Published in final edited form as:

Trends Cell Biol. 2001 December ; 11(12): 512–519.

Triggering the all-or-nothing switch into mitosis

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Abstract

The past decade of cell cycle investigations has identified many roads not taken. The kinase that drives mitosis can be modulated by cyclins, by activating phosphorylation, by inhibitory phosphorylation and by binding of inhibitors, but one of these regulatory options controls the transition from G2 phase to mitosis in most circumstances. A switch-like mechanism integrates signals of cellular status and commits the cell to mitosis by abruptly removing inhibitory phosphate from preformed cyclin:Cdk1 complexes. The pathways that flip this switch alter the balance of modifying reactions to favor dephosphorylation, thereby generating a flood of mitotic kinase.

The past ten years of cell cycle analysis have led to a consensus that has not yet been conveyed by portrayals of the cell cycle in textbooks. The textbook 'understanding' is that the accumulation of cyclins drives the cell cycle. However, at least as regards the control of mitosis, the data suggest something different: discrete, irreversible transitions drive the initiation of S phase, the entry into mitosis and the transition to anaphase (Fig. 1). These milestones of cell cycle progress control processes – DNA replication and chromosome segregation – whose success requires great fidelity. Even slight inaccuracies in these processes can lead to cell death or genetic destabilization that can promote tumor formation. These are not processes to be pursued halfheartedly. Molecular circuitries that are capable of an all-or-nothing switch-like output create the decisive transitions that commit the cell to these events. In this article, I focus on two issues: the molecular nature of the switch that creates the all-or-nothing transition into mitosis and the signals that flip this switch.

Ten years ago, I reviewed this same transition in *Trends in Cell Biology*¹, but, at that time, there was much less information directing us to the regulatory inputs that actually trigger the mitotic switch. I consequently emphasized the open-minded option that there are several different regulatory inputs affecting the mitotic kinase (Fig. 2), and that the cell cycles in different organisms and at different developmental stages might use any of the available options – complex formation with cyclins, release of cyclin:kinase inhibitory proteins (CKIs), activating phosphorylation or reversal of inhibitory phosphorylation. However, as more situations have been studied, it has become apparent that entry into mitosis is usually regulated by a subset of the regulatory options involving a particular avenue of regulation of Cdk1. In organisms as diverse as frog (*Xenopus*) and fission yeast (*Schizosaccharomyces pombe*), and developmental situations ranging from oocyte maturation to terminal differentiation in an epithelium, cells control entry into mitosis by controlling the enzymes that add or remove inhibitory phosphates to Cdk1. In contrast to dogma, cyclins accumulate to levels in excess of requirements for mitosis and consequently do not limit the final flipping of the switch that triggers mitosis. Here, I present several cases in which the processes that flip the switch are substantially understood, and I relate our current understanding of the events triggering mitosis to the notion that cyclin accumulation drives the cell cycle.

Mitotic cyclin and cyclin-dependent kinase

Arguably, the most important advance in the study of the cell cycle came with the recognition that a complex of cyclin and cyclin-dependent kinase (CDK) promotes mitosis. Large families of cyclins and CDKs are now recognized, and these interact in many combinations to form cyclin:CDK complexes with a variety of roles². Many, but not all, of the known roles of cyclin:CDKs are in cell cycle regulation and, even within cell cycle control, there is specialization. In particular, some cyclin:CDKs are described as S-phase cyclin:CDKs and others as mitotic cyclin:CDKs.

The categorization of cyclin:CDKs as S phase or mitotic gives the appearance of support for a concept that remains to be tested. It is recognized that cyclin:CDKs drive S phase and mitosis, a duality of function that is most easily understood if there is specialization. Accordingly, one might propose that the type of cyclin:CDK that is activated would determine the outcome, mitosis or S phase. However, a single B-type cyclin and Cdk1 can support a complete cell cycle in both the budding yeast *Saccharomyces cerevisiae* and in *S. pombe*^{3,4}. In *Drosophila*, cyclin A:Cdk1 can drive either mitosis or S phase, depending on the status of the cell^{5,6}. Thus, there is a mechanism that can define the outcome of cyclin:Cdk1 activation independent of cyclin:CDK specialization. Rather than dictating overall outcome, one of the important contributions of the specializations of cyclin isoforms might be to provide opportunities for more finely tailored regulation of the switches that initiate S phase and mitosis. Accordingly, different mitotic cyclins might play different roles in triggering the switch into mitosis.

