Cloning of Human Ubiquitin-conjugating Enzymes UbcH6 and UbcH7 (E2-F1) and Characterization of Their Interaction with E6-AP and RSP5*

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E6-AP, a 100-kDa cellular protein, was originally identified through its interaction with the E6 protein of the oncogenic human papillomavirus types 16 and 18. The complex of E6-AP and E6 specifically interacts with p53 and mediates ubiquitination of p53 in concert with the E1 ubiquitin-activating enzyme and the E2 ubiquitin-conjugating enzyme UbcH5. Recent results suggest that E6-AP is representative of a family of putative ubiquitin-protein ligases. Members of this family are characterized by a conserved C-terminal region, termed hect domain. In this paper, we describe the isolation of two human E2s, designated as UbcH6 and UbcH7, that in addition to UbcH5 can interact with E6-AP. UbcH6 is a novel member of an evolutionally conserved subfamily of E2s that includes UbcH5 and Saccharomyces cerevisiae UBC4. Although UbcH7 does not appear to be a member of this subfamily, UbcH7 efficiently substitutes for UbcH5 in E6-AP-dependent ubiquitination. Surprisingly, UbcH6 was only weakly active in this particular assay. In addition, UbcH5 but not UbcH6 or UbcH7 efficiently interacts with the hect protein RSP5. These results indicate that E6-AP can interact with at least two species of E2 and that different hect proteins may interact with different E2s.

The ubiquitin system represents a major pathway involved in selective protein degradation (1–4). This pathway first requires the covalent attachment of ubiquitin, a highly conserved 76-amino acid protein, to defined lysine residues of substrate proteins. Ubiquitin-protein conjugates are then recognized and degraded by a specific protease complex, the 26 S proteasome. Protein ubiquitination involves three classes of enzymes. These are the ubiquitin-activating enzyme E1, the ubiquitin-conjugating enzymes E2, and the ubiquitin-protein ligases E3. Ubiquitin is first activated by E1 via formation of a thioester bond between the carboxyl-terminal glycine of ubiquitin and a cysteine residue of E1. The activated ubiquitin is then transferred to one of a number of E2s preserving the high energy thioester bond. The E2s have then been thought to catalyze the final attachment of ubiquitin to a substrate protein, often in concert with E3s. E3s have been proposed to function by specifically binding to substrate proteins that are otherwise not recognized by E2s. Recent results, however, suggest that at least some E3s may also be directly involved in the final transfer of ubiquitin to a substrate protein (5).

Only two genes encoding proteins with E3 activity have been cloned so far. These are UBR1 of Saccharomyces cerevisiae (6) and human E6-AP (7). E6-AP was originally identified through its interaction with the E6 oncoprotein of the cancer-associated human papillomavirus types 16 and 18 (8). The E6-E6-AP complex specifically binds to the tumor suppressor protein p53 and induces its ubiquitination and subsequent degradation (7, 9, 10). An essential intermediate step in E6-AP-dependent protein ubiquitination is the formation of a thioester complex between ubiquitin and E6-AP (5). Furthermore, the direction of ubiquitin transfer is from E1 to E2 and then from E2 to E6-AP. This suggests that in this particular system, the E3 catalyzes the final attachment of ubiquitin to a substrate protein, rather than the E2 as previously assumed. The cysteine residue of E6-AP involved in thioester formation has been mapped to the carboxyl terminus. The carboxyl-terminal regions of several proteins from different organisms show significant similarity to the carboxyl terminus of E6-AP (5, 11). The cysteine residue necessary for thioester formation of E6-AP with ubiquitin is conserved among all of these proteins. Because of this similarity these proteins have been termed hect proteins, for homologous to E6-AP C Terminus (11). Although their function is presently unknown, an intriguing possibility is that these proteins have E3 activity, similar to E6-AP. In support of this hypothesis, it has been shown that two of the hect proteins, namely S. cerevisiae RSP5 and a rat 100-kDa protein, can form thioester complexes with ubiquitin (11).

