THE ROYAL SOCIETY

Mechanisms and regulation of the degradation of cyclin B

Avram Hershko

Unit of Biochemistry, B. Rappaport Faculty of Medicine and Rappaport Institute for Research in the Medical Sciences, Technion—Israel Institute for Technology, Haifa 31096, Israel

The degradation of the cyclin B subunit of protein kinase Cdk1/cyclin B is required for inactivation of the kinase and exit from mitosis. Cyclin B is degraded by the ubiquitin pathway, a system involved in most selective protein degradation in eukaryotic cells. In this pathway, proteins are targeted for degradation by ligation to ubiquitin, a process carried out by the sequential action of three enzymes: the ubiquitin-activating enzyme E1, a ubiquitin-carrier protein E2 and a ubiquitin–protein ligase E3. In the system responsible for cyclin B degradation, the E3-like function is carried out by a large complex called cyclosome or anaphase-promoting complex (APC). In the early embryonic cell cycles, the cyclosome is inactive in the interphase, but becomes active at the end of mitosis. Activation requires phosphorylation of the cyclosome/APC by protein kinase Cdk1/cyclin B. The lag kinetics of cyclosome activation may be explained by Suc1-assisted multiple phosphorylations of partly phosphorylated complex. The presence of a Fizzy/Cdc20-like protein is necessary for maximal activity of the mitotic form of cyclosome/APC in cyclin–ubiquitin ligation.

Keywords: cyclin degradation; cyclosome; ubiquitin; anaphase-promoting complex (APC); Cdk1; Suc1

1. INTRODUCTION

In this paper I briefly describe past work from our laboratory, as well as from other laboratories, that has led to the identification of the components of the system that degrades cyclin B. I also discuss the present state of knowledge of the molecular mechanisms involved in the regulation of this system in the relatively simple early embryonic cell cycles.

2. IDENTIFICATION OF THE COMPONENTS OF THE SYSTEM THAT DEGRADES CYCLIN B

I have become involved in the problem of the mode of the degradation of mitotic cyclins because of my long-standing interest in the biochemical mechanisms of the degradation of cellular proteins. The dynamic state of cellular proteins (Schoenheimer 1942) and the important roles of protein degradation in the control of cellular enzyme concentrations (Schimke & Doyle 1970) have been recognized for a long time, but the underlying molecular mechanisms remained unknown. A clue to an unusual mechanism was provided by observations indicating that the degradation of cellular proteins required metabolic energy (Simpson 1953; Hershko & Tomkins 1971). In 1978, we fractionated an ATP-dependent cell-free proteolytic system from reticulocytes and found that a small, heat-stable protein was required for its activity (Ciechanover *et al.* 1978). In 1980, we found that this protein (subsequently identified as ubiquitin) was covalently ligated to protein substrates, and proposed that ubiquitin ligation targeted proteins for degradation (Hershko *et al.* 1980). Our further fractionation–reconstitution work has shown (Hershko *et al.* 1983) that ubiquitin–protein ligation involves the sequential action of three enzymes. First, a ubiquitin-activating enzyme, E1, uses the energy of ATP hydrolysis to form a thiolester bond with ubiquitin. Next, activated ubiquitin is transferred to a ubiquitin-carrier protein, E2. Finally, ubiquitin is transferred to the protein substrate by the action of a ubiquitin–protein ligase, E3 (Hershko *et al.* 1983). Further work by several laboratories has shown that there is a single E1, but there are multiple species of E2s and E3s, involved in the ligation to ubiquitin of different proteins. The selectivity of protein degradation is mainly determined by the specificity of the binding of a certain class of cellular proteins to a specific E3 enzyme. Proteins ligated to polyubiquitin chains are degraded by the 26S proteasome complex and free ubiquitin is released by the action of ubiquitin-C-terminal hydrolases or isopeptidases (reviewed in Hershko & Ciechanover 1998).

