Lecture 14. Cell cycle I

Outline

I. Overview of the cell cycle
II. The cell cycle control system
III. S phase

Reminder: cell theory

Cell comes from cell

I. Overview of cell cycle

- ♥ Phases of cell cycle
- ♥ Model systems for cell cycle studies
- ♥ Common methods to study cell cycle

1. Cell cycle

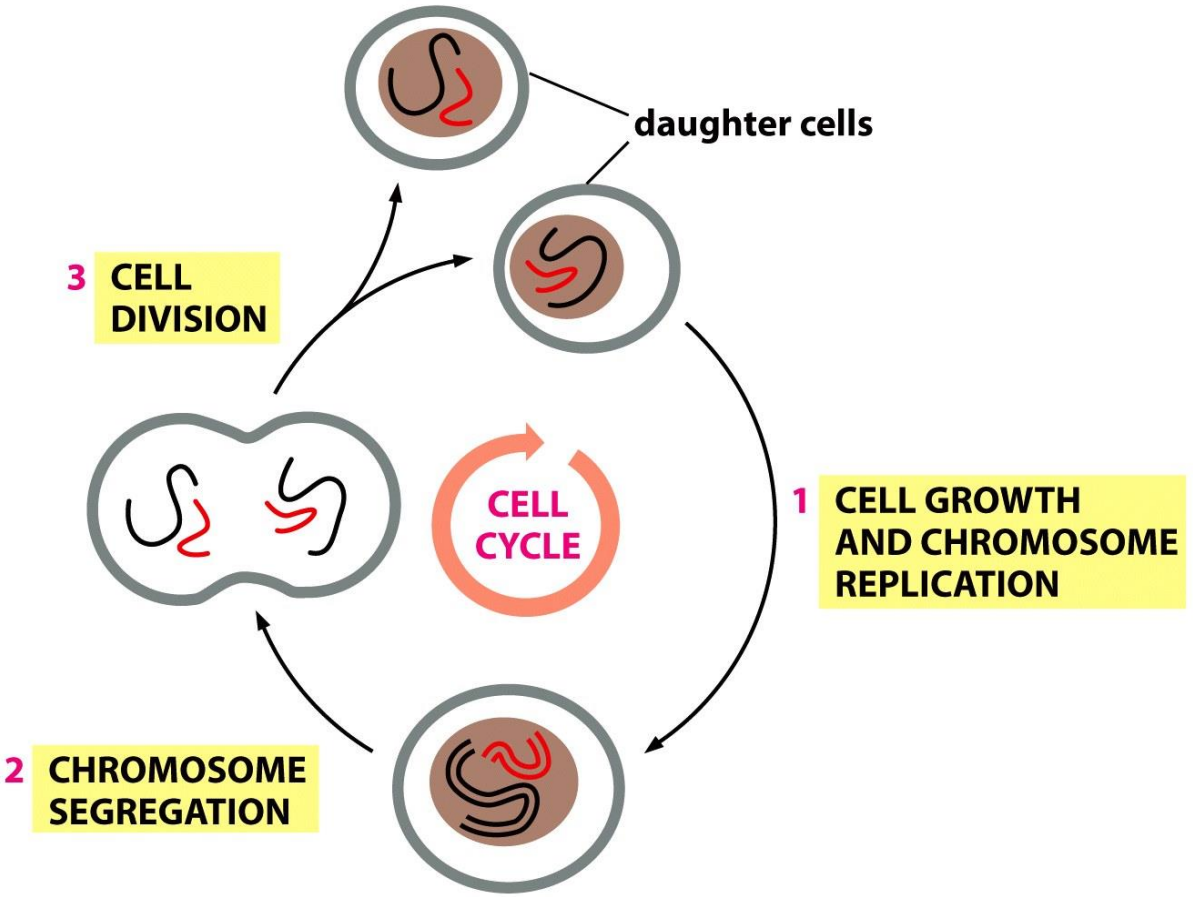
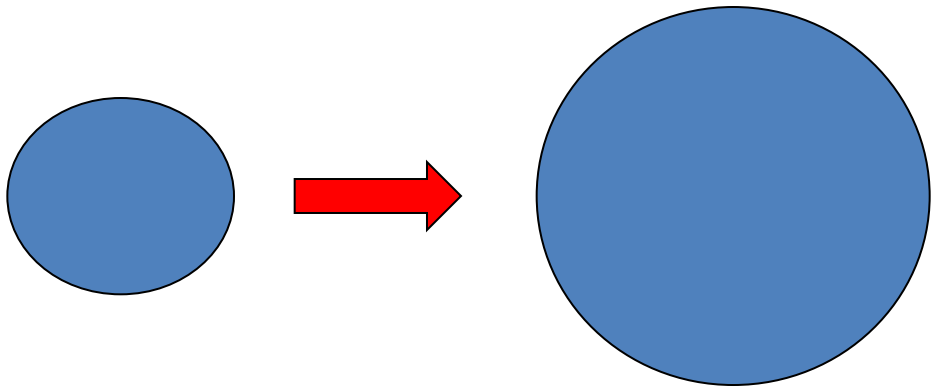
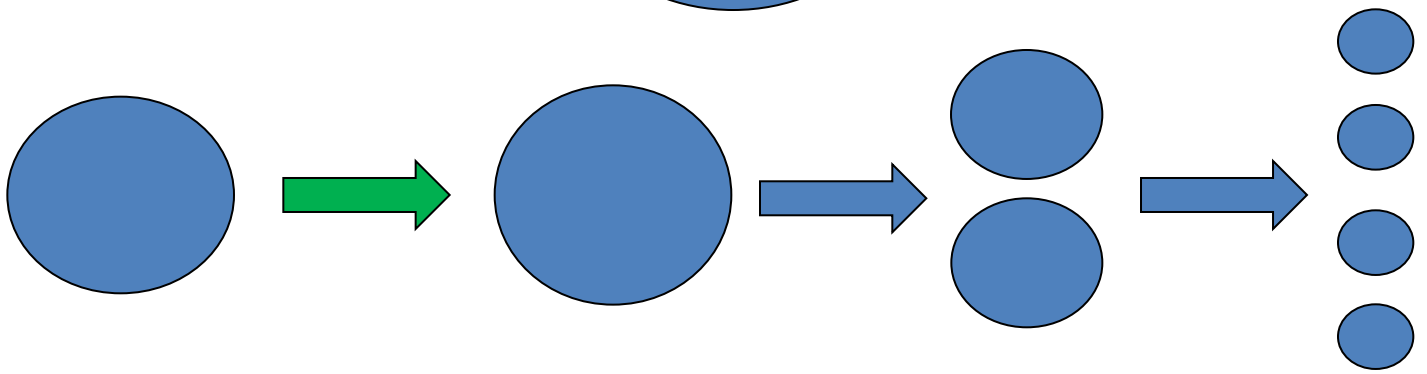


Figure 18-1 Essential Cell Biology 3/e (© Garland Science 2010)

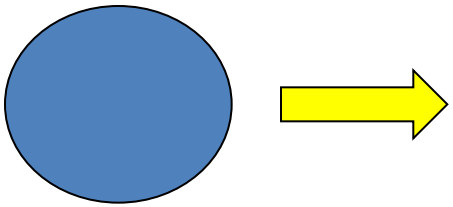
What happens if this is dyregulated?



No division



No growth



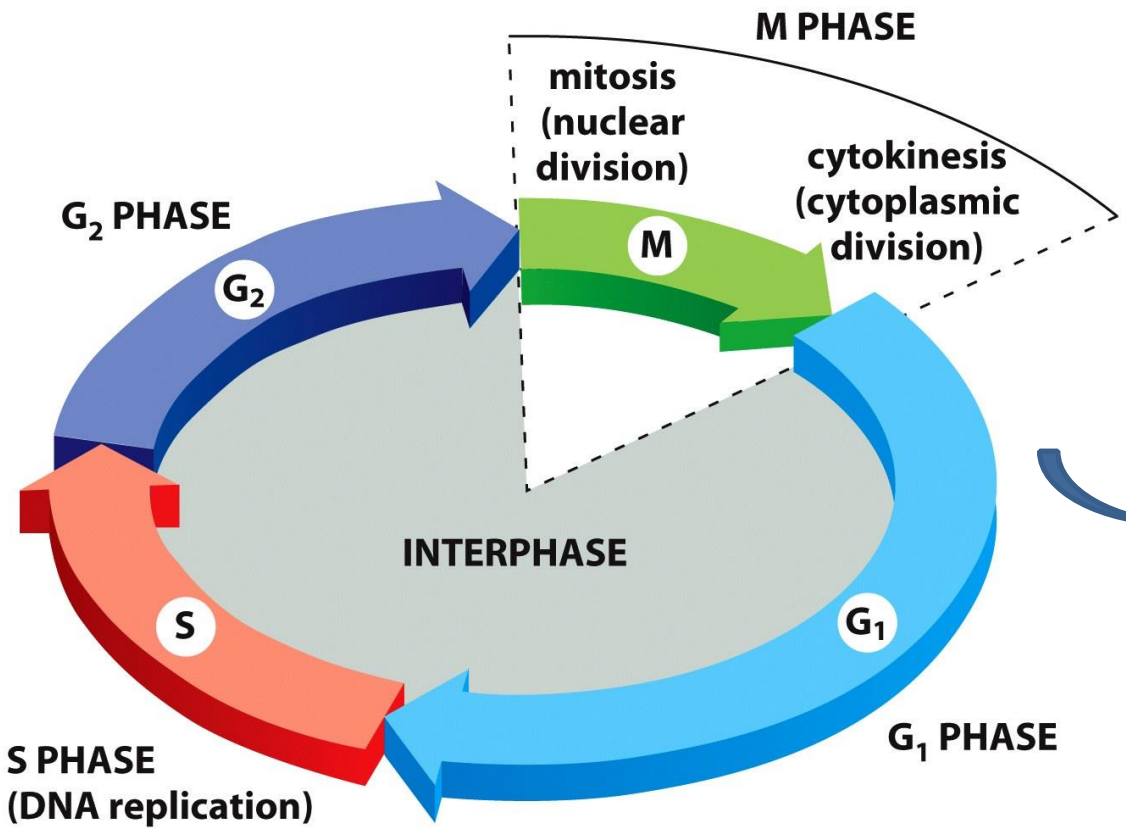
Normal organ

Division numbers are controlled

Tumor mass

Division numbers are out of control

The major events in cell cycle



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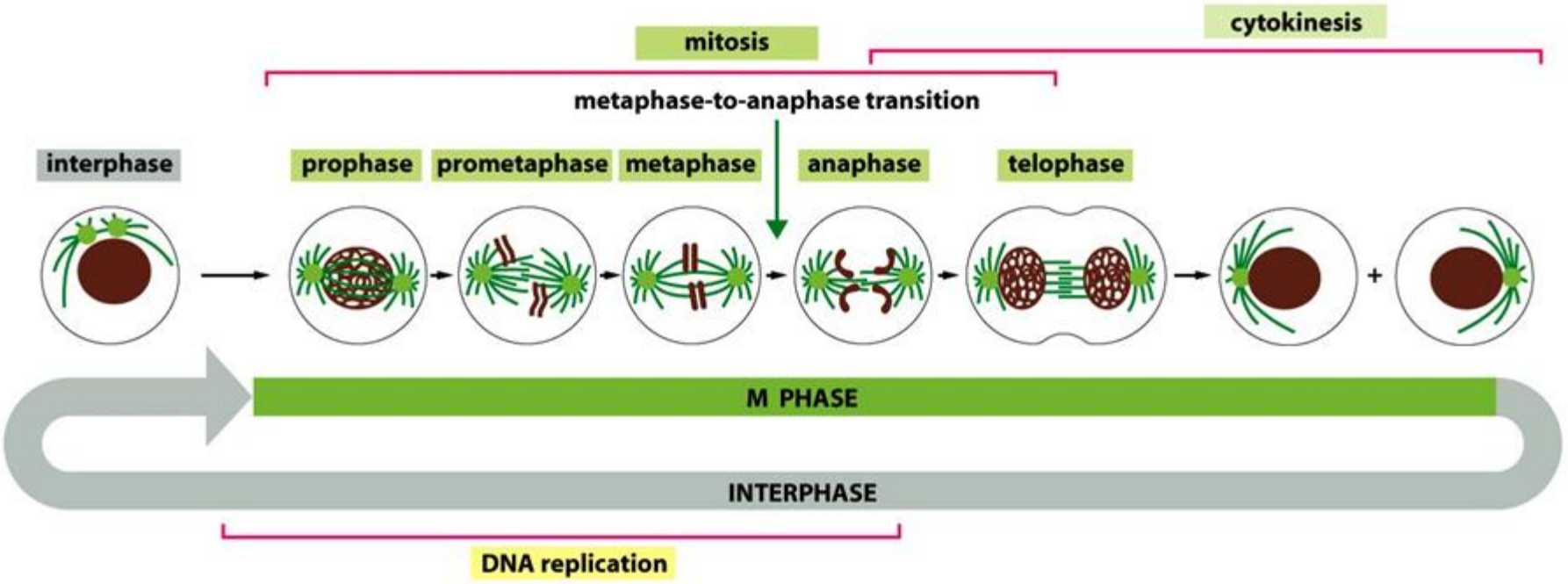
M phase can be further divided into: mitosis and cytokinesis



