The Role of HIV-1 genome on biological mechanisms, Molecular function, Interactions between viral and host cell components, Mechanisms of viral entry, Chromosomal integration, and Transcription as potential target for new Preventive HIV-1 Vaccine Development (Part Sixteen)

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Abstract

The human immunodeficiency virus (HIV) genome encodes a total of three structural proteins, two envelope proteins, three enzymes, and six accessory proteins.

Three-dimensional molecular structures can provide detailed information on biological mechanisms and, for cases in which the molecular function affects human health, can significantly aid in the development of therapeutic interventions.

Moreover, structural analyses of the interactions between viral and host cell components have yielded key insights into the mechanisms of viral entry, chromosomal integration, transcription and egress from cells

Studies over the past ten years have provided high resolution three-dimensional structural information for all of the viral enzymes, structural proteins and envelope proteins, as well as for three of the accessory proteins.

Peptide complexes with two regulatory RNA fragments and a protein complex with an RNA recognition/encapsidation element have also been structurally characterized.

Early studies used both synthetic and promoter expressed small interfering RNAs (siRNAs) or expressed short hairpin RNAs (shRNAs) to demonstrate that this virus was susceptible to RNAi.

RNAi-mediated down-regulation of cellular targets that encode receptors required for viral entry also proved to be effective.

In this article, I discuss Molecular Virology of HIV, Cellular Reservoirs in HIV-1 Pathogenesis, Immunological Response, Inhibition of Fusion, Accessory Proteins and Vaccine Development

Key Words: HIV-1, Molecular Virology of HIV, HIV-1 Pathogenesis, Immunological Response, Therapeutical Approach, Inhibition of Fusion, Accessory Proteins and Vaccine Development

The Table of Contents

1. Introduction

Virus enters through successive interactions with CD4 and CXC chemokine receptor type 4 (CXCR4) or CC chemokine receptor type 5 (CCR5); as a consequence HIV-1 core (diploid single strand positive sense RNA, tRNA primers, viral protease, retrotranscriptase, and integrase) is released into cytoplasm (1),(2).
Activation of host cells induces the binding of transcriptional preinitiation complex to enhancer elements in the 5_LTR proximal promoter that gathers essential host transcription factors, such as NF-κB, NFAT, AP-1 and SP1 which transmit activation signals to basal transcription machinery and promote the binding of RNA polymerase II to the TATA box to initiate transcription (3),(4),(5). Transactivation response element (TAR), a 59-nucleotide stem loop structure, is then formed at 5_ end of nascent viral transcript that creates the binding site for viral transactivator (Tat) which promotes efficient elongation of viral transcripts by recruiting

the positive transcription elongation factor b (PTEFb), thereby enhancing the functional capacity of RNAPII (6). Viral regulatory protein (Rev) regulates the processing, nuclear cytoplasmic transport, and splicing of HIV mRNA. HIV-1 large precursor proteins assemble to create viable particles budding off the cell and are processed into mature proteins (7). HIV-1 replicates preferentially in activated CD4+ T cells but these cells generally survive only for few days after infection. Hardly, an infected CD4+ lymphoblast survives long enough to revert back to a resting memory state (8),(9),(10). Efforts to control the AIDS epidemic have focused heavily on studies of the biology, biochemistry, and structural biology of HIV and on interactions between viral components and new drug candidates (10),(11),(12). Although these drugs delay the progression of the disease, they do not prevent it, as infection readily leads to drug-resistant mutants (13). Recently developed ``drug cocktails'' containing combinations of PR and RT inhibitors can reduce viral loads to undetectable levels, and these low levels can be maintained for periods of two years or more (14).Advances in structural biology can aid the development of next-generation compounds that are active against previously exploited targets, and can also help define new drug targets and boost the effectiveness of vaccination strategies. Much evidence indicating that these gene products, collectively referred to as auxiliary proteins, are capable of regulating viral replication and infectivity has accumulated in the past decade. HIV type 1 (HIV-1) possesses at least six such auxiliary proteins, namely, Vif, Vpr, Tat, Rev, Vpu, and Nef. The Tat, Rev, and Nef proteins are synthesized early from Rev-independent multiply spliced mRNAs (-2 kb), while Vif, Vpr, and Vpu are expressed late from Rev-dependent singly spliced mRNAs (-4 kb). Mutations affecting either Tat or Rev severely impair viral replication, indicating that these two auxiliary proteins are essential for viral replication (15). Though accessory proteins are not required for viral replication, they are nonetheless capable of modulating replication events, even in vitro, and accordingly, phenotypes associated with their expression have been recognized. Importantly, accumulating evidence suggests that these proteins may modulate viral pathogenesis in vivo, thus affecting disease progression and outcome.

