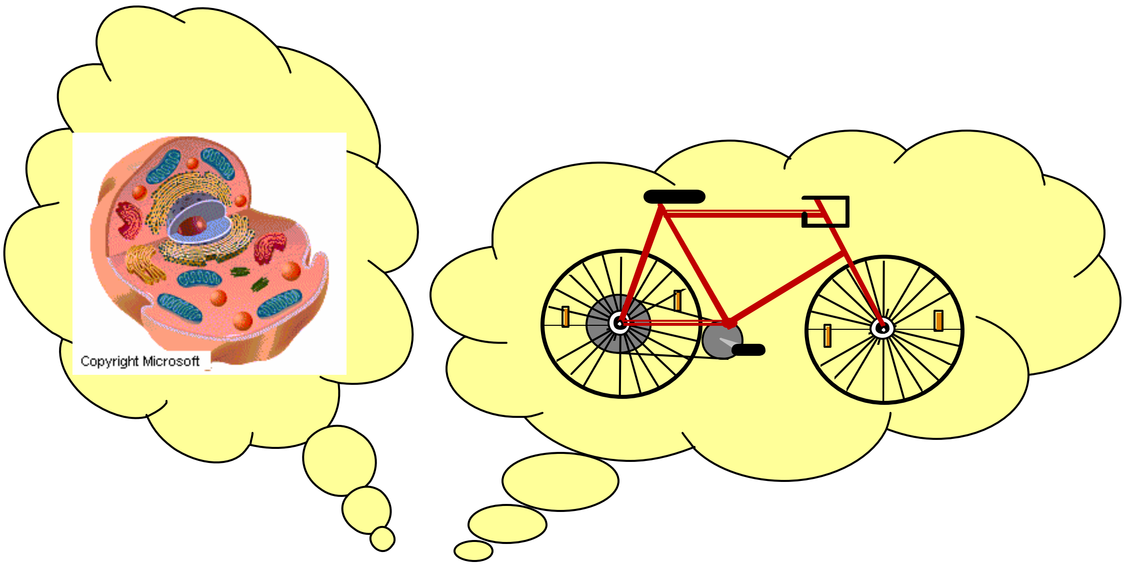


Cell-Cycle!

If this is what you can imagine on hearing this word then you have been ignorant of one of science's most interesting facts. Don't worry, here is a special report about cell-cycle to unveil all the secrets behind this fascinating concept!

-by

Karthik Venkatesh.R

20002319

II semester, I.B.T.

Cell Cycle and Cyclins.

