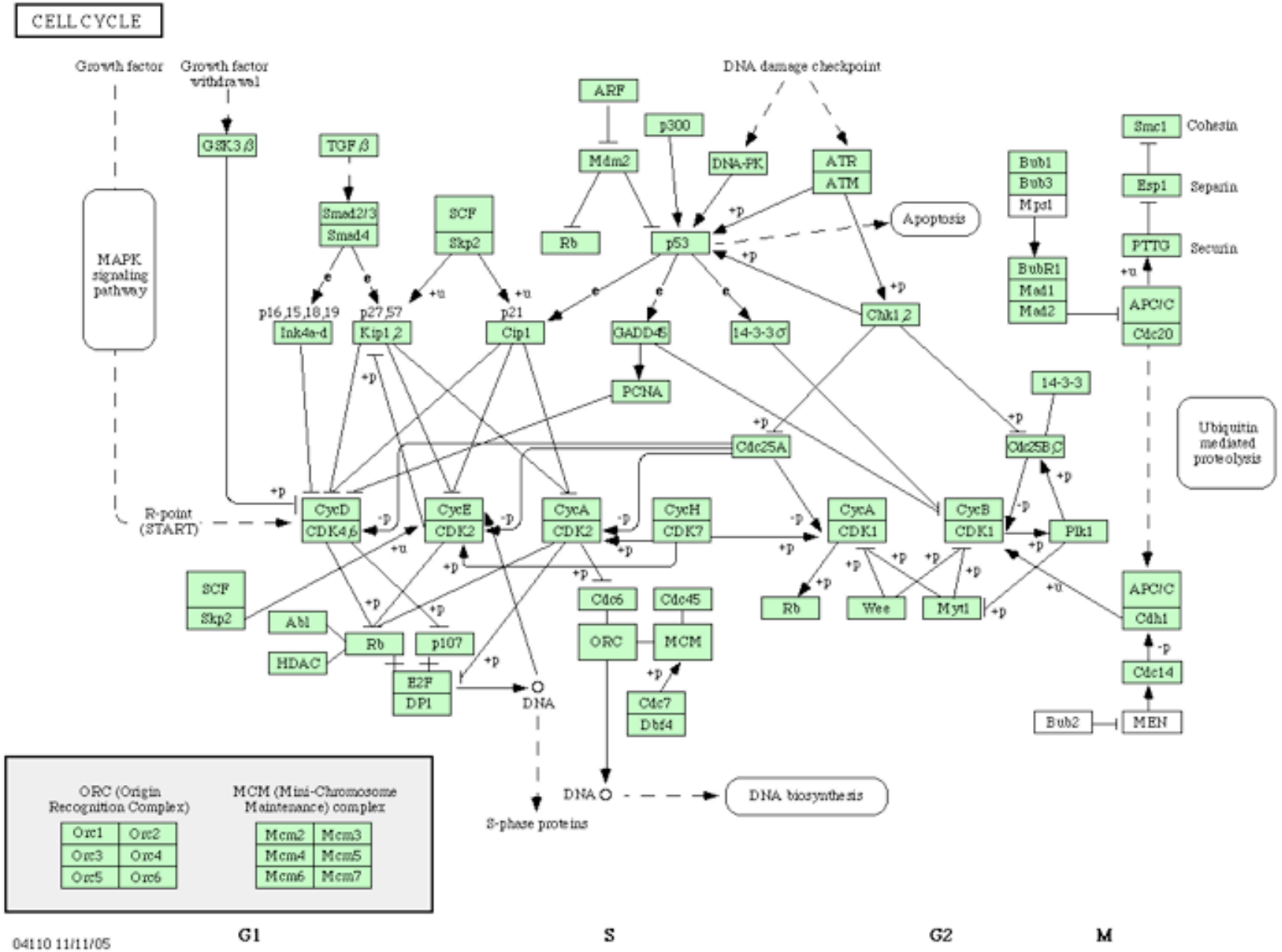
Lecture 9
Regulation of the Cell Cycle

Outline:

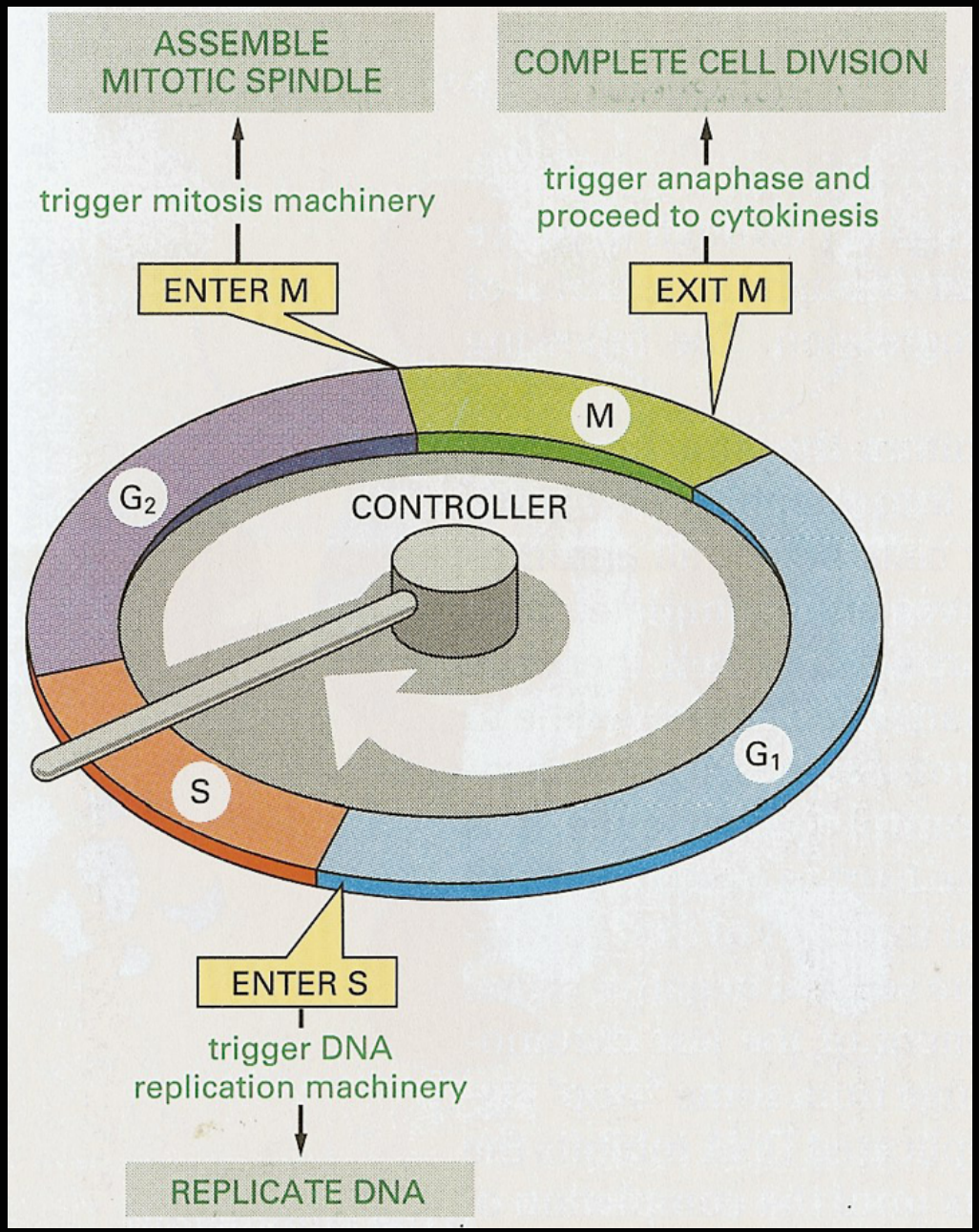
- Overview
- Identification of MPF, Cyclins and CDKs
- Identification of the controllers
- Regulation of Mitosis by Phosphorylation and Degradation

Paper:

CC Regulation is VERY Complex

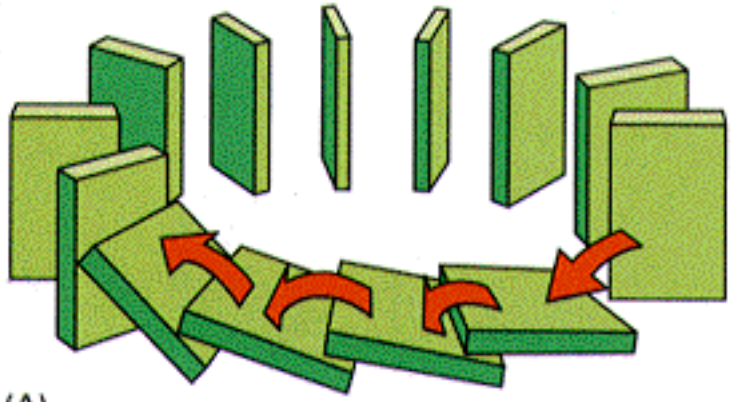


Overview of CC Regulation

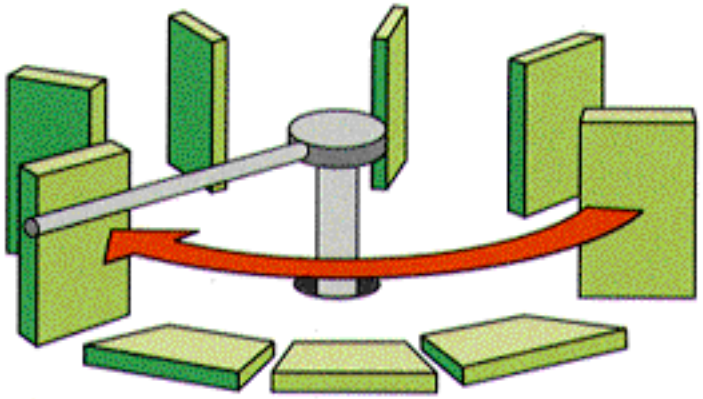


Two Views of CC Regulation Logic

‘Dominos’
sequential, dependent events

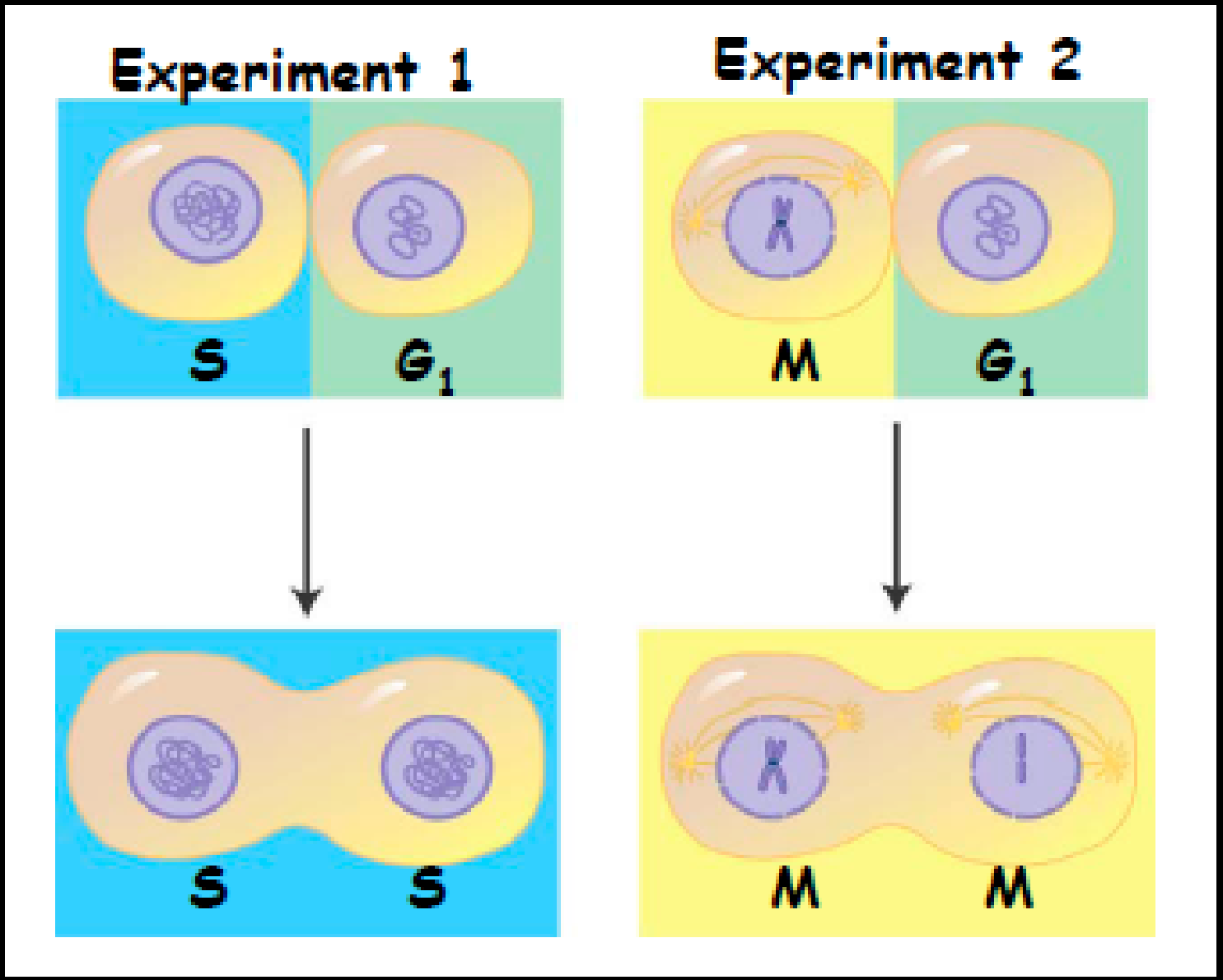


‘Oscillator’
central controller



both are involved

Evidence for ‘Master Controllers’

