Minireview

# HIV: master of the host cell

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## Abstract

The human immunodeficiency virus has evolved various mechanisms to exploit its host cells, including the interruption and augmentation of signal transduction pathways. Recently, two DNA microarray studies have illustrated a remarkably broad-based perturbation in host transcriptional responses, which is in part mediated by the HIV-encoded Nef protein. HIV therefore seems to function as a 'master regulator' of cellular gene expression.

The human immunodeficiency virus (HIV) infects $CD4^+$ T lymphocytes and macrophages, eventually inducing the depletion of $CD4^+$ T cells, which is the defining feature of the acquired immune deficiency syndrome (AIDS). It is not clear precisely how the virus exploits the host cell to maximize viral particle production, but evidence is accumulating that HIV activates the cellular transcription machinery to achieve this aim. While biochemical approaches have been extensively employed to study the intracellular response to HIV infection, the advent of lymphocyte microarrays has provided a powerful new tool to help illuminate the extensive effects of HIV on host-cell transcriptional responses.

Biochemical studies have demonstrated that HIV is capable of modulating a variety of signal transduction pathways in the host cell at multiple stages in the infection process, beginning at entry when it engages two transmembrane receptors, CD4 plus either of the chemokine receptors CCR5 or CXCR4, thereby activating intracellular protein tyrosine kinases [1]. Indication that HIV gene products influence signaling processes in host cells also comes from analyses of transgenic mice that express portions of the HIV genome and display a variety of abnormalities, ranging from altered T-cell maturation [2] to the development of a systemic disease similar to AIDS [3]. Because the long terminal repeats (LTRs) of HIV contain consensus recognition motifs for the NF-κB and NFAT families of transcriptional transactivators, it has been speculated that HIV may have evolved mechanisms to potentiate cellular activation pathways, thereby augmenting expression of its own genome. Until now, however, there has been limited understanding of how HIV exerts control over specific transactivation responses of the host cell.

Because HIV employs host factors that are vital for its replication cycle, the virus may have evolved means of modulating their expression levels during infection, so as to favor its own replication. One crucial host factor is the well-characterized transcription-elongation factor complex pTEFb, which is recruited to the nascent HIV transcript by the RNA-binding Tat protein encoded by the virus [4]. This complex contains the cyclin-dependent kinase CDK9 and cyclin T1, and it phosphorylates the carboxy-terminal-domain repeats of RNA polymerase II, activating the polymerase and thus allowing processive transcription of the HIV genome. Other host proteins are required to facilitate transport of unspliced viral RNA from the nucleus to the cytoplasm and to enhance viral assembly and release [5,6]. The identity of most of the host proteins involved in facilitating HIV replication has yet to be determined, however. Recent studies suggest that HIV infection can influence the expression of many host genes, and some of these may indeed have critical roles in the HIV replication cycle.

## Nef as a modulator of host-cell signal transduction

Among the various HIV gene products implicated in modulation of cell signaling, Nef appears to be the most potent. The *nef* gene, expressed rapidly and abundantly following infection, is a major virulence factor both *in vitro* and *in vivo*. Rhesus macaques infected with simian immunodeficiency virus (SIV), a close relative of HIV, rarely progress to disease if the viral *nef* gene is deleted [7]. It has also been shown in infected macaques that there is very strong selective pressure for SIVs with *nef* open reading frames [7]. In humans, members of a cohort of individuals infected with a *nef*-deleted form of HIV have remained disease-free for many years [8]. In primary T-cell cultures, HIVs with intact *nef* genes replicate much better than *nef*-defective viruses [9]. While the precise function of the Nef protein has remained elusive, the presence of an amino-terminal myristoyl linkage and a proline-rich SH3-binding domain suggest that it may interact with host proteins at the plasma membrane. Recent work has confirmed that these two regions of Nef are required for its association with lipid rafts, cholesterol-rich membrane microdomains that concentrate potent signaling mediators [10]. One functional consequence of Nef expression in T cells, as elucidated from *in vitro* studies, may be to enhance the levels of secreted interleukin-2 (IL-2, a growth factor) during activation [10]. Nef has also been shown to associate with the ζ-chain of the T-cell-receptor complex (TCR-ζ) and concomitantly to induce expression of Fas ligand, one of the mediators of apoptosis in differentiated cells, an outcome that may account for the high levels of apoptosis associated with HIV infection [11]. In this and other studies, Nef was also found to complex with a serine/threonine protein kinase, which in some cases has been identified as belonging to the PAK (p21-activated kinase) family.