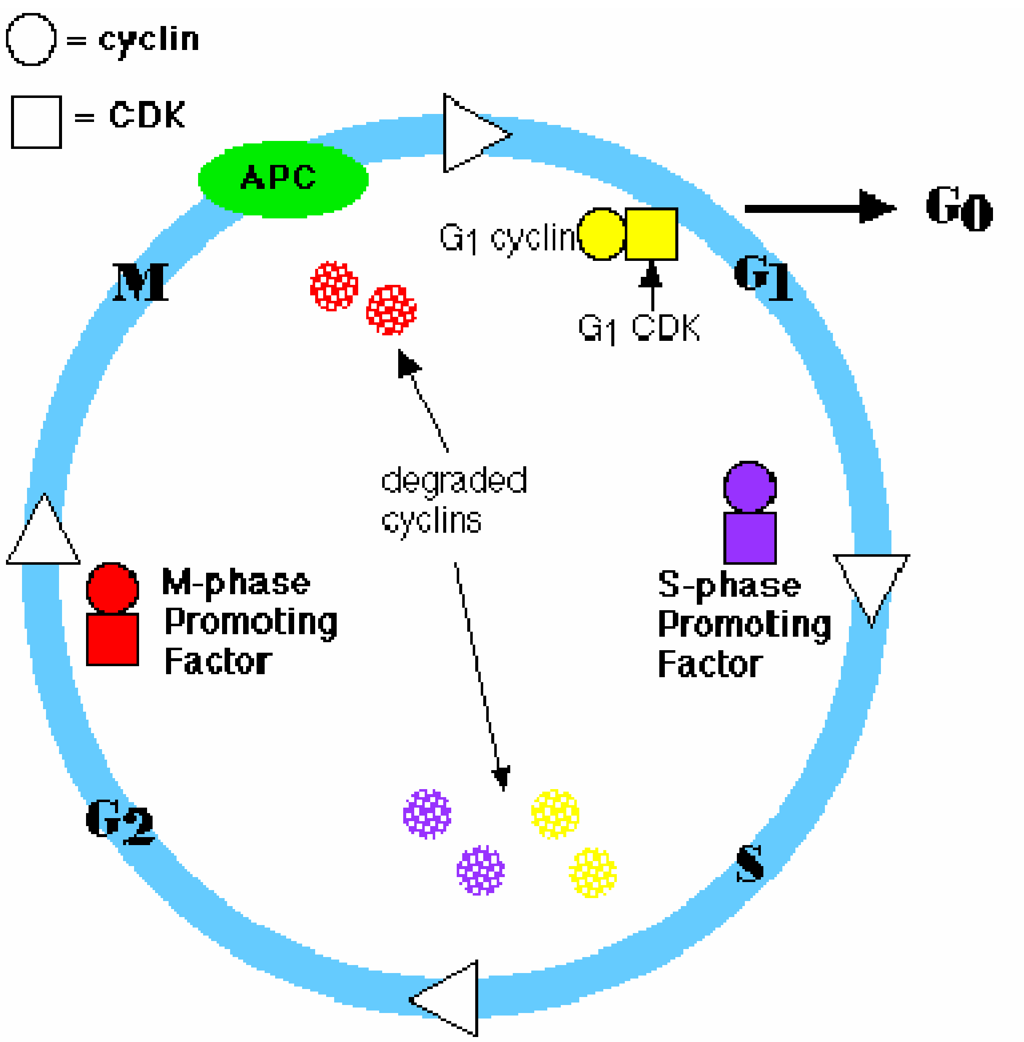
Self - Reproduction is perhaps the **'MOST FUNDAMENTAL '** characteristics of cells-as may be said for all living organisms. All cells reproduce by dividing into two with each parental cell, giving rise to two daughter cells on completion of each cycle of cell division. These newly formed daughter cells can themselves grow and divide, giving rise to a new cell population formed by the growth and division of a single parental cell and its progeny. In the simplest case, such cycles of growth and division allow a single bacterium to form a colony consisting of millions of progeny cells during overnight incubation on a plate of nutrient agar medium. In a more complex case, repeated cycles of cell growth and division result in the development of a single fertilized egg into the more than TEN TRILLION (10000000000000 or 10^{13}) cells that make up the human body.

Regulation of cell divisions is critical for the normal development of multicellular organisms. Loss of control ultimately leads to cancer .In the late 1980s it became clear that the molecular processes regulating chromosome replication and cell division are fundamentally similar in all eukaryotic cells.

PHASES OF CELL CYCLE

The following is the current model of the cell cycle and it also describes key experiments revealing information about cell cycle regulation. It also explains key experiments that have provided information about cell-cycle regulation.

