Published in final edited form as:

Proteomics Clin Appl. 2015 February ; 9(0): 221–234. doi:10.1002/prca.201400101.

Proteomics in the investigation of HIV-1 interactions with host proteins

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Abstract

Productive HIV-1 infection depends on host machinery, including a broad array of cellular proteins. Proteomics has played a significant role in the discovery of HIV-1 host proteins. In this review, after a brief survey of the HIV-1 host proteins that were discovered by proteomic analyses, we focus on analyzing the interactions between the virion and host proteins, as well as the technologies and strategies used in those proteomic studies. With the help of proteomics, the identification and characterization of HIV-1 host proteins can be translated into novel antiretroviral therapeutics.

Keywords

Proteomics; Mass Spectrometry (MS); HIV-1; Viral Protein; Host Protein; Cellular Factor

INTRODUCTION

Despite great effort and much progress, Human Immunodeficiency Virus (HIV)/Acquired Immune Deficiency Syndrome (AIDS) remains a major threat to global health. Currently, almost all antiretroviral drugs target viral proteins, which include reverse transcriptase, protease, and integrase, to achieve their inhibitory effects on HIV replication. However, the virus is capable of quickly developing resistance to these drugs [1]. The introduction of Antiretroviral Therapy (combining multiple antiretroviral drugs) has largely overcome viral resistance, but it associates with significant side effects and high cost. Evidently, the current HIV treatments, which focus on repressing viral proteins, have limitations. Novel approaches and new therapeutic targets need to be explored.

It is well known that HIV-1 encodes only 9 (18 if all matured proteins are counted) viral proteins [2] and must depend on host cellular machinery to support its replication. So far, various host proteins have been associated with HIV-1 and some play key roles during the viral life cycle [3]. For example, CD4 [4] and co-receptors, CXCR4 or CCR5 [5], are needed during viral entry; lens epithelium-derived growth factor (LEDGF) [6–7] is essential for HIV-1 integration into host DNA; CDK9/Cyclin T1 [8] is important for HIV-1 transcription,

and TSG101 [9] is involved in the HIV-1 budding process. Due to the lower probability of spontaneous mutations in these proteins compared to those of the virus, they provide attractive targets for antiretroviral treatments [10]. Based on this notion, one drug called Maraviroc (a host CCR5 co-receptor antagonist, which prevents viral entry by blocking interaction between cellular CCR5 and viral gp120 [11]) was developed and is currently clinically available.

Even with the aforementioned advances, due to the complex HIV-1 life cycle and its deep dependence on host machinery, many important host proteins (targets) likely remain to be discovered. To explore a complete set of cellular factors involved in HIV-1 infection, three genome-wide siRNA screenings were performed [12–14]. Altogether, these studies identified a total of 842 host genes associated with HIV-1 infections. However, the overlaps among the siRNA screens were poor [15]. It has become clear that studies on the genome and/or transcriptome cannot be translated directly into knowledge of proteins, because alternative RNA splicing, post translational modifications (PTMs) and proteolytic processing give rise to a very diverse and dynamic proteome. In many cases, proteins are the ultimate effectors of most cellular functions. Thus, direct examination of protein expression and PTMs by proteomics is critical for understanding cellular responses to HIV-1 infection. Mass spectrometry (MS) based proteomic analyses have significantly matured in the last few years. The sufficient sensitivity and high level throughput of modern proteomics have greatly facilitated investigations of host proteomes during HIV-1 infection [16–17].

SIGNIFICANT ROLES OF PROTEOMICS IN DISCOVERING HIV-1 ASSOCIATED CELLULAR FACTORS

In the last decade, numerous proteomic investigations on HIV-1 associated cellular factors have been carried out with substantial discoveries. Due to limited space, only some exemplary studies are summarized below: (1) *Host proteomes involved in signaling/metabolic pathways*: An early two-dimensional (2-D) gel based proteomic analysis showed that multiple pathways, including glycolysis, mitochondrial oxidation, apoptotic signaling, and protein transport, were significantly influenced by HIV-1 infection [18]. Along with the transition of proteomics from qualitative to quantitative, Chan et al found more than 20% of proteins changed in abundance at the peak of viral replication. Not surprisingly, the differential expression of proteins was concentrated in select biological pathways [19]. Another proteomic analysis showed that HIV-1 modulates production of proteins that affect lipid-metabolic pathways [20]. In addition to the aforementioned studies on T cells, Kraft-Terry et al, using a pulsed stable isotope labeling of amino acids in cell culture (SILAC) based proteomic method, found that HIV-1 regulates a range of host proteins in macrophages that affect cell survival and ability to contain infection [21]. All these studies showed that HIV-1 infection has profound effects on the host proteome. By selectively targeting a certain set of upstream cellular proteins, HIV-1 can affect cellular pathways to its advantage. (2) *Time-course proteomic studies*: Taking the shot gun liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach, distinct proteomic profiles were revealed between 8 and 24 hours post-infection [22]. Furthermore, Navare et al examined the host proteome response upon HIV-1 infection at 4, 8, and 20 hours post-infection by using

iTRAQ (isobaric tag for relative and absolute quantification) based proteomics, and found that the majority of changes in protein abundance occurred at an early stage of infection, well before the *de novo* production of viral proteins [23]. These studies clearly showed that HIV-1 infection is a quick, constantly evolving process with wide dynamics. These dramatic proteomic changes at various time points reminds researchers that studies at different time points are often needed to get a complete picture of HIV-1 infection. (3) *Epigenetic proteomes*: In addition to the apparent abundance of changes to various proteins, the epigenetic regulation induced by HIV-1 infection is more subtle, yet not insignificant. Wojcechowskyj et al employed a SILAC based quantitative phosphoproteomics approach to examine the CXCL12/CXCR4 signaling axis in the CEM cell line [24]. Later, the same group also found significant numbers of phosphorylation sites on numerous proteins during HIV-1 entry [25]. The Histone posttranslational modification (PTM) of HIV-1 infection was also examined by nano-LC-MS/MS. Major changes in histone PTM abundances were linked to massive fluctuations in mRNA expression of associated chromatin enzymes [26]. Unlike the numerous epigenetic studies performed for many diseases, especially in cancer, the investigation of HIV-1 induced PTM proteomic regulation has been rather limited. Further study in this direction is needed and will likely bring insights into HIV-1 epigenetic regulation. (4) *Subcellular proteomes*: A comprehensive proteomic study on early HIV-1 nucleoprotein complexes (reverse transcription and preintegration complex) revealed at least 54 and 52 host proteins were enriched in the infected and control samples respectively, adding additional novel candidates to the growing HIV-1 host protein list [27]. As to the possible functions of enriched proteins, a T-cell nuclei proteome profiling upon HIV-1 infection suggested that these protein sets are related to nuclear architecture, RNA regulation, cell division, and cell homeostasis [28]. A separate study further showed that HIV-1 infection induces extensive changes in the proteome of the nuclear envelope (NE), one notable aspect of which is a significant decrease in the abundance of nucleoporins [29]. As a specific stage of viral life cycle is often concentrated in a certain subcellular compartment, rather than doing proteomic screening on the whole cell, subcellular proteomic study can provide targeted and more precise information. (5) *Secretome/exosomal proteome*: It is logical for most studies to focus on virions, the ultimate effectors of infection. However, the profile of secreted proteins from infected cells can offer valuable information as to the disease stage, indications of transmission and even mechanistic insights. Ciborowski et al used LC-MS/MS to analyze the secretome of HIV-1 infected human monocyte-derived macrophages. Among the total 110 identified proteins, some were differentially expressed [30]. Another similar proteomic analysis showed that cytoskeletal proteins actin and profilin 1 were rearranged and secreted in conjunction with productive viral replication and affected cytopathicity [31]. These different secretome profiles can be traced back to the fact that HIV-1 hijacks the host exosome release pathway to facilitate its budding [32–33]. A SILAC based proteomic analysis confirmed that HIV-1 can induce differential exosomal protein expression and suggested that HIV-1 may be able to induce an extracellular environment that favors cell-to-cell transmission and infection [34].

All these proteomic studies have dramatically increased our understanding of how HIV-1 affects the host proteome to its advantage. However, it is unrealistic to cover all the

proteomic studies on HIV-1 in this review. Here, we focus on dissecting the interactions between host proteins (cellular factors) and viral proteins, and discuss the therapeutic potential of harnessing the host proteins to treat HIV-1 infection.

CELLULAR FACTORS ASSOCIATED WITH THE HIV-1 GENOME AND ITS PRODUCTS

The HIV-1 genome encodes a very limited number of viral proteins, which include three indispensable structural proteins (Gag, Pol and Env), two essential regulatory elements (Tat, Rev) and four accessory regulatory proteins (Nef, Vif, Vpr and Vpu). To complete its life cycle, HIV-1 must interact with many cellular factors, often directly (physically). For examples: CypA, a peptidyl-prolyl isomerase that binds to HIV-1 CA [35], UNG2 (uracil DNA glycosylase 2), a cellular DNA repair enzyme that binds HIV-1 integrase (IN) [36], and clathrin, which is vital for the spatial organization of Gag and Pol proteins [37]. Identifying the physical interactions between cellular factors and viral proteins at a global scale is crucial for a comprehensive understanding of how HIV-1 uses the host's cellular machinery during the course of infection.

