JOURNAL OF VIROLOGY, Apr. 1979, p. 116-123
0022-538X/79/04-0116/08$02.00/0

Vol. 30, No. 1

Assignment of the Large Oligonucleotides of Vesicular Stomatitis Virus to the N, NS, M, G, and L Genes and Oligonucleotide Gene Ordering Within the L Gene

JON P. CLEWLEY AND DAVID H. L. BISHOP*

Department of Microbiology, The Medical Center, University of Alabama in Birmingham, Birmingham, Alabama 35294

Received for publication 9 November 1978

Analyses of prototype vesicular stomatitis (VSV, Indiana serotype) mRNA-^{32}P-labeled viral RNA duplexes have established the assignments of 65 of the 72 large oligonucleotides that are recovered by two-dimensional electrophoresis of RNase T_1 digests of the viral RNA. Fifty of the oligonucleotides are recovered in the L RNA duplex, four each in the N, M, and NS duplexes, and three in the G RNA duplex. Studies of three small defective-particle RNA species indicate that they have only L gene oligonucleotides in addition to three of the seven unassigned oligonucleotides. Some L gene ordering of oligonucleotides can be postulated from the defective-particle RNA sequence analyses. Analyses of naturally occurring alternate isolates of VSV Indiana have established that by comparison to the prototype virus strain, the alternate isolates minimally have genome sequence differences in L, G, N, NS and/or unassigned regions of the genome. Changes in the genome have also been induced by in vitro high-level mutagenesis of the prototype virus.

The genome of the rhabdovirus vesicular stomatitis virus, Indiana serotype (VSV Indiana), consists of a negative-sense, single-stranded RNA molecule with a molecular weight of 3.8 $\times 10^6$ (14). The five viral complementary mRNA species of VSV Indiana code for a glycoprotein G, a membrane protein M, a major nucleocapsid protein N, and two components of the virion transcriptase, L and NS (4, 20). The gene order of the virion RNA has been shown to be: 3' ... N-NS-M-G-L ... 5' (1, 2).

We have previously compared various rhabdoviruses by oligonucleotide fingerprinting their genomes, using two-dimensional polyacrylamide gel electrophoresis of the RNase T_1-resistant oligonucleotides derived from ^{32}P-labeled genome RNA (6, 18; J. P. Clewley and D. H. L. Bishop *In* D. H. L. Bishop (ed), *Rhabdoviruses*, in press). In addition to prototype VSV Indiana, we have fingerprinted the genomes of alternate isolates of that virus (the Colorado, San Juan, and New Mexico topotypes), other rhabdovirus serotypes, including VSV New Jersey (four isolates), Chandipura (two isolates), spring viremia of carp virus (one isolate), Cocal virus (one isolate), and three attenuated strains of rabies virus (6, 18; Clewley and Bishop, in press). From these analyses it was shown that the genome of each rhabdovirus isolate has a characteristic fingerprint. A total of 71 large oligonucleotides were identified in the fingerprints of the genome of prototype VSV Indiana.

In the study reported here we have isolated five mRNA-^{32}P viral RNA duplexes and determined: (i) which of the large oligonucleotides of the genome of the prototype strain of VSV Indiana correspond to the N, NS, M, G, and L duplexes and (ii) which are not found in these duplexes and presumably represent genome or mRNA end or intercistronic sequences. Furthermore, by comparing the fingerprints of the alternate isolates of VSV Indiana and mutagenized derivatives of the prototype strain, we have been able to document where genome changes have occurred.

Oligonucleotide fingerprint analyses of various defective interfering (dI) derivatives (T particles) of cloned prototype VSV Indiana (12), have allowed us to postulate some oligonucleotide ordering of the L gene.

MATERIALS AND METHODS

Virus. The source of the virus prototype strain of VSV Indiana used in this study has been described (6, 12).

Infection of cells and virus purification. The methods used for the preparation and purification of ^{32}P-labeled virus have been described previously (6).

Oligonucleotide fingerprinting of RNA. Oligonucleotide fingerprinting utilized the method of de Wachter and Fiers (9) as modified by Clewley and

associates (7). Double-stranded RNA samples were heat denatured at 100°C for 2 min in the presence of 10 U of RNase T_1, quick frozen, then incubated at 37°C for 30 min after the addition of a further 10 U of the enzyme. Before loading on the first-dimension gel, the digest was incubated at 60°C for 3 min. After the second-dimension electrophoresis, gels were exposed to Kodak X-Omat R film with Du Pont Hi-Plus intensifier screens and stored at −70°C (19).

