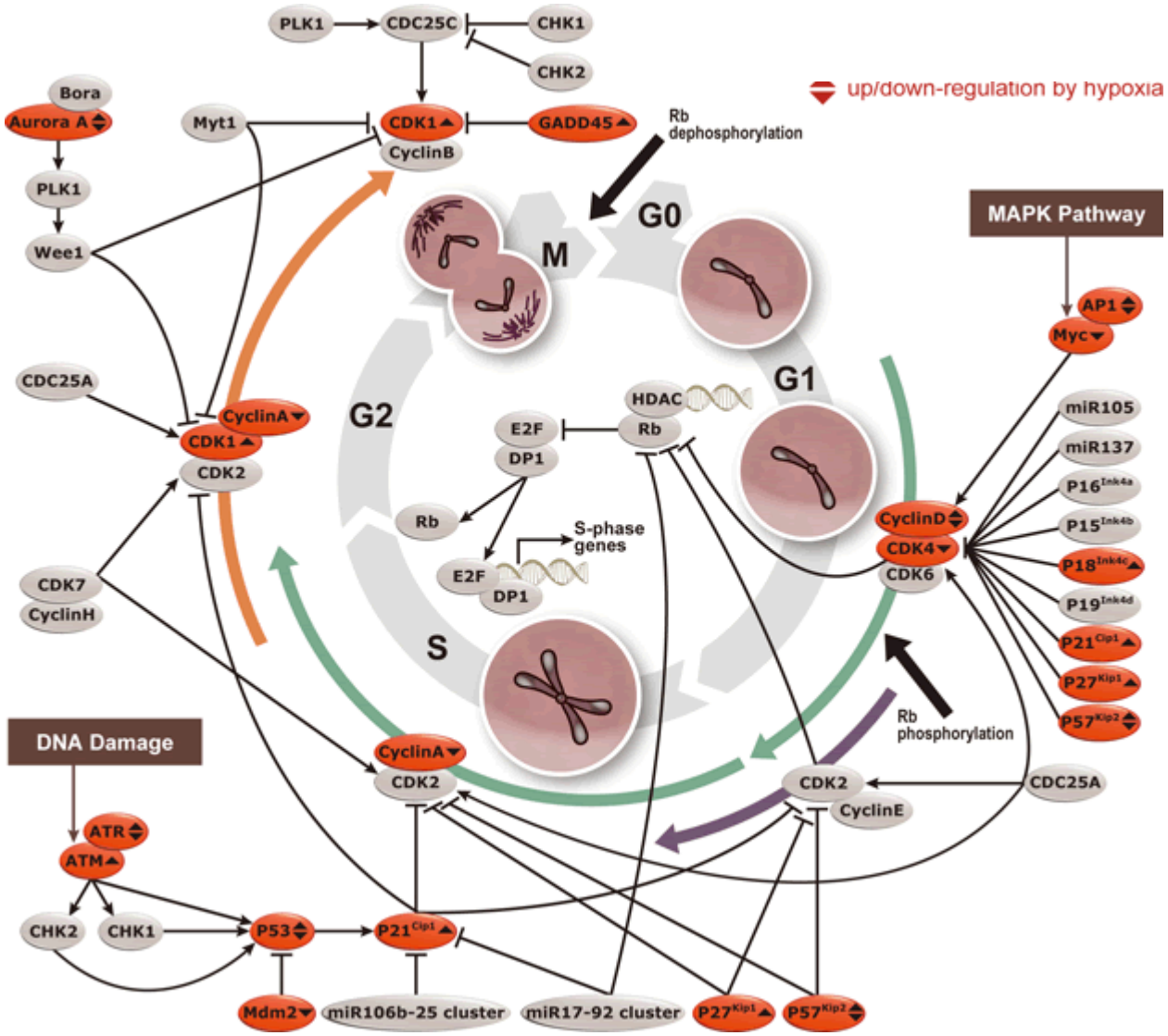
MUNI
SCI

Cell cycle

RNDr. Jan Škoda, Ph.D.
Department of Experimental Biology



Outline

- Definition and phases of the cell cycle
- The cell-cycle control system
- Molecular regulation of the cell cycle
- Defects in the cell cycle
- Models to study the cell cycle

The cell cycle

Omnis cellula e cellula

- **Law of cell lineage: *each cell (stems) from another cell***
- *Pathologic conditions result from defects in cells*

- 1857 – Rudolf Virchow

- 1852 – Robert Remak

The cell cycle

- Well-organized sequence of events in which the cell replicates by duplicating its content and dividing in two
  - Biogenesis of cell structures (and organelles)
  - Their distribution within the cell and to daughter cells
  - Unicellular organisms (bacteria, yeasts, protists) – new organism; Multicellular organisms: development, growth and tissue renewal/regeneration

- **A minimum set of spatiotemporally-organized processes** that a cell must perform **to pass the genetic information to the next generation of cells**: replication of genome, essential macromolecules and organelles; proper distribution into daughter cells

The cell cycle

Requires a precise control

All organisms

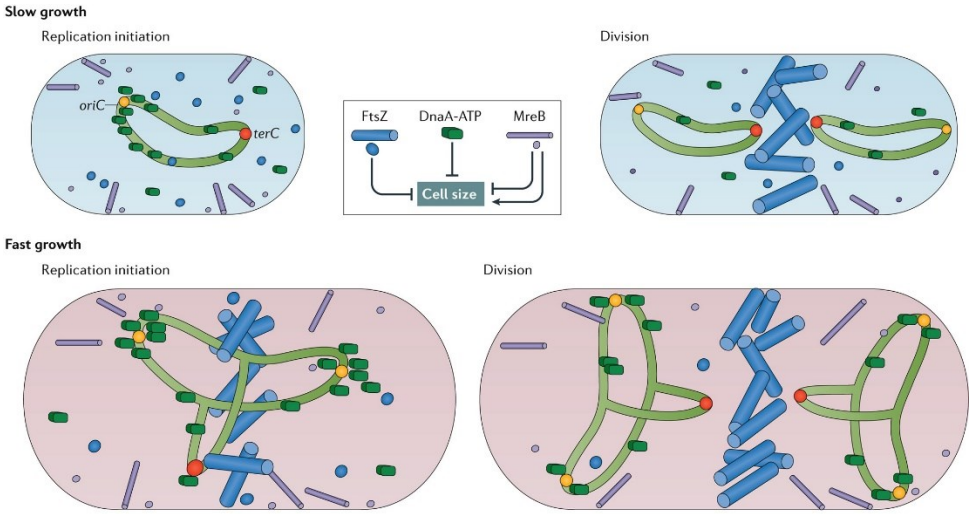
- Orchestrates **cell growth and division**: functional cell size

Multicellular organisms

- Orchestrates **cell division with development/renewal**
- Homeostasis and a proper function of tissues
- Prevents uncontrolled proliferation

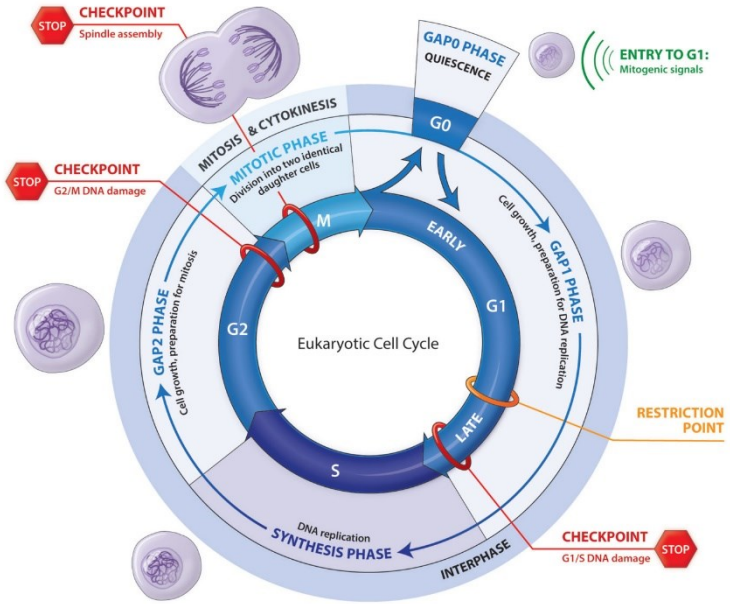
The cell cycle: growth and division

- **Prokaryotic cells**: **cell growth** affects spatial localization of **cell cycle determinants**



In media that support slow growth, replication initiation and Z-ring assembly occur sequentially (top panel), whereas in media that support fast growth, FtsZ can form the Z-ring a few minutes after cell birth, potentially coinciding with replication initiation (bottom panel).

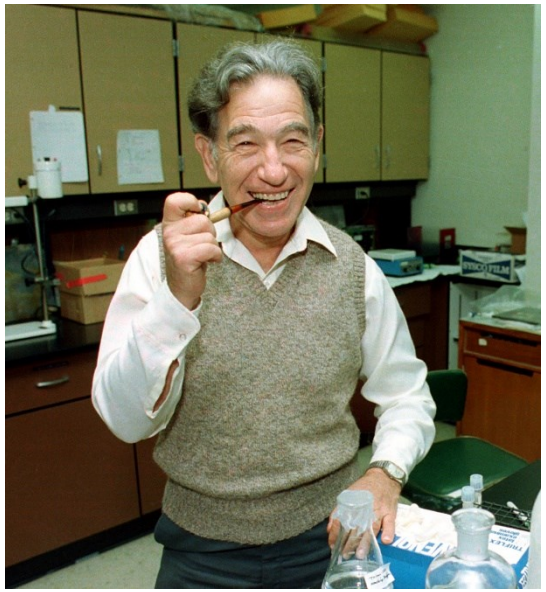
- **Eukaryotic cells**: cell cycle entry regulated by **growth factors** (restriction point: "point of no return")



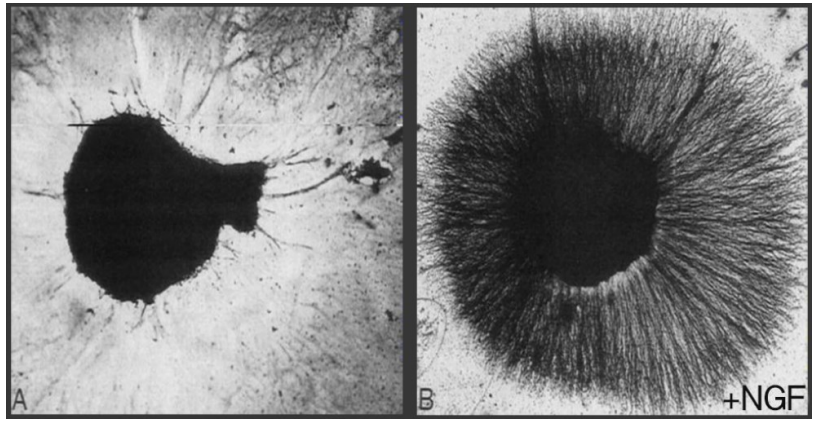
Growth factors and cell cycle

Rita Levi-Montalcini and **Stanley Cohen**

- 1950s discovered *nerve growth factor* (NGF) and *epidermal growth factor* (EGF)
- Nobel Prize in 1986 for “discoveries which are of fundamental importance for our understanding of the mechanisms which regulate cell and organ growth.”



