

# The self-destructive personality of a cell cycle in transition

Raymond J Deshaies

California Institute of Technology, Pasadena, USA

The transition from G<sub>1</sub> to S phase, sister chromatid separation in anaphase, and the exit from mitosis are driven by the destruction of cell cycle regulatory proteins by distinct ubiquitin-dependent proteolytic pathways. The components and targets of these key degradation pathways are now becoming clear. Genetic and biochemical dissections of these extremely specific and well regulated destruction pathways are providing fundamental insights into the mechanisms of control of the cell division cycle.

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## Introduction

Since the discovery that cyclin B destruction is required for exit from mitosis, it has become apparent that protein degradation plays a pervasive role in the regulation of the cell division cycle. Entry into S phase, separation of sister chromatids during anaphase, and exit from mitosis all require the degradation of specific proteins. This degradation is mediated by the ubiquitin/26S proteasome dependent system. Several key cell cycle regulatory proteins are unstable (see Table 1), including cyclins A and B, budding yeast G<sub>1</sub> cyclins, and the cyclin-dependent kinase (Cdk) inhibitor p40<sup>SIC1</sup>. For each of these proteins, ubiquitin-mediated degradation plays a prominent role both in the regulation of the protein's activity and in the regulation of cell physiology. Mutations that block cyclin A and cyclin B degradation arrest the cell cycle in mitosis [1,2], whereas mutations that block p40<sup>SIC1</sup> degradation block both exit from G<sub>1</sub> phase and the initiation of DNA replication [3•]. In contrast, cells with stabilized G<sub>1</sub> cyclins continue to cycle but are unable to regulate correctly progression through G<sub>1</sub> phase [4–6].

For an introduction to the mechanism and regulation of ubiquitin-dependent protein degradation, I direct you to the recent review by Hochstrasser [7]. To recap briefly, the proteasome degrades proteins that contain covalently linked multi-ubiquitin chains. Ubiquitin is attached to proteins in a multistep process. First, ubiquitin is attached via its carboxyl terminus to the ubiquitin-activating (E1) enzyme. E1-bound ubiquitin is then transferred to ubiquitin-conjugating (E2) enzymes. Whereas most eukaryotic cells are thought to contain a single E1, there are at least 12 genes in budding yeast that encode E2 enzymes. In many cases ubiquitin can be transferred directly from a ubiquitin-charged E2 to a substrate protein. Nevertheless, many physiological ubiquitination events may require the activity of a ubiquitin ligase

referred to as E3. Two distinct classes of E3 are known, and they are not homologous to one another. Thus it may be impossible to deduce by molecular approaches whether there are few or many E3s present in the cell as a common sequence motif is not shared by all E3s. Substrate proteins bearing covalently attached multi-ubiquitin chains are selectively recognized by the 26S proteasome, which then completely degrades the ubiquitinated polypeptide and releases free ubiquitin. This free ubiquitin can then go on to participate in further cycles of ubiquitination and degradation.

In this review I discuss advances from the past 18 months that highlight the fundamental role played by the ubiquitin system in the regulation of the basic cell division program. Thanks to a propitious convergence of biochemical and genetic approaches, much progress has been made recently in identifying components and substrates of the ubiquitin pathways that control the entry into S phase, the initiation of sister chromatid segregation, and the exit from mitosis. For earlier reviews on the mechanism and regulation of cyclin B degradation please refer to King *et al.* [8] and Murray [9]. For a discussion of the role of p53 degradation in the regulation of cell proliferation, see [10].

## The role of ubiquitin-dependent proteolysis in the G<sub>1</sub>/S transition

### The CDC34 pathway

An important role for ubiquitin-mediated proteolysis in cell cycle control was suggested by the discovery seven years ago that the *CDC34* gene of *Saccharomyces cerevisiae* encodes an E2 enzyme [11]. *CDC34* is required for the exit from G<sub>1</sub> phase, and temperature-sensitive mutants (*cdc34<sup>ts</sup>*) incubated at the non-permissive temperature show high levels of G<sub>1</sub> phase cyclin–CDC28 kinase activity (CDC28 is the *S. cerevisiae* homolog of the ubi-

### Abbreviations

APC—Anaphase-Promoting Complex; Cdk—Cyclin-dependent kinase; CSF—cytostatic factor; E1—ubiquitin-activating enzyme; E2—ubiquitin-conjugating enzyme; E3—ubiquitin ligase; MAP—mitogen-activated protein; PP1—protein phosphatase 1; UBC4—human ubiquitin-conjugating enzyme 4.

