of ligand, though the receptor dissociation constants were indistinguishable. It seems highly unlikely that the mitogenic response is due to residual receptor internalization and intracellular targeting beneath our level of detection given the increased sensitivity of cells carrying low numbers of this mutant to subsaturating ligand concentrations. Although, theoretically, any receptor mutation could alter the kinase specificity, analysis with antibodies to phosphotyrosine did not reveal significant alterations in substrate phosphorylation with the c'973 receptor (*11*). In addition, previous studies suggest that an altered kinase activity of the c'973 mutant, if any, is not biologically relevant (*6*).

Removal of the entire region COOH-terminal to the kinase domain, a 213–amino acid truncation, resulted in an enzyme not subject to the normal mechanisms of attenuation. Previous reports have suggested that the COOH-terminal sequences of the EGF receptor facilitate the mitogenic effects of receptor activation (*16*). Elimination of the autophosphorylation sites present in the terminal 69 amino acids does not result in enhanced growth. This contrasts with other tyrosine kinases in which removal of these sites results in activation (*17*). The explanation for this difference appears to be that in addition to the autocatalytic substrates, which function as competitive inhibitors (*18*), the EGF receptor tail contains a second element responsible for quenching receptor signaling, an internalization domain (*6*). A receptor lacking both control elements would be expected to maintain ligand-induced signaling. Cells presenting such a receptor should be more sensitive than WT cells at low levels of ligand and exhibit the transformed phenotype. Our results imply that the activation of the tyrosine kinase (*3*) at the plasma membrane is sufficient for cell division. As a consequence the membrane-bound forms of the pro-EGF (*19*) and pro–TGF-α (*20*) should be capable of eliciting cell growth in neighboring receptor-bearing cells. Internalization and degradation of the receptor appear to abrogate the long-term actions of the EGF receptor. In the absence of this attenuation mechanism, low concentrations of ligand would result in uncontrolled proliferation; that is, the transformed phenotype. It may be speculated that mutations disabling the endocytic pathway for growth factor receptors would result in neoplastic transformation.

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22. Cells were plated on fibronectin-coated plates and grown in minimum essential medium α (MEMα) plus 10% dialyzed fetal bovine serum. After 48 hours the medium was replaced by MEMα plus bovine serum albumin (1 mg/ml), and the cells were incubated overnight. The specific internalization rates of the EGF receptors were determined as described [H. S. Wiley, *J. Cell Biol.* **107**, 801 (1988)]. The ^{125}I-labeled EGF concentration was 0.5 n*M* at a specific activity of 250,000 cpm/ng. Nonspecific binding was determined at all time points in the presence of 1 μ*M* unlabeled EGF and was <2% of total binding.
23. Internalization of the WT and truncated receptors was investigated by immunofluorescence. Cells were treated with 100 n*M* EGF for 30 min at 37°C and then fixed. The receptors were visualized by a monoclonal antibody to the EGF receptor [J. R. Glenney *et al.*, *Cell* **52**, 675 (1988)]. Cells expressing the truncated c'973 receptors were identical before and after the EGF treatment, whereas those expressing the WT receptors displayed a relocation of the receptors to the intracellular compartment.

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Requirement for Cotolerogenic Gene Products in the Clonal Deletion of I-E Reactive T Cells

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T cells that express the T cell receptor V_β5.2 domain react with the class II major histocompatibility complex (MHC) molecule I-E, and V_β5.2$^+$ T cells are deleted in mouse strains that express I-E glycoproteins. By examination of genetically defined recombinant inbred (RI) mouse strains, it was found that the deletion was dependent on the expression of I-E and one of a limited number of non-MHC gene products (cotolerogens). The gene encoding one of these cotolerogens maps to chromosome 12 and is linked to the endogenous provirus Mtv-9. These observations suggest that the I-E–mediated and minor lymphocyte-stimulating antigen (Mls)–mediated deletions of αβ T cells from the repertoire are similar; both require the expression of a class II MHC glycoprotein and a second non-MHC gene product.

