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HIV-1 NUCLEAR IMPORT: IN SEARCH OF A LEADER

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1. ABSTRACT

The ability of HIV-1 to use host cell nuclear import machinery to translocate the viral preintegration complex into the cell nucleus is the critical determinant in the replication of the virus in non-dividing cells, such as macrophages. In this review, we describe the viral and cellular factors involved in this process. The available data suggest that the process of HIV-1 nuclear import is driven by interaction between nuclear localization signals (NLSs) present on viral proteins matrix and integrase and the cellular NLS receptor, karyopherin alpha. However, this interaction by itself is weak and insufficient to insure effective import of the preintegration complex. Viral protein R (Vpr) functions to increase the affinity of interaction between viral NLSs and karyopherin alpha, thus substantially enhancing the karyophilic potential of the preintegration complex. Interestingly, some cells, in particular HeLa, seem to contain a factor which can substitute for the Vpr's activity, making HIV-1 replication in such cells Vpr-independent. We also describe a class of novel anti-HIV compounds which target the NLSs of HIV-1 and effectively block viral replication in T cells and macrophages.

2. INTRODUCTION

Intense research into the fundamental processes of human immunodeficiency virus type 1 (HIV-1) replication has yielded knowledge that in many aspects equals or exceeds that of the oncogenic retroviruses. As a clearer picture of the pattern of HIV-1 replication evolves,it becomes apparent that HIV-1 biology is distinct from that of the prototypic oncogenic retroviruses in several key aspects, particularly with regard to host cell range and determinants of viral permissiveness. The most striking feature of HIV-1 biology is its ability to replicate in non-dividing cells (1-4). Non-dividing cells of the monocyte/macrophage lineage are supposed to be among the first targets of HIV infection (5,6) and are likely to contribute significantly to HIV-1 persistence (7,8) and the complications of AIDS (9). Early results (10) indicated that the process of nuclear import of the HIV-1 genome is ATP-dependent, thus implying an active, energy-dependent import mechanism, rather than passive diffusion. This is in contrast to oncogenic retroviruses which lack this mechanism and have to rely on the dissolution of the nuclear envelope during mitosis for delivery of their genome into the nucleus (3,11). Such an energy-dependent mechanism is characteristic for the nuclear import of cellular proteins and ribonucleoproteins (RNPs), thus suggesting that the virus is exploiting the cellular nuclear import machinery.

An indirect confirmation of this hypothesis came from the demonstration that nuclear import of HIV-1 could be greatly impaired by mutations in a short stretch of basic amino acids within the viral matrix antigen (MA) with a high similarity to nuclear localization signals (NLSs) identified in many cellular nuclear proteins (4,12).

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Subsequent studies also added integrase (13) and viral protein R (Vpr) (2,12,14) to the list of viral karyophilic proteins. All these proteins have been shown to be components of the viral preintegration complex (PIC) (14,15), and the evolving hypothesis postulated that they function as adapters which connect the HIV-1 PIC to the nuclear import machinery.

In this article, we review the mechanisms of interaction between the HIV-1 PIC and the cellular nuclear import machinery and discuss the role of HIV-1 karyophilic proteins in this interaction.

3. CELLULAR NUCLEAR IMPORT MACHINERY

Nuclear transport of macromolecules occurs through the nuclear pore complexes and is controlled by the nuclear localization signals (NLSs). The most common type of NLSs is a short stretch of basic amino acids that introduce an overall net positive charge crucial for nuclear targeting properties of these sequences (reviewed in (16)). Import of NLS-containing proteins across the nuclear pore complex is mediated by karyopherin alphabeta heterodimers (also termed NLS receptor/importin) which bind NLS-containing proteins in the cytosol and target them to the nucleus (17-23). Karyopherin alpha binds the NLS (17) whereas karyopherin beta enhances the affinity of alpha for the NLS (24) and mediates docking of karyopherin-NLS protein complexes to nucleoporins (a collective term for nuclear pore complex proteins) that contain FG peptide repeats (20,24,25). A different import pathway is mediated by the M9 domain, which is the import signal for hnRNP such as A1, and employs the transportin- (a homologue of karyopherin beta), rather than karyopherin alphabeta-mediated, pathway (26-29).