In 1983, Hunt and co-workers discovered the first cyclin, cyclin B, a protein that is destroyed at the end of each cell cycle in early embryos of marine invertebrates, such as sea urchins and clams (Evans *et al.* 1983). This discovery not only opened up a new era in cell-cycle research, but also kindled my interest in the following questions. Why is cyclin B stable in the interphase and yet rapidly degraded at the end of mitosis? What is the machinery that degrades cyclin B and how is its action regulated in the cell cycle? Although cell-cycle researchers at that time were mentioning a putative 'cyclin protease', I thought that there might exist a specific ubiquitin ligase that acted on cyclin B (and thus committed it for degradation) only at the end of mitosis. However, experimental evidence for this notion only began to be obtained eight

 © 1999 The Royal Society

years later. In 1991, independent work by Glotzer *et al.* (1991) and from our laboratory (Hershko *et al.* 1991) indicated that cyclin B was degraded by the ubiquitin system. Both laboratories employed biochemical approaches, using cell-free systems from early embryonic cells that faithfully reproduced the selectivity and regulation of cyclin B degradation *in vitro*. With a cell-free system from *Xenopus* eggs, Glotzer *et al.* (1991) showed that cyclin B is degraded and is ubiquitinylated only in mitosis, but not in the interphase. These investigators have also shown that a nine-amino-acid, partially conserved motif at the N-terminal region of mitotic cyclins, the 'destruction box', is required for both the degradation and the ubiquitinylation of cyclin B in mitotic extracts. These correlations between cyclin degradation and the formation of cyclin–ubiquitin conjugates strongly suggested that cyclin B is degraded by the ubiquitin system. This conclusion was in agreement with our independent study in which we found that in extracts of fertilized clam oocytes the degradation of both cyclin A and cyclin B was inhibited by methylated ubiquitin, a derivative of ubiquitin that blocks the formation of polyubiquitin chains (Hershko *et al.* 1991). The specificity of the action of this agent was indicated by the finding that the supplementation of excess ubiquitin completely overcame the inhibitory action of methylated ubiquitin on cyclin degradation.

The studies described above have suggested that the degradation of mitotic cyclins is carried out by the ubiquitin pathway, but they have not identified the components responsible for the specificity and regulation of cyclin degradation. For this purpose, the fractionation of extracts and the isolation of the active components was required. Initial fractionation was achieved by our laboratory (Hershko *et al.* 1994). Fractionation of extracts of clam oocytes showed that in addition to E1, two novel components were required to reconstitute cyclin–ubiquitin ligation *in vitro*. These were a specific E2, called E2-C, and an activity associated with particulate material. Alhough E2-C specifically acts on this system, and homologues of E2-C are present in many (though not all) eukaryotic organisms (reviewed in Hershko 1997; Townsley & Ruderman 1998), its activity is not regulated in the cell cycle. By contrast, the activity of the component associated with particulate material was cell-cycle regulated: it was inactive in the interphase, and became active in mitosis (Hershko *et al.* 1994). The particulate material with which this specific ubiquitin ligase activity is associated in clam oocytes has not been identified. In other organisms, it is not associated so strongly with particulate structures or organelles (see below).

This slow start has paved the way to rapid progress in our knowledge of this system that took place in 1995. This progress was due to convergence of information from biochemical work with genetic analysis in yeasts. In collaboration with Joan Ruderman, we have dissociated the cell-cycle-regulated component from the particulate fraction of oocytes and have partially purified and characterized it (Sudakin *et al.* 1995). It was found to be a large (*ca.* 1500 kDa) complex that has destruction-box-specific cyclin–ubiquitin ligase activity. The activity of the isolated complex remained cell-cycle regulated. Furthermore, the inactive complex from interphase cells could be converted *in vitro* to the active form by incubation with protein kinase Cdk1/cyclin B. We have called this complex the cyclosome, to denote its large size and important role in cell-cycle regulation (Sudakin *et al.* 1995). A similar biochemical approach of fractionation of *Xenopus* egg extracts by King *et al.* (1995) identified a similar complex that was termed anaphase-promoting complex, or APC. The identification of the subunits of the cyclosome/APC was made possible by the work of Irniger *et al.* (1995), who used an elegant screen to isolate yeast mutants defective in cyclin B proteolysis. The products of some of these genes, CDC16, CDC23 and CDC27, had been previously shown to be required for the onset of anaphase in budding and fission (Goebl & Yanagida 1991) yeasts, and to form a multiprotein complex (Lamb *et al.* 1994). The availability of antibodies directed against vertebrate homologues of these gene products (Tugendreich *et al.* 1995) has enabled their identification as subunits of the *Xenopus* cyclosome/APC (King *et al.* 1995). Further work has identified eight subunits of the cyclosome/APC in *Xenopus* eggs (Yu *et al.* 1998) and 12 subunits in budding yeast (Zachariae *et al.* 1998). Thus far, the specific functions of the different subunits have not been identified.

