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Review

# The ribosome-bound Hsp70 homolog Ssb of *Saccharomyces cerevisiae*

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ABSTRACT

The Hsp70 homolog Ssb directly binds to the ribosome and contacts a variety of newly synthesized polypeptide chains as soon as they emerge from the ribosomal exit tunnel. For this reason a general role of Ssb in the *de novo* folding of newly synthesized proteins is highly suggestive. However, for more than a decade client proteins which require Ssb for proper folding have remained elusive. It was therefore speculated that Ssb, despite its ability to interact with a large variety of nascent polypeptides, may assist the folding of only a small and specific subset. Alternatively, it has been suggested that Ssb's function may be limited to the protection of nascent polypeptides from aggregation until downstream chaperones take over and actively fold their substrates. There is also evidence that Ssb, in parallel to a classical chaperone function, is involved in the regulation of cellular signaling processes. Here we aim to summarize what is currently known about Ssb's multiple functions and what remains to be ascertained by future research.



## 1. Hsp70 homologs — common principles

A canonical Hsp70 (70 kDa heat-shock protein) consists of a ~45 kDa N-terminal ATPase, a ~15 kDa peptide binding, and a ~10 kDa variable C-terminal domain. Hsp70s perform a broad range of different tasks based on one simple principle: the C-terminal peptide binding domain dynamically interacts with hydrophobic segments of substrate polypeptides and this substrate binding and release cycle is controlled by the N-terminal ATPase domain, which alternates between the low affinity ATP, and the high affinity ADP state. Co-chaperones regulate Hsp70 function via modulation of the ATPase cycle. One family of co-chaperones is the J-domain proteins, which transiently interact with the ATPase domain and stimulate ATP hydrolysis. Via this mechanism J-domain proteins induce tight substrate binding [1,2]. The other major class of Hsp70 co-chaperones is the nucleotide exchange factors (NEFs), which enhance the exchange of ADP with ATP and by this means promote the release of substrate peptides [1].

## 2. Cytosolic Hsp70 homologs of yeast — chaperones and co-chaperones

Four subfamilies of Hsp70s localize to the cytosol of yeast. The Ssa-family, consisting of the closely related but differentially expressed *SSA1–4* genes, the Ssb-family, encoded by the constitutively expressed close homologs *SSB1* and *SSB2*, the Sse-family, consisting of constitutively expressed *SSE1* and the stress-inducible *SSE2*, and constitutively expressed *SSZ1* [3–6]. Like all members of the Hsp70 family, the cytosolic Hsp70s of yeast show a high degree of conservation, especially within their N-terminal ATPase domains [7] (Fig. 1). The cytosolic Hsp70s form an interconnected network in which Ssa and Ssb are the central chaperones with distinct and non-overlapping functions [3,8] while Sse [9–14], and Ssz1 [15–17] act as their co-chaperones. Expression of at least one of the *SSA* and *SSE* family members is essential for the life of yeast, while strains lacking *SSB* or *SSZ1* are viable [3,11,18,19]. *SSB* and *SSZ1* are genetically linked, because Δ*ssb1*Δ*ssb2*, Δ*ssz1*, or Δ*ssb1*Δ*ssb2*Δ*ssz1* strains suffer from a similar set of specific growth defects, such as slow growth, cold sensitivity, and aminoglycoside sensitivity [15,16], and overexpression of Ssb1 partly suppresses growth defects of a Δ*ssz1* strain [16,20]. Ssz1 binds to ATP but does not hydrolyze the nucleotide; nucleotide binding is not strictly required for its *in vivo* function [17,20]. Consistently, even overexpression of a Ssb mutant, which cannot hydrolyze ATP can partly suppress the phenotype of a Δ*ssz1* strain [20].

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A

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Ssb1   MAEGVFQGAI GIDLGTTYSC VATYESSVEI IANEQGNRVT PSFVAFTPEE RLIGDAAKNQ AALNPRNTVF DAKRLIGRRF
Ssb2   MAEGVFQGAI GIDLGTTYSC VATYESSVEI IANEQGNRVT PSFVAFTPQE RLIGDAAKNQ AALNPRNTVF DAKRLIGRRF

Ssb1   DDESVQKDMK TWPFKVIDVD GNPVIEVQYL EETKTFSPQE ISAMVLTKMK EIAEAKIGKK VEKAVITVPA YFNDAQRQAT
Ssb2   DDESVQKDMK TWPFKVIDVD GNPVIEVQYL EETKTFSPQE ISAMVLTKMK EIAEAKIGKK VEKAVITVPA YFNDAQRQAT

Ssb1   KDAGAISGLN VLRIINEPTA AAIAYGLGAG KSEKERHVLI FDLGGGTFDV SLLHIAGGVY TVKSTSGNTH LGGQDFDTNL
Ssb2   KDAGAISGLN VLRIINEPTA AAIAYGLGAG KSEKERHVLI FDLGGGTFDV SLLHIAGGVY TVKSTSGNTH LGGQDFDTNL

Ssb1   LEHFKAEFKK KTGLDISDDA RALRRLRTAA ERAKRTLSSV TQTTVEVDSL FDGEDFESSL TRARFEDLNA ALFKSTLEPV
Ssb2   LEHFKAEFKK KTGLDISDDA RALRRLRTAA ERAKRTLSSV TQTTVEVDSL FDGEDFESSL TRARFEDLNA ALFKSTLEPV

Ssb1   EQVLKDAKIS KSQIDEVVLV GGSTRIPKVQ KLLSDFFDGK QLEKSINPDE AVAYGAAVQG AILTGQSTSD ETKDLLLLDV
Ssb2   EQVLKDAKIS KSQIDEVVLV GGSTRIPKVQ KLLSDFFDGK QLEKSINPDE AVAYGAAVQG AILTGQSTSD ETKDLLLLDV

Ssb1   APLSLGVGMQ GDMFGIVVPR NTTVPTIKRR TFTTCADNQT TVQFPVYQGE RVNCKENTLL GEFDLKNIPM MPAGEPVLEA
Ssb2   APLSLGVGMQ GDIFGIVVPR NTTVPTIKRR TFTTVSDNQT TVQFPVYQGE RVNCKENTLL GEFDLKNIPM MPAGEPVLEA

Ssb1   IFEVDANGIL KVTAVEKSTG KSSNITISNA VGRLSSEEIE KMVNQAEEFK AADEAFAKKH EARQRLESYV ASIEQTVTDP
Ssb2   IFEVDANGIL KVTAVEKSTG KSSNITISNA VGRLSSEEIE KMVNQAEEFK AADEAFAKKH EARQRLESYV ASIEQTVTDP

Ssb1   VLSSKLKRGS KSKIEAALSD ALAALQIEDP SADELRKAEV GLKRVVTKAM SSR
Ssb2   VLSSKLKRGS KSKIEAALSD ALAALQIEDP SADELRKAEV GLKRVVTKAM SSR
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B

