DETERMINING THE EFFECT OF SSB ON [*LSB*$^{+}$] PRION-BASED STRESS MEMORY

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Quincy Faber

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DETERMINING THE EFFECT OF SSB ON [*LSB*$^{+}$] PRION-BASED STRESS MEMORY

Approved by:

Dr. Yury Chernoff, Advisor
School of Biological Sciences
Georgia Institute of Technology

Dr. Michael Goodisman
School of Biology
Georgia Institute of Technology

Dr. Tatiana Chernova
School of Medicine
Emory University

Date Approved: May 2nd, 2019

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LIST OF SYMBOLS AND ABBREVIATIONS

DNA Deoxyribonucleic Acid

WT Wild-type

SD Standard Deviation

μL microliter

mL milliliter

ADE adenine

YPD yeast extract peptone dextrose media

PGAL galactose promoter

RAC ribosome associated complex

QN glutamine/asparagine

UPS ubiquitin-proteasome system

GuHCl Guanidine Hydrochloride

ABSTRACT

Prions are self-perpetuating protein isoforms, that are usually based on ordered fibrous protein aggregates (amyloids), cause disease in humans and control non-Mendelian heritable traits in yeast. Formation and loss of yeast prions are modulated by environmental and physiological conditions, including heat stress. [*LSB*$^{+}$], a metastable prion generated by the cytoskeleton-associated protein Lsb2 and influencing aggregation of other proteins, is induced by heat stress and persists in a fraction of yeast cells for a number of cell generations after stress, thus generating a cellular memory of stress. Chaperone proteins control protein folding, play an important role in adaptation during stress conditions, and are involved in prion formation and propagation. Ssb is member of the Hsp70 chaperone family and is normally associated with translating ribosomes. Previous studies in our lab indicated that Hsp70-Ssb has an anti-prion effect. We show that the formation and mitotic stability of the [*LSB*$^{+}$] prion are greatly increased in the absence of Ssb. This links the ribosome-associated chaperone machinery to the cellular memory of stress.

CHAPTER 1. INTRODUCTION

1.1 Prions

Proteins are complex molecules made of amino acids that are essential for cells. Amyloids and their transmissible forms, prions, are protein aggregates rich in β-sheets that cause neurological diseases. Prions, or proteinaceous infectious particles, are a form of misfolded proteins (Prusiner, 1982). They are self-perpetuating proteins that induce other proteins to take on their alternative shape and transmit infectious or heritable patterns through proteins. Prions are associated with many human and animal diseases such as Creutzfeldt-Jacob disease and "mad cow" disease (Colby and Prusiner, 2011). However, amyloids and prions have recently been associated with a variety of biological processes that are beneficial, like protection to stress and long-term memory in animals (Si et al., 2003). Remarkably, Cpeb3 (cytoplasmic polyadenylation element-binding protein) associated to long-term memory has prion-like features in mammals (Fioriti et al., 2015). Likewise, prions could play a role in protecting proteins from degradation under unfavorable conditions (Chernoff, 2007).

In yeast, prion formation and loss are modulated by environmental and physiological conditions, such as nutrient limitation and heat stress. Some prions have been considered beneficial because aggregate formation during unfavorable conditions may protect proteins from degradation. High temperatures cause proteins to misfold, which affects the functions of the protein. Prion formation could be an adaptive response to avoid misfolded proteins during heat shock (Newman et al, 2011). This is of great interest because prions propagate and may be able to pass on stress memory as an epigenetic factor.

Prions are often studied in yeast because they have a detectable phenotype and are inherited through protein conformation in the cytoplasm rather than through DNA. Proteins that form prions in yeast contain domains that are QN-rich, or have large amounts of Glutamine and Asparagine, that are necessary to form the prion state. Amino acid composition has been shown to be important for prion properties. Loss of function mutations in proteins can lead to prion formation. Overproduction of proteins like Ure2 (transcriptional co-repressor in nitrogen regulation) and Sup35 (the translation termination factor Sup35 (eRF3)) and can also cause proteins to convert to prion forms, [*URE3*] and [*PSI*$^{+}$] respectively (Liebman and Chernoff, 2012).

1.2 Chaperones

Chaperone proteins play an important role in protein quality control and protecting cells against proteotoxic stress (Mayer and Bakau, 2005). They also control a protein's "life cycle" by modulating protein folding, as well as refolding, degradation or aggregation. Some chaperones are involved in a powerful adaptation mechanism, protecting proteins from thermal damage Verghese et al., 2012). Increased expression of some proteins during thermal stress led to the designation of whole chaperone families as Heat Shock Protein (Hsp). One of the most abundant chaperone families is Hsp70, which has many subfamilies. In yeast, two major subfamilies working in the cytoplasm are Ssa (coded by 4 partly divergent genes, *SSA1-4*) and Ssb (coded by 2 almost identical genes, *SSB1* and *SSB2*) (Lopez-Buesa et al., 1998). Ssa and Ssb play an important role in adaptation during stress conditions and prion propagation. Ssa is a chaperone that is essential for cell viability and participates in protein disaggregation and refolding. Ssb is a co-translational chaperone that binds to the Ribosome-Associated Complex (RAC), consisting of Zuo1 and Ssz1, which assists in protein folding (Sharma and Masison, 2006).

Previous studies have examined the effect of chaperones on the yeast prion, [*PSI*$^+$]. This provides a model for the impact of chaperones on other prions. Chernoff et al. 1999 showed that Ssa and another chaperone, Hsp104, participate in prion propagation and Ssb-RAC promotes protein folding (Chernoff et al., 1999). However, in cells lacking one or both RAC components, Ssb is released from the ribosome to the cytosol causing the protein to misfold. This leads to prion formation, but interferes with Ssa, preventing prion fragmentation and causing loss of prions in the daughter cell during cell division. This data suggests that Ssb has an "anti-prion" effect because it destabilizes the cycle of prion propagation of [*PSI*$^+$] (Kiktev et al., 2014). Following this model, there is potential for the creation of a more stable form of other prions that are easier to study.