## Gene targets of Nef in T cells

In their recent *Immunity* paper, Simmons *et al.* [12] describe results of an extensive microarray survey of expression levels of Nef-responsive loci. As a model system, they have developed a clone of the T-cell-leukemia cell line Jurkat that expresses Nef in a tetracycline-inducible manner, thus minimizing the cytopathic effects of Nef in long-term cultures. Using arrays containing 3,528 genes, Simmons *et al.* observed that a panoply of loci were up-regulated at multiple time points after Nef induction (Figure 1a). Most remarkably, they observed significant induction of mRNAs encoding many transcription factors that positively regulate the viral LTR, including NFATc, NF-κB p52 and p100, IRF-1 and IRF-2, c-Fos, and Jun-D. The cytokines transforming growth factor β (TGF-β) and IL-4, which may function in an autocrine and/or paracrine manner to promote LTR transactivation, were also elicited, along with MIP-1α and MIP-1β, chemokines previously shown to be up-regulated by Nef expression in macrophages [13]. Because these soluble factors are likely to influence host gene responses, it will be interesting in future studies to distinguish transactivation cascades that are autonomous from those requiring new protein synthesis. Host cofactors implicated in potentiating other stages of the HIV life cycle, from the processing of viral transcripts to virion budding, were also elicited in response to Nef expression. It was also confirmed that the Tat-cofactor CDK9 was elevated in Nef-expressing cells. That these findings point to a role for Nef in enhancing some of the earliest stages of the viral life cycle is particularly intriguing in the context of a recent study showing that *nef* transcripts are produced prior to integration of the virus into the genome of resting T cells, resulting in accumulation of Nef protein that primes viral synthesis upon T-cell activation [14].

In view of previous work implicating Nef in signaling at the plasma membrane, the ability of Nef to influence expression of many loci suggested that it might function as an upstream regulator of multiple divergent cascades. To explore this issue, Simmons *et al.* [12] compared Nef-responsive genes with those modulated when the T-cell line was activated with antibodies against the T-cell antigen-receptor (TCR) complex. Surprisingly, the spectrum of genes exhibited 97% overlap, indicating that a major function of Nef may be to trigger the conventional T-cell activation program. When Nef was induced during antibody stimulation, the same loci were activated even more potently, with the exception of targets unique to either inducing factor. Among the targets triggered only by Nef are several genes that may aid viral progression, including those encoding the transcription-elongation factor TAT-SF1, the transcription factor IRF-2, and the small nuclear riboprotein U1 SNRNP A. In contrast, stimulation with anti-TCR antibody but not with Nef induced two factors, the cytokine IL-16 and the transcription factor YY1, that are thought to negatively regulate viral transcription. How a single viral gene can achieve such remarkable specificity will need to be addressed in future studies. But, as indicated in supplemental data accompanying the Simmons *et al.* paper [12], Nef expression also results in down-modulation of numerous positive effectors, including the kinases PKC-ε and ZAP-70, the phospholipase PLC-γ2, and 40S ribosomal protein.

One of the advantages of the *in vitro* system of Simmons *et al.* [12] is that it is highly amenable to manipulation. For example, numerous Jurkat mutants that lack the expression of proteins involved in T-cell activation have been generated. Building on previous studies, Simmons *et al.* [12] inducibly expressed Nef in two such lines, one that lacked TCR-ζ and another deficient in ZAP-70, a tyrosine kinase recruited to the TCR-ζ chain upon activation. Expression profiling revealed that both proteins are required for the full-spectrum Nef response; in each of the mutant lines approximately half of the gene targets were not induced. A similar magnitude of inhibition was achieved in wild-type Jurkat cells in which Nef was expressed in the presence of the drug

