BHARATHIDASAN UNIVERSITY

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Programme: M.Sc., Biomedical science

Course Title : Cancer Biology

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Unit-II

TOPIC: MAJOR CELL CYCLE REGULATORY CHECKPOINTS

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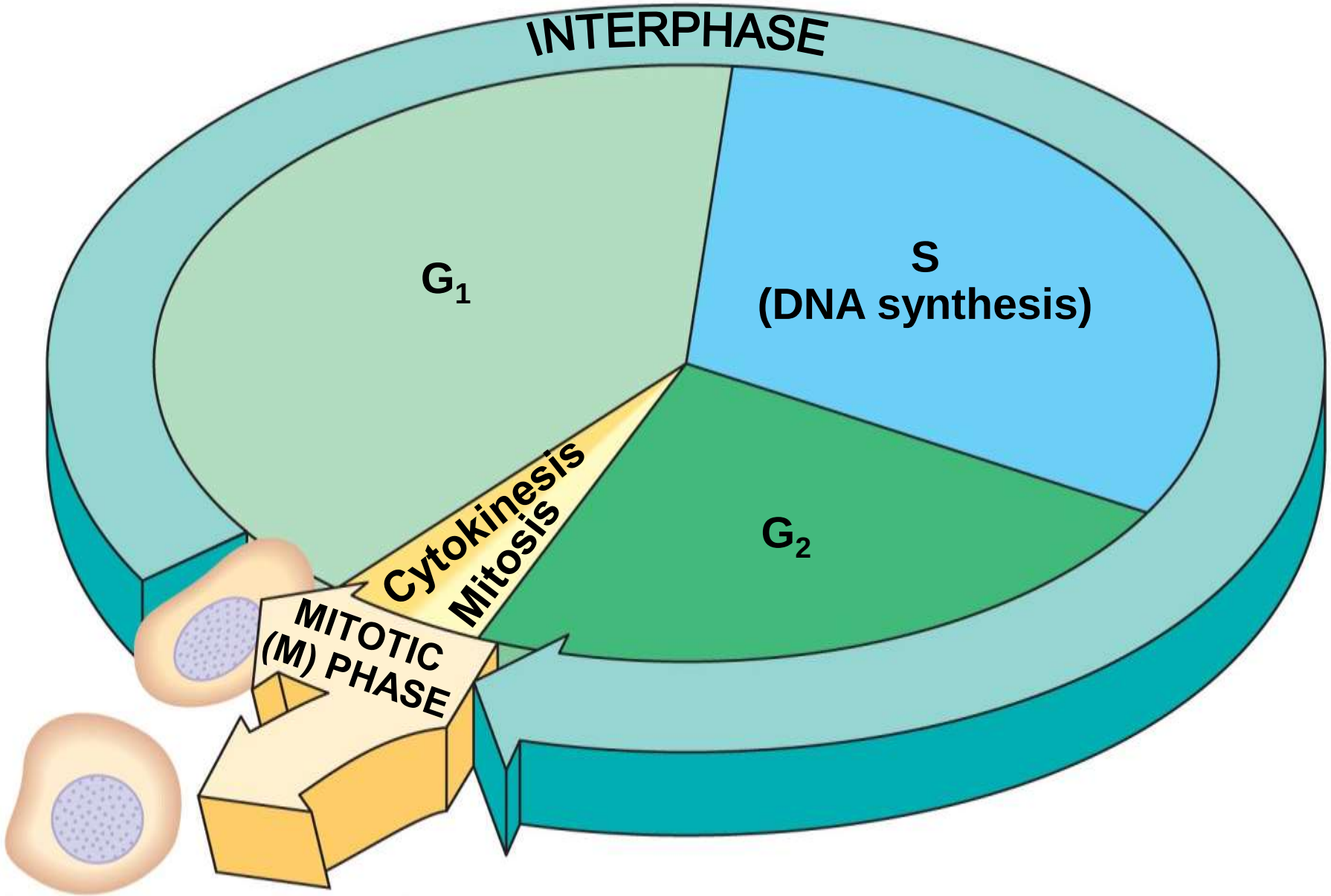
Cell Cycle Phases

- Cell cycle consist of following four phases:
- (i) G1 Gap phase for growth and preparation of the chromosomes for replication.
- (ii) S Synthesis of DNA
- (iii) G2 Gap phase for growth and preparation for mitosis
- (iv) mitosis (nuclear division) and cytokinesis (cell division)



The Cell Cycle Clock/Checkpoints

2 Types of Regulatory Molecules, together act as a checkpoint

1) Kinases

- Amount doesn't fluctuate
- enzymes that phosphorylates molecules
- Cyclin dependent kinase (Cdk) – inactive (G1 & G2) until cyclin are present

