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CELL CYCLE

# It takes three to find the exit

**Mitotic cell division separates chromosome pairs into two genetically identical daughter cells. A study in fission yeast reveals that this separation is guided by the sequential activation of three phosphatase enzymes.**

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The ultimate aim of most cells is to become two cells[1], through a cell-division process that occurs following phases of cell growth and chromosome duplication. Cell division requires the ordered assembly, disassembly and reorganization of numerous cellular components. These structural rearrangements are coordinated by kinase and phosphatase enzymes, which regulate proteins by attaching phosphate molecules or removing them, respectively. Many proteins are phosphorylated during early cell division, and most are dephosphorylated again at the exit from division, but it is unclear how the phosphatases involved in this process are regulated. In a paper published on *Nature*'s website today, Grallert *et al.*[2] show that the end of cell division in fission yeast (*Schizosaccharomyces pombe*) is driven by three phosphatases that are organized in a relay, whereby one enzyme sequentially activates another.

During the first part of cell division, called mitosis, chromosomes from each chromosome pair are pulled towards opposite poles of the cell, culminating in the formation of two separate nuclei. The ensuing cytoplasmic division generates two daughter cells. Work over the past two decades[3] has revealed that, in budding yeast, the phosphatase Cdc14 opposes the activity of Cdk1 kinase, the master regulator of mitosis. However, this mitotic function of Cdc14 is not evolutionarily conserved in fission yeast and animals. Instead, complexes of protein phosphatases 1 (PP1) and 2A (PP2A) have emerged as key regulators of the exit from mitosis in these organisms[3].

Grallert and colleagues report that PP1 and PP2A are largely inactive at the beginning of mitosis in fission yeast. PP1 inactivity comes about because it is phosphorylated by Cdk1, which not only reduces the phosphatase's activity, but also promotes its degradation. The authors did not investigate the mechanism underlying the low PP2A activity in early mitosis. However, they did examine how these phosphatases are activated at mitotic exit, and uncovered an unexpected regulatory cascade (Fig. 1).

PP2A complexes comprise a catalytic and scaffolding subunit and a regulatory B subunit. The researchers discovered unanticipated docking sites for PP1 on two PP2A complexes that have structurally unrelated B subunits — B55 and B56. They found that, just before the start of chromosome separation, when Cdk1 becomes inactive, PP2A-B55-associated PP1 removes its own inhibitory phosphate group and then activates and releases the PP2A-B55 to which it is bound. Subsequently, PP2A-B55 removes a phosphate group from the PP1-binding site on PP2A-B56. The efficient dephosphorylation of this site is delayed, however, because PP2A-B55 activity is counteracted by an opposing kinase, Plk1. When Plk1 is inactivated in late mitosis, the B56 subunit is efficiently dephosphorylated and PP1 binds, resulting in the activation of PP2A-B56 and its release from PP1. Grallert and co-workers provide evidence that interference with this regulatory cascade causes chromosome-segregation defects, highlighting its importance for cell division.

These findings raise exciting questions. What are the mechanisms by which the relay-controlled pool of PP2A-B55 and PP2A-B56 is inactivated during early mitosis, and exactly how are these complexes activated in late mitosis? Because PP1 is implicated in the activation process, it seems likely that these mitotic PP2A complexes are activated by dephosphorylation of their B subunits[4], but the authors did not define the sites at which these modifications occur. Studies in other model organisms[5] indicate that mitotic PP2A can be restrained by inhibitory proteins that are removed by dephosphorylation at mitotic exit, but it is not known whether this applies to fission yeast.

How can the B55 and B56 subunits form a complex with PP1, given that their PP1-binding sites are buried deep in the hydrophobic core of well-folded protein domains[6]? With the structural data available[6], it is difficult to envisage how this can be achieved without disrupting the folding of the B subunits. The authors speculate that B55 and B56 can adopt alternative conformations that expose their PP1-binding sites. Some proteins are indeed known to undergo substantial conformational changes following ligand binding[7,8], but it is unclear whether this also applies to the PP1–PP2A-B55 and PP1–PP2A-B56 interactions. Furthermore, given the strong associations between PP1 and the PP2A complexes, it is somewhat counterintuitive that

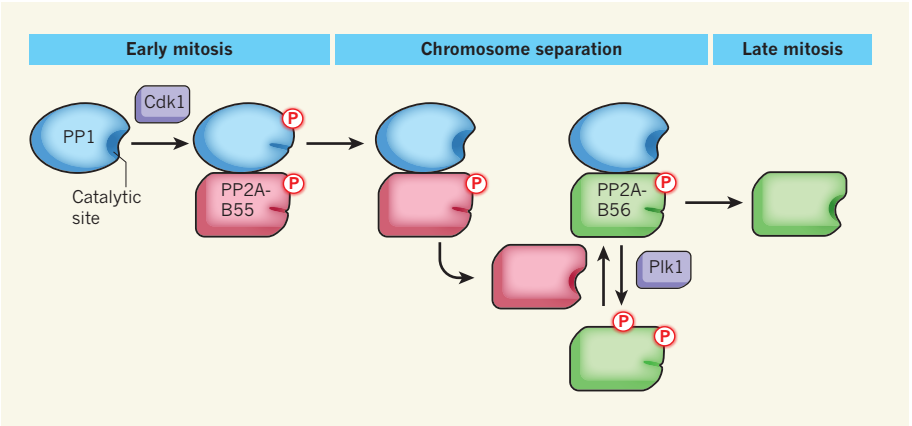


**Figure 1 | A mitotic enzyme relay.** Grallert *et al.*[2] report that during early mitosis, the kinase enzyme Cdk1 adds a phosphate group (P) to PP1, a phosphatase enzyme, thereby inactivating it. Active and inactive phosphatases are indicated by an open (half circle) or closed catalytic site, respectively. Cdk1 is inactivated just before the beginning of chromosome separation, causing PP1 that is bound by another phosphatase, PP2A-B55, to dephosphorylate and activate both itself and the bound PP2A-B55. Subsequently, PP2A-B55 dephosphorylates an amino-acid residue in the B56 subunit of PP2A-B56 phosphatase. This dephosphorylation is inefficient during chromosome separation, because Plk1 kinase opposes the action of PP2A-B55. At the end of chromosome separation, Plk1 is inactivated, and the resulting efficient dephosphorylation of B56 enables this subunit to recruit PP1, which subsequently activates PP2A-B56. Activation of the PP2A phosphatases results in their dissociation from PP1.

PP2A activation coincides with dissociation from PP1. How is this achieved? One possibility is that PP1 release occurs because of a reversal of the conformational change in B55 or B56, burying the PP1-binding sites in the hydrophobic core once again.

The elegance of the reported phosphatase relay is that it functions as a timer for the orderly dephosphorylation of proteins at the end of division. The cascade may be evolutionarily conserved, because Grallert and colleagues demonstrated that PP1 and PP2A-B56 can also interact in human cells. The relay affects only part of the cellular pool of PP1 and PP2A, explaining why other sources of these phosphatases fulfil independent functions throughout cell division[5]. In fact, recent data[9] hint at the existence of another relay system, which controls a cell-division step that occurs before chromosome separation, and which involves the recruitment of a chromosome-associated pool of PP1 by PP2A-B56. In animals, an additional layer of regulation of phosphatase relays may come from 'scaffolding' proteins that bind and coordinate local pools of both PP1 and PP2A[5]. Overall, this research implies that although budding yeast and other organisms, including fission yeast, employ distinct phosphatases to coordinate the mitotic exit, control of this process in the different organisms shares a similar precision. ■

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