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Restriction of the *Xenopus DEADSouth* mRNA to the primordial germ cells is ensured by multiple mechanisms

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ABSTRACT

DEADSouth mRNA encoding the RNA helicase DDX25 is a component of the germ plasm in *Xenopus laevis*. We investigated the mechanisms underlying its specific mRNA expression in primordial germ cells (PGCs). Based on our previous findings of several microRNA miR-427 recognition elements (MREs) in the 3′ untranslated region of the mRNA, we first examined whether *DEADSouth* mRNA was degraded by miR-427 targeting in somatic cells. Injection of antisense miR-427 oligomer and reporter mRNA for mutated MREs revealed that *DEADSouth* mRNA was potentially degraded in somatic cells via miR-427 targeting, but not in PGCs after the mid-blastula transition (MBT). The expression level of miR-427 was very low in PGCs, which probably resulted in the lack of miR-427-mediated degradation. In addition, the *DEADSouth* gene was expressed zygotically after MBT. Thus, the predominant expression of *DEADSouth* mRNA in the PGCs is ensured by multiple mechanisms including zygotic expression and prohibition from miR-427-mediated degradation.

1. Introduction

In *Xenopus*, the germline is generated by the inheritance of a special cytoplasm, called 'germ plasm', which is present at vegetal cortex of the fertilized egg (Ikenishi, 1998). Recently, using cytoplasmic transfer, we demonstrated that the germ plasm contains a determinant necessary and sufficient for germline development in *Xenopus laevis* (Tada et al., 2012). *DEADSouth* mRNA has been identified as a component of the germ plasm (MacArthur et al., 2000). Expression of the *DEADSouth* gene begins in early diplotene oocytes during oogenesis. The transcript is initially present in the ooplasm and perinuclear mitochondrial aggregates in pre-stage I oocytes, accumulates in the mitochondrial cloud at stage I and then localizes to the germ plasm in the vegetal cortex of mature oocytes. After fertilization, *DEADSouth* mRNA is present in the germ plasm and is detectable in the primordial germ cells (PGCs) at least until the tailbud stage embryo (Kataoka et al., 2006). Thus, the expression profile of *DEADSouth* is similar to that of other germ plasm components including *nanos1* mRNA (Zhou and King, 2004). Such persistent expression in PGCs suggests that *DEADSouth* is very important for PGC development.

Previously, we demonstrated that the *DEADSouth* 3′ untranslated region (UTR) was involved in the PGC-specific expression of a reporter protein (Kataoka et al., 2006). This was initially detected in embryos around stage 7 and then appeared to be restricted to PGCs in the mid-blastula transition

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(MBT, around stage 9) and onwards, with loss of expression in somatic cells. This suggested that PGC-specific expression might result from clearance of the reporter mRNA in somatic cells. Dissection of the *DEADSouth* 3′ UTR allowed to define region A as a region required for the PGC-specific expression. In addition, we found three possible microRNA miR-427 recognition elements (MREs) in region A. Because it is well known that miR-427 in *Xenopus* and miR-430 in zebrafish—an equivalent to miR-427—are involved in the clearance of multiple maternal mRNAs with MREs at the MBT (Giraldez et al., 2006; Lund et al., 2009), *DEADSouth* mRNA might also be a target of miR-427-mediated mRNA clearance. In zebrafish, germline-specific mRNAs including *nanos1* and *TDRD7* (Tudor-domain-containing protein 7) have MREs for miR-430 and degrade in somatic cells after the MBT (Giraldez et al., 2006). However, in PGCs, they are protected from miR-430-mediated degradation by the DAZL protein (Mishima et al., 2006; Takeda et al., 2009). In *Xenopus*, *dead end* (*dnd*) mRNA is a component of germ plasm and is detected in a PGC-specific manner until tailbud stage (Horvay et al., 2006). Recently, it was shown that *dnd* mRNA was also degraded in somatic cells by targeting of miR-18, but not of miR-427 (Koebernick et al., 2010).

