Current Topics in Microbiology and Immunology

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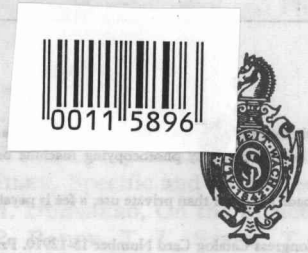

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Paramyxovirus Replication[1]

David W. Kingsbury[2,3]

With 2 Figures

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I. Introduction

Paramyxoviruses, long-recognized agents of disease (Chanock and Parrott, 1965; Henle and Enders, 1965; Katz and Enders, 1965), have been sharing in the recent rapid growth of knowledge about molecular events in virus replication. Probably the most distinctive fundamental property of paramyxoviruses is possession of the largest genomes among viruses which have a single piece of RNA as their genetic material (Duesberg, 1968), but other features of paramyxoviruses, though not unique to the group, also make them worthwhile subjects for studies on virus structure and replication.

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3 Laboratories of Virology and Immunology, St. Jude Children's Research Hospital and The University of Tennessee Medical Units, Memphis, Tennessee 38101.

Two other virus groups which have some similarities to paramyxoviruses are the influenza viruses and the rhabdoviruses (MELNICK and MCCOMBS, 1966; MELNICK, 1970). Some paramyxoviruses agglutinate erythrocytes and contain neuraminidase, like the influenza viruses (CHANOCK and COATES, 1964). Largely on morphological grounds, however, these two virus groups were distinguished by WATERSON (1962). His designation of "subgroup 2" of the myxoviruses served until recently, when the term "paramyxovirus" has come into vogue. This term reflects more accurately current recognition of a fundamental difference between paramyxoviruses and influenza viruses with respect to organization of the genetic material: namely, that each influenza virus genome is divided among several pieces of RNA, while each paramyxovirus genome is a single piece of RNA (ROTT and SCHOLTISSEK, 1967; ROBINSON and DUESBERG, 1968; KINGSBURY, 1970a; BLAIR and DUESBERG, 1970; SHATKIN, 1971).

WATERSON and ALMEIDA (1966) argued that viruses which lack demonstrable hemagglutinin or neuraminidase, or both, such as measles virus or canine distemper virus, should not be included among the paramyxoviruses, despite morphological or biochemical similarities to viruses which have those surface activities, because the etymology of the root "myxo-" would thereby be violated. This seems too narrow a view, and pertinent observations on such viruses will be included in this review.

The bullet-shaped rhabdoviruses, of which vesicular stomatitis virus (VSV) is the best studied representative, do not have hemagglutinins or neuraminidases, but resemble paramyxoviruses in possessing virion transcriptase (BALTIMORE et al., 1970) and generating genome transcripts in infected cells (SCHAFFER et al., 1968). In the following, I will point out these and some other important parallels between these virus groups.

II. Virus Structure

Reliable chemical analyses of virions require pure virus preparations. ROBINSON and DUESBERG (1968) discussed uncertainties which existed *a priori* about establishing the purity of virus particles which have physical properties similar to cell membrane fragments. Newer methods of virus purification, especially centrifugation in density gradients (ROBINSON and DUESBERG, 1968; BLAIR and DUESBERG, 1970) undoubtedly helped to produce preparations of adequate purity; in any case, numerous investigators have been able to obtain paramyxovirus preparations which reproducibly contain a few characteristic protein molecules (e.g., MOUNTCASTLE et al., 1971) although cell membranes contain many proteins (KIEHN and HOLLAND, 1970). This has given confidence to chemical analyses of virion components, such as virion lipids, where differences from cell membranes are slight (KLENK and CHOPPIN, 1969). Parallel progress with virions of other complex enveloped viruses (LAVER, 1964; DUESBERG et al., 1968; WAGNER et al., 1969; KANG and PREVEC, 1969) has bolstered this confidence.

