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Cyclin Ubiquitination: The Destructive End of Mitosis

Minireview

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The problem of how cells make rapid but orderly transitions between different phases of the cell cycle has fascinated biologists since they first observed mitosis late in the nineteenth century. Physiological experiments first suggested that progress through particular phases of the cell cycle might depend on the accumulation of critical "periodic" proteins, and the discovery that cyclin B accumulates during interphase of embryonic cell cycles later provided a molecular candidate for the inducer of mitosis. In addition, the disappearance of cyclin at the end of mitosis suggested the novel possibility that the destruction of particular proteins is needed to progress from one stage of the cell cycle to the next (Evans et al., 1983).

We now know that both of these ideas are true. An appropriately phosphorylated complex of Cdc2 (the prototypic member of the cyclin-dependent kinase family) and cyclin B is an active protein kinase (often called M phase–promoting factor [MPF]) that can induce mitosis. Molecular dissection of cyclin B revealed the existence of the destruction box, a short amino acid sequence near the N-terminus that targets the protein for destruction at the transition between metaphase and anaphase (Glotzer et al., 1991). Removing the destruction box makes cyclin indestructible and reveals that cyclin destruction is required for many of the events that occur as cells leave mitosis (Murray et al., 1989). These events include chromosome decondensation, nuclear envelope reformation (in bigger eukaryotes), and cytokinesis, but not sister chromatid segregation, which can still occur in cells (Surana et al., 1993) or extracts (Holloway et al., 1993) that have high levels of cyclin B. In contrast, fragments of cyclin that contain the destruction box slow down both endogenous cyclin B destruction and sister chromatid separation, suggesting that some unknown protein involved in the linkage of sister chromatids must be destroyed by the same machinery that destroys cyclin B (Holloway et al., 1993).

How do cells destroy cyclin B, and how is destruction regulated so that in 1 or 2 min the half-life of cyclin falls from more than 1 hr to less than 1 min? The short answer is ubiquitin-mediated proteolysis (Glotzer et al., 1991; Hershko et al., 1991), a process that has been biochemically well understood for several years, but whose biological function was unclear. In this complex reaction, several molecules of the small protein ubiquitin are attached to a lysine on the substrate protein, thus directing it to the proteasome, a complex proteolytic machine that degrades the polyubiquitinated protein into small peptides (reviewed by Ciechanover, 1994). Ubiquitin-mediated proteolysis is hierarchical (see Figure 1). The first enzyme in the pathway, E1, uses the energy of ATP hydrolysis to form a thioester bond between itself and a ubiquitin molecule. This complex then reacts with one of many different E2 enzymes, transferring the bound ubiquitin from E1 to E2. The final transfer of ubiquitin to the protein substrate can be mediated by an E2 alone or by an E2 acting in concert with an E3. In the latter situation, it is not yet clear whether the ubiquitin is passed from E2 to E3 to target protein or whether the E3 acts to bring the E2 and target protein close to each other.

The regulation of cyclin proteolysis takes at least two forms: a temporal one that imposes a minimum lag between the activation of MPF and the activation of cyclin destruction, and a cell cycle checkpoint that prevents the destruction of cyclin B in cells that have not yet assembled a functional spindle (Minshull et al., 1994). The development of fractionated systems that can ubiquitinate cyclin (Hershko et al., 1994; King et al., 1995 [this issue of *Cell*]) and the identification of the specific E2 and E3 enzymes required for this reaction are the first steps toward understanding how the destruction of cyclin B and the hypothetical sister cohesion protein are controlled.

