VIROLOGY

HIV Infection of T Cells: Actin-in and Actin-out

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Three studies shed light on the decade-old observation that the actin cytoskeleton is hijacked to facilitate entry of HIV into its target cells. Polymerization of actin is required to assemble high concentrations of CD4 and CXCR4 at the plasma membrane, which promote viral binding and entry in both the simple model of infection by free virus and the more physiologically relevant route of infection through the virological synapse. Three types of actin-interacting proteins—filamin, ezrin/radixin/moesin (ERM), and cofilin—are now shown to play critical roles in this process. Filamin binds to both CD4 and CXCR4 in a manner promoted by signaling of the HIV gp120 glycoprotein. ERM proteins attach actin filaments to the membrane and may promote polymerization of actin. Early in the process of viral entry, cofilin is inactivated, which is proposed to facilitate the early assembly of actin filaments, but cofilin is reported to be activated soon thereafter to facilitate postentry events. This complex role of cofilin may help to reconcile the paradox that actin polymerization promotes initial binding and fusion steps but inhibits some subsequent early postentry events.

Although cytoskeletal proteins do not often get "top billing" in models of viral infection, there is a growing body of evidence that highlights their importance (*1*, *2*). Subversion of the cytoskeleton by HIV was first reported in a short seminal paper published in 1998 (*3*) that made two key observations: (i) exposure of T lymphocytes to the viral envelope glycoprotein gp120 results in induction of an actin-rich polarized cap of the co-receptors, CD4 and CXCR4, that mediate viral binding and entry; and (ii) inhibition of actin polymerization profoundly inhibits HIV entry and infection. This paper continues to be cited to this day, which underscores both the importance of the finding and that the underlying mechanisms remain to be elucidated. Three studies (*4–6*) now shed some light on this decade-old observation.

The process of formation of the actin cap needs to be interpreted in light of a major paradigm shift in the field of retrovirology that involves two distinct, but related, realizations. First, there is now convincing evidence that the bulk of infection by retroviruses (including HIV) occurs not through the infection of cells by free virus but by cell-to-cell transmission of virus (*7*, *8*).

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Second, the cell-cell contact point at which HIV transfer occurs has fundamental similarities to the immunological synapse formed between an antigen-presenting cell and a T cell (*9*, *10*), with the "recipient" T lymphocyte that acquires the virus playing the role of a lymphocyte that is activated by encounter with antigen.

The original observation that gp120 induces the formation of an actin cap was made in an infection system involving free virus. Is the observation still relevant? Indeed it is, for at least two reasons. First, evidence indicates that at the virological synapse, HIV first buds from the infected donor cell and then binds to and fuses with the recipient cell, as in a free virus system. Second, there are fundamental similarities between the cytoskeletal changes seen in the free virus system and those observed at the virological synapse. Specifically, the features of HIV-dependent interaction between infected and uninfected primary T cells strongly parallel the processes of gp120-induced cap formation: gp120-dependent recruitment of CD4 and CXCR4 in an actin-dependent fashion (*11*). So, we have adopted the view that studies of cytoskeletal events in these two systems should be integrated into a single conceptual model.

What does the actin-dependent cap do? The most appealing hypothesis is that it promotes formation of the high local concentration of the relevant co-receptors (CD4 and CXCR4) as proposed initially (*3*) and thus favors virus binding [and plausibly therefore fusion, which in itself is a complex process (*12*) and which for HIV occurs at the plasma membrane]. High concentrations of receptors and ligands are especially useful when cooperativity of multiple interactions is involved, as is suggested, for example, by high-resolution electron tomographic images of the three-dimensional architectures of virions binding to T cells (*13*).

How does actin polymerization mediate local enrichment of CD4 and CXCR4? The work of Jimenez-Baranda and colleagues has supplied a key missing piece to this puzzle, the actin-crosslinking protein filamin (*5*). Filamin bound to both CD4 and to CXCR4 (as well as to actin), and this binding was promoted by gp120 signaling. Knockdown and transfection studies showed that filamin played a critical role in the actin-mediated concentration of CD4 and CXCR4. But the problem of what molecular process concentrates actin, CD4, and CXCR4 into a cap or synapse is still unsolved. Based on analogy to the immunological synapse (*14*), one model is that centripetal flow of actin into the evolving cap drags CD4 and CXCR4 along with it. Such centripetal flow has yet to be demonstrated in models of virus infection and, if observed, is likely to be at the later stages of a well-formed virological synapse.

