Abstract
The DNA damage checkpoint coordinates a block in cell proliferation with the DNA repair process that follows when lesions are inflicted on the genome. However, we do not know exactly how cell division can recommence following a DNA damage--induced arrest. Recent work from our lab has identified Polo-like kinase-1 and Cdc25B as two essential components of the machinery that sets the cell division process back in motion when the checkpoint response is abrogated. Here, we discuss these novel insights and discuss their possible implications for the treatment of cancer. (Cancer Res 2005; 65(16): 7037-40)

DNA Damage Checkpoints
Cells continuously encounter DNA lesions caused either by damaging agents present within the cell (i.e., oxygen radicals), replication errors, stalled replication forks, or by extracellular factors such as UV light or ionizing radiation. Such DNA lesions can be fixed by a variety of DNA repair mechanisms that serve to protect the genomic integrity of a cell. To efficiently repair DNA damage, lesions have to be recognized and ongoing cell cycle progression has to be stopped to prevent propagation of a mutated genome that may contribute to malignant transformation. Therefore, DNA damage checkpoints act at several points in the cell cycle to trigger a cell cycle arrest and activate DNA damage repair. These DNA damage--induced signaling cascades consist of (a) DNA damage sensors, (b) checkpoint transducers, and (c) checkpoint effector proteins (reviewed in ref. 1).

Mitotic entry depends on activity of the cyclin-dependent kinase-1 (Cdk1) in complex with its regulatory subunit cyclin B. Cyclin B-Cdk1 activity is determined through phosphorylation events on Cdk1 as well as subcellular localization of the cyclin B-Cdk1 complex (2). During G2, Cdk1 is kept inactive via inhibitory phosphorylation by the Wee1 and Myt1 kinases (2). During mitotic onset, these inhibitory phosphorylations are removed by one of the members of the Cdc25 phosphatases (Cdc25A, Cdc25B, and Cdc25C; reviewed in ref. 3). Furthermore, DNA damage results in sequestration of the inhibitory phosphorylation or via ubiquitin-mediated degradation (3). Activating phosphatases Cdc25A, Cdc25B, and Cdc25C are efficiently inhibited in response to DNA damage, either through inhibitory phosphorylation or via ubiquitin-mediated degradation (3). Additionally, DNA damage results in inactivation of the cyclin B-Cdk1 complex in the cytoplasm (2) and catalytic inhibition of Plk1 (6).

Cell Cycle Resumption after a DNA Damage--Induced Checkpoint Arrest
Once DNA damage is repaired, the DNA damage checkpoint is silenced so that cell cycle progression is allowed to resume (a process called "recovery"; Fig. 1). Alternatively, in budding yeast the checkpoint can be actively abrogated (a process called "adaptation"; Fig. 1) when a small amount of damage fails to be repaired (7). Cell cycle resumption following a checkpoint arrest may seem as easy as setting a car in motion on a downhill track; simply removing the break is enough to get it going. However, work in yeast has shown that several factors are required to actively promote such a cell cycle restart, even when the damage is fully repaired. Genetic screens in budding yeast cells with a defined, nonrepairable double-strand break have identified a range of proteins that are required for checkpoint adaptation, including the polo homologue Cdc5, Casein kinase II, Srs1, and the phosphatases Ptc2 and Ptc3 (8–10). Although acting at different levels in the checkpoint signaling cascade, all adaptation mutants ultimately fail to inactivate the checkpoint kinase Rad53 (8, 10, 11). Similarly, in fission yeast, the Dis2 phosphatase and Crb2 were shown to play a role during recovery (12, 13). Again, both these proteins control mitotic entry after a checkpoint arrest through direct modulation of checkpoint kinases (12, 13).

Little is known about the mechanism of checkpoint inactivation in mammalian cells. In our lab, we recently investigated the requirements for a cell cycle restart in G2 DNA damage--arrested cells. By inhibition of the checkpoint kinases ATM and ATR with caffeine, one can abrogate a DNA damage--induced arrest and allow a cell cycle restart in a highly synchronous fashion. Using this method, we found that both Plk1 and Cdc25B are essential for mitotic entry when cells recover from a DNA damage checkpoint--induced arrest (14). In contrast, both Plk1 and Cdc25B are dispensable for mitotic entry in unperturbed cell cycles (14). These results are different from those in budding yeast cells, which only require Cdc5 for adaptation and not for recovery (9, 10). The requirement for
Plk1 during recovery was lost in Wee1-depleted cells, suggesting that Plk1 in human cells might function as an upstream inhibitor of Wee1 (14). Indeed, Wee1 phosphorylation by Plk1 was shown to promote proteosomal degradation of Wee1 (15). We could show that undamaged Plk1-depleted cells enter mitosis with high levels of Wee1, indicating that the effect of Plk1 on Wee1 is not essential to allow mitotic entry in an unperturbed cell cycle. However, Plk1-dependent Wee1 degradation becomes rate limiting during recovery from a DNA damage-induced checkpoint arrest (14). An explanation for this apparent switch may lie in the difference in Cdc25 phosphatase activity before and after checkpoint activation. Whereas in undamaged cells several (redundant) Cdc25 isoforms can act to promote mitotic entry, all isoforms are efficiently inhibited in damaged cells. Recovery may therefore depend on more stringent removal of the Cdc25-counteracting Wee1 activity. Alternatively, the requirement for Plk1 may also reflect a role for Plk1 in modulation of DNA damage checkpoint components. Although the most upstream checkpoint kinases are inhibited by caffeine, it is unknown whether a full shutdown of the downstream pathway may still require active silencing in this specific situation. If so, Plk1 may play a role in the inactivation of Claspin, Chk1, and/or Chk2, analogous to the roles of Cdc5 and Plx1 in checkpoint inactivation during adaptation in budding yeast and *Xenopus* (11, 16).

