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Spatiotemporal localization of germ plasm RNAs during zebrafish oogenesis

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Abstract

In zebrafish, primordial germ cells (PGCs) are determined by a specialized maternal cytoplasm, the germ plasm, which forms at the distal ends of the cleavage furrows in 4-cell embryos. The germ plasm includes maternal mRNAs from the germline-specific genes such as *vasa* and *nanos1*, and vegetally localized *dazl* RNA is also incorporated into the germ plasm. However, little is known about the distributions and assembly mechanisms of germ plasm components, especially during oogenesis. Here we report that the germ plasm RNAs vasa, nanos1, and dazl co-localize with the mitochondrial cloud (MC) and are transported to the vegetal cortex during early oogenesis. We found that a mitochondrial cloud localization element (MCLE) previously identified in the 3′ untranslated region (3′UTR) of *Xenopus Xcat2* gene can direct RNA localization to the vegetal cortex via the MC in zebrafish oocytes. In addition, the RNA-binding protein Hermes is a component of the MC in zebrafish oocytes, as is the case in *Xenopus*. Moreover, we provide evidence that the *dazl* 3′UTR possesses at least three types of *cis*-acting elements that direct multiple steps in the localization process: MC localization, anchorage at the vegetal cortex, and localization at the cleavage furrows. Taken together, the data show that the MC functions as a conserved feature that participates in transport of the germ plasm RNAs in *Xenopus* and zebrafish oocytes. Furthermore, we propose that the germ plasm components are assembled in a stepwise and spatiotemporally-regulated manner during oogenesis and early embryogenesis in zebrafish.
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1. Introduction

In many animals, including *Caenorhabditis elegans*, *Drosophila*, and *Xenopus*, maternal mRNA localization plays a crucial role in germ cell formation. Primordial germ cells (PGCs) are formed via the inheritance of a specialized cytoplasm called the germ plasm, which contains an electron-dense, non-membrane bound structure called the germinal granule (reviewed in Eddy, 1975; Wylie, 1999). In *Drosophila*, *oskar* and *nanos* mRNAs, which are important for germ plasm (pole plasm) assembly and germ cell function, are localized to the posterior pole of the oocyte (Ephrussi et al., 1991; Kim-Ha et al., 1991; Wang and Lehmann, 1991). Similarly, in frogs, the germ plasm is present at the vegetal pole of oocytes (Ikenishi et al., 1974; Mahowald and Hennen, 1971; Williams and Smith, 1971).

There are two major pathways for vegetal localization of maternal mRNAs during *Xenopus* oogenesis, the early and late pathways (Kloc and Etkin, 1995). *Vg-1* and *VegT* mRNAs, which encode germ layer determinants, are transported to the vegetal cortex via the late pathway in a microtubule-dependent manner. In contrast, maternal mRNAs incorporated into the germ plasm, such as Xcat2 (Zhou and King, 1996), Xpat (Hudson and Woodland, 1998), and Xdazl (Houston et al., 1998), utilize the early or messenger transport organizer (METRO) pathway, by associating with a structure called the mitochondrial cloud (MC), also known as the Balbiani body (reviewed in King et al., 2005; Kloc and Etkin, 2005). The germ plasm RNAs localize by non-directed movement and entrapment within the MC (Chang et al., 2004). Many studies have shown that

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the 3′ untranslated region (3′UTR) governs mRNA localization. For example, the *Xcat2* 3′UTR possesses a mitochondrial cloud localization element (MCLE) and a germinal granule localization element (GGLE) (Kloc et al., 2000; Zhou and King, 1996). Interestingly, Betley et al. (2002) reported that clusters of CAC-containing motifs are a ubiquitous signal for RNA localization in the early and late pathways. The *Xcat2* MCLE contains several copies of the UGCAC motif. Deletion of the motifs from the *Xcat2* MCLE abolishes localization (Betley et al., 2002). However, little is known about the *trans*-acting factors involved in targeting the germ plasm RNAs to the MC, although a few protein constituents of the MC have been identified (King et al., 2005). Recently, it was reported that Xpat protein is involved in germ plasm assembly, although homologs have not been identified outside the genus *Xenopus* (Machado et al., 2005).

