Biosynthesis of Mumps Virus F Glycoprotein: Non-fusing Strains Efficiently Cleave the F Glycoprotein Precursor

By DAVID CHARLES MERZ, ALFRED C. SERVER, M. NEAL WAXHAM AND JERRY S. WOLINSKY*

Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, U.S.A.

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SUMMARY

Mumps virus infection of the CV-1 cell line results either in no cytopathic effect or extensive cell fusion, depending upon the infecting mumps virus strain. Growth cycle analyses indicated that both types of infection were the result of multiple cycle replication of mumps virus. Intracellular virus-specific polypeptide synthesis was examined by pulse- and pulse-chase-labelling with radioactive amino acids and sugars. The major polypeptides seen on SDS-polyacrylamide gels were NP (69,000 mol. wt.), P (45,000 mol. wt.) and M (40,000 mol. wt.); a non-structural polypeptide (22,000 mol. wt.) was also present in infected cell lysates. The HN (74,000 to 79,000 mol. wt.) glycopolypeptide was detected in [3H]glucosamine- and [3H]mannose-labelled infected cells. A 65,000 mol. wt. species that had incorporated these precursors was seen in pulse-labelled infected cell lysates, and this glycopolypeptide vanished during the chase interval with the concomitant appearance of two glycopolypeptides (59,000 mol. wt. and 14,000 to 15,000 mol. wt.) which represented the F1 and F2 subunits of the F glycoprotein. Immunological data confirmed the relatedness of the 65,000 mol. wt. glycopolypeptide to the F glycoprotein and identified it as the precursor F0. The F0 precursor glycopolypeptide was seen in cells infected with both fusing and non-fusing strains, and F0 was processed completely to F glycoprotein for all infections. Thus, the lack of cell fusion after infection with certain mumps strains is not the consequence of incomplete processing of the F0 precursor.

INTRODUCTION

Paramyxoviruses possess a fusing activity which enables initiation of an infection by fusion of the viral envelope with cellular membranes and enhances the development of an infection by promoting cell-to-cell spread via syncytium formation (Choppin & Scheid, 1980). Fusion activity is primarily mediated by the viral F glycoprotein. The active F glycoprotein, which consists of two disulphide-linked polypeptides, F1 and F2, arises from an inactive precursor F0 by protease activation (Scheid & Choppin, 1974, 1977).

The cytopathic effects and virulence attributable to a paramyxovirus have been shown to be in part related to F glycoprotein activity. This conclusion is based on several elegant studies using precisely defined F protein mutants of Sendai virus (Scheid & Choppin, 1976) and naturally occurring strains of Newcastle disease virus (NDV) (Nagai et al., 1976, 1979; Madansky & Bratt, 1981). These studies have shown that persistence of the inactive F0 form, either due to an inability of the host cell to cleave F0 or to an insensitivity of the F0 precursor to proteolytic activation, results in a limited, non-cytopathic infection. Such an infection can be transformed to a cytopathic infection capable of further propagation, by the addition of a protease capable of cleaving F0 to the active F glycoprotein.

† Present address: The University of Texas Health Science Center at Houston, Department of Neurology, P.O. Box 20708, Houston, Texas 77025, U.S.A.
Mumps virus is a paramyxovirus that infects humans. Like other paramyxoviruses it possesses both a haemagglutinin–neuraminidase (HN) and an F glycoprotein (Jensik & Silver, 1976; Örvell, 1978a, b; McCarthy & Johnson, 1980; Server et al., 1982). One of the interesting properties of mumps virus is that some strains cause extensive cell fusion in tissue culture, while others can productively infect tissue cultures without causing cell fusion. The fusing mumps virus strains are more neuropathogenic in that they are neuroinvasive, i.e. disseminate to brain after peripheral inoculation (Wolinsky & Stroop, 1978), and neurovirulent, i.e. capable of infecting neurons once the central nervous system has been penetrated (Wolinsky et al., 1974; McCarthy et al., 1980). Since aberrations in the conversion of F₀ to active F glycoprotein have been associated for other paramyxoviruses with the loss of cell fusion potential and diminished virulence, we investigated F glycoprotein biosynthesis for the various mumps virus strains in order to understand better the strain-dependent variation in fusion phenotype in culture. Unlike the Sendai and Newcastle disease viruses, avirulent, non-fusing strains of mumps virus possess an F₀ protein that is completely processed to F glycoprotein prior to virus maturation. This occurs in a manner indistinguishable from that of the neuropathogenic, fusing mumps virus strains and in a cell system where different fusion cytopathologies are manifest. Thus, factors other than proteolytic processing of mumps virus F glycoproteins must determine mumps virus cytopathogenicity and virulence.