Different Cell cycle time (doubling time) for some eukaryotic cells

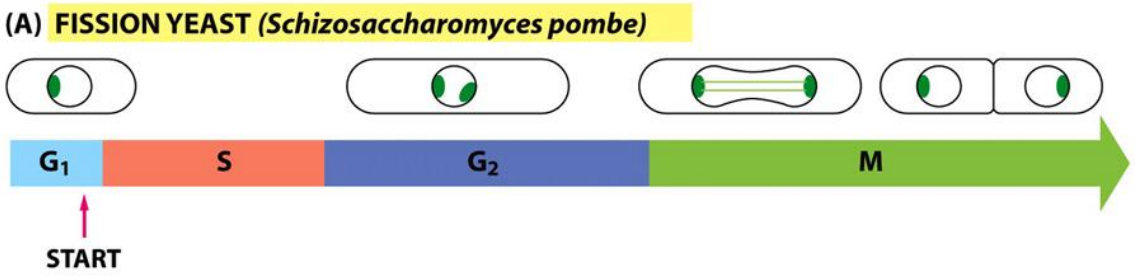
Fertilized xenopus oocytes	30 min
Yeast cell	1.5-3 hours
Mammalian intestine epithelial cells	~12 hours
Mammalian fibroblasts	20 hours
Human liver cell	~ 1 year

2. Model systems to study cell cycle

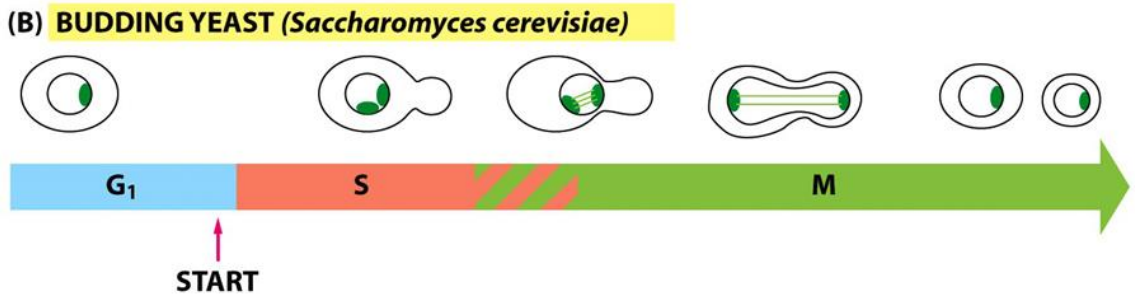
Cell cycle control mechanisms are conserved during evolution

- Yeast:
  - fission yeast (*S.pombe*)
  - budding yeast (*S.cerevisiae*)
- Xenopus oocyte
- Mammalian cells

1) Yeast



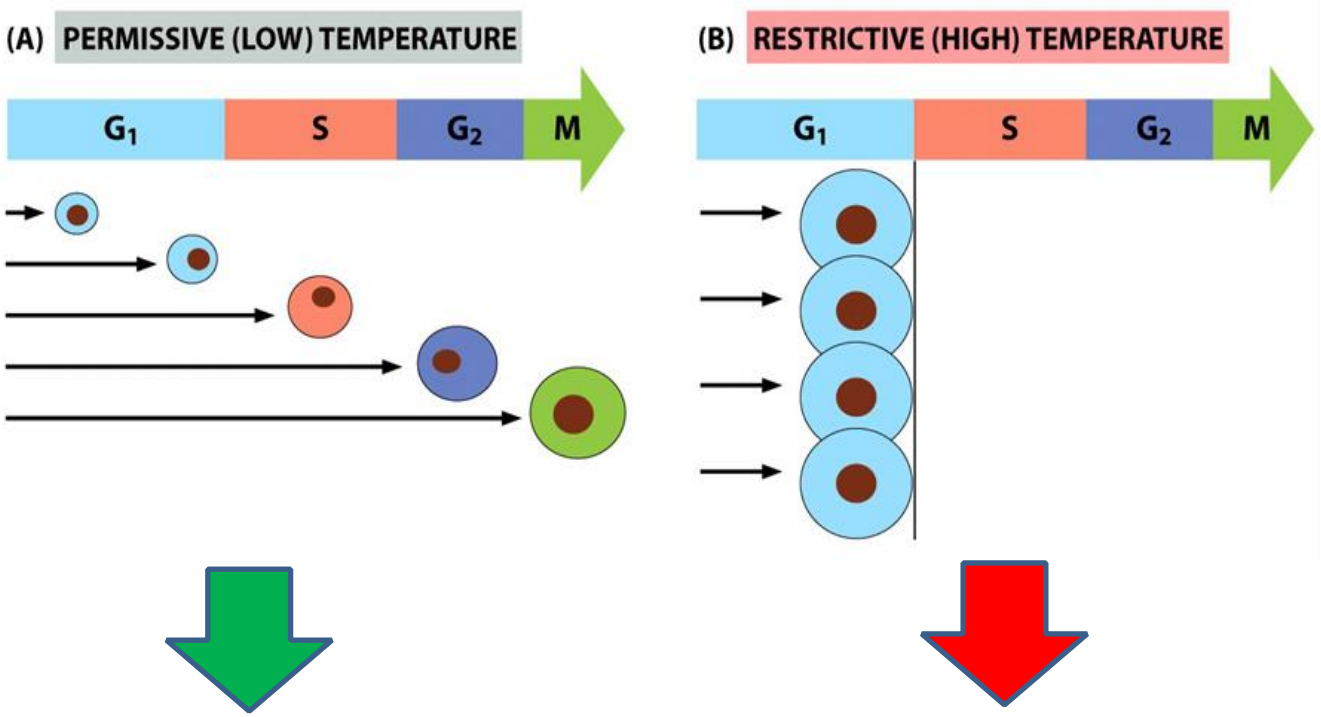
Fission yeasts are rod, septum
Divides into two daughter cells



Budding yeasts are oval, daughter cell buds from mother cell.

