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Mutations in the Cytoplasmic Tail of Influenza A Virus Neuraminidase Affect Incorporation into Virions

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The significance of the conserved cytoplasmic tail sequence of influenza A virus neuraminidase (NA) was analyzed by the recently developed reverse genetics technique (W. Luytjes, M. Krystal, M. Enami, J. D. Parvin, and P. Palese, Cell 59:1107–1113, 1989). A chimeric influenza virus A/WSN/33 NA containing the influenza B virus cytoplasmic tail rescued influenza A virus infectivity. The transfectant virus had less NA incorporated into virions than A/WSN/33, indicating that the cytoplasmic tail of influenza virus NA plays a role in incorporation of NA into virions. However, these results also suggest that the influenza A virus and influenza B virus cytoplasmic tail sequences share common features that lead to the production of infectious virus. Transfectant virus was obtained with all cytoplasmic tail mutants generated by site-directed mutagenesis of the influenza A virus tail, except for the mutant resulting from substitution of the conserved proline residue, presumably because of its contribution to the secondary structure of the tail. No virus was rescued when the cytoplasmic tail was deleted, indicating that the cytoplasmic tail is essential for production of the virus. The virulence of the transfectant viruses in mice was directly proportional to the amount of NA incorporated. The importance of the NA cytoplasmic tail in virus assembly and virulence has implications for use in developing antiviral strategies.

The neuraminidase (NA) of influenza A virus is a type II membrane glycoprotein with an uncleaved amino-terminal signal/anchor domain and a cytoplasmic tail that is presumed to be intracellular (1). The NA is thought to facilitate the mobility of virions by removing sialic acid residues from virus and infected cells during both entry and release from the cells (31). The three-dimensional structure of the NA has been determined (9, 42) and indicates that the protein is a tetramer consisting of four identical disulfide-linked subunits. The ectodomain of NA consists of a stalk and a globular head (1), with the latter containing the enzyme-active center and major antigenic sites. The hydrophobic transmembrane domain of NA serves as a signal sequence for membrane insertion and anchors the protein in the lipid bilayer (3). The stalk and the transmembrane domain sequences are highly variable among the nine NA subtypes (10). In contrast to the rest of the NA, however, the six-amino-acid cytoplasmic tail is highly conserved within the NA subtypes of influenza A virus. This extreme sequence conservation suggests that the sequence is critical to the function of this domain. However, the biological role of the NA cytoplasmic tail sequence has not been studied in detail.

The cytoplasmic tails of integral membrane proteins are of considerable interest because they may interact with cytoplasmic factors during protein synthesis, maturation, or intracellular transport. In the case of viral transmembrane glycoproteins, the cytoplasmic tails have the potential to interact with host and/or viral components and play a role in virus assembly. The cytoplasmic domains of viral surface glycoproteins may be structurally fixed against the cytoplasmic face of the membrane bilayer, providing potential binding sites for nucleocapsids and matrix proteins in initiating assembly. Direct interaction between the nucleocapsid and a peptide corresponding to the cytoplasmic domain of the viral glycoprotein has been shown for Semliki Forest virus (26). Similarly, a peptide corresponding to the cytoplasmic domain sequence of influenza virus hemagglutinin (HA) inhibits virus formation, suggesting virus-specific interactions between the HA and other viral proteins during viral assembly (7). However, such interactions for NA have not yet been defined.

Reverse genetics systems to rescue cloned genes in negative-strand RNA viruses have recently been established (8, 23, 32, 36). Palese and colleagues have rescued influenza A virus containing the chloramphenicol acetyltransferase gene (23) or influenza virus genes derived from cDNA (12–14, 28). In the present study, we used the influenza virus reverse genetics system to analyze the functional significance of the highly conserved cytoplasmic tail sequence of influenza A virus NA.

