Mammalian Dicer finds a partner

RNA interference (RNAi) is a complex process that uses small RNAs of 21–23 nucleotides to guide sequence-specific silencing of genetic information at both the transcriptional and post-transcriptional levels. There are two main post-transcriptional gene-silencing pathways in metazoans: the microRNA (miRNA)-mediated inhibition of translation and the small interfering RNA (siRNA)-mediated targeted degradation of RNAs (reviewed in Hannon & Rossi, 2004; He & Hannon, 2004; Filipowicz et al., 2005; Tomari & Zamore, 2005). miRNAs are generally characterized by imperfect helices both in the precursor (pre-miRNA) and in the binding of the miRNA antisense strand to the target, which usually takes place within the 3’ untranslated region of the mRNA. siRNAs generally have near-perfect Watson–Crick base pairs (bp), both in the double-stranded precursors and in the pairing of the antisense strand with the target mRNA, which can occur throughout the message. RNAi is active in organisms ranging from the fission yeast Schizosaccharomyces pombe through to humans. An important aspect of RNAi is the strong evolutionary conservation of many of the components involved. The most highly conserved proteins include members of the Argonaute family and the RNase III family of double-stranded ribonucleases (Filipowicz et al., 2005; Tomari & Zamore, 2005) that cleave the precursors of miRNAs and siRNAs into 21–23 bp duplexes with two-base 3’ overhangs. Despite the strong conservation of these proteins between kingdoms, there are important differences that relate to their specialized functional activities. For instance, in Drosophila, there are two distinct RNase III family members called Dicer. Dcr1 specializes in processing endogenous hairpin RNA precursors into miRNAs, whereas Dcr2 specializes in cleavage of double-stranded RNAs (dsRNAs) that are destined to function as siRNAs (Tomari & Zamore, 2005). In plants, there are multiple forms of the Dicer protein that are specialized in either nuclear or cytoplasmic processing of dsRNAs (Baulcombe, 2004). By contrast, Caenorhabditis elegans and mammals have only a single species of Dicer, which is involved in processing precursor dsRNAs into both miRNAs and siRNAs (Filipowicz et al., 2005). In Drosophila, another RNase III family member, Drosha—which functions in a complex with a dsRNA-binding protein, Pasha (Denli et al., 2004)—processes primary-miRNA transcripts into individual pre-miRNAs. In mammals, primary-miRNA precursors are also processed by a Drosha homologue, which functions in combination with the Pasha homologue DGC8 (Han et al., 2004).

As investigators probe more deeply into the mechanism of RNAi, new combinations of functionally interacting proteins are being revealed. In Drosophila, Dcr2 forms a complex with a dsRNA-binding protein called R2D2 (Liu et al., 2003). This protein combination senses differential thermodynamic stabilities of the two ends of siRNAs and delivers the antisense strand to the Argonaute-containing RNA-induced silencing complex (RISC). It is not known in mammalian systems whether both strands or only a single strand are transported to RISC. Once the antisense strand is selected in the RISC, it guides sequence selection of the target for cleavage.
In mammals, Dicer was only known to interact with the Argonaute 2 (Ago2) protein, a key component of RISC. Two recent studies have now revealed a new and important Dicer-interacting protein in mammalian systems: TRBP (Fig 1; Chendrimada et al., 2005; Haase et al., 2005). As reported in the 4 August 2005 issue of Nature, Chendrimada et al. used antibodies directed against Ago2 to identify its interacting partners. They identified both Dicer and TRBP and reciprocal antibody immunoprecipitates confirmed this tri-partite association. In this issue of EMBO reports, Haase et al. reported their use of monoclonal antibodies directed at human Dicer to pull down Dicer-interacting partners, and they identified the Argonaute proteins and TRBP. In addition to their anti-Dicer antibody assays, Haase et al. used gradient sedimentation, Flag-tagged TRBP and yeast two-hybrid analyses to confirm the interaction between Dicer and TRBP.

The two groups went on to investigate the functional role of the TRBP–Dicer interaction by using siRNAs to knock out either Dicer or TRBP in cell culture. Both groups showed that knockdown of either TRBP–Dicer interaction by using siRNAs to knock out either Dicer or miRNAs. Chendrimada et al. observed that cell extracts prepared from cells treated with siRNAs against TRBP had reduced processing activity of pre-miRNAs. Chendrimada et al. observed that depletion of TRBP in cell culture led to the instability of Dicer, but this was not observed by Haase et al. Chendrimada et al. also found that TRBP knockdown reduced endogenous miRNA levels in cell culture, whereas Haase et al. saw no such reduction, nor did they observe the accumulation of premiRNAs. These contradictory results probably reflect the different methodologies used in the two studies. Haase et al. used total cell extracts from anti-TRBP siRNA-treated cells to monitor the levels of Dicer and TRBP, whereas Chendrimada et al. used antibody-mediated pulldown of the Ago2 complex as a source of these proteins. The loss of Dicer in the Ago2 complex makes sense if TRBP is required for the association of Dicer with Ago2, and thus one would expect to see a reduction in co-immunoprecipitated Dicer when TRBP is depleted. By contrast, the analysis by Haase et al. suggests that this loss of TRBP does not globally affect Dicer stability. The contradictory results of TRBP depletion and miRNA levels are more difficult to resolve. Perhaps the observed differences are also due to the method used to deplete TRBP. Haase et al. used tetracycline-inducible plasmids expressing short hairpin RNAs (shRNAs) that target TRBP, whereas Chendrimada et al. depleted TRBP with transfected synthetic siRNAs. In the latter case, the intracellular concentrations of the siRNAs are probably greater than the siRNAs processed from shRNAs, thereby effecting a faster and more potent knockdown of the target TRBP mRNA. The subsequent loss of TRBP would then affect processing of pre-miRNAs into miRNAs.

