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Functional Studies of the Sendai Virus HN Gene Product in BHK-21 Cells

Maqsood Ali Ansari[1] and Nancy L. McQueen
Department of Biology/Microbiology, California State University, Los Angeles, California, USA
[1]Current address: Department of Genetics, University of Karachi, Pakistan

Abstract: We have earlier reported on the expression of Sendai virus HN gene in BHK-21 cells (Ansari and McQueen 1999). As expression gives no idea about the functional activity of the protein therefore we are reporting here on the functional activities of the expressed HN protein. HN is a multi functional molecule having hemagglutinin activity and neuraminidase activity. We tested both the activities separately and found both the activities in the *in vitro* expressed HN protein. The results confirmed that the HN gene not only is synthesized but is functionally active too.

Keywords: Sendai virus. HN gene, Functional activity

Introduction

The HN protein of sendai virus has 575 amino acids and is encoded by 1725 nucleotides. The protein has a molecular weight of 63,514 daltons (Ranjit *et al.,* 1991). The HN protein is a class II transmembrane glycoprotein with a very long ectodomain, a relatively short endodomain and transmembrane domain near the amino terminus. Its amino terminus faces inside of the cell and carboxyl terminus faces outside. The HN protein is a multi functional molecule that is responsible for binding of the virus to sialic acid containing receptors on host cells, agglutination of erythrocytes, neuraminidase activity and antigenic properties. It is also believed to play a possible role in fusion (Mostov *et al*., 1992). The hemagglutinin part of the HN binds the virus to sialic acid containing receptors on the host cell and neuraminidase enzymatically cleaves sialic acid residues. The binding of the virus to the host cell is necessary for the Fusion (F) protein to be sufficiently close to allow initiation of infection (Scheid and Choppin, 1974). The precise location of the HA and NA activities of HN have not been identified. There is some evidence that a single site in the molecule is responsible for both the activities (McQueen *et al.,* 1986). Other evidence suggests that the cell binding and neuraminidase activities are associated with independent sites (Scheid and Choppin, 1974). Earlier we have reported on the expression of sendai virus HN gene in BHK-21 cells using recombinant vaccinia virus, but as expression alone does not indicate anything about the functional activities of the protein, we report here experiments conducted to determine the functional activity of the protein.

Materials and Methods

Bacteria, viruses, plasmid and cells. *E. coli* strain MV1190, Wild type Sendai virus (Z strain) and the F1-R variant of Sendai were provided by Dr. Seto, California State University Los Angeles. Baby Hamster kidney cells (BHK-21) used for the expression of the HN gene was provided by Dr. Nayak. University of California Los Angeles and were grown as described earlier (McQueen *et al.,* 1984).

Recombinant KS^+ plasmid having T7 and T3 polymerase promoters flanking the polylinker region used for transcribing HN gene and vTF7-3 a recombinant vaccinia virus that expresses T7 polymerase that binds to T7 promoters to begin transcription were provided by Dr. Nayak, UCLA. Sendai HN cDNA with *EcoR1* linkers was given by Elizabeth Cal. State LA.

Infection and transfection for HN gene expression: BHK-21 cells were grown in 60 cm^2 tissue culture plates to 80 percent confluency as described earlier (McQueen *et al.,* 1984). Recombinant vaccinia virus (vTF 7-3) and wt Sendai virus (for + ve control) were trypsinized with equal volume of 0.25 mg/ml trypsin for 30 minutes. One plate was infected with Sendai virus (1 pfu/cell) and two plates were infected with recombinant vaccinia virus (1 pfu/cell). The viruses were allowed to adsorb for 1 hour at 37°C with rocking after every 15 minutes. During the last 15 minutes recombinant plasmid DNA was precipitated with lipofectin (15 μg of plasmid DNA in 70 μl volume was added to 30 μl lipofectin (1 unit/μl) in a polystyrene tube followed by a 15 minutes incubation at room temperature. This was then used for transfecting the cells. The transfected cells were incubated for 5 hours with serum free DMEM at 37°C in a humidified CO_2 incubator followed by addition of 3 ml DMEM containing 20 percent FCS and 12 hours incubation.

