Dissecting the roles of *X. laevis* miRNAs and Ago proteins in RISC activity

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MicroRNAs function by guiding RNP complexes, called RNA Induced Silencing Complexes (RISCs), to targeted mRNAs. Interaction of RISCs with mRNAs results in silencing of mRNA expression through inhibition of translation initiation or elongation, or through destabilization of the mRNA via deadenylation or direct cleavage. These processes are mediated by Argonaute (Ago) proteins, members of a class of proteins that form the core of RISCs, which recruit facilitating proteins such as specific deadenylases, adapter or silencing factors. Thus, the fate of the mRNA is likely to be determined by proteins that are associated with the miR•RISC. It is unclear what causes one of these activities to predominate, although a perfectly base-paired miR-mRNA interaction is needed for cleavage of the mRNA (RNAi) by the inherent endonuclease activity of Ago2.

We have found that in *Xenopus laevis* embryos, one of the earliest zygotic Pol II transcripts is pri-miR-427, which is processed in a step-wise manner into pre-miRNA-427 (a short imperfect hairpin structure) and mature miR-427 (23 ntd) by the RNaseIII-type endonucleases Drosha and Dicer, respectively. Up to 10^6 copies/cell of miR-427 accumulate by ~ 8 hours after fertilization, at the **m**id**b**lastula **t**ransition (MBT). A **m**iR-427 **r**ecognition **e**lement (MRE_{427}) present in the 3' UTRs of several maternally inherited mRNAs is necessary and sufficient to support miR-427-directed rapid deadenylation and destabilization shortly after MBT. An unrelated sequence, $MRE_{let\text{-}7}$ can also direct deadenylation if the cognate miRNA, let-7, is furnished exogenously (by injection of pre-let-7 RNA), showing that the identity of proteins in embryonic RISCs are determinants for deadenylation, rather than the miRNA itself.

Curiously, the profile of Ago proteins, in *Xenopus* early embryos appears to be unusual. Ago-2, a major RISC component in many cells, is absent, so embryos are unable to cleave mRNAs, even using endogenous miR-427 as a guide. Moreover, **s**hort **i**nterfering RNA duplexes (siRNAs), which ordinarily can contribute one strand to RISCs, actually inhibit processing of pre-miRNAs, and they do so independent of sequence. This inhibition can be suppressed by the introduction of exogenous human Ago-2, which also restores the capacity of miR-427 (or $siRNA_{427}$) to function in RNAi. Dicer activity, but not Dicer protein, increases during egg maturation, in parallel with increases in over-all amounts of Ago proteins. We conclude that early *Xenopus laevis* embryos are deficient in Ago-2, and perhaps other Ago proteins, and that expression of Ago proteins is subject to developmental control.