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The *ubc-2* Gene of *Caenorhabditis elegans* Encodes a Ubiquitin-Conjugating Enzyme Involved in Selective Protein Degradation

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The ubiquitin-protein conjugation system is involved in a variety of eukaryotic cell functions, including the degradation of abnormal and short-lived proteins, chromatin structure, cell cycle progression, and DNA repair. The ubiquitination of target proteins is catalyzed by a ubiquitin-activating enzyme (E1) and ubiquitin-conjugating enzymes (E2s) and in some cases also requires auxiliary substrate recognition proteins (E3s). Multiple E2s have been found, and these likely possess specificity for different classes of target proteins. Here we report the cloning and characterization of a gene (*ubc-2*) encoding a ubiquitin-conjugating enzyme which is involved in the selective degradation of abnormal and short-lived proteins in the nematode *Caenorhabditis elegans*. The nematode *ubc-2* gene encodes a 16.7-kDa protein with striking amino acid sequence similarity to *Saccharomyces cerevisiae UBC4* and *UBC5* and *Drosophila UbcD1*. When driven by the *UBC4* promoter, *ubc-2* can functionally substitute for *UBC4* in yeast cells; it rescues the slow-growth phenotype of *ubc4 ubc5* mutants at normal temperature and restores their ability to grow at elevated temperatures. Western blots (immunoblots) of *ubc4 ubc5* yeast cells transformed with *ubc-2* reveal a protein of the expected size, which cross-reacts with anti-*Drosophila UbcD1* antibody. *C. elegans ubc-2* is constitutively expressed at all life cycle stages and, unlike yeast *UBC4* and *UBC5*, is not induced by heat shock. Both *trans* and *cis* splicing are involved in the maturation of the *ubc-2* transcript. These data suggest that yeast *UBC4 and UBC5*, *Drosophila UbcD1*, and *C. elegans ubc-2* define a highly conserved gene family which plays fundamental roles in all eukaryotic cells.

The ubiquitin-mediated proteolysis system is the major pathway for the selective, controlled degradation of intracellular proteins in eukaryotic cells (12, 15). Targeted proteins undergoing selective degradation are covalently tagged with ubiquitin through the formation of an isopeptide bond between the C-terminal glycyl residue of ubiquitin and a specific lysyl residue in the substrate protein. This process is catalyzed by a ubiquitin-activating enzyme (E1) and one of the ubiquitin-conjugating enzymes (E2s) and in some cases also requires auxiliary substrate recognition proteins (E3s) (12). Following the linkage of the first ubiquitin chain, additional molecules of ubiquitin may be attached to lysine 48 of the previously conjugated moiety to form branched multi-ubiquitin chains. Studies suggest that the formation of high-molecular-weight ubiquitin-protein conjugates is a prerequisite for degradation of target proteins (2).

One major function of the ubiquitin-mediated pathway is to control the half-lives of cellular proteins (15). The known half-lives of individual proteins can vary from several minutes to a few days. Previous studies have shown that abnormal proteins induced by environmental stresses such as heat shock and amino acid analogs are quickly degraded via the ubiquitin system (6). In addition, several key regulatory proteins are known to be degraded through the ubiquitin-dependent pathway (24); these include the *Saccharomyces cerevisiae* transcriptional repressor MATα2 (13, 14), the cell cycle-controlling protein cyclin in *Xenopus* eggs (8), the tumor suppressor p53 (27), and the far-red light-absorbing phytochrome in higher plants (31). Ubiquitin-mediated protein degradation also seems to play a role in developmentally programmed cell degeneration and death (28).

The molecular genetics of the ubiquitin system have been studied most extensively in *S. cerevisiae*. This work has revealed that genes encoding ubiquitin-conjugating enzymes (the *UBC* genes) are involved in surprisingly diverse cellular functions (16). *UBC4* and *UBC5* encode the major ubiquitin-conjugating enzymes of the protein degradation pathway. Together with UBC1, these proteins form an essential enzyme family in yeast cells, as demonstrated by the inviability of *ubc1 ubc4 ubc5* triple mutants (30). In *ubc4 ubc5* double mutants, the turnover of short-lived and abnormal proteins is impaired, the cells grow poorly at normal temperatures, and they are inviable under stress conditions, e.g., at 37°C or in the presence of amino acid analogs such as canavanine (29). *UBC2* (*RAD6*) is involved in DNA repair, induced mutagenesis, and sporulation (17, 18). It has recently been shown that RAD6 mediates E3-dependent protein degradation (4, 34). *UBC3* (*CDC34*) encodes a protein which is required for progression through the cell cycle (9). *UBC10* (*Pas2*) is required for peroxisome biogenesis (37). *UbcD1*, a *Drosophila* homolog of the *UBC4* and *UBC5* genes, has recently been cloned and found to exhibit striking structural and functional similarity to the yeast genes (35). Indeed, the expression of *UbcD1* in yeast cells rescues the phenotypic defects of *ubc4 ubc5* double mutants (35).

