

The Host Cell Co-Chaperonin Hsp10 is an Interacting Partner for the Legionella pneumophila Multifunctional Chaperonin HtpB

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Abstract

L. pneumophila is an intracellular bacterium that replicates inside a membrane-bound vacuole called Legionella-Containing Vacuole (LCV), where it profusely releases its HtpB chaperonin. From LCV, HtpB reaches the host cell cytoplasm, where it interacts with SAMDC, a cytoplasmic protein required for synthesis of host polyamines that are important for intracellular growth of *L. pneumophila*. Moreover, cytoplasmic expression of HtpB in *S. cerevisiae* induces pseudohyphal growth, and in mammalian cells recruits mitochondria to LCV and modifies actin microfilaments organization. This led us to hypothesize here that HtpB recruits a protein(s) from eukaryotic cells that is involved in emergence of the aforementioned phenotypes. To identify this protein, a commercially available HeLa cDNA library was screened using a yeast two-hybrid system. Approximately 5 x 10⁶ yeast clones carrying HeLa cDNA library plasmid were screened. Twenty one positive clones were identified. DNA sequence analysis revealed that all of these positive clones encoded the mammalian small heat shock protein Hsp10. Based on the fact that chaperonins are required to interact with co-chaperonins to function properly in protein folding, thus, HtpB may recruit the host cell Hsp10 to appropriately interact with SAMDC and to induce the cellular phenotypes deemed important for *L. pneumophila* pathogenesis.

Keywords: *Legionella pneumophila*; Chaperonin HtpB; Hsp10 co-chaperonin

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Introduction

L. pneumophila is a Gram-negative environmental bacterial that naturally replicates in freshwater amoebae within a specialized vacuole called the Legionella-Containing Vacuole (LCV). Upon inhalation of *L. pneumophila*-contaminated water aerosols by susceptible individuals, *L. pneumophila* would reach the alveoli and replicates within the LCV of the human alveolar macrophages resulting in a severe atypical pneumonia known as Legionnaires' disease [1].

Group 1 Chaperonins, 60 kDa Heat Shock Protein (Hsp60), are a family of structurally and functionally conserved and essential proteins (known as Cpn60, GroEL, Hsp60, or HtpB) that are present in bacteria, and in eukaryotic organelles such as chloroplasts and mitochondria (known as CCT, TRiC or c-cpn) [2]. The remarkable amino acid sequence and structural conservation across these chaperonins highlights their

functional importance [3]. A comparison of the aligned amino acid sequences of the eukaryotic and bacterial chaperonins, (including HtpB), revealed that these proteins have high degrees of amino acid homology that sometimes exceed 70% [4-6]. To function properly in protein folding, bacterial or eukaryotic chaperonins must work in conjunction with a small heat shock protein (~10 kDa) known as Hsp10 or Cpn10 (also known as GroES in bacteria and HtpA in *L. pneumophila*) [2, 7, 8]. The amino acid sequences homology of the eukaryotic and bacterial co-chaperonins is also highly conserved [6, 9, 10]. It has been shown that eukaryotic chaperonins cpn60 bind to bacterial, mitochondrial, and chloroplast cpn10 homologs with an different affinity, suggesting that chaperonins from bacteria can interact with co-chaperonin from eukaryotic cells and vice versa [11].

The fundamental functions of chaperonins are to assist other proteins to fold properly after translation, protect proteins from denaturation, and help denatured proteins to refold after stress. However, it has been found that some chaperonins, such as the *L. pneumophila* HtpB have cellular roles independent of protein folding [3]. For instance, the expression of HtpB is upregulated upon contact with L929 murine cells or human monocytes [12]. High levels of HtpB expression are maintained during the course of intracellular infection [12], leading to its accumulation in the lumen of the LCV, as has been shown in infected HeLa cells [13]. In addition, HtpB has been found in association with the *L. pneumophila* cytoplasmic membrane [14, 15], as well as on the bacterial cell surface [7, 13], where it can mediate attachment to and invasion of HeLa cells [16]. We have reported that that micro beads coated with purified HtpB were sufficient to attract mitochondria and transiently modify the organization of actin microfilaments in mammalian cells. These two post-internalization events of HtpB coated beads mimic the early trafficking events of virulent *L. pneumophila* [17]. Recently, we have found other evidence clearly demonstrate that HtpB plays multifunctional role in *L. pneumophila* life cycle. For instance, a small portion of the accumulated HtpB in LCV can reach the cytoplasm of the

