A Human Ubiquitin Carboxyl Extension Protein Functions in Yeast*

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The ability of the human ubiquitin carboxyl extension protein (HUBCEP80) to functionally replace its yeast homolog was determined in a ubi3 mutant of Saccharomyces cerevisiae. Expression of HUBCEP80 in ubi3 mutants resulted in processing of the fusion protein to produce free ubiquitin and extension protein, the latter of which localized specifically with the 40 S ribosomal subunit. Furthermore, expression of the human fusion protein completely alleviated the phenotypic deficiencies found in ubi3 mutants, including slow growth, abnormal ribosomal RNA processing, and correspondingly low levels of 40 S ribosomal subunits. Finally, expression of the extension protein alone was much less efficient in complementing the ubi3 mutant phenotype as compared with expression of the normal ubiquitin-fused extension protein. In the latter case, cells were found to contain at least 5-fold more extension protein, suggesting that ubiquitin either increased translational efficiency of the HUBCEP80 transcript or increased the stability of the processed extension protein.

Ubiquitin is a 76-amino acid, highly conserved, eukaryotic protein that exists in cells both free and covalently conjugated to itself and other cellular proteins as a post-translational modification. Many functions of the ubiquitin system have been proposed and have been the topic of several reviews (1-3). The best characterized function of ubiquitin is in the selective, ATP-dependent, degradation of cellular proteins (1-3). In the currently accepted model, proteins are marked for degradation by the covalent attachment of ubiquitin's carboxyl terminus to ε-amino groups of lysine residues of target proteins. This event is believed to signal ubiquitin-dependent proteases for target protein degradation in a reaction requiring ATP (1-3).

Ubiquitin is encoded in the eukaryotic genome by a multi-gene family of natural gene fusions. This gene family can be divided into two classes. The first class is comprised of polygenes (polyubiquitin) in which direct repeats of the 76-amino acid encoding unit exist in a contiguous manner (4-12). The final repeat at the 3' end of the polyubiquitin gene is usually followed by a codon encoding a non-ubiquitin carboxy-terminal amino acid which is not conserved among different species. Although polyubiquitin genes are all generally similar, considerable variation exists in the number of ubiquitin-encoding repeats found in each gene and in the number of polyubiquitin-encoding loci in each genome (4-12).

A second class of ubiquitin genes has been identified in eukaryotic cells in which a single ubiquitin-encoding unit exists fused in frame to sequences encoding a stretch of unrelated amino acids at the carboxyl-terminal side of ubiquitin (4, 12-16). The putative translation products of these genes are referred to as ubiquitin carboxyl extension proteins (UBCEPs) (17). Two types of UBCEP genes have been identified. The first type encodes ubiquitin fused to a carboxyl extension protein (CEP) of 52 amino acids (CEP52) (4, 13, 15, 16). The second encodes an extension, depending upon the species, of 76-80 amino acids (CEP76-80) (4, 12, 14). Although CEP52 and CEP76-80 are not homologous to each other or to ubiquitin, they are both rich in basic amino acids and contain an arrangement of cysteine and histidine residues resembling a common structural motif present in many DNA and RNA binding regulatory proteins known as the "zinc-finger" motif (18). These observations provided the initial basis of proposals suggesting cellular roles for CEPs involving nucleic acid interactions (4, 17, 19).

Another interesting feature of CEPs is their high degree of amino acid conservation among organisms as evolutionarily diverged as yeast, Dictyostelium, mouse, and human (4, 12-16). For example, CEP52 shows 80% amino acid conservation between these organisms and all contain relatively long stretches of perfect identity (4, 12, 15, 16). Genes encoding CEP76-80 have also been identified in yeast (76 amino acids), Dictyostelium (78 amino acids), and human (80 amino acids) (4, 13, 14). Although not as marked as their 52-amino acid counterpart, the longer CEPs show significant conservation of amino acid sequence between these organisms, with the 76-amino acid extension of yeast being 59% and 62% identical with its Dictyostelium and human homologs, respectively.

Four ubiquitin genes comprise the yeast ubiquitin gene family (4). UBI1 and UBI2 both code for the ubiquitin-CEP52 (UBCEP52) fusion protein, UBI3 encodes the ubiquitin-CEP76 (UBCEP76) fusion protein, and UBI4 encodes the pentamer polyubiquitin (4). These genes have recently been chromosomally mapped and shown to be dispersed throughout the yeast genome (20). Furthermore, expression of ubiquitin genes in yeast has been shown to be regulated differentially depending on cellular conditions (4, 21). In general polyubiquitin expression is associated with conditions of stress whereas UBCEP expression is associated with conditions amenable to rapid cell growth (4, 21). Similar observations for the differential expression of polyubiquitin genes and UBCEP genes in plants (9), Dictyostelium (12), protozoa (10), and mammalian cells have been made and suggest that both...

