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Insights into Cellular Factors That Regulate HIV-1 Replication in Human Cells[†]

Andrew M. L. Lever[‡] and Kuan-Teh Jeang*[,§]

[‡]*Addenbrooke's Hospital, University of Cambridge, Cambridge CB2 0QQ, U.K., and*
[§]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-0460, United States*

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ABSTRACT: Retroviruses integrate into the host cell's chromosome. Accordingly, many aspects of the life cycle of retroviruses like HIV-1 are intimately linked to the functions of cellular proteins and RNAs. In this review, we discuss in brief recent genomewide screens for the identification of cellular proteins that assist HIV-1 replication in human cells. We also review findings for other cellular moieties that help or restrict the viral life cycle.

Within the past 10 years, with the assistance of the new screening technologies for analyzing cellular gene expression, it has become apparent that the interaction between HIV[1] and its host cell is a vastly more sophisticated and intimate duet than had previously been surmised. Here we review the results of such approaches pertaining to HIV-1. First, we summarize the recent genomewide screens that have begun to reveal how extensively the virus interacts with and utilizes its host cell. We then survey the virus life cycle, highlighting the identified cellular chaperones of the virus and, where known, their role in viral replication. Finally, we describe in some detail the functional attributes of certain of the best characterized innate defense molecules of the cell, the so-called "restriction factors".

GENOMEWIDE EVIDENCE OF POSITIVE AND RESTRICTIVE FACTORS REGULATING HIV-1 REPLICATION

As in a number of other viral systems, such as influenza (*1*)and hepatitis C (*2*), the replication cycle of HIV-1 and its dependence on cellular factors have been studied by large-scale knockdown experiments using interfering RNA (siRNA and shRNA). These effectively identify factors necessary for replication and do not reveal cellular inhibitors of replication unless they are specifically designed to do so. Four such genomewide studies have been published. At first glance, the two surprising features of these screens are the fact that so many cellular proteins are apparently involved in HIV-1 replication and how little overlap there is between the factors identified in different screens. Some of the latter disparity may be ascribed to methodological differences in the cell lines, reporter genes, assay times, and methods, as well as the nature of the infectious construct analyzed.

In the first of these screens, which assessed the whole viral life cycle, Brass et al. (*3*) transfected pools of siRNAs targeting more than 20000 host proteins into a HeLa-derived cell line expressing both the cellular receptors for HIV-1 and an long-terminal repeat-driven reporter construct. Viral infection with the IIIB strain of HIV-1 followed 72 h later. Virus production was analyzed 48 h later by direct fluorescent staining of the cells for capsid protein and by measuring the infectivity of the supernatant from the cells; 273 cellular factors were identified as having an effect on HIV-1, and 36 of those had previously been shown to play a role.

The second siRNA screen (*4*) targeted a similar number of proteins, focusing more on earlier stages of infection. In this case, the 293T cells were infected at 48 h with a pseudotyped HIV carrying the firefly luciferase gene within the viral RNA. Luciferase expression was a surrogate marker for successful infection, integration, and gene expression. From 295 initial hits, a combination of quantitative polymerase chain reaction (PCR) and detailed in silico analyses reduced this to 40 proteins with possible roles in uncoating and reverse transcription and 15 more affecting integration or nuclear import.

The third screen (*5*), again using siRNA, targeted 20000 gene products, and like the first of these screens, wild-type virus was used to infect a HeLa cell line expressing a reporter gene driven by an HIV-1 long-terminal repeat (LTR). In this case, wild-type virus was used to infect the cells 24 h after transfection, and the reporter gene was assayed 24 and 72 h later. siRNAs that affected cell viability were filtered out and potential targets reanalyzed with a second siRNA pool. The final list of likely genes was determined by further in silico screening, limiting it to those genes expressed in activated T cells or macrophages; 311 targets resulted from this combined screen, and 44 of those had previously been implicated in HIV replication.

In total, these screens identified 842 genes as capable of assisting HIV replication. This amounts to around 3.3% of the known human protein coding genes (*6*). The overlap, however, was small, with 34 genes identified in two or more screens and only three, MED6, MED7, and RELA, common to all three (Figure 1). Another surprise was the absence of some genes well-established as being pivotal for HIV replication. LEDGF/p75, an essential cofactor for proviral integration (*7*), did not appear at

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*To whom correspondence should be addressed: Building 4, room 306, 9000 Rockville Pike, Bethesda, MD 20892. Telephone: (301) 496-6680. Fax: (301) 480-3686. E-mail: kjeang@nih.gov.

[1]Abbreviations: HIV, human immunodeficiency virus; siRNA, small interfering RNA; shRNA, small hairpin RNA.

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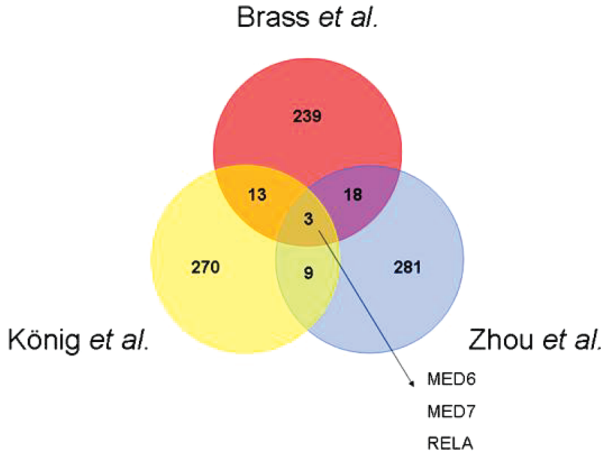

FIGURE 1: Diagram showing unique gene products and gene products common to two or all three siRNA screens (*3*−*5*).

all, and TSG101 (*8*) and CRM1 (*9*), which are known to be essential, appeared in only one screen.

