[Frontiers in Bioscience, 2, d283-297, June 1, 97]

INHIBITION OF THE HIV REV TRANSACTIVATOR: A NEW TARGET FOR THERAPEUTIC INTERVENTION

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TABLE OF CONTENTS

1. ABSTRACT

The viral transactivator Rev is essential for HIV replication, since it allows the nuclear export of unspliced and partially spliced viral mRNAs that encode the structural proteins. Rev is an RNA binding protein that interacts with a highly structured RNA element, the RRE, found within the envelope sequences. This viral protein also interacts with cellular proteins, termed nucleoporins, and acts as an adaptor between the viral mRNAs and the cellular nuclear export machinery. Both interactions are specific, and required for Rev function. Because of its crucial role in the HIV replication cycle, and its novel mechanism of action, Rev represents an ideal target for therapeutic intervention. This review describes the efforts towards Rev inhibition. Gene therapy approaches, including the expression of trans-dominant mutants and RNA decoys, as well as antisense therapies and small molecule inhibitors of Rev-RRE binding or Rev interaction with the cellular machinery will be discussed.

2. INTRODUCTION

HIV-1, the etiologic agent of Acquired Immunodeficiency Syndrome (AIDS), displays a complex regulation of viral gene expression during its life cycle. Unlike many "simple" retroviruses (i.e. avian and murine leukemia viruses), which express only three viral genes, the genome of HIV-1 encodes nine genes whose expression patterns are tightly regulated during the HIV-1 replication cycle (Figure 1, for reviews see 1, 2, 3). In the infected host cell, HIV expresses over 20 distinct mRNA species (reviewed in 4). The early stage of regulation of the HIV-1 life cycle is marked by the appearance of the viral regulatory molecules Tat, Rev, and Nef, encoded by the fully spliced 2 kb class of viral mRNAs. The late viral life cycle gene expression is characterized by the cytoplasmic appearance of the 4 kb class of single spliced and 9.2 kb unspliced mRNAs, that encode the proteins required for the assembly of infectious virions. The viral transactivator Rev allows this transition into the late cycle (5, 6), and is therefore essential for viral replication. In effect, proviral mutants that do not express Rev fail to produce structural proteins and therefore cannot form new infectious viral particles (5, 7).

Because of its essential role in HIV replication, Rev constitutes an excellent target for therapeutic intervention. Its mode of action and specific interactions with its target RNA and cellular proteins have been extensively studied and elegantly elucidated, and this body of knowledge adds to the attractiveness of Rev as a target. The purpose of this article is to briefly review the latest developments on Rev, and how this knowledge can be used for development of anti-viral strategies, as well as to

Received 6/2/97 Accepted 6/4/97

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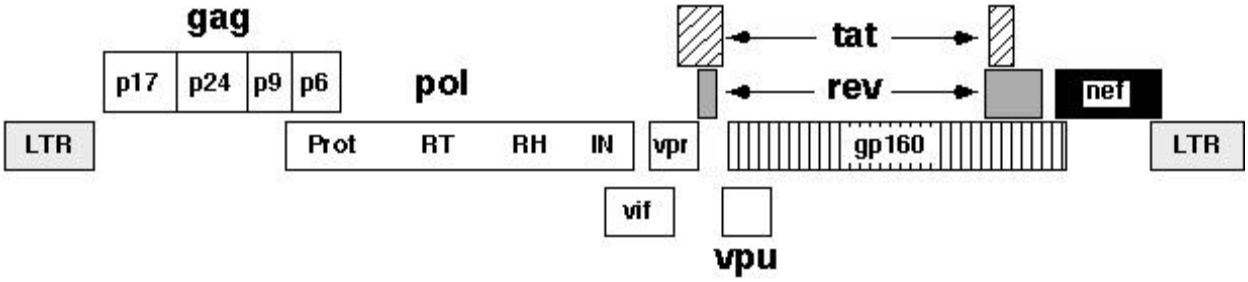


Figure 1. Schematic representation of the HIV-1 genome. Gag-pol and Envelope (gp 160) are the classical retroviral proteins. Note the overlapping reading frames. In addition to these structural proteins and enzymes, HIV has a number of accessory proteins that have crucial functions in the replication cycle and pathogenesis: Tat, Rev, Nef, Vif, Vpr and Vpu. Rev is expressed very early in the life cycle, and is the product of a fully spliced mRNA.

review current efforts in this field.