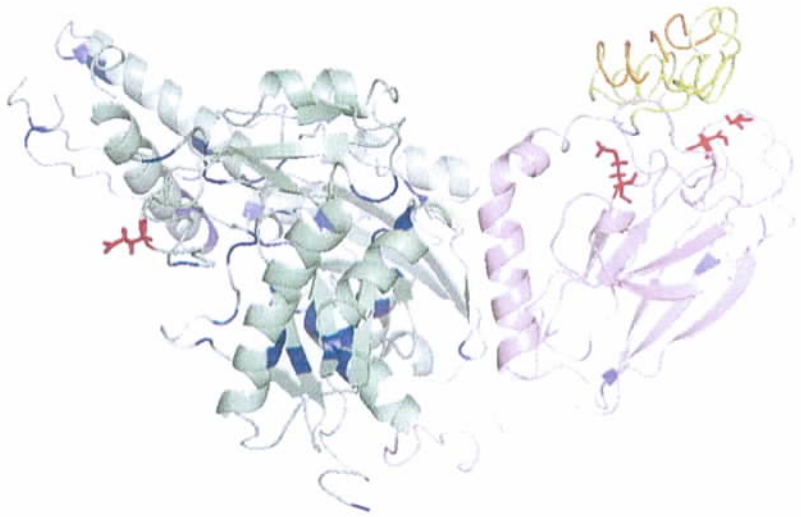

**Fig. 1.** The Ssb1 and Ssb2 homologs of *Saccharomyces cerevisiae*. (A) The ATPase domain (1–384) is shown in green, the peptide binding domain (385–558) is shown in pink, and the C-terminal domain is shown in yellow (559–613). Amino acid residues conserved in the cytosolic Hsp70 homologs of yeast (Ssa1–4, Ssb1–2, Sse1–2, Ssz1) are shown in blue. Amino acid residues which differ between Ssb1 and Ssb2 are shown in red, and the nuclear export signal (NES) is shown in orange. For details compare text. (B) Model of the Ssb1 structure generated by I-TASSER protein structure and function prediction (http://zhang.bioinformatics.ku.edu/I-TASSER/ [142–144]). The colour code is as described in (A).

Ssz1 is intimately connected to the Hsp40 co-chaperone family because it is a component of the Ssb specific J-domain partner termed RAC (ribosome-associated complex) [19]. RAC, which binds ribosomes close to the tunnel exit [21] consists of Ssz1 and the J-domain protein Zuo1 [19], which is required for binding of the complex to ribosomes [22]. The function of this unusual heterodimeric chaperone complex is conserved from yeast to higher eukaryotes [23,24]. Both subunits of RAC are required for its function as a J-domain partner of Ssb *in vivo* and *in vitro* [15–17,20]. Genetic evidence suggests that Ssb1 may also partner with the Hsp40 homolog Sis1 [25] and also binding of Ssb to Sis1 has been reported [26]. On the other hand neither Sis1, nor the cytosolic Hsp40s Ydj1 and Jjj1 stimulate the rate of ATP hydrolysis by Ssb as would be expected from partner co-chaperones [17,27–30]. In which way Sis1 affects the function of Ssb1 remains to be determined. According to a recent report the ATPase activity of Ssb can be stimulated by Ydj1 when prion fibers are added to the reaction [31].

Sse1, Fes1, and Snl1 can act as NEFs for Ssb *in vitro* [9–12,32,33]. In contrast to RAC, which is bound to ribosomes and functions specifically in concert with Ssb, Fes1 [34], Snl1 [32], and Sse1 [9,10] are predominantly soluble in the cytosol and also act as NEFs for Ssa1 [34,35]. Fes1 and Sse1 accelerate the release of nucleotide from Ssb *in vitro* [11,12,33]. However, when the ATPase activity of Ssb was tested in the presence of both co-chaperones, RAC and Fes1, it was inhibited rather than stimulated [33]. This is unexpected because the stimulation of ATP hydrolysis (via RAC) in combination with enhanced nucleotide release (via Fes1) should lead to an accelerated rate of ATP hydrolysis at steady state. Binding of a $His_6$-tagged, C-terminal Snl1-fragment to Ssb has been demonstrated in yeast lysates, but Snl1 does not significantly affect the rate of nucleotide exchange on Ssb. Snl1 may primarily function in combination with Ssa, while the affinity of Ssb for nucleotides might be so low that it does not require NEF function *in vivo* [32]. Similar considerations apply for nucleotide

exchange on Ssb in general. If Ssb would strictly depend on the NEFs *in vivo* one would expect growth defects of Δ*fes1* and Δ*sse1* strains to overlap with those of a Δ*ssb1*Δ*ssb2* strain. However, the Δ*fes1* and Δ*sse1* mutants resemble strains with defects related to Ssa and Ssa's J-domain partners [34,36–38]. In summary, the *in vivo* role of NEFs for Ssb's function has so far remained unclear. Possibly Ssb does not require NEFs *in vivo*; alternatively, Ssb may not discriminate between different NEFs, which then could functionally replace each other.

## 3. Ssb is encoded by the closely related genes *SSB1* and *SSB2*

*SSB1* and *SSB2* are transcribed with similar efficiencies and the Ssb1 and Ssb2 proteins differ in only four amino acids (Fig. 1). The single deletion strains Δ*ssb1* and Δ*ssb2* do not display significant growth defects indicating that at least in many respects the two copies of *SSB* are functionally interchangeable and a steady state level of about 50% is sufficient to sustain cellular functions [3,39]. However, there is an indication that the *in vivo* roles of Ssb1 and Ssb2 might differ in at least some aspects. *SSB1*, but not of *SSB2*, acts as a multicopy suppressor in yeast mutants lacking mitochondrial DNA [40]; Ssb1, but not Ssb2, was found to bind to calmodulin [41]. Interestingly, a recent genome scan aimed at the identification of signatures of selection for paralogous functional amino acids revealed that three of the amino acids which differ between Ssb1 and Ssb2 have been protected by selection against gene conversion for a long time not only in *S. cerevisiae* but also in other yeast species [42]. The finding suggests that the three amino acids, M413, C435, A436 in Ssb1 and I413, V435, and S436, in Ssb2, which are localized within the peptide binding domains (Fig. 1) have an important effect on the function of Ssb.

### 3.1. Transcriptional regulation of *SSB*

The two genes encoding *SSB* are regulated in a similar fashion and it was noticed early that transcriptional regulation of *SSB1* and *SSB2* closely resembles regulation of genes encoding ribosomal proteins and other core components of the translation machinery [6,35,39,43]. Regulation of the translational machinery reflects that the preferred energy source of yeast is glucose, which it utilizes via fermentation even under aerobic conditions. Consequently, in the presence of glucose genes required for respiration and alternative carbon source utilization are strongly repressed. At the same time transcription of genes encoding components of the translational machinery is strongly enhanced, because fermentation of glucose presents the opportunity for most efficient mass accumulation [44–46]. When glucose is depleted, e.g. in an older batch culture, glucose repression is relived and genes involved in respiration are now transcribed while genes encoding components required for protein synthesis are turned off. The transition between turning on and off glucose repression requires a massive transcriptional re-programming and involves a number of major signaling pathways [44–46].

