The murine N-*ras* gene is not essential for growth and development

(gene targeting/embryonic stem cells/oncogenes)

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ABSTRACT The mammalian *ras* gene family encodes key cell-signaling, cell growth-related proteins that have been highly conserved in species from yeast to man. Specific point mutations in the *ras* genes are associated with various mammalian tumors. To understand the developmental role of the N-*ras* protooncogene in the mouse, we have disrupted its gene function by homologous recombination in embryonic stem cells. Mice derived from these cells that are homozygous for the N-*ras* mutation do not produce any detectable N-Ras protein and are morphologically and histologically indistinguishable from their heterozygous and wild-type siblings. Since N-*ras* is expressed at high levels in hematopoietic cells, we examined different populations of cells in peripheral blood and found no differences between mutant and normal animals. Our results show that N-*ras* gene function is dispensable for normal mouse development, growth, and fertility.

The mammalian *ras* genes encode a family of highly related proteins that play a critical role in a signaling pathway and transmit extracellular signals for cellular proliferation and differentiation (1–4). Members of the *ras* family of genes have been identified in several eukaryotic organisms and the function of the Ras proteins is similar in all of these organisms (5). In addition, mutant forms of *ras* were observed in many tumors.

A large number of genetic, cell biological, and biochemical approaches have provided significant information about the *ras* family of genes and their role in normal growth and oncogenesis. The *ras* genes encode a M_r 21,000 protein (p21) that is located on the inner surface of the plasma membrane (6) and acts as a biological switch. The proteins bind guanine nucleotides GTP and GDP and have GTPase activity (for reviews see refs. 6–9). Cellular Ras proteins exist in a state of equilibrium between an active and inactive state. The inactive state is characterized by binding to GDP. Upon appropriate stimuli, the proteins exchange GDP for GTP followed by a conformational change to an active state. Alterations of conditions, including gene mutations, may allow stabilization of Ras proteins in their active state leading to malignant transformation.

There are three prominent members of the *ras* gene family that were initially identified in their oncogenic forms: Ha-*ras* and Ki-*ras*, corresponding to the Harvey and Kirsten rat sarcoma virus oncogenes, and N-*ras*, which was initially isolated from a human neuroblastoma (8). The three genes are unlinked and map to different chromosomes (Ha-*ras* to chromosome 11, Ki-*ras* to chromosome 12, and N-*ras* to chromosome 1 in humans and to chromosomes 7, 6, and 3, respectively, in the mouse; ref. 8).

Although the role of p21 in signal transduction mechanisms involving cell division and cellular differentiation is being

elucidated (2, 10, 11), the individual roles of different members of the *ras* gene family in mammals are not understood. The expression of *ras* genes is ubiquitous; all cell types analyzed have been found to express p21 (12, 13). Cell proliferation is not a prerequisite for *ras* gene expression. Indeed, one of the organs that shows maximal *ras* gene expression is brain, where there is little or no cell proliferation (12, 13). In yeast, there are two *ras* genes, *ras1* and *ras2*, and although inactivation of either of them shows some metabolic effects, only mutations in both genes result in spore inviability (14). These results suggest that individual *ras* gene function is dispensable in yeast. However, several different observations suggest unique roles for the three *ras* genes in mammals. Although all three genes are expressed in all tissues, it has been observed that the mRNA levels of each of these products vary among tissues (12). In addition, oncogenic forms of each of the three genes are consistently associated with specific cell and tissue types [e.g., Ha-*ras* in skin and mammary tumors (12, 15); Ki-*ras* in colon and pancreatic tumors (16–18); and N-*ras* in hematologic malignancies (18–21)], suggesting a preeminent role for each of these genes in particular tissue types.

To understand the role of individual *ras* genes in mammalian development, we have initiated an effort to generate mice carrying null mutations in each of the *ras* genes. Using gene targeting mediated by homologous recombination in mouse embryonic stem (ES) cells, we have developed a cell line in which one copy of the mouse N-*ras* gene was disrupted. This disruption results in a null mutation of the gene. We have successfully transmitted this disrupted gene through the germ line. We present data illustrating that mice that are heterozygous (HET) and homozygous (HOM) for the N-*ras* mutation appear to be normal. These observations suggest that, despite its somewhat unique patterns of expression and oncogenic specificity, N-*ras* function is completely dispensable for normal development, growth, and fertility in mice.

