may provide a new mechanism for mutation accumulation during tumor progression. We need to understand this mechanism since tumors accumulate mutations over decades. Even a modest decrease in the rate of mutation accumulation may effectively prevent these cancers by simply delaying their onset sufficiently.

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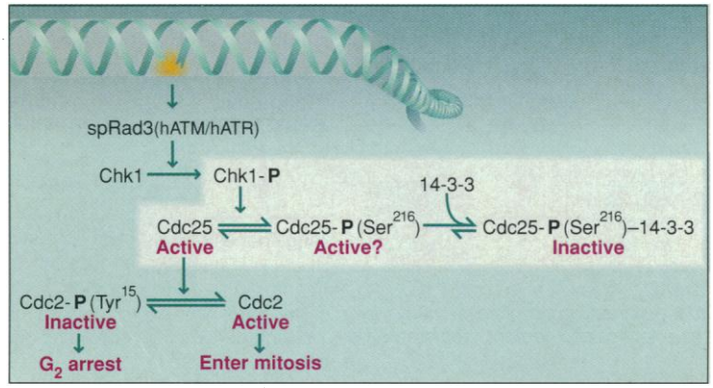
CELL CYCLE

A DNA Damage Checkpoint Meets the Cell Cycle Engine

Ted Weinert

Cell cycle regulators govern key transitions in the life of a cell—when to begin DNA replication and when to enter mitosis and divide. Preeminent among cell cycle regulators is the family of cyclin-dependent kinases ($p34^{CDK}$) and their partner cyclins, which together form heterodimer protein kinases (*1*). Reflecting that preeminence, Cdk–cyclin was crowned the cell cycle's "engine" (*2*): How goes Cdk-cyclin, so goes the cell cycle.

Changes in cell physiology, particularly damage to DNA, stop the cell cycle either before DNA replication in G_1 (termed the G_1 checkpoint) or before mitosis in G_2 [the G_2-M checkpoint (*3*, *4*)]. In many cell types DNA damage response pathways cause arrest by regulation of Cdk-cyclin through checkpoint proteins, which sense damage and transduce an inhibitory signal (*3*). Until recently it was unclear whether Cdc2, a prominent member of this Cdk family and a major mitotic activator in yeast, even plays a role in arrest in G_2 after damage. Nor was it clear how the checkpoint proteins transmit a signal to cause arrest. In the last year, the functions of both Cdc2 and checkpoint proteins have become clearer, and an ever more detailed hypothesis for a checkpoint pathway has emerged, culminating in three reports (pages 1495, 1497, and 1501) in this week's issue from fission yeast, human, and mouse (*5–7*).



A DNA break (a single-strand gap highlighted in yellow) activates the protein kinase Rad3 in fission yeast (and probably Rad3-like proteins ATM and ATR in human cells) (*3*). Activation of Rad3 probably occurs through association with other checkpoint proteins not shown (*3*). Active Rad3 then somehow activates the protein kinase Chk1 (Rad3 is required for phosphorylation of Chk1, but the exact mechanism of activation is unknown) (*13*). Activated Chk1 phosphorylates the phosphatase Cdc25 on Ser^{216} that then binds to and is sequestered by 14-3-3 protein. Sequestered Cdc25 is prevented from activating Cdc2. Cells arrest in G_2 when inhibitory phosphorylation of Cdc2 is intact. The dotted line highlights the aspects of this pathway discussed in three reports in this issue (*5–7*).

Evidence from several cell types has indicated that the G_2 arrest is caused by regulation of Cdc2; DNA damage results in the phosphorylation of inhibitory sites on the Cdc2 catalytic subunit in the filamentous fungi *Aspergillus*, in human cells, and in fission yeast (*8–10*). This inhibitory phosphorylation is required for arrest at the G_2-M checkpoint: in all three organisms nonphosphorylatable mutants of Cdc2 fail to fully arrest. How is this inhibitory phosphorylation of Cdc2 achieved? This question is addressed in three reports in this issue, which forge an attractive model explaining how upstream checkpoint proteins mediate the inactivation of Cdc25, a key activator of Cdc2.

The elegant model (see the figure) derived from these three reports seems destined for textbooks—regardless of whether it ultimately proves correct in detail. As yet, the single proposed pathway has not been demonstrated in its entirety in any one cell type but is instead synthesized from experiments in evolutionarily distant organisms—human, mouse, and yeast. Nevertheless, the mosaic nature of this model seems justified because the pathways in different cell types thus far have been found to be conserved.