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1 The abbreviations used are: UBCEP, ubiquitin carboxyl extension protein; CEP, carboxyl extension protein; HUBCEP, human UBCEP; HCEP, human CEP; YCEP, yeast CEP; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; kb, kilobase(s).

polyubiquitin and UBCEP gene products function similarly in all eukaryotes.

Recently, the structure, subcellular location, and function of UBCEPs has been investigated by a number of laboratories (13, 17, 19, 23, 24). In Dictostelium, UBCEP52 has been reported to exist exclusively as an unprocessed ubiquitin fusion protein and as a component of the small (40 S) ribosomal subunit (25). However, in yeast, CEP52 has been shown to exist exclusively in the free, deubiquitinated form and as a component of the large (60 S) ribosomal subunit (24). Recent studies from our laboratory using CEP52-specific antisera have revealed that human CEP52 also exists in cells free from ubiquitin and as a component of the large (60 S) ribosomal subunit. In contrast, CEP76-80 has been shown to exist in the free, deubiquitinated form in all organisms examined and as a component of the small (40 S) ribosomal subunit (17, 23, 24). Furthermore, rat CEP80 has been shown to be the previously characterized 40 S ribosomal protein S27a and yeast CEP76 appears to be the previously identified 40 S ribosomal protein S37 (23, 26).

UBCEP function has been investigated in yeast using mutants bearing engineered deletions of the UBCEP-encoding genes, UBI1, UBI2, and UBI3 (24). Deletions of any of these genes conferred a slow growth phenotype to cells, with the UBI3 mutant (ubi3) being most severe. Mutants deficient in UBCEP2 expression (ubi1, ubi2) were inviable. Biochemical analysis of these mutants revealed deficiencies in ribosomal structure, such as a substoichiometric ratio of 18 S to 25 S ribosomal RNA (ubi3), deficiency in 40 S subunits (ubi3), and deficiency in 60 S subunits (ubi1). Interestingly, expression of the UBI3 gene product (CEP76) without an amino-termi-nally fused ubiquitin was very inefficient in complementing the ubi3 mutant phenotype, indicating that the function of CEP76 is significantly enhanced by its transient covalent association with ubiquitin (24).

Human ubiquitin carboxyl extension proteins (HUBCEPs) have previously been shown to undergo rapid and complete processing to produce free ubiquitin and CEP when heterologously expressed in yeast (17). Furthermore, the processed products were shown to be stable in this host (17). The objective of the present study was to determine if human CEPs are functionally interchangeable with their yeast homologs. To test this, we identified the cellular location of the human CEP80 protein in yeast during heterologous expression and determined the ability of human CEP80 to functionally complement the yeast ubi3 mutant phenotype.

**EXPERIMENTAL PROCEDURES**

**Materials**—Chemicals including buffers, solvents, and dyes were obtained from Sigma. Nitrocellulose (0.2 μm) was purchased from Schleicher & Schuell.

**Strains and Expression Plasmids**—Saccharomyces cerevisiae strains F762, Sub62 (wild type), and ubi3 (ubi3 deletion mutant) were employed for expression and complementation analysis (17, 24). Sub62 is the congenic wild type relative of ubi3 mutants and was employed in experiments comparing normal and mutant cells. Human UBCEP80 (HUBCEP80) expression plasmids were constructed and expressed under control of the Cu"+-inducible metallothionein promoter as described previously (17).

**SDS-PAGE, Protein Blotting, and Immunodetection**—Equal amounts of total or fractionated cell proteins were quantitated by the BCA method (Pierce) and separated on 18% SDS-polyacrylamide gels, and resolved proteins were either stained with Coomassie Brilliant Blue or blotted to nitrocellulose (Trans-Blot, Bio-Rad) followed by immunodetection. Methods for protein transfer and immunode-tection were as previously described (17).

**RNA Analysis**—Total RNA was extracted from yeast cells as follows. Aliquots of yeast cultures (10–50 ml) were pelleted, washed in diethylpyrocarbonate-treated water, and resuspended in 0.5 ml of diethylpyrocarbonate containing vanadium ribonuclease inhibitor.

**Fig. 1.** Distribution of HCEP80 among yeast ribosomal subunits. HUBCEP80 was expressed in S. cerevisiae strain F762, and ribosomal subunits were purified. Top, absorbance across the sucrose gradient. Sedimentation was from right to left. Bottom, individual fractions were analyzed by SDS-PAGE, and the location of HCEP80 was determined by immunoblot using the human-specific, anti-HCEP80 antisera c80-3. HCEP80 migrates on SDS-PAGE as a 16-kDa protein (17, 19).