MATERIALS AND METHODS

Vector Construction, Transfection, and Selection of Homologous Recombinants. An 11.7-kbp N-*ras* gene replacement vector was constructed by cloning fragments of the mouse N-*ras* gene into the plasmid vector pWE2.1. pWE2.1 is derived from pUC19 and contains a modified polylinker and a 1.1-kb *Xho* I–*Sal* I fragment from pMC1neopolA containing the bacterial neomycin phosphotransferase (neo) expression cassette (22) cloned into the *Att* II site. N-*ras* sequences were derived from offspring of a cross between AKR and RF mice. The 1.4-kbp *Hae* II–*Pvu* II N-*ras* fragment containing the 3' end of exon 1 and 5' end of exon 2 was subcloned into the *Eco*RV site of pWE2.1; the *Xho* I–*Xba* I fragment containing

3.5 kbp of the *N-ras* gene (19) was subcloned into pWE2.1, previously digested with *Sal* I and *Xba* I. Finally, the 1.8-kbp herpes simplex virus thymidine kinase (HSV-TK) gene from pMC1TK (23), flanked by *Sfi* I adapters, was cloned into an *Sfi* I site of pWE2.1 for negative selection. The resulting *N-ras* gene replacement vector, designated pNras, is shown in Fig. 1A.

pNras was linearized at the unique *Not* I site prior to transfection. Twenty-five micrograms of linearized vector was transfected into 10^8 E14 ES cells using a Bio-Rad Gene Pulser set at 250 V and 500 μ F. Transfected cells were transferred onto ≈ 20 10-cm feeder plates containing mitomycin-treated, neomycin-resistant embryonic fibroblasts and 9 ml of ES medium prepared according to Robertson (24) except that the only serum was fetal calf (15%), supplemented with leukemia inhibitory factor at 1000 units/ml. Twenty-four hours after transfection the neomycin analogue G418 (Geneticin, GIBCO/BRL) was added to the medium at 100 μ g/ml (active concentration); 36 hr after transfection, ganciclovir (GANC, 2 μ M final concentration, kindly provided by Syntex, Palo Alto, CA) was added to the medium. Drug selection was continued for 10–14 days.

PCR, Southern Analysis, Transfer of Modified ES Cells, and Tail DNA Analysis. Colonies that were resistant to G418 and GANC were isolated and expanded in 24-well plates. Portions of each transfectant clone were pooled with 12 clones to a pool. DNA from each of the pools was prepared and tested for homologous recombinants by PCR using an *N-ras* targeting primer pair. Primer 189 is a 21-mer complementary to *N-ras* sequences, which start at the third nucleotide following the ATG in exon 1. The sequence of this primer is 5'-TGAG-TACAACTGGTGGTGGT-3'; primer 188 is a 21-mer complementary to pUC19 sequences, which start at bp 367. The sequence of this primer is 5'-AGTCACGACGTTGTA-AAACGA-3'. Amplification of the modified locus with these primers is expected to result in a 1520-bp diagnostic fragment. Amplification conditions included preheating at 94°C for 2 min, 45 cycles of melting at 94°C for 1 min, annealing at 56°C for 30 sec, extension at 72°C for 1.5 min, and a final extension at 72°C for 10 min in the last cycle. Individual members of a positive pool were tested to identify clones that carry the desired modification.

Clones that yielded the expected PCR product were expanded in 6-cm dishes and DNA was prepared for Southern analysis (25). DNA was digested with the appropriate restriction enzyme, fractionated on agarose gels, denatured, transferred to a Hybond-N (Amersham) membrane, and hybridized with probes shown in Fig. 1A.

A targeted clone was expanded and injected into C57BL/6 host blastocysts obtained from 3.5-day postcoitus females. Injected blastocysts were transferred into the uterus of CD-1 foster mothers for the generation of chimeras (24). Chimeras were crossed to C57BL/6 or 129/SJ females to produce outbred or inbred heterozygotes, respectively. Genotyping of wild-type (WT), HET, and HOM offspring was carried out on respective tail DNA preparations by Southern analysis.

