

# Genes and screens: the search for novel chemotherapeutic targets

**Ann M Sheehy**

College of the Holy Cross,  
Department of Biology,  
Worcester, MA 01610, USA  
Tel.: +1 508 793 2255;  
Fax: +1 508 793 2696;  
asheehy@holycross.edu

**Evaluation of: Brass AL, Dykxhoorn DM, Benita Y et al.: Identification of host proteins required for HIV infection through a functional genomic screen. *Science* 319, 921–926 (2008).** HIV, like all viruses, must hijack critical cellular machinery in a bid to survive, replicate and spread. The compact genome of HIV is in stark contrast to the sophistication of the retroviral life cycle, suggesting the essential involvement of a myriad of host factors at distinct stages of the viral life cycle, from entry to integration to budding. Identification of these host components is critical to assemble a full spectrum of the potential points of therapeutic intervention and, in the last two decades, meticulous research by a long list of talented individuals has yielded the identity of dozens of these important factors. Brass *et al.* have recently added an incredible 237 unique cellular proteins, which they term HIV-dependency factors, to the list. Characterizing and understanding the role each of these cellular proteins plays in the interplay between host and virus promises to reveal previously unidentified cellular pathways important to viral pathogenesis. The obvious hope is that one or several of these newly identified players may ultimately be manipulated in a therapeutically meaningful context.

The complexity and sophistication of the retroviral life cycle of HIV is underscored by the virus's genetic simplicity; with only nine genes, encoding 15 proteins, to call its own, HIV has, of necessity, evolved to utilize and exploit a broad array of complex intracellular processes. In the past two decades, as the discipline of molecular biology has evolved, a number of host factors that are usurped by HIV have been identified [1]. However, as the recent report by Brass *et al.* [2] reveals, there is a significant lack of specific understanding of the network of host proteins that are exploited by HIV during its invasion of the human cell. This assembled group of researchers combined the strengths of basic virology, genetics and biotechnology to generate a multistep assay designed to identify host proteins that significantly contribute to the ability of HIV to infect a cell.

The transfection of a siRNA library composed of four siRNAs per gene into the Hela-derived cell line, TZM-bl, allowed a genome-wide assessment of host factors important to the initial and early stages of HIV pathogenesis. Subsequent qualitative analysis of virus produced in this context enabled a follow-up examination of the influence of host proteins on the later stages of HIV virion production. Using this type of two-tiered approach, Brass *et al.* were able to describe hundreds of host proteins that participate in viral pathogenesis. The validity and reliability of the approach was verified with the identification of host factors known to participate in HIV pathogenesis, CD4, CXCR4,

Rab9p40 and Rel-A, to name but a few [3–6]. In addition, it was quite clear from some of the extensive validation performed on the data set that a significant percentage of the genes (over 30%) exhibited elevated expression in immune cells. This is an important aspect to consider as one of the obvious caveats with the experimental design is the choice of the TZM-bl cell line; although this cell line is easily manipulated and amenable to experimental analysis, it is not representative of a natural target for HIV infection. Therefore, it becomes critically important to confirm the relevance of the achieved data set.

In addition, unlike a pure differential expression approach, such as subtractive hybridization, this group used a powerful functional screen. Thus, the myriad of novel factors identified have already been shown to impact HIV pathogenesis in a tissue culture model. Therefore, the field can initiate the analysis of the proteins with reasonable confidence. Several of the genes identified were further scrutinized by Brass *et al.*, providing not only some preliminary insights into the novel HIV-dependency factors (HDFs) but also initial glances at the several cellular pathways that have not been previously highlighted as essential to viral infection. Specifically, an interesting emerging hypothesis suggests that the retrograde vesicular transport pathways are important for HIV entry; this importance was observed in both the original TZM-bl cells and, perhaps more critically, in Jurkat cells, a T-cell

**Keywords:** antiretroviral therapy, forward genetics, genome-wide screening, host proteins, host–virus protein interactions

future medicine part of fsg

line, representing a somewhat better approximation of an *in vivo* target of HIV. Finally, Brass *et al.* put forth several interesting hypotheses centered on pathways that have not yet been described as playing defined roles in HIV pathogenesis; for instance, the process of autophagy, while hinted at as significant, remains virtually unexplored [7]. These somewhat provocative postulates serve as examples of the scientific trains of thought that may now begin to initiate directed experimentation. Now that such a cataloging has been reported there exist incredible opportunities for innovative projects.

