MUF1, A Novel Elongin BC-interacting Leucine-rich Repeat Protein That Can Assemble with Cul5 and Rbx1 to Reconstitute a Ubiquitin Ligase*

The heterodimeric Elongin BC complex has been shown to interact in vitro and in mammalian cells with a conserved BC-box motif found in a growing number of proteins including RNA polymerase II elongation factor Elongin A, SOCS-box proteins, and the von Hippel-Lindau (VHL) tumor suppressor protein. Recently, the VHL-Elongin BC complex was found to interact with a module composed of Cullin family member Cul2 and RING-H2 finger protein Rbx1 to reconstitute a novel E3 ubiquitin ligase that activates ubiquitylation by the E2 ubiquitin-conjugating enzymes Ubc5 and Cdc34. In the context of the VHL ubiquitin ligase, Elongin BC functions as an adaptor that links the VHL protein to the Cul2/Rbx1 module, raising the possibility that the Elongin BC complex could function as an integral component of a larger family of E3 ubiquitin ligases by linking alternative BC-box proteins to Cullin/Rbx1 modules. In this report, we describe identification and purification from rat liver of a novel leucine-rich repeat-containing BC-box protein, MUF1, which we demonstrate is capable of assembling with a Cullin/Rbx1 module containing the Cullin family member Cul5 to reconstitute ubiquitin ligase activity. In addition, we show that the additional BC-box proteins Elongin A, SOCS1, and WSB1 are also capable of assembling with the Cul5/Rbx1 module to reconstitute potential ubiquitin ligases. Taken together, our findings identify MUF1 as a new member of the BC-box family of proteins, and they predict the existence of a larger family of Elongin BC-based E3 ubiquitin ligases.

The mammalian Elongin BC complex is a heterodimeric complex composed of the 112-amino-acid Elongin C protein and the 118-amino-acid, ubiquitin-like Elongin B protein. The Elongin BC complex was initially identified as a positive regulator of RNA polymerase II elongation factor Elongin A, which is one of several transcription factors capable of stimulating the rate of elongation by RNA polymerase II in vitro (1). The Elongin BC complex was subsequently found to be a component of the multiprotein von Hippel-Lindau (VHL) tumor suppressor complex (2, 3). Interaction of Elongin BC with Elongin A and VHL depends on binding of Elongin C to an 10-amino acid degenerate sequence motif, referred to as the BC-box, which has the consensus sequence (A,P,S,T)LXXXVXXVX(A,I,L,V) and which is the only sequence shared between Elongin A and VHL (2–4). Analysis of the crystal structure of the VHL-Elongin BC complex revealed that binding of Elongin BC to the BC-box is governed by interaction of the highly conserved leucine at position 2 in the N terminus of the BC-box motif with a hydrophobic pocket created by residues in the C-terminal half of Elongin C (5). Elongin B binds to a short N-terminal Elongin C region and does not appear to interact directly with the BC-box.

In addition to Elongin A and VHL, Elongin BC binds to a large number of additional proteins including members of the SOCS-box protein family (6, 7), each of which includes an Elongin BC-binding site linked to protein-protein interaction motifs such as SH2 domains, ankyrin repeats, WD repeats, SPRY domains, or Ras-like domains (8). A role for the Elongin BC complex in ubiquitylation was brought to light by the discovery that the VHL tumor suppressor complex is an E3 ubiquitin ligase (9, 10) that targets the alpha subunits of the hypoxia-inducible transcription factors HIF1 and HIF2 for ubiquitylation (11–14). In the context of the VHL complex, the VHL protein functions as a substrate recognition subunit (12), which binds directly to HIF1α or HIF2α that is hydroxylated at a critical proline within the oxygen-dependent degradation domain (15, 16). Elongin BC functions as an adaptor that links the VHL protein to a Cul2/Rbx1 module that can function as a potent activator of HIFα ubiquitylation by the E2 ubiquitin-conjugating enzyme Ubc5 (12). Notably, the VHL complex bears a striking resemblance to SCF-ubiquitin ligase complexes, which contain one of a number of F-box protein

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substrate recognition subunits linked to a Cul1/Cdc53/Rbx1 module by the Elongin C-like Skp1 protein (17–22). Taken together, these observations raise the possibility that the Elongin BC complex could function as an integral component of a larger family of E3 ubiquitin ligases by linking additional BC-box containing substrate recognition subunits to Cullin/Rbx1 modules.

