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The overall goal of this project is to develop and maintain a resource of mouse models for breast cancer research. In the 03 year 12 induced mutant strains have been identified and accepted for importation into The Jackson Laboratory (TJL) Induced Mutant Resource (IMR) repository for breast cancer research models. The importation process frees mice of infectious pathogens. Embryos or gametes are cryopreserved. Correct nomenclature is assigned, efficient breeding strategies are developed, and genotyping protocols are modified for optimal efficiency and accuracy. Strain availability is announced in several media, including a page on the IMR's World Wide Web site, accessed through TJL's WWW home page.

A principal aim of this project is to transfer relevant mutations to a defined genetic background. Nine mutations are being transferred, including 7 to the FVB/NJ inbred background. Reports by others and our own observations suggest that tumor characteristics may be altered as a consequence of background strain modifiers. Congenic strains require characterization of tumor onset and type.

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FOREWORD

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Muriel J. Davison 6/26/97
PI - Signature Date

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INTRODUCTION

Mouse mutants that provide models for human breast cancer and model systems to study the function of genes implicated in breast cancer are being produced in large numbers in many research laboratories, world-wide. Genetically engineered models are powerful tools for a) determining gene function in normal mammary gland development; b) understanding mechanisms of mammary carcinogenesis; and c) testing new therapies.

This grant supports a collection of mutant mouse strains relevant to breast cancer research within the Induced Mutant Resource (IMR) at The Jackson Laboratory (TJL). The purpose of the repository for breast cancer-related mutants is to make genetically defined mice of assured health status available to the world research community.

The specific aims of this project are to:

1. Select mutants with importance to breast cancer research for importation into the IMR. Selection involves:
   A) Identifying relevant strains
   B) Determining criteria for selection
   C) Encouraging participation by investigators holding transgenic and targeted mutants
   D) Addressing legal considerations
   E) Cooperating to avoid duplication of efforts

2. Import (by hysterectomy rederivation) transgenic and targeted mutant mice with importance for breast cancer research into defined health status breeding rooms at The Jackson Laboratory;

3. Maintain and expand breeding colonies of imported strains for cryopreservation, strain development, and distribution;

4. Develop accurate and rapid methods for typing stocks for inclusion of transgenes or targeted mutations;

5. Develop improved mouse models for breast cancer research by transferring mutant genes to selected inbred backgrounds conferring specific experimental advantages;

6. Distribute mutant and control mice to scientific investigators on a cost recovery basis;

7. Maintain data on imported mutants and subsequently developed new strains in a computerized database for maintenance of nomenclature, information on mutants held in the resource, and tracking information of mice.

BODY OF THE REPORT

SPECIFIC AIM 1: SELECT MUTANTS WITH IMPORTANCE TO BREAST CANCER RESEARCH FOR IMPORTATION INTO THE IMR.

A. Identifying Relevant Strains

Drs. Sharp and Tennent are responsible for identifying relevant strains for the breast cancer repository. Table 1 lists the strains accepted to the repository in the 03 year. Several of the strains accepted this year carry mutations in key cell cycle regulatory genes, which have been implicated in human breast cancer. The effects of these mutations on mammary gland morphology has not been systematically investigated. By including them in the repository, we can bring them to the attention of breast cancer researchers who may not be aware of them. Where appropriate, they will be

backcrossed to a standard inbred strain, so that their actions in combination with other mutations maintained on the same background can be accurately assessed.

For one strain that was accepted by the Genetic Resources Committee, a request to the investigator to submit it is still pending.

Table 1. Strains accepted for importation to the transgenic repository for breast cancer research in 03 year.

Strain Designation	Type of mutation	JR #	Ref	Distribution Status
FVB-TgN(MMTVCCND1)*	transgenic	2951	[1]	Accepted - not yet available
129/SvEv-*Atm*^{tm1Awb}	KO	JR 2753	[2]	Minimal distribution
C57BL/6J-*Cdkn1b*^{tm1M}	KO	JR 2781	[3]	Accepted - not yet available
FVB/N-TgN(MtTPRMET)773*	transgenic	JR 2775	[4]	Limited distribution
B6,129-*Oxt*^{tm1Wsy}	KO	JR 2713	[5]	Limited distribution
B6,129-*Stat5a*^{tm1Ma}	KO	JR 2833	[6]	Accepted - not yet available
FVB/N-TgN(WapInt3)#Rnc*	transgenic	JR 2755	[7]	Accepted - not yet available
FVB/N-TgN(TIE2LacZ)182Sato	transgenic	JR 2856	[8]	Minimal distribution
BALB/c-*Tcfap2a*^{tm1Jae}	KO	JR 2794	[9]	Accepted - not yet available
C57BL/6J-*Plg*^{tm1*}	KO	JR 2830	[10]	Accepted - not yet available**
B6,129-*E2f1*^{tm1Meg}	KO	JR 2785	[11]	Accepted - not yet available
B6,129-Mdm2^{tm1*}	KO	JR 2968	[12]	Accepted - not yet available

* Nomenclature is incomplete pending confirmation with investigators

Brief descriptions of strains accepted (from literature cited in Table 1 and personal communications with original investigators)

FVB-TgN(MMTVCCND1)*

Cyclin D1 is a cell-cycle regulator essential for G1 phase progression and a candidate protooncogene implicated in pathogenesis of several types of human cancers, including breast carcinomas. It is a component of the chromosome 11q13 amplicon involved in an estimated 15-20% of human breast cancers, and overexpression/accumulation of cyclin D1 protein was found in about half of 170 primary human breast carcinomas analyzed in one study. Mutations in cyclin D1 may be an early event in carcinogenesis, as concordance between level of expression and carcinomas *in situ*, invasive carcinomas, and metastatic lesions was > 80%.