Detection of Ras Proteins. Protein extracts were prepared by homogenization of testes, thymus, lung, liver, or spleen in the presence of aprotinin (1 μ g/ml) and phenylmethylsulfonyl fluoride (PMSF) (100 μ g/ml). Disrupted tissues were washed in 5 ml of phosphate-buffered saline (PBS) and the cell pellet was resuspended in 1.5 ml of lysis buffer, prepared according to the manufacturer's instructions (Santa Cruz Biotechnology, Santa Cruz, CA) with aprotinin (1 μ g/ml) and PMSF (100 μ g/ml). The tissue lysate was homogenized with a Dounce instrument 10 times and transferred to a 1.5-ml centrifuge tube; cellular debris was cleared by centrifugation at 4°C, $15,600 \times g$ for 20 min. The supernatant was immunopurified by passage through a Santa Cruz Biotechnology pan-Ras immunoaffinity column and the resulting Ras-enriched fraction

was eluted from the column in 100 mM glycine (pH 2.5) according to manufacturer's instructions.

The immunopurified extracts were fractionated on 15% SDS/PAGE gels (25) and electroblotted onto Immobilon-P (Millipore) membranes according to manufacturer's instructions (Novablot, LKB). Membranes were blocked in 5% Blotto, 6% sheep serum, and 0.05% Tween 20 in Tris-buffered saline (pH 7.6). An *N-Ras* specific monoclonal antibody (mAb), IgG1, or Ki-ras specific mAb, IgG2a (both from Santa Cruz Biotechnology), was incubated with immobilized Ras proteins for 1 hr at room temperature at a 1:100 dilution. The membrane was incubated with a secondary anti-mouse immunoglobulin horseradish peroxidase-labeled antibody, used at a 1:1000 dilution. Washing and the luminol-based detection of Ras proteins were carried out according to manufacturer's (ECL kit, Amersham) instructions.

Histological analysis of major mouse organs was carried out using standard procedures.

Preparation of Lymphocytes and Macrophages from Whole Blood Samples, mAb Staining, and Fluorescence-Activated Cell Sorting Analysis. Approximately 0.5-ml whole blood samples were drawn from the retroorbital sinus of genotyped offspring. Ammonium chloride lysis of red blood cells was carried out and, after washing, cells were resuspended in 600 μ l of PBS (pH 7.2). Aliquots of the washed cells (100 μ l; $\approx 5 \times 10^5$ cells) were transferred to 1.5-ml centrifuge tubes and incubated with one or two dye-conjugated mAbs to appropriate cell surface markers at the manufacturer's recommended concentrations. mAbs included fluorescein isothiocyanate (FITC)-conjugated CD45R/B220, which detects B cells; goat anti-mouse IgM (Jackson ImmunoResearch) conjugated to phycoerythrin (PE), which detects pre-B cells; CD8 α -FITC (Pharmingen), which recognizes thymocytes; MAC-1-FITC (Caltag, South San Francisco), which recognizes macrophages and granulocytes; and R-PE-conjugated CD4 and CD45PE (both from Pharmingen), which recognize specific subpopulations of T cells. Incubation with mAbs was for 45 min at 4°C, in the dark. Cells were then washed, resuspended in 500 μ l of 0.5% paraformaldehyde solution, and analyzed on a FACscan (Becton Dickinson) flow cytometer using LYSIS II software (version 1.1).

RESULTS

Gene Targeting in ES Cells. The mouse *N-ras* locus encompasses 13 kbp, including six exons, of which the last two are untranslated (26). The 11.7-kbp *N-ras* gene replacement vector (pNras) contained the cassette for the bacterial neo gene (pMC1neopola) sequence nested within 4.9 kbp of homology to the *N-ras* genomic locus (1.4 kbp of homology at the 5' end and 3.5 kbp at the 3' end). The vector also contained a flanking expression cassette for the herpes thymidine kinase gene (pMC1 HSV-TK) for negative selection (Fig. 1A). Homologous recombination between the input and target sequences will result in the disruption of the *N-ras* gene in the second exon. The event will produce a modified *N-ras* allele such that the 2.2-kb region between the *Pvu* II and *Xba* I sites is replaced by 3.8 kb of vector and *neo* DNA. The modified gene is expected to encode a nonfunctional product as a result of the removal of two-thirds of its C-terminal amino acids.

pNras DNA was transfected by electroporation into E14 cells and subjected to selection with Geneticin (G418) and GANC. Cells from individual colonies were pooled (12 clones per pool) and tested for gene targeting events by PCR. Fig. 1 shows the location of the PCR primers used for amplification. Each primer failed to amplify any DNA fragment and only a small set of pools yielded the 1520-bp fragment, which is expected from a gene-targeted event. Although the enrichment achieved by the use of GANC varied from experiment to experiment, we achieved an average enrichment of 5.3-fold (range, 2.3- to 10-fold). Two hundred and sixty-nine pools representing 3178 doubly resis-

