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ORIGINAL PAPER

Alleviation of deleterious effects of protein mutation through inactivation of molecular chaperones

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Abstract Molecular chaperones recognize and bind destabilized proteins. This can be especially important for proteins whose stability is reduced by mutations. We focused our study on a major chaperone system, RAC-Ssb, which assists folding of newly synthesized polypeptides in the yeast cytosol. A sensitive phenotypic assay, the red color of Ade2 mutants, was used to screen for variants with metabolic activity dependent on RAC-Ssb. None of the Ade2 mutants were found to exhibit lower metabolic activity after inactivation of RAC-Ssb. In order to explicitly test the relationship between protein instability and activity of chaperones, a series of temperature sensitive Ade2 mutants were tested in the presence or absence of RAC-Ssb. The growth of Ade2(ts) mutants at elevated temperatures was enhanced if chaperones were missing. Similar pattern was found for thermally sensitive mutants of several other genes. Because RAC-Ssb normally supports the folding of proteins, it appears paradoxical that catabolic activity of mutants is reduced when these chaperones are present. We suggest that under non-stressful conditions, molecular chaperones are tuned to support folding of native proteins, but not that of mutated ones.

Keywords *Saccharomyces cerevisiae* · Molecular chaperone · Mutation · Phenotypic buffering · Genetic robustness

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Introduction

The phenotypic effects of mutations are often conditional. One of the possible explanations for this can be the structural instability of mutated proteins. Instability results in excessive misfolding and aggregation and thus in inadequate metabolic activity of proteins (Pakula and Sauer 1989). Physical and chemical conditions either exacerbate or alleviate protein misfolding and therefore can determine the extent of phenotypic damage caused by mutation (Chakshusmathi et al. 2004). Mutation and environmental stress tend to reinforce each other because they both lead to similar abnormalities in protein structure, that is, the exposure of normally hidden hydrophobic residues of amino acids. Atypical hydrophobic surfaces of proteins attract molecular chaperones (Rüdiger et al. 1997; Horwich et al. 1999). The chaperones block the hydrophobic patches protecting them from contacts within and between polypeptides, which would lead to misfolding and aggregation, respectively. Chaperones are potent agents of stabilization at the time of stress; this earned them their original name of heat-shock proteins, Hsps (Estruch 2000; Gasch et al. 2000). It was hypothesized that under normal conditions the protective role of chaperones is directed towards the excessively unstable mutated proteins. In this way molecular chaperones could potentially mask phenotypic expression of mutations destabilizing the proteins. If cryptic mutants that are normally hidden under the chaperone-mediated phenotypic cover-up were occasionally unmasked, a wealth of novel variants would be produced of which some may be fixed as adaptations (Rutherford and Lindquist 1998; Queitsch et al. 2002). The buffering role of chaperones may thus contribute evolutionary novelty. Besides long term evolutionary scenarios, it seems intuitive that any alleviation of harmful phenotypic effects would be of

immediate benefit to bearers of mutations. Indeed, some reports suggest that the activity of molecular chaperones may mitigate disease states, especially those linked to protein misfolding and aggregation (Warrick et al. 1999; Auluck et al. 2002).

Empirical evidence for chaperone-mediated masking of mutations has been sought in different experimental systems. In bacteria, there are examples of mutations that likely destabilize the proteins, but their phenotypic effects are apparently alleviated by chaperones. Nonetheless, chaperones typically need to be overexpressed to fulfill their buffering role (Van Dyk et al. 1989; Fares et al. 2002). Overexpression may interfere with other cellular processes and while being tolerable under laboratory conditions, and in some very special ecological settings, it does not normally occur in nature (Moran 1996; Maisnier-Patin et al. 2005). In eukaryotes, several experiments demonstrated that insufficient activity of molecular chaperones resulted in heritable diversification of phenotypes (Rutherford 2003; Sangster et al. 2004). The underlying molecular mechanisms have not yet been pinpointed (Sangster and Queitsch 2005). Research is impeded by abundant pleiotropic effects caused by malfunctioning of genes coding for molecular chaperones (Sollars et al. 2003). These agents are engaged in many cellular processes and therefore alteration of their activity typically leads to multidirectional phenotypic changes obscuring those potentially associated with the destabilization of mutated proteins (Milton et al. 2003). In a former study, we found mutations that were synthetically harmful with the deletion of genes coding for RAC-Ssb. However, neither the mutated genes nor the nature of the genetic lesions implied that chaperones could alleviate the effects of mutation through supporting the stability of defective polypeptide chains. We concluded that a general effect of molecular chaperones was to stabilize the functioning of networks of (non-mutated) proteins, making them more resistant to malfunction or absence of particular elements (Bobula et al. 2006). Although this result indicated that chaperones can stabilize genetic networks (Soti et al. 2005), it left unsettled whether chaperones can stabilize individual proteins. It has been shown that the latter is possible. In bacteria, a protein from the coat of phage P22 normally folds efficiently without visible assistance of the bacterial chaperone GroEL. Maturation of mutated polypeptides requires the chaperone because it prevents excessive interaction between slowly folding chains and thus averts their aggregation (Nakonechny and Teschke 1998). This interesting example suggests that well focused and sensitive screens are needed to discover chaperone-mediated masking of mutations destabilizing proteins. The goal of the present study was to perform an exhaustive screen for potential interactions between a cytosolic protein and a major chaperone system in the yeast cell.