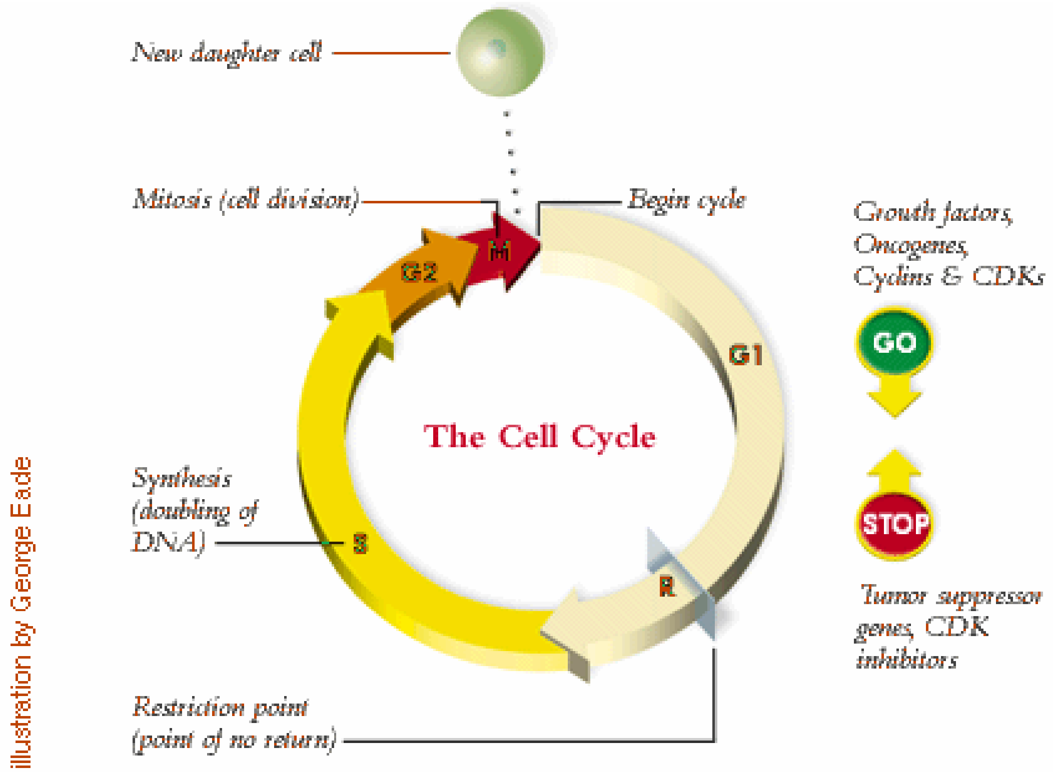
THE CELL CYCLE IS A SERIES OF EVENTS LEADING TO REPLICATION OF CELLS.



CURRENT MODEL OF EUKARYOTIC CELL CYCLE

As illustrated in the figure the cell cycle is divided into four major phases. In cycling somatic cells chromosomes are replicated during the *'S (Synthetic) phase'*

After progressing through the *'G_2 (Gap) phase'*, cells begin the complicated process of *'Mitosis'* or *'Meiosis'* also called the *'M phase'* which is further subdivided.



REGULATION OF CELL CYCLE.

Following mitosis the cycling cells enter the *'G_1 phase'*, the period before DNA synthesis is reinitiated in the S phase.

Time taken for one cycle.

In human cell cycle the various stages take place in the following manner.

STAGE	TIME
G_1 phase	9 hours
S phase	10 hours
G_2 phase	4.5 hours
Mitosis	30 minutes

But in yeast the entire cycle completes in about 90 minutes.

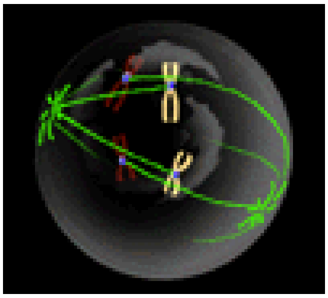
The G_0 phase.

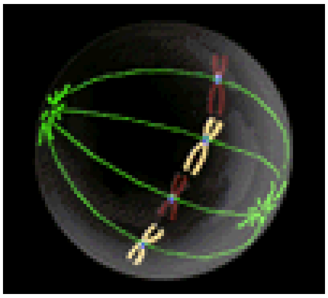
Postmitotic cells in multicellular organisms can 'exit' the cycle and remain dormant for days, weeks or in some cases (e.g. nerve cells & cells of eye lens) even the lifetime of organisms without proliferating further. Most postmitotic cells in vertebrates exit the cell cycle in G_1, entering a phase called G_0 phase. G_0 cells returning to the cell cycle enter into the S phase. This re-entry is regulated thereby providing control of cell proliferation.

