BACTERIOLOGICAL REVIEWS, Dec. 1977, p. 811-821

Vol. 41, No. 4
Printed in U.S.A.

Viral Pathogenesis and Molecular Biology†

ALICE S. HUANG

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

INTRODUCTION 811
STRUCTURE OF VSV DI PARTICLES 812
INTERFERENCE BY DI PARTICLES 812
EFFECT OF AGE ON DI PARTICLE-MEDIATED INTERFERENCE 812
Encephalitis Caused by VSV in Young or Old Mice 813
Assay for DI Particles 814
Infection of Cells in Culture from Young or Old Mice 815
Cell Cultures for Studies on Viral Pathogenesis 815
ORIGIN OF VSV DI GENOMES 815
Subgenomic and Extragenomic Sequences 815
Complementary Sequences 816
Generation of DI Genomes During Replication of RNA 817
Significance of Complementary Termini in Viral Genomes 818
CONCLUSION 819
LITERATURE CITED 820

INTRODUCTION

Molecular biology applied toward understanding viral diseases has come of age. An exciting future is in store when questions of viral pathogenesis can be answered by biochemical analysis of animal viruses and their infected cells. This is not, however, a one-way street. Our experience has been that in designing experiments which attempt to answer questions related to disease processes new information is often gathered that contributes significantly to our thinking about general problems in molecular biology. This two-way exchange between molecular biology and viral pathogenesis will be illustrated here by recent examples in our work as well as in that of others.

Studies on the structure of animal viruses have been most rewarding. Identification and separation of viral structural proteins have led to an understanding of the components on the virion surface that determine attachment and penetration into host cells. An example of the importance of these viral surface proteins is that of paramyxoviruses. For this group of viruses, the envelope is covered by glycoproteins that must be cleaved before the virus particle can become fully infectious (14, 51). This suggests that attachment and penetration of this virus into new tissues and new hosts may be determined by the presence or availability of specific proteases. A recent study positively correlates cleavage of the hemagglutinating and fusing proteins of Newcastle disease virus to its virulence for chickens (25).

† Eli Lilly Award Address, May 1977.

Another way to study virulence is in the manipulation of conditional lethal mutants both genetically and biochemically. Among the many examples is influenza virus, where some of the genes responsible for virulence have been identified and physically separated (37). Knowing the gene product(s) that accounts for virulence may provide a new direction for viral prophylaxis or for treatment of a virus disease while it is in progress.

The utility of analyzing viral structural proteins is elegantly demonstrated by Arnon and colleagues (28) where a crucial oligopeptide of only 20 amino acids, derived from the coat protein of the bacteriophage ms-2, has been found to be essential for bacteriophage attachment to its host cell. When this oligopeptide is linked to a carrier, neutralizing antibodies against the bacteriophage are elicited. Modification of the antigenicity of this oligopeptide with other carriers and the ability to synthesize vast amounts of it chemically in the laboratory indicate a whole new era of antiviral vaccines.

Another potent tool for probing virus-infected cells is the ability to copy viral messenger ribonucleic acid (RNA) into highly labeled deoxyribonucleic acid (DNA) in vitro by using a virion-associated polymerase (2, 56). Labeled DNA is a very sensitive probe for detecting common sequences both in cells and among related viruses. Such an approach has opened up a whole era of study of endogenous viruses—viruses that are integrated into the genetic information of their hosts and are vertically transmitted from parent to offspring (32). The finding of identical or related sequences in different species has re-

vealed a history of the persistence and spread of endogenous viruses among different animal species during the evolution of these species. These endogenous viruses usually reside in a cryptic form without any expression of their information. What role thay play in viral diseases is only beginning to be understood.

These are just a few examples to indicate the areas where animal virology may bear fruit in the next few years. The precision of biochemical techniques can now be applied to questions of direct relevance to disease processes. My introduction to molecular animal virology began in the laboratory of Robert R. Wagner at Johns Hopkins University. We used rate-zonal centrifugation to separate infectious standard vesicular stomatitis virus (VSV) from its deletion mutants (18). This resulted in the first isolation and characterization of a defective interfering (DI) particle. Since then DI particles have been shown to be an important determinant of the outcome of viral infections (see reference 16).

STRUCTURE OF VSV DI PARTICLES

DI particles have all the same structural proteins of the standard virus from which they are derived and are, therefore, antigenically indistinguishable (18, 22, 58). Figure 1 illustrates the VSV structural proteins. There are only five proteins, one of which is glycosylated and two of which are phosphorylated. All five proteins are specified by the genome of standard VSV. The virions of VSV resemble bullets. Deletion mutants that form shorter bullets are readily separated from the standard virus. Analysis of DI particles suggests that the length of the bullet corresponds well with the length of the nucleocapsid and the length of the genome RNA (20). Recently, particles equal in length to standard VSV have been detected, which contain RNA with only one-third the complexity of the standard genome, suggesting that several equivalents of a DI RNA may become encapsulated into one long, bullet-shaped particle (D. Rao and A. Huang, unpublished observations).

The organizations of the nucleotide sequences of these DI RNAs are peculiar (see below). In this paper, a molecular model to account for the reorganization of the VSV genome into DI genomes will be presented. The potential significance of those rearrangements will be discussed.

INTERFERENCE BY DI PARTICLES

The finding of a DI agent immediately raises the possibility that such an agent might be useful in the prophylaxis of viral disease. A step towards achieving this goal is understanding the mechanism of interference. Over the years the important features of interference mediated by DI particles have been uncovered by using VSV as a prototype system for study. These features are applicable to other virus systems as well (see reference 16) and are summarized below: (i) interference occurs intracellularly and not at the cell surface; (ii) it is specific in that it is homotypic, i.e., interference occurs most strongly against the standard virus from which the DI particle was derived; with closely related viruses interference occurs to a lesser degree, and with unrelated viruses it does not occur at all; (iii) this phenomenon can exist independently of the interference mediated by interferon. The critical step during multiplication of VSV, Sindbis, polio, and simian virus 40 affected by DI particles is that of genome replication (see reference 16). Since these viruses have different strategies for replicating their nucleic acids, the molecular details of interference by specific DI particles are likely to vary for each individual virus group. This will also be reflected in other effects that may be secondary to interference with genome replication. For instance, those steps that occur after genome replication, or are dependent on prior genome replication, are likely to be inhibited also. Thus, during morphogenesis, there may be competition for limiting structural proteins.

