strains available is limited, this pattern of $V_\beta 5.2$ deletion is similar to that observed in B × D RI strains. Spleen cells treated with LPS and IL-4 from one-half of the I-E^k–expressing B × H strains stimulated 5Q12; the strain distribution pattern again was in concordance with the B6 viral marker Mtv-9 on chromosome 12 (*12*). A comparison of $V_\beta 5.2$ expression and ability to stimulate 5Q12 revealed a correlation consistent with our hypothesis that the hybridoma 5Q12 recognizes a gene product involved in the strong deletion of $V_\beta 5.2$-bearing T cells.

Our data show that the deletion of I-E–reactive ($V_\beta 5.2$-bearing) T cells requires the expression of the MHC molecule I-E and one of a limited number of cotolerogens. One of the B6-derived cotolerogens appears to be recognized by the T cell hybridoma 5Q12, and the gene encoding this ligand is closely linked to the endogenous virus Mtv-9 on chromosome 12. The requirement for B cell–derived gene products in the recognition of I-E by $V_\beta 17^+$ hybridomas has been described (*14*). However, the number of non-MHC gene products involved and their possible relationship to the deletion of $V_\beta 5.2^+$ T cells is not known. The expression of multiple non-MHC (*15–24*) gene products (from the Mls loci) have been shown to have a role in the deletion of several V_β elements. The I-E cotolerogens described here resemble Mls products in that they associate with class II molecules and delete families of V_β-bearing T cells. For these reasons, the Mls antigens and I-E cotolerogens may belong to the same family of proteins. However, unlike Mls antigens, I-E cotolerogens function exclusively in association with I-E molecules and do not induce proliferative responses in mixed lymphocyte cultures (*25*).

A limited number of background genes apparently can dramatically alter the T cell repertoire. $V_\beta 5^+$ T cells are involved in the development of diabetes in I-E^- nonobese diabetic (NOD) mice (*26*). NOD mice that express I-E molecules are protected from developing diabetes, presumably because $V_\beta 5$ cells are clonally deleted. It seems likely that I-E cotolerogens participate in the resistance displayed by some strains of mice to diabetes and other autoimmune diseases.

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Mini-Mouse: Disruption of the Pygmy Locus in a Transgenic Insertional Mutant

XIN XIANG,* KATHLEEN F. BENSON,* KIRAN CHADA

A founder transgenic mouse harbored two different integration patterns of a transgene at the same locus, each of which gave rise to a similar autosomal recessive mutation. Mice of the mutant phenotype were of small stature but had normal levels of growth hormone. The disrupted locus was cloned, and a genetic and molecular analysis showed that the insertional mutants were allelic to a spontaneous mutant, pygmy. The mice should be a useful model for the growth hormone–resistant human dwarf syndromes and could lead to a greater understanding of the pathways involved in growth and development.

THERE ARE MORE THAN 1000 MOUSE mutants that express a plethora of aberrant developmental phenotypes (*1*), but further analysis of these mutations has been limited because of the difficulty in characterizing them at the molecular level. Incorporation of exogenous DNA into the germ line of mice overcomes this shortcoming because the foreign DNA, or transgene, can be used as an insertional mutagen and then as a probe to clone the disrupted locus. A number of transgenic insertional mutants (*2*) have been isolated by this approach (*2*) and characterized at a molecular level (*2, 3*). In this report we describe a founder transgenic mouse containing a 2.8-kb human globin gene fragment; homozygous progeny were significantly retarded in their overall growth and development compared to both nontrans-

Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854.

*X. Xiang and K. F. Benson contributed equally to this manuscript.

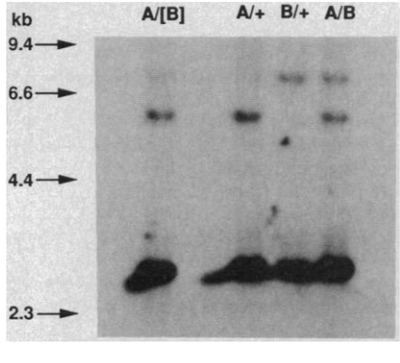

Fig. 1. DNA analysis of transgene transmission from the founder transgenic mouse. The founder (A/[B]) harbored a 2.8-kb human globin gene fragment; when bred to a wild-type mouse, it produced progeny of genotypes A/+ and B/+. Intercrosses between A/+ and B/+ mice produced mice of genotype A/B. Equal amounts of genomic DNA (7.8 μg) were applied; the procedure was as described (*16*), with the hybridization probe being the transgene.

