Acta virol. **34** : 220—227, 1990

MONOCLONAL ANTIBODIES AGAINST THE NUCLEOPROTEIN OF MUMPS VIRUS: THEIR BINDING CHARACTERISTICS AND CROSS-REACTIVITY WITH OTHER PARAMYXOVIRUSES

MASATO TSURUDOME[1], AKIO YAMADA[2], MICHIKO HISHIYAMA[2], YASUHIKO ITO[1]

[1]Department of Microbiology, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie 514, and [2]Department of Measles Virus, National Institute of Health Gakuen, Musashi-Murayama, Tokyo, Japan

Received January 4, 1989

Summary. — Twelve monoclonal antibodies (MoAbs) directed against the nucleoprotein (NP) of mumps virus were analysed for their binding characteristics. Competitive binding in enzyme-linked immunosorbent assay divided them into eight groups. Two of the MoAbs recognized exclusively 66 kD polypeptide of NP, two recognized 66 kD and 60 kD, and one recognized 66 kD (and 60 kD to a lesser extent) in Western blot assays under either denaturing or partially denaturing conditions. Under partially denaturing condition, another five MoAbs reacted faintly but the remaining two did not react at all. Under denaturing condition, on the other hand, these seven MoAbs showed little reactivity with any polypeptide. Furthermore, denaturation resulted in formation of other polypeptides 55 kD, 50 kD, and 43 kD which all were detected by MoAbs reacting with 66 kD and/or 60 kD. Previously demonstrated antigenic cross-reactivity among the NPs of mumps virus and those of human parainfluenza viruses type 2 and type 4 in radioimmunoprecipitation assay using polyclonal antisera was confirmed by an anti-NP MoAb which showed little reactivity in denaturing Western blot assay.

Key words: *mumps virus*; *nucleoprotein*; *monoclonal antibody*

Introduction

The genus Paramyxovirus belongs to the family *Paramyxoviridae* and consists of human parainfluenza viruses type 1 (PIV1), type 2 (PIV2), type 3 (PIV3), type 4 (PIV4), mumps virus bovine parainfluenza virus type 3 (bovine PIV3), Newcastle disease virus (NDV) and Sendai virus (Kingsbury *et al.*, 1978). Simian virus 5 (SV5) have long been considered as an animal type of PIV2.

Antigenic interrelationships among the paramyxoviruses have been extensively investigated (Cook *et al.*, 1959; Van der Veen and Sondercamp

1965; Goswami and Russel, 1982, 1983; Julkunen, 1984; Ray and Compans, 1986). There is not enough information, however, about the relationships with respect to each structural protein. Recently, Ito *et al.* (1987*b*) using the immunoprecipitation technique have shown that the NP of mumps virus was most cross-reactive, especially with those of PIV2 and PIV4 and that of SV5, and thus, possible common epitopes among the NPs of these viruses were assumed. A panel of MoAbs against the NP of mumps virus was reported in a previous study (Tsurudome *et al.*, 1986) but these MoAbs were not further characterized. In the present study, the MoAbs were analysed for their binding characteristics and examined for the cross-reactivity with the NPs of other paramyxoviruses, revealing antigenic relatedness among the NPs of mumps virus, PIV2, and PIV4.

Materials and Methods

Cells and viruses. Vero, LLCMK2 and primary monkey kidney cells were grown in Eagle's minimum essential medium (EMEM) supplemented with 5% foetal calf serum. Vero cell-adapted Enders strain (EY strain) of mumps virus, Toshiba strain of PIV2, 21004-WR strain of SV5 and Nagoya strain of Sendai virus were grown in Vero cells. Toshiba strain of PIV1, Toshiba strain of PIV3, Miyadera strain of NDV were grown in LLCMK2 cells. Toshiba strain of PIV4 subtype A (PIV4A) and 62-M786 strain of PIV2 were grown in primary monkey kidney cells. These stock viruses were frozen at −80 °C until use. An egg-grown Enders strain (EA strain) of mumps virus was purified as described previously (Yamada *et al.*, 1984).

Antibody. A male Balb/c mouse (Charles River Inc., Atsugi, Japan) was immunized with the EA strain of mumps virus and hybridoma clones were obtained by fusing the immune spleen cells and SP2/OAg14 myeloma cells and the specificity was determined as described previously (Tsurudome *et al.*, 1986). The isotypes of the MoAbs were determined as described by Sato *et al.* (1985).