MMTV-cyclin D1 transgenic females develop proliferative disturbances in mammary tissue correlated with age of animal, number of pregnancies, and level of transgene expression. Lobulo-alveolar development was more pronounced in nulliparous transgenic mammary glands following puberty, including extensive side bud formation. Adenocarcinomas developed with a mean age at onset between 511 days and 630 days of age, including multiple independent tumors.

129/SvEv-*Atm*^{tm1Awb}

Mutations in the human *ATM* gene are responsible for ataxia telangiactasia (AT), an autosomal recessive syndrome characterized by progressive neurologic degeneration, lymphoreticular malignancies, immunodeficiency, incomplete sexual maturation, endocrine abnormalities, and extreme sensitivity to ionizing radiation. The pleiotropic characteristics of AT suggest that the primary defect may be a defect in double stranded break repair, leading to chromosomal instability. The gene product is likely part of a signal transduction cascade regulating cell cycle progression after DNA damage. Some studies have found an increased susceptibility to breast cancer among women heterozygous for *ATM* mutations, indicating a potential involvement of this gene in the etiology of breast cancer.

Mice deficient in *Atm* display many of the characteristics of AT, including growth retardation, neurologic dysfunction, infertility, defects in T lymphocyte maturation, and extreme sensitivity to γ-irradiation. Most homozygous animals develop thymic lymphoma between 2 and 4 months of age. Heterozygous mice displayed no abnormalities through eight months of age. At the time of publication, they were still being followed for tumor incidence. This strain appears to be a good model for human AT and will be useful for defining the involvement of *Atm* in breast cancer.

C57BL/6J-*Cdkn1b*^{tm1M}

p27^{kip}, encoded by *Cdkn1b*, is a member of the Kip/Cip family of cyclin-cyclin dependent kinase (CDK) inhibitors. These regulatory proteins inhibit the CDK complexes essential for G1 progression and S phase entry. Although mutations in *Cdkn1b* are rare in breast cancers, reduced expression of *Cdkn1b* in primary breast carcinomas is prognostic for reduced disease-free survival, particularly in younger women, in whom *Cdkn1b* expression was also prognostic for reduced overall survival. Loss of p27^{kip} activity may result in loss of sensitivity to negative growth regulatory factors, altered proliferative potential, differentiation, and intercellular adhesion.

Mice deficient in p27^{kip} are viable and larger than normal littermates, with increased cellularity of all tissues. The thymus, spleen, and pituitary glands are particularly enlarged. Female mice are infertile, apparently due to a resistance of the ovarian granulosa cells to luteinizing hormone stimulation. Large doses of exogenous gonadotropin induced ovulation, but the uterine environment was unable to support implantation. No data was provided on the appearance of the mammary glands, however, these mice will be useful for examining the involvement of p27^{kip} on mammary gland development and differentiation.

FVB/N-TgN(MtTPRMET)773*

This strain was accepted in the 02 year, but is included in this year's report as well because we have imported an additional lineage. The protooncogene *MET* encodes a tyrosine kinase receptor for the hepatocyte growth factor ligand (HGF). HGF, which acts as a mitogen and morphogen, is believed to be necessary for the normal growth and development of several tissues and organs. It has been hypothesized that the HGF receptor/ligand system mediates cross-talk between epithelial and stromal cells within tissues. The oncogenic form of *MET,* tpr-*MET* was identified in a chemical-carcinogen-treated human osteosarcoma cell line. In this line, a chromosomal rearrangement resulted in a chimeric gene in which an upstream promoter-containing sequence (tpr)

from Chr 1 was fused to the carboxyl terminus of *MET* on Chr 7. Receptor dimers are constitutively phosphorylated. Amplified, abnormally processed, or overexpressed *MET* is frequently found in various transformed cell lines and human tumors.

Mammary tumors developed at around a year of age in continuously mated females. Multiple tumors classified as independent arose in several mice. No metastases were observed. Hyperplastic alveolar nodules were identified in most multiparous mice and several also had foci of microscopic carcinoma. Tumors exhibited scirrhous, papillary, or nodular histologic patterns. Other tumor types seen included diffuse lymphoblastic lymphomas involving the mammary gland and lymph nodes, thymic lymphoma, spindle cell sarcoma, and orbital giant cell osteosarcoma.

B6,129-*Oxt*^{tm1Wsy}

Oxytocin participates in the regulation of parturition and lactation, and may have a role in mating and maternal behaviors. Oxytocin is released by the posterior pituitary in response to the stimulation of suckling, and acts on myoepithelial cells to cause contraction and milk ejection.

Oxytocin-deficient mice are viable and fertile. No defect in parturition or early maternal behaviors are observed, but females do not lactate. Milk ejection was restored by treatment with exogenous oxytocin. The morphology of the mammary gland appeared unaffected. This strain will be useful for examining the interactions of oxytocin with other hormones orchestrating milk production.

B6,129-*Stat5a*^{tm1Ma}

STAT5A is a member of the signal transducer and activation of transcription family which interacts with the Janus kinase (JAK) family to mediate a number of responses to cytokines and growth factors. Each cytokine activates a subset of STATs, and many STATs can be activated by more than one cytokine. Prolactin, an important inducer of mammary gland differentiation and lactation, activates genes through the JAK-STAT5A pathway.

STAT5A-deficient mice are viable, develop normally, and are fertile, but do not lactate. Mammary ductal development through pregnancy is normal, but lobuloalveolar development is severely reduced and there was no milk secretion even after prolonged suckling. Levels of the closely related STAT5B signalling molecule were also markedly reduced in STAT5A-deficient mice, but levels increased and phosphorylation was evident after 3 days of suckling. Expression of several milk protein genes was unaffected in STAT5A-deficient mice, whereas whey acidic protein expression was severely reduced.