2. Molecular Virology of HIV

The HIV virion's diploid genome consists of 2 single-stranded RNA molecules within a host-derived lipid bilayer. Trimers of surface glycoproteins gp41 and gp120 protrude from the virion. The latter's outermost part contains a variable region known as a V3 loop, and is responsible for inducing a strong immune response.2 A p9 nucleocapsid protein interacts noncovalently
with the viral genome, a p17 matrix protein anchors the internal face of the viral envelope, and a
p24 capsid protein encloses the genome. Within the capsid of the virus particle are enzymes essential to reverse transcription and integration within the host.

3. Molecular Insights into HIV-1 Latency

Two forms of viral latency have been seen on the basis whether or not HIV-1 genome has integrated into the host cell genomes: preintegration and postintegration latency (16). Preintegration latency results in partial or complete blockade of viral life cycle prior to integration of virus genome into host genome. It could result from incomplete reverse transcription or from restriction by factors such as APOBEC3G (cellular

deoxycytidinedeaminase whose function can be counteracted by viral vif protein) (17),(18). Further the preintegration latency does not appear to be of clinical significance because unintegrated forms persist in the cytoplasm for only one day and cannot account for long-term latently infected CD4+ T-cell reservoirs but this unintegrated form of DNA remains stable for at least one month in nondividing metabolically active macrophages (19),(20). Postintegration latency occurs when HIV-1 genome integrated into host genome is reversibly silenced and is limited only by the life span of the infected cells and its progeny. Most mechanisms to maintain HIV-1 latency operate at transcriptional level.

3.1. The Site and Orientation of Integration. HIV-1 latency

Mostly operates at the transcriptional level; for example, the chromosome environment at the site of integration and availability of viral and host factors can have influence on viral latency (21). HIV-1 integrates into the host chromosomal DNA in nonrandom manner. Specific sequences at the ends of dscDNA are required to target PIC predominantly to the intronic regions of the actively transcribed genes (22). One study of the integration sites in purified resting CD4+ T cells fromthe patients on HAART found majority of provirus (93%) located within the coding regions of host gene, probably owing to the increased chromatin accessibility of these regions (23). The finding that latent HIV-1 proviruses integrate in actively transcribed regions may seem paradoxical considering the establishment of transcriptional latent state (24). However, the viral replication from these proviruses can suffer from intense transcriptional interference because of the orientation of the proviruses or their proximity to a stronger host gene promoter (25). The steric hindrance occurs: when the provirus integrates in the same transcriptional orientation as the host gene, read through transcription from upstream promoter displaces key transcription factors from HIV-1 promoter and prevents the assembly of the preinitiation complex on the viral promoter, thereby hindering HIV-1 transcription (26),(27). These transcriptional interferences could be reversed by inhibiting the upstream transcription or by cooperatively activating viral transcription initiation and elongation (28). Furthermore, integrated provirus suffering from transcriptional interference becomes transcriptionally active following Tat expression, and this provirus can switch off the transcription of the host genes within which it has integrated or can allow the coexistence of expression of both host and viral genes (29). Convergent transcription may also allow for the elongation of both viral DNA strands which results in the formation of double-stranded RNAs, might lead to RNA-interference, RNA directed DNA methylation or generation of antisense RNA (30),(31). Furthermore, the phenomenon of enhancer trapping can occur when enhancer of one gene is placed out of context near the promoter of the second gene. Taken together, the orientation-dependent regulation is highly variable and relies on the 5_LTR occupancy and on the rate of host gene elongation (32),(33).

