

Staging a recovery from mitotic arrest Unusual ways of Cdk1

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Checkpoint controls, the surveillance pathways that impose “an order of execution” on the major cell cycle events, are critical to the maintenance of genome stability. When cells fail to execute a cellular event or do so erroneously due to misregulation or exposure to genotoxic stresses, these evolutionarily conserved regulatory circuits prevent passage to the subsequent event, thus bringing the cell cycle to a halt. Once the checkpoint stimulus is removed, cells recover from the arrest and eventually resume cell cycle progression. While the activation, execution and maintenance, the three major aspects of the checkpoint controls, have been investigated in detail, the recovery process remains underexplored. It is not clear if cells recover passively upon dissipation of the checkpoint signals or require an active participation by specific effectors. A recent study in the yeast *Saccharomyces cerevisiae* uncovered two previously unsuspected functions of Cdk1 in efficient recovery from the spindle assembly checkpoint (SAC) imposed arrest. An inability to fulfil these requirements in the absence of Cdk1 makes it virtually impossible for cells to recover from the mitotic arrest. Given the conserved nature of the SAC, these findings may have implications for vertebrate cells.

cycle events that tethers them in a temporal order and partly from the negative regulation imposed by the surveillance pathways called the checkpoint controls (or simply checkpoints).¹ Checkpoints are particularly important in situations where cells fail to initiate or complete an event appropriately, due either to intracellular aberrations or an exposure to damage inducing agents. Such circumstances result in the activation of checkpoint controls which prevent the onset of subsequent events and bring the division cycle to a grinding halt. These arrest-states can last for extended periods, giving cells sufficient time to repair the damage, so that normalcy is restored and cells can resume cell cycle progression. The DNA damage checkpoint was the first ‘checkpoint control’ to be conceptually defined based on studies in the budding yeast *Saccharomyces cerevisiae*.¹ It is activated in response to DNA damage and causes cells to arrest in the G2 phase until the damage is repaired. Subsequent studies, also on the budding yeast, helped formulate the DNA replication checkpoint² and Spindle assembly checkpoints (SAC).³ While the replication checkpoint prevents premature onset of mitosis when replication fork progression is impeded, the SAC functions during mitosis and monitors the attachment of duplicated chromosomes to the mitotic spindle before sister-chromatid separation can be initiated. Thus, these checkpoints collectively maintain the integrity of the genome through the division cycles. Consistent with the fundamental role they serve at the cellular level, the regulatory schemes and the main effectors of the three checkpoints are evolutionarily conserved from yeast to man.

Keywords: mitosis, Cdk1, spindle, checkpoint, recovery, yeast, cell cycle, cell division

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Background

Highly coordinated execution of cellular events is one of the most conspicuous features of the eukaryotic cell division cycle. This strict order emerges partly from the functional dependence among the cell

The general framework in which the checkpoints operate involves (1) sensing of the checkpoint stimulus (cellular aberration or damage) (2) activation of the checkpoint (3) execution and maintenance of the cell cycle arrest (4) switching-off of the checkpoint after the repair is performed and (5) recovery and resumption of the cell cycle. While many of these aspects have been studied in detail for all the major checkpoints, the mechanics of recovery from checkpoint-imposed arrest has remained largely unexplored. The reason for this neglect may partly lie in the presumption that recovery is a passive process, in that once the repairs are made and the checkpoint stimulus dissipates, cells gradually return to the “ground state”, normalcy is restored and cell cycle progression is resumed. An assumption inherent in such a consideration is that during cell cycle arrest, most facets of the cellular physiology relevant to the division cycle are “naturally” in transient suspension and an active participation by specific effectors is not required for maintenance of a cellular state from where cells can make an efficient return to the cycle. However, it is possible that during cell cycle arrest, the cellular processes, though relevant to the arrest-state but not directly regulated by the checkpoint effectors, may continue to follow their temporal course. During a prolonged arrest, this state of dynamics may drive cells to a physiological state from where recovery becomes increasingly difficult. In such a scenario, it may be imperative for cells to actively maintain a cellular context in which a return to normalcy can be mounted efficiently.

