Fv-1 Host Restriction of Friend Leukemia Virus: Oligonucleotide Analysis of Host Range Variants

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The *Fv-1* murine gene controls predominantly the replication of leukemia viruses of murine cells. Forced passage by B-tropic Friend leukemia virus in the restrictive host cells (NIH, *Fv-1^{n/n}*) results in viral progeny capable of replicating efficiently in murine cells of any *Fv-1* type, which are denoted as NB-tropic virus. We have studied the RNase T₁-resistant oligonucleotide pattern of a series of NB-tropic Friend virus isolates and have been able to show changes from the parental B-tropic virus which occur at the 5' end of the genome. Cloned NB-tropic virus falls into three classes, demonstrating either four, one, or no apparent changes in the genome. These results suggest the possibility that conversion to NB tropism occurs by a recombination mechanism but, since change to NB tropism can occur without any observable oligonucleotide alteration, they do not confirm that any single oligonucleotide is diagnostic of NB tropism.

The efficiency with which murine leukemia virus (MuLV) can infect mouse cells is predominantly controlled by the murine gene *Fv-1*. This dominant gene exists in two allelic forms, *n* and *b*, which, in the homozygous state, result in the restriction of growth of B-tropic and N-tropic viruses, respectively (1, 13). N-tropic viruses are restricted 100- to 10,000-fold on *Fv-1^{b/b}* cells, an effect which is known to occur intracellularly at an early phase of viral replication (9, 10, 16, 17). B-tropic viruses exhibit a similar restriction of growth in *Fv-1^{n/n}* cells. Biological studies of *Fv-1* host restriction with murine sarcoma virus pseudotyped with N, B, or NB-tropic MuLV or phenotypically mixed N- and B-tropic MuLV suggest that host cells recognize a viral structural component to effect an *Fv-1*-mediated restriction of viral replication (1, 11, 14).

As part of an overall approach to the study of the *Fv-1* restriction, we generated a series of host range variants by in vitro forced passage of B-tropic Friend MuLV through NIH cells (*Fv-1^{n/n}*). Progeny derived in this way exhibit altered host range (NB tropism), that is, they plate equally well on cells of either *Fv-1* type. We analyzed the primary structure of *gag* gene proteins p15, p12, p30, and p10 of the parental B virus and several cloned NB-derived viruses. Our data (S. Duttagupta and R. Soeiro, Proc. Natl. Acad. Sci. U.S.A., in press) show that primary structural changes occur in the p15, p12, and p30 of the NB-tropic viruses, but that the p10 of the NB variants and the parental B appear to be identical. Our interpretation of the pattern of these changes led to the conclusion

that the altered host range viruses were derived by recombination between the exogenous parental B-tropic virus and, presumably, complete or partial sequences of endogenous murine viruses.

The studies to be presented here represent structural analysis of the RNA genomes of these same isolates. Cells infected with stock virus were radiolabeled with ³²P, released virus was harvested, and 70S viral RNA was purified by sucrose gradient sedimentation. The 70S viral RNA complex is composed of two genomes joined at the 5' end. As normally isolated, a fraction of the genomic RNA contains breaks which are revealed only on denaturation of the complex (6). Polyadenylic acid-containing viral RNA segments of heat-denatured 70S RNA were selected on oligodeoxythymidylic acid columns and separated according to size by sucrose gradient sedimentation. Viral genomic (35S) RNA and subgenomic fractions were collected, and each was digested to completion with RNase T₁. Analysis of the resultant oligonucleotides by two-dimensional polyacrylamide gel electrophoresis was carried out by a modification of the method of de Wachter and Fiers (3).

Figure 1a shows an autoradiogram of parental B-tropic Friend MuLV. At least 50 large oligonucleotides can be identified. Figure 1b represents a schematic of the major spots. For reference, the solid spots in Fig. 1b represent those larger oligonucleotides which are found exclusively in the 5' end of the genome; that is, they are absent in the fingerprints obtained with subgenomic-length RNAs.

