Role of the SCF<sup>Skp2</sup> Ubiquitin Ligase in the Degradation of p21<sup>Cip1</sup> in S Phase*

Received for publication, February 19, 2003, and in revised form, April 10, 2003
Published, JBC Papers in Press, May 2, 2003, DOI 10.1074/jbc.M301774200

Gil Bornstein‡, Joanna Bloom§, Danielle Sitry-Shevaž, Keiko Nakayama†, Michele Pagano§, and Avram Hershko¶

From the ³Unit of Biochemistry, the B. Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel, the ¤Department of Pathology and Kaplan Comprehensive Cancer Center, New York University School of Medicine, New York, New York 10016, and the ‡Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan

The cyclin-dependent kinase inhibitor p21<sup>Cip1</sup> has important roles in the control of cell proliferation, differentiation, senescence, and apoptosis. It has been observed that p21 is a highly unstable protein, but the mechanisms of its degradation remained unknown. We show here that p21 is a good substrate for an SCF (Skp1-Cullin1-F-box protein) ubiquitin ligase complex, which contains the F-box protein Skp2 (S phase kinase-associated protein 2) and the accessory protein Cks1 (cyclin kinase subunit 1). A similar ubiquitin ligase complex has been previously shown to be involved in the degradation of a related cyclin-dependent kinase inhibitor, p27<sup>Kip1</sup>.

The levels of Skp2 oscillate in the cell cycle, reaching a maximum in S phase. The ubiquitylation of p21 <em>in vitro</em> required the supplementation of all components of the SCF complex as well as of Cks1 and Cdk2-cyclin E. The protein kinase Cdk2-cyclin E acts both by the phosphorylation of p21 on Ser-130 and by the formation of a complex with p21, which is required for its presentation to the ubiquitin ligase. As opposed to the case of p27, the phosphorylation of p21 stimulates its ubiquitylation but is not absolutely required for this process. Levels of p21 are higher in Skp2<sup>−/−</sup> mouse embryo fibroblasts than in wild-type fibroblasts in the S phase, and the rates of the degradation of p21 are slower in cells that lack Skp2. It is suggested that SCF<sup>Skp2</sup> participates in the degradation of p21 in the S phase.

The cyclin-dependent kinase (Cdk) inhibitor p21<sup>Cip1</sup> has important roles in the control of cell proliferation, differentiation, senescence, and apoptosis. The amino-terminal domain of p21 binds to and inhibits cyclin-Cdk complexes, while the carboxyl-terminal domain binds to proliferating cell nuclear antigen and inhibits its function as a processivity factor of DNA polymerase. Levels of p21 increase dramatically under conditions such as p53-induced growth arrest, early stages of differentiation, and senescence (1–4). Much of the regulation of p21 levels is achieved by transcriptional control mechanisms. However, it appears likely that post-transcriptional processes may also regulate p21 levels. p21 is a highly unstable protein (5–7), and thus modulation of the rate of its degradation may provide an additional level of regulation. However, not much is known about the mechanisms of the degradation of p21 or its regulation. It has been reported that p21 is degraded by a ubiquitylation-independent but proteasome-dependent mechanism since a lysine-less derivative of p21 was still degraded rapidly in cells (8). It was later reported that a carboxyl-terminal region of p21 is required for its binding to the C8 subunit of the 20 S proteasomes as well as for its degradation by purified 20 S proteasomes (9). However, it has also been repeatedly observed that ubiquitylated derivatives of p21 accumulate when cells are treated with proteasome inhibitors (5–8). This suggests that at least some fraction of p21 in cells is targeted for ubiquitylation by proteasomal degradation. It was furthermore reported that interaction of p21 with Cdk is necessary for its degradation <em>in vivo</em> (6). This is in contrast to the <em>in vitro</em> degradation of p21 by 20 S proteasomes that occurs in the absence of Cdk (9). On the other hand, the need for Cdk interaction for p21 degradation is reminiscent of a similar requirement for the degradation of a related Cdk inhibitor, p27<sup>Kip1</sup> (10, 11).

