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Two distinct ubiquitin-proteolysis pathways in the fission yeast cell cycle

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The SCF complex (Skp1–Cullin-1–F-box) and the APC/cyclosome (anaphase-promoting complex) are two ubiquitin ligases that play a crucial role in eukaryotic cell cycle control. In fission yeast F-box/WD-repeat proteins Pop1 and Pop2, components of SCF are required for cell-cycle-dependent degradation of the cyclin-dependent kinase (CDK) inhibitor Rum1 and the S-phase regulator Cdc18. Accumulation of these proteins in *pop1* and *pop2* mutants leads to re-replication and defects in sexual differentiation. Despite structural and functional similarities, Pop1 and Pop2 are not redundant homologues. Instead, these two proteins form heterodimers as well as homodimers, such that three distinct complexes, namely $SCF^{Pop1/Pop1}$, $SCF^{Pop1/Pop2}$ and $SCF^{Pop2/Pop2}$, appear to exist in the cell. The APC/cyclosome is responsible for inactivation of CDK/cyclins through the degradation of B-type cyclins. We have identified two novel components or regulators of this complex, called Apc10 and Ste9, which are evolutionarily highly conserved. Apc10 (and Ste9), together with Rum1, are required for the establishment of and progression through the G1 phase in fission yeast. We propose that dual downregulation of CDK, one via the APC/cyclosome and the other via the CDK inhibitor, is a universal mechanism that is used to arrest the cell cycle at G1.

Keywords: anaphase-promoting complex (APC); cyclosome; Cullin; F-box; fission yeast; WD-repeat

1. INTRODUCTION

The SCF complex (Skp1–Cullin-1–F-box) and the APC/cyclosome (anaphase-promoting complex) are two ubiquitin ligases (E3s) that play a pivotal role in cell cycle progression. Both complexes are composed of multiple protein subunits, most of which are evolutionarily conserved from vertebrates to yeasts. In fission yeast Pop1 and Pop2, members of an F-box/WD-repeat family are components of SCF. Pop1 and Pop2 are required for ubiquitin-dependent degradation of the cyclin-dependent kinase (CDK) inhibitor Rum1 and the S-phase regulator Cdc18. Mutations in the *pop1* and/or *pop2* genes result in stabilization of these two cell cycle regulators, which leads to re-replication and failure to differentiate. Despite structural and functional similarities, Pop1 and Pop2 are not redundant homologues. Instead, these two proteins act independently and form heterodimers as well as homodimers in the cell. Therefore, there appear to be three distinct complexes, namely $SCF^{Pop1/Pop1}$, $SCF^{Pop1/Pop2}$, and $SCF^{Pop2/Pop2}$, by which SCF might evolve a sophisticated mechanism to control a cell-cycle-dependent fluctuation of Rum1 and Cdc18. As in other eukaryotes, Cullin-1 (called Pcu1) comprises a platform for $SCF^{Pop1, Pop2}$. We have found that cell-cycle-regulating proteins other than Rum1 and Cdc18 are also degraded in a Pop1- and Pop2-dependent manner. Therefore, it appears that $SCF^{Pop1, Pop2}$ is required for ubiquitin-dependent degradation of a broader range of substrates than originally thought. Furthermore, we have evidence that shows fission yeast SCF is composed of F-box-containing proteins other than Pop1 and Pop2, as proposed in budding yeast SCF.

The APC/cyclosome is responsible for inactivation of CDK/cyclins through ubiquitin-dependent degradation of mitotic cyclins, and required for sister chromatid separation through the degradation of Cut2. In frogs, it has been shown that the APC/cyclosome forms the 20S complex, consisting of eight stoichiometric subunits that are conserved through evolution. We have identified a novel regulator of this complex, called Apc10. Homologues of Apc10 are found from budding yeast to humans. In budding yeast, Apc10 (also called Doc1) is also required for the degradation of mitotic cyclins and has been proposed to be a stoichiometric component of the APC/cyclosome. In fission yeast, our data suggest that Apc10 is rather a positive regulator for this complex as Apc10 does not co-sediment with the 20S complex. Nonetheless, Apc10 does co-immunoprecipitate with this complex. We have shown that the APC/cyclosome, together with the CDK inhibitor Rum1, is required for the establishment of and progression through the G1 phase in fission yeast. We propose that dual downregulation of CDK, one via the APC/cyclosome and the other via the CDK inhibitor, is a universal mechanism that is used to arrest cell-cycle progression at G1.