THE MATURATION OF T LYMPHOcytes in the thymus involves two selective steps, the positive selection of self-MHC–restricted T cells and the negative selection (deletion) of self-reactive T cells (*1*). Several murine T cell receptor (TCR) V_β gene segments (V_β5.1, V_β5.2, V_β11, and V_β17) encode reactivity with the class II MHC antigen I-E, and mice expressing I-E delete the majority of these T cells from the peripheral repertoire (*2–6*). However, it is not known whether the expression of additional non-MHC genes is required for the deletion of I-E–reactive T cells. We have previously described the variation in V_β11 expression among the B6 × DBA/2 (B × D) RI strains (*4*). Although the genetic background has a role in determining the

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frequency of $V_\beta11^+$ cells, the variation seen in the B × D RI strains is not due solely to the segregation of non-MHC genes involved in deleting $V_\beta11^+$ T cells. Rather, subsequent experiments have shown that the differences between the B × D RI strains are due to differences in the positive selection of $V_\beta11$-bearing cells in H-2^d mice (7).

In contrast, the frequencies of $V_\beta5.2^+CD8^+$ cells in DBA/2 and B10.D2 mice are significantly different, despite the fact that both strains express I-E^d and result from differences in the clonal deletion of $V_\beta5.2$-bearing cells (Fig. 1). Whereas 2.5 ± 0.15% (mean ± SE) of $CD8^+$ T cells in DBA/2 mice express $V_\beta5.2$-encoded antigen receptors (moderate deletion), only 0.7 ± 0.2% (mean ± SE) of $CD8^+$ T cells express $V_\beta5.2$ in B10.D2 mice (strong deletion). Analysis of $V_\beta5.2^+CD8^+$ expression in 16 H-2^d B × D RI strains (*8*) revealed an unusual pattern of deletion, and three phenotypes of $V_\beta5.2$ expression are apparent (Fig. 1, top). The majority of the B × D strains (11/16) were strong deletors of $V_\beta5.2^+CD8^+$ T cells and were phenotypically similar to B10.D2 (solid bars). Two strains, B×D 12 and B×D 32, displayed a moderate deletion of $V_\beta5.2^+$ T cells similar to the DBA/2 phenotype (open bars). Finally, three of the B × D strains, B×D 18, B×D 28, and B×D 31, expressed high frequencies of $V_\beta5.2^+$ T cells (weak deletion) similar to that found in I-E^- strains (shaded bars). A similar pattern of $V_\beta5.2$ expression in B × D RI mice was observed in $CD4^+$ T cells (7). However, the magnitude of the differences in the CD4 subpopulation was less dramatic because of the general underrepresentation of $V_\beta5.2^+$ T cells in the $CD4^+$ subset, even in I-E^- haplotypes (*6*). The similar patterns of deletion in both $CD4^+$ and $CD8^+$ T cell subsets is consistent with deletion occurring at the double positive ($CD4^+CD8^+$) stage of thymocyte development.

Several mechanisms can be invoked to explain the inability of I-E molecules to tolerize $V_\beta5.2^+$ cells in some I-E–bearing B × D strains (B×D 18, B×D 28, and B×D 31): (i) The clonal deletion of $V_\beta5.2$-bearing T cells may depend on the degree of I-E expression in B × D RI strains. However, cytofluorographic analysis with a monoclonal antibody (MAb) to I-E^d (MAb 14-4-4) (*9*) showed that the surface concentration of I-E^d on lymph node cells did not vary significantly between different H-2^d B × D RI strains (Fig. 1, bottom). (ii) The clonal deletion of $V_\beta5.2^+$ T cells occurs normally in all of the H-2^d B × D RI strains, but in some strains there may be an aggressive positive selection of the remaining $V_\beta5.2^+$ cells. (iii) The deletion of $V_\beta5.2^+$ cells is dependent on the expression of both I-E and a cotolerogen that is absent from these "weak deletor" strains. To distinguish between the second and third hypotheses, we