Competitive binding assay. Competitive binding assay in ELISA was carried out as described by Sato *et al.* (1985). The inhibition ratio of competitors was calculated as the percentage of the absorbance value of their maximal inhibition compared with the absorbance value given by a peroxidase-conjugated MoAb in the absence of any competitor.

Polyacrylamide gel electrophoresis (PAGE) and Western blot assay. Samples were disrupted with equal amount of PAGE sample buffer [125 mmol/l Tris-HCl, pH 6.6; 4% Tris-dodecyl sulphate (Tris-DS; Katayama Chemical, Osaka, Japan); 10% (v/v) 2-mercaptoethanol, 20% (v/v) glycerol] at 4 °C for 4 hr (partially denaturing condition). When sodium dodecyl sulphate (SDS) was used as a substitute for Tris-DS, the disruption was performed by boiling for 5 min (denaturing condition). The resulting samples were subjected to PAGE, in which Tris-buffered systems (Laemmli, 1970) were used. The upper electrode buffer and the gels were added with 0.1% Tris-DS or SDS corresponding to the sample buffer. All processes of PAGE were performed at 4 °C when Tris-DS was employed (partially denaturing condition), whereas at room temperature (RT) in the case of SDS (denaturing condition). The electrophoretic transfer of polypeptides from the gels to nitrocellulose membranes was performed at 4 °C as the method of Towbin *et al.* (1979). After blocking with normal horse serum, the membranes were treated with MoAbs (diluted 1 : 10), biotinylated horse anti-mouse IgG (Vector Laboratories, Cal., U.S.A.) and avidin-biotin-peroxidase complex (Vector Laboratories). Then the membranes were immersed in methanol/PBS (1 : 4) containing 4-chloro-1-naphthol (0.3%) and hydrogen peroxide (0.009%).

Radioimmunoprecipitation assay (RIPA). RIPA was done as described by Ito *et al.* (1987*b*). In brief, Vero cells were infected with viruses at a multiplicity of infection of 0.1 to 10 $TCID_{50}$ per cell, labelled with [^{35}S]methionine (40 μCi/ml) and cell extracts were prepared. The precipitates with MoAbs were subjected to SDS-PAGE as described above and the gels were processed for fluorography as described by Bonner and Laskey (1974).

Table 1. Competitive binding assay for anti-NP MoAbs

Competitor		Isotype	Peroxidase-conjugated MoAbs											
			7	88	87	62	48	106	11	41A	55	39B	45B	23D
I	7	IgG2a	+[a]	+	(+)[b]	−[c]	−	−	−	−	−	−	−	−
II	88	IgG1	−	+	−	+	+	−	−	−	−	−	−	−
	87	IgG2a	−	+	+	+	+	−	−	−	−	−	−	−
	62	IgG2a	−	+	(+)	+	+	−	−	−	−	−	−	−
	48	IgG2a	−	−	(+)	+	+	−	−	−	−	−	−	−
III	106	IgG3	−	+	(+)	+	+	+	(+)	−	−	−	−	−
IV	11	IgG3	−	−	−	−	(+)	−	+	−	−	−	−	−
V	41A	IgG2b	−	(+)	−	(+)	(+)	−	−	+	+	−	−	−
	55	IgG2a	−	−	−	(+)	(+)	−	−	+	+	−	−	−
VI	39B	IgG2a	−	−	−	(+)	(+)	−	−	−	−	+	−	−
VII	45B	IgG1	−	−	−	−	(+)	−	−	−	−	−	+	(+)
VIII	23	IgG1	−	−	−	−	−	−	−	−	−	−	−	+

[a] Complete inhibition (<25%).
[b] Partial inhibition (25 to 50%).
[c] No inhibition (>50%).