infected cells, as judged by the Cya-A reporter assay [18]. To identify potential functions of the HtpB present in the eukaryotic cytoplasm, HtpB was ectopically expressed in *Saccharomyces cerevisiae*. HtpB expression induced Pseudohyphal Growth (PHG) in yeast, suggesting it interacts with eukaryotic targets. Using a yeast two-hybrid screen (Y2H), we showed that HtpB interacted with S-Adenosyl Methionine Decarboxylase (SAMDC), an essential conserved enzyme required for synthesis of polyamines [18]. Over expression of the yeast gene encoded SAMDC, SPE2, also induces PHG in *S. cerevisiae*. Thus, overexpression of either HtpB or its interacting protein, SAMDC, induces the same phenotype in *S. cerevisiae*. Surprisingly, a strong correlation exists between elevated polyamine levels and hyphal formation in many yeast species [19 - 21]. Moreover, using an in vitro binding assay, we confirmed that HtpB also interacts with SAMDC from amoeba, and macrophages, confirming the importance of this host protein in *L. pneumophila* pathogenesis. It turned out that host polyamines are crucial for optimal intracellular growth of *L. pneumophila* [18].

In the above study, we could not fully explain of how HtpB can interact with SAMDC from the host cells or *S. cerevisiae* without the presence of the *L. pneumophila* co-chaperonin HtpA. In this study, based on the facts that (i) there is a high degree of homology between eukaryotic and bacterial co-chaperonins, (ii) bacterial chaperonin can interact with eukaryotic co-chaperonin [11], we hypothesized that HtpB could recruit the host cell Hsp10 as co-chaperonin in order to (i) appropriately interacts with SAMDC and to (ii) mediate mitochondrial attraction and actin microfilaments reorganization in host cells [17]. Indeed, in this study, using yeast two-hybrid (Y2H) screen of HeLa cell cDNA library, we showed that HtpB strongly interacts with the small heat shock protein Hsp10 of HeLa. The Hsp10-HtpB interaction might be crucial for the aforementioned HtpB-induced phenotypes.

Materials and Methods

Culture condition, strains, and plasmids

Strains and plasmids used in this study are described in [Table 1]. *Escherichia coli* strain DH5 α was grown at 37°C on Luria-Bertani (LB) media containing ampicillin (100 μ g/mL). DH5 α transformants harboring the plasmids of interest were stored at -70°C in nutrient broth containing 10% dimethyl sulfoxide. *S. cerevisiae* strains were cultured at 30°C on YEP-Dextrose agar or in YM-1 broth [22], containing the appropriate supplements (all at a concentration of 10 μ g/mL) to compensate for strain auxotrophies. Sugars added to yeast culture media were used at 2% (w/v). Synthetic Complete (SC) or synthetic defined (SD) medium (Guthrie & Fink 1991) was used to select and grow plasmid-carrying prototroph's. SD media containing kanamycin (40 μ g/mL) and appropriate amino acid combination was used to select and grow kanamycin-resistant auxotroph yeast transformants [18, 23]. Yeast strains were stored at -70°C in YM-1 broth with 15% glycerol.

Yeast transformation

Lithium acetate transformation was used to introduce yeast shuttle plasmids into yeast cells according to a standard protocol [3, 24]. A culture of *S. cerevisiae* cells was grown overnight to a density of 2 X 10⁷ cells/mL (enumerated in a Coulter particle counter, ZM, Coulter Electronics, Mississauga, Ontario) in YEP-Dextrose. Approximately 2 X 10⁸ cells were harvested by centrifugation and resuspended to 2 x 10⁶ cells/mL in 100 mL pre-warmed YEP-Dextrose medium and grown to a final density of 2 x 10⁷ cells/mL. The freshly grown cells (~10⁹) were washed with 10 mL ddH₂O, resuspended in 1 mL ddH₂O and transferred to 1.5 mL micro centrifuge tube. The cells were then washed with 1 mL of freshly diluted 1X TE/lithium acetate solution [100 mM lithium acetate in 1.0X TE (0.01 M Tris-HCl, 0.01 M EDTA), pH 7.5] and resuspended to a cell density of ~2 X 10⁹ cells/mL in 1X TE/lithium acetate. Fifty μ L of the yeast cell suspension was mixed with 1 μ g transforming DNA, 50 μ g single stranded salmon sperm DNA and 300 μ L polyethylene glycol (PEG 3350) solution (50 % PEG (w/v) in 10X TE/lithium acetate solution). The cells were

