

Molecular Cloning and Complete cDNA Sequence of *UBH1* in Mouse Testis

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We have identified a full-length mouse *UBH1* cDNA, encoding a putative deubiquitinating enzyme, from the testis by RT-PCR using primers prepared from sequences conserved amongst deubiquitinating enzymes. Sequence analysis predicts that the *UBH1* cDNA encodes a 355 amino acid polypeptide with the molecular weight of approximately 39 kDa containing the highly conserved Cys, Asp, and His domains characteristic of the ubiquitin-specific processing proteases. Biochemical assay revealed that the mouse *UBH1* has deubiquitinating enzyme activity and sequence analysis showed 98.3% amino acid identity with human *UBH1*.

Keywords: Deubiquitination; DUB-1; *UBH1*; Ubiquitin

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A number of biological metabolisms are regulated by ubiquitination and deubiquitination. Ubiquitin (Ub), consisted of 76 amino acids, encodes an 8.5 kDa polypeptide and is found either free or covalently conjugated to cellular proteins (Varshavsky, 1997). Conjugation of ubiquitin is modulated by a series of cascade reactions involving ubiquitin-activating E1 enzymes, ubiquitin-conjugating E2 enzymes, and ubiquitin ligases (E3) (DeSalle and Pagano, 2001). These ubiquitinated proteins are recognized and degraded by the 26S proteasome (Hochstrasser, 1996). The conjugation of ubiquitin is a reversible process, which is mediated by deubiquitinating enzymes. We have recently demonstrated that conserved aspartic acid is required for deubiquitinating enzyme activity (Baek *et al.*, 2001; Lee *et al.*, 2001). Using the conserved amino acid residue and its adjacent residues, we screened putative murine deubiquitinating enzymes registered on GenBank.

We report here that the *UBH1*, identified in murine testis, encodes a deubiquitinating enzyme, showing functional enzymatic activity.

In order to screen putative deubiquitinating enzymes that contain an aspartic acid motif in mouse, databases from GenBank were accessed and searched using the BLAST algorithm (Altschul *et al.*, 1997) at the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/BLAST/>). The amino acids for the motif used for searching is QEDAHEFL (amino acids between 131–138 of DUB-1, DUB-2, and DUB-2A) (Zhu *et al.*, 1996; Zhu *et al.*, 1997; Baek *et al.*, 2001). We found a total number of 11 proteins including DUB-1, DUB-2, and DUB-2A. The *UBH1* cDNA, encoding one of these proteins, was isolated by RT-PCR from murine testis and subcloned into an expression vector, which was subjected to further analysis. We performed RT-PCR using a pair of primers designed with reference to sequences from cDNA library clone (GenBank accession number AK006739). The nucleotide sequences of these primers are following: 5'-CGCG-GATCCTCCATCTGTACC-3' and 5'-CTCGAGCGT-GGCTCCTCTCAG-3'. Total RNA isolated from mouse testis using TRIzol (Gibco BRL) served as the template for reverse transcription. The PCR amplified fragment with a predicted size was subcloned into pGEM-T Easy vector (Promega) and introduced into *Escherichia coli* DH5 α . *Escherichia coli* DH5 α harboring pGEM-*UBH1* clone was cultured to prepare supercoiled recombinant plasmid DNA using a MaxiPrep kit (Qiagen). The DNA sequences were established by automated sequencing (ABI Prism). Both strands were analyzed, confirming the fidelity of the sequence information.