A. Virion RNA

RNA molecules isolated from paramyxovirions have the distinction of sedimenting faster than any other single-stranded RNA virus genomes. Virion RNA sedimenting at 40 to 60S was first found in NDV (ADAMS, 1965; DUESBERG and ROBINSON, 1965; KINGSBURY, 1966a; SOKOL et al., 1966; NAKAJIMA and OBARA, 1967). Similar RNA has been obtained from SV5 (COMPANS and CHOPPIN, 1968) Sendai virus (IWAI et al., 1966; BARRY and BUKRINSKAYA, 1968; BLAIR and ROBINSON, 1968), measles virus (SCHLUEDERBERG, 1971; BUSSELL and ROBINSON, 1971; WINSTON and BRATT, 1971) mumps virus (EAST and KINGSBURY, 1971), and Yucaipa virus (KINGSBURY, 1970b). Because the sedimentation rate of single-stranded RNA varies with conditions (SPIRIN, 1963), different workers have obtained somewhat different numerical values for sedimentation coefficients of paramyxovirion RNAs on different occasions. Because these values have been used as convenient designations, confusion may arise as to whether the "57S" molecules referred to by some authors are equivalent to the "50S" molecules referred to by others. However, it has generally been the practice to compare the sedimentation rate of a new paramyxovirus RNA with the RNA of a previously characterized member of the group by cosedimentation, usually in a double-label experiment, and in no cases have differences been found[4]. For convenience in this review, I will continue to designate the RNA from paramyxovirions as "50S".

The slow migration of NDV and Sendai virion RNAs in acrylamide gels (DUESBERG, 1968; KINGSBURY et al., 1970), and their exclusion from 2% agarose columns (KINGSBURY, 1966a; PORTNER and KINGSBURY, 1972) indicate that the molecules are larger than other virion RNAs (ERIKSON and GORDON, 1966; ROY and BISHOP, 1970). DUESBERG (1968) showed that the rapid sedimentation of NDV virion RNA reflected an uncommonly large size, and was not the result of an especially compact conformation. When NDV RNA was denatured and sedimented in dimethyl sulfoxide, its sedimentation rate indicated a molecular weight of about 6×10^6. It might be worth the effort to apply classical hydrodynamic methods of molecular weight determination to paramyxovirus genomic RNA, since other molecular weight estimates, based on a variety of more or less doubtful criteria, exist in the literature (DUESBERG and ROBINSON, 1965; COMPANS and CHOPPIN, 1968; NAKAJIMA and OBARA, 1967; LOMNICZI et al., 1971), and it might be useful to put ideas about the coding potential of these viruses on a firmer basis.

The 50S RNA molecules isolated from paramyxovirions are thought to represent complete viral genomes, even though they have not been shown to be infectious (KINGSBURY, 1966a). Lack of infectivity could have a variety of trivial explanations, but the existence of virion transcriptases, and the idea that they are essential in paramyxovirus replication (see below) adequately explains the failure of isolated RNA to be infectious. Small amounts of very slowly sedimenting RNA species (about 4S) obtained on occasion from NDV

4 For what may be an important exception, see SCHLUEDERBERG, 1971.

virions (DUESBERG and ROBINSON, 1965) were shown not to be encapsidated, and are probably contaminants (ADAMS, 1966; KINGSBURY and DARLINGTON, 1968). Encapsidated RNA species which sediment slower than 50S RNA have been seen in Sendai virions (KINGSBURY et al., 1970). These appear to be defective viral genomes and they will be discussed later.

A peculiar property of the virion 50S RNAs is their ability to self-hybridize. ROBINSON (1970) converted up to 60% of the 50S RNA from Sendai virions to ribonuclease-resistant form when he annealed it by itself. He showed that the ribonuclease-resistant material melted sharply and had a low buoyant density characteristic of double-stranded RNA (ROBINSON, 1971a). Since the self-hybridization was concentration dependent, he suggested that the 50S RNA population consisted of two kinds of molecules, both the same size, one being completely complementary to the majority species (ROBINSON, 1970). However, he was unable to separate populations of Sendai virus 50S RNA molecules with mutually complementary base compositions on the basis of buoyant density (ROBINSON, 1971a). He reported that NDV 50S RNA also self-hybridized, but no more than 30% (ROBINSON, 1970).

Self-hybridization of Sendai 50S RNA was confirmed by PORTNER and KINGSBURY (1970), though they did not obtain more than 30% ribonuclease-resistant material with any of their preparations. Mumps virus 50S RNA self-hybridized about 20% (EAST and KINGSBURY, 1971). PORTNER and KINGSBURY (1970) found little evidence of self-hybridization with the "C" strain of NDV under conditions which gave significant self-hybridization with Sendai virus RNA, in agreement with earlier reports (BRATT and ROBINSON, 1967; KINGSBURY, 1967). Thus, the self-hybridization phenomenon, though real, and reproducibly obtained with a given batch of viral RNA, appeared to vary in magnitude from virus to virus and batch to batch.