Several parameters known to affect the degree of interference exerted by DI particles have been summarized (16). Most likely, others will be uncovered, and a knowledge of them may help in manipulating DI particles in prophylaxis of viral diseases. An important factor is the virus strain. Different isolates of a virus, which may be antigenically indistinguishable, show quantitative and qualitative differences with respect to the generation of DI particles. They may also show a variable degree of susceptibility to interference caused by a given amount of DI particles. Of particular importance is the species of host in which the virus is grown. Thus, the generation of DI particles and their ability to interfere are species specific and can even be seen in different tissues from one animal. In certain cases it has been shown that the degree of interference is affected by a single genetic locus within an inbred strain of mice (7). This host function affecting DI interference is thought to act on viral nucleic acid replication.

EFFECT OF AGE ON DI PARTICLE-MEDIATED INTERFERENCE

In the 1930's Sabin and Olitsky (47–50) published a series of papers on VSV in which they showed that mice 2 months or older are relatively resistant to acute encephalitis caused by

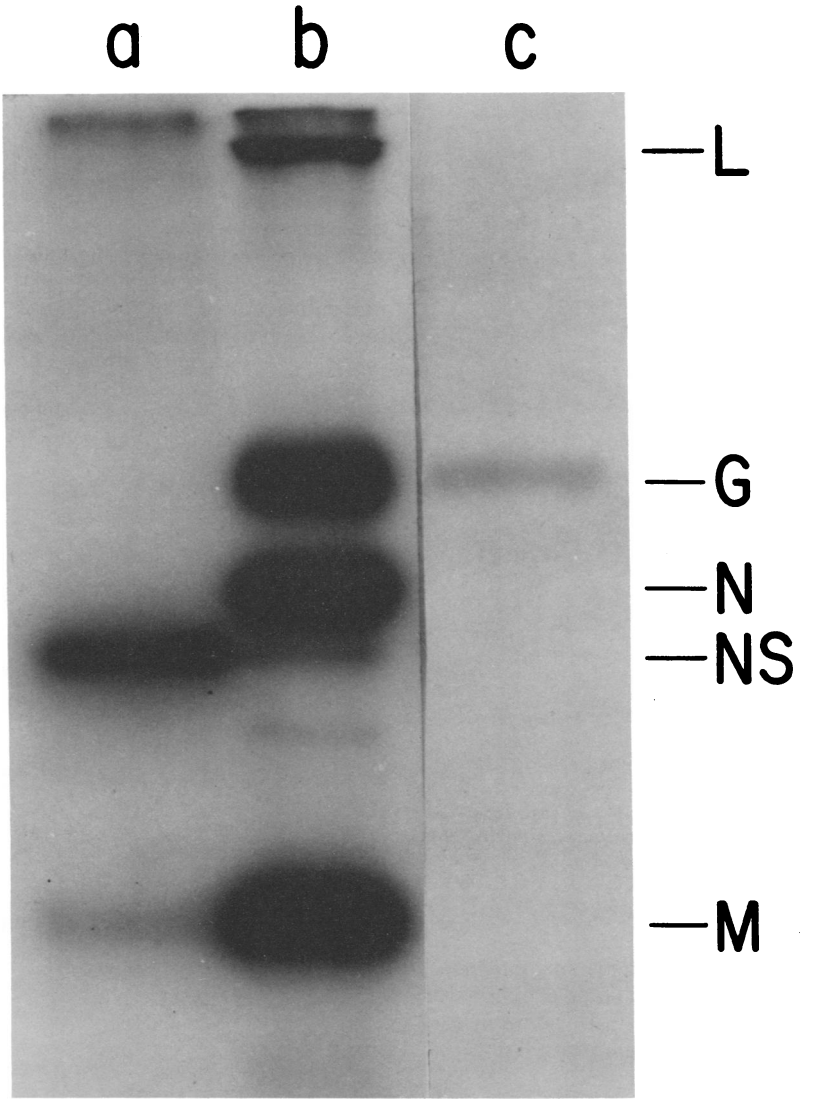

FIG. 1. *Structural proteins of VSV. Purified standard VSV grown in suspended Chinese hamster ovary cells (54) in the presence of three different isotopes was disrupted, and the polypeptides were separated on a 10% acrylamide gel containing sodium dodecyl sulfate (27). The VSV-specific protein-containing bands are labeled L, G, N, NS, and M. (a) ^{32}P; (b) [^{35}S]methionine; (c) [^{14}C]glucosamine.*

this virus. In contrast, VSV causes an encephalitis resulting in paralysis and rapid death in mice that are only a few weeks old. Sabin and co-workers were unable to detect in these animals any general systemic host response against VSV that occurs rapidly enough to neutralize the virus or to inhibit virus replication (36). They proposed the existence of "localized barriers" that develop immediately after virus infection and surround the sites of virus introduction so that the virus does not spread to the central nervous system. One mechanism for the development of such barriers may be production of DI particles.

Encephalitis Caused by VSV in Young or Old Mice

P. Sinarachatanant has confirmed these findings in a recent study of VSV-caused encephalitis in mice. Figure 2 shows the lethal dose (LD_{50}) of VSV necessary to cause a 50% mortality in young or old mice plotted against the days after intranasal inoculation. Young mice were 3 weeks old and old mice were 2 months old. The

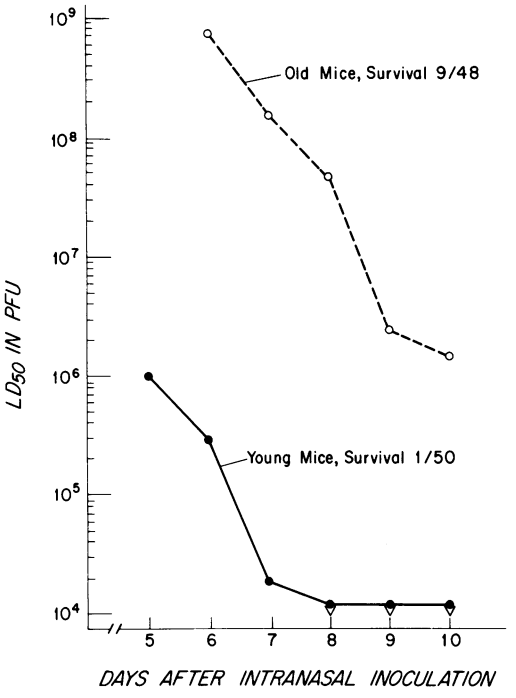

FIG. 2. *In vivo assay of VSV in young and old mice. CD-1 outbred female mice, 50 at 3 weeks of age and 48 at 8 weeks of age, were each divided into groups containing 8 to 10 mice. Each group was inoculated intranasally with purified standard VSV (54) at the indicated concentrations. The day that 50% of the mice in a group died (LD_{50}) is plotted versus the concentration of virus inoculated. (●) 3-week-old mice; (○) 8-week-old mice.*

old mice not only expired later than young mice, but a higher dose of purified standard virus was necessary to cause mortality. In addition, the overall survival rate was higher for old mice.

