

Lin28 is Required for Single Niche Development in the *Drosophila* Male Gonad

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Conflict of interests

The authors declare no potential conflict of interest.

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Authors' contributions

Conceptualization: Sreejith P.

Data curation: Sreejith P.

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Writing-review & editing: Sreejith P, Kim C.

Ethics approval

This article does not require IRB/IACUC approval because there are no human

Abstract

A stem cell niche provides an environment that governs stem cell maintenance and division. Thus, the development of a proper niche is of prime importance to stem cell behaviors. Mechanisms of niche development are beginning to be revealed in the *Drosophila* male gonad. Niche cells are initially dispersed throughout the gonad, then assemble at its apical tip through the anterior migration of posteriorly located niche cells. The molecular mechanisms of this migration and assembly are still poorly understood. Here we show evidence suggesting that Lin28, an RNA-binding protein and regulator of *let7* genesis, might be an intrinsic factor for the anterior migration of niche cells. We found that a dispersed, ectopic niche, a phenotype observed with anterior migration defects, occurs in *lin28* mutant gonads. This phenotype is rescued by expression of *lin28* in the niche cells. These findings suggest that Lin28 might be required for the anterior migration of niche cells.

Keywords: *lin28*, niche, *drosophila*, stem cells, testis

INTRODUCTION

The maintenance of adult organs depends on the intricate regulation of stem cell self-renewal and differentiation. Stem cell behavior is governed by its local microenvironment, termed the stem cell niche (Morrison & Spradling, 2008), which plays a pivotal role in adult organ maintenance. Niche signaling to stem cells is relatively well studied, but how a niche is established during development remains poorly understood.

In the *Drosophila* male gonad, a single niche comprised of hub cells is located at the apical tip of the testis, wherein it contacts and regulates the behaviors of adjacent stem cells of two types, germline stem cells (GSCs) and cyst stem cells (CySCs) (Gönczy & DiNardo, 1996; Kiger et al., 2001; Greenspan et al., 2015). GSCs self-renew to produce a gonoblast, which undergoes four mitotic cell divisions to produce 16 germline cells, while CySCs self-renew to produce somatic cyst cells that enwrap the germline cells (Zoller & Schulz, 2012). The niche secretes signaling molecules, including unpaired, decapentaplegic, glass bottom boat and hedgehog that govern stem cell behaviors and stemness (Greenspan et al., 2015; Eslahi et al., 2024). However, the mechanism by which this single apical niche is formed is largely unknown.

The apical location of the gonad niche is established during embryogenesis (Le Bras & Van Doren,

and animal participants.

2006; Dinardo et al., 2011; Zamfirescu et al., 2022). Niche cells are specified from somatic gonadal precursor cells of mesodermal origin, and in mid-embryos are initially dispersed throughout the spherical gonad (Boyle & DiNardo, 1995; Le Bras & Van Doren, 2006; Kitadate & Kobayashi, 2010; Okegbe & DiNardo, 2011). Subsequently, posteriorly located niche cells migrate anteriorly to assemble with the anteriorly located niche cells, thereby forming a single niche at the gonad anterior (Anllo et al., 2019; Anllo & DiNardo, 2022). Defects in the signaling directing this migration result in the dispersed formation of an extra niche (Anllo & DiNardo, 2022); however, the molecular mechanisms of anterior migration and niche assembly are still poorly understood.

Lin28 is an evolutionarily conserved RNA-binding protein and also a regulator of let-7 microRNA genesis (Krsnik et al., 2022). In the adult *Drosophila* testis, Lin28 is exclusively expressed in niche cells, wherein it has been demonstrated to be required for the maintenance of the number of niche cells and niche architecture (Sreejith et al., 2019). Here, we find that Lin28 is required in niche cells for the formation of a single niche in the embryonic gonad.