Conditional mutation in yeast cells for cell cycle studies

Start with haploid cells, perform genetic mutation

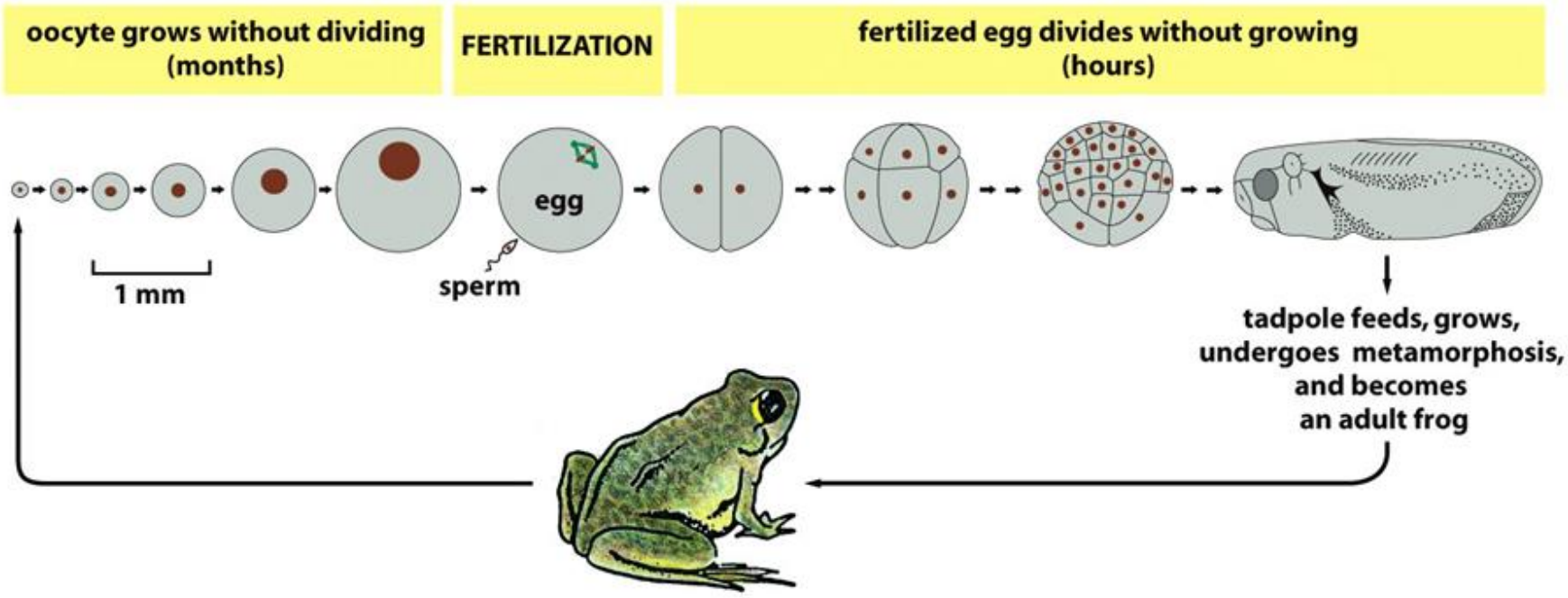


Proliferation

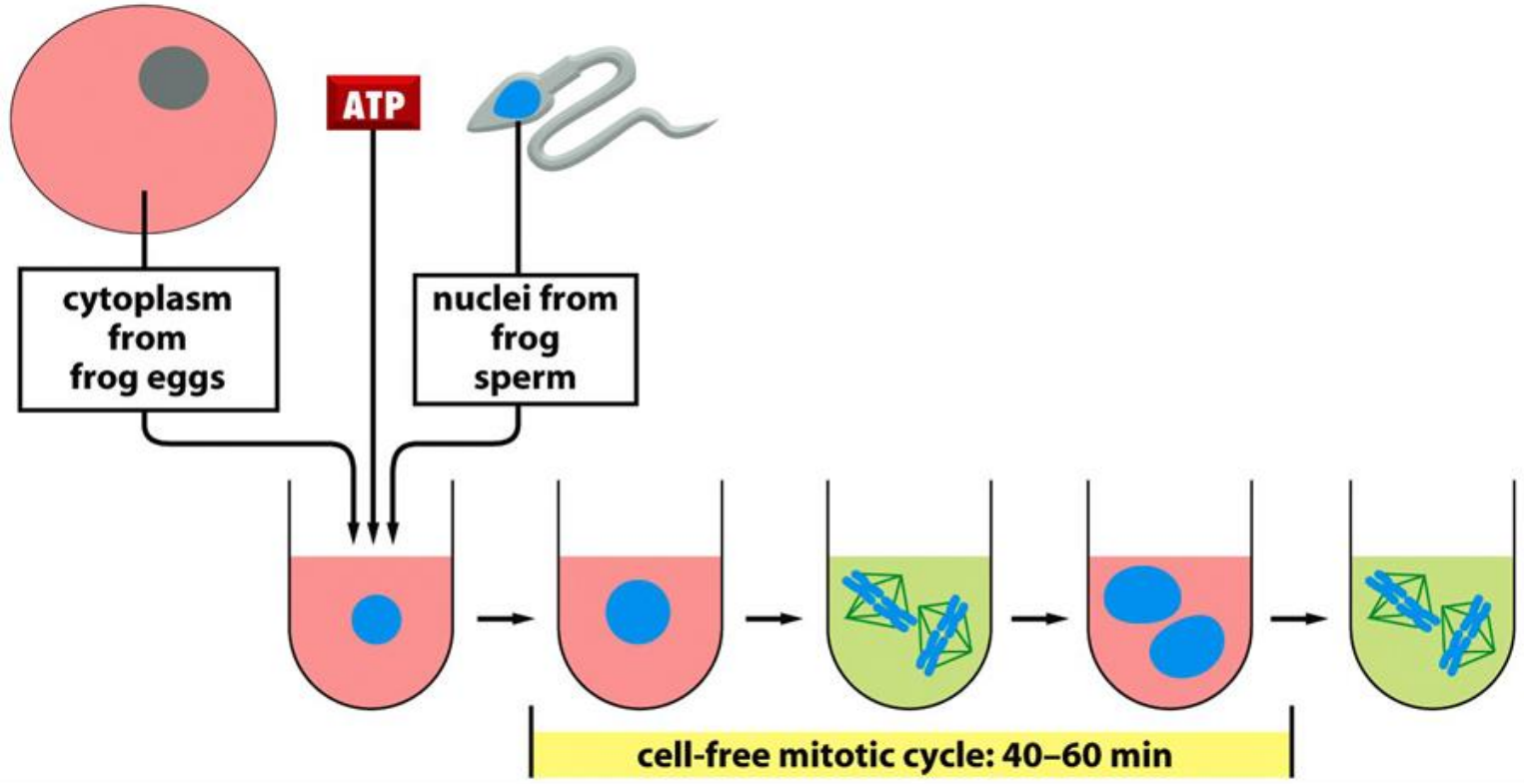
no proliferation

2). Xenopus oocytes

Xenopus oocytes have rich source of cell division proteins.

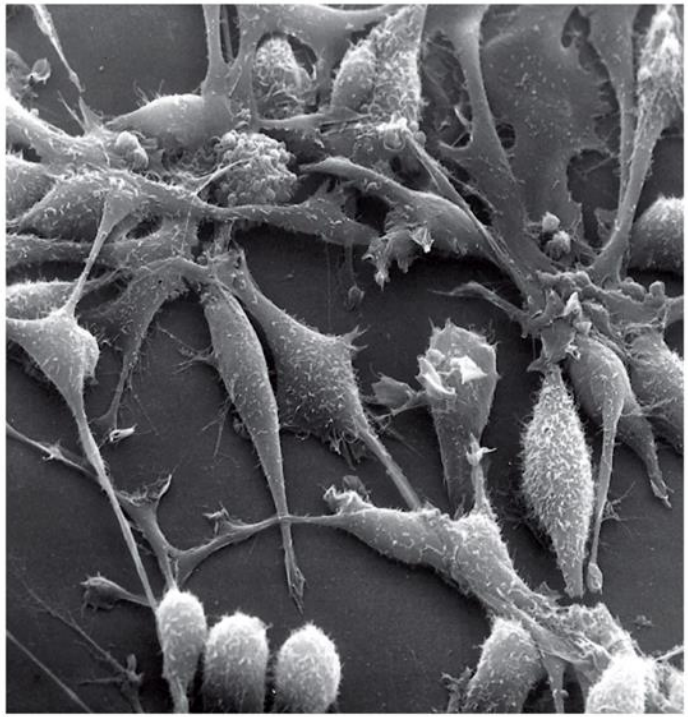


Xenopus oocytes are frequently used in cell-free system for *in vitro* studies



3). Cultured mammalian cells

- ♥ Normal primary cell culture
- ♥ Transformed immortal culture
- ♥ Cancer cell lines

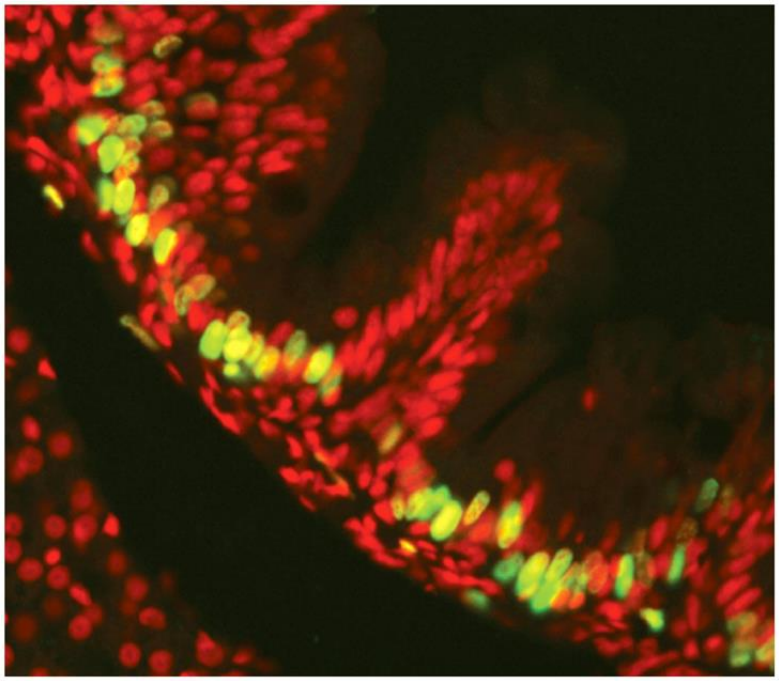


3. Various methods to study cell cycle

- ♥ Visualization under microscope
- ♥ BrdU/EdU incorporation assay
- ♥ Cell cycle distribution assay

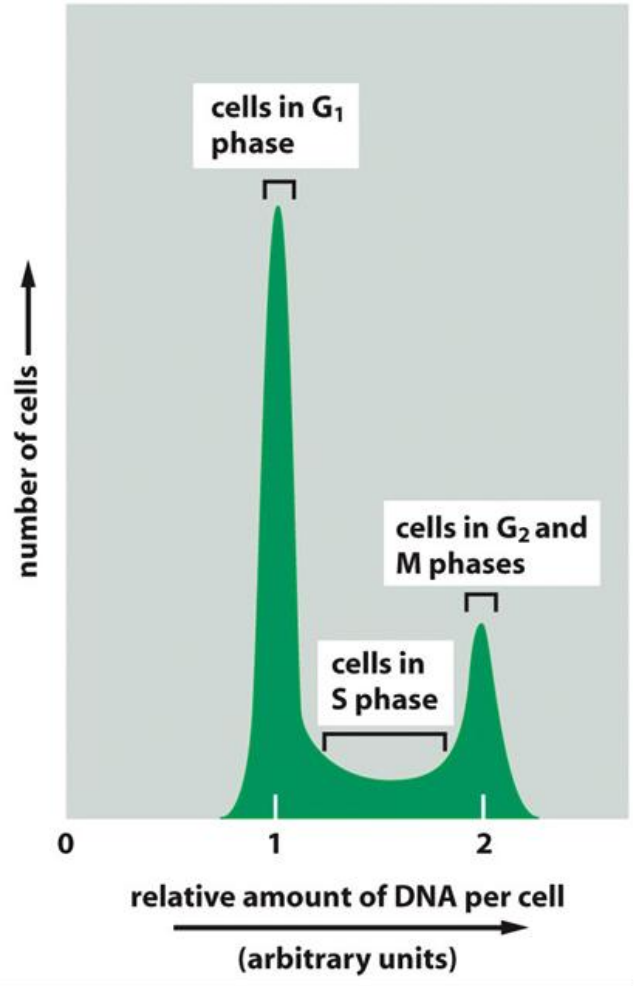
T analog incorporation analysis

treat cells with T analog BrdU (or EdU)
Stain by BrdU antibody (or with Click labeling)



Mitotic index:
mitosis cell numbers/
Total cell numbers

Flow cytometry to detect cell cycle phase



Use fluorescence dye to bind to DNA quantitatively, followed by flow cytometry

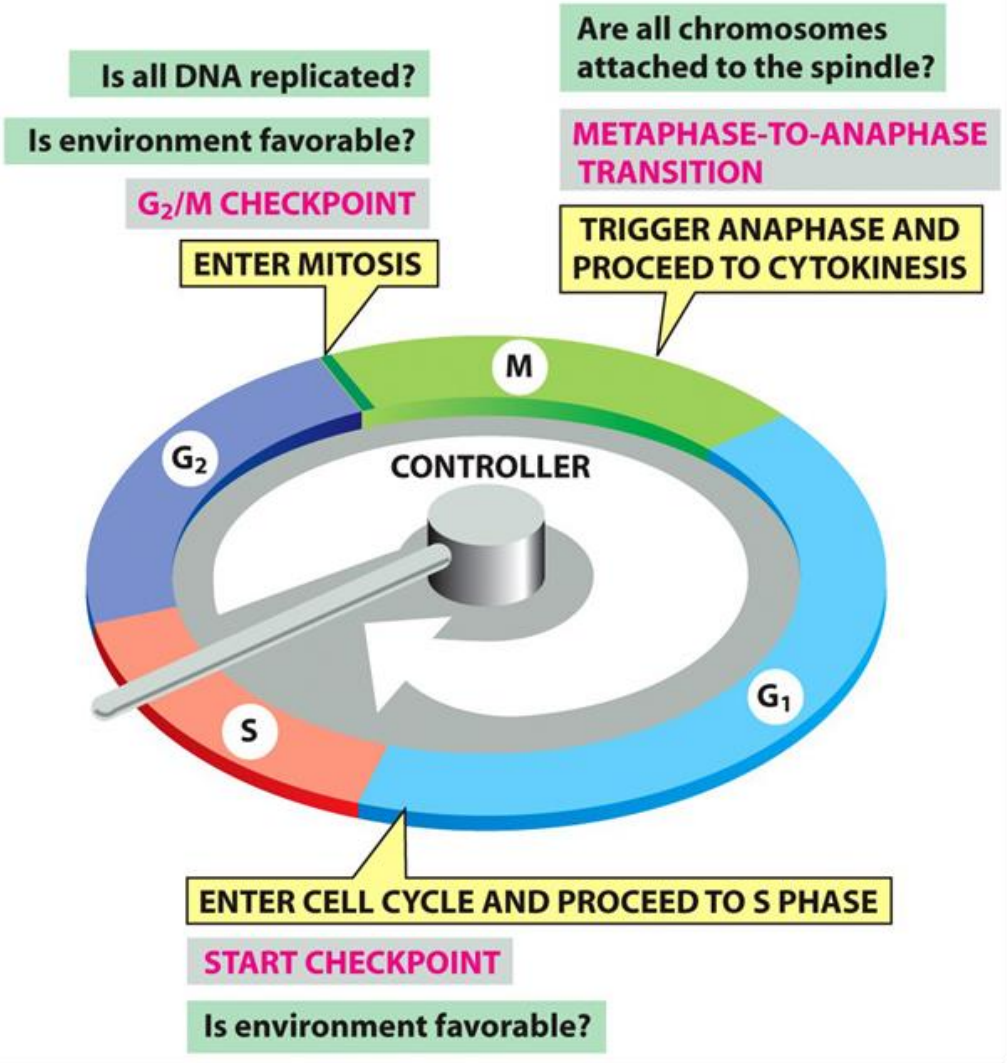
II. The cell cycle control system

Three major control checkpoints:

