CHAPTER 5

Membrane Insertion and Intracellular Transport of Influenza Virus Glycoproteins

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I. INTRODUCTION

While the major features of the intracellular route traveled by the hemagglutinin (HA) and neuraminidase (NA) glycoproteins of influenza virus were established more than a decade ago by a synthesis of studies of virus morphogenesis with investigations of the secretory pathway in mammalian cells, our understanding of the biosynthesis of these viral envelope glycoproteins has expanded dramatically during the past 5 years. This progress has depended in part on the availability of detailed information on the structure of the HA and NA glycoproteins, and on the ability to express cloned genes encoding these polypeptides. These advances rest on a foundation of more than half a century of investigation of the nature of the surface antigens of a virus that remains one of the uncontrolled pathogens of man.

The envelope glycoproteins of influenza viruses were first identified as hemagglutinating and neuraminidase activities associated with the vir-
ion surface (Hirst, 1941, 1942, 1943; McClelland and Hare, 1941; Gottschalk, 1957). In some of the earliest applications of electron microscopy to the study of virus-infected cells, it was discovered that influenza viruses matured by the then novel process of budding from the cell surface (Murphy and Bang, 1952) and that influenza virions were covered with a fuzzy coat (Morgan et al., 1956). As preservation techniques improved, this outer fuzz was seen to be composed of spikelike projections (Horne et al., 1960), which were later shown to be embedded in a lipid bilayer derived from areas of the plasma membrane modified by the virus (Bachi et al., 1969; Compans and Dimmock, 1969). That the NA and HA activities resided on separate proteins was established by analyzing viruses with reassorted genomes that express the HA of one antigenically distinct parent and the NA of the other (Tumova and Pereira, 1965; Laver and Kilbourne, 1966). Through the use of such viruses, antisera specific for each spike protein were prepared, and HA was identified as the antigen responsible for eliciting neutralizing antibodies (Seto and Rott, 1966; Webster and Laver, 1967).

The molecular analysis of HA and NA began with the discovery that influenza viruses could be dissociated with sodium dodecyl sulfate (SDS) into subunits that retained HA or NA activity. These subunits could be purified from certain virus strains by electrophoresis (Laver, 1963, 1964; Laver and Webster, 1968). Using electron microscopy, Laver and Valentine (1969) were able to show correspondence between these isolated subunits and two kinds of functionally and morphologically distinct spikes, each anchored to the virus envelope by a terminal hydrophobic domain. Soon afterward, experiments that employed controlled proteolysis of the virion surface established that HA was attached to the viral membrane by its carboxy-terminus (Compans et al., 1970; Brand and Skehel, 1972). Several groups used electrophoretic and immunochimical techniques to show that the HA and NA polypeptides were glycosylated as well as to determine their molecular weights (Dimmock, 1969; Schultz, 1975; Compans et al., 1970; Haslam et al., 1970; Webster, 1970; Skehel and Schild, 1971). Comparison of the molecular weights of the HA and NA polypeptides with the size and morphology of the intact spikes observed by electron microscopy led to the idea that HA was most likely a trimer (Laver, 1973; Griffith, 1975; Schultz, 1975) and NA a tetramer (Wrigley et al., 1973). As the details of the structure of the influenza virus glycoproteins became known, awareness increased that these proteins were interesting not only for the role they played in viral infection, but also as model cell-surface proteins that were easy to isolate and characterize biochemically.

Four key observations led to the understanding that influenza virus morphogenesis, and in particular the biosynthetic pathway of the envelope proteins, reflected the traffic patterns of normal membrane proteins within animal cells. First, the viral spike proteins were observed at the plasma membrane before budding of virus particles began, and thus were