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Recombinant bacteriophages containing the integrated transforming provirus of Gardner–Arnstein feline sarcoma virus

(transforming genes/molecular cloning/DNA transfection/restriction mapping/heteroduplexing)

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ABSTRACT **The integrated DNA provirus of the Gardner–Arnstein (GA) strain of feline sarcoma virus (FeSV) was molecularly cloned in a bacteriophage λ vector. The cloned DNA fragment is 14.4 kilobase pairs long and contains a 6.7-kilobase provirus flanked by cellular sequences derived from nonproductively transformed mink cells. Transfection of mouse NIH/3T3 cells with the cloned DNA fragment induced foci of transformation at efficiencies of 10^4 focus-forming units/pmol of sarcoma virus DNA. Restriction endonuclease mapping and heteroduplex analyses were used to compare the GA-FeSV provirus with that of Snyder–Theilen (ST)-FeSV, a second strain that contains homologous transformation-specific sequences (v-*fes*). Both viruses have the general structure 5′-*gag-fes-env-c region*-3′, each having retained portions of the feline leukemia virus (FeLV) *gag* and *env* genes. In addition to segments shared by the two sarcoma viruses, GA-FeSV contains 1.7 kilobases of extra sequences not found in ST-FeSV. Of these, at least 400–500 base pairs located near the 5′ end of *v-fes* encode a portion of the GA-FeSV polyprotein; the remaining 1.2 kilobases are derived from the FeLV *env* gene but do not appear to encode any detectable product related to the FeLV envelope glycoprotein. The close homology of the v-*fes* sequences shows that GA- and ST-FeSV were formed by recombination of FeLV with similar portions of a cat cellular gene (c-*fes*).**

Three independent isolates of feline sarcoma virus (FeSV), which include the Snyder–Theilen (ST) (1), Gardner–Arnstein (GA) (2), and McDonough (3) strains, produce fibrosarcomas in domestic cats and acutely transform fibroblasts in culture. The RNA genomes of these viruses contain nucleotide sequences derived from feline leukemia virus (FeLV), as well as other sarcoma viral-specific sequences derived from the cellular DNA of normal uninfected cats (4). By analogy to other retroviral systems (5–8), the latter sequences are presumed to be responsible for morphological transformation. The sarcoma viral-specific regions of ST- and GA-FeSV (v-fes^{ST} and v-fes^{GA}, respectively) share homologous nucleotide sequences unrelated to those in McDonough-FeSV (4). The ST- and GA-FeSV strains encode gag-*x* polyproteins of M_r 85,000 and 95,000, respectively, that contain the NH_2-terminal segment of the FeLV *gag* gene precursor linked to a sarcoma viral-specific polypeptide ("*x*") (9, 10). The *x* portions of the two polyproteins are antigenically related (9–12) and exhibit a cyclic AMP-dependent protein kinase activity capable of phosphorylating tyrosine residues (11, 12). Thus, it appears that these two FeSV strains arose by recombination between FeLV sequences and the same cat cellular gene (c-*fes*).

Although v-fes^{ST} sequences are only ≈1.5 kilobase pairs (kb) long, (13, 14) the cat c-*fes* gene is at least 4.5 kb long and contains intervening sequences that are not represented in ST-FeSV proviral DNA (15). The formation of the ST-FeSV genome must, therefore, have involved the retention of only certain c-*fes* coding regions critical for transformation. Differences between ST- and GA-FeSV genomes could therefore reside in sequences derived from c-*fes*, FeLV, or both. To compare the structures of these FeSV strains, GA-FeSV proviral DNA was molecularly cloned in a bacteriophage λ vector and compared with previously cloned ST-FeSV DNA (14, 16) by heteroduplex and restriction enzyme analyses. The data show that ST- and GA-FeSV contain similar v-*fes* regions and that other differences in the structures of the two proviruses reside in helper viral-derived sequences.

