

Linxia Song · Shouyi Chen · Xinbing Yu
Zhongdao Wu · Jin Xu · Guang Yang · Nancai Zheng
Xuchu Hu · Lingchen Guo · Jianfeng Dai · Jian Xu
Chaoneng Ji · Shaohua Gu · Kang Ying

Molecular cloning and characterization of cDNA encoding a ubiquitin-conjugating enzyme from *Clonorchis sinensis*

Received: 6 July 2004 / Accepted: 3 August 2004 / Published online: 1 September 2004
© Springer-Verlag 2004

Abstract The ubiquitin–proteasome system is an essential mechanism for protein degradation in eukaryotes. Protein ubiquitination is composed of a series of enzymatic reactions. The ubiquitin-conjugating enzyme (E2) is one of the important enzymes involved in the process. A cDNA encoding an E2 enzyme was cloned from a *Clonorchis sinensis* cDNA library by large-scale sequencing. This new cDNA contains 862 bp with a putative open reading frame of 156 amino acids. The deduced amino acid sequence is 77% identical to the human E2, HHR6A and HHR6B. The coding region of this cDNA was expressed in *E. coli* as a GST-tagged protein, and was purified to electrophoretic homogeneity. Enzymatic assays showed that this E2 had the capacity to form a thiolester linkage, and could conjugate ubiquitin to histone H2A in an E3-independent manner in vitro, which indicated that the expressed protein was functionally active. The nucleotide sequence reported in this paper has been submitted to the Genbank Database with accession number AY632078.

Introduction

Clonorchis sinensis (Cs) is one of the most important trematode parasites that cause human clonorchiasis in China, Korea, Japan and Southeast Asia. The *C. sinensis* parasitizes in the biliary passages of humans, and is capable of destroying the biliary and hepatic tissues (Rim 1986). There are about 7 million people reported to be infected with *C. sinensis* in east Asian countries (Crompton 1999).

The ubiquitin-dependent proteolytic system is of great importance for the maintenance of homeostasis of a broad range of eukaryotic proteins. It is a major pathway for protein degradation in eukaryotes, and plays an essential role in the control of numerous cellular processes, including cell cycle progression (Tyers and Jorgensen 2000), signal transduction (Strous et al. 1997), transcriptional regulation (Hochstrasser et al. 1991), organelle biogenesis (Finley et al. 1989), and the inflammatory response (Chen et al. 1995).

The conjugation of ubiquitin (Ub) to a target protein is a cascade enzymatic reaction involving a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2 or UBC), and a ubiquitin–protein ligase (E3). Initially, ubiquitin is activated by the E1 in an ATP-dependent manner, resulting in the formation of a high-energy thiolester bond between the C-terminal glycine residue of ubiquitin and a conserved cysteine residue within E1. The activated ubiquitin is then transferred from E1 to the active-site cysteine residue of E2 by trans-thiol esterification. Finally, E2 donates ubiquitin monomers or multiubiquitin chains either directly or with the assistance of E3 to an ϵ -amino group of a lysine residue in the target proteins, where a covalent isopeptide bond is formed. The ubiquitinated proteins are subsequently delivered to and degraded by the 26S proteasome (Hershko and Ciechanover 1998).

In contrast to the single E1 ubiquitin-activating enzyme, E2 exists as a family of related isozymes. In

L. Song · S. Chen · X. Yu (✉) · Z. Wu · J. Xu · G. Yang
N. Zheng · X. Hu
Department of Parasitology, Medical School,
Sun Yat-Sen University,
510089 Guangzhou, P.R. China
E-mail: slxch@163.com
Tel.: +86-20-87330566
Fax: +86-21-65642502

L. Song · S. Chen · X. Yu · Z. Wu · J. Xu · G. Yang
N. Zheng · X. Hu
Key Laboratory of Tropical Diseases Control,
Ministry of Education,
510089 Guangzhou, P.R. China

L. Guo · J. Dai · J. Xu · C. Ji · S. Gu · K. Ying
State Key Laboratory of Genetic Engineering,
School of Life Science,
Institute of Genetics,
Fudan University, 200433 Shanghai, P.R. China