Isolation and purification of ^{3}H-mRNA. Confluent monolayers of BHK cells in Blake bottles were incubated for 1 h at 37°C in the presence of 5 μg of actinomycin D per ml of medium. The medium was removed, and the cells were overlaid with 0.5 ml of a suspension of sucrose gradient-purified virus (multiplicity of infection, 50 PFU/cell) and 100 μg of DEAE-dextran. After 30 min at room temperature, the inoculum was removed and replaced with 30 ml of Eagle medium containing 5% (vol/vol) calf serum, 150 μg of actinomycin D, and 50 μCi of [^{3}H]uridine. Infected cells were incubated at 37°C for 6 h. The medium was then removed, the monolayers were washed twice with phosphate-buffered saline, and the final traces of liquid were removed by aspiration. The cells were then frozen at −20°C and, after thawing, removed from the glass with 5 ml of 0.01 M Tris-hydrochloride, 0.4 M NaCl, 1% sodium dodecyl sulfate (pH 7.4), and RNA extracted as described previously (6, 7). After the addition of ethanol to the final aqueous phase, DNA was spooled out of solution. RNA was recovered from the remaining ethanol mixture by centrifugation. It was further purified by dissolving in 1 M NaCl–0.01 M Tris-hydrochloride (pH 7.4), frozen in a dry ice–ethanol bath, brought to 0°C, then immediately centrifuged at 10,000 rpm for 30 min to recover single-stranded RNA (19). Poly(A)-containing mRNA was selected by oligo-dT cellulose chromatography as previously described (8).

Formamide gel electrophoresis of single-stranded RNA. Formamide gels were prepared and run as described by Duesberg and Vogt (10). The gels were impregnated with 2,′5-diphenyloxazole for fluorography, dried, and exposed to X-ray film at −70°C as described elsewhere (5).

Annealing of viral RNA species and generation of double-stranded RNA duplexes. ^{3}H-labeled mRNA and ^{32}P-labeled virion RNA were annealed together, and the RNase resistance was determined by using procedures described elsewhere (3). After determining the optimum conditions for complete annealing within 2 h, a preparative annealing reaction in a volume of 1 ml of 0.4 M NaCl–0.05 M Tris-hydrochloride–1 mM EDTA (pH 8.0) was incubated for 2 h at 60°C. RNA was recovered by precipitation with ethanol, dissolved in 1 ml of 5% (vol/vol) glycerol–2.5 mM $ZnCl_2$, 0.1 M NaCl–0.07 M sodium acetate (pH 4.5), containing 10 U of nuclease S_1, 25 U of RNase T_2, 20 U of RNase T_1, and 0.2 μg of RNase A, and incubated at 37°C for 30 min. Nucleases were removed by phenol extraction, and duplex RNA was separated from nucleotides by gel filtration on Sephadex G-50 (17). The RNA was precipitated from the void volume with ethanol and resolved by discontinuous gel electrophoresis by the method of Laemmli (13). For this electrophoresis, the resolving gel concentration was 12%, whereas the stacking gel concentration was 3%. Electrophoresis was at 20 mA for 18 h. The positions of the RNA bands were determined by autoradiography; they were then cut out, and the RNA was recovered as described previously (19).

RESULTS

Oligonucleotide fingerprints of VSV Indiana viral RNA sequences recovered in individual mRNA-^{32}P viral RNA duplexes. Purified total ^{3}H-labeled mRNA preparations obtained from prototype VSV Indiana-infected cells, as described in Materials and Methods, were analyzed by formamide gel electrophoresis either before or after selection of poly(A)-containing species by oligo-dT cellulose chromatography (Fig. 1). For both samples, four major

