COMMD1 (Copper Metabolism MURR1 Domain-containing Protein 1) Regulates Cullin RING Ligases by Preventing CAND1 (Cullin-associated Nedd8-dissociated Protein 1) Binding

Xicheng Mao¹, Nathan Gluck²*, Baozhi Chen³, Petro Starokadomskyy¹, Haiying Li¹, Gabriel N. Maine³, and Ezra Burstein⁴

From the Departments of Internal Medicine and Molecular Biology, University of Texas Southwestern Medical Center, Dallas, Texas 75390, the Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109, the Department of Biochemistry, School of Medicine, Hebrew University, Jerusalem, Israel, and the Department of Clinical Pathology, Royal Oak, Michigan 48073

Cullin RING ligases (CRLs), the most prolific class of ubiquitin ligase enzymes, are multimeric complexes that regenerate a wide range of cellular processes. CRL activity is regulated by a variety of factors, including the dissociation of substrate receptor components from the CRL. We demonstrate here that COMMD1 (copper metabolism MURR1 domain-containing 1), a factor previously found to promote ubiquitination of various substrates, regulates CRL activation by antagonizing CAND1 binding. We show that COMMD1 interacts with multiple Culins, that the COMMD1-Cul2 complex cannot bind CAND1, and that, conversely, COMMD1 can actively displace CAND1 from CRLs. These findings highlight a novel mechanism of CRL activation and suggest that CRL regulation may underlie the pleiotropic activities of COMMD1.

Ubiquitin, a highly conserved 76-amino acid polypeptide, can be conjugated to substrate proteins through an enzymatic machinery present in all eukaryotic cells (1). Ubiquitination can lead to a variety of outcomes, but a particularly notable event is the degradation of the target protein by the proteasome. Ubiquitination of the target protein involves a multistep enzymatic process that requires a ubiquitin ligase, also referred to as an E3 enzyme (2). It is estimated that there are ~500–1,000 ubiquitin ligases (3–5), and among them, a particularly prolific group are the Cullin RING ligases (CRLs) (6). The core CRL complex contains a Cullin protein (such as Cul1, 2, 3, 4A, 4B, or 5 in mammals), and a RING box protein (Rbx1 or Rbx2). In addition, a variety of substrate binding subunits specific to each Cullin contributes to a large repertoire of complexes, estimated at ~300 distinct ligases (7–9). These complexes are designated by the Cullin and the specific substrate subunit that they contain. For example, the well known ligase complex that targets IκB-α, which contains Cul1 and the substrate receptor protein βTrCP, is designated as CRL1-βTrCP.

Structurally, the carboxyl-terminal globular domain of the Cullin protein binds to Rbx1 or Rbx2, whereas the amino-terminal region is a rigid stalk formed by three unique five–helix bundle structures called Cullin repeat 1, 2, and 3 (10). The amino-terminal Cullin repeat 1 is responsible for recruiting the substrate receptor complex (SRC). Displacement of this complex is a major mechanism of inhibition of these ligases and is mediated by CAND1 (Cullin-associated Nedd8-dissociated), a large protein that interacts with all three Cullin repeats and the carboxyl-terminal domain (11–13). Formation of the CRL-CAND1 complex is in turn regulated by the ubiquitin-like protein Nedd8, which upon conjugation to the carboxyl-terminal domain prevents CAND1 binding to the Cullin protein (11, 12). On the other hand, the assembled Culli-CAND1 complex cannot be effectively neddylated because of sterio occlusion of the lysine acceptor site by CAND1. Therefore, the mechanism of dissociation of the Culli-CAND1 complex remains poorly understood, and the existence of cellular factors that control CRL-CAND1 interactions has been proposed (13).

