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Expression of microRNAs during embryonic development of *Xenopus tropicalis*

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ABSTRACT

microRNAs (miRNAs) are short, non-coding RNAs that regulate gene expression and have prominent roles during early embryo development and organogenesis. We set out to determine the expression pattern of miRNAs in the developmental model system, *Xenopus tropicalis*. We made probes to predicted primary-miRNA transcripts and performed in situ hybridization. Our data show conserved and novel tissue-specific expression patterns during embryogenesis that suggest functional roles during development.

1. Results and discussion

microRNAs are a recently discovered class of small, non-coding RNAs encoded by the genome that regulate genes post-transcriptionally. They are transcribed by RNA polymerase II as primary microRNAs (pri-miRNAs), which are then processed by the enzymes Drosha and Dicer to generate the mature single-stranded miRNA of ~22 nucleotides, which is incorporated into the RNA-induced silencing complex (RISC), characterized by the presence of the Argonaute family of proteins (Pasquinelli et al., 2005). This complex is responsible for the regulatory function of the miRNAs. Though microRNAs are present in all metazoan taxa (Hertel et al., 2006), few studies have addressed the *in vivo* function of these molecules during development. In order to understand the function of miRNAs during embryogenesis of the developmental model organism *Xenopus*, it is important to know the spatiotemporal expression profiles of these genes throughout development. Though other groups have cloned miRNAs from *Xenopus laevis* embryos (Watanabe et al., 2005), there is as yet no data on the spatiotemporal expression of miRNAs during embryogenesis. Therefore, we generated approximately 1-kb digoxigenin-labeled antisense probes to 60 predicted *Xenopus tropicalis* miRNAs and hybridized them to neurula, tailbud, and tadpole stage embryos. Probes to 18 of the miRNAs gave spatially distinct patterns above background, and these are shown in Fig. 1.

Many of the *X. tropicalis* miRNA expression patterns are conserved across animal species. miR-1a-1 is expressed in mesoderm in *Drosophila* (Aboobaker et al., 2005; Chen et al., 2006), zebrafish (Chen et al., 2006), and mouse (Zhao et al., 2007) and can clearly be seen in the trunk mesoderm of the neurula stage embryo and in somites at the tailbud stage (Fig. 1B′). Similarly, miR-133 is expressed in muscle tissue (Chen et al., 2006) (Fig. 1O′). Among other conserved expression patterns (Arora et al., 2007; Deo et al., 2006; Mansfield et al., 2004), miR-124 is highly expressed in the entire central nervous system (Fig. 2N–R), miR-9 is expressed in the brain (Fig. 1D–F′ and Fig. 2A–G), miR-7 is expressed in the eye (Fig. 1C′), and miR-10 is expressed in the posterior region of the tailbud embryo (Fig. 1G). These expression patterns are conserved across large evolutionary distances.

For the 42 probes that failed to detect distinct patterns of expression above background, we cannot say that this is due to a lack of expression. A negative result could be the result of very rapid processing of the primary transcript, low levels of expression, expression at a different stage of development, or a non-optimized hybridization probe or procedure. Some probes gave ubiquitous expression patterns, but we have not eliminated the possibility that these probes caused non-specific background staining. Probes that gave tissue-specific expression patterns were more enriched for miRNAs that are intergenic (17/18, 94%) than the total collection of probes (48/61, 79%), though the significance of this is not clear. miR-133b is the only intronic miRNA whose expression is investigated here. It is found in the second intron of a homolog of Pkhd1, whose expression in *Xenopus* is unreported, and where no ESTs have been found among the 1.2 million in genbank. In mice, however, this gene is expressed in the developing kidney (Nagasawa et al., 2002), a tissue in which miR-133b is not detected.