3. REV STRUCTURE AND FUNCTION

3.1 Rev domains

Rev is a 116 amino acid RNA binding phosphoprotein that binds a cis-acting RNA regulatory element contained within the *env* mRNA, termed the Rev response element (RRE) (8, 9, 10, 11). Mutational analyses of Rev have revealed several discrete domains: i) an amino terminal domain that determines RRE binding and nuclear localization, ii) an oligomerization domain, flanking the RRE binding domain, and iii) a carboxy terminal domain that acts as a nuclear export signal (NES) and binding site for cellular proteins, known as the activation domain (Figure 2).

The arginine-rich motif, located between amino acids 35 and 50 in the Rev protein, is responsible for nuclear localization as well as for the sequence-specific interaction with the RRE (10, 12, 13, 14, 15). A 17 amino acid peptide from this highly basic domain has been shown by circular dichroism to form an α-helix that binds the RRE with the same affinity as the full-length protein (15). The sequences immediately adjacent to this basic domain are critical for Rev oligomerization, which is required for full activity *in vivo* (13, 16, 17, 18). Subsequent to binding, Rev monomers multimerize on the RRE, in a process mediated by both protein-protein interactions, and protein-RNA interaction (13, 19, 20). Cellular cofactors binding to the activation domain facilitate multimerization (21, 18).

In addition to the nuclear localization and RNA binding domain, a protein activation domain which is required to mediate Rev effector functions *in vivo* is located at the carboxy terminus (22-26). A leucine-rich region has been identified as the critical part of this domain, which is required for interaction with cellular protein(s) involved in the transport of HIV mRNAs (24, 25). This domain also acts as a nuclear export signal (NES) (27, 28, 29, 30). NES have been identified in Rev proteins from non-primate lentiviruses (30), as well as in several cellular proteins: the inhibitor of cAMP-dependent protein kinase (PKI) (28), the fragile X mental retardation protein (FMRP) (31), and the amphibian transcription factor IIIA (32). Unlike the better known nuclear localization sequences (NLS), this domain contains critical hydrophobic residues (28), and like the NLS, all these peptide domains are functionally interchangeable (28, 30, 31, 32) and capable of directing the export of unrelated proteins (27, 28).

3.2 Rev-RRE interactions

Rev represents a paradigm for the arginine-rich family of RNA binding proteins, and one of the best studied. The target for Rev binding, the RRE, is a highly structured 234 nucleotide RNA that forms an array of stem-loops (33, 34, 35). It has been demonstrated that the Rev binding site is located in a 13-nucleotide bulge structure in stem-loop IIB, shown in Figure 3 (11, 13, 14, 19, 20, 36, 37) (Figure 3). The secondary and tertiary structure of the RRE has been deduced from the *in vitro* selection of randomized RREs (38,39) and variation of these sequences (40). Two purine-purine pairs within the internal bulge of stem-loop IIB have been identified (37, 39- 42). These non-canonical base pairs open the major groove of the A-form RNA double helix, making the bases more accessible to the arginine-rich, positively- charged Rev peptide (39, 41).

In addition to these *in vitro* studies, a genetic strategy has been used to isolate Rev "suppressor" mutations that alleviated the deleterious effects of mutations in stem-loop IIB of the RRE (43). Taken together, these studies suggested that the arginine-rich α-helix of Rev docked into the major groove of the RNA double helix in the bulge of stem-loop IIB. The three dimensional structure of the high affinity RRE site (stem-loop IIB) complexed with the arginine-rich Rev peptide has recently been determined by nuclear magnetic resonance (NMR) techniques (44, 45). These studies confirm the purine-purine base pairs, separated by a non-