False negatives are not too surprising because in a high-throughput screen there is no validation of successful knockdown of the gene product. In addition, the knockdown may be only partial, the target may be essential for cell viability, or there may be functional redundancy, or a combination of these.

In addition, complementarity of as few as seven nucleotides with an unrelated mRNA sequence may suffice for knockdown (*10*); hence, bystander interference with nontargeted genes, so-called off-target effects, may also muddy the waters.

The cell line used must also be taken into account. So, for example, DDX53, identified in the Brass screen as affecting HIV replication, is expressed only in testicular and some malignant tissues (*11*), making it an unlikely candidate HIV cofactor in vivo.

shRNA has also been used as a screen for cellular cofactors of HIV-1 (*12*). The fact that these are transcribed within the cell and processed to siRNA means that they have a more sustained duration of action. In a fourth genomewide screen, shRNAs that target 54509 human transcripts were expressed as an FIV lentivirus vector library pseudotyped with the VSV-G envelope and transduced into Jurkat cells. Antibiotic selection was used to pick out constitutively expressing cell clones; 9357 shRNA-expressing clones survived selection, and these clones were then infected with HIV. Any cells surviving after 4 weeks of HIV infection were assumed to be expressing a shRNA that knocked down the expression of a protein necessary for viral replication. The findings from this study suggested that only 18.2% of the cell's total transcriptome can be knocked down without affecting Jurkat cell viability in tissue culture, implying that the durable knockdown of 82% of cellular transcripts is incompatible with cell survival in culture. This screen identified 252 transcripts enriched in surviving cells and thus presumed to be required for HIV replication. Again, there was trivial overlap with the three siRNA screens, with only three genes in common between this and each of the latter two, although combining all four screens revealed 40 genes common to at least two of them. The critical methodological difference is that the shRNA study incorporated a "selection" process built into the "screening" while the three siRNA studies are purely screening assays (Figure 2).

A comprehensive meta-analysis of the overlap between the three siRNA screens and other large-scale screens has been published. It concludes that potentially 2410 protein coding genes (9.5% of all human genes) may be involved in the replication of HIV. The analyses also showed that variations between replicates, in time points, and in filtering thresholds all influence the readouts from the various siRNA screens. This article concludes that these type of approaches are better at identifying essential common cellular pathways rather than at pinpointing specific proteins within these pathways.

More recently, a transcriptome analysis of unseparated cells from lymph nodes of HIV-infected individuals was published (*13*), seeking mRNAs that exhibited a correlation with viral load. The vast majority (~95% of 592 transcripts) showed a negative correlation. A significant number of those with identifiable function were involved in downregulating immune activation or cellular transcription, reflecting the enhancing effect on virus replication associated with activated immune cells. Surprisingly, of the 5% (32 genes and two unknown transcripts) that were positively associated with viral load, many were components of the innate and adaptive immune response, with a particular emphasis on the interferon pathway (which might be expected to inhibit HIV replication). Novel candidate restriction factors were identified. Perhaps because of the nature of the tissue of derivation being a mixed population of primary cells, there were only five genes identified that overlapped with the previous screens: GOLGA9P, MED31, TCFL5 (Cha), ACADSB, and CYCS

KNOWN CELLULAR FACTORS INVOLVED IN THE HIV-1 LIFE CYCLE

In contrast to the genomewide screens described above, there have been many studies focusing on particular stages of the viral life cycle and the cellular processes and individual cellular factors identified as being associated with each of these phases of HIV replication.

VIRAL ENTRY

Surface Receptor Molecules for Cell Entry. The primary receptor for HIV, the CD4 protein, found on macrophages and T-lymphocytes (*14*) is a member of the immunoglobulin superfamily. The second major receptor (coreceptor) for HIV-1 is one of two molecules: CCR5 on macrophages (*15*, *16*) and T-lymphocytes or CXCR4 found on T-lymphocytes (*17*). Both of these are chemokine receptors. CXCR4 is an α-chemokine receptor specific for the ligand stromal derived factor 1 (SDF-1 or CXCL12). CCR5 is a *β*-chemokine receptor and like CXCR4 belongs to the seven-transmembrane family of cell surface receptors. Its ligands make up a small family of molecules, including MIP-1α (CCL3), MIP-1*β* (CCL4), and RANTES (CCL5) (*18*) and variants, including CCL3L1 and CCL4L1 (*19*). All of these ligands can compete with HIV and inhibit the entry of the virus into the cell. The latter two have varying genetic copy numbers, and for CCL3L1, the number of gene copies and hence the level of chemokine were thought to influence disease progression (*20*), although subsequent studies failed to substantiate this (*21*, *22*).

In vivo chemokine receptors are signal transduction molecules, and there is evidence that this process is involved in HIV entry (*23*−*25*). There is accumulating evidence that infection involves disruption and rearrangement of the cytoskeletal molecules, particularly actin. Downstream signaling from CXCR4, induced by HIV, dephosphorylates and activates cofilin, leading to depolymerization of F-actin (*26*). Molecules inhibiting this process can impair HIV replication significantly. As yet, a similar functional pathway has not been demonstrated for CCR5.

