Interaction of the RING Finger-related U-box Motif of a Nuclear Dot Protein with Ubiquitin-conjugating Enzymes*

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The U-box domain has been suggested to be a modified RING finger motif where the metal-coordinating cysteines and histidines have been replaced with other amino acids. Known U-box-containing proteins have been implicated in the ubiquitin/proteasome system. In a search for proteins interacting with the ubiquitin-conjugating enzyme UbcM4/UbcH7, we have identified a novel U-box containing protein, termed UIP5, that is exclusively found in the nucleus as part of a nuclear dot-like structure. Interaction between UbcM4 and UIP5 was observed in vivo and in vitro with bacterially expressed proteins. In addition to UbcM4, several other ubiquitin-conjugating enzymes (E2s) that share the same sequence within the L1 loop bind to UIP5. Mutational analysis showed that the U-box, like the RING finger in other proteins, forms the physical basis for the interaction with E2 enzymes. Further support for the structural similarity between U-box and RING finger comes from the observation that, in both cases, the same regions within the UbcM4 molecule are required for interaction. Our results establish at the molecular level a link between the U-box and the ubiquitin conjugating system and strongly suggest that proteins containing U-box domains are functionally closely related to RING finger proteins.

The RING finger motif (for real interesting new gene) is a cysteine-rich structure that has been found in many functionally distinct proteins. Because of their widespread occurrence and their link to human diseases, RING finger proteins have attracted considerable interest (for reviews, see Refs. 1 and 2). Until recently, very little was known about the molecular function of the RING domain, except that it was involved in mediating protein-protein interactions. However, recent results from several laboratories strongly suggest that RING finger proteins play critical roles in mediating the transfer of ubiquitin to target proteins (3–5). The ubiquitin pathway generally involves three types of enzymes (for reviews, see Refs. 6–8). In an initial step, the ubiquitin-activating enzyme (E1 or Uba) forms a thioester bond with the C terminus of ubiquitin, which is then transferred to a specific cysteine residue of a ubiquitin-conjugating enzyme (E2 or Ubc).1 In the final step the E2 enzyme donates ubiquitin to a lysine residue of the target protein, either directly or with the assistance of ubiquitin protein ligase (E3 or Ubr). Following formation of the polyubiquitin chain, the protein moiety is in most cases degraded by the 26 S proteasome complex, and free ubiquitin is released.

The E3 ubiquitin protein ligases play a key role in recognition and selection of proteins targeted for ubiquitination and subsequent degradation. Over the past year many RING finger proteins have been shown to act as E3s, either by themselves (3–5) or as part of a multisubunit E3 protein complex (9). The RING proteins are thought to act as scaffolds that bring together the E2 enzyme and the target protein. In most cases the RING motif itself is needed for the E3 activity and interacts specifically with E2 enzymes. The biological significance of this interaction is underlined by the fact that mutations of the RING motif that prevent interaction with E2s can contribute to oncogenic transformation (10), familial Parkinson disease (11) and abnormal development (11).

We have recently described a family of proteins, termed UIPs (UbcM4-interacting proteins), that interact with two closely related E2 enzymes, UbcM4 (identical to UbcH7) and UbcH8 (12). In all cases interaction occurs through the RING domain of the UIPs, the only exception being UIP5, where UbcM4 binds to a sequence that has several amino acids in common with the RING motif; however, the metal-coordinating cysteine and histidine residues are replaced with other amino acids. As this modified RING finger motif was first found in the yeast UFD2 gene, it has been designated U-box (13, 14). Recently, a database search initiated with the U-box of UDF2 revealed the presence of the U-box motif in several other proteins from eukaryotic organisms (15). In the present report we have characterized the UIP5 protein and show that the U-box domain can directly interact with several E2 enzymes and, therefore, is likely to function similar to the RING finger in the ubiquitination pathway.