MATERIALS AND METHODS

1. Immunohistochemistry

Immunostaining was done as previously reported (Fidler et al., 2014). Briefly, third instar male larvae were dissected in Schneider's media. The posterior region of the dissected larvae, containing the testis, was fixed in 4% formaldehyde in phosphate-buffered saline for 20 minutes. The larvae were then incubated in primary antibodies overnight at 4°C. The next day, secondary antibody treatment was performed, and then the testis was carefully detached from the posterior region using tungsten needles and mounted with an antifade reagent. The following primary antibodies were used at the indicated concentrations: rat anti-VASA 1:400 (Developmental Studies Hybridoma Bank, DSHB) and guinea pig anti-Traffic Jam 1:10,000 (Dorothea Godt, Toronto, ON, Canada). The corresponding secondary antibodies were used at 1:500 concentration (Alexa Series, Invitrogen, Carlsbad, CA, USA).

2. *Drosophila* stocks

Flies were reared in standard cornmeal/agar medium at 25°C with 50% humidity and a 12 h light/dark cycle. *W^{#118}* (used as wild-type) and *lin-28^{EP915}* were obtained from the Bloomington *Drosophila* Stock Center. *esg-GAL4* was a kind gift from S. Dinardo. *UAS-lin28* was generated in the lab (Sreejith et al., 2019).

RESULTS AND DISCUSSION

We marked niche cells using antibodies against Traffic jam (Tj), a Maf transcription factor (Li et al., 2003; Sinden et al., 2012; Fairchild et al., 2016), which is expressed in niche cells and germline cells using Vasa (Sinden et al., 2012; Sreejith et al., 2019). Immunohistochemistry of wild-type larval male gonads showed a single niche, while *lin28* mutant gonads (*lin28^{EP915}*) (Sreejith et al., 2019) demonstrated dispersed, ectopic niches (Fig. 1). These findings suggest that Lin28 is required for the formation of a single niche in the gonads. To test this potential requirement, we sought to express Lin28 specifically in niche cells within the *lin28* mutant male gonad. We employed a Gal4/UAS binary expression system in which the UAS-downstream gene is expressed in specific cells under the Gal4 driver (Brand & Perrimon, 1993). To express Gal4 in the niche cells, we used the *esg-Gal* driver (Voog et al., 2008, 2014; Sênos Demarco et al., 2022). The *UAS-lin28* line was previously generated in our lab (Sreejith et al., 2022). With this system, we generated a rescue

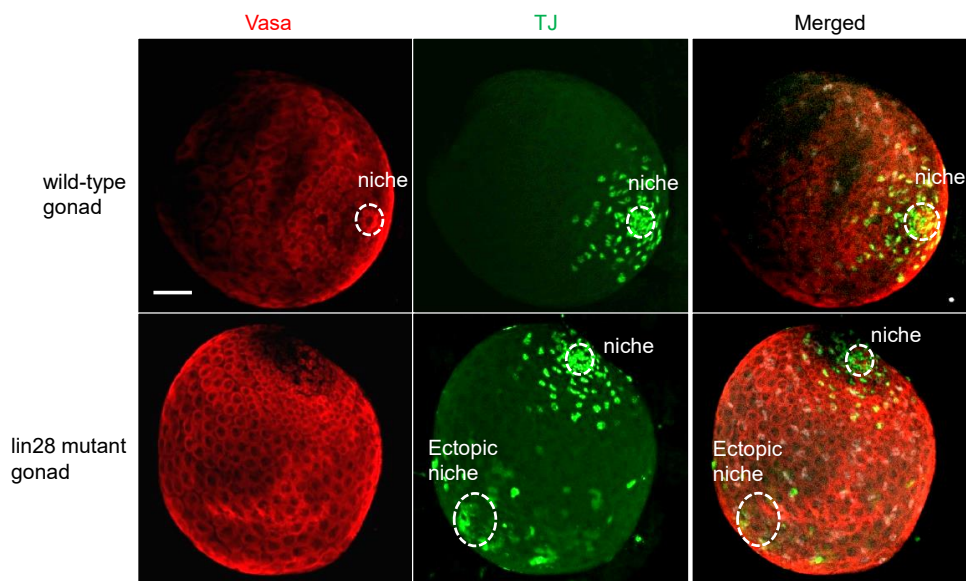


Fig. 1. Ectopic niche in the *lin28* mutant gonad. Confocal images of male gonads from 3rd instar larvae stained with Tj (niche marker) and vasa (germline marker) antibodies. A single niche is formed in the wild-type gonad ($n=5$). An ectopic niche is formed in the *lin28* mutant gonad (detected in three out of five gonads examined). Scale bar, 5 μ m.

