Chaperoning ribosome assembly

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Chaperones help proteins fold in all cellular compartments, and many associate directly with ribosomes, capturing nascent chains to assist their folding and prevent aggregation. In this issue, new data from Koplin et al. (2010. *J. Cell Biol.* doi: 10.1083/jcb.200910074) and Albanèse et al. (2010. *J. Cell Biol.* doi: 10.1083/jcb.201001054) suggest that in addition to promoting protein folding, the chaperones ribosome-associated complex (RAC), nascent chain–associated complex (NAC), and Jjj1 also help in the assembly of ribosomes.

Many proteins require molecular chaperones to fold into their native conformations (Hartl and Hayer-Hartl, 2009). A subset of conserved chaperones are associated with ribosomes, where they are thought to bind nascent peptides to prevent their aggregation during completion of protein synthesis and aid in folding once the protein is released from ribosomes. In fact, a recent proteomic study identified >3,400 nonchaperone-interacting partners for the eukaryotic chaperone RAC in yeast (Gong et al., 2009), demonstrating the broad importance of chaperones for cellular protein folding.

Although many proteins need to avoid nonnative conformations to fold into complex tertiary structures, thereby requiring molecular chaperones, their folding is relatively simple compared with that of ribosomes. Ribosome assembly involves the coordinated transcription, maturation, and folding of ribosomal RNA (rRNA) as well as the binding of ribosomal proteins (r-proteins). In eukaryotes, this process is facilitated by >200 RNAs and proteins (Strunk and Karbstein, 2009). Some of these assembly factors help rRNA fold correctly. Such factors include DEAD box RNA helicases and also RNAs, whose base pairing with pre-rRNA prevents the premature formation of rRNA contacts (Strunk and Karbstein, 2009). Previous studies have also shown that the J-protein Jjj1 is required for 60S subunit assembly, where it has been implicated in removal of the export adaptor Arx1 from pre-60S subunits (Demoinet et al., 2007; Meyer et al., 2007, 2010).

In this issue, Koplin et al. and Albanèse et al. report that the chaperones RAC, NAC, and the J-protein Jjj1 cooperate to facilitate ribosome assembly in *Saccharomyces cerevisiae* (Albanèse et al., 2010; Koplin et al., 2010). Although NAC deletions alone have no discernible phenotype, NAC/RAC double deletions accumulate aggregates consisting mostly of r-proteins but also pre-rRNAs and some nuclear ribosome assembly factors. Strikingly, 52 out of 78 r-proteins were identified in the aggregates, and many of the missing r-proteins were the smallest ones, which can be hard to identify by mass spectrometry. Furthermore, both RAC/NAC and RAC/Jjj1 double-deletion strains show decreased levels of 40S and 60S subunits. Additionally, using a GFP-labeled r-protein from the large subunit, RpL25-GFP, both groups demonstrate defects in export of nascent 60S subunits from the nucleus into the cytoplasm. Furthermore, Albanèse et al. (2010) use microarrays to show deficiencies in pre-18S and pre-25S rRNA processing in strains with RAC or Jjj1 deletions. Interestingly, although deletions of different RAC subunits (Zuo1 and Ssb) result in very similar processing defects, these differ from those observed upon Jjj1 deletion. This finding is consistent with only a partially redundant function of these chaperones. Finally, coimmunoprecipitation experiments show that Jjj1 and Zuo1 associate with several nuclear and cytoplasmic 60S subunit assembly intermediates.

Although these data strongly support a role for protein chaperones in ribosome assembly, their exact function is much less clear. A tempting model is that the chaperones accompany newly made r-proteins into the nucleus and release them directly onto rRNA (Fig. 1 A). R-proteins are highly charged, and many are not strictly globular but have unstructured extensions that protrude into the ribosome's RNA core. As a result, many r-proteins are insoluble when overexpressed in bacteria (Culver and Noller, 2000), which can be overcome by the recombinant addition of fusion tags (Lamanna and Karbstein, 2009). Ubiquitin is a naturally occurring fusion tag (Baker et al., 1994), and in yeast, the r-proteins RpS31, RpL40A, and RpL40B are expressed as ubiquitin fusion proteins (Finley et al., 1989). For RpS31, this tag is required for protein stability and normal growth (Lacombe et al., 2009). Interestingly, both RpS31 and RpL40A or B were not found in aggregates upon NAC and RAC deletion (Koplin et al., 2010), possibly indicating that the ubiquitin fusion stabilizes these proteins such that they bypass the need for chaperone assistance. Perhaps the ubiquitin fusion evolved for these exceptionally small, charged proteins, as they have insufficient hydrophobic surface for efficient chaperone binding. Supporting the model that chaperones promote r-protein folding, recent work has shown that the bacterial ribosome-associated chaperone trigger factor (TF) also binds

