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Host restriction of Friend leukemia virus: gag proteins of host range variants

(*Fv-1* gene/genetic variation/tryptic peptides/retrovirus/thin-layer chromatography)

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ABSTRACT **The host response to murine ecotropic leukemia viruses is mainly controlled by the mouse *Fv-1* gene. This locus controls virus replication at an intracellular stage and prevents provirus integration. Biological studies suggest that the *Fv-1* effector molecule recognizes at least one virion structural protein. We have produced host range variants of B-tropic Friend murine leukemia virus in order to study the primary structure of potential viral target proteins. Our results show that conversion of B-tropism to NB-tropism is associated with changes in the primary structure of three gag proteins—p15, p12, and p30. These results suggest that host range conversion is due to a recombinational event, presumably between the parental virus and an endogenous murine virus. They also open the possibility that p12 and p30 may be involved in host range restriction.**

The response of the mouse to infection by ecotropic murine leukemia viruses is controlled by several genes, but one, the *Fv-1* locus, predominates (1). This gene, which maps to chromosome 4, exists in two allelic forms, $Fv\text{-}1^n$ and $Fv\text{-}1^b$. NIH Swiss mice represent the prototype of the homozygous N-genotype ($Fv\text{-}1^{n/n}$), and BALB/c mice represent the homozygous B-genotype ($Fv\text{-}1^{b/b}$). The phenotype of both primary and tissue culture lines derived from these animals faithfully reproduces their genotype (2, 3). N-tropic viruses grow well on N-type cells and poorly on B-type. B-tropic murine leukemia virus (MuLV) exhibit a reversed host range. Growth on the restricting cell type varies from 1/100th to 1/10,000th that on the permissive cell type, depending upon the exact cell and virus used. Forced passage of B-tropic MuLV through NIH Swiss mouse cells results in viral progeny that have lost the host range restriction—that is, virus that grows equally in cells of either genotype (NB-tropic) (4, 5).

Recently, evidence has been presented by Benjers *et al.* (6) showing that NIH Swiss mouse cells may possess an additional gene that appears to modify the *Fv-1* gene of these cells. This modified *Fv-1* gene effect renders these cells even more restrictive than can be accounted for purely on the genotype of the $Fv\text{-}1^{n/n}$ locus (2).

Although the mechanism of action of *Fv-1* gene is not completely understood, it is known to restrict virus replication intracellularly after adsorption, penetration, and "uncoating" (7, 8). The gene effect is known to prevent successful integration of proviral DNA. It has been shown (9, 10) that, in *Fv-1* restricted infections, proviral nonintegrated positive- and negative-strand DNA is made (as detected by molecular hybridization) in amounts essentially equivalent to those made in a permissive infection. Additionally, in permissive infections, nonintegrated linear double-stranded DNA (form III) as well as covalently closed circular DNA (form I) may be found. These closed circles, which may be the precursors to the viral DNA integrated into the host genome, appear to be decreased or absent in a restrictive infection (11, 12).

Although a major effect of the *Fv-1* gene is on the nucleic acid phase of viral replication, evidence has been presented which implicates viral structural protein as a determinant in this host–virus interaction. Initially, it was shown that phenotypic mixing of a MuLV helper virus with murine sarcoma virus (MSV) imparted a host range determinant to the otherwise genotypically nonrestricted MSV (13, 14). Similarly, Rein *et al.* (15) and Kashmiri *et al.* (16) showed that coinfection of mouse cells with N-tropic plus B-tropic MuLV results in a phenotypically mixed virus progeny that exhibits restricted growth on either NIH Swiss or BALB/c cells. These viruses, however, were capable of growing on $Fv\text{-}1^{-/-}$ 3T3 FL cells and, when recloned, were found to be genotypically either N- or B-tropic. In both these experiments, a viral structural protein appeared to be a host range determinant.

Using a combined genetic and biochemical analysis, Hopkins and colleagues (17) showed that there is a correlation between the major viral core protein p30 and viral host range. Similar studies revealed a lack of such a relationship for the envelope protein gp69/70 or the core protein p15. In addition, Gautsch *et al.* (18) demonstrated by tryptic peptide pattern that the primary structure of p30 was specific for the viral host range. Furthermore, structural changes in the 5′ end of the genome—that is, in the general region of the *gag* gene which includes p30—have been found to correlate with host variation (19).

