MOLECULAR AND CELLULAR BIOLOGY, Dec. 1989, p. 5491–5499
0270-7306/89/125491-09$02.00/0
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Vol. 9, No. 12

Lymphoid and Mesenchymal Tumors in Transgenic Mice Expressing the v-*fps* Protein-Tyrosine Kinase

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Received 12 April 1989/Accepted 1 September 1989

***src*, *abl*, and *fps/fes* are prototypes for a family of genes encoding nonreceptor protein-tyrosine kinases. The oncogenic potential of the v-*fps* protein-tyrosine kinase was investigated by introduction of the *gag-fps* coding sequence of Fujinami sarcoma virus into the mouse germ line. Transgenic mice with v-*fps* under the transcriptional control of a 5′ human β-globin promoter (GF) or with both 5′ and 3′ β-globin regulatory sequences (GEF) were viable. Unexpectedly, both GF and GEF transgenes were expressed in a wide variety of tissues and induced a spectrum of benign and malignant tumors. These tumors, which included lymphomas, thymomas, fibrosarcomas, angiosarcomas, hemangiomas, and neurofibrosarcomas, developed with various frequencies after latent periods of 2 to 12 months. The majority of lymphoid neoplasms appeared to be of T-cell origin and were monoclonal, as judged by rearrangements of the T-cell receptor β or immunoglobulin genes. Some tissues that expressed the v-*fps* oncogene, such as heart, brain, lung, and testes, developed no malignant tumors. The v-*fps* protein-tyrosine kinase therefore has a broad but not unrestricted range of oncogenic activity in cells of lymphoid and mesenchymal origin. The incomplete penetrance of the neoplastic phenotype and the monoclonality of lymphoid tumors suggest that tumor formation in v-*fps* mice requires genetic or epigenetic events in addition to expression of the P130$^{gag\text{-}fps}$ protein-tyrosine kinase.**

Nonreceptor protein-tyrosine kinases (PTKs) encoded by genes such as *src*, *abl*, and *fps/fes* are generally associated with the inner surface of the plasma membrane or cytoskeleton but have no transmembrane or extracellular domains. The cellular *fps/fes* gene encodes a 92 to 98-kilodalton cytoplasmic PTK that is specifically expressed in immature and differentiated myeloid cells and, to a lesser extent, in B cells and is therefore implicated in myelopoiesis and lymphopoiesis (8, 22, 25). Oncogenic alleles of *fps/fes* are the most frequently isolated retroviral transforming genes and have been identified in a variety of avian and feline viruses, including Fujinami avian sarcoma virus (FSV) (11, 12, 37). FSV induces the rapid formation of fibromyxosarcomas in infected chickens (21) and also transforms erythroid cells in vivo (17). The FSV oncoprotein is a 130-kilodalton cytoplasmic PTK (P130$^{gag\text{-}fps}$) with N-terminal retroviral *gag* sequences fused to a v-*fps* polypeptide (13, 21, 29, 37).

In addition to their activation as transforming genes of acutely oncogenic retroviruses, structural alterations to cellular genes encoding cytoplasmic PTKs may contribute to the development of some human cancers. In human chronic myelogenous leukemia and acute lymphocytic leukemia, the c-*abl* oncogene is activated as a direct result of a reciprocal translocation between chromosomes 9 and 22 resulting in the juxtaposition of c-*abl* sequences 3′ to a sequence called *bcr* (6, 18, 19, 38). *src* and *fps/fes* have also been implicated in tumorigenesis in humans, although not decisively (5, 40). This circumstantial evidence calls for specific models to test the extent to which such PTKs can contribute to tumor formation.

Transgenic mice have been effectively used to study the mechanisms underlying tumor development. Introduction of dominantly acting oncogenes into the mouse genome has used genes for nuclear oncoproteins such as *myc* and simian virus 40 (SV40) large T antigen or *ras* GTP-binding proteins (1, 14, 20, 30, 31, 39, 42, 43). Tumor induction by these transgenic oncogenes appears to require additional genetic or epigenetic events, although the receptorlike *neu* PTK is reported to induce single-step transformation of mammary epithelial cells (28). *fos* and polyomavirus middle T antigen have also induced specific developmental abnormalities in transgenic mice (33, 48).

Genetic modification of the mouse germ line has the potential to address both the normal functions of cytoplasmic PTKs and their roles in oncogenesis. We have generated transgenic mice that harbor and widely express the v-*fps* PTK. We report here that these mice develop a variety of tumors of lymphoid or mesenchymal origin.

MATERIALS AND METHODS

DNA constructions. A 3.8-kilobase (kb) *Hin*dIII-*Nru*I fragment containing the *gag-fps* coding sequence was obtained from the pIV2 vector (34). The *Nru*I site of this fragment was modified by ligation of a *Bgl*II linker and was then subcloned into pUC18 carrying a *Bgl*II linker at the *Hin*cII site. The adult human β-globin promoter was isolated as a 1.6-kb *Bgl*II-*Nco*I fragment, and the *Nco*I site was removed with S1 nuclease and ligated with a *Hin*dIII linker. This β-globin fragment was then cloned 5′ to the *gag-fps* coding sequence. The poly(A) signal of pGF was obtained from SV40 as a *Bcl*I-*Eco*RI fragment that was cloned 3′ of the *gag-fps* sequence. For pGEF, the poly(A) signal was included in a 2.7-kb *Bam*HI-*Xba*I 3′ fragment of the human β-globin gene. This fragment was subcloned into pUC19 carrying a *Bgl*II linker at the *Hin*cII site. The globin sequence was then excised from the plasmid by using *Bam*HI and *Bgl*II and cloned downstream of the *gag-fps* sequence. pGBF1 was generated by subcloning a 2.75-kb *Bam*HI fragment of

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TABLE 1. Transgenic founders and lineages

Construct	Founder (sex)[a]	Lineage	Copy no.	Age (wk)[b]	Tumor pathology	Other phenotype(s)
GF	I2 (M)	GF2	10–20	12	Lymphoma	Tremor
	L8 (F)	GF3	1–5	36	Lymphoma	None
	R7 (F)	GF6	30–40	54	Fibrosarcoma	None
GEF	A2 (F)	GEF1	10–20	48	None	Tremor, cardiomegaly
	H2 (F)	GEF3	40–50	23	Ganglioneuroma	Tremor, cardiomegaly
	H4 (M)	GEF4	30–40	12	None[c]	Tremor, cardiomegaly
	I1 (F)	GEF6	30–40	10	Neurofibroma	Tremor, cardiomegaly

[a] M, Male; F, female.
[b] Age of founder at death.
[c] Founder was sacrificed for in vitro fertilization to maintain the lineage.

gag-fps into pGEM2. The orientation of the *Bam*HI insert was such that antisense v-*fps* transcripts could be generated with T7 RNA polymerase.

