Developmental Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Role of maternal *Xenopus syntabulin* in germ plasm aggregation and primordial germ cell specification

Denise Oh, Douglas W. Houston*

The University of Iowa, Department of Biology, 257 BB, Iowa City, IA 52242-1324, USA

ARTICLE INFO

Keywords:
Xenopus
Germ plasm
Primordial germ cells
Germline
Mitochondrial transport

ABSTRACT

The localization and organization of mitochondria- and ribonucleoprotein granule-rich germ plasm is essential for many aspects of germ cell development. In Xenopus, germ plasm is maternally inherited and is required for the specification of primordial germ cells (PGCs). Germ plasm is aggregated into larger patches during egg activation and cleavage and is ultimately translocated perinuclearly during gastrulation. Although microtubule dynamics and a kinesin (Kif4a) have been implicated in Xenopus germ plasm localization, little is known about how germ plasm distribution is regulated. Here, we identify a role for maternal Xenopus Syntabulin in the aggregation of germ plasm following fertilization. We show that depletion of *sybu* mRNA using antisense oligonucleotides injected into oocytes results in defects in the aggregation and perinuclear transport of germ plasm and subsequently in reduced PGC numbers. Using live imaging analysis, we also characterize a novel role for Sybu in the collection of germ plasm in vegetal cleavage furrows by surface contraction waves. Additionally, we show that a localized kinesin-like protein, Kif3b, is also required for germ plasm aggregation and that Sybu functionally interacts with Kif3b and Kif4a in germ plasm aggregation. Overall, these data suggest multiple coordinate roles for kinesins and adaptor proteins in controlling the localization and distribution of a cytoplasmic determinant in early development.

1. Introduction

Cell polarity and intracellular localization in the egg are important for many aspects of normal embryonic development, including overall body patterning and proper germ cell development. The role of a localized “germ plasm” in the formation of the germline has been particularly well studied. Germ plasm was first identified as a histologically distinct region localized within eggs and embryonic germline cells of various organisms and is composed mainly of cytoplasmic aggregates of mitochondria and other organelles, dense ribonucleoprotein germ line granules, and a collection of proteins and RNAs (reviewed in Beams and Kessel, 1974; Houston and King, 2000; Extavour and Akam, 2003; Aguero et al., 2017). The presence of germ plasm and certain localized RNAs is required for germline development in organisms such as *Drosophila*, *Xenopus* and zebrafish. Although maternal germ plasm per se is notably absent from the mammalian egg and early blastocyst (and also from salamander/newt eggs), the differentiating germ cells of these organisms are known to contain a “nuage” substance that is similar in ultrastructure to germ plasm (Eddy, 1975). Nuage also houses a similar set of localized proteins and RNAs and functions during later aspects of germ cell differentiation (Houston and King, 2000; Voronina et al., 2011).

Whereas the overall role of the germ plasm in germ cell biology is well appreciated, the mechanisms controlling its assembly and localization in vertebrates are less well understood. In *Xenopus* eggs, germ plasm is found dispersed in small islands throughout the vegetal cortex (Czołowska, 1969; Whitington and Dixon, 1975). Germ plasm is thought to originate in the mitochondrial cloud/Balbiani body of previtellogenic oocytes, which accumulates germ plasm-specific mRNAs, including *nanos1, dazl,* and *vasa homologs*. The mitochondrial cloud is fragmented during early oogenesis (stage II in *Xenopus*) and its material localizes to the future vegetal pole of the growing oocyte (Kloc and Etkin, 1995; Kloc et al., 1998; Choo et al., 2005). Following oocyte maturation and subsequent fertilization, the germ plasm undergoes additional local clustering and further aggregates into larger masses at the vegetal pole. Aggregation continues during the cleavage stages, mediated in part by surface contraction waves (SCWs) in the egg and blastomeres undergoing mitosis, and is followed by “ingression” of germ plasm along the vegetal cleavage furrows (Ressom and Dixon, 1988; Savage and Danilchik, 1993). At these stages, germ plasm is asymmetrically inherited, resulting in an initial PGC population of about four cells. During gastrulation, the germ plasm transitions from a

* Corresponding author.
E-mail address: douglas-houston@uiowa.edu (D.W. Houston).

http://dx.doi.org/10.1016/j.ydbio.2017.10.006
Received 5 June 2017; Received in revised form 20 September 2017; Accepted 11 October 2017

peripheral position to a perinuclear localization and is subsequently inherited symmetrically by daughter cells, presumptively increasing the number of cells specified toward the germline (reviewed in Houston and King, 2000).