To address this possibility, we sought to determine whether additional BC-box proteins might also function as components of ubiquitin ligases. In a previous study, we identified and partially purified multiple, chromatographically distinct Elongin BC-containing species from rat liver (6). Here we report purification of one of these species to near homogeneity and characterization of its potential role as an individual component of a larger family of E3 ubiquitin ligases.

**Identification and Characterization of Elongin BC-box Protein MUF1**

**EXPERIMENTAL PROCEDURES**

**Materials**—Male Harlan–Sprague Dawley rats (200–300 g) were purchased from Harlan-Sprague Dawley. Phenylmethylsulfonyl fluoride, antipain, leupeptin, and anti-Flag (M2) monoclonal antibody were obtained from Sigma. Anti-VHL (Ig32) monoclonal antibody was from Roche Molecular Biochemicals. Anti-T7 monoclonal antibody was from Invitrogen, and anti-GST (4C10) monoclonal antibody was from Covance.

**Purification of MUF1-Elongin BC-containing Complex from Rat Liver**—A postnuclear supernatant was prepared from the livers of ~350 male Harlan–Sprague Dawley rats and fractionated into a postnuclear supernatant fraction with 38% (NH4)2SO4, as previously described (23). Protein fractions containing Elongin B were identified by Western blotting using an anti-Elongin B polyclonal antibody (6). The 0 to 38% (NH4)2SO4 fraction was resuspended in Buffer A (40 mM Hepes-NaOH (pH 7.9), 1 mM DTT, 0.5 mM EDTA, and 10% (v/v) glycerol) containing 0.5 mM phenylmethylsulfonyl fluoride, antipain, and 10 µg/ml leupeptin, and brought to a conductivity equivalent to that of Buffer A containing 0.1 M KCl by a 3-h dialysis against buffer A containing 0.5 mM phenylmethysulfonyl fluoride and dilution with the same buffer. Following centrifugation for 30 min at 28,000 × g, the resulting supernatant was mixed with 0.8 liter of phosphocellulose (P1, Whatman) pre-equilibrated in Buffer A containing 0.1 M KCl and 0.5 mM phenylmethylsulfonyl fluoride. After 45 min, the slurry was filtered at 500 ml/h in a 10.5-cm diameter column and then washed at the same flow rate with Buffer A containing 0.1 M KCl. The phosphocellulose column was eluted stepwise at 800 ml/h with Buffer A containing 0.3 M KCl, and 160-ml fractions were collected. Fractions containing Elongin B were concentrated by precipitation with 0.3 g/ml (NH4)2SO4, resuspended in ~10 ml of Buffer B containing 10 µg/ml antipain and 10 µg/ml leupeptin, and dialyzed against Buffer A containing 0.3 M KCl to a conductivity equivalent to that of Buffer A containing 0.4 M KCl. Following centrifugation for 15 min at 12,000 × g, the resulting supernatant was applied to 5 ml/min to a 21.5 × 150-mm SpheroGel TSK DEAE 5-PW HPLC column (Toso Haas) pre-equilibrated in Buffer C containing 80 mM KCl. The TSK DEAE 5-PW column was eluted at the same flow rate with a 250-kDA linear gradient from 80 to 500 mM KCl in Buffer C, and 5-ml fractions were collected. Fractions containing Elongin C, which eluted between 100 and 140 mM KCl, were pooled and dialyzed against Buffer A to a conductivity equivalent to that of Buffer A containing 40 mM KCl.