FVB/N-TgN(WapInt3)#Rnc*

The *Int* loci are sites of mouse mammary tumor proviral (MMTV) insertion. They lack homology with known proviral oncogenes. It is thought that MMTV induces tumorigenesis through insertional mutagenesis and activation of the *Int* protooncogenes. The *Int3* locus was identified in the wild-derived *Mus musculus musculus* strain Czech II, where it is the usual site of MMTV integration. *Int3* is a signaling molecule and member of the *Notch* gene family.

In transgenic mice carrying the WAP/Int3 construct, mammary ductal growth was unaffected in virgin females, but growth and differentiation of secretory lobules during gestation was profoundly inhibited. Mammary dysplasia and tumorigenesis occurred in all breeding females by 25 weeks of age. In non-breeding WAP/Int3 females mammary tumor incidence also reached 100% but only after 70 weeks. The WAP/Int3 mammary tumors were highly malignant and most tumor-bearing females, irrespective of breeding history developed metastatic lung lesions. WAP/Int3 females are unable to lactate.

This phenotype is different from that of the FVB-TgN(MMTVInt3)3Rnc already accepted to the breast cancer repository. In virgin and multiparous mice of that strain, mammary ductal growth and secretory lobule development were both curtailed. Mammary adenocarcinomas were observed as early as 7 weeks, and salivary adenocarcinomas were also observed. Males were sterile. Differences in the phenotype are attributed to the tissue-specific promoters used. The WAP promoter directs expression of the transgene to the secretory epithelium, whereas the MMTV LTR is more widely expressed and at an earlier stage of mammary ductal development.

FVB/N-TgN(TIE2LacZ)182Sato

TIE2 is a vascular endothelial-specific receptor tyrosine kinase essential for the regulation of vascular network formation and remodeling. A construct was made combining the TIE2 promoter and an enhancer element that is autonomous and endothelial-specific with a β-galactosidase reporter gene. Transgenic mice were produced in which all vascular endothelial cells carry the reporter gene. These mice provide an *in vivo* assay system for examining gene expression during neovascularization of tumors. When mated to mice susceptible to mammary carcinogenesis, they will be an attractive model for investigating neovascularization and for testing therapeutic agents aimed at retarding angiogenesis.

BALB/c-*Tcfap2a*^{tm1Jae}

Activator protein-2 (AP2) is a DNA-binding transcription factor which is believed to be involved in signalling terminal differentiation. Several genes implicated in breast cancer are reportedly regulated by AP2, including *MYC* and *ERBB2*. AP2 also transcriptionally activates p21$^{WAF1/CIP1}$, which is implicated in growth arrest and differentiation of breast cancer cells.

Mice homozygous for this mutation die perinatally due to failure of cranial closure. Severe defects in morphogenesis of the face, skull, sensory organs, and cranial ganglia are also evident. There is increased apoptosis in the midbrain of day 9 embryos, coinciding with failure of cranial closure. These developmental mutants provide tractable models for determining molecular pathways in which AP2 functions.

C57BL/6J-*Plg*^{tm1*}

Activation of the zymogen plasminogen (PLG) to the active serine proteinase, plasmin, is commonly involved in extracellular proteolytic activity. Plasmin is thought to be involved in mammary gland involution following lactation, and is associated with tumor cell invasion in several cancers. Plasminogen is activated by urokinase type activator (uPA), which is overexpressed in many human breast cancers. uPA overexpression is under investigation as a prognostic indicator for metastatic potential of primary breast cancer cells.

Mice homozygous for a nonfunctional *Plg* gene are viable and fertile. Mutant mice were susceptible to severe thrombosis, developing thrombotic lesions in several tissues. Fibrin deposition in the liver was observed in 5 to 21 week animals. No details of mammary gland morphology were provided. *Plg* deficient mice mated to transgenic mice with early onset mammary carcinomas may be useful for examining the requirement for plasmin-mediated proteolysis in tumor induction and progression. Tissue explants or cell lines prepared from *Plg*-deficient mice could be used for longer-term studies.

B6,129-*E2f1*^{tm1Meg}

The E2F family of transcription factors act as transcription activators by sequence specific DNA-binding. They are important for moving cells into the S phase of the cell cycle. E2F1 is inactivated by binding to RB1 and may stimulate proliferation in RB-1 deficient cells. The RB/E2F1 complex suppresses transcription of certain cell cycle regulatory genes, suggesting that this complex may be important in suppressing progression through G1 until RB1 is phosphorylated and released from E2F1. *Rb1* is mutated in a substantial proportion of human primary breast cancers, suggesting that this cell-cycle regulatory pathway is important for controlling normal mammary epithelial cell proliferation.

The binding domain and part of the dimerization domain of *E2F1* were disrupted. Mice homozygous for defective *E2F1* are viable and fertile. They show thymocyte maturation defects due to a failure of apoptosis, eventually resulting in increased proliferation and increased tumorigenesis. As mutant mice age, they show exocrine gland dysplasia and testicular atrophy. Mutant mice develop a broad spectrum of cancers, although mammary carcinomas were not observed on the 129 X C57BL/6J hybrid background.

B6,129-Mdm2^{tm1*}

The MDM2 protein forms a complex with TRP53, inhibiting TRP53-mediated regulation of gene expression. It is thought that MDM2-TRP53 binding autoregulates *Mdm2* expression and modulates TRP53 activity. Amplification of *MDM2* is found in a low percentage of human primary breast cancers, and is correlated with aggressive, aneuploid tumors.

Mice homozygous for a null mutation in *Mdm2* die early in gestation, but are rescued in the absence of *Trp53*. *Mdm2/Trp53*-double null mice share the same phenotype as *Trp53* mice. These mice will be useful for examining the role of the MDM2-TRP53 complex in TRP53 mediated mammary epithelial apoptosis and carcinogenesis.