Preparation of DNA for microinjection. Plasmid DNAs were digested with *Bgl*II and *Eco*RI (pGF) or *Bgl*II (pGEF) and electrophoresed through a 0.6% agarose gel. Fragments for injection were excised and electroeluted in TBE (89 mM Tris borate, 89 mM boric acid, 2 mM EDTA). The eluate was extracted once each with phenol, phenol-chloroform (1:1), and chloroform. The DNA was precipitated with ethanol, and the pellet was washed once with 70% ethanol, dried, and suspended in TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA). DNA concentration was determined by ethidium bromide staining of agarose gels containing serial dilutions of samples with known quantities of DNA. All microinjection and oviduct transfer procedures were carried out as described by Hogan et al. (15). All embryos for microinjection were derived from CD-1 mice (Charles River Breeding Laboratories, Inc.).

Identification of transgenic animals. Tail biopsies were obtained from 2.5- to 3-week-old mice, minced, and digested with 100 μg of proteinase K per ml in buffer containing 50 mM Tris hydrochloride (pH 8.0), 150 mM NaCl, 5 mM EDTA, and 1% sodium dodecyl sulfate (SDS) at 55°C overnight. The supernatants were extracted twice with phenol-chloroform (1:1) and once with chloroform. DNAs were spooled with a glass rod, washed twice with 70% ethanol and once with 95% ethanol, dried, and suspended in 0.3 ml of TE buffer. DNA (5 μg) was digested with restriction endonuclease and subjected to electrophoresis in a 0.6% agarose gel. Transgenes were detected by Southern blot analysis (41) with a 2.75-kb *gag-fps Bam*HI fragment of *gag-fps* radiolabeled with [α-^{32}P]dCTP by nick translation (32). All mice used in this study were hemizygous for the transgene.

RNA isolation and protection analysis. Freshly dissected tissues were either used immediately or flash frozen in liquid N_2 and stored at −70°C until use. RNA was prepared as described by Auffray and Rougeon (2), with slight modifications. Initially, tissues were disrupted by using a Polytron (Brinkmann Instruments, Inc.) at full speed for 1 to 2 min in lysis buffer containing 6 M urea, 3 M LiCl, and 0.2% SDS. The homogenates were then sonicated at maximum setting two times for 30 s. The samples were incubated at 0°C for 16 h and centrifuged for 30 min at 3,000 × *g*. The pellets were suspended in buffer containing 10 mM Tris (pH 8.0), 1 mM EDTA, and 0.2% SDS and sequentially extracted with phenol, phenol-chloroform (1:1), and chloroform. RNA was then precipitated with ethanol and suspended in sterile H_2O. RNA concentrations were determined by UV absorption at 260 nm.

The RNA protection assay was performed as described by Melton et al. (26). The template for the RNA protection probe was pGBF1 linearized at a unique *Not*I site within the v-*fps* sequence. Transcription from the T7 promoter generated a 131-nucleotide antisense transcript consisting of 94 nucleotides of v-*fps*-specific sequence and 37 nucleotides of pGEM sequence. Total RNA (5 μg) was hybridized overnight at 50°C to an excess of ^{32}P-labeled RNA probe and then digested with RNases A and T_1. The final products were fractionated in 8 M urea–5% polyacrylamide sequencing gels. The gels were dried on Whatman 3MM paper and exposed to Kodak XAR-5 X-ray film with an intensifying screen overnight at −70°C.

Assays for P130$^{gag\text{-}fps}$ kinase activity. Freshly excised mouse tissues were washed with phosphate-buffered saline, disrupted with a Polytron (Brinkmann) in RIPA buffer (50 mM Tris hydrochloride [pH 7.5], 150 mM NaCl, 0.1% [wt/vol] SDS, 1% [vol/vol] sodium deoxycholate, 1% [vol/vol] Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 100 μg of leupeptin per ml, 100 μM sodium orthovanadate), and centrifuged for 15 min at 3,000 × *g* to remove insoluble debris. The supernatants were then precleared by incubation at 4°C for 1 h in the presence of rabbit anti-mouse immunoglobulin antibody and heat-inactivated, formalin-fixed *Staphylococcus aureus*. *S. aureus* cells were collected by centrifugation, and 10 μl of supernatant was removed for protein determination with the BCA protein assay (Pierce Chemical Co.). An equal amount of protein (5 to 10 mg) was then used for each immunoprecipitation. Samples were divided into two, immunoprecipitated with either R254E anti-p19^{gag} mouse monoclonal antibody or a control mouse monoclonal antibody, and assayed in immune complex kinase reactions containing 50 μCi of [γ-^{32}P]ATP for P130$^{gag\text{-}fps}$ autophosphorylation as described previously (34). The final products were analyzed by 7.5% SDS-polyacrylamide gel electrophoresis. The gels were fixed in 10% methanol–10% acetic acid, rinsed with two changes of H_2O, and incubated in 10 vol of 1 M KOH at 55°C for 1.5 h with gentle shaking to enrich for phosphotyrosine. The gels were washed with two changes of H_2O, stained, and destained before drying. The dried gels were exposed to Kodak XAR-5 X-ray film with an intensifying screen at −70°C.