MATERIALS AND METHODS

Viruses and cells. Influenza virus A/WSN/33 (H1N1) (WSN) was obtained from Thomas Chambers (University of Kentucky, Lexington, Ky.). Masahiro Ueda (The Institute of Public Health, Tokyo, Japan) provided a helper virus [WSN-HK (H1N2)] that contained the NA gene from influenza virus A/Hong Kong/1/68 (H3N2) and all other genes from WSN (41), which was used to rescue the WSN NA gene. Madin-Darby bovine kidney (MDBK) and HeLa T4 cells were maintained in Eagle's minimal essential medium in the presence of 10% fetal calf serum. Madin-Darby canine kidney (MDCK) cells were maintained in Eagle's minimal essential medium in the presence of 5% calf serum.

Reverse genetics. Construction of pT3WSN(NA15), a plasmid containing the WSN NA gene flanked by an upstream T3 RNA polymerase promoter sequence and a downstream *Ksp*632I site, has been described previously (5). Cytoplasmic tail mutants were constructed from pT3WSN(NA15) by oligonucleotide-directed mutagenesis (20). An in vitro ribonucleo-

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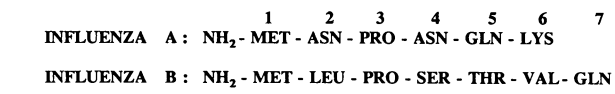


FIG. 1. Alignment of the cytoplasmic tail sequences of influenza virus A and B NAs.

protein complex of NA was prepared by transcription of pT3WSN(NA15) or its derivatives with T3 RNA polymerase in the presence of nucleoprotein and polymerase proteins (33) after these plasmids had been digested with *Ksp*632I and filled in with Klenow fragment, as described elsewhere (12, 13). The NA ribonucleoprotein complex was then transfected into 70 to 90% confluent MDBK cells infected 1 h before transfection with WSN-HK at a multiplicity of infection of 1. Eighteen hours after transfection, transfectants in the supernatant were identified by plaque formation on MDBK cells (41) and then plaque purified five times in MDBK cells. The plaque sizes for the transfectant viruses in MDBK and MDCK cells were similar and, therefore, are reported as MDBK plaque sizes in the text. RNAs were extracted from plaque-purified transfectants, used for cDNA synthesis, and sequenced to confirm the identity of each transfectant.

Glycoprotein incorporation analysis. MDBK cells were infected with wild-type or mutant virus. Four hours postinfection, cells were starved of glucose for 30 min and labelled with 0.2 mCi of [^{3}H]mannose for 2 or 14 h. Virus in the culture supernatant was partially purified by centrifugation at 130,000 $\times$ *g* through 30% sucrose. The virus pellet was disrupted with lysis buffer (50 mM Tris-HCl [pH 7.5], 600 mM KCl, 0.5% Triton X-100). Cell lysates were prepared at 6 h postinfection. Cell lysates and virus were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (21).

Experimental infection of mice. Six-week-old female BALB/c mice, anesthetized with methoxyflurane, were infected intranasally with 70 μl of virus at different dilutions (three mice per dilution). The minimal lethal dose for 50% of the mice (MLD_{50}) for each virus was determined 14 days postinfection.

Transient expression of cytoplasmic tail mutants. Mutant NA genes were expressed from the T7 promoter of the Bluescript vector (Stratagene) in the vaccinia virus T7 polymerase transient assay system (15). Briefly, HeLa T4 cells were infected with vaccinia virus expressing T7 RNA polymerase (vTF7-3), and then the Bluescript NA plasmids were transfected by lipofectin (Bethesda Research Laboratories) as described by Nuss and Air (30).

The cells were fixed with 3.7% formaldehyde at various time points and subjected to indirect immunofluorescence as described earlier (43) with a monoclonal antibody to WSN NA. The transfected cells were also assayed for enzyme activity by preparing cell lysates with lysis buffer and performing standard NA assays with fetuin as the substrate (2).

RESULTS

Rescue of NA cytoplasmic tail mutants. To understand the significance of the sequence conservation of the cytoplasmic tail of the influenza A virus NA, we conducted a mutational analysis of the cytoplasmic tail of A/WSN/33 NA. Our strategy was to mutate the influenza A virus NA cytoplasmic tail sequence to that of influenza B virus NA (38). The seven-amino-acid influenza B virus NA tail sequence differs from that of influenza A virus NA at all positions except for the initiating methionine and third-position proline (Fig. 1). Our rationale for choosing the influenza B virus NA sequence was to use a closely related virus which may share common structural features so as not to severely impair cytoplasmic tail function. The mutations included single-amino-acid substitutions, deletions, and insertions and substitution of influenza A virus NA tail with influenza B virus NA. The results obtained with each mutant are discussed individually below.