Earlier studies had shown that about 30% of 50S NDV-specific RNA isolated from infected cells hybridized with added virion RNA (BRATT and ROBINSON, 1967). This indicates that infected cells contain 50S RNA molecules complementary to genomes, possibly having a template function in genome replication. A mechanism for discriminating against such genome complements in virus assembly apparently exists, as the low self-hybridization levels of some NDV strains attest, but this mechanism may be less effective for some viruses, and might vary in effectiveness for a given virus. The variation in self-hybridization from virus to virus and in different preparations of a virus could thus reflect differences in amounts of complementary strand encapsidation. On the other hand, the complementary strand might enter virions unencapsidated. What is needed to resolve these alternatives is an examination of the self-hybridization capacity of RNA derived from virion nucleocapsids. It would also be desirable if further efforts were made to separate genomic from complementary 50S strands. If succesful, this would finally rule out the possibility of extensive intramolecular self-complementarity in viral genomes (a complication with unfathomable biological consequences) and two useful reagents

would be obtained: clean genomic RNA and clean complements representing the entire genome.

Even though clean genomes (in the above sense) are not available from mumps or Sendai virions, the fact remains that 50S RNA from these viruses, when sufficiently concentrated, converts most of the virus-specific RNA in infected cells to double-stranded forms on annealing. This indicates that self-hybridization of virion RNA need not be an obstacle to determining that the predominant virus-specific RNAs in cells are complementary in base sequences to the predominant species of 50S RNA in virus particles (PORTNER and KINGSBURY, 1970; EAST and KINGSBURY, 1971). This last point needs emphasis, because the existence of self-hybridization has caused some confusion about what is complementary to what in infected cells (BLAIR and DUESBERG, 1970).

The availability of single-stranded RNA molecules complementary in base sequence to paramyxovirus genomes, as generated by infected cells (see below), makes it relatively easy to test whether different paramyxoviruses have any base sequences in common. So far, this has been examined with NDV, Sendai virus, measles virus and mumps virus. No cross-hybridization was obtained when complementary RNA species generated in cells infected by each of these viruses was annealed with genomic RNA from one of the others (BLAIR and ROBINSON, 1968; EAST and KINGSBURY, 1971; BUSSELL and ROBINSON, 1971). These experiments do not completely rule out common base sequences among these viruses, since there is some uncertainty about how much of the genome is represented by complementary sequences in infected cells (BRATT and ROBINSON, 1967; KINGSBURY, 1967), but clearly, with regard to the majority of sequences, there is no commonality. This might have been expected in view of the long period presumably separating these viruses from a common ancestor. In contrast, different strains of NDV cross-hybridized indistinguishably (KINGSBURY, 1966b). It remains to be seen whether cross-hybridization tests of other paramyxoviruses will uncover relationships in base sequences.

B. Nucleocapsids from Virions

The helical structure of paramyxovirus nucleocapsids was shown early, using negative staining techniques, and such basic quantities as the diameter (17 nm) and periodicity (5 nm) of the helix were measured (HORNE et al., 1960).

There was early uncertainty about whether the helices were single- or double-stranded (HORNE and WATERSON, 1960), but this was resolved in a number of ways to indicate a single-stranded helix (CHOPPIN and STOECKENIUS, 1964; FINCH and GIBBS, 1970). Despite tendencies of nucleocapsids from various paramyxoviruses to fragment or aggregate end to end, it has been the rule to find modal length distributions around 1 μm (HOSAKA et al., 1966; COMPANS and CHOPPIN, 1967a, b; HOSAKA and SHIMIZU, 1968; KINGSBURY and DARLINGTON, 1968; FINCH and GIBBS, 1970). Buoyant densities in the range of 1.27 to 1.32 gm/cc have been reported (COMPANS and CHOPPIN, 1967a; GITELMAN and BUKRINSKAYA, 1971; BLAIR and ROBINSON, 1970), and RNA contents

