

Semiconserved Regulation of Mesendoderm Differentiation by microRNAs

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MicroRNAs are known to play important roles in many different processes. However, their roles in shaping the early steps in embryogenesis have remained largely hidden. In this issue of *Developmental Cell*, Rosa et al. show that the miR-430/427/302 family of microRNAs has a distinct effect on Nodal signaling, affecting mesendoderm differentiation in *Xenopus* embryos and human embryonic cell lines.

MicroRNAs (miRNAs) are small RNA molecules that affect gene expression through lowering mRNA stability and translation efficiency. They are derived from long primary transcripts that are converted into 60- to 70-nucleotide-long hairpin intermediates, named pre-miRNAs, which can be processed further by the Dicer enzyme. The resulting double-stranded RNA molecule is then loaded into a member of the Argonaute (Ago) protein family, which removes in a nonrandom fashion one of the two strands, and remains bound to the mature miRNA molecule (Carthew and Sontheimer, 2009). The Ago protein exposes part of the miRNA, the seed region, so that it is available for basepairing to mRNAs. In animal systems, the seed region, although only six or seven bases long, is all that is required for a miRNA to recognize a target site, which is most often located within the 3' untranslated regions (3'UTRs) of an mRNA (Bartel, 2009). Interestingly, many miRNAs consist of families in which all the members share a common seed sequence, but vary widely in their sequence elsewhere. Because the seed region is the main determinant of specificity, it is believed that most members of a given miRNA family will regulate a very similar set of target mRNAs.

Many different types of genes can be targets of miRNAs. Therefore it is remarkable that miRNAs appear to be not widely used in early stages of embryogenesis. One notable exception to this is the miR-430 family of miRNAs, which is expressed and required before gastrulation in zebrafish (Giraldez et al., 2005). The main function ascribed to this miRNA family is clearance of maternal mRNAs: many

maternally provided mRNAs contain miR-430 target sites in their 3'UTR, and these sites trigger rapid mRNA destabilization once miR-430 expression starts (Giraldez et al., 2006). Embryos lacking both maternal and zygotic Dicer therefore suffer from the extended expression of many maternal messengers. However, this does not rule out the possibility that miR-430 family members are involved in tuning specific pathways as well. In fact, a more detailed analysis of miR-430 in zebrafish revealed a role in fine-tuning Nodal signaling through targeting two diffusible ligands acting in this pathway: the agonist *squint* (Nodal), and the antagonist *Lefty* (Choi et al., 2007).

In this issue of *Developmental Cell*, Rosa et al. now show that this mode of Nodal regulation is strongly conserved at the level of *Lefty* expression. First, they show that the miR-430 homolog in *Xenopus* (miR-427) can regulate both *Lefty* genes (*Lefty A* and *B*). As in zebrafish, loss of miR-427 function leads to an inhibition of Nodal signaling, with concomitant loss of mesendodermal cell fates. Very similar, if not identical, phenotypes can be induced by specifically blocking miR-427 target sites in both *Lefty* genes, suggesting that these are the most relevant targets for the observed phenotypes. Interestingly, Rosa et al. find that in human ESCs (hESCs) the miR-430 family member miR-302 also has a very strong effect on Nodal signaling: by blocking *Lefty*, miR-302 strongly affects the differentiation potential of hESCs. It should be mentioned that hESCs express at least two other miR-430 family members: miR-372 and 373 (Suh et al., 2004). It is therefore not clear why inhibition of miR-302 alone elicits such a strong response.

It is possible that basepairing outside the seed region is relevant in this scenario, or that a difference in expression levels among the different miRNAs accounts for this result. Alternatively, the antisense miRNA inhibitory oligos that were used to target miR-302 could perhaps also inhibit miR-372 and 373.

