

Chk1: A Double Agent in Cell Cycle Checkpoints

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Two cell cycle surveillance systems—the DNA damage checkpoint and the spindle checkpoint—guard against genomic instability. The protein kinase Chk1 is a well-established signal transducer in the DNA damage checkpoint. In this issue of *Developmental Cell*, Zachos et al. (2007) present evidence to indicate that Chk1 also plays a critical role in the spindle checkpoint, suggesting an interplay between the DNA damage and spindle checkpoints.

The cell division cycle is driven by fluctuating activities of cyclin-dependent kinases (Cdk) and consists of a series of tightly coordinated and interdependent processes, including DNA replication in S phase and sister-chromatid separation in mitosis. Cell cycle checkpoints are surveillance mechanisms that halt the cell cycle at appropriate stages in response either to exogenous damages to DNA or the mitotic spindle or to the incompleteness of a given cell cycle process, such as DNA replication or chromosome alignment (Figure 1). Upon DNA damage caused by ionizing radiation (IR), ultraviolet light (UV), chemicals, or replication stress, the DNA damage checkpoint blocks the cell cycle at multiple junctures, including the G1-S and G2-M transitions (Kastan and Bartek, 2004). This affords cells windows of opportunities to repair their DNA or commit apoptosis, thus preventing the passage of genetic alterations to the next generation. The spindle checkpoint senses the existence of sister chromatids that have not achieved proper attachment to the mitotic spindle and delays the onset of anaphase, thus ensuring the fidelity of chromosome segregation (Bharadwaj and Yu, 2004). Malfunctions of these cell cycle checkpoints result in genomic instability and promote tumorigenesis.

In the DNA damage checkpoint, two apical kinases, ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3 related), are activated following recruitment to nuclei foci that are believed to be sites of DNA damage and repair (Kastan and Bartek, 2004). ATR and ATM then phosphorylate and activate two signal-transducing kinases,

Chk1 and Chk2, which in turn phosphorylate key downstream effectors, including p53 and the Cdc25 family of phosphatases (Figure 1). Chk1/2-mediated phosphorylation of p53 causes its stabilization and accumulation, leading to the elevated transcription of p21, a Cdk inhibitor. Phosphorylation of Cdc25 proteins leads to their degradation, sequestration, and/or inhibition, preventing them from removing inhibitory phosphorylation on Cdk1 or Cdk2. Collectively, these mechanisms block the activation of Cdk1 or Cdk2 and critical cell cycle transitions.

In the spindle checkpoint, the Aurora B-INCENP kinase complex lies at the top of a cascade that recruits other spindle checkpoint proteins to kinetochores that are not attached by microtubules and/or not under tension, including kinases such as Mps1, Bub1, and BubR1 (Figure 1) (Vigneron et al., 2004). The kinetochore recruitment of the checkpoint proteins is believed to facilitate the binding of BubR1 and Mad2 to Cdc20 (Bharadwaj and Yu, 2004) and to activate the kinase activity of Bub1 toward Cdc20 (Tang et al., 2004). Mad2/BubR1 binding and Bub1-mediated phosphorylation of Cdc20 inhibit the ubiquitin ligase activity of the anaphase-promoting complex or cyclosome (APC/C) (Tang et al., 2001, 2004), resulting in the stabilization of securin and cyclin B1. This delays the activation of separase, cleavage of cohesin, and the onset of anaphase.

Both the DNA damage and spindle checkpoints are intracellular signal transduction pathways and share several common operating principles. Most notably, both checkpoints con-

tain protein kinase cascades that receive and propagate checkpoint signals, although relatively less is known about the kinase cascade in the spindle checkpoint. The findings of Zachos et al. (2007) represent an important step forward in this area and indicate that the two major cell cycle checkpoints not only share mechanistic parallels in their operations, but they also share certain common components.

Zachos et al. started their investigation by examining the possible mitotic functions of Chk1, a key component of the DNA damage checkpoint (Zachos et al., 2007). They showed that genetic ablation of Chk1 (Chk1^{-/-}) in chicken DT40 cells or depletion of Chk1 from human BE colon cancer cells by RNA interference (RNAi) causes chromosome missegregation, resulting in abnormal karyotypes. To further probe the potential function of Chk1 in the spindle checkpoint, they treated Chk1^{-/-} DT40 cells or Chk1 RNAi human cells with two spindle poisons—Taxol, which stabilizes microtubules and reduces tension across paired kinetochores, or nocodazole, which depolymerizes microtubules and renders all kinetochores unattached. While the Chk1-deficient cells undergo mitotic arrest when treated with nocodazole, these cells fail to arrest in mitosis in the presence of Taxol. Importantly, ectopic expression of the wild-type Chk1, but not its kinase-inactive mutant, in Chk1^{-/-} DT40 cells restores mitotic arrest in the presence of Taxol, indicating that the kinase activity of Chk1 is required for Taxol-triggered mitotic arrest. Consistent with a role of Chk1 in the spindle checkpoint, the Chk1-GFP