(1) Cellular factors associated with the whole virion

Early proteomic studies of HIV-1 cellular factors mainly focused on investigating proteins isolated from whole virions. In 2002, Misumi et al applied purified HIV-1 particles, obtained systemically by ultracentrifugation, subtilisin treatment and Sepharose chromatography, to 2D gel electrophoresis. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry identified 24 proteins inside the virions isolated from a T cell line (CEM). Most interestingly, three isoforms of cyclophilin A (CyPA), an essential host protein for HIV-1 infectivity [38], were found to physically interact with the viral membrane [39]. In addition to T lymphocytes, macrophages are also known to host HIV-1 infection. Are there any different host proteomic responses between macrophages and T cells? Chertova et al purified HIV-1 virions from infected macrophages by coupling density centrifugation and CD45 immunoaffinity depletion. Besides confirming many previously reported HIV-1 cellular factors, their proteomic analysis showed that a significant number of proteins are involved in membrane organization, late endosomal function, as well as exosomes, consistent with the idea that HIV-1 uses the late endosome/multivesicular body pathway during virion budding from cells [40]. Recently, Linde et al developed an alternative high-yield virion purification technique using cholesterol that differentially modulates the density of virions and microvesicles. Purified virions were analyzed using iTRAQ based MS/MS. Besides many previously documented HIV-1 associated proteins, such as HLA proteins, chaperones, and actins, they identified additional HIV-1 associated proteins, among them ERM proteins and the dynamin domain containing protein EH4 [41]. With better yield and simplified steps, the recent virion purification coupled proteomic screens further confirmed that HIV-1 infection affects numerous processes in the host. It is logical to believe that focused analysis of interactions between individual viral protein and cellular factors would offer better resolution and deeper understandings as to how viral proteins explore host proteome.

(2) Viral structural proteins

1. The gag (group-specific antigen) gene encodes Gag precursor polyprotein, which is processed by viral protease during maturation into CA (capsid protein, p24), NC (nucleocapsid protein, p7), MA (matrix protein, p17), P6, SP1 (spacer peptide 1, p2), and SP2 (spacer peptide 2, p1) [2]. In general, Gag polyprotein plays the central role during membrane binding, nucleic acid binding and interaction with the host cell derived budding machinery [42–43]. Specifically, the CA protein forms the core of virion. The NC is responsible for facilitating reverse transcription as well as recognizing the packaging signal of HIV-1. MA is mainly responsible for stabilizing viral particle as well as escorting the viral DNA to the nucleus. The p6 mediates the incorporation of Vpr into assembling virions and aids efficient release of budding virions from infected cells [44].

Although several Gag-binding proteins, including TSG101 (tumor susceptibility gene 101) [45], APOBEC3G (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G) [46] and Trim5 α (tripartite motif-containing protein 5 α) [47], have been previously characterized, our understanding of Gag-host interactions remains incomplete. In order to identify bona fide interaction partners with Gag, Engeland et al performed a series of six independent (to minimize the false positive/negative) affinity purification screens to characterize the host interactome with HIV-1 Gag. Other than confirming various previously documented host proteins, translation factors, cytoskeletal and motor proteins and factors involved in RNA degradation were found to be enriched. And significantly, components of cytoplasmic RNA transport granules were co-purified with Gag [48], suggesting that HIV-1 may hijack RNA transport during trafficking of viral RNA. This rigorously designed study provided additional novel HIV-1 cellular factors for further verification.

So far, only a few host proteins have been identified by mass spectrometry, assigned associations with Gag (or one of its matured forms), and received extensive study. (1) *LYRIC*, Lysine-rich CEACAM1 co-isolated protein, also known as astrocyte elevated gene-1 (AEG-1) or metadherin (MTDH), is a ubiquitously expressed 64 kDa protein that has been associated with various signaling pathways [49]. In a series of independent affinity purification coupled MS experiments, Engeland et al consistently identified the cellular protein LYRIC to be an HIV-1 Gag-interacting protein. Molecular dissection revealed that Gag interacts with LYRIC via its MA and NC domains. This interaction requires Gag multimerization and LYRIC amino acids 101 to 289. The expression level of LYRIC is also consistent with Gag expression level and viral infectivity. This proteomic study identified LYRIC as a cellular interaction partner of HIV-1 Gag that has roles in regulating infectivity [50]. (2) *RHA*, RNA helicase A, is a member of the DEAH family of proteins that are capable of unwinding double-stranded RNA structure. RHA has been shown to play roles in Rev-mediated gene expression [51] and modulates translation of HIV-1 and infectivity of progeny virions [52]. Using tandem affinity purification (TAP) coupled mass spectrometry, RHA was isolated and identified from the Gag associated host protein complex. Further evidence showed that RHA participates in HIV-1 particle production and promotes viral reverse transcription [53]. (3) *aPKC*, atypical protein kinase C, unlike conventional or novel kinase isoforms, does not require diacylglycerol or calcium for activation. Various cellular pathways can be affected by PKC phosphorylation of serine and threonine induced signal

cascades [54]. In terms of HIV-1, however, PKC has primarily been suggested to activate latent HIV-1 [55]. The Gag C-terminal p6 domain mediates incorporation of the viral Vpr protein and has been found to be phosphorylated during HIV-1 infection. However, the kinase that directs the phosphorylation of Gag p6 remains elusive. Using a proteomic approach, Kudoh et al identified 22 kinase candidates that have potent interaction with Gag. Among these 22 kinases, aPKC was found to phosphorylate HIV-1 Gag p6. Further LC-MS/MS phosphorylation site mapping and immunoblotting analysis confirmed that aPKC phosphorylates HIV-1 Gag at Ser487. Finally, structural modeling and cell-based assay revealed that this phosphorylation is necessary for the interaction between Gag and Vpr [56]. (4) *ALIX*, HIV-1 release requires a series of cellular factors to help it bud from the plasma membrane. Among them, TSG101, a cellular factor that functions in the biogenesis of the multivesicular body (MVB), plays a central role [57]. To gain better understanding of MVB biogenesis, 22 candidate human class E proteins were identified by mass spectrometry. Among them, ALIX, also known as (PDCD6IP) programmed cell death 6-interacting protein, were found to play a key role in linking complexes at early and late stages of viral budding. The binding between ALIX and Gag was also identified [58]. A later proteomic analysis specifically showed that the Gag p6 domain contains a region that binds ALIX. And most interestingly, ALIX also interacts with Tsg101 [59]. Taken together, these observations identified ALIX as a critical component of the viral budding machinery. Although the study subject and focus varies among the aforementioned four representative proteomics studies, they all used viral Gag protein as the “bait” to isolate the potential host protein targets and verified the protein identities with mass spectrometry. This approach has proved very effective in studying the interactions between Gag and host proteins.

Alternatively, there are some cases in which the known host protein(s), works as a linker between viral protein(s) and cellular factors yet to be discovered, can also serve as “bait”. These proteins are usually well-characterized, have proved binding with viral protein(s) and also comprise potential high affinity to cellular factors that need to be discovered. A good example is a study on the Staufen1 ribonucleoprotein (RNP) proteome. Staufen1, a double-stranded RNA-binding protein [60], along with Gag is a prominent part of the RNP, which interacts with viral genomic RNA (vRNA) during the process of export from the nucleus to the cytoplasm for the synthesis of viral proteins [61–62]. Staufen1-binding proteins were purified using TAP from HIV-1 infected cells and identified by mass spectrometry. Four viral proteins, including Gag, Gag/Pol, as well as over 200 host proteins, were identified in these RNPs. Moreover, HIV-1 induces both qualitative and quantitative differences in host protein content in these RNPs. This comprehensive view of Staufen1-containing HIV-1 RNPs and their molecular characterization will lead to the elucidation of the function of these RNPs in the transport of Gag, and the fate of the vRNA in host cells [63].

2. The pol (DNA polymerase) gene encodes Pol polypeptide, which is processed into protease (PR), reverse transcriptase (RT), integrase (IN) and RNase H [64]. HIV-1 PR is required to cleave the precursor Gag and Gag-Pol polyprotein during virion maturation [44]. RT is involved in making a double-stranded DNA from the HIV-1 genomic RNA template [65]. IN is necessary to integrate the proviral double stranded DNA into the host genome [66].

