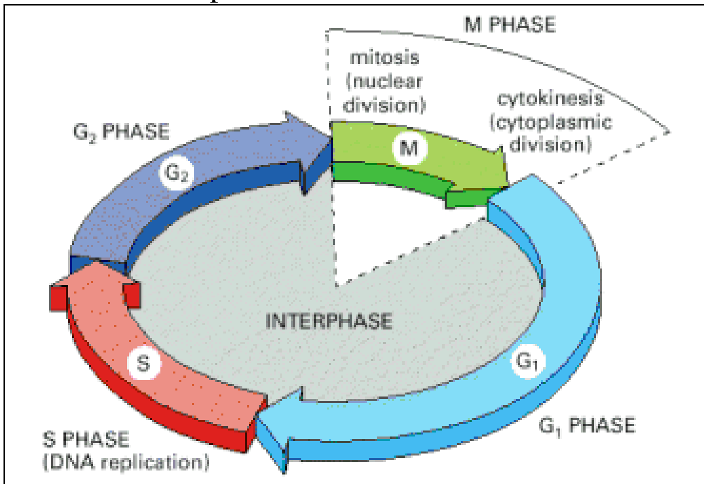
The cell cycle in eukaryotes Jason Kahn, Biochem 465 Spring 2006

(Mostly from Alberts et al., *Molecular Biology of the Cell*, 2002).
The book is available for free on line at http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mboc4 but navigation is only via searching.

The cell cycle is tightly controlled, both in terms of initiation and progression. The key features are that the cycle is <u>ordered</u> and <u>unidirectional</u> and there are <u>checkpoints</u> to make sure that each step is completed before the next one begins.

There are four phases:

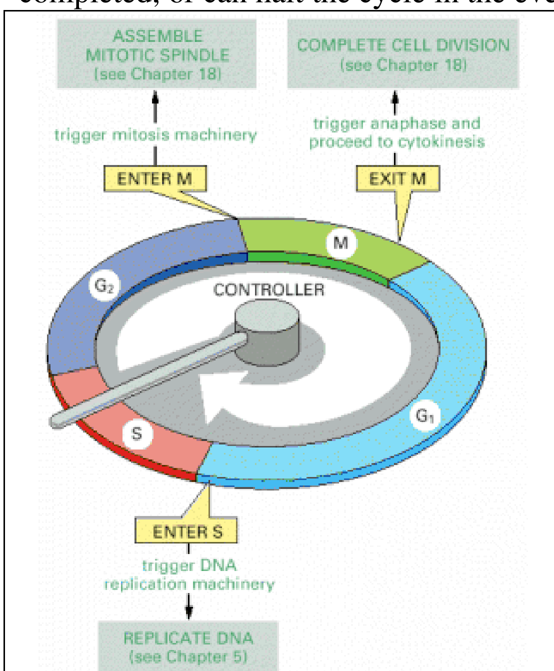


M = mitosis is when chromosomes become condensed and separate and the cell divides: visible changes. ~ 1 hr

G = Gap

G0 or resting state (can be forever) enters and exits via G1
S = DNA Synthesis, ~10 hrs in 24 hr cell cycle
G2 is short gap before mitosis

Two systems:
The timer and controls that move the cycle forward and ensure unidirectionality and irreversibility (the cell does not want to synthesize DNA during mitosis!). These are mainly the cyclins and cyclin-dependent kinases (Cdk's).
The checkpoints, which prevent taking the next step unless the previous step has been successfully completed, or can halt the cycle in the event of DNA damage.