Analysis of DNA rearrangements. Tumor DNA was digested with the appropriate restriction enzyme and then subjected to Southern blot analysis. To identify the T-cell receptor β-chain (TcRβ) locus, we used two probes. $J_{\beta 1}$ is a 3.2-kb *Hin*dIII-*Bam*HI fragment, and $J_{\beta 2}$ is a 4.5-kb *Hin*dIII-*Bam*HI fragment (23). To analyze immunoglobulin genes, we used an immunoglobulin heavy-chain (IgH) probe, J_H, which corresponds to a 1.9-kb *Bam*HI-*Eco*RI fragment containing the genomic J_H3-4 region and including the heavy-chain enhancer. The immunoglobulin kappa light-chain [IgG(κ)] probe, J_κ, represents a 2.76-kb *Hin*dIII fragment containing J_κ1-5. These probes hybridize to 6.4-kb *Pvu*II ($J_{\beta 1}$), 5.3-kb

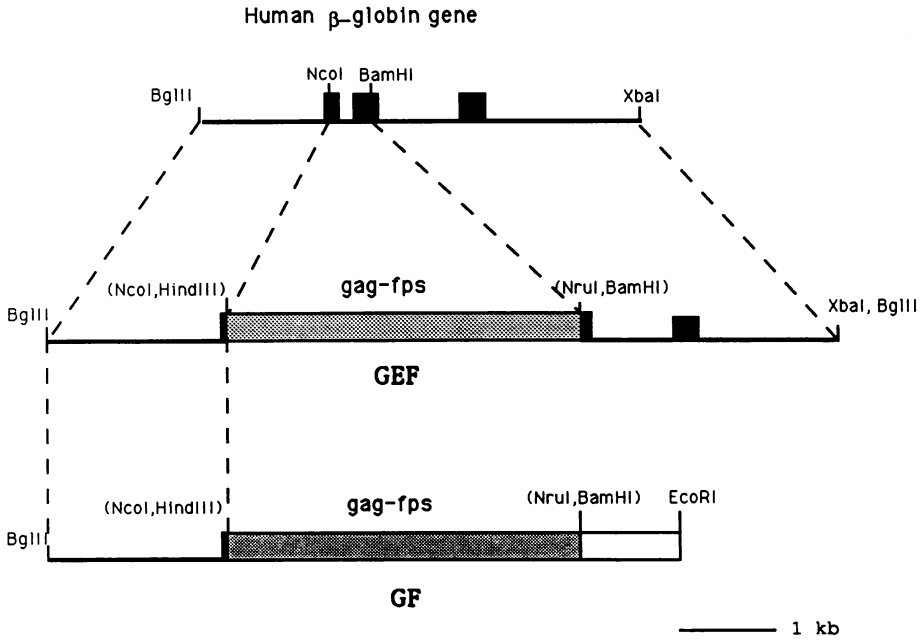


FIG. 1. Structures of the GF and GEF transgenes. Symbols: ——, human β-globin sequences; ■, β-globin exons; ▩, *gag-fps* coding sequences; □, SV40 sequence. Restriction sites within parentheses were lost during the cloning procedure.

*Hin*dIII ($J_{\beta 2}$), 6.7-kb *Eco*RI (J_H), and 4.3- and 2.7-kb *Bgl*II (J_κ) fragments in unrearranged germ line DNA.

Cell lines. Clone 10 is a Rat-2 fibroblast line transformed by FSV that produces $P130^{gag\text{-}fps}$. The FS1 line was derived from a poorly differentiated fibrosarcoma of a GEF1 mouse. The tumor was minced, digested with trypsin, and plated in Dulbecco modified Eagle medium in 10% fetal bovine serum. FS1 is an established cell line that grew out of this culture.

Histopathology. Tissues were fixed in 10% neutral buffered Formalin, embedded in paraffin, sectioned at 7 or 3 μm, and stained with hematoxylin and eosin. Where applicable, specific stains were used to illustrate special features of tumor samples.

RESULTS

v-*fps* transgenic mice. Seven lines of transgenic mice that carry the *gag-fps* coding sequence were generated by DNA microinjection of single-cell embryos (Table 1). The two transgenes (GF and GEF) contain the human β-globin promoter and are distinguished by the presence of a 3′ SV40 polyadenylation signal (GF) or 3′ human β-globin regulatory sequences (GEF) (Fig. 1). Three founder animals with the GF transgene (GF2, GF3, and GF6) and four founders with the GEF transgene (GEF1, GEF3, GEF4, and GEF6) successfully transmitted their respective transgenes to the F1 generation to establish lines.