MATERIALS AND METHODS

Purification of Cellular DNA Fragments. Mink F_1 clone 10 cells nonproductively transformed by GA-FeSV (17, 18) were obtained from George Todaro of the National Cancer Institute. The extracted cellular DNA (13) was digested with *Eco*RI and the digest was extracted with phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol), and concentrated under ethanol. The DNA fragments (2 mg) were electrophoretically separated in 0.8% agarose (19), and fragments of 14-kb mean length were electroeluted from the gels (20). The DNA was further fractionated by velocity sedimentation in a 15–30% neutral sucrose gradient (17 hr at 38,000 rpm in an SW 41 rotor), and aliquots containing GA-FeSV sequences were identified by the Southern blotting technique (19). The contents of a single gradient tube were taken for recombinant DNA procedures.

Molecular Cloning of GA-FeSV. Purified cellular DNA fragments (0.3 μg) were ligated in a 20-μl reaction mixture (3 hr at 15°C) to 1.5 μg of purified vector arms from λgtWES.λB (21). An aliquot containing 0.5 μg of DNA was packaged *in vitro* into phage particles (22), yielding 6×10^5 phage plaque-forming units. Recombinant phages (1.5×10^5 plaque-forming units) were plated on *Escherichia coli* LE392 cells, and two phages containing v-*fes* sequences were identified by plaque blotting; the two hybrid phages (designated λGA-FeSV, clones 1 and 2) were amplified, and phage DNA was purified (23).

Hybridization Reagents and Conditions. The v-*fes* sequences of ST-FeSV proviral DNA include several cleavage sites for *Pst* I (14, 15). Two *Pst* I fragments, each ≈0.5 kb long, contain the majority of v-fes^{ST} sequences (see Fig. 1, line 3). These fragments were purified by electroelution from a *Pst* I digest of molecularly cloned ST-FeSV DNA and separately subcloned into the ampicillin-resistance gene of the plasmid vector pBR322 (24). The recombinant plasmids were identified by colony-hybridization techniques and grown in an *E. coli* host, and

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Abbreviations: FeSV, feline sarcoma virus; ST and GA, Snyder–Theilen and Gardner–Arnstein strains of FeSV, respectively; FeLV, feline leukemia virus; kb, kilobase pair(s); LTR, long terminally redundant.

their DNA was extracted and purified (15, 24). The DNAs of plasmids containing the two different *Pst* I inserts were mixed in a 1:1 molar ratio, labeled with ^{32}P by nick translation (specific activity, 1×10^8 dpm/μg; 1 Bq = 60 dpm) (25), and used for Southern or plaque-blotting analyses.

Infectious DNA Assay. The biological activity of λGA-FeSV DNA was assayed by transfection using mouse NIH/3T3 cells and the calcium precipitation method of Graham and van der Eb (26) as described (23). The phage DNA was cleaved with both *Eco*RI and *Bam*HI before analysis and mixed with carrier DNA at 12 μg/ml before coprecipitation. Recipient cultures were trypsinized and replated 1 day after infection, and foci were scored in confluent cultures after 14–19 days. Randomly selected foci were subcultured and tested for the presence of rescuable FeSV genomes and for FeSV-coded polyprotein by described methods (27). Cloned Moloney murine sarcoma virus DNA (λ ml-MSV) (23) generously provided by George Vande Woude (National Cancer Institute) was used as a positive control.

Heteroduplex Analyses. DNA was mounted for microscopy by using the formamide technique essentially as described by Davis *et al.* (28). Grids were examined in a Siemens Elmeskop 101 electron microscope at 40 kV accelerating voltage. Electron micrographs were taken on Kodak electron image plates at ×6000. The magnification was calibrated for each set of plates with a grating replica (E. F. Fuller, cat. no. 1000), and contour lengths were measured with a Numonics graphics calculator interphased to a Wang 2200 computer.