then washed and incubated at 30°C with agitation for 30 min. The cells were heat shocked at 42°C for 15 min and were then pelleted by centrifugation at 4000 x g for 5 min. The cells were then resuspended in 1X TE and plated onto appropriate selective media.

Pseudohyphae formation and Invasive Growth

PHG was performed as described in [18]. Briefly Yeast cells grown at 30°C with agitation (150 rpm) in YEP-Dextrose or SD medium with 2% dextrose, including appropriate selection, were harvested in exponential phase. Cells (~10⁷) were washed in water, diluted to 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶ in inducing medium, spotted in duplicate 100 μ L drops on solid inducing medium plates, and incubated at 30°C in a humid chamber. Cell elongation and unipolar budding were scored 15 to 20 h after inoculation (while the inoculum drops were still wet) by light microscopy using the 40X objective of a Nikon DIAPHOT-TMD inverted microscope. To test for invasive growth, plates were incubated for five days, surface-washed with a stream of ddH₂O, and observed as above. Photographs were captured with a Nikon 2000 camera using 35-mm Fuji film, or digitally with a Pro-series monochrome camera and Image-Pro 4.0 software (Media Cybernetics Inc., Silver Springs MD).

Y2H screen of a HeLa cDNA Library

The haploid yeast strain Y187 [Table 1] was the host strain for the MATCHMAKER GAL4 HeLa cDNA library (BD Bioscience, Palo Alto, CA). The HeLa cDNA library was cloned into plasmid pGADT7 [Table 1] to create plasmids that encode Gal4-AD-HeLa cell fusion proteins. (For simplicity, the HeLa library plasmid was named pGADT7::cDNA_x). For the Y2H assay, the yeast strains Y187, bearing the cDNA library and strain AH109 [Table 1], bearing plasmid pGBD-C1::htpB and were mated and diploids selected by plating on SD medium with appropriate supplements as described in the MATCHMAKER two-hybrid system user manual (Clontech). Blue colonies that grew on media lacking leucine and tryptophan (to select for the mated AH109-Y187 diploid

strains), and lacking histidine and adenine (to select for positive interaction via activation of HIS3 and ADE2 reporter genes), and supplemented with X- α -gal (to select for positive interaction via activation of MEL1 reporter gene) were considered to express HeLa cell proteins that interacted with HtpB. The pGADT7:c-DNAx library plasmids [Table 1] were isolated and transformed into E. coli strain DH5 α for plasmid

amplification. The amplified plasmids were isolated using a kit (Qiagen miniprep) and sent for DNA sequence determination (Genome Quebec, Canada) of the yeast library DNA fragment. The nucleotide BLAST tool (NCBI website), was used to identify proteins encoded by the positive Y2H library fragments.

