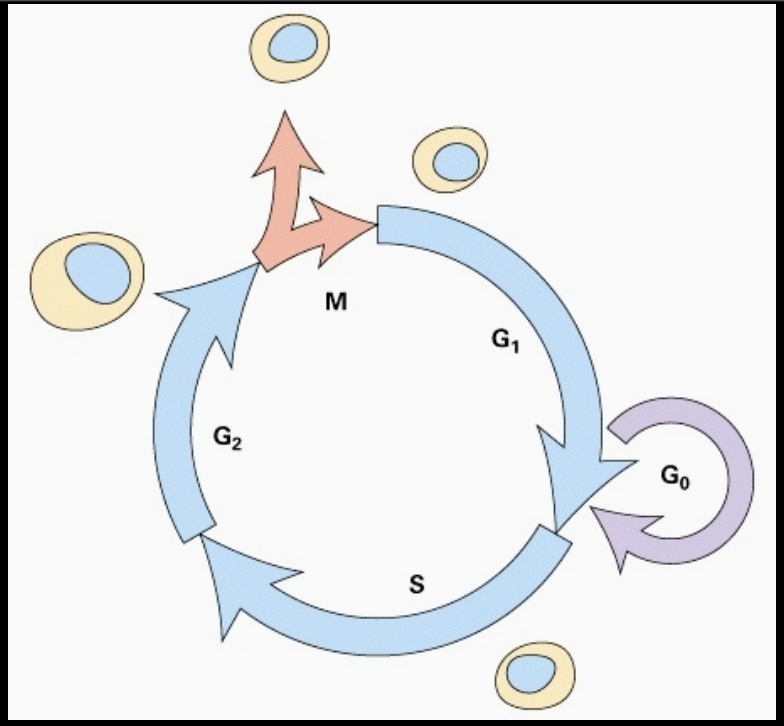
Lecture 10
G1/S Regulation and Cell Cycle Checkpoints

Outline:

- G1/S regulation and growth control
- G2 repair checkpoint
- Spindle assembly or mitotic checkpoint

Paper: The roles of Fzy/Cdc20 and Fzr/Cdh1 in regulating the destruction of cyclin B in space and time



G1/S is the major control point:

in budding yeast

in mammalian tissue culture cells

regulated by:

cell growth

nutrient availability

signals from other cells

Budding Yeast G1/S Regulation

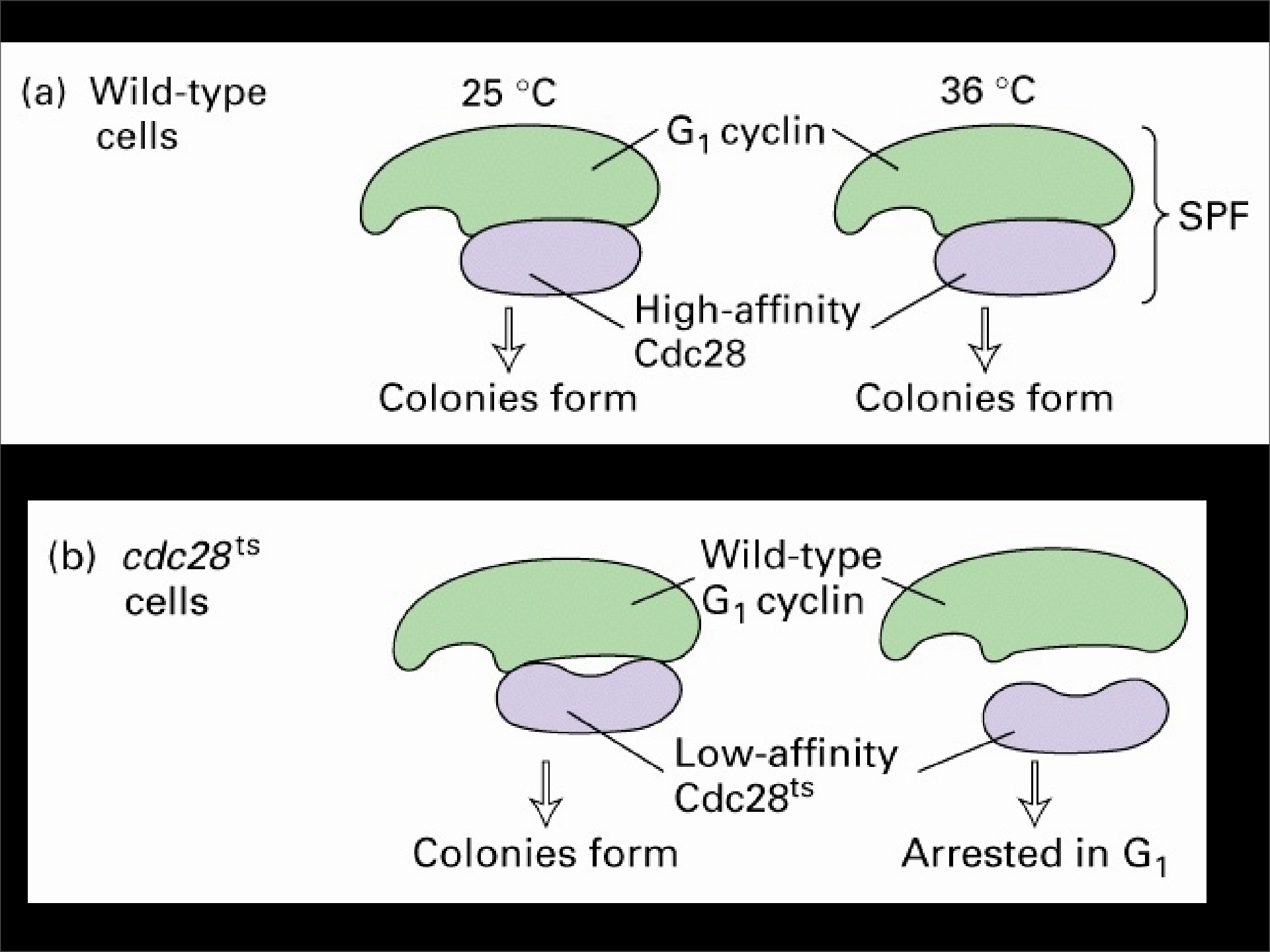
Cdk1 = CDC28 = cdc2

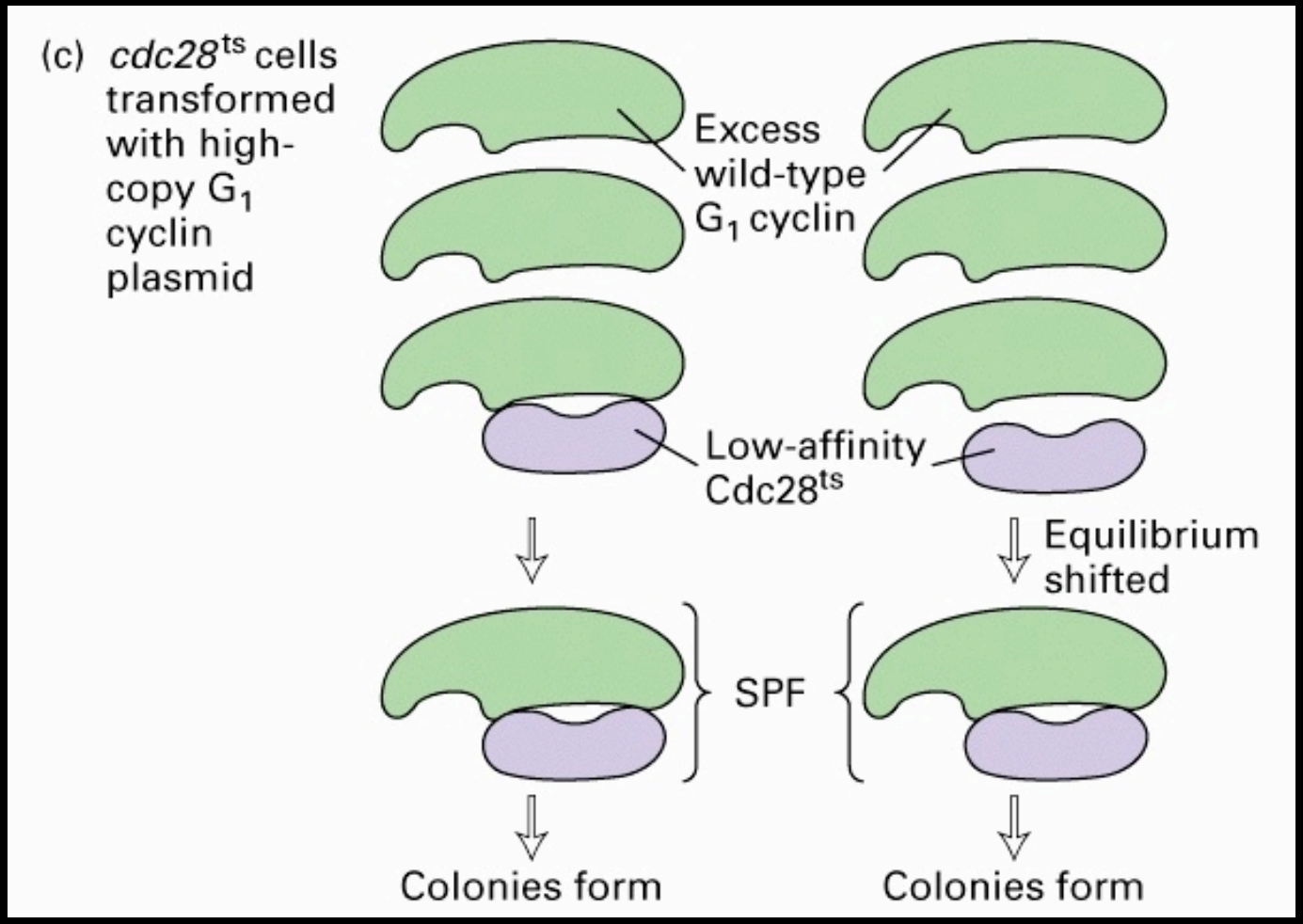
cdc28 mutations - arrest with no bud

analogous to MPF:
SPF = S-phase Promoting Factor
Cdc28/cyclin heterodimer

how to identify cyclin partner?

look for high copy suppressors of $cdc28^{ts}$ mutations


(a) Wild-type cells
25 °C
36 °C
G1 cyclin
SPF
High-affinity Cdc28
Colonies form
Colonies form
(b) cdc28ts cells
Wild-type G1 cyclin
Low-affinity Cdc28ts
Colonies form
Arrested in G1



CLN1, CLN2, CLN3 genes identified

homology to mitotic cyclins (“cyclin box”)

one CLN gene sufficient, triple knockout lethal

Cln necessary and sufficient to induce passage through start

Sic1-CDK inhibitor

Induced by Cdc14 phosphatase during exit from mitosis

inhibits MPF/ -type cyclins, promtes exit from mitosis

Binds and inhibits S-phase Clb-Cdc28 complexes

Clb 5 and 6- S phase cyclins

no effect on G1 Cln-Cdc28 complexes

Sic1 degradation causes S-phase onset

recognized by ubiquitination machinery when phosphorylated by G1 Cln-Cdc28

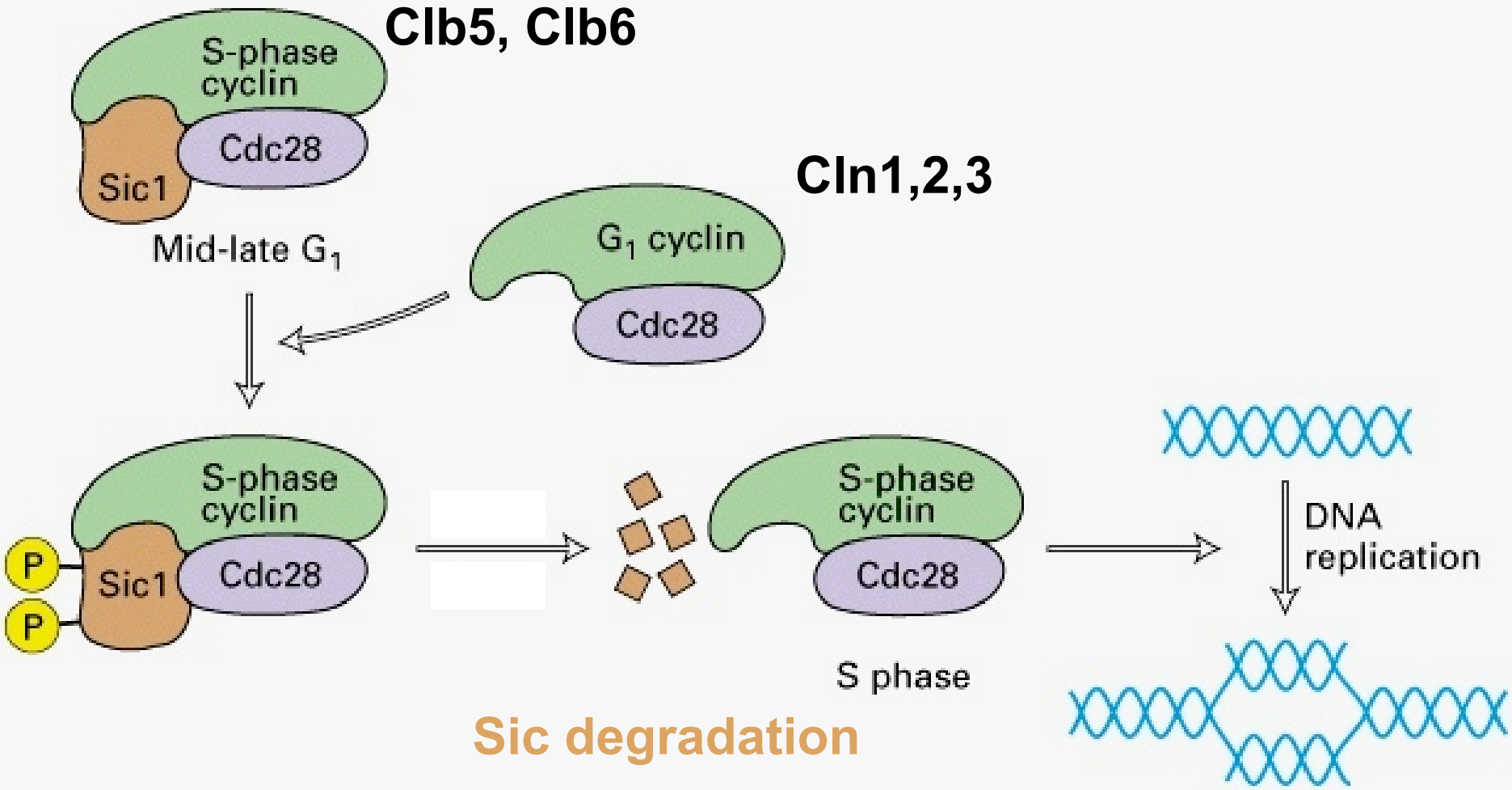
Two Roles for Sic1-CDK inhibitor

promotes one transition:

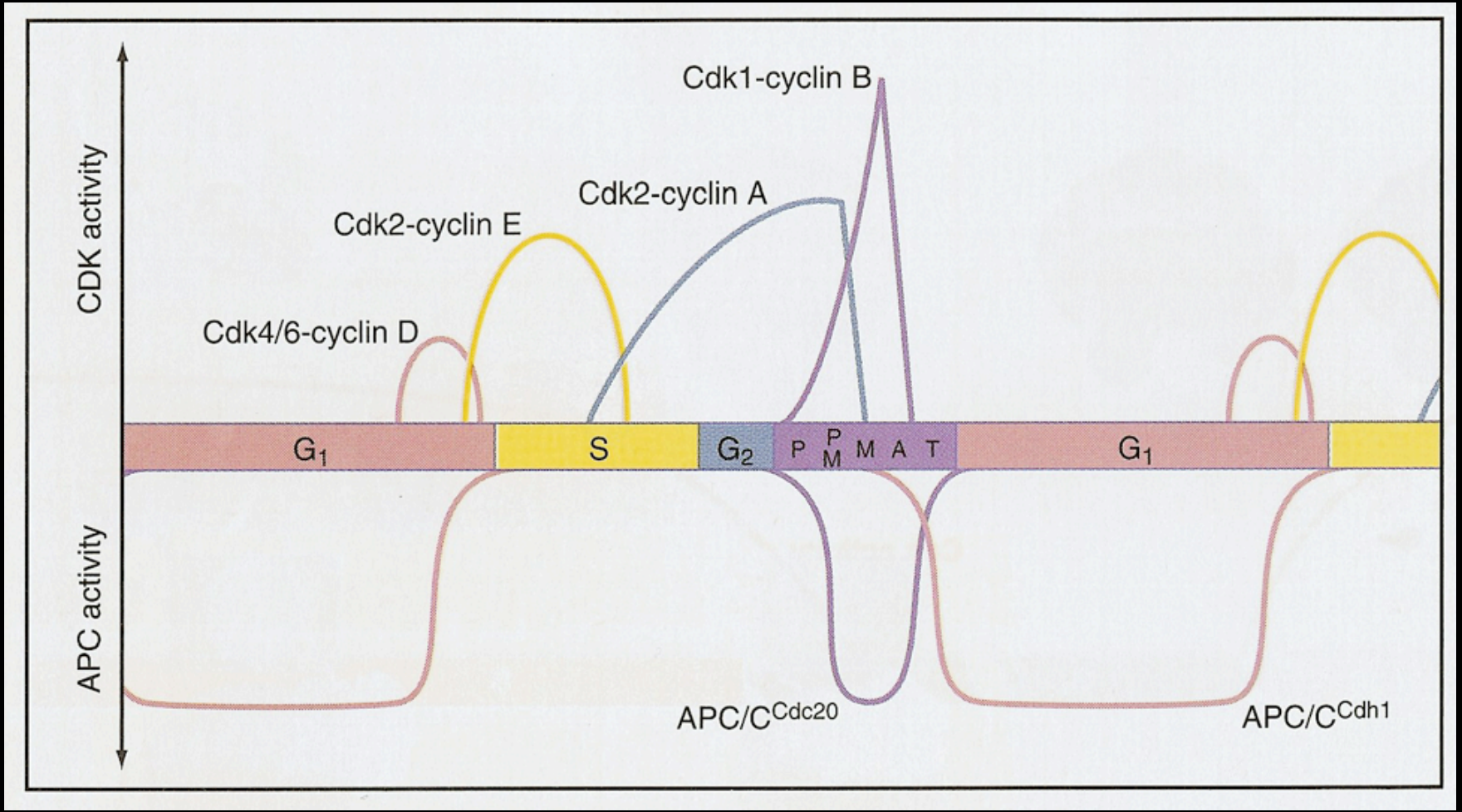
induces exit from M-phase by **inhibiting B-type cyclins**

inhibits another transition:

provides **barrier to S-phase** that must be overcome

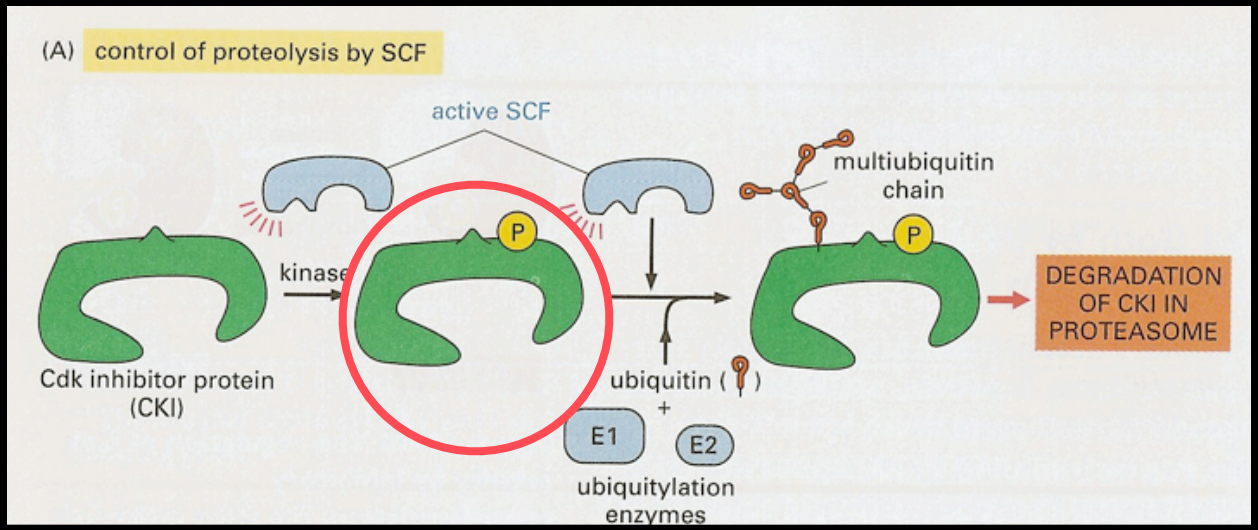


Something other than APC must degrade Sic1 in S

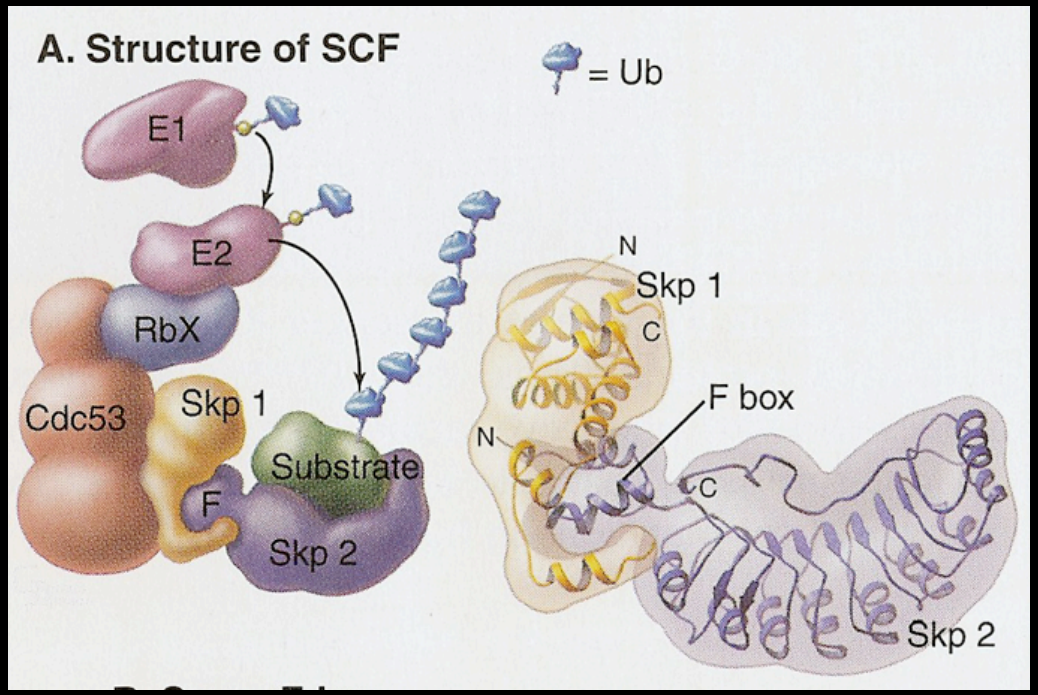


SCF = Skp1-cullen-F-box-protein complex

Ubiquitin E3 ligase-analogous to APC

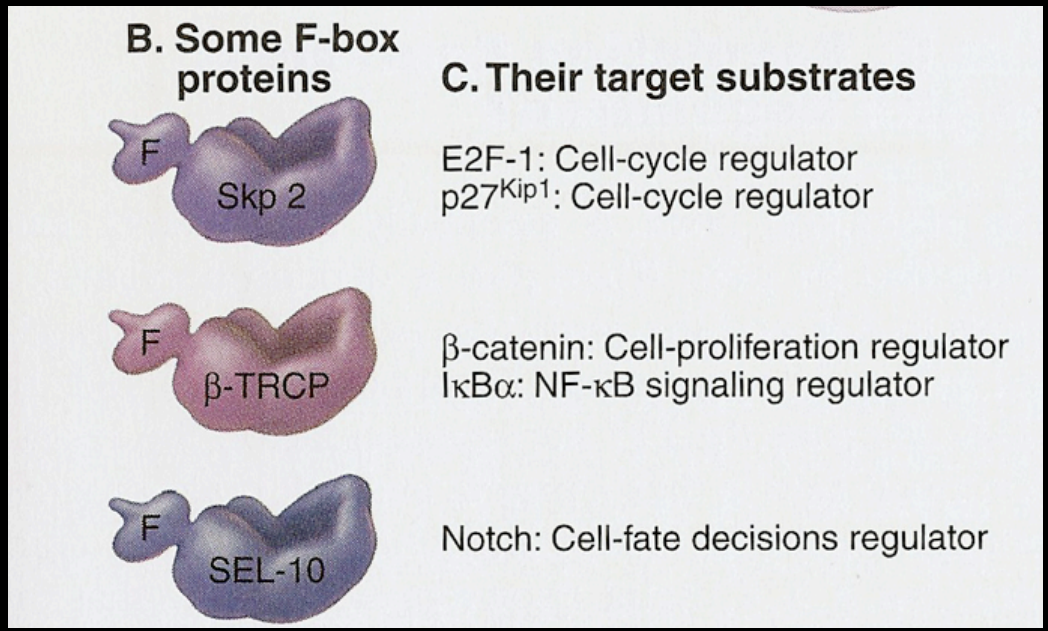


SCF Structure and Targets



Skp2 provides specificity for substrate

not just for cell cycle regulation, also signal transduction



What generates specificity?

APC
Accessory factors:

Cdc20 kinase?:
Clb3, Clb5, Pds1

Cdh1 kinase?:
Clb1, Clb2, Clb3

SCF
F-box proteins:

Cdc4 kinase:
Sic1

Grr1 kinase:
Cln1, Cln2

model for progression through G1/S

Cdc28-Cln3 induced at proper cell size

phosphorylates and activates

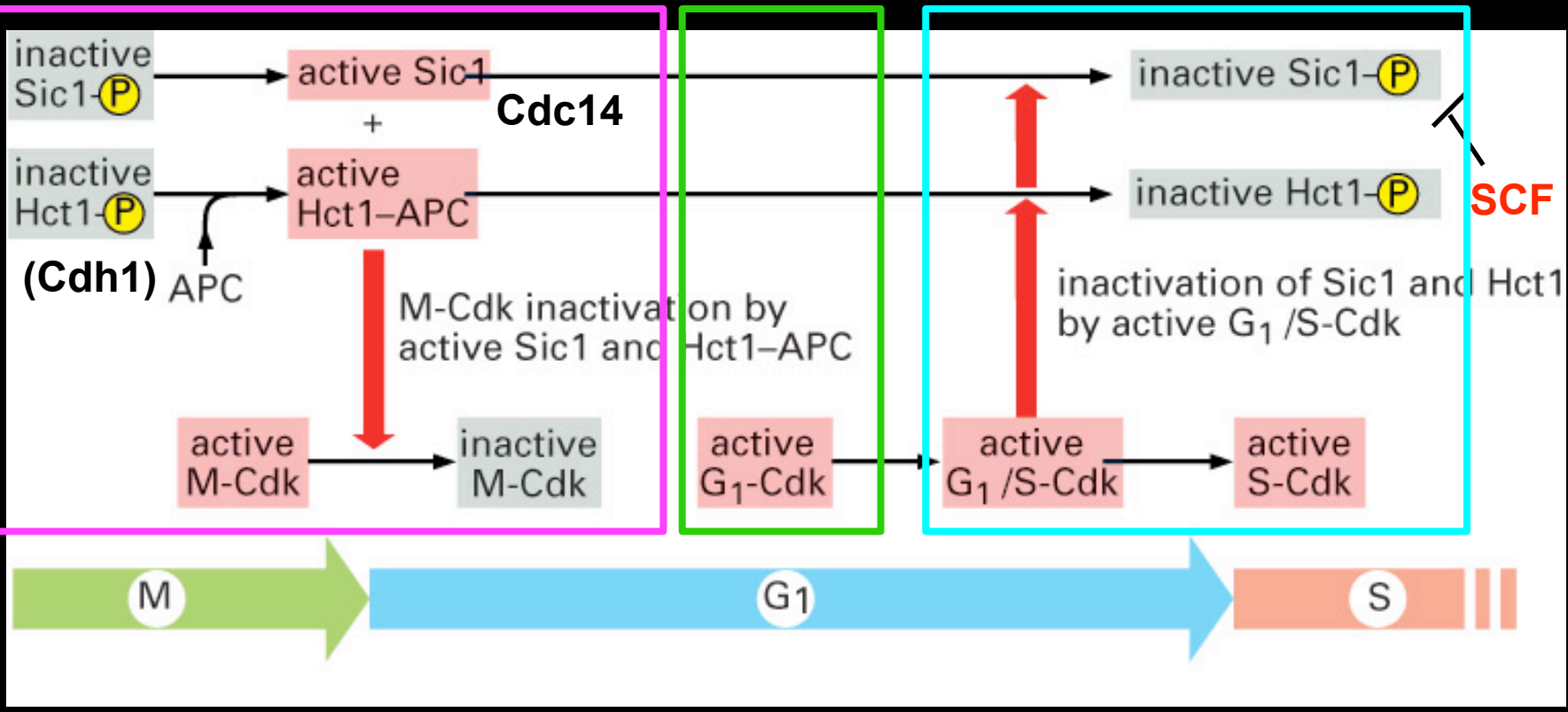
- transcription factors, CLN1 and CLN2, and DNA replication enzymes
- late G1 - CLB5 and CLB6

APC phosphorylated and inactivated

- Clb5 and Clb6 (S-phase cyclins)

Cdc28-Clb5, Cdc28-Clb6 immediately inactivated by Sic 1

mitotic exit and G1 progression in budding yeast



M-Cdk inactivation by Sic1 and APC

G1-Cdk activity increases - not susceptible to Sic1

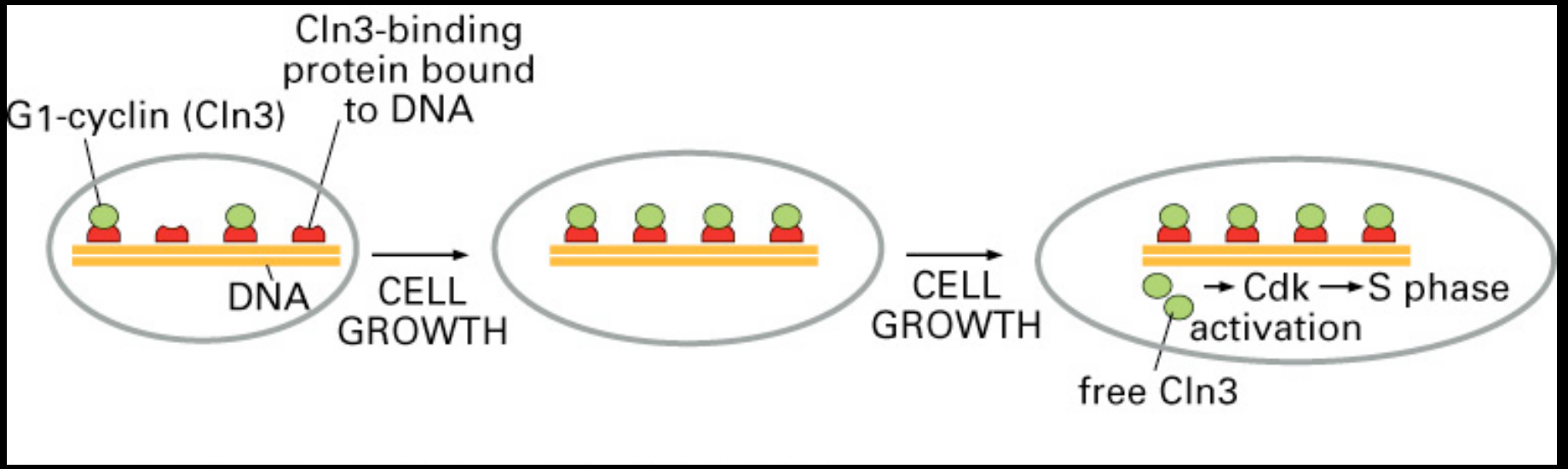
S Cyclin synthesis induced

S-Cdk inactive until phosphorylation of Sic1 and Cdh1 by G1/S-Cdks

Sic1 degraded by SCF

active S-Cdk initiates DNA replication

Possible mechanism to coordinate cell growth and cell cycle progression



Cln3 synthesized in parallel with cell growth
How is threshold level reached?
Cells inherit fixed amount of inhibitor (DNA?)

