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Structure of a c-Cbl–UbcH7 Complex: RING Domain Function in Ubiquitin-Protein Ligases

Ning Zheng,*† Ping Wang,*† Philip D. Jeffrey,* and Nikola P. Pavletich*†‡
*Cellular Biochemistry and Biophysics Program
†Howard Hughes Medical Institute
Memorial Sloan-Kettering Cancer Center
New York, New York 10021

Summary

Ubiquitin-protein ligases (E3s) regulate diverse cellular processes by mediating protein ubiquitination. The c-Cbl proto-oncogene is a RING family E3 that recognizes activated receptor tyrosine kinases, promotes their ubiquitination by a ubiquitin-conjugating enzyme (E2) and terminates signaling. The crystal structure of c-Cbl bound to a cognate E2 and a kinase peptide shows how the RING domain recruits the E2. A comparison with a HECT family E3-E2 complex indicates that a common E2 motif is recognized by the two E3 families. The structure reveals a rigid coupling between the peptide binding and the E2 binding domains and a conserved surface channel leading from the peptide to the E2 active site, suggesting that RING E3s may function as scaffolds that position the substrate and the E2 optimally for ubiquitin transfer.

Introduction

Ubiquitin-dependent protein degradation is involved in the regulation of various cellular processes, including cell cycle progression, signal transduction, transcription, DNA repair, and protein quality control (Koepp et al., 1999; Laney and Hochstrasser, 1999). Ubiquitination involves the successive actions of the ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-protein ligase enzymes (E3) (Hershko and Ciechanover, 1998). The E1 activates free ubiquitin and transfers it to an E2 through a thioester linkage between the ubiquitin COOH terminus and an E2 active site cysteine. Over 30 E2s have been identified in humans, and they all contain a conserved ~160 amino acid catalytic core.

E3s represent a much larger and diverse superfamily. They are defined as proteins or protein complexes that are needed in addition to E1 and E2 for the ubiquitination of specific substrates (Hershko and Ciechanover, 1998). Known E3s share in common the ability to bind both the substrate and a specific E2. However, there are at least two different E3 types that mediate substrate ubiquitination in functionally distinct ways. HECT E3s, which contain a conserved catalytic HECT domain, first form a ubiquitin-E3 thioester intermediate and then transfer the ubiquitin to lysine side chains of the substrate (Scheffner et al., 1995; Schwarz et al., 1998). E3s lacking the HECT domain are divergent at the level of subunit composition and amino acid sequence, but most share a zinc-stabilized RING domain that binds the E2. RING domain–containing E3s (RING E3s) are thought to promote the ubiquitination of substrate lysines directly by the E2 in a process that is not yet understood. RING E3s, which represent the largest E3 family known to date, include the c-Cbl proto-oncogene (Joazeiro et al., 1999; Levkowitz et al., 1999; Yokouchi et al., 1999), the multi-subunit SCF and APC cell cycle-regulatory complexes (Yu et al., 1998; Zachariae et al., 1998; Kamura et al., 1999; Ohta et al., 1999), the MDM2 proto-oncogene that regulates the p53 tumor suppressor (Fang et al., 2000), and members of the IAP family of antiapoptotic proteins (Yang et al., 2000).

The c-Cbl protein attenuates signaling by the activated PDGF, EGF, and CSF-1 receptor tyrosine kinases (RTKs) by inducing their ubiquitination and subsequent degradation by the proteasome (Miyake et al., 1998; Joazeiro et al., 1999; Lee et al., 1999; Levkowitz et al., 1999). c-Cbl also negatively regulates the immune receptor–coupled ZAP-70 and Syk tyrosine kinases, but it is not known whether it induces their ubiquitination (Lupher et al., 1997; Ota and Samelson, 1997). c-Cbl recognizes activated RTKs and the ZAP-70/Syk kinases by binding a phosphotyrosine sequence motif through its SH2-containing tyrosine kinase binding (TKB) domain, and it binds an E2 through its conserved RING domain (Joazeiro et al., 1999; Levkowitz et al., 1999; Yokouchi et al., 1999). The RING domain has a central role in c-Cbl function, because its deletion or disruption, as observed in the murine retrovirus-encoded v-Cbl and the pre-B cell lymphoma 70Z/3 c-Cbl dominant oncogenic mutants, abolishes the function of c-Cbl (Blake et al., 1991; Andoniou et al., 1994). The TKB and RING domains are connected by an ~40-residue conserved intervening sequence (hereafter referred to as linker). The E2s that can support c-Cbl-mediated ubiquitination include the closely related Ubc4, UbcH5B/C, and UbcH7, but it is not yet clear which E2 functions with c-Cbl in vivo. Here we describe the crystal structure of a c-Cbl–UbcH7-ZAP70 peptide ternary complex and discuss the implications this structure has for understanding E2-E3 specificity and the mechanism by which RING E3s promote the ubiquitination of substrates by the E2.