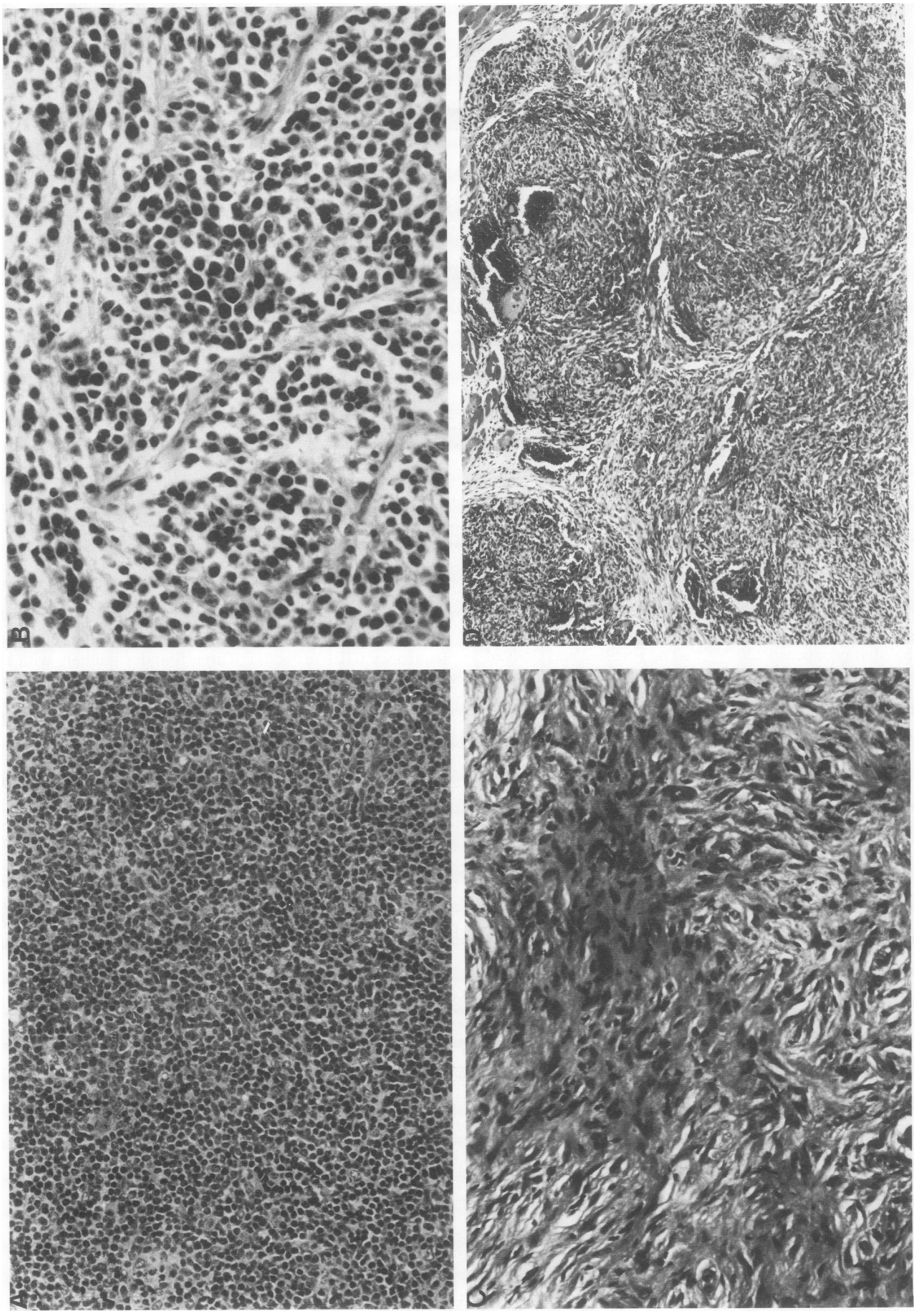
Development of lymphoid tumors by v-*fps* mice. The phenotypes of the transgenic founders are summarized in Table 1. The types and incidence of tumors observed in all mice of the GF and GEF lines are detailed in Table 2.

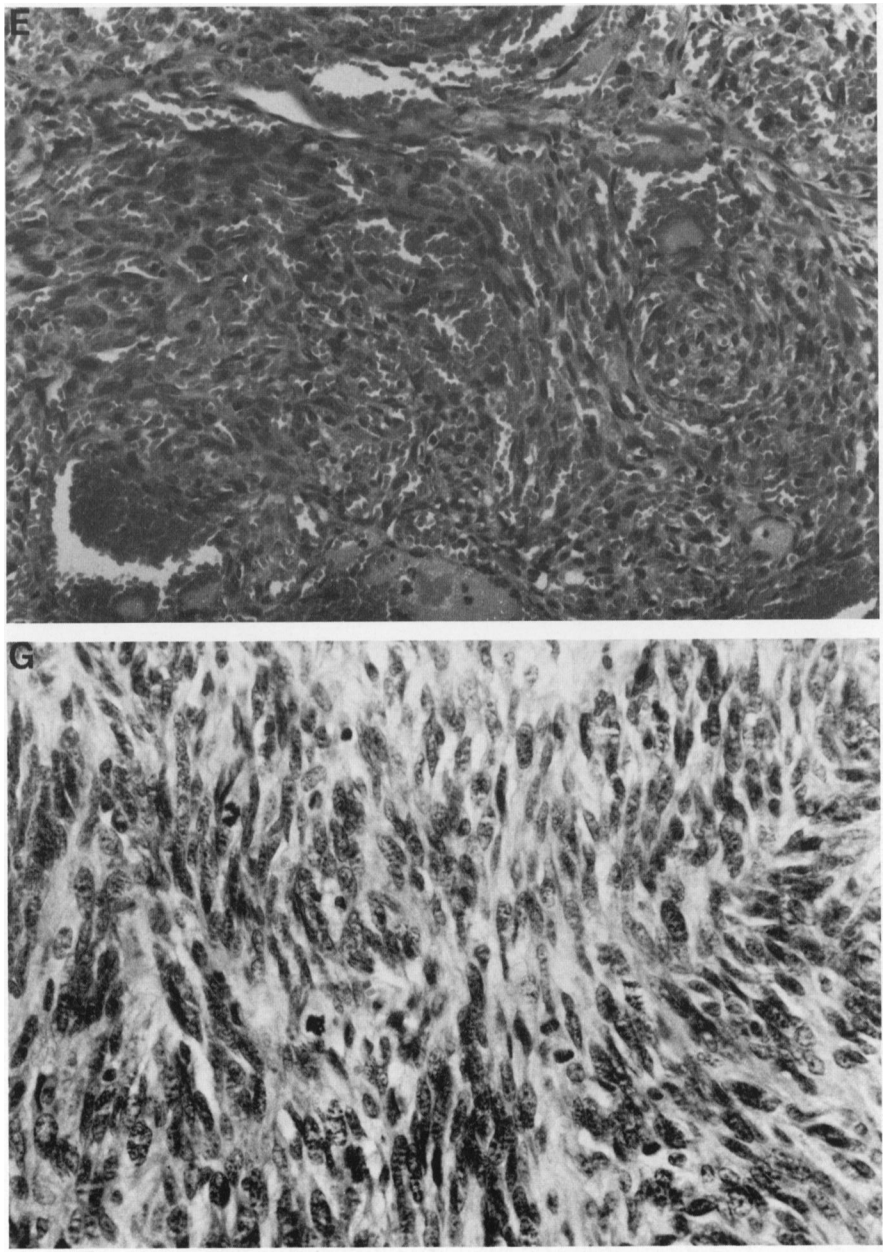
The founder animal of the GF2 line developed palpably enlarged axial and cervical lymph nodes at 12 weeks of age. Histological examination revealed a lymphoma involving multiple lymph nodes. All of the lymphoid tumors observed in the founder and subsequent mice of the GF2 line were well differentiated, destroying the internal architecture of af-