S. cerevisiae, there are at least 13 different E2s (Hochstrasser 1996). Functions of E2s have been extensively studied in yeast, and they appear to play distinct roles in a variety of cell events. UBC2/RAD6 is a requirement for DNA repair, damage-induced mutagenesis, and sporulation (Jentsch et al. 1987). UBC3/CDC34 participates in the progression of the cell cycle from the G1 into the S phase (Goebel et al. 1988). UBC4 and UBC5 play important roles in the turnover of short-lived and abnormal proteins (Seufert and Jentsch 1990). UBC1 is able to functionally overlap with UBC4/5 but appears to act primarily in the early stages of growth after the germination of spores (Seufert et al. 1990). Members of E2 enzyme genes that are homologous to yeast have also been characterized in other species, such as humans (Koken et al. 1991b; Jensen et al. 1995), rabbits (Wing et al. 1992), *Caenorhabditis elegans* (Zhen et al. 1993), *Drosophila melanogaster* (Koken et al. 1991a), and many plants (Girod and Vierstra 1993; Girod et al. 1993; Feussner et al. 1997; Zhang et al. 2003).

In this study, we describe the cloning, sequence analysis, and the characterization of a cDNA encoding a ubiquitin-conjugating enzyme from *Clonorchis sinensis*. This is the first report of parasite E2 to be overexpressed in an active form.

Materials and methods

cDNA library construction and sequencing

Adult *Clonorchis sinensis* worms were collected from the livers of cats 5~10 months after oral infection with the metacercariae. An adult *C. sinensis* cDNA library was constructed in a modified pBluescriptIISK(+) vector. After mRNA extraction, double-strand cDNA was synthesized using the SMART cDNA Library Construction Kit (Clontech), following the manufacturer's instructions. After digestion with SfiI and size fractionation on a Sepharose CL-2B column, the cDNA fragments longer than 500 bp were ligated into SfiI A and SfiI B sites of the modified pBluescriptIISK(+) vector. Then, the constructs were transformed into *E. coli* DH5 α cells. Individual clones were cultured overnight in LB broth with 100 μ g/ml ampicillin, and plasmids were isolated using a QIAwell plasmid purification system (Qiagen). The cDNA inserts were sequenced on an ABI PRISM 377 DNA sequencer (Perkin-Elmer), using the BigDye Terminator Cycle Sequencing Kit and the BigDye Cycle Sequencing Kit (Perkin-Elmer).

Sequence analysis of the *C. sinensis* ubiquitin-conjugating enzyme (CsUBC) gene

DNA and the deduced protein sequence comparisons were carried out using the BLASTN and BLASTP at the NCBI Web Server (<http://www.ncbi.nlm.nih.gov/blast>). Sequence alignment was carried out using GeneDoc software.

Expression of CsUBC cDNA in *E. coli*

Primers were designed according to the putative open reading frame (ORF) of the *C. sinensis* ubiquitin-conjugating enzyme (CsUBC). The sense primer was 5'CGGGATCCATGTCCACTCCGGCCCAAC3', and the anti-sense primer was 5'CCGCTCGAGTTCATGTCATCTTCCTCCTC3', with a BamHI site and an XhoI site incorporated, respectively. PCR was carried out for 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s. The reaction was continued for 10 min at 72°C after the last cycle. The amplified PCR fragment was digested with BamHI/XhoI and cloned into the expression vector pGEX4T1. The ligation mixture was transformed into *E. coli* BL21 (DE3). After being cultured overnight in an LB plate containing 100 μ g/ml ampicillin, plasmids were isolated and sequenced to confirm the correct insertion of the cDNA fragment. After sequencing, a correct transformant was selected and cultured at 37°C in LB media containing 100 μ g/ml ampicillin. Induction was carried out by final concentration of 1 mmol/l IPTG when the bacterial cells grew to OD₆₀₀ = 0.4~0.6, and continuously cultured for 3 h.

