REVIEW

Germline Stem Cell Transplantation and Transgenesis

Ralph L. Brinster

The recently developed testis cell transplantation method provides a powerful approach to studying the biology of the male germline stem cell and its microenvironment, the stem cell niche. The technique also is being used to examine spermatogenic defects, correct male infertility, and generate transgenic animals.

Germline stem cells have been studied in most detail in mice, in which they first can be identified at 7.0 to 7.5 days of gestation as a group of about 100 primordial germ cells (PGCs) arising from embryonal ectoderm. Over the next few days, PGCs multiply to about 20,000 cells while migrating to the genital ridges. In females, they undergo meiosis and become oocytes, thereby ending their stem cell potential. In males, they enter fetal seminiferous tubules, become gonocytes, and cease dividing (*1*). In contrast to oocytes, gonocytes retain their stem cell potential. Following birth in mammals, gonocytes migrate to the seminiferous tubule basement membrane and differentiate into spermatogonial stem cells (SSCs). As male germline stem cells, SSCs share two characteristics with other adult stem cells; they can both self-renew and provide daughter cells, which differentiate into one or more terminal cell types (*2*, *3*). Because stem cells traditionally have been defined by function, unequivocal identification rests on the development of an assay demonstrating the ability to regenerate the appropriate system of the body (e.g., hematopoiesis, spermatogenesis, etc.). Transplantation assays have been developed fully only for hematopoietic stem cells (HSCs) and SSCs; adult stem cells of other self-renewing systems are tentatively identified by a variety of characteristics, including morphology, surface markers, and the ability to differentiate into two or more cell types (*2*, *3*).

Testis cell transplantation as a functional assay for spermatogonial stem cells was first reported in 1994 using mice (*4*, *5*). The basic procedure consists of harvesting testis cells from a fertile male and microinjecting the cell suspension into seminiferous tubules of an infertile recipient, in which colonies of donor-derived spermatogenesis are established (Fig. 1). The apparent simple nature of this assay belies the complexity of the spermatogenic process and the interactions that exist among cells of the seminiferous tubule. The primary somatic cells of the tubules are myoid and Sertoli cells that support and nourish germ cells, form the tubule wall, and define a niche for the stem cell (Fig. 2). The spermatogenic process is complex, tightly regulated, and extremely productive (*1*, *6*). In rats, the differentiation and meiotic process that begins with division of a single stem cell theoretically can produce 4096 spermatozoa, although as a result of apoptoses the efficiency is only 25 to 50% (*6*). In the adult human male, this process generates about 1000 spermatozoa each time the heart beats, and every spermatozoa contains a different complement of paternal genes, thereby generating the male half of species diversity.

Because transplantation of testis cell populations results in generation of individual colonies that represent the products of a single stem cell, quantitative analysis of these clonal events is possible. Therefore, this assay provides a powerful mechanism to study the biology of the stem cell. These studies are difficult because only about 1 in 5000 mouse testis cells is a stem cell, and they have no distinguishing morphological or biochemical characteristics (*1*, *6*). However, purification and characterization of the stem cell have been facilitated by use of specific antibodies and fluorescence-activated cell sorting, followed by transplantation of selected cell populations (*7*). Stem cell transplantation also provides an opportunity to study the stem cell niche (*8*, *9*). Despite the multiple layers of differentiating germ cells (Fig. 2), a stem cell transferred to the lumen of a normal wild-type seminiferous tubule is able to migrate through these layers and Sertoli cell tight junctions to find and occupy its niche on the basement membrane. There is competition among stem cells for these niches, and the number of both stem cells and available niches increases with age and testis growth (*8*, *9*). Studies indicating a nonrandom distribution of undifferentiated spermatogonia in mouse seminiferous tubules suggest a regulatory mechanism for niche position (*10*). The interaction between stem cell and niche, particularly the signals that determine whether a cell will remain self-renewing or differentiate, is a critical area for investigation.

Xenogeneic transplantation of rat testis cells to immunodeficient mice results in production of rat spermatogenesis in mouse tubules, and