Although inhibition of miR-430 members leads to a block in mesendoderm differentiation in all three systems described above, the strength of the phenotype is different in each case (Rosa et al., 2009). In hESCs the block in mesendoderm differentiation is very strong; it is somewhat weaker in *Xenopus* and only mild in zebrafish. A plausible explanation is the extent to which Nodal is regulated by miR-430 family members. Humans only have one Nodal gene and this gene is not a target of miR-302. In contrast, zebrafish have three Nodal genes, and perhaps the most relevant Nodal gene in this context, *squint*, is itself a miR-430 target (Choi et al., 2007). *Xenopus* has at least five Nodal genes, of which only *Xnr5* and *Xnr6b* are targets of miR-427. *Xnr1*, *Xnr2*, and *Xnr4* are not targets, while they are relevant to the phenotypes observed. Thus it appears that the impact of miR-430/427/302 on Nodal signaling strongly depends on the relative amount of targeting of both agonist and antagonist (Figure 1).

Why then would different Nodal genes be differently affected by miRNAs? The role of miR-430 in clearing maternal mRNAs may provide a clue. Zebrafish *squint* is maternally provided and after onset of zygotic transcription, the maternal pool, which is uniformly distributed over the embryo, may need to be removed to establish proper ratios between relatively local *squint* activity

and long-range Lefty activity (Branford and Yost, 2004). The switch to zygotic transcription happens much earlier in human development, long before Nodal signaling becomes important. So although miRNAs are thought to affect maternal messengers (Murchison et al., 2007), maternal Nodal mRNA does not need to be removed by miR-302. In *Xenopus*, none of the Xnr transcripts are transmitted maternally; however, the two Xnr genes that are under control of miR-427 are expressed significantly earlier, before the Mid-Blastula-Transition (pre-MBT), than the others (Yang et al., 2002). Therefore it appears that miR-430/427/302-guided regulation is preferentially imposed on those Nodal genes that are present at very early stages, either through pre-MBT transcription or by maternal transmission, while it is dispensable for those transcribed at later stages.

Rosa et al. have set the stage for investigations on the roles of miRNAs in tuning early differentiation pathways in multiple systems,

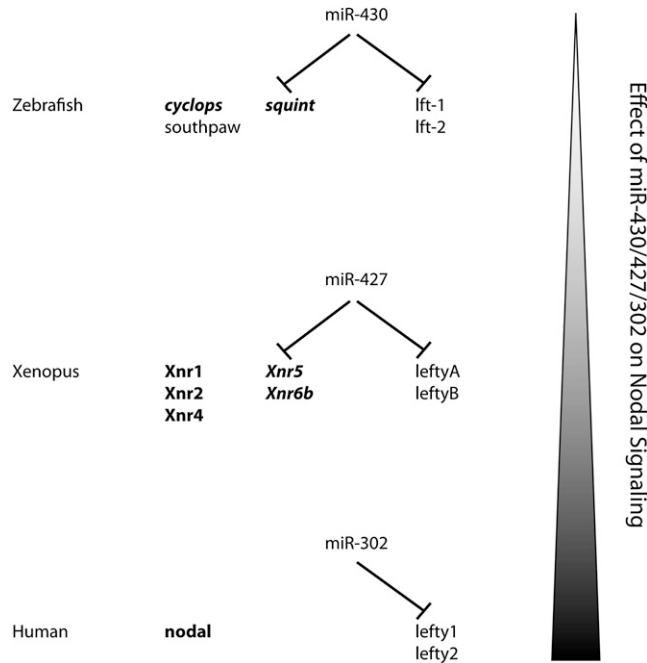


Figure 1. Differential Effects of miR-430/427/302 on Nodal Signaling in Different Species

When moving from zebrafish to *Xenopus* to human, relatively more Nodal genes (on the left side) escape regulation by miR-430/427/302, while in all systems both Lefty genes (on the right side) are strong targets. The change in target range results in differential sensitivity of the pathways to miR-430/427/302. Nodal genes in bold are expressed during the time window relevant for the observed phenotypes. Nodal genes in italics are provided maternally or transcribed pre-MBT.

including hESCs. It will be very exciting to learn to what extent we can use

miRNAs to steer ESCs in the directions we want them to go.

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