Assay for DI Particles

To measure DI particles from mouse brains, an assay system was developed that depends on the ability of DI particles to inhibit uridine incorporation into virus-specific RNA. It was demonstrated earlier that DI particles dramatically inhibit RNA synthesis by standard virus (19). Figure 3 shows the results of a reconstruction experiment. Different concentrations of DI particles were added to cells infected with a given concentration of standard VSV. The sensitivity of this assay, at 4.5 h after infection, indicates that DI particles at a ratio of 1:100 to standard VSV produced a significant degree of inhibition (30%). This ratio represents a lower limit of detection for DI particles of 2×10^5 per ml. Standard plaque-forming VSV was readily detected in the infected animal by making brain homogenates, especially if care was taken to include the rhinencephalon of the mouse along with the rest of the brain. Unfortunately, this assay was unable to detect DI particles from a single brain. While this work was in progress, Holland and Villarreal (13) reported that they were able to detect in vivo synthesized DI particles of VSV only if they pooled the brains of six mice. It became clear in our measurements that as the titer of standard virus rose in the individual brains, death of the animal was presaged. Sinarachatanant was unable to detect DI particles in individual brains throughout the course of these VSV infections, whether the mice had expired or not. Neutralizing antibody in the brain was detected after day 12 in the older mice.

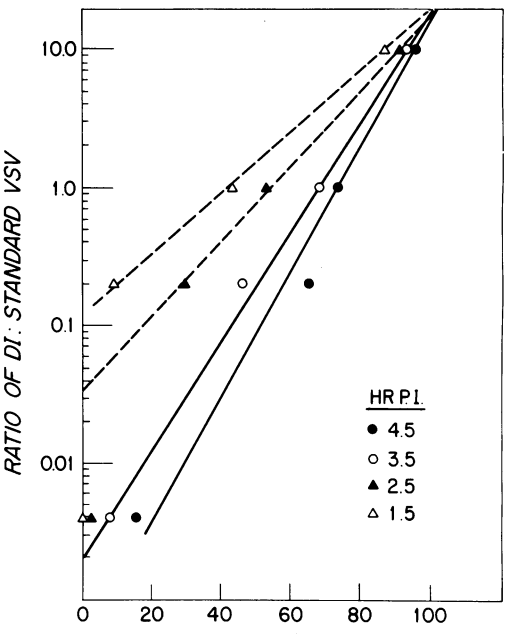

FIG. 3. *In vitro radioactive assay of VSV DI particles. Chinese hamster ovary cells (8×10^6) were infected with standard VSV at a multiplicity of 20 plaque-forming units and different concentrations of DI particles (19). The infected cells were incubated at 34°C in medium containing 10 μg of actinomycin D and 0.6 μCi of [^{14}C]uridine. Virus-specific RNA synthesis was measured as previously described (19). A control sample infected with only standard VSV gave the maximal amount of virus-specific RNA synthesis at each time period after infection (p.i.). The percentage of inhibition of RNA synthesis by DI particles is based on a comparison between VSV RNA synthesis in the presence of increasing concentrations of DI particles and VSV RNA synthesis in the absence of DI particles.*

A recent attempt to detect DI particles of lymphocytic choriomeningitis virus made in vivo was more successful. Popescu and Lehmann-Grube (45) were able by a very sensitive negative-plaque assay, based on the protection of cells by DI particles, to detect significant amounts of DI particles in various infected mouse tissues. The distribution and amounts of DI particles increased with age and led to the conclusion that DI particles indeed play a role in chronic, persistent infections.

Infection of Cells in Culture from Young or Old Mice

To adequately test whether or not DI particles formed the localized barriers in mice to VSV, cell cultures from mice were prepared. Johnson et al. found that fibroblast cells obtained from young and old mice grown in culture still maintain their relative susceptibility or resistance to infection by a togavirus (21). Infection of cell cultures made from the hind limb muscles of young and old mice with purified standard VSV at different multiplicities showed that cells from old mice, in contrast to cells from young mice, were more resistant, produced less infectious VSV per culture, and developed a cytopathic response more slowly, if at all (P. Sinarachatanant, unpublished observations). When these cells were infected with different ratios of DI to standard particles, it was shown that interference of VSV growth by DI particles as measured by RNA synthesis is more evident in cells from old animals than in cells from young animals (Fig. 4). As a control, it was determined that VSV attaches and penetrates equally well in both types of cell cultures.

The difference in the degree of interference exerted by a given concentration of DI particles between these two cell types is consistently 30% as measured by inhibition of RNA synthesis. This inhibition is significant because it is only an indirect measure of the inhibition of VSV replication; a 30% inhibition of RNA could reflect a reduction of 99% in the production of infectious standard virus during one cycle of growth (38). Therefore, these preliminary experiments indicate that DI particles may play a role, particularly during the initial stages of an infection, in limiting the spread of VSV in old animals. Considerable work along these lines is needed to define the precise relation between age and the synthesis of DI particles.

Cell Cultures for Studies on Viral Pathogenesis

Studies at the interface of molecular virology and viral pathogenesis are difficult to perform in the whole animal. There are too many parameters that the experimenter cannot control. Therefore, development of such systems, where cells from the animal can be put into culture and their environment can be carefully controlled, offers a great potential for these types of studies. A similar approach is to establish long-term cultures by transformation of fibroblast cells with simian virus 40 (7).