TABLE 1. Sequences of cytoplasmic tail mutants of influenza A virus NA[a]

Mutant	Cytoplasmic tail sequence						
WSN	NH_2	Met	Asn	Pro	Asn	Gln	Lys
CYT 2B			Leu				
NO PRO				Ala			
CYT 4B					Ser		
CYT 5B						Thr	
CYT 6B							Val
CYT 7B								Gln
FLUBCYT		Met	Leu	Pro	Ser	Thr	Val	Gln
NO TAIL		Met	–	–	–	–	–	–

[a] Amino acids are numbered left to right from positions 1 to 7. The sequences of CYT 2B through CYT 7B and of NO PRO are identical to the WSN sequence except for the positions indicated in the table. The dashes in the NO TAIL sequence indicate deletions.

FLUBCYT. The WSN NA cytoplasmic tail was replaced by the influenza B virus NA cytoplasmic tail (Table 1). This chimeric NA rescued influenza A virus infectivity, indicating that the influenza B virus NA sequence can substitute for the conserved influenza A virus NA sequence in the production of virus. However, the FLUBCYT transfectant virus produced smaller plaques than wild-type WSN (diameter, 0.5 versus 1.3 mm) yet grew to titers comparable to those with WSN (Table 2). These results suggest that the influenza A and influenza B virus NA cytoplasmic tails share common structural features that lead to the production of infectious virus.

CYT 2B, 4B, 5B, and 6B. To systematically assess the significance of the conservation of influenza A virus NA residues, each amino acid in the WSN cytoplasmic tail sequence was individually changed to that of the influenza B virus NA sequence. The name of each mutant indicates the position that contains the corresponding influenza B virus NA residue (Table 1). All of these single-amino-acid mutants rescued influenza A virus infectivity. The transfectant viruses produced plaques in MDBK cells similar in morphology and size to those of WSN (diameter, 1.3 mm) and grew to titers equivalent to that of wild-type WSN (Table 2).

CYT 7B. The seventh residue (Gln) of the influenza B virus NA tail was inserted between the influenza A virus NA tail and

TABLE 2. Growth of NA cytoplasmic tail mutants in MDBK cells

Mutant	MDBK titer (log_{10} of PFU/ml)[a]
WSN	6.87
FLUBCYT	6.28
CYT 2B	5.96
CYT 4B	6.20
CYT 5B	6.20
CYT 6B	6.97
CYT 7B	4.23
NO PRO	NA[b]
NO TAIL	NA[b]

[a] Transfectant virus was plaque purified five times in MDBK cells, and the titers of MDCK grown virus were determined in MDBK cells.

[b] NA, not applicable, i.e., transfectant virus was not rescued.

TABLE 3. Virulence of NA cytoplasmic tail mutants in mice

Virus	MLD_{50} (log_{10} of PFU)[a]
WSN	2.8
CYT 2B	4.7
CYT 4B	3.6
CYT 5B	4.1
CYT 6B	2.9
CYT 7B	≥ 4.5[b]
FLUBCYT	5.7

[a] Six-week-old female BALB/c mice were infected intranasally with 70 μl of virus at different dilutions (three mice per dilution), and MLD_{50} values were calculated on day 14.

[b] No mice died; therefore, endpoint titers could not be determined.