We propose that much of the accumulation of co-receptors at the cap results from a simple diffusion-capture process. Signaling by gp120 (see below) induces the local polymerization of actin in the nascent cap similarly to that in the lamellipod (*15*) or in blebs (*16*). Once a favorable form of actin filaments is assembled, filamin will likewise (through a diffusion-facilitated encounter) accumulate on the actin (Fig. 1). Finally, diffusion of CD4 and CXCR4 in the membrane will result in their encounter with and binding to filamin in the complex (assuming that their diffusion is not unduly restricted). Thus, local signal generation, coupled with diffusion, and not the ordered transport of molecules, would be sufficient for assembly of a cap rich in actin, filamin, CD4, and CXCR4.

Some key signaling events that mediate the gp120-induced formation of the cytoskeletal cap are identified by the work of Jimenez-Baranda (*5*). Engagement of CD4 by gp120 induced filamin-dependent activation of the small guanosine triphosphatase
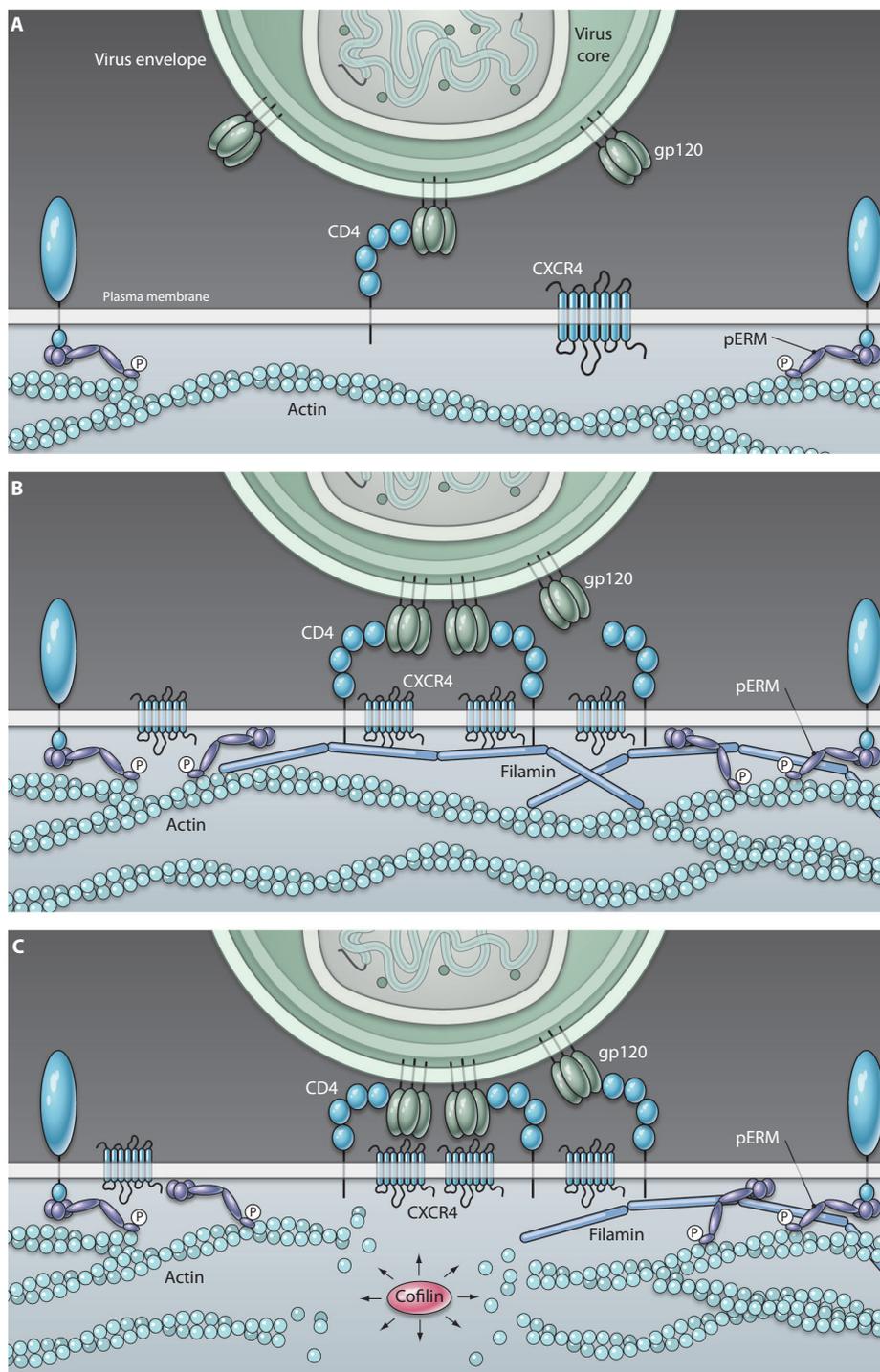
Fig. 1. Diagram representing the highly simplified sequence of events involved in the binding of HIV to lymphocyte membrane. (**A**) The initial state of the lymphocyte at the time of initial engagement of HIV gp120 with CD4. gp120 is randomly distributed on the virus, and CD4 and CXCR4 are not yet enriched at the contact point. A loose weave of cortical actin is held at the membrane by sparsely phosphorylated ERM proteins (pERM). (**B**) Binding of gp120 to CD4 signals reorganization of the cytoskeleton. The number of actin filaments is increased due to local actin polymerization, and