TABLE 2. Neoplasia in GF and GEF transgenic mice

Line	No. of animals examined	Tumor occurrence (avg age [wk] when identified)						Total tumor incidence (%)
		Lymphoma	Thymoma	Fibrosarcoma	Angiosarcoma	Neurofibrosarcoma	Other	
GF2	48	10 (24)		11 (39.6)				43.8
GF3	8	2 (44)	1 (20)				1[a] (51)	50
GF6	32	1 (38)	1 (6)	7 (36.1)	1 (36)			31
GEF1	32	1 (20.5)	4 (19)	2 (42)			1[b] (16)	25
GEF3	29		3 (22.5)	1 (15)	7[c] (32.8)	2[d] (35.3)	1[e] (22.7)	44.8
GEF4	33	4 (15.4)		1 (33)	1 (33)			18.2
GEF6	17	2[f] (27)				2[g] (23)	1[b] (10)	17.6

[a] Mucous cystadenoma (benign tumor of the salivary gland).
[b] Cell origin of the tumor could not be identified because of autolysis.
[c] Two were hemangiomas.
[d] One animal also had an angiosarcoma.
[e] Ganglioneuroma.
[f] One animal also had a neurofibroma.
[g] Benign neurofibromas.





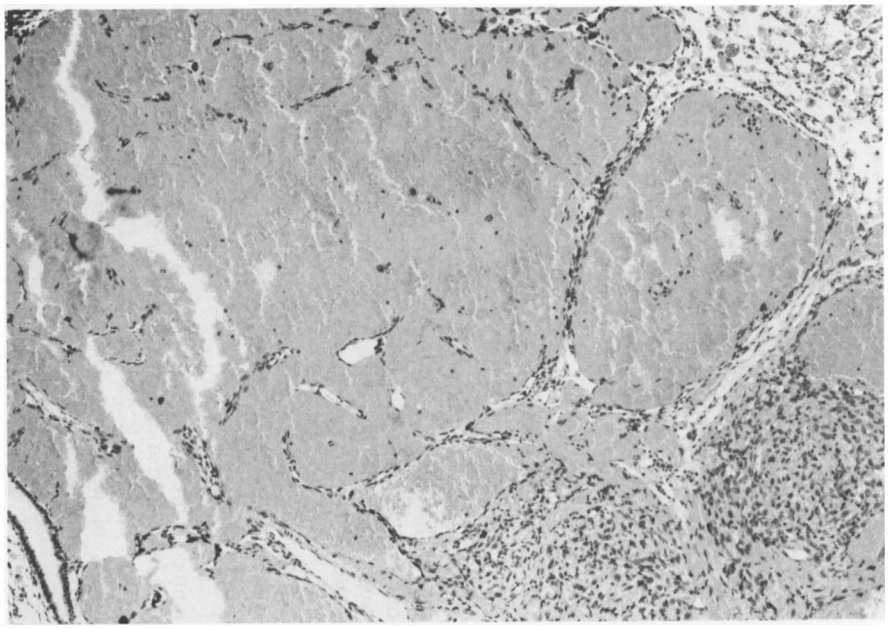


FIG. 2. Histopathology of tumors induced by v-*fps* transgenes. (A) Lymphoma. Axillary lymph nodes from a 12-week-old GF2 mouse were 1 cm in greatest diameter. Histology showed complete effacement of the nodal architecture and replacement with atypical lymphocytes. Magnification, ×210. (B) Thymoma. The thymus gland of an 11-week-old GEF1 mouse was grossly enlarged but remained circumscribed. Histologically, it consisted of large epithelioid cells with occasional aggregates of lymphocytes. Magnification, ×330. (C) Fibrosarcoma. The photomicrograph shows a typical area from a fibrosarcoma that arose in the axillary region of a 31-week-old GF2 mouse. It consists of irregular arrays of atypical hyperchromatic spindle-shaped cells with intercellular collagen. Magnification, ×210. (D) Angiosarcoma. The low-power photomicrograph shows a lobulated tumor mass from a 30-week-old GEF3 mouse consisting of atypical hyperchromatic spindle-shaped cells forming crude vascular channels and infiltrating adjacent muscle. Magnification, ×85. (E) High-power photomicrograph of the same angiosarcoma. Note the cellular pleomorphism. Magnification, ×210. (F) Hemangioma. The lung of a 24-week-old GEF3 mouse contained multiple angiomatous proliferations consisting of erythrocyte-filled sinusoidal spaces lined by endothelial cells. Magnification, ×85. (G) Neurofibrosarcoma. The tumor was associated with the trigeminal nerve overlying the region of the foramen ovale in the middle cranial fossa. This photomicrograph shows that the tumor consisted of a mass of plump, spindle-shaped cells arranged in swirling fasicles. The tumor was not circumscribed and contained infiltrating fat causing resorption of the adjacent calvarium. A moderately large nerve was noted at the periphery of the tumor, and the tumor cells were in continuity with the nerve sheath. Magnification, ×330.