Results

General characterization

The specificity of anti-NP MoAbs was determined by RIPA followed by SDS-PAGE and fluorography using [^{35}S]methionine-labelled extract of HeLa cells infected with the EY strain as described previously (Tsurudome *et al.*, 1986). In order to analyse topographically the antigenic determinants of the Enders strain NP, competitive binding using ELISA was measured (Table 1). The anti-NP MoAbs were classified into eight groups recognizing different

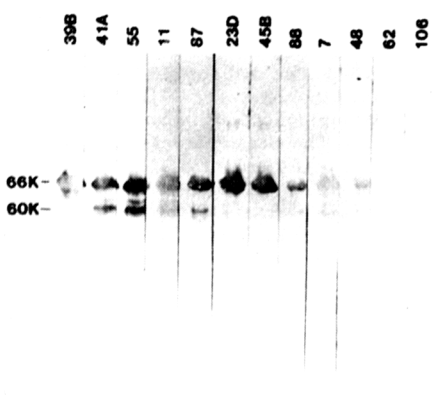

Fig. 1
Partially denaturing Western blot assay with anti-NP MoAbs
The EA strain of mumps virus was disrupted at 4 °C in the presence of Tris-DS and subjected to PAGE at 4 °C, electroblotted onto nitrocellulose membranes and reacted with MoAb (indicated above each lane) as described in Materials and Methods.

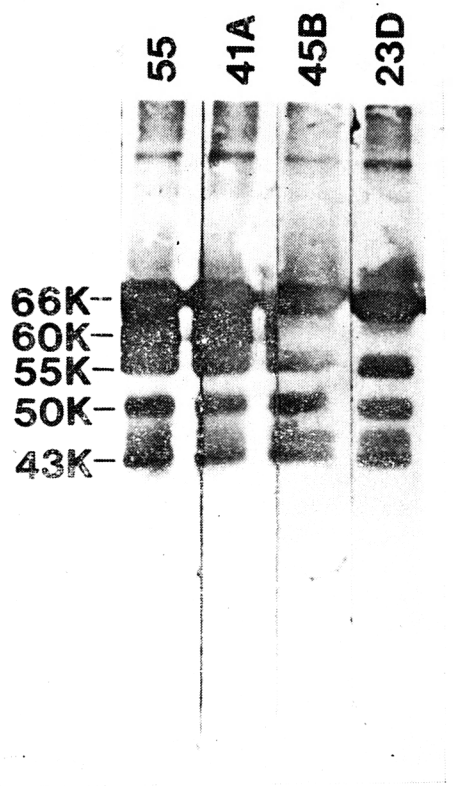

Fig. 2
Denaturing Western blot assay with anti-NP MoAbs
The EA strain was disrupted by boiling for 5 min in the presence of SDS, subjected to PAGE at RT and reacted with MoAbs after electroblotting.

antigenic sites. On the other hand, it has been reported that the SBL-1 strain NP contained at least ten separate antigenic sites (Örvell, 1984).

Antigen recognition of MoAbs in Western blot assays

Fig. 1 shows the results of immunostaining with anti-NP MoAbs in partially denaturing Western blot assay using Tris-DS. MoAbs 23D and 45B exclusively reacted with the polypeptide whose molecular weight was approximately 66 kD corresponding to the authentic size of mumps virus NP, whereas MoAbs 55 and 41A reacted with 66 kD, 60 kD, and a few polypeptides migrated between 66 kD and 60 kD. MoAb 87 reacted with 66 kD and to a lesser extent with 60 kD. Other five MoAbs (39B, 11, 88, 7, and 48) reacted with 66 kD and 60 kD to lesser extents, whereas the remaining two MoAbs (62 and 106) showed little reactivity. In denaturing Western blot assay using SDS (described later), none of these MoAbs (39B, 11, 88, 7, 48, 62, and 106) reacted with any polypeptide (data not shown).