Purification of recombinant CsUBC

A single transformant was inoculated into 2 ml LB media containing 100 μ g/ml ampicillin and cultured overnight. Then, the overnight culture was diluted into 200 ml LB containing 100 μ g/ml ampicillin, and grew at 37°C for 3 h. The bacterial cells were induced with 1 mmol/l IPTG for 3 h before harvesting at 5,000 g for 10 min at 4°C. The cell pellets were resuspended in PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄) containing 0.5 mM PMSF and 2 mM β -mercaptoethanol, for sonication for 10 \times 1 min with 1-min pauses. The homogenate was centrifuged at 12,000 g at 4°C for 15 min, and the supernatant was collected and loaded onto glutathione Sepharose 4B resin (Ambion Biotech). GST-CsUBC fusion protein was cleaved with 2U thrombin (Pharmacia) at 22°C overnight. The cleaved protein was loaded again onto the glutathione Sepharose 4B resin for the purified CsUBC protein. Protein concentration was determined using the Bradford method (Bradford 1976).

Thiolester formation and histone ubiquitination assays

CsUBC activity was assessed with some modifications according to a previously described approach (Sun et al. 1997). The thiolester formation reaction mixture contained 1 μ g purified recombinant CsUBC, 0.3 μ g rabbit E1 (Calbiochem), and 1 μ g ubiquitin His-tag (Calbiochem) incubated in a total volume of 20 μ l of 50 mM Tris-HCl, pH 7.5, 0.2 mM DTT (dithiothreitol), 5 mM ATP, and 5 mM MgCl₂ at 37°C for 30 min.

The reaction was stopped with Laemmli sample buffer without β -mercaptoethanol. Protein samples were separated on 12% SDS-PAGE (SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis), and transferred to a nitrocellulose membrane. The membrane was incubated in PBST (0.1% Tween-20 in PBS) supplemented with 0.5% (w/v) commercial skim milk powder overnight at 4°C, washed twice with PBST on a rocker platform, and incubated for 2 h at room temperature with mouse anti-penta histidine monoclonal antibody (Qiagen), 1:2,000 diluted. After three washes with PBST, the membrane was incubated for 2 h at room temperature with goat anti-mouse IgG labeled with horseradish peroxidase (Santa Cruz), 1:2,000 diluted. After washing the membrane with PBST three times, we added ECL plus Western blotting detection reagents (Amersham Pharmacia Biotech) onto the membrane surface and incubated for 5 min at room temperature, then wrapped the membrane with a fresh piece of SaranWrap and exposed it to an X-ray film for 10~30 min. Thiolester linkage was confirmed by the observed instability of the product after boiling the sample prior to electrophoresis for 5 min in 4% β -mercaptoethanol.

To monitor ubiquitination of histone H2A, 3 μ g of H2A was added to the thiolester formation reaction mixture, and we continued to incubate at 37°C for 30 min. Samples were boiled for 4–5 min in the presence of β -mercaptoethanol, and resolved on a 12% SDS-polyacrylamide gel. Ubiquitination of histone H2A was also determined by the method of Western blotting plus ECL as above.