EXPERIMENTAL PROCEDURES

Binding Assay Using the Yeast Two-hybrid System—Yeast expression plasmids containing the UbcH1/HHR66B (16), UbcM2 (17), UbcM3 (17), UbcM4 (18), UbcH5a (19), UbcH8 (20), or UbcM9 (21) cDNAs fused in frame to the LexA binding domain were obtained by PCR amplification of the complete coding regions of the respective cDNAs followed by ligation into vector pBBL23 (22) or vector pBTML116. The latter vector was derived from pBTMI16 (23) by insertion of a linker region, consisting of two repeats of four glycine and one serine residue, adjacent to the LexA protein, thus giving the bait protein greater flexibility. The plas-

1 The abbreviations used are: E2/Ubc, ubiquitin-conjugating enzyme; E3/Ubr, ubiquitin-protein ligase; E1/Uba, ubiquitin-activating enzyme; GFP, green fluorescent protein; GST, glutathione S-transferase; HA, hemagglutinin; PAG, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; UIP, UbcM4 interacting protein; DAPI, 4',6'-diamidino-2-phenylindole.
mid expressing a fusion protein between the VP16 activation domain and a mouse UIP5 fragment extending from amino acid 241 to 335 and containing the U-box has been described earlier (12). A construct containing the complete translated region of hUIP5 fused to the LexA binding domain was obtained by PCR amplification of pKIAA0860 (a gift from Dr. T. Nagase, Kazusa DNA Research Institute, Kisarazu, Japan) followed by cloning of the amplified fragment into the vector pBTML116. The fusion protein will be referred to as LexA-hUIP5. Mutations of UbcM4 and hUIP5 were carried out using the Stratagene QuickChange kit (Stratagene, Amsterdam, Holland). All constructs were sequenced to confirm their structure. Sequences of oligonucleotides used for amplification of cDNAs and for site-directed mutagenesis are available upon request. Expression of VP16 or LexA fusion proteins in yeast was analyzed by SDS-PAGE of total protein extracts and Western blotting. Fusion proteins were detected by using anti-VP16 or anti-LexA antibodies (Santa Cruz Biotechnology, Heidelberg, Germany), respectively.

Plasmids containing the proteins of interest fused to the LexA DNA binding or the VP16 activation domain were co-transformed into the yeast YRN 974 strain. In this strain, a reporter gene encoding the green fluorescent protein (GFP), is chromosomally integrated downstream of a LexA binding site (24). Interaction of two proteins, one with a LexA binding domain, the other with a transcriptional activation domain, results in activation of GFP, which was quantified by flow cytometric analyses. Approximately 20,000 cells of at least three independent transformants were analyzed for fluorescence intensity using a Becton Dickinson FACSscan flow cyrometer.

In Vivo Binding Assay with Tagged Proteins in 293 Cells—Construction of (His<sub>6</sub>)-tagged UbcM4 has been described previously (12). The plasmid pUIP5-HA, containing the hemagglutinin (HA) tag at the C-terminus of the full-length protein, was obtained by standard PCR techniques using as template the plasmid pKIAA0860. The amplified fragment was cloned in the EcoRI site of vector pHCMV-G, from which the insert was removed (25). Transfection of human 293 kidney cells, purification of (His<sub>6</sub>)-tagged proteins by metal ion chromatography, SDS-PAGE, and Western blotting were performed exactly as described previously (12).

In Vitro Binding Assay with Bacterially Expressed Proteins—UbcM4 with a C-terminal (His<sub>6</sub>)-tag was amplified by PCR and cloned in frame with glutathione S-transferase (GST) of the bacterial expression vector pGEX-5X-1 (Amersham Pharmacia Biotech, Freiburg, Germany). To obtain GST-hUIP5 (241–335), a fragment encompassing amino acids 241–335 of hUIP5 was amplified by PCR and cloned in the vector pGEX-XX-1 in frame with GST. GST fusion proteins were expressed in Escherichia coli DH<sub>5</sub>α induced with 0.1 mM isopropyl-β-D-thiogalacto- pyranoside. Cell extracts were prepared by resuspending the bacterial pellet in lysis buffer (1% Nonidet P-40, 50 mM sodium phosphate, pH 7.0, 150 mM NaCl, protease inhibitor mixture (Complete, EDTA-free, Roche Molecular Biochemicals)), and sonication. Cell debris was removed by centrifugation and the clarified lysate stored at −70 °C.