3. REGULATION OF THE ACTION OF CYCLOSOME/APC

Our present knowledge of the molecular mechanisms responsible for the regulation of cyclosome action is very limited. Regulation is less complex in early embryonic cell cycles (which consist of rapidly alternating S and M phases, without G1 and G2 intervals) than in those of somatic cells or yeasts. In addition, it seems that several levels of regulation exist in both cases: a basal mechanism that turns on the activity of the cyclosome/APC at the end of mitosis, and ancillary factors that allow its action on various substrates at different times of the cell cycle. I shall describe mainly regulation in the less complex early embryonic cell cycle, on which we have been working in the clam oocyte biochemical system.

In early embryonic cells, a basal regulatory mechanism is the reversible phosphorylation of the cyclosome/APC. We found that the active, mitotic form of the clam cyclosome was inactivated by incubation with an okadaic-acid-sensitive phosphatase. Addition of protein kinase Cdk1/cyclin B to a phosphatase-treated cyclosome preparation restored activity after a time lag that reproduced that seen in intact cells (Lahav-Baratz *et al.* 1995). Similarly, phosphatase treatment inactivated purified cyclosome/APC from *Xenopus* eggs (Peters *et al.* 1996). Alhough protein kinase Cdk1/cyclin B is undoubtedly the upstream activator of embryonic cyclosome/APC, it is not clear whether it acts by direct phosphorylation in all cases, or by the phosphorylation and activation of an intermediary protein kinase. It has been reported that the action of a polo-like protein kinase, which can be activated by phosphorylation by Cdk1/cyclin B, is required for the Ca^{2+}-induced transition of extracts of cytostatic-factor-arrested *Xenopus* eggs into the interphase, a process that includes cyclin B degradation (Descombes & Nigg 1998). However, it is not clear from these experiments which stage of this complex process is affected by the kinase, and whether the polo-like kinase is also required in the

subsequent embryonic cell cycles. It has also been reported that polo-like kinase activated by Cdk1/cyclin B can phosphorylate and activate cyclosome/APC, whereas Cdk1/cyclin B cannot do so (Kotani *et al*. 1998). However, these experiments were done with APC/cyclosome purified from mammalian fibroblasts, so it is not clear whether this mechanism occurs in the embryonic system. By contrast, we have found that highly purified, dephosphorylated preparations of clam cyclosome/APC are activated by purified Cdk1/cyclin B (M. Shteinberg and A. Hershko, unpublished observations). Similarly, Patra & Dunphy (1998) have reported that Cdk1/cyclin B directly phosphorylates cyclosome/APC purified from interphase *Xenopus* eggs, provided that a Suc1/Cks protein was also present (see also below). By contrast, active polo-like kinase had no such effect in this preparation. It thus seems that at least in early embryonic cells, the cyclosome/APC can be directly phosphorylated and activated by protein kinase Cdk1/cyclin B.

