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The Yeast HtrA Orthologue Ynm3 Is a Protease with Chaperone Activity that Aids Survival Under Heat Stress

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Ynm3 is the only budding yeast protein possessing a combination of serine protease and postsynaptic density 95/disc-large/zona occludens domains, a defining feature of the *h*igh *t*emperature *r*equirement *A* (HtrA) protein family. The bacterial HtrA/DegP is involved in protective stress response to aid survival at higher temperatures. The role of mammalian mitochondrial HtrA2/Omi in protein quality control is unclear, although loss of its protease activity results in susceptibility toward Parkinson's disease, in which mitochondrial dysfunction and impairment of protein folding and degradation are key pathogenetic features. We studied the role of the budding yeast HtrA, Ynm3, with respect to unfolding stresses. Similar to *Escherichia coli* DegP, we find that Ynm3 is a dual chaperone-protease. Its proteolytic activity is crucial for cell survival at higher temperature. Ynm3 also exhibits strong general chaperone activity, a novel finding for a eukaryotic HtrA member. We propose that the chaperone activity of Ynm3 may be important to improve the efficiency of proteolysis of aberrant proteins by averting the formation of nonproductive toxic aggregates and presenting them in a soluble state to its protease domain. Suppression studies with Δ*ynm3* led to the discovery of chaperone activity in a nucleolar peptidyl-prolyl *cis*-trans isomerase, Fpr3, which could partly relieve the heat sensitivity of Δ*ynm3*.

INTRODUCTION

Environmental stresses like heat lead to cell cycle arrest and subsequent cell death due to a variety of reasons, the major one being the accumulation of unfolded proteins. Denatured or unfolded proteins may themselves be toxic or may interfere with cellular detoxification processes. The accumulation of denatured proteins sequesters important cellular proteins or protein complexes into aggregates as in the case of disorders such as Parkinson's disease, in which the unfolded protein, α-synuclein, forms aggregates in the cytosol along with components of the ubiquitin proteasome machinery, thus disrupting the normal functioning of the cellular protein degradation pathway (Krüger *et al*., 2002; Sakahira *et al*., 2002; Snyder *et al*., 2003). Cells cope with heat stress by enhancing the synthesis of thermoprotectant factors; by upregulating the expression of molecular chaperones, which aid in the refolding of misfolded proteins; or by activating proteases involved in eliminating irreversibly unfolded proteins (Imai *et al*., 2003). One of the major classes of heat-shock proteins essential for cell survival at higher temperatures in *Escherichia coli* belongs to the HtrA (*h*igh *t*emperature *r*equirement *A*) family (Lipinska *et al*., 1989; Strauch *et al*., 1989). The HtrA family includes proteins that have a characteristic combination of a protease domain with at least one postsynaptic density 95/disc-large/zona occludens (PDZ) domain (Pallen and Wren, 1997). The bacterial HtrA/DegP protein is the best-characterized member of this family. Its role in handling misfolded proteins in the periplasm of *Escherichia coli* becomes prominent under heat stress (Sklar *et al*., 2007); at normal temperature, the same protein acts as a chaperone (Spiess *et al*., 1999).

Members of the HtrA family are present in many but not all eukaryotic genomes (Vande Walle *et al*., 2008). The human homologues are believed to be involved in arthritis, cell growth, apoptosis, and aging (Ponting, 1997; Faccio *et al*., 2000; Baldi *et al*., 2002; Hegde *et al*., 2002). The mammalian HtrA family member, HtrA2/Omi, resides in the intermitochondrial space, which corresponds to the periplasmic localization of DegP in bacteria (Verhagen *et al*., 2002). Cell culture studies suggest a proapoptotic role for HtrA2/Omi. During apoptosis, HtrA2/Omi is released into the cytosol where it binds inhibitor of apoptosis proteins (IAPs) via the IAP binding domain present in the N terminus of HtrA2/Omi (Suzuki *et al*., 2001; Hegde *et al*., 2002; Verhagen *et al*., 2002). IAPs are proteins that inhibit caspases. Binding of HtrA2/Omi to IAPs presumably triggers its serine protease activity, leading to the cleavage of IAPs, thereby promoting apoptosis (Verhagen *et al*., 2000; Hegde *et al*., 2002; Martins *et al*., 2002). However, the *m*otor *n*euron *d*egeneration 2

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Abbreviations used: CS, citrate synthase; PPIase, peptidyl prolyl *cis-trans* isomerase; Ynm3, yeast HtrA homologue.

(*mnd2*) mutant mice, in which the corresponding gene encodes an intact IAP binding domain but carries a protease inactivating point mutation (S276C), suffer neurodegeneration leading to juvenile death (Jones *et al*., 2003). Interestingly, the HtrA2 knockout mice show a similar phenotype, thus reinforcing the physiological relevance of the serine protease activity of this protein (Jones *et al*., 2003; Martins *et al*., 2004). Cells from these mice are more susceptible to apoptotic stimuli. A certain percentage of cells from HtrA2/Omi knockout mice exhibit abnormal mitochondrial morphology combined with a decreased mitochondrial density (Martins *et al*., 2004). This suggests a more protective than a proapoptotic role for mammalian HtrA2/Omi under physiological conditions, which is more reminiscent of its bacterial homologues. Mutations in the gene encoding HtrA2/Omi have been identified in patients suffering from Parkinson's disease (Strauss *et al*., 2005). This gene has been allocated to the locus PARK13. Proteolytic stress due to the accumulation of unfolded proteins as in Parkinson's disease leads to neuronal damage. One possible role of HtrA2/Omi might be to play a protective role in relieving mitochondria from the toxicity resulting from the accumulation of misfolded proteins. However, no definitive role for HtrA2/Omi in protein quality control has been so far described.

The eukaryotic model organism, *Saccharomyces cerevisiae* encodes an HtrA-like protein called Ynm3 or Nma111. It has an HtrA-like serine protease domain followed by two PDZ domains (Apweiler *et al*., 2000), one domain present immediately proximal to the protease domain (PDZ1) and the other domain at the C-terminal end of the protein (PDZ2). The role of this HtrA-like Ynm3/Nma111 in yeast is still obscure, because seemingly contradictory functions have been ascribed to it in previous reports. It was originally proposed to be a proapoptotic serine protease and hence called Nma111, which stands for *n*uclear *m*ediator of *a*poptosis 111-kDa protein. It was reported that the absence of the corresponding gene *NMA111* rendered yeast resistant to apoptosis induced by H_2O_2. Its overexpression was shown to induce apoptosis (Fahrenkrog *et al*., 2004). The same protein, termed Ynm3, was described as a modulator of fatty acid metabolism. Furthermore, deletion of the *YNM3* locus in the yeast strain YB322 was shown to result in the inability to use nonfermentable carbon sources pointing to a possible mitochondrial role for Ynm3 (Tong *et al*., 2006).

Here, we functionally characterize Ynm3, the budding yeast homologue of mammalian HtrA2/Omi. We address the importance of its serine protease activity and provide evidence for chaperoning activity. This study also led to the identification of a chaperoning ability in the yeast peptidyl-prolyl *cis-trans* isomerase (PPIase) Fpr3.

MATERIALS AND METHODS

Yeast Strains and Growth Conditions

All yeast strains used in this study are listed in Table 1. All yeast strains are congenic to the wild-type YB322 strain (Johnson *et al*., 1994) unless otherwise stated. Standard methods for genetic crosses and transformations were used (Ito *et al*., 1983). The yeast strain RH3340 was obtained by replacing the *YNM3* genomic locus with a module conferring kanamycin resistance. The deletion was verified by polymerase chain reaction (PCR). The yeast strain RH3343 was obtained by PCR-based tagging of the *YNM3* genomic locus at its 5′ region with a yeast-enhanced green fluorescent protein (yeGFP)-encoding module that was PCR amplified from the plasmid pymN25 as described previously (Janke *et al*., 2004). RH3343 expresses yeGFP-Ynm3 under the control of the *GAL1* promoter from its chromosomal locus. The yeast strains RH3344 and RH3345 were obtained by replacing the genomic *FPR3* locus in the wild-type YB322 or in RH3340 with the nourseothricin-resistant module amplified from the plasmid pFA6a-natNT2 (Janke *et al*., 2004), respectively. The strains were grown in standard yeast extract-peptone-dextrose (YPD: 1% yeast extract, 2% peptone, and 2% dextrose) or yeast extract-peptone-galactose (YPGal: 1% yeast extract, 2% peptone, and 2% galactose) supplemented with adenine or in synthetic complete (SC) media (YNB: 1.5 g/l yeast nitrogen base lacking amino acids, 5 g/l ammonium sulfate, 2% dextrose or galactose, and supplemented with amino acids).