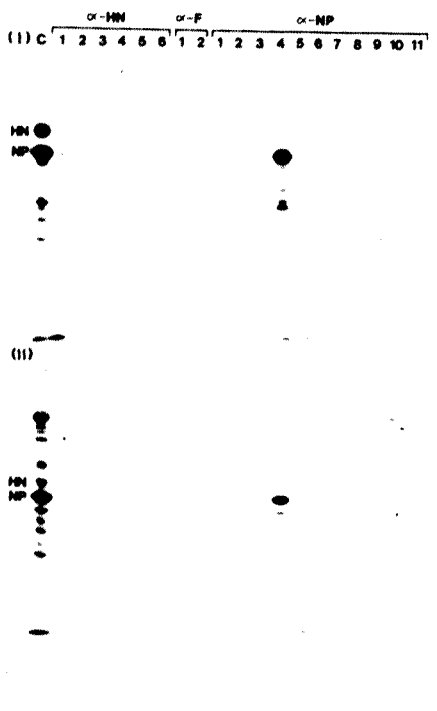

Fig. 3
Fluorogram of SDS-PAGE gels after RIPA using the 62-M786 strain of PIV2 (I) and the Toshiba strain of PIV4A (II) Virus infected Vero cells extracts labelled with (^{35}S) methionine were immunoprecipitated with MoAbs and the precipitates were analysed by SDS-PAGE. Lanes HN-1 to HN-6, anti-HN MoAbs 30B, 3-1, 85, 54, 8C, and 73; lanes F-1 and F-2, anti-F MoAbs 101 and 84; lanes NP-1 to NP-11, anti-NP MoAbs, 45B, 88, 7, 39B, 55, 87, 41A, 11, 106, 23D and 48; c, polyclonal antiserum to the corresponding virus.

In vitro proteolysis by boiling with SDS-containing sample buffer

As shown in Fig. 1 in partially denaturing Western blot assay, MoAbs 55 and 41A recognized both 66 kD and 60 kD, whereas MoAbs 23D and 45B recognized only 66 kD. This result was confirmed in denaturing Western blot assay, in which the virions were disrupted by boiling in the presence of SDS (Fig. 2). Intriguingly, additional polypeptides (55 kD, 50 kD, and 43 kD) were detected by either of the four MoAbs under such denaturing condition.

Binding of MoAbs with other paramyxoviruses

Ito *et al.* (1987*b*) have reported that polyclonal antisera against mumps virus also reacted with PIV2, PIV4, and SV5. The polypeptide involved in the cross-reactivity was mainly NP. In this study, a panel of anti-NP MoAbs together with anti-HN and anti-F MoAbs (Tsurudome *et al.*, 1986) was examined for the cross-reactivity with the paramyxoviruses and one of anti-NP MoAbs (MoAb 39B) clearly precipitated the NPs of PIV2 (62-M786 strain) and PIV4A (Toshiba strain) (Fig. 3-I and Fig. 3-II, respectively) and that of SV5 (data not shown). The NPs of other paramyxoviruses, that is, PIV1 and PIV3, Sendai virus, NDV, and unexpectedly Toshiba strain of PIV2, were not recognized by all the MoAbs tested (data not shown).

Discussion

It has been reported that mumps virus nucleocapsid derived from an egg-grown Enders strain contained major polypeptides 66 kD and 61 kD (Huppertz and ter Meulen, 1977). Although nucleocapsid fraction should contain polymerase (45—47 kD) and large (180—200 kD) proteins in addition to NP (Wolinsky and Server, 1985), the amounts of the former two proteins are small in the nucleocapsid. Since the relative amounts of 66 kD and 61 kD were similar (Huppertz and ter Meulen, 1977) and the molecular weight of 61 kD is too large if it is the polymerase protein and too small if it is the large protein, one could assume that 61 kD may be a cleaved form of 66 kD. It is known that mumps virus NP is readily susceptible to proteolytic degradation by cellular proteases (McCarthy and Lazzarini, 1982; Server *et al.*, 1982). In the present study, an unconventional Western blot assay using Tris-DS (Kubo and Takagi, 1986) was employed in order to analyse the NP with minimum denaturation, and to examine sensitively the binding characteristics of the MoAbs. In partially denaturing Western blot assay using Tris-DS, it was shown that an egg-grown Enders strain contained almost equal amount of 66 kD and 60 kD. The 60 kD polypeptide detected by MoAbs 55 and 41A seemed to be a proteolytically cleaved form of 66kD by some cellular protease. If it could be postulated that the N-termini of 66 kD and 60 kD are retained as those of other paramyxoviruses after cleavage by several proteases (Heggeness *et al.*, 1981; Mountcastle *et al.*, 1974), these MoAbs may recognize 60 kD containing the N-terminus of 66 kD. On the other hand, MoAbs 23D and 45B reacted exclusively with 66 kD and the inability of these MoAbs to react with 60 kD could be explained by the loss of 6 kD, from the C-termimus which should contain antigenic sites recognized by these MoAbs.

