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Single-Particle Tracking Demonstrates that Actin Coordinates the Movement of the Ebola Virus Matrix Protein

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ABSTRACT The Ebola virus causes severe hemorrhagic fever and has a mortality rate that can be as high as 90%, yet no vaccines or approved therapeutics, to our knowledge, are available. To replicate and egress the infected host cell the Ebola virus uses VP40, its major matrix protein to assemble at the inner leaflet of the plasma membrane. The assembly and budding of VP40 from the plasma membrane of host cells seem still poorly understood. We investigated the assembly and egress of VP40 at the plasma membrane of human cells using single-particle tracking. Our results demonstrate that actin coordinates the movement and assembly of VP40, a critical step in viral egress. These findings underscore the ability of single-molecule techniques to investigate the interplay of VP40 and host proteins in viral replication.

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The actin cortex below the plasma membrane of mammalian cells is essential for maintenance of cell shape and for cell movement. This cortex has also been found to play an essential role in the replication process of a number of viruses including West Nile virus (1), respiratory syncytial virus (2), influenza (3), and vaccinia virus (4). Additionally, actin has been found to play a central role in the assembly and budding of HIV-1 (5) whereas Marburg virus has been shown to use actin-enriched filopodia to exit the host cell (6). Actin has also been found to be packaged into Ebola-virus-like particles (VLPs) (7). Ebola virus, which causes severe hemorrhagic fever, harbors a single-stranded negative-sense RNA genome encoding seven proteins. Of these seven proteins, VP40 is the most abundantly expressed and has been found to play a central role in the budding of the virus from the plasma membrane (8). Whereas actin has been found in Ebola VLPs (7), the role of actin in Ebola VP40 assembly is still seemingly unknown. Here, we have used Raster image correlation spectroscopy (RICS) (9) and three-dimensional single-particle tracking (see Fig. S1 in the Supporting Material) (10) to investigate the dynamics of Ebola VP40 and actin. We report that preassembled VLPs (pVLPs) of Ebola VP40 require actin for directed movement and assembly.

Ebola VP40 has been demonstrated to colocalize with actin and actin is found in VP40 VLPs (7), suggesting an important role for actin in the replication cycle of the virus. To confirm the colocalization between VP40 and actin in HEK293 and CHO-K1 cells, we used confocal microscopy to examine the distribution of EGFP-VP40 and mCherry-actin. EGFP-VP40 and mCherry-actin displayed colocalization at the plasma membrane of HEK293 and CHO-K1 cells (see Fig. S2 *A*), which was markedly reduced in response to treatment with LAT-A (see Fig. S2 *B* and Fig. S3 *A*), an actin polymerization inhibitor. VP40 plasma membrane localization was not disrupted by LAT-A treatment (not unexpected, as VP40 is a lipid-binding protein (11) where high affinity for the PM drives its cellular localization (E. Adu-Gyamfi and R. V. Stahelin, unpublished)). To test whether this VP40-actin interaction is important to viral egress, we detected EGFP-VP40 with an anti-EGFP antibody used to measure VLPs formed from cells expressing EGFP-VP40. This was also performed to assess the effect of pharmacological treatment on EGFP-VP40-expressing cells with LAT-A or with the microtubule polymerization inhibitor nocodazole (see Fig. S3 *B*). LAT-A treatment led to a significant reduction in VLP formation whereas nocodazole did not display detectable effects.

