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A NOVEL REVIEW OF HEAT SHOCK PROTEIN 110 KDA: A BASIS FOR
RESEARCH AND CONTINUED EXPERIMENTATION THROUGH BIOCHEMICAL
ANALYSIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at Virginia Commonwealth University.

by

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List of Abbreviations

ATP - Adenosine triphosphate

C. albicans – *Candida albicans*

DNA – Deoxyribonucleic acid

HSP(s) – Heat Shock Protein(s)

Hsp110 – Heat Shock Protein 110 kDa

LB – Luria broth

NBD – Nucleotide-binding domain

NEF – Nucleotide exchange factor

P. falciparum – *Plasmodium falciparum*

PCR – Polymerase chain reaction

SBD - Substrate-binding domain

S. cerevisiae – *Saccharomyces cerevisiae*

SDS PAGE – Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Abstract

Heat shock protein 110 kDa, Hsp110, is a distinct cellular protector, different in form and function from Hsp70, a close relative of Hsp110. Functioning primarily as a holdase or in tandem with other molecular chaperones, a review of current accomplishments elucidates the uniqueness of this protein and the continued mysteries that surrounds it. Found only in eukaryotes, Hsp110 has been linked to many diseases, ranging from parasitic infection to neurodegenerative disorders. While still lacking, studies of this protein have provided much in the realm of speculation on the mechanisms behind Hsp110s' contribution to different pathologies. This review will serve as an introduction to present thinking and provide preliminary data investigating the form and function of this chaperone.

Chapter 1 Introduction

Introduction

Since their discovery in *Drosophila*, Heat Shock Proteins (HSPs) have been heavily studied and are a continued topic of interest (Ritossa, 1962). Proteins of this class aid in the folding and or refolding of proteins, prevention of protein aggregates, and in the process of protein degradation through ubiquitination and are called molecular chaperones (Balchin et al., 2016; Hartl et al., 2011; Kandasamy et al., 2018; Kuo et al., 2013; Lang et al., 2021; Mayer et al., 2013; Nillegoda et al., 2015; Yam et al., 2005). The penultimate aim of HSPs as we know it is to maintain cellular homeostasis in the face of stressors, commonly exemplified by temperature flux outside of ideal conditions, such as in the first discovery of these proteins. Heat Shock Protein 70 kDa, Hsp70, is the most ubiquitous of these proteins, existing in most organisms in some form or another, however, other HSPs remain of interest for their implications in human disease and virulence in human pathogens (Batista et al., 2018; Becherelli et al., 2013; Berestoviy et al., 2021; Bhartiya et al., 2015; Bielecka-Dabrowa et al., 2009; Dorard et al., 2011; Eroglu et al., 2010; Gong et al., 2017; Mayer et al., 2013; Mehta et al., 2005; Moran et al., 2015; Muralidhara et al., 2013). This review will serve to provide current evidence for continued research on Hsp110s, distant homologues of Hsp70s.

Heat Shock

The Heat Shock Response is the cellular reaction to insults or stressors. First described by Dr. Ritossa when he noticed puffing differences in the glands of fruit flies after an accidental change in the temperature of their incubator, heat shock as a cellular response and event has been heavily studied (Ritossa, 1962). Maintaining homeostasis and the subsequent viability by organisms depends on the ability to adapt less than ideal conditions and spontaneous events. These cellular insults can range from toxic solutes to temperature and electric stimuli that all can cause degradation and lower the viability of an organism. HSPs act as the stewards of organisms in the face of these insults (Morimoto et al., 1997).

HSP70

Hsp70 is a major HSP and can be thought of as the classic HSP. It plays a variety of roles in its function as a chaperone. As a protein, Hsp70 is widely conserved and exists in many different species in an analogous or homologous forms. The major role of Hsp70 as a HSP has been widely studied and its importance and necessity of proper function has been linked to the pathologies of different diseases, ranging from cancer to neurodegenerative diseases (Murphy, 2013; Shrestha et al., 2016; Zuiderweg et al., 2017). While Hsp70 is thought of as the main HSP, many other HSPs interact with it as cochaperones and can assist it with their functions to maintain homeostasis. An HSP cochaperone of note that will be detailed here is Hsp110.

Chapter 2 Hsp110

Structure

A study of the Hsp110 from *Saccharomyces cerevisiae* has given insight into this protein's function and helped to elucidate its structure (Fig. 1) (Liu et al., 2007). Similar to Hsp70, Hsp110 has two major domains, a substrate-binding domain (SBD) at the carboxy terminus and a nucleotide-binding domain (NBD) at the amino terminus with a short linker segment between the two domains (Fig. 1A). The SBD is attracted to hydrophobic polypeptide