METHODS

Cells and viruses. A subclone of the CV-1 line of African green monkey kidney cells was used for all infections (Merz & Wolinsky, 1981). Six strains of mumps virus underwent three cycles of plaque purification in CV-1 cells prior to use. The RW and O'Take strains produce microplaques under agar and cause little cytopathic effect in tissue culture and no cell fusion. The Kilham, MD, MJ and Jeryl Lynn B strains produce larger plaques and cause extensive cell fusion. Each strain is unique as judged by HN protein activities (Merz & Wolinsky, 1981). Infectivity titrations were performed on CV-1 monolayers using a 2% agarose overlay in Eagle's minimal essential medium (MEM) supplemented with 4% newborn calf serum (Gibco).

Monoclonal antibody. Hybridoma cell cultures were obtained by fusing X63-Ag8.653 myeloma cells with lymphocytes from spleens of BALB/c mice immunized with the Kilham strain. Antibody-secreting hybrids were detected and subsequently cloned as detailed by Server et al. (1982) before purifying their secreted IgG by Protein A immunoadsorption (Protein A-Sepharose CL4B; Pharmacia). Assays of haemagglutination inhibition and infectivity neutralization (Server et al., 1982) and haemolysis inhibition (Merz et al., 1981) were performed using purified monoclonal antibody.

Infections. Confluent monolayers in 60 mm plastic Petri dishes were inoculated with mumps virus at a multiplicity of infection (m.o.i.) of 10 to 50, except the Jeryl Lynn B strain which was inoculated at a m.o.i. of 2. Inocula were removed after 2 h adsorption and the cultures further incubated in MEM. At 18 h post-infection, culture medium was replaced with MEM lacking the appropriate amino acid and the cultures were further incubated for 45 min. Deficient MEM was then replaced by the same medium made hypertonic with NaCl to a final excess concentration of 150 mM, and incubation was continued for 15 min. Pulse-labelling was accomplished by incubation with 1 ml hypertonic deficient MEM and either [35S]methionine (1200 Ci/mmol; Amersham), [35S]cysteine (1350 Ci/mmol; Amersham) or [3H]leucine (120 Ci/mmol, Amersham) at 100 to 200 μCi/ml for 1 h at 37 °C. Pulse-labelled products were chased for various time intervals with Dulbecco's reinforced minimal essential medium (REM). Oligosaccharide labelling was performed similarly with glucose-free MEM supplemented with fructose and [3H]mannose (15-8 Ci/mmol) and [3H]glucosamine (30 Ci/mmol) at 100 μCi/ml. Pulse and pulse-chase labelling were terminated after rinsing the monolayers with phosphate-buffered saline by lysing with either (i) 1% SDS, 1% dithiothreitol, 35% glycerol in 0.0625 M-Tris–HCl pH 6.8 (SDS lysis buffer), or (ii) 1% Zwittergent 3-10 (Calbiochem-Behring), 0.1% Triton X-100, 0.001% L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK; Sigma) in 0.01 M-Tris–HCl pH 7.4, 0.5 M-NaCl (immunoprecipitation buffer), and these samples were then stored at -70 °C. Samples in SDS lysis buffer were boiled at 100 °C for 3 min before freezing.

Immunoprecipitation and gel electrophoresis. Precipitation of viral proteins was performed using virus-specific antisera and a staphylococcal Protein A–agarose adsorbent (Server et al., 1982). Rabbit anti-mumps virus serum was obtained by immunization with the egg-grown Enders strain of virus. The anti-HN monoclonal antibody has been described by Server et al. (1982) and the anti-F monoclonal antibody will be described below. The monospecific anti-F antisera (Örvell, 1978a) was a gift of Dr E. Norrby (Karolinska Institute, Stockholm, Sweden).