We have isolated a series of NB-tropic variants in order to study possible structural changes that might be produced in genome or protein. Our earlier work (unpublished) using Dowex 50 column separation of p30 tryptic digests revealed minor but reproducible changes in p30 primary structure when host range was converted from B- to NB-tropism. Here, we describe a further analysis of *gag* gene proteins p15, p12, and p10, as well as p30, by means of thin-layer chromatography of tryptic digests. Our results indicate that the conversion of host range from B- to NB-tropism is accompanied by primary structural changes in viral p15 and p12 as well as the previously described changes in p30. Analysis of p10, however, failed to reveal such changes. These results suggest that a recombinational event has occurred in the generation of virus with the expanded host range. They also open the possibility that at least one other *gag* gene protein, p12, may be involved as a viral determinant of tropism.

MATERIALS AND METHODS

Cells and Viruses. NIH, BALB/c, and XC cells were obtained from J. Hartley and W. Rowe. DBA/2 cells were donated by R. Bassin. N- and B-tropic F-MuLV were from F.

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Abbreviations: MuLV, murine leukemia virus; MSV, murine sarcoma virus; F-MuLV, Friend MuLV.

Lilly. Cell maintenance and virus infection have been described (12).

Cloning Method. Viruses were plated at limiting dilutions as judged by XC assay (19). Virus from monolayers with one plaque were similarly recloned and passaged for stock.

Polyacrylamide Gel Electrophoresis. Viral proteins were separated as described (20, 23).

***In Vitro* Iodination of Protein and Peptide Chromatography.** Iodination of virus protein in gel slices was as described by Elder *et al.* (21), with 300 μCi of ^{125}I (Amersham) and 5 μl of freshly prepared chloramine-T (Eastman; 1 mg/ml) (1 Ci = 3.7×10^{10} becquerels). Electrophoresis and chromatography were as described (21).

RESULTS

Generation of NB-Tropic Variants. Cloned B virus produced in BALB/c cells was used to generate NB-tropic variants according to the scheme outlined in Fig. 1. Independently produced variants were derived by infection of 2×10^5 NIH cells at multiplicity of infection between 2 and 5. Progeny from such an infection were passaged (multiplicity not determined), as 0.5 ml of medium, into NIH cells and the process was repeated for a total of three viral passages. When assayed for host range, passage 3 virus routinely exhibited NB-tropism. Cloning of such NB-tropic virions by end-point dilution demonstrated that this did not result from mixtures of N-tropic and B-tropic virus. Cloned NB-tropic isolates maintained their host range when passaged in NIH cells for further analysis.

The independence of these isolates was confirmed in part by the observation, in one case, of a small-plaque variant and further by the demonstration of differences in the oligonucleotide pattern of their genomes (unpublished data). The results of p30 structure analysis (see below) also indicate differences among the five NB-tropic variants that have been generated. The analysis of the structural core proteins is described below.

Identification of Viral Proteins. Low molecular weight *gag* gene proteins of N-tropic and B-tropic virions cannot be identified exclusively on the basis of apparent molecular weight as determined by polyacrylamide gel electrophoresis (22). The p15 of N-tropic F-MuLV migrated more slowly than p12 in such a system. However, p15 of our strain B-tropic F-MuLV and its NB-tropic derivatives migrated more rapidly than p12. Fig. 2*a* shows an acrylamide gel preparation stained with Coomassie blue to demonstrate viral proteins. Identification of p12 in each case was made by radiolabeling virus with [^{32}P]orthophosphate followed by autoradiography. Additionally, with Coomassie blue, p12 stains a distinctive magenta compared with the blue color of all the other protein bands. p15, p10, and p30 were identified by immunoprecipitation with monospecific antibody of [^{3}H]leucine-labeled virus proteins followed by polyacrylamide gel electrophoresis of the immunoprecipitate (23).

After identification and localization by staining, the protein band was sliced from the gel, and the gel was minced and subjected to radioiodination (21). Trypsin-digested protein was eluted from the gel as peptides after free iodine had been eliminated by a modification of their procedure. In the case of p30, reanalysis by polyacrylamide gel electrophoresis of radioiodine-labeled but nondigested protein revealed a single band (autoradiography) with the apparent molecular weight of noniodinated p30 (not shown).