B. Criteria for Selection

Drs. Sharp and Tennent present identified strains to the Genetic Resources Committee, chaired by Dr. Kenneth R. Johnson, for a decision regarding selection. Criteria for selection of mutants is based on existing guidelines for importing mice to the Laboratory's Genetic Resources. These are 1) the immediate need for use in biomedical research; 2) the numbers of requests for mice being received by the investigators who created them; 3) the potential for future research; 4) the time and effort needed to replace or recreate the mutant; and 5) the uniqueness of the mutation. This year we have begun accepting strains to be cryopreserved directly, without maintaining a breeding colony for distribution of live mice. These are strains that carry a scientifically valuable mutation of interest to a small number of investigators, for which we do not expect a large demand. Orders for mice will be filled by recovering animals from cryopreserved gametes or embryos. Institutions that are equipped to recover mice from cryopreservation may request shipment of frozen embryos or gametes. We expect that this method of distribution will increase, as more institutions establish cryopreservation laboratories. Mice recovered from cryopreservation have effectively gone through a rederivation process and can be introduced into high level barrier facilities at institutions where rederivation is required.

C. Encouraging Participation by Investigators Holding Transgenic Mice

Several approaches are being used to expand the current level of contributions to the IMR. These include notices about the IMR in general and specific notices about the breast cancer repository placed as described below. Many of the mutant stocks submitted to the IMR through these mechanisms are relevant to breast cancer research. This informational program also widely distributes information on available stocks to potential research users.

1) An IMR presentation is given at all courses and workshops held at The Jackson Laboratory. These courses include the *Short Course in Medical and Experimental Mammalian Genetics*, given annually in association with the Johns Hopkins University School of Medicine; *Experimental Genetics of the Laboratory Mouse*, a graduate and post-graduate level course led by an international faculty; the *Cryopreservation* course, and special workshops and conferences focused on animal models. In October, 1997, a meeting on *The Mouse in Mammary Carcinogenesis Research* will be held at TJL for an expected 100 participants. A presentation on the repository will be made, as well as sessions introducing participants to online sources of information about mutant mice. The meeting, which will be the first comprehensive discussion about genetically engineered mouse models for breast cancer research, should be an excellent forum for focusing community attention and receiving community input on the breast cancer repository.

2) IMR personnel accept all relevant speaking and writing opportunities to disseminate information and invite participation in the program. In the 03 year, these included:

- Report on the Induced Mutant Resource: Prospects for the Future. *'Harold Varmus Workshop - Manipulating the Mouse Genome: The Next Phase'*, NIH. Bethesda MD. June 1996.
- The Jackson Laboratory Induced Mutant Resource. *'Second Meeting on Animal Models and Biomedical Tools: Mouse Skin and Hair Mutations'*. The Jackson Laboratory and The Hair Research Society, Bar Harbor ME. Sept 1996.
- 49th Annual Symposium on Fundamental Cancer Research: Regulatory Mechanisms in Growth and Differentiation, Houston TX, October 1996
- Genetic Resources and Genetic Maintenance of Induced Mutants. *National AALAS Meeting*, Minneapolis, MN November, 1996.

Publications about the Induced Mutant Resource include:

Sharp JJ, Mobraaten LE. 1996. To Save or Not To Save: The Role of Repositories in a Period of Rapidly Expanding Development of Genetically Engineered Strains of Mice. *Transgenic Animals - Generation and Use*. Houdebine LM (ed), Harwood Academic Publishers GMBH, Switzerland, pp 525-532

Tennent BJ, Sharp JJ, Washburn LL, Schweitzer P, Sundberg JP, Silva KA, Davisson MT. 1997. Repository of Induced Mutant Mice for Breast Cancer Research. *Proceedings of Breast Cancer Research Program, USAMRMC,* in press.

3) In July, 1996 all users of JAX mice (a mailing list of 7000) received an issue of the quarterly publication, *JAX Notes*, which included a listing of strains in the breast cancer repository and an announcement that we are continually seeking new mutants.

4) A list of mutants in the breast cancer repository is available through The Jackson Laboratory home page on the World Wide Web (WWW; http://www.jax.org/). Information on the gene (through links with *Mouse Genome Database*), strain background, availability, phenotype,

references, and ordering information are provided. Genetic typing protocols for some strains are posted on the WWW as well.

5) The IMR breast cancer mutant strain list is linked to the Biology of the Mammary Gland homepage maintained by Dr. Lothar Hennighausen (http://alice.dcrt.nih.gov/~mammary/). This Website offers written descriptions and scanned images of histomorphology from mice carrying induced or spontaneous mutant mutations that affect mammary gland development and mammary cancer.

6) IMR personnel and Dr. Carol Linder, Technical Services Advisor for TJL, distribute information about the IMR program in general and the breast cancer repository in particular at selected scientific meetings where The Laboratory trade booth is exhibited. In the 03 year of this grant, the booth has been exhibited at 5 meetings. The exhibit booth is a valuable forum for exchanging information about new mutants presented at these meetings and for disseminating information about mutants already in the breast cancer repository.

7) Associated Board members refer investigators holding new mutants to the breast cancer repository and suggest mutants that we should pursue.

D. Addressing Legal Considerations

TJL has formulated a general approach to enter negotiations, based on over four years' experience. TJL encourages participation in the IMR program and attempts to discourage institutions from imposing licensing or royalty requirements. When necessary, however, distribution agreements have been negotiated requiring royalty payments by the Laboratory and/or notices to for-profit companies that a commercial license may be required from the originating institution. The Jackson Laboratory, as a policy matter, will not be a party to any distribution agreement that restricts the ability of the investigator to breed animals for research purposes, or restricts the Laboratory's ability to distribute mice on a first come-first served basis. The legal negotiations at The Jackson Laboratory are the responsibility of David Einhorn, Esq., The Jackson Laboratory in-house counsel. Two strains accepted to the breast cancer repository this year have required that new agreements be negotiated. Three strains accepted in previous years are not being distributed due to ongoing legal negotiations.