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2. RESULTS AND DISCUSSION

(a) *E3-ubiquitin ligase as a crucial regulator for selective proteolysis*

In the ubiquitin–proteasome-dependent proteolysis pathway, a ubiquitin ligase (E3), as well as a ubiquitin-conjugating enzyme (E2), is responsible for substrate selectivity and timing of degradation. Novel E3s, SCF and the APC/cyclosome play a pivotal role in cell-cycle progression. Recent studies in a variety of organisms and systems have begun to uncover the importance of these novel E3s in cell-cycle regulation. We have been investigating the molecular mechanisms underlying cell-cycle progression via ubiquitin-dependent selective proteolysis by using fission yeast, *Schizosaccharomyces pombe* as a convenient model system.

(b) *Skp1–Cullin-1–F-box (SCF)*

(i) *Identification of* pop1^{+} *as a gene required for degradation of the CDK inhibitor and S-phase regulator*

Studies of the fission yeast SCF E3-complex began when we isolated and characterized a mutant (designated *pop1*) that increased genome ploidy. Polyploidization in the *pop1* mutants takes place via the occasional bypass of M phase, resulting in re-replication (S-phase–S-phase) of genomic DNA (Kominami & Toda 1997; Maekawa *et al.* 1998). In fission yeast, three distinct mechanisms are known to lead to polyploidization. One is a bypass of M phase, which arises from either mutations in genes encoding components of mitotic CDK/cyclins (*cdc2* and *cdc13*), or overproduction of the CDK inhibitor Rum1, both of which result in inhibition of activities of mitotic CDK/cyclins without preventing S-phase progression (Broek *et al.* 1991; Hayles *et al.* 1994; Moreno & Nurse 1994; Correa-Bordes & Nurse 1995; Fisher & Nurse 1996; Jallepalli & Kelly 1996; Mondesert *et al.* 1996). The second mechanism is overproduction of the S-phase regulator Cdc18, which results in successive S phases (Nishitani & Nurse 1995; Jallepalli *et al.* 1997). The third is failure in proper chromosome segregation at anaphase, which is caused by mutations in genes involved in sister chromatid separation (Creanor & Mitchison 1990; Broek *et al.* 1991; Yanagida 1998).

Phenotypic similarities between the *pop1* mutants and strains in which Rum1 or Cdc18 is overproduced prompted us to examine the level of these two proteins in the *pop1* mutants. It was found that Rum1 and Cdc18 accumulate to a high level in this mutant, suggesting that Pop1 is involved in the stability of these two proteins. Subsequent analysis showed that (i) Rum1 and Cdc18 are normally degraded through the ubiquitin–proteasome pathway; (ii) ubiquitination of Rum1 and Cdc18 does not occur or is dramatically reduced in the *pop1* mutants; and (iii) the reason that the *pop1* mutants re-replicate is mainly due to the accumulation of Rum1. The *pop1*$^{+}$ gene was cloned and found to encode a protein that contains an F-box motif and seven WD40-repeats (Neer *et al.* 1994; Bai *et al.* 1996). In budding yeast, it was found that Cdc4, which shows the highest homology to Pop1, is required for ubiquitin-dependent degradation of the CDK inhibitor Sic1 and Far1 (Schwob *et al.* 1994; Henchoz *et al.* 1997) and also of the S-phase regulator Cdc6, the budding yeast homologue of Cdc18 (Piatti *et al.* 1996; Drury *et al.* 1997). It was also reported in mammalian cells that the CDK inhibitor p27^{Kip1} was degraded through the ubiquitin–proteasome pathway (Pagano *et al.* 1995). Taken together, these results suggest that the molecular mechanisms underlying cell-cycle progression via proteolysis of the CDK inhibitors and S-phase regulators are evolutionarily conserved.