*SSB* is most highly expressed on glucose, followed by galactose, and ethanol [47]. *SSB* is induced during the lag- and early exponential phase of growth in glucose medium when yeast generates energy via fermentation [48]. During the diauxic shift, or upon transfer from glucose- to glycerol-containing medium, *SSB* transcript levels decrease in abundance [6,39,49]. As many other genes encoding components of the translational apparatus *SSB* is also down-regulated upon amino acid starvation [6,39]. Despite Ssb's classification as a heat shock protein, the expression of *SSB* is strongly and rapidly repressed upon temperature up shift [3,6]. Only 5 min after a shift from 23 °C to 39 °C, the level of *SSB* mRNA decreases by approximately 80% [49].

## 4. Cellular localization of Ssb *versus* Ssa

Ssb (~300000 molecules per cell [50,51]) and Ssa (~600000 molecules per cell [51]) are highly abundant Hsp70s which are more than 60% identical on the amino acid level [7]. While both localize to the cytosol their distribution differs significantly. First, only Ssb is excluded from the nucleus at steady state. To that end, Ssb contains a functional nuclear export signal (NES) in its C-terminal variable domain [52] (Fig. 1). It has been hypothesized that Ssb plays a role in the nucleus, which might be related to ribosome biogenesis, proteasome-mediated protein degradation, or regulation of gene expression [52]. As Ssb lacking the C-terminal variable domain is active, but no longer excluded from the nucleus, active export at least is not essential for its cellular function [52,53]. In the cytosol a large fraction of Ssb is directly associated with ribosomes, the remainder is soluble [8,50,54]. In contrast, Ssa does not directly interact with ribosomes, and only a small fraction co-sediments with translating ribosomes [8,35,55]. Ribosome association of Ssa is thought to be mediated via interaction with nascent polypeptides, and/or interaction with factors involved in translation initiation [55].

After cell lysis about half of the Ssb molecules co-purify with ribosomes [50,54]. As the number of ribosomal particles in a cell approximately equals the number of Ssb molecules this suggests that about 50% of the ribosomes are occupied by Ssb [8,50,54]. Most likely, the situation is not static and Ssb cycles on and off ribosomes, however, the dynamics of the interaction has not yet been determined. Also, the Ssb binding site on the ribosome has not been identified so far. Ssb is thought to bind close to the ribosomal tunnel exit. This localization is suggested first, because of Ssb's close proximity to even short nascent polypeptides [50,56,57], and second, because of its functional interaction with RAC [15–17,20], which binds in proximity of Rpl31 at the tunnel exit [21]. RAC, however, does not affect the binding of Ssb to ribosomes and *vice versa* [58]. Ssb binds to ribosomes in at least two different modes, which can be distinguished by the stability of Ssb-ribosome complexes in the presence of high salt concentrations. When bound to non-translating ribosomes, Ssb can be stripped off with high salt; when bound to ribosomes exposing a nascent polypeptide, Ssb is resistant to high salt treatment [50,56]. Protein-nascent chain interactions that persist conditions of high ionic strength are predicted to involve hydrophobic surfaces [59]. Thus, a possible explanation for the salt-resistance is that ribosome-bound Ssb establishes additional interactions with hydrophobic stretches of a nascent polypeptide. Unexpectedly, however, Ssb was found to bind in a salt-resistant manner even in the presence of 10 mM ATP [56]. As ATP releases Hsp70s from their polypeptide substrates, this should reduce salt-resistance caused by interactions between Ssb and the nascent polypeptide. Another mechanism that may induce salt-resistance is a conformational change within the actively translating ribosome affecting the interaction of Ssb with the ribosome itself. Such a conformational change would have to be transmitted from the peptidyl transferase center to the ribosomal binding site of Ssb.

## 5. Biochemical properties of Ssb *versus* Ssa

The question how the different domains of Ssb and Ssa contribute to their localization and functional specificity has been addressed with a series of chimeras containing all combinations of the ATPase, peptide binding, and variable domains of Ssb (BBB) and Ssa (AAA) [8,53,60,61].

Purified Ssa1 has an ATPase activity very similar to that of other canonical Hsp70s, with a *K*m for ATP in the order of 0.2 μM and a *k*cat of 0.04 ATP $min^{-1}$ at physiological potassium concentrations [62]. Purified Ssb has a *K*m of ~150–300 μM and a *k*cat of approximately 1 $min^{-1}$ [60]. Thus, compared to the unstimulated ATPase of Ssa, Ssb displays a 1000× higher *K*m but hydrolyzes ATP about 50-times faster. These unusual properties are determined by the peptide binding and variable C-terminal domains of Ssb because the ABB chimera was found to have very similar kinetic properties compared to wild type Ssb. Moreover, the isolated ATPase domains of Ssb and Ssa are

kinetically similar [60]. The data indicate that the peptide binding and C-terminal domains of Ssb have a significant effect on the ATPase domain they are fused to.

However, the situation is more complicated and the specialization of Ssb is not simply a consequence of its special peptide binding domain. A chimera in which the peptide binding domain of Ssb is replaced with the peptide binding domain of Ssa (the BAB chimera) efficiently rescues the major growth defects of a *Δssb1Δssb2* strain, while wild type Ssa does not [8,53]. The observation suggests that the peptide binding domains of Ssa and Ssb display a considerable degree of flexibility [1], which allows the Ssa peptide binding domain in the BAB chimera to accommodate Ssb substrates. This interchangeability of the Ssa and Ssb peptide binding domains is intriguing because none of the peptides or permanently unfolded proteins which readily bind to Ssa and stimulate its ATPase bind to, or stimulate Ssb [53,56,60]. Chimeras BAA and AAB fail to rescue aminoglycoside sensitivity of a *Δssb1Δssb2* strain and do not bind to polysomes, while ABB as well as BAB chimeras confer aminoglycoside resistance and interact with ribosomes [8]. The observations suggest that the C-terminal domain of Ssb is required for ribosome binding and that ribosome binding is relevant to prevent aminoglycoside sensitivity of yeast. In any case, it is not understood what allows Ssb to interact with nascent peptides in the context of the ribosome [16,50,56,57,60,63], and how this interaction can even persist ribosome release [9,35], while Ssb on its own has no significant affinity for peptides or unfolded proteins in solution. Recently it was discovered that even in the context of the ribosome Ssb interacts with many, but not all types of nascent chains. If a nascent chain contains a signal anchor sequence which recruits the ER targeting factor SRP [64], this nascent chain does not bind to Ssb [50,63]. Whether this is due to intrinsic properties of the nascent chain or results from sterical hindrance between Ssb and SRP at the tunnel exit in respect to interaction with the nascent chain is currently not known.

