Minireview

Cyclin destruction in mitosis: a crucial task of Cdc20

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Abstract Proteolytic destruction of cyclins is a fundamental process for cell division. At the end of mitosis, degradation of mitotic cyclins results in the inactivation of cyclin-dependent kinases. Cyclin proteolysis is triggered by the anaphase-promoting complex/cyclosome (APC/C), a multi-subunit complex which contains ubiquitin ligase activity. Recent data in yeast demonstrated that a partial degradation of the mitotic cyclin Clb2, mediated by APC/C and its activator protein Cdc20, is essential and sufficient for the mitotic exit. Remarkably, a complete inactivation of cyclin-dependent kinases seems to be not essential. This review discusses recent novel insights into cyclin destruction and its implications for the mitotic exit.

Key words: Anaphase-promoting complex; Clb2; Cyclin B; Mitotic exit; Ubiquitin-dependent proteolysis

1. Cyclin degradation: a historical overview

Twenty years have passed now since Tim Hunt and his coworkers set a milestone in cell cycle research. When studying protein synthesis in sea urchin embryos, they discovered a novel protein which is destroyed every time cells divide [1]. Due to its cyclic appearance this protein was termed cyclin. In the last two decades, cyclins have been identified in all eukaryotes and their functions as regulatory subunits of cyclin-dependent kinases (CDKs) have been intensively studied.

But why do cells need to get rid of cyclins and how do they do this? The former of these questions was first addressed by Marc Kirschner's lab in the late 1980s. They demonstrated the importance of cyclin destruction by constructing a truncated version of cyclin B [2]. When the N-terminal region of sea urchin cyclin B was deleted, these cyclins could not be degraded and CDKs remained active. As a consequence, cells were arrested in mitosis. Subsequent experiments in yeast revealed that high amounts of a stabilised version of the mitotic cyclin Clb2 specifically blocked very late events in mitosis, the mitotic exit [3]. These cells arrested with chromosomes segregated to opposite poles and with elongated mitotic spindles. These results demonstrated that the inactivation of CDKs, mediated by destruction of the regulatory cyclin subunit, is needed for spindle disassembly, cytokinesis and the transition into G1 phase.

The early 1990s then brought the breakthrough in identifying the mechanism of cyclin destruction and of the cellular machinery which triggers this process. Glotzer et al. [4] found that cyclin degradation was dependent on a specific sequence element in the N-terminal region, the degenerate but highly conserved motif RxxLxxxxN, termed the destruction box. They further showed that cyclin B is degraded by the ubiquitin pathway. The ubiquitination reaction involves multiple steps and finally results in the formation of ubiquitin chains on the target protein. These modified proteins are then recognised and degraded by the 26S proteasome.

The next key step in understanding cyclin destruction was the identification of the anaphase-promoting complex/cyclosome (APC/C), an 11-subunit complex which contains ubiquitin ligase activity [5–9]. Ubiquitin ligases catalyse the last step in the ubiquitination reaction, the transfer of ubiquitin to the target proteins. APC/C is now known as a central regulator of mitosis (reviewed in [10,11]). In addition to cyclins, it also triggers destruction of many other proteins, such as securins, polo kinases and spindle-associated proteins. Securin proteolysis is essential for sister chromatid separation and thus, securin and mitotic cyclins are now known to be the main targets of APC/C in mitosis.

But how does this ubiquitin ligase trigger proteolysis of the right protein at the right time? A clue to an answer was the discovery of two proteins, known as Cdc20 and Cdh1 [12,13]. Both proteins contain WD40 repeats and are, like APC/C, highly conserved in eukaryotes (Table 1). The association of APC/C with either Cdc20 or Cdh1 is essential for its activity. It now appears that these WD40 proteins bind directly to target proteins and recruit them to the APC/C core complex [14–17]. Importantly, Cdc20 and Cdh1 bind APC/C in a sequential manner during mitosis. At the metaphase/anaphase transition, APC/C^{Cdc20} is activated and triggers securin degradation, thereby allowing separases to become active and dissolve the cohesion between sister chromatids [18]. Cdh1 binds APC/C in telophase and participates in the destruction of mitotic cyclins. APC/C^{Cdh1} remains active in the subsequent G1 phase. The temporal control of APC/C activation is mainly achieved by phosphorylation of Cdh1, thereby preventing its association with APC/C until telophase (Fig. 1). Furthermore, both complexes are regulated by spindle checkpoints [11,19]. The spindle assembly checkpoint controls APC/C^{Cdc20} activity, whereas a spindle orientation checkpoint regulates the activity of Cdc14 phosphatase, which promotes Cdh1 dephosphorylation and APC/C^{Cdh1} complex formation.

