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Mini Review

Stage-specific control of stem cell niche architecture in the *Drosophila* testis by the posterior *Hox* gene *Abd-B*

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ABSTRACT

A fundamental question in biology is how complex structures are maintained after their initial specification. We address this question by reviewing the role of the *Hox* gene Abd-B in *Drosophila* testis organogenesis, which proceeds through embryonic, larval and pupal stages to reach maturation in adult stages. The data presented in this review highlight a cell- and stage-specific function of Abd-B, since the mechanisms regulating stem cell niche positioning and architecture at different stages seem to be different despite the employment of similar factors. In addition to its described role in the male embryonic gonads, sustained activity of Abd-B in the pre-meiotic germline spermatocytes during larval stages is required to maintain the architecture of the stem cell niche by regulating βPS-integrin localization in the neighboring somatic cyst cells. Loss of Abd-B is associated with cell non-autonomous effects within the niche, leading to a dramatic reduction of pre-meiotic cell populations in adult testes. Identification of Abd-B target genes revealed that Abd-B mediates its effects by controlling the activity of the sevenless ligand Boss via its direct targets *Src42A* and *Sec63*. During adult stages, when testis morphogenesis is completed with the addition of the acto-myosin sheath originating from the genital disc, stem cell niche positioning and integrity are regulated by Abd-B activity in the acto-myosin sheath whereas integrin acts in an Abd-B independent way. It seems that the occurrence of new cell types and cell interactions in the course of testis organogenesis made it necessary to adapt the system to the new cellular conditions by reusing the same players for testis stem cell niche positioning in an alternative manner.

Contents

Abbreviations: Abd-B, abdominal-B; CySCs, somatic cyst stem cells; ECM, extracellular matrix; GSCs, germline stem cells; SCCs, somatic cyst cells; SGPs, somatic gonadal precursors; wt, wild type; L3, 3rd instar *Drosophila* larvae.

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1. Introduction

Hox genes are master regulators of morphogenesis that code for homeodomain-containing transcription factors with a high conservation in different metazoans. Studying their function during embryogenesis in animals as diverse as insects and vertebrates revealed their critical role in establishing the identity of segmental structures along the anterior-posterior (A/P) body axis of these organisms [66]. More recent research emphasizes the role of *Hox* genes as cell-type switches [8,55,79] that control local cell behaviors resulting in the development of segment-specific structures and organs [3,43,66]. *Hox* genes are expressed throughout an animal's life [66], suggesting that they control different aspects of morphogenesis in a stage-dependent manner. However, due to the deleterious effects of *Hox* gene mutations, which normally result in the death of the organism at the end of embryogenesis, later Hox functions have rarely been studied [2,61,62,74]. Even more important, it has not been successfully addressed if and how *Hox* genes control the development and maintenance of structures and organs throughout the life of an organism, from embryogenesis to adulthood when new cell types and interactions emerge in the various stages. To answer this question, we use the fruitfly *Drosophila melanogaster*, a well-established model system with a wealth of available genetic tools for conditional, cell-type and stage-specific knockdown of genes to investigate stem cell function in a highly precise way.

Adult stem cells are the lifetime source of many differentiated cell types that maintain homeostasis of a tissue and respond to injury when challenged. They reside in microenvironments, the stem cell niches, that have an important role in stem cell behavior [85]. The stem cell niche (thereafter referred to simply as the "niche") relies on a subset of differentiated cells or extracellular substrates that recruits the stem cells and promotes stem cell maintenance in vivo through physical contacts and diffusible signals [107]. In this review we discuss how the *Drosophila* male stem cell niche is maintained after its initial specification, we review the current state of the art on stage-specific niche architecture and function, and explain how the posterior Hox gene *Abd-B* controls, as an upstream regulator, niche positioning and integrity in a cell-type and stage specific way.

2. *Drosophila* testis and the male stem cell niche

In all adult tissues harboring stem cells, the stem cell niche has a critical function as an organizer, which recruits the stem cells and provides the microenvironment required for stem cell maintenance. Much of the knowledge we have on testis stem cells and their niche comes from studies in *Drosophila*, a well-characterized system to study the biology of the stem cell niche, the germline stem cells and spermatogenesis [69]. Organogenesis of the *Drosophila* testis, a structure first made by the coalesce of germ cells and somatic gonadal cells at stage 14 of embryogenesis, continues throughout embryonic and larval stages, and goes through a second wave of organ shaping in the pupae, 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 gonad coalesce [4,20,21,25,40,53].