## 6. The ribosomal function of Ssb can be partly replaced by Ssa recruited to the ribosome via J-domain proteins

As outlined above, growth defects of the *Δssb1Δssb2* deletion cannot be complemented by overexpression of its close homolog Ssa [8]. However, overexpression of the ribosome-bound Hsp40 Jjj1, which serves as a partner of Ssa but not Ssb [29], quite efficiently rescues the slow growth and cation-sensitive phenotype of a *Δssb1Δssb2* and even a *Δssb1Δssb2Δzuo1Δssz1* strain [29]. Based on the data it was suggested that Jjj1, which is about 40-fold less abundant compared to Zuo1, recruits Ssa to ribosomes when overexpressed. When localized to the ribosome via Jjj1 Ssa is able to take over the function(s) of Ssb [29]. Such a model is supported by the observation that overexpression of the mammalian Zuo1 homolog MPP11 partially suppresses cation and aminoglycoside sensitivity not only of a *Δzuo1* [23,24] strain but also of a *Δssb1Δssb2Δzuo1* strain [23]. MPP11 can stimulate the ATPase activity of Ssa and thus likely enables Ssa to take over Ssb's ribosomal function [23]. Finally, even two prokaryotic folding chaperones unrelated to the Hsp70 and Hsp40 families were found to suppress growth defects of a *Δssb1Δssb2* strain. *E. coli* Trigger Factor (TF) is structurally unrelated to Ssb, however, like Ssb binds to non-translating and translating ribosomes as well as to nascent chains [65,66]. When expressed in yeast, TF interacts with ribosomes and forms a crosslink to nascent polypeptides. TF partly complements the aminoglycoside sensitivity of a *Δssb1Δssb2Δzuo1Δssz1* strain, while it does not complement slow growth or cold sensitivity [67]. Thus, TF which is structurally entirely unrelated to Ssb specifically suppressed the defects related to translational fidelity. The common theme of the *Δssb1Δssb2* suppressors Jjj1, MPP11, and TF is their ability to directly interact with the ribosome. A remarkable exception of this rule is bacterial GroEL, which serves as a post-translational folding chamber for an essential subset of client proteins in bacteria [66,68]. Overexpression of GroEL was found to fully rescue growth defects of a *Δssb1Δssb2* strain [35]. Unexpectedly, in this case GroEL was a suppressor of *Δssb1Δssb2*, but failed to suppress in a *Δzuo1* or *Δssz1* background.

## 7. The role of Ssb in *de novo* protein folding — evidence awaiting positive proof

The idea that Ssb aids nascent polypeptide folding emerged early, when it was discovered that Ssb interacts with ribosomes [54]. The view is supported by the finding that Ssb does not only interact with ribosomes directly but also contacts a variety of nascent polypeptide chains [16,35,50,56,57,63]. Based on these findings models viewing Ssb as a co-translational folding helper have been presented in a number of reviews on the topic of cytosolic protein folding [69–73]. The models predict that ribosome-bound Ssb plays an early and general role in protein folding and Ssa, or other cytosolic chaperones, take over after synthesis is complete (Fig. 2). Based on experimental data it was alternatively suggested that Ssb might assist the folding of only a small and specific subset of nascent chains [74,75] or serve as a passive holdase rather than foldase [33]. However, to date a protein which requires Ssb for successful *de novo* folding has not been identified.

An unbiased approach aiming on the identification of Ssb's folding substrates did not settle the issue. To identify proteins which fold less efficiently in the absence of Ssb, the soluble proteome of *Δssb1Δssb2* and wild type strains was directly compared [76]. While differences in the expression level of specific proteins exist, these are not related to translational or post-translational events – like folding – but turned out to result from transcriptional deregulation in the *Δssb1Δssb2* strain [76]. A caveat in respect to the proteome approach is that only the most abundant, approximately 1000, yeast proteins are detected. Folding substrates of Ssb might be hidden among the proteins expressed with lower efficiency. Also, other chaperones may take over Ssb's function in the *Δssb1Δssb2* strain. In this respect it is interesting that in a *Δssb1Δssb2* strain the co-translational association of Ssa with nascent chains is enhanced, suggesting that Ssa can partly replace Ssb [9]. Enhanced interaction of Ssa in the *Δssb1Δssb2* background on the other hand disfavors a model in which Ssb is required to hand over nascent chains to Ssa (Fig. 2). What is interesting in this context is that the expression level of Ssa is not increased in a *Δssb1Δssb2* strain [76,77] and overexpression of Ssa does not suppress defects of a *Δssb1Δssb2* strain [3,8].

A recent *in vivo* screen addressed the question whether Ssb is required for the *in vivo* folding of a set of mutant proteins with decreased structural stability [78]. The hypothesis behind the approach was that destabilized client proteins require chaperone function even more strictly than their stable wild type counterparts. Unexpectedly the outcome was the opposite: in the absence of Ssb (or also its co-chaperone RAC) the activity of structurally destabilized proteins was enhanced rather than reduced. *De novo* folding seemingly is not only independent of Ssb and RAC but even furthered in the absence of the chaperones. Importantly, the expression level of the destabilized proteins is not affected in the *Δssb1Δssb2*, *Δssz1*, or *Δzuo1* strains compared to wild type. This indicates that the increase in steady state activity of the destabilized proteins is not due to their escape from degradation in the absence of the chaperones. Based on these findings, the authors suggest that Ssb and RAC might be optimized to assist the folding of native proteins *only*, while folding of point mutants with reduced stability is not supported [78]. However, positive evidence that any of the proteins tested, native or destabilized by mutations, folds less efficiently in a *Δssb1Δssb2* or *Δzuo1* strain is not available [78,79]. The interesting but puzzling observation rather seems to challenge the idea of a role of Ssb and RAC in protein folding.