RESULTS

Cloning of a Transforming GA-FeSV Provirus. The integrated DNA provirus of GA-FeSV lacks cleavage sites for the restriction endonuclease *Eco*RI (see below). After digestion of DNA from FeSV-transformed cells with this enzyme, the viral DNA-containing fragments are longer than the provirus itself and their lengths are determined by sites of *Eco*RI cleavage in cellular DNA. By using the Southern blotting procedure, the DNA of nonproductively transformed mink F_1 clone 10 cells was found to contain a single proviral DNA-containing fragment of ≈14 kb. Digested DNA enriched for these fragments was recombined into a bacteriophage λ vector, and two hybrid phages (λGA-FeSV, clones 1 and 2) containing proviral DNA sequences were isolated.

DNA preparations from both recombinants were assayed for focus-inducing activity by transfection on NIH/3T3 cells (Table 1). The DNA of both clones induced foci of transformation at an efficiency similar to that obtained with molecularly cloned Moloney murine sarcoma virus DNA. The number of foci was a linear function of DNA dose, and the efficiency of focus induction was unaltered after cleavage of λ-GA-FeSV with both *Eco*RI and *Bam*HI, two restriction enzymes that do not recognize cleavage sites in GA-FeSV proviral sequences (see below). Infection of transformed NIH/3T3 cells with Moloney murine leukemia virus led to the rescue of viral genomes that induced foci in subsequent rounds of infection. The transformed NIH/3T3 subclones were also found to synthesize the 95,000-dalton polyprotein encoded by GA-FeSV (data not shown). Thus, both λ phage recombinants were biologically active and contained FeSV proviral DNA sequences.

Table 1. Infectivity of λ GA-FeSV DNA

DNA dose, ng per plate	Restriction enzyme cleavage	Foci, no.	Efficiency of transformation*
320	No	121	4.0
32	No	9	3.9
250	Yes	54	3.8
125	Yes	28	3.8
25	Yes	8	4.0

Phage DNA was digested with both *Eco*RI and *Bam*HI as indicated prior to transfection. The digested DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1), concentrated under ethanol, and suspended in buffer at the appropriate concentration before admixture with carrier DNA. The transformation efficiency obtained for cloned λ m1-MSV in the same experiments was 4.2. M_r of the GA-FeSV provirus was taken as (6.8 kb × 622/base pair equal to) 4.2×10^6.
* Expressed as log (focus-forming units/pmol).

Restriction Mapping of the Cloned DNA Fragment. A restriction map of the 14.4 kb *Eco*RI fragment in λ GA-FeSV clone 1 is shown in Fig. 1. Sites of cleavage for different enzymes were positioned with respect to one another and in relation to known cleavage sites in the λ vector arms. The endonucleases *Xba* I, *Bam*HI, *Bgl* II, and *Sal* I recognize single cleavage sites in the cloned DNA sequence. More complex patterns of digestion for other enzymes were deduced from studies of electroeluted restriction fragments; in certain cases, these were subcloned in the plasmid pBR322 for verification of critical map assignments in a different vector. From these studies, it was concluded that all mapped cleavage sites in the inserted sequences of λ-GA-FeSV clones 1 and 2 are identical and that the cloned fragment is oriented in opposite directions with respect to the vector arms in the two clones. The phages must therefore have originated by independent recombinational events between the vector and a single cellular sequence.

Comparison of the cleavage patterns obtained from λ GA-FeSV clone 1 with that of previously cloned ST-FeSV DNA (14)