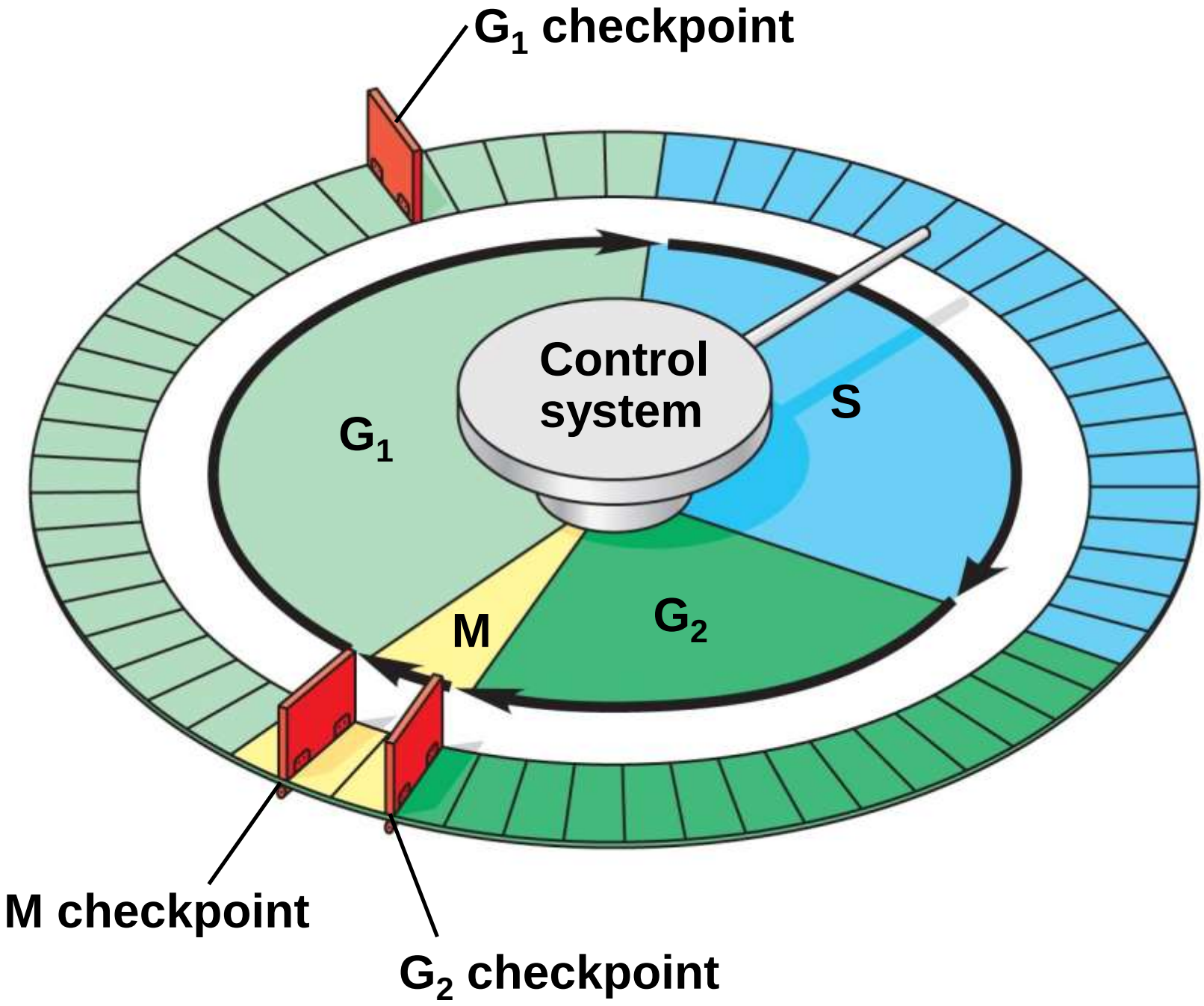
2) Cyclins

- Molecule concentration fluctuates (unlike kinase)
- Bonds w/ Kinase and serves a checkpoint
- Cyclin-Cdk complex (MPF M-phase promoting factor) promotes certain activities that eventually lead to the next stage of the cell cycle
- Having the minimum concentration of these complexes help the cell cycle proceed through the checkpoints

- There are four classes of cyclins involved in cell cycle:
- G1/S cyclins bind CDKs at the end of G1 and commit the cell to DNA replication
- S cyclins bind CDKs during S phase and are required for the initiation of DNA replication
- M cyclins promote the events of mitosis
- G1 cyclins help promote passage through start or the restriction of DNA replication

CELL CYCLE CHECK POINT

- Cyclin–CDK complexes are themselves negatively regulated by two classes of CDK inhibitors.
- These proteins – many of which are known to be tumor suppressors are able to inhibit cell cycle entry and progression and prevent replication of abnormal DNA, by allowing cells to stall at appropriate points during the cell cycle so that DNA damage can be repaired.
- These so-called “checkpoints”.



- The cell has several options for arresting the cell cycle if something goes wrong – the so-called checkpoints. For instance, if a cell sustains DNA damage following exposure to radiation or chemical agents, or replication errors have occurred during the cell cycle, then the duplication of that cell might pose a potential cancer risk to the organism.