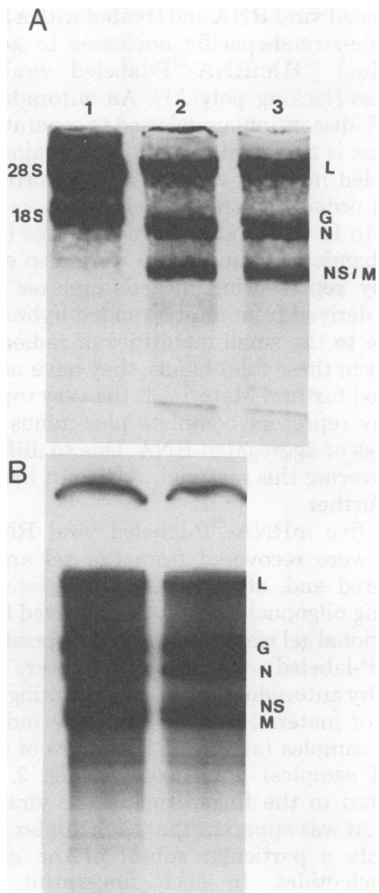

FIG. 1. *Electrophoresis of ^{3}H-labeled VSV mRNA's on a 3.5% polyacrylamide gel in 99% formamide. (A) Lane 1, BHK 28S and 18S rRNA markers; lane 2, VSV mRNA before chromatography on oligo (dT)-cellulose; lane 3, VSV mRNA after chromatography on oligo (dT)-cellulose. (B) Electrophoresis of mRNA-^{32}P-labeled viral RNA duplexes on a 12% discontinuous polyacrylamide gel.*

bands of RNA were observed. By reference to the marker 28S and 18S ribosomal RNA species (lane 1), a 31S L mRNA was identified as well as the faster-moving 17S G and 15S N mRNA species. The 12S NS and M RNA species were not resolved from each other (16). Material at the top of the gel of the unselected RNA sample (lane 2), but absent from that selected by oligo-dT cellulose (lane 3), was presumed to be 42S viral RNA. It was not analyzed further.

The viral complementary nature of the oligo-dT-selected [^{3}H]RNA species was determined by annealing portions of it to low-specific-activity, ^{32}P-labeled virion RNA. It was found that under conditions of [^{32}P]RNA excess, the ^{3}H label was rendered almost completely RNase resistant. An excess of the poly(A)-containing mRNA was annealed to high-specific-activity ^{32}P-labeled viral RNA and treated with a battery of single-strand-specific nucleases to generate individual [^{3}H]mRNA-^{32}P-labeled viral RNA duplexes [lacking poly(A)]. An autoradiograph of a 12% discontinuous gel used to separate these duplexes is shown in Fig. 1B. Five major bands of labeled material were observed, corresponding (in order of increasing electrophoretic mobility) to L, G, N, NS, and M duplexes (11, 15). Faint bands of radioactivity were also evident, possibly representing nicked duplexes or duplexes derived from multistranded hybrids (Fig. 1). Due to the small quantities of radioactivity present in these faint bands, they have not been analyzed further. Material at the very top of the gel may represent complete plus-minus strand duplexes or aggregated RNA. Due to difficulties in recovering this material, it has not been analyzed further.

The five mRNA-^{32}P-labeled viral RNA duplexes were recovered from the gel and heat denatured and, after RNase T_1 digestion, the resulting oligonucleotides were resolved by two-dimensional gel electrophoresis. The positions of the ^{32}P-labeled oligonucleotides were determined by autoradiography. The resulting fingerprints of material derived from the individual duplex samples (as well as a mixture of the NS and M samples) are shown in Fig. 2. When compared to the fingerprint of the viral RNA (Fig. 3), it was apparent that each duplex sample had only a particular subset of the genomic oligonucleotides. In each fingerprint, minor amounts of other genomic oligonucleotides were evident, presumably derived from contaminating, partially degraded duplexes (Fig. 1).

In previous fingerprint studies of the genome RNA of prototype VSV Indiana (6), 71 large oligonucleotides were assigned arbitrary numbers (Fig. 3). Although most appear to be unique by both visual inspection and pancreatic RNase digestion (unpublished data), it has been determined that there are two oligonucleotides which were assigned no. 44 (i.e., 44A, 44B), giving a total of 72 oligonucleotides (no. 1–43, 44A, 44B, 45–71). However, whether there are more than six oligonucleotides in the region where oligonucleotides 18 through 23, or seven where oligonucleotides 46 through 52 are recovered cannot be determined due to overlap of the oligonucleotides in those regions (Fig. 4). Pancreatic RNase analyses of recovered oligonucleotides from those regions of the gel have not resolved these issues.

Using the nomenclature proposed previously, it has been possible to assign 65 of the 72 viral oligonucleotides to the 5 VSV Indiana genes (Fig. 5). The remaining 7 oligonucleotides were not recovered in these genes and presumably either represent end or intercistronic sequences that are removed by the procedure used to derive duplexes (see Discussion).