In zebrafish, maternal mRNAs of germline-specific genes (i.e. *vasa*, *nanos1*, *dead-end (dnd)*, *askopos (kop)* and *TDRD7*) are localized to the distal ends of the first and second cleavage planes at 4-cell stage (Blaser et al., 2005; Köprunner et al., 2001; Mishima et al., 2006; Weidinger et al., 2003; Yoon et al., 1997). Knock-down experiments using antisense morpholino oligos have shown that *nanos1* and *dnd* genes are essential for proper development of germ cells (Köprunner et al., 2001; Weidinger et al., 2003). Our previous study showed that at this stage, *DAZ-like (dazl)* and *bruno-like (brul)* mRNAs are also localized to the cleavage furrows in addition to the vegetal pole of oocytes and fertilized eggs (Hashimoto et al., 2004). Electron-microscopic analyses showed that germinal granule-like structures are localized at the furrows of 4-cell embryos, and *vasa* transcripts are embedded in these structures (Knaut et al., 2000). Moreover, ablation of the cytoplasm at the sites results in the loss of PGCs (Hashimoto et al., 2004). These findings demonstrate that the maternally supplied cytoplasm containing localized mRNAs at the cleavage furrows in 4-cell stage embryos functions as germ plasm in zebrafish.

It is of great interest to learn the mechanisms that govern localization and assembly of the germ plasm-forming RNAs in zebrafish. Recently, Theusch et al. (2006) reported that a first class of germ plasm RNAs, *vasa*, *nanos1*, and *dnd*, are enriched in a wide cortical band at the animal pole in the freshly laid zebrafish egg, whereas a second class of RNAs that includes vegetally localized *dazl* mRNA translocate along the plane of the cortex towards the animal pole. After recruitment to the cleavage furrows, these two classes of RNAs occupy overlapping but distinct regions of the germ plasm (Theusch et al., 2006).

Less is known about the distributions of the germ plasm RNAs during oogenesis. Here, we focused on localization of the germ plasm components, *vasa*, *nanos1*, and *dazl* mRNAs, during zebrafish oogenesis. To our surprise, we found that both classes of germ plasm RNAs co-localize with the mitochondrial cloud (MC) and are transported to the vegetal cortex during early oogenesis, and that their distributions change during late oogenesis. We provide evidence that in zebrafish and *Xenopus*, vegetal localization of germ plasm RNAs is directed by the METRO pathway: *Xcat2* mRNA is localized to the MC when expressed in zebrafish oocytes and the RNA-binding protein Hermes, a constituent of the MC in *Xenopus* (Zearfoss et al., 2004), is also observed at the MC in zebrafish. The localization of the germ plasm mRNAs is directed by their 3′UTRs, and by analyzing transgenic fish expressing GFP mRNA fused with various truncated forms of *dazl* 3′UTR, we found that *dazl* 3′UTR possesses multiple *cis*-elements required for localization. These independent elements direct MC localization, anchorage at the vegetal cortex, and localization to embryonic cleavage furrows. Thus, the results of our study suggest that germ plasm components are assembled in a stepwise and spatiotemporally-regulated manner during oogenesis and early embryogenesis in zebrafish.

2. Results

2.1. Germ plasm RNAs co-localize with the mitochondrial cloud in zebrafish oocytes

To identify in detail the distribution patterns of germ plasm RNAs during oogenesis, we performed *in situ* hybridization on serial sections of the zebrafish ovary. As we previously described (Maegawa et al., 1999; Suzuki et al., 2000), *dazl* RNA can be observed adjacent to the germinal vesicle at stage I and localized to the vegetal cortex of stage II oocytes (Fig. 1). To our surprise, the distributions of *vasa* and *nanos1* transcripts were quite similar to that of *dazl* RNA during early oogenesis. In stage I oocytes, *vasa* and *nanos1* RNAs were tightly co-localized with *dazl* RNA, forming an aggregate adjacent to the germinal vesicle (Fig. 1a–d). Subsequently, these maternal RNAs became restricted to the vegetal cortex (Fig. 1e–h).

However, at stage II, the distribution patterns of *vasa*, *nanos1* and *dazl* became distinct (Fig. 1i–l). By stage II, *dazl* RNA was localized strictly at the vegetal cortex, whereas *vasa* RNA formed small particles broadly around the vegetal cortex. *nanos1* RNA became to be distributed throughout the oocyte. Later in oogenesis, *vasa* mRNA is present at the cortex as reported previously (Braat et al., 1999; Howley and Ho, 2000) (Fig. 1m). However, we could not observe localization of *nanos1* (Fig. 1o). Co-localization of *vasa*, *nanos1* and *dazl* mRNAs in stage I oocytes suggests that the same pathway directs each of these germ plasm RNAs to the vegetal cortex during early oogenesis.

The distributions of *vasa*, *nanos1* and *dazl* RNAs in early oocytes are reminiscent of the METRO (messenger transport organizer) pathway, which is involved in germ plasm formation in *Xenopus* (Kloc and Etkin, 1995). We therefore hypothesized that a METRO-like pathway exists in zebrafish oocytes and controls vegetal localization of maternal RNAs. To address this hypothesis, we first stained zebrafish oocytes with Mitotracker Red, a

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