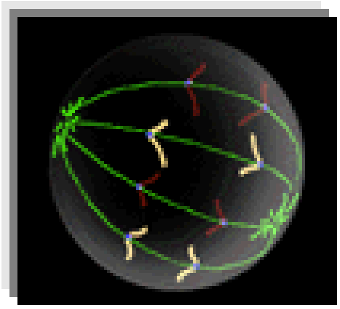
Mitosis.

This stage is more complicated and has many sub-stages. They are as follows.

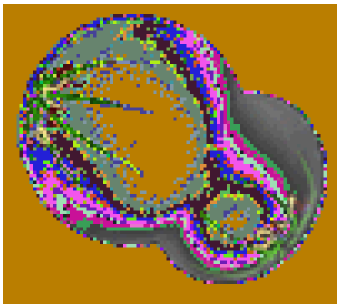
Chromosomes condense during the *'Prophase'* period of mitosis, by

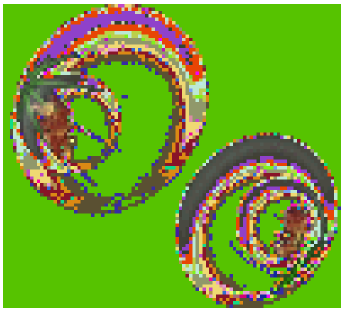

PROMETAPHASE


METAPHASE


ANAPHASE

length and become aligned at the center of the cell during *'Metaphase'*. During the *'Anaphase'* portion of mitosis, sister chromatids separate and move to opposite poles of the *mitotic apparatus or 'Spindle'* segregating one of the two sister chromatids to each daughter cell. In most cells from higher eukaryotes nuclear envelope breaks down into multiple small vesicles early in


TELOPHASE


CYTOKINESIS

mitosis and reforms around the segregated chromosomes as they decondense during *'Telophase'*, the last mitotic stage.

Then *'cytokinesis'* yields two daughter cells. The golgi complex and endoplasmic reticulum also vesiculate during mitosis and reforms into the two daughter cells after cell division.

MEIOSIS:

This type of division has two substages. It is seen only in germ cells.

Meiosis I.

This is a reduction division. It has the following stages.

a) Prophase I.

It includes Leptotene, Zygotene, Pachytene, Diplotene & Diakinesis.

b) Metaphase I

c) Anaphase I

d) Telophase I

Meiosis II.

