Host IQGAP1 and Ebola Virus VP40 Interactions Facilitate Virus-Like Particle Egress

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We have identified host IQGAP1 as an interacting partner for Ebola virus (EBOV) VP40, and its expression is required for EBOV VP40 virus-like particle (VLP) budding. IQGAP1 is involved in actin cytoskeletal remodeling during cell migration and formation of filopodia. The physical interaction and the functional requirement for IQGAP1 in EBOV VP40 VLP egress link virus budding to the cytoskeletal remodeling machinery. Consequently, this interaction represents a novel target for development of therapeutics to block budding and transmission of filoviruses.

Ebola virus (EBOV) and Marburg virus (MARV) are enveloped, negative-sense RNA viruses belonging to the family Filoviridae which cause hemorrhagic syndromes with high mortality rates in humans (1, 2). There are currently no licensed vaccines or therapeutics to control filovirus infection and transmission. The filovirus VP40 matrix protein plays a central role in virion assembly and egress such that independent expression of VP40 leads to the production of virus-like particles (VLPs) that accurately mimic budding of live virus (3–7). Late (L) budding domains of VP40, which recruit host proteins (e.g., Tsg101) required for efficient virus-cell separation (or “pinching-off”), consist of core consensus amino acid motifs such as PPxY, P(T/S)AP, YxxL, or FPIV (x = any amino acid). The conservation of L-domains within matrix proteins of many RNA viruses suggests that they are generally important and required for efficient RNA virus budding (8), although they are not absolutely required for viral replication (9).

Unlike the events that contribute to the late stages of filovirus budding, little is known about the regulation of early stages of filovirus budding, but this likely involves cellular mechanisms that control cytoskeletal remodeling and membrane deformation/curvature. For example, filopodia significantly increase the ability of filoviruses to spread from cell to cell, thereby contributing to pathogenesis (10). One multifunctional host protein that plays key roles in regulating cell motility, cytoskeletal architecture, actin polymerization, and formation of filopodia is IQGAP1 (11–16). Indeed, IQGAP1 is a widely expressed scaffolding protein with multiple protein-protein interaction domains, including a WW-domain that may interact with viral PPxY type L-domains (12). Intriguingly, IQGAP1 has been detected in purified HIV-1 virions (17) and has been shown to interact with the Gag protein of Moloney murine leukemia virus (MuLV) (18) and the core protein of classical swine fever virus (CSFV) (19) as well as host Tsg101 (20).

Here we investigated whether endogenous IQGAP1 interacts with EBOV VP40 and whether this interaction regulates efficient egress of EBOV VP40 VLPs. We found that EBOV VP40 interacts with endogenous IQGAP1 and that the L-domain region of VP40 mediates this interaction. Importantly, we found that egress of EBOV VP40 VLPs from IQGAP1-suppressed cells is reduced. Together, our findings identify a functional requirement for IQGAP1 interactions with EBOV VP40 during budding and suggest that IQGAP1-regulated proteins/pathways may be generally important for filovirus egress. As such, an EBOV VP40–IQGAP1 interaction represents a novel target for therapeutics to block filovirus budding and transmission.

IQGAP1 (Fig. 1) is a ubiquitously expressed scaffolding pro-

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**FIG 1** Schematic diagram of IQGAP1. The amino acid (aa) numbers are shown for the following IQGAP1 domains: calponin homology (CHD), polyproline binding domain (WW), IQ domain containing four IQ motifs (IQ), Ras GTPase-activating protein-related domain (GRD), and RasGAP C-terminal domain (RGCT). Amino acids 7 to 13 from EBOV VP40 (PTAPPEY) are shown. Solid arrows indicate reported interactions between Tsg101 and the RGCT domain of IQGAP1 (20) as well as with the PTAP motif of EBOV VP40 (25). The dashed arrow linking the PPEY motif of EBOV VP40 and the WW domain of IQGAP1 indicates a putative interaction.
EBOV VP40 interacts with endogenous IQGAP1. Cell proteins from antiserum (Fig. 3, top panel). EBOV VP40 was detected in precipitates were quantified by Western analysis using VP40-specific IQGAP1-specific monoclonal antibody, and VP40 levels in pre-nondenaturing buffer 30 h posttransfection. Cell proteins were transfected with vector alone or pCAGGS-VP40 and then lysed in interaction between IQGAP1 and VP40. To test this, cells were transfected with random small interfering RNAs (siRNAs) or were mock transfected for 12 h. Cells were then transfected with the indicated siRNAs and pCAGGS-VP40 for an additional 12 h. Cell extracts and supernatants containing released VLPs were harvested, and proteins were analyzed by SDS-PAGE and Western analysis using IQGAP1- or VP40-specific antisera (Fig. 2). Expression of endogenous IQGAP1 was reduced by approximately 90% in cells receiving IQGAP1-specific siRNAs (Fig. 2, lane 1) compared to the expression seen after transfection with random siRNAs (lane 2) or mock transfection (lane 3) in control cells. Importantly, VP40 budding in IQGAP1-suppressed cells was reduced by approximately 10-fold (Fig. 2, bottom panel, lane 1) relative to control results (Fig. 2, bottom panel, lane 1 versus lanes 2 and 3), indicating that IQGAP1 is crucial for efficient egress of EBOV VP40 VLPs from HEK293T cells.