- **Growth factors stimulate the proliferation of cells** (and regulate differentiation during development)



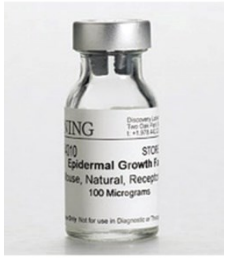
Growth factors and cell cycle

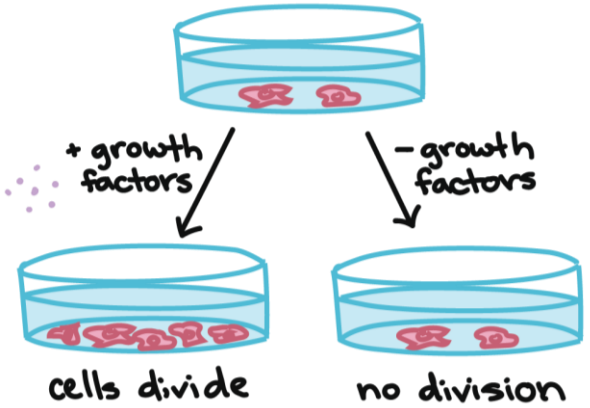
– **Mammalian cell culture** protocols include growth factors in media:

blood serum

OR

defined growth factors

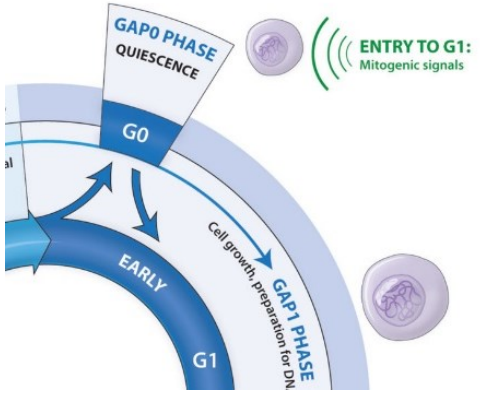




Phases of the eukaryotic cell cycle

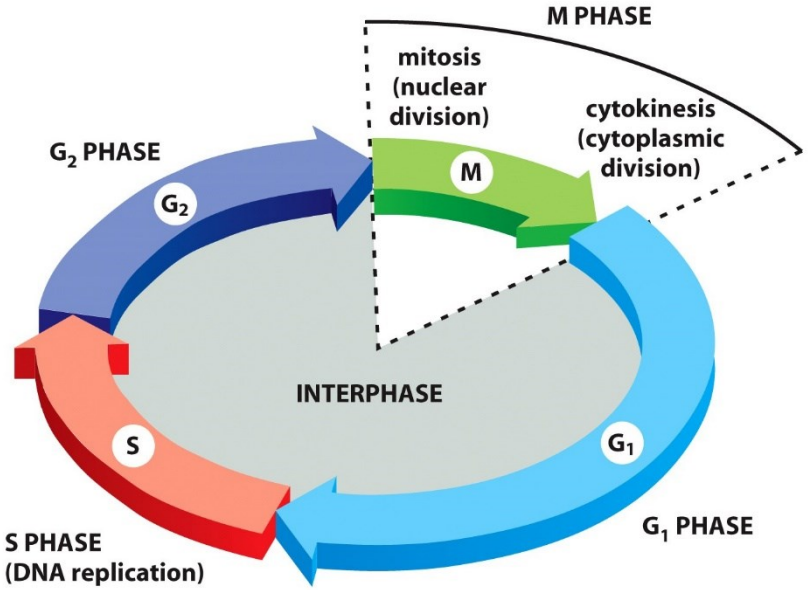
Interphase

- **(G0 phase)**
  - Resting state (quiescence)
  - Functional/metabolically active
  - Terminally differentiated cells
- **G1 phase**
  - Metabolic activity, cell growth
  - Biosynthesis, doubling of organelles
- **S phase**
  - Replication of DNA
- **G2 phase**
  - Biosynthesis of proteins essential for nuclear division and cytokinesis



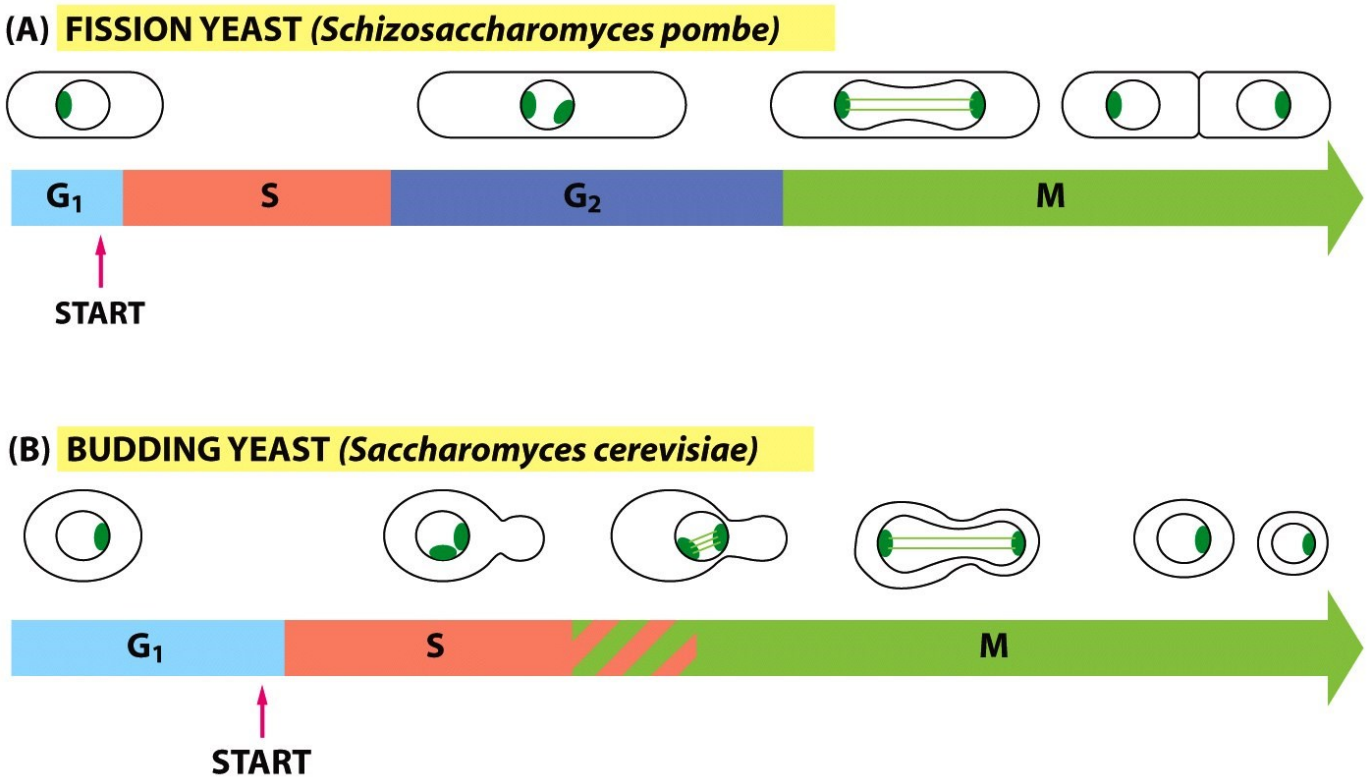
M phase

- Nuclear division (mitosis/meiosis)
- Cytokinesis



("G" phase comes from "gap" phase)

Not all eukaryotic organisms follow the typical eukaryotic cell cycle



- Budding yeasts: mitotic spindle forms during late S phase (no G2 phase)

The cell-cycle control system

Transition through different phases must be well controlled

Maintenance of genome integrity

- Proper sequence of different phases
  - DNA replication followed by nuclear division (not replicated twice, prevents gene amplification)
- Fully replicated error-free DNA
  - Nuclear division must not start before DNA replication is completed
- Proper segregation of chromatids/chromosomes

Homeostasis

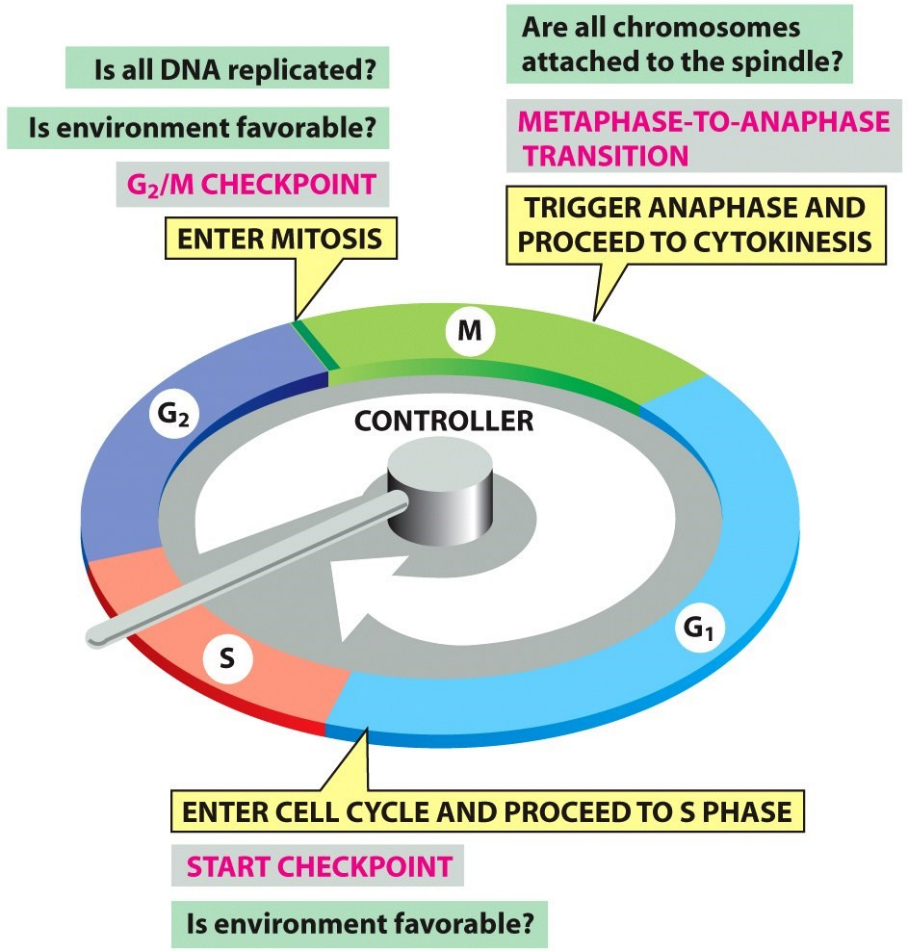
- In coordination with developmental programs

Cell cycle checkpoints

- **Regulate the cell cycle progression**

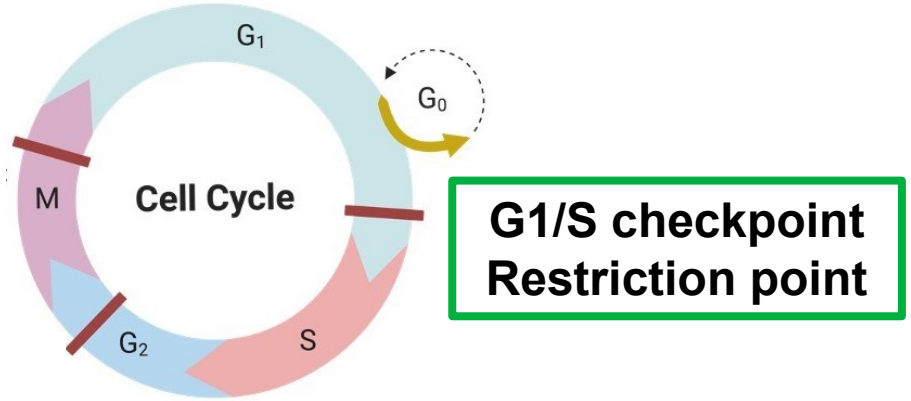
Three major checkpoints:

- **Late G1 (G1/S):**
  Restriction point (mammalian cells)
  Start (yeasts)
- **Late G2 (G2/M)**
- **Metaphase-to-anaphase transition**



G1/S checkpoint – Restriction point

- **Favorable environment?**
  - Enough nutrients, nucleotides
  - **Extracellular mitogenic signals** (growth factors; homeostasis-related)
- **DNA undamaged?**



YES:

- **"Point of no return"** → committed to proceed through the cell cycle

NO:

- **Progress delayed**
  - e.g., DNA repair
- **Or enters G0 phase**
  - Terminally differentiated cells
  - Quiescent cells (adult stem cells)
  - Senescent (aged) cells
- **Or undergoes apoptosis**

G2/M and metaphase-to-anaphase

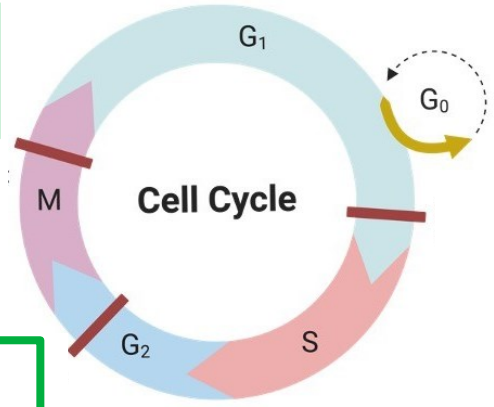
G2/M checkpoint

- **DNA fully replicated, no damage?**
- Sufficient cell size? (Yeasts)

Metaphase-to-anaphase transition **(spindle checkpoint)**

- Chromosomes (chromatids) attached to spindle?
- Aligned in the metaphase plate?