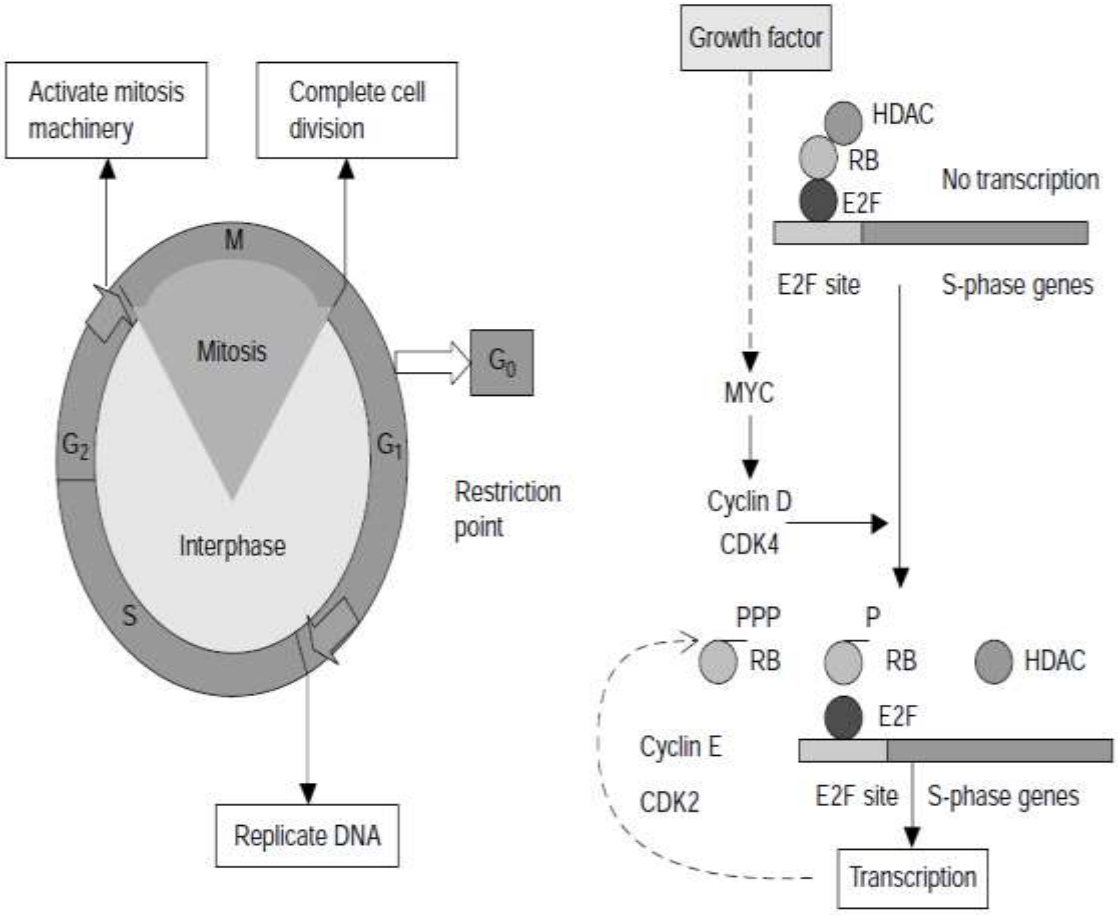
- Far better for the organism is to eliminate the cell by death (apoptosis) or for it to permanently exit from the cell cycle (senescence), unless the DNA damage can be efficiently repaired. For these reasons, there exist various checkpoints throughout the cell cycle:

CHECKPOINTS – PUTTING BREAKS ON THE CELL CYCLE ENGINE

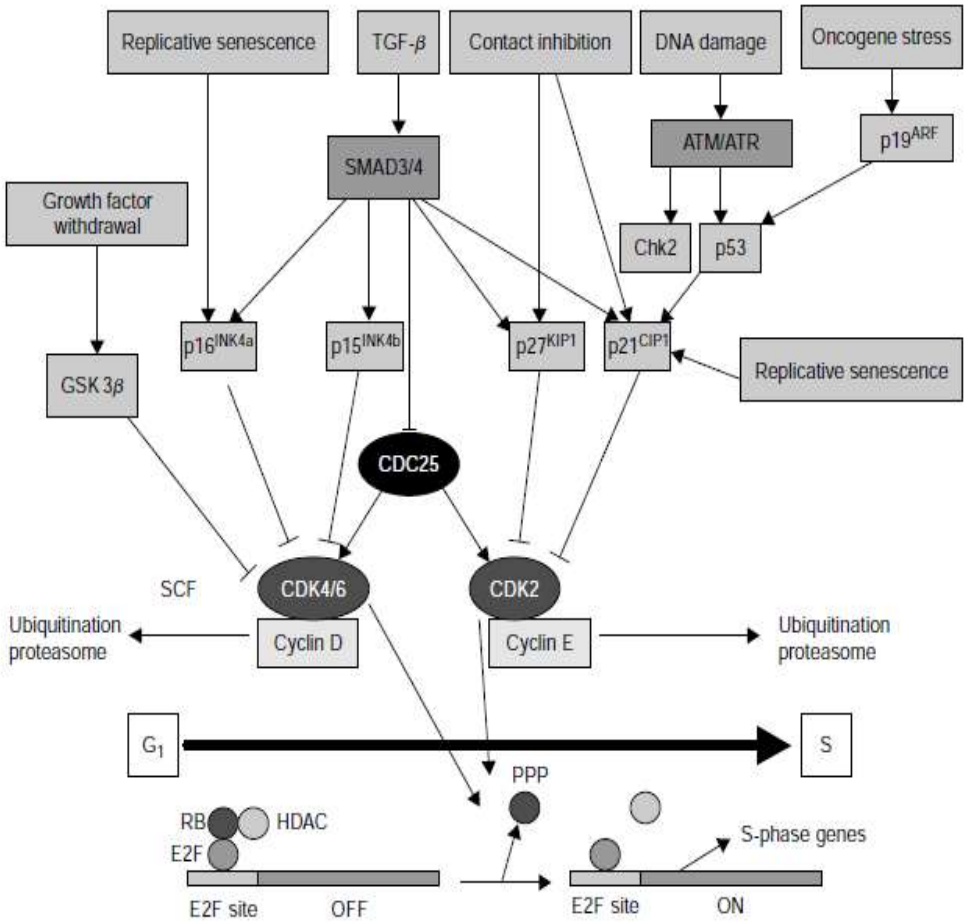
- DNA damage checkpoints operate before S phase (G1/S checkpoint), during S phase, and after DNA replication (a G2/M checkpoint)
- These checkpoints serve to detect DNA damage and stall G1 or G2 until the damage is repaired, or else trigger the elimination of the cell by apoptosis.
- In addition, mitotic (or spindle) checkpoints arrest the cell in metaphase if chromosomes are not properly aligned on the spindle prior to cell division (cytokinesis).

G1S CHECKPOINT

- One of the key players in the G1/S checkpoint is the p53 tumor-suppressor protein. A key player in stress responses and in the DNA damage response, p53 indirectly senses DNA damage and can either arrest the cell cycle in G1 by inducing expression of p21CIP1, until the damage is repaired, or if repair is not possible can trigger apoptosis, via induction of various proapoptotic factors (PUMA, BAX, NOXA).
- Two checkpoint pathways involved in DNA damage have been described.
- In the first, double-stranded breaks (DSBs) activate the ATM (ataxia telangiectasia mutated) pathway, which can arrest the cell cycle in G1 in a p53-dependent manner.



The G1/S cell cycle checkpoint controls the passage of eukaryotic cells from the first "gap" phase (G1) into the DNA synthesis phase (S). Two cell cycle kinases, CDK4/6-cyclin D and CDK2-cyclin E, and the transcription complex that includes RB and E2F, are key regulators of this checkpoint.



- In G1, the RB–HDAC repressor complex binds to the E2F transcription factors, inhibiting transcription of S phase genes. Phosphorylation of RB by CDK4/6 andCDK2 dissociates the RB–repressor complex, permitting transcription of S-phase genes (encoding proteins that amplify the G1 to S-phase switch and that are required for DNA replication). Different stimuli exert checkpoint control including (i) TGF-β, (ii) DNA damage, (iii) contact inhibition, (iv) replicative senescence, (v) oncogenic stress, and(vi) growth factor withdrawal.