The already defined cell lineage of the nematode *Caenorhabditis elegans*, as well as the wealth of genetic and developmental information available for this organism (39), makes it an excellent choice for studies of the roles of

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ubiquitin conjugation in the development of a multicellular eukaryote. Here we report the cloning and characterization of an E2-encoding gene from *C. elegans* which is homologous to yeast *UBC4* and *UBC5*.

MATERIALS AND METHODS

Culturing of *C. elegans* and yeast strains. Wild-type *C. elegans* Bristol (N2) was grown on nutrient agar plates spread with a lawn of *Escherichia coli* OP50 as described previously (1). Wild-type *S. cerevisiae* cells (YWO1) and *ubc5*, *ubc4 ubc5* (YWO17, YWO22, [29]), and *ubc4*::*UbcD1 ubc5* (YWO56 [35]) mutants are congenic to the strain used for the *ubc-2* transformation. Ycuc217 is the resulting recombinant (*ubc4*::*ubc-2 ubc5*::*LEU2*). Standard protocols (25) were followed for growth and transformation of yeast cells.

Gene cloning and analysis. Two degenerate oligodeoxynucleotides, OPC9 [5′ GAGGATCCTA(T,C)GA(A,G)GG(T,A)GG(T,A)GT(T,C)TT(T,C)TT 3′] and DJ2 [5′ CTGTCGACAC(A,G,C,T)GC(G,A)TA(C,T)TT(C,T)TT(A,G,C,T)GTCCA(C,T)TC 3′], were designed based on conserved regions of the yeast UBC4 and UBC5 (29) and *Drosophila* UbcD1 amino acid sequences, respectively. First-strand cDNA was synthesized by using mouse mammary leukemia virus reverse transcriptase (GIBCO-BRL). The second strand was synthesized, and the resulting cDNA was amplified by using the polymerase chain reaction (PCR). PCRs were performed in 50-μl reaction mixtures containing first-strand cDNA, 50 pmol of each oligonucleotide primer, 50 μM each deoxynucleoside triphosphate, and 1 U of *Taq* DNA polymerase (Promega) in PCR buffer as described by the manufacturer (Promega). The amplification consisted of 35 cycles of 94°C for 30 s, 55°C for 90 s, and 72°C for 90 s. An additional 10-min incubation at 72°C followed the final cycle. PCR products were separated on 2% agarose gels, and the fragments of the expected sizes were eluted and subcloned into pBluescript SK vectors. Sequencing of the fragment was done with a Sequenase kit (U.S. Biochemical). DNA probes were ^{32}P labeled by nick translation (23) and used to screen a λZAP cDNA library from *C. elegans* embryos. Positive plaques were amplified and subcloned into pBluescript for sequencing. The genomic clone was isolated from a λCharon4 genomic library by using the cDNA as probe.

Replacement of yeast *UBC4* by *C. elegans ubc-2*. *Nco*I and *Spe*I sites were created at the initiator ATG and the terminator TAA, respectively, of *UBC4* as previously described (35). Similar restriction sites were introduced into the open reading frame (ORF) of the *ubc-2* cDNA by a PCR. The sequence of the PCR-amplified gene fusion was confirmed by DNA sequencing. After the exchange of the *Nco*I-*UBC4*-*Spe*I ORF fragment with the *Nco*I-*ubc-2*-*Spe*I ORF fragment, a linear DNA fragment carrying the *ubc-2* gene and *UBC4* upstream and downstream sequences of about 1,000 and 700 bp, respectively, was used to transform haploid yeast *ubc4 ubc5* double-mutant cells (strain YWO22). The *ubc-2* recombinants were selected by growth at 37°C on YPD medium (yeast extract-peptone-glucose [25]). Since *UBC4* was disrupted with the yeast *HIS3* marker (strain YWO22), *ubc-2* recombinants acquired histidine auxotrophy. The correct integration of the *C. elegans* sequence at the *UBC4* locus was confirmed by Southern hybridization (data not shown).