TABLE 4. Surface expression of NA cytoplasmic tail mutants

Source of DNA	% Cells positive (mean ± SD) with anti-WSN NA monoclonal antibody at the following times[a]:			
	9 h	12 h	15 h	18 h
WSN	3.8 ± 0.97	8.1 ± 1.3	19.4 ± 5.3	18.4 ± 4.5
FLUBCYT	2.3 ± 0.93	9.1 ± 3.5	20.0 ± 4.0	31.3 ± 4.6
CYT 2B	9.0 ± 6.3	15.4 ± 1.2	29.8 ± 2.8	35.8 ± 0.9
CYT 4B	3.4 ± 1.5	12.6 ± 2.2	23.3 ± 1.9	23.9 ± 5.2
CYT 5B	3.3 ± 0.37	10.3 ± 0.3	23.9 ± 2.2	36.9 ± 3.7
CYT 6B	3.7 ± 0.26	15.5 ± 3.7	29.2 ± 3.7	30.2 ± 1.2
CYT 7B	5.1 ± 3.1	18.3 ± 3.1	21.8 ± 4.5	24.4 ± 3.8
NO PRO	4.5 ± 2.2	8.6 ± 3.8	15.6 ± 3.5	13.1 ± 3.1
NO TAIL	2.5 ± 1.12	5.9 ± 2.13	19.6 ± 0.29	20.3 ± 4.3

[a] Times indicated are hours posttransfection; no NA was detectable at the surface at 3 and 6 h posttransfection.

the transmembrane domain, thus increasing the length by one residue (Table 1). CYT 7B NA also rescued influenza A virus infectivity. However, the transfectant virus produced smaller plaques in MDBK cells than WSN (diameter, 0.5 versus 1.3 mm) and grew to lower titers (Table 2).

NO PRO. The proline in the third position is conserved in both influenza A virus NA and influenza B virus NA (Fig. 1), suggesting an important role for this residue, presumably in maintaining the secondary structure of the cytoplasmic tail. To test the significance of this residue, we substituted the proline with alanine (NO PRO; Table 1). Virus containing this mutant NA was not rescued, suggesting that the proline may be a critical determinant of the conformation of the cytoplasmic tail.

NO TAIL. A mutant NA with a deleted cytoplasmic tail (NO TAIL) was constructed to test whether a transfectant virus containing a tailless NA could be obtained (Table 1). The NO TAIL mutant was not rescued, suggesting that the cytoplasmic tail is necessary for production of infectious virus (Table 2).

Virulence in mice. The differences in growth characteristics and plaque sizes observed in tissue cultures for the transfectant viruses suggested that the mutations in the cytoplasmic tail of the NA may alter virus infectivity in vivo. Therefore, all of the transfectant viruses were tested for virulence in mice. WSN transfectant virus was highly virulent in mice (Table 3). CYT 2B, 4B, and 5B were virulent, but only CYT 6B was as virulent as WSN. In contrast, CYT 7B and FLUBCYT were highly attenuated. CYT 7B did not kill any mice; therefore, no endpoint MLD_{50} titer was determined.

Cell surface expression of NA cytoplasmic tail mutants. Transfectant virus was not obtained with NO PRO and NO TAIL NAs. To determine whether these NAs as well as the NAs from the attenuated viruses were transport defective or functionally aberrant relative to WSN, the mutant NAs were transiently expressed by the T7-vaccinia virus system and analyzed for cell surface expression.

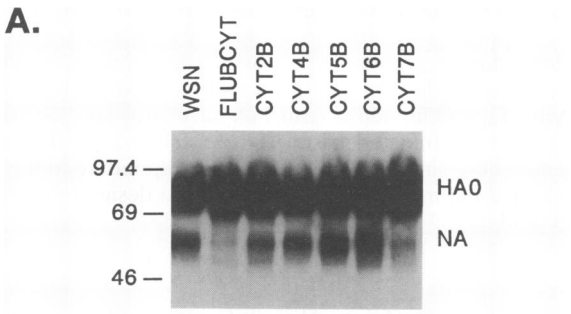
Mutant NA genes under the control of the bacteriophage T7 RNA polymerase promoter were transfected into HeLa T4 cells that were infected with a recombinant vaccinia virus expressing the T7 RNA polymerase (vTF7-3). The cells were fixed and examined for NA surface expression by indirect immunofluorescence at various time intervals (3, 6, 9, 12, 15, and 18 h posttransfection). All of the mutant NA proteins were expressed at the cell surface and at levels comparable to those with WSN in reproducible experiments. The rates of appearance of the mutant NAs at the cell surface were similar to that of WSN NA (Table 4). The expression of NO PRO and NO TAIL NAs at the cell surface indicates that the reason for their rescue deficiency was not because of a lack of surface expression.