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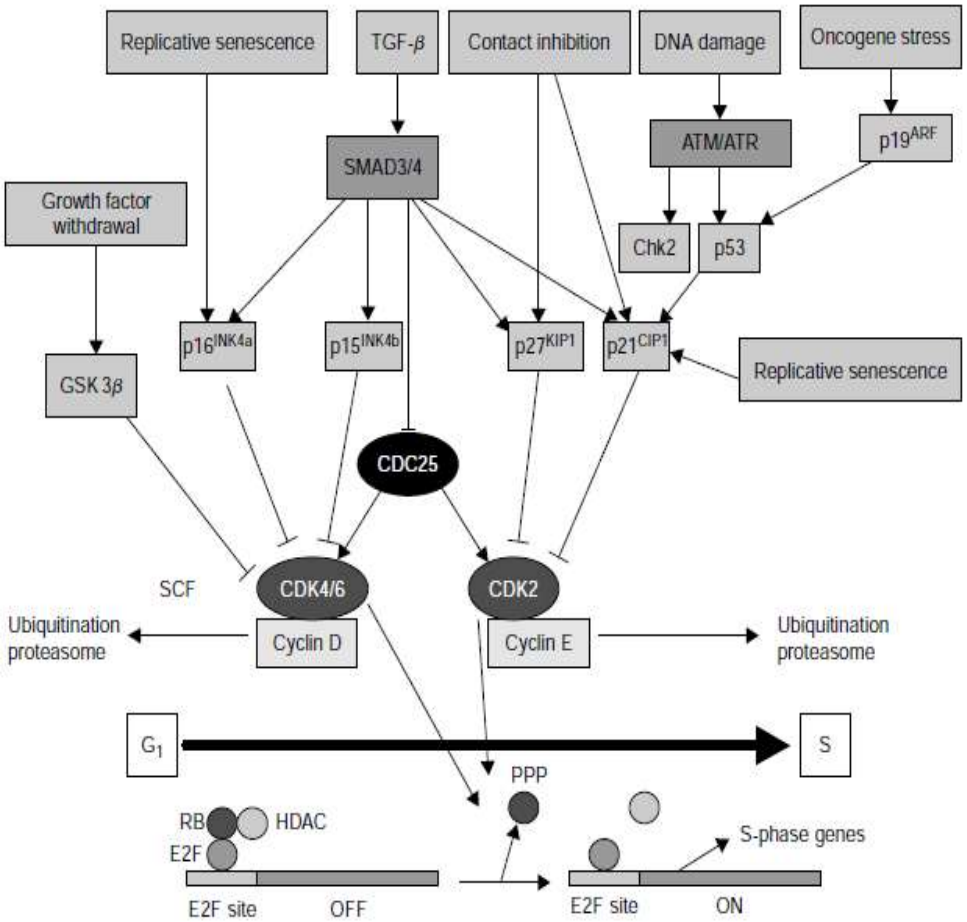
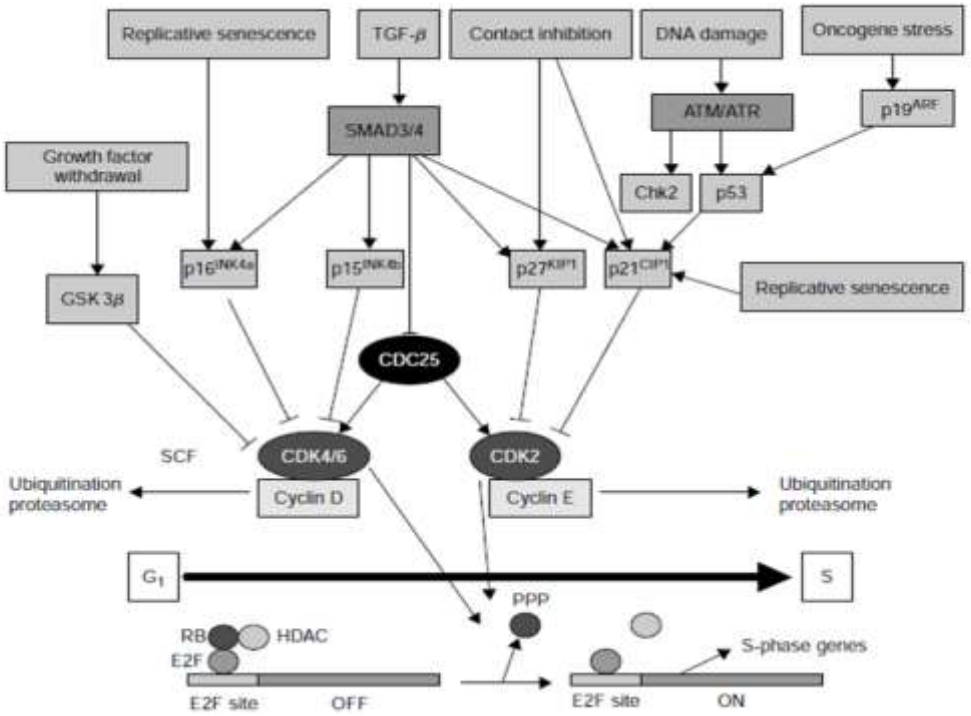
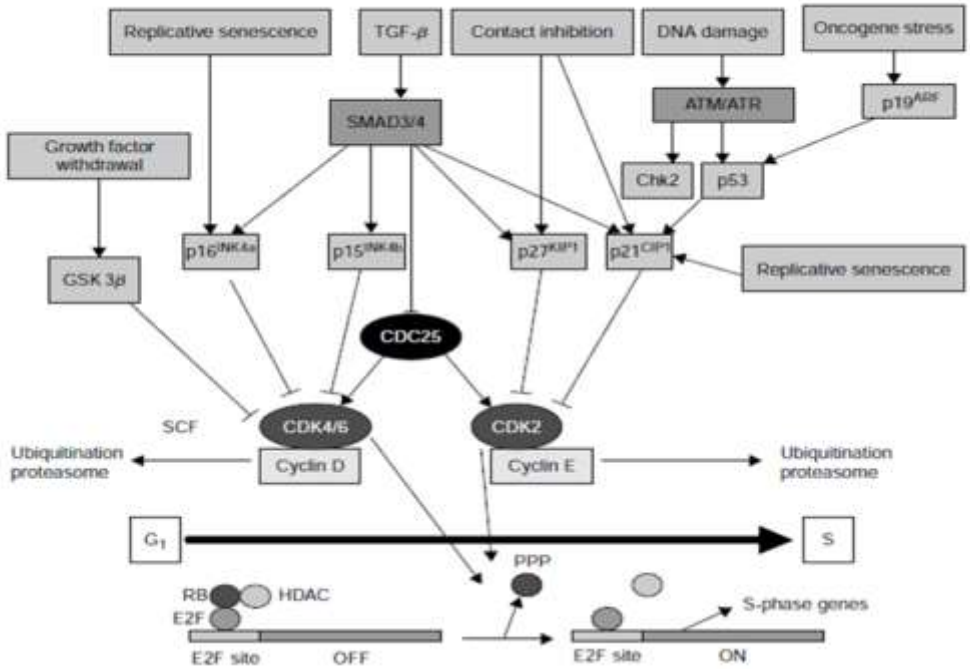


