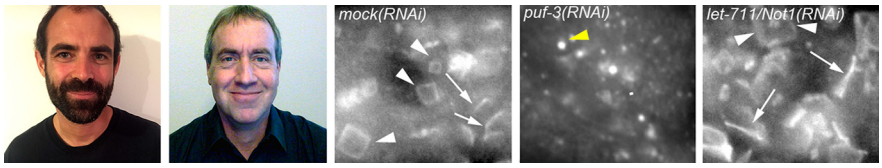
Text by Ben Short
bshort@rockefeller.edu

Solidifying the view of RNP dynamics

An RNAi screen provides new insights into the regulation of RNP granules and mRNA repression.

FOCAL POINT



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Arnaud Hubstenberger (left), Tom Evans (right), and colleagues screened for genes that modulate the assembly of RNP granules in *C. elegans* germ cells. grPBs are liquid-like RNP granules that form in arrested oocytes. In the absence of the RNA helicase CGH-1, however, they form solid, square-shaped aggregates (left). Additional depletion of some factors, such as the RNA-binding translational repressor PUF-3 (center), results in dispersal of these square granules, whereas the removal of other proteins, such as the deadenylation factor LET-711/Not1 (right) enhances solid granule formation. Many of these regulatory factors collaborate to control mRNA translation, and also control the assembly of normal grPBs, a process that may rely on the interactions between glutamine-rich repeats.

Ribonucleoproteins (RNPs), consisting of mRNAs bound to multiple regulatory proteins, can assemble into larger granules that can behave like liquid droplets or solid aggregates in the cell. RNP condensation is thought to be driven by both specific and nonspecific, multivalent interactions between proteins and RNAs, but, because RNP granules are dynamic and have distinct compositions, the process must be tightly regulated in vivo. In the *C. elegans* germline, for example, two types of RNP bodies—P granules and grPBs—assemble with different dynamics during oogenesis. Hubstenberger et al. now describe some of the factors that regulate grPB assembly, and how these factors combine to repress mRNA translation (1).

grPBs are large structures that assemble in arrested oocytes and contain numerous mRNAs repressed by specific RNA binding proteins (RBPs) (2, 3). In 2013, Tom Evans, Arnaud Hubstenberger, and their colleagues at the University of Colorado discovered that grPB assembly is induced by these repressive RBPs. Although grPBs usually behave like liquid droplets, grPB components condense into solid, square-shaped aggregates in the absence of an RNA helicase called CGH-1, and solid formation also depends on RBP function (4). "That made us wonder what other proteins are required to form these aberrant solid granules," says Evans, especially because, in humans, the assembly of large, solid RNP aggregates is associated with several neurodegenerative diseases.

> **"It suggests that many mRNAs are suppressed by multiple mechanisms."**

Hubstenberger et al. therefore conducted an RNAi screen and identified 66 factors that affected the size, shape, or abundance of square granules in oocytes lacking CGH-1 (1). Many of these factors were required not only for the formation of square granules, but also for the assembly of normal, liquid-like grPBs in wild-type worms. "But there was also a set of genes that limited granule assembly and prevented solidification," Evans explains.

The screen identified several factors that weren't expected to regulate RNP bodies, including membrane trafficking proteins, chaperones, and components of the Ras signaling pathway. Most of the screen's hits, however, were factors known, or predicted, to be involved in various aspects of RNA regulation, and Hubstenberger et al. were able to draw a number of conclusions about how different classes of regulator influence RNP dynamics.

Given that polyadenylation generally promotes mRNA translation, one surprising finding was that the poly(A) polymerase GLD-2 and the poly(A) tail effector PAB-1 promote the formation and solidification of repressive grPBs. Deadenylation factors, meanwhile, were found to limit granule assembly. Evans says that it's not yet clear how these proteins regulate RNP dynamics.

Hubstenberger et al. also found that many of the proteins regulating grPB assembly or solidification contain glutamine-rich (polyQ) motifs, whereas polyQ motifs were not enriched in proteins previously identified as modulators of P granules. Several proteins known to modulate the aggregation of polyQ-containing proteins were also found to regulate grPB dynamics, suggesting that polyQ interactions are critical to grPB assembly. PolyQ/N motifs have been implicated in the assembly of yeast RNP granules (5, 6), and abnormal expansion of glutamine-rich repeats is associated with many different neurodegenerative disorders.

Finally, and in agreement with their previous study (4), the researchers found that numerous proteins involved in RNA repression were required for grPB assembly and solidification. These repressors function in different ways, but Hubstenberger et al. determined that they often acted on the same targets. "It suggests that many mRNAs are suppressed by multiple mechanisms," Evans says. "They could be interdependent pathways, or they could be independent, partially redundant mechanisms."

Overall, Hubstenberger et al.'s study reveals the diversity of factors that regulate RNP assembly and dynamics. "We're not going to be able to study them all, but we hope that others will," says Evans. "We're interested in how some of the key regulators induce and control RNP assembly and how this process impacts mRNA fate."

1. Hubstenberger, A., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201504044
2. Jud, M.C., et al. 2008. *Dev. Biol.* 318:38–51.
3. Noble, S.L., et al. 2008. *J. Cell Biol.* 182:559–572.
4. Hubstenberger, A., et al. 2013. *Dev. Cell.* 27:161–173.
5. Decker, C.J., et al. 2007. *J. Cell Biol.* 179:437–449.
6. Reijns, M.A., et al. 2008. *J. Cell Sci.* 121:2463–2472.