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Filamentous morphology of recombinant A/WSN/33 (H1N1) virions detected with novel electron microscopy protocols

Content

Pleiomorphic morphology (spherical, ellipsoidal, filamentous or irregular) of virions is one of the characteristics of Influenza virus. Filamentous phenotype is thought to be favorable for virus transmission in nature. As was shown for several virus strains, it is supported by specific amino acid changes in the matrix M1 protein and might be influenced by some host cellular components. The method of the sample preparation for the electron microscopy analysis might also change the virions shape. Finally, it is not known whether the recombinant virus produced from eight plasmids via the wide-spread reverse genetics technique demonstrate quite the same morphology as the native virus or the laboratory strain. Now we compare the morphology of recombinant A/WSN/33 (H1N1) virus grown in various hosts with that of the laboratory strain. We analyzed both wild type recombinant virus and two M1 mutants. To avoid the virus morphology distortion, we used a number of sample preparation protocols including (1) gentle concentration of virions by low speed filtration of the virus-containing allantoic fluid/ cell culture medium through Amicon 100K filters or (2) fixation of virus particles within the allantoic fluid/ cell culture medium with glutaric aldehyde before ultracentrifugation. We found that (1) the M1 of control recombinant virus contains two amino acid substitutions, Ser126Cys and Ile219Val, compared to the Flu database and laboratory strain. This control recombinant virus gathered in the mixed HEK 293T/ MDCK cell culture and further passaged twice in embryonated chicken eggs demonstrated 11% filamentous phenotype (the remainder particles are spherical/ellipsoidal); (2) the control recombinant virus collected from MDCK cells instead of chicken embryos is 98% spherical; (3) the reverse substitutions Cys126Ser/Val219Ile in M1 got via site-directed mutagenesis raised the portion of filamentous particles till 22% if the virus was collected from chicken embryos; (4) an amino acid substitution Ala209Thr in M1 decreased the portion of filamentous particles accumulated in chicken embryos till 3%. In conclusion, (1) the pleiomorphic morphology of recombinant influenza virus is affected by both the M1 protein sequence and combination of some host components; (2) the recombinant A/WSN/33 (H1N1) virus may demonstrate (partially) filamentous phenotype in contrast to the well-known “spherical” (over 98%) phenotype of A/WSN/33 (H1N1) laboratory strain; (3) the amino acid substitutions Ser126Cys/ Ile219Val observed in the M segment of recombinant virus are not the reason of the recombinant virus abnormal phenotype compared to the laboratory strain.

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Visualising Flu

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Virus host cell interaction

Contribution Type : Paper presentation