Aspartyl-prolyl peptide bonds can be cleaved by heating in PAGE sample buffer containing SDS (Rittenhouse and Marcus, 1984). In the present study, heating mumps virions in the presence of SDS (the denaturing Western blot assay) resulted in additional cleavage of 66 kD into 55 kD, 50 kD, and 43 kD, all of which were recognizable by MoAbs 55, 41A, 23D, and 45B. That these smaller polypeptides were recognized by MoAbs 23D and 45B implies that these polypeptides should contain at least a portion of 6 kD which was not included in 60 kD. MoAbs 55 and 41A seem to recognize antigenic sites located between the cleavage sites of 60 kD and 43 kD.

Radioimmunoprecipitation assay, one of the most sensitive techniques for antigen detection, revealed the cross-reactivity of an MoAb specific for the NP of mumps virus with the NPs of PIV2 and PIV4A. The inability of this MoAb to react with the NP of the Toshiba strain of PIV2, despite of its reactivity with that of the 62-M786 strain of PIV2 was intriguing, since polyclonal anti-mumps virus serum reacted with the NPs of either strains (Ito *et al.*, 1987*a*). The molecular weight of the NP of the former strain was larger than that of the latter. The NP of PIV4A was shown to be cleaved immediately after its synthesis in Vero cells and this MoAb reacted with the cleaved polypeptide, namely NP in this study, and not with its larger

precursor (Komada *et al.*, 1989). One type of antibody recognizes the primary structure of polypeptides (continuous amino acid sequence) and the other type of antibody recognizes conformational structure (discontinuous amino acid sequence). This MoAb did not react with mumps virus NP in the denaturing Western blot assay using SDS, showing that it could not recognize a denatured form of NP and reacted with conformational structure which might be preserved in immunoprecipitation assay. Thus, it could be assumed that the 62-M786 strain (PIV2) has an NP, which may be deleted form of that of the Toshiba strain (PIV2), and that such deletion may lead to a conformational alteration, resulting in an exposure of a de novo appeared epitope on the molecule. Another possibility is that the epitope is unique to the 62-M786 strain being independent of the deletion. The primary structures of the NPs of the both strains of PIV2 were now under determination to elucidate these problems.

Antigenic relatedness between the NPs of mumps virus and PIV2 could not be detected with a large set of anti-NP MoAbs for mumps virus (Örvell *et al'*, 1986). That an MoAb recognizing mumps virus NP also reacted with NPs of PIV2 and PIV4A in this study indicate that a common antigenic site was conserved among these viruses probably as a site on the tertiary structure on the NPs. In our recent study (Tsurodome *et al.*, 1989), furthermore, it was shown that an MoAb directed against PIV2 (Toshiba strain) NP also reacted with the NPs of PIV2 62-M786 strain and mumps virus either in RIPA or Western blot assay, indicating another common epitope consisting of continuous amino acids among these viruses.

Acknowledgements. We wish to thank Dr. A. Sugiura for his continuous advice and encouragement. We are also grateful to Miss M. Nishio for her assistance in preparing the manuscript. This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan.

References

Bonner, W. M., and Laskey, R. A. (1974): A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* **46**, 83–88.

Cook, M. K., Andrews, B. E., Fox, H. H., Turner, H. C., James, W. D., and Chanock, R. M. (1959): Antigenic relationships among the "newer" myxoviruses (parainfluenza). *Amer. J. Hyg.* **69**, 250–264.

Goswami, K. K. A., and Russel, W. C. (1982): A comparison of paramyxoviruses by immunoprecipitation. *J. gen. Virol.* **60**, 177–183.

Goswami, K. K. A., and Russel, W. C. (1983): Monoclonal antibodies against human parainfluenza virus type 3 and against SV5 virus: preparation and preliminary characterization. *J. gen. Virol.* **64**, 1663–1672.

Heggeness, M. H., Scheid, A., and Choppin, P. W. (1981): The relationship of conformational changes in the Sendai virus nucleocapsid to proteolytic cleavage of the NP polypeptide. *Virology* **114**, 555–562.

Huppertz, H. I., and ter Meulen, V. (1977): Polypeptide composition of mumps virus. *Med. Microbiol. Immunol.* **163**, 251–259.

Ito, Y., Tsurudome, M., and Hishiyama, M. (1987*a*): The polypeptides of human parainfluenza type 2 virus and their synthesis in infected cells. *Arch. Virol.* **65**, 211–224.

Ito, Y., Tsurudome, M., Hishiyama, M., and Yamada, A. (1987*b*): Immunological interrelationships among human and non-human paramyxoviruses revealed by immunoprecipitation. *J. gen. Virol.* **68**, 1289–1297.