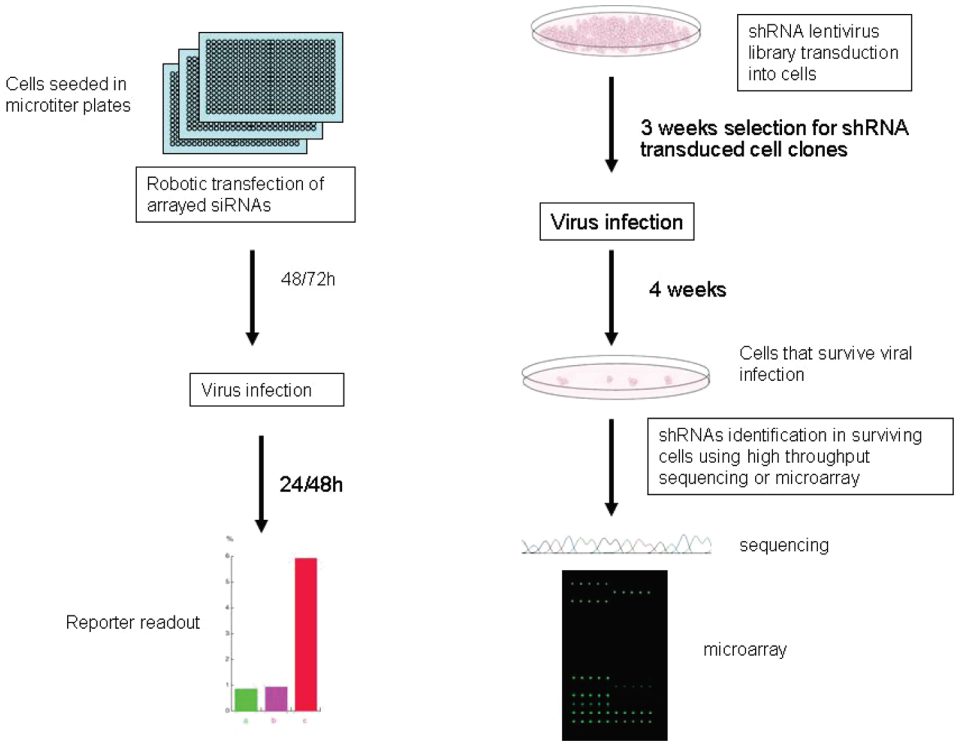

FIGURE 2: Schematic representations of general siRNA-based (left) and shRNA-based (right) screening approaches for cellular factors that assist viral replication. A more detailed explanation is given in the text.

A more direct interaction has been implicated through the gp41 molecule directly recruiting p115-RhoGEF, a guanine nucleotide exchange factor (*27*). This would facilitate remodeling of actin by GTPase activity.

Other cell surface molecules can also participate in HIV binding and entry. Integrin α4β7 can bind gp120, activating LFA-1, which is involved in viral synapse formation and cell to cell transmission (*28*).

Transport of the Viral Preintegration Complex to the Nucleus. Subsequent to fusion and entry into the target cell, the steps required for the virus to traffic to the cell nucleus are relatively less well documented. Control of actin polymerization and depolymerization is apparently important, and the actin polymerization nucleator Arp2/3 is involved (*29*). It is suggested that this may facilitate a short-range trafficking toward the microtubule network that is then responsible for transport to the nucleus. The actin cytoskeleton was also specifically assessed as playing a role in early HIV replication in one of the large siRNA screens. Proteins including AKAP13, another guanine exchange factor, were identified in this screen together with factors that regulate actin nucleation and organization as well as proteins affecting microtubule formation and function. Each of these candidates needs to be validated by a specific study.

Entry to the nucleus involves the ability to translocate across an intact nuclear membrane as, unlike other retrovirus families, lentiviruses can infect and integrate into nonmitotic cells. A number of the viral proteins that form the preintegration complex (PIC) have been shown to bind to members of the importin α family (*30*). They dock with importin β at the nuclear pore and facilitate entry of molecules bearing a nuclear localization signal. Several cellular proteins have been identified as being associated with the PIC, including barrier to autointegration factor (BAF) (*31*, *32*), Lap 2α (*33*), and HMG I(Y) protein (*34*). The viral accessory protein Vpr, which is a component of the PIC, binds to the nuclear pore complex protein Pom 121 (*35*). As the PIC is larger than a nuclear pore, it is unsurprising that various other nuclear pore complex proteins have also been identified in nuclear entry, including Nup 98 (*36*), Nup 124p (*37*), Nup 358 (*38*), and Nup 153 (*39*). Importin 7 has recently been shown to enhance the nuclear entry of HIV-1 (but not HIV-2) (*40*), correlating with its ability to bind to the viral integrase proteins that also are components of the PIC. Transportin 2, which was identified by two of the siRNA screens (*3*, *4*), has also been shown to enhance nuclear import of the PIC (*41*). Perhaps most intriguing has been the observation that tRNA molecules themselves can act as nuclear entry chaperones for the HIV PIC. Because most HIC cellular movement involves hijacking cellular processes, it will be interesting to see how widespread this mode of nuclear targeting is (*42*).

Proteins Influencing Reverse Transcription. AKAP14, a regulator of PKA in response to signal transduction from the G protein-coupled receptor, has been implicated in HIV infection, and there is evidence of direct interaction of this protein with the reverse transcriptase enzyme (*43*). Other proteins have proven to be more controversial. There are conflicting reports that the A/U binding protein HuR, known to stabilize mRNA, does (*44*), and does not (*45*), have a role in interacting with reverse transcriptase. Again the large scale siRNA screens have identified novel and plausible candidates, including DHX15, a helicase, and RBM17, a nucleic acid binding protein, among others. Evidence of their direct interaction with the preintegration complex is awaited.