Following centrifugation for 20 min at 60,000 × g, the resulting supernatant was applied at 1 ml/min to a 7.5 × 75-mm TSK SP 5-PW HPLC column (TSK) pre-equilibrated in Buffer A containing 40 mM KCl. The TSK SP 5-PW column was eluted at the same flow rate with a 30-ml linear gradient from 40 to 500 mM KCl in Buffer A, and 1-ml fractions were collected. Fractions containing Elongin B, which eluted between 220 and 280 mM KCl, were pooled and diluted with an equal volume of Buffer B containing 2.0 mM (NH4)2SO4. Following centrifugation for 20 min at 60,000 × g, the resulting supernatant was applied at 0.5 ml/min to a 7.5 × 75-mm TSK phenyl 5-PW HPLC column (TSK) pre-equilibrated in Buffer C containing 1.0 M (NH4)2SO4. The TSK phenyl 5-PW column was eluted at the same flow rate with a 20-ml linear gradient from 1.0 to 0 mM (NH4)2SO4 in Buffer C, and 0.5-ml fractions were collected. Fractions containing Elongin B, which eluted between 60 and 30 mM (NH4)2SO4, were pooled and dialyzed against Buffer C containing 30 mM KCl to a conductivity equivalent to that of Buffer C containing 40 mM KCl. Following centrifugation for 20 min at 60,000 × g, the resulting supernatant was applied at 0.2 ml/min to a 4.6 × 35-mm TSK DEAE-NPR HPLC column (Toso Haas) pre-equilibrated in Buffer C containing 40 mM KCl. The TSK DEAE-NPR column was eluted at the same flow rate with a 4-ml linear gradient from 40 to 350 mM KCl in Buffer C, and 0.2-ml fractions were collected. Fractions containing Elongin B eluted between 70 and 110 mM KCl.

**Cloning of MUF1 cDNA**—The MUF1-Elongin BC-containing complex was fractionated by 13% SDS-polyacrylamide gel electrophoresis. Proteins were visualized by Coomassie staining, excised, and subjected to in-gel reduction, S-carboxamidomethylation, and tryptic digestion. Peptide sequences were determined in a single run by microsequencing. Peptide sequences were determined in a single run by microsequencing. Sequences were entered into the algorithm SEQUEST and by programs developed in the Harvard Microchemistry and Proteomics Analysis Facility (24). IMAGE Consor-
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FIG. 2. Sequence of MUF1. The MUF1 ORF was derived from mouse expressed sequence tags (accession numbers AA023471 and AA111647). MUF1 peptide sequences determined by ion trap mass spectrometry are underlined. An asterisk (*) is placed above amino acids that fall within the LXXLXX consensus sequence for leucine-rich repeats (38). The BC-box is boxed.

FIG. 3. MUF1 contains a BC-box resembling the SOCS-box. Panel A, alignment of sequences surrounding the MUF1 BC-box with representative SOCS-box sequences. Sequences of SOCS-boxes shown in the figure are from Hilton et al. (8). SOCS3, SH2 domain-containing SOCS-box protein 3; WSB1, WD repeat-containing SOCS-box protein 1; ASB2, ankyrin repeat-containing SOCS-box protein 2; RAR, Ras-related SOCS-box protein; SSB1, SPRY domain-containing SOCS-box protein. Panel B, interaction of Elongin BC with MUF1 depends on the BC-box. Sf21 cells were co-infected with the baculoviruses indicated in the figures. Anti-Flag immunoprecipitations were performed as described under “Experimental Procedures.” Total cell lysates (left) and anti-Flag immunoprecipitates (right) were subjected to SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting as described under “Experimental Procedures.”