line that harbors *esg-Gal4* and *UAS-lin28* in the *lin28* mutant background (*esg-Gal4*, *UAS-lin28*; *lin28^{EP915} / lin28^{EP915}*), and therefore expresses *lin28* in niche cells within a *lin28* mutant male gonad. Immunohistochemistry demonstrated that individuals of this rescue line exhibit a single niche, indicating that Lin28 is required in niche cells to form a single niche in the gonads (Fig. 2).

The *Drosophila* testis contains a single niche at the apical tip of the gonad (Kiger et al., 2001). How this single niche is formed is largely unknown, despite such mechanisms being of prime interest in the stem cell field. Here we show that Lin28 is required in gonad niche cells for this process of single niche formation. Firstly, we show that the formation of a single niche is aberrantly disrupted in *lin28* mutant gonads. Secondly, specific expression of *lin28* in niche cells in the *lin28* mutant background restores the single niche. These data highlight a requirement of Lin28 in niche cells for the formation of a single niche.

In the development of this singular niche, niche cells are initially dispersed throughout the gonad (Le Bras & Van Doren, 2006; DeFalco et al., 2008; Anllo et al., 2019; Anllo & DiNardo, 2022). Those located posteriorly then migrate anteriorly and assemble with their anteriorly located counterparts to produce the single niche (Anllo et al., 2019; Anllo & DiNardo, 2022). Defects in this anterior migration process are known to result in a dispersed, ectopic niche in the gonads (Anllo & DiNardo, 2022). The observation of similar phenotypes in *lin28* mutant gonads supports the proposition that Lin28 might play a role in the anterior migration of niche cells (Fig. 3).

This raises the question: What function does Lin28 perform in niche cells? Studies have shown that Lin28 mediates the stability of niche-specific mRNAs in niche cells (Sreejith et al., 2019; To et al., 2021). Thus, Lin28 might stabilize mRNAs that are themselves involved in the anterior migration of niche cells. Candidate targets of Lin28 include the transcription factor *islet*, which was recently shown to be expressed in niche cells and required for anterior niche cell migration (Anllo & DiNardo, 2022), and also regulators and components of the actin cytoskeleton, which mediate cell migration in diverse cellular contexts. Alternatively, Lin28 is also known to regulate the genesis of let7 microRNAs, which might play a role in the anterior migration. Future research is required