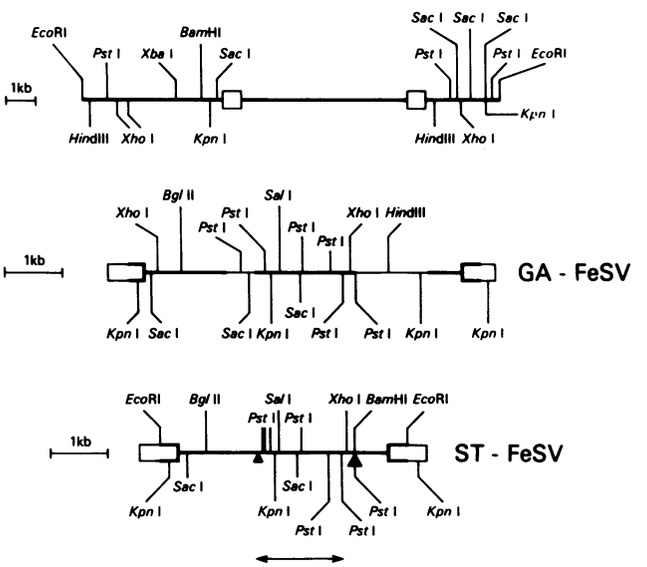

FIG. 1. Physical maps of GA-FeSV and ST-FeSV genomes. The top line shows the cloned 14.4-kb *Eco*RI fragment containing the GA-FeSV provirus. The position of proviral DNA is indicated by the boxes at each terminus, which depict the LTR segments. Sites of restriction endonuclease cleavage in mink cellular sequences flanking the provirus are indicated. In λ GA-FeSV clone 1 (as shown), the virus is in 5′ to 3′ orientation both with respect to the left and right arms of λ and in relation to the canonical order of genes in viral RNA. The two lower line drawings are maps of GA- and ST-FeSV DNA proviruses, respectively. Boxes indicate LTR sequences. Heavy solid lines designate regions of homology between the two sarcoma viral DNAs, as determined by heteroduplex analyses. The lengths of the various segments (drawn to scale) are indicated by bar standards at the left. Additional sequences in GA-FeSV are represented by the thin horizontal lines in line 2 and by the triangles in line 3. The horizontal bar at the bottom of the figure defines the approximate position and complexity of homologous sarcoma viral-specific sequences in the two genomes.

suggested that GA-FeSV proviral DNA sequences were localized near the center of the 14.4-kb fragment in 5′ to 3′ orientation with respect to the left and right vector arms (Fig. 1). This predicted that the single site of *Sal* I cleavage corresponded to a restriction site within the v-*fes* sequences. Labeled recombinant plasmids containing v-*fes*ST sequences were therefore hybridized to electroeluted restriction fragments representing different portions of the cloned DNA insert (see schematic, Fig. 2). These included (from 5′ to 3′) (*i*) the left-hand 4.1-kb *Eco*RI/*Bam*HI fragment, presumed to contain only mink cellular DNA; (*ii*) a 4.2-kb *Bam*HI/*Sal* I fragment containing the 5′ half of the FeSV provirus; and (*iii*) a 7.1-kb *Sal* I fragment containing the remaining proviral DNA, 2.6 kb of mink cellular sequences, and ≈1 kb of the right vector arm. Because the 7.1-kb fragment was purified after sequential digestion of λ GA-FeSV clone 1 with *Bam*HI and *Sal* I, these preparations included two partial digestion products (7.6 and 8.9 kb) containing additional right-vector-arm sequences (Fig. 2). Of the three major fragments representing different portions of the cloned DNA (lanes 1, 2, and 4, respectively), two contained sequences homologous to the v-*fes*ST probe. The homologous sequences were further localized to the right of the *Bgl* II site in the 1.75-kb *Bgl* II/*Sal* I fragment (lanes 3) and in the adjacent 1.85-kb *Sal* I/*Hin*dIII fragment (lanes 5). These studies chemically verified the nature of the cloned DNA sequences and localized the v-*fes*ST-related sequences in a 3.6-kb region flanking the *Sal* I site.