Expression of Recombinant Proteins in Escherichia coli—Human Ub5a containing an N-terminal 6-histidine tag and a C-terminal Flag tag, Saccharomyces cerevisiae Uba1 containing an N-terminal Myc tag and a C-terminal Flag tag, and mammalian GST-ubiquitin were previously described (6, 12).

RESULTS AND DISCUSSION

Expression of Recombinant Proteins in Sf21 Insect Cells—cDNA encoding wild type mouse MUF1 and mouse MUF1 double point mutant MUF1(L24P;C28F) containing N-terminal 6-histidine and Flag tags, mouse WSB1 containing N-terminal 6-histidine and Flag tags, and human Cul1 and Cul2 containing N-terminal HA tags were subcloned into pBacPAK 8. cDNA encoding rat Elongin A containing an N-terminal Flag tag and human Cul3, mouse Cul4, and human Cul5 containing N-terminal HA tags were subcloned into pBacPAK 9. Recombinant baculoviruses were generated with the BacPAK baculovirus expression system (CLONTECH). Baculoviruses encoding human VHL, human Elongin B, human Elongin C, and mouse Rbx1 containing an N-terminal Myc tag, and mouse SOCS1 containing N-terminal 6-histidine, T7, and Xpress tags were previously described (6, 12).

Sf21 cells were cultured at 27 °C in SF-900 II SFM with 5% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). Sf21 cells were infected with the recombinant baculoviruses indicated in the figures. Sixty h after infection, cells were collected and lysed in ice-cold buffer containing 40 mM Hepes-NaOH (pH 7.9), 150 mM NaCl, 1 mM DTT, 0.5% (v/v) Triton X-100, 10% (v/v) glycerol, 5 μg/ml leupeptin, 5 μg/ml antipain, 5 μg/ml pepstatin A, and 5 μg/ml aprotinin.

Ubiquitination Assays—Sf21 cells co-infected with the baculoviruses indicated in the figures were lysed in ice-cold buffer containing 40 mM Hepes-NaOH (pH 7.9), 150 mM NaCl, 1 mM DT1, 0.5% (v/v) Triton X-100, 10% (v/v) glycerol, 5 μg/ml leupeptin, 5 μg/ml antipain, 5 μg/ml pepstatin, and 5 μg/ml aprotinin. After centrifugation at 10,000 × g for 20 min at 4 °C, the supernatants were immunoprecipitated with 2 μg of anti-Flag antibody and 10 μl of protein A-Sepharose. The beads were mixed with ~50 μg of Uba1, ~100 ng of hUb5a, and ~3 μg of GST-ubiquitin in a 20-μl reaction containing 40 mM Hepes-NaOH (pH 7.9), 60 mM potassium acetate, 2 mM DTT, 5 mM MgCl2, 0.5 mM EDTA (pH 7.9), 10% (v/v) glycerol, and 1.5 mM ATP. Reaction mixtures were incubated for 30 min at 26 °C. Reaction products were subjected to SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting with anti-GST antibodies.

Identification and Characterization of Elongin BC-box Protein MUF1—A multiprotein Elongin BC-containing complex was purified from a post-nuclear supernatant prepared from ~3 kg of rat liver by 0–38% (NH4)2SO4 fractionation, followed by chromatography on consecutive phosphocellulose, Ultrogel AcA 34, TSK DEAE 5-PW, TSK SP 5-PW, TSK phenyl-PW, and TSK DEAE-NPR columns (Fig. 1A). Purification of the Elongin BC-containing complex was monitored by Western blotting of aliquots of column fractions using anti-Elongin B antibodies. Analysis of the final TSK DEAE-NPR column fractions by
Identification and Characterization of Elongin BC-box Protein MUF1