Results

Cloning and analysis of the *C. sinensis* UBC gene

During the large-scale cDNA sequencing of the adult *C. sinensis* cDNA library, a novel *C. sinensis* cDNA was obtained with an open reading frame of 471 bp that encodes a protein of 156 amino acids (Fig. 1). DNA sequence analysis using BLASTN showed that no DNA sequence was found being highly homologous to the *C. sinensis* cDNA, while the protein sequence analysis using BLASTP showed that many E2s of different species were found being highly homologous to the putative CsUBC. It has 77% amino acids identity with the human ubiquitin-conjugating enzymes HHR6A and HHR6B, and 64% identity with *S. cerevisiae* RAD6. There are three in-frame stop codons upstream of the ORF, and a polyadenylation signal at the 3'-end of the sequence. Comparison of the CsUBC protein with some other members of this family showed that the conserved amino acids in other species were also conserved in *C. sinensis* UBC (Fig. 2). The putative molecular mass of the deduced peptide of the CsUBC was calculated to be 17.76 kDa, and the isoelectric point to be 4.33.

```

1 GGACAGTTTGGTTGGCGGAATCTCCCCTGGCCGTTGGTCTCTTCGTACCACCGTATC
61 TAGCCCTTGATCTCTCGGTTGGCTGGCAGGTGTGTTGGC TAGCGTTTGTGACG TGAGC
121 CGAGAAATGGAGCTGCGCTCGTAGTTATGTCCTCACTCCGCCCAACGGCGCTGTGGCGC
      M S T P A Q R R L L R
181 GACCTAAAGCGTCTGCAGACCGATCCGCCCGCAGGTATATGTGGTCTCCTGTGGATGAC
  D L K R L Q T D P P P G I C G S P V D D
241 AACGTAATGGTGTGGAATGCCGTTATATTCGGTCCAGAAGACACTGCTTTTGAAGACGGA
  N V M V W N A V I F G P E D T A F E D G
301 ACTTCAAACACTACGATTGGACTTACCGAAGAGTACCCCAATAAGCCTCCACGAGTGT
  T F K L R L D F T E E Y P N K P P R V V
361 TTCTTTCGAAGATGTTCCATCCCAACGTTTACACGGACGGAAGCATGCGCTGGATATC
  F L S K M F H P N V Y T D G S I C L D I
421 TTGTCAAGTCTTGGAGCCCAACGTACGATGATTGGCTATTCTCACCTCTATCCAGTCC
  L S S A W S P T Y D V L A I L T S I Q S
481 CTCTCGATGAGCCTAATCCAAACTACCAGCCCAACAGTCTCGCCGCCCAACTTACGTA
  L L D E P N P N S P A N S L A A Q L Y V
541 GAGAACAGACGTGAATACGAAAAACGTGACGTGCCTGTGTTGAGGAGAGCATGTACGAG
  E N R R E Y E K R V R A C V E E S M Y E
601 GAGGAAGATGACATGTAACCTCGCACGCTCAGTCCAAGTTGACTTATGTTTATGATACA
  E E D D M *
661 TACTTATTTTGTAAAAATTTATCGACAACCCAGTATTAGTGTGTTGCCCATTTGTTACTG
721 AATTTTATTTTGGCGTCCAGGTACCTCCTAAATGAGCGGTGCTATCATTCTGTGTAGA
781 TGCTGCTCTTACCTGTGATTGAGATATCTATAGCGCTTGTGTTCTCTACTCTTCAGT
841 GAATAAAGATAATCGTAAAAA

```

Fig. 1 Nucleotide sequence and the deduced amino acid sequence of the *C. sinensis* UBC cDNA. The putative translation start codon is in *bold letters* and the stop codon is indicated by an *asterisk*. The three stop codons in the 5'-flanking region are in *italics*, and the polyadenylation signal at the 3'-end is *underlined*

Expression and purification of the CsUBC protein

The putative CsUBC cDNA ORF was amplified by PCR and cloned into the expression vector pGEX4T1 (Fig. 3). A recombinant pGEX4T1 containing CsUBC cDNA was sequenced with pGEX4T1 forward primer, and revealed that the ORF of the CsUBC was in frame to the Sj26GST (*Schistosoma japonicum* 26-kDa glutathione S-transferase) tag of the vector. The recombinant CsUBC was induced by IPTG and overexpressed as a fusion protein to Sj26GST in *E. coli* BL21 (DE3). The fusion protein, with a molecular mass of about 43 kDa, was purified to homogeneity with a single round of glutathione Sepharose 4B affinity chromatography. After digestion with thrombin, a pure recombinant CsUBC was produced. The concentration of the purified protein was 0.93 mg/ml, and it was used for the analysis of the UBC enzymatic activity.

Thiolester and conjugation assays

The activity of the purified recombinant CsUBC was detected by its ability to accept a ubiquitin from a ubiquitin-activating enzyme (E1), forming a thiolester bond with a conserved cysteine residue, and transferring the ubiquitin to either a substrate or a cognate ubiquitin protein ligase (E3). The ubiquitin we used here was a

Fig. 2 Amino acid sequence comparison of *Clonorchis sinensis* E2 (CsUBC) with other members of E2 proteins from humans (HHR6A and HHR6B), *Saccharomyces cerevisiae* (ScRAD6), *Caenorhabditis elegans* (CeUBC1), *Drosophila melanogaster* (Dhr6), and *Arabidopsis thaliana* (AtUBC1). Highly conserved amino acids are represented by shaded type. The putative active cysteine is marked with an arrowhead