In Fig. 1c, the autoradiogram of one of the

FIG. 1. Oligonucleotide analysis of genomic RNA. B-tropic Friend leukemia virus (kindly donated by Frank Lilly) was cloned by endpoint dilution, and stock virus was prepared by growth in BALB/c cells. NB-tropic variants were produced by infection of NIH cells at a multiplicity of infection of 1 to 5. The progeny of a confluent monolayer of such infected NIH cells was harvested 16 h after "refeeding." This released progeny, which is considered passage 1, was passed blindly in 0.5 ml of medium (multiplicity not determined) onto a second dish of subconfluent NIH cells. This process was repeated for a total of three passages. The host range of the resultant progeny was determined by the relative plating on NIH versus BALB/c cells by the UV-XC assay. Intact 35S as well as polyadenylic acid-containing subgenomic RNA of ^{32}P metabolically labeled 70S viral genome was selected by an oligodeoxythymidylic acid affinity column. After separation of 35S intact from subgenomic RNA by sucrose gradient velocity sedimentation, the RNA species were digested with RNase T_1 . RNase T_1 -digested oligonucleotides were separated in two dimensions by polyacrylamide gel electrophoresis. The first dimension (left to right) was carried out in a cylindrical gel with a 6 M urea buffer. After completion, the cylindrical gel was equilibrated in second-dimension electrophoresis buffer to reduce the concentration of urea. The cylindrical gel was then polymerized in a slab gel apparatus with 4% polyacrylamide stacking gel and 0.75% agarose. The second-dimension (top to bottom) gel (21% polyacrylamide) was subjected to electrophoresis until the bromophenol blue tracking dye migrated approximately 11 cm. The figure shows the autoradiogram of the separated oligonucleotides and a diagrammatic sketch of each. (a, b) B-tropic; (c, d) NB₁; (e, f) NB₃; (g, h) NB₄₂.

NB-tropic variants (NB₁) is shown. The overall pattern reveals that 37 out of 41 numbered oligonucleotides are, by electrophoretic criteria, identical to those of the parental B-tropic MuLV. However, several significant changes have occurred. In Fig. 1d, the schematic of the NB₁ oligonucleotides, four distinct changes are noted. Spots 22a, 17a, and 8a (see arrows) are clearly new oligonucleotides not found in the parental B virus. Furthermore, spot 15, present in the parental virus, is missing in NB₁ (see shaded spot marked by arrow). Analysis of subgenomic fragments of this host range variant reveals that spots 17a and 22a (solid spots) exist only in the complete 35S genome. Oligonucleotide 8a, although not at the extreme 5' end of the genome, appears to map in the 5' half. Spot 15, missing from the parental virus, is also found exclusively on the complete genome (Fig. 1b). Therefore, analysis of three of the four oligonucleotides found altered in the selection of NB₁ revealed that they are located at the extreme 5' end of the viral genome.

By comparison, in Fig. 1e and g, are shown similar analyses of two other NB-tropic isolates, NB₃ and NB₄₂, respectively. In the NB₃ isolate, only a single new oligonucleotide can be found, and there are no spots clearly missing from parental B. This is shown in the schematic for NB₃ (Fig. 1f) as the single oligonucleotide shown as a solid spot (see arrow). The NB₃ new oligonucleotide is not present in the analysis of the subgenomic fragments (not shown) and, therefore, as in NB₁, the change in genome structure appears localized to the 5' end. This single new spot (22a) appears to migrate identically with one of the new spots found in NB₁ (see arrow, Fig. 1d). However, a similar analysis of isolate NB₄₂ (Fig. 1g and h) reveals that this variant differs from both NB₁ and NB₃ in that there are no oligonucleotides observed which differ from parental B-tropic Friend MuLV.

The compilation of the analysis of seven such NB-tropic isolates is shown in Table 1. These host variants fall into three classes. NB₁, which contains four oligonucleotide changes, represents the sole isolate of class A. NB₃, containing a single spot change, represents a prototype of four separately isolated variants (NB₁', NB₂, and NB₄) grouped as class B. In the grouping with NB₄₂ (class C) are two others, NB₄₁ and NB_D, which fail to show any apparent spot changes.

These results, which demonstrate changes in the genome on conversion to NB tropism, confirm the studies of Hopkins and co-workers, in that they demonstrate significant changes in the 5' end of the genome (5, 6, 15). Our data differ from theirs, however, in showing that within a series of independently isolated variants derived

from a single cloned B-tropic stock, the change in the genome observed may not be a unique one. That is, more than one pattern of change is possible and, for at least three cases, no apparent oligonucleotide spot diagnostic of NB tropism could be identified. Since only an estimated 5% of the genome may be analyzed by this method, significant genome changes may be lost by this approach and would be resolved only by direct sequence analysis.