After DNA damage, the inhibitory phosphorylation of Cdc2 that causes G_2 arrest occurs on Tyr^{15}. Genetic studies in fission yeast imply that this phosphorylation is maintained by the activity of protein kinases spWee1 [and the redundant spMik1 (here the prefix sp refers to *Schizosaccharomyces pombe*] and the simultaneous inactivity of phosphatase spCdc25 (*10*). In one of the new reports, human Cdc25 (here designated hCdc25) is shown to become phosphorylated in vivo on Ser^{216} after DNA damage in mammalian cells (*7*), and all three reports suggest that this is how Cdc25 (the *S. pombe* or human protein) is kept inactivated (*5–7*, *10*). This phosphorylation is clearly functionally important because the nonphosphorylatable allele hCdc25(S216A) (in which Ser^{216} is mutated to Ala) is defective for G_2-M arrest.

Phosphorylation of spCdc25 and hCdc25 is achieved by Chk1 protein kinase (*5–7*), a protein kinase required for arrest after DNA damage at least in fission yeast (*11–13*). From elegant genetic studies in fission yeast, Furnari *et al.* argue that Chk1 acts primarily through Cdc25 and not through Wee1 and Mik1 protein kinases, although they are required to maintain arrest (*6*, *10*). [The rel-

The author is at the Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721, USA. E-mail: ted_weinert@tikal.biosci.arizona.edu

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evance of the association of spChk1 with spWee1 is as yet unknown (*14*)]. Chk1 probably acts directly on Cdc25: spChk1 and hChk1 proteins (*5–7*) can bind to and phosphorylate spCdc25 and hCdc25 in vivo and in vitro. The sites of phosphorylation on hCdc25 by hChk1 is the same Ser^{216} (*5*, *7*) that is required for arrest in vivo (*7*).

How does phosphorylation inhibit Cdc25? Cdc25 phosphorylation itself does not appear to directly inactivate Cdc25 (*7*). Rather, Peng *et al.* found that only the species of Cdc25 phosphorylated on Ser^{216} (and not the unphosphorylated species) binds to 14-3-3 proteins, a class of molecules that bind to signaling molecules including phosphatases (*15*). Peng *et al.*, therefore, propose that the 14-3-3 protein sequesters Ser^{216}-phosphorylated Cdc25, thereby preventing it from activating Cdc2 by dephosphorylation. Supporting this scheme is the observation that 14-3-3 proteins encoded by the *rad24* and *rad25* genes are involved in arrest after damage in fission yeast (*16*, *17*).

Taken together, these results suggest that after damage, Chk1 becomes active, phosphorylates Cdc25 on Ser^{216}, which promotes the binding of Cdc25 to 14-3-3 protein and therefore its sequestration. In this state, Cdc25 cannot activate Cdc2. The activation of Chk1 after DNA damage appears to involve phosphorylation mediated by other checkpoint proteins, including spRad3 in fission yeast (*13*) and probably related Rad3-like proteins in other cell types (*3*).

This hypothesis now connects the function of checkpoint protein kinase Chk1 to cell cycle regulators Cdc25 and Cdc2. Because this model is based on a number of inferences and depends on the conservation of pathways in different cell types, several points remain to be directly tested. The roles of the putative Chk1 and 14-3-3 proteins in mammalian cells in arrest have yet to be established. The model predicts that the fission yeast Rad24 and Rad25 proteins will bind to and sequester Cdc25 phosphorylated on Ser^{216} and thereby somehow prevent activation of Cdc2. If and how that occurs is unknown. The mechanism of activation of Chk1 remains to be determined as well.

These findings suggesting a specific mechanism for cell cycle arrest at G_2 (see the figure) are a substantial advance in the field. Nevertheless, additional mechanisms of regulation for G_2 arrest apparently exist. Human cells with a nonphosphorylatable Cdc2 subunit are only partially defective in G_2 arrest, leading to speculation that other mechanisms (such as localization of cyclin B) may also be involved in arrest (*9*). In budding yeast, inhibitory phosphorylation of Cdc28, the homolog of Cdc2, is not sufficient to explain G_2 arrest (*20*, *21*). Finally, arrest in S phase when DNA replication is blocked may in some cell types also require Cdc2 phosphorylation (*7*), but in some cases S-phase arrest involves additional mechanisms unrelated to Cdc2 phosphorylation (*18–20*). Indeed, arrest in G_1 after DNA damage in mammalian cells may be regulated by both an inhibitor of Cdk activity, p21 (*23*, *24*), and an inhibitory tyrosine phosphorylation of Cdk4 (*25*) (possibly by the same Chk1-dependent pathway discussed here).

Next, the field will want to inactivate the checkpoint pathways for cancer therapy by targeting components like Chk1 (*26*). Cancer cells treated with drugs that inactivate Chk1, for example, may render those cells more sensitive to DNA damaging agents already widely used in therapy. Whether the resulting increase in sensitivity will affect cancer cells more than normal cells in one of several important issues. Ultimately, to manipulate checkpoint pathways we will need to know yet more molecular details of those pathways, how the pathways are changed in cancer cells, and what else the specific proteins in those pathways do in a cell.

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Edited by David Voss

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