Checkpoint recovery after DNA damage: a rolling stop for CDKs

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The cyclin-dependent kinases (CDKs) must be inhibited when DNA damage occurs to prevent cell-cycle progression and to allow for repair. A paper in the June issue of *EMBO reports* from Rene Medema's group now suggests that some CDK activity must actually be retained during arrest for efficient checkpoint recovery to occur (Alvarez-Fernández *et al*, 2010).

Cells have evolved several mechanisms to ensure accurate DNA replication and to prevent genomic instability and cancer. A crucial role is played by checkpoints, which sense DNA damage and subsequently inhibit cell-cycle progression. If the DNA damage can be repaired, cells will resume cell-cycle progression, a process known as checkpoint recovery. If the DNA damage cannot be repaired, the checkpoint activates programmes that result in permanent cell-cycle arrest, apoptosis or senescence.

CDKs drive cell division, and their tightly regulated expression, stability and activity is vital to cell-cycle progression. It is therefore not surprising that these kinase complexes are targets of the DNA-damage checkpoint. In G2 phase, the DNA-damage-mediated arrest of cell-cycle progression requires direct inhibition of CDK1–cyclin B, the CDK–cyclin complex that is required for mitotic entry (Linqvist *et al*, 2009). The regulation of CDK activity is controlled largely by inhibitory phosphorylation of the CDK subunit, which is carried out by the kinase Wee1. To activate CDK complexes, this phosphate group must be removed by CDC25 phosphatases. The G2 checkpoint initially establishes cell-cycle arrest by modulating this phosphorylation, both by degradation and inactivation of CDC25 and by activation of WEE1 (Fig 1; Bartek & Lukas, 2007).

It is clear from many studies that several pathways converge on inhibition of CDK activity following DNA-damage-mediated

checkpoint activation. However, an apparent paradox also arises as recent data suggests that active CDK–cyclin complexes might also have a role in directing the DNA-damage response (Wohlbold & Fisher, 2009). For instance, the resection step of double-stranded break repair by homologous recombination depends on CDK activity, which might help restrict the processing to S and G2 phases of the cell cycle when recombination is possible. This CDK dependence is at least partly due to regulation of CtIP, as CDK-mediated phosphorylation of CtIP is required for resection (Huertas & Jackson, 2009).

How then does one achieve the CDK activity needed for repair and other events, while also preventing cell-cycle progression? One possibility is that repair factors such as CtIP are activated immediately after DNA damage, before full inhibition of CDK activity. Initial phosphorylation could be sustained throughout the damage response, possibly through downregulation of other factors such as phosphatases. A second possibility is that proteins required for repair are constitutively phosphorylated in certain cell-cycle phases so that they are poised for repair when damage occurs. Interestingly, Alvarez-Fernández *et al* now provide evidence that a small fraction or subset of CDK complexes remain active after DNA damage, thereby revealing another mechanism by which CDK activity can exert control during the DNA-damage response.

Their paper focuses on FoxM1, a transcription factor that controls a subset of genes essential for the G2/M transition, including PLK1, cyclin A and cyclin B. In previous studies it was shown that cell-cycle-dependent phosphorylation of FoxM1 by CDK–cyclin A results in activation of FoxM1 (Laoukili *et al*, 2008). Intriguingly, Alvarez-Fernández *et al* now find that FoxM1 retains transcriptional activity after DNA-damage-induced G2

arrest. Furthermore, they show that reduction of FoxM1 protein levels, as well as inhibition of CDK activity, impedes checkpoint recovery from a DNA-damage-induced G2 arrest. These findings suggest strongly that there is a need for functional FoxM1 and some CDK activity during the DNA-damage response for recovery to occur. Indeed, the authors demonstrate that expression of a constitutively active FoxM1 mutant can partly overcome the reduced recovery competence when CDK activity is inhibited. Thus, Alvarez-Fernández *et al* show for the first time that residual CDK activity is required for efficient recovery from a DNA-damage-induced G2 arrest (Fig 1).