1.3 [*LSB*$^+$] prion

A study by Dr. Chernova in 2011 introduces a stress induced protein, Las17-binding protein (Lsb2), that is the first short-lived protein known to have a role in prion induction (Chernova et al., 2011). Lsb2 increases significant its levels by stress and promotes the formation *de novo* of [*PSI*$^+$] prion in the presence of excess from its protein form, Sup35 (Fig. 1).

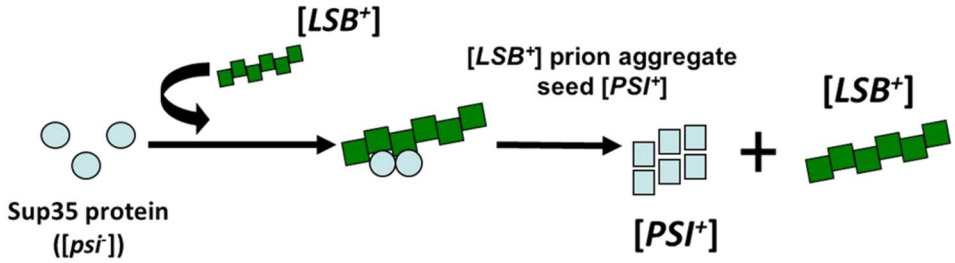


Figure 1. Model of Heterologous prion cross-seeding of [*LSB*$^+$] prion. [*LSB*$^+$] directly seeds formation of [*PSI*$^+$] prion in the presence of excess Sup35, which is easily detectable by growth on media lacking Adenine (See Fig. 2).

Lsb proteins were shown to be ubiquinated and most were degraded during heat shock, but some of the Lsb proteins accumulated, causing prion induction. Ubiquination is when ubiquitin peptides link to lysine residues in proteins to mark misfolded proteins for degradation. The proteosomal mutation, *doa3-1*, reduced the degradation of Lsb2 and led to the accumulation of proteins. This explains why the protein accumulates during thermal stress.

Another study by Chernova et al. 2017 found that overproduction of Lsb2 actually forms [LSB^+], which is a heat-induced and metastable prion (Chernova et al., 2017). [LSB^+] based stress memory is maintained only in a small fraction of the population and is difficult to study because about 3% of cells have this phenotype and of those, 2-13% maintain the prion form in subcolonies. Another issue is that the [LSB^+] prion degrades quickly and is only present at high levels during heat shock. Creating a more stable form of [LSB^+] that results in more colonies having the prion phenotype would allow us to expand research on the prion.

1.4 Goals

In this study, we will research the effects of deletions of *SSB1* and *SSB2* genes in a *Saccharomyces cerevisiae* strain on [LSB^+]. Since *ssb1/2* deletions have been proven to stabilize the [PSI^+] prion, we hypothesize that these deletions will also increase the stability of the [LSB^+] prion. As previously stated, heat shock induces [LSB^+], which induces the formation of [PSI^+] prion, so further characterization of the prion inducing [PSI^+] is needed. Until this is confirmed, the prion inducing [PSI^+] will be designated as [PRN^+]. A more stable form of the [PRN^+] prion will allow us to characterize the features of this prion.

Two genetic features unique to prions are reversible curability and increased frequency of prion formation by overproduction of prion protein (Wickner et al., 1994). A previous paper

already shows that increasing the amount of Lsb2 protein leads to the prion phenotype (Chernova et al., 2017). We will study the curability of the [*PRN*$^{+}$] prion by Guanidine Hydrochloride because it is an Hsp104 inhibitor and Hsp104 is necessary for most yeast prions (Liebman and Chernoff, 2012). We will also analyze the protein by Western blot to confirm the presence of the Lsb2 protein in samples hypothesized to have the [*LSB*$^{+}$] prion. [*LSB*$^{+}$] is hypothesized to protect cells from stress and pass that trait onto further generations. Prion-based stress memory could be a strong adaptive tool to transfer stress resistance to the next generation in yeast cells connecting the heat shock non-inducible chaperone Ssb in the machinery for cellular memory of stress.

CHAPTER 2. MATERIALS AND METHODS

2.1 Prion Induction and Monitoring

2.1.1 Yeast media

YPD (Rich Yeast Media) was made with 1% yeast extract, 2% dextrose, and 2% peptone (Sherman 2002). Solid media was made with agar and liquid media was made without. Media lacking one or more amino acids was made to grow yeast that had plasmids with amino acid markers. Media was also made with galactose instead of dextrose for yeast with plasmids with the galactose promoter.

2.1.2 Transformation

Yeast was transformed following the protocol previously described (Gietz et al., 1992). Strains (See appendix A.1) were grown overnight in 10mL of liquid YPD media. The preculture was diluted to OD_{600}=0.1 in 50ml YPD then incubated for 2 hours at 30°C with good aeration. The cells were centrifuged at 3000 rpm for 10 minutes, then the supernatant was removed and resuspended in 10ml of 0.1M LiAc-TE. The cells were incubated for 1 hour at 30°C. The cells were spun down again, the supernatant was discarded, and the cells were resuspended in 0.1M Lithium acetate Tris-EDTA buffer (LiAc-TE). 100uL cells, 3uL carrier DNA, and 10uL transforming DNA from P_{GAL}-SUP35N (pFL39) (See appendix A.2) were incubated together for 30 minutes at 30°C, then 700uL of Lithium Acetate-Polyethylene glycate-Tris-EDTA buffer (LiAc-PEG-TE) was added and it was incubated for 60 minutes. The cells were then heat shocked at 42°C for 5 minutes and place at 4°C overnight. The next day, cells were centrifuged for 2

minutes at 3000 rpm and 100uL was plated on synthetic media with specific marker for that plasmid, this case -Trp plates.