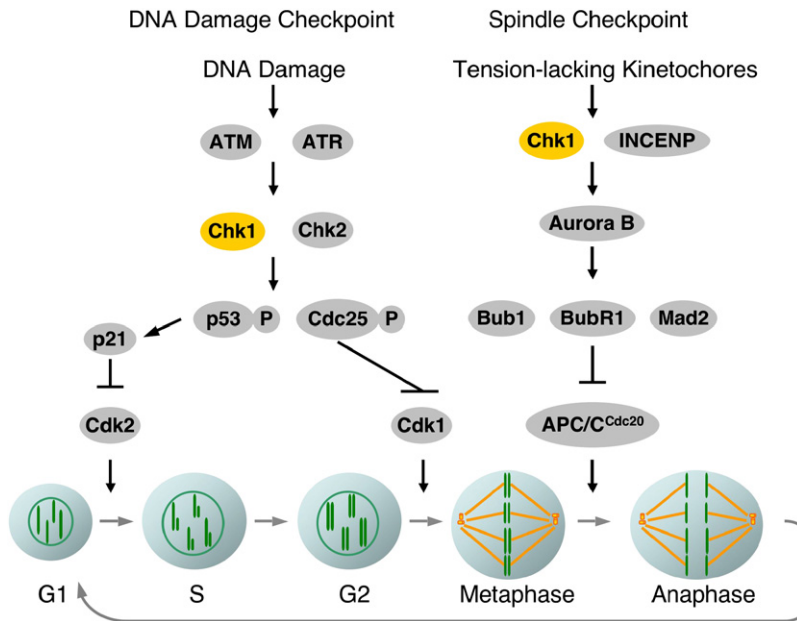


Figure 1. Simplified Schemes of the DNA Damage and Spindle Checkpoints that Highlight the Functions of Chk1

In the DNA damage checkpoint, Chk1 acts downstream of ATR and ATM to phosphorylate p53 and Cdc25A/C among other effectors, thus blocking G1-S and G2-M transitions. In the spindle checkpoint, Chk1 contributes to the activation of Aurora B in response to kinetochores that are not under tension. Aurora B then signals through the Bub and Mad proteins to inhibit APC/C^{Cdc20} and delays the metaphase-anaphase transition.

fusion protein localizes to kinetochores in DT40 cells in prometaphase. Furthermore, the kinetochore localization of BubR1 is diminished in Chk1-deficient chicken DT40 and human BE cells during unperturbed mitosis and in the presence of Taxol, whereas BubR1 and Mad2 localize normally to kinetochores in nocodazole-treated Chk1-deficient cells. These observations suggested that Chk1 is specifically required for the tension-sensing branch of the spindle checkpoint.

As a similar function has been ascribed to Aurora B (Ditchfield et al., 2003; Hauf et al., 2003), Zachos et al. next examined whether Chk1 and Aurora B lie in the same pathway (Zachos et al., 2007). Though Chk1 is phosphorylated at sites that are distinct from the conventional ATM/ATR sites in Taxol-treated mitotic cells, the kinase activity of Chk1 does not appear to increase in mitosis. Inhibition of Aurora B kinase activity in DT40 cells with a chemical inhibitor (VX-680) does not affect the kinetochore localization of Chk1-GFP or mitotic phosphorylation of Chk1. In con-

trast, despite its proper kinetochore localization, Aurora B is not fully activated in Chk1-deficient cells in the presence of Taxol, as judged by the reduced phosphorylation of CENP-A and BubR1 in cells and by the reduced kinase activity of immunoprecipitated Aurora B toward histone H3 *in vitro*. Chk1 phosphorylates and activates Aurora B *in vitro* independently of INCENP. Therefore, Chk1 appears to lie upstream of Aurora B in the tension-sensing branch of the spindle checkpoint. On the other hand, inhibition of Aurora B by VX-680 further exacerbates the checkpoint defects of Chk1-deficient cells, indicating that Aurora B can be activated in Chk1-independent manners, presumably through INCENP (Vader et al., 2006).

While this elegant work by Zachos et al. clearly establishes a role of Chk1—a well-known DNA damage checkpoint kinase—in the spindle checkpoint (Zachos et al., 2007), it also raises many interesting questions. For example, how does phosphorylation of Aurora B by Chk1 activate the kinase activity of Aurora B? Does this

occur in living cells? How is Chk1 recruited to mitotic kinetochores? What is the kinase that mediates phosphorylation of Chk1 in mitosis? What are the functions of these mitotic phosphorylation events on Chk1, as they do not appear to enhance its kinase activity? Are ATR and to a lesser extent ATM that act upstream of Chk1 in the DNA damage checkpoint also involved in the spindle checkpoint? Recent studies have shown that the DNA damage response is chronically activated in cancer cells (Bartkova et al., 2005), possibly including those used in the study by Zachos et al. (2007). It will be interesting to determine whether it is this chronic activation of the DNA damage response that sustains the kinase activity of Chk1 and contributes to spindle checkpoint signaling and whether Chk1 is dispensable for the spindle checkpoint in normal untransformed cells. Future studies aimed at answering these questions may reveal a closer than expected kinship between the DNA damage and spindle checkpoints.

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