The replication checkpoint is activated every cell cycle and shares a signaling cascade with the DNA damage checkpoint. As we find that Plk1 is not essential for mitotic entry in undamaged cells, there does not seem a requirement for Plk1 in general checkpoint recovery. This ambiguity might be explained by the differences in checkpoint responses provoked by replication stress or double-strand breaks. Whereas double-strand breaks primarily activates ATM-Chk2 signaling that also inhibits Plk1, the replication checkpoint predominantly activates ATR-Chk1 (and at least in *Xenopus* does not inhibit Plx1; ref. 16). Alternatively, the amount of DNA damage might explain why Plk1 is apparently not essential for inactivation of the intrinsic replication checkpoint. The level of checkpoint signaling in response to replication stress might be far less when compared with checkpoint signaling in response to high levels of double-strand break–inducing reagents. In addition, adaptation experiments in budding yeast already showed that a possible cell cycle restart critically depends on the amount of DNA lesions (11).
Checkpoint Recovery and Cancer

The significance of intact checkpoint signaling is illustrated by the fact that mutations in checkpoint or repair genes cause a marked predisposition for tumorogenesis (for instance, germ line p53 and CHEK2 mutations in Li Fraumeni syndrome, inactivating ATM mutations in ataxia telangiectasia, defective DNA damage repair with Brca1/2 and Nbs1 mutations in familial breast cancer patients, and Nijmegen breakage syndrome patients, respectively; refs. 17–21). In addition, a causal role for other checkpoint genes, such as 53BP1 and H2AX in protection from cancer susceptibility was shown in animal models (22, 23). However, whereas most research on DNA damage control has focused on (in)adequate activation of the DNA damage checkpoint, a scenario in which cells can abrogate the implemented checkpoint might pose an equally dangerous threat to genomic integrity. Although the process of premature checkpoint abrogation (or “adaptation,” as originally identified in yeast; ref. 7) is not recognized in mammalian cells yet, evidence for adaptation in Xenopus was recently presented (16). Interestingly, adaptation in Xenopus was shown to depend on the Plk1 homologue Pxl1 (16). Whether deregulated checkpoint recovery and/or adaptation are actually responsible for altered genomic integrity and tumor susceptibility remains to be determined. A hint that these processes do play a role comes from the observation that both Pxl1 and Cdc25B are frequently overexpressed in cell lines as well as primary tumors (3, 4). Elevated levels of Pkl1 correlate to metastatic potential and poor prognosis (4). In addition, for both genes, it was shown that deregulated expression promotes transformation (3, 4). Overexpression of Cdc25B, for instance, enhances the development of hyperplasia in mammary gland after carcinogen exposure (3), whereas constitutive expression of Plk1 caused transformation of mouse 3T3 fibroblasts (4). Likely, overexpression of Cdc25B or Plk1 in tumor cells will result in differential kinetics of checkpoint recovery. Indeed, expression of a (mutated) active form of Plk1 was previously shown to cause unscheduled mitotic entry in cells that were treated with DNA-damaging agents (6). Whether these specific results actually represented premature checkpoint recovery or an inability to activate the DNA damage checkpoint remains to be elucidated, but these results do indicate that the activity of Plk1 influences the outcome of DNA damage checkpoint signaling.

Treatment with DNA-damaging agents is an often-used method to eliminate cancer cells. Excessive DNA lesions, through irradiation or radio-mimicking chemotherapy, trigger checkpoint responses leading to stalled cell division and subsequent cell death. However, such approaches exploit cellular mechanisms that are often defective in human tumors, as discussed above. Dysfunctional p53 for instance (as seen in ~50% of all human cancers) results in the loss of the typical G1 arrest in response to DNA damage that is seen in primary cells. For this reason, these cells will display a stronger dependency on the G2 DNA damage checkpoint for protection against genotoxic insults. Because impairing checkpoint recovery could possibly serve to achieve a more pronounced G2 arrest in response to DNA-damaging agents, it is of interest to study whether inhibition of Plk1 or Cdc25B would be useful as adjuvant anticancer therapy.

From their roles in cell cycle regulation, one might expect that inhibition of Plk1 and Cdc25B causes gross proliferation defects in primary as well as malignant cells. Surprisingly, Cdc25B deficiency does not markedly alter growth characteristics of somatic mouse cells (24), making Cdc25B an attractive target for cancer therapy. However, most of the known Cdc25B inhibitors also inhibit Cdc25A and Cdc25C. Consequently, these inhibitors provoked a cell cycle arrest, both in the G1 and G2 phase of the cell cycle. Very recently, a novel Plk1 inhibitor was presented that caused a typical mitotic arrest, as also observed after interference with Plk1 using antibody microinjection or RNA interference (25). More importantly, treatment of tumor cells with this inhibitor caused a dramatic decrease of tumor cell growth and this Plk1 inhibitor could elicit a potent antitumor response in tumor-injected mice. Based on our findings on the role of Plk1 following checkpoint abrogation, one would predict that inhibition of Plk1 is an efficient tool to establish an irreversible DNA damage–induced G2 arrest. Hence, Plk1 inhibition would serve as a potent adjuvant therapy when combined with a DNA-damaging regimen. Intriguingly, a strong synergy between Plk1 inhibition and DNA-damaging agents was indeed observed for tumor cell growth (25). Although the underlying mechanism responsible for this synergy will have to be addressed, Plk1 might prove to be a promising antitumor target, due to its role during checkpoint recovery.

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References

Restarting the Cell Cycle When the Checkpoint Comes to a Halt

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