Results and Discussion

Overall Structure of the c-Cbl–UbcH7–ZAP-70 Peptide Complex

The complex consists of an ~46 kDa portion of c-Cbl (residues 47 to 447) bound to the UbcH7 E2 and an 11-residue peptide containing the c-Cbl recognition sequence from the ZAP-70 protein kinase. This c-Cbl portion, which contains both the TKB and RING domains, has been shown to be functional as an E3 in vivo (Yokouchi et al., 1999; Ota et al., 2000) and is similar in size to the 448-residue *Drosophila* homolog (Hime et al., 1997).

The complex has a compact overall structure, with UbcH7, the TKB and the RING domains of c-Cbl all interacting with each other across multiple interfaces (Figure 1A). The structure of the TKB domain consists of a four-helix bundle (4H), two EF hand domains, and a variant SH2 domain, and is unchanged compared to

‡To whom correspondence should be addressed (e-mail: nikola@xray2.mskcc.org).

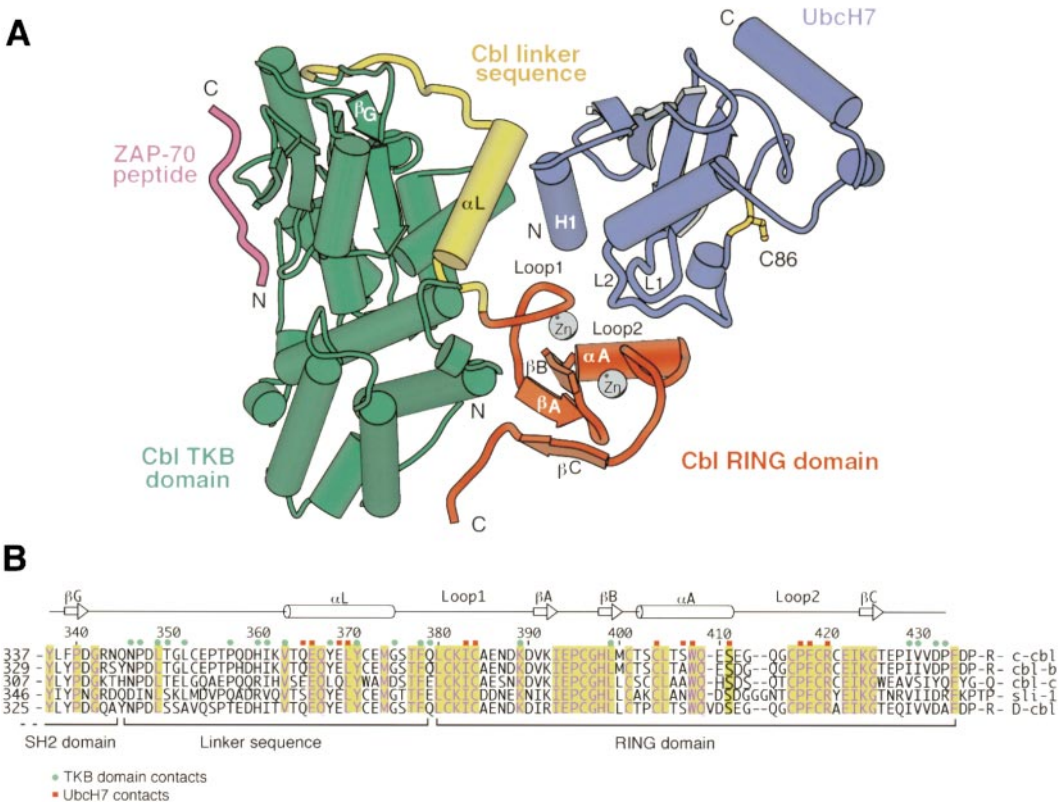

Figure 1. The Ternary Complex of c-Cbl, UbcH7, and Phosphorylated ZAP-70 Peptide Has a Compact Structure

(A) Overall view of the ternary complex. The TKB domain is colored green, the RING domain red, and the linker region of c-Cbl yellow. UbcH7 is colored in cyan and its active-site cysteine in orange. The two zinc ions are shown as gray spheres. (B) Alignment of the RING domain and linker sequences of human c-Cbl, human cbl-b, human cbl-c, *C. elegans* sli-1, and *Drosophila* cbl. Secondary structure elements are indicated on top of the sequences. Invariant residues are highlighted in yellow. Green dots mark the residues that contact the TKB domain and red squares mark the residues that contact UbcH7. The sequence of the TKB domain is not shown as its structure has been described previously (Meng et al., 1999).

the crystal structure of the isolated TKB domain bound to the ZAP-70 peptide (Meng et al., 1999). The RING domain is anchored onto the TKB domain by interacting with the 4H bundle. The linker sequence, which was absent from the structure of the isolated TKB domain, packs on the TKB domain forming an ordered loop and an α helix (linker helix; Figures 1A and 1B). The linker thus is an integral part of the TKB domain.

