EDITORIAL

P-bodies and RNAi: The missing link?

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For many years studies on the regulation of gene expression have focused on the control of transcription, thinking that once the gene was expressed as mRNA it would be translated and when its time was up it would simply be degraded in the cytoplasm. However, recent years have seen increasing interest in mechanisms involved in the post-transcriptional regulation of gene expression. An important aspect of this explosion in interest has been the discovery that small regulatory RNA molecules, small interfering (si) RNAs and micro (mi) RNAs, are capable of inhibiting gene expression by either directing the degradation of homologous mRNA targets or inducing the repression of translation of mRNA targets which have incomplete complementarity. Extensive biochemical and genetic analysis has helped to shed light on the mechanism by which siRNAs are incorporated into the RNA-induced silencing complex (RISC), recognize the target mRNA and directing its cleavage. This has been greatly aided by recent studies on the crystal structure of a siRNA guide strand associated with Piwi, the Piwi domain of Argonaute 2 (Ago2) being responsible for recognition and endonucleolytic cleavage of the target mRNA. Despite being the first post-transcriptional gene silencing mechanism to be identified, much less is known about the mechanism of translational repression compared to the targeted degradation of mRNA. It is possible to envision a variety of potential mechanisms by which RISC could induce the inhibition of translation, including the impairment of steps in the process of translation (for example, blocking the progress of ribosomes along the mRNA) or the "tagging" of newly formed proteins for degradation. In fact, an alternative mechanism of translation repression, that of sequestering the target mRNA into distinct sites within the cytoplasm and away from the translational machinery, has been the focus of a number of recent studies.

These sites in the cytoplasm, referred to by a variety of names including processing (P)-, cytoplasmic-, GW-, Dcp-, or Lsm-bodies, serve as foci for the accumulation of mRNAs that are destined for storage or degradation. In addition to the mRNA component, these site contain the mRNA decapping enzymes (Dcp1/Dcp2), essential components of the mRNA degradation pathway, as well as, additional proteins, including the 5'-3' exonuclease, Xrn1, Dhh1p, Pat1p and in mammalian cells, GW182 (Coller and Parker, 2004; Sen and Blau, 2005). The first hint of the interaction of the miRNA machinery with these sites of mRNA turnover was the demonstration that the mammalian Ago proteins (Ago1, -2, -3 and -4) were concentrated into discrete foci in the cytoplasm which colocalized with components of the mammalian P-bodies, GW182, Dcp1a and 2 (Sun and Blau, 2005; Liu et al, 2005a). Interestingly, members of a second family of Argonaute proteins, the Piwi family, that have not been found to be associated with miRNAs did not show this localization pattern (Liu et al, 2005a). Biochemical analysis demonstrated a direct physical interaction between components of RISC and the mammalian P-bodies since Dcp1 and 2 could co-immunoprecipitate with Ago1 and Ago2. In the absence of RNA, these factors retained the ability to co-immunoprecipitate (Liu et al, 2005a), however, they failed to localize to the P-bodies (Sun and Blau, 2005). This data indicates that the Ago proteins could interact with the P-body components independently of their incorporation into the discrete foci. In addition, mutations in the PAZ domain of Ago2 that inhibit its ability to bind to siRNAs and miRNAs did not affect the interaction of Ago2 with Dcp1a but did prevent their localization to P-bodies, demonstrating that the localization of Ago2 into the P-bodies was a miRNA-/siRNA-dependent process. This was further confirmed by following the fate of reporter mRNAs containing multiple MS2 binding sites that can be visualized by the introduction of a fluorescently tagged MS2 protein (MS2-YFP) and sites for either the endogenously expressed let-7 miRNA or an exogenously introduced siRNA (CXCR4) that mimics miRNA function by binding to

multiple imperfectly complementary sites on the mRNA. The tagged mRNAs were localized in the P-bodies in the presence of their respective miRNA, but not in the absence of the miRNA or in reporter mRNAs that lacked the miRNA binding sites. Although this data showed a linkage between the components of the silencing machinery and localization to the P-bodies it provided no evidence of the functional necessity of this localization for gene silencing. This was quickly remedied by two studies that provided a functional link between P-bodies and RNAi-mediated silencing (Liu et al, 2005b; Jakymiw et al, 2005). They found that the silencing of GW182 by treatment with siRNAs impaired the formation of P-bodies. In fact, there was a striking lack of P-bodies in the GW182 siRNA treated cells, demonstrated the critical role that GW182 plays in the organization and architecture of these structures. More importantly for the determination of the mechanism of RNAi-mediated silencing, the disruption of the P-bodies significantly impaired both miRNA- (translational repression) and siRNA- (mRNA cleavage) mediated silencing. This was found to be the case for both the silencing of exogenous miRNA and siRNA reporter gene (Liu et al, 2005b) and for endogenous gene expression (Jakymiw et al, 2005).

The physical and functional link between the sites of mRNA turnover/storage and miRNA-/siRNA-mediated silencing raises interesting questions not only about the mechanism of RNAi-mediated silencing but the potential role that RNAi or components of the RNAi machinery may play in other translational regulation mechanisms. P-bodies, far from being the site of mRNA degradation alone, are increasingly thought to be sites for the storage of translationally repressed mRNAs, with mRNAs being able to move between the active and inactive pool as needed. One hypothesis put forward by the Parker and Hannon groups suggests that miRNAs may mediate their repressive function by selectively transporting and possibly even maintaining their mRNA targets in these sites of translational repression segregated from the translational machinery. Though far from providing a complete answer these studies represent an important step in our understanding of the miRNA-mediated repression of translation.

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