Full-length integrated proviruses are characterized by long terminally redundant (LTR) sequences that flank the viral structural genes (29, 30). In recombinant phages, crossing over between the LTR regions can result in excision of intervening proviral sequences and retention of a single LTR region flanked by host cellular information. The deleted progeny phages contain DNA fragments that are shorter than parental molecules by a value corresponding to the complexity of the viral RNA genome (31). Hybridization of λ GA-FeSV plaques with the v-*fes*ST probe showed that 10–20% of the phages in plaque-purified amplified lysates had segregated proviral sequences. By selecting five such phages, we obtained deleted recombinant clones all containing 8.3-kb inserts. Fig. 3 shows the distribution of the small *Kpn* I fragments generated from λ GA-FeSV clone 2 (lane 2) and a deleted derivative (lane 1). The deleted phage lacked three contiguous parental *Kpn* I fragments (designated B, D, and F) measuring 6.1–6.2 kb in aggregate length. DNA from parental phage (lane 5), from a mixture of phages arising during passage of the parental stock (lane 4), and from a plaque-purified deleted derivative (lane 3) were analyzed after *Xho* I cleavage. The deleted phage lacked the contiguous *Xho* I bands designated A, B, and C (aggregate length 11.4 kb) but contained a 5.2-kb fusion fragment (designated a + b in lane 3). The latter band was also detected at various intensities with DNA from chronically passaged phage stocks containing mixtures of parental and segregant genomes (lane 4). These results confirmed that the complexity of the deleted proviral DNA sequence was 6.1–6.2 kb, showed that *Kpn* I cleaves at sites within the LTR regions while *Xho* I does not, and localized proviral sequences within the parental 14.4-kb insert as shown in Fig. 1. Given that the LTR sequences of FeLV are estimated to be 0.5–0.7 kb long, (13, 32), we conclude that the length of the integrated GA-FeSV provirus is 6.7–6.8 kb.

Comparison of GA-FeSV with ST-FeSV and FeLV by Heteroduplex Analyses. The lengths and positions of various segments in the cloned 14.4-kb fragment were determined from heteroduplexes formed between λ GA-FeSV and previously cloned ST-FeSV and FeLV phage recombinants (14). The cloned ST-FeSV and ST-FeLV DNA fragments are 4.3 kb and 7.8 kb long, respectively, and lack sequences distal to the two *Eco*RI sites in the LTR regions (See Fig. 1, line 3). Hybridizations were performed by using phage DNA containing proviral

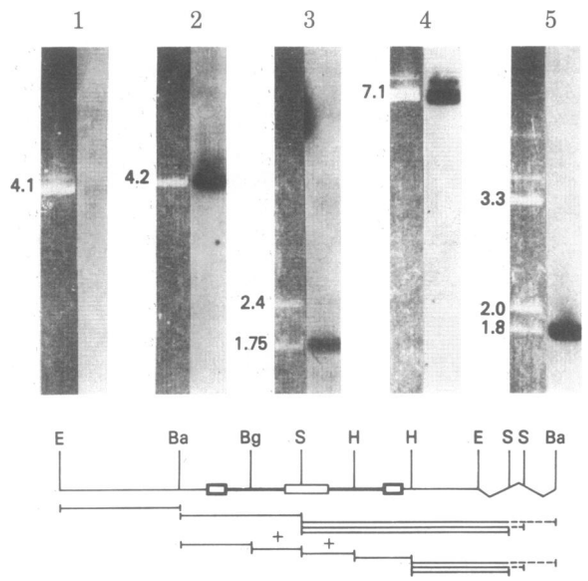

FIG. 2. Localization of sequences homologous to v-*fes*ST in λ GA-FeSV clone 1. Purified restriction fragments representing different portions of the cloned DNA were resolved on gels (left lanes); numbers on left indicate their lengths in kb. Fragments containing sequences hybridizing with the v-*fes* probe are indicated by autoradiograms (right lanes). The schematic below the gel composite indicates the positions of the various fragments within the insert. Sites of restriction: E, *Eco*RI; Ba, *Bam*HI; Bg, *Bgl* II; S, *Sal* I; H, *Hin*dIII. Dashed lines at the right of the schematic indicate the sizes of partially restricted *Sal* I/*Bam*HI and *Sal* I/*Sal* I fragments that contaminated the major 7.1-kb *Sal* I fragment. Lanes: 1, 2, and 4, results with the 4.1-*Eco*RI/*Bam*HI fragment, the 4.2-kb *Bam*HI/*Sal* I fragment, and the 7.1-kb *Sal* I/*Sal* I fragment, respectively; lanes 3 and 5, results after cleavage of the 4.2-kb fragment with *Bgl* II and after cleavage of the 7.1-kb fragment with *Hin*dIII.

