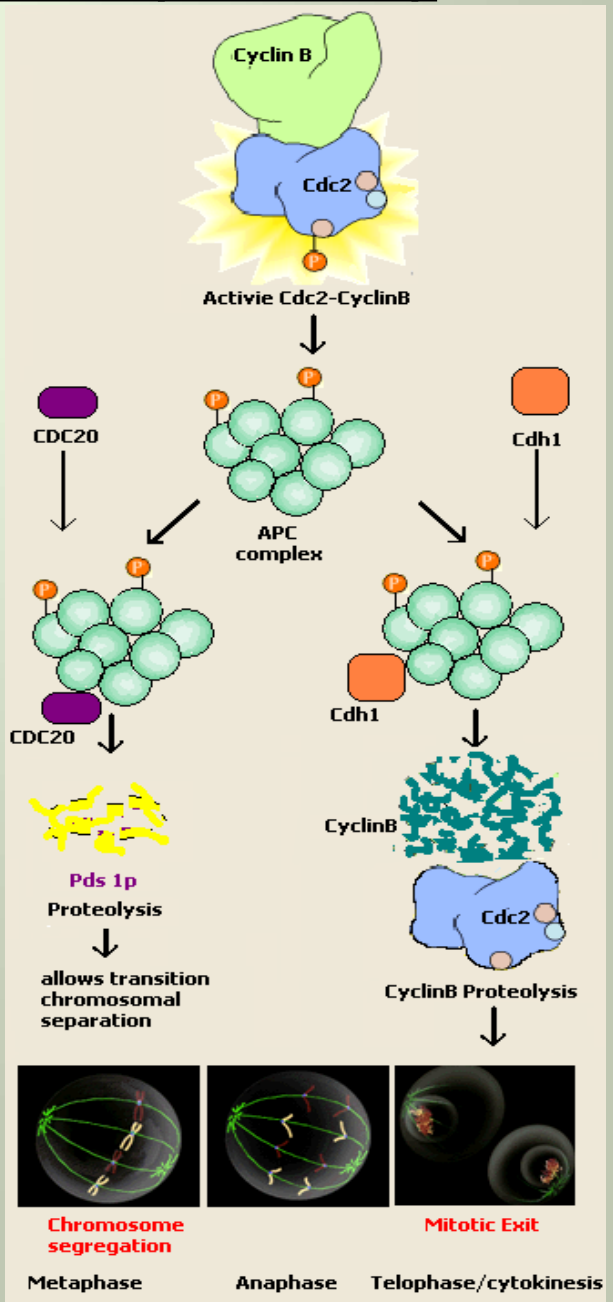


M-phase CYCLIN DEGRADATION & Activation of Anaphase-promoting complex

- **MPF** activities drives the cell to cross the commitmental point and enter **metaphase** of mitosis
- **MPF** activates the **Anaphase-promoting complex** (**APC/ or Cyclosome**)
- **Anaphase-promoting complex promotes the** degradation of cyclins and other proteins as the cell progresses through mitosis
- Several degradation mechanisms operate simultaneously, which play different roles during mitotic progression

Anaphase-promoting complex (APC/C)

- **APC** large complex of 8 subunits and functions as an E3 ubiquitin ligase
- **APC** binds to the target protein and marks it with **ubiquitin**.
- The **ubiquitinated** substrate is then degraded by the **proteosomes**.



Anaphase-Promoting Complex (APC) activities
Degradation of Cyclin A

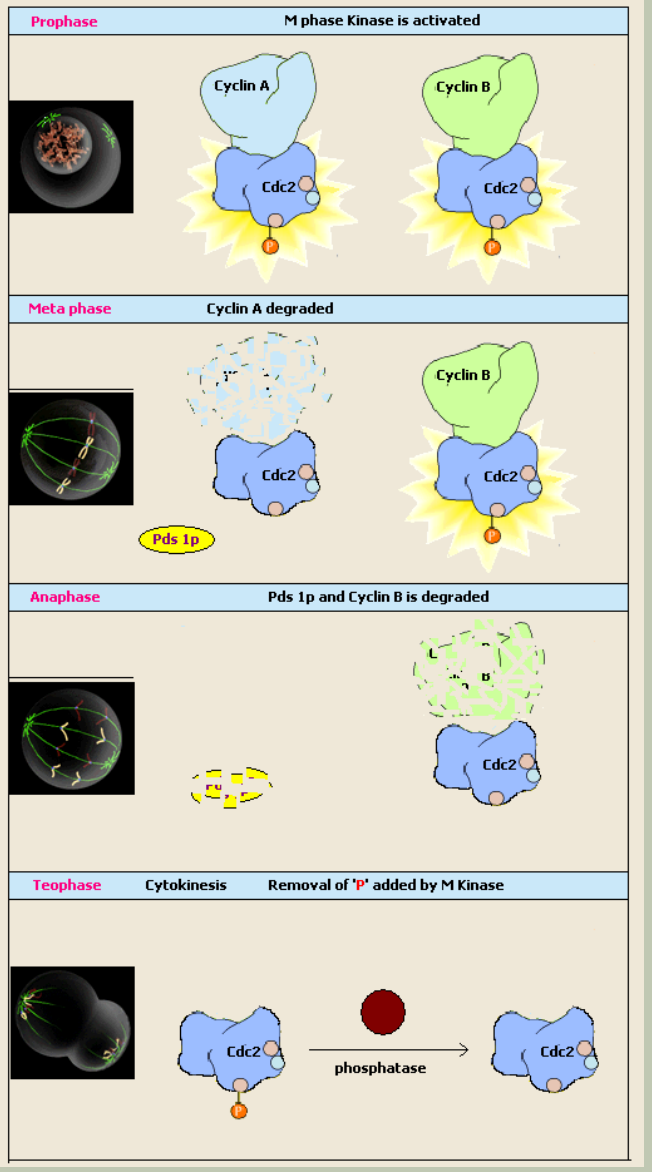
- The first event to occur is the degradation of **cyclin A**
  - Triggers destruction of **cohesins** thus allowing the sister chromatids to separate;

- **Cohesins** associates with sister chromatids during S-phase and keep them togather

- **Separin,** another protein has the ability to releases **cohesins** and thus allow chromatids to move toward the poles

- **Securin (Pds1p)** sequesters **separin,** and make it inactive.

- **Pds1p** degradation activates **separin** which degrades **cohesins** and thus facilitates the separation of sister chromatids & to move toward the poles



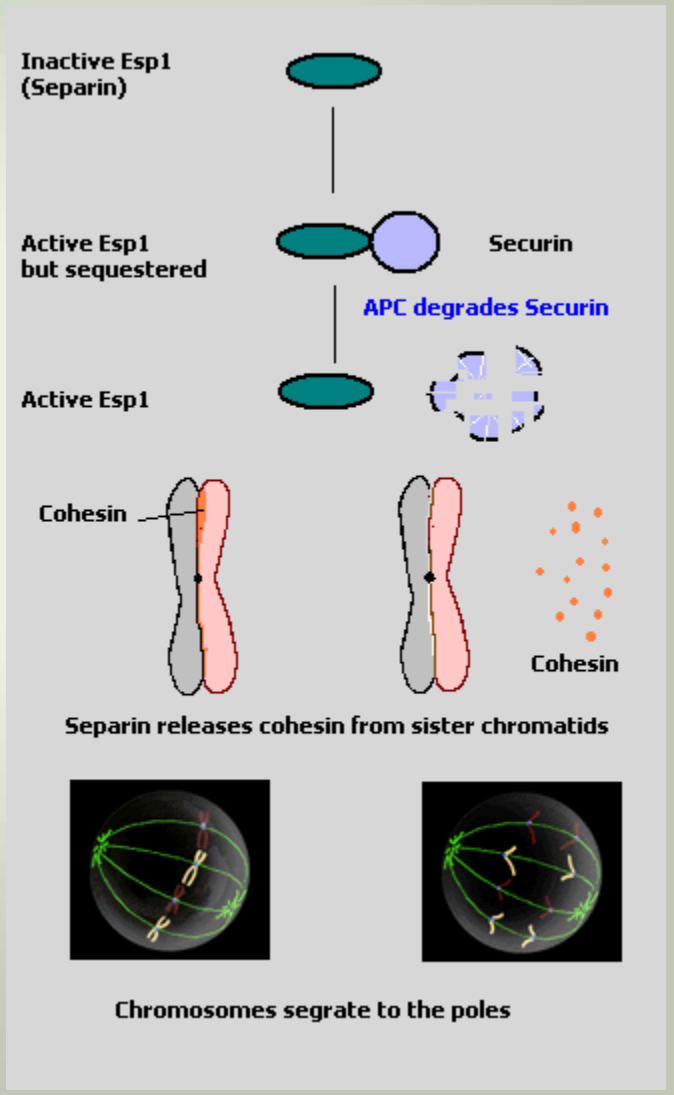
Cohesins & Separation of Chromosomes

Cohesin molecules are complex heterodimers

Cohesins comprises of **Scc1p**, **Scc3p**, and 2 SMC (structural maintenance of chromosomes) proteins **Smc1p** and **Smc3p.**

Degradation of **Scc1p** is sufficient to abosish the ability to hold sister chromatids togather

Cohesins also contain a protein called **Rec8p** the degredation of which allows the separation of homologous chrmosomes during anaphase 1 of meosis.



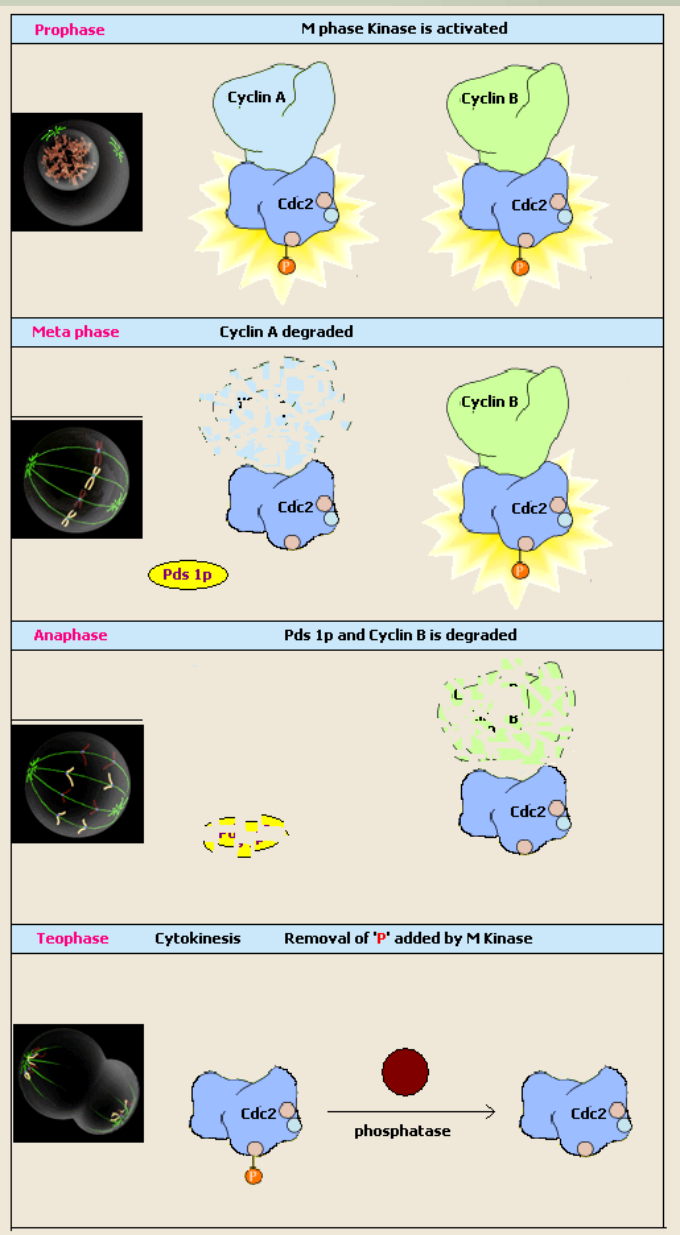
Anaphase-Promoting Complex (APC) activities
Degradation of Cyclin B

APC^{Cdh1} complex targets the degradation of **Cyclin B**

Inactivation of **cyclin B** allows the reversal of events of phosphorylation promoted by M phase kinase

Cyclin B undergoes destruction to inactivate the **M phase kinases** by ubiqutination
Degrades **geminin**, a protein that has kept the freshly-synthesized DNA in S phase from being re-replicated before mitosis

Turns on synthesis of G1 cyclin for the next turn of the cycle



Apart from cyclin and phosphorylation mediated regulation, the activity of CDKs is regulated by inhibitory proteins.

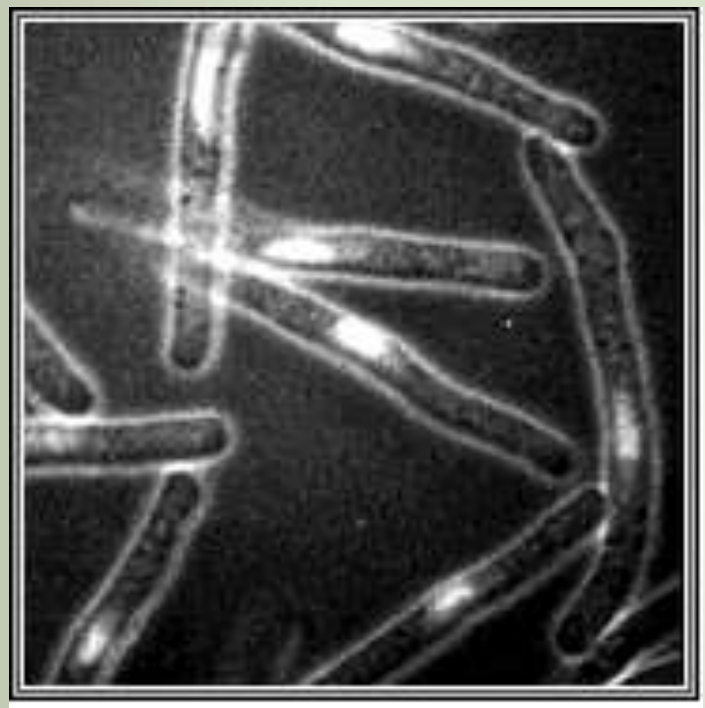
- **Rum1** is a protein which is synthesized to inhibit CDK-cyclin complex and is synthesized through out the G1 and S phase and prevents the cycle skipping DNA replication and entering mitosis prematurely.

- The **FAR1** is another protein in *S cerevisiae* which senses the mating pheromones and inhibits the activity of CDK cyclin complex at START thus arresting the cells at G1 for mating purpose.

- In animals, two families of CDK cyclin inhibitors (CKIs) are found. One blocks all CDK-cyclin activity and the other specifically inhibits D-cyclin complexes containing CDJ04 and CDJ-6.