**Fig. 2.** Growth rates of S. cerevisiae ubi3 containing various expression plasmids. Fresh, midlog cultures of cells were inoculated in YPD (rich) media to an A600 of 0.2, and growth was monitored spectrophotometrically at a wavelength of 600 nm for up to 35 h. Symbols: ○, wild type; ○, ubi3 + HUBCEP80 plasmid; ●, ubi3 + ubiquitin plasmid and HCEP80 plasmid; □, ubi3 + HCEP80 plasmid; ▲, ubi3 + control plasmid; △, ubi3 alone.
Ubiquitin Carboxyl Extension Proteins

**RESULTS**

**Localization of Expressed Human CEP80 to the 40 S Ribosomal Subunit in Yeast**—Previous investigations on the heterologous expression of human ubiquitin carboxyl extension proteins (HUBCEPs) in yeast demonstrated that the expressed proteins are stable in these cells and that the fusion proteins undergo rapid cleavage during or after translation to generate free ubiquitin and CEP80 (17). As a first step in determining if the 80-amino acid human extension protein (HCEP80) can function in yeast, the subcellular location of HCEP80 was determined in these cells following expression of HUBCEP80. Yeast 60 S and 40 S ribosomal subunits were purified on sucrose gradients, and ribosomal subunit proteins were resolved by SDS-PAGE, transferred to nitrocellulose, and subjected to immunodetection using antisera selective for HCEP80 (14). As shown in Fig. 1, expression of HUBCEP80 in yeast resulted in the specific localization of HCEP80 to the 40 S ribosomal subunit. In addition, a significant amount of expressed HCEP80 was detected in the region of the sucrose gradient containing free, nonribosomally associated proteins (Fig. 1, Fractions 4–19). This observation may be explained by the saturation of available HCEP80 binding sites on 40 S subunits during high level expression of the protein.

**Preparation of Ribosomes**—Ribosomes and ribosomal subunits were prepared from yeast as described previously by Otaka and Kobata (26).

**Analysis of yeast ribosomal RNA was performed by ethidium bromide staining as described previously (24). For Northern transfer analysis, equal amounts of total RNA (10–20 μg) were separated on 1.5% agarose gels containing 0.2 M formaldehyde and transferred to positively charged nylon sheets by capillary action for 12–24 h. Following transfer, blots were baked at 80 °C for 1.5 h, prewashed (0.1 × SSC, 0.01% SDS) for 1 h and prehybridized (5 × SSC, 2 × Denhardt's, 0.01% SDS, 50 μg/ml salmon sperm DNA) overnight. Following prehybridization, blots were hybridized with 32P-labeled DNA probes in prehybridization buffer containing 10% dextran sulfate for 20–24 h. The oligonucleotide probe UBI3, corresponding to the complementary sequence of nucleotides 1116–1143 of the yeast UBI3 gene (41, was synthesized on an Applied Biosystems 380B DNA Synthesizer using cyanoethyl phosphoramidite chemistry (17) and was used at an activity of 4–5 × 10^6 cpm/ml. Full length DNA probes to yeast polyubiquitin (4) or human CEP80 (14) were used at an activity of 1–2 × 10^6 cpm/ml. Following hybridization, blots were washed and autoradiographed.

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HUBCEP80. This result is somewhat surprising since many ribosomal proteins have been reported to be short-lived if not assembled within ribosomes (26). Nevertheless, these results demonstrate that, following expression of HUBCEP80 in yeast, HCEP80 localizes to the correct subcellular structure, the 40 S ribosomal subunit.