Cyclin B is presented as the quintessential mitotic cyclin, but this categorization belies the complexity of the relationships among cyclins – a relationship that includes overlapping but not identical functions. A *Drosophila* mutant lacking cyclin B is viable, albeit with a slightly disturbed mitosis⁷. Further genetic analysis has led to the demonstration that there are three distinct cyclins that make overlapping contributions to mitosis^{5,7,8}. Cyclin A is the only cyclin that is essential for mitosis in *Drosophila*: cyclin A mutants arrest in G2, indicating that this cyclin has a role in triggering entry into mitosis^{5,9}. Cyclin B3 (which, despite its name, is not a subtle variant of cyclin B but a separate, more distantly related, cyclin type that is conserved from *Caenorhabditis elegans* to vertebrates^{10,11}) also has a mitotic role⁷. Like cyclin B, it is dispensable for the life of a fly, but double mutants of cyclin B and cyclin B3 die as embryos at the stage when maternal cyclin contributions decline. Notably, at the onset of the phenotype, the cells of the double mutant embryos still enter mitosis, but mitotic progression is grossly disturbed⁷. Without discounting the importance of the distinctions and redundancies, I here focus on the simplifying generalization that cyclins complexed with Cdk1 are essential for entry into mitosis, and, in several experimental models, activation of such complexes is sufficient for mitosis.

Controlling inhibitory phosphorylation of Cdk1

Cyclin:Cdk1 complexes drive mitosis, and so the status of this complex immediately before mitosis is particularly germane to a consideration of the event that triggers mitosis. During G2, the cyclin:Cdk1 of *S. pombe*, *Drosophila*, vertebrates, echinoderms and plants is held inactive by inhibitory phosphorylation of residues Y15 and T14 of Cdk1^{12–17}. Maintenance of this inhibitory phosphorylation is required for G2 quiescence^{18–20}. In *S. pombe*, Wee1 is the major (but not exclusive) contributor to Y15 phosphorylation (Box 1): in its absence, G2 is shortened and cells divide when they are smaller, whereas Wee1 overproduction prolongs G2 (Refs 21 and 22). Reciprocally, loss of function of Cdc25, the phosphatase that removes the inhibitory phosphate, arrests cells in G2, and its overexpression drives G2 cells into mitosis²³. Finally, expression of a mutant form of Cdk1 that is immune to inhibitory phosphorylation bypasses the need for Cdc25 and causes cells to escape G2 prematurely¹². With the exception of *S.*

cerevisiae, which lacks a G2 phase (Box 2), similar experiments in a variety of organisms have had a similar outcome, confirming that inhibitory phosphorylation of cyclin:Cdk1 is required for G2 and that the dephosphorylation of Cdk1 drives mitosis.

The homologs of the *S. pombe* inhibitory kinase, Wee1, that were initially identified in metazoans are nuclear enzymes that, like their yeast namesake, phosphorylate Y15 with high specificity²⁴. However, metazoan Cdk1, unlike its yeast counterpart, is phosphorylated at T14 as well as Y15. A second inhibitory kinase found in metazoans, Myt1, is also homologous to Wee1, but it has several distinctions, including an ability to efficiently phosphorylate T14 as well as Y15 (Ref. 25). The Myt1 kinase appears to have a very significant role because its activity can suffice for a G2 arrest, at least in some circumstances. *Xenopus* oocytes, which are arrested in G2 before meiosis I, lack Wee1 and rely on Myt1 to hold Cdk1 inactive²⁶. *Drosophila*, which relies on inhibitory phosphorylation for cell cycle control, nonetheless survives when mutant for *Wee1*. *Drosophila Wee1* mutants still exhibit T14 and Y15 phosphorylation, indicating that there is a second significant Cdk1 kinase activity apparently contributed by the *Drosophila* MYT1 (Ref. 27). It is currently thought that Wee1 and Myt1 are partially redundant in metazoans, meaning that, when both are present, both enzymatic activities need to be suppressed or overridden to allow progression to mitosis.