The small GTP binding protein, Ran (30,31), is a key regulator of the import process. Ran switches between the GDP- and GTP-bound states by nucleotide exchange and GTP hydrolysis. Because the intrinsic rates of these reactions are very low, the nucleotide associated with Ran, and hence the state of Ran's activation, is determined by the presence of RCC1 (Ran's major nucleotide exchange factor (32)) and RanGAP1 (the only known RanGTPase-activating protein (33,34)). RCC1 is chromatin-bound (35), while RanGAP1 is excluded from the nucleus (36). Therefore, the concentration of Ran-GTP is high in the nucleus and low in the cytoplasm. It is believed that this gradient is used to provide direction to the nucleo-cytoplasmic exchange. In particular, by directly binding to karyopherin beta in the nucleoplasm, Ran-GTP disassembles the import complex (24) and thus terminates the import process (37). It also stimulates assembly of the karyopherin alpha complex with CAS, a recently discovered export factor (38), thus promoting re-export of karyopherin alpha into the cytoplasm for recycling. A family of Ran-binding proteins facilitates the function of Ran. These include RanBP1, which activates RanGTPase (39,40) and also stabilizes the interaction of Ran-GDP with karyopherin beta during translocation through the pore (41), and RanBP2 (Nup358), which may be the initial docking site for nuclear protein import (42,43).

In addition to karyopherins and Ran, several other soluble proteins are involved in nuclear import, although their mechanism of action is less defined. The nuclear import factor p10 (also termed NTF2) (44,45) appears to coordinate the activity of Ran by binding Ran-GDP into a complex with nucleoporin-docked karyopherins (46). Heat-shock protein 70 (Hsp70, Hsc70), as well as some as yet uncharacterized cytoplasmic factors, may act to facilitate the interaction between the NLS and karyopherin alpha (47,48). The ectopic expression of human Hsp70 in mouse cells complemented the defective import of a mutant SV40 large T antigen (49), and the depletion of Hsp70 from cytosolic extracts prevented import (50,51).

4. HIV-1 NUCLEAR IMPORT

4.1. Role of active nuclear import in HIV-1 life cycle

Oncogenic retroviruses enter the nuclear compartment of target cells during mitosis, when the nuclear envelop is resolved (3,11). Although HIV-1 was shown to infect and replicate in non-dividing cells by utilizing the active nuclear import mechanism (4,10,12), it was hypothesized that in dividing cells, such as activated T lymphocytes, entry of the virus into the nucleus occurred during mitosis, and thus did not require active nuclear importation. This concept was based primarily on the ability of import-deficient mutants to replicate in $CD4^+$ T cell lines (4,10,14). Nevertheless, published evidence indicated that active nuclear import mechanism can be functional in HIV-1 infection of immortalized T cells, at least under certain conditions. For instance, while HIV-1 does not replicate in quiescent (G_0) T lymphocytes (52,53), it can productively infect T cells arrested in either G_1-S (54) or G2 (55) phases of the cell cycle, suggesting that cell activation, but not cell division, is necessary for virus replication in T cells. Several lines of evidence support the notion that HIV-1 infection *in vivo* may occur primarily in activated but non-dividing cells. Firstly, $CD4^+$ T lymphocytes replicate very slowly *in vivo*. For instance, naïve T lymphocytes divide once every 3.5 years, while memory T lymphocytes divide once every 22 weeks (56). Secondly, cell cycle analysis performed on peripheral blood mononuclear cells freshly collected from HIV-1 infected individuals detected approximately 98% of cells in G_0-G_1, 2% in S and G_2 combined, and almost no mitotic cells (57). Furthermore, the combined length of the G_1, S, and G_2 phases covers most of the cycle span of a T cell, while mitosis lasts for only 2-3 hr. Finally, evaluation of HIV-1 replication dynamics *in vivo* revealed rapid turnover rate for free plasma virus ($t_{1/2}$ 6 hr), and rapid loss of virus-producing cells ($t_{1/2}$ = 1.6 d) (58). High number of infected cells and a rapid dynamics of virus replication combined with a low number of proliferating T cells argue against HIV-1 replication in dividing cells.