L duplex. Of the 72 large oligonucleotides of the prototype VSV Indiana genome, 50 were recovered as major components in the L mRNA-viral RNA duplexes (Fig. 5). That most of the viral large oligonucleotides were recovered in this duplex in part reflects the fact that this mRNA codes for the largest viral polypeptide (3, 20). It is also possible that the rest of the genome has more guanylic residues than the L gene, resulting in fewer large oligonucleotides from these sequences. The faint spots in the L (and other) duplexes probably represent contaminating viral RNA or, in the case of the other duplexes, partially degraded larger duplexes.

G duplex. Although three large genomic oligonucleotides (no. 31, 57, and 61) were assigned to the G gene, other unique oligonucleotides were evident (e.g., above oligonucleotide 44). None of these oligonucleotides were evident as major spots in any of the other duplex patterns.

N duplex. Four large oligonucleotides of the viral genome (no. 11, 13, 58, and 65) were assigned to the N gene. None of the four oligonucleotides were evident as major spots in the other duplex patterns.

M duplex. Four large oligonucleotides (no. 32, 35, 52, and 71) were identified in the M duplex pattern. None of these oligonucleotides were present as major spots in the L, G, or N patterns. However, all four were present in the pattern obtained for the NS sample, as indicated by the NS and M coelectrophoresis. In the coelectrophorogram, it also appears that some N-type oligonucleotides were present, presumably derived from degraded N duplexes.

NS duplex. In addition to the four M oligo-

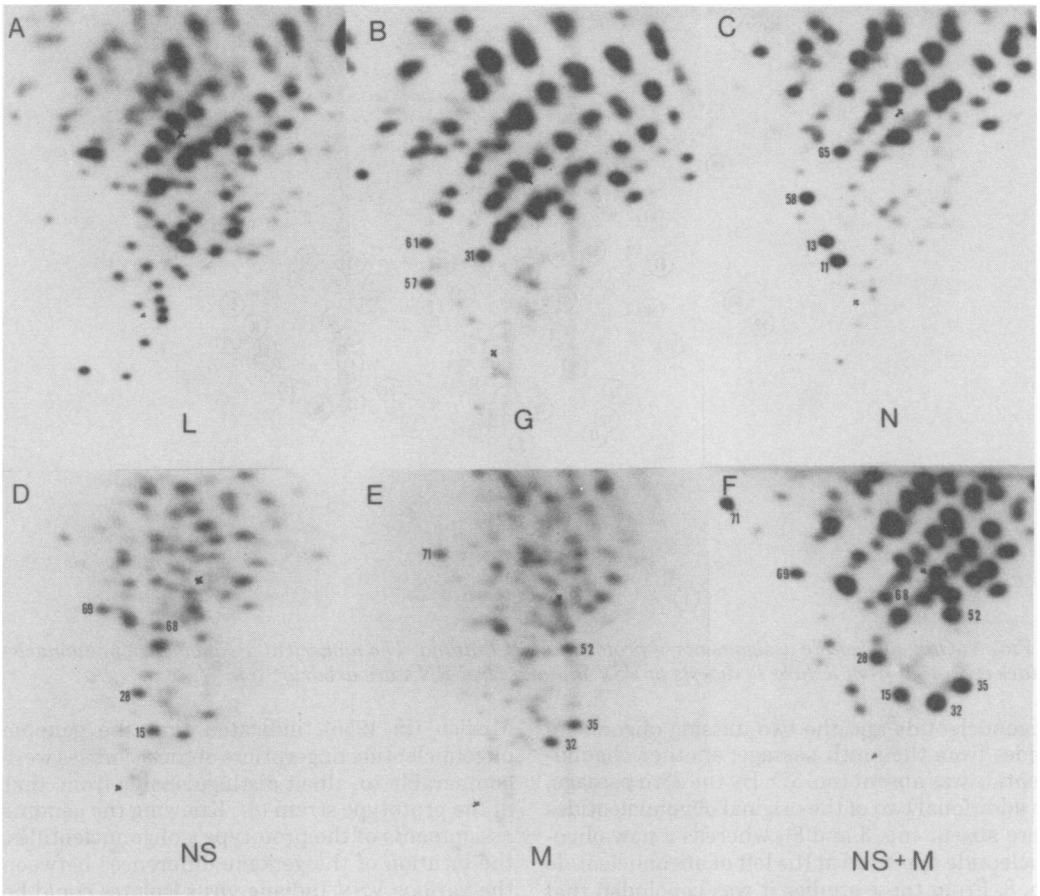

FIG. 2. *Oligonucleotide fingerprints of ^{32}P-labeled oligonucleotides obtained from mRNA-^{32}P viral RNA duplexes. (A) L duplex. (B) N duplex. (C) G duplex. (D) NS duplex. (E) M duplex. (F) Coelectrophoresis of NS and M duplexes. In this and in all other fingerprints, electrophoresis in the first dimension is from left to right, and in the second dimension it is from bottom to top. X indicates position of two dye markers. (Bromophenol blue, top right; xylene cyanol FF, lower left).*

nucleotides, the NS sample gave a pattern containing four other genomic oligonucleotides (no. 15, 28, 68, and 69). These latter four oligonucleotides were not found in the M, G, N, or L patterns. Due to the difficulty in separating NS and M duplexes (Fig. 1B), it is probable that the M oligonucleotides present in the NS pattern represent contaminating M duplexes.

