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Commentary

Spermatogonial stem cells of the testis

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Recent studies by Brinster and colleagues reported in this issue of the *Proceedings* (1, 2) have shown that the injection of spermatogonial stem cells into the testes of sterile recipient mice gives rise to functional sperm in the recipients. These results provide a new technique for the investigation of the physiology of the spermatogonial stem cells and, more importantly, may eventually lead to new approaches for the construction of transgenic animals. In this commentary, I will first review the physiology of the spermatogonial stem cells and then consider the implications of spermatogonial transplants.

Spermatogenesis, the process by which the type A spermatogonial stem cells divide and differentiate to produce the mature spermatids (3), occurs in the seminiferous tubules of the testis. The spermatogonial type A stem cells proliferate actively to produce two types of cells: other spermatogonial stem cells and differentiating spermatogonia. The differentiating cells (intermediate and type B spermatogonia) divide and give rise to the more specialized meiotic spermatocytes, but they do not divide to produce new stem cells. Among the type A spermatogonia there are distinct subpopulations, types A_1–A_4, which were considered the renewing stem-cell spermatogonia, and type A_0, which was considered a reserve type of stem cell (4). The type A_4 cells divide to give rise to differentiating spermatogonia, the intermediates, as well as to new type A_1 cells, to maintain the stem cell population. The type A_0 cells divide slowly and are kept in reserve to repopulate the testis after an injury such as irradiation damage or as a finely tuned mechanism to maintain normal numbers of types A_1–A_4 (Fig. 1). A similar scheme of spermatogonial renewal was proposed for the monkey (5) and for man (6). Other investigators in the early seventies proposed that in the rat and mouse the types A_1–A_4 spermatogonia were already differentiated cells and thus not stem cells but that the A_s (A_0) cells were the true stem cells (7, 8). During the past 20 years, very little new information has been added on the topic of stem-cell renewal in the testis. Unfortunately, there is no general agreement on the identity of the true stem cell in the testis or on the validity of either stem-cell renewal model (9, 10).

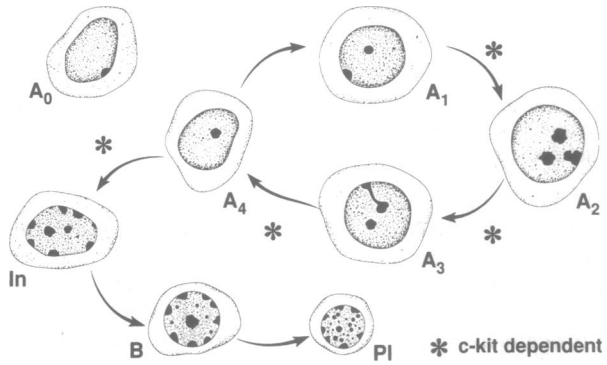

FIG. 1. Stem-cell renewal model modified from ref. 4. The types A_1–A_4 spermatogonia are considered to be the renewing stem cells. The spermatogenic process is initiated by the divisions of type A_1 cells to form the A_2 cells; the type A_2 cells divide to yield type A_3, which in turn give rise to type A_4. The type A_4 cells have the capacity to give rise to new type A_1 cells as well as to the more differentiated intermediate (In) spermatogonia. The type A_0 spermatogonia are reserve stem cells which normally divide only rarely. The asterisks depict the divisions that are dependent upon the c-Kit receptor according to Yoshinaga and colleagues (31).