The ability of HIV-1 to replicate in interphasic CD4$^+$ T lymphocytes suggests that the virus may utilize the same active nuclear importation pathway as used during infection of primary macrophages. The importance of this pathway for the virus is further underscored by the ability of virion-packaged Vpr to arrest infected T cells in the G_2 phase (M. Emerman, personal communication). This effect would make active nuclear import crucial for the survival of the virus in an infected target cell.

These considerations clearly indicate that while active nuclear import may not be required for HIV-1 infection of immortalized T cell lines or rapidly dividing mitogen-activated primary T cells *in vitro*, in the environment of chronic infection *in vivo* where antigen-driven cell activation is slow and tightly regulated, this cellular pathway may be critical for establishment of HIV-1 infection and propagation of the virus.

4.2. Viral proteins that regulate nuclear import

4.2.1. Matrix antigen (MA)

Early studies recognized the karyophilic properties of MA (59-63). The MA protein was the first to be identified as a participant from the viral side in the process of HIV-1 nuclear import (4). Its role turned out to be also the most controversial one. The work by Bukrinsky et al. (4) and by Nadler et al. (47) demonstrated that a basic region in the MA protein encompassing amino acids 25-33, G^{25}KKKYKLKH, functions as an NLS when conjugated to BSA. Compared to the NLS of SV40 large T antigen, this MA NLS was a weak one, requiring the presence of multiple peptides per BSA molecule to achieve partial nuclear localization. Another basic region in the C-terminal part of the MA protein, N^{109}KSKKKA, was found to be an even weaker NLS, although it was still capable of targeting the BSA-NLS conjugate into the nucleus (47). Given such an incomplete effect of peptides corresponding to the MA NLS when compared to the effect of strong NLSs, such as the SV40 large T antigen NLS, it is not surprising that Fouchier et al. (64) interpreted their results as negative when analyzing the nuclear import function of the MA NLS peptide. These authors further analyzed the intracellular localization of MA fused to pyruvate kinase or maltose binding protein, and detected the proteins only in the cytoplasm. Again, this result is in contrast to results of a similar experiment performed by Gallay et al. (13) who detected nuclear localization of the GST-MA fusion protein. Given the weakness of the MA NLS, these differences may reflect the way that the MA NLS is presented in a particular fusion protein, or simply the size of such protein. In any case, the value of these experiments for assessing the role of the MA protein in HIV-1 nuclear import is at least questionable, since there is no evidence that MA functions in the import process as a fusion protein.

A more direct analysis of the MA NLS role in HIV-1 nuclear import came from mutagenesis experiments. These studies clearly demonstrated that mutations introduced into the MA NLS substantially diminished HIV-1 replication in non-proliferating cells (2,4,12,64,65). Surprisingly, the replication defect of the MA NLS mutants was observed to some extent in proliferating cells, such as T cell lines or activated PBLs (64,65). Because viral replication in proliferating cells was considered to be independent of nuclear import, these results were interpreted as an evidence for the lack of MA role in HIV-1 nuclear import (64,65). Recent studies, however, demonstrated significance of the active nuclear import process for effective replication of the virus in activated, proliferating T lymphocytes (see a special section of this review on the role of nuclear import in HIV-1 life cycle). In addition, some of those results (64) were obtained with viruses that carry a functional Vpr gene, thus masking the effect of mutations in the MA NLS (see below). Finally, mutagenesis of the MA NLS was usually limited to substitution of threonines for lysines in positions 26 and 27, while earlier analysis (4) clearly demonstrated that replacement of lysines in positions 26, 27, 30, and 32 was required for complete inactivation of the NLS activity. In addition, the second functional NLS identified in the C-terminal part of MA (47) can partially substitute for the defective N-terminal NLS (M.I.B. and O.K.H., unpublished data).

