Reversal of polarization
Cell surface at the stimulated point

CELL CYCLE

Cell cycle is the cyclical process of growth and Cellular reproduction in unicellular and multicellular eukaryotes. The cycle includes two basic parts: Mitosis (containing Karyokinesis & Cytokinesis) & interphase (the period between mitoses.

• PHASES OF THE CELL CYCLE

Basically there are two parts in Cell cycle:

i) Mitosis: It is the most dramatic stage of cell cycle, corresponding to the separation of daughter chromosomes & usually ending with Cell devision via Cytokinesis.

ii) Interphase: It is the preparatory stage for Cell devision during which both Cell growth & DNA replication occur in an orderly manner.

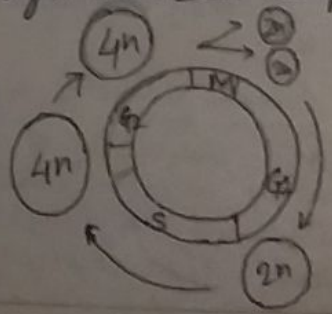
The timing of DNA synthesis, divides the interphase into three discrete phases →

a) **G_1 phase (gap 1):** This phase corresponds to the interval (gap) between mitosis and initiation of DNA replication. During G_1, the cell is metabolically active and continuously grows but does not replicate its DNA.

b) **S phase (Synthesis):** G_1 phase is followed by S phase during which DNA replication takes place. Genome content of a Cell doubled here.

c) **G_2 Phase (Gap 2):** The completion of DNA synthesis is followed by the G_2 phase during which Cell growth continues & proteins are synthesized in preparation for mitosis.

For a typical rapidly proliferating human cell with a total cycle time of 24 hrs G_1 phase last about 11 hrs.
S " " 8 hrs
G_2 " " 4 "
M " " 1 "



• Check Points Of Cell Cycle :-

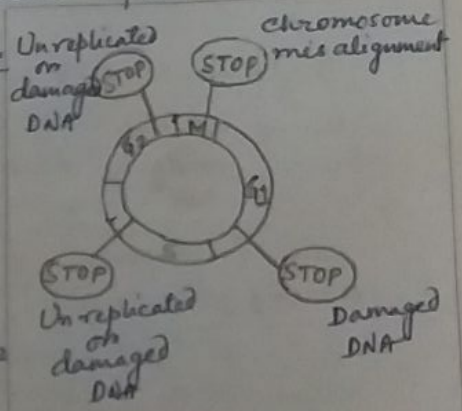
Checkpoints are the stages in cell cycle at which progression of a cell through the cycle is blocked if there is damage to the genome or the mitotic machinery.

Several cell cycle checkpoints function to ensure that incomplete or damaged chromosomes are not replicated & passed on to daughter cells. These checkpoints sense unreplicated or damaged DNA and coordinate further cell cycle progression with the completion of DNA replication or repair. For example →

i) G1 Checkpoint : Sense ~~DNA~~ damaged DNA in G1 phase of cell cycle, arrest the cell & allow repair of damage to take place before the cell enters S phase.

ii) S Check point : Provides continual monitoring of the integrity of DNA to ensure that damaged DNA is repaired before it is replicated during S phase of Cell Cycle.

In addition, the S phase check point provides a quality control monitoring to promote the repair of any errors that occur during DNA replication. Such as the incorporation of Incorrect bases or Incomplete replication of segments of DNA.



Cell cycle check points

iii) G2 Check point : It prevents the initiation of mitosis until DNA replication is complete. G2 check point senses unreplicated DNA & generates a signal that leads to cell cycle arrest during G2 phase.