(ii) *Identification of components of SCF and novel substrates*

Extensive studies from various laboratories mainly performed on budding yeast have established the notion that SCF is composed of Skp1, Cullin-1 (in budding yeast Cdc53) and F-box-containing proteins like Cdc4, together with Cdc34/Ubc3 (E2-ubiquitin-conjugating enzyme) (Schwob *et al.* 1994; Bai *et al.* 1996; Mathias *et al.* 1996; Willems *et al.* 1996; Feldman *et al.* 1997; Skowyra *et al.* 1997). The F-box is a motif that is responsible for an interaction with Skp1. There are a number of proteins containing an F-box even within the same organisms (15 in budding yeast and at least five in fission yeast) and experimental data suggest that SCF comprises multiple populations depending on which F-box-containing proteins form a complex (Patton *et al.* 1998*a*). For clarity, to show which F-box-containing proteins bind SCF, SCF complexed with particular F-box-containing proteins is referred to in the form of a superscript suffix, such as SCF^{Cdc4}. SCF is conserved in vertebrates as Skp1 is a highly conserved protein; in fact, the gene was originally cloned from human cells (Zhang *et al.* 1995) and Skp2, F-box-containing protein, forms a complex with Skp1 and Cullin-1 (Lisztwan *et al.* 1998; Lyapina *et al.* 1998; Yu *et al.* 1998), although cell-cycle regulators that are degraded via SCF in human cells still remain to be established.

In fission yeast, we have identified *pop2*$^{+}$, which encodes another F-box/WD-repeat protein with significant homology to Pop1 and Cdc4 (overall identity of amino-acid sequence is around 35% to both proteins). Deletion of *pop2*$^{+}$ results in defective phenotypes similar to the *pop1* mutants; re-replication and sterility (Kominami *et al.* 1998*a*; Jallepalli *et al.* 1998). Immunoblotting analysis indicates that as in the *pop1* mutants, in the *pop2* mutants the level of Rum1 and Cdc18 increases to a high level. Interestingly, the profile of the accumulation of these two proteins between *pop1* and *pop2* mutants differs in that more Rum1 accumulates in the *pop1* mutants, while more Cdc18 accumulates in the *pop2* mutants. The double *pop1pop2* mutants are viable, but result in even more accumulation of Rum1 and Cdc18. Pop1 and Pop2 bind Cdc18 (Kominami & Toda 1997; Jallepalli *et al.* 1998). This suggests that there is a difference in specificity of substrate recognition between Pop1 and Pop2. Importantly, despite structural and functional similarities, Pop1 and Pop2 are not redundant homologues. Overproduction of Pop1 fails to rescue defects of the *pop2* mutants and vice versa. Binding experiments using the tagged products derived from multicopy plasmids show that Pop1 and Pop2 form a heterocomplex in the cell. Furthermore, Pop1 and Pop2 also form homocomplexes. Therefore, it appears that three distinct complexes exist (SCF$^{Pop1/Pop1}$, SCF$^{Pop1/Pop2}$ and SCF$^{Pop2/Pop2}$), that are responsible for the elaborate regulation of the level of cell-cycle regulators through SCF (figure 1; Kominami *et al.* 1998*a*).