E. Cooperating to Avoid Duplication of Efforts

We have found that duplication of effort is best avoided by contact with the investigator holding the mice requested. Investigators who initiate the contact usually have offered their mutants only to The Jackson Laboratory. To screen for potential duplication, a form is sent to each potential provider of mutant animals asking if the animal is being offered to other institutions. Investigators are also asked for their knowledge of any similar animal being produced elsewhere. Investigators holding mice relevant for breast cancer research are very helpful in suggesting mice for the repository and discussing the specific experimental advantages of similar models.

SPECIFIC AIM 2. IMPORT (BY HYSTERECTOMY REDERIVATION) TRANSGENIC AND TARGETED MUTANT MICE WITH IMPORTANCE FOR BREAST CANCER RESEARCH INTO DEFINED HEALTH STATUS BREEDING ROOMS AT THE JACKSON LABORATORY

Importation is completed for 17 of the 20 of the strains accepted in the 01 and 02 years. The FVB/N - TgN(WapMyc)212Bri strain has not been imported pending completion of legal negotiations with a third party holding patent rights. The CD1-TgN(MtTGFA)42Lmb strain was imported but rederived progeny were sacrificed before breeding colonies could be established because they developed mega-esophagus by 2 months of age. Additional breeding pairs are

scheduled for importation. Progeny from the B6,SJL-TgN(WapIGFBP3)67Dlr strain have been rederived and colonies are being established. There was a delay in importation because the genotyping protocol did not work in our hands initially, but this is now resolved.

The status of strains accepted in the 03 year is shown in Table 2. Four strains have cleared importation and are being distributed. Six strains are in the importation process; progeny from most have been recovered but vigorous breeding colonies are not yet established. One strain is awaiting importation pending legal negotiations, and a second strain accepted in May, 1996, is scheduled for importation when isolator space is available.

Table 2. Importation of strains accepted in the 03 year

Strain	Status
FVB-TgN(MMTVCCND1)*	legal negotiations
129/SvEv-*Atm*^{tm1Awb}	cleared importation
C57BL/6J-*Cdkn1b*^{tm1M}	in importation
FVB/N-TgN(MtTPRMET)773*	cleared importation
B6,129-*Oxt*^{tm1Wsy}	cleared importation
B6,129-*Stat5a*^{tm1Ma}	in importation
FVB/N-TgN(WapInt3)#Rnc*	in importation
FVB/N-TgN(TIE2LacZ)182Sato	cleared importation
BALB/c-*Tcfap2a*^{tm1Jae}	in importation
C57BL/6J-*Plg*^{tm1*}	in importation
B6,129-*E2f1*^{tm1Meg}	in importation
B6,129-Mdm2^{tm1*}	scheduled for importation

SPECIFIC AIM 3. MAINTAIN AND EXPAND BREEDING COLONIES OF IMPORTED STRAINS FOR CRYOPRESERVATION, STRAIN DEVELOPMENT AND DISTRIBUTION

Linda Washburn, Senior Professional Assistant, is manager of the Induced Mutant Resource Colony (IMRC). Kathleen Silva, Biomedical Technologist, is responsible for maintaining the strains in the breast cancer repository.

Breeding colonies are established for 21 of the 32 strains accepted in the 01-03 years. One strain is maintained solely as frozen embryos.

Ten of the strains accepted in the 01-02 years have been or are being cryopreserved. Since many of the strains are difficult to establish in breeding colonies, we have used most of the available mice from strains in demand to fill requests from investigators before cryopreserving them. The FVB/N-TgN(MMTVInt3)3Rnc strain was the first for which cryopreservation of ovaries was attempted. It was impossible to import this strain by hysterectomy derivation, our usual method, because males were infertile and females developed tumors as early as 7 weeks of age. Ovaries were collected from tumor-bearing females in the isolators, halved, and cryopreserved until they could be transferred to a recipient female. Live pups were recovered from ovary-transfer recipients, and a breeding colony is now being established, but animals are not yet ready for distribution. If there are sufficient orders for mice of this strain, it will likely be maintained by routine ovary transfer. If there is minimal demand, the strain will be preserved by cryopreservation of ovaries. Ovary cryopreservation is a useful addition to our methods for preserving difficult strains.

Mutations that arrive on a nonstandard genetic background may be transferred to an inbred strain background by repeated backcross. At present 9 mutations are being transferred to other backgrounds conveying specific experimental advantages. Strain development is discussed more fully under Specific Aim 5.

Most of the strains in the breast cancer repository must be maintained as heterozygotes or hemizygotes. In several strains transgenic females do not lactate, requiring that the strain be maintained either by breeding carrier males to inbred, wild-type females, or by fostering litters, or both. When strains are maintained using hemizygous breeders, all offspring must be genotyped to identify carriers. Blood samples are obtained primarily by retro-orbital sampling, and DNA extracted and typed as described in Specific Aim 4 below.

SPECIFIC AIM 4. DEVELOP ACCURATE AND RAPID METHODS FOR TYPING STOCKS FOR INCLUSION OF TRANSGENES OR TARGETED MUTATIONS

The IMR allele typing program is the responsibility of Dr. Sharp. Virtually all mouse strains in the IMR require genetic typing to confirm the presence of the transgene in transgenic strains, or the genotype of targeted mutants. Genetic typing is required to identify carrier animals (heterozygotes) in strains where the mutation is being backcrossed onto a defined genetic background, to identify hemizygous animals for those transgenic strains supplied as hemizygotes, and to identify heterozygotes for those strains where the homozygous mutants are embryonic lethals or do not reproduce. Allele typings are carried out using the polymerase chain reaction (PCR) because it is rapid, the reaction conditions may be standardized, it does not require the use of radioisotopes, and it is adaptable to automation.