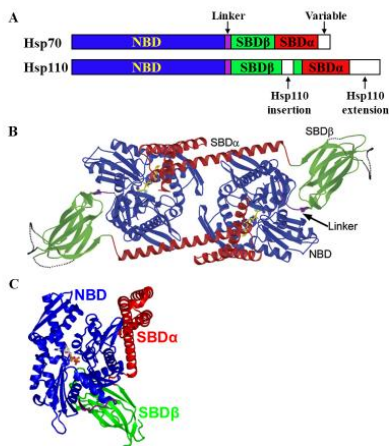


Figure 1: The Structure of Hsp70 and Sse1, a Hsp110 from yeast. (A) Schematics of Hsp70 and Hsp110 sequences and prototypic Hsp70 domain structures. Coloring is NBD (blue), interdomain linker (purple), SBD β (green), SBD α (red). (B) Ribbon diagram of the dimer. Protomer A is left, and B is right. Missing loops are dotted. Protein coloring is as in (A). (C) Ribbon diagram of protomer B in the canonical, front-face NBD view. *Made possible by a generous gift from Dr. Liu and with permission to reproduce.

segments while the NBD binds ATP. Hsp70 carries out its function in a cycle that has two major forms: an open and closed conformation. When ATP is bound to the N-terminal NBD, a conformational change occurs that opens the SBD and allows for binding of protein substrate. ATP hydrolysis causes a closed conformation to occur that prevents the release of substrate. Nucleotide Exchange Factors (NEFs), a function of Hsp110, allow for the release of ADP from the NBD and the release of substrate

from the SBD to allow for this process to repeat as a cycle (Abrams et al., 2014; Alderson et al., 2016; Andréasson et al., 2008, Batista et al., 2018; Becherelli et al., 2013; Bösl et al., 2006; Dragovic et al., 2006; Goeckeler et al., 2008; Kaimal et al., 2017; Kityk et al., 2018; Liu et al., 2007; Mattoo et al., 2013; Mayer et al., 2013; Raviol et al., 2006; Shaner et al., 2006; Shaner et al., 2005; Shorter, 2011; Yam et al., 2005). While Hsp110 is a cochaperone and may have a similar conformational cycle to Hsp70, recent studies suggest Hsp110 may bind substrates without ATP and may have its own intrinsic functions besides assisting Hsp70 (Bhartiya et al., 2015; Kumar et al., 2020; Matoo et al., 2013; Yakubu et al., 2018).

Hsp110 as a Nucleotide Exchange Factor

Hsp110 is a cochaperone that assists Hsp70 in its functions. In order for Hsp70 to perform its cyclic function, the binding and release of substrates, it will form complexes with other HSPs or cochaperones. One of these cochaperones is Hsp110. It has been demonstrated that the binding order of cofactors for HSPs affects conformation and the ability to form distinct complexes (Nillegoda et al., 2015). For Hsp110, the binding of ATP at the NBD allows for a complex with Hsp70 and the refolding of protein aggregates (Bhartiya et al., 2015; Matoo et al., 2013; Nillegoda et al., 2015; Yakubu et al., 2018).

HSP110 as Distinct Proteins

Hsp70(s) is the classical HSP and the first to be discussed. As previously mentioned, this super family of proteins activates during time of cellular stress and acts to maintain protein homeostasis. In order to function, Hsp70 has the ability to bind polypeptide substrates and ATP (Mayer et al., 2005; Young, 2010). Hsp110 cochaperones help Hsp70 and can act as NEFs and

assist in ATP hydrolysis and subsequent release of ADP. HSP70s are ATP dependent and rely on NEFs to maintain their rate of activity. HSP110s, however, in recent studies have been shown to do more than be mere NEFs and have their own intrinsic activities and function.

Normally, proteins achieve their three-dimensional confirmation in order to carry out intended functions. The environment, of a cell, however, is less than ideal. The cytoplasm of a human cell is extremely crowded environment which, when under added stress, can have a polypeptide chain become denatured and expose hydrophobic amino acids to the aqueous environment of the cytoplasm. This denaturing can cause the formation of protein aggregates. It has been demonstrated that while Hsp110 is a NEF, it also has its own ATP binding activity that is tied to the prevention of protein aggregates or a “holdase function” (Shaner et al., 2004).

Hsp110 has been implicated to prefer aromatic amino acids as a substrate in contrast to Hsp70s preference for aliphatic residues (Xu et al., 2012). While this does not truly set Hsp110 alone as a chaperone versus cochaperone solely, other studies have shown that Hsp110 alone is able to prevent protein aggregation and deletion of Hsp110 can be detrimental, causing a lack of thermotolerance and growth at high temperatures or cell death (Kuo et al., 2013; Moran et al., 2013; Muralidharan et al., 2012; Yakubu, et al., 2021). ATP is required for this holdase activity. Paradoxically, while Hsp110s are able to act as a holdase independent of ATP hydrolysis, the NBD of Hsp110 has recently been shown to have an intrinsic ATPase function that is normally suppressed in normal function (Kumar et al., 2020).

Chapter 3 Hsp110 Implicated in Diseases

Hsp110 of Yeast Linked to Disease

Candida Albicans is a fungus that is part of the normal human biome. In most cases, *C. albicans* will never cause illness or an infection but in some cases, it is the cause of human yeast infections. These infections can occur in the body in warm, moist environments to include the mouth, with most infections occurring in immunocompromised individuals but still possible in healthy adults.