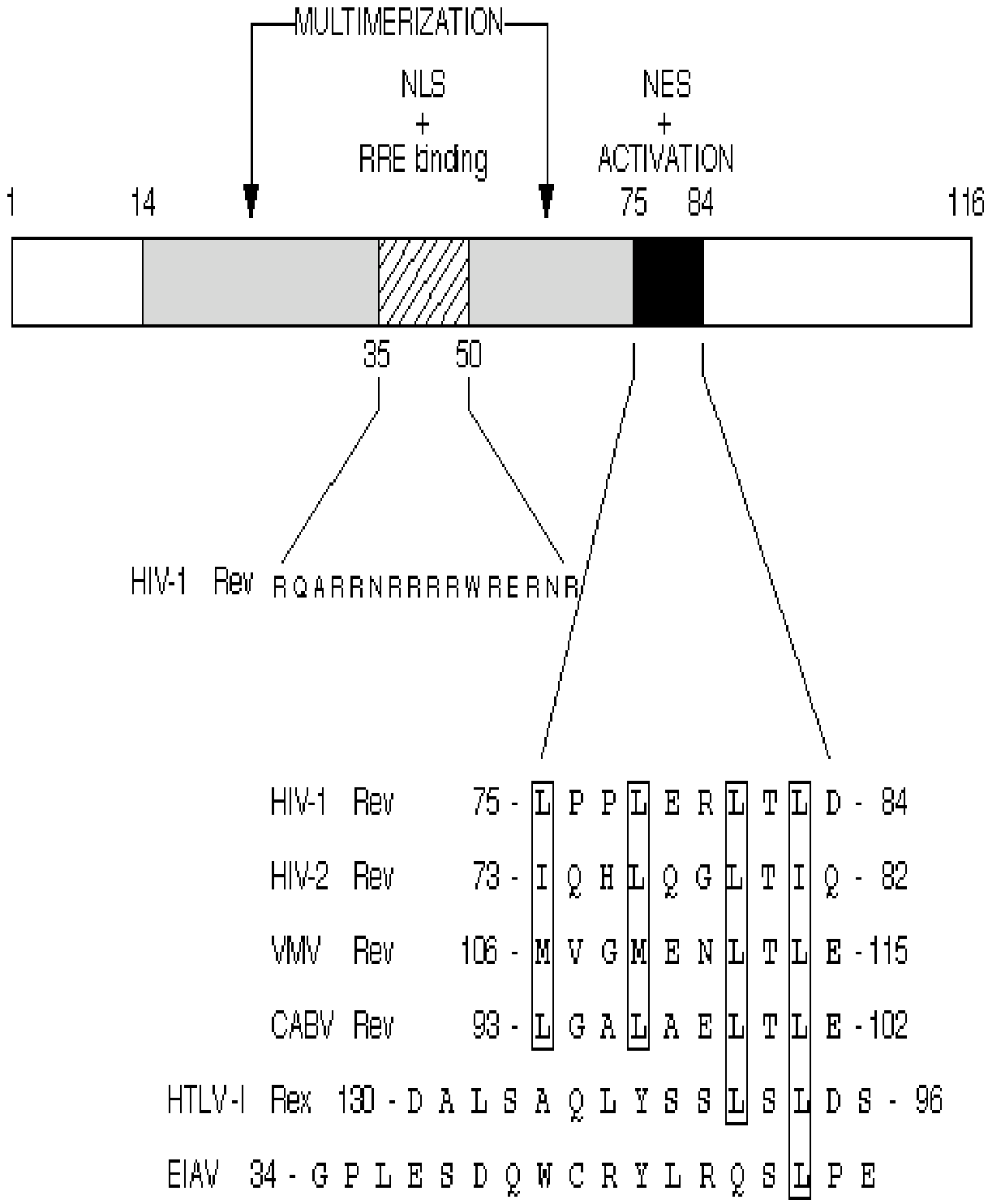


Figure 2. Rev domains. The RRE binding domain (hatched box) is located between amino acid 35-50 in HIV-1 Rev, and is rich in arginine residues. This domain is flanked by sequences important for oligomerization (shaded). The black box represents the activation domain or nuclear export signal (NES). The sequences of other NES in related lentiviruses are shown in the insert.

