An Endoplasmic Reticulum Cyclophilin Cpr5p Rescues Z-type α_1 -Antitrypsin from Retarded Folding

Chan-Hun Jung, Jeong Hun Lim, Kyunghye Lee,[†] and Hana Im^{*}

Department of Molecular Biology, Sejong University, Seoul 143-747, Korea. *E-mail: hanaim@sejong.ac.kr

[†]Department of Chemistry, Sejong University, Seoul 143-747, Korea

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Human α_1 -antitrypsin (α_1 -AT) is a natural inhibitor of neutrophil elastases and has several dozens of genetic variants. Most of the deficient genetic variants of human α_1 -AT are unstable and cause pulmonary emphysema. However, the most clinically significant variant, Z-type α_1 -AT, exhibits retarded protein folding that leads to accumulation of folding intermediates. These aggregate within the endoplasmic reticulum (ER) of hepatocytes, subsequently causing liver cirrhosis as well as emphysema. Here, we studied the role of an ER folding assistant protein Cpr5p on Z-type α_1 -AT folding. Cpr5p was induced > 2-fold in Z-type α_1 -AT-expressing yeast cells compared with the wild type. Knockout of *CPR5* exacerbated cytotoxicity of Z-type α_1 -AT, and re-introduction of *CPR5* rescued the knockout cells from aggravated cytotoxicity caused by the α_1 -AT variant. Furthermore, Cpr5p co-immunoprecipitated with Z-type α_1 -AT and facilitated its protein folding. Our results suggest that protein-folding diseases may be suppressed by folding assistant proteins at the site of causal protein biosynthesis.

Key Words : α_1 -Antitrypsin, Cpr5p, Peptidyl-prolyl isomerase, Protein folding

Introduction

α_1 -Antitrypsin (α_1 -AT) is a prototypical member of the serine protease inhibitor (serpin) superfamily¹ that includes several protease inhibitors, such as α_1 -antichymotrypsin, antithrombin-III, C1 inhibitor, and plasminogen activator inhibitor-1. Serpin shares a common tertiary structure composed of a mobile reactive center loop (RCL), three β -sheets, and several α -helices.² When these enzymes inhibit target proteases, the metastable native form of serpins facilitates insertion of the RCL into β -sheet A.³ However, incorporation of metastable intramolecular interactions during the folding process makes serpin molecules vulnerable to folding defects,⁴ and more than 100 genetic variants of serpins have been reported.⁵

α_1 -AT is synthesized in the liver and secreted into plasma to protect tissues against indiscriminate proteolytic attacks from neutrophil elastases.⁶ Many dysfunctional genetic variants of α_1 -AT cause an imbalance between serum proteases and their inhibitors, causing serious clinical problems, such as emphysema.³ Most of the dysfunctional α_1 -AT variants show conformational instability and fold into loosely packed structures prone to aggregation.⁷ During inflammation, elevated body temperature can exacerbate α_1 -AT polymerization, leading to the onset of clinical symptoms indicative of α_1 -AT deficiency.⁸ Z-type α_1 -AT (Glu 342 $\rightarrow$ Lys) is the most clinically significant α_1 -AT variant, affecting 1 in 1,800-2,000 live births, and 8-10% of homozygotes develop liver cirrhosis.^{9,10} In contrast to other variants, the molecular basis of the Z-type variant deficiency was identified as severely retarded protein folding,¹¹ which leads to the accumulation of folding intermediates with a high tendency to polymerize

at the site of biosynthesis in the endoplasmic reticulum (ER) of hepatocytes, causing liver cirrhosis as well as emphysema. Once folded, the native form of the Z-type α_1 -AT variant showed considerable stability and activity. Thus, facilitating folding of this α_1 -AT variant might be a viable approach to prevent onset of clinical symptoms.

