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The Biology of Novel Animal Genes: Mouse APEX Gene Knockout

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Abstract

This is the final report of a one-year, Laboratory Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). The controlled breeding of novel genes into mice, including the 'gene knockout' (KO), or conversely by adding back 'transgenes' provide powerful genetic technologies that together suffice to determine in large part the biological role(s) of novel genes. Inbred mouse remains the best understood and most useful mammalian experimental system available for tackling the biology of novel genes. The major mammalian apurinic/apyrimidinic (AP) endonuclease (APE), is involved in a key step in the repair of spontaneous and induced AP sites in DNA. Efficient repair of these lesions is imperative to prevent the stable incorporation of mutations into the cellular genome which may lead to cell death or transformation. Loss or modulation of base excision repair activity in vivo may elevate the spontaneous mutation rate in cells, and may lead to a substantial increase in the incidence of cancer. Despite extensive biochemical analysis, however, the significance of these individual APE functions in vivo has not been elucidated. Mouse embryonic stem (ES) cells heterozygous for a deletion mutation in APE have been generated and whole animals containing the APE mutation have been derived from these ES cells. Animals homozygous for the APE null mutation die early in gestation, underscoring the biological significance of this DNA repair gene.

1. Background and Research Objectives

The overall goal of this project is to continue competency development at LANL for combined whole animal genetic and biological studies. The essential question: "What does a gene do in animals and humans?" drives the biomedical sciences today. Among animals, the laboratory mouse is uniquely amenable to produce alterations in any available gene sequence, and then to generate new mutant mice [1, 2]. The controlled breeding of novel genes into mice, including the 'gene knockout' (KO), or conversely by adding back

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'transgenes' provide powerful genetic technologies that together suffice to determine in large part the biological role(s) of novel genes. Although the basic functions of conserved mouse and human genes may be nearly identical, we realize that the patho-physiological consequences of a mutation in mice would not necessarily be identical or even similar to that in humans. Still, the inbred mouse remains the best understood and most useful mammalian experimental system available for tackling the biology of novel genes.

2. Importance to LANL's Science and Technology Base and National R&D Needs

This project relates to LANL core competencies in biotechnology and biosciences, and in complex experimentation of potential interest for industrial and government partnerships. Competency in solving a number of gene-biology problems is crucial to our future competitive position in the Laboratory's civilian national mission in health research. New candidate genes of potential importance for DNA repair and biomarkers of individual disease susceptibilities are now being identified by highly automated technologies [15]. Their biological and genetic roles remain problematic.

Our principal aim was to conduct preliminary genetic, biological, and cellular sensitivity characterizations of a homozygous KO mouse for the **AP exonuclease (APEX)** gene. This remarkable protein has putative dual functions: as a DNA repair enzyme for oxidative- or ionizing radiation- induced DNA base damages, and as a redox gene regulatory protein for transcription factors [3 - 7]. The APEX gene itself exhibits marked cell-type specific expression in rodent and human brain [8, 9; pers. comm. M. R. Kelly]. APEX therefore represents an excellent and challenging test of the mouse genetics approach to solving the biology of novel genes. LANL co-investigators have isolated and determined the structure of the mouse APEX gene [10]. This structure was used to create a complete knockout gene mutation in the mouse (see below).

Mice with KO mutations in DNA repair genes have shown dramatic in vivo sensitivities to UV radiation and chemical carcinogens [11 - 13]. Some DNA repair mutations in mice may also have detrimental impacts on growth and reproductive development (for examples see refs. 11, 14). Given the putative dual functions and developmental regulation of the APEX gene [5, 8, 9], we suggest that developmental or neurological disorders may well emerge in the APEX KO mice, in addition to cellular sensitivities to exogenous agents.