UbcH7 adopts an α/β structure characteristic of the E2 fold (Cook et al., 1992; Huang et al., 1999) and binds to c-Cbl with one end of its elongated structure burying a total of 1850 Å^2 surface area. Two UbcH7 loops pack in and around a shallow groove on the c-Cbl RING domain and a UbcH7 helix interacts with the c-Cbl linker helix (Figure 1A). The UbcH7 active site cysteine is located on the side of the complex opposite from where the ZAP-70 recognition peptide binds and is separated by ~60 Å from the peptide. The lysine residues of the receptor tyrosine kinases that are ubiquitinated have not yet been identified, and the ZAP-70 peptide used in the crystallization does not contain any lysine residues.

The c-Cbl RING domain structure consists of a three-stranded β sheet, an α helix, and two large loops, and is stabilized by two tetrahedrally coordinated zinc ions (Figure 1B). The overall structure is similar to the crystal structure of the RAG1 RING domain (Bellon et al., 1997). RAG1 is not known to be ubiquitin-protein ligase and its RING domain lacks the groove of the c-Cbl that binds the E2.

Previous studies have suggested that the RING domain of RING E3s might allosterically activate the E2 enzyme (Joazeiro et al., 1999; Skowyra et al., 1999). However, UbcH7 in the complex has a structure that is very similar to the structures of other isolated E2s and does not appear to have undergone any significant conformational changes. The closest distance between the UbcH7 active site cysteine and any RING domain residue is about 15 Å, indicating that the RING domain does not provide reactive groups to the active site. However, the structure does not rule out the possibility that c-Cbl may induce conformational changes or contribute additional contacts when UbcH7 is conjugated to ubiquitin.

The RING Domain and Linker Sequence Pack Extensively with the TKB Domain

The RING domain is anchored on the 4H bundle of the TKB domain through hydrogen bond networks and van der Waals contacts involving 7 RING domain residues and 11 4H bundle residues (Figures 1B and 2A). Most of these residues are highly conserved in the *Drosophila* and *C. elegans* Cbl orthologs, suggesting that the integrity of this interface and the precise relative arrangement of the two domains are important for c-Cbl function.

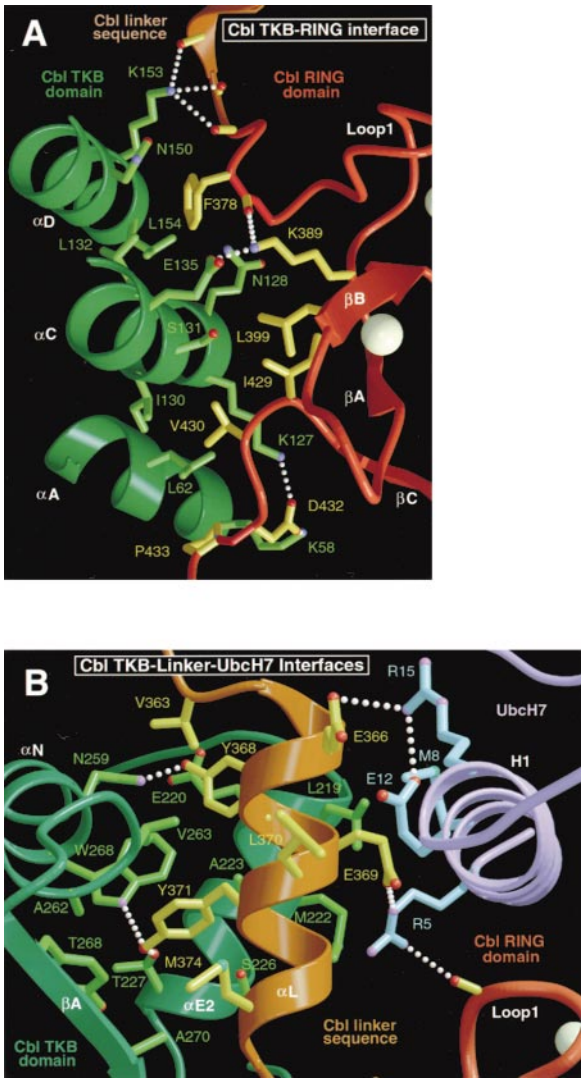

Figure 2. Both the RING Domain and the Linker Sequence Interact Extensively with the TKB Domain in c-Cbl

