Chk-ing in and Chk-ing out: Kinase Compartmentalization Comes to Checkpoint Control

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Checkpoints are the sentinels of cell-cycle progression. In this issue of *Molecular Cell*, Yaffe and colleagues (Reinhardt et al., 2010) show that spatial and temporal resolution of Chk1 and MK2, checkpoint kinases with identical substrate specificity, are necessary to signal different aspects of DNA damage signaling.

In enzymology, protein compartmentalization is often considered the "last refuge of a scoundrel." Yet the spatial segregation of enzymes is a fundamental tenet of cell biology (Scott and Pawson, 2009). The union of these two seemingly contradictory ideologies comes into sharp focus during cell division. Progression through the G_1, S, G_2, and M phases of the cell cycle is a complex molecular dance that requires an extraordinarily high degree of fidelity in space and time. Such meticulous synchronization of numerous enzyme activities is one way to ensure accurate transfer of the genetic code. Conversely, failure to complete DNA replication, chromosome condensation, or cell division causes the cell to descend into biochemical limbo in the form of checkpoint arrest. This favors recovery from genotoxic stress and protects against mutations, chromosomal aberrations, or defects in genome maintenance that accumulate as cells venture down a perilous path toward malignancy. Although at least five independent molecular complexes sense and sort DNA damage, two protein kinase-signaling units modulate the key elements of checkpoint control (Harper and Elledge, 2007).

Chk1 and Chk2 are functionally redundant protein kinases that respond to checkpoint signals emanating from the phosphatidylinositol 3-kinase family members ATM (ataxia-telangiectasia mutated) and ATR (Ataxia-telangiectasia and Rad-3 related). A concerted research effort has revealed many mechanistic details of how Chk1 and Chk2 keep a lid on the Pandora's box of DNA damage. Chk1 is activated by bulky DNA lesions and in response to replication fork collapse during S phase of the cell cycle. In contrast, Chk2 responds primarily to DNA double-strand breaks in DNA. The Yaffe group and others have implicated a third kinase pathway, p38/MK2, downstream of ATM and ATR that elicits checkpoint arrest (Bulavin et al., 2001; Manke et al., 2005; Reinhardt et al., 2007). Adding to the intrigue, Chk1, Chk2, and MK2 share identical phosphorylation site preferences and target the same substrates in vitro (O'Neill et al., 2002). So what is the point of having these seemingly redundant checkpoint kinase pathways? This conundrum set the stage for the next chapter in the story.

In this issue, Yaffe, Reinhardt, and colleagues shed new mechanistic light on this element of checkpoint control. This elegant, comprehensive, and carefully controlled study demonstrates that Chk1 and MK2 perform different spatial and temporal roles in the establishment of the G_2/M checkpoint in p53-deficient cells. This work began with an examination of how each kinase drives cell cycle re-entry following checkpoint release. Upon depletion of Chk1 with shRNA in doxorubicin-treated cells, cells failed to establish a complete G_2 checkpoint. Conversely, depletion of MK2 led to disruption of long-term G_2 checkpoint maintenance. Based on these observations, the authors speculated that Chk1 was necessary for the initiation (and/or early maintenance) of the G_2 checkpoint, whereas MK2 is required to sustain this checkpoint at later times. The authors next asked whether these different temporal responses might be due to dynamic changes in subcellular localization of Chk1 or MK2. This turned out to be the case. Following doxorubicin treatment, GFP-tagged MK2 translocated from the nucleus to the cytoplasm while the nuclear localization of GFP-Chk1 was unchanged. These results were substantiated by other techniques including immunofluorescence detection of the endogenous enzymes and a series of convincing cell fractionation studies. Moreover, using a clever reciprocal chimera strategy, Reinhardt and colleagues demonstrated that the Chk1 kinase domain can compensate for the loss of MK2 if targeted to the proper cellular compartment or vice versa. The conclusion of these experiments was that Chk1 and MK2 regulate different temporal phases of checkpoint control through phosphorylation of spatially distinct substrates (Figure 1).