COMMD (copper metabolism MURR1 domain-containing) proteins are pleiotropic factors present in a wide range of eukaryotic organisms (14) and are defined by the presence of the carboxyl-terminal COMMD domain (15, 16). The best studied of these factors is COMMD1, which participates in copper metabolism (17), NF-κB-mediated transcription (18), adaptation to hypoxia (19), and electrolyte transport (20, 21). A large deletion in the canine COMMD1 gene, which abolishes protein expression, leads to pathologic copper accumulation, cirrhosis, and liver failure in Bedlington terriers (17). Although humans with pathologic copper accumulation caused by COMMD1 mutations have not been identified (22, 23), a role for this gene in modulating the phenotype of Wilson’s disease has been proposed (24). Moreover, COMMD1 has been found to have copper binding activity in vitro (25) and can modulate the matura-
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The expression vectors for Cullin proteins, CAND1, and Skp1 (all of human origin) were generated by PCR amplification of the corresponding coding sequence, which was then introduced into the pEBB-FLAG and pEBG vectors. For Cul1, Pallino-FLAG-Cul1 was used as template (kindly provided by Dr. Michele Pagano). The following IMAGE cDNA clones were obtained from Open Biosystems and used as templates for Cul3, Cul4A, Cul4B, Cul5, Cul7, CAND1, and Skp1: 5784147, 3537176, 5269392, 30331132, 5580027, 5265409, and 6672613, respectively. COMMD-EYFP constructs were generated by subcloning EYFP into pEBB-FLAG expression vectors previously described (15). The plasmid pEBB-COMMD1 (M110A/H134A)-GST was generated by site-directed mutagenesis. Deletion constructs for Cul2 were generated by PCR using pEBB-FLAG-Cul2 as template (16), with the amino acid boundaries of the encoded mutant proteins being: Cul2 1–415, 109–745, 415–745, 1–150 (R1), 151–270 (R2), 271–386 (R3), and 151–386 (R2, R3). His6-tagged versions of COMMD1 and Skp1 were subcloned from the corresponding pEBB vectors into pET30a using the BamHI and NotI sites. All other plasmids have been previously described (15, 16, 28, 29).

Cell Culture and Transfection—HEK 293 cells and HeLa cells were obtained from ATCC. U2OS cells with stable shRNA-mediated repression of COMMD1 have been previously reported (16). HEK 293 cells stably expressing GST or GST-Cul2 were generated by lentiviral infection and selection as previously described (16). All of the cell lines were cultured in DMEM supplemented with 10% FBS and l-glutamine (2 mM). Biotin (Sigma; 4 μM) was added to the medium when fusion proteins with the biotinylation target peptide were expressed. A standard calcium phosphate transfection protocol was used to transfect HEK 293 cells (29). HeLa cells were transfected using FuGENE (Roche Applied Science) according to the manufacturer’s instructions.

Confocal and Fluorescence Microscopy—HeLa cells were plated in chambered coverglass plates and transfected with the indicated COMMD-EYFP plasmids (0.5 μg/well). The cells were stained with Hoechst 33342 (8 μM) for 30 min, and images were obtained with a Zeiss LSM 510 META confocal microscope equipped with a Chameleon XR NIR laser.

EXPERIMENTAL PROCEDURES

Plasmids—Expression vectors for Cullin proteins, CAND1, and Skp1 (all of human origin) were generated by PCR amplification of the corresponding coding sequence, which was then introduced into the pEBB-FLAG and pEBG vectors. For Cul1, Pallino-FLAG-Cul1 was used as template (kindly provided by Dr. Michele Pagano). The following IMAGE cDNA clones were obtained from Open Biosystems and used as templates for Cul3, Cul4A, Cul4B, Cul5, Cul7, CAND1, and Skp1: 5784147, 3537176, 5269392, 30331132, 5580027, 5265409, and 6672613, respectively. COMMD-EYFP constructs were generated by subcloning EYFP into pEBB-FLAG expression vectors previously described (15). The plasmid pEBB-COMMD1 (M110A/H134A)-GST was generated by site-directed mutagenesis. Deletion constructs for Cul2 were generated by PCR using pEBB-FLAG-Cul2 as template (16), with the amino acid boundaries of the encoded mutant proteins being: Cul2 1–415, 109–745, 415–745, 1–150 (R1), 151–270 (R2), 271–386 (R3), and 151–386 (R2, R3). His6-tagged versions of COMMD1 and Skp1 were subcloned from the corresponding pEBB vectors into pET30a using the BamHI and NotI sites. All other plasmids have been previously described (15, 16, 28, 29).