Hemadsorption: After the Infection of BHK-21 Cells with Recombinant Vaccinia Virus and Transfection with Recombinant KS^+ plasmid with HN DNA as described in the earlier paper (Ansari and McQueen, 1999), the monolayer of BHK-21 cells was washed twice with 3 mls of PBS^{++} (containing $MgCl_2$ and $CaCl_2$). This was followed by the addition of 0.5 percent suspension of Guinea Pig erythrocytes in PBS^{++}. After 30 minutes incubation at 4°C it was washed 4 times with cold PBS^{++}. The plate was then observed under the microscope and photographed.

Neuraminidase assay: After the Infection and Transfection as described earlier, the assay for neuraminidase was done as described by Aymard-Henry *et al.* (1973). The cells were washed twice with 3 mls of cold PBS and were scrapped in the presence of 500 μl 0.9 percent NaCl. The suspension was then sonicated for 30 seconds. Saline solution was used as a negative control and Sendai virus as a positive control. 50 μl of the cell lysate was mixed with an equal volume of fetuin (48 g/L diluted 1:2 with phosphate buffer containing 6 mM $CaCl_2$), mixed and allowed to stand at 37°C for 18 hours. The tubes were cooled to 20°C and 100 μl of periodate reagent (4.28 g of $NaIO_4$ in 38 ml of ddH_2O + 62 ml of syrupy

orthophosphoric acid) was added to each sample, mixed and allowed to stand at 20°C for exactly 20 minutes. 1 ml of arsenite reagent (10 percent Na_2SO_4, 7.1% Na_2SO_4 with 0.3 ml of H_2SO_4) was added so that a brown color developed. The mixture was shaken until brown color disappeared. 2.5 ml of thiobarbituric acid (0.6 percent thiobarbituric acid + 7.1% Na_2SO_4in H_2O heated to dissolve) was added, mixed thoroughly and the tubes were placed in a boiling waterbath for 15 minutes and looked for the appearance of red color which indicates neuraminidase activity.

Results

Hemadsorption: The functional activity of HN protein was determined using a hemadsorption assay. A positive result proves that HN protein is synthesized in the cells, transported to the cell surface and has the ability to bind guinea pig erythrocytes. The results were compared with positive (Sendai virus infection) and negative (vaccinia virus infection) controls. The plates were observed under the microscope and were photographed. The positive results obtained suggest that the expressed HN protein is transported to the cell surface and has hemagglutinating activity.

Neuraminidase assay: Neuraminidase is an enzyme that cleaves sialic acid residues. It is thought that the neuraminidase protein is involved in the release of the virus from infected cells by preventing virus aggregation (Palese *et al.,* 1974). The experiment for neuraminidase activity was based on the following principles: release of free N-acetyl neuraminic acid from the fetuin substrate by the action of neuraminidase, conversion of N-acetyl neuraminic acid to β-formyl pyruvic acid by periodate oxidation, and formation of chromophore by thiobarbituric acid. The red color developed at the end indirectly indicated neuraminidase activity.