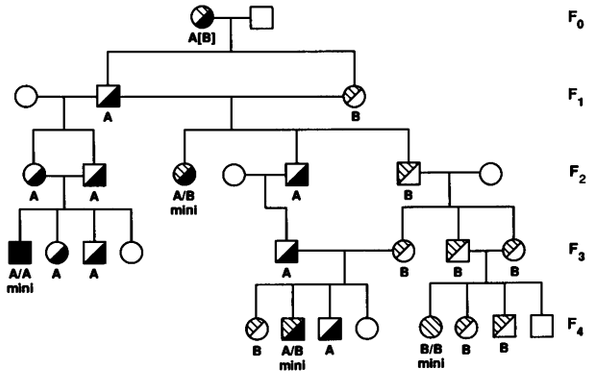

Fig. 2. A representative portion of the pedigree derived from the founder transgenic mouse A/[B]. Females are represented by circles and males by squares. Open circles and squares represent mice that do not harbor the transgene. Solid and shaded circles or squares represent mice of line A and line B, respectively. Half-filled circles or squares represent transgenic hemizygotes (A or B), and filled circles or squares represent transgenic homozygotes (A/A or B/B). The genotype of the mice was determined as in (*16*), and the transgene was the hybridization probe. Mice of small stature are indicated as mini.

genic and hemizygous littermates. These homozygous mice were called mini-mice.

The founder transgenic mouse was of a normal phenotype, and DNA hybridization revealed the 2.8-kb repeat unit of the tandem array (*4*) and two additional bands at 6.0 kb and 8.6 kb (Fig. 1). The founder, however, was chimeric for the transgene because its progeny harbored either the diagnostic band at 6.0 kb (designated line A) or 8.6 kb (designated line B), in addition to the 2.8-kb band (Fig. 1). Because the 8.6-kb band was more intense in the progeny than in the founder (Fig. 1), the founder mouse was probably mosaic for this integration pattern and hence its genotype is designated A/[B].

Intercrosses were set up between hemizygous mice in either line A or line B (Fig. 2), and the mini-mouse phenotype was segregated as an autosomal recessive mutation (*5*). In addition, all 28 mini-mice tested were found to be homozygous, demonstrating that the mutation co-segregates with the transgene. When hemizygous mice of line A and line B were mated (Fig. 2), they also produced mini-mice (genotype A/B). When 12 of these mini-mice were analyzed, they all contained both the 6.0- and 8.6-kb diagnostic bands (Fig. 1). This finding suggested that the disrupted locus in line A is allelic to that of line B.

The most prominent feature of the mutation is the small size of the mice (Fig. 3). Except for their short ears, the mini-mice appear to be proportionately reduced overall in size and can be distinguished by visual inspection from their littermates during the first week of birth. By 10 weeks of age, they are approximately 40% of the weight of their negative littermates and are fully viable (*6*). However, the circulating levels of growth hormone and somatostatin were normal (*7*).

To further our understanding of the mutation, the next step was to clone the disrupted locus. The 6.0-kb diagnostic band in line A (Fig. 1) was hypothesized to be a junction fragment between the tandem array and genomic DNA and was therefore cloned (*8*). After further analysis, a 500-bp Apa I–Apa I single copy sequence that was devoid of any transgene sequences was isolated (Fig. 4A). The 500-bp fragment hybridized to a 10.0-kb fragment on wild-type DNA; to two fragments of 10.0 and 6.0 kb on hemizygous A DNA, which are indicative of the normal and disrupted chromosomes, respectively; and to a 6.0-kb fragment with homozygous A DNA (Fig. 4B). A similar pattern has been obtained with six different homozygotes and two other restriction enzymes, Hpa I and Bgl I (*9*). This finding therefore demonstrates that the 500-bp fragment is derived from the disrupted locus.