appear to be around 4% (COMPANS and CHOPPIN, 1967a; HOSAKA, 1968; KINGSBURY and DARLINGTON, 1968). When it has been possible to isolate unaggregated or unfragmented nucleocapsids, sedimentation coefficients of 200 to 300 S have been obtained (HOSAKA, 1968; KINGSBURY and DARLINGTON, 1968; BLAIR and ROBINSON, 1970), the value from analytical ultracentrifugation (300 S) (HOSAKA, 1968) presumably being the most accurate. Although x-ray diffraction has not been applied to paramyxovirus nucleocapsid structure, FINCH and GIBBS (1970) were able to obtain some analogous information by applying optical diffraction methods to electron micrographs. They calculated that there are 11 or 13 structure units per turn of the helix in Sendai virus. HOSAKA (1968) had estimated 15 structure units per turn, using a less accurate method. Thus, there are 2400 to 3000 structure units per nucleocapsid. Calculated molecular weights of structure units agreed well with values obtained from acrylamide gel electrophoresis (see below). FINCH and GIBBS (1970) suggested that the RNA helix resides at a radius of 5 nm, which would place it more symmetrically within the polypeptide helix than the 8 nm suggested by HOSAKA and SHIMIZU (1968). FINCH and GIBBS (1970) confirmed that the polypeptide subunits of paramyxovirus nucleocapsids are not perpendicular to the long axis of the particle as in the rod-shaped plant viruses, but angled about 60° to it. This may explain the ability of paramyxovirus nucleocapsids to bend into curved shapes (a prerequisite for being wrapped in an envelope with a diameter as small as one-fifth the length of each helix).

The total length of nucleocapsid in a single paramyxovirus particle is frequently much more than 1 μm (WATERSON, 1964). In a careful study, HOSAKA et al. (1966) showed that Sendai (HVJ) virions were separable by centrifugation into populations with different amounts of nucleocapsid per virion. These amounts were integral multiples of 1 μm, indicating the existence of more than one genome in some virions. This fits with GRANOFF's (1959b; 1962) model for paramyxovirus heterozygotes: two independent genomes in a single virus particle. The idea was pursued further by DAHLBERG and SIMON (1969b), using radiobiological methods. They separated NDV virion populations into groups sedimenting at different rates and determined the inactivation rate of each population by UV light. More rapidly sedimenting, presumably larger, virus particles, thought to contain more than one unit nucleocapsid, were inactivated in a manner which appeared to conform to multihit kinetics. On this basis, they calculated the fraction of the whole virus population which was multiploid, and found that this value compared favorably with the number of heterozygotes obtained in infection with two plaque types in cell culture. On the contrary, KINGSBURY and GRANOFF (1970) adduced evidence that heterozygotes had physical properties indistinguishable from all other virus particles. Even though the NDV population derived from chick embryo fibroblast cells in culture sedimented heterogeneously on sucrose gradients, all sedimenting classes of NDV particles contained about the same percentage of heterozygotes. Likewise, GRANOFF (1962) could not separate heterozygotes from homozygotes by isopycnic centrifugation. KINGSBURY and GRANOFF

(1970) repeated an experiment performed by GRANOFF (1959b) in which the UV sensitivity of heterozygotes was compared with the UV sensitivity of homozygous particles. If each genome in a heterozygote were inactivated independently, the ability of a particle to give rise to mixed progeny should be inactivated at a much greater rate than its ability to be infectious; clumped virus particles would behave in a similar manner. As before, heterozygotes and homozygotes were inactivated at the same rate, indicating that the genomes in NDV heterozygotes are not independently inactivable. One way this could occur would be if both genomes were covalently linked in a single strand of RNA, that is, if 50S virion RNA were redundant in nucleotide sequences. Alternatively there could be incomplete, but extensive, redundancy of the genetic information in a single RNA strand. A specialized kind of genetic recombination, solely between redundant regions, would be required to produce heterozygotes of this type (KINGSBURY and GRANOFF, 1970). This idea should be viewed in the light of evidence that recombination or multiplicity reactivation, rarely, if ever, occur with NDV (GRANOFF, 1959a; DRAKE, 1962; BARRY, 1962; GRANOFF, 1962; KIRVAITIS and SIMON, 1965; DAHLBERG and SIMON, 1969a). When the sizes of all viral gene products are known and compared with the total coding capacity of genomic RNA, this question of possible redundancy in the genome may be resolved. What is learned about NDV will presumably apply to the other paramyxoviruses, since all have the same size genome.

There is only one reasonably well-documented claim for a successful demonstration of infectivity of paramyxovirus nucleocapsids (BUKRINSKAYA et al., 1968). A major difficulty in this type of experiment is ruling out trace amounts of intact virus as th actual infective entities. If virion transcriptase is needed to make paramyxovirus RNA infectious (see above), and if this transcriptase resides in paramyxovirus nucleocapsids (ROBINSON, 1971c), then nucleocapsids should be infectious, provided the method used to obtain them does not inactivate the enzyme. In this connection, it might not have been expected that CARTWRIGHT and co-workers (1970) could demonstrate infectivity for vesicular stomatitis virus (VSV) nucleocapsids, using sodium deoxycholate to isolate them, since the detergent inactivates the virion transcriptase (BALTIMORE et al., 1970).