What is required for cells to maintain such a context during mitotic arrest? A recent study in yeast investigates the process of recovery from SAC-imposed mitotic arrest and uncovers two previously unknown roles of Cdk1, the main regulator of the cell cycle in yeast.⁴

Chromosome segregation and the SAC. Accurate segregation of sister-chromatids into two daughter compartments requires proper loading of the duplicated chromosomes onto the mitotic spindle. The kinetochores are the main contact-interface between the spindle and the chromosomes. During mitosis,

sister-kinetochores are captured by the kinetochore microtubules such that each member of the sister-kinetochore pair is attached to the microtubules emanating from the opposite spindle pole. This arrangement is termed bi-orientation or amphitelic attachment.^{5,6} In vertebrate cells, each kinetochore is attached to multiple kinetochore microtubules, whereas in yeast each kinetochore is occupied by only one microtubule.⁷ The integrity and the proper functioning of this entire assembly are essential for accurate segregation of chromosomes during anaphase. Another important element in this assemblage is sister-chromatid cohesion. The sister chromatids are held together by the cohesin complex until anaphase.⁸ At anaphase onset, separase, a cysteine protease, cleaves the cohesin complex subunit Scc1 and dissolves the sister-chromatid cohesion.⁹ However, separase is inhibited by securin until anaphase, preventing it from dissolving sister chromatid cohesion prematurely. Upon initiation of anaphase, E3 ubiquitin ligase APC^{Cdc20} (anaphase promoting complex activated by Cdc20) catalyzes proteolytic degradation of securin,¹⁰ thus setting separase free, which in turn dissolves cohesion between the sister-chromatids and allows their progressive separation.

Chromosome bi-orientation generates tension in the chromosome-spindle assemblage as the spindle microtubules pull the sister-chromatids toward the opposite pole while sister-chromatid cohesion, mediated by the cohesin complex, resists the pole-ward pull. Cellular defects or treatment with extraneous agents (such as microtubule poisons) that result in unoccupied kinetochores and a loss of tension in the chromosome-spindle assemblage can activate SAC.¹¹ In yeast, activation of the SAC signaling requires a number of highly conserved effectors including Mad1, Mad2, Mad3 (orthologs of human Mad1, Mad2 and BubR1, respectively), Bub1, Bub3 (orthologs of human Bub1 and Bub3), Mps1 (ortholog of human Mps1) and Ipl1 (ortholog of human aurora B kinase).¹² The sensor kinases Ipl1, Bub1 and Mps1 are activated in response to bi-orientation defects and trigger recruitment and phosphorylation

of Mad1, Mad2, Mad3 and Bub3.¹³⁻¹⁵ These proteins assemble in various complexes at the unoccupied kinetochores and eventually inhibit Cdc20, an activator of the APC and an essential mediator of chromosome segregation. The central piece of this inhibition is the mitotic checkpoint complex (MCC) that contains Mad2, Mad3 (BubR1), Bub2 and also Cdc20; it binds to APC and inhibits its ubiquitin ligase activity, preventing it from triggering the dissolution of sister-chromatid cohesion.¹² Thus unoccupied kinetochores not only initiate the checkpoint signal but also serve as the catalytic platform for assembly of the inhibitory complexes.

Cdk1 and the recovery from SAC arrest. Treatment with the microtubule disrupting agent nocodazole leads to SAC activation and causes yeast cells to arrest in a preanaphase state (loosely referred to as just “metaphase”) with high Cdk1/Clb mitotic kinase activity. Upon removal of nocodazole, the checkpoint signal dissipates and cells undergo recovery during which the spindle is rapidly reassembled, chromosomes bi-orient, the checkpoint is switched off and anaphase ensues. Whether recovery is a passive process in the absence of the checkpoint-inducing signals or requires active participation of specific effectors has not been explored extensively. A recent report⁴ uncovers unsuspected roles of Cdk1 kinase in the recovery from SAC-induced mitotic arrest. This study in yeast shows that cells attempting to recover from SAC-induced arrest in the absence of Cdk1 activity assemble a short spindle but are unable to turn off the checkpoint signaling as indicated by persistent Mad1 phosphorylation. Consequently, the cohesion subunit Scc1 is not cleaved, sister-chromatids do not segregate and cells remain arrested in metaphase. The failure to switch off the checkpoint control despite nocodazole removal is due to the cells’ inability to establish bi-orientation in the absence of Cdk1 activity. Interestingly, the mitotic spindle extends dramatically during this period raising the possibility that untimely spindle elongation in the absence of Cdk1 drastically reduces bi-orientation efficiency. Indeed, restraining spindle extension by introducing