Similar comparison of the 3' half of parental B-tropic versus NB₁ virus (data not shown) shows that 26 large oligonucleotides which mapped to this region appear to be identical. This is of importance, since selection for retroviruses which differ immunologically in the envelope protein (2) reveals extensive changes in the RNase T₁-derived oligonucleotides from the envelope region of the genome, whereas those from the 5' (*gag*) sequence region of the genome were not changed. Such results were interpreted by Clements as the selection of random mutants in surface glycoprotein due to the presence of neutralizing antibody. Our data, which show oligonucleotide changes restricted to the 5' end of the genome, therefore imply that these changes are selected by the presence of *Fv-1* resistance, but do not clearly indicate a mechanism, either mutation or recombination, for these results.

However, our analysis of the pattern of *gag* gene proteins observed in NB variants (Duttagupta and Soeiro, in press) has led us to the conclusion that host range variation occurs by a recombinational mechanism. Additionally, the fact that one variant (NB₁) has been shown to contain at least four oligonucleotide spot differences from its parental B-tropic Friend MuLV is consistent with a recombination model. The single new oligonucleotide which appears in the class B NB-tropic variants is, by electrophoretic migration criteria, identical to one of the new spots found in the class A group. Since in class C this new oligonucleotide is not observed, it cannot by itself be classified as diagnostic of conversion to NB tropism.

We favor a model in which recombination has occurred between the parental B-tropic virus and an endogenous virus, which results in NB

TABLE 1. Analysis of NB-tropic isolates

Class	NB no.	No. of oligonucleotide differences	Location on genome	No. of isolates
A	NB ₁	4	5' end	1
B	NB ₃ (NB ₁ ', NB ₂ , NB ₄)	1	5' end	4
C	NB ₄₂ (NB ₄₁ , NB _D)	0	— ^a	3

^a —, Not determined.

variation. Thus far, all naturally occurring retroviruses isolated have been found to exhibit either N or B tropism (8; F. Lilly, personal communication). NB tropism may only be associated with laboratory-passaged isolates. These facts suggest that the appearance of an NB-tropic variant may be due to a recombinational event between B-tropic virus and an endogenous virus of either N- or B-tropic host range. Alternatively, recombination with an endogenous xenotropic virus is possible. A mechanism for the generation of B-tropic virus by recombination between an N-tropic MuLV and a xenotropic virus has recently been suggested (7). The studies of Ellis et al. (4) have also suggested the possibility of recombination between eco- and xenotropic virus in MuLV isolated during the generation of radiation-induced leukemia in mice. Their work also emphasizes variation of both the protein and the RNA structure of the 5' end of the genome and suggests the possibility that recombination may be responsible for such virus polymorphism. Our protein studies reveal several tryptic peptide patterns of p30 among the different classes of NB variants defined in this report. However, the patterns of NB p12 and p15, although changed from the parental B-tropic type, appear identical with each other. The model which these data suggested is that of a single crossover event somewhere within the region of the p30 gene. These current RNA studies are consistent with those results. We would suggest that class A NB-tropic variants result from a crossover to the 3' direction of that observed in class B. This might result both in the appearance of a new shared oligonucleotide and also explain the new nonshared spot differences. In class C, the recombination could fall to the 5' direction of class B, apparently resulting in the appearance of no new oligonucleotide spots.

In conclusion, these studies confirm that oligonucleotide changes within the 5' end of the genome occur on host range conversion from B to NB tropism. However, they reveal the new information that, associated with host range variation, more than one pattern of genomic RNA structure has been observed. Either single, multiple, or no apparent oligonucleotide alterations have been demonstrated. These studies suggest the possibility, consistent with our protein analyses, that recombination at any one of several sites within the 5' region of the genome, perhaps within the p30 gene region, can result in host range variation. As with our protein data, these oligonucleotide studies suggest that more than one potential site for crossover in the 5' end of the genome can result in NB conversion. Such a conclusion, however, remains for identification of the viral genome with which recombination

may have occurred and a sequence analysis of the *gag* gene regions.

This work was supported by grants MV40 from the American Cancer Society and T30-CA1330 from the National Cancer Institute. S.D. is supported by training grant 532-CA09060 from the National Cancer Institute.

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