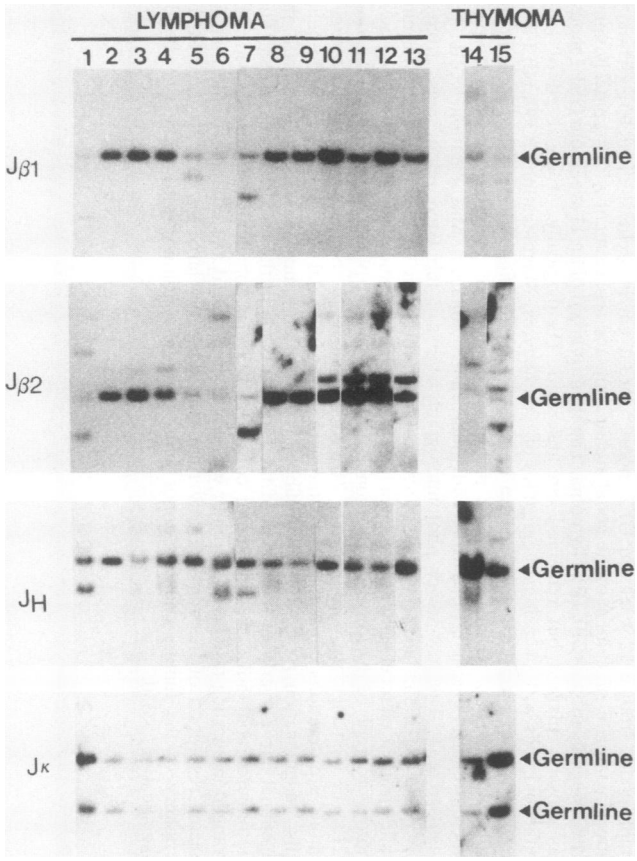


FIG. 3. TcRβ and immunoglobulin gene rearrangements in GF and GEF lymphomas. DNAs from lymphomatous tissues were isolated and analyzed for TcRβ ($J_{\beta 1}$ and $J\beta_2$), IgH (J_H) and IgG(κ) (J_κ) rearrangements by Southern blot hybridization, using probes described in Materials and Methods.

fected lymph nodes without any incidence of extranodal involvement (Fig. 2A). Pathological analysis of the GF3 founder animal, which was moribund at 36 weeks of age, detected an aggressive lymphoma infiltrating major organs such as liver and kidney. Lymphomas were detected in approximately 25% of GF2 and GF3 animals (Table 2) and typically involved multiple lymph nodes. Additionally, six GF2 mice and three animals from other lines subjected to histopathological examination showed a marked lymph node hyperplasia that may represent a premalignant stage. Lymphomas have been identified with various frequencies in all lines except GEF3. Furthermore, thymomas have been detected in animals of four lines (Table 2; Fig. 2B).

To characterize these lymphoid tumors, we analyzed the TcRβ and IgH and IgG(κ) genes in lymphomas from 13 animals and thymomas from 2 animals. Genetic rearrangements normally occur at these loci during the differentiation of T and B cells (16) and can therefore be used as diagnostic markers for T- and B-cell lineages. Specific rearrangements of the TcRβ gene were detected in tumors from 12 mice (10 lymphomas and the 2 thymomas) by Southern analysis (Fig. 3). Of these, three also contained rearrangements within the IgH locus (Fig. 3, lanes 1, 6, and 7). IgH rearrangements are frequently found in murine T-cell lymphomas. No rearrangement of the IgG(κ) gene were detected in any of the mice examined. On the basis of previous comparisons of TcRβ and immunoglobulin gene rearrangements and the histopathology of mouse lymphomas (27), these tumors appear to be derived from cells of the T-cell lineage. Three mice exhibited lymphomas in which the TcRβ and immunoglobulin genes were detected only in the germ line configuration (Fig. 3, lanes 2, 8, and 9). As previously noted by Mucenski et al. (27), such tumors may contain rearranged sequences that are not detectable with the hybridization probes or that comigrate with the germ line restriction fragments. Alternatively, they might be derived from early stem cells or myeloid cells.

The identification of rearranged TcRβ or immunoglobulin alleles at single copy in the majority of tumors indicated that they were monoclonal in origin. Furthermore, when lymphomatous tissue was taken from distinct sites of the same animal, the patterns of gene rearrangement were identical (data not shown). These disseminated lymphomas therefore appear to have arisen from a single cell.

Mesenchymal tumors in v-*fps* mice. Fibrosarcomas were identified in a relatively high proportion of GF2 and GF6 mice as well as in mice from three GEF lines (Table 2). They were primarily located on the ear, body wall, tail, and footpad. The majority of fibrosarcomas were locally aggressive and were composed of well-differentiated cells (Fig. 2C). However, three poorly differentiated fibrosarcomas were more highly invasive and in one instance had metastasized to the kidney.

The most common tumors in GEF3 mice were angiosarcomas, which have also been seen in mice of other lines (Table 2). These malignant endothelial tumors, which were located in the neck, chest cavity, and body wall, consisted of sheets of cells that formed vascular channels lined by hyperchromatic, somewhat pleomorphic spindle-shaped cells (Fig. 2D and E). Two GEF3 mice were found at autopsy to contain benign hemangiomas in the lung (Fig. 2F). Neurofibrosarcomas associated with the trigeminal nerve were identified in two GEF3 mice (Fig. 2F), and benign neurofibromas were seen in two GEF6 mice. In addition to tumors that have been identified several times in animals of different lines, some abnormal growths were observed only once (Table 2).

This broad spectrum of tumorigenesis suggested that expression of the transgenes might be widespread. We therefore investigated the distribution of v-*fps* RNA and $P130^{gag\text{-}fps}$ tyrosine kinase activity in transgenic animals.

v-*fps* RNA expression. Figure 4 shows an RNA protection analysis of v-*fps* RNA from a variety of tissues from a GEF3 mouse. Expression was highest in heart, which contained a level of v-*fps* RNA similar to that of the clone 10 v-*fps*-transformed Rat-2 cell line. v-*fps* RNA was also readily detectable in brain, lymph nodes, thymus, spleen, lung, and testes, with lower levels in other tissues. Qualitatively similar patterns of expression were observed in mice of other GEF and GF lines, with the exception that little v-*fps* RNA was detected in the brains of GF3 and GF6 mice or in the hearts in any of the GF lines (data not shown). Surprisingly, given the presence in both the GF and GEF transgenes of β-globin transcriptional regulatory sequences, little v-*fps* RNA was detected in peripheral blood cells from any transgenic animals examined.

