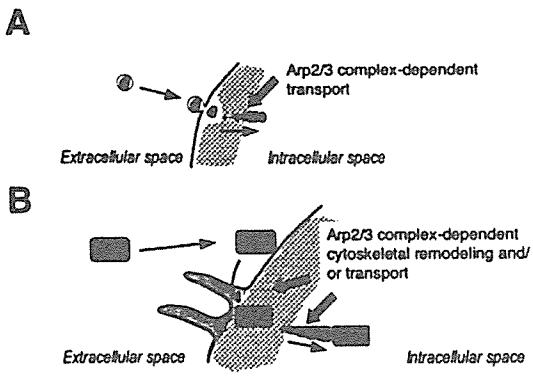

Figure 6. Models in which the Arp2/3 complex supports infection of both primate lentiviruses and IMV. (A) Primate lentiviruses enter cells via membrane fusion at cell surface. After membrane fusion, viral gene products might initiate activation of Arp2/3 complex-dependent actin polymerization (red) behind the viral core to cross the cortical layer (gray). (B) IMV also enters cells via membrane fusion at the cell surface. At or soon after the attachment, the dynamic actin cytoskeletal reorganization takes place that depends partly on the Arp2/3 complex-mediated actin polymerization (red), which may facilitate viral entry. Alternatively, the Arp2/3 complex-mediated actin polymerization (red) powers the viral core (green) to migrate toward the cytoplasmic compartment in which vaccinia virus replicates.

number of Arp2/3 complex should participate. This may account for the less efficient block of IMV's infection by both GFP-A and siRNA directing against Arp2 (Figure 4, C and E).

Our finding suggested a new therapeutic target to control HIV-1 replication in AIDS patients. We should be able to limit HIV-1 replication by inhibiting the HIV-1's ability to activate Arp2/3 complex by a small chemical compound. It is underway to determine which viral gene product is responsible to activate Arp2/3 complex.

ACKNOWLEDGMENTS

We thank Drs. Hironori Sato, Bill Sugden, and Tsutomu Murakami for critical reading of the manuscript and Yuko Futahashi for the technical assistance. We also thank Drs. T. Takenawa, A. Yamashita, J. Young, E. Freed, P. G. Spear, Y. Takai, S. Takase-Yoden, W. Sugiura, and K. Mori for generously sharing reagents and equipments. This work was partly supported by both the Japan Health Science Foundation and a grant from Japanese Ministry of Health, Labor, and Welfare.

REFERENCES

Aoki, Y., Aizaki, H., Shimoike, T., Tani, H., Ishii, K., Saito, I., Matsuura, Y., and Miyamura, T. (1998). A human liver cell line exhibits efficient translation of HCV RNAs produced by a recombinant adenovirus expressing T7 RNA polymerase. Virology *250*, 140–150.

Bukrinskaya, A., Brichacek, B., Mann, A., and Stevenson, M. (1998). Establishment of a functional human immunodeficiency virus type 1 (HIV-1) reverse transcription complex involves the cytoskeleton. J. Exp. Med. *188*, 2113–2125.

Cantarelli, V.V., Takahashi, A., Akeda, Y., Nagayama, K., and Honda, T. (2000). Interaction of enteropathogenic or enterohemorrhagic *Escherichia coli* with HeLa cells results in translocation of cortactin to the bacterial adherence site. Infect. Immun. *68*, 382–386.

Castellano, F., Le Clainche, C., Patin, D., Carlier, M.F., and Chavrier, P. (2001). A WASp-VASP complex regulates actin polymerization at the plasma membrane. EMBO J. *20*, 5603–5614.

Cudmore, S., Reckmann, I., and Way, M. (1997). Viral manipulations of the actin cytoskeleton. Trends Microbiol. *5*, 142–148.

Dean, H.J., Terhune, S.S., Shieh, M.T., Susmarski, N., and Spear, P.G. (1994). Single amino acid substitutions in gD of herpes simplex virus 1 confer resistance to gD-mediated interference and cause cell-type-dependent alterations in infectivity. Virology *199*, 67–80.

Dehio, C., Prevost, M.C., and Sansonetti, P.J. (1995). Invasion of epithelial cells by *Shigella flexneri* induces tyrosine phosphorylation of cortactin by a pp60c-src-mediated signalling pathway. EMBO J. *14*, 2471–2482.