This is an equation division. It is similar to mitosis. It includes,

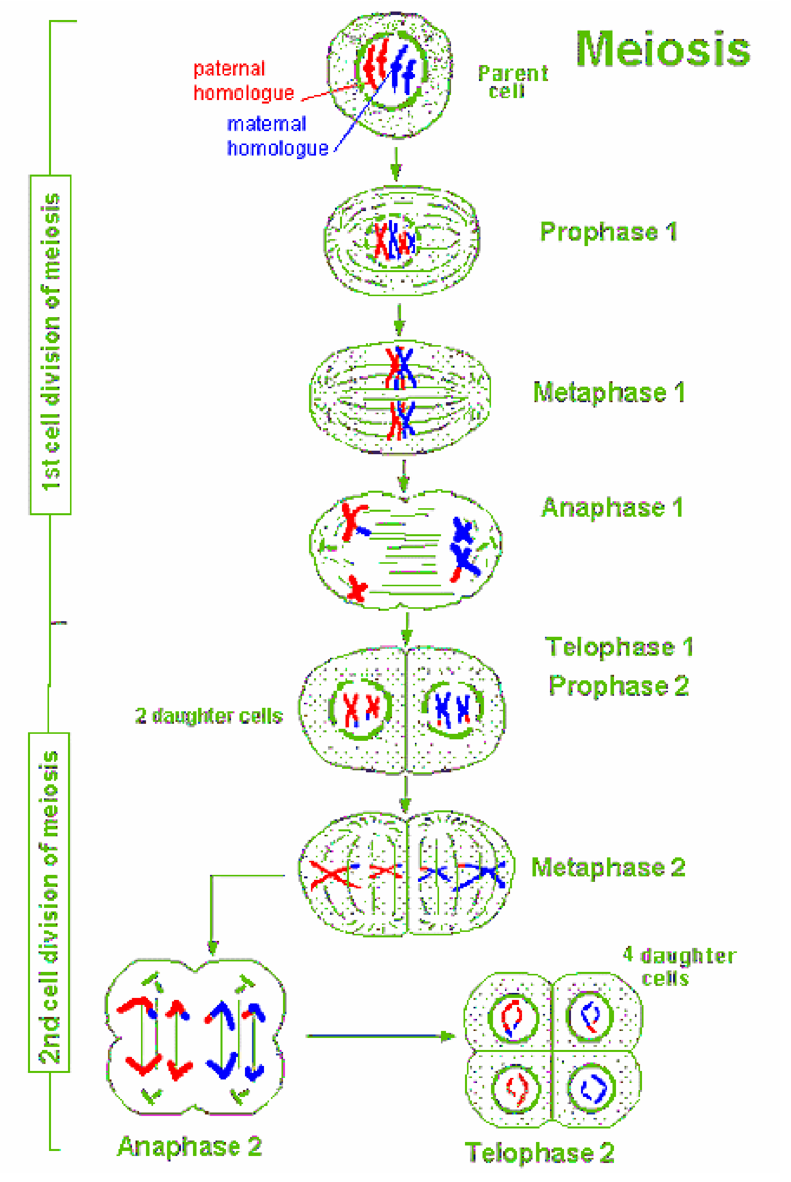
a) Prophase II.

b) Metaphase II.

c) Anaphase II.

d) Telophase II.

Schematic representation of meiosis.



Current model of eukaryotic cell cycle regulation.

The cell cycle is controlled by a small number of heterodimeric protein Kinases. It includes a regulatory subunit called cyclin and a catalytic subunit called cyclin dependent kinase (cdks). During an eukaryotic cell cycle the series of cdks are expressed in the following order.

G1 cdk is expressed first.

- Activate transcription factors that cause expression of enzymes required for DNA synthesis.
- Activate genes encoding S phase cdk complex.
- Induces degradation of S phase inhibitor.

S phase cdk complex is expressed next.

S phase cdk phosphorylate regulatory sites in the proteins that form DNA pre replication complexes. This not only initiates DNA replication but also prevents re-assembly of pre replication complex (thus each chromosomes replicates only once). Mitotic cdk is synthesized during S phase and G_2 but until DNA synthesis is completed its activity is kept in check.

Mitotic cdk is expressed third.

On activation it induces chromosome condensation, breakdown of nuclear envelope, assembly of the mitotic spindle apparatus and alignment of condensed chromosomes at the metaphase plate. After proper association of all chromosomes with spindle microtubules it activates APC (anaphase promoting factor).

Anaphase promoting factor.

- It initiates anaphase.
- Degradation of mitotic cyclins takes place.
  - This results in chromosome decondensation, nuclear envelope reforms and cytoplasm to divide at cytokinesis.

*Phosphorylation of APC by G_1 cdk complex in late G_1 inactivates it allowing subsequent accumulation of mitotic cdks.

Is cell cycle reversible?

- Experiment: If a G_1 cell is fused with a cell in S phase, DNA synthesis begins in the G_1 cell. However similar changes are not seen when a G_2 cell is fused with S phase cell. This shows that the cell cycle is not reversible.

Where are these factors which control the cell cycle present in the cell?

- Experiment: If G_1, G_2 or S stage of any cell cycle were fused to cells in mitosis, their nuclear envelopes vesiculate and chromosomes condense (characteristics of mitosis). This shows that *some diffusible component in* *cytoplasm of the mitotic cells forced interphase nuceli to undergo many of the process associated with mitosis.*

- Large protein complexes also mark specific inhibitors of cell cycle events for proteolytic degradation by proteasomes.

Discovery of MPF.

