Virology 476 (2015) 141–150

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Actin-myosin network is required for proper assembly of influenza virus particles

Michiko Kumakura, Atsushi Kawaguchi *, Kyosuke Nagata *

Department of Infection Biology, Faculty of Medicine, University of Tsukuba, Tsukuba 305-8575, Japan

ARTICLE INFO

Article history:
Received 28 August 2014
Returned to author for revisions
2 December 2014
Accepted 8 December 2014
Available online 24 December 2014

Keywords:
Blebbistatin
Influenza virus assembly
Non-muscle myosin II

ABSTRACT

Actin filaments are known to play a central role in cellular dynamics. After polymerization of actin, various actin-crosslinking proteins including non-muscle myosin II facilitate the formation of spatially organized actin filament networks. The actin-myosin network is highly expanded beneath plasma membrane. The genome of influenza virus (vRNA) replicates in the cell nucleus. Then, newly synthesized vRNAs are nuclear-exported to the cytoplasm as ribonucleoprotein complexes (vRNPs), followed by transport to the beneath plasma membrane where virus particles assemble. Here, we found that, by inhibiting actin-myosin network formation, the virus titer tends to be reduced and HA viral spike protein is aggregated on the plasma membrane. These results indicate that the actin-myosin network plays an important role in the virus formation.

Introduction

Actin filaments play essential roles in cytokinesis, cell motility, cell adhesion, and formation of membrane architecture (Diz-Muñoz et al., 2013; Giannone et al., 2007; Mishra et al., 2013). The core component of the actin filaments is monomeric actin that self-assembles into filamentous actin. A various actin-crosslinking proteins facilitate the formation of spatially-organized networks of actin filaments. Among these proteins, non-muscle myosin II (NMII) is known to be a crucial factor for the fundamental actin dynamics. NMII is a motor protein consisting of two identical heavy chains and two pairs of light chains. The heavy chains are subunits forming bipolar filaments for self-association of NMII through the interaction in an anti-parallel manner (Conti and Adelstein, 2008). Through the heavy chains, NMII binds to and walks along the actin filaments in its ATPase activity-dependent manner. The actin-myosin network is highly expanded beneath the plasma membrane, where a variety of membrane morphogenesis events occur, including receptor clustering, membrane protrusion and secretion (Chichili and Rodgers, 2009; Goswami et al., 2008; Jaqaman et al., 2011; Lillemeier et al., 2006; Papadopulos et al., 2013; Porat-Shliom et al., 2013; Vicente-Manzanares et al., 2007).

It has been known that a variety of viruses utilize cellular cytoskeleton for their efficient replication. After the attachment of viruses to the cell surface, viruses such as human immunodeficiency virus (HIV), vesicular stomatitis virus, and vaccinia virus, move along cellular filopodia and microvilli toward the cell body, which is called “virus surfing”. The actin-myosin network located beneath the plasma membrane mediates this movement (Lehmann et al., 2005; Mercer and Helenius, 2008). The virus surfing makes virions move toward the entry site on the plasma membrane, where endocytosis regulated by the actin-myosin network for virus entry occurs. Microtubules are generally exploited for a long-distance transport. It is known that microtubules and kinesin motor protein KIF4 serve to transport Gag proteins of retroviruses to the plasma membrane (Tang et al., 1999). Subsequently, the assembly and budding of HIV-1 occur in a lipid raft-dependent manner, which is regulated by underlying actin filaments as well as microtubules (Jolly et al., 2007).

The influenza viral genome (vRNA) consists of eight single-stranded RNA segments of negative polarity. These vRNA segments exist as ribonucleoprotein complexes (vRNPs) with viral RNA-dependent RNA polymerases and nucleoprotein (NP). After attachment of virions to host cells, vRNPs are released into the cytoplasm through uncoating process of virions. Then, vRNPs translocate to the nucleus, where transcription and replication of the virus genome occur. At late phases of infection, newly synthesized vRNPs are exported to the cytoplasm and transported to the apical plasma membrane. Consequently, vRNPs are enveloped by the plasma membrane

* Correspondence to: Department of Infection Biology, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan.
Tel./fax: +81 29 853 3233.
E-mail addresses: ats-kawaguchi@md.tsukuba.ac.jp (A. Kawaguchi), knagata@md.tsukuba.ac.jp (K. Nagata).

