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The expression profile of purified *Drosophila* germline stem cells

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Abstract

We developed a method to highly purify germline stem cells (GSCs) from the *Drosophila* ovary, one of the best understood types of adult stem cell. GSCs express variant isoforms of general transcriptional components, translation initiation factors, and several variant ribosomal proteins, including RpL22, a protein enriched in several mammalian stem cells. These novel isoforms may help regulate stem cell gene expression because a reversion assay indicated that at least four were specific for GSCs. By comparative analysis, we identify additional genes enriched in GSCs, including Psc, the *Drosophila* homolog of the Bmi-1 Polycomb group gene, as well as genes that may delay cytokinesis in pre-meiotic germ cells. By comparing GSCs arrested by BMP over-expression and *bam* mutation, we hypothesize that mRNA utilization is modulated in differentiating GSC daughters. Our findings suggest that *Drosophila* and mammalian stem cells utilize at least two regulatory mechanisms in common.

Keywords: Germ cell; Stem cell; Expression profile; *Psc*; *Drosophila*; RpL22

Introduction

Stem cells maintain and replenish the tissues of multicellular organisms, a function that when compromised may lead to deficiency, premature aging, or cancer. Despite their importance, however, the rarity of stem cells and their location within complex tissues continues to limit our knowledge (reviewed by Fuchs et al., 2004; Ohlstein et al., 2004). A potentially powerful way to gather information about stem cells is to determine their gene expression profiles (reviewed by Kawasaki, 2004). Such studies identify genes and pathways whose function in stem cell biology can subsequently be tested. Recently, based on such comparisons, stem cells have been proposed to share a suite of common characteristics that contribute to their ability to serve as long-term cellular progenitors (Ramalho-Santos et al., 2002; Ivanova et al., 2002). The evolutionary conservation of stem cell mechanisms can also be probed by comparing the gene expression patterns of similar stem cells from diverse species.

Among of the most accessible stem cell types for functional and molecular studies are the germline stem cells (GSCs) of the *Drosophila* ovary (reviewed in Lin, 2002; Ohlstein et al., 2004). 2–3 GSCs normally reside at the tip of each ovariole within the ovary where under optimal nutritional conditions they divide asymmetrically every 20 h to produce a stem cell and a daughter cystoblast (Fig. 1A). Cystoblasts turn on the differentiation gene *bag-of-marbles* (*bam*) and divide synchronously to generate 16-cell germ cell cysts interconnected by ring canals that become surrounded by somatic cells to form a new follicle. Cystoblasts also initiate a complex process of cytoskeletal and cytoplasmic differentiation that is manifest by changes in the germ cell structure known as the fusome (de Cuevas and Spradling, 1998). Non-dividing somatic cells known as cap cells support a niche by adhering to GSCs and by

Abbreviations: GSC, germline stem cell; PGC, primordial germ cell; BMP, bone morphogenetic protein; FACS, fluorescence-activated cell sorting; PI, propidium diiodide; Prc1, Polycomb repressive complex 1; Tbps, TATA-binding proteins; TAFs, TATA-associated factors; hTR, human telomerase RNA.

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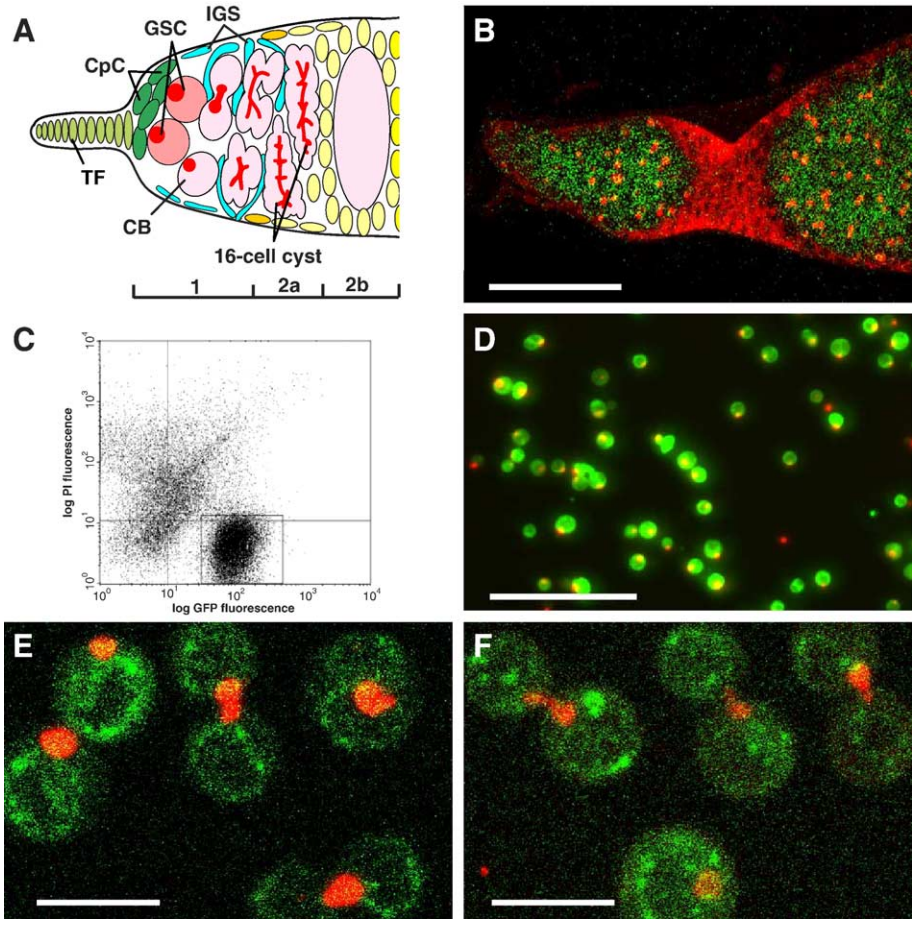

