Review

# Regulation of mitosis via mitotic kinases: new opportunities for cancer management

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## Abstract

**Mitosis, a critical and highly orchestrated event in the cell cycle, decides how cells divide and transmit genetic information from one cell generation to the next. Errors in the choreography of these events may lead to uncontrolled proliferation, aneuploidy, and genetic instability culminating in cancer development. Considering the central role of phosphorylation in mitotic checkpoints, spindle function, and chromosome segregation, it is not surprising that several mitotic kinases have been implicated in tumorigenesis. These kinases play pivotal roles throughout cellular division. From DNA damage and spindle assembly checkpoints before entering mitosis, to kinetochore and centrosome maturation and separation, to regulating the timing of entrance and exit of mitosis, mitotic kinases are essential for cellular integrity. Therefore, targeting the mitotic kinases that control the fidelity of chromosome transmission seems to be a promising avenue in the management of cancer. This review provides an insight into the mechanism of mitotic signaling, especially the role of critical mitotic kinases. We have also discussed the possibilities of the use of mitotic kinases in crafting novel strategies in cancer management. [Mol Cancer Ther 2007;6(7):1920–31]**

## Introduction

Cancer accounts for ~560,000 deaths each year in the United States, making it second only to heart disease as the most common cause of death (1). A unifying feature of cancer is the uncontrolled proliferation of cells, which disrupts the normal function of surrounding or even distant tissues, ultimately leading to organ failure and death. Cell proliferation and division is a series of coordinated events that compose a cell division cycle in the eukaryotes. The progression of cell cycle is a complex but extremely orchestrated process and defects in this process may result in an uncontrolled proliferation of cells that may lead to the development of cancer.

In a normal cell cycle, a cell possesses a single copy of its DNA in $G_1$ phase. After sufficient growth, the cell duplicates its DNA content from 2N to 4N in synthesis, or S, phase. After DNA duplication, the cell undergoes another gap phase, $G_2$, and the DNA damage checkpoint. If no DNA damage is detected, the cell enters mitosis and undergoes multiple major cellular changes before and during actual division. First, during prophase, the chromosomes condense to the center of the cell and nuclear envelope breakdown occurs. Prometaphase follows where chromosomes attach to microtubules, or spindles, connecting chromosomes to the centrosomes at opposite ends of the cell. During this stage, the cell undergoes a ''wait anaphase'' signal until all chromosomes are attached to the centrosomes. This is also known as the spindle assembly checkpoint. After proper spindle attachments are made, the anaphase-promoting complex (APC), a multisubunit E3 ubiquitin ligase that targets cell cycle–related proteins for degradation by the 26S proteasome, becomes activated, degrading multiple cell cycle regulators, and promotes entry into anaphase. The sister chromosomes separate and enter telophase where new nuclear envelopes form and cytokinesis occurs forming two identical sister cells. From here, a cell may then reenter the cell cycle at $G_1$ or it may enter $G_0$ and further differentiate and go on to perform its cellular functions.

The process of mitosis is tightly regulated and disrupted passage through mitosis often leads to chromosome missegregation and aneuploidy, which is a frequent characteristic of cancer cells and believed to be a possible cause of tumorigenesis. The use of antitumor tubulin drugs, such as the *Vinca* alkaloids and taxanes, has given promising results for the treatment of cancer. These drugs inhibit proper microtubule dynamics leading to mitotic arrest and/or cell death. However, there is an additional burden to healthy cells as microtubules are required for proper molecular transport. This has led to the exploration of specific targets for the management of cancer. Studies have suggested that drugs that target mitotic spindle assembly may be useful in the management of a variety of neoplasms as they lead to chronic mitotic arrest from

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sustained activation of the mitotic checkpoint. The list of targets consists of cell cycle–specific, and quite often mitosis-specific, proteins and structures. In addition to the structures of microtubules and centrosomes themselves, ongoing research is exploring the potential of targeting essential mitosis-specific kinases, phosphatases, and kinesin motor proteins to combat the proliferation of cancerous cells. Here, we review and discuss the mitosis-specific kinases, their function in the cell cycle, and their potential as targets for the development of approaches for cancer management. The other important and potential areas of cancer drug development are the antimicrotubule agents and the mitosis-specific kinesin motor proteins; these research areas are reviewed by several investigators, such as by Attard et al. (2) and Jiang et al. (3), respectively.

## Requirement for the Regulation of Mitosis

After DNA synthesis and before the $G_2$-M transition, if DNA damage is detected, two important kinases [i.e., ataxia-telangiectasia mutated (ATM) and ATM and Rad3-related (ATR)] are activated, initiating the DNA damage checkpoint (4, 5). These proteins initiate a cascade of kinase activation that ultimately leads to a $G_2$-M cell cycle arrest and prevents mitotic progression of cells with damaged DNA (Fig. 1). This is done via activation of multiple kinases, including the checkpoint kinases (Chk) 1 and 2 and Polo-like kinase (Plk) 3, which all are able to phosphorylate Cdc25C at $Ser^{216}$, leading to increased 14-3-3 binding and nuclear exclusion (6, 7). Prohibiting nuclear localization of Cdc25C leads to an inhibition of cyclin-dependent kinase (Cdk) 1/cyclin B1 activities and cell cycle progression. ATM/ATR also inhibits Plk1 activity to achieve the same end point indicating paralleling pathways (8).

A second checkpoint controlled by kinases is the spindle assembly checkpoint during mitosis. During prometaphase, the spindles connect the centrosomes and chromosomes. Two different complexes function to ensure proper attachment at the chromosomes. At the spindle-chromosome interface, a complex containing Aurora B, inner centromere protein (INCENP), survivin, and borealin functions to detect improper spindle-chromosome attachments and severs these connections, maintaining a cell cycle delay until proper attachments are made (Fig. 2A; refs. 9, 10). Whereas this complex directly acts on the spindle-chromosome interface to ensure proper connections are formed, a second complex works indirectly to stall cell cycle progression. A complex consisting of BubR1, Bub3, Mad2, and Cdc20 is recruited to unattached spindles and quickly releases (Fig. 2B; ref. 11). This complex binding to Cdc20 along with an independent binding of Emi1 to Cdc20 prevents APC-controlled degradation of cyclin B1 and securin, preventing cell cycle progression (11, 12). On proper spindle-chromosome attachment, the BubR1/Bub3/Mad2 complex loses its activity and Plk1 phosphorylates Emi1, releasing its binding to Cdc20, allowing Cdc20 to bind to APC. This binding initiates APC-dependent degradation of cyclin B1 and securin as well as Skp1-Cullin-F-box ubiquitin ligase complex-dependent degradation of Emi1 and the cell begins its exit from mitosis (11, 12).