Box 1

The family of Wee1 kinases

In addition to the Wee1 kinase, *Schizosaccharomyces pombe* has a related Wee1-like kinase called Mik1 that also phosphorylates Cdc2 on Tyr15. The two kinases, although they are partially redundant, are specialized to some extent, with Mik1 providing the predominant input for checkpoint control of entry into mitosis and Wee1 providing the primary regulation of growth^a. The sequences of Wee1 and Mik1 are more similar to each other than they are to related kinases from other species. Because the Wee1:Mik1 divergence is likely to be relatively recent, I have not distinguished their roles, which might be viewed as a private specialization of fission yeast (the text refers to generic Wee1/Mik1 function of *S. pombe* as Wee).

Most metazoans also have two distinct Wee1-related kinases, called Wee1 and Myt1. The presence of distinct Wee1 and Myt1 sequences in species from *Drosophila* to human suggests that the divergence of the two kinase types occurred early in metazoan radiation. In contrast to my treatment of the *S. pombe* Wee1-related kinases, I have commented on the specializations of these kinases.

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Myt1 and Wee1 have additional distinctions that are likely to influence the switch to mitosis. Whereas Wee1 is nuclear, Myt1 is membrane bound and cytoplasmic²⁵. More surprisingly, in addition to its action as an inhibitory kinase, Myt1 can inhibit Cdk1 by a kinase-independent route that appears to involve direct interaction and perhaps recruitment to a locale where it is ineffective^{25,28,29}. Although there is no indication that metazoan Wee1 has this activity, the evolutionary predecessors of these enzymes might have had this kinase-independent activity as a similar activity has been demonstrated in the inhibitory kinase of *S. cerevisiae*³⁰.

To summarize, the inhibitory actions of two kinases, Wee1 and Myt1, during G2 phase allow the accumulation of a large reserve of inactive cyclin:Cdk1 complexes before any commitment to mitosis. Inhibitory phosphorylation of already accumulated G2 cyclin:Cdk1 complexes

appears to be a hallmark of G2 in all systems. The abrupt dephosphorylation of these sites by a dual-action phosphatase, Cdc25, creates a flood of cyclin:Cdk1 kinase activity and drives mitosis. The circuitry that creates the abruptness of this transition constitutes the mitotic switch. I now focus on this switch and the factors that trigger its activation.

Triggering all-or-nothing activation of the mitotic kinase

The fundamental feature of a switch is that, under the influence of a fairly small input, it rapidly changes from one state to another without intermediates (Box 3). Rapid conversion of inactive phosphorylated forms of Cdk1 to active forms switches cells into mitosis. Our understanding of this switch is based on *in vitro* studies of extracts that show switch-like activation of cyclin:Cdk1 kinase activity. Two positive-feedback loops contribute to a directional switch (Fig. 3)³¹. Cdc25 is activated by mitotic phosphorylation, whereas Wee1 and Myt1 are inactivated by mitotic phosphorylation. Although the changes in activity of the kinases and the phosphatase have complex inputs (Box 4), the Cdk1 activity can initiate these changes. Hence, Cdk1 regulates its regulators to create positive-feedback loops. Once these loops are engaged, the switch is thrown. The suppression of Wee1 and Myt1 kinases, and the activation of Cdc25 kinase abruptly give the phosphatase the upper hand, and the accumulated reserve of cyclin:Cdk1 is rapidly activated. The inferred *in vivo* relevance of the feedback loops that have been defined in extract systems is supported by evolutionary conservation of the regulatory interactions and the autoamplification of 'maturation-promoting factor' [or 'mitosis-promoting factor' (MPF) as it came to be called; see below], but we still lack a direct demonstration that Cdk1 makes a rate-limiting contribution to its own dephosphorylation *in vivo*.