3. Scientific Approach and Accomplishments

Genetics of the APEX KO. We have completed the production of the APEX KO gene knockout mouse in collaboration with Roger Pedersen (UCSF). The 'KO' mutant gene construct was fabricated and screened at LANL by Takiguchi and Chen. Embryonic manipulations were done at UCSF. Physiologically, all APE^{+/-} animals appeared normal and there have been no apparent physiological problems observed to date (>3months age). Animals heterozygous for the APE mutation were bred to nontransgenic animals to expand the colony and to heterozygous siblings to obtain animals homozygous for the APE mutation (APE^{-/-}). Several matings for homozygosity were carried out and pups allowed to mature to birth. After 1 week, tail DNA was analysed from the viable pups. Genotype analysis determined that no viable pups were APE^{-/-}. The segregation ratio of homozygous wild type (APE^{+/+}) to heterozygous (APE^{+/-}) animals was 1:2. This fit the segregation pattern for an embryonic lethal null genotype for APE. The expected segregation pattern for a viable null genotype would be 1:2:1. If the APE^{-/-} animals were inviable, the ratio would instead be 1:2:0, as observed. Additional full term litters are currently being generated to statistically verify this frequency. To determine if the embryos were inviable during early or late gestation, litters from heterozygous matings were harvested prior to maturity and embryos genotyped. The gestation period for a mouse is 19 to 20 days. Attachment of the embryo to the uterine wall occurs on day 4.5 of gestation. (Note: Because fertilization occurs during the nocturnal period, time periods postcoitum (p.c.) are typically given in half days.) We therefore selected mid-gestation embryos for initial analysis. This is the period at which embryos null for c-jun become inviable and the loss of APE function, in this case redox, may effect a similar developmental pattern. Embryos were harvested at day 11.5 or 13.5 p.c. and genotyped for the APE locus. There were no apparent morphological abnormalities associated with any embryo, but several spontaneous abortion (empty sites) were evident. Again, no viable APE null embryos were detected by Southern hybridization. The observed ratio was (1:2:0), consistent with an embryonic lethal genetic defect prior to mid-gestation. Recent histochemical evidence has identified embryonic remnants, within the immediate post-implantation period of 5 to 7 days, likely associated with loss of APE. Additional serial sections of these tissues are currently being assayed for the presence of APE by in situ fluorescent antibody staining. It is possible that the accumulation of mutations within the embryo, as a result of a failure of cells to repair spontaneous abasic lesions, effects an induction of apoptosis. In order to test this hypothesis, sections will be screened for the presence of apoptotic cells using a TdT based fluorescent labeling kit (ApopTag, Boehringer Mannheim).

In support of results above suggesting that embryonic lethality occurs shortly after implantation, PCR-based genotyping of pre-implantation (3.5 day) blastocyst stage embryos identified viable null embryos at Mendelian frequencies in populations isolated from pregnant females of heterozygous matings. Essential to the completion of our experimental objectives is the generation of transgenic mice containing transgenes of APE mutated in regions known to modulate either its DNA repair function or its transcriptional activator redox regulatory activity. As a result of a collaborative effort with the University of New Mexico, a mouse containing the full length genomic copy of APE possessing a C92A mutation was generated and characterized by Southern hybridization. This mutation effects constitutive redox activity to the expressed gene product. Genetic complementation with this transgene of null embryos will be achieved in two generations. These animals are expected to be viable; however, possible mosaicism of expression of the transgene within these animals may result in the appearance of null cell populations in some tissues. C92A animals will be monitored for the appearance of spontaneous tumors, as a result of an effect on AP-1 activity.

In order to generate an embryonic stem-cell line completely deficient in APE, the hygromycin targeting vector for ablation of the second APE allele has been completed. This vector has been transfected into APE[±] ES cells and clones resistant to both hygromycin and G418 have been isolated. A total of 313 clones were picked and grown in culture. The first 84 clones have been screened by Southern analysis for secondary gene targeting and no double mutant lines were identified. However, due to the possibility that loss of APE may lead to reduced growth rate of cells, colonies of varying degree of size were isolated. The smaller colony isolates are only now being screened. Based on information regarding the viability of blastocyst stage APE^{-/-} embryos above, it is anticipated that null ES cells will also be viable. Should secondary gene targeting fail to yield APE null cells, blastocyst stage embryos will be utilized as the alternative method of isolating this line. In summary, we have made significant advances in the preparation for detailed characterization of in vivo functions of APE in cells and transgenic animals.

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