2.1.3 Heat shock

A preculture was made with colonies grown on a YPD plate and 10mL liquid YPD. This was grown overnight at 25°C with shaking, then diluted to OD_{600}=0.1 in 50ml of YPD and incubated for 2 more hours. Then, cultures were placed in a 39°C water bath with shaking. Samples were taken at 0h and 2h (approximately 300 cells per culture), then streaked out on YPD plates and grown at 25°C. After 5-6 days of growth, individual colonies were patched on -Trp media and velveteened to -Trp+Gal media to induce formation of excess Sup35, and then to a -Ade plate (Fig. 2).

2.1.4 Monitoring of [PSI$^+$] induction

The presence of [*PSI*$^+$] was monitored by its ability to suppress the reporter *ade1-14* (containing the premature stop codon, UGA), resulting in growth on media lacking adenine (-Ade) (Kushnirov et al., 2007) (Fig. 3).

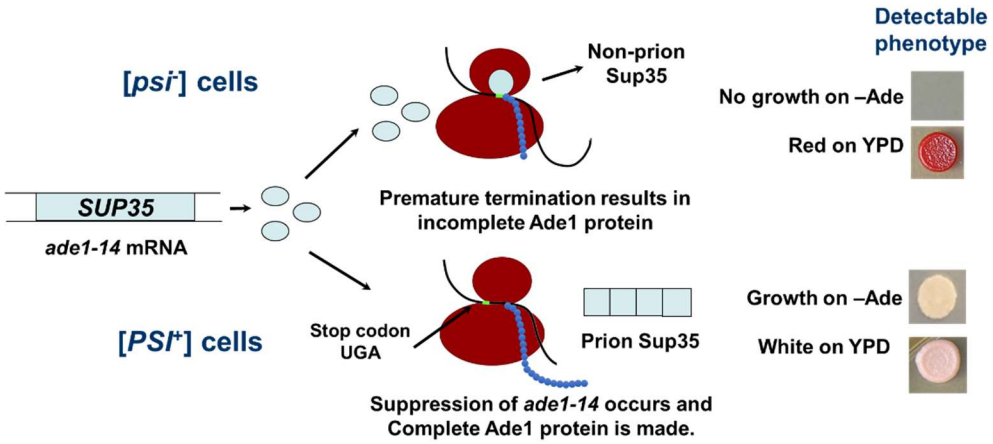


Figure 2. The [*PSI*$^+$] phenotype is detected by white growth on YPD and growth on media lacking Adenine. The Sup35 release factor causes the ribosome to stop at the premature stop codon in the *ade1-14* mutation preventing the cell from synthesizing Adenine. In the presence of the [*PSI*$^+$] prion, the ribosome can read-through the stop codon, allowing cells to make some full-length Ade1.

2.1.5 Curability of [PSI$^+$]

Colonies that grew on -Ade media were checked for curability by guanidinium hydrochloride (GuHCl), an Hsp104 inhibitor (Jung and Masison 2001). Colonies were patched onto 2 mM GuHCL for *ssb1/2Δ* strain and 5mM GuHCL for wild-type strain in 3 consecutive passages and then streaked out on YPD plates and veleveteened to -Ade plates (Fig. 2). Colonies were curable if they did not grow on -Ade media.

2.1.6 Curability of [PRN$^+$]

Prior to induction of the galactose promoter, colonies that were shown to grow on Adenine plates were patched in GuHCl according to the protocol above, *2.1.3*. Then they were streaked out on –Trp plates, patched on -Trp media and velveteened to -Trp+Gal. then to a -Ade plate. Colonies were considered not curable for if they grew on -Ade media.

2.1.7 Stability of [PRN$^+$]

Colonies that were Ade+ were selected from the Gal-Trp plate and were streaked out in –Trp. These colonies were then checked if they had the [*PSI*$^+$] phenotype as described above.

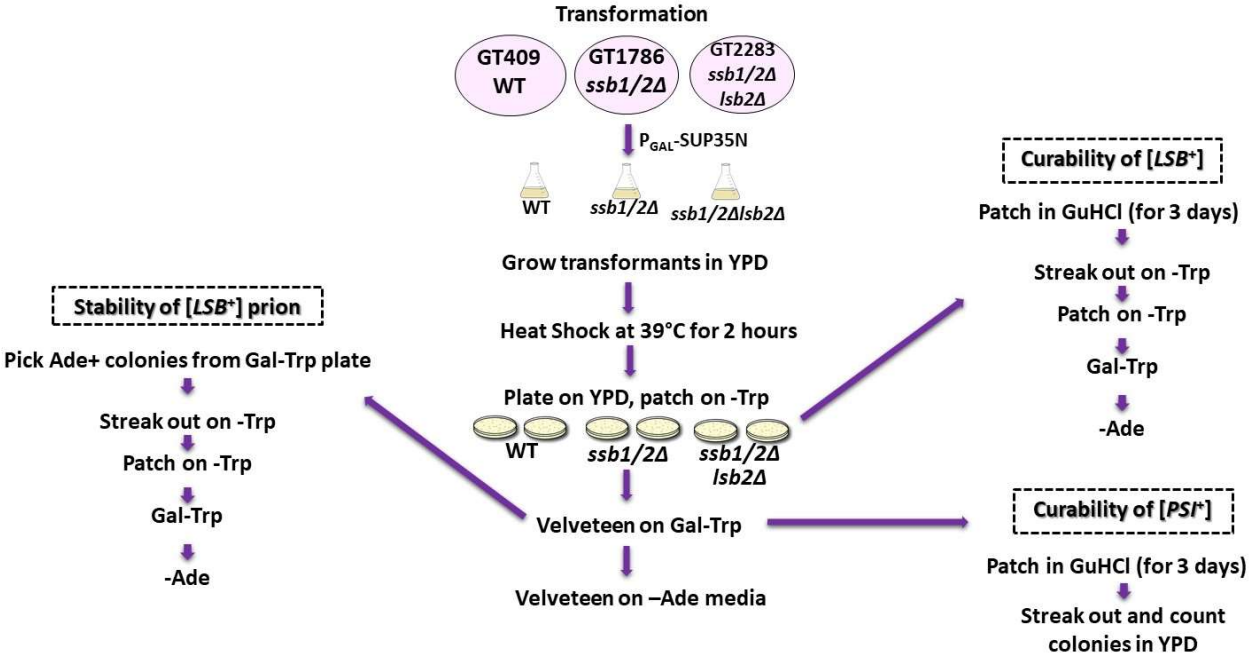


Figure 3. Diagram of experimental design. After transformation with P$_{GAL}$-SUP35N TRP1, some colonies from each strain were selected and heat shocked. Later, the inducibility of [*PSI*$^+$] by [*LSB*$^+$] prion was studied along with the stability of the [*LSB*$^+$] prion. The curability of both prions, [*PSI*$^+$] and [*LSB*$^+$], was also studied.