Table 1. *S. cerevisiae* strains used in this study

Yeast strain	Genotype	Source
RH3339	YB322 wildtype, *Mat a,ura3,his3, ade2,lys2,leu2, trp1* YB322, *Mat a,ura3,his3,ade2,lys2, leu2,trp1*	Johnson *et al.* (1994)
RH3340	*ynm3Δ::G418* YB322, *Mat a,ura3,his3,ade2,lys2, leu2,trp1*	This study
RH3343	$GAL1_{UAS}$*eGFP::YNM3* YB322, *Mat a,ura3,his3,ade2,lys2, leu2,trp1*	This study
RH3344	*fpr3Δ::NAT* YB322, *Mat a,ura3,his3,ade2,lys2, leu2,trp1*	This study
RH3345	*ynm3Δ::G418; fpr3Δ::NAT*	This study

Plasmid Constructions

All plasmids used in this study are listed in Table 2. All plasmids are derived from p416*MET25* or p426*MET25* unless otherwise indicated. The plasmid pME3325 was constructed by introducing the PCR product containing the *YNM3* open reading frame (ORF) amplified from YB322 chromosomal DNA into BamH1/Xho1-restricted plasmid p416*MET25* (Mumberg *et al*., 1994). The plasmids pME3449, pME3451, and pME3453 were constructed by cloning the *YNM3* ORF or the mutants *YNM3S235A* and *YNM3S236A*, respectively, into the Spe1/Sma1 restriction sites and GFP sequence into the Sma1/Cla1 restriction sites of p416*MET25*. Similarly, the plasmids pME3450, pME3452, and pME3454 were constructed by cloning *YNM3* ORF or the mutants *YNM3S235A* or *YNM3S236A*, respectively, and the GFP encoding sequence into p426*MET25*. The mutations were introduced using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The plasmids pME3455 and pME3457 were constructed by cloning the PCR product encoding Ynm3 without the amino acids 290–375, which constitutes the first PDZ domain (PDZ1) or by inserting a PCR product encoding Ynm3 without the amino acids 779–868, which constitute the second PDZ domain (PDZ2), respectively, into the Spe1/Sma1 restriction sites and GFP sequence into the Sma1/Cla1 restriction sites of p416*MET25*. Plasmids pME3456 and pME3458 were constructed similarly by cloning DNA encoding Ynm3ΔPDZ1 or Ynm3ΔPDZ2, respectively, and GFP encoding sequence into p426*MET25*. The plasmid pME3459 contains GFP-encoding sequence in the Sma1/Cla1 restriction sites of p416*MET25*. The plasmids pME3460, pME3461, or pME3462 were obtained by inserting *YNM3*, *YNM3S236C*, or *YNM3S236A* into BamH1/Xho1-restricted pGEX-6P1. pME3363 and pME3463 contain the *FPR3* ORF cloned in their BamH1/Xho1 sites. pME3364 was constructed by introducing DNA encoding Ynm3 without its first 100 amino acids (Ynm3ΔN100aa) into the Spe1/Sma1 sites of pME2564.

Growth Tests

For spot dilution assays, 10-fold dilutions of overnight cultures of each strain starting with an OD_{600} of 0.5 were made in sterile water. Then, 10 μl of each dilution was spotted on plates containing solid SC medium or appropriate selective media (SC-Ura or SC-Met-Ura) or rich medium containing either glucose or galactose and incubated at the temperatures indicated.

Western Blotting

Overnight cultures of yeast strains were grown in liquid SC-Ura. For induction from the *MET25* promoter, cells were pelleted and resuspended in SC-Met-Ura and incubated for two more hours. Cell extracts were prepared and the amount of protein estimated using the Bradford method. Extracts containing equal amounts of protein from each strain were separated by 12% SDS-polyacrylamide gel electrophoresis (PAGE) followed by transfer on to nitrocellulose membranes. The membranes were probed with a 1:500 dilution of mouse anti-GFP monoclonal antibody (Clontech, Mountain View, CA) or 1:2000 dilution of rabbit polyclonal antiserum against Cdc28. Peroxidase-coupled rabbit anti-mouse and goat anti-rabbit immunoglobulin G were used as secondary antibodies (Dianova, Hamburg, Germany).

Table 2. Plasmids used in this study

Plasmid	Description	Source
pRS416	*URA3, CEN, Amp^R (bla), ori*	Sikorski and Hieter (1989)
pRS426	*URA3, 2 μ, Amp^R (bla), ori*	Sikorski and Hieter (1989)
pFA6a-*natNT2*	Contains nourseothricin resistance cassette	Janke *et al.* (2004)
pYM-N25	Contains *GAL1-yeGFP-natNT2* promoter module	Janke *et al.* (2004)
pRS416*MET25*	pRS416 containing *MET25* promoter and *CYC1* terminator	Mumberg *et al.* (1994)
pME3325	pRS416*MET25* containing *YNM3* ORF	This study
pME3363	pRS416*MET25* containing *FPR3* ORF	This study
pME3364	*YNM3ΔN100aa*-GFP fusion in pME2564	This study
pME3449	pRS416*MET25* containing *YNM3-GFP*	This study
pME3450	pRS426*MET25* containing *YNM3*-GFP	This study
pME3451	pRS416*MET25* containing *YNM3S235A-GFP*	This study
pME3452	pRS426*MET25* containing *YNM3S235A-GFP*	This study
pME3453	pRS416*MET25* containing *YNM3S236A-GFP*	This study
pME3454	pRS426*MET25* containing *YNM3S236A-GFP*	This study
pME3455	pRS416*MET25* containing *YNM3ΔPDZ1-GFP*	This study
pME3456	pRS426*MET25* containing *YNM3ΔPDZ1-GFP*	This study
pME3457	pRS416*MET25* containing *YNM3ΔPDZ2-GFP*	This study
pME3458	pRS426*MET25* containing *YNM3ΔPDZ2-GFP*	This study
pME3459	pRS416*MET25* containing *GFP*	This study
pME3460	pGEX-6P1 containing *YNM3* ORF	This study
pME3461	pGEX-6P1 containing *YNM3S236C*	This study
pME3462	pGEX-6P1 containing *YNM3S236A*	This study
pME3463	pGEX-6P1 containing *FPR3*	This study
pME3358	Plasmid encoding mitochondria-targeted BFP	Kindly provided by Dr. Stefan Jakobs (MPI-BPC, Goettingen)

Protein Purification

Ynm3, Ynm3S236C, and Ynm3S236A were expressed from the constructs pME3460, pME3461, and pME3462, respectively, and Fpr3 from pME3463. Recombinant proteins were expressed as glutathione transferase (GST) fusions containing a PreScission protease site in the Rosetta2 strain (Novagen, an Diego, CA) of *E. coli* for ~12 h at 20°C after induction with 250 μl of 1 mM isopropyl β-D-thiogalactoside in a total culture volume of 1 l of 2X-YT medium (16 g of tryptone, 10 g of yeast extract, and 5 g NaCl) containing 2% glucose with 100 μg/ml ampicillin and 100 μg/ml chloramphenicol. Cells were pelletted after centrifugation at 4000 × *g* for 30 min at 4°C. The cell pellets were resuspended in 30 mM phosphate-buffered saline (PBS). Cells were disrupted by passing the suspension through a microfluidizer five times at 80 psi and then centrifuged at 10,000 × *g* for 15 min. The supernatant containing the GST fusion protein was applied on to glutathione (GSH)-Sepharose, washed with PBS, and eluted with a buffer containing 20 mM Tris, 100 mM NaCl, and 25 mM GSH. The fusion proteins were cleaved overnight with PreScission protease at 4°C. After concentration, the samples were passed through either Superdex 200 26/60 for the Ynm3 variants or Superdex 200 16/60 for purifying Fpr3 and eluted in a buffer containing 20 mM Tris and 100 mM NaCl. For the Ynm3 variants, additional purification using GSH-Sepharose matrix was performed.