While there are many virulence factors responsible for infection of individuals, one of note is the ability of *C. albicans* to form a biofilm. Biofilms are polypeptides that are secreted from the yeast into its surrounding to increase its rate of survival. Yeast cells that originate from a mature biofilm have been shown as a direct contribution to virulent, being more virulent in a mouse model of infection (Dadar et al., 2018; Mayer et al., 2013; Moyes et al., 2015). Recent studies have linked HSPs to the production of the biofilm, with increased expression of selected HSPs being noticed and Msi3p, the Hsp110 homologue, being linked to antifungal drug resistance (Becherelli et al., 2013; Gong et al., 2017; Kadosh, 2016).

Hsp110 of Malaria and a Link to Virulence

Malaria is a disease that ravages the world. Many third world and developing countries devote resources every year to combat this threat. This disease is caused by the *Plasmodium falciparum* parasite, a single cellular organism that thrives in tropical environments. It is hallmarked by malarial fevers, intense fevers that afflicts those infected with the parasite.

As the disease is characterized by temperature fluctuations and high fevers, it is not surprising that HSPs have been implicated to play a major role in the virulence of the pathogen. The genome of *Plasmodium falciparum* is characterized by regions of DNA with tandem repeats and AT rich sequences (Muralidharan et al., 2012; Muralidharan et al., 2013). The AT rich regions encode for asparagine residues. While hydrogen bonds between A and T nucleotides are fewer than those of made between C and G nucleotides and therefore have a lower melting temperature, the asparagine residues of *Plasmodium falciparum* proteins help it to survive at higher temperatures, i.e., live through the human body's response to its invasion during the "malarial fever" (Muralidharan et al, 2012; Muralidharan et al., 2013). Not by coincidence, the mean and median lengths of *Plasmodium falciparum* proteins in comparison to other eukaryotic and prokaryotic organism showed that the *Plasmodium falciparum* proteome had the largest variation and protein lengths (Carlton et al., 2005). While the entirety of the *Plasmodium falciparum* genome has been sequenced, its intricacies are still elusive, however, the importance of the *Plasmodium falciparum* Hsp110 cannot be ignored as a potential target for therapeutics. The asparagine rich proteome of *Plasmodium falciparum* coupled with the lengths of protein makes it surprisingly prone to error and misfolding despite the ability of *Plasmodium falciparum* to remain a global threat. This virulence can be attributed to the Hsp110 of *Plasmodium*

falciparum as it has evolved to stabilize asparagine rich regions of proteins and the lack of it causes temperature sensitivity and the eventual perishing of mutant protozoans (Bhartiya et al., 2015; Muralidharan et al., 2012; Muralidharan et al., 2013).

Human Hsp110 in Disease

Hsp110 is of interest not only because of pathogens that malady humans, but also as HSPs and the Hsp110s of humans have been implicated in diseases. Cancerous cells not only grow at rapid rates but seem to thrive in hostile environments, metastasizing from one location to spread to another. It is not surprisingly then that mutant Hsp110 profiles have been associated with a poor prognosis. Recently, a study has demonstrated that human Hsp110 of colorectal cancer cells will translocate to the nucleus and perform DNA repair, providing a possible avenue for the correlation between Hsp110 profile and outcome (Causse et al., 2019). Acting as a foil but in the same vein, a study has been able to demonstrate truncated Hsp110s in colorectal cancer cells improve outcome and chemotherapy efficacy (Dorard et al., 2011).

If we look at another human disease, Alzheimer's, Hsp110 is also implicated in disease progression and prognosis. Alzheimer's is a neurodegenerative disease hallmarked but memory loss and difficulty thinking leading to eventual death. Underlying the condition is the accumulation of Beta-amyloid plaques in the brain, caused by aggregation of hyperphosphorylated tau protein into neurofibrillary tangles. Lack of Hsp110 causes an early onset accumulation of Beta-amyloid plaques or a disease state reminiscent of Alzheimer's in mice (Eroglu et al., 2010). Even outside the realm of human pathogens, Hsp110 remains a target of interest for scientific research in the field of medicine as it is distinguishable from other possible pathogen Hsp110 (Raviol et al., 2006).

Chapter 4 Materials and Methods

CaCl₂ competent cell preparation

A single colony of appropriate *E. coli* strains was inoculated into LB (Lauria broth) medium with no antibiotic and grew overnight at 37 °C. A 1:100 dilution was done into 1 L of LB medium with no antibiotic and grown at 37 °C to an O.D.600 of 0.375. The culture was divided into centrifuge vessels and incubated on ice for 5 minutes. The culture was centrifuged at 3,000 rpm for 7 minutes. The supernatant discarded and cells resuspended in 200 ml of cold 50 mM CaCl₂. The resuspended culture was centrifuged at 2,500 rpm for 5 minutes. Supernatant was discarded and cells resuspended in 200 ml of cold 50 mM CaCl₂. Incubated cells on ice for 30 minutes. The culture was centrifuged at 3,000 rpm for 7 minutes. Supernatant was discarded and cells resuspended in 10 ml of cold 50 mM CaCl₂. The resuspended cells were incubated at 4 °C for 4 hours. After incubation, 15 ml of cold 80% glycerol solution was added per 5 ml of cells, gently mixed well, and then cell suspension aliquoted into prechilled tubes. Tubes were flash frozen in liquid nitrogen and stored at -80 °C in a freezer.

