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Influenza neuraminidase is delivered directly to the apical surface of MDCK cell monolayers

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The aim of this study was to investigate whether influenza neuraminidase travels directly from the Golgi complex to the apical domain of the plasma membrane in virally infected epithelial (MDCK) cell monolayers, or whether it passes transiently through the basolateral domain. Using a new assay for the delivery of neuraminidase to the plasma membrane, we found that the time course of transport of this protein from the Golgi complex to the apical surface of MDCK cell monolayers was very similar to that for influenza haemagglutinin, which is known to be delivered directly to its destination. In addition, a similar time course of neuraminidase transport was found in BHK cells, which are not asymmetric and in which delivery must therefore be direct. Finally, basolateral exposure of MDCK cell monolayers grown on nitrocellulose filters to an anti-neuraminidase antibody was shown to have no effect on the delivery of active neuraminidase to the apical surface. We conclude from these results that neuraminidase, like haemagglutinin, is delivered directly to the apical surface.

Influenza virus; Neuraminidase; Epithelial polarity; (MDCK cell)

1. INTRODUCTION

Ideas about the sorting of plasma membrane proteins in MDCK cell monolayers, an extensively investigated model epithelium, have been based largely on the behaviour of two proteins, the apically targetted influenza haemagglutinin and the basolaterally targetted vesicular stomatitis virus (VSV) G-protein [1,2]. After synthesis, these proteins move together through the Golgi complex and are apparently segregated as they leave this organelle, for delivery to their different destinations [3–6]. A different sorting mechanism operates in the hepatocyte. Here proteins destined for both the basolateral and apical domains of the plasma membrane are transported initially to the basolateral domain, from where apical proteins are moved on to their final location [7].

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In order to strengthen the argument that the sorting mechanisms in these two cell types are fundamentally different, we have examined the route taken between the Golgi complex and the plasma membrane of MDCK cell monolayers by influenza neuraminidase, another protein that is targetted to the apical surface. We present evidence that neuraminidase, like haemagglutinin, is delivered directly to the apical surface.

2. MATERIALS AND METHODS

MDCK cells (strain II) obtained initially from Dr K. Simons (EMBL, Heidelberg) were grown as monolayers in Eagle's minimum essential medium (EMEM) supplemented with 10% (v/v) newborn calf serum, non-essential amino acids and 50 IU/ml penicillin/50 μg/ml streptomycin. Baby hamster kidney (BHK-21) cells, obtained from Flow Labs (Rickmansworth, England), were grown in Glasgow's minimum essential medium (GMEM) supplemented with 10% newborn calf serum, 10% tryptose phosphate broth and penicillin/streptomycin. All media and supplements were obtained from Flow Labs.

The WSN strain of influenza virus was obtained from Dr

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S.C. Inglis (Department of Pathology, University of Cambridge). The X-31 strain of influenza virus, and a monoclonal antibody (as ascites fluid) to the X-31 neuraminidase, were obtained from Dr A. Hay (NIMR, Mill Hill, London). Semliki Forest virus (SFV) was obtained from Dr T.R. Hesketh (Deparment of Biochemistry, University of Cambridge). All viruses were propagated as described [8]. [^{3}H]SFV was prepared in the same way as SFV, except that post-infection maintenance medium contained *N*-[^{3}H]acetyl-D-mannosamine (30 Ci/mmol; NEN, Stevenage, England). The envelope proteins of progeny virions consequently contained [^{3}H]sialic acid [9].

Neuraminidase at the cell surface was detected through its ability to cleave [^{3}H]sialic acid from [^{3}H]SFV. Confluent monolayers of MDCK cells, growing either on 3 cm diameter plastic dishes or on 2.5 cm diameter nitrocellulose filters (0.45 μm pore size; Millipore, Harrow, England) in perspex chambers, were washed twice with 2 ml EMEM containing 0.2% (w/v) bovine serum albumin (BSA) and infected with > 10 plaque-forming units/cell of influenza virus in the same medium for 1 h at 37°C. The medium was then replaced with EMEM containing 2% newborn calf serum. At various times after infection monolayers were washed twice with 2 ml ice-cold phosphate-buffered saline containing 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$ (PBS) and exposed to [^{3}H]SFV (approx. 13000 dpm) in 0.5 ml PBS containing 2 mg/ml BSA for 30 min at 4°C (WSN strain) or 37°C (×31 strain) with gentle rocking. Protein was precipitated by addition of 10% (w/v) trichloroacetic acid, followed by incubation on ice for 30 min. Protein was pelleted by centrifugation for 2 min in an Eppendorf microfuge. Supernatants were removed and radioactivity was counted by liquid scintillation spectroscopy. We have shown previously [8] that the release of [^{3}H]sialic acid from [^{3}H]SFV is linear over both the 30 min incubation period and the range of neuraminidase activities used in this study.

