Role of Cell Cycle Checkpoints In Cell Division Regulation

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Abstract- Cell cycle is the regulatory mechanism of regulated cell division process in the body. The cell cycle checkpoints such as regulatory protein and genes work together in different stages during interphase stage of the cell cycle that regulates the cell division in regulatory manner. Some of the regulatory proteins and gene such as p53, CDK family, cyclin family MAPK etc. responsible for proper functioning of the cell division processes such as unreplicated sections of DNA blocks progression in the cell cycle, DNA replication, cell growth, growth factors, cell proliferation, differentiation etc. in both the cells prokaryotic as well as eukaryotic cell. A proto-oncogene is a normal gene having the potential to become an oncogene by mutations, while oncogene is a gene that when mutated, gains a function or is over-expressed, abnormally resulting in the conversion of a normal cell into a cancer cell and the CDKIs are commonly mutated or suppressed in many human malignancies. For instance, germline mutations of p16 causes melanoma. At the single cell level, each cell either had entirely phosphorylated MAPK or no phosphorylated MAPK, confirming that it acts as a switch-like mechanism in each cell. While cyclins activate the CDKs, their inhibitors (CDKIs) suppress the CDKs, exerting negative control over the cell cycle. Somatically acquired inactivation or deletion of p16 causes carcinoma pancreas, glioblastomas, cancer of esophagus, acute lymphocytic leukemia (ALL), Non-small-cell lung carcinomas (NSCLC), soft-tissue sarcomas, and urinary bladder cancers.

KEYWORDS: Cell cycle checkpoints, p53 gene, CDK proteins, MAPK, Non-small-cell lung carcinomas (NSCLC).

I. INTRODUCTION

Cell cycle is genetically controlled series of changes that occur in a newly formed cell by which it supplicates ions contents, undergoes, growth and division to form two daughter cells. The process consists of two stages in which first is cell cycle and second is cell division and these two stages/phases are long non-dividing Interphase (I-phase) and a short dividing mitotic phase (M-phase) [1]. Cells are the basic units of all living things which make from single cell structure to continuous regulatory division into leaves, roots, stems, and flowers in plants but the same way cells are responsible for bones, muscles, skin, and blood in animals. As the organism grows, the cells must reproduce.

Cell Cycle is the sequence of series of events that occur within cell, by which a cell duplicates its genome and synthesizes the other cell constituents. These events include the duplication of its DNA and its organelles and subsequently the partitioning of its cytoplasm and other components into two daughter cells. The cell cycle process is similar in prokaryotic cells and eukaryotic cells but it is more complex in eukaryotic cells type. Cell Cycle is the series of events which occur in a cell that includes the division and growth of a cell. The division of a mother cell into two or more daughter cells is known as Cell Division. Cell division is the key to growth, repair, and reproduction. Cell division is generally referred to as cellular replications.

II. PHASES OF CELL CYCLE

The Cell cycle process consist of two main phases such ass interphase and mitotic phase which occurs simultaneously in cell division process specially in mitosis cell division.

A. ***Interphase-*** The interphase also called the resting phase in the meantime; the cell is preparing for division by undergoing both cell growth and DNA replication in an orderly manner. Interphase lasts quite 95% of the cell cycle. In Interphase, a newly formed cell and its nucleus enquiring a series of changes before it becomes capable of division again. It is further divided into four phases-

1. **G0 Phase (Quiescent stage)-** In this phase, cells remain metabolically active but do not undergo division. The G0 phase is a reversible stage where a cell can enter the cell cycle again to divide and the cells in this phase have different regulators that ensure the proper functioning of the cell. Sometimes, cells might enter the G0 phase from the checkpoint in the G1 phase because of the lack of growth factors or nutrients. Nerve cells and muscle cells are examples of cells that enter the G0 phase when they reach maturity. Some cells like the liver and kidneys' parenchymal cells enter the G0 phase semi-permanently and can be induced to divide (Figure 1).
2. **G1 phase (Gap 1)-** It is a part of the interphase and it is a termination of previous mitotic phase and continues till the starting of DNA replication, so it is known as the first growth phase/post-mitotic gap phase. The cell is metabolically active and continuously grows and the transcriptions of all three sets of RNA such as mRNA, tRNA and rRNA are complete and proteins are synthesized. The duration of the G1 phase is also highly variable among different cells. The importance of G1 phase is the G1/S checkpoint determining. At this point, events like detecting DNA damage and nutrient concentration are performed to make sure that the cell has enough machinery to undergo cell division (Figure 1).

3. **S-Phase (Synthetic Phase)-** The S-phase is known as the synthesis phase take place in between G1 and G2 phases and in this phase DNA replication takes place on the template of the existing DNA to form chromatin and chromatids. The formation of protein especially histone proteins are important in this phase as the newly replicated DNA molecules need histone proteins to form nucleosomes. The entry into the S phase is regulated by the G1/S checkpoint that only enables cells with enough nutrients and healthy DNA to enter the next phase. It is moderately long and having about 30% of the total cell cycle time. Each chromosome carries a duplicate set of genes. A haploid cell becomes diploid, and a diploid cell becomes tetraploid at the end of the S phase (Figure 1).
4. **G2 Phase (Gap 2)-** G2 phase is also known as the second growth phase or pre mitotic gap phase because the cell collects nutrients and releases proteins to prepare the cell for the M phase. This phase essentially checks for DNA damage (during replication) to make sure that the cell is in proper condition to undergo division and also regulates the synthesis of DNA stops, synthesis of RNAs and proteins continues [2]. Organelles and spindle formation start in this phase and the entry of the cell from the G2 phase to the M phase is regulated by the G2 checkpoint, where different proteins and complexes are involved. In case of DNA damage or insufficient nutrients, the cell remains in the G2 phase and is not passed for cell division (Figure 1).

