# The neuraminidase of bat influenza viruses is not a neuraminidase

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Influenza A viruses are classified into subtypes according to their two viral glycoproteins, hemagglutinin (HA) and neuraminidase (NA). These viruses are known to infect multiple animal species, including humans, pigs, horses, dogs, sea mammals, chickens, ducks, and other birds. Although humans are sporadically infected with avian influenza virus strains belonging to multiple subtypes, only H1, H2, and H3 HA subtypes and N1 and N2 NA subtypes have been documented to cause pandemics and annual epidemics of influenza in humans. By contrast, all H16 and N9 subtypes of influenza A viruses are found circulating in birds. The recent discovery of influenza A viruses clearly distinct from any known avian influenza A virus subtype in little yellow-shouldered bats from Guatemala has been the first example of an influenza A virus subtype not found in avian species (1). Bat influenza viruses have glycoproteins related to the HA and NA of influenza A virus, which prompted to the classification of these viruses as H17N10 influenza A virus. The crystal structure of the N10 glycoprotein is now solved independently by Li et al. (2) and Zhu et al. (3) in PNAS, and these works provide important clues on the characteristics of these newly discovered influenza viruses.

The HA of the H17N10 bat influenza viruses is clearly related to the other 16 previously described subtypes of influenza A virus HAs based on sequence identity and phylogenetic analysis. However, the NA of the H17N10 viruses is less related to the nine previously described subtypes of influenza A virus NAs, and also different from the NA of influenza B virus, a distant relative of influenza A virus found circulating only in humans. Since the first crystal structure of the N2 NA of influenza A virus was reported (4), crystal structures have been generated for N1, N4, N5, N8, and N9, and for the influenza B virus NA (5–8). All these NAs are characterized by being tetrameric with a mushroom-like shape, anchored at the viral membrane by an N-terminal transmembrane domain, with each monomer head consisting of a propeller-like structure made by six-bladed β-sheets. The N10 protein shares the same structural features, but lacks conserved amino acids involved in sialic acid binding and sialic acid cleavage (2, 3). Consistent with this, N10 lacks NA activity. These intriguing, distinctive features beg the question whether N10 has any enzymatic activity (2, 3). More in general, the specific N10 features suggest that bat influenza viruses do not use sialic acids as their receptors, and indicate a possible different mechanism of viral entry and exit for bat influenza viruses compared with influenza A and B viruses.

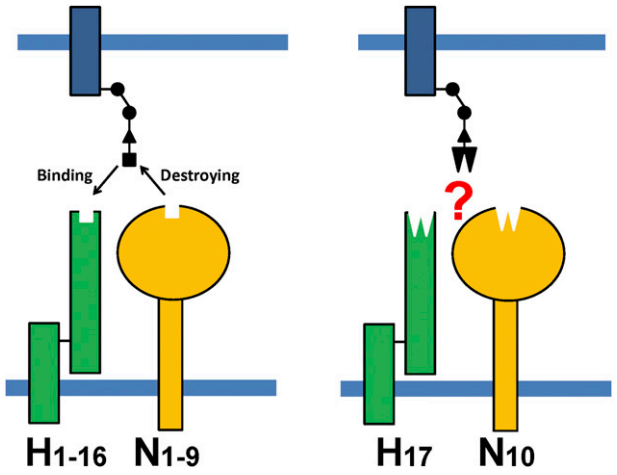


**Fig. 1.** H1 to H16 HA subtypes bind to sialic acid-containing receptors and mediate influenza A virus entry. N1 to N9 NA subtypes cleave sialic acids and destroy the receptor, mediating virus spread. The structure of the N10 subtype from the recently discovered bat influenza viruses (2, 3) shows profound changes in the active site of this NA-like molecule that prevent sialidase activity. It remains to be determined (*i*) whether the H17 HA from bat influenza viruses binds sialic acids, (*ii*) what the receptor of bat influenza viruses is, and (*iii*) whether the N10 NA-like protein from bat influenza virus has receptor-destroying activity.

The HA and NA glycoproteins of influenza A and B viruses have opposite roles during the viral life cycle (Fig. 1). The HA binds to sialic acid-containing receptors, mediating attachment and subsequent entry of the virus into the host cell. By contrast, the NA is a receptor-destroying enzyme, and, by virtue of its enzymatic activity, cleaves sialic acids from their sugar backbones. This allows efficient spread of the virus, as otherwise viruses will remain attached to nonfunctional receptors, such as sialic acids present in neighboring virions during viral release. In the absence of NA activity, influenza viruses clamp to each other and remain unreleased from the surface of infected cells (9). This characteristic is the basis of the antiviral activity of NA inhibitors used in the clinic for the treatment of influenza (10). The opposite functions of HA and NA result in a delicate balance between HA and NA activities that is required for the fitness of the virus: influenza A viruses with HA proteins with higher affinity for their receptors require NA proteins with stronger enzymatic activity, and vice versa (11). The combination of receptor binding and receptor destroying activities in the same virion is not a unique property of influenza viruses, as multiple parainfluenza viruses also possess sialic acid binding and NA activities, although, in this case, both activities are found in the same viral protein, HN, and not in different viral proteins. Influenza C virus binds to 9-O-acetylated sialic acids and destroys this receptor through an esterase activity by virtue of its HA-esterase glycoprotein (12).

As the HA of the bat influenza viruses is closely related to the HA of influenza A viruses, it is likely that bat influenza viruses also use a combination of receptor binding and receptor-destroying activities for optimal entry and exit from infected cells. However, the lack of NA activity by the

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N10 protein would suggest that the H17 HA binds to a receptor different from the conventional sialic acid receptors of all other influenza A virus subtypes. This might explain the lack of growth of bat influenza viruses in conventional substrates for replication of influenza viruses. However, N10 might or might not have receptor-destroying activity and might even have acquired a new function. It will be important to continue the biochemical and structural characterization of the H17 and N10 glycoproteins of bat influenza viruses to find the receptors and to determine the mechanism by how these viruses enter and spread in their hosts.

Bats are known to be the reservoir for multiple zoonotic viruses infecting humans, such as rabies virus, coronaviruses, henipaviruses, and ebolaviruses (13). More recently, it has become clear that bats harbor a vast diversity of viruses (14). Although not all of these viruses are going to represent a threat, the zoonotic potential of most bat viruses is unclear. In this respect, influenza A viruses are notoriously known to jump from host to host and start pandemics. Do bat influenza viruses represent a threat for humans? The sequence analysis of the viral polymerase makes unlikely that these viruses would be able to reassort with influenza A viruses, bringing into question whether they should be considered influenza A viruses. It will be important to investigate whether bat influenza viruses are able to reassort with other influenza A viruses and exchange viral RNA segments, which would increase their zoonotic potential. The identification of the receptor of the bat influenza virus will also be important for the proper evaluation of the zoonotic potential of these viruses to infect other hosts that may share the same receptor. In this respect, the studies of Li et al. (2) and Zhu et al. (3) represent a first step. Future studies on bat influenza viruses are needed to address these questions and to reveal the ways how influenza viruses can adapt to different hosts.


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