Minireview

Distinct yet linked: chaperone networks in the eukaryotic cytosol

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Abstract

The terms chaperone and heat-shock protein are frequently used as synonyms, but this is an oversimplification. Although one subset of chaperones is induced by heat stress, a distinct group fails to respond in the same manner. Recent work reveals that this latter group is linked to the translational apparatus and functions in co-translational processes.

A decade ago it was recognized that chaperone systems in bacteria form a lateral network of cooperating proteins [1]. The idea of chaperones acting in parallel, with the capacity to replace each other, turned out to be a best-seller and is now generally accepted. Recent work by Albanese *et al*. [2] in the yeast *Saccharomyces cerevisiae* now modifies this concept and suggests the existence of distinct and independent chaperone networks in eukaryotes. One network consists of heat-inducible chaperones that can rescue or dispose of proteins in response to various environmental stresses. The other is thought to be required specifically during *de novo* protein folding.

A chaperone is not always a heat-shock protein as well

Some years ago Brown and co-workers [3] analyzed the transcriptional profiles of yeast in response to environmental changes, including a variety of stress conditions. Now, Albanese *et al*. [2] have performed clustering analysis of these datasets for chaperone-encoding genes, discovering that transcription of a defined group is co-regulated with the 138 yeast ribosomal protein genes. The authors termed this subgroup 'chaperones linked to protein synthesis' (CLIPS).

Ribosome biogenesis is strictly controlled, and ribosomal protein genes form one of the most prominent clusters in studies of the yeast transcriptome. One important characteristic of the ribosomal gene cluster is that heat stress leads to its downregulation [3]. This means that CLIPS mRNA levels are changing in the opposite direction of the 'classic' heat shock factor-dependent chaperones [2]. Prominent examples of chaperones co-regulated with the ribosomal protein genes are TRiC (chaperonin-containing T-complex), prefoldin, NAC (nascent-polypeptide associated complex), RAC (ribosome-associated complex), and the Hsp70 homolog Ssb. Consistent with the lack of induction of CLIPS by heat stress, yeast strains lacking nonessential CLIPS are not specifically sensitive to elevated temperatures [4-7], although exceptions have been reported [8,9].

CLIPS interact with polysomes and cope with specific stress conditions

In their comprehensive survey, Albanese *et al*. [2] used sucrose gradient fractionation to investigate which cytosolic chaperones have the propensity to interact with polysomes. When these results are combined with previous analyses it becomes clear that the extent of ribosome association is characteristic for each chaperone [4-6,10-13]. For example, among the yeast homologs of Hsp70 classified as CLIPS [2], only a minor fraction of Ssa [2,11] and Sse1 [2], about half of Ssb [4], and virtually all of Ssz1 [6] is ribosome-associated. These differences suggest that some CLIPS are confined to co-translational processes, whereas others serve multiple functions in the cell.

Stimulated by the transcriptome data, the polysome association, and the lack of temperature sensitivity, Albanese *et al*. [2]

tested the idea that CLIPS specifically mediate *de novo* protein folding. The question was tackled using the imino acid analog azetidine-2-carboxylic acid (AZC), which is incorporated into proteins competitively with proline and affects *de novo* folding. Indeed, yeast strains lacking CLIPS such as Ssb were hypersensitive to AZC. On the basis of these findings the authors propose a model in which CLIPS chaperone polypeptides during their synthesis but fail to handle misfolding of preexisting proteins induced by heat stress. Consistent with this model, Albanese *et al.* [2] find that toxic misfolded protein species cause growth defects in yeast strains lacking Ssb. To that end they used the so-called GroEL trap, which is an elegant molecular device that captures unfolded polypeptides but is unable to mediate folding [14]. When GroEL trap was expressed in a yeast strain lacking Ssb, growth defects were attenuated, suggesting that simple capturing of misfolded polypeptides can suppress growth defects in the absence of Ssb.

In this context it is worth noting that AZC also affects the stability of proteins [15]. The drug is known to selectively repress expression of ribosomal protein genes while heat shock factor-regulated genes are strongly induced [15]. Furthermore, it has been reported that defects in the disposal of misfolded proteins result in hypersensitivity to this drug [16]. More than a decade ago, Ssb was discovered as a multicopy suppressor of a yeast strain carrying a temperature-sensitive mutation in an essential proteasome subunit [17]. One possible scenario would thus be that Ssb and other CLIPS are involved in the degradation of proteins that fail to fold correctly. Earlier observations by Frydman and coworkers [18] had indicated, however, that the degradation of the VHL tumor suppressor was independent of Ssb. From the new data one may now speculate that high cellular concentrations of Ssb reduce *de novo* misfolding, alleviating the pressure on the malfunctioning proteasome.

Functional overlap of distinct chaperone networks

On the basis of its interaction with polysomes, Albanese *et al.* [2] classify Ssa, the housekeeping Hsp70 in the yeast cytosol, as a CLIPS. In contrast to most CLIPS, however, *SSA* is regulated in a heat shock factor-dependent manner and is also involved in the rescue of proteins denatured after an upshift in temperature [2,19]. In folding, Ssa is thought to act predominantly posttranslationally, and may ensure that nascent polypeptides that have initiated folding on the ribosome complete the process after their release [20]. Ssa's regulation and function thus overlaps with the CLIPS as well as with the heat shock factor-regulated chaperone network.

Is it possible to assign clear-cut functions to Ssb and Ssa, the major cytosolic Hsp70s in yeast? To date, only limited information is available. Ssa-dependent folding of a few proteins has been demonstrated *in vivo*. These Ssa substrates did not require Ssb for folding [20,21]. Instead, Ssb was found to cooperate with the TRiC machinery, which is engaged in the folding of a specific set of substrates [13]. Interestingly, Albanese *et al.* [2] find that Ssb is the most efficient binder of nascent polypeptides among the chaperones compared in this study. Whether this interaction is functionally confined to the delivery of folding-competent polypeptides to TRiC awaits further investigation.

Chaperone networks in yeast and higher eukaryotes

Most components of the yeast chaperone networks are present also in higher eukaryotes, suggesting that the mechanisms of protein biogenesis are conserved in eukaryotes. Some of the ribosome-associated chaperones have been discovered only recently. The Hsp40 homolog MPP11 [22,23] and the Hsp70 homolog Hsp70L1 [23] form a heterodimer functionally equivalent to yeast RAC [6,23]. In yeast, both subunits of RAC are tightly connected to Ssb and the three chaperones form a functional triad [12,24]. Ssb, the central player of the yeast CLIPS system [2], does not, however, seem to have an obvious counterpart in mammalian cells. This has led to the suggestion that mammalian Hsc70, a close homolog of yeast Ssa, serves a dual function and mediates processes that in yeast are divided between Ssa and Ssb [22]. In agreement with this, Hsc70 cooperates with TRiC, a function that in yeast is performed by Ssb [13,25]. Thus, compared with Ssa, Hsc70 even more intimately connects with cytosolic and ribosome-associated chaperone networks. The question of how interconnections are established and what distinguishes yeast and mammalian chaperone networks will certainly continue to be a central topic for researchers in the field.

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