If there is no loss of proper cell cycle control, either the cell can repair both DNA and spindle assembly damage or it may undergo a variety of end points, including apoptosis, mitotic catastrophe, or senescence, if the damage is too extensive and irreparable. However, if these regulation mechanisms are damaged themselves, the cells may proceed through cytokinesis with DNA or spindle errors and the cells may inherit unrepaired mutations or gain an abnormal number of chromosomes (aneuploidy). What happens to a cell with these errors is complicated by many factors, including the type and severity of damage. However, the pathways from which the fate of a cell is determined during mitotic arrest are poorly understood. Therefore, it is difficult to anticipate the effects of antimitotic agents on both normal and cancerous cells. This is particularly true for distinguishing between apoptosis and mitotic catastrophe, which shares similar characteristics but are distinct cellular processes (13). Most aneuploid cells die due to the loss of certain necessary genes or through functional cellular detection pathways that kill the cell. However, some aneuploid cells survive, which may be due to gaining mutations in genes that control cellular integrity (11). An aneuploid cell can either contain extra chromosomal material, which may contribute to tumorigenesis by increasing expression of oncogenes or, if the aneuploid cell lost chromosomal material, may contribute to tumorigenesis by a loss of heterozygosity of various tumor suppressor genes. It is not currently known if aneuploidy is the cause or consequence of tumorigenesis, but it is known that aneuploidy is one of the most common characteristics of all solid tumors. Finally, uncontrolled proliferation may still be halted through senescence of dysregulated cells if the cells undergo a prolonged stall in the cell cycle or after mitotic exit the cells never reenter the cell cycle or differentiate. But similar to apoptotic and mitotic catastrophe signaling pathways, the molecular pathways that direct a cell toward senescence are not well understood.

## Mitotic Kinases in Regulation of Mitosis and Cell Cycle

It is now well known that the regulation of M-phase progression relies on two posttranslational mechanisms: protein phosphorylation and proteolysis. These are intimately intertwined as the proteolytic machinery is controlled by phosphorylation, whereas several mitotic kinases are down-regulated by degradation. These kinases play major roles in the two checkpoints both before entry into and during mitosis. The most prominent mitotic kinase is the Cdk1, the founding member of the Cdk family of cell cycle regulators. Mitosis begins and ends with the activity of Cdk1 and its binding partner cyclin B1. Proper control of Cdk1/cyclin B1 activity is absolutely essential for

appropriate cell cycle progression and exit. First studied in fission yeast (*Saccharomyces cerevisiae*) more than 30 years ago, Nurse (14) identified a gene that controlled entry into mitosis, named *cdc2* or *Cdk1*. Before Cdk1 identification, Masui and Smith separately identified a complex later termed the maturation-promoting factor, named due to its ability to induce M-phase when introduced into *Xenopus* oocytes (6, 15, 16). Maturation-promoting factor was later purified and found to consist of Cdk1 and cyclin B1 (17).

With mitosis relying on the activity of a single complex, maturation-promoting factor, it is not surprising that maturation-promoting factor is controlled by multiple counteracting kinases and phosphatases to ensure normal cell cycle progression. With current cancer therapies not being able to effectively manage the disease, strategies targeting mitosis regulators could be a potentially useful option, which may improve the therapeutic index when used alone or in combination with current regimens. The nature of mitotic kinases makes them a suitable target for antitumor therapy; because they are only expressed during the cell cycle (i.e., in actively dividing cells), differentiated cells should not be a viable target. Additionally, dysregulation of mitotic kinases has been associated with uncontrolled and improper cell cycle progression both *in vitro* and *in vivo*, which presents a potential for specific diagnosis and treatment. Finally, the mitotic kinases possess relatively specific targeting sites for small-molecule inhibitors with little to no homology outside their respective kinase families. In the following pages, we have discussed the mechanisms of the major mitotic kinases involved in the cell cycle and how targeting these kinases may be used for the management of cancer.

### Cyclin-Dependent Kinase 1

Cdk1 is involved in many stages of the cell cycle, including mitosis (Table 1). Cdk1 can bind with cyclin A or cyclin B depending on the stage of the cell cycle. Cdk1 binding to cyclin A controls entry and progression through the $G_2$ phase, whereas Cdk1-cyclin B1 binding regulates the $G_2$-M transition. Once bound and activated in the nucleus, Cdk1/cyclin B1 phosphorylates multiple targets that initiate mitotic entrance, regulates its progression, and controls mitotic exit whereupon cyclin B1 degradation is necessary. Cdk1 regulation is too extensive to be discussed here; a detailed review is available elsewhere (18). Briefly, Cdk1 protein levels are expressed ubiquitously throughout the cell cycle; therefore, Cdk1 activity is controlled directly by phosphorylation and indirectly through regulation of its cyclin binding partners (Fig. 1). Inactivation of Cdk1 is maintained by phosphorylation of $Thr^{14}$ and $Tyr^{15}$ by the kinases Myt1 and Wee1, respectively (18). These residues reside in the ATP-binding site of Cdk1; thus, phosphorylation inhibits ATP binding (18). Myt1 and Wee1 are active during the S and $G_2$ phases of the cell cycle (18). At the mitotic transition, Myt1 is phosphorylated by Plk1 and Cdk1 *in vitro*, resulting in lowered kinase activity, indicating a possible feedback loop (18). Wee1 is phosphorylated by the kinases Chk1 and Cds1, resulting in kinase inactivation and decreased 14-3-3 binding and protein stability (18).