Spindle checkpoint



G2/M checkpoint

Delay progression until problems solved / Chronic activation leads to cell death

Molecular regulation of the cell cycle

Central regulatory system

Cyclins & cyclin-dependent kinases (Cdks)

Cyclins

- Undergo **cycles of synthesis and degradation**
- **Regulate Cdk activity – positive regulators: bind to and activate Cdks**

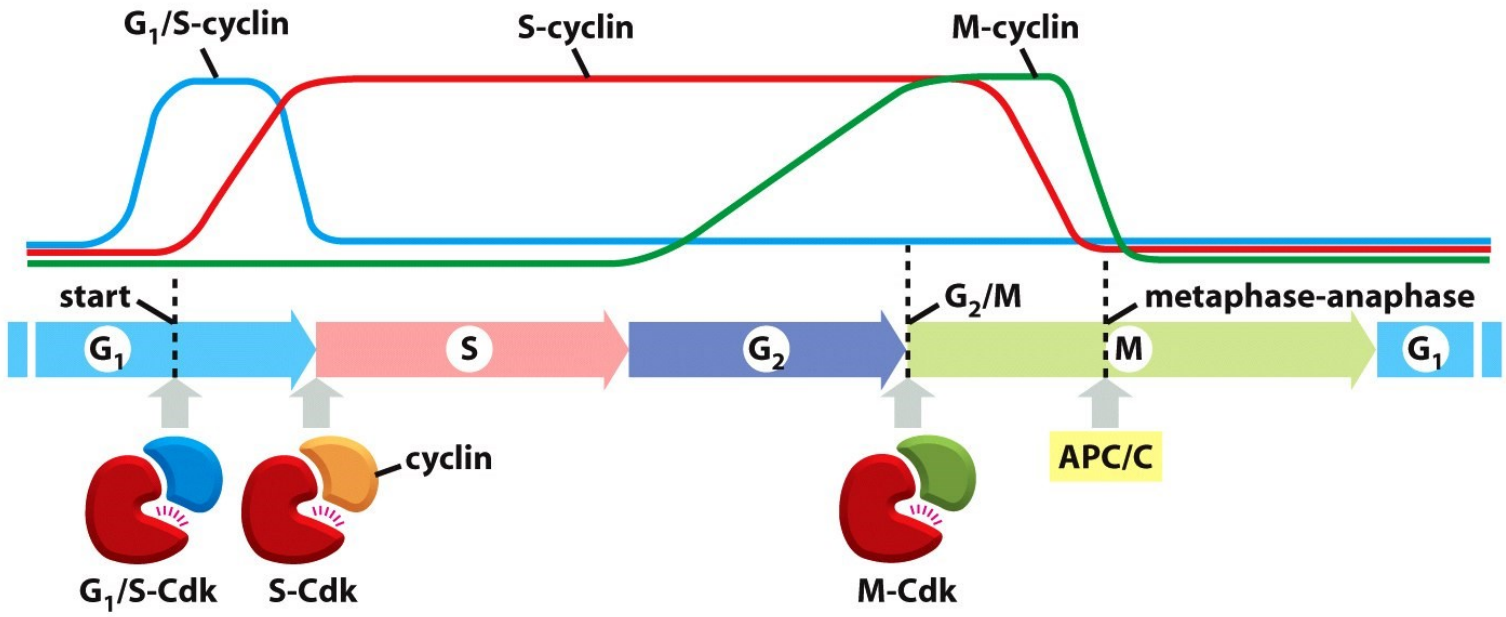
Cdks

- **Protein kinases: active Cdks phosphorylate target proteins**
- **Govern progression through the cell cycle**

Different cyclin-Cdk complexes = different target proteins

Central regulatory system

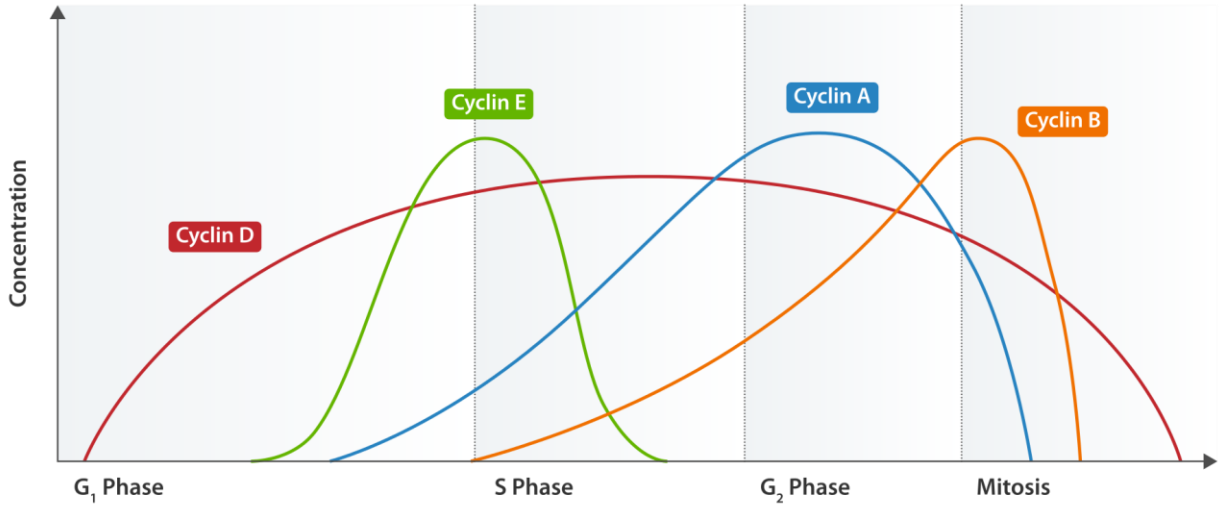
- **Cdks constantly present**
- Cyclins synthesized and degraded during cell cycle
- Regulated by **cell signaling** and **degradation systems**



Cyclin-Cdks complexes specific for each phase

CYCLIN–CDK COMPLEX	VERTEBRATES CYCLIN	VERTEBRATES CDK PARTNER
G_1-Cdk	cyclin D*	Cdk4, Cdk6
G_1/S-Cdk	cyclin E	Cdk2
S-Cdk	cyclin A	Cdk2, Cdk1**
M-Cdk	cyclin B	Cdk1

- Help progression through G1 (Rb phosphorylation)
- **Progression through Restriction point**
- Mainly promote DNA replication
- Stimulate entry into the M-phase – **progression through G2/M checkpoint**



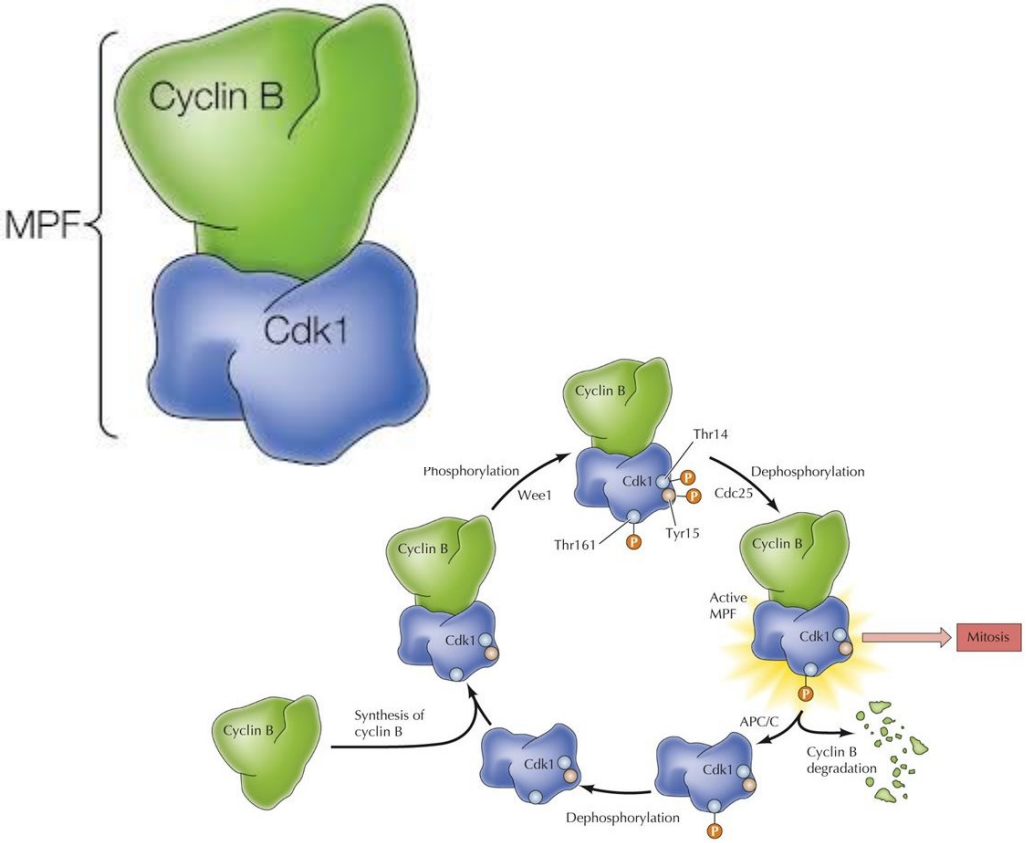
Evolutionary conserved

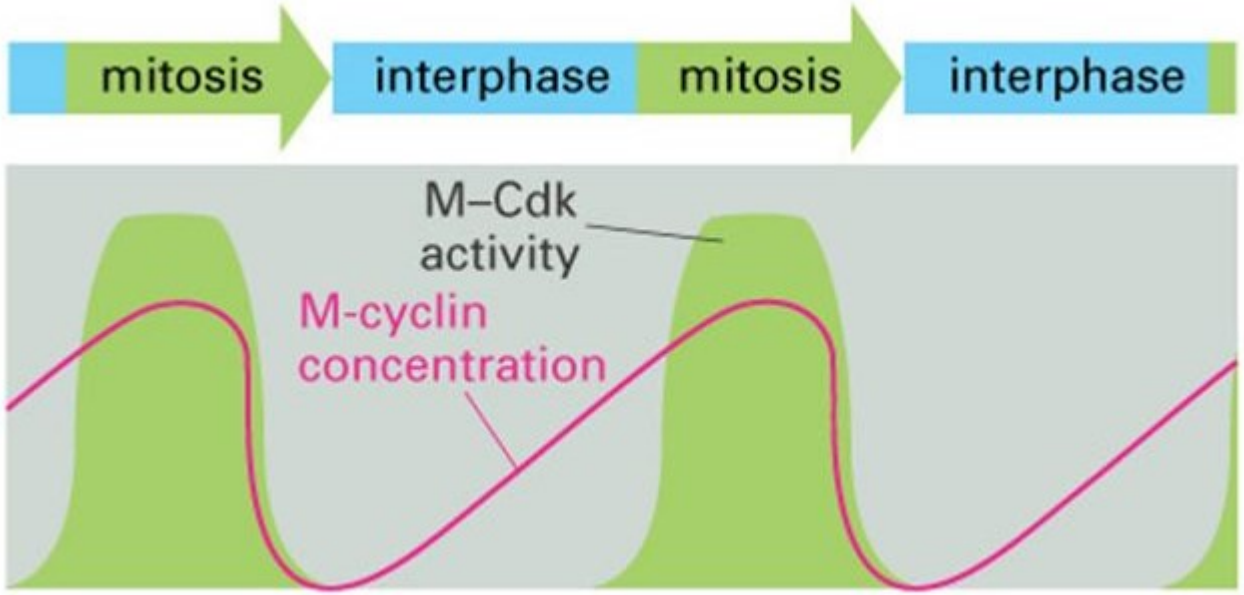
- Yeast cyclins/Cdks can be compensated by human homologs