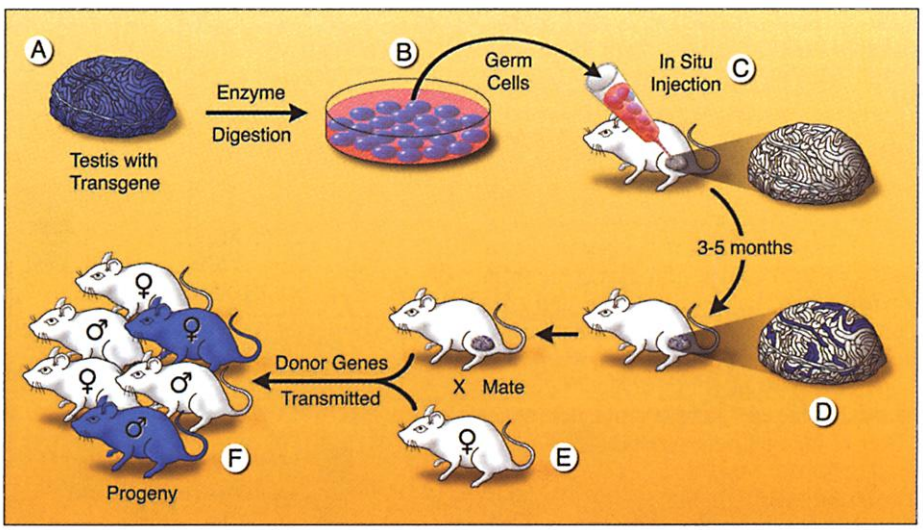

Fig. 1. Testis cell transplantation method. A single-cell suspension is produced from a fertile donor testis (**A**). The cells can be cultured (**B**) or microinjected into the lumen of seminiferous tubules of an infertile recipient mouse (**C**). Only a spermatogonial stem cell can generate a colony of spermatogenesis in the recipient testis. When testis cells carry a reporter transgene that allows the cells to be stained blue, colonies of donor cell–derived spermatogenesis are identified easily in recipient testes as blue stretches of tubule (**D**). Mating the recipient male to a wild-type female (**E**) produces progeny (**F**), which carry donor genes. Genetic modification can be introduced while the stem cells are in culture.

Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, 3850 Baltimore Avenue, Philadelphia, PA 19104, USA.

the rat germ cells are nourished by mouse Sertoli cells (*11*). Thus, the cell surface–recognition molecules and growth factors necessary for movement of transplanted stem cells from the lumen through Sertoli cell tight junctions to the basement membrane, followed by differentiation and movement of rat germ cells in the opposite direction, have been conserved for 10 million years, since the evolutionary divergence of rats and mice. Testis cell transplantation from other species, (e.g., dog, cow, pig, and baboon) into immunodeficient mice results in colonies of spermatogonia on the basement membrane, which are sustained for 6 months or longer (*12*, *13*). However, differentiation of germ cells to spermatozoa, as seen in rat-to-mouse transplantation, does not occur for these species that diverged 50 to 100 million years ago. Nevertheless, primitive spermatogonial stages can survive and replicate for long periods in the seminiferous tubules of distantly related species. The environment required for stem cell and undifferentiated spermatogonial cell maintenance appears to be highly conserved, whereas factors necessary for the differentiation process diverge more quickly.

Because Sertoli cells support and virtually engulf germ cells during the differentiation process (Fig. 2), it was thought that the somatic cell exerted considerable control over germ cell maturation. However, donor rat germ cells supported by recipient mouse Sertoli cells follow precisely the cell cycle timing and organizational pattern of rat germ cells, although spermatogenesis requires 52 days in rats and only 35 days for mice (*14*). Thus, the rigid species timing and pattern of germ cell differentiation reflects the intrinsic genetic program of the germ cell and is not influenced by somatic supporting cells.

Testis cell transplantation also provides a valuable approach to determine the role of the germ cell and Sertoli cell in natural or induced defects of spermatogenesis. Using this technique, the juvenile spermatogonial depletion (*jsd*) mutation of mice was shown to be a germ cell defect (*15*). On the other hand, in males with a targeted mutation of the estrogen receptor alpha or that lack functional androgen receptors, the defect is in supporting cells (*16*, *17*). Germ cell defects could be corrected by genetic modification of the stem cell during the transplantation procedure (Fig. 1), and fertility in mice with functional stem cells and a Sertoli cell defect already has been restored by microinjecting a viral vector with a corrective gene into the seminiferous tubules (*18*).

Using testis cell transplantation, the immense regenerative capability of the spermatogenic process was demonstrated by experiments in which fewer than 200 transplanted stem cells, less than 1% of the number present in a normal wild-type testis, restored fertility in a mouse with a stem cell defect (*19*). This efficiency and the generation of normal spermatogenesis after testis cell transplantation between rats that are not immunologically compatible (*20*, *21*) underlies the potential clinical application of the technique in animals and humans. Initial studies in animals (monkeys and pigs), have demonstrated that germ cells transplanted between males of the same species can be identified in recipient tubules 4 weeks later, although donor-derived differentiated germ cells have not yet been demonstrated for these species (*22*, *23*). In humans, men undergoing stem cell–destroying irradiation or chemotherapy for cancer have few options to safeguard fertility. Thus, clinical trials based on testis cell transplantation already have been initiated (*24*). This approach is possible because spermatogonial stem cells can be frozen and will regenerate spermatogenesis when transplanted (*25*). A testis biopsy is obtained and frozen and, after eradication of malignant cells from the patient, the testis cells are thawed and reintroduced into his seminiferous tubules (*24*). The general approach of spermatogonial stem cell cryopreservation and serial transplantation has the potential of making an individual male germline immortal.