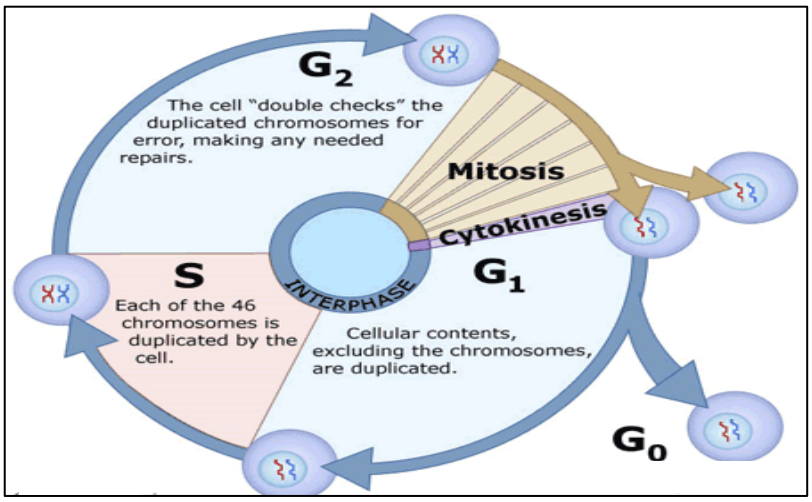

Figure 1: The Cell Cycle and their phases.

(B) **M Phase (Mitotic Phase)-** Mitotic phase is most dramatic multi-step process in which actual cell division occurs. In this phase the chromosomal movement occurs rapidly from center to poles by the contraction and relaxation of spindle fibers which are originated from centrioles and become separated to form double cell structure with actual number of chromosomes. This phase starts with the nuclear division, corresponding to the separation of the daughter chromosome (Karyokinesis) and usually ends with the division of cytoplasm (Cytokinesis). It is also called an equational division because the number of chromosomes in parent and daughter cells remain the same (Figure 2). The cellular division is called somatic division**.** Cell division resulting in the assembly of diploid cells for growth and development. In plants, mitosis happens in both haploid, diploid cells and there are two major events happen in mitosis- first is Karyokinesis and second is cytokinesis. Mitosis is accomplished during the expansion and development of the organisms. The Karyokinesis process complete in four phases-

1. ***Prophase***- It is the first phase of mitosis where the chromosomes become shorter and thicker and have clear visibility. Chromosomes are become identical to paired chromatids. The sister chromatids link to each other at a central little region called centromere and the centrioles begin moving apart and reach opposite poles. Spindle fibers appear in the middle of daughter centrioles forming the achromatic spindle. Various cell organelles such as Golgi complex, Endoplasmic reticulum are not throughout this stage and the nucleolus and nuclear membrane also vanish from this site (Figure 2).

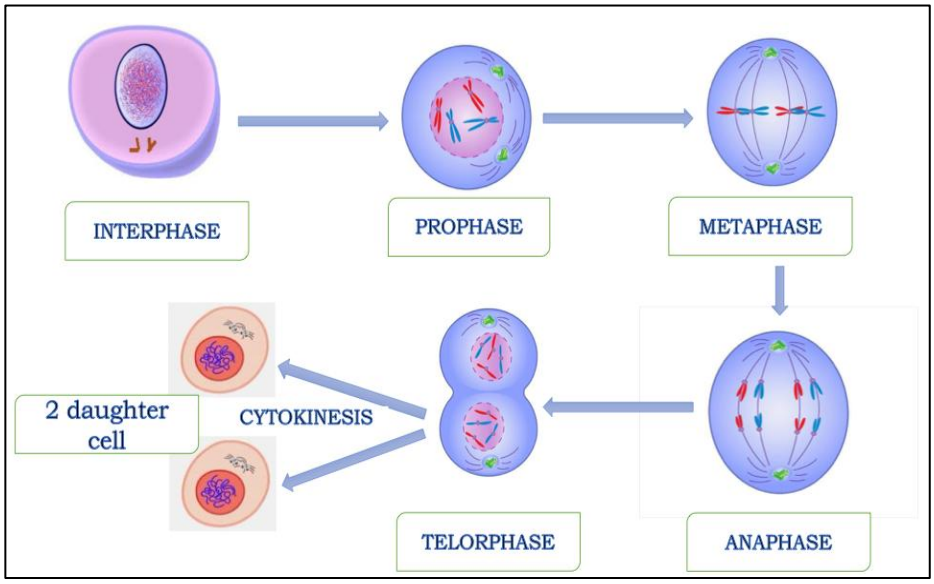

Figure 2- Stages in the mitosis cell division during M-phase.

2. *Metaphase-* In this phase each chromosome gets attached to the spindle fibers by its centromere. In each chromosome, both chromatids are connected to the spindle fibre from both opposite poles. Chromosomes line up in one plane at the equator. The plane of alignment of chromosomes during this phase so called the metaphase plate (Figure 2).