In this study, we focus on regulatory mechanisms for PGC-specific expression of *DEADSouth* mRNA. We suggest that even if it is in somatic cells, *DEADSouth* mRNA is degraded by miR-427 targeting, but not in PGCs. In addition, zygotic expression also ensures persistent presence of this mRNA in PGCs after the MBT.

2. Results

2.1. *miR-427 targets DEADSouth mRNA degradation in somatic cells*

Our previous deletion analysis of the 3′ UTR of *DEADSouth* mRNA showed that a 368-nucleotide sequence, designated region A, regulates its PGC-specific expression (Fig. 1A; Kataoka et al., 2006). In addition, we found three possible target sites for miR-427 (microRNA recognition element: MRE) in region A (Fig. 1B). Therefore, we speculated that *DEADSouth* mRNA might be degraded by miR-427 in the somatic cells. First, we examined the stability of the reporter mRNA encoding the fluorescent protein Venus fused with the *DEADSouth* 3′ UTR (*v-DS*, Kataoka et al., 2006) in the somatic cells of miR-427-depleted embryos (Fig. 2). To deplete miR-427, we injected an antisense miR-427 locked nucleic acid oligomer (anti-miR427 LNA) together with reporter *v-DS* mRNA into the animal hemisphere of fertilized *X. laevis* eggs. As a control, embryos were also coinjected with a mutated LNA oligomer (anti-miR427mut LNA). Embryos at stages 11 and 30 were subjected to reverse transcription polymerase chain reaction (RT-PCR) analysis to monitor the level of the injected *v-DS* mRNA. Although the injected *v-DS* mRNA remained at stage 11 regardless of the coinjected LNA oligomers, it was detected only in the miR-427-depleted embryos at stage 30 by RT-PCR (Fig. 2A). This was also confirmed by the observation of Venus fluorescence from *v-DS* mRNA (Fig. 2B–D).

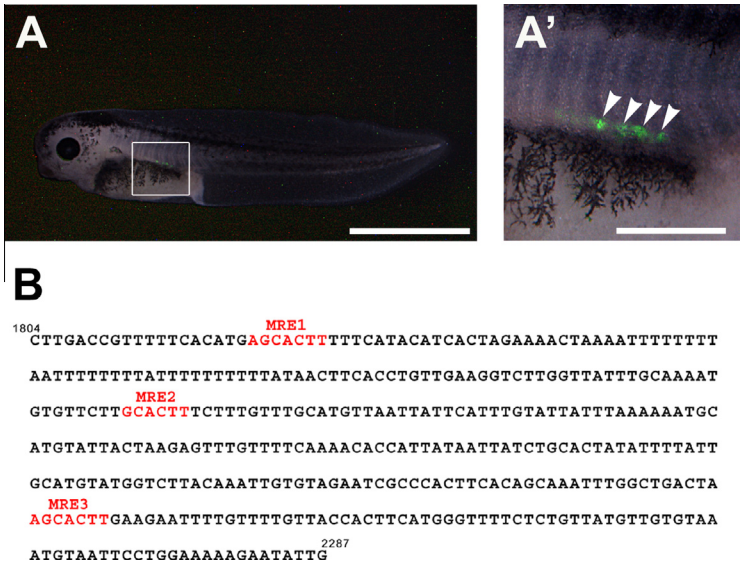

Fig. 1 – Expression pattern of *venus* fused with region A of *DEADSouth* 3′ UTR in the middle of 3′ UTR of *Xenopus* β-globin (A and A′) and the sequence of region A (B). (A) Whole-mount lateral view of stage 41 tadpole. Scale bar: 1 mm. (A′) High-magnification of the area indicated in (A). Arrowheads indicate PGCs. Scale bar: 250 μm. (B) The possible target sites for miR-427 (MRE, in red) found in the region A of *DEADSouth* 3′ UTR (DDBJ/GenBank/EMBL accession No. AF190623 nucleotides 1804–2287).