A Dominant, GOF allele of *CDC2* revealed a *Wee phenotype*

cdc2^{ts} LOF



Cdc2^{wee2} GOF

From Susan Forsburg, Salk Institute

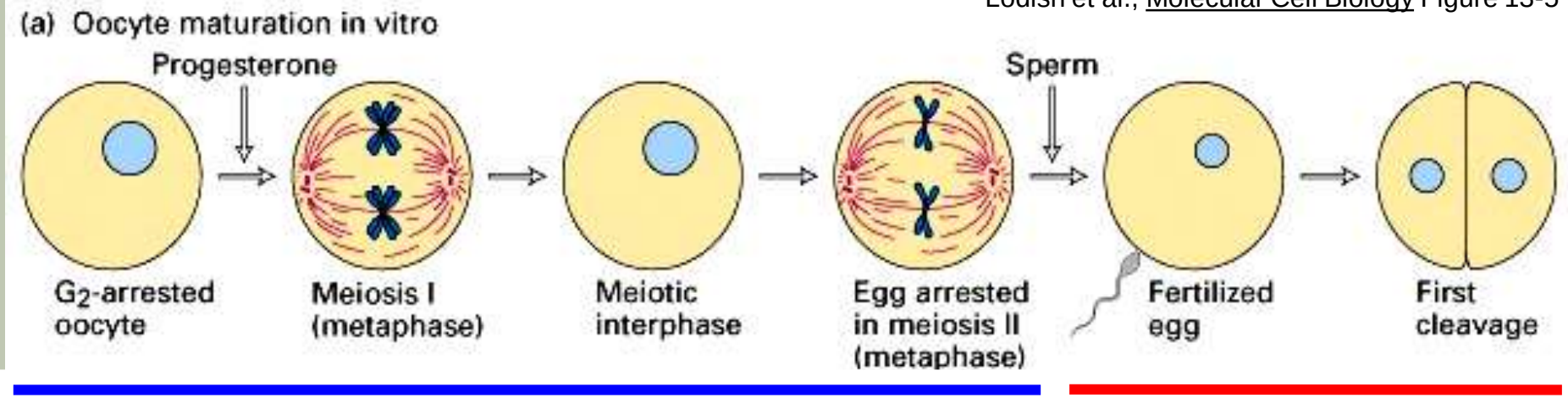
These phenotypes imply that *CDC2* is an important gene

- LOF mutations are cdc (big, fail to enter mitosis)
  - Cdc2 function is required to initiate mitosis
- GOF mutations are wee, implying that Cdc2 is a regulator

Oocyte cytoplasm can be obtained in quantity and manipulated, allowing both experimental and biochemical studies

Maturation of frog eggs is induced by progesterone, induction of embryogenesis is induced by a sperm

Lodish et al., Molecular Cell Biology Figure 13-5



Maturation

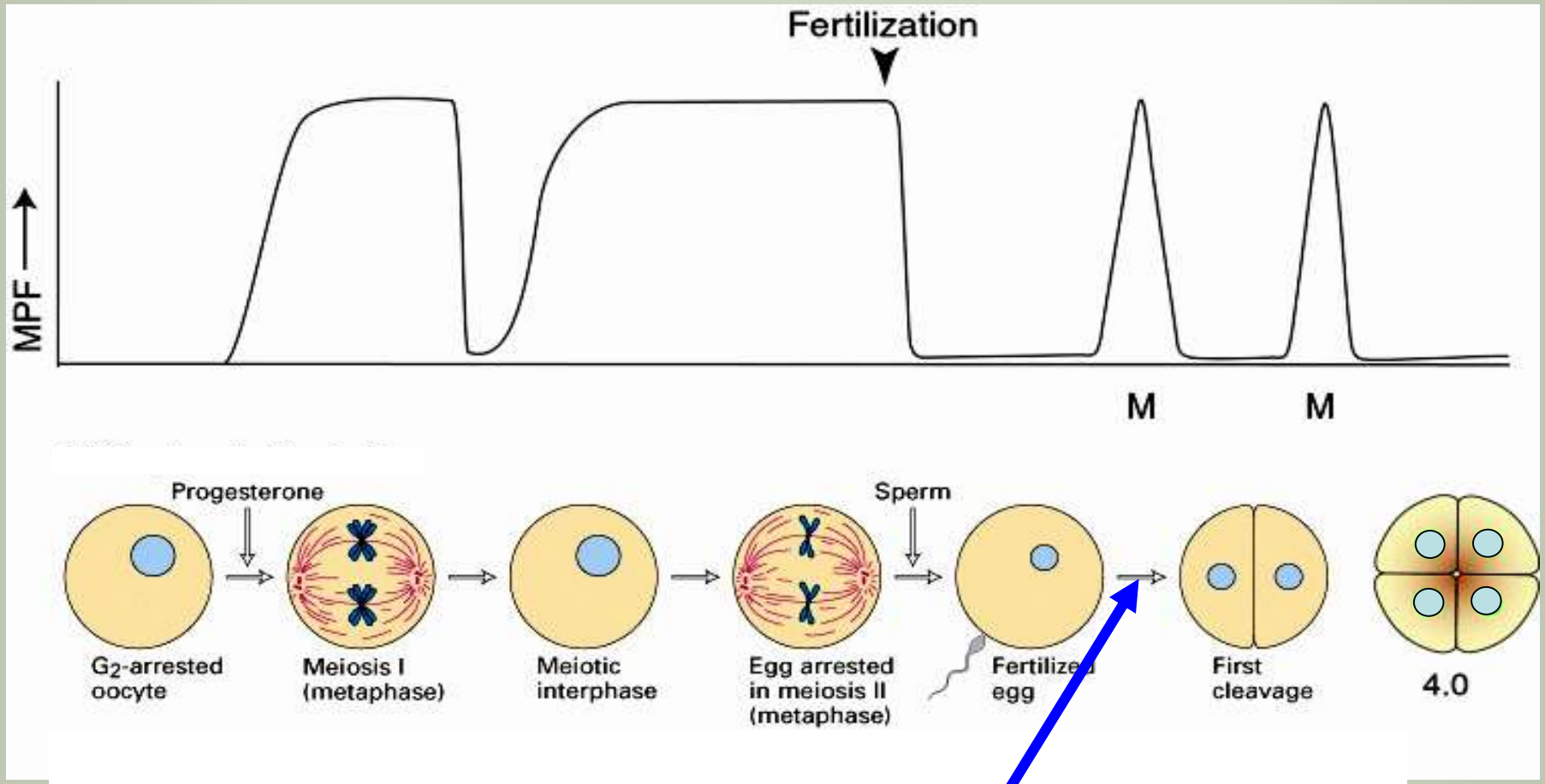
Activation

Yoshio Masui:

Use Oocyte maturation to study a simple, synchronized cell cycle

- Hormone can drive G2 - M
- Activation can drive M-G1

MPF turns out to regulate the embryonic Cell Cycle as well



MPF Regulates Mitosis as well as Meiosis

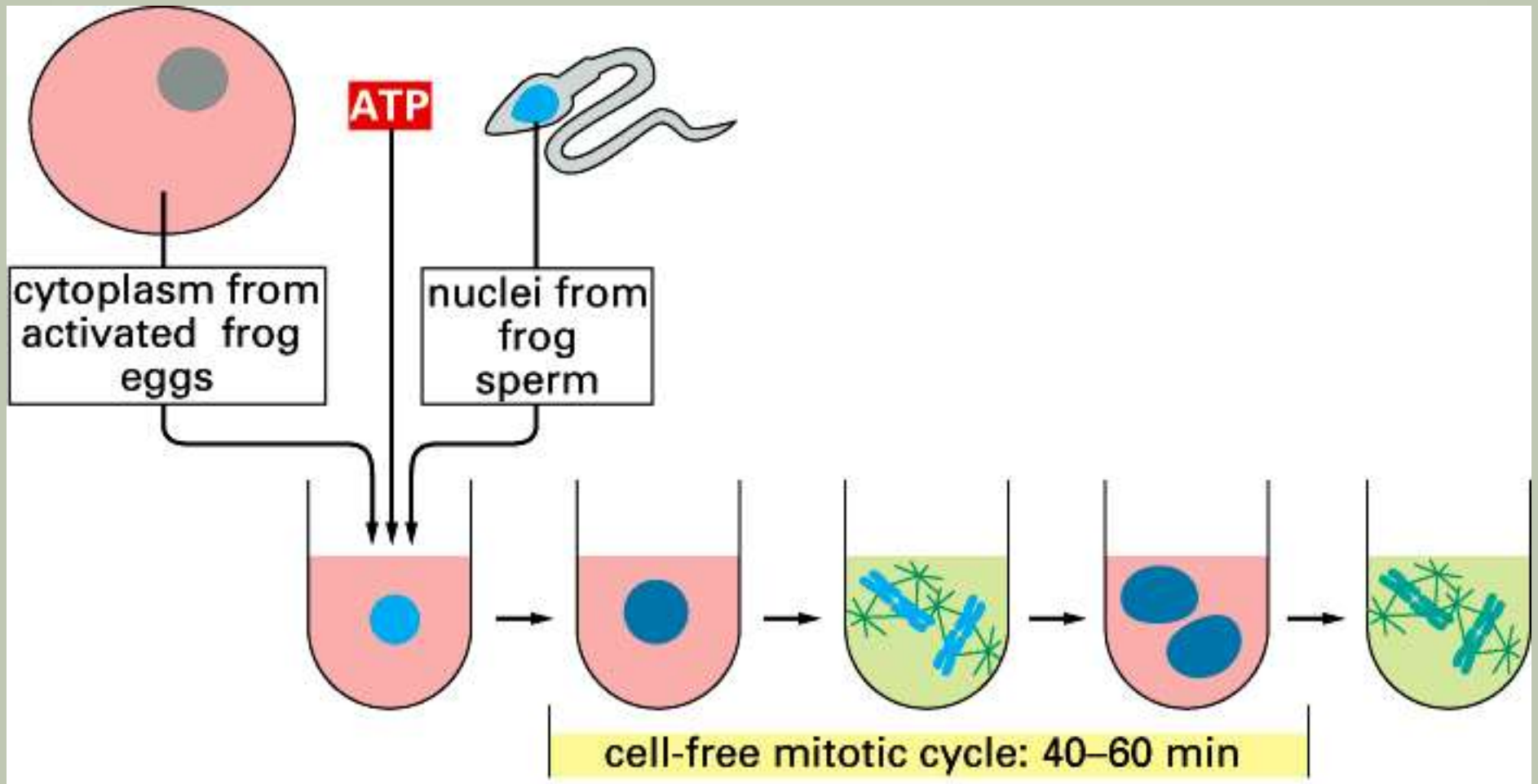
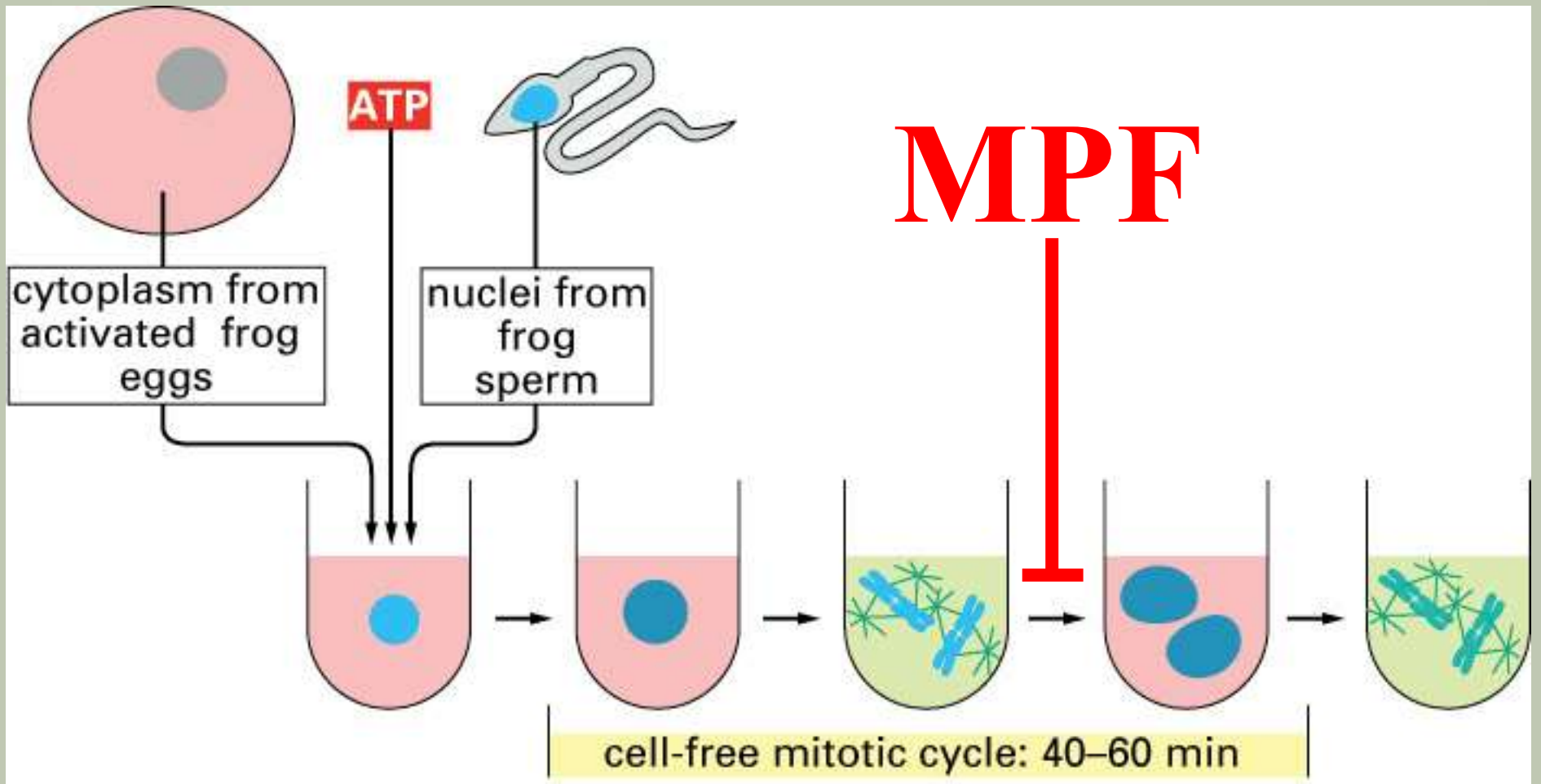