Proteolysis Assay

Reaction mixtures containing 1 μM purified Ynm3, Ynm3S236C, or Ynm3S236A were incubated with 1.6 μM heat denatured β-casein in a total volume of 250 μl of 50 mM HEPES-KOH, pH 7.3. Then, 20-μl samples were withdrawn at the beginning of the reaction and after overnight incubation (16 h) at 37°C. The samples were separated by 15% SDS-PAGE, and the protein bands were visualized by staining with Brilliant Blue G-Colloidal Coomassie (Sigma Chemie, Deisenhofen, Germany) and quantified using the Kodak 1D image analysis software (Eastman Kodak, Rochester, NY).

Chaperone Activity Assay

The assay is based on the thermal aggregation of citrate synthase (Buchner *et al*., 1998). The reaction mixture containing 0.15 μM CS in the presence or absence of the indicated amounts of chaperones or a negative control protein such as chymotrypsinogen was subjected to 43°C temperature with constant stirring. Aggregation was monitored on a Hitachi F-4500 spectrofluorometer with both excitation and emission wavelengths set to 500 nm at a spectral bandwidth of 2.5 nm. Data points were recorded every 0.5 s for 20 min. The assay for thermal inactivation of CS also was performed as described previously (Buchner *et al*., 1998). In brief, thermal denaturation of CS was performed as described above. Then, 20 μl of the reaction mixture was withdrawn at several time points, and the specific activity of CS was determined using an assay based on the first step of the citric acid cycle. The CoA formed in this assay stoichiometrically reduces the Ellmann's reagent dithio-1,4-nitrobenzoic acid, resulting in an increased absorbance at 412 nm. The linear slope of the initial increase in absorbance recorded online spectrophotometrically (UV-1601; Shimadzu Europe, Duisberg, Germany) was used to calculate specific activity. The specific activity obtained before the start of the thermal inactivation was considered as 100% specific activity. The calculated specific activities during the course of each reaction were expressed as percentage of this value.

Genetic Suppressor Screen

For the gain of function screen, the pRS202 genomic DNA library was used (Connelly and Hieter, unpublished, June 1990). Inserts from this library are ~6–8 kb. The Δ*ynm3* strain was transformed with the library, plated on SC-Ura, and incubated for a week at 38°C. Colonies that looked large were picked, and growth tests were performed at 38°C to avoid any false positives. Plasmids contained in the colonies that grew better at 38°C were isolated and retransformed into the Δ*ynm3* strain for a further verification of their ability to induce better growth at 38°C. The inserts contained in the plasmids were sequenced.

Fluorescence Microscopy

Yeast cultures were grown in synthetic complete medium (SC-Met-Ura) containing glucose supplemented with amino acids. Cells were viewed with either the GFP filter or 4,6-diamidino-2-phenylindole (DAPI) filter by using an Axiovert S100 microscope (Carl Zeiss, Jena, Germany). Images showing co-localization of Ynm3-GFP with mito-blue fluorescent protein were taken using a DM 6000 widefield microscope (Leica, Wetzlar, Germany).

RESULTS

The S. cerevisiae Strain YB322 Requires Ynm3 for Survival under Heat Stress

The bacterial periplasmic HtrA/DegP acts by degrading denatured cell envelope proteins when subjected to heat stress. It is required for survival of *E. coli* at temperatures >40°C (Lipinska *et al*., 1990). To test whether the budding yeast HtrA-like protein, Ynm3, has a similar role in cell survival at higher temperatures, we deleted the *YNM3* gene in the yeast strain YB322. Growth of the wild-type and Δ*ynm3* was compared at both ambient growth temperature

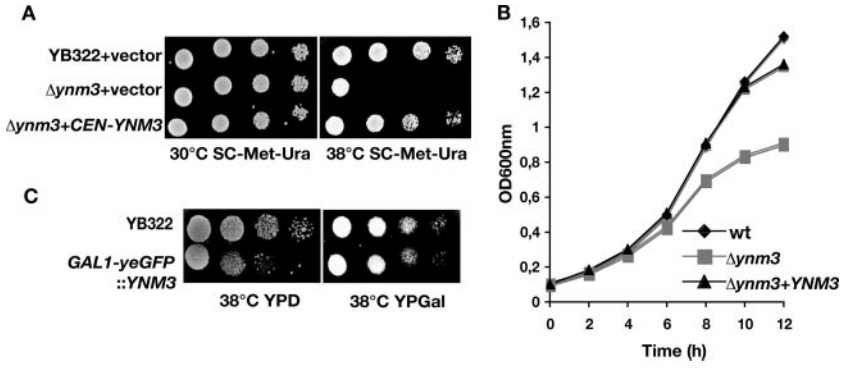

Figure 1. Deletion of *YNM3* in the YB322 yeast strain results in reduced growth under sublethal heat stress. (A) Reduced growth of a Δ*ynm3* strain at 38°C was complemented by a single copy plasmid carrying *YNM3* under the control of the *MET25* promoter. (B) Growth of the indicated strains was also assessed in liquid SC-Met-Ura medium at 39°C. (C) The yeast strain *GAL1*$_{UAS}$-*yeGFP::YNM3*, in which the chromosomal copy of *YNM3* is under the control of the inducible-repressible *GAL1* promoter, exhibited reduced growth on YPD at 38°C due to complete repression of the *GAL1* promoter. This was rescued on YPGal due to the induction of the *GAL1* promoter.

(30°C) and at a sublethal higher temperature (38°C) on solid medium. At 30°C, growth of the wild-type strain and Δ*ynm3* was comparable. At 38°C, growth of Δ*ynm3* was reduced compared with the wild-type strain (Figure 1A). We cloned the *YNM3* gene from the genomic DNA of the wild-type YB322 yeast strain, into a *CEN* yeast vector under the control of the *MET25* promoter. To prove that the above-mentioned phenotype was indeed due to the absence of Ynm3, growth of the Δ*ynm3* strain carrying this construct was compared with that of the wild-type YB322 strain and the Δ*ynm3* strain transformed with empty vector at both 30 and 38°C. As shown in Figure 1A, the reduced growth of Δ*ynm3* at 38°C could be rescued by the construct carrying wild-type *YNM3*. The difference in the growth pattern of wild-type YB322 and Δ*ynm3* in liquid culture was most pronounced at 39°C (Figure 1B).

In an alternate approach, the endogenous promoter of the chromosomal copy of the *YNM3* gene was replaced with the *GAL1* repressible-inducible promoter. This was achieved by the PCR based epitope-tagging method using the cassette *natNT2-GAL1*$_{UAS}$*-yeGFP* (Janke *et al*., 2004). Growth of this strain was compared with that of the wild-type on rich medium containing either glucose (YPD), which completely represses the *GAL1* promoter or galactose (YPGal), which induces the *GAL1* promoter. As expected, growth of the above-mentioned strain was reduced compared with that of the wild type at 38°C on YPD, due to repression of the *GAL1* promoter in the presence of glucose (Figure 1C). This growth defect was absent on the YPGal plate because galactose present in the medium led to the induction of the *GAL1* promoter and therefore expression of the yeGFP-Ynm3 fusion protein (Figure 1C).