Identification of all the components necessary and sufficient for E6-AP-induced ubiquitination of p53 revealed that E6-AP interacts only with a distinct E2 activity (10). This E2 activity is represented by members of an evolutionally conserved subgroup of E2s that includes human UbcH5, S. cerevisiae UBC4 and UBC5, and Arabidopsis thaliana UBC8 (12–14). Recently, another mammalian E2 activity was described that appeared to be involved in E6-E6-AP-dependent ubiquitination of p53 (15, 16). This E2, termed E2-F1, is different from UbcH5, as shown by comparison of the partial amino acid sequence of proteolytic fragments of biochemically purified E2-F1 and the sequence of UbcH5. This indicated that there may be more than one species of E2 that can interact with E6-AP. Here we report the isolation of two human cDNAs encoding E2s. One of these, termed UbcH6, represents a novel
and sequenced. Using a 32P-labeled fragment comprising nucleotides 1 to 21 of the respective open reading frame, an Ndel site at the 5′-end. The PCR products were cloned into pET-3a and sequenced. Underlined (15). Amino acids are presented in capital letters. The previously reported peptide sequences derived from direct protein sequencing of biochemically purified E2-F1, now referred to as UbcH7, are underlined (3), and amino acids are given in the single-letter code.

![Fig. 1. Complete nucleotide and amino acid sequence of UbcH6 and UbcH7](image)

The complete nucleotide and amino acid sequences of UbcH6 and UbcH7 are presented. UbcH6 and UbcH7 are members of the subfamily of E2s mentioned above. The amino acid sequence of the other E2, termed UbcH7, includes the previously reported sequences of the proteolytic fragments of E2-F1, indicating that UbcH7 is identical to E2-F1. In E6-AP-dependent ubiquitination, UbcH7 is as active as UbcH5, whereas UbcH6 shows only little activity. However, only UbcH5 interacts efficiently with RSP5. This indicates that E6-AP can interact with different species of E2s and that different het proteins may interact with different E2s.

**EXPERIMENTAL PROCEDURES**

**cDNA Cloning—Cytoplasmic RNA was prepared from the human papillomavirus-negative cell line C-33A, and from the human papillomavirus-positive cell line HeLa by standard procedures (17). Source and maintenance of the cell lines has been described previously (18).**

For cloning of UbcH6, two degenerate primers that were used previously to clone a cDNA encoding UbcH5 (12) were used for reverse transcription followed by PCR amplification. Reactions were performed using the RNA PCR kit from Cetus. PCR products were cloned into pGEM-1. The sequence of several clones derived from independent PCR reactions was determined using T7 DNA polymerase (Sequenase, U.S. Biochemical Corp.). The 5′-end and the 3′-end of the open reading frame were determined as described previously (10). Preparation of E6-AP and RSP5C6 from Si9 cells infected with a recombinant baculovirus expressing E6-AP and RSP5C6, respectively, has been described elsewhere (10, 11, 21).

**RESULTS**

Cloning of UbcH6—Human UbcH5 has been cloned by PCR using degenerate oligonucleotide primers corresponding to highly conserved regions of S. cerevisiae UBC4 and A. thaliana UBC8 (12). S. cerevisiae as well as A. thaliana encode for several E2s, which are highly related to UBC4 and UBC8 at the amino acid sequence level (3, 14). To test whether human cells also encode a UbcH5-like subfamily of E2s, cDNA was synthesized from cytoplasmic RNA and was amplified by PCR using the same set of primers. This approach yielded a 325-basepair PCR product.

Cloning and Characterization of UbcH6 and UbcH7

UbcH6

| Amino Acid Sequence |
|---------------------|
| PHE LYS THR PHE SER |
| LYS PHE SER LEU LYS |
| LEU LYS PHE SER LEU |
| LEU LYS PHE SER LEU |

UbcH7

| Amino Acid Sequence |
|---------------------|
| LEU LYS PHE SER LEU |
| LEU LYS PHE SER LEU |
| LEU LYS PHE SER LEU |
| LEU LYS PHE SER LEU |

