

Cyclin B–Cdk1 activates its own pump to get into the nucleus

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The transition to mitosis requires extensive nuclear and cytoplasmic rearrangements that must be spatially and temporally coordinated. In this issue, Gavet and Pines (2010a. *J. Cell Biol.* doi:10.1083/jcb.200909144) report on a simple yet elegant mechanism as to how this is achieved. By monitoring the activity of cyclin B–Cdk1 in real time, the authors show that concomitant with its activation in the cytoplasm, the kinase complex is rapidly imported into the nucleus by modifying the activity of the nucleocytoplasmic transport machinery. Thus, cyclin B–Cdk1 activates its own pump to get into the nucleus.

Cdk1 in complex with cyclin B phosphorylates hundreds of target proteins to promote the transition from interphase to mitosis. The cyclin B–Cdk1-mediated phosphorylations lead to dramatic cellular rearrangements, including the assembly of a mitotic spindle to enable equal separation of the genetic material to two daughter cells. The assembly of a mitotic spindle requires strict coordination of cytoplasmic and nuclear events: cytoplasmic microtubules and nuclear chromatin both need to be reorganized before nuclear envelope breakdown (NEBD) to ensure efficient capture of chromosomes by microtubules once the nuclear envelope breaks down (Fig. 1). Because cyclin B–Cdk1 is responsible for these reorganizations, the mechanism by which its activity is regulated spatially and temporally has been the subject of intense investigation for two decades.

Although it shuttles back and forth between the nucleus and cytoplasm, cyclin B–Cdk1 is mainly localized to the cytoplasm in interphase. During prophase, cyclin B–Cdk1 translocates to the nucleus, but exactly how the nuclear translocation is regulated and how it correlates to activation of cyclin B–Cdk1 has remained unclear. Based in part on circumstantial evidence, a model has emerged in which cyclin B–Cdk1 is first activated in the cytoplasm, presumably on centrosomes. The prevailing model suggests that phosphorylation of cyclin B then regulates nuclear translocation by altering its interactions with import and export factors (Li et al., 1997; Hagting et al., 1999). Considerable efforts have been devoted to identify the phosphorylation sites on cyclin B responsible for nuclear translocation and to find the kinases that control phosphorylation of these sites.

Specifically, Polo-like kinase 1 (Plk1) has been suggested to promote nuclear translocation of cyclin B–Cdk1 by blocking nuclear export, but whether Plk1 phosphorylates a residue that potentially can block nuclear export or a neighboring residue has remained controversial (Toyoshima-Morimoto et al., 2001; Yuan et al., 2002; Jackman et al., 2003).

In this issue, Gavet and Pines use a novel biosensor that is specifically phosphorylated by cyclin B–Cdk1 to simultaneously quantitate the kinase activity as well as its subcellular distribution pattern as the cell progresses into mitosis. The biosensor consists of two fluorophores connected by a sequence that can be phosphorylated by cyclin B–Cdk1 and a phospho-binding domain. Upon phosphorylation by cyclin B–Cdk1, the phospho-binding domain binds to the phosphorylated sequence, which brings the two fluorophores closer to each other. The decreased distance between the fluorophores results in an increased efficiency of Förster resonance energy transfer (FRET), which can be used as readout for when the biosensor becomes phosphorylated. Gavet and Pines (2010a) monitored the FRET efficiency of the biosensor in live cells and found that it was phosphorylated with similar kinetics in both the nucleus and the cytoplasm during mitotic entry. This is a surprising result

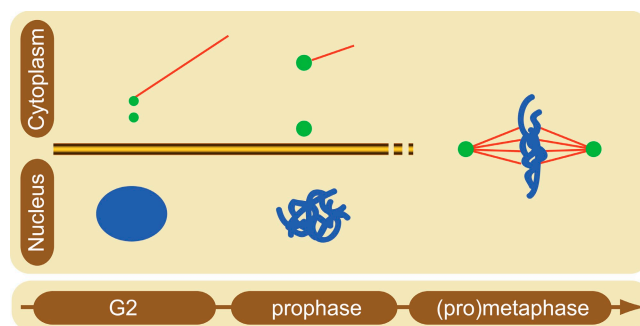


Figure 1. The coordination problem. Condensation of nuclear chromatin (blue), maturation and separation of cytoplasmic centrosomes (green), and altered dynamics of cytoplasmic microtubules (red) need to be synchronized in prophase to ensure timely assembly of a bipolar mitotic spindle once the nuclear envelope (yellow) breaks down.