Transformation

Competent cells were thawed on ice. Cell lines used consisted of: XL1-Blue, BL-21(DE3), Stella and Rosetta2(DE3)pLysS cell lines. 100 μ l of thawed competent cells was added to a chilled 14 ml tube. 1 μ l of plasmid DNA added to tube and gently mixed. Tube with plasmid and cells incubated on ice for 30 minutes. Chilled tube submerged in 42 °C water for 45 sec to heat shock cells. Submerged tube placed on ice for 2 minutes after heat shock. 1 ml of LB media added to tube and tube incubated on ice for 37 °C for 1 hour. The transformants were spread on a LB agar plate with appropriate antibiotics and grown overnight at 37 °C to check for viable colonies.

Construct Creation

The *P. falciparum* Hsp110 was amplified using a pair of primers: forward primer (sequence: ACA GAT TGG TGG ATC CAT GTC GGT TTT AGG TAT AGA TAT AGG AAA TGA C) and reverse primer (sequence: GGT GGT GGT GCT CGA GTT AAT TAA TAT TAT TTA AGA ATT TCG TTG TTC T). The primers were ordered from Fisher Scientific. The PCR product was run on a 1% agarose gel at 120V for 60 minutes and the PCR product was purified from the gel band. The pSMT3 vector was digested with BamHI and XhoI then the digested vector was run on a 1% agarose gel and purified (Mossessova et al., 2000). The purified PCR product and purified linearized vector were mixed at a 2:1 molar ratio and then incubated at 37 °C for 15 minutes in a proprietary In-Fusion tube. After incubating, the In-Fusion reaction was inactivated by heating at 50 °C for 15 minutes. The In-fusion reaction mixture was transformed into Stella competent cells and plated on an LB agar plate containing Kanamycin at 25 µg/ml. Single colonies were inoculated into 5 ml LB medium containing Kanamycin at 25 µg/ml and grew overnight. Plasmid was extracted using a miniprep procedure as described below.

Crosslinking procedure

Experimental proteins (Sse1, Sse2, Msi3) were diluted to a concentration of 2 mg/ml in buffer A (25mM Hepes-KOH, pH 7.5, 150mM KCl, 10mM Acetate, 2mM Dithiothreitol and 10% Glycerol) and kept on ice. Glutaraldehyde was diluted to varying concentrations with buffer A (0.00625, 0.0125, 0.025, 0.05, and 0.1%). 10 μ l of the protein dilution was combined with 10 μ l of a glutaraldehyde solution. The protein and glutaraldehyde solution were mixed through gentle pipetting only and incubated on ice for 30 minutes. 5 μ l of 0.5 M Tris-HCl, pH7.5 was added to quench the reaction. The quenched reaction was then kept on ice for 15 minutes then underwent a SDS polyacrylamide gel electrophoresis (SDS- PAGE). The SDS gel was visualized with Coomassie blue stain.

Protein Expression and Purification

Protein was expressed and prepared as detailed in Wang, Ying et al (Wang et al., 2021).

Plasmid Miniprep

Successful transformants with desired plasmid were inoculated into LB Medium with appropriate antibiotics. The culture was spun at 5,000 g for 7 minutes to generate a pellet. The pellet supernatant was decanted, and the remaining pellet was then spun at 5,000 g again for 7 minutes. Resulting residual supernatant removed immediately and the pellet was resuspended in 400 μ l buffer P1. The resuspension was transferred to 2 ml Eppendorf tubes. 400 μ l buffer P2 was added to the resuspension and mixed by inverting the tubes thoroughly. After mixing, 560 μ l buffer N3 was added and mixed by shaking. The resuspension with buffer N3 added was then spun for 10 minutes at 13,000 rpm and ~700 μ l of the resulting supernatant was applied to a spin column. The column with supernatant was spun for 30 seconds to 1 minute at 13,000 rpm and flow-through discarded, this was repeated to the same column until all supernatant was passed through the spin column. 750 μ l buffer PE (with ethanol) was added to the spin column and spun 30 second to 1 minute at 13,000 rpm and the flow through was discarded. The spin column was spun for 1 minute at 13,000 rpm to remove PE buffer. Then, 50 μ l of sterile double distilled H₂O was added to the center of the spin column. A new collection tube was placed under each spin column in case of flow through and the column was incubated at 37 °C for 15 minutes. After the incubation, the spin column was spun for 1-2 min at 13,000 rpm and the elution was collected. The DNA elution was vortexed and DNA concentration checked using OD 260.

All solutions listed are property solutions from Qiagen (P1, P2, N3, PE).

Malachite Green ATPase Assay

800 μ l of Malachite Green Dye solution (36 ml of a .045% Malachite green solution combined with 12 ml of 4.2% ammonium molybdate and 1 ml of 1% Triton X-10 and filtered) was combined with 50 μ l of 1x TEA buffer (50 mM triethanolamine-HCl (TEA), 50 mM KCl, 20 mM $MgCl_2 \cdot 6H_2O$ at pH 7.5). Then 50 μ l of varying phosphate standard solution (varying amounts of H_3PO_4 into H_2SO_4) was added vortexed to mix and incubated for 60 seconds at room temperature. 100 μ l of 34% citric acid solution was added after the 60 seconds incubation and mixture was vortexed again. Three readings of the phosphate standard at 660 nm were taken and this was repeated for each phosphate standard. An ATPase reaction with a final concentration of 50 μ M ATP (100 mM ATP, pH 7.5), 7.25 μ M Protein (Sse1, DnaK, Msi3) was raised to 100 μ l volume with Hepes buffer. The reaction was monitored over three hours with a sample taken at time zero and wanted time points to be measured reaction at 660nm.