CYCLIN–CDK COMPLEX	VERTEBRATES CYCLIN	VERTEBRATES CDK PARTNER	BUDDING YEAST CYCLIN	BUDDING YEAST 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

Mitosis promoting factor (MPF): M-Cdk

- Cyclin B + Cdk1 (Cdc2)

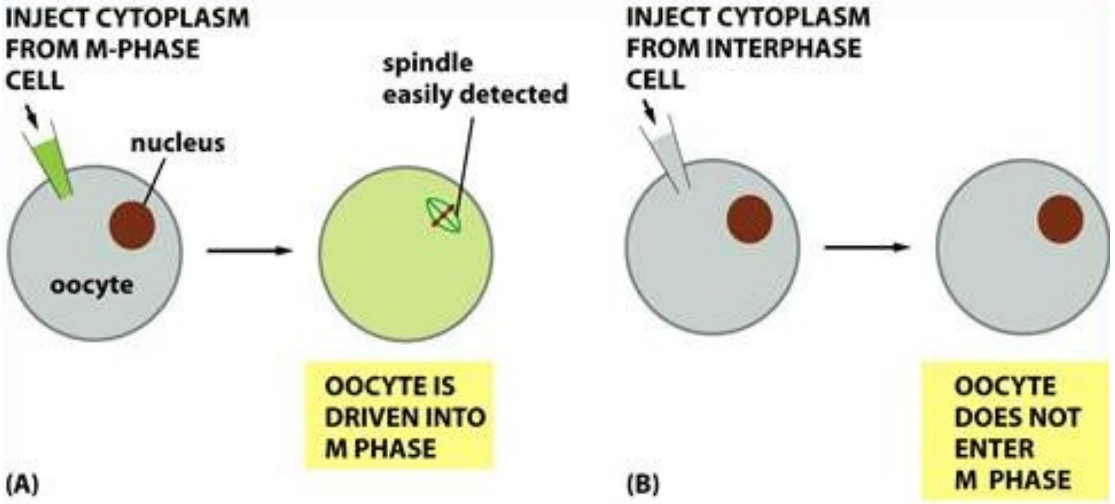




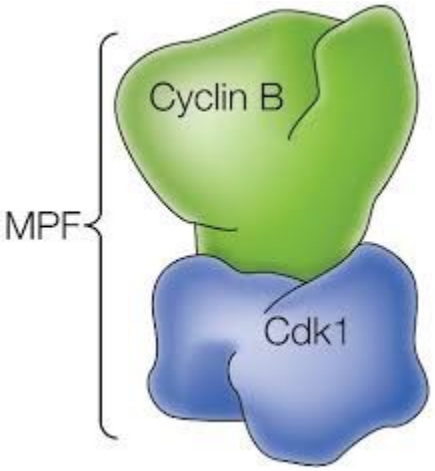
- M-Cdk (MPF) activity triggers progression to M-phase

Discovery of MPF

- 1971: Masui & Markert, Smith & Ecker
- Microinjection of M-phase vs. interphase cytoplasm into oocytes of frog (*Rana pipiens*, northern leopard frog)
- **M-phase cytoplasm contains a factor inducing meiosis (M-phase) → MPF**



Function of MPF (M-Cdk)



- **Phosphorylates target proteins → initiates processes essential for M-phase**
  - **Nuclear envelope breakdown**: phosphorylates nuclear pore complex subunits & proteins of nuclear lamina
  - **Initiates and promotes spindle assembly**: phosphorylation of motor proteins associated with microtubules
  - **Increases microtubule dynamics**
  - **Chromosome condensation**: phosphorylation of condensin proteins

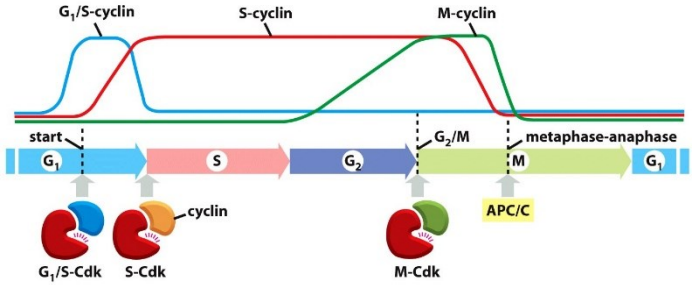
- Progression through G2/M checkpoint

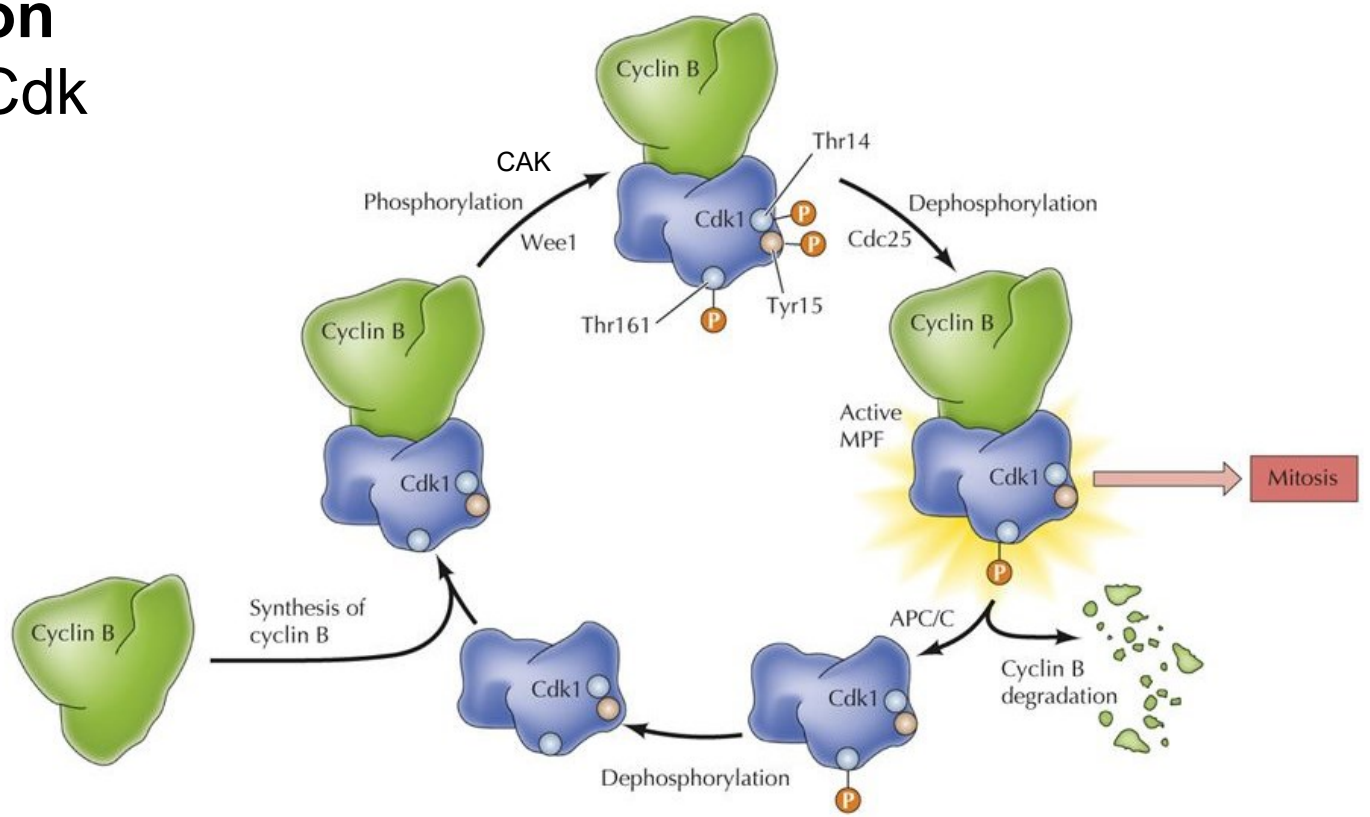
Regulation of M-Cdk activity

- **Cyclin B levels & protein kinases**
  - Wee1: **Inhibitory phosphorylation**
  - **Activating phosphorylation** by Cdk activating kinase (CAK)
  - Cdc25: **Activating dephosphorylation**

→ **Active M-Cdk: M-phase**

- Tagged for **proteasomal degradation** by **APC/C**





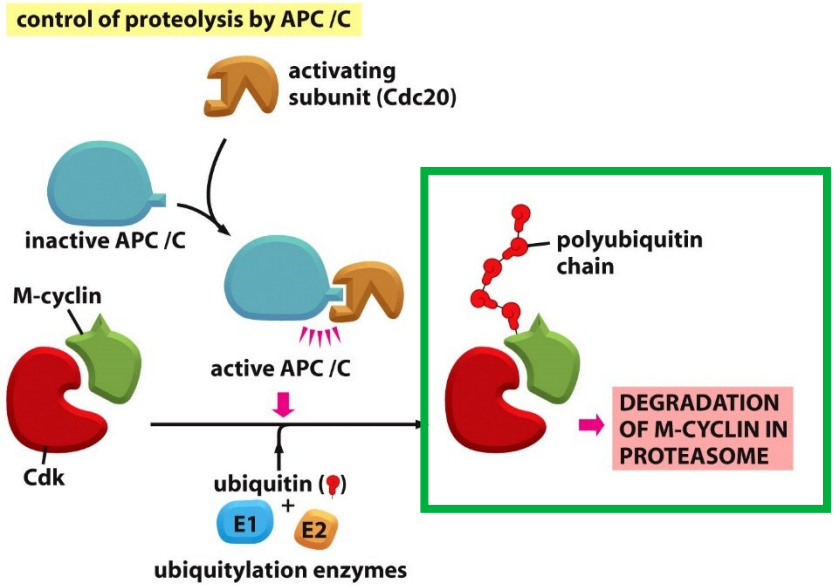
APC/C (anaphase promoting complex/cyclosome)

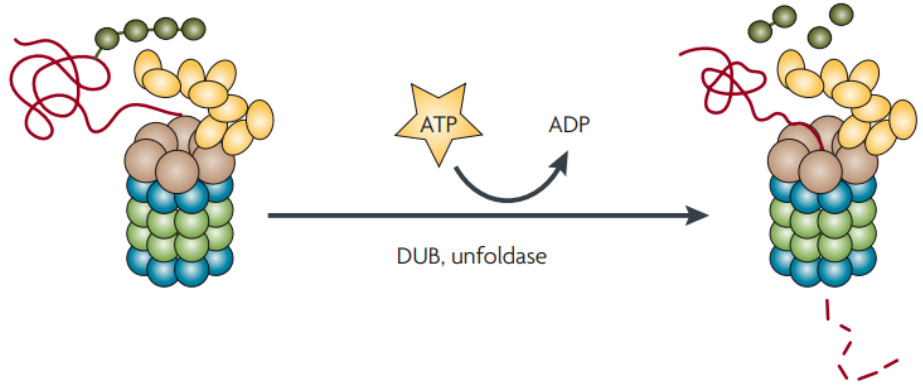
- **Ubiquitin ligase:** adds multiple ubiquitin molecules to target proteins
- Polyubiquitylated proteins tagged for **degradation by proteasome**
- APC/C target specificity regulated by activating subunits
- Degradation targets
  - Securin (holds sister chromatid pairs) → promotes transition to anaphase
  - S- and M-cyclins → dephosphorylation and inactivation of Cdks (APC/C active to early G1)