Allele typing protocols for IMR strains are first developed in the IMR Development Laboratory under the supervision of Peter Schweitzer, Ph.D. Dr. Schweitzer is responsible for developing and testing all genetic typing protocols for each new strain and is also responsible for overseeing the correct use of these protocols. He has contacted all of the researchers supplying mice to the breast cancer repository and is optimizing protocols for the strains already received. He reviews all typing results and provides these protocols to researchers requiring assistance. The protocols are regularly posted on the World Wide Web.

The IMR genotyping lab has evaluated several DNA isolation systems. Among these were QIAGEN's QIAamp 96 DNA isolation system, Gentra's Generation DNA isolation system, Schleicher and Schuell's Isocode DNA isolation system, as well as several different published protocols. While most commercially available systems were found to be too expensive and often no less labor intensive than standard DNA isolation protocols, several are considered very good candidates for automation when such procedures are developed. The most promising technologies are paper-based DNA isolation systems, and whenever automated systems are developed using such technologies, these will be examined closely for use by the IMR.

In the interim, the IMR lab has adapted existing protocols for the isolation of DNA from peripheral blood samples to a 96-well plate format. These new procedures dramatically reduce the time and labor spent on DNA isolation prior to the actual genotyping assays.

The IMR genotyping lab is continually involved in developing PCR assays to replace Southern blotting protocols to identify carriers of transgenes and to distinguish wild type, heterozygous, and homozygous knockout mice. Approximately 30% of the strains imported to the IMR require development of new PCR assays. These new assays are designed with a standard set of conditions, where possible. In addition, the majority of assays are being adapted for multiplexed primers. For some assays, such multiplexing allows all genotypes to be detected in a single reaction tube, thereby lowering overall costs. Also, by multiplexing primers most PCR assays

performed in the IMR contain an internal control for presence and integrity integrity of genomic DNA, thereby lowering the rate of false negative results.

The IMR lab is constantly monitoring the performance of their PCR assays and, where needed, are improving their assays. This is becoming increasingly important as mutations are backcrossed onto different genetic backgrounds, where a pair of PCR primers which works on the 129 background, for example, may not work well on a C57BL/6 background.

The evaluation of new technologies for genotyping has been based on making a priority of maintaining the highest confidence in the results of our assays as well as minimizing costs. Emerging technologies for detection of PCR products have been evaluated, and to date no system has been deemed superior to gel analysis subsequent to PCR amplification. This decision was based on the advantages of gel-based detection systems: the ability to multiplex primers in a single reaction; and the additional information given by the molecular size of the DNA fragments amplified during the PCR reaction. It is crucial to the IMR's operation to obtain the most accurate genotyping information possible on mice in the colonies. The consequences of mis-typing mice are great, and conventional PCR and gel analysis minimizes both false negatives and false positives. False negative results, e.g. the absence of a DNA product due to lost DNA, failed PCR amplification, or ambiguous results (faint bands, etc) could have devastating effects on the IMR colonies and researchers' experiments. As a consequence of the large number of samples processed weekly by the Molecular Genotyping Labs, a small percentage of DNA samples are lost. The chance of such samples being mis-scored is lower using a conventional PCR and gel analysis, where multiplexing primers is possible. Because many strains are maintained by using wild type sibs of mutant mice as breeders, false negative results may interfere with proper maintenance of the colonies, leading to breeding errors. Similarly, many control mice also are obtained from breeding pairs that produce mutant mice, and mis-typing mice may lead to mutant mice in control groups of mice. False positive genotypings can be equally devastating to a colony or research experiment. Having the added information of DNA product size acts as an internal check that the PCR product is, in fact, the correct product.

The use of robotics in the IMR typing lab has been examined, and we are currently using an ABI Catalyst DNA robot for routine PCR for genetic quality control examining SSLPs using fluorescent primers. Robotics have not been used for routine genotyping of IMR mice for importation, colony management, strain development, distribution, genetic quality control, and cryopreservation. Such genotyping usually involves small numbers of any individual strain and, as such, robotics have not proved worthwhile. The routine use of robotics will be valuable in aliquoting reagent mixes for generating PCR plates stored for future use.

SPECIFIC AIM 5. DEVELOP IMPROVED MOUSE MODELS FOR BREAST CANCER RESEARCH BY TRANSFERRING MUTANT GENES TO SELECTED INBRED BACKGROUNDS CONFERRING SPECIFIC EXPERIMENTAL ADVANTAGES

Because of the marked strain differences in susceptibility to spontaneous, hormonally-induced, and chemically induced mammary adenocarcinomas and the number of as yet unidentified "background" modifying genes (including MMTV proviral insertions) participating in susceptibility, it is essential to place transgenes and targeted mutant genes on defined, inbred backgrounds. Appropriate selection of mutant alleles and inbred backgrounds will increase the utility of these models for breast cancer research. Seven mutations (Table 3) are being transferred to the FVB/NJ background because of the common use of this strain to make transgenic mice, low incidence of spontaneous mammary tumors, and lack of milk-transmitted MMTV or replication-competent endogenous MMTV provirus. At the fifth generation of backcrossing, matings will be expanded as needed, carrier females will be identified and examined for tumor development. If the tumor phenotype is preserved, backcrossing will then be resumed. The transgene is also maintained on the background on which it was originally received.