Table 1: Bacterial and plasmids used in this study

Plasmids	Selection marker	Characteristics	Reference or source
pBluescript II KS	Amp ^R	High-copy plasmid used as a general cloning vector in E. coli	Stratagene
pGBD-C1	Amp ^R , Trp ⁺	Yeast two-hybrid plasmid encoding the GAL4 DNA-binding domain (DBD) controlled by a modified alcohol dehydrogenase promote PADH1	(James et al. 1996)
pGBD-C1::htpB	Amp ^R , Trp ⁺	pGBD-C1 carrying the GAL4DBD::htpB gene fusion, GALDBD fused to htpB	Nasrallah et al. 2011b)
pGADT7	Amp ^R , Leu ⁺	Yeast two-hybrid plasmid encoding the GAL4 trans-activating domain, controlled by a modified S. cerevisiae PADH1 promoter	Clontech
pGADT71::cDNAx	Amp ^R , Leu ⁺	pGADT7C1 carrying highly representative cDNA genomic libraries from HeLa cell	Clontech
pSE1111	Amp ^R , Trp ⁺	pGAD-C1 positive control plasmid encoding the yeast Snf1 protein fused to the GAL4 DNA binding domain. Snf1 is an interacting protein partner for Snf2	Durfie et al.1993
pSE1112	Amp ^R , Leu ⁺	pGAD-C1positive control plasmid encoding the yeast Snf2 protein fused to the GAL4 DNA binding domain. Snf2 is an interacting protein partner for Snf1	Dufree et al. 1993
Strains			
W303		MAT α leu2-3112 ura3-1 his3-11 15 trp1-1 ade2-1	G. Jonston
AH109		MAT α , trp1-901, leu2-3, 112, ura3-52, his3-200, gal4D, gal80D, LYS2 : : GAL1UAS-GAL1TATA-HIS3, GAL2UAS-GAL2TATA-ADE2, URA3 : : MEL1UAS-MEL1 TATA-lacZ	Clontech
Y187		MAT α ura3-52his3-200 ade2-101trp1-901leu2-3, 112gal4 Δ met-gal80 Δ URA3::GAL1UAS-GAL1TATA-lacZ	Clontech
DH5 α		F- Φ 80 Δ lacZAM15 Δ thi-1 relA1	Clontech

Rapid plasmid isolation from yeast

Two mL of overnight broth-grown yeast cells were pelleted and resuspended in 200 μ L of solution I (100 mM NaCl, 10 mM Tris-HClpH 8.0, 1 mM EDTA, 0.1% SDS, w/v). Acid-washed glass beads (0.4 mm diameter, sterilized at 160°C overnight) were added until just below the level of the liquid and vortexed at maximum speed for 2 min. 200 μ Lice cold solution II (0.2 M NaOH, 1% Triton X-100, w/v) was then added. Following a 5 min incubation on ice, the sample was

then treated with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, v/v). This mixture was briefly vortexed and centrifuged for 2 min. The aqueous upper phase was transferred to a clean tube then used to electroporate electrocompetent DH5 α [Table 1]. Plasmids were isolated from DH5 α using a kit (Qiagen miniprep).

Protein electrophoresis and immunoblotting

Protein electrophoresis and immunoblotting was done as described [25]. Bacterial cell pellets from 1-mL suspensions with an OD₆₂₀ of 1.0 unit, were solubilized in 100 µL of Laemmli sample buffer, and 10 µL per lane were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 12 % (w/v) acrylamide vertical slab mini-gel. For yeast samples, 10⁸ pelleted cells (800 x *g* for 5 min) were resuspended in 200 µL of sample buffer containing the α -yeast protease inhibitor cocktail (Sigma), and mechanically broken by adding ~100 µL of acid-washed and baked glass beads (BT-5 high impact beads, 40-50 µm diameter, Supply America Company Inc., Norfolk, Virginia) and vortex mixing at 4°C for 15 min. Samples were then boiled for 5 min, unbroken cells and cell wall debris pelleted at 15,000 x *g*, and 10 µL of the supernatant per lane subjected to SDS-PAGE. For immunoblotting [26], proteins resolved by SDS-PAGE were transferred onto nitrocellulose membranes using a BIO-RAD electro transfer apparatus (Model Mini-Protean® II), To visualize transferred proteins, nitrocellulose membranes were stained with a 0.2% (w/v) solution of Ponceau-S (Allied Chemical Co., New York, NY) prepared in 3% (w/v) trichloroacetic acid, 3% (w/v) sulfosalicylic acid, and a reference digital image was acquired. Immunostaining was done with a appropriate primary monoclonal antibody (MAb) [GW2X4B8B2H6 (165) for HtpB. diluted 1:1,000 in Tris buffer solution (TBS) containing 0.1% (w/v) bovine serum albumin (BSA). Secondary antibodies were alkaline phosphatase conjugates of either anti-mouse or anti-

rabbit IgG (Cedarlane Laboratories Ltd.) diluted 1:5,000 in TBS containing 0.1% (w/v) BSA.