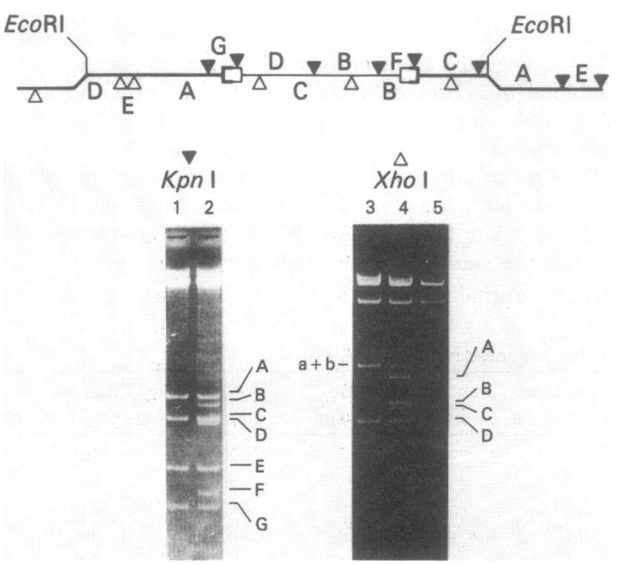

FIG. 3. Restriction fragments from λ GA-FeSV clone 2 and deleted progeny phages. Cleavage of λ recombinant DNAs was performed with either *Kpn* I or *Xho* I as indicated. Lanes: 2 and 5, results with parental phage; 4, results with a mixed population of parental phage and deleted segregants arising in a chronically passaged stock; 1 and 3, results with plaque-purified variants. The positions of the fragments (designated by letters) are noted on the schematic at the top. Sites of restriction: ▼, *Kpn* I; △, *Xho* I.

sequences in 5′ to 3′ orientation, both with respect to viral RNA and to the vector arms. Representative structures are shown in Fig. 4, and the lengths of the various segments are given in its legend.

When λ ST-FeSV was hybridized with λ GA-FeSV clone 1, the combined lengths of all the duplexed segments (designated b, d, and f in Fig. 4*A*) equaled the length of cloned ST-FeSV DNA. Thus, all of the ST-FeSV sequences were homologous to sequences in the heterologous GA-FeSV recombinant. Deletion loops corresponding to additional unpaired sequences in λ GA-FeSV DNA delineated each of the three proviral homology regions. The two largest loops (segments g and a) were 5.1 and 2.7 kb long, respectively; these included all the mink cellular sequences flanking the GA-FeSV provirus, as well as 0.2–0.4 kb from each proviral LTR region that was unable to base pair with ST-FeSV DNA. A stem loop in the 5.1-kb single-stranded region (segment g) indicated the presence of base-paired, inverted repeat sequences in the mink cellular sequences at the 5′ end of the provirus. Two smaller deletion loops, measuring 1.2 and 0.5 kb (designated c and e, respectively), were localized within GA-FeSV proviral DNA sequences. These segments correspond to "extra" sequences in the GA-FeSV provirus that lack homology to sequences in ST-FeSV; their positions are indicated in Fig. 1 by the thin linear segments (line 2) and by triangles (line 3).