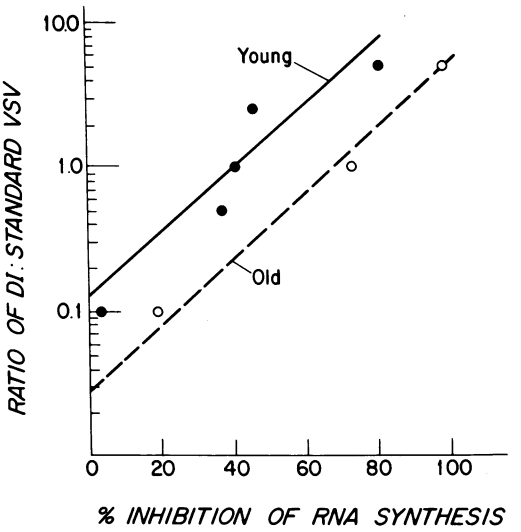

FIG. 4. *Interference by VSV DI particles in fibroblast cells from young and old mice. Fibroblast cell cultures were made from the hind limb muscles of two-week-old or one-year-old CD-1 outbred mice, according to Johnson et al. (21). Infection of these cells and RNA synthesis were measured as shown for Fig. 3. Only the results at 4 h postinfection were plotted.*

ORIGIN OF VSV DI GENOMES

Subgenomic and Extragenomic Sequences

The central issue, as well as the most intriguing aspects of interference by DI particles, lies in the particles' effect on viral nucleic acid synthesis (19, 39, 41). Most of the workers in the field suspect that inhibition during nucleic acid replication depends on competition between DI RNA and RNA for limiting enzymes. The success of this competition depends very much on the sequence organization along the two genomes. VSV DI particles contain less RNA than does standard virus (20). The three nucleotides at the 5′ and 3′ termini of the standard VSV genome are inverted complementary sequences, pppApCpGp . . . $CpGpU_{OH}$ (4, 5, 11). If defective RNA in DI particles were generated by internal deletions, one would expect that the two ends of the RNA would be identical to those of the standard virus RNA. On the other hand, there

could be end deletions in which one or the other of the 5′ or 3′ ends of the RNA molecule would differ from the parental standard virus RNA.

Hybridization studies by Reichmann and co-workers (29, 52) have shown that deletions in the DI RNA tend to be largely from the 3′ end of the genome. However, Lazzarini and co-workers (23) found that the three nucleotides at the 3′ ends of RNA from several strains of DI particles are identical to those of standard RNA.

To examine more than just the nucleotide sequences at the termini, D. Rao and G. Freeman in my laboratory have compared the T1 ribonuclease-generated oligonucleotides of a DI RNA to its standard RNA (Fig. 5). There are fewer spots in the fingerprint of the DI RNA compared with that of the standard virus RNA. This finding supports the idea of a lower complexity and confirms the finding that the molecular weight of the DI particle RNA is about one-third the size of a standard virus RNA (20). This analysis would, also, indicate whether or not the DI RNA contained only subgenomic fragments of the standard virus RNA. Most of the oligonucleotide spots, at or below the bromophenol blue dye marker, generated from DI RNA appeared to be included among the spots for standard virus RNA. However, the appearance of two extra spots in the DI RNA not seen in the B particle was quite striking. One spot is at the far right of the fingerprint for DI RNA, and the other is near the center, one-fourth of the way down from the X to the arrow marking the dye, bromophenol blue. Similar observations have been made by Coffin and Kang with other preparations of VSV DI particles (personal communications).

These fingerprints indicate clearly that DI RNA not only contains certain portions of the genome of the standard virus RNA, but that additional sequences have been included. Where do these additional sequences come from? Do they come from other virus-specified RNAs? Or are the extra nucleotide sequences obtained from nucleic acid molecules of the host?

Complementary Sequences

Several recent results are pertinent here. To repeat, Banerjee's group (4) and Lazzarini's group (23) have shown by an analysis of only three nucleotides that the 3′ ends contain the identical three nucleotides in both standard and DI genomes. Since the sequence of these three nucleotides is complementary to that found at the 5′ end of standard RNA, it is expected that some complementary sequences may be found in all RNAs of VSV DI particles. Jacques Perrault, in John Holland's laboratory, has been able to observe, in the electron microscope, circularized DI RNA and, also, dimer linear struc-

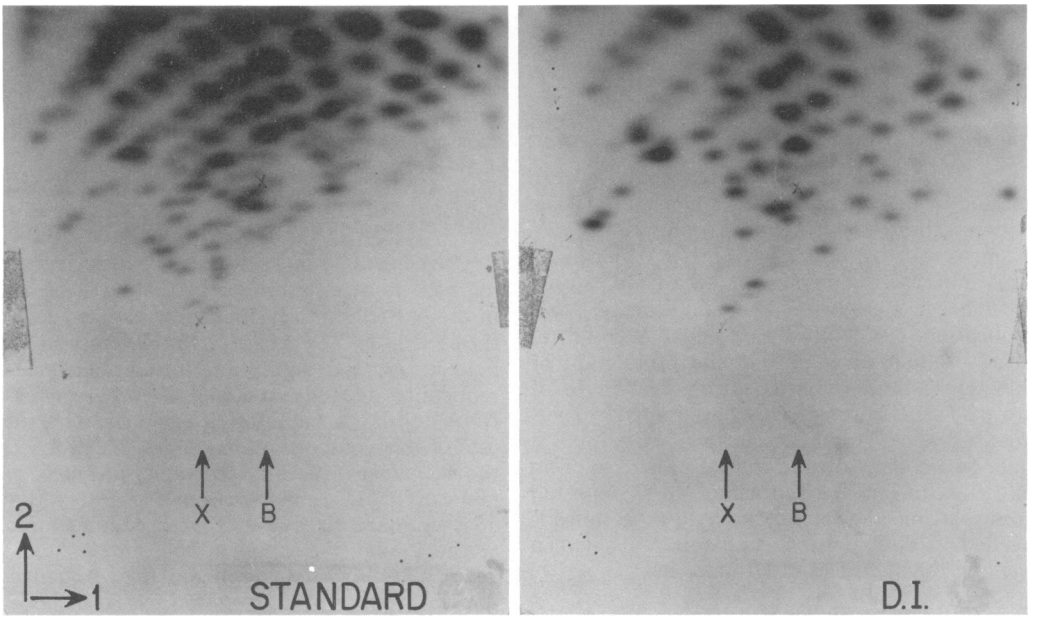

FIG. 5. *Ribonuclease T1-generated oligonucleotides of RNA from standard and DI particles of VSV. ^{32}P-labeled, purified standard VSV and DI particles were extracted and their RNAs purified on sucrose gradients. The RNAs were digested by T1 ribonuclease and fingerprinted on two-dimensional gels (8). (X) Xylene cyanol dye marker; (B) bromophenol blue dye marker.*

tures (42, 43). Similar circularization of Sindbis virus RNA (15) and Sendai virus DI RNAs (26) as well as the segmented bunyavirus RNA (12) has been detected. Such circularization suggests complementarity at the termini of these RNAs. In some cases, the RNA circles included "panhandle" structures, suggesting extensive inverted complementary sequences at the RNA termini (26, 42, 43).