A classical example of discovering important Pol associated host proteins by proteomics was carried out by Cherepanov et al in 2003. They co-immunoprecipitated the HIV-1 integrase (IN) complexes from cells expressing FLAG-tagged IN. The IN was found to be associated with a 76 kDa protein, which was later identified by LC-MS/MS as LEDGF/p75. The IN and LEDGF/p75 were further found to be co-localized in the nuclei [6]. Numerous later studies have shown that LEDGF/p75 is essential for strand transfer activity of viral IN [66–67]. Before it integrates into the host genome, HIV-1 must convert to double stranded DNA. This process is catalyzed by viral RT with the help of host cell tRNA Lys as a primer [68]. Lysyl-tRNA synthetase (KRS), a tRNA Lys -binding protein, was found to be packaged into newly formed HIV-1 virions [69]. Combining co-immunoprecipitation with an MS-based functional proteomics approach, Meerschaert et al found that Syntenin-1, a tandem PDZ protein, directly interacts with KRS and modulates its activity [70]. Evidence showed that Syntenin-1 also plays a regulatory role in the dynamics of HIV-1 entry [71]. Coupled with a novel in vivo biotinylation and capture approach [72], Schweitzer et al employed mass spectrometry to analyze affinity purified HIV-1 integrase and matrix protein complexes. Leucine-rich PPR-motif containing (LRPPRC) protein, a cellular protein involved in mitochondrial function, cell metabolism, and cell-cycle progression was found to be associated with HIV-1 during the early steps of infection. They identified LRPPRC as a cellular factor that is involved in HIV-1 replication through multiple mechanisms [73].

In addition to cellular factor discovery, coupled with molecular modeling experiments, mass spectrometry was also applied to uncover novel intra- and inter-protein-protein contacts in the full-length IN-LEDGF complex. It was revealed that LEDGF stabilized these contacts, promoted IN tetramerization and in turn, this IN tetramer interface is important for enzymatic activities and high affinity binding [74].

Unlike other viral proteins, Protease is distinctive. Rather than forming stable or loose contacts with host proteins, it degrades proteins. It is well known that Protease is essential for the maturation and infectivity of HIV-1 [64, 75]. Although there were some reports regarding the cleavage of human proteins by Protease [76–77], global scale HIV-1 Protease substrate study is lacking. Impens et al used positional proteomics (SILAC coupled with COFRADIC proteomics technology [78]) to identify protein processing by the HIV-1 Protease. Over 120 cellular HIV-1 Protease substrates, including previously reported, as well as novel Protease substrates, were identified. Cleavage site alignments revealed a specificity profile well correlated with previous studies [79]. This unique degradomics study provided a list of interesting host proteins that need further molecular dissection.

Sometimes, a detailed identification and characterization of HIV-1 associated host proteins is hindered by the technical limitation of acquiring a sufficient amount of functional material. This is especially the case for studying cellular factors associated with catalytically active PIC (Pre-integration complex). To overcome this problem, Raghavendra et al used biotinylated target DNA to isolate catalytically active PICs, and the proteins selectively co-purified with PICs were then subjected to MS. Their study revealed at least 19 host proteins that are associated with HIV-1 PICs, 18 of which have not been previously described with respect to HIV-1 integration. Molecular functions of the identified proteins range from chromatin organization to protein transport [80].

3. The env (Envelope) gene codes for a precursor protein (gp160), which is processed to give an external glycoprotein gp120 and a transmembrane glycoprotein gp41 [2]. Gp120 mediates the interaction between HIV-1 and CD4 receptors on the surface of host cells [81], while gp41 enables the fusion of viral and host membranes, thus allowing the delivery of virion contents into the newly infected cells [82].

Like many other proteins expressed on the cell surface, gp120 and gp41 are extensively glycosylated. This glycosylation is important for their immunogenicity and structural integrity [83]. A mass spectrometry based approach has been used to study inter-clade and intra-clade glycosylation variations [84], the structure [85], and the site-specific N-linked glycosylation of gp120 [86]. However, very few proteomic analyses have been done on Env associated cellular factors. In search of the molecular mechanisms of Env induced bystander apoptotic effects, Molina et al did comparative proteomic analyses on two types of uninfected immune cells after their co-culture with cells that expressed, or not, Env. They found over 30 proteins were differentially expressed. The majority of these proteins are involved in various processes, such as degradation, and linked to mitochondrial functions [87]. More MS based investigations on Env host proteins are clearly needed, as identification of novel host proteins essential to this early recognition process could provide effective and economical therapeutic targets to block the virus at the beginning of infection.

4. Essential regulatory elements

(1). Rev (Regulator of expression of virion proteins) is a 116-residue (13 kDa) RNA-binding protein. Rev shuttles between the nucleus and the cytoplasm, mediating the nuclear export of unspliced viral RNA through interaction with the Rev Response Element (RRE) in viral RNA [88–89], and is thus essential for synthesis of major viral proteins in cytoplasm.

Together with Rev, host cellular proteins, including eukaryotic initiation factor 5A (eIF-5A) [90], and some nucleoporins [91], have been found to participate in viral RNA export. Naji et al employed multidimensional protein identification technology (MudPIT) [92] and statistical analysis to study the host cell interactome of HIV-1 Rev. From 250 significant candidates, Eight DEAD/H box proteins of the RNA helicase family, a top ranking group, were further validated. Among them, DDX3X, DDX5, DDX17, or DDX21 distinctively impact HIV-1 viral expression level and cellular distribution. This study suggests host DEAD proteins play an important role in viral mRNA production [93]. Kula et al generated cell lines harboring an integrated provirus carrying RNA binding sites for the MS2 protein. Flag-tagged MS2 was then used for affinity purification of RNP. Nuclear matrix component MATR3 (Matrin 3) was also shown to bind viral RNA and to be required for the Rev/RRE mediated nuclear export of unspliced HIV-1 RNAs [94]. In another proteomic study, the hnRNP E family of proteins was recognized as a modulator of viral RNA metabolism and expression. The modulation of hnRNP E1 expression alters Rev Expression [95]. After transcription, HIV-1 pre-mRNA needs to be spliced into different forms. Viral RNA and multiple cellular factors are believed to be involved in these processes [96]. 33 host proteins were identified by a study using affinity chromatography coupled LC-MS/MS. Among them, hnRNP K, was confirmed as a participant in this RNA splicing process by various biochemical analyses [97].

(2). Tat (Trans-activator of transcription) is a viral regulatory protein that functions primarily to enhance the efficiency of HIV-1 transcription [98] and mediate pleiotropic effects on various other viral functions [99]. Two forms (Tat-1 exon of 72-residues and Tat-2 exon of 86–101-residues) of Tat are expressed. Uniquely, Tat binds to stem-loop secondary structure (the transactivation response element, TAR) at the 5' terminus of HIV-1 RNAs to activate viral transcription [100].

Coiras et al started to examine the effects of the Tat protein on cellular gene expression by using 2-D DIGE (Differential in Gel Electrophoresis) based MS analysis. They identified decreased expression of several cytoskeletal proteins such as actin, beta-tubulin, and annexin II, as well as gelsolin, cofilin and the Rac/Rho-GDI complex [101]. Human nucleosome assembly protein-1 (hNAP-1), a histone chaperone, was also identified as a novel cellular protein interacting with HIV-1 Tat. The expression level of hNAP-1 is positively correlated with Tat-mediated viral expression [102]. Taking an immunoaffinity based MS/MS approach, a study conducted by Timani et al showed Y-box binding protein 1 (YB-1) potentiates the Tip110/Tat-mediated transactivation of the HIV-1 LTR promoter [103].

In order to obtain a comprehensive nuclear interaction map of Tat in T-cells, Gautier et al designed a proteomic strategy based on affinity chromatography coupled with MS. They identified a total of 183 candidates as Tat nuclear partners, 90% of which have not been previously characterized. Interactome analyses showed that their dataset is enriched for domains mediating protein, RNA and DNA interactions, and helicase and ATPase activities [104]. To further dissect the interaction between Tat and the nucleolus, where viral transcription takes place, Jarbouli et al investigated the nucleolar proteome of Jurkat T-cells stably expressing tagged Tat. Using an SILAC based organelle proteomics approach, they found 49 proteins showing significant changes in abundance upon Tat expression. Pathway analysis and network reconstruction suggested that Tat expression in the nucleolus favors host biosynthetic activities and may contribute to the creation of a cellular environment supporting robust HIV-1 production [105].

Tat released by infected cells can affect bystander uninfected cells and induce various biological responses. Liao et al conducted a comprehensive investigation of Tat-related metabolic changes in Jurkat cells using combined gas chromatography-MS, reversed-phase LC-MS and a hydrophilic interaction LC-MS based metabolomics approach. They revealed that HIV-1 Tat caused significant and comprehensive metabolic changes, as represented by significant changes of 37 metabolites. Ten relevant enzymes in HIV-1 Tat-treated cells and 11 pathways were also acutely perturbed [106]. Assisted by LC-MS/MS, a separate study found that intracellular expression of HIV-1 Tat in T cells stabilized the mitochondrial membrane and reduced caspase activation. This discovery suggested that Tat-mediated protection against apoptosis may be a mechanism for HIV-1 persistence [107].

Finally, it is also known that posttranslational modification of Tat affects its activity on viral transcription. Berro et al used streptavidin bead pull-down assay coupled with MALDI-TOF to investigate proteins that preferentially bound acetylated Tat. P32, among a number of proteins, was identified and found to associate with viral splicing inhibition induced by Tat acetylation [108].

Taken together, mass spectrometry based methods have been successfully applied to identify and characterize the corresponding host binding partners in different aspects of Tat function, ranging from metabolic changes to bystander effects.

5. Accessory regulatory proteins

1. Nef (Negative regulatory factor) is a 206-residue (27 kDa) N-terminally myristoylated protein [109]. It has multiple functions that include modulation of cellular signaling pathways, alteration of intracellular trafficking and enhancement of viral replication and infectivity [110].