Julkunen, I. (1984): Serological diagnosis of parainfluenza virus infections by enzyme assay with special emphasis on purity of viral antigens. *J. med. Virol.* **14**, 177–187.

Kingsbury, D. W., Bratt, M. A., Choppin, P. W., Hanson, R. P., Hosaka, Y., ter Meulen, V., Norrby, E., Plowright, W., Rott, R., and Wunner, W. H. (1978): Paramyxoviridae. *Intervirol.* **10**, 137–152.

Komada, H., Tsurudome, M., Bando, H., Nishio, M., Yamada, A., Hishiyama, M., and Ito, Y. (1989): Virus specific polypeptides of human parainfluenza virus type 4 and their synthesis in infected cells. *Virology* **171**, 254–259.

Kubo, K., and Takagi, T. (1986): Marked effect of cations on the separation of spinach thylakoid chlorophyll-proteins on polyacrylamide gel electrophoresis at 0 °C in the presence of alkanolammonium dodecyl sulfates. *J. Biochem.* **99**, 1545–1548.

Laemmli, U. (1970): Cleavage of proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**, 680–685.

McCarthy, M., and Lazzarini, R. A. (1982): Intracellular nucleocapsid RNA of mumps virus. *J. gen. Virol.* **58**, 205–209.

Mountcastle, W. E., Compans, R. W., Lackland, H., Choppin, P. W. (1974): Proteolytic cleavage of subunits of the nucleocapsid of the paramyxovirus simian virus 5. *J. Virol.* **14**, 1253–1261.

Örvell, C. (1984): The reactions of monoclonal antibodies with structural proteins of mumps virus. *J. Immunol.* **132**, 2622–2629.

Örvell, C., Rydbeck, R., and Löve, A. (1986): Immunological relationship between mumps virus and parainfluenza viruses studied with monoclonal antibodies. *J. gen. Virol.* **67**, 1929–1939.

Ray, R., and Compans, R. W. (1986): Monoclonal antibodies reveal extensive antigenic differences between the hemagglutinin-neuraminidase glycoproteins of human and bovine parainfluenza 3 virus. *Virology* **148**, 232–236.

Rittenhouse, J., and Marcus, F. (1984): Peptide mapping by polyacrylamide gel electrophoresis after cleavage at aspartyl-prolyl peptide bonds in sodium dodecyl sulfate-containing buffers. *Anal. Biochem.* **138**, 442–448.

Sato, T. A., Fukuda, A., and Sugiura, A. (1985): Characterization of major structural proteins of measles virus with monoclonal antibodies. *J. gen. Virol.* **66**, 1397–1409.

Server, A. C., Merz, D. C., Waxham, M. N., and Wolinsky, J. S. (1982): Differentiation of mumps virus strains with monoclonal antibody to the HN glycoprotein. *Infect. Immun.* **35**, 179–186.

Towbin, H., Staehelin, T., and Gordon, J. (1979): Electrophoretic transfer of proteins from polyacrylamide gels to nitro-cellulose sheets: procedure and some applications. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354.

Tsurudome, M., Nishio, M., Komada, H., Bando, H., and Ito, Y. (1989): Extensive antigenic diversity among human parainfluenza type 2 virus isolates and immunological relationship among paramyxoviruses revealed by monoclonal antibodies. *Virology* **171**, 38–48.

Tsurudome, M., Yamada, A., Hishiyama, M., and Ito, Y. (1986): Monoclonal antibodies against the glycoproteins of mumps virus: fusion inhibition by anti-HN monoclonal antibody. *J. gen. Virol.* **67**, 2259–2265.

Van der Veen, J., and Sondercamp, H. A. (1965): Secondary antibody response of guinea pigs to parainfluenza and mumps viruses. *Arch. ges. Virusforsch.* **15**, 721–734.

Wolinsky, J. S., and Server, A. C. (1985): Mumps virus, pp. 1255–1284. In B. N. Fields, D. M. Knipe, R. M. Chanock, J. L. Melnick, B. Roizman, and R. E. Shope (Eds): *Virology*, Raven Press, New York.

Yamada, A., Tsurudome, M., Hishiyama, M., and Ito, Y. (1984): Inhibition of host cellular ribonucleic acid synthesis by glycoprotein of mumps virus. *Virology* **135**, 299–307.