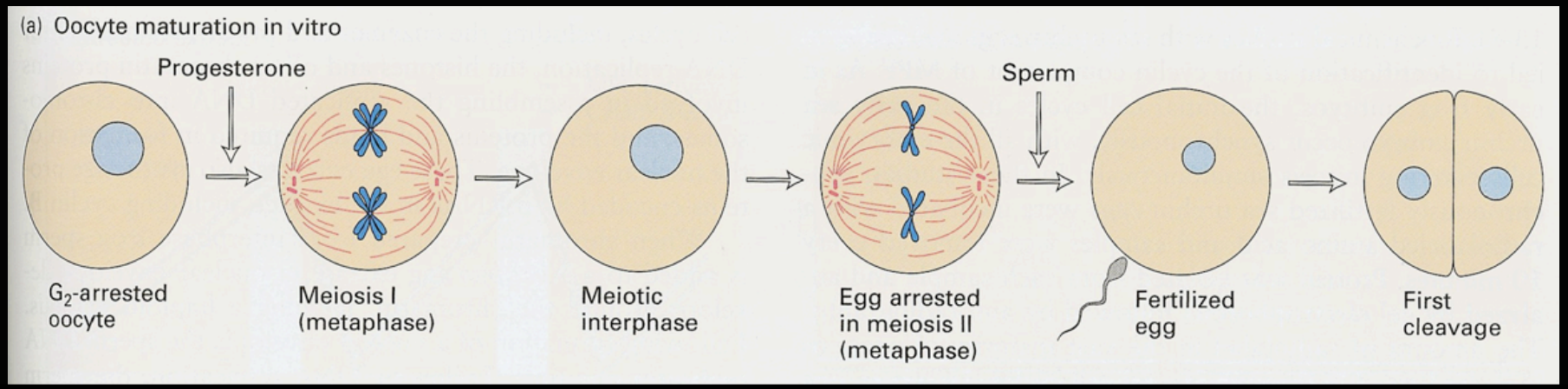


S phase ‘cytoplasm’ dominant over G1

M ‘cytoplasm’ dominant over interphase

The Discovery of MPF

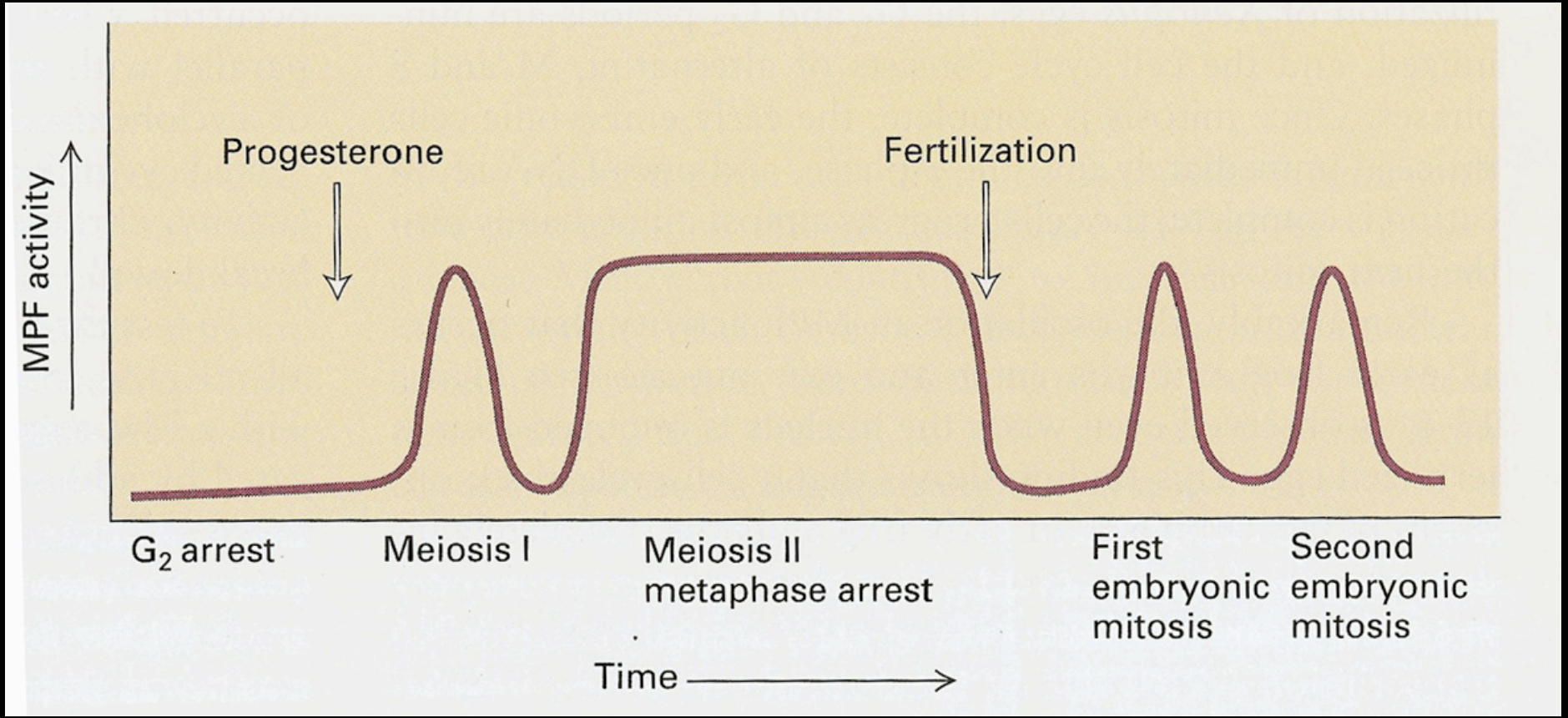
Xenopus and sea urchin egg extracts



transfer of mitotic cytoplasm induces mitosis

MPF- Maturation Promoting Factor, now Mitosis Promoting Factor

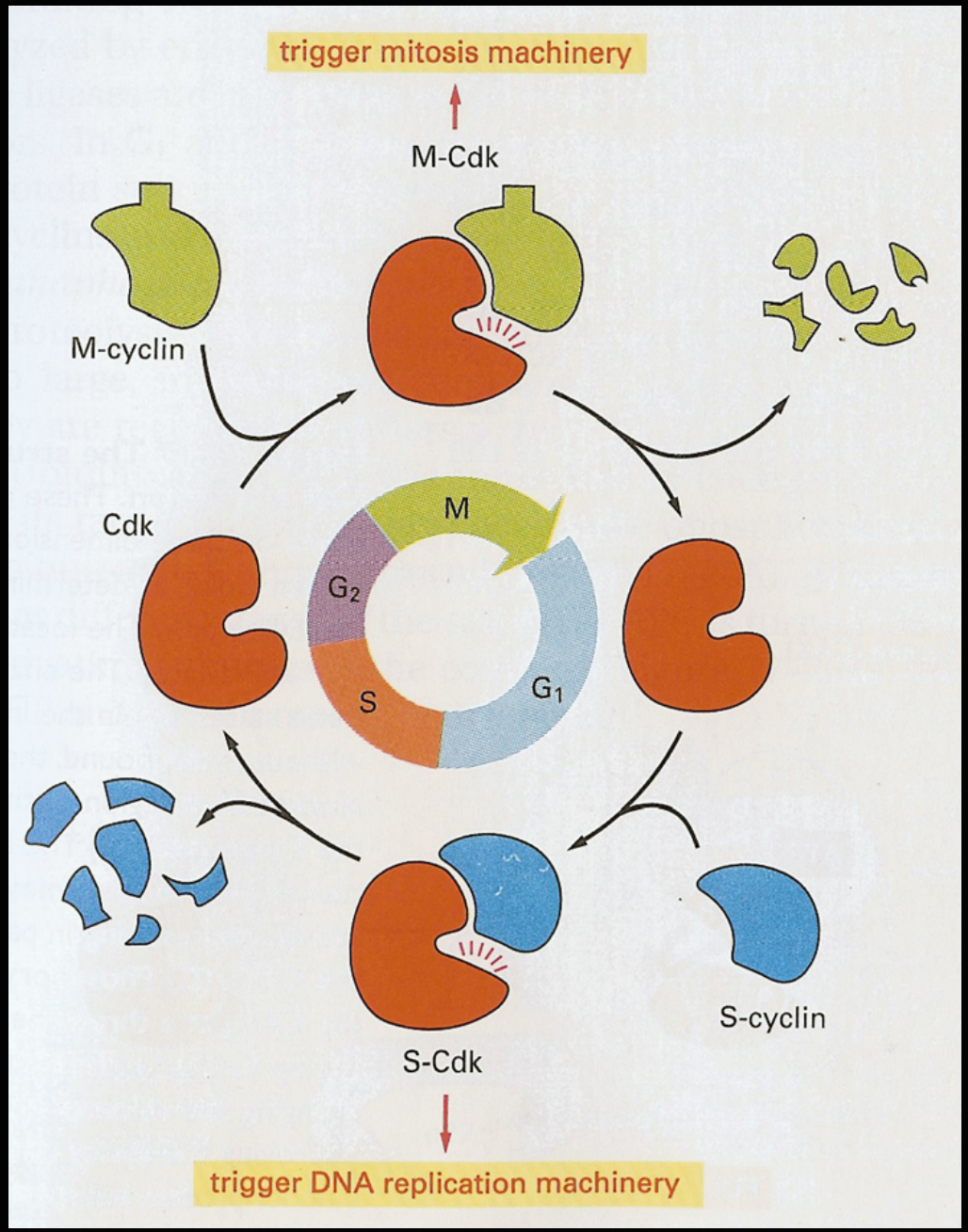
MPF activity oscillates during the cell cycle



activity correlates with meiotic and mitotic divisions

suggested that CC regulated by central controller

MPF (and SPF) are Cyclin-CDK complexes



CDKs

Cyclin Dependent Kinases

S/T kinases

phophorylate factors that drive CC processes

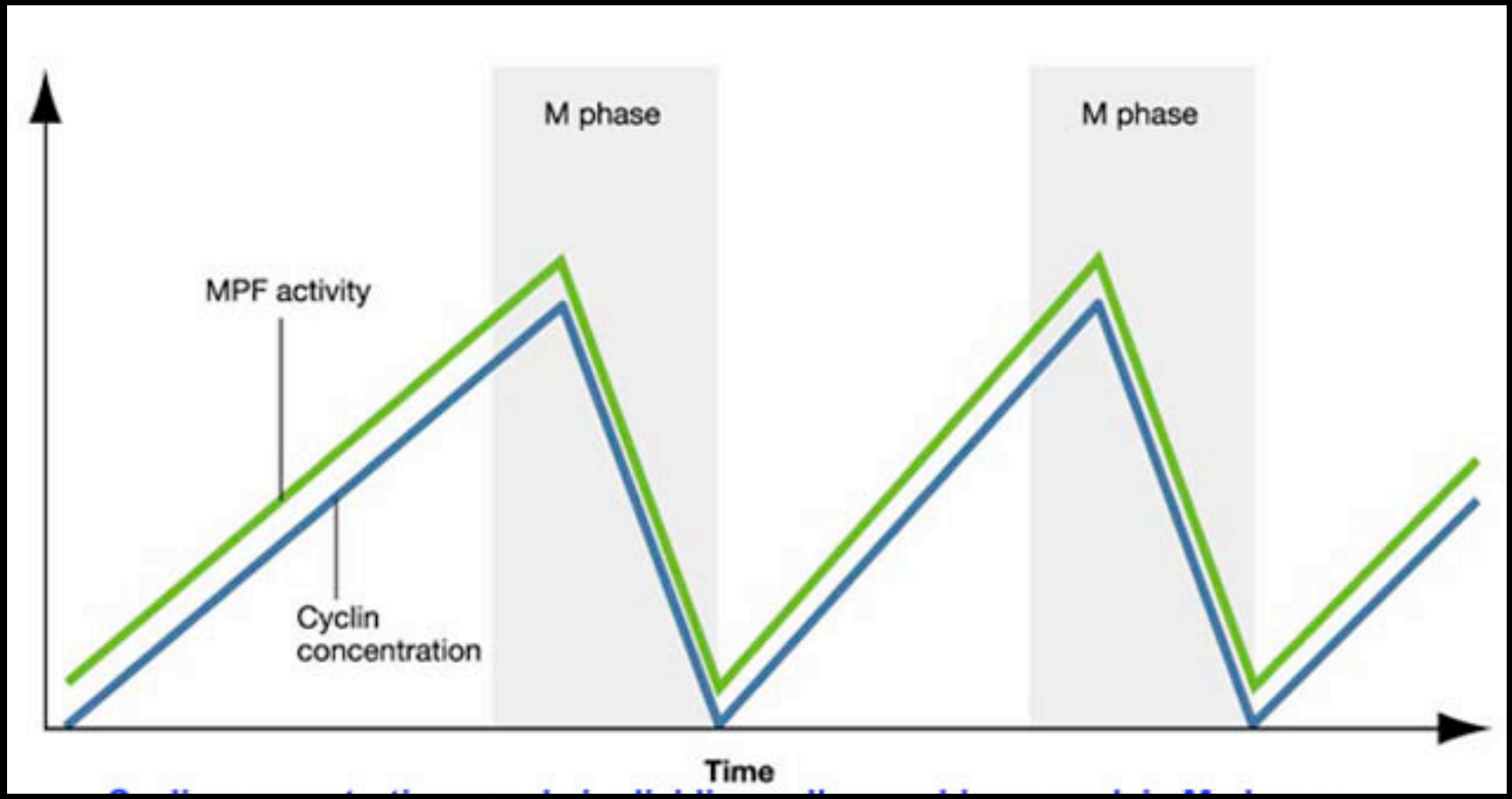
Cyclins

CDK activators

different cyclins for S and M

degradation inactivates CDKs

Cyclin concentration regulates MPF activity



Cyclins and CDKs in yeasts and metazoans

TABLE 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

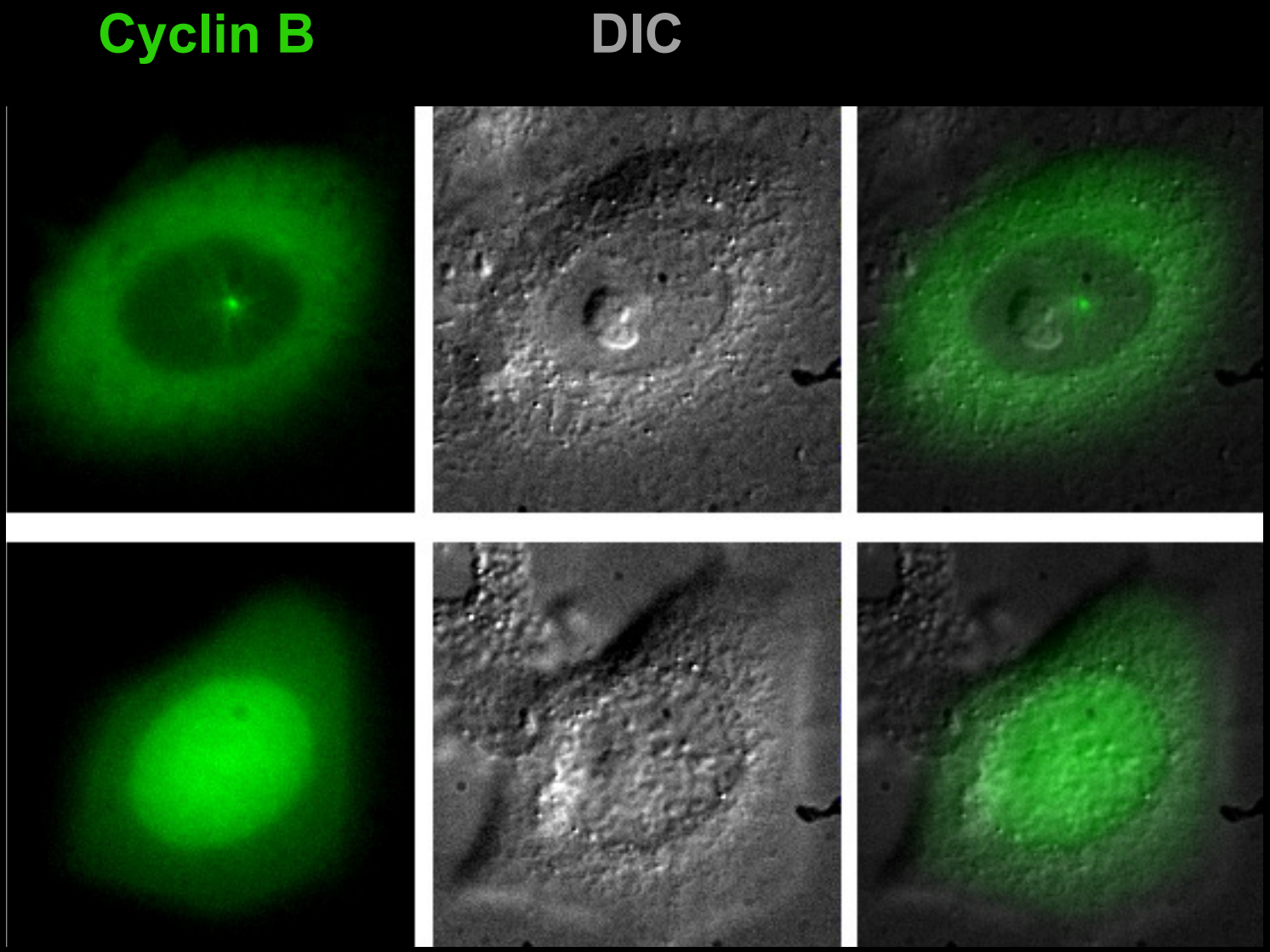
CYCLIN–CDK COMPLEX	VERTEBRATES		BUDDING YEAST	
	CYCLIN	CDK PARTNER	CYCLIN	CDK PARTNER
G_1-Cdk	cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	cyclin A	Cdk2	Clb5, 6	Cdk1
M-Cdk	cyclin B	Cdk1**	Clb1, 2, 3, 4	Cdk1