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Table 1
Cdc20 and Cdh1 family members in different eukaryotes

Family	Name	Organism	Substrate recognition motif	Period of APC/C activation/ substrate recruitment
Cdc20 family	Cdc20	*Saccharomyces cerevisiae*	D box	Anaphase
	Slp1	*Schizosaccharomyces pombe*		
	Fzy	*Drosophila melanogaster*		
	hCDC20/ $p55^{CDC20}$	*Homo sapiens*		
Cdh1 family	Cdh1/Hct1	*Saccharomyces cerevisiae*	D box+KEN box	Telophase+G1 phase
	Ste9/Srw1	*Schizosaccharomyces pombe*		
	Fzr	*Drosophila melanogaster*		
	hCDH1	*Homo sapiens*		

2. Job sharing for cyclin destruction by Cdc20 and Cdh1

The initial characterisation of Cdc20 and Cdh1 in budding yeast provoked a simple model for substrate recognition. It was proposed that Cdc20 recognises securin and thereby promotes the onset of anaphase, whereas Cdh1 recruits cyclins and triggers the mitotic exit [12,13]. However, subsequent findings revealed that Cdc20 is also needed for the exit from mitosis. A deletion of yeast securin *PDS1* suppressed the defect of *cdc20* mutants at the metaphase to anaphase transition, but these cells now arrested with elongated spindles indicating that they failed to exit mitosis [20]. These results implied that Cdc20 is required for degradation of additional target proteins. A genetic screening revealed that a deletion of Clb5, a B-type cyclin known to be important for S phase, suppressed the telophase arrest and lethality of *cdc20*Δ *pds1*Δ cells [21]. This was the first hint that Cdc20 participates in cyclin proteolysis in budding yeast.

Are then mitotic cyclins specifically recognised by Cdh1? Further studies clearly contradicted this assumption, by showing that these cyclins get degraded during anaphase independently of Cdh1. Clb2, the major yeast mitotic cyclin, was partially destroyed in a destruction box-dependent manner, as soon as APC/C^{Cdc20} was activated at the metaphase/anaphase transition [22,23]. In parallel to Clb2, Cdk1 activity also dropped to a lower level. It was estimated that approximately 50% of Clb2 normally present in metaphase cells gets degraded in a Cdc20-dependent manner [23]. Intriguingly, a second fraction of Clb2 remained stable during anaphase suggesting that it was protected from APC/C^{Cdc20}. Proteolysis of this Clb2 fraction was dependent on APC/C^{Cdh1} activation in telophase. The conclusion from these results was that Cdc20 initiates destruction of mitotic cyclins, but it can only partially fulfil this task. Cdh1 is required to take over Cdc20's job to finally complete Clb2 destruction. Thus, Clb2 is degraded in mitosis by a division of labour between Cdc20 and Cdh1, but until recently, it was unknown whose job is relevant for the mitotic exit.

3. An essential role of Cdc20: destruction of Clb2

Initial results suggested that Clb5 is the only important target of APC/C^{Cdc20} among the cyclins, but a recent paper by Wäsch and Cross [24] came to completely new conclusions. They showed that Cdc20 performs a crucial task by targeting Clb2 for degradation, by analysing Clb2 and Clb5 proteins lacking their cyclin destruction boxes, Clb2ΔDB and Clb5ΔDB. *CLB2ΔDB* expressed from its own promoter effi-