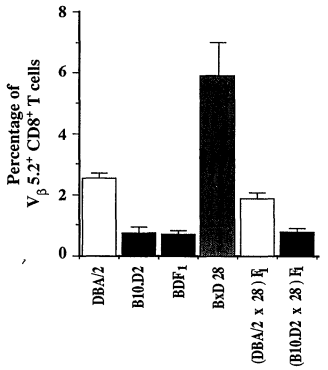

Fig. 2. Frequency of $CD8^+$ T cells expressing the $V_\beta5.2$ domain from I-E^d-bearing DBA/2, B10.D2, B×D 28, and F_1 strains. Three different phenotypes are represented, strong deletion (solid bars), moderate deletion (open bars), and weak deletion (shaded bars). $V_\beta5.2$ expression was determined as described in Fig. 1. Data from each strain represent the mean and SE of determinations from three animals.

bred F_1 mice from B×D 28 (I-E^d, weak deletor) and DBA/2 (I-E^d, moderate deletor) or B10.D2 (I-E^d, strong deletor) parents and measured the frequencies of $V_\beta5.2^+CD8^+$ T cells in (DBA/2 × B×D 28)F_1 and (B10.D2 × B×D 28)F_1 mice (Fig. 2). In each of the F_1 strains, the phenotype of strong or moderate deletion (B10.D2 or DBA/2) was dominant. The high frequency of $V_\beta5.2^+CD8^+$ T cells in B×D 28 mice is consistent with the absence of cotolerogenic gene products encoded by non-MHC genes.

Approximately three-quarters of the RI strains (11/16) were strong deletors of $V_\beta5.2^+$ T cells; thus, this phenotype is likely dependent on the segregation of two genes, either of which can mediate strong deletion (B10.D2 phenotype). These genes must be derived from the B6 genome, since DBA/2 mice are only moderate deletors of $V_\beta5.2^+$ cells. Approximately one-quarter (5/16) of the B × D strains have lost both of the B6-derived genes by segregation. These five strains fall into two groups that expressed either moderate deletion of $V_\beta5.2^+$ cells (DBA/2 phenotype) or the weak deletion of $V_\beta5.2^+$ cells (B×D 28 phenotype). The two strains that were moderate deletors of $V_\beta5.2^+$ cells (B×D 12 and B×D 32) have likely inherited a single DBA/2-derived locus that mediates the moderate deletion characteristic of DBA/2 mice. The remaining three B × D strains (B×D 18, B×D 28, and B×D 31) that were weak deletors of $V_\beta5.2^+$ T cells have likely lost all three of these loci (two derived from B6 and one derived from DBA/2) by segregation. Thus, the extent of deletion of $V_\beta5.2^+$ T cells in the H-2^d B × D RI strains is determined by the segregation of a limited number of non-MHC genes. Our interpretation of the dele-

