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Commentary

The Elusive Spermatogonial Stem Cell Marker?[1]

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Spermatogonial stem cells (SSCs) are undifferentiated male germ cells that have the potential to self-renew and differentiate into committed progenitors that maintain spermatogenesis throughout adult life. Rodent SSCs can be identified in whole-mount preparations of testicular seminiferous tubules (initially described by Clermont and Bustos-Obregon [1]) as isolated A_{single} spermatogonia. These A_{single} SSCs are present on the basement membrane of seminiferous tubules and can be distinguished from committed, transit-amplifying progenitor spermatogonia (some A_{paired} and $A_{aligned}$ chains of 4–16 cells) because committed cells are clonally arranged and connected by intercellular cytoplasmic bridges. Here we define progenitors as undifferentiated spermatogonia that are committed to differentiate and can undergo a finite number of self-renewing divisions. SSCs can be definitively identified in a retrospective manner by observing their ability to produce and maintain spermatogenesis in a functional transplantation assay as initially described by Brinster et al. [2, 3]. It has been widely assumed that all $A_{singles}$ (as the classically-defined SSCs) possess stem cell properties [4–7]. However, it has never been shown whether all or only some $A_{singles}$ are bona fide SSCs, and cells with a particular morphological/clonal arrangement (e.g., A_{single}) may or may not comprise the entire SSC pool [8].

Antibody-based studies using intact (whole-mount) seminiferous tubules have provided an avenue for defining proteins with expression patterns that are limited to classically described undifferentiated spermatogonia and, by extension, spermatagonial stem cells (SSCs). Such studies have revealed that CDH1, GFRA1, LIN28, NANOS2, NANOS3, NEUROG3, ZBTB16, and POU5F1 are expressed by undifferentiated stem and progenitor spermatogonia, including A_{single}, A_{paired}, and $A_{aligned\ 4–16}$ [9–16]. However, a unique marker that distinguishes A_{single} spermatogonia and perhaps spermatogonial stem cells has been elusive.

In the present issue, Oatley et al. identified ID4 as a marker restricted to A_{single} spermatogonia in the testis and a protein essential for normal spermatogonial stem cell renewal both in vitro and in vivo [17]. The authors found that only a portion of ID4+ spermatogonia also stained for ZBTB16 (i.e., PLZF) in seminiferous tubule cross sections, indicating the presence of both ID4+/PLZF– and ID4+/PLZF+ A_{single} spermatogonia (Fig. 1) and perhaps distinct subpopulations of SSCs. From the data provided, it is not known what proportion of total A_{single} spermatogonia are marked by either ID4 or PLZF, so the possible existence of ID4–/PLZF+ or ID4–/PLZF– $A_{singles}$ cannot be excluded (Fig. 1). These ID4 data support a growing theory in the field that the pool of rodent A_{single} spermatogonia (and SSCs) is phenotypically heterogeneous, which may define unique subpopulations of these cells with potential functional differences. Heterogeneity among $A_{singles}$ has been observed for CDH1, LIN28, NANOS2, and NANOS3 [13, 16, 18, 19]. Heterogeneity among transplantable SSCs has been revealed by studies on NEUROG3 and GFRA1 [10, 20]. While the significance of this heterogeneity is not well understood, it seems clear that a one-size-fits-all definition of A_{single} spermatogonia and SSCs oversimplifies the stem cell system underlying spermatogenesis.

Oatley et al. used two approaches to posit a functional role for ID4 in SSCs. First, ID4 knockdown in cultured SSCs resulted in stunted stem cell renewal without changes in germ cell amplification, suggesting a role for ID4 in maintenance of the SSC pool (i.e., self-renewal). Second, ID4 null mice showed progressive spermatogenic failure characteristic of a SSC self-renewal defect. Based on these data, Oatley's group suggested a model whereby the phenotypically distinct subpopulations of A_{single} spermatogonia may represent functionally discrete populations. For example, ID4+/PLZF– A_{single} spermatogonia may represent $A_{singles}$ that are quiescent (Fig. 1, red nuclei) and/or that have self-renewing capacity and that these cells acquire PLZF expression on entering the cell cycle (green/yellow nuclei). Under this scenario, presence or persistence of ID4 at the subsequent cell division would favor a self-renewal fate decision, while absence or loss of ID4 might favor commitment to differentiation (e.g., production of A_{paired} and $A_{aligned}$ spermatogonia).