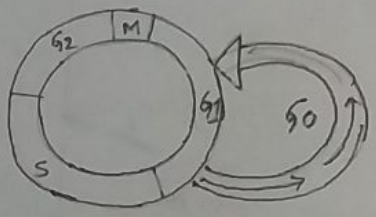
In addition to sensing unreplicated DNA the G2 checkpoint senses DNA damage, such as that resulting from irradiation. It can arrest the cell to allow time for the damage to be repaired, rather than being passed on to daughter cells.

iv) Spindle Check point : Maintains the integrity of the genome occurs toward the end of mitosis. This checkpoint also monitors the alignment of chromosomes on the mitotic spindle, Thus ensuring that a complete set of chromosomes is distributed accurately to the daughter cells. Improper alignment of chromosome cause mitosis to arrest at spindle checkpoint of M phase until a complete complement of chromosomes has been organized for distribution to each daughter cell.

Qura

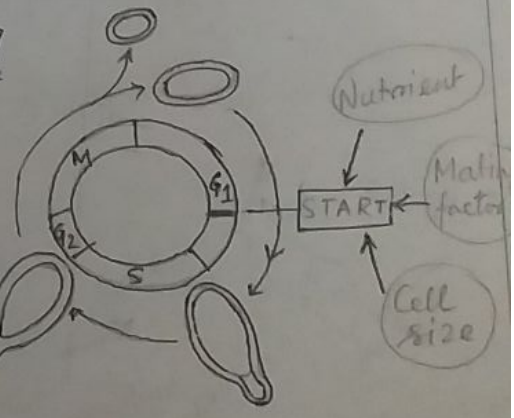
G0 phase of Cell Cycle :- The availability of growth factors controls the animal cell cycle at a point in late G1 called restriction point. If growth factors are not available during G1, the cell enters a quiescent stage of the cycle called G0 where the cell remain metabolically active but no longer proliferate unless called on to do so by appropriate extracellular signals.

Eg: Nerve cells in Adult animals cease division altogether & remain in G0 phase of Cell cycle
Skin fibroblasts, cell of liver divide only occasionally & exit G1 to enter G0.



- **'START'- a regulatory point in budding yeast**

A major cell cycle regulatory point ~~in many types~~ occurs late in G1 and controls progression from G1 to S was first defined by studies of budding yeast (Saccharomyces cerevisiae), where ~~Known~~ it is known as START.



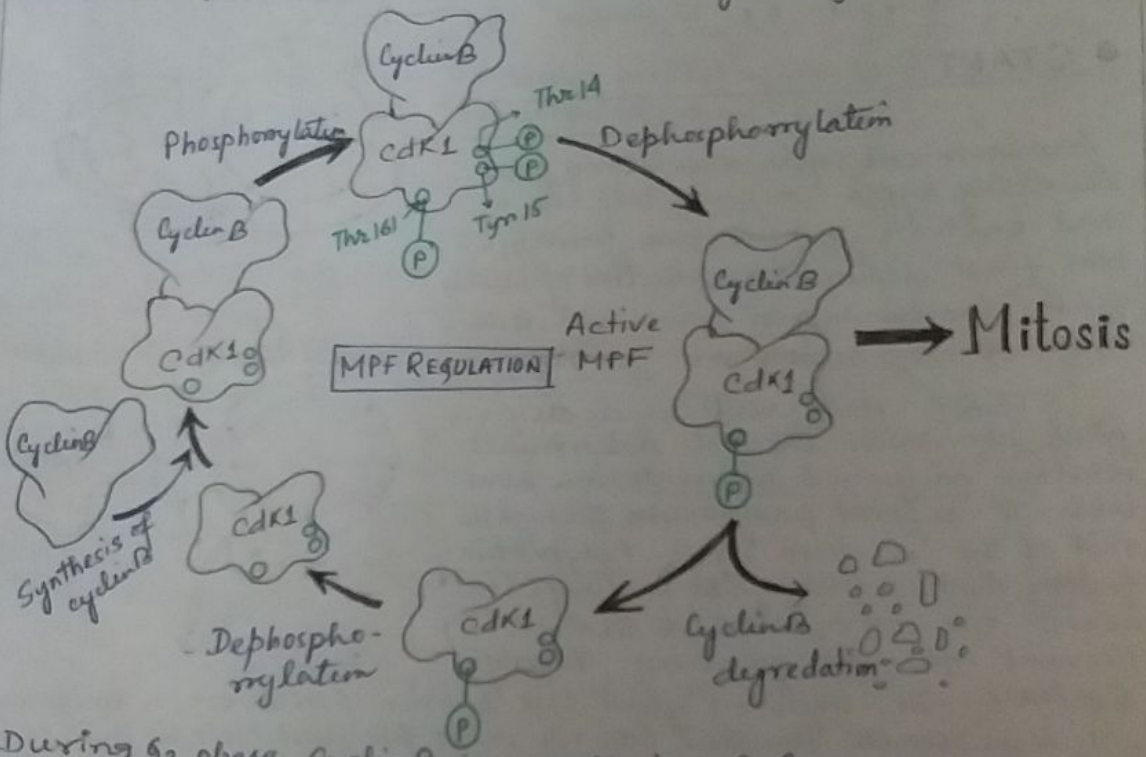
START represents a decision point at which the cell determines whether sufficient nutrients are available to support progression through the rest of the division cycle. Polypeptide factors that signal yeast mating also arrest the cell cycle at ~~@~~ START & prevent the progression through S phase. In budding yeast Cell division produces a large mother cell & a small daughter cell. In order for yeast cells to maintain a constant size, the small daughter cell must grow more than the large mother cell does before they deviide again. These Cell size must be monitored in order to coordinate Cell growth with other cell cycle event. This regulation is accomplished by a control mechanism that requires each cell to reach a minimum size before it can pass START.