Transcription and Chromatin. HIV-1 transcription is regulated by the viral promoter located in the 5′ LTR of the provirus. The LTR contains binding sites for several transcription factors such as Sp1 and NF-κB, NFAT, LEF-1, COUP-TF, Ets1, USF, and AP-1. Among these factors, Sp1 and NF-κB have been studied best, and through detailed mutagenesis of their binding sites in the LTR, their contributions to HIV-1 replication in

human cells have been well-delineated (*46*, *47*). Besides Sp1 and NF-κB, the roles of the other transcription factors are believed to contribute differentially to transcription under varying conditions of stimuli and in different cell types such as in primary T cells versus macrophages (*48*, *49*). Because the activities of LTR-interacting DNA-binding factors in basal LTR transcriptional initiation and elongation have been well-reviewed elsewhere (*50*, *51*), they will not be further discussed here.

In recent years, perhaps the strongest impetus for understanding the transcriptional regulation of HIV-1 arises from a need to address transcriptional mechanisms of proviral latency (*52*). Latently infected cells form a reservoir of antiretroviral treatment (ART) resistant cells that prevent curative therapy of HIV-1 (*53*, *54*). These latent cells arise stochastically as a small population from productively HIV-1-infected cells that have integrated proviral DNA. To comprehend transcriptional latency, one needs to study the nucleosomally organized structure of the integrated provirus. HIV-1 integration is generally random but tends to favor active genes (*55*); however, in a manner independent of the site of integration in human chromosomes, two nucleosomes, named nuc-0 and nuc-1, are precisely organized in the 5′ LTR. In particular, the histone-organized nuc-1 structure (located at positions −2 to 140 of the LTR) normally serves to downmodulate basal transcription.

Because the nuc-1 nucleosome presents a barrier to HIV-1 transcription, it stands to reason that the HIV-1-encoded transcriptional activator Tat would have evolved mechanisms for resolving this block. Indeed, Tat is known to associate with histone acetyl transferase (HAT) proteins whose activities remodel nucleosomes to allow transcriptional access. Tat has been found to bind several different HATs: CBP/p300, p/CAF, GCN5, Tip60, and $TAF_{II}250$ (*56*−*60*). By binding to the HAT proteins, Tat then relieves chromatin repression at the HIV-1 LTR. Recently, Tat has also been found to bind histone chaperone protein hNAP-1 (*61*) that acts with ATP-dependent chromatin remodeling complexes to facilitate transcription.

Countering the effect of HATs are the histone deacetylase proteins (HDACs) that remove the acetyl group from HAT-acetylated histones to enforce transcriptional silencing. In the HIV-1 LTR, it is thought that the LSF protein binds positions −10 to 27 of the LTR to recruit the YY1 factor, which further binds HDAC-1 to silence viral transcription. Tat expression downregulates HDAC-1, serving to remove this repression of transcription. Indeed, this scheme of removal of repressive activity has been verified through treatment with several HDAC inhibitors (HDACIs) such as trichostatin A (TSA), trapoxin (TPX), valproic acid (VPA), sodium butyrate (NaBut), and other compounds that have been shown to activate integrated proviruses in latently infected cells (*62*, *63*). The clinical importance of these findings lies in the potential use of HDACi in HIV-1-infected patients undergoing ART; this use could possibly activate the latent viral reservoirs, allowing for the potential purging of the in vivo latently infected cells.

Post-Transcriptional Regulation of Incompletely Spliced HIV-1 RNAs. The expression of unspliced and partially spliced HIV-1 RNAs is regulated post-transcriptionally by the viral Rev protein. Rev modulates the export of unspliced and/or partially spliced viral RNAs from the nucleus into the cytoplasm (*64*). This is an important property because unspliced and partially spliced viral RNAs serve as the moieties for the synthesis of Gag, Pol, and Env proteins, and the unspliced RNA is also the genomic RNA that is packaged into progeny virions. Because cellular RNAs are normally retained in the nucleus and do not exit into the cytoplasm, there must be a target specificity by Rev for unspliced and partially spliced HIV-1 RNAs. This specificity is conferred on unspliced and partially spliced HIV-1 RNAs by a highly secondarily structured RNA element [the Rev-responsive element (RRE)], which is a binding site for the RNA-binding Rev protein. The current view is that Rev binds to the RRE and interacts with CRM1 [chromosome maintenance region 1 (*65*−*67*)] protein. This interaction then directs the viral ribonucleoprotein complex to a nuclear−cytoplasmic shuttling pathway that is normally used for the export of cellular small nuclear RNAs, and rRNAs. The RRE−CRM1 pathway is distinct from that used to export fully spliced HIV-1 mRNA and cellular mRNAs from the nucleus (*68*, *69*). There are recent comprehensive reviews about the export of unspliced and/or partially spliced HIV-1 RNA from the nucleus to the cytoplasm (*70*), about the possible role of Rev in the translation of HIV-1 transcripts (*71*), about Rev activity in RNA encapsidation (*72*), and about the effect of Rev on proviral integration (*73*). Rather than repeating those summaries, below, we will highlight selectively two classes of proteins, RNA helicases and RNA cap methylase, as examples of cellular factors that cooperate with Rev to regulate post-transcriptional HIV-1 RNA expression.