Fawaz, F.S., van Ooij, C., Homola, E., Mutka, S.C., and Engel, J.N. (1997). Infection with *Chlamydia trachomatis* alters the tyrosine phosphorylation and/or localization of several host cell proteins including cortactin. Infect. Immun. *65*, 5301–5308.

Flanagan, L.A., Chou, J., Falet, H., Neujahr, R., Hartwig, J.H., and Stossel, T.P. (2001). Filamin A, the Arp2/3 complex, and the morphology and function of cortical actin filaments in human melanoma cells. J. Cell Biol. *155*, 511–517.

Frischknecht, F., Moreau, V., Rottger, S., Gonfloni, S., Reckmann, I., Superti-Furga, G., and Way, M. (1999). Actin-based motility of vaccinia virus mimics receptor tyrosine kinase signalling. Nature *401*, 926–929.

Fuerst, T.R., Niles, E.G., Studier, F.W., and Moss, B. (1986). Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. Proc. Natl. Acad. Sci. USA *83*, 8122–8126.

Fuller, A.O., and Spear, P.G. (1987). Anti-glycoprotein D antibodies that permit adsorption but block infection by herpes simplex virus 1 prevent virion-cell fusion at the cell surface. Proc. Natl. Acad. Sci. USA *84*, 5454–5458.

Gournier, H., Goley, E.D., Niederstrasser, H., Trinh, T., and Welch, M.D. (2001). Reconstitution of human Arp2/3 complex reveals critical roles of individual subunits in complex structure and activity. Mol. Cell *8*, 1041–1052.

Gruenheid, S., and Finlay, B.B. (2003). Microbial pathogenesis and cytoskeletal function. Nature *422*, 775–781.

Gundlach, B.R. *et al.* (1997). Construction, replication, and immunogenic properties of a simian immunodeficiency virus expressing interleukin-2. J. Virol. *71*, 2225–2232.

Harlander, R.S., Way, M., Ren, Q., Howe, D., Grieshaber, S.S., and Heinzen, R.A. (2003). Effects of ectopically expressed neuronal Wiskott-Aldrich syndrome protein domains on *Rickettsia rickettsii* actin-based motility. Infect. Immun. *71*, 1551–1556.

Higgs, H.N., Blanchoin, L., and Pollard, T.D. (1999). Influence of the C terminus of Wiskott-Aldrich syndrome protein (WASp) and the Arp2/3 complex on actin polymerization. Biochemistry *38*, 15212–15222.

Higgs, H.N., and Pollard, T.D. (2001). Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. Annu. Rev. Biochem. *70*, 649–676.

Ichihashi, Y., and Oie, M. (1996). Neutralizing epitope on penetration protein of vaccinia virus. Virology *220*, 491–494.

Iyengar, S., Hildreth, J.E., and Schwartz, D.H. (1998). Actin-dependent receptor colocalization required for human immunodeficiency virus entry into host cells. J. Virol. *72*, 5251–5255.

Kizhatil, K., and Albritton, L.M. (1997). Requirements for different components of the host cell cytoskeleton distinguish ecotropic murine leukemia virus entry via endocytosis from entry via surface fusion. J. Virol. *71*, 7145–7156.

Krause, M., Sechi, A.S., Konradt, M., Monner, D., Gertler, F.B., and Wehland, J. (2000). Fyn-binding protein (Fyb)/SLP-76-associated protein (SLAP), Ena/vasodilator-stimulated phosphoprotein (VASP) proteins and the Arp2/3 complex link T cell receptor (TCR) signaling to the actin cytoskeleton. J. Cell Biol. *149*, 181–194.

Li, E., Stupack, D., Bokoch, G.M., and Nemerow, G.R. (1998). Adenovirus endocytosis requires actin cytoskeleton reorganization mediated by Rho family GTPases. J. Virol. *72*, 8806–8812.

Locker, J.K., Kuehn, A., Schleich, S., Rutter, G., Hohenberg, H., Wepf, R., and Griffiths, G. (2000). Entry of the two infectious forms of vaccinia virus at the plasma membrane is signaling-dependent for the IMV but not the EEV. Mol. Biol. Cell *11*, 2497–2511.

Machesky, L.M., and Insall, R.H. (1998). Scar1 and the related Wiskott-Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. Curr. Biol. *8*, 1347–1356.

Machesky, L.M., Mullins, R.D., Higgs, H.N., Kaiser, D.A., Blanchoin, L., May, R.C., Hall, M.E., and Pollard, T.D. (1999). Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. Proc. Natl. Acad. Sci. USA *96*, 3739–3744.