So the passage through START is controlled by —
- Availability of nutrients.
- Mating factors
- Cell size.

• **MPF** : Maturation Promoting Factor first studied in frog oocyte, act as a general regulation of the transition from G2 to M phase of cell cycle of eukaryotes.

It is composed of two key subunits CdK1 & Cyclin B. Cyclin B is a regulatory subunit required for catalytic activity of the CdK1 protein kinase.

MPF activity is controlled by the periodic accumulation & degredation of cyclin B during cell cycle progression. Cyclin B play important role in phosphorylation & dephosphorylation of CdK1 which is the key regulatory mechanism of MPF



→ During G2 phase Cyclin B is synthesized & forms complexes with CdK1.

→ As these complex forms. CdK1 is phosphorylated at two critical regulatory posion position, one is on Threonine 161 required for CdK1 activity & another is on Tyrosine 15 (In vertebrates it is on Threonine 14).

→ Phosphorylation on Tyrosine 15 on Threonine 14 catalyzed by protein Kinase Wee1, which inhibits CdK1 activity.

→ Active CdK1/Cyclin B complex is formed by dephosphorylation of Tyrosine 15 & Threonine 14 by the protein phosphatase Cdc 25C.

→ Active CdK1/Cyclin B complex promote G2 to M transition

→ MPF activity is then terminated toward the end of the mitosis by proteolytic degradation of cyclin B.

→ Dephosphorylation of CdK1 on Threonine 161 position makes it ready to bind with cyclin B again.