- The first five [(i) – (v)] act by inducing INK4 or KIP/CIP families of CKIs. TGF-β additionally inhibits the transcription of CDC25. Growth factor withdrawal activates GSK3β, which phosphorylates cyclin D, leading to its rapid ubiquitination and proteosomal degradation. Ubiquitination, nuclear export, and degradation are mechanisms often employed to rapidly reduce the concentration of cell cycle control proteins.



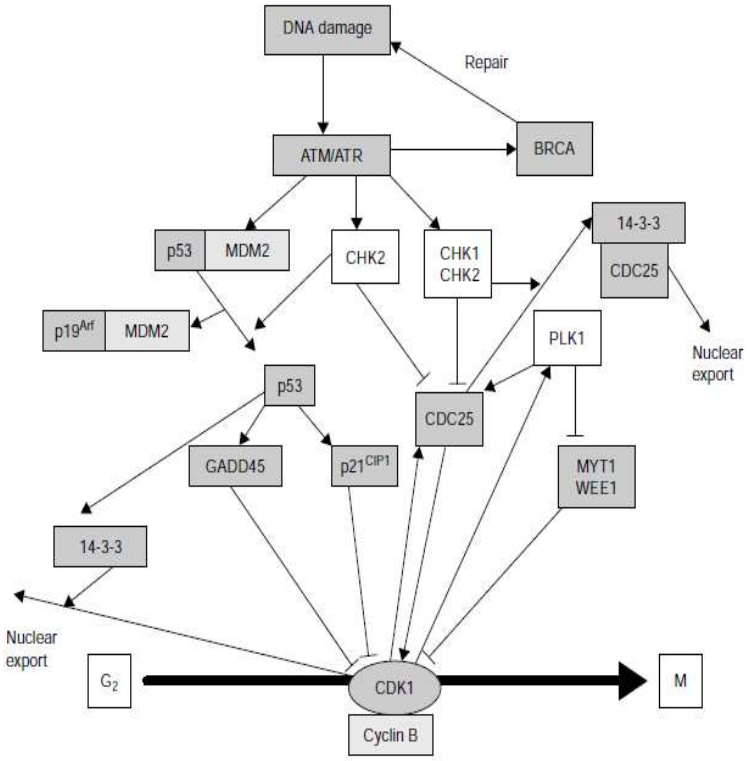
- The second involves the ATR (ATM- and RAD3-related) andCHK1 protein, and respond s to “less extreme” DNA damage by inducing G2 arrest, independently of p53.
- Importantly, p53 is the most frequently mutated tumor-suppressor gene in human cancer cells lacking p53 can survive levels of DNA damage and mitogenic stimulation that would otherwise kill the cell and can enter and progress through the cell cycle thus propagating DNA damage.
- The p53 protein is also activated via a complex system that responds to “oncogenic stress”.



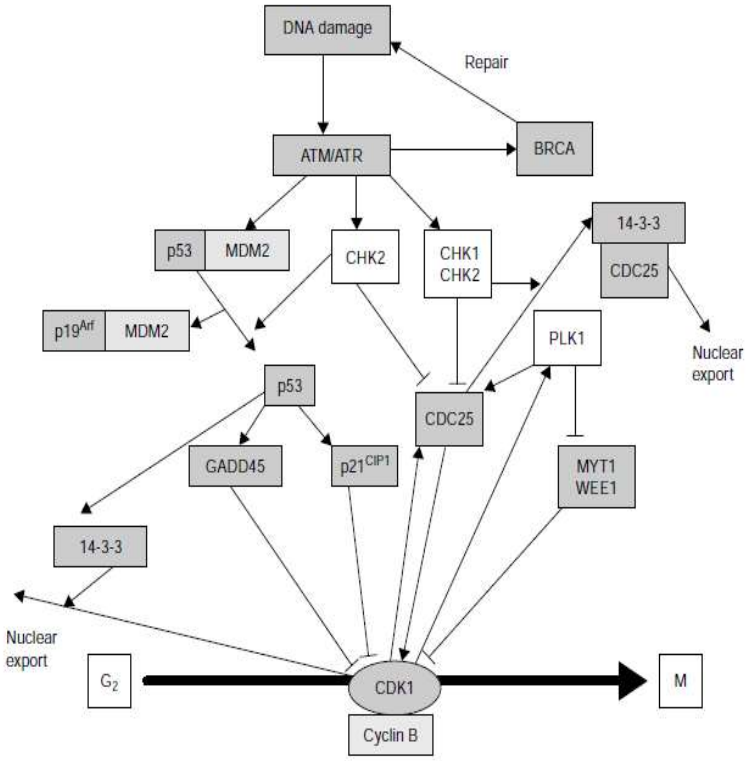
- In oncogenic c-MYC, the cell activates the p19ARF tumor suppressor that in turn can both indirectly, via MDM2 and p53, and probably directly promote apoptosis or cell cycle arrest.
- Another key tumor-suppressor pathway involves the RB pathway. The protein p16INK4a inhibits CDK4 activity in G1 and thereby induces cell cycle arrest and may also contribute to replicative senescence in response to oncogenic RAS or other oncogenes.