As a multifaceted modulator, Nef must interact with multiple cellular factors in order to exert its effects [111]. To dissect the underlying interactions, by analyzing immune-purified Nef-associated protein complex with mass spectrometry, Janardhan et al identified the association of Nef with DOCK2-ELMO1, a key activator of Rac. This association enables Nef to influence multiple aspects of cell function through Rac regulated chemokine- and antigen-initiated signaling pathways [112].

Nef is known for stimulating the formation of tunneling nanotubes and virological synapses, and can be transferred to bystander cells via intercellular contacts/ extracellular vehicles (EVs). To find the fundamental mechanism, Mukerji et al employed LC-MS/MS to analyze Nef immunocomplexes from Jurkat cells expressing wild-type Nef or its mutants. Only the wild-type Nef was associated with the exocyst complex, which regulates exocytosis and recruits proteins required for nanotube formation. Further biochemical verifications and bioinformatics analyses suggested that exocyst complex proteins are likely a key effector of Nef-mediated enhancement of nanotube formation/ microvesicle secretion [113]. On the other hand, the existence of secretion modification region (SMR; AA₆₆₋₇₀) on Nef itself is also required for Nef to be secreted. The secreted Nef can induce apoptosis in uninfected cells, and understanding this significant event could be a key insight for HIV-1 treatment. By using MS/MS and co-immunoprecipitation with a novel SMR-based peptide that blocks Nef secretion and HIV-1 virus release, Shelton et al identified mortalin as an SMR-specific cellular protein [114].

Nef is also recognized for increasing viral infectivity as Nef-deleted viruses cannot efficiently complete the early steps of replication. Bregnard et al combined DIGE and iTRAQ to identify differences between the proteomes of WT and Nef-deleted viruses. Host protein Ezrin and EHD4 were discovered to be involved in the ability of Nef to increase virus infectivity. Molecular dissection showed that Ezrin behaves as an inhibitory factor counteracted by Nef, while EHD4 is a cofactor required by Nef to increase virus infectivity [115].

The cellular factors, especially those participate in Nef's intracellular trafficking, might serve as attractive targets to "confine" HIV-1 infection and lead to eventual clearance of the viruses by host immune system.

2. Vif (Viral infectivity factor) is a 192-residue (typically 23 kDa) protein that is important for the infectivity of HIV-1 in certain cell types [2]. One significant role of Vif is to disrupt

the antiviral activity of the cellular protein APOBEC3G (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G) by targeting it via cellular Cullin5 E3 ubiquitin ligase for proteasomal degradation [116]. In the absence of Vif, APOBEC3G causes a detrimental level of mutation in the nascent viral genome [117–118].

A series of topological studies employing mass spectrometry were carried out to find the highly adaptive structures of Vif. Combining chemical cross-linking and mass spectrometry, Auclair et al found that HIV-1 Vif is in a dynamic equilibrium between the various oligomers potentially allowing it to interact with other binding partners [119]. With the help of hydrogen exchange mass spectrometry (HX MS), Marcsisin et al first found that a short fragment of Vif, consisting of the viral suppressor of cytokine signaling box, was likely responsible for interacting with Elongin BC [120]. Later, HX MS analysis of Vif alone from the same group revealed that the N-terminal portion of Vif contains structured or protected elements, while the C-terminal portion of Vif is unstructured in the absence of cellular interacting proteins. Several regions within Vif are likely binding sites for cellular factors [121]. These structural studies showed the power of using HX MS to dissect proteins that are difficult to study using X-ray crystallography and/or nuclear magnetic resonance.

In terms of finding critical host Vif binding partners, mass spectrometry also played a significant role. One early work performed by Yu et al revealed, through Immunoprecipitation coupled with MALDI-TOF identification, that Vif interacts with cellular proteins Cullin5, Elongins B and C, and Rbx1 to form an Skp1-cullin-F-box (SCF)-like complex; this complex is essential for Vif induced ubiquitination and degradation of APOBEC3G [122]. Recently, Jager et al, using an affinity tag/purification plus mass spectrometry, revealed that Vif additionally recruits the transcription cofactor CBF-beta to the ubiquitin ligase complex [123]. They also demonstrated that CBF-beta is required for Vif-mediated degradation of APOBEC3G.

3. Vpr (Viral protein R) is 96 -residue (14 kDa) nucleocytoplasmic shuttling regulatory protein. It facilitates nuclear import of the preintegration complex [124]. Vpr also induce host cells to arrest in the G2 phase [125]. It is recognized that Vpr is essential for viral infection itself, but how the host responds is unclear. A SILAC based LC-MS/MS analysis showed that Vpr over-expression in macrophages significantly altered over 20% proteins that were quantified. The notable enrichment of enzymes in the glycolytic pathway suggested that HIV-1 hijacks the glucose metabolism pathway to facilitate viral replication and biogenesis [126].

4. Vpu (Viral protein unique) is a 81-residue (16 kDa) oligomeric integral membrane phosphoprotein [2]. It has two major biological functions: induction of the degradation of CD4 through the ubiquitin proteasome pathway [127] and enhancement of virion release from the surface of an infected cell [128]. In the search of additional Vpu targets, Douglas et al performed SILAC based quantitative proteomics analyses using the plasma membrane fraction of HeLa cells expressing either wild-type Vpu or a Vpu mutant. BST-2 (bone marrow stromal antigen 2, also known as Tetherin or CD317), which had been shown to impede viral release by retaining newly budded HIV-1 virions on the surface of cells [129], was underrepresented in the wild-type Vpu proteome [130]. Similar to Vpu's role in CD4

degradation, acting as an adapter linking BST-2 to the host ubiquitination machinery via beta transducin repeat-containing protein (β TrCP), Vpu leads to the degradation of BST-2, thereby weakening the block to release of nascent virions [131]. We can expect that Vpu uses a similar mechanism to degrade unexplored host proteins in order to maintain HIV-1's infectivity.

In all, a large amount of information has been gained about the interaction of viral proteins and the host proteome. However, considering the complex nature of these interactions (sometimes involving counteractions between the host and certain viral accessory proteins), more host proteins essential in HIV-1/host interaction still need to be discovered and characterized in order for us to have a better understanding of this process. Mass spectrometry can play a central role in this discovery process.

GENERAL COMMENTS

Mass spectrometry (MS) based identification of the components of protein complexes has become one of the most powerful and routinely used technologies for high-throughput detection of protein interactions [132]. The studies of protein interactions by MS give insights into protein function, binding partners and cellular pathways [133]. Several popular MS based approaches have been applied to the study of HIV-1, host proteins and their interactions. These approaches include co-immunoprecipitation (Co-IP) [134], tandem affinity purification (TAP) [135], and quantitative stable isotope label, such as iTRAQ [136], SILAC [137], and label-free, e.g MRM (multiple reaction monitoring) [138]. Co-IP works by isolating larger complexes out of solution by using an antibody against a known protein that is a member of the complex. Through Co-IP, unknown proteins are isolated in their native states. However, this method is only good for relatively stable interactions and can not specify whether interaction is direct. TAP is commonly referred as a two-step affinity purification method that involves creating a fusion protein with a TAP tag. This method is relatively simple and can be done quantitatively; however, there is a possibility that the added TAP tag might obscure protein binding, and this method is also unsuitable for screening transient protein interactions. Nevertheless, without requiring prior knowledge of the likely interacting partners, both Co-IP and TAP are powerful techniques that have been widely used to study HIV-1/host protein interactions. In addition to isolation, proteins often need to be labeled before mass spectrometric analysis. Chemical labeling based iTRAQ is a powerful method that can be used to determine the amount of protein from multiple sources in a single experiment. However, time-constraints and labeling variability hinder its wide usage. On the other hand, metabolic based SILAC is simple to perform and has low technical variation. SILAC has been widely adopted since its introduction, but also has its limitations. It is best for labeling actively proliferative cell lines, less suitable for primary cells. The label-free MRM method has been commonly used for the targeted quantitation of proteins/peptides in biological samples. Eventually, proteome(s) needs to be identified, characterized and possibly quantified. Helped by rapid advances in mass spectrometry instruments and analysis tools, LC-MS/MS is a fairly standard approach for nowadays proteomic research. It offers high resolution and low variability.

It is encouraging that proteomic studies on HIV-1 have greatly improved our understanding of host proteins and their relations with the virus and we can expect that proteomics will continue to generate new insights on HIV-1. However, we need to recognize that there are some challenges which need to be addressed: 1. variations among proteomic samples (studies). To increase the chance of identifying valid targets, it would be useful to perform analogous experiments with different methods and back up with stringent technical controls. 2. The abundance of the host proteins in the samples. Less abundant proteins are obviously harder to identify, but one often neglected aspect is that some proteins may evade identification just because their relative abundance is overwhelmed by the complexity of the samples. Increasing sample input, optimizing mass spectrometric instruments and pre-fractionation of complex samples are some of the steps to address this issue. 3. An even more practical issue is how to translate many snapshot datasets obtained from various sources into a mechanistic understanding of HIV-1 /host protein interactions.