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Case of displacement of overexpressed FLAG-CAND1 (see Fig. 4, C and D), the cells were similarly transfected but lysed in a Triton X-100-containing buffer (25 mM HEPES, pH 7.9, 100 mM NaCl, 1% Triton X-100). All of the buffers were supplemented with protease inhibitors (Roche Applied Science) and DTT (10 mM). Cul1 or Cul2 complexes were purified by GSH-Sepharose (GE Healthcare). The GSH beads were aliquoted and incubated at 30 °C for 30 min with ATP in dissociation buffer (40 mM HEPES, pH 7.9, 60 mM potassium acetate, 10% glycerol), with the indicated recombinant proteins (Skp1 or COMMD1). Thereafter, the beads were washed twice with Triton X-100 lysis buffer (25 mM HEPES, 100 mM NaCl, 1 mM EDTA, 10% glycerol, 1% Triton X-100). The leftover material was utilized for Western blot analysis.

RESULTS

COMMD1 Associates with CRL Complexes—Given the diverse activities of COMMD1 (32), we initially explored the possibility that this factor might play a broader role in the regulation of ubiquitination of various targets by CRLs. To this end, we examined in more detail the interaction between COMMD1 and other Cullin proteins. We found that endogenous COMMD1 could be co-precipitated with endogenous Cul1, Cul2, or Cul3 (Fig. 1A). The Cul1-COMMD1 interaction was best visualized in a detergent-free buffer, whereas the Cul2 and Cul3 interactions were best seen with a glycerol-free buffer containing low amounts of Nonidet P-40 (see “Experimental Procedures”). These interactions were also evident using a buffer containing 1% Triton X-100 (supplemental Fig. S1). Utilizing expression vectors for all five canonical Cullins (Cul1, 2, 3, 4A, 4B, and 5), we could easily recapitulate the interaction between COMMD1 and all of the Cullin family members tested (Fig. 1B).

Other COMMD Proteins Also Interact with CRLs—Other COMMD proteins have largely undefined functions at this point. Using a similar approach, it was found that other COMMDs may also interact with CRLs (Fig. 1C). Interestingly, their patterns of Cullin preference were not identical.

Several COMMDs are known to display differential tissue expression (15), yet several of them are ubiquitous and form heterodimers. To try to understand the potential redundancy in this system, we examined the cellular distribution of COMMD proteins expressed with a fused EYFP tag (Fig. 1D). Although some similarities were found for several family members, certain COMMD proteins displayed specific and unique cellular distribution. For example, in the case of COMMD2, the protein was consistently excluded from the nucleus, whereas COMMD7 demonstrated a unique punctuate pattern.

COMMD1 Associates with Active CRLs in a Copper-independent Manner—Consistent with the interaction between COMMD1 and CRLs, we found that COMMD1 could precipitate ubiquitin ligase activity when extracted from mammalian cells. COMMD1 was expressed and affinity-purified from HEK 293 cells or E. coli, and the recovered proteins were offered to an in vitro reaction containing the E1 and E2 (UbcH5a) enzymes, recombinant ubiquitin, and an ATP regenerating buffer. Polyubiquitin chain formation in the presence of ATP was readily detectable when COMMD1 precipitated from mammalian cells was provided as a source of ubiquitin ligase activity (Fig. 2A, left panels). However, if COMMD1 was precipitated and washed in RIPA buffer, this activity was lost, consistent with the fact that COMMD1-CRL interactions are abolished in this buffer (data not shown). On the other hand, recombinant COMMD1 prepared in E. coli was devoid of ligase activity, but when the protein was incubated with a mammalian lysate and then washed, ligase activity was reconstituted (Fig. 2A, right panels). These results suggested that COMMD1 interacts with cellular factors that provide ligase activity.

Next, we observed that the COMM domain of COMMD1, which is responsible for binding to Cul2 (16), was also necessary and sufficient for recovering ligase activity (Fig. 2B, COMM Domain or CD lane). Given the conservation of the COMM domain across all COMMD proteins and our finding of COMMD-CRL interactions, we speculated that other family members may be similarly linked to active ubiquitin ligases. Indeed, when precipitated from mammalian cells, several COMMD proteins provided a source of ubiquitin ligase activity in vitro (Fig. 2C). The ligase activity varied as a function of the E2 offered to each COMMD protein (supplemental Fig. S2), consistent with their differential binding to Cullin family members.