C. Virion Proteins

The proteins in virions of three paramyxoviruses, NDV (EVANS and KINGSBURY, 1969; HASLAM et al., 1969; BIKEL and DUESBERG, 1969), SV5 (CALIGUIRI et al., 1969; KLENK et al., 1970a), and Sendai virus (CONTENT and DUESBERG, 1970; MOUNTCASTLE et al., 1971) have been examined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS). As might be expected for virions which have similar morphology and viral genomes with the same apparent coding capacity, the gel patterns of proteins from these viruses have many similarities. However, differences exist to the extent that

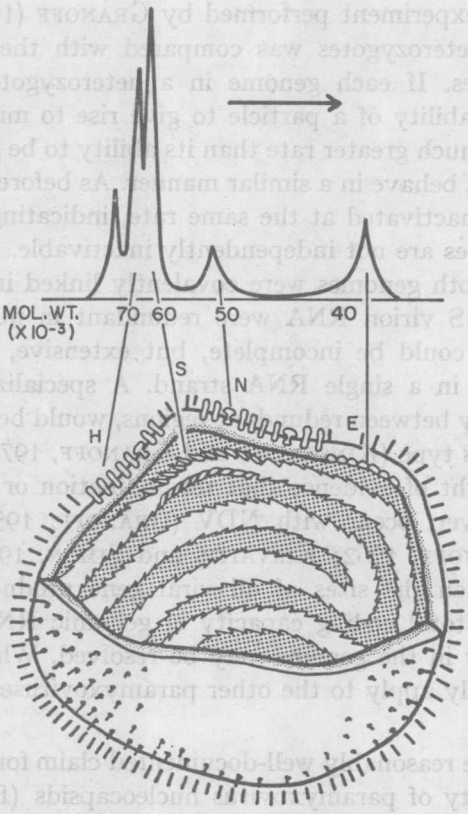

Fig. 1. Presumed relationships between paramyxovirion proteins resolved by electrophoresis in SDS-polyacrylamide gels and virion components. In the upper part of the figure, the electrophoretic profile of Sendai virion proteins (unpublished data of H. O. Stone, A. G. Kasselberg, and D. W. Kingsbury) is represented. The arrow indicates the direction of migration. A diagram of a virus particle is in the lower part of the figure (not to scale). For documentation, see the text. *H* hemagglutinin, *N* neuraminidase; *S* nucleocapsid structure unit, *E* putative inner envelope protein

each of these viruses could be said to have a distinctive and characteristic acrylamide gel protein pattern (Mountcastle et al., 1970; Mountcastle et al., (1971). Shapiro and Bratt (1971) even found differences in polypeptides among different strains of the same virus (NDV).

Virion proteins of the rhabdovirus, VSV, have some parallels with paramyxovirion proteins (Kang and Prevec, 1969; Wagner et al., 1969), which may reflect convergent evolution or, alternatively, common ancestry (Choppin and Compans, 1970).

The following will deal with those proteins which are present in largest amounts and whose structural roles in virions have been established or seem strongly indicated (Fig. 1).

Only one kind of polypeptide structure unit has been found in paramyxovirus nucleocapsids; it has a molecular weight about 60,000 and is not a

glycoprotein (MOUNTCASTLE et al., 1971). The nucleocapsid polypeptide is very abundant in virions, and is in fact the most abundant in aggregate mass, correlating with the evidence that a large number of structure units encapsidate a single RNA strand. The existence of a few molecules of an additional polypeptide in nucleocapsids, such as a virion transcriptase, is not ruled out by any of the data available. A few percent by weight of a minor component would be hard to see in acrylamide gel patterns.