(A) Close-up view of the interface between the RING domain, colored in red, and the 4H bundle of the TKB domain, colored in green. Residues of the RING domain and the 4H bundle that are involved in the interaction are colored in yellow and light green, respectively. White dotted lines indicate hydrogen bonds and salt bridges. The hydrogen bond networks involve Lys-153, Glu-135, Lys-58, and Lys-127 of the 4H bundle and Lys-389 and Asp-432 as well as several backbone carbonyl groups of the RING domain. Van der Waals contacts are made by the Phe-378, Ile-429, Val-430, and Pro-433 side chains of the RING domain and the Leu-132, Leu-62, Leu-154, Gln-128, Ile-130, Ser-131, and Gln-150 side chains of the 4H bundle. (B) Close-up view of the linker helix–TKB domain interface and the interface between the linker helix and UbcH7 H1 helix. The linker helix, the TKB domain, and UbcH7 are colored in orange, green, and purple, respectively. The side chains of the residues involved in the interfaces are colored in yellow (from linker helix), green (from the TKB domain), and cyan (from UbcH7). The zinc ions are indicated with white spheres. Prepared with the programs MOLSCRIPT (Kraulis, 1991) and RASTER3D (Merritt and Bacon, 1997).

The linker sequence that connects the TKB and RING domains is an integral part of the TKB domain and plays a key structural role in c-Cbl. Half of the sequence is highly conserved among c-Cbl homologs and forms an α helix that packs with the TKB domain and also with UbcH7 (Figures 1B and 2B). The linker–TKB interactions are centered on the conserved Tyr-368 and Tyr-371 residues from the linker. These residues, which are in a buried environment, make multiple van der Waals contacts with hydrophobic TKB residues and also hydrogen bond with Asn-259 from the SH2 domain and Thr-227 from the EF hand, respectively (Figure 2B). The deletion of either tyrosine causes c-Cbl to become oncogenic (Andoniou et al., 1994), indicating that the integrity of the linker–TKB interface is necessary for c-Cbl's function (Andoniou et al., 1994).

It has been suggested that phosphorylation of the linker residue Tyr-371 may be required for the E3 ligase activity of c-Cbl (Levkowitz et al., 1999). This was based, in part, on the inactivation of c-Cbl by the mutation of Tyr-371 to phenylalanine. The crystal structure, however, indicates that Tyr-371 has a structural role and that this mutation would affect the integrity of the linker–TKB interface. The structure also indicates that if Tyr-371 were to be phosphorylated, this would have to be preceded by a major conformational change in the linker helix to make Tyr-371 solvent accessible, and would also result in a significant structural change in the linker–TKB and linker–E2 interfaces.

The c-Cbl–UbcH7 Interface

The crystal structure reveals that c-Cbl binds UbcH7 using both its RING domain and its linker helix (Figure 1A). The RING domain makes the most extensive contacts, and this is consistent with the ability of the isolated RING domain to bind E2s in a yeast two-hybrid assay (Yokouchi et al., 1999). The RING domain provides a shallow groove into which the tips of the L1 and L2 loops of UbcH7 bind. The groove is formed by the α helix and the two zinc-chelating loops (loop 1 and loop 2) of the RING domain (Figure 3A). The structure of the RAG1 RING domain, which is not known to be an E3, does not contain this groove, as its loops are closer together and the space in between is occupied by amino acid side chains. This c-Cbl groove accommodates the most significant contacts from UbcH7. These involve Phe-63 of the UbcH7 L1 loop and Pro-97 and Ala-98 of the UbcH7 L2 loop (Figure 3A). The side chain of Phe-63, buttressed by Pro-62, makes multiple van der Waals contacts with Ile-383 from loop 1 of the RING domain, and with Trp-408, Cys-404, Ser-407, and Ser-411 from the α helix of the RING domain. Pro-97 of UbcH7 contacts Ile-383, Trp-408, and Pro-417 of c-Cbl and Ala-98 of UbcH7 contacts Phe-418 of c-Cbl. Ala-98 of UbcH7 also makes a hydrogen bond between its backbone carbonyl group and the backbone amide group of Pro-417 of c-Cbl.

The linker helix of c-Cbl packs with the H1 helix of UbcH7 at a perpendicular angle. This portion of the interface contains primarily polar and charged residues that form intermolecular hydrogen bond contacts (between c-Cbl Glu-369 and UbcH7 Arg-5, and between c-Cbl Glu-366 and UbcH7 Arg-15; Figure 2B). The involvement of the c-Cbl linker helix in E2 binding helps explain the observation that certain RING E3s may need portions of their polypeptide chain in addition to their RING domain for E2 binding (Xie and Varshavsky, 1999).

