



## Cell Cycle Short Lecture

### 1) What is the cell division cycle?

- a. Cells must be able to proliferate.
  1. during development
  2. wound healing
  3. stem cells in blood, small intestine, immune system
- b. For cells to copy themselves they need to do 4 things for this.
  1. Grow; make more stuff; e.g. protein, lipid, mitochondria
  2. Copy their genetic material; i.e. DNA and chromosomes
  3. Segregate contents to daughter cells, especially...
  4. Segregate replicated chromosomes to daughter cells
- c. This occurs through an ordered series of events called the **cell division cycle (cell cycle)**

**\*Two things to keep in mind when thinking about cancer and the cell cycle:**

1. The cell cycle is a highly controlled process; disruptions of this control are a major contributing cause of cancer.
2. A hallmark of cancer is hyper-proliferation; the changes that occur in cancer cells ultimately must affect the cell cycle.

### 2) Phases of the Cell Cycle

1. **INTERPHASE** cells duplicate chromosomes.
  2. **MITOSIS** cells segregate duplicated chromosomes into two **daughter cells**.
- a. **Interphase** has 3 periods: G1, S, G2
- In **G1** cells decide whether to divide or not.
  - This is called the **Restriction Point** in mammals and **START** in yeast.
  - Execution of these decisions commits a cell to complete a full division cycle.
  - During G1 a cell prepares for S by asking two questions.
    - 1) Have I grown big enough to enter the cycle?
    - 2) Am I OK? e.g. is my DNA damaged?
  - During S phase chromosomes are duplicated
  - During **G2** a cell prepares to enter mitosis by asking two questions
    - 1) Have I completed DNA synthesis properly?
    - 2) Am I OK? e.g. is my DNA damaged?



b. **Mitosis** has 4 periods:

1. chromosomes condense in **prophase**
2. duplicated chromosomes align on the spindle in **metaphase**
3. sister chromatids separate in **anaphase**
4. chromosomes decondense in **telophase**

c. **Cytokinesis**: the physical division into two new daughter cells at the end of mitosis

**\*\*The main job of the cell cycle is to accurately transmit the genetic information**

1. Introduce no mistakes
2. Maintain diploidy; No aneuploidy
3. What is aneuploidy? additions or subtractions of one or more chromosomes.  
e.g.  $2n + 1$ ,  $2n - 1$ , or other variations of normal chromosome number

### 3) The S (“synthesis”) phase of the cell cycle

a. Chromosomes must have 4 properties in order to be propagated during the cell cycle:

1. One and only one centromere
2. A telomere at both ends
3. Chromosomes must be linear (i.e. fully replicated)
4. They cannot be too large or too small

b. Semiconservative DNA replication begins at discrete places on the chromosome.

These places are called origins of replication (or autonomously replicating sequence (ARS) if you’re a yeast). An origin consists of a nucleotide sequence to which trans acting factors bind.

Trans acting factors come in two flavors.

1. Those that bind to the origin; e.g. ORC=origin recognition complex
2. Those that are recruited by ORC and are needed to make DNA

- DNA synthesis occurs bi-directionally on the chromosome.
- In eukaryotes DNA replication begins at many places; i.e. there are many origins of replication spread throughout the chromosome

c. Each origin initiates DNA synthesis once and only once during a normal S phase. This is very important and necessary to proliferate and to maintain proper ploidy.



#### 4) The mitotic phase of the cell cycle

- a. The “spindle” is a very complex molecular machine needed to separate sister chromatids during mitosis. The spindle is a bipolar structure, with a **centrosome** at either end
- b. The centrosome nucleates microtubules with the “+” or growing ends pointing out  
A microtubule is a polymer of heterodimers of  $\alpha$ - and  $\gamma$ -tubulin.
- c. There are two types of spindle microtubules
  1. **Kinetochores microtubules**
  2. **Polar microtubules**
- d. The kinetochore is a protein complex that assembles at the centromere and captures microtubules to attach the chromosomes to the spindle.
- e. Anaphase does not begin until all chromosomes are attached in a bipolar manner; i.e. sister chromatids attached to opposite centrosomes. This is again necessary to maintain normal diploidy.
- f. Motor proteins that move along microtubules pull the chromatids during anaphase.
- g. Taxol is an anti-cancer drug that stabilizes microtubules.

#### 5) Cell cycle control points

There are two major control points in the cell cycle

1. The G1-S transition
  2. The G2-M transition
- a. Cell fusion experiments identified S phase and M phase promoting activities
    1. Fuse a cell in mitosis with a cell in interphase: the interphase nucleus enters mitosis. Therefore there must be an “M phase” promoting factor.
    2. Fuse a cell in S phase with a cell in G1: the G1 nucleus enters S phase. Therefore there must be an “S phase” promoting factor.
    3. Fuse an S phase cell with a G2 cell, then the G2 nucleus does not enter S phase. This is because of “once and only once” replication control during the cell cycle.
    4. It took ~15 more years to identify that cyclin dependent kinases (CDK) are the molecular components of these two activities.



## 6) How do you identify and study the molecules controlling cell cycle progression?

- a. The identification and analysis of cell cycle regulatory proteins was achieved using diverse model experimental systems. *Most of the major breakthroughs were made in non-mammalian systems like yeast, frogs, and sea urchins.*
- b. The identification of CDKs occurred via many experimental approaches
  1. Genetics: We can understand the function of genes by making mutations in them, and asking what happens to the cell cycle.
  2. Biochemistry: We can physically purify certain activities
- c. *The cell biology learned in model systems is directly applicable to human cells!*

## 7) Isolation of *cdc* mutants in yeast

- a. The labs of Lee Hartwell and Paul Nurse collected a bunch of *cdc* mutants *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively.  
Cdc mutants are temperature sensitive (ts): they work at a low temp, but do not work at a high temp. You can isolate *cdc* mutants by looking at the shape of the cell at the high or restrictive temp. If they all look the same, they arrested in the same phase of the cell cycle
- b. Many different proteins were identified in this manner, acting at all cell cycle stages
  - cdc2*: prototype for a family of kinases
  - cdc13*: a cyclin
  - replication proteins

## 8) The discovery of cyclins in sea urchin embryos

- a. The early sea urchin embryo consists of **S/M cycles**  
These cycles can be activated simultaneously in many eggs and occur synchronously. The proteins made can be identified radioactively on separation gels
- b. Cyclins are synthesized during interphase (S) and destroyed during mitosis.  
Protein activity can be controlled this way: i.e. if it's not there it can't function, so turn it on and off by synthesis and destruction

## 9) Putting it all together: Purification of MPF from frogs

- a. **Oocytes** mature into eggs in response to hormones  
Maturation means the oocytes grow and then enter **meiosis**. They remain arrested in metaphase of meiosis II until fertilization.



- b. Maturation is promoted if you transfer cytoplasm from an MII arrested egg into an oocyte by microinjection; hence “**MPF**”. MPF activity peaks as cell enter either a meiotic or mitotic division.
  
- c. Purified MPF consists of a cdk called cdc2 and a cyclin .