Lysates of the transfected cells were used in standard NA assays to determine whether the expressed mutant NAs were functional. All of the mutant NAs, including NO PRO and NO TAIL NAs, had activities comparable to that of WSN (data not shown). These results demonstrate that none of our mutations, including deletion of the cytoplasmic tail, affected transport to the cell surface or abolished NA activity.

Incorporation of NA cytoplasmic tail mutants into virions. Deletion of the cytoplasmic tail did not abrogate transport to the cell surface or the enzymatic activity of NA, as shown by transient expression of NO TAIL NA. However, transfectant virus was not obtained with NO TAIL NA. Furthermore, no differences in the other transiently expressed cytoplasmic tail mutant NAs were seen that would suggest why biological differences among the transfectant viruses exist. These observations raise the possibility that the cytoplasmic tail has a role in the assembly of virus. To directly analyze the incorporation of NA into virions, transfectant viruses were grown in MDBK cells in the presence of [^{3}H]mannose. Lysates of infected cells were prepared 5 h postinfection, or ^{3}H-labelled virus was partially purified by being pelleted through 30% sucrose at 18 h postinfection. Cell lysates and disrupted virus were analyzed by SDS-PAGE. The relative amounts of HA and NA in cell lysates and virus particles were determined by scanning densitometry of autoradiographs (Fig. 2B).

WSN NA was efficiently incorporated into virions (Fig. 2A). CYT 2B, 4B, 5B, and 6B had levels of incorporated NA that were similar to that of WSN (Fig. 2A), whereas FLUBCYT and CYT 7B had significantly reduced amounts of incorporated NA (Fig. 2A). Similar results were observed with MDCK cells. The similar ratios of HA to NA in cell lysates (Fig. 2B) suggest that the drastic ratio differences observed in virions are due not to reduced levels of NA expression but to incorporation efficiency. These results suggest that the virulence of FLUBCYT and CYT 7B in mice is attenuated because of poor incorporation of NA into the virion.

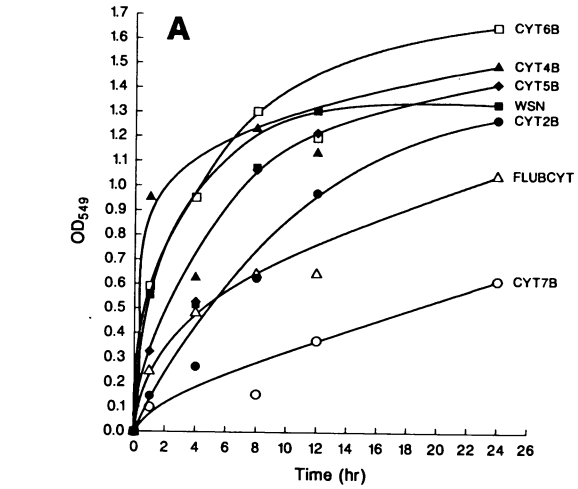
NA activities of transfectant viruses. Standard NA assays were performed in order to correlate the amounts of incorporated NA with the enzymatic activity for each of the purified transfectant viruses. Identical amounts of purified virus based on protein concentrations were incubated with fetuin, and NA activities were compared at various time points. Figure 3A is representative of replicate assays depicting the NA activities of WSN and the mutants over a 24-h time course. The NA activities of CYT 4B, 5B, and 6B were similar to that of WSN throughout the duration of the reaction. CYT 2B displayed the same level of activity as WSN by 24 h, despite a lag in the early stages of the reaction (Fig. 3B). However, FLUBCYT and



B.

