NANOS is known to be required for germline cell development in a variety of animal species and for the maintenance of germline stem cells in Drosophila. The recent study by Sada et al. has demonstrated that NANOS2, one of the three mammalian homologues, is required intrinsically for maintaining adult mouse spermatogonial stem cell self-renewal.

Spermatogonial stem cells (SSCs) in the mammalian male, also known as male germline stem cells (GSCs) in Drosophila and other species, are responsible for continuously generating functional male gametes—sperms. Like GSCs in Drosophila, SSCs are also maintained in their niche within the seminiferous tubule, but the niche’s physical structure is not well-defined. On the basis of physical locality, the niche is likely composed of basal membrane and Sertoli cells that SSCs are physically associated with (Brinster, 2007). Perhaps, Sertoli cells are the key niche component based on the finding that they provide the most critical extrinsic niche signal, GDNF, for maintaining SSC self-renewal. Following SSC division, it generates a self-renewing SSC and a differentiating A single (A single), which then further divides to generate A spermatogonia (A paired, A aligned, A1, A2, A3 and A4) and B spermatogonia (Brinster, 2007). As expected, true SSCs represent a small fraction of A cells within the tubule, and have not been definitively identified at the cellular level due to a lack of unique molecular markers and distinct morphological features. However, most of A cells, A pr and A al cells are often referred to as undifferentiated spermatogonia because they still have some SSC properties and retain the ability to revert back to true SSCs when placed in right microenvironments (Nakagawa et al., 2007). Due to poorly defined SSCs, many critical intrinsic factors and niche factors that control SSC development remain to be identified (Oatley and Brinster, 2008). In a recent issue of Science, Sada et al. (2009) demonstrate that NANOS2 is an intrinsic factor both necessary and sufficient for SSC self-renewal.

NANOS2 belongs to a family of evolutionarily conserved zinc-finger motif-containing RNA-binding proteins. The first NANOS family member was identified as one of the posterior fate determinant of the embryo in Drosophila by cooperating with its partner PUMILIO to directly suppress ‘hunchback’ expression. It also functions in the subsequent migration of primordial germ cells (PGCs), which form at the posterior end of the embryo, into gonads during gastrulation and also prevents their differentiation after reaching the developing gonad (Asaoka-Taguchi et al., 1999; Gilboa and Lehmann, 2004). Like many other evolutionarily important genes, NANOS has three homologues in mammals, NANOS1, NANOS2 and NANOS3. Only NANOS2 and NANOS3 expression is restricted to the germ cell lineage. NANOS2 is predominantly expressed in male germ cells starting from E13.5 for maintaining PGCs, whereas NANOS3 is expressed in migrating PGCs and is required for their maintenance in both sexes (Tsuda et al., 2003). Drosophila NANOS has recently been shown to be important for maintaining GSCs by preventing differentiation in the adult ovary (Gilboa and Lehmann, 2004; Wang and Lin, 2004). The recent study by Sada et al. elegantly demonstrates that NANOS2 is required in mouse SSCs for maintaining their self-renewal by preventing differentiation.

The study by Sada et al. (2009) utilized three approaches to investigate the role of NANOS2 in the control of SSC development in the adult mouse testis. First, they took advantage of a Tamoxifen (TM) inducible Cre/LoxP cell-lineage tracing system to examine if NANOS2 is expressed in true adult SSCs. The mice carrying a Nanos2 enhancer driven a TM-inducible CreERT (a fusion between Cre with the mutant estrogen receptor gene) transgene and a LacZ-based Cre reporter were subjected to TM induction at the adult stage. After LacZ-positive transiently amplifying clones derived from marked differentiating spermatogonia cells gradually moved away from the seminiferous tubule by forming mature sperms, the persistent LacZ-positive clones spanned all stages of spermatogenic cells after 40 days (the time for an A to differentiate into sperms), representing true SSC clones. Interestingly, the frequency of marked SSC clones generated by Nanos2-CreERT was much higher than that induced by Ngn3-CreERT, since Ngn3 was proposed to be expressed in SSCs. Further careful analysis showed that some Ngn3-GFP-positive cells also express NANOS2 protein, but some of NANOS2-positive A spermatogonial cells are negative for Ngn3-GFP expression, suggesting that NANOS2-positive Ngn3-GFP-negative spermatogonial cells must include true SSCs. The Ngn3-creERT induced persistent marked SSC clones are likely due to dedifferentiation of transit amplifying stem cells (Nakagawa et al., 2007). Therefore, these findings have excluded the possibility...
that Ngn3 is expressed in mouse SSCs, and have further confirmed that NANOS2 is an intrinsic factor for SSCs.