A large variety of DI particles of VSV have now been generated (44, 46). Several groups have noted the ability of RNA molecules from these DI particles to rapidly self-anneal from a few to as much as 80% (30, 40, 42, 43). These results indicate that the RNA may have long regions of nucleic acid homology that are covalently linked within one molecule. This property of rapid intramolecular self-annealing has resulted in the term "snap-back" RNA and suggests very strongly that extra oligonucleotide sequences found in DI RNA may come from complementary RNA sequences.

Generation of DI Genomes During Replication of RNA

During replication of VSV, there is an ordered synthesis of nucleic acids. The strategy of RNA synthesis of VSV begins with the incoming virion that contains a 40S RNA genome defined as a minus strand (17, 35). The virion becomes uncoated to the nucleocapsid stage, and the nucleocapsid serves as template for the virion-associated polymerase during transcription of messenger RNA (3). The messenger RNA strands are of opposite polarity, complementary to virion RNA, and smaller than genome RNA (17, 35). These messenger RNAs are designated plus strands. When virus-specific protein synthesis is accomplished, replication of the genome can proceed (19). This procedure is thought to occur by synthesis of a complete complement of the genome into a plus strand of 40S RNA, which serves as template for the synthesis of minus-strand 40S RNA genome (34, 53). How in this scheme of synthesis can short RNA pieces be generated that contain sequences from both minus and plus strands?

Our hypothesis for generation of these DI genomes is presented in Fig. 6. The standard VSV genome RNA is represented by the alphabet with A and A′ at the 3′ and 5′ ends, respectively. A and A′ designate complementary sequences at the termini. Under the usual circumstances of replication, the minus strand is copied into its complement. Upon completion, another full-sized molecule, the plus strand, is synthesized with the termini A and A′ at the 3′ and 5′ ends, respectively (Fig. 6, no. 1).

If replication of this RNA is abortive and the 3′ end of the nascent strand forms a hairpin (Figure 6, no. 2), the polymerase can then continue synthesis by copying the nascent strand, displacing the template, and synthesizing in the direction of the 5′ end of the nascent molecule. This is demonstrated by the two "panhandle" structures in Fig. 6, no. 3. What is generated is a plus strand that has, not only original termini A and A′ from the standard RNA molecule, but also other complementary sequences, such as B′ and B and C′ and C (Fig. 6, no. 4). When a minus strand is copied from this defective plus strand, a defective minus-strand genome is generated with the same A and A′ terminal sequences and the additional complementary sequences of B and B′ as well as C and C′ (Fig. 6, no. 5).

Other complementary sequences can readily be generated by introducing a second hairpin loop during synthesis of this minus-strand-defective genome. This would result in sequences from the standard genome, the minus strand, interspersed by complementary sequences from the plus strand (Fig. 6, no. 6). Such extensive complementarity will allow considerable self-annealing within the RNA molecule itself.

In general, however, most DI particles of VSV contain RNA with a majority of their sequences from the 5′ end of the standard genome (51). The model shown in Fig. 6 generates defective RNA with sequences from the 3′ end of the standard genome. Synthesis of RNA during VSV replication is asymmetrical in that much more minus-strand 40S RNA is made than plus-strand 40S RNA. A model for generation of VSV DI genomes from the 5′ end is formally similar to that shown in Fig. 6 and is more accurately depicted in Fig. 7. Formation of the hairpin loop in the nascent strand occurs during synthesis of the minus strand while full-sized, plus-strand 40S RNA is utilized as the template (Fig. 7, no. 2). The defective genome generated in this way would then contain the sequences T, U, V, W, X, Y, Z, and A′, all from the 5′ end of the standard genome, as well as sequences Y′, Z′, and A, which would be complementary to the sequences from the 5′ end of standard RNA. Gene order of the various cistrons of VSV, as determined by Ball and White (1), is shown on the bottom of Fig. 7, no. 5. Comparing the VSV gene order to the defective RNA generated in Fig. 7, complementary sequences will exist between the defective genome and the plus-strand messenger RNA for the L protein. Such results have been found by Reichmann and coworkers (29, 52).