The most abundant cytosolic chaperones in yeast are Ssa1–4 and Ssb1–2, all of the Hsp70 class. Ssas are essential because they are engaged in numerous processes both under normal and stressful conditions (James et al. 1997). Ssbs are nonessential and unresponsive to stress (Nelson et al. 1992). Their activity is strongly correlated with protein biosynthesis. The majority, if not all, of the polypeptide chains interact with Ssb1/2p at the time of translation and immediately afterwards (Albanèse et al. 2006). The co-translational activity of Ssb1–2 depends on the ribosome associated complex (RAC) of Zuo1 (Hsp40) and Ssz1 (modified Hsp70) (Conz et al. 2007; Sahi and Craig 2007). Deletion of either the Ssbs or any of the RAC partners leaves the cell viable, although slow growing (Gautschi et al. 2001; Gautschi et al. 2002). The main function of the RAC-Ssb chaperone system is probably to assist the folding of newly translated chains (Hartl and Hayer-Hartl 2002). The universal role of the RAC-Ssb system and the fact that it can be inactivated without causing cell death makes it a good candidate for the study of chaperone-mediated masking of mutations. The yeast gene *ADE2* is an especially attractive target of mutation. Both complete and partial malfunctioning of its product, Ade2, is readily detectable because inactivation of this enzyme leads to the accumulation of a red pigment in vacuoles. Partial (leaky) mutants grow at a reduced rate on media lacking adenine. This phenotype results from both insufficient level of adenine within a cell and a moderately toxic effect of the accumulating pigment (Ugolini and Bruschi 1996).

In the present experiment, we used the color assay to find a correlation between the metabolic activity of Ade2 mutants and the activity of the RAC-Ssb system. Despite repeated screens, we were unable to find mutants whose color indicated higher metabolic activity in the presence of chaperones, implying their assistance. In an alternative approach, we first collected heat sensitive (structurally unstable) mutants of Ade2 and then tested their functioning in cells that either possessed or lacked the RAC-Ssb system. Contrary to the hypothesis of the stabilizing effect of chaperones, we found that the damage to adenine metabolism was more readily seen when Ssb-RAC was active. We showed that mutated molecules of Ade2 were in physical contact with the chaperones but this interaction apparently did not result in enhancement of the protein's metabolic activity. Our results suggest that under normal metabolism the activity of co-translational chaperones may be optimized to assist folding of native proteins instead of supporting chains of reduced stability (Tomala and Korona 2008).

Materials and methods

Strains and plasmids

BY4742 MATα his3Δ leu2Δ lys2Δ ura3Δ met15Δ was a precursor of all yeast strains used in this study. Additional gene deletions were performed by replacing the genes with the *MX4* cassette. The cassette contained *kan*, *nat* or *hph* genes providing resistance to 200 mg/l geneticin, 100 mg/l nourseothricin or 200 mg/l hygromycin B, respectively. One of the deleted genes, *ade2*::*natMX4*, was subsequently replaced by a series of thermosensitive mutants, *ade2(ts)*. Restoration of the adenine prototrophy at 23°C was used as an initial sign of successful replacement. Subsequently, the DNA sequence of inserted alleles was determined to ensure that mutations other than the known substitutions leading to thermosensitivity were not accidentally introduced.