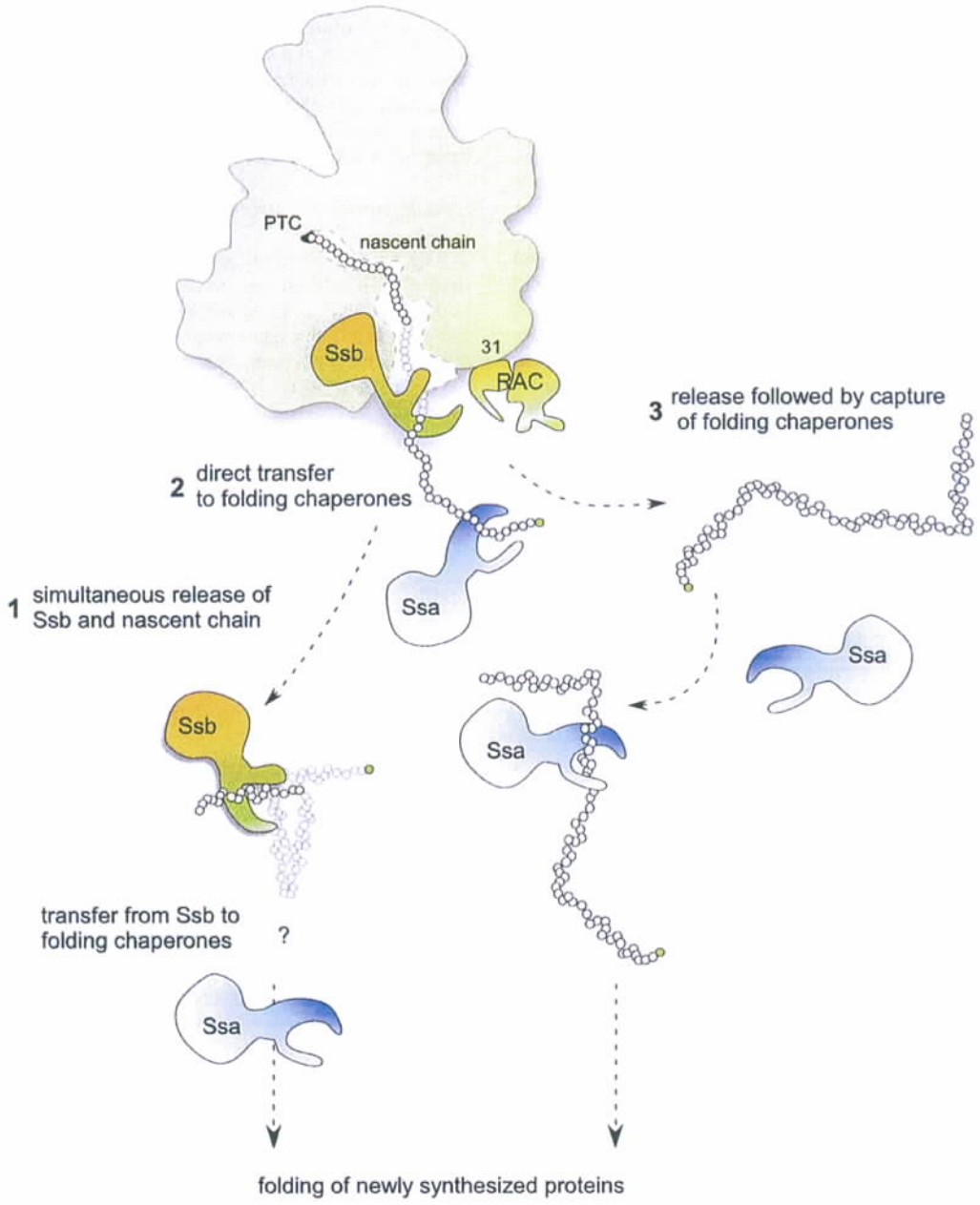


**Fig. 2.** Different models for the transfer mechanism of nascent chains from the ribosome to chaperones mediating their folding. Newly synthesized polypeptides (nascent chains) exit the ribosome through a tunnel, which spans the large ribosomal subunit from the peptidyl transferase center (PTC) to the exit. At the platform surrounding the exit, Ssb and its co-chaperone RAC bind. In the case of RAC the binding site involves Rpl31 (31), in case of Ssb the binding site is unknown. Ssb contacts nascent chains directly, the interaction depends on RAC. (1) *Ssb may be an intermediate holdase on the way to the folded state of its client proteins.* In this model Ssb interacts with nascent chains during their synthesis and the interaction persists when the synthesis is complete and the nascent chain is released from the ribosome. In a next step the nascent chain is transferred to chaperones like Ssa which actively mediate folding. (2) *Ssb may facilitate nascent chain transfer to folding chaperones.* In this model Ssb interacts with nascent chains during their synthesis and presents them to folding chaperones like for example Ssa, which also interact co-translationally with the nascent chain, however, do not interact directly with the ribosome. Without Ssb the transfer to Ssa is affected and as a consequence folding is less efficient. (3) *Ssb may serve an as yet unknown function during translation.* Ssb interacts with nascent chains during their synthesis, however, this interaction does not mediate folding or transfer to other chaperones. Instead, folding chaperones like Ssa bind either co- or post-translationally and mediate folding to the native state. The role of Ssb may be related to maintaining translational fidelity via assisting transport of the nascent chain through the exit tunnel, degradation of nascent chains which stall the ribosome, co-translational targeting to specific cellular compartments, or presentation of nascent chains to modifying enzymes. Models 1–3 are not mutually exclusive. For details and references compare the text.

## 8. Phenotypic characteristics of yeast lacking Ssb – hints on *in vivo* functions

### 8.1. Carbon source dependent slow growth

Despite homology to the heat-shock protein family of Hsp70s Ssb is not induced by heat shock (compare above). Cells lacking Ssb fail to grow below 20 °C, display moderate slow growth at 30 °C, and grow with a rate very similar to wild type at 37 °C [3,54,76]. Only recently it was recognized that slow growth of the Δ*ssb1*Δ*ssb2* strain depends on the carbon source supplied for growth. The Δ*ssb1*Δ*ssb2* strain displays slow growth on glucose containing media, while on ethanol or other media utilized via respiration slow growth is significantly less pronounced [76].

### 8.2. A ribosome-independent function of Ssb in glucose repression

Consistent with a carbon source specific growth defect it was recently discovered that glucose-grown Δ*ssb1*Δ*ssb2* cells suffer from deregulation on the transcriptional level similar to mutants affected in glucose repression [76]. Microarray analysis comparing transcript levels of wild type and Δ*ssb1*Δ*ssb2* growing logarithmically on glucose, revealed that up-regulated transcripts in Δ*ssb1*Δ*ssb2* were highly overrepresented among the ones involved in oxidative phosphorylation and the citrate cycle. In the same study, down-regulated transcripts in Δ*ssb1*Δ*ssb2* were found to be overrepresented among the ones involved in processes related to amino acid and sulphur metabolism and also in transcripts encoding ribosomal proteins [76]. The Δ*ssb1*Δ*ssb2* strain displays additional similarities with mutants affected in glucose sensing. The Δ*ssb1*Δ*ssb2* strain is unable to respond to sudden glucose depletion with a transient shut-off of translation initiation [76]. One of the originally described mutants defective in translational shut-off induced glucose depletion is the Δ*reg1* mutant [80]. Reg1 regulates the interaction of the type 1 protein phosphatase Glc7 with specific substrates [81]. Most importantly, Glc7 is targeted to the heterotrimeric kinase SNF1 via the Reg1 regulatory subunit [82,83] (Fig. 3). SNF1, which is regulated via phosphorylation/dephosphorylation of a specific threonine residue of its Snf1 subunit, on its part is a central player of glucose signaling in yeast [84,85]. In the presence of glucose Snf1 is dephosphorylated and inactivated by Glc7/Reg1. In a Δ*reg1* strain, Snf1 remains phosphorylated in the presence of glucose and glucose repression is not established [81–83] (Fig. 3). For recent reviews on glucose sensing in yeast compare [44–46].