It is perhaps not surprising that RNA helicases could serve as cofactors for Rev-directed export of HIV-1 RNAs (*74*). In this respect, DDX3, a cellular RNA helicase, was found to enhance Rev-dependent nuclear−cytoplasmic export of HIV-1 RNAs (*75*). DDX3 is a nuclear−cytoplasmic shuttling protein that binds CRM1, Rev, and nuclear pore proteins. Thus, one notion is that this RNA helicase may function with Rev and CRM1 to remodel the HIV-1 ribonucleoprotein complex to "thread" the attached RNA through the nuclear pore, facilitating its release into the cytoplasm. A second RNA helicase, DDX1, has also been reported to bind the N-terminus of Rev and to participate in the export of unspliced HIV-1 RNA from the nucleus to the cytoplasm (*76*). There is additional evidence that two other RNA helicases, RHA and RH116, also regulate HIV-1 expression (*77*, *78*). The mechanisms for these latter helicases in viral replication appear to be different from the nuclear−cytoplasmic regulation of RNA export. Knockdown of another helicase, DDX24, appears to reduce the level of viral RNA encapsidation possibly by its negative effects on the enhancement of RNA packaging, which is now a recognized property of the Rev protein. It is likely that additional RNA helicases that interact with HIV-1 will be discovered.

Besides RNA-binding proteins, the inherent characteristics of an RNA may also dictate its post-transcriptional fate. An early RNA modification of many cellular transcripts is the formation of a 7-methylguanosine (m7G) cap. The m7G cap facilitates the initiation of translation in mammalian cells, and uncapped RNAs are generally unstable (*79*, *80*). The cap status of HIV-1 RNAs had not been well-understood. Recently, it was found that different HIV-1 RNAs are either m7G-capped or hypertrimethylated TMG (trimethylguanosine)-capped at their 5′ ends (*81*). Viral transcripts containing RRE (i.e., unspliced or partially spliced HIV-1 RNAs) appear to be bound by Rev, which then recruits a cap hypermethylating enzyme, PIMT (peroxisome proliferator-activated receptor-interacting protein with methyltransferase), to modify the m7G cap to a TMG cap on these RNAs. The acquisition of a TMG cap by these HIV-1 RNAs facilitates their recognition by CRM1 and directs the RNAs to the CRM1 nuclear−cytoplasmic export pathway. Accordingly,

the PIMT-mediated TMG modification increases selectively the cytoplasmic expression and translation of HIV-1 mRNAs encoding proteins like Gag and Env.

The two classes of Rev cofactors described above illustrate the complexities of post-transcriptional HIV-1 gene regulation. A potential benefit of characterizing these and other cellular cofactors for HIV-1 replication is that some of the proteins could be potentially targeted by small molecule inhibitors that might repress viral propagation. Initial candidate inhibitors for RNA helicases and cap hypermethylases and their possible utility for inhibiting HIV-1 have been reported previously (*82*).

Viral Assembly and Export. The intact viral particle consists of two copies of the virus RNA and a group of carefully ordered structural proteins. It appears that an early preassembly complex trafficking to the plasma membrane consists of a combination of a single RNA molecule with a small number of associated Gag proteins (*83*). The site of initial interaction of these two is not established, although there is some evidence that this occurs at the microtubule organizing center (*84*, *85*). Cellular proteins likely bind both the protein and RNA components of this complex to facilitate its trafficking. In the case of the RNA, a relatively small number of proteins have been implicated, some of which appear to be associated with the viral RNA in both nuclear and cytoplasmic subcellular compartments. Members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family, in which A1 and A2 possess nucleocytoplasmic shuttling capability, have been identified as playing a role (*86*, *87*). A1 appears to enhance Gag production possibly by increasing the rate of nuclear export of the genomic RNA, although this is controversial. A2 binds to two response RNA elements within the genomic RNA, A2RE-1 and A2RE-2 found in the Gag and Vpr coding sequences, respectively. Mutation of A2RE-2 leads to mislocalization of gRNA in and around the nucleus. Knockdown of hnRNPA-2 leads to accumulation of gRNA in the perinuclear microtubule organizing center (MTOC); however, this RNA appeared to be derived from the cytoplasm. Conflicting results have been obtained upon comparison of knockdown of A2 with mutation of A2RE-A2, but with its suggested links to the microtubule system, one could speculate that the protein is involved in ensuring the RNA takes the appropriate pathway as it moves through the cell.

The RNA binding protein Staufen appears to act as a chaperone with respect to the RNA and has been detected in viral particles (*88*). Similarities between this and the known HIV TAR RNA binding protein TRBP (*89*) may be pertinent.

There is a growing body of evidence that the microtubule network is involved in the cytoplasmic transport of the early assembly intermediates of HIV. Knockdown of KIF4, a kinin involved in cytokinesis that is also known to bind Gag, altered localization of the latter, and expression of the dominant negative form of KIF4 decreased Gag levels globally but led to an accumulation in the perinuclear region. SOCS1 is induced during HIV infection and can stimulate late steps in HIV replication and can bind the MA and NC domains of Gag. Knockdown slows trafficking and assembly and again results in the appearance of perinuclear aggregates of Gag. Other transport proteins, including Arf and GGA, may also play roles in trafficking and viral release (*90*).