An interesting process that can be conveniently studied in the relatively simple early embryonic system is the lag kinetics of the activation of the cyclosome/APC by protein kinase Cdk1/cyclin B. This time lag is presumably important to prevent premature self-inactivation of Cdk1/cyclin B before this protein kinase completes to induce all events of mitosis. This lag can be reproduced *in vitro* in extracts of *Xenopus* eggs (Felix *et al*. 1990) and in preparations of clam cyclosome/APC (Hershko *et al*. 1994; Sudakin *et al*. 1995). Our recent findings indicate that Suc1 (also called Cks), an essential cell cycle protein that associates with Cdk1 and with phosphate-containing compounds (Pines 1996), has a role in this process (Shteinberg & Hershko 1999). We have previously found that the active, phosphorylated form of the clam cyclosome binds to Suc1 via the phosphate-binding site of this protein, and used this interaction for the affinity purification of the cyclosome (Sudakin *et al*. 1997). We now find that low, physiological concentrations of Suc1 stimulate the activation of the interphase form of the cyclosome/APC by protein kinase Cdk1/cyclin B. Examination of the time-course of the activation process showed that when Suc1 was present from the start of the incubation together with protein kinase Cdk1/cyclin B, activation of the cyclosome occurred with the normal lag kinetics. However, when interphase cyclosome was first incubated with protein kinase Cdk1/cyclin B without Suc1, the subsequent addition of Suc1 caused a rapid burst of cyclosome activation and the lag was completely abolished. These findings are consistent with the interpretation that, after initial slow phosphorylations of the cyclosome by the protein kinase, Suc1 accelerates multiple phosphorylations that culminate in the activation of the cyclosome. In support of this interpretation, we have found that Suc1 accelerates the phosphorylation of several proteins in the preparation of the interphase cyclosome, and that the effect of Suc1 on phosphorylation was augmented by prior incubation of interphase cyclosome with protein kinase Cdk1/cyclin B (Shteinberg & Hershko 1999). It is possible that, because Suc1 has binding sites to both Cdk1 and phosphate groups (Pines 1996), the binding of Suc1 to both Cdk1/cyclin B and partly phosphorylated cyclosome may increase the affinity of the protein kinase for the cyclosome and may thus accelerate multiple phosphorylations required for the full activation of the cyclosome. Very recently, Patra & Dunphy (1998) have reported that, in extracts of *Xenopus* eggs, a Suc1/Cks protein is required for the activation of the degradation of cyclin B and for the hyperphosphorylation of the Cdc27 subunit of the cyclosome/APC by Cdk1/cyclin B. These authors have also shown that Suc1 stimulates the direct phosphorylation of purified cyclosome/APC by the protein kinase. These findings are in agreement with our results, although the influence of Suc1 on the lag kinetics of cyclosome activation was not addressed in this study.

If phosphorylation of the cyclosome/APC was the sole mechanism for the regulation of its action, it would be expected that all substrates of the cyclosome/APC would be degraded at the same time at the end of mitosis. However, it was known that some other destruction-box-containing substrates of the cyclosome/APC, such as cyclin A or anaphase inhibitory proteins, are degraded earlier in the cell cycle than cyclin B (reviewed in Hershko 1997). Therefore, the existence of additional levels of regulation of cyclosome action was suspected for a long time, but some of the molecules involved have begun to be identified only recently. Genetic work has identified two WD40-repeat-containing proteins, called Cdc20 and Cdh1/Hct1 in the budding yeast, or Fizzy (Fzy) and Fizzy-related (Fzr) in *Drosophila*, respectively, that are required for the degradation of different substrates of the cyclosome/APC (reviewed in Wolf & Jackson 1998). In the budding yeast, these two proteins seem to confer different substrate specificity on cyclosome/APC-dependent protein degradation (Schwab *et al*. 1997; Visintin *et al*. 1997). In *Drosophila* they facilitate the degradation of the same substrates, but at different times in the cell cycle: Fzy is required for the degradation of cyclins A and B in mitosis, whereas Fzr promotes the degradation of the same proteins in G1 (Sigrist & Lehner 1997).

These genetic findings can now be reproduced in biochemical systems. During the purification of clam cyclosome by affinity chromatography on Suc1–Sepharose, we noticed a great loss of cyclosome activity after purification. However, activity could be restored by the addition of the flow-through fraction not adsorbed to Suc1–Sepharose (Sudakin *et al*. 1997). More recently, we have found that the activity of the flow-through fraction is due to a Fzy-like protein, since it could be replaced by recombinant human Fzy (Shteinberg *et al*. 1999). In the course of this work, two other groups have reported similar findings. Lorca *et al*. (1998) have reported that antibodies directed against Fzy block the degradation and ubiquitinylation of cyclin B in *Xenopus* extracts. Fang *et al*. (1998) have shown that human Cdc20 (Fzy) and Cdh1 (Fzr) stimulate the activity of purified cyclosome/APC from both mitotic and interphase *Xenopus* eggs. By contrast, we find that Fzy stimulates only mitotic, but not interphase, clam oocyte cyclosome (Shteinberg *et al*. 1999). The reason for this discrepancy in results is not known.