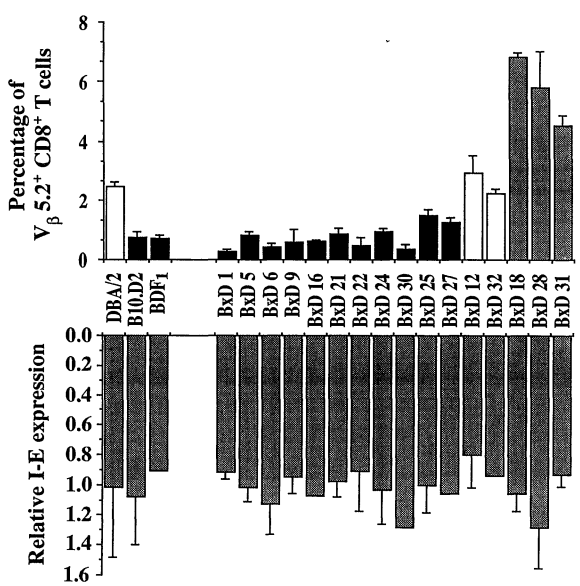

Fig. 1. (**Top**) Frequency of $CD8^+$ T cells expressing $V_\beta5.2$ antigen receptors in DBA/2, B10.D2, (B6×DBA/2)F_1 (BDF_1), and 16 H-2^d B×D RI strains. Three different phenotypes are represented: strong deletion (solid bars), moderate deletion (open bars), and weak deletion (shaded bars). (**Bottom**) Relative expression of I-E^d on class II–positive lymph node cells from the same set of strains. To assess $V_\beta5.2$ expression, from each strain, lymph node cells that did not adhere to nylon wool were stained first with biotinylated MR9-4 (specific for $V_\beta5.1$ and $V_\beta5.2$) or biotinylated MR9-8 (specific for $V_\beta5.1$ only) MAbs (*6*). Cells were washed and counterstained with phycoerythrin-conjugated streptavidin (Tago) and fluoresceinated 53-6-72 (antibody to CD8). Fluorescence intensity was determined with an EPICS C flow cytometer (Coulter Electronics). The percentage of $V_\beta5.2^+$ cells was determined by calculating the percentage of $CD8^+$ cells that express the MR9-8 epitope ($V_\beta5.1^+$ only) and subtracting this value from the MR9-4^+ $CD8^+$ fraction ($V_\beta5.1^+$ and $V_\beta5.2^+$). The data for each strain represent the mean and SE of determinations from at least three mice. I-E expression was determined on whole lymph node cells from each strain with a primary MAb specific for I-E^d (14-4-4) (*9*) and fluoresceinated goat antibodies to mouse immunoglobulin (Ig) Fc (Jackson Immunoresearch Labs), which does not bind Ig on the surface of Ig^+ B cells. Usually 20 to 50% of the cells stained positive for I-E^d under these conditions. Relative I-E expression was determined by calculating the linear mean channel fluorescence (I-E^d expression in the DBA/2 strain is arbitrarily set at 1.0). Data from each strain represent the mean of determinations from two or three animals. The SE is shown when the data from three determinations were available.

tion patterns seen in B × D RI mice has been confirmed in a backcross experiment (*10*).

To analyze these genes further, we screened a panel of T cell hybridomas for reactivity with I-E molecules. One of these hybridomas, 5Q12 (*4*), responds strongly to I-E^d and I-E^k molecules on spleen cells by secreting interleukin-2 (IL-2). The hybridoma only responded to antigen-presenting cells that had been treated with lipopolysaccharide (LPS) and IL-4. The reactivity of the 5Q12 hybridoma to I-E^d is inhibited by antibodies directed against I-E^d (MAb 14-4-4) (*9*), but is not inhibited by antibodies reactive against either I-A^d (MAb MK-D6) (*11*) or H-2^d class I (MAb 27-11-13) molecules (*7*). The 5Q12 cells responded to I-E^d presented on BALB/c, NZB, B10.D2, and BDF_1 spleen cells, but not to I-E^d on DBA/2 spleen cells (Fig. 3). This lack of response was not caused by the expression of fewer I-E molecules on DBA/2 stimulator cells, since the relative amount of I-E^d on the surface of spleen cells induced with LPS and IL-4 from B10.D2, BALB/c, NZB, BDF_1, and DBA/2 mice was similar as determined by cytofluorometry. The 5Q12 hybridoma was probably responding to a peptide or protein presented by I-E^d that was not produced in DBA/2 mice. We tested the ability of spleen cells treated with LPS and IL-4 from I-E^d–bearing B × D RI strains to stimulate 5Q12. Approximately one-half (9/16) of the H-2^d B × D RI strains stimulated 5Q12 (Fig. 3, bottom). This strain distribution pattern is consistent with the inheritance of a single locus. A comparison of this strain distribution pattern with other known gene distributions in B × D RI mice revealed a perfect concordance with the endogenous mouse mammary tumor virus integrant Mtv-9 on chromosome 12 (*12*). The response of 5Q12 to the chromosome 12–encoded surface antigen requires induction of the antigen-presenting cells with LPS and IL-4 (*13*). It is unclear whether this stimulus is required to increase the density of surface I-E or to induce the production of the antigen.

