Text by Ben Short
bshort@rockefeller.edu

PP6 puts the brakes on spindle assembly

The phosphatase limits Aurora A's kinase activity during mitosis.

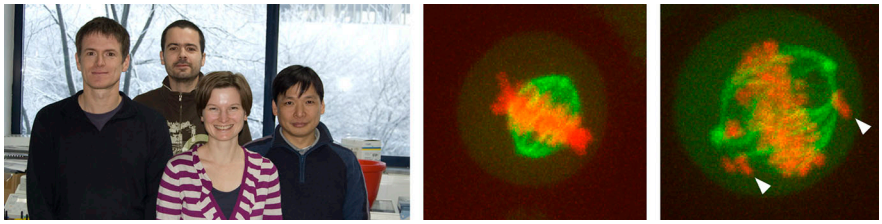
Different protein kinases control the assembly and function of the mitotic spindle. Although much is known about how these kinases are activated, what switches them off is less well understood. Zeng et al. now reveal that protein phosphatase 6 (PP6) inactivates the mitotic kinase Aurora A to achieve the precise level of activity required for accurate chromosome segregation (1).

"Kinases are often thought of as being switched on when cells enter mitosis and switched off at the end of cell division," says Ulrike Gruneberg from the University of Liverpool in the UK. "But their regulation is probably more dynamic than that."

Many kinases are turned on by binding to specific activator proteins, which promote the phosphorylation of a threonine residue in a regulatory domain of the kinase called the T-loop (2). Little is known, however, about the phosphatases that reverse this activation step. "That's partly because many people think phosphatases are boring," explains Francis Barr, also from the University of Liverpool. "It's like with cars: people will talk about how big the engine is but won't discuss how powerful the brakes are."

Barr and Gruneberg—along with their colleagues Kang Zeng and Ricardo Nunes Bastos—therefore set out to identify phosphatases that regulate mitosis by inactivating mitotic kinases. Their initial approach was simple: the researchers knocked down individual phosphatases by RNAi and looked for cells with abnormally shaped nuclei that arose from defective mitoses. The strongest effect was seen upon depletion of PP6, a phosphatase complex previously implicated in NFκB signaling and DNA damage repair (3, 4). Cells lacking PP6 formed disorganized mitotic spindles and aligned their chromosomes poorly at the metaphase plate, resulting in frequent mis-segregations. "That told us that there was a defect in spindle formation," says Barr, "which suggested that PP6 might dephosphorylate either Plk1 or Aurora A, [two kinases that are critical to mitotic spindle assembly]."

Focal Point



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(Left to right) Francis Barr, Ricardo Nunes Bastos, Ulrike Gruneberg, and Kang Zeng find that the phosphatase PP6 regulates mitotic spindle assembly by limiting the kinase activity of Aurora A. Cells lacking PP6 (far right) show disorganized spindles (green) and misaligned chromosomes (red) due to unchecked Aurora A activity, which results in chromosome mis-segregation.

Zeng et al. found that, in the absence of PP6, Aurora A retained its T-loop phosphorylation and remained associated with its activating protein TPX2. This suggested that the mitotic defects associated with PP6 depletion might be due to Aurora A hyperactivation. Indeed, treating cells with an Aurora A inhibitor reduced the number of abnormal nuclei induced by PP6 knockdown.

In vitro experiments revealed that PP6 dephosphorylates Aurora A bound to TPX2. "That's the physiologically active form of Aurora A," says Gruneberg, which means that PP6 modulates an active pool of the kinase, rather than simply maintaining Aurora A in an inactive state.

"The level of Aurora A activity is critical for spindle assembly," Barr says. "The balance of TPX2 and PP6 produces the right amount of activity for the cell." In combination with the Ran GTPase, Aurora A controls spindle assembly by regulating downstream spindle assembly factors such as NuMA and the kinesin motor Eg5 (5). In the absence of PP6, neither of these factors was strongly recruited to spindle poles, but their localization was restored by the addition of an Aurora A inhibitor.

Excess Aurora A activity resulting from gene amplification is associated with genomic instability and tumorigenesis. "Mutations in PP6 would also amplify Aurora A activity, so it would be interesting to look at the status of PP6 in different cancers," says Gruneberg. Indeed, a melanoma sample with a homozygous mutation in the PP6 catalytic site has previously been identified (6). On the other hand, PP6 might be an Achilles heel for tumors that already have elevated Aurora A activity. Although genomic instability provides adaptive advantages to tumors, too many mitotic defects can be catastrophic. "It's like a speeding car with no brakes," says Barr. "It's going to crash and burn. Similarly, if we take away PP6 from cells with amplified Aurora A, they go straight into apoptosis." PP6 may therefore represent a new therapeutic target for tumors with amplified Aurora A.

> **"The balance of TPX2 and PP6 produces the right amount of [Aurora A] activity."**

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