Viral Budding. In contrast to the relative sparsity of proteins known to be involved with transcytoplasmic trafficking, there is an abundance of information about those involved in the later stages of assembly and viral budding. This reflects the fact that the virus hijacks a complex of proteins within the cell that are usually used for budding and export into the endosomal system, the endosomal sorting complex required for transport (ESCRT) machinery. A full description and analysis of all of the ESCRT and ESCRT-associated proteins involved in viral export is beyond the scope of this review. The reader is referred to excellent recent reviews on the subject (*91*−*93*). In brief, it is known that the Gag protein of HIV can bind specifically to a number of cellular proteins. In particular, the PTAPP motif in the P6 region of Gag is able to interact directly with the ESCRT I protein TSG 101, and the YSPTL motif from P6 interacts with ALIX from ESCRT III. In a manner analogous to their role in budding of cell membranes into the endosome, these proteins facilitate the assembly of Gag monomers in an array at the plasma membrane and the evagination of a Gag-containing particle away from the cell. The final process of membrane scission allowing the enveloped viral particle to escape from the cell surface is still a matter of debate but involves proteins of the CHMP family whose in vivo role is in the final separation of cell membranes during cell division.

Intracellular Defenses against HIV. Clearly, the cell is not a passive participant in virus replication. However, apart from those multifarious pathways subverted by the virus for its own use, there are inhibitory factors within cells that act as intracellular defenses and whose presence inhibits or "restricts" the virus (Figure 3). The first of these to be identified in retroviruses was Fv1, which restricts ecotropic murine leukemia viruses (*94*). This paradigm prepared the way for the identification of similar factors restricting HIV. Because of their potential importance in novel antiviral approaches, they have been extensively investigated in recent years.

*TRIM5*α. Simian immunodeficiency viruses are able to replicate in the cells of Old World monkeys (*95*−*99*), but HIV fails to do so (*100*) despite successful binding and cell entry. The inhibitory factor, initially identified and termed Lv1 (*101*−*104*), was saturable with an excess of viral cores. From a rhesus macaque cDNA library expressed in HeLa cells, clones resistant to HIV-1 but sensitive to SIVmac were isolated and found to express simian TRIM5α (*105*). siRNA knockdown of this protein rendered the cells permissive to HIV. TRIM5α interacts with the viral capsid through a region also associated with the binding of a cellular factor cyclophilin A (CypA) (*106*−*108*), a cellular peptidyl/prolyl isomerase that isomerizes a peptide bond at this locus on the HIV-1 capsid. This was substantiated by identification of an unusual fusion protein in the owl monkey named TRIMCyp comprising a chimera of TRIM5α and cyclophilin (*109*, *110*) [a similar insertion event producing a chimeric protein has been noted in monkeys of the *Macaca* genus (*111*)]. The owl monkey is a New World monkey yet still restricts HIV, and TRIMCyp was hypothesized to target the TRIM effector component to the viral core, because restriction could be blocked by the cyclophilin inhibitor cyclosporin A.

TRIM stands for tripartite motif, a term used for a family of ~70 proteins sharing three polypeptide domains: an N-terminal RING domain, a B-box, and a coiled coil. They carry out diverse functions within the cell, including roles in development as well as antiviral activity, and they have been implicated in oncogenesis (*112*−*115*). TRIM5α is the longest splice variant of the TRIM5 family with a unique C-terminal B30.2/SPRY domain. It is expressed constitutively but also upregulated by type I interferons (*116*−*118*). The RING domain has a zinc binding motif associated with E3 ubiquitin ligase activity, and TRIM5α can