In some of our strains, epitope tags were used to obtain *ADE2TAP* or *ade2(ts)TAP*. A template for amplification of the tag was obtained from the strain S288C *ADE2TAP* (Open Biosystems). Selection for successful tagging was done on synthetic medium lacking histidine. All tag insertions were confirmed by positive results of PCR amplifications that spanned over the expected sites of recombination, both upstream and downstream of the involved genes.

A centromeric plasmid, pRS416 *URA3*, with *ZUO1* controlled by its own promoter, was described in a former study (Bobula et al. 2006). This plasmid, designated here as p*ZUO1 URA3*, was amenable for both positive or negative selection by use of synthetic medium lacking uracil or synthetic medium supplied with 1 g/l of 5-fluoroorotic acid (5-FOA). Another centromeric plasmid, pRS412 *ADE2*, was cut at *Xho*I i *Sac*I restriction sites. A fragment containing *LEU2* together with its promotor and terminator sequences was inserted to obtain a plasmid with two trophic markers, p*ADE2 LEU2*.

Plasmid pCu*SSB2* was obtained from J. Frydman. It contains the *SSB2* gene tagged with HA at the N-terminus. Expression of this construct is controlled by a promoter sensitive to copper ions. Plasmid p*ADE2*-mORF (Open Biosystems) was used to overexpress Ade2 tagged with HA; the gene is controlled by the galactose sensitive *GAL1* promoter.

Strains with thermally sensitive alleles in genes other than *ADE2* were obtained from the laboratories of T. R. Hughes and C. Boone. The strains were strictly isogenic to our *BY4742* except that they contained thermally sensitive alleles of 17 different genes obtained and tested in a number of former studies (Davierwala et al. 2005). We added one more modification by replacing *ZUO1* for the *hphMX4* cassette.

Mutagenesis and phenotypic assays

Ten micrograms of p*ADE2 LEU2* was suspended in 0.5 ml solution of 1 M hydroxylamine, 15 mM $Na_4P_2O_7$, 100 mM NaCl and 2 mM EDTA (pH 6.0). After 2 h of incubation at 65°C, samples were chilled on ice. The plasmid was then purified on Sephadex G25 coarse columns and used to transform *E. coli*. Transformation reactions resulted in no fewer than 10,000 small bacterial colonies per plate. All colonies obtained after one transformation were washed out of the agar surface. Plasmids were isolated from the cell suspension and used to transform yeast strains.

Synthetic complete agar media without leucine were used in screens for red-colored colonies of *ade2* mutants. To boost the red color of mutant colonies, adenine was added at 20% of its standard concentration. Before assessing the intensity of red pigmentation, plates were chilled for 24 h at 4°C.

Biochemical procedures

Cells in exponential growth phase (5 ml) were harvested, washed and suspended in 0.6 ml of a lysing buffer (50 mM Tris–Cl pH 7.5, 1 mM EDTA, 0.5% SDS, 1 mM DTT, protease inhibitors: 1 mM PMSF and 1 μg/ml pepstatin). Cells were destroyed with glass beads (diameter 0.5 mm) by vortexing for 30 min at 4°C. Lysates were boiled in a reductive buffer (62.5 mM Tris–HCl pH 6.8, 25% glycerol, 2% SDS, 0.25% (v/v) β-mercaptoethanol, 0.1% bromophenol blue). The resulting samples were used in Western blot analyses. Samples of proteins prepared for centrifugation in 25% sucrose cushions were lysed in another buffer (50 mM Tris pH 7.5, 10 mM HEPES, 100 mM KCl, 5 mM $MgCl_2$, 10% glycerol, 0.1% Triton X 100) and then subjected to bead-beating. Ade2 protein was visualized by Western blot using anti-TAP rabbit polyclonal antibody (Open Biosystems).

For cross-linking studies, cells were suspended in a buffer composed of 50 mM HEPES and 0.15 mM NaCl (pH 7.4). DSP was then added to a final concentration of 0.2 mM and bead-beating was applied. Ade2 was precipitated by an overnight incubation on ice with anti-TAP. Gel-immobilized A and G proteins (Pierce) were then added and incubated for 3 h at 4°C. The gel was washed three times and suspended in the PBS buffer with 0.3% Tween 20. The reductive buffer was added and the samples were boiled to dissociate protein–protein and protein–antibody complexes. After electrophoresis and Western blot, anti-HA rabbit polyclonal antibody (Open Biosystems) was used to detect HA-tagged Ssb2.