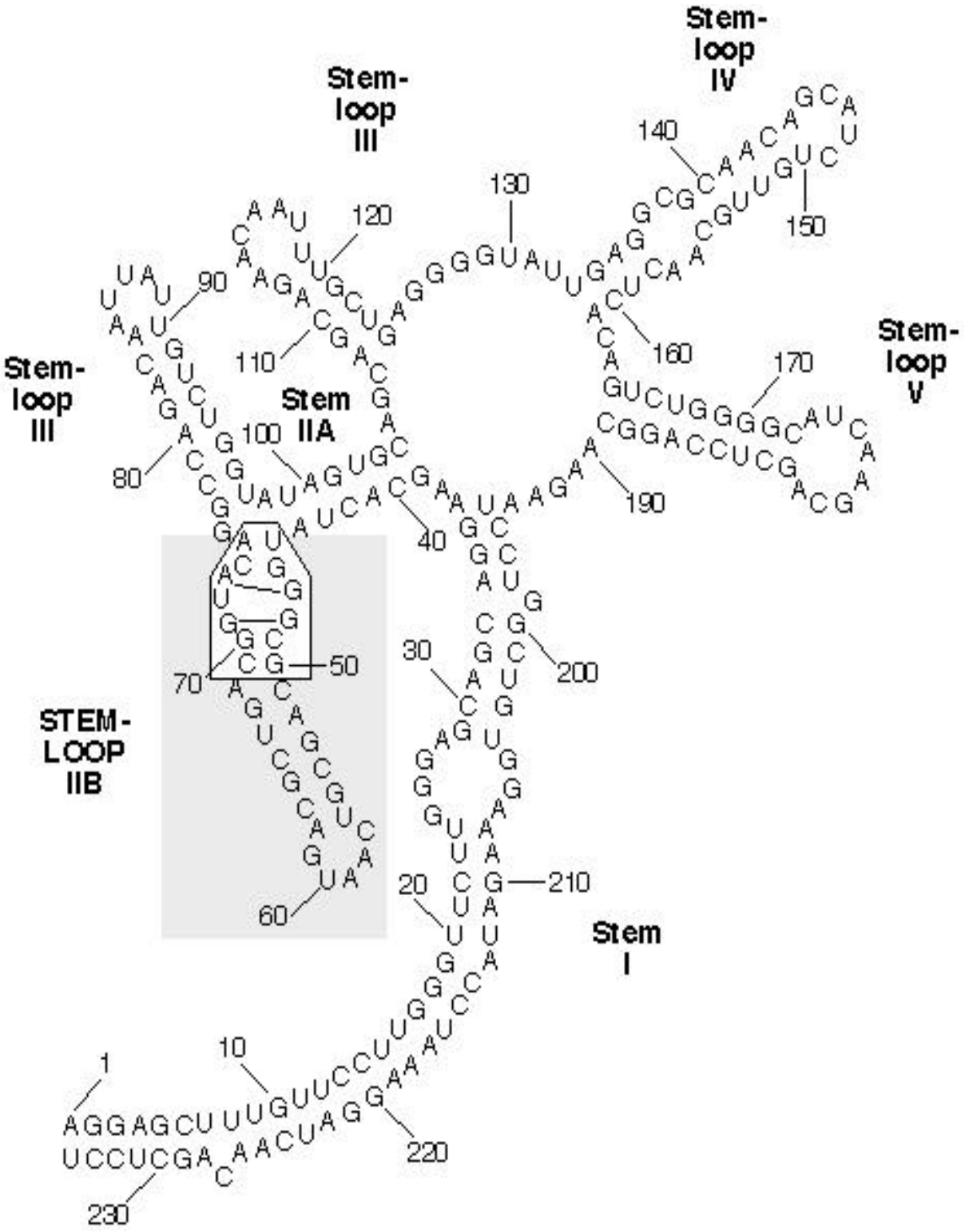


Figure 3. Structure of the Rev Responsive element (RRE). The stem-loop structure of the HIV-1 RRE is depicted here. Stem-loop IIB (SLIIB) (shaded) contains the Rev binding site (RBE), shown within the box. The two non-canonical purine-purine base pairs are indicated.

conserved residue in the bulge, that cause the backbone to twist in an S-shaped fold. As predicted, the major groove is doubled in width, allowing the arginine-rich α-helix to fit, making contacts with the phosphate backbone and with purine residues (46).

3.3 Cellular factors that interact with Rev

Rev is functional in a wide variety of eukaryotic cells, including yeast, *Drosophila*, *Xenopus* oocytes, and mammals (25, 47, 48, 49). Thus, the Rev cellular cofactor(s) may be evolutionary conserved proteins, essential for the function of normal cells. Several cellular factors have been described to interact with Rev. The murine protein YL2 and its human homologue p32 were shown to interact with the basic domain of Rev, the same domain that interacts with the RRE and contributes to the oligomerization process (50, 51, 52). The p32 protein associates with ASF/SF2, an essential splicing factor (53), and is thought to function as a link to the cellular splicing machinery. Rev has been shown to recruit ASF/SF2 itself to the Rev-RRE complex *in vitro*, thus causing inhibition of splicing (52). However, ASF/SF2 is not specific for Rev, since it also binds to the basic RNA binding domain of Tat (54), and it does not bind to the activation or effector domain of Rev, which has been shown to be essential for Rev function (22-26).