**Table 1.** A partial list of unstable cell cycle regulatory proteins.

Protein	Remarks	References
<i>cdc18</i> , <i>CDC6</i>	Presumed to be unstable because <i>cdc18<sup>+</sup></i> and <i>CDC6</i> functions disappear rapidly in promoter shut-off experiments	[34**,63]
<b>CENP-E*</b>	Degraded in mitosis after cyclin B; contains destruction box-like sequence and two PEST elements	[45]
<b>CLB2</b>	Destruction in G <sub>1</sub> and mitosis requires a destruction box and APC; stabilized CLB2 blocks exit from mitosis	[34**,56**]
<b>CLB5</b>	Unstable throughout cell cycle; role of destruction box untested	[33*]
<b><i>CLN2</i>, <i>CLN3</i></b>	Maximal degradation requires <i>CDC34</i> , <i>CDC28</i> ; contain PEST elements; stabilized <i>CLN2</i> and <i>CLN3</i> deregulate START	[19*,20*,21]
<b><i>c-Mos</i></b>	Degraded upon fertilization of <i>Xenopus</i> eggs; destruction requires second and third codons, but doesn't occur by N-terminal rule pathway	[64]
<b><i>cyclin A</i></b>	Destruction in mitosis requires a destruction box, and 'cyclosome' (APC) stabilized cyclin A blocks exit from mitosis	[32*]
<b><i>cyclin B</i></b>	Destruction in mitosis requires a destruction box and APC/cyclosome; stabilized cyclin B blocks exit from mitosis	[30**,32*]
<b>FAR1</b>	Degradation occurs only after START and requires amino terminus	[18*]
<b>NIMA</b>	Degraded during mitosis; contains destruction box and PEST elements; deletion of carboxy-terminal PEST elements stabilizes NIMA and blocks exit from mitosis; destruction box not yet tested	[46*]
<b>OHO-31</b>	<i>Drosophila</i> tumor suppressor protein related to nuclear import factor 'importin'; contains multiple copies of destruction box like sequence; disappearance from nucleus between prophase and anaphase may be the result of regulated transport or destruction	[48,49]
<b>p40<sup>SIC1</sup></b>	Degradation after START requires <i>CDC34</i> , <i>CDC4</i> , and <i>CDC53</i> ; contains PEST sequences; p40 degradation required for S phase	[3**]
<b>p53</b>	Naturally unstable, but degradation accelerated by interaction with human papilloma virus (HPV) E6 protein and cellular E6-associated protein in HPV-infected cells	[10]
sister chromatid cohesion factor ('RESIST')	A hypothetical protein; postulated to hold sister chromatids together until it is degraded by destruction box dependent pathway in mitosis	[44]
<b>Thymidine kinase</b>	Degraded in mitosis; intriguingly, degradation persists in early G <sub>1</sub> see [56**]; carboxyl terminus required for degradation	[47]

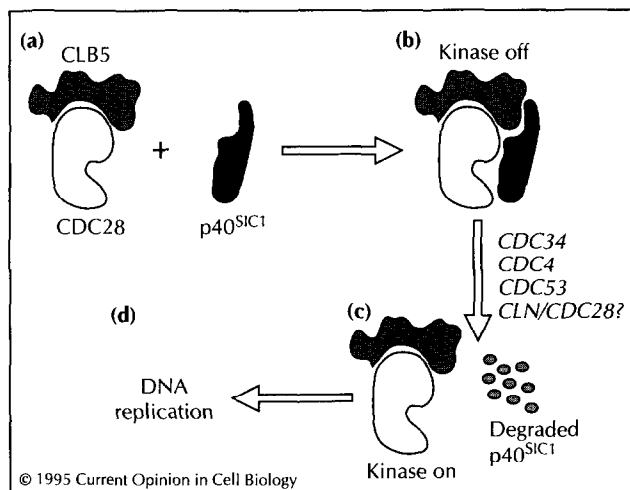
\*Proteins in boldface have been shown to be unstable; proteins in bold italics are known to be ubiquitinated.

quitos p34<sup>cdc2</sup> cyclin-dependent kinase). Such mutants also continue to produce new buds and fail to initiate DNA replication. In addition to *CDC34*, *CDC4* and *CDC53* are also required for the exit from G<sub>1</sub> phase. Any combination of *cdc4<sup>ts</sup>*, *cdc34<sup>ts</sup>* and *cdc53<sup>ts</sup>* double mutants is synthetically lethal, and overexpression of

*CDC4* suppresses *cdc53<sup>ts</sup>* and vice versa [12]. Similar genetic interactions have often been detected between genes that encode physically interacting polypeptides. The DNA sequence of *CDC4* [13] predicts an 86 kDa protein that contains eight copies of the WD-40 repeat [14]. The DNA sequence of *CDC53* predicts a 94 kDa

protein with no discernible sequence motifs (M Goebel, personal communication).