Electrophoresis of immunoprecipitated products was performed in polyacrylamide gels in a discontinuous Tris–glycine buffer system (Laemmli, 1970). The different acrylamide : bisacrylamide compositions (A/B) of the
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RESULTS

Replication of mumps virus in tissue culture

Representative growth curves for two mumps virus strains are shown in Fig. 1. At a m.o.i. of 10, both the Kilham and RW strains showed initial bursts of infectious progeny virions at 30 h post-infection (Kilham: approx. 4 p.f.u./cell; RW: approx. 6 p.f.u./cell). The first signs of infection, namely cell fusion for Kilham, and increased cell refractility for RW, coincided with the initial bursts. Shortly after the initial burst, exponential production of virus ceased in Kilham strain-infected cultures; cell fusion encompassed these monolayers by 48 h post-infection. The RW strain-infected cultures continued to produce infectious virus with very little destructive cytopathic effects.

Cultures were infected at a m.o.i. of 0.01 to determine whether the fusing and non-fusing strains were capable of multiple cycle growth in CV-1 cells. The Kilham strain-infected cultures again showed fusion foci at 28 h post-infection, but the initial burst was delayed to 50 h post-infection. The RW strain showed a similar delay of the initial burst, to 60 h post-infection at a time when cytopathic effects were first apparent. The burst size was the same for both high and low input multiplicity RW infections. Growth curves for the O'Take strain were similar in shape to those for the RW strain (data not shown). Since the yields and kinetics of virus production were the same at both input multiplicities, although delayed 20 to 30 h at the lower m.o.i., both fusing and non-fusing strains had undergone multiple cycle replication in CV-1 cells.

Fig. 1. Growth curves of (a) Kilham (fusing) and (b) RW (non-fusing) strains of mumps virus. CV-1 cell cultures (2.8 × 10⁶ cells) were infected at a m.o.i. of 10 (●) or 0.01 (○). Samples at various times after infection were assayed for infectivity in duplicate.
Fig. 2. Identification of mumps virus glycoproteins. Disrupted [3H]leucine-labelled Kilham (a to c) and O'Take (d to j) virus were subjected to immunoprecipitation with monoclonal anti-HN (b, d, g, i) and monoclonal anti-F (c, e, h, j) antibody and polypeptides were identified by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). Untreated virus is shown in (a) and (j). Samples shown in (a to c) and (d, e, i, j) were treated with 1% dithiothreitol; those in (d, e, i, j) were not treated with reducing agent. All immunoprecipitates contained trace amounts of non-specifically precipitated NP polypeptide. The gels are linear 7.5 to 15% acrylamide gradient gels (A/B = 37.5).

**Definition of mumps virus F glycoprotein**

Immunoprecipitation of disrupted radiolabelled virus using monoclonal antibodies was used to identify the mumps virus structural glycoproteins. Fig. 2 shows the results obtained for the Kilham and O'Take strains of mumps virus. Seven or eight major polypeptides are found in disrupted virus (Fig. 2a, f). The major polypeptide with an apparent mol. wt. of 69000 and another of 45000 are the NP and P polypeptides respectively; these are associated with nucleocapsid structures (Huppertz et al., 1977; M. N. Waxham et al., unpublished observations). The 40000 mol. wt. polypeptide is soluble in 2% non-ionic detergent-2 M-KCl, but precipitates in low ionic strength solutions (Scheid & Choppin, 1973), and is thus the mumps virus M protein (Jensik & Silver, 1976; D. C. Merz et al., unpublished observations). Two polypeptides can be labelled with [3H]glucosamine: the HN glycopolypeptide, and another of 59000 mol. wt., the F1 glycopolypeptide (Server et al., 1982). As shown in Fig. 2(b, g), the anti-HN monoclonal antibody precipitated a single polypeptide of 79000 mol. wt. for Kilham and 74000 mol. wt. for O'Take. When the same immunoprecipitates were subjected to electrophoresis without reduction of disulphide bonds, larger forms consistent with dimeric and tetrameric HN were seen (Fig. 2d, i).