Unassigned oligonucleotides. Seven viral oligonucleotides (no. 3, 38, 42, 43, 44B, 59, and 70) could not be assigned to one or other of the mRNA-viral RNA duplexes.

In vitro evolution of the genome of prototype VSV Indiana by consecutive high-level mutagenesis. To evaluate the effect of high-level mutagenesis on the stability of the genome of prototype VSV Indiana, a series of experiments were undertaken involving two independent series of consecutive high-level mutagenesis, plus control nonmutagenized series. The protocol involved growth for 2 days at 38°C of cloned stocks of VSV Indiana in the presence of 400 μg of 5-fluorouracil per ml of growth medium, plating at 38°C the viable progeny (representing two logs of progeny virus) in the absence of mutagen, selecting a clone at random, recloning, and growing a stock of virus from the secondary clone. The control series was processed identically, except that no mutagen was employed. Fingerprints were obtained for various passages of two series of consecutively mutagenized virus as well as for the nonmutagenized control series (Fig. 4).

It was found that, by the ninth passage of the first mutagenesis series, oligonucleotides 71 and 64 were absent (Fig. 4), and a new oligonucleotide to the right of no. 71 was present (Fig. 4). By the 21st passage, in addition to the new

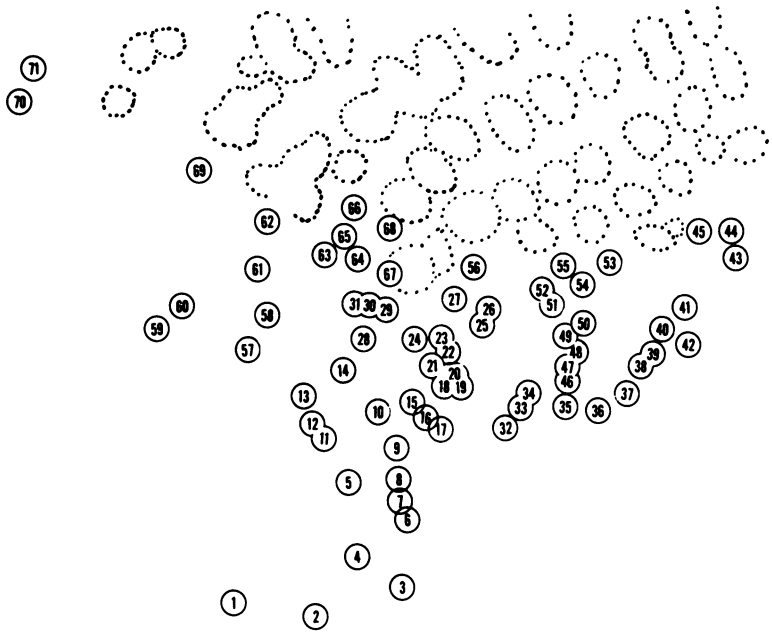

FIG. 3. *Oligonucleotide assignments of prototype VSV Indiana. The numerical assignments of oligonucleotides recovered from RNase T_1 digests of VSV Indiana viral RNA are arbitrary (6).*

oligonucleotide and the two missing oligonucleotides from the ninth passage, another oligonucleotide was absent (no. 57). By the 23rd passage, an additional two of the original oligonucleotides were absent (no. 3 and 8), whereas a new oligonucleotide appeared at the left of oligonucleotide no. 8. From these studies it was concluded that not only could the genome fingerprint of VSV Indiana be changed by high-level consecutive mutagenesis but also that the loss or addition of oligonucleotides that occurred during earlier mutageneses was inherited.

From the gene assignments described above it was concluded that, by passage 9, sequence changes had occurred involving oligonucleotides in the M gene (no. 71) and L gene (no. 64). By passage 21, a change had occurred in the G gene (no. 57) whereas, by passage 23, additional changes had occurred in the L gene (no. 8), as well as in an unassigned sequence (no. 3).

