The role of Cdc14 phosphatases in the control of cell division

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Abstract

The periodicity of CDKs (cyclin-dependent kinases) regulates most cell cycle transitions including cytokinesis. High Cdk1 activity promotes cytoskeletal rearrangements necessary for cell division while at the same time ensuring that cytokinesis does not begin before the separation of sister chromatids during anaphase. The conserved Cdc14 (cell division cycle 14)-family of phosphatases reverses Cdk phosphorylation events and therefore Cdc14 phosphatases promote the process of cytokinesis. Here, we review the elucidated roles of Cdc14 phosphatases in cytokinesis and the current outstanding questions regarding their function in this process.

Dynamics of Cdc14 (cell division cycle 14) localization

Like several important mitotic regulators, Cdc14 family phosphatases localize dynamically to a variety of cellular structures in a cell cycle-dependent manner. During interphase, they concentrate in the nucleolus and/or on the spindle pole bodies of yeast, or centrosomes of higher eukaryotes, where catalytic activity is prevented. Indeed, intracellular sequestration is a major mechanism of Cdc14 phosphatase inhibition [1]. During mitosis, Cdc14 proteins decorate centrosomes, kinetochores, the mitotic spindle, the site of cell division, and the midbody, depending on the species [1]. At these different sites they presumably dephosphorylate their substrates, including those that participate in cytokinesis.

Cdc14 in budding yeast cytokinesis

Because *Saccharomyces cerevisiae* Cdc14's role in Cdk1 (cyclin-dependent kinase 1) inactivation is essential, cells lacking *S. cerevisiae* Cdc14 arrest in anaphase. Although this arrest obscures Cdc14's later function in cytokinesis, Cdc14 has been implicated in cytokinesis through several studies in which its requirement for Cdk1 inactivation was bypassed [1]. Cdc14 was also shown to specifically affect actomyosin ring constriction through analysis of a mutation in the Cdc14 NES (nuclear export sequence) [2]. This mutation traps Cdc14 in the nucleus and, as a result, mitosis occurs on schedule but actomyosin ring constriction fails. The cytoplasmic target(s) of Cdc14 activity important for cytokinesis is, however, not clear. Given that Cdc14 localizes to the *S. cerevisiae* bud neck [2], it is likely that a protein(s) participating in the mechanics of cytokinesis is a substrate.

Key words: Cdc14 (cell division cycle 14), cyclin-dependent kinase (CDK), cytokinesis, phosphatase.

Abbreviations used: Cdc, cell division cycle; Cdk, cyclin-dependent kinase; CR, contractile ring; FEAR, fourteen early anaphase release; MEN, mitotic exit network; SIN, septation initiation network.

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Cdc14 cytokinetic roles in multicellular organisms

A role for Cdc14 in cytokinesis is likely to be general but its influence on this process varies between organisms. In *Caenorhabditis elegans* embryogenesis, for example, a role for Cdc14 in cytokinesis was only revealed in animals producing a tagged allele of a central spindle component, Zen-4, that is also required for cytokinesis [3,4]. This genetic interaction probably reflects the resilience of cytokinesis to perturbation and underscores the difficulty in teasing apart the respective roles of converging regulatory pathways on the process. In mammalian tissue culture cells, the Cdc14 phosphatase appears to play a more central role in cytokinesis. Cells overexpressing or depleted for Cdc14A, one of two characterized paralogues, fail at cytokinesis (reviewed in [5]).

Cytokinetic role of Cdc14 phosphatase in fission yeast

Our laboratories have concentrated on understanding the *Schizosaccharomyces pombe* Cdc14 enzyme known as Clp1/Flp1, hereafter referred to as Clp1 [5]. An experimental advantage of studying Clp1 is that it is not an essential gene, although Clp1 is important for many aspects of cell cycle regulation including cytokinesis [5–8]. Cells lacking Clp1 fail regularly at cytokinesis and are sensitive to mutation or perturbation of other cytokinetic proteins, making *S. pombe* an attractive model for dissecting Clp1/Cdc14 cytokinetic functions [5].