Analysis of p30 of B- and NB-Tropic Virions. The electrophoretic migration of p30 of five NB-tropic variants relative to B- and N-tropic F-MuLV is shown in Fig. 2*b*. B-tropic p30 migrated more slowly than the p30 of N-tropic F-MuLV. The p30 of all the NB-tropic variants showed a similar faster migration pattern than that of the parental B-tropic virus. In this experiment the electrophoresis was continued until the p30 had migrated to the end of the gel in order to maximize the small differences in migration among the NB-tropic variants.

The pattern of tryptic peptides produced by thin-layer two-dimensional analysis of iodinated p30 of B-tropic F-MuLV is shown in Fig. 3. These data, which represent multiple analyses of at least three preparations for each protein, demonstrate that host range conversion from B- to NB-tropism is associated with a significant alteration in primary structure. Although several

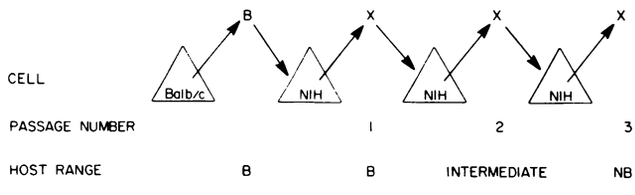

FIG. 1. Production of NB-tropic variants. B-tropic virus derived from chronically infected BALB/c cells was used to infect 10^5 NIH cells at a multiplicity of infection of 5 in 60-mm dishes. The progeny of the confluent monolayer of NIH cells as an overnight harvest of freshly fed cells is considered a single passage. A 0.5-ml portion of this medium was passed blindly (multiplicity unknown) into a second dish of NIH cells; the process was repeated for a total of three passages. The host range was determined by the relative plating on NIH vs. BALB/c cells.

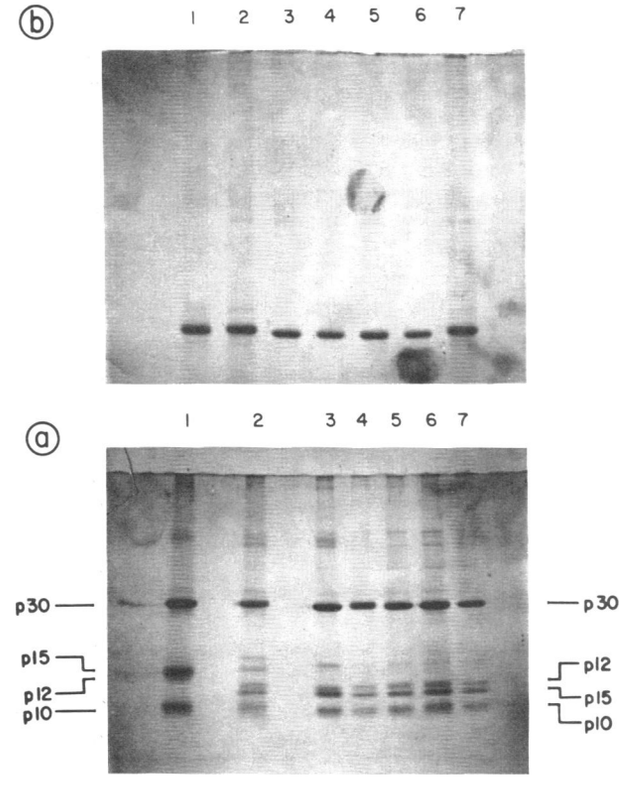

FIG. 2. Polyacrylamide gel electrophoresis of viral proteins. (*a*) Total viral proteins. Proteins from gradient-purified virus were subjected to electrophoresis in a 10–20% exponential gel. The pattern of Coomassie blue–stained proteins is shown. Lanes (in terms of the tropism of each virus): 1, N; 2, B; 3, NB'_1; 4, NB'_1; 5, NB_2; 6, NB_3; 7, NB_4. (*b*) Viral p30. To maximize the migrational differences in p30 among the several NB-tropic isolates, viral proteins were subjected to polyacrylamide gel electrophoresis. This gel represents a 5–15% linear gradient gel in which p30 protein migrated to the bottom of the slab gel (100 *V*, constant voltage; 7 hr). Lanes (in terms of the tropism of each virus): 1, N; 2, B; 3, NB_1; 4, NB_2; 5, NB_3; 6, NB_4; 7, B.

