A Novel Function of the Human Chaperonin CCT Epsilon Subunit in Yeast

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A malfunction in the yeast HAC1 causes the unfolding-protein response in the endoplasmic reticulum, resulting in stress-sensitive and inositol auxotrophic phenotypes. Chaperonin-containing TCP1 (CCT) is necessary for the folding of actin and tubulin in the cytosol. The introduction of the truncated human CCT epsilon subunit into yeast cells of which hac1 was disrupted clearly suppressed not only its inositol auxotrophic phenotype but also its stress-sensitive phenotype.

Key words: unfolded-protein-response; yeast HAC1; inositol auxotrophic phenotype; human chaperonin

In eukaryotic cells, all proteins that enter the secretory pathway must pass through the endoplasmic reticulum (ER) to be folded properly, and this process is aided by molecular chaperones. The accumulation of miss-folded proteins in the lumen of the ER causes the so-called unfolded-protein response (UPR). In yeast, Ire1, an ER-resident transmembrane kinase/RNase, senses the protein folding status inside the ER. When activated, Ire1 undergoes autophosphorylation and oligomerization, which activates its RNase activity and initiates non-conventional mRNA splicing of HAC1 mRNA. Splicing results in the production of transcription factor Hac1, which induces UPR target gene expression. The expression of these genes, including those of ER resident chaperones and critical protein-folding enzymes, restores ER homeostasis by increasing the protein folding capacity, and causes attenuation of UPR signaling.

Experimentally, the UPR can be triggered in yeast by blocking glycosylation with drugs such as tunicamycin (Tm) or by preventing disulfide-bond formation with reducing agents such as 2-mercaptoethanol. In ire1 and hac1 mutants, the UPR is completely blocked, and hence the mutants cannot grow in the presence of Tm or 2-mercaptoethanol. ire1 and hac1 mutants also do not grow in the absence of inositol in the growth medium. Hac1 and Ire1 have been found to be necessary for the expression of INO1, which encodes inositol 1-phosphate synthase. The SCS2 gene was first identified as a multi-copy suppressor gene for the inositol auxotrophic phenotype of the hac1 mutation (also known as the ire15 mutation). Op1 and Scs2 were found to be involved in the activation of INO1 by Brickner and Walter. They obtained evidence that positive transcription activators Ino2 and Ino4 constitu-

tively associate with the INO1 promoter, which is kept transcriptionally inactive by Op1. Triggering of the UPR leads to the production of Hac1, which promotes Op1 dissociation from chromatin, and Ssc2 prevents re-association of Op1 with chromatin. Hence Hac1, on activation of the UPR, triggers INO1 expression in addition to binding to a 23-bp UPR element in the promoter of UPR-regulated genes, which produces chaperone molecules, but the precise mechanism by which Hac1 promotes Op1 dissociation from chromatin is not yet known.

Folding of proteins in the cytosol is also assisted by several kinds of molecular chaperones. One family of these, the chaperonins, is conserved in all organisms, eukaryotes, archae-bacteria, and eubacteria. Chaperonin-containing TCP1 (CCT) (also known as TRiC) is an eukaryotic chaperonin. CCT was originally discovered and characterized due to its essential role in the folding cytoskeletal proteins actin and tubulin. It is the only chaperonin identified to date in the eukaryotic cytosol. It is made up of two rings, each of which consists of eight different subunits, known as CCTα-CCTβ in mammals and CCT1-CCT8 in yeast. Each CCT subunit-encoding gene is as old as the origin of eukaryotes and is highly conserved from mammals to yeasts, suggesting independent roles of the various subunit species in chaperonin function.

In previous work, we isolated several human and yeast genes that suppress the inositol auxotrophic phenotype of a yeast Hac1-malfunction strain. They include cDNAs for TGFβ receptor, 14–3–3 protein, protein phosphatase type Ia subunit A, and protein arginine methyl transferase from a human cDNA library. From a yeast genomic DNA library, we isolated CIN5 and YLR149c as multi-copy suppressor genes, in addition to SCS2, as described above. We also confirmed that yeast gene counterparts for human cDNA, viz., those for 14–3–3 protein and protein arginine methyl transferase, suppress the inositol auxotrophic phenotype of a hac1 mutant when introduced as a multi-copy. However, none of the human cDNAs and yeast genes identified to date suppresses the Tm-sensitive phenotype, another characteristic phenotype of the hac1 mutant.

To gain more insight into the inositol auxotrophic phenotype caused by UPR dysfunction and to elucidate the details of the mechanism by which Hac1 activates INO1 expression, we attempted to isolate new suppressor genes from a human cDNA library.

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Abbreviations: ER, endoplasmic reticulum; CCT, chaperonin-containing TCP1; Tm, tunicamycin
From the same human cDNA library and by the same method as that used previously, the \( \Delta \text{hac1} \) strain (HU1) and a cDNA library obtained from a human glioblastoma, we obtained a new clone and named it p#7. Sequence analysis revealed that the clone contained a cDNA fragment of the CCT epsilon subunit: 15 nucleotides of the 5' untranslated region and 774 nucleotides of the coding region. The expected gene product comprised 258 amino acids of the original CCT epsilon, about one half of the entire protein (541 amino acids). It contained consensus ATP-binding motif GXGXXG (X is any amino acid).