Next, we examined whether CRL interactions were responsible for COMMD1-associated ligase activity. To test this possibility, COMMD1 and Rbx1 (the main RING finger subunit in CRLs) were co-expressed in HEK 293 cells, and Rbx1 was immunoprecipitated prior to assessing the E3 activity associated to COMMD1. Depletion of Rbx1 led to a significant drop in COMMD1-associated ubiquitin ligase activity (Fig. 2D), consistent with the notion that ligase activity associated with COMMD1 is due to its interaction with CRLs.

Finally, a copper binding activity for COMMD1 has been found in vitro, and residues that coordinate copper have been similarly mapped (25). We examined whether copper could modulate COMMD1-CRL interactions. A double mutation in COMMD1 (M110A/H134A) targeting copper-binding residues did not affect Cul2 binding or COMMD1-associated E3 activity (Fig. 2E). Similarly, the addition of copper to the growth medium did not affect these interactions (data not shown), indicating that CRL binding is copper-independent.

The Cul2-COMMD1 Complex Excludes the CRL Inhibitor CAND1—Altogether, the data indicated that COMMD1 associates with CRLs, a property that seems to be shared by other COMMD proteins. Nevertheless, the specific function for such interactions was not immediately apparent. To address the latter question, we utilized a bi-molecular tandem affinity purification scheme (29) to isolate a purified Cul2-COMMD1 complex from mammalian cells (Fig. 3A). Cul2 was purified using a glutathione-Sepharose column (fraction A); after elution, this material was offered to a streptavidin-agarose column for purification of the COMMD1 subfraction (fraction B). As expected, COMMD1 and Cul2 were readily demonstrated in fraction B (Fig. 3B), and based on the amounts recovered, this complex was estimated to represent 2–5% of all cellular Cul2. In addition, fractions A and B provided ubiquitin ligase activity in vitro (not shown here), and known components of the active ligase, such as Elongin C, were also present in both fractions (Fig. 3C).
Interestingly, the CRL inhibitor CAND1 was readily identified in fraction A but was absent in Cul2-COMMD1 complexes (Fig. 3C).

Next, we sought to ascertain whether the lack of CAND1 in the Cul2-COMMD1 complex was truly due to the purification of a distinct cellular fraction of Cul2 rather than an artifact of the purification scheme itself. To address this question, Cul2 was first purified, eluted, and subsequently offered to a second column for precipitation of either COMMD1 or CAND1. Although COMMD1 and CAND1 were readily co-purified with Cul2 in the initial fraction, the second purification step of either COMMD1 or CAND1 resulted in the depletion of the other protein (Fig. 3D), consistent with the existence of mutually exclusive pools of COMMD1 and CAND1 when bound with Cul2 in cells.

**COMMD1 Promotes CRL E3 Activity**—Given the exclusion of CAND1 from Cul2-COMMD1 complexes, we reasoned that COMMD1 might bind to or promote a more active CRL state.
To test this notion, Cul2 was co-expressed with COMMD1 and subsequently precipitated from cell lysates directly or through COMMD1. The CRL inhibitor CAND1 was included as a negative control. At the end of the precipitation and after adjusting for equivalent Cul2 recovery, we utilized the isolated complexes as a source for E3 ligase activity in an *in vitro* ligase reaction. These experiments demonstrated that Cul2 associated with COMMD1 was the most active isolate, whereas the CAND1 fraction was relatively devoid of enzymatic activity (Fig. 3E).