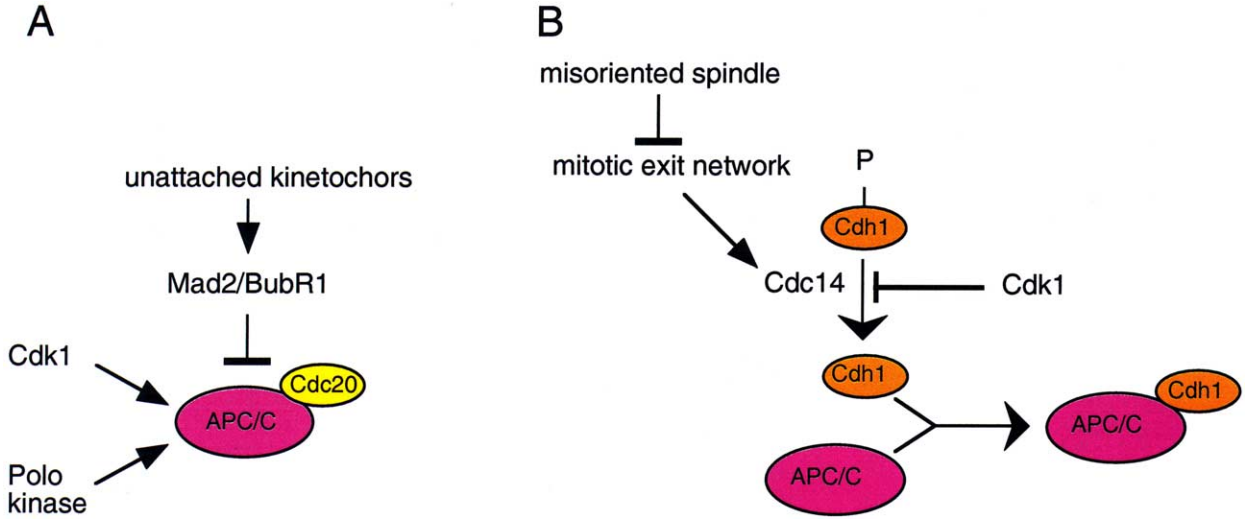

Fig. 1. Regulation of APC/C^{Cdc20} and APC/C^{Cdh1}. A: APC/C^{Cdc20} activation in metaphase requires phosphorylation of specific APC/C subunits by cyclin-dependent kinase 1 (Cdk1) and polo kinase. Kinetochores not attached to the mitotic spindle (or not under tension) activate the spindle checkpoint. Two spindle checkpoint proteins, Mad2 and BubR1, bind Cdc20 and inhibit its activity until all kinetochores achieve a bipolar attachment [11]. B: Cdh1 is phosphorylated by Cdk1 and thereby its binding to APC/C is prevented. In late anaphase/telophase, Cdc14 dephosphorylates Cdh1 and allows APC/C^{Cdh1} complex formation and activation. Cdc14 activation requires a group of proteins termed the 'mitotic exit network' (MEN), which triggers the release of Cdc14 from the nucleolus. The MEN is controlled by a spindle checkpoint monitoring the orientation of the mitotic spindle (for review see [31]).