3. *Anaphase-* Centromere attaching the two chromatids divides. The two sister chromatids of each chromosome separate and are drawn apart towards opposite poles pulled by shortening of spindle fibers. The chromosomes which are separated and moves towards their opposite poles. This phase is called anaphase stage (Figure 2).

4. ***Telophase-*** The two sets of daughter chromosomes reach opposite poles and the spindle fibers vanish from site. When spindle fibers become disappeared. It induced the appearance of nuclear membrane around the separated chromosomal clusters. The other cell organelles such as nucleolus**,** Golgi complex and endoplasmic reticulum are formed. The cleavage lines begin deepening within the animal cell. Karyokinesis is followed by cytokinesis (Figure 2).

C. Cytokinesis

In the late telophase, an invagination appears within the cell membrane in the middle, which deepens and eventually splits the cytoplasm into two parts and produce two new cells. In plant cells, cell wall formation begins within the center by the formation of the cell plate. This grows outwards to meet the existing lateral walls, and thus, the cytoplasm is split into two parts. This cell plate becomes the center lamellae for the plant cells [3]. In some organism's cytokinesis is not immediately followed by karyokinesis, such as fungi, algae and plant cells and therefore, the multinucleate stage is created, referred to as a syncytium. Cytokinesis process occurs in both animal and plant cell but in slightly different in manner (Figure 3 and 4).

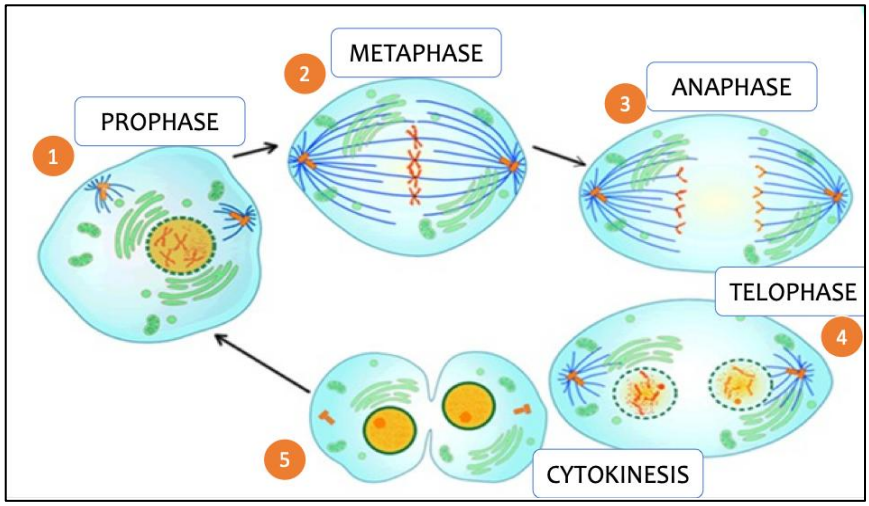

Figure 3- Cytokinesis in animal cell

i. **Animal cytokinesis-** The spindle gets charged into dense fibrous and vesicular structure on equator called mid body. In the middle region of cell, microfilaments start to collected which induces the cell membrane to invaginate and

furrow forms and deepens centripetally and finally cleaves the parent cell into two daughter cells. This method of cytokinesis is known as cleavage method. All cell organelles such as mitochondria, Golgi complex, lysosomes., ER, ribosomes etc.) are also distributed nearly equal between two daughter cells (Figure 3).

ii. **Plant cytokinesis**- The plant cytokinesis are differs from animal cytokinesis due to presence of rigid cell wall. In lower plants cytokinesis occurs by cleavages method (like animal cell) and in higher plants it takes place by cell plate method. During plant cytokinesis small vesicles of Golgi complex are collected at the equator. Here spindle persists for some time called phragmoplast. All vesicles fuse to form two sheets which enclose a matrix/film which solidified to form cell plate or middle lamella. It grows centrifugally and finally phragmoplast disappear. Cellulose, hemicelluloses and pectin are deposited on either side of cells plate. It forms primary wall (Figure 4).