The Rev leucine-rich effector domain was considered a more likely candidate for interaction with cellular factors specific for Rev function, since mutations in this domain (Figure 2) abrogate Rev function whether fused to its own RRE-binding domain or to heterologous RNA binding sequences (55). In further support of this model, non-functional Rev mutants in the activation domain which contain an intact RNA binding domain, exhibit a potent dominant-negative effect (12, 23, 25). At least two cellular proteins have been shown to bind to the activation domain of Rev: the eukaryotic initiation factor 5A (eIF-5A) (56), and a novel class of nuclear pore-associated proteins (57, 58, 59, 60). Although the role of eIF5A in mediating Rev function is not completely understood, it has been shown that non-functional mutants of this protein that still retain their ability to bind Rev inhibit Rev-mediated nuclear export, in yeast and in human T cells (61). A novel yeast cellular protein that is part of the nuclear pore complex, called Rip1p (59), and its mammalian homologue , hRIP/Rab (57, 58) were found to bind to the activation domain of Rev, as well as to that of HTLV-I Rex (58), as required for a true cofactor of HIV-1 Rev function, since Rev and Rex, together with the Rev proteins from other lentiviruses have functionally equivalent activation domains (58). The RIP/Rab protein contains a series of repeats containing the amino acids phenylalanine and glycine, known as FG repeats. These repeats are characteristic of a class of nuclear pore proteins called FG nucleoporins (62). Rev has also recently been shown to interact with multiple FG nucleoporins in yeast and in mammalian cells (60), and the ability of Rev mutants to interact with these proteins correlates with their ability to promote nuclear export of RNA (60). These cellular proteins are important in the nuclear export process, and they have been shown to bind other NES in cellular proteins, such as PKI (29, see Section 3.1).

3.4 Mechanism of action of Rev

Two main hypotheses have been proposed for the mechanism by which Rev causes the relocalization of unspliced or partially spliced viral mRNAs in the cytoplasm: 1. inhibition of some aspect of pre-mRNA splicing by Rev, leading to increased mRNA transport to the cytoplasm, and 2. direct effect of Rev to increase the nuclear export of pre-mRNA species.

Most of the evidence in favor of the role of Rev in inhibition of splicing was originated in *in vitro* experimental systems. These studies showed that inefficient splicing is a pre-requisite for Rev function and that Rev inhibits the splicing of RRE-containing introns (63, 64). An arginine-rich peptide from the NLS/RNA binding domain of Rev has been shown to block the entry of the essential U4/U6.U5 small ribonuclear protein complex in the spliceosome assembly *in vitro* (65). However, this block does not require the presence of the Rev activation domain, that has been shown to be essential for Rev function *in vivo*. In addition, it has not been demostrated that this *in vitro*-observed inhibition of splicing is required *in vivo* for Rev function.

Although both models are plausible and not necessarily mutually exclusive, a recent large body of data points to RNA export rather than splicing as the mechanism of action of Rev, and a direct effect of Rev on the cellular nuclear transport machinery has now been demonstrated (49, 57-60). Earlier evidence in support of a role of Rev in nuclear export stemmed from the fact that no incompletely spliced viral mRNAs are exported to the cytoplasm in the absence of Rev, in human T cells containing stably integrated proviruses (66). Moreover, a sequence from an unrelated retrovirus, the Mason-Pfizer monkey virus, was shown to enable Rev-independent HIV replication, possibly by interacting with a cellular factor that plays a role in mRNA transport analogous to that of the Rev protein (67). More recently, the simultaneous discovery of the nucleoporin RIP/Rab by three independent laboratories (57-59) as a cellular cofactor for Rev function confirmed that Rev plays a direct role in the nuclear export of pre-mRNAs. As described in Section 3.1, several cellular proteins have been shown to contain nuclear export signals (NES) functionally homologous to that of Rev (28, 31, 32). Taken together, this evidence indicates that Rev acts as an adaptor to allow RRE-containing viral mRNAs to access a pre-existing cellular export pathway (29).

