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# To exploit the exploitation of actin by HIV?

'The emergence of an anti-HIV therapeutic agent that targets actin-dependent processes in the coming years will not be surprising.'

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The AIDS epidemic has been a formidable global health challenge ever since its emergence, and the identification of human immunodeficiency virus as its causative agent, more than twenty years ago. Although highly active antiretroviral therapy (HAART) has been effective in reducing the mortality and morbidity in recent years, adverse side effects of the chemotherapy, patient non-compliance and the development of viral resistance remain major problems [1]. Current HAART treatments for HIV infection include the use of nucleoside and non-nucleoside reverse transcriptase inhibitors, in combination with protease inhibitors. The gp41 fusion inhibitor, enfuvirtide, is a new addition to the list, being approved by the FDA in March of 2003. All of these inhibitors, together with the emerging integrase inhibitors, target essential, viral encoded proteins.

Potential alternative approaches might involve the disruption of important interactions between viral components and host cell proteins that are implicated in the HIV life cycle. Such notions will inevitably draw skepticism and might easily be dismissed due to concerns about the potential for poor selectivity and toxicity, although significant metabolic, CNS and hematologic side effects are not uncommon with HAART. Antagonists of CCR5 and CXCR4 HIV entry co-receptors are currently in clinical trials, thus, indicating that targeting select host cell proteins might be a viable strategy to control HIV infection. As HIV relies on the host cell machinery for infection, replication and transmission, basic research in HIV biology will continue to furnish novel molecular targets that will be indispensable in the search for new and more effective treatments for AIDS [2]. Our understanding of HIV biology is the only limitation to the opportunities and creativity of devising anti-HIV therapies.

## Taking advantage of the cell network

Upon infection, the functions of several cellular proteins succumb to the control of HIV, particularly by the viral accessory proteins Nef, Vpr, Vif and Vpu. Accumulating evidence suggests that HIV is relentless in its exploitation of actin to sustain its infectivity. The Nef protein is capable of binding to actin – an interaction that is dependent on the N-terminal myristoylation of Nef. This modification targets Nef to cellular membranes and is important for its signaling activity. Initiation of the Vav-Rac1/Cdc42 signaling cascade by Nef induces subsequent binding of PAK kinase to Nef. The activated kinase then regulates actin organization directly, through the activation of downstream JNK kinase [3]. Additionally, Rac1 and Cdc42 can activate WASP, which is an important signaling protein for controlling actin polymerization. The Vpr protein also increases F-actin polymerization and induces its subsequent reorganization, and increases the expression of the membrane–actin linking protein, ezrin [4]. This phenomenon correlates with the observed up-regulation of cell surface adhesion molecules, for example, cadherin and integrins. In particular, overexpression of integrin α5 leads to an up-regulation of the cell death suppressor bcl-2. These particular effects of Vpr contribute to virus persistence during infection, and represent one mechanism by which HIV resists the inhibitory effect of host cells through apoptosis.

Actin has also been identified as a co-factor in key processes of HIV infection. For example, following membrane fusion and viral core disassembly, the HIV reverse transcription complex rapidly associates with the host cell actin cytoskeleton. This interaction with an intact actin microfilament, which is mediated by the matrix protein, is a prerequisite for the efficient synthesis of proviral DNA [5]. It has also been demonstrated, *in vitro*, that the monomeric p66 subunit of the reverse transcriptase heterodimer and the pol precursor polyprotein bind to β-actin. Another important example is the Rev-dependent nuclear export pathway of unspliced RRE-containing viral



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RNA. This process requires the formation of a viral RNA-Rev nucleoprotein complex that consists of eukaryotic initiation factor 5A (eIF-5A), export receptor CRM-1/exportin-1 and RanGTP, at the C-terminal nuclear export signal region of Rev. Recent studies have shown that eIF-5A binds to nuclear actin, which is functionally responsible for both the transportation of the nucleoprotein complex through the nucleoplasm to the nuclear pore complex, and its translocation to the cytoplasm [6, 7]. Interestingly, after leaving the nucleus, RNA-Rev nucleoproteins form perinuclear clusters with β-actin and the synthesis of viral structural proteins at polysomes begins.

