PP1 inactivates Greatwall to release PP2A-B55 from mitotic confinement

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Entry into and exit from mitosis are brought about by the increase and decrease, respectively, in the activity of cyclin-dependent kinases (CDKs). Many examples are known of how the properties of particular proteins can be altered by phosphorylation, promoting processes like nuclear envelope breakdown or assembly of the mitotic spindle. The regulation of protein phosphatases is shedding new light on how this quantitative change of protein phosphorylation is achieved by a tight linkage between CDK activity and CDK-antagonizing phosphatases. On entering mitosis, increasing CDK activity ignites a repressive pathway that acts on PP2A-B55, one of the major phosphatases for CDK substrates in higher eukaryotes. This repression allows rapid and near complete substrate phosphorylation. But this raises a serious bootstrapping problem at mitotic exit. Because the phosphatase responsible for CDK substrates has been shut off, how can the repression pathway, which was activated by CDK, be reversed? In the current issue, Heim and colleagues propose an answer to this question [1]. Their data show that dephosphorylation of Greatwall kinase (Gwl) at its auto-phosphorylation site(s) is targeted by PP1, which leads to significant decrease in Gwl kinase activity. This early action by PP1 seems to be a prerequisite for PP2A-B55 to escape from repression and to return Gwl back to its inactive hypophosphorylated interphase state. This study provides an important piece of evidence for how the repression mechanism of PP2A-B55 is made reversible, and offers a solution to the bootstrap problem.

See also: A Heim *et al* (November 2015)

Quantitative phosphorylation of CDK substrates is the biochemical basis of mitosis. After the discovery of CDK, its regulation by union with a cyclin subunit and tyrosine-15 phosphorylation has been extensively studied. But even the full activation of CDK (10 times or so, [2]) cannot solely explain the switch-like change of phosphorylation level of CDK substrates on mitotic entry. (Think about simple equilibrium. As protein phosphorylation increases, the dephosphorylation reactions speed up and the level of phosphorylation reaches a plateau.) Advances in understanding the regulation of protein phosphatases confirm this issue. In budding yeast, for example, Cdc14 (the main phosphatase for CDK substrates) is repressed during mitosis by nucleolar confinement. In higher eukaryotes, PP2A-B55 has been reported to dephosphorylate a subset of CDK substrates. PP2A-B55 activity is repressed on entering mitosis and reactivated after cyclin destruction. Such a combination of CDK activation together with repression of antagonizing phosphatases can well account for the rather complete switch in the phosphorylation level of CDK substrates.

Repression of PP2A-B55 is achieved by a sequence of four distinct steps (Fig 1) [3]: (1) CDK phosphorylates Gwl; (2) CDK-phosphorylated Gwl phosphorylates itself (auto-phosphorylation) for its full activation [4,5]; (3) Fully active Gwl phosphorylates ARPP-19 (ARPP) and/or α -endosulfine (ENSA); (4) Phosphorylated ARPP/ENSA binds to and inhibit PP2A-B55. As a result, the activity of PP2A-B55 is reduced by 10 times or more. How does PP2A-B55 get reactivated upon

the return to interphase? Since its repression procedure is as described above, we would expect the following events to occur as cells exit mitosis; (5) CDK is inactivated by cyclin destruction; (6) Gwl is dephosphorylated at its autophosphorylation site(s) and inactivated; (7) ARPP/ENSA are dephosphorylated, resulting in reactivation of PP2A-B55; (8) Gwl is dephosphorylated at its CDK sites.

Steps 6, 7, and 8 present a problem, of course, depending on which protein phosphatase(s) is responsible for dephosphorylating Gwl and ARPP/ENSA. Since PP2A-B55 dephosphorylates CDK substrates, it has been suggested that the CDK sites on Gwl are dephosphorylated by PP2A-B55 (step 8) [6]. Williams and colleagues showed that ARPP/ENSA are substrates as well as inhibitors of PP2A-B55 (step 7) [7]. So the remaining question is which enzyme acts on the autophosphorylation (and activating) site(s) of Gwl, whose importance was predicted by a mathematical modeling [8]. Step 6 should come prior to steps 7 and 8, because as long as Gwl remains active, ARPP/ENSA will be rephosphorylated faster than their dephosphorylation by PP2A-B55, and PP2A-B55 can only target Gwl after ARPP/ENSA dephosphorylation is complete. There is, in short, a bootstrapping problem.