FIG. 1. Targeted disruption of the *N-ras* genomic locus. (**A**) The *N-ras* genomic locus, depicted in the upper line, extends 13 kbp. A targeting vector, depicted in the middle line, was constructed lacking 5' exon 1 sequences and containing bacterial DNA and the pMC1neo⁺ cassette disrupting 3' exon 2 sequences. The pGK-TK cassette was included for negative selection. A homologous recombination event within the regions indicated by X will result in the targeted locus depicted in the lower line. Targeted transfectants were screened in pools by PCR using the indicated primers and then by Southern hybridization using the indicated probes. A PCR positive pool, using the *Hae* II 750-bp probe. Clone 2a6 carried the modified *N-ras* locus, indicated by the 5.5-kbp *Eco* RI fragment. MUT, mutant. (**B**) Southern blot of *Eco* RI-digested cellular DNA of two transfectants from a PCR positive pool, using the *Hae* II 750-bp probe. Clone 2a6 carried the modified *N-ras* locus, indicated by the 5.5-kbp *Eco* RI fragment. MUT, mutant. (**C**) Genotypic analysis of offspring resulting from intercrossing *N-ras* heterozygotes. *Bgl* II-digested tail DNAs were blotted and hybridized with the *Hae* II 750-bp probe. Lanes 4–9 show signals from one litter including two WT, two heterozygotes, and two homozygotes. The WT *Bgl* II fragment is 10 kbp; the mutant fragment is 11.6 kbp.

tant colonies were examined. We obtained three pools that gave the appropriate PCR product. Individual members of each of these three pools were analyzed by PCR and only one member of each pool gave the expected 1520-bp PCR fragment. This corresponds to a targeting efficiency of 1/1059 doubly resistant clones or 1/6889 G418-resistant clones.

Each of the three clones that was determined to be the result of homologous recombination by PCR was expanded, and the DNA from these cell lines was subjected to Southern blot analysis. The blots were analyzed with a *Hae* II 750-bp probe that can detect the modified and unmodified *N-ras* alleles as shown in Fig. 1. *Eco* RI digestion of the WT *N-ras* allele yields a 7.5-kb fragment, while the modified gene generates a 5.5-kb fragment. The results of this analysis are shown in Fig. 1B. The

cell line 2a6 (Fig. 1B, lane b) contained the 7.5-kb and 5.5-kb fragments, confirming that this cell line had undergone a homologous recombination event on one *N-ras* allele. The two other cell lines also gave similar results (not shown).

Generation of Mice Carrying Disrupted *N-ras* Allele. To generate chimeras, cells from each of the three targeted clones were injected into C57BL/6 host embryos and transferred to the uteri of CD-1 foster mothers. One cell line, 14a2, was effective in generating chimeric mice. The chimerism of the animals ranged from 35% to 100% as determined by examination of coat color. Of the nine chimeric animals obtained, we chose the seven strongest chimeras for subsequent crosses. These animals were mated with either C57BL/6 or 129/SV females.

Four of the seven chimeric animals transmitted the ES cell genome through their germ line as determined by the transmission of the agouti coat color marker. The proportion of the offspring that carried this dominant coat color marker was 97%, 90%, 87%, and 62%. To ascertain if the transmission of the modified *N-ras* gene followed Mendelian rules, DNA from the tails of agouti offspring was analyzed by Southern hybridization. DNA was digested with *Bgl* II and probed with the 750-bp *Hae* II fragment (Fig. 1A). The WT *N-ras* locus yields a 10-kb *Bgl* II fragment, while the modified locus yields an 11.6-kb *Bgl* II fragment. As expected, some of the agouti animals carried the 10-kb fragment and others contained 10-kb and 11.6-kb fragments. Of the total 37 agouti animals examined, 17 animals carried the WT allele and the rest carried the WT and modified alleles. This ratio is consistent with a Mendelian pattern of transmission of the two alleles.