CsUBC :	MSTPARRLLRDEKRLQEDFPAGVSCAPSENNIMQWNAVIRGPEEDTFFBDGTFKLSLEFSEBYFN :	65
HHR6A :	MSTPARRLLMRDEKRLQEDFPAGVSCAPSENNIMQWNAVIRGPEEDTFFBDGTFKLSLEFSEBYFN :	65
HHR6B :	MSTPARRLLMRDEKRLQEDFPAGVSCAPSENNIMQWNAVIRGPEEDTFFBDGTFKLSLEFSEBYFN :	65
ScRAD6 :	MSTPARRLLMRDEKRLQEDFPAGVSCAPSENNIMQWNAVIRGPEEDTFFBDGTFKLSLEFSEBYFN :	65
CeUBC1 :	MTTFSRRLLMRDEKRLQEDFPAGVSCAPSENNIMQWNAVIRGPEEDTFFBDGTFKLSLEFSEBYFN :	65
Dhr6 :	MSTPARRLLMRDEKRLQEDFPAGVSCAPSENNIMQWNAVIRGPEEDTFFBDGTFKLSLEFSEBYFN :	65
AtUBC1 :	MSTPARRLLMRDEKRLQEDFPAGVSCAPSENNIMQWNAVIRGPEEDTFFBDGTFKLSLEFSEBYFN :	65
▼		
CsUBC :	KPPRVVFLSKMPFHNVYADGSIICLDILQNSPQYDVAAILTSTIQSLLEDENPNPNSPANSAAAGLY :	130
HHR6A :	KPPRVVFLSKMPFHNVYADGSIICLDILQNSPQYDVAAILTSTIQSLLEDENPNPNSPANSAAAGLY :	130
HHR6B :	KPPRVVFLSKMPFHNVYADGSIICLDILQNSPQYDVAAILTSTIQSLLEDENPNPNSPANSAAAGLY :	130
ScRAD6 :	KPPRVVFLSKMPFHNVYADGSIICLDILQNSPQYDVAAILTSTIQSLLEDENPNPNSPANSAAAGLY :	130
CeUBC1 :	KPPRVVFLSKMPFHNVYADGSIICLDILQNSPQYDVAAILTSTIQSLLEDENPNPNSPANSAAAGLY :	130
Dhr6 :	KPPRVVFLSKMPFHNVYADGSIICLDILQNSPQYDVAAILTSTIQSLLEDENPNPNSPANSAAAGLY :	130
AtUBC1 :	KPPRVVFLSKMPFHNVYADGSIICLDILQNSPQYDVAAILTSTIQSLLEDENPNPNSPANSAAAGLY :	130
▼		
CsUBC :	VENRREYBKRVRAQVESSMYEEEDDM----- :	156
HHR6A :	QENKREYBKRVSAIVEQSRDC----- :	152
HHR6B :	QENKREYBKRVSAIVEQSNDS----- :	152
ScRAD6 :	KDHRSQYVKKVKETVEKSEDDMDMDDDDDDDDDDDDEAD----- :	172
CeUBC1 :	QENRREYBKRVQQIVGQSWLNFGENEGDAVLKDDVEIEEIAAPGANDADDDRRMDEGASGNA :	192
Dhr6 :	KENRREYBKRVKACVEQSEID----- :	151
AtUBC1 :	SESKREYNRVRDVEQSEITAD----- :	152

fusion with 6×His tag, so the protein bonds containing ubiquitin could be detected using anti-penta-histidine antibody. The thiolester linkage is stable under nonreducing conditions but is cleaved under reducing conditions. As can be observed in Fig. 4a, the reaction mixture containing all the UBC, E1 and ATP treated with Laemmli sample buffer without β -mercaptoethanol results in the formation of the thiolester bond in E1–Ub and E2–Ub, while the thiolester bond of E2–Ub could not be formed in the absence of either E1 or ATP, because the formation of E1–Ub is ATP-dependent.

Addition of histone H2A to the thiolester reaction mixture showed that CsUBC can ubiquitinate histones in vitro. This reaction does not require additional factors (such as E3) but depends on the E1 and ATP. The results can be seen in Fig. 4b—both the monoubiqui-

tinated and the multiubiquitinated histones were detected.

Discussion

A cDNA encoding a ubiquitin-conjugating enzyme was cloned from *Clonorchis sinensis* by a cDNA library construction and large-scale sequencing. This is a rapid and efficient procedure for generating a *C. sinensis* EST database, and cloning cDNAs of great importance in growth and development. We determined the E2 sequence from many different clones. In the sequence analysis of E2, we obtained different results with the DNA and protein homologous research. Further analysis of the open reading frame of *C. sinensis* cDNA and human HHR6A showed that their different bases mostly lie in the third position of codons (data not shown). This suggests that *C. sinensis* and humans have different codon preferences for the same amino acid in the evolution progress.