Anti-UbcD1 serum and Western blot (immunoblot) analysis. For preparation of proteins, yeast cells were harvested, resuspended in 50 mM Tris-HCl (pH 7.5)–10 mM Na-EDTA–0.5 mM phenylmethylsulfonyl fluoride, and vigorously mixed with an equal volume of glass beads for 3 min. For Western analysis, proteins were separated on 18% polyacrylamide-sodium dodecyl sulfate (SDS) gels and electrophoretically blotted onto a polyvinylidene difluoride membrane (Millipore). Blotted proteins were probed with anti-UbcD1 serum (11) diluted 1:100 in 20% fetal calf serum–1× phosphate-buffered saline (PBS), washed with 1× PBS containing 0.2% Brij 58, and developed with ^{125}I-protein A (5 μCi per blot; Amersham).

Nucleic acid hybridizations. Southern and Northern (RNA) hybridization analyses were performed by standard methods (23). ^{32}P-labeled DNA probes were prepared by nick translation or random primer extension (23). Background nonspecific hybridization was eliminated by the inclusion of 5 μg of heparin (sodium salt; Sigma) per ml in hybridization solutions.

S1 nuclease analysis. S1 nuclease protection experiments were performed as described previously (19) with the following modifications. Instead of a universal forward primer, an oligodeoxynucleotide (oZM7) complementary to nucleotides 486 to 504 of the *ubc-2* gene sequence was used as the primer to synthesize single-stranded DNA probes. The annealing of primer to template was done by heating to 65°C for 10 min and then cooling to room temperature for 15 min. Primer extension with Klenow fragment was done at 37°C for 10 min.

RESULTS

Cloning and sequencing of *ubc-2*, a gene encoding a ubiquitin-conjugating enzyme (E2) in *C. elegans*. Among the 10 E2s identified in *S. cerevisiae*, UBC4 and UBC5 have been shown to be most important for bulk protein degradation in yeast cells. UBC4 and UBC5 and their *Drosophila* homolog, UbcD1, share 85% amino acid sequence identity. To clone the homologous genes in *C. elegans*, we designed two degenerate oligonucleotides based on conserved protein sequences and used them to amplify cDNA synthesized from *C. elegans* RNA (see Materials and Methods).

This approach yielded a 312-bp PCR fragment which encoded part of an E2 (amino acids 45 to 145 of ubc-2; see Fig. 3). Using this fragment as a probe to screen a *C. elegans* cDNA library, we isolated a complete cDNA clone encoding a ubiquitin-conjugating enzyme. The corresponding gene

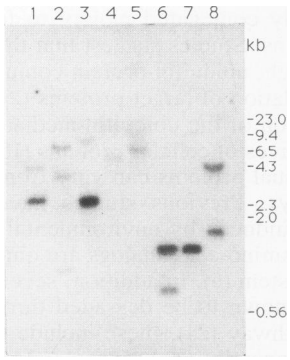

FIG. 1. Genomic Southern blot analysis. DNA (1 to 2 μg) prepared from *C. elegans* embryos was digested with *Xba*I, *Xho*I, *Pst*I, *Apa*I, or *Bam*HI (lanes 1 to 5, respectively), *Bgl*II plus *Eco*RI (lane 6), *Bgl*II (lane 7), or *Eco*RI (lane 8). The hybridization probe was a nick-translated 312-bp PCR fragment encoding amino acids 45 to 145 of *ubc-2*.

was named *ubc-2*. A genomic clone corresponding to *ubc-2* was obtained from a *C. elegans* genomic library by using the cDNA as a probe. Genomic Southern analysis with the 312-bp PCR fragment suggests the existence of more than one E2 gene in *C. elegans* (Fig. 1). The sequence of the *ubc-2* gene is shown in Fig. 2.

***ubc-2* shares striking amino acid identity to its yeast and *Drosophila* homologs.** Sequencing of the *ubc-2* cDNA indicated that it encodes a 16.7-kDa type I ubiquitin-conjugating enzyme (16). The cysteine at position 66 corresponds to the residue which is required for conjugation to ubiquitin and which is conserved among all ubiquitin-conjugating enzymes. *C. elegans ubc-2* shares 85% amino acid identity with yeast *UBC4* and *UBC5* and 95% identity with *Drosophila UbcD1* (Fig. 3). The amino acid similarities between ubc-2 and UBC4 and UBC5 or UbcD1 are 90 and 98%, respectively. This high degree of conservation suggests that this type of E2 probably plays essential and fundamental roles in eukaryotic cells.

The *ubc-2* gene has been physically mapped to chromosome V by Coulson et al. (3) as part of the *C. elegans* genome mapping and sequencing project.