- Progression through spindle checkpoint
- **Resets the cell-cycle control system (Cdks inactivation)**

Proteolysis by proteasome

- Ubiquitination (ubiquitylation) – tag for **proteasomal degradation**
- **Important regulatory role: Rapid degradation of cyclins** of preceding phases

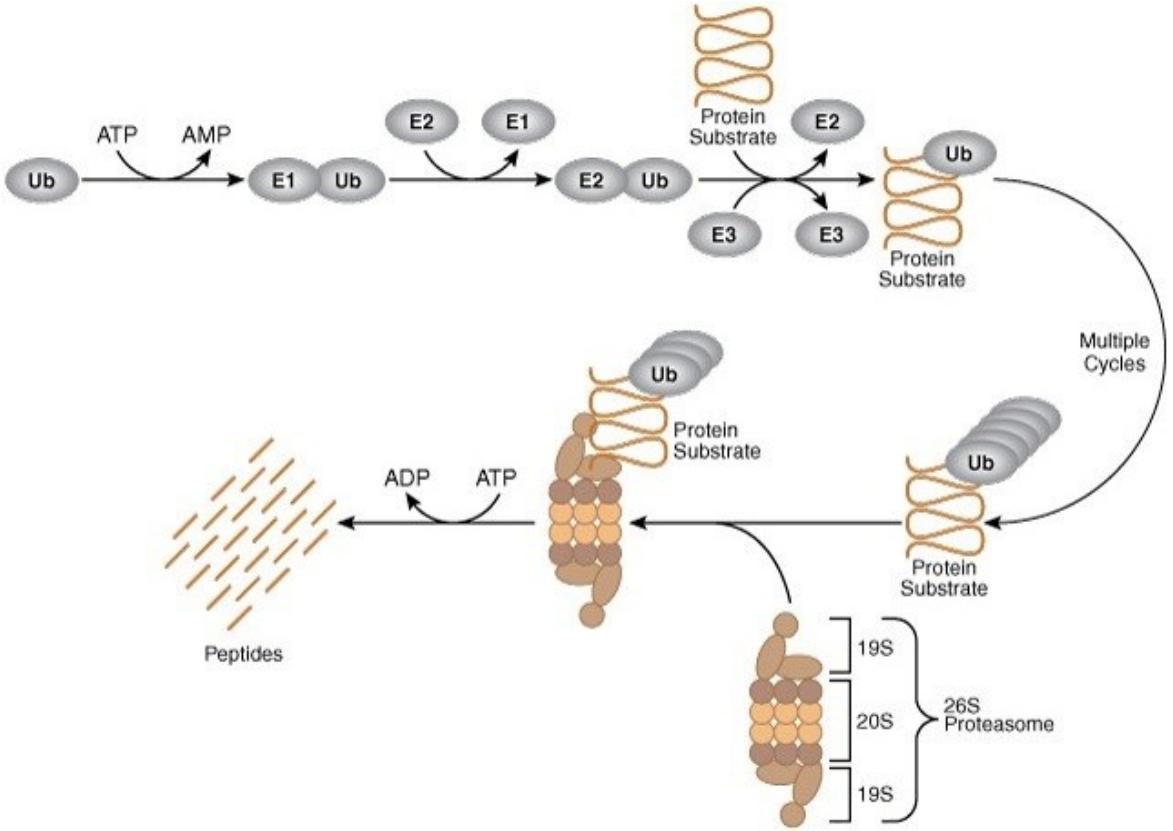




Degraded protein (peptides)

Ubiquitin-dependent proteolysis

- **Multiple cycles of ubiquitination**
- Ubiquitin transferred from ubiquitin conjugating enzyme, E2, to target protein by **E3 ubiquitin ligase**
- **Ubiquitin covalently attached**
- **Polyubiquitinated proteins transferred to proteasome → Cleavage**



Signals regulating cell cycle entry

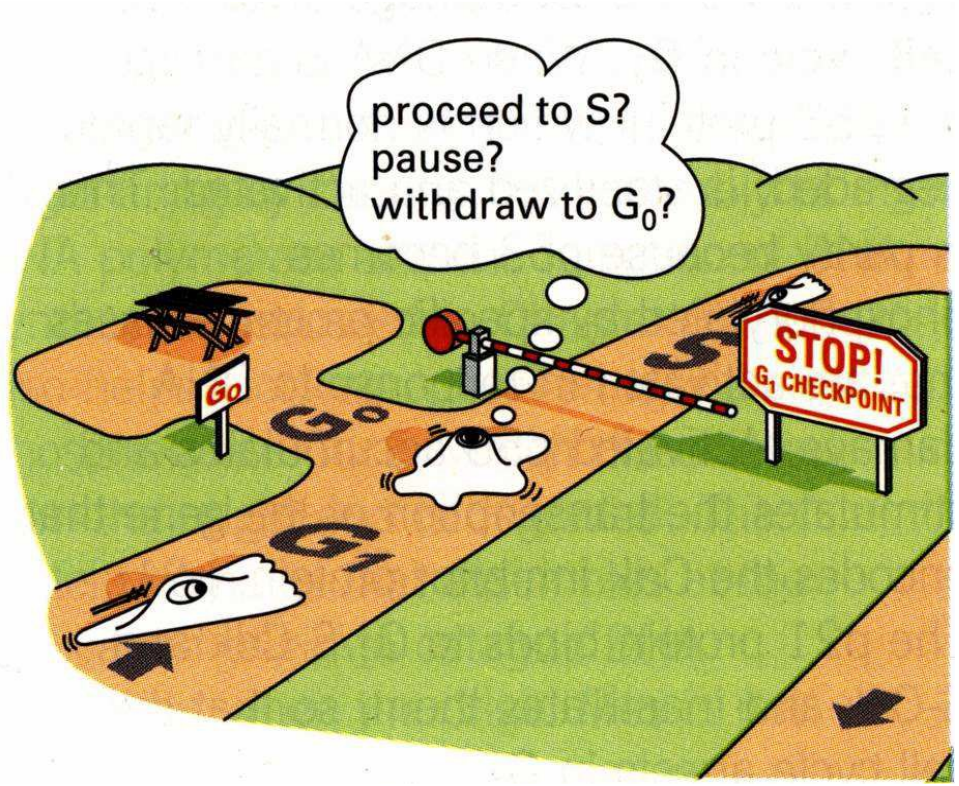
Unicellular

- Determined by nutrients availability (similar to prokaryotes)

Multicellular

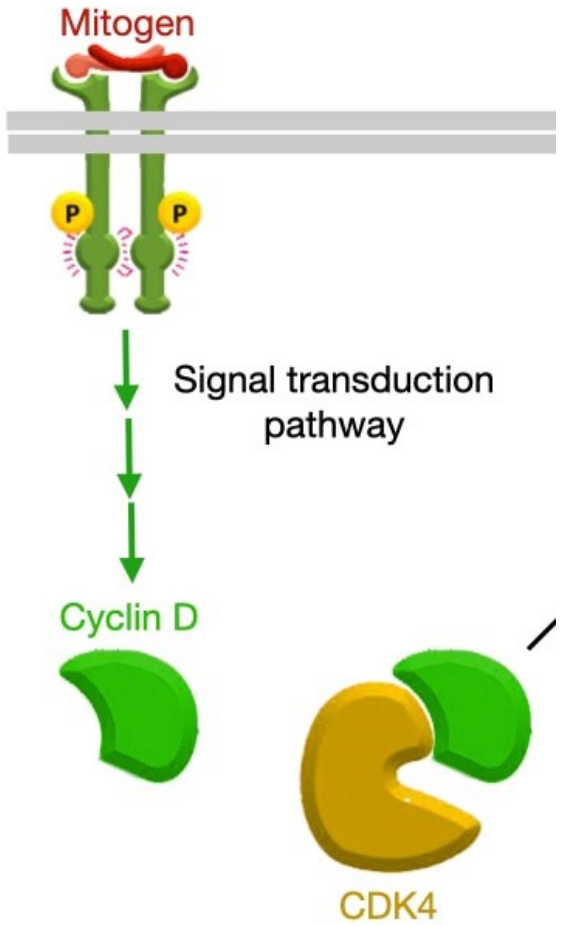
- **Mitogenic signals (growth factors)**
  - Produced by surrounding or distant cells within the organism
  - Homeostasis – **only when necessary to proliferate**: growth, renewal
- **Lack of mitogenic signaling**
  - Cell cycle halted/blocked (G0) – intrinsic braking mechanisms prevail

Cell cycle arrest in G0



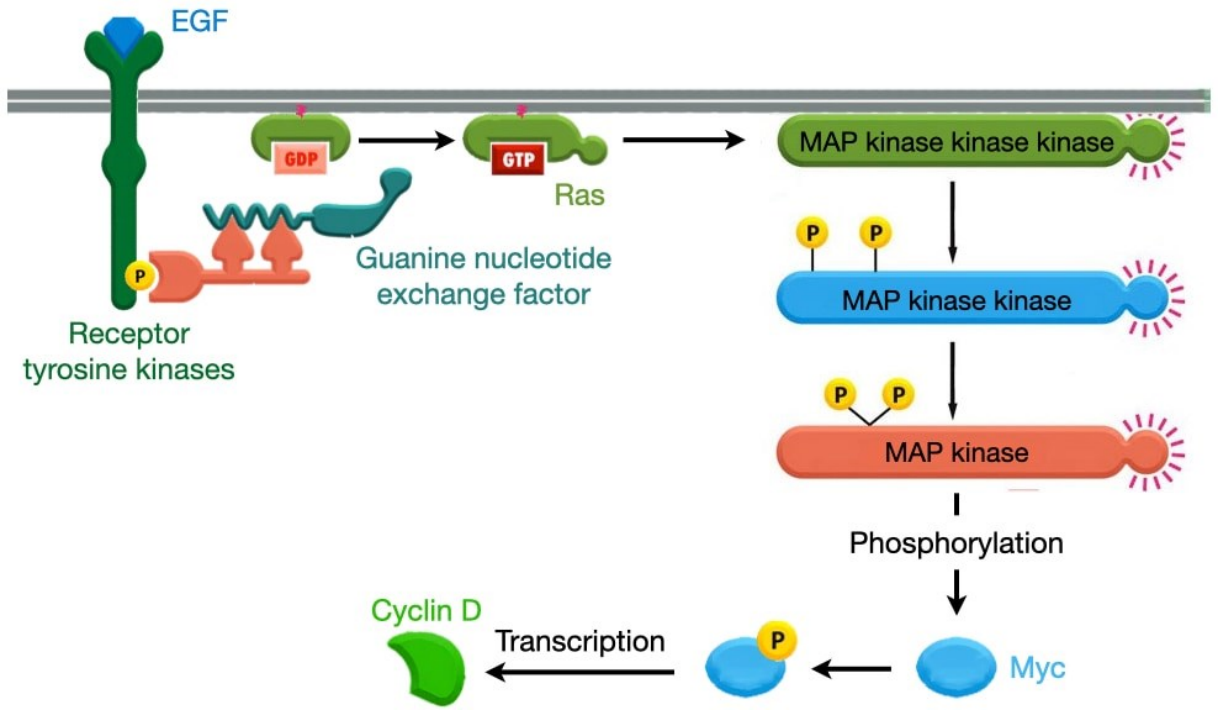
- **Lack of mitogenic signaling**
  - May be restored by mitogens
- **Terminally differentiated cells**
  - Expression of Cdks and cyclins permanently turned off – cell cycle not responsive to mitogens
  - e.g., neurons, skeletal muscle cells
  - Reprogramming: dedifferentiation restores cell cycle
- **Replicative senescent cells**
  - Shortened telomeres (DNA damage) prevent progression through G1/S checkpoint
  - Restricts number of cell divisions