Maddon, P.J., McDougal, J.S., Clapham, P.R., Dalgleish, A.G., Jamal, S., Weiss, R.A., and Axel, R. (1988). HIV infection does not require endocytosis of its receptor, CD4. Cell *54*, 865–874.

Marchand, J.B., Kaiser, D.A., Pollard, T.D., and Higgs, H.N. (2001). Interaction of WASP/Scar proteins with actin and vertebrate Arp2/3 complex. Nat. Cell Biol. *3*, 76–82.

May, R.C., Caron, E., Hall, A., and Machesky, L.M. (2000). Involvement of the Arp2/3 complex in phagocytosis mediated by FcgammaR or CR3. Nat. Cell Biol. *2*, 246–248.

McClure, M.O., Sommerfelt, M.A., Marsh, M., and Weiss, R.A. (1990). The pH independence of mammalian retrovirus infection. J. Gen. Virol. *71*, 767–773.

McDonald, D., Vodicka, M.A., Lucero, G., Svitkina, T.M., Borisy, G.G., Emerman, M., and Hope, T.J. (2002). Visualization of the intracellular behavior of HIV in living cells. J. Cell Biol. *159*, 441–452.

McGee, K., Zettl, M., Way, M., and Fallman, M. (2001). A role for N-WASP in invasin-promoted internalisation. FEBS Lett. *509*, 59–65.

Miki, H., Miura, K., and Takenawa, T. (1996). N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. EMBO J. *15*, 5326–5335.

Miki, H., and Takenawa, T. (1998). Direct binding of the verprolin-homology domain in N-WASP to actin is essential for cytoskeletal reorganization. Biochem. Biophys. Res. Commun. *243*, 73–78.

Moreau, V., Frischknecht, F., Reckmann, I., Vincentelli, R., Rabut, G., Stewart, D., and Way, M. (2000). A complex of N-WASP and WIP integrates signalling cascades that lead to actin polymerization. Nat. Cell Biol. *2*, 441–448.

Nussbaum, O., Broder, C.C., and Berger, E.A. (1994). Fusogenic mechanisms of enveloped-virus glycoproteins analyzed by a novel recombinant vaccinia virus-based assay quantitating cell fusion-dependent reporter gene activation. J. Virol. *68*, 5411–5422.

Nussbaum, O., Roop, A., and Anderson, W.F. (1993). Sequences determining the pH dependence of viral entry are distinct from the host range-determining region of the murine ecotropic and amphotropic retrovirus envelope proteins. J. Virol. *67*, 7402–7405.

Panchal, S.C., Kaiser, D.A., Torres, E., Pollard, T.D., and Rosen, M.K. (2003). A conserved amphipathic helix in WASP/Scar proteins is essential for activation of Arp2/3 complex. Nat. Struct. Biol. *10*, 591–598.

Pantaloni, D., Le Clainche, C., and Carlier, M.F. (2001). Mechanism of actin-based motility. Science *292*, 1502–1506.

Ragheb, J.A., and Anderson, W.F. (1994). pH-independent murine leukemia virus ecotropic envelope-mediated cell fusion: implications for the role of the R peptide and p12E TM in viral entry. J. Virol. *68*, 3220–3231.

Rietdorf, J., Ploubidou, A., Reckmann, I., Holmstrom, A., Frischknecht, F., Zettl, M., Zimmermann, T., and Way, M. (2001). Kinesin-dependent movement on microtubules precedes actin-based motility of vaccinia virus. Nat. Cell Biol. *3*, 992–1000.

Robinson, R.C., Turbedsky, K., Kaiser, D.A., Marchand, J.B., Higgs, H.N., Choe, S., and Pollard, T.D. (2001). Crystal structure of Arp2/3 complex. Science *294*, 1679–1684.

Rohatgi, R., Ma, L., Miki, H., Lopez, M., Kirchhausen, T., Takenawa, T., and Kirschner, M.W. (1999). The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. Cell *97*, 221–231.

Rosenthal, K.S., Perez, R., and Hodnichak, C. (1985). Inhibition of herpes simplex virus type 1 penetration by cytochalasins B and D. J. Gen. Virol. *66*, 1601–1605.

Rozelle, A.L. *et al.* (2000). Phosphatidylinositol 4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP-Arp2/3. Curr. Biol. *10*, 311–320.