We have isolated a hybridoma cell line which secretes an IgG2a(κ) that precipitates the other glycoprotein of mumps virus, the F glycoprotein. Two or three polypeptides were detected in immunoprecipitates of [3H]leucine- or [3H]glucosamine-labelled Kilham and O'Take virus with this monoclonal antibody (Fig. 2c, h; glucosamine results not shown). For Kilham virus, the apparent mol. wt. were 59000 and 14200, while for O'Take virus the glycopolypeptides were 58000, 54500 and 15000 mol. wt. In a separate experiment, using O'Take virus 3H-labelled with amino acids, it was possible to determine that the molar ratio of the 58000 mol. wt. polypeptide to the 54500 mol. wt. polypeptide was 2:6, and this ratio was constant with prolonged incubation in tissue culture. Immunoprecipitates of [35S]methionine-labelled virus revealed only the larger glycopolypeptides, indicating a lack of methionine residues in the 14000 to 15000 mol. wt. polypeptide (data not shown).
Assays of function in the presence of this monoclonal antibody suggested that these glycopolypeptides corresponded with the F1 and F2 subunits of the F glycoprotein found for other paramyxoviruses (Scheid & Choppin, 1977; Hall et al., 1980). Specifically, the monoclonal antibody failed to inhibit haemagglutination but did inhibit haemolysis to a limited extent. Furthermore, the monospecific anti-mumps virus F glycoprotein antibody of Orvell (1978a) which inhibits haemolysis without inhibiting haemagglutination also immunoprecipitated the same glycopolypeptides (data not shown). Thus, the Kilham and O'Take strains, and other mumps virus strains (data not shown) contain two glycopolypeptides, F1 and F2. The 54500 mol. wt. glycopolypeptide of O'Take virus is termed Fx.

The disulphide linkage of the mumps virus F1 and F2 glycopolypeptides was demonstrated by electrophoresis without reduction (Fig. 2e, j). A single glycopolypeptide was detected in Kilham virus–anti-F monoclonal antibody immunoprecipitates with a mol. wt. of 69000 when compared to cross-linked, non-reduced molecular weight markers. Electrophoresis of excised gel slices containing these glycopolypeptides under reducing conditions yielded the F1 and F2 patterns shown in Fig. 2 (c, h). O'Take virus–anti-F monoclonal antibody immunoprecipitates contained two glycopolypeptides of mol. wt. 69000 and 65000 (data not shown). Thus, the mumps virus F glycoprotein is composed of F1 and F2 glycopolypeptides linked by disulphide bonds.

Detection of the precursor of mumps virus F glycoprotein

Cells infected at a m.o.i. of 10 were pulse-labelled at various times after infection in order to determine the time course of mumps virus polypeptide synthesis. Using a range of multiplicities from 5 to 50, only the NP, P and M polypeptides could be detected above background cellular polypeptide synthesis (data not shown). The initial appearance of the NP polypeptide was followed by the P and then the M polypeptide as the infection progressed.

In order to enhance detection of mumps virus-specific polypeptides, cells were treated with hypertonic medium immediately before and during the pulse interval (Peluso et al., 1977; Saborio et al., 1974) and incorporated label was subsequently chased in standard medium. A representative experiment is shown in Fig. 3(a). The NP, P and M polypeptides were readily seen in the RW mumps virus-infected cell lysate. The HN polypeptide migrated at the trailing edge of NP and was not readily seen unless the oligosaccharide moieties were labelled (Fig. 3b). A small polypeptide (22000 mol. wt.) was found in abundance, and a larger polypeptide (95000 mol. wt.) was also seen in infected cell lysates. The former was considered to be a non-structural (NS) polypeptide since anti-mumps virus sera failed to immunoprecipitate it (see Fig. 7) and limited proteolysis of the polypeptide generated a spectrum of fragments which showed no similarities to digest fragments of any of the other structural polypeptides (data not shown). The 95000 mol. wt. polypeptide may be one of the glucose transport polypeptides shown to be induced by other paramyxovirus infections (Peluso et al., 1978). Finally, a unique high molecular weight polypeptide was seen in all infected cell lysates. This polypeptide may correspond to the putative L polypeptide observed in purified mumps virus (Fig. 2a, f).