Results

Screen for mutants dependent on RAC-Ssb

A haploid strain of yeast, *zuo1Δ ura3Δ ade2Δ leu2Δ*, was used. It contained a counter-selectable plasmid, p*ZUO1*

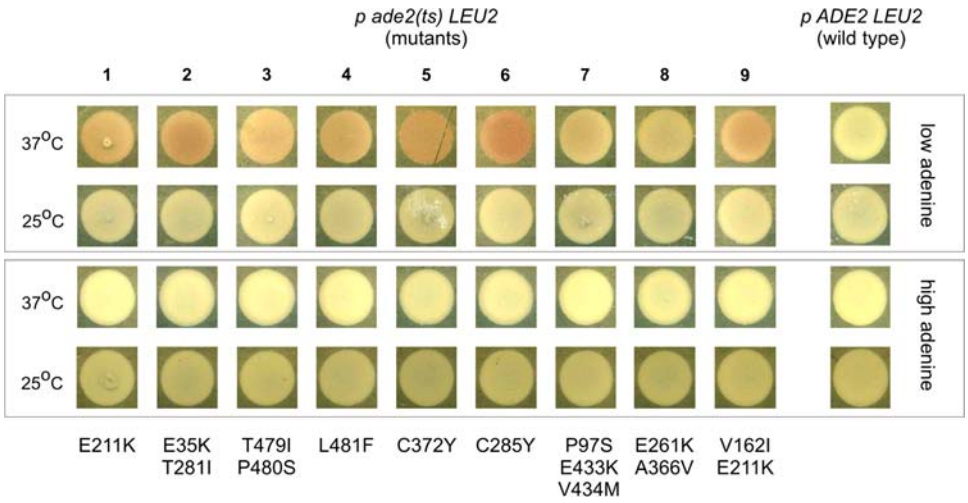

Fig. 1 Ade2(ts) mutants. Nine mutants (*left*) are compared with a wild type allele (*right*). Amino acid substitutions present in every mutant are listed in the *bottom panel*

URA3, which enabled activation or inactivation of RAC-Ssb because the presence or absence of the plasmid-coded Zuo1 determined the activity the whole system. Another plasmid, *p ADE2 LEU2,* was mutagenized in vitro and then introduced into the host strain. In this way, we obtained tens of thousands of yeast colonies with two plasmids, of which one carried random base substitutions. The colonies were replica-plated onto medium lacking adenine but supplemented with 5-FOA (to select for maintenance of *p ADE2 LEU2* and loss of *p ZUO1 URA3*). Our goal was to find colonies that were normal (white) when the plasmid was present but turned red or pink, or even stopped to grow, after losing it. Of 30,000 colonies screened, we found about 200 that showed signs of the phenotypic switch. For all presumptive mutants, original colonies (with two plasmids) were streaked to single cells on the medium with 5-FOA added and adenine dropped out. Only in 29 cases were the new colonies again red or reddish. However, their sizes and colors varied. This was seen in repeated trials of streaking and re-streaking, suggesting the heterogeneity of mutant colonies. A possible explanation for this effect was that the efficiency of adenine metabolism was violated not because of mutation in *ADE2*, but because of the decreased stability of *p ADE2 LEU2*. To test this, we streaked original colonies on medium containing both 5-FOA and adenine but lacking leucine. All 29 strains formed colonies clearly smaller and more variable than the non-mutagenized control strain. Thus, the phenotype of poor growth was the same irrespective whether *ADE2* or *LEU2* were tested implying that in all 29 cases stability of the plasmid was reduced. Returning to the main hypothesis, we were unable to find mutations whose negative effect on the activity of Ade2 was aggravated after inactivation of RAC-Ssb. Thus, our data do not support the idea that chaperones can mask phenotypic expression of genetic damage. An evidence that our experiment was sufficiently sensitive and saturated comes from the next screen in which the same pool of mutagenized plasmids was used. Thermosensitive mutants of Ade2, indicating destabilization of protein's spatial structure, were found without difficulty.