Folding assistant proteins, including molecular chaperones, facilitate proper folding of newly synthesized polypeptide chains, assembly of subunits and transport of the polypeptide into the correct cellular compartments. Some folding assistant proteins, such as protein disulfide isomerases and peptidyl-prolyl isomerases (PPIases), have enzymatic activity that accelerates the rate limiting steps of protein folding.¹² Since α_1 -AT has no disulfide bond, the enzymatic activity of protein disulfide isomerases is not likely to contribute to the folding of Z-type α_1 -AT. However, α_1 -AT has 17 X-Pro peptide bonds out of 393 total peptidyl bonds, which may limit the folding rate of the protein, and the level of PPIases was shown to be elevated in Z-type α_1 -AT expressing yeasts.

PPIases accelerate the rate-determining proline *cis-trans* isomerization steps in protein folding.^{13,14} They are classified into three major groups: the cyclophilins, the FK506-binding proteins (FKBPs), and the parvulins. Cyclophilins were originally discovered as cellular targets that bind with high affinity to the immunosuppressive drug cyclosporin A,^{15,16} and these complexes inhibit PPIase activity and calcineurin-mediated immune responses.¹⁶⁻²⁰ Cyclophilins are highly conserved proteins found in all organisms examined and in all subcellular compartments, including the cytosol, nucleus, ER, and mitochondria,²¹ implying that they have critical roles. Humans have > 10 cyclophilins, including the seven major cyclophilins hCypA, hCypB, hCypC, hCypD, hCypE,

hCyp40, and hCypNK.²² After cytosolic hCypA, hCypB is the second most abundant cyclophilin, with an *N*-terminal signal sequence that targets the protein to the ER, where it localizes mainly in the lumen.²³ An ER stress response element (ERSE), a consensus sequence necessary for transactivation of genes encoding ER chaperones during the unfolded protein response (UPR), is located upstream of the hCypB ORF. Indeed, ER protein misfolding induced by tunicamycin, an inhibitor of *N*-linked protein glycosylation, increased the expression of hCypB approximately two-fold.²⁴ Meanwhile, overexpression of hCypB attenuated ER stress-induced apoptosis.²⁴ There are several studies showing that ER cyclophilin(s) are required for proper folding of secreted or membrane proteins, including *Drosophila* cyclophilin *ninaA*, which is required for the secretion of rhodopsin.²⁵ In addition, treatment of chicken embryo tendon fibroblasts with cyclosporin A inhibits the proper folding of procollagen I.²⁶

Yeast is a good model system to study the function(s) of a particular protein, due to the availability of high throughput genetic screening and accumulation of large amounts of biochemical and genetic information. *Saccharomyces cerevisiae* has eight different cyclophilins (Cpr1p-Cpr8p),²² and Cpr5p, a yeast homologue of hCypB, is also localized in the ER.^{27,28} To study whether the folding defects of Z-type α_1 -AT could be remedied by Cpr5p, human wild-type and Z-type α_1 -AT were each overexpressed in *S. cerevisiae*. Expression of Cpr5p was induced more than two-fold in cells expressing Z-type α_1 -AT compared with those expressing wild-type α_1 -AT. If the protein is involved in the response to Z-type α_1 -AT-induced ER stress, knockout of the coding gene should increase the susceptibility of the cells to Z-type α_1 -AT-induced cytotoxicity. In this study, the effects of *CPR5* knockout on cell viability upon Z-type α_1 -AT overexpression were examined. Physical interaction between Cpr5p and Z-type α_1 -AT was confirmed by co-immunoprecipitation analysis, and the contributions of Cpr5p in Z-type α_1 -AT protein folding were analyzed.