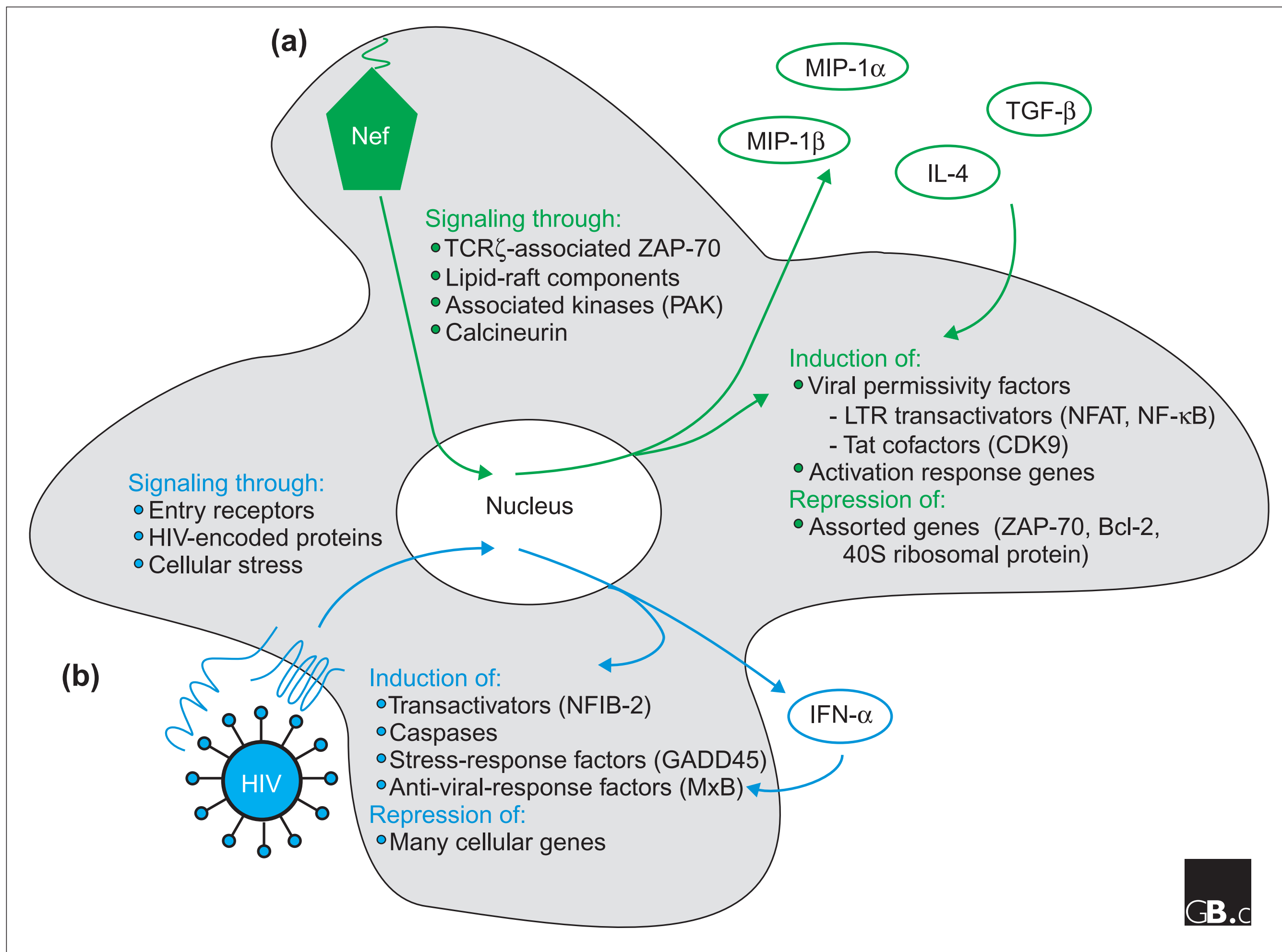


**Figure 1**
Modulation of the gene responses of host T cells by HIV. **(a)** Expression of Nef in T cells results in the induction of numerous viral permissivity factors as well as genes associated with T-cell activation. The induction of secreted factors, such as IL-4 and TGF-β, may generate secondary signals that favor viral replication, while the up-regulation of chemokines such as MIP-1α and MIP-1β may attract cells to the site of infection, perhaps aiding transmission of the virus. Although the precise mechanisms through which Nef modulates signaling have not been elucidated, it has been implicated in exerting controls on a number of membrane-proximal pathways. **(b)** Infection of T cells with intact HIV induces gene responses that are more complex and include the up-regulation and suppression of functionally diverse host genes. As infection proceeds, host-cell genes are increasingly suppressed, and there is a shift toward the induction of factors associated with a cytopathic response, including those regulating apoptosis and responses to genotoxic stress. The anti-viral gene IFN-α is induced relatively early in infection, and may modulate a variety of response cascades. HIV may elicit these transcriptional changes through a wide variety of mechanisms, either directly (though viral factors such as Nef, Env, and Tat), or indirectly, by inducing cellular stress during the acute phase of infection. Other factors included in the figure are discussed elsewhere in the text.