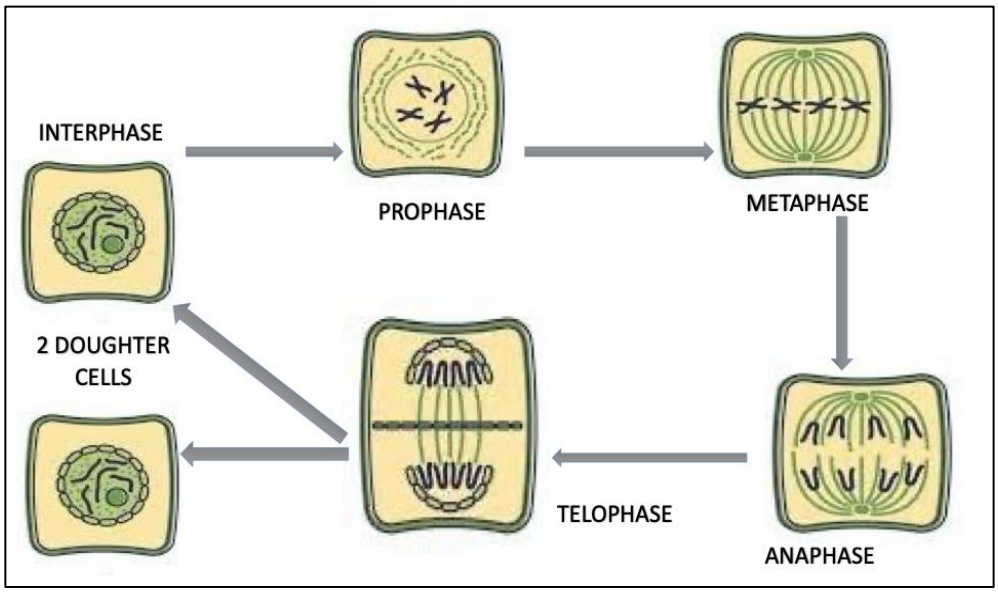

Figure 4- Cytokinesis in plant cells.

III. CELL CYCLE CHECKPOINTS

Cell cycle checkpoints are controlling mechanisms in the eukaryotic as well as prokaryotic cell cycle for ensuring proper progression. Each checkpoint serves as a potential termination point along the cell cycle [4], during which the conditions of the cell are assessed, with progression through the various phases of the cell cycle occurring only when favorable conditions are met. There are many checkpoints in the cell cycle [5], but there three major ones are: the G1 checkpoint, also known as the start or restriction checkpoints. The metaphase-to-anaphase transition, also known as the spindle checkpoints [6]. Progression through these checkpoints is largely determined by the activation of cyclin-dependent kinases by regulatory protein subunits called cyclins which are produced at each stage of the cell cycle to control the specific events that occur therein [7]. The protein cyclins are differentiated into many forms such as cyclin A, B, D, E. All cyclins are work at different phase in interphase and control the cell cycle (Figure 5).

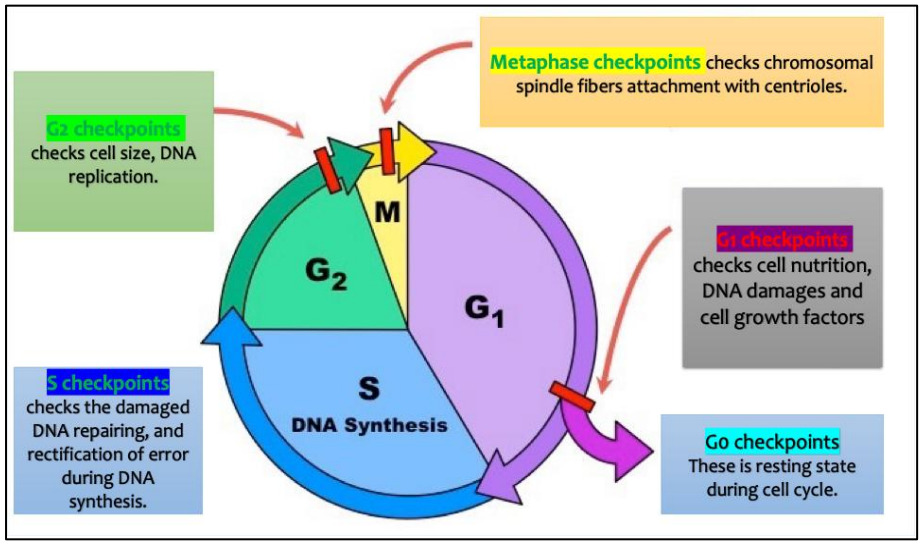

Figure 5: Cell Cycle Checkpoints at every stage during cell division.

1. **G1 checkpoints**

The G1 checkpoint, also known as the restriction point in mammalian cells and the start point in yeast. It is the point at which the cell becomes committed to entering the cell cycle, cell progresses through G1, depending on internal and external conditions. G1 checkpoints can either delay G1, G0 or proceed past the restriction points. G1 checkpoint also check and regulates size of cells its nutrition, growth factors and repair DNA damages. DNA damage is the main indication for a cell to restrict and not enter the cell cycle [8]. The decision to commit to a new round of cell division occurs when the cell activates cyclin-CDK-dependent transcription which promotes entry into S phase. This check point ensures the further process [9] (Figure 5). There are two checkpoint work at G1 phase by its different mechanisms.

a. ***MAPK signaling cascade***- During early G1, there are three transcriptional repressors, known as pocket proteins, that bind to E2F transcription factors. These gene are a group of transcription factors that target many genes which are important for control of the cell cycle, including cyclins, CDKs, checkpoint regulators, and DNA repair proteins. Mis-regulation of the E2F family is often found in cancer cases, providing evidence that the E2F family is essential for the tight regulation of DNA replication and division. The three pocket proteins are retinoblastoma (Rb), p107, and p130, binds to E2F transcription factors to prevent progression past the G1 checkpoint. These gene family contains some proteins with activator mechanisms and some proteins with repressing mechanisms. P107 and p130 act as co-repressors for E2F 4 and E2F 5, which work to repress transcription of G1-to-S promoting factors. The third pocket protein, Rb, binds to and represses E2F 1, E2F 2, and E2F 3, which are the E2F proteins with activating abilities [9] (Figure 5).

b. ***Cyclin-dependent kinase inhibitor 1B (CDKN1B)-*** Cyclin-dependent kinase inhibitor 1B (CDKN1B), also known as p27 proteins which binds and prevents the activation of cyclins E and inhibit the action of Cdk2. However, as Cyclin A accumulates and binds to Cdk2, they form a complex and inhibit p27 protein activity. The G1 phase cyclin-dependent kinase works together with S phase cyclin-dependent kinase targeting p27 for degradation and allows for full activation of Cyclin A: Cdk2, a complex which phosphorylates E2F 1-3 initiating their disassociation from the DNA promoter sites [10]. This allows E2F 6-8 to bind to the DNA and inhibit transcription. The negative feedback loop used to successfully inhibit the inhibitor, p27, is another essential process used by cells to ensure mono-directional movement and no backtrack through the cell cycle [11].