1. G1/S transition

2. G2/M Transition

3. Metaphase-to-anaphase transition



Cyclins and Cyclin-dependent kinase (Cdk) are two major players in cell cycle control

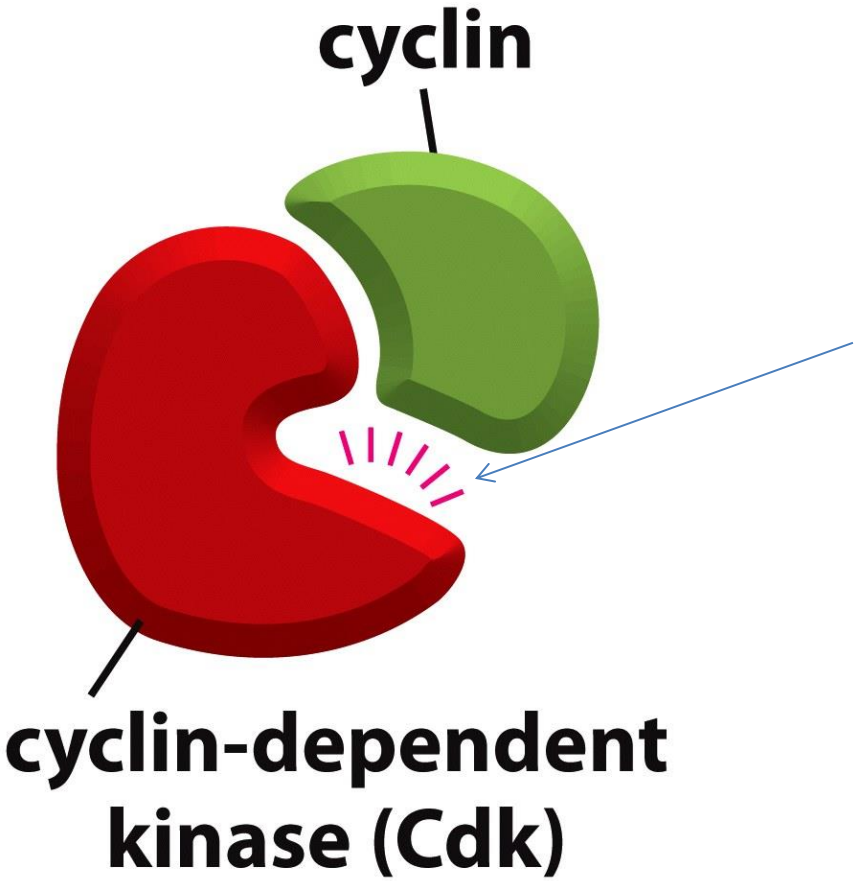
- **Cyclins**:

  --- different cyclins ocsillate in cell cycle and bind/control different cdk activity; it decides cdk substrates specificity and activates cdk.

- **Cdk**:

  --- protein kinase, phosphorylates a subset of substrates to control cell cycle progression at specific checkpoint.

Cyclin/cdk complex



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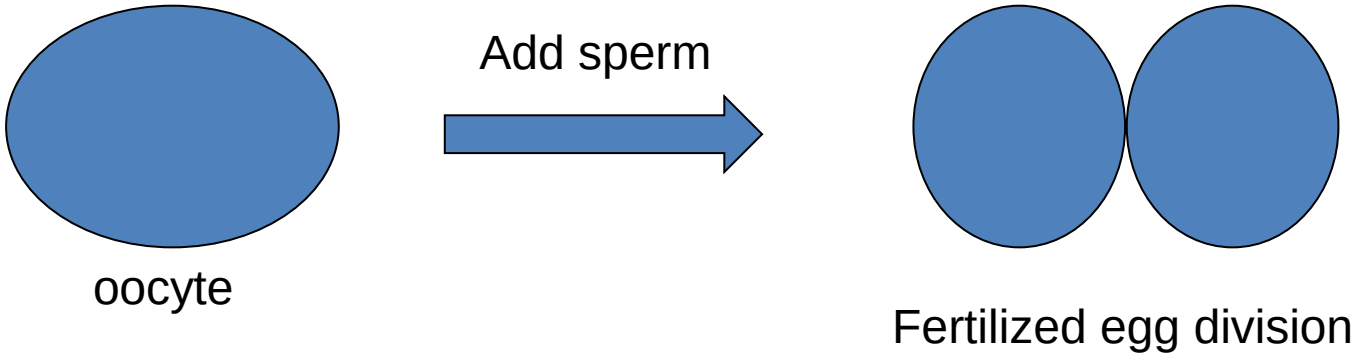
The discovery of cyclins

- 1983, by Joan Ruderman and Tim Hunt
- Use oocytes from sea urchins and surf clams



Addition of sperm causes synchronized division for oocytes

♥Synchronized

cells start from the same point in cell cycle

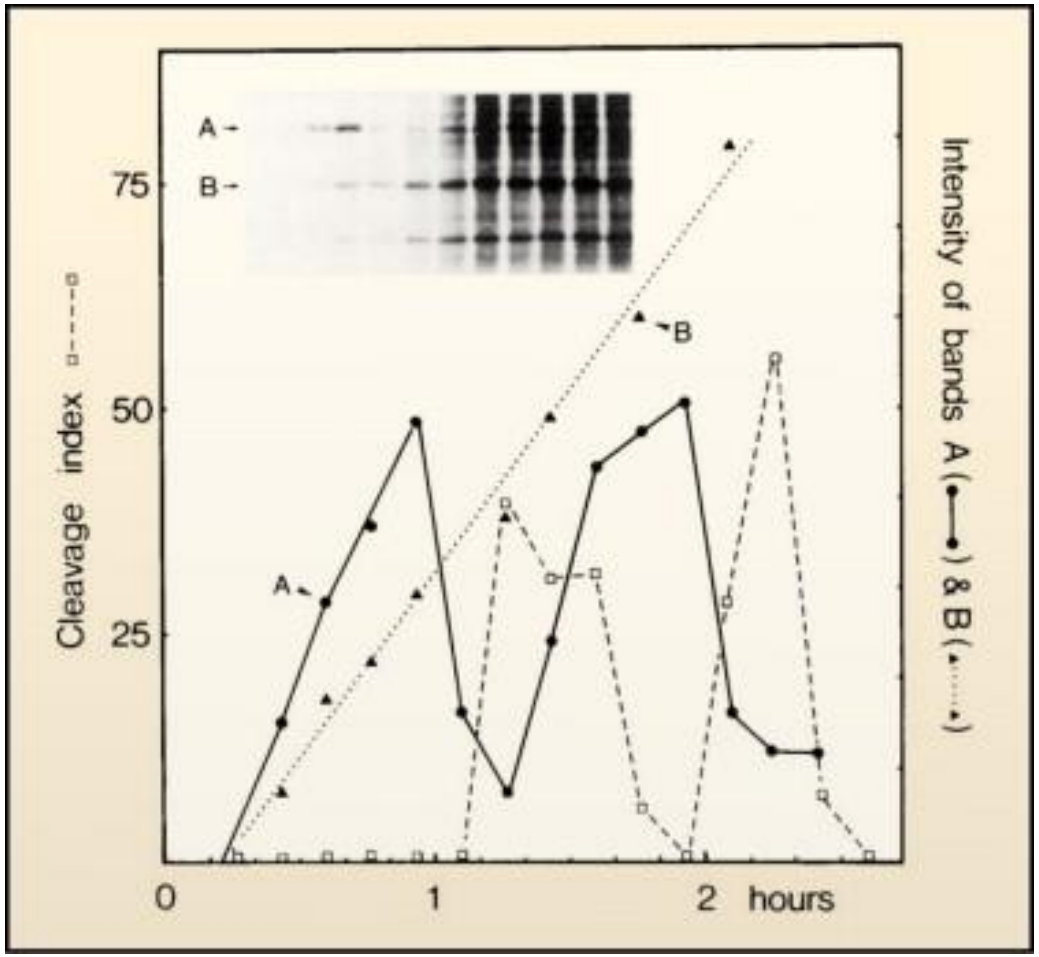
♥Unsynchronized

cells start cell cycle differently

In oocytes: many mRNAs were not translated.

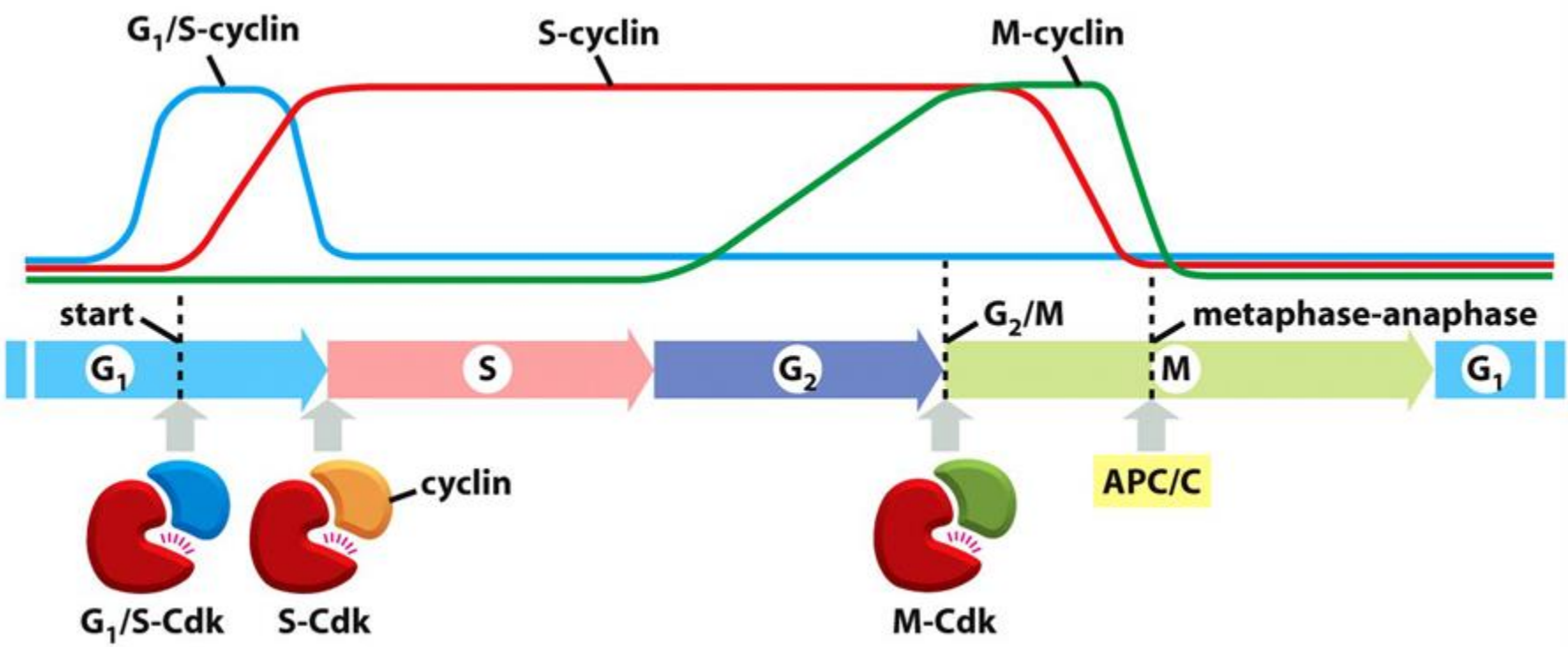
In fertilized eggs: many mRNA began to be translated.