On the other hand, beside proteomics, various tools are available to define the biological relevance of proteins, including western blotting, immunoprecipitation, and immunohistochemistry. In addition, RNA interference screens and animal models can also be used to complement proteomic discovery. As each method has its strength and limitations, it is wise to choose them carefully. For example: RNA interference allows rapid examination of the biological consequences of depleting a particular gene product in an organism and it can be accomplished in a high throughput and unbiased manner. However, it has its own caveats: a. depletion of essential host proteins lead to cytotoxicity; b. off-target effects associated with false positives and negatives. Clearly, multiple experimental strategies are needed to ultimately insure the biological significance of host proteins.

CONCLUDING REMARKS

A recent comprehensive proteomic study on interacting cellular factors with all 18 tagged HIV-1 proteins provides a blueprint of how to combine proteomic screening with targeted biochemical verifications [139]. A few highlights can be learned from this research: (1) employing a proved affinity tagging and purification coupled mass spectrometry method; (2) minimizing variations by cross-checking two different cell lines; (3) combining rigorous statistical analysis to achieve high confidence in identification and quantification; (4) performing thorough biochemical studies on high ranking targets.

It is obvious that proteomic approaches have provided powerful insights into the interactions between HIV-1 and its hosts. By incorporating proteomic and traditional biochemical studies, optimizing the pipeline composed of high throughput proteomic and targeted non-proteomic approaches, various hurdles can be overcome. And we can expect, by taking the aforementioned approaches, a better understanding of how HIV-1 exploits host proteomes to complete its lifecycle can be achieved.

ACKNOWLEDGMENTS

Work in the author's laboratory is supported by Lifespan Pilot Research Fund (#701-5857), Rhode Island Foundation Medical Research Grant (#20133969) and NIH COBRE URI/RIH Pilot Research Grant (P20GM104317). I apologize to the authors of studies that were not cited due to space constraints.

REFERENCES

1. Clavel F, Hance AJ. HIV drug resistance. *N Engl J Med*. 2004; 350:1023–1035. [PubMed: 14999114]
2. Frankel AD, Young JA. HIV-1: fifteen proteins and an RNA. *Annu Rev Biochem*. 1998; 67:1–25. [PubMed: 9759480]
3. Friedrich BM, Dziuba N, Li G, Endsley MA. Host factors mediating HIV-1 replication. *Virus Res*. 2011; 161:101–114. [PubMed: 21871504]
4. Sattentau QJ, Weiss RA. The CD4 antigen: physiological ligand and HIV receptor. *Cell*. 1988; 52:631–633. [PubMed: 2830988]
5. Moore JP, Kitchen SG, Pugach P, Zack JA. The CCR5 and CXCR4 coreceptors--central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Res Hum Retroviruses*. 2004; 20:111–126. [PubMed: 15000703]
6. Cherepanov P, Maertens G, Proost P, Devreese B, et al. HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. *J Biol Chem*. 2003; 278:372–381. [PubMed: 12407101]
7. Llano M, Saenz DT, Meehan A, Wongthida P, et al. An essential role for LEDGF/p75 in HIV integration. *Science*. 2006; 314:461–464. [PubMed: 16959972]
8. Wei P, Garber ME, Fang SM, Fischer WH, Jones KA. A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. *Cell*. 1998; 92:451–462. [PubMed: 9491887]
9. Garrus JE, von Schwedler UK, Pornillos OW, Morham SG, et al. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell*. 2001; 107:55–65. [PubMed: 11595185]
10. Flexner C. HIV drug development: the next 25 years. *Nat Rev Drug Discov*. 2007; 6:959–966. [PubMed: 17932493]
11. Dorr P, Westby M, Dobbs S, Griffin P, et al. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. *Antimicrob Agents Chemother*. 2005; 49:4721–4732. [PubMed: 16251317]
12. Brass AL, Dykxhoorn DM, Benita Y, Yan N, et al. Identification of host proteins required for HIV infection through a functional genomic screen. *Science*. 2008; 319:921–926. [PubMed: 18187620]
13. Konig R, Zhou Y, Elleder D, Diamond TL, et al. Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication. *Cell*. 2008; 135:49–60. [PubMed: 18854154]
14. Zhou H, Xu M, Huang Q, Gates AT, et al. Genome-scale RNAi screen for host factors required for HIV replication. *Cell Host Microbe*. 2008; 4:495–504. [PubMed: 18976975]
15. Bushman FD, Malani N, Fernandes J, D'Orso I, et al. Host cell factors in HIV replication: meta-analysis of genome-wide studies. *PLoS Pathog*. 2009; 5:e1000437. [PubMed: 19478882]
16. Aebersold R, Mann M. Mass spectrometry-based proteomics. *Nature*. 2003; 422:198–207. [PubMed: 12634793]
17. Han X, Aslanian A, Yates JR 3rd. Mass spectrometry for proteomics. *Curr Opin Chem Biol*. 2008; 12:483–490. [PubMed: 18718552]
18. Ringrose JH, Jeeninga RE, Berkhout B, Speijer D. Proteomic studies reveal coordinated changes in T-cell expression patterns upon infection with human immunodeficiency virus type 1. *J Virol*. 2008; 82:4320–4330. [PubMed: 18287243]
19. Chan EY, Qian WJ, Diamond DL, Liu T, et al. Quantitative analysis of human immunodeficiency virus type 1-infected CD4+ cell proteome: dysregulated cell cycle progression and nuclear transport coincide with robust virus production. *J Virol*. 2007; 81:7571–7583. [PubMed: 17494070]
20. Rasheed S, Yan JS, Lau A, Chan AS. HIV replication enhances production of free fatty acids, low density lipoproteins and many key proteins involved in lipid metabolism: a proteomics study. *PLoS One*. 2008; 3:e3003. [PubMed: 18714345]

21. Kraft-Terry SD, Engebretsen IL, Bastola DK, Fox HS, et al. Pulsed stable isotope labeling of amino acids in cell culture uncovers the dynamic interactions between HIV-1 and the monocyte-derived macrophage. *J Proteome Res.* 2011; 10:2852–2862. [PubMed: 21500866]
22. Chan EY, Sutton JN, Jacobs JM, Bondarenko A, et al. Dynamic host energetics and cytoskeletal proteomes in human immunodeficiency virus type 1-infected human primary CD4 cells: analysis by multiplexed label-free mass spectrometry. *J Virol.* 2009; 83:9283–9295. [PubMed: 19587052]
23. Navare AT, Sova P, Purdy DE, Weiss JM, et al. Quantitative proteomic analysis of HIV-1 infected CD4+ T cells reveals an early host response in important biological pathways: protein synthesis, cell proliferation, and T-cell activation. *Virology.* 2012; 429:37–46. [PubMed: 22542004]
24. Wojcechowskyj JA, Lee JY, Seeholzer SH, Doms RW. Quantitative phosphoproteomics of CXCL12 (SDF-1) signaling. *PLoS One.* 2011; 6:e24918. [PubMed: 21949786]
25. Wojcechowskyj JA, Didigu CA, Lee JY, Parrish NF, et al. Quantitative phosphoproteomics reveals extensive cellular reprogramming during HIV-1 entry. *Cell Host Microbe.* 2013; 13:613–623. [PubMed: 23684312]
26. Britton LM, Sova P, Belisle S, Liu S, et al. A proteomic glimpse into the initial global epigenetic changes during HIV infection. *Proteomics.* 2014
27. Schweitzer CJ, Jagadish T, Haverland N, Ciborowski P, Belshan M. Proteomic analysis of early HIV-1 nucleoprotein complexes. *J Proteome Res.* 2013; 12:559–572. [PubMed: 23282062]
28. DeBoer J, Jagadish T, Haverland NA, Madson CJ, et al. Alterations in the nuclear proteome of HIV-1 infected T-cells. *Virology.* 2014; 468:409–420. [PubMed: 25240327]
29. Monette A, Pante N, Moulard AJ. HIV-1 remodels the nuclear pore complex. *J Cell Biol.* 2011; 193:619–631. [PubMed: 21576391]
30. Ciborowski P, Kadiu I, Rozek W, Smith L, et al. Investigating the human immunodeficiency virus type 1-infected monocyte-derived macrophage secretome. *Virology.* 2007; 363:198–209. [PubMed: 17320137]
31. Kadiu I, Ricardo-Dukelow M, Ciborowski P, Gendelman HE. Cytoskeletal protein transformation in HIV-1-infected macrophage giant cells. *J Immunol.* 2007; 178:6404–6415. [PubMed: 17475870]
32. Nguyen DG, Booth A, Gould SJ, Hildreth JE. Evidence that HIV budding in primary macrophages occurs through the exosome release pathway. *J Biol Chem.* 2003; 278:52347–52354. [PubMed: 14561735]
33. Kadiu I, Narayanasamy P, Dash PK, Zhang W, Gendelman HE. Biochemical and biologic characterization of exosomes and microvesicles as facilitators of HIV-1 infection in macrophages. *J Immunol.* 2012; 189:744–754. [PubMed: 22711894]
34. Li M, Aliotta JM, Asara JM, Tucker L, et al. Quantitative proteomic analysis of exosomes from HIV-1-infected lymphocytic cells. *Proteomics.* 2012; 12:2203–2211. [PubMed: 22807456]
35. Hatzioannou T, Perez-Caballero D, Cowan S, Bieniasz PD. Cyclophilin interactions with incoming human immunodeficiency virus type 1 capsids with opposing effects on infectivity in human cells. *J Virol.* 2005; 79:176–183. [PubMed: 15596813]
36. Willetts KE, Rey F, Agostini I, Navarro JM, et al. DNA repair enzyme uracil DNA glycosylase is specifically incorporated into human immunodeficiency virus type 1 viral particles through a Vpr-independent mechanism. *J Virol.* 1999; 73:1682–1688. [PubMed: 9882380]
37. Zhang F, Zang T, Wilson SJ, Johnson MC, Bieniasz PD. Clathrin facilitates the morphogenesis of retrovirus particles. *PLoS Pathog.* 2011; 7:e1002119. [PubMed: 21738476]
38. Thali M, Bukovsky A, Kondo E, Rosenwirth B, et al. Functional association of cyclophilin A with HIV-1 virions. *Nature.* 1994; 372:363–365. [PubMed: 7969495]
39. Misumi S, Fuchigami T, Takamune N, Takahashi I, et al. Three isoforms of cyclophilin A associated with human immunodeficiency virus type 1 were found by proteomics by using two-dimensional gel electrophoresis and matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Virol.* 2002; 76:10000–10008. [PubMed: 12208976]
40. Chertova E, Chertov O, Coren LV, Roser JD, et al. Proteomic and biochemical analysis of purified human immunodeficiency virus type 1 produced from infected monocyte-derived macrophages. *J Virol.* 2006; 80:9039–9052. [PubMed: 16940516]