G1 — **Sense cell size, Commit to division**

S — **Activate replication origins**

M — **Spindle assembly, anaphase, exit**

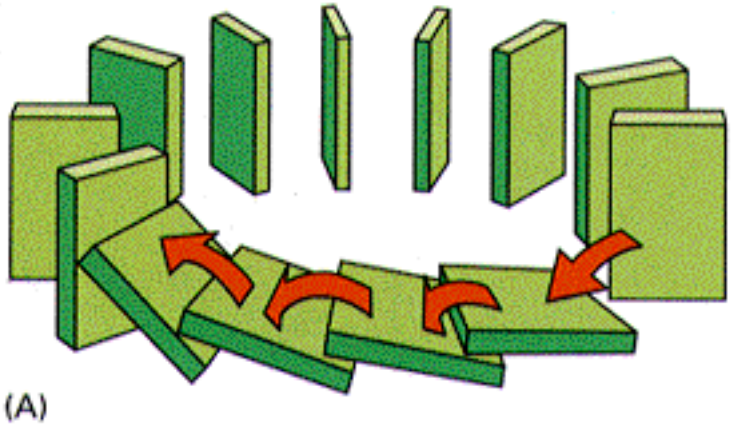
Nuclear location of Cyclin B regulated at onset of Mitosis



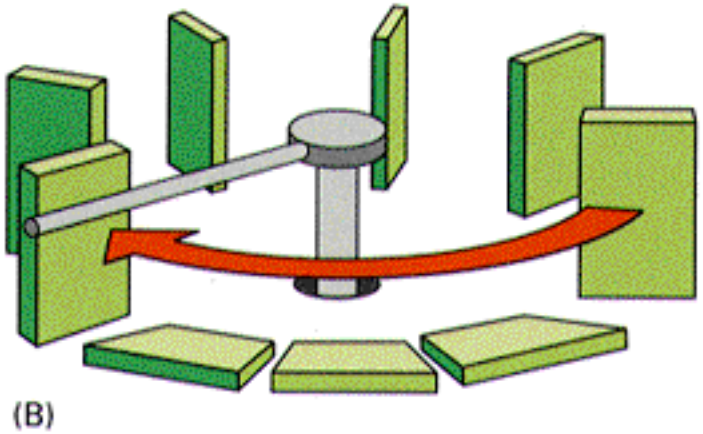
due to Nuclear Envelope Breakdown (NEB)

The Identification of CC Regulator Proteins

‘Dominos’
sequential, dependent events



‘Oscillator’
central controller



Genetic Screens: Yeast ‘Cell Division Cycle’ (CDC) Mutants

can individual protein mutations block steps or whole process ?

provided evidence that both are involved

The Identification of CC Regulator Proteins using YEASTS

Advantages:

rapidly growing, divides every 90 min

powerful genetics

cell cycle progress easy to follow

Disadvantages:

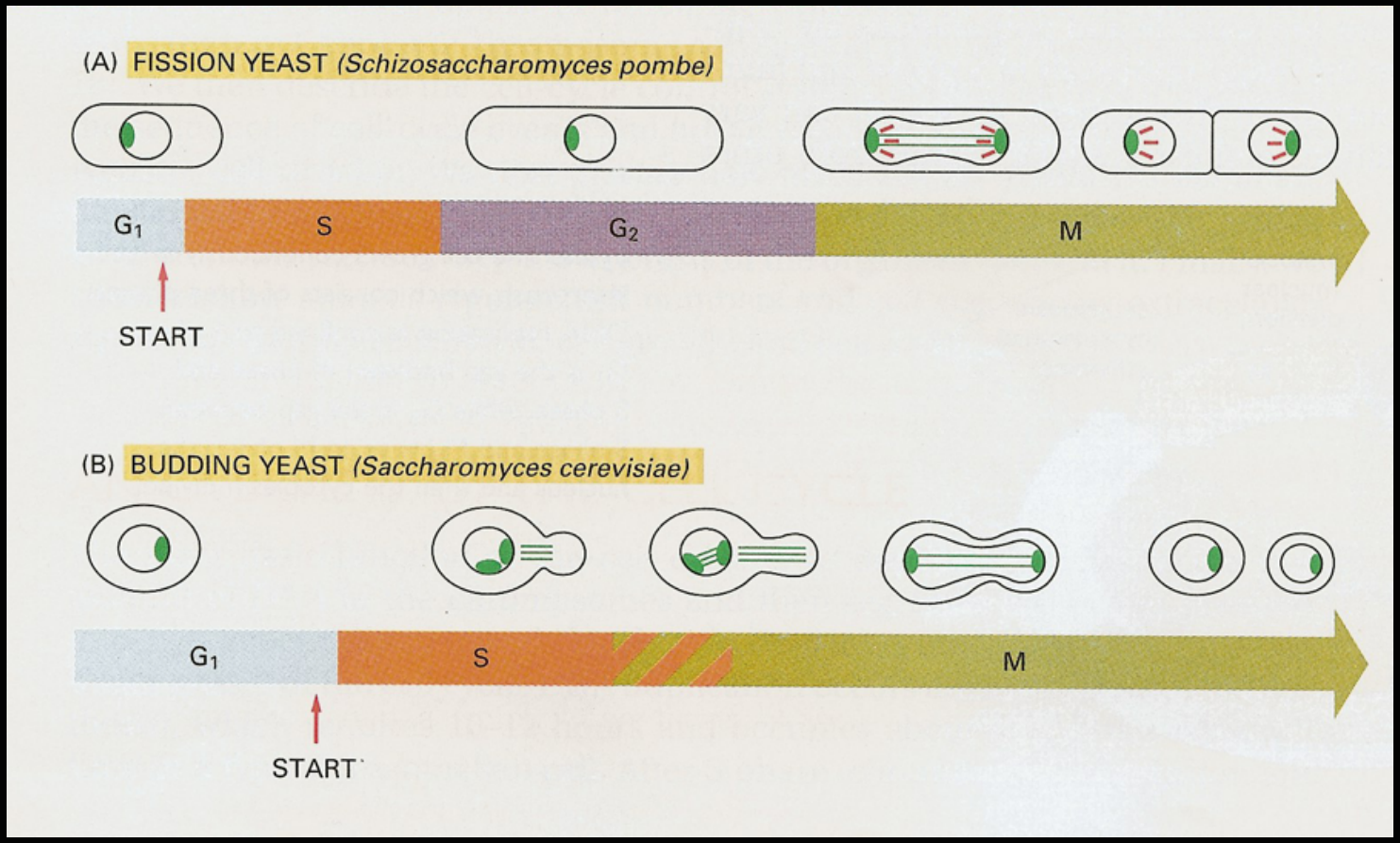
different from higher eukaryotes

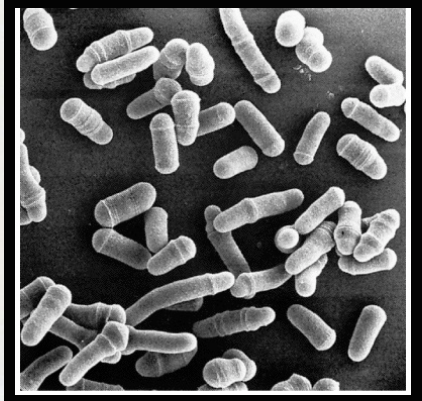
nuclear envelope does not break down in M-phase

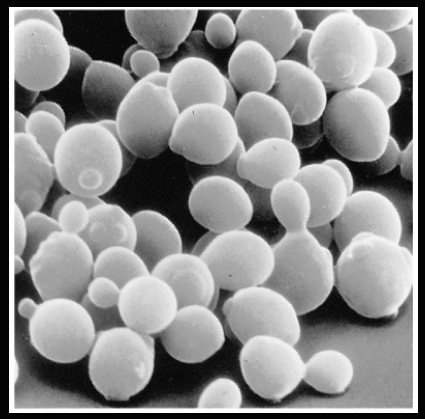
spindle assembly occurs during DNA replication (budding yeast)

little or no chromosome condensation in M-phase

Pombe and cerevisiae life cycles







Cell cycle coordinated with growth at two points: G1/S and G2/M

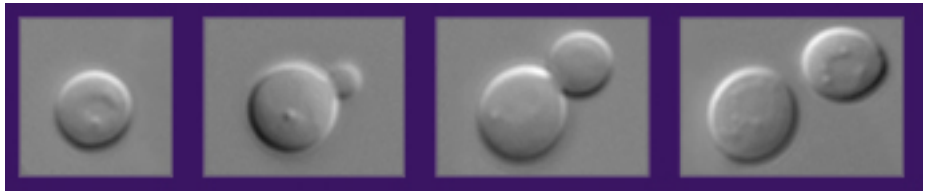
WT fission yeast: cells born large enough to pass start

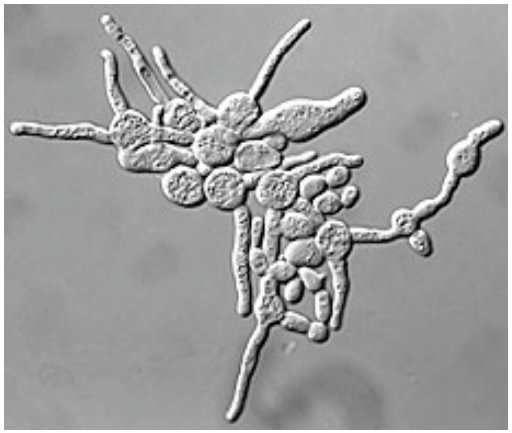
G1/S transition less visible - long G2

WT budding yeast: cells big enough to pass start and to enter mitosis (committed to divide even in absence of nutrients)

G2/M transition less visible - long G1

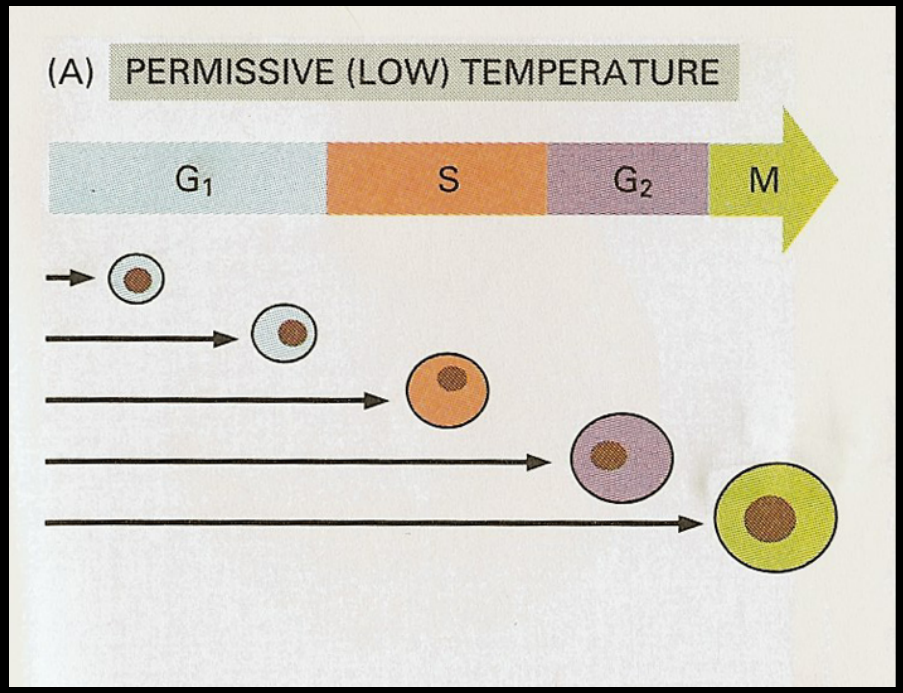
Genetic Screens: Yeast 'Cell Division Cycle' (CDC) Mutants