Cell cycle control in mammalian cells

quiescent cells in G_0 phase

"mitogens" required to stimulate proliferation
= growth factors (in serum)

multiple Cdks

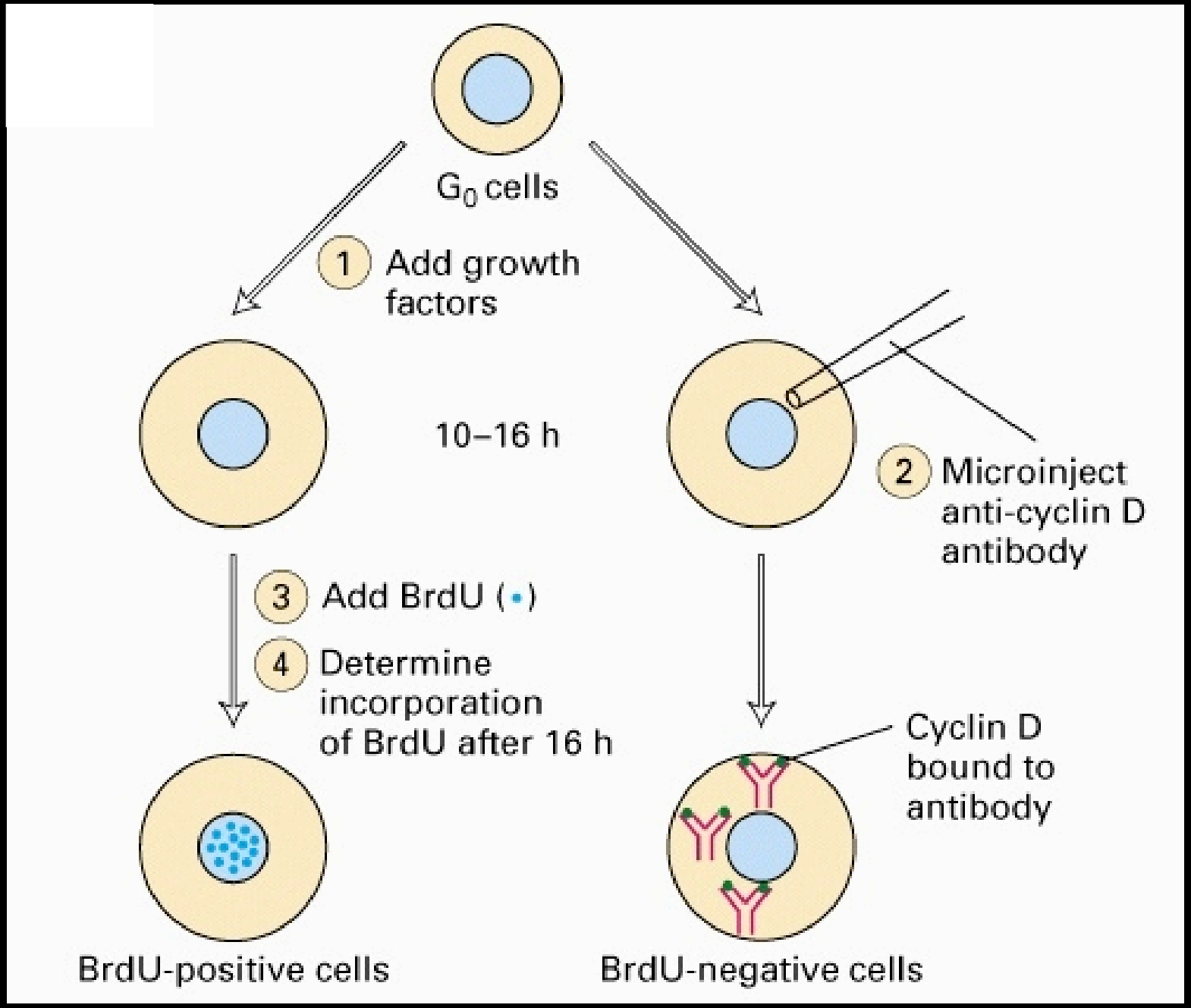
multiple Cyclins

many cloned on the basis of their ability to complement yeast mutations

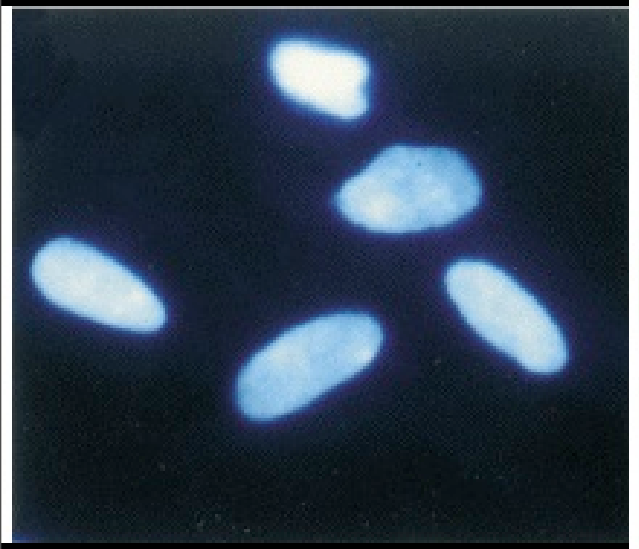
Phase	Cdk	Cyclin
G1	Cdk4, Cdk6	D Cyclins
G1-S	Cdk2	Cyclin E
S	Cdk2	Cyclin A
G2/M	Cdk1	B Cyclins

G1/S

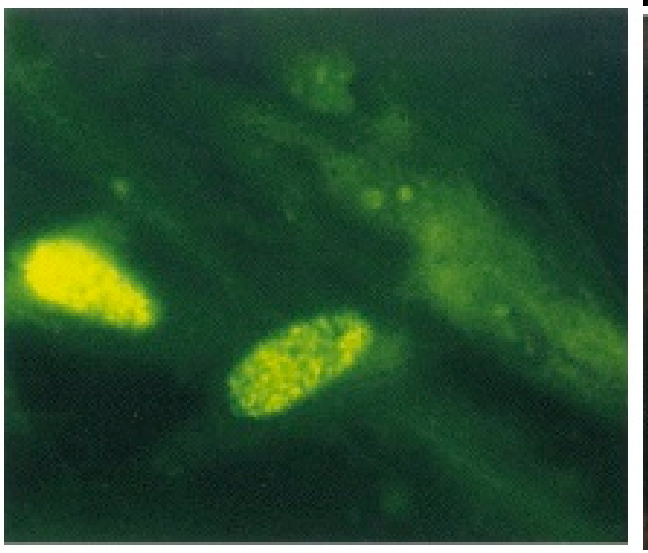
D-type cyclin required



DNA



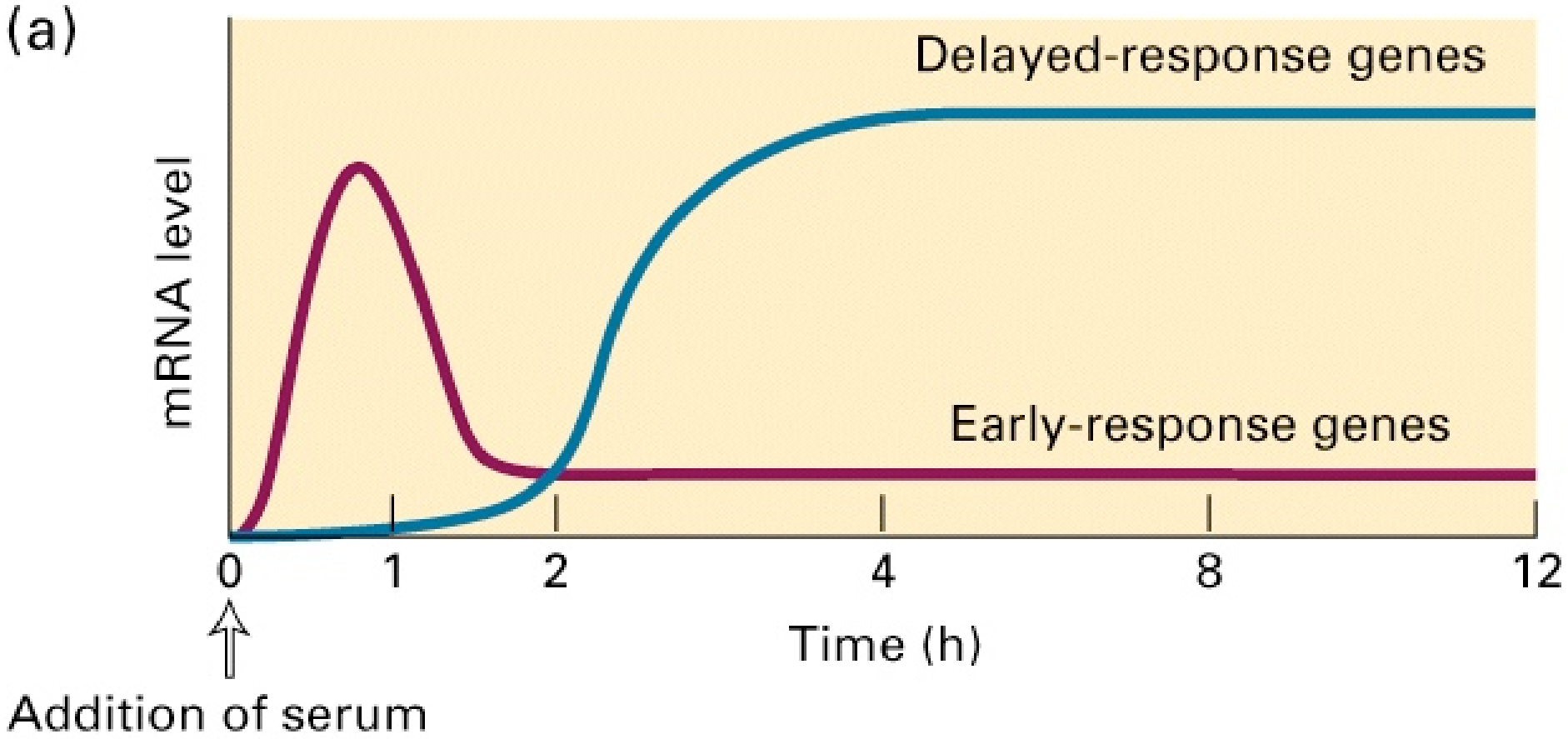
BrdU



Cyclin D antibody

initiation of DNA synthesis blocked by antibody

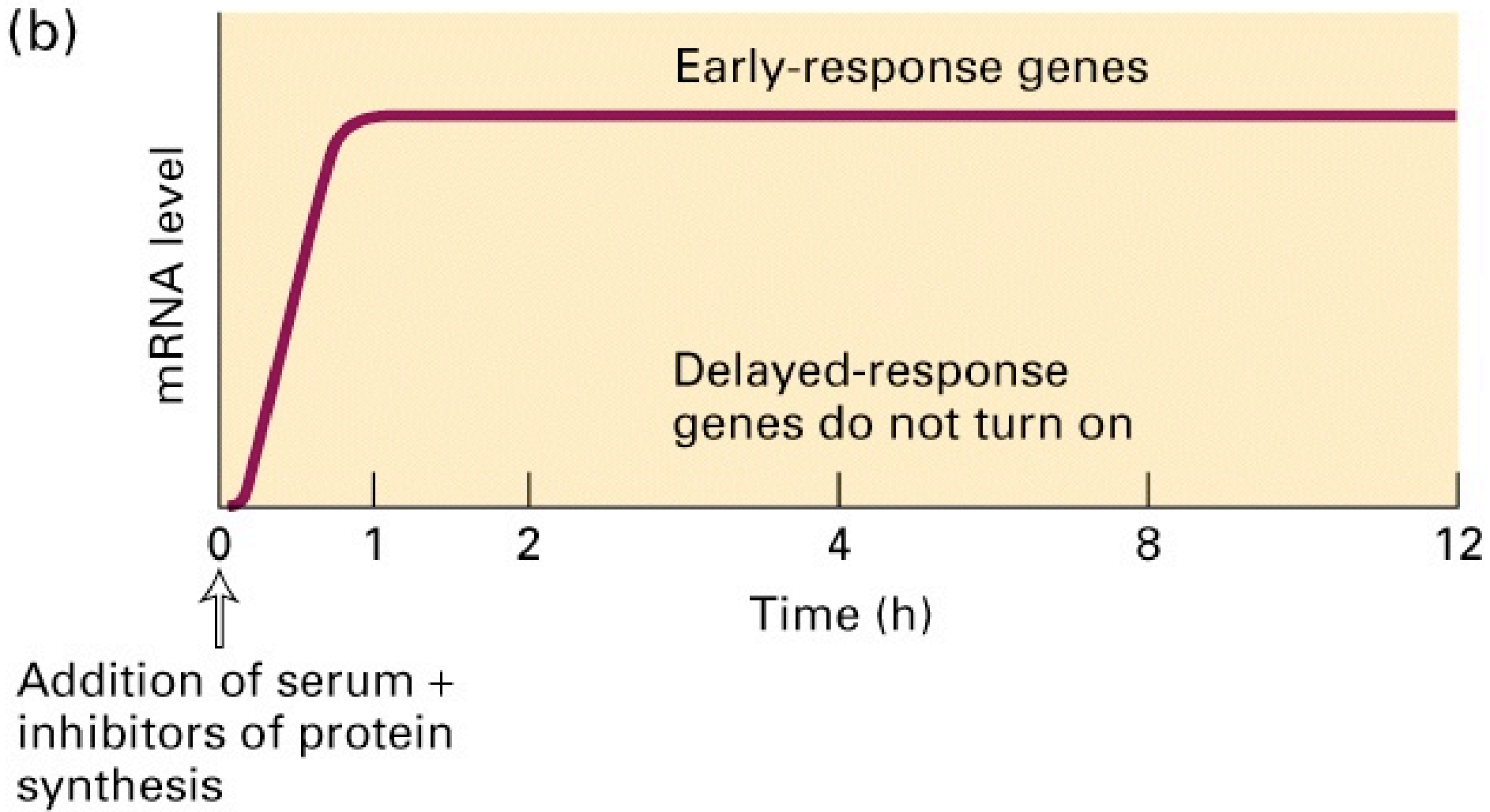
What happens downstream of growth factors?



