CHAPTER 5

PRESERVATION OF GENOMIC INTEGRITY IN MOUSE EMBRYONIC STEM CELLS

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Abstract

Embryonic stem (ES) cells and germ cells have the potential to give rise to an entire organism. A common requirement is that both must have very robust mechanisms to preserve the integrity of their genomes. This is particularly true since somatic cells have very high mutation frequencies approaching 10^-4 in vivo that would lead to unacceptable levels of fetal lethality and congenital defects. Notably, between 70% and 80% of mutational events monitored at a heterozygous endogenous selectable marker were loss of heterozygosity due to mitotic recombination, a mechanism that affects multiple heterozygous loci between the reporter gene and the site of crossing over. This chapter examines three mechanisms by which mouse embryonic stem cells preserve their genomic integrity. The first entails suppression of mutation and recombination between chromosome homologues by two orders of magnitude when compared with isogenic mouse embryo fibroblasts which had a mutation frequency similar to that seen in adult somatic cells. The second renders mouse ES cells hypersensitive to environmental challenge and eliminates damaged cells from the self-renewing population. Mouse ES cells lack a G1 checkpoint so that cells damaged by exogenous insult such as ionizing radiation do not arrest at the G1/S phase checkpoint but progress into the S phase where the damaged DNA is replicated, the damage exacerbated and the cells driven to apoptosis. The third mechanism examines how mouse ES cells repair double strand DNA breaks. Somatic cells predominantly utilize error prone nonhomologous end joining which, from a teleological perspective, would be disadvantageous for ES cells since it would promote accumulation of mutations. When ES cells were tested for the preferred pathway of double strand DNA break repair, they predominantly utilized the high fidelity homology-mediated repair pathway, thereby minimizing the incidence of mutations during the repair process. When mouse ES cells are induced to differentiate, the predominant repair pathway switches from homology-mediated repair to nonhomologous end joining that is characteristic of somatic cells.

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INTRODUCTION AND HISTORICAL PERSPECTIVE

The capacity of a single cell to give rise to multiple different cell types of an organism has been an area of investigative interest for many years. A question that lingers is that of nuclear equivalence, (i.e., whether all cells of a multicellular organism have genomes that are quantitatively and qualitatively equivalent). Nuclear equivalence was tested as early as 1902 when Spemann separated the two blastomeres following the first cleavage division of a newt embryo and showed that both blastomeres could develop into a complete embryo, supportive of functional equivalence (cited in ref. 1). This work was presaged eleven years earlier by Hans Driesch who showed that separated blastomeres of sea urchin embryos were capable of developing into normal, albeit smaller, sea urchins (cited in ref. 1).

Half a century later, a period replete with many important and insightful discoveries, two seminal experiments were described. The classic and elegant experiments by Hämmerling, using the green alga Acetabularium as a model organism, showed that the nucleus contained all of the information necessary to dictate cell morphology. Acetabularium is a single cell organism that has a base that contains the nucleus, a stalk and a cap. Using two species of Acetabularium, A. mediterrania and A. crenulata, the former with a smooth cap and the latter with a wrinkled cap, Hämmerling showed that when the stalk and cap of A. mediterrania was grafted to the base of A. crenulata, the grafted cap was transformed from smooth to wrinkled. The reciprocal experiment also held true, indicating that the genetic information determining cap morphology was dictated by the nucleus within the grafted base. At about the same time, Briggs and King expanded on the findings of Driesch and Spemann by successfully transferring the nucleus of an undifferentiated frog blastula cell to an enucleated fertilized egg. In about one third of their attempts, the transplanted nucleus was capable of directing development to a normal embryo. When using nuclei from later staged embryos (neurula or tailbud), however, they found that none of the recipient eggs developed normally and that the majority failed to complete gastrulation, suggesting a restriction in the potential to differentiate as cells mature.

In 1962, the question of nuclear equivalence and the potential pluripotency of nuclei from differentiated cells in a multicellular organism was reignited by the findings of John Gurdon. He reported that the nucleus from a Xenopus tadpole intestinal cell, when introduced into an enucleated Xenopus egg, was able to support the development of a fully-formed feeding tadpole, demonstrating that the intestinal cell nucleus retained the genetic information necessary to produce all of the cells of a complete multicellular organism. It should be noted, however, that of the large number of nuclear transplant experiments performed, only slightly more than one percent of recipient eggs successfully produced a mature tadpole, leading to discussions as to whether the nuclei with demonstrated pluripotency are derived from stem cells or come from truly differentiated cells.

A major advance in cloning technology occurred in 1981 when Martin Evans in Cambridge and Gail Martin in San Francisco concomitantly and independently succeeded in culturing embryonic stem (ES) cells from mouse blastocysts. The pluripotency of ES cells coupled with the ability to target specific gene sequences to their cognate sites by targeted homologous recombination led to cloning of mice with very specifically and selectively inactivated or modified genes. Oliver Smithies and his colleagues first used targeted homologous recombination to correct a mutant Hprt gene in mouse ES cells and independently Capecchi and colleagues inactivated an Hprt gene in a similar manner. Three years later reports of genetically modified mice produced by introduction