RNAs as chaperones

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Recently, we found that RNA is a remarkably powerful chaperone that can bind to unfolded proteins and transfer them to Hsp70 for refolding. Combined with past studies on RNA-chaperone interactions, we propose a model for how chaperone RNA activity may contribute to the cellular response to stress.

**Molecular chaperone function**

In the cell, molecular chaperones prevent protein aggregation and help proteins to fold. Preventing protein aggregation is important to the cell; if left unchecked, protein aggregation can lead to cellular death. Additionally, aggregation of proteins into amyloids is linked to a number of disease states including Alzheimer’s and Huntington’s. ATP-independent chaperones, often called holdases, bind to unfolded or misfolded proteins in the cell and directly prevent aggregation. These holdases usually do not actively refold unfolded or misfolded proteins. Instead, they work in combination with ATP-dependent chaperones, often called foldases, which directly help proteins to fold. Together, holdases and foldases optimize the protein folding environment in the cell and maintain a favorable equilibrium of folded proteins, termed proteostasis.

For several decades, the study of protein folding in vivo and the role of chaperones has focused on how protein chaperones aid other proteins, despite suggestions that many proteins in the cell do not regularly use the services of ATP-dependent refolding machinery. However, recent studies have begun to suggest that other types of molecules, including polyphosphate and nucleic acids, could also play important roles in proteostasis. Certain RNAs are known to help fold RNA binding proteins, and fusions containing RNA-binding proteins. Ribosomes are recognized as powerful chaperones that aid in the folding of polypeptide chains as they emerge during protein synthesis. Intriguingly, certain RNAs from the ribosome, especially domain V of 23S RNA, appear to have substantial chaperone activity on their own. These studies led to an idea that RNAs in general might possess chaperone activity.

**Nucleic acids are powerful molecular chaperones**

Recently, we tested a wide variety of nucleic acids for chaperone activity and found that many differing DNA and RNA molecules possess potent molecular chaperone activity. We found that nucleic acids were very effective in preventing the aggregation of a number of classic chaperone substrates, including luciferase and citrate synthase. Indeed, we found that RNA was about 300-fold more effective at preventing protein aggregation than the well-studied protein chaperone GroEL. Circular dichroism experiments at elevated temperatures demonstrated that the nucleic acids bound to luciferase in a non-native state, suggesting that DNA and RNA can bind to unfolded proteins and lower their tendency to aggregate. Single stranded nucleic acids were generally more effective than duplex nucleic acids, with polyU RNA the most effective nucleic acid tested. Length played a less important role than sequence or strandedness, as nucleic acids longer than about 20 bases had similar effectiveness.

RNA was tested for its ability to cooperate with the foldase DnaK, the bacterial homolog of Hsp70, to help proteins refold. polyU RNA was incubated at high temperature with luciferase, and added to the DnaK, DnaJ, GrpE protein folding system at room temperature. The presence of RNA substantially enhanced luciferase folding and activation, suggesting that the RNA was able to effectively transfer the unfolded protein to DnaK for refolding. This activity was comparable in strength to that measured for small heat shock proteins in performing the same task of transferring bound clients to DnaK.

The strong in vitro chaperone activity of multiple RNAs suggests that RNA could also be important for protein folding in vivo. Even though the majority of RNA in the cell is bound by proteins, RNA is an order of magnitude more abundant under stress conditions than heat shock proteins, and is far more effective on a weight basis in preventing aggregation.

Thus, we are willing to put forward the proposal that it is RNA, not proteins, that plays the dominant role in preventing protein aggregation in the cell (Fig. 1).
Chaperone: A+U rich element interactions

Our observation that polyU RNA could enhance the chaperone activity of DnaK is particularly interesting given studies over the past decade on how chaperones interact with RNA. In particular, Hsp70 interacts with A+U rich elements (AREs) both in vitro and in vivo.16-19. AREs are poly-uridine rich repeats containing interspersed adenines present in the 3’UTR of ~10% of all mRNAs in eukaryotes.20 RNAs containing AREs are transcribed constitutively, but are rapidly degraded under non-stress conditions. However, upon heat shock or other cellular stresses, the cell stabilizes ARE-containing mRNAs via a variety of protein interactors.21 The presence of AREs in the messages for many important stress-response proteins suggest that AREs may be a mechanism to promote fast response to stress by stabilizing specific mRNAs, and thus ensuring their greater translation during periods of stress.21

Although Hsp110 and Hsp60 also interact with AREs,16,22 Hsp70s are especially effective ARE binders. For example, human Hsp70 binds to a central ARE repeat with a Kd of 12 nM in vitro.17 Many Hsp70 variants also bind RNA, including bacterial homolog DnaK.19 The strong binding arises from the polyU portion of AREs, as polyU RNA bound Hsp70 with the tighter affinity than an ARE with interspersed adenesines.18,19 Not unsurprisingly, this binding also occurs in cells.17 Of note, addition of GrpE and DnaJ, or ATP can modulate RNA binding.19,22 These results suggest that the intricacies of Hsp70: RNA interactions and their effects on chaperone function could be strongly context dependent.

Possible mechanisms of Hsp70-assisted refolding by RNA

As discussed above, we found in our recent study that polyU RNA could transfer unfolded luciferase to DnaK for refolding.6 The observations that polyU RNA binds directly to DnaK and the subsequent modulation of binding by co-chaperones suggests several possible mechanisms for how the RNA helps protein folding. In the simplest mechanism, the RNA binds to the unfolded luciferase strongly enough to prevent its aggregation, but much weaker than the 12 nM Kd measured between the RNA and DnaK. Thus, when DnaK comes in contact with the RNA:luciferase complex, the RNA:DnaK binding outcompetes the luciferase: RNA binding, and the RNA releases the luciferase. The newly released luciferase can then bind to DnaK for refolding. More complicated or regulated mechanisms relying on the binding and unbinding of co-chaperones and ATP are also possible. Delineation of these mechanisms will require detailed analysis of how RNA affects each stage of the chaperone cycle and its interactions with clients.

Stress granules

How exactly could these RNA:Hsp70 interactions affect protein folding in the cell? Hints have come from recent studies on stress granules. These dynamic particles form when cells are subjected to various stress conditions, including heat and oxidative stress.23,24 Many stress conditions cause the disruption of translation, dramatically increasing the concentration of free mRNA. These freed mRNAs proceed to bind to a variety of proteins and form stress granules.25,26 The purpose of stress granules remains unclear. Although stress granules were originally thought to serve as centers for mRNA storage and processing, recent studies suggest that this may not be their primary role.27,28 Consistent with our findings, stress granules could alternatively protect the cell from unregulated protein aggregation.26,29 In this model, mRNA binds misfolded proteins and targets them toward stress granules. These mRNA chaperones could then work with ATP-dependent chaperones that are abundant in stress granules, such as Hsp70 and CCT.30 The stress granules are then disassembled in an Hsp70-dependent process upon stress relief.31 The role of Hsp70:RNA binding in this process is currently unclear, but it is possible that the
simple competition mechanism described above could apply in the context of Hsp70 binding RNAs in stress granules, in which Hsp70 exploits its high affinity of RNA to target stress granules. Although the RNA transcripts present in stress granules are not well known, ARE-binding proteins are commonly found in stress granules, suggesting that polyU segments in AREs could serve as a hub for protein refolding.

Conclusions

That nucleic acids may play roles in protein folding in addition to their other well-known roles may come as a surprise. However, the potency of chaperone RNAs as chaperones in vitro suggests that molecules other than traditional chaperones could be important for folding in vivo, including nucleic acids.

Disclosure of potential conflicts of interest

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