Screen for thermosensitive *ade2* mutants

Procedures of mutagenesis and transformation were the same as in the former experiment. The yeast strain was not deleted for any chaperone-coding gene and did not contain *p ZUO1 URA3*. To screen for mutants, master plates were replica-plated onto agar plates lacking both adenine and leucine, and then incubated at 37°C. Of the 40,000 colonies tested, 86 were red or pink at a high temperature. Of these, 16 showed the same phenotype in repeated assays. Analysis of DNA sequence showed that all 16 mutants contained base substitutions in *ADE2*. Unique substitutions were found in nine mutants. The mutations are listed and their phenotypic effects are presented in Fig. 1. The substitutions caused change of charge (resulting in alteration of ionic bonds), exchange of one hydrophobic amino acid for another (less tight filling of protein core), and exchange of proline for a non-cyclic amino acid (altered conformation of loops). These modifications are typically associated with moderate destabilization of protein structure. They lead to thermal sensitivity and increased rates of protein aggregation (Varadarajan et al. 1996; Chiti et al. 2003).

Genetic interaction between *ade2(ts)* and RAC-Ssb genes

Plasmids containing *ade2(ts)* mutants were used to transform *ade2Δ* strains in which either all the genes coding for RAC-Ssb were present or some were deleted, namely, *zuo1Δ*, *ssz1Δ*, or *ssb1Δ ssb2Δ*. To compare the phenotypic effects of *ade2(ts)* mutants on these four genetic backgrounds, stationary phase cultures of relevant strains were serially diluted and deposited as dots on media lacking adenine. Of the nine tested mutants, none grew better than

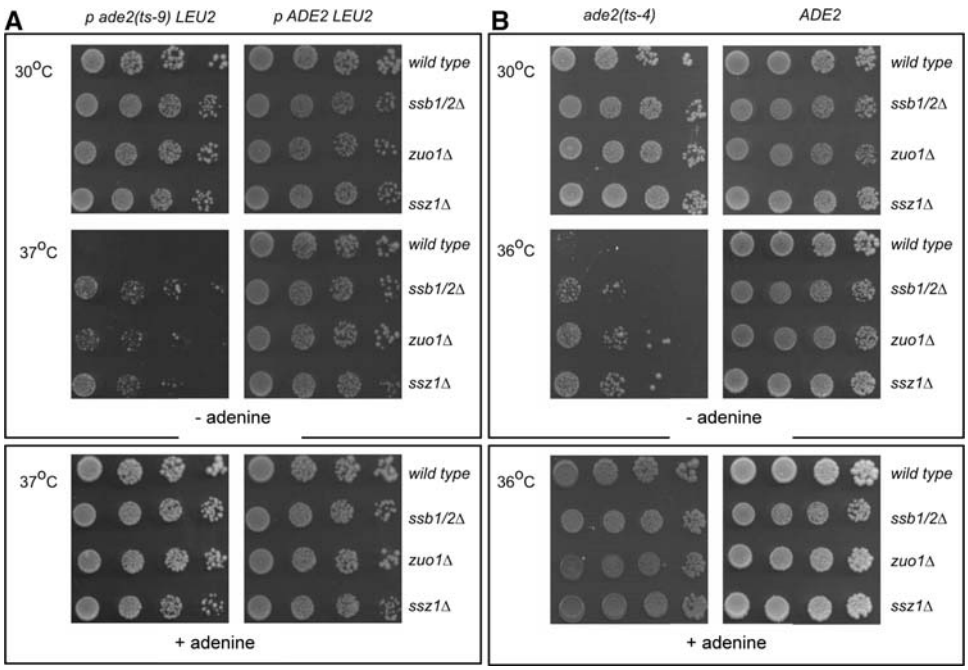

Fig. 2 Growth of Ade2(ts) mutants. Examples include mutant 9 residing on a plasmid (**a**) and mutant 4 residing in its chromosomal locus (**b**). Growth of a strain with complete RAC-Ssb system (wild type) is compared with growth of three strains in which one of the system's components was lacking. Note that strains with an active RAC-Ssb system grew poorly at non-permissive temperature when medium was deprived of adenine

when the RAC-Ssb system was proficient. On the contrary, strains with deletions of *ZUO1, SSZ1,* or *SSB1* and *SSB2* grew as well or better than the wild type strain when adenine was present in the medium. Note that deletion of any of the chaperone-coding genes results in a considerable growth effect on plain media (Fig. 2a). A possible caveat was that the number of copies of the plasmid with *ade2(ts)* could vary among the compared strains. To circumvent this potential source of error, we moved these alleles to their chromosomal locus preceded by an intact promoter. Generally, growth on media lacking adenine was halted at lower temperatures, indicating that sensitivity to temperature increased. The simplest explanation is that the centromeric plasmid *p ADE2 LEU2* was often present in more than one copy per cell. In contrast, only one copy of *ADE2* was present after insertion into a chromosome. Most importantly for this experiment, *ade2(ts)* mutants were again more sensitive to high temperature when RAC-Ssb was active. The phenotypes of *zuo1Δ*, *ssz1Δ*, and *ssb1Δssb2Δ*were similar, implying the functional interdependence of Ssbs, Ssz1, and Zuo1 (Fig. 2b).