In pulse-labelled RW strain-infected cell lysates, a polypeptide with an apparent mol. wt. of 65000 was seen which migrated slightly faster than the NP polypeptide (Fig. 3a, b). This polypeptide rapidly disappeared in the subsequent chase period. This polypeptide had also incorporated [3H]glucosamine and mannose, and as it disappeared in the chase interval, there was a concomitant increase in the amount of F1 and F2 glycopolypeptides (Fig. 3b). These results suggest that the 65000 mol. wt. glycopolypeptide is the mumps virus F glycoprotein precursor F0.

RW virus-infected cell lysates pulse- and pulse-chase-labelled with [3H]glucosamine and mannose were treated with anti-mumps virus immunoadsorbent (Fig. 4a) and monospecific anti-F immunoadsorbent (Fig. 4b) to identify the mumps F0 precursor. Immunoprecipitates of pulse-labelled cell lysates contained a glycopolypeptide of 65000 mol. wt. as well as F1 and F2, while pulse-chase-labelled lysates contained only the F1 and F2 glycopolypeptides. Immunoprecipitates of mock-infected cell lysates treated with the same immunoadsorbent contained no radiolabelled material. These data show that the 65000 mol. wt. glycopolypeptide is

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immunologically related to the mumps virus F glycoprotein and identify it as the mumps virus precursor F₀ glycoprotein. Treatment of the same infected cell lysates with the monoclonal anti-F antibody failed to precipitate the 65000 mol. wt. glycopolypeptide.

Identification of the F₀ glycoprotein of other mumps virus strains

The occurrence and lability of the F glycoprotein precursor for the other mumps virus strains was investigated in an attempt to determine whether the fusing or non-fusing character of a strain was related to precursor-product processing. A comparison of Kilham strain- and RW strain-infected CV-1 cell lysates pulse-labelled with [³⁵S]methionine is shown in Fig. 5. Kilham strain virus-infected CV-1 cells contained a 65000 mol. wt. polypeptide which labelled and migrated similarly to the RW F₀ glycopolypeptide. Lysates of Vero cells infected with either Kilham or RW virus also contained a 65000 mol. wt. polypeptide which disappeared during the chase interval (data not shown).

Pulse- and pulse-chase-labelled infected cell lysates were treated with anti-mumps virus antiserum to extend these findings. Fig. 6 shows such an experiment performed with O'Take virus-infected cells; [³⁵S]cysteine was chosen so that the F₂ glycopolypeptide could be readily

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Fig. 3. Polypeptide synthesis in mumps virus-infected CV-1 cells. (a) RW virus-infected cultures (m.o.i. = 10) were pulse-labelled with [³H]leucine (100 µCi/ml) in hypertonic medium for 1 h and subsequently chased for the indicated time intervals (min). Cell lysates were subjected to SDS-PAGE in 10% acrylamide gels (A/B = 76:9). (b) RW-infected cultures (m.o.i. = 10) were pulse-labelled with [³H]leucine (100 µCi/ml) in hypertonic medium (Leu) for 1 h or [³H]glucosamine and [³H]mannose (100 µCi/ml each) (GlcN, Man) for 1-5 h and then chased for 2 h. Cell lysates were subjected to SDS-PAGE in a linear 7-5 to 15% acrylamide gel (A/B = 37:5). U = mock-infected cell lysates.

Fig. 5. Comparison of polypeptide synthesis in CV-1 cells infected with the RW (non-fusing) and Kilham (fusing) mumps virus strains. CV-1 cells infected at a m.o.i. of 10 were pulse-labelled with [³⁵S]methionine (100 µCi/ml) in hypertonic medium for 1 h and subsequently chased. Electrophoresis of cell lysates was done in 10% acrylamide gels (A/B = 76:9). U = mock-infected cell lysate.

