tenyl pyrophosphate and farnesyl pyrophosphate has been purified from pig liver (*5*).

It will be important to identify the geranylgeranyl-modified proteins and study their structures in view of current information regarding farnesylated proteins. The cDNA-derived amino acid sequences of those farnesylated proteins that have been identified contain a conserved CAAX motif at the COOH-terminus (where C is cysteine and A and X refer to aliphatic and any amino acid, respectively). Furthermore, prenylation occurs on the cysteine in this motif (*4, 6*), and ras proteins may be similarly modified (*6–8*).

Several of the farnesylated proteins also have been shown to be further modified. The last three amino acids (AAX) are removed from the COOH-terminus, and the newly exposed cysteine α-carboxyl group is converted to the methyl ester (*7, 8*). The CAAX motif may be the critical recognition element in this modification pathway because engineering of this sequence onto the COOH-terminus of a heterologous, cytosolic protein is sufficient to direct prenylation of the protein (*7*). In the cases so far examined, the isoprenoid group seems to be required for membrane localization and biological activity (*6–8*).

Similar possibilities warrant consideration in the case of geranylgeranyl-modified proteins. Therefore, a major task is to determine whether these proteins contain a directing structural motif such as the CAAX sequence, whether further posttranslational modifications occur, and whether these modifications play an important biological role.

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A Dominant Mutation That Predisposes to Multiple Intestinal Neoplasia in the Mouse

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In a pedigree derived from a mouse treated with the mutagen ethylnitrosourea, a mutation has been identified that predisposes to spontaneous intestinal cancer. The mutant gene was found to be dominantly expressed and fully penetrant. Affected mice developed multiple adenomas throughout the entire intestinal tract at an early age.

AN UNDERSTANDING OF NEOPLASIA is enhanced by the identification of genes that positively or negatively affect this process. Transgenic mice carrying activated oncogenes provide one animal model system that is proving to be useful (*1*). A complementary approach is to identify animals carrying single germline mutations that result in a susceptibility to tumor formation. The efficient production of mice carrying such mutations is possible by use of germline mutagenesis with ethylnitrosourea (Enu) combined with screening for a defined mutant phenotype. Ethylnitrosourea induces forward germline mutations at a frequency up to 1 in 700 per locus (*2*). Usually, these are single base pair changes (*3*). Either gain-of-function or loss-of-function mutations can be induced; this broadens the set of genes that can be detected by a mutational analysis.

We report a mutation in the mouse genome that leads to multiple intestinal tumors in all carriers. The mutation was identified in a pedigree established during a mutagenesis project in which C57BL/6J (B6) males were treated with Enu and then mated to AKR/J (AKR) females (*4*). One offspring from such a cross, an (AKR × B6)F_1 female that exhibited a circling behavior, was mated to a B6 male to test for the heritability of the circling trait. A progressive, adult-onset anemia was noted in some of the progeny. The anemia appeared to be transmitted as an autosomal dominant trait. The circling behavior was also shown to be heritable, but genetically unlinked to the anemia, and is no longer carried in this pedigree.

The hematological status of the animals was assessed by hematocrit determinations at regular intervals. Animals that showed a progressive decrease in hematocrit value were classified as anemic; those animals with normal hematocrit values (45 to 55%) were classified as unaffected. Blood counts revealed a progressive anemia characterized by a decreasing red cell count and an increasing proportion of reticulocytes, symptoms consistent with an anemia due to chronic blood loss. Moribund mice usually had hematocrit values in the range of 10 to 20%. This severe chronic anemia is thought to be the cause of lethality. In addition, nearly all anemic animals exhibited lipemia.

Anemic animals frequently passed bloody feces and had numerous visible tumors of the large and small intestine. The smallest visible tumors were about 1 mm in diameter, the largest up to 8 mm. Histological analysis revealed that these tumors were primarily polypoid, papillary, or sessile adenomas (Fig. 1). In older animals, locally invasive tumors were seen, but no visible metastatic tumors were observed at necropsy. Small areas of carcinoma in situ (*5*) were sometimes found in sections of tissues from anemic animals. By contrast, no significant abnormalities were seen in tissues of nonanemic animals from this pedigree. The primary phenotype of mice carrying this mutation appears to be the development of multiple adenomas which progress to adenocarcinomas of the intestine in older mice; the anemia is presumably secondary. Therefore, we named the mutant gene multiple intestinal neoplasia (*Min*).