Box 2

Budding yeast usually lack a G2 phase

The cell cycle of the budding yeast *Saccharomyces cerevisiae* differs from that of fission yeast and metazoans in having no G2 phase, and the role of inhibitory phosphorylation of cyclin:Cdk1 is modified accordingly^a. In the budding cycle, bud growth, mitotic spindle assembly and DNA replication are initiated together upon a G1/S-associated rise in cyclin:CDK activity. Ordinarily, these processes continue without interruption. However, if an osmotic stress disturbs the organization of the cytoskeleton, thereby disrupting polarized bud growth, then the *S. cerevisiae* homolog of Wee1, Swe1, is activated^b. The activated Swe1 phosphorylates and inhibits Cdc28 kinase, resulting in an arrest of the nuclear cycle and an opportunity to reorganize the cytoplasmic structures. Thus, as in other systems, inhibitory phosphorylation controls activation of cyclin:Cdk1, but its action is only called for in response to stress. Although the absence of a G2 phase in the *S. cerevisiae* cell cycle sets it apart, the mechanisms that control Swe1 in this organism might nonetheless be relevant to those operating in organisms that have a G2 phase. Indeed, the decision whether or not to activate Swe1 and engage the checkpoint in *S. cerevisiae* is mediated by a group of kinases that are related to nim1/cdr1 kinase of *S. pombe*^c. In *S. cerevisiae*, these kinases appear to be constitutively active after G1/S unless something is awry, whereas, in *S. pombe*, they act to shut off the Wee1 kinase only at the end of G2. Thus, whereas *S. cerevisiae* normally lacks a G2:M transition, the regulation of Swe1 defined in this powerful experimental system is likely to contribute to our understanding of the G2:M transition in other organisms.

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Box 3

The biological design of switching circuits

Cells use switches to integrate graded inputs to create decisive and appropriate outputs. Switches are fundamental to information processing. Because Michaelian biochemical reactions dampen or at best linearly transmit input signals, molecular switches require special design features that promote all-or-nothing responses. Two of the better known such design features are cooperativity, whereby binding of one molecule promotes a conformational change to improve the binding of second molecule, and positive feedback, whereby the product of a pathway feeds back to promote the same reaction. In addition to these strategies, if a signal molecule modulates multiple steps in a circuit, the circuit is very responsive to the levels of the signal (in the extreme, the response is a power function of the number of modulated steps). Also, two opposing reactions, such as phosphorylation and dephosphorylation, whose kinetics define the steady-state amount of a component can be placed in precarious balance so that even a subtle change in the rate of one of the modification reactions causes a large swing in the balance and hence the levels of the products^a. All of these strategies promote an abrupt change at a threshold concentration of input signal, but robust and effective switches are likely to include several such features to ensure a truly all-or-nothing response^b.

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Even though the complexity of the feedback loops has left us without a full appreciation of the enzyme kinetics underlying the switch, one feature of a switch that relies on positive-feedback loops is general and relevant. Positive-feedback loops act directionally, in that, once activated, positive reinforcement hinders backwards movement. Consequently, the on and off states are not in equilibrium, and switching occurs from a metastable state. For example, an increase in Cdc25 phosphatase expression ought to promote activation of Cdk1, but Cdc25 cannot easily throw the switch because it is inactive. However, any basal activity of Cdc25 or cyclin:Cdk1 would fluctuate with time and eventually initiate the positive-feedback cascade and throw the switch. Accordingly, switching would be stochastic. Indeed, an experimentally designed positive-feedback switch shows just such stochastic behavior³². This characteristic of positive-feedback switches seems at odds with the observed temporal precision of stereotyped mitotic programs. Perhaps such precise programs require specialized inputs to flip the switch promptly.