Although representing different sizes, structures and functions, all E2s identified have a conserved domain of roughly 16 kDa (called the UBC domain). This domain exhibits at least 35% sequence identity in all known E2 enzymes, and includes a centrally located cysteine residue required for ubiquitin–enzyme thiolester formation (Jentsch 1992). There are three cysteines in the positions 25, 88, and 144 of the CsUBC sequence. Based on the homology to the E2 active-site motif HXN(I/V)X3/4GX(I/V/L)C(I/L)X(I/V), Cys88 is most likely to be the active site where the thiolester bond between ubiquitin and CsUBC forms (Fig. 2). The amino acids sequence surrounding the putative active-site cysteine residue is highly conserved. It is indicated that this region may form a conserved domain for accepting ubiquitin from E1–Ub thiolester adducts.

Histone ubiquitination is an important mechanism of regulating the cell cycle. Our results showed that the

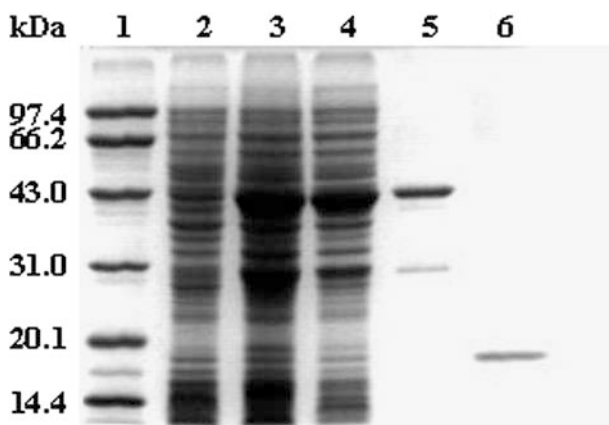


Fig. 3 Expression and purification of recombinant *C. sinensis* UBC. Proteins were resolved on 12% SDS-PAGE and stained with Coomassie blue. Lane 1 Protein marker, lane 2 uninduced pGEX–CsUBC fusion, lane 3 pGEX–CsUBC fusion induced for 3 h, lane 4 soluble fraction of the induced fusion, lane 5 fusion protein purified with glutathione sepharose column, lane 6 recombinant CsUBC digested with thrombin

