Ubiquitin-conjugating enzymes catalyze the covalent attachment of ubiquitin to cellular substrates. Here we describe the isolation of a novel ubiquitin-conjugating enzyme from human placenta and the cloning of the corresponding cDNA. DNA sequencing revealed that this gene, UbcH2, encodes a protein with significant sequence similarity to yeast Ubc8. In contrast to a previous report (Qin, S., Nakajima, B., Nomura, M., and Arfin, S. M. (1991) J. Biol. Chem. 266, 15549-15554), we discovered that Ubc8 is interrupted by a single intron bearing an unusual branch point sequence. The revised amino acid sequence of yeast Ubc8 exhibits 54% amino acid sequence identity to human UbcH2. Moreover, full-length UbcH2 and Ubc8 enzymes expressed from their cDNAs show similar enzymatic activities in vitro by catalyzing the ubiquitination of histones, suggesting that the two enzymes may fulfill similar functions in vivo. Interestingly, comparison of the enzymatic activities of a truncated Ubc8 (Qin, S., Nakajima, B., Nomura, M., and Arfin, S. M. (1991) J. Biol. Chem. 266, 15549-15554) and of the full-length enzyme (this report) suggests, that the first 12 amino-terminal residues of Ubc8 are required for ubiquitination of histones in vitro but not for thiolester formation with ubiquitin. This suggests that the NH2 terminus of Ubc8 may be necessary either for substrate recognition or for the transfer of ubiquitin onto substrates. The UbcH2 gene is located on chromosome 7 and shows a complex expression pattern with at least five different mRNAs.

A major pathway for protein degradation in eukaryotes is ubiquitin-dependent. Substrate specific ubiquitin-conjugating enzymes (E2 enzymes) and accessory factors recognize specific signals on proteolytic substrates and attach ubiquitin, a small and highly conserved protein, to defined lysine residues of substrate proteins. Ubiquitin-protein conjugates are then degraded by the 26S protease. Ubiquitin conjugation is highly specific and is required for a surprising variety of cellular functions. Genetic studies in yeast showed that ubiquitin-conjugating enzymes are required for DNA repair, sporulation, repression of retrotransposition, cell cycle progression, heat shock resistance, cadmium tolerance, and peroxisome biogenesis (1-3).

Several in vivo substrates of the ubiquitin system have been identified, including histones (4-6), actin (7), cell surface receptors (8-11), the MATa2 transcriptional repressor (12), the Mos kinase (13), and cyclins (15). Substrate selectivity by the ubiquitin-conjugating system is thought to be mediated by the recognition of degradation signals on proteolytic substrates by ubiquitin-conjugating enzymes or accessory substrate recognition proteins known as E3s. Previous studies indicated that conjugation of ubiquitin to the model substrate histone in vitro does not depend on an E3 protein. Studies with the yeast enzymes UBC2/RAD6 (16) and UBC3/CDC34 showed that the highly acidic carboxyl (COOH)-terminus of the two enzymes is required for this in vitro activity (17, 18).

In this report we describe the isolation and characterization of a human ubiquitin-conjugating enzyme. cDNA cloning revealed that this enzyme, UbcH2, is structurally homologous to the yeast Ubc8 enzyme (19). Both UbcH2 and Ubc8 have COOH-terminal extensions enriched in acidic residues, and we show that both enzymes are capable of conjugating ubiquitin to histones in vitro. Interestingly, we found that amino-terminal sequences of Ubc8 appear to be required for ubiquitin-histone conjugation but not for ubiquitin thiolester formation.

EXPERIMENTAL PROCEDURES

Cloning of the UBC8 Gene and cDNA—The UBC8 gene was isolated from a yeast genomic DNA library in EMBL3A using a radiolabeled UBC8 probe (plasmid kindly provided by S. Arfin). The DNA sequence of a 562-base pair EcoRI-SphI fragment carrying the 5' portion of the UBC8 gene was determined after subcloning into M13mp18/19 vectors. UBC8 cDNA was synthesized with M-MuLV reverse transcriptase (New England Biolabs, Beverly, MA) under conditions recommended by the supplier after annealing the antisense primer W53 (5'-GGACGCTTCTTATCAAGG-3') to total yeast RNA (50 μg). A UBC8 cDNA clone was amplified by PCR1 with primers W52 (5'-GGAATAT-TGGAAGAAAGGAGCG-3') and W53, rendered blunt end, and cloned in the Smal site of M13mp18 for sequencing.