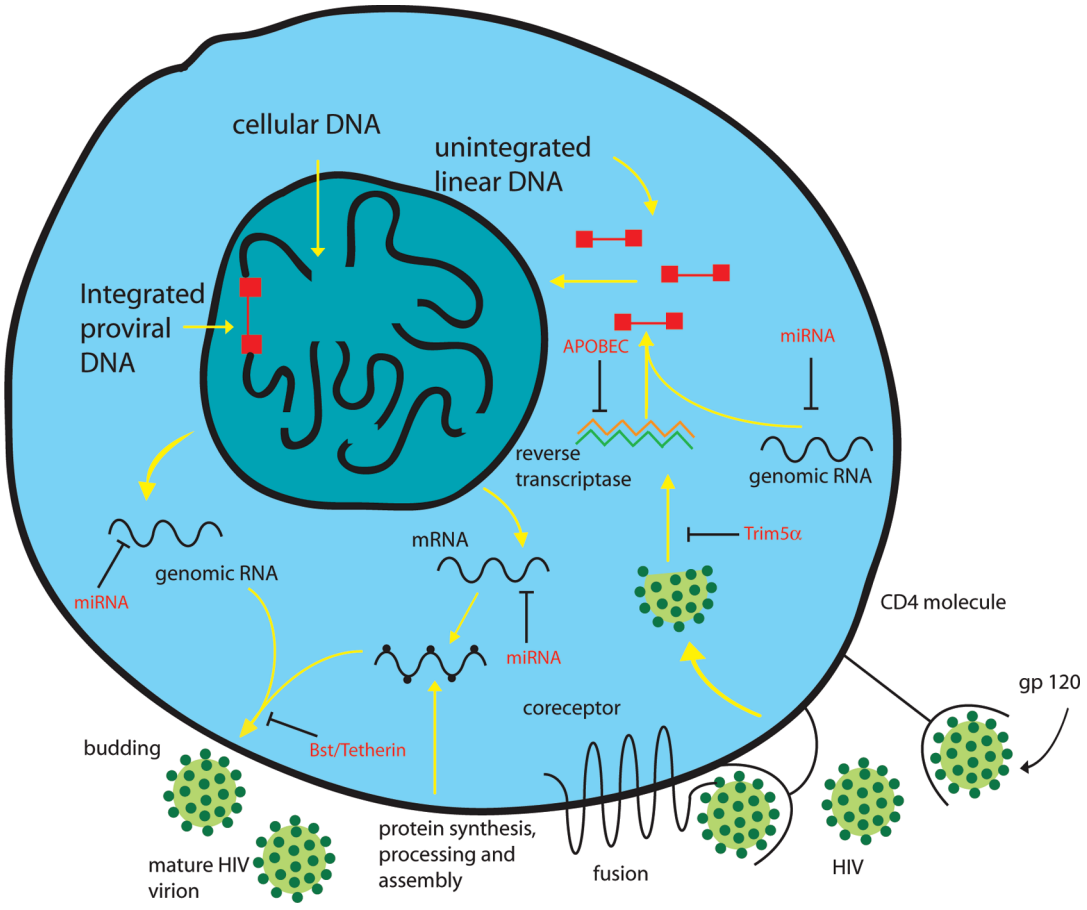

FIGURE 3: Drawing highlighting different infection processes used by HIV-1 and the various cellular factors that restrict viral replication. Modified from the work of Wainberg and Jeang (*216*).

ubiquitinate other proteins and itself (*119*, *120*). The B-box is a B2 form (*121*, *122*) and is essential for the restriction activity of TRIM5α as shown by the inactivating effect of point mutations (*123*, *124*). It also contains a zinc binding motif with homology to the RING domain, although its function is still not fully elucidated.

The coiled coil domain, which occurs in other proteins such as the HIV envelope transmembrane glycoprotein, facilitates oligomerization, and the functional form of TRIM5α is thought to be either a dimer or a trimer (*125*).

The B30.2 domain contains a sequence with a PRYSPRY motif associated with exposed loops with three highly variable regions (V1−V3) (*126*, *127*). These are thought to confer the virus binding specificity of the TRIM5α protein. As one of a number of examples, a single-amino acid substitution in the V1 region substituting a nonpositively charged amino acid for arginine at position 332 renders human TRIM5α as effective as owl monkey TRIMCyp in inhibiting HIV-1 (*128*, *129*).

The exact mode of action of TRIM5α in restricting HIV-1 remains to be fully elucidated. Mutational analysis of the RING domain has produced inconsistent results. Ubiquitination of the viral capsid, followed by degradation in the proteasome, is suggested by the reduction in the level of inhibition seen in the presence of proteasome inhibitors (*130*, *131*), and it has been postulated that TRIM5α binds to capsid and that its own autoubiquitination capability flags the complex for degradation (*132*). However, other studies have suggested a RING-dependent proteasome-independent mechanism (*133*) possibly involving direct disassembly of the capsid.

Despite a high level of species specificity and diversity of TRIM5α between species and polymorphisms within a species, the human sequence is almost invariant (*134*). This has been thought to have contributed to the near universal susceptibility of humans to HIV (*135*). A small number of single-nucleotide polymorphisms have been identified; the significance of these is unclear, although one, the H43Y mutation, actually abolishes the moderate level of restriction achieved against HIV-2 (*136*, *137*). Attempts to exploit this natural antiviral system to protect against HIV are being explored.

APOBEC. APOBEC3 proteins make up a family of DNA editing proteins (*138*), named because of their homology to APOBEC1, an mRNA editing enzyme. There are seven in humans (APOBEC 3A−3H) that have arisen through gene duplication on chromosome 22. Overall, this family of cytidine deaminases acts to defend the cell against exogenous and endogenous retro elements and a variety of other viruses, including HBV (*139*, *140*) and HCV (*141*). APOBEC 3G and 3F are expressed in primary T-cell lines and in monocytes and macrophages and are the major ones involved in HIV restriction. They have target sequence preferences: 3F targeting 5′-TC and 3G specific for 5′-CC, both deaminating the 3′ C residue. Their importance in HIV infection came to light during studies on the Vif protein, a 23 kDa protein accessory protein of the virus that is required for replication in certain cell lines (*142*−*144*). APOBEC 3G was found to be an endogenous restriction factor that could be overcome by the Vif protein (*145*). APOBEC 3G is incorporated into the viral particle in the producer cell line and can exert its effect

whether the virus subsequently infects APOBEC 3-expressing cells or nonexpressing cells.

APOBEC 3F/G has cytidine deaminase activity and binds to the nucleocapsid protein and the viral genomic RNA (*146*), where, during the process of reverse transcription, it acts to deaminate cytidine to uridine on the negative strand of the viral cDNA as it is synthesized. The mutated cDNA now acts as a template for the second DNA strand, producing A to G mutations. Thus, the provirus will contain multiple nonsense and stop codons and is nonfunctional. The mutated DNA will also be subject to destruction by the cell prior to integration (*147*−*154*).

APOBEC appears to have a number of other actions that are unrelated to the cytidine deaminase activity, and active site mutants still display an inhibitory effect on HIV. The nature of this deaminase-independent restriction is not fully elucidated (*155*−*161*). The deaminase activity is in the C-terminus of the protein, yet surprisingly, mutants with an intact N-terminal genomic RNA binding domain but a mutated C-terminus can still cause A to G mutations.