There is evidence for physical interaction between Ssb and Reg1 from two-hybrid as well as pull-down experiments [86,87] (Fig. 3). It was suggested that the interaction between Ssb and Reg1 may serve as a metabolic sensor to modulate the activity of Glc7/Reg1 towards Snf1 [87]. Several observations support the idea that Ssb effects the activity of Glc7/Reg1 towards its target Snf1 [76]. i) Snf1 is hyperphosphorylated in the presence of glucose in the Δ*ssb1*Δ*ssb2* strain, ii) Δ*ssb1*Δ*ssb2* and Δ*reg1* strains display similar patterns of deregulated protein expression iii) overexpression of Ssb efficiently reduces the level of Snf1 hyperphosphorylation in a Δ*reg1* strain, and iv) overexpression of Ssb efficiently suppresses the severe growth defects of a Δ*reg1* strain [76]. But how can Ssb influence the interplay between SNF1 and Glc7/Reg1? It is unlikely that Ssb is involved in the folding/stabilization of Glc7 in the absence of the targeting subunit Reg1, because the steady state level of Glc7 is not decreased in a Δ*reg1* or Δ*ssb1*Δ*ssb2* strain [76] and Glc7, in the absence of Δ*reg1*, functions normally in combination with other targeting subunits [44–46]. An interesting possibility is that Ssb contributes to SNF1 signaling via regulating the transient interactions between SNF1 and Glc7/Reg1 (Fig. 3). SNF1 and its mammalian homolog AMP-activated kinase (AMPK) are central energy sensors in all eukaryotic cells [88]. Unlike mammalian AMPK, and besides the high homology between yeast SNF1 and mammalian AMPK, SNF1 does not seem to bind AMP directly, and thus must sense the energy status of the cell by a different mechanism [89]. It is intriguing, but at present speculative, that Ssb may act as a sensor of the cellular ATP status in the context of the SNF1 signaling pathway. The unusually high Km value for ATP hydrolysis (compare above) and the fact that the reported cellular ATP concentration in yeast vary in the range of 0.04–4.8 mM [90] would predestine Ssb for such a function. Alternatively, Ssb and SNF1 might be connected via the regulation of the heat-shock transcription factor (HSF) which responds to heat stress as well as glucose starvation [91,92]. Induction of HSF target genes in response to glucose starvation, but not heat shock, depends on Snf1 [91,92]. Snf1 interacts with HSF *in vivo* and HSF was shown to be a substrate of Snf1-mediated phosphorylation *in vitro* [92]. The combined data suggest that SNF1 may directly phosphorylate HSF in response to glucose starvation in a manner distinct from that occurring in response to heat shock [91]. Interestingly, Ssb was shown to interact with HSF directly and it was suggested that Ssb binding may directly influence the activation of HSF [93]. Alternatively, SNF1-dependent phosphorylation of HSF might be enhanced in the Δ*ssb1*Δ*ssb2* strain because of the irregular hyperphosphorylation/activation of SNF1 occurring in the absence of the chaperone [76].

### 8.3. Salt sensitivity — Ssb may affect membrane protein biogenesis

The Δ*ssb1*Δ*ssb2* strain suffers from sensitivity to a set of specific supplements and drugs. One of the strains characteristics is hypersensitivity to increased salt concentrations, including NaCl, LiCl, and guanidinium chloride [74,75]. Salt sensitivity does not simply reflect a general problem in respect to increased osmolarity, because the Δ*ssb1*Δ*ssb2* strain is not sensitive towards the uncharged, but osmotically active, supplement sorbitol [75]. Rather, the defect seems to be due to an enhanced influx of all kinds of cations. Enhanced cation uptake is a characteristic of mutants with an increased negative potential across the plasma membrane. A well studied example of such a mutant is Δ*trk1*Δ*trk2*, which lacks the plasma membrane $K^+$-transporters Trk1 and Trk2 [75,94]. Based on phenotypic similarities it was suggested that Ssb might, directly or indirectly, affect the biogenesis of membrane proteins such as Trk1 or Trk2, because proper co-translational insertion of the transporters into the membrane of the ER is hampered in the Δ*ssb1*Δ*ssb2* strain [75]. Such an effect of Ssb on membrane protein biogenesis, however, does not seem to be general because for example ABC transporter function is normal in a Δ*ssb1*Δ*ssb2* strain [74].

### 8.4. Sensitivity to translational inhibitors — Ssb affects translational fidelity

The Δ*ssb1*Δ*ssb2* strain is hypersensitive also to a number of translational inhibitors. Most striking is the exceptionally high sensitivity of the Δ*ssb1*Δ*ssb2* strain towards the aminoglycoside class of antibiotics as for example paromomycin, hygromycin B, and G418 [54,58,75]. Aminoglycosides bind close to the decoding center of the small ribosomal subunit and affect translational fidelity [95]. Via this mechanism aminoglycosides can induce phenotypic suppression of nonsense as well as missense mutations [96]. A number of yeast mutants in proteins or rRNA localized close to the decoding center display enhanced sensitivity to paromomycin [97–101]. In the case of Δ*ssb1*Δ*ssb2* at least two, possibly additive mechanisms, seem to contribute to the hypersensitivity against aminoglycosides.

First, hypersensitivity towards aminoglycosides is a consequence of an increased intracellular accumulation of the drugs, which are cationic in nature [75]. As discussed above this could be connected to a role of Ssb in the biogenesis of membrane transporters required for ion homeostasis [75]. Alternatively, defects in cation homeostasis might be related to hyperphosphorylation of Snf1 in the Δ*ssb1*Δ*ssb2* strain. Specifically the non-phosphorylated form of Snf1 is required for mediating tolerance to toxic cations and activation of potassium transport [102]. Also Glc7 mutations, which affect the phosphorylation status of Snf1, lead to sensitivity against cations as well as aminoglycosides [103].