Protein Purification and Peptide Sequencing—Components of the ubiquitin-conjugating system were purified from human term placenta by covalent affinity chromatography on an ubiquitin-Sepharose column, and peptide sequencing was done as described (20).

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) Z229328-Z229331.

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cDNA Cloning and Sequencing—Plasmids were isolated as described (21). Cloning, screening, and PCR methods were according to standard protocols (22).

We obtained the following peptide sequence by protein sequence analysis of the purified human UbcH2: PEIGEGTYPGE. The underlined residues were designed to optimize the oligonucleotide probe used for cloning [25]. Expression and purification of the recombinant fusion proteins completed after 2 h at room temperature.

...[Partial text is redacted for brevity]...

UbcH2 or recombinant yeast UBC8, 100 ng of affinity purified human placental El, 1 pg of biotinylated ubiquitin, and in case of histone ubiquitination 2 pg of histone H2A (Boehringer Mannheim) were incubated for at least 2 h with alkaline phosphatase conjugated to streptavidin (Boehringer Mannheim) diluted 1:5000 in TBS. Unbound streptavidin was removed during incubation with TBS/milk powder for 1 h. After a rinse in phosphate-buffered saline, pH 7.5 (137 mM NaCl, 2 mM KCI, 4.6 mM Na,HP04, 1.5 mM NaH,HPO,). After 4 h of labeling at room temperature, the reaction mixture was dialyzed overnight against phosphate-buffered saline, pH 7.5 (137 mM NaCl, 2 mM KCI, 4.6 mM Na,HP04, 1.5 mM NaH,HPO,). After 4 h of labeling at room temperature, the reaction mixture was dialyzed against TBS/milk powder. A partial cDNA clone with striking similarity to the wheat germ E223p (23) was isolated. Further screening using this cDNA as a radiolabeled probe succeeded in the isolation of clone 23k

...[Partial text is redacted for brevity]...
UbhcH2 is Structurally Homologous to Yeast UBC8—The open reading frame of UbhcH2 predicts a protein of 183 amino acids with a molecular mass of 20.6 kDa. The difference between the calculated size of UbhcH2 and the size estimated by its migration in SDS gels may be caused by an abnormal running behavior in gels due to the high proline content of the protein. Comparison of the deduced amino acid sequence of UbhcH2 with sequences in current data bases revealed extensive sequence similarities to all published ubiquitin-conjugating enzymes, but the strongest similarity was with the yeast UBC8 (19) and Calmodulin. The reason for the alleged discrepancy became apparent when we inspected the published nucleotide sequence of UBC8 revealing an out-of-frame ATG codon only two bases upstream of the initiator codon. Isolation of the predicted UBC8 sequence shows a good match with the amino acid sequence of yeast UBC8 (19). In contrast to the yeast UBC8 reaction in vitro, we noticed the appearance of two closely spaced bands in the range of 30 kDa that were both sensitive to β-mercaptoethanol as predicted for a thiolester complex of UbhcH2 and ubiquitin. Using recombinant UbhcH2 in these assays, we noticed the appearance of two closely spaced bands in the range of 30 kDa that were both sensitive to β-mercaptoethanol as predicted for a thiolester complex of UbhcH2 and ubiquitin. Using recombinant UbhcH2 in these assays, we noticed the appearance of two closely spaced bands in the range of 30 kDa that were both sensitive to β-mercaptoethanol as predicted for a thiolester complex of UbhcH2 and ubiquitin.
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**FIG. 4.** Comparison of deduced amino acid sequences of *Saccharomyces cerevisiae* UBC8, wheat germ E2, and human UbcH2. The unique cysteine residues are shadowed, and the identical residues of the novel homologous NH₂-terminal part of yeast UBC8 are highlighted in black. Amino acids that are identical or chemically similar (D = E, R = Y, I = L = V = M) are boxed.

|                | yeast UBC8 | Wheat E2 (23kDa) | human UbcH2 |
|----------------|------------|-----------------|-------------|
|                | M S S - S  | - K A H I E T D Y M K L L M S D H Q  | 21          |
|                | M S S P S  | - K R R - E M D L H K L M M S D Y K  | 21          |
|                | M S S P S P | G K R R M D T D V V K L I E B H E  | 24          |
|                | V D L I N D | S M Q E F H V K F | 56          |
|                | V D M I N D G | M H E F F V | 56          |
|                | V T I L G - G | L N E F V | 58          |