Molecular interaction between Ade2(ts) and co-translational chaperones

Using a cross-linking technique, we showed that Ssb2 co-precipitated with Ade2, indicating direct physical contact. Ssb2 did not bind Ade2(ts) more intensely than Ade2, which would suggest a mechanistic basis of reduced enzymatic activity of the mutant (Fig. 3). In general, there are two explanations for the improved functioning of *ade2(ts)* mutants in strains lacking any component of RAC-Ssb.

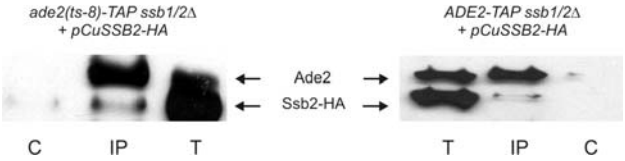

Fig. 3 Physical interaction of Ade2 and Ssb2. Cross-linking studies suggest that both Ade2ts (*left*) and wild-type Ade2 (*right*) are bound by tagged Ssb2. *C* Control, *IP* immunoprecipitation, *T* total protein

Either the total amount of Ade2(ts) was higher, or enzymatically active molecules of this protein were relatively more frequent. To test this, TAP (tandem affinity protein) tags were inserted behind the wild type and mutated *ADE2* alleles. The thermal sensitivity of all tagged mutants increased. It continued to be higher in strains with active RAC-Ssb. This was best seen in case of a mutant whose thermosensitive phenotype was assessed as relatively mild in an original screen (Fig. 4a).

Western blot analysis showed that the overall cellular amount of Ade2(ts) were roughly constant irrespective of whether the chaperones were active or inactive (Fig. 4b). It decreased after 11 h of incubation at a non-permissive temperature; the decline was similar in all tested strains. We then asked whether the mutated chains formed aggregates of metabolically inactive polypeptides and whether the intensity of aggregation differed between the tested strains. Samples of protein extracts were subject to centrifugation through a sucrose cushion followed by Western blot analysis. Aggregation of Ade2(ts) was possibly somewhat more pronounced in strains with active chaperones but the difference was small (Fig. 4c). Thus, neither rapid removal nor