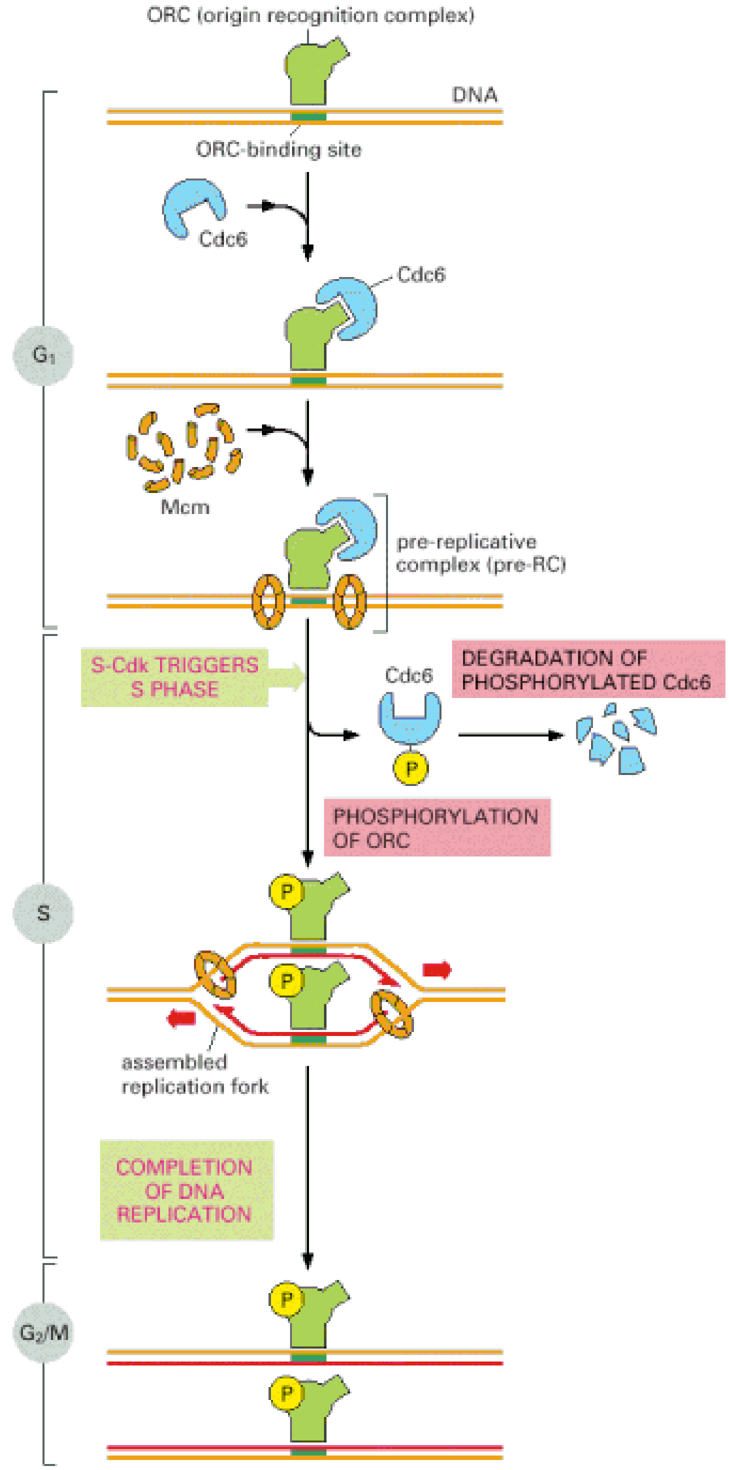
Alberts uses the analogy of a washing machine timer. The checkpoints are sensors that regulate e.g. tub filling.
Triggering of each step is accompanied by inactivation of the cyclins or other components needed for the previous step. This can be done by phosphorylation and dephosphorylation or by proteolysis via ubiquitination and subsequent digestion in the proteasome. This is an expensive way to regulate! But any expense is better than an unregulated cell cycle.

Focus on control of DNA replication:

The proteins at the replication origins (ORC, analogous to DnaA in E. coli + Cdc6+Mcm proteins) can assemble only during G1. They cannot "fire." The S-phase Cdk then causes the origin to fire and also prevents reassembly by phosphorylating Cdc6, which is then targeted for destruction.