Figure 1 | Model for control of Clp1 intracellular localization
Localization of Clp1 is controlled by phosphorylation (P) by the Sid2–Mob1 kinase complex, which promotes binding of the 14-3-3 protein Rad24. See the text for further details.

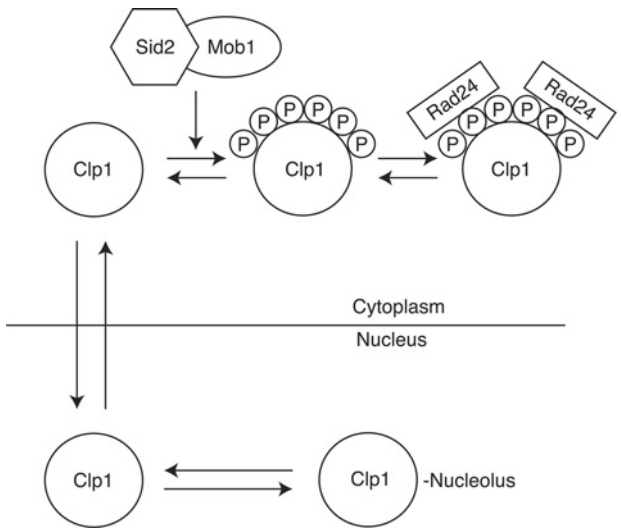
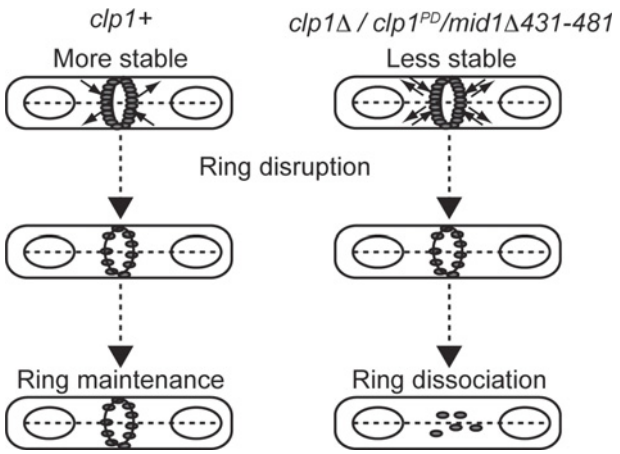


Figure 2 | Model for regulation of CR stability by Clp1
In the presence of Clp1 at the CR, indicated by grey ovals, the ring is more stable to disruption. In cells lacking (*clp1Δ*), in the Clp1 phosphatase-dead (PD) mutant, or in cells producing a Mid1 deleted for amino acids 431–481 of Mid1, Clp1 is not recuited to the CR and ring stability is compromised.



Regulation of Cdc14 phosphatases by signalling pathways

As mentioned above, intracellular sequestration is a major mechanism of Cdc14 phosphatase regulation. In *S. cerevisiae*, Net1 tethers Cdc14 at the nucleolus and acts as a competitive inhibitor to keep it inactive during interphase [1]. Cdc14 release from Net1 occurs in two stages. First, early anaphase release depends upon a set of genes termed the FEAR (fourteen early anaphase release) network [1]. Then, the MEN (mitotic exit network) sustains Cdc14 release [1]. A tether comparable to Net1 has not been described in other organisms and it is unclear how Cdc14 phosphatases are generally released from the nucleolus. For example, in other organisms, Cdc14 phosphatases are freed from nucleolar sequestration during earlier stages of mitosis and in *S. pombe*, genes analogous to FEAR components do not affect Clp1 localization or function [9].