To obtain purified UbcM4(His<sub>6</sub>), the GST-UbcM4(His<sub>6</sub>) fusion protein was bound to glutathione-Sepharose. After several washes, with 50 mM Tris-HCl, pH 8.5, 150 mM NaCl and then with 1× PBS, UbcM4(His<sub>6</sub>) was released from the GST tag by incubating the resin with 50 units of factor Xa (Amersham Pharmacia Biotech) in PBS followed by several washes with lysis buffer. The eluted UbcM4(His<sub>6</sub>) was further purified by absorption to TALON resin (CLONTECH, Heidelberg, Germany) and elution with imidazole. Binding assays were performed by incubating GST-hUIP5 (241–335) bound to glutathione-Sepharose with UbcM4(His<sub>6</sub>) in 300 μl of PBS. The bound proteins were then eluted with a buffer containing 50 mM Tris-HCl, pH 8.0 and 10 mM reduced glutathione and analyzed by SDS-PAGE and Western blotting. The GST-tagged hUIP5 protein was detected using a goat polyclonal anti GST antibody (Amersham Pharmacia Biotech, Freiburg, Germany). For detection of the (His<sub>6</sub>)-tagged UbcM4, BMG-His-1 (Roche Molecular Biochemicals, Mannheim, Germany) was used as the primary antibody. Primary antibodies were visualized by peroxidase-conjugated donkey antibodies directed against goat or rabbit immunoglobulins (Dianova, Hamburg, Germany) and chemiluminescence detection (ECL kit, Amersham Pharmacia Biotech).

RNA Preparation and Analysis—Total cellular RNA was isolated by using the RNeasy total RNA isolation according to the manufacturer’s protocol (Qiagen GmbH, Hilden, Germany), fractionated (10 μg per lane) by electrophoresis in 1% agarose gels containing formaldehyde and transferred by capillary blotting onto Ny lon Plus membranes (Qiagen GmbH, Hilden, Germany). Preparation of 32P-labeled radioactive probe, hybridization, and washing of filters were described previously (26). The cDNA insert from the UIP5 plasmid initially isolated from the mouse embryo cDNA (12) library was used for preparing the radioactive probe.

Transfection of HeLa Cells and Immunohistochemical Staining—HeLa cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. The plasmids phUIP5-HA and pSGS-LINK-Sp100, encoding Sp100 (27), were introduced into HeLa cells by use of Effectene Transfection Reagent (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. For indirect immunofluorescence staining, cells were grown on coverslips and fixed for 5 min in methanol and for 30 s in acetone. The HA-tagged hUIP5 was detected using a 1:200 dilution of the rat monoclonal anti-HA high affinity antibody 3F10 (Roche Molecular Biochemicals, Mannheim, Germany), and Sp100 was detected using a 1:200 dilution of a rabbit anti-Sp100 antibody (27). Antibodies were incubated with substrate for 1 h at room temperature and unbound antibodies removed by three successive washes with PBS. To visualize the primary antibodies, rhodamine RedX-conjugated goat anti-rat IgG antibodies or dichlorotriazinylfluorescein-conjugated goat anti-rabbit IgG antibodies (Dianova, Hamburg, Germany) were diluted 1:200 in PBS. DAPI (4',6'-diamidino-2-phenylindole) (Roche Molecular Biochemicals, Mannheim, Germany) was used for staining of nuclear DNA. Cell imaging was performed with a Leica DMRA Microscope (Leica, Bensheim, Germany) and the Spot Fluorescence Imaging Program (Intas, Heidelberg, Germany).

RESULTS

Structure, Expression, and Cellular Location of hUIP5—UIP5 was originally isolated as a partial cDNA clone from a mouse embryonal mouse and second-hybrid library using UbcM4 as a bait (12). The amino acid sequence predicted from its open reading frame is 84% identical with the protein encoded by the human gene KIAA0860 (GenBank™ accession number AB020667), for which the complete amino acid sequence is known. This protein, which will be referred to in this paper as human UIP5 (hUIP5), was used in all subsequent experiments where the
complete UIP5 protein was analyzed. As shown schematically in Fig. 1, hUIP5 contains both a RING-HC domain and an U-box domain. Based on sequence profile analysis, the U-box of hUIP5 (KIAA0860) has been suggested to be a modified RING finger domain that lacks the hallmark metal-chelating residues of the latter but is likely to adopt a RING finger-like conformation (15). The amino acids replacing the metal-chelating cysteines and histidines of the RING finger are indicated by asterisks in the U-box sequence of Fig. 1.