Chapter 5 Results

Plasmid Construction

In order to begin a biochemical characterization of *P. falciparum* Hsp110 to gain insights into the specific mechanisms for its robust abilities, a vector was created for future use in protein expression and purification. The gene was amplified and then cloned into the vector pSMT3 (Fig. 2).

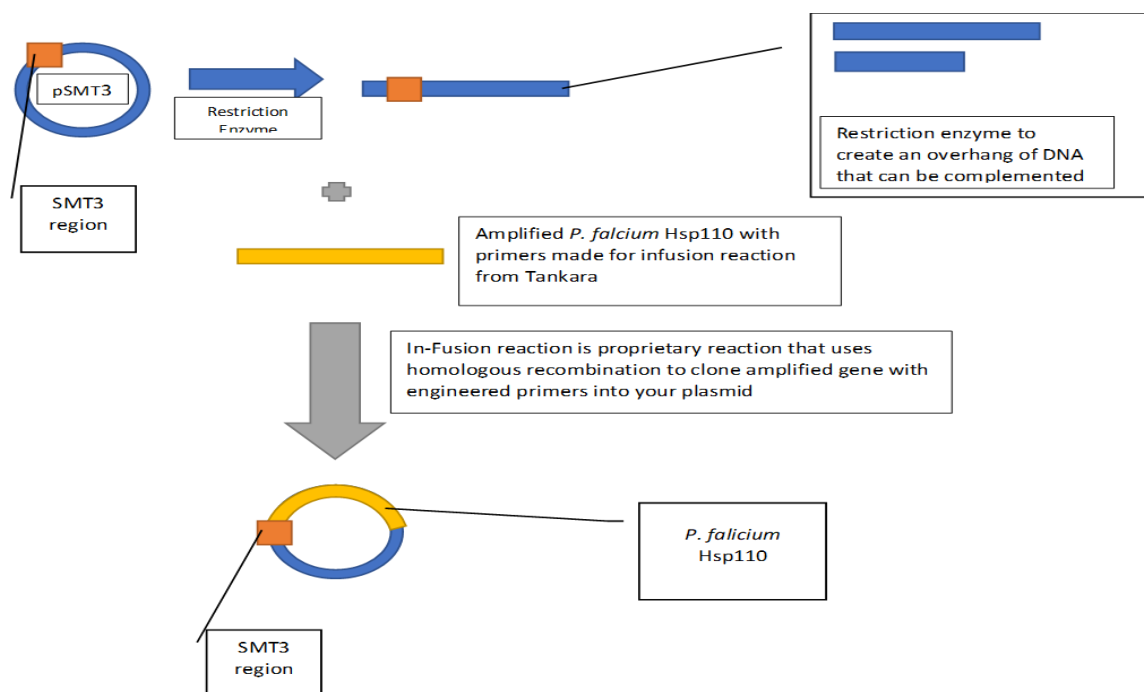


Figure 2: Plasmid Construct. pSMT3 is a plasmid from pET28 with *SMT3* inserted for gene expression in *E. coli*. The proprietary infusion reaction used homologous recombination, specifically at the 3 ends of the linearized plasmid to incorporate our gene sequence.

A restriction enzyme digest as a preliminary test suggested that our pSMT3 plasmid was correct due to the *XhoI* site within the *P. falciparum* Hsp110 gene sequence in addition to the plasmid restriction enzyme sites (Fig. 3). DNA sequencing of the plasmid returned 99% and 98% consensus with NCBI gene: 2655069, the accepted Hsp110 of *P. falciparum* (Table 1).