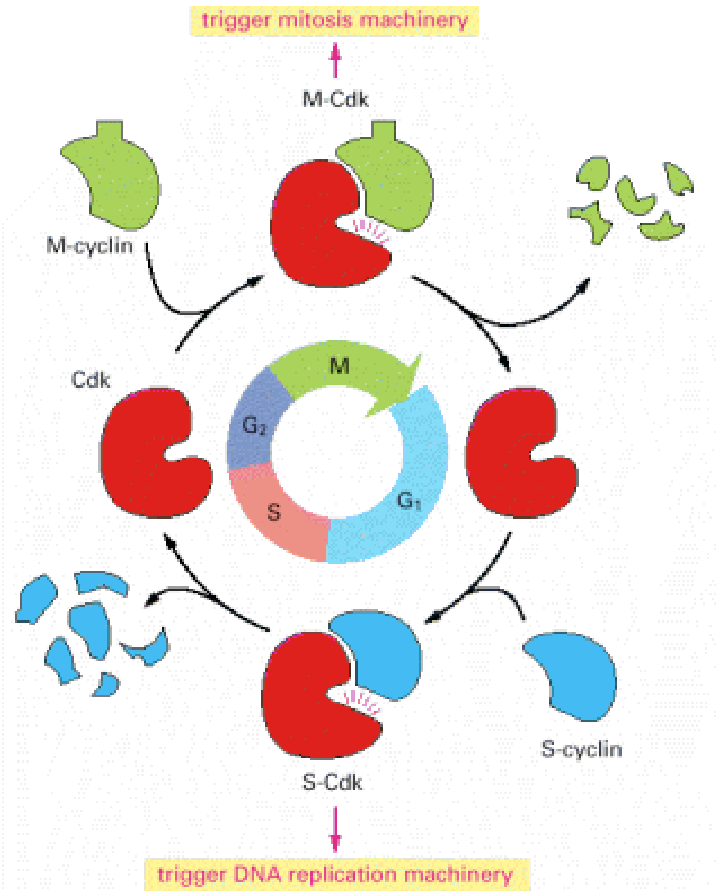
The Mcm proteins are the replication fork helicases, analogous to DnaB.

Origins cannot assemble until after M phase is complete, at which point all cyclin activity is destroyed and everything resets. G1 cyclins then gradually accumulate and eventually allow another round.



"Once and only once upon a time"

Regulation of Cyclin Activity



The cyclic synthesis and destruction of the S phase cyclins and the M phase cyclins.

There are four cyclin-cdk complexes
G1-Cdk
G1/S-Cdk
S-Cdk
M-Cdk

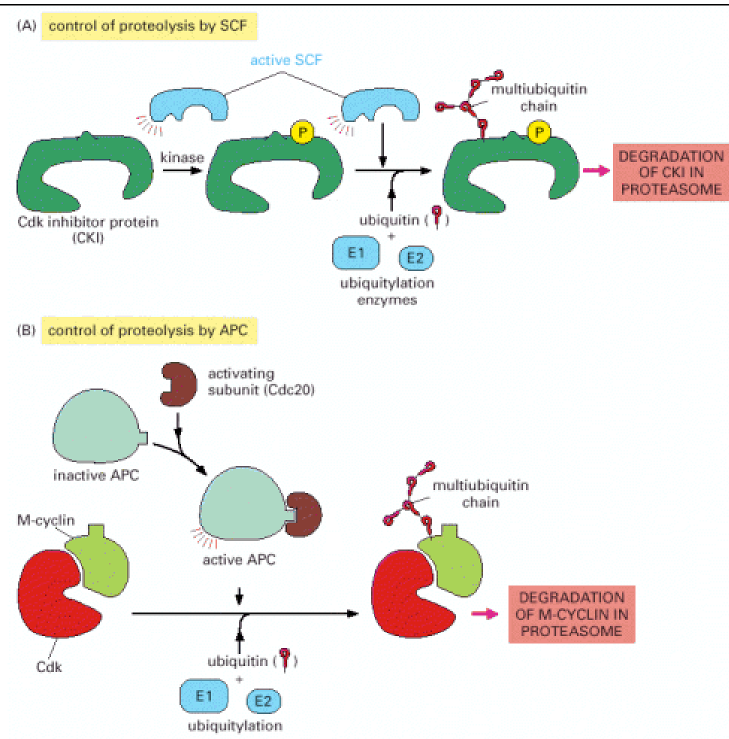
In vertebrates there are four cyclins, in yeast just one.

Cyclins control activity and specificity of the Cdks. The complexes are also regulated by other kinases and phosphatases.

Note the cyclic activation and destruction of the cyclins: Irreversibility.

