

# How Cells Cycle

At each of these checkpoints, the presence of **extracellular growth factors** or **mitogens** is required to allow the cycle to go past this checkpoint

For example, if we starve the cell of mitogens in G1 before the checkpoint, it'll go into G0. If it's after the checkpoint, they'll replicate once more before going into G0

There are a series of **checkpoints** in the cell cycle

Discovered through various experiments

**Proof of the checkpoints - cell fusion**

- Get two nuclei in S phase
- Therefore, factor inducing S phase is dominant
- It is in fact SPF
- Fuse a cell in S phase with one in G1 phase
- Get two nuclei in M phase
- Therefore, the factor driving M phase is dominant
- It is in fact MPF
- Fuse a S (or G1 or G2) cell with one in M phase

**Nature of the factors**

**Frogs**

- Maturation of frog oocytes into eggs is accompanied by release of **maturation promoting factor (MPF)**
- Putting it into more oocytes causes them to enter meiosis rapidly
- The signal auto-amplifies
- Similar activity in humans in mitosis (but not G1 or S phase)
- Fractionates biochemically with a protein kinase
- MPF is notoriously difficult to purify

**Yeast**

- Mutants which arrested at specific points in the cell cycle were collected (by Hartwell - *S. Cervisiae* - and Nurse - *S. pombe*)
- Analysis of mutants revealed crucial genes (**CDC28** in *S. Cervisiae* and **cdc2** in *S. Pombe*) as vital for entry into mitosis
- cdc2** was cloned and sequenced and found to encode for a **protein kinase** (34 000 Daltons)
- A human homologue was found

**Sea urchin & clam embryos**

- We can perform time course of protein synthesis, by incubating in 35S methionine
- We would expect all proteins to gradually give stronger and stronger bands on the gel
- They disappeared each time the cell divided, and then gradually built-up again
- However, they found two proteins which cycled
- They named them **cyclins** - cyclin B had a molecule weight of ~55 000 Daltons

**Finally...**

- Lohka developed an *in vitro* assay for MPF and used it to purify it
- Binds enzymes raised against *S. Pombe* cdc2
- There is the kinase
- 34 kd subunit
- This is **cyclin B**
- 45 kd subunit
- It's size depends on the gel formula used
- MPF is in fact a dimer of the cdc2 kinase and cyclin

**Mechanism for MPF**

- When the cdc2 kinase becomes active, mitosis occurs
- But why is it inactive during interphase?
- Bound to cyclin B
- Not phosphorylated at conserved tyrosine 15
- This blocks the ATP binding site
- Two conditions are needed for activity
- One (**cdc25**) encodes **tyrosine phosphatase** which activates cdc2
- This is an important checkpoint
- There is an associated kinase which deactivates cdc2
- Genes which regulate cdc2 in *S. Pombe* have been identified
- The cyclins are then destroyed - this is essential for entrance into anaphase

**Other checkpoints**

- Other checkpoints exist
- In other words, taking the cell out of G0
- Choice between replication and quiescence
- Implicated in many forms of cancer
- Regulated by other factors, like Cdk2
- Entry into S-phase

## Control of the cycle

## Introduction

The main stages of division are **replication of cellular DNA** and **division of the cell**

It's an ordered and regular process - it would be catastrophic if, for example, a cell decided to split before its DNA was replicated

The cell cycle can be divided into four stages

- Interphase
  - G1 - The longest **gap phase** during which cells are preparing for replication
  - G2 - A short gap phase
  - S - DNA synthesis phase
- M (mitosis)
  - Prophase** (chromosomes condense)
  - Metaphase** (sister chromatids become aligned)
  - Anaphase** (system chromatids separate)
  - Telophase** (cells separate)

Cells can also enter **G0** after mitosis (this is a **resting state**, also known as **quiescence**)

## Molecular mechanisms

Two distinct cytoskeletal machines are assembled in sequence to perform mitosis and cytokinesis

**Mitotic spindle**

- Responsible for allocating one copy to each daughter cell

**Cytokinesis**

- Contractile ring (animals)**
  - Contains both actin and myosin filaments
  - Forms around the equator of the cell, just under the plasma membrane
  - As the ring contracts, it pulls the membrane inwards, dividing the cell into two
- Phragmoplast (plants)**
  - Contractile rings can't work, because there's a cell wall to contend with!
  - Phragmoplast constructs a new cell wall within the cell between the two sets of replicated chromosomes

**Ensuring that mitosis precedes cytokinesis**

- Inhibited by proteins required for mitosis (this is why cytokinesis cannot occur until M-Cdk is inactivated)
- The residual central region of the spindle is required to maintain a functional contractile ring
- So until all the chromosomes have moved out of the way and a **central spindle** is formed, cytokinesis can't happen