Mechanisms of Development xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Mechanisms of Development

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

Stage-specific control of niche positioning and integrity in the *Drosophila* testis

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ARTICLE INFO

Article history:
Received 7 May 2015
Received in revised form 23 June 2015
Accepted 24 July 2015
Available online xxxx

Keywords:
Abd-B
Drosophila testis
Integrin
Talin
Niche positioning
Hox genes

ABSTRACT

A fundamental question is how complex structures are maintained after their initial specification. Stem cells reside in a specialized microenvironment, called niche, which provides essential signals controlling stem cell behavior. We addressed this question by studying the *Drosophila* male stem cell niche, called the hub. Once specified, the hub cells need to maintain their position and architectural integrity through embryonic, larval and pupal stages of testis organogenesis and during adult life. The *Hox* gene *Abd-B*, in addition to its described role in male embryonic gonads, maintains the architecture and positioning of the larval hub from the germline by affecting integrin localization in the neighboring somatic cyst cells. We find that the AbdB-Boss/Sev cascade affects integrin independent of Talin, while genetic interactions depict integrin as the central downstream player in this system. Focal adhesion and integrin-adaptor proteins within the somatic stem cells and cyst cells, such as Paxillin, Pinch and Vav, also contribute to proper hub integrity and positioning. During adult stages, hub positioning is controlled by Abd-B activity in the outer acto-myosin sheath, while Abd-B expression in adult spermatocytes exerts no effect on hub positioning and integrin localization. Our data point at a cell- and stage-specific function of Abd-B and suggest that the occurrence of new cell types and cell interactions in the course of testis organogenesis made it necessary to adapt the whole system by reusing the same players for male stem cell niche positioning and integrity in an alternative manner.

1. Introduction

In all adult tissues harboring stem cells, the niche has a critical function as an organizer, which recruits the stem cells and provides the microenvironment that supports stem cell identity. Organogenesis of the *Drosophila* testis, initiated by the coalescence of germ cells and somatic gonadal cells late in embryogenesis, proceeds throughout embryonic and larval stages, and culminates in a second wave of organ shaping in pupal stages to reach maturation in adult stages. The *Drosophila* male stem cell niche, called the hub, is a cluster of non-dividing cells specified in the anterior most somatic gonadal cells already before the gonads coalesce (Boyle and DiNardo, 1995; DeFalco et al., 2008; DeFalco et al., 2004; Dinardo et al., 2011; Hatini et al., 2005; Le Bras and Van Doren, 2006).

The first signs of testis organogenesis are detected in late embryogenesis, once the specified hub cells recruit the anterior-most germ cells to become the germline stem cells (GSCs) (Sheng et al., 2009). A testis with a mature stem cell niche and all pre-meiotic cell types is detected at 3rd instar larvae (L3) (Fig. 1A). The *Drosophila* testis contains two types of stem cells arranged in stereotypic manner: the germline stem cells (GSCs) and the somatic cyst stem cells (CySCs). Each GSC is flanked by two CySCs and both stem cell identities are sustained through their association with the hub. Upon asymmetric cell division, each GSC produces a new GSC attached to the hub and a distally located gonialblast. The CySCs also divide with an asymmetric outcome, to generate a CySC remaining associated with the hub and a distally located post-mitotic daughter somatic cyst cell (SCC) (Fuller and Spradling, 2007). CySCs and SCCs are collectively called here cyst cells. Two SCCs enclose each gonialblast thereby forming a cyst that is "sealed" outside by the extracellular matrix (ECM) (Fig. 1A) (Papagiannouli et al., 2014). The gonialblast divides mitotically four more times to give rise to 16 interconnected spermatogonial cells, which then undergo pre-meiotic DNA replication and become spermatocytes. The cyst cells co-differentiate with the germ cells they enclose, grow enormously in size, elongate and accompany them throughout their differentiation steps up to individualization and sperm production in the adult testis (Fuller, 1993). Spermatocytes turn on the transcription program required for terminal differentiation and undergo meiotic divisions. During pupal stages, testis morphogenesis is completed with the addition of the acto-myosin sheath originating from the genital disc (Kozopas

Abbreviations: Abd-B, Abdominal-B; CySCs, somatic cyst stem cells; ECM, extracellular matrix; GSCs, germ-line stem cells; L, larval stage; SC, spermatogonial cysts; SCCs, somatic cyst cells; wt, wild type.

