Published in final edited form as:

Nat Cell Biol. 2008 October ; 10(10): 1129–1130. doi:10.1038/ncb1008-1129.

Stressed out? Make some modifications!

Angela Hilliker and Roy Parker

Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, University of Arizona, Tucson, AZ, 85721-0106, USA.

Angela Hilliker: ; Roy Parker: rrparker@u.arizona.edu

Abstract

Stress granules and processing bodies are related mRNA-containing granules implicated in controlling mRNA translation and decay. A genomic screen identifies numerous factors affecting granule formation, including proteins involved in *O*-GlcNAc modifications. These results highlight the importance of post-translational modifications in translational control and mRNP granule formation.

Control of translation and mRNA degradation is an important aspect of gene expression. Two conserved cytoplasmic mRNA-containing ribonucleoprotein (mRNP) granules, stress granules and processing (P) bodies, have been implicated in translational control and mRNA degradation. Stress granules are formed in response to a wide variety of stresses that compromise translation initiation. They contain non-translating mRNA, some translation initiation factors and RNA-binding proteins, and may contribute to translation repression and mRNA triage, when mRNAs are sorted for decay, degradation or re-initiation¹. However, cells that cannot form stress granules can still repress translation², suggesting that granule formation *per se* has some other role. One possibility is that stress granules may concentrate translation initiation factors and mRNA, thereby promoting the assembly of translation initiation complexes³.

P-bodies also contain non-translating mRNAs, as well as factors involved in translation repression and mRNA decay. Protein factors that accumulate in P-bodies function in mRNA decapping, nonsense-mediated decay, miRNA-mediated repression and general translation repression³. P-bodies are generally present in eukaryotic cells but their levels increase during stress responses that increase the pool of non-translating mRNAs³. mRNAs that accumulate in P-bodies can exit P-bodies and return to translation, indicating that these foci can contain stored mRNAs^{4,5}. Stress granules and P-bodies share some protein components, can dock or overlap with one another and can contain the same mRNAs⁶, suggesting that mRNAs may move between these granules (Fig. 1).

There are a number of unresolved questions about P-bodies and stress granules. For example, the function of mRNP aggregation into these granules is unknown. Moreover, the mechanisms by which stress granules and P-bodies are assembled are poorly understood. Both are dependent on a pool of non-translating mRNPs, which assemble into the larger stress granules and P-bodies by an emerging set of redundant, direct protein–protein interactions that are thought to link individual mRNPs into larger structures^{1,7}. However, the complete composition of cellular factors that control and influence the formation of stress granules and P-bodies is not clear. On page 1224 of this issue, Ohn *et al.* provide an important step in identifying factors that affect stress granule and P-body assembly⁸.

Ohn *et al.* created a stable human cell line that expresses fluorescently tagged stress granule and P-body markers. Using this strain, they performed a siRNA screen based on Dharmacon's

druggable genome (about 7000 genes that are probable therapeutic targets) to identify genes that, when knocked down, decrease stress granule and P-body formation after stress induction by arsenite treatment. They found 101 factors that affect stress granule formation, 39 that affect P-body formation and 31 that affect both.

Some factors identified in this screen were expected to influence stress granule and P-body formation. For example, knockdown of six subunits of the translation factor eIF3 prevents stress granule assembly, suggesting that eIF3 may be directly involved in stress granule formation. Knockdown of some ribosomal proteins, translation elongation factors or translation termination factors reduce stress granule and/or P-body formation, perhaps by limiting the ability of ribosomes to complete translation and disengage from the mRNA.

Interestingly, many of the factors identified in this screen have no obvious connection to stress granules or P-bodies. The value of an open-ended genetic screen is that it is likely to identify new factors that affect the formation and function of these granules. However, as the authors suggest, knockdown of some of these factors may affect granule formation indirectly through a variety of pathways, including inhibition of the cellular response to arsenite, altered expression of important granule components or by stalling cells in mitosis, as stress granule and P-body formation are markedly reduced in mitotic cells⁹. Thus, further detailed analysis of these factors is essential to understand how they affect stress granule and P-body assembly and function.

Ohn *et al.* decided to focus on sortilin, a factor that promotes protein modification and is important for both stress granule and P-body assembly. Sortilin is a membrane protein that regulates the vesicular trafficking of the GLUT4 glucose transporter. Intracellular glucose can generate a substrate of the hexosamine biosynthetic pathway, which allows the reversible addition of *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) to proteins. *O*-GlcNAc modification of proteins is important for cell survival during stress¹⁰. Interestingly, arsenite treatment, which induces stress granules, also induces *O*-GlcNAc modification of many proteins¹⁰. This finding led to the hypothesis that *O*-GlcNAc modification promotes stress granule and/or P-body formation.

Ohn *et al.* provide several additional observations that connect *O*-GlcNAc modification to stress granule assembly (Fig. 1). First, knockdown of other components in the hexosamine biosynthetic pathway, including *O*-GlcNAc transferase, reduces stress granule formation. Second, several components of stress granules, including ribosomal proteins, are shown to be *O*-GlcNAc modified during arsenite-induced stress. Third, an antibody against the *O*-GlcNAc modification detects *O*-GlcNAc in stress granules, suggesting that at least some proteins within stress granules are *O*-GlcNAc modified. Finally, knockdown of sortilin or *O*-GlcNAc transferase still allows polysome disassembly in response to arsenite, suggesting that *O*-GlcNAc modification is required for the subsequent assembly of non-translated mRNPs into larger and visible stress granules, not the initial translation repression triggered by stress.