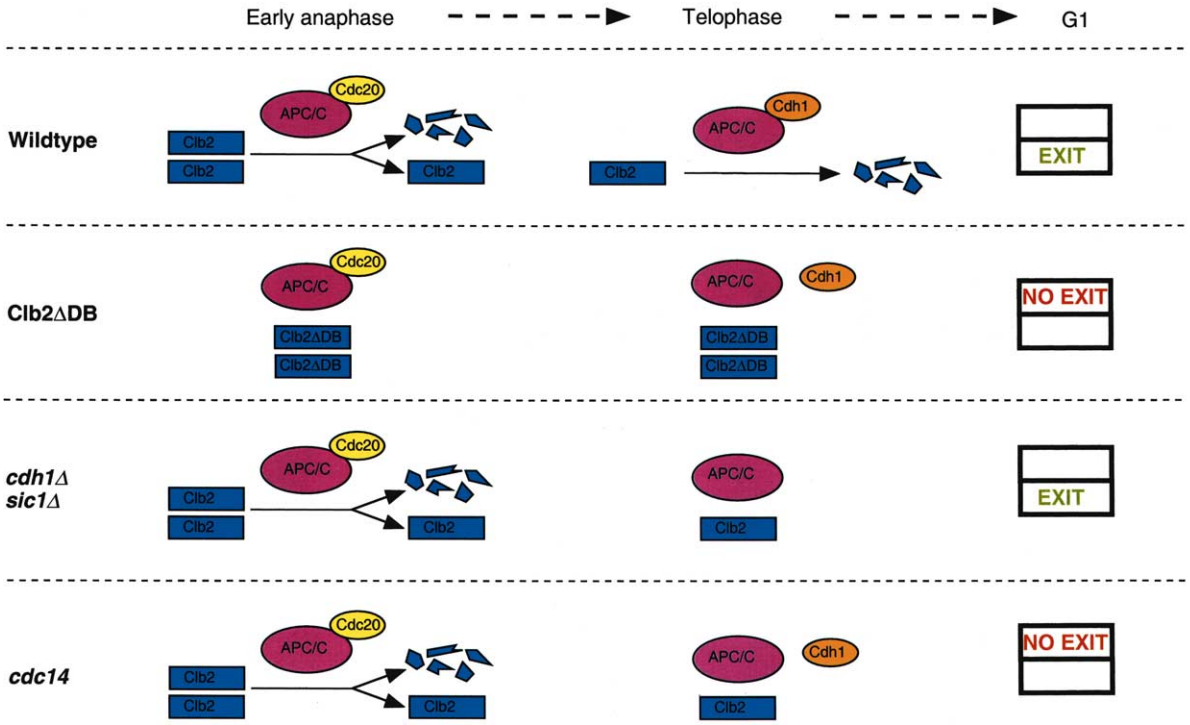

Fig. 2. Clb2 degradation and the exit from mitosis in budding yeast. In wild-type yeast cells, Clb2 is degraded in a biphasic manner: in early anaphase by APC/C^{Cdc20} and in telophase by APC/C^{Cdh1} [22,23]. Cyclin degradation results in Cdk1 inactivation and mitotic exit. Clb2 with a destruction box deletion (Clb2ΔDB) is stable throughout mitosis and blocks the transition into G1 phase [24]. In cells lacking both Cdh1 and Sic1 (*cdh1Δ sic1Δ*), a fraction of Clb2 is degraded by APC/C^{Cdc20}. The second fraction of Clb2 is not degraded and Cdk1 remains partially active, but nevertheless, these cells exit mitosis. In contrast, APC/C^{Cdc20}-mediated degradation of Clb2 is not sufficient for the mitotic exit in *cdc14* mutants, indicating that Cdc14 has essential targets other than Cdh1 and Sic1.

ciently blocked the mitotic exit demonstrating that Clb2 degradation is essential for this cell cycle transition (Fig. 2). In contrast, *CLB5ΔDB* expressed from either its own or a strong inducible promoter failed to arrest cells in telophase [24,25].

How do these results fit with the viability of *cdc20Δ pds1Δ clb5Δ* cells, which suggested that Clb5 is an essential target of APC/C^{Cdc20} [21]? A possible explanation was provided by Surana and co-workers [26]. In agreement with similar data from David Pellman's lab [27], they showed that Clb5-associated Cdk1 participates in turning off APC/C^{Cdh1} in early S phase. In *clb5Δ* strains, APC/C^{Cdh1} did not become completely inactivated, as manifested by less phosphorylated forms of Cdh1. As a consequence, Clb2 did not accumulate to normal levels. This may indicate that Cdc20 is not essential for cyclin destruction in *clb5Δ* strains because APC/C^{Cdh1} is abnormally active in these cells. Thus, a major role of Clb5 is likely APC/C^{Cdh1} inactivation in early S phase rather than controlling the mitotic exit.

4. Mitotic exit without Cdk1 inactivation

The results of Wäsch and Cross [24] imply that a partial Cdk1 inactivation caused by APC/C^{Cdc20}-mediated Clb2 proteolysis is essential and sufficient for most aspects of the mitotic exit. In contrast, proteolysis of the remaining Clb2 protein by APC/C^{Cdh1} and complete Cdk1 inactivation are dispensable. It was already known that cells lacking Cdh1 are viable, because yeast cells have a redundant mechanism for Cdk1 inactivation [12]. The Cdk1 inhibitor protein Sic1 accumulates in late mitosis and contributes to kinase inactivation [28]. Thus, in the absence of Cdh1, Sic1 alone is sufficient to turn off Cdk1. Cells lacking both Cdh1 and Sic1 were found to be non-viable and it was thought that this is due to a failure to inactivate Cdk1 in late mitosis [12]. These double mutants, kept alive by a *GAL-SIC1* construct, were now reinvestigated [24]. Intriguingly, *cdh1Δ sic1Δ* mutants were able to exit from mitosis, albeit with a slight delay and some abnormal morphologies (Fig. 2). Kinase assays confirmed that this cell cycle transition occurred in the presence of active Cdk1/Clb2 kinase suggesting that a complete inactivation of this kinase is not essential for the mitotic exit.