3.2. Availability of Host Cell Transcription Factors and HIV-1

HIV-1 gene expression is strongly dependent on host cell transcription machinery and the lack of host transcriptional activator or the presence of host transcription repressors also influences the viral latency. The 5_LTR functions as an HIV-1 promoter and contains several DNA binding sites for various cellular transcription factors such as SP1 and NF-κB which are required for

viral replication, whereas other sites, such as those binding NFAT, LEF-1, COUP-TF, ets-1, USF, and AP-1, enhance transcription without being indispensable (34),(35),(36). The p50/p65 NF-κB heterodimer is sequestered into the cytoplasm in unstimulated cells through its interaction with an inhibitory protein of the family of NF-κB inhibitors (IκBs) (37). Following cellular activation, phosphorylation of IκB by IκB kinase (IKK) results in its dissociation from NF-κB, NF-κB translocation into the nucleus, and transcription of NF-κB-dependent genes (38). Following T-cell activation, p50/p50 homodimers are uprooted by the p50/p65 heterodimers, which recruit histone acetyltransferases (CBP and p300) to enhance the viral replication (38). Furthermore, the p65 subunit of NF-κB stimulates transcriptional elongation by interacting with RNAPII complexes of cdk7/TFIIH and pTEFb which in turn phosphorylate the serine-5 and serine-2 residues, respectively, in the carboxyl terminal domain (CTD) of RNAPII for the efficient transcription elongation (39),(40). As far as NFAT is concerned, T-cell activation dephosphorylates cytoplasmic NFAT via PKC pathway and translocates into the nucleus where it interacts with 5_LTR at the sites overlapping the U3 NF-κB binding site and thus promotes the chromatin remodeling by recruiting transcriptional coactivator like CBP and p300 (41),(42). Further the AP-1 complex, composed of Jun, Fos, and ATF family members, having three binding sites in HIV-1 5_LTR, cooperates with NFAT to activate HIV-1 transcription through U3 NF- κB/NFAT binding sites (43),(44). In addition to host cell transcription factors, HIV-1 transcription is boosted by viral protein like Tat (45). Tat interacts with the cis-acting RNA element TAR (transacitivation response element) present at the 5_ of viral transcripts. The inhibition of Tat also induces latency because in its absence, transcription is initiated but blocked at the promoter in the early stage of elongation due to the repressive chromatin environment (46),(47). Tat activity is regulated mainly through the acetylation of Lys28 and Lys50 (48). Tat acetylation by PCAF on Lys28 enhances the recruitment of pTEF at 5_ end of nascent viral transcripts promoting efficient elongation, whereas acetylation of Lys50 by CBP promotes the dissociation of Tat from Tat-cyclin T complex, allowing its interaction with PCAF and Tat-PCAF complex recruiting to the elongating RNAPII (49),(50),(51). Some cellular protein affects the acetylation state of Tat modulating its activity. Sirtuin 1, a class III HDAC, acts as specific Tat deacetylase, thus increasing the quantity of Tat that is available to act as a transcriptional activator (52). Further CDK9, a component of pTEFb, is acetylated by hGCN5 and PCAF, reducing the transcriptional activity of pTEFb and promoting HIV-1 latency (53). APOBEC3G strongly inhibits HIV-1 replication in CD4+ T cells by inducing C to U conversions in the viral strand DNA during reverse transcription (54). This viral replication inhibitory effect of APOBEC3G is only present in resting cells, where it exists as an active, low molecular mass ribonuleoprotein complex (55). T-cell activation induces the shift from an active low molecular mass to inactive high molecular mass form of APOBEC3G that cannot restrict viral infection. This inactive form of APOBEC3G can be found in tissue resident na¨ıve ormemory CD4+ T cells, which are permissive to HIV- 1 infection (56).