To examine the possibility that the B6-derived gene product recognized by the hybridoma 5Q12 is a cotolerogen responsible for the deletion of $V_\beta5.2^+$ cells, we compared the strain distribution of 5Q12 reactivity with $V_\beta5.2$ tolerance among the H-2^d B × D RI strains (Fig. 3). All of the H-2^d strains that express the gene product involved in the stimulation of 5Q12 were strong deletors of $V_\beta5.2^+$ cells (B10.D2 phenotype, solid bars). Similarly, those strains that express either the DBA/2 (moderate deletor, open bars) or B×D 28 (weak deletor, shaded bars) phenotypes did not stimulate the 5Q12 hybridoma. The concordance is perfect except for two strains, B×D 25 and B×D 27, that are strong deletors of $V_\beta5.2^+$ cells (B10.D2 phenotype) but that did not stimulate the 5Q12 hybridoma. We propose that the hybridoma 5Q12 recognizes one of two B6-derived gene products that associate with I-E and are responsible for the strong deletion of $V_\beta5.2^+$-bearing cells in B10.D2 and BDF_1 mice. B×D 25 and B×D 27 likely represent strains that have inherited the second B6-derived gene

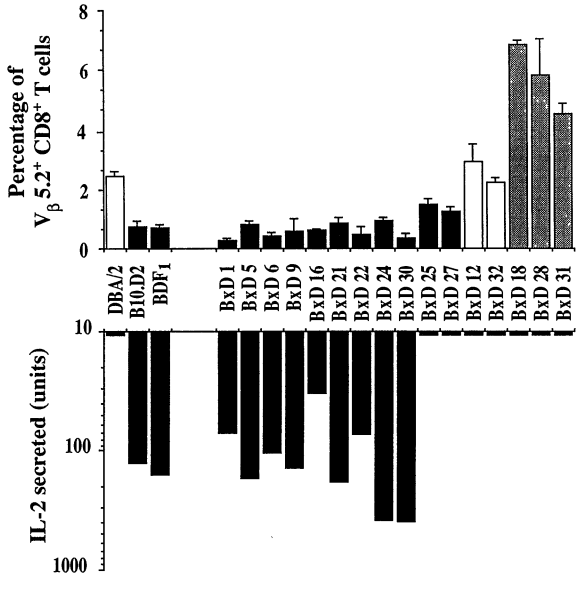

Fig. 3. (**Top**) Frequency of $CD8^+$ T cells expressing $V_\beta5.2$ antigen receptors in various mouse strains (see the legend to Fig. 1). (**Bottom**) Response of the T cell hybridoma 5Q12 to splenocytes treated with LPS and IL-4 from DBA/2, B10.D2, B10.D2, BDF_1, and 16 H-2^d B × D RI strains. Splenocytes from appropriate strains were depleted of red blood cells, washed, and cultured (2×10^6 cells per milliliter)) in medium (*27*) supplemented with LPS (30 μg/ml) and recombinant mouse IL-4 (25 U/ml) (Genzyme, Boston, Massachusetts). After 16 hours, these cells were washed four times and used for stimulation assays. The 5Q12 stimulation assays were performed in triplicate 250-μl cultures containing 5Q12 cells (1×10^5) and LPS plus IL-4–treated splenocytes (1×10^6). After 24 hours, dilutions of culture supernatants were assayed for their ability to support the growth of 5×10^3 IL-2–dependent HT-2 cells in microtiter plates. Cell growth was determined by incorporation of MTT [3-(4,5-demethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] after 20 to 24 hours of culture (*28*). Units of IL-2 were determined by comparison to a standard curve generated with a known quantity of IL-2. The data for each strain represent the mean and SE of determinations from three animals.

