pattern of B lymphocyte induced maturation protein-1 (Blimp-1) during mouse embryonic development. Mech. Dev. *117*, 305–309.

14. de Souza, F.S., Gawantka, V., Gomez, A.P., Delius, H., Ang, S.L., and Niehrs, C. (1999). The zinc finger gene Xblimp1 controls anterior endomesodermal cell fate in Spemann's organizer. EMBO J. *18*, 6062–6072.

15. Baxendale, S., Davison, C., Muxworthy, C., Wolff, C., Ingham, P.W., and Roy, S. (2004). The B-cell maturation factor Blimp-1 specifies vertebrate slow-twitch muscle fiber identity in response to Hedgehog signaling. Nat. Genet. *36*, 88–93.

16. Anderson, R., Copeland, T.K., Scholer, H., Heasman, J., and Wylie, C. (2000). The onset of germ cell migration in the mouse embryo. Mech. Dev. *91*, 61–68.

17. Starz-Gaiano, M., and Lehmann, R. (2001). Moving towards the next generation. Mech. Dev. *105*, 5–18.

18. Raz, E. (2004). Guidance of primordial germ cell migration. Curr. Opin. Cell Biol. *16*, 169–173.

19. Balkwill, F. (2004). Cancer and the chemokine network. Nat. Rev. Cancer *4*, 540–550.

20. Lapidot, T., Dar, A., and Kollet, O. (2005). How do stem cells find their way home? Blood, DOI: 10.1182/blood-2005-04-1417.

Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11 37070, Goettingen, Germany.

MicroRNAs: Loquacious Speaks out

In *Drosophila*, Dicer-2 requires the double-stranded RNA binding protein R2D2, to mediate the assembly of short interfering RNAs into the RNA-induced silencing complex. New data show that Dicer-1 also requires a double-stranded RNA binding protein called Loquacious for efficient microRNA-mediated gene silencing.

Philipp J. F. Leuschner, Gregor Obernosterer and Javier Martinez

A major breakthrough in molecular biology was the finding that eukaryotic cells harness mechanisms of RNA interference (RNAi) to regulate the expression of endogenous genes [1,2]. Just as in the 'canonical' RNAi pathway, short single-stranded RNA molecules called microRNAs (miRNAs) serve as sequence specific guides to target silencing complexes containing an Argonaute (Ago) protein to cognate sequences — typically in the 3′-untranslated region of target messenger RNAs [3].

miRNAs originate from long primary transcripts (pri-miRNAs) [4], which are processed in the nucleus by the RNase III-like enzyme Drosha and its cofactor Pasha/DCGR8 into ~65 nt, hairpin shaped precursors, or pre-miRNAs [5–8]. Pre-miRNAs are exported to the cytoplasm, where another RNase III-like enzyme, Dicer, liberates a ~22 nt long miRNA duplex from the hairpin. One strand of the duplex is integrated into an active RNA-induced silencing complex (RISC) [9].

While human Dicer is able to process long double-stranded RNA (dsRNA) as well as pre-miRNAs, the two pathways are separated in *Drosophila* (Figure 1). Here, Dicer-2 does not play a role in miRNA biogenesis, but is required to cleave long dsRNAs into short interfering RNAs (siRNAs), which are then assembled into siRISCs (Figure 1A). Dicer-1 processes pre-miRNAs and loads the resulting miRNAs into miRISC containing Ago-1 (Figure 1B). However, Dicer-1 also seems to be required downstream of siRNA-production in siRISC assembly [10–12]. It has been shown that the activities of Drosha and Dicer-2 absolutely depend on the auxiliary dsRNA binding domain (dsRBD) proteins, Pasha and R2D2, respectively [5–8,13]. Now Saito *et al*. [14] and Förstemann *et al.* [15] provide a missing piece in the RNAi versus miRNA puzzle: they conclusively demonstrate that Dicer-1 also requires a dsRBD protein to efficiently process pre-miRNAs into miRNA duplexes. Saito *et al*. [14] relied on an RNAi-based functional screen for *Drosophila* dsRBD proteins that affect miRNA biogenesis, while Förstemann *et al.* [15] searched a database for conserved dsRBDs containing proteins. Both laboratories identified the same candidate — a paralogue of *Drosophila* R2D2, featuring two canonical and one non-canonical dsRBDs. This candidate was baptized *loquacious* (*loqs*), as endogenous RNA-mediated silencing is lost in mutant flies.

