DIVISION OF GERM CELL BIOLOGY

Professor
YOSHIDA, Shosei

Assistant Professors:	*HARA, Kenshiro* *KITADATE, Yu*
NIBB Research Fellow:	*NAKAMURA, Yoshiaki*
Technical Staff:	*MIZUGUCHI-TAKASE, Hiroko*
Graduate Students:	*IKAMI, Kanako* *TOKUE, Moe*
Technical Assistants:	*ICHIKAWA, Rie* *INADA, Kana* *MARUYAMA, Ayumi* *SUGIMOTO, Ryo*
Secretary:	*KUBOKI, Yuko*

Mammalian testes produce numerous sperm for a long period in a constant manner, which is supported by the robust behaviors of the stem cell population. Decades of research, including morphological examinations, post-transplantation repopulation, and in vitro culture, have made it one of the most intensively studied mammalian tissue stem cell systems. However, the nature of the stem cells and their control, as well as their niche, remains largely unknown.

The Division of Germ Cell Biology aims to fully understand the mouse sperm stem cell system in vivo, i.e., in the context of testicular tissue architectures. We have revealed a number of characteristics of this potent stem cell system, including: 1) Cells believed to have irreversibly committed for differentiation still retain the self-renewing potential and can contribute to stem cell pool maintenance ("potential stem cells"). 2) "Reversion" from potential stem cells occurs at a higher frequency when testicular tissue is damaged and regeneration is induced. 3) The undifferentiated spermatogonia (A_{undiff}) population that includes both "actual" and "potential" stem cells localized to vasculature (vascular-associated niche). 4) Stem cells turn over frequently and stochastically even under steady-state situations. (Nakagawa et al., Dev. Cell 2007, Science 2010; Yoshida et al., Science 2007; Klein et al., Cell Stem Cell 2010)

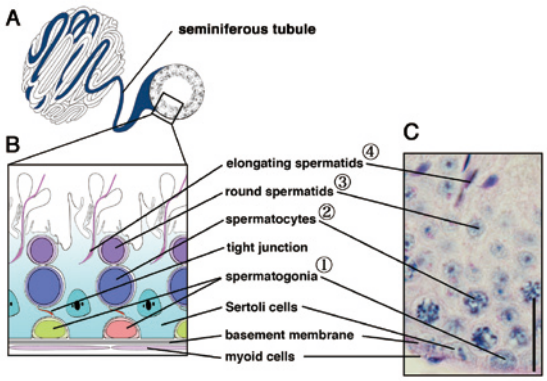

Figure 1. Architecture of seminiferous tubules and seminiferous epithelium. The seminiferous epithelium exhibits a stratified organization of the differentiating germ cells (numbers in circle), which are nourished by Sertoli cells. Bar, 20 μm.

In 2011, we revealed the molecular mechanisms underlying the temporal regulation of differentiation and self-renewal of stem cells (A_{undiff}) in mouse seminiferous epithelium, which are comprised of germ cells –both stem and differentiating cell types- and supporting somatic cells. Our study proposed that local retinoic acid (RA) metabolism plays important roles for the timed stem cell differentiation and coordination with somatic cells that support the differentiation process to spermatozoa (Sugimoto et al., Mech. Dev. 2011).

I. Architecture and cyclic dynamics of seminiferous epithelium

Spermatogenesis occurs inside the seminiferous tubule of the testis, where differentiating germ cells and supporting somatic Sertoli cells comprise a composite epithelium, termed seminiferous epithelium (Fig. 1). Sertoli cells are huge cells that form typical epithelium. All the stages of germ cells (from stem cells to spermatozoa) are nourished by Sertoli cells in a striated manner. This stratification is established as a result of the periodic differentiation of A_{undiff} into A_1 spermatogonia with an interval of 8.6 days, which is followed by programmed differentiating process toward spermatozoa that takes 35 days (Fig. 2). Consequently, the resultant combination of the differentiating germ cell observed at a particular region appears in an 8.6-day cyclic manner, known as 'seminiferous epithelial cycle'. In mice, this cycle is divided into stages I to XII, while A_{undiff}-to-A_1 differentiation occurs at stage VII to VIII.