Like much of our knowledge about the cell cycle, the recent information about cyclin proteolysis comes from combining genetics and biochemistry. Genetic analysis was complicated because cells must satisfy two conditions to leave mitosis: the spindle must be properly assembled, and the cyclin proteolysis machinery must be activated. Thus ,although mutants that damage the cyclin destruction apparatus would arrest in mitosis, this phenotype could also be due to lesions in the spindle that prevent cyclin destruction by activating the spindle assembly checkpoint. Irniger et al. (1995 [this issue of *Cell*]) elegantly circumvented this problem by capitalizing on the observation that cyclin B destruction in budding yeast is active from the onset of anaphase until the cells pass START, the point in G1 that commits them to DNA synthesis (Amon et al., 1994). By placing cyclin B synthesis under the control of an inducible promoter and arresting cells before START, they were able to identify temperature-sensitive mutations in three genes that stabilized cyclin B. Two of these, *cdc16* and *cdc23*, represent new alleles of genes that had been identified in Hartwell's original screen for cell cycle mutants. Since the original mutants and the new alleles all arrest in metaphase, CDC16 and CDC23 are likely to be required for the destruction of sister chromatid cohesion proteins as well as cyclin B. If *cdc23* cells are genetically tricked into entering mitosis with unreplicated DNA, the spindle elongates and the chromatin separates into two masses (Irniger et al., 1995). This work reinforces the earlier conclusion that it is sister chromatid separation, rather than MPF inactivation, that induces the transition between metaphase and anaphase (Holloway et al., 1993). Without linkage to a sister, a chromosome cannot achieve a position midway between the two spindle poles, the length of the spindle cannot be restrained, and the spindle eventually breaks in two.

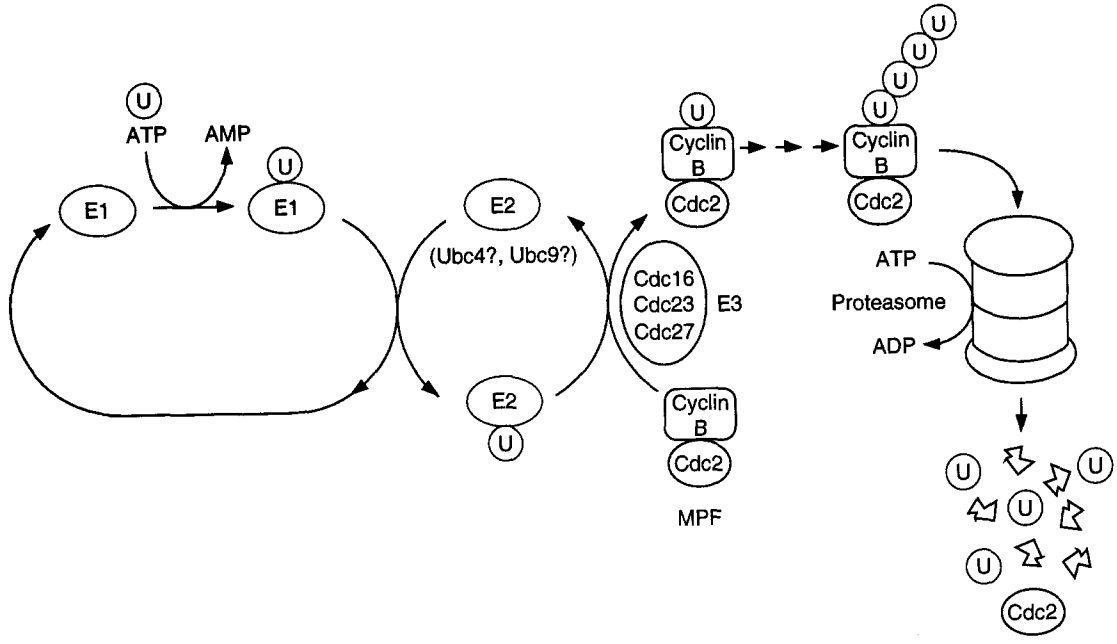

Figure 1. The Biochemistry of Cyclin Destruction
See text for further details. U represents ubiquitin.