***C. elegans ubc-2* can functionally substitute for *UBC4* in yeast cells.** To test the hypothesis that ubc-2 is functionally equivalent to the yeast UBC4 enzyme, we introduced the *C. elegans ubc-2* gene into yeast cells and tested it for complementation of the phenotypic deficiencies of a *ubc4 ubc5* double mutant. To eliminate the possibility that high-level expression of a distantly related enzyme might result in complementation (29), we integrated the *C. elegans* gene

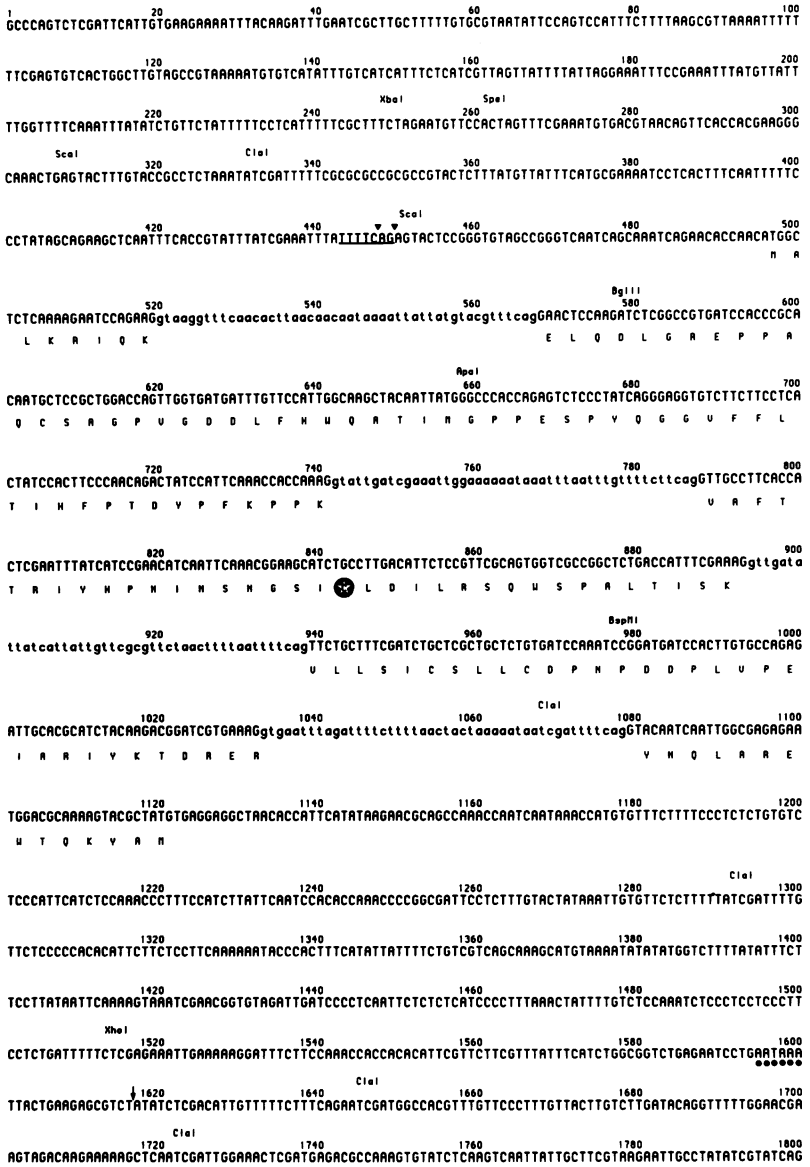

FIG. 2. Nucleotide and deduced amino acid sequences of *ubc-2*. Introns are shown in lowercase. The consensus sequence of the *trans*-splicing signal is underlined. Two solid triangles denote the 5′ endpoints of transcripts determined from S1 nuclease protection experiments. The polyadenylation signal is underlined with dots, and the site of polyadenylation is shown by an arrow. The putative active-site cysteine is starred.

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                    1                   20                  40                  60
ubc-2   C. elegans  MAL KRIQKELQDLGREPPAQCSAGPVGDDLFHWQATIMGPPESPYQGGVFFLTIHFPTDYPFKPPKVAFTTRI
UbcD1   Drosophila  --- ---M----------------------------------D------------------------------
UBC4    Yeast       -SSS---A---S--------S----------Y----S----AD---A------S--------------S---K-