Mitogenic signaling leads to cyclin D expression



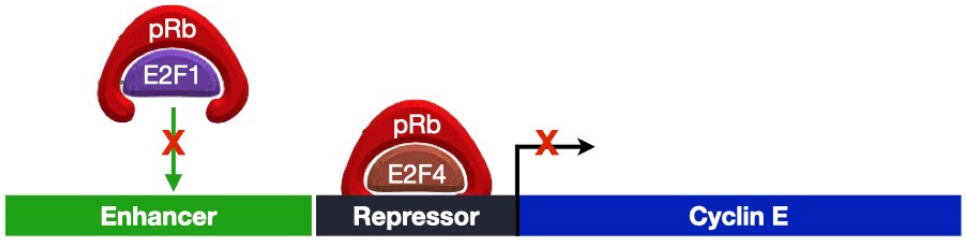
Helps to initiate cell cycle progression

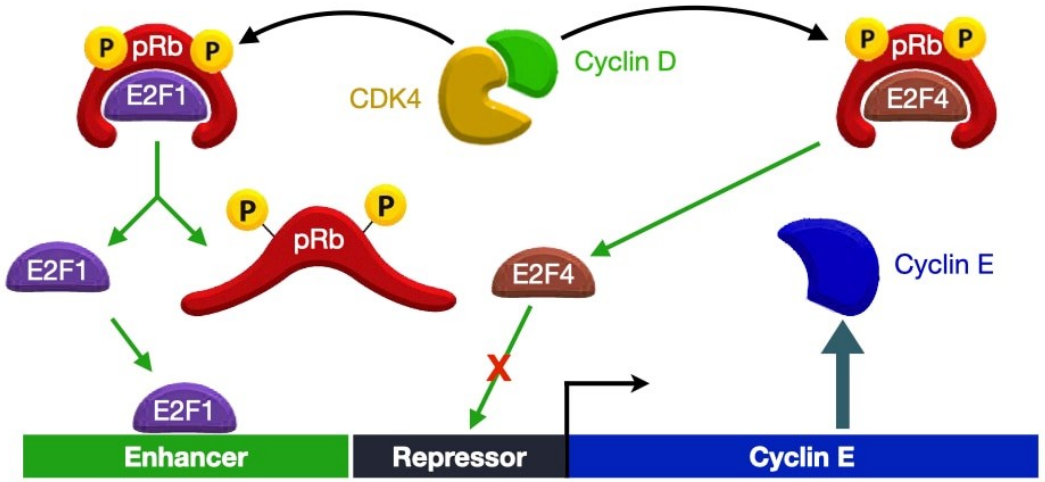
– Epidermal growth factor (EGF) induced expression of cyclin D



How cyclin D helps to overcome restriction point?

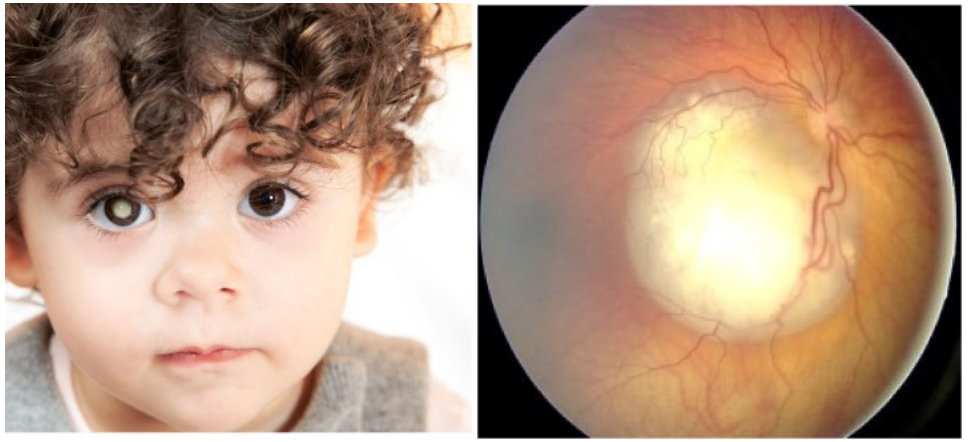
– Cyclin D/CDK4 phosphorylates protein Rb – release of E2F transcription factors – expression of cyclin E and other S-phase proteins

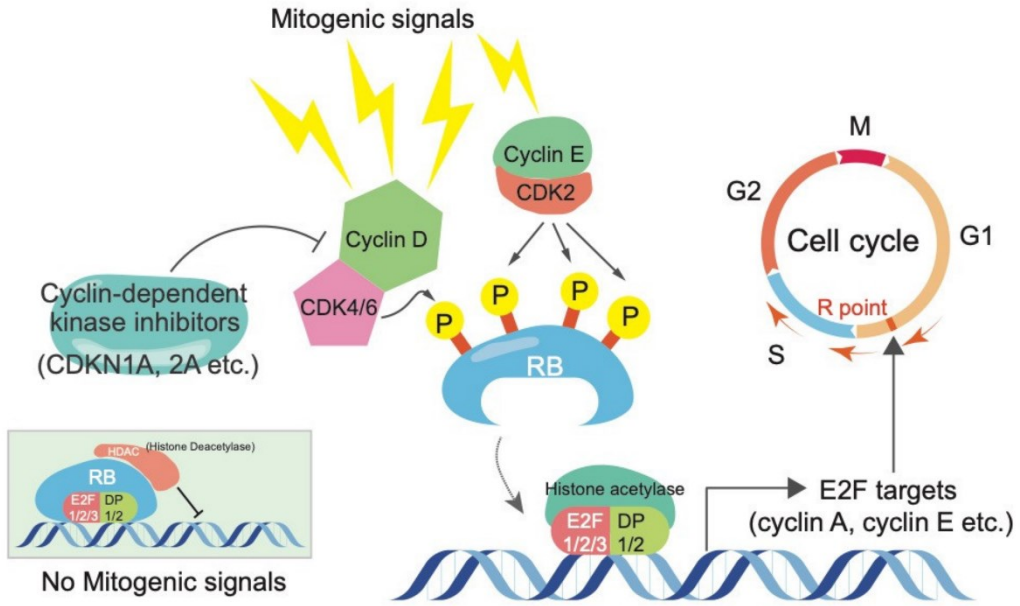




Protein Rb (retinoblastoma)

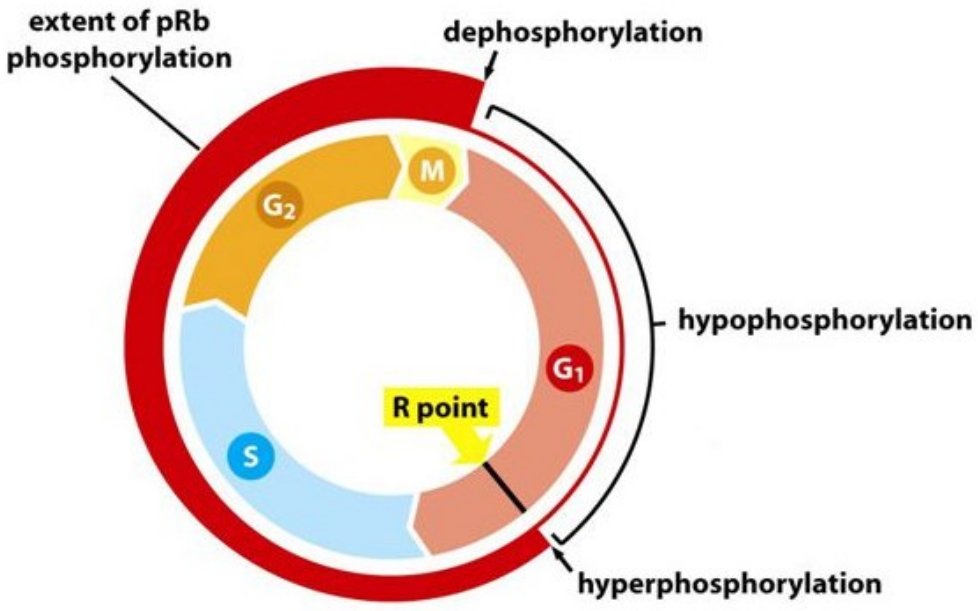
- **Tumor suppressor**
- Intrinsic negative cell cycle regulator
- Nuclear protein binding E2F
- **Prevents transcription of E2F targets**: genes encoding proteins involved mainly in DNA replication and cell cycle progression
- Rb phosphorylation – binding inhibited, E2F released



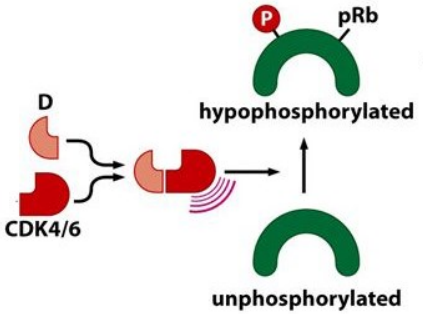


Protein Rb phosphorylation & dephosphorylation

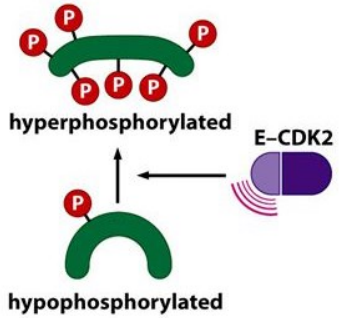
- **Hyperphosphorylation – overcomes restriction point**
- Dephosphorylated at late M-phase (protein phosphatase 1)



Hypo-phosphorylation is catalyzed by cycD-CDK4/6



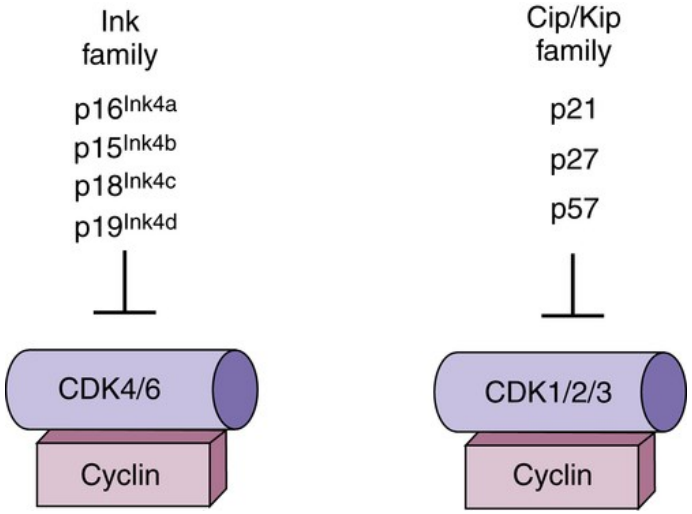
Hyper-phosphorylation is catalyzed by cycE-CDK2