Experimental

Strains and Reagents. Human α_1 -AT was overexpressed in *S. cerevisiae* BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and the derived *cpr5Δ* strain (Open Biosystems Inc., Huntsville, AL, USA). *Escherichia coli* BL21 (DE3) was from Novagen Inc. (Madison, WI, USA). Bio-Rad detergent-compatible (DC) protein assay kit was purchased from Bio-Rad Laboratories Inc. (Hercules, USA). Rabbit anti-human α_1 -AT antibody and goat anti-rabbit IgG antibody conjugated to peroxidase were purchased from Sigma (St. Louis, MO, USA). Rat anti-Cpr5p antibody was from Aprogen Co. (Daejeon, Korea). Protein A/G PLUS-Agarose was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Q-sepharoseTM Fast Flow column, HybondTM ECLTM nitrocellulose membrane, and PD-10 desalting column were purchased from GE Healthcare (Piscataway, NJ, USA). Ni²⁺-NTA (nitrilo-tri-acetic acid) agarose was

from Peptron Co. (Daejeon, Korea). Curix CP-BU, a medical X-ray film, was purchased from Agfa Co. (Ridgefield Park, NJ, USA). All other chemicals were reagent grade.

α_1 -AT Expression in Yeasts. pYInu-AT (for human wild-type α_1 -AT expression) and pYInu-ATZ (for human Z-type α_1 -AT expression) was used to express human α_1 -AT in *S. cerevisiae*.²⁹ These plasmids contain an inulinase (Inu) signal sequence under the control of the yeast *GAL10p* promoter. Wild-type BY4741 and the derived *cpr5Δ* yeast strain were transformed with pYInu-AT or pYInu-ATZ using the standard lithium acetate method.³⁰ Transformants were selected by growing cells in drop-out medium lacking uracil at 30 °C for 3 days. The yeast strains transformed with either pYInu-AT or pYInu-ATZ were cultured in YPD (1% yeast extract, 2% peptone, and 2% glucose) liquid medium at 30 °C overnight. Cultured cells were harvested and serially diluted to reach adequate cell densities. Ten μ L of each dilution was spotted on both YPD plate (which did not induce α_1 -AT expression) and YPGal (1% yeast extract, 2% peptone, and 2% galactose) plate (which induced α_1 -AT expression). The plates were further incubated at 30 °C for 2-3 days, and cell growth was observed.

Complementation Analysis. The *CPR5* gene was amplified from *S. cerevisiae* Y2805 (*MATa pep4::HIS3 prb1-d can1 GAL2 his3 ura3-52*) genomic DNA by polymerase chain reaction (PCR) using *Pfu* polymerase (Promega Co., Madison, WI, USA). The forward primer was 5'-CTCCAAGCTTATGAACTTCAATTTTTTTCCTTTATTACCTTATTTGCTT-3' and the reverse primer was 5'-TCCAAAGCTTTTAGAGTTCATCGTGGGCTGCTTCG-3'. The PCR products were digested using *Hind*III and cloned into the *Hind*III sites of a yeast expression vector, pACT2 AD (Clontech Laboratories Inc., Mountain View, CA, USA), for the constitutive expression of Cpr5p. The resulting plasmid was named pACT2-CPR5. The *cpr5Δ* yeast strain was co-transformed with pYInu-ATZ and pACT2-CPR5, and the co-transformants were selected in drop-out medium lacking leucine and uracil at 30 °C for 3-4 days. Cell growth upon the expression of Z-type α_1 -AT was monitored by a spotting assay, as described above.

Co-immunoprecipitation of Cpr5p and Z-type α_1 -AT from Cell Extracts. The *cpr5Δ* yeast strain harboring pYInu-ATZ and/or pACT2-CPR5 was cultured in YPGal liquid medium at 30 °C for 48 h. The cultured cells were harvested and resuspended in 1 mL of lysis buffer (50 mM HEPES, pH 7.0, 1% Triton X-100, 1 mM PMSF, and 1 μ M aprotinin). Cells were lysed by vigorous vortex with glass beads (425-600 μ m in diameter), and the cell extracts were precleared with Protein A/G PLUS-Agarose beads at 4 °C for 2 h. Crude lysates were then incubated with polyclonal rat anti-Cpr5p antibodies (1:100 dilution) at 4 °C overnight. Immune complexes were incubated with Protein A/G PLUS-Agarose beads for 2 h then collected by centrifugation. The immunoprecipitates were washed four times with IP wash buffer (50 mM HEPES, pH 7.0, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride), and the pellet was resuspended in sodium dodecyl sulfate (SDS) sample buffer. Protein concentrations were