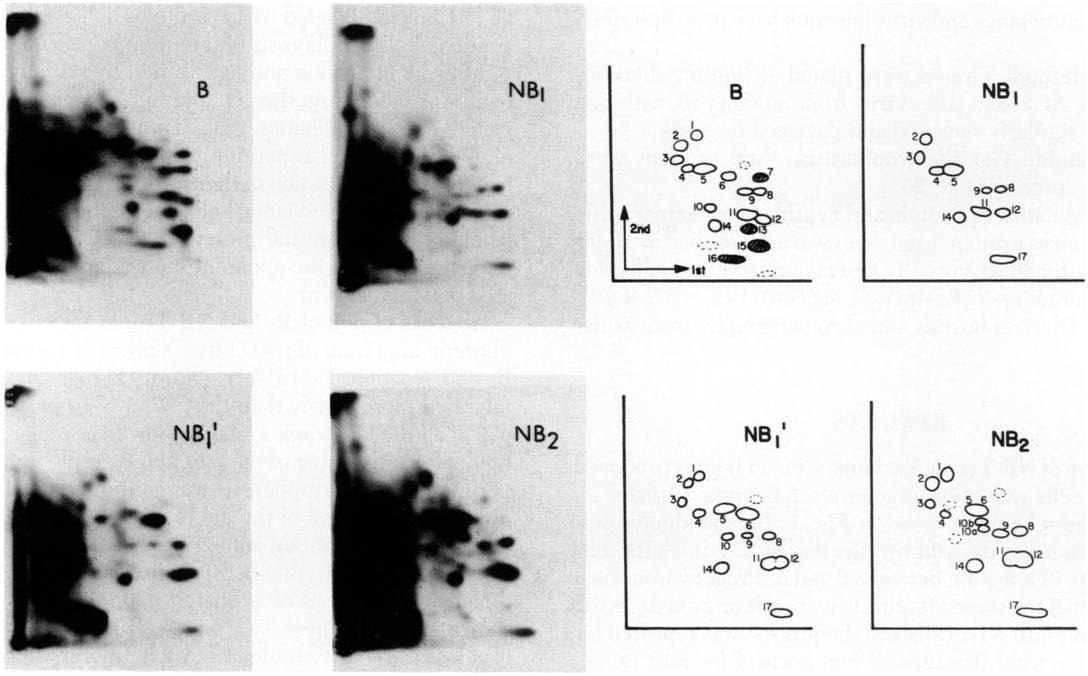

FIG. 3. Tryptic peptides of B-tropic and NB-tropic p30. Virus proteins were prepared and isolated for tryptic peptide analysis essentially as in Fig. 2*a*. Individual proteins were iodinated in gel slices, trypsinized, eluted, and concentrated. Purified peptides were analyzed by thin-layer chromatography according to Elder *et al.* (21). The first dimension (horizontal, from left to right) electrophoretic separation was carried out at 750 V for approximately 30 min or until the major dye spot had migrated to the edge of the gel. The second dimension (vertical, from bottom to top) utilized ascending chromatography until the solvent front reached 0.5 cm from the top of the chromatogram. The autoradiograph of the dried chromatogram and the outlines of the major spots are shown; hatching of a spot indicates that it is found only in the B-tropic parental virus. Minor spots are outlined.

spots appear to be shared between B- and NB-tropic p30, all five NB-tropic isolates appeared to lack the B-tropic-specific spots 7, 13, 15 and 16 but had one new spot, no. 17. Additionally, differences among the patterns of the individual NB-tropic variants could be seen. Spots 1, 6, and 10, in addition to the B-tropic specific spots, appeared to be absent from isolate NB_1. Analysis of the NB_2-tropic variant showed two spots (10_A and 10_B) in the area of spot 10, which appeared to distinguish this from other variants. NB_3 and NB_4 resembled NB_1 closely and were not clearly distinguishable from one another on mixing.

