A virtual cycle: theory and experiment converge on the exit from mitosis

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Fl000 Biology Reports 2010, **2**:33 (doi:10.3410/B2-33)

The electronic version of this article is the complete one and can be found at: <http://f1000.com/reports/biology/content/2/33>

Abstract

The cell division cycle can be modelled as a series of quantitative thresholds of cyclin-dependent kinase (CDK) activity. DNA synthesis has a lower threshold requirement for CDK than does entry into mitosis, and mitotic exit and re-setting of replication origins occur upon collapse of CDK activity below both thresholds, so that the simple rise and fall of CDK with each cell cycle might suffice to ensure repeated alternation of chromosome duplication and segregation. Recent experimental dissections of mitotic exit, which have both guided and been informed by computational modelling, suggest a more complicated mechanism, in which unidirectional progression is ensured by systems-level control of CDK function and the balance between mitotic CDK and phosphatase activities.

Introduction and context

Eukaryotic cells enter mitosis with high levels of active cyclin-dependent kinase (CDK), which phosphorylates numerous proteins, leading to an array of cellular changes [1]. High kinase levels are also important for maintaining the mitotic state; inhibition of CDK in cells arrested in mitosis with spindle poisons causes aberrant progression into interphase [2-4]. At anaphase onset, the anaphase-promoting complex (APC) becomes active and polyubiquitinates proteins to target them for degradation. Among the proteins tagged for destruction is cyclin, with consequent lowering of mitotic CDK (Cdk1) activity. Supraphysiologic levels of a non-degradable cyclin can prevent mitotic exit, which led to the hypothesis that cyclin degradation is necessary and sufficient to drive cells out of mitosis [1,5].

In *Saccharomyces cerevisiae*, mitotic exit also depends on the phosphatase Cdc14, which dephosphorylates many Cdk1 substrates. Cdc14 is sequestered by the Net1 protein in the nucleolus until anaphase, when it is released by the sequential action of the Cdc14 early

anaphase release (FEAR) and mitotic exit network pathways [6]. In metazoans, as in yeast, dephosphorylation of mitotic substrates is a hallmark of the M/G1 transition [7], but the responsible phosphatases are only now beginning to emerge.

Major recent advances

A central prediction of the simple CDK threshold model was that mitotic exit would be effectively inhibited by levels of Cdk1 activity normally attained during metaphase [8]. To test this, Cross and colleagues [9] developed a system that allowed precisely controlled expression of a fluorescently labelled, non-degradable mitotic cyclin, Clb2, in budding yeast. Surprisingly, Clb2 expression sustained at the peak level reached during a normal mitosis delayed but did not prevent the events of mitotic exit. Because Cdc14 was released from the nucleolus with normal efficiency and kinetics in cells expressing non-degradable Clb2, the authors hypothesized that mitotic exit is controlled by the ratio of Cdk1 to Cdc14, not by simply crossing a threshold of Cdk1 activity. A mathematical model incorporating this additional complexity predicted experimental results of

expressing non-degradable Clb2 or high levels of Sic1, a CDK inhibitor involved in mitotic exit, more accurately than did the simple threshold model [9].

Although cells expressing non-degradable Clb2 could exit mitosis, they showed abnormalities during the following cycle in the response to mating factor and in nucleation of the metaphase spindle [9]. This suggested that the persistence of stable Cdk1-Clb2 complexes might disturb the smoothly unidirectional progression of the cell cycle even when mitotic exit *per se* could be accomplished. Irreversibility of mitotic exit was widely assumed to be the inevitable result of mitotic cyclin destruction, but experiments by Uhlmann and colleagues [4] exposed the logical flaw in this reasoning (which had been pointed out by Novak, Tyson, and colleagues [10]) by showing that irreversibility of Cdk1 inhibition and mitotic exit are instead a consequence of systems-level feedback. Also working with budding yeast, they asked if destruction of Clb2 was sufficient to drive irreversible mitotic exit. Within about 50 minutes after release from a metaphase block, Clb2 was degraded almost completely, mitotic phosphoproteins became dephosphorylated, and mitotic spindles broke down. Nevertheless, if the APC was then inactivated, these events could be reversed (Figure 1a). Their interpretation: Clb2 destruction is not sufficient for irreversible mitotic exit because its degradation can be balanced by re-synthesis, which, like proteolysis, is a thermodynamically irreversible process [4].

When the APC was allowed to remain active for just 10 minutes longer, mitotic exit became essentially irreversible. This correlated with the accumulation of Sic1 and, in *sic1Δ* cells, mitotic exit remained reversible for up to 90 minutes. This behavior was simulated by a mathematical model; irreversibility occurred once Sic1 accumulated to a threshold level, whereas below this threshold Clb2 re-synthesis could drive cells back into a mitotic state. Expression and stability of Sic1 are regulated by the balance of Cdk1 and Cdc14 activity, suggesting that the switch for mitotic exit proposed by Cross and colleagues might be built to ensure irreversibility (Figure 1b).

Future directions

Less is known about the control of mitotic exit in higher eukaryotes, but several studies suggest that its basic regulatory logic is conserved. Gorbsky and colleagues [2,11] reported that mitotic exit caused by chemical inhibition of Cdk1 was fully reversible if mitotic cyclin degradation was prevented in cultured *Xenopus* and human cells. As in budding yeast, there is a finite

window in which reversal can occur. This interval of reversibility appears to end with activation of the kinases that phosphorylate Cdk1 to inhibit its activity, Wee1 and Myt1 [11], which are themselves antagonized by Cdk1 in a double-negative feedback loop [1]. Modelling suggests that this loop could function analogously to the one comprising Cdk1 and its antagonists in yeast to prevent mitotic re-entry [12]. Some caution in accepting these conclusions is still warranted, however, because of another study in which mitotic exit did not occur in human cells when Cdk1 and the proteasome were simultaneously inhibited [3].