If such a model (Fig. 7) were true for VSV, it

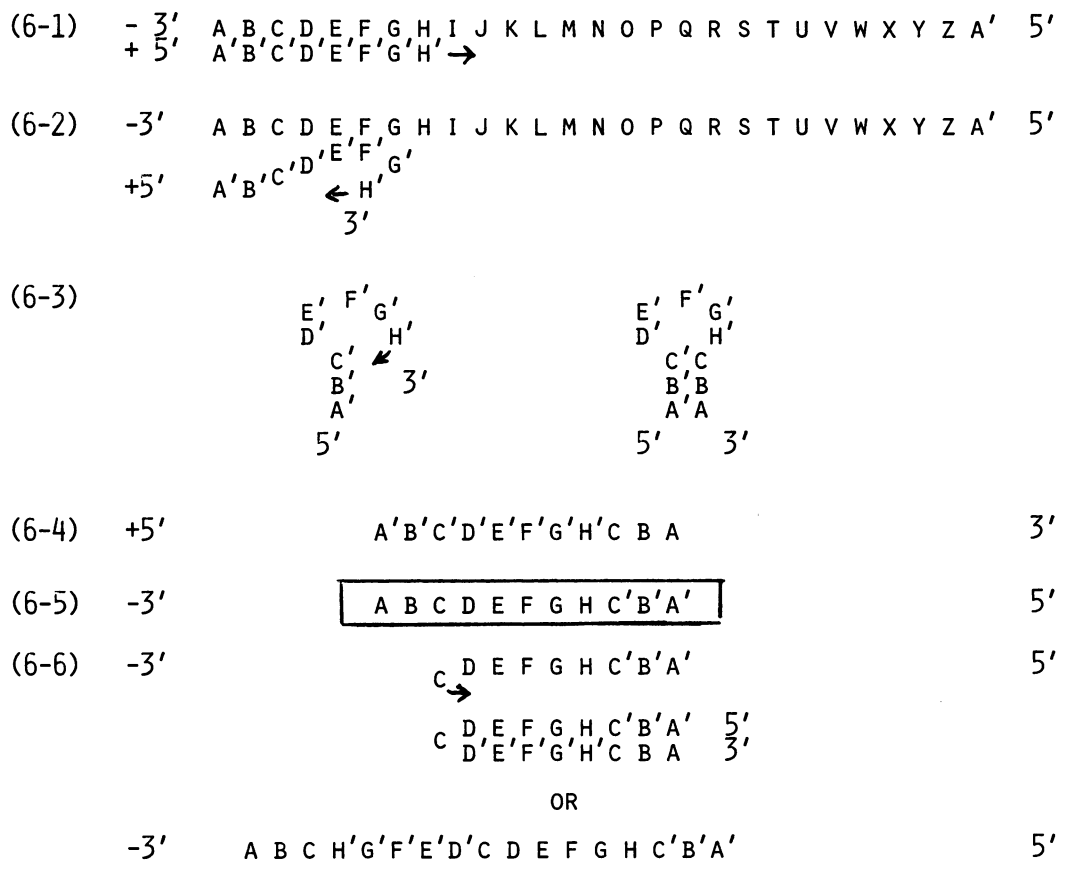

FIG. 6. *Model for the generation of VSV DI RNA from the 3′ end.*

would be expected (i) that some of the extragenomic, or complementary, sequences would reside near the 3′ terminus of the RNA extracted from the DI particle and (ii) that self-annealed RNA sequences would be enriched for sequences at both the 3′ and the 5′ ends. We have obtained preliminary evidence indicating that one of the extragenomic oligonucleotides of DI RNA, shown in Fig. 5, is indeed near the 3′ end and anneals intramolecularly to form ribonuclease-resistant fragments (G. Freeman, D. Rao, and A. Huang, unpublished observations).

The model for generation of VSV DI genomes presented in Fig. 6 and 7 may be applicable to all linear viral genomes, whether they are double stranded or single stranded, and whether they contain DNA or RNA. A similar method has been discussed for the observed generation of defective adenovirus DNA by Daniell (6). It is tempting to speculate that viruses from subacute sclerosing panencephalitis which contain information in excess from the standard measles genome (10) and that Sindbis virus deletion mutants (9, 24) are generated in a similar manner. In addition, this model would predict that not only smaller defective genomes are generated, but that defective genomes larger than the standard virus genome could be generated if the hairpin occurred more than halfway from the terminus initiating replication of the nucleic acid and if most of the nascent strand is copied into complementary sequences covalently linked to the defective genome.

Significance of Complementary Termini in Viral Genomes

The panhandle structures depicted in Fig. 6 and 7 are of potential significance. When the 3′ and the 5′ ends are juxtaposed, the sequences A and A′ are annealed to each other. The formation of similar double-stranded termini with A and A′ annealed to each other can occur not only within one molecule by the formation of a panhandle, but plus and minus 40S strands of VSV annealed together contain ends which also have A and A′. In addition, nascent chain synthesis, once it is under way, immediately presents an end with the A′ sequences of the nascent strand 5′ terminus and the A sequences of the template 3′ terminus annealed together. Such a

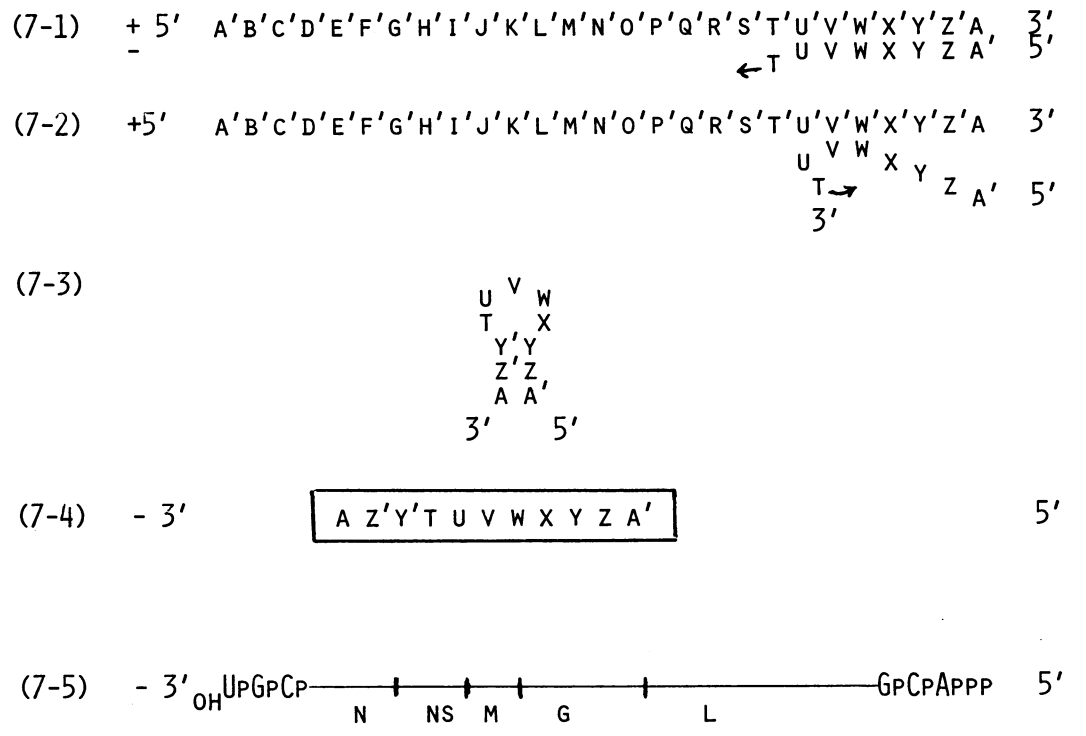

FIG. 7. *Model for the generation of VSV DI RNA from the 5' end.*

juxtaposition of the 3' and 5' termini by complementarity between A and A' may serve as a recognition site for polymerase attachment and genome replication. Therefore any RNA molecule, or molecules together, that can present double-stranded termini consisting of a 3' and a 5' terminus annealed to each other would have the potential of being recognized by the polymerase and would be copied at the 3' end with concomitant displacement of the 5' end. Such a model for polymerase recognition of double-stranded or single-stranded templates has been suggested by Lechner and Kelly (31) for initiating replication of adenovirus DNA (55).

It is also highly likely that the extent of complementarity, as well as the base composition internal to the termini, determines in some way stability of the binding of the polymerase or rate of replication of the molecule. By increasing the extent of homology at the termini, VSV DI RNA may compete more or less effectively for the polymerase than standard VSV RNA. These complementary termini may be important for other functions. For instance, Marcus and Sekellick (33) have found that DI particles with a great deal of genome complementarity are the most effective inducers of interferon.

