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By Stephen Bradt

Scientists at the School have successfully used a retrovirus to modify genes in spermatogonial stem cells in a mouse — the first instance, in any species, of a transgenic animal created by inserting a gene into male germ-line stem cells.

The inserted gene subsequently appeared in approximately 4.5 percent of offspring of mice transplanted with the altered stem cells, and was transmitted to at least three succeeding generations.

The work was the cover story in the Nov. 6 issue of the Proceedings of the National Academy of Sciences. The findings should enable the creation of transgenic individuals in a wide range of species, permitting scientists to develop research models for study of numerous human diseases.

Led by **Ralph L. Brinster**, Richard King Mellon Professor of Reproductive Physiology at the School, the scientists succeeded in inserting a foreign gene — in this case the common reporter gene lacZ, whose product is the enzyme fl-galactosidase — into 2 to 20 percent of mouse spermatogonial stem cells in laboratory experiments, a tenfold improvement over previous attempts.

"These results indicate that there is no intrinsic barrier to the genetic engineering of spermatogonial stem cells using retroviruses, and that once inserted, the foreign genes will continue to be transmitted and expressed from one generation to the next," said Dr. Brinster. All male mammals harbor many spermatogonial stem cells, key repositories of genetic material whose daughter cells give rise after puberty to sperm. In the human male, approximately 1,000 sperm cells, each carrying a different combination of genetic material, are generated in this manner with each heartbeat. Whereas the female germ cell, the egg, stops dividing before birth, male germ-line stem cells continue to divide throughout life.

Of the various types of stem cells, only two — spermatogonial and hematopoeitic stem cells — can be positively identified using functional assays. This makes them valuable models for other types of stem cells, such as those that give rise to skin, the lining of the intestines, brain, muscle and liver.

Spermatogonial stem cells are of additional interest for transgenics applications because they are the only cells, including all other stem cells, that undergo self-renewal throughout an animal's lifetime and contribute genes to subsequent generations. Previous attempts to genetically alter this unique type of stem cell, either through retroviruses or other methods, have met with little success.

Brinster's work is unusual in that his transgenic mice were created using cells from male animals. Transgenic animals are generally produced by inserting foreign DNA into cells derived from females, such as oocytes, eggs and blastocysts. Brinster's group has demonstrated that the efficiency of generating transgenic mice through spermatogonial stem cells is roughly equivalent to that of female-derived cells.

"Questions had been raised regarding whether male germ-line stem cells could be transduced with a retroviral vector and whether any gene introduced would be silenced," Brinster said. "Our work clearly demonstrates that the stem cell can be transduced at relatively high efficiency and expression is not silenced. About 10 percent of stem cells carry active genes that are transmitted for at least three generations."

Retroviruses are the most common vehicles for introducing genes in human somatic cell gene therapy, and some scientists had expressed concern that this approach might result in genetic alterations to germ-line cells. Brinster's paper indicates that the germ cells are indeed susceptible to insertion of foreign genes via retroviruses, although the somatic cells that surround stem cells in the body most likely provide a protective shield.

Brinster was joined in the work by Makoto Nagano, now at McGill University, and Clayton J. Brinster, Kyle E. Orwig, Buom-Yong Ryu and Mary R. Avarbock, all of the Department of Animal Biology at the School. Their work was supported by the National Institutes of Health, the Commonwealth and General Assembly of Pennsylvania and the Robert J. Kleberg Jr. and Helen C. Kleberg Foundation.