However, in both yeasts, sustained release of Cdc14 phosphatases from the nucleolus depends upon analogous pathways, the *S. cerevisiae* MEN and the *S. pombe* SIN (septation initiation network) [1,5]. These pathways ensure that the phosphatase remains untethered and able to counteract Cdk1 phosphorylation events during anaphase. Mechanistically, how this occurs in *S. cerevisiae* is not known. In *S. pombe*, the SIN kinase Sid2 promotes phosphorylation events on Clp1 that produce binding sites for the 14-3-3 protein Rad24 [10]. Clp1-Rad24 interaction promotes cytoplasmic retention of Clp1 that in turn promotes cytokinesis [10,11] (Figure 1).

What prevents Cdc14 phosphatases from reversing Cdk1 phosphorylation during early mitosis if they are not tethered in the nucleolus? In *S. pombe*, Clp1 activity is attenuated by Cdk1 phosphorylation. When Cdk1 activity is high, Clp1 does not promote mitotic exit events [12]. As Cdk1 activity decreases during anaphase, Clp1 auto-dephosphorylates and becomes fully active. Plk1 (polo-like kinase 1) has also been shown to phosphorylate hCdc14A during anaphase to reverse intramolecular inhibition of the phosphatase [13].

Mechanisms of Cdc14 influence over cytokinetic events

How does Clp1/Cdc14 promote cell division? Through a proteomics approach, Mid1 was identified as a Clp1-interacting protein. Mid1 serves as a stable scaffold for CR (contractile ring) assembly in the medial region of the cell by bridging an interaction with the plasma membrane to CR components [14,15]. In its absence, CR assembly is not restricted to the medial region and CRs form at inappropriate angles and positions [14]. Mid1 binds Clp1 directly through a small stretch of amino acids (431–481). Cells lacking Mid1 altogether or producing only a deletion of *mid1*, *mid1-Δ431–481,* fail to recruit Clp1 to the CR. In these *mid1* mutations, in *clp1*-deleted cells and in cells producing only catalytically inactive Clp1-286S which localizes to the CR, the key CR components Cdc15 and myosin II become more dynamic as determined by FRAP (fluorescence recovery after photobleaching) analyses, and all of these mutant strains show negative genetic interactions with a range of other cytokinesis mutants. In addition, the phosphorylation state of the CR protein, PCH family member Cdc15 is altered in all of these mutant strains. These findings indicate that Cdk1 activity antagonizes cytokinesis in part by destabilizing CR assembly and, conversely, Clp1 promotes cytokinesis by helping to stabilize the CR (Figure 2).

Like Cdc14 phosphatases, the SIN and MEN function in cytokinesis and modulation of SIN/MEN activity is another mechanism by which Clp1/Cdc14 is likely to affect CR dynamics. Thus far, only one MEN component, protein kinase Cdc15, is known to be a Cdc14 substrate and Cdc15 dephosphorylation alone is not essential for cytokinesis [1]. Based on genetic data, however, competing influences of Cdk1 and Cdc14 are likely to be a recurring theme in SIN/MEN regulation. Because protein kinases of the SIN/MEN localize to the CR, it is anticipated that CR components will prove to be SIN/MEN substrates. Thus far however, SIN/MEN substrates at the CR remain unidentified and, as with Cdk1 and Clp1/Cdc14 substrates, there are likely to be several. Unravelling the complexity of phosphoregulation in CR assembly and function has only begun.

Concluding remarks

In conclusion, Cdc14 phosphatases promote cytokinesis in several eukaryotic organisms, most likely by helping to relieve the brake on cell division imposed by high Cdk1 activity. Thus far, our clearest understanding of the detailed role(s) this phosphatase family plays in the final step of cell division derives from studies in *S. pombe*. Clp1 enhances cytokinesis; first by directly binding the CR and influencing CR dynamics, and secondly by influencing SIN activity, which also controls CR function. Future studies will define specific substrates of Clp1 and SIN at the CR and their relative importance in promoting cytokinesis. Such studies will help to unravel the complex regulatory loops that co-ordinate the final steps in cell division.

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