An interesting artifact arose in early work on the nucleocapsid polypeptide of SV5. The polypeptide of nucleocapsids isolated from infected cells had a mobility like that of the fastest moving virion polypeptide, indicating a molecular weight of about 40,000 (CALIGUIRI et al., 1969), A molecular weight of 61,000 for the polypeptide in nucleocapsids from virions was later established, and it was learned that traces of trypsin, which had been used to release the nucleocapsid-containing cells from the surface on which they had been growing, cleaved each nucleocapsid structure unit, leaving 40,000 molecular weight fragments still attached to the RNA (MOUNTCASTLE et al., 1970). Concomitantly, the nucleocapsids became more rigid, in agreement with earlier observations of HOSAKA (1968) on trypsin-treated nucleocapsids of Sendai virions. Nucleocapsids from cells infected by NDV and Sendai virus also yielded split products with molecular weights about 40,000 when the cells were trypsin-treated before disruption (MOUNTCASTLE et al., 1970). Attempts to reproduce the phenomenon with nucleocapsids isolated from virions were considered successful, but the products may be somewhat more heterogeneous than the split products from infected cells. Whether the splitting of nucleocapsid structure units by proteolytic enzymes has any relationship to viral RNA uncoating (see below) conjectural.

There appear to be at least two glycoprotein species represented in each paramyxovirus, the larger and more abundant of the pair having a molecular weight in the range of 65,000 to 74,000, the smaller from 53,000 to 56,000 (MOUNTCASTLE et al., 1971). These molecular weight estimates are probably too large, since SDS does not bind well to carbohydrates, causing a glycoprotein to migrate slower than an unsubstituted polypeptide of the same mass (SEGREST et al., 1971). The glycoproteins are on the surfaces of the virions and appear to be the only proteins removed by proteolytic enzyme treatment of virions (CHEN et al., 1971), but the existence of surface proteins which are not glycoproteins has not been entirely ruled out. The surface spikes of paramyxovirions disappear with proteolytic enzyme treatment (CALBERG-BACQ et al., 1967; MAENO et al., 1970), indicating that the glycoproteins make up the spikes. Since hemagglutinin and neuraminidase activities are removed from virions as well, it is logical to connect the glycoproteins with these functions (MAENO et al., 1970; CHEN et al., 1971). Although no definite identification of either hemagglutinin or neuraminidase has been made, it has been suggested that the larger glycoproteins, with molecular weights greater than 65,000, represent viral hemagglutinins, since hemagglutinins are thought likely to be the most abundant surface proteins and since the larger glycoprotein is the principal

one that adsorbs to erythrocytes (EVANS and KINGSBURY, 1969; HASLAM et al., 1969). The smaller glycoprotein could be the neuraminidase, or it could be a subunit of the hemagglutinin (cf. LAVER, 1971).

No gross differences were detected in carbohydrate contents of virion glycoproteins of SV5 grown in different host cells (KLENK et al., 1970a), but it is thought that cellular enzymes add the carbohydrate ersidues to the virus-specified proteins, as indicated for other enveloped viruses (BURGE and HUANG, 1970; COMPANS et al., 1970; STRAUSS et al., 1970). Paramyxovirion glycoproteins, like influenza virus glycoproteins, are distinctive in lacking neuraminic acid (KLENK et al., 1970a) although this moiety is abundant in cell surface membranes (COOK, 1968) and in glycoproteins from viruses which lack neuraminidase (STRAUSS et al., 1970; BURGE and HUANG, 1970). It is logical to think that the virion neuraminidase distinctive to these two groups of viruses is responsible for this, and the opinion has been expressed that the removal of neuraminic acid somehow facilitates virus assembly (KLENK et al. 1970a).

The smallest major paramyxovirion protein, with a molecular weight about 40,000, is not a glycoprotein and may be an important structural element of the interior of the virus envelope (MOUNTCASTLE et al., 1971) by analogy with a similar protein which exists in influenza virions (COMPANS et al., 1970). On the contrary, HASLAM et al., (1969) thought that this polypeptide was the viral neuraminidase. However, the susceptibility of viral neuraminidase to proteolytic enzyme digestion (see above) and the resistance of the smallest paramyxovirion polypeptide to such treatment indicate that the smallest polypeptide is neither neuraminidase nor a virion surface protein (CHEN et al., 1970).

D. Virion Lipids

Extensive studies of paramyxovirion lipids have been made. BLOUGH and co-workers (BLOUGH and LAWSON, 1968; TIFFANY and BLOUGH, 1969a, b) found that three influenza virus strains and two paramyxoviruses grown in the same host system had somewhat different lipid compositions and suggested that virion envelope proteins influence virus lipid composition by steric effects. However, they confirmed previous indications that the bulk of the lipids in an enveloped virion are like those in cell surface membranes from which the virus buds (KATES et al., 1962). Comparisons of cell surface membrane and virion lipids by KLENK and CHOPPIN (1969) confirmed that the virus lipid composition reflected the lipid composition of the cell in which it grew. However, these workers also found evidence for an influence of the virus on its lipid composition (KLENK and CHOPPIN, 1970a). They suggested also that the lipid composition of the cell surface membrane might influence the ability of a cell to produce virus. Corresponding to the absence of neuraminic acid from virion proteins was its absence from virion glycolipids, presumably for the same reason, removal by virion neuraminidase (KLENK and CHOPPIN, 1970b; KLENK et al., 1970a).