Sakamoto, T., Ushijima, H., Okitsu, S., Suzuki, E., Sakai, K., Morikawa, S., and Muller, W.E. (2003). Establishment of an HIV cell-cell fusion assay by using two genetically modified HeLa cell lines and reporter gene. J. Virol. Methods *114*, 159–166.

Sakisaka, T. *et al.* (2001). Requirement of interaction of nectin-1alpha/HveC with afadin for efficient cell-cell spread of herpes simplex virus type 1. J. Virol. *75*, 4734–4743.

Scaplehorn, N., Holmstrom, A., Moreau, V., Frischknecht, F., Reckmann, I., and Way, M. (2002). Grb2 and Nck act cooperatively to promote actin-based motility of vaccinia virus. Curr. Biol. *12*, 740–745.

Smith, G.L., Vanderplasschen, A., and Law, M. (2002). The formation and function of extracellular enveloped vaccina virus. J. Gen. Virol. *83*, 2915–2931.

Stein, B.S., Gowda, S.D., Lifson, J.D., Penhallow, R.C., Bensch, K.G., and Engleman, E.G. (1987). pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. Cell *49*, 659–668.

Takase-Yoden, S., and Watanabe, R. (1999). Contribution of virus-receptor interaction to distinct viral proliferation of neuropathogenic and nonneuropathogenic murine leukemia viruses in rat glial cells. J. Virol. *73*, 4461–4464.

Takenawa, T., and Miki, H. (2001). WASP and WAVE family proteins: key molecules for rapid rearrangement of cortical actin filaments and cell movement. J. Cell Sci. *114*, 1801–1809.

Uruno, T., Liu, J., Zhang, P., Fan, Y., Egile, C., Li, R., Mueller, S.C., and Zhan, X. (2001). Activation of Arp2/3 complex-mediated actin polymerization by cortactin. Nat. Cell Biol. *3*, 259–266.

Vanderplasschen, A., Hollinshead, M., and Smith, G.L. (1998). Intracellular and extracellular vaccinia virions enter cells by different mechanisms. J. Gen. Virol. *79*, 877–887.

Wang, K., Huang, S., Kapoor-Munshi, A., and Nemerow, G. (1998). Adenovirus internalization and infection require dynamin. J. Virol. *72*, 3455–3458.

Weaver, A.M., Karginov, A.V., Kinley, A.W., Weed, S.A., Li, Y., Parsons, J.T., and Cooper, J.A. (2001). Cortactin promotes and stabilizes Arp2/3-induced actin filament network formation. Curr. Biol. *11*, 370–374.

Weaver, A.M., Young, M.E., Lee, W.L., and Cooper, J.A. (2003). Integration of signals to the Arp2/3 complex. Curr. Opin. Cell Biol. *15*, 23–30.

Weed, S.A., Karginov, A.V., Schafer, D.A., Weaver, A.M., Kinley, A.W., Cooper, J.A., and Parsons, J.T. (2000). Cortactin localization to sites of actin assembly in lamellipodia requires interactions with F-actin and the Arp2/3 complex. J. Cell Biol. *151*, 29–40.

Weed, S.A., and Parsons, J.T. (2001). Cortactin: coupling membrane dynamics to cortical actin assembly. Oncogene *20*, 6418–6434.

Welch, M.D., Rosenblatt, J., Skoble, J., Portnoy, D.A., and Mitchison, T.J. (1998). Interaction of human Arp2/3 complex and the *Listeria monocytogenes* ActA protein in actin filament nucleation. Science *281*, 105–108.

Wittels, M., and Spear, P.G. (1991). Penetration of cells by herpes simplex virus does not require a low pH-dependent endocytic pathway. Virus Res. *18*, 271–290.

Zalevsky, J., Grigorova, I., and Mullins, R.D. (2001). Activation of the Arp2/3 complex by the *Listeria* acta protein. Acta binds two actin monomers and three subunits of the Arp2/3 complex. J. Biol. Chem. *276*, 3468–3475.

Zhang, J. *et al.* (1999). Antigen receptor-induced activation and cytoskeletal rearrangement are impaired in Wiskott-Aldrich syndrome protein-deficient lymphocytes. J. Exp. Med. *190*, 1329–1342.

Zhang, J., Shi, F., Badour, K., Deng, Y., McGavin, M.K., and Siminovitch, K.A. (2002). WASp verprolin homology, cofilin homology, and acidic region domain-mediated actin polymerization is required for T cell development. Proc. Natl. Acad. Sci. USA *99*, 2240–2245.