The mode of action of Fzy and Fzr in the stimulation of the activity of the cyclosome/APC is not known. They both bind to cyclosome/APC, and the affinity of binding to mitotic cyclosome/APC appears to be somewhat higher than that to the interphase form (Fang *et al*. 1998).

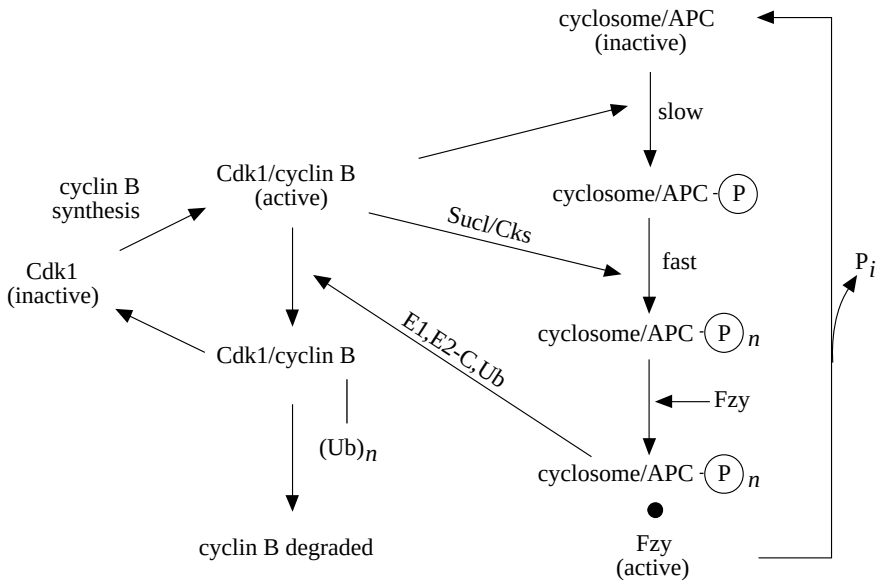

Figure 1. Model for the regulation of cyclin B degradation in the early embryonic cell cycles. See the text for details. Abbreviations: Ub, ubiquitin; Fzy, Fizzy/Cdc20-like activator of cyclosome/APC.

However, direct binding of cyclin B or of other cyclosome substrate to Fzy or Fzr has not been demonstrated. Therefore, they do not seem to be simply 'specificity factors' that bind specific substrates to the cyclosome/APC. Rather, their binding to the cyclosome may alter the structure of the complex in a way that allows other subunits or ancillary factors to become active in the ligation of ubiquitin to specific substrates.

Figure 1 summarizes our present model of the mechanisms and regulation of cyclin B degradation in the early embryonic cell cycles. According to this model, the active protein kinase Cdk1/cyclin B activates the inactive, interphase form of the cyclosome/APC at the end of mitosis by direct phosphorylation. The lag kinetics of the activation process may be explained, at least in part, by the assumption that the initial slow phosphorylations are succeeded by Suc1-assisted multiple phosphorylations. In addition to phosphorylation, the presence of the Fzy protein is also necessary for full activation of the cyclosome. The fully active cyclosome ubiquitinylates cyclin B, a process that involves the action of E1 and E2-C. Ubiquitinylated cyclin B is degraded by a constitutively acting 26S proteasome, and thus Cdk1 is converted back to the inactive, monomeric form. Subsequently, the cyclosome/APC is converted back to the inactive interphase form by phosphatase action.