The function of hUIP5 or its mouse homolog, mUIP5, is presently unknown. As a first step to characterize the gene, we analyzed its transcription pattern in mouse and the cellular location of its protein. At least two transcripts, about 4.5 and 4.2 kilobases in length, can be detected by Northern blot analysis. They occur predominantly in embryonic stem cells, testis, and embryos and placentas at day 14.5 postcoitus (Fig. 2). However, mUIP5-specific RNA was also found in other tissues, in particular in brain, when the more sensitive reverse transcriptase-PCR method was used (data not shown). The presence of a potential nuclear localization signal (1366PSQKRKKI), which is related to that of simian virus 40 large T antigen (28), suggested that hUIP5 might be a nuclear protein. To investigate this, a plasmid encoding HA-tagged hUIP5 was transiently transfected into HeLa cells and the protein stained with anti-HA antibody. As shown in Fig. 3, hUIP5 (red fluorescence) was localized exclusively in the nucleus where it is associated with nuclear body-like structures. As the staining pattern was very similar to that of the previously described promyelocytic leukemia protein PML-Sp100 nuclear bodies (reviewed in Ref. 29), we determined the localization of hUIP5 with respect to Sp100. For this purpose HeLa cells co-transfected with HA-tagged hUIP5, and Sp100 were double-immunostained with anti-HA (red fluorescence) and anti-Sp100 (green fluorescence) antibodies. An overlay of both pictures showed only very limited co-localization of the two dot-like structures, indicating that the nuclear bodies containing hUIP5 are different from those containing Sp100 (Fig. 3).

Interaction of UIP5 with E2 Enzymes—A mouse UIP5 fragment containing little more than the U-box motif was previously shown to bind to UbcM4 (12). Here we show that several other E2 enzymes can also interact with the U-box domain of this protein when analyzed by the yeast two-hybrid system using GFP as a reporter (Fig. 4). Among the E2s tested, only UbcH1 and UbcM9 did not bind, the latter being a conjugating enzyme for the ubiquitin-related SUMO-1 (30, 31).

To determine whether hUIP5 interacts with UbcM4 in mammalian cells, (His₆)₆-tagged UbcM4 and (HA)-tagged hUIP5 were transiently co-expressed in human 293 kidney cells. After lysis of cells, (His₆)₆-tagged UbcM4 was purified by metal affinity chromatography and the presence of hUIP5 associated with UbcM4 analyzed by SDS-PAGE, Western blotting, and immunostaining with HA antibodies. The results in Fig. 5 show that hUIP5 co-elutes with the metal resin-bound UbcM4 (lane 5). In the absence of (His₆)₆-tagged UbcM4, hUIP5 is not bound to the resin (lane 4). These results clearly indicate that hUIP5 can associate with UbcM4 in mammalian cells.

U-box of hUIP5 Is Required for Interaction with E2 Enzyme—As described above, results obtained with partial cDNA clones indirectly suggested that the U-box of UIP5 and not the RING-HC domain was involved in the interaction with UbcM4. To directly show that the U-box provides the physical basis for binding to UbcM4, some of the amino acid residues in positions occupied by cysteines and histidine in classical RING fingers (marked by asterisks in Fig. 1) were mutated by site-directed mutagenesis. Two mutant hUIP5 proteins were generated, one with both, Asp-265 and Thr-268, mutated to Gly (hUIP5[D265G,T268G]) and the other with Ser-280 converted

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**Fig. 2. Expression of the UIP5 gene.** Northern blot containing total RNA from the CCE embryonal stem cell line, from mouse embryos and placentas from day 14.5 of gestation, and from the indicated tissues of adult animals were hybridized with a radioactive probe derived from a partial mouse UIP5 cDNA. The positions of the 18 and 28 S rRNA are depicted on the right. The amount of RNA was controlled by hybridization with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe.

**Fig. 3. Cellular location of hUIP5.** HeLa cells, transiently co-transfected with plasmids expressing HA-tagged hUIP5 and Sp100, were stained with anti-HA antibody (A, red fluorescence) or anti-Sp100 antibody (B, green fluorescence). C, DAPI staining. D, for co-localization the red fluorescence of hUIP5, the green fluorescence of Sp100, and the DAPI staining were merged.
to Leu and Lys-282 to Gly (hUIP5[S280L,K282G]). Both mutant proteins did not interact with UbcM4 when analyzed in the yeast two-hybrid system using GFP as a reporter (Fig. 6). Western blot analysis showed that the mutant UIP5 proteins are synthesized at levels comparable with that of the wild-type protein. Therefore, lack of interaction is not due to the absence or to reduced amounts of the mutant protein. Mutation of the U-box also prevented binding to other E2 enzymes (data not shown). These results clearly show that the U-box is required for hUIP5/E2 interaction.

Interaction of hUIP5 with UbcM4 in Vitro—To test if there is a direct interaction between UIP5 and UbcM4, hUIP5 was expressed as a GST fusion protein in *E. coli*. As the complete protein was not soluble under nondenaturating conditions, a 95-amino acid-long fragment containing the U-box motif was synthesized as a GST fusion protein (GST-hUIP5-(241–335)) and bound to glutathione-Sepharose beads. Binding to UbcM4 was analyzed by adding (His6)-tagged UbcM4 also purified from bacterial extracts. As shown in Fig. 7, lane 3, UbcM4 bound to hUIP5 under these *in vitro* conditions.