these filaments are attached to the plasma membrane by enrichment of phosphorylated ERM proteins. gp120 becomes enriched at the interface by mutual co-capping with CD4. CD4 and CXCR4 are tethered to filamin and are also locally enriched. (**C**) As the interaction progresses, gp120 undergoes conformational changes to expose its binding site for CXCR4. The binding of gp120 to CXCR4 modifies its signaling cascade. Cofilin is activated by dephosphorylation, which contributes to further reorganization, including the severing of actin. Viral fusion is also occurring at this stage.

(GTPase) RhoA. RhoA induced the phosphorylation, and thus inactivation, of cofilin, thereby promoting the polymerization of actin, because cofilin is an actin-severing protein. (Caution should be noted in this model, however, because cofilin-mediated severing can also have the opposite effect of promoting actin polymerization by creating an increased number of short actin fragments that act as nucleation sites for new filaments).

Another family of cytoskeletal proteins, ezrin/radixin/moesin (ERM), now seems to play a key role in the formation of the gp120-induced cap (*6*). ERM proteins attach cortical actin filaments to the plasma membrane, and they are abundant in eukaryotic cells (*17–19*). ERM proteins are autoinhibited in the cytosol and become activated at the membrane, in part, by phosphorylation at a C-terminal threonine residue (designated pERM). Barrero-Villar *et al.* showed that HIV gp120 induced phosphorylation-dependent activation of ERM proteins in lymphocytes and that one of them (moesin) was recruited into the actin-rich cap (*6*). The study concludes that ERM proteins are facilitators of early events in the infection of T lymphocytes by HIV (these being virion binding to or fusion with the plasma membrane). The experimental evidence for this conclusion is robust because the study used multiple functional readouts of HIV infection and two approaches to perturb the function of ERM proteins: transfection and knockdown. ERM proteins participate in many normal cellular processes that involve actin and membrane, such as mitosis, phagocytosis, and peripheral processes such as the formation of microvilli, filopodia, and neurites (*17–20*). A plausible role for active ERM molecules in this context is in facilitating the assembly of actin at the plasma membrane (*20*) and retaining its tight linkage to the membrane, which facilitates the diffusion-capture process described above (Fig. 1). It is important to note that ERM proteins have not yet been confirmed to play such a role at the virological synapse. Moreover, ERM proteins

are largely excluded from the immunological synapse by mechanisms that include induced dephosphorylation of ERM (21). Thus, if phosphorylated ERM accumulates at the virological synapse, it seems to represent a dramatic deviation from the paradigm that the virological synapse closely mimics the immunological synapse.

A provocative issue raised by Barrero-Villar (6) is the kinetic and spatial association of ERM phosphorylation with cap formation and enhanced viral infection. If phosphorylation of ERM proteins proves to be not just associated with cap formation but also a causative factor, it becomes a high priority for further study. A potential involvement of RhoA is of interest because gp120 induced the activation of RhoA (5), which induces the phosphorylation of ERM proteins in many cell types, including lymphocytes (22, 23). It is increasingly clear that multiple kinases are involved in the phosphorylation of ERM proteins (24). Of note, the kinase that is responsible for the majority of ERM phosphorylation at the lymphocyte plasma membrane is specific to hematopoietic cells (24). It is possible, therefore, that inhibitors of ERM kinases could contribute to combinatorial approaches in HIV therapy, because kinases are "druggable," and despite worries about toxicity or off-target effects, treatment of other disease states with kinase inhibitors is exceeding expectations.