Thus, the evidence available, briefly summarized above, indicates that lipids play an essentially passive role in virus structure. However, there are indications of enhanced phospholipid synthesis in Sendai virus infected cells which might reflect a specific effect on turnover of cell membrane constituents (SHIBUTA et al., 1969; SHIBUTA et al., 1971).

E. Adventitious Enzymes in Virions

Virion neuraminidase, already discussed, and transcriptase, which will be discussed below, are probably virus-coded. A number of other enzymes have been described in enveloped viruses, such as ATPases (NEURATH and SOKOL, 1963) and phosphotransferases (ROY and BISHOP, 1971; STRAND and AUGUST, 1971). Paramyxoviruses have not always been examined for these enzymes, but may be expected to have them, since these enzymes are probably not virus-coded but carried into virions from cell membranes (NEURATH, 1965). They have no obvious role in virus replication. It is probable that none of these enzymes is present in sufficient quantity to appear as a peak in acrylamide gel patterns of paramyxovirion proteins.

III. Attachment, Penetration and Uncoating

Although influenza viruses have been the most widely used models, paramyxoviruses have shared in the development of ideas about cell surface glycoprotein receptors for viruses. This aspect of virus-cell interaction has been judiciously reviewed by COHEN (1963). It is interesting that viral neuraminidase, an enzyme in search of a function, while recently considered to play a role in myxovirus release (SETO and ROTT, 1966; WEBSTER and LAVER, 1967), had also been put forward as an important factor in virus penetration (RUBIN, 1957). The arguments developed on these points must be categorized as circumstantial or correlative at this point.

The idea of "viropexis", or the phagocytic engulfment of virus particles, as a means of virus entry into cells, was first suggested for influenza viruses (FAZEKAS DE ST. GROTH, 1948), and then was extended to a general theory of virus entry (DALES, 1965). A number of studies have documented the passage of paramyxovirions into cytoplasmic vacuoles (COMPANS et al., 1966; MUSSGAY and WEIBEL, 1962; SILVERSTEIN and MARCUS, 1964). This mechanism was questioned early, however (RUBIN, 1957; RUBIN and FRANKLIN, 1957), and when looked for, fusion of virion envelopes with cell surface membranes has also been observed, leading to the alternative proposal that infection is effected by virus-cell fusion (ADAMS and PRINCE, 1957; HOYLE, 1962; MEISELMAN et al., 1967; MORGAN and HOWE, 1968; DOURMASHKIN and TYRRELL, 1970). Related phenomena which may be mediated by the same mechanism are hemolysis (GRANOFF and HENLE, 1954), the erythrocyte presumably being unable to repair punctures in its membrane caused by imperfect fusion (HOWE and MORGAN, 1969), and polykaryon formation by added virus, "fusion from without" (BRATT and GALLAHER, 1969) as distinguished from the "fusion

from within" (KOHN, 1966) occurring late in infection, which will be discussed below.

A number of hypotheses have been advanced about the identity of the "fusion factor" (CASCARDO and KARZON, 1965; POSTE, 1970). The lipid content of virion envelopes and viral hemolytic activity appear to be the parameters most closely related to capacity for cell fusion (HENLE et al., 1954; HOSAKA, 1970; YOUNG and ASH, 1970). KOHN and co-workers (KOHN, 1965; KOHN and KLIBANSKY, 1967; KOHN and FUCHS, 1969) took pains to evaluate these and other possibilities and agreed that lipids were involved, but they could establish no definite relationship between cell fusing capacity and any other separately measurable virion function. The fusion of virion envelopes with cell membranes appears to require metabolic activity, since artificial membranes, to which enveloped viruses including paramyxoviruses attach, have not provided any evidence of fusion (TIFFANY and BLOUGH, 1970; TIFFANY and BLOUGH, 1971).