3.3. The Chromatin Organization and Epigenetic Regulation

HIV-1 promoter activity depends on the chromatin environment where two nucleosomes, namely, nuc-0 and nuc-1, are precisely positioned at the viral promoter in latently infected cell lines and impose a block to transcriptional elongation. Nuc-1 nucleosome, located immediately downstream the transcription initiation site, impedes the LTR activity (57),(58).. Epigenetic

modification and disruption of nucleosome, nuc-1, are required for LTR driven transcription activation and viral gene expression (59). The chromatin organization can be modulated through a variety of mechanisms, including posttranslational covalent modifications of histone tails and ATP-dependent, chromatin remodeling events (60),(61). Histone modification (i.e., acetylation, methylation, phosphorylation, sumoylation, ADP-ribosylation and ubiquitination) can influence the gene expressions, which are all reversible and localized to N- and C-terminus of histone tails (62),(63). Hypoacetylation of histones by histone deacetylase (HDACs) correlates with transcription repression, whereas hyperacetylation by histone acetyltransferase (HATs) induces the transcription activation (64). The silent proviral 5_LTR can be activated from postintegration latency by cell treatment with a variety of stimuli, including cytokines like TNF-*a* and IL-6, antibodies (anti- CD3 and -CD28 stimulation) phorbol esters (PMA, PHA, prostratin), or by viral proteins (Tat and Nef). The nucleosome nuc-1, located immediately downstream of transcription start site, is specifically remodeled following IL-6, TNF, or PMA treatment, and this event is specifically correlated with the activation of HIV-1 gene expression (57),(59). Furthermore, HIV-1 transcriptional activation was shown to occur following treatment with HDAC inhibitors (HDACIs) such as trichostatin A (TSA), trapoxin (TPX), valproic acid (VPA), and sodium butyrate (Na But), suggesting that during latency nuc-1 is constitutively deacetylated by HDACs (65),(66). The HDACI-mediated transcriptional activation is accompanied by specific remodeling of nuc-1 and by an increased acetylation of H3K4 and H4K4 in the promoter region (67). Several transcription factors such as ying and yang (YY1) and late SV40 factor (LSF; also known as TFCP2) repress the HIV-1 replication by recruitingHDAC1 to repressor complex sequence located at position −10 to +27 nucleotides in the LTR (68),(69). Other host transcription factors, such as AP-4 (activating protein-4), NF-κB p50/p50 homodimers, and CBF-1 (C-promoter binding factor-1), can also recruit HDACs to the LTR and inhibit viral transcription (70). Tat and several cytokines and HDAC inhibitors decrease HDACs occupancy at the repressor complex sequence and activate the transcription at 5_LTR by recruiting factors with HAT activity such as CREB binding protein (CBP), CBP-associated factors (PCAFs), and human general control of amino acids synthesis protein 5, which induces nucleosome hyperacetylation in cell lines (71), Similarly, in the absence of Tat, LTR-associated nucleosomes are hypoacetylated, and viral gene expression is silenced, contributing to viral latency. HDAC inhibitors are not sole factors to induce transcription; host factors such as NF-κB, NFAT, and SP-1 must also be recruited to the 5LTR (72),(73). Generally, the histone acetylation is associated with gene activation while histone methylation can be associated with both activation and silencing (74). The histone 3 at lysine 9 methylation that is mediated by SUV39H1 (suppressor of variegation 3–9 homologue 1) has been correlated with heterochromatin assembly by recruiting HP1Y (heterochromatin protein 1 homologuegama), resulting in HIV-1 silencing (71), The transcription factor COUP-TF interacting protein 2 (CTIP2) plays an essential role in promoting viral latency in microglial cells by recruiting a chromatin modifying enzyme complex and by establishing a heterochromatic environment at the HIV-1 promote r (75). Actually, the CTIP2 recruits HDAC1, HDAC2, SUV39H1, and HP1 proteins to establish a heterochromatic environment that leads to HIV-1 silencing in several cell lines (76).