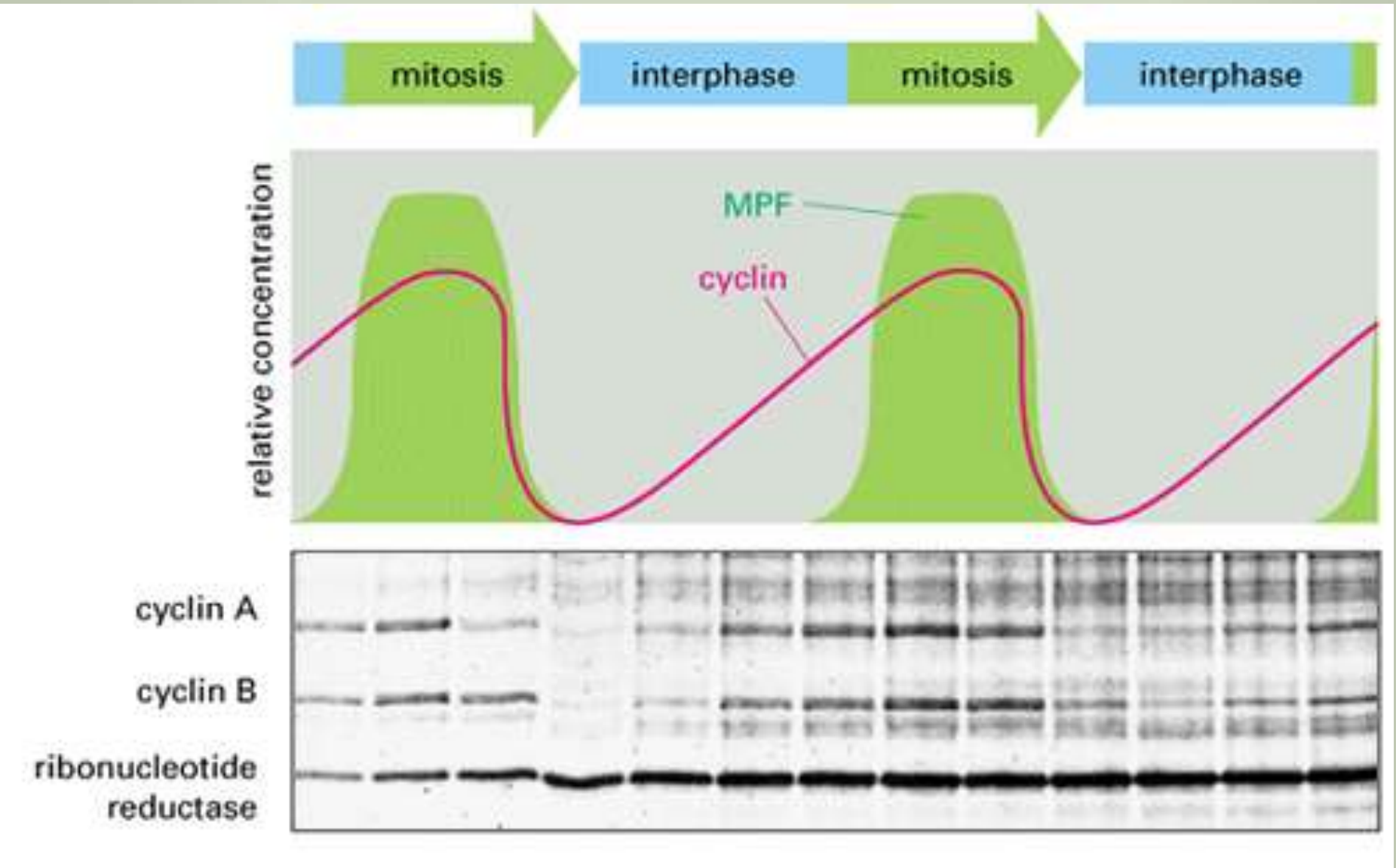
Figure 17–9. Molecular Biology of the Cell, 4th Edition.



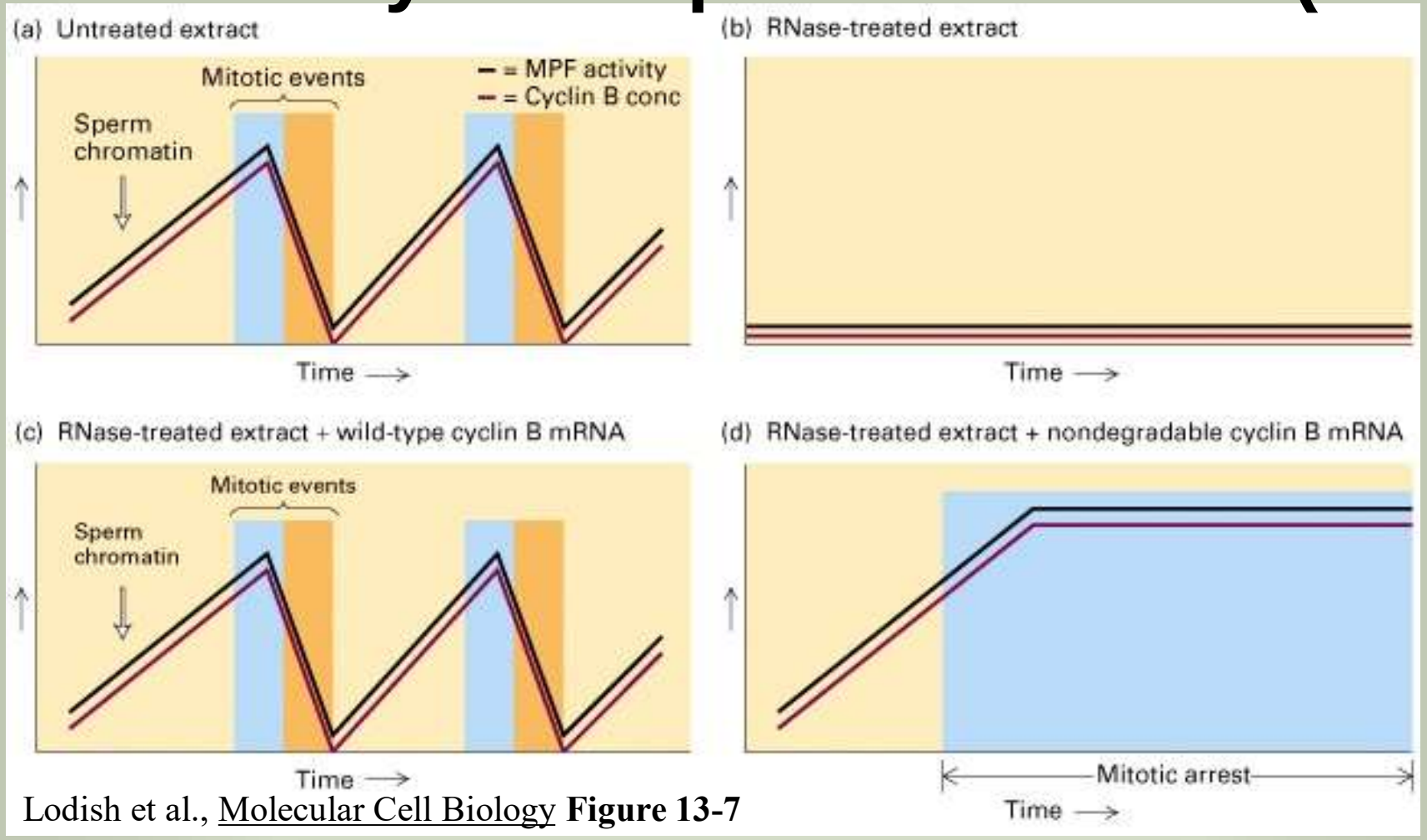
Manfred Lohka and Jim Maller (UCHSC)

- used cell free assay to purify MPF
- tested various cytosolic fractions for MPF activity

MPF regulation was elucidated by Tim Hunt's discovery of Cyclins



MPF is a cyclin-dependent kinase (CDK)



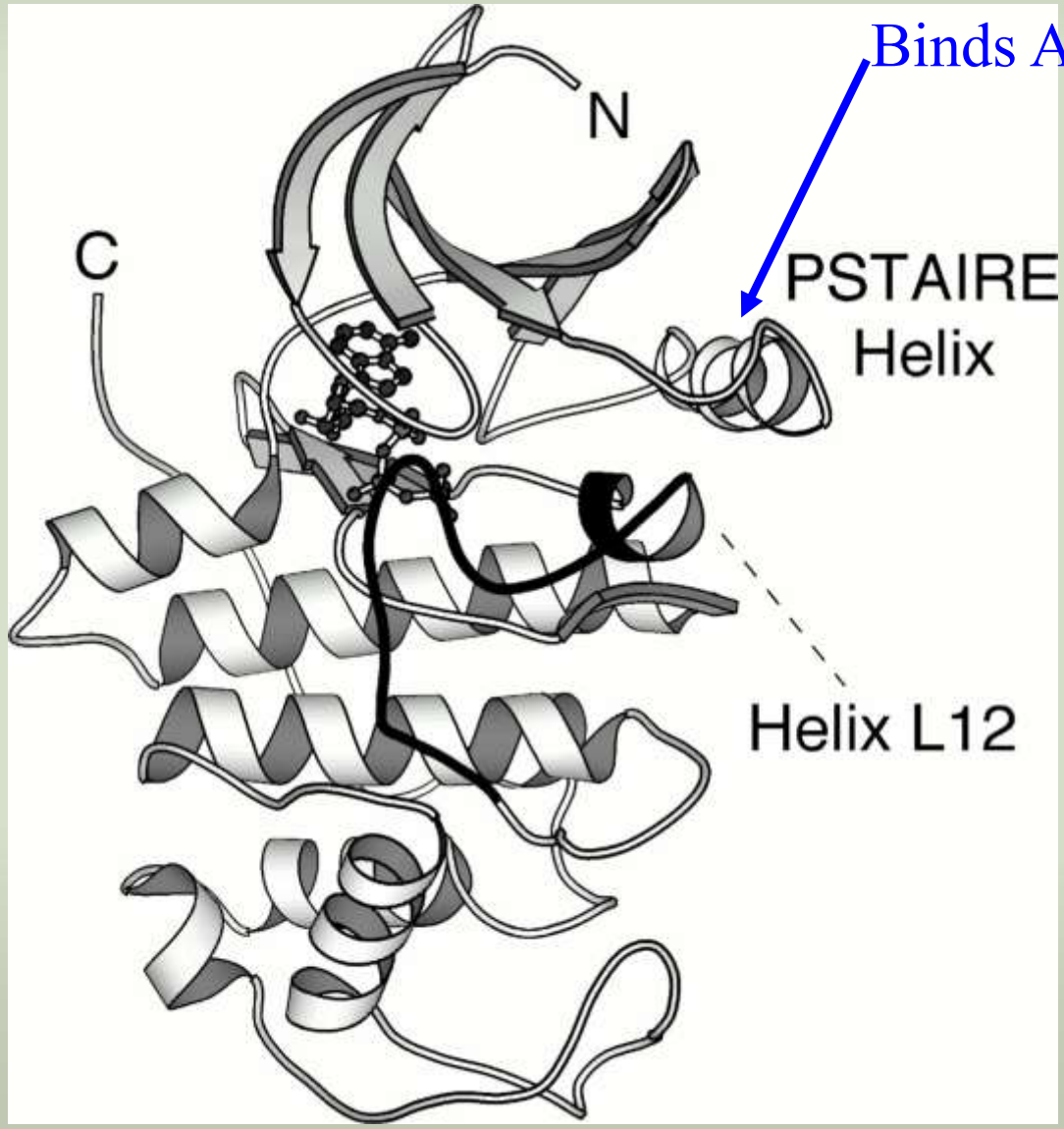
Lodish et al., Molecular Cell Biology **Figure 13-7**

1) MPF Activity Requires Cyclin B Production by Oocyte
   - Cyclin B ONLY Protein Synthesis Required to Initiate Development

2) Suggests that 45 kDa MPF subunit is Cyclin B…

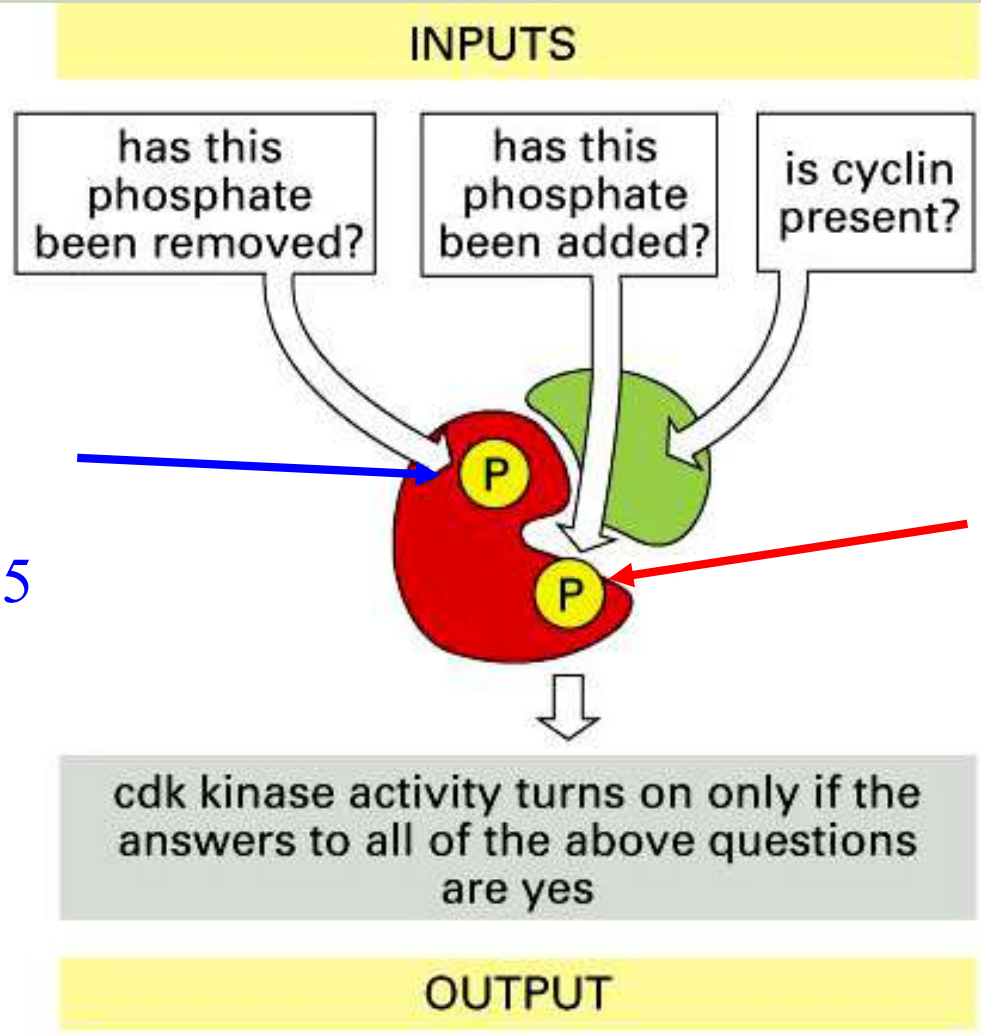
Structure of a CDK (Human CDK2)

PSTAIRE antibody
Binds ALL CDK's



Tyr 15

Inhibitory:
Added byWee1
Removed by Cdc25



Thr 160
Activating:
CAK adds after
Cyclin binding

Figure 3–66. Molecular Biology of the Cell, 4th Edition.