Flipping the switch

General considerations

There are three categories of mechanism that could trip the switch that controls activation of cyclin:Cdk1 complexes. First, in the ‘bursting-the-dam’ type of mechanism, the accumulation of potentially active cyclin:Cdk1 complexes essentially overwhelms the switch mechanism. The ‘pressure’ of the accumulating cyclin:Cdk1 might exert its influence if, for example, the phosphorylated cyclin:Cdk1 has a low kinase activity. In this scenario, the pool might eventually drive sufficient activation of some Cdc25 to initiate the reinforcing cascade of the

switch. Second, in the 'lowering the bar' type of mechanism, the threshold level of active Cdk1 needed to activate the positive-feedback loop is reduced. Third, in the 'changing the rules' type of mechanism, a new or altered factor is produced that does not obey the rules that held the system inactive during G2. For example, the switch could be triggered by the expression of a different isoform of the Cdc25 phosphatase that does not require mitotic phosphorylation for high activity. Expression of such a rule-breaking phosphatase might be triggered by either developmental or cell cycle inputs.

In the examples discussed below, the major *in vivo* mode of flipping the switch appears to be a lowering of the bar by modulation of the relative levels of inhibitory kinase and activating phosphatase. However, experimental manipulations suggest that the switch can also be flipped by the other types of mechanism.

Box 4

The reactions underlying the mitotic switch

On the surface, the positive-feedback loops that underlie the mitotic switch appear to be simple. Cdc25 phosphatase is activated upon entry into mitosis, whereas both Wee1 and Myt1 are inhibited. All three enzymes become multiply phosphorylated upon entry into mitosis, and phosphatase treatment of isolated Wee1 and Cdc25 reverses the mitotic inhibition and activation, respectively^{a,b}. Furthermore, cyclin:Cdk1 phosphorylates each of the enzymes directly. Nonetheless, there are many indications that the feedback pathways are more complex than the direct action of cyclin:Cdk1 on the inhibitory kinases and the activating phosphatase. Indeed, *in vitro* phosphorylation of Wee1 and Myt1 by cyclin:Cdk1 is not sufficient to suppress their enzymatic activity^{a,c}. *In vitro* phosphorylation of Cdc25 by cyclin:Cdk1 does lead to activation, but this does not appear to be the full story. Other kinases, such as the polo-related kinase, contribute to the extensive mitotic phosphorylation of Cdc25, and a two-step activation time course suggests at least a couple of tiers of activation^{d,e}. Other factors modifying the activities of the kinase and/or phosphatase include the cyclin:Cdk1 accessory factor Cks1, chaperones such as Hsp90, which have dramatic influences on Wee1 activity, and a prolyl isomerase that acts specifically on the phosphorylated (thr/ser) pro sites that are produced by cyclin:Cdk1. Full understanding of the kinetic features of the mitotic switch will require a more complete description of its components and modifiers.

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Xenopus oocytes

The oocytes of *Xenopus* arrest in a premeiotic G2 phase until induced by progesterone to mature and progress into meiosis. The initiation of meiosis I is triggered by a rise in cyclin-B:Cdk1 kinase activity in an event that has been a very important model of the G2:M transition. Injection

of cytoplasm from activated eggs induces the maturation of G2-arrested oocytes (autoamplification), and this constituted the early assay for MPF (Ref. 33). The arrested oocyte has an abundant pool of cyclin-B:Cdk1 that is inactive owing to inhibitory phosphorylation by Myt1 (Ref. 26). A variety of agents that impinge on the mitotic switch can trigger activation of this pool of cyclin-B:Cdk1. Injection of even a small amount of active cyclin:Cdk1 can initiate the feedback loops, thereby flipping the switch and activating the endogenous supply of cyclin:Cdk1 (Ref. 33). Substantial levels of cyclin also activate mitosis, presumably by a version of the 'burst the dam' mechanism³⁴. Introduction of Cdc25 phosphatase alters the balance of activating phosphatase and inhibitory kinase and thereby lowers the bar to activate the switch³⁵.

In addition to integral components of the mitotic switch, other proteins also flip the switch: mos, a kinase that stimulates the MAP kinase cascade; p90rsk, a downstream kinase activated by the MAP kinase cascade; and polo-like kinase, a cell cycle kinase with roles in mitotic progression³⁵. Reassuringly, *in vitro* studies have demonstrated how these kinases might flip the switch by acting on known switch components. For example, p90rsk phosphorylates and inactivates Myt1, which would allow endogenous Cdc25 to activate the switch without the opposition of this inhibitory kinase³⁵. Activated p90rsk bypasses a need for mos and activation of the MAP kinase pathway, suggesting that its activation mediates these inputs³⁵. The pololike kinase phosphorylates and activates the Cdc25 phosphatase to flip the switch³⁵. Although this might seem like a bewildering array of kinases, the results strongly support the conclusion that it is inhibitory phosphorylation that blocks progress to meiosis I and that manipulations that drive activation of the mitotic switch relieve the block. However, what is the real pathway of activation of the switch in the oocyte?