CDC16 and CDC23 are members of the tetratricopeptide repeat family of proteins, named after the characteristic presence of several copies of a loosely conserved 34 amino acid repeat. Genetic and physical evidence suggests that three members of this family, CDC16, CDC23, and CDC27, exist together as a large complex whose presence is required for the metaphase to anaphase transition (Lamb et al., 1994). The small size and poor cytology of budding yeast make determining the localization of these proteins difficult. The pace of genome sequencing offers a simple solution to this problem: find a homolog in an organism with bigger cells and better cytology. This strategy revealed human homologs of CDC16 and CDC27, and antibodies to the human proteins stain the spindle poles and microtubules (Tugendreich et al., 1995 [this issue of *Cell*]). Injecting anti-CDC27 antibodies into interphase cells arrests them in metaphase of their next mitosis. Injecting metaphase cells also prevents the onset of anaphase, suggesting that the antibody blocks a late step in the reactions that induce cyclin B destruction. Injection of anti-CDC16 antibodies has no effect. Although this discrepancy may reflect differences in the strength of the antibodies, it is consistent with observations from budding yeast, in which some *cdc16* alleles cannot block cyclin degradation in G1, even though they do arrest cells in metaphase (Irniger et al., 1995).

Why should components required for cyclin destruction be physically associated with the spindle? In the earliest cell cycles of the fly embryo, there is no obvious global destruction of cyclin B at the end of metaphase, although cycles of abundance do become visible as the density of nuclei increases (Edgar et al., 1994). One interpretation of these observations is that in this organism the presence of a spindle locally concentrates the destruction machinery to a point where the rate of cyclin destruction exceeds new synthesis, thus triggering local destruction. This is an appealing model for an organism with no membranes between nuclei, since it could allow adjacent spindles some independence from each other in the timing of anaphase. In contrast, frog egg extracts can globally destroy cyclin B in the absence of both spindle poles and microtubules (Minshull et al., 1994). This difference probably reflects the very different life cycles of frogs and flies. Frog eggs are enormous cells that physically divide, requiring that MPF be inactivated at the cleavage furrow, hundreds of microns away from the spindle, whereas early fly embryos lack cytokinesis and thus do not need to inactivate MPF anywhere other than around the nuclei. The ability of fly nuclei to replicate their DNA while swimming in a cytoplasmic sea of MPF suggests that MPF activity in the nucleus and cytoplasm can be independently regulated.

The direct biochemical approach to cyclin B ubiquitination was initiated by Hershko et al. (1994), who fractionated mitotic clam egg extracts that would specifically degrade cyclin B. They showed that the ubiquitination activity could be separated into three components: E1, an E2, and a large complex that was needed to allow the other two enzymes to add ubiquitin to cyclin B and that therefore meets the definition of an E3 (Sudakin et. al, 1995). By preparing the different factors from either interphase or mitotic extracts, Hershko et al. (1994) showed that the activity of the large complex varied between interphase and mitosis, but that of the E1 and E2 did not. King et al. (1995) adopted the same approach, using frog egg extracts to fractionate

the activities needed for cyclin B ubiquitination into E1, either of two E2s, and a large E3 complex whose activity differs between interphase and mitosis. Treating extracts with anti-CDC27 antibodies can deplete the large complex, and material immunoprecipitated from complete extracts by these antibodies can substitute for the large complex. Unfortunately, since this material has not yet been fully purified, it is impossible to know what else the E3 complex contains in addition to CDC27 and CDC16.

Which E2s are responsible for conjugating ubiquitin to cyclin B? Biochemistry and genetics give conflicting answers. One of the E2s identified in frog eggs reacts with antibodies to the human homolog of a yeast E2 called UBC4 (King et al., 1995). But, although there is a genetic interaction between *ubc4* and *cdc23* mutations (Irniger et al., 1995), removing both UBC4 and its close relative UBC5 fails to arrest budding yeast in mitosis (Seufert and Jentsch, 1990). In contrast, the temperature-sensitive *ubc9* mutation arrests cells in G2 or mitosis (the two stages are not morphologically distinguishable in budding yeast) and prevents cyclin B destruction in G1-arrested cells (Seufert et al., 1995). Although a variety of complicated models can accommodate all these observations, further experiments are clearly required to determine which E2s are the physiological mediators of cyclin B destruction.

