ABSTRACT

Functional differences between the yeast Hsp70 molecular chaperones Ssb1 and Ssb2

Protein folding is a complex process that is mediated in the cell by molecular chaperones. Errors in protein folding can lead to aggregation of misfolded proteins, causing diseases such as Alzheimer’s disease and Parkinson’s disease. Understanding the regulation and mechanism of action of chaperones could help us better understand how to treat these diseases. One of the major chaperones in fungi involved in the folding of nascent polypeptides is the Hsp70 protein Ssb (Pfund C. et al., 1998). There are two Ssb homologs, Ssb1 and Ssb2. Ssb1 and Ssb2 differ by only four amino acids. These differences have been maintained in many fungal species, suggesting they may be functionally important (Takuno S. et al., 2009). To test this, I created chimera constructs to determine whether the coding sequence or in the promoter sequence of the genes is responsible for difference in function.
Functional differences between the yeast Hsp70 molecular chaperones Ssb1 and Ssb2

Lucas Gu, Department of Biochemistry, Spring 2011

Abstract

Protein folding is a complex process that is mediated in the cell by molecular chaperones. Errors in protein folding can lead to aggregation of misfolded proteins, causing diseases such as Alzheimer’s disease and Parkinson’s disease. Understanding the regulation and mechanism of action of chaperones could help us better understand how to treat these diseases. One of the major chaperones in fungi involved in the folding of nascent polypeptides is the Hsp70 protein Ssb (Pfund C. et al., 1998). There are two Ssb homologs, Ssb1 and Ssb2. In a subset of fungi, including the budding yeast Saccharomyces cerevisiae, Ssb1 and Ssb2 differ by only four amino acids. These differences have been maintained in many fungal species, suggesting they may be functionally important (Takuno S. et al., 2009). To test this idea, I created and tested constructs to determine whether differences in strains lacking Ssb1 or Ssb2 function were due to differences in the coding sequence or in the promoter sequence of the genes. I conclude that the four unique amino acid differences between the Ssb homologs are important for their differential rescue of salt and cold sensitivity.

Introduction

Ssb1 and Ssb2 are molecular chaperones that act in complex with Ssz1 and Zuo1 to aid in the folding of nascent polypeptide chains. The amino acid sequence between Ssb1 and Ssb2 are highly conserved, they differ by only 4 amino acid residues. However we have observed overexpression of Ssb1 and Ssb2 show differences in their ability to rescue salt and cold sensitivity phenotype in Δssz1 strain. To test whether the promoter sequence or the coding sequence of the chaperones are responsible for their functional difference we have created chimeric Ssb constructs. This research will elucidate whether the regulatory promoter sequences or the coding sequence of Ssb1 and Ssb2 is responsible for the functional differences observed between Ssb1 and Ssb2.
The function of Zuo1 in the chaperone complex is to stimulate the hydrolysis of ATP by Ssb, facilitating the folding of proteins. Zuo1’s homolog in C. elegans has been linked to asymmetrical cell division (Hatzold et al., 2008). Our lab has found that Zuo1 is involved in longevity regulation in yeast. Zuo1 is thought to facilitate asymmetric cell division once a certain cell density is reached to maximize survival. Growth of strains lacking Ssb1 and Ssb2 will be assayed to determine if these chaperones also function in asymmetric cell division in yeast.