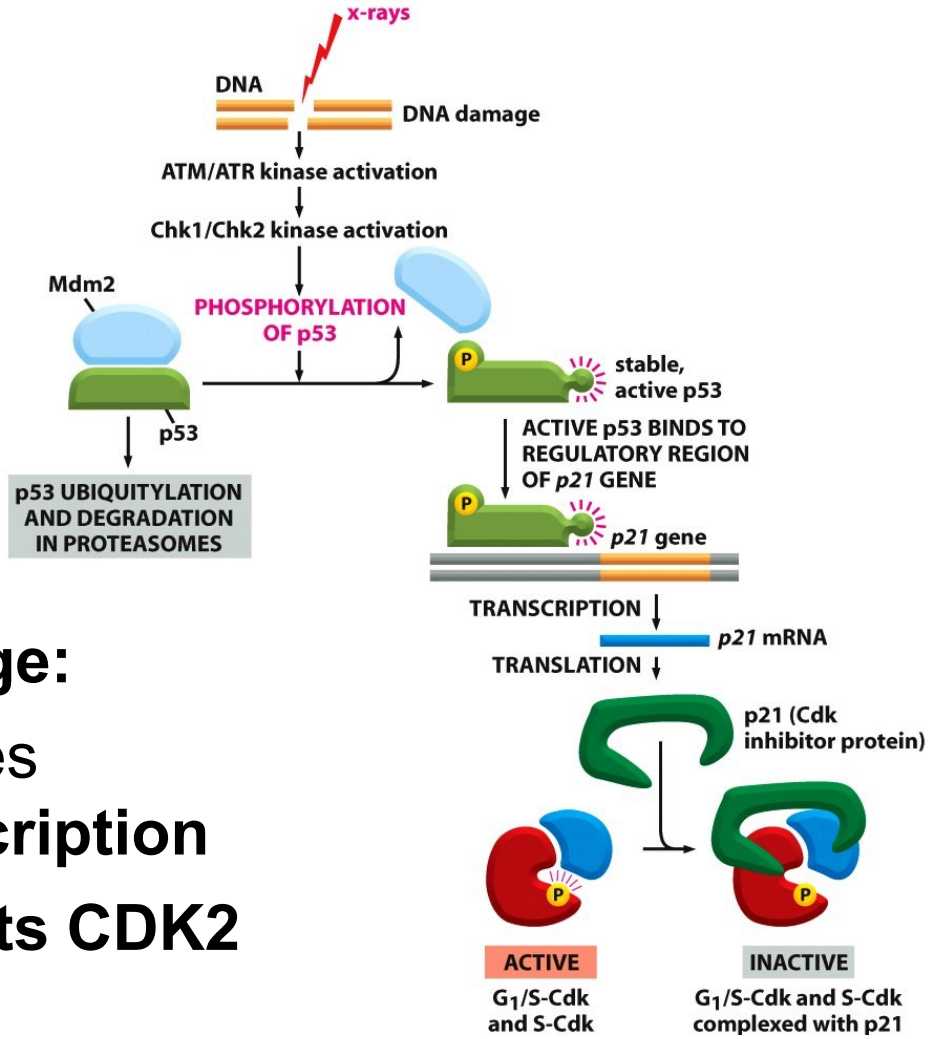


Defects in the cell cycle

Cell cycle arrest: Cdk inhibitor proteins

- Cell intrinsic Cdk inhibitors
- **Transcription induced upon stress stimuli**, e.g., DNA damage



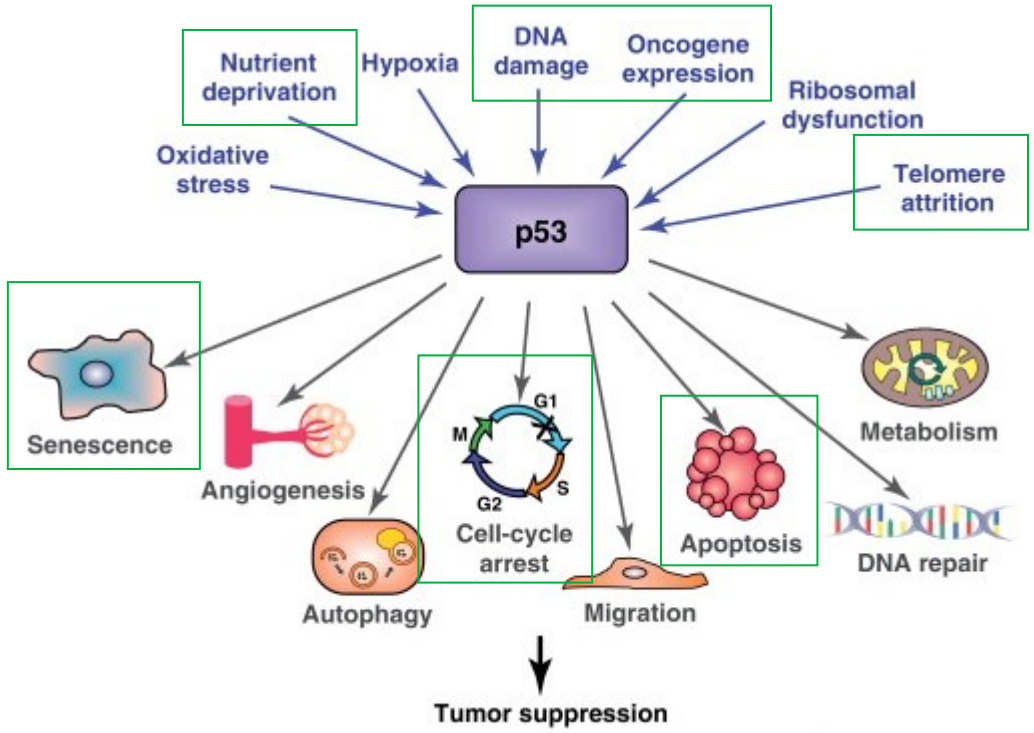


DNA damage:

- **p53** induces **p21 transcription**
- **P21 inhibits CDK2 and CDK1**

p53 – guardian of the genome

- **Tumor suppressor**
- Integrates various stress signals
- Regulates expression of downstream targets
- Cell context-dependent response
  - Repair mechanisms, normal function restored, cell-cycle arrest, or apoptosis



- **p53 mutated in ~50% of human cancers**

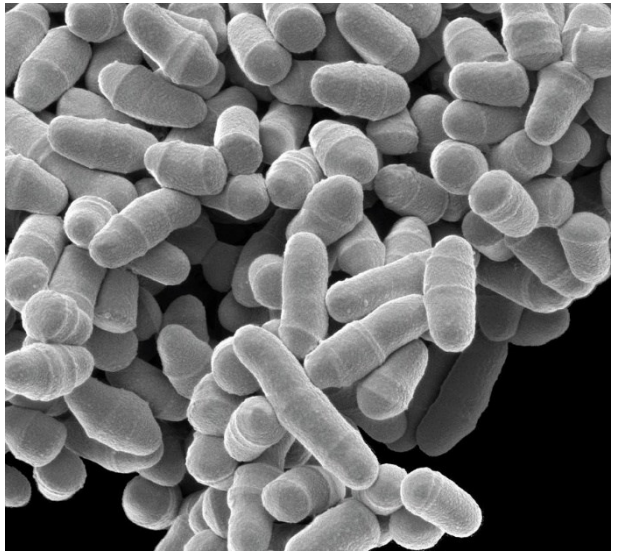
Cancer: A cell cycle defect

- Cell cycle positive regulators – typical **proto-oncogenes**
  - **Cyclin D/E, growth factors,** mitogenic signaling kinases and transcription factors (all promote cyclin D/E expression)
  - Amplification, overexpression, constitutive activity of kinases → **oncogenes: promote (uncontrolled) cell cycle progression**

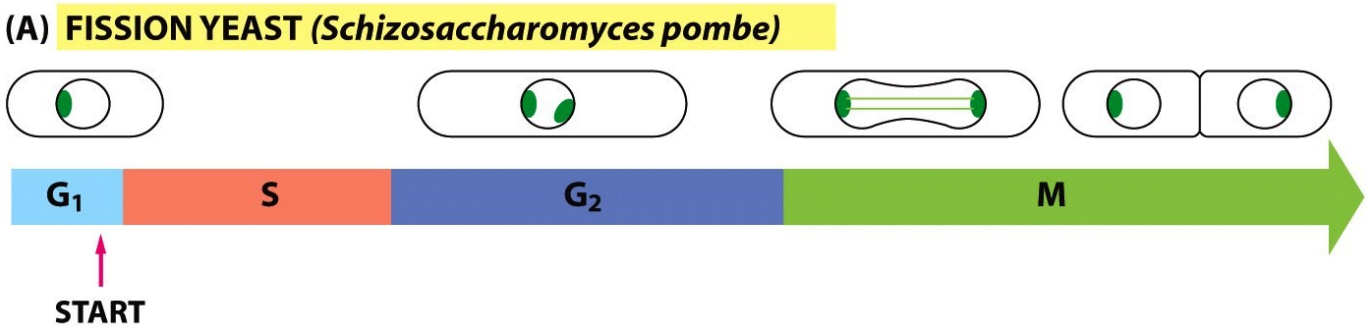
- Cell cycle negative regulators – typical **tumor suppressors**
  - **Intrinsic Cdk inhibitors, protein Rb, protein p53** and DNA damage recognizing kinases
  - Inactivating mutations, deletions → **cell cycle checkpoint mechanisms disabled**

Models to study the cell cycle

Schizosaccharomyces pombe

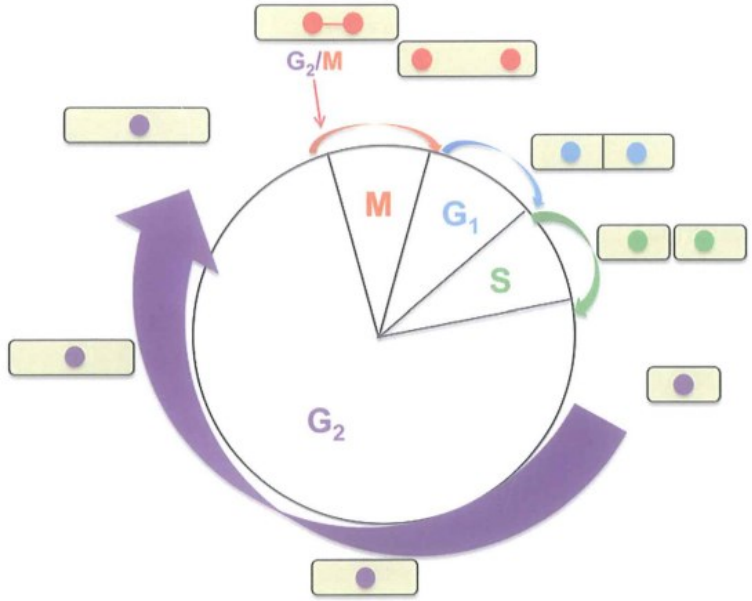


- Fission yeast; Cell cycle: 4 phases



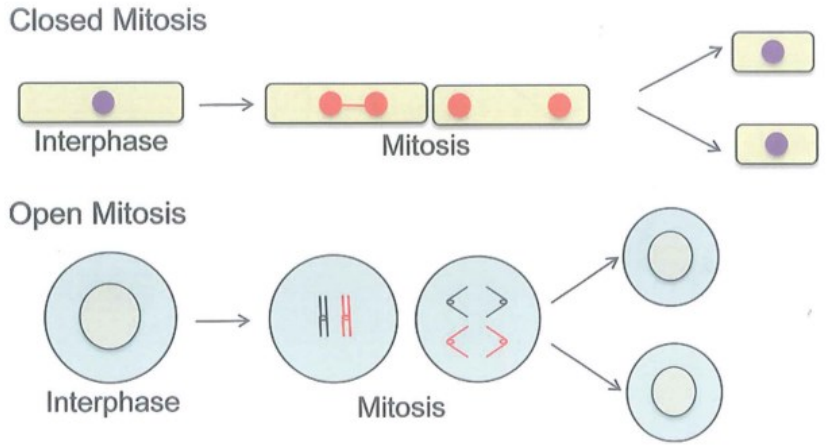
- **Small genome**: 4,824 genes, **~70% human orthologs**
- Non-pathogenic, **fast cell cycle** (90 min)
- Can exist as haploid: **easy loss-of-function studies** (only one allele)

Schizosaccharomyces pombe

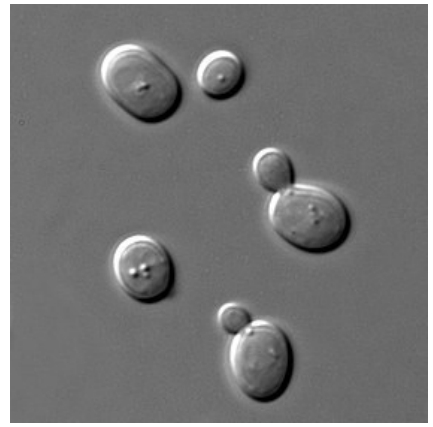


- **Optimal to study G2/M transition**
  - G2 is the longest – size is a parameter of progression
  - **Cdc2 (Cdk1), Wee1, Cdc25 identified in *S. pombe***