Overall, it appears that although HIV-1 MA carries an NLS(s), it is a rather weak one. How then can it target to the nucleus a large macromolecular complex, such as the HIV-1 pre-integration complex? To some extent the weakness of the MA NLS is compensated by the presence of multiple (1,000) copies of MA in the HIV-1 PIC (66). Presence of multiple NLSs has been shown to improve substantially nuclear import (67). In addition, other proteins within the PIC (e.g. integrase, see below) may contribute their NLSs to the process of HIV nuclear import. However, multiplicity of NLSs on the HIV-1 PIC is not sufficient to make it a strong karyophile without involvement of another viral protein, Vpr. This protein regulates interaction between the viral NLSs and karyopherin alpha, thus effectively enhancing the karyophilic potential of the PIC (see below).

4.2.2. Integrase (IN)

A role for integrase in HIV-1 nuclear import has been suggested recently by Gallay and co-workers (13). They demonstrated that IN associates with karyopherin alpha and can target a fusion GST-IN protein into the nucleus of microinjected COS cells. This result contradicts a previously published report (68) in which no karyophilic activity of IN-beta-galactosidase fusion protein was identified. Although the explanation for this disparity suggested by Gallay et al. (68), namely, that the configuration of a particular fusion construct may influence the availability of the NLS, is quite credible, it appears that the inherent weakness of the IN NLS may be another important factor influencing the outcome of these experiments.

The contribution of IN to HIV-1 nuclear import is even harder to evaluate on the basis of published results. One of the major problems is incomplete inactivation of the MA NLSs in mutants which are considered to be MA NLS-defective. Indeed, as discussed above, inactivation of Lys^{26} and Lys^{27} in the MA NLS is not sufficient to destroy its karyophilic activity, and nuclear import observed after infection with HIV-1 carrying these mutations may be driven by the residual NLS activity of MA. In addition, the multiplicity of infection plays an important role in the outcome of experiments on nuclear import, as demonstrated in a response by Trono and Gallay to a letter by Freed et al. (69). Therefore, experiments with pseudotyped constructs (which carry selected HIV-1 determinants) carrying envelopes of MLV or VSV (70-72) are difficult to interpret given a different route (in case of VSV G protein pseudotyped constructs) and undetermined multiplicity of infection. The only convincing evidence for the role of IN in the import process provided so far is found in the report by Gallay et al. (68) who demonstrated that mutation in the IN gene combined with mutations in Vpr and the MA NLS eliminates nuclear import of HIV-1 PICs in P4 cells, while import of a virus defective in Vpr and the MA NLS is only partially reduced. However, it remains unclear whether Vpr-like cellular proteins (see below) are participating in the nuclear import of HIV-1 in this cell line.

4.2.3. Viral protein R (Vpr)

The first glimpse of the Vpr's role in HIV-1 nuclear import came when it was realized that T cell line-adapted HIV-1 strains used in the initial experiments contained a frame-shift mutation in the *vpr* gene (2). When strains with a functional *vpr* were used, the effect of inactivating mutations in the MA NLS on nuclear import was greatly diminished (2,14,73). Vpr rescued replication of an MA NLS mutant in macrophages by providing sufficient, although reduced by about 60-80% compared to wild-type virus, nuclear translocation of viral DNA. Studies performed with the cloned *vpr* gene demonstrated nuclear localization of Vpr after transfection (74). Also consistent with a nuclear import role of this protein is the finding that Vpr is dispensable for HIV-1 replication in dividing cells, such as transformed T cell lines, while being critically required in non-dividing macrophages (75,76).