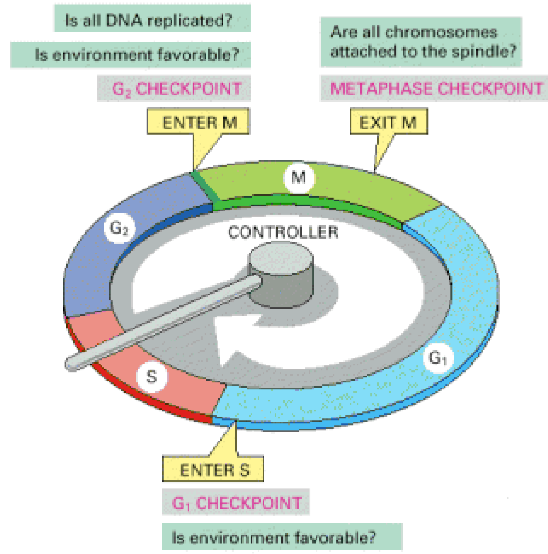
APC = anaphase-promoting complex is responsible for targeting M-Cdk for destruction.

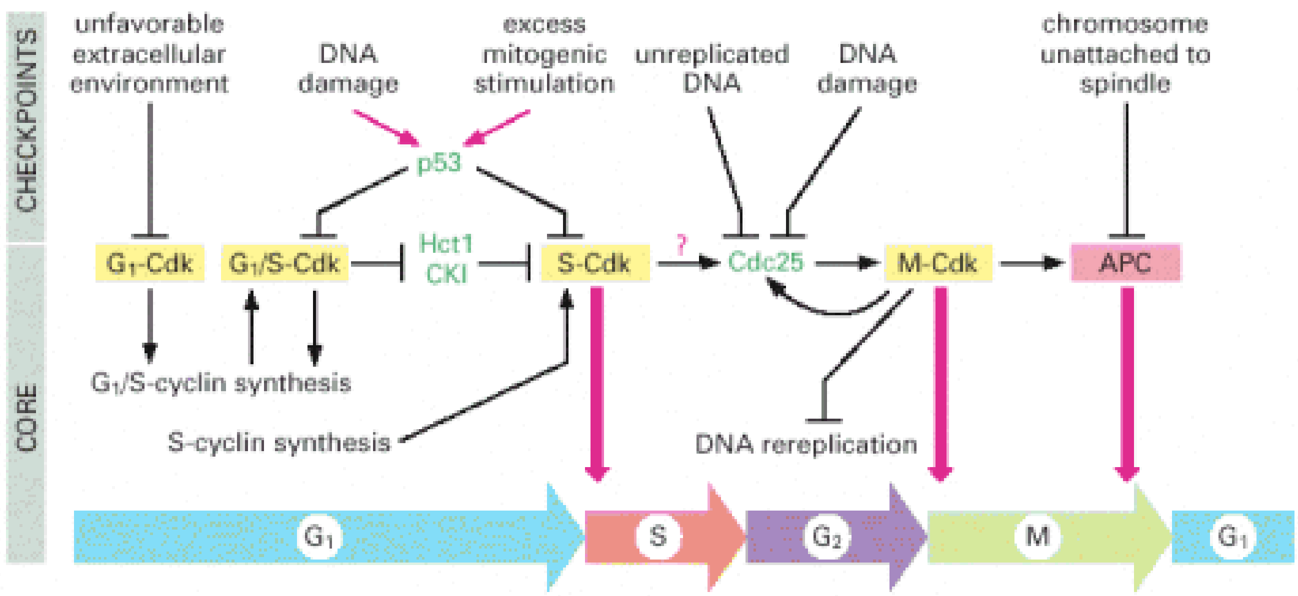
The proteasome is a large, highly regulated trash can for proteins. It is becoming more and more clear that many regulatory factors are turned over rapidly through ubiquitination and proteolysis: turning signals off is just as important as turning them on.



Checkpoints:

These are an overlay on the core machinery: if everything is going well, many checkpoints are dispensable.