Experimental definition of the normal pathway of activation of the mitotic switch is surprisingly difficult. We have three tools available to define the pathway. (1) We can activate it ectopically by the introduction of activities. (2) We can try to block it by inhibiting activities. (3) We can determine which components change during the normal process of activation. All these approaches have been tried, but ambiguities remain. Because the switch is metastable and remains in the off state because of the balance in the activities of several components, it is not surprising that there are several activities that can activate the switch. Nor is it surprising that there are several components that are necessary for the normal control of the switch. Finally, because the operation of the switch involves a circuit in which the various components influence each other, it is also not surprising to find that several implicated components change during oocyte activation. Despite these limitations, there has been progress. When p90rsk is activated *in vivo*, it makes an essential contribution to the progression of meiosis, and it can activate meiosis in the quiescent oocyte³⁵. However, there appears to be redundancy because activation can occur without p90rsk activity, at least in some oocytes³⁶. Despite remaining ambiguities, these results highlight an important point that I would like to make in this review – of the several possible avenues of regulation of Cdk1 that might be used (Fig. 2), the results argue strongly that the one that is actually used involves regulation of the activities of enzymes that add and remove inhibitory phosphates.

***Drosophila* G2:M transition**

During development, the cell cycle often follows stereotyped programs that require precise temporal control. The spatial patterning of mitosis 14 in the *Drosophila* embryo provides a dramatic example of the accuracy of such programs³⁷. The cells of the embryo arrive in G2 of cycle 14 at the same time after the preceding 13 synchronous cycles. The G2 quiescent cells have high levels of cyclins A, B and B3, and all the Cdk1 is inactivated by phosphorylation on T14 and/or Y15 (Refs 5, 7 and 14). These quiescent G2 cells are induced to enter mitosis according to a rigid schedule that reveals itself as detailed and stereotyped patterns of mitotic

cells displayed over the surface of the embryo³⁷. Entry into mitosis is associated with and requires transcriptional activation of CDC25^{string}, and this expression is sufficient to induce dephosphorylation of Cdk1 and to trigger mitosis^{13,19}. These experiments define CDC25^{string} as the limiting component that controls progress to mitosis and show that the stereotyped program of mitosis is secondary to a program that induces CDC25^{string} transcription in stereotyped patterns that anticipate the mitotic patterns. Reversal of inhibitory phosphorylation by CDC25^{string} expression also controls later embryonic cycles as well as the timing of mitosis in the epithelium of the imaginal discs at pupariation³⁸. These findings show that the mitotic switch integrates developmental inputs to control the timing of mitosis.

Checkpoint control

Progression through mitosis with damaged or incompletely replicated DNA could wreak genetic havoc, and special checkpoint mechanisms prevent this outcome by blocking cells in G2. Such checkpoint-arrested cells have high levels of cyclin:Cdk1 whose activity is held in check by inhibitory phosphorylation. Work in *S. pombe* has defined two kinases that mediate this arrest. The Chk1 and Cds1 kinases are activated by DNA damage and unreplicated DNA, respectively, whereupon they phosphorylate Wee and Cdc25 (Ref. 39). Unlike mitotic phosphorylation of these switch components, this phosphorylation activates Wee and suppresses Cdc25 to maintain the mitotic switch in the off state. These actions of checkpoint phosphorylation are, at least in part, mediated by the binding of phosphorylated-epitope-specific binding proteins called 14-3-3 proteins. 14-3-3 proteins bind to Wee1 and to Cdc25 upon their phosphorylation, activating or inactivating them, respectively⁴⁰. The components of this checkpoint system are conserved from *S. pombe* to vertebrates, and its operation in these highly diverged systems is very similar in format, albeit different in some of its details. Importantly, the regulation invariably operates by manipulating the mitotic switch.