Results

Expression of HtpB in a form of fusion protein GAL4DBD-HtpB

Before performing a Y2H screen using the bait plasmid pGBD-C::HtpB [Table 1], we wanted to test whether the GAL4DBD-HtpB fusion chimera protein, which is encoded by pGBD-C::HtpB is expressed in the Y2H strain Y187, and the expression of this fusion protein does not inhibit yeast growth. It should be noted that the expression of the GAL4DBD-HtpB fusion protein by this Y2H plasmid is controlled by a modified alcohol dehydrogenase promoter (*P_{ADHI}*), which is constitutively expressed in a non-overwhelming quantity throughout the yeast growth cycle when it grows in non-inducing glucose medium [27, 18]. Ectopic expression of the GAL4DBD-HtpB (80 kDa) from the pGBD-C:: *htpB* in yeast cells grown in medium containing glucose was confirmed by immunoblot [Figure. 1]. No significant growth differences were noticed between two the HtpB -expressing clones pGAD-C:: HtpB and the empty plasmid control pGAD-C1 that grown in glucose-containing medium (data not shown). These results suggest that GAL4DBD-HtpB fusion protein is expressed in a quantity that seems appropriate to perform a Y2H screen.

Figure: 1

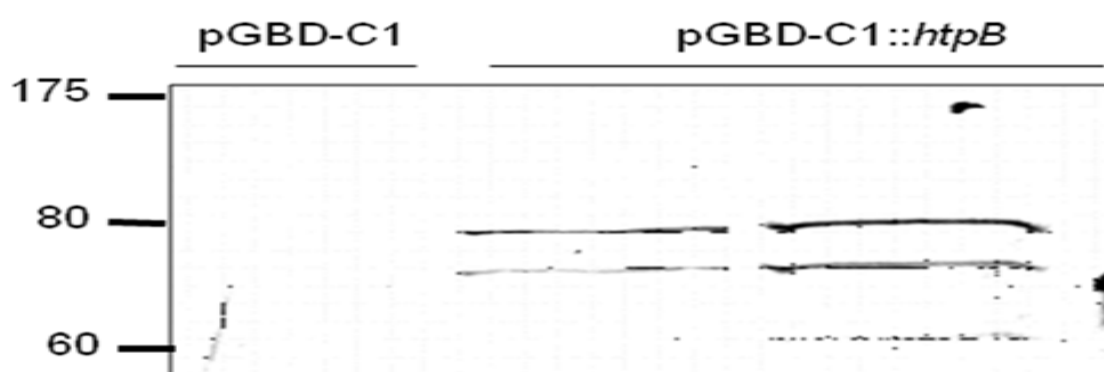


Figure 1: Expression of the Gal4BD-HtpB chimera detected by immunoblotting. Y187 carrying the indicated vectors were grown in SD-glucose medium without tryptophan. Whole cell lysates from these yeast strains were separated by SDS-PAGE, transferred to a nitrocellulose membrane, and immunostained with monoclonal antibody specific for HtpB. The sizes (kDa) and position of protein standards are marked at the left side of the blot. The presence of more than one band under pGBD-C:htpB resulted from degradation products of the GAL4DBD-HtpB (~ 78 kDa)

pGBD-C1::HtpB expressing GAL4DBD-HtpB fusion protein is an ideal bait plasmid to be used in Y2H screen

Next, we wanted to confirm that the expression of the HtpB in the context of GAL4DBD-HtpB fusion protein does not affect the functionality of HtpB, and that pGBD-C1::htpB can be used as a bait to conduct a Y2H screen. We have reported previously [18] that ectopic expression of HtpB in the yeast

cells strain W303 grown in inducing medium from a high-copy plasmid pEMBLyex4:: HtpB or pPP389::HtpB (in which HtpB expression is controlled by a galactose-inducible hybrid promoter GAL1-CYC1) strongly induces PHG. Based on this finding, we hypothesized that if the fusion of GALDBD to the N-terminal end of the HtpB does not affect HtpB functionality, then the expression of GAL4DBD-HtpB fusion protein should also be able to induce PHG. As shown in [Figure.2], we found that GAL4DBD-HtpB expression from the pGBD-C1::HtpB is also able to induce the yeast strain W303-1b to form pseudohyphae after 15-20 h inoculation on synthetic define (SD) solid medium with glucose. The PHG is characterized by elongation and budding in a unipolar direction, and after 5 days at 30°C, they produced some agar-invasive filamentous colonies [Figure. 2]. W303-1b yeast cells carrying the empty vector pGBD-C1 stayed ovoid when grown on the same medium and were not agar-invasive [Figure. 2] Collectively, with the immunoblot results, these findings strongly suggest that our Y2H plasmid bait pGBD-C1::HtpB should be ideal to perform a Y2H screen.