41. Linde ME, Colquhoun DR, Ubaida Mohien C, Kole T, et al. The conserved set of host proteins incorporated into HIV-1 virions suggests a common egress pathway in multiple cell types. *J Proteome Res.* 2013; 12:2045–2054. [PubMed: 23432411]
42. Freed EO. HIV-1 gag proteins: diverse functions in the virus life cycle. *Virology.* 1998; 251:1–15. [PubMed: 9813197]
43. Bell NM, Lever AM. HIV Gag polyprotein: processing and early viral particle assembly. *Trends Microbiol.* 2013; 21:136–144. [PubMed: 23266279]
44. Sundquist WI, Krausslich HG. HIV-1 assembly, budding, and maturation. *Cold Spring Harb Perspect Med.* 2012; 2:a006924. [PubMed: 22762019]
45. VerPlank L, Bouamr F, LaGrassa TJ, Agresta B, et al. Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag). *Proc Natl Acad Sci U S A.* 2001; 98:7724–7729. [PubMed: 11427703]
46. Cen S, Guo F, Niu M, Saadatmand J, et al. The interaction between HIV-1 Gag and APOBEC3G. *J Biol Chem.* 2004; 279:33177–33184. [PubMed: 15159405]
47. Stremlau M, Perron M, Lee M, Li Y, et al. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5 α restriction factor. *Proc Natl Acad Sci U S A.* 2006; 103:5514–5519. [PubMed: 16540544]
48. Engeland CE, Brown NP, Borner K, Schumann M, et al. Proteome analysis of the HIV-1 Gag interactome. *Virology.* 2014; 460–461. 194–206.
49. Emdad L, Das SK, Dasgupta S, Hu B, et al. AEG-1/MTDH/LYRIC: signaling pathways, downstream genes, interacting proteins, and regulation of tumor angiogenesis. *Adv Cancer Res.* 2013; 120:75–111. [PubMed: 23889988]
50. Engeland CE, Oberwinkler H, Schumann M, Krause E, et al. The cellular protein lyric interacts with HIV-1 Gag. *J Virol.* 2011; 85:13322–13332. [PubMed: 21957284]
51. Li J, Tang H, Mullen TM, Westberg C, et al. A role for RNA helicase A in post-transcriptional regulation of HIV type 1. *Proc Natl Acad Sci U S A.* 1999; 96:709–714. [PubMed: 9892698]
52. Bolinger C, Sharma A, Singh D, Yu L, Boris-Lawrie K. RNA helicase A modulates translation of HIV-1 and infectivity of progeny virions. *Nucleic Acids Res.* 2010; 38:1686–1696. [PubMed: 20007598]
53. Roy BB, Hu J, Guo X, Russell RS, et al. Association of RNA helicase a with human immunodeficiency virus type 1 particles. *J Biol Chem.* 2006; 281:12625–12635. [PubMed: 16527808]
54. Spitaler M, Cantrell DA. Protein kinase C and beyond. *Nat Immunol.* 2004; 5:785–790. [PubMed: 15282562]
55. McKernan LN, Momjian D, Kulkosky J. Protein Kinase C: One Pathway towards the Eradication of Latent HIV-1 Reservoirs. *Adv Virol.* 2012; 2012:805347. [PubMed: 22500169]
56. Kudoh A, Takahama S, Sawasaki T, Ode H, et al. The phosphorylation of HIV-1 Gag by atypical protein kinase C facilitates viral infectivity by promoting Vpr incorporation into virions. *Retrovirology.* 2014; 11:9. [PubMed: 24447338]
57. Martin-Serrano J, Zang T, Bieniasz PD. Role of ESCRT-I in retroviral budding. *J Virol.* 2003; 77:4794–4804. [PubMed: 12663786]
58. von Schwedler UK, Stuchell M, Muller B, Ward DM, et al. The protein network of HIV budding. *Cell.* 2003; 114:701–713. [PubMed: 14505570]
59. Strack B, Calistri A, Craig S, Popova E, Gottlinger HG. AIP1/ALIX is a binding partner for HIV-1 p6 and EIAV p9 functioning in virus budding. *Cell.* 2003; 114:689–699. [PubMed: 14505569]
60. Villace P, Marion RM, Ortin J. The composition of Staufen-containing RNA granules from human cells indicates their role in the regulated transport and translation of messenger RNAs. *Nucleic Acids Res.* 2004; 32:2411–2420. [PubMed: 15121898]
61. Mouland AJ, Mercier J, Luo M, Bernier L, et al. The double-stranded RNA-binding protein Staufen is incorporated in human immunodeficiency virus type 1: evidence for a role in genomic RNA encapsidation. *J Virol.* 2000; 74:5441–5451. [PubMed: 10823848]
62. Chatel-Chaix L, Clement JF, Martel C, Beriault V, et al. Identification of Staufen in the human immunodeficiency virus type 1 Gag ribonucleoprotein complex and a role in generating infectious viral particles. *Mol Cell Biol.* 2004; 24:2637–2648. [PubMed: 15024055]