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ATGGGCGCCAATGCGTCCGGCATAGAGAAAGAGATTGGTCCAGAACAGTTCCGGTCAAC 60
M G A N A S A L E K E I G P E Q F P V N
GAGCACTATTTTGGATTAGTCAATTTTGGGAATACCTGCTACTGCAACTCAGTCTTCAA 120
E H Y F G L V N F G N T C Y C N S V L Q
GCACTTTATTTTGTGTCGTCATTTCGGGAAAAAGTTCTAGCATACAAAAGT CAGCCAGAG 180
A L T F C R P F R E K V L A Y K S Q P R
AAAAGGAGAACCTTCTCACCTGCCTGGCAGACCTCTTCCACAGCATAGCCACCCAGAA G 240
K K E N L L T C L A D L F H S I A T Q K
AAGAAGGT CGGCGT GATCCCGCCCAAGAAGTTCA TCACAAGGTT GCGGAAAGAAAACGAG 300
K K V G V I P P K K F I T R L R K E N E
CTGTTTGACAACTACATGCAACAAGATGCCATGAATTCCTAAATTACCTACTCAATACA 360
L F D N Y M Q Q D A H E F L N Y L L N T
ATTGCTGATATTCTACAA GAAGAAGAA AACAGGAGAAGCAAATGGCCGATTACGGAAC 420
I A D I L Q E E R K Q E K Q N G R L R N
GGA GACGT GGACAACGAGGATAA CAACA GCACACCAGACCCAACCTGGGTCCACGAGAT C 480
G D V D N E D N N S T P D P T W V H E I
TTT CAGGGAACAT TAACT AATGAACCA GGTGTCTTACTTGTGAAACTATAAGCAGCAAAA 540
F Q G T L T N E T R C L T C E T I S S K
GATGAAGATTTTTTAGACCTTTCTGTTGACGTGGAACAAAACACATCAATTA CTCACTGC 600
D E D F L D L S V D V E Q N T S I T H C
TTAAGAGGGTT CAGT AACACAGAAACTCTGTGCA GTGAATATAAGTACTACTGTGAAGAG 660
L R G F S N T E T L C S E Y K Y Y C E E
TGCCGGAGCAAGCAGGAA GCACA CAAGCGGATGAAGGTTAAGAAACTGCCCTGATCCTG 720
C R S K G E A H K R M K V K K L P L I L
GCTCTGCACCTGAAGAGTTTAAAGTATA TGGACCAGCTT CACCGGTACA CGAAGCTTTCT 780
A L H L K R F K Y M D Q L H R Y T K L S
TATCGCGTGGTCTTTCCTTTAGAGCTCCGTCTGT TCAACACGT CAGGAGATGCGACCAAC 840
Y R V V F P L E L R L F N T S G D A T N
CCTGACAGAATGTATGACCTGGT CGCCGTTGTGGTTCACTGTGGAAGTGGTCCCAACCGA 900
P D R M Y D L V A V V V H C G S G P N R
GGCCATTATATTGCAATAGTTAAGAGTCATGATT TCTGGCTGTTGT TTGATGATGACATT 960
G H Y I A I V K S H D F W L L F D D D I
GTAGAAAAAATCGATGCCAAGCTATTGAAGAAT TCTACGGATTGACATCTGACATCTCC 1020
V E K I D A Q A I E E F Y G L T S D I S
AAGAAGT CCGAGT CTGGCTACAT CCTTTCTATCAGTCTCGGACTGA 1068
K N S E S G Y I L F Y Q S R D *

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FIGURE 1 Nucleotide and predicted amino acid sequences for the mouse *UBH1* cDNA. This sequence was screened using databases from GenBank (GenBank accession number AK006739) and confirmed with RT-PCR followed by direct sequencing.

To determine whether *UBH1* has deubiquitinating enzyme activity, *Escherichia coli* MC1061 cells containing pACYC184-*Ub-β-gal* and pGEX-4T-1-*UBH1* were grown, induced with IPTG (isopropyl-β-thiogalactopyranoside), and lysed in lysis buffer (0.01 M phosphate (pH 7.4), 8 M urea, 1% SDS, and 1% β-mercaptoethanol). The assay with these whole cell lysates, based on the cleavage of ubiquitin-β-galactosidase fusion protein, was performed by immunoblotting with a rabbit anti-β-gal antiserum (Cappel, Durham, NC), a rabbit anti-GST antiserum (Santa Cruz Biotechnology, CA), and the ECL system (Amersham, Buckinghamshire, UK).

A potentially significant gene, *UBH1*, was identified in the mouse testis using RT-PCR with primers designed based on the conserved aspartic acid and

adjacent amino acids among deubiquitinating enzymes. The complete 1068bp ORF and the deduced amino acid sequence of 355 amino acids are shown as Fig. 1. The amino acid sequence revealed that *UBH1* contains not only conserved Asp domain, but also conserved Cys and His domains (Fig. 2), suggesting that this *UBH1* is a putative deubiquitinating enzyme. The amino acid sequence is highly homologous to human *UBH1* (98.3% amino acid similarity) and a partial sequence of mouse *UBH1* has been identified (Hansen-Hagge *et al.*, 1998). As shown by immunoblot analysis, a cDNA clone encoding GST-*UBH1* fusion protein showed the cleavage of Ub-Met-β-gal similar to that observed with GST-DUB-1 (Fig. 3, lanes 2 and 3). As a control, cells transformed with pGEX vector

