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Reversal of *Fv-1* Host Range by In Vitro Restriction Endonuclease Fragment Exchange Between Molecular Clones of N-Tropic and B-Tropic Murine Leukemia Virus Genomes

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We molecularly cloned unintegrated viral DNA of the BALB/c endogenous N-tropic and B-tropic murine leukemia retroviruses and in vitro passaged N-tropic Gross (passage A) murine leukemia retroviruses. Recombinant genomes were constructed in vitro by exchanging homologous restriction enzyme fragments from N- or B-tropic parents and subsequent recloning. Infectious virus was recovered after transfection of these recombinant genomes into NIH-3T3 cells and cocultivation with the *Fv-1* nonrestrictive SC-1 cells. XC plaque assays of recombinant virus progeny on *Fv-1*^{n} and *Fv-1*^{b} cells indicated that the *Fv-1* host range was determined by sequences located between the *Bam*HI site in the p30 region of the *gag* gene (1.6 kilobase pairs from the left end of the map) and the *Hin*dIII site located in the *pol* gene (2.9 kilobase pairs from the left end of the map).

The biological properties and molecular mechanism of murine leukemia virus (MuLV) host range restriction governed by the mouse *Fv-1* gene have been studied in many laboratories in recent years (reviewed in reference 18). There are two clearly defined alleles, *Fv-1*^{n} and *Fv-1*^{b}, which dominantly restrict B-tropic and N-tropic MuLVs, respectively (24, 26). Another allele, *Fv-1*^{nr}, has been described that is distinct from *Fv-1*^{n} in that in addition to restricting all B-tropic MuLVs, this allele also restricts some N-tropic MuLVs (Rowe and Hartley, cited in references 22 and 30). *Fv-1* restriction is not absolute; it is usually observed in cell culture as a 100- to 1,000-fold reduction in infectivity relative to permissive cells (15), and it is characterized by a two-hit infectivity curve (9, 25, 32). The two-hit phenomenon is best explained as an abrogation of the *Fv-1* restriction by the initial restricted virus (10). It has been proposed that the incoming virion RNA serves as mRNA to produce an excess of the *Fv-1* target, thus titrating out the available *Fv-1* gene product and allowing subsequent virus to establish a productive infection (10).

It is now clear that a major consequence of the *Fv-1* restriction is a failure of the viral DNA to integrate into the host genome (19, 31). This may be due to a block in the formation of covalently closed supercoiled DNA (form I), the presumed precursor to integration (20, 33). In some cases, the earlier linear DNA (form III) synthesis is restricted, suggesting a polymorphism in *Fv-1* action (33). Integration, rather than circularization alone, has also been suggested as the event which is blocked in *Fv-1* restrictive cells (8).

The virion determinant which is responsible for *Fv-1* host range can be phenotypically acquired and contributes a dominant sensitivity to the *Fv-1* gene (21, 23, 27). A likely candidate for the virion target is the major core protein p30. Although p30 is highly conserved among MuLV isolates, subtle differences in this protein have been observed that correlate with *Fv-1* host range (7, 13, 16, 29). Specific p30 tryptic peptides are known to be associated with B-tropic to NB-tropic conversion (16) and to segregate with the *Fv-1* tropism phenotype in recombinants of N- and B-tropic viruses (29) and recombinants of N- and xenotropic viruses (12). Naturally occurring B-tropic MuLVs are thought to arise by recombination of an ecotropic (N-tropic) genome and a xenotropic (cryptic B-tropic) genome (1, 2, 12). Recent analysis of NB-tropic variants created by forced in vitro passage of B-tropic MuLV in nonpermissive cells demonstrates differences between the NB tropic variants and the original B-tropic virus in the adjacent p15 and p12 in addition to p30 (11). This observation suggests a recombinational origin

for these NB-tropic variants and the possibility that p12 (or p15, or both) is involved in *Fv-1* action.

It has been shown that N-, B-, and NB-tropism is genetically linked to (and possibly determined by) a distinct nucleotide sequence identified from a RNase T1 oligonucleotide characteristic of each of the tropism phenotypes (28). The 5′ location of these nucleotides is consistent with the idea that p30 (and possibly other *gag* gene products) is involved in *Fv-1* restriction.

We have used recombinant DNA cloning to exchange precisely defined regions of N- and B-tropic MuLV genomes and have determined the *Fv-1* host range of the resulting recombinant viruses. The results showed that exchanging a 1.3-kilobase pair (kbp) fragment (15% of genome) which included the 3′ end of *gag* and the 5′ end of *pol* reversed the N- and B-tropism and indicated the presence of genetic determinants for the *Fv-1* restriction in this fragment of the MuLV genome.

MATERIALS AND METHODS

Viruses and cells. The BALB/c MuLV isolates WN1802N (N-tropic) and WN1802B (B-tropic) and the in vitro passaged Gross (passage A) MuLV were originally obtained from W. P. Rowe and J. W. Hartley of the National Institutes of Health, Bethesda, Md. Protocols for infection and maintenance of stocks and sources and culture conditions of NIH-3T3, C57BL, SC-1, and XC cells have been described elsewhere (23, 32).