63. Milev MP, Ravichandran M, Khan MF, Schriemer DC, Mouland AJ. Characterization of staufen1 ribonucleoproteins by mass spectrometry and biochemical analyses reveal the presence of diverse host proteins associated with human immunodeficiency virus type 1. *Front Microbiol.* 2012; 3:367. [PubMed: 23125841]
64. Hill M, Tachedjian G, Mak J. The packaging and maturation of the HIV-1 Pol proteins. *Curr HIV Res.* 2005; 3:73–85. [PubMed: 15638725]
65. Hu WS, Hughes SH. HIV-1 reverse transcription. *Cold Spring Harb Perspect Med.* 2012; 2
66. Craigie R, Bushman FD. HIV DNA integration. *Cold Spring Harb Perspect Med.* 2012; 2:a006890. [PubMed: 22762018]
67. Maertens G, Cherepanov P, Pluymers W, Busschots K, et al. LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells. *J Biol Chem.* 2003; 278:33528–33539. [PubMed: 12796494]
68. Jiang M, Mak J, Ladha A, Cohen E, et al. Identification of tRNAs incorporated into wild-type and mutant human immunodeficiency virus type 1. *J Virol.* 1993; 67:3246–3253. [PubMed: 8497049]
69. Cen S, Khorchid A, Javanbakht H, Gabor J, et al. Incorporation of lysyl-tRNA synthetase into human immunodeficiency virus type 1. *J Virol.* 2001; 75:5043–5048. [PubMed: 11333884]
70. Meerschaert K, Remue E, De Ganck A, Staes A, et al. The tandem PDZ protein Syntenin interacts with the aminoacyl tRNA synthetase complex in a lysyl-tRNA synthetase-dependent manner. *J Proteome Res.* 2008; 7:4962–4973. [PubMed: 18839981]
71. Gordon-Alonso M, Rocha-Perugini V, Alvarez S, Moreno-Gonzalo O, et al. The PDZ-adaptor protein syntenin-1 regulates HIV-1 entry. *Mol Biol Cell.* 2012; 23:2253–2263. [PubMed: 22535526]
72. Belshan M, Schweitzer CJ, Donnellan MR, Lu R, Engelman A. In vivo biotinylation and capture of HIV-1 matrix and integrase proteins. *J Virol Methods.* 2009; 159:178–184. [PubMed: 19490971]
73. Schweitzer CJ, Matthews JM, Madson CJ, Donnellan MR, et al. Knockdown of the cellular protein LRPPRC attenuates HIV-1 infection. *PLoS One.* 2012; 7:e40537. [PubMed: 22808186]
74. McKee CJ, Kessl JJ, Shkriabai N, Dar MJ, et al. Dynamic modulation of HIV-1 integrase structure and function by cellular lens epithelium-derived growth factor (LEDGF) protein. *J Biol Chem.* 2008; 283:31802–31812. [PubMed: 18801737]
75. Brik A, Wong CH. HIV-1 protease: mechanism and drug discovery. *Org Biomol Chem.* 2003; 1:5–14. [PubMed: 12929379]
76. Nie Z, Phenix BN, Lum JJ, Alam A, et al. HIV-1 protease processes procaspase 8 to cause mitochondrial release of cytochrome c, caspase cleavage and nuclear fragmentation. *Cell Death Differ.* 2002; 9:1172–1184. [PubMed: 12404116]
77. Castello A, Franco D, Moral-Lopez P, Berlanga JJ, et al. HIV-1 protease inhibits Cap- and poly(A)-dependent translation upon eIF4GI and PABP cleavage. *PLoS One.* 2009; 4:e7997. [PubMed: 19956697]
78. Gevaert K, Goethals M, Martens L, Van Damme J, et al. Exploring proteomes and analyzing protein processing by mass spectrometric identification of sorted N-terminal peptides. *Nat Biotechnol.* 2003; 21:566–569. [PubMed: 12665801]
79. Impens F, Timmerman E, Staes A, Moens K, et al. A catalogue of putative HIV-1 protease host cell substrates. *Biol Chem.* 2012; 393:915–931. [PubMed: 22944692]
80. Raghavendra NK, Shkriabai N, Graham R, Hess S, et al. Identification of host proteins associated with HIV-1 preintegration complexes isolated from infected CD4+ cells. *Retrovirology.* 2010; 7:66. [PubMed: 20698996]
81. Capon DJ, Ward RH. The CD4-gp120 interaction and AIDS pathogenesis. *Annu Rev Immunol.* 1991; 9:649–678. [PubMed: 1910691]
82. Blumenthal R, Durell S, Viard M. HIV entry and envelope glycoprotein-mediated fusion. *J Biol Chem.* 2012; 287:40841–40849. [PubMed: 23043104]
83. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science.* 1998; 280:1884–1888. [PubMed: 9632381]
84. Go EP, Chang Q, Liao HX, Sutherland LL, et al. Glycosylation site-specific analysis of clade C HIV-1 envelope proteins. *J Proteome Res.* 2009; 8:4231–4242. [PubMed: 19610667]

85. Wang L, Qin Y, Ilchenko S, Bohon J, et al. Structural analysis of a highly glycosylated and unliganded gp120-based antigen using mass spectrometry. *Biochemistry*. 2010; 49:9032–9045. [PubMed: 20825246]
86. Honarmand Ebrahimi K, West GM, Flefil R. Mass Spectrometry Approach and ELISA Reveal the Effect of Codon Optimization on N-Linked Glycosylation of HIV-1 gp120. *J Proteome Res*. 2014
87. Molina L, Grimaldi M, Robert-Hebmann V, Espert L, et al. Proteomic analysis of the cellular responses induced in uninfected immune cells by cell-expressed X4 HIV-1 envelope. *Proteomics*. 2007; 7:3116–3130. [PubMed: 17676665]
88. Pollard VW, Malim MH. The HIV-1 Rev protein. *Annu Rev Microbiol*. 1998; 52:491–532. [PubMed: 9891806]
89. Cullen BR. Nuclear mRNA export: insights from virology. *Trends Biochem Sci*. 2003; 28:419–424. [PubMed: 12932730]
90. Hofmann W, Reichart B, Ewald A, Muller E, et al. Cofactor requirements for nuclear export of Rev response element (RRE)- and constitutive transport element (CTE)-containing retroviral RNAs. An unexpected role for actin. *J Cell Biol*. 2001; 152:895–910. [PubMed: 11238447]
91. Zolotukhin AS, Felber BK. Nucleoporins nup98 and nup214 participate in nuclear export of human immunodeficiency virus type 1 Rev. *J Virol*. 1999; 73:120–127. [PubMed: 9847314]
92. Washburn MP, Wolters D, Yates JR 3rd. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat Biotechnol*. 2001; 19:242–247. [PubMed: 11231557]
93. Naji S, Ambrus G, Cimermancic P, Reyes JR, et al. Host cell interactome of HIV-1 Rev includes RNA helicases involved in multiple facets of virus production. *Mol Cell Proteomics*. 2012; 11:M111 015313. [PubMed: 22174317]
94. Kula A, Guerra J, Knezevich A, Kleva D, et al. Characterization of the HIV-1 RNA associated proteome identifies MatrIn 3 as a nuclear cofactor of Rev function. *Retrovirology*. 2011; 8:60. [PubMed: 21771346]
95. Woolaway K, Asai K, Emili A, Cochrane A. hnRNP E1 and E2 have distinct roles in modulating HIV-1 gene expression. *Retrovirology*. 2007; 4:28. [PubMed: 17451601]
96. Fukuhara T, Hosoya T, Shimizu S, Sumi K, et al. Utilization of host SR protein kinases and RNA-splicing machinery during viral replication. *Proc Natl Acad Sci U S A*. 2006; 103:11329–11333. [PubMed: 16840555]
97. Marchand V, Santerre M, Aigueperse C, Fouillen L, et al. Identification of protein partners of the human immunodeficiency virus 1 tat/rev exon 3 leads to the discovery of a new HIV-1 splicing regulator, protein hnRNP K. *RNA Biol*. 2011; 8:325–342. [PubMed: 21368586]
98. Debaisieux S, Rayne F, Yezid H, Beaumelle B. The ins and outs of HIV-1 Tat. *Traffic*. 2012; 13:355–363. [PubMed: 21951552]
99. Romani B, Engelbrecht S, Glashoff RH. Functions of Tat: the versatile protein of human immunodeficiency virus type 1. *J Gen Virol*. 2010; 91:1–12. [PubMed: 19812265]
100. Feng S, Holland EC. HIV-1 tat trans-activation requires the loop sequence within tar. *Nature*. 1988; 334:165–167. [PubMed: 3386755]
101. Coiras M, Camafeita E, Urena T, Lopez JA, et al. Modifications in the human T cell proteome induced by intracellular HIV-1 Tat protein expression. *Proteomics*. 2006; 6(Suppl 1):S63–S73. [PubMed: 16526095]
102. Vardabasso C, Manganaro L, Lusic M, Marcello A, Giacca M. The histone chaperone protein Nucleosome Assembly Protein-1 (hNAP-1) binds HIV-1 Tat and promotes viral transcription. *Retrovirology*. 2008; 5:8. [PubMed: 18226242]
103. Timani KA, Liu Y, He JJ. Tip110 interacts with YB-1 and regulates each other's function. *BMC Mol Biol*. 2013; 14:14. [PubMed: 23822148]
104. Gautier VW, Gu L, O'Donoghue N, Pennington S, et al. In vitro nuclear interactome of the HIV-1 Tat protein. *Retrovirology*. 2009; 6:47. [PubMed: 19454010]
105. Jarboui MA, Bidoia C, Woods E, Roe B, et al. Nucleolar protein trafficking in response to HIV-1 Tat: rewiring the nucleolus. *PLoS One*. 2012; 7:e48702. [PubMed: 23166591]