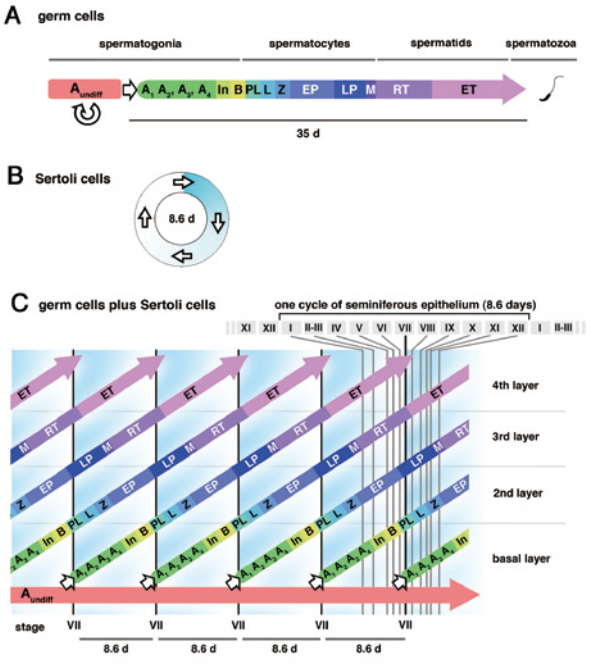

Figure 2. Coordination of periodical events in mouse seminiferous epithelium. (A) Germ cell differentiation, which takes 35 days from A_{undiff} to spermatozoa. (B) 8.6-day cycle of gene expression in Sertoli cells. (C) Coordination of germ cell differentiation and Sertoli cell cycle.

Sertoli cells, while quiescent in their cell cycle, cyclically change their gene expression so that they can nourish the appropriate stages of germ cells. It is an interesting question but remains a mystery how this periodic differentiating of A_{undiff} is achieved and the cycle of Sertoli cells is coordinated with germ cell differentiation.

II. Role of RA and regulation of RA metabolism

It has been known that RA plays important roles in both differentiation of A_{undiff} and control of the seminiferous epithelial cycle. This notion was primarily derived from observations made after RA signaling blockade by deficiency of the dietary vitamin A (VA) -the only precursor of RA- and by disruption of retinoic acid receptor genes. However, results of these and other preceding studies were somewhat puzzling and it remained a challenge to reveal how RA is involved in the genesis of the coordinated cycling of the seminiferous epithelium.

We revisited the classical VAD model and discovered that artificial elevation of RA signaling elicits not only differentiation of A_{undiff} into A_1 spermatogonia but also a reset of the cycle of Sertoli cells' function to the appropriate stage that supports the A_1 spermatogonia in transition between A_{undiff} and A_1 (stage VII). Then we asked how the RA-related metabolism is regulated in the seminiferous epithelium based on the expression of genes encoding enzymes involved in the synthesis of RA from VA and inactivation of RA. Based on the data from a previous study of Manuel Mark's group and our own analyses, it was strongly suggested that the modes of RA metabolism dynamically shift in accordance with the seminiferous epithelial cycle, with a prominent increase of RA concentration across stage VII (Fig. 3). This is also supported by the fact that artificially increased RA induced A_{undiff}-to-A_1 differentiation and reset Sertoli cells to stage VII.

Interestingly, RA metabolism involves multiple enzymes (Fig. 3A) that are expressed separately among different cell types including meiotic and haploid germ cells and Sertoli cells. Given that RA and its precursors are able to translocate across adjacent cells rather freely, the different cell types seem to cooperate in the regulation of RA metabolism. Then, the next question was raised: How is coordinated expression of these genes among different cell types achieved? We discovered that RA metabolism-related genes expressed in Sertoli cells are regulated by RA signaling, forming positive and negative feedback loops; while those expressed in meiotic and haploid germ cells hardly respond to RA signaling: Perhaps a differentiation step-related mechanism controls their expression. Thus, control of RA metabolism-related genes show a good contrast between Sertoli cells and germ cells.