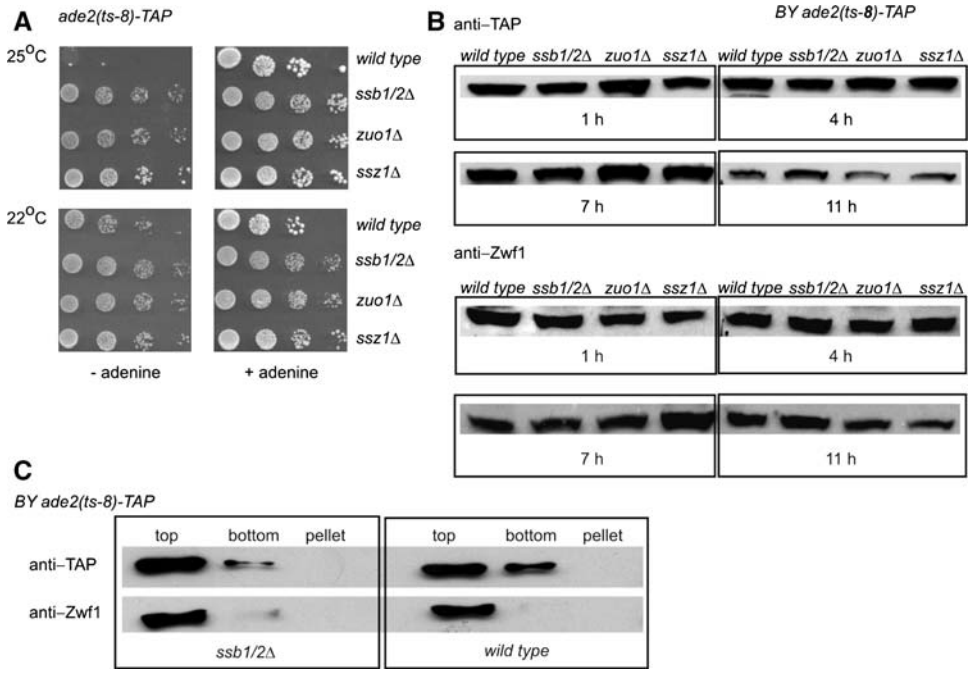

Fig. 4 The cellular level of Ade2 protein. **a** Tagging with TAP did not change the pattern of poorer growth of a strain with proficient RAC-Ssb chaperones (wild type). **b** Western blot analysis showed that the cellular level of Ade2(ts) protein was not visibly different in strains with active and inactive RAC-Ssb. Zwf1 serves as a control. **c** Centrifugation through a sucrose cushion followed by Western blot showed that most of the Ade2 protein was not aggregated (*top of tube*). Large aggregates were not formed in strains either with or without chaperones (absence of Ade2 protein in pellets). Moderate aggregates were tentatively more abundant in the strain with active RAC-Ssb (*bottom of tube*)

intense aggregation was a plausible explanation of the low activity of Ade2(ts) in strains with complete RAC-Ssb.

Genetic interaction between RAC-Ssb and mutations in other genes

We tested 17 thermally sensitive alleles located in loci other than *ADE2*. We used a collection of ts mutants prepared for another study (Davierwala et al. 2005). We found that eight of them grew better at elevated temperatures after inactivation of RAC-Ssb (Fig. 5). In some cases, phenotypic differences between strains with and without active chaperones were stronger than that detected for the *ade2(ts)* mutants.

Discussion

In the crowded interior of the cell, the assistance of chaperones is important for the folding of many proteins and may be critical for those with reduced stability (Zimmerman and Trach 1991; Ellis 2001). Misfolding and aggregation is especially likely when synthesis of proteins is still under way or has just been completed. Newly synthesized polypeptides are locally dense and only partly folded and therefore susceptible to intra- and intermolecular aggregation, making the assistance of chaperones essential (Fedorov and Baldwin 1997; Thulasiraman et al. 1999; Wegrzyn and Deuerling 2005). We explored an experimental system in which the effects of inactivation of co-translationally acting chaperones on cellular activity of mutated proteins could be relatively easily detected. Our test protein was Ade2, the malfunctioning of which was manifested by the accumulation of a red pigment. We were unable to show that the RAC-Ssb chaperone system can support the proper folding of mutated Ade2. We then used preselected mutants of Ade2 whose thermal sensitivity marked structural instability. These mutants appeared in fact less active metabolically in cells in which the chaperone system was working. Better growth of thermally sensitive mutants in absence of active RAC-Ssb was also observed for 8 of 17 other genes. This suggests that negative interaction between thermal stability of mutated proteins and activity of co-translational chaperones is a widespread phenomenon.

The question of how RAC-Ssb interferes with the folding of the intrinsically unstable polypeptides remains unanswered. The cellular function of chaperones attached to a ribosome must in some way relate to synthesis and maturation of proteins, however, for a long time the exact metabolic role of RAC-Ssb was unknown (Craig et al. 1993). It was then found that other eukaryotes also have ribosome-associated chaperones which activate cytosolic Hsp70 in order to bind newly synthesized polypeptides (Otto et al. 2005; Rauch et al. 2005; Conz et al. 2007). It is increasingly evident that their main function is not to aid translation (Rakwalska and Rospert 2004; Muldoon-Jacobs and Dinman 2006), but to assist the initial folding of polypeptides (Craig et al. 2003; Albanèse et al. 2006). What happens if the folding of a polypeptide is ineffective? It is still insufficiently understood how new polypeptides are held by Ssb1/2 and then released or passed on to other chaperones or enzymes (Young et al. 2004; Wegrzyn and Deuerling