These data show that host range conversion results in significant changes in the primary structure of p30 as revealed in the trypic peptide pattern. Several deletions of prototype B-tropic p30 specific peptide spots occurred, and one new p30 spot

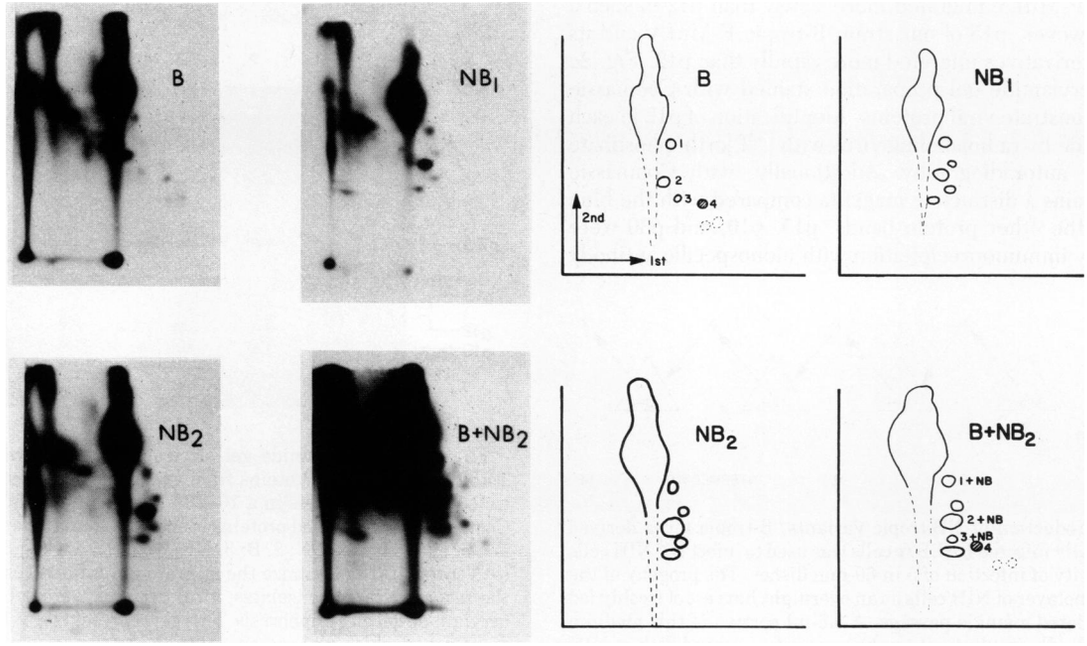

FIG. 4. Tryptic peptides of B-tropic and NB-tropic variant p15. Peptides were analyzed as in Fig. 3.