**Complementation of the Yeast Mutant ubi3 by HUBCEP80**—The ability of HUBCEP80 to functionally replace its yeast homolog during heterologous expression was tested using the *S. cerevisiae* mutant ubi3, which is devoid of genes encoding for YCEP76 (24). This strain grows extremely slowly and has a severe deficiency in 40 S ribosomal subunits and a substoichiometric ratio of 18 S/25 S ribosomal RNA (24). Four expression plasmids were employed: 1) a control plasmid (pYSK136), which does not express a gene product; 2) a ubiquitin expression plasmid (YEP-46); 3) a plasmid expressing HCEP80 without amino-terminally fused ubiquitin (pYSK-HCEP80); and 4) a plasmid expressing the HUBCEP80 fusion protein (pYSK-HUBCEP80). Construction schemes and expression analysis of all of these plasmids have previously been described (17, 27). Plasmids were transformed into *ubi3* mutants, transformants were selected and grown in rich media (YPD), and cell growth was monitored (17, 24). As shown in Fig. 2, *ubi3* mutants expressing HUBCEP80 grew at a rate similar to that of wild type cells, both displaying a doubling time of approximately 2 h. In contrast, *ubi3* mutants containing the control plasmid grew at an extremely slow rate, displaying a doubling time of approximately 12 h. Growth rates were monitored for two other transformants; one containing the HCEP80-expression plasmid (without amino-terminally fused ubiquitin) and a second transformant containing two expression plasmids: one expressing HCEP80 and the other expressing ubiquitin. As shown in Fig. 2, these transformants displayed virtually identical growth rates that were intermediate between wild type and *ubi3* cells (approximately 8-h doubling times). This result indicates only partial complementation of the *ubi3* mutant phenotype by these expression plasmids.

Transformants were next subjected to biochemical analysis. Fig. 3 shows the results of a Northern blot in which total RNA was extracted from transformants and hybridized to specific DNA probes complementary to the various plasmid messenger RNA products. The ratio between polyubiquitin transcripts and UBCEP transcripts varied considerably between the different transformants (Fig. 3, panel A). Wild type and *ubi3* cells expressing HUBCEP 80 showed low levels of polyubiquitin transcripts and high levels of UBCEP transcripts whereas *ubi3* cells alone displayed the opposite pattern: high levels of polyubiquitin transcripts and low levels of UBCEP transcripts. Cells expressing HCEP80 alone displayed intermediate levels of both polyubiquitin and UBCEP transcripts. These results are consistent with the reciprocally coordinated expression of polyubiquitin genes and UBCEP genes (3, 4, 9, 12). Since *ubi3* mutants are not significantly ubiquitin-deficient (24), it is unlikely that the augmented expression of the polyubiquitin gene in these cells is due to a ubiquitin-mediated negative feedback mechanism. These observations can perhaps be explained by the stress inducibility of the yeast polyubiquitin gene (21). *ubi3* mutants may be stressed because of translational errors resulting from malfunctioning of their small subunits, which lack the UB13 protein. Alternatively, the low levels of small subunits in this mutant could possibly induce stress simply because they can support only very low rates of protein synthesis.

The results of Fig. 3 indicate that expression of HCEP80 in *ubi3* mutants, either alone or as a ubiquitin fusion, attenuates polyubiquitin gene expression. This observation can be explained by partial (HCEP80) or complete (HUBCEP80) complementation of the “stress phenotype” of *ubi3* cells by the human extension protein, although a direct effect on polyubiquitin gene expression by HCEP80 cannot be ruled out. Probes directed to *ubi3* transcripts and HCEP80-encoding transcripts were also employed in the analysis described above. As shown in Fig. 3B, only wild type cells expressed *ubi3* transcripts, demonstrating that none of the *ubi3* mutants analyzed above reverted to the wild type *UB13* genotype. Furthermore, expression of HCEP80 transcripts and HUBCEP80 transcripts was observed only in the appropriate transformants and were present at approximately equal levels (Fig. 3C).

Analysis of the ratios of 18 S and 25 S ribosomal RNAs in *ubi3* mutants generated results in agreement with those found for growth rates described above. As shown in Fig. 4, *ubi3* mutants displayed a dramatic substoichiometric ratio of 18 S to 25 S ribosomal RNA, with the amount of 18 S ribosomal RNA being virtually undetectable. This deficiency was alleviated by expression of HUBCEP80 in these cells (Fig. 4). Expression of HCEP80 without amino-terminally fused ubiquitin also resulted in a significant increase in the levels of 18 S ribosomal RNA in *ubi3* mutants, but the ratio between
18S and 25S still remained substoichiometric.

Analysis of HCEP80 production in ubi3 mutants expressing either the HCEP80 plasmid or the HUBCEP80 plasmid was performed by immunoanalysis and yielded surprising results. As shown in Fig. 5A, the levels of mRNA encoding HCEP80 or HUBCEP80 in ubi3 mutants were approximately equal. However, as shown in Fig. 5B, the amount of HCEP80 protein produced for the HUBCEP80-encoding transcripts was at least 5-fold higher compared to the HCEP80-encoding transcripts. These results may explain the inability of HCEP80, expressed without amino-terminally fused ubiquitin, to complement the ubi3 mutant phenotype completely.