early: Fos, Jun

delayed: E2F, D Cyclins, Cyclin E, Cdk2, 4, 6

early genes induce transcription of delayed genes



transcription factors activated post-translationally

Regulation of E2F transcription factors

E2Fs induce:

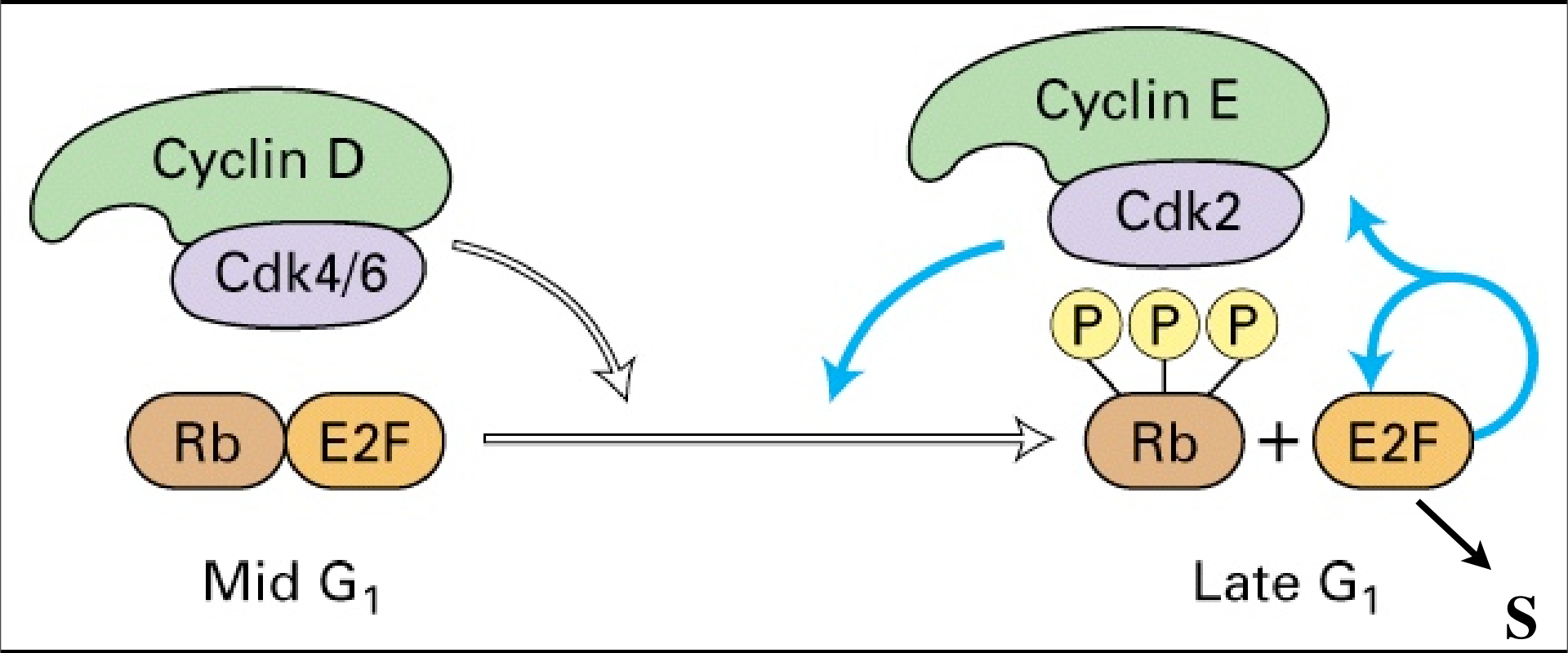
- themselves
- Cdk2
- Cyclin E, Cyclin A

E2F Inhibited by retinoblastoma protein (Rb), p107, p130

Rb inhibited by phosphorylation

- target of Cdk4,6-Cyclin D

E2F and Rb are tumor suppressors



positive feedback loop

Rb phosphorylation maintained until Cyclin B destruction

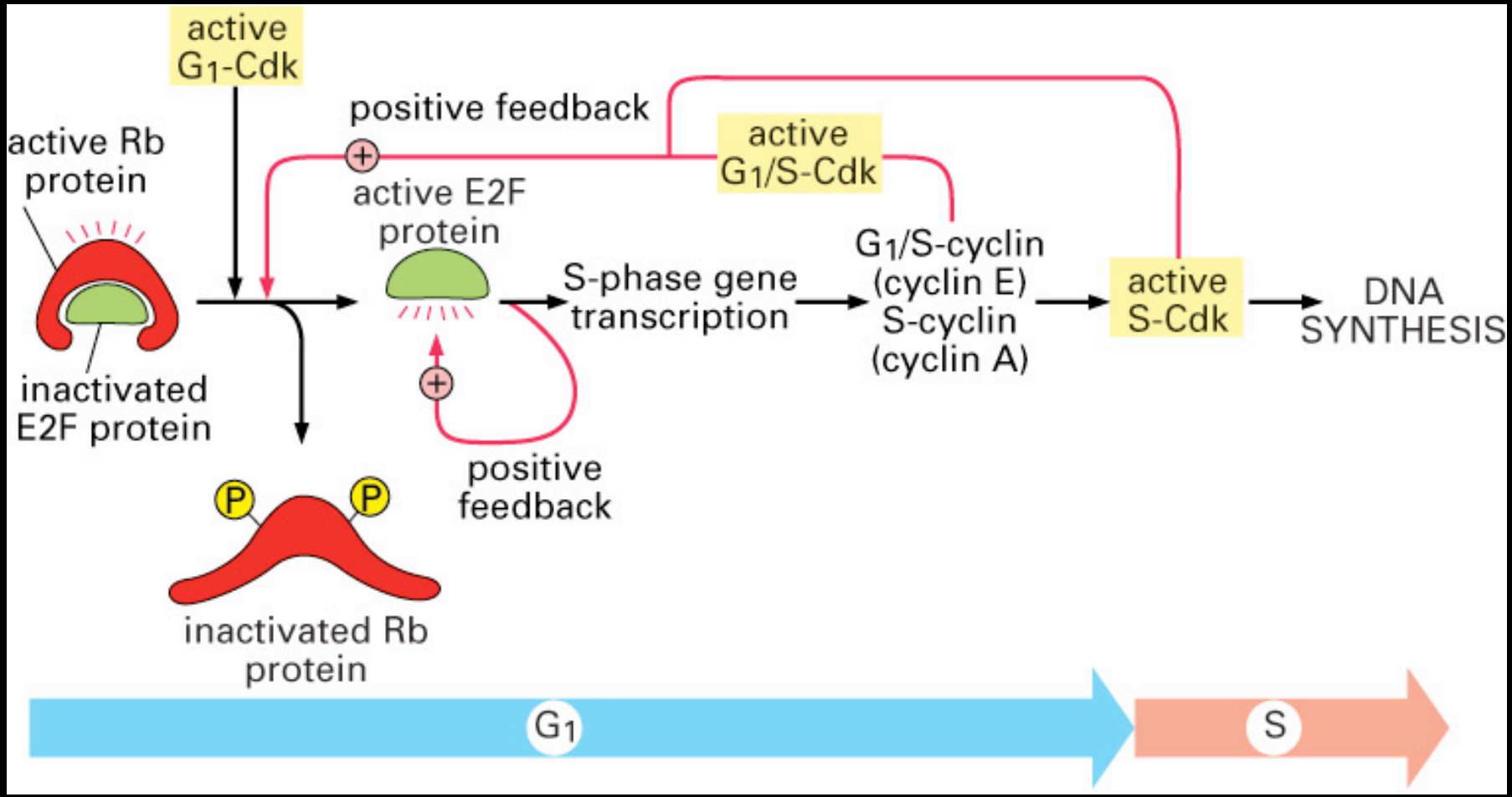
Cdk2-Cyclin A

Required to progress through S phase

Required for Cyclin B accumulation

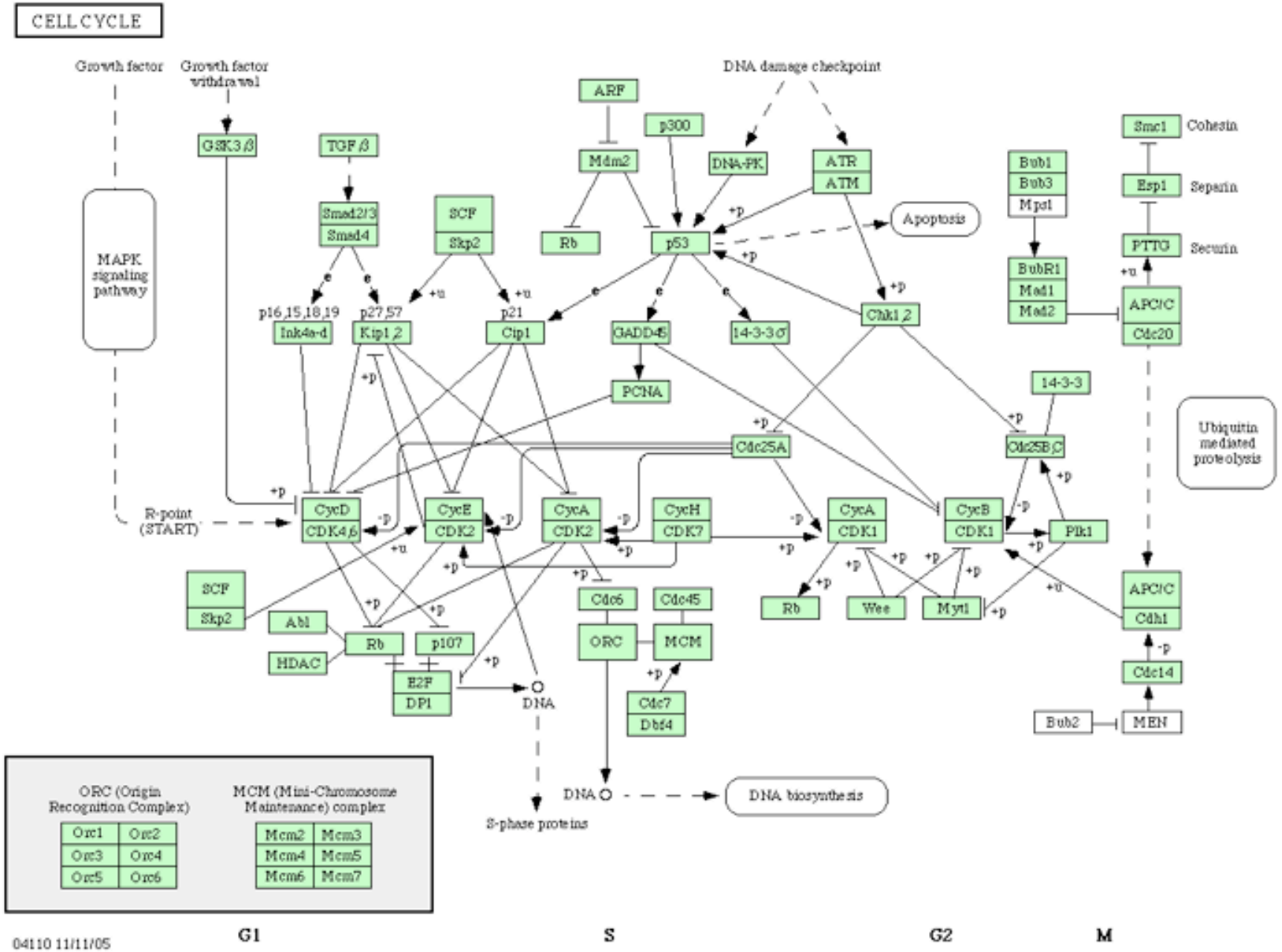
- phosphorylates and inactivates Cdh1
- blocks cyclin B degradation by proteosome

Control of G1 progression in mammalian cells



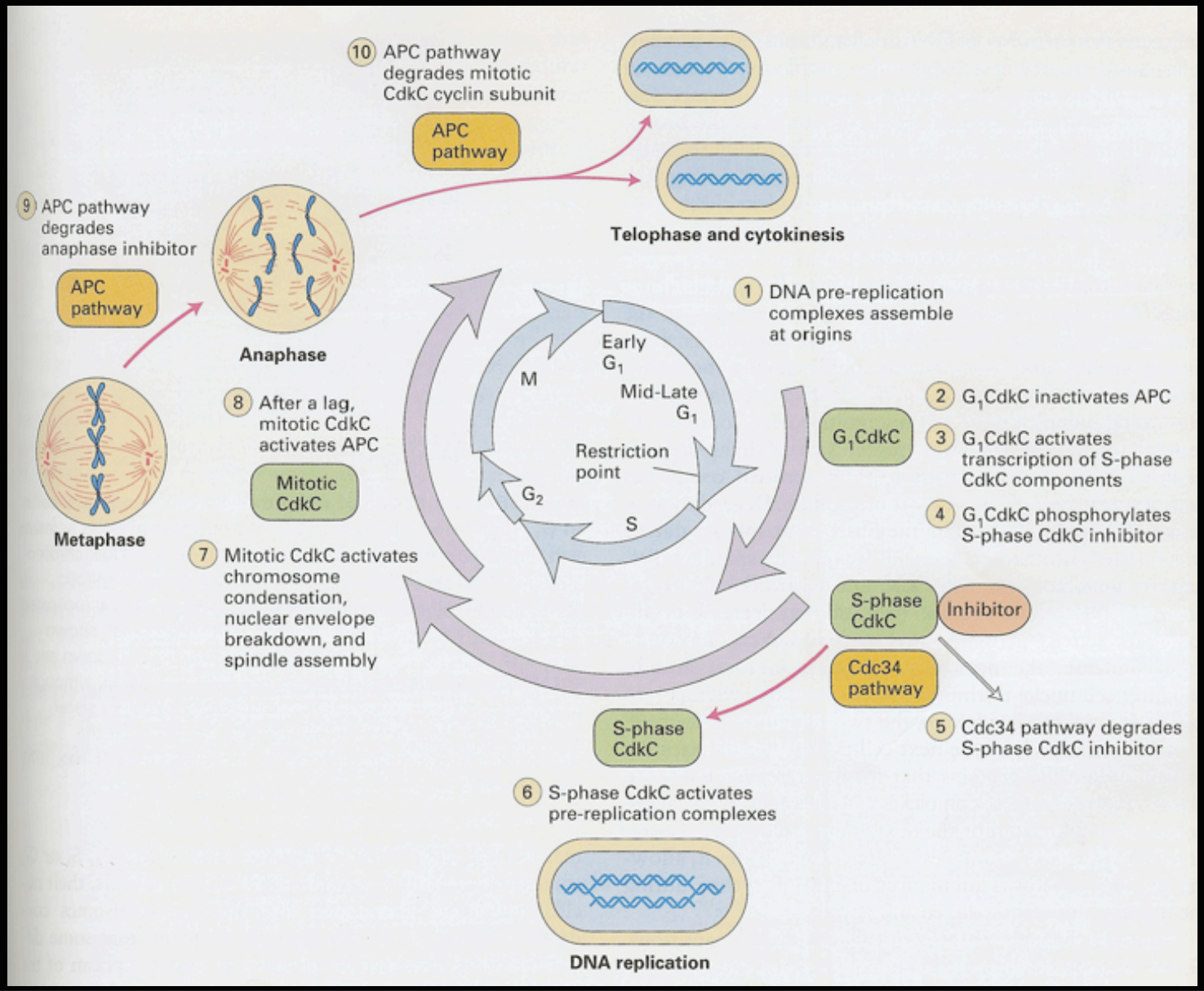
G1-Cdk induced by growth factor, phosphorylates Rb
E2F induces more of itself, and S phase Cyclins (E, A)
S-Cdks further phosphorylate Rb
more S-Cdks accumulate - DNA replication