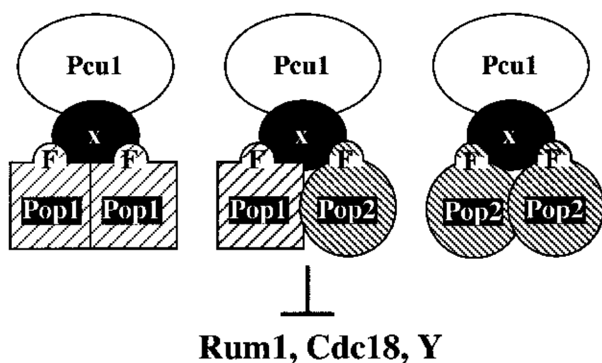

Figure 1. Model of fission yeast SCF$^{Pop1, Pop2}$ complexes. The SCF$^{Pop1, Pop2}$ complex comprises three distinct forms in terms of compositions of Pop1 and Pop2—SCF$^{Pop1/Pop1}$, SCF$^{Pop1/Pop2}$, SCF$^{Pop2/Pop2}$—which are responsible for the recognition of substrates (Rum1, Cdc18 and something else also, denoted by Y) and subsequent ubiquitination and degradation via the 26S proteasome. Most probably a Skp1 homologue (denoted by X) is also a component of SCF.

We have some evidence that Rum1 and Cdc18 are not the sole substrates to be degraded through SCF$^{Pop1, Pop2}$. These include B-type cyclins other than Cdc13 (Bueno & Russell 1993; Connonlly & Beach 1994; Obara-Ishihara & Okayama 1994). B-type cyclins are believed to be degraded through the APC/cyclosome (Stern & Nurse 1998). These results raise the interesting possibility that some cell-cycle regulators are degraded via two distinct E3 pathways, and suggest that there is cross-talk between SCF and the APC/cyclosome.

Cullin-1 was first identified as a mutation (*cul-1*) in *Caenorhabditis elegans* that is defective in G1-phase regulation (Kipreos *et al.* 1996). It is part of a large protein family: at least six members exist in humans (Cullin-1, -2, -3, -4A, -4B and -5). Cullin-1, both in budding yeast (called Cdc53) and human cells, acts as a platform for components of SCF (Feldman *et al.* 1997; Skowyra *et al.* 1997). We have identified a fission yeast homologue of *cullin-1* and *cullin-3* (designated *pcu1*$^+$ and *pcu3*$^+$, respectively) in the *S. pombe* genome database (which is located at the Sanger Centre, Cambridge, UK). Gene-disruption experiments of the *pcu1*$^+$ gene showed that cells deleted for *pcu1*$^+$ re-replicate, consistent with the idea that Pcu1 is a component of fission yeast SCF$^{Pop1, Pop2}$ (Kominami *et al.* 1998*a*). In fact, Pcu1 forms a complex with Pop1 and Pop2. In contrast to Pcu1, Pcu3 is not a component of SCF^{Pop1}, nor does it regulate genome ploidy. Instead, Pcu3 appears to be involved in a stress-responsive signal transduction pathway, as the loss of Pcu3 results in hypersensitivity to various stress conditions including DNA damage and drugs (Kominami *et al.* 1998*a*). It is of note that Pcu3 is nonetheless involved in, either directly or indirectly, cell cycle progression, as *pcu3*-deleted cells are delayed at G2/M-transition. Recently, Cullin members other than Cullin-1 have started to be analysed in other organisms and it will be interesting to see the biological role of each Cullin and the molecules with which they interact (Pause *et al.* 1997; Du *et al.* 1998; Lonergan *et al.* 1998; Osaka *et al.* 1998).

It should be noted that, unlike *pop1*$^+$ and *pop2*$^+$, *pcu1*$^+$ is essential for cell viability. Therefore, it appears that fission yeast SCF, in which Pcu1 acts as a platform, consists of F-box-containing components other than Pop1 and Pop2, which play a role essential to cell viability. This situation is similar to that of budding yeast SCF, in which at least three distinct F-box-containing proteins are known to be components of SCF (SCF^{Cdc4}, SCF^{Grr1} and SCF^{Met30}: Jaquenoud *et al.* 1998; Kishi *et al.* 1998; Li & Johnston 1997; Patton *et al.* 1998*a*). This complexity of regulation through F-box-containing subunit exchange is called 'combinatorial control' (Patton *et al.* 1998*b*).