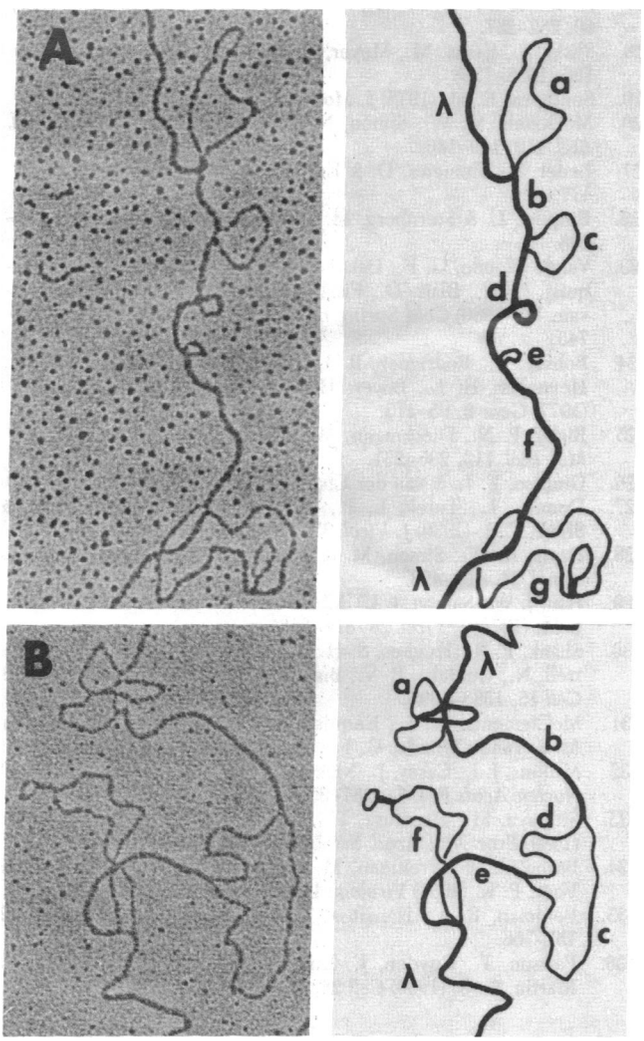

FIG. 4. Electron micrographs of representative heteroduplex molecules formed between GA-FeSV and ST-FeSV (*A*) or ST-FeLV (*B*). Heteroduplexes were formed by mixing equal quantitites (5–10 μg) of each DNA, denaturing with alkali, and renaturing for 4 hr at 25°C in 50% formamide/0.1 M Tris base, pH 8.5/1 mM EDTA. Contour lengths of the hybridized and nonhybridized regions in kb (mean ± SD) are (*A*; 16 molecules) a, 2.7 + 0.3; b, 0.9 + 0.3; c, 1.3 + 0.3; d, 1.8 + 0.1; e, 0.5 + 0.1; f, 1.7 + 0.2; and g, 5.1 + 0.5; (*B*; 15 molecules) a, 2.9 + 0.5; b, 2.4 + 0.3; c, 3.3 + 0.5; d, 1.7 + 0.2; e, 2.1 + 0.2; and f, 4.9 + 0.3.

To determine whether the extra nonhomologous sequences of GA-FeSV were derived from FeLV, heteroduplexes formed between λ GA-FeSV and a cloned helper provirus were examined (Fig. 4*B*). The large deletion loop (segment f) corresponding to mink host-cell sequences in λ GA-FeSV again demarcated the 5′ end of the proviral DNA. An adjacent 2.1-kb base-paired region (segment e) contained the sequences represented by the 5′ 1.7-kb homology region detected in heteroduplexes between the two sarcoma viruses and appeared to include at least some of the additional sequences represented in the contiguous 0.5-kb deletion loop (Fig. 4*A*, segments f plus e, respectively). This duplexed segment was followed by a region of nonhomology containing 3.3 kb of FeLV DNA (segment c) and 1.7 kb of GA-FeSV sequences (segment d). The latter single-stranded region, representing sequences unique to the sarcoma virus, defines the complexity of the v-*fes*^{GA} gene. At its 3′ end, another 2.4-kb homology region (segment b) was seen that terminated at the 2.9-kb deletion loop (segment a) marking the end of proviral DNA sequences.