To test whether the VP40 and actin are engaged in synchronized movement, we performed time-lapse imaging in both the green and red channels. We observed that the pVLPs move with actin fibers extending from the plasma membrane (see Movie S1 in the Supporting Material). The movement was rapid, and caused smaller particles to merge into larger filamentous forms. To further demonstrate that the motion of actin and VP40 spatially overlapped, we used RICS to obtain correlation maps of EGFP-VP40 and mCherry-actin (Fig. 1). The spatial cross-correlation map indicated significant overlap of VP40 and actin movement (Fig. 2, *A–C*) at the plasma membrane (Fig. 1 and see Fig. S6), but not in the cytosol

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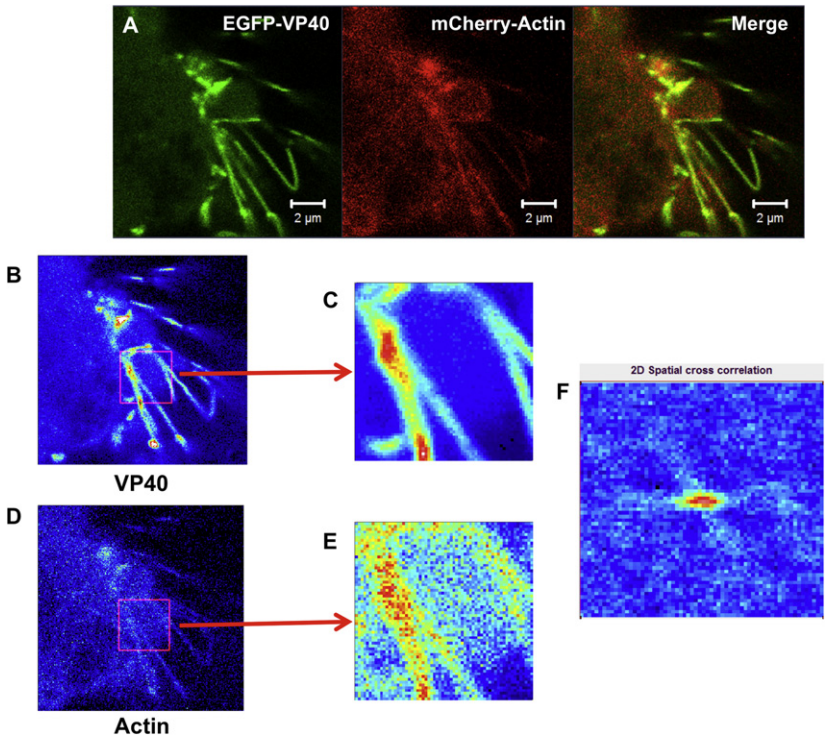

FIGURE 1 EGFP-VP40 and mCherry-actin RICS analysis at the membrane. (*A*) HEK293 cells expressing EGFP-VP40 and mCherry-actin were imaged for 100 frames at 256 × 256 pixels. (*White scale bar* = 2 μm.) (*B*) Average intensity image of EGFP-VP40 across the 100 collected frames. (*Pink box*) Used to select a region of interest to yield the (*C*) average EGFP-VP40 intensity image. (*D*) Average intensity image of mCherry-actin taken for 100 frames at 256 × 256 pixels was used to select the same region of interest as in panel *B* (*pink box*) to yield the (*E*) average intensity image of the mCherry-actin signal in this region. (*F*) The two-dimensional spatial cross-correlation analysis of panels *C* and *E* demonstrates significant cross-correlation of VP40 and actin signals.

(see Fig. S5 and Fig. S7). In contrast, EGFP-VP40 and mCherry-α-tubulin (see Fig. S8, Fig. S9, and Fig. S10) displayed no significant spatial cross-correlation at the plasma membrane (Fig. S11) or other regions of the cell (see Fig. S12), supporting the VLP egress data where inhibition of microtubule polymerization did not influence viral egress.