When the 500-bp fragment was hybridized to DNA from mice of the genotype B/+, only the 10.0-kb fragment indicative of the normal chromosome was observed (Fig. 4B). DNA from homozygous B mice

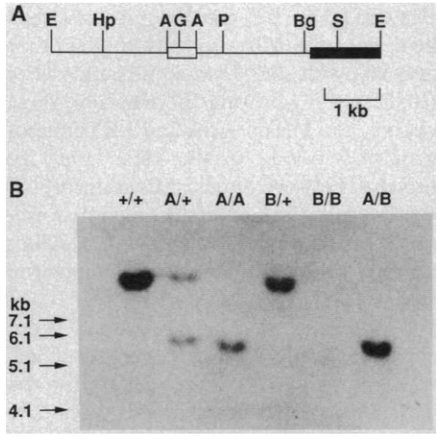

Fig. 4. (**A**) Restriction map of the 6.0-kb Eco RI junction fragment containing flanking sequences adjacent to the foreign DNA. The solid region denotes a portion of the transgene, and the open box represents the 0.5-kb Apa I–Apa I single copy region. Abbreviations: E, Eco RI; Hp, Hpa I; A, Apa I; G, Bgl I; P, Pvu II; Bg, Bgl II; and S, Sph I. (**B**) Various amounts of DNA were isolated from mice of the indicated genotypes and hybridized by the methods in (*16*). The +/+ represents wild type. The 0.5-kb Apa I–Apa I fragment in (A) that was used as a probe is not polymorphic for the two parental strains, CBA and C57/Bl (*9*), from which the transgenic mice are derived.

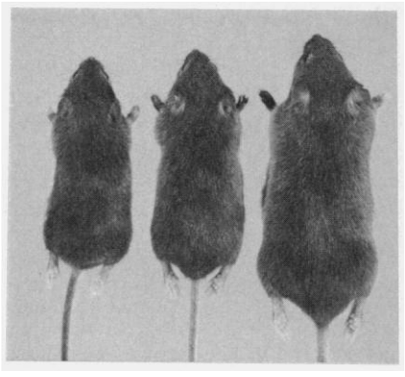

Fig. 3. Comparison of mice of differing genotypes. From left to right, a pg/pg spontaneous mutant, an A/A mini-mouse, and a mouse of the A/+ genotype. The mice were between 10 to 14 weeks old.

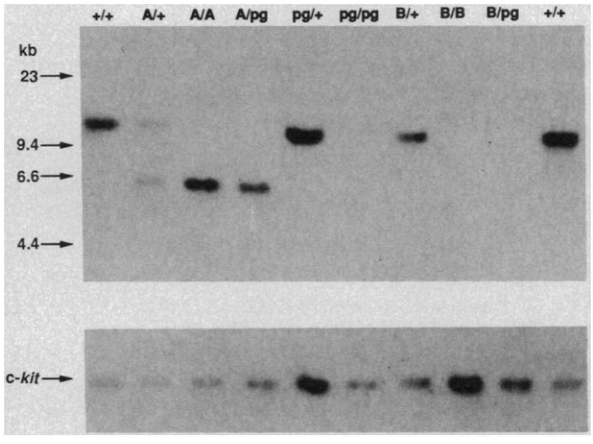

Fig. 5. DNA hybridization of the indicated genotypes probed with the 0.5-kb Apa I–Apa I (Fig. 4A) and c-*kit* fragments. Mice of genotypes A/pg and B/pg were of small stature and were produced from matings between hemizygous A or B mice, respectively, with heterozygous pg mice. They had been shown to harbor the same quantity of transgene as hemizygous mice (*13*).

did not show a hybridization signal (Fig. 4B); this was not a result of DNA degradation because the same blot reprobed with the transgene revealed the expected pattern of hybridization (*9*). Therefore, in line B, the 500-bp genomic sequence has been deleted, and subsequent experiments have revealed a deletion of at least 40 kb of the normal locus (*9*). In mice of the A/B genotype, only the indicative band at 6.0 kb of line A was present, confirming the conclusion that A and B are at the same locus.

Mutations in a number of previously identified loci, localized to various chromosomes, produce mice of small stature as their predominant phenotype (*1*). One of these mutants, pygmy (pg) (*10*), was mapped to chromosome 10 and has many of the characteristic features of the mini-mutation (Fig. 3). Because the 500-bp Apa I–Apa I fragment also mapped to chromosome 10 (*11*), the pygmy and mini loci were tested for allelism. Twelve out of 48 progeny from A/+ and pg/+ matings and 6 out of 35 progeny from B/+ and pg/+ matings were found to be of small stature, which is consistent with the insertional and spontaneous mutants being allelic (*12*).