It should be emphasized that the above model is applicable only to the relatively simple early embryonic cell cycles. In somatic cells and yeasts the basic machinery is apparently similar, but regulation is much more complex, presumably owing to the need to maintain an ordered sequence of events in the G1 and G2 phases of the cell cycle and to respond to environmental changes and checkpoint controls. Thus, activation of cyclosome/APC at the end of mitosis in yeasts requires not only the action of Cdk1/cyclin B, but also that of three other protein kinases, a phosphatase and several other gene products (reviewed in Nasmyth 1996). In addition, the window of cyclosome action is much longer than in embryonic cells, and is turned off only at the end of G1 (Amon *et al*. 1994; Brandeis & Hunt 1996). At least in part, this may be due to the action of Fzr/Cdh1/Hct1 in G1 (Sigrist & Lehner 1997), a protein that is absent in embryonic cells (Lorca *et al*. 1998). Finally, checkpoint controls operate much more efficiently in somatic than in early embryonic cells. The mode by which the spindle-assembly checkpoint control system (Rudner & Murray 1996) inhibits the action of cyclosome/APC has begun to be elucidated by the observation that a component of this system, Mad2, interacts with and inhibits the action of homologues of Fzy in fission and budding yeast (Kim *et al*. 1998; Hwang *et al*. 1998). Apparently, we are now having only our first glimpses into the intricate mechanisms by which controls of the action of the cyclosome/APC regulate the somatic cell cycles.

Our work described in this paper was supported by the United States–Israel Binational Science Foundation, the Israel Science Foundation and a grant from the Ministry of Science, Israel.

REFERENCES

Amon, A., Irniger, S. & Nasmyth, K. 1994 Closing the cell cycle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cell cycle. *Cell* **77**, 1037–1050.

Brandeis, M. & Hunt, T. 1996 The proteolysis of mitotic cyclins in mammalian cells persists from the end of mitosis until the onset of S phase. *EMBO J.* **15**, 5280–5289.

Ciechanover, A., Hod, Y. & Hershko, A. 1978 A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes. *Biochem. Biophys. Res. Commun.* **81**, 1100–1105.

Descombes, P. & Nigg, E. A. 1998 The polo-like kinase Plx1 is required for M phase exit and destruction of mitotic regulators in *Xenopus* egg extracts. *EMBO J.* **17**, 1328–1335.

Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D. & Hunt, T. 1983 Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* **33**, 389–396.

Fang, G., Yu, H. & Kirschner, M. W. 1998 Direct binding of CDC20 family members activates the anaphase-promoting complex in mitosis and G1. *Mol. Cell* **2**, 163–171.

Felix, M.-A., Labbe, J.-C., Doree, M., Hunt, T. & Karsenti, E. 1990 Triggering of cyclin degradation in interphase extracts of amphibian eggs by cdc2 kinase. *Nature* **346**, 379–382.

Glotzer, M., Murray, A. W. & Kirschner, M. W. 1991 Cyclin is degraded by the ubiquitin pathway. *Nature* **349**, 132–138.

Goebl, M. & Yanagida, M. 1991 The TPR snap helix: a novel motif from mitosis to transcription. *Trends Biochem. Sci.* **16**, 173–177.

Hershko, A. 1997 Roles of ubiquitin-mediated protein degradation in cell cycle control. *Curr. Opin. Cell Biol.* **9**, 788–799.

Hershko, A. & Ciechanover, A. 1998 The ubiquitin system. *A. Rev. Biochem.* **67**, 425–479.

Hershko, A. & Tomkins, G. M. 1971 Studies on the degradation of tyrosine aminotransferase in hepatoma cells in culture. Influence of the composition of the medium and adenosine triphosphate dependence. *J. Biol. Chem.* **246**, 710–714.

Hershko, A., Ciechanover, A., Heller, H., Haas, A. L. & Rose, I. A. 1980 Proposed role of ATP in protein breakdown: Conjugation of proteins with multiple chains of the polypeptide of ATP-dependent proteolysis. *Proc. Natl Acad. Sci. USA* **77**, 1783–1786.

Hershko, A., Heller, H., Elias, S. & Ciechanover, A. 1983 Components of ubiquitin-protein ligase system: resolution, affinity purification and role in protein breakdown. *J. Biol. Chem.* **258**, 8206–8214.