2.2 Yeast Mating

Multiple strains were transformed (as shown in Appendix A.1), and colonies were streaked out on YPD plates in several lines. Mat a and Mat α strains were streaked out perpendicularly to each other on separate YPD plates. After several days, plates were velveteened together onto media that was selective for markers in both strains, so that only diploids would grow. The diploids were

then patched onto -Trp media and velveteened to Gal-Trp and -Ade media to check for the presence of the [LSB^+] prion.

2.3 Biochemical analysis of proteins

2.3.1 Protein Extraction

Yeast cells were isolated and analyzed to find protein levels. Cells were precipitated by centrifugation at 3,000rpm. The pellet was diluted in 300 µl Protein Isolation Buffer (25 mM Tris-Cl pH 7.5, 0.1mM NaCl, 10mM EDTA, 100µg/ml cycloheximide, 2mM Benzamide, 20µg/ml Leupeptin, 4µg/ml Pepstain A, 1mM N-Ethylmaleimide, 1X complete mini protease inhibitor cocktail from Roche, and 3 mM phenylmethanesulfonylfluoride). Glass beads were added to the samples and vortexed for 8 min at 4°C. The cells were spun down again at 8,0000rpm at 4°C and a Bradford assay (Biorad) was used to determine protein concentration. The concentrations of each sample were then normalized using Protein Isolation Buffer.

2.3.2 SDS-PAGE

Protein samples were denatured by incubation with 4X loading buffer (240 mM Tris-HCl pH 6.8, 8% SDS, 40% glycerol, 12% 2-mercaptoethanol and 0.002% bromophenol blue) and being placed in a 100°C water bath for five minutes. The samples were then run on sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels in running buffer (Tris-Glycine) using electrophoresis at 85V for 2h. Proteins were then transferred to a Hybond-ECL nitrocellulose membrane (GE Healthcare Life Sciences) and blocked in 5% non-fat milk. The membrane was washed and incubated with the primary antibodies (Lsb) overnight, then washed 3 times the membrane with TBS buffer (0.05M Tris-HCl pH 7.5, 0.15M NaCl, and 0.1% Tween 20) and

incubated with the anti-rabbit HRP antibody per 1 hour. The nitrocellulose was washed 3 times with TBS buffer and reaction was detected by using the chemiluminescent detection reagents as described in the GE Healthcare protocols.

CHAPTER 3. DETERMINING THE EFFECT OF SSB1/2 DELETIONS ON [*LSB*$^+$] PRION

3.1 Inducibility of [*PRN*$^+$]

Previous unpublished studies in the Chernoff Lab show that *ssb1/2Δ* increased induction of the [*PRN*$^+$] prion during heat shock. We presumed that [*PRN*$^+$] prion is [*LSB*$^+$], as described previously. In order to explore this hypothesis, we used three isogenic strains (Wild-type, *ssb1/2Δ*, and *ssb1/2Δlsb2Δ* strains (See appendix A.1). These strains were transformed with P_{GAL}-SUP35N and then were heat shocked. The inducibility of [*PSI*$^+$] was checked for each strain after overexpression of Sup35N. Our results indicated that [*PSI*$^+$] induced by [*LSB*$^+$] was low in wild-type (1.59 %), while *ssb1/2Δ* deletions was around 33.57% inducible. The strain with a triple deletion was slightly inducible (4.7%) (Fig. 4).

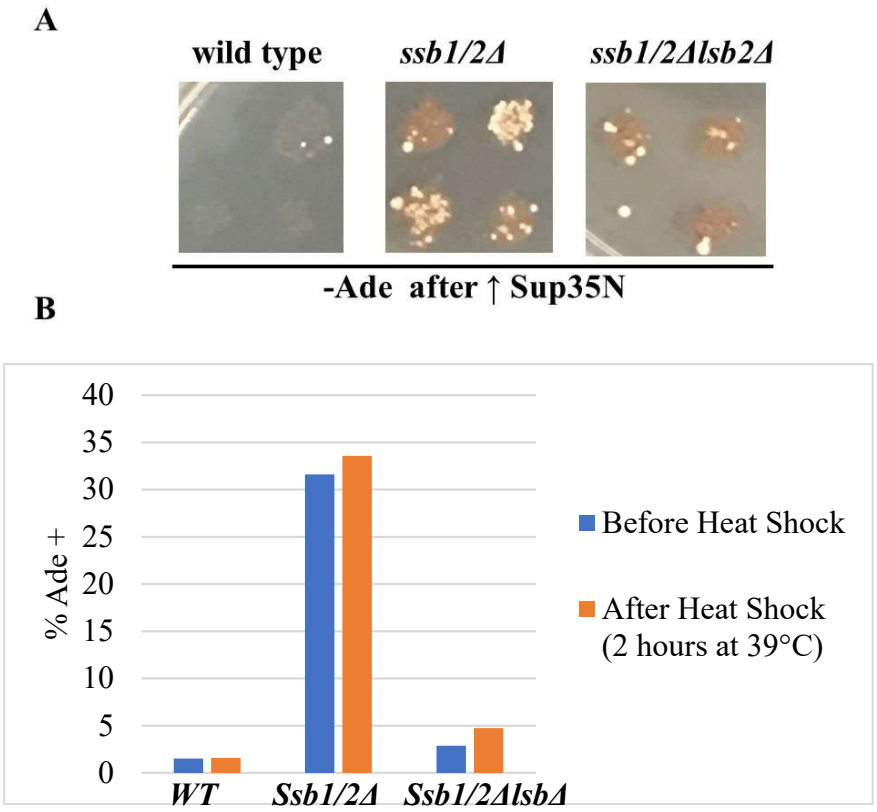


Figure 4. [*PSI*$^+$] is mostly induced by [*LSB*$^+$]. **A**. Plate assays after colonies were patched and velveteened after heat shock for 2 hours at 39°C. **B.** Quantitative assay. They were checked for [*PSI*$^+$] inducibility by growth of -Ade media. Strains with *ssb1/2* deletions had the most [*PSI*$^+$] colonies.