(iii) *Mechanisms underlying substrate recognition by SCF*$^{Pop1, Pop2}$

Detailed molecular mechanisms concerning how SCF, in particular F-box-containing proteins, binds substrates for ubiquitination and transfers them to the proteasome for degradation in a temporally and spatially specific manner remain to be determined. It has been shown that, at least in the case of Cdc4, Pop1 and Pop2, substrates such as Sic1, Far1, Rum1 and Cdc18 need to be phosphorylated by CDK/cyclins for recognition and degradation. In other words, phosphorylation via CDK/cyclins is a prerequisite for these substrates to be degraded by SCF. In fact, mutant forms of Sic1, Rum1 and Cdc18, in which the consensus CDK-phosphorylation sites are either substituted or deleted, become highly stable and fail to bind SCF (Lanker *et al.* 1996; Schneider *et al.* 1996; Henchoz *et al.* 1997; Jallepalli *et al.* 1997, 1998; Verma *et al.* 1997*a*,*b*; Baum *et al.* 1998; Benito *et al.* 1998; Nishizawa *et al.* 1998).

(c) *APC/cyclosome*

(i) *A role of the APC/cyclosome in G1 cell-cycle arrest*

It has been shown unambiguously that the APC/cyclosome is required for the onset of anaphase (King *et al.* 1996; Hershko 1997). It has also been shown that activity of the APC/cyclosome persists from anaphase throughout the G1 phase in various organisms, including yeast and animal cells (Amon *et al.* 1994; Brandeis & Hunt 1996; Yamano *et al.* 1996). This suggests not only that the APC/cyclosome is required for the onset of anaphase, but also that it might be crucial for the maintenance of the G1 phase.

We have proposed previously the existence of the molecular mechanisms underlying G1 cell-cycle arrest upon nutritional deprivation in fission yeast (Kumada *et al.* 1995). A novel genetic screen for identifying factors required for this G1 cell-cycle arrest has been undertaken and has revealed the following simple mechanism. G1 cell-cycle arrest is achieved through dual downregulation of CDK/cyclins, one via direct physical inhibition of the CDK/cyclins by the Rum1 CDK inhibitor and the other via protein degradation of cyclins by the APC/cyclosome (figure 2; Kominami *et al.* 1998*b*). We propose that dual downregulation of CDK is a universal mechanism that is used to arrest cell-cycle progression in G1 not only in fission yeast but also in other eukaryotes. As described before, the level of Rum1 is regulated by SCF$^{Pop1, Pop2}$. Thus G1 cell-cycle arrest is regulated independently by two distinct E3s, another example of cross-talk between SCF and the APC/cyclosome. However, it should be noted that in this case, these two E3s function in an

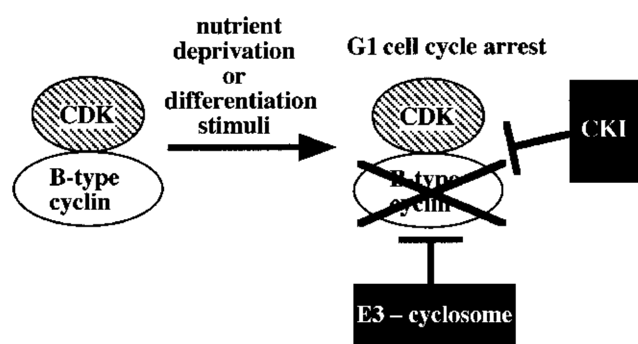

Figure 2. Dual downregulation of CDK/cyclins to achieve G1 cell-cycle arrest and differentiation. The APC/cyclosome plays a role in not only the onset of anaphase during mitotic cycle but also the establishment and maintenance of the G1 phase upon nutritional deprivation or differentiation conditions. To achieve proper G1 cell-cycle arrest, CDK/cyclins need to be downregulated in a dual manner, one via physical inhibition by the CDK inhibitor and the other via degradation of cyclins by the APC/cyclosome.

antagonistic manner: SCF inhibits the occurrence of G1 via degradation of Rum1, while the APC/cyclosome promotes it via degradation of B-type cyclins.