4. Rev as target for therapeutic intervention

As described above, Rev function is essential for viral replication. No cellular homologs of Rev have been

described so far. Several steps are required for Rev function: binding to the RRE, oligomerization of Rev monomers, and interaction with cellular factors from the nuclear transport machinery. Each of these steps provide potentially specific targets for therapeutic intervention, and the fact that the structural contacts for RRE binding, as well as the role of Rev in nuclear export and its interaction with cellular proteins are now well understood makes this protein a very attractive therapeutic opportunity for the treatment of HIV infection and AIDS. Even though Rev represents an excellent viral target, no anti-Rev compounds have yet entered clinical trials, although some clinical studies have been initiated using gene therapy approaches involving Rev (see 68 for review of these efforts). In this Section, I will review the results of gene therapy, antisense and drug discovery efforts focused on the Rev protein.

4.1 Rev as a target for gene therapy

The resistance of HIV to anti-viral drugs, especially in the early days of single-drug regimes, has prompted a search for alternative methods of therapy. One approach has been gene therapy, meaning the transfer of antiviral genes to infected cells. This strategy is based on the notion that these "therapeutic" genes will render target cells resistant to HIV replication. Gene therapy can be based on the expression of suppressor proteins, or on expression of anti-viral RNA or DNA molecules. Some excellent review articles on gene therapy of AIDS have been published in the past few years (68-71). An extensive review of the anti-HIV gene therapy approaches is beyond the scope of this article, and I will focus on strategies involving Rev.

4.1.1 Protein-based suppressors of Rev function

One of the most advanced protein-based approaches involves the Rev mutant M10, a trans-dominant negative mutant with amino acid substitutions at positions 78 and 79 in the NES/activation domain, that retains the ability to bind to the RRE and multimerize, but is unable to effects its role in transport of pre-mRNAs (25, 72). Because of its trans-dominant negative phenotype, the M10 protein inhibits HIV replication when expressed in stable cell lines (73-76). In HIV infected patients Rev M10-transduced T cells showed increased survival compared to T cells transduced with a vector expressing a deletion mutant of Rev M10 (77). High levels of M10 are required to inhibit viral replication in primary cells, and the choice of vectors is therefore critical to the success of gene therapy (78). A Phase I clinical trial taking into account these parameters has been initiated by Systemics, Inc. (Palo Alto, CA).

Another protein-based strategy that has been explored is expression of an anti-Rev single-chain antibody (79). This single-chain antibody, or SFv, was expressed from a construct consisting of both light and heavy chain variable regions of an anti-Rev monoclonal antibody. Intracellular expression of this SFv resulted in a level of inhibition of HIV replication comparable to that shown with the Rev M10 transdominant mutant (79). This antibody appears to sequester Rev in the cytoplasm, thus preventing it from exerting its function in nuclear transport.

4.1.2 Intracellular expression of RNA-based Rev inhibitors

A large portion of the anti-HIV gene therapy efforts is based on RNA-based suppressors of viral replication, like ribozymes and RNA decoys. Ribozymes are RNA molecules that can be engineered to cleave RNA at specific sites (80). Retroviral vectors expressing hammerhead ribozymes targeted against different regions of the HIV genome have been shown to inhibit viral replication in transduced cells (81-83). A hammerhead ribozyme targeting the common exon of the Tat and Rev genes has been shown to inhibit HIV replication in a human T cell line (84). Because ribozymes are extremely sequence specific, mutations in the virus would rapidly result in resistance. To address this concern, combination strategies with ribozymes that target different sites, or with ribozymes together with other antiviral genes, such as RNA decoys, have been proposed. In fact, a fusion molecule consisting of a ribozyme targeting the U5 region of the HIV LTR and an RNA decoy representing stem-loop IIB of the RRE, has been shown to be more efficient than ribozymes or RNA decoys alone (85, 86). The expression of antisense RRE decoys in retroviral vectors is also being explored as of potential therapeutic value (87-89). The effects of stable expression of antisense RNA targeting the Rev, Tat, and Vpu genes on viral replication has also been investigated, and showed to be of limited efficacy (90, 91).

Although gene transfer for the treatment of HIV infections is an attractive alternative or complement to the use of antiviral drugs, it is still not a reality, and many problems related to gene delivery and level of expression remain to be solved (69). More classical antiviral approaches, such as drug discovery, are being pursued, extending the efforts towards other viral targets, and one of these is Rev. The next Sections will describe antisense and drug screening targeting the Rev protein, that do not involve gene transfer.