III. Model for the seminiferous epithelial cycle

Based on these observations, we have proposed a model that can explain the coordinated expression of RA metabolism-related genes that appears to cause the cyclic change of the RA concentration (Fig. 4, 5). Importantly, this model suggests that meiotic and haploid germ cells regulate the local RA metabolism to increase and decrease the RA

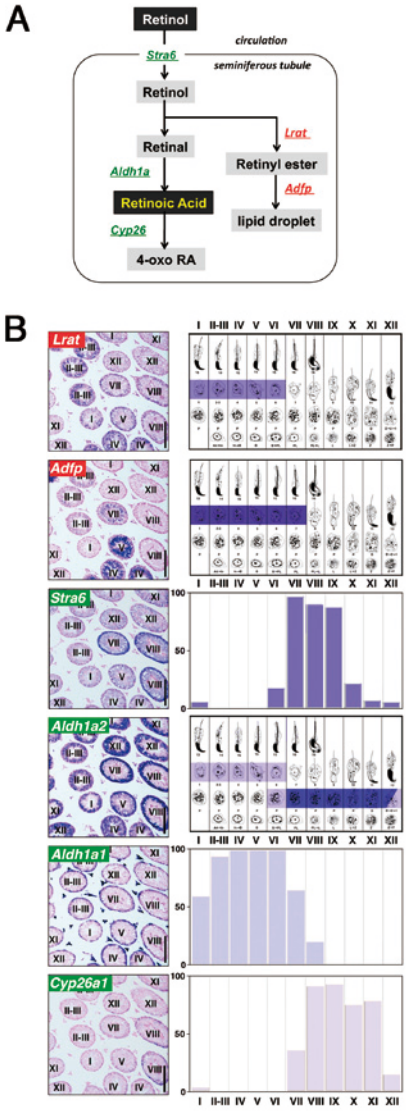

Figure 3. RA metabolism-related genes and their cyclic expression. (A) Multiple genes act together in the RA metabolic pathways, starting from retinol (VA). (B) These genes are expressed in different cell types and in different stages of the seminiferous epithelial cycle.

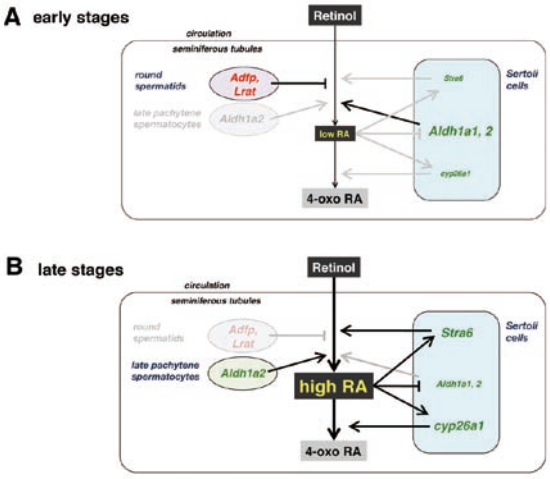

Figure 4. Regulation of RA metabolism-related gene expression. Expression patterns of these genes (Fig.3) in early (A) and late (B) stages are explained as a result of feedback regulation in Sertoli cells but not in germ cells.

concentration, respectively (Fig. 4). Given that RA signaling causes the induction of A_{undiff}-to-A_1 differentiation and reset of Sertoli cell's cycle, it can be said that these differentiating germ cells play a central role in the coordination of the seminiferous epithelial cycle by sending 'go' and 'wait' signals to the A_{undiff} and Sertoli cells (Fig. 5). Therefore, the differentiation program of germ cells appears to determine the timing of stem cell differentiation and makes the local environment appropriate for germ cell differentiation.

This model nicely explains the beautiful orchestration between germ and Sertoli cells in the seminiferous epithelium. Generally speaking, regulation of stem cell differentiation by their differentiating progeny that modulate the local environment may be a common strategy of stem cell control in other systems.