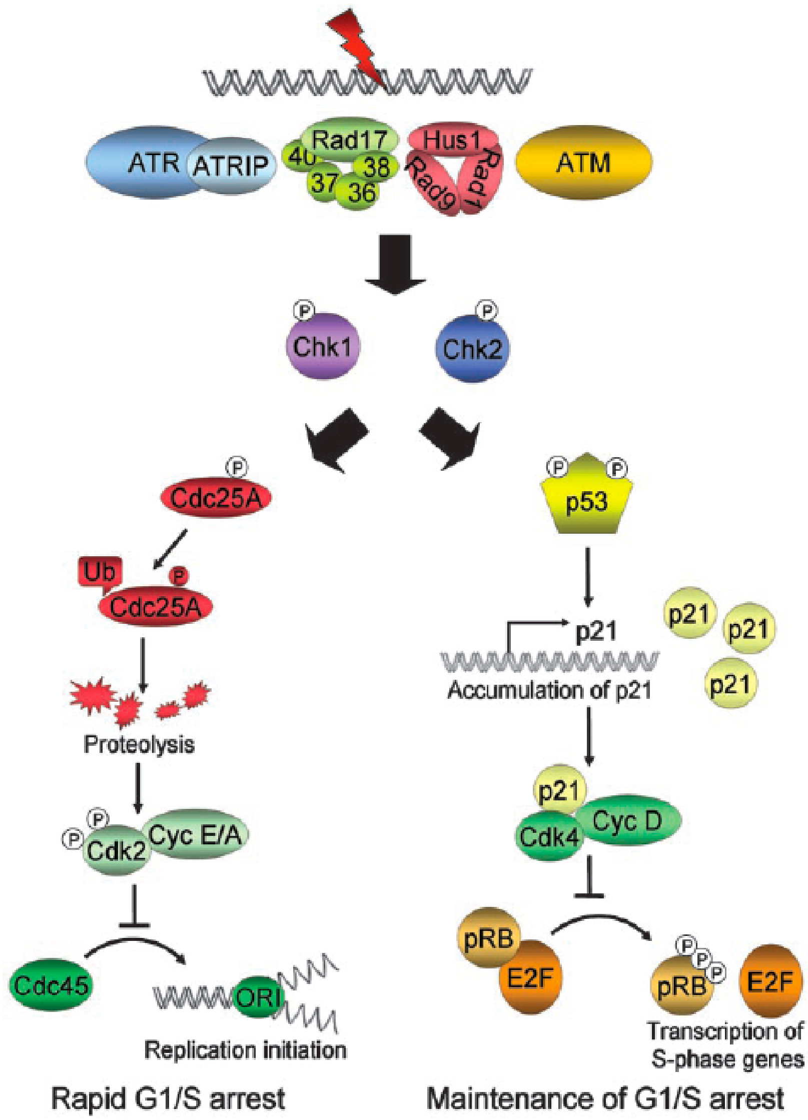




One of the critical checkpoints is the G1/S transition. If there is excess DNA damage, a phosphatase (Cdc25A) which would otherwise release the inhibited S-Cdk is destroyed instead, halting progression of the cycle. The halt is maintained by p53, a tumor suppressor protein. p53 is phosphorylated and thereby stabilized. It is a transcription factor. It up-regulates DNA damage repair proteins and also the p21 protein, which blocks the cell cycle.

If there is too much DNA damage, p53 can initiate apoptosis, programmed cell death. In a multicellular organism, sacrificing the cell is preferable to risking cancer. Therefore p53 mutations predispose cells to become cancerous.

Checkpoints for completion of synthesis and spindle assembly are less well understood, but it is clear that repressive signals are sent because of incomplete synthesis/separation. It is much easier to detect the cessation of inhibition than the total level of a positive signal.



(figure on p53 and the G1/S checkpoint from Sancar et al., Annu. Rev. Biochem., 2004)

Bottom Lines:

- **Irreversible progression through the cell cycle is ensured by cyclin-Cdk kinase relays to move forward, proteolysis or other inactivation of cyclins to prevent moving backward.**
- **Progression through the cell cycle also requires transcription of new cyclins. (10% of all genes in yeast oscillate with the cell cycle.)**
- **The proteins needed to initiate DNA replication can be assembled at origins during G1 but cannot initiate. They can initiate but not assemble during S, and cannot initiate again until G1. Called licensing models.**
- **Checkpoints regulate the cell cycle by inhibiting progression unless the previous steps have completed successfully.**