Supplementary Results and Discussion

Wang and coworkers found an abundance of X-linked genes expressed by spermatogonia [1]. In contrast, we found that the 88 candidate genes in stem/progenitor spermatogonia cluster 1 were evenly distributed among chromosomes (Supplementary Fig. 1A). The discrepancy between stem/progenitor spermatogonia cluster 1 results in the current study and Wang et al. (2001), with respect to X chromosome genes is intriguing and may highlight important differences in the target cell population. In a comprehensive study of sex-biased genes, Khil and coworkers confirmed a nonrandom chromosomal distribution, but found that this phenomenon was developmentally regulated [2]. While premeiotic germ cells and somatic cells express a greater number of X-linked genes than expected by chance, meiotic germ cells express a lower than expected number of X-linked genes. This later phenomenon has been attributed to a process called meiotic sex chromosome inactivation (MSCI), which involves the condensation and transcriptional silencing of sex chromosomes during meiosis [3, 4]. Our results are consistent with these observations because X chromosome genes are under represented in the germ cell cluster (representing primarily differentiated germ cells, $p=6.5 \times 10^{-9}$) and over represented in the somatic cell cluster ($p=1.4 \times 10^{-9}$, Supplementary Fig. 1B, Supplementary online material). Thus, we conclude that X chromosome genes are not differentially expressed in stem/progenitor spermatogonia, but may be activated upon differentiation to type A1, A2, A3, A4, Intermediate and B spermatogonia. These differentiating spermatogonia were not assayed in the current experimental design (the cryptorchid testis contains only stem

[A_s] and progenitor [A_p, A_{al}] spermatogonia), but were included in the isolated spermatogonia studied by Wang and coworkers [1].

References

1. Wang PJ, McCarrey JR, Yang F et al. An abundance of X-linked genes expressed in spermatogonia. Nat Genet 2001;27:422-426.

2. Khil PP, Smirnova NA, Romanienko PJ et al. The mouse X chromosome is enriched for sex-biased genes not subject to selection by meiotic sex chromosome inactivation. Nat Genet 2004;36:642-646.

3. Solari AJ. The behavior of the XY pair in mammals. Int Rev Cytol 1974;38:273-317.

4. McKee BD, Handel MA. Sex chromosomes, recombination, and chromatin conformation. Chromosoma 1993;102:71-80.