Molecular cloning. Covalently closed circular DNA of WN1802N, WN1802B, and Gross MuLVs was isolated by cesium chloride-ethidium bromide centrifugation of Hirt supernatant DNA prepared 48 h after infection of SC-1 cells. Viral DNA was digested with *Hin*dIII, which had a single recognition site approximately 3 kbp from the left end of each genome (left to right orientation of the map corresponds to the 5′ to 3′ orientation of the RNA genome) and cloned in λ Charon 9 DNA (4; supplied by F. Blattner, University of Wisconsin). In vitro packaging, and replication in *Escherichia coli* DP50 Sup F, were performed as described by Blattner et al. (4). Recombinants were screened by the plaque hybridization protocol of Benton and Davis (3), using ^{32}P-labeled cDNA prepared from WN1802B 70S RNA. Infectious inserts were subcloned into the *Hin*dIII site of pBR322 (for WN1802N and Gross MuLVs) or pBR325 (for WN1802B MuLV) vectors (5) which had been previously treated with *Bam*HI and S1 nuclease to destroy the *Bam*HI recognition sequence. This was done to facilitate later exchange of *Bam*HI fragments in the insert without affecting the vector. Subclones and recombinants between N- and B-tropic clones were constructed as illustrated in Fig. 2 and 4 and as described elsewhere in the text. The National Institutes of Health guidelines for recombinant DNA research were followed throughout this work.

Plasmid amplification and DNA isolation. The protocol for amplification of pBR322 clones and pBR325 clones was essentially as described in reference 5. Amplified 200-ml cultures were centrifuged, and the washed cell pellet was suspended in 2.5 ml of 15% sucrose–50 mM EDTA–50 mM Tris-hydrochloride (pH 6.8) and treated with 2 mg of lysozyme for 5 min on ice. Cells were lysed with 2.5 ml of 0.5% Triton X-100–50 mM EDTA–50 mM Tris-hydrochloride (pH 6.8), held on ice for 10 min, and centrifuged at 30,000 rpm for 90 min. The supernatant was carefully removed, adjusted to 2% sodium dodecyl sulfate, and heated to 60°C for 5 min. Then 5 ml of 5 M NaCl was added, and the solution was mixed, held on ice overnight, and centrifuged at 24,000 rpm for 30 min. DNA in the superantant was precipitated by the addition of polyethylene glycol (6,000 molecular weight) to 10% (wt/vol) and was held on ice for at least 1 h. The DNA was pelleted by centrifugation at 20,000 rpm for 20 min, suspended in 2.5 ml of 8 M urea–0.24 M $NaPO_4$ (pH 6.8)–0.8% sodium dodecyl sulfate, and loaded onto a hydroxylapatite column (DNA grade Bio-Gel HTP, Bio-Rad Laboratories) equilibrated in the same buffer. The column was washed in 8 M urea–0.24 M $NaPO_4$ (pH 6.8) followed by 0.01 M $NaPO_4$ (pH 6.8), and DNA was eluted in 0.3 M $NaPO_4$. DNA was extensively dialyzed against 10 mM Tris-hydrochloride (pH 7.4)–10 mM NaCl–1 mM EDTA.

DNA transfection. Plasmid DNA was purified, digested with *Hin*dIII, and ligated overnight (~16 h) at 30 to 50 μg/ml with T4 DNA ligase. The transfection technique of Graham and Van der Eb (14), as previously modified (17), was used. Samples were diluted to a final concentration of 3 μg/ml in HEPES buffer (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) containing 30 μg of calf thymus DNA per ml. The solution was adjusted to 0.125 M $CaCl_2$ and incubated for 15 min at room temperature, and 0.1 ml was placed onto 4×10^4 NIH-3T3 cells per 16-mm well. After holding at room temperature for 10 min, 1 ml of standard growth medium was added, and cells were incubated at 37°C as usual. After 1 day, the cells were cocultured with SC-1 cells, and the medium was supplemented with 1 μM hydrocortisone and 0.1 IU of insulin and 2 μg of polybrene per ml to facilitate expression and spread of the virus (17). At 3- to 4-day intervals, each culture was trypsinized and replated into duplicate cultures. One set of cultures was assayed for infection by the XC plaque assay, and the other set was used for continuous passage.

Assay of recovered virus. Culture medium was saved from each passage after transfection and was stored at −70°C. Media from cultures which were positive by the XC plaque assay were subsequently assayed for *Fv-1* host range by the standard XC plaque assay in NIH-3T3 (*Fv-1*^{n}) and C57BL (*Fv-1*^{b}) fibroblast cultures.

Enzymes and reagents. Restriction endonucleases were purchased from Bethesda Research Laboratories and were used as recommended by the supplier.