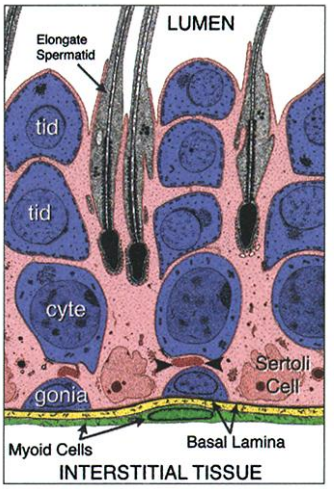

Fig. 2. Organization of germ cells and somatic cells in a seminiferous tubule. Germ cell differentiation proceeds through multiple stages, including spermatogonia (gonia), spermatocyte (cyte), spermatid (tid), and finally spermatozoa, which are released into the lumen. Sertoli cells are joined continuously around the tubule by tight junctions (denoted by the arrowheads) that regulate passage of cells and large molecules between the basal compartment, containing spermatogonia, and the luminal compartment, containing differentiating germ cells. Only a small fraction (~1%) of spermatogonia are stem cells; the remainder have begun differentiating. [Adapted with permission from (*28*)]

Maintenance of any cell type in culture, particularly a germline cell or stem cell, is enormously valuable to understand factors that control its survival, replication, and differentiation. Although spermatogonial stem cells can survive in vitro for longer than 3 months and generate spermatogenesis when transplanted to a recipient, generally only 10 to 20% of stem cells remain after 7 days in culture (*26*, *27*). Nonetheless, the transplantation assay has enabled a systematic approach to study requirements necessary for long-term maintenance and replication of stem cells in vitro, which resulted in improved conditions and permitted experimental genetic modification. When testis cells are cultured with a retroviral vector carrying a reporter gene, before the transplantation procedure (Fig. 1), spermatogonial stem cells are transduced readily, and the vector is integrated into chromosomal DNA of the stem cell (*27*). After transplantation, the transduced stem cells generate colonies of spermatogenesis, and the transgene can be transmitted through the germline to progeny of the recipient (Fig. 1). Moreover, expression of the transgene is not silenced, as often occurs after retroviral transduction of HSCs or embryonic stem cells. Other viral vectors also can be used to transduce spermatogonial stem cells and, combined with sophisticated somatic cell gene therapy techniques, will provide a powerful approach to generate gain-of-function and loss-of-function transgenic animals in many species. Further improvement in culture techniques will permit introduction or modification of genes by more selective methods, possibly leading to the ability to correct human genetic defects. One implication of these viral transduction studies is that male germline stem cells are potentially subject to permanent alteration by viral vectors commonly used for human somatic cell gene therapy.

Spermatogonial stem cells are the only cells in the postnatal animal that undergo self-renewal throughout life and transmit genes to subsequent generations. Thus, the ability to cryopreserve, culture, and transplant these unique cells provides a powerful system to study stem cell biology, preserve individual genomes, and modify germ lines.

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29. I apologize to colleagues whose work was not cited because of space limits. I thank R. Behringer, R. Davies, K. Orwig, and E. Sandgren for valuable comments on the manuscript; M. Avarbock, C. Freeman, R. Naroznowski, and C. Pope for contributions to experiments; and J. Hayden for help with photography and figures. Supported by the National Institute of Child Health and Human Development grant 36504, the Commonwealth and General Assembly of Pennsylvania, and the Robert J. Kleberg Jr. and Helen C. Kleberg Foundation.

REVIEW

Unique Chromatin Remodeling and Transcriptional Regulation in Spermatogenesis

Paolo Sassone-Corsi

Most of our knowledge of transcriptional regulation comes from studies in somatic cells. However, increasing evidence reveals that gene regulation mechanisms are different in haploid germ cells. A number of highly specialized strategies operate during spermatogenesis. These include a unique chromatin reorganization program and the use of distinct promoter elements and specific transcription factors. Deciphering the rules governing transcriptional control during spermatogenesis will provide valuable insights of biomedical importance.