When DNA damage occurs and cell detects any defects which necessitate it to delay and halt the cell cycle in G1 phase, arrest occurs through several mechanisms. The rapid response involves phosphorylation events that initiate with either kinase ATM (Ataxia Telangiectasia mutated) or ATR (Ataxia Telangiectasia and rad3 related). Both are act as sensors, depending on the type of damage [12]. These kinases phosphorylate and activate the effector kinases Chk-2 and Chk-1 respectively. As Cdc25A activates the previously mentioned cyclin E-CDK2 complex by removing inhibitory phosphates from CDK2.

2. ***P53 activity both in G1 and S phase-*** The p53, also known as Tumor protein P53, cellular tumor antigen p53 or transformation-related protein 53 (TRP53) is a regulatory transcription factor protein that is often mutated in human cancers. The p53 proteins are crucial in vertebrates, where they prevent cancer formation and as the guardian of the genome, because of its role in conserving stability by preventing genome mutation [13]. Hence TP5 is classified as a tumor suppressor gene [14, 15]. To maintain the arrest, another response is initiated, by which Chk2 or Chk1 phosphorylate p53, a tumor suppressor, and this stabilizes p53 by preventing it from binding Mdm2, a ubiquitin ligase which inhibits p53 by targeting it for degradation. The stable p53 then acts a transcriptional activator of several target genes, including p21, an inhibitor of the G1-to-S promoting complex cyclin E-CDK2 [16]. In addition, another mechanism by which p21 is activated is through the accumulation of p16 in response to DNA damage [17]. The p16 genes disrupts cyclin D-CDK4 complexes, thus causing the release of p21 from the complexes, which leads to the dephosphorylation and activation of Rb, which allows Rb to bind and inhibit E2F 1-3, thus keeping the cell from transitioning to S phase. Recently, some aspects of this model have been disputed [15].

3. **G2 checkpoints**

Similar to S Phase, G2 experiences a DNA damage checkpoint. The cell is once more examined for sites of DNA damage or incomplete replication. ATR and ATM both kinases are recruited to damage sites. Activation of Chk1 and Chk2 also transpire, as well as p53 activation, to induce cell cycle arrest and halt progression into mitosis. An additional component of S phase, the Pre-Replicative Complex (Pre-RC), must be inactivated via cyclin B-Cdk1 phosphorylation [6].

c. ***Cyclin-dependent kinase 2-*** Cyclin-dependent kinase 2, also known as cell division protein kinase 2, (Cdk2), is an enzyme that encoded by CDK-2 gene in humans [18]. The protein is a member of the cyclin-dependent kinase family of Ser/Thr protein kinases. This kinase is highly similar to the gene products of *S. cerevisiae* cdc28, and *S. pombe* cdc2, also known as Cdk1 in humans. The catalytic subunit of the cyclin-dependent kinase complex, whose activity is restricted to the G1-S phase of the cell cycle, where cells make proteins necessary for mitosis and replicate their DNA.

This protein associates with and is regulated by the regulatory subunits of the complex including cyclin E or A. Cyclin E binds G1 phase Cdk2, which is required for the transition from G1 to S phase while binding with Cyclin A is required to progress through the S phase [19]. Its activity is also regulated by phosphorylation. Multiple alternatively spliced variants and multiple transcription initiation sites of this gene have been reported [20].

The mechanism of activated cyclin B to CDK-1 with G2 protein accumulation. CyclinA-Cdk2 activates Cdc25, an activator of cyclinB-Cdk1, which then deactivates the cyclinB-Cdk1 inhibitor Wee1. This results in a positive feedback loop, significantly increasing cyclin B expression and Cdk1 activation. As the cell progresses through G2 and reaches the G2/M transition, the kinase Plk1 phosphorylates Wee1, which targets Wee1 for degradation via the SCF ubiquitin ligase complex [21]. An additional function of Plk1 is to activate Cdc25 through phosphorylation. The compound effect of Wee1 degradation and Cdc25 activation is the net removal of inhibitory phosphorylation from cdc2, which activates cdc2. Plk1 is activated at the G2/M transition. which accumulate during G2 and form an activation complex.