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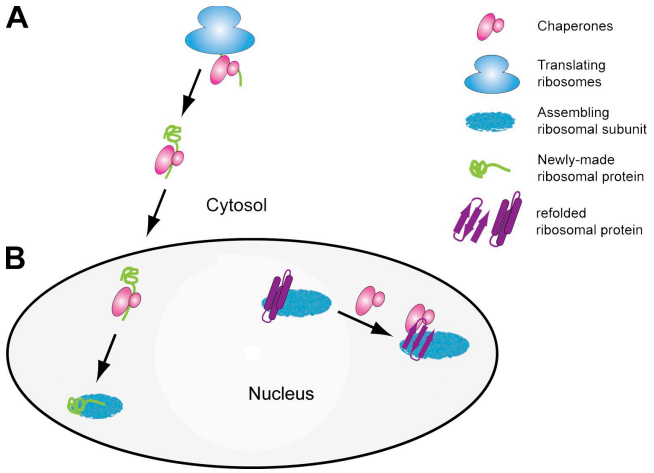

Figure 1. **Models for the function of chaperones in ribosome assembly.** (A) Chaperones might prevent the aggregation of the highly charged r-proteins, many of which have unstructured tails, and deliver newly made r-proteins to nascent ribosomes. (B) Chaperones might also act directly upon assembling ribosomes by refolding r-proteins or assembly factors (purple) within preribosomal particles. This is shown as a change from helices to sheets.

to r-proteins, even when its ribosome-binding site is mutated (Martinez-Hackert and Hendrickson, 2009). A subset of these r-proteins (and others) aggregate in the absence of TF, and TF deletion strains have reduced levels of 30S subunits. Furthermore, the ribosome-binding–deficient TF fully rescues the phenotype from TF deletion. Together, these data indicate a function for TF in ribosome assembly independent of its translation-associated chaperone function. Extending the parallels between TF and the eukaryotic ribosome–associated chaperones, Albanèse et al. (2010) now show that the Jjj1 mutant Jjj1ΔC, which binds very weakly to ribosomes and at equilibrium is not bound to ribosomes, fully complements the phenotype from Jjj1 deletion and also rescues the RAC deletion when overexpressed. These results support a function for Jjj1 that is independent of binding to translating ribosomes. However, note that a similar study for the RAC subunit Zuo1 has not yielded mutants that complement its deletion but no longer bind ribosomes (Yan et al., 1998).

An alternative (or additional) role for chaperones in ribosome assembly could be the remodeling of protein structures within preribosomal particles (Fig. 1 B). Upon binding to preribosomes, proteins could undergo a slow structural change that requires unfolding (Fig. 1 B, shown as a hypothetical transition from helices to sheets). Indeed, prior work in bacteria has shown that the Hsp70/40 pair DnaK/J promotes the conversion of a small subunit assembly intermediate to native 30S subunits (Maki et al., 2002). DnaK/J promotes ribosome assembly in a reconstituted system, substituting for an otherwise required heating step. This heating step accelerates a rearrangement of the assembly intermediate (Traub and Nomura, 1969), suggesting that DnaK/J can act directly upon the assembly intermediate. Additionally, however, DnaK binds some r-proteins, and overexpression of the r-protein S4 can rescue the slow growth phenotype of a DnaK mutant (Maki et al., 2002), indicating perhaps that DnaK/J can also chaperone the folding of at least some r-proteins, thereby also carrying out both suggested functions in ribosome assembly. Although future experiments will be required to elucidate the exact function of protein chaperones in ribosome assembly, these new data show that neither RNA nor protein can be left alone to assemble into ribosomes.

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