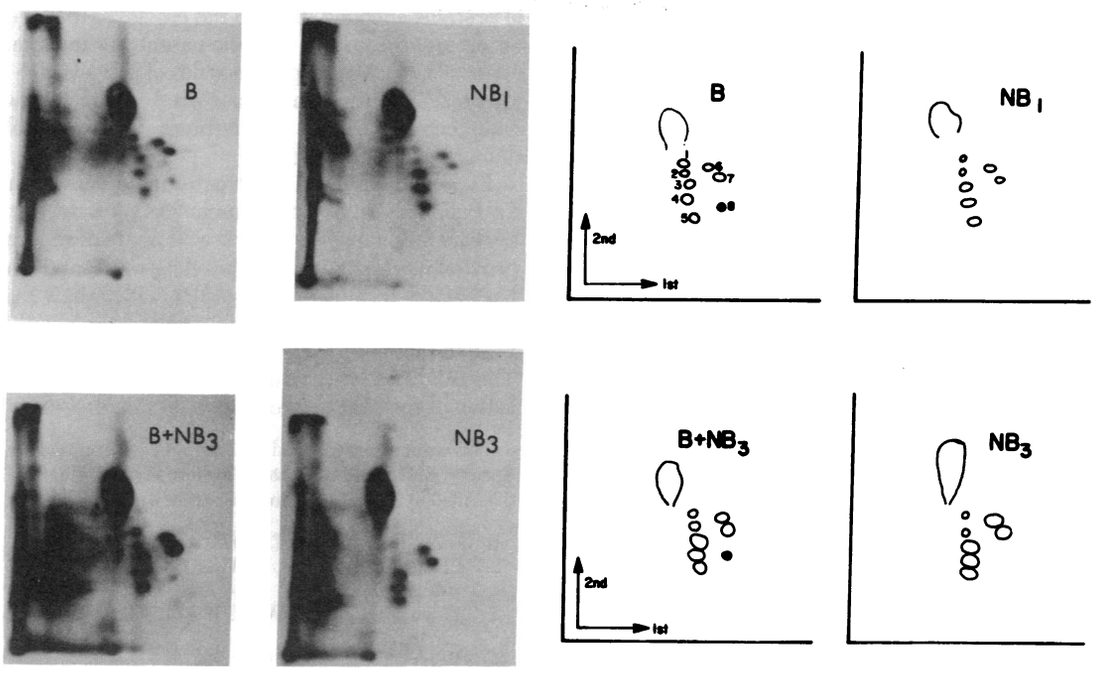

FIG. 5. Tryptic peptides of B-tropic and NB-tropic variant p12.

appeared on NB-tropic conversion. Among the several NB-tropic isolates, three patterns of tryptic peptide spots were found.

Analysis of p15 of B- and NB-Tropic Virus. No measurable differences in the migration of the p15 of B-tropic and of the NB-tropic variants could be seen on polyacrylamide gel electrophoresis (Fig. 2*a*). However, the exponential gradient gel used in this analysis would minimize small differences. In the analysis of the tryptic peptides shown in Fig. 4, in each case, and with many different preparations, the pattern produced by p15 had a large smear. However, the pattern of the well-delineated spots demonstrated changes between the parental B-tropic virus and the NB-tropic variants. Spot 4 (hatched) was the only B-tropic p15 spot that could be resolved unequivocally from spots of the NB-tropic variants. The differences in migration of the individual spots have been verified by cochromatography of B-tropic with NB_2-tropic p15. Spots 1, 2, and 3 either comigrated or failed to separate from the parental B-tropic spots. At least two spots not found in the B-tropic p15 were identified by analysis of cochromatographed samples (see Fig. 4). In the case of each NB-tropic variant, five spots were generated. There were slight differences in the distribution of this five-spot pattern among the NB-tropic isolates. It is unclear whether this represents slight differences in the spot migration due to the method of analysis used or subgrouping of structure within the variants. We favor the former explanation because cochromatography of two NB-tropic variants (NB_1 and NB_2) failed to show double spots (not shown).

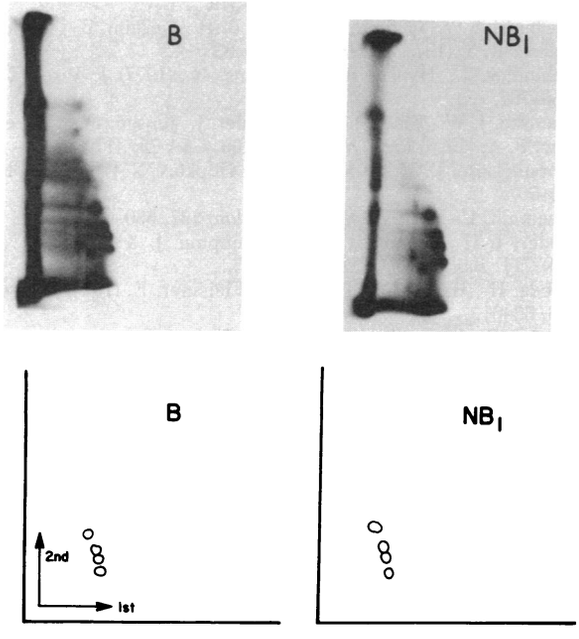

FIG. 6. Tryptic peptides of B-tropic and NB-tropic variant p10.

Analysis of p12 from B- and NB-Tropic F-MuLV. No clear electrophoretic differences were seen between B-tropic and NB-tropic p12. However, tryptic peptide analysis (Fig. 5) demonstrated a single spot difference (spot 8) between B-tropic viral p12 and all its NB-tropic derivatives. Mixing studies showed that all of the common spots of B-tropic and NB-tropic p12 comigrated. Furthermore, all our NB-tropic variants appeared to possess the same primary structures, and no subsets could be detected. This may suggest a strong conservation of structure for this core viral protein.