These differences in the results of TRBP knockdown need to be reconciled to understand the functional role of TRBP in RNAi. In one model, TRBP is required both for Dicer cleavage and passage of the siRNA/miRNA to Ago2 and RISC. In the other model, TRBP is only required for the functional passage of siRNA/miRNAs to RISC. Another important problem is whether TRBP functions in a manner analogous to R2D2 in sensing the relative thermodynamic stabilities of the two ends of siRNAs and miRNAs (Tomari et al., 2004). Haase et al. suggest that TRBP does not have the same functional role as R2D2, leaving open the question of how the antisense strand is selected in mammalian cells.

Plasma retinol-binding protein (PRBP) is the murine homologue of TRBP, but what are the potential TRBP homologues in other systems? Haase et al. point out that there are three possible isoforms of this protein in human cells. The splice variant TRBP2, which interacts with Dicer, differs from the variant TRBP1 in its amino-terminus, and TRBP3 in its carboxy-terminal domain. Alignments of TRBP2 with other dsRNA-binding proteins reveal high similarity with Drosophila Loqs1 and the human and murine protein kinase R (PKR)-activating protein (PACT). Interestingly, the Drosophila protein R2D2 is not a homologue of TRBP. A genetic knockout of PRBP gives a mild phenotype (Zhang et al., 1999) in contrast to the embryonic-lethal phenotype of a Dicer knockout (Bernstein et al., 2003). It is therefore possible that other dsRNA-binding proteins have parallel functions to PRBP (TRBP), especially with respect to the loading of miRNAs into the RISC.

An interesting paradox exists between TRBP and PACT, which have 42% identity. TRBP inhibits the dsRNA-activated PKR, whereas PACT activates this protein—both use their C-terminal domains for these functions (Gupta et al., 2003). The C-terminal domain of TRBP is also required for interaction with Dicer (Haase et al., 2005), raising the intriguing possibility that TRBP and PACT modulate PKR differentially in response to dsRNAs in the cytoplasm. For instance, it would be disadvantageous to the cell to have pre-miRNA precursors activating PKR, as this may ultimately lead to cell death. Thus, TRBP might be responsible for keeping PKR inactive. As TRBP binds the HIV TAR element, it might prevent the double-stranded region of TAR from activating PKR. It is also possible that TRBP binding to HIV TAR could result in the recruitment of Dicer to this double-stranded element. The recent finding that Tat is a suppressor of RNAi, and in particular an inhibitor of Dicer (Bennasser et al., 2005), raises the intriguing possibility that in the absence of Tat, TRBP could recruit Dicer to TAR, resulting in cleavage of the TAR stem and the functional destruction of HIV transcripts. When Tat is bound to TAR it might inhibit the interaction of Dicer with TRBP, thus preventing such cleavage. This scenario requires further experimental testing.

As PACT has opposing activity to TRBP, it will be interesting to determine how the balance between these two activities is regulated. It is not known whether PACT also binds to Dicer. If so, binding to Dicer might functionally block PACT activation of PKR. The recent identification of a role for TRBP in RNAi has fuelled some important new problems for investigation. The potential link between dsRNA-binding proteins, RNAi and PKR is important as pre-miRNAs are potential substrates for PKR activation, and yet they are essential in a variety of cellular processes both during development and in differentiated tissues.

These new studies pose an interesting dilemma about the discrimination between pre-miRNAs and pre-siRNAs by Dicer. In Drosophila, two forms of Dicer use different interactions with dsRNA-binding proteins Loqs1 and R2D2 to discriminate between pre-miRNAs and siRNAs, respectively. In mammals, with a single species of Dicer, this discrimination must occur in a different manner. The newly defined interaction of Dicer with TRBP2 opens up the possibility that Dicer might also interact with one of the other TRBP isoforms, or even PACT, to modulate inhibition or activation of PKR and the discriminatory recognition of pre-miRNAs versus pre-siRNAs. Finally, the recently discovered role of small RNAs in transcriptional gene silencing in humans (Kawasaki et al., 2005) suggests that these small RNAs might be generated by another Dicer–dsRNA-binding protein partnership. Look for some interesting new stories to be unravelled in the months to come.
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REFERENCES


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