Transfectant virus	Ratio of HA:NA	
	Cell lysate	Virions
WSN	7.7	5.5
FLUBCYT	16.7	67.9
CYT2B	10.8	7.1
CYT4B	9.1	7.1
CYT5B	7.9	4.9
CYT6B	7.2	5.5
CYT7B	9.3	65.6

FIG. 2. (A) Comparison of glycoprotein incorporation of NA mutants. Cytoplasmic tail mutants were grown in the presence of [^{3}H] mannose in MDBK cells. Partially purified virus was disrupted in lysis buffer and analyzed by SDS-PAGE. Molecular mass size markers are indicated in kilodaltons on the left. HAO, uncleaved HA. (B) Relative amounts of HA and NA in infected cell lysates and partially purified virus. Ratios were determined by scanning densitometry of autoradiographs.



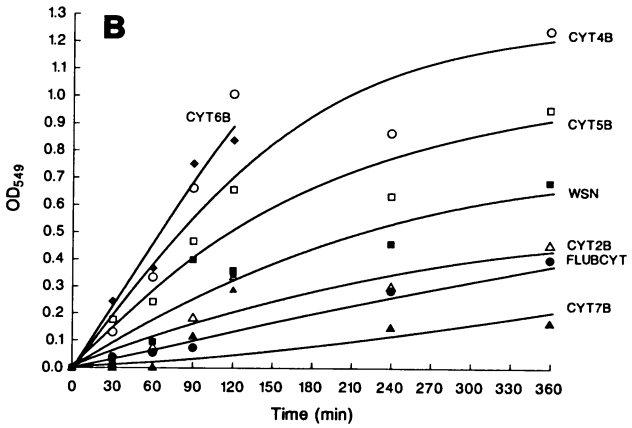


FIG. 3. (A) NA activities of cytoplasmic tail mutants. Identical amounts of purified virus (4.5 μg) were incubated with fetuin in standard NA assays (2). NA activities were determined at the indicated time points. (B) Initial rates for NA activity. Purified virus (2.5 μg) was incubated with fetuin as described above. NA activities were determined from 30 to 360 min. OD_{549}, optical density at 549 nm.

CYT 7B did not deplete the substrate by 24 h, as had all of the other viruses, suggesting different enzymatic rates. Therefore, the NA activities were measured at earlier time points to compare the initial rates (Fig. 3B). FLUBCYT and CYT 7B showed significantly less NA activity than WSN. The decrease in NA activity observed for CYT 7B and FLUBCYT in the initial stages of the reaction correlates with the reduction in the incorporation of NA into virions.

DISCUSSION

To understand the role of the conserved residues in the cytoplasmic tail, cytoplasmic tail mutants of NA were used to rescue influenza A virus infectivity. Our results indicate that the cytoplasmic tail is essential for the production of infectious virus but that the entire conserved sequence is not critical. Rescue of the chimeric virus FLUBCYT, in which the WSN NA cytoplasmic tail is replaced by the influenza B virus NA tail, indicates that the cytoplasmic tails of influenza viruses A and B have common structural features that lead to incorporation of NA during viral assembly. However, the amount of FLUBCYT NA incorporated into released virus was drastically reduced compared with the amount of WSN NA, suggesting that the homologous influenza A virus cytoplasmic tail is more efficient in incorporation of NA into influenza type A virus. Specific interactions between the NA cytoplasmic tail and other viral proteins may be required during assembly, in which case the homologous tail could be more compatible with those proteins.

In mice, CYT 7B and FLUBCYT viruses were attenuated compared with wild-type WSN virus. The mechanism of attenuation appears to be due to the decreased incorporation of NA of these two viruses relative to that of WSN. When the NA activities of purified FLUBCYT and CYT 7B virus are compared with that of WSN, the initial rates of NA activity for FLUBCYT and CYT 7B are less than that of WSN, reflecting the reduced amount of incorporated NA. FLUBCYT and CYT 7B NAs appear to be functionally comparable to WSN NA, with decreased incorporation of NA into virions accounting for the attenuated phenotypes. A critical level of NA activity during release from infected cells may be necessary in vivo for the spread of virus through the mucosal layer for efficient virus production. Recently, it was shown with an NA-lacking mutant of influenza virus that the NA is not required during entry but is important in the release of virus from infected cells (22). FLUBCYT and CYT 7B may be less efficient in spreading because of reduced NA activity, accounting for their reduced virulence in mice.