SDS-polyacrylamide gel electrophoresis revealed that the ~19-Da Elongin B protein copurified with five additional polypeptides of ~90, ~45, ~30, ~20, and ~15 kDa (Fig. 1B). The identities of the ~19- and ~15-Da proteins as Elongin B and Elongin C were confirmed by ion trap mass spectrometry (data not shown). The ~45-, ~30-, and ~20-kDa polypeptides were identified by ion trap mass spectrometry as the EAP45, EAP30, and EAP20 proteins, which had previously been independently purified in association with RNA polymerase II elongation factor ELL and shown to assemble into an EAP20/30/45 subcomplex in vitro and in cells (26). Sequence analysis of the ~90-Da polypeptide by ion trap mass spectrometry revealed that it was a previously uncharacterized mammalian protein. Two overlapping mouse expressed sequence tags (accession numbers AA023471 and AA111647), which included all of the peptide sequences from the ~90-kDa polypeptide, were combined to generate a 790-amino acid ORF (Fig. 2). 5’-Rapid amplification of cDNA ends carried out with several cDNA libraries failed to identify any longer ORFs. Because a search of the GenBank™ nonredundant data base identified an expressed sequence tag designated MUF1 (accession no. CAA60013), which codes for a C-terminal portion of the predicted ORF from amino acids 226 to 776, we refer to the ~90-kDa polypeptide as MUF1. Further analysis revealed that MUF1 is a leucine-rich repeat protein (Fig. 2) that is highly conserved in mammalian species, but failed to identify potential MUF1 orthologs in lower eukaryotes.

MUF1 Binds Directly to the Elongin BC Complex through an N-terminal BC-box Motif Resembling the SOCS-box—A search of the Conserved Domain Database with reverse position specific BLAST (27) revealed that MUF1 contains an N-terminal sequence motif resembling the SOCS-box (Fig. 3A), which was previously shown to contain a functional BC-box and to bind the Elongin BC complex (6, 7). The SOCS-box is an ~50-amino acid sequence motif composed of an N-terminal Elongin BC-box and a short C-terminal proline-rich region of unknown function (6–8). The SOCS-box was originally identified as a conserved sequence motif in the C terminus of the SH2 domain-containing suppressors of cytokine signaling (SOCS) proteins, which act as negative regulators of cytokine-induced Jak/STAT signaling (28–30). SOCS proteins appear to inhibit phosphorylation and activation of STATs by binding to and inhibiting Jak or receptor tyrosine kinases. In addition, recent studies have shown that overexpression of SOCS1 in mammalian cells can increase the rate of ubiquitin-dependent proteolysis of Vav, TEL-JAK2, and JAK2, consistent with the possibility that SOCS1 is a component of a ubiquitin ligase (31, 32). Subsequently, a larger collection of SOCS-box proteins was identified and found to include nearly 30 previously uncharacterized members of the SH2, WD-40 repeat, ankyrin repeat, SPRY, and Ras-like protein families (8). The Elongin BC complex has been shown to interact in vitro and in cells with representative members of each of these families (6, 7).

To determine which of the proteins in the MUF1-Elongin BC complex is capable of interacting with the Elongin BC complex, Sf21 insect cells were co-infected with baculoviruses encoding Elongins B and C and Flag-MUF1, T7-EAP20, HA-EAP30, or Myc-EAP45. As expected from the presence of a SOCS-box-like motif in the N terminus of MUF1, the Elongin BC complex could be specifically co-immunoprecipitated with MUF1 from Sf21 cell lysates (Fig. 3B). The Elongin BC complex did not co-immunoprecipitate with EAP20, EAP30, or EAP45 (data not shown).

To determine whether MUF1 binds the Elongin BC complex through its N-terminal SOCS box-like motif, a MUF1 double

Fig. 4. The MUF1-Elongin BC complex assembles with a Cul5/Rbx1 module. Sf21 cells were co-infected with the baculoviruses indicated in the figure. Anti-Flag and anti-HA immunoprecipitations were performed as described under “Experimental Procedures.” Total cell lysates and anti-Flag and anti-HA immunoprecipitates were subjected to SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting as described under “Experimental Procedures.”