3.4. Posttranscriptional Latency and MicroRNAs

MiRNAs are single-stranded noncoding RNAs of 19 to 25 nucleotides in length that function as posttranscriptional regulator and introduce a new level of complexity to virus-host interplay

(77),(78). Further miRNAs can also regulate the gene expression at the epigenetic level by remodeling chromatin surrounding (79). Several cellular miRNAs (miR-28, miR- 125b, miR-150, miR-223, and miR-382) control HIV-1 replication by targeting all spliced or unspliced HIV-1 mRNA except Nef coding mRNA (80). These cellular miRNAs are enriched in resting CD4+ T cells and inhibit the translation of almost all HIV-1-encoded proteins contributing to viral latency (81). Furthermore, viral genome produces viral interferences RNAs that can target the viral RNAs, cellular mRNAs, and host miRNAs (82),(83). Moreove, HIV-1 can also suppress the miRNAs-mediated silencing pathway by reducing the expression of miRNA-17, miRNA-5p, and miRNA20a that results in increased expression of Tat cofactor PCAF ultimately enhancing the viral transcription (84). HIV-1 products interfere directly with the cellular RNAi machinery through different mechanisms. Firstly, Tat physically interacts with the helicase domain of Dicer and partially represses the ability of Dicer to process precursor dsRNA into small interfering RNAs (siRNAs) (85),(86). Further, the viral TAR sequence prevents the formation of a functional RNA-induced silencing complex (RISC) by sequestering the Dicer-interacting protein TAR RNA-binding protein 2 (TRBP2) (87).

4. Cellular Reservoirs in HIV-1 Pathogenesis

The macrophages, dendritic cells (DCs), and CD4+ T lymphocytes are considered reservoirs for HIV-1 infection. In CD4+ T cells, the viral replication is dependent upon the cell cycle of the host cell and HIV-1 entry into activated CD4+ T lymphocytes leads to productive infection. Virions found with in monocyte-derived macrophages persist and retain infectivity for weeks, thus providing an environment for viral persistence. Dendritic cells capture and internalize extracellular virions via DC-SIGN which can be subsequently transmitted to T cells *in trans*. HIV-1 hidden in DCs and macrophages certainly play an important role for viral spread and cell-to-cell transmission, and its involvement in long-term viral persistence

4.1. Monocyte-Macrophage Lineage as Viral Reservoirs

Cells of myeloid lineage including monocytes, macrophages, and dendritic cells are the first line of defense against pathogenesis, because these cells are critical immune cells responsible for a wide range of both innate and adaptive immune functions (88),(89). Circulating monocytes are recruited to different tissues, differentiate into macrophages, and form the HIV-1 reservoirs. Furthermore, a minor monocyte subset, the CD16+ is more permissive to infection than the more abundant CD14+ CD16− monocytes subset, which account for less than 1%circulating monocytes (90). Macrophages contain the CD4 receptor and CCR5 and CXCR4 coreceptors which are early cellular targets for HIV-1. These cells are able to produce and harbor the virus for longer period of time due to high resistance to cytopathic effects (91). The resident macrophages of central nervous system like microglial cells are involved in the pathogenesis of HIV-1-associated dementia, survive for many years, and are potential reservoirs for HIV-1 (92),(93). Macrophages can harbor large quantities of unintegrated viral DNA in circular form, which remains unstable for up to two months in non-dividing macrophages (94),(95). Further the viral protein Vpr is important for viral replication in monocyte macrophages lineage but not in nondividing CD4+ T cells. The deletion of Vpr decreases the transcription from unintegrated HIV-1 DNA up to 10 times (96),(97). A recent finding shows that infected human macrophages