The Vpr protein does not contain a canonical NLS, but does have a cluster of 6 arginine residues at the carboxyl terminus which could be a candidate NLS. This region was initially reported to be both necessary and sufficient to direct Vpr to the nucleus (74). However, later studies provided convincing evidence that the nuclear targeting determinant is likely to reside in the amino-terminal alpha helical half of the protein (77,78). This portion of the protein is also involved in mediating Vpr interactions with cellular protein(s) (79,80). Mutations in the alpha–helix domain of Vpr that abolished protein-protein interactions also affected nuclear localization of Vpr (78,81). It appeared therefore that karyophilic properties of Vpr are mediated by a cellular Vpr-interacting protein.

This protein was recently identified in our lab (Popov et al., submitted). Not surprisingly, it turned out to be karyopherin alpha. A previous study by Gallay et al. (2869} failed to identify Vpr-karyopherin alpha interaction and concluded that Vpr is imported by a karyopherin alpha-independent mechanism. The reason for this disparity lies in an unusual mode of interaction between Vpr and karyopherin alpha. While binding of MA to karyopherin alpha is mediated by the NLS of MA, binding of Vpr to alpha does not involve an NLS. Therefore, interaction of Vpr and karyopherin alpha could not be competed with an excess of NLS peptide (Gallay *et al*., 1996). The binding site of Vpr on karyopherin alpha does not appear to overlap with the NLS or karyopherin beta binding sites of alpha; in fact, karyopherin alpha, karyopherin beta, Vpr, and MA can assemble into a tetramer.

As a result of Vpr binding to karyopherin alpha the affinity of interaction between the NLS and alpha is increased 5-10 fold. This effect explains the enhancing activity of Vpr on HIV-1 nuclear import. It appears that Vpr regulates the nuclear import of HIV-1 preintegration complexes by binding to karyopherin alpha and increasing its affinity for viral NLSs, including the NLS of MA. This binding interaction may allow the PIC to compete efficiently for karyopherin alphabeta heterodimers in the cytosol, and may facilitate docking and movement of the PIC across the nuclear pore complex. Such an activity of Vpr explains why mutation of the MA NLS had only a modest effect on HIV-1 nuclear import (64,65,73). Indeed, other weak NLSs in the HIV-1 PIC can substitute for the MA NLS in the presence of Vpr. The role of Vpr, therefore, is to make the HIV-1 PIC a strong karyophile by enhancing the interaction of its NLSs with karyopherin alpha. These results implicate Vpr as a key regulator of HIV-1 nuclear import.

Even though all published reports (75,76,82,83) agree on the role of Vpr in HIV-1 infection of non-dividing cells, the magnitude of this effect clearly differs between experimental systems. Discrepancies may be explained by differences between cell types used or methods of cell cultivation. Nevertheless, the fact that substantial replication of HIV-1 with a mutation in the *vpr* gene was observed in macrophages (14,65) and growth-arrested HeLa cells (2), while no nuclear import of such a mutant was detected in an *in vitro* system (Popov *et al*., submitted), suggests that a cellular protein expressed in those cells can partially substitute for the function of Vpr.

The existence of such proteins is also suggested by a conservation of Vpr-binding site on karyopherin alpha from different species. Indeed, both human and yeast karyopherin alpha bind Vpr (Popov *et al*, submitted), despite their only 40-50% similarity (84). It appears likely that a high-level expression of such proteins in certain cells (e.g. neurons) makes them susceptible to transduction by Vpr-defective lentiviral vectors (72).

5. NULEAR IMPORT OF HIV-1 AS A DRUG TARGET

The protein-protein interaction between the NLSs of the HIV-1 PIC and karyopherin alpha presents an attractive new target for drug development. Interrupting the process of viral replication at the step of nuclear importation of the HIV-1 PIC may be accomplished by developing inhibitors to either of the interacting proteins. In contrast to inhibitors that target viral NLS-proteins, such as MA, inhibitors that interact with karyopherin alpha may affect normal cell function and may be less specific.