Fig. 6. Immunoprecipitation of O'Take-infected cell lysates. Infected cells (m.o.i. = 10) were pulse-labelled with [³⁵S]cysteine (20 µCi/ml) in hypertonic medium for 1 h and subsequently chased for 2 h. Lysates were treated with anti-mumps virus immunoadsorbents and the precipitates were applied to a linear 7-5 to 15% acrylamide gel (A/B = 37:5).
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Fig. 4. Immunological identification of intracellular forms of mumps virus glycoproteins. (a) [³H]glucosamine/mannose- and [³H]leucine-labelled RW-infected cell lysates with or without chase (see Fig. 3b) were treated with anti-mumps virus immunoadsorbent and the resulting precipitates subjected to SDS-PAGE on a linear 7.5 to 15% acrylamide gel (A/B = 37.5). U = mock-infected cell lysates treated with anti-mumps virus immunoadsorbent. (b) [³H]glucosamine/mannose-labelled RW-infected cell lysates with or without chase were treated with monospecific anti-F immunoadsorbent and subjected to SDS-PAGE on a linear 7.5 to 15% acrylamide gel (A/B = 37.5).

Fig. 5

Fig. 6
Fig. 7. Comparison of polypeptide synthesis in cells infected with various mumps virus strains. CV-1 cells infected at a m.o.i. of 10 were pulsed-labelled with $[^{35}S]$methionine (200 µCi/ml) in hypertonic medium for 1 h and then chased for 2 h. Cell lysates were treated with an anti-mumps virus immunoadsorbent and the precipitates analysed in 10% acrylamide gels (A/B = 76-9).

identified by its disulphide linkage to the F1 glycopolypeptide. A 65000 mol. wt. protein was seen in pulse-labelled lysates; it disappeared during the chase interval with the concomitant appearance of the F1 and F2 glycopolypeptides. Similar results were obtained with $[^{3}H]$leucine-labelled infected cells (data not shown).

Fig. 7 shows immunoprecipitates prepared with anti-mumps virus serum from various mumps virus strain-infected cell lysates labelled with $[^{35}S]$methionine. In each instance a 65000 mol. wt. polypeptide was seen in the pulse-labelled infected cell lysate, and this polypeptide disappeared with the simultaneous appearance of the F1 glycopolypeptide during the chase interval. The Jeryl Lynn B strain also directed the synthesis of a 65000 mol. wt. precursor polypeptide which yielded the F1 polypeptide over the chase interval (data not shown). Therefore, all of the mumps virus strains direct the synthesis of a 65000 mol. wt. precursor to the F glycoprotein, which in each case is cleaved within a 2 h chase interval.

**DISCUSSION**

Our results indicate that mumps virus-infected cells contain seven virus-specified polypeptides as has been described for other paramyxoviruses (Lamb *et al.*, 1976; Peluso *et al.*, 1977; Hightower & Bratt, 1974). The 69000 mol. wt. NP protein is the most abundant polypeptide in infected cells. Polypeptides corresponding to the nucleocapsid-associated polypeptide P (45000 mol. wt.) and membrane (M) protein (40000 mol. wt.) are also present. A polypeptide of mol. wt. 22000 is found in infected cells which does not precipitate with anti-mumps virus antiserum and has a unique limited proteolysis peptide map; this is consistent with it being a non-structural polypeptide (termed NS) like those seen in other paramyxovirus infections (Lamb *et al.*, 1976; Lamb & Choppin, 1978; Peluso *et al.*, 1977).

Two glycopolypeptides are seen in mumps virus-infected cells. One, whose apparent mol. wt. varies from 74000 to 79000 between strains, corresponds to the HN glycoprotein and is recognized by a well characterized anti-HN monoclonal antibody (Server *et al.*, 1982). No evidence of a precursor (HN0) to the HN glycopolypeptide (Nagai & Klenk, 1977; Nagai *et al.*, 1976) was obtained for any of the mumps virus strains examined. The other glycopolypeptide appears in pulse-labelled cells initially as a 65000 mol. wt. polypeptide. In subsequent chase
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intervals, this glycopolypeptide disappears with the concomitant appearance of two glycopolypeptides with mol. wt. of 59000 and 14000 to 15000. By both immunological and chemical methods we have shown that these glycopolypeptides represent the precursor F0, and the F1 and F2 subunits of the mumps virus F glycoprotein (Fig. 2). Conformational differences between the F0 and F glycoproteins, like those found for Sendai and Newcastle disease viruses (Hsu et al., 1981; Kohama et al., 1981), are likely to be present for mumps virus, since the monoclonal antibody specific for the F glycoprotein did not recognize the F0 form which was recognized by the monospecific anti-F antibody.

