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PROTEIN AGGREGATION

The secret recipe for RNA granules

> reversible polymerization may drive the inclusion and exclusion of RBPs in RNA granules

RNA granules, including stress granules of mammalian cells and neuronal granules in dendrites, are membrane-free cytoplasmic structures composed of RNA molecules and RNA-binding proteins (RBPs). RNA granules have been implicated in the control of mRNA localization, and, although they were first observed more than 100 years ago, the mechanism underlying their formation has been elusive. Here, McKnight and colleagues describe cell-free RNA granule formation and propose a set of principles that may drive the formation of RNA granules *in vivo*.

The chemical isoxazole has been reported to induce differentiation of stem cells, and the authors used a biotinylated derivative (b-isox) to characterize this function. However, the observation that the addition of b-isox to cell lysates resulted in the formation of protein- and mRNA-containing precipitates shifted the focus of the authors. As the b-isox-induced precipitates were significantly enriched in mRNAs and RBPs that are found in RNA granules, the authors investigated which structural components of mRNAs and RBPs trigger the formation of RNA granule-like b-isox-induced precipitates.

Interestingly, the RNA granule-like structures were enriched for mRNAs with extended 3′ untranslated region sequences and RBP-binding sites. However, ribonuclease treatment did not inhibit the formation of RNA granule-like precipitates, suggesting that it is the RBP component that may lead to the formation of such structures.

Computational studies revealed that low-complexity polypeptide sequences (which are often observed in RBPs and DNA-binding proteins) are frequent within both RNA granule RBP constituents and b-isox-precipitated proteins. Moreover, low-complexity domains (and not domains involved in RNA binding) were required for b-isox-induced precipitation. Analysis of the X-ray structure of b-isox microcrystals led the authors to suggest that their wavy surface enables the reversible organization of low-complexity domains, which are disordered in soluble proteins, into an extended β-strand. So, such a transition in the organization of low-complexity domains may also occur *in vivo*.

Strikingly, the presence of a low-complexity domain in the stress granule constituent FUS (fused in sarcoma) was essential and sufficient for the cell-free formation of RNA granule-like protein hydrogels at high protein concentrations even in the absence of b-isox. In addition, mutational analyses in U2OS2 cells indicated that the Tyr residues of the 27 (Gly/Ser)-Tyr-(Gly/Ser) triplet repeats of the FUS low-complexity domain were required for the inclusion of FUS in endogenous stress granules.

Moreover, cell-free experiments suggested that low-complexity domain-containing proteins polymerize into amyloid-like fibres that differ from pathogenic prion-like amyloids in that they can be reversibly depolymerized. Such amyloid-like fibres could be extended by the addition of homotypic and heterotypic low-complexity-domain-containing proteins, and this depended on the Tyr residues of the low-complexity domains. By contrast, phosphorylation of this domain at multiple Ser residues inhibited further low-complexity domain-dependent protein polymerization. Thus, this reversible polymerization may drive the inclusion and exclusion of RBPs in RNA granules in a manner that can be regulated by local RBP concentration and post-translational modifications such as phosphorylation.

Together, these findings reveal the previously unappreciated role of low-complexity polypeptide sequences in the formation of membrane-free subcellular structures such as RNA granules. The authors speculate that locally controlled formation of such structures may underlie many cellular functions, including RNA splicing and gene transcription.

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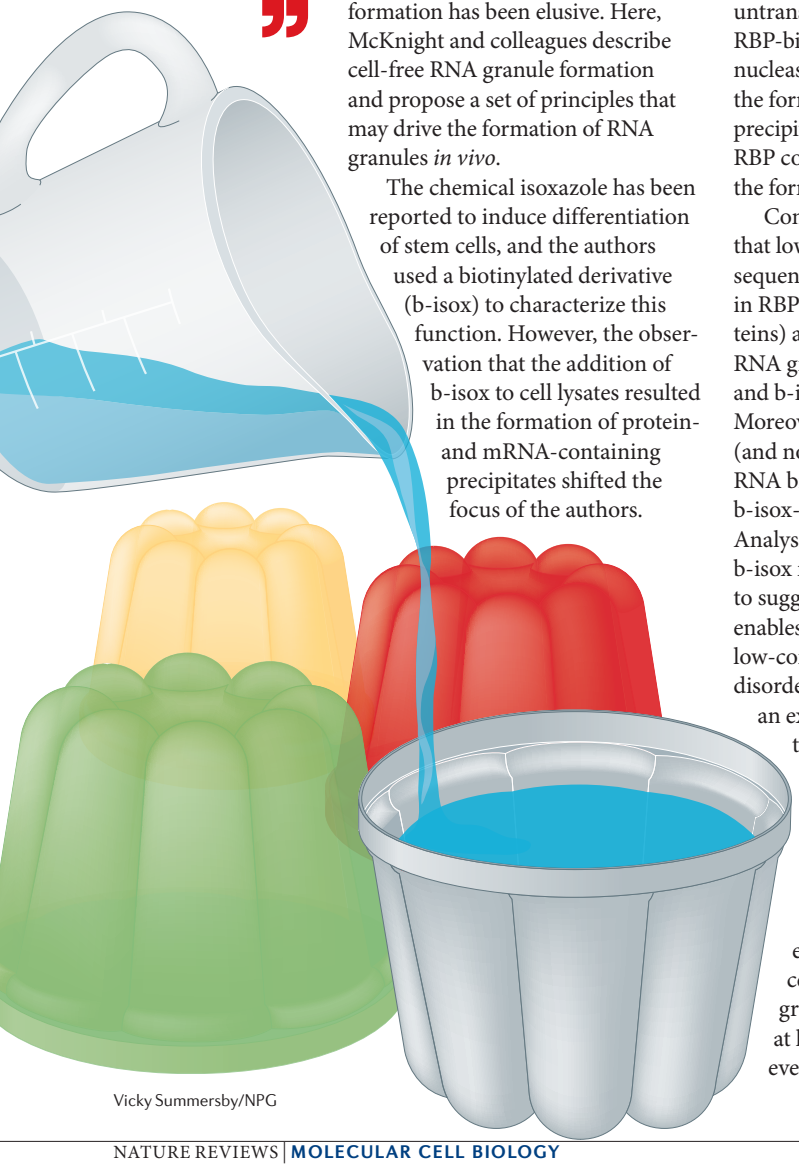

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ORIGINAL RESEARCH PAPERS Kato, M. *et al.* Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers with hydrogels. *Cell* **149**, 753–767 (2012) | Han, T. W. *et al.* Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. *Cell* **149**, 768–779 (2012)