Both groups show that reduced levels of Loqs result in the accumulation of endogenous pre-miRNAs. The same phenotype is observed when cells are depleted of Dicer-1, but not Dicer-2 or R2D2. The physical association of Loqs and Dicer-1 was confirmed by reciprocal co-immunoprecipitation, and did not depend on a pre-miRNA substrate. Saito *et al.* [14] demonstrate that both proteins also co-immunoprecipitate with Ago1, providing further evidence that miRNA processing may be directly linked to the assembly of miRISC. If assayed for processing activity *in vitro*, immunoprecipitates of Dicer-1 or Loqs readily generated mature miRNAs from synthetic precursors and were also found to associate with pre- and mature miRNAs *in vivo.*

So what is the actual function of Loqs in this complex? It is certainly not simply the stabilization of Dicer-1, because Dicer-1 protein levels did not decrease significantly in the absence of Loqs. According to Saito *et al.* [14], Loqs confers substrate specificity for pre-miRNAs to Dicer-1. Surprisingly, Dicer-1 processes long dsRNA as well as pre-miRNA substrates, if Loqs is removed from the complex. Re-addition of Loqs inhibited dsRNA processing and enhanced pre-miRNA processing. But how is this achieved? Three splice variants of *loqs* are known, of which only two isoforms interact with Dicer-1. Interestingly, the third isoform lacks the non-canonical dsRBD, suggesting that this domain may be essential for association with Dicer-1. Loqs could enhance the binding
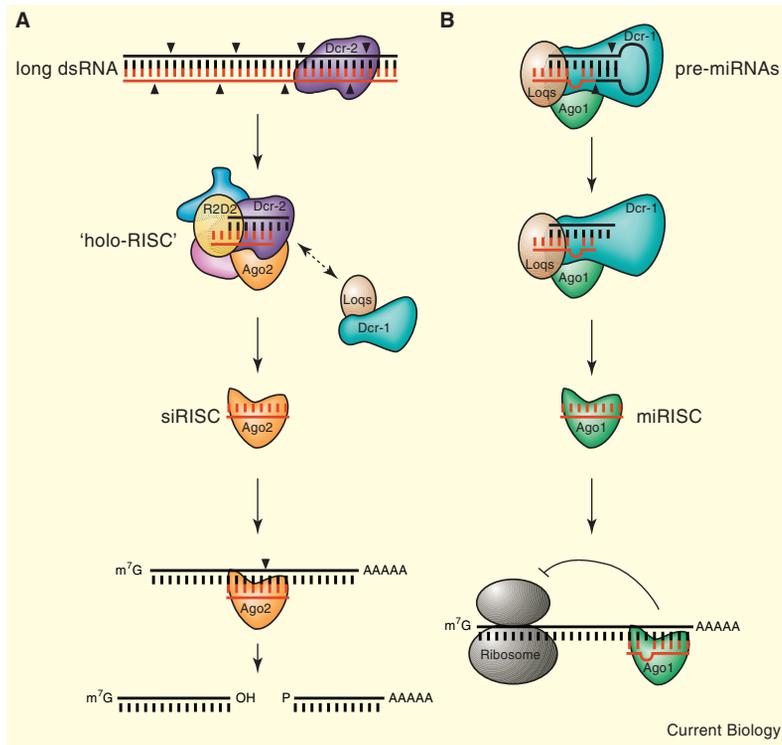
Figure 1. Model of the Dicer-1 and Dicer-2 pathways in *Drosophila*.

(A) The RNase III-like enzyme Dicer-2 (Dcr-2) is needed to process long dsRNAs into duplex siRNAs. R2D2, a dsRBD protein, helps to sense duplex asymmetry and, together with Dicer-2, facilitates the assembly of an 80S 'holo-RISC' [16], a multi-protein complex. Both Dicer-1 and Loqs play a role downstream of siRNA production, probably as components of the putative 'holo-RISC' complex. Upon binding to complementary sequences in target RNAs, RISC cleaves target mRNAs. (B) A protein complex consisting of the RNase III-like enzyme Dicer-1 (Dcr-1), the dsRBD protein Loqs and Ago1 is able to convert pre-miRNA hairpins into ~22 nt miRNA duplexes, which are then loaded into the miRISC, composed of a single stranded miRNA bound to Ago1. This silencing complex inhibits translation by binding to complementary sequences in the 3′ untranslated region of cognate messenger RNAs.

efficiency of Dicer-1 to pre-miRNAs, thus stabilizing this interaction. Alternatively, it could position Dicer-1 in such a way that allows efficient processing, or introduce conformational changes in Dicer-1 leading to an enhancement of pre-miRNA cleavage.