The discovery of cyclins



Cyclin upheaval: gene transcription
Cyclin down: protein degradation

Cyclin-cdk complexes of the cell-cycle control system



Major cyclins and cdks of vertebrates and budding yeast

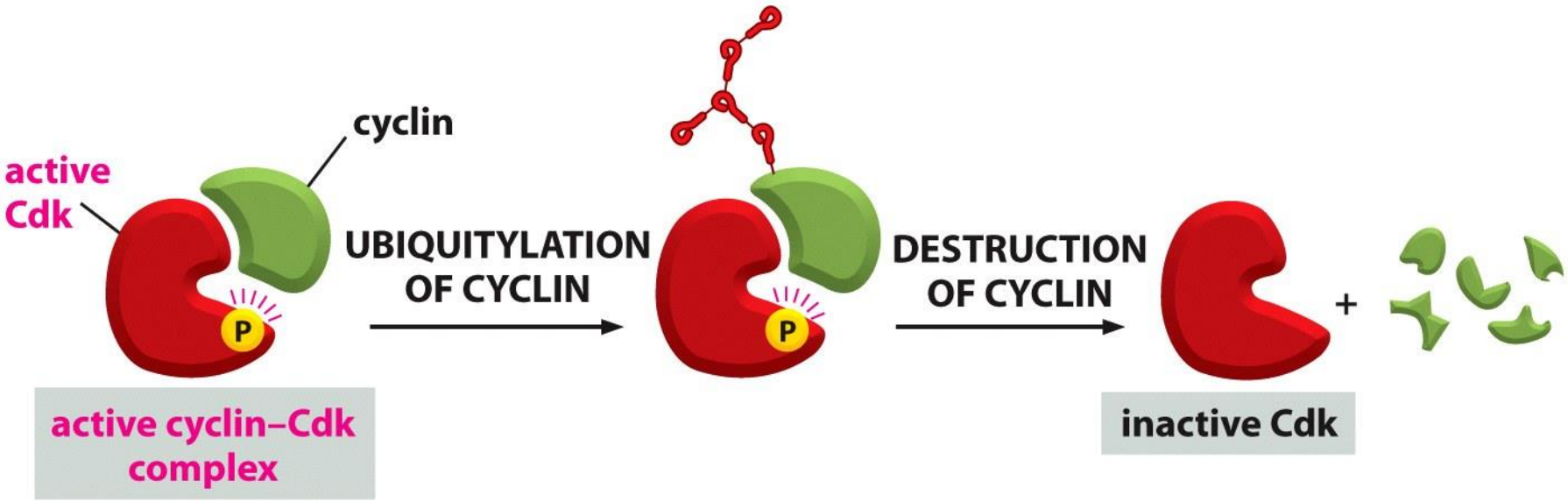
Table 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

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, Cdk1**	Clb5, 6	Cdk1
M-Cdk	cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

A fourth class of cyclin, cyclin G1, helps to govern the activities of the G1/S cyclins.

Cyclin degradation through ubiquitination



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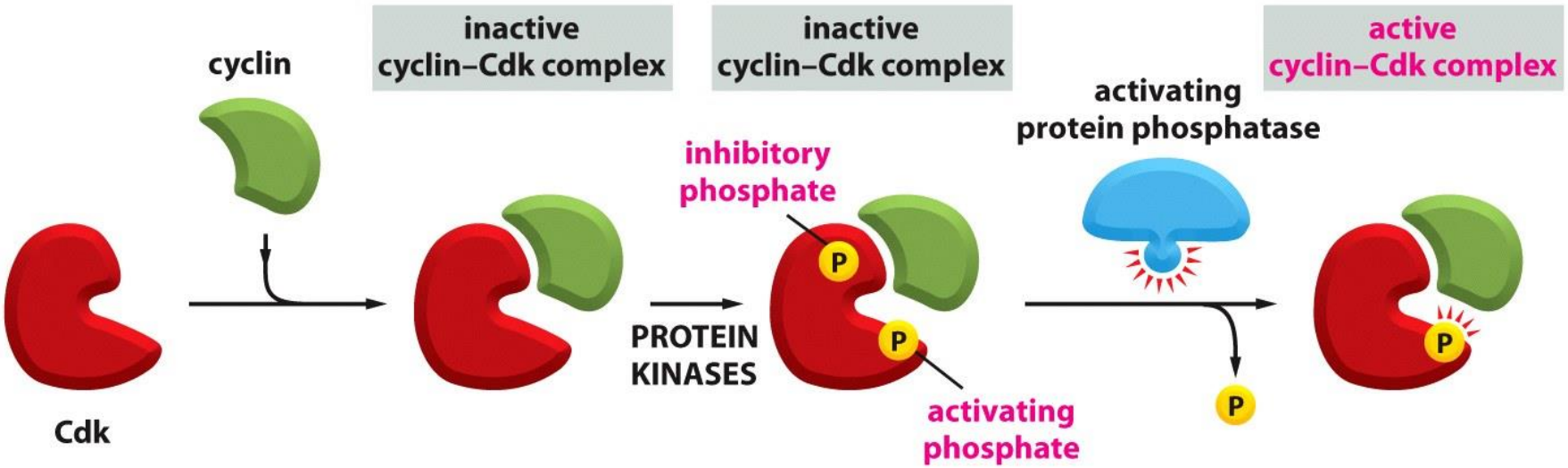
Activation of cdk is additionally controlled by phosphorylation



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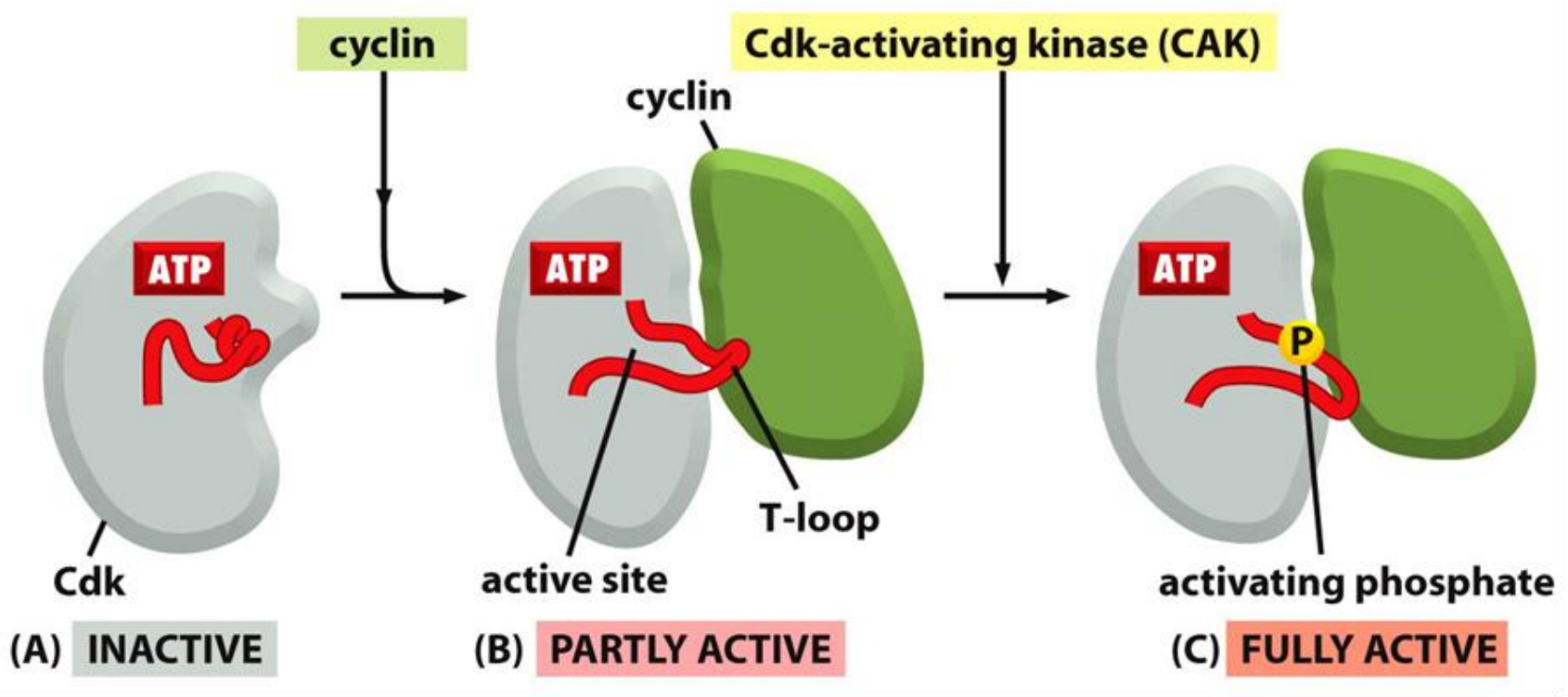
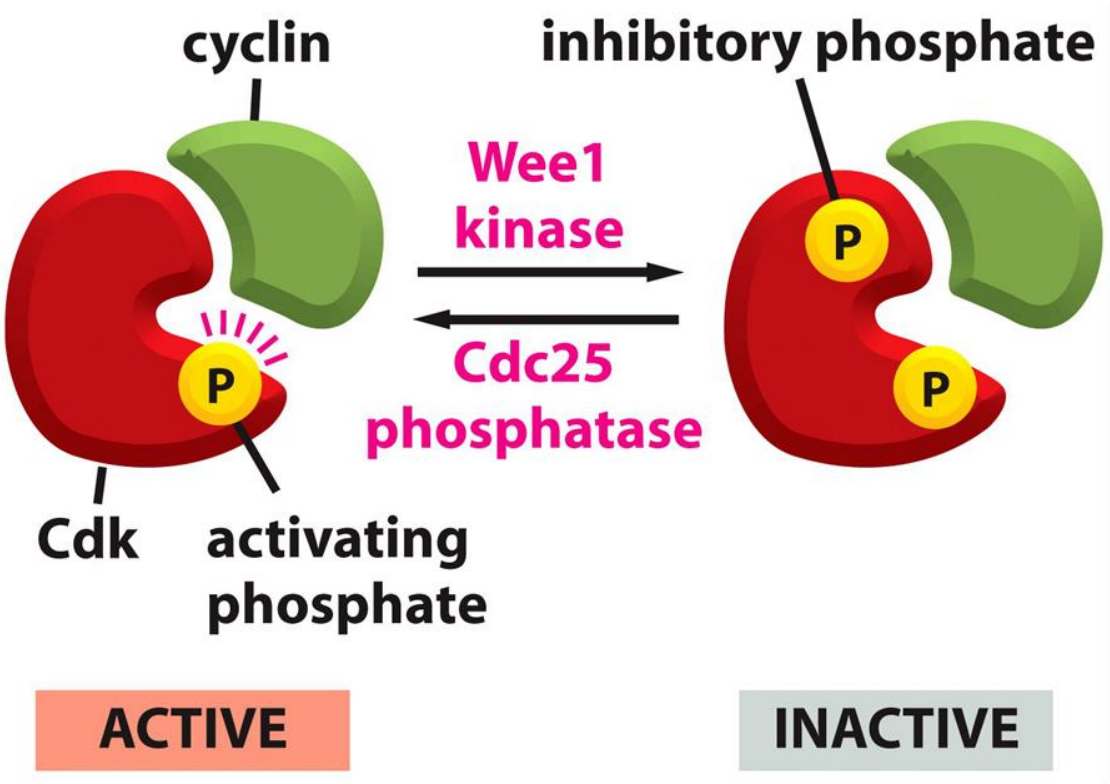
Protein kinases and protein phosphatases that modify Cdks	
Cdk-activating kinase (CAK)	phosphorylates an activating site in Cdks
Wee1 kinase	phosphorylates inhibitory sites in Cdks; primarily involved in suppressing Cdk1 activity before mitosis
Cdc25 phosphatase	removes inhibitory phosphates from Cdks; three family members (Cdc25A, B, C) in mammals; primarily involved in controlling Cdk1 activation at the onset of mitosis