The concept of a quiescent population of SSCs is similar to the A_0/A_1 model that was originally advanced for mouse and rat spermatogenesis [1, 21–23]. The A_0/A_1 model holds that normal spermatogenesis is maintained by an ''active'' pool of SSCs (A_1) and that a quiescent ''reserve'' pool of A_0 is

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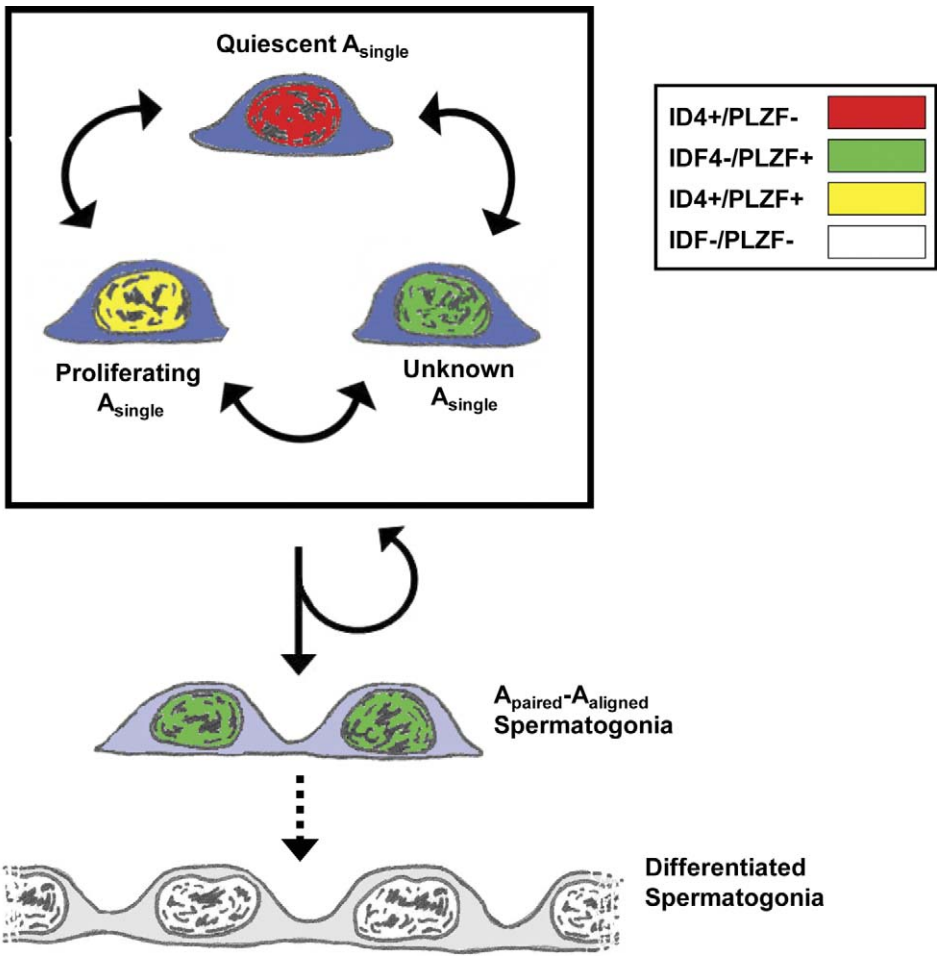

FIG. 1. Phenotypic and functional heterogeneity among rodent SSCs. Within the pool of A_{single} spermatogonia, some express only ID4 (red nuclei), while other $A_{singles}$ also express PLZF (yellow nuclei), suggesting subpopulations of $A_{singles}$ and SSCs. It is not known whether some $A_{singles}$ express PLZF only (green nuclei) or neither marker (not shown). It is also not known whether all or some A_{single} spermatogonia (or cells beyond $A_{singles}$) make up the SSC pool. Based on results of in vitro knockdown and in vivo knockout studies that suggest that ID4 participates in SSC self-renewal, Oatley et al. propose that ID4 delineates different functional subpopulations of SSCs (i.e., quiescent and proliferating).

mobilized following an insult to spermatogenesis. This model is also consistent with the A_{dark}/A_{pale} ''reserve stem cell'' model of primate SSCs [24]. Ultimately, the A_0/A_1 model was supplanted by the A_{single} model [25, 26], in which a single population of stem cells (A_{single} spermatogonia) divides regularly but infrequently and gives rise to the spermatogenic lineage. Recent pulse-chase experiments lend support for continual, steady-state renewal of the SSC pool in mice [27] but do not rule out the possibility of a phenotypically distinct, quiescent component. A model of rodent SSC contribution to normal spermatogenesis that incorporates a quiescent intermediate (whether truly ''reserve'' or simply ''long cycling'' [28]) would help unify our disparate understanding of spermatogenic lineage development in rodents and primates.

ID4 has the most restricted pattern of expression in undifferentiated spermatogonia observed to date and clearly delineates subpopulations of A_{single} spermatogonia in the mouse testis. It is likely that molecular heterogeneity in the pool of A_{single} spermatogonia have functional correlates that will be the focus of ongoing investigations. Whether the stem cell pool resides entirely in the population of A_{single} spermatogonia or might be extended to include some A_{paired} or possibly larger chains, as suggested by Nakagawa et al. [8], is the subject of ongoing debate.

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