Fig. 1. Purification of GSCs from adult ovaries. (A) Diagram of normal anterior germarium. 2–3 GSCs are located in a niche composed of CpCs (cap cells: dark green) and TF cells (terminal filament: light green). BMP signaling (dark pink) is highly activated only in GSCs while cystoblasts (CBs) and cyst cells exhibit low levels (light pink). Both GSCs and their daughter CBs contain a round fusome (red). Cysts develop an elongated fusome (red) in region 1 and completed 16-cell cysts move through regions 2a and 2b. (B) An ovariole tip from a *bam, vasa-GFP* adult contains hundreds of GSCs as shown by fusome morphology (red, anti-Hts) and vasa content (green, anti-GFP). (C) A FACs profile of cells released from *bam, vasa-GFP* ovaries such as those in panel B. GSCs were selected as cells with high GFP and low propidium iodide fluorescence (box). (D) Low power microscopic view of the purified cells reveals that >99% display intense green labeling of the cytoplasm as well as a characteristic round fusome labeled with anti-Hts (red). (E and F) Higher magnification of purified GSCs from *dpp*-expanded (E) or *bam*-expanded (F) ovaries. Single cells and cell pairs joined by a fusome are seen. Scale bar = 50 μm (B and D) and 10 μm (E and F).

activating BMP signaling that is essential for GSCs to repress *bam* transcription and remain stem cells (Ohlstein and McKearin, 1997; Xie and Spradling, 1998, 2000; Song et al., 2002; Chen and McKearin, 2003; Casanueva and Ferguson, 2004). Recently, many of the same mechanisms that regulate adult ovarian GSCs have been shown to maintain the late embryonic and larval populations of primordial germ cells (PGCs) (Zhu and Xie, 2003; Gilboa and Lehmann, 2004; Kai and Spradling, 2004).

Germline stem cells have also been widely studied in male gonads. In the *Drosophila* testis, GSCs reside in an apical niche adjacent to non-dividing hub cells. The regulation of male and female GSCs has much in common, including niche cell adhesion (Yamashita et al., 2003), *bam* repression by BMPs (Shivdasani and Ingham, 2003; Kawase et al., 2004; Schulz et al., 2004; Song et al., 2004; Bunt and Hime, 2004), and ring canal/fusome morphogenesis (Lin et al., 1994; Hime et al., 1996; de Cuevas and Spradling, 1998), although details differ between the sexes. In both sexes, germline cystocytes were recently shown to be capable of reverting into functional GSCs in vivo (Kai and Spradling, 2004; Brawley and Matunis, 2004). However, unlike the situation in female GSCs, JAK-STAT signaling functions as the major male GSC regulatory signal (Tulina and Matunis, 2001; Kiger et al., 2001).

Mouse testes also contain niches that support large numbers of GSCs along the basal surface of the seminiferous tubules (reviewed in Zhao and Garbers, 2002). Mouse GSCs give rise to cells that divide asymmetrically to form clusters of interconnected A-type spermatogonia that are analogous to cystocytes. Mouse GSCs can be cultured with other testicular cells in vitro on feeder cells and retain the ability to function when re-introduced into a host animal. Recently, conditions have been found that either maintain enriched mouse or rat GSC populations, or cause them to differentiate into clusters of spermatogonia-like cells. This has allowed a profile to be determined of genes associated with GSC maintenance in culture (Hamra et al., 2004).