Purified ribosomes from ubi3 mutants, containing either the HCEP80 expression plasmid or the HUBCEP80 expression plasmid, were dissociated, and subunits were purified and subjected to immunoanalysis using anti-HCEP80 antisera. As shown in Fig. 6 (top), cells expressing HCEP80 without amino-terminally fused ubiquitin contained a significantly lower level of 40S subunits as compared to cells expressing the HUBCEP80 fusion gene. These results are consistent with the substoichiometric ratio of 18S to 25S ribosomal RNA observed in ubi3 mutants expressing HCEP80 (Fig. 4). Furthermore, immunoanalysis of 40S and 60S subunit proteins demonstrated that HCEP80 localized to the 40S ribosomal subunit in cells expressing either HCEP80 or HUBCEP80 (Fig. 6, bottom). Thus, amino-terminally fused ubiquitin is not a strict requirement for the localization of HCEP80 to the 40S ribosomal subunit in yeast.

**DISCUSSION**

The experimental results presented in this manuscript support the hypothesis that ubiquitin carboxyl extension proteins (UBCEPs) evolved early during eukaryotic evolution and that they function similarly in all eukaryotes. As reported previously, the mutant yeast strain ubi3, which is devoid of genes encoding the long ubiquitin carboxyl extension protein (YUBCEP76), displayed marked abnormalities in both growth properties and ribosome structure (24). Expression of the human homolog of YUBCEP76 (HUBCEP80) in ubi3 mutants efficiently reversed these defects, demonstrating functional interchangeability between the yeast and human proteins. Furthermore, the human extension protein was found to associate with the correct subcellular structure during expression in yeast (the 40S ribosomal subunit) and the efficiency of complementation of ubi3 mutants by the human protein was dramatically increased by its fusion with ubiquitin. Although yeast CEP76 (YCEP76) and human CEP80 (HCEP80) display significant sequence conservation at the protein level (61% identity) (4, 15), these results are still surprising in view of the considerable differences that exist in the primary sequences of these proteins. Furthermore, since the short carboxyl extension proteins of yeast and human (YCEP52 and HCEP52) are even more conserved at the amino acid level than CEP76–80 (>80% identity) (4, 15), it is likely that these proteins are also functionally interchangeable.

Although HUBCEPs are rapidly processed to free ubiquitin and CEP when expressed in yeast (17), expression of HCEP80 alone or simultaneously with ubiquitin from separate plasmids did not complement the ubi3 mutant phenotype as efficiently as expression of plasmids encoding HUBCEP80. Thus, although amino-terminal fusion with ubiquitin is not a strict requirement for CEP function, it is required for efficient extension protein function. This conclusion is consistent with the proposed role of ubiquitin in this capacity as a “molecular chaperone” in ribosome biogenesis (24).

Although it is clear that CEPs are ribosomal proteins which are involved in certain aspects of ribosome assembly, their precise function and mechanism of action remain unknown. Do CEPs have strictly structural roles in the ribosome or are they also involved in certain aspects of ribosomal RNA processing or messenger RNA translation? Based on their highly basic nature and the presence of a zinc-finger motif, it is reasonable to speculate that CEPs associate with ribosomes.
by binding specifically to ribosomal or messenger RNA. However, specific interactions of CEPs with other ribosomal proteins cannot be ruled out. It has previously been demonstrated that the \textit{UB13} gene product in yeast, YCEP76, is required for the final processing steps in the formation of 18S ribosomal RNA (24). However, whether YCEP76 is directly involved in the processing of ribosomal RNA precursors or whether it participates in processes upstream of these events which subsequently affect the production of 18S ribosomal RNA and its precursors is unknown.

Although expression of HCEP80 and HUBCEP80 in yeast resulted in the production of comparable levels of their respective mRNAs, the amount of HCEP80 protein detected during expression of the HUBCEP80 fusion mRNA was at least 5-fold more than that of HCEP80 mRNA. These observations suggest that ubiquitin plays a role in enhancing HCEP80 synthesis or stability. One possible explanation for these findings is that fusion with ubiquitin aids in the proper folding of carboxyl extension proteins during translation, which may be required for the stabilization of these proteins following processing. Alternatively, the ubiquitin-encoding portion of the UBCEP mRNA may serve to enhance the efficiency of CEP mRNA translation, increasing CEP production to levels that are required for normal ribosome assembly. Although the mechanism by which ubiquitin fusion enhances CEP production in cells is unknown, reports that the presence of ubiquitin, or its encoding mRNA, is the single requirement for enhanced expression of ubiquitin gene fusions (28, 29). Mutagenesis studies directed to portions of the ubiquitin molecule known to be important for ubiquitin structure and function (30) should provide important insights toward the mechanism of enhanced CEP production by ubiquitin fusion and the role of ubiquitin in CEP function and ribosome assembly.

REFERENCES