In this issue, Heim and his colleagues report that protein phosphatase 1 (PP1) holds the key to reactivation of PP2A-B55 [1]. They first noticed that, even in the absence of PP2A-B55 activity, Gwl is half-dephosphorylated and almost inactivated when CDK is suppressed. This half-dephosphorylated Gwl returned to the fully phosphorylated and active form if PP1 was inhibited, although CDK was still inactive,

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Figure 1. Repression (left) and reactivation (right) sequences of PP2A-B55.

There are three kinds of phosphorylation to repress PP2A-B55: Gwl phosphorylation by CDK (orange), Gwl auto-phosphorylation (yellow) and ARPP/ENSA phosphorylation by Gwl (blue). On mitotic exit, all these phosphorylations should be removed with the highest priority for Gwl auto-phosphorylation site (step 6), to reactivate PP2A-B55. Because, as long as Gwl is fully active, PP2A-B55 cannot escape from the repression by ARPP/ENSA. Although PP1 is also repressed by CDK (green), PP1 reactivation is auto-catalyzed, allowing PP1 to target Gwl auto-phosphorylation site(s) on mitotic exit.

suggesting that PP1 dephosphorylates the auto-phosphorylation site(s) of Gwl, which is critical for the full activity of Gwl. Another well-controlled experiment showed that PP1 activity was essential only at a very early stage of Gwl dephosphorylation and inactivation. All these data support the idea that the Gwl-ARPP/ENSA pathway, once it is activated, cannot be overridden by repressed (ENSA-inhibited) PP2A-B55. Most likely, the Gwl auto-phosphorylation site(s) is a very poor substrate for PP2A-B55, leading to the requirement of PP1 for timely mitotic exit.

It has long been known that PP1 activity is repressed by phosphorylation of its C-terminus by CDK1 and that PP1 can auto-dephosphorylate this site and reactivate itself once CDK is inactivated [3]. Hence, auto-reactivation of PP1 would be the first event (Fig 1, between step 5 and 6) to initiate the reactivation of PP2A-B55 after CDK inactivation.

Thus, PP1 comes into sharp focus, but as usual, questions remain. PP1 has a long list of regulatory partners, making a variety of different holocomplexes with distinct functions [9]. So identification of the PP1

regulatory subunit(s) for Gwl inactivation is very important for cell cycle control. This kinase-phosphatase balancing mechanism composed of Gwl, ARPP/ENSA, PP2A-B55 and PP1 is probably broadly utilized as a functional unit, although a partner kinase, corresponding to CDK, might be different in each case, because PP2A-B55 plays versatile roles in many other biological phenomena, where mitotic CDK not believed to be operating (for example, in the nutrient response in budding yeast [10]). We see this as a useful plug-in module whenever switch-like changes in phosphorylation states are required in Nature.

References

1. Heim A, Konietzny A, Mayer TU (2015) *EMBO Rep* 16: 1501–1510
2. Fang F, Newport JW (1991) *Cell* 66: 731–742
3. Wurzenberger C, Gerlich DW (2011) *Nat Rev Mol Cell Biol* 12: 469–482
4. Blake-Hodek KA, Williams BC, Zhao Y et al (2012) *Mol Cell Biol* 32: 1337–1353
5. Vigneron S, Gharbi-Ayachi A, Raymond AA et al (2011) *Mol Cell Biol* 31: 2262–2275
6. Mochida S, Hunt T (2007) *Nature* 449: 336–340
7. Hegarat N, Vesely C, Vinod PK et al (2014) *PLoS Genet* 10: e1004004
8. Williams BC, Filter JJ, Blake-Hodek KA et al (2014) *Elife* 3: e01695
9. Vinod PK, Novak B (2015) *FEBS Lett* 589: 667–671
10. Bontron S, Jaquenoud M, Vaga S et al (2013) *Cell Rep* 3: 16–22