                   80                  100                 120                 140
ubc-2   C. elegans  YHPNINSNGSICLDILRSQWSPALTISKVLLSICSLLCDPNPDDPLVPEIARIYKTDRERYNQLAREWTQKYAM
UbcD1   Drosophila  ----------------K-----------------------------------------K--E------R----
UBC4    Yeast       ------A--M------KD-------------------T-A----------Q------PK-EAT-----K---V
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FIG. 3. Comparison of the proteins encoded by *C. elegans ubc-2*, *Drosophila UbcD1*, and yeast *UBC4*. The arrowhead marks the cysteinyl residue which is essential for enzyme activity. Identical residues are indicated by dashes.

into the yeast genome. A precise ORF replacement of *UBC4* by *ubc-2* was performed as outlined in Fig. 4. Using site-directed mutagenesis and PCR, we introduced *Spe*I and *Nco*I sites at the beginning and end, respectively, of the ORFs of the *ubc-2* cDNA and *UBC4* genomic DNA. The chimeric gene carrying a *UBC4* control region and the *ubc-2* ORF was transformed into a yeast *ubc4 ubc5* double mutant, in which *UBC4* was disrupted by a *HIS3* marker (see Materials and Methods). Homologous recombinants were selected by their viability at 37°C, since if *ubc-2* can functionally replace *UBC4*, the recombinants should be phenotypically similar to *ubc5* single mutants, which grow at 37°C. Integration of the *C. elegans* sequences at the *UBC4* locus was indicated by histidine auxotrophy and confirmed by Southern analysis (data not shown).

As shown in Table 1 and Fig. 5, yeast *ubc4 ubc5* mutants do not grow at 37°C and display impaired growth at 30°C (29, 30); *ubc4* or *ubc5* mutants show normal growth at 30°C and are viable at 37°C. When driven by the *UBC4* promoter, the expression of *C. elegans ubc-2* in recombinant strains rescues the growth defect of *ubc4 ubc5* mutants at 30 and 37°C. The *ubc4*::*ubc-2 ubc5* strain is phenotypically similar to *ubc5* single-mutant strains. Its growth rate is very close to that of the wild type. This suggests that *C. elegans ubc-2* can functionally substitute for *UBC4*, a gene which is responsible for the selective degradation of abnormal and short-lived proteins in *S. cerevisiae*.

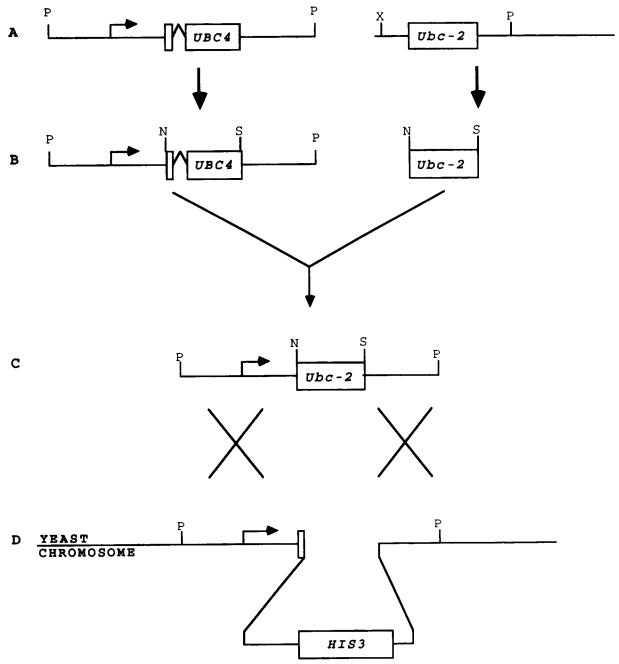

FIG. 4. Strategy for ORF replacement of *UBC4* by *ubc-2* in yeast cells. (A and B) *Nco*I (N) and *Spe*I (S) sites were introduced at the beginning and end, respectively, of the ORFs of the *ubc-2* cDNA and *UBC4* genomic DNA. (C) Chimeric gene carrying a *UBC4* control region and the *ubc-2* ORF was transformed into a yeast *ubc4 ubc5* double mutant, in which *UBC4* was disrupted by a *HIS3* marker. Homologous recombinants were selected by their viability at 37°C.

The expression of *C. elegans ubc-2* in yeast cells was further confirmed by Western blotting analysis (Fig. 6). Protein extracts from wild-type, *ubc4 ubc5*, *ubc4*::*ubc-2 ubc5*, and *ubc4*::*UbcD1 ubc5* cells were prepared, separated by SDS-gel electrophoresis, blotted, and reacted with a polyclonal antiserum prepared against *Drosophila* UbcD1 (see Materials and Methods). This antiserum recognized not only the *Drosophila* UbcD1 and *C. elegans* ubc-2 proteins expressed in the transformed strains but also yeast UBC4 in wild-type cells.