Mapping of Regions of UbcM4 That Are Required for Interaction with the U-box of UIP5—To determine which amino acids of UbcM4 are necessary for binding to the U-box, mutations were introduced into the UbcM4 molecule and their effect on interaction analyzed with the help of the yeast two-hybrid system. The amino acid sequence of UbcM4 and an alignment of secondary structure features is shown in Fig. 8A. UbcM4, which consists of little more than the conserved UBC domain, has an α/β structure similar to the structures of other E2s (32). The loop region connecting the third and fourth strand of the β-sheet and the loop located between the fourth β-strand and the second α-helix will be referred to as loop L1 and loop L2, respectively, in accordance with the nomenclature used in a previous publication (33). Based on the crystal structure analysis of complexes between UbcH7 and the Hect domain protein E6-AP or the RING finger protein c-Chl, the N-terminal α-helix H1 and loops L1 and L2 of UbcH7 are involved in interaction with these proteins (33, 34). Therefore, in our initial experimen-
DISCUSSION

The most significant result of the present report is the observation that the U-box domain can directly interact with E2 enzymes. No other proteins seem to be necessary for this interaction, as bacterially expressed E2 and UIP5 proteins can associate with each other in vitro. Therefore, for the first time a molecular link has been established between the U-box motif and the ubiquitin-conjugating system. Our results strongly suggest that proteins containing U-box domains are functionally closely related to RING finger proteins, whose importance for the ubiquitin-conjugating system has been firmly estab-

Fig. 7. Interaction of hUIP5 with UbcM4 in vitro. GST-hUIP5(241–335) bound to glutathione-Sepharose was incubated alone (lane 2) or with UbcM4(His6) (lane 3). As a control glutathione-Sepharose was incubated with UbcM4(His6) (lane 1). After incubation proteins were eluted with reduced glutathione and analyzed by SDS-PAGE and Western blotting. Lower panel, the blot was hybridized with an antibody directed against the (His6)-tag of UbcM4. Upper panel, the blot shown in the lower panel was rehybridized with an antibody directed against GST to detect UIP5. No binding was observed when GST bound to glutathione-Sepharose was incubated with UbcM4(His6) (data not shown).

Fig. 6. Mutation of U-box of hUIP5 prevents interaction with UbcM4. Interaction of UbcM4 fused in frame to the VP16 activation domain with wild-type or mutant hUIP5s fused to the LexA binding domain was analyzed in the yeast two-hybrid system using GFP as a reporter and the fluorescence quantified by flow cytometry. A, yeast cells expressing either VP16/UbcM4 (continuous line) or LexA/hUIP5 (shaded peak). B, yeast cells co-expressing VP16/UbcM4 + LexA/hUIP5 (shaded peak) or VP16/UbcM4 + LexA/hUIP5[D265G,T268G] (continuous line) or VP16/UbcM4 + LexA/hUIP5[S280L,K282G] (dotted line). C, synthesis of LexA fusion proteins in yeast cells transformed with plasmids encoding LexA/hUIP5 (lane 1), LexA/hUIP5[D265G,T268G] (lane 2), or LexA/hUIP5[S280L,K282G] (lane 3). As a control, untransformed cells were analyzed (lane 4). Fusion proteins were detected on Western blots by using anti-LexA antibodies.

ments, amino acids in these regions were mutated or deleted and the effect on interaction with the U-box of UIP5 tested. The results are summarized in Fig. 8C. When the N-terminal 12 amino acids comprising the H1 α-helix of UbcM4 were deleted (UbcM4(13–154)), interaction with UIP5 was abolished. However, as shown by the hybrid protein UbcH5-(1–14)/UbcM4-(16–154) interaction could be restored by adding the corresponding region of UbcH5. At the C terminus, deletion of 35 residues (UbcM4(4–119)) did not affect binding; however, larger deletions like in UbcM4-(2–110) abolished interactions. As shown in Fig. 8C, the truncated UbcM4 proteins that do not interact with UIP5 are synthesized at levels comparable with wild-type UbcM4, ruling out the possibility that lack of interaction is due to the absence of these proteins.