can support persistent transcription from this unintegrated DNA which suggests that these circular forms of episomal DNA may therefore account for persistence and expression in nondividing cells such as macrophages (98),(99). Another strategy that allows the virus to infect and persist in macrophages is the resistance to apoptosis. The NF-κB pathway is activated upon HIV-1 infection in primarymonocytes and macrophages (100),(101). TNF-a-induced NF-κB activity might be involved in the inhibition of apoptosis and the survival of monocytes and macrophages. NF-κB-mediated resistance to TNF-a-induced apoptosis might result in a decreased susceptibility to apoptosis of macrophages versus T cells in the context of chronic immune activation during HIV-1 infection (102). Further, the absence of apoptosis in HIV-1-infected primary macrophages has been correlated with an increase in antiapoptotic Bcl-2 and Bcl-XL proteins and a decrease of proapoptotic Bax and Bad proteins (103). Furthermore, macrophages express 10 times lower number of cell surface CD4 receptor than CD4+ T cells and therefore are less susceptible to HIV-1 superinfection (104). High number of CD4 receptors in HIV-1-infected CD4+ T cells induce a dramatic reduction in the infectivity of release virions by sequestering the viral envelope by CD4, while the less number of CD4 on the cell surface of the macrophages might favor the release of infectious virions from infected cells and thereby could optimize the transmission of virions to cells present in the vicinity (105). Dendritic cells are also involved in HIV-1 propagation, through capture of viruses by receptor DC-SIGN (DC-specific ICAM3-grabbing non integrin) as well as through efficient HIV-1 transmission to T cells at the virological synapse (106). Follicular dendritic cells in lymphoid tissues are specialized in trapping and retaining the antigens, including HIV-1 virions, on their surface in the forms of immune complexes (107),(108). Further, mature myeloid dendritic cells located in lymph nodes can sustain the very low virus replication and therefore have potential role in HIV-1 latency (109). The mechanism of viral persistence in these cells is not yet clearly understood (110). CD34+ haematopoetic cells (HPCs) also serve as a viral reservoir, since a subpopulation of CD34+ HPCs expresses CD4 and CCR5 and/or CXCR4 and these cells are susceptible to HIV-1 infection (111),(112). HIV-1-infected CD34+ HPCs have been detected in some patients where these HPCs are associated with impaired growth and development (113).

4.2. Lymphocytes: Source of Latently Infected Cells

The most T lymphocytes in the body are in a resting G0 state, and following activation, these resting na¨ıve T cells, in response to antigen, undergo a burst of proliferation and differentiation in response to antigen and give rise to effector T cells. These lymphocytes persist as memory cells with different pattern of gene expression for the long-term survival and rapid response to the relevant antigen in the future (114),(115). Indeed, the activated CD4+ T cells are highly susceptible to HIV-1-infection and die quickly as a result of cytopathic effects either of the virus or of the host immune system. However, a subset of HIV-1-infected CD4+ T cells revert back to a resting state and survive for longer period of time (16). Both na¨ıve and memory subpopulation of resting lymphocytes provide an extremely restrictive environment for HIV-1 replication due to low CCR5 expression, low nucleotide pools and ATP level, and cytoplasmic APOBEC3G (54),(116). In addition to macrophages or dendritic cells, a stable form of latency also occurs in CD4+ T cells that carry integrated provirus (117). Certain chemokines CCL19, CXCL9/ CXCL10, and CCL20 activate the cofilin and actin dynamics necessary for the development of latency in resting CD4 T cells (118). Since the HIV-1 integration requires cell activation to allow efficient reverse transcription and nuclear import of preintegration complex (119), the postintegration latency occurs when infected activated T cells return to quiescent

ormemory cells. The phenotypes of these resting T cells carrying a nonproductive HIV-1 infection have specific set of surface markers such as CD4+, CD25−, CD69−, and HLA-DR− (120).