**Methods**

The coding sequence of Ssb1 was cloned in between the Ssb2 promoter and terminator sequence using quickchange. PCR primers with homology to the Ssb1 coding sequence and Ssb2 promoter and terminator sequence were used to amplify Ssb1. The product of this PCR reaction contains Ssb1 coding sequence flanked by partial Ssb2 promoter and terminator sequence. The PCR product was then used as primers to amplify pRS316-Ssb2 plasmid, the end product of this reaction would contain chimera Ssb1 coding sequence flanked Ssb2 promoter and terminator sequences. To clone Ssb2 coding sequence into Ssb1 promoter and terminator sequence restriction enzymes BamHI and AgeI were used to cut pRS316-Ssb1 and pRS314-Ssb2 plasmids. The insert containing the coding sequence of Ssb2 was integrated into the plasmid containing the promoter and terminator sequence of Ssb2. The correct integration of the two chimera constructs were confirmed by sequencing. To test the rescue of these constructs, the plasmids were transformed into Δssb1/2 and Δssz1 strains and their ability to grow on salt media and cold were analyzed by drop test.
**Figure 1.** The two chimera constructs created by cloning, the coding sequence of Ssb1 is flanked by promoter and terminator sequences of Ssb2, while the coding sequence of Ssb2 is flanked by Ssb1 promoter and terminator sequences.

To test the viability of yeast cells, cultures were grown in minimal media and diluted to an optical density of 0.2. Growth in minimal media was monitored using spectrophotometry over a 33 hour time period.

**Results and Discussion**

Drop test of Δssb1/2 transformants showed similar ability of Ssb2 and Ssb2p/Ssb1c constructs in rescuing salt and cold sensitivity. This suggests that the coding sequence is not critical and that Ssb1 and Ssb2 are interchangeable in function when rescuing in a Δssb1/2 strain. Drop test of Δssz1 transformants however showed Ssb2 aggravated the salt and cold sensitivity phenotype while chimera Ssb2p/Ssb1c was able to rescue salt and cold sensitivity. This result implies the coding sequence differences in Ssb1 and Ssb2 are functionally important when rescuing the phenotype in Δssz1. Protein analysis by Western blot show similar levels in expression of Ssb2 and Ssb2p/Ssb1c being expressed in the cell, indicating that expression levels of Ssb2 and Ssb2p/Ssb1c do not contribute to their ability to rescue.

**Figure 2.** Δssb1/2 overexpressing Ssb2 and chimera Ssb2p/Ssb1c shows comparable ability to rescue salt and cold sensitivity in Δssb1/2 strain.
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**Figure 3.** Δssz1 overexpressing Ssb2 shows aggravated salt and cold sensitivity. Δssz1 overexpressing chimera Ssb2p/Ssb1c shows rescue of salt and cold sensitivity phenotype.

Viability assay of Δssb1 and Δssb2 strains and have found that deletion of Ssb2 chaperone significantly increases the optical density of cells after diauxic shift. The optical density of Δssb2 strain after 32.5 hours of growth in minimal media is 4.82 compared to the optical density of 4.36 of Δssb1 strain. Optical density of wildtype after 32.5 hours of growth in minimal media is 4.3. This suggests that although Ssb1 and Ssb2 are similar in their sequences they may have different substrate or have different non-chaperone related functions. I have observed increased cell growth in Δzuo1 strains after the diauxic shift and an increase in cell viability.

**Future direction**

Future research could include confirming the rescue of salt and cold sensitivity of Ssb1 and Ssb1p/Ssb2c chimera transformants in both Δssb1/2 and Δssz1 strain using strains with the chimera Ssb proteins integrated into the yeast genome to test the rescue by genomic expression. Viability of chimeric chaperons constructs can also be test with using the viability assay. The ability of the Ssb chaperones to rescue heat sensitivity in Δtom1 can also be analyzed. Tom1 is an ubiquitin ligase that is involved in the transport of mRNA from the nucleus to the cytosol, deletion of Tom1 has been shown to be heat sensitive. The defective growth phenotype of Δreg1 has been shown to be rescued by overexpression of Ssb. Reg1 is protein phosphatase that is part of the energy regulation pathway. Chimera constructs can be transformed into Δreg1 strain to test rescue of Ssb.
and chimeric chaperones in the rescue of defective growth phenotype and heat sensitivity in Δreg1 strain. These experiments will help to further characterize which phenotypes are dependent on Ssb1 and which phenotype are dependent on Ssb2.

References