The preferential binding of COMMD1 to a CAND1-free pool of Cul2 could be responsible for these findings; alternatively, COMMD1 could promote an active conformation for the complex. Therefore, we explored further whether COMMD1 could potentiate CRL E3 activity in *in vitro*. GST–Cul1 that was immunoprecipitated from mammalian lysate and its E3 activity was examined *in vitro* as before. In these assays, its substrate receptor adaptor Skp1 or COMMD1 were added to the *in vitro* reaction. As can be seen in Fig. 3F, the addition of COMMD1 or Skp1 led to enhanced Cul1 E3 activity. This was not due to additive E3 activity precipitated by COMMD1 or Skp1, because there was no associated E3 activity bound to COMMD1 or Skp1 when these preparations were made with RIPA buffer (Fig. 3F, right half of the gel). Altogether, this indicated that COMMD1 binds to active CRL complexes and can lead to their activation *in vitro*. However, cellular deficiency of COMMD1 did not substantially affect endogenous CRL2 activity (supplemental Fig. S3), potentially because of redundant effects by other COMMDs expressed in these cells.

**COMMD1 Promotes the Dissociation of CAND1 from CRL Complexes**—Akin to the distinct fractionation between CAND1 and substrate receptor complexes (11–13), our data indicated that CAND1 and COMMD1 do not coexist in the same CRL complexes. To test this notion further, we examined whether the purified Cul2-COMMD1 complex was capable of binding CAND1 *in vitro*. To that end, after purification of fraction B, unbound complexes were offered again to a glutathione-agarose resin and eluted with glutathione. A COMMD1-GST pull-down assay was performed as described before. This revealed that COMMD1, but not CAND1, was able to pull down Cul2 (Fig. 3E, left half of the gel). Together, these results suggest that COMMD1 promotes the dissociation of CAND1 from CRL complexes, leading to their activation *in vitro*.
FIGURE 3. The Cul2-COMMD1 complex excludes the CRL inhibitor CAND1. A, purification strategy used to isolate the Cul2-COMMD1 complex. Cul2 fused to GST (G) and COMMD1 fused to a biotinylation tag (B) were expressed in HEK 293 cells. Cul2 was purified through a GSH-Sepharose column (fraction A) and subsequently eluted. COMMD1 was precipitated from the eluted material using streptavidin (SA)-agarose beads (fraction B). Cul2 remaining in the flow-through after the streptavidin-agarose column was reprecipitated with a GSH column (Re-PD: Cul2 (GSH)) resulting in fraction C. B, Cul2-COMMD1 complex purification. Proteins from fractions A and B were separated by SDS-PAGE and stained with SYPRO Ruby; the identity of major bands was determined by paired Western blot analysis (nonspecific bands indicated by open arrowheads). C, the Cul2-COMMD1 complex excludes CAND1. Fractions A and B were immunoblotted for Cul2, COMMD1, Elongin C, and CAND1. Endogenous Cul2 and GST-Cul2 are noted by arrowheads to the left of the top panel. D, COMMD1 and CAND1 exist in separate Cul2 cellular pools. GST-Cul2, COMMD1 fused to a biotinylation tag, and FLAG-CAND1 were co-expressed in HEK 293 cells. Cul2 was purified through a GSH affinity column (PD: Cul2) and subsequently eluted. COMMD1 or CAND1 were then precipitated from the eluate using SA agarose beads or FLAG antibody, respectively. The resulting fractions were immunoblotted for Cul2, CAND1, or COMMD1. E, Cul2 complexes containing COMMD1 are active. GST-Cul2 was precipitated from transfected HEK 293 cells utilizing GSH beads (Cul2) or using protein G beads as a control (Control). FLAG-COMMD1 or FLAG-CAND1 co-expressed with GST-Cul2 were precipitated utilizing the FLAG antibody. After adjusting for equal Cul2 recovery, the resulting material was added to an in vitro ubiquitination reaction as in Fig. 2A and immunoblotted for ubiquitin. F, purified COMMD1 potentiates Cul1 E3 activity in vitro. GST-tagged Cul1 was expressed in HEK 293 cells and purified using GST affinity matrix. Purified HA-Skp1 or HA-COMMD1 prepared from mammalian lysates was then added to the recovered Cul1 complex, and this preparation was used as a source for E3 activity as before.
Next we examined whether COMMD1 could facilitate the dissociation of CAND1 from CRLs. To test this notion, GST-tagged Cul1 or Cul2 was precipitated from transfected HEK 293 cells resulting in the co-precipitation of endogenous CAND1. Next, the precipitated Cul1 or Cul2 complexes were mixed with purified COMMD1 or their respective substrate receptor adaptors, Skp1 or Elongin C, respectively. After incubation at 30 °C and thorough washing, the presence of CAND1 still associated to either Cul1 or Cul2 was assessed by immunoblotting. As has been reported before, substrate receptor adaptors were able to promote the dissociation of CAND1 (12, 33), particularly when the reaction was supplemented with ATP (Fig. 4B). Importantly, the same was true of COMMD1, which promoted CAND1 dissociation from either Cul1 or Cul2 (Fig. 4B). Interestingly, this displacement reaction was abrogated when COMMD1 had been heat-inactivated (Fig. 4C). In addition, recombinant COMMD1 made in E. coli was devoid of activity compared with a mammalian preparation (Fig. 4D). This is in contrast with Skp1, which, as previously described (12), was active irrespective of it being prepared from E. coli lysates (data not shown).