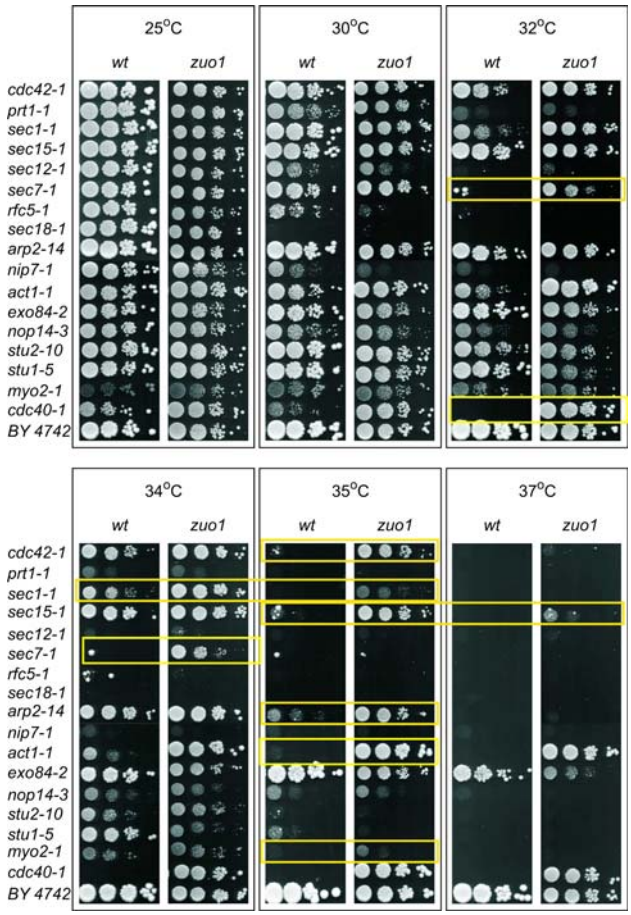

Fig. 5 Thermally sensitive mutants in presence (*wt*) and absence (*zuo1*) of active co-translational chaperones. *Frames* indicate temperatures at which better growth of strains with inactivated chaperones is best visible

2005). It is therefore difficult to hypothesize how the inactivation of RAC-Ssb affects cellular systems of protein quality control. Aberrant polypeptides can be passed on from a Hsp70 chaperone to proteases and then degraded, as shown by several examples involving different proteins destabilized by mutation in eukaryotic chaperone systems (Esser et al. 2004; McClellan et al. 2005a; McClellan et al. 2005b; Bukau et al. 2006; Wang et al. 2006; Brodsky 2007). Our results do not provide evidence that the function of RAC-Ssb is to detect aberrant polypeptides and direct them to degradation. We did show that this protein interacts physically with Ssbs, but this was also demonstrated for a substantial fraction of non-mutated cytosolic protein (Hartl and Hayer-Hartl 2002). There is no evidence showing that Ssbs interact differently with stable and unstable proteins. For example, we were unable to demonstrate that RAC-Ssb was responsible for rapid degradation of Ade2(ts). However, currently accessible assays are insufficiently precise to detect fine changes in relative abundance of free molecules, molecules bound by chaperones, and aggregates of different sizes. Even moderate alteration of a proportion of metabolically active Ade2(ts) molecules could be responsible for detectable growth effects. Therefore the hypothesis that the co-translational chaperones act as initial guards against cyto-toxicity of misfolded proteins cannot be rejected (Craig et al. 2003). Indeed, slow-growth of strains lacking Ssb1/2 has been recently linked to accumulation of misfolded chains (Albanèse et al. 2006).

An alternative explanation of our results is based on the well known observation that expression of molecular chaperones is highly dependent on stress. When environment is benign and genes are not mutated, then a bulk of newly synthesized chains fold at their standard pace. Notoriously aberrant structures are formed chiefly by chains containing translational errors (Ogle and Ramakrishnan 2005). They have to be "sanitized" to prevent their potential cytotoxic effects. We hypothesize that since mutated proteins resemble defective products of translation, they likely share their fate. The situation is much different if RAC-Ssb is inactive because this leads to increased polyubiquitination of ribosome-bound polypeptides and upregulation of those chaperones that normally react to heat and other stresses (Albanèse et al. 2006). These are signs of massive misfolding of non-mutated proteins. We suggest that under these conditions the mutated proteins fall into a broad class of defective molecules that are allowed to re-gain their functional conformations, often with the help of chaperones. This would explain the seemingly paradoxical observation that Ade2(ts) mutants were more active as catalysts in strains with inactivated RAC-Ssb.

In our experiments, co-translational chaperones did not mask the phenotypic expression of mutation. On the contrary, the negative effects of mutation were magnified in the presence of chaperones. This implies the strengthening of selection against mutations, an effect that is in opposition to the postulated phenotypic buffering of genetic variation (Barton and Partridge 2000). This is an interesting evolutionary role for molecular chaperones that remains largely unexplored. Examples of chaperone mediated phenotypic masking or unmasking are at present too scarce to decide which role might dominate (Tomala and Korona 2008).

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