Expression of $P130^{gag\text{-}fps}$ protein-tyrosine kinase in GF and GEF transgenic mice. The pathological changes induced by the GF or GEF transgenes are likely the result of aberrant phosphorylation induced by the v-*fps* PTK. To determine whether the v-*fps* RNA transcripts were translated into enzymatically active $P130^{gag\text{-}fps}$ oncoprotein, we used an immune complex kinase assay. Tissue samples, tumors, or cell lines were lysed and immunoprecipitated with an anti-

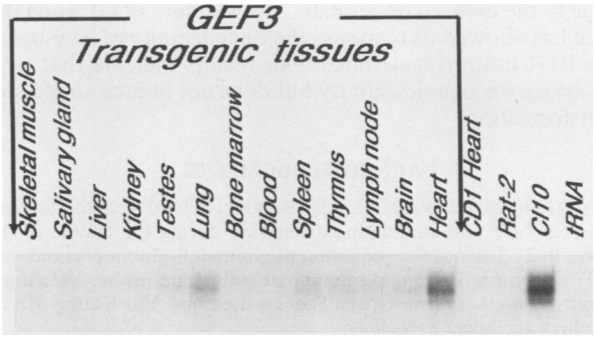


FIG. 4. Expression of v-*fps* RNA in a GEF3 mouse. RNA was isolated from the indicated transgenic tissues of a single 5-month-old GEF3 mouse, from the heart of a nontransgenic sex-matched litter mate (CD1 Heart), from Rat-2 fibroblasts (Rat-2), or from v-*fps*-transformed Rat-2 fibroblasts (Cl10). Yeast tRNA (tRNA) was used as a negative control. RNA was annealed to ^{32}P-labeled v-*fps* antisense RNA probe and then digested with RNases as described in Materials and Methods. The final products were electrophoresed in an 8 M urea–5% polyacrylamide gel. *gag-fps* RNA protects a 94-nucleotide fragment of the antisense RNA probe.

gag monoclonal antibody that recognizes P130$^{gag\text{-}fps}$ or with a control monoclonal antibody. Immune complexes were incubated with [γ-^{32}P]ATP and $MnCl_2$ to allow autophosphorylation of precipitated P130$^{gag\text{-}fps}$. Preliminary results showed that catalytically active P130$^{gag\text{-}fps}$ could be detected in a cell line established from a poorly differentiated fibrosarcoma from a GEF1 mouse (Fig. 5, lane 18), and this analysis was then extended to include different tissues of GF and GEF animals. Figure 5 shows P130$^{gag\text{-}fps}$ kinase activity from a 30-week-old GEF3 mouse. This animal was found on autopsy to contain a tumor that was diagnosed histologically as an angiosarcoma. P130$^{gag\text{-}fps}$ autophosphorylating activity was detectable in brain, thymus, spleen, and testes. Relative to these samples, the P130$^{gag\text{-}fps}$ activity immunoprecipitated from the heart was strikingly elevated. This distribution of kinase activity corresponds to the expression of GEF RNA. The P130$^{gag\text{-}fps}$ kinase activity recovered from the angiosarcoma considerably exceeded that from the heart or any other tissue.

DISCUSSION

GF and GEF transgenic mice hemizygous for the v-*fps* oncogene developed a range of malignant tumors. These included thymomas and lymphomas, apparently of T-cell origin, as well as fibrosarcomas, neurofibrosarcomas, and angiosarcomas. Indeed, some mice had two distinct tumors. The P130$^{gag\text{-}fps}$ cytoplasmic PTK therefore has a broad spectrum of oncogenic activity in hematopoietic and mesenchymal cells when inserted into the mouse germ line. Considerable variation was observed in the latent periods for tumor formation, in the histopathology and malignancy of specific tumors, and in the presence of the oncogenic phenotype (Table 2). Although all tumors analyzed expressed v-*fps* RNA or P130$^{gag\text{-}fps}$ kinase activity, expression of the GF and GEF transgenes could also be detected in organs such as thymus and lymph nodes before tumor formation. Furthermore, premalignant, hyperplastic lymph nodes were frequently observed in GF2 mice, which demonstrated a propensity to develop lymphomas. Malignant lymphoid tumors had specific rearrangements of TcRβ or immunoglobulin genes, indicating that they were monoclonal. These data are consistent with the notion that malignancies in GF and GEF mice require multiple events, of which one (expression of the v-*fps* PTK) is inherited, whereas subsequent somatic changes occur at random. The observation that v-*fps* alone is not sufficient for tumor formation in v-*fps* transgenic mice can be contrasted with the finding that some of these lines develop cardiovascular and neurological abnormalities rap-