5.1. Compounds which bind to the MA NLS

Arylene bis(methyl ketone) compounds modified with a pyrimidine side chain were the first small molecules shown to associate with MA, inhibit binding of the HIV-1 PIC to karyopherin alpha, and block viral replication (85,86). These small molecules are represented by the prototypic compound CNI-H0294. CNI-H0294 forms Schiff-base adducts via its carbonyl moieties with the lysine residues in the MA NLSs (85). This interaction inactivates the MA NLS and represents the primary mechanism of action of CNI-H0294. The specificity of the compound for HIV appears to be derived from the poly-lysine nature of the MA NLS which allows the formation of Schiff base adducts with two neighboring residues. This property of the compound is dictated by the spatial separation of the carbonyl moieties. Additionally, CNI-H0294 associates with the viral RT via the pyrimidine side chain (86). RT is a component of the PIC and may be located in proximity to MA in the HIV-1 PIC (14,15). The exact site on RT where CNI-H0294 binds remains to be identified, however, the presence of fully reverse transcribed nascent cDNA in treated infected cells suggests that it is outside the active site of RT. Consistent with this assumption, CNI-H0294 and its analogues are not antagonistic to AZT or 3TC (see below). Binding to RT appears to stabilize the otherwise reversible Schiff base adducts between CNI-H0294 and lysines in the MA NLS. The mechanism of interaction between CNI-H0294 and the PIC proteins was confirmed using analogues of CNI-H0294 with modifications in either the bis(methyl ketone) group or the pyrimidine ring (86). For instance, removing either the pyrimidine ring or one of the carbonyl moieties reduced the activity of the inhibitor by approximately 200-fold.

CNI-H0294 and its functional analogues specifically inhibited nuclear importation of the HIV-1 PIC as determined by reduction in the levels of 2-LTR circles, a nuclear form of the HIV-1 DNA (85), while binding and entry of the virus into target cells and reverse transcription of the viral RNA were not affected. These results supported the hypothesis that the compounds specifically target the nuclear import step of HIV-1 replication. The CNI compounds inhibited infection of primary macrophage cultures with clinical or lab-adapted isolates of HIV-1 (85). Similarly, these compounds inhibited acute infection of activated PBMC cultures, as well as virus replication in endogenously infected PBMCs collected from HIV-1 seropositive individuals (O.K.H. *et al*., submitted). Since these individuals commonly carry a swarm of virus quasispecies, this result suggests that the inhibitory effect of the CNI compounds is virus strain-independent. In addition, this latter finding provided an independent confirmation for the role of active nuclear import mechanism in HIV-1 replication in activated T lymphocytes (see above). CNI-H0294 and analogues were also evaluated *in vitro* in combination with AZT and 3TC. The nuclear importation inhibitors had at least an additive effect when combined with these RT inhibitors (M.I.B. and O.K.H., manuscript in preparation). Furthermore, an analogue of CNI-H0294 inhibited virus replication in activated PBMCs collected from an HIV-1 infected individual resistant to AZT and ddC therapies (O.K.H. and M.I.B., unpublished). Although this represents a single observation, it does suggest that these novel compounds may prove useful for treating HIV-infected cohorts where resistance to established therapies is on the rise.

A distinct NLS in IN has been recently identified, and proposed to play a role in nuclear importation of the HIV-1 PIC (13). Given the poly-lysine nature of this new NLS, and the anti-HIV-1 properties of CNI-H0294 and its analogues, it is plausible that these compounds may also bind to the IN NLS. It would be necessary to address this question to fully delineate the mechanism of action of this class of inhibitors.