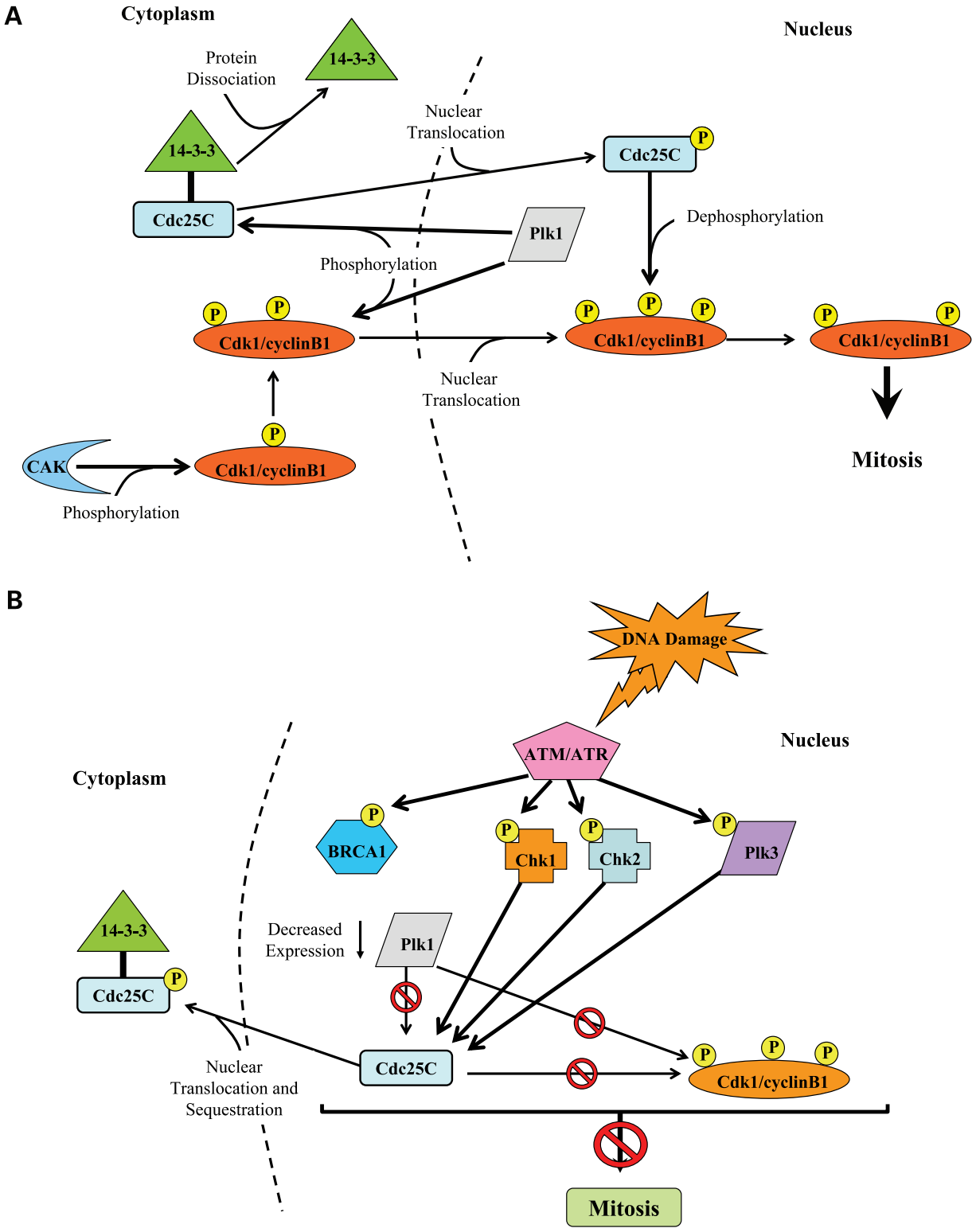
Activation of Cdk1 occurs through the combination of three required steps. First, phosphorylation of $Thr^{161}$ opens up the catalytic region of Cdk1. Phosphorylation of $Thr^{161}$ is done by Cdk-activating kinase (CAK), which occurs late in $G_2$ and is not removed until after cyclin B1 degradation late in mitosis (19). Second, nuclear localization of Cdk1/cyclin B1 is promoted by Plk1 phosphorylation of $Ser^{147}$ on Cdk1 (18). The final activation step of Cdk1 involves dephosphorylation of $Thr^{14}$ and $Tyr^{15}$ by members of the Cdc25 phosphatase family, Cdc25A, Cdc25B, and Cdc25C (18). Cdc25A seems to be involved in the $G_1$-S phase transition, whereas Cdc25B and Cdc25C are involved in dephosphorylation and activation of Cdk1 in $G_2$, with Cdc25C being the primary phosphatase at the $G_2$-M transition.

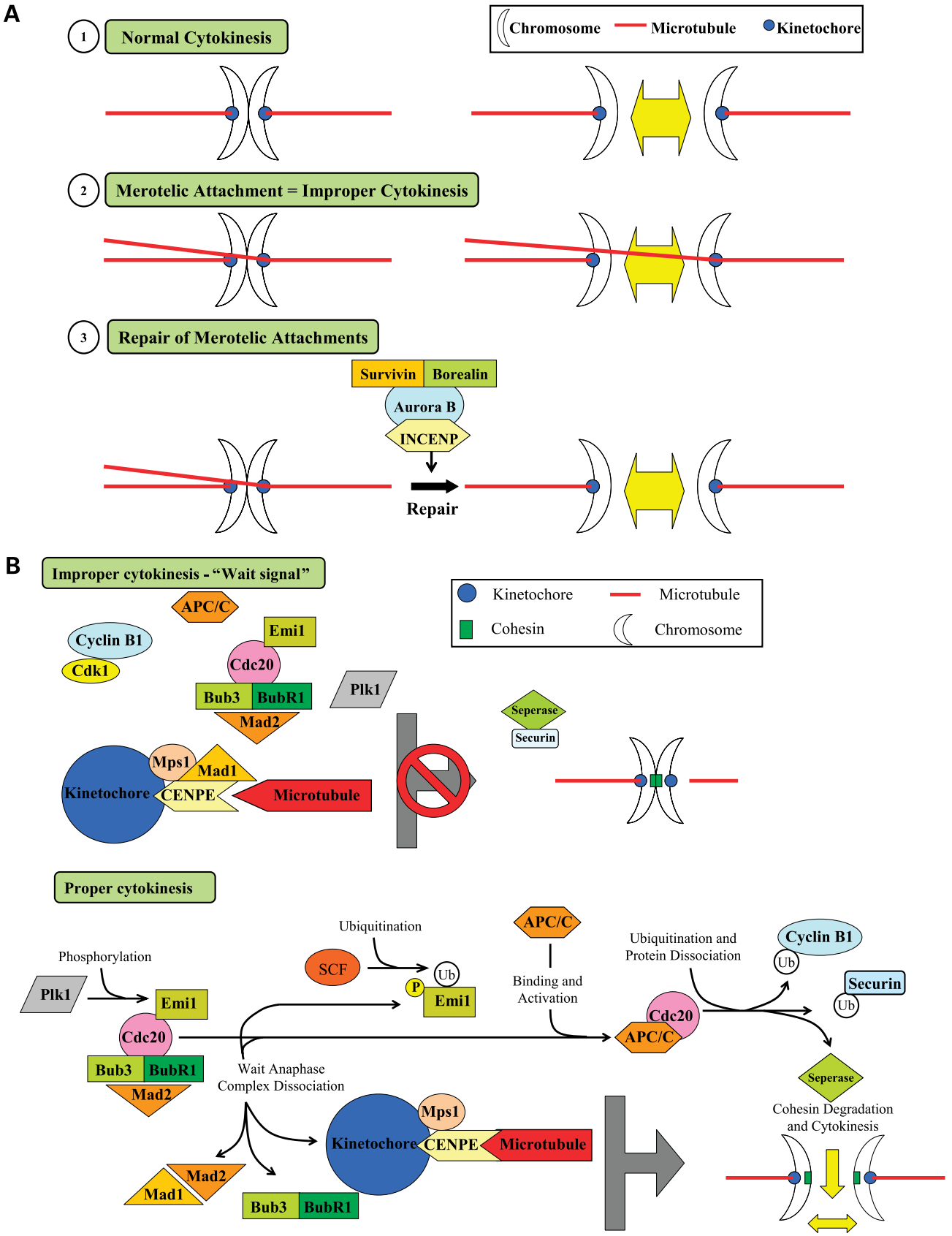
Activation of Cdc25C is achieved by phosphorylation of $Ser^{198}$ by Plk1 (20). $Ser^{198}$ lies within the nuclear export signal of Cdc25C, and phosphorylation of this residue promotes localization of Cdc25C to the nucleus. Once Cdc25C is activated and localized to the nucleus, it becomes hyperphosphorylated by Cdk1/cyclin B1, creating a positive feedback loop, increasing Cdc25C and Cdk1/cyclin B1 activities. Inactivation of Cdc25C is achieved through phosphorylation of $Ser^{216}$, creating a binding site for 14-3-3 protein, sequestering Cdc25C in the cytoplasm, and preventing Cdc25C and Cdk1 interaction. Phosphorylation of $Ser^{216}$ has been attributed to Chk1, Chk2, C-TAK1, and Plk3 (18, 21).