To test whether the motion of the pVLPs is directed by actin, we applied the three-dimensional orbital tracking method first introduced by Levi et. al. (10). Tracking of isolated particles (Fig. 3 *A*) in five different cells allowed determination of the pVLPs trajectories (Fig. 3 *D*), which suggested that the VP40 particles undergo a directed motion. To verify this, we plotted the mean-square displacement (MSD) curves for the pVLPs (Fig. 3 *C*), which confirmed the trajectory was characteristic of directed motion. Analysis of the intensity profile of the dynamic VP40 particles suggested that the intensity of the particle changes with respect to time. Bleaching is expected if the molecule is exposed to the laser beam for an extended period of time; however, an increase in intensities was observed along the trajectory of the green channel due to addition of VP40 molecules. This suggests that the movement of the particles along actin fibers promote multimerization and maturation of the pVLPs. When actin polymerization was inhibited in four different cells with LAT-B, the rapid movement (see Fig. S13) and the directed trajectories of the pVLPs were lost (Fig. 3, *E* and *F*). This was reflected in a change from directed motion to movement indicative of random then constrained diffusion (Fig. 3, *E* and *F*).

Taken together, our findings demonstrate that the movement of the pVLPs is driven by actin. Analysis of the pVLPs trajectories also suggests that the motion of pVLPs on actin enables further addition of VP40 molecules. These findings raise important questions regarding contemporary

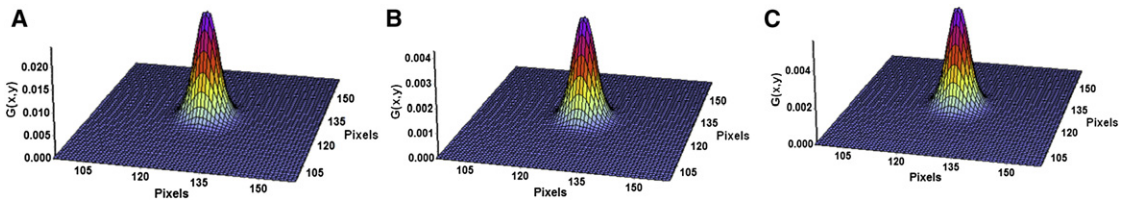

FIGURE 2 Three-dimensional RICS correlation maps of VP40 and actin cross-correlate at the plasma membrane. (*A*) EGFP-VP40 and (*B*) mCherry-actin (Fig. 1 and see Fig. S6 in the Supporting Material) RICS autocorrelation functions. (*C*) Appreciable cross-correlation is observed for EGFP-VP40 and mCherry-actin at the plasma membrane.