RESULTS

Molecular clones of the endogenous BALB/c MuLVs WN1802N (N-tropic) and WN1802B (B-tropic), and the in vitro passage isolate of the leukemogenic Gross passage A MuLV (N-tropic), were constructed as described above. We have shown previously that within a population

TABLE 1. Titration of virus recovered after transfection

Group[a]	Molecular clone	XC plaques ($\times 10^{-2}$) of recovered virus		N/B ratio	*Fv-1* tropism
		NIH-3T3 (*Fv-1*^{n})	C57BL (*Fv-1*^{b})		
A	pWN41	15	0.2	75	N
	pGN104	50	0.06	833	N
	pWB5	0.05	15.7	0.003	B
B	pWN/b(*X-H*)1	<0.01	>0.09	<0.1	B
	pWB/n(*X-H*)1	3,200	6.9	464	N
	pWB/n(*X-H*)3	2.6	<0.1	>26	N
	pWB/n(*X-H*)4	4.0	<0.1	>40	N
	pGN/b(*X-H*)15	<0.03	0.8	<0.037	B
	pGN/b(*X-H*)17	<0.03	2.1	<0.014	B
	pGN/b(*X-H*)53	<0.01	0.3	<0.033	B
C	pWN/b(*B-H*)9	0.6	73	0.008	B
	pWN/b(*B-H*)21	0.02	2.4	0.008	B
	pWN/b(*B-H*)28	1.0	36	0.028	B
	pWB/n(*B-H*)40	450	0.4	1,125	N
	pWB/n(*B-H*)66	450	0.5	900	N
	pWB/n(*B-H*)69	860	0.8	1,075	N

[a] Groups: A, parental N- and B-tropic molecular clones; B, recombinant genomes involving *Xor*II-*Hin*dIII exchange; C, recombinant genomes involving *Bam*HI-*Hin*dIII exchange.

of molecularly cloned unintegrated WN1802N MuLV genomes, there is a long terminal repeat (LTR) size heterogeneity (6). We took advantage of this by choosing λWN41, which was an isolate with two LTRs of the basic 527-base pair (bp) size and was easily distinguished (with the appropriate restriction enzymes) from the B-tropic isolate, λWB5, which had two slightly larger LTRs of 580 bp (53 bp larger due to a direct repeat in the U3 region). The molecular clone of Gross passage A virus, λGN104, was easily distinguished from the other two since it contained a single LTR of 563 bp (36 bp larger due to an additional sequence in the U3 region).

As an additional aid to distinquish these viral genome clones, in which most restriction fragments appeared to be identical, we subcloned λWN41 and λGN104 (both N-tropic) into pBR322 and subcloned λWB5 (B-tropic) into pBR325. These vectors could be distinguished by the chloramphenicol resistance gene (and subsequent 1.1-kbp size increase) present in pBR325 but not in pBR322 (5). The parental origin of all restriction fragments used in in vitro recombination experiments was distinguishable on the basis of these properties (i.e., LTR size and vector) or by construction of deletion subclones.

The parental N- and B-tropic molecular clones were infectious by DNA transfection, and the virus recovered after transfection and subsequent passage in SC-1 cells exhibited the host range of the original virus as expected (Table 1). Restriction fragments from parental N- and B-tropic genomes were exchanged to map the determinant of the *Fv-1* host range. Figure 1 is a schematic representation of the parental and recombinant MuLV genomes to illustrate the location of the fragments exchanged in these experiments. The first experiment involved exchanging the 2.4-kbp *Xor*II to *Hin*dIII fragment

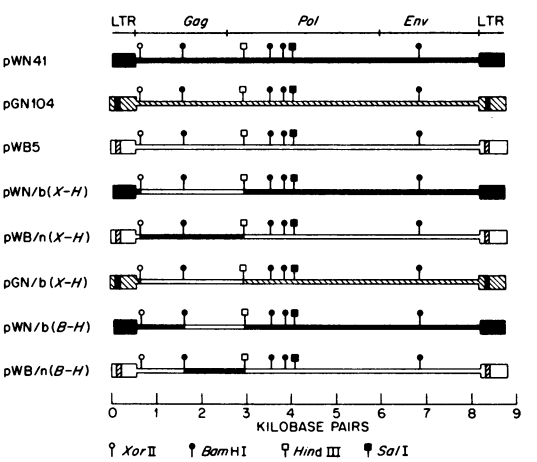

FIG. 1. Schematic representation of in vitro constructed recombinant MuLV molecular clones. Map is aligned as nonpermuted linear viral DNA. Parental genomes and recombinants between N-tropic pWN41 and B-tropic pWB5, and between N-tropic pGN104 and pWB5, are shown. Each hatched region in the LTRs of pWB5 indicates the position of a distinctive 53-bp repeat; each solid region in the LTRs of pGN104 indicates the position of a distinctive 36-bp insertion. (Although shown here with two LTRs, the molecular clone pGN104 has a single LTR.)