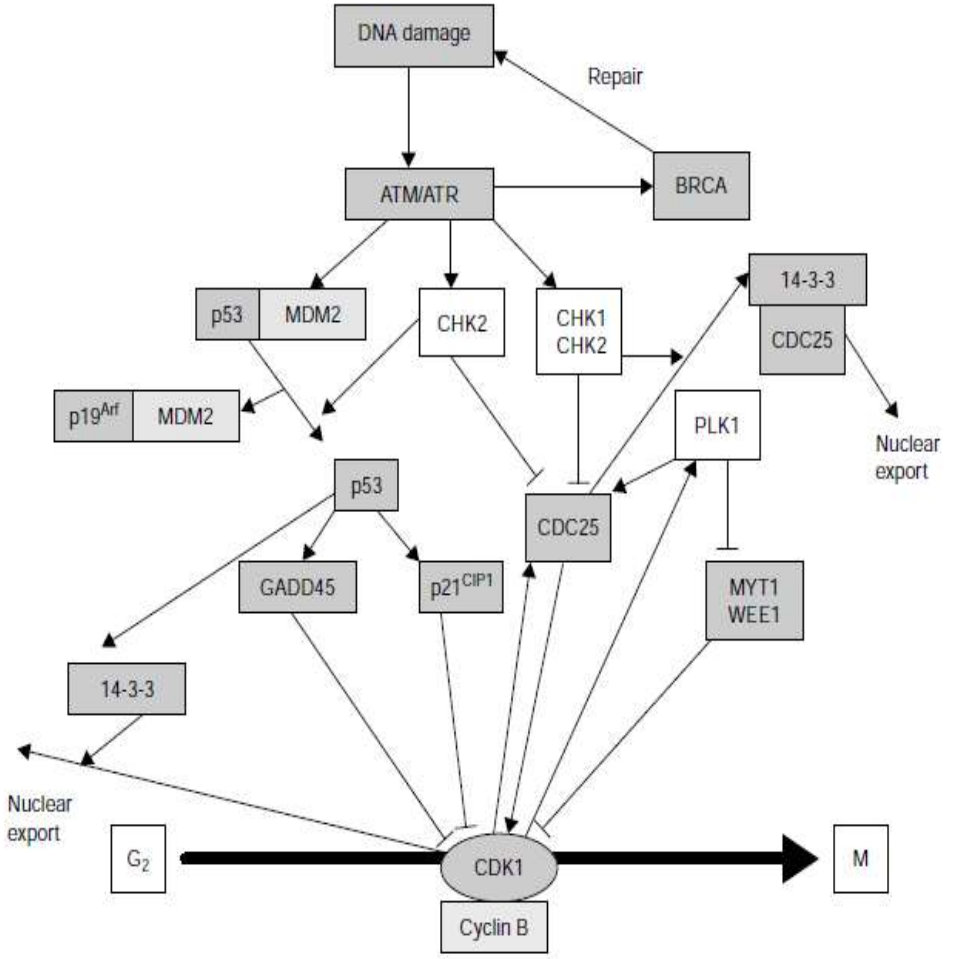
G2/M CHECKPOINT

- The G2/M DNA damage checkpoint prevents the cell from entering mitosis (M phase) if the genome is damaged . The cyclin B CDK1(CDC2) complex acts as a regulator of this transition. During G2, the kinases CHK1, WEE1, and MYT1 act directly or indirectly to phosphorylate CDK1 (CDC2), thereby inhibiting activity.
- As M phase approaches, the CDC25 phosphatases are activated by the polo like kinase PLK1. CDC25 then activates CDK1, in part by reversing inhibitory phosphorylation, thus establishing a feedback amplification loop that efficiently drives the cell into mitosis.