Second, there is evidence for a direct connection between aminoglycoside sensitivity and a role of Ssb in translational fidelity. *In vivo* read through of a reporter construct containing an *in frame* stop codon is enhanced in a Δ*ssb1*Δ*ssb2* strain even in the absence of error inducing drugs. Thus, Δ*ssb1*Δ*ssb2* causes a defect in translational fidelity independent of its defect in cation uptake [58]. Support for a direct effect of Ssb on translational fidelity comes from several additional observations. In an unbiased screen *SSB* was identified as an efficient multicopy enhancer of termination efficiency [104]. In the Δ*ssb1*Δ*ssb2* strain inhibition of −1 programmed ribosomal frame shifting and defects in Killer virus maintenance were observed [105]. Also, the Δ*ssb1*Δ*ssb2* mutation leads to the accumulation of flock

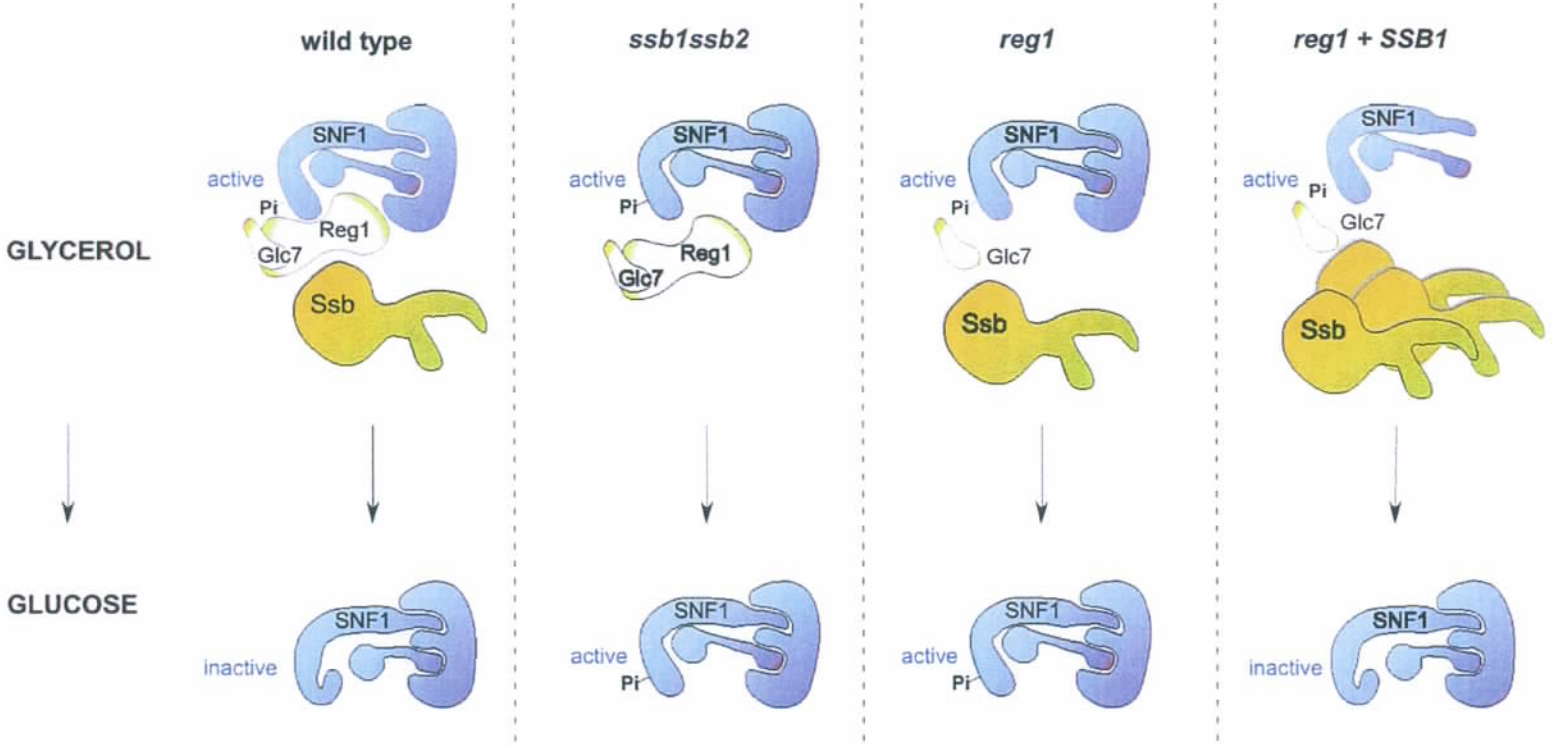


**Fig. 3.** Model for an involvement of Ssb in the regulation of the SNF1 kinase. In the wild type strain a transfer from a respiratory medium (glycerol) to glucose results in the dephosphorylation of the catalytic subunit Snf1 of the heterotrimeric SNF1 complex. Then dephosphorylated Snf1 adopts an auto inhibited conformation and is inactive. Dephosphorylation requires the type 1 protein phosphatase Glc7 and its regulatory subunit Reg1, which targets Glc7 to Snf1. Besides Reg1 also Ssb is required for efficient dephosphorylation. As a consequence in a Δ*ssb1*Δ*ssb2* as well as in a Δ*reg1* strain Snf1 remains phosphorylated and active when glucose is present in the growth medium. In a Δ*reg1* strain dephosphorylation of Snf1 in glucose containing medium can be restored by overexpression of Ssb. For details and references compare the text.

house virus RNA expressed in yeast and it was hypothesized that the effect might be related to Ssb's role in maintaining translational fidelity [106]. Finally, the fidelity of translation termination *in vitro* is significantly lower when Ssb is absent from the translation reaction. When aminoglycosides are added to the *in vitro* reaction the error rate in the absence of Ssb increases dramatically, while the error rate in the presence of Ssb is much less affected [58]. How Ssb, localizing far from the decoding center at the exit of the ribosomal tunnel can affect the fidelity of translation is currently not understood. However, the phenomenon is not without precedence and other mutations within ribosomal proteins of the large subunit localized at the tunnel exit have similar consequences on translational fidelity. For example, ribosomal proteins Rpl39 and Rpl31, which localize close to the tunnel exit, affect decoding [21,107,108].

## 9. Impact on [*PSI*$^+$] — Ssb prevents prion propagation

The yeast prion [*PSI*$^+$] is a self-propagating, prion-like form of the translation termination factor subunit Sup35 (eRF3). Formation of Sup35 prions reduces the concentration of soluble Sup35. As a consequence yeast strains which are [*PSI*$^+$] display increased stop codon read through (for recent reviews on the topic compare [109–113]). The chaperones Hsp104, Hsp90, various Hsp70s and Hsp40s play crucial roles in the maintenance and propagation of [*PSI*$^+$]. Hsp104 is the most important chaperone in the context of [*PSI*$^+$] formation and propagation. Both, its overexpression and absence result in the loss of [*PSI*$^+$] [114]. Ssb and Ssa influence the action of Hsp104, however, with opposite effects. Overexpression of Ssa1 protects [*PSI*$^+$] from curing by Hsp104 [115], while Ssb has a negative effect on [*PSI*$^+$] propagation [58,77,116,117]. These opposing *in vivo* effects are mediated by the Ssa and Ssb substrate-binding domains [61]. Evidence suggests that Ssb either stimulates refolding of Sup35 into its native conformation or targets misfolded Sup35 intermediates for degradation [61]. Consistent with such a role, direct interaction between Ssb and Sup35 was reported [61,118]. Ssb, in concert with its co-chaperone RAC, inhibits the spontaneous formation of prion fibrilles *in vitro* [31]. The exact role of Ssb in [*PSI*$^+$] propagation still has to be clarified. The process is complex and *in vivo* studies are impeded because the well established detection methods for the [*PSI*$^+$] status of a cell involves determination of stop codon read through efficiency. As outlined above, Ssb affects read through also in a [*PSI*$^+$] independent manner [58,74,104].