Similar results were obtained for the second mutagenesis series with inherited losses of other oligonucleotides and acquisition of additional oligonucleotides. By contrast, through 24 consecutive passages no changes were observed in any of the control nonmutagenized series (data not shown).

Analyses of alternate isolates of VSV Indiana. Previous studies of VSV Indiana isolates representing topotypes of the virus obtained originally in New Mexico (in 1966), Colorado (in 1942), and at the San Juan reservation, New Mexico (in 1956), indicated that the genome oligonucleotide fingerprints of these viruses were comparable to, albeit distinguishable from, that of the prototype strain (6). Knowing the genome assignments of the prototype's oligonucleotides, the location of the genome differences between the various VSV Indiana virus isolates could be deduced.

By comparison with the fingerprint of the prototype virus, the Colorado strain of VSV Indiana lacks six oligonucleotides (no. 9, 42, 46, 57, 59, and 61). Of these oligonucleotides, two are L gene oligonucleotides (no. 9 and 46), two are G gene oligonucleotides (no. 57 and 61) and two are unassigned (no. 42 and 59). The New Mexico variant of VSV Indiana does not have 11 oligonucleotides that are present in the prototype strain (no. 7, 10, 15, 16, 24, 25, 49, 57, 58, 63, and 65). Of these oligonucleotides, seven have been assigned to the L gene (no. 7, 10, 16, 24, 25, 49, and 63), two to the N gene (no. 58 and 65), and one each to NS gene (no. 15) and G gene (no. 57). The San Juan isolate of VSV Indiana does not have 11 oligonucleotides (no. 1, 2, 3, 16, 26, 37, 57, 58, 62, 63, and 69). Of these, seven are assigned to the L gene (no. 1, 2, 16, 26, 37, 62, and 63) and one each to NS (no. 69), N (no. 58), and G (no. 57), whereas oligonucleotide no. 3 is unassigned.

DISCUSSION

From the fingerprints of the mRNA-viral

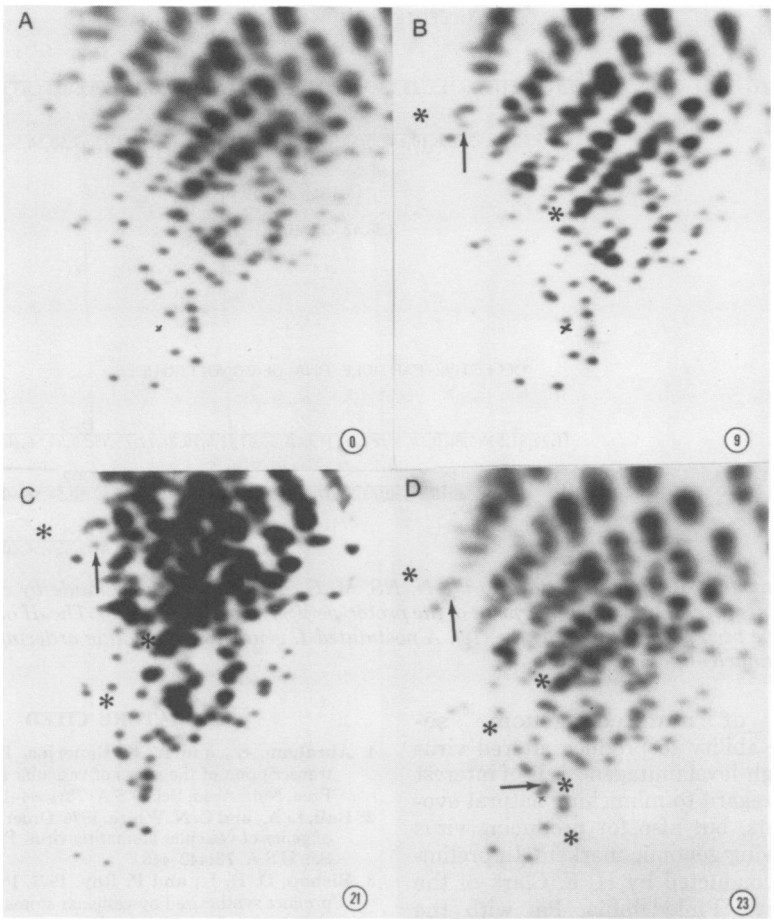

FIG. 4. *Effect of consecutive high-level mutagenesis on the genome oligonucleotide fingerprints of VSV Indiana. (A) Starting clone; (B) 9th passage; (C) 21st passage; (D) 23rd passage. Oligonucleotides that by comparison to the original clone are not present in the mutagenized virus passages are indicated by stars, new oligonucleotides are indicated by arrows.*

RNA duplexes generated using ^{32}P-labeled virion RNA, we have been able to assign 65 of the 72 large oligonucleotides of VSV Indiana genome RNA. The seven unassigned oligonucleotides may either be present in nontranscribed end or intercistronic sequences, or represent sequences that are only partially transcribed (e.g., at the ends of the mRNA species), or be present near the ends of the duplexes but are removed during their selection.