We then questioned whether the observed decrease in VP40 VLP egress from IQGAP1-suppressed cells correlated with a direct interaction between IQGAP1 and VP40. To test this, cells were transfected with vector alone or pCAGGS-VP40 and then lysed in nondenaturing buffer 30 h posttransfection. Cell proteins were immunoprecipitated with either mouse preimmune serum or an IQGAP1-specific monoclonal antibody, and VP40 levels in precipitates were quantified by Western analysis using VP40-specific antisera (Fig. 3, top panel). EBOV VP40 was detected in precipitates from IQGAP1, VP40, and Tsg101 immunoprecipitates but not in precipitates produced with preimmune antiserum (Fig. 3, top panel; compare lanes 1 and 2). As expected, control cells receiving pCAGGS vector alone were negative for VP40 (Fig. 3, top panel, lanes 3 and 4). Although these findings are consistent with a direct IQGAP1-VP40 interaction, they do not rule out the possibility that these proteins are linked through other intermediates such as the actin cytoskeleton and/or Tsg101.

Because IQGAP1 has been shown to interact with host Tsg101 (20), we sought to determine whether endogenous Tsg101 regulates IQGAP1 interactions with EBOV VP40. To test this, we performed an analysis similar to that described above in control and Tsg101-suppressed cells (Fig. 4). Suppression of endogenous Tsg101 resulted in a difference of <2-fold in the amount of VP40 pulled down by IQGAP1 (Fig. 4, top panel; compare lanes 1 and 3), and VP40 was not pulled down by preimmune control serum (Fig. 4, top panel, lanes 2 and 4). Taken together, these data strongly suggest that EBOV VP40 interacts directly with endogenous IQGAP1 and that this association is not mediated by Tsg101.

We next used BiMC (21–24) to visualize IQGAP1-VP40 complex formation in live mammalian cells and to confirm that IQGAP1 and VP40 directly interact. To do this, both an N-terminal enhanced yellow fluorescent protein (EYFP) fragment (residues 1 to 173, denoted NYFP) joined in-frame to human IQGAP1 and a previously generated C-terminal EYFP fragment (residues 174 to 239, denoted CYFP) joined to either the wild type (WT) (CYFP-eVP40-WT) or an L-domain deletion mutant of EBOV VP40 (CYFP-eVP40-ΔPT/PY) that produces at least 10-fold less VLPs than CYFP-eVP40-WT (23, 24) were coexpressed in 293T cells. Expression of all YFP fusion proteins was confirmed by Western analysis, and CYFP-eVP40-WT was confirmed to bud as a VLP (Y. Liu and R. N. Harty; data not shown) (23, 24). Cells were fixed 24 h posttransfection with acetone-methanol and stained with 4',6-diamidino-2-phenylindole (DAPI), and YFP fluorescence, which is indicative of an interaction between the expressed protein pairs, was examined by confocal microscopy (Fig. 5). A strong fluorescent signal was observed in cells expressing NYFP-
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FIG 5 Use of BßMC to detect and visualize IQGAP1–VP40 interactions. Human 293T cells were cotransfected with the indicated plasmid pairs: NYFP–IQGAP1 plus CYFP–VP40-WT (top left), NYFP–IQGAP1 plus CYFP–VP40-DPT/PY (top right), NYFP–VP40-WT plus CYFP–VP40-DPT/PY (bottom left), and NYFP plus CYFP–VP40–WT (bottom right). Cells were fixed at 24 h posttransfection, stained with DAPI, and examined by confocal microscopy for YFP fluorescence. Bar = 2.0 µm.

IQGAP1 plus CYFP–VP40–WT (Fig. 5, top left); however, little to no fluorescence was observed in cells expressing NYFP–IQGAP1 and CYFP–VP40–DPT/PY (Fig. 5, top right). To prove that CYFP–VP40–DPT/PY was indeed expressed in transfected cells, we cotransfected 293T cells with NYFP–VP40–WT and CYFP–VP40–DPT/PY (Fig. 5, bottom left). As expected, we observed a strong fluorescent signal in these cells, since deletion of the L-domain region of VP40 does not abolish a VP40–VP40 self-interaction (Fig. 5, bottom left). As an additional negative control, 293T cells were cotransfected with a plasmid expressing just the NYFP fragment alone plus CYFP–VP40–WT (Fig. 5, bottom right). These data confirm that EBOV VP40 and IQGAP1 interact directly and also demonstrate that the L-domain region of EBOV VP40 is critical for mediating this virus-host interaction.

Although IQGAP1 has been shown to interact with MuLV Gag during infection (18), our findings here are the first to demonstrate that IQGAP1 associates with VP40 and furthermore are the first to link IQGAP1 expression to filovirus egress. IQGAP1 is a central and crucial scaffolding protein that assembles, coordinates, and regulates formation of a multiprotein complex that includes actin, calmodulin, and Cdc42 to promote cell migration and actin microspikes and formation of filopodia (12). Our working model is that IQGAP1 is required by VP40 to initiate recruitment of other proteins (e.g., Cdc42) that promote early (e.g., bud protrusion) virus budding. We speculate that filoviral L-domains may recruit host proteins in a temporal and/or sequential manner to promote both early (e.g., bud protrusion) and late (e.g., pinching-off) stages of virion egress. This requirement of IQGAP1 for virus budding suggests that cellular proteins/pathways linked to IQGAP1 and involved in formation of filopodia/cytoskeletal rearrangements/cell motility are important for efficient EBOV egress. We further speculate that the IQGAP1 WW-domain region is involved in mediating interactions with EBOV VP40, although a full understanding of these domains and the mechanism by which IQGAP1 regulates the budding process of EBOV particles requires visualization of the spatial and temporal dynamics and trafficking of IQGAP1–VP40 complexes in live mammalian cells.