3. RESULTS AND DISCUSSION

We used two approaches in investigating the route taken by influenza neuraminidase from the Golgi complex to the apical domain of the plasma membrane. First, we measured the time taken for the enzyme to travel between the two compartments, and compared this time with the transfer time previously reported for haemagglutinin in the same cells and also with the time taken for neuraminidase to move between the same two compartments in a non-asymmetric cell (BHK), in which the transfer must be direct. Second, we examined the effect of an inactivating antibody to neuraminidase, placed on the basolateral side of an MDCK cell monolayer, on the rate of delivery of active enzyme to the apical surface. We reasoned that if neuraminidase travels to the apical surface via the basolateral surface, then the presence of an antibody on the basolateral side should either cause the neuraminidase to be retained at this surface or result in the delivery of inactivated enzyme to the apical surface.

In order to examine the time course of transit of neuraminidase between the trans elements of the Golgi complex and the plasma membrane, we exploited the observation that reduction of the incubation temperature to 20°C causes a reversible block in the intracellular transport of several plasma membrane proteins [10,11], including neuraminidase in MDCK cells [6], at the point at which they normally leave the Golgi complex. The compartment in which the proteins are blocked has been termed the trans Golgi network [12]. Our aim was to stack neuraminidase in this late Golgi compartment and then release the block by warming the cells to 37°C, thus allowing a bolus of enzyme to move synchronously out to the plasma membrane. To keep this bolus as compact as possible, we inhibited further synthesis of neuraminidase by addition of cycloheximide after the low-temperature block was applied.

The effect of incubation at 20°C on the rate of delivery of neuraminidase to the apical domain of the plasma membrane in MDCK monolayers grown on plastic dishes is shown in fig.1. In control cells, neuraminidase activity at the cell surface is just detectable at 3 h post-infection. Surface ac-

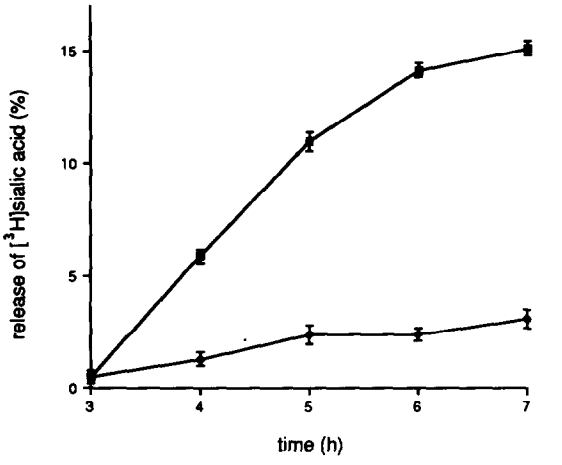

Fig.1. Effect of low-temperature incubation on the rate of delivery of neuraminidase to the cell surface in MDCK cell monolayers. MDCK cell monolayers, grown on plastic dishes, were infected with influenza virus (WSN) and incubated from 3 h post-infection at either 37°C (□) or 20°C (◆), and surface neuraminidase activity determined at 4°C. Values are means ± SE (n = 3).

tivity then rises in an approximately linear manner between 3 and 5 h and eventually reaches a plateau after 7 h, presumably when the rate of delivery to the surface is balanced by the rate of loss through viral budding [8]. In cells incubated at 20°C from 3 h post-infection, there is very little increase in neuraminidase activity at the cell surface. At 7 h post-infection, for example, [^{3}H]sialic acid release is 3.1% compared with 15.2% in control cells. Hence, low-temperature incubation causes an almost complete block in the delivery of neuraminidase to the cell surface.

To measure the time course of delivery of neuraminidase to the cell surface following a low-temperature block, MDCK cell monolayers grown on plastic dishes were incubated at 37°C for 3 h and then transferred to 20°C. After 1 h at low temperature, cycloheximide (20 μg/ml) was added to inhibit protein synthesis. After a further 30 min at 20°C, cells were warmed to 37°C and the activity of neuraminidase at the cell surface was measured at various times after warm-up. The results obtained are shown in fig.2. Delivery of neuraminidase to the cell surface was almost complete after 30 min, as has previously been shown for haemagglutinin [5]. Between 60 and 90 min, surface neuraminidase activity declined to baseline, because enzyme was lost through viral budding. No significant neuraminidase activity was detected at the surface of monolayers that had not been warmed to 37°C.

A similar experiment to that described above was carried out with BHK cells. The results obtained are shown in fig.3. In these cells, surface neuraminidase activity rises linearly over the first 60 min after warm-up, and then reaches a plateau. There is no detectable decline in activity between 60 and 90 min, in contrast to the situation in MDCK cells. Again, neuraminidase is not delivered to the cell surface in cells kept at 20°C.

