Chaperoning ribosome assembly

Katrin Karbstein¹,²

¹Department of Chemistry and ²Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109

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.201002102) 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 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...
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.

I thank members of my laboratory for comments on the manuscript.

Submitted: 17 February 2010
Accepted: 15 March 2010

References

Albanèse, V., S. Reissmann, and J. Frydman. 2010. A ribosome-anchored network that facilitates eukaryotic ribosome biogenesis. J. Cell Biol. 189:69–81.

Baker, R.T., S.A. Smith, R. Marano, J. McMee, and P.G. Board. 1994. Protein expression using cotranslational fusion and cleavage of ubiquitin. Mutagenesis of the glutathione-binding site of human Pi class glutathione S-transferase. J. Biol. Chem. 269:25381–25386.

Culver, G.M., and H.F. Noller. 2000. In vitro reconstitution of 30S ribosomal subunits using complete set of recombinant proteins. Methods Enzymol. 318:446–460. doi:10.1016/S0076-6879(00)18069-3

Demenet, E., A. Jacquier, G. Luftalla, and M. Fromont-Racine. 2007. The Hsp40 chaperone Jj1 is required for the nucleo-cytoplasmic recycling of preribosomal factors in *Saccharomyces cerevisiae*. RNA. 13:1570–1581. doi:10.1261/rna.585007

Finley, D., B. Bartel, and A. Varshavsky. 1989. The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis. Nature. 338:394–401. doi:10.1038/338394a0

Gong, Y., Y. Kikihara, N. Krogan, J. Greenblatt, A. Emili, Z. Zhang, and W.A. Houry. 2009. An atlas of chaperone-protein interactions in *Saccharomyces cerevisiae*: implications to protein folding pathways in the cell. Mol. Syst. Biol. 5:275. doi:10.1038/msb.2009.26

Hartl, F.U., and M. Hayer-Hartl. 2009. Converging concepts of protein folding in vitro and in vivo. Nat. Struct. Mol. Biol. 16:574–581. doi:10.1038/nsmb.1591

Koplin, A., S. Preisler, Y. Ilina, M. Koch, A. Scior, M. Erhardt, and E. Deuerling. 2010. A dual function for chaperones Ssb–RAC and the NAC nascent polypeptide-associated complex on ribosomes. J. Cell Biol. 189:57–68.

Lacombe, T., J.J. García-Gómez, J. de la Cruz, D. Roux, E. Hut, P. Linder, and D. Kressler. 2009. Linear ubiquitin fusion to Rps31 and its subsequent cleavage are required for the efficient production and functional integrity of 40S ribosomal subunits. Mol. Microbiol. 72:69–84. doi:10.1111/j.1365-2958.2009.06622.x

Lamanna, A.C., and K. Karbstein. 2009. Nob1 binds the single-stranded cleavage site D at the 3′-end of 18S RNA with its PIN domain. Proc. Natl. Acad. Sci. USA. 106:14259–14264. doi:10.1073/pnas.0905403106

Maki, J.A., D.J. Schnobrich, and G.M. Culver. 2002. The DnaK chaperone system facilitates 30S ribosomal subunit assembly. Mol. Cell. 10:129–138. doi:10.1016/S1097-2765(02)00562-2

Martinez-Hackert, E., and W.A. Hendrickson. 2009. Promiscuous substrate recognition in folding and assembly activities of the trigger factor chaperone. Cell. 138:923–934. doi:10.1016/j.cell.2009.07.044

Meyer, A.E., N.J. Huang, P. Yang, A.W. Johnson, and E.A. Craig. 2007. The specialized cytosolic J-protein, Jj1, functions in 60S ribosomal subunit biogenesis. Proc. Natl. Acad. Sci. USA. 104:1558–1563. doi:10.1073/pnas.0610704104

Meyer, A.E., L.A. Hoover, and E.A. Craig. 2010. The cytosolic J-protein, Jj1, and Rei1 function in the removal of the pre-60 S subunit factor Arx1. J. Biol. Chem. 285:961–968. doi:10.1074/jbc.M109.038349

Strunk, B.S., and K. Karbstein. 2009. Powering through ribosome assembly. RNA. 15:2083–2104. doi:10.1261/rna.1792109

Traub, P., and M. Nomura. 1969. Structure and function of *Escherichia coli* ribosomes. VI. Mechanism of assembly of 30 S ribosomes studied in vitro. J. Mol. Biol. 40:391–413. doi:10.1016/0022-2836(69)90161-2

Yan, W., B. Schilke, C. Pfund, W. Walter, S. Kim, and E.A. Craig. 1998. Zuotin, a ribosome-associated DnaK molecular chaperone. EMBO J. 17:4809–4817. doi:10.1093/emboj/17.16.4809

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.