Although CDC34 was one of the first ubiquitin pathway components to be implicated in cellular regulation, its role remained elusive until last year, when Schwob *et al.* [3\*\*] demonstrated that CDC34 is required for the cell cycle regulated destruction of the cyclin-dependent kinase inhibitor p40<sup>SIC1</sup> (Fig. 1). The gene encoding p40<sup>SIC1</sup> was independently identified by two groups. Nugroho and Mendenhall [15\*] purified p40<sup>SIC1</sup> and then cloned SIC1 on the basis of the ability of p40<sup>SIC1</sup> to serve as a tightly bound substrate of CDC28. Johnston's group [16\*\*] isolated the SIC1 gene (which they dubbed SDB25) as a multicopy suppressor of a mutant, *dbf2<sup>ts</sup>*, which fails to exit mitosis at the non-permissive temperature. DNA sequencing and gene disruption experiments revealed that p40<sup>SIC1</sup> is a novel protein that is dispensable for cell viability. In wild-type cells p40<sup>SIC1</sup> begins to accumulate during late mitosis, persists throughout early G<sub>1</sub> phase, and is degraded following the execution of START in late G<sub>1</sub> phase [3\*\*,16\*\*]. In *cdc4<sup>ts</sup>*, *cdc34<sup>ts</sup>* and *cdc53<sup>ts</sup>* mutants, however, p40<sup>SIC1</sup> persists and the cells fail to traverse from G<sub>1</sub> to S phase [3\*\*]. Because recombinant p40<sup>SIC1</sup> potently inhibits the activity of a specific cyclin B–CDC28 complex required for the initiation of DNA replication, the accumulation of p40<sup>SIC1</sup> in *cdc4<sup>ts</sup>*, *cdc34<sup>ts</sup>* and *cdc53<sup>ts</sup>* mutants would be expected to block the initiation of DNA synthesis. In fact, deletion of SIC1 suppresses the G<sub>1</sub>→S arrest phenotype of these *cdc* mutants, indicating that p40<sup>SIC1</sup> is a crucial target of the CDC4/34/53 pathway [3\*\*]. A simple model for the role of p40<sup>SIC1</sup> and CDC34 in the G<sub>1</sub>→S transition is presented in Figure 1.



**Fig. 1.** A molecular model for the G<sub>1</sub>→S transition. The activity of CLB5–CDC28 heterodimers (a) produced during G<sub>1</sub> phase is quenched by p40<sup>SIC1</sup>, which binds to and inhibits the kinase complex (b). The action of the CDC4, CDC34 and CDC53 genes leads to the disappearance of p40<sup>SIC1</sup> (c), thereby freeing CLB5–CDC28 to phosphorylate replication proteins and trigger the initiation of DNA synthesis (d). The destruction of p40<sup>SIC1</sup> by the CDC4/34/53 pathway may require the activity of G<sub>1</sub> phase cyclin–CDC28 complexes that are immune to the repressive influence of p40<sup>SIC1</sup>.

### Other substrates of CDC34

Although *cdc34 sic1* Δ double mutants fail to arrest at the G<sub>1</sub>→S transition, they do remain temperature-sensitive for division, suggesting that the accumulation of a CDC34 substrate besides p40<sup>SIC1</sup> can block cell division in G<sub>2</sub> or M phase [3\*\*]. Other candidate substrates for CDC34 include the transcription factor GCN4 [17\*], the G<sub>1</sub> cyclin–Cdk inhibitor FAR1 [18\*] and the G<sub>1</sub> cyclins CLN2 [19\*] and CLN3 [20\*]. Note that G<sub>1</sub> cyclin stability in *cdc34<sup>ts</sup>* mutants may be modulated by strain background: other reports claim that CLN2 [21] and CLN3 [6] are not stabilized in *cdc34<sup>ts</sup>* cells.