Invisible inhibitory phosphorylation

The early mitotic cycles of most embryos are very fast and lack G1 or G2 phases⁴¹. In *Drosophila* and *Xenopus*, inhibitory phosphorylation is not detected during these rapid cycles^{14,42}. Despite the lack of detected inhibitory phosphorylation in frog eggs, it is detected in cycling extracts prepared from these eggs. In addition, Chk1- and Wee1-stimulated phosphorylation promotes cell cycle arrest in fly embryos and in a frog embryonic extract system upon inhibition of DNA replication^{43,44}. Importantly, Chk1^{grapes} and Wee1 function are required to regulate the normal progression of the early embryonic cycles that exhibit no inhibitory phosphorylation^{14,27,44}. Hence, inhibitory phosphorylation of a small fraction of Cdk1, presumably a special and perhaps nuclear pool, appears to be required for the regulation of these cycles⁴⁵. Although its role in these cycles is incompletely understood, the finding that inhibitory phosphorylation makes an important regulatory contribution to cycles that lack a G2 phase underscores the widespread use of this mitotic switch in the control of mitosis. Furthermore, the genetic interactions in *Drosophila* suggest that checkpoint functions and the Wee1 kinase play a more important role in controlling the mitotic switch than does the level of cyclin, even though these rapid early embryonic cycles are usually described as being driven by a cyclin oscillator.

Throwing light upon flipping the switch

Findings over the past decade have supported early work in *S. pombe* and in *Drosophila* that implicated the regulation of inhibitory phosphorylation as the key factor in controlling entry into mitosis. The components governing inhibitory phosphorylation have been shown to be conserved and to function in an interacting network to create a switch whose activation governs the transition from G2 to mitosis. The mechanisms that govern the activation of this conserved mitotic switch are responsible for controlling entry into mitosis.

In each of the cases reviewed above, the input that flips the mitotic switch influences the activities of the inhibitory kinases or the Cdc25 phosphatase. Other studies offer further support for a generalization that the mitotic switch is regulated by inputs at this level. For example, studies in yeast show that the Nim1/Cdr1 kinase and its relatives phosphorylate and suppress the activity of Wee1 to promote mitosis (Box 4). All of the recognized inputs feeding into the mitotic switch, whether polo-like kinase activation of Cdc25, Nim1 suppression of Wee1, p90rsk suppression of Myt1, checkpoint regulation of Wee1 and Cdc25, or transcriptional control of CDC25^{string}, are themselves regulated by a complex signaling pathway (Fig. 2). These inputs provide the information about the status of the cell, and, based on these inputs, the mitotic switch produces a decisive output: whether or not to proceed to mitosis.

A priori, it did not seem that the regulation of the inhibitory kinase and the opposing phosphatase would be the predominant pathway governing entry into mitosis. The destruction of cyclins at mitosis had encouraged the attractive idea that cyclin accumulation would provide a timer of cell cycle progress and cells would enter mitosis at a threshold of cyclin level. This idea was supported by tests in an extract system in which translation limited progress of the cycle and by experiments showing that oocyte maturation could be promoted by the introduction of ectopic cyclins^{34,46}. When the importance of the positive-feedback loops governing the removal of inhibitory phosphorylation were first recognized, it was suggested that the switch-like behavior acted to interpret the rising levels of cyclin, which would burst the dam at a threshold concentration to induce abrupt activation of cyclin:Cdk1 and entry into mitosis³¹. However, the results of *in vivo* analysis suggest that cyclin levels are generally not key inputs into the decision to enter mitosis. The switch successfully prevents relatively large amounts of cyclin:Cdk1 from driving mitosis until some other signal alters the relative activities of inhibitory kinases and opposing Cdc25 phosphatase to lower the bar for flipping the switch.