Figure: 2

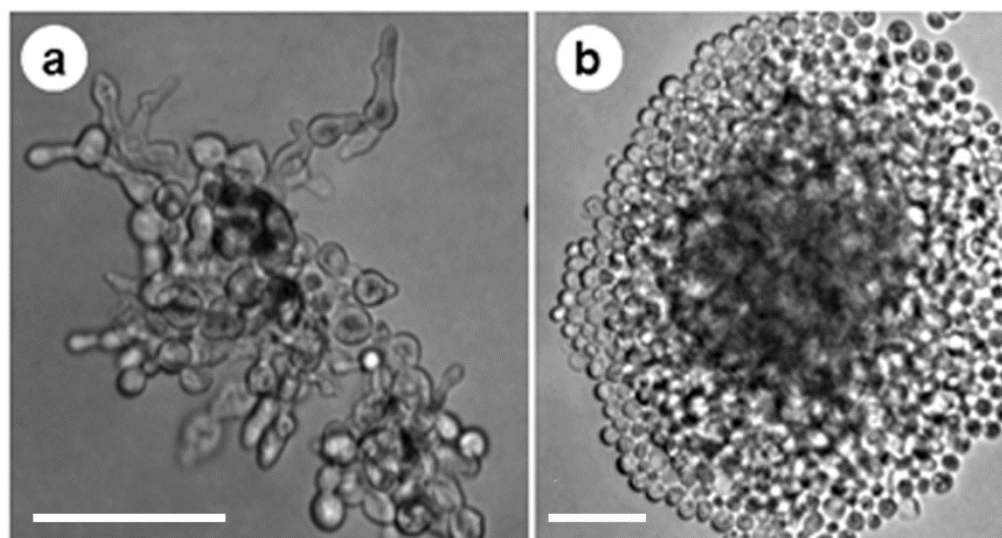


Figure 2: The Gal4DBD-HtpB chimera induces PHG on glucose-replete medium. *S. cerevisiae* strain W303-1b within microcolonies was grown on non-inducing SD-glucose solid medium for 5 days (a) and (b). This strain carrying either

pGBD-C1:: htpB for expression of HtpB (a), or the empty vector pGBD-C1 (b) as a negative control. The filamentous colony shown in (a) had penetrated the agar. Size bars represent 20 μm

The eukaryotic small heat shock protein (Hsp10) interacts with HtpB

We hypothesized that there is a specific interaction between HtpB and a mammalian protein(s) that involve in the interaction of HtpB with SAMDC and the multiphenotype induction in mammalian cells. To test this hypothesis, we conducted a Y2H screen using, as bait, the Gal4DBD-HtpB chimera to test for HeLa cell proteins (Gal4AD-HeLa proteins) that interacts with HtpB. Approximately 5×10^6 yeast clones carrying HeLa c-DNA library plasmid (pGADT7::cDNAx) were screened, and 21 positive clones were identified [Figure 3]. DNA sequence determination revealed that all of the 21 positive clones encoded the mammalian small heat shock protein Hsp10. One of these positive clones was chosen and the encoding plasmid was named pGADT7::HSP10. Hsp10 is the co-chaperonin that together with the mitochondrial chaperonin (Hsp60p), mediates protein folding in the mitochondrial matrix [2]. The eukaryotic Hsp10 and Hsp60 proteins are synthesized in the cytoplasm and imported through the mitochondrial membranes into the mitochondrial lumen. Additionally, Hsp10 has been detected on the surface of mitochondria [28]. Therefore, the HtpB-Hsp10 interaction could be relevant to the recruitment of mitochondria to the LCV.