The Plk1-Cdc2-cdc25 complex then initiates a positive feedback loop which serves to further activate Cdc2, and in conjunction with an increase in cyclin B levels during G2, the resulting cdc2-cyclin B complexes then activate downstream targets which promote entry into mitosis. The resultant Cdk1 activity also activates expression of Mem1-Fkh, a G2/M transition gene. The rapid surge in cyclinB-Cdk1 activity is necessary, as M phase initiation is an all-or-nothing event engaging in hysteresis. Hysteresis of Cdk1 activity via cyclin B drives M phase entry by establishing a minimum threshold of cyclin B concentration. This exists at a level higher than the minimum needed for the continuation of M phase after entry, acting to safeguard the all-or-nothing event. This entry concentration is further increased in the case of incomplete DNA replication, adding another regulatory mechanism at the G2/M transition point. The presence of hysteresis allows for M phase entry to be highly regulated as a function of cyclinB-Cdk1 activity [22].

d. G2–M transition in Xenopus oocytes

To know the regulatory mechanism of G2-M transition into mitosis, At the end of G2, the cell transitions into mitosis, where the nucleus divides. The G2 to M transition is dramatic; there is an all-or-nothing effect, and the transition is irreversible. This is advantageous to the cell because entering mitosis is a critical step in the life cycle of a cell. If it does not fully commit, the cell would run into many issues with partially dividing, ultimately likely leading to the cell's death [23].

In frog oocytes, the signal cascade is induced when progesterone binds to a membrane bound receptor. Downstream, Mos is activated. Mos then phosphorylates MEK1, which phosphorylates MAPK. MAPK serves two roles: activating the CyclinB-Cdk1 complex to initiate entrance into mitosis and activating Mos. The activation of Mos leads to a positive feedback loop and therefore acts as toggle switch to create the all-or-nothing entrance into mitosis.

e. ***Activity of MAPK-P (phosphorylated MAPK)-*** This feedback loop was first found by showing that MAPK-P (phosphorylated MAPK) concentrations increased in response to increasing levels of progesterone. At the single cell level, each cell either had entirely phosphorylated MAPK or no phosphorylated MAPK, confirming that it acts as a switch-like mechanism in each cell. It was additionally shown that blocking Mos protein synthesis makes the MAPK-P responses more graded, showing that Mos protein synthesis is necessary for the all-or-none character of MAPK activation (Figure 6).

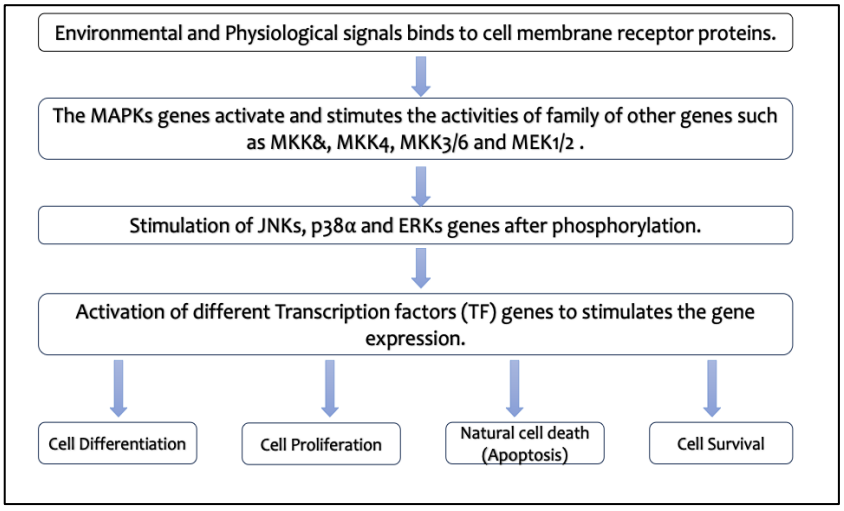

Figure 6- flow diagram of MAPKs genes and transcription factors (TF) on the gene expression that stimulates the proliferation, differentiation, apoptosis and survival of cells.

IV. REPLICATION STRESS RESPONSE

Since entering mitosis is a large and costly commitment for the cell, it is logical that systems would be in place to prevent premature entrance into this step. It has been shown that mistakes in previous steps, such as having unreplicated sections of DNA blocks progression in the cell cycle. The Novak–Tyson model predicts this occurs via raising the level of cyclin B necessary for entrance into mitosis.

Sha et al. 2003, investigated whether this was true in *Xenopus* egg extracts. They used aphidicolin (APH) to inhibit DNA polymerase and prevent DNA replication [23]. When treated with Cyclin B in interphase, the threshold of activation

increased to between 80 and 100 nM, as predicted by the Novak–Tyson model. In 1990 Bela Novak and John Tyson, identified and characterized the dynamic relationship between cyclin and MPF in interphase arrested in Xenopus species of frog [24]. Cyclin activation of P34cdc2 and triggering of cyclin degradation in interphase of amphibian eggs of cdc 2 kinases [25, 26]. So, these experiments confirm that the stress of unreplicated DNA in the cell affect the hysteresis loop and result in a much higher cyclin B threshold to enter into mitosis.

V. METAPHASE CHECKPOINTS

The mitotic spindle checkpoints at metaphase stage of cell division where all the centrosomes arranged in aligned at the mitotic plate and be under bipolar tension. The tension created by this bipolar attachment is what is sensed, which initiates the anaphase entry. To do this, the sensing mechanism ensures that the anaphase-promoting complex (APC/C) is no longer inhibited, which is now free to degrade cyclin B which harbors a D-box (destruction box), and to break down securin and also inhibit separase, which in turn cuts cohesins, the protein composite responsible for cohesion of sister chromatids. Once this inhibitory protein is degraded via ubiquitination and subsequent proteolysis, separase then causes sister chromatid separation. After the cell has split into its two daughter cells, the cell enters G_1 (Figure 9).