4.2 Inhibiting Rev function via antisense oligonucleotides and other nucleic-acid molecules

The antisense RNA strategy was inspired by a naturally occurring mechanism of gene regulation in prokaryotes (92). The specificity of Watson-Crick base pairing made antisense molecules very attractive as potential therapeutic agents. A vast amount of literature exists on the application of this strategy to human diseases, including viral infections (reviewed in 93). Both unmodified and modified antisense oligonucleotides directed against various HIV RNA sequences have been shown to inhibit viral replication, both in a sequence-specific and in a non-sequence specific manner. A synthetic phosphorothioate oligodeoxynucleotide targeting Rev mRNA has been shown to have antiviral activity in chronically infected cells, inhibiting HIV replication by

80% at 25 μM (94), possibly through inhibition of translation. Since very early on, oligonucleotides complementary to the RRE sequences were shown to have the capability of disrupting Rev-RRE binding in vitro (95), several modified oligonucleotides targeting different stem-loops of the RRE have been tested for inhibition of viral replication (96, 97), and found to inhibit viral replication in a specific manner.

A novel nucleic acid-based approach towards inhibition of HIV infection by blocking Rev function has been the use of decoy RNA-DNA chimeric oligonucleotides containing the high affinity 13 nucleotide "bubble" structure of stem-loop IIB (see Figure 3) (98). These chimeric decoy bound the RRE with high affinity in vitro and were shown to inhibit HIV replication 40-70% at ~10 mM, using various assays (98).

In spite of the enthusiasm generated by the use of phosphorothioate oligonucleotides in the area of viral diseases, to date these strategies have met with limited success and significant issues remain in their potential use as therapeutic agents, including efficacy, cell permeability, delivery and cost. Because of the present limitations of both gene therapy and nucleic-acid-based antivirals, it is important that traditional approaches, such as screening for compounds with anti-Rev activity, are explored. The next Section will review the low molecular weight compounds and natural products that inhibit Rev.

4.3 Low-molecular weight compounds and natural products that inhibit Rev

Rev has been considered a promising target for therapeutic intervention of HIV infections since it was proven to be essential for HIV replication. The earliest attempts at intefering with its function were based on antisense technology, in the late 1980s (see previous Section). In the past few years, knowledge of the mechanism of action of Rev has increased rapidly, and it became clear that Rev offers several molecular targets for drug discovery. The very specific Rev-RRE interactions have been a preferred target for drug discovery, since it has no cellular counterpart. At the same time, other groups have focused on cell-based assays that would allow discovery of a drug that acts at the level of the interaction of Rev with the cellular transport machinery, as well as at the RNA-binding level. The next sections will describe the compounds and natural products that have been found to interfere with Rev function, as well as their potential usefulness as therapeutic agents. A list of these agents is presented in Table 1**. 4.3.1. Intercalating agents and other RNA-binding compounds**

The first approaches towards anti-Rev drug discovery focused on the Rev-RRE interaction. Because Rev binds to an RNA target, intercalating agents with specificity or preference towards RNA were investigated

as potential Rev-RRE inhibitors. The intercalating dye pyronin Y was reported to completely block the formation of the Rev-RRE complex *in vitro*, at low μM concentrations (99). In agreement with previous reports that Rev-RRE binding is a pre-requisite for oligomerization (13, 19, 20) this intercalating agent also block the formation of multimeric complexes. Despite these strong *in vitro* effects, the dye failed to inhibit HIV replication in cytoprotection assays, in part because of its high levels of cellular toxicity (99). This result was not altogether surprising since pyronin Y is known to intercalate DNA in addition to RNA. Other intercalating agents, derivatives of diphenylfuran, were also reported to inhibit Rev-RRE interaction, by causing a conformational change in the RRE (100). Although these agents can be useful as probes to investigate the precise mechanism of Rev-RRE binding, intercalating agents are clearly not attractive molecules from a therapeutic point of view, because of their many toxic and mutagenic effects.