If the *N-ras* gene product plays a critical role during mouse development, it is to be expected that embryos that carry two mutant alleles will fail to develop properly. To assess the need for *N-ras* function during development, we interbred the HET animals. Each of the matings was productive and the litter sizes were indistinguishable from WT matings. DNA derived from tail tissues of 71 progeny from these matings was examined by Southern blot analysis. Results of this experiment are presented in Fig. 1C. Southern blots of *Bgl* II-digested tail DNAs were probed with the 750-bp *Hae* II fragment (Fig. 1C). As expected, WT F₂ offspring carried only the 10-kb WT fragment (e.g., lanes 5 and 7), heterozygotes contained the WT and modified 11.6-kb fragment (e.g., lanes 2, 6, and 8), and homozygotes contained only the modified fragment (lanes 1, 3, 4, 9, 10, and 11). The results from the analysis of offspring from HET crosses are summarized in Table 1. Of 70 F₂ animals examined, 15 carried the WT allele, 42 carried one copy of the modified allele, and 13 carried two copies of the modified allele. The ratio of the three classes of animals is not significantly different from the expected values of normal transmission of the WT and mutant alleles. The HOM mutant animals were indistinguishable from their HET and WT HOM littermates. This suggests that the modified *N-ras* locus did not impair normal development of the mice.

We also ascertained if the breeding pattern of HOM *N-ras* mutant mice is different by setting up matings between HOM mutant animals. These matings yielded normal litter sizes and the progeny appear normal. We now have a breeding colony of such HOM animals.

Detection of Ras Proteins. To ensure that the modification of the *N-ras* gene results in a null mutation, we examined several tissues from the WT, HET, and HOM animals for N-Ras protein expression. Initially, protein extracts from lung were enriched for Ras proteins and fractionated on polyacrylamide gels. The proteins were transferred to membranes and incubated with mAbs specific to Ki-ras and N-ras. Results of this experiment are shown in Fig. 2.

Table 1. Quantitation of genotypes of offspring from HET crosses

Cross	Segregation of modified <i>N-ras</i>		
	No. of WT	No. of HET	No. of HOM
61 × 62, 64	7	16	6
61 × 63, 68	3	14	3
71 × 69, 72	3	5	1
13 × 1, 19	0	3	1
10 × 4	2	4	2
Total	15	42	13

One male was crossed with two females except for one cross in which only one female was present. The numbers for each cross identify the animals involved. The numbers of WT, HET, and HOM mice obtained for each cross combine results from several litters. The χ^2 statistic obtained from these data supports a Mendelian inheritance pattern.

FIG. 2. Western analysis of lung extracts from WT, HET, and HOM animals. Lung tissues from two animals of each genotype were combined, homogenized, and lysed. Crude extracts were purified on a pan-Ras affinity column and the Ras-enriched fraction was collected. Purified extracts were resolved on 15% SDS/PAGE gels, transferred to nylon membranes, and probed using N- and Ki-Ras specific mAbs. Ras proteins were detected using an ECL chemiluminescent kit (Amersham).

The Ki-Ras antibody recognized the expected-size protein from extracts of all three classes of mice (Fig. 2 Right). The N-Ras antibody recognized the same size protein from extracts of WT and HET animals (Fig. 2 Left) but failed to detect a protein from the extracts derived from HOM animals. Since the antibodies were tested against all extracts at the same time, under identical conditions, we conclude that the modification introduced into the *N-ras* gene indeed results in a null mutation.

Phenotypic Analysis. The oldest HET and HOM mice in our colony are >1 year old. Although we have observed some premature death, inflammation of the penis, and skin rash in some HET and HOM mutant animals, we were unable to establish any cause and effect relationship between the mutation and these phenotypes. We did not observe any other abnormalities in the mutant animals.

We have sacrificed some of the HOM mutant animals at 6–9 months of age and examined them for pathological or abnormal histological features. Testis, thymus, lung, spleen, kidney, gut, heart, and liver were examined and the three genotypic groups of animals were indistinguishable by this examination.

Since *N-ras* is expressed at high mRNA levels in the hematopoietic compartment (12) we examined the three genotypic groups of animals to determine if there were any differences in the lymphocyte populations in the mice by flow cytometric methods. Lymphocytes were incubated with antibodies that distinguish between B cells, CD4⁺ and CD8⁺ T cells, and macrophages (see *Materials and Methods*). Although variations between individual animals in the relative ratios of these cells were observed, no consistent differences between the different genotypic classes were observed.

