# Research

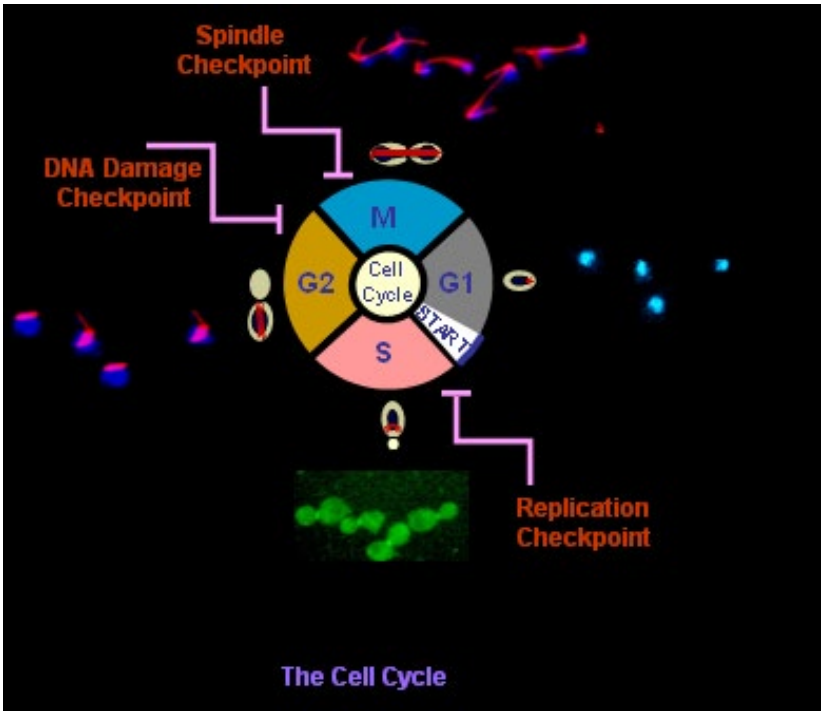
## Regulation of the Cell Division Cycle

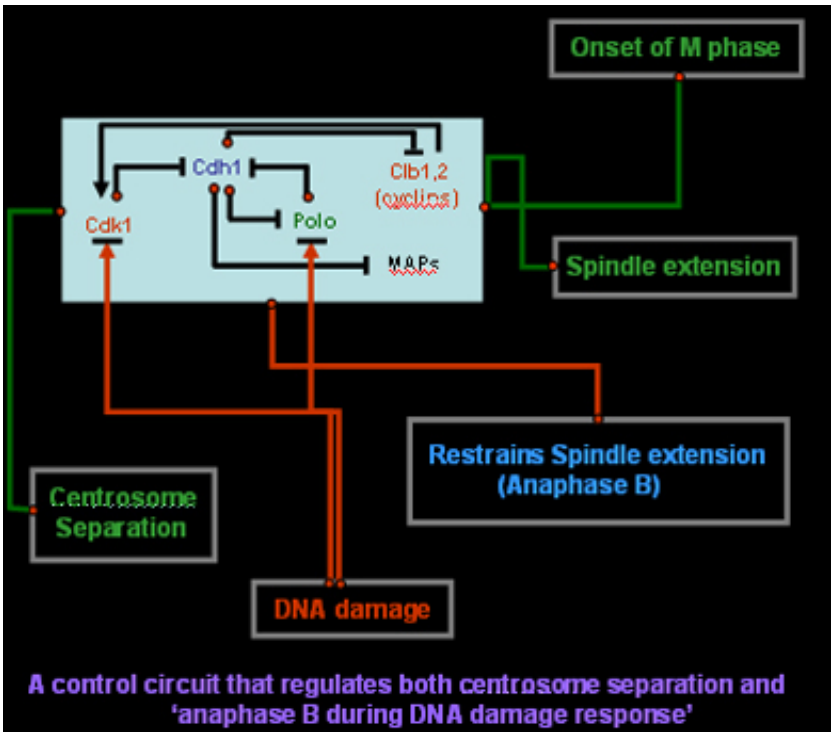
Cellular activities leading to division are highly regulated both in time and space. Violation of this precision can lead to genomic instability which may result in reduced cellular fitness, unbridled proliferation (cancerous growth) or death of parent and progeny cells. Valuable insights into the way cell division is controlled in complex systems, such as human cells, have come from investigations of relatively simple eukaryotes. The budding yeast *Saccharomyces cerevisiae*, (the model system we use for our investigations) is one such organism that has served remarkably well due to its amenability to genetic manipulations and because many of the pathways that regulate cell division are highly conserved from yeast to man.