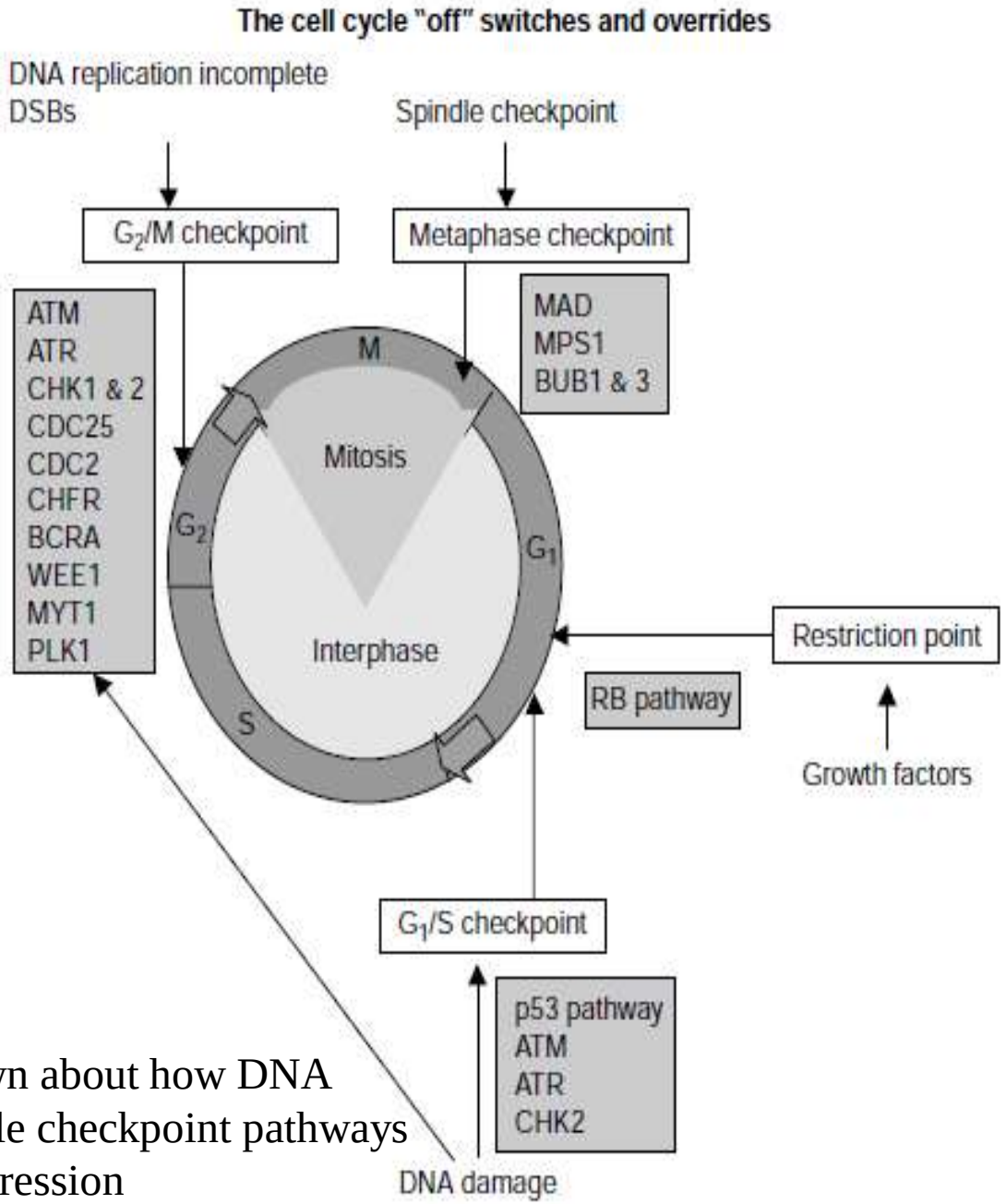


- DNA damage activates the ATM/ATR kinases, initiating two parallel cascades (the ATM–CHK2 and ATR–CHK1). These pathway negatively regulate cyclin B–CDK1 thus arresting the cycle; CHK kinases phosphorylate and inactivate CDC25, preventing both progression of S phase and entry into M phase.
- The ATM pathway is largely responsible for detecting major damage such as DSBs and arrests cells in G1, in a large part through activation of the p53–p21CIP1 pathway.
- The ATR–CHK1 pathway seems largely responsible for detecting less pronounced DNA damage, possibly triggered by stalling of the DNA polymerase during DNA replication.





- Phosphorylation of p53 dissociates it from MDM2, activating its DNA binding activity. Acetylation by p300/PCAF further activates its transcriptional activity. The genes that are activated by p53 encode proteins including 14-3- 3s, which bind phosphorylated CDK1-cyclin B promoting nuclear export; GADD45, which apparently binds to and Dissociates the CDK1-cyclin B kinase; and p21CIP1, an inhibitor of a subset of the CDKs including CDK1.



Much is therefore known about how DNA damage triggers multiple checkpoint pathways to arrest cell cycle progression

Acknowledgement

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- Thanks are due to all the original contributors and entities whose pictures were used in the creation of this presentation.