Cloning and sequencing of this product revealed that two classes of cDNAs were isolated. The DNA sequence of one class of cDNAs was identical to the sequence of UbcH5 (data not shown). The other class of DNA sequences encoded an amino acid sequence similar to amino acids 52–140 of UbcH5 (for numbering see Fig. 2), but not identical (62% identity). To obtain the sequence of the complete open reading frame other
PCR approaches, 5'-RACE and 3'-RACE, were used (22). This resulted in the specific accumulation of PCR products encoding portions of the 5'-region as well as of the 3'-region of the E2 open reading frame. The cDNA sequence shown in Fig. 1 (UbcH6) contains only those sequences that were present in multiple clones from independent PCRs. It encodes a protein of 193 amino acids with a predicted molecular mass of 21.3 kDa. The E2 was termed UbcH6 because, to our knowledge, at the time of isolation this was the sixth E2 isolated from human cells (12, 23–27). It should be noted, however, that it is not clear whether the methionine codon at position 1 represents the initiating codon since a stop codon located upstream of the methionine codon could not be found in the 5'-RACE PCR products. In addition, cDNA library screening has not yet resulted in the isolation of a clone that expands in the 5'-direction of this putative E2.

Sequence comparison suggests that UbcH6 is related to the E2 subfamily including UbcH5 and S. cerevisiae UBC4 (Fig. 2). However, UbcH6 appears to be unique among these E2s since it contains an amino-terminal extension of approximately 40 amino acids (see Fig. 1). The functional significance of this extension is presently unknown.

Cloning of UbcH7 (E2-F1)—To obtain a cDNA that encodes for E2-F1, two degenerate oligonucleotide primers were designed based on two peptide sequences derived from direct protein sequencing of biochemically purified E2-F1 (15). The primers were used to amplify cDNAs synthesized from cytoplasmic RNA isolated from human cells. A 162-base pair product was isolated, cloned, and sequenced. The DNA sequence was identical in all clones and encoded an amino acid sequence containing the sequence of a third peptide derived from E2-F1 (see Fig. 1). Since the sequence of the third peptide was not encoded by the primers used, this strongly suggests that the cDNA cloned indeed corresponds to E2-F1. Using the 162-base pair PCR fragment as a probe, a cDNA clone encoding the complete open reading frame was isolated from a cDNA library made from primary human keratinocytes (Fig. 1, UbcH7). The cDNA contains an open reading frame that encodes 154 amino acids with a predicted molecular mass of 17 kDa. Since there is a stop codon located upstream of the putative start codon (data not shown) the initiating methionine is presumably present within this cDNA. In keeping with the nomenclature introduced above, this E2, which most likely is identical to E2-F1, was termed UbcH7.

Cloning and Characterization of UbcH6 and UbcH7

![Figure 2: Comparison of amino acid sequences of UbcH6 and UbcH7 with other E2s.](image)

![Figure 3: Thioester adduct formation of bacterially expressed UbcH6 and UbcH7 with ubiquitin.](image)
UbcH5 with respect to the ability to mediate E6-AP-dependent ubiquitination.

UbcH7 But Not UbcH6 Can Mediate E6-AP-dependent Ubiquitination In Vitro—A common feature of all E2s characterized to date is the ability to form thioester adducts with ubiquitin in the presence of E1 (3, 28–30). To demonstrate that UbcH6 and UbcH7 are indeed E2s, the respective cDNAs were cloned into the presence of E1(3,28–30). To demonstrate that UbcH6 and UbcH7 are indeed E2s, the respective cDNAs were cloned into the pET-3a vector and the proteins were expressed in E. coli. The proteins could form thioester complexes with ubiquitin, demonstrating that UbcH6 as well as UbcH7 have the properties of an E2.

To test whether UbcH7 may indeed represent the E2 activity described by Ciechanover and colleagues (15, 16), its ability to mediate the E6-AP-dependent ubiquitination of an E6-E7 fusion protein or of p53 was assayed. As a negative control A. thaliana UBC1 was used which was previously shown to be inactive in E6-AP-dependent ubiquitination (10). In the presence of E6-AP, UbcH7 as well as UbcH5 could efficiently mediate ubiquitination of the E6-E7 fusion protein (Fig. 4) as well as of p53 (data not shown), with UbcH5 being slightly more active than UbcH7 under the conditions used. This strongly suggests that UbcH7 represents the E2 activity described previously.