The persisting Cdk1/Clb2 activity in *cdh1Δ sic1Δ* cells indicates that no other factor is able to replace Cdh1 and Sic1 in the inactivation of this kinase. Cdc6, a factor required for DNA replication, was earlier shown to contribute to Cdk1 inhibition in late mitosis [29]. One possibility could be that Cdc6 inhibits Cdk1 only locally, perhaps at origins of replication. Such a model would be consistent with the findings that *cdh1Δ sic1Δ* mutants are able to re-initiate DNA replication in the presence of partially active Cdk1 [24]. Since this process is dependent on low Cdk1 activity [30], a localised kinase inactivation at origins of DNA replication may allow the reloading of pre-replicative complexes.

5. A new role of Cdc14 in the mitotic exit?

The mitotic exit of *cdh1Δ sic1Δ* mutants has also posed new questions about Cdc14, a phosphatase essential for the exit from mitosis. Cdc14 is kept inactive in the nucleolus for most of the cell cycle and is released during anaphase [31]. It was

thought that Cdc14's key role is to inactivate cyclin-dependent kinases, by dephosphorylating Cdh1 and Sic1 [32,33], but the dispensability of these Cdc14 targets for the mitotic exit enforces a modification of this model. Unlike *cdh1*Δ *sic1*Δ deletions strains, *cdc14* mutants are blocked in telophase at their restrictive temperature (Fig. 2), suggesting that Cdc14 has at least one additional and essential target. Further known substrates of Cdc14 phosphatase are Cdc15 [34] and Bfa1 [35], two factors of the mitotic exit network, and Swi5 [32], a transcription factor whose dephosphorylation allows its nuclear entry. It remains to be shown whether the essential role of Cdc14 in the mitotic exit is dephosphorylation of one of these substrates or of a new one.

The persistent telophase arrest caused by the non-destructible Clb2ΔDB protein further implies that a partial Clb2 degradation is required for Cdc14 function. It is known that highly active Cdk1 does not inhibit the release of Cdc14 phosphatase from the nucleolus, but rather antagonises Cdc14 activity [36]. Cdc14's capacity to dephosphorylate its targets appears to be insufficient in the presence of a highly active kinase. It seems that Cdc14 phosphatase can only win the competition against Cdk1/Clb2, if this kinase is partially inactivated by Cdc20-mediated Clb2 destruction. This dependence of Cdc14 on partial Cdk1 inactivation ensures that Cdc20 function always precedes Cdc14 activation and thereby may help to coordinate sister chromatid separation with the mitotic exit.

6. Conserved roles of Cdc20 and Cdh1 in cyclin destruction

The recent new data on cyclin destruction in yeast imply that the roles of Cdc20 and Cdh1 are much more evolutionarily conserved than initially thought. In most eukaryotes, Cdc20 is obviously the key factor for cyclin degradation during mitosis, whereas Cdh1 is dispensable for mitosis, but important during G1 phase.

The involvement of mammalian Cdc20 in cyclin proteolysis was first demonstrated with in vitro experiments showing that APC/C^{Cdc20} is able to trigger ubiquitination of cyclin B [37,38]. Strong evidence for an in vivo role of Cdc20 in this process was just recently provided [39]. Human cells transfected with a modified cyclin B, containing a substitution of the destruction box by a KEN box, arrested in telophase, although this modified cyclin was shown to be a substrate of Cdh1. Since Cdh1 cannot fulfil Cdc20's function, human Cdc20 is obviously essential for cyclin B degradation in mitosis.

Cyclin B degradation is also triggered by APC/C^{Cdc20} in *Xenopus* and *Drosophila* embryos. Embryonic cell cycles are composed of alternating S and M phases lacking intervening gap phases. Importantly, *CDH1* is not expressed in early embryonic cells of these organisms [40,41]. A recent paper demonstrated that the *Drosophila* Cdh1 homologue Fzr is indeed absent and not required for mitosis [42]. By excluding the possibility that small amounts of maternally derived Fzr/Cdh1 contribute to the mitotic exit, the authors showed that the Cdc20 homologue Fzy alone is sufficient for the completion of mitosis in *Drosophila* embryos and that Fzr/Cdh1 is needed exclusively for the subsequent G1 phase.

This function of Cdh1 in G1 phase is obviously also highly conserved in eukaryotes. Yeast cells and *Drosophila* embryos depleted of Cdh1 fail to arrest the cell cycle in G1 phase and ultimately initiate DNA replication [12,40,43]. A primary function of Cdh1 in G1 cells is probably to inhibit abnormal cyclin accumulation and CDK activation, thereby preventing an unscheduled entry into S phase. Cdh1 is also responsible for degradation of non-cyclin proteins, such as polo kinase, spindle-associated proteins and kinesins [11]. The viability of yeast *cdh1*Δ mutants implies that proteolysis of none of these substrates is essential, but their removal is probably important for a proper coordination of events in the subsequent cell cycle. APC/C^{Cdh1} is also expressed during G1 and G0 in mammalians cells, as well as in terminally differentiated cells such as neurones [44,45]. How Cdh1 regulates differentiation is still unknown, but there is now increasing evidence that APC/C^{Cdh1} has non-cell cycle functions, such as the regulation of transforming growth factor-β signalling [10,11].