Dysregulation or mutations of *Cdk1* itself have not been reported in any cancers. However, inhibition of Cdk1 has been shown to induce cell cycle arrest and apoptosis. Cdk inhibitors may impart their effects through either inhibiting the catalytic subunit, preventing downstream phosphorylation, or through inhibiting the ability of Cdk1 to control transcription through phosphorylation of RNA polymerase

**Figure 1.** Mitotic kinases at the DNA damage checkpoint and $G_2$-M transition. The regulation patterns of mitotic kinases differ greatly in healthy cells (**A**) versus cells with DNA damage (**B**). **A,** Cdc25C activity is regulated by Plk1. Cdc25C is inhibited by binding to 14-3-3, sequestering it in the cytoplasm. At mitotic entry, Plk1 phosphorylates $Ser^{198}$, inhibiting Cdc25C-14-3-3 binding, allowing for localization of Cdc25C to the nucleus where it can dephosphorylate and activate Cdk1/cyclin B1. Before entering mitosis, Cdk1/cyclin B1 is in an inactive state by phosphorylation of $Thr^{14}$ and $Tyr^{15}$. Activation of Cdk1/cyclin B1 occurs in steps. The first step is phosphorylation of $Thr^{161}$ by Cdk-activating kinase (CAK) opening up the catalytic region of Cdk1. The second step involves phosphorylation of $Ser^{147}$ by Plk1 within the nuclear export signal of cyclin B1, promoting Cdk1/cyclin B1 nuclear localization. Finally, dephosphorylation of both $Thr^{14}$ and $Tyr^{15}$ by Cdc25C fully activates Cdk1/cyclin B1, which is essential for mitotic exit and progression. **B,** DNA damage within a cell inhibits progression through mitosis. This is initiated by the ATM/ATR complex that phosphorylates and activates multiple targets, including BRCA1, Chk1, Chk2, and Plk3. These proteins work to inhibit Plk1 activity, thereby preventing the phosphorylation of Cdc25C at $Ser^{198}$ and cyclin B1 at $Ser^{147}$ by Plk1. Chk1, Chk2, and Plk3 further prevent mitotic progression by phosphorylating Cdc25C at $Ser^{216}$, promoting Cdc25C-14-3-3 binding and sequestering Cdc25C in the cytoplasm, preventing Cdc25C-mediated dephosphorylation of Cdk1/cyclin B1 at $Thr^{14}$ and $Tyr^{15}$. The sum of these processes prevents Cdk1/cyclin B1 activation and inhibits the mitotic progression.

II. Many of the Cdks, including Cdk1, phosphorylate the COOH-terminal domain of RNA polymerase II, inhibiting mRNA production during mitosis (22). Tumor cells seem to be particularly sensitive to RNA polymerase II inhibition, indicating that the proapoptotic response to Cdk inhibition may be due to both blocking its kinase activity on traditional targets and also through altering its affects on transcription (23).





There are many known small-molecule inhibitors that target Cdks. Flavopiridol and UCN-01 were the first two pharmacologic inhibitors of the Cdks to be used in clinical trials. Flavopiridol has been shown to inhibit Cdk1, Cdk2, Cdk4, Cdk6, Cdk7, and Cdk9 by targeting the ATP-binding site, whereas UCN-01 is more specific toward Cdk1 and Cdk2 (24). However, both inhibitors do show nonselective inhibition of other kinase families, although with a weaker affinity, which is a major downfall of many of the Cdk inhibitors. Some other important Cdk inhibitors are *R*-roscovitine, E7070, olomoucine, bryostatin-1, and BMS-387032, although these inhibitors show more potent selectivity toward Cdk2 (24, 25).

