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# Cell–cell contact promotes Ebola virus GP-mediated infection

Chunhui Miao [a], Minghua Li [a], Yi-Min Zheng [a], Fredric S. Cohen [b], Shan-Lu Liu [a,*]

[a] Department of Molecular Microbiology and Immunology, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA
[b] Department of Molecular Biophysics and Physiology, Rush University Medical Center, Chicago, IL 60612, USA

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ABSTRACT

Ebola virus (EBOV) is a highly pathogenic filovirus that causes hemorrhagic fever in humans and animals. Here we provide evidence that cell–cell contact promotes infection mediated by the glycoprotein (GP) of EBOV. Interestingly, expression of EBOV GP alone, even in the absence of retroviral Gag-Pol, is sufficient to transfer a retroviral vector encoding Tet-off from cell to cell. Cell-to-cell infection mediated by EBOV GP is blocked by inhibitors of actin polymerization, but appears to be less sensitive to KZ52 neutralization. Treatment of co-cultured cells with cathepsin B/L inhibitors, or an entry inhibitor 3.47 that targets the virus binding to receptor NPC1, also blocks cell-to-cell infection. Cell–cell contact also enhances spread of rVSV bearing GP in monocytes and macrophages, the primary targets of natural EBOV infection. Altogether, our study reveals that cell–cell contact promotes EBOV GP-mediated infection, and provides new insight into understanding of EBOV spread and viral pathogenesis.



## Introduction

Ebola virus (EBOV) belongs to the filovirus family and causes severe hemorrhagic fever in humans and animals. The fatality rates of the disease induced by EBOV can reach up to 90%, with currently no effective antiviral drug or FDA-licensed vaccine available (Feldmann and Geisbert, 2011; Hoenen and Feldmann, 2014a). A better understanding of EBOV infection, especially the early stage of viral transmission, would facilitate the development of novel therapeutic approaches to combat this deadly disease.

EBOV infection of host cell is mediated by its sole glycoprotein, known as GP. GP is synthesized as a precursor (GP0) in the endoplasmic reticulum, cleaved into GP1 and GP2 in the Golgi apparatus, and eventually targeted to the plasma membrane for viral incorporation (Takada et al., 1997; White et al., 2008; Wool-Lewis and Bates, 1999). During this process, EBOV GP is modified by N- and O-linked glycosylation, the exact functions of which are still not well understood (Dowling et al., 2007; Simmons et al., 2002). In mature virions, EBOV GP exists as a homotrimer, with each monomer composed of GP1 and GP2 subunits that are linked by a disulfide bond and non-covalent interactions (Jeffers et al., 2002; Lee et al., 2008). As is the case for many class I viral fusion proteins, GP1 is responsible for interacting with cellular receptors or cofactors, whereas GP2 is directly involved in fusion of EBOV with target cell membranes (Brindley et al., 2007; Gregory et al., 2011; Hood et al., 2010; Lee et al., 2008; Manicassamy et al., 2005; Wang et al., 2011; Watanabe et al., 2000; Wool-Lewis and Bates, 1999).

The detailed molecular mechanism underlying EBOV GP-mediated infection is currently unknown (White and Schornberg, 2012). However, sufficient evidence has indicated that EBOV enters host cells through macropinocytosis (Hunt et al., 2011; Nanbo et al., 2010; Saeed et al., 2010), a process that is initiated by binding of EBOV GP to attachment factors, such as DC-SIGN and TIM-1 (Alvarez et al., 2002; Kondratowicz et al., 2011; Lin et al., 2003; Marzi et al., 2006; Nanbo et al., 2010). Following the uptake of viral particles into late endosomes and lysosomes, GP1 is cleaved by cellular cysteine proteases, especially cathepsins B (CatB) and L (CatL), resulting in the production of a fusion-competent intermediate (Chandran et al., 2005; Dube et al., 2009; Schornberg et al., 2006) that binds to the recently identified intracellular receptor, Niemann-Pick type C1 (NPC1) (Côté et al., 2011; Kaletsky et al., 2007; Miller et al., 2012). Studies from several groups have shown or suggested that NPC1, low pH, and possibly mild reduction of GP are important for EBOV GP-mediated infection (Bale et al., 2011; Brecher et al., 2012; Gregory et al., 2011; Miller et al., 2012; Schornberg et al., 2006).

Cell-to-cell transmission has been shown to play important roles in the dissemination and pathogenesis of many pathogenic viruses, including HIV and HCV (Brimacombe et al., 2011; Dale et al., 2013; Roberts et al., 2015; Zhong et al., 2013). In this work, we provide evidence that cell–cell contact facilitates infection mediated by EBOV GP and this process requires cellular cathepsins and NPC1. Our work supports the idea that cell-to-cell infection

* Corresponding author.
*E-mail address:* liushan@missouri.edu (S.-L. Liu).

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may be another means of EBOV spread and could serve as a potential target of viral therapeutics.