a deficiency of kinesin motor Cin8 (which mediates spindle elongation during anaphase B) restored bi-orientation even in the absence of Cdk1 activity, suggesting that (1) spindle length is inversely correlated with bi-orientation efficiency and (2) the absence of Cdk1 activity during recovery results in unrestrained recruitment of Cin8 to the spindle midzone, resulting in premature spindle elongation. It has been reported that Cin8 recruitment to spindle midzone (where it functions) is mediated by another microtubule binding protein Ase1¹⁶ whose activity is negatively regulated by Cdk1 such that phosphorylation of Ase1 inhibits its ability to mediate Cin8 localization to the spindle midzone.^{17,18} Accordingly, Ase1 was found to be hypophosphorylated in cells recovering from SAC-induced arrest in the absence of Cdk1. Moreover, expression of phosphorylation-resistant Ase1 resulted in dramatic spindle elongation even in the presence of Cdk1 and severely compromises the cells' ability to establish bi-orientation. Thus, one critical role of Cdk1 during recovery from SAC-mediated arrest is to prevent premature spindle elongation, via phosphorylation of Ase1, to allow efficient bi-orientation.

In an extension to these investigations, the study⁴ also found that a lack of Cdk1 activity during recovery leads to another major defect. Cells recovering from SAC-induced arrest in the absence of Cdk1 activity exhibit a precipitous drop in intracellular levels of the APC activator Cdc20. Cdc20 is degraded throughout the cell cycle¹⁹ and therefore also during the SAC-induced arrest. The *CDC20* gene must be actively transcribed during the arrest to maintain a constant level of Cdc20 protein,²⁰ so that during recovery, cells can rapidly degrade securin, clearing the way for dissolution of sister-chromatid cohesion and progression to anaphase. *CDC20* transcription is under the control of a transcription repressor Yox1^{21,22} which keeps Cdc20 levels very low during S phase. However, upon mitotic onset, Yox1 abundance declines and *CDC20* gene is actively transcribed. This study shows that Cdk1 negatively regulates *YOX1* transcription, thus keeping the intracellular levels of Yox1 low and, in turn, promoting the

transcription of *CDC20*. In cells recovering from the SAC-induced arrest in the absence of Cdk1 activity, Yox1 begins to accumulate and Cdc20 levels rapidly decline, causing a depletion of APC^{Cdc20} activity. Thus Cdk1 maintains Cdc20 levels through a 'transcription-regulation relay' system during recovery. Taken together, this study ascribes two new critical functions to Cdk1 during the recovery process (Fig. 1): (1) the coordination of spindle dynamics to facilitate chromosome bi-orientation and (2) the maintenance of Cdc20 levels via suppression of *YOX1* transcription. In the absence of Cdk1 activity, it would be virtually impossible for cells to recover from the SAC-induced arrest and resume cell cycle progression.

A renewed perspective. Posttranslational regulation is generally thought to be the most effective way of eliciting a rapid response to changing cellular landscape during mitosis. It is, therefore, interesting that Cdk1 ensures efficient recovery from SAC-induced arrest by

mediating the maintenance of Cdc20 via a transcriptional cascade. This transcription regulation is effected through the Cdk1-mediated suppression of the transcription of *YOX1*, itself a transcriptional suppressor of *CDC20*.^{21,22} Yox1, like Cdc20, is also an unstable protein such that suppression of its transcription results in rapid loss of Yox1 protein, which in turn leads to sustained Cdc20 expression. Hence it is a combination of proteolytic destruction and transcription 'relay-regulation' that sustains the level of Cdc20 during the mitotic arrest for an efficient recovery at a later time.