Although UbcH6 appears to be more similar to UbcH5 than UbcH7 on the amino acid sequence level, UbcH6 was only weakly active in this particular assay (Fig. 4). A possible explanation for this observation was that the amino-terminal extension of UbcH6, which distinguishes it from UbcH5 and UbcH5-related proteins of other organisms, negatively interferes with the activity of UbcH6. However, a deletion mutant of UbcH6, which initiates at the second methionine and therefore lacks the amino-terminal extension, was as inefficient in ubiquitination of the E6-E7 fusion protein as the full-length UbcH6 (data not shown).

UbcH6 and UbcH7 Interact Differentially with E6-AP and RSP5—An essential intermediate step in E6-AP-dependent protein ubiquitination is the formation of thioester adducts of E6-AP with ubiquitin in the presence of E1 and a specific E2 (5). Therefore a possible explanation for the inability of UbcH6 to efficiently support E6-AP-dependent ubiquitination is that UbcH6 may be impaired in its ability to transfer an activated ubiquitin molecule to E6-AP. Alternatively, besides transferring ubiquitin to E6-AP, UbcH5 and UbcH7 may play an active role in the final attachment of ubiquitin to the E6-E7 fusion protein, a function that may not be exerted by UbcH6. To distinguish between these possibilities, thioester formation of E6-AP with ubiquitin was assayed in the presence of the different E2s as indicated as well as the running positions of molecular size markers.

shown in Fig. 5, UbcH5 and UbcH7 could transfer ubiquitin to E6-AP with a similar efficiency. In the presence of UbcH6, significantly less ubiquitin thioester complexes of E6-AP could be detected, indicating that UbcH6 is not capable to efficiently transfer ubiquitin to E6-AP.

Recently it was reported that UbcH5 can also interact with RSP5, a member of the huct family of proteins, as measured by UbcH5-dependent formation of ubiquitin thioester adducts of a deletion mutant of RSP5 lacking the carboxyl-terminal 6 amino acids (RSP5ΔC6). It was therefore tested whether UbcH7 or UbcH6, or both, could substitute for UbcH5 in this reaction (Fig. 6). In contrast to the experiments with E6-AP, both UbcH6 and UbcH7 were significantly less active than UbcH5, as judged by the appearance of thioester complexes of RSP5ΔC6 with ubiquitin.

DISCUSSION

The ubiquitin system plays a major role in selective protein degradation (1–4). Selective protein degradation requires the specific targeting of many different proteins, often at particular
Fig. 6. Ubiquitin thioester adduct formation of RSP5ΔC6. A deletion mutant of RSP5 lacking the carboxyl-terminal 6 amino acids (RSP5ΔC6) was expressed in the baculovirus system as described previously (11). Thioester complex formation of RSP5ΔC6 with ubiquitin was assayed as described in Fig. 5. A, reactions were terminated in the absence of dithiothreitol; B, reactions were terminated in the presence of 100 mM dithiothreitol. Running positions of the ubiquitin thioester adducts of the different E2s and of RSP5ΔC6, respectively, are indicated as well the running positions of molecular size markers.

Stages of the cell cycle or differentiation. The selectivity of the ubiquitin system appears to be mediated by E2s, often in conjunction with E3s. It was previously shown that E6-AP and RSP5, two members of the hect family of putative E3 proteins, interact specifically with a subgroup of human E2s represented by UbcH5 (11, 12). We have now cloned two additional human E2s, designated UbcH6 and UbcH7, that can interact with E6-AP and RSP5. However, whereas RSP5 interacts only weakly with UbcH6 and UbcH7 compared with UbcH5, UbcH7 is as active as UbcH5 in E6-AP-dependent ubiquitination. This indicates that different hect proteins may require different E2 activities for protein ubiquitination. The differential interaction between E3s and E2s may further contribute to ensure the high specificity of protein ubiquitination.