***ubc-2* is constitutively expressed at all life stages of *C. elegans*.** The expression of *ubc-2* in *C. elegans* was studied by Northern analysis. Total RNA prepared from the various life cycle stages of *C. elegans* was separated on 1% denaturing agarose gels (see Materials and Methods). To avoid the detection of other possible E2 transcripts, we used a fragment from the 3′-untranslated region of the *ubc-2* cDNA as a probe. A transcript close to the predicted size, 1.2 kb, was detected at all *C. elegans* stages (Fig. 7). As a control, the same blot was stripped and reprobed with the *C. elegans* actin 1 gene, which is constitutively expressed throughout the nematode life cycle (22). No significant differences in the levels of the *ubc-2* transcripts were observed among different stages.

Expression of *ubc-2* is unaffected by heat shock. In yeast cells, the levels of *UBC4* and *UBC5* transcripts are increased during a heat shock (29). Total cellular RNA was prepared from *C. elegans* mixed cultures and from cultures of selected stages, following either maintenance at normal temperature or a 2-h heat shock at 33°C. The latter condition is used routinely in this laboratory for the induction of heat shock proteins in *C. elegans* (26, 32). The RNA samples were subjected to Northern analysis with a *ubc-2* cDNA probe,

TABLE 1. Growth rates of *ubc* mutants and *ubc-2*-expressing *ubc* mutants

Mutant	Doubling time (h)
Wild type	1.5
ubc4	2.0
ubc5	1.5
ubc4 ubc5	6.0
ubc4::*UbcD1 ubc5*	1.8
ubc4::*ubc-2 ubc5*	1.8

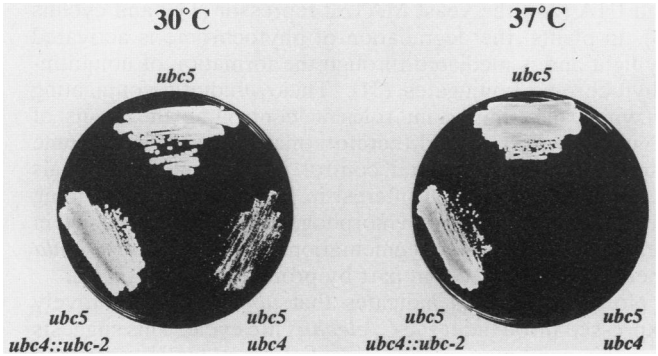

FIG. 5. Growth on plates of yeast *ubc5* mutants, *ubc4 ubc5* double mutants, and ubc-2-expressing double mutants (*ubc5 ubc4*::*ubc-2*) at normal growth temperature (30°C) and heat shock temperature (37°C).

and the blot was then stripped and reprobed with an actin 1 gene probe, as a control for equal RNA loading. No significant change in the level of the *ubc-2* transcript was seen after heat shock (Fig. 8). The same blot was also stripped and rehybridized with a probe for the *hsp16-1* gene, a strictly inducible heat shock gene (26); this showed the expected high levels of induction in the heat shock RNA samples, while no signal was seen with the RNA from unshocked animals at any stage (data not shown).

***ubc-2* transcript undergoes both *trans* and *cis* splicing.** Many *C. elegans* gene transcripts are known to undergo *trans* splicing, a process in which a short leader from a distinct species of small nuclear RNA is attached to the 5′ end of the mRNA precursor at a position corresponding to a 3′ splice site consensus sequence (36). Comparison of the *ubc-2* genomic and cDNA sequences reveals that a splice leader sequence (SL1) is present at the 5′ end of the *ubc-2* cDNA. A consensus splicing signal (TTTTCAG) is found at the relevant position in the genomic DNA (nucleotides 443 to 449, Fig. 2). The 5′ sequence of the *ubc-2* cDNA is as follows, where underlining indicates identity with the gene sequence: 5′ *AAGTTTGAG*AGTACTCCGGGT. . . . Italicized nucleotides represent bases derived from SL1. Except for the lack of introns in the cDNA, this was the only difference seen between the gene sequence and the complete cDNA sequence. This indicates that the *ubc-2* transcript undergoes *trans* splicing.