Our results show that induction of the [*PSI*$^+$] prion is increased when *SSB1* and *SSB2* genes are deleted. A third strain with deletions of *SSB1*, *SSB2*, and *LSB2* was also studied. Wild type colonies induced the [*PSI*$^+$] prion at a very low rate and *ssb1/2Δ* colonies had a much higher rate of induction. *Ssb1/2Δlsb2Δ* had a higher rate of induction that wild type but was much lower than for *ssb1/2Δ*. This shows that the [*PSI*$^+$] prion is mostly induced by [*LSB*$^+$], but there may be another protein involved in induction.

3.2 Stability of the [*PRN*$^+$] prion

In previous studies, the [*LSB*$^+$] prion was passed on to subcolonies (Chernova et al., 2017). *Wild-type, ssb1/2Δ*, and *ssb1/2Δlsb2Δ* strains were tested to see if the prion was passed on more without Ssb. [*PSI*$^+$] colonies were streaked out in -Trp, patched, then velveteened to Gal-Trp and -Ade to see if they were inducible for [*PSI*$^+$]. Results show that the 100% of colonies from the strain with deletions of *SSB1* and *SSB2* (GT1786) are stable after heat shock, meaning that all subcolonies were [*PSI*$^+$] (Table 1, Fig. 5). All of the subcolonies of strains with deletions *SSB1*, *SSB2*, and *LSB2* were also [*PSI*$^+$].

Time	Strain	High stability	Low stability	Total
Before 2 hours heat shock (39°C)	GT409 wild type	0	1	**1**
	GT1786 *ssb1/2Δ*	3	0	**3**
	GT2283 *ssb1/2Δ, lsb2Δ*	8	0	**8**
After 2 hours heat shock (39°C)	GT409 wild type	0	1	**1**
	GT1786 *ssb1/2Δ*	3	0	**3**
	GT2283 *ssb1/2Δ, lsb2Δ*	8	0	**8**

Table 1. *Ssb1/2Δ* increases the stability of [*PRN*$^+$] in quantitative assays. The subcolonies of [*PSI*$^+$] colonies were checked for induction of [*PSI*$^+$]. In both the double deletion and triple deletion strains, 100% of the colonies were highly stable, meaning that *SSB1* and *SSB2* deletions increase the stability of [*LSB*$^+$] and [*PRN*$^+$].

Figure 5. *Ssb1/2Δ* increases the stability of [*PRN*$^+$] in plate assays. [*PSI*$^+$] Subcolonies were checked for induction of [*PSI*$^+$]. *Ssb1/2Δ* and *ssb1/2Δlsb2Δ* still induced [*PSI*$^+$].

This data shows that deletions of *SSB1* and *SSB2* increase the inducibility of [*PSI*$^+$] by subcolonies. Subcolonies can still form the prion even though they did not undergo heat shock because this trait is passed onto them. Following heat shock, none of the wild type colonies had high stability but all the colonies with *ssb1/2Δ* and *ssb1/2Δlsb2Δ* had high stability. This shows that *SSB1* and *SSB2* deletions increased the ability of cells to pass on [*LSB*$^+$] to daughter cells. It also increased stability of the [*PRN*$^+$] prion in strains with *SSB1*, *SSB2*, and *LSB2* deletions.

3.3 Curability of Prions

Guanidine Hydrochloride, an Hsp104 inhibitor, is used to cure [*PSI*$^+$] colonies. Hsp104 is required for most yeast prions (Liebman and Chernoff, 2012). Wild-type, *ssb1/2Δ*, and *ssb1/2Δlsb2Δ* strains were tested to see if [*PSI*$^+$] induced by [*LSB*$^+$] is curable by guanidine hydrochloride as expected. These strains were also tested to see if the [*LSB*$^+$] prion is curable by guanidine hydrochloride to determine if Hsp104 is necessary for the prion.

Cells were heat shocked to induce the formation of [*LSB*$^+$]. Then individual colonies were velveteened onto Gal-Trp and -Ade to check for [*PSI*$^+$]. These colonies from Gal-Trp were patched onto plates with GuHCl for three days. Then the colonies were grown on YPD and the color was

used to determine if the [*PSI*$^{+}$] prion was still present. Most colonies tested were curable for GuHCl (Fig. 6).