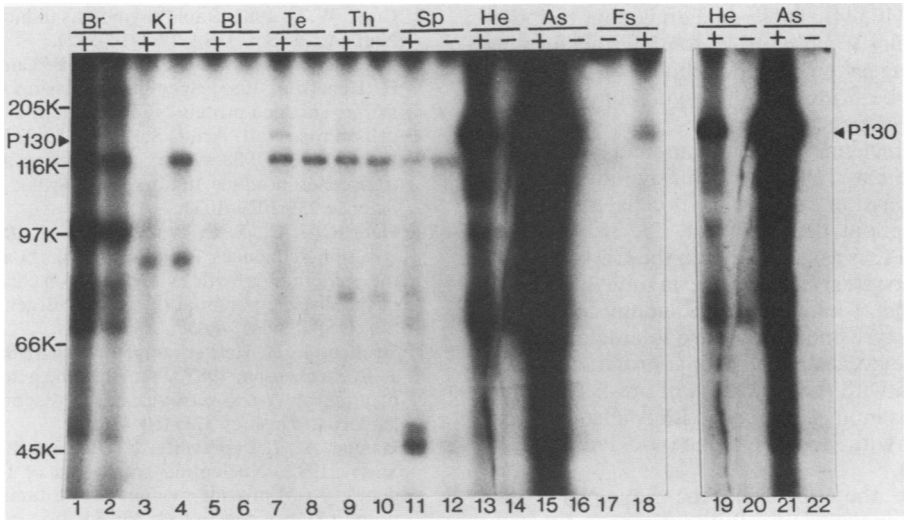


FIG. 5. P130$^{gag\text{-}fps}$ kinase activity in normal and malignant tissues of a GEF3 transgenic mouse. Tissues from a GEF3 mouse were extracted in RIPA buffer, normalized for total protein, and immunoprecipitated with anti-*gag* monoclonal antibody (+) or a control monoclonal antibody (−). Tissues examined were brain (lanes 1 and 2), kidney (lanes 3 and 4), blood (lanes 5 and 6), testes (lanes 7 and 8), thymus (lanes 9 and 10), spleen (lanes 11 and 12), and heart (lanes 13, 14, 19, and 20), as well as an angiosarcoma (lanes 15, 16, 21, and 22). The FS1 fibrosarcoma cell line served as a positive control (lanes 17 and 18). Immunoprecipitates were incubated with [γ-^{32}P]ATP to allow autophosphorylation of P130$^{gag\text{-}fps}$, and the kinase reaction products were separated in a 7.5% SDS-polyacrylamide gel that was alkali treated before exposure to film for 18 h (lanes 1 to 18). Lanes 19 to 22 are shorter (5 h) exposures of lanes 13 to 16 to show the relative activities in the heart and the angiosarcoma. The mobilities of size markers and of P130$^{gag\text{-}fps}$ are indicated. K, Kilodaltons.

idly and with complete penetrance, suggesting that these latter events only require expression of $P130^{gag\text{-}fps}$ (49).

All GEF lines show a cardiac enlargement and cardiomyopathy associated with $P130^{gag\text{-}fps}$ expression in the heart (49). In addition, all GEF lines and GF2 mice display neurological disorders, manifested as trembling and hyperactivity. Although some GEF mice, especially from the GEF3 line, die from congestive heart failure, the incomplete penetrance of the oncogenic phenotype cannot be ascribed to premature death from these cardiac or neurological syndromes. Many mice, other than from the GEF3 lineage, lived at least 18 months with no signs of tumor formation. Furthermore, GF3 and GF6 mice had no obvious physiological abnormalities other than oncogenesis. Although v-*fps* gene products were widely distributed in a variety of tissues, not every organ that expressed the v-*fps* transgene developed tumors. For example, although v-*fps* was expressed to relatively high levels in the hearts of the GEF animals, no cardiac malignancies were observed. In contrast, expression of SV40 large T antigen in the heart induces an unusual hyperplasia of cells in the right atrium (4, 9). Furthermore, v-*fps* transgenes were expressed in organs such as the lung, brain, and testes and to lower levels in other tissues such as salivary gland, liver, and kidney, which developed only occasional benign tumors or were tumor free (Table 2). The transforming potential of v-*fps* is therefore not unrestricted. It will be of particular interest to define the phenotypic properties of different cells that render them susceptible or refractory to v-*fps* transforming activity.

The relatively wide range of tumors induced by v-*fps* is in contrast to polyomavirus middle T antigen, which activates endogenous *src*- or *src*-like kinases and specifically produces benign hemangiomas in transgenic mice or chimeric embryos (3, 48). Interestingly, the $P130^{gag\text{-}fps}$ PTK induced a malignant, invasive proliferation of vascular endothelial cells resulting in the formation of angiosarcomas. Angiosarcomas were particularly evident in GEF3 mice, which also yielded two hemangiomas. Three avian angiosarcoma viruses carry *erbB* oncogenes, whose tyrosine kinases have extensive C-terminal deletions (10, 44). These data argue that endothelial cells are particularly susceptible to the transforming activity of PTKs, suggesting the possibility that tyrosine kinase function may be critical to the regulation of normal vascular growth and angiogenesis.

Similarly, the high incidence of thymomas and lymphomas in GF and GEF mice may reflect an extensive involvement of PTKs in the regulation of normal T cells and in the development of lymphoid neoplasia (24, 35, 36, 46). The $p56^{lck}$ cytoplasmic PTK is associated with the CD4 and CD8 antigens of T lymphocytes and may be involved in T-cell activation (45), and the 21-kilodalton zeta subunit of the TcR is phosphorylated on tyrosine in response to antigenic stimulation (36). The *abl* cytoplasmic PTK oncoprotein has been commonly associated with the formation of pre-B lymphoid tumors and thymomas in mice infected with Abelson murine leukemia virus and with acute lymphocytic leukemia in humans (6, 7, 19, 47).

As described above, the v-*fps* transgenes have pleiotropic effects not limited to oncogenicity. Without care, a substantial number of newborn animals in lines such as GF2 died within a week without obvious pathological abnormality. Despite repeated attempts with all lines, only GEF6 mice, which have the most modest phenotype (see Table 2 for example), have been successfully bred to homozygosity. These results suggest that the widespread expression of activated cytoplasmic PTKs in transgenic mice is potentially toxic to the embryo or neonate. The viability of GF and GEF mice has allowed us to assess the oncogenicity of a cytoplasmic PTK in transgenic mice. Our results indicate that v-*fps* has broad oncogenic activity but does not induce single-step transformation.

ACKNOWLEDGMENTS

We are indebted to Alain Lavigueur and Richard Renlund for their generous help and stimulating discussion during the course of this work, to T. Townes for providing the human β-globin plasmid, and to T. Simon for TcRβ and immunoglobulin gene probes. We thank Karen Hewlett, Laurie Gray, Theresa Lee and Yiu-Keung Ma for excellent technical assistance.

This work was supported by a Terry Fox Programme project grant from the National Cancer Institute of Canada. P.G. is a postdoctoral fellow of the Cancer Research Society, Inc. S.-P.Y. is a postdoctoral fellow, and T.P. and J. R. are Terry Fox Scientists of the National Cancer Institute of Canada.

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