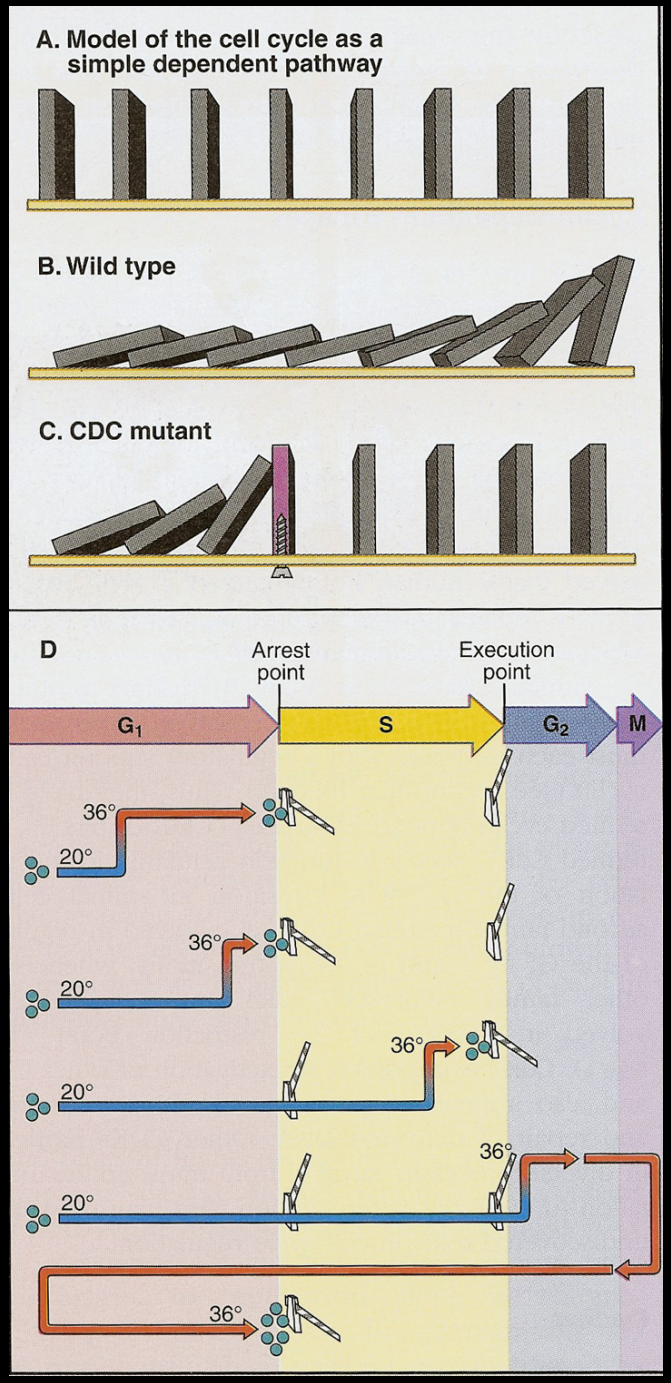




- Lee Hartwell (cerevisiae); Paul Nurse (pombe)
- Goal: find mutants unable to transit the cell cycle
- Why yeast?
  - Cell shape --> cell cycle stage
  - Grow as haploids (easier to find mutants), or diploids (can do genetics)
- Problem:
  - the screen is for cells that can't grow
- Solution:
  - temperature sensitive mutants
  - Replica plating

CDC Screen: TS Cell cycle arrest after mutagenesis





clone genes and identify proteins by transfecting random plasmids and looking for rescue

Note on yeast nomenclature

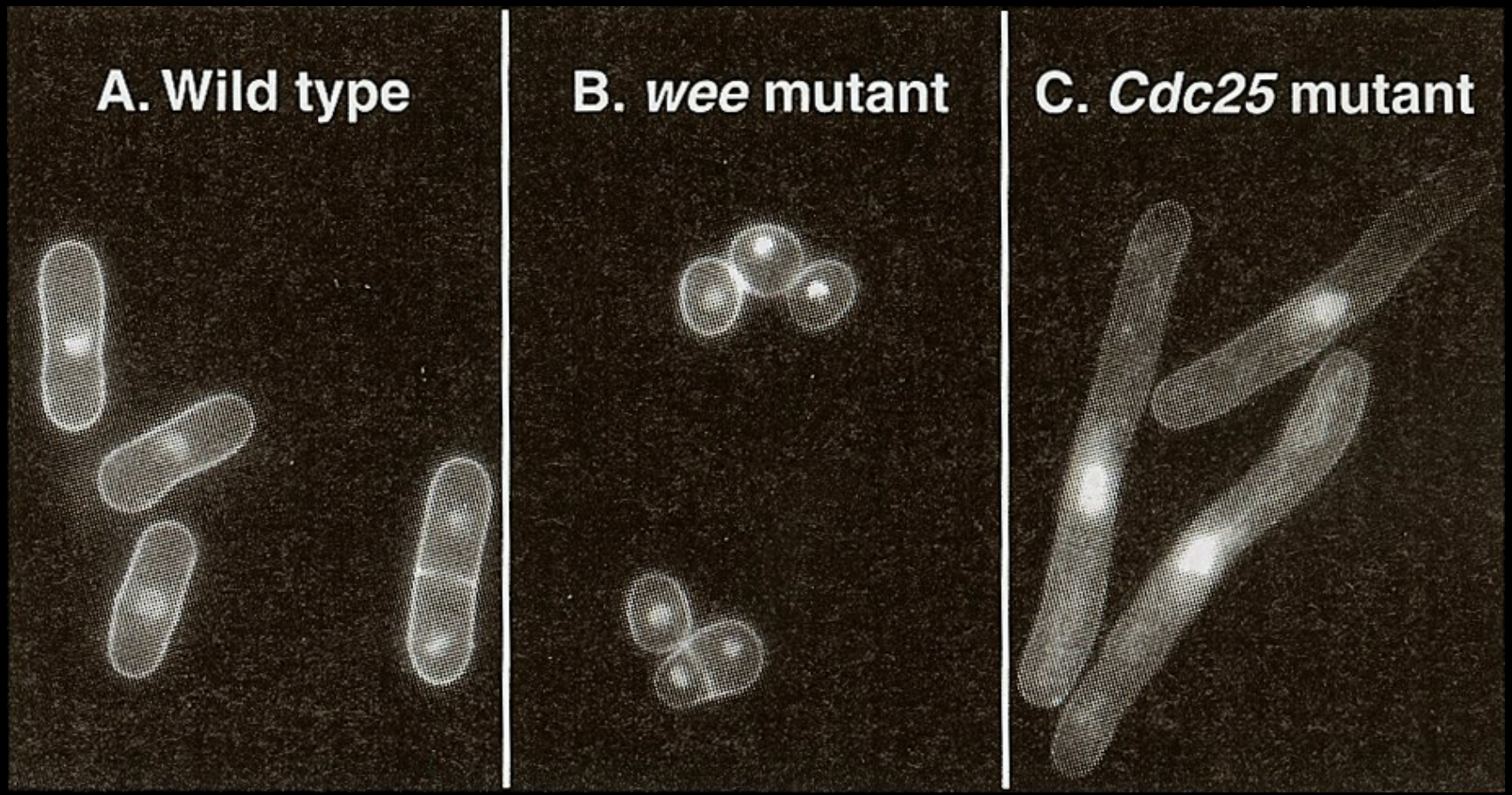
S. cerevisiae (budding yeast)

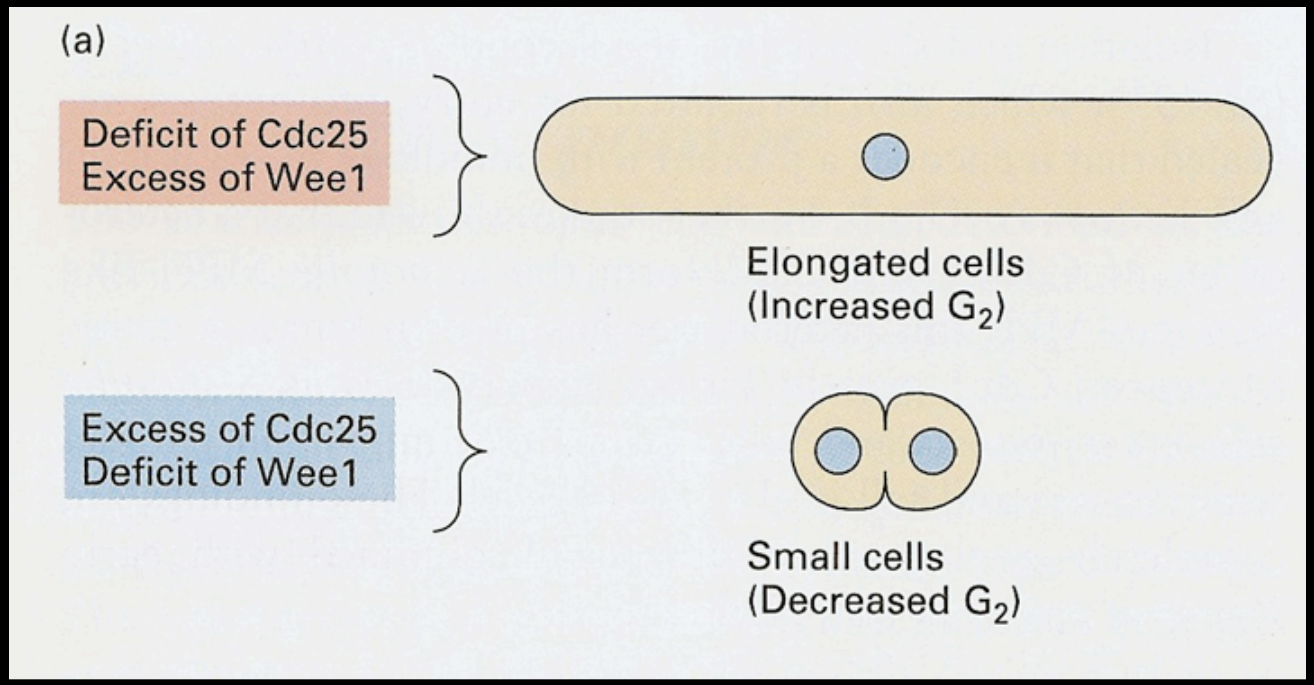
wild type or dominant	CDC28
mutations	cdc28-4^{ts}, Δcdc28
protein	Cdc28

S. pombe (fission yeast)

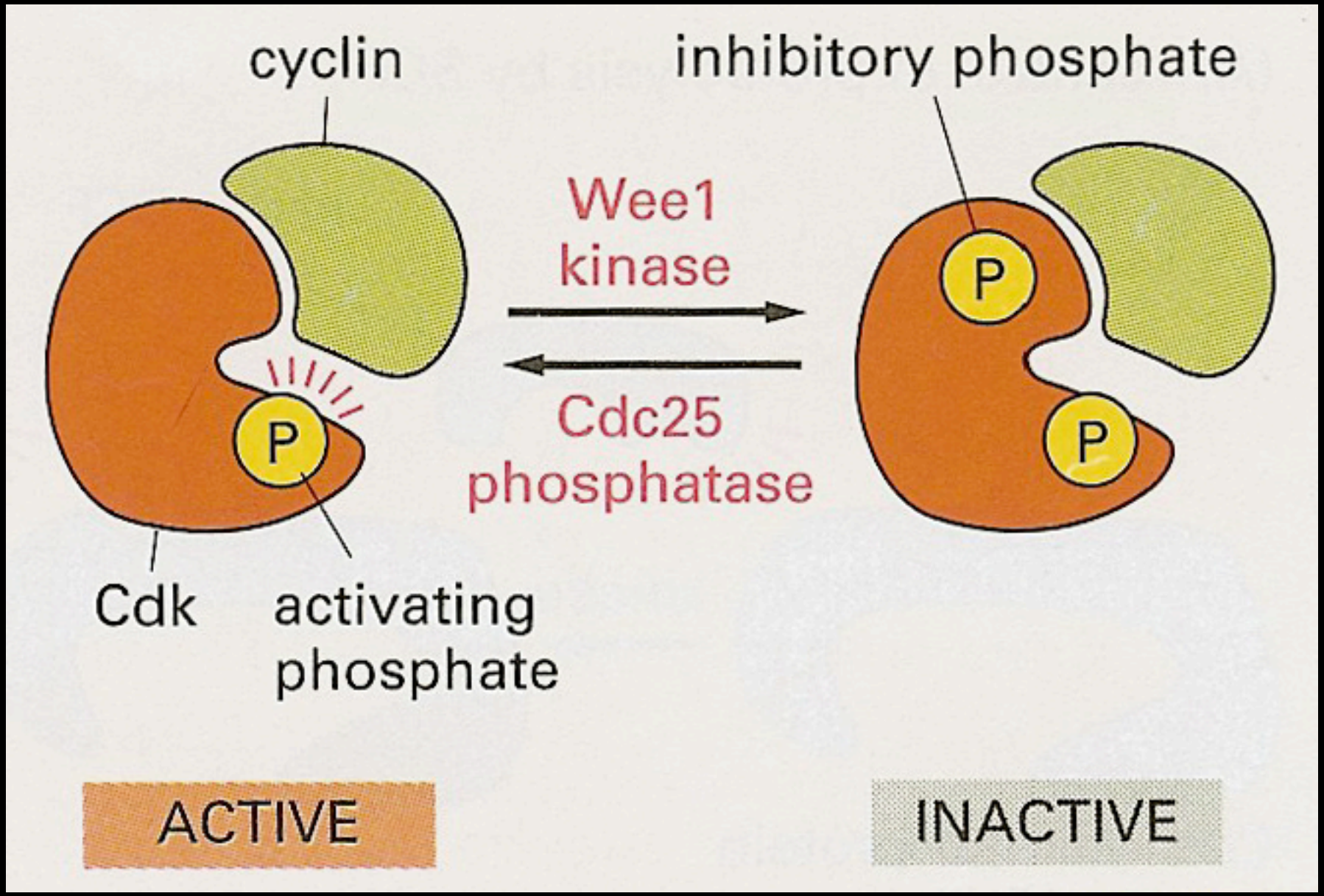
wild type or dominant	cdc2^{+} cdc2^{D}
mutations	cdc2^{ts} cdc2^{-}
protein	Cdc2

Pombe mutant phenotypes



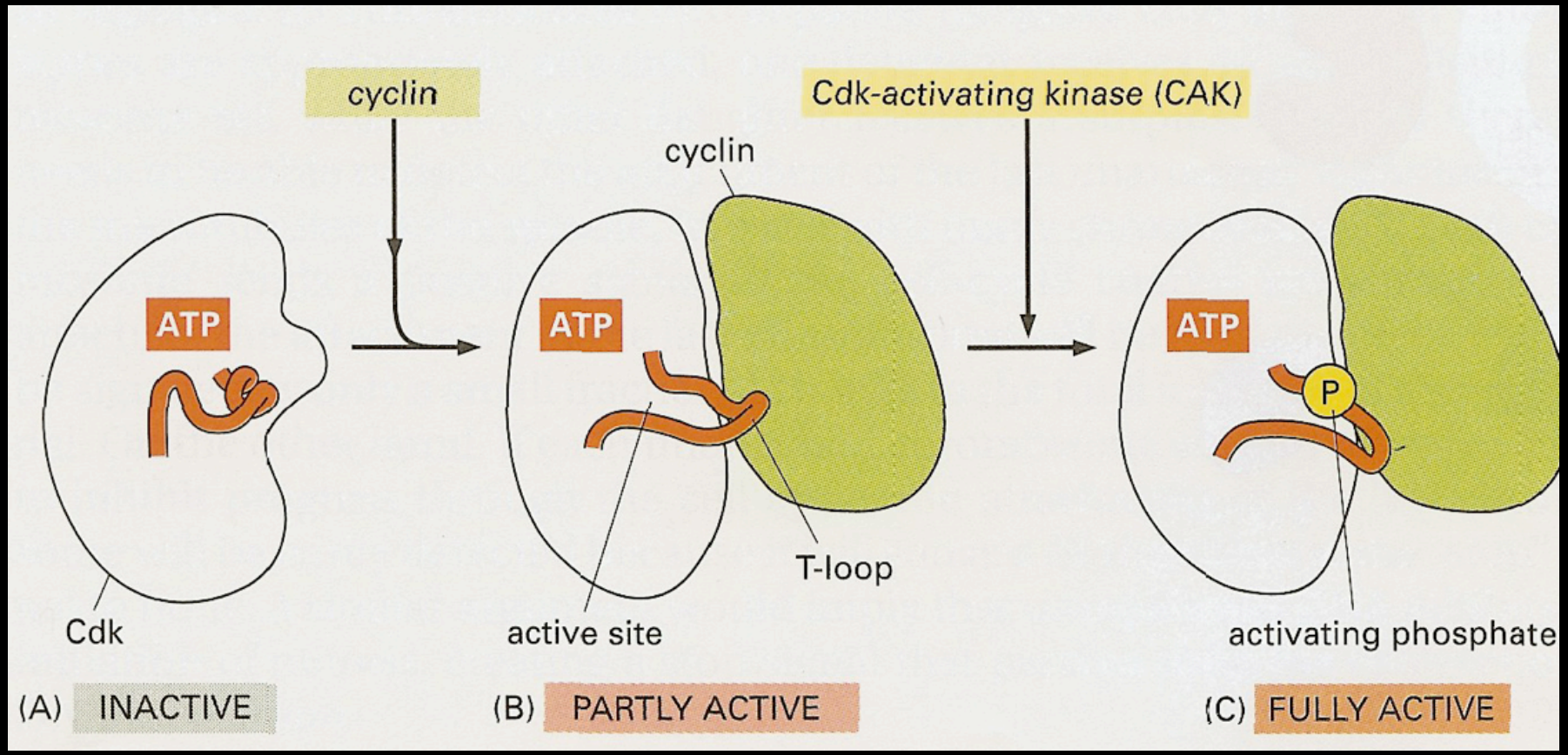

(a)
Deficit of Cdc25
Excess of Wee1
Elongated cells
(Increased G_2)
Excess of Cdc25
Deficit of Wee1
Small cells
(Decreased G_2)