determined using a Bio-Rad DC protein assay kit (Hercules, CA, USA). The proteins were resolved by 10% SDS-polyacrylamide gel electrophoresis (PAGE), then transferred to a nitrocellulose membrane. The blots were probed with rabbit anti-human α_1 -AT antibodies, diluted 1:1,000 in PBS containing 0.03% Tween 20 (PBST), and then with horseradish peroxidase-conjugated goat anti-rabbit IgG antibodies diluted 1:10,000 in PBST. Bound antibodies were visualized by enhanced chemiluminescence on X-ray film using luminol as the substrate.

Purification of Cpr5p Expressed in *E. coli*. To express Cpr5p in *E. coli*, the *CPR5* gene without a signal sequence was amplified from *S. cerevisiae* Y2805 genomic DNA by PCR. The forward primer was 5'-CTCAGAATTCAAAGAGGACACGGCAGAAGATCCTGAG-3' and the reverse primer was 5'-TCCACTCGAGTTAGAGTTCATCGTGGGCTGCTTCG-3'. After digestion with *EcoRI* and *XhoI*, the *CPR5*-containing fragment was subcloned into the *EcoRI* and *XhoI* sites of the pET28a vector containing a polyhistidine-tag at the 5' end of the coding region, generating pET28a-CPR5. *E. coli* BL21 (DE3) cells were transformed with pET28a-CPR5 and grown at 37 °C in LB medium containing 50 μ g/ml kanamycin to an optical density at 600 nm of approximately 0.6. Cpr5p expression was then induced by adding 0.1 mM isopropyl β -D-thiogalactoside (IPTG) followed by a 3-h incubation at 37 °C. The cells were harvested and disrupted in buffer (0.1 mM phenylmethylsulfonyl fluoride, 50 mM Tris-Cl, 250 mM NaCl, and 8 mM imidazole, pH 7.9) using a Bandelin sonicator at 43% power and 90% pulse for 2.5 min. The cell lysates were cleared by centrifugation at 14,000 rpm for 30 min in a Hanil Micro 17R+ centrifuge (Hanil Science Industrial Co., Seoul, Korea). The supernatants were loaded on a Ni²⁺-NTA agarose column pre-equilibrated with binding buffer (50 mM Tris-Cl, 250 mM NaCl, and 8 mM imidazole, pH 7.9) and eluted by a linear gradient of 30-1000 mM imidazole in 50 mL of binding buffer. Purified Cpr5p protein was buffer-exchanged to IP wash buffer using a PD-10 desalting column. The concentrations of the purified proteins were measured with a Bio-Rad DC protein assay kit.

Refolding Assay for Z-type α_1 -AT. pFEAT30-ATZ was used for Z-type α_1 -AT expression as inclusion bodies in *E. coli* BL21 (DE3).³¹ The inclusion bodies were dissolved in 6 M urea and Z-type α_1 -AT was quickly purified at 4 °C on a Q-Sepharose™ Fast Flow column equilibrated with refolding buffer (10 mM phosphate, pH 6.5, 50 mM NaCl, and 1 mM EDTA) as previously described.²⁹ To achieve the native conformation of α_1 -AT, 20 μ g of α_1 -AT protein was incubated at 30 °C with/without an equimolar amount of purified Cpr5p. α_1 -AT folding was monitored by 10% nondenaturing gel electrophoresis in a Tris-glycine buffer system.