UbcH6 was cloned by PCR using degenerate oligonucleotide primers that correspond to highly conserved regions of a subfamily of E2s, which includes UbcH5 and S. cerevisiae UBC4 and UBC5 (11, 13). Accordingly, amino acid sequence comparison with other E2s revealed that UbcH6 has the highest similarity to the members of this subfamily. Nevertheless, UbcH6 appears to be a unique member of this subfamily in that it contains an amino-terminal extension of approximately 40 amino acids, in contrast to all other members of the subfamily described to date (11, 13, 14, 31, 32). Despite the similarity, UbcH6 could not efficiently substitute for UbcH5 or S. cerevisiae UBC4 in E6-AP-dependent protein ubiquitination. It seemed possible that the amino-terminal extension masks a domain of UbcH6 necessary for interaction with E6-AP or induces a conformation of UbcH6, which is not properly recognized by E6-AP. However, a deletion mutant of UbcH6 lacking the amino-terminal extension was not more efficient in E6-AP-dependent ubiquitination than the full-length protein. The amino-terminal extension contains an unusual high number of serines. Therefore, it is conceivable that modification of the amino-terminal extension, such as phosphorylation, may result in a form of UbcH6 that, like UbcH5, can efficiently interact with E6-AP. Alternatively, UbcH6 and UbcH5 may interact with a different set of E3s. In support of the latter hypothesis is the recent observation by S. Jentsch and co-workers that a murine E2, which is almost identical to UbcH6 at the amino acid sequence level, could only partially substitute for S. cerevisiae UBC4 and UBC5 in genetic experiments.

UbcH7 was cloned based on the published sequences of proteolytic fragments derived from a chromatographically purified E2 activity termed E2-F1 (15). Similar to UbcH5, E2-F1 was reported to function in E6-AP-dependent ubiquitination (16). Since UbcH5 and E2-F1 have similar chromatographic properties,3 however, it seemed possible that the E2-F1 preparation used was contaminated by UbcH5. This possibility can now be excluded since bacterially expressed UbcH7 was indeed functional in E6-AP-dependent ubiquitination. Amino acid sequence comparison of UbcH7 with UbcH5 suggests that UbcH7 is not more related to UbcH5 than E2s, which are inactive in E6-AP-dependent ubiquitination. This indicates that E6-AP can interact with different species of E2s. Whether there are additional E2s that may interact with E6-AP is not clear at present. Such E2s, however, should have chromatographic properties similar to UbcH5 and UbcH7 because only fractions obtained by cation-exchange chromatography of cellular extracts that contain UbcH5 and UbcH7 can support E6-AP-dependent ubiquitination (10).5 An intriguing but purely speculative hypothesis is that in the presence of UbcH7, E6-AP has an altered substrate specificity from that in the presence of UbcH5. However, although E6-AP interacts with both UbcH5 and UbcH7 in vitro, further experiments will be necessary to determine whether these interactions also exist in vivo.

Similar amounts of thioester complexes of E6-AP and ubiquitin were detected in the presence of UbcH5 and UbcH7, respectively. In contrast, transfer of ubiquitin to RSP5 was much less efficient in the presence of UbcH7 than in the presence of UbcH5. The reason for this apparent difference between RSP5 and E6-AP is not known. As mentioned above, UbcH7 is apparently not a member of the UbcH5-like subfamily of E2s. Nevertheless it seems likely that the regions (or structures) of UbcH5 and UbcH7 that are necessary for interaction with E6-AP and RSP5 are similar to each other. Construction of deletion and point mutants as well as of chimeric proteins of UbcH5 and UbcH7 or UbcH6, respectively, should allow definition of those regions.

Both E6-AP and RSP5 are members of the family of hect proteins, which are putative E3s (11). E3s are presumably

2 S. Jentsch, personal communication.

3 M. Scheffner, unpublished.
involved in mediating the substrate selectivity of protein ubiquitination, indicating that there may be a large number of proteins with E3-like activities. Recent database searches suggest that human cells encode for 10 or more E3-like proteins. Assuming that all of these proteins interact with the ubiquitin system, it will be interesting to see whether these proteins in general share the property of E6-AP and RSP5 to interact with members of the UbcH5-like subfamily of E2s or whether some of these may interact with other E2s. Characterization of these proteins and their interplay with E2s should contribute to define the precise role of E2s and E3s in mediating substrate recognition in E3-dependent protein ubiquitination.