DISCUSSION

The GA- and ST-FeSV strains are presumed to have arisen by independent recombination events involving FeLV sequences and a cat cellular *onc* gene (now designated c-*fes*) (4, 13, 15). Because c-*fes* contains intervening sequences not found in its v-*fes*ST counterpart (15), the two FeSV strains must have each retained portions of the cellular gene necessary for transformation. Comparison of the cloned GA-FeSV provirus with ST-FeSV DNA shows that the v-*fes* sequences of both sarcoma viruses contain similar sites of restriction endonuclease cleavage and exhibit uninterrupted homology over at least 1.3 kb in heteroduplexes formed between the different recombinant DNAs. GA-FeSV contains ≈1.7 kb of additional genetic information that has been localized to two discontiguous regions in the provirus. One segment of 0.4–0.5 kb is located near the 5′ end of the v-*fes*^{GA} gene. Based on the results of heteroduplex analyses, this segment could be derived entirely from *gag* sequences at the 5′ end of the helper virus. However, two sites of cleavage for *Pst* I and *Sac* I assigned to this region do not correspond to sites mapped within the *gag* sequences of two previously cloned FeLV strains (14, 32) or to sites detected in the segmented cat c-*fes* gene (15). Given the precision of these measurements, the short segment directly preceding the v-*fes* homology region could therefore correspond to (*i*) additional sequences derived from cat cellular DNA, (*ii*) a discontiguous "upstream" portion of an FeLV genome, or (*iii*) sequences derived from an FeLV strain other than the two subgroup B viruses analyzed to date.

The additional sequences near the 5′ end of v-*fes*^{GA} must encode a portion of the GA-FeSV polyprotein. Although the gag-*x* polyproteins of ST- and GA-FeSV contain p15, p12, and some p30 antigenic determinants, as well as immunologically and functionally related *x*-portions (9–12), the two polyproteins differ by 10,000–15,000 daltons (9, 10). This represents 100–150 amino acids or an additional coding capacity of 0.3–0.45 kb for the GA-FeSV genome. These differences in the structures of the ST- and GA-FeSV proviruses do not affect the polyprotein-associated kinase activity attributed to the v-*fes* coding region (11, 12) and do not appear essential for transformation.

Although sequences near the 5′ end of the v-*fes* gene are different in the two FeSV strains, the helper virus-derived se-

quences adjacent to the 3′ end of v-*fes* appear to be homologous. For ST-FeSV, the 3′ end of v-*fes* is joined to a sequence derived from a region close to the 5′ end of the FeLV *env* gene (14, 16). This FeLV-derived segment includes sites of cleavage for *Pst* I, *Xho* I, and *Bam*HI; nucleotide sequence analysis shows that the 3′ end of v-fes^{ST} is ≈100 base pairs to the left of the *Xho* I site (unpublished data). Several homologous sites of restriction were identified in GA-FeSV proviral DNA and are contiguous with 1.2 kb of additional *env*-derived sequences found in GA- but not ST-FeSV. The *env*-derived sequences have not been shown to encode any detectable product antigenically related to the envelope glycoprotein of FeLV (9–12).

The homologous FeLV-derived segments of ST- and GA-FeSV exhibit some differences in sites of restriction endonuclease cleavage. This is consistent with the hypothesis that these viruses represent independent genetic recombinants between different FeLV strains and the same cat cellular gene. The fact that both strains have acquired similar subsets of c-*fes* sequences that have each been joined at the 3′ end to homologous helper viral-derived segments shows that the mechanisms for formation of these viruses could be quite precise. The latter FeLV-derived segment is also partially homologous to sequences that flank the *onc* gene of Moloney MSV (designated v-*mos*) at its 5′ end (14). Thus, certain sequences derived from the helper virus *env* region (or the *pol-env* junction) may be necessary for recombination with cellular *onc* elements or confer collaborative functions necessary for transformation. As the cloned GA-FeSV provirus is biologically active and transforms cultured indicator cells at high efficiency, it is now possible to test the requirements for different FeLV-derived sequences in the FeSV transformation process. Such studies should help to define the mechanisms that activate cellular transforming genes and formally define the viral genetic elements necessary for transformation.

The *onc* sequences of two avian sarcoma viruses, the Fujinami and PRC II strains, have recently been reported to be homologous to sequences in GA- and ST-FeSV (33). Indeed, all of these viruses encode antigenically related polyproteins (34) that have associated tyrosine-specific protein kinase activities (11, 12, 35, 36). If these avian viruses contain sequences homologous to v-*fes*, the number of transforming genes transduced by different replicating retroviruses may, in fact, be few and their modes of action may be similar.

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