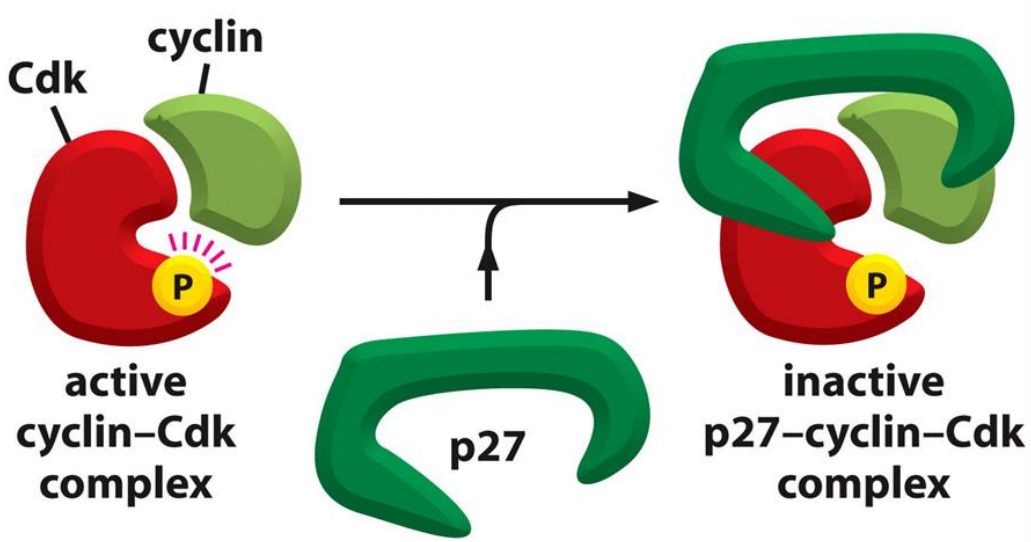
CAK activates the activation loop of Cdk through phosphorylation



Cdk phosphorylation can be regulated by Wee1/Cdc25



Cdk inhibitor proteins (CKI) inhibits Cdk kinase activity



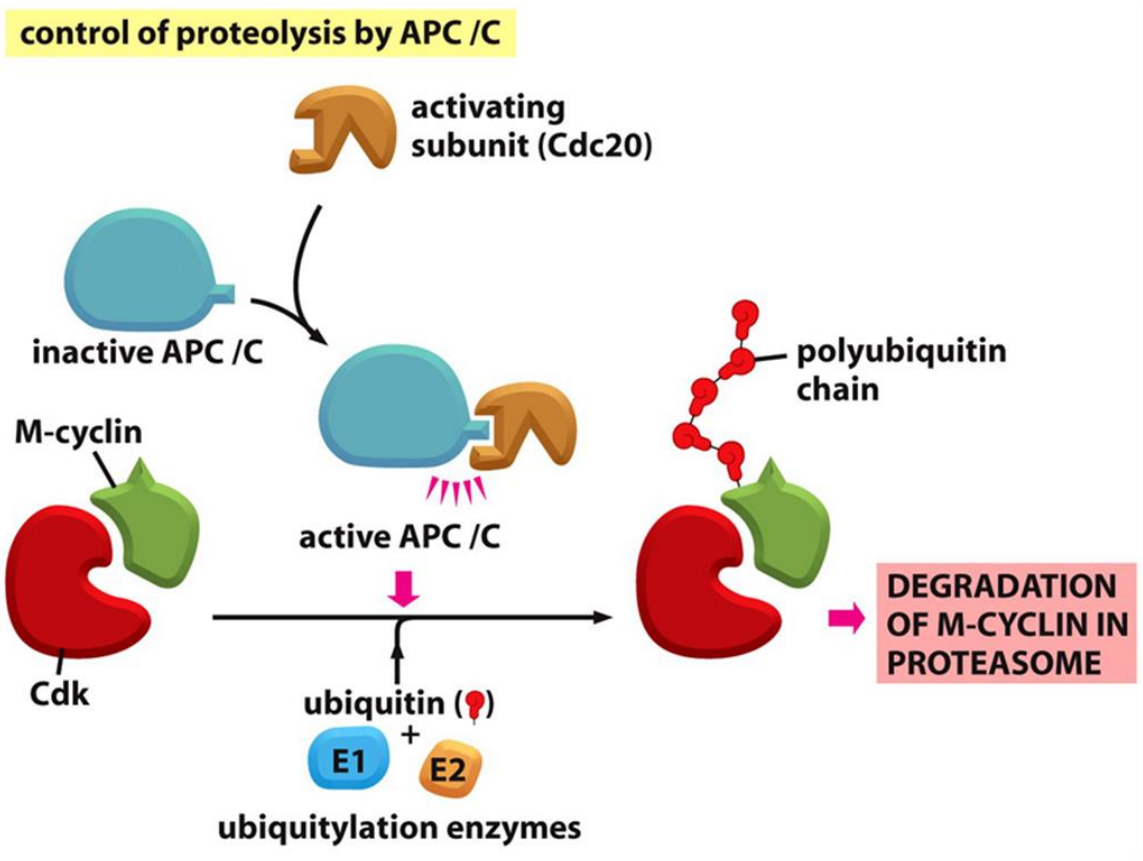
Cdk inhibitor proteins (CKIs)	
Sic1 (budding yeast)	suppresses Cdk1 activity in G_1; phosphorylation by Cdk1 at the end of G_1 triggers its destruction
p27 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities in G_1; helps cells withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities following DNA damage
p16 (mammals)	suppresses G_1-Cdk activity in G_1; frequently inactivated in cancer

Metaphase to anaphase transition in controlled by proteolysis

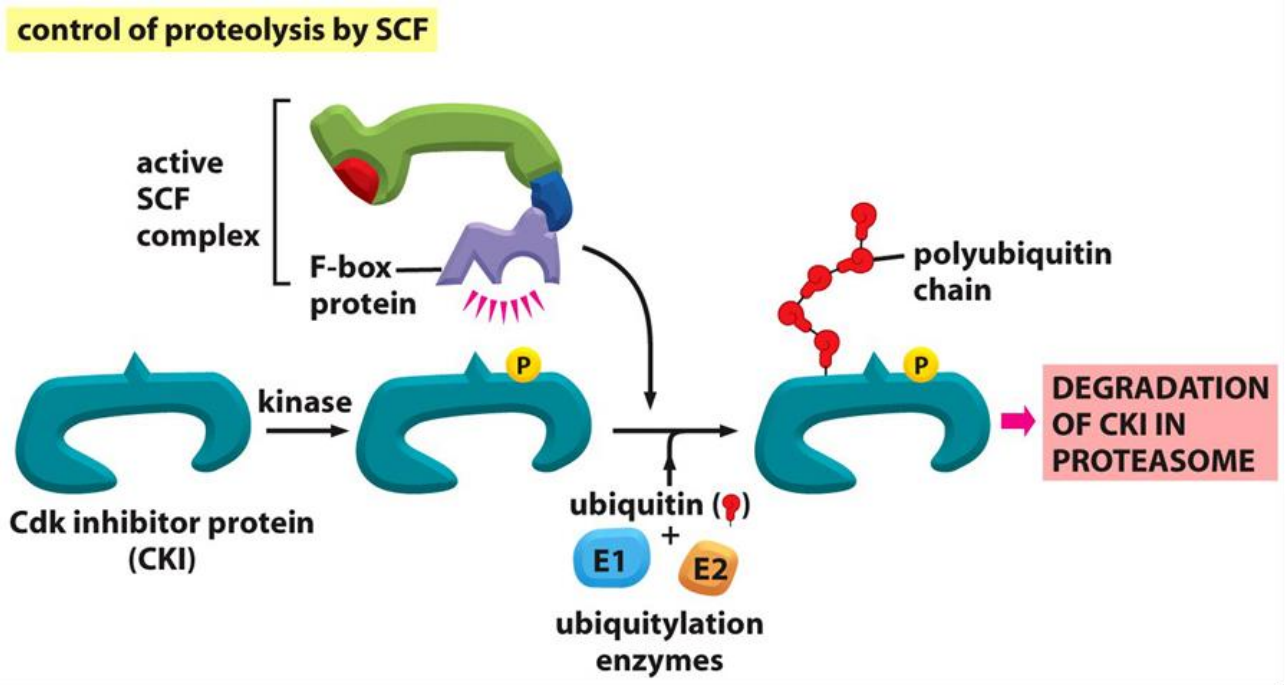
- One Key player: anaphase-promoting-complex, or cyclosome (APC/C), a ubiquitin ligase

Reminder: protein degradation mediated by uniquitination

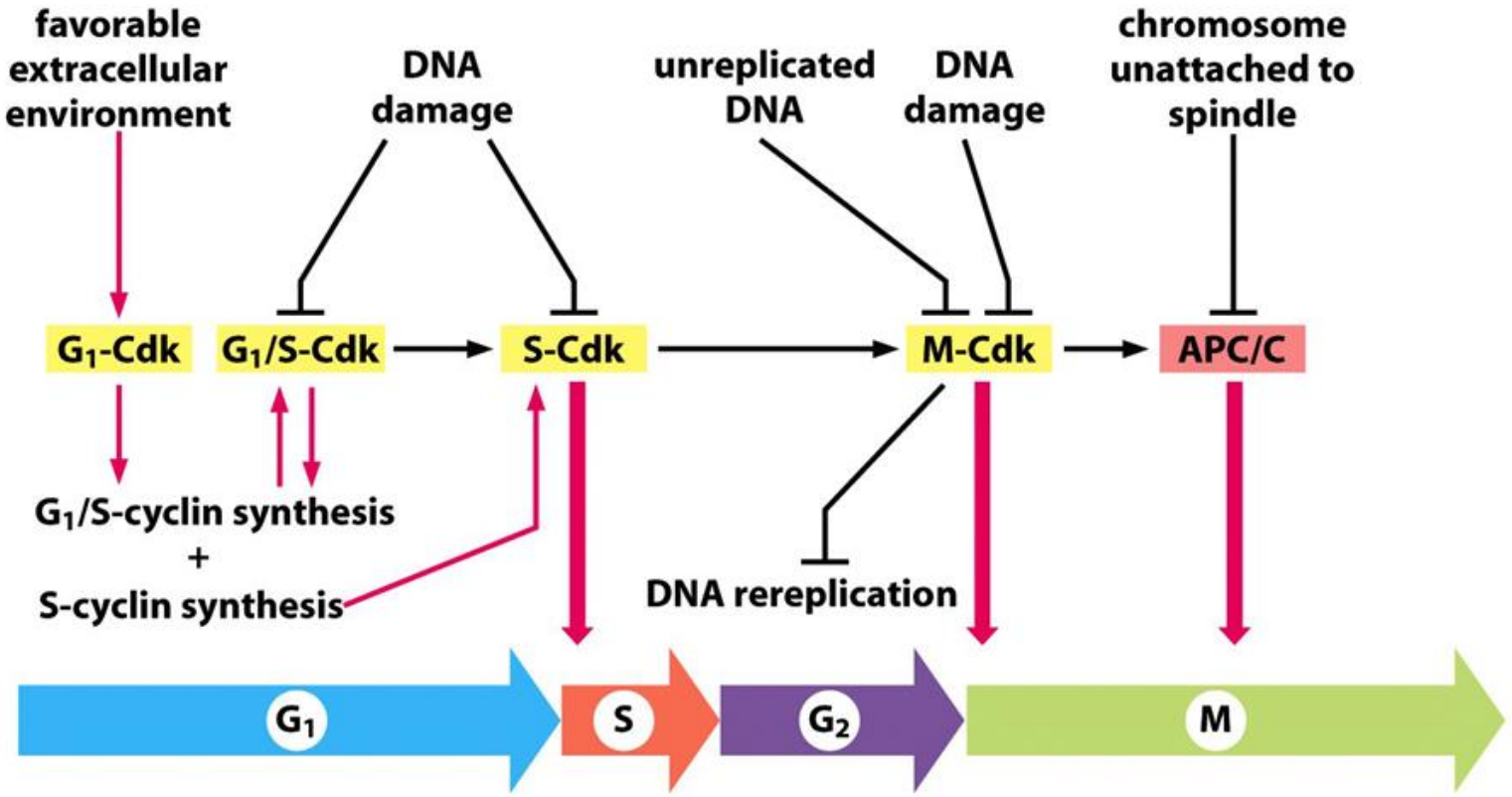
APC/C functions as ubiquitination ligase