Further work will be required to understand how *O*-GlcNAc modifications promote stress granule assembly. One possibility is that the *O*-GlcNAc modification of proteins within stress granules enhances protein–protein interactions that drive stress granule formation. On the other hand, *O*-GlcNAc modifications may be reciprocal to phosphorylation¹¹ and may block or compete with phosphorylation events that prevent stress granule assembly. Alternatively, *O*-GlcNAc modifications may be required for the trafficking of mRNPs to stress granules, a process thought to involve microtubules^{2,12}, as microtubule-associated proteins that promote microtubule assembly are GlcNAcylated¹³. Finally, because *O*-GlcNAc modifications can prevent the aggregation of some proteins¹⁴, stress-induced *O*-GlcNAc modifications may limit

non-specific protein aggregation, thereby allowing specific aggregation mechanisms to assemble stress granules.

The role of *O*-GlcNAc modification in stress granule assembly adds to the growing list of protein modifications involved in mRNP granule formation and translational control. For example, methylation of the fragile X mental retardation protein (FMRP) correlates with increased recruitment into large stress granules, increased association with its mRNA targets and decreased translation of the target mRNAs¹⁵. Phosphorylation of Grb7, a component of stress granules, promotes disassembly of stress granules¹⁶. Moreover, the cytoplasmic deacetylase HDAC6 localizes to stress granules and is required for their formation². HDAC6 mutants that are defective in either deacetylase activity or ubiquitin binding both block stress granule formation, suggesting that deacetylation and ubiquitin modifications are important for stress granule formation; consistent with this view, ubiquitin was detected in stress granules by immunostaining². Future work will be required to understand the mechanistic role of these modifications. However, one intriguing possibility is that, analogous to the ‘histone code’¹⁷, the combination of modifications on proteins bound to a specific mRNA may dictate the localization, translation and degradation rate of individual mRNAs.

Post-translational modification is an ideal mechanism for modulating mRNA function in response to changing cellular conditions. As many stresses trigger translational arrest and polysome disassembly¹, the cell requires means of adaptation that do not involve new protein synthesis. Regulated, rapid and reversible protein modifications allow quick adaptation to stress without a large commitment in energy or time and also allow the cell to return to its initial state without the need to degrade newly synthesized proteins. As protein modifications have the ability to change the function of a protein by altering its stability, its binding partners or its enzymatic activity, protein modifications are capable of driving granule formation and influencing the control of translation.

References

1. Anderson P, Kedersha N. Trends Biochem. Sci 2008;33:141–150. [PubMed: 18291657]
2. Kwon S, Zhang Y, Matthias P. Genes Dev 2007;21:3381–3394. [PubMed: 18079183]
3. Parker R, Sheth U. Mol. Cell 2007;25:635–646. [PubMed: 17349952]
4. Brengues M, Teixeira D, Parker R. Science 2005;310:486–489. [PubMed: 16141371]
5. Bhattacharyya SN, et al. Cell 2006;125:1111–1124. [PubMed: 16777601]
6. Kedersha N, et al. J. Cell Biol 2005;169:871–884. [PubMed: 15967811]
7. Decker CJ, Teixeira D, Parker R. J. Cell Biol 2007;179:437–449. [PubMed: 17984320]
8. Ohn T, et al. Nature Cell Biol 2008;10:1224–1231. [PubMed: 18794846]
9. Sivan G, Kedersha N, Elroy-Stein O. Mol. Cell Biol 2007;27:6639–6646. [PubMed: 17664278]
10. Zachara NE, et al. J. Biol. Chem 2004;279:30133–30142. [PubMed: 15138254]
11. Copeland RJ, Bullen JW, Hart GW. Am. J. Physiol. Endocrinol. Metab 2008;295:E17–E28. [PubMed: 18445751]
12. Ivanov PA, Chudinova EM, Nadezhkina ES. Exp. Cell Res 2003;290:227–233. [PubMed: 14567982]
13. Arnold C, Hart GW. Trends Glycosci. Glycotech 1999;11:355–370.
14. Sohn KC, Lee KY, Park JE, Do SI. Biochem. Biophys. Res. Commun 2004;322:1045–1051. [PubMed: 15336570]
15. Dolzhanskaya N, Merz G, Aletta JM, Denman RB. J. Cell Sci 2006;119:1933–1946. [PubMed: 16636078]
16. Tsai NP, Ho PC, Wei LN. EMBO J 2008;27:715–726. [PubMed: 18273060]
17. Krebs JE. Mol. Biosyst 2007;3:590–597. [PubMed: 17700858]

Figure 1.

A model of the possible mRNA transitions among the translating pool, P-bodies (pink) and stress granules (green). During stress, mRNAs exit the translating pool to enter stress granules and P-bodies, but the order in which the mRNA populates each granule is unclear. Ohn *et al.* demonstrate that O-GlcNAc modifications promote stress granule assembly.