The yeast study also brings to light a negative correlation between spindle length and bi-orientation efficiency, which had been hinted at by an earlier report.²³ The shorter spindles are more efficient in establishing bi-orientation than longer spindles and the physical distance between the SPBs and the kinetochores appear to be the determining factor. The yeast study also reveals that the short spindle (~2 μM in length)

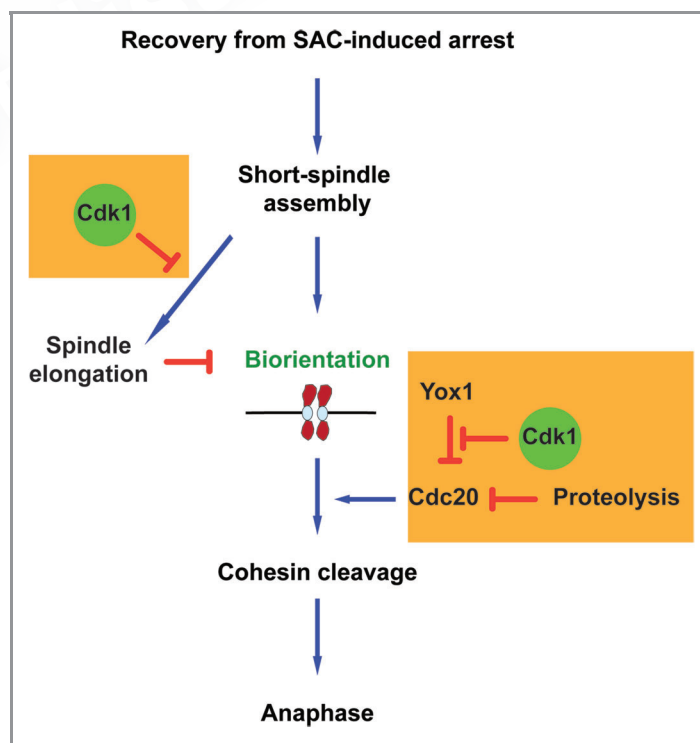


Figure 1. Cdk1 in the recovery from spindle assembly checkpoint-mediated arrest. Cdk1 plays two important roles during the cell's recovery from SAC-induced arrest in yeast. It (1) prevents untimely extension of the mitotic spindle to allow efficient establishment of chromosome bi-orientation and (2) maintains adequate cellular abundance of Cdc20 (required for APC activation) by inhibiting the transcription of transcriptional repressor *YOX1* (see text for details).

assembled during the initial phase in recovery has a propensity to elongate and must be actively suppressed by Cdk1 if cells were to efficiently establish bi-orientation. However, Cdk1's role in restraining spindle elongation in the initial phase to facilitate bi-orientation is surprising in view of the well-established notion that Cdk1 promotes spindle elongation during anaphase.²⁴ How can the elongation suppressive activity of Cdk1 be reconciled with its elongation-conducive role during anaphase B? It has been shown that Ase1 is dephosphorylated by Cdc14 in early anaphase;¹⁷ as a result, Cin8 is continually recruited to the spindle midzone where it catalyzes spindle elongation. During this time, Cdc14-mediated dephosphorylation also progressively sets in motion the activation of Cdh1,²⁵ an activator of APC which targets

Cin8 for proteolytic destruction.²⁶ Cdk1 is a potent inhibitor of Cdh1 and, in combination with polo-like kinase Cdc5, is known to mediate Cin8 accumulation.²⁷ Therefore, Cdk1's role in spindle elongation during anaphase B may lie in its ability to inhibit Cdh1 and stabilize Cin8. Thus, what appears to be a switch from Cdk1's elongation-suppressive to elongation-conducive role is not really a switch but is a change in the physiological manifestation of its activity caused by the emerging inter-locking regulatory relationships between various regulators as cells traverse anaphase. Interestingly, Cdk1's involvement in promoting bi-orientation by restricting spindle elongation is only required during recovery from SAC-induced arrest but not during an unperturbed division cycle.⁴ It may be due to low Cin8 abundance during

late S phase when bi-orientation is established, posing no threat of untimely spindle elongation.

What implications do these findings in yeast have for vertebrate cells? Previous studies reporting the role of p31 (comet)²⁸ and Cdc20 ubiquitylation²⁹ in the reversal of checkpoint-induced arrest clearly indicate that, as in yeast, recovery from SAC-induced arrest in vertebrate cells is also an active process. Given that the checkpoint controls and the mitotic regulatory circuits are evolutionarily conserved, investigations into the cellular contexts required for efficient recovery from SAC-induced arrest may yield novel insights into mitotic regulation in vertebrate cells. Since mitotic inhibitors are being actively used in cancer therapy, such studies may also have important implications for therapeutic efficacy of anti-mitotic agents.

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