Until recently, research on stem-cell renewal in the testis has been limited because of technical difficulties in isolating and identifying these cell populations. In 1977, a procedure was described to isolate individual germ-cell types from the mouse testis, using a gradient of bovine serum albumin and velocity sedimentation at unit gravity (11). With this technique, it was possible to obtain a fraction of type A spermatogonial cells from immature mice which was about 90% pure. A number of other investigators (12–14) have used various techniques to isolate the type A spermatogonia from the mouse, but research on the stem cells did not progress very far, mostly because there were no adequate markers which could conclusively establish the identity of the type A cells after isolation. This latter problem was partially solved in the late 1980s and early 1990s with the identification of a new growth factor/receptor system (Kit ligand/c-Kit receptor) important in stem cells regulating melanogenesis, hematopoiesis, and gametogenesis (15, 16). In the testis, the Kit ligand (stem-cell factor, steel factor, mast-cell growth factor) is produced by the Sertoli cells (17) and its receptor, the c-Kit receptor, is present in the type A spermatogonial population (18). In addition, it has been demonstrated that spermatogonial proliferation is regulated by the Sertoli cell-produced Kit ligand (17). The c-Kit receptor could now be used as a marker for the type A cells. Highly purified populations of these type A cells were isolated from rat and characterized by demonstrating the expression of the c-Kit receptor at the gene and protein level (19). Furthermore, the c-Kit receptor was phosphorylated in response to Kit ligand, suggesting that the receptor was indeed an active receptor (19). It should now be possible to develop methods that allow for the long-term culture of the type A spermatogonial stem cells.

One of the most important unanswered questions in cell biology today is the identification of the factor(s) directing the stem cells in the testis towards meiosis—i.e., the meiotic inducer. The regulation of stem-cell renewal and/or differentiation can be addressed more adequately *in vitro* with purified populations of type A cells. *In vivo*, the type A cells can be directed to one of three fates (Fig. 2). They will either remain as type A stem cells, differentiate to form the intermediate and type B spermatogonia and eventually the preleptotene spermatocytes, or die by the process of apoptosis. Indeed, it has been shown *in vivo* that at least two-thirds of the type A cells undergo programmed cell death (20). Primordial germ cells respond to a mixture of three growth factors: Kit ligand, basic fibroblast growth factor, and leukemia inhib-

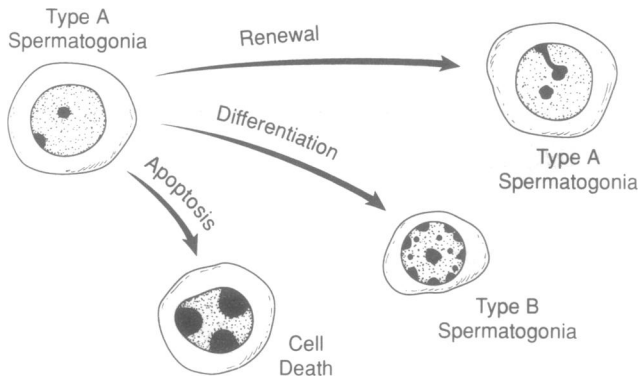

FIG. 2. Fate of type A spermatogonial stem cells. They can give rise to other type A cells or to the differentiated intermediate and type B spermatogonia, or they can die by the process known as programmed cell death or apoptosis.

itory factor (21). It will be interesting to determine whether the type A spermatogonial stem cells *in vitro* also respond to these same growth factors and whether one or more of the growth factors can direct the cells to different fates. The isolated cells could also be used to examine the signal transduction events in the spermatogonial population in response to various growth factors. Furthermore, until now it was not possible to identify which growth factor receptors are present on the type A cells. Thus, a series of fundamental studies which could shed information regarding the regulation of the stem cells in the testis can now be carried out.

The spermatogonial type A stem cells in the testis can be considered as a kind of "eternal" germ cell because they are present from birth to death and have the capacity to give rise to new type A cells and to spermatozoa which can pass genetic material from generation to generation (2, 22, 23). It will be interesting to attempt to actually immortalize these type A spermatogonial stem cells by well-characterized procedures such as described by Millán and colleagues (24) for other testicular cell types. Of particular value would be immortalized type A stem cells that retained the capacity to differentiate into early spermatocytes and to divide and maintain their own numbers. Furthermore, the retention of the c-Kit receptor in the immortalized cells would be important, since this growth factor receptor is probably essential for their normal behavior.

These stem cells may also open up new avenues of research on early stages of development (25). In the past, human embryo experiments have generally been limited to the first 2 weeks after fertilization. During this time, the zygote develops into a morula and finally a blastocyst which normally implants itself in the lining of the mother's uterus by the 14th day after fertilization. Although federal funding on the preimplantation embryo was prohibited between 1980 and 1993, the National Institutes of Health is now finalizing guidelines for the resumption of embryo research. Manipulation of the male and female gametes prior to fertilization (i.e., the spermatozoon and the egg and their respective progenitor cells) has not received a great deal of attention until recently.