Cdc2 Activates Cdc25 and Inhibits Wee1:

Positive Feedback Loop

A little Cdc2 goes a long way…

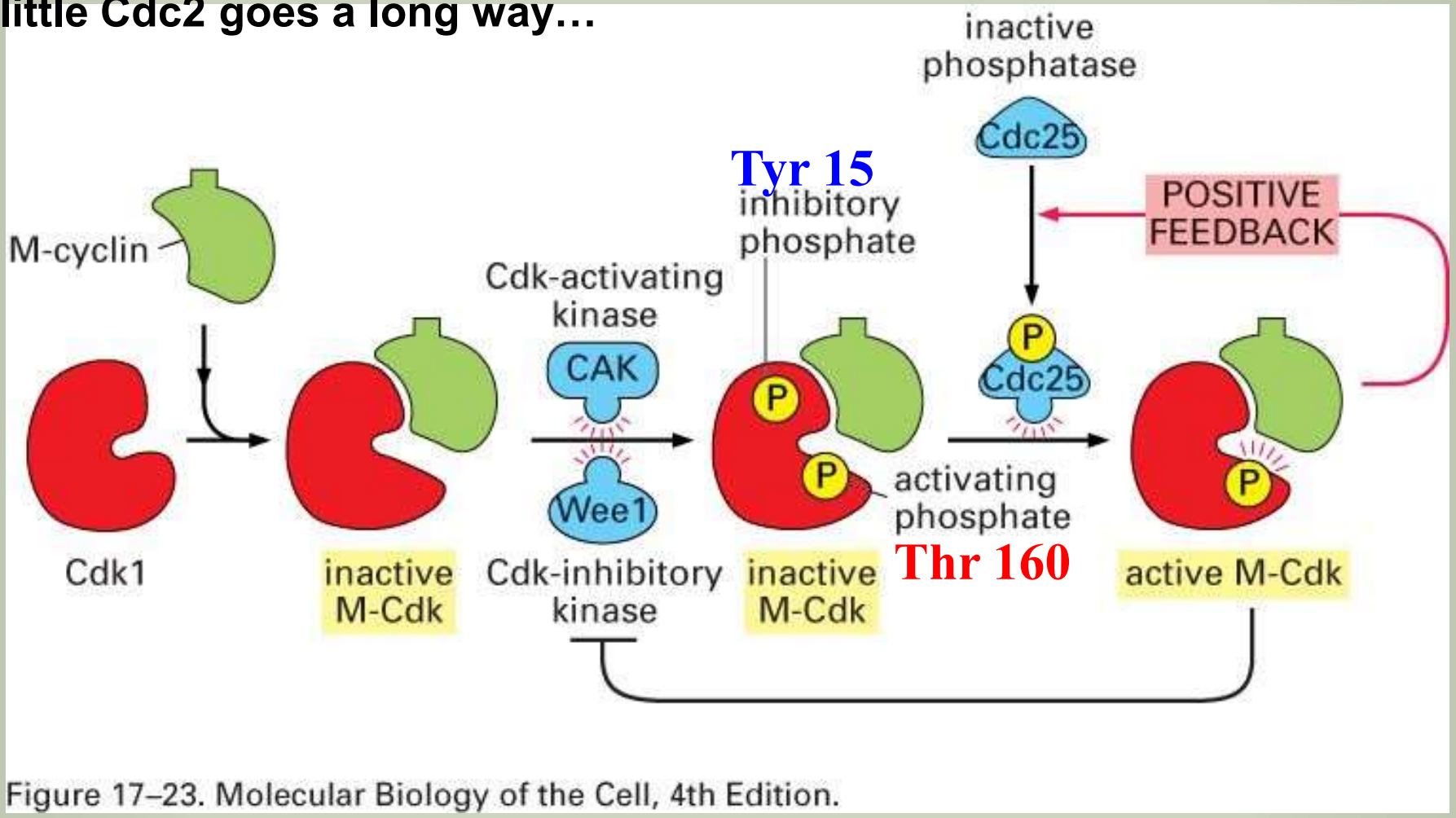
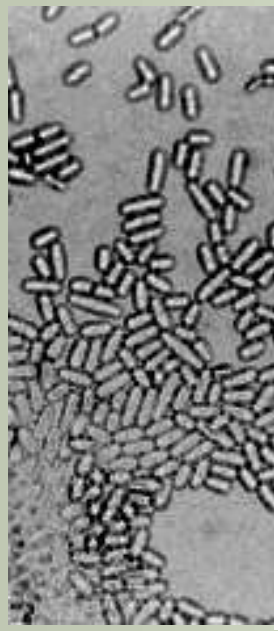


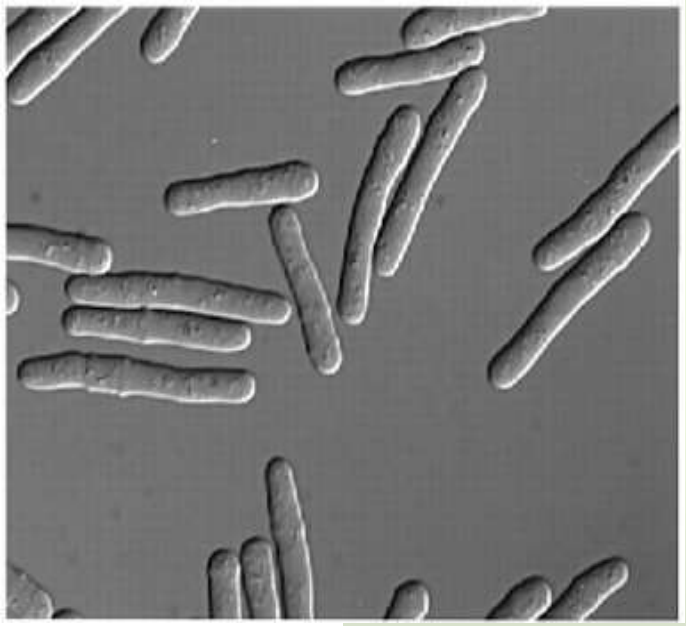
Figure 17–23. Molecular Biology of the Cell, 4th Edition.

This means **Cdc25** Must also be regulated…

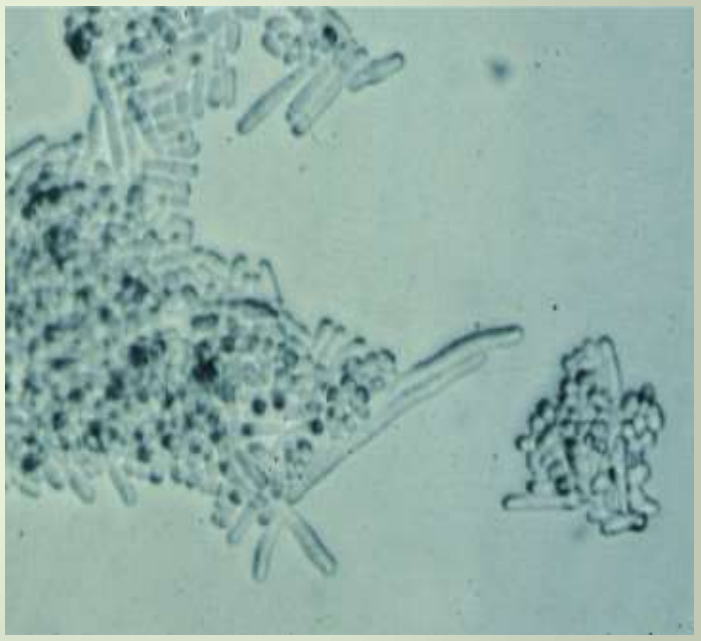
Wild-Type Cells



cdc2^{ts} Cells

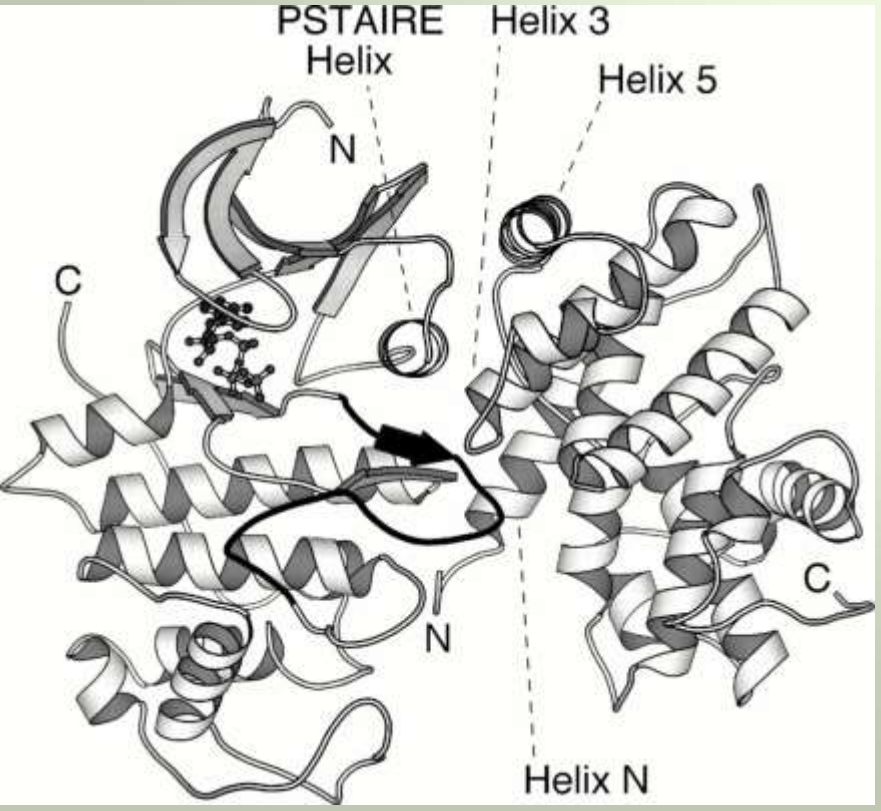


cdc2^{ts} Cells
+ Human *CDC2* plasmid



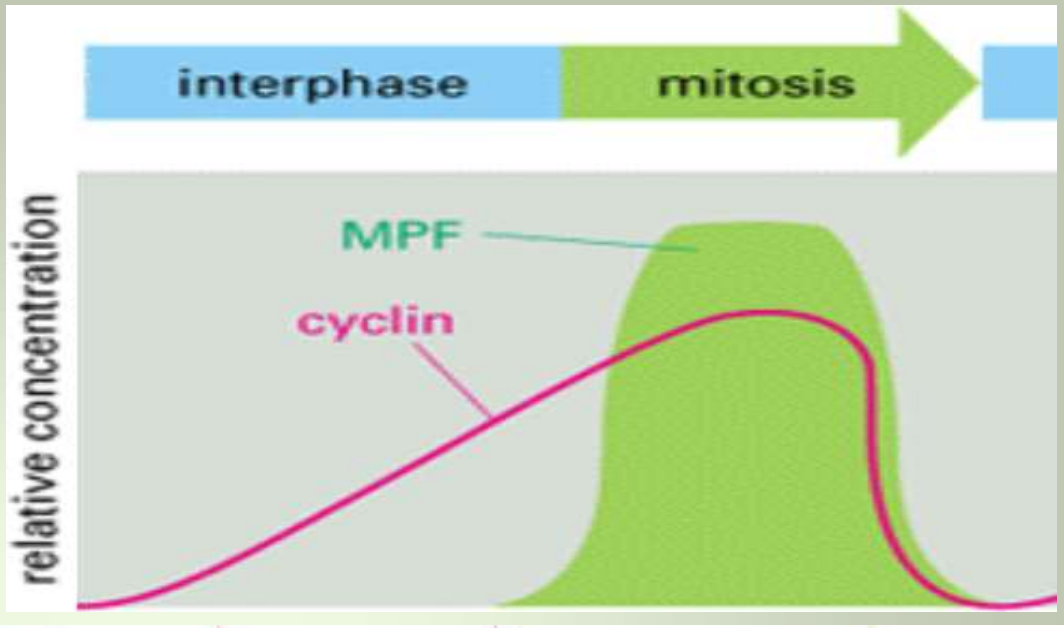
CDK Control of Mitosis is Universal

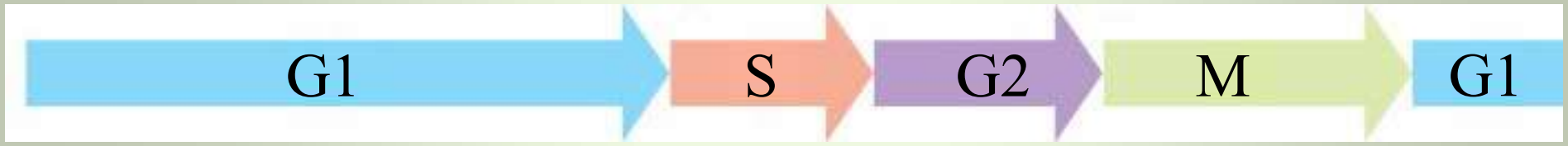
Fission Yeast Cdc2-Cdc13 heterodimer is equivalent to *Xenopus* MPF

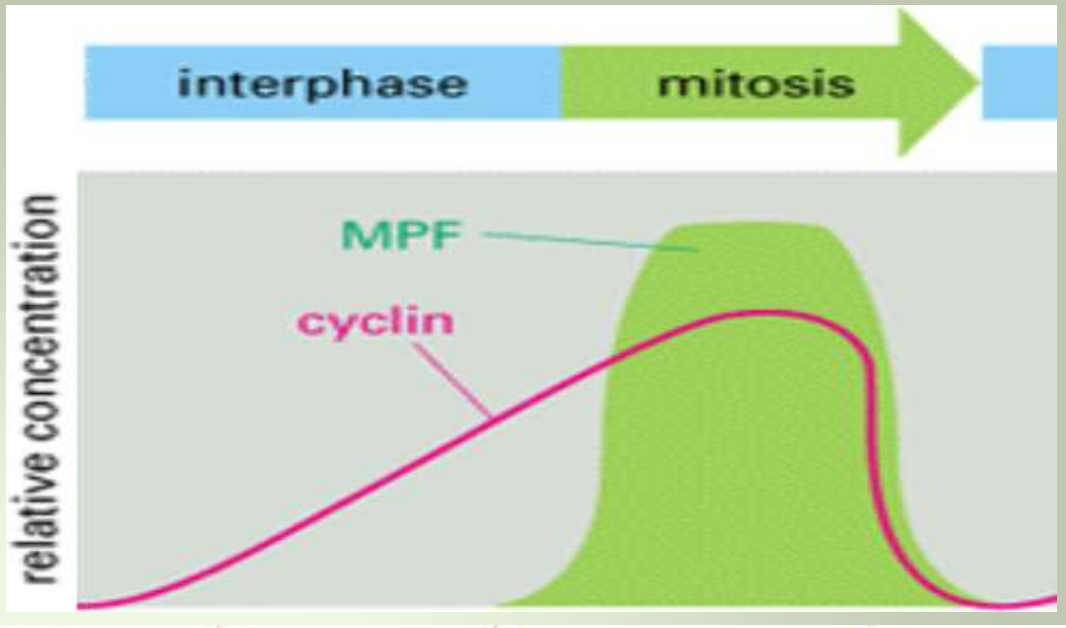


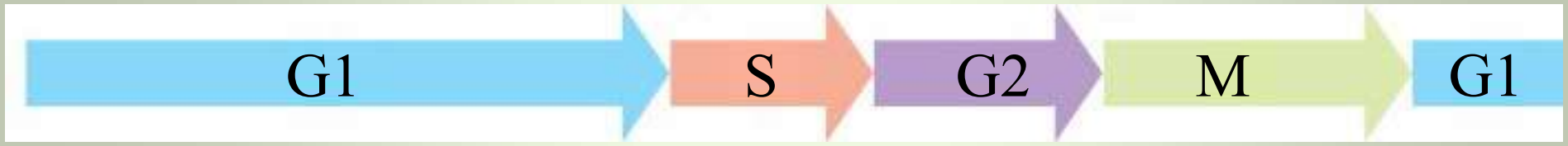
PSTAIRE antibody:

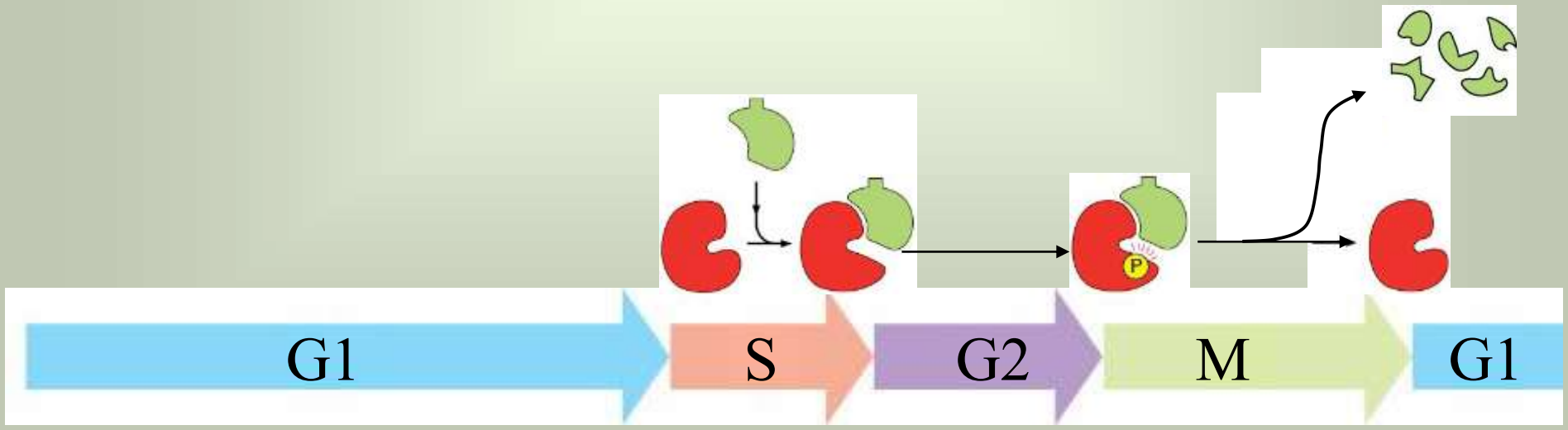
- cross reacts with ALL CDK's
- cross reacts with MPF
- removes MPF activity from *Xenopus* Oocyte extracts