RING E3-E2 Specificity

The structure indicates that the Phe-63 residue of the UbcH7 L1 loop and the Trp-408 and Ile-383 residues

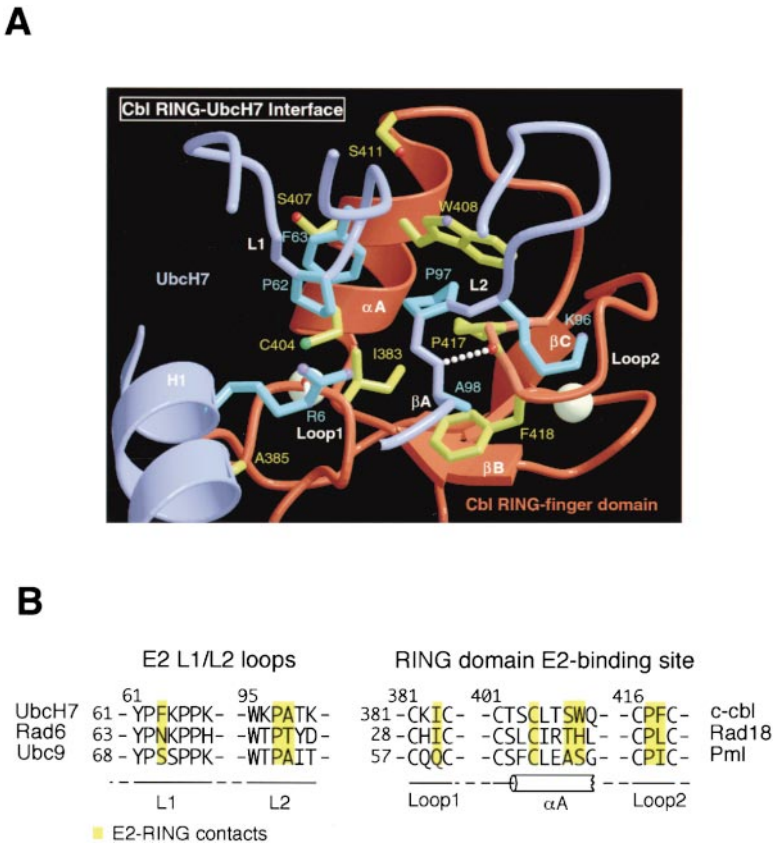

Figure 3. UbcH7 Uses Its L1 and L2 Loops to Bind a c-Cbl Site Formed by Loop 1, Loop 2, and the Helix of the RING Domain

(A) Close-up view of the interface between UbcH7 (purple) and the RING domain of c-Cbl (red) by looking down to the RING domain from the center of UbcH7. The residues from c-Cbl and UbcH7 at the interface are colored yellow and cyan, respectively. White dash lines represent hydrogen bonds. White spheres represent the zinc ions. In addition to the c-Cbl–UbcH7 contacts discussed in the text, the side chains of Arg-6, Pro-62, and Lys-96 of UbcH7 pack against the polypeptide backbone of the RING domain, and Ala-385 of the c-Cbl RING domain makes van der Waal contacts with Arg-6 and Lys-9 on the H1 helix of UbcH7. (B) On the left side, alignment of the sequences corresponding to the L1 and L2 loops in three different E2s: human UbcH7, yeast Rad6, and human Ubc9. On the right side, alignment of the sequences corresponding to the loop 1, loop 2, and the helix in the RING domain of three E3s that bind the E2s on the left side, namely, human c-Cbl, yeast Rad18, and human Pml. Residues that are involved in E2/E3 interaction are highlighted in yellow.

that demarcate the RING groove have a central role in determining the specificity of the c-Cbl E3 for the E2. This is supported by Phe-63 being invariant among the E2s that have been shown to support c-Cbl-dependent ubiquitination, and by previous studies showing that mutation of Trp-408 to alanine reduces c-Cbl's affinity for the E2 and eliminates its ubiquitin-protein ligase activity in vitro (Joazeiro et al., 1999).