To facilitate the propagation and study of mutant animals, we needed a method for the detection of *Min*/+ mice other than scoring for tumors at necropsy. All of the anemic mice examined had intestinal tumors. In addition, if a mouse was not anemic by 150 days of age, then no tumors were visible on necropsy at 300 days of age. Therefore, anemia was used as a provisional marker with which to recognize *Min* carriers for breeding. This classification was confirmed for each mouse in the *Min* breeding colony by examination of the intestines at autopsy.

As scored by anemia, *Min* is transmitted by affected mice to 50% of progeny with an unbiased sex distribution, as is characteristic of a fully penetrant autosomal dominant trait. As an explicit test of penetrance, 20 nonanemic males from the pedigree were

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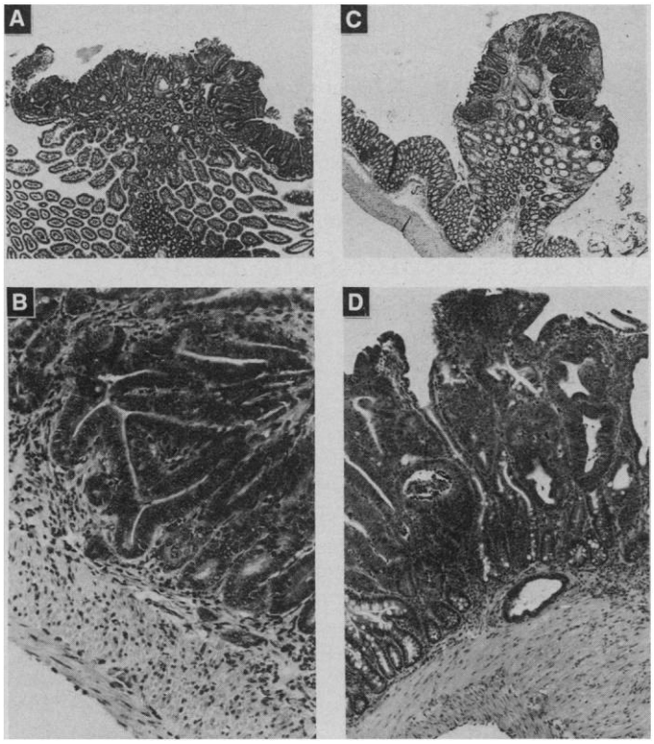

Fig. 1. (**A**) Section of an adenoma of the small intestine from a *Min*/+ mouse, showing the area compatible with carcinoma in situ (×100). (**B**) Area of marked epithelial dysplasia of small intestine compatible with carcinoma in situ (×450). (**C**) Section of a polyp of the large intestine exhibiting areas compatible with carcinoma in situ (×100). (**D**) Section of marked epithelial dysplasia and carcinoma in situ of the large intestine exhibiting glandular growth into muscularis (×450).

crossed with B6 females, and 144 progeny were screened for anemia. All offspring had normal hematocrits at 150 days of age, indicating that the phenotypically normal parents were not carrying *Min*.

The mutation is currently maintained by crossing *Min*/+ males with B6 females; it is now at the ninth backcross generation. At each generation, half of the progeny are affected; this supports the hypothesis that the propensity for tumor development is dependent on a single dominant mutant allele. *Min*/+ mice from this pedigree are referred to as B6-*Min*/+, with the backcross generation designated. B6-*Min*/+ mice from the eighth backcross generation are usually anemic by 60 days of age and rarely survive beyond 120 days of age. This early lethality makes the mutation difficult to maintain, but the average life-span has not decreased after the sixth generation of backcrossing. Therefore, we expect to be able to maintain a B6-*Min*/+ congenic line.

It is difficult to obtain progeny from an intercross of two affected mice: female B6-*Min*/+ mice are rarely healthy enough to maintain a pregnancy. Only 17 progeny from intercrosses between B6-*Min*/+ mice have been monitored to detect a viable *Min*/*Min* class exhibiting a more extreme phenotype. Of 12 affected progeny, none developed anemia more quickly or exhibited a greater number of tumors than B6-*Min*/+ mice from similar generations. Only 3 of the 12 affected animals were successfully progeny tested to determine genotype; all 3 proved to be *Min*/+ heterozygotes. Therefore, mutant homozygotes either are phenotypically indistinguishable from heterozygotes or else are inviable.