Figure: 3

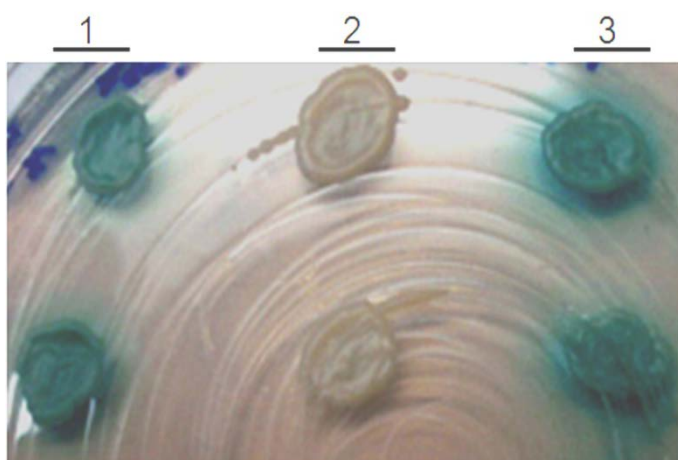


Figure 3: The HeLa cell Hsp10 interacts with HtpB. Yeast strain AH109 transformed with pGBD-C1::htpB was mated

with yeast strain Y187 transformed (1) with one of the 21 isolated HeLa cDNA positive clones that interact with HtpB (pGADT7::HSP10), or (2) Y187 transformed with empty vector pGADT7 (serves as negative control), or (3) AH109 transformed with pSE1111 (Table.1) was mated with Y187 transformed with pSE1112 (serves as positive control). All of these clones grew on media lacking leucine and tryptophan and supplemented with X- α -gal (indicator for activation of MEL1 reporter gene). Negative Y2H interactions (AH109 contains pGBD-C1::htpB plasmid mated with Y187 contains pGAD-C1::cDNAx in which cDNA encode HeLa cell protein that does not interact with HtpB) looks as in (2). Positive Y2H interacting library clones were originally grown on SD plates lacking leucine and tryptophan (to select for mating) and histidine and adenine (to select for positive interaction via activation of HIS3 and ADE2 reporter genes). Negative interacting clone did not grow on this media.

Discussion

We previously reported using two different protein-protein interaction assays that HtpB interacts with a eukaryotic partner protein SAMDC from yeast, amoeba, L929 mouse fibroblasts, and U937 macrophages [18, 25]. In this study, using a Y2H screen we showed that HtpB interacts with the Hsp10 of host cells. In this study, it was unexpected that in spite of screening $\sim 5 \times 10^6$ yeast clones of the HeLa cDNA library by Y2H, SAMDC was not identified as a partner protein for HtpB. In theory, this HeLa Y2H screen should be sufficient to cover 250X the estimated 2×10^4 protein-encoding mRNAs present in the human genome. Therefore, it can be concluded that, despite the many advantages of the Y2H system, it also has limitations [29, 30]. The failure in identifying SAMDC as prey for HtpB could be due to one of the following reasons: (i) the fusion proteins in the Y2H system must be targeted to the nucleus to activate the Gal4 transcription factor in order to activate the expression of reporter genes. The complex of mammalian SAMDC fused to Gal4AD and HtpB fused to Gal4BD may not be able to enter the nucleus to activate the reporter genes. (ii) SAMDC is expressed as proenzyme and undergoes post-

translational modification to become an active enzyme. The SAMDC-HtpB interaction may thus depend upon posttranslational modifications of the human SAMDC that do not occur in yeast. (iii) The mammalian SAMDC fused to Gal4AD is unable to interact with HtpB fused to Gal4BD. (iv) SAMDC cDNA is not represented in the MATCHMAKER HeLa cDNA library.

The interaction of HtpB with the mitochondrial co-chaperonin Hsp10 could have potential implications for the already identified effects of HtpB on mammalian cells. The HtpB-Hsp10 interaction could be relevant to mitochondrial recruitment because Hsp10 has been detected on the surface of mitochondria, as well as in other extra-mitochondrial locations where Hsp10 moonlights as the early pregnancy factor [28]. Finding Hsp10 in extra-mitochondrial locations is not entirely surprising because Hsp10 is a mitochondrial protein whose encoding gene resides in the cell nucleus, and it is synthesized in the eukaryotic cytosol, from where Hsp10 needs to be imported into the mitochondria. While the import of proteins into mitochondria is mostly co-translational, it is possible that some Hsp10 molecules could stay on the mitochondrial surface (bound to the import apparatus) after translation [3].