BIOCHEMICAL STUDIES WITH OOCYTES, EGGS AND EARLY EMBRYOS.

Before moving on with the experiment it is essential to know about the oocyte maturation

OOCYTE MATURATION.

Oocytes replicate their DNA and become arrested in G_2 for about eight months as they grow in size to a diameter of 1mm, stockpiling all materials needed for the multiple cell division required to generate a swimming feeding tadpole. When stimulated by a male, an adult female's ovarian cells secrete the steroid hormone progesterone, which induces G_2 arrested oocytes to enter meiosis I. They undergo meiosis I, interphase and arrest during the second meiotic metaphase (here the cells are called eggs). Only when fertilized by sperm the egg nucleus is released from its metaphase arrest and completes meiosis.

Resulting haploid egg nucleus fuses with haploid sperm producing 'Zygote'. Then mitosis of early embryogenesis begins.

Experiment: When cytoplasm from eggs arrested in metaphase of meiosis II (eggs) is microinjected into G_2 arrested oocytes, the oocytes mature into eggs in the absence of progesterone. This identified 'maturation promoting factor (MPF)' a factor present in the cytoplasm off egg, which promotes maturation even in the absence of egg. MPF activity is increased during mitosis and decreased during other time. Though first discovered from frog MPF is seen in mitotic cells from all species assayed.

Is MPF present in mammals?

Experiment: Mammalian cells can be arrested in mitosis by treatment with compounds (e.g.colchicine) that inhibit assembly of microtubules. When cytoplasm of this cell is injected into G_2 arrested xenopus oocytes, the oocytes mature to eggs. This shows that the mammalian somatic mitotic cells contained a cytosolic factor that exhibited frog's MPF activity. When cytoplasm of mammalian mitotic arrested cells are injected into mammalian interphase cells, interphase cells entered mitosis. This shows that MPF controls the entry of mammalian somatic cells into mitosis as well as the entry of frog oocytes into meiosis.

Composition of MPF.

MPF is a heterodimer complex composed of a 'cyclin' and 'cyclin dependent protein kinase (cdks)'.

Identification of cyclins.

Mitotic cyclin was first identified in early sea urchin embryos. As in frog, the initial cell cycles in early sea urchin embryos occur simultaneously. Here synchronously fertilized sea urchin eggs were incubated with a radiolabelled amino acid and samples were removed every ten minutes. Protein was isolated from each sample and analyzed by gel electrophoresis followed by autoradiography. The amount of radiolabel in the vast majority of proteins increased steadily through several cell cycles. However one protein peaked in intensity early in mitosis, fell abruptly during anaphase and then slowly accumulated during following interphase to peak early in the next mitosis. Careful analysis showed that this protein named 'cyclin B' is synthesized continuously during the embryonic cell cycles and is abruptly destroyed at the onset of anaphase.

Later C DNA clone encoding sea urchin cyclin B was used as a probe to isolate a homologous cyclin B C DNA from xenopus. Western blotting of MPF purified from xenopus egg using antibody prepared against the protein encoded by cyclin B C DNA, showed that one subunit of MPF is indeed cyclin B. The other is cdk subunit.

The cell cycle clock.

The oscillation in the MPF activity that occurs as early frog enter and exit mitosis is observed even when the nucleus is removed from a fertilized egg. This finding shows that a 'cell cycle clock' operates in cytoplasm of early embryos completely independent of nuclear events. It occurs in synchronously dividing cells of early animal embryos. No transcription occurs during these rapid cycles, indicating that all the cellular components required for progress through the truncated cell cycles are stored in the unfertilized egg.

Cyclins and mitosis.

Experiment 1:When chromatid prepared from interphase frog sperm is added to a xenopus egg extract, a nuclear envelope develops around the chromatin forming a haploid nucleus. Following formation of nuclear envelope sperm DNA replicates once. Then sperm chromosomes condense and the nuclear envelope breaks down. Later the concentration of cyclin B suddenly falls down. Simultaneously the sperm chromosomes decondense and a nuclear envelope reforms around. After twenty minutes cyclin B accumulates, chromosomes condense and nuclear envelope breaks.