5. Immunological Response

B cells decline in number and function12, and, because of the toxicity of HIV antigens, cytokine regulation is distorted causing a decrease in CD4+ T-cells (121). There is a distinct interplay between HIV and the immune defenses. Typical non-progressors (those who have been infected with HIV but do not show symptoms) display several responses that are different than those of progressors. Non-progressors show more TH1-type cytokines like IL-2 and IFN-ã, and an elevated response by CD4+ T-cells and cytotoxic CD8+ T-cells towards HIV is observed. The HIV virus counters these defenses by varying antigenic sites (122), (preventing an effective immune response and overwhelming the immune system) and by reducing MHC on the surface of cells, and reducing the number of CD8+ T-cells (123).

6. Targeting HIV-1 Reservoirs: A New Therapeutical Approach

The implementation of HAART therapy has improved the survival and quality of life of HIV-1-infected individual, but it has unable to eradicate the virus from latently infected reservoirs like memory CD4+ T cells and macrophages constituting a major obstacle in HIV-1 eradication (124). The frequency of HIV-1-infected cells, in the patients on HAART, has been reduced to less than one cell per 106 resting CD4 T cells, but after many years of treatment, the frequency of these infected cells is not decreasing further (120),(125). Moreover, some reservoirs are found in tissue sanctuary sites, like the brain, that are protected from drug penetration (126).

6.1. Current Antiviral Strategies

Many methods of combating the HIV virus have been investigated over the last two decades. Most of these strategies involve inhibiting normal viral functions. Ideally, drugs that are developed will provide effective resistance for as long as possible, be safe with minimal side effects, and be chemically stable and inexpensive. The following techniques are examples of newer methods for combating HIV, and are currently entering or undergoing clinical trials.

6.2. Antiretroviral Drugs

Inhibiting viral replication gives the host immune system a chance to recover from infection. Although they inhibit a crucial step in viral replication, these drugs do not address latent viral reservoirs and are therefore only a temporary solution. The efficacy of these drugs increases when used in tandem with similar drugs, the dosage required of each drug is lowered, and the side effects are minimized. There are two main categories of antiretroviral drugs currently under investigation. The first type, nucleoside inhibitors, inhibits RTase by binding the enzyme's active site and adding to the growing DNA chain. As a result, normal 5′ to 3′ synthesis halts. The second category of antiretroviral drugs are the non-nucleoside inhibitors. These drugs bind RTase at a site that is distal from the active site, inducing a detrimental conformational change within the enzyme. These drugs show high antiviral activity and low toxicity *in vitro*, but are too highly specific. Drugs which block the protease substrate site

prevent the cleavage of gp160 at the cell surface, preventing virion maturation. Currently, patients infected with HIV-1 undergo a treatment called Highly Active Anti-Retroviral Therapy (HAART) (127).

6.3. Inhibition of Fusion

Although, in theory, any of the proteins mediating viral fusion could be potential drug targets, viral glycoprotein gp41 has recently proven a worthy target *in-vivo*. A synthetic peptide named T-20 targets two consensus motifs in gp41 which occur commonly in hydrophobic alphahelices. The sequences targeted by T-20 have been shown to be flexible and important in mediating movement of gp41 upon binding of gp120 to the host chemokine receptor. *In-vivo* studies in humans saw a reduced viral-load in all participants, even those that had undergone HAART. Side effects were minimal, and because the sequence targeted by T-20 is highly conserved, mutations are not likely to negate the drug's effects. Other efforts to inhibit fusion have included soluble chemokine receptor ligands, which cause receptor internalization, thereby preventing gp120 from binding.