To explain the mechanism by which HtpB induces PHG in yeast, we proposed the following model [Figure 4].

SAMDC is a rate-limiting enzyme essential in the polyamines biosynthesis, which is strongly regulated in eukaryotic cells [31]. Like other rate-limiting enzymes, SAMDC has a short half-life, its basal activity is low, and it is rapidly induced by different stimuli [31-33]. Additionally, SAMDC is synthesized as an inactive proenzyme that in response to different stimuli undergoes an intramolecular cleavage reaction to form the active enzyme [31, 34]. Thus, having said that HtpB is essentially a chaperonin that is important in protecting proteins from denaturation, it is possible that one or more of the processes that influence the function of SAMDC could be enhanced upon interaction with HtpB. Yet, HtpB needs a co-chaperonin to function properly in protein folding. Therefore, based on the high degree of homology between co-chaperonin, we believe here that Hsp10 of the host cells can complement the function of the *L. pneumophila* HtpA. Consequently, the interaction of Hsp10-HtpB with the SAMDC should in theory protect SAMDC from early degradation leading to increase activity of SAMDC. This in turn results in an increase in the amount of intracellular polyamines that are required for triggering PHG, optimal intracellular growth of *L. pneumophila*, and perhaps other HtpB-induced phenotypes. Finally, this study constitutes a good example of how the yeast functional model has enabled us to uncover unique functions of HtpB and a novel aspect of chaperonin biology.

Figure: 4

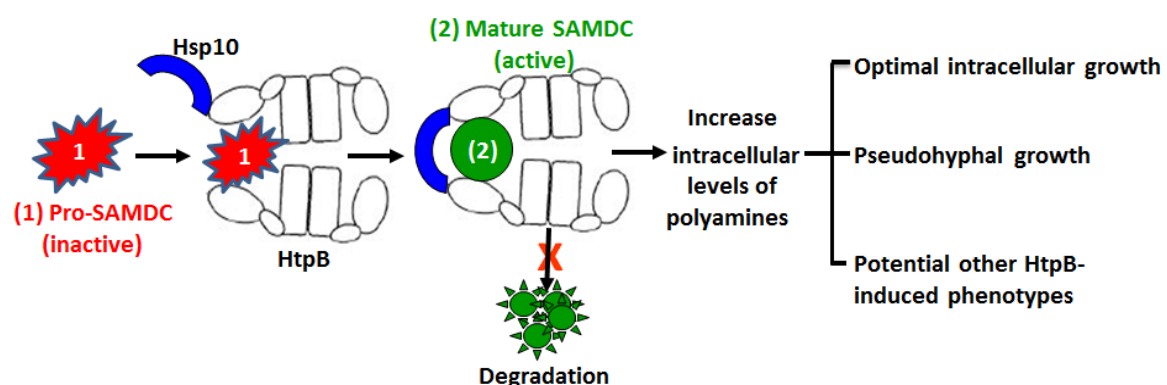


Figure 4: Model illustrating the potential consequences of the interaction of Hsp10-HtpB with SAMDC. We propose that Hsp10-HtpB interaction induces PHG through enhancing SAMDC activity. SAMDC is synthesized as an inactive proenzyme (1) that undergoes intramolecular cleavage to form the active enzyme (2). The interaction of SAMDC with HtpB could have a number of consequences. Because of its protein folding activity, HtpB could simply enhance the proper folding of SAMDC proenzyme which in turn could accelerate its rate of cleavage into active SAMDC. Alternatively, the interaction of active SAMDC with HtpB could extend its half-life by preventing its degradation. Finally, HtpB could increase the enzymatic activity of active SAMDC. This model assumes that the Hsp10-HtpB-bound SAMDC is still capable of interacting with its substrate. These potential scenarios would lead to an increase in the level of intracellular polyamines that are important for induction of PHG in *S. cerevisiae*, optimal intracellular growth of *L. pneumophila*, and other HtpB-induced phenotypes

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