Cdc25 and Wee1 regulate Cdk phosphorylation and activity

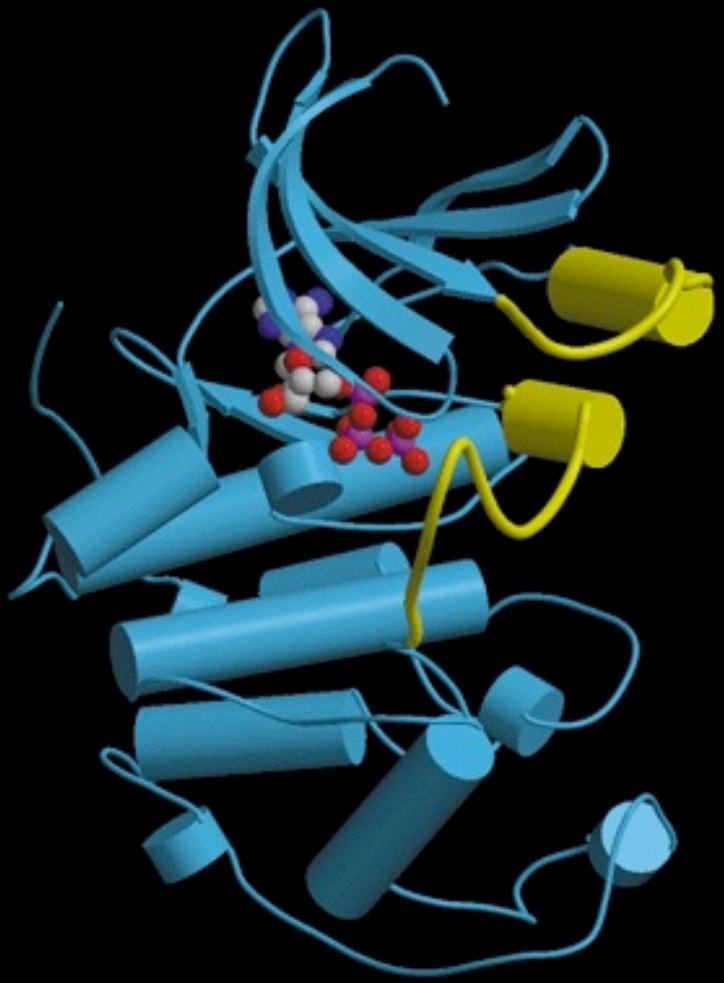


CDKs are activated by phosphorylation (CAKs)

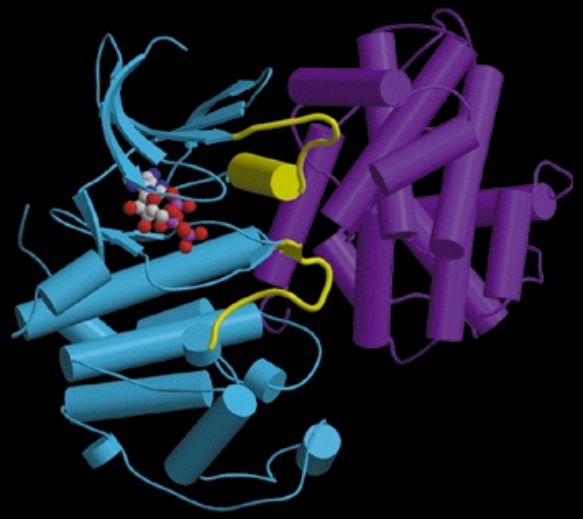
requires binding of Cyclins to CDKs



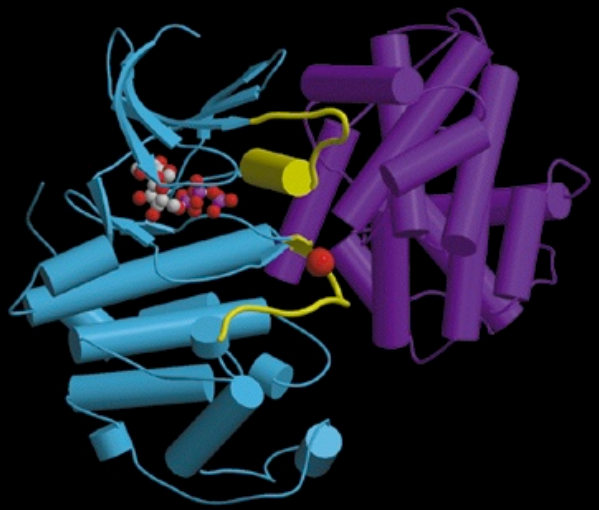
Cdk



Cdk + Cyclin

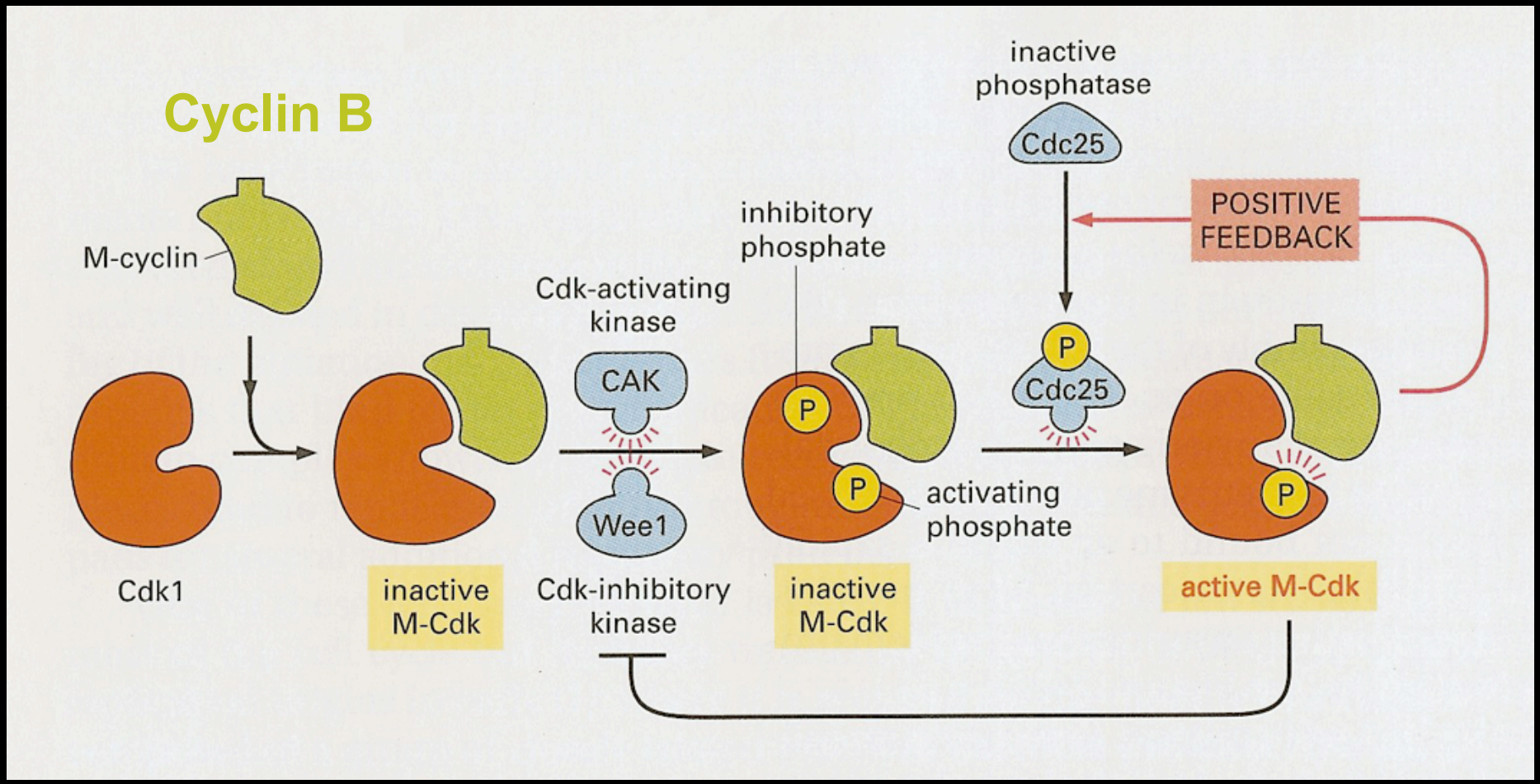


partially active



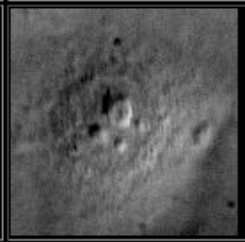
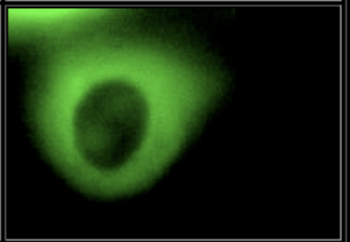
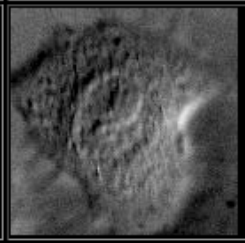
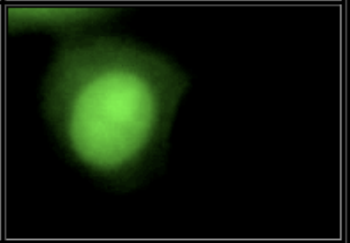
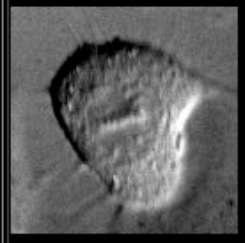
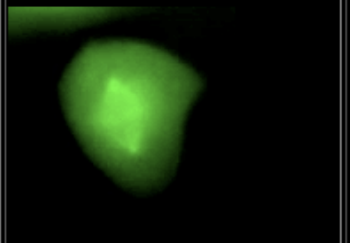
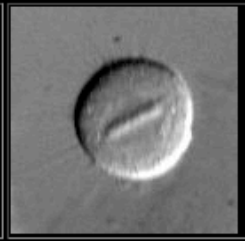
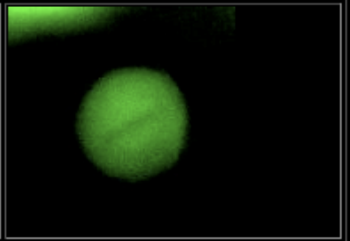
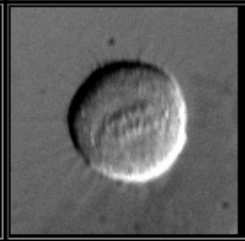
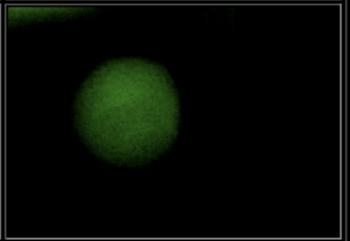
active

Feedback Loops maintain M-Cdk Activity



Active M-Cdk inhibits Wee inhibitory kinase and activates Cdc25 activating kinase

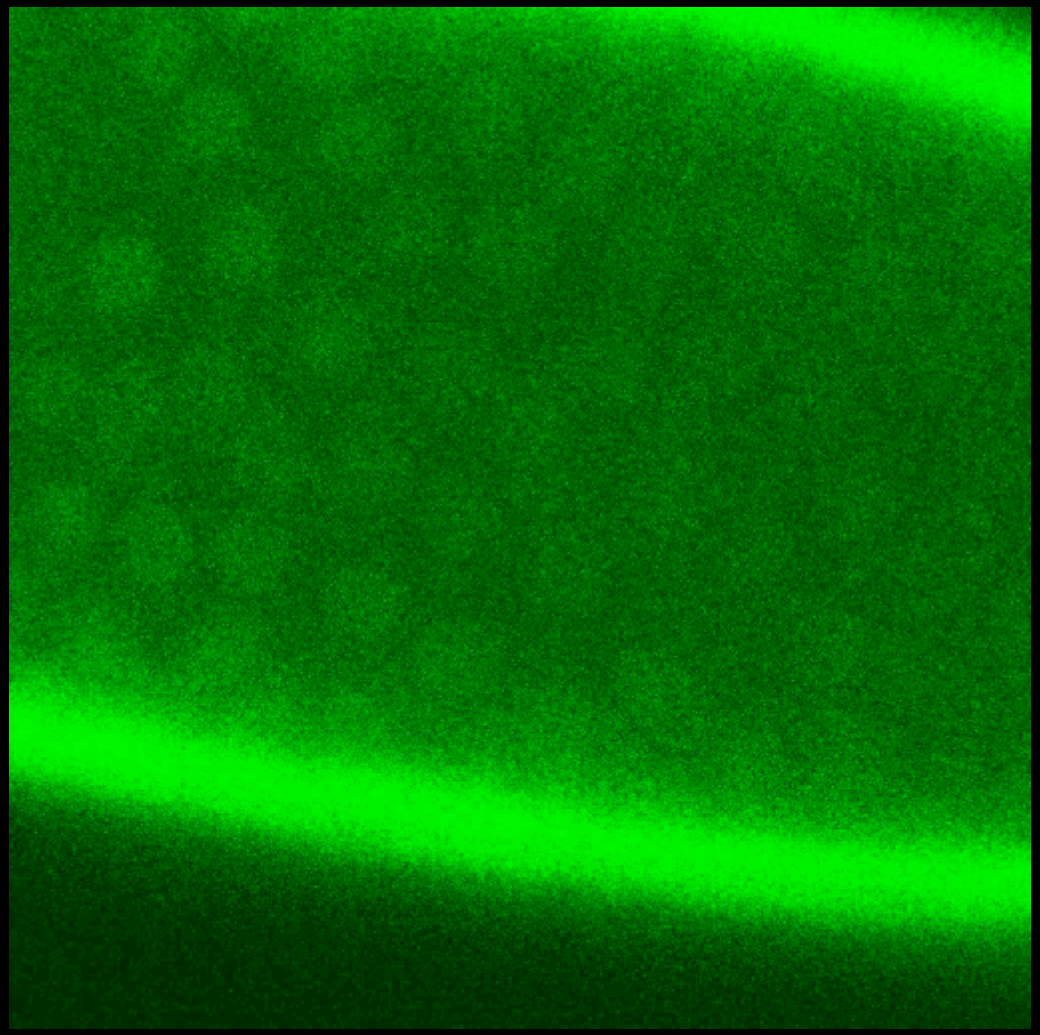
Cyclin levels are regulated during the cell cycle