Is there a reason why the seemingly straightforward mechanism of cyclin-level-based trigger might not be used? Perhaps it is because specialized mechanisms are required to flip the switch with temporal precision. Developmental programs demonstrate the temporal accuracy of cell cycle control that prevails. For example, during mitosis 14 in the *Drosophila* embryo, cells programmed to divide at the same time do so with an accuracy of better than one minute, even when the cells are distant from one another (in different segments) and when the cell cycle is relatively long (~2 h). The timer must be accurate and the switch must respond promptly, but a switch that is based on positive feedback is not easily engaged. Although it is difficult to predict the kinetic features of cyclin-driven transition of the mitotic switch⁴⁷, the switch does not seem well designed to give temporally stereotyped outputs from such a signal. Indeed, the expectation is that a bursting the dam mechanism would result in a stochastic transition to mitosis and might not have the precision that biological control exhibits. I propose that the difficulties inherent in flipping a switch with high temporal precision necessitate a sophisticated mechanism that itself has switch-like properties (Box 3). Such features might be incorporated into the conserved system that regulates inhibitory phosphorylation of Cdk1. The utility of precise switching might explain why this pathway of Cdk1 regulation is chosen in so many diverse situations.

A cell must not only make a decisive commitment to mitosis – it must also make a wise decision if it is to avoid the hazards of entering the process unprepared. Consequently, the regulatory switch governing entry into mitosis responds to a wide range of inputs. Indeed, the circuitry governing the mitotic switch is a beautiful model for molecular mechanisms of information processing.

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Fig. 1.

Major cell cycle transitions show all-or-nothing regulation. Switch-like events regulate the accumulation, activity or destruction of cyclin:CDK complexes to drive the key transitions of the cell cycle. The circuitry that creates switch-like behavior is incompletely understood, but the G1:S phase transition and the initiation of anaphase are associated with abrupt and important changes in the activity of the ubiquitination complexes APC and SCF that target proteins for degradation. The partially characterized switch that governs the G2:M transition changes the activities of Cdk1-modifying enzymes to abruptly relieve the inhibition of Cdk1 activity that characterizes G2.

Fig. 2.

Tiers of Cdk1 regulation. The signals controlling Cdk1 flow through several regulatory tiers (colors). At the most immediate level (orange), Cdk1 is regulated by cyclin association, by activating phosphorylation on T161, by association with cyclin:kinase inhibitory proteins (CKIs) and by inhibitory phosphorylation on T14 and/or Y15. These immediate regulators include inducers, whose action is indicated with pointed arrows, or inhibitors, whose action is indicated by 'arrows' ending in a bar. In turn, these immediate regulators are regulated by upstream factors (turquoise). Cyclin levels are controlled at the levels of synthesis and APC-promoted destruction. The CKIs that have important regulatory inputs into Cdk1, such as Sic1 of *Saccharomyces cerevisiae*, Rum1 of *Schizosaccharomyces pombe* and RUX1 of *Drosophila*, are inactivated by CDK activity (mutual antagonism).

The components that are involved in the mitotic switch are clustered towards the top of the diagram. Regulators of inhibitory phosphorylation (Myt1 and Wee kinases, and Cdc25 phosphatase) are subject to elaborate regulation (gold arrows). These pathways flip the mitotic switch at the appropriate time. Importantly, in organisms from yeast to humans, the predominant regulatory paths used to govern entry into mitosis act through the regulators of inhibitory phosphorylation. These regulators appear to be specialized for controlling this transition. They have conserved regulatory inter-relationships that impart the switch-like behavior on the transition into mitosis. Note that specialization of some gene family members (e.g. Wee1 and Mik1 of *S. pombe*) complicates some of the generic generalizations made here. Abbreviation: MAP kinase, mitogen-activated protein kinase.

Fig. 3.

Positive feedback contributes to the mitotic switch. Activation of Cdc25 phosphatase and inhibition of the inhibitory kinase Wee1 contribute to the all-or-nothing behavior of Cdk1 activation at the entry to mitosis. Although Cdk1 phosphorylates its own modifying enzymes, the details of the regulatory loops are still incompletely understood (Box 3). The basic relationships described in this diagram have been conserved from yeast to human and apply to a second inhibitory kinase, Myt1, as well as to Wee1.