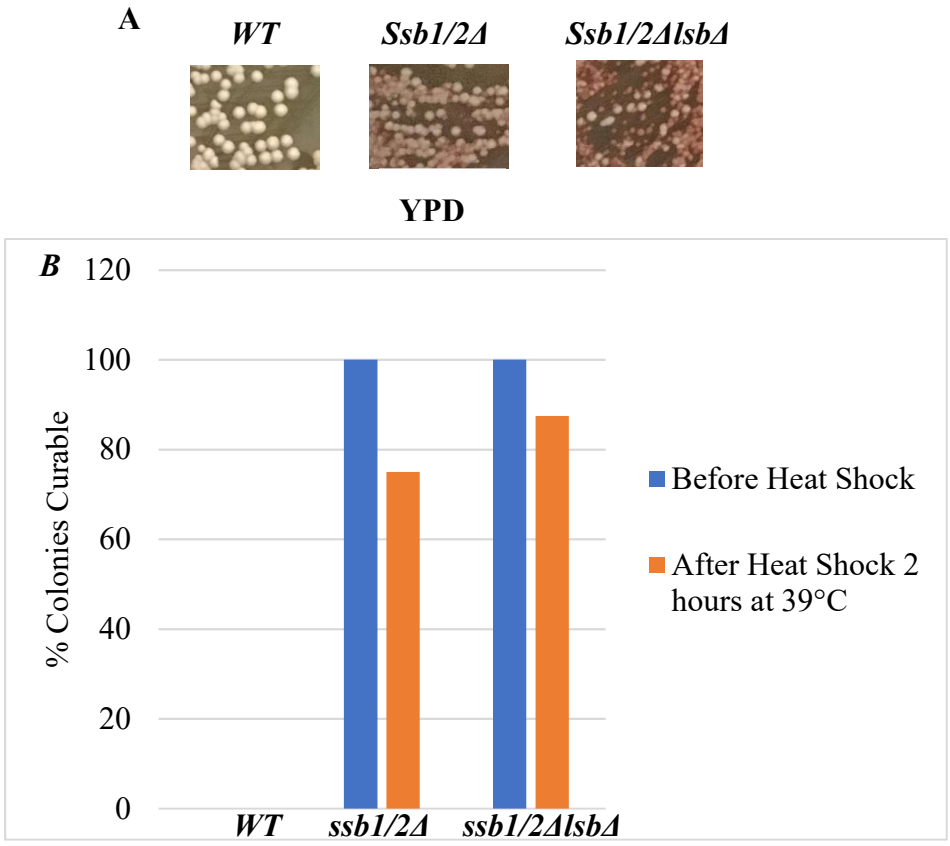


Figure 6. [*PSI*$^{+}$] colonies induced by [*LSB*$^{+}$] are curable for GuHCl. A. Plate assays after [*PSI*$^{+}$] colonies were patched in GuHCl. **B.** Quantitative assay. They were checked for [*PSI*$^{+}$] by color in YPD. [*PSI*$^{+}$] colonies induced by both [*LSB*$^{+}$] and [*PRN*$^{+}$] are curable by Guanidine Hydrochloride.

The curability of [*LSB*$^{+}$] prion was determined by taking [*LSB*$^{+}$] colonies before Sup35N induction and patched GuHCl. In *ssb1/2Δ* and *ssb1/2Δlsb2Δ*, 70% of colonies before heat shock and around 50% of colonies after heat shock had the [*PSI*$^{+}$] prion after curing, showing that [*LSB*$^{+}$] prion was not cured by GuHCl (Fig. 7). The wild type strain was too unstable to test.

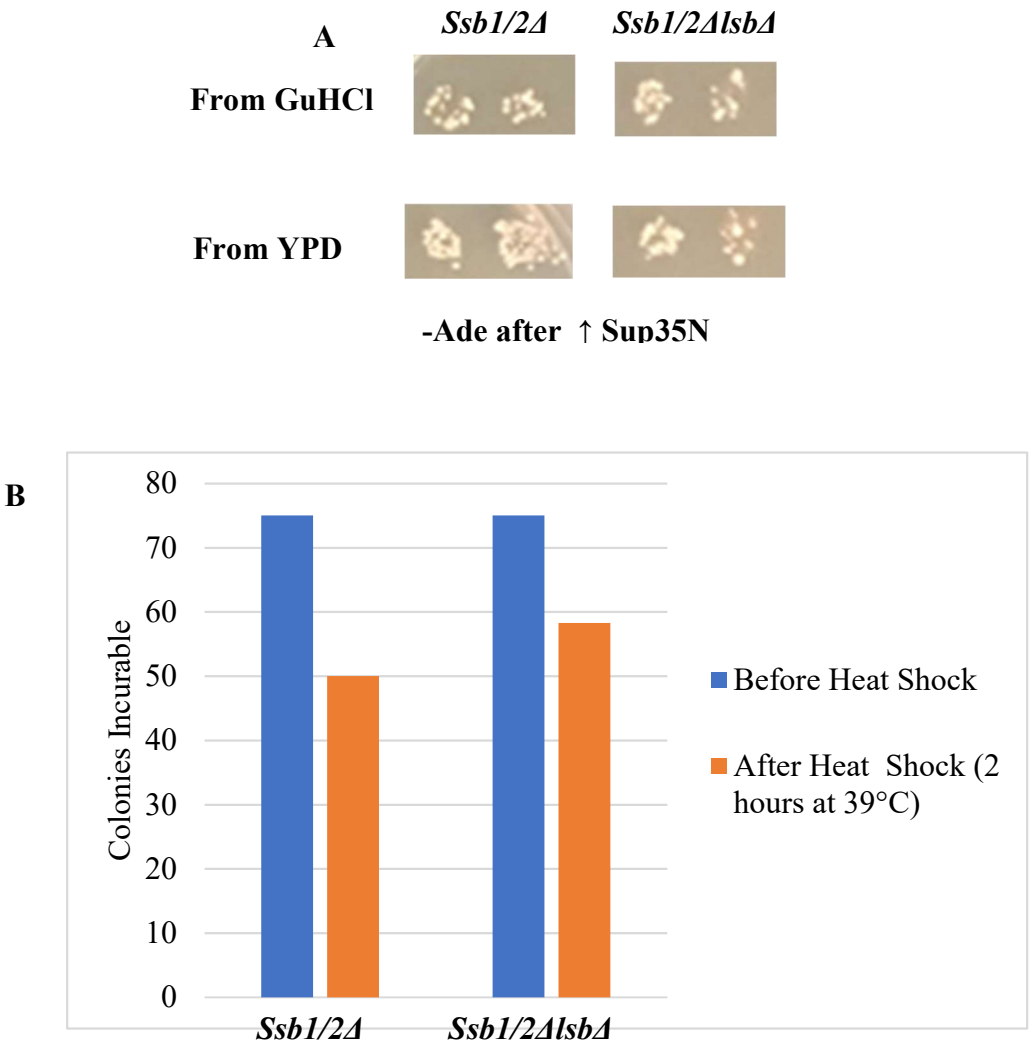


Figure 7. [*LSB*$^+$] is not curable by GuHCl. A. Plate assays after [*PSI*$^+$] induction before and after strains were treatment with GuHCl. **B.** Quantitative assay. [*PSI*$^+$] colonies were patched in GuHCl prior to overproduction of Sup35. When Sup35 was overproduced, colonies were still [*PSI*$^+$], showing that [*LSB*$^+$] and [*PRN*$^+$] are both incurable by Guanidine Hydrochloride.

