

# A Dead End for MicroRNAs

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**Dead end (Dnd1) is an RNA-binding protein that mediates germ-cell viability and suppresses the formation of germ-cell tumors. Kedde et al. (2007) now provide evidence that Dnd1 mediates these effects by counteracting microRNA-mediated silencing of mRNAs.**

Despite the fact that germ cells are specified through very different mechanisms in various species, the genes that are required for their survival and further development appear to be remarkably similar. One such highly conserved gene encodes the RNA-binding protein Dead end (Dnd1). Dnd1 has been shown to regulate germ-cell viability and suppress the formation of germ-cell tumors, yet how it exerts these effects has remained unresolved. Work by Kedde et al. (2007) presented in this issue of *Cell* now suggests that by binding to target mRNAs, Dnd1 blocks their interaction with microRNAs (miRNAs) and thereby protects mRNAs from miRNA-mediated repression.

Dnd1 was first characterized as a gene required for germ-cell viability in a large-scale screen in zebrafish aimed at identifying mRNAs that are specifically found in germplasm (also known as nuage), a granular structure characteristic of germ cells (Weidinger et al., 2003). Following this, a premature stop codon in Dnd1 was identified in Ter mutant mice, which display germ-cell loss in addition to a high frequency of testicular germ-cell tumors in one genetic background (Youngren et al., 2005). Yet, apart from the fact that Dnd1 most likely affected some aspect of RNA biology, a molecular understanding of Dnd1 function was completely lacking.

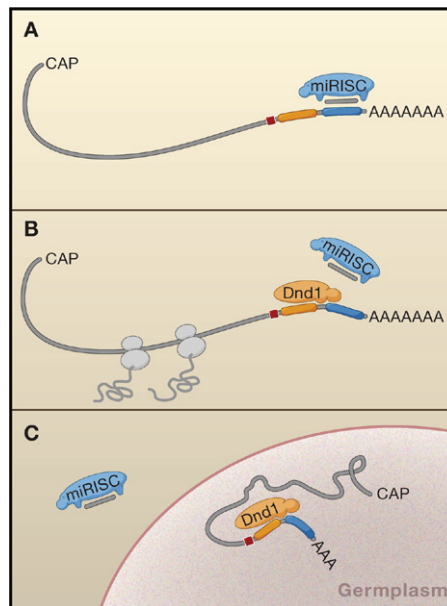
Increasing evidence suggests that miRNAs play an important role in germ-cell biology. In general, miRNAs silence

mRNA translation and often affect the stability of the targeted mRNAs (Valencia-Sanchez et al., 2006). MicroRNAs carry out these effects by binding to homologous regions in the mRNA, usually in the 3' untranslated regions (UTRs) as part of a ribonucleoprotein complex named miRISC (Figure 1A). Voorhoeve et al. (2006) showed that miRNAs may act as oncogenes in human germ cells. In zebrafish, *nanos* and *tdrd7*

are maternally derived mRNAs that are cleared from somatic cells by the miR-430 family (Mishima et al., 2006). Yet, in germ cells, *nanos* and *tdrd7* mRNAs persist despite the presence of miR-430. Kedde et al. now establish a mechanism that connects these two observations by revealing a role for Dnd1 in repressing miRNA-mediated silencing.

Using cell culture-based assays the authors find that Dnd1 is able to elevate the expression of reporter genes carrying 3'UTRs of the genes encoding the tumor suppressors *LATS2* and *p27*. Previous work showed that miR-372 and miR-373 may act as oncogenes in germ cells by targeting the *LATS2* 3'UTR (Voorhoeve et al., 2006). Importantly, the ability of Dnd1 to elevate expression of these reporters depends on the presence of a functional miRNA-binding site in the reporter (miR-372 in the case of the *LATS2* reporter and miR-221 for the *p27* reporter). Agami and colleagues then go on to show that Dnd1 binds to U-rich regions that are located close to the miRNA recognition sites in the *p27* 3'UTR. Without these U-rich regions Dnd1 cannot affect miRNA action. Yet, the *LATS2* 3'UTR does not have a clearly recognizable U-rich region and still is affected by Dnd1.

Importantly, the authors extend their findings to settings where endogenous Dnd1 protein could be analyzed. They show that Dnd1 is required in zebrafish for the remarkable resistance of *nanos* and *tdrd7* mRNA to miR-



**Figure 1. Dnd1 Promotes mRNA Stability**

(A) MicroRNAs (miRNAs) are part of a multisubunit complex, often referred to as miRISC. This complex can bind to mRNAs when a region in the mRNA (blue box) is homologous to the miRNA. MicroRNAs inhibit translation and decrease the stability of target mRNAs.

(B) When the Dnd1 protein binds to a U-rich region in the mRNA (orange box), it can block recognition of the mRNA by miRISC, allowing translation of the mRNA.

(C) Alternatively, Dnd1 may translocate the bound mRNA to structures that are inaccessible to miRISC, such as the germplasm of germ cells.