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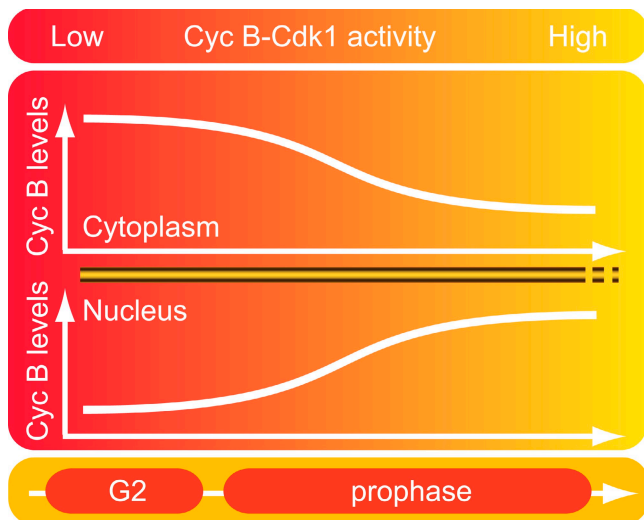


Figure 2. **Cyclin B–Cdk1 activity is similar in the nucleus and in the cytoplasm.** The kinetics of phosphorylation of a nuclear and a cytoplasmic cyclin B–Cdk1 target (color gradient) is similar despite large differences in the nuclear and cytoplasmic concentrations of cyclin B–Cdk1 (white lines) during mitotic entry.

because the majority of cyclin B–Cdk1 resides in the cytoplasm when its activation starts in late G2, and because of nuclear translocation, a large part of cyclin B–Cdk1 resides in the nucleus when the activation continues in late prophase (Fig. 2).

Apparently, there must be a mechanism that ensures a distribution of active cyclin B–Cdk1 between the nucleus and the cytoplasm. In search for such a mechanism, Gavet and Pines (2010a) revisited the regulation of cyclin B–Cdk1 nuclear translocation in prophase and found that nuclear translocation depends on a dramatic increase in nuclear import of cyclin B rather than a decrease in nuclear export. Moreover, Plk1, which has been a prime suspect of inhibiting cyclin B nuclear export in prophase (Toyoshima-Morimoto et al., 2001), does not affect the rate of cyclin B–Cdk1 nuclear translocation. In fact, cyclin B–Cdk1 nuclear translocation in prophase cannot be explained by phosphorylation of the previously identified residues in cyclin B (Li et al., 1997; Hagting et al., 1999), although these sites do determine the apparent localization of cyclin B throughout interphase by regulation of cyclin B nucleocytoplasmic shuttling. Rather, Gavet and Pines (2010a) found that cyclin B–Cdk1 activity itself directly regulates nuclear translocation by dramatically increasing its own nuclear import.

This finding has three important implications for the regulation of mitotic entry. First, as the translocation depends on increased nuclear import rather than decreased nuclear export of active cyclin B–Cdk1, this strongly suggests that cyclin B–Cdk1 is first activated in the cytoplasm. Cytoplasmic activation is also supported by the findings that autophosphorylated cyclin B–Cdk1 can first be detected on centrosomes and that cyclin B–Cdk1 activation depends on a high local concentration of cyclin B–Cdk1 complexes, which is present on centrosomes in G2 (Solomon et al., 1990; Jackman et al., 2003). Second, it is not possible to restrict the active pool of cyclin B–Cdk1 to the cytoplasm because active cyclin B–Cdk1 will immediately promote its own translocation. Thus, either the entire cell enters mitosis

or the entire cell does not enter mitosis, as the cytoplasm will not convert to a mitotic state on its own. This finding both couples back and gives a new meaning to the classic study “Cytoplasmic control of nuclear behavior...” by Masui and Markert (1971) in which cytosol from a metaphase oocyte was shown to induce metaphase when injected into the cytoplasm of an interphase oocyte. Third, coupling cyclin B–Cdk1 activity to its own nuclear import is an elegant mechanism to make sure that once activated, active cyclin B–Cdk1 will be present throughout the cell. This provides spatial coordination of nuclear and cytoplasmic rearrangements during early mitosis to ensure that the cell is prepared to divide once the nuclear envelope breaks down (Fig. 1).