Coordinated execution of cell cycle events is, in-part, imposed by checkpoint controls that ensure that a given event is not initiated until the preceding event is successfully completed. During S phase the genome is monitored by two such major control pathways, namely, DNA replication checkpoint and DNA damage checkpoint. When cells incur replication stress or DNA damage, these pathways prevent initiation of chromosome segregation until the damage is repaired. We have focused our investigations on the mechanisms by which checkpoint pathways prevent execution of mitotic events. Using various genetic and biochemical analysis, we have discovered that the replication checkpoint prevents premature chromosome segregation by directly regulating spindle dynamics, thereby inhibiting precocious spindle elongation. This has led us to closely examine the process of spindle assembly itself. We have uncovered a novel mechanism by which duplicated centrosomes are separated and moved apart to assemble a bipolar spindle. Our findings suggest that cyclin dependent kinase (Cdk1) and polo kinase act synergistically (with Cdk1 serving as a priming kinase for polo kinase) to inactivate the ubiquitin ligase APCCdh1 that mediates the proteolytic destruction of Cin8 and Kip1 kinesins essential for centrosome separation. An extension of these studies has revealed that the regulatory circuit involving Cdk1, Polo kinase, Cdh1 and kinesins is also utilized by the DNA damage checkpoint to prevent segregation of damaged chromosomes. Hence mitotic spindle has emerged as a novel target of S phase checkpoints. To take these studies forward we are investigating the mechanism by which hyper-activation of Rad53 (Chk2) during G2/M leads to mitotic spindle collapse

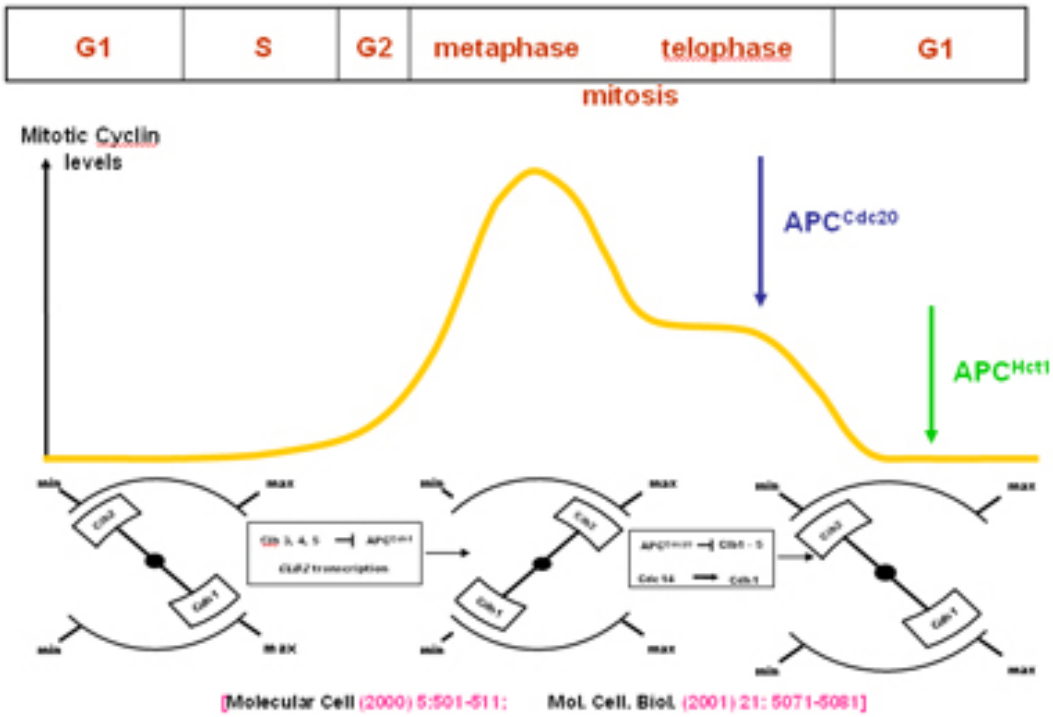
Recovery from (resumption of cell cycle progression after repair) or adaptation to (escape from arrest in the absence of repair) checkpoint-imposed arrest are also critical aspects of checkpoint regulation and have important implications for chromosome stability. Efforts are now underway to delineate the "adaptation pathway" utilized by cells to escape from DNA-damage-induced arrest

By studying the coordination of various cellular events, we hope to understand the molecular circuits through which eukaryotic cells exercise temporal and spatial control over their activities during cell division. In addition to providing clues to the organizing principles of living cells, such efforts may also lead to the identification of key regulator that could serve as targets for the therapeutic interventions against growth of cancerous cells. We are currently conducting screens to identify anti-proliferative compounds targeting key cell cycle regulators.