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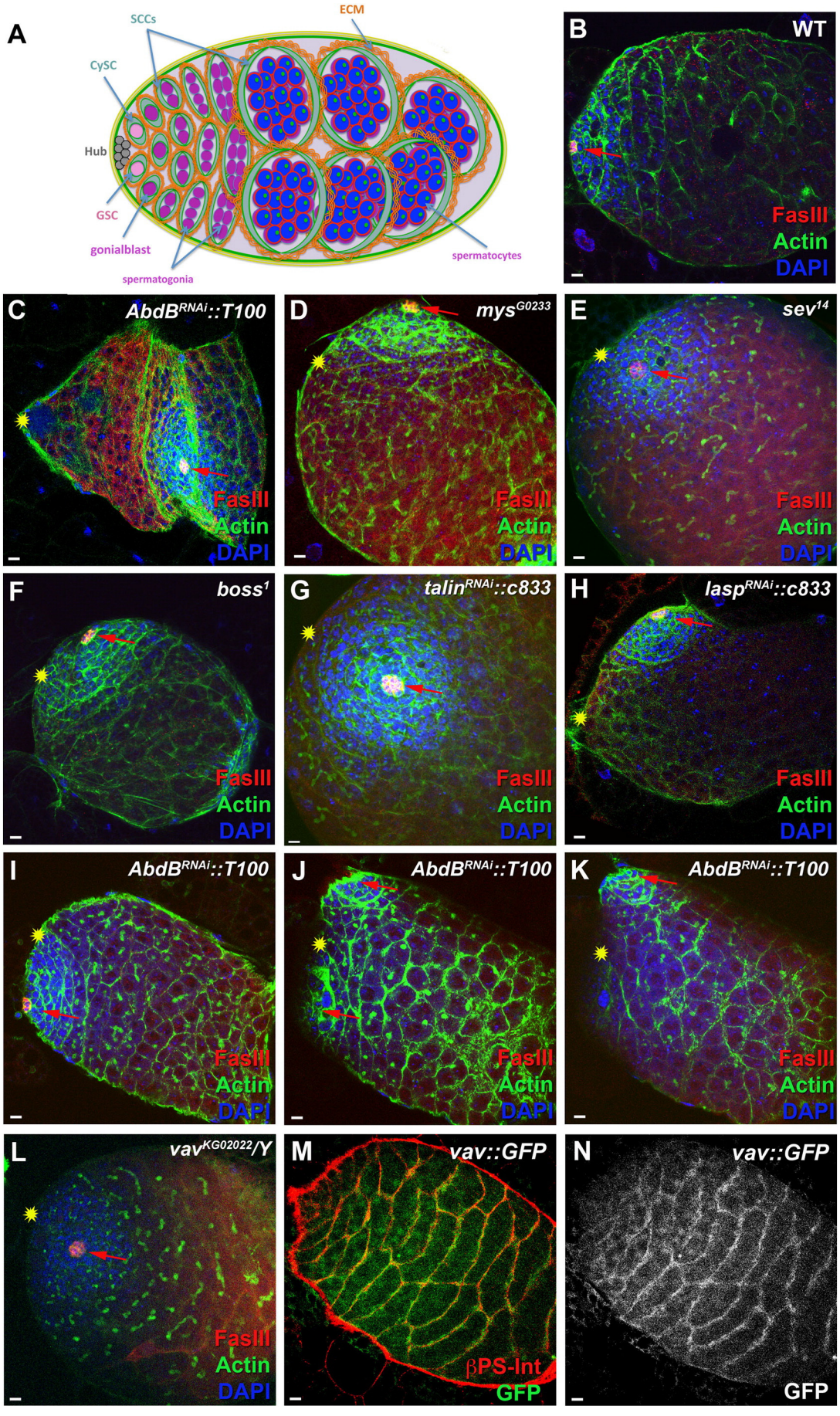
http://dx.doi.org/10.1016/j.mod.2015.07.009



Please cite this article as: Schardt, L., et al., Stage-specific control of niche positioning and integrity in the *Drosophila* testis, Mechanisms of Development (2015), http://dx.doi.org/10.1016/j.mod.2015.07.009