CONCLUSION

The study of inverted terminal complementary sequences may have potential impact in several important areas. For viral diseases, these studies may reveal the underlying principles by which certain defective genomes can compete and successfully inhibit synthesis of standard virus. For molecular biology, these studies may reveal the link between linear viral genomes that can circularize and the mechanism by which their nucleic acids are replicated.

For generating DI genomes, this model, based on the presence of inverted complementary termini on linear molecules, precludes random recombination between RNA molecules as a means of generating DI genomes. This model predicts that the DI genomes will arise only from one or the other end of the genome. Complementary or extragenomic sequences arise by hairpin formation during synthesis. How internal hairpin loops are made during nucleic acid replication is not clearly understood, but such events are thought to occur when the reverse transcriptase functions in vitro in the absence of actinomycin D (57; I. Verma, personal communication). Therefore, these studies on the genomes of VSV DI particles, which were initiated because of the intrinsic relevance of interference to viral diseases, are likely to lead to answers basic for the replication of nucleic acids and for the generation of deleted and inverted sequences.

ACKNOWLEDGMENTS

Previously unpublished material has been contributed by members of my laboratory: Pantipa Sinarachatanant, Gail M. Clinton, Sheila P. Little, Gordon Freeman, and Donald Rao. I thank Tom Kelly and John Coffin for stimulating discussions and Jacques Perrault, Ellen Daniell, and C. Y. Kang for preprints.

This work was supported by Public Health Service research grants AI 10100 from the National Institute of Allergy and Infectious Diseases and grant VC 63 from the American Cancer Society. Alice S. Huang is a Public Health Service Research Career Development awardee.

LITERATURE CITED

1. **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.
2. **Baltimore, D.** 1970. RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature (London) **226**:1209–1210.
3. **Baltimore, D., A. S. Huang, and M. Stampfer.** 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. II. An RNA polymerase in the virion. Proc. Natl. Acad. Sci. U.S.A. **66**:572–576.
4. **Banerjee, A., and D. P. Rhodes.** 1976. 3′ terminal sequence of vesicular stomatitis virus genome RNA. Biochem. Biophys. Res. Commun. **68**:1387–1394.
5. **Colonno, R. J., and A. K. Banerjee.** 1976. A unique RNA species involved in initiation of vesicular stomatitis virus RNA transcription in vitro. Cell **8**:197–204.
6. **Daniell, E.** 1976. Genome structure of incomplete particles of adenovirus. J. Virol. **19**:685–708.
7. **Darnell, M. B., and H. Koprowski.** 1974. Genetically determined resistance to infection with group B arboviruses. II. Increased production of interfering particles in cell cultures from resistant mice. J. Infect. Dis. **129**:248–256.
8. **DeWachter, R., and W. Fiers.** 1972. Preparative two-dimensional polyacrylamide gel electrophoresis of ^{32}P-labeled RNA. Anal. Biochem. **49**:184–197.
9. **Guild, G., and V. Stollar.** 1977. Defective interfering particles of Sindbis virus. V. Sequence relationships between SV_{STD} 42S RNA and intracellular defective viral RNAs. Virology **77**:175–188.
10. **Hall, W. W., and V. terMeulen.** 1976. RNA homology between subacute sclerosing panencephalitis and measles viruses. Nature (London) **264**:474–477.
11. **Hefti, E., and D. H. L. Bishop.** 1975. The 5′ nucleotide sequence of vesicular stomatitis viral RNA. J. Virol. **15**:90–96.
12. **Hewlett, M. J., R. F. Pettersson, and D. Baltimore.** 1977. Circular forms of Üükünieni virion RNA: an electron microscopic study. J. Virol. **21**:1085–1093.
13. **Holland, J. J., and L. P. Villarreal.** 1975. Purification of defective interfering T particles of vesicular stomatitis and rabies viruses generated in vivo in brains of newborn mice. Virology **67**:438–449.
14. **Homma, M.** 1972. Trypsin action on the growth of Sendai virus in tissue culture cells. I. Restoration of the infectivity for L cells by direct action of trypsin on L cell-borne Sendai virus. J. Virol. **8**:619–629.
15. **Hsu, M. T., H. J. King, and N. Davidson.** 1971. An electron microscope study of Sindbis virus RNA. Cold Spring Harbor Symp. Quant. Biol. **38**:943–950.
16. **Huang, A. S., and D. Baltimore.** 1977. Defective interfering animal viruses, p. 73–116. *In* H. Fraenkel-Conrat and R. R. Wagner (ed.), Comprehensive virology, vol. 10. Plenum Publishing Corp., New York.
17. **Huang, A. S., D. Baltimore, and M. Stampfer.** 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. III. Multiple complementary messenger RNA molecules. Virology **42**:946–957.
18. **Huang, A. S., J. W. Greenawalt, and R. R. Wagner.** 1966. Defective T particles of vesicular stomatitis virus. I. Preparation, morphology, and some biologic properties. Virology **30**:161–172.
19. **Huang, A. S., and E. K. Manders.** 1972. Ribonucleic acid synthesis of vesicular stomatitis virus. IV. Transcription by standard virus in the presence of defective interfering particles. J. Virol. **9**:909–916.
20. **Huang, A. S., and R. R. Wagner.** 1966. Comparative sedimentation coefficients of RNA extracted from plaque-forming and defective particles of vesicular stomatitis virus. J. Mol. Biol. **22**:381–384.
21. **Johnson, R. T., H. F. McFarland, and S. E. Levy.** 1972. Age-dependent resistance to viral encephalitis: studies of infections due to Sindbis virus in mice. J. Infect. Dis. **125**:257–262.
22. **Kang, C. Y., and L. Prevec.** 1969. Proteins of vesicular stomatitis virus. I. Polyacrylamide gel analysis of viral antigens. J. Virol. **3**:404–413.
23. **Keene, J. D., M. Rosenberg, and R. A. Lazzarini.** 1977. Characterization of the 3′ terminus of RNA isolated from vesicular stomatitis virus and from its defective interfering particles. Proc. Natl. Acad. Sci. U.S.A. **74**:1353–1357.
24. **Kennedy, S. I. T., C. J. Bruton, and S. Schlesinger.** 1976. Defective interfering passages of Sindbis virus: nature of the defective virion. J. Virol. **19**:1034–1043.
25. **Klenk, H.-D., and R. Rott.** 1977. Virus infection and the cell surface. *In* G. Poste and L. G. Nicholson (ed.), Cell surface reviews, vol. 2. Elsevier-North Holland Publishing Co., New York.
26. **Kolakofsky, D.** 1976. Isolation and characterization of Sendai virus DI-RNAs. Cell **8**:547–555.
27. **Laemmli, W. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–682.
28. **Langbeheim, H., R. Arnon, and M. Sela.** 1976. Antiviral effect on ms-2 coliphage obtained with synthetic antigen. Proc. Natl. Acad. Sci. U.S.A. **73**:4636–4640.
29. **Leamnson, R. N., and M. E. Reichmann.** 1974. The RNA of defective vesicular stomatitis virus particles in relation to viral cistrons. J. Mol.