Vif has been shown to target APOBEC 3F/G to the proteasome through ubiquitination via linkage to the ELONGINB/C-CULLIN5 E3 ubiquitin ligase (*162*). There is also evidence that Vif can inhibit APOBEC translation, reducing levels in the producer cell (*163*, *164*). It is highly species specific. A single-point mutation at position 128 switches HIV-1 Vif restriction activity from HIV to SIVmac (*165*−*167*). The concentration dependence of Vif activity and the selective advantage to HIV of mutational escape have fostered the concept that limited APOBEC activity may actually be advantageous to the virus in enhancing its own capabilities for sequence variation (*168*).

BST/Tetherin. The viral accessory protein Vpu had long been known to be essential for efficient virus assembly and export in some cell lines but not others (*169*−*171*). Mutating or deleting Vpu led, in nonpermissive cells, to a phenotype with a reduced level of viral production and accumulation of virus particles in the endosomes and at the cell surface (*172*). Initially, Vpu was thought to interfere with an unwanted premature interaction of the virus surface (SU) protein gp120 with its cognate receptor CD4, as both were synthesized in the endoplasmic reticulum (*173*). However, accumulating evidence showed that Vpu enhances virion release and overcomes a dominant but protease sensitive inhibitor that retains virions associated with the cell membrane (*174*, *175*). Electron microscopy studies showed mature virions tethered to the plasma membrane (*176*). The cellular protein responsible for this restriction was only recently identified as CD317 or BST, also termed tetherin (*177*). Tetherin is a 30−36 kDa heterogeneously glycosylated type II membrane protein that is an interferon inducible protein with an unusual topology. It has an N-terminal cytoplasmic tail, a transmembrane domain, an extracellular coiled coil domain, and a C-terminal glycosylphosphatidylinositol (GPI) membrane anchor. The intracellular domain of tetherin contains a variety of important motifs, specifically, a YxY domain mediating clathrin-linked endocytosis, a KxxK motif required for degradation by the KSHV K5 protein, and, in non-human primates, a DDIWK sequence targeted by Nef and also resulting in degradation. The coiled coil consists of two α-helices containing three cysteines mediating disulfide bonding and two asparagines representing putative glycosylation sites in the ectodomain. Tetherin is enriched in lipid rafts of the plasma membrane because of its GPI anchor (*178*), where it can be incorporated into assembling virions and subsequently prevent their budding away from the cell surface. The mechanism is believed to involve bridging of the virion to the cell surface by the two membrane binding domains of the protein (*179*). The bridging complex appears to be an antiparallel dimer (*180*). Vpu counteracts the effect of tetherin by inducing its downregulation from the cell surface and its subsequent degradation (*1*). One model suggests an interaction between the two proteins' transmembrane domains and subsequent ubiquitination of the tetherin moiety leading to proteasome degradation (*181*−*183*). Tetherin restricts the budding of a number of enveloped viruses, including a variety of retroviruses, Kaposi's sarcoma herpesvirus (*184*), and Ebola virus (*185*). Vpu is species and/or virus specific.

MicroRNAs. Small noncoding RNAs play important roles in the regulation of mammalian genes. It has been suggested that more than 30% of all human genes are regulated by microRNAs (miRNAs). To date, more than 1000 human miRNAs have been identified (http://www.microrna.org). While recent genomewide siRNA and shRNA screenings (see above) have shown that several hundred host cell proteins contribute to the regulation of HIV-1 infection in human cells, how miRNA-mediated regulation complements this picture is poorly understood. The biogenesis and currently accepted mechanisms of action for miRNAs have recently been reviewed (*186*). We briefly outline below findings relevant to miRNA regulation of HIV-1.

Plants and lower eukaryotic cells use miRNAs as a form of RNA interference (RNAi) to restrict infecting viruses (*187*). While mammals conserve the same functional miRNA repertoire and RNA-silencing machinery, some have debated whether they employ a miRNA-based antiviral strategy (*12*, *188*−*190*). For endogenous mammalian retroviruses, there is a large body of literature demonstrating that a variety of small noncoding RNA forms are employed to silence these elements (*191*−*193*). In silico analyses have also indicated that exogenous mammalian viruses may be similarly susceptible to miRNA-based restriction (*194*, *195*). Indeed, several investigators have demonstrated independently that a number of human miRNAs specifically influence productive HIV-1 infection in human cells (*196*−*199*). These results agree with findings that the knockdown of either the Dicer protein (*200*) or the RISC components (*201*), each necessary for miRNA-mediated gene silencing, has resulted in enhanced HIV-1 replication in cells. The notion that miRNAs restrict viruses in mammals as they do in invertebrate or plant cells is additionally supported by an increasing number of examples of RNAi-silencing suppressors encoded by mammalian viruses such as adenovirus (*202*−*204*), HCV (*205*), Ebola (*206*), influenza A virus (*207*−*209*), primate foamy virus (*210*), HIV (*211*−*213*), SARS corona virus (*214*), and HTLV-1 (*215*). Further investigation is needed to understand how RNA-based and protein-based viral restriction mechanisms cooperate together in human cells.

CONCLUDING REMARKS

Our understanding of the extent of interaction and dependence of a virus like HIV-1 on cellular factors continues to increase as do the number of factors involved. Apart from giving us insights into the roles of these factors in the normal cell, they provide an array of novel drug targets. HIV-1 is such a mutable virus that drug treatments targeting pure virion processes rapidly produce escape mutations. Drugs that target processes that involve interactions with nonmutable cell proteins will also target regions of the virus whose variability is constrained by the conservation

of their cellular partner. These present exciting new therapeutic opportunities in HIV-1 and other viruses.

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