The present investigation was undertaken to examine whether p21 may be targeted for degradation by a ubiquitin ligase system similar to that involved in the degradation of p27. It is now well established that p27 is ubiquitylated by an SCF (Skp1-Cullin1-F-box protein) ubiquitin ligase complex (for reviews, see Refs. 12 and 13), which contains Skp2 (S phase kinase-associated protein 2) as its specific F-box protein (14–17). Levels of Skp2 in cultured fibroblasts rise in the G<sub>0</sub>/G<sub>1</sub> phase transition coincident with the timing of the degradation of p27 (14, 18). The ubiquitylation of p27 by SCF<sup>Skp2</sup> requires its binding to Cdk2-cyclin A/E (10, 11) and its phosphorylation by Cdk2 on Thr-187 (10, 11, 19). The ubiquitylation of p27 by SCF<sup>Skp2</sup> also requires the participation of an accessory protein, Cks1 (20, 21). Cks1 binds to Skp2 and to phosphorylated p27 and thus promotes their tight interaction (20–22). Concerning the possible involvement of SCF<sup>Skp2</sup> in the degradation of p21, it has been reported that inhibition of the expression of Skp2 with antisense oligonucleotides increased the levels of p21 and of cyclin D, suggesting their stabilization (23). However, in the same paper it was reported that similar ablation of Skp2 had no effect on levels of p27 (23), which is not consistent with the results described above. We now report that SCF<sup>Skp2</sup> promotes the ubiquitylation of p21 <em>in vitro</em> and that Skp2 is involved in p27 degradation <em>in vivo</em> during the S phase of the cell cycle.
EXPERIMENTAL PROCEDURES

Reagents—His6-Cdc43, His6-UbcH5c, His6-p27, and His6-p21 were expressed and were purified by chromatography on nickel-agarose. p21 expressed in the bacteria was not soluble and was renatured prior to nickel chromatography as follows. The pellet was dissolved in a solution containing 50 mM Tris-HCl (pH 8.0), 6 mM guanidine hydrochloride, 5 mM EDTA, and 0.5% Triton X-100. The sample was allowed to stay for 30 min at room temperature and then was dialyzed against 50 mM Tris-HCl (pH 8.0), 20% (v/v) glycerol, and 1 mM dithiothreitol. His6-Skp1, His6-Skp2, His6-Skp1-Skp2, His6-Skp1-Skp2-TRCP, His6-Skp1-Skp2-Cks1 (M, 63,000 form), His6-Cull1-Roc1, and His6-cyclin E-Cdk2 (either wild type or catalytically inactive kinase Cdk2m (K33T,K34S; Ref. 11)) were produced by co-infection of 5B insect cells with baculoviruses encoding the corresponding proteins and were purified by nickel-agarose chromatography as described previously (11, 14). The approximate concentrations of the proteins (representing the protein present at lower concentration) in the preparation of this preparation was 0.3 μM. Cdk inhibitor p21 was prepared as described previously (24). The Cdk inhibitor p21Cip1 is a protein with translation-independent but proteasome-dependent manner (8). It has been reported that p21 is degraded in a ubiquitylation-dependent pathway. Since a number of inhibitors (5–8), indicating that ubiquitylation-dependent pathways are also involved in p21 degradation. Therefore, we have examined the possible involvement of this complex in p21 degradation. In the experiment shown in Fig. 1A, the ubiquitylation of in vitro translated 35S-labeled p21 by purified components of the SCFp21 system was examined. In the presence of ubiquitin, high molecular weight ubiquitylated derivatives of p21 were formed (lane 7), while with methylated ubiquitin (that prevents the formation of polyubiquitin chains, Ref. 24) lower molecular weight conjugates accumulated (lane 2). We prefer to use methylated ubiquitin for these assays because of the easy detection of the distinct low molecular weight products. The ubiquitylation of p21 in vitro required the suplementation of Skp2-Skp1 (Fig. 1A, lane 3), Cks1 (lane 5), and Cdk2-cyclin E (lane 6). The omission of Cul1-Roc1 resulted in partial in p21 ubiquitylation (Fig. 1A, lane 4). This could be due to the presence of Cul1 and Roc1 in reticulocyte lysates, added with in vitro-translated 35Sp21. In fact, significant amounts of Cul1 and were detected in reticulocyte lysates by immunoblotting with a specific antibody (data not shown). That indeed all components of the SCFSkp2 complex are required for p21 ubiquitylation was verified by a similar assay in which bacterially expressed, purified p21 was the substrate and reaction products were detected by immunoblotting with an anti-p21 antibody (Fig. 1B). In this completely purified system, robust ligation of p21 to ubiquitin (Fig. 1B, lane 8) or to methylated ubiquitin (lane 2) was observed, and p21 ubiquitylation required Cul1-Roc1 (lane 4) as well as all other components such as Skp2-Skp1, Cks1, and Cdk2-cyclin E (Fig. 1B). Cdk2-cyclin A could effectively replace Cdk2-cyclin E for p21 ubiquitylation (data not shown). The selectivity of p21-ubiquitin ligation for Skp2 was indicated by the observation that Skp2 could not be replaced in this reaction by some other mammalian F-box proteins such as TRCP or hCdc4 (Fig. 1C). Control experiments indicated that the preparations of βTRCP and of hCdc4 used in these experiments were active in the ubiquitylation of their known substrates IκBα (25) and cyclin E (26), respectively (data not shown). These properties of p21-ubiquitin ligation by SCFSkp2 are similar to those observed previously for the ubiquitylation of p27 (14, 20). These results indicated that p21 is a good substrate for the SCFSkp2-Cks1 ubiquitin ligase complex at least in vitro.