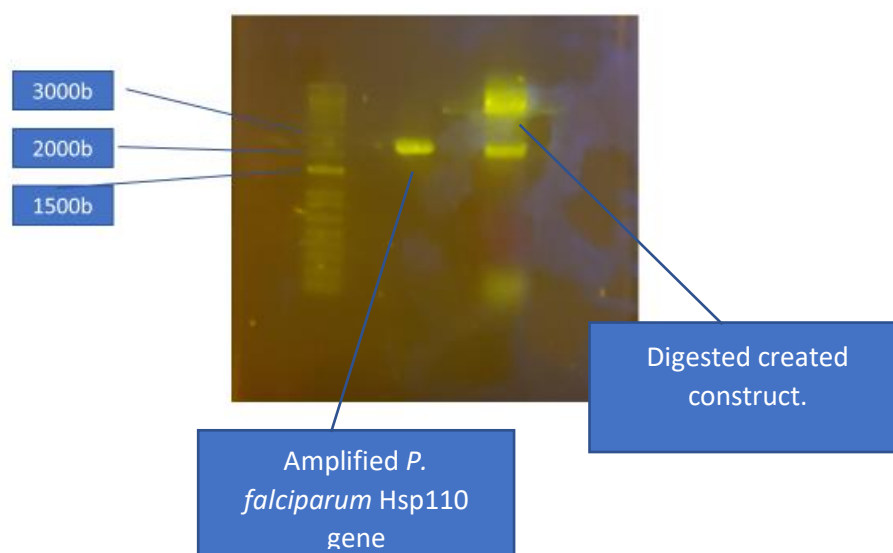


Figure 3: 1% Agarose gel of Hsp110 from *P. falciparum* and plasmid construct digested with BamHI and XhoI. PCR product is around 2 kb. Banding pattern of construct is representative of the three restriction enzyme sites present.

absorbances eliciting a linear curve (Table 2). An inconsistent curve was able to be demonstrated with the use of Hsp110s: Sse1 and Sse1 I163D, a mutant suspected to have increased hydrolytic activity (Table 3, Fig. 4). While this method has proven successful for reporting on the ATP hydrolysis and ATPase activity of other proteins, this method was unsuccessful for Hsp110.

A	
Phosphate Concentration in uM	Absorbance at 660nm
20	0.14
40	0.937666667
60	1.351666667
80	1.771
100	2.016333333
B	
Phosphate Concentration in uM	Absorbance at 660nm
0	0.083066667
20	0.080333333
40	0.127333333
60	0.094333333
80	0.161666667
100	0.225333333
120	0.698
C	
Phosphate Concentration in uM	Absorbance at 660nm
0	0
4	0.363666667
8	0.719
12	1.041666667
16	1.371666667
20	1.761666667
40	1.894666667

Table 2: Phosphate Curve

Phosphate Concentration uM	Absorbance 660nm	
0	0.045333333	
4	0.116333333	
8	0.183333333	
12	0.247666667	
16	0.322333333	
24	0.452	
Sse1 Reaction	Absorbance 660nm	Phosphate Concentration in uM
0	0.164666667	6.941275333
1	0.078333333	1.849421667
2	0.137333333	5.329182667
3	0.109666667	3.697430333
I163D Reaction	Absorbance 660nm	Phosphate Concentration in uM
0	0.194	8.671326
1	0.064333333	1.023715667
2	0.145	5.781355
3	0.189666667	8.415750333