Defective interfering particle RNA oligonucleotide sequences. During studies involving the generation of small defective interfering (dI) particles, three dI RNA species were identified and characterized by oligonucleotide fingerprinting (12). With the information generated in the duplex analyses described here, we can correlate the oligonucleotide assignments with the oligonucleotides we have described previously (12) for three dI RNA species (Fig. 5).

Except for three oligonucleotides, all of the oligonucleotides identified in the dI RNA fingerprints are L gene oligonucleotides. The three other oligonucleotides are no. 38, 59, and 70, and they are unassigned. Since the largest dI examined (dI-3) has all the oligonucleotides found in dI-2 (as well as 4 additional ones), whereas the dI-2 RNA has all the oligonucleotides found in dI-I (as well as 10 additional ones), it is probable that oligonucleotide no. 38, 59, and 70 are end sequence oligonucleotides. Although we cannot exclude the possibility that these three oligonucleotides are derived from intercistronic regions (e.g., between the G and L genes), or the 3′ end of the genome, it is probable that they originate from the 5′ end of the L gene. Both 3′ and 5′ end sequencing of the dI and viral RNA species will be needed to resolve this issue.

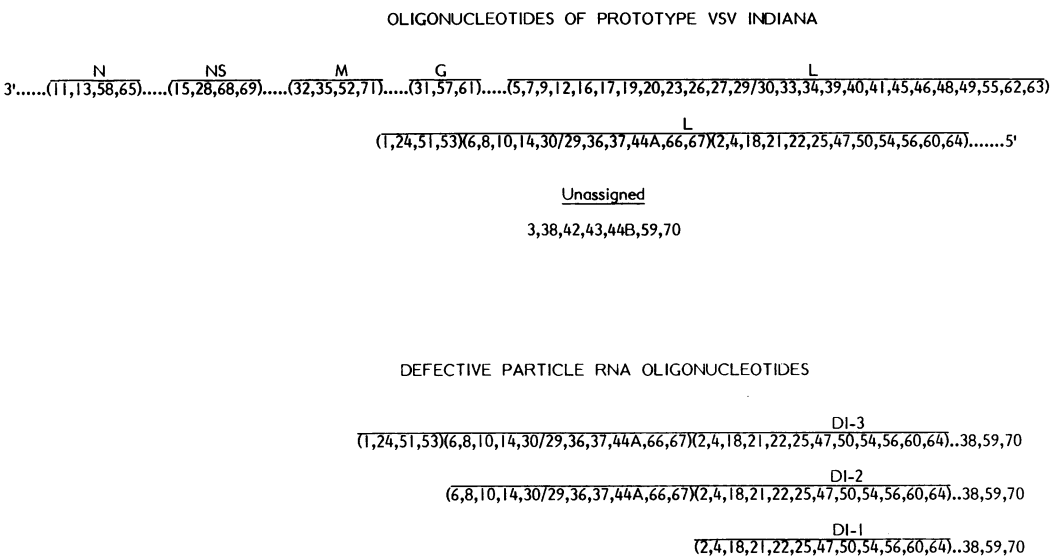

FIG. 5. *Assignments of oligonucleotides to the N, NS, M, G, and L genes were made by comparing the duplex fingerprints (Fig. 2) with the fingerprint of the prototype VSV Indiana virus (6). The dI oligonucleotide assignments have been published elsewhere (12). A postulated L gene oligonucleotide ordering is presented from the dI oligonucleotide data.*

Derivation of mutated genome sequences. The ability to produce altered virus genomes by high-level mutagenesis is of interest not only with regard to mimicking natural evolutionary events, but also for producing virus derivatives having genomic markers. In preliminary studies conducted by H. F. Clark of the Wistar Institute, Philadelphia, Pa. with the highly mutagenized virus (passage 27 of the series shown in Fig. 4), it appears that despite the fact that the mutagenized virus grows in tissue culture as efficiently as the original, or control, nonmutagenized virus stock, its ability to kill 15- to 20-day-old mice inoculated by intraperitoneal or intraplantar routes is significantly lower (ratios of 50% lethal doses to PFU decreased by 3 to 4 logs). Whether high-level mutagenesis can be used to produce attenuated vaccine strains of virus remains to be determined.