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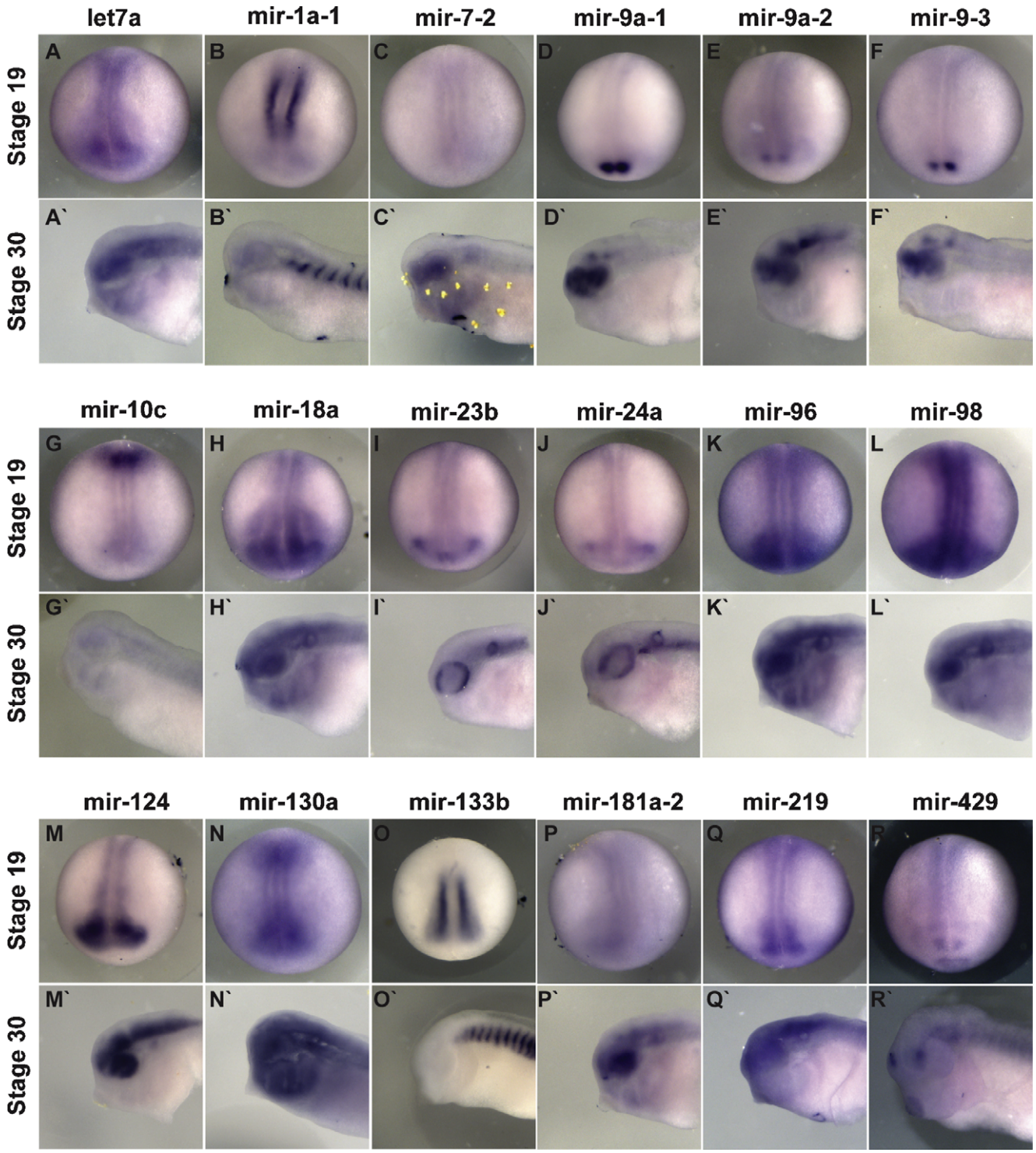

Fig. 1. Expression patterns of *X. tropicalis* miRNAs at neurula (A–R, dorsal view) and early tadpole stages (A′–R′, lateral view, anterior to the left).

Using probes for the entire primary-miRNA transcript allowed us to differentiate between the expression patterns of genes that have identical mature miRNA sequences. The three paralogous miR-9 genes show unique but overlapping expression patterns (Fig. 1D–F′). The most highly expressed at all stages is miR-9a-1. miR-9a-2 has very low levels of expression in the anterior-most portion of the neural plate at the neurula stage, but expression increases at tailbud stages. miR-9-3 displays intermediate expression levels at both these stages. Interestingly, during the tailbud stage, miR-9a-2 has a broader expression pattern than the other paralogs, encompassing not just the eye and forebrain, but also the hindbrain. However, by the tadpole stage, all show similar expression patterns to miR-9a-1 (Fig. 2A and data not shown).

A cluster of miRNAs containing miR-23b and miR-24a is found in the *X. tropicalis* genome, along with miR-27b (Griffiths-Jones et al., 2008). Non-overlapping probes to miR-23b and miR-24a revealed identical novel expression patterns, first appearing at stage 19 in the eye anlagen and posterior cells around the blastopore