In a further set of experiments, the loop regions were mutated. The sequence of loop L1 is highly conserved, and Phe-63 at the tip of L1 has previously been shown to be essential for interaction with Hect domain proteins (35) and with RING finger proteins.2 The results in Fig. 8C show that mutation of Phe-63 to Asn (UbcM4[N94D]) had no effect, mutation of the adjacent Trp-95, which is present in all E2 enzymes, to Gly (UbcM4[W95G]) and mutation of Pro-97 to Gly (UbcM4[P97G]) abolished binding to hUIP5, clearly indicating that these residues are required for interaction with the U-box. Mutant proteins that do not interact with UIP5 were shown to be present in yeast protein extracts at levels comparable with wild-type UbcM4 (Fig. 8D). Binding was not affected when Lys-96, Thr-99, and Lys-100 were changed to Ser, Leu, and Thr, respectively (UbcM4[K96S,T99L,K100T]), so that loop L2 of UbcM4 looked like that of UbcH5. In contrast to the effects of mutations in loops L1 and L2, no interference with binding to UIP5 was observed when either the catalytically active cysteine residue (Cys-86) was mutated to alanine (UbcM4[L33F]). In summary, amino acids in loops L1 and L2, no interference with binding to UIP5 was observed when either the catalytically active cysteine residue (Cys-86) was mutated to alanine (UbcM4[L33F]), Leu-33 was changed to Phe (UbcM4[L33F]), Leu-33 was changed to Phe (UbcM4[L33F]), or Leu-33 was changed to Phe (UbcM4[L33F]).

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were detected on Western blots by using anti-LexA antibody. UbcM4[2–110] (lane 5), or UbcM4[13–154] (lane 6), or UbcM4[W95G] (lane 4), or mutant UbcM4 proteins were formed with plasmids encoding LexA fused to UbcM4 (lane 2), UbcM4[W95G] (lane 4), or UbcM4[13–154] (lane 6), or UbcM4[13–154] (lane 6). Fusion proteins were detected on Western blots by using anti-LexA antibody.

**Interaction between U-box and E2 Enzymes**

Evidence from several laboratories suggests that U-box proteins are involved in the ubiquitin/proteasome pathway. The most prominent example is UFD2 of Saccharomyces cerevisiae, which was originally identified as a component of the ubiquitin fusion degradation pathway (13) and which was recently shown to act as a so-called E4 enzyme, which is required for multiquitination of ubiquitin fusion proteins, a process necessary for efficient degradation of such proteins by the proteasome (14). In yeast, UFD2 is needed for cell survival under stress conditions, suggesting that it mediates degradation of stress-induced aberrant proteins. The UFD2 homolog in Dictyostelium discoideum, NOSA, is required for normal differentiation as disruption of the nosA gene causes developmental arrest at the aggregate stage (37). Another example for an U-box protein is CHIP, which negatively regulates chaperone activity (38).

Our observation that U-box domains can bind to E2 enzymes strongly suggests that it is involved in the ubiquitin/proteasome pathway. The function of UIP5 is presently unknown. Most likely it is not a substrate for ubiquitination as it is not ubiquitinated in vitro (data not shown). However, the fact that it can associate with E2 enzymes strongly suggests that it is involved in the ubiquitination of as yet unidentified target proteins. Of particular interest is the observation that hUIP5 is found exclusively in proteins involved in the ubiquitin/proteasome pathway. The most prominent example is UFD2 of Saccharomyces cerevisiae (13) and which was recently shown to act as a so-called E4 enzyme, which is required for multiquitination of ubiquitin fusion proteins, a process necessary for efficient degradation of such proteins by the proteasome (14). In yeast, UFD2 is needed for cell survival under stress conditions, suggesting that it mediates degradation of stress-induced aberrant proteins. The UFD2 homolog in Dictyostelium discoideum, NOSA, is required for normal differentiation as disruption of the nosA gene causes developmental arrest at the aggregate stage (37). Another example for an U-box protein is CHIP, which negatively regulates chaperone activity (38). Our observation that U-box domains can bind to E2 enzymes supports the suggestion that CHIP mediates interactions between the chaperone and the ubiquitin/proteasome system.

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teins like PLZF (39). Very little is known about the physiological role of nuclear bodies. Some indirect evidence suggests that they might be involved in various aspects of transcriptional regulation and are targets of viral infection (reviewed in Ref. 29). In view of the recently described observation that ubiquitin-mediated proteolysis can be restricted to a certain cellular compartment (36), one could speculate that UIP5 is needed specifically for the ubiquitination and degradation of regulatory proteins in the nucleus. Experiments are under way to test this hypothesis.

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