**FIG. 5.** UbcH2-ubiquitin thiolester formation. Thiolester reactions contained purified human E1, recombinant human UbcH2, biotinylated ubiquitin, and ATP as indicated. Samples were separated on a 12.5% SDS-polyacrylamide gel, and ubiquitin conjugates were detected as described under “Experimental Procedures.” Thiolesters were identified by their lability to boiling for 5 min in the presence of 4% β-mercaptoethanol (+ indicates samples which were boiled in the presence of β-mercaptoethanol; - indicates samples without boiling). Lane 1, recombinant human UbcH2, E1, and ATP; lane 2, same as lane 1 but without ATP; lane 3, same as lane 1 but recombinant UbcH2 omitted. The arrow and the arrowhead indicate migration of E1- and UbcH2 thiolester adducts, respectively.

**DISCUSSION**

In this paper we report the cDNA cloning and characterization of a human ubiquitin-conjugating enzyme. This enzyme, UbcH2, is structurally homologous to wheat E2₂₂₃ (23) and yeast UBC8 with more than 50% amino acid identity. Moreover, these three related proteins have COOH-terminal extensions enriched in acidic residues. Comparable acidic COOH-terminal tails are found in the UBC2/RAD6 (16) and UBC3/CDC34 (18) gene products where they were shown to be involved in the recognition of basic substrates, such as histones. UBC8 is the third member of yeast ubiquitin-conjugating enzymes possessing acidic tails. The human UbcH2 is the only known human E2 showing this feature, so far.

The ability of the UbcH2 expressed in *E. coli* to catalyze thiolester formation, as well as to transfer ubiquitin to histone...
H2A and H2B in an E3-independent reaction, was tested in vitro using a novel nonradioactive assay. In contrast to published results (19), which were based on studies using a truncated UBC8, we demonstrated that the full-length yeast UBC8 also catalyzes the transfer of ubiquitin moieties to histones in vitro. Obviously, the first 12 NH2-terminal amino acids, missing in the recombinant UBC8 used by Qin et al., are important for ubiquitination of histones in vitro. The NH2-terminal amino acids are highly homologous between the human and yeast enzymes and may be involved in substrate recognition or enzyme function. The importance of the NH2 terminus of ubiquitinating enzymes was recently demonstrated for UBC8 in vitro.

Intron-containing cDNAs corresponding to the three shorter transcripts were isolated, and comparison of their primary structures revealed that in this case the NH2 terminus is important for RAD6 function in sporulation and DNA repair (32).

The yeast UBC8 gene contains an intron which separates the first five translated nucleotides from the remaining coding sequence by 123 spliced nucleotides. Within this noncoding sequence we localized a 5' and a 3' splice site and an unusual branchpoint sequence. Interestingly, the introns in UBC8 and UBC9 contain non-consensus splice or branchpoint sites. The non-consensus splice signals have been implicated in the regulation of yeast genes (34, 35).

The human UbcH2 gene shows a complex expression pattern. We detected at least five UbcH2-specific cDNAs of different size by Northern blot analysis. The length of these transcripts are between 800 and more than 5000 nucleotides. cDNAs corresponding to the three shorter transcripts were isolated, and comparison of their primary structures revealed that they are generated from a single primary transcript by the usage of alternative polyadenylation sites. The corresponding gene is located on chromosome 7, as shown by using hamster-human hybrid cells. The biological function for this complex transcription pattern might reflect post-transcriptional regulation by differing RNA stability.

Ubiquitinated histones (H2A and H2B) are the most abundant ubiquitin conjugates known in higher eukaryotes. Despite extensive studies in the past 15 years the function of this chromatin modification remains elusive. The capacity of the UBC8/Ubch2 enzymes to ubiquitinate histones in vitro raises the intriguing possibility that these enzymes may be involved in this process in vivo. Support for an important cellular function of these enzymes comes from the finding that they are remarkably conserved during evolution. Surprisingly, however, the yeast ubc8 deletion mutant does not exhibit a detectable deleterious phenotype. This may indicate that functionally overlapping enzymes may exist in yeast or that this yeast enzyme has a more specialized function. Studies addressing these possibilities are currently underway.

Acknowledgments—We are indebted to B. Auer for helpful discussion and oligonucleotide synthesis and to M. Hirsch-Kaufmann and B. Gruber for supplying HeLa cells. We thank S. Arfin (University of California) for providing DNA clones and yeast strains.

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