(ii) *Novel regulators of the APC/cyclosome*

Upon screening of mutants that are defective in G1 cell cycle arrest, we have identified, in addition to *rum1*$^{+}$ encoding the CDK inhibitor, two other genes that are important for the APC/cyclosome activities. One gene encodes Ste9/Srw1, a member of the WD-repeat proteins (Yamaguchi *et al.* 1997; Kitamura *et al.* 1998). Ste9/Srw1 is evolutionarily conserved and is the homologue of Hct1/Cdh1 in budding yeast (Schwab *et al.* 1997; Visintin *et al.* 1997) and Fzr in *Drosophila* (Sigrist & Lehner 1997), which has been shown to be a positive regulator of the APC/cyclosome. The other gene, called *apc10*$^{+}$, which has been identified in our screen, is also highly conserved in the evolution of yeast to human. In budding yeast, the homologue (*APC10/DOC1*) has been proposed to be a subunit of the APC/cyclosome (Hwang & Murray 1997; Zachariae *et al.* 1998). Our analysis in fission yeast, however, suggests that Apc10 is a regulator, rather than a subunit of the APC/cyclosome, as Apc10 does not co-sediment with the 20S APC/cyclosome (figure 3). Furthermore, in the temperature-sensitive *apc10* mutations, this 20S complex is still intact. This clearly contrasts with the situation of mutant stoichiometric subunits of this complex (*cut4*, *cut9* and *nuc2*) in this organism, where the 20S complex is physically disrupted (Yamashita *et al.* 1996; Yamada *et al.* 1997). It has been shown that the native size of the budding yeast APC/cyclosome is somewhat larger (36S: Zachariae *et al.* 1996) than that reported both for animals (20S: King *et al.* 1995; Sudakin *et al.* 1995) and for fission yeast (20S: Yamashita *et al.* 1996; Yamada *et al.* 1997). It is possible that during evolution, Apc10, which is essential for the activity of APC/cyclosome, is incorporated into the APC/cyclosome as a core complex in budding yeast; or, conversely, in animal and fission yeast cells, Apc10 gains a regulatory role, so the binding between Apc10 and the APC/cyclosome becomes more loose. It should be noted that although Apc10 does not co-sediment with the 20S core complex, it does co-immunoprecipitate with the APC/cyclosome (Kominami *et al.* 1998*b*).

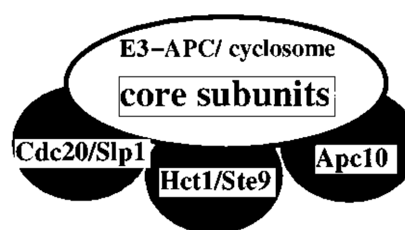

Figure 3. Subunits and regulators of the APC/cyclosome. The APC/cyclosome comprises stoichiometric core subunits (in frogs Apc1–8) and a number of regulators. These include two distinct WD-repeat proteins Cdc20/Slp1 (the names used in reference to budding yeast/fission yeast) and Hct1/Ste9, and Apc10. Cdc20/Slp1 (called Fizzy in *Drosophila* and vertebrates) is a positive regulator of the APC/cyclosome, which binds directly and activates this complex (Matsumoto 1997; Fang *et al.* 1998; Kramer *et al.* 1998; Lim *et al.* 1998; Lorca *et al.* 1998; Wolf & Jackson 1998).