Experimental Procedures.

FIG. 5. The MUF1-Elongin BC-Cul5-Rbx1 complex has ubiquitin ligase activity. Panel A, the MUF1-Elongin BC-Cul5-Rbx1 complex activates formation of polyubiquitin conjugates by the E2 ubiquitin-conjugating enzyme Ubc5. The MUF1 complex was purified by anti-Flag immunoprecipitation from insect cells infect with baculoviruses encoding Flag-MUF1, Elongin B, Elongin C, Cul5, and Rbx1. Immunoprecipitated complexes were assayed for their abilities to activate ubiquitylation by Ubc5 as described under “Experimental Procedures.” Panel B, the MUF1L24P,C28F mutant does not support assembly of the MUF1-Elongin BC-Cul5-Rbx1 complex or activation of ubiquitylation by Ubc5. Sf21 cells were co-infected with the baculoviruses indicated in the figure. Anti-Flag immunoprecipitations were performed as described under “Experimental Procedures.” Total cell lysates and anti-Flag immunoprecipitates were subjected to SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting as described under “Experimental Procedures.” Anti-Flag immunoprecipitates were assayed for their ability to activate ubiquitylation by Ubc5 as described under “Experimental Procedures.” HC, heavy chain.
point mutant MUF1[L24P;C28F] containing BC-box mutations shown previously to disrupt binding of Elongin BC to SOCS1 (6) was coexpressed in SF21 cells with Elongins B and C. As shown in Fig. 5B, binding of MUF1 to Elongin BC was strongly dependent on the presence of an intact BC-box within the MUF1 N-terminal SOCS box-like motif; whereas wild type MUF1 was co-immunoprecipitated from SF21 cell lysates with Elongins B and C, the MUF1 mutant was not.

The MUF1-Elongin BC Complex Is Capable of Assembling with a Cul5/Rbx1 Module to Reconstitute a Ubiquitin Ligase—In light of evidence that the Elongin BC-containing VHL tumor suppressor complex is a ubiquitin ligase (9, 10, 13, 14) and, furthermore, that Elongin BC functions in the context of the VHL complex as an adaptor that links the VHL protein to a Cul2/Rbx1 module that can activate formation of polyubiquitin chains by the E2 ubiquitin conjugating enzymes Ubc5 and Cdc34 (12), we asked whether Elongin BC could also function as an adaptor to link MUF1 to a Cullin/Rbx1 module to reconstitute ubiquitin ligase activity. In mammalian cells the Cullin protein family includes at least 5 members referred to as Cul1, Cul2, Cul3, Cul4, and Cul5 (33). Each of these Cullin proteins has been shown to assemble with Rbx1 to reconstitute a module that is capable of activating ubiquitylation by E2 ubiquitin conjugating enzymes (20, 21). To determine whether Elongin BC is capable of recruiting a Cullin/Rbx1 module to the MUF1-Elongin BC complex, anti-Flag and anti-HA immunoprecipitations were carried out on lysates of SF21 cells co-infected with baculoviruses encoding Flag-MUF1, Elongins B and C, Rbx1, and individual Cullin proteins containing N-terminal HA epitope tags. In contrast to the VHL-Elongin BC complex, the MUF1-Elongin BC complex did not interact stably with the Cul2/Rbx1 module. In addition, the MUF1-Elongin BC complex did not interact stably with Cul5/Rbx1 modules containing Cul1, Cul3, or Cul4. The MUF1-Elongin BC complex did, however, assemble stably with a Cul5/Rbx1 module to reconstitute a multiprotein complex containing MUF1, Elongins B and C, Cul5, and Rbx1 (Fig. 4).