The number and distribution of tumors in the intestinal tract were determined for 104 mice from the fourth through eighth backcross generations. Tumors were usually present throughout both the small and large intestine, although the distribution varied from animal to animal. The distribution of the number of tumors in each of four regions of the intestine, representing about one-third of the small intestine and most of the large intestine, is shown in Fig. 2. The average total number of tumors for these four regions is 29 ± 10 (the SD of the sample). The average life-span within this group of mice was 119 ± 31 days. Since the mice were killed when moribund, this average tumor number probably represents the lethal tumor load.

The *Min* mutation exerts a decisive effect in a pathway to intestinal tumor formation. All mice carrying the mutation developed multiple intestinal adenomas. Is the *Min* mutation sufficient for intestinal carcinogenesis? The fact that all cells of a *Min*/+ mouse carry this predisposing germline alteration, but only a limited number of tumors develop, indicates that somatic events are also necessary for tumor formation. Therefore, *Min* may release a key step in the process of carcinogenesis, but it is not sufficient for the process. This requirement for somatic alteration is also seen for tumor formation in most transgenic mice carrying activated proto-oncogenes (*1*).

The *Min* mutation may involve a loss of function in a gene important for normal intestinal development, or a gain of function in a gene of unstated normal function. Our results do not eliminate either of these general possibilities. If the *Min* mutation inactivates a gene controlling the process of nor-

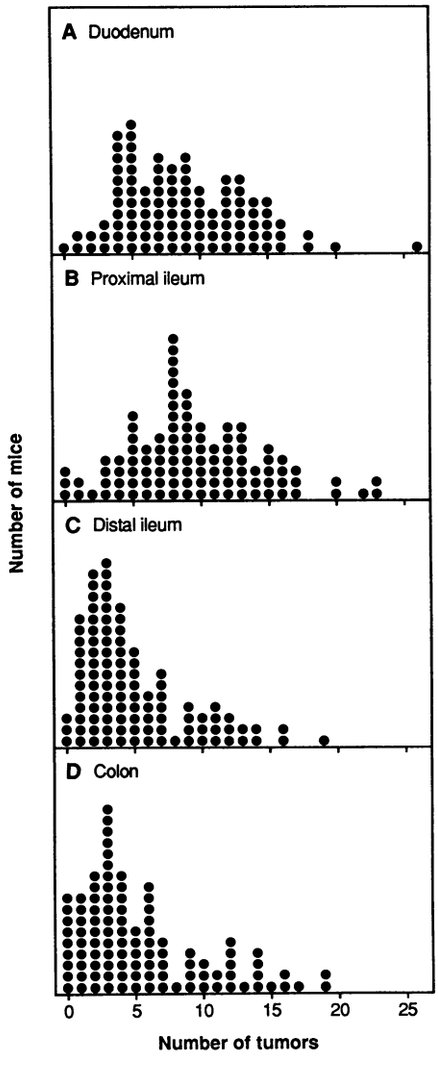

Fig. 2. The distribution of the number of visible tumors in four regions of the intestine of B6-*Min*/+ mice (from the fourth through the eighth backcross generation). Animals were killed when moribund, and the entire intestinal tract was removed, washed with phosphate-buffered saline, and opened longitudinally. The tumors were counted at ×10 magnification by use of a dissecting microscope. The smallest scorable tumors were about 1 mm in diameter. Within each panel, each circle represents 1 of the 104 mice examined. The mice were 78 to 260 days of age. The minimum number of tumors recorded for a mouse was 3; the maximum was 58. (**A** to **C**) A 4-cm-long segment was taken from each of three regions of the small intestine. (**D**) The entire large intestine (~6 cm) was scored.

mal development, the dominant mode of action could arise from somatic segregation or mutation that eliminates or alters the normal allele at the *Min* locus or affects dosage of another locus (*6*). The question of whether loss of the normal allele at the *Min* locus is a necessary step in the process of tumorigenesis can be investigated when the *Min* locus has been mapped and linked probes are available. Then, analysis of tumor tissue for evidence of somatic segregation at this locus will be possible. By contrast, a gain-of-function mutation could generate a true dominant allele capable of inducing the transformation of intestinal cells.