CKI can be degraded by SCF complex

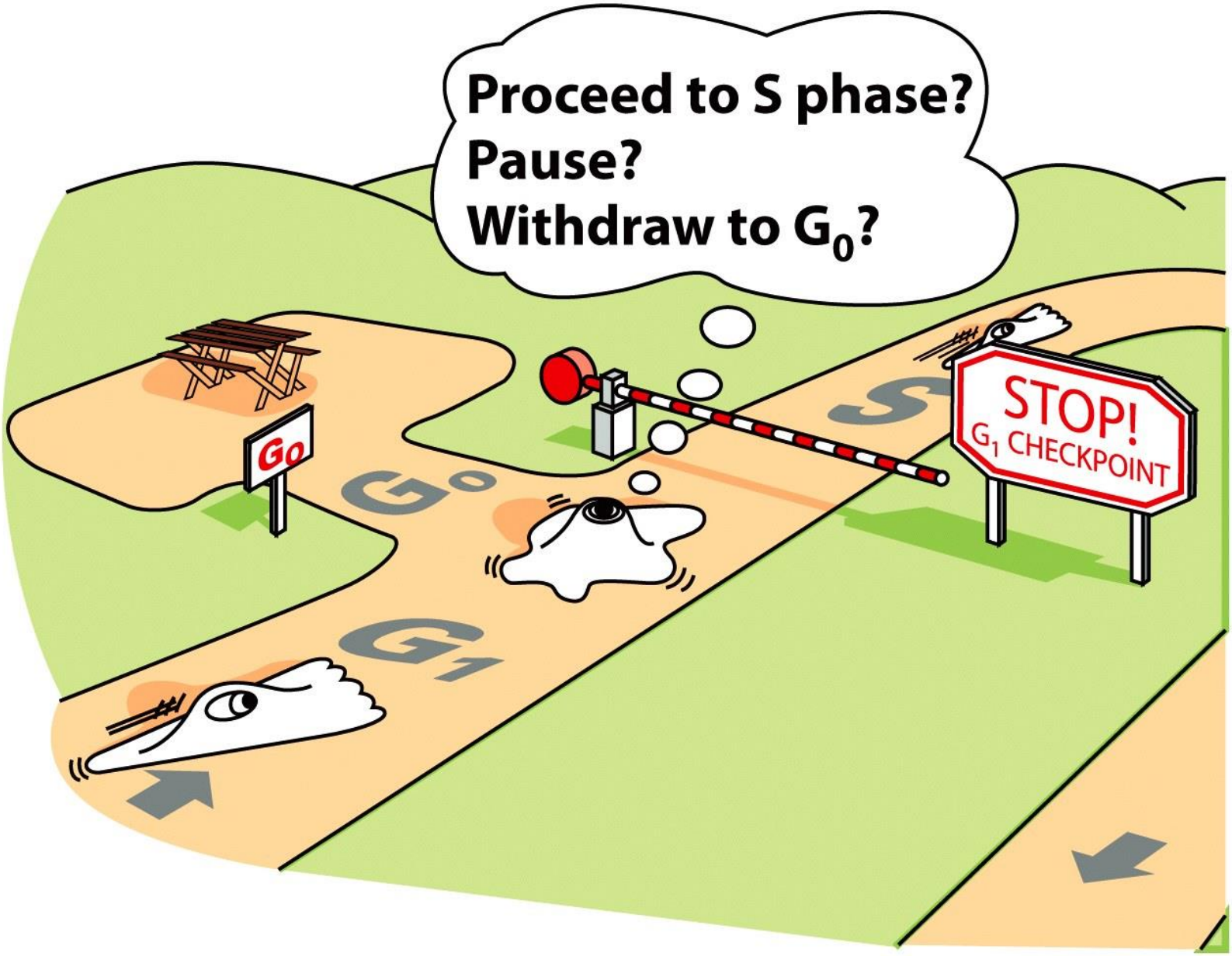


The overview of the cell cycle control system



III. S phase

1. **DNA replication once per cycle**
2. Chromatin protein replication
3. Chromatin structure duplication (histone modification, heterochromain, euchromain packaging)
4. Cohesions hold sister chromatids.
5. Centrosome duplication : semiconservative manner, triggered by Cyclin E/cdk2.



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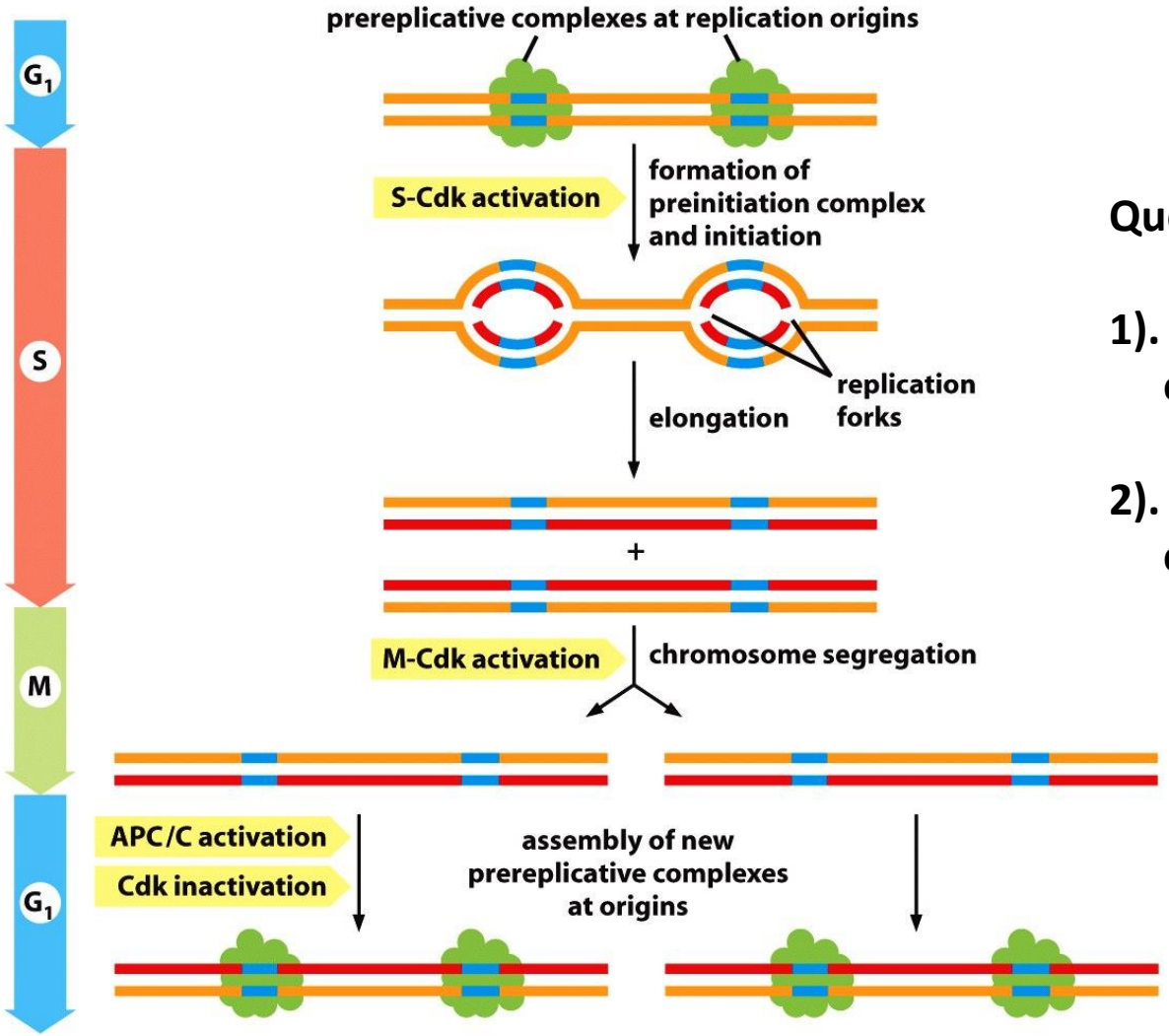
Chromosome duplication in cell cycle



Questions to be asked:

1). How to ensure DNA is replicated only once?

2). How do Cdks and APC/C control timed replication?

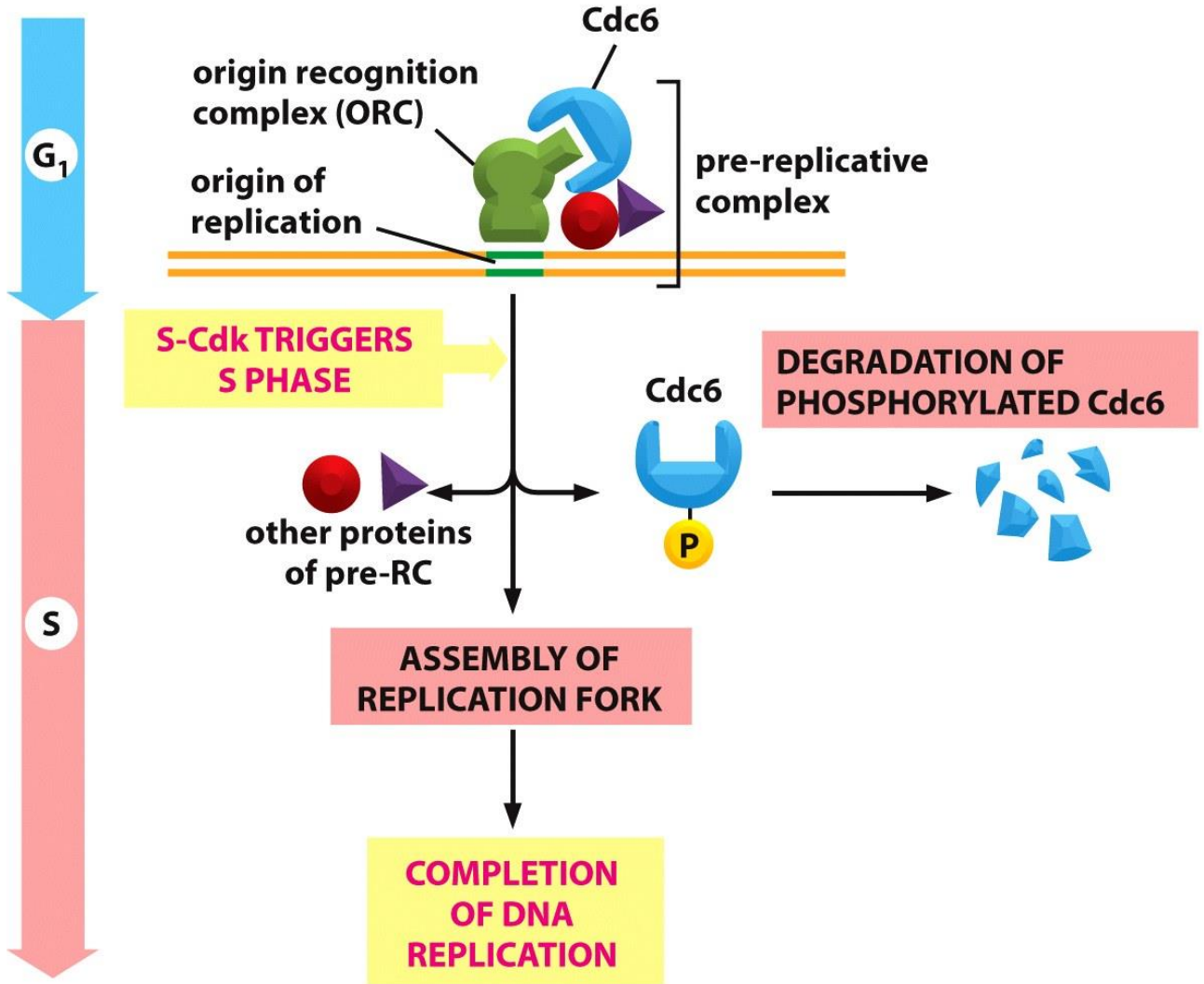
Question 1. how to ensure DNA replication once per cycle?