The disparate results reported by the Gorbsky and Margolis groups [2,3,11] have yet to be reconciled. There is general agreement, however, that inhibiting Cdk1 with small molecules can trigger mitotic exit in the absence of spindle function in cells with working APCs and proteasomes. In the same paper that questioned the occurrence of mitotic exit in the absence of proteasome activity, Margolis and colleagues [3] found another way to block exit triggered by Cdk1 inhibition – addition of okadaic acid, an inhibitor of protein phosphatases PP1 and PP2A. Kornbluth and colleagues [13] later reported that PP1 is the major activity responsible for dephosphorylating mitotic phosphoproteins in *Xenopus* egg extracts and HeLa cells. Cdk1 phosphorylates and inhibits PP1, and the degradation of mitotic cyclin partially relieves that inhibition to allow PP1 auto-dephosphorylation and subsequent inactivation of a PP1-inhibitory protein. This is reminiscent of the regulation of Cdc14 and its fission yeast ortholog Clp1, in that the phosphatase participates in a positive feedback loop to ensure its own robust activation during mitotic exit [6,14]. There appears to be something missing from the metazoan circuitry, however. In *S. cerevisiae*, high Cdk1 levels at anaphase initially promote Cdc14 release by phosphorylating Net1 (Figure 1b), whereas in metazoans Cdk1 activity must decrease before PP1 becomes active. This difference raises two possibilities: (a) the model of Drapkin *et al.* [9] (in which the CDK:phosphatase ratio is the determining factor for mitotic exit) is incompletely conserved in metazoans; or (b) there remains to be discovered an additional phosphatase promoting mitotic exit in metazoans, which can become active before mitotic cyclin is destroyed.

PP2A is a candidate to be the 'missing' phosphatase *a priori* because of its known sensitivity to okadaic acid, which, as mentioned above, can block mitotic exit. Moreover, Hunt and colleagues [15] recently demonstrated a role for PP2A in promoting mitotic exit in *Xenopus* egg extracts. They detected a phosphatase that was regulated during the cell cycle and active towards a

Figure 1. Models of mitotic exit in yeast**(a)** **(b)**

(a) A balance between Cdk1 and Cdc14 activities governs mitotic exit in budding yeast. Early in mitosis, Cdk1 activity is at its peak while Cdc14 activity is very low. As cells progress through anaphase, Cdk1 activity drops and Cdc14 activity rises to trigger mitotic exit. This transition is reversible – despite mitotic cyclin degradation – until Sic1 protein accumulates beyond a threshold level [discussed in (b)]. **(b)** The circuitry controlling an irreversible switch. Early in mitosis, phosphorylation by Cdk1-Clb leads to downregulation of the Cdh1-activated form of the anaphase-promoting complex (APC), the transcription factor Swi5, and the cyclin-dependent kinase (CDK) inhibitor Sic1. As cells enter anaphase, Cdk1 phosphorylates Net1, triggering an initial, partial release of Cdc14 by the Cdc14 early anaphase release (FEAR) pathway (which also requires APC function). Concomitantly, the Cdc20-bound form of the APC (which is positively regulated by CDK activity) ubiquitylates mitotic cyclins to target them for degradation. Later in anaphase, the remaining Cdc14 is released due to activation of the mitotic exit network (MEN) pathway, allowing for more extensive dephosphorylation of mitotic phosphoproteins. Unphosphorylated Swi5 can trigger transcription and accumulation of Sic1, which is now able to evade phosphorylation-directed ubiquitylation and degradation, leading to further Cdk1 inhibition. Activation of APC-Cdh1 leads to further destruction of mitotic cyclins as cells complete exit from mitosis. It is through this system of feedback loops that irreversibility of mitotic exit is ensured.

CDK substrate, and identified it as PP2A associated with the B55 δ regulatory subunit. Immunodepletion of B55 δ from extracts accelerated entry to, and prevented exit from, mitosis. Interestingly, depletion of PP2A/B55 δ was effective at delaying mitotic entry only when performed on an interphase extract (i.e., prior to mitotic entry); depletion of the phosphatase from extracts already in mitosis had little or no effect on the rate of dephosphorylation of an APC subunit phosphorylated by CDK. This implies that other phosphatases (such as PP1) can dephosphorylate the bulk of mitotic phosphoproteins, and that PP2A/B55 δ must execute its required function earlier, either in interphase before mitotic CDK activation or at mitotic entry [15]. However, activity of this PP2A isoform is abruptly downregulated upon entry to mitosis, which might argue against a role strictly analogous to that of the 'early release' form of Cdc14 in budding yeast.

Here we have summarized recent advances in understanding mitotic exit and in building mathematical models that accurately predict behavior of the mitotic control system *in vivo*. These studies have made it clear that features such as irreversibility are the product of systems-level feedback, not the thermodynamic irreversibility of any one reaction – a generalization likely to hold true throughout evolution [10]. It is also possible that yeast-like feedback loops containing CDKs and opposing phosphatases make mitotic exit robust and switch-like in metazoan cells. Before we can be certain of that conclusion, however, more of the relevant phosphatases probably need to be identified, their roles must be more precisely defined, and the regulation of their localization and activity will need to be elucidated.

Abbreviations

APC, anaphase-promoting complex; CDK, cyclin-dependent kinase.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We thank members of the Fisher lab for helpful discussions. Work in the lab is supported by National Institutes of Health grants GM056985 and GM076021.

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