In order to assess the *in vivo* role of *loqs* in greater detail, Förstemann *et al.* [15] took advantage of a hypomorphic *loqs* mutant. These flies showed reduced silencing of a miRNA-controlled transgene as well as defects in silencing triggered by long dsRNA. Thus, Loqs is not only required for efficient miRNA-mediated silencing, but also cooperates with Dicer-1 in order to enhance silencing by siRNAs, emphasising the idea of a crosstalk between the Dicer-1 and Dicer-2 pathways [10,16]

(Figure 1A). Furthermore, the authors show that *loqs* plays a critical role in the maintenance of germ-line stem cells. While the two Loqs isoforms interacting with Dicer-1 are present throughout the adult fly, they show sex-specific expression in the gonads. Mutant female flies are sterile and lack germ-line stem cells. Mutations in *piwi*, a member of the Argonaute protein family, result in a similar phenotype [17], implying that *dicer-1* and *loqs* might function together with *piwi*.

With the discovery of *loqs*, it now seems a general feature of RNase III enzymes to cooperate with dsRBD-proteins in RNA silencing. This raises a couple of exciting questions. Both groups highlight the homology of Loqs with the human dsRBD protein HIV TAR RNA binding protein (TRBP), which binds to a

hairpin-shaped TAR RNA, implicated in the response to HIV infection [18]. Moreover, TAR RNA has recently been suspected to be a viral pre-miRNA [19]. The homology between Loqs and TRBP is especially intriguing, as there is no clear human homolog for R2D2. Furthermore, human Dicer is more closely related to *Drosophila's* Dicer-1. As Dicer-1 and Loqs are required for miRNA- and siRNA-mediated gene silencing, and Dicer-1 even shows weak siRNA-generating activity in the absence of Loqs, it seems plausible that human Dicer could function in a fashion similar to Dicer-1. Dicer-2 may play a unique role in *Drosophila*. Thus, findings in *Drosophila* siRISC biogenesis should be applied carefully to the human system.

Beyond that, Loqs may be involved in processing other types of non-coding RNAs. Further studies on other binding partners will certainly help to characterize its function in greater detail. There are more than 70 miRNAs known in *Drosophila* to date [20], of which all pre-miRNAs fold into precursor hairpins which do not share common sequence motifs. So how does the Dicer-1–Loqs complex specifically recognize its substrates? By now, this question cannot be answered. Analyses of the double-stranded stem as well as the terminal loop structure of pre-miRNAs will make it possible to elucidate the precise molecular mechanism of substrate recognition.

References
1. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature *391*, 806–811.
2. Meister, G., and Tuschl, T. (2004). Mechanisms of gene silencing by double-stranded RNA. Nature *431*, 343–349.
3. Ambros, V. (2004). The functions of animal microRNAs. Nature *431*, 350–355.
4. Lee, Y., Kim, M., Han, J., Yeom, K.H., Lee, S., Baek, S.H., and Kim, V.N. (2004). MicroRNA genes are transcribed by RNA polymerase II. EMBO J. *23*, 4051–4060.
5. Gregory, R.I., Yan, K.P., Amuthan, G., Chendrimada, T., Doratotaj, B., Cooch, N., and Shiekhattar, R. (2004). The Microprocessor complex mediates the genesis of microRNAs. Nature *432*, 235–240.
6. Denli, A.M., Tops, B.B., Plasterk, R.H., Ketting, R.F., and Hannon, G.J. (2004). Processing of primary microRNAs by the

Microprocessor complex. Nature *432*, 231–235.

7. Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Radmark, O., Kim, S., *et al.* (2003). The nuclear RNase III Drosha initiates microRNA processing. Nature *425*, 415–419.

8. Landthaler, M., Yalcin, A., and Tuschl, T. (2004). The human DiGeorge syndrome critical region gene 8 and its *D. melanogaster* homolog are required for miRNA biogenesis. Curr. Biol. *14*, 2162–2167.

9. Schwarz, D.S., Hutvagner, G., Du, T., Xu, Z., Aronin, N., and Zamore, P.D. (2003). Asymmetry in the assembly of the RNAi enzyme complex. Cell *115*, 199–208.

10. Lee, Y.S., Nakahara, K., Pham, J.W., Kim, K., He, Z., Sontheimer, E.J., and Carthew, R.W. (2004). Distinct roles for Drosophila Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. Cell *117*, 69–81.

11. Bernstein, E., Caudy, A.A., Hammond, S.M., and Hannon, G.J. (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature *409*, 363–366.

12. Okamura, K., Ishizuka, A., Siomi, H., and Siomi, M.C. (2004). Distinct roles for Argonaute proteins in small RNA-directed RNA cleavage pathways. Genes Dev. *18*, 1655–1666.

13. Liu, Q., Rand, T.A., Kalidas, S., Du, F., Kim, H.E., Smith, D.P., and Wang, X. (2003). R2D2, a bridge between the initiation and effector steps of the Drosophila RNAi pathway. Science *301*, 1921–1925.