The findings of the Medema group fine-tune the generally accepted view of how the cell-cycle machinery is inhibited after genotoxic stress. It is not hard to imagine that recovery from a DNA-damage-induced cell-cycle arrest will be easier if some aspects of the cell-cycle machinery involved in the G2/M transition remain poised for action. However, a little danger comes from not 'braking' completely. So, how is it that danger is kept under control and how does the cell make sure it does not roll into mitosis before the damage is fixed? Interestingly, Alvarez-Fernández *et al* show that under conditions of genotoxic stress, FoxM1 transcriptional activity depends on cyclin A, whereas cyclin B is dispensable. Thus, one answer to this question might be that only the cyclin-A-bound CDKs have residual activity, whereas the cyclin-B-bound CDKs that are essential for entry into mitosis are more completely blocked. Differential regulation of cyclin-A- and cyclin-B-containing complexes could be accomplished by specific regulation of the CDK subunit, as cyclin A can interact with both CDK2 and CDK1, whereas cyclin B only forms an active complex with CDK1. Significantly,

Fig 1 | Model for regulation of cyclin-dependent kinase and FoxM1. Feedback cycles after (A) DNA damage, (B) checkpoint recovery and (C) recovery with reduced levels of CDK or FoxM1. See text for details. Arrow size represents strength of the signal. CDK, cyclin-dependent kinase; P, phosphate group.

WEE1-mediated phosphorylation of the CDK subunit seems to have a bigger role in inhibition of CDK1 than CDK2 (Chow *et al*, 2003). Thus, it is possible that CDK1 complexes are more strongly inhibited than are CDK2 complexes during checkpoint activation through WEE1 (Fig 1), although other regulators of CDK activities, such as MYT1, could also be involved.

Another interesting implication of the work by Alvarez-Fernández *et al* is that cyclin A might be required for recovery from a DNA-damage-induced G2 arrest. Cyclin A is required for FoxM1 activation, which regulates G2 target genes, so there is at least an indirect role for cyclin A in recovery. However, cyclin A is also needed for entry into mitosis during a normal cell cycle and might have a direct role in activation of CDK1–cyclin B. Although, the exact role of cyclin A in the induction of mitosis is not clear, a recent study suggests that cyclin A weakens the WEE1 negative feedback, thereby priming CDK1–cyclin B activity (Li *et al*, 2010). Thus, residual CDK–cyclin A activation might contribute to recovery not only by regulating FoxM1 but also by directly activating CDK1–cyclin B.

One outstanding question is whether the amount of DNA damage affects the level of CDK inhibition and whether this correlates with the efficiency of recovery. And is it possible that a higher dose of damage completely inhibits CDK activity and that this

triggers programmes in the cell that result in permanent growth arrest or cell death? There is some evidence that different levels of CDK activity are needed for different events in S phase. Activation of new replication clusters is more sensitive to CDK inhibition than is initiation of replication within a cluster (Thomson *et al*, 2010). Ultimately, more quantitative approaches are needed to dissect how different CDK levels and complexes act in normal cell-cycle progression and under conditions of genotoxic stress.

The work of Medema and colleagues introduces FoxM1 as a novel factor required for recovery from the G2 checkpoint. Interestingly, there might be ties between FoxM1 and other mediators of recovery. A central player in recovery from a G2 arrest is Plk1 kinase, which phosphorylates both WEE1 and claspin, a factor involved in checkpoint activation and CDC25 degradation (Bartek & Lukas, 2007). PLK1-mediated phosphorylation of both WEE1 and claspin during damage recovery results in their degradation, ultimately leading to reactivation of CDK complexes through removal of the inhibitory CDK phosphorylation. Interestingly, PLK1 also regulates FoxM1 in normal cell cycles, promoting entry into mitosis through regulation of FoxM1 transcripts, and positive feedback in this process leads to further upregulation of PLK1 (Fu *et al*, 2008). How PLK1 is activated after DNA repair remains enigmatic, and whether

the links between PLK1 and FoxM1 are maintained under these conditions is not clear. Because PLK1-mediated phosphorylation of FoxM1 requires initial priming by CDK activity, this could be a key event regulated by the low levels of CDK activity now known to be needed for checkpoint recovery.

The many feedback loops and pathways that regulate mitotic entry are only starting to be unravelled, and the work of Medema and colleagues makes it clear that DNA-damage-induced events add another level of complexity to this network.

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