All putative Cdc34p substrates share two features in common. First, Cdc34p substrates contain segments rich in proline, glutamic acid, serine, and threonine. These PEST sequences (single-letter code for amino acids) are commonly found in unstable proteins. Mutational analyses have shown that specific PEST segments of CLN2 [21], CLN3 [20\*], and GCN4 [17\*] are important for maximum rates of proteolysis but are neither essential nor sufficient for degradation. Second, all CDC34 substrates except for GCN4 (which is possibly phosphorylated by a distinct kinase) are phosphorylated in a CDC28-dependent manner before their degradation. Substrate phosphorylation may play an important role in CDC34-mediated degradation as the rate of both CLN2 [19\*] and CLN3 [20\*] degradation is diminished in *cdc28<sup>ts</sup>* mutants. Indeed, a putative CDC28 phosphorylation site is required for degradation of a hybrid protein that contains a small fragment of CLN3 fused to β-galactosidase. It remains unclear, however, how CDC28 promotes the degradation of intact CDC34 substrates. CDC28-dependent phosphorylation may reveal a cryptic ubiquitination site on the substrate and/or activate a component of the CDC4/34/53 pathway. CDC34 is phosphorylated by an unknown kinase on serine residues *in vivo*, but the function of this modification and when it occurs have not yet been examined [22].

### Is the CDC34 pathway regulated by a checkpoint?

The p40<sup>SIC1</sup> protein is not crucial for the timing of G<sub>1</sub> phase, as cells lacking p40<sup>SIC1</sup> do not enter S phase precociously [3\*\*]. Why then has the G<sub>1</sub>→S transition evolved to depend upon proteolysis of this non-essential protein? In mammalian cells double-stranded breaks in DNA provoke cell cycle arrest at the G<sub>1</sub>→S transition by a p53-dependent mechanism [23]. Yeast cells with damaged DNA also pause during G<sub>1</sub> phase at START and at or shortly after the CDC4-dependent step [24]. Perhaps p40<sup>SIC1</sup> functions in a checkpoint pathway to delay the initiation of DNA synthesis in cells that have suffered DNA damage, or in haploid yeast cells which are undergoing mating-type switching (which is initiated by an HO endonuclease dependent double-stranded break at the mating-type locus). It will be interesting to see whether the phosphorylation or destruction of p40<sup>SIC1</sup> is inhibited in cells containing damaged DNA.

### Is the *CDC34* pathway evolutionarily conserved?

To borrow J Monod's famous sound-bite, what is true for yeast is true for humans (cell cycle researchers have yet to investigate the biology of *Elephas maximus* cells). A human *CDC34* homolog was isolated in a screen for human genes that could rescue a yeast mutant that fails to arrest cell division in the presence of unreplicated DNA [25]. Yeast and human *CDC34* may perform analogous functions: human *CDC34* suppresses the thermosensitive growth of *cdc34<sup>ts</sup>* yeast strains [25], and human *CDC34* can ubiquitinate the G<sub>1</sub> phase Cdk inhibitor p27<sup>KIP1</sup> [26]. It seems likely that the entire *CDC4/34/53* pathway is conserved amongst eukaryotes, as *CDC4* and *CDC53* are homologous to the *Caenorhabditis elegans* *lin-23* and *lin-19* genes, respectively (E Kipreos, W-W He, E Hedgecock, abstract p.83, 10th International *C. elegans* Meeting, 1995). In contrast to the cell division arrest of *cdc4<sup>ts</sup>* and *cdc53<sup>ts</sup>* mutants in yeast, *lin-19* and *lin-23* mutants exhibit hyperplasia of multiple tissues, suggesting that their wild-type counterparts normally restrain cell division. *CDC53* and *lin-19* are members of a conserved multigene family: five homologs have been detected in humans, four in *C. elegans*, and two in *Arabidopsis*.

### The role of ubiquitin-dependent proteolysis in the metaphase/anaphase transition

#### Cyclin degradation and the APC pathway

Cyclin B was first identified on the basis of its cyclic accumulation in clam eggs; cyclin B steadily accumulates during interphase and is rapidly destroyed by a ubiquitin-dependent pathway during anaphase [27]. Cyclin B is targeted for destruction in mitosis because it possesses a sequence near its amino terminus referred to as the 'destruction box'; cyclin A also has this sequence. Mutant proteins with deletions or point mutations in the destruction box are neither ubiquitinated nor degraded, and the persistent cyclin locks the cell in a mitotic state. In most cases the presence of the destruction box alone is insufficient to ensure cyclin B degradation, and cyclin B mutants that fail to bind p34<sup>cdc2</sup> are stable [28,29]. Following cyclin degradation the cell returns to an interphase state and the cyclin degradation machinery is shut off, allowing cyclins to reaccumulate for the next mitosis.