Hershko, A., Ganoth, D., Pehrson, J., Palazzo, R. E. & Cohen, L. H. 1991 Methylated ubiquitin inhibits cyclin degradation in clam oocyte extracts. *J. Biol. Chem.* **266**, 16 376–16 379.

Hershko, A., Ganoth, D., Sudakin, V., Dahan, A., Cohen, L. H., Luca, F. C., Ruderman, J. V. & Eytan, E. 1994 Components of a system that ligates cyclin to ubiquitin and their regulation by protein kinase cdc2. *J. Biol. Chem.* **269**, 4940–4946.

Hwang, L. H., Lau, L. F., Smith, D. L., Mistrot, C. A., Hardwick, K. G., Hwang, E. S., Amon, A. & Murray, A. W. 1998 Budding yeast Cdc20: a target of the spindle checkpoint. *Science* **279**, 1041–1044.

Irniger, S., Piatti, S., Michaelis, C. & Nasmyth, K. 1995 Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. *Cell* **81**, 269–277.

Kim, S. H., Lin, D. P., Matsumoto, S., Kitazono. A. & Matsumoto, T. 1998 Fission yeast slp1: an effector of the mad2-dependent spindle checkpoint. *Science* **279**, 1045–1047.

King, R. W., Peters, J.-M., Tugendreich, S., Rolfe, M., Hieter, P. & Kirschner, M. W. 1995 A 20S complex containing *CDC27* and *CDC16* catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* **81**, 279–288.

Kotani, S., Tugendreich, S., Fujii, M., Jorgensen, P.-M., Watanabe, N., Hoog, C., Hieter, P. & Todokoro, K. 1998 PKA and MPF-activated polo-like kinase regulate anaphase-promoting complex activity and mitosis progresion. *Mol. Cell* **1**, 371–380.

Lahav-Baratz, S., Sudakin, V., Ruderman, J. V. & Hershko, A. 1995 Reversible phosphorylation controls the activity of cyclosome-associated cyclin–ubiquitin ligase. *Proc. Natl Acad. Sci. USA* **92**, 9303–9307.

Lamb, J. R., Michaud, W. A., Sikorski, R. S. & Hieter, P. A. 1994 Cdc16p, Cdc23p and Cdc27p form a complex essential for mitosis. *EMBO J.* **13**, 4321–4328.

Lorca, T., Castro, A., Martinez, A.-M., Vigneron, S., Morin, N., Sigrist, S., Lehner, C., Doree, M. & Labbe, J.-C. 1998 Fizzy is required for activation of the APC/cyclosome in *Xenopus* egg extracts. *EMBO J.* **17**, 3565–3575.

Nasmyth, K. 1996 At the heart of the budding yeast cell cycle. *Trends Genet.* **12**, 405–412.

Patra, D. & Dunphy, W. G. 1998 Xe-p9, a *Xenopus* Suc1/Cks protein, is essential for the Cdc2-dependent phosphorylation of the anaphase-promoting complex at mitosis. *Genes Dev.* **12**, 2549–2559.

Peters, J.-M., King, R. W., Hoog, C. & Kirschner, M. W. 1996 Identification of BIME as a subunit of the anaphase-promoting complex. *Science* **274**, 1199–1201.

Pines, J. 1996 Reaching for a role for the Cks proteins. *Curr. Biol.* **6**, 1399–1402.

Rudner, A. D. & Murray, A. W. 1996 The spindle assembly checkpoint. *Curr. Opin. Cell Biol.* **6**, 773–780.

Schimke, R. T. & Doyle, D. 1970 Control of enzyme levels in animal tissues. *A. Rev. Biochem.* **39**, 929–976.

Schoenheimer, R. 1942 *The dynamic state of body constituents.* Cambridge, MA: Harvard University Press.

Schwab, M., Lutum, A. S. & Seufert, W. 1997 Yeast Hct1 is a regulator of Clb2 proteolysis. *Cell* **90**, 683–693.

Schteinberg, M. & Hershko, A. 1999 Role of Suc1 in the activation of the cyclosome by protein kinase Cdk1/cyclin B. *Biochem. Biophys. Res. Commun.* **257**, 12–18.