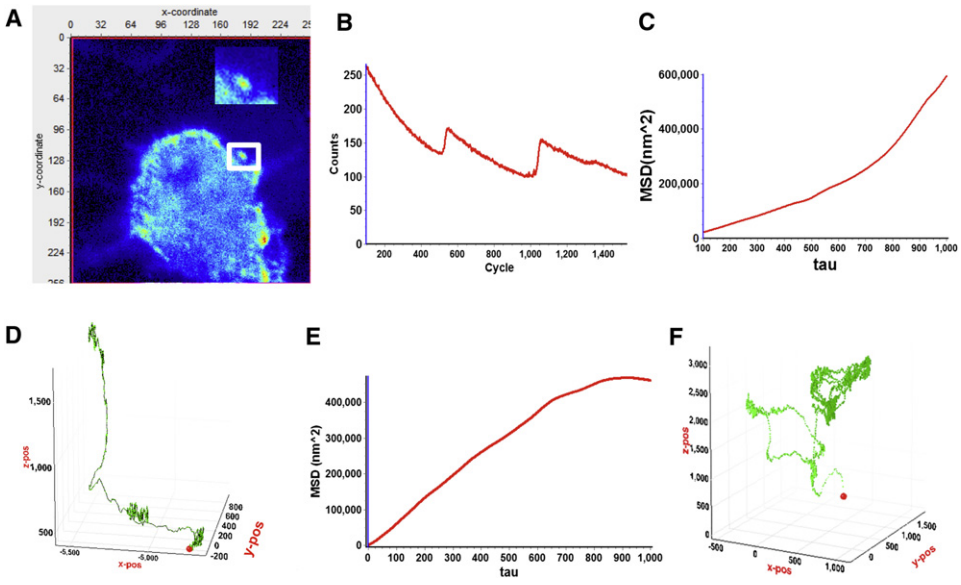

FIGURE 3 Actin directs the movement of VP40 particles. HEK293 cells transfected with EGFP-VP40 were imaged with an electronic zoom of 2000 mV, corresponding to 72 nm/pixel in both *X* and *Y*. (*A*) An isolated and representative VP40 particle (highlighted by *white box*, *inset*) was tracked as described in the Supporting Material. (*B*) Intensity profile of the pVLP in A demonstrates increases in EGFP-VP40 intensity along the trajectory. (*C*) MSD of the pVLP, which follows a ballistic motion with a velocity of 0.067 ± 0.01 $\mu m^2\ s^{-1}$. (*D*) The three-dimensional trajectory of the particle shown in panels *A*–*C*. (*E*) MSD curve of VP40 particles yields random then constrained diffusion after LAT-B treatment with a mean velocity of 0.017 ± 0.006 $\mu m^2\ s^{-1}$ ($p < 0.001$). (*F*) Three-dimensional trajectory of the same particle shown in panel *E* displays a random then constrained diffusion.

understanding of Ebola assembly and egress. VP40 lacks a consensus actin-binding motif, suggesting an adaptor protein such as an actin motor protein may function in this process. For instance, Myo10 has been found to be essential to Marburg virus release (6); however, Marburg VP40-Myo10 direct interactions were not observed, suggesting other cellular adaptor proteins may function in this process. Given the pathogenic nature of the Ebola virus and the necessity of VP40 to the assembly and egress of the virus (8), the VP40-actin coordination represents, to us, a novel target for therapeutic development.

SUPPORTING MATERIAL

Materials and Methods, thirteen figures, and one movie are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(12)01064-8.

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REFERENCES and FOOTNOTES

1. Chu, J. J., B. G. Choo, …, M. L. Ng. 2003. Actin filaments participate in West Nile (Sarafend) virus maturation process. *J. Med. Virol.* 71:463–472.
2. Kallewaard, N. L., A. L. Bowen, and J. E. Crowe, Jr. 2005. Cooperativity of actin and microtubule elements during replication of respiratory syncytial virus. *Virology.* 331:73–81.
3. Simpson-Holley, M., D. Ellis, …, P. Digard. 2002. A functional link between the actin cytoskeleton and lipid rafts during budding of filamentous influenza virions. *Virology.* 301:212–225.
4. Cudmore, S., P. Cossart, …, M. Way. 1995. Actin-based motility of vaccinia virus. *Nature.* 378:636–638.
5. Gladnikoff, M., E. Shimoni, …, I. Rousso. 2009. Retroviral assembly and budding occur through an actin-driven mechanism. *Biophys. J.* 97:2419–2428.
6. Kolesnikova, L., A. B. Bohil, …, S. Becker. 2007. Budding of Marburgvirus is associated with filopodia. *Cell. Microbiol.* 9:939–951.
7. Han, Z., and R. N. Harty. 2005. Packaging of actin into Ebola virus VLPs. *Virol. J.* 2:92–99.
8. Harty, R. N. 2009. No exit: targeting the budding process to inhibit filovirus replication. *Antiviral Res.* 81:189–197.
9. Digman, M. A., and E. Gratton. 2009. Analysis of diffusion and binding in cells using the RICS approach. *Microsc. Res. Tech.* 72:323–332.
10. Levi, V., Q. Ruan, and E. Gratton. 2005. 3-D particle tracking in a two-photon microscope: application to the study of molecular dynamics in cells. *Biophys. J.* 88:2919–2928.
11. Adu-Gyamfi, E., M. A. Digman, …, R. V. Stahelin. 2012. Investigation of Ebola VP40 assembly and oligomerization in live cells using number and brightness analysis. *Biophys. J.* 102:2517–2525.