Ubiquitination and degradation of a fragment of cyclin B containing a destruction box have been reconstituted in both frog [30••] and clam [31,32•] egg extracts. Fractionation experiments indicate that destruction box dependent ubiquitination requires three components: E1 enzyme, a specific E2 enzyme, and a 1000–1500 kDa complex referred to as either the 'cyclosome' or 'Anaphase-Promoting Complex' (APC) [30••,32•]. Whereas the E1 and E2 enzymes are equally active in mitotic and interphase extracts, the APC is only active in mitotic extracts. At least two distinct *Xenopus* E2s, one of which is related to human ubiquitin-conjugating enzyme 4 (UBC4), can

catalyze ubiquitination of cyclin B [30••]. In *S. cerevisiae* the UBC9 ubiquitin-conjugating enzyme has been implicated in the destruction of two distinct B-type cyclins, CLB2 and CLB5 [33•]. Surprisingly, a *Xenopus* homolog of UBC9 is neither necessary nor sufficient for mitosis and destruction box specific ubiquitination *in vitro* [30••]. There is no direct evidence that budding yeast UBC9 is involved in destruction box dependent proteolysis, and it is possible that UBC9 indirectly regulates cyclin B stability, or that CLB2 and CLB5 are degraded by multiple pathways in intact yeast cells.

Genes involved in cyclin B proteolysis in *S. cerevisiae* were recently identified by screening for mutants that accumulate a cyclin B- $\beta$ -galactosidase chimera in yeast cells that have been depleted of G<sub>1</sub> cyclins [34••]. Whereas wild-type cells fail to accumulate detectable  $\beta$ -galactosidase because the cyclin B- $\beta$ -galactosidase fusion, like natural cyclin B, is destroyed during G<sub>1</sub> phase, cells with *cdc16<sup>ts</sup>*, *cdc23<sup>ts</sup>* and *cse1<sup>ts</sup>* mutations accumulate high levels of  $\beta$ -galactosidase activity and cyclin B protein. *CDC16*, *CDC23* and *CSE1* were previously identified as being required for or involved in chromosome segregation and anaphase. Co-immunoprecipitation studies indicate that CDC16 and CDC23 assemble into a complex with CDC27 (which is another protein implicated in chromosome segregation) [35]. Befitting their fundamental role in anaphase these proteins are highly conserved, and human homologs of CDC16 (CDC16Hs) and CDC27 (CDC27Hs) have been identified in sequence databases [36•]. *CDC27* and *CDC16* homologs required for anaphase progression have also been identified in *Schizosaccharomyces pombe* [37•] and in *Aspergillus nidulans* [38], suggesting that an anaphase-promoting complex that contains CDC16–CDC23–CDC27 may be present in all eukaryotes. Immunofluorescence experiments indicate that CDC16Hs and CDC27Hs are components of the mitotic spindle and centrosomes, and microinjection of anti-CDC27Hs antibodies into human cells blocks mitosis in metaphase [36•]. Anti-CDC27Hs and anti-CDC16Hs sera also detect related proteins in *Xenopus* extracts, and anti-CDC27Hs antibodies immunodeplete a factor from *Xenopus* egg extracts that is required for destruction box dependent ubiquitination [30••]. Besides *Xenopus* CDC27, anti-CDC27Hs immunoprecipitates contain *Xenopus* CDC16 and six other unidentified polypeptides. To underscore its fundamental role in promoting the transition from metaphase to anaphase in eukaryotes ranging from yeast to man, the name 'Anaphase-Promoting Complex' has been bestowed upon the large complex containing *Xenopus* CDC16, CDC27, and (presumably) CDC23.

#### Other substrates of the APC pathway

Whereas non-degradable cyclin B blocks the budding yeast cell cycle in late anaphase [39], cells with thermosensitive *CDC16*, *CDC23* [34••] or proteasomal [40–42] gene products arrest in metaphase at the non-permissive temperature. Furthermore, in *Xenopus* egg

extracts, non-degradable cyclin elicits a post anaphase mitotic arrest, whereas competitive inhibition of cyclin B degradation (by peptides containing destruction boxes) blocks chromosome segregation instead [43]. Taken together, these observations suggest that the APC pathway promotes the degradation of both cyclin B and an unidentified protein involved in sister chromatid cohesion (which I'll refer to here, for brevity, as 'RESIST', for REgulator of SISTer chromatid cohesion) [43]. I favor the notion that RESIST is a factor that stoichiometrically inhibits an enzyme required for sister chromatid separation, as the failure to degrade a small number of RESIST subunits would thereby only diminish the rate at which sisters disjoin. If RESIST were to fasten sisters together directly, anything less than complete destruction of the entire pool of RESIST would elevate the risk of chromosome fragmentation.

The putative kinetochore microtubule motor CENP-E [44], the mitotic inducer NIMA [45•], and thymidine kinase [46] are all stable during interphase and are rapidly degraded during progression through mitosis, and the *Drosophila* tumor suppressor protein OHO-31 rapidly disappears from the nucleus between prophase and anaphase [47,48]. Each of these proteins contains sequences that resemble the destruction box to varying degrees. The effects of mutations in these putative destruction boxes have not been reported, however, and it remains to be seen whether there are multiple pathways for mitosis-specific protein degradation.