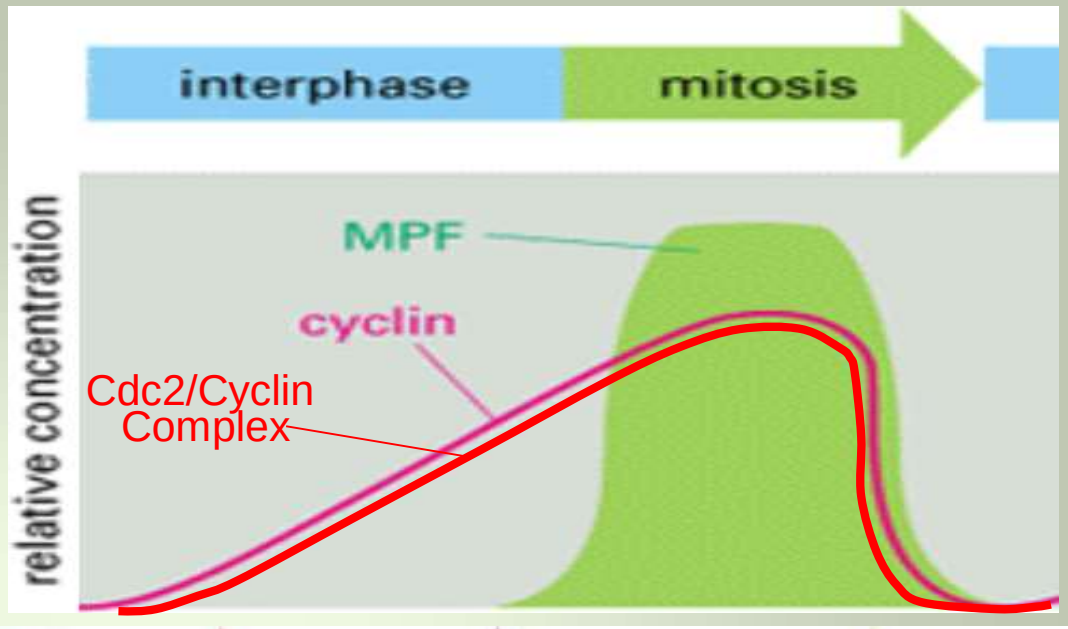

interphase
mitosis
relative concentration
MPF
cyclin

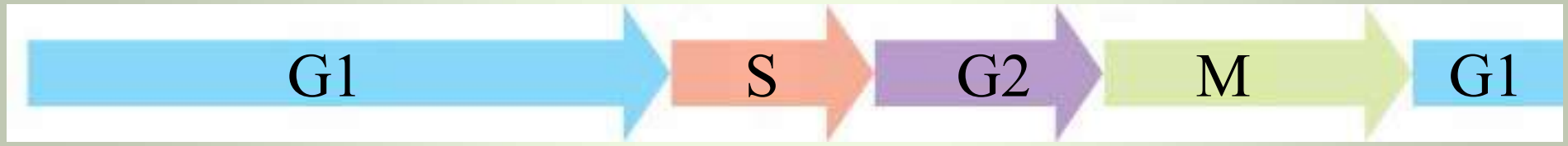

G1
S
G2
M
G1

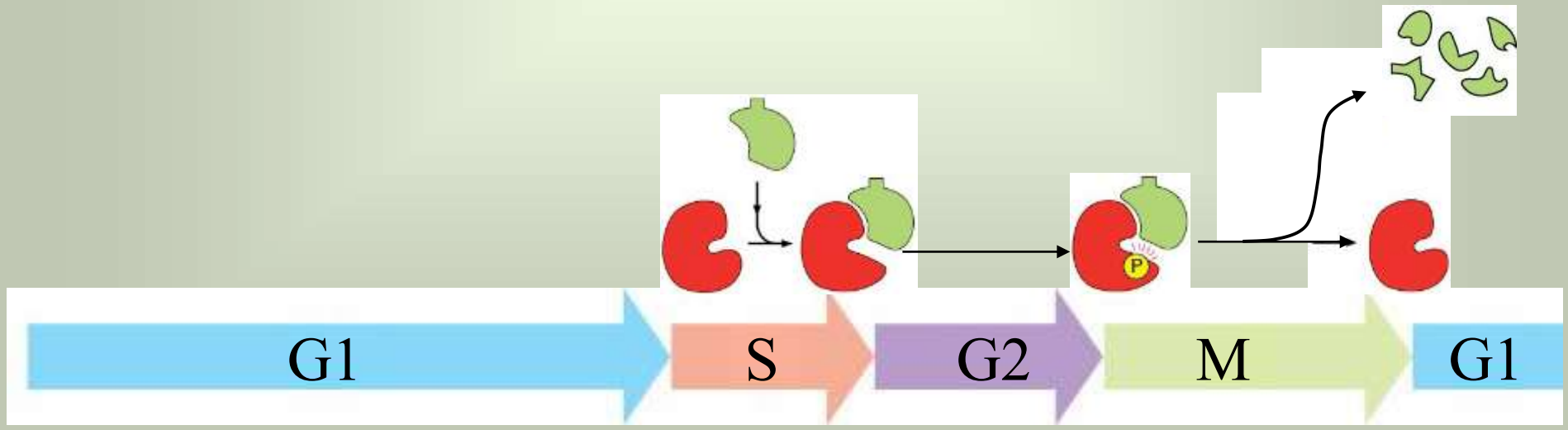

interphase
mitosis
MPF
cyclin
relative concentration

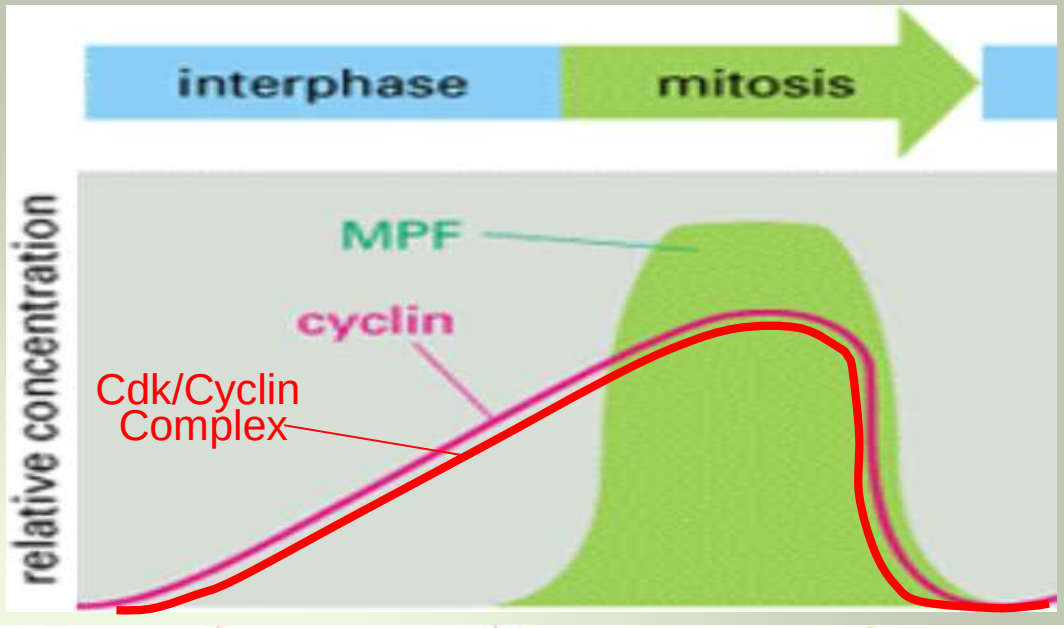

G1
S
G2
M
G1


P
G1
S
G2
M
G1

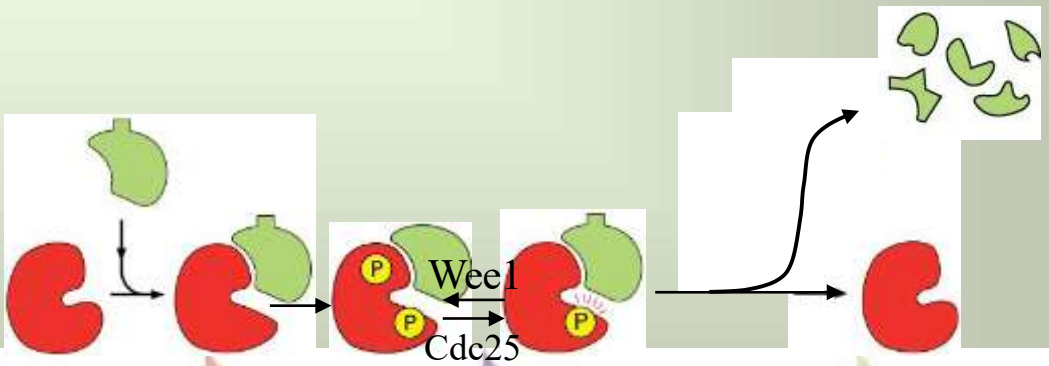

interphase
mitosis
relative concentration
MPF
cyclin
Cdc2/Cyclin
Complex


G1
S
G2
M
G1


P
G1
S
G2
M
G1



G1 S G2 M G1



G1 S G2 M G1

Cyclin Destruction Drives Mitotic Exit

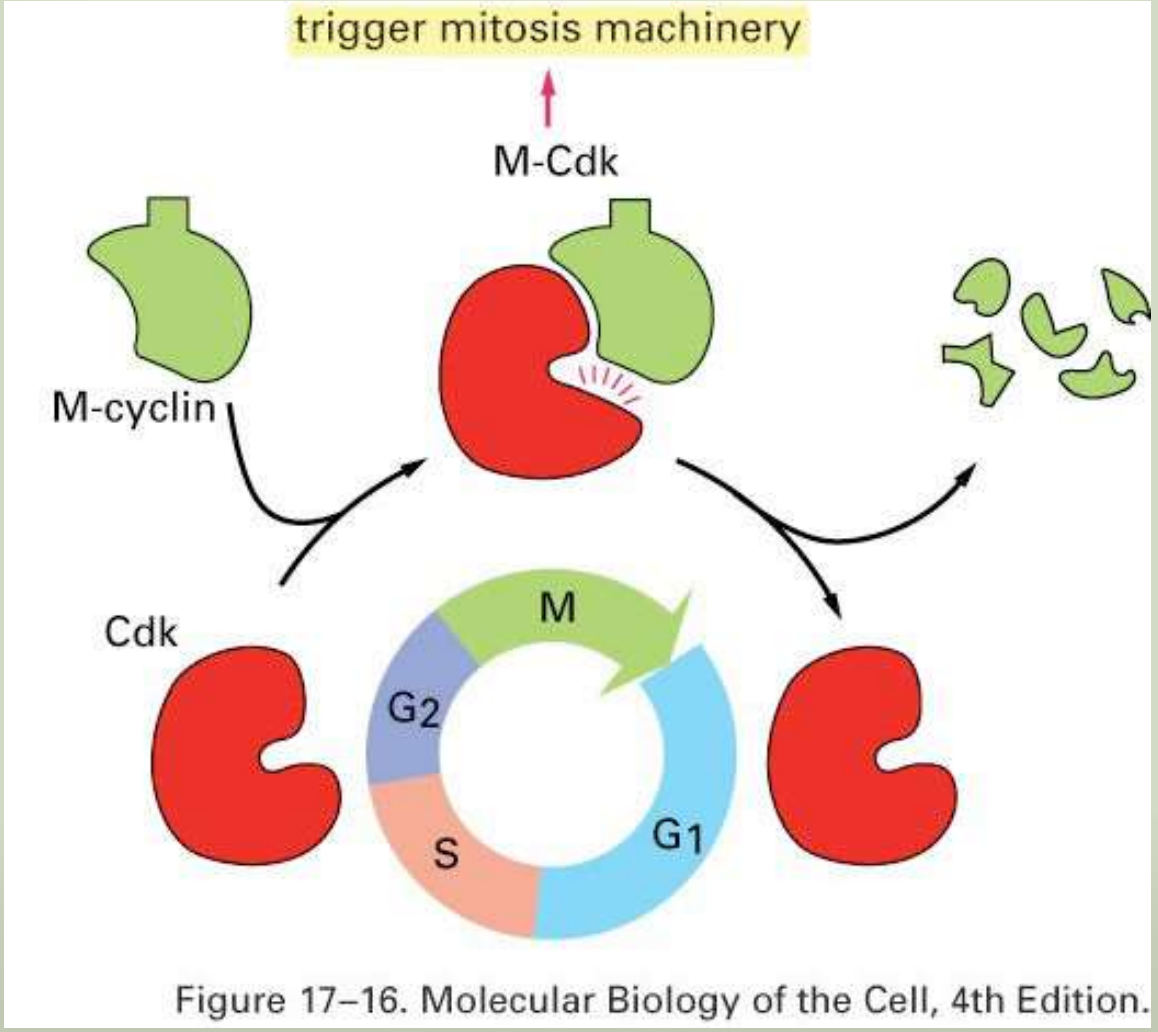
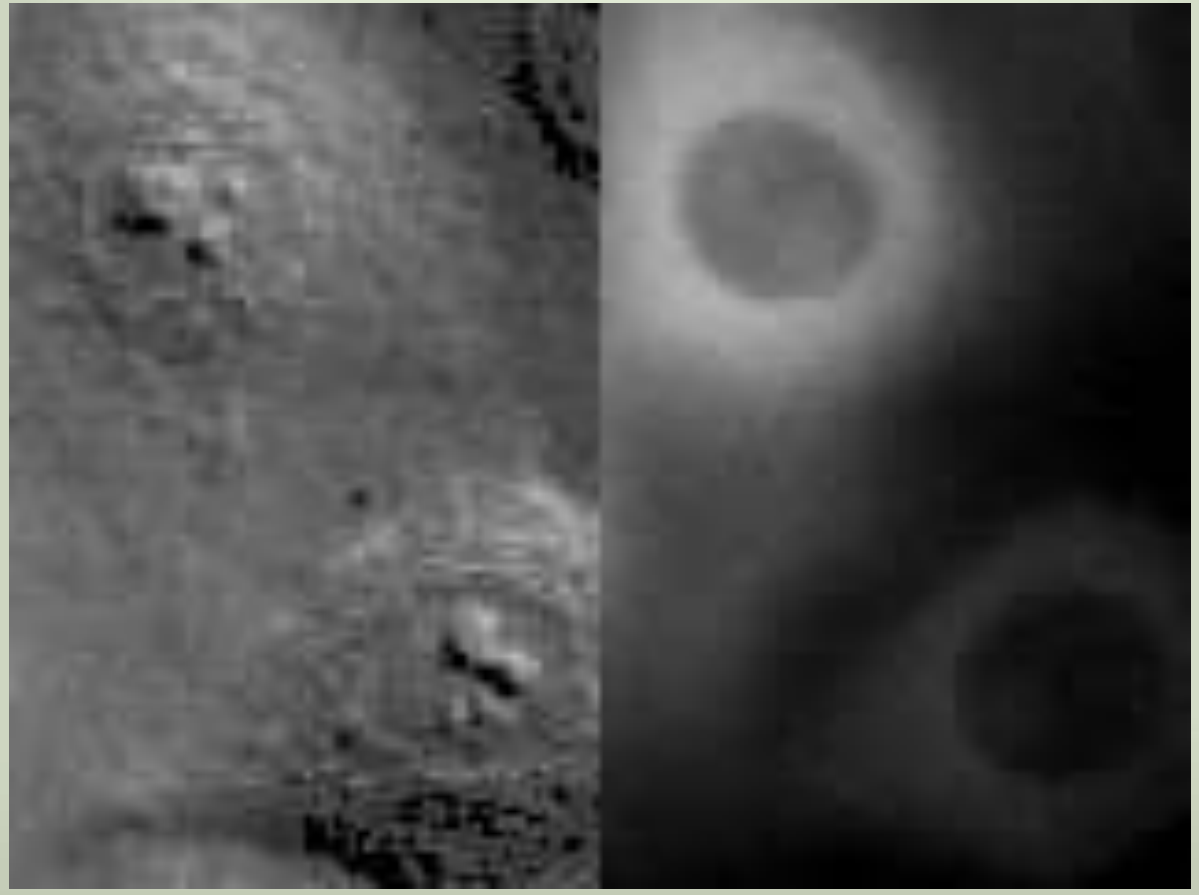


Figure 17–16. Molecular Biology of the Cell, 4th Edition.

Cyclin B degradation in Live Cells (HeLa)

Jonathan Pines' lab



http://www.welc.cam.ac.uk/~jplab/Movies/b1-degradationmovie.htm

Mitotic Cyclins are Destroyed by Ubiquitin-Dependent Proteolysis

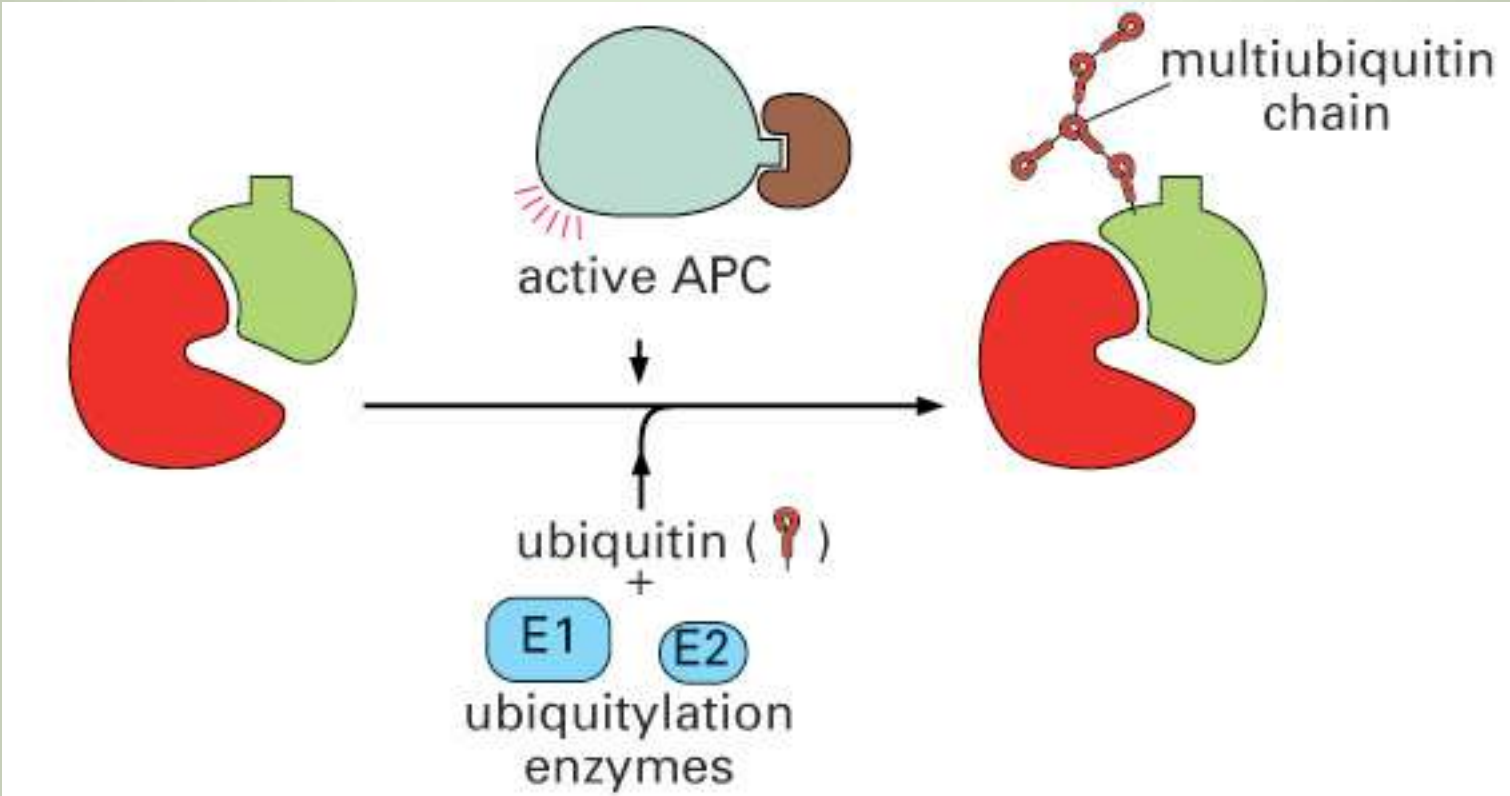


Figure 17–20 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Ubiquitin:

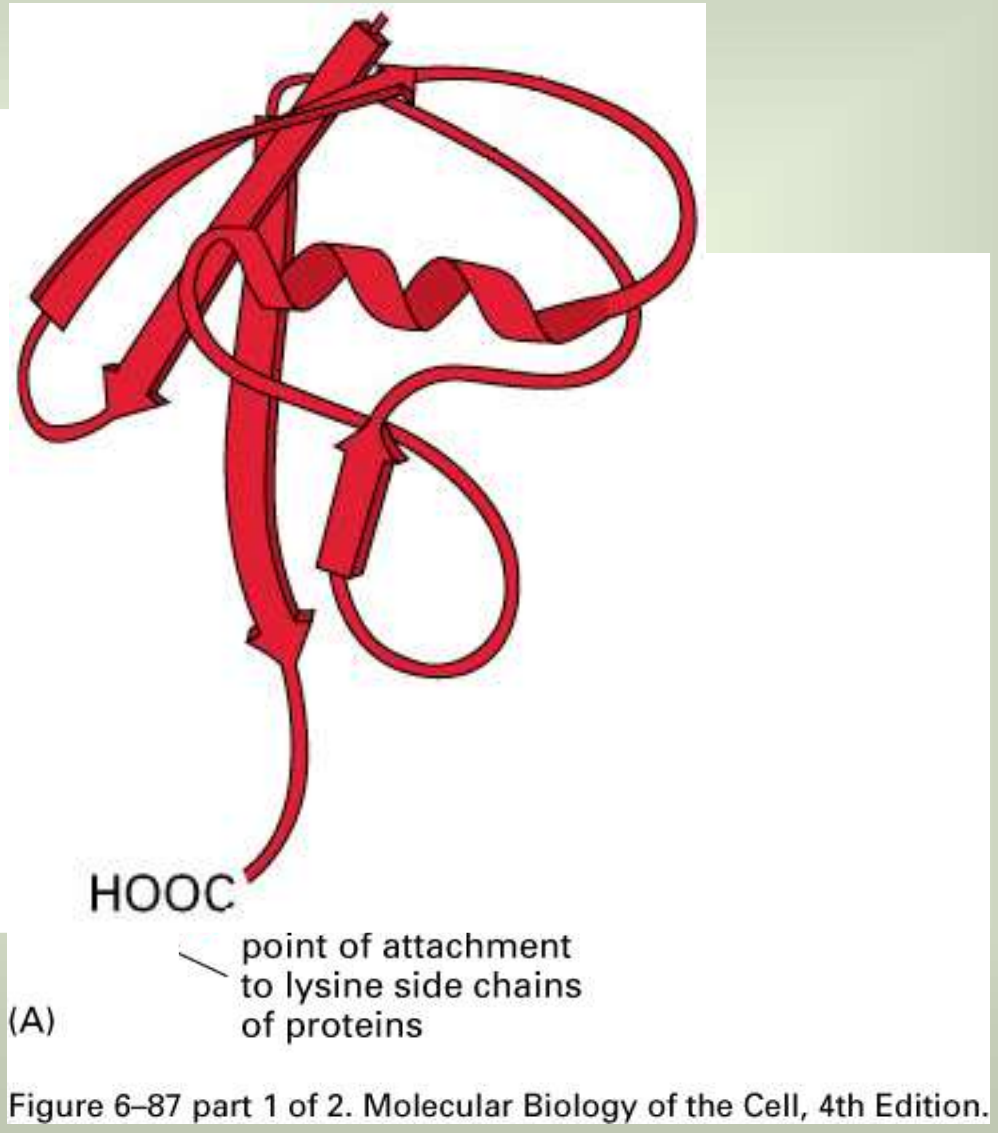
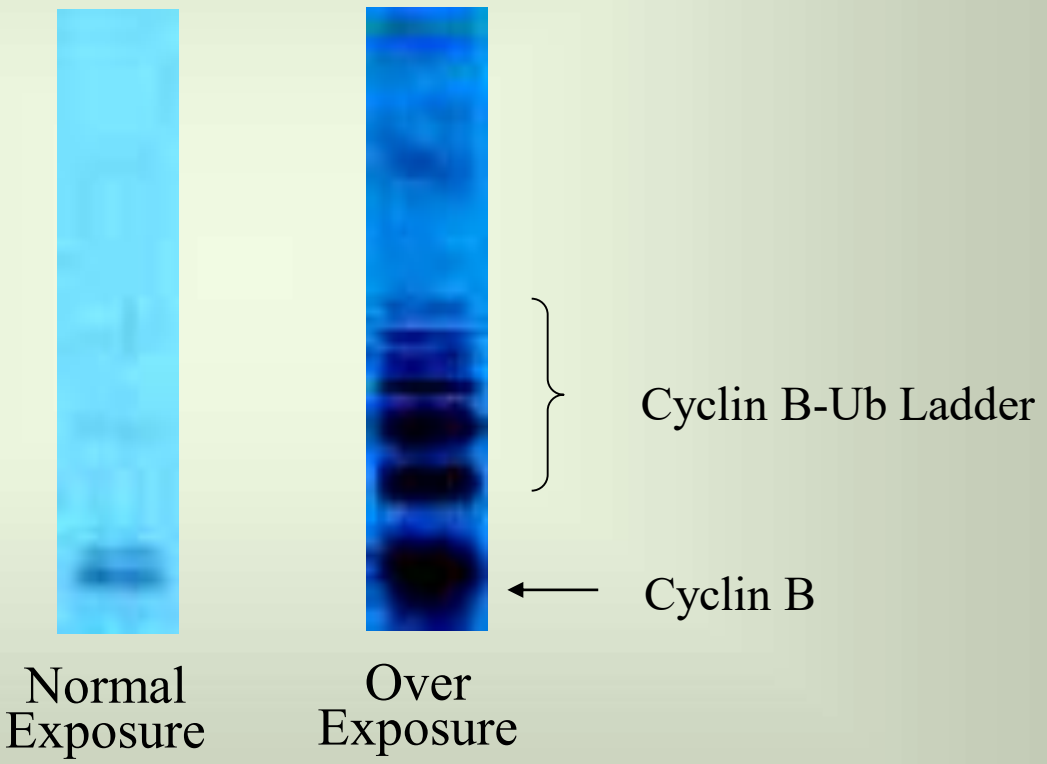


