Acid-sensitive molecular changes contribute to the emergence of pandemic influenza

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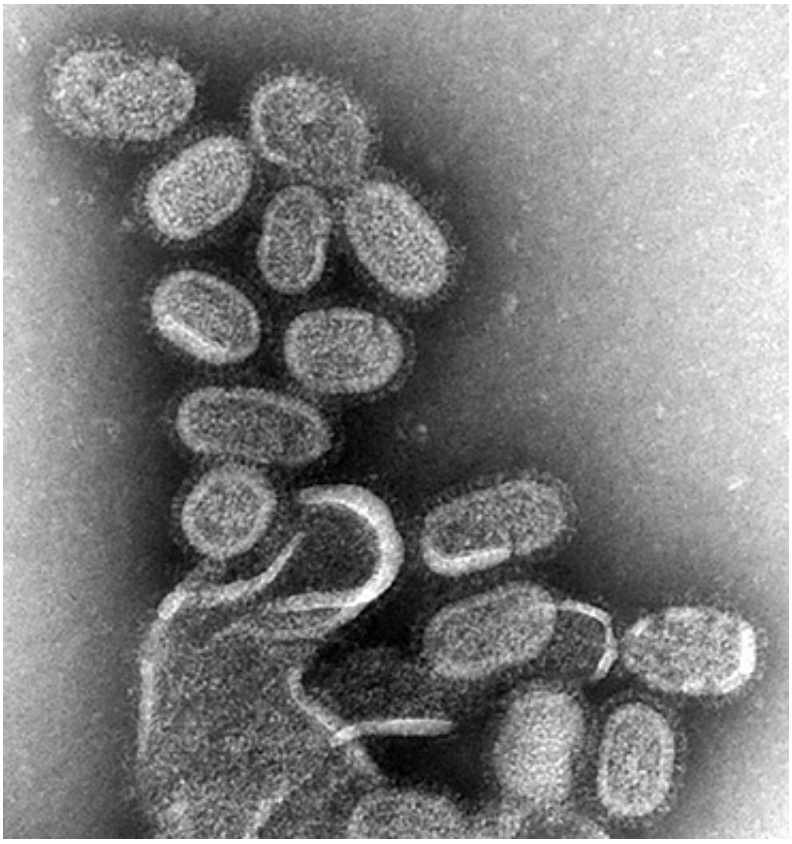

Electron microscopy of influenza virus. Credit: CDC

St. Jude Children's Research Hospital scientists have identified a molecular property of the hemagglutinin protein that contributed to the emergence of the 2009 H1N1 pandemic influenza virus. The findings may help officials recognize and control flu viruses that pose the greatest

risk to humans. The study appears today in the online, early edition of the scientific journal *Proceedings of the National Academy of Sciences* (*PNAS*).

Hemagglutinin is carried on the surface of the flu virus. The virus needs the protein for binding to and infecting host cells.

The researchers showed that hemagglutinin became more stable in an acidic environment as the H1N1 virus shifted from swine to humans. The adaptation increased the protein's stability in the acidic conditions of the human respiratory tract and lowered the pH at which hemagglutinin was activated. Activation triggers an irreversible change in the protein's molecular shape that fuses the virus and target cell.

Investigators demonstrated in the laboratory that the hemagglutinin adaption was essential for airborne viral transmission in ferrets, the animal model of the human disease. Mutating hemagglutinin to reduce the protein's stability in acid, and increase the pH of activation, blocked the virus' ability to spread through airborne particles.

"We have identified hemagglutinin acid stability as an essential property of pandemic viruses," said Charles Russell, Ph.D., an associate member of the St. Jude Department of Infectious Diseases who led the research. "These findings should aid pandemic preparedness by helping officials recognize and prioritize circulating animal viruses for surveillance, vaccine production and other measures."

The pH of activation differs for different flu viruses. Avian and swine viruses are activated at pH 5.5-6.0 compared to pH 5.0-5.5 for human flu viruses. Previous studies by Russell and others showed that changing the hemagglutinin to reduce the protein's stability in acid, and lower its pH of activation, led to transmission of avian flu viruses in mice and ferrets. Now researchers have linked such changes to a human pandemic

virus.

In this study, researchers tracked the pH of activation in H1N1 swine flu viruses before and during the 2009 flu pandemic. Prior to the pandemic, H1N1 swine viruses were activated at pH 5.5-6.0. The H1N1 virus that infected humans early in the pandemic was activated at pH 5.5. In later cases, the activation pH of the H1N1 pandemic virus declined to 5.2-5.4.

"The hemagglutinin protein plays a central role in human flu pandemics, yet until now the molecular properties required for pandemic viruses have remained largely undefined," Russell said. "Our findings suggested that one requirement for a pandemic influenza A virus was an acid-stabilized protein with an activation pH of 5.5 or less, which was sufficient to allow airborne human-to-human transmission at the start of the 2009 H1N1 pandemic."

When scientists mutated the 2009 H1N1 pandemic virus to make it more like swine flu virus and give it a hemagglutinin with a higher activation pH, the virus caused less illness and spread more slowly in mice and ferrets.

The virus responded to the loss-of-function mutation by quickly altering hemagglutinin in the laboratory to a lower activation pH of 5.3. The spontaneous change restored airborne transmissibility of the virus in ferrets. An analysis of pandemic H1N1 samples collected from more than 21,000 human cases worldwide found a small number with the same H1 mutation.

"The hemagglutinin shape change that causes the membrane fusion and gets the viral genetic material into target cells is triggered by a drop in pH," Russell said. "The virus can be exposed to low pH in the mildly acidic upper respiratory tract of humans and other mammals. If

hemagglutinin is too easy to trigger, it is like a mouse trap triggered prematurely, and the virus is inactivated before reaching the target cell."

More information: Molecular requirements for a pandemic influenza virus: An acid-stable hemagglutinin protein, *PNAS*, www.pnas.org/cgi/doi/10.1073/pnas.1524384113

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