The role of the cytoplasmic tail in the incorporation of NA into virions is unknown. The overall structure of the cytoplasmic tail appears to be important in the assembly process. CYT 7B and FLUBCYT have significantly less NA incorporated

than WSN, which is reflected in the low level of NA activity observed for both mutants. The influenza B virus cytoplasmic tail in FLUBCYT and the insertion in CYT 7B may each result in a less stably integrated NA. Mutations in the amino end of the transmembrane domain have previously been shown to affect NA stability (40). However, the mutations may also alter the structure of the cytoplasmic tail and have adverse effects on incorporation into virus. The configuration of the cytoplasmic tail in the NA tetramer is not known; presumably, however, it must be able to interact with other viral proteins and/or nonviral factors during the assembly process. The significance of the constraints on the cytoplasmic tail structure can be seen by altering the proline that is conserved between influenza A virus tail and influenza B virus tail. The NA containing an alanine instead of proline (NO PRO) did not rescue infectious virus. However, the transiently expressed NO PRO NA was transported to the cell surface and was enzymatically functional. The proline residue may maintain the cytoplasmic tail in a proper conformation along the membrane to interact with the components of the budding virion. A/New Jersey/8/76 (27) and N1 swine isolates (18a) contain a threonine instead of the proline accompanied by a lysine-to-arginine change in the sixth residue of the cytoplasmic tail. The effect of those residues on the rescue of WSN NA has not yet been determined.

All of the NA cytoplasmic tail mutants, including NO TAIL and NO PRO, were expressed at the cell surface with rates of appearance similar to that of WSN in a transient vaccinia virus expression system. These results indicate that the signals for intracellular transport of NA are not contained within the cytoplasmic tail, in agreement with previous studies that mapped the translocation signals and membrane anchor to the hydrophobic transmembrane domain (3, 40). The oligomeric state of the NA cytoplasmic tail mutants was not directly addressed; however, the transiently expressed mutant NAs were functional, indicating that they had attained the proper oligomeric structure. Only tetrameric forms of NA are thought to be enzymatically active (10, 19, 34).

The requirement for cytoplasmic tails in viral surface glycoproteins varies. Deletion of the cytoplasmic tail of the Rous sarcoma virus envelope glycoprotein has no effect on assembly or virus infectivity (37). However, the cytoplasmic domain of the human immunodeficiency virus type 1 envelope glycoprotein appears to play a role in viral entry (16), which may in part be due to inefficient incorporation of the glycoprotein into virus (11). In other enveloped viruses, the cytoplasmic domain of the vesicular stomatitis virus glycoprotein (G) is required for incorporation of G protein into viral particles (44). In contrast, the tailless mutant of influenza virus HA is incorporated into virions but is blocked in infectivity (39). Our NO TAIL NA was transported to the cell surface, but no transfectant virus was obtained. This suggests that the cytoplasmic tail may be essential for the incorporation of NA into virus. Alternatively, the tail may be expendable for incorporation into virions but may be required subsequently for the virus to initiate a new round of infection.

Recent data with Sindbis virus E2 glycoprotein revealed that the E2 tail is important for virus budding (17). Specific interactions between viral glycoproteins and components of the budding virus have been suggested for alphaviruses (26), Mason-Pfizer monkey virus (4), Rous sarcoma virus (18) and vesicular stomatitis virus (24). The influenza virus matrix protein M1 is presumed to mediate the interaction between glycoproteins and nucleocapsids during virus assembly (25), and it has been suggested that specific signals required for influenza virus assembly may be contained in M, NP, or NA (35, 39). A recent study has suggested that the cytoplasmic domains of the envelope proteins may serve in binding to other viral components in order to avoid being excluded from the envelope during assembly (29). Synthetic peptides matching the cytoplasmic domains of Sindbis virus E2, vesicular stomatitis virus G, and influenza virus HA have virus-specific antiviral activities in infected tissue culture cells (6, 7). The data presented here suggest that peptides corresponding to the NA cytoplasmic tail may also be a target in the design of antiviral compounds.

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