Paradoxically, gp120-induced assembly of an actin-rich cap at the viral entry site also creates potential problems for the virus. That cortical actin is a barrier to events such as secretory processes at the plasma membrane was observed long ago in a study of mast cell degranulation (25). Since then, it has become clear that cortical actin modulates a wide variety of processes at the plasma membrane (and is in turn modulated by them). This is also true for HIV, for which early postentry events are inhibited by polymerized cortical actin (4, 26). This inhibition is overcome by a process of depolymerization of actin that involves cofilin induced by engagement of CXCR4 by gp120 (4). The mechanism by which polymerized actin inhibits infection is incompletely defined. It is not simply due to steric hindrance of the movement of the viral core, because the spacing of the actin meshwork is larger than the size of the virus core. Wu and co-workers suggest possible retention of the preintegration complex on the actin cytoskeleton (4). Although it has not yet been shown that ERM proteins contribute to the inhibitory

roles of filamentous actin, we consider it plausible because active ERM proteins are usually cleared from the inner aspect of signaling interfaces such as the immunological synapse (21). Moreover, moesin inhibits postentry retroviral transport by destabilizing microtubules (27). Thus, actin and ERM proteins play dual roles in HIV infection: facilitating and inhibiting. There is insufficient data to confidently reconcile these observations; however, we anticipate that understanding of the temporal and spatial complexity is necessary to resolve the paradoxes. Temporal complexity has already been observed. For example, phosphorylation of cofilin is proposed to contribute to early polymerization of actin (5), whereas dephosphorylation of cofilin contributes to the actin remodeling that is necessary soon thereafter (4). Spatial complexity of the actin caps, and especially the virological synapse, are to be expected. Thus, viral binding may occur at actin- and ERM-rich subregions of the membrane, but penetration of the cell may occur at regions that are relatively devoid of actin and ERM proteins.

References and Notes

1. M. H. Naghavi, S. P. Goff, Retroviral proteins that interact with the host cell cytoskeleton. *Curr. Opin. Immunol.* **19**, 402–407 (2007).
2. O. T. Fackler, H. G. Krausslich, Interactions of human retroviruses with the host cell cytoskeleton. *Curr. Opin. Microbiol.* **9**, 409–415 (2006).
3. S. Iyengar, J. E. Hildreth, D. H. Schwartz, Actin-dependent receptor colocalization required for human immunodeficiency virus entry into host cells. *J. Virol.* **72**, 5251–5255 (1998).
4. A. Yoder, D. Yu, L. Dong, S. R. Iyer, X. Xu, J. Kelly, J. Liu, W. Wang, P. J. Vorster, L. Agulto, D. A. Stephany, J. N. Cooper, J. W. Marsh, Y. Wu, HIV envelope-CXCR4 signaling activates cofilin to overcome cortical actin restriction in resting CD4 T cells. *Cell* **134**, 782–792 (2008).
5. S. Jimenez-Baranda, C. Gomez-Mouton, A. Rojas, L. Martinez-Prats, E. Mira, R. Ana Lacalle, A. Valencia, D. S. Dimitrov, A. Viola, R. Delgado, A. C. Martinez, S. Manes, Filamin-A regulates actin-dependent clustering of HIV receptors. *Nat. Cell Biol.* **9**, 838–846 (2007).
6. M. Barrero-Villar, J. R. Cabrero, M. Gordon-Alonso, J. Barroso-Gonzalez, S. Alvarez-Losada, M. A. Munoz-Fernandez, F. Sanchez-Madrid, A. Valenzuela-Fernandez, Moesin is required for HIV-1 induced CD4-CXCR4 interaction, F-actin redistribution, membrane fusion and viral infection in lymphocytes. *J. Cell Sci.* **122**, 103–113 (2009).
7. Q. Sattentau, Avoiding the void: Cell-to-cell spread of human viruses. *Natl. Rev.* **6**, 815–826 (2008).
8. D. McDonald, L. Wu, S. M. Bohks, V. N. Kewal-Ramani, D. Unutmaz, T. J. Hope, Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science* **300**, 1295–1297 (2003).
9. C. Haller, O. T. Fackler, HIV-1 at the immunological and T-lymphocytic virological synapse. *Biol. Chem.* **389**, 1253–1260 (2008).
10. C. Jolly, Q. J. Sattentau, Retroviral spread by induction of virological synapses. *Traffic* **5**, 643–650 (2004).
11. C. Jolly, K. Kashefi, M. Hollinshead, Q. J. Satten-