### Chk1 and Chk2

Chk1 and Chk2 play essential roles in controlling progression through the cell cycle (Table 1). Although structurally unrelated, Chk1 and Chk2 seem to have some overlapping functions. It was originally thought that Chk1 activation relied solely on ATR-dependent detection of DNA damage caused by UV light or chemotherapeutics and, similarly, Chk2 activity depended on ATM detection of double-strand breaks. Now, more evidence is indicating a redundant role, particularly in the downstream targets, between the two kinases. This is further reinforced by the response of Chk1 to ionizing radiation–induced double-strand breaks, which was once thought to be solely an ATM-Chk2 response (26). For the sake of simplicity, the remainder of the review will treat the Chk1/Chk2 responses as a singular entity. If DNA damage is detected by ATM/ATR during the $G_1$-S transition, $G_1$ arrest is initiated by ATM/ATR phosphorylation of Chk1 and Chk2, increasing their kinase activity that then inactivates Cdc25A by nuclear exclusion or degradation through the ubiquitin pathway, resulting in reduced Cdk1 activity and $G_1$-S phase arrest (27). During the $G_2$-M transition, DNA damage again promotes ATM/ATR activity and phosphorylation of Chk1 and Chk2 (Fig. 1B). However, the Chk targets not only include Cdc25A but also Cdc25C and Wee1 (18). Again, Chk1/Chk2 phosphorylation of Cdc25A promotes its degradation through the ubiquitination pathway (18). Phosphorylation of Wee1 by Chk1 and Chk2 increases Wee1 activity, resulting in increased $Tyr^{15}$ phosphorylation of Cdk1; conversely, phosphorylation of $Ser^{216}$ by Chk1 and Chk2 on Cdc25C down-regulates Cdc25C activity by promoting 14-3-3 binding and nuclear exclusion. Both phosphorylation events lead to reduced Cdk1/cyclin B1 activity and mitotic arrest (18).

Chk1 and Chk2 have also been implicated in pathways involving other players in the $G_2$-M transition. Plk3 activity is also increased by ATM in response to DNA damage, and Plk3 phosphorylation of Chk2 fully activates Chk2, indicating a second pathway to Chk2 activation (7, 28). Chk2 may also be involved in stabilization of p53 in response to DNA damage (29). Active BRCA1 is essential for activation of Chk1, which in turn blocks Plk1 activity blocking mitotic progression (30).

Cancer-associated defects of *Chk1* are rare but have been found in cancers of the colon, lung, stomach, and endometrium (31). The majority of mutations result in a catalytically inactive truncated protein. *Chk2* mutations are also not a common characteristic of many cancers but have been found in carcinomas of the breast, lung, vulva, bladder, colon, and ovary, with the majority being missense or truncation mutations (31). Additionally, two *Chk2* variants, 1100delC and I157T, found in families with Li-Fraumeni syndrome predispose patients to breast and colon cancers (32). 1100delC mutations lead to a loss of catalytic function where I157T variants may work through a dominant-negative effect (27, 32). Both cases support the idea of Chk2 acting as a tumor suppressor, where loss of function predisposes an individual to cancer.

In the recent past, many pharmaceutical companies have put their efforts in developing a wide range of Chk inhibitors; however, a majority of them are weak in terms of inhibition of the kinase or its specificity toward other kinases. A detailed review of these inhibitors can be found by Tao and Lin (33). The majority of these inhibitors have been designed and tested against Chk1, although many also show inhibition of Chk2. Some of these inhibitors include UCN-01, scytonemin, and PD0166285, all of which show efficacy against other kinases reviewed here. The effect of many of these inhibitors is achieved through blocking the ATP-binding domain of Chk kinases. The use of Chk inhibitors would most likely be used as a sensitizer to other anticancer treatments because inhibiting Chk kinases allows the cell to progress with damage induced

**Figure 2.** Mitotic kinases involved in regulation of the spindle assembly checkpoint. There are two main stages of the spindle assembly checkpoint during mitosis. **A,** the first stage consists of the regulation of spindle attachments to the chromosomes to ensure proper cytokinesis and ploidy by Aurora B, survivin, borealin, and INCENP. **B,** the second stage controls microtubule binding to kinetochores before cytokinesis to maintain proper cell ploidy. **A,** during normal spindle-chromosome attachment and cytokinesis, microtubules from one centrosome attach to a single corresponding kinetochore on one sister chromatid. Spindles from the opposing pole attach to the opposite kinetochore. *1,* when proper attachment is achieved, cytokinesis begins and the sister chromatids are separated to form identical cells. *2,* if merotelic orientation occurs, where spindles of both poles attach to a single kinetochore, and is not repaired, it is possible for one sister cell to obtain both sister chromatids. This creates aneuploidy in both cells, one with extra DNA content and other lacking a chromatid. *3,* the complex of Aurora B, INCENP, survivin, and borealin detects improper spindle attachments and severs these attachments, prolonging the ''anaphase wait'' signal before cytokinesis. **B,** until proper spindle attachment, an anaphase wait signal prevents improper cytokinesis and aneuploidy. Unattached kinetochores recruit Plk1, Mps1, BubR1, Bub1, Bub3, Mad1, Mad2, and CENPE. This grouping activates a complex consisting of BubR1, Bub3, and Mad2, which binds Cdc20, preventing Cdc20-APC/C binding and activation. This prevents APC/C-dependent degradation of securin and cyclin B1. On spindle binding to CENPE, the activity of the anaphase wait signal is lost and Emi1 is phosphorylated by Plk1-releasing Cdc20, which binds and activates APC/C. APC/C-dependent degradation of securin and cyclin B1 enhances mitotic exit, whereas Emi1 is targeted by Skp1-Cullin-F-box for degradation. Degradation of securin releases the protease seperase, which in turn cleaves cohesin, binding the two sister chromatids, allowing for cytokinesis. Cyclin B1 degradation is then necessary for the exit of the cell from mitosis.

**Table 1. Targets and functions of mitotic kinases and associated ongoing clinical trials**

| Kinase | Known substrate(s) | Role(s) | Inhibitor(s) in ongoing clinical trials* |
|---|---|---|---|
| Cdk1 | Cdc25 family, CAK, cyclin B1, Myt1, Wee1 | Mitotic entrance, chromosome condensation, bipolar spindle assembly, nuclear envelope breakdown, APC/C regulation | AG-024322, PD-0332991, SNS-032, flavopiridol, UCN-01, *R*-roscovitine (seliciclib) |
| Chk1/Chk2 | ATM, ATR, Cdc25 family, Wee1, Plk3, p53, BRCA1 | DNA damage checkpoint, mitotic entrance | UCN-01 |
| Plk1 | Cdc25 family, Cdk1, cyclin B1, p53, ATM/ATR, BRCA1, Chk1, Emi1 | Mitotic entrance, centrosome maturation, bipolar spindle formation, APC/C regulation | BI 2536 |
| Plk2 | p53 | Centriole duplication, spindle damage checkpoint? | None |
| Plk3 | ATM, Cdc25 family, Chk2, p53 | DNA damage checkpoint, mitotic entrance | None |
| Plk4 | Not known | Centriole duplication APC/C regulation | None |
| Aurora A | TPX2, p53 | Spindle formation, centrosome separation | MK-0457, MLN8054 |
| Aurora B | INCENP, survivin, borealin | Spindle assembly checkpoint, cytokinesis | MK-0457, AZD1152 |
| Bub Family | Mps1, Mad1, Mad2, CENPE, Cdc20 | Spindle assembly checkpoint, APC/C regulation | None |
| NIMA Family | C-Nap1 | Centrosome assembly, maturation and separation, mitotic entrance | None |