In *S. cerevisiae*, Pds1p (securin) regulates sister chromatids cohesion, because it binds and inhibits the protease Esp1p also known separin or separase. When anaphase onset is triggered. The anaphase-pramoting complex (APC). This anaphase promoting complex also known Cyclosome that degrades securin. It is a ring E3 ubiquitin ligase that recruits an E2 ubiquitin-conjugating enzyme loaded with ubiquitin. When securin, Cdc20, and E2 are all bound to APC/C E2 ubiquitinates securin and selectively degrades it. Securin degradation releases the protease Esp1p/separase, which degrades the cohesin rings that link the two sister chromatids, therefore promoting sister chromatids separation [27] and also shown that Polo/Cdc5 kinase phosphorylates serine residues next to the cutting site for Scc1, and this phosphorylation would facilitate the cutting activity [28]. On the other hand, some experiments show that sister chromatids cohesion in the arms is lost gradually after sister centromeres have separated, and sister chromatids move toward the opposite poles of the cells [29, 30].

VI. ROLE OF CYCLIN: CDK COMPLEX IN CANCER

Cancer or malignancy is the unchecked and uncontrolled multiplication of cells. Cell cycle regulator genes or the checkpoint genes are either mis regulated or mutated in cancer, for example as seen in case of overexpression of cyclin D in cancer of breast, esophagus, liver, and a subset of lymphomas. However, amplification of the CDK4 gene leads to malignancies like melanomas, sarcomas, and glioblastomas [31]. In the regulation of cell cycle and mutations in the genes coding cell-cycle regulatory proteins result in unrestrained proliferation of cells leading to formation of tumors [32, 33]. Accordingly, modulation of activity of these proteins by therapeutic agents has been suggested as a promising strategy for treatment of cancers [34]. At the single cell level, each cell either had entirely phosphorylated MAPK or no phosphorylated MAPK, confirming that it acts as a switch-like mechanism in each cell. While cyclins activate the CDKs, their inhibitors (CDKIs) suppress the CDKs, exerting negative control over the cell cycle. The CDKIs are commonly mutated or suppressed in many human malignancies [35]. For instance, germline mutations of p16 causes melanoma.

Somatically acquired inactivation or deletion of p16 causes carcinoma pancreas, glioblastomas, cancer of esophagus, acute lymphocytic leukemia (ALL), non-small-cell lung carcinomas (NSCLC), soft-tissue sarcomas, and urinary bladder cancers [31, 22]. A proto-oncogene is a normal gene having the potential to become an oncogene by mutations, while oncogene is a gene that when mutated, gains a function or is over-expressed, abnormally resulting in the conversion of a normal cell into a cancer cell [36]. It also causes a rise in gene transcription by kinases. The proto-oncogene works normally when cyclins are excess in the cells induces normal cell cycle in the body, but it also inhibits the work of p53 gene and these genes causes stopping natural cell death. The second important gene known as tumor suppressor gene work antagonistic against proto-oncogenes. It inhibits the cyclin molecules which causes cell cycle in restricted manner and also promoting p53 gene which promotes the natural cell death. These kinases are called transcription factors which usually affect G_1/S transition, causing increased cyclin-CDK expression with decreased inhibition at an inappropriate time of cell cycle [37, 38] (Figure 7).

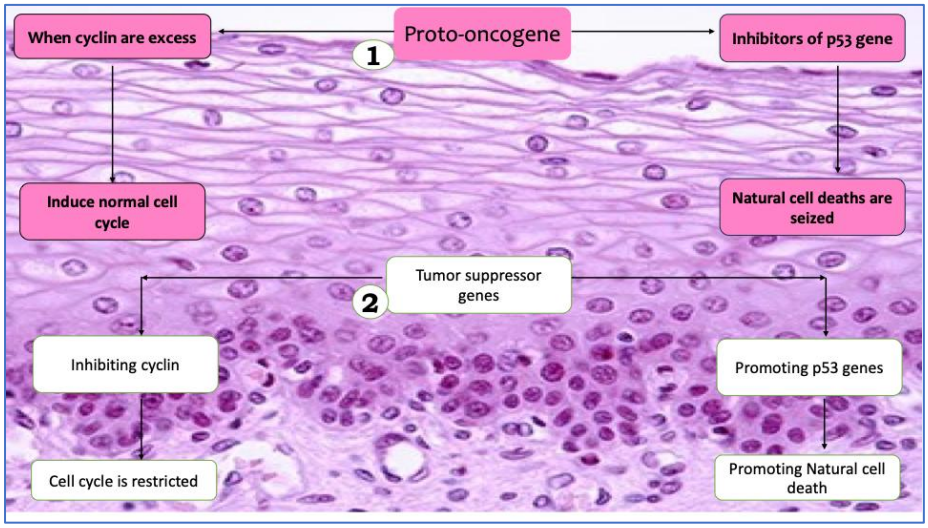

Figure 7- Flow diagram showing proto-oncogene and tumor suppressor gene.