As shown in Fig. 1, the introduction of plasmid p#7 into the HU1 strain clearly suppressed the inositol auxotrophic phenotype, whereas vector plasmid pADNS did not. All the strains grew on minimal medium supplemented with inositol. The cells into which plasmid p#7 was introduced also showed Tm resistance. Ire1 is epistatic to Hac1, and hence it is possible that the truncated version of CCT suppresses the phenotype caused by Ire1 disruption. To determine the nature of the CCT epsilon further, we constructed a plasmid that produces the full-length CCT epsilon subunit under the control of the yeast ADH1 promoter.

Plasmid KIAA0098, which contains the full-length cDNA for CCT epsilon (GeneBank accession no. D43950; cDNA length, 1,891) was obtained from the Kazusa DNA Research Institute (Kazusa, Japan). KIAA0098 was digested with HinDIII and NotI, and an approximately 1.9-kbp fragment was inserted between the HinDIII and NotI sites of pADNS to produce pAD-hCCT5. We also constructed a plasmid that harbored part of the yeast CCT5 gene, which encodes yeast CCT subunit 5, Cct5. The DNA region of yeast CCT5 for 279 amino acid residues, corresponding to truncated human CCT epsilon of p#7, was amplified by the PCR method with primers 5'-ATGGCTGCTCGTCCACAAC-3' and 5'-AGGAGTTTCAAAATGGACAC-3', with genomic DNA as a template. The approximately 0.8-kbp PCR fragment thus obtained was cloned with a DynaExpress TA PCR Cloning Kit (BioDynamics Laboratory, Tokyo) to yield pT-yCCT5m. Plasmid pT-yCCT5m was digested with EcoRI and HindIII, and then an approximately 0.8-kbp DNA fragment was inserted between the EcoRI and HindIII sites of pADNS, to generate pAD-yCCTm. pADNS, which is a vector derived from pADNS from which the EcoRI site in the LEU2 gene has been removed.

Fig. 1. Effects of Human cDNA on the Growth Phenotype of the \( \Delta \text{hac1} \) Strain.

The wild-type strain (D452-2) and the \( \Delta \text{hac1} \) strain (HU1) were transformed with pADNS (vector) or plasmid p#7. The transformant cells were streaked onto minimal media with (+Ino) and without inositol (−Ino), and with Tm (+Tm) and without Tm (−Tm), in addition to histidine and uracil. Inositol, histidine, and uracil were added each to the medium to a concentration of 20 μg/mL. Tm was added to a final concentration of 0.5 μg/mL. The cells were grown at 30 °C for 3 d.

Fig. 2. Effects of Full-Length Human cDNA and Yeast Truncated CCT5 on the Growth Phenotype of the \( \Delta \text{hac1} \) Strain.

The \( \Delta \text{hac1} \) strain (HU1) was transformed with pADNS (vector), plasmid p#7 (truncated human CCT epsilon), pAD-hCCT5 (full-length human CCT epsilon) or pAD-yCCTm (truncated yeast CCT5). The transformant cells were 10-fold serially diluted and then spotted onto minimal medium with (+Ino) and without inositol (−Ino), in addition to histidine. Inositol and histidine were added to the medium each to a concentration of 20 μg/mL. The cells were grown at 30 °C for 2 d (+Ino) or 3 d (−Ino).

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pAD-hCCT5, pAD-yCCTm, and vector plasmid pADNS were introduced into the Δhac1 strain (HU1). As shown in Fig. 2, all the transformants grew on the medium supplemented with inositol, but the introduction of the full-length human CCT epsilon cDNA (pAD-hCCT5) did not suppress the inositol auxotrophic phenotype of the Δhac1 strain (HU1). This suggests that the C-terminal region of the human CCT epsilon subunit has a negative effect on rescue of the UPR defect, unless otherwise modified, as by phosphorylation.

On the other hand, the introduction of yeast truncated CCT5 (pAD-yCCTm) slightly but clearly suppressed the inositol auxotrophic phenotype. The doubling time of the Δhac1 strain (HU1) harboring plasmid pAD-yCCTm was 20 h in minimal medium without inositol, whereas that of the Δhac1 strain (HU1) harboring p#7 was 7 h. This suggests that some difference in amino acid length or nature is necessary for suppression of the UPR dysfunction, as between the human CCT epsilon subunit and yeast Cct5, though they are similar in amino acid sequence (46% of the amino acids are identical).

The results presented here indicate that human CCT epsilon is not merely a subunit of CCT, but has a unique function in the protein folding process in the ER. It has been reported that a mutation in the epsilon subunit causes autosomal recessive mutilating sensory neuropathy with spastic paraplegia.20) CCT functions in the cytosol, whereas UPR works in the ER. The epsilon subunit might play a role in cross-talk between protein folding in the ER and in the cytosol.

It is also noteworthy that the truncated epsilon subunit is the first example of suppressors that is involved in protein folding, and that it suppresses both the yeast inositol auxotrophic phenotype and the Tm-sensitive phenotype caused by UPR malfunction. Suppresser genes obtained previously elevated the expression of INO1,21) and thus produced more inositol. Thus the human CCT epsilon gene should prove a useful tool to elucidate further the mechanisms by which the inositol auxotrophic phenotype is caused in Hac1-defect yeast cells.

References