Although the biochemical and genetic dissection of cyclin B destruction has identified important players in this complicated reaction, it raises a number of questions. At the mechanistic level, how does the CDC16- and CDC27-containing E3 complex stimulate cyclin ubiquitination, and how is the activity of this complex regulated during the cell cycle? More importantly, how is the activity of this complex controlled, and what role does this control play in the physiological regulation of proteolysis? The discrete lag between the activation of MPF and cyclin B destruction suggests that MPF acts indirectly to activate the CDC16–CDC27 complex, thus guaranteeing cells that lack an effective spindle assembly checkpoint (such as early frog embryos) a brief period for spindle assembly before the onset of anaphase. Three lines of evidence suggest that cell cycle regulation occurs at the level of cyclin itself, in addition to changes in the activity of ubiquitinating enzymes. First, even though the same activities catalyze the destruction of both cyclin B and the S phase cyclin, cyclin A (Sudakin et al., 1995), the in vivo degradation of cyclin A precedes that of cyclin B, and the spindle assembly checkpoint can protect cyclin B but not cyclin A (Hunt et al., 1992; Minshull et al., 1994; Whitfield et al., 1990). Second, certain cyclins can be degraded only if they are physically associated with Cdc2 (Stewart et al., 1994). Third, kinases that are activated by the spindle assembly checkpoint can phosphorylate cyclin B, although so far this reaction has only been demonstrated under very artificial conditions (Minshull et al., 1994). Deciphering regulation at the level of cyclin and determining the relative importance of modifications on the ubiquitination machinery compared with those on its substrates will probably require another dive into the murk of crude cell cycle extracts.

How general a mechanism is proteolysis for regulating biology? One of the first genetically understood examples of induction, the activation of lysogenic bacteriophages, depends on the proteolytic destruction of the phage repressor. Despite this lesson and the abundant regulatory possibilities that the complexity of ubiquitin-mediated proteolysis offers, eukaryotic biology is just starting to discover the richness and ubiquity of control by regulated proteolysis. One emerging principle, which follows the original paradigm of phage induction, is that ubiquitin-mediated proteolysis often produces its effects by destroying repressors. Thus, anaphase appears to require the destruction of a protein that somehow prevents the separation of sister chromatids, and the transition from G1 to S phase in budding yeast is dependent on ubiquitin-mediated proteolysis of a protein that inhibits the activity of complexes between CDC28 and S phase cyclins (Schwob et al., 1994). Dissection of the genes that papillomaviruses use to transform mammalian cells reveals that one of the viral gene products (E6) recruits a cellular factor that acts as an E3 to stimulate the destruction of p53, which can act as a repressor of the G1 to S transition (Scheffner et al., 1993). In the immune system, a wide range of stimuli leads to the nuclear localization of a transcriptional activator (NF-κB) by inducing the ubiquitin-dependent proteolysis of a binding protein (IF-κB) that keeps it in the cytoplasm (Palombella et al., 1994), and homologs of this system are used in the control of dorsal–ventral polarity in the fly embryo (Whalen and Steward, 1993). In other circumstances, ubiquitin-mediated proteolysis can be used to remove activating signals. In plants the activated form of phytochrome that mediates the diverse responses to long wavelength light is subject to ubiquitin-mediated proteolysis, whereas the inactive form is not (Jabben et al., 1989). This scheme allows plants to respond rapidly to darkness by quickly removing the active phytochrome and to respond to light by activating the stable but inactive form of the phytochrome. The wide range of these examples suggests that, like Cdk–cyclin complexes, the combinatorial possibilities of E2s and E3s allowed evolution to diversify an invention into so many different areas of regulation that we will be uncovering new ones until well into the twenty-first century.

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