The Serine Protease Activity of Ynm3 Is Important to Mediate Its Thermoprotective Function

The serine protease activity of the bacterial HtrA homologue DegP executes its protective heat-stress–responsive function (Lipinska *et al*., 1990; Spiess *et al*., 1999). Ynm3 has an HtrA-like serine protease domain and two PDZ domains (Apweiler *et al*., 2000). We asked whether the serine protease activity of Ynm3 is required for conferring its thermoprotective function. We exchanged both S235 and S236 to alanines because these residues have been described in the literature as putative active site serine residues (Fahrenkrog *et al*., 2004; Walter *et al*., 2006; Rawlings *et al*., 2008). According to an earlier version of the MEROPS Peptidase Database (http://merops.sanger.ac.uk), the serine at position 236 was predicted to be the catalytic serine. We used GFP-tagged fusions of the above-mentioned variants to enable verification of the level of protein expressed using Western blot. *YNM3-GFP*, *YNM3S235A-GFP*, and *YNM3S236A-GFP* were cloned into a single copy yeast vector under the control of the *MET25* promoter. The Δ*ynm3* strain was transformed with these constructs for further experiments. Growth tests of strains carrying these constructs in comparison with the wildtype YB322 and Δ*ynm3* encoding GFP alone were performed under inducing conditions at both 30 and 38°C on plates containing SC-Met-Ura. The growth defect of Δ*ynm3* at 38°C was completely rescued by the construct encoding *YNM3-GFP* and *YNM3S235A-GFP* (Figure 2A). However, the construct encoding *YNM3S236A-GFP* could not rescue the growth defect of the Δ*ynm3* strain (Figure 2A). Because, there was no marked difference in the level of expression of the Ynm3 variants (Figure 2B), it is evident that the serine protease activity of the budding yeast Ynm3 plays an important role in executing its protective function at higher temperatures and that the serine residue at position 236 is the catalytic serine.

Although the expression of Ynm3 from a single copy vector is beneficial for cell survival under heat stress, overexpression of Ynm3 or its protease active S235A variant from a multicopy vector is deleterious even at ambient temperature (30°C), but stronger at higher temperatures (38°C). The overexpression of the Ynm3S236A-GFP variant did not lead to any growth impairment, unlike as for the S235A variant or the native protein. So, the observed growth im-

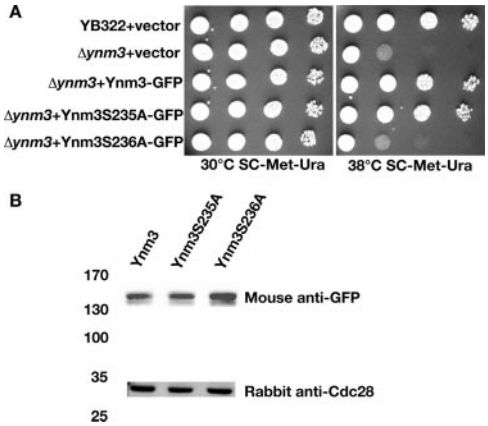

Figure 2. The serine protease activity of Ynm3, mediated by the catalytic serine residue at position 236, is required to execute its thermoprotective function. (A) The reduced growth of Δ*ynm3* strain at higher temperatures was completely rescued by a single copy plasmid expressing either Ynm3-GFP or Ynm3S235A-GFP under the control of the *MET25* promoter. Ynm3S236A-GFP could not confer thermoprotection. (B) All these variants were expressed to similar levels as seen in the Western blot.

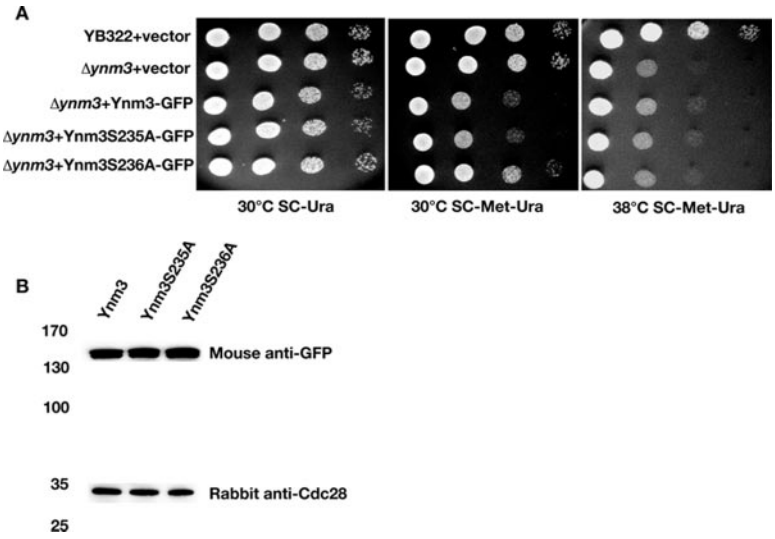

Figure 3. Overexpression of Ynm3 is deleterious. (A) Ynm3-GFP or its S235A variant, when overexpressed led to growth impairment even at 30°C and therefore could not confer thermoprotection at 38°C. The overexpression of the S236A variant did not lead to growth impairment. (B) The fusion proteins were expressed to similar levels as seen in the Western blot.

pairment was due to excessive proteolytic activity of Ynm3 conferred by the serine at position 236 (Figure 3A) because all these variants were expressed to similar levels (Figure 3B).

Although purified Ynm3 showed no effect on a nonnative substrate like β-casein, it underwent slow autocatalysis, which could be monitored by SDS-PAGE. After 16 h of incubation at 37°C, the amount of native Ynm3 in the reaction was reduced to <17% as shown by the intensity of the protein band on a Coomassie-stained gel, whereas the S236A variant was significantly stable, with >86% remaining intact (Figure 4). This clearly validates our in vivo finding that the serine at position 236 confers protease activity to Ynm3. It has been documented that exchange of active site serines to cysteines does not necessarily abolish proteolytic activity, which remains to varying degrees in different proteases (Hahn and Strauss, 1990; Tautz *et al*., 2000). In Ynm3, exchanging serine at position 236 to alanine significantly enhanced its stability, but exchange to cysteine was not so effective, with only 65% of the protein left in the reaction after 16 h. Together, there is sufficient evidence that the serine residue at position 236 confers catalytic activity to Ynm3.

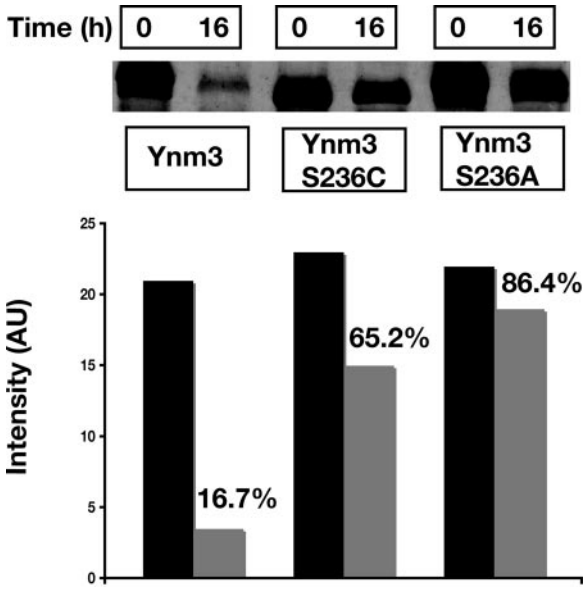

Figure 4. Ynm3 undergoes slow autocatalysis in vitro due to its serine protease activity. On incubation of a reaction mixture containing Ynm3 in the presence of heat denatured β-casein at 37°C, it underwent slow autocatalysis which was dramatically reduced in the S236A variant. Exchange of the catalytic serine to cysteine was not as effective. The protein bands were quantified and the net intensities plotted. The percentage intensity that remained after 16 h of incubation is shown.