It is now reported that stem cells from the testis survive transplantation and multiply in the testis of the recipient animals. In an initial series of novel and exciting experiments, Brinster and Zimmerman (1) have demonstrated the feasibility of transplanting a mixture of enzyme-dispersed mouse testis cells, isolated from immature normal male mice, directly into the seminiferous tubules of adult sterile mice (sterile mutants and busulfan-treated mice). Remarkably, the testicular stem cells, the type A spermatogonia, in the mixture of cells obtained from the donor mice, seeded the recipient seminiferous tubules, then proliferated and differentiated, and eventually proceeded to repopulate the recipient testes with spermatozoa. In the research reported, about one-third of the recipient testes demonstrated apparently normal spermatogenesis with the production of mature spermatids in about 10% of the seminiferous tubules. In a second study reported in this issue, Brinster and Avarbock (2) used recipient mice that were treated with busulfan but still maintained some endogenous spermatogenesis; strikingly, they discovered that up to 80% of the progeny sired by the recipient mice came from donor-derived spermatozoa. In these latter experiments, spermatogenesis was present in >70% of the recipient mice. Thus, the transplanted diploid spermatogonial stem cells among the mixture of the donor testicular cells proliferated and differentiated to form the early meiotic spermatocytes, which then completed the two meiotic reduction divisions resulting in the formation of immature haploid spermatids. Finally, the immature spermatids differentiated to form the mature spermatids or the spermatozoa. The spermatozoa were capable of fertilizing eggs in female mice, and normal live offspring were produced. The success rate for the transplants could be very much improved with the use of purified isolated spermatogonial stem cells. Brinster points out that the next step in this research is the development of a culture system for stem-cell spermatogonia, the analysis of various agents that could modify the cells, and the selection of specifically modified clones of individual cells.

Extension of this work to humans could have far-reaching clinical consequences, since it may offer a means of treating male infertility. Furthermore, there is also the potential to correct defective genes in the spermatogonial stem cells prior to transplant in order to prevent the transmission of certain illnesses to offspring. This technology offers enormous potential for eradicating a number of catastrophic genetic diseases. Although many ethical, technical, and scientific issues regarding germ-line gene modifications and subsequent transplant to a recipient testis remain to be clarified, Brinster's research very elegantly directs attention to the importance of the spermatogonial stem-cell population in the testis and, in particular, to the importance of gaining a thorough understanding of their behavior.

The development of the spermatogonial stem-cell transplantation technique and the possibility of manipulating the genetic makeup of the spermatogonia provide many potential opportunities for agricultural, biological, and medical research. Indeed, the University of Penn-

Table 1. Some ethical arguments for and against germ-line gene modification

Arguments in favor
Moral obligation of health professions to use best available treatment methods
Parental autonomy and access to available technologies for purposes of having a healthy child
Germ-line gene modification more efficient and cost-effective than somatic cell gene therapy
Freedom of scientific inquiry and intrinsic value of knowledge
Arguments against
Expensive intervention with limited applicability
Availability of alternative strategies for preventing genetic diseases
Unavoidable risks, irreversible mistakes
Inevitable pressures to use germ-line gene modification for enhancement

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sylvania (Brinster's home institution) has applied for a patent on such a technique from the European Patent Office and this issue will be decided shortly. Although somatic cell gene therapy is a generally accepted means of preventing disease, this procedure affects only the individual undergoing treatment. Germ-line gene therapy, on the other hand, affects not only the patient but subsequent generations as well. However, the technology required to introduce a corrective gene to a specific site on a chromosome has not been perfected and the risk of error remains great (26). Several reviews (26–30) described a number of arguments in favor of and against germ-cell gene modification. A table from one publication (27) is reproduced here (Table 1).

The general consensus at this time seems to be that it is much too early to consider germ-line gene modifications and human stem cell transplantation, but ultimately it is likely that this type of approach will be of immense benefit to mankind. Of particular value at the moment would be to obtain a thorough understanding of the factors regulating the type A stem cell population in the testis. This could also lead to a better understanding of how other types of stem cells function.

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