NOTE: This table lists a variety of known functions that mitotic kinases are involved in throughout the cell cycle. It also lists current small-molecule inhibitors currently undergoing recruitment for testing in clinical trials.
Abbreviation: CAK, Cdk-activating kinase.
*http://www.clinicaltrials.gov

by a second drug, increasing the potency of the second drug. If Chk kinases are not inhibited, chemotherapeutic-induced cell toxicity may be allowed to repair, at least enough to survive, possibly leading to increased aneuploidy and possibly contribute to drug resistance. The use of Chk inhibitors alongside other treatments, such as gemcitabine, cisplatin, and paclitaxel, has been shown to increase their potency (33).

### Plk1, Plk2, Plk3, and Plk4

*Plks*, named after the *Drosophila melanogaster* homologue *Polo*, are a family of conserved serine/threonine kinases with multiple distinct and overlapping roles (Table 1). There are currently four identified Plks, named Plk1 (Plk), Plk2 (Snk), Plk3 (Fnk, Prk), and Plk4 (Sak). Each member contains a conserved $NH_2$-terminal kinase domain and one (Plk4) or two (Plk1, Plk2, and Plk3) highly conserved noncatalytic COOH-terminal polo-box domains that have been implicated in kinase localization. Plk1 is the most extensively characterized mammalian Plk family member. *Plk1* gene expression is tightly controlled with mRNA accumulation beginning in S phase and peak mRNA levels detected at the $G_2$-M transition and through mitosis with a corresponding increase in kinase activity paralleling the mRNA increase (34). At both the $G_2$-M transition and during mitosis, Plk1 contributes to multiple processes, including centrosome maturation, bipolar spindle formation, the activation of the Cdk1/cyclin B1 cascade by phosphorylating cyclin B1 and Cdc25C targeting it toward the nucleus, and regulation of the APC-targeted degradation through Emi1, which is necessary for mitotic exit (Figs. 1 and 2B; refs. 12, 20, 35–38). Plk1 is also able to directly bind to and phosphorylate p53, destabilizing and reducing the activity of p53 (7). With the role of Plk1 outlined above, it is not surprising that Plk1 activity is negatively correlated with DNA damage (39). Plk1 activity is tightly controlled by ATM/ATR during the $G_2$-M transition, with ATM/ATR inhibiting Plk1 activity in response to DNA damage, preventing mitotic entry (8). Plk1 is also indirectly controlled by ATM/ATR through both increased BRCA1 and Chk1 activities (40).

Deregulation of Plk1 has been shown to result in the formation of abnormal centrosomes, which have been correlated with aneuploidy and chromosomal instability leading to tumor development. Therefore, it is not surprising that Plk1 expression is up-regulated in a variety of tumors and could possibly be used as a prognostic marker for many neoplasms. Elevated Plk1 levels have been found in breast cancer, colorectal cancer, endometrial carcinomas, esophageal carcinoma, head/neck squamous cell carcinomas, melanomas, non–small cell lung cancer, oropharyngeal carcinomas, ovarian cancer, pancreatic

cancer, papillary carcinomas, and prostate carcinomas (41, 42). The complex mechanism of Plk1 control and overexpression in multiple tumors implicates Plk1 as a possible oncogene involved in tumorigenesis.

Targeting Plk1 *in vitro* and *in vivo* through multiple mechanisms has shown promising results. Knockdown of Plk1 using antisense oligonucleotides or small interfering RNA shows a decrease in cellular proliferation and a corresponding increase in apoptosis in multiple cell lines (43–46). Small interfering RNA against Plk1 also inhibited the growth of bladder cancer cells *in vivo* in a mouse model (46). Microinjection of anti-Plk1 antibodies into both transformed and nontransformed cells results in an inhibition of cell cycle progression (36). Various small-molecule inhibitors have also shown activity against Plk1 (reviewed in refs. 3, 47). Scytonemin, wortmannin, and LY294002 are compounds that inhibit Plk1 activity nonselectively. A more specific ATP-competitive inhibitor of Plk1 is BI 2536, which shows a 10,000-fold level of selectivity for Plk1 over other tyrosine and serine/threonine kinases. Finally, ON01910 is a compound that inhibits Plk1 at concentrations 10- to 20-fold lower than its other targets, including Cdk1 and Plk2. ON01910 has been shown to inhibit cell growth in each of the 151 cancer cell lines that it was tested on through an accumulation of cells in $G_2$-M arrest and apoptosis.

*Plk2* is a serum-inducible immediate-early response gene and may function primarily as a regulator of $G_1$ progression in mammalian cells (48, 49). Plk2 mRNA and corresponding protein levels transiently increase following serum stimulation of quiescent NIH3T3 fibroblasts peaking during $G_1$. Plk2 is a centrosomal kinase involved in centriole duplication (50). Studies have suggested that Plk2 is not required for cell growth; however, Plk2 inhibition is associated with a decrease in cellular proliferation. $Plk2^{-/-}$ mice show no embryonic lethality but grow slower than their wild-type counterparts due to a slower progression through S phase (51). Studies have shown that Plk2 mRNA is rapidly induced in human thyroid cells in response to X-ray irradiation, and a p53-binding homology element has been identified in the promoter region of Plk2 (52). Plk2 responds to spindle damage by paclitaxel in a p53-dependent manner, and the loss of Plk2 function sensitizes cells to paclitaxel, indicating a possible role in the spindle damage checkpoint where Plk2 halts cell cycle progression in response to spindle damage (53). Although Plk2 is not essential for cell growth, its loss might render the cells more susceptible to stress, indicating that Plk2 may be promising target in conjunction with spindle-damaging chemotherapeutics such as paclitaxel (53).