Table 3. Strains backcrossed to FVB/NJ strain background

Original strain	Generation
B6,129-*Ccnd*^{tmi}	N4
B6D2-TgN (MMTVTGFA)254Rjc	N3
B6D2-TgN (MMTVTGFA)29Rjc	N4
B6,129-*Trp53*^{tm1Tyj}	N5
B6, 129-*Rb1*^{Tm1Tyj}	N5
B6D2 TgN(MMTVTGFB1)46Hlm	N4
SJL-TgN(Wnt1)1Hev	N5

Strain Characterization

Wnt1

Preliminary observations on tumor latency in successive backcross generations of SJL-TgN(Wnt1)1Hev mice to FVB/NJ suggest that strain background may influence tumorigenesis in this model. Animals were inspected during weekly cage changing for visually observable tumors. Mice were necropsied by TJL's Pathology Service when they were judged "sick", as determined by tumor size, behavior, or scruffy pelt. Data presented below are based on small numbers observed as part of routine colony maintenance, and will be further tested by a more rigorous comparison of palpable tumor latency, incidence, and number of mammary glands affected between SJL-TgN(Wnt1)Hev and N5 FVB backcross progeny. This study will be conducted in the 04 year.

Figure 1 Comparative tumor incidence and lifespan in strains transgenic for MMTV-Wnt1.

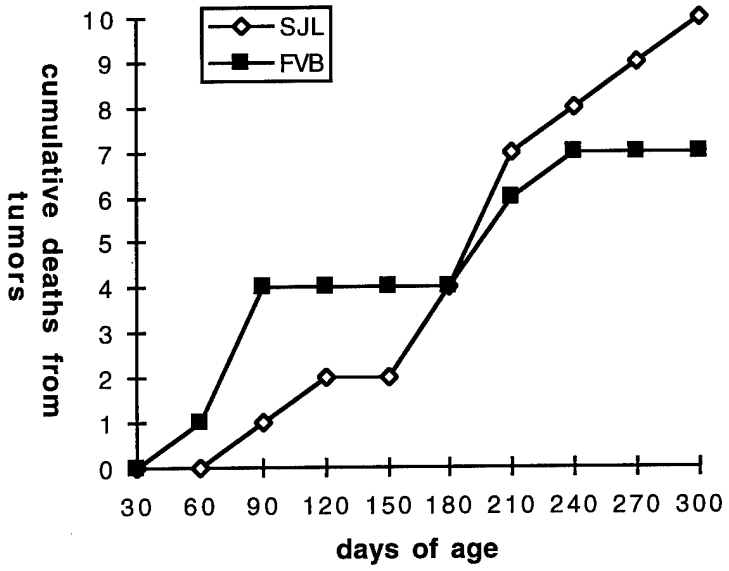
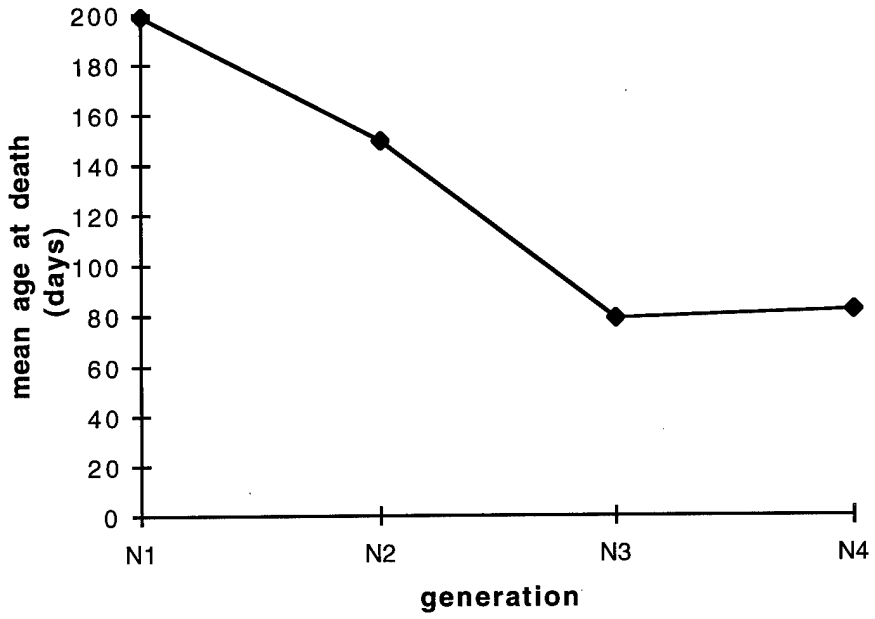


Figure 2 Lifespan in successive backcross generations to FVB/NJ



The SJL-TgN(Wnt1)1Hev strain was originally produced on a C57BL/6J X SJL/J hybrid background and has been maintained by repeated backcross to SJL/J. Because of the very poor reproductive performance of SJL-TgN(Wnt1)1Hev mice, we made an outcross to C57BL/6J, and progeny will be backcrossed to SJL/J for five generations, when the line will be fixed. We anticipate that the N5 SJL/J mice may be better breeders than the original strain we imported.

We have seen a marked improvement in reproduction with the first generation and have been able to fill back orders for this mutation.

Trp53

The *Trp53* and *Rb1* null mutations have been transferred to the C57BL/6J strain background (N10) and are being distributed. We are also transferring the *Trp53* and *Rb1* null mutations to the BALB/cJ and C3H/HeOuJ backgrounds. The BALB/cJ strain was chosen because spontaneous papillary adenocarcinomas with histomorphology closely resembling the human infiltrating ductal carcinoma have been observed in the TJL Animal Resource colony of this strain. *Trp53*-deficient BALB/cJ mice will also be useful to investigators using chemical or MMTV-induced mammary tumors.