## 10. Ssb in cellular protein turnover

Incorrectly folded proteins need to be eliminated since exposure of hydrophobic patches, which are usually buried inside native proteins and protein complexes, leads to aggregation in the aqueous cellular environment. The consequences for the cell are detrimental and thus cytosolic aggregates are normally disposed by the proteasome system [119]. The coordination between protein synthesis and protein degradation is only incompletely understood, however, chaperones are predestined to play an important role.

Early data suggested that *in vivo* a large fraction of newly synthesized polypeptides are degraded by the proteasome [120]. Because one of Ssb's characteristics is the interaction with nascent polypeptides as well as with the ribosome it would be ideally suited to act at this gateway between co-translational folding and degradation. However, protein synthesis is expensive from an energetic point of view and a futile cycle of synthesis and degradation seems sumptuary. Indeed, it was later shown in a well controlled set of experiments that the earlier results suggesting turnover of a large fraction of newly synthesized polypeptides were due to an experimental artifact [121]. However, specific biologically relevant situations can produce nascent polypeptides, which need to be delivered co-translationally for degradation. This is the case, for example, if an mRNA lacks an *in frame* stop codon and translation proceeds into the poly(A) sequence, or if read through of a stop codon occurs. As a consequence a stretch of consecutive lysines will be added to the C-terminus of the protein. The positively charged lysine stretches can result in transient arrest of translation and stalling of ribosomes [122]. Interestingly, it was found that consecutive lysine residues cause degradation of nascent polypeptides by the proteasome [123,124]. Based on that it was proposed that proteins C-terminally tagged with poly-lysine stretches cause translational repression of the encoding mRNA [123]. In the light of these observations the question arises if Ssb might be involved in these processes at the interface between translation and degradation. Direct evidence is not available, however, circumstantial evidence points into this direction. Overexpression of Ssb1, however not the overexpression of Ssa1, efficiently suppresses the temperature sensitive growth defect caused by a mutation in a proteasome subunit [125]. Moreover, the Δ*ssb1*Δ*ssb2* strain is sensitive towards the proline analog, azetidine-2-carboxylic acid (AZC) [26,35]. AZC causes reduced thermal stability or misfolding of the proteins into which it is incorporated competitively with proline [126]. Many yeast mutants sensitive to AZC are related to the ubiquitin proteasome system. Among them mutations in the E3 ubiquitin ligase *RSP5* [127], the Δ*ubc4*Δ*ubc5* E2 ubiquitin ligase double mutant [128], and a large class of cycloheximide resistant mutants originally termed the *crl* mutants [129], which were later found to localize within genes encoding subunits of the proteasome [130]. Δ*ssb1*Δ*ssb2* and the *crl* mutants display a budding arrest defect when they enter stationary phase [76,129]. Similar to the Δ*ssb1*Δ*ssb2* mutant, the *crl* mutants are sensitive towards aminoglycosides as well as high salt concentrations [129]. However, it should be noted that only the *crl* mutations but not Δ*ssb1*Δ*ssb2* causes temperature sensitivity [3,54,129]. In respect to AZC sensitivity another genetic interaction with the Δ*ssb1*Δ*ssb2* mutant was recently reported. *BUD27*/*URI1* encodes an unconventional member of the prefoldin family, which participates in the regulation of the nutrient-sensitive TOR-dependent transcriptional programs [131]. Unexpectedly, the AZC sensitivity of a Δ*ssb1*Δ*ssb2* strain was slightly reduced in a Δ*ssb1*Δ*ssb2*Δ*uri1* strain [26]. Because to date mechanistic details on a role of Ssb in degradation are missing, it is possible that the observed genetic interactions as well as drug effects are more indirectly linked to Ssb. For example, in the Δ*ssb1*Δ*ssb2* strain several amino acid permeases are severely deregulated on the transcriptional level [76] and the uptake of the non-natural amino acid analog AZC might be enhanced. In this context it would be interesting to compare the AZC incorporation into cellular proteins in Δ*ssb1*Δ*ssb2* and wild type strains. In general, surprisingly little is known about AZC incorporation into proteins *in vivo*. Studies on the effect of AZC on protein conformation and stability have been performed mainly with the collagen helix, which is a special case because of its structure and high proline content. Computational analysis revealed that even in collagen a high level of substitution of prolines with AZC is required to cause effects [132].

Finally, it should be mentioned that effects of Ssb on protein degradation have been directly tested in a number of studies and the outcome suggests that the chaperone is not a general component of the protein degradation machinery. Ssb is dispensable for the degradation of ER-import defective mutants of carboxypeptidase yscY, whereas Hsp90 and Ssa, and Ydj1p are required [133]. Ssb is dispensable for the retro-translocation and elimination of mutated versions of prepro-α-factor via ER mediated degradation (ERAD) [134]. While Ssa together with Sse1 and Hsp90 promotes the degradation of the von Hippel–Lindau (VHL) tumor-suppressor, the level of VHL tumor-suppressor is not affected by Ssb [135]. Apolipoprotein B (apoB) is targeted for degradation co-translationally when translocation into the ER comes to halt [136]. Ssa1 and Sse1, each influence the turnover of apoB in a distinct manner, however, Ssb1 does not [137,138].

## 11. Open questions and future prospects

*Δssb1Δssb2* strains suffer from a combination of different growth defects which allude to possible cellular roles of the chaperone. The situation is complicated as Ssb is expected to be involved in various cellular processes. Multiple malfunctions may contribute to what is finally assumed to be a specific phenotype. It thus does not come as a surprise that some growth defects of the *Δssb1Δssb2* strain can be explained by different models. To date, none of the defects observed in the *Δssb1Δssb2* strain can be stringently explained on a molecular level.

The most surprising conclusion from the literature survey is how little experimental evidence indicates a role of Ssb in *de novo* folding. Insufficient data available on an active role of Ssb in folding has more recently led to models which focus on a function of Ssb in transfer of nascent peptides from the ribosome to downstream folding chaperones [65,66]. A provoking hypothesis is that Ssb on the ribosome is not involved in protein folding at all (Fig. 2), but required for other, currently for the most part enigmatic processes. Positive proof of a model in which Ssb is a component of the cellular folding machinery will involve the identification of its *in vivo* substrates and the reconstitution of an *in vitro* system in which Ssb is required for the folding of a nascent model protein. Such evidence has firmly established, for example, that Ssa is a central player of the cytosolic protein folding machinery [30,139–141]. It will also be of eminent importance to further define the role of Ssb in maintaining cellular energy homeostasis. If Ssb is involved in regulating the interaction between components of the SNF1 signaling pathway, such a function may also regulate dynamic interactions within other functional protein networks.

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