cyclosporin A, which blocks the more downstream NFAT effector calcineurin. Intriguingly, the genes inhibited by cyclosporin A only partially overlapped with those inhibited in the mutant Jurkat lines. Caveats that must be considered when interpreting these results, however, are that the mutant lines may have undergone adaptive changes and the levels of Nef protein in different Jurkat lines may not be identical. Different levels of *nef* expression, and different *nef* alleles, may elicit dramatically different outcomes, as suggested by studies using both *in vitro* [10] and *in vivo* [2,3] models.

## Complex effects of HIV on gene responses

Whereas the work of Simmons *et al.* [12] paints a picture of Nef as a 'master switch' of cellular activation, a parallel study by Corbeil *et al.* [15] has produced more complex results. This group infected the CEM T-cell line for various lengths of time (eight time points, from 0 to 72 hours) with high levels of replication-competent HIV. After analyzing microarrays containing 6,800 loci, they found that productive infection was associated with complex patterns of up- and down-regulated genes (Figure 1b). On average, more

genes were induced early in infection (up to 24 hours) than later, when the cytotoxic effects of the virus resulted in dramatic repression of approximately one third of expressed host genes (33% of cells were apoptotic 72 hours after infection). Among the genes augmented at consecutive early time points were *interferon-α* (*IFN-α*) and its target *MxB*, which serve anti-viral functions. *NFIB-2*, which encodes a factor involved in the transcription of both viral and cellular genes, was up-regulated, as confirmed by real-time PCR. Perusal of the supplementary data [15] reveals that a multitude of host genes are strongly up-regulated at individual time points, although the relevance and reproducibility of these findings remain uncertain until confirmed. Other activated loci appeared to reflect a state of genotoxic stress, including the gene *Gadd45*, which is induced by DNA damage. Both the mRNA encoding the proapoptotic mediator Bax and the protein itself were up-regulated in infected cells, as were numerous caspases. It is worth noting that an earlier survey of HIV-1-induced genes by differential display revealed a variety of up- and down-regulated host genes, including some responses consistent with a cytopathic outcome [16]. Moreover, in the supplemental data of Simmons *et al.* [12], it is apparent that Nef down-regulated the anti-apoptotic *Bcl-2* gene while up-regulating the proapoptotic mediator *BAD*.

Utilization of replication-competent HIV to study effects on host genes has advantages as well as disadvantages. The most obvious merit is that the system is likely to reflect *in vivo* outcomes better (although certainly the *in vivo* cellular microenvironment will exert a profound influence on gene responses). The data obtained by this method are more difficult to interpret and dissociate from stress responses associated with apoptosis, however. Additional studies are now needed to dissect out the contributions of individual viral proteins by employing a variety of mutated HIV strains. One obvious experiment will be to examine differences among viruses lacking the *nef* gene. Given that the envelope glycoprotein of HIV is cytotoxic, it will also be interesting to compare strains lacking expression of this product. Replication-defective HIV coated with envelopes specific for CCR5 or CXCR4 can be compared to replication-defective HIV enveloped with vesicular stomatitis virus (VSV) glycoprotein to address the provocative question of whether viral entry by means of different receptors may itself influence expression of various genes.

HIV exerts profound effects on the transcriptional responses of host T cells. Whereas viral products such as Nef may have adapted to activate loci that favor viral progression, the acute phase of infection induces a cellular stress program associated with a generalized dampening of host-cell transcription and a shift towards the induction of the proapoptotic machinery. The effects on host gene responses of defined *nef* mutations, changes in *nef* expression, and substitution of *nef* alleles can be powerfully addressed in future microarray experiments. Moreover, it will be crucial to examine the effects of clinical HIV isolates in primary T cells, as well as macrophages, in which the virus also perturbs activation cascades [13,17]. As enlarged panels of gene arrays become available, more comprehensive genome scanning will be possible. These studies might ultimately be extended to examine the effects of human genetic polymorphisms on HIV gene responses. Together, these approaches will prove crucial in developing new therapies that seek to suppress and eliminate HIV.

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