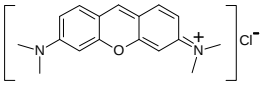
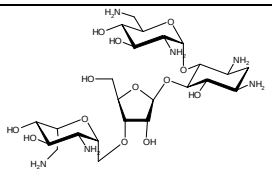
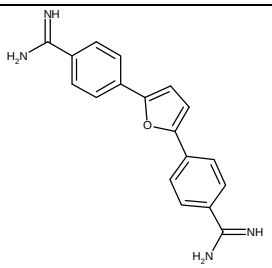
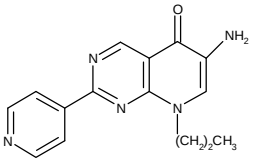
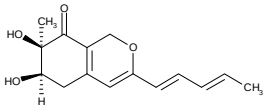
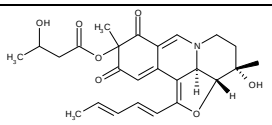
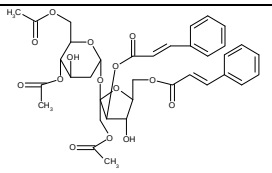
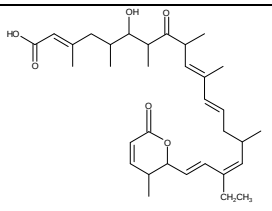
Non-intercalating compounds with previously known RNA binding properties were also candidates for inhibition of Rev-RRE binding. In this group of molecules, the aminoglycoside antibiotic neomycinB and some of its analogs were reported to disrupt Rev binding to the RRE in a specific manner (40, 101). Aminoglycoside antibiotics are known to act at the level of prokaryotic ribosomes, disrupting mRNA translation by binding to 16S RNA (102,103). In addition to binding to bacterial 16S RNA and to the RRE, these antibiotics have been also reported to interfere with splicing (104) and to bind to hammerhead ribozymes (104). The binding affinities of the aminoglycosides for their RNA targets are not very high, they are in the low μM range (40, 101, 104, 105), and therefore not surprisingly this binding has been shown to have a low degree of specificity or selectivity (106). Because of this, it is expected that a large number of cellular RNA molecules will bind to these compounds in the μM range. In fact, aminoglycoside antibiotics are known to be quite toxic to human (107). As with intercalating agents, these molecules are also not very interesting from a therapeutic point of view, due to toxicity and lack of specificity.

4.3.2 Screening approaches

A classical approach towards drug discovery has been the random screening of a vast number of synthetic organic compounds or fungal/plants extracts. This method of discovery, combined with the use of medicinal chemistry, has been very successful in discovering new activities resulting in the development of therapeutic agents. Not surprisingly, this approach has been utilized to discover compounds capable of inhibiting Rev function.

A small-molecule inhibitor of Rev was discovered at Sterling-Winthrop (now Sanofi-Winthrop), using a 96-well plate assay to measure Rev function in transfected cells (108). The assay measured production of the p24 protein from the HIV *gag-pol* gene as a result of Rev expression, in COS-1 cells. A series of structurally related compounds, 8-alkyl-2-(4-pyridyl)pyrido[2,3-

Table 1. Low molecular weight inhibitors of Rev function. The structure of the compounds described so far as Rev inhibitors is shown, as well as the molecular target and their effect on HIV replication assays.

Compound		Molecular target	Inhibition of HIV replication
Pyronin Y		RRE binding (Intercalating agent)	No
Neomycin B		RRE binding	85% at 2.5 mM
Diphenylfuran Derivatives		RRE binding (Intercalating agent)	Not tested
WIN-49611		Unknown, NOT RRE binding	Yes, IC_{50}=3.5 μM
Harziphilone		RRE binding	NO
Fleephilone		RRE binding	NO
Niruriside		RRE binding	NO
Leptomycin B		Inhibition of nuclear export of Rev	Yes, IC_{50}=2-6 nM

d]pyrimidin-5(8H)-ones, were found to inhibit Rev-dependent p24 production with an IC_{50} in the low μM range. These compounds were also found to inhibit HIV-1 replication in a human T lymphoma line in the same concentration range. Because cytotoxicity was observed at concentrations of ~25 μM, these compounds are not likely to be of therapeutic use in their present form, although they could be considered leads for the design of less toxic, more potent derivatives. At the same time, this effort has provided proof that inhibitors of Rev can be found using classical screening approaches.

A screening of natural products using an *in vitro* Rev-RRE binding assay was carried out at Bristol-Myers Squibb. Three novel natural products, one from a plant and two from fungi, were discovered and isolated by bio-assay guided fractionation (109, 110). The plant metabolite, niruriside