Answer

- **Pre-replication complex** (pre-RC) - “prime and licensing”
  activated by APC/C in late M and early G1 when APC/C activity is high.

- **Pre-initiation complex** – DNA unwinding, replication
  activated by S-Cdk in late G1 when APC/C activity is low, pre-RC is partially dismantled.

  S-Cdks and M-Cdks remain high until after late mitosis, when APC/C regains its activity, and start the next round of pre-RC formation.



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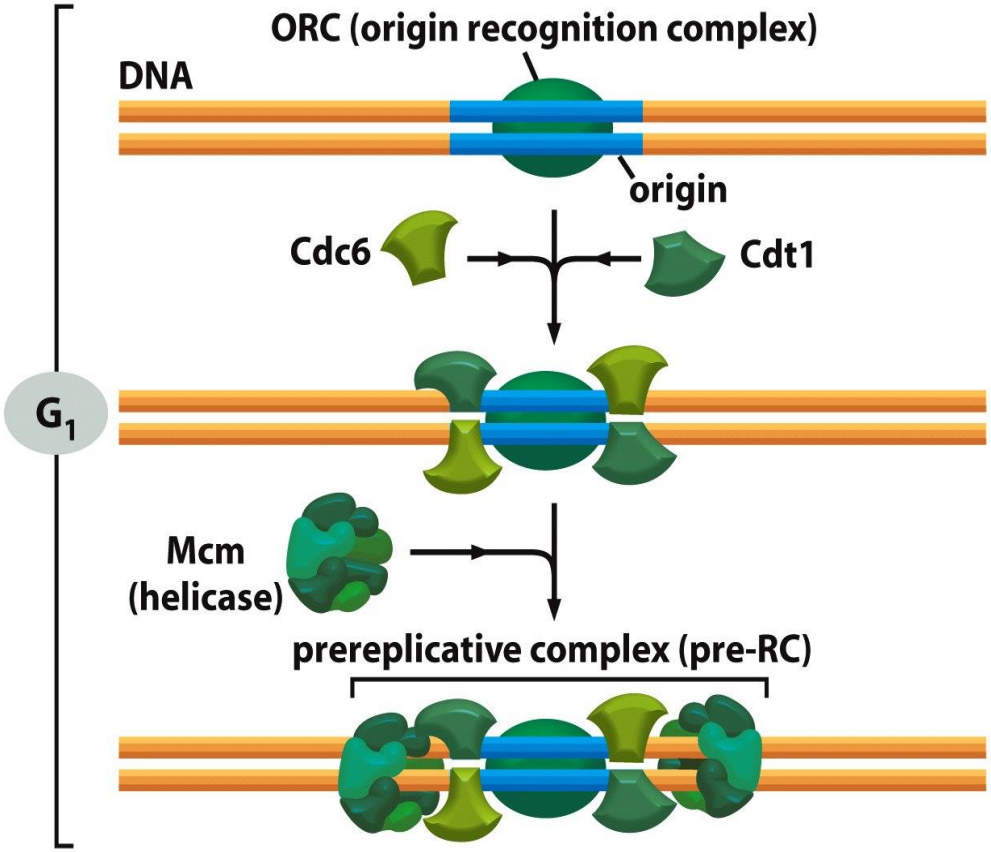
Question 2: how do Cdks and APC/C control DNA replication in a timed manner?

Stage 1: formation of pre-RC



Pre-RC is inhibited by Cdks
And promoted by APC/C

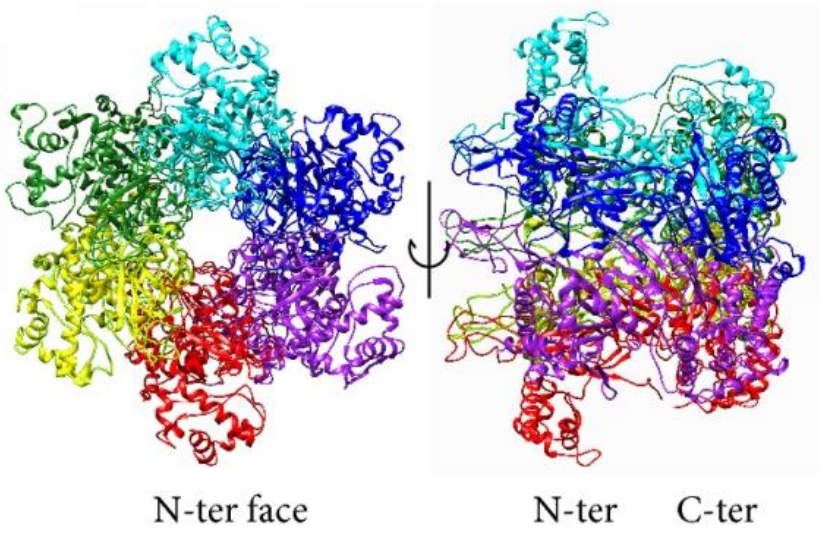
Late M/Early G1: low Cdk activity,
And high APC/C activity, pre-RC
Is formed.

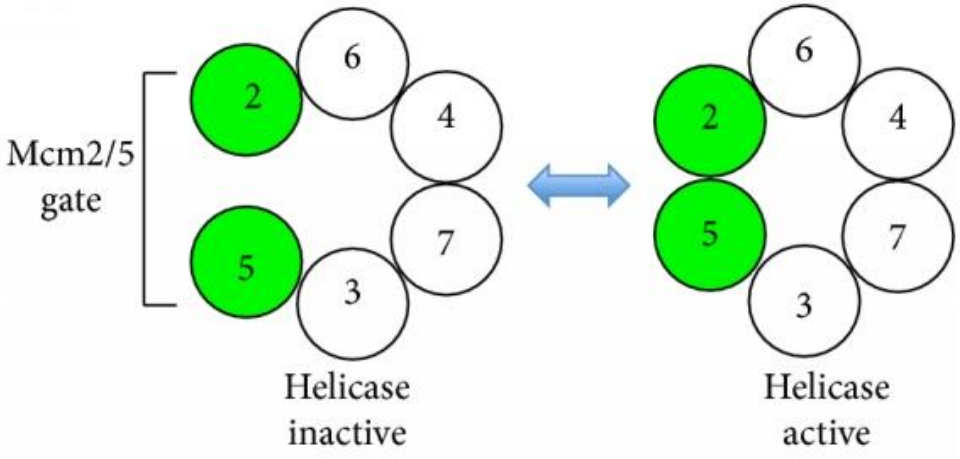
Firstly, cdc6 and cdt1 associate with ORC

Secondly, MCM ring load onto DNA

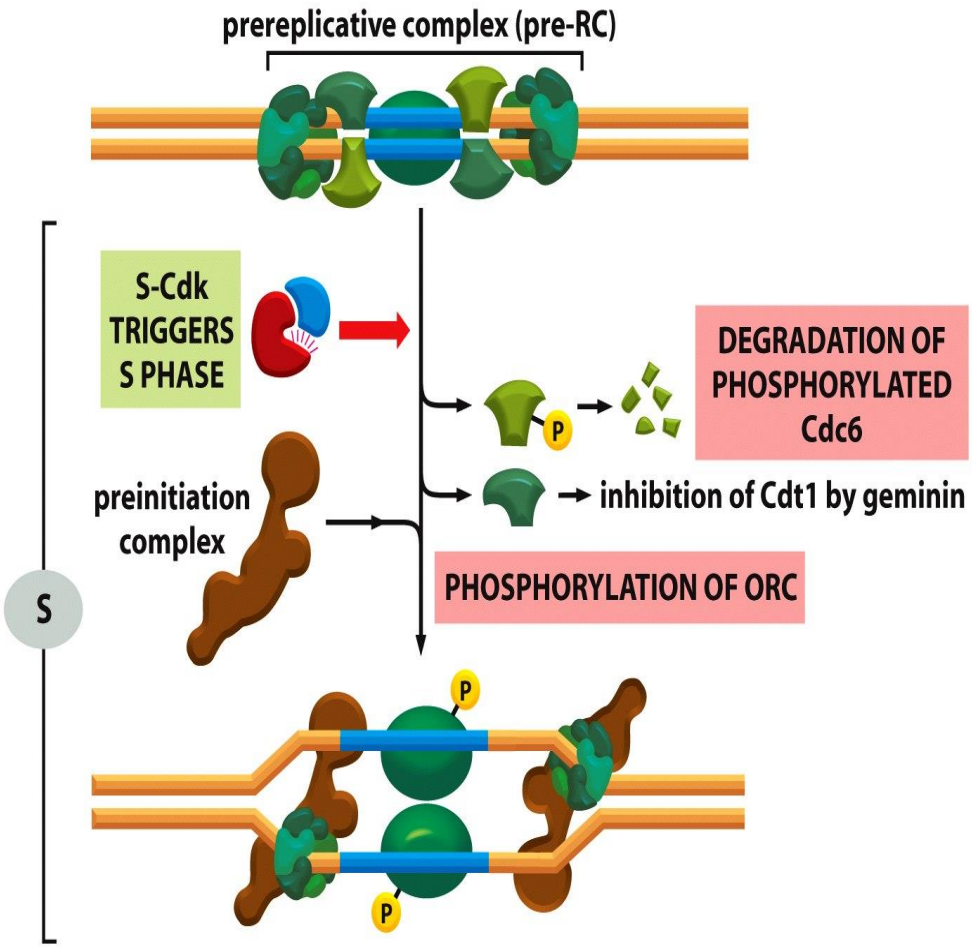
Thirdly, pre-RC is formed:
MCM/cdc6/cdt1/ORC

MCM2-7 ring complex





Stage 2: formation of Pre-initiation complex



Firstly, S-Cdk Trigger inactivation
Of Cdc6 AND Cdt1, dismantles pre-RC

Secondly, APC/C triggers degradation of geminin, but when S-Cdk is high and APC/C is low in activity, geminin is stabilized.

Thirdly, S-Cdk phosphorylates ORC
And load preinitiation complex onto ORC to initiate DNA replication.

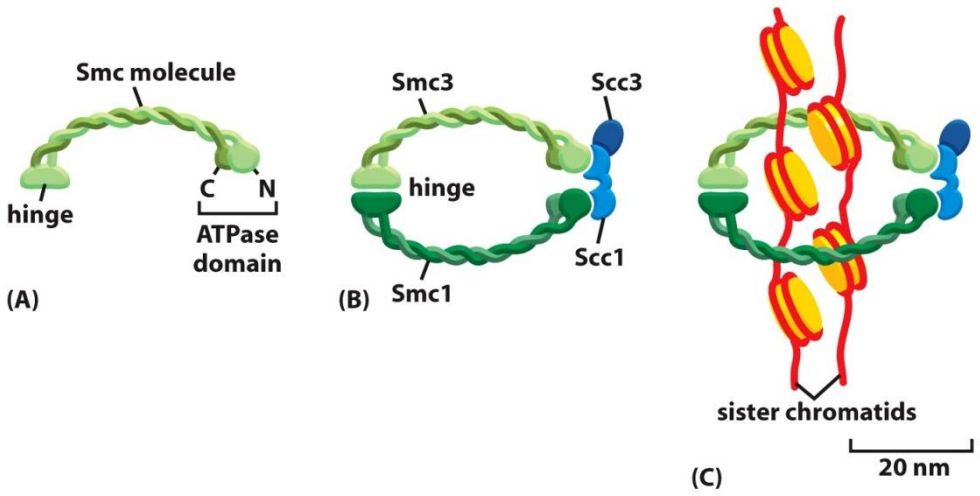
Cohesins and DNA catenation help hold sister chromatids together

♥ Cohesins are deposited at many locations along the length of each sister chromatids

♥ Prevent drifting apart for sister chromatids after DNA replication.

♥ DNA catenation is interwining of sister DNA molecules which can be resolved by DNA topoisomerase II

Cohesins



Centriole replication happens during S phase,