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REFERENCES
1. Finley, D., and Chau, V. (1991) Annu. Rev. Cell Biol. 7, 25–69
2. Hershko, A., and Ciechanover, A. (1992) Annu. Rev. Biochem. 61, 761–807
3. Jentsch, S. (1992) Annu. Rev. Genet. 26, 177–205
4. Hochstrasser, M. (1995) Curr. Opin. Cell Biol. 7, 215–223
5. Scheffner, M., Nuber, U., and Huibregtse, J. M. (1995) Nature 373, 81–83
6. Bartel, B., Wünning, I., and Varshavsky, A. (1990) EMBO J. 9, 3179–3189
7. Huibregtse, J. M., Scheffner, M., and Howley, P. M. (1993) Mol. Cell. Biol. 13, 775–784
8. Huibregtse, J. M., Scheffner, M., and Howley, P. M. (1991) EMBO J. 13, 4129–4139
9. Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J., and Howley, P. M. (1990) Cell 63, 1129–1136
10. Scheffner, M., Huibregtse, J. M., Vierstra, R. D., and Howley, P. M. (1993) Cell 75, 495–505
11. Huibregtse, J. M., Scheffner, M., Beaudenon, S., and Howley, P. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2563–2567
12. Scheffner, M., Huibregtse, J. M., and Howley, P. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8797–8801
13. Seufert, W., and Jentsch, S. (1990) EMBO J. 9, 543–550
14. Girod, P.-A., Carpenter, T. P., van Nocker, S., Sullivan, M. L., and Vierstra, R. D. (1993) Plant J. 3, 545–552
15. Blumenfeld, N., Gonen, H., Mayer, A., Smith, C. E., Siegel, N. R., Schwartz, A. L., and Ciechanover, A. (1994) J. Biol. Chem. 269, 9574–9581
16. Ciechanover, A., Shkedy, D., Oren, M., and Bercovitch, B. (1994) J. Biol. Chem. 269, 9582–9589
17. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Scheffner, M., Münger, K., Byrne, J. C., and Howley, P. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5523–5527
19. Hatfield, P. M., and Vierstra, R. D. (1992) J. Biol. Chem. 267, 14799–14803
20. Sullivan, M. L., and Vierstra, R. D. (1991) J. Biol. Chem. 266, 23878–23885
21. Huibregtse, J. M., Scheffner, M., and Howley, P. M. (1993) Mol. Cell. Biol. 13, 4918–4927
22. Frohman, M. A., Dush, M. K., and Martin, G. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8998–9002
23. Schneider, R., Eckerskorn, C., Lottspeich, F., and Schweiger, M. (1990) EMBO J. 9, 1431–1435
24. Koken, M. H., Reynolds, P., Jakowics-Dekker, I., Prakash, L., Prakash, S., Bootsma, D., and Hoeijmakers, J. H. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8965–8969
25. Liu, Z., Diaz, L. A., Haas, A. L., and Giudice, G. J. (1992) J. Biol. Chem. 267, 15829–15833
26. Pion, S. E., Leppig, K. A., Do, H.-N., and Groudine, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10484–10488
27. Kaiser, P., Seufert, W., Höfferer, L., Köfler, B., Sachsenmaier, C., Herzog, H., Jentsch, S., Schweiger, M., and Schneider, R. (1994) J. Biol. Chem. 269, 8777–8802
28. Haas, A. L., Warm, J. V. B., Hershko, A., and Rose, I. A. (1982) J. Biol. Chem. 257, 2543–2548
29. Pickart, C. M., and Rose, I. A. (1985) J. Biol. Chem. 260, 1573–1581
30. Haas, A. L., and Bright, P. M. (1988) J. Biol. Chem. 263, 13258–13267
31. Treier, M., Seufert, W., and Jentsch, S. (1992) EMBO J. 11, 367–372
32. Zhen, M., Hantelin, R., Jönes, D., Jentsch, S., and Candido, E. P. M. (1993) Mol. Cell. Biol. 13, 1717–1377