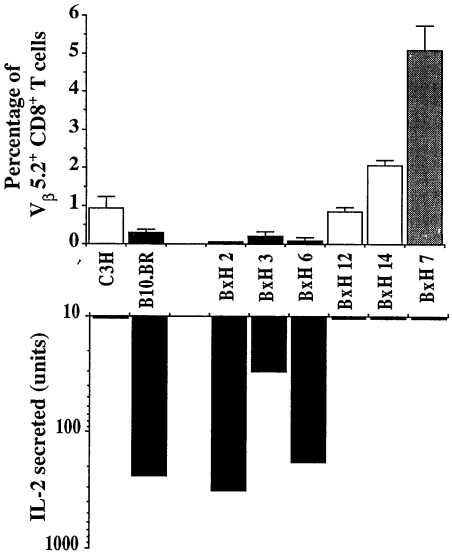

Fig. 4. (**Top**) Frequency of $CD8^+$ T cells expressing $V_\beta5.2$ antigen receptors in C3H, B10.BR, and six I-E^k–expressing B × H RI strains. $V_\beta5.2$ expression was determined as described in Fig. 1. (**Bottom**) Response of the T cell hybridoma 5Q12 to LPS plus IL-4–treated splenocytes from C3H, B10.BR, and six I-E^k–expressing B × H strains. Responses were determined as described in Fig. 3. Data from each strain represent the mean and SE of determinations from three animals.

mediating the strong deletion of $V_\beta5.2^+$ lymphocytes, but not the chromosome 12 gene that encodes the ligand recognized by 5Q12. The existence of two B6-derived genes, either of which can mediate the strong deletion of $V_\beta5.2^+$ cells, is consistent with the observation that approximately three-fourths of the I-E^d–expressing B × D RI strains are strong deletors.

We also analyzed $V_\beta5.2$ expression in I-E^k–bearing B6 × C3H recombinant inbred (B × H RI) mice (*8*). The frequencies of $V_\beta5.2^+CD8^+$ T cells in the parental strains of this RI set were substantially different. Whereas 0.95 ± 0.3% of $CD8^+$ T cells expressed the $V_\beta5.2$ gene in C3H mice (moderate deletion), only 0.29 ± 0.1% of $CD8^+$ T cells expressed $V_\beta5.2$ in B10.BR mice (strong deletion). $V_\beta5.2$ expression in the six I-E^k–bearing B × H strains followed a pattern similar to that observed in B × D strains (Fig. 4, top). Three (of six) B × H strains were strong deletors of $V_\beta5.2^+CD8^+$ T cells, similar to the B10.BR phenotype. Two strains, B×H 12 and B×H 14 were moderate deletors (C3H phenotype) and one strain, B×H 7, was a weak deletor of $V_\beta5.2^+CD8^+$ T cells. The F_1 that resulted from the cross between the B×H 7 and B10.BR strains inherited the B10.BR (strong deletor) phenotype, indicating that the "weak deletor" phenotype (B×H 7) results from the absence of I-E cotolerogens (*7*). Although the number of B × H RI

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

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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-

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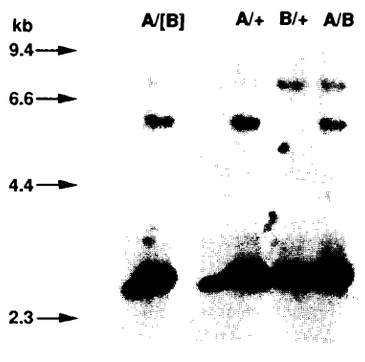

Fig. 1. DNA analysis of transgene transmission from the founder transgenic mouse. The founder (A/[B]) harbored a 2.8-kb human globin gene fragment; when bred to a wild-type mouse, it produced progeny of genotypes A/+ and B/+. Intercrosses between A/+ and B/+ mice produced mice of genotype A/B. Equal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