Results

Knockout of *CPR5* Aggravated Cytotoxicity of Z-type α_1 -AT Expression in Yeast. To evaluate the effects of Cpr5p on cytotoxicity induced by Z-type α_1 -AT over-

expression, human wild-type and Z-type α_1 -AT proteins were expressed under the control of the yeast *GAL10p* promoter in *S. cerevisiae*. Differential protein expression between the wild-type and Z-type variant-expressing yeast cells was analyzed using two-dimensional differential gel electrophoresis (2D-DIGE) and matrix-assisted laser desorption/ionization-time of flight tandem mass spectroscopy (MALDI-TOF-TOF). The Cpr5p protein level was 2.2-fold higher in the cells expressing Z-type α_1 -AT-compared with those expressing wild-type α_1 -AT (Shin, unpublished data). The increased expression of Cpr5p in Z-type α_1 -AT-expressing cells was also confirmed by Western blotting (Suppl. Figure 1). If Cpr5p was involved in the cellular response to misfolded Z-type α_1 -AT accumulation within cells, deletion of the gene should impede the cellular adjustment and exacerbate cytotoxicity of Z-type α_1 -AT expression. Wild-type BY4741 and the derived *CPR5* knockout yeast strains (Open Biosystems Inc.) were transformed with pYInu-AT and pYInu-ATZ to express the wild-type and Z-type α_1 -AT proteins, respectively. Expression of α_1 -AT was induced by growing cells in medium containing 2% galactose, and cell growth was visually monitored on plates spotted with serially diluted cultures. Overexpression of Z-type α_1 -AT caused only moderate cytotoxicity in wild-type yeast, but knockout of *CPR5* aggravated cytotoxicity of Z-type α_1 -AT by reducing cell viability down to 47% (Figure 1). To confirm that the increased cytotoxicity in the *cpr5* Δ strain was due to

Figure 1. Aggravated cytotoxicity of Z-type α_1 -AT in *CPR5* knockout yeast. (a) The yeast cells containing either wild-type (pYInu-AT) or Z-type α_1 -AT (pYInu-ATZ) expression vector, were serially diluted, so that aliquots of 10 μ L of each dilution contains approximately 1500, 150, 75 cells. Ten μ L of each dilution were spotted onto both YPD plates (not to induce α_1 -AT) and YPGal plates (to induce α_1 -AT). The plates were incubated at 30 °C for 2-3 days and cell growth was observed. (b) Densitometric scanning of the boxed spots in Figure 1(a) showed that cell growth of Z-type α_1 -AT-expressing cells was significantly reduced. (**wt**) Expression of Z-type α_1 -AT induced mild cytotoxicity in the wild-type yeast. (***cpr5* Δ**) Expression of Z-type α_1 -AT increased cytotoxicity in the *cpr5* Δ strain, compared to that in the wild-type yeast.

Figure 2. Reintroducing *CPR5* recovered the *cpr5Δ* yeast strain from the aggravated Z-type α_1 -AT-induced cytotoxicity. The *cpr5Δ* yeast strain was co-transformed with α_1 -AT expression vector (either pYInu-AT or pYInu-ATZ) and pACT2-CPR5. (a) The co-transformed cells were serially diluted, and aliquots of 10 μ L taken from the final three dilutions were spotted on both YPD and YPGal plates. After incubation at 30 °C for 2–3 days, cell growth was monitored. (b) Densitometric scanning of the boxed spots in Figure 2(a) showed that reintroduction of *CPR5* recovered cell growth of Z-type α_1 -AT-expressing cells.

knockout of the gene, *CPR5* was reintroduced into the knockout strain, and cell survival recovery was monitored. When the *cpr5Δ* strain was co-transformed with pYInu-ATZ and pACT2-CPR5, cell survival upon Z-type α_1 -AT-expression returned to the level seen in the wild-type strain (Figure 2). These results indicate that *CPR5* is involved in cellular responses to Z-type α_1 -AT-mediated cytotoxicity.