As actins are highly concentrated in the cell cortex below the plasma membrane and, together with ezrin, support cell surface-protruding cholesterol-rich microdomains (microvilli, rafts), it is not surprising that they are implicated in the HIV entry and virion budding/release processes. CD4, CCR5 and CXCR4 are preferentially clustered in these microdomains on human macrophages and T cells [8]. The colocalization of CD4 and CXCR5, induced by gp120, can be inhibited by the actin-depolymerizing agent, cytochalasin, which also reversibly inhibits the HIV envelope-mediated fusion process. However, the exact mechanism of the influence of such localization on viral entry and the role of cholesterol in this process are still subjects of active debate [9]. HIV also buds from membrane protusions on activated primary T cells and from monocytes. The release of HIV-1 virions from infected cells can be inhibited by a myosin light-chain kinase inhibitor with submicromolar potency, indicating that the actin–myosin motor complex is essential for the release process [10]. Actin, and actin binding proteins, such as ezrin, as well as elongation factor-1α (EF1α), are incorporated into HIV virions [11]. In the presence of viral RNA, EF1α binds to Gag protein *in vivo*, via both the matrix and the nucleocapsid domains. More importantly, the actin cross-linking function of EF1α is important for translation. Gag polyprotein, itself, binds to actin and Gag-expressing macrophages exhibit enhanced motility [12].

Thus, a coherent picture of the roles of this important class of cellular proteins in the HIV life cycle, and of the control of actin organization by HIV accessory proteins, is emerging. Other hitherto unknown and specific functions of actin and its binding proteins that are essential for HIV infection will be identified in the future, just as actin has been revealed as a cofactor of adenovirus protease, and the actin binding protein, profilin, has been shown to be a cofactor during the actin-dependent transcription of the RNA genome of respiratory syncytial virus. However, the wealth of information that has been generated in this area has not attracted much attention, and has certainly not been exploited in the drug discovery arena.

## Prospects for drug discovery

Actin is a major component of the cytoskeleton that plays crucial roles in cell shaping, motility, division, adhesion and intracellular transportation. Since its discovery in the 1940s, important and previously unknown cellular functions of actin continue to be unveiled. A diverse bonanza of actin binding proteins have also been well characterized, with respect to their structural and functional interactions with actin. However, this array of knowledge has yet to be used in disease intervention. This is in stark contrast to other classes of extensively studied cellular proteins, for example, the human G-protein coupled receptors that are considered viable drug targets.

Owing to the ubiquitous presence of actin and its diverse functions inside the cell, achieving selectivity in a particular pathway will be a daunting task in treating disorders that are caused by cell malfunctioning. In the anti-HIV area, several different approaches toward disrupting the manipulation of actin by the foreign pathogen can be envisaged. Specific interference of the binding of viral proteins to actin and to actin binding proteins, and inhibition of binding of viral proteins to the signaling molecules that are involved in pathways controlling actin organization (e.g. Nef-Van and Nef-PAK interactions), will constitute an unusual and general anti-viral strategy. Present indications of the potential for attaining selectivity are encouraging. For example, studies of the inhibition of the nuclear export of Gag mRNA by latrunculin B strongly suggest that the inhibition was not due to non-specific disruption of cellular structures. Viral proteins might also possess actin-binding motifs that are different from mammalian counterparts. Future research will need to define the nature of these interactions, determine the actual forms of actin involved (F-actin, G-actin or its short polymeric form) and establish precise contributions to the viral replication in certain areas.

New natural product molecular probes that have more potent effects and different modes of action on actin, and the X-ray structure of uncomplexed G-actin are now available for studies in this area. The development of HTS assays for the identification of inhibitors of processes that involve actin (e.g. those that inhibit actin signaling pathways) is underway. The studies and assays will help to further our understanding of the biochemical pathways involved and will detail the molecular interactions that involve actin and HIV viral proteins, and thus, contribute to the identification of new targets and their inhibitors. Research efforts from different perspectives will, collectively, form an early-phase drug discovery process. The emergence of an anti-HIV therapeutic agent that targets actin-dependent processes in the coming years will not be surprising.

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