Previous inhibitor-based studies in *Xenopus* have indicated that both local and SCW-mediated germ plasm aggregation is microtubule dependent and likely independent of actin microfilaments (Ressom and Dixon, 1988; Savage and Danilchik, 1993). Correspondingly, the germ plasm is enriched in microtubules and depletion of maternal *kif4a* mRNA (*Xklp1*; Robb et al., 1996) inhibited local and SCW-mediated germ plasm aggregation, in the latter case by seemingly inhibiting SCW propagation. Depletion of *kif15* (*Xklp2*) did not affect germ plasm aggregation (Robb et al., 1996), suggesting that different kinesin family members may play discrete roles in germ plasm localization. It is unclear whether Kif4a-mediated germ plasm aggregation is conserved in vertebrates. In zebrafish, germ plasm assembly appears to occur at the cleavage stages and involves a two-stage localization of RNAs to the blastodisc at the animal pole, followed by actin and microtubule mediated mechanisms acting at cleavage furrows (Theusch et al., 2006; Eno and Pelegri, 2016). It is thus likely that the germ plasm assembly process has been modified during evolution of the teleost egg/embryo morphology, and despite the similarity in overall composition and role of the cytoskeleton, the specific mechanisms regulating germ plasm aggregation in either organism are not well understood.

Another conserved cytoplasmic localization event occurring in fertilized eggs is the putative translocation of determinants by microtubule-mediated cortical rotation leading to dorsal axis formation (reviewed in Houston, 2013, 2017). Interestingly, several maternal genes involved in *Xenopus* axis formation exhibit at least partial mRNA localization to the germ plasm in eggs and embryos. These include *trim36*, *dead end homolog 1 (dnd1)* and *plin2*. Importantly, mRNA depletion of maternal stores of these molecules leads to loss of cortical rotation and to defects in dorsal axis formation (Chan et al., 2007; Cuykendall and Houston, 2009; Mei et al., 2013). In zebrafish, two maternal-effect mutants deficient in axis formation have been identified that encode mRNAs localized to the mitochondrial cloud and vegetal cortex of the oocyte and egg (*tokkebi (tkk)/syntabulin (sybu)*, Nojima et al., 2010; *hecate/grip2a*, Ge et al., 2014). Both gene products are potentially involved in microtubule organization and/or trafficking. However, both Sybu and Grip2a protein and RNA fail to accumulate in the aggregating germ plasm following its transport to the forming blastodisc and thus do not localize to embryonic germ plasm. By contrast, in *Xenopus*, *sybu* and *grip2a* do associate with germ plasm in oocytes (Colozza and De Robertis, 2014; Kaneshiro et al., 2007; Tarbashevich et al., 2007) but also remain localized to the germ plasm throughout early embryogenesis. Depletion of Grip2a in fertilized embryos using morpholino antisense oligos in *Xenopus* can result in PGC migration defects (Kirilenko et al., 2008) but a role in axis formation was not specifically tested by depletion of maternal *grip2a* mRNA in oocytes. Maternal depletion of *sybu* by the host-transfer procedure does indeed result in axis deficient embryos (Colozza and De Robertis, 2014) but it remains unclear to what extent Sybu is acting similarly in fish and frogs. Nevertheless, the localization of *sybu* mRNA to the germ plasm in cleavage stage embryos prompted us to examine its roles in the germ plasm.