The first signs of testis organogenesis are already detected in late embryogenesis (stages 14-17), once the specified hub cells recruit the anterior-most germ cells to become the germline stem cells (GSCs) [88]. A testis with a mature stem cell niche and all pre-meiotic stages is detected at 3rd instar larvae (L3) (Fig. 1A). The *Drosophila* testis contains two types of stem cells: the germline stem cells (GSCs) and the somatic cyst stem cells (CySCs). Each GSC is flanked by two somatic cyst stem cells (CySCs) and both types of stem cells are maintained through their association to the hub cells, a cluster of non-dividing cells forming the niche organizer. Upon asymmetric cell division, each GSC produces a new GSC attached to the hub and a distally located gonialblast. The CySCs also divide asymmetrically to generate a CySC remaining associated with the hub and a distally located post-mitotic daughter somatic cyst cell (SCC) [33]. Two SCCs enclose each gonialblast forming a testicular cyst "sealed" from the outside by the extracellular matrix (ECM) (Fig. 1) [74]. The gonialblast divides mitotically four more times to give rise to 16 interconnected spermatogonial cells, which then undergo pre-meiotic DNA replication, become spermatocytes, turn on the transcription program for terminal differentiation and undergo meiosis. During pupal stages testis morphogenesis is completed with the addition of the acto-myosin sheath originating from the genital disc [50]. The SCCs 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 [32].

2.1. Specification of the testis stem cell niche

Specification of the hub cells is a prerequisite for establishment of the testis stem cell niche per se. Hub cells are somatic cells specified before gonad formation from a subpopulation of the lateral mesoderm, the somatic gonadal precursor cells (SGPs), in bilateral clusters of the abdominal parasegments 10 to 13 [4,22,53,68,72,76]. The different SGP populations joining the embryonic male gonad orchestrate testis morphogenesis at this initial stage, since the germ cells represent a uniform population at this time. *zinc-finger homeodomain 1* (*zfh-1*), a key player in SGP specification, is initially expressed in cell clusters of the lateral mesoderm (PS2-14) whereas at a later stage *zfh-1* expression in parasegments 10-13 correlates with the specification of these cells as SPGs [9,68,92].

However, not only the hub cells but also the cyst cells are specified from the SGPs. The common origin between the hub and CySCs has been shown by lineage tracing experiments [25]. Hub cell fate vs. cyst cell fate is specified prior to gonad coalescence in a subset of SGPs upon Notch signaling activation [25]. Specification of CySCs vs. hub cell fate is further shaped by the antagonistic function of the cytoplasmic protein lines (Lin) and the transcription factor Brother of odd with entrails limited (Bowl) [40,111]. Bowl promotes hub cell fate and lines CySCs fate, evidenced by fewer hub cells in *bowl* mutant gonads and increased number of hub cells in *line*s mutant gonads. Also, *line*s depleted CySCs acquired some hub-like properties and markers [25]. This is further supported by the fact that both cell types can be traced with the same cell markers such as Zfh-1 and Traffic Jam (TJ) [111]. In the posterior SGPs, the epidermal growth factor receptor (EGFR) represses hub formation and allows its formation only at the anterior part of the gonad [46].

Before gonad coalescence, the *Hox* genes *abdominal-A* (*Abd-A*) and *abdominal-B* (*Abd-B*) pattern the anterior-posterior (A/P) axis of the male embryonic gonad (Fig. 2): *Abd-A* specifies the anterior most SGPs giving rise to the hub, a combination of *Abd-A* and *Abd-B* specifies the posterior SGPs, and *Abd-B* alone specifies the male-specific SGPs [4,20,21,53]. Thus, *Abd-A* and *Abd-B* pattern the A/P axis of the formed gonad. Once specified, the hub cells are able to recruit the anterior-most germ cells to become the GSCs [88], giving rise to the male stem cell niche [11].

2.2. Testis stem cell niche positioning: state of the art

Stem cell niche and subsequent testis morphogenesis is a stepwise process based on the physical contact and diffusible signals exchanged between the germline and somatic cell populations [107]. In order to ensure normal niche function the hub cells of the *Drosophila* testis not only need to be properly specified but also need to be correctly placed and the architectural integrity of the system has to be maintained. Proper niche function in terms of hub positioning and integrity is tightly coupled to adhesion and cell communication, with βPS-integrin (encoded by the *myospheroid* (*mys*) gene) and the bride of sevenless