	DIC	*GFP*
G2 Phase		
Prophase		
Pro-Meta phase		
Metaphase		
Anaphase		

Cyclin B

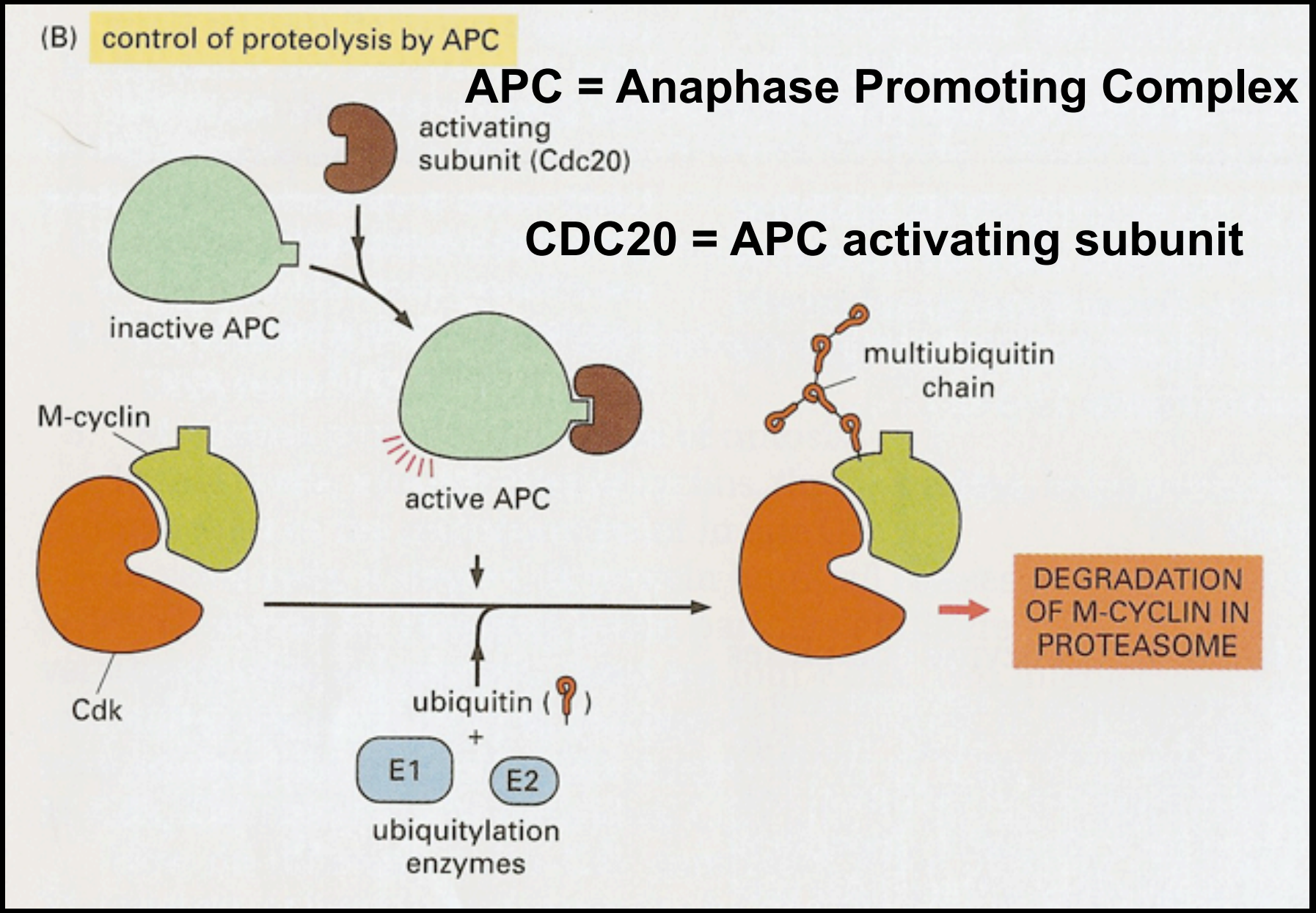
Cyclins are regulated during the cell cycle

