A Place for RNAi

Processing bodies (P bodies) are discrete cytoplasmic foci to which mRNA is routed for degradation. In mammalian cells, they are also associated with miRNA-induced translational silencing and siRNA-induced mRNA degradation. In a recent issue of *Molecular Cell*, Ding and coworkers described an argonaute-interacting protein that appears to promote the assembly of P bodies in *C. elegans* (Ding et al., 2005).

The deconstruction of mRNA into its component parts requires several enzymatic activities: a deadenylase, a decapping enzyme, and an exonuclease. Recent results have revealed that representatives from each enzymatic class are concentrated at discrete cytoplasmic foci that have variously been called processing, cytoplasmic, GW, or Lsm bodies (henceforth referred to as P bodies). In yeast, the finding that mRNA degradation intermediates are localized at P bodies suggests that these structures are sites at which mRNA is degraded (Sheth and Parker, 2003). In mammalian cells, the RNA-mediated knockdown of the P body-associated exonuclease enhances the accumulation of poly(A)^+ RNA at P bodies, further supporting the contention that these foci are sites of mRNA degradation (Cougot et al., 2004). In addition to RNA degradative enzymes, P bodies contain an Lsm1-7 heptamer, an RNA helicase (rck/p54), and several RNA binding proteins, including eIF4E, TTP, BRF1, and GW182, suggesting that they may play additional roles in the regulation of mRNA metabolism. Recently, P bodies have been found to harbor components of the RNA-induced silencing complex (RISC) (Liu et al., 2005; Sen and Blau, 2005). Thus, P bodies may also be sites at which miRNA-induced translational silencing and siRNA-induced RNA destruction occur. Consistent with this suggestion, reporter mRNAs targeted by miRNAs are concentrated at P bodies in a miRNA-dependent manner (Liu et al., 2005). These results suggest that P bodies repress mRNA expression, either by inhibiting translation or promoting mRNA degradation. Studies employing genetic or pharmacologic inhibitors of protein translation reveal that P bodies are in a dynamic equilibrium with polysomes, indicating that mRNA can move in and out of these structures (Sheth and Parker, 2003). Thus, recruitment of mRNA to the P body may not be irreversible. Selected transcripts might be released from the P body to translate again.

The dynamic nature of the P body is reminiscent of a related RNP granule that is observed in mammalian cells subject to environmental stress. These “stress granules” harbor untranslated mRNAs that accumulate during stress (Kedersha et al., 2005). mRNA and protein rapidly shuttle in and out of stress granules, indicating that these structures are not simply storage depots for untranslated mRNA but may be sites of mRNA triage at which mRNP complexes are remodeled and then routed to sites of reinitiation, degradation, or storage. Real-time microscopy using GFP and RFP-tagged proteins reveals that stress granules are relatively fixed within the cytosol, whereas P bodies rapidly move about in what appears to be a random walk (Kedersha et al., 2005). Occasionally, mobile P bodies will “dock” at stress granules where they remain for several minutes before disengaging to resume their cytosolic survey. RNA binding proteins that promote mRNA degradation (e.g., TTP, BRF-1, and BFR-2) promote docking of P bodies at stress granules, suggesting that selected mRNAs can move from stress granules to P bodies for degradation. In this capacity, the stress granule appears to be an intermediate between the polysome and the P body.

In *C. elegans*, germ cells contain an RNP granule that is compositionally and functionally related to the P body. These so-called “P granules” are thought to store specific maternal mRNAs that are required for germline specification. Like P bodies, P granules are composed of RNA binding proteins, RNA helicases, and an isoform of eIF4E, suggesting that they regulate some aspect of RNA metabolism (Wang et al., 2005). A germline RNA helicase (CGH-1, an ortholog of the rck/p54 helicase characteristically found in P bodies and SGs) found in P granules is also found in somatic cells during early embryogenesis, where it is concentrated at discrete cytoplasmic foci that may correspond to P bodies (Navarro and Blackwell, 2005). Consistent with the possibility that other *C. elegans* somatic cells possess P bodies, Ding et al. (2005) have identified AIN-1, a *C. elegans* protein that interacts with the RISC components argonaute and dicer, as well as selected miRNAs. Genetic analysis indicates that *ain-1* has complex functional effects that regulate the timing of multiple aspects of *C. elegans* development. A clue to its molecular function came from sequence analysis which identified a 144 amino acid glycine/tryptophan (GW)-rich domain that is homologous to GW182, an RNA binding protein found in mammalian P bodies (Eystathioy et al., 2003), and an AIN-1::GFP fusion protein colocalizes with *C. elegans* orthologs of the P body-associated decapping enzyme complex DCP1/2. Moreover, biochemical analysis shows that AIN-1 interacts with the RISC components argonaute and dicer, as well as selected miRNAs. Surprisingly, the *C. elegans* argonaute protein (ALG-1), when expressed as a DsRed2::ALG-1 fusion protein, has a diffuse cytosolic distribution. This is in contrast to mammalian cells, where argonaute proteins are constitutively found within P bodies (Liu et al., 2005; Sen and Blau, 2005). However, in a *C. elegans* strain expressing AIN-1::GFP and DsRed2::ALG-1, both fusion proteins colocalize at cytoplasmic foci, suggesting that AIN-1 may be required to recruit ALG-1 to P bodies.

Although these results are intriguing, there are many gaps that remain to be filled. The structure and function...
of AIN-1::GFP-containing foci remains to be defined. It is not certain that these foci have the same composition as endogenous P bodies. Indeed, it is possible that P body-like structures may be compositionally and functionally heterogeneous. Different cell types may use different classes of P body-like structures to regulate specific aspects of RNA storage/metabolism. It will be important to compare the composition of P bodies and P granules to define the relationship between these structures. The recent demonstration that retinoblastoma mutants exhibit somatic misexpression of P granules together with enhanced RNAi function suggests that components of P granules and RISC may be coordinately regulated (Wang et al., 2005). It will be important to determine whether RISC-mediated RNAi contributes to the general process of translational silencing at RNP granules.

The importance of C. elegans in unraveling the mechanisms of RNAi makes the discovery of its site of action all the more exciting. Although much remains to be learned, these initial steps underscore the importance of subcellular localization in the regulation of mRNA metabolism.

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Selected Reading