Our results are in general agreement with several recent reports concerning polypeptide synthesis in mumps virus-infected cells (Rima et al., 1980; Naruse et al., 1981; Herrler & Compans, 1982). The polypeptide we have designated NS corresponds nicely with Rima's C polypeptide (Rima et al., 1980). However, we did not observe the glycopolypeptide migrating between HN and F0 seen by Rima et al. (1980). The electrophoretic mobilities observed for F0 and F1 were similar to those reported by both the Rima and Naruse groups; Herrler & Compans (1982) described an F0 which migrated markedly more slowly. This latter discrepancy might represent strain-dependent rather than host-dependent variation, since we found no differences in F0 electrophoretic mobility when infected CV-1 and Vero cell lysates were compared directly.

Paramyxoviruses mediate the fusion of lipid bilayers through the action of the F glycoprotein. This fusion activity is manifested in two general ways: (i) externally mediated fusion which can result in haemolysis, 'fusion from without', and virus penetration of the cell and (ii) internally mediated post-infection cell fusion and syncytia formation ('fusion from within').

Past studies of paramyxoviruses have shown that proteolytic cleavage of the F0 precursor confers infectivity, haemolytic and cell-fusing activities on virions which correlate with virulence (Scheid & Choppin, 1974, 1976; Nagai et al., 1976, 1979; Nagai & Klenk, 1977; Fujinami & Oldstone, 1981). Our results show that the mumps F0 precursor is completely cleaved in infected cells, resulting in its absence in purified infectious virions of both non-fusing and fusing strains (Fig. 2). This enables both strain types to undergo multiple cycle growth in CV-1 cells.

Cell-associated active F glycoprotein is necessary for cell fusion during productive infection (Merz et al., 1980, 1981). Nagai et al. (1976) found that cells infected with NDV which did not develop syncytia contained the F0 precursor, whereas infected cells which underwent cell fusion activated the F0 precursor to the F glycoprotein. The various mumps virus strains we studied are like NDV in that within the same tissue culture system, some strains cause cell fusion following infection, while others do not. It was possible that cells infected with the non-fusing strains contained a disproportionate amount of F0 precursor compared to cells similarly infected with the fusing strains. However, our results show that regardless of strain, mumps virus F0 glycoprotein is completely processed to F glycoprotein in infected cells. This result is in marked contrast to the persistence of the NDV F0 glycoprotein in non-fusing infections (Nagai et al., 1976).

Our results indicate that the ability of a particular mumps virus strain to cause cell fusion during the course of an infection must result from some mechanism(s) in addition to proteolytic cleavage of the F glycoprotein. It remains possible that internally mediated cell fusion activities of the different F glycoproteins of mumps virus strains are distinct. Proteolytic cleavage of virus glycoprotein precursors may not always generate products with the same range of activities. Lazarowitz & Choppin (1975) reported that several different proteases could cleave the influenza virus haemagglutinin precursor to haemagglutinin molecules composed of polypeptide subunits of indistinguishable electrophoretic mobilities. Yet only one protease rendered the virion infectious. By analogy the lack of internally mediated cell fusion for the non-fusing mumps virus strains could represent differential sensitivity of the F0 glycoprotein to host cell proteases. The demonstration of the F5 glycopolypeptide in the non-fusing O'Take strain is an example of altered cleavage. Alternatively, the activity of HN glycoprotein may modify the expression of the active F glycoprotein function since the tissue culture cell fusion activity of mumps virus has been shown to correlate with virus-associated neuraminidase activity (Merz & Wolinsky, 1981).
The expression of mumps virus fusing activity in tissue culture correlates with neuropathogenicity, and similar observations with respect to neurovirulence and cell fusion have been made for parainfluenza virus 3 (Shibuta et al., 1982) and canine parainfluenza virus (Evermann et al., 1980; Baumgartner et al., 1981). It therefore remains important to understand fully the complex factors which govern paramyxovirus-mediated cell fusion, particularly as they relate to central nervous system pathogenicity.

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REFERENCES


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