Table 3: ATPase Assay of Hsp110s: Sse1 and I163D

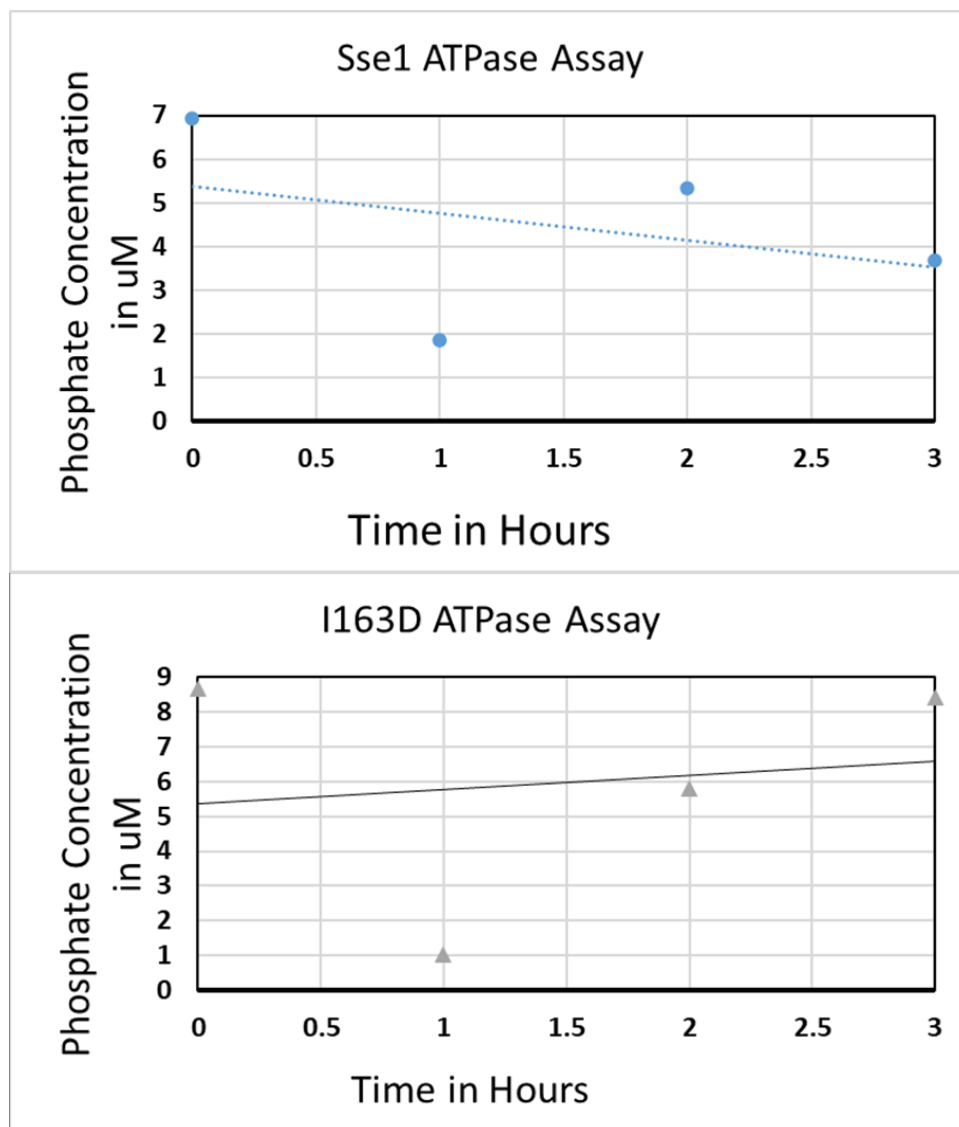


Figure 4: Linear Trendline for ATP Hydrolysis from Table 3. Curve did not eliminate time point zero due to absorbance not equaling zero.

Crosslinking

To investigate the possibility that Hsp110 forms a dimer, glutaraldehyde was used to crosslink purified protein in hopes of exhibiting signs of dimer formation. Two preliminary crosslinking experiments were performed, one on *C. albicans* Msi3 with glutaraldehyde concentrations at 0.00625, 0.0125, 0.025, 0.05, and 0.1% and the other with Sse1, Sse2 and Msi3 at 0.025, 0.05% glutaraldehyde. The crosslinking experiment showed preliminary evidence of bands that originate around 168 kDa on an 8% SDS PAGE gel around 190 kDa and 273 kDa (Fig. 5,6)

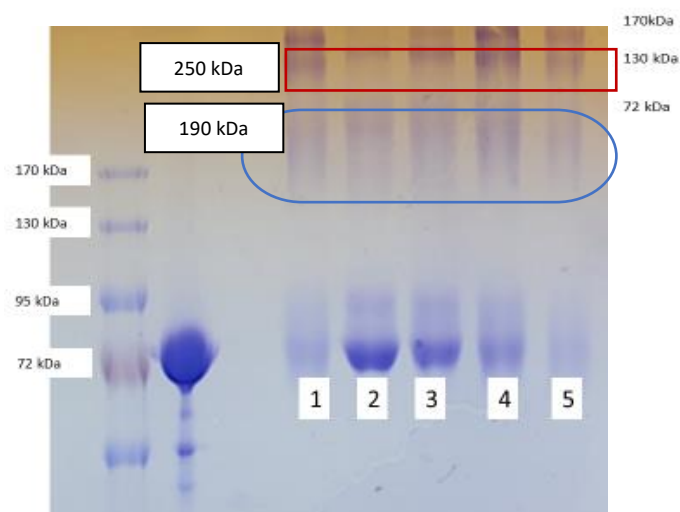


Figure5: *C. albicans* Hsp110 Crosslinking.

Crosslinking of *C. albicans* Sse1 using glutaraldehyde at concentrations: 0.00625, 0.0125, 0.025, 0.05, and 0.1%, with a possible dimer at 168 kDa or around 250 kDa.

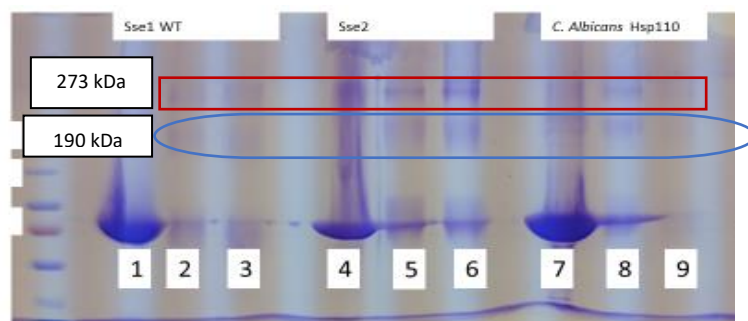


Figure 6 Crosslinking of Various Hsp110s. Crosslinking done at 0.025 and 0.05% glutaraldehyde. Lanes 1, 4, 7 control. Lanes 2, 5, 8 are at 0.025% glutaraldehyde. Lanes 3, 6, 9 are at 0.05% glutaraldehyde. Two bands can be seen above the normal 70 kDa weight of Hsp110 at around 190 kDa and 273 kDa respectively.

Chapter 6 Discussion

Hsp110 is a HSP that stands as more than a cochaperone. While most literature does not support it acting alone as a *de novo* protein folder, it has been identified as having a discrete holdase activity and can function as a cochaperone in complex with Hsp70. Its implications in diseases and maladies are not something that can be ignored. The virulence that pathogens have that can strictly be attributed to Hsp110 makes Hsp110 a protein and chaperone of interest. *P. falciparum*, even with recent advancements in the fight against malaria, malaria is still a disease that is a threat to many developing and tropical nations. Removing the robust Hsp110 could potentially destabilize the viability of the parasite during malarial fevers and could serve as a potent therapy (Muralidharan et al., 2012; Yakubu, et al., 2021; Zininga et al., 2016). Furthermore, understanding the role that Hsp110s play in human cancers and neurodegenerative disease may be a key for future treatments. A few studies have demonstrated how targeting Hsp110 may be a potent treatment and are currently targeting human Hsp110 for treatments (Gozzi et al., 2020). In order to effectively use Hsp110 as a human therapeutic target, all facets must be understood as Hsp110 has been implicated to be both an activator and inhibitor of disease, its presence or lack thereof contributing to different disease states. Future research on Hsp110 as a therapeutic target as such must include the minimization of potential side effects. As