5.2. Compounds which bind karyopherin alpha

Inhibition of nuclear importation can also be accomplished by compounds which associate with karyopherin alpha, the cellular NLS receptor. Gulizia *et al*. (87) and Gallay *et al*. (88) utilized the prototypic NLS peptide of the SV40 large T antigen as an inhibitor of karyopherin alpha-PIC binding, and demonstrated the critical importance of this protein-protein interaction in nuclear importation of the HIV genome and HIV-1 infection of target cells. The inhibitory effects of the SV40 NLS peptide were attributed to its ability to compete with the HIV-1 PIC for binding to karyopherin alpha. This was confirmed by Gallay *et al*. (88) who showed by biochemical analysis that binding of Rch1, one form of the human karyopherin alpha, to the MA NLS was competitively inhibited by the SV40 NLS peptide. One limitation however, for the application of NLS peptides as therapeutic compounds for HIV-1 infection is the high levels of peptides necessary to achieve the inhibitory effect. For instance, approximately 100 µM of SV40 NLS was required to inhibit HIV-1 infection, and no inhibition was observed at concentrations below 20 µM (87). Similarly, a very high concentration of the NLS peptide (500 µM) was required to inhibit binding of recombinant karyopherin alpha and PIC in cell free assays (88). These observations may be a result of two distinct events. First, the NLS peptide of SV40 is rich in lysine residues and is therefore highly charged, which limits its uptake into cells across the plasma membrane. Second, it is estimated that each karyopherin alpha protein contains eight NLS binding sites (89). Although, it is not yet clear whether all of these binding sites can be occupied at the same time, this observation suggests that efficient inhibition of all NLS-binding sites on karyopherin alpha may be difficult to achieve. In addition, given the central role that this class of proteins plays in cell activation and transport of transcriptional factors to the nucleus, it remains to be determined whether a significant therapeutic index can be achieved using this approach.

5.3. Perspectives for development of nuclear import inhibitors as anti-HIV drugs

Unlike the classical approach to anti-viral therapeutic drug development which targets the viral enzymes, such as RT, protease, or integrase, targeting nuclear importation represents a novel paradigm for therapeutic intervention in HIV-1 infection. The availability of new inhibitory compounds validates the potential of nuclear importation as a target for drug development. Importantly, these novel compounds inhibit HIV-1 infection in both primary macrophages and activated primary T lymphocytes, the principle target cells of the virus *in vivo*. Given the demonstrated efficacy of a combination therapy approach to treatment of HIV infection, it is expected that these and other new compounds will be administered together with the available approved therapies. The ability of the nuclear importation inhibitors to synergize with nucleoside analogues *in vitro*, suggests a promising potential for success of such therapy *in vivo*.

6. SUMMARY AND CONCLUSIONS

Intensive research into the mechanisms of HIV nuclear import revealed new levels of complexity that could not be anticipated in the beginning. Nevertheless, recent findings provided reasonable explanations for the controversial observations regarding the role of various HIV proteins in the import process, and the hypothesis compatible with most published findings seems to have emerged. It appears that the HIV-1 PIC is a relatively weak karyophile driven to the nucleus via a karyopherin alphabeta-dependent pathway. Stable interaction between karyopherin alpha and the HIV-1 PIC is mediated by a coordinated action of Vpr and NLSs present on multiple copies of MA and IN. Using the automobile analogy, the NLS-containing proteins (MA and IN) constitute the engine that drives the complex to the nucleus, while Vpr is the computer that regulates the work of the engine. In the absence of Vpr, nuclear import of HIV is very inefficient, unless a cell has Vpr-substituting proteins. The nature of such proteins is unclear, but one of the possibilities is that they belong to a family of 70 kDa heat shock proteins (reviewed in (90)). On the other hand, without NLS-containing proteins, Vpr would not target the complex to the nucleus. The ultimate test of this hypothesis would be to eliminate all potential NLSs within the PIC, while leaving Vpr intact, although it is unlikely that such an experiment can be done, given a likely perturbation of other viral functions by such extensive mutagenesis.

However, it may be that a similar experiment has been already performed using a different approach. Indeed, bis(methyl ketone) compounds targeting the NLS have been shown to effectively block replication of Vpr^+ HIV-1 strains (85). Since such compounds do not affect Vpr/karyopherin alpha interaction, this result suggests that functional NLSs are required for the Vpr activity. Although the MA NLS was proposed as a target for bis(methyl ketone) compounds (85,86), it may be that other NLSs within the pre-integration complex are also affected. A low-level residual nuclear import activity observed in the presence of these compounds (85,86) may reflect incomplete inactivation of the NLSs. Clearly, Vpr/karyopherin alpha interaction presents a good target for second generation inhibitors of HIV nuclear import.

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