IV. Perspectives and ongoing research

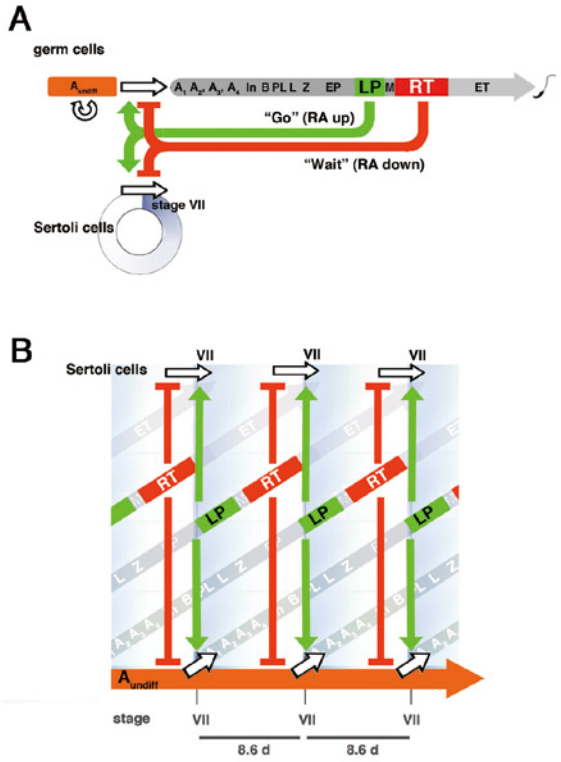

Figure 5. Coordination of germ cell differentiation and Sertoli cells' cycle. Particular stages of meiotic cells (LP) and haploid cells (RT) send the *'go'* and *'wait'* signals to A_{undiff} and Sertoli cells, by regulating the local RA metabolism to increase and decrease the RA concentration, respectively (A). These signals will occur reciprocally and periodically to maintain the seminiferous epithelial cycle. All the figures are reproduced from Sugimoto *et al*., Mech. Dev. (2011) with permission.

The suggested model for the role of RA in the regulated stem cell differentiation warrants farther evaluation: While the central role of RA is in no doubt, it is also clear that RA solely cannot regulate the very complex and highly orchestrated events that occur in seminiferous epithelial cycle. In addition, stem cells should behave in response to the cyclically changing microenvironment. We hope that our model and future investigations will clarify the details of the testicular environment and the stem cell response.

Besides the seminiferous epithelial cycle, we are also investigating a number of yet-to-be elucidated problems in spermatogenesis, especially with regard to its stem cells. In addition to temporal regulation described here, the spatial regulation of stem cells is also a very important issue. We previously observed that A_{undiff} preferentially localize to the vasculature-proximal region, and dispatch this region when they differentiate into A_1 at stage VII (Yoshida et al., Science 2007), which is likely to occur in response to RA signaling. We are also investigating the cellular and molecular nature of this 'vasculature-associated niche'. We are also investigating the behavior of A_{undiff} taking advantages of live imaging that we have developed (Yoshida et al., Science 2007, Nakagawa et al., Science 2010). We hope that these studies will throw light to a better understanding the mouse sperm stem cell system.

Publication List

〔Original paper〕

- Sato, T., Aiyama, Y., Ishii-Inagaki, M., Hara, K., Tsunekawa, N., Harikae, K., Uemura-Kamata, M., Shinomura, M., Zhu, X. B., Maeda, S., Kuwahara-Otani, S., Kudo, A., Kawakami, H., Kanai-Azuma, M., Fujiwara, M., Miyamae, Y., Yoshida, S., Seki, M., Kurohmaru, M., and Kanai, Y. (2011). Cyclical and patch-like GDNF distribution along the basal surface of sertoli cells in mouse and hamster testes. PLoS ONE 6, e28367.

〔Original papers (E-publication ahead of print)〕

- Gely-Pernot, A., Raverdeau, M., Célébi, C., Dennefeld, C., Feret, B., Klopfenstein, M., Yoshida, S., Ghyselinck, N.B., and Mark, M. Spermatogonia differentiation requires retinoic acid receptor γ. Reproduction 2011 Nov. 1.
- Sugimoto, R., Nabeshima, Y., and Yoshida, S. Retinoic acid metabolism links the periodical differentiation of germ cells with the cycle of Sertoli cells in mouse seminiferous epithelium. Mechanisms of Development 2011 Dec. 19.

〔Review articles〕

- Spradling, A., Fuller, M.T., Braun, R.E., and Yoshida, S. (2011). Germline stem cells. In Cold Spring Harbor Perspectives in Biology Collection, "Germ Cells", Cold Spring Harbor Laboratory Press, 1-20.
- Yoshida, S. (2011). Stem cell niche system in mouse spermatogenesis. In Stem Cell Biology and Regenerative Medicine Series, "Male Germline Stem Cells: Developmental and Regenerative Potential", Humana Press, 159-175.