Second, Sada et al. used a conditional knockout strategy to demonstrate that NANOS2 is required to maintain SSCs in the adult mouse testis. In the study, a Nanos2 conditional knockout mouse strain was generated by putting a Cre-dependent removable Nanos2 rescue construct into the Nanos2 knockout strain background. Without TM injection, Nanos2 expression driven by its own promoter could fully rescue Nanos2 mutant phenotype. Following TM injection in adult mice, the Cre-dependent removal of functional Nanos2 resulted in the depletion of the SSC pool, which was evidenced by the absence of expression of undifferentiated SSC markers such as PLZF and GFR. Consistently, differentiated germ cells started to appear in the locations which were normally occupied by undifferentiated germ cells. It was further shown that loss of the SSC pool was due to differentiation rather than apoptosis. These findings demonstrate that NANOS2 is required intrinsically to maintain postnatal SSC self-renewal, and further confirms that NANOS has a conserved role in GSC maintenance in different organisms.

Third, Sada et al. also used an overexpression strategy to show that NANOS2 is sufficient to block SSC differentiation. In addition to its expression in migrating PGCs during early development, NANOS3 is expressed in more differentiated long-chained cells. Forced Nanos2 overexpression in the Nanos3-expressing differentiated spermatogonial cells prevented their differentiation based on expression of markers for differentiated and undifferentiated germ cells, causing the accumulation of undifferentiated spermatogonial cells. Interestingly, such Nanos2 overexpression decreased proliferation rates of the undifferentiated spermatogonial cells. Thus, the accumulated undifferentiated spermatogonial cells could not be attributed to the increased proliferation of the undifferentiated cells. As expected, overexpression of Nanos2 in Ngn3-expressing cells using the same strategy generated similar results. Taken together, these findings demonstrate that NANOS2 is sufficient for maintaining adult SSCs by preventing their differentiation and thus maintaining their self-renewal, further supporting the idea that NANOS is a master regulator of SSC self-renewal. Although Nanos is necessary for GSC self-renewal in the Drosophila ovary, it is not sufficient for repressing germ cell differentiation when overexpressed (Wang and Lin, 2004), indicating that there is a subtle difference in how NANOS controls GSC self-renewal in different organisms.

Although the study by Sada et al. has clearly established the central role of NANOS2 in SSC self-renewal, many outstanding questions related to NANOS2 functions remain to be addressed. Although its homologue has been shown to be a translational regulator in Drosophila, its critical target mRNAs in stem cells have not been identified yet. The most important question is what are the critical target mRNAs and interacting partners of NANOS2 in SSCs. The next important question is how its expression and function are regulated in SSCs and their differentiated progeny because its function is restricted to undifferentiated spermatogonial cells and its expression is turned off in differentiated spermatogonial cells. The GDNF signal from Sertoli cells is necessary and sufficient for preventing SSC differentiation, although NANOS2 is an intrinsic factor also necessary and sufficient for preventing SSC differentiation. Another important question is whether and how GDNF signaling and NANOS2 intersect in SSCs to control their self-renewal. NANOS2 was previously shown to be capable of substituting for NANOS3 during early PGC development, while overexpression of NANOS2 in NANOS3-positive differentiated spermatogonial cells can prevent their differentiation, indicating that they function differently in different development stages of germ cells. The next exciting question is how two related NANOS proteins can have distinct functions in different cellular contexts. The answers to these questions will surely help better understand how mammalian GSCs are regulated, and the findings should have important implication in understanding stem cell biology and the future treatment of male infertility.

References