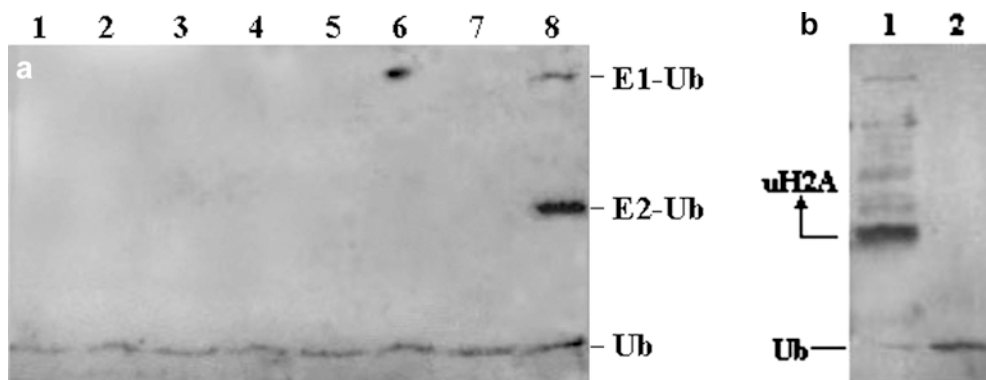


Fig. 4a, b Thiolester bond and histone conjugation assays. **a** Thiolester bond formation between recombinant CsUBC and His tag ubiquitin was at 37°C for 30 min in a 20- μ l mixture containing rabbit E1, recombinant CsUBC (E2), and ATP under reducing and nonreducing conditions (lanes 7, 8). Control reactions were performed in the absence of E1 (lanes 1, 2), ATP (lanes 3, 4) or E2 (lanes 5, 6), to verify that the emergence of the CsUBC-ubiquitin (E2-Ub) band depends on the presence of all three of these components. As seen, no band corresponding to E2-Ub forms when any of these components is missing. Samples were heated to 100 °C for 5 min in Laemmli sample buffer with (lanes 1, 3, 5, 7) or without (lanes 2, 4, 6, 8) β -mercaptoethanol prior to resolution on SDS-PAGE. **b** Histone-ubiquitin conjugation reaction was carried out at 37 °C for 30 min with (lane 1) or without (lane 2) 3 μ g bovine histone H2A. As indicated in lane 1, monoubiquitination and multiubiquitination of histone H2A were formed in the reaction (uH2A adducts of ubiquitin and histone H2A)

recombinant CsUBC expressed in *E. coli* has the ability to form a thiolester linkage with ubiquitin, as well as to transfer ubiquitin to histone H2A in an E3-independent manner. Previous studies showed that some E2s could transfer ubiquitin to the model substrate histone in vitro without the participation of E3s, such as yeast RAD6/UBC2 and CDC34/UBC3, because the highly acidic C-terminus of the two enzymes is required for this in vitro activity (Sung et al. 1988; Goebel et al. 1988). Studies on human UBCH2 also revealed that its C-terminal acid domain was responsible for interacting with substrate proteins (Kaiser et al. 1995). For UBC8, however, the homolog of human UBCH2 in yeast with a C-terminal acidic extension, the amino-terminal residues are required for the ubiquitination of histones in vitro (Kaiser et al. 1994). *C. elegans* UBC1 is a ubiquitin-conjugating enzyme homologous to yeast RAD6/UBC2. Despite its possession of an acidic carboxy-terminal tail, it is unable to transfer ubiquitin to histone H2B in vitro (Leggett et al. 1995). These structure differences in the ubiquitination of histones may be explained by different E2s having their own individual sites for substrate recognition.

In conclusion, we isolated a gene encoding a new member of ubiquitin-conjugating enzymes. The high homology between the UBC in *Clonorchis sinensis* and human RAD6 suggests that they may have a similar role in vivo. Our results demonstrate that the CsUBC has the

basic functions of thiolester formation and histone ubiquitination, which are consistent with the properties of E2s in other species.

Acknowledgement This research was funded by a grant from the Office of Health and the Office of Science and Technology of Guangdong Province, P.R. China (2002B31005). The experiments comply with the current laws of the country in which the experiments were performed.

References

- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Chen Z, Hagler J, Palombella VJ, Melandri F, Scherer D, Ballard D, Maniatis T (1995) Signal-induced site-specific phosphorylation targets I κ B α to the ubiquitin-proteasome pathway. *Genes Dev* 9:1586–1597
- Crompton DW (1999) How much human helminthiasis is there in the world? *J Parasitol* 85:397–403
- Feussner K, Feussner I, Leopold I, Wasternack C (1997) Isolation of a cDNA coding for an ubiquitin-conjugating enzyme UBC1 of tomato—the first stress-induced UBC of higher plants. *FEBS Lett* 409:211–215
- Finley D, Bartel B, Varshavsky A (1989) The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis. *Nature* 338:394–401
- Girod PA, Vierstra RD (1993) A major ubiquitin conjugation system in wheat germ extracts involves a 15-kDa ubiquitin-conjugating enzyme (E2) homologous to the yeast UBC4/UBC5 gene products. *J Biol Chem* 268:955–960
- Girod PA, Carpenter TB, van Nocker S, Sullivan ML, Vierstra RD (1993) Homologs of the essential ubiquitin conjugating enzymes UBC1, 4, and 5 in yeast are encoded by a multigene family in *Arabidopsis thaliana*. *Plant J* 3:545–552
- Goebel MG, Yochem J, Jentsch S, McGrath JP, Varshavsky A, Byers B (1988) The yeast cell cycle gene CDC34 encodes a ubiquitin-conjugating enzyme. *Science* 241:1331–1335
- Hershko A, Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* 67:425–479
- Hochstrasser M (1996) Ubiquitin-dependent protein degradation. *Annu Rev Genet* 30:405–439
- Hochstrasser M, Ellison MJ, Chau V, Varshavsky A (1991) The short-lived MAT α 2 transcriptional regulator is ubiquitinated in vivo. *Proc Natl Acad Sci USA* 88:4606–4610
- Jensen JP, Bates PW, Yang M, Vierstra RD, Weissman AM (1995) Identification of a family of closely related human ubiquitin conjugating enzymes. *J Biol Chem* 270:30408–30414
- Jentsch S (1992) The ubiquitin-conjugation system. *Annu Rev Genet* 26:179–207