S1 nuclease protection experiments (not shown) were performed in an attempt to localize the site of transcript initiation. Two main protected probe fragments were seen, corresponding to S1 cleavage at nucleotides 447 and 449 of the gene, adjacent to the 3′ splice site consensus sequence (Fig. 2). These results are similar to those seen with *ubiA*, another *trans*-spliced gene (10), and likely arise because the 3′-most six nucleotides of SL1 are identical to the 3′ splice site sequence of the *ubc-2* gene, with just one mismatch. Thus, the cleavage at nucleotide 447 may represent protection of the 3′ portion of the SL1 sequence joined to the *ubc-2* 3′ splice site. The start site of the transcript could not be determined, probably because of the extremely low levels of unspliced *ubc-2* heterogeneous nuclear RNA present.

Sequence analysis of *ubc-2* shows that the gene is made up of five exons divided by four introns (Fig. 2). Thus, both *trans* and *cis* splicing are involved in the maturation of the *ubc-2* transcript. The single introns of yeast *UBC4* and *UBC5* are located at nonconserved positions with respect to the introns of *ubc-2*.

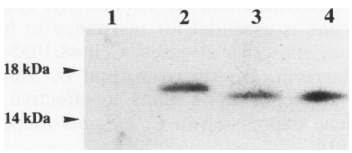

FIG. 6. Immunodetection of yeast UBC4, *C. elegans* ubc-2, and *Drosophila* UbcD1 proteins in yeast cells. Protein extracts were prepared from the *ubc4 ubc5* double-mutant strain YWO22 (lane 1), wild-type YWO1 (lane 2), and *ubc4 ubc5* mutant strains expressing ubc-2 (Ycuc217, lane 3) or UbcD1 (YWO56, lane 4). Identical amounts of protein (10 μg per lane) were separated on an 18% polyacrylamide-SDS gel, blotted onto a polyvinylidene difluoride membrane, and reacted with an antiserum raised against UbcD1. The blot was developed with ^{125}I-protein A and then autoradiographed.

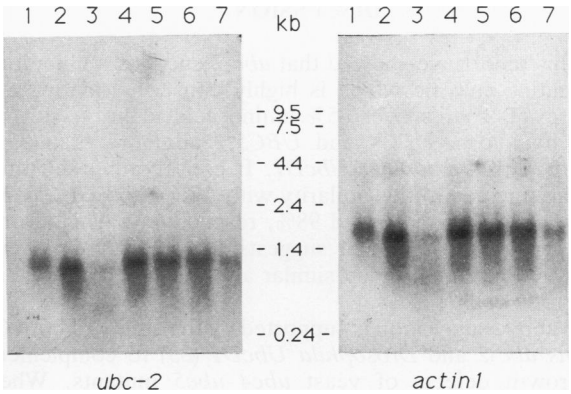

FIG. 7. Northern blot analysis. Total cellular RNA (5 to 20 μg) was prepared from the major stages of development: egg, adult, L1, L2, L3, L4, and dauerlarva (lanes 1 to 7, respectively), separated by electrophoresis on a 1% formaldehyde-agarose gel, and probed with a 423-bp cDNA fragment specific to *ubc-2* (left panel) or with the actin 1 gene (right panel).

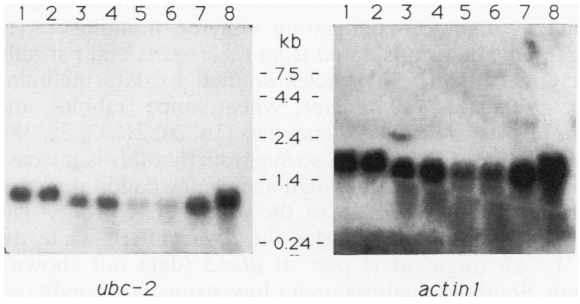

FIG. 8. Northern blot analysis. Total cellular RNA (5 to 20 μg) was prepared from control and heat-shocked *C. elegans* in different developmental stages: mixed culture (lanes 1 and 2), egg (lanes 3 and 4), L1 (lanes 5 and 6), and L2 (lanes 7 and 8). Lanes 1, 3, 5, and 7 are from control cultures; lanes 2, 4, 6, and 8 are from heat-shocked cultures (33°C, 2 h). Electrophoresis and hybridization probes were as described in the legend to Fig. 7. When the same blot was stripped and rehybridized with a probe for *hsp16-1*, a strictly inducible heat shock gene (26), the heat shock RNA lanes showed the expected strongly hybridizing band, while RNA from unshocked animals gave no detectable signal (data not shown).

DISCUSSION

In this report, we showed that *ubc-2* encodes a ubiquitin-conjugating enzyme which is highly conserved among eukaryotes. It shares over 85% amino acid identity with its yeast homologs, *UBC4* and *UBC5*, and more than 95% identity with *Drosophila UbcD1*. If conservative substitutions are included, the similarity with *UBC4* and *UBC5* and *UbcD1* increases to 90 and 98%, respectively. This strong evolutionary conservatism suggests that the enzymes encoded by these genes play similar and fundamental roles in eukaryotic cells.