DISCUSSION

We have generated mice that carry a null mutation in the *N-ras* gene. The modified cells were initially identified by a PCR based assay and we confirmed the modification by Southern blot analysis. Other *N-ras* modified ES cell lines have been generated independently (27), although, to our knowledge, mice with mutations in this gene have not been reported previously. It is known that mice and humans carry only one *N-ras* locus. Southern blot analysis of mouse DNA with an *N-ras* specific probe recognizes a single appropriate band with two different restriction enzymes and DNA from the modified ES cells as well as the derivative mice. These results show that we have disrupted the only functional *N-ras* locus in the mouse genome.

There are several lines of evidence that suggest that the modification in the N-*ras* gene results in a null mutation. The gene targeting construct replaced parts of exon 2 and intron 2 with the selectable gene (neo) cassette. Based upon the nucleotide sequence of the junction (not shown), the modified gene codes for a peptide of 60 amino acids, of which the first 58 correspond to the normal N-Ras protein. Since amino acids at regions 77–92, 109–123, and 186–189 are required for normal function (28), the modified protein would be nonfunctional. It is conceivable that the entire second exon could be spliced out from the message, resulting in a protein that contains the N- and C-terminal ends of the protein. The N-Ras specific antibody, used in the Western blot analysis, recognizes one or more epitopes in the region corresponding to amino acids 81–189 and no protein was detected with the antibody. If a cryptic splice site within the neo cassette was used, the resulting protein would lack amino acids 59–97 that encompass one of the regions critical for N-Ras function. Based upon these considerations, we conclude that in the HOM animals, no functional N-Ras protein is made.

Each of the three *ras* genes is expressed ubiquitously, but tissue-specific and developmental stage-specific quantitative differences in the levels of expression of different *ras* mRNAs have been described (11, 13). This fact, together with the observation that different *ras* genes are predominantly activated in different tumors, suggests specific roles for the members of the *ras* gene family. Based upon these facts, we expected that a null mutation at the N-*ras* locus, in the HOM state, would result in embryonic lethality. However, interbreeding of HET animals yielded HOM mutant animals at the expected ratio. These results show that N-*ras* gene function is not necessary for normal development of mice. The growth rates of HOM mice are indistinguishable from WT and HET mice and the mutant mice reproduce normally. We have established a breeding colony of N-*ras* null animals and the breeding behavior and litter sizes of these animals are normal. Despite the observation that N-*ras* gene expression is high in testis, lack of N-*ras* does not affect the fertility of the mice. Histological analysis of various adult tissues and examination of different lymphocytic cell populations failed to reveal any differences among the three genotypic classes of animals. Therefore, we conclude that N-*ras* gene expression is completely dispensable for mouse development, growth, and fertility.

Because the Ras proteins play a critical role in the signal transduction pathway, we can conclude that there is at least some redundancy in the function of the three *ras* gene products. It has been observed that in certain types of malignancies, such as colonic tumors and hematological malignancies, activation mutations involved either Ki- or N-*ras* genes. Therefore, it is possible that in the N-*ras* mutant mice, Ki-*ras* substitutes for N-*ras* gene function.

If one *ras* gene function is not essential or can be compensated by another, it is possible that loss of Ha-*ras* or Ki-*ras* function alone might not result in any untoward phenotypes in the animals. This view can be tested by generating mice with mutations in Ki- or Ha-*ras* genes.

Precedents for redundancy and for the essential nature of *ras* function in eukaryotes have been established. In yeast, there are two *ras* genes (*ras1* and *ras2*) and, although there are subtle differences between *ras1* and *ras2* knockouts, loss of function of both genes is necessary to elicit the spore lethal phenotype (14). However, with respect to the genetics of *ras*, differences exist in *Drosophila* and *Caenorhabditis elegans*. *Drosophila* contains one *ras* gene (*Dras1*), which is highly homologous to human c-Ki- and c-Ha-*ras* genes, as well as two other *ras* related genes (10). Mutant alleles in *Dras1* have been isolated that are homozygous lethal (10). Similarly, the only characterized *ras* gene of *C. elegans*, and the homologue to *Dras1* of

Drosophila, *let60*, exhibit recessive lethality in the loss of function mutant (2).

It has been shown that injection of Ras antibodies or use of agents that inhibit posttranslational modification of Ras proteins lead to reversal of the transformed phenotype. A corollary of this observation is that the lack of a *ras* gene might result in reduced levels of malignancy. Since N-*ras* activation mutations are seen in many different tumors, it would be of interest to examine if the incidence of such tumors in the N-*ras* null mice is severely reduced.

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