Hsp104 is required for the formation of [*PSI*$^+$] prions (Jung et al., 2001). Our results prove that this is true for [*PSI*$^+$] induced by [*LSB*$^+$] because most of the colonies were cured using Guanidine Hydrochloride, which is an Hsp104 inhibitor. Our results also show that [*LSB*$^+$] is not curable by Guanidine Hydrochloride, which indicates that it may not be dependent of Hsp104. This is significant because most prions rely on Hsp104 for propagation.

3.4 Checking protein levels of Lsb2

A western blot was done to confirm that Lsb2 was present in samples during heat shock. Proteins were extracted from wild type, *ssb1/2Δ*, and *ssb1/2Δlsb2Δ* cells at time 0 hours and 2 hours of heat shock, then ran on an SDS-PAGE gel. Wild type and *ssb1/2Δ* strains with artificial overexpression of Lsb2 was used as positive control. Lsb2 protein was detected on *ssb1/2Δ* strains. (Fig. 8).

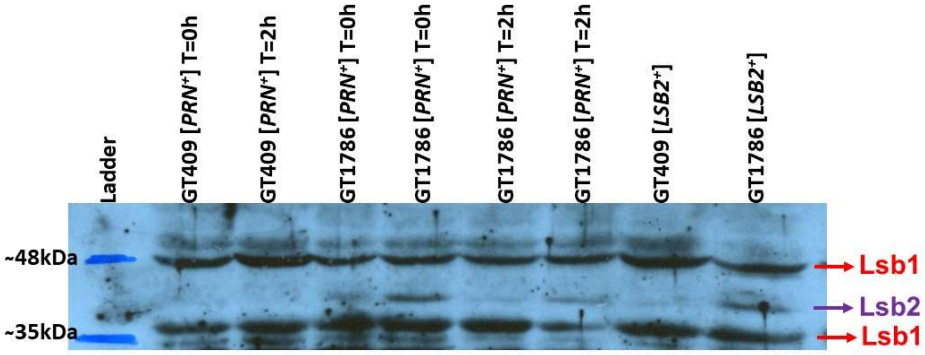
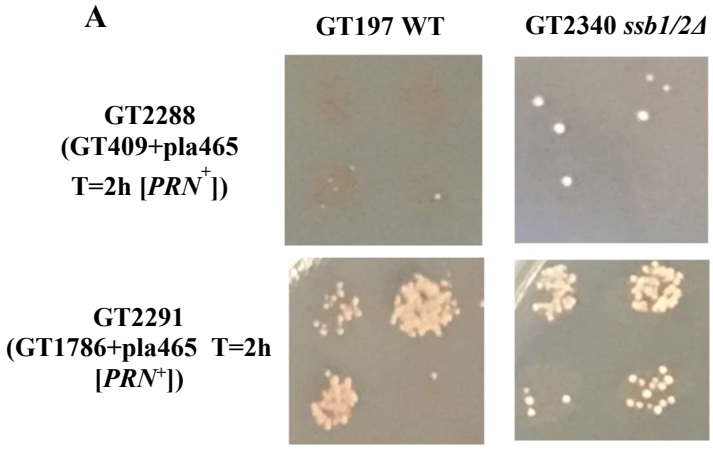


Figure 8. [*LSB*$^+$] samples from ssb1/2Δ strains have bands of Lsb2. Samples after 5 minutes of exposure. Proteins were extracted and run on an SDS-PAGE gel. Then a Western Blot was done with a lsb2 primary antibody. Overexpression of Lsb2 forms [*LSB*$^+$] and has bands of Lsb2.

3.5 Checking the effect of Ssb on [*LSB*$^+$] prion after mating

Wild type and *ssb1/2Δ* mat a strains that have the [*LSB*$^+$] prion were mated with *wild type* and *ssb1/2Δ* mat α strains to see if reintroduction of Ssb would decrease prion stability in diploids. The [*LSB*$^+$] prion in the GT409 WT strain was unstable when it was mated with another WT strain or a *ssb1/2Δ* strain, as expected. The [*LSB*$^+$] prion is stable when the GT1786 *ssb1/2Δ* strain is mated with another WT strain or a *ssb1/2Δ* strain (Fig. 9 and Table 2). We hypothesized that the

reintroduction of Ssb would destabilize the prion. These results must be confirmed before any conclusions are made.



B

GT2287→ GT409+pla465 T=0h [*PRN*$^{+}$]
GT2288 → GT409+pla465 T=2h [*PRN*$^{+}$]
GT2289→ GT1786*ssb1/2Δ* +pla465 T=0h [*PRN*$^{+}$]
GT2290 → GT1786*ssb1/2Δ* +pla465 T=0h [*PRN*$^{+}$]
GT2291→ GT1786*ssb1/2Δ* +pla465 T=2h [*PRN*$^{+}$]
GT2292 → GT1786*ssb1/2Δ* +pla465 T=2h [*PRN*$^{+}$]

Figure 9. Reintroduction of Ssb does not destabilize [*LSB*$^{+}$] in *ssb1/2Δ* strain. A. Plate assay of mating experiment. **B.** List of strains.