- Jentsch S, McGrath JP, Varshavsky A (1987) The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. *Nature* 329:131–134
- Kaiser P, Seufert W, Hofferer L, Kofler B, Sachsenmaier C, Herzog H, Jentsch S, Schweiger M, Schneider R (1994) A human ubiquitin-conjugating enzyme homologous to yeast UBC8. *J Biol Chem* 269:8797–8802
- Kaiser P, Mandl S, Schweiger M, Schneider R (1995) Characterization of functionally independent domains in the human ubiquitin conjugating enzyme UbcH2. *FEBS Lett* 377:193–196
- Koken M, Reynolds P, Bootsma D, Hoeijmakers J, Prakash S, Prakash L (1991a) Dhr6, a *Drosophila* homolog of the yeast DNA-repair gene RAD6. *Proc Natl Acad Sci USA* 88:3832–3836
- Koken MH, Reynolds P, Jaspers-Dekker I, Prakash L, Prakash S, Bootsma D, Hoeijmakers JH (1991b) Structural and functional conservation of two human homologs of the yeast DNA repair gene RAD6. *Proc Natl Acad Sci USA* 88:8865–8869
- Leggett DS, Jones D, Candido EP (1995) *Caenorhabditis elegans* UBC-1, a ubiquitin-conjugating enzyme homologous to yeast RAD6/UBC2, contains a novel carboxy-terminal extension that is conserved in nematodes. *DNA Cell Biol* 14:883–891
- Rim HJ (1986) Current pathobiology and chemotherapy of clonorchiasis. *Korean J Parasitol* 24 (Suppl):67–69
- Seufert W, Jentsch S (1990) Ubiquitin-conjugating enzymes UBC4 and UBC5 mediate selective degradation of short-lived and abnormal proteins. *EMBO J* 9:543–550
- Seufert W, McGrath JP, Jentsch S (1990) UBC1 encodes a novel member of an essential subfamily of yeast ubiquitin-conjugating enzymes involved in protein degradation. *EMBO J* 9:4535–4541
- Strous GJ, van Kerkhof P, Govers R, Rotwein P, Schwartz AL (1997) Growth hormone-induced signal transduction depends on an intact ubiquitin system. *J Biol Chem* 272:40–43
- Sun B, Jeyaseelan K, Chung MC, Tan TW, Chock PB, Teo TS (1997) Cloning, characterization and expression of a cDNA clone encoding rabbit ubiquitin-conjugating enzyme, E2_{32 k}. *Biochim Biophys Acta* 1351:231–238
- Sung P, Prakash S, Prakash L (1988) The RAD6 protein of *Saccharomyces cerevisiae* polyubiquitinates histones, and its acidic domain mediates this activity. *Genes Dev* 2:1476–1485
- Tyers M, Jorgensen P (2000) Proteolysis and the cell cycle: with this RING I do thee destroy. *Curr Opin Genet Dev* 10:54–64
- Wing SS, Dumas F, Banville D (1992) A rabbit reticulocyte ubiquitin carrier protein that supports ubiquitin-dependent proteolysis (E2_{14 k}) is homologous to the yeast DNA repair gene RAD6. *J Biol Chem* 267:6495–6501
- Zhang XD, Jenkins JN, Callahan FE, Creech RG, Si Y, McCarty JC, Saha S, Ma DP (2003) Molecular cloning, differential expression, and functional characterization of a family of class I ubiquitin-conjugating enzyme (E2) genes in cotton (*Gossypium*). *Biochim Biophys Acta* 1625:269–279
- Zhen M, Heinlein R, Jones D, Jentsch S, Candido EP (1993) The *ubc-2* gene of *Caenorhabditis elegans* encodes a ubiquitin-conjugating enzyme involved in selective protein degradation. *Mol Cell Biol* 13:1371–1377