Drosophila embryos



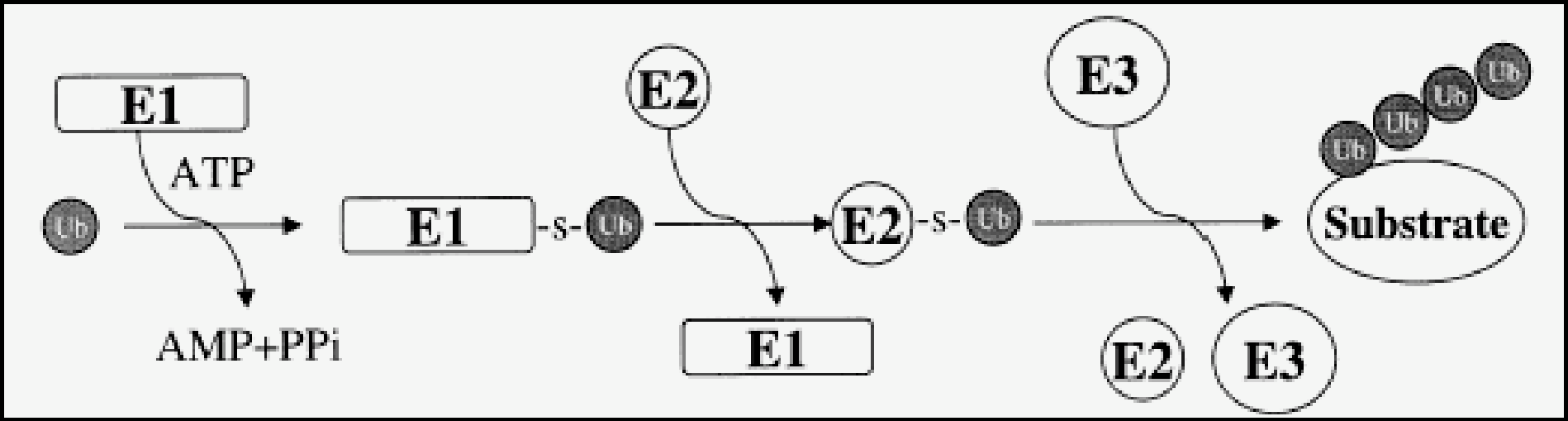
Cyclin B

Cyclins are regulated by proteolysis



Two modular complexes that direct proteolysis

E3 ubiquitin ligases



APC

anaphase start & M exit

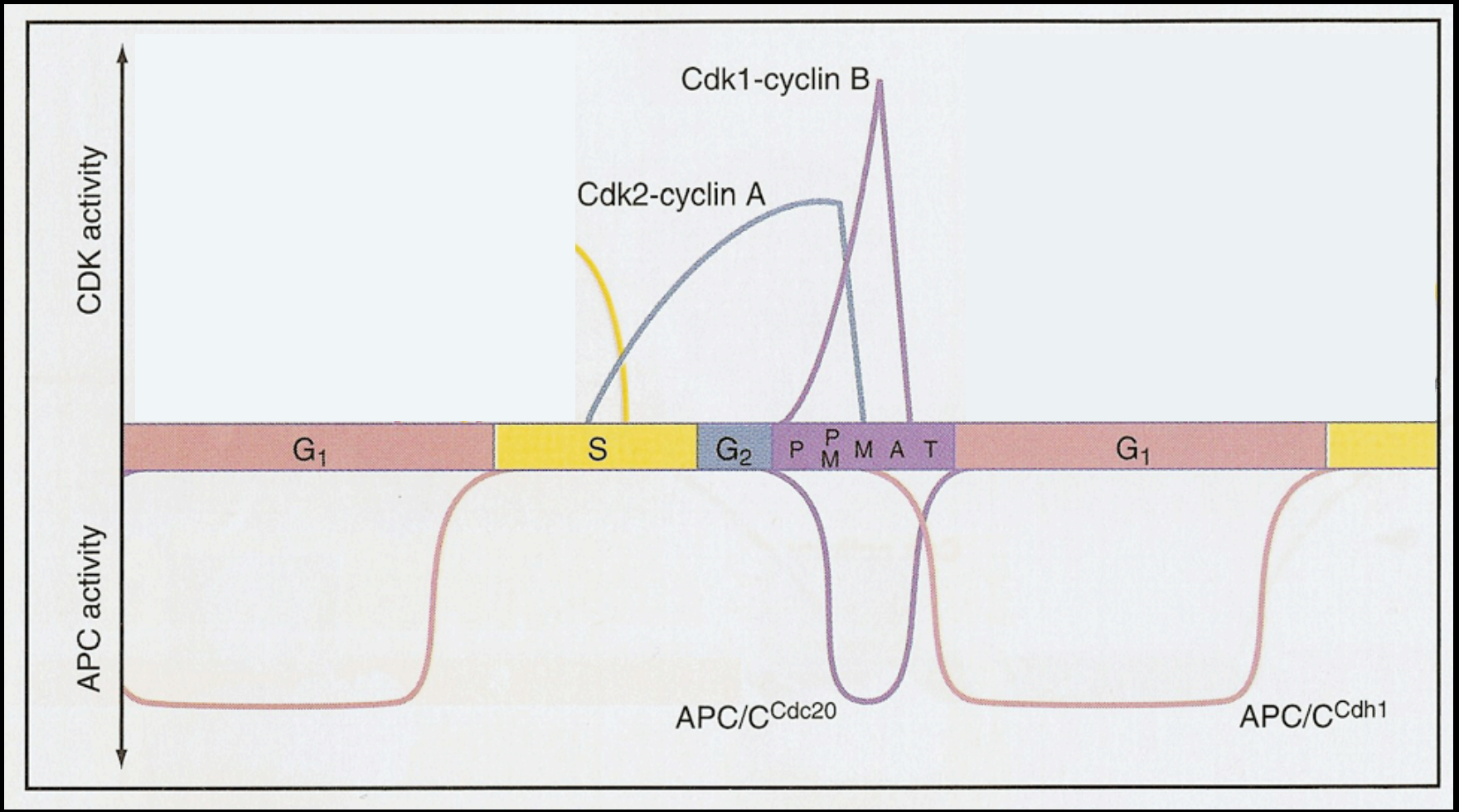
subunits phosphorylated

SCF

G1/S

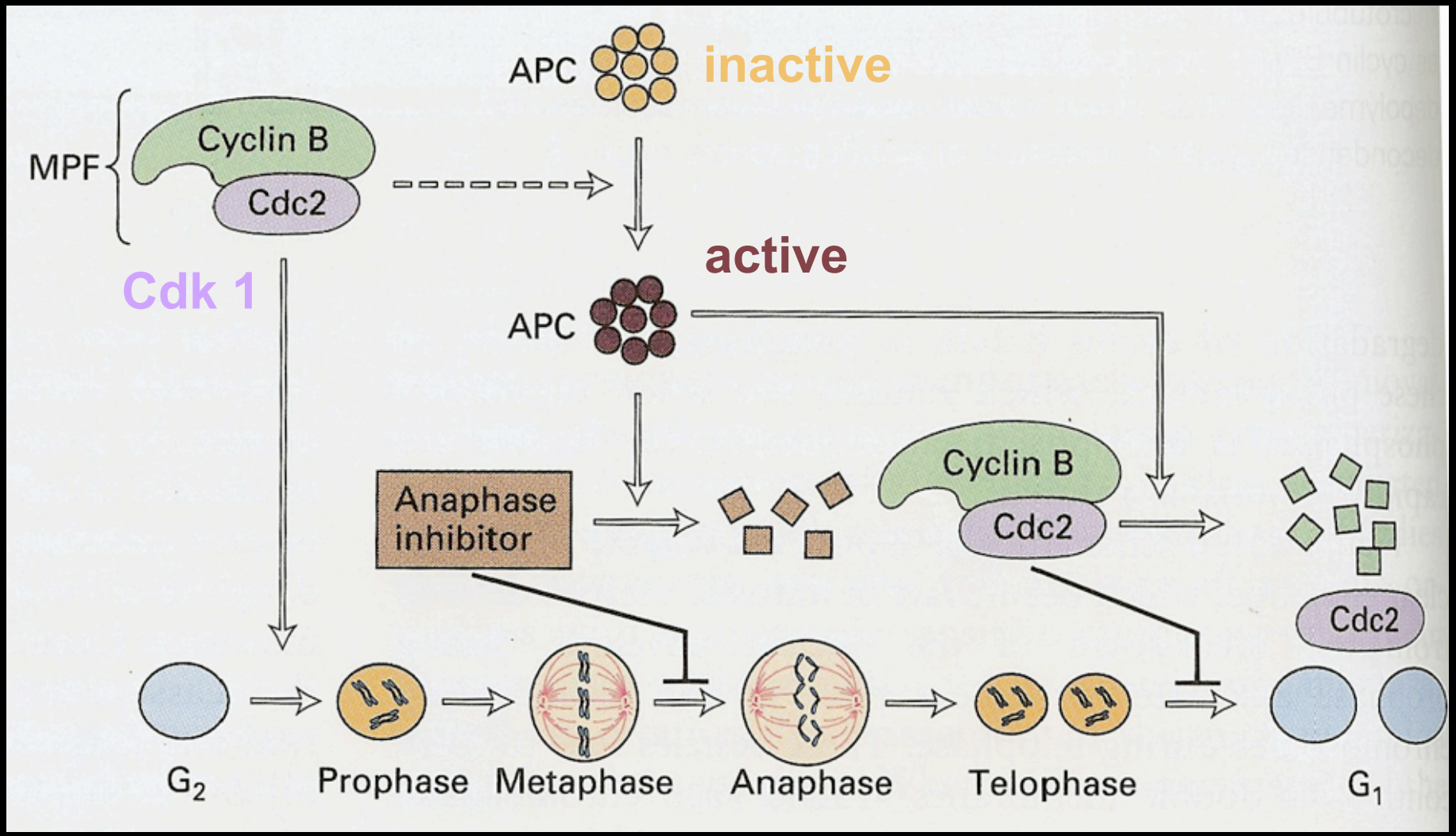
substrates phosphorylated

CDK activity and Cyclin levels are correlated with APC levels

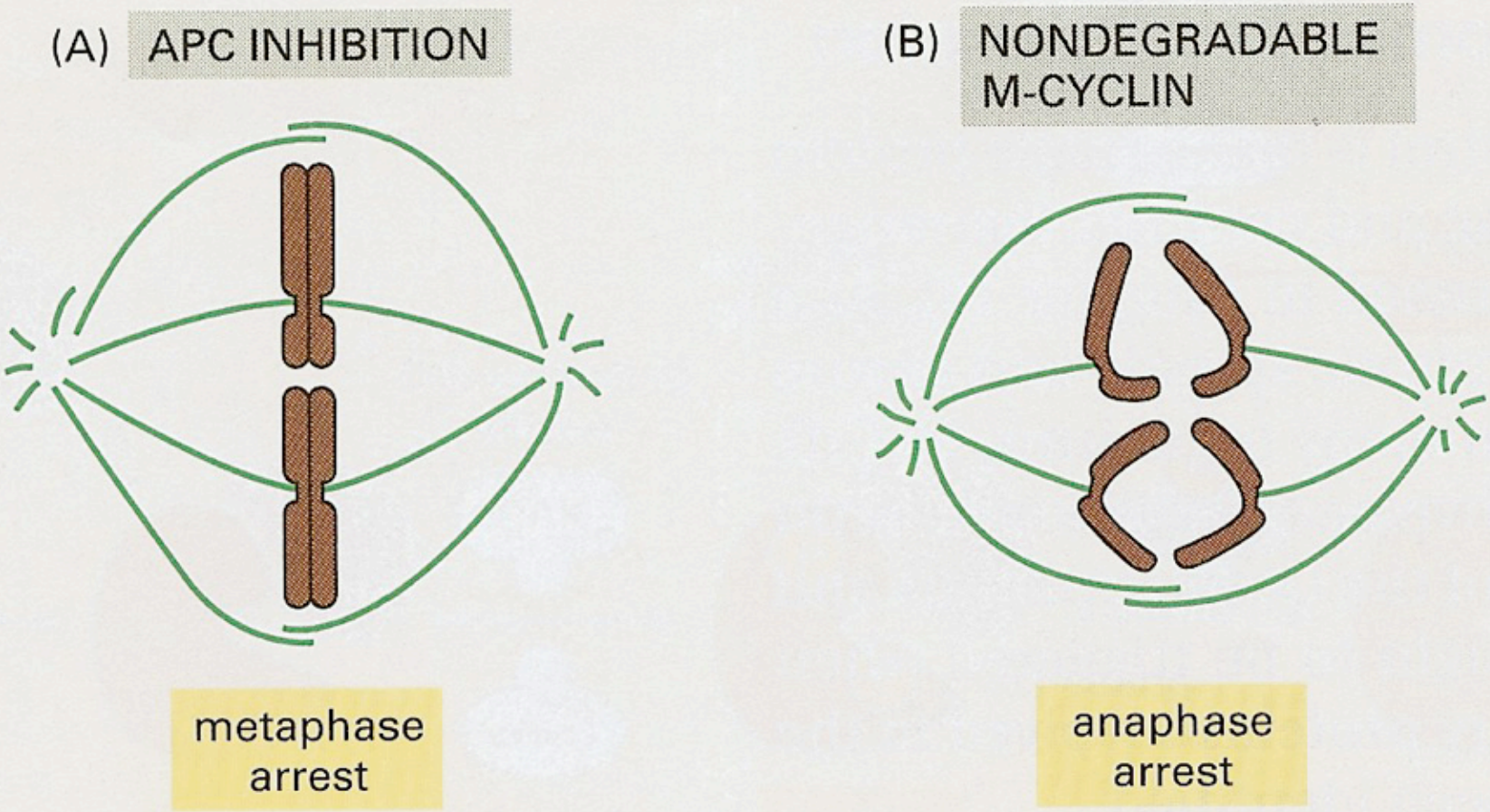


active APC correlates with loss of Cyclins and CDK inactivity

The APC is required for anaphase entry and exit from mitosis



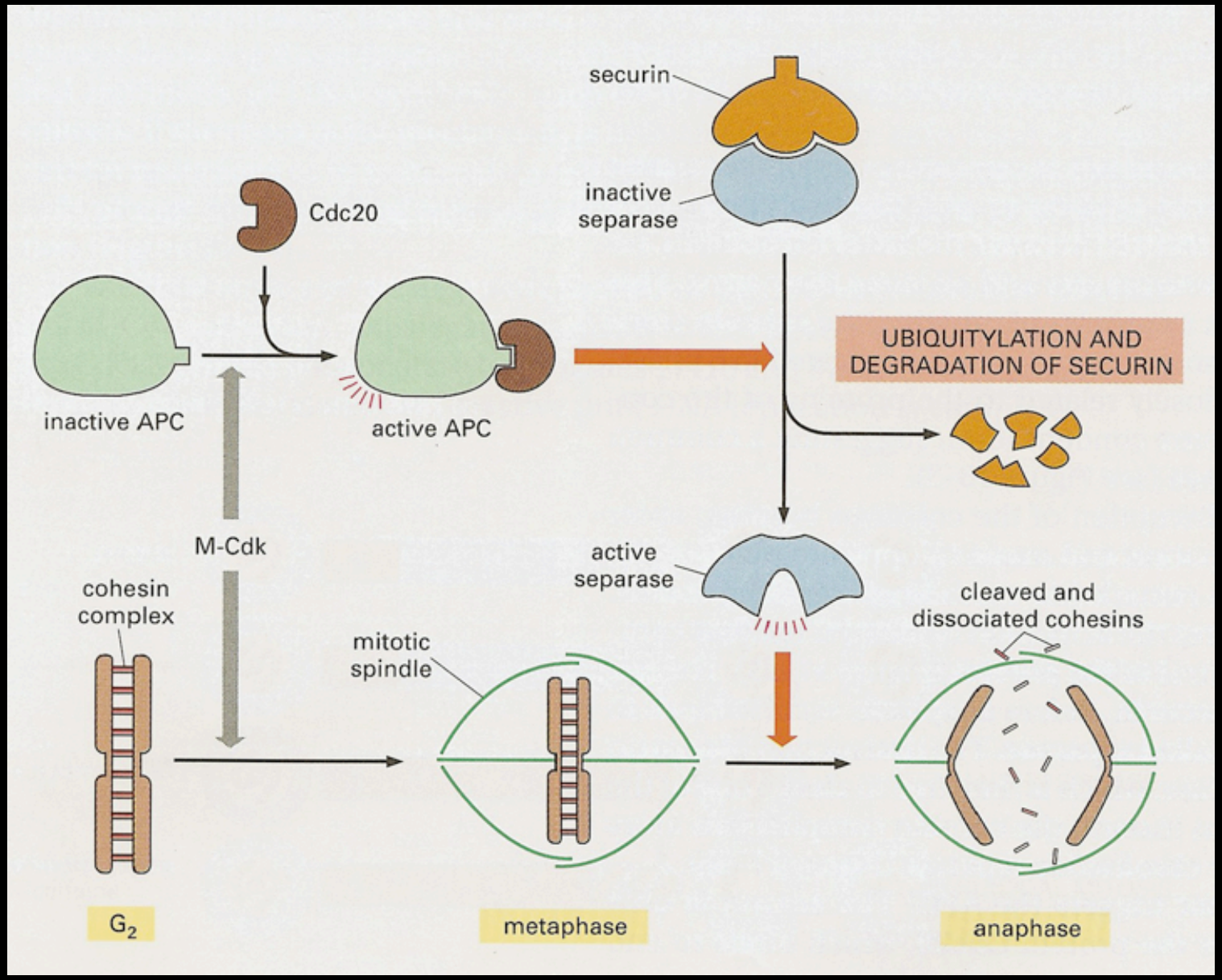
Inhibition of APC and Cyclin degradation result in different phenotypes