This allelism was demonstrated by Southern blot analysis of DNA isolated from mice of the indicated genotypes (Fig. 5). Pygmy heterozygotes (pg/+) revealed only a single band at 10 kb, which was representative of the normal locus, and no other bands were apparent. However, in pygmy homozygotes (pg/pg), there was no detectable hybridization, and the same blot rehybridized with c-*kit* as a control probe revealed the appropriate bands. Additionally, mice of genotype B/pg also showed no hybridization signal, whereas A/pg mice revealed only the 6.0-kb disrupted band. Thus, there is a deletion of the 500-bp genomic sequence in the spontaneous mutant, which extends to at least 40 kb (*9*); this finding establishes that there is a disruption at the same locus as in the two insertional mutants. This 500-bp Apa I–Apa I fragment is now routinely used in our laboratory to distinguish between wild-type and heterozygous mice of the spontaneous mutant line rather than the more laborious method of test breeding.

This study describes the molecular characterization of two transgenic insertional mutants at the pygmy locus derived from one founder mouse. The simplest explanation for the pattern of integration is that a single integration event took place that ultimately gave rise to line A. After a few cell divisions, a recombination event occurred in one cell; this event resulted in the deletion of a portion of the tandem array and flanking genomic locus so as to give rise to line B. This would explain the reduced number of copies of the foreign DNA (*13*), the absence of the 500-bp genomic sequence, and the apparent mosaicism of the founder for integration B. Since these initial events no further rearrangements have been observed in line A after ten generations.

Although the pygmy spontaneous mutant has existed for 45 years, it has remained somewhat enigmatic. Unlike the well-characterized dwarf syndromes in mice (*1*) and humans (*14*), the pygmy mutant has normal levels of circulating growth hormone (*15*). However, its usefulness as a model to elucidate the unknown biochemical nature of the African pygmy phenotype and the growth hormone–resistant human dwarf syndromes (*15*) has been limited by the lack of any molecular analysis. Cloning of the pg locus and the eventual identification of the gene product will assist in defining the utility of the pygmy mutant for these studies. In addition, the mutant mouse could be used as a molecularly authentic version of the relevant human dwarf syndrome to develop various therapeutic regimes to alleviate the symptoms of that disease.

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6. A more detailed histological and morphological analysis of the mutant is given by K. Benson and K. Chada (in preparation).
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8. Preparative gel electrophoresis was performed on 160 μg of Eco R1–digested hemizygous A DNA, and the region containing the 6.0-kb fragment was eluted from the gel and cloned into the lambda ZAP vector (Stratagene). Then, 3×10^5 phage were screened with the transgene, and two positive phage were isolated. The phage were then converted to plasmids, ZAP1 and ZAP2, by "zapping" (Stratagene), and only ZAP1 was further characterized.
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Atrionatriuretic Peptide Transforms Cardiac Sodium Channels into Calcium-Conducting Channels

LISA ANN SORBERA AND MARTIN MORAD*

The atrionatriuretic peptide (ANP) is released from atrial cells in response to increased extracellular fluid volume and reduces sodium absorption by the kidney, thus reducing the blood volume. In this report, ANP suppressed the calcium and sodium currents in rat and guinea pig ventricular myocytes. The suppression of sodium current was caused by enhanced permeability of the sodium channel to calcium without significant changes in the kinetics or the tetrodotoxin sensitivity of the channel. Thus, ANP may regulate the sodium channel by altering its cationic selectivity site to calcium, thereby repressing the sodium current. The suppression of sodium and calcium channels and the resultant depressed excitability of the atrial cells may help to regulate ANP secretion.

THE SODIUM CHANNEL IS ESSENTIAL for the excitability of nerve, muscle, and heart cells (*1, 2*). The modulation of cardiac Na^+ channels by pharmacological agents has served as the basis for clinical treatment of myocardial arrhythmias (*3*). The physiological regulation of the cardiac Na^+ channel is not well understood, although phosphorylation of the Na^+ channel by an adenosine 3′,5′-monophosphate