106. Liao W, Tan G, Zhu Z, Chen Q, et al. Combined metabonomic and quantitative real-time PCR analyses reveal systems metabolic changes in Jurkat T-cells treated with HIV-1 Tat protein. *J Proteome Res.* 2012; 11:5109–5123. [PubMed: 23025307]
107. Lopez-Huertas MR, Mateos E, Sanchez Del Cojo M, Gomez-Esquer F, et al. The presence of HIV-1 Tat protein second exon delays fas protein-mediated apoptosis in CD4+ T lymphocytes: a potential mechanism for persistent viral production. *J Biol Chem.* 2013; 288:7626–7644. [PubMed: 23364796]
108. Berro R, Kehn K, de la Fuente C, Pumfery A, et al. Acetylated Tat regulates human immunodeficiency virus type 1 splicing through its interaction with the splicing regulator p32. *J Virol.* 2006; 80:3189–3204. [PubMed: 16537587]
109. O'Neill E, Kuo LS, Krisko JF, Tomchick DR, et al. Dynamic evolution of the human immunodeficiency virus type 1 pathogenic factor, Nef. *J Virol.* 2006; 80:1311–1320. [PubMed: 16415008]
110. Abraham L, Fackler OT. HIV-1 Nef: a multifaceted modulator of T cell receptor signaling. *Cell Commun Signal.* 2012; 10:39. [PubMed: 23227982]
111. Geyer M, Fackler OT, Peterlin BM. Structure--function relationships in HIV-1 Nef. *EMBO Rep.* 2001; 2:580–585. [PubMed: 11463741]
112. Janardhan A, Swigut T, Hill B, Myers MP, Skowronski J. HIV-1 Nef binds the DOCK2-ELMO1 complex to activate rac and inhibit lymphocyte chemotaxis. *PLoS Biol.* 2004; 2:E6. [PubMed: 14737186]
113. Mukerji J, Olivieri KC, Misra V, Agopian KA, Gabuzda D. Proteomic analysis of HIV-1 Nef cellular binding partners reveals a role for exocyst complex proteins in mediating enhancement of intercellular nanotube formation. *Retrovirology.* 2012; 9:33. [PubMed: 22534017]
114. Shelton MN, Huang MB, Ali SA, Powell MD, Bond VC. Secretion modification region-derived peptide disrupts HIV-1 Nef's interaction with mortalin and blocks virus and Nef exosome release. *J Virol.* 2012; 86:406–419. [PubMed: 22013042]
115. Bregnard C, Zamborlini A, Leduc M, Chafey P, et al. Comparative proteomic analysis of HIV-1 particles reveals a role for Ezrin and EHD4 in the Nef-dependent increase of virus infectivity. *J Virol.* 2013; 87:3729–3740. [PubMed: 23325686]
116. Sheehy AM, Gaddis NC, Malim MH. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat Med.* 2003; 9:1404–1407. [PubMed: 14528300]
117. 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.* 2002; 418:646–650. [PubMed: 12167863]
118. Zhang H, Yang B, Pomerantz RJ, Zhang C, et al. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature.* 2003; 424:94–98. [PubMed: 12808465]
119. Auclair JR, Green KM, Shandilya S, Evans JE, et al. Mass spectrometry analysis of HIV-1 Vif reveals an increase in ordered structure upon oligomerization in regions necessary for viral infectivity. *Proteins.* 2007; 69:270–284. [PubMed: 17598142]
120. Marcsisin SR, Engen JR. Molecular insight into the conformational dynamics of the Elongin BC complex and its interaction with HIV-1 Vif. *J Mol Biol.* 2010; 402:892–904. [PubMed: 20728451]
121. Marcsisin SR, Narute PS, Emert-Sedlak LA, Kloczewiak M, et al. On the solution conformation and dynamics of the HIV-1 viral infectivity factor. *J Mol Biol.* 2011; 410:1008–1022. [PubMed: 21763503]
122. Yu X, Yu Y, Liu B, Luo K, et al. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Science.* 2003; 302:1056–1060. [PubMed: 14564014]
123. Jager S, Kim DY, Hultquist JF, Shindo K, et al. Vif hijacks CBF-beta to degrade APOBEC3G and promote HIV-1 infection. *Nature.* 2012; 481:371–375. [PubMed: 22190037]
124. Heinzinger NK, Bukrinsky MI, Haggerty SA, Ragland AM, et al. The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. *Proc Natl Acad Sci U S A.* 1994; 91:7311–7315. [PubMed: 8041786]

125. Jowett JB, Planelles V, Poon B, Shah NP, et al. The human immunodeficiency virus type 1 vpr gene arrests infected T cells in the G2 + M phase of the cell cycle. *J Virol*. 1995; 69:6304–6313. [PubMed: 7666531]
126. Barrero CA, Datta PK, Sen S, Deshmane S, et al. HIV-1 Vpr modulates macrophage metabolic pathways: a SILAC-based quantitative analysis. *PLoS One*. 2013; 8:e68376. [PubMed: 23874603]
127. Margottin F, Bour SP, Durand H, Selig L, et al. A novel human WD protein, h-beta TrCp, that interacts with HIV-1 Vpu connects CD4 to the ER degradation pathway through an F-box motif. *Mol Cell*. 1998; 1:565–574. [PubMed: 9660940]
128. Klimkait T, Strebel K, Hoggan MD, Martin MA, Orenstein JM. The human immunodeficiency virus type 1-specific protein vpu is required for efficient virus maturation and release. *J Virol*. 1990; 64:621–629. [PubMed: 2404139]
129. Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature*. 2008; 451:425–430. [PubMed: 18200009]
130. Douglas JL, Viswanathan K, McCarroll MN, Gustin JK, et al. Vpu directs the degradation of the human immunodeficiency virus restriction factor BST-2/Tetherin via a {beta}TrCP-dependent mechanism. *J Virol*. 2009; 83:7931–7947. [PubMed: 19515779]
131. Mangeat B, Gers-Huber G, Lehmann M, Zufferey M, et al. HIV-1 Vpu neutralizes the antiviral factor Tetherin/BST-2 by binding it and directing its beta-TrCP2-dependent degradation. *PLoS Pathog*. 2009; 5:e1000574. [PubMed: 19730691]
132. Mann M, Hendrickson RC, Pandey A. Analysis of proteins and proteomes by mass spectrometry. *Annu Rev Biochem*. 2001; 70:437–473. [PubMed: 11395414]
133. Ranish JA, Yi EC, Leslie DM, Purvine SO, et al. The study of macromolecular complexes by quantitative proteomics. *Nat Genet*. 2003; 33:349–355. [PubMed: 12590263]
134. Bonifacino JS, Dell'Angelica EC, Springer TA. Immunoprecipitation. *Curr Protoc Mol Biol*. 2001; Chapter 10(Unit 10):16. [PubMed: 18265056]
135. Krogan NJ, Cagney G, Yu H, Zhong G, et al. Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature*. 2006; 440:637–643. [PubMed: 16554755]
136. Ross PL, Huang YN, Marchese JN, Williamson B, et al. Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. *Mol Cell Proteomics*. 2004; 3:1154–1169. [PubMed: 15385600]
137. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, et al. Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Mol Cell Proteomics*. 2002; 1:376–386. [PubMed: 12118079]
138. Anderson L, Hunter CL. Quantitative mass spectrometric multiple reaction monitoring assays for major plasma proteins. *Mol Cell Proteomics*. 2006; 5:573–588. [PubMed: 16332733]
139. Jager S, Cimermancic P, Gulbahce N, Johnson JR, et al. Global landscape of HIV-human protein complexes. *Nature*. 2012; 481:365–370. [PubMed: 22190034]

Figure 1.

Selected HIV-1 host proteins discovered by mass spectrometry based approaches and their associations with viral proteins. Viral protein is shown in blue, host protein is shown in black and the arrow indicates their established association. Gag, group-specific antigen; Pol, DNA polymerase; Env, envelope; Rev, regulator of expression of virion proteins; Tat, transactivator of transcription; Nef, negative regulatory factor; Vif, viral infectivity factor; Vpr, viral protein R; Vpu, viral protein unique. The complete host protein names are listed in Table 1.

Table 1

Proteomic strategies employed in discovering HIV-1 interacting host proteins. The specific proteomic methods, the viral proteins and their associated host proteins (the abbreviated and complete names) are listed. The reference for each study is also given. AP, affinity purification; TAP, tandem affinity purification; Co-IP, co-immunoprecipitation; MALDI, matrix-assisted laser desorption/ionization; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MudPIT, multidimensional protein identification technology; DIGE, differential in gel electrophoresis; iTRAQ, isobaric tag for relative and absolute quantitation.

Techniques	Viral Binding Partner	Host Protein Name	Reference
Multiple TAP LC-MS/MS	Gag	LYRIC (Lysine-rich CEACAM1 co-isolated protein)	50
TAP LC-MS/MS	Gag	RHA (RNA helicase A)	53
LS-MS/MSPhosphoproteomics	Gag (P6)	aPKC (atypical protein kinase C)	56
Co-IP LS-MS/MS	Gag	ALIX (Programmed cell death 6-interacting protein)	59
TAP LC-MS/MS	Gag	Staufen1	63
Co-IP LS-MS/MS	Pol (IN)	LEDGF/p75 (Lens epithelium-derived growth factor)	6
TAP/Co-IP MALDI & LS-MS/MS	Pol	Syntenin-1	70
AP LC-MS/MS	Pol	LRPPRC (Leucine-rich PPR-motif containing)	73
MudPIT Proteomics	Rev	DDX3X, DDX5, DDX17, or DDX21 (DEAD/H box proteins)	92
Subcellular Co-IP LC-MS/MS	Rev (RNA)	Matrin 3 (Nuclear matrix component MATR3)	94
AP LC-MS/MS	Rev (RNA)	hnRNP E1 (Heterogeneous nuclear ribonucleoprotein E1)	95
AP LC-MS/MS	Rev (RNA)	hnRNP K (Heterogeneous nuclear ribonucleoprotein K)	97
Co-IP LC-MS/MS	Tat	hNAP-1 (Human nucleosome assembly protein-1)	102
Co-IP LC-MS/MS	Tat	YB-1 (Y-box binding protein 1)	103
AP MALDI	Tat	Splicing regulator p32	108
Co-IP LC-MS/MS	Nef	DOCK2-ELMO1 (Dedicator of cytokinesis 2-Engulfment and cell motility protein 1)	112
Co-IP LC-MS/MS	Nef	Mortalin	114
DIGE/iTRAQ LC-MS/MS	Nef	Ezrin & EHD4 (EH-domain containing 4)	115
Co-IP MALDI	Vif	Cullin-5	122
AP LC-MS/MS	Vif	CBF-beta (Core-binding factor subunit beta)	123
Co-IP LC-MS/MS	Vpu	BST-2 (bone marrow stromal antigen 2)	130