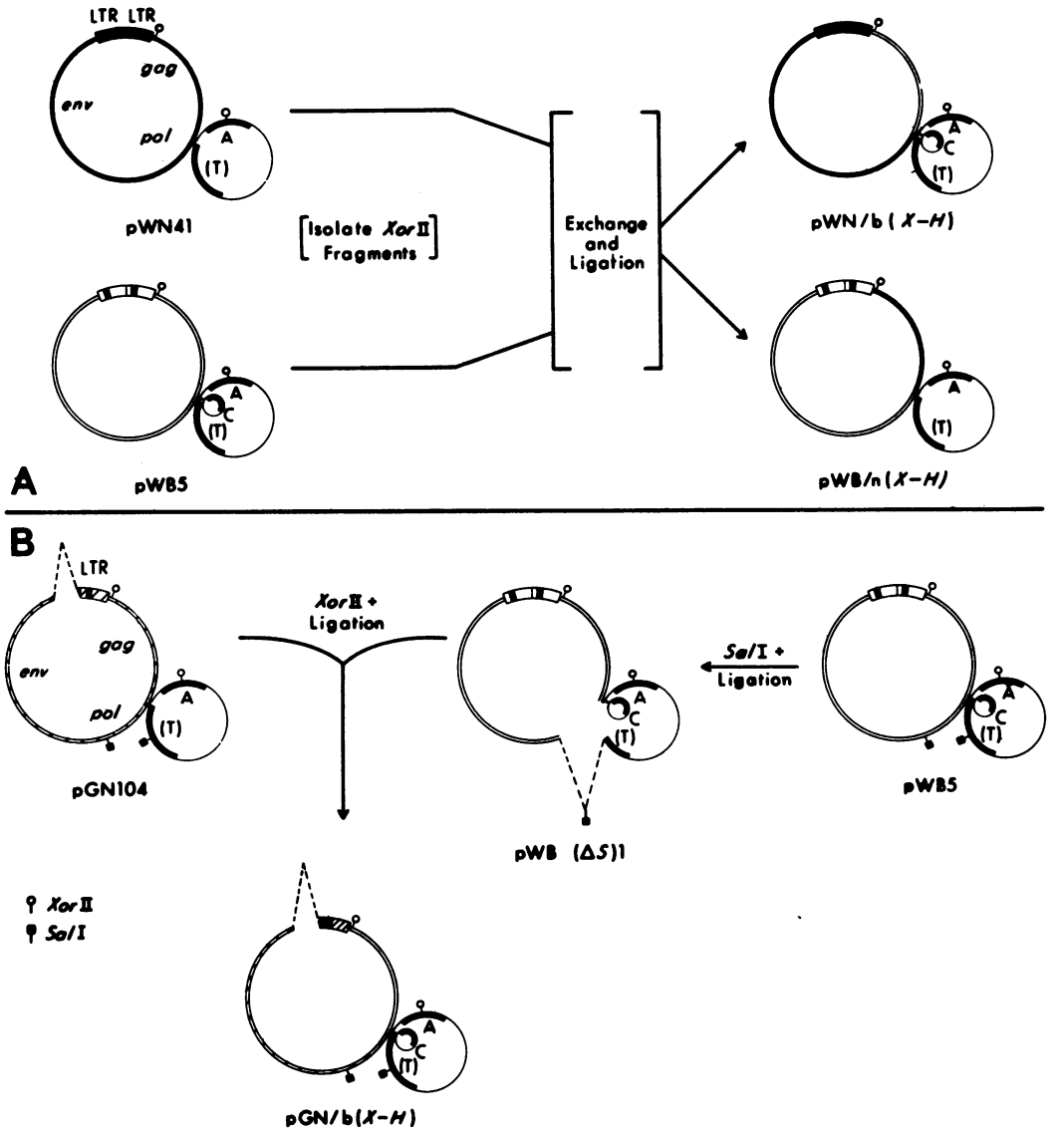

FIG. 2. Schematic representation of *Xor*II to *Hin*dIII fragment exchange. (A) Exchange between N-tropic pWN41 and B-tropic pWB5. (B) Recombination of N-tropic pGN104 and pWB(Δ*S*)1, a subclone of B-tropic pWB5 (see Fig. 4). Abbreviations: A, ampicillin resistance gene; C, chloramphenicol resistance gene; (T), inactivated tetracycline resistance gene (due to removal of *Bam*HI site; see the text).

that includes the entire *gag* gene. This is shown schematically in Fig. 2. In the exchange between N-tropic pWN41 and B-tropic pWB5 (Fig. 2A), parental clones were digested with *Xor*II, and the large and small fragments were isolated by preparative gel electrophoresis. Reciprocal mixes were made, and fragments were ligated and used to transform *E. coli* C600. Recombinant plasmids were analyzed, and the parental origin of the small *Xor*II fragment was established by the chloramphenicol resistance gene of pWB5 that was not present on the homologous small fragment of pWN41. The parental origin of the large *Xor*II fragment was established by determining the LTR size (pWN41 has two 527-bp LTRs and pWB5 has two LTRs of 580 bp, 53 bp larger due to a direct repeat).

For the recombination of N-tropic pGN104 and pWB(Δ*S*)1, a subclone of B-tropic pWB5 (Fig. 2B), parental clones were also digested with *Xor*II, mixed and ligated, and used to transform *E. coli* C600. Recombinant plasmids were analyzed, and the parental origin of the small *Xor*II fragment was established by the

chloramphenicol resistance gene of pWB(ΔS)1 absent in pGN104. The parental origin of the large *Xor*II fragment was established by the LTR size and number (pGN104 contains a single 563-bp LTR, and pWB(ΔS)1 contains two 580-bp LTRs) and by the presence (in pGN104) or absence [in pWB(ΔS)1] of the 1.75-kbp *Sal*I fragment [see Fig. 4 for construction of pWB(ΔS)1].