The structure also helps explain the preference of other RING E3s for their cognate E2s. The nature of the amino acid side chains corresponding to Phe-63 of UbcH7 and Ile-383 and Trp-408 of c-Cbl varies in a correlated fashion in other E2-RING E3 pairs (Figure 3B). In the Rad6-Rad18 E2-RING E3 pair, for example, the residue corresponding to the UbcH7 Phe-63 is an asparagine (Asn-65), and the residue corresponding to the c-Cbl Trp-408 is a histidine (His-55). The structure suggests that the Rad6 Asn-65 and the Rad18 His-55 may form a hydrogen bond contact in that interface. Similarly, in the case of the Ubc9-Pml E2-E3 complex that may be involved in protein modification by the ubiquitin-like molecule SUMO-1 (Duprez et al., 1999), the residues corresponding to Phe-63 of UbcH7 and Trp-408 and Ile-383 of c-Cbl are replaced by Ser-70, Gln-59, and Ser-84, respectively. The structure, in conjunction with these sequence correlations, provides a framework to help

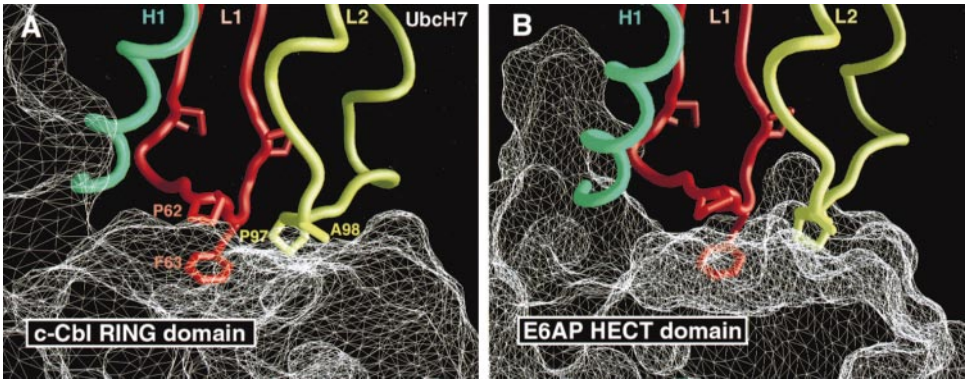

Figure 4. The RING Domain of c-Cbl and the HECT Domain of E6AP Recognize the Same Structural Elements of UbcH7

The H1 helix, L1, and L2 loops of UbcH7 are colored in green, red, and yellow, respectively. Portions of the molecular surfaces of the RING domain of c-Cbl (left) and the HECT domain of E6AP (right) are shown as a white net. The Phe-63 residue of UbcH7 inserts its side chain into a groove formed by the E3 in both cases. (Prepared with the program GRASP; Nicholls et al., 1991)

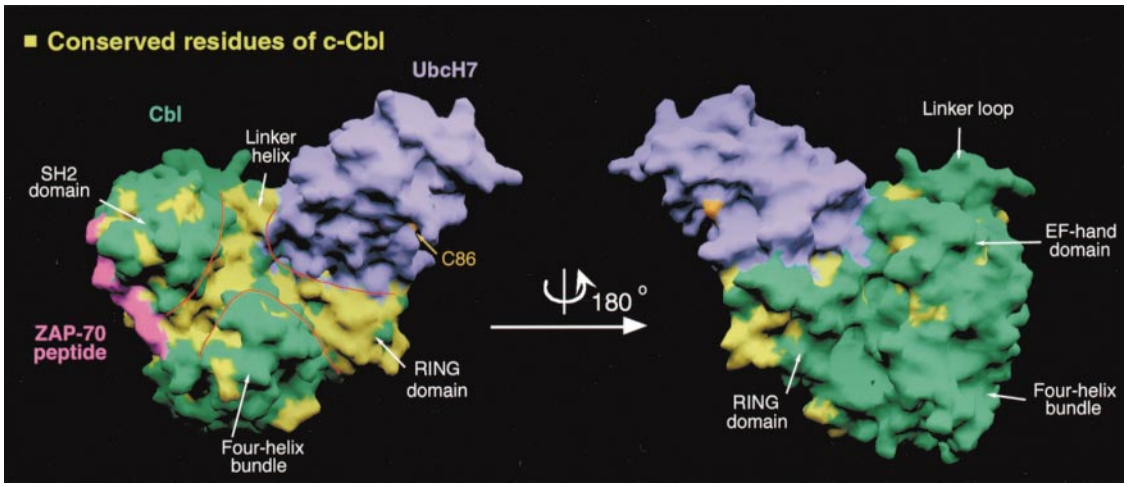

Figure 5. c-Cbl Has a Conserved Surface Channel Leading from the Peptide to the E2 Active Site

