

## MAINTENANCE OF GENETIC INTEGRITY IN EMBRYOS AND STEM CELLS

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Methodological advances over the past 2-3 decades have facilitated remarkable advances in clinical technologies that allow otherwise infertile couples to conceive their own biological children, and related technical advances now promise to foster a new era of stem cell-based therapeutic approaches to the treatment of previously intractable diseases and debilitating conditions. While these methodologies have and will continue to provide solutions to clinical challenges that previously seemed insurmountable, they also involve significant manipulations of cells that afford opportunities for the development of aberrations in either epigenetic or genetic programming of the resulting embryos, stem cells or their derivatives. While numerous studies have focused on the former (epigenetic aberrations), very few have investigated the latter (genetic aberrations), and of those studies that have addressed genetic abnormalities in either offspring produced by assisted reproductive technologies (ARTs) or stem cells or their derivatives, most have examined the occurrence of gross chromosomal aberrations such as aneuploidies. This is despite the fact that point mutations, which result in single-base substitutions or small insertions or deletions of only a few base pairs, are the most frequently occurring type of mutation, and are responsible for the majority of genetic diseases in humans (Crow, 2000).

I will describe a novel model transgenic mouse system that has allowed us to investigate the frequency of spontaneous mutations in various cell types in the body, including germ cells and somatic cells, as well as in offspring produced by natural reproduction, ARTs or cloning by somatic cell nuclear transfer (SCNT), and, most recently, in undifferentiated and differentiated embryonic stem (ES) cells. This transgenic mouse, known as the "Big Blue Mouse<sup>®</sup>", produced and marketed by Stratagene (La Jolla, California, USA) (Kohler et al., 1990), carries as an integrated transgene in each cell, a tandem array of multiple copies of a lambda phage shuttle vector that includes the *lacI* gene encoding a repressor of the lac operon. When DNA is recovered from transgenic mouse cells and mixed with packaging extract, functional bacteriophage particles are formed, with each carrying a single copy of the shuttle vector, including a copy of the *lacI* gene. The bacteriophage can then be plated on a lawn of *Escherichia coli* cells, leading to the formation of plaques in the bacterial lawn. If the *lacI* gene has remained un-mutated while in the mouse, it will continue to encode a functional repressor that will inhibit expression of the lac operon in the plaque. However, if, while in the mouse, the *lacI* gene has undergone a point mutation at any of ~38% of the bases in this gene encoding the repressor, the repressor will have lost its function and will no longer inhibit expression of the lac operon in the plaque.  $\beta$ -galactosidase is one of the enzymes encoded by the lac operon, and its expression is easily detectable following addition of the correct substrates that result in the formation of a blue deposit. Thus, in those plaques in which the packaged *lacI* gene is un-mutated, the lac operon will fail to be expressed and no blue color will form (these plaques appear clear), whereas in those plaques in which the packaged *lacI* gene carries a point mutation, the lac operon will be expressed and a blue deposit will form. Normally spontaneous point mutations occur only very rarely ( $10^{-4}$  -  $10^{-6}$ ), but by recovering and plating large numbers of bacteriophage particles, each carrying a unique copy of the lambda shuttle vector, it is straight forward to detect a mutant *lacI* gene because of the blue colored plaque it forms. In this way we can determine the frequency of mutant transgenes (# blue plaques/total # plaques), which is indicative of the frequency of spontaneous point mutations in the tissue or cell type from which the transgenes were recovered. In addition, we can then sequence each mutant *lacI* gene to determine the spectrum of point mutations that have occurred in that cell type or tissue.

We have used this approach to examine the frequency of point mutations in different cell types of the mouse, and have discovered that this frequency is typically 5-10 fold lower in germ cells compared to various somatic cell types from the same individual (Walter et al., 1998). We also found that methods associated with ARTs, including in vitro fertilization, intracytoplasmic sperm injection, round spermatid injection, culture of preimplantation embryos and embryo transfer do not lead to any increases in the frequency of spontaneous mutations in the resulting offspring (Caperton et al., 2007). Although well over 3 million human babies have been conceived to date by one or another form of ART, this was the first direct analysis of the occurrence of point mutations as a result of the use of these methods, and was necessarily conducted in an animal model system.

Recently, we found that cloned offspring produced by SCNT also show no greater frequency of point mutations than do naturally conceived offspring (Murphey et al., 2009). This is despite the fact that cloning involves the use of genomes derived from somatic cells that, on average, carry 5-10 fold more spontaneous point mutations than do germ cells. We ascribe this result to the fact that, because of the relatively low frequency of spontaneous mutations in any cell type, any single cell selected at random for use as a nuclear donor for cloning is very unlikely to carry a spontaneous mutation at the test locus. However, this also allowed us to determine that epigenetic mechanisms that normally regulate the level of genetic integrity in a cell-type specific manner are subject to reprogramming during the cloning process. This, in turn, suggested the possibility of a mechanistic relationship between pluripotency and enhanced maintenance of genetic integrity. To test this hypothesis, we have begun to use the Big Blue Mouse<sup>®</sup> transgenic mouse system to examine the frequency of spontaneous point mutations in undifferentiated ES cells and their differentiated derivatives. Recently acquired data describing these frequencies will be presented and discussed.

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