VII. DISCUSSION

There are many checkpoints in the cell cycle [5], but there three major ones are: the G1 checkpoint, also known as the start or restriction checkpoints. The G1 checkpoints are MAPK signaling cascade and Cyclin-dependent kinase inhibitor 1B (CDKN1B). The activation of cyclins E and inhibit the action of Cdk2. In which cyclin-A accumulates and binds to Cdk2, they form a complex and inhibit p27 protein activity. Cyclin activation of P34cdc2 and triggering of cyclin degradation in interphase of amphibian eggs of cdc 2 kinases [25, 26]. So, these experiments confirm that the stress of unreplicated DNA in the cell affect the hysteresis loop and result in a much higher cyclin B threshold to enter into mitosis. The G1 checkpoint, also known as the restriction point in which the cell becomes committed to entering the cell cycle, cell progresses through G1, depending on internal and external conditions. DNA damage is the main indication for a cell to restrict and not enter the cell cycle [8]. The G1 phase cyclin-dependent kinase works together with S phase cyclin-dependent kinase targeting p27 for degradation and allows for full activation of Cyclin A: Cdk2, a complex which phosphorylates E2F 1-3 initiating their disassociation from the DNA promoter sites [10]. During early G1, there are three transcriptional repressors, known as pocket proteins, that bind to E2F transcription factors. These gene are a group of transcription factors that target many genes which are important for control of the cell cycle, including cyclins, CDKs, checkpoint regulators, and DNA repair proteins.

To know the regulatory mechanism of G2-M transition into mitosis, At the end of G2, the cell transitions into mitosis, where the nucleus divides. The G2 to M transition is dramatic; there is an all-or-nothing effect, and the transition is irreversible. This is advantageous to the cell because entering mitosis is a critical step in the life cycle of a cell. The anaphase-promoting complex (APC/C) is no longer inhibited, which is now free to degrade cyclin B which harbors a D-box (destruction box), and to break down securin and also inhibit separase, which in turn cuts cohesins, the protein composite responsible for cohesion of sister chromatids.

In this study, we discuss about the essential principle and factors responsible for the cell cycle checkpoint. There are various factors work together for controlling the cell division and cell cycle checkpoints. These checkpoints factors work and control the cell cycle and cell division process in regular and controlling manner through the cell cycle program, which in turn sends signals forward to change the oscillation of CDK activity in order to further control the cell cycle events. Some aspects of checkpoint signaling still remain to be clearly understood either as the basic underlying principle or within the context of diseases. The various check points factors such as MAPK, CDK, p53, cyclin dependent kinase-2 and cyclin-dependent kinase inhibitors 1B work at different stages of cell cycle such as G0-phase, G1-phase, S-phase and M-phase to control and regulate the cell division process. These factors are responsible for preventing the normal cell to become cancer cell. Undoubtedly, the hidden aspects of checkpoint signaling can be further explored with the help of an ever-growing arsenal of highly sophisticated experimental tools and techniques, which will enable us to get a more clear and complete picture of the remarkable fidelity of the cell cycle.

Cancer or malignancy is the unchecked and uncontrolled multiplication of cells. Due to the over production of cyclin D in cancer of breast, esophagus, liver, and a subset of lymphomas. However, amplification of the CDK4 gene leads to malignancies like melanomas, sarcomas, and glioblastomas [31]. In the regulation of cell cycle and mutations in the genes coding cell-cycle regulatory proteins result in unrestrained proliferation of cells leading to formation of tumors [32, 33]. Accordingly, modulation of activity of these proteins by therapeutic agents has been suggested as a promising strategy for treatment of cancers [34]. At the single cell level, each cell either had entirely phosphorylated MAPK or no phosphorylated MAPK, confirming that it acts as a switch-like mechanism in each cell. While cyclins activate the CDKs, their inhibitors (CDKIs) suppress the CDKs, exerting negative control over the cell cycle. The CDKIs are commonly mutated or suppressed in many human malignancies [35]. For instance, germline mutations of p16 causes melanoma.

The p16 genes causes pancreatic carcinoma cancer, glioblastoma, acute lymphocytic leukemia, non-small cells lung cancer, soft-tissue sarcomas, and urinary bladder cancers due to the mutational changes [31, 22]. A proto-oncogene is a normal gene having the potential to become an oncogene by mutations, the activated oncogene induces over-expressed, abnormally resulting in the conversion of a normal cell into a cancer cell [36]. It also causes a rise in gene transcription by kinases. The proto-oncogene works normally when cyclins are excess in the cells induces normal cell cycle in the body, but it also inhibits the work of p53 gene and these genes causes stopping natural cell death. The second important gene known as tumor suppressor gene work antagonistic against proto-oncogenes. It inhibits the cyclin molecules which causes cell cycle in restricted manner and also promoting p53 gene which promotes the natural cell death. So due to know the importance of cell cycle checkpoints regulates the normal cell division process in organism, we have described the basic principles about the cell cycle checkpoints and their action mechanism how they control and regulates the cell division process in regulatory manner. The feature of detecting a defect in the cell cycle checkpoints, some of them signals about this defect to other normal cells to repair them. When all these defects which already takes place in cells are not repaired by them induces the malignancy in the body at the particular site and created cancer in the body. so the cell cycle checkpoints play an important role in the regulation of unwanted cell growth (malignancy) in the body of organisms [39].

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CONFLICT OF INTEREST

Author has no conflict of interest.

VII. REFERENCES

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