One side of the c-Cbl–UbcH7 complex is characterized by a surface channel formed by residues that are solvent exposed and generally conserved (Lys-78, Asn-79, Ser-80, Arg-148, Arg-149, Lys-153, Asp-275, Glu-276, Lys-278, Ala-279, Arg-280, Gln-267, Leu-370, Glu-373, Met-374, and Lys-382). The molecular surfaces of c-Cbl and UbcH7 are colored in green and blue, respectively. On c-Cbl, the surface of the residues completely conserved in human c-Cbl, human cbl-b, human cbl-c, *C. elegans* sli-1, and *Drosophila* cbl are colored in yellow. The surface of the UbcH7 active-site cysteine is colored orange. The channel is outlined by three solid red lines. The right panel shows that the molecular surface of the complex opposite to the side containing the channel is not conserved. The view on the left has an orientation similar to that of Figure 1A.

identify the cognate E2s for the large number of recently isolated RING E3 proteins.

Common Structural Elements of E2s Recognized by Both RING and HECT E3s

The only other known structure of an E3-E2 complex is of the catalytic domain of E6AP from the HECT E3 family bound to UbcH7 (Huang et al., 1999). In vitro, both E6AP and c-Cbl work with the Ubc4 subfamily of E2s, to which UbcH7 belongs. Comparison of the c-Cbl–UbcH7 and E6AP-UbcH7 E3-E2 interfaces reveals a striking similarity in how the UbcH7 E2 binds the two functionally and structurally distinct E3 families (Figure 4). In both cases, the tips of the L1 and L2 loops of UbcH7 bind into a shallow groove on the E3, and the UbcH7 H1 helix contributes additional minor contacts. In both cases, the most extensive contacts are provided by Phe-63, Pro-97, and Ala-98 of UbcH7 even though the RING domain and HECT domain structural elements and residues that make up the grooves into which the UbcH7 residues bind are entirely unrelated in the two E3s. Since the E2 binds the same way to the representatives of the two E3 families, it is likely that most other E2-E3 complexes from each family would form in a similar way. This should allow the classification of the large number of known E2s into different specificity families based on the sequences of their L1 and L2 loops.

Implications for RING E3–Mediated Ubiquitin Transfer

The mechanism of ubiquitination by the RING family E3-E2 complexes is not yet understood. It is conceivable that the only function of the RING E3 is to recruit both the E2 and the substrate protein. This could in principle promote substrate ubiquitination by the E2 by increasing the effective local concentration of the substrate around the E2 active site. The structure of the c-Cbl–UbcH7 complex is consistent with this model, but it suggests that the precise relative position and orientation of the substrate binding and E2 binding domains are also important. UbcH7 and the TKB and RING domains of c-Cbl are tightly associated by interactions across multiple interfaces, and these involve conserved c-Cbl residues. Furthermore, the sequence connecting the TKB and RING domains is an integral part of the structure and contributes to the rigid arrangement of the multiple domains. A mutation (Tyr-368 deletion) that would disrupt the structure of the linker helix and affect the position and orientation of UbcH7 relative to the TKB domain abolishes c-Cbl's function without significantly affecting its ability to bind either receptor tyrosine kinases or E2s (Joazeiro et al., 1999; Meng et al., 1999). This supports the hypothesis that the precise relative arrangement of the TKB domain and the E2 is important for the E3 activity.

The structure also reveals a deep surface channel made up of portions of the c-Cbl TKB, linker and RING domains, and UbcH7 (Figure 5). The solvent-exposed residues that line this channel are highly conserved, and this is in sharp contrast to the rest of the molecular surface (Figure 5). The conservation of these residues, which are mostly polar or charged and do not have any apparent structure stabilizing roles, indicates that the channel is important for c-Cbl function. This channel marks a continuous, ~60 Å path between the N terminus of the ZAP-70 recognition peptide and the E2 active site. The location of this channel suggests that it may provide additional sites of interaction with the polypeptide substrate.

Taken together, the rigid arrangement of the subunits in the Cbl-UbcH7 complex and the conserved surface channel suggest that the function of c-Cbl is more than the simple recruitment of the substrate and the E2. It is conceivable that c-Cbl may also contribute to the selection of lysine residues to be ubiquitinated. It is not yet known whether ubiquitination of c-Cbl's substrates occurs at specific lysine residues. Recent studies have

Table 1. Statistics from the Crystallographic Analysis

Beam line	MacCHESS A1
Resolution (Å)	2.9
Observations	156,681
Unique reflections	38,512
Data coverage (%)	96.8
R_{sym} (%)	6.2
Refinement statistics	
Reflections ($\|F\| > 2\sigma$)	34,351
Total atoms	5322
R factor (%)	22.7
R_{free} (%)	26.2
rmsd bonds (Å)	0.011
rmsd angles (°)	1.92
rmsd B factor (Å^2)	2.4

$R_{sym} = \Sigma_h\Sigma_i|I_{h,i} - I_h|/\Sigma_h\Sigma_i I_{h,i}$ for the intensity (I) of i observations of reflection h.