14. Saito, K., Ishizuka, A., Siomi, H., and Siomi, M.C. (2005). Processing of pre-microRNAs by the Dicer-1-Loquacious complex in *Drosophila* cells. PLoS Biol. *3*, e235.

15. Förstemann, K., Tomari, Y., Du, T., Vagin, V.V., Denli, A.M., Bratu, D.P., Klattenhoff, C., Theurkauf, W.E., and Zamore, P.D. (2005). Normal microRNA maturation and germ-line stem cell maintenance requires Loquacious, a double-stranded RNA-binding domain protein. PLoS Biol. *3*, e236.

16. Pham, J.W., Pellino, J.L., Lee, Y.S., Carthew, R.W., and Sontheimer, E.J. (2004). A Dicer-2-dependent 80s complex cleaves targeted mRNAs during

RNAi in Drosophila. Cell *117*, 83–94.

17. Cox, D.N., Chao, A., Baker, J., Chang, L., Qiao, D., and Lin, H. (1998). A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. Genes Dev. *12*, 3715–3727.

18. Gatignol, A., Buckler-White, A., Berkhout, B., and Jeang, K.T. (1991). Characterization of a human TAR RNA-binding protein that activates the HIV-1 LTR. Science *251*, 1597–1600.

19. Bennasser, Y., Le, S.Y., Yeung, M.L., and Jeang, K.T. (2004). HIV-1 encoded candidate micro-RNAs and their cellular targets. Retrovirology *1*, 43.

20. Griffiths-Jones, S. (2004). The microRNA registry. Nucleic Acids Res. *32*, D109–D111.

Cell Division: SIN, Cytokinesis and Ethanol Dependency

A novel mutant screen in fission yeast has identified the 'ethanol dependent' protein etd1p as a potential link between the septation initiation network (SIN), which initiates cytokinesis, and the actomyosin contractile ring that drives separation of the two daughter cells at the end of mitosis.

Andrea Krapp and
Viesturs Simanis

Cells of the fission yeast *Schizosaccharomyces pombe* are rod-shaped, grow mainly by elongation at their tips and divide by means of a medially placed division septum. The site of division is established early in mitosis by the formation of an actomyosin-based contractile ring at the cell cortex. The ring assembles throughout mitosis and contracts at the end of anaphase, coincident with breakdown of the mitotic spindle [1].

The initiation of septum formation is signaled from the spindle pole body by the septation initiation network, or SIN. The core components of the SIN are three protein kinases — cdc7p, sid1p, sid2p, in presumed order of action — and their associated regulatory/targeting subunits — spg1p, cdc14p and mob1p, respectively. SIN signaling also requires the protein kinase plo1p, which stands at the head of the signaling network. The proteins assemble at the spindle pole body on a scaffold composed of the coiled-coil proteins sid4p and cdc11p. The nucleotide status of the GTPase spg1p is regulated by the byr4p–cdc16p GTPase activating protein (GAP) complex. Constitutive signaling gives rise to multiseptated cells; in contrast, loss-of-function mutants for any of the core components of the SIN or the anchoring scaffold, or function-specific mutants of *plo1*, fail to signal septum formation, becoming multinucleated as growth and the nuclear cycle continue in the absence of cytokinesis (for reviews see [2,3]).

How do the SIN and the contractile ring communicate? Of the known core SIN proteins and regulators, only plo1p, sid2p–mob1p and the negative regulator dma1p associate with both the ring and the spindle pole body. Association of sid2p–mob1p with the ring is thought to be part of the trigger for its contraction. However, the identity of the ring component(s) that might link the SIN and the ring has remained elusive.

A new study by Daga *et al.* [4] has identified a novel protein, etd1p, that may link the SIN and the contractile ring. The 'ethanol dependent' *etd1* gene was identified in a screen for *S. pombe* mutants that were either hypersensitive to, or dependent upon, ethanol in the medium [5]. Interestingly, *etd1* has not been identified in any screen for thermosensitive mutants, suggesting that this novel selection scheme may allow isolation of mutants in proteins with structures that do not accommodate changes conferring thermosensitivity.

Etd1 is an essential gene: both the *etd1* mutation and a null give rise to elongated, multinucleated cells which resemble mutants with defects of the SIN or contractile ring. Unfortunately, etd1p's sequence gives no clue to its biochemical function, and there are no obvious homologs in other eukaryotes.

GFP-tagged etd1p localises to the cell cortex in interphase, with a bias for the growing cell tips. Though it lacks any obvious membrane-targeting sequences, the protein seems to associate with sterol-rich membrane domains. At the onset of mitosis it forms a broad band overlying the