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Editorial

Bim vanishes in the light of a mitotic Aurora

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Mitosis is biochemically peculiar, as in this phase of the cell cycle a large fraction of the proteome appears modified by CDK1 (Cyclin dependent kinase 1)-dependent phosphorylation.[1] Many of the mitotic phosphoproteins are direct CDK1 targets, but various other mitotic kinases act in concert with CDK1, altering protein function and enforcing changes in cell physiology that are instrumental to achieve faithful partitioning of cellular components, most prominently chromosomes.

Not surprisingly, the apoptotic machinery is also regulated in a CDK1-dependent fashion in mitosis at several layers: most interestingly, phosphorylation of the prosurvival Bcl-2 family members facilitates apoptosis either by direct inhibition of protein function (as shown for Bcl-xL and Bcl-2 itself[2–4]) or via phosphorylation-dependent proteasomal degradation (as documented for Mcl-1[5,6]). Such findings help to explain how temporal extension of mitosis can be proapoptotic itself, regardless of the actual trigger (see Topham and Taylor,[7] for a recent review). In stark contrast, direct CDK1 phosphorylation of initiator Caspases inhibits their ability to autoprocess, believed to result in apoptosis inhibition.[8–10] Collectively, these findings assign CDK1 as the designated driver in this 'Gas & Brake' model and highlight a complex interplay between mitotic arrest and the cell death machinery (Figure 1a). So far, however, the mitotic regulation of the upstream antagonists of Bcl-2 and its prosurvival homologs, that is, the 'BH3-only' proteins, remained enigmatic.

In a paper by Moustafa-Kamal and colleagues,[11] in a recent issue of *Cell Death and Differentiation*, the mitotic regulation of the BH3-only protein Bim, an initiator of apoptosis in response to numerous apoptotic stimuli, is investigated. Previous work had already highlighted that Bim is a mitotic phosphoprotein, but the relevant kinase(s) and the functional impact of the phosphorylation events remained a matter of debate.[12–17] By using two different synchronization approaches, Moustafa-Kamal and colleagues[11] show that the longest and most abundant splice variant of Bim (BimEL) is not only phosphorylated, but also undergoes proteasomal degradation during normal mitosis. As inhibition of the proteasome in mitosis stabilizes the phosphorylated form of BimEL, the authors hypothesize that phosphorylation and proteolysis of BimEL in mitosis might be coupled.

Using two different phospho-specific antibodies for known Bim phospho-sites that impact on its stability, the authors show that both the P-S69 and P-S93/94/98 phospho-epitopes become more abundant in mitosis and decline upon mitotic exit. Forcing a sudden mitotic exit by inhibiting CDK1 in prometaphase induced rapid dephosphorylation of the two sites in BimEL and this correlated with increased protein stability, suggesting that coupled phosphorylation/proteolysis occurs only in mitosis. Conversely, extending the mitotic duration by interfering with the functionality of the mitotic spindle using the microtubule-stabilizing agent Taxol, promoted sustained BimEL phosphorylation at S93/94/98, S69 and BimEL degradation.

The authors went on to show that Bim is a target of polyubiquitination in mitosis and that such modification only affects the longest splice variant, BimEL, which goes well along with the observation that interfering with phosphorylation of S94/98 (exclusively present in BimEL) by mutation to alanine abolished the degradation, whereas avoiding phosphorylation of S69 and other described CDK1 target sites across Bim had minor or no impact on Bim mitotic stability.

BimEL is a substrate of the phosphorylation-dependent E3 Ubiquitin ligase SCF-βTrCP1 in response to phosphorylation of S93/94/98.[18] Furthermore, Wee1, also a substrate of SCF-βTrCP1, displayed similar decay kinetics to BimEL in synchronized cells. Therefore, the authors set out to look for the impact of βTrCP1 knockdown on BimEL abundance that appeared readily increased. The synchronization of cells in late G2 by a reversible CDK1 inhibitor revealed that the binding of βTrCP1 to BimEL was absent in G2, but increased sharply as soon as 30 min after inhibitor wash out. Binding not only correlated with S93/94/98 phosphorylation but mutation of S94/98 to alanine abolished it, showing that mitotic phosphorylation of S94/98 is necessary for βTrCP1 binding to BimEL. Using okadaic acid and specific RNAi knockdowns, the authors also show that BimEL phosphorylation of S94/98 (and subsequent degradation) are counteracted by protein phosphatase 2A (PP2A).

Of note, S94 matches the phosphorylation consensus motif of Aurora kinases, having Aurora A and B as major players with non-redundant functions. Dissecting which kinase is required for the modification of the phosphodegron proved to be difficult: although the evidence provided argues for a crucial contribution of Aurora A, it remains possible that Aurora B might also play a role. Although the use of an Aurora A-specific inhibitor as well as Aurora A siRNAs reduced the phoshorylation of S93/94/98 and led to BimEL stabilization, it also simultaneously impacted on the mitotic index of the cell population, as judged by Cdc27 phospho-shift