*Plk3*, like *Plk2*, is an immediate-early gene showing low kinase activity levels in $G_1$ phase, increasing through S phase and peaking at $G_2$ phase (54). In response to DNA damage, Plk3 is directly phosphorylated by ATM, leading to increased Plk3 kinase activity at the DNA damage checkpoint and during $G_2$-M transition but in an opposing manner to Plk1 (Fig. 1B; ref. 28). Whereas Plk1 phosphorylates Cdc25C on $Ser^{198}$ leading to Cdk1/cyclin B1 activation and mitotic entry, Plk3 phosphorylates Cdc25C on $Ser^{216}$ in response to DNA damage (20, 21). Phosphorylation on this residue leads to nuclear exclusion of Cdc25C, inhibiting its activation of Cdk1/cyclin B1 and halting mitotic entry. Plk3 phosphorylation of Chk2 fully activates Chk2, increasing the cellular response to DNA damage (28). Likewise, Plk3 also phosphorylates p53 on $Ser^{20}$ in response to DNA damage, leading to increased p53 stability, increasing the cellular response to DNA damage (7). Further supporting a counteracting role to Plk1 is the fact that Plk3 expression levels are commonly decreased in various cancer tissues. These include head and neck squamous cell carcinomas, lung carcinomas, uterus carcinomas, and bladder carcinomas (55–57). Furthermore, overexpression of Plk3 induces similar end points to Plk1 knockdown, including a decrease in cellular proliferation, chromatin condensation, and apoptosis (58, 59). Surprisingly, overexpression of the Plk3 polo-box domain alone is sufficient to induce this response (59).

Plk4 is essential for cell division, and $Plk4^{-/-}$ mouse embryos are arrested after gastrulation at E7.5, with a marked increase in mitotic and apoptotic cells (60). These embryos also displayed cells in late anaphase or telophase and continued to express cyclin B1 and phosphorylated histone H3, indicating a possible role for Plk4 in APC-dependent destruction of cyclin B1 and cellular exit from mitosis (60). Analyzing Plk4 mRNA expression levels through the cell cycle supported this notion. Plk4 mRNA levels are low in $G_1$ and begin to rise through S and $G_2$, eventually peaking during mitosis (61). Plk4 is also necessary for proper centriole duplication (62, 63). Silencing of Plk4 using RNA interference leads to disorganized mitotic spindles and apoptosis (62). Overexpression of Plk4 leads to multiple centrosome formation, which could lead to aneuploidy if apoptosis does not occur (63, 64). One study reported Plk4 overexpression in colorectal tumors possibly contributing to chromosomal instability (65).

### Aurora Kinases 1 and 2

Similar to Plks, Aurora kinases are a conserved family of serine/threonine kinases that play multiple and critical roles in the cell cycle, especially in mitosis (Table 1). Three mammalian members of the Aurora family have been identified: Aurora A (Aurora 2), Aurora B (Aurora 1), and Aurora C (Aurora 3; reviewed in ref. 66). The Aurora kinases all consist of a catalytic COOH-terminal domain, with Auroras A and B sharing 71% homology in their COOH terminus. Even with this high level of similarity, the Aurora kinases have vastly differing localizations and functions. Very limited studies have been conducted to explore and define the role of Aurora C thus far; therefore, in this review, we will limit our discussion to Auroras A and B.

Studies have shown that Aurora A is primarily associated with the centrosomes and the microtubules in close proximity to the centrosomes beginning in late S-$G_2$. The binding of Aurora A with TPX2, a required spindle assembly factor in higher eukaryotes, is shown to be

responsible for Aurora A activation and localization to the microtubules (67). In mitosis, Ran-GTP, a GTPase involved in polarity of nuclear transport and mitotic spindle assembly, releases TPX2 from importin-α and importin-β, allowing TPX2 to bind to Aurora A, targeting it to the microtubules near the poles (67). TPX2 is also involved in regulation of Aurora A kinase activity by both counteracting a phosphatase of Aurora A, PP1, and stimulating autophosphorylation of Aurora A at $Thr^{295}$, an essential amino residue in the activation loop of Aurora A (68, 69).

In addition, Aurora A activity is shown to be required for centrosome separation and spindle formation at the onset of mitosis. Recruitment of several components of the pericentriolar material to the centrosome has been found to be deficient in the absence of Aurora A, leading to a microtubular mass reduction of ~60% (70). Inhibition of Aurora A by RNA interference has been shown to delay mitotic entry in human cells (71). Conversely, overexpression of Aurora A led to mitotic abnormalities, ending in failure of cytokinesis and aneuploid cells. Mitotic abnormalities were found to be increased in the absence of p53, which could bind to and inactivate Aurora A (72). *Aurora A* resides on chromosomal region *20q13.2*, which is amplified in many cancer cell lines and primary tumors, including breast, cervical, colorectal, pancreatic, ovarian, prostate, and gastric carcinomas (66).

Aurora B acts much differently than Aurora A. Aurora B acts as a chromosomal passenger protein whose expression peaks at the $G_2$-M transition, with maximum kinase activity in mitosis. Aurora B works in association with INCENP, survivin, and borealin (Fig. 2A; ref. 73). These proteins target, help activate, and aid in localization of Aurora B in a multiprotein complex. Aurora B localizes to the chromosomes early in mitosis and has a role in kinetochore-microtubule interactions. Aurora B is also responsible for phosphorylation of histone H3 on $Ser^{10}$ and $Ser^{28}$ during mitosis (74, 75). Aurora B also plays a critical role in cytokinesis; overexpression of a kinase inactive Aurora B has been shown to cause defects in complete cytokinesis in a variety of cell types (76). Interference of Aurora B with RNA interference, antibodies, or small-molecule inhibitors has been found to result in defects in chromosome congression (66). With the many critical roles required for promoting progression through mitosis, it is not surprising that several studies have shown that Aurora B is overexpressed in a variety of cancer cell lines and tissues promoting an increased progression through the cell cycle, including colorectal and prostate carcinomas (9, 10).

In the recent past, multiple small-molecule inhibitors targeting Aurora kinases have been created; most notably, these include hesperadin, MK-0457, ZM447439, MLN8054, and AZD1152 reviewed by Carvajal et al. (77). The number of Aurora small-molecule inhibitors is constantly growing and the review by Carvajal et al. (77) thoroughly discusses other Aurora inhibitors, which are presently in their infancy. These inhibitors have been shown to function via targeting the enzymatic activity by occupying the catalytic ATP-binding site (77). Further, these compounds have been found to inhibit histone H3 phosphorylation at $Ser^{10}$ that resulted in cell cycle arrest and/or apoptosis (77). However, following the treatment with the inhibitors, some cells continued to replicate but showed a very polyploid state, possibly because of the p53 status differences among the cell lines (77). All three of these small-molecule inhibitors seem to be selective for the Aurora kinases, thereby adding to a stronger rationale of possible usefulness in chemotherapeutics.