Schteinberg, M., Protopopov, Y., Litovsky, T., Brandeis, M. & Hershko, A. 1999 Phosphorylation of the cyclosome is required for its stimulation by fizzy/Cdc20. *Biochem. Biophys. Res Commun.* **260**, 193–198.

Sigrist, S. J. & Lehner, C. F. 1997 Drosophila *fizzy-related* down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles. *Cell* **90**, 671–681.

Simpson, M. V. 1953 The release of labeled amino acids from the proteins of rat liver slices. *J. Biol. Chem.* **201**, 143–154.

Sudakin, V., Ganoth, D., Dahan, A., Heller, H., Hershko, J., Luca, F. C., Ruderman, J. V. & Hershko, A. 1995 The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. *Mol. Biol. Cell* **6**, 185–198.

Sudakin, V., Shteinberg, M., Ganoth, D., Hershko, J. & Hershko, A. 1997 Binding of activated cyclosome to $p13^{suc1}$. Use for affinity purification. *J. Biol. Chem.* **272**, 18 051–18 059.

Townsley, F. M. & Ruderman, J. V. 1998 Proteolytic ratchets that control progression through mitosis. *Trends Cell Biol.* **8**, 238–234.

Tugendreich, S., Tomkiel, J., Earnshaw, W. & Hieter, P. 1995 CDC27Hs colocalizes with CDCl6Hs in the centrosome and mitotic spindle and is essential for the metaphase to anaphase transition. *Cell* **81**, 261–268.

Visintin, R., Prinz, S. & Amon, A. 1997 *CDC20* and *CDH1*: a family of substrate-specific activators of APC-dependent proteolysis. *Science* **278**, 460–463.

Wolf, D. A. & Jackson, P. K. 1998 Oiling the gears of anaphase. *Curr. Biol.* **8**, 636–639.

Yu, H., Peters, J.-P., King, R., Page, A. M., Hieter, P. & Kirschner, M. W. 1998 Identification of a cullin homology region in a subunit of the anaphase-promoting complex. *Science* **279**, 1219–1222.

Zachariae, W., Shevchenko, A., Andrews, P. D., Ciosk, R., Galova, M., Stark, M. J. R., Mann, M. & Nasmyth, K. 1998 Mass spectrometric analysis of the anaphase-promoting complex from yeast. *Science* **279**, 1216–1219.

Discussion

J. Pines (*Wellcome—CRC Institute, Cambridge, UK*). Do you think the initial phosphorylation has any connection with polo kinase?

A. Hershko. No. We tried polo and this didn't work in our hands. But there is evidence that polo activates the cyclosome in mammalian somatic cells. So this could be a difference between embryonic and somatic cell regulation.

M. Tyers (*Mount Sinai Hospital, Toronto, Canada*). Can you see recruitment of Cdc2p to the cyclosome, for example by sucrose gradient sedimentation in a Suc1p-dependent manner?

A. Hershko. We haven't tried this yet.

D. F. X. Diffley (*Imperial Cancer Research Fund, South Mimms, UK*). One of the popular models at the moment is that Fizzy and Fizzy-related act to recruit the substrate to the cyclosome. So, do you know if the stimulation of APC activity by fizzy works by decreasing the K_M for substrate or by increasing the V_{MAX} of the enzyme?

A. Hershko. I do think Fizzy increases the affinity of the cyclosome for its substrate because you can see much higher molecular weight conjugates. That is, the cyclosome is more processive with Fizzy. High processivity usually means high affinity.

M. Yanagida (*CREST Research Project, Kyoto University, Kyoto, Japan*). Suc1p assembles 'basket-like' structures, but in your model, you present it as a monomer. Can you accommodate the Suc1 structure in your model?

A. Hershko. Although there was some initial excitement about these 'basket-like' structures, I think they actually block the Cdc2p binding site. So now most people think the monomeric form is the active form.

J. Endicott (*University of Oxford, Oxford, UK*). Yes, from looking at the *S. pombe* Suc1 it appears that only the monomeric form would have both sites accessible. With the *pombe* protein, we never see any hexameric forms of the protein.