6.4. Accessory Proteins

Viral accessory proteins play numerous critical roles in the HIV life cycle. Strategies to combat the virusfrom this standpoint are currently under development. One method of targeting accessory proteins involves the use of lentivirus vectors, which can be packed with plasmids. One such use of transducing vectors includes the murine retrovirus vector, which can carry defective transdominant-negative *Rev* (*TdRev*) genes. *TdRev* inhibits nuclear export of *Rev* by forming useless multimers. *Rev* serves to export un-spliced mRNA from the nucleus. Blocking this function causes the accumulation of transcription products within the nucleus, preventing viral assembly (128). In addition to using vectors, synthetic analogues of a 99 amino acid N-terminal stretch of *Vif* have been shown to bind HIV-1 protease *in-vitro* and prevent viral replication. *Vif* normally functions to prevent processing of *gag* and *gag-pol* precursors until viral assembly, so it stands to reason that too much of this protein would prevent protease from ever carrying out its normal function (129).

6.5. Vaccine Development

HIV vaccines aim to reduce the spread of HIV and eliminate viral existence in the host. The development of immunostimulatory vaccines has been a priority in the fight against HIV, primarily because of their low cost, simplicity of administration, and low storage requirements. Many have been proven unsafe and unable to elicit strong CD4+ and CD8+ responses. Only about a third of all vaccines have been shown to elicit strong cytotoxic T-cell responses, albeit with weak neutralizing antibody responses. Many antigens have been tried in vaccine development, including *env*, gp120, gp160, 120 multimers, V3 peptides, and recombinant envelope proteins. Many vectors have also been investigated for their abilities to deliver the antigens, such as alpha viruses, polioviruses, adenoviruses, herpes viruses, and Venezuelan equine encephalitis viruses to name a few. Several vaccines are currently going through phase I clinical trials. One method involves a plasmid that encodes *env* and *Rev* genes, and has been shown to elicit strong T-cell response and chemokine secretion in non-HIV volunteers (130). In

addition to antigen-encoding plasmids, immunostimulatory DNA sequences and cytokine-encoding plasmids can be coadministered.

7. Conclusions and Remarks

Completing structural studies of isolated viral components, future efforts will need to focus on functional intermolecular interactions. Although the molecular determinants of several key interactions have been addressed, such as those associated with target cell recognition and penetration, genome recognition and packaging, and the regulated transcription of the integrated viral DNA, complete understanding of the structural biology associated with these and other processes has not been revealed. Structural biology will continue to have a significant impact on HIV/AIDS research by providing high-resolution glimpses of target protein– drug complexes and virus–host interactions, such as CA–TRIM5α, Vif–APOBEC3G or Vpu–tetherin, and this will reveal novel druggable sites. Despite decades of research, the interactions between HIV-1 and host proteins that underlie some steps in the viral life cycle — for example, import of the pre-integration complex into the nucleus are only now being illuminated. The simian immunodeficiency virus Vpx protein was recently shown to counteract SAMHD1, the restriction factor that inhibits HIV-1 reverse transcription and infection of monocytic cells (131),(132), indicate ng that these protein complexes could also define new paradigms for antiviral drug development. Further to the ongoing work with PR inhibitors, it will be interesting to see whether structure-based substrate–inhibitor envelope hypotheses will apply to the development of other HIV-1 inhibitors. Three-dimensional structures of new drug targets as well as inhibitor- or antibody-bound targets will predictably increase the pace of antiviral development and help guide vaccine development efforts (133),(134). Several lines of evidence suggest that the accessory proteins encoded by HIV play an important role in the viral life cycle and disease pathogenesis: accessory protein reading frames are highly conserved among HIV strains and usually among distant lentivirus relatives, a finding indicative of a selective pressure to foster these ORFs. Viruses carrying mutations in one or more of these genes are severely impaired in cell types, such as macrophages, that may play a pivotal role in viral pathogenesis (135),(136),(137). While it is likely that the phenotypes observed in vitro are operational in vivo, it is possible that these proteins may have thus far unrecognized, and possibly indispensable, functions in vivo, not ascertainable by restrictive in vitro assays that have been employed to study them. Tracking the presence of new HIV strains is important for surveillance purposes, effective chemo- therapy, diagnosis and disease monitoring including vaccine design and development.

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