430. Also, in these cases, the miRNA-binding site is flanked by a U-rich region that is required for Dnd1 to function. Does this fully explain the germ-cell defects observed upon knocking down Dnd1 in zebrafish? This remains an open question, as the experiment required to test this has not been done: if the germ-cell defects that arise when Dnd1 is knocked down are due to miRNA-mediated silencing, then taking away Dicer, a key enzyme in miRNA generation, should rescue the germ-cell defect inflicted by loss of Dnd1. The authors also found that Dnd1 protects *LATS2* from being targeted by miR-372 in a human teratoma-derived cell line (Tera1). In fact, miR-372 is highly expressed in many testicular germ-cell tumors (Voorhoeve et al., 2006). Hence, overexpression of miR-372 may be selected during tumorigenesis to overcome the inhibitory effect of Dnd1. Unfortunately, an extensive correlation between the presence of Dnd1 and miRNA expression in these tumors is not available at the moment.

Together, these results strongly suggest that endogenous Dnd1 affects miRNA-mediated repression of specific mRNAs. How does Dnd1 do this? As the authors show that Dnd1 prevents the association of miR-221 with the *p27* 3'UTR, there are at least two possibilities that come to mind, which are not mutually exclusive (Figure 1). First, Dnd1 may physically block access to an miRNA target site. In fact, the U-rich regions in the *nanos* and *tdrd7* 3'UTRs are located directly 5' of the miR-430 seed match (the seed match is the most 5' part of the miRNA, which is very important for target-site recognition). Although pairing between the miRNA and the bases upstream of the seed match

may not be required for miRNA activity, it seems likely that miRISC will be sterically hindered by a protein binding to that region. However, Kedde et al. also show that a single U-rich region in the 3'UTR of *p27* that is not directly flanking an miRNA target site can still prevent miRNA-mediated silencing. Clearly, at present, we know too little about the structural aspects of both miRISC target recognition and Dnd1 binding to RNA to address these issues. Another possibility is that Dnd1 may change the subcellular localization of an mRNA, taking it out of reach of miRNAs. In agreement with this, Dnd1 localizes to discrete perinuclear granules in primordial germ cells (Weidinger et al., 2003). Although the absence of antibodies to Dnd1 has prohibited analysis of its subcellular distribution during early embryogenesis, it may, like the *nanos* and *tdrd7* mRNAs, also localize to germplasm at the early cleavage stages of zebrafish development. Whether these mRNAs are translated at that time is not clear, but in general these granular structures are thought to be repressive in nature. Later, when the germ-cell precursors have reached the future gonad, *nanos* and *tdrd7* mRNAs become cytoplasmic. Around this time miR-430 levels drop (Giraldez et al. 2005) and storage of these mRNAs in protective structures may no longer be required.

As 3'UTRs of mRNAs are targeted by numerous RNA-binding proteins, only one of which is miRISC, it is not surprising to see evidence accumulating that miRNA-mediated silencing is tuned by RNA-binding proteins that are not directly part of the miRNA complex. For example, the RNA-binding protein HuR can relieve miRNA-mediated repression in human hepatocarcinoma cells

(Bhattacharyya et al., 2006), and miRNA activity at synapses appears to be regulated in a similar manner (Schratt et al., 2006; Ashraf et al., 2006). It was only a matter of time before we would hit upon modulators of miRNAs that may have medical relevance. Kedde et al. have shown that such endeavors have surely not met a dead end.

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#### REFERENCES

- Ashraf, S.I., McLoon, A.L., Sclarsic, S.M., and Kunes, S. (2006). *Cell* 124, 191–205.
- Bhattacharyya, S.N., Habermacher, R., Martine, U., Closs, E.I., and Filipowicz, W. (2006). *Cell* 125, 1111–1124.
- Giraldez, A.J., Cinalli, R.M., Glasner, M.E., Enright, A.J., Thomson, J.M., Baskerville, S., Hammond, S.M., Bartel, D.P., and Schier, A.F. (2005). *Science* 308, 833–838.
- Kedde, M., Strasser, M.J., Boldajipour, B., Oude Vrielink, J.A.F., Slanchev, K., le Sage, C., Nagel, R., Voorhoeve, P.M., van Duijse, J., Andersson Örom, U., et al. (2007). *Cell*, this issue.
- Mishima, Y., Giraldez, A.J., Takeda, Y., Fujiwara, T., Sakamoto, H., Schier, A., and Inoue, K. (2006). *Curr. Biol.* 16, 2135–2142.
- Schratt, G.M., Tuebing, F., Nigh, E.A., Kane, C.G., Sabatini, M.E., Kiebler, M., and Greenberg, M.E. (2006). *Nature* 439, 283–289.
- Valencia-Sanchez, M.A., Liu, J., Hannon, G.J., and Parker, R. (2006). *Genes Dev.* 20, 515–524.
- Voorhoeve, P.M., le Sage, C., Schrie, M., Gillis, A.J.M., Stoop, H., Nagel, R., Liu, Y., van Duijse, J., Drost, J., Griekspoor, A., et al. (2006). *Cell* 124, 1169–1181.
- Weidinger, G., Stebler, J., Slanchev, K., Dumstrei, K., Wise, C., Lovell-Badge, R., Thisse, C., Thisse, B., and Raz, E. (2003). *Curr. Biol.* 13, 1429–1434.
- Youngren, K.K., Coveney, D., Peng, X., Bhattacharya, C., Schmidt, L.S., Nickerson, M.L., Lamb, B.T., Deng, J.M., Behringer, R.R., Capel, B., et al. (2005). *Nature* 435, 360–364.