MATE A	Mate α GT197 (WT) + pla96 URA3				Mate α GT2340 (ssb1/2Δ) + pla96 URA3			
	# Colonies Tested	[PRN$^+$] colonies	[prn$^-$] colonies	% of [PRN$^+$]	# Colonies Tested	[PRN$^+$] colonies	[prn$^-$] colonies	% of [PRN$^+$]
[PRN$^+$] # 1	10	2	8	20	6	1	5	16.67
(GT2287)	11	0	11	0	12	2	10	16.67
	11	4	7	36.36	10	8	2	80
	7	1	6	14.29	10	4	6	40
	9	0	9	0	10	0	10	0
	10	1	9	10	4	1	3	25
	0				12	3	9	25
	0				12	3	9	25
Total	**58**	**8**	**50**	**13.44**	**76**	**22**	**54**	**28.54**
[PRN$^+$] # 2	11	2	9	18.18	12	3	9	25
(GT2288)	9	1	8	11.11	12	3	9	25
	12	1	11	8.33	12	3	9	25
	12	2	10	16.67	12	2	10	16.67
	12	1	11	8.33	12	2	10	16.67
	11	1	10	9.09	12	2	10	16.67
	0				2	0	2	0
	0				12	3	9	25
Total	**67**	**8**	**59**	**11.95**	**86**	**18**	**68**	**18.75**

Mate A	Mate α GT197 (WT) + pla96 URA3				Mate α GT2340 (ssb1/2Δ) + pla96 URA3			
	# Colonies Tested	[PRN+] colonies	[prn-] colonies	% of [PRN+]	# Colonies Tested	[PRN+] colonies	[prn-] colonies	% of [PRN+]
[PRN+] # 3	10	7	3	70	11	3	8	27.27
(GT2289)	10	8	2	80	10	2	8	20
	9	2	7	22.22	9	3	6	33.33
	9	3	6	33.33	10	4	6	40
	12	8	4	66.67	8	2	6	25
					10	3	7	30
					12	6	6	50
					12	6	6	50
Total	**50**	**28**	**22**	**54.44**	**82**	**29**	**53**	**34.45**
[PRN+] # 4	12	9	3	75	11	7	4	63.64
(GT2290)	11	4	7	36.36	12	6	6	50
	12	2	10	16.67	12	5	7	41.67
	12	7	5	58.33	12	9	3	75
	12	10	2	83.33	12	7	5	58.33
					12	5	7	41.67
Total	**59**	**32**	**27**	**53.94**	**71**	**39**	**32**	**55.05**
[PRN+] # 5	12	9	3	75	12	6	6	50
(GT2291)	12	8	4	66.67	12	6	6	50
	12	7	5	58.33	12	8	4	66.67
	12	4	8	33.33	12	9	3	75
	12	6	6	50	12	4	8	33.33
					12	4	8	33.33
					12	4	8	33.33
					12	9	3	75
Total	**60**	**34**	**26**	**56.67**	**96**	**50**	**46**	**52.08**
[PRN+] # 6	12	7	5	58.33	12	4	8	33.33
(GT2292)	12	3	9	25	11	5	6	45.45
	12	4	8	33.33	12	8	4	66.67
	12	9	3	75	12	8	4	66.67
	12	7	5	58.33	12	6	6	50
					8	3	5	37.5
Total	**60**	**30**	**30**	**50**	**67**	**34**	**33**	**49.94**

Table 2. Reintroduction of Ssb does not destabilize [*LSB*$^+$] in *ssb1/2Δ* strain. Quantitative assay. Mat a and Mat α strains were mated, then diploids were checked for the presence of [LSB+] as described in Fig. 2. There is no significant difference between Mat a *ssb1/2Δ* strain mated with WT or *ssb1/2Δ* Mat a strains.

3.6 Conclusions

Our work utilized the deletion of *SSB1* and *SSB2* genes to create a more stable form of [*LSB*$^+$] in order to characterize the prion. Our results confirm that it is mostly the [*LSB*$^+$] prion that is inducing the formation of [*PSI*$^+$]. We show that the *SSB1* and *SSB2* deletion increases the both the formation and stability of the [*LSB*$^+$] prion. The *SSB1* and *SSB2* deletion also increases the stability of the [*PRN*$^+$] prion. We show that [*LSB*$^+$] is not curable by Guanidine Hydrochloride, which suggests that the prion is not dependent on Hsp104.

The western blot shows that [*LSB*$^+$] colonies had bands of Lsb2. The positive controls was formed through overproduction of Lsb2. The reintroduction of Ssb in diploid cells with *SSB1* and *SSB2* deletions did not destabilize the [*LSB*$^+$]. The mating experiment must be repeated with a larger sample size.

3.7 Future Work

The biochemical characterization of the [*LSB*$^+$] prion needs to be continued. The presence of Lsb2 in samples during heat shock need to be confirmed by SDS-PAGE. SDD-AGE will also be used to confirm that there is aggregation of Lsb2 proteins during heat shock. The mating experiment also needs to be repeated with a larger number of cells in order to determine the effect of the reintroduction of Ssb in a *ssb1/2Δ* strain on [*LSB*$^+$].

APPENDIX A. TABLES OF STRAINS AND PLASMIDS

A.1 Strains

Strain	**Genotype**
GT409	Mat a ade1-14 his3-Δ200 or 11,15 leu2-3,112 lys2 trp1-Δ ura3-52 [*psi*$^-$] [*pin*$^-$]
GT197	GT409 MATα
GT1786	GT409 with *ssb1Δ::HIS3 ssb2Δ::ura3*
GT2340	GT409 MATα ssb1/2Δ [psi-] [pin-]
GT2283	GT409 with *ssb1Δ::HIS3 ssb2Δ::ura3 lsb2Δ::kanMX4*

The Saccharomyces cerevisiae strains used in this study are listed in Table 1. The strain GT409 is part of Dr. Chernoff lab's collection of strains and was previously described (Chandramowlishwaran et al. 2018). The GT1786 and GT2283 strains were obtained from GT409

after delete the genes *SSB1*, *SSB2* and *LSB2* using the Pringle method. The plasmid construction used in this study is shown in Table 2.

A.2 Plasmids

Plasmid name	**Plasmid number**	**Yeast marker**	**Promoter**	**Expression**
pFL39	465	TRP1	P_{GAL}	*SUP35N*
	96	URA3	Empty	

A.3 Antibodies

Ssb antibody was a gift from the Craig lab at the University of Wisconsin-Madison and the Lsb2 antibody was a gift from Dr. Wilkinson's lab at Emory University. Secondary Anti-rabbit antibodies were purchased from Sigma.

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