Biol. 85:551–568.
30. **Lazzarini, R. A., G. H. Weber, L. D. Johnson, and G. M. Stamminger.** 1975. Covalently linked message and anti-message (genomic) RNA from a defective vesicular stomatitis virus particle. J. Mol. Biol. 97:289–308.
31. **Lechner, R. L., and T. J. Kelly.** 1977. The structure of replicating adenovirus-2 DNA molecules. Cell, in press.
32. **Lowy, D. R., W. P. Rowe, N. Teich, and J. W. Hartley.** 1971. Murine leukemia virus: high-frequency activation in vitro by 5-iododeoxyuridine and 5-bromodeoxyuridine. Science 174:155–156.
33. **Marcus, P. I., and M. J. Sekellick.** 1977. Defective interfering particles with covalently linked [±]RNA induced interferon. Nature (London), in press.
34. **Morrison, T. G., M. Stampfer, H. F. Lodish, and D. Baltimore.** 1975. In vitro translation of vesicular stomatitis virus messenger RNAs and the existence of a 40S "plus" strand, p. 293–306. *In* B. W. J. Many and R. D. Barry (ed.), Negative strand viruses. Academic Press Inc., New York.
35. **Mudd, J. A., and D. F. Summers.** 1970. Polysomal ribonucleic acid of vesicular stomatitis virus-infected HeLa cells. Virology 42:958–968.
36. **Olitsky, P. K., A. B. Sabin, and H. R. Cox.** 1936. An acquired resistance of growing animals to certain neurotropic viruses in the absence of humoral antibodies or previous exposure to infection. J. Exp. Med. 64:723–737.
37. **Palese, P., and J. L. Schulman.** 1976. Differences in RNA patterns of influenza A viruses. J. Virol. 17:876–884.
38. **Palma, E. L., and A. S. Huang.** 1974. Cyclic production of vesicular stomatitis virus caused by defective interfering particles. J. Infect. Dis. 129:402–410.
39. **Palma, E. L., S. M. Perlman, and A. S. Huang.** 1974. Ribonucleic acid synthesis of vesicular stomatitis virus. VI. Correlation of defective particle RNA synthesis with standard RNA replication. J. Mol. Biol. 85:127–136.
40. **Perrault, J.** 1976. Cross-linked double-stranded RNA from a defective vesicular stomatitis virus particle. Virology 70:360–371.
41. **Perrault, J., and J. J. Holland.** 1972. Absence of transcriptase activity and transcription-inhibiting ability in defective interfering particles of vesicular stomatitis virus. Virology 50:159–170.
42. **Perrault, J., and R. W. Leavitt.** 1977. Characterization of snap-back RNAs in vesicular stomatitis defective interfering virus particles. J. Gen. Virol., in press.
43. **Perrault, J., and R. W. Leavitt.** 1977. Complementary terminal repeat sequences in single-stranded RNAs and snap-back RNAs from vesicular stomatitis virus defective interfering particles. J. Gen. Virol., in press.
44. **Petric, M., and L. Previc.** 1970. Vesicular stomatitis virus: a new interfering particle, intracellular structures, and virus-specific RNA. Virology 41:615–630.
45. **Popescu, M., and F. Lehmann-Grube.** 1977. Defective interfering particles in mice infected with lymphocytic choriomeningitis virus. J. Gen. Virol., in press.
46. **Reichmann, M. E., C. R. Pringle, and E. A. C. Follett.** 1971. Defective particles in BHK cells infected with temperature-sensitive mutants of vesicular stomatitis virus. J. Virol. 8:154–160.
47. **Sabin, A. B., and P. K. Olitsky.** 1937. Influence of host factors on the neuroinvasiveness of vesicular stomatitis virus. I. Effect of age on the invasion of the brain by virus instilled in the nose. J. Exp. Med. 66:15–34.
48. **Sabin, A. B., and P. K. Olitsky.** 1937. Influence of host factors in neuroinvasiveness of vesicular stomatitis virus. II. Effect of age on the invasion of the peripheral and central nervous systems by virus injected into the leg muscles or the eye. J. Exp. Med. 66:35–56.
49. **Sabin, A. B., and P. K. Olitsky.** 1938. Influence of host factors on neuroinvasiveness of vesicular stomatitis virus. III. Effect of age and pathway of infection on the character and localization of lesions in the central nervous system. J. Exp. Med. 67:201–227.
50. **Sabin, A. B., and P. K. Olitsky.** 1938. Influence of host factors on neuroinvasiveness of vesicular stomatitis virus. IV. Variations on neuroinvasiveness in different species. J. Exp. Med. 67:229–249.
51. **Scheid, A., and P. W. Choppin.** 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology 57:475–490.
52. **Schnitzlein, W. M., and M. E. Reichmann.** 1976. The size and the cistronic origin of defective vesicular stomatitis virus particle RNAs in relation to homotypic and heterotypic interferences. J. Mol. Biol. 101:307–325.
53. **Soria, M., S. P. Little, and A. S. Huang.** 1974. Characterization of vesicular stomatitis virus nucleocapsids. I. Complementary 40S RNA molecules in nucleocapsids. Virology 61:270–280.
54. **Stampfer, M., D. Baltimore, and A. S. Huang.** 1971. Absence of interference during high multiplicity infection by clonally purified vesicular stomatitis virus. J. Virol. 7:409–411.
55. **Sussenbach, J. S., and M. G. Kuijk.** 1977. Studies in the mechanism of replication of adenovirus DNA. V. The location of termini of replication. Virology 77:149–157.
56. **Temin, H., and S. Mitzutani.** 1970. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature (London) 226:1211–1213.
57. **Varmus, H. E., W. E. Levinson, and J. M. Bishop.** 1971. Extent of transcription by the RNA-dependent DNA polymerase of Rous sarcoma virus. Nature (London) New Biol. 233:19–21.
58. **Wagner, R. R., T. C. Schnaitman, R. M. Snyder, and C. A. Schnaitman.** 1969. Protein composition of the structural components of vesicular stomatitis virus. J. Virol. 3:611–618.