The results presented so far indicate that neuraminidase is transported directly from the Golgi complex to the apical domain of the plasma membrane. We cannot, however, exclude the possibility that neuraminidase does pass through the basolateral domain but moves so quickly through the cell that it arrives at the apical domain at the same time as haemagglutinin, which travels directly to its destination. To test this possibility directly, we exposed confluent MDCK cell monolayers, grown on nitrocellulose filters, to an anti-neuraminidase monoclonal antibody, either on the apical side or the basolateral side, and examined its effect on the delivery of neuraminidase to the apical surface. Antibody (ascites fluid

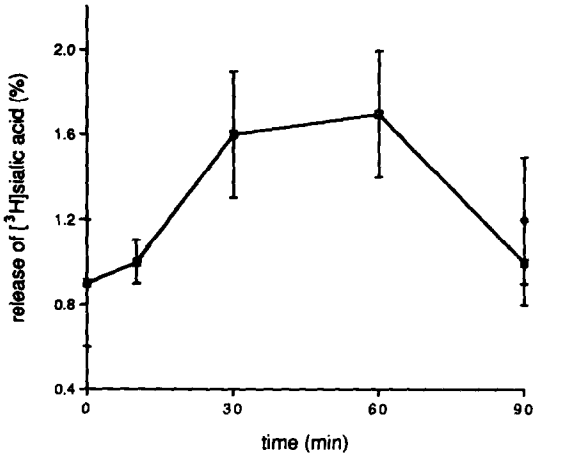

Fig.2. Time course of transport of neuraminidase from the trans Golgi network to the cell surface in MDCK cell monolayers. MDCK cell monolayers, grown on plastic dishes, were infected with influenza virus (WSN) and incubated at 37°C until 3 h post-infection. The temperature was then reduced to 20°C. After 1 h, medium containing cycloheximide (20 μg/ml) was added and the low-temperature incubation was continued for a further 30 min. The cells were then warmed to 37°C for various times (□) or kept at 20°C (♦), and surface neuraminidase activity determined as described in fig.1. Values are means ± SE (n = 3).

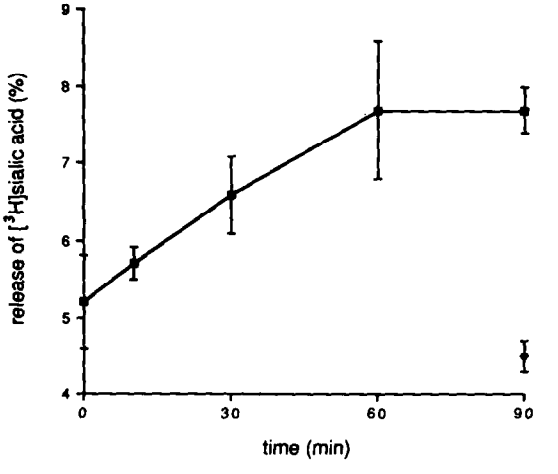

Fig.3. Time course of transport of neuraminidase from the trans Golgi network to the cell surface in BHK cells. Methods as described in fig.2. Values are means ± SE (n = 3).

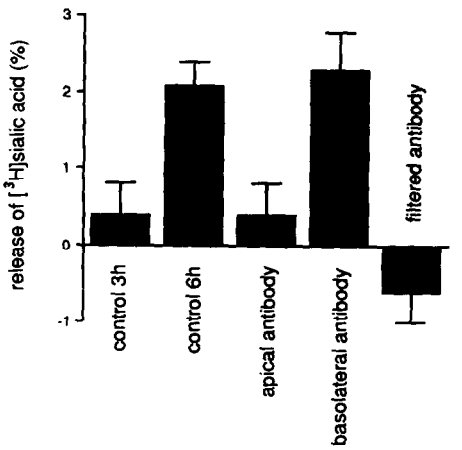

Fig.4. Effect of an anti-neuraminidase antibody on neuraminidase activity at the apical surface of MDCK cell monolayers. MDCK cell monolayers, grown on nitrocellulose filters, were infected with influenza virus (X-31) and exposed from 3 h post-infection to either normal medium or medium containing a monoclonal antibody to neuraminidase. Antibody was present on either the apical or the basolateral side of the monolayer. In addition, some monolayers were exposed on the apical side to antibody-containing medium that had been previously passed through a filter. Neuraminidase activity at the apical surface was determined 6 h post-infection at 37°C. Values are means ± SE ($n \geq 9$, except for the filtered medium condition, where $n = 3$).

diluted 1:1000) was present in the medium bathing the cells from 3 to 6 h post-infection. We found that in control cells there was an increase in surface neuraminidase activity between 3 and 6 h, and that this increase was abolished by the presence of the antibody in the apical bathing medium (fig.4). Antibody in the basolateral medium, on the other hand, had no effect on the increase in enzyme activity at the apical surface. Fig.4 also shows that medium containing antibody that had been allowed to pass through a filter, was still able to inhibit neuraminidase activity. Hence, antibody in the basolateral bathing medium, although able to pass freely through the filter, does not affect the delivery of neuraminidase to the apical surface.

We have shown, therefore, that influenza neuraminidase, like haemagglutinin, is delivered directly to the apical domain of the plasma membrane in MDCK cell monolayers. This result indicates that the delivery of apically targetted membrane proteins directly to their destination may be a general mechanism in MDCK cells, and strengthens the argument that these cells differ fundamentally from, for example, hepatocytes in the way in which plasma membrane proteins are processed. Whether MDCK cells handle endogenous apical membrane proteins in the same way is at present unknown.

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