Previous studies have demonstrated that MK2 can stabilize mRNAs with AU-rich elements (AREs) in the 3′ untranslated region (UTR) (Gaestel, 2006; Janes et al., 2008; Neininger et al., 2002). Reinhardt and colleagues extended these findings by screening for molecules involved in cell-cycle regulation that contain 3′ AREs. This led to the identification of Gadd45α. DNA damage by doxorubicin led to the accumulation of Gadd45α mRNA, and this increase was blunted by shRNA depletion of MK2. Furthermore, loss of Gadd45α resulted in a checkpoint

maintenance failure tightly mirroring the effect seen with the loss of MK2. Subsequent searches for RNA-binding proteins (RBPs) that interact with the 3′UTR of Gadd45α led to the identification of hnRNP A0. The authors then linked posttranscriptional RNA regulation back to kinase signaling by demonstrating that MK2 can phosphorylate hnRNP A0, causing it to bind to the 3′UTR of Gadd45α mRNA stabilizing message levels and resulting in increased Gadd45α proteins levels. At the same time, MK2 phosphorylation of the ribonuclease PARN blocks Gadd45α mRNA degradation. Finally, this manuscript reveals a positive feedback loop where Gadd45α functions through p38/MK2 to maintain Cdc25B/C in the cytoplasm, effectively blocking mitotic entry prior to completion of DNA damage repair (Figure 1).

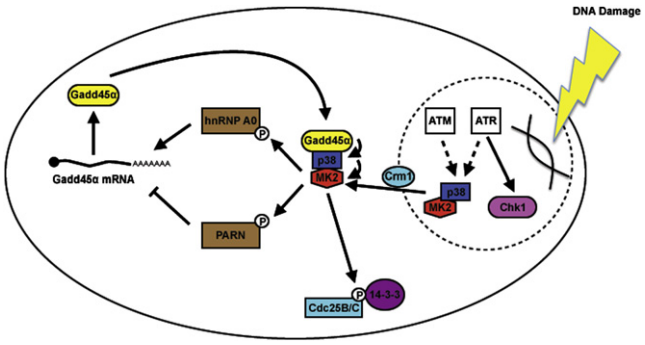

Figure 1. The Roles of Chk1 and MK2 in DNA Damage-Induced Checkpoint Arrest

In response to DNA damage, ATR and ATM signal through the p38/MK2 stress kinase cascade, leading to the rapid translocation of MK2 from the nucleus to the cytoplasm. MK2 then phosphorylates hnRNP A0, leading to stabilization of Gadd45α mRNA, at the same time preventing Gadd45α mRNA degradation by phosphorylating the ribonuclease PARN. Gadd45α protein can then participate in a positive feedback loop to p38/MK2, thereby preventing mitotic entry during DNA damage repair by retaining Cdc25B/C in the cytoplasm.

So what can we glean from this illuminative foray into how Chk1 and MK2 participate in checkpoint control? First and foremost, this work reveals that cells deficient in the tumor suppressor p53 contain two spatially distinct G_2/M phase checkpoint control kinase networks. This provides compelling evidence to support the notion that kinase targeting creates order out of chaos by clustering enzymes with their preferred substrates. Second, a combination of biochemical and genetic techniques show that local MK2-mediated phosphorylation of the targets involved in RNA regulation potentiates the response by stabilizing the Gadd45α mRNA. This not only uncovers a vital link between checkpoint control and gene expression but emphasizes how localized protein kinase activity can definitively act at the posttranscriptional level. Finally, this study illustrates how p53-deficient cells, or cells that lack a functional p53/p21 pathway, rewire their G_2/M checkpoint mechanisms to depend on Gadd45α/p38/MK2. This finding could have significant implications for understanding cell-cycle changes in cancer cells. The next chapter in this intriguing story could assess the differential impact that local and dynamic changes in Chk1 and MK2 activity exert on the posttranscriptional control and protein stability of addition target molecules. This may reveal further consequences of "Chk-ing in" and "Chk-ing out" of the nucleus.

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