**COMMD1 Binds to CRLs in a Manner Distinct from Substrate Receptors**—Structural information indicates that Skp1 and CAND1 bind to an overlapping region of Cullin repeat 1 (R1) in Cul1 (13). CAND1 also binds to the carboxyl-terminal domain of Cul1 over its neddylation site and makes extensive contacts with R2 and R3 (13, 34) (Fig. 5F). This information explains why CAND1 does not bind to neddylated Cullins and why CAND1 and SRC binding are mutually exclusive.

Next, we tried to ascertain whether competitive binding for a shared domain on Cul2 may be involved in the ability of COMMD1 to displace CAND1. First, it was noted that neither the amino terminus nor the carboxyl terminus of Cul2 were required for COMMD1 binding (Fig. 5A). Instead, amino acids 109–415 were found to be sufficient to mediate the COMMD1 interaction. Utilizing an alignment between Cul2 and Cul1 and the published crystal structure of Cul1 as a framework (10), we predicted the boundaries of Cul2 R1, R2, and R3 (Fig. 5F). COMMD1 was found to bind preferentially to R2 and not to R1 (Fig. 5B).

Based on this finding, we predicted that COMMD1-CRL binding occurs independently of the SRC. To test this notion, we introduced a previously reported point mutation in R1 of Cul1 (10), which disrupted Skp1 binding (Fig. 5D). Interestingly, the T47A mutant lost the ability to interact with Skp1 but bound to CAND1 (Fig. 5C), consistent with the incomplete overlap between CAND1 and Skp1 binding surfaces (10, 13). Importantly, this Cul1 mutant retained its ability to interact with COMMD1 (Fig. 5D), indicating that this interaction is independent of the SRC.

Consistent with the competitive nature of the COMMD1-CAND1 interactions with CRLs, we observed that siRNA-mediated reduction of CAND1 expression increased the Cul2-COMMD1 endogenous binding (Fig. 5E). On the other hand, depletion of COMMD1 by siRNA had little effect on the CAND1-Cul1 interaction (data not shown), which may be explained by the potential redundancy of the nine other COMMD family members.
COMMD1 Is Required for the Ubiquitination of Specific Targets in Vivo—We next set out to address the role of COMMD1 on the ubiquitination of endogenous proteins in vivo. We examined this question in the context of the NF-κB signaling pathway. As reported previously, we found that COMMD1 is required for NF-κB/RelA ubiquitination (16, 35, 36). Cells with decreased levels of COMMD1 (Fig. 6A) demonstrated a profound decrease in the ubiquitination of endogenous RelA (Fig. 6B). In addition, the pro-inflammatory cytokine TNF stimulated the binding of COMMD1 to Cul2 (Fig. 6C), consistent with its role in promoting NF-κB/RelA ubiquitination and degradation as a mechanism to terminate signaling (16, 35, 36). Interestingly, TNF stimulation had only modest effects on total CRL2 activity (Fig. 6D), suggesting that the TNF induction of COMMD1/Cul2 binding more likely activates specific CRL2 complexes, such as those targeting RelA and possibly other targets.

DISCUSSION

Altogether, our studies indicate that COMMD1 can modulate CRL activity through its ability to displace CAND1.
finding provides a mechanism for the ability of COMMD1 to promote protein ubiquitination and degradation (16, 36, 37). The data also suggest that COMMD1 activates specific CRL complexes involved in the turnover of specific products, such as RelA. This activation seems to be regulated by the inducible interaction between COMMD1 and certain CRL complexes under specific conditions, as in the case of induced COMMD1–Cul2 interactions after TNF treatment.