BALB/cJ-*Trp53*^{tm1Tyj} N5 progeny homozygous for the null mutation become moribund between 3 and 4.5 months of age. In a small group (N=6) that were examined by The Jackson Laboratory's Pathology Resource, hemangiosarcomas, generalized lymphosarcomas, and a poorly differentiated rhabdomyosarcoma were observed between 3 and 4.5 months of age. One mouse had a severe cortical cataract. N5 progeny heterozygous for the *Trp53* null mutation are being observed for tumor latency and incidence. To date, 3 mice have become moribund between 8 and 11 months of age and were necropsied. Two cases of generalized lymphosarcoma, a bilateral Leydig cell tumor with pulmonary metastases, and an anaplastic carcinoma of unknown origin were observed. Backcrossing will continue to N10, when further characterization of tumor types and latencies will be completed.

The C3H/HeOuJ strain was chosen because mice develop a high frequency of MMTV-induced mammary adenocarcinomas and are widely used in breast cancer research. Currently, however, a pronounced decrease in mammary tumorigenesis has been detected in the Animal Resource colony of this strain. We have fixed the backcross lines generated to transfer the *Trp53* and *Rb1* at N5. Seven C3H/HeOuJ-Trp53+/- mice were followed for tumor incidence. Four developed mammary carcinomas at 11-12 months. One female developed a hepatocellular carcinoma, two were killed at 15 months with no tumors detected. There was no difference in tumor latency or frequency in *Trp53*+/- and +/+ females. The experiment was terminated until MMTV is reintroduced.

Tailshort

We have also investigated a potential new spontaneous model for mammary carcinoma. Mice carrying a spontaneous mutation, tailshort (*Ts*), were previously imported to The Jackson Laboratory, where the mutant stock was crossed to C57BL/6J, C57BR/cd, and BALB/cSn mice, then sibmated to produce the strain TSJ/Le. Anecdotal evidence suggested that *Ts*/+ mice developed a high incidence of mammary tumors. Groups (N=40) of *Ts*/+ and wild type mice were set up in breeding pairs and followed for tumor incidence. To date, 15.8% of wild type and 21% of *Ts*/+ mice have developed mammary tumors, after at least two pregnancies and between 13 and 18 months. Simple and mixed tubular adenocarcinomas as well as simple solid mammary adenocarcinomas were observed. Pulmonary metastases were observed in only one mouse. These data suggest that the TSJ/Le strain represents a new model for susceptibility to mammary carcinomas that resemble certain human carcinomas. Although the BALB/c progenitor strain is also susceptible to mammary tumors, a papillary morphology is more commonly observed.

SPECIFIC AIM 6. DISTRIBUTE MUTANT AND CONTROL MICE TO SCIENTIFIC INVESTIGATORS ON A COST RECOVERY BASIS

The scientific community is made aware of the availability of strains through the informational program described in Specific Aim 1 C. In total, 27 strains are now being distributed from the

Breast Cancer Repository, some of which are different inbred strains carrying the same mutation. Over 1000 mice have been distributed to more than 130 investigators. Many of these are breeding pairs to establish new colonies.

SPECIFIC AIM 7. MAINTAIN DATA ON IMPORTED MUTANTS AND SUBSEQUENTLY DEVELOPED NEW STOCKS IN A COMPUTERIZED DATABASE FOR MAINTENANCE OF NOMENCLATURE, INFORMATION ON MUTANTS HELD IN THE RESOURCE AND TRACKING INFORMATION OF MICE

The IMR database is maintained by Phyllis Mobraaten, Information Specialist, to track internal management information on all IMR strains. This year, the INGRES-based database using a Netscape interface was launched. The database allows multiple-user access with both JAX-only and public views. The IMR database contains strain information such as gene description, phenotype, husbandry, typing methods, and nomenclature. All the genetic databases at The Jackson Laboratory use correct nomenclature following the guidelines of the International Committee on Standardized Genetic Nomenclature for Mice. When strains are accepted for importation to the breast cancer repository, appropriate nomenclature is agreed upon with the original investigator, approved by the Mouse Genome Database Nomenclature Coordinator, and a Laboratory Registration Code is obtained from the International Central registry maintained by the Institute for Laboratory Animal Resources (ILAR, NRC, NAS). Strain information from the IMR database is routinely posted on the World Wide Web as described in Specific Aim 1C.

CONCLUSIONS

This year, twelve induced mutant strains relevant to breast cancer research have been identified and accepted for importation into The Jackson Laboratory Induced Mutant Resource repository for breast cancer research models. Correct nomenclature has been assigned to each strain, an important step for disseminating information about mutant strains and reducing duplication of effort. Efficient breeding strategies for each strain have been developed, and typing protocols have been obtained and are being modified for optimal efficiency and accuracy. The availability of these strains is being announced in several media, including a dedicated page in the IMR strain list accessed through The Jackson Laboratory's WWW home page. This page is linked to other sites that convey genetic and phenotypic information.

Many of the strains accepted to the repository have impaired reproductive performance as a consequence of the transgene or strain background. We have implemented ovary transfer and ovary cryopreservation to speed colony establishment and preservation of strains. We have initiated outcrosses where necessary to preserve the mutation. In one case, we requested shipment of additional mice to the importation facility.

Many induced mutant stocks of particular relevance to breast cancer research have been created and maintained on mixed genetic backgrounds, which limits their usefulness for most genetic studies. At present, many of these models for breast cancer are transgenic stocks carrying oncogenes or growth factors with expression directed to the mammary gland. We are transferring mutant genes to the FVB/NJ background by repeated backcross, while monitoring for any changes in phenotype.

We continue our outreach to the breast cancer research community. A meeting on mouse models for breast cancer will be held at The Jackson Laboratory this fall, at which community input to the repository will be sought. The mouse promises to be a vital tool for functional genetics research on the normal and diseased mammary gland. Interest in genetically engineered models for mammary carcinogenesis continues to grow, and several targeted mutants are now available to examine gene function in the developing mammary gland. There is clearly a continuing need for the Breast Cancer Repository.

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