Deletion of Either of Its PDZ Domains Destabilizes Ynm3

The HtrA family of proteins is characterized by the presence of an N-terminal serine protease domain followed by at least one C-terminal PDZ domain. PDZ domains are modular protein–protein interaction domains that possess unique structural features, which enable interaction with C-terminal residues of ligand proteins. The PDZ domains of the bacterial homologues and the mammalian HtrA2 homologue of this family are important for regulating their protease activity and are also required for substrate binding and multimerization (Clausen *et al*., 2002; Krojer *et al*., 2002; Li *et al*., 2002). We designated the PDZ domain present immediately proximal to the protease domain (residues 290–375) as PDZ1 and the other predicted PDZ domain at the C-terminal end of the protein as PDZ2 (779–868). To investigate whether the predicted PDZ domains of the budding yeast HtrA homologue Ynm3 have any aforementioned essential roles, we deleted either PDZ1 or PDZ2 encoding regions. The Δ*ynm3* strain was transformed with *CEN* vectors encoding either Ynm3ΔPDZ1-GFP or Ynm3ΔPDZ2-GFP fusion proteins. Growth tests of strains carrying these constructs in comparison with the wild-type YB322 and Δ*ynm3* carrying empty vector were performed under inducing conditions in plates containing SC-Met-Ura at 30 and 38°C. As shown in Figure 5A, only full-length Ynm3 was able to completely rescue the heat sensitivity of the Δ*ynm3* strain at higher temperatures. The lack of PDZ1 or PDZ2 domains drastically reduced its protective function. This was because the absence of either of the PDZ domains, especially PDZ1 dramatically reduced the stability of the protein as seen in the Western blot (Figure 5B) without affecting its localization in the nucleus (Figure 5C). We identified the nuclear localization signal of Ynm3 to

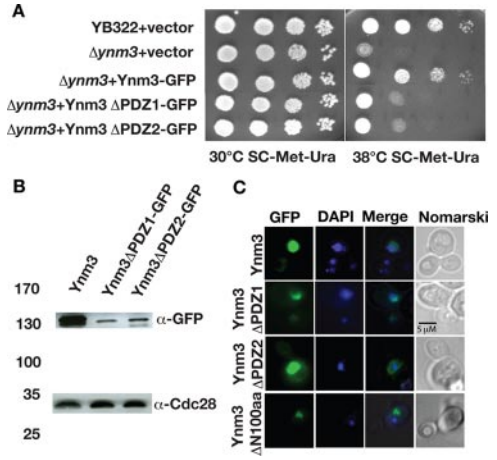

Figure 5. The lack of either of its two PDZ domains destabilizes Ynm3. (A) Deletion of either of its two PDZ domains drastically reduced Ynm3's function. (B) The lack of either PDZ domain, especially PDZ1 led to destabilization as seen in the Western blot. (C) Lack of PDZ domains did not mislocalize the protein. Ynm3ΔPDZ1-GFP and Ynm3ΔPDZ2-GFP were predominantly localized in the nucleus, whereas Ynm3ΔN100aa lacking the first 100 N-terminal amino acids was localized entirely outside the nucleus. This indicates that the nuclear localization signal of Ynm3 lies in the N-terminal 100 amino acids.

be located in the first 100 N-terminal amino acids because the variant lacking these residues (Ynm3ΔN100aa) fused with GFP C-terminally was localized entirely outside the nucleus (Figure 5C). Thus, the lack of PDZ domains markedly reduced the stability of Ynm3 rendering it insufficient for conferring thermoprotection, although it did not affect its nuclear localization significantly.

Ynm3 Exhibits General Chaperone Activity In Vitro

The prokaryotic HtrA proteins are dual chaperone-proteases. Chaperone activity in any eukaryotic HtrA has not been recognized so far. We tested whether the budding yeast Ynm3 exhibits chaperone activity by using an assay based on the thermal aggregation of citrate synthase (CS), a nonnative substrate (Buchner *et al*., 1998). As seen in Figure 6A, in the absence of chaperone, the reaction mixture containing 0.15 μM CS scattered light at 500 nm, which increased with time when subjected to a temperature of 43°C. The increase in the amount of light scattered with time was due to both increase in size and the number of aggregates formed by thermally denaturing CS as described previously (Buchner *et al*., 1998). Presence of equimolar amounts of Ynm3 in the reaction mixture completely suppressed light scattering, whereas another protein, chymotrypsinogen, did not (Figure 6A). This indicated that Ynm3 possesses ATP-independent general chaperone activity due to its ability to bind unfolding intermediates of a nonnative substrate such as CS at 43°C, thereby preventing its aggregation. Several known chaperones such as GroEL, Hsp70, Hsp90, and small heat-shock proteins have been shown to possess this activity (Buchner *et al*., 1998). The *E. coli* HtrA/DegP is a chaperone at low temperatures and switches to being a protease only at higher temperatures (Spiess *et al*., 1999). In contrast, Ynm3 acts as a chaperone at 43°C in vitro, a condition similar to heat-shock temperatures in vivo. But the protease dead S210A variant of bacterial DegP retains chaperoning ability even at higher temperatures, which is sufficient to rescue the heat sensitivity of a *degP* mutant when overexpressed

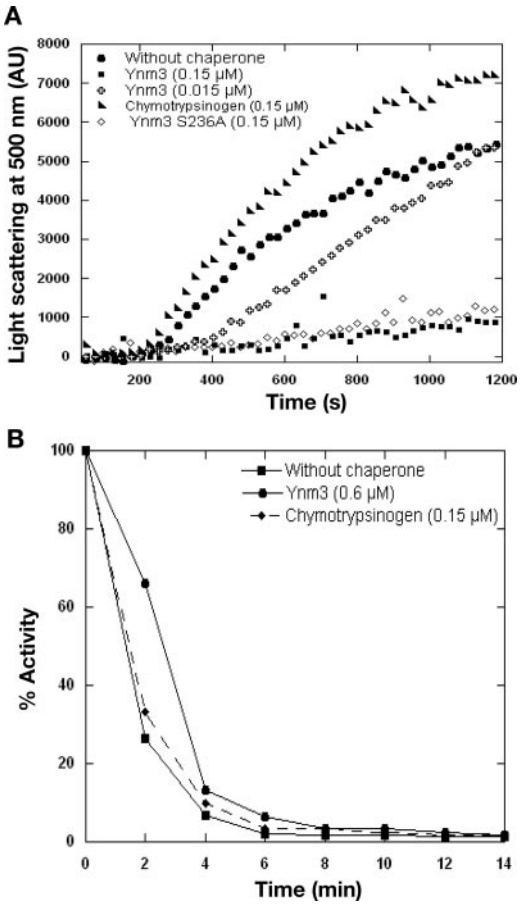

Figure 6. Ynm3 exhibits chaperone activity in vitro. (A) In the absence of chaperones, the reaction mixture containing 0.15 μM CS scattered light in an increasing manner at 43°C due to thermal aggregation of CS. In the presence of equimolar amounts of Ynm3 or Ynm3S236A, this light scattering was completely suppressed. (B) Presence of Ynm3 did not affect the thermal inactivation kinetics of CS.

(Spiess *et al*., 1999). This does not seem to be the case with the budding yeast Ynm3. Although the protease dead variant Ynm3S236A exhibited chaperone activity at 43°C (Figure 6A), its overexpression could not compensate for the lack of proteolytic activity (Figure 3A), which is indispensable for protection under heat stress. Ynm3 could prevent aggregation of CS, yet it had no significant influence on the thermal inactivation of CS at 43°C (Figure 6B), which shows that Ynm3 tightly binds unfolding intermediates of CS, which are no more in equilibrium with the native state.