#### Conclusion & future perspective

Without doubt there are essential host factors that this screen did not identify. As with every approach, experimental bias is an inevitable obstacle. For instance, the screen does easily distinguish between constitutive and HIV-inducible factors; factors that may be essential and unique to an R5-using virus may not have been identified; factors expressed exclusively in immune cells may not appear. In spite of these shortcomings, Brass *et al.* have generated, via a remarkable *tour de force*, a Who's Who cataloging of cellular factors important for HIV pathogenesis. Certainly, countless laboratories have already begun to examine whether their pet proteins have made the A-list. While prophylaxis research has had a difficult year, with the dismal failures of both the Phase IIb Merck vaccine and the Phase III Carraguard microbicide trial being

reported in the last 9 months, there is a current of excitement accompanying this report [8,9]. Unlike innovative antiretrovirals, many of which are ultimately doomed to fail due to the mutagenic capacity of the virus, HDFs represent a more difficult, perhaps insurmountable, obstacle for HIV escape mutants. Similar to considering the potential of exploiting the intracellular host defense proteins APOBEC3G, TRIM5 $\alpha$  and Tetherin for their intrinsic abilities to restrict HIV infection, the HDFs represent unique potential points of therapeutic intervention [10–12]. Only extensive investigation that must be performed at the bench will reveal which, if any, of these 237 candidates can be safely manipulated to therapeutic advantage.

As a final note of encouragement, the potential that the ensuing experimentation will reveal information regarding fundamental cellular processes should also not be overlooked. As intracellular parasites, viruses must intimately connect with basic cellular processes, and investigating the viral life cycle has often yielded cellular secrets as well. Ultimately, the legacy of Brass *et al.*, in addition to fueling the careers and enthusiasm of countless graduate students and postdoctoral fellows, should be one of collaboration. Merging the powerful forces of bioinformatics and empirical 'wet-bench' experimentation (and manual curation), Brass *et al.* have created a fresh starting point in the search for novel therapeutics that can be directed at one of the most dreaded viruses in documented history.

### Executive summary

#### Objectives of the study

- To identify novel cellular host proteins essential to HIV infection (HIV-dependency factors [HDFs]).
- Delineate previously unidentified cellular pathways critical to viral infection.
- Suggest a unique role for a subset of identified host factors.

#### Methods

- Two-part genome-wide functional screen examining the ability of siRNAs to impact HIV infection in a tissue culture system.
- A library of siRNAs was used to suppress expression of more than 21,000 individual genes; the effect of this gene silencing on viral infection was evaluated by immunofluorescence and a single-round infectivity assay.
- The initial identification of 273 genes was examined via bioinformatics technology and 237 were confirmed as uniquely identified proteins important to HIV infection.

#### Initial characterization

- Based on the identity of the reported HDFs, a number of previously unknown cellular pathways are exploited by HIV.
- The retrograde vesicular transport pathway appears to be essential to HIV entry.

#### Conclusion

- Although generating the list of candidates is a remarkable achievement, extensive investigation will be required to determine the feasibility of targeting any of the HDFs in an effective therapeutic strategy.

## Financial &amp; competing interests disclosure

*The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes*

*employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

*No writing assistance was utilized in the production of this manuscript.*

## Bibliography

- Goff SP: Host factors exploited by retroviruses. *Nat. Rev. Microbiol.* 5, 253–263 (2007).
- Brass AL, Dykxhoorn DM, Benita Y *et al.*: Identification of host proteins required for HIV infection through a functional genomic screen. *Science* 319, 921–926 (2008).
- Lifson JD, Engleman EG: Role of CD4 in normal immunity and HIV infection. *Immunol. Rev.* 109, 93–117 (1989).
- Dimitrov DS: How do viruses enter cells? The HIV coreceptors teach us a lesson of complexity. *Cell* 91, 721–730 (1997).
- Murray JL, Mavrakis M, McDonald NJ *et al.*: Rab9 GTPase is required for replication of human immunodeficiency virus type 1, filoviruses, and measles virus. *J. Virol.* 79, 11742–11751 (2005).
- Griffin GE, Leung K, Folks TM, Kunkel S, Nabel GJ: Activation of HIV gene expression during monocyte differentiation by induction of NF- $\kappa$ B. *Nature* 339, 70–73 (1989).
- Espert L, Codogno P, Biard-Piechaczyk M: What is the role of autophagy in HIV-1 infection? *Autophagy* 4, 273–275 (2008).
- Sekaly RP: The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? *J. Exp. Med.* 205, 7–12 (2008).
- Cohen J: AIDS research. Microbicide fails to protect against HIV. *Science* 319, 1026–1027 (2008).
- Sheehy AM, Gaddis NC, Choi JD, Malim MH: Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 418, 646–650 (2002).
- Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J: The cytoplasmic body component TRIM5 $\alpha$  restricts HIV-1 infection in Old World monkeys. *Nature* 427, 848–853 (2004).
- Neil SJ, Zang T, Bieniasz PD: Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451, 425–430 (2008).

## Affiliation

- Ann M Sheehy  
College of the Holy Cross, Department of Biology,  
Worcester, MA 01610, USA  
Tél.: +1 508 793 2255;  
Fax: +1 508 793 2696;  
asheehy@holycross.edu