CC Regulation is VERY Complex

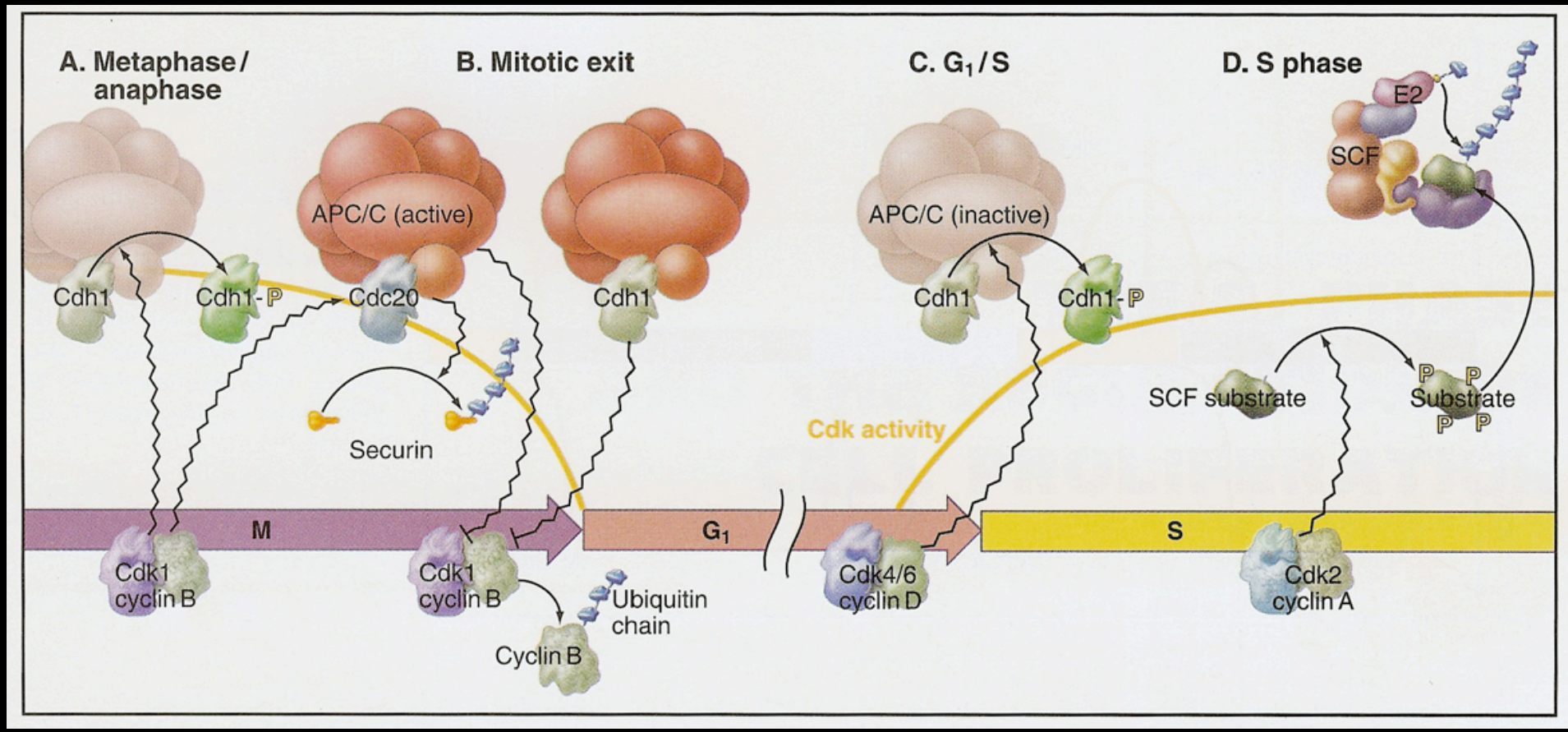


really a bit simpler...

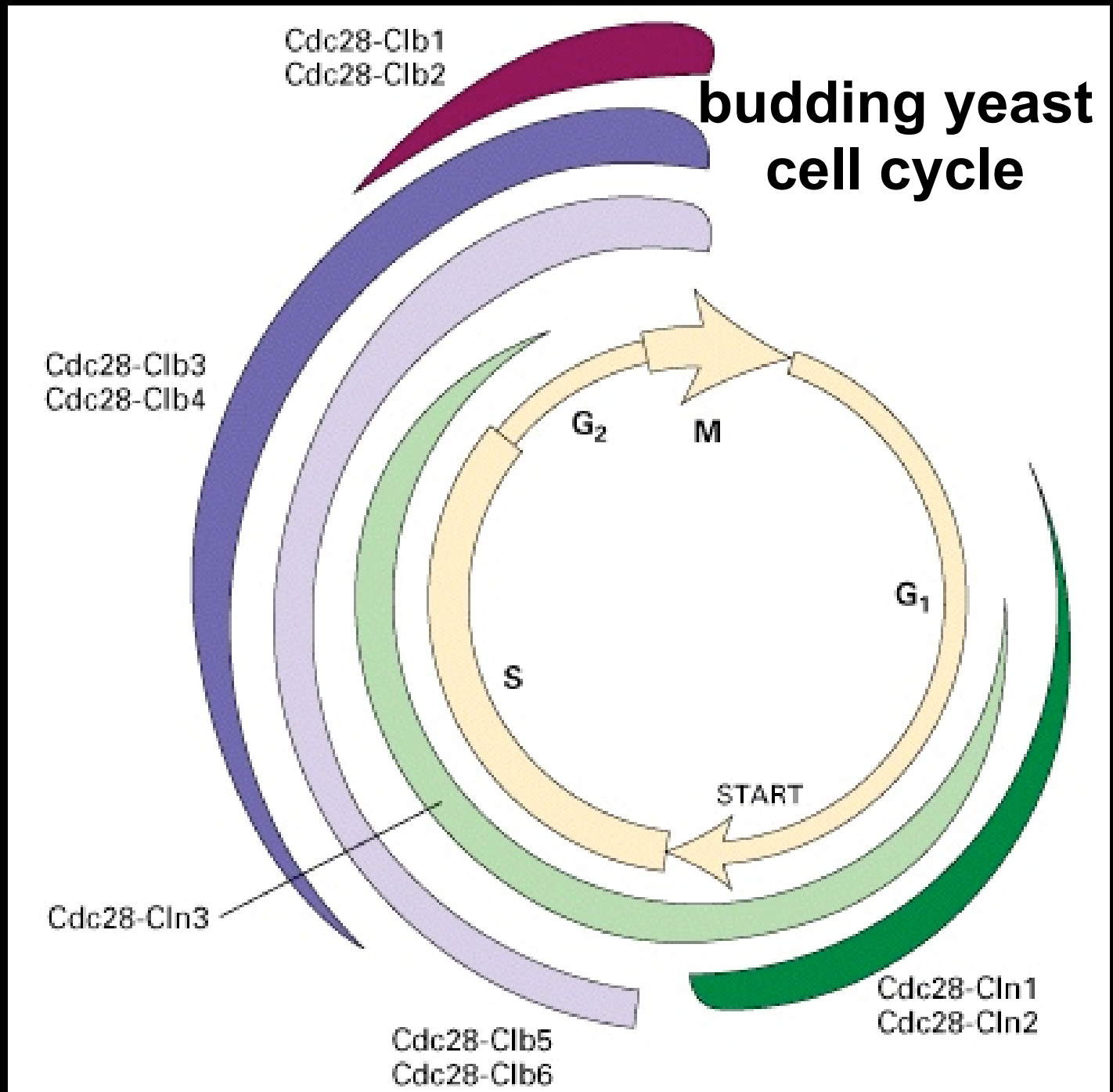
yeast



Cyclins are regulated by proteolysis



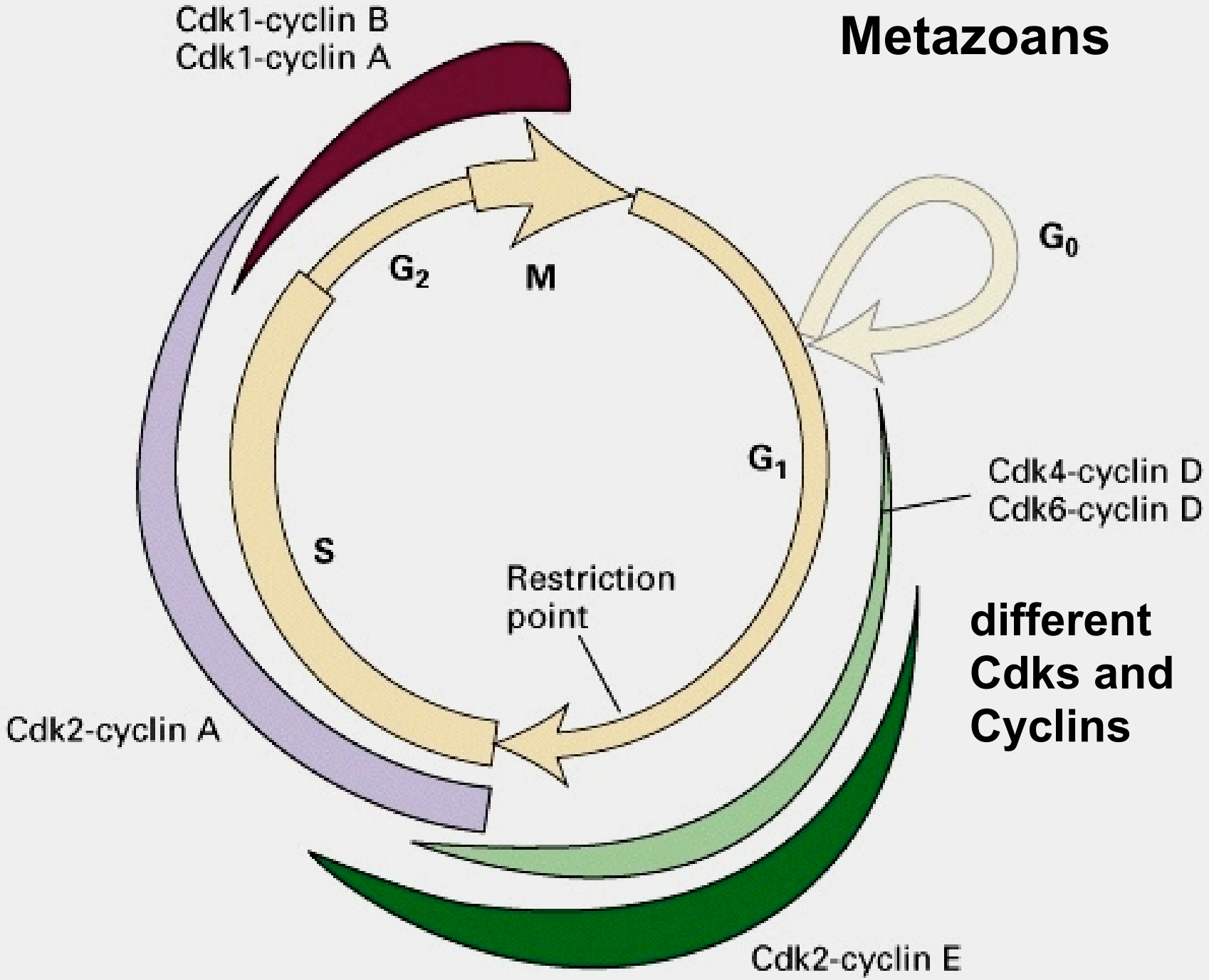
many cyclins,
one CDK



Cyclin Expression in Budding Yeast

Cln3 Cln1, Cln2	G1	Sense cell size Commit to division
Clb5, Clb6 Clb4, Clb3	S	Activate replication origins
Clb1, Clb2	M	Spindle assembly, anaphase

Metazoans



different Cdks and Cyclins

What generates Cdk/cyclin substrate specificity?

how are diverse events induced?

Cdk subunit?

only slight differences in substrate specificity

both Cdk1 and Cdk2 can rescue cdc28 mutations

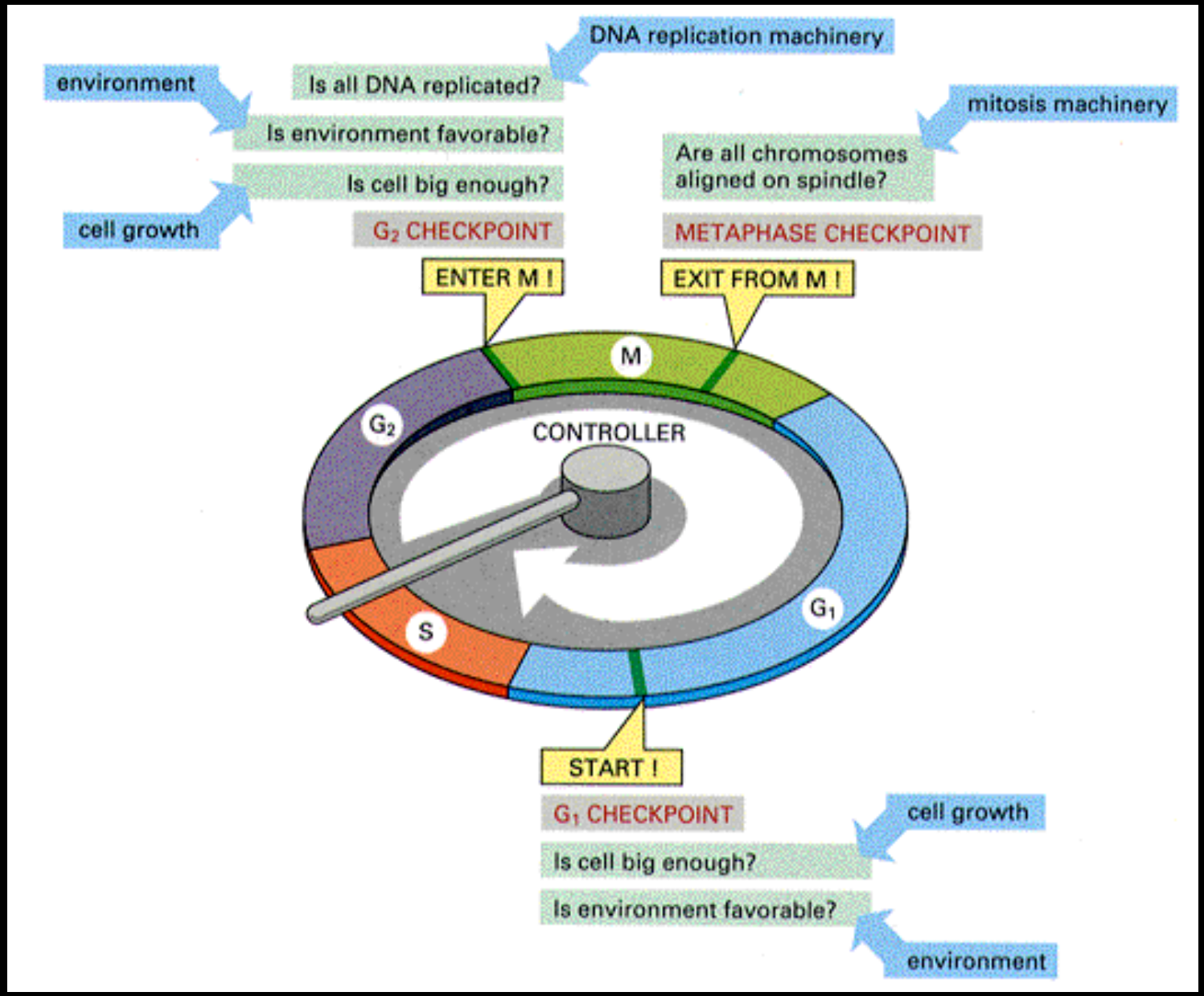
Cyclin subunit?

some mediate interaction with substrates
Cyclin D-Rb

different affinity for inhibitors

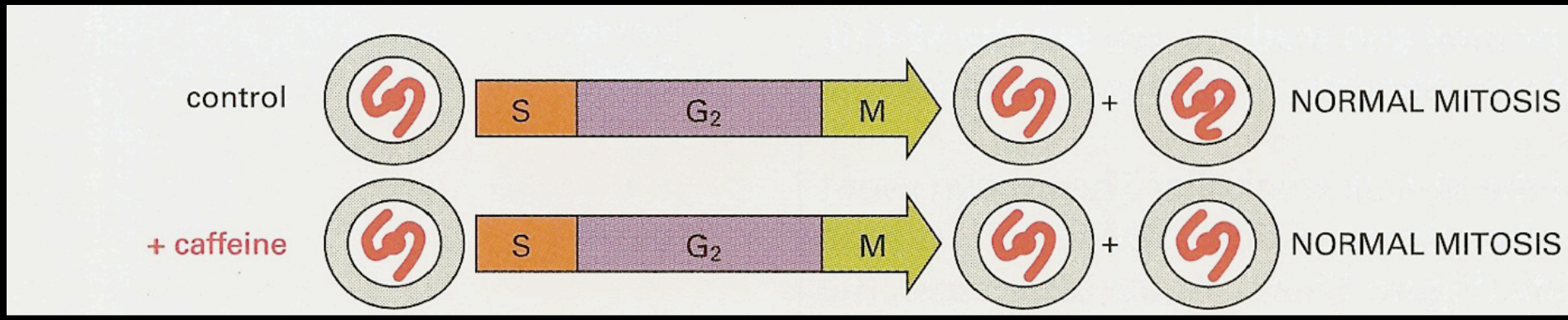
But: a single Cyclin can allow fission yeast to grow normally

Cell Cycle Checkpoints



Early Evidence for a DNA Damage / Defective Replication Checkpoint

tissue culture cells - 1974



DNA damage causes arrest in S

caffeine blocks arrest and allows abnormal entry into mitosis

How to identify genes involved in checkpoint function?