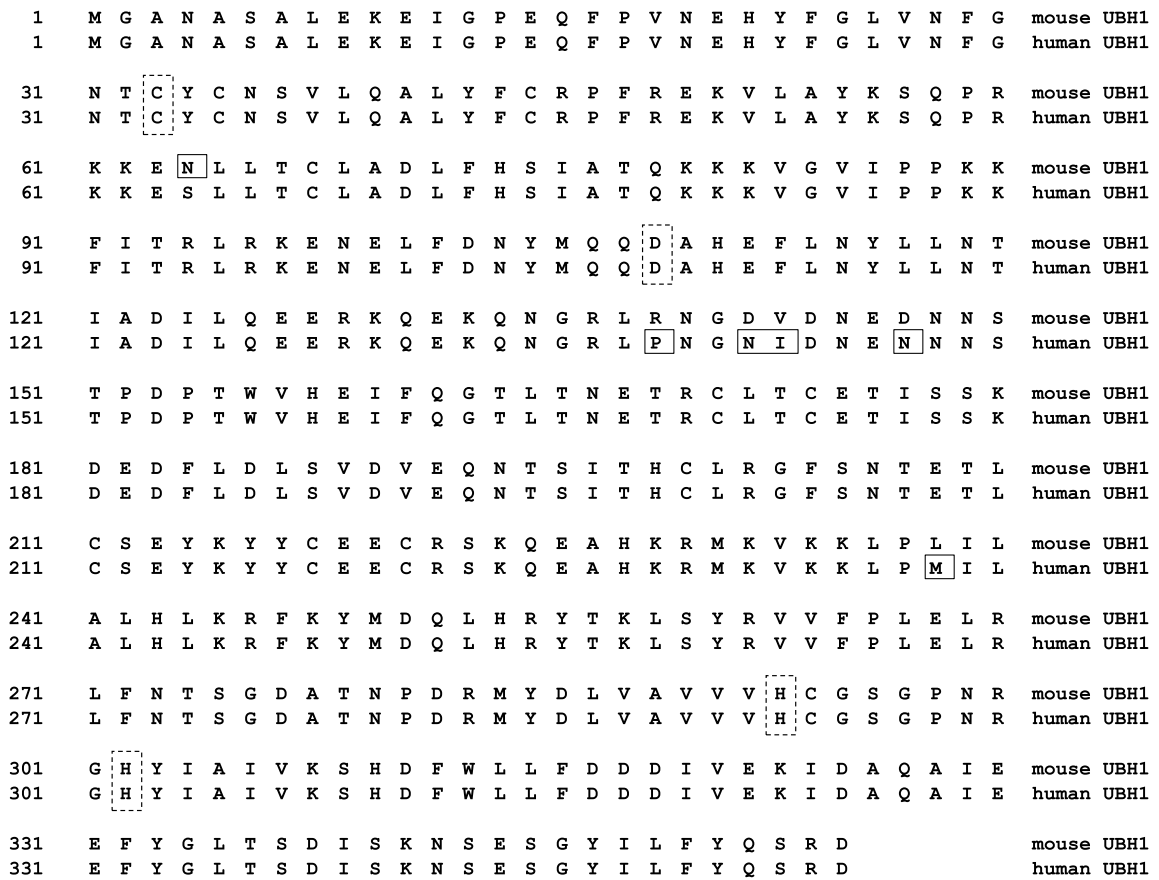


FIGURE 2 Alignment of amino acid sequences between mouse UBH1 and human UBH1 using MegAlign software (clustal method) from DNASTAR (LASERGENE). Different amino acids are blocked and conserved amino acids (Cys, Asp, and 2 His) are in dotted boxes.

failed to cleave Ub-Met-β-gal. Therefore, these results demonstrate that UBH1 has deubiquitinating enzyme activity. An anti-GST immunoblot confirmed that the GST-DUB-1 and UBH1 proteins synthesized

at comparable levels (Fig. 3, lower blot). In the present study, we have isolated the murine *UBH1* cDNA from adult male testis and the predicted protein revealed conserved domains (Cys, Asp, and

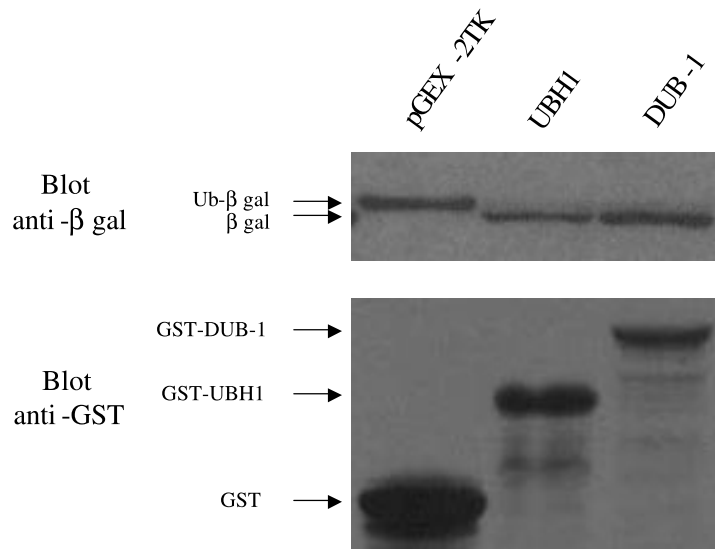


FIGURE 3 UBH1 is a functional deubiquitinating enzyme. A, the deubiquitination of the Ub-Met-β-gal fusion protein by GST-DUB proteins coexpressed in *E. coli*. Immunoblot analysis was performed with anti-β-gal antiserum. Coexpressed plasmids were pGEX-4T-1 (lane 1, negative control); pGEX-UBH1 (lane 2); and pGEX-DUB-1 (lane 3). B, GST-DUB fusion proteins were analyzed by an immunoblot with an anti-GST monoclonal antibody (Santa Cruz Biotechnology).

His) and the deubiquitinating activity. The nucleotide sequence of mouse *UBH1* has been submitted to the GenBank nucleotide databases under the accession number AF441835. While the biological function of the *UBH1* cDNA remains unknown, the isolation of the full-length *UBH1* cDNA should facilitate investigations of cellular functions in mammalian systems. In addition, finding its substrates and the molecular mechanism of deubiquitination by this enzyme will clarify to understand its biological roles.

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