### Bub-Related Kinases

Some lesser-defined, yet critical, kinases for the spindle checkpoint during mitosis are the Bub family of kinases (Table 1). These kinases are involved in the spindle assembly checkpoint, which prevents anaphase until the chromosomes have aligned correctly and spindle tension is correct (Fig. 2B). The checkpoint is a complex mechanism involving many regulators and is not yet fully understood, but a current working model has been proposed (11). Studies have suggested that chromosome connections are maintained through cohesins that are cleaved by the protease seperase, which is inhibited by binding to securin (78). Before proper spindle assembly to the chromosomes, unattached kinetochores rapidly recruit many proteins, including Mps1, BubR1, Bub1, Bub3, Mad1, Mad2, and CENPE (79, 80). This congregational grouping activates a complex consisting of BubR1, Bub3, Mad2, and Cdc20 that quickly releases from the kinetochore and inhibits APC degradation of cyclin B1, securin, etc. (81). Cdc20 is also bound by Emi1, prohibiting Cdc20 binding to APC (12). On spindle binding to CENPE and the kinetochore, the activity of these kinases is blocked and the inhibitory complex formation halted and Plk1 phosphorylates Emi1, blocking Emi1/Cdc20 binding (12). This allows Cdc20 and APC to bind and ubiquitination and degradation of cyclin B1 and securin by APC and Skp1-Cullin-F-box–dependent degradation of Emi1 to begin (11, 12). On APC-mediated ubiquitination of securin, seperase is released, cleaving the cohesins that bind the two sister chromatids together; thereby allowing anaphase to proceed. Altered expression or activity of these proteins may have serious consequences contributing to aneuploidy and cancer. It has been shown that the mouse cells with insufficient CENPE possess kinetochores that cannot recruit normal levels of Mad1, Mad2, and BubR1, leading to an increase in unattached chromosomes without a delay of anaphase entry and an increase in missegregated chromosomes (82). Mice with reduced levels of Mad2, BubR1, or Bub3 showed an increase in aneuploid fibroblasts and an increase in multiple forms of tumors (82). Therefore, it is not surprising that multiple human tumor cell lines contain mutations in these genes as well (11). The checkpoint function in these cell lines has not all been established but, with many being aneuploid, a strong link between the spindle checkpoint and aneuploidy can be made. However, the reported instances of mutations or dysregulation of these genes in primary cancers is rare, which supports their potential as a chemotherapeutic target in a similar

situation to that of Cdk1 where the benefit would be inhibiting an essential mitotic component in proliferating cells, only leading to severe cellular damage and ideally apoptosis (11).

### Never in Mitosis A – Related Kinase

Never in mitosis A (NIMA) is an essential mitotic kinase first described in the filamentous fungus *Aspergillus nidulans* (83). Temperature-sensitive mutants of NIMA were found to arrest in $G_2$ at the restrictive temperature, whereas overexpression resulted in the rapid entry into mitosis (83, 84). This quickened mitotic entry also results in premature nuclear envelope breakdown, chromatin condensation, and mitotic spindle formation. There are currently at least 11 NIMA-related kinases (Nrk or Nek) found in humans (85). It is not clear which, if any, of the NIMA-related kinases is a true homologue to NIMA; however, Nek2 has gained a lot of interest based on its structural and functional homology to NIMA (Table 1).

Two spliced variants of Nek2 (i.e., Nek2A and Nek2B) have been identified (86). Nek2B is a shortened version of Nek2A, missing the KEN box and the destruction box found at the COOH terminus of the protein. Due to these lost destruction-targeting motifs, Nek2B levels are maintained longer through mitosis than Nek2A, which is quickly eliminated at the onset of mitosis. Both variants contain 44% homology to NIMA in the catalytic domain, which resembles a serine/threonine kinase domain. A leucine zipper motif on Nek2 was shown to promote homodimerization and autophosphorylation within the COOH-terminal region. Nek2 expression increases at the $G_1$-S transition, peaking during S and through $G_2$, and both Nek2 variants are shown to be associated with centrosomes throughout the cell cycle, although the signal is shown to weaken as Nek2A is degraded through mitosis.

At present, the exact role and mechanism of Nek2 in the cell cycle is not known, but through various overexpression of wild-type and catalytically inactive Nek2 variants, some possible roles have been proposed (83, 84, 87, 88). These include (*a*) functioning in centrosome assembly and maturation, (*b*) centrosome separation in association with C-Nap1, and (*c*) contributing to the $G_2$-M transition and mitotic progression. Nek2 inhibition through RNA interference has been shown to inhibit centrosome maturation and separation, chromosome segregation, and delay mitotic exit, supporting Nek2 as a possible chemotherapeutic drug target (88). Elevated Nek2 protein levels have been reported in cell lines derived from breast, cervical, and prostate carcinomas (89). An amplification of *1q32*, the locus containing *Nek2*, has also been reported in breast and gastric cancers (89). Further research needs to be done to evaluate the prevalence of Nek2 dysregulation to determine its potential as a target for therapy.

## Conclusion

The research efforts to define the mitotic signaling pathways and the role of mitotic kinases in the process are still in their infancy. In this review, we have discussed the regulation of important mitotic kinases, which seems to have a potential of providing novel targets and biomarkers and therefore opportunities for the management of cancer. The fact that mitotic kinases are only expressed in actively dividing cells presents unique targets against cancer cells, although this is also a major side effect of current drugs that target these kinases where nonspecific targeting of normal dividing tissues can occur. Therefore, there is a need for concerted research efforts, especially *in vivo* in appropriate preclinical models, to define the role of mitotic kinases in mitotic signaling and their usefulness as targets before we can actually embark on translational studies in human. Development of appropriate reagents (i.e., activation state–specific antibodies) will be extremely useful for future studies on mitotic kinases. This would facilitate our learning of exact mechanism about the function of mitotic kinases. In addition, targeting the mitotic kinases may be very useful in conjunction with other chemotherapeutic agents, especially in case of drug resistance. Finally, modulating mitotic kinases in a chemoprevention setting with natural compounds may be useful at safer doses for the prevention of primary cancers as well as for the prevention of recurrence.

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