Xenopus egg extracts can thus mediate several cycles. Using the above said experimental system, researchers found that MPF activity assayed by its ability to phosphorylate histone H1, rises and falls in synchronously with concentration of cyclin B. During mitosis if cyclohexamide (an inhibitor to protein synthesis) is added synthesis of cyclin B is prevented. Also rise in MPF activity, chromosome condensation and nuclear envelope breakdown also doesn't take place.

Experiment 2: if RNAse is added in mild concentrations mRNA gets degraded. Thus production of cyclin B (protein) is stopped. Here the DNA is replicated but mitosis didn't occur. If cyclin B mRNA produced in vitro is added mitosis is seen. If a non-degradable cyclin B is added, the reformation of nuclear envelope, recondensation of chromatin didn't occur.

> This shows that cyclin B should be produced be for mitosis and should be degraded to end mitosis.

Exit from mitosis.

Ubiquitin-mediated degradation of mitotic cyclins promotes exit from mitosis. All the encoded proteins contain a homologous sequence near the end terminals called the 'destruction box'. In intact cells cyclin degradation begins shortly after the onset of anaphase. Mitotic cyclins are modified by the addition of Ubiquitin, a 76 residue protein. Covalent attachment of ubiquitin is called polyubiquitinisation. Addition of ubiquitin to a mitotic cyclin requires three types of enzymes. They are ubiquitin-activating enzyme E1, ubiquitin conjugating enzyme E2, ubiquitin ligase E3.this E3 that targets mitotic cyclin for polyubiquitimisation is the APC. The APC targets E2 complex to the destruction box in the mitotic cyclin and then stimulates transfer of ubiquitin to a lysin residue on the C-terminal side of the destruction box. Further cycles of ubiquitinisation result in chains of polyubiquitin, which are recognized by proteasomes. APC isolated from eggs arrested in the metaphase has low activity for stimulating polyubiquitination of cyclin B. in contrast APC isolated from eggs stimulated to complete mitosis has a high ubiquitin stimulating activity. When MPF activity reaches its peak at metaphase it activates APC. Polyubiquitination of cyclin B then occurs leading to degradation of cyclin B. since cyclin B is an essential subunit of MPF its degradation causes inactivation of MPF activity. APC is deactivated in late G_1, and thus the cycle continues. This mechanism accounts for the variation of cyclin B levels.

Cell cycle control in mammalian cells.

Checkpoints in cell-cycle regulation.

- Checkpoints controls function to ensure that chromosomes are intact and that critical stages of the cell cycle are completed before the following stage is initiated.
- One checkpoint operates during Sand G_2 to prevent the activation of MPF before DNA synthesis is complete.
- Another checkpoint operates during early mitosis to prevent activation of APC and the initiation of anaphase until the mitotic spindle apparatus is completely assembled and all chromosome kinetochores are properly attached to spindle fibres.
- Checkpoints that function in response to DNA damage prevent entry into S or M until the damage is repaired.

Perspectives for the future.

Although the general logic of cell cycle regulation now seems well established, many critical details remain to be discovered. The components of the pre-replication complex that must be phosphorylated by S phase cdk cyclin complexes to initiate DNA replication remain to be determined, as does the mechanism of initiation. Current understanding of the structure of condensed chromosomes remains vague. Much remains to be learned about how the APC is activated during anaphase and how its activity is first directed towards anaphase inhibitors and only subsequently towards mitotic cyclins.

Significance of cell cycle in the field of medicine.

Understanding these concepts will have significant consequences especially in the field of cancer treatment. Radiation therapy works because it causes DNA damage in the target cells that induces their

'apoptosis'.

Bibliography

Books

1. Moleclar Cell Biology by Lodish, Baltimore, Darnell ...
2. Genes by Lewin
3. Cell Biology by Kimball

Websites

1. www.nobel.se
2. www.vlib.org
3. www.geocities.com
4. www.mitosis.com