The alterations produced by 23 consecutive mutageneses involve a minimum of five changes in the viral genome. Since the oligonucleotide fingerprint of the prototype strain of VSV Indiana corresponds to approximately 10% of the VSV genome (6), the 5 changes may reflect alterations involving on the order of 50 genome nucleotide changes.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant A1 13402 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Abraham, G., and A. K. Banerjee.** 1976. Sequential transcription of the genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **73**:1504–1508.
2. **Ball, L. A., and C. N. White.** 1976. Order of transcription of genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. **73**:442–446.
3. **Bishop, D. H. L., and P. Roy.** 1971. Properties of the product synthesized by vesicular stomatitis virus particles. J. Mol. Biol. **58**:799–814.
4. **Bishop, D. H. L., and M. S. Smith.** 1977. Rhabdoviruses, p. 169–280. *In* D. P. Nayak (ed.), The molecular biology of animal viruses. M. Dekker Inc., New York.
5. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
6. **Clewley, J. P., D. H. L. Bishop, C.-Y. Kang, J. Coffin, W. M. Schnitzlein, M. Reichmann, and R. E. Shope.** 1977. Oligonucleotide fingerprints of RNA species obtained from rhabdoviruses belonging to the vesicular stomatitis virus subgroup. J. Virol. **23**:152–166.
7. **Clewley, J. P., J. Gentsch, and D. H. L. Bishop.** 1977. Three unique viral RNA species of snowshoe hare and La Crosse bunyaviruses. J. Virol. **22**:459–468.
8. **Clewley, J. P., and S. I. T. Kennedy.** 1976. Purification and polypeptide composition of Semliki Forest virus RNA polymerase. J. Gen. Virol. **32**:395–411.
9. **de Watcher, R., and W. Fiers.** 1972. Preparative two dimensional polyacrylamide gel electrophoresis of ^{32}P-labeled RNA. Anal. Biochem. **49**:184–197.
10. **Duesberg, P. H., and P. K. Vogt.** 1973. Gel electrophoresis of avian leukosis and sarcoma viral RNA in formamide: comparison with other viral and cellular RNA species. J. Virol. **12**:594–599.
11. **Freeman, G. J., J. K. Rose, G. M. Clinton, and A. S. Huang.** 1977. RNA synthesis of vesicular stomatitis virus VII. Complete separation of the mRNA's of vesicular stomatitis virus by duplex formation. J. Virol. **21**: 1094–1104.

12. **Kang, C. Y., T. Glimp, J. P. Clewley, and D. H. L. Bishop.** 1978. Studies on the generation of vesicular stomatitis virus (Indiana Serotype) defective interfering particles. Virology **84**:142–152.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (London) **227**:680–685.
14. **Repik, P., and D. H. L. Bishop.** 1973. Determination of the molecular weight of animal RNA viral genomes by nuclease digestions. I. Vesicular stomatitis virus and its defective T particles. J. Virol. **12**:969–983.
15. **Rhodes, D. P., G. Abraham, R. J. Colonno, W. Jelinek, and A. K. Banerjee.** 1977. Characterization of vesicular stomatitis virus mRNA species synthesized in vitro. J. Virol. **21**:1105–1112.
16. **Rose, J. K., and D. Knipe.** 1975. Nucleotide sequence complexities, molecular weights, and poly(A) content of the vesicular stomatitis virus mRNA species. J. Virol. **15**:994–1003.
17. **Roy, P., and D. H. L. Bishop.** 1973. Initiation and direction of RNA transcription by vesicular stomatitis virus virion transcriptase. J. Virol. **11**:487–501.
18. **Roy, P., and J. P. Clewley.** 1978. Spring viremia of carp virus RNA and virion-associated transcriptase activity. J. Virol. **25**:912–916.
19. **Vezza, A. C., J. P. Clewley, G. P. Gard, N. Z. Abraham, R. W. Compans, and D. H. L. Bishop.** 1978. Virion RNA species of the arenaviruses Pichinde, Tacaribe, and Tamiami. J. Virol. **26**:485–497.
20. **Wagner, R. R.** 1975. Reproduction of rhabdoviruses, p. 1–93. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 4. Plenum Press, New York.