This work focuses on Sybu, previously characterized as a kinesin motor linker protein involved in the transport of mitochondria, syntaxin-associated vesicles and other cargoes in neuronal axons (Su et al., 2004; Cai et al., 2005, 2007; Frederick and Shaw, 2007). We therefore hypothesized that Sybu would function in linking germ plasm to microtubule motors via mitochondria or other vesicles. Here we show that maternal *sybu* is required for PGC formation in Xenopus, likely by mediating kinesin-dependent aggregation in the early embryo. We also define the roles of additional kinesins in germ plasm aggregation and show that germ plasm aggregation at the vegetal pole is coupled to the surface contraction waves occurring after vegetal cleavage furrow formation.

2. Results

2.1. Syntabulin is associated with germ plasm in Xenopus

In Xenopus and zebrafish, *sybu* is expressed in the mitochondrial cloud/Balbiani body (Bb) of pre-vitellogenic oocytes and in the vegetal

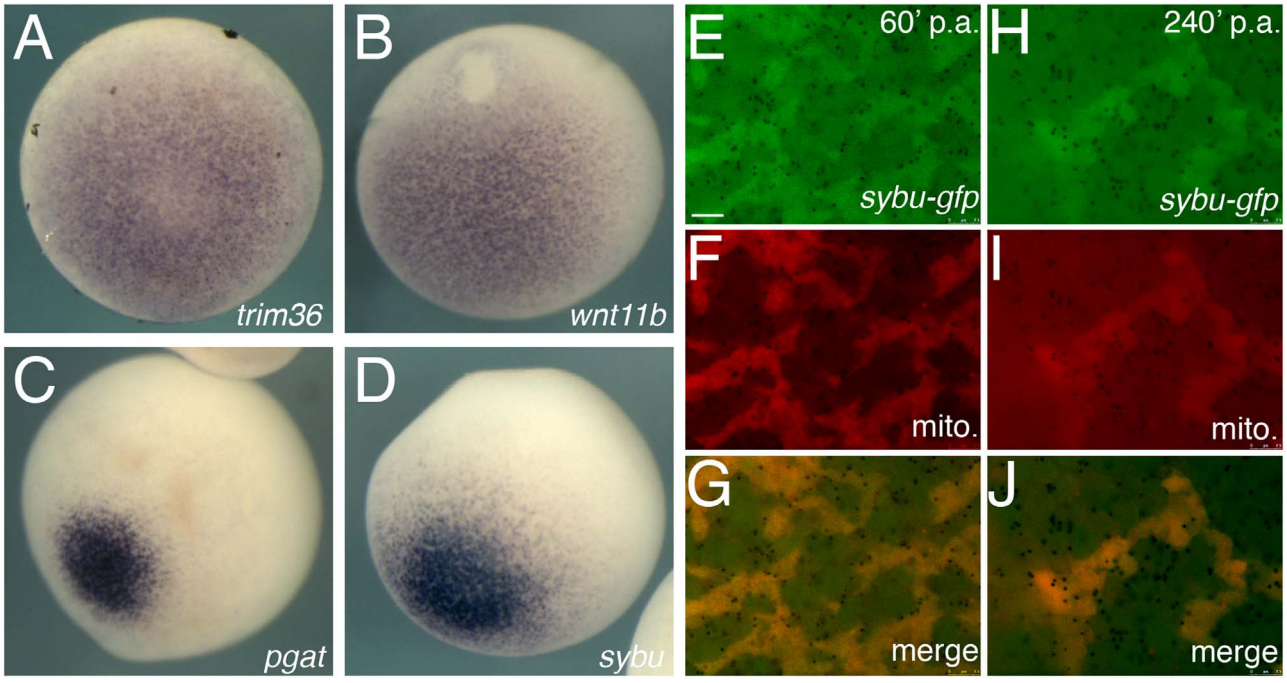

Fig. 1. Germ plasm enrichment of Sybu mRNA and protein in oocytes and early embryos. (A-D) Representative images of whole-mount in situ hybridization on stage VI oocytes comparing expression of (A) *trim36*, (B) *wnt11b*, (C) *pgat* and (D) *sybu*. Vegetal views are shown. (E-J) Localization of Sybu-GFP to germ plasm. (E-G) Overlap of Sybu-GFP with clustered mitochondria (mito., MitoTracker) in pricked eggs 60-min post-activation (p.a.) and at 240-min post-activation (p.a.). Scale bars represent 7.5 μm.