Although the physically recombined fragment in these constructions was *Xor*II-*Xor*II, the "net exchange" of viral insert was the 2.4-kbp *Xor*II-*Hin*dIII fragment. An example of an in vitro recombinant genome from each exchange, and the parental DNA, each digested with *Xor*II, are shown in Fig. 3. It is obvious from the restriction enzyme analysis in Fig. 3A that the pWN/b(*X-H*)1 recombinant (lane 2) contains the small *Xor*II fragment of the B-tropic parent, pWB5 (lane 4), and that the pWB/n(*X-H*)1 recombinant (lane 3) contains the small *Xor*II fragment of the N-tropic parent, pWN41 (lane 1). The parental origin of the large *Xor*II fragment (indistinguishable on this gel) was verified by LTR size analysis, using *Pst*I digestion (6; other data not shown). It is readily apparent in Fig. 3B that the pGN/b(*X-H*)53 recombinant (lane 2) consists of the large *Xor*II fragment of the N-tropic parent pGN104 (lane 1), and the small *Xor*II fragment of the B-tropic parent subclone, pWB5(ΔS)1 (lane 3).

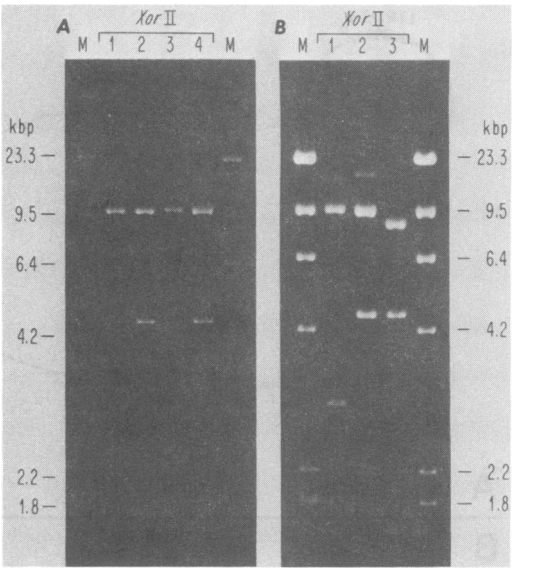

FIG. 3. Agarose gel electrophoresis of parental and in vitro recombinant clones of *Xor*II-*Hin*dIII exchange. Cloned DNA was digested with *Xor*II, subjected to electrophoresis for 16 h at 50 V, and stained with ethidium bromide. (A) Lane 1, pWN41; lane 2, pWN/b(*X-H*)1; lane 3, pWB/n(*X-H*)1; lane 4, pWB5. Size markers (M) are *Hin*dIII fragments of λ. B Lane 1, pGN104; lane 2, pGN/b(*X-H*)53; lane 3, pWB(ΔS)1. Size markers (M) are *Hin*dIII fragments of λ.

All pWN/b(*X-H*), pWB/n(*X-H*), and pGN/b(*X-H*) isolates (identical to the examples shown in Fig. 3) were examined for infectious activity by DNA transfection on NIH-3T3 cells and were cocultivated with SC-1 cells to amplify any B-tropic virus. Not all recombinant clones were infectious. Virus recovered from transfection-positive cultures were assayed for *Fv-1* host range by titration on *Fv-1*^{n} and *Fv-1*^{b} cells. Some isolates were passed in SC-1 cells several times and thus have a higher titer. The results (Table 1) indicated that *Fv-1* tropism was determined by a sequence residing in the 2.4-kbp *Xor*II to *Hin*dIII fragment. The large XC plaque-forming ability of WN1802B MuLV was observed in pWB5 and the N-tropic pWB/n(*X-H*) isolates, suggesting that plaque morphology was not affected by exchange of this fragment.

The B-tropic pWN/b(*X-H*)1 and pGN/b(*X-H*)53 isolates, however, produced XC plaques distinct from their N-tropic parent. These were difficult to score on C57BL, due to lifting off of the XC syncitium, but they were easy to detect on SC-1 cells (data not shown).

A *Bam*HI cleavage site which exists approximately in the middle of the 2.4-kbp *Xor*II-*Hin*dIII fragment (see Fig. 1) was used to create a smaller fragment exchange. Subcloned intermediates with restriction fragments deleted were constructed, and the in vitro recombination between N- and B-tropic parents was conducted in two steps. First, the ~3.5-kbp *Bam*HI fragment of one type was cloned into the double deletion subclone (ΔSΔB) of the opposite tropism to create a *Bam*HI fragment recombinant. Then the final recombinant (in which only the 1.3-kbp *Bam*HI-*Hin*dIII fragment is from the parent of opposite tropism) was constructed by a *Xor*II exchange with the original (major) parental clone. The parental origin of the small *Xor*II fragment was established by the chloramphenicol resistance of pWB5 (and subclones) and sensitivity of pWN41 (and subclones). The parental origin of the large *Xor*II fragment was established by the presence (in the original parental clone) or absence (in the *Bam*HI fragment recombinant) of the 1.75-kbp *Sal*I fragment.