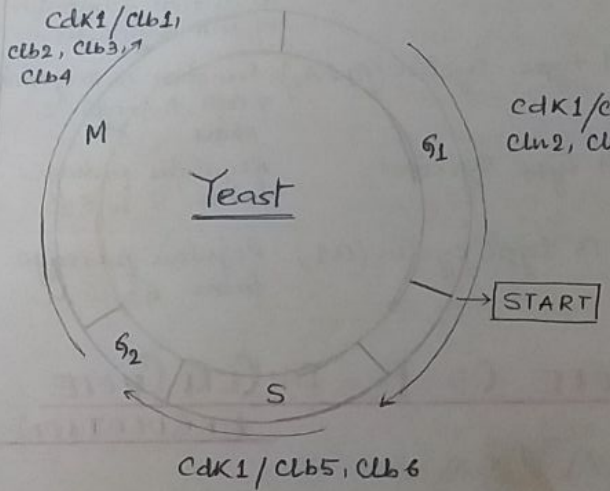
Short

Role of Cyclin and Cyclin Dependent Kinase in Cell Cycle Regulation :-

In Prokaryotes :-

The cell cycles of prokaryotes are controlled by multiple types of cyclin & also by multiple cyclin dependent kinases (CdKs). Spe. Each type of cyclin associates with specific type of CdK & makes a complex, needed to progress through cell cycle. different point of cell cycle. ~~As in Yeast~~

As in Yeast two types of cyclin (G1 cyclin & B-type cyclin) combine with CdK1 & make the passage through cell cycle.



- In Yeast passage through START is controlled by CdK1 in association with G1 cyclin (Cln1, Cln2, Cln3)
- Complex of CdK1 with distinct ~~same~~ B type cyclin (Clb5, Clb6) regulate progression through S phase.
- Complex of CdK1 with distinct B type cyclin (Clb1, Clb2, Clb3, Clb4) regulate entry of the cell into mitosis.

In Eukaryotes :-

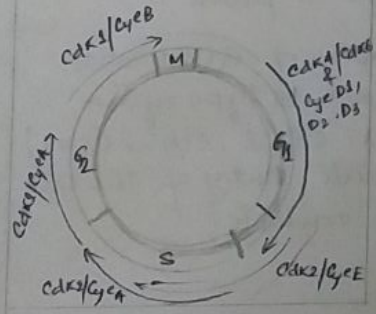
Cyclins are the proteins, accumulated ~~periodically~~ & destructed periodically & responsible for controlling the entry & exit of cell through the different phase of cell cycle.

CdKs are protein kinases & encoded by specific genes, has been shown to be a conserved cell cycle regulator. ~~in all euk~~ Phosphorylation & Dephosphorylation of CdK's are controlled by Cyclin thats why CdK's are Cyclin dependent Kinase.

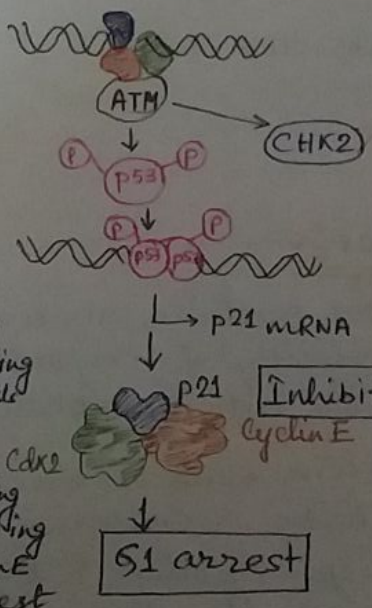
In different phase of Cell cycle specific type of CdK get phosphorylated by specific type of cyclin & makes active CdK - Cyclin complex, which is essential for progression of a cell through Cell Cycle.

~~There~~ There are many types of CdK & cyclin, specifically in Eukaryotes multiple members of CdK family associate with specific cyclins to drive progression through different stages of Cell cycle. For example →

• Complex of CdK4 & CdK6 with D type cyclin (Cyclins D1, D2, D3)	Control progression through G1 restriction point.
• Complex of CdK2, ~~CdK4, CdK6~~ & E type cyclin (E1 & E2)	Required for G1 to S transition & initiation of DNA synthesis.
• Complex of CdK2 with A type cyclin (A_1 & A_2)	Function in progression of cells through S phase
• Complex of CdK1 with A type cyclin	Regulate passage form S to G2
• Complex of CdK1 with B type cyclin (B1, B2, B3)	Regulate passage from G2 to M.