To determine whether the MUF1-Elongin BC-Cul5/Rbx1 complex possesses ubiquitin ligase activity, the complex was immunopurified and assayed for its ability to activate formation of polyubiquitin chains by the E2 ubiquitin conjugating enzyme Ubc5 in the presence of ATP, the E1 ubiquitin activating enzyme Uba1, and GST-ubiquitin\(^{K48R}\) As shown in Fig. 5A, the MUF1 complex stimulated formation of a ladder of GST-ubiquitin\(^{K48R}\) conjugates by Ubc5. In GST-ubiquitin\(^{K48R}\), ubiquitin lysine 48 is mutated to arginine. As a consequence, GST-ubiquitin K48R is unable to form multi-ubiquitin chains linked through lysine 48. At the present time, we do not know whether the observed ladder of GST-ubiquitin\(^{K48R}\) conjugates is due to formation of alternate forms of polyubiquitin linked through lysines 6, 11, 29, or 63 of ubiquitin (34) or to attachment of single GST-ubiquitin\(^{K48R}\) molecules to multiple sites within the GST portion of the fusion protein, to subunits of the MUF1 complex, or to the E1 or E2 enzymes.

In a previous study, we observed that VHL BC-box mutants that do not bind Elongin BC or assemble with the Cul2/Rbx1 module do not activate ubiquitylation by Ubc5 (12). Likewise, the MUF1 BC-box mutant MUF1[L24P,C28F], which does not bind Elongin BC, did not efficiently assemble with the Cul5/ Rbx1 module and did not activate ubiquitylation by Ubc5 (Fig. 5B).

The Elongin BC Complex Can Link the Additional BC-box Proteins Elongin A, SOCS1, and WSB1 to the Cul5/Rbx1 Module—Our finding that Elongin BC is capable of recruiting Cullins Cul2 and Cul5 into multiprotein VHL and MUF1 complexes possessing ubiquitin ligase activity raised the possibility that Elongin BC could also function as an adaptor to link additional BC-box proteins to an E2-activating Cullin/Rbx1 module. To investigate this possibility, anti-HA immunoprecipitations were carried out on lysates of SF21 insect cells co-infected with baculoviruses encoding Flag-MUF1, Elongins A, SOCS1, or WSB1, Elongins B and C, Rbx1, and either HA-Cul2 or HA-Cul5. As shown in Fig. 6, the VHL-Elongin BC complex was capable of assembling with both the Cul2/Rbx1 and Cul5/Rbx1 modules; in contrast, the Elongin A-Elongin BC, SOCS1-Elongin BC, and WSB1-Elongin BC complexes all failed to assemble efficiently with the Cul2/Rbx1 module, but were able to assemble with the Cul5/Rbx1 module. Like the MUF1 complex, all of these complexes are capable of activating formation of GST-ubiquitin conjugates by the E2 ubiquitin-conjugating enzyme Ubc5 (data not shown).

In summary, in this report we have identified the novel Elongin BC-box protein MUF1 and demonstrated that it is capable of assembling with the Ubc5-activating Cul5/Rbx1 module to reconstitute a multiprotein complex with ubiquitin ligase activity. In addition, we have shown that the additional BC-box proteins VHL, Elongin A, SOCS1, and WSB1 are also capable of assembling with the Cul5/Rbx1 module. Cul5 is the founding member of the Cullin protein family and was originally identified as a cytoplasmic arginine-vasopressin receptor, referred to as the vasopressin-activated calcium-mobilizing-1 protein (35). Subsequently Cul5 (vasopressin-activated calcium-mobilizing-1) was shown to have a role in signal transduction and to be capable of mobilizing calcium, stimulating d-
proteins are encoded by class E
functionality relationship between MUF1 and the EAP20, EAP30,
components of the RNA polymerase II transcriptional machin-
at least one of the functions of Cul5 may be to participate
(Fig. 6, upper right panel) raises the intriguing possibility that
at least one of the functions of Cul5 may be to participate
cAMP production in mammalian cells (36). Although it is pres-
their genetic interactions with the
genes, which have recently been linked to cellular ubiquityla-
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