### Regulation of the APC pathway

#### Turning it on

Because cyclin B-p34<sup>cdc2</sup> activity sows the seeds of its own destruction, it seems logical that there would be a temporal delay interposed between the activation of cyclin B-p34<sup>cdc2</sup> and the initiation of cyclin proteolysis to allow cyclin B-p34<sup>cdc2</sup> sufficient time to trigger the panoply of events that occur during mitosis. In fact, active cyclin B-p34<sup>cdc2</sup> complexes trigger cyclin degradation in *Xenopus* extracts only after a lag period [49]. How does cyclin B-p34<sup>cdc2</sup> kinase turn on cyclin degradation, and what is the nature of the lag? Overexpression of a dominant form of the mitotic NIMA protein kinase triggers cyclin B degradation in G<sub>2</sub>-arrested *A. nidulans* cells [45•], suggesting that cyclin B-p34<sup>cdc2</sup> may normally activate NIMA, which then activates cyclin degradation. Biochemical experiments suggest that APC is the only component of the cyclin degradation machinery whose activity is regulated by cell cycle phase. *Xenopus* CDC16 and CDC27 both show an apparent increase in molecular weight upon entry into mitosis [30••]; this is consistent with the simple notion that the activation of cyclin degradation in mitosis is triggered by the phosphorylation of APC subunits such as CDC16 and CDC27. Alternatively, activation of APC may require both kinase and phosphatase activities: protein phosphatase I (PP1) is required for progression from metaphase to anaphase in *Drosophila* [50], *S. cerevisiae* [51], *S. pombe* [52], and *A.*

*nidulans* [53]; also, PP1-deficient cells accumulate high levels of cyclin-p34<sup>cdc2</sup> protein kinase. Two competing hypotheses can be advanced to explain the (perhaps universal) requirement for PP1 in anaphase: either it directly participates in triggering anaphase (possibly via APC activation), or a deficit in PP1 activity triggers a checkpoint that restrains chromosome segregation.

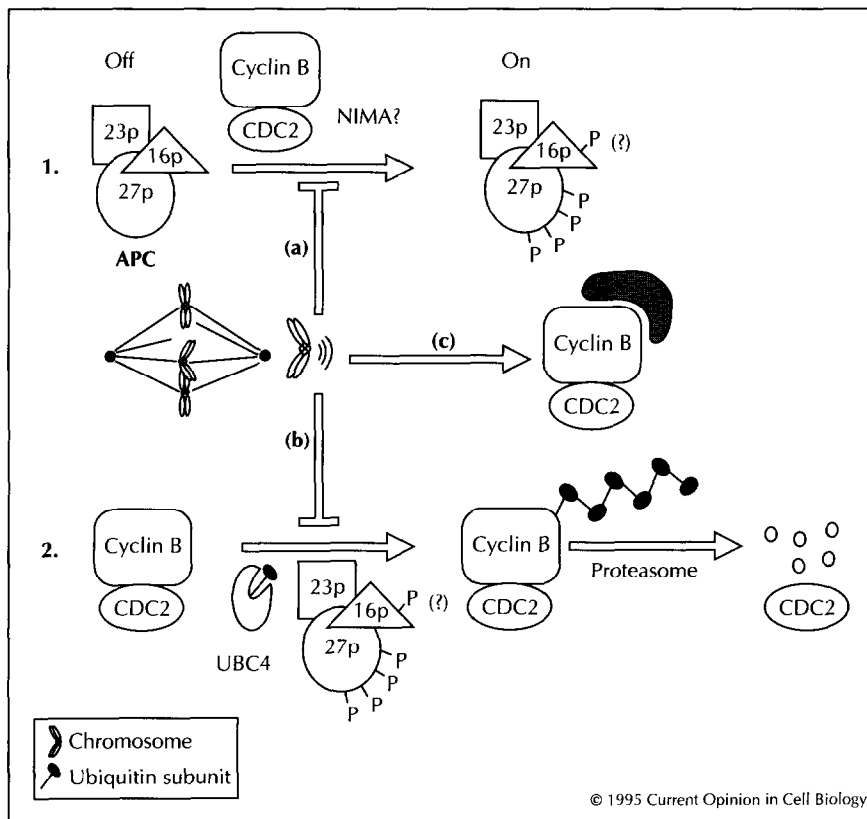
A set of three genetically interacting protein kinase genes (*CDC5*, *CDC15*, and *DBF2*) [54] is required for execution of post anaphase events in the *S. cerevisiae* cell cycle. The mutants *cdc15<sup>ts</sup>* [39] and *dbf2<sup>ts</sup>* [55•] accumulate cyclin B-p34<sup>cdc2</sup> activity, although about half of the mitotic complement of cyclin B is degraded at the *cdc15<sup>ts</sup>* block [34••]. The *cdc5<sup>ts</sup>*, *cdc15<sup>ts</sup>*, and *dbf2<sup>ts</sup>* strains are not deficient in the activation of APC, as sister chromatids efficiently segregate from each other at the non-permissive temperature in these mutants. Rather, it has been proposed that CDC15 may inactivate a molecule that shields cyclin B from APC-dependent destruction [34••].