YEAST

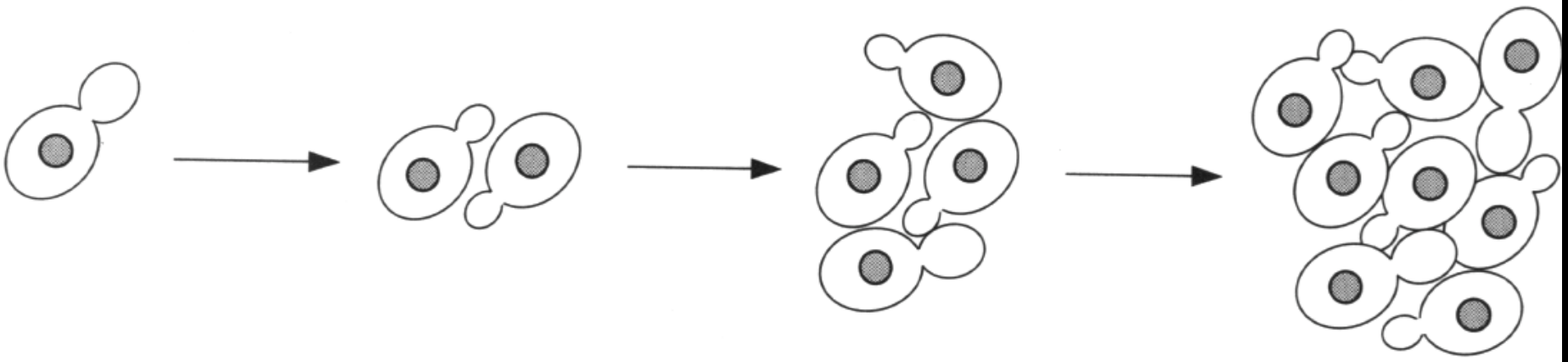
Identify mutants that cannot recover from DNA damage

2 classes:

repair deficient

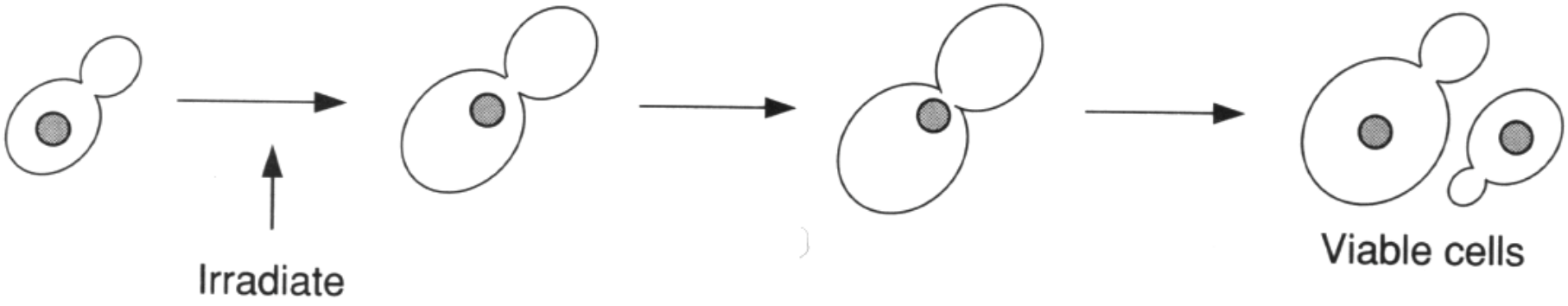
arrest deficient

WILD-TYPE YEAST, NO IRRADIATION



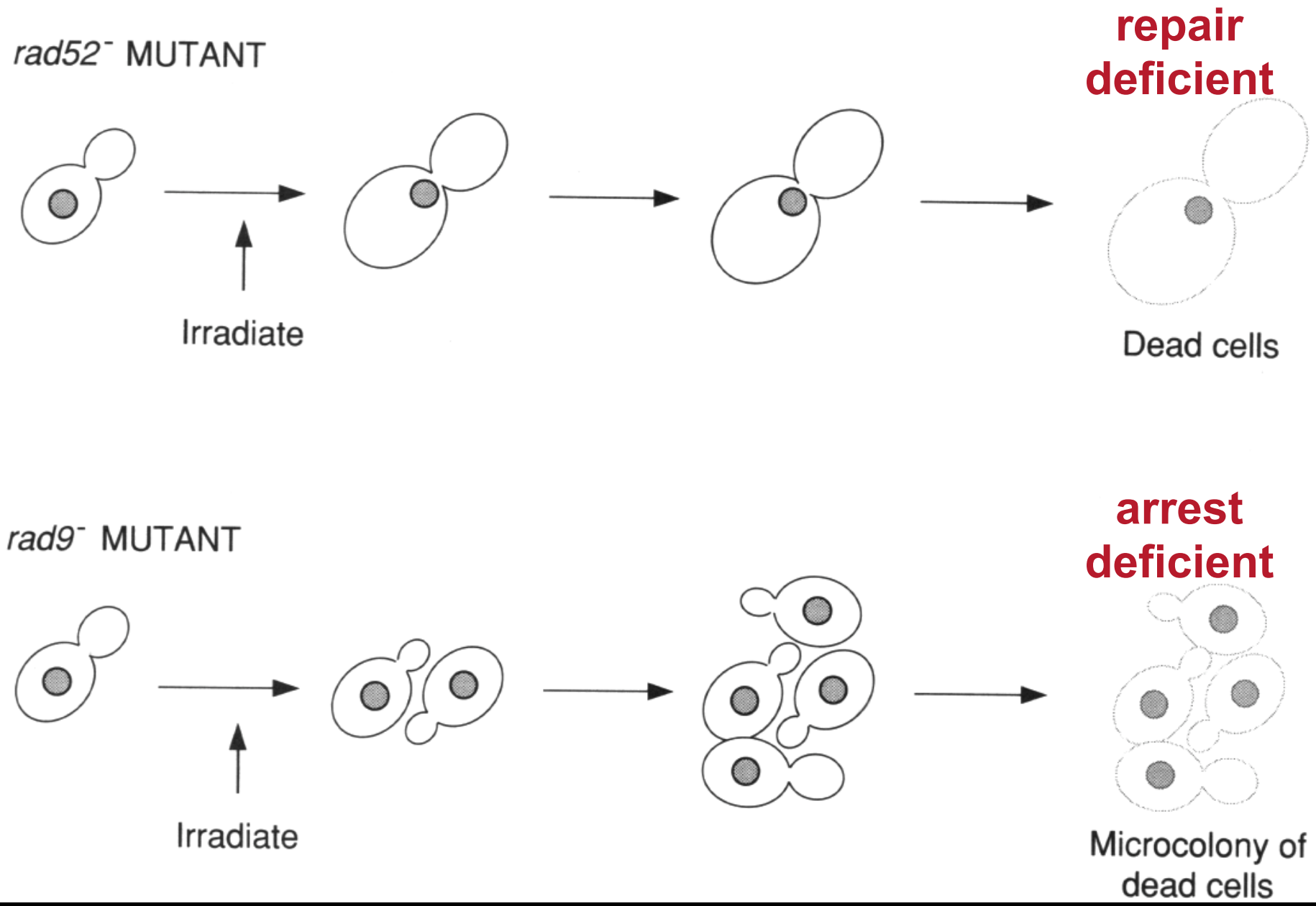
Viable cells

WILD-TYPE YEAST



Irradiate

Viable cells

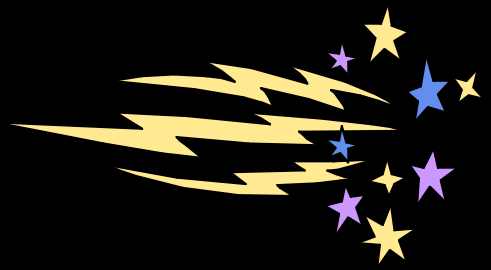

rad52⁻ MUTANT
repair deficient
Irradiate
Dead cells
rad9⁻ MUTANT
arrest deficient
Irradiate
Microcolony of dead cells

Conserved elements of DNA damage and replication checkpoints

I. Sensors	Proteins with functional analogs in DNA replication	recognize damage load onto DNA
II. Transducers: = kinases	ATM and ATR (inhibited by caffeine) Chk1 and Chk2	phosphorylate substrates affecting protein activity or stability
III. Effector output:	cell cycle arrest activate DNA repair maintain arrest until repair complete re-initiate cell cycle progression or APOPTOSIS	

Examples of pathways that block the cell cycle:

DNA damage pathway in budding yeast



ionizing radiation

RAD9

MEC1 (ATR kinase)

CHK1

PDS1

cohesins

anaphase entry

RAD53

CDC5 (polo kinase)