3. FUTURE QUESTIONS

In mammalian cells, counterparts for yeast Cdc4 and Pop1 have not been identified yet. In view of the high degree of conservation of other components in SCF such as Cullin-1 and Skp1, it is likely that mammalian cells also have F-box/WD-repeat proteins as components of SCF. In fission yeast, by forming three distinct complexes, $SCF^{Pop1/Pop1}$, $SCF^{Pop1/Pop2}$ and $SCF^{Pop2/Pop2}$, SCF has appeared to evolve a sophisticated mechanism to control the level of Rum1 and Cdc18. It is of interest to see whether this degree of regulation by hetero- and homo-dimerization between F-box-containing proteins in a single SCF complex is conserved in evolution. Also, as there are a number of distinct F-box-containing proteins in the same organism, how SCF specifically forms a complex with distinct F-box-containing proteins is an important question. Finally, the identification of more substrates that are degraded via each form of SCF and the molecular mechanisms underlying substrate recognition by SCF need to be explored. One of the important functions of the APC/cyclosome is the establishment and maintenance of the G1 phase. As the mutants we have isolated in fission yeast are impaired in differentiation due to failure to arrest at G1, some mutations in genes encoding subunits or regulators of the APC/cyclosome in human cells might result in the occurrence of cancerous cells. How the APC/cyclosome is regulated through the cell cycle is, therefore, a crucial question to be addressed in terms of eukaryotic growth control.

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Note added in proof. After submission of the manuscript, a number of papers have been published which describe substrates and F-box proteins of metazoan SCF.

These include β-catenin, IκBα and HIV-1 Vpu (for the F-box β-TrCP protein, reviewed in Laney & Hochstrasser (1999 *Cell* **97**, 427–430) and Maniatis (1999 *Genes Dev.* **13**, 505–510)), and E2F-1 and $p27^{Kip1}$ (for the F-box Skp2 protein, Marti *et al.* (1999 *Nature Cell Biol.* **1**, 14–19) and Tsvetkov *et al.* (1999 *Curr. Biol.* **9**, 661–664)).

Discussion

M. Hochstrasser (*University of Chicago, USA*). You mentioned that Cig2 has a destruction box. Is this required for its degradation and, if not, is there a PEST sequence in the protein?

T. Toda. The destruction box of Cig2 is important for its degradation by the APC. We don't know what sequence in Cig2 is required for its degradation by the Pop1 pathway.

R. T. Hunt (*ICRF Clare Hall Laboratories, Hertfordshire, UK*). Have homologues of Cdc34 or Skp1 been found in *S. pombe*?

T. Toda. Not yet.

K. A. Nasmyth (*Research Institute for Molecular Pathology, Vienna, Austria*). I would like to make a comment on the Apc10. I wonder whether you have tried to 'pull down' the APC from extracts to see the other stoichiometric subunits and failed to have seen Apc10. The reason I say this is because the budding yeast results are very clear—Apc10 is present in stoichiometric amounts.

T. Toda. We think that the fact that the 20S complex is not affected in the temperature-sensitive mutants suggests that Apc10 might not be an integral component of the APC.

T. Humphrey (*MRC, Harwell, Didcot, Oxon, UK*). Your analysis of the *S. pombe* Cullin disruption showed a clear 4C peak of DNA content. If this is the sole Cullin involved in Rum1 and Cdc18 degradation, then you should get quite a high level of expression of these proteins and, therefore, you should see more re-replication.

T. Toda. In the *pcu1* mutants, other things like Cig2 are being overexpressed, which might counteract the effects of overproduction of a single protein.

M. Tyers (*Samuel Lunenfeld Research Institute, Toronto, Canada*). I am interested in the Pop1–Pop2 heterodimers. If you overexpress either Pop1 or Pop2, do you see any dominant phenotypes similar to the loss of either protein?

T. Toda. Overproduction of either *pop1* or *pop2* produces only very mild phenotypes. However, in the *pop2* mutant background, for example, overexpression of $pop1^+$ has a more severe effect.

M. Yanagida (*CREST Research Project, Kyoto University, Japan*). Why are only Nuc2 and Apc10 found to be required for arresting cells in G1? There are many other *apc* mutants which do not have this phenotype.

T. Toda. The simplest explanation is that our screen is not saturated.

M. Yanagida (*CREST Research Project, Kyoto University, Japan*). Is it possible that Apc10 is only bound to the APC during G1?

T. Toda. We have tried to examine this but we don't see any significant increase in the amount of APC-associated Apc10 in G1.