#### Turning it off

Following the degradation of cyclin and exit from mitosis, APC activity must be extinguished to allow cyclins A and B to reaccumulate for the next mitosis. Unexpectedly, the cyclin B degradation machinery remains active during most of G<sub>1</sub> phase in budding yeast, and is only inactivated following the assembly of an active G<sub>1</sub> cyclin-CDC28 kinase at START [56••]. This finding suggests an appealing model for maintaining the orderly progression of phases of the cell cycle in yeast. Cyclin B accumulation promotes both mitosis and the activation of APC. Upon destruction of cyclin B and other crucial cell cycle regulators, cells divide and enter G<sub>1</sub> phase. Neither cyclin B accumulation nor mitosis can occur, however, until the G<sub>1</sub> cyclin-CDC28 complex switches off APC. Thus mitosis cannot possibly occur until the G<sub>1</sub> cyclins have executed their functions [56••]. Inactivation of cyclin B proteolysis has not yet been examined in larger eukaryotes, so it remains to be determined whether this model is broadly applicable.

#### Checkpoints and developmental regulation

Mutations or drugs that perturb the microtubule spindle block the degradation of cyclin B and RESIST, thereby maintaining the cell at metaphase until a competent spindle is assembled. The signaling pathway that links spindle function to the cyclin destruction machinery has recently been reviewed by Murray [57,58] and will not be discussed further here. Three general models can be used to account for the failure to degrade cyclin B in checkpoint-arrested cells (Fig. 2): activation of the cyclin destruction machinery might be blocked (Fig. 2a); an inhibitor activated by checkpoint-dependent signaling might attenuate the activity of the cyclin destruction machinery (Fig. 2b); or an inhibitor protein activated by checkpoint-dependent signaling might shield cyclin B from active destruction machinery (Fig. 2c). Cyclin B and RESIST are stable during checkpoint-induced



**Fig. 2.** Regulation of cyclin B degradation. Reaction 1: APC is inactive during interphase, but p34<sup>cdc2</sup> (CDC2) activity in mitosis either directly or indirectly triggers APC activity, perhaps via phosphorylation of APC subunits; p34<sup>cdc2</sup>-dependent activation of APC may proceed via the mitotic NIMA protein kinase. CDC27 (27p) is extensively phosphorylated (P) during mitosis, and CDC16 (16p) may be phosphorylated during mitosis. Reaction 2: cyclin B is ubiquitinated by the UBC4 ubiquitin-conjugating enzyme in conjunction with the APC, which contains CDC16 and CDC27 homologs. APC probably contains predominantly CDC23 (23p) in addition to other unidentified proteins. Ubiquitinated cyclin B is subsequently degraded by the proteasome. Unattached chromosomes can block cyclin degradation and the exit from mitosis. This block could conceivably be achieved in at least three ways: (a) activation of APC may be blocked; (b) mitotic activation of APC may proceed normally, but active APC or UBC4 is directly inhibited; (c) APC and UBC4 remain active, but cyclin B is shielded from ubiquitination.

mitotic arrest, but cyclin A degradation proceeds unabated ([8] and references therein). Whereas this observation favors the proposal that checkpoint arrest is achieved by substrate-specific shielding, it is possible that there are several distinctly regulated degradation pathways involving the destruction box. An alternative explanation is that cyclin A degradation proceeds by a destruction box independent pathway while cells linger at the metaphase/anaphase boundary; a role for the destruction box in cyclin A proteolysis has not, to my knowledge, been demonstrated in checkpoint-arrested cells.