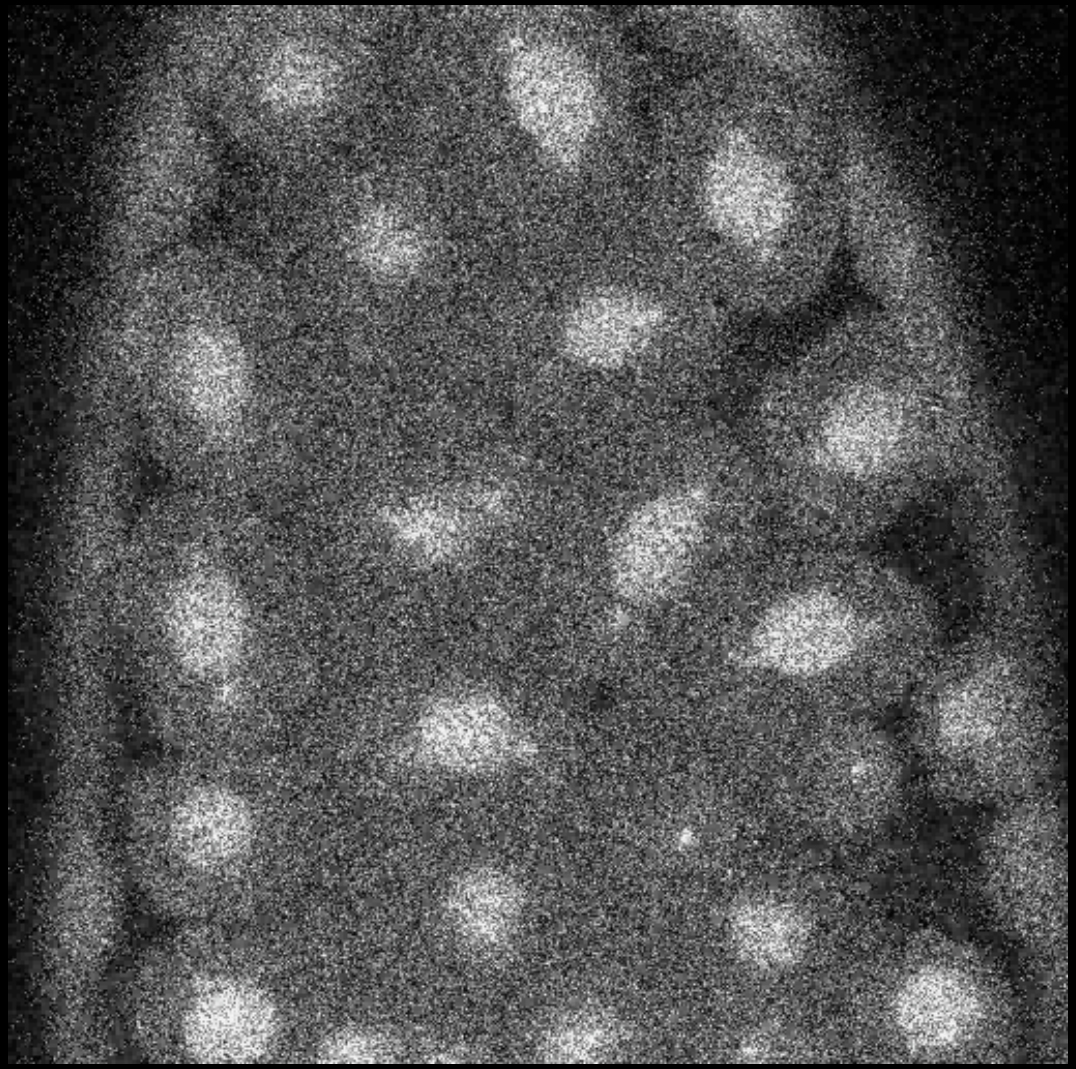
need active APC to enter anaphase

need to degrade CycB to exit mitosis

APC mediated degradation of securin and chromatin separation

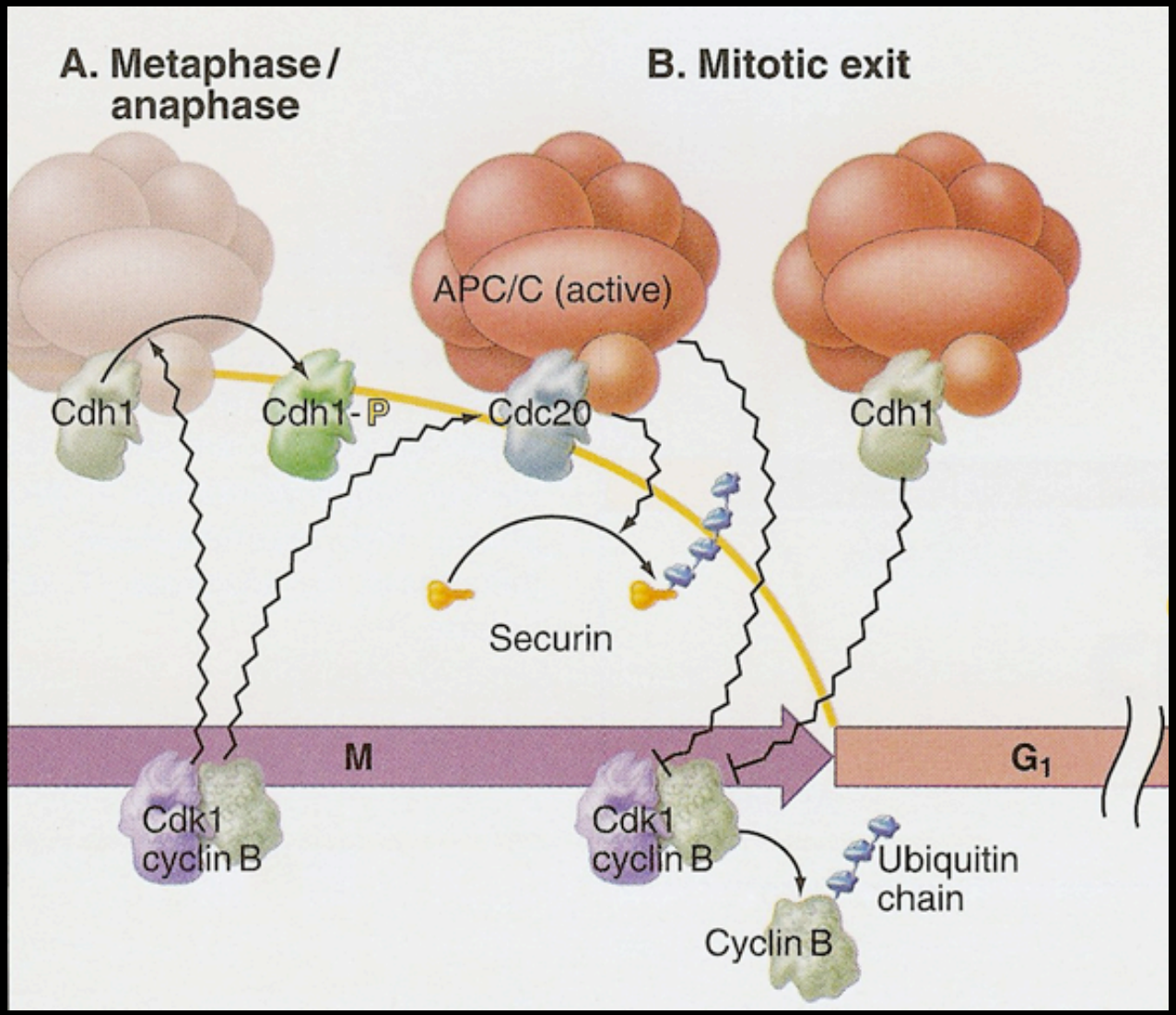


A non-destructible form of Cyclin B delays exit from mitosis

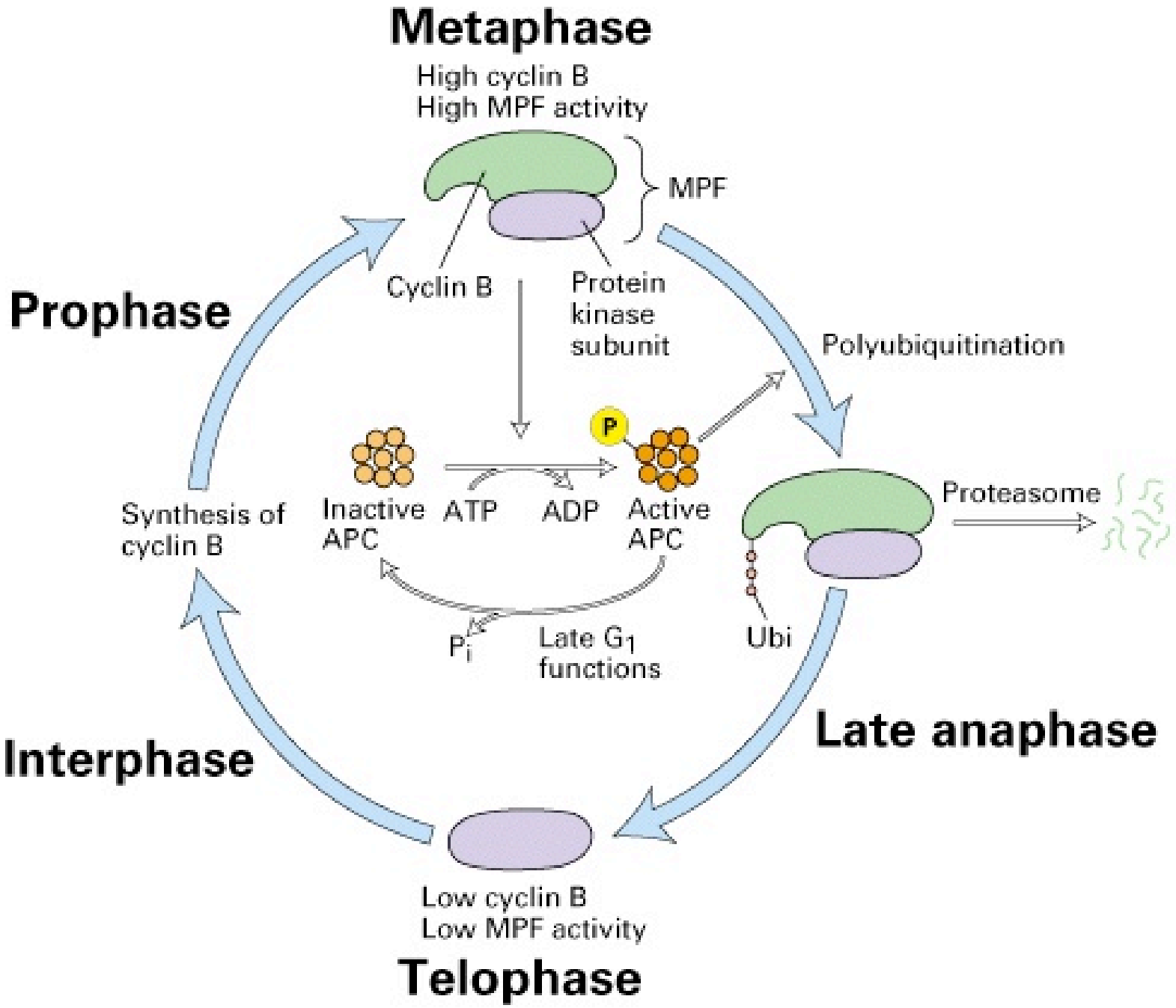


Cyclin B

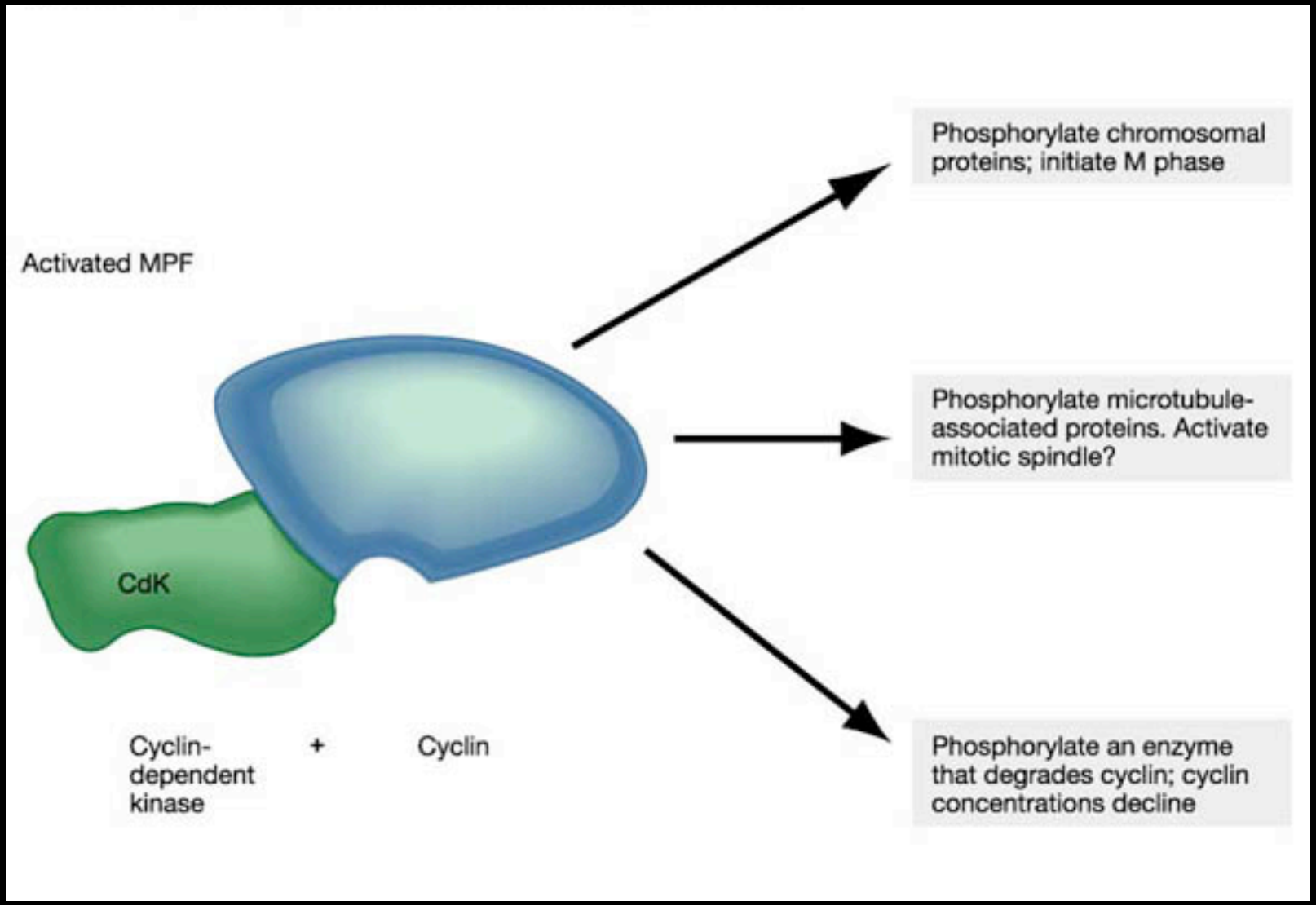
APC is regulated by Cdh1 phosphorylation



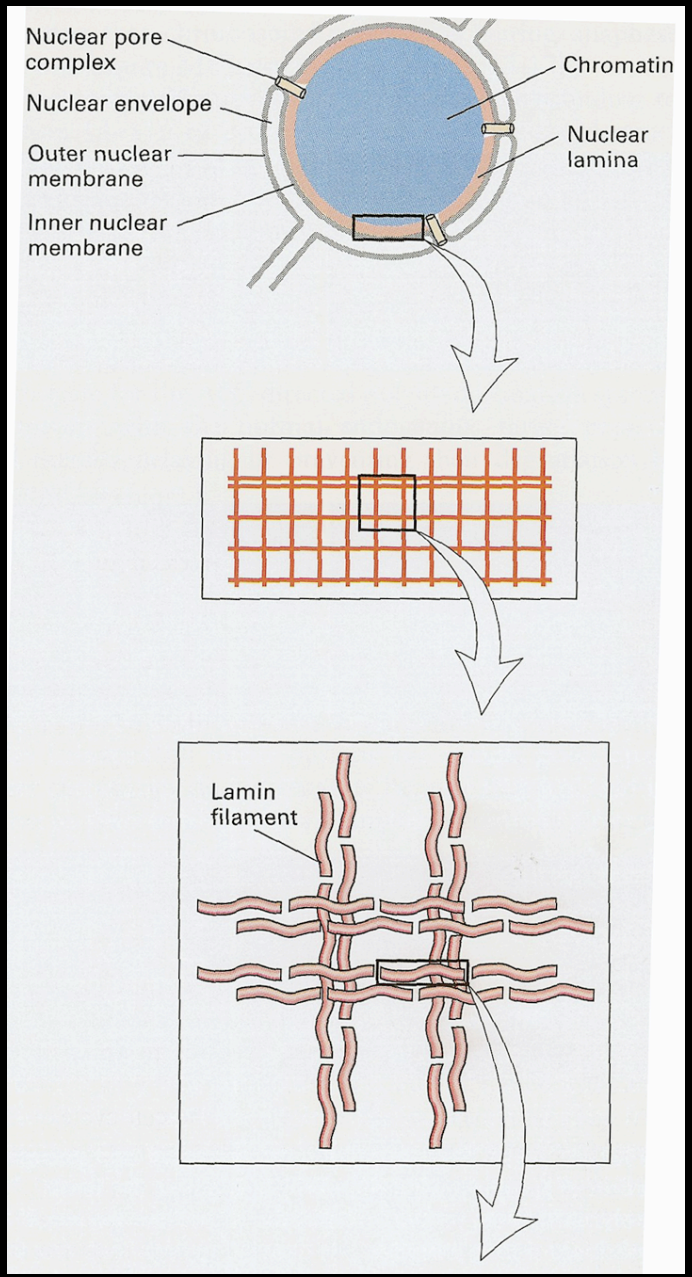
feedback between Cdk/Cyclins and APC

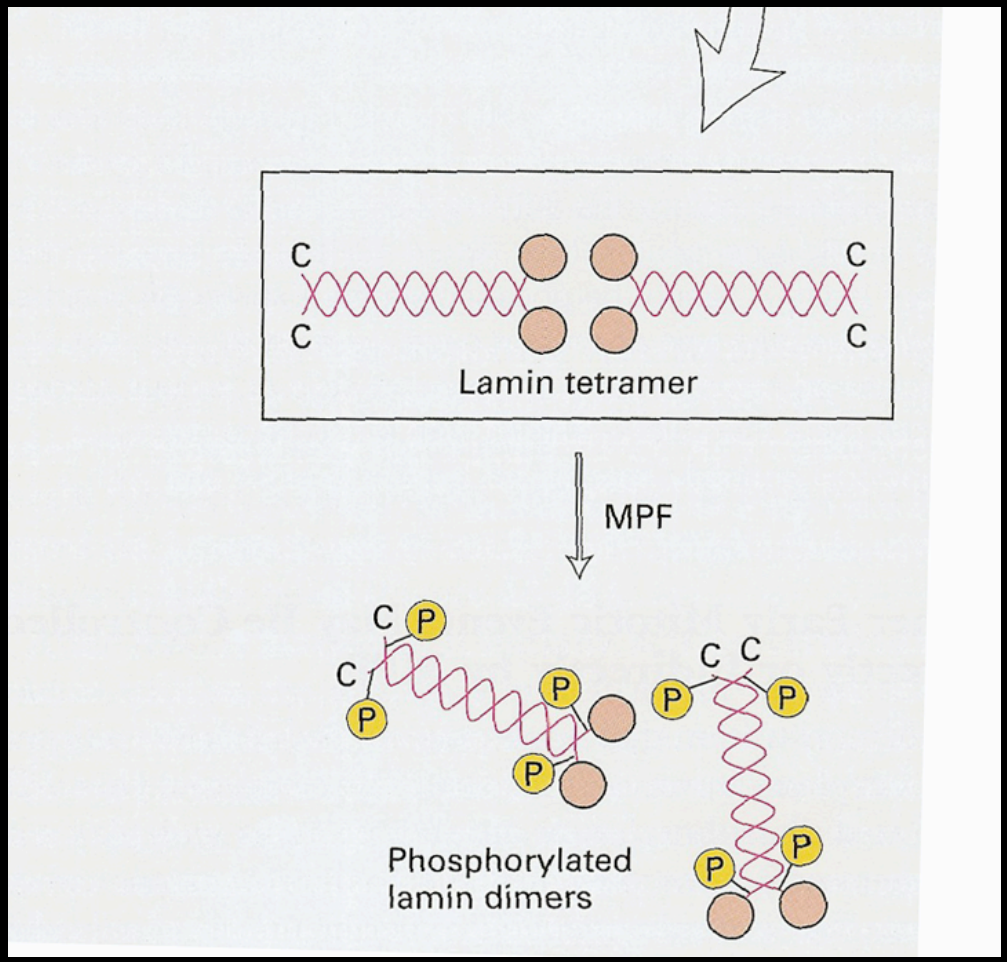

Metaphase
High cyclin B
High MPF activity
MPF
Cyclin B
Protein kinase subunit
Prophase
Polyubiquitination
P
Synthesis of cyclin B
Inactive APC
ATP
ADP
Active APC
Proteasome
P_i
Late G_1 functions
Ubi
Late anaphase
Interphase
Low cyclin B
Low MPF activity
Telophase

CDK functions: Downstream effects of MPF activation



Lamins are phosphorylation prior to NE breakdown





at onset of mitosis

Lamins phosphorylation is required for NE breakdown

IP

Pro

Meta