So how does cyclin B–Cdk1 activation trigger its own nuclear translocation? One clue comes from the observation that a GFP-tagged nuclear import substrate translocated to the cytoplasm at similar time points as cyclin B–Cdk1 translocated to the nucleus in prophase. Although clearly not all proteins are affected at this time point, including the cyclin B–Cdk1 regulator Cdc25C, this indicates that there is a general change in parts of the transport machinery that coincides with cyclin B–Cdk1 activation. The relevant targets of cyclin B–Cdk1 in the transport machinery remain unclear, although there are several candidates for such a function (Li and Zheng, 2004; Swaminathan et al., 2004). Importantly, a general effect on the transport machinery opens up the possibility that other proteins also translocate at the same time as cyclin B–Cdk1. Thus, cyclin B–Cdk1 may partly direct cellular rearrangements by redistributing proteins during mitotic entry. For example, by changing the nucleocytoplasmic transport machinery, cyclin B–Cdk1 could enable a nuclear protein that can modify microtubule dynamics access to the cytoplasmic microtubules in prophase without directly modifying that protein (Fig. 3). Allowing proteins that are spatially separated from their targets in interphase to translocate before NEBD could potentially provide an additional mechanism to coordinate nuclear and cytoplasmic events and ensure that the cell is prepared for mitosis when NEBD occurs.

If cyclin B–Cdk1 activation regulates its nuclear translocation to ensure spatial coordination, how can temporal coordination be achieved? A long-standing concept in the cell cycle field has been that a gradual increase in Cdk activity can ensure that different processes occur in the correct order. The general idea is that different substrates will require different thresholds of Cdk activity to become phosphorylated. By gradually increasing Cdk activity, some substrates will therefore become phosphorylated before others, providing a mechanism for temporal coordination through the cell cycle (Stern and Nurse, 1996). Previous results have suggested that this may also be the case for mitosis in human cells because the level of Cdk1 activity required for triggering mitotic entry is not sufficient to ensure later mitotic progression (Lindqvist et al., 2007). In a recent study, Gavet and Pines (2010b) revisited this concept by correlating cyclin B–Cdk1 activity measured by the biosensor to different early mitotic events. They found that centrosome separation (in unperturbed cells), cell rounding, and NEBD occur at set thresholds of cyclin B–Cdk1 activity (Gavet and Pines, 2010b). Expressing a mutant Cdk1, which leads to oscillations

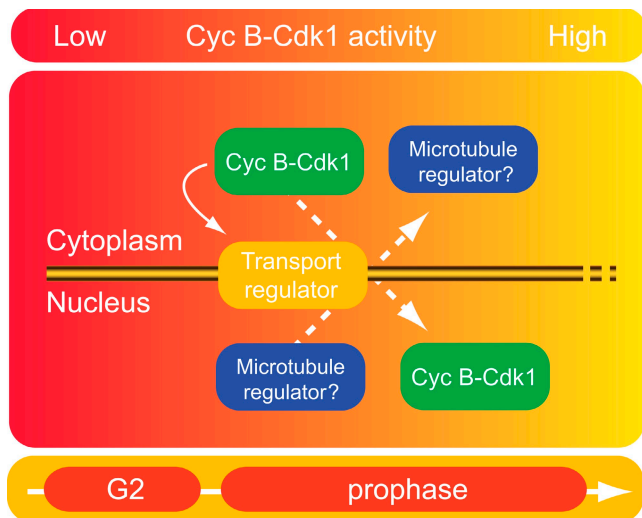


Figure 3. **Coupling between cyclin B-Cdk1 activity and nuclear translocation ensures spatial coordination of early mitotic events.** Cyclin B-Cdk1 activity causes a general change in the transport machinery that triggers cyclin B-Cdk1 nuclear import. Cyclin B-Cdk1-dependent changes of the transport machinery may also lead to coordinated redistribution of other mitotic regulators, e.g., by enabling nuclear microtubule regulators access to cytoplasmic microtubules.

in cyclin B-Cdk1 activity, leads to execution of the events that fit with the resulting level of cyclin B-Cdk1 target phosphorylation, indicating that different thresholds of cyclin B-Cdk1 activity will determine what mitotic event is executed. Thus, a gradual activation of cyclin B-Cdk1 is likely to underlie temporal coordination, whereas cyclin B-Cdk1 activity being linked to its nuclear translocation ensures spatial coordination during mitotic entry. Although we have a long way to go before we understand how mitotic entry is regulated and coordinated, these concepts are likely to form the basis for future models of the transition to mitosis.

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