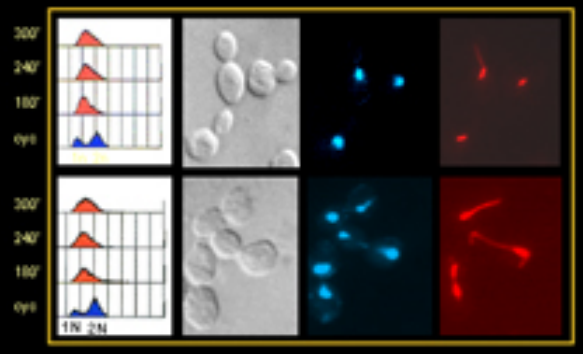



## A two stroke Engine




[Molecular Cell (2000) 5:501-511; Mol. Cell. Biol. (2001) 21: 5071-5081]

## Replication Checkpoint in Yeast:




( Checkpoint + )

( Checkpoint - )

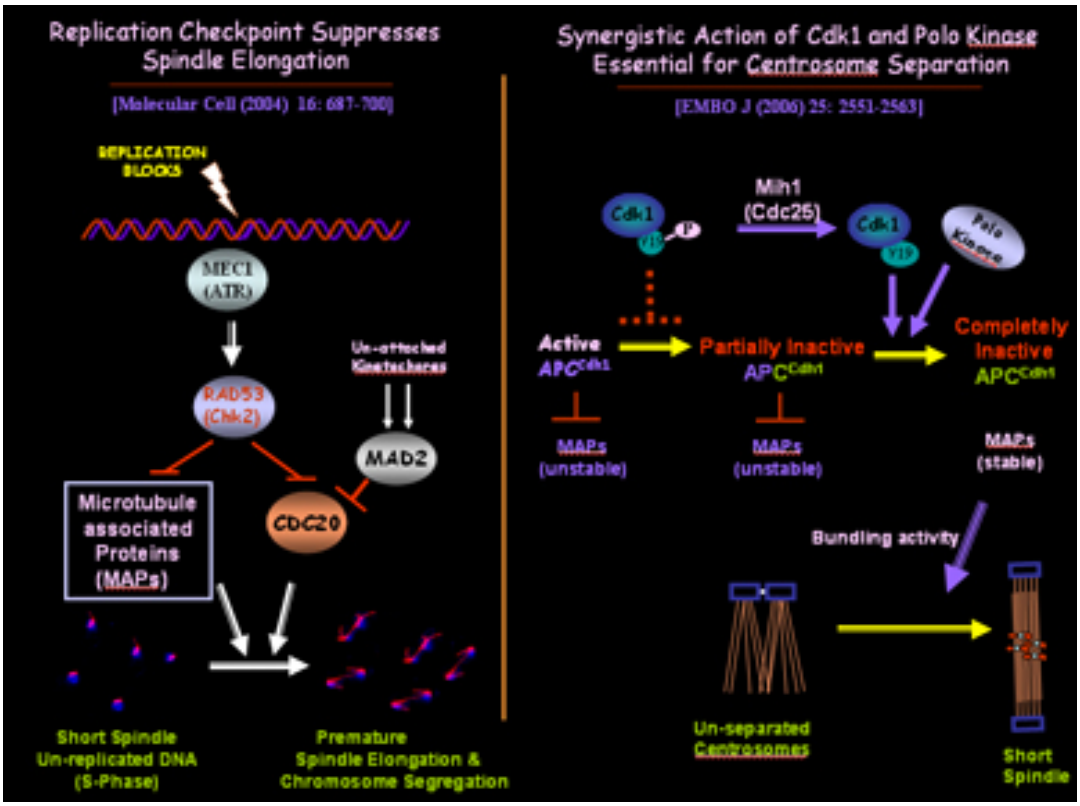
**Mec1 (ATR)**

**Rad53 (Chk2)**

**Mitotic Entry (?)**

**Maintains Replication Fork Integrity**

**Mec1: Mitotic Entry Checkpoint**




DNA damage checkpoint regulates both Anaphase A and Ananphase B