In the human, a number of hereditary syndromes include intestinal tumor formation; examples are familial adenomatous polyposis, Gardner's syndrome, Lynch syndrome, and several other nonpolyposis cancer syndromes (*7*). The identification of the *Min* mutation demonstrates that mutant genes resulting in similar phenotypes can be recovered in mice. Mouse germline mutagenesis may also allow the identification of mutant genes that are not frequent in natural populations. The tumors in *Min*/+ mice arise as the result of an induced germline mutation and, unlike many mouse tumors, do not have a known viral etiology. Mouse models such as the *Min* mutant should provide a major resource for study of pathways of tumorigenesis and for the molecular identification and manipulation of genes that can control particular neoplastic processes.

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Cloning of an Interleukin-3 Receptor Gene: A Member of a Distinct Receptor Gene Family

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Interleukin-3 (IL-3) binds to its receptor with high and low affinities, induces tyrosine phosphorylation, and promotes the proliferation and differentiation of hematopoietic cells. A binding component of the IL-3 receptor was cloned. Fibroblasts transfected with the complementary DNA bound IL-3 with a low affinity [dissociation constant (K_d) of 17.9 ± 3.6 n*M*]. No consensus sequence for a tyrosine kinase was present in the cytoplasmic domain. Thus, additional components are required for a functional high affinity IL-3 receptor. A sequence comparison of the IL-3 receptor with other cytokine receptors (erythropoietin, IL-4, IL-6, and the β chain IL-2 receptor) revealed a common motif of a distinct receptor gene family.

INTERLEUKIN-3, PRIMARILY PRODUCED by activated T cells, stimulates colony formation of multiple lineages (multi-CSF), maintains spleen colony forming units (CFU-S), stimulates mast cell growth and histamine release, induces 20-α-steroid dehydrogenase and the Thy1 antigen, serves as a growth factor for megakaryocytes, and acts on pre–B cells and potentially on pre–T cells (*1*). Bone marrow stromal cells probably do not produce IL-3 (*2*); IL-3 may preferentially participate in the expansion of hematopoietic cells in response to inflammatory stimuli rather than in the constitutive hematopoiesis of the bone marrow (*3*).

Murine IL-3 binds to its receptor with both high and low affinities (*4*). Although the molecular size of the IL-3 receptor was initially reported as 60 to 70 kD (*5*), recent cross-linking results indicate the presence of additional binding proteins of 140 and 120 kD (*4*). A protein tyrosine kinase seems to participate in IL-3 signal transduction. Expression of oncogenes with tyrosine kinase activity abrogates IL-3 dependence (*6*). Particularly, a mutant v-*abl* with a temperature-sensitive tyrosine kinase abrogates IL-3 dependence in a temperature-dependent manner (*7*). Tyrosine phosphorylation of a set of cellular proteins is induced within a minute after addition of IL-3, which suggests the close association of a tyrosine kinase with the IL-3 receptor (*8*). IL-3 induced the tyrosine phosphorylation of a 140-kD IL-3 binding protein (*9*). Thus, either the IL-3 binding protein contains an intrinsic tyrosine kinase activity, or the IL-3 binding protein and the tyrosine kinase are distinct molecules.

Using IL-3–dependent murine cells as an immunogen, we obtained a monoclonal antibody (MAb), anti-Aic2, to a 105-kD cell surface protein present on various IL-3–dependent cell lines (*10*), whose expression correlates with IL-3 binding. The antibody partially inhibits the binding of IL-3 to its receptor and immunoprecipitates several ^{125}I-IL-3 cross-linked proteins. Thus, the Aic2 antigen is probably a component of the IL-3 receptor (*10*). We now have cloned and expressed the Aic2 cDNA, shown that the Aic2 antigen is a binding component of the IL-3 receptor, and identified a family of

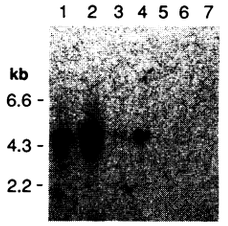

Fig. 1. An RNA blot analysis of IL-3 receptor mRNA. Polyadenylated RNA was isolated from MC/9 (lane 1), PT18 (lane 2), NFS60.8 (lane 3), DA3.15 (lane 4), HT-2 (lane 5), D9 (lane 6), and total liver cells (lane 7). The RNA (2 μg) was denatured and subjected to blotting analysis with the AIC2-2 cDNA. The numbers in the left lane indicate the position of Hind III fragments of λ phage DNA (in kilobases).

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