Cpr5p Co-immunoprecipitates with Z-type α_1 -AT. To determine whether Cpr5p interacts with Z-type α_1 -AT in yeast cells, a co-immunoprecipitation study was performed in *cpr5Δ* cells co-transformed with pYInu-ATZ and pACT2-CPR5. Cell extracts were prepared from co-transformed cells grown in YPGal medium, followed by incubation with polyclonal rat anti-Cpr5p antibodies. Bound complexes were isolated using Protein A/G PLUS-Agarose beads, and co-precipitated Z-type α_1 -AT was detected by immunoblotting using rabbit polyclonal anti- α_1 -AT antibodies. Cpr5p co-immunoprecipitated with Z-type α_1 -AT when both Cpr5p and Z-type α_1 -AT were overexpressed (Figure 3, lane 3), but not in the absence of pYInu-ATZ or pACT2-CPR5 transformation (Figure 3, lanes 1 & 2). This result shows that Cpr5p selectively interacts with Z-type α_1 -AT in co-transformed yeast cells.

Cpr5p Facilitates Folding of Z-type α_1 -AT *in vitro*. For *in vitro* refolding assays, Cpr5p was overexpressed in *E. coli* and purified. *CPR5* without the signal sequence was amplified

Figure 3. Co-immunoprecipitation of Cpr5p and Z-type α_1 -AT. The *cpr5Δ* yeast strains harboring pYInu-ATZ and/or pACT2-CPR5 were cultured in YPGal liquid medium. The cell extracts were incubated with polyclonal anti-Cpr5p antibodies (diluted 1:100), and immune complexes were precipitated using Protein A/G PLUS-Agarose beads. The precipitated proteins were resolved on 10% SDS-polyacrylamide gels and analyzed by immunoblotting using rabbit polyclonal anti-human α_1 -AT antibodies.

Figure 4. Purification of Cpr5p. Samples were taken at various steps during purification, and analyzed on a 12% SDS-polyacrylamide gel. Proteins were stained with Coomassie Brilliant Blue. The migration position of Cpr5p is indicated by an arrowhead. Lanes: MW, molecular weight markers (Bio-Rad, standards); C, whole cell lysate before induction of Cpr5p; I, whole cell lysate after induction of Cpr5p; Ni²⁺, Cpr5p purified by Ni²⁺-NTA-agarose column chromatography.

from yeast Y2805 genomic DNA by PCR and cloned onto the pET28a plasmid, which has an N-terminal poly-His-tag. Overexpression of Cpr5p was induced in the *E. coli* BL21 (DE3) strain by addition of IPTG, and the expressed (His)_n-tagged Cpr5p was purified by liquid column chromatography using Ni²⁺-NTA agarose beads. The purification of Cpr5p was analyzed by 12% sodium-dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (Figure 4). The purified Cpr5p retained PPIase activity, which was confirmed using a traditional protease-coupled assay³² (data not shown). The results of the PPIase activity assay and co-immunoprecipitation analysis suggest that Cpr5p reduces Z-type α_1 -AT-induced cytotoxicity by interacting with Z-type α_1 -AT and assisting protein folding.

To further explore this possibility, Z-type α_1 -AT was overexpressed in inclusion bodies and purified in its unfolded form. Unfolded Z-type α_1 -AT was allowed to refold in the absence/presence of Cpr5p, and its folding was assessed by native gel electrophoresis. As previously reported, folding of the wild-type α_1 -AT occurred immediately (Figure 5, lane 6), while that of Z-type α_1 -AT was very slow¹¹ (Figure 5, lanes 2–5). However, addition of Cpr5p accelerated the folding of Z-type α_1 -AT into its native form (Figure 5, lanes

Figure 5. Accelerated refolding of Z-type α_1 -AT by Cpr5p. Recombinant α_1 -AT was expressed in inclusion bodies in *E. coli* BL21 (DE3) and refolded in refolding buffer (10 mM phosphate, pH 6.5, 50 mM NaCl, and 1mM EDTA) at 30 °C for various times with/without the addition of Cpr5p. The α_1 -AT folding was monitored on 10% non-denaturing gels. Migration position of native, intermediate, and dimeric form are indicated by arrow heads.