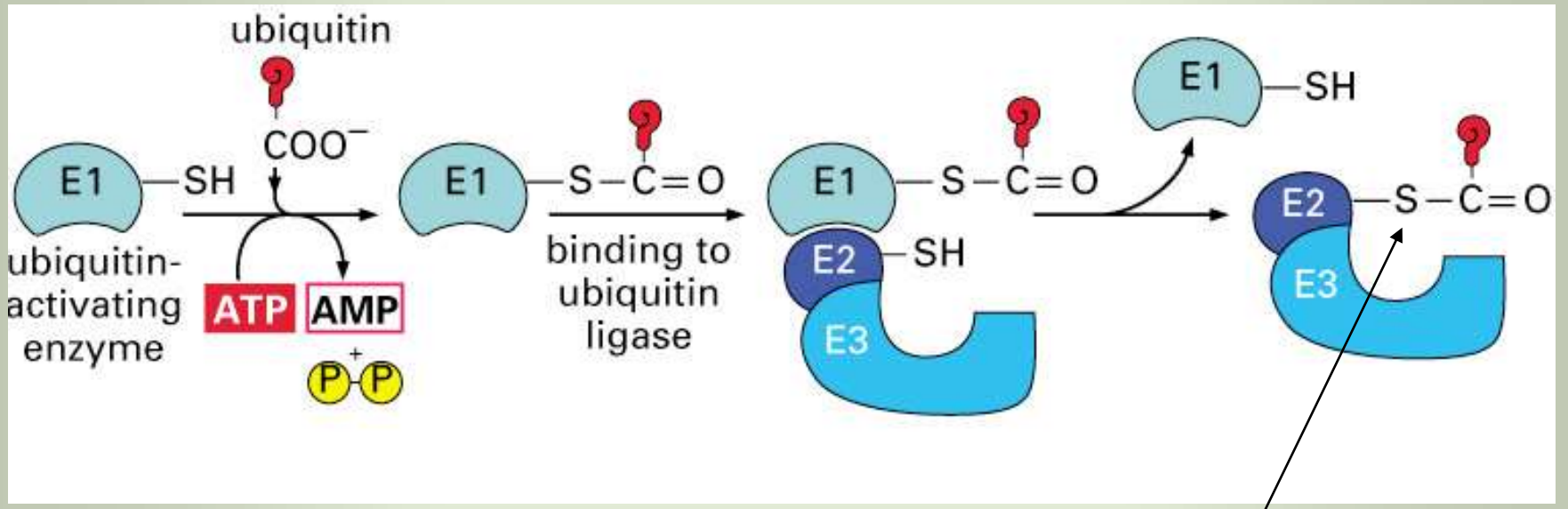
Figure 6–87 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Fortuitous Discovery of Cyclin B Ubiquitination

IP of ^{35}S-Labeled Cyclin B:



Ubiquitin is Transferred via Thioester Bonds



High Energy Thioester Bond,
can be transferred from E1 to E2, E3, or substrate

Substrate Ubiquitination is Processive

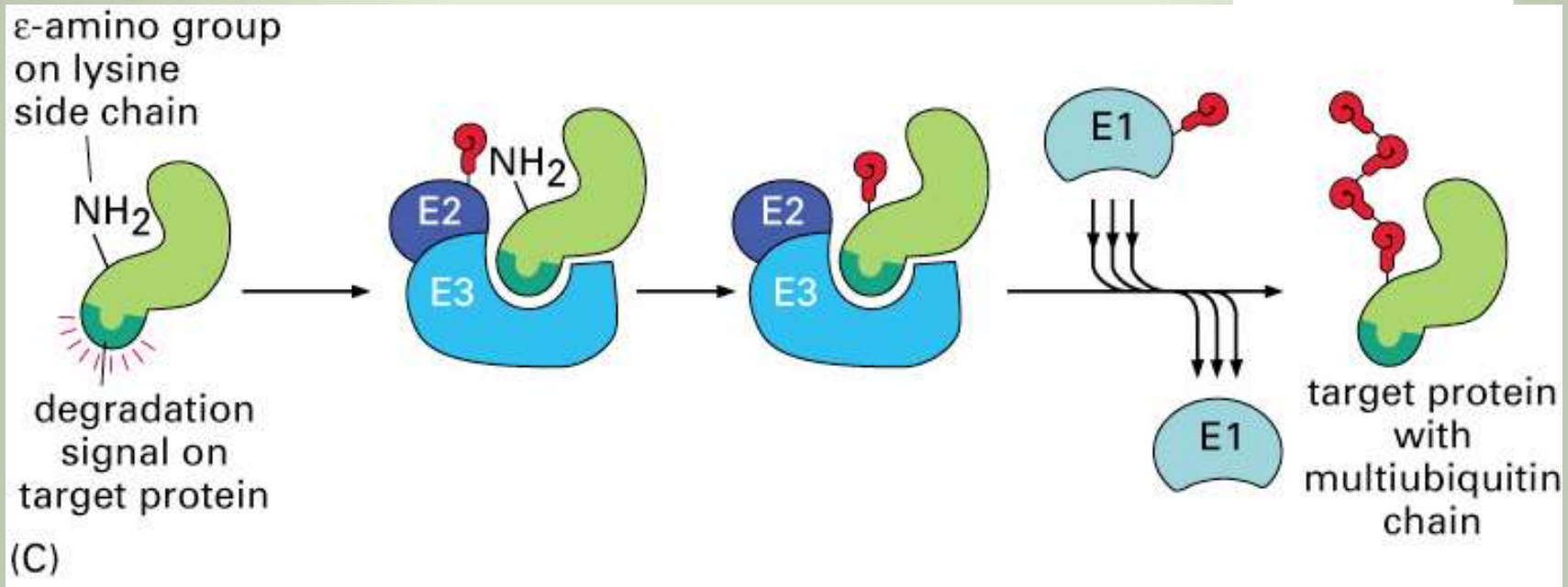


Figure 6–87 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Substrate Ubiquitination is Processive

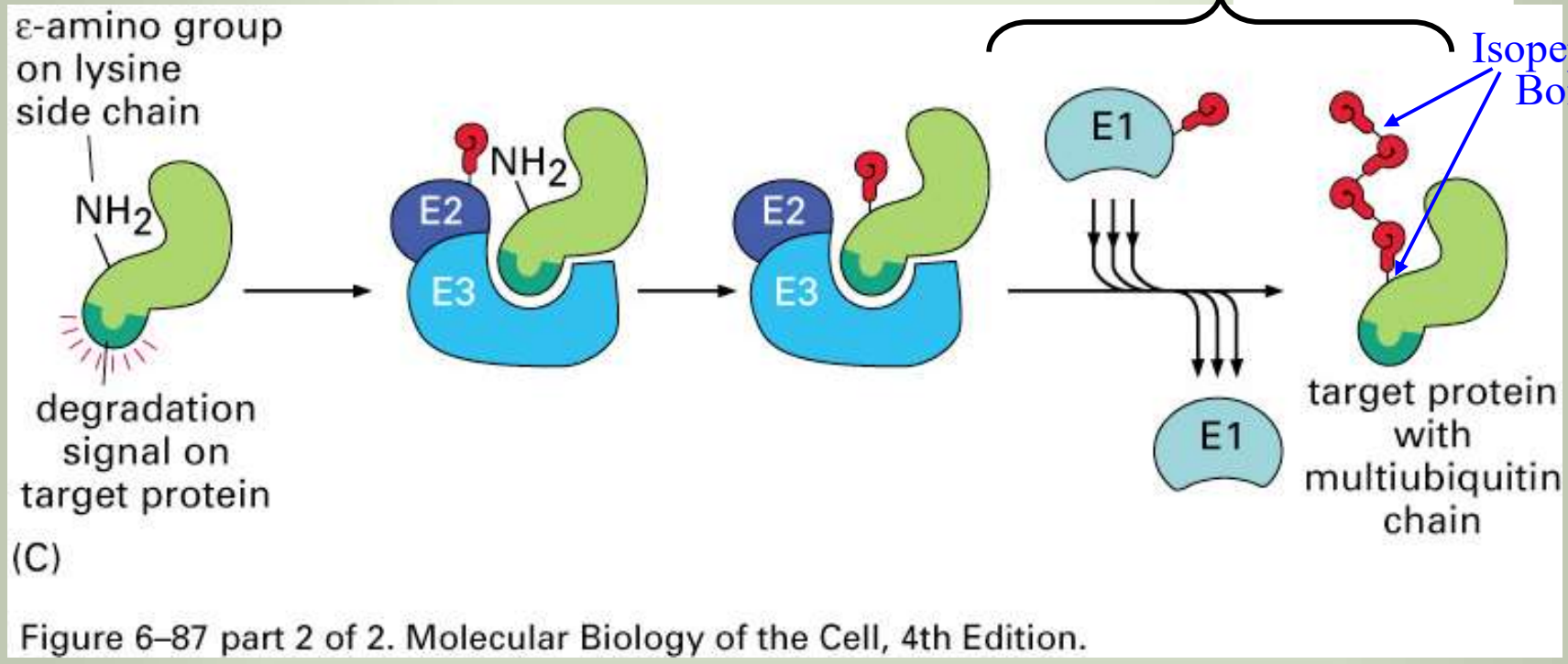
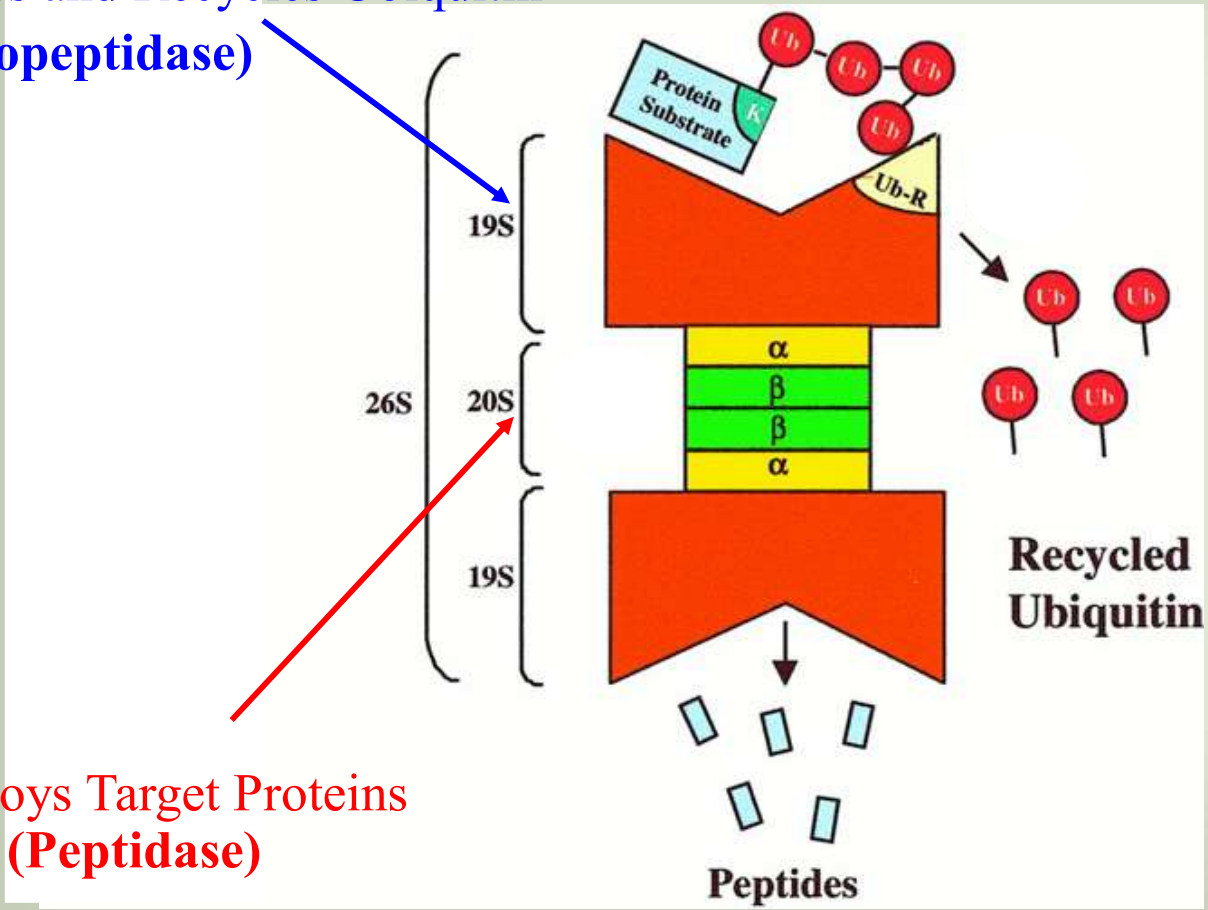


Figure 6–87 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Ubiquitinated Proteins are Degraded by the Proteosome