The *Bam*HI to *Hin*dIII fragment of pWN41 was replaced with the homologous fragment from pWB5, as shown schematically in Fig. 4. DNAs of the parental clones and intermediates digested with the appropriate enzymes are shown in Fig. 6A. One of the final recombinants, pWN/b(*B-H*)28, is shown in Fig. 6A, lane 9, and the parental origin of both *Xor*II fragments is clearly demonstrated. Construction of the reciprocal exchange to produce the pWB/n(*B-H*) series is shown schematically in Fig. 5, and restric-

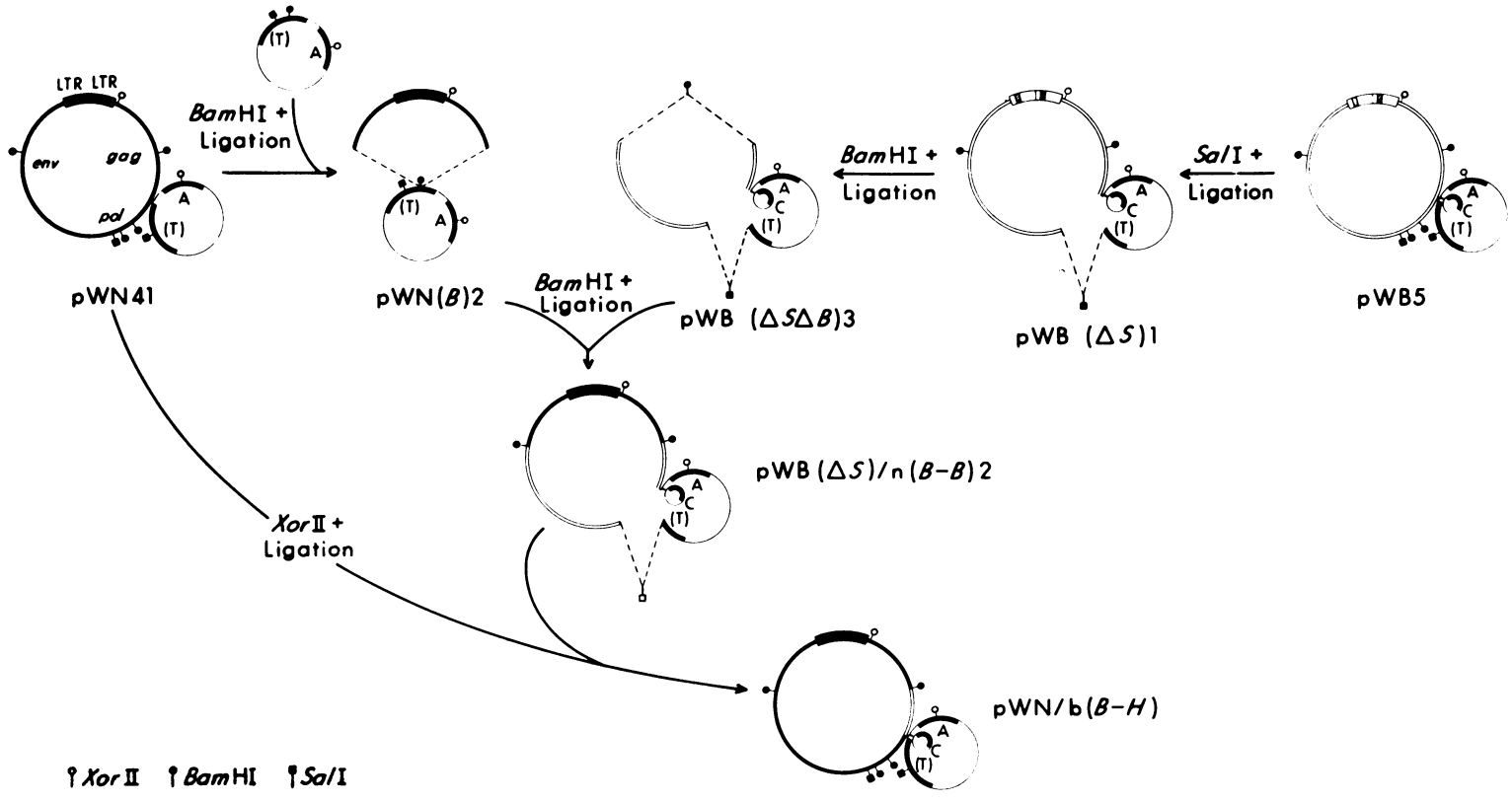

FIG. 4. Schematic representation of construction in which the 1.3-kbp *Bam*HI-*Hin*dIII fragment of N-tropic pWN41 was replaced with the homologous fragment from B-tropic pWB5. Subcloned intermediates with restriction fragments deleted (designated ΔS for deleted *Sal*I fragment and ΔB for deleted *Bam*HI fragment) were constructed by digestion with the appropriate restriction enzyme and self-ligation at low DNA concentration. The deleted fragments are indicated by a dashed line which connects at the deletion boundary. Symbols are the same as in Fig. 2.