The developmental process of spermatogenesis relies on a number of distinct regulatory programs involving sophisticated hormonal control from the hypothalamic-pituitary axis (*1*). This review concentrates on recent advances about the unique rules governing postmeiotic transcription in male germ cells. One very special feature concerns the process of chromatin remodeling, which involves various steps that are unlike those in somatic cells (*2*). Many generally expressed genes use alternative promoters in male germ cells, and several genes have a homolog whose expression is specific for the male germ line. Transgenesis experiments have revealed that various cis-acting regulatory elements direct expression exclusively to the testis, demonstrating the presence of germ cell–specific factors (*2*, *3*–*5*).

Chromatin Dynamics

In somatic cells, specific chromatin remodeling events have been directly coupled to transcriptional activation and silencing (*6*–*8*). Are the same events operating in male germ cells? During spermatogenesis, the haploid genome undergoes extensive reorganization through meiosis and DNA compaction. Meiosis involves homologous chromosome pairing at synapsis and meiotic recombination (Fig. 1). After desynapsis and completion of meiosis, gene transcription increases, but then the haploid genome is compacted within the sperm head to a volume of about 5% of that of a somatic cell nucleus. This remarkable repackaging event is achieved by replacing histones with protamines (*9*, *10*), arginine- and cysteine-rich proteins that organize the haploid male genome into a highly specialized, doughnut-shaped chromatinic structure that is fundamentally different from the classical nucleosomal architecture (*9*, *10*). The reason for the histone-protamine transition is probably related to the high compaction potential of nucleoprotamines and the requirement for a unique chromatin architecture that would enable a specific transcription schedule after fertilization.

In mammals, histones are not replaced directly by protamines (Fig. 1). Transition proteins (TP1 and TP2) are small, basic nuclear proteins that appear when histones are displaced and chromatin condensation initiates. Targeted mutation of each transition protein suggests a redundant role for the transition proteins (*11*, *12*). Both TP1- and TP2-mutant mice are fertile and display only minor spermiogenesis abnormalities, indicating that histone replacement and chromatin compaction are transition protein–independent processes. Indeed, precocious chromatin con-

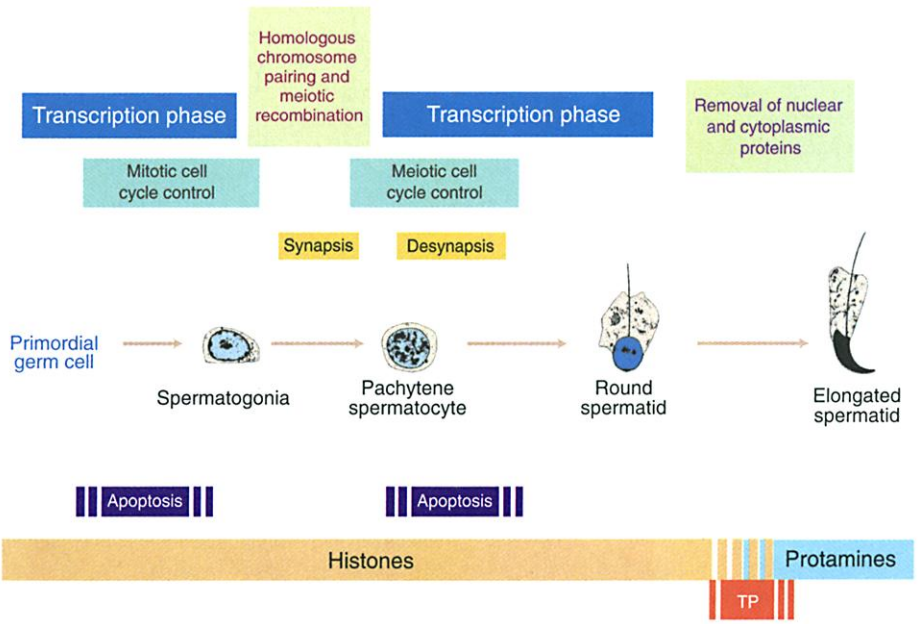

Fig. 1. Spermatogenesis is a cyclic developmental process by which spermatogonia cells generate the mature spermatozoon. These events are characterized by important modifications in chromatin organization, basically during two periods, meiosis—which includes the synapsis and desynapsis of the chromosomes—and the histone-protamine transition. Postmeiotically, a powerful wave of transcription occurs in haploid cells, which is governed by highly specialized molecular mechanisms. Specific genes operate at distinct steps of the spermatogenic process.

Institut de Génétique et de Biologie Moléculaire et Cellulaire, B. P. 10142, 67404 Illkirch, Strasbourg, France. E-mail: paolosc@igbmc.u-strasbg.fr