Role Of P53 In Cell Cycle Regulation



The protein P53 plays a key role in cell cycle arrest at G1 checkpoint in mammalian cells. Phosphorylation by ATM & CHK2 stabilize P53, resulting in rapid increases in P53 levels in response to DNA damage. The Protein P53 then activates transcription of the gene encoding the CdK Inhibitor P21, leading to inhibition of CdK2/CyclinE Complexes & cell cycle arrest in G1. Mutation in P53 gene results loss of P53 function, as a result G1 arrest does not occur in response to DNA damage, so damaged DNA is replicated & passed to daughter cells instead of being repaired.

CANCER

Dhera 15

■ TUMOR SUPPRESSOR GENE

• In normal cell, gene product of few genes has the ability to suppress the uncontrolled cell proliferation i.e characteristic of cancer cell. The genes are called tumor suppressor gene.

• Mutation of tumor suppressor gene produce nonfunctional gene product; as a result uncontrolled cell proliferation takes place.

• Mutations of tumor suppressor genes are recessive that is; cell proliferation can be affected only if both alleles are inactivated.

■ RETINOBLASTOMA (RB) — a tumor suppressor gene

i) The human RB tumor suppressor gene has been mapped to 13q14.1 — q14.2; to produce 928 aa containing 4.7 Kb mRNA which translated encodes nuclear phosphoprotein pRB

ii) pRB is involved in regulating cell cycle at the G1 to S checkpoint.

iii) Two CdK-cyclin complexes are formed during G1, are CdK4 - cyclin D and CdK2 - cyclin E. These complexes cause the phosphorylation & dephosphorylation of pRB protein & regulate G1 to S checkpoints.

~~Unphosphorylated pRB protein~~

In unphosphorylated state, pRB bind to a transcription factor named E2F. & make a complex

↓

Activity of E2F is inhibited

↓

Inhibited E2F does not promote the transcription of gene essential for DNA synthesis.

In G1 phase CdK-cyclin complex phosphorylate pRB protein

↓

Phosphorylated pRB cant bind to E2F

↓

Free E2F becomes active & transcribe the essential genes for DNA synthesis.

↓

G1 to S progress

↓

cyclin degraded after the G1 to S progression; so pRB no longer being phosphorylated.

↓

Unphosphorylated pRB again bind to E2F & prevent transcription of gene for DNA synthesis

In a cell with
So the phosphorylated & un phosphorylated state of PRB regulate Cell cycle by activating & inactivating the E2F respectively.

In a cell with two mutant rb alleles, PRB often is truncated & unstable & does not bind to E2F; activation & inactivation and inactivate it no more. So, E2F becomes active for all time & it activate DNA synthesis gene. Cell devision becomes unprogrammed.

■ THE TP53 — TUMOR SUPPRESSOR GENE :

The tumor suppressor gene TP53 encodes a protein of molecular weight 53 KDa called p53. When both alleles are mutated, TP53 may involved in development of perhaps 50% of all human cancers.

Individuals who inherit one mutant copy of TP53 develop Li-Fraumeni Syndrome; when second copy of TP53 becomes mutated, cancer develops in a number of tissues.

FUNCTION OF p53

i) When DNA damage occurs, p53 initiates a cascade of events leading to arrest in G1.

ii) p53 provides some protection against oncogenes.

iii) p53 also plays a role in progammed cell death (apoptosis) a process by which a cell with a high level of DNA damage commits suicide.

In this process →

p53 does not induce DNA repair genes

↳ activates the BAX gene for apoptosis pathway

↓

BAX protein blocks the function of the BCL-2 protein

↓

BCL2 is a repressor for apoptosis pathway; without an active BCL-2 repressor apoptotic pathway activated and the cell commits suicide.

If both alleles of TP53 carry loss of function mutations, no active p53 can be produced. Thus no p21 is available to block CdK activity, so the cell is unable to arrest in G1. Similarly, a cell with high level of DNA damage will not be able to undergo apoptosis. Uncontrolled cell devision will take place.