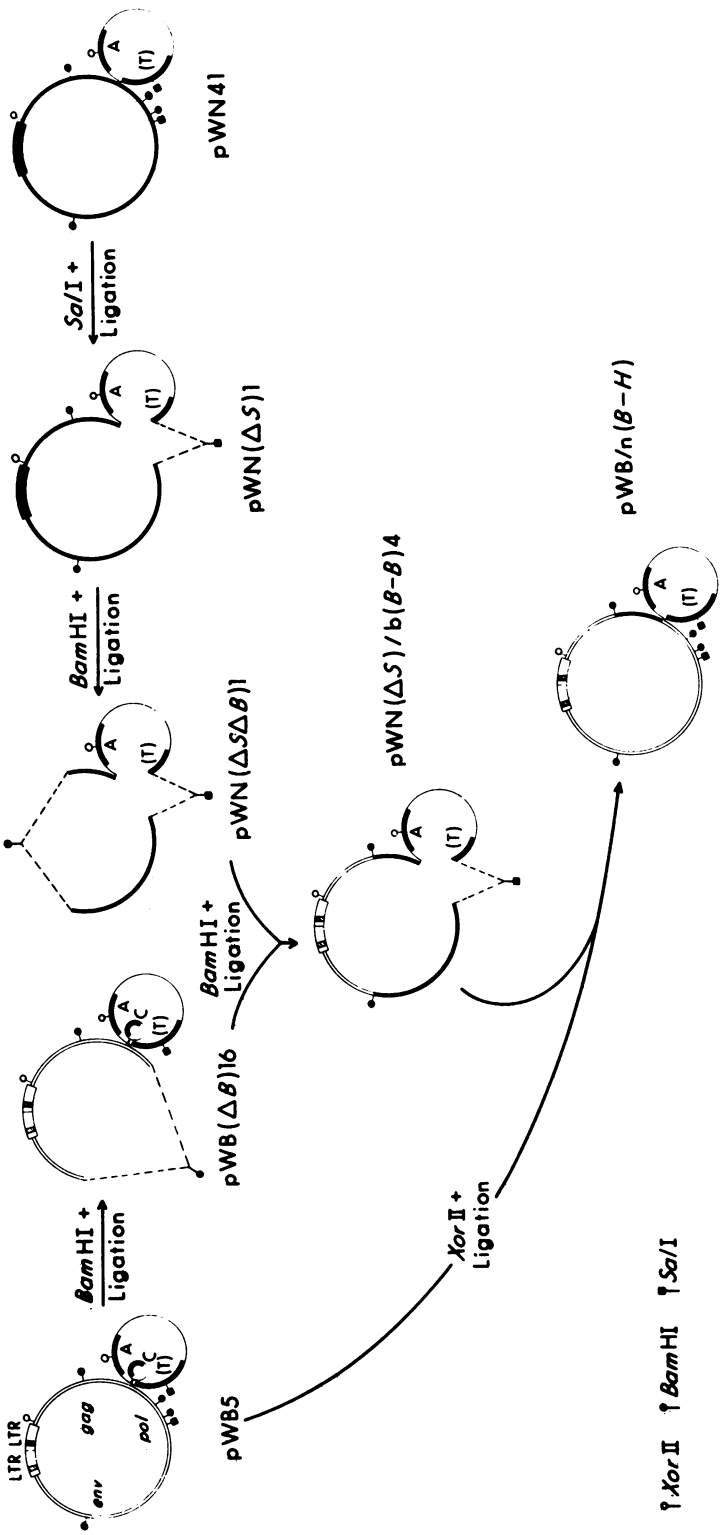

FIG. 5. Schematic representation of construction in which the 1.3-kbp *Bam*HI-*Hin*dIII fragment of B-tropic pWB5 was replaced with the homologous fragment from N-tropic pWN41. Symbols are the same as in Fig. 2 and 4.