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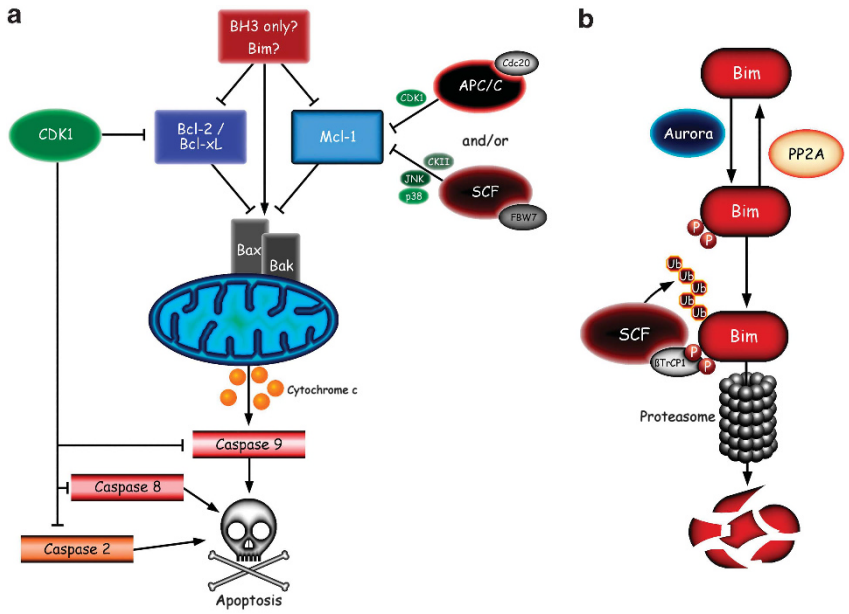

Figure 1 Modulation of mitochondrial apoptosis in mitosis. (**a**) Central to mitochondrial apoptosis is the permeabilization of mitochondrial outer membrane by Bax and/or Bak multimers, leading to Cytochrome *c* release in the cytoplasm and subsequent activation of Caspase-9. This process is on one hand inhibited in mitosis by CDK1 (by direct phosphorylation of Caspase-2/8/9) and on the other hand promoted by inhibition of 'Bax/Bak inhibitors', that is, Bcl-2, Bcl-xL and Mcl-1. This latter phenomenon can occur either by direct inactivation of Bcl-2 and Bcl-xL by phosphorylation or by phosphorylation-dependent degradation of Mcl-1. Such degradation can have CDK1 and/or CKII/JNK/p38 as priming kinases and the APC/C-Cdc20 and/or SCF-FBW7 as phosphorylation-dependent Ubiquitin ligases, respectively. (**b**) Aurora A (and/or possibly Aurora B) kinase can reversibly phosphorylate Bim in mitosis and this modification is counteracted by the action of PP2A phosphatase. Phosphorylated Bim at Ser94/98 is selectively bound by SCF-βTrCP1, polyubiquitinated and degraded via the proteasome. Although the role of Bim in mitotic death remains to be fully understood, its regulation at the level of protein stability reveals how Aurora inhibitors might be used to promote cell death in mitosis

(a readout for CDK1 activity), confounding the analysis. Aurora kinases also share the consensus motif,[19] suggesting that both Aurora A and B might be able to phosphorylate the same substrate *in vitro*, leaving open the possibility that Aurora B might also serve as a kinase for the phosphodegron on BimEL.

Regardless of these issues, long-term inhibition of Aurora A appears to be proapoptotic in a Bim-dependent fashion, suggesting that lack of mitotic degradation of BimEL in an aberrant mitosis tips the balance of pro- and anti apoptotic signals toward cell death.

In summary, this paper convincingly shows that BimEL is a target for Aurora kinases in mitosis, a crucial event for inducing the recognition of a phosphodegron by SCF-βTrCP1, polyubiquitination and subsequent proteasomal degradation of BimEL (Figure 1b). This work thus provides the first solid evidence for a prosurvival impact of extended mitotic arrest at the BH3-only protein level. Inhibition of Aurora kinases during mitotic arrest has the potential to rewire the apoptotic circuitry, favoring cell death over cell survival in mitosis. It will be interesting to define whether the increased BimEL activity exerts its proapoptotic function by neutralizing residually active Bcl-2 prosurvival molecules or as a direct activator of Bax/Bak.

Conflict of Interest

The authors declare no conflict of interest.

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1. Dephoure N *et al. Proc Natl Acad Sci USA* 2008; **105**: 10762–10767.
2. Terrano DT, Upreti M, Chambers TC. *Mol Cell Biol* 2010; **30**: 640–656.
3. Upreti M *et al. J Biol Chem* 2008; **283**: 35517–35525.
4. Vantieghem A *et al. J Biol Chem* 2002; **277**: 37718–37731.
5. Harley ME *et al. EMBO J* 2010; **29**: 2407–2420.
6. Wertz IE *et al. Nature* 2011; **471**: 110–114.
7. Topham CH, Taylor SS. *Curr Opin Cell Biol* 2013; **S0955-0674**: 00117–00118.
8. Allan LA, Clarke PR. *Mol Cell* 2007; **26**: 301–310.
9. Andersen JL *et al. EMBO J* 2009; **28**: 3216–3227.
10. Matthess Y *et al. Mol Cell Biol* 2010; **30**: 5726–5740.
11. Moustafa-Kamal M *et al. Cell Death Differ* 2013; **20**: 1393–1403.
12. Czernick M, Rieger A, Goping IS. *Biochem Biophys Res Commun* 2009; **379**: 145–150.
13. Gilley R *et al. Cell Signal* 2012; **24**: 170–180.
14. Girio A *et al. Cell Signal* 2007; **19**: 1964–1972.
15. Graos M, Almeida AD, Chatterjee S. *Biochem J* 2005; **388**(Pt 1): 185–194.
16. Kutuk O, Letai A. *Cell Death Differ* 2010; **17**: 1624–1635.
17. Mac Fhearraigh S, Mc Gee MM. *Cell Cycle* 2011; **10**: 3886–3896.
18. Dehan E *et al. Mol Cell* 2009; **33**: 109–116.
19. Alexander J *et al. Sci Signal* 2011; **4**: ra42.