There is no way to know, on available evidence, whether viropexis, or fusion, or both mediate infection. But as COHEN (1963) has pointed out, viropexis does not explain very much, because a virus particle in a vacuole is still separated by a cell membrane from the cytoplasmic matrix of the cell, and a mechanism must be envisioned for transport of at least the viral nucleic acid across that membrane. One mechanism for transport out of a vacuole could again be simply fusion, the vacuole membrane behaving like the cell surface membrane. Another possibility, suggested by advocates of viropexis, would involve partial digestion of the virus by lysosomal enzymes (DALES, 1965; DURAND et al., 1970).

Attempts to trace the fate of virus structural elements in cells by biochemical methods have been hampered by a lack of effective methodology. The critical difficulty in this area is to distinguish virions which are nonspecifically degraded by the cell from virions which are engaged in the infectious process. This difficulty may be compounded by the need for high infecting multiplicities to achieve measurable concentrations of isotopically labeled virion components. LERNER et al. (1969) traced changes in the susceptibility of labeled NDV genomes to added ribonuclease at intervals after adding virus to cells. Some virion RNA became susceptible to ribonuclease about one hour after infection, but became resistant again by about four hours. The first event was attributed to uncoating, and the second to a shift of the uncoated RNA from a single- to a double-stranded form. Uncoating was not inhibited by puromycin treatment of the cells, suggesting that new proteins are not necessary for this function, in agreement with data of WHEELOCK (1962). Other observations indicate that nucleocapsids are only very gradually, and possibly not completely, divested of their protein in infected cells (BUKRINSKAYA et al., 1969). DURAND et al. (1970) found that isolated lysosomes solubilized some protein and RNA when mixed with labeled virions, and suggested that this might relate to the process of uncoating, but they cautioned that separating viral RNA from its protective proteins entirely in lysosomal vesicles might be risky in view of the presence

of ribonucleases in lysosomes. As will be discussed later, paramyxovirion transcriptase appears to function *in vitro* in structurally intact nucleocapsids, so it is conceivable that genome transcription at least, and perhaps also genome replication, occur without the RNA being completely uncoated.

IV. Macromolecule Synthesis

The distinctive feature of paramyxovirus replication that divorces it from the classical scheme of RNA virus replication is the production of large amounts of single-stranded RNA molecules smaller than viral genomes and complementary in base sequences to viral genomes. (For related findings with VSV, see SCHAFFER et al., 1968; PETRIC and PREVEC, 1970; SCHINCARIOL and HOWATSON, 1970; HUANG et al., 1970; MUDD and SUMMERS, 1970). While a direct demonstration of messenger functions for this complementary RNA has not been made, circumstantial evidence for this is abundant and the idea has been supported dramatically by the discovery of RNA transcriptases in paramyxovirions.

A. Virion Transcriptase and Early Synthetic Events

The search for a transcriptase in paramyxovirions would be predicated on two assumptions. First, that none of the viral genetic information essential to the early stages of infection is available as message in virion RNA; rather, genome transcripts would function as such messages. Second, that an enzyme capable of transcribing these messages does not pre-exist in uninfected cells. This situation would be formally analogous to that obtaining in poxvirus and reovirus replication where neither DNA nor double-stranded RNA can function as message, and the essential event of genome transcription is mediated not by cellular enzymes but by virion enzymes (KATES and MCAUSLAN, 1967; SHATKIN and SIPE, 1968). The inability, discussed above, of isolated RNA from paramyxoviruses to be infectious supports the idea that another substance from virus particles is essential. Similar notions about rhabdovirus replication led to the discovery of an RNA transcriptase in VSV particles (BALTIMORE et al., 1970), which appears to be virus-coded (CORMACK et al., 1971). The VSV enzyme is especially active, so the relatively low activity (about 2% of the VSV enzyme) demonstrable in NDV particles seemed insignificant (BALTIMORE et al., 1970). However, later, when the NDV reaction was examined more closely, RNA molecules of respectable size (sedimenting at about 16S) were seen, and these were complementary in base sequence to the viral genome (HUANG et al., 1971). Part of the product was associated with template virion 50S RNA in a partially base-paired form. Thus, a paramyxovirus contained an enzyme functionally similar to the VSV enzyme, although the paramyxovirus enzyme was much less active. (As chance would have it, among rhabdoviruses, VSV possesses an exceptionally active enzyme [AASLESTAD et al., 1971]). A similar activity was later detected in virions of Sendai virus (ROBINSON, 1971c; STONE et al., 1971) and SV5 (CHOPPIN, 1971). The Sendai virus enzyme