A Genetic Screen Identified FPR3 as a Suppressor of the Heat Sensitivity of Δynm3

We performed a suppressor screen using a yeast genomic DNA library to identify other genes that could suppress the heat sensitivity of a Δ*ynm3* strain. From the screen, we identified a plasmid containing the full ORF of *FPR3* along with its upstream and downstream sequences, which was able to partially rescue the heat sensitivity of a Δ*ynm3* strain. *FPR3* encodes a PPIase localized to the nucleolus of the budding yeast (Shan *et al*., 1994). We cloned the *FPR3* ORF into a single copy vector under the control of the *MET25* promoter. At 37°C, the above-mentioned construct partially rescued the growth defect of the Δ*ynm3* strain carrying it,

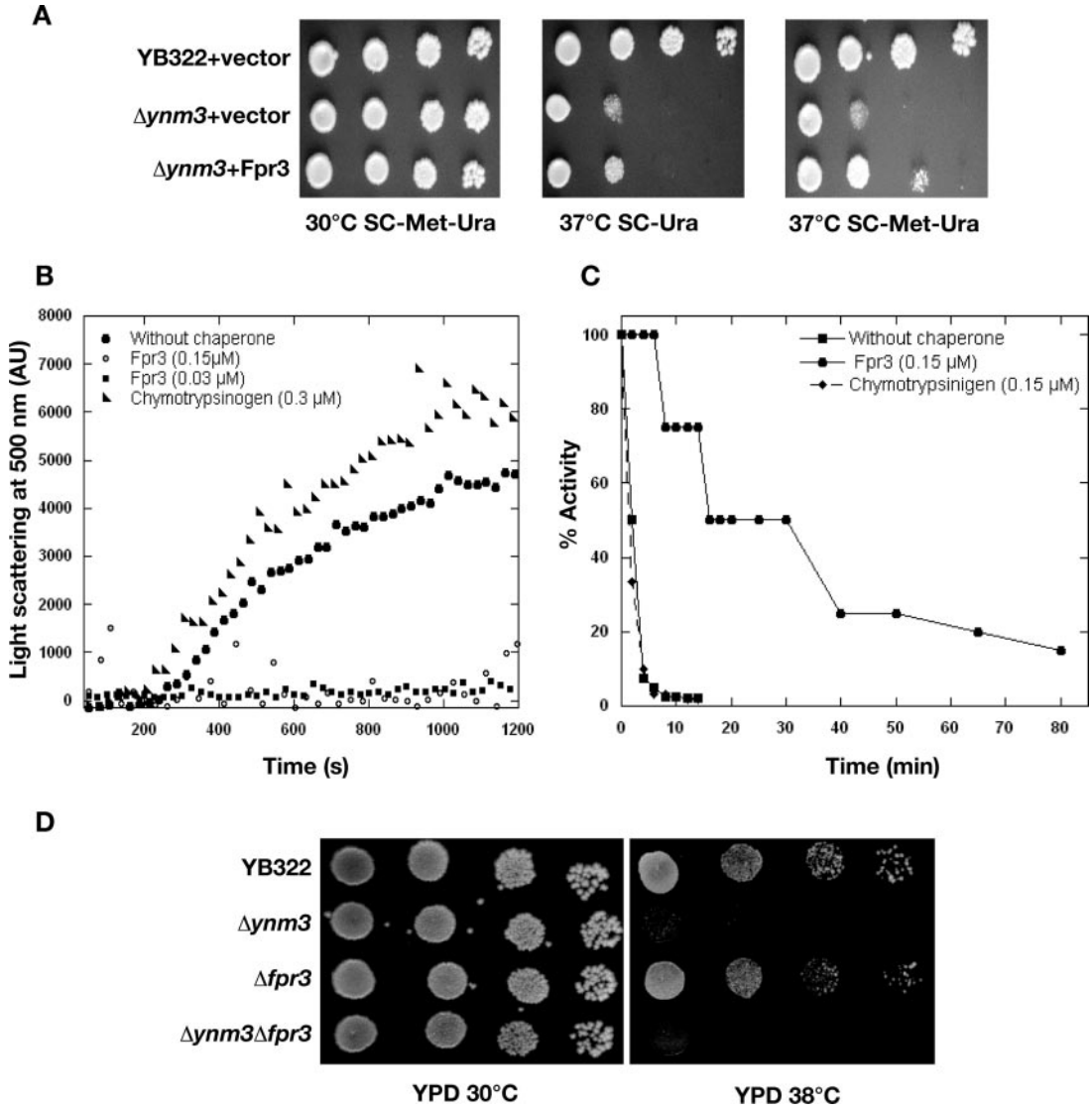

Figure 7. Fpr3 possesses chaperone activity and can suppress the heat sensitivity of Δ*ynm3*. (A) A single copy plasmid carrying *FPR3* under the control of the *MET25* promoter partially rescued the heat sensitivity of a Δ*ynm3* strain under inducing conditions. (B) Fpr3, in substoichiometric amounts (0.03 μM) completely suppressed light scattering due to thermal aggregation of CS. (C) Equimolar amounts of Fpr3 dramatically reduced the rate of thermal inactivation of CS by ~18-fold. (D) Deletion of *FPR3* alone did not lead to heat sensitivity and double deletion of *YNM3* and *FPR3* was not synthetic lethal.

confirming the result of the genetic screen (Figure 7A). In *E. coli*, too, a functional relationship exists between DegP and the periplasmic PPIase SurA, which is mainly responsible for the maturation of outer membrane proteins. DegP along with another periplasmic chaperone Skp can substitute for the absence of SurA (Sklar *et al*., 2007); therefore, *surA/degP* double deletion is synthetic lethal (Rizzitello *et al*., 2001).

PPIases are thought to accelerate the rate of conformational interconversions around proline residues in polypeptides, thereby hastening protein folding by chaperones. Several PPIases such as SurA are known to exhibit chaperone-like activities. In vitro, SurA exhibits chaperone activity where a 64-fold molar excess of SurA could completely suppress the thermal aggregation of a nonnative substrate such as CS (Behrens *et al*., 2001). We tested whether Fpr3 also exhibited any such activity. We expressed and purified Fpr3 from *E. coli*. Substoichiometric amounts of Fpr3 could completely suppress the thermal aggregation of CS (Figure 7B), indicating that Fpr3 has excellent ATP-independent chaperoning ability, which most likely contributes to the suppression of the heat sensitivity of the Δ*ynm3* strain. Equimolar amounts of Fpr3 reduced the rate of thermal inactivation of CS at 43°C. The thermal inactivation of CS follows apparent first-order kinetics (Buchner *et al*., 1998). In the experiment depicted in Figure 7C, the calculated rate constant of thermal inactivation of CS in the absence of chaperones was $\sim 7.2 \times 10^{-3}$. In the presence of equimolar amounts of Fpr3, it was reduced to $\sim 4.0 \times 10^{-4}$, an 18-fold decrease (Figure 7C). Whereas just an additional copy of *FPR3* could suppress the heat sensitivity of a Δ*ynm3* strain, deletion of *FPR3* alone did not lead to detectable heat sensitivity and double deletion of *YNM3* and *FPR3* did not lead to synthetic lethality (Figure 7D). This suggests that the chaperoning activity of Fpr3 simply substituted for the absence of Ynm3. Such compensatory chaperoning mechanisms in the yeast nucleus resembles that among DegP, SurA, and Skp in the bacterial periplasm (Sklar *et al*., 2007).

DISCUSSION

Prokaryotic HtrAs are dual-chaperone proteases, but the function of eukaryotic HtrAs is less well understood. The role of mammalian mitochondrial HtrA2/Omi is controversial: the neurodegenerative phenotype due to progressive mitochondrial dysfunction in mice lacking HtrA2/Omi or expressing a protease-deficient variant (Jones *et al*., 2003; Martins *et al*., 2004) is inconsistent with the proapoptotic function concluded from cell culture studies (Suzuki *et al*., 2001; Hegde *et al*., 2002; Verhagen *et al*., 2002). Mitochondria are evolutionary derivatives of ancestral α-proteobacteria. Therefore, it is tempting to suspect that HtrA2/Omi and other eukaryotic HtrAs have conserved their original bacterial function i.e., protection against unfolding stresses.