R factor $= \Sigma|F_{obs} - F_{calc}|/\Sigma|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively. $R_{free} = R$ factor calculated using 5% of the reflection data chosen randomly and omitted from the start of refinement.

rmsd: root mean square deviations from ideal geometry and root mean square variation in the B factor of bonded atoms.

shown that ubiquitination of several other RING E3 substrates is highly selective for specific lysine residues. For example, ubiquitination of IκB by the SCF complex occurs with high specificity at two neighboring lysine residues positioned 9 and 10 residues from the sequence motif recognized by this RING E3 (Hochestrasser and Kornitzer, 1998). In the N-end rule ubiquitination pathway, which determines protein stability based on the N-terminal residue, ubiquitination and degradation of synthetic substrates has been shown to be most efficient when lysines are located at specific distances relative to the N terminus that is recognized by the Ubr1 RING E3 (Suzuki and Varshavsky, 1999). Since no general consensus sequence is known for ubiquitination sites, this specificity for lysines may be governed by steric and distance restraints. c-Cbl may provide such restraints through the rigid arrangement of its domains that bind the receptor tyrosine kinase and the E2, and also by its surface channel that may interact with additional portions of the receptor tyrosine kinase. RING E3s may thus not only recruit the substrate and the E2, but also serve as scaffolds that position and orient them optimally for ubiquitin transfer.

Experimental Procedures

Protein Overexpression and Purification

Residues 47 to 447 of human c-Cbl were overexpressed as a GST fusion protein in *E. coli*. The C-terminal region of c-Cbl (448 to 906) missing from this construct appears to be nonessential for c-Cbl's function as an E3 ligase based on deletion studies (Yokouchi et al., 1999; Ota et al., 2000), and is also absent from the 448-residue *Drosophila* and 585-residue *C. elegans* c-Cbl homologs (Yoon et al., 1995; Hime et al., 1997). The GST-c-Cbl fusion protein was isolated from the soluble cell lysate by glutathione affinity chromatography and cleaved by thrombin. The c-Cbl fragment was further purified by cation exchange and gel filtration chromatography and concentrated by ultrafiltration. The human UbcH7 protein was prepared as described before (Huang et al., 1999). The ZAP-70 phosphorylated peptide (Meng et al., 1999) was synthesized chemically and purified by reversed phase chromatography.

Crystallization and Data Collection

The purified c-Cbl fragment (27 mg/ml), UbcH7 (32 mg/ml), and ZAP-70 peptide (40 mg/ml) were mixed at a 1:1:3 molar ratio in a solution of 20 mM 2-(N-Morpholino) ethanesulfonic acid, 150 mM NaCl, 5 mM dithiothretiol, pH = 6.5. Crystals of the ternary complex were grown at 4°C by the hanging drop vapor diffusion method by mixing the complex with an equal volume of reservoir solution containing 100 mM sodium citrate, 1.9 to 2.1 M ammonium sulfate, pH = 5.6. The crystals form in space group $P4_132$, with $a = b = c = 219.0$ Å and contain one complex in the asymmetric unit. The native data set was collected at the A1 beamline ($\lambda = 0.909$ Å) of the Cornell High Energy Synchrotron Source (MacCHESS) using crystals flash frozen in crystallization buffer supplemented with 30% ethylene glycol at −170°C. Reflection data were indexed, integrated, and scaled using DENZO and SCALEPACK (Otwinowski and Minor, 1997).

Structure Determination and Refinement

The structure of the complex was determined by a combination of molecular replacement and single isomorphous replacement (SIR) with an Hg derivative (data not shown, Table 1). The c-Cbl TKB domain and the UbcH7 were located with the program AmoRe (CCP4, 1994) using the structures of the isolated c-Cbl TKB domain (Meng et al., 1999) and of the E6AP-bound UbcH7 (Huang et al., 1999) as search models. The structure was built with the program O (Jones et al., 1991) using electron density maps calculated with phases derived from the rigid-body refined model of the TKB domain and UbcH7 and with the SIR phases. The model was refined with the program CNS (Brünger et al., 1998). The refined model contains residues 47–434 of c-Cbl, residues 4–147 of UbcH7, residues 289–297 of ZAP-70, and three sulfate molecules. UbcH7 makes only a small number of crystal packing contacts, and the portion of UbcH7 distal from the c-Cbl–UbcH7 interface has poor electron density and high temperature factors.

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Protein Data Bank ID Code

The coordinates for the structure described in this work have been deposited with the ID code 1FBV.