In the present model of CRL regulation, CAND1 exerts its inhibitory function on all Cullins, potentially regulating different ligase complexes by preventing the full assembly of the active ligase (9). This broad activity would have a myriad of effects on cellular physiology, and therefore factors that control CAND1–CRL regulation in a more restricted fashion would make physiologic sense. Indeed, others have proposed the existence of such regulation based on the unexplained variable affinity of CAND1 for different Cullins in different tissues (8).

Based on the data presented, our view is that the COMMD protein family, with its variable pattern of tissue and cellular expression and the unique Cullin binding preferences of each COMMD family member, could provide fine tuning of CRL activation by countering CAND1 binding.

Given the myriad of activities regulated by the ~300 CRL complexes existent in mammals, it is anticipated that COMMD proteins would likely affect many physiological processes. Indeed, consistent with this notion, COMMD1 has been implicated in a variety of seemingly unrelated processes such as NF-κB regulation, sodium and electrolyte transport, hypoxia responses, and copper excretion. In most instances, the mechanism has been linked to protein ubiquitination, in agreement with the notion that COMMD1 and other COMMD proteins regulate CRL ubiquitin ligase complexes. Our view is that the biochemical events dissected in this study provide a mechanistic explanation for the various biological activities identified for COMMD1.

Similar to what has been reported for substrate receptor complexes (11, 12, 33), COMMD1 binding to CRLs displaces CAND1, leading to an enzymatically active complex. Based on the structure of Cul1, we speculate that this is mediated by competitive binding for the highly conserved R2 region (Fig. 6).
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5F), which interacts extensively with CAND1 (13). The competitive nature of COMMD1/CAND1 binding to CRLs is extensively demonstrated using various in vitro systems, and the relevance of these findings is validated by the increased binding of COMMD1 to Cul2 in CAND1-deficient cells.

It is important to note that the biochemical events that lead to CRL-CAND1 dissociation remain quite enigmatic. In the case of both COMMD1 and the SRC components Skp1 or Elongin C, the reaction requires ATP supplementation, yet the basis for this ATP requirement is not evident, because none of these factors have ATPase or kinase activity. In addition, a CRL1-CAND1 complex formed by E. coli-expressed recombinant proteins cannot be dissociated by Skp1 or neddylation co-factors (13), and this reaction only takes place when the CRL-CAND1 complex is isolated from mammalian cells (12, 33). For all of these reasons, we speculate that additional factors present in the mammalian preparations are required for CAND1 dissociation, and these factors may explain the requirement for ATP in the reaction. Similarly, we found that COMMD1 was active in this reaction only when extracted from mammalian cells, suggesting either that post-translational modifications of COMMD1 are necessary or that co-factor(s) associated with COMMD1 in mammalian cells are required for this process.

Recent data indicate that the recycling of CRLs between the CAND1-bound and SRC-bound states facilitates the loading of rare SRCs that would otherwise be outcompeted by more abundant co-factors (38). Although the SRC can dissociate the Cul1-SRC complex formation (39), various co-factors (38). Although the SRC can dissociate the Cul1-SRC complex formation (39), various experiments indicate that other factors regulate this interaction. Disrupting Cul1-SRC complex formation in vivo has limited effects on CRL1-CAND1 interactions (39). Similarly, inhibiting CRL neddylation in cells did not promote CRL1-CAND1 interactions, nor did it destabilize CRL1-SRC complexes (40). These observations support the notion that other factors regulate CRL-CAND1 interactions in vivo, which is in keeping with the findings of this study.

At the present time, it remains unclear whether COMMD1 acts to synergize the SRC recycling or whether it constitutes a parallel activation pathway. Until the precise mechanisms of CAND1 displacement are further elucidated, addressing these questions will remain problematic with our current systems. Nevertheless, the fact that COMMD1 binds to Culmins in a manner that is distinct from that used by SRCs suggests that these two mechanisms of CAND1 displacement may be related but are still independent from each other.

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