We addressed this issue for Ynm3, the budding yeast HtrA representative and could demonstrate a chaperone-protease activity reminiscent of prokaryotic HtrAs. The serine protease dead variant Ynm3S236A could not rescue the growth defect of Δ*ynm3* at higher temperatures whereas native Ynm3 or Ynm3S235A resulted in full complementation. Thus, the lack of Ynm3 or its proteolytic activity adversely affected growth at higher temperatures, which is characteristic to the absence of quality control chaperones or proteases. Ynm3 presumably degrades toxic unfolded proteins that result from sublethal heat stress in a manner similar to bacterial HtrA/DegP. Although Ser235 was proposed to be the catalytic serine (Walter *et al*., 2006), we found Ynm3S235A to be fully active, whereas Ynm3S236A was inactive.

An optimal amount of Ynm3 was beneficial to the cell, but excessive amounts were deleterious as is often the case with overproduction of quality control proteases. At higher levels, Ynm3 might target not only damaged but also properly folded proteins for proteolysis in an unsupervised manner. HtrA/DegP protects *E. coli* from cytotoxicity resulting from heat stress by proteolytically cleaving irreversibly unfolded proteins in the periplasm (Strauch *et al*., 1989). In vitro, DegP cleaves a heterogenous group of unfolded proteins (Clausen *et al*., 2002). Ynm3 had no effect on heat denatured nonnative substrates such as β-casein, but it underwent slow autocatalysis in vitro. Perhaps, a specific modification triggers Ynm3's proteolytic activity in the physiological mileu. Alternatively, Ynm3 may target only a specific class of proteins. Finding native substrates of Ynm3 will be an interesting area for work ahead. It was speculated that Ynm3 might proteolytically cleave acyl-CoA synthetases such as Faa1 and Faa4, thereby modulating lipid metabolism (Tong *et al*., 2006). However, we found no decrease in the endogenous levels of Faa1 or Faa4 upon overexpression of Ynm3 (data not shown). It still cannot be excluded that the lack of Ynm3 may alter membrane lipid composition and fluidity leading to enhanced thermosensitivity either directly or indirectly due to protein denaturation upon heat stress.

The PDZ domains of bacterial HtrAs and mammalian HtrA2/Omi play regulatory roles (Krojer *et al*., 2002; Li *et al*., 2002; Iwanczyk *et al*., 2007). Lack of either of the two PDZ domains of Ynm3 was destabilizing. Therefore, the PDZ domains might be required to maintain proper folding or oligomerization. However, Δ*ynm3* expressing Ynm3ΔPDZ1 or Ynm3ΔPDZ2 showed slightly improved growth under heat stress, suggesting that the PDZ domains may be dispensable for Ynm3's basic function. Future structural studies will reveal both the intra- and intermolecular interactions of Ynm3 and the role of the PDZ domains.

In addition to its proteolytic activity, which is indispensable for growth at higher temperature, Ynm3 also exhibited robust general chaperone activity in vitro. Ynm3 in equimolar amounts was able to prevent the thermal aggregation of a nonnative substrate such as CS. Ynm3 exhibited chaperone activity even at higher temperatures in contrast to bacterial DegP, which is a chaperone only at ambient growth temperatures. Ynm3 did not influence the thermal inactivation kinetics of CS, suggesting that it functions by sequestering and preventing the aggregation of unfolding intermediates, which are unable to refold to their native state. The protease dead Ynm3S236A could not rescue the heat sensitivity of Δ*ynm3* even in excessive amounts despite being a robust chaperone in vitro unlike in bacteria in which DegPS210A in excessive amounts could do so (Spiess *et al*., 1999). This implies that the protease activity of Ynm3 is crucial for handling irreversibly unfolded proteins, which may otherwise continue to accumulate forming toxic aggregates refractory to proteolysis. Evolution has probably preserved the chaperone activity of Ynm3 so that it recognizes heat-denatured proteins, preventing aggregation and delivering them in a more soluble state to its protease domain, thereby increasing the efficiency of proteolysis. Consequently, in the absence of its protease activity, the chaperone activity of Ynm3 alone is unable to tackle unfolding stresses. Thus Ynm3 plays a role analogous to the bacterial DegP in the nucleus of the budding yeast. Although Ynm3 is primarily nuclear, a subpopulation was associated with mitochondria and probably also plays a role in mitochondrial homeostasis during ageing because Δ*ynm3* expressed signs of poor oxidative growth upon prolonged incubation (Supplemental Data).

A screen to find suppressors of the heat sensitivity of Δ*ynm3* resulted in the identification of Fpr3 as a strong chaperone. This FKBP-type nucleolar PPIase is implicated in maintaining meiotic recombination checkpoint activity (Hochwagen *et al*., 2005). PPIases in general are protein-folding catalysts, known to catalyze the rate-limiting *cis-trans* conversion of prolines, accelerating protein folding. Many of these foldases are known to possess additional chaperone activities. In *E. coli*, the periplasmic PPIase, SurA, aids in the folding of cell envelope proteins (Rouviere and Gross, 1996). Cooperation between DegP and the PPIase, SurA, in the periplasm is exemplified by the synthetic lethality of a *surA/degP* double deletant (Rizzitello *et al*., 2001; Sklar *et al*., 2007). Surprisingly, we found a similar scenario in yeast where an additional copy of *FPR3* was sufficient to partially rescue the heat sensitivity of Δ*ynm3*. Our data provide strong evidence for chaperone activity in Fpr3 in vitro, a novel function for this yeast nucleolar FKBP-type PPIase. We have shown that Fpr3, in substochiometric amounts, could strongly prevent the thermal aggregation of CS, a nonnative substrate. Equimolar amounts of Fpr3 slowed the rate of thermal inactivation of CS, suggesting that Fpr3 transiently binds to CS intermediates that are able to refold to their native state. The chaperoning ability of Fpr3 probably substitutes for the loss of the chaperone-protease activity of Ynm3, although not completely. Such compensatory mechanisms are well exemplified in the bacterial periplasm (Sklar *et al*., 2007). Deletion of Fpr3 alone did not result in heat sensitivity. Therefore, Fpr3, owing to its chaperoning ability, might partly compensate for the absence of Ynm3 or more so for the absence of the Tom1 ubiquitin ligase (Utsugi *et al*., 1999), providing some relief from unfolding stresses arising due to higher temperatures.

In summary, we show for the first time that a eukaryotic HtrA member has retained, through evolution, the chaperone-protease function of its prokaryotic counterparts. Knowledge of the mechanism by which Ynm3 recognizes

toxic misfolded conformers, preventing their aggregation and facilitating their degradation will significantly enhance our understanding of the function of eukaryotic HtrAs with respect to protein quality control. This will have noteworthy implications in the understanding of the molecular pathways underlying aggregopathies such as Parkinson's disease. Interestingly, during the course of this study, we also identified a previously unknown chaperone activity in the budding yeast nucleolar PPIase Fpr3 that could partially compensate for the lack of Ynm3.

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REFERENCES

Apweiler, R. *et al*. (2000). InterPro—an integrated documentation resource for protein families, domains and functional sites. Bioinformatics *16*, 1145–1150.

Baldi, A. *et al*. (2002). The HtrA1 serine protease is down-regulated during human melanoma progression and represses growth of metastatic melanoma cells. Oncogene *21*, 6684–6688.

Behrens, S., Maier, R., de Cock, H., Schmid, F. X., and Gross, C. A. (2001). The SurA periplasmic PPIase lacking its parvulin domains functions in vivo and has chaperone activity. EMBO J. *20*, 285–294.

Buchner, J., Grallert, H., and Jakob, U. (1998). Analysis of chaperone function using citrate synthase as nonnative substrate protein. Methods Enzymol. *290*, 323–338.

Clausen, T., Southan, C., and Ehrmann, M. (2002). The HtrA family of proteases: implications for protein composition and cell fate. Mol. Cell *10*, 443–455.

Faccio, L., Fusco, C., Chen, A., Martinotti, S., Bonventre, J. V., and Zervos, A. S. (2000). Characterization of a novel human serine protease that has extensive homology to bacterial heat shock endoprotease HtrA and is regulated by kidney ischemia. J. Biol. Chem. *275*, 2581–2588.

Fahrenkrog, B., Sauder, U., and Aebi, U. (2004). The *S. cerevisiae* HtrA-like protein Nma111p is a nuclear serine protease that mediates yeast apoptosis. J. Cell Sci. *117*, 115–126.