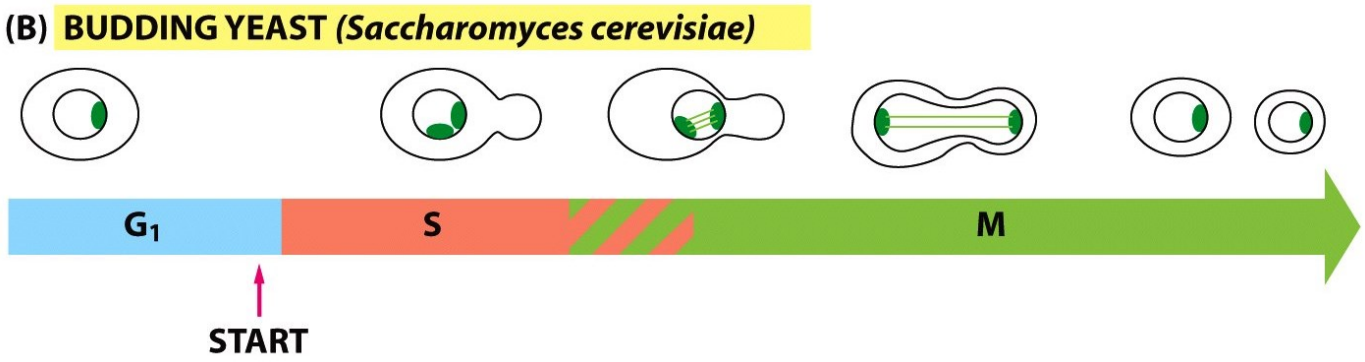
- ! Differences from multicellular organisms
- **Closed mitosis**
  - **Nuclear envelope does not break down**



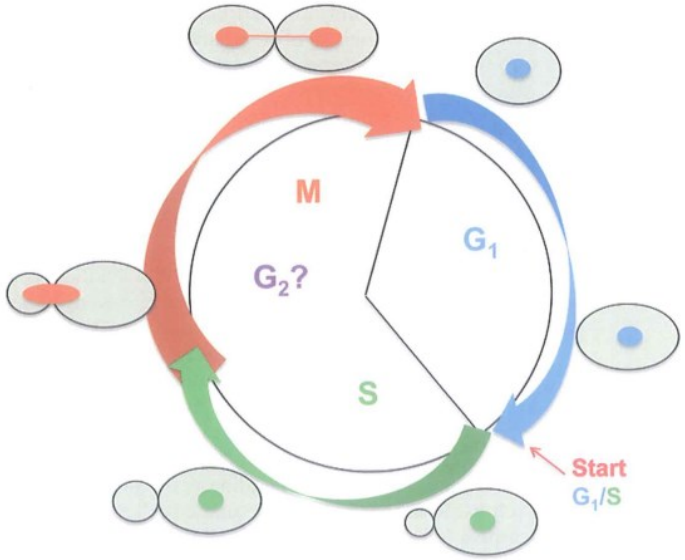
Saccharomyces cerevisiae



- Budding yeast

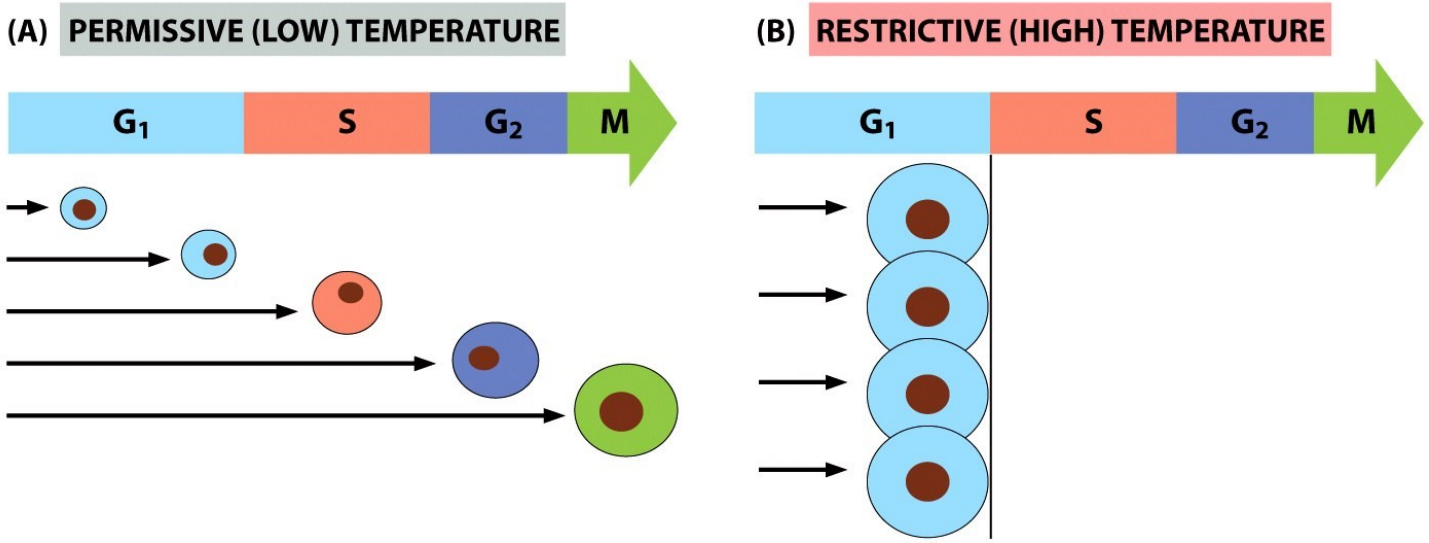


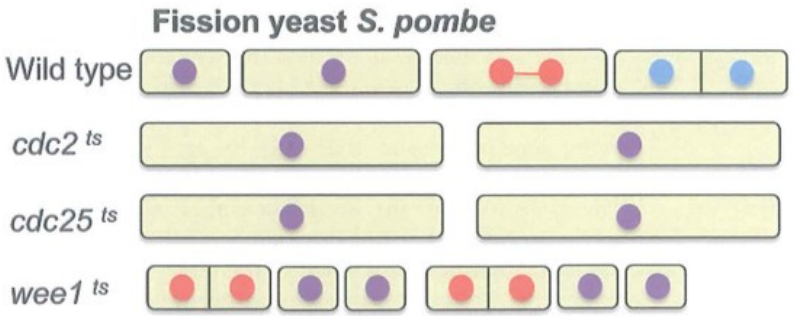
- Bud appears in S-phase, grows through cell cycle
- **Optimal to study G1/S transition**



How to study loss-of-function effects

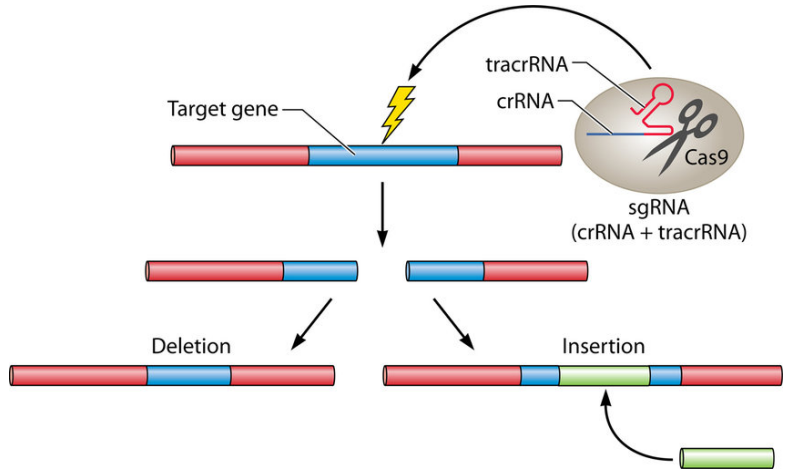
- Loss of cell cycle – no cell division, non-viable culture
- **Conditional mutants – temperature sensitive**
  - Low temperature – protein functional vs. High temperature – protein not functional

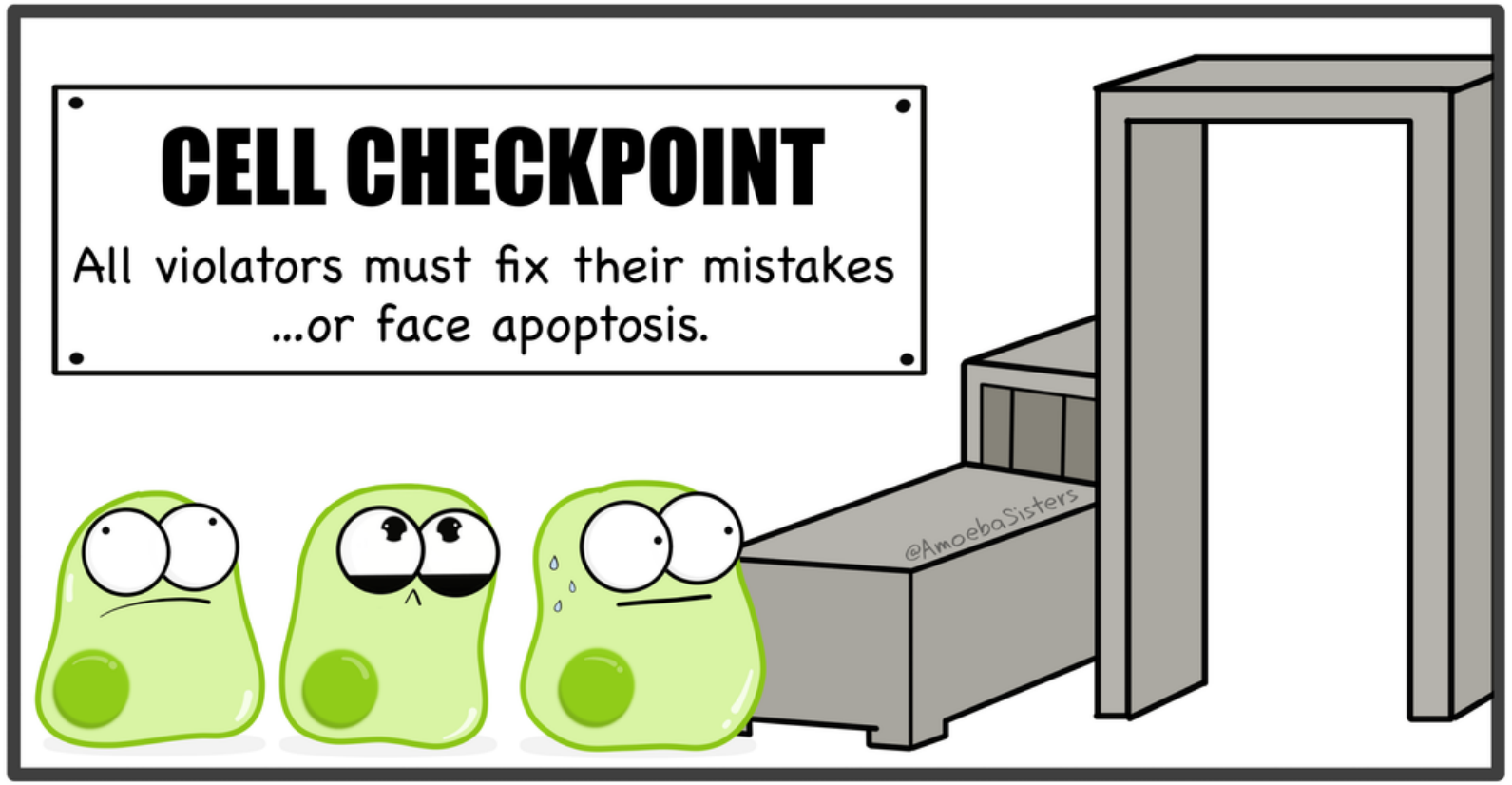




Mammalian cell cultures

- **Primary (non-cancerous) cells**
  - Replicative senescence studies
  - Normally 25-50 (max. 80) divisions – telomere shortening
- **Immortalized/cancer cell lines**
  - Not restricted by replicative senescence
  - Approximate mimic of in vivo regulation in normal healthy cells
- **Genetic manipulation by RNA silencing, CRISPR-Cas9 editing**





The cell checkpoints were always a site of intense scrutiny.