7. Spatial and temporal control of cyclin B degradation in *Drosophila*

A biphasic degradation of mitotic cyclins has also been observed in organisms other than yeast. In *Drosophila* cells, proteolysis of cyclin B initiates at centrosomes and then spreads along the mitotic spindle towards the spindle equator [46]. Remarkably, in *Drosophila* mutants in which centrosomes are detached from the mitotic spindle, cyclin B disappeared from centrosomes, but not from the spindle [47]. Cytoplasmic cyclin B was degraded slightly later than spindle-associated cyclins. Thus, cyclin B is degraded in two waves, first on spindles and then in the cytoplasm. Real-time studies in living human cells also demonstrated temporally and spatially separable phases of cyclin B destruction [48]. As soon as the last chromosome aligned on the metaphase plate, cyclin B was first eliminated from spindle poles and chromosomes and only later from other regions of the cell.

Jordan Raff and colleagues [49] recently analysed the roles of Fzy/Cdc20 and Fzr/Cdh1 in the spatial and temporal destruction of cyclin B in *Drosophila* embryos. They found that both green fluorescent protein-tagged Fzy/Cdc20 and Fzr/Cdh1 proteins were associated with spindles in vivo and bound microtubules in vitro. In living *Drosophila* embryos, Fzy/Cdc20 localised predominantly at centrosomes, kinetochores and on spindles in early mitosis. Fzr/Cdh1 was found at centrosomes throughout the cell cycle. They then monitored cyclin B destruction in living early, syncytial embryos, which lack detectable Fzr/Cdh1, and in cellularised embryos, which contain normal levels of Fzr/Cdh1. Intriguingly, only spindle-associated cyclin B was degraded at the end of mitosis in syncytial embryos. Levels of cytoplasmic cyclin B remained unchanged suggesting that Fzy/Cdc20 triggers destruction of cyclin B only on spindles. In cellularised embryos cyclin B was degraded throughout the cell.

These findings with living embryos are consistent with earlier data showing that cyclin B is only partially degraded at the end of mitosis in syncytial embryos [50]. Furthermore, it was shown that cyclin-dependent kinase Cdk1 was only locally inactivated near the centrosomes, but remained active in other parts of the embryo [51]. It appears that cyclin B degradation on mitotic spindles, mediated by Fzy/Cdc20, and localised Cdk1 inactivation is sufficient for the mitotic exit. This function of Fzy/Cdc20 is essential, because a modified cyclin B lacking a functional destruction box was not degraded on spindles in syncytial embryos and blocked cells in mitosis [49].

Raff et al. [49] proposed the following model for the biphasic degradation of cyclin B: Fzy/Cdc20 catalyses destruction of cyclin B exclusively on mitotic spindles and this localised inactivation of Cdk1 allows Fzr/Cdh1 activation at centrosomes. Cdh1/Fzr then spreads throughout the cell and triggers proteolysis of the remaining cyclin B. How Fzy/Cdc20 activity is restricted to the spindle is unknown, but it was proposed that only the pool of Fzy/Cdc20 which has been loaded onto the spindles via kinetochores is competent to degrade cyclin B.

8. Conclusions

It remains to be shown whether yeast Cdc20 and Cdh1 are localised in a similar manner as their homologues in *Drosophila* and whether the biphasic Clb2 degradation is due to a restriction of Cdc20 function to spindles. However, the last few months have provided some unexpected similarities in cyclin destruction among eukaryotes. In both yeast and *Drosophila*, mitotic cyclins are degraded in two steps, and in both organisms, only the first, Cdc20-dependent step is essential for the exit from mitosis. The mitotic exit of *Drosophila* syncytial embryos and of yeast *cdh1*Δ *sic1*Δ mutants suggests that complete Cdk1 inactivation is dispensable for this cell cycle transition. It will be an interesting task to find out how specifically Cdk1 associated with the Cdc20-sensitive fraction of cyclins affects the exit from mitosis.

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