tau, HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. *J. Exp. Med.* **199**, 283–293 (2004).
12. M. Marsh, A. Helenius, Virus entry: Open sesame. *Cell* **124**, 729–740 (2006).
13. R. Sougrat, A. Bartesaghi, J. D. Lifson, A. E. Bennett, J. W. Bess, D. J. Zabransky, S. Subramaniam, Electron tomography of the contact between T cells and SIV/HIV-1: Implications for viral entry. *PLoS Pathog.* **3**, e63 (2007).
14. A. L. DeMond, K. D. Mossman, T. Starr, M. L. Dustin, J. T. Groves, T cell receptor microcluster transport through molecular mazes reveals mechanism of translocation. *Biophys. J.* **94**, 3286–3292 (2008).
15. J. Condeelis, How is actin polymerization nucleated in vivo? *Trends Cell Biol.* **11**, 288–293 (2001).
16. G. T. Charras, C. K. Hu, M. Coughlin, T. J. Mitchison, Reassembly of contractile actin cortex in cell blebs. *J. Cell Biol.* **175**, 477–490 (2006).
17. A. Neisch, R. G. Fehon, FERMing up the plasma membrane. *Dev. Cell* **14**, 154–156 (2008).
18. A. Ivetic, A. J. Ridley, Ezrin/radixin/moesin proteins and Rho GTPase signalling in leucocytes. *Immunology* **112**, 165–176 (2004).
19. A. Bretscher, K. Edwards, R. G. Fehon, ERM proteins and merlin: Integrators at the cell cortex. *Nat. Rev. Mol. Cell Biol.* **3**, 586–599 (2002).
20. H. Defacque, M. Egeberg, A. Habermann, M. Diakonova, C. Roy, P. Mangeat, W. Voelter, G. Marriott, J. Pfannstiel, H. Faulstich, G. Griffiths, Involvement of ezrin/moesin in de novo actin assembly on phagosomal membranes. *EMBO J.* **19**, 199–212 (2000).
21. J. Delon, K. Kaibuchi, R. N. Germain, Exclusion of CD43 from the immunological synapse is mediated by phosphorylation-regulated relocation of the cytoskeletal adaptor moesin. *Immunity* **15**, 691–701 (2001).
22. S. Faure, L. I. Salazar-Fontana, M. Semichon, V. L. Tybulewicz, G. Bismuth, A. Trautmann, R. N. Germain, J. Delon, ERM proteins regulate cytoskeleton relaxation promoting T cell-APC conjugation. *Nat. Immunol.* **5**, 272–279 (2004).
23. R. Nijhara, P. B. van Hennik, M. L. Gignac, M. J. Kruhlak, P. L. Hordijk, J. Delon, S. Shaw, Rac1 mediates collapse of microvilli on chemokine-activated T lymphocytes. *J. Immunol.* **173**, 4985–4993 (2004).
24. N. V. Belkina, Y. Liu, J. J. Hao, H. Karasuyama, S. Shaw, LOK is a major ERM kinase in resting lymphocytes and regulates cytoskeletal rearrangement through ERM phosphorylation. *Proc. Natl. Acad. Sci. USA* **106**, 4707–4712 (2009).
25. J. R. Pfeiffer, J. C. Seagrave, B. H. Davis, G. G. Deanin, J. M. Oliver, Membrane and cytoskeletal changes associated with IgE-mediated serotonin release from rat basophilic leukemia cells. *J. Cell Biol.* **101**, 2145–2155 (1985).
26. E. M. Campbell, R. Nunez, T. J. Hope, Disruption of the actin cytoskeleton can complement the ability of Nef to enhance human immunodeficiency virus type 1 infectivity. *J. Virol.* **78**, 5745–5755 (2004).
27. M. H. Naghavi, S. Valente, T. Hatziioannou, K. de Los Santos, Y. Wen, C. Mott, G. G. Gundersen, S. P. Goff, Moesin regulates stable microtubule formation and limits retroviral infection in cultured cells. *EMBO J.* **26**, 41–52 (2007).
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