## Results

### *Cell–cell contact promotes EBOV GP-mediated retroviral infection*

EBOV is a BSL-4 agent; thus, in this study we employed several systems, including retroviral pseudotypes, virus-like particles (VLPs), as well as rVSV that bear GP to determine cell-to-cell infection. For the retroviral system, we co-transfected 293T cells with the pQCXIP retroviral vector encoding a tetracycline-controlled transcription factor (tTA, referred to as Tet-off hereafter), along with plasmids that encode EBOV GP and murine leukemia virus (MLV) Gag-Pol. Following 24 h transfection, donor 293T cells producing pseudovirions were co-cultured with target 293FT cells stably expressing tetracycline-responsive element (TRE)-driven *Gaussia* luciferase (293FT/TRE-GLuc); cell-to-cell infection efficiency was assayed by measuring the GLuc activity

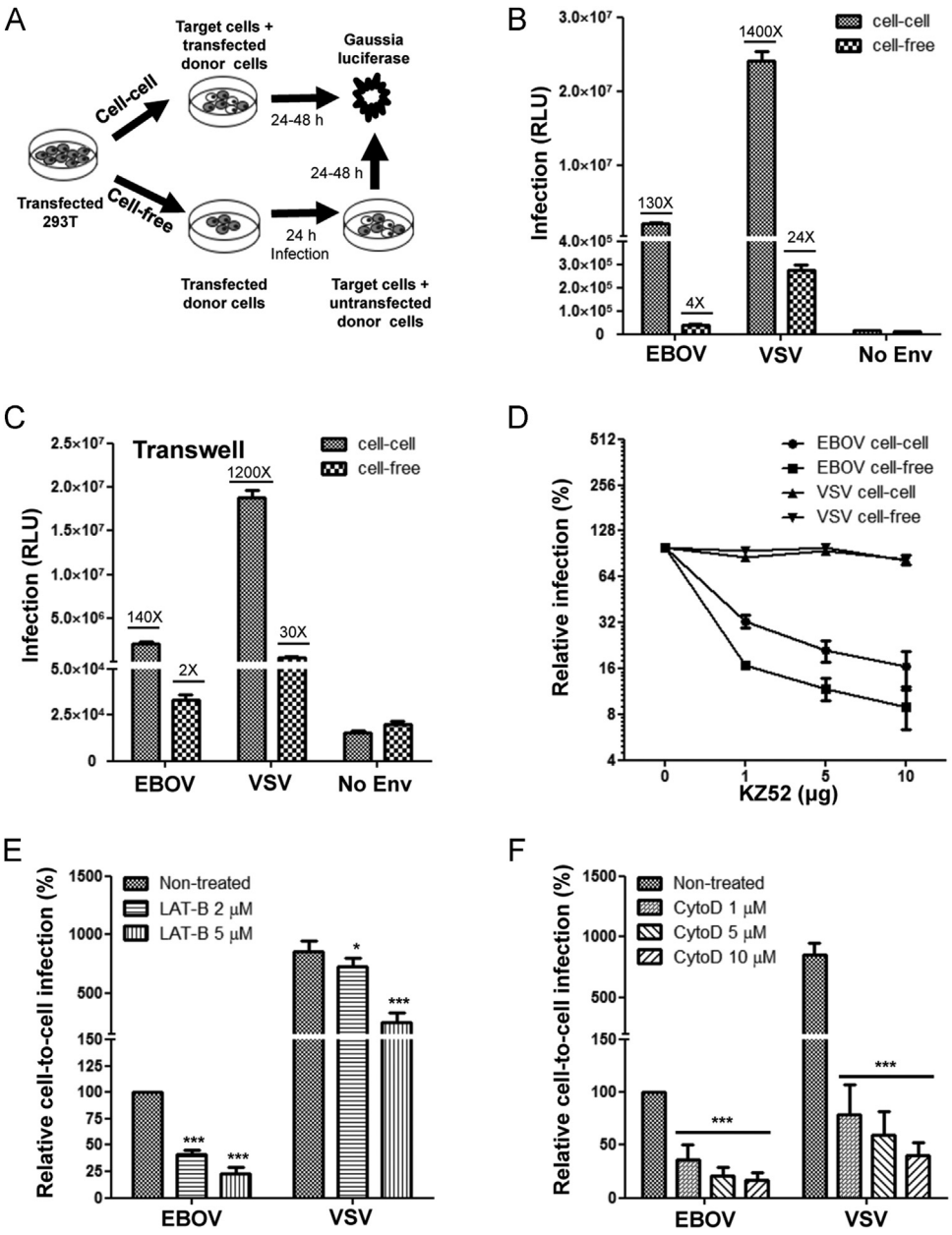


**Fig. 1.** EBOV GP mediates cell-to-cell infection of retroviral pseudotypes. (A) Schematic representation of cell-to-cell vs. cell-free infections. See details in Methods and Results. (B) Comparisons between cell-to-cell and cell-free infections mediated by EBOV GP and VSV-G. Results shown are averages of three independent experiments measured 24 and 48 h after co-culture. "No Env" indicates the background Gluc activity derived from co-culture of 293T donor cells transfected with "Tet-off" alone. The fold differences in Gluc activity above the corresponding "No Env" background are indicated. (C) Comparisons between cell-to-cell and cell-free infections in *Transwell* plates. The fold differences in Gluc activity above the corresponding "No Env" background are indicated. (D) Effect of KZ52 on cell-to-cell and cell-free infections. For cell-to-cell infection, KZ52 was added during co-culture. For cell-free infection, KZ52 was incubated with viral supernatants for 2 h at 37 °C prior to infection and maintained during infection. The efficiencies of cell-to-cell or cell-free infection of EBOV GP and VSV-G without KZ52 were set to 100%, respectively, and relative activities at different doses were calculated and plotted. (E) Effect of LAT-B on cell-to-cell infection mediated by EBOV GP or VSV-G. Two concentrations of LAT-B (i. e., 2 μM and 5 μM) were applied to co-culture, and Gluc activity was measured 24–48 h later. (F) Effect of CytoD on cell-to-cell infection mediated by EBOV GP or VSV-G. Three concentrations of CytoD were used during cell co-culture. Results are from at least three independent experiments. $^{*}p < 0.05$; $^{***}p < 0.001$.