CLB/CDC28

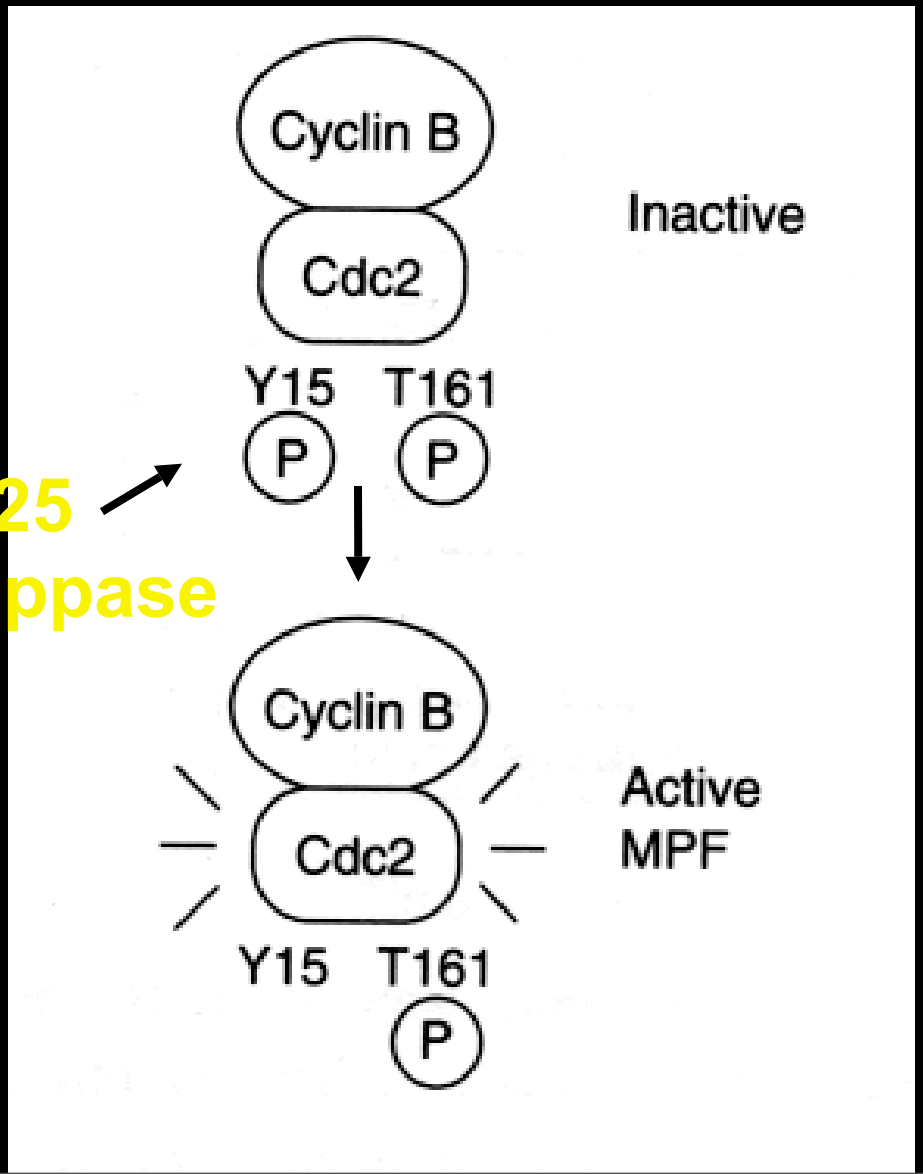
mitosis

G2 or M arrest

Another feedback pathway in fission yeast: DNA damage results in G2 arrest

Two genes identified:
chk1, rad24

Both act through cdc25



Mechanism: Sequester Cdc25 away from Cdc2

DNA damage

↓

Cdc25 phosphorylated by Chk1

↓

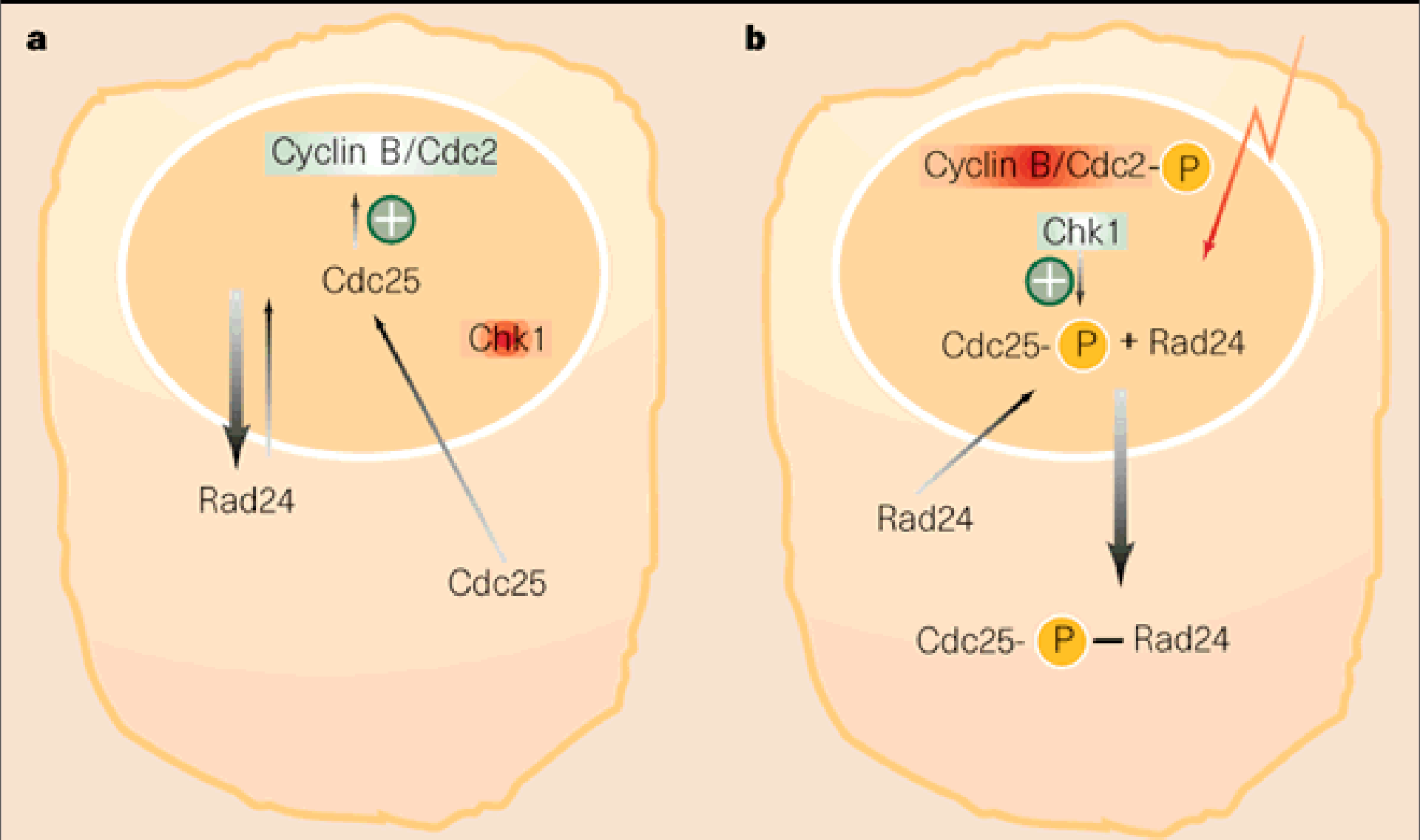
P-Cdc25 recognized by Rad24 (14,3,3 protein with NES)

↓

Rad24 transports P-Cdc25 out of nucleus

↓

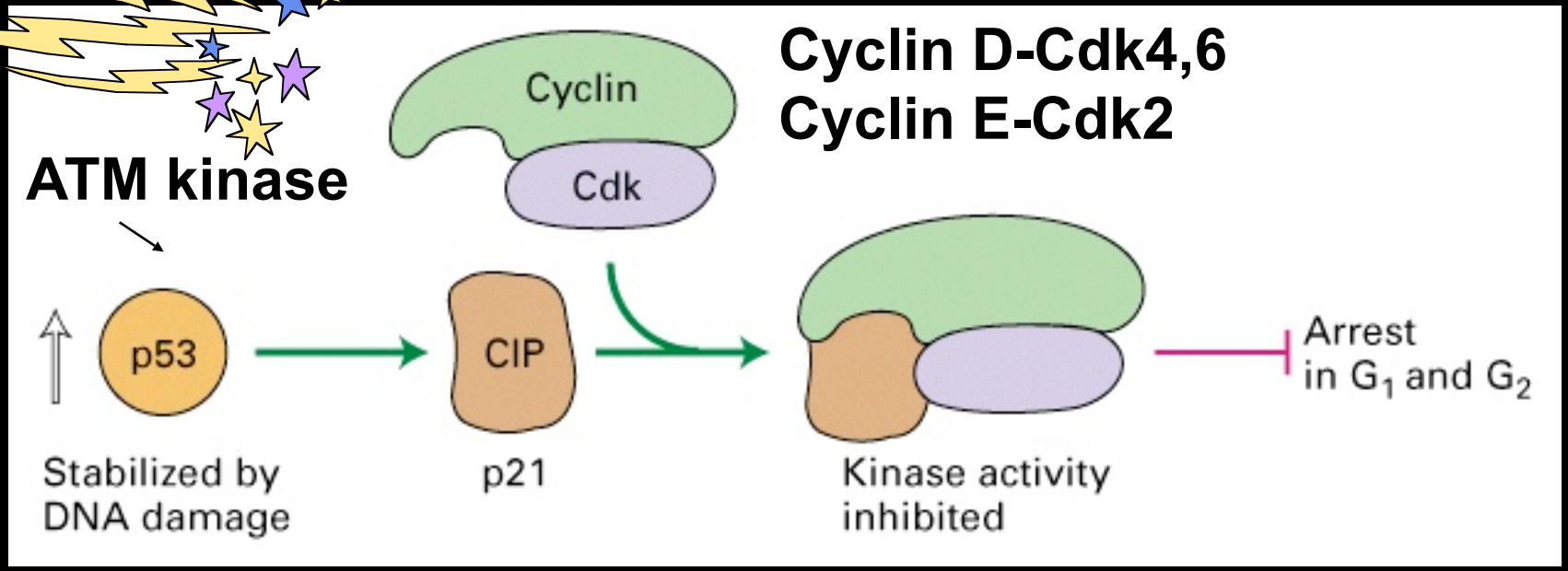
Cdc25 cannot dephosphorylate nuclear Cdc2