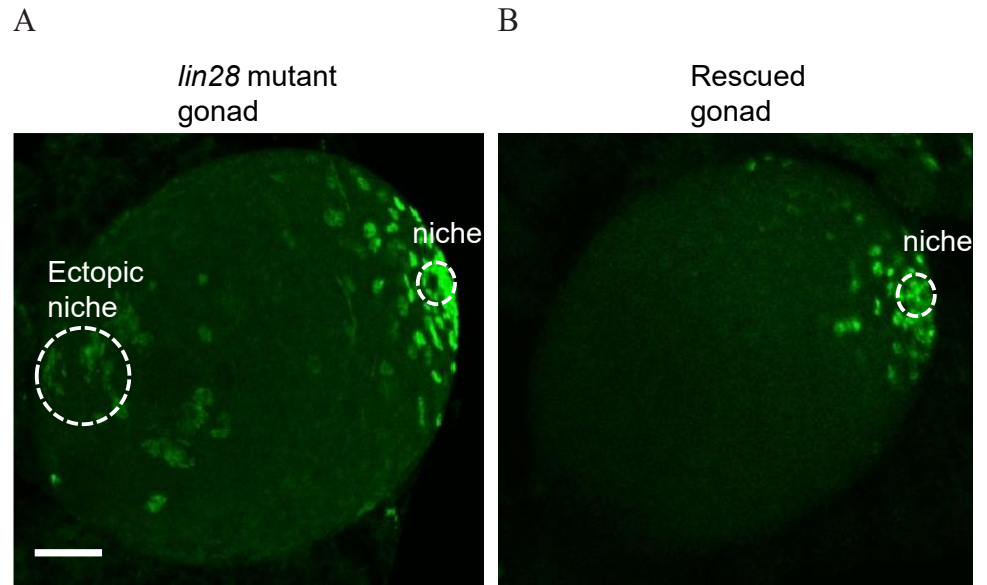


Fig. 2. Rescue of ectopic niche. Confocal images of male gonads from 3rd instar larvae stained with Tj (niche marker) antibody. (A) An ectopic niche in *lin28* mutant gonad (*esg-Gal4/+; lin28^{EP915}*), detected in three out of five gonads examined. (B) Single niche formation in the rescue line gonad (*esg-Gal4/UAS-lin28; lin28^{EP915}*), detected in all gonads examined (n=5). Scale bar, 5 μ m.

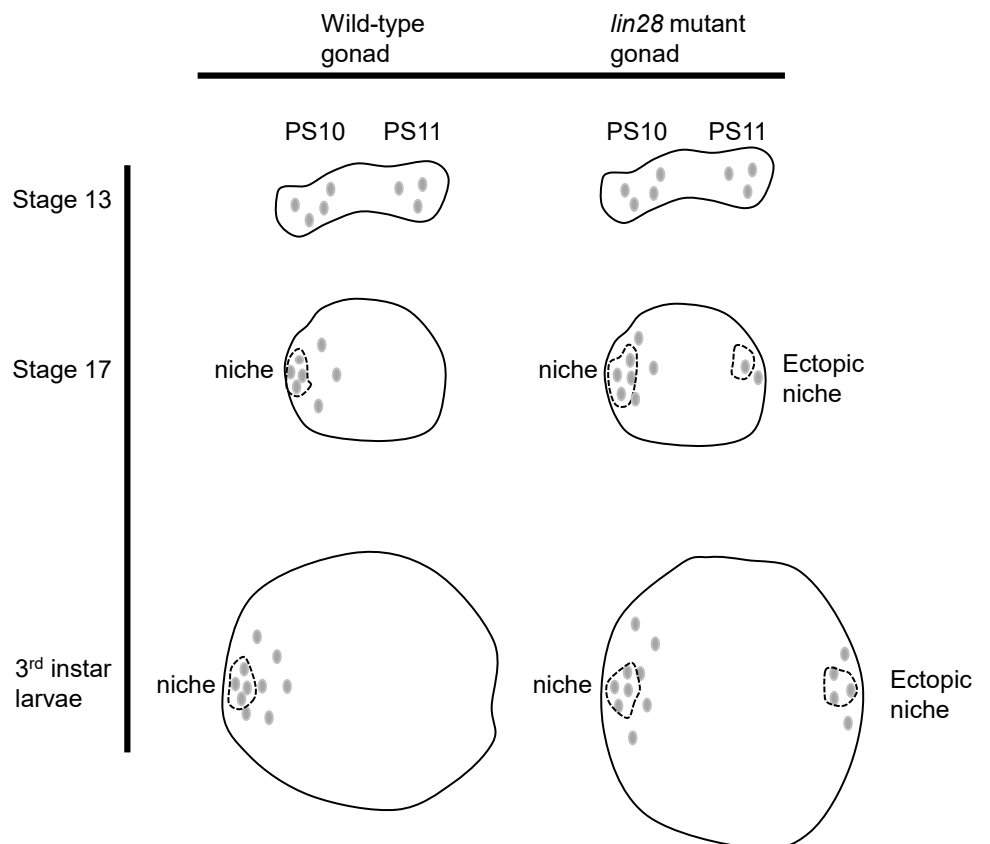


Fig. 3. A model of defective anterior migration in the *lin28* mutant gonad. Anterior migration of wild-type niche cells yields a single niche in the wild-type gonad. Defects in anterior migration yield an ectopic niche in the *lin28* mutant gonad. PS10, PS11 denote parasegments 10 and 11.

to reveal the detailed mechanism by which Lin28 regulates the development of a single niche in the *Drosophila* male gonad.

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