stated, since Hsp110 has been implicated as an activator of colon cancer and as a protector against Alzheimer's, ensuring that the appropriate effect of Hsp110 is elicited to improve one outcome and not induce or worsen another disease state is paramount to ensure its safe use as a therapeutic target. Further understanding of the process through which Hsp110 acts as a disaggregase may elucidate the answers to the duality of Hsp110.

The Hsp110 of *P. falciparum* remains a prime target of interest for therapeutics as it is the sole Hsp110 that allows for the parasite's survival during malarial fevers (Muralidharan et al., 2012). Specifically targeting this chaperone could serve as an effective treatment for malaria. This use of therapeutics is supported with the similarities that has been shown in yeast. Yeast naturally produce prions that prove toxic to their own viability if not dealt with. Affecting Hsp110 and impairment of the holdase activity proves to be detrimental to cells and removal of malarial protections may cause a similar fate (Kuo et al., 2013; Mayer, 2013). Interestingly, some evidence suggests it is the linker region or the tail of the C-terminus that allows for *P. falciparum* Hsp110's robustness (Chakafana et al., 2021; Yakubu, et al., 2021). The Hsp110 stands apart from other HSPs as it has been implicated to have more evolutionary roles, doing more than being a holdase or cochaperone folder; possibly interacting with RNA and playing a regulatory role in its native organism. Whether or not Hsp110 from *P. falciparum* stands as an evolutionary offset or is not as unique, with functions that remain hidden in other Hsp110s is a mystery that could unlock advancements in therapeutics and human diseases. There has been evidence that Hsp110 from *P. falciparum* may form a dimer, does a different

oligomerization state lead to different and new functions of this protein (Zininga et al., 2016)? When and what causes a monomer versus dimer to form? If other Hsp110s are indeed able to form dimers as preliminarily investigated here, what does this mean a more conserved function can exist? These mysteries hold the ability to grant greater understanding into another major protector of a cell through understanding their mechanisms. Similarly, while we have a crystal structure of Sse1, a Hsp110 from *S. cerevisiae*, it is still unclear what role ATP hydrolysis plays exactly in protein regulation and function. Whether the ‘holdase’ activity of Hsp110 is a distinct function separate from Hsp110 in complex or independent of confirmation and how exactly ATP is linked to this function is still unclear.

To investigate some of these preliminary questions, some experimental procedures were carried out to varying degrees of success. While the malachite green assay is usable for more reactive proteins, a trendline was not able to be confidently demonstrated, however, future experiments may use a purchased phosphate standard and reagents that are certified may yield results with better confidence. Understandably, glutaraldehyde was used because it is nonspecific, but this also may have caused crosslinking at protein interfaces that would not occur otherwise. Using a more specific reagent may yield either similar results if all Hsp110s are able to crosslink or it may show some specificity. The use of a negative stain and electron microscopy could be a potential avenue to show the potential for different oligomerization states for Hsp110 in a native form, however, with out the need for extra chemical interactions. The Hsp110 from *P. falciparum* was not codon harmonized and this may cause future difficulties in gene expression, requiring the creation of another construct to ensure the ability for future protein expression and purification but this is yet to be investigated.

As we speculate into the nature of Hsp110, it has been suggested through experimentation that Hsp110 has a secondary binding site for substrate and that the holdase function of Hsp110 operates in an ATP dependent manner (Goekeler et al., 2002; Wang et al., 2021; Yakubu, et al., 2021; Zininga et al., 2016). Therefore, it could be feasible at some base level Hsp110 operations through allosteric interactions outside the realm of protein folding or aggregation prevention. It has been repeatedly shown that the holdase function of Hsp110 is ATP dependent but the ATPase activity of Hsp110 is heavily regulated and normally suppressed. A rudimentary explanation could be that separate from Hsp110 being alone or as part of a construct, *in vivo* an accumulation of substrate could unleash the lytic abilities of Hsp110 to perform a new function through possible allosteric modulation. Currently, it is unknown how ATP hydrolysis fits into the function of Hsp110. Alternatively, could there be a new, undiscovered protein that is interacting with Hsp110 which is the reason Hsp110 can act as either an activator or protector of disease in humans, similar to the network of proteins that coevolved specifically with the *P. falciparum* Hsp110 (Bhartiya et al., 2015). Cryptically, recent research has demonstrated the ability of the Hsp110 C-terminus contributes to holdase activity in some fashion, but what new findings this could lead to can only be imagined without further work (Yakubu, et al., 2021). By focusing research efforts on Hsp110 as an individual protein, and as a family, may potentially lead to advancements furthering the fields of medicine and disease and therefore Hsp110 continues to remain a prime target for study.

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