Other signals besides crippled spindles can restrain cyclin B degradation. Unfertilized *Xenopus* eggs are naturally maintained at metaphase of meiosis II by an activity referred to as cytosolic factor (CSF) [59••]. Although cyclin B-p34<sup>cdc2</sup> is active in CSF-arrested eggs, the cyclin B degradation machinery is maintained in a quiescent state [59••]. Upon penetration of the egg membrane by sperm, CSF is inactivated, resulting in rapid degradation of cyclin B and entry into the first interphase of embryonic development. Both CSF and damaged spindles inhibit cyclin B proteolysis in *Xenopus* extracts through the action of a mitogen-activated protein (MAP) kinase homolog, implying that both of these physiological effectors block cyclin B proteolysis by signaling through a common pathway [59••]. The availability of purified APC, hUBC4, and cyclin substrate now makes it feasible to examine the biochemical circuits that link both CSF activated and spindle activated MAP kinase to the cyclin destruction machinery.

### Exceptions to the standard view

During the first seven mitotic divisions of *Drosophila* embryogenesis cycles of entry into, and exit from, mitosis occur even though neither the levels of cyclin B nor the activity of cyclin B-p34<sup>cdc2</sup> appear to oscillate [60•]. A possible explanation for this result is that degradation of cyclin B may be restricted to the vicinity of spindle microtubules and centrosomes, thereby leaving unscathed the bulk of cyclin B that is present in the cytosol of developing *Drosophila* embryos. The localization of APC subunits to the centrosome and mitotic spindle in fungal and human cells supports the notion that cyclin degradation is spatially [36•,38], and possibly allosterically [61], regulated by microtubules.

In contrast to the results obtained in *Drosophila*, the activity of p34<sup>cdc2</sup> protein kinase rises and falls as plasmodia of *Physarum polycephalum* traverse mitosis, even though cyclin B remains constant [62]. Overexpression of the cyclin B-p34<sup>cdc2</sup> inhibitor p40<sup>SIC1</sup> (p40<sup>SDB25</sup>) can suppress the mitotic arrest of a yeast mutant that arrests late in M phase with high levels of cyclin B-p34<sup>cdc2</sup> protein kinase [16••]. This implies that the need to degrade cyclin B may be supplanted in some organisms by alternative mechanisms for inactivating p34<sup>cdc2</sup> protein kinase.

### Conclusions

Three basic principles emerge from the studies reviewed here. First, protein degradation provides a very effective tool for regulating cell cycle progression. Ubiquitin-

dependent destruction is both selective and irreversible, making it well suited for the remodeling of multiprotein cyclin-Cdk-Cdk inhibitor complexes or for the triggering of vectorial transitions. Second, protein destruction is highly regulated during the cell cycle. In all of the cases discussed here, degradation must be activated by modification of either the substrate or the proteolytic machinery. Third, protein destruction pathways are complex; degradation of p40<sup>SIC1</sup> requires the activities of at least five genes, and the APC complex may contain seven or more subunits. This complexity may facilitate temporally and spatially restricted destruction of cell cycle regulatory proteins.

Investigation of the role of protein degradation in cell cycle regulation is entering an exciting period. In the past year tremendous strides have been made in elucidating the specific proteolytic events that drive the traversals from G<sub>1</sub> to S phase and from metaphase to anaphase. Many key questions are now accessible thanks to this progress. For example, what is the identity of the complete set of cellular proteins that carry out the specific, regulated ubiquitin-dependent degradation reactions that drive these transitions, and what are their mechanisms of action? How are these reactions switched on to trigger cell cycle transitions, and how are they extinguished prior to subsequent cell cycles? How do developmental and checkpoint signals modulate the activity of degradation pathways? Is the role of *CDC34* in G<sub>1</sub>→S progression conserved? I suspect we will not have to wait long for answers to these important questions. The identification of components and substrates for both pathways and the availability of genetic approaches together with *in vitro* reconstitution systems provide all the tools necessary for a rapid expansion of knowledge.

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Several important points are established here. First, cyclin B (CLB) activity is essential for DNA replication. Second, the temperature-sensitive

*cdc34<sup>ts</sup>*, *cdc4<sup>ts</sup>* and *cdc53<sup>ts</sup>* mutants arrest at the G<sub>1</sub>→S transition as a result of accumulation of the CLB–CDC28 inhibitor p40<sup>SIC1</sup>, suggesting that ubiquitin-dependent proteolysis of p40<sup>SIC1</sup> is the physiological trigger for entry into S phase. Third, p40<sup>SIC1</sup> is the only substrate that the *CDC34* pathway needs to degrade to complete the G<sub>1</sub>→S transition, but is not the only substrate of *CDC34* that needs to be degraded in order for cells to grow. Overall, this represents a major advance in our understanding of the molecular basis of the G<sub>1</sub>→S transition.

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RJ Deshaies, Division of Biology, California Institute of Technology, Pasadena, California 91125, USA.

E-mail: deshaiesr@starbase1.caltech.edu