Hahn, C. S., and Strauss, J. H. (1990). Site-directed mutagenesis of the proposed catalytic amino acids of the Sindbis virus capsid protein autoprotease. J. Virol. *64*, 3069–3073.

Hegde, R. *et al*. (2002). Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction. J. Biol. Chem. *277*, 432–438.

Hochwagen, A., Tham, W. H., Brar, G. A., and Amon, A. (2005). The FK506 binding protein Fpr3 counteracts protein phosphatase 1 to maintain meiotic recombination checkpoint activity. Cell *122*, 861–873.

Imai, J., Yashiroda, H., Maruya, M., Yahara, I., and Tanaka, K. (2003). Proteasomes and molecular chaperones: cellular machinery responsible for folding and destruction of unfolded proteins. Cell Cycle *2*, 585–590.

Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. *153*, 163–168.

Iwanczyk, J., Damjanovic, D., Kooistra, J., Leong, V., Jomaa, A., Ghirlando, R., and Ortega, J. (2007). Role of the PDZ domains in *Escherichia coli* DegP protein. J. Bacteriol. *189*, 3176–3186.

Janke, C. *et al*. (2004). A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. Yeast *21*, 947–962.

Johnson, D. R., Knoll, L. J., Levin, D. E., and Gordon, J. I. (1994). Saccharomyces cerevisiae contains four fatty acid activation (FAA) genes: an assessment of their role in regulating protein N-myristoylation and cellular lipid metabolism. J. Cell Biol. *127*, 751–762.

Jones, J. M. *et al*. (2003). Loss of Omi mitochondrial protease activity causes the neuromuscular disorder of mnd2 mutant mice. Nature *425*, 721–727.

Krojer, T., Garrido-Franco, M., Huber, R., Ehrmann, M., and Clausen, T. (2002). Crystal structure of DegP (HtrA) reveals a new protease-chaperone machine. Nature *416*, 455–459.

Krüger, R., Eberhardt, O., Riess, O., and Schulz, J. B. (2002). Parkinson's disease: one biochemical pathway to fit all genes? Trends Mol. Med. *8*, 236–240.

Li, W., Srinivasula, S. M., Chai, J., Li, P., Wu, J. W., Zhang, Z., Alnemri, E. S., and Shi, Y. (2002). Structural insights into the pro-apoptotic function of mitochondrial serine protease HtrA2/Omi. Nat. Struct. Biol. *9*, 436–441.

Lipinska, B., Fayet, O., Baird, L., and Georgopoulos, C. (1989). Identification, characterization, and mapping of the *Escherichia coli htrA* gene, whose product is essential for bacterial growth only at elevated temperatures. J. Bacteriol. *171*, 1574–1584.

Lipinska, B., Zylicz, M., and Georgopoulos, C. (1990). The HtrA (DegP) protein, essential for *Escherichia coli* survival at high temperatures, is an endopeptidase. J. Bacteriol. *172*, 1791–1797.

Martins, L. M. *et al*. (2002). The serine protease Omi/HtrA2 regulates apoptosis by binding XIAP through a reaper-like motif. J. Biol. Chem. *277*, 439–444.

Martins, L. M. *et al*. (2004). Neuroprotective role of the Reaper-related serine protease HtrA2/Omi revealed by targeted deletion in mice. Mol. Cell. Biol. *24*, 9848–9862.

Mumberg, D., Muller, R., and Funk, M. (1994). Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res. *22*, 5767–5768.

Pallen, M. J., and Wren, B. W. (1997). The HtrA family of serine proteases. Mol. Microbiol. *26*, 209–221.

Ponting, C. P. (1997). Evidence for PDZ domains in bacteria, yeast, and plants. Protein Sci. *6*, 464–468.

Rawlings, N. D., Morton, F. R., Kok, C. Y., Kong, J., and Barrett, A. J. (2008). MEROPS: the peptidase database. Nucleic Acids Res. *36*, D320–325.

Rizzitello, A. E., Harper, J. R., and Silhavy, T. J. (2001). Genetic evidence for parallel pathways of chaperone activity in the periplasm of *Escherichia coli*. J. Bacteriol. *183*, 6794–6800.

Rouviere, P. E., and Gross, C. A. (1996). SurA, a periplasmic protein with peptidyl-prolyl isomerase activity, participates in the assembly of outer membrane porins. Genes Dev. *10*, 3170–3182.

Sakahira, H., Breuer, P., Hayer-Hartl, M. K., and Hartl, F. U. (2002). Molecular chaperones as modulators of polyglutamine protein aggregation and toxicity. Proc. Natl. Acad. Sci. USA 99 (suppl 4), 16412–16418.

Shan, X., Xue, Z., and Melese, T. (1994). Yeast NPI46 encodes a novel prolyl cis-trans isomerase that is located in the nucleolus. J. Cell Biol. *126*, 853–862.

Sikorski, R. S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics *122*, 19–27.

Sklar, J. G., Wu, T., Kahne, D., and Silhavy, T. J. (2007). Defining the roles of the periplasmic chaperones SurA, Skp, and DegP in *Escherichia coli*. Genes Dev. *21*, 2473–2484.

Snyder, H., Mensah, K., Theisler, C., Lee, J., Matouschek, A., and Wolozin, B. (2003). Aggregated and monomeric alpha-synuclein bind to the S6′ proteasomal protein and inhibit proteasomal function. J. Biol. Chem. *278*, 11753–11759.

Spiess, C., Beil, A., and Ehrmann, M. (1999). A temperature-dependent switch from chaperone to protease in a widely conserved heat shock protein. Cell *97*, 339–347.

Strauch, K. L., Johnson, K., and Beckwith, J. (1989). Characterization of degP, a gene required for proteolysis in the cell envelope and essential for growth of *Escherichia coli* at high temperature. J. Bacteriol. *171*, 2689–2696.

Strauss, K. M. *et al*. (2005). Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. Hum. Mol. Genet *14*, 2099–2111.

Suzuki, Y., Imai, Y., Nakayama, H., Takahashi, K., Takio, K., and Takahashi, R. (2001). A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. Mol. Cell *8*, 613–621.

Tautz, N., Kaiser, A., and Thiel, H. J. (2000). NS3 serine protease of bovine viral diarrhea virus: characterization of active site residues, NS4A cofactor domain, and protease-cofactor interactions. Virology *273*, 351–363.

Tong, F., Black, P. N., Bivins, L., Quackenbush, S., Ctrnacta, V., and DiRusso, C. C. (2006). Direct interaction of *Saccharomyces cerevisiae* Faa1p with the Omi/HtrA protease orthologue Ynm3p alters lipid homeostasis. Mol. Genet. Genomics *275*, 330–343.

Utsugi, T., Hirata, A., Sekiguchi, Y., Sasaki, T., Toh-e, A., and Kikuchi, Y. (1999). Yeast *tom1* mutant exhibits pleiotropic defects in nuclear division, maintenance of nuclear structure and nucleocytoplasmic transport at high temperatures. Gene *234*, 285–295.

Vande Walle, L., Lamkanfi, M., and Vandenabeele, P. (2008). The mitochondrial serine protease HtrA2/Omi: an overview. Cell Death Differ. *15*, 453–460.

Verhagen, A. M., Ekert, P. G., Pakusch, M., Silke, J., Connolly, L. M., Reid, G. E., Moritz, R. L., Simpson, R. J., and Vaux, D. L. (2000). Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell *102*, 43–53.

Verhagen, A. M. *et al*. (2002). HtrA2 promotes cell death through its serine protease activity and its ability to antagonize inhibitor of apoptosis proteins. J. Biol. Chem. *277*, 445–454.

Walter, D., Wissing, S., Madeo, F., and Fahrenkrog, B. (2006). The inhibitor-of-apoptosis protein Bir1p protects against apoptosis in *S. cerevisiae* and is a substrate for the yeast homologue of Omi/HtrA2. J. Cell Sci. *119*, 1843–1851.