http://dx.doi.org/10.1016/j.virol.2014.12.016
0042-6822/

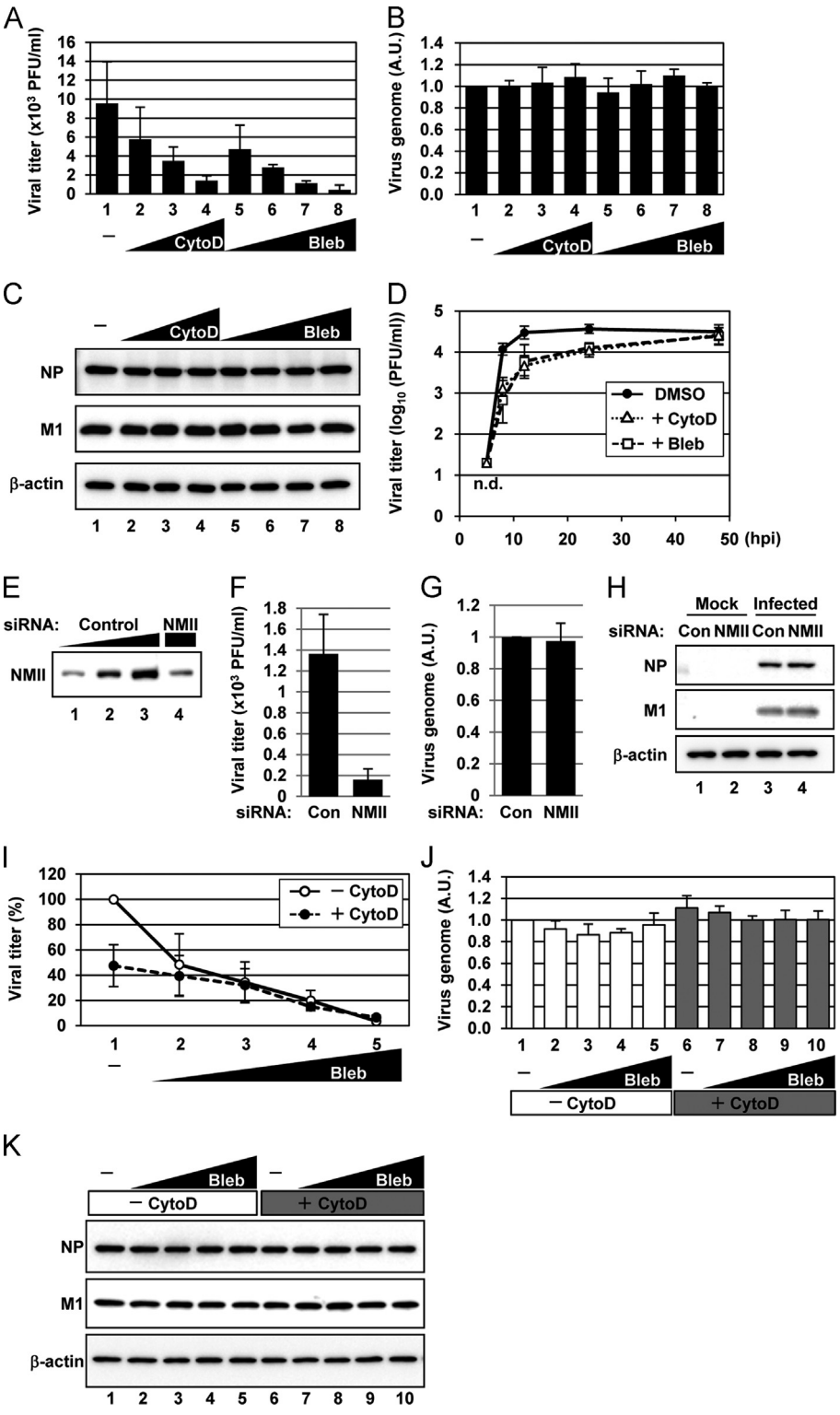

Fig. 1. Effects of inhibitors and siRNA for actin cytoskeleton on influenza virus replication. In panels A, B, and C, MDCK cells were infected with PR8 virus at an MOI of 3. The cells were untreated (lane 1) or treated with 10, 33, or 100 μM of CytoD (lanes 2 to 4), or 5, 15, 50, or 150 μM of Bleb (lanes 5 to 8) at 5 hpi. At 8 hpi, infected cells and their medium fractions were collected for further analyses. (A) Viral titer. Medium fractions of infected cells were collected and subjected to plaque assays. Viral titers were shown as means with *SD* ($n=3$). (B) Quantitative detection of vRNA. Total RNA of infected cells was purified, and the synthesis level of segment 5 vRNA was quantified by real-time PCR using corresponding primers. Results were normalized as the ratio to those of untreated cells and presented as means with *SD* ($n=3$). (C) Expression level of viral proteins. Infected cells were subjected to Western blotting with anti-NP (upper panel), anti-M1 (middle panel), and anti-β-actin (lower panel) antibodies. (D) Time course analysis of the production of infectious virions. MDCK cells infected with PR8 virus at an MOI of 3 were mock-treated (DMSO) or treated with 100 μM of CytoD or 150 μM of Bleb at 5 hpi. At 5, 8, 12, 24, and 48 hpi, the viral titers in the supernatant fractions were determined by plaque assay. n.d.: Not detected (less than 20 PFU/ml). (E) Expression level of NMII in knocked-down cells. HeLa cells transfected with nontargeting (Control; lanes 1 to 3) or NMHC-IIA (NMII; lane 4) siRNA were lysed at 48 h post transfection. Then, the lysates equivalent to 1.25 μg (lane 1), 2.5 μg (lane 2), and 5 μg (lanes 3 and 4) of total protein were subjected to SDS-PAGE followed by Western blotting with anti-NMHC-IIA antibody. (F) The viral titers at 12 hpi were determined by plaque assays. Viral titers were shown as means with *SD* ($n=3$). (G) The level of segment 5 vRNA was quantified as described in the legend of panel B. (H) The lysates of mock-infected (lanes 1 and 2) and infected (lanes 3 and 4) cells were subjected to Western blotting assay with anti-NP (upper panel), anti-M1 (middle panel) and anti-β-actin (lower panel) antibodies, respectively. In panels I, J, and K, MDCK cells were treated with 0, 5, 15, 50, or 150 μM of Bleb in the absence or presence of 20 μM of CytoD (−CytoD and +CytoD, respectively). Then, the samples were collected at 8 hpi. (I) Viral titers. Medium fractions of infected cells were subjected to plaque assays. Results in the absence (−CytoD, solid line) or presence of 20 μM of CytoD (+CytoD, dash line) are represented. Results were normalized as the ratio to those in the absence of any inhibitors and shown as means with *SD* ($n=4$). (J) Quantitative detection of vRNA. Results were shown as means with *SD* ($n=4$). (K) Expression level of viral proteins. Infected cells were subjected to Western blotting with anti-NP (upper panel), anti-M1 (middle panel), and anti-β-actin (lower panel) antibodies.