The above suggestion is supported by the ability of both *C. elegans ubc-2* and *Drosophila UbcD1* (35) to complement the growth defects of yeast *ubc4 ubc5* mutants. When expressed from the *UBC4* promoter, the ubc-2 protein rescues the growth defect of a yeast *ubc4 ubc5* mutant at both normal and elevated temperatures. The doubling time of a *ubc5 ubc4::ubc-2* yeast strain is identical to that of a *ubc5* single mutant and very close to that of wild-type cells. UBC4 and UBC5 are thought to function in the ubiquitin-dependent degradation of both abnormal proteins and normal short-lived proteins (29), by mediating the formation of high-molecular-weight ubiquitin-protein conjugates. Most such conjugates are missing from *ubc4 ubc5* mutants and are restored in cells expressing *Drosophila* UbcD1 (35). Given the high degree of sequence conservation, it is therefore likely that ubc-2 functions in the elimination of abnormal proteins and in the degradation of short-lived proteins in *C. elegans*.

In contrast to the situation with yeast *UBC4* and *UBC5*, the transcript level of *ubc-2* does not change significantly after heat shock. This result was somewhat unexpected given the ability of *ubc-2* to functionally substitute for *UBC4*, its presumed role in the elimination of damaged proteins, and the inducibility of the related yeast enzymes. One possible explanation for this is that the *ubc-2* transcript may be abundant enough under normal conditions to cope with the increased demand for ubiquitin-dependent proteolysis under stress conditions. Alternatively, there may exist other E2 genes in *C. elegans* whose expression is heat inducible. In this regard, we have recently cloned a gene (named *ubc-3*) which weakly cross-hybridizes with *ubc-2*. Characterization of this gene will test the second possibility.

Besides *ubc-2* and the above-mentioned *ubc-3*, a gene encoding a ubiquitin-conjugating enzyme homologous to yeast *RAD6* has been isolated from *C. elegans* and partially characterized (22a). All species studied to date including yeasts, *Drosophila melagaster*, wheat, mice, rabbits, and humans possess multiple E2 enzymes (16, 20, 21, 33, 35, 38). Genomic Southern analysis also suggests that this is the case in *C. elegans* (Fig. 1). Under high-stringency conditions, two *Eco*RI fragments hybridize to the *ubc-2* PCR probe. The upper 5.8-kb fragment encodes the *ubc-2* ORF, while the lower 1.8-kb fragment is part of *ubc-3* (data not shown). Genomic Southern analysis under low-stringency conditions reveals additional hybridizing bands, suggesting the existence of more distantly related E2 genes (data not shown). Although other E2-encoding genes clearly exist in *C. elegans*, on the basis of its degree of similarity to *UBC4* and its high level of expression, *ubc-2* may be the major one responsible for ubiquitin-mediated protein degradation in this species.

The ubiquitin-mediated proteolysis system is involved in determining the half-lives of several regulatory proteins, including the nuclear oncoproteins N-Myc, c-Myc, c-Fos, and E1A (24), the yeast MATα2 repressor (14), and cyclins (8). In plants, the degradation of phytochrome is activated by light and is mediated through the formation of ubiquitin-phytochrome conjugates (31). Thus, ubiquitin-conjugating enzymes play important roles in controlling the levels of regulatory proteins and therefore may be involved in some aspects of developmental control in multicellular animals such as *C. elegans*. Of interest in this regard is the finding that the levels of bicoid, a morphogenetic protein involved in defining the anterior segmentation pattern in *Drosophila* species, are regulated in part by protein degradation (5).

Northern analysis indicates that *ubc-2* is constitutively expressed throughout the *C. elegans* life cycle. This suggests that this gene may encode essential functions in many and perhaps all cells. Experiments are in progress to determine the tissue specificity of this expression by in situ immunofluorescence and by transformation of *ubc-2-lacZ* fusion genes into the animal. The well-defined cellular anatomy and transparency of *C. elegans* should permit the precise determination of the cell types which express *ubc-2*, which in turn may provide useful clues to the functions of the ubiquitin-dependent proteolysis system in multicellular animals.

Disruption of *ubc-2* expression by transformation with antisense constructs (7) or by mutation may now be feasible and would open the way to a more detailed analysis of its function. If *ubc-2* is essential, antisense constructs could be expressed from an inducible promoter such as that derived from the heat-inducible *C. elegans hsp16* gene (26, 32).

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