planation of this pattern is that blue patches reflect the colonization of the tubule by a stem cell, which then generates more stem cells and spermatogonia. It is unclear whether more than 1 stem cell contributes to an individual blue patch, but given the small number of stem cells injected and no obvious reason for the stem cells to aggregate in 1 area, each patch may probably represent colonization by a single stem cell. Differentiation to more mature germ cell stages appears to begin after the initial colonization and in the center of the colony. Lateral spread of the colony continues for several months, although the rate of spread may decrease with time and generally does not extend to the entire length of an individual tubule.

The extent of donor stem cell colonization and spermatogenesis necessary to allow the transgene to be transmitted to progeny is unknown. In general, ca. 15% of the seminiferous tubules must contain spermatogenesis for a mouse to be fertile. However if endogenous spermatogenesis returns in the recipient animal because of a decreased busulfan effect, the resulting spermatozoa will act as carrier cells to facilitate the passage of donor cell-derived spermatozoa through the epididymis and into the ejaculate.[4] The first recipient male to transmit a donor cell transgene to progeny contained a significant level of endogenous spermatogenesis. In this recipient, the donor cells colonized the seminiferous tubules in a random manner; in some areas of the testes little staining appeared, but other locations contained almost 10% of tubule cross-sections with donor cell-derived spermatogenesis. Areas of donor cell colonization typically show cellular associations characteristic of mouse spermatogenesis.[6] When this male was mated to wild-type control females, 1 in 120 progeny males had testes that stained blue.[4] Excellent colonization can be achieved by using donor cell populations with high numbers of stem cells, increasing injected cell density and employing recipients that have a low level of endogenous spermatogenesis regeneration. In some experiments, a mixture of donor cells harvested from the testes of 2 different transgenic mouse lines was transplanted, which can be distinguished by the number of integrated transgene copies. Analysis of progeny from fertile recipients demonstrated that stem cells from the testes of both transgenic mouse lines colonized individual recipients and produced spermatozoa.[2] Thus the transplantation technique allows a comparison within the same testis or recipient of mixed populations of donor cells that have received separate treatments. This provides a powerful system to study experimental effects on stem cells and spermatogenesis.

Cultivation of Spermatogonial Stem Cells

Several studies in male germ cells culture have been reported, but these have shown limited success.[1] Some specific sequences in the entire spermatogenic process, e.g., the initiation of meiosis or spermatid maturation was assessed in vitro. In general, it is thought that these processes are facilitated by interaction with Sertoli cells, and that male germ cell development in vitro is short-lived, at a few days to a few weeks. However, it is difficult to assess the actual state of the spermatogonial stem cell in vitro.

In recent experiments, the status of spermatogonial stem cells recovered from neonatal and adult testes of ZF*lacZ* transgenic mice cultured for various periods of time either with or without STO feeders was examined.[2] Testis cells were recovered as described for transplantation and placed in culture plates or flasks, by using standard tissue culture methods and a basic medium of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Spermatogonial stem cells taken from both adult and neonatal testes were maintained in culture for periods up to 111 days, and following transplantation, they established normal donor cell-derived spermatogenesis in recipient seminiferous tubules. No colonization of testes (0 of 24) occurred in recipients that received cells cultured without STO feeders, whereas almost 50% of the testes that received cells cultured on STO feeders established spermatogenesis.[2] Furthermore the stem cell concentration in the cultured cell population may have been more than 10 times that found in newly collected testis cells, suggesting replication had occurred during the culture period.[2] The results of these experiments demonstrate that spermatogonial stem cells can be maintained in culture for long periods of time and probably