Discussion

Since expression alone does not indicate anything about the functional activities of the protein, assays were conducted to determine if the protein is functionally active. The HN protein produced in Sendai virus infected cells has been shown to posses both receptor binding (hemagglutinating) and enzymatic (neuraminidase) activities. Adsorption of the virus to host cells, erythrocytes or soluble molecules, involves a receptor containing neuraminic acid residues. There is a considerable variation among virus strains as to the number of neuraminic acid residues required for the adsorption. Adsorption is temperature independent, requires cations ad occur over a broad range of pH. Recently it has bee found that chloride and other halide ions inhibit the neuraminidase activity of some paramyxoviruses, but not their hemagglutinin activity (Choppin and Scheid, 1980). Neuraminidase promotes the efficient spread of the virus by allowing virions to become dissociated from neuraminic-acid containing glycoproteins. The glycoproteins of paramyxoviruses normally do not contain neuraminic acid residues because of the action of viral neuraminidase. Temperature sensitive mutants of influenza virus that under nonpermissive temperature contain neuraminic acid, form large aggregates and such aggregates limit the spread of the virus. Thus one role of the viral neuraminidase is to prevent aggregation of progeny virus by removing potential virus receptors from their own glycoproteins (Palese *et al.*, 1974) (Fig. 1).

A role of HN in fusion is suggested by studies in which various

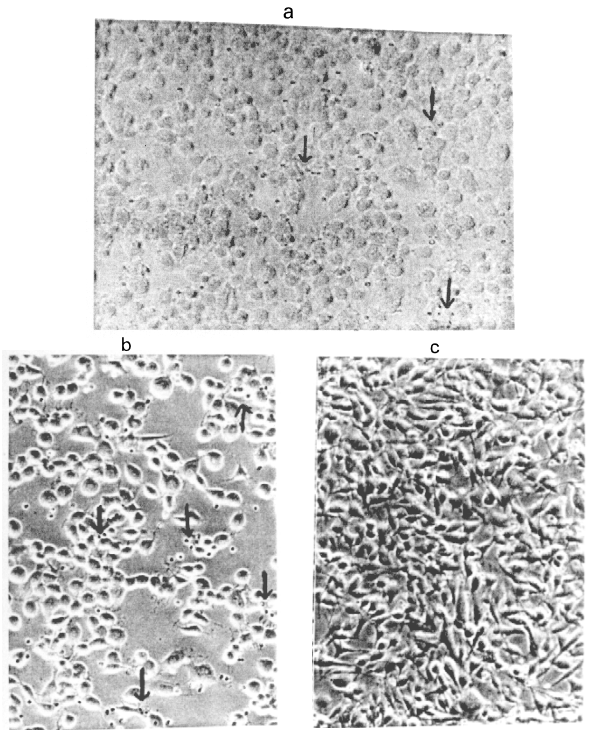

Fig. 1: Pictures showing the hemadsorption by the HN protein. A) Sendai virus as a positive control. B) hemadsorption by BHK-21 cells transfected with HN gene. C) Vaccinia virus as a negative control. Arrows indicating guinea pig erythrocytes attached to BHK-21 cells indicating Hemagglutinin activity.

biochemical and immunological treatments of HN caused the loss of fusion, but did not inhibit hemagglutination nor neuraminidase activity. Studies using monoclonal antibodies that only inhibit fusion, identified a Sendai virus escape mutant with a substitution in the HN protein at residue 420. Another report says that the bovine parainfluenza virus 3 (PIV3) fusion protein expressed from a vaccinia vector will not mediate fusion unless the cells are coinfected with a vector containing HN gene (Sakai and Shibuta, 1989). In addition, a single amino acid change in the HN sequence at a position 539, severely diminishes the ability of cells to fuse furthermore if both F and HN envelope proteins are attached to their specific receptors then the fusion rate is enhanced (Bagai *et al.*, 1993) . On the other hand studies using Simian virus 5 and measles virus indicate that expression of the F protein alone results in cell fusion (Hu *et al.*, 1992). Further studies are necessary to determine if the HN protein plays some yet undefined role in fusion process.

Hemadsorption activity was determined by adding guinea pig erythrocytes to the infected/transfected cells. Erythrocytes were found bound to the cell surface for both the test and the positive control. This result indicated that the HN protein was synthesized and was then transported to the cell surface. The infected/transfected cell was also analyzed for the neuraminidase activity. The results showed that neuraminidase activity was also present in the synthesized HN protein.

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