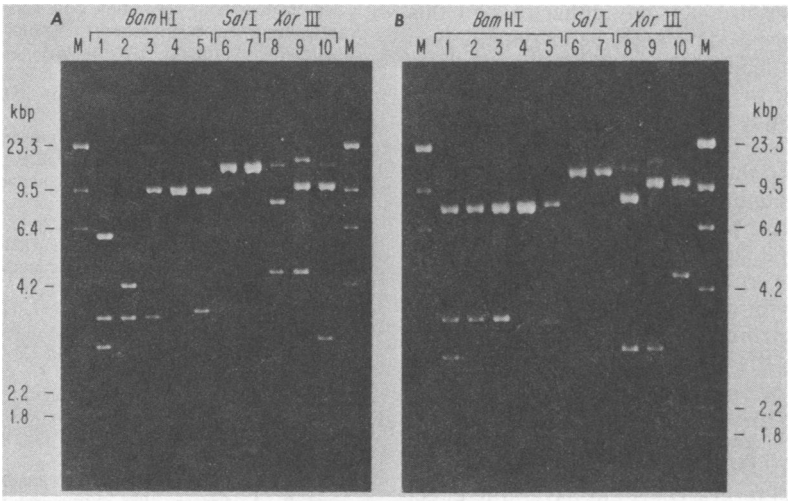

FIG. 6. Agarose gel electrophoresis of intermediates and in vitro recombinants of *Bam*HI-*Hin*dIII exchange. Cloned DNAs were digested with restriction enzymes (indicated above lane numbers) and subjected to electrophoresis as in Fig. 3. (A) pWN41 (lanes 1 and 10); pWN(*B*)2 (lane 2); pWB(Δ*S*)/n(*B-B*)2 (lanes 3 and 8); pWB(Δ*S*Δ*B*)3 (lane 4); pWB(Δ*S*)1 (lanes 5 and 6); pWB5 (lane 7); pWN/b(*B-H*)28 (lane 9). Size markers (M) are as in Fig. 3. (B) pWB5 (lanes 1 and 10); pWB(Δ*B*)16 (lane 2); pWN(Δ*S*)/b(*B-B*)4 (lanes 3 and 8); pWN(Δ*S*Δ*B*)1 (lane 4); pWN(Δ*S*)1 (lanes 5 and 6); pWN41 (lane 7); pWB/n(*B-H*)69 (lane 9). Size markers (M) are as in Fig. 3.

tion enzyme digests of intermediates and a final recombinant are shown in Fig. 6B. The resulting recombinant clones from both series (identical to those shown in Fig. 6) were tested for transfection ability, and positive isolates were then tested for *Fv-1* host range. As with the *Xor*II-*Hin*dIII recombinants, not all isolates have been passaged to the same extent, and absolute titers vary. The relative restriction in one host or the other is evident, and the results shown in Table 1 indicate that the 1.3-kbp *Bam*HI to *Hin*dIII fragment contained the determinant of *Fv-1* tropism.

DISCUSSION

The N- and B-tropic MuLVs of BALB/c mice are extremely similar in molecular and biochemical characteristics, yet they are very different biologically. The difference between the genomes is subtle, and their recombination with each other is difficult to map precisely. In vitro recombination by exchange of homologous restriction endonuclease fragments is a powerful approach to genetic mapping in this situation. The experiments reported here demonstrated that the determinant of *Fv-1* host range resided in a 1.3-kbp *Bam*HI to *Hin*dIII fragment. Recent nucleotide sequence analysis indicated that this fragment extended from ~120 nucleotides beyond the beginning of the p30 region of the *gag* gene to ~380 nucleotides inside the *pol* gene (C. Y. Ou, L. R. Boone, and W. K. Yang, manuscript in preparation). These results are consistent with the idea that p30 is the virion target for the *Fv-1* gene product (1, 2, 7, 12, 13, 16, 28, 29), although involvement of the remaining *gag* gene region (p10) and amino terminal 139 amino acids of *pol* remains to be determined. Although it remains a possibility that p12 (or p15, or both) is involved in NB-tropism (i.e., loss of sensitivity to *Fv-1*) (11), our results indicate that no N-tropic or B-tropic determinants reside outside the 1.3-kbp *Bam*HI to *Hin*dIII fragment.

The large XC plaque morphology of virus recovered from pWB5 was not affected by conversion to N-tropism with either *Xor*II-*Hin*dIII or *Bam*HI-*Hin*dIII fragments. This is consistent with the idea that the *env* gene is responsible for XC plaque morphology. The conversion of pWN41 (small XC plaque) to B-tropism by the *Xor*II-*Hin*dIII fragment exchange resulted in altered XC plaque morphology, suggesting that other factors also contribute. The level of virus production, for example, may be affected in these recombinants.

The mechanism of *Fv-1* gene action is still unclear; however, a complete definition of the target in the genome will help focus our ideas and experimental approaches to understanding the biochemistry of this phenomenon. Comparison of the nucleotide sequence of this 1.3-kbp *Bam*HI to *Hin*dIII fragment from the N- and B-tropic genomes is in progress. Our working hypothesis is that the *Fv-1* target is involved in maintaining the viral genome termini in close proximity to facilitate "jumping" during reverse transcription and subsequent circle formation.

The *Fv-1* gene product may either disrupt this association or interfere with the enzymatic processes which lead to circularization and supercoiling. How the *Fv-1* gene product interacts with the virion target remains to be determined. Although it is likely that the determinants for tropism are on the p30 molecule, the functional target may be a minor component of the virion, possibly a larger precursor molecule which contains the same sequences. This could possibly explain why even though p30 is the major structural protein of the virus core, only a small number of functional target molecules per virion are indicated by some biological experiments (21, 27).

We have previously demonstrated a polymorphism of *Fv-1* gene action on viral DNA (33). For example, DBA/2 cells (*Fv-1^n*) inhibit the appearance of form I DNA but have little effect on form III DNA of infecting WN1802B B-tropic virus, whereas NIH-3T3 cells (*Fv-1^n*) severely inhibit both forms of DNA. Examination of the DNA forms synthesized by in vitro constructed "reverse tropism" viruses may indicate whether the block in form III viral DNA synthesis is due to the *Fv-1* determinant or is a separate restriction.

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