Analysis of p10 from B-Tropic and NB-Tropic Virus. No difference in the structural analysis of viral p10 was observed between B-tropic and any of the NB-tropic variant proteins. Fig. 6 shows the same four-spot pattern for each of these proteins; one N-tropic F-MuLV also had this pattern (not shown).

DISCUSSION

Previously reported biological experiments involving the donation of host range by way of phenotypic mixing suggested strongly that viral structural protein(s) contribute at least part of the viral determinant(s) of host range. We elected to start with a single cloned B-tropic MuLV to obtain host range variants in

order to analyze protein changes. Two major kinds of results have been generated. First, electrophoretic migrational differences in the p30 of NB-tropic variants could be found on polyacrylamide gel electrophoresis. Although the migration resembles that of N-tropic F-MuLV, the primary structure of N-tropic p30 (not shown) differs significantly from that of the NB-tropic variants. Because our N- and B-tropic F-MuLV are not clearly derived one from the other, this result would be expected (24).

However, the change in host range is associated with changes in the primary structure of several of the viral core proteins. p30 changes are quite dramatic and confirm the differences in p30 primary structure observed by Gautsch *et al.* (18) who observed structural correlation of p30 and viral host range. However, we are unable to confirm definitely that single spot changes are associated with host range variation.

Our data further show minor differences within the primary structure of p30 among the several NB-tropic variants we analyzed. However, the primary structure of both B- and NB-tropic variants revealed significant overlap. This is to be expected from the data of Gautsch *et al.* (18) indicating a conservation of primary structure among the proteins of MuLV.

Additionally, we observed changes in primary structure of both p15 and p12 as born out by comparisons of individual protein peptides and by mixing studies.

The literature has not clearly resolved the genetic mechanism by which the host range variants are generated. Hopkins and coworkers (19) postulated that NB-tropic variants derive from B-tropic virus by mutation. This conclusion was based on extensive analysis of the sequence of an oligoribonucleotide which appears to correlate with tropism. This RNase T1-derived oligonucleotide demonstrated a single base change from its B-tropic progenitor. However, the single base change was at the 5′ end of the sequence. These workers commented upon the possibility that changes in adjoining nucleotides might not be detected by their method of analysis and that other undetected changes might have occurred. Their data could not rule out the recombinational origin of the NB-tropic variant.

Our data, which show changes in the primary structure not only of p30 but also of the adjoining gag proteins p12 and p15 toward the 5′ end of the genome, would suggest that the NB-tropic variants arise by recombination, presumably with an endogenous virus of the cells in which they are propagated. Furthermore, our data show no changes in p10 on host range variation. The order of proteins within the *gag* gene from 5′ to 3′ is: p15, p12, p30, and p10. The simplest possible model to explain our results would be that NB-tropic variants arise by a recombination between the B-tropic parent and an endogenous virus. Furthermore, if we postulate that the recombination occurs at any one of several locations within the p30 gene region, it would explain these results. The finding of several tryptic peptide patterns for NB-tropic p30 could occur by a crossover at different sites within the p30 gene. This mechanism could result in deletions of B-tropic spots, as observed, and the appearance of several p30 peptide patterns. Furthermore, a single crossover within p30 would predict that the primary structure of proteins encoded distal to the crossover (p12 and p15) could be different from the B-tropic parent but invariant from each other. That is, the tryptic peptides of p12 and p15 all would be identical if recombination occurred within p30 with a single endogenous provirus. Furthermore, by this model, p10 would demonstrate no changes.

Finally, the role of viral structural proteins as "targets" in the *Fv-1* gene restriction phenomenon as yet is unclear. Restriction appears to involve the nucleic acid phase of replication prior to proviral integration. As yet, no clear role for p30 in this process has been determined. Our results, which show changes in viral p12 on host range conversion, suggest the possibility that this protein could be a target, either alone or in addition to viral p30. The validity of these conclusions must await a further clarification of the precise mechanism of the *Fv-1* effect.

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