translocation has not been confirmed in other organisms

Higher Eukaryotes: G1 arrest mediated by p53

p53 = tumor suppressor/transcription factor: stabilized in damaged cells



induces expression of Cdk inhibitor $p21^{CIP}$

Cyclin E inhibition and Cyclin D degradation by APC

apoptosis

Also a faster response recently identified,independent of p53

Spindle / Mitotic Checkpoints

Kinetochore-mediated

senses when all chromosomes have been attached and properly aligned

regulates sister separation

cross-talk

MTOC-mediated

senses proper spindle position

regulates exit from mitosis

Budding yeast

mutants that fail to arrest in the presence of microtubule depolymerizing drugs

Hoyt lab
bub:
budding uninhibited by benomyl

Bub1, Bub2, Bub3

Murray lab
mad:
mitotic arrest deficient

Mad1, Mad2, Mad3

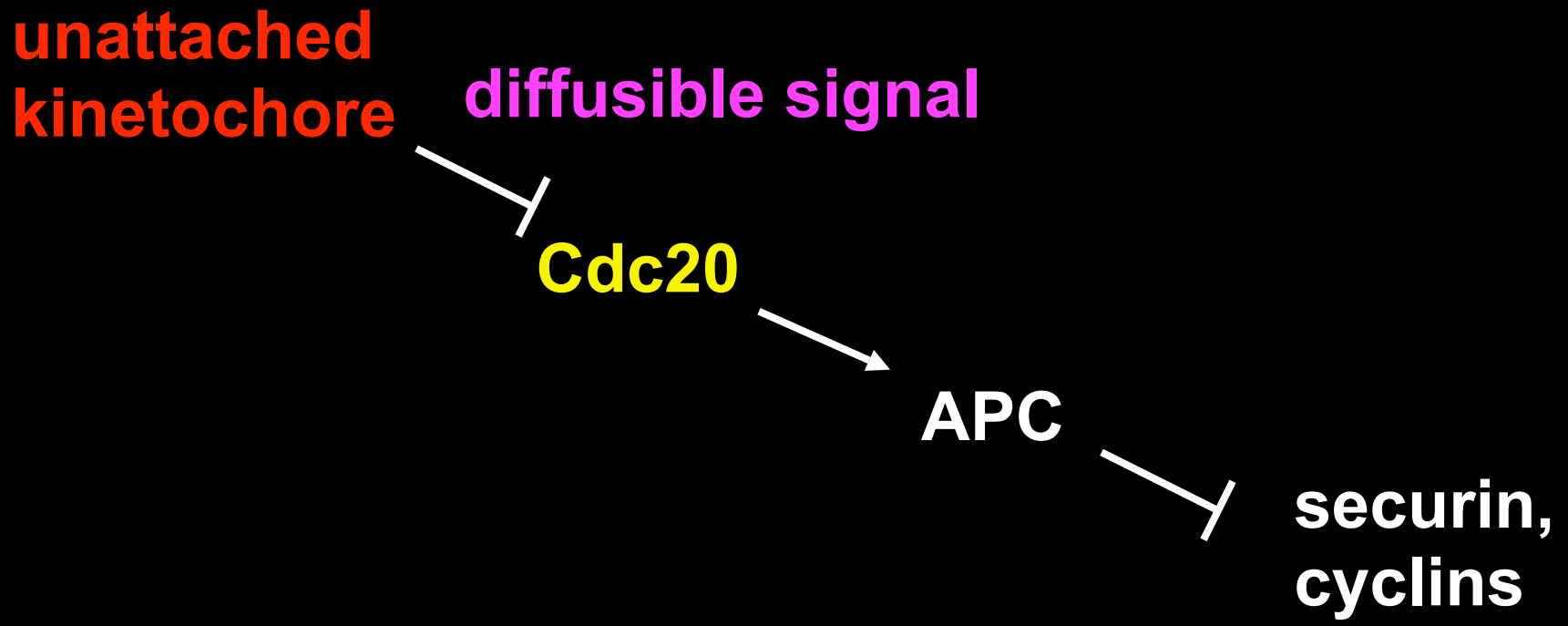
highly conserved

Kinetochore checkpoint

Target = Cdc20

activator of APC-mediated destruction of Pds1(securin), B Cyclins

activates chromosome segregation

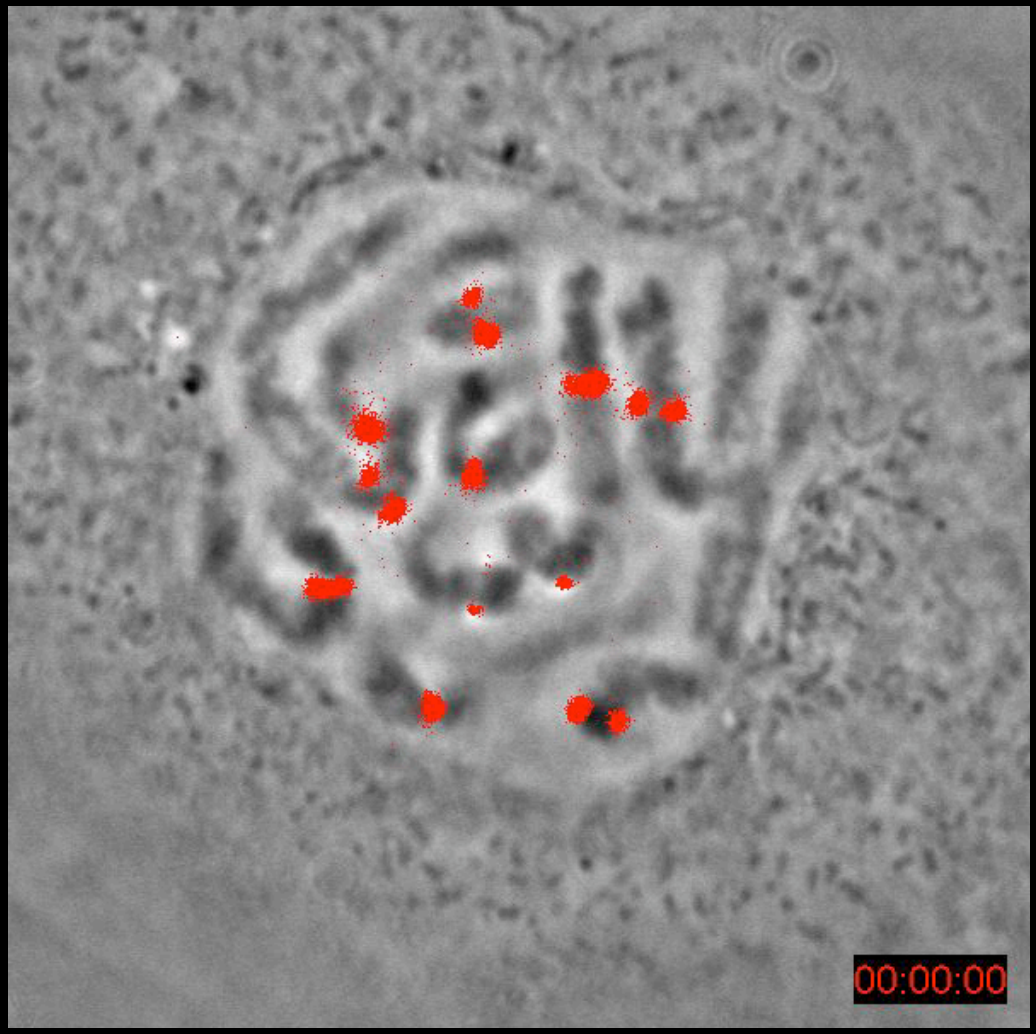


focus on Mad2

associates only with unattached kinetochores

inhibits Cdc20-APC

mouse knockout embryonic lethal

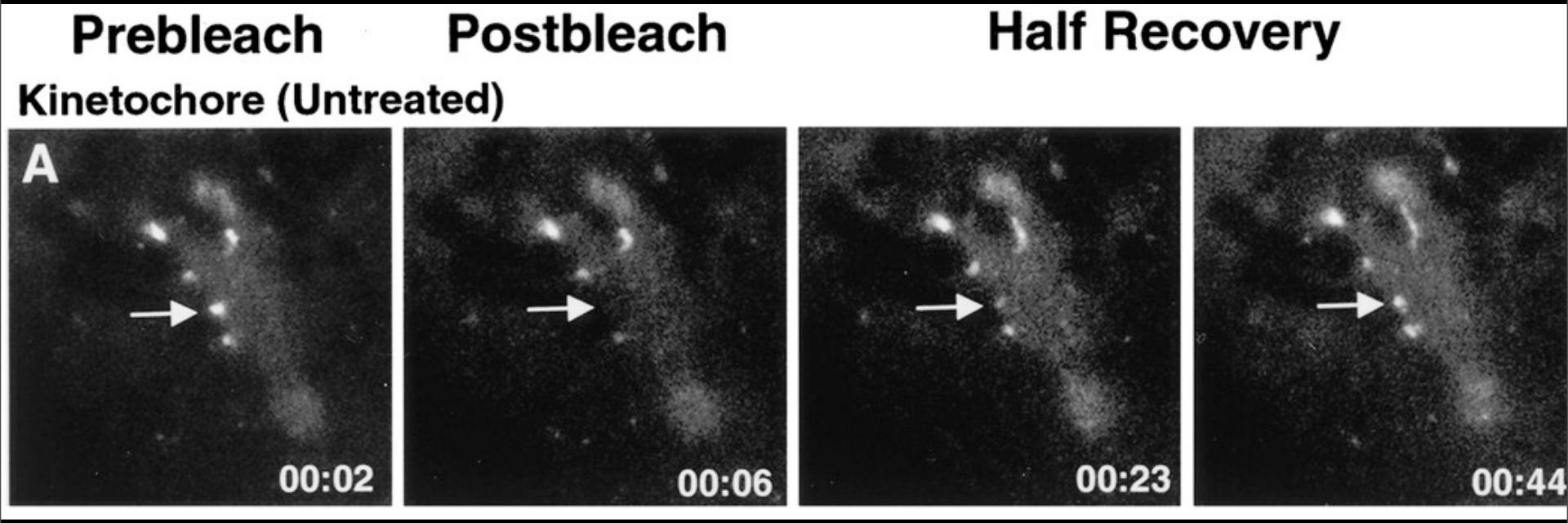


Model

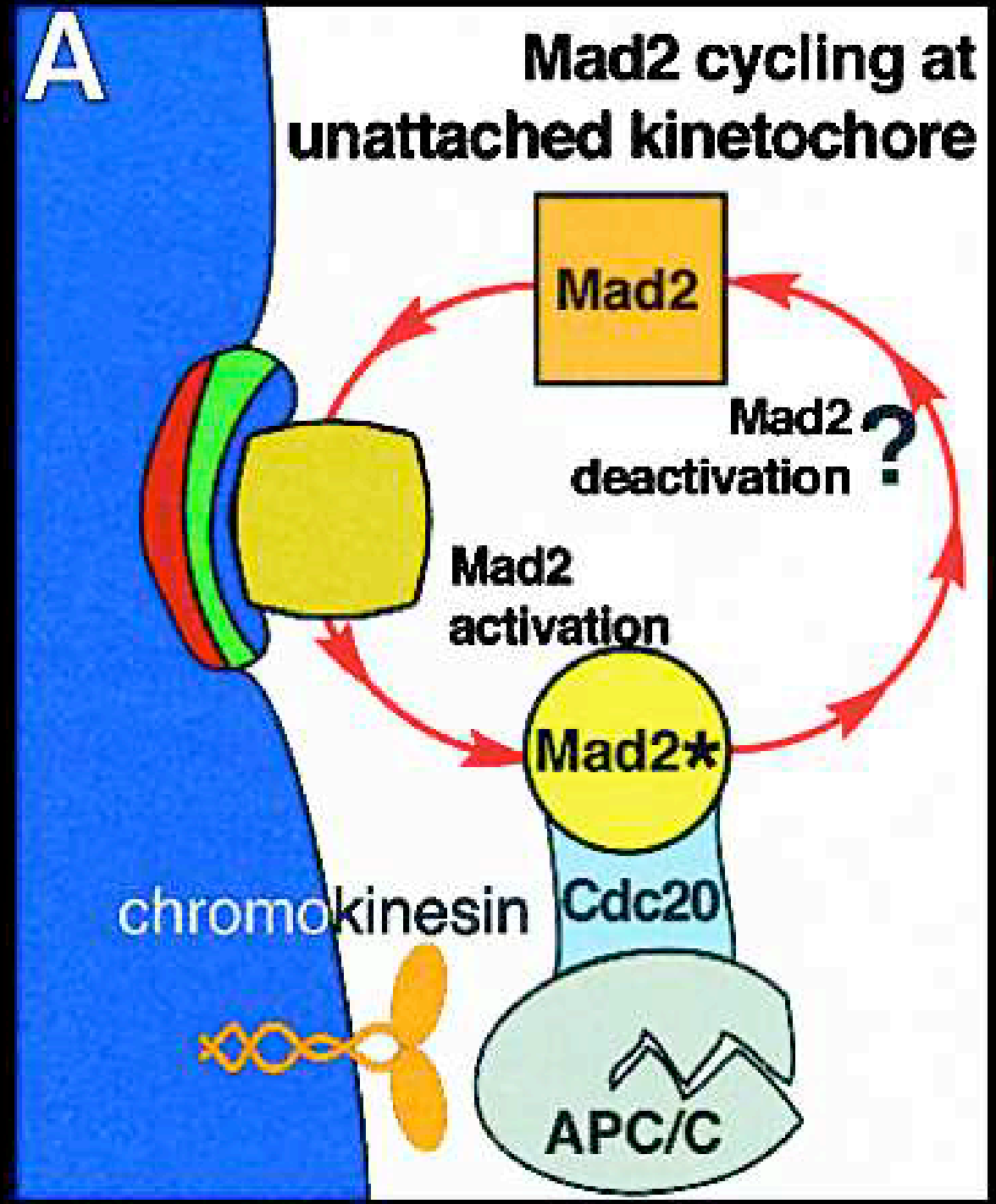
unattached kinetochores “activate” Mad2

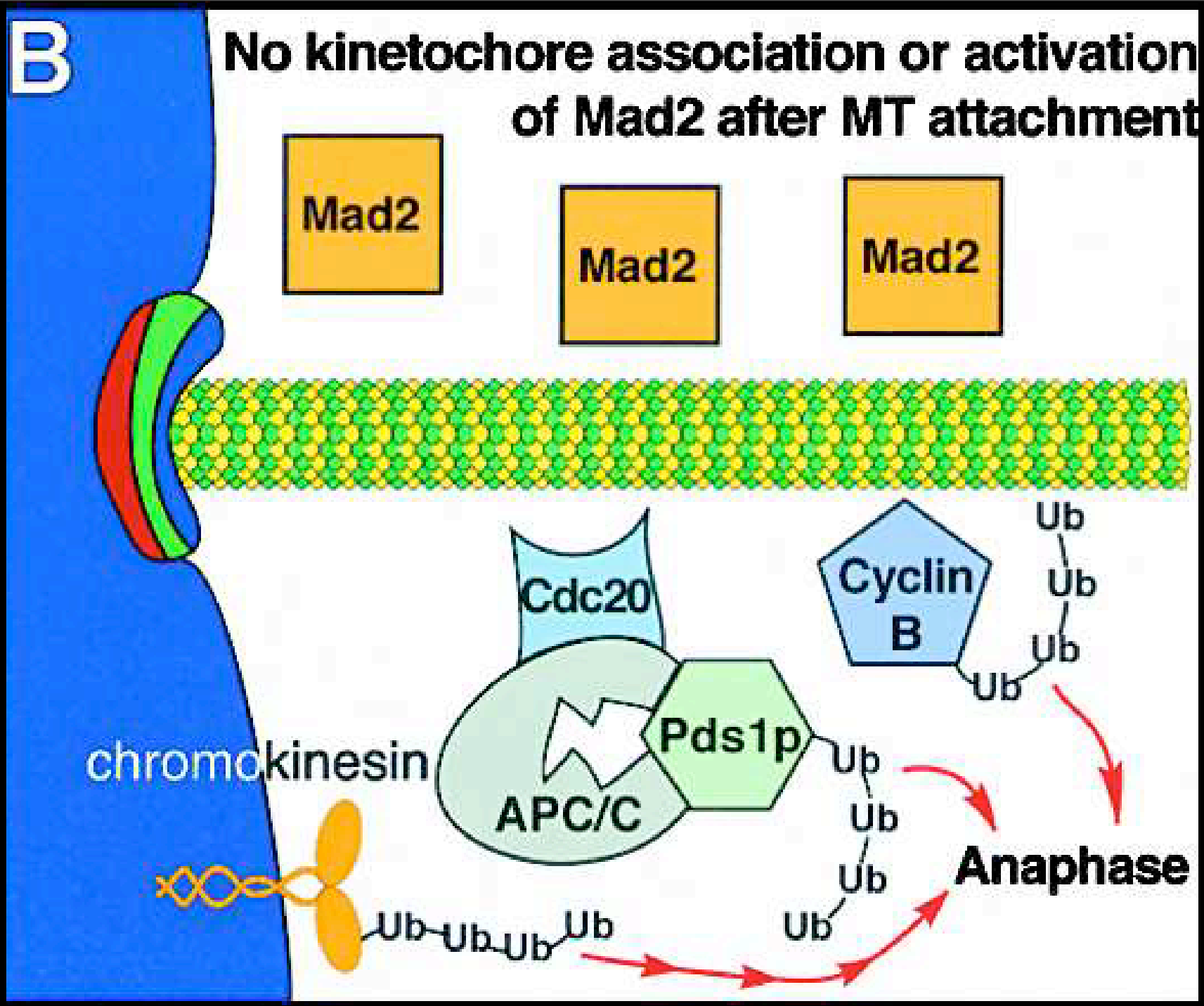
Mad2* diffuses away and inhibits Cdc20-APC

FRAP experiment: Mad2 turns over rapidly



MT destabilization: more kinetochores with MAD2, still turnover


A
Mad2 cycling at unattached kinetochore
Mad2
Mad2 deactivation
?
Mad2 activation
Mad2*
chromokinesin
Cdc20
APC/C


B
No kinetochore association or activation of Mad2 after MT attachment
Mad2
Mad2
Mad2
Cdc20
Cyclin B
Ub
Ub
Ub
Ub
Pds1p
APC/C
chromokinesin
Ub
Ub
Ub
Ub
Ub
Ub
Ub
Anaphase

Questions

What activates/inactivates Mad2?

binding partners: Mad1, Mad3, BubR1, Bub3?

What is Mad2*

complex? oligomer?

What generates and stops the signal???

somatic cells - MT attachment
meiotic cells - tension

How transduced??

dynein-dependent transport to spindle poles may turn it off

Different checkpoint proteins in higher eukaryotes

No Bub2

Mad3 homolog = BubR1, acquired kinase domain

CENP-E

ZW10/Rod (dynein interactors)

All behave like Mad2:

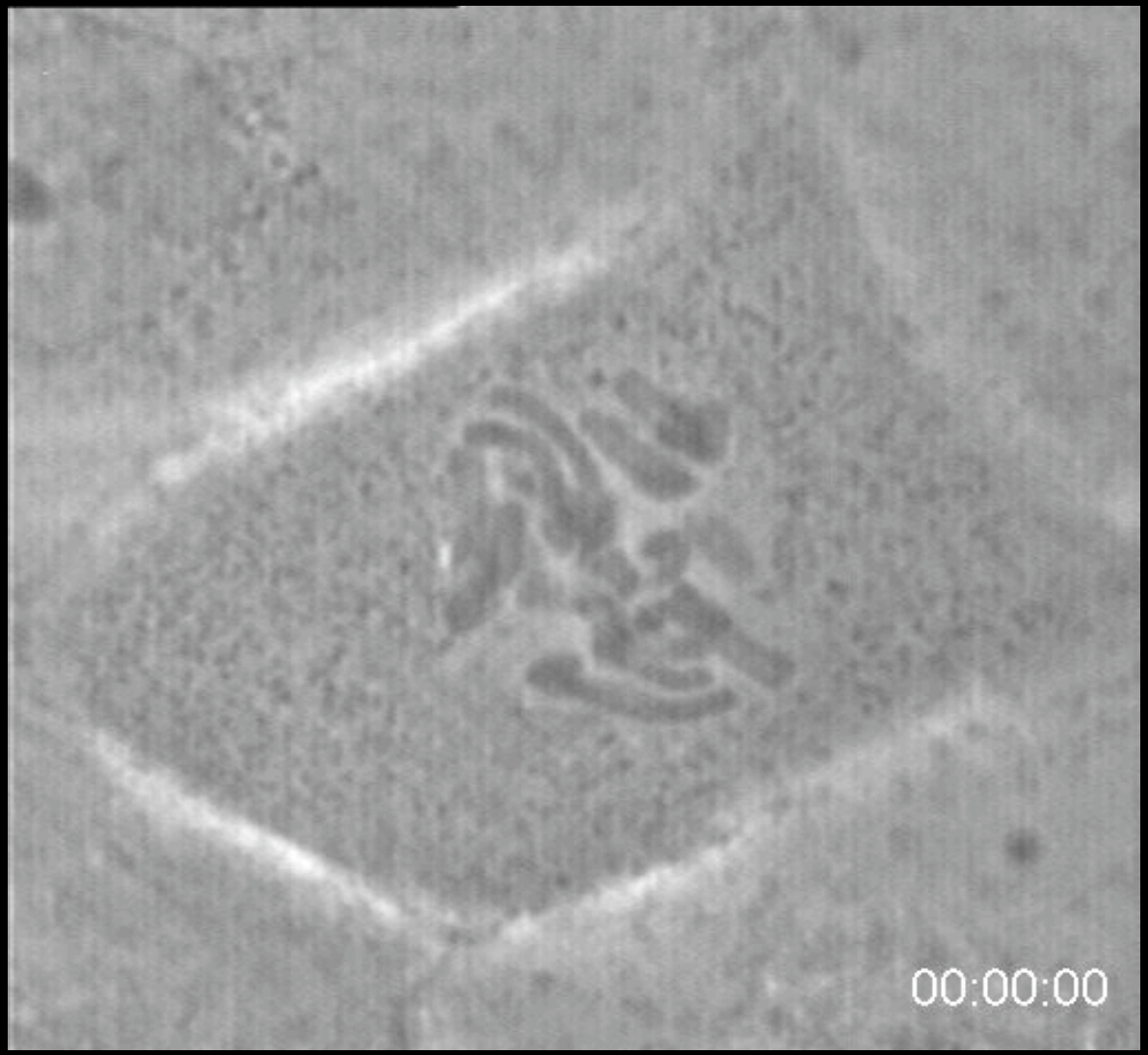
associate with unattached kinetochores

required for checkpoint signaling

Dynamitin (dynein inhibitor) induces arrest

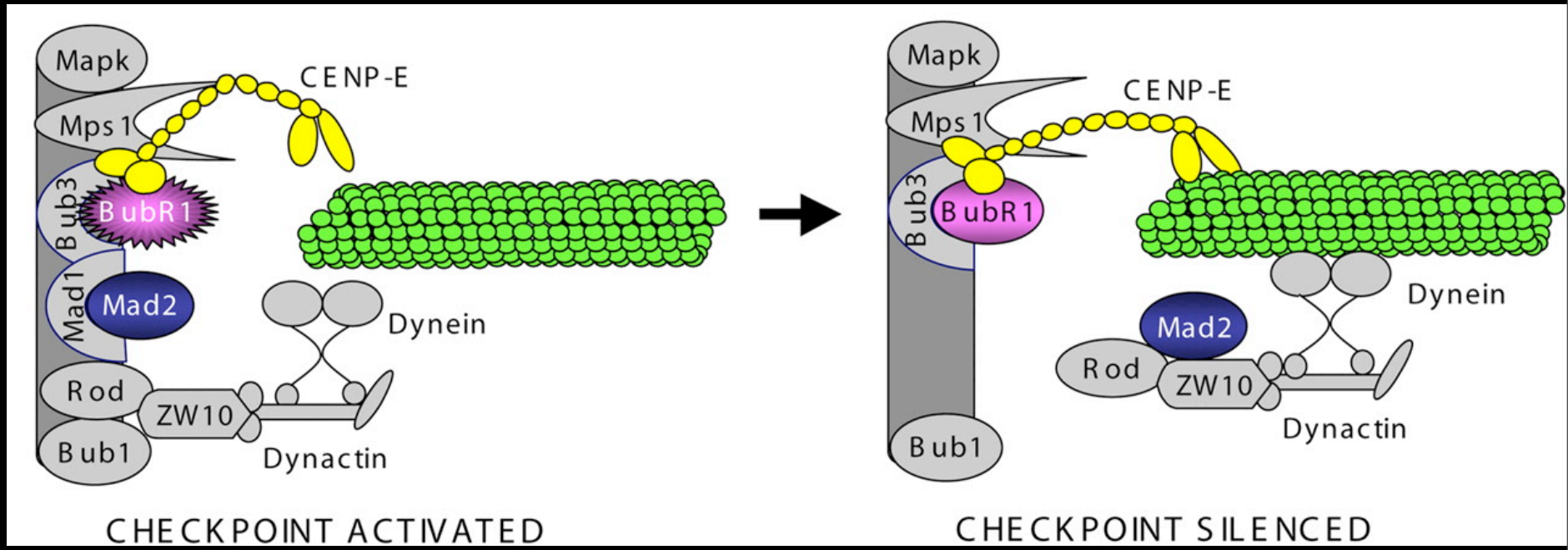
inhibition of Dynein-fails to turn off signal

Dynamitin then Mad 2



addition of Mad2 overcomes block, enters anaphase

Motor-dependent mechanism for checkpoint signaling?



CENP-E activates BubR1 kinase activity until attached to microtubules - creates "wait" signal

After attachment, complexes are carried off the kinetochore by dynein

Mao, Desai and Cleveland, JCB 170, 873-80 (2005)

Cell Cycle Checkpoints