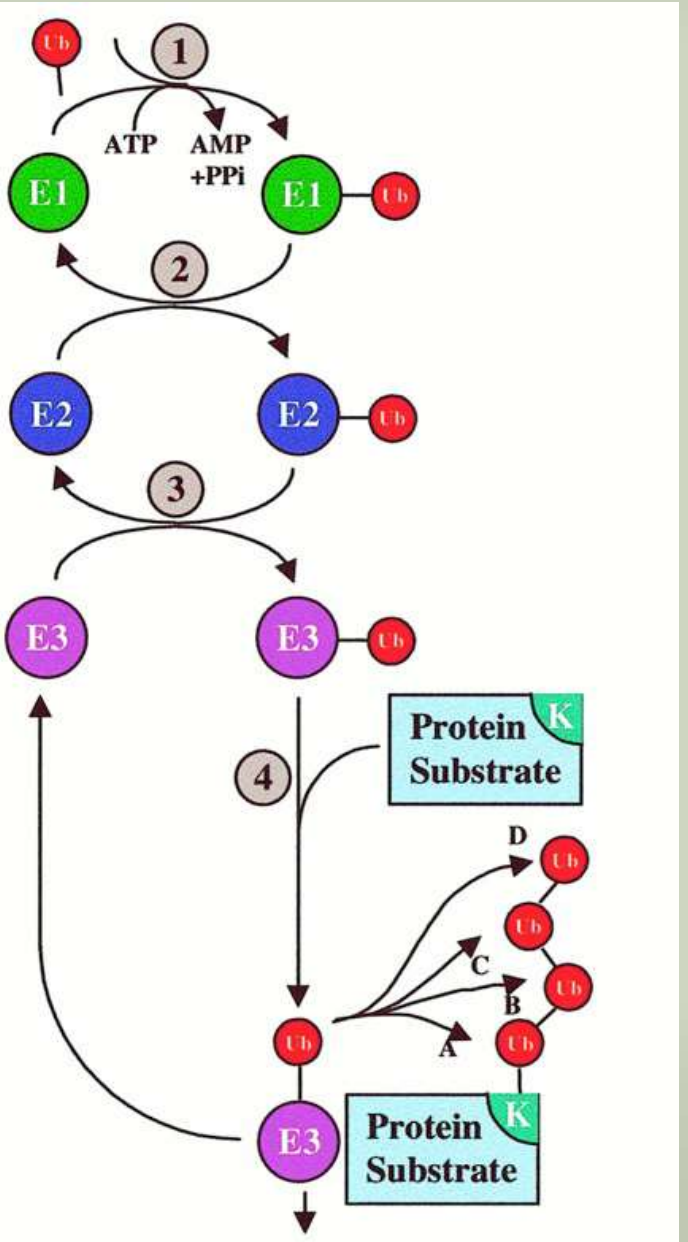
Binds to Ubiquitinated Proteins,
Cleaves and Recycles Ubiquitin
(Isopeptidase)



Destroys Target Proteins
(Peptidase)

Ubiquitination of Protein Substrates

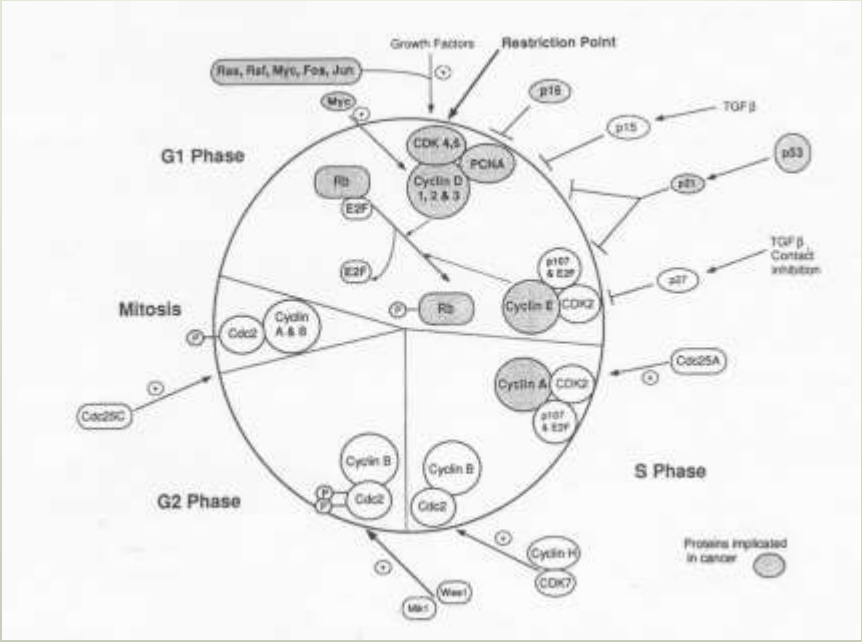
E3 Enzymes:
responsible for specificity
and diversity of Ub system



CYCLINS: cell cycle phase specific ?FUNCTION IN CELL CYCLE

- CYCLIN B = G2/M with cdc2; =cdc13 ; =CLB1-4
- CYCLIN A = S + G2 with cdk2; ?cig2 ; =CLB5,6
- CYCLIN E = G1/S with cdk2; ?cig1 ?puc1; =CLN1,2,3
- CYCLIN D = G1 with cdk4,5,6; ? in yeast (early vs late G1)
- CYCLIN C = present through cycle: CTD of RNA pol II
- CYCLIN F = peaks like A; ?cyclin stability and proteolysis
- CYCLIN G = induced 3 hr growth stim and by p53: ?function
- CYCLIN H = part of CAK (with cdk7)
- CYCLIN J = sorry, it exists

Cell Cycle Regulators and Cancer



Flow cytometry gives a histogram of no. of cells vs. Amount of DNA/cell. Gives % cells in cycle phases.

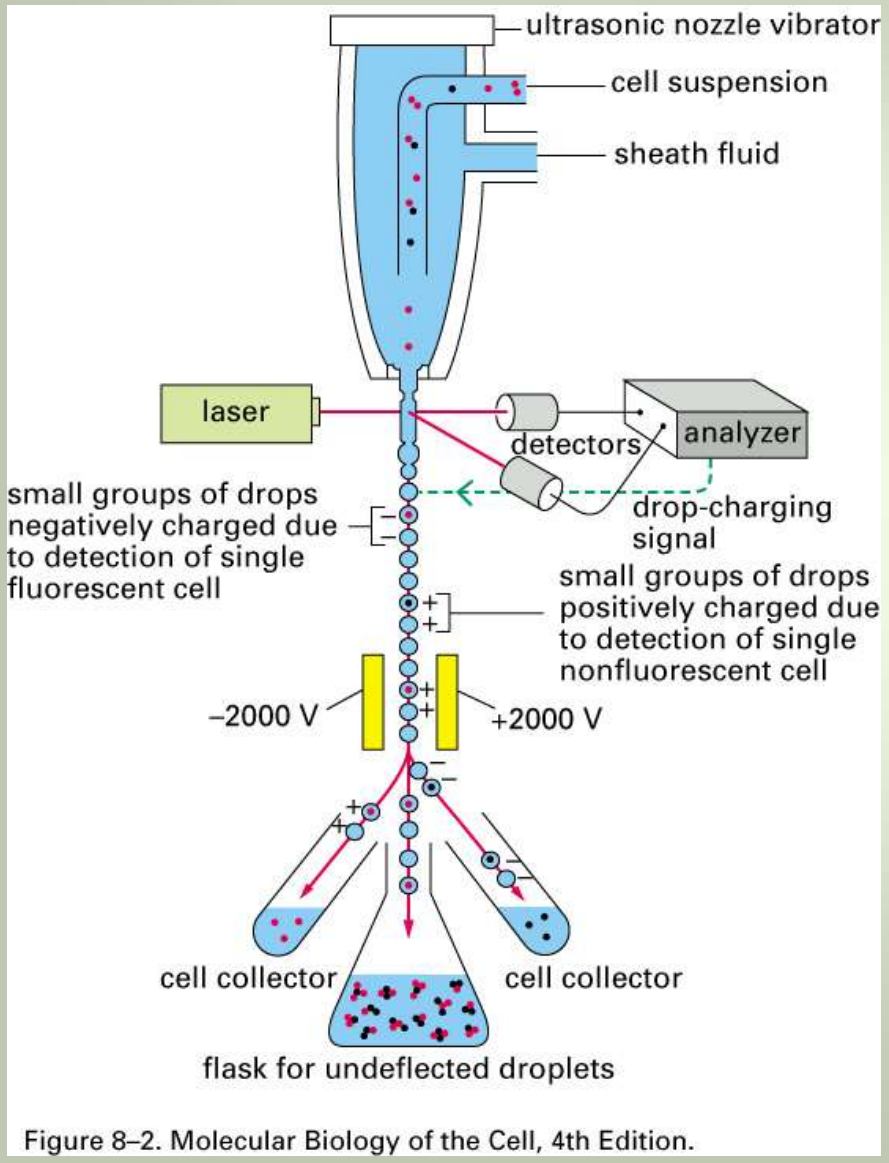


Figure 8–2. Molecular Biology of the Cell, 4th Edition.

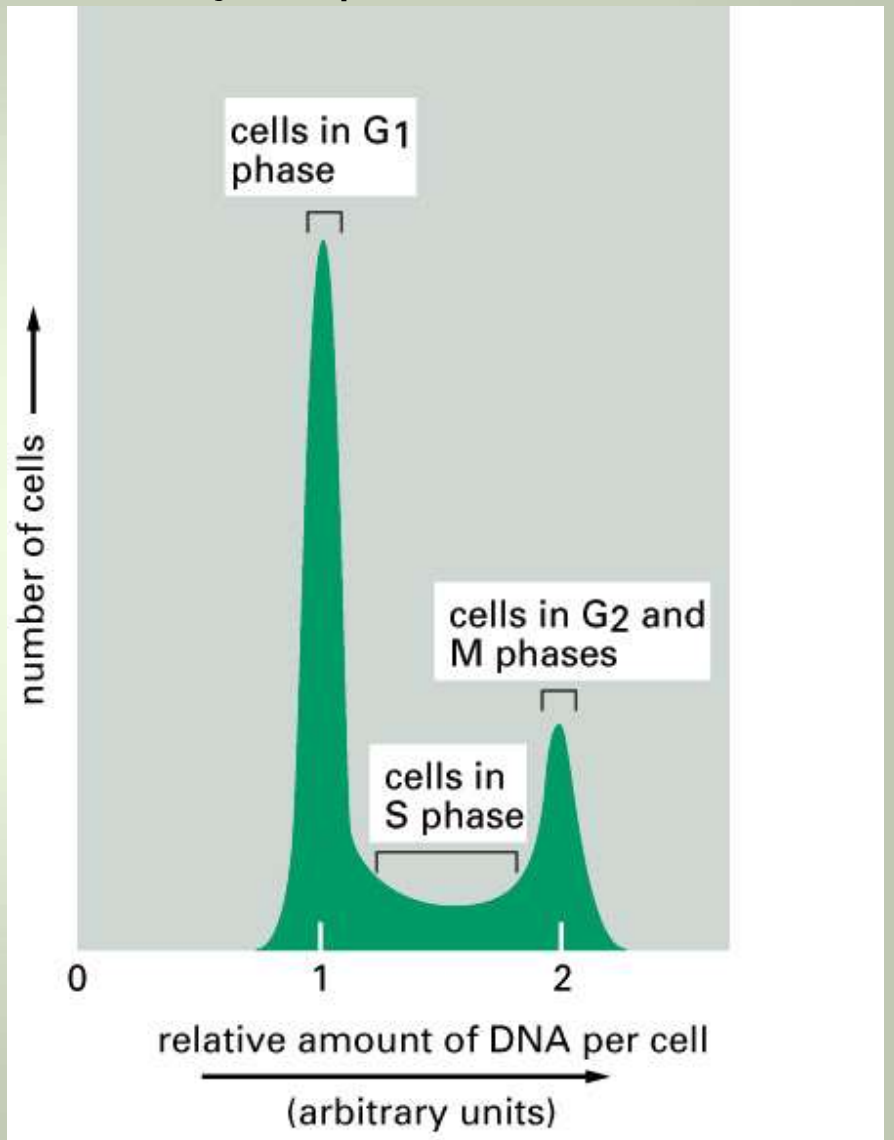


Figure 17–12. Molecular Biology of the Cell, 4th Edition.

Also, Wee 1 a kinase that inhibits Cdk by P04ing a tyr, & Cdc 25, a phosphatase that stimulates Cdk by removing that P04

p27 a direct-binding inhibitor of Cdk

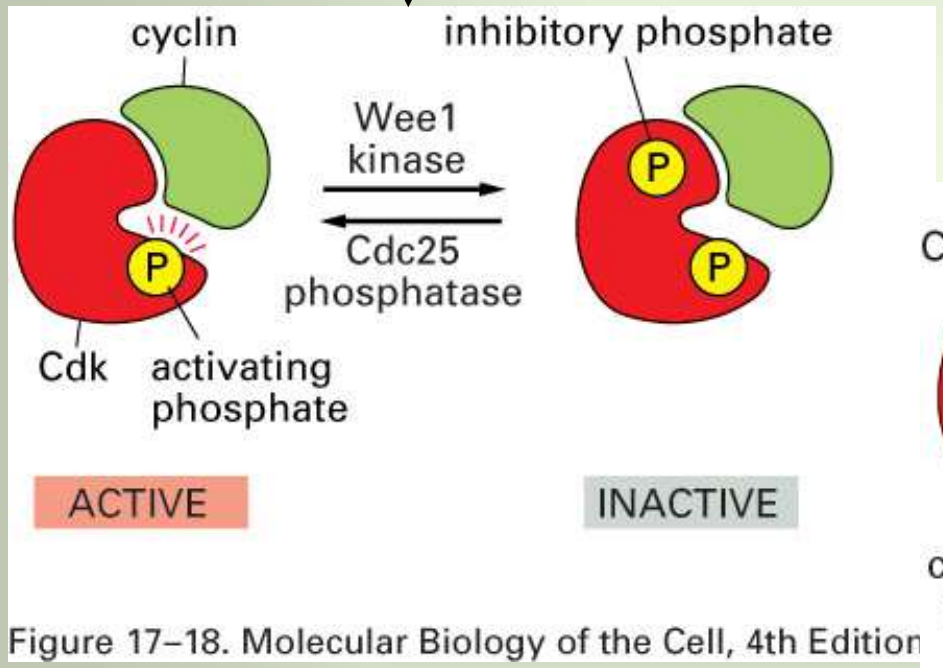


Figure 17–18. Molecular Biology of the Cell, 4th Edition

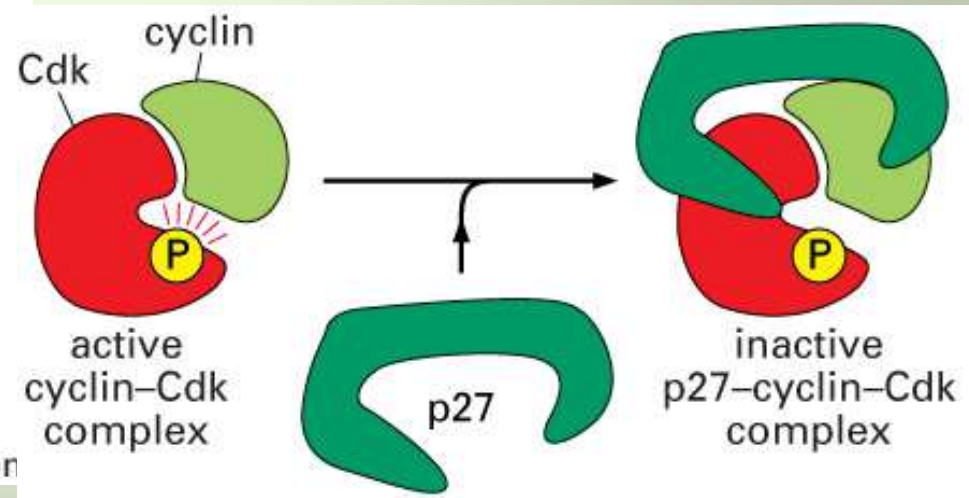


Figure 17–19. Molecular Biology of the Cell, 4th Edition.