7-10), suggesting that Cpr5p rescued the Z-type α_1 -AT variant from retarded protein folding.

Discussion

The lumen of the ER is a specialized subcellular compartment for folding of secreted or membrane proteins. Quality control machinery in the ER allows for the advancement of properly folded proteins only via secretory pathways. Misfolded proteins are either retained in the ER or redirected to the cytosol for ER-associated degradation (ERAD). Most unstable α_1 -AT variants are degraded via ERAD and consequently fail to reach the plasma, leading to pulmonary emphysema. Meanwhile, the Z-type α_1 -AT variant has been reported to fold very slowly and to accumulate as folding intermediates prone to intermolecular loop-sheet polymerization.¹¹ Accumulation of misfolded Z-type α_1 -AT in the ER induces cell death, leading to liver cirrhosis in addition to pulmonary emphysema. The native form of Z-type α_1 -AT, once folded, exhibits significant stability and activity. Thus, in this study, we pursued an approach to resolve the slow folding issues associated with Z-type α_1 -AT by aids from folding assistant proteins. Cpr5p was induced by Z-type α_1 -AT expression in yeast, and knockout of *CPR5* aggravated the cytotoxicity of Z-type α_1 -AT (Figure 1), suggesting that Cpr5p is involved in the cellular response to Z-type α_1 -AT accumulation. Interaction between Cpr5p and Z-type α_1 -AT was confirmed by co-immunoprecipitation (Figure 3). Purified Cpr5p accelerated folding of Z-type α_1 -AT *in vitro* (Figure 5), though to a lesser extent compared with another ER cyclophilin, Cpr2p.²⁹ The result suggests the presence of other components of folding complex which are required for full-folding activity. Indeed, the human ortholog of Cpr5p, hCypB, exists in a complex with other chaperones,³³ suggesting that it cooperates with other folding assistant proteins to protect cells against ER stress. *In vitro* folding assays without other cooperating chaperones

might limit the degree of facilitation of Z-type α_1 -AT folding by Cpr5p. However, exacerbated cytotoxicity in the *CPR5* knockout strain (Figure 1) and recovery from the sensitized phenotype upon Cpr5p expression (Figure 2) suggest that Cpr5p plays significant role(s) *in vivo* in the response to Z-type α_1 -AT accumulation, possibly by facilitating folding through cooperation with other chaperones in the complex.

Cis-trans isomerization of X-Pro peptide bonds is extremely slow and considered to be a rate limiting step in protein folding,³⁴ which can be accelerated by PPIases. There are several previous studies suggesting that Cpr5p homologues are involved in folding of secreted or membrane proteins: *Drosophila ninaA* was shown to be essential in the secretion of rhodopsin Rh1 from photoreceptor cells;²⁵ the folding of procollagen I and subsequent production of secreted collagen I were decreased by cyclosporine A, an inhibitor of hCypB, in chick embryonic fibroblast cultures;²⁶ reduction of hCypB levels by siRNA-mediated knockdown or cyclosporin A treatment decreased the secretion of active ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type 1 repeat);³⁵ and siRNA-mediated knockdown of hCypB also significantly increased ER stress-induced apoptosis upon tunicamycin treatment.²⁴ Considering that hCypB is the most abundant cyclophilin in the ER and has highly conserved sequences among organisms, it is likely to play a crucial role in the management of protein folding in the ER.

ER stress has been implicated in the pathology of several protein folding diseases, such as Alzheimer's disease, Parkinson's disease, and polyglutamine-induced aggregation diseases.³⁶⁻³⁸ Further studies would provide a better understanding of the biological function of ER chaperones and their possible contribution to the proper folding of these disease-associated proteins, which might enable the development of novel therapeutic strategies. The knowledge obtained through this study might aid in the identification of new therapeutic targets for prevention/treatment of folding diseases, including Z-type α_1 -AT-induced liver cirrhosis.

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