Properties of p21-Ubiquitin Ligation by SCFSkp2—Further examination of the properties of p21-ubiquitin ligation by SCFSkp2 showed additional similarities to those of p27-ubiquitin ligation but also revealed some significant differences. p27 ubiquitylation requires its phosphorylation on Thr-187 by Cdk2-cyclin E or Cdk2-cyclin A (11, 14). As described above, the complementation of these protein kinases was also absolutely required for the ubiquitylation of p21 in vitro. However, Cdk2-cyclin E/A is required not only for the phosphorylation of p27 but also for its binding in a trimeric complex necessary for the presentation of the Cdk inhibitor to the ubiquitylation machinery (10, 11). To distinguish between these actions of Cdk2, we have used the protein kinase inhibitor staurosporine or replaced Cdk2 with a catalytically inactive mutant (K33T,K34S) of this enzyme (11). A control experiment showed that staurosporine or mutant Cdk2 prevented most of the ligation of p27 to ubiquitin (Fig. 2A, lanes 3 and 4). The residual rate of p27-ubiquitin ligation observed in the absence of protein kinase activity did not exceed 10% of the control values (Fig. 2B, left panel). Surprisingly, however, about one-half the rate of p21-
ubiquitin ligation persisted in the presence of these agents (Fig. 2, A, lanes 7 and 8, and B, right panel). This was not due to phosphorylation of p21 by some other protein kinase from reticulocyte lysate used for in vitro translation of [35S]p21 because similar results were obtained with bacterially expressed, purified p21 (data not shown). We conclude that phosphorylation by Cdk2 stimulates p21-ubiquitin ligation by SCF-Skp2 but is not absolutely required for this process. The absolute requirement for the presence of Cdk2-cyclin E/A is presumably due to obligatory formation of a trimeric complex with p21 for its presentation to the ubiquitin ligase.

We next asked whether the phosphorylation of a specific component of the SCF-Skp2-Cks1 complex for the ligation of [35S]p21 to ubiquitin. The ligation of [35S]p21 to methylated ubiquitin (MeUb) or to native ubiquitin (Ub) was determined as described under “Experimental Procedures” in the presence of the indicated components. Numbers on the right side indicate the position of molecular mass marker proteins. B, ubiquitylation of bacterially expressed p21 by SCF-Skp2. Reaction conditions were similar to those described in A except that [35S]p21 was replaced with 15 ng of bacterially expressed, purified p21. C, F-box protein specificity of the SCF complex that ubiquitylates p21. Reaction conditions were as described under “Experimental Procedures.” Where indicated, Skp2 was replaced by the other F-box proteins. All recombinant, purified F-box proteins were co-expressed with Skp1 and were supplemented at 2 nM. Similar results were obtained when Cdc34 was replaced by UbcH5 (not shown). Cyc, cyclin.

FIG. 2. Phosphorylation of p21 on Ser-130 stimulates, but is not absolutely required for, its ligation to ubiquitin. A, effects of phosphorylation and S130A mutation on p21-ubiquitin ligation. The ligation of methylated ubiquitin to 35S-labeled p27 (lanes 1–4), wild-type p21 (lanes 5–8), or S130A mutant p21 (lanes 9–12) was determined as described under “Experimental Procedures.” Where indicated, native Cdk2-cyclin E was replaced by a similar concentration of its catalytically inactive mutant Cdk2m-cyclin E. Staurosporine (10 μM) was added prior to the addition of Cdk2-cyclin E. Numbers on the right side indicate the position of molecular mass marker proteins (in kDa). B, time course of ubiquitylation of [35S]p27 (left panel) or of [35S]p21 (right panel) in the absence (Control, open circles) or in the presence of 10 μM staurosporine (closed circles). Results were quantified as described under “Experimental Procedures.” C, alignment of carboxyl-terminal residues of p21 and p27. Identical amino acid residues are boxed. Ub, native ubiquitin; WT, wild type; Cyc, cyclin.
zymes with which the SCFSkp2 complex acts. In all previous reports, ubiquitylation was observed concerning the specificity for E2 enzyme UbcH5c. By contrast, in the case of p21, UbcH5c was much less effective than Cdc34 (Fig. 3B). Since the affinities of E2 enzymes for SCF complexes are increased by Nedd8 ligation to the Cullin1 subunit (27), we have examined these E2 specificities in the presence or absence of the Nedd8 ligation system. The supplementation of the Nedd8 ligation system stimulated ubiquitylation of p21 in a manner similar to its effect on the ubiquitylation of p27 (28) in the presence of either E2 enzyme. However, even with the Nedd8 ligation system, activity with UbcH5c in p21 ubiquitylation remained low relative to that observed with Cdc34 (Fig. 3). We conclude that the selectivity for E2 of the same SCF<sub>Skp2</sub> complex is affected by the identity of the protein substrate.

**Role of Skp2 in p21 Degradation in the S Phase**—The above-described experiments established that p21 is a good substrate for the SCF<sub>Skp2</sub>-Cks1 ubiquitin ligase complex in vitro. We next tried to examine whether this system is actually involved in the degradation of p21 in intact cells. Levels of Skp2 are very low in G<sub>0</sub>, rise in late G<sub>1</sub>, and are maximal in S phase of the cell cycle (14, 18). Thus, it seemed reasonable to assume that the contribution of SCF<sub>Skp2</sub> to p21 degradation may be maximal in S phase. Wild-type and Skp2−/− (17) MEFs in culture were synchronized by serum starvation and then were stimulated to re-enter the cell cycle by the addition of serum. When cells were in S phase (20–24 h after serum stimulation), cycloheximide was added to inhibit protein synthesis, and the rate of the

---

**TABLE I**

<table>
<thead>
<tr>
<th>Cks1</th>
<th>Percentage of p21 ligated to ubiquitin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cdk2-cyclin E</td>
</tr>
<tr>
<td>Wild type</td>
<td>39.5</td>
</tr>
<tr>
<td>R20A</td>
<td>5.7</td>
</tr>
<tr>
<td>W54F</td>
<td>21.4</td>
</tr>
<tr>
<td>R71A</td>
<td>4.7</td>
</tr>
</tbody>
</table>

---

Residue of p21 is responsible for stimulation of p21-ubiquitin ligation. Alignment of the carboxyl-terminal regions of p21 and p27 revealed a similarity in several amino acid residues surrounding a Cdk phosphorylation site at Ser-130 in p21 to those in the vicinity of Thr-187 in p27 (Fig. 2C). We mutated Ser-130 in p21 to Ala and found that the rate of the ubiquitylation of this mutant decreased to about one-half of that obtained with the wild-type protein (Fig. 2A, lane 10). With the S130A p21 mutant, inhibition of Cdk2 activity with staurosporine or its replacement with catalytically inactive Cdk2 did not decrease further ubiquitin ligation (Fig. 2A, lanes 11 and 12). These results suggested that phosphorylation of p21 at Ser-130, but not at other sites, is responsible for the stimulation of its ubiquitylation by SCF<sub>Skp2</sub>.

SCF<sub>Skp2</sub> is unique among SCF ubiquitin ligases in its requirement for an accessory protein, Cks1 (20, 21). Previously it was shown that three different binding sites of Cks1 are required for its action to promote p27 ubiquitylation: a Skp2-binding site, a Cdk-binding site, and an anion-binding site (22). The exact role of the anion-binding site of Cks1 remains unknown, although it was shown that this site is required for the binding of the Skp2-Cks1 complex to the carboxyl-terminal region of Thr-187-phosphorylated p27 (22). The possibility that the anion-binding site of Cks1 binds directly to the phosphate group of the substrate could not be addressed in that study. The present results showing that Cks1 is required for p21 ubiquitylation (Fig. 1, A and B) and that p21 phosphorylation is not absolutely required for this process (Fig. 2A) allowed us to examine this problem. In the experiment shown in Table I, we have examined the effects of mutations in three highly conserved residues in the anion-binding site of Cks1 (Arg-20, Trp-54, and Arg-71) on p21-ubiquitin ligation in the presence of active or enzymatically inactive Cdk2-cyclin E. If the anion-binding site was required only for interaction with the phosphate group of the substrate, it would be expected that mutations in the phosphate-binding site would affect only the part of ubiquitylation activity that is stimulated by substrate phosphorylation. However, as shown in Table I, all three mutations in the anion-binding site of Cks1 inhibited p21-ubiquitin ligation activity in the absence of substrate phosphorylation to extents similar to those observed in the presence of p21 phosphorylation. Similar results were observed when bacterially expressed, purified p21 was used as the substrate (data not shown). These results indicate that, at least in the case of p21, the anion-binding site of Cks1 has some other role such as interaction with acidic groups of the substrate (see “Discussion”).

Another difference in the properties of p21 versus p27 ubiquitylation was observed concerning the specificity for E2 enzymes with which the SCF<sub>Skp2</sub> complex acts. In all previous experiments, we have used the Cdc34 E2 enzyme, which works well with SCF<sub>Skp2</sub> (14). As shown in Fig. 3A, Cdc34 can be effectively replaced for the ubiquitylation of p27 by the E2 enzyme UbcH5c. By contrast, in the case of p21, UbcH5c was

---

**FIG. 3. Differences in selectivity for E2 in the ubiquitylation of p21 or p27 by SCF<sub>Skp2</sub>.** Reaction conditions were as described under “Experimental Procedures” except that 35S-labeled substrates were replaced with 15 ng of bacterially expressed, purified p27 (A) or p21 (B). Where indicated, 15 pmol of Cdc34 or 25 pmol of UbcH5c were supplemented. The Nedd8 system (Nedd8 syst) consisted of 24 pmol of Nedd8, 0.03 pmol of APP/BP1-Uba3, and 0.05 pmol of Ub12. Reaction products were separated by SDS-PAGE and were immunoblotted by antibodies against p27 (A) or p21 (B).
degradation of p21 was followed by immunoblotting. As shown in Fig. 4A, p21 was degraded rapidly in wild-type cells with a half-life of 50 min. In Skp2−/− cells, the initial levels of p21 in S phase were significantly higher, and the rate of p21 degradation was much slower than in cells with a half-life of approximately 2–4 h.

The rate of growth of Skp2−/− MEFs in culture is much lower than that of wild-type cells. It was possible that the p21 degradation in Skp2−/− cells is secondary to other alteration caused by growth retardation, which is in agreement with the observation that the initial levels of p21 in S phase are significantly lower in Skp2−/− cells than in wild-type cells. This also suggests that Skp2 is involved in the degradation of p21 in S phase.

The slow but noticeable degradation of p21 in Skp2-deficient cells indicates the existence of additional systems involved in the degradation of this protein.

FIG. 4. Skp2 is required for the degradation of p21 in mouse embryo fibroblasts. Early passage mouse embryo fibroblasts of the indicated genotypes were synchronized in S phase and were then treated with cycloheximide for the time periods indicated. Cell extracts were separated by SDS-PAGE and were blotted with antibodies directed against p21 (A and B) and α-tubulin (B). C, the immunoblot shown in B was quantified as described under “Experimental Procedures.” Results were expressed as the ratio of p21 to α-tubulin.

A. WT Skp2−/−

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>p21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tubulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. p27−/− Skp2−/−; p27−/−

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>p21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-tubulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C. p27−/−; Skp2−/−

The results were quantified and expressed as p21/α-tubulin ratio (Fig. 4C). The addition of cycloheximide, the rate of decline of p21 in Skp2−/−; p27−/− MEFs was much lower than that in control p27−/− cells. Based on these experiments, we concluded that Skp2 is involved in the degradation of p21 in S phase. The slow but noticeable degradation of p21 in Skp2-deficient cells indicates the existence of additional systems involved in the degradation of this protein (see “Discussion”).

DISCUSSION

The data reported in this paper indicate that p21Cip1 is a substrate for the SCFSkp2 ubiquitin ligase complex. This conclusion is supported by the observations that all components of the SCFSkp2-Cks1 system are required for the ubiquitination of p21 in vitro and that the rate of degradation of p21 in Skp2−/− mouse embryo fibroblasts in S phase is considerably lower than that in wild-type cells. These properties resemble those observed previously for the SCFSkp2-mediated degradation of p27 (14, 17, 20). However, as opposed to the case of p27 degradation, it appears reasonable to assume that this is one of several systems involved in the degradation of p21. In cells at G0, low levels of Skp2 are accompanied by high levels of p21 caused by marked retardation in the rate of p21 degradation (14). By contrast, levels of p21 are not very high in G0, and p21 is still rapidly degraded in diploid fibroblasts arrested in G0 by serum deprivation. In addition, the observation that lysine-less p21 is rapidly degraded in a proteasome-dependent manner (8) still awaits explanation. It is possible that p21 is subject to degradation by several systems, some of which are predominant only under certain physiological situations or at certain stages of the cell cycle.

The properties of the ubiquitylation of p21 by SCFSkp2 are similar to those of p27 with respect to the requirement for all components of this complex (Fig. 1, A and B), selectivity for Skp2 as the F-box protein (Fig. 1C), and requirements for the presence of Cks1 and of Cdk2-cyclin E/A (Fig. 1). They are also similar in that phosphorylation at a specific site, Thr-187 in p27 (11) and Ser-130 in p21 (12 Fig. 2), stimulates the ubiquitylation of both proteins. They are different in the extent at which ubiquitylation can take place in the absence of phosphorylation, which is much more prominent with p21 than with p27.

This is unusual for substrates of SCF complexes, most of which are recognized for ubiquitylation only following phosphorylation (12, 13). Furthermore, the anion-binding site of Cks1, which is necessary for the interaction of the Skp2-Cks1 complex, is required for the ubiquitylation of p21Cip1 by SCFSkp2. However, the exact mechanism by which Skp2-Cks1 stimulates the ubiquitylation of p21Cip1 is not clear.

REFERENCES


complex with phosphorylated p27, is still required for p21 ubiquitylation in the absence of phosphorylation (Table I). It is notable that the highly conserved anion-binding site of Cks proteins interacts not only with phosphate groups but also with negatively charged Glu residues in proteins (32). A possible explanation for the above observations is that p21 contains additional negatively charged regions recognized by the Skp2-Cks1 complex at an efficiency similar to the recognition of the region of the phosphorylated amino acid residue.

Another observation that may be of interest for the mode of the action of SCF ubiquitin ligases in general is the influence of the identity of the substrate on the action of the same SCF complex with different E2 enzymes: UbcH5 replaces Cdc34 much less efficiently in the ubiquitylation of p21 than in that of p27 (Fig. 3). The crystal structure of the SCFSkp2 complex (33) shows a gap of ~100 Å between Roc1, which binds E2, and Skp2, which binds the substrate together with Cks1. This gap has to accommodate both E2 (Cdc34 or UbcH5) and the substrate (trimeric complex of p27 or p21 with Cdk2-cyclin A/E). The exact structure of the substrate may determine the efficiency with which different E2 enzymes fit into this gap at a correct orientation for ubiquitin transfer to the substrate. Thus, exact selectivity for an E2 enzyme may be influenced not only by the identity of its E3 partner but also by that of the protein substrate.

The results of the present study add p21Cip1 to the expanding list of protein substrates of the SCFSkp2 complex. It has been recently reported that the pRb-related p130 protein is targeted for degradation in the G0 to S phase transition by the SCFSkp2-Cks1 system (34). Other possible substrates are hORC1p, the large subunit of the origin replication complex (35), B-Myb (36), and cyclin E (17). It is not clear, however, what features of the protein substrate are recognized by SCFSkp2. Although p21 and p27 share several similar amino acid residues near their phosphorylation sites (Fig. 2B), no such similarity can be detected around Ser-672 in p130, the phosphorylation of which is necessary for its ubiquitylation (34). This is different from the case of SCFTrCP, which recognizes two phosphorylated Ser residues in the context of a defined amino acid motif (25). It is possible that the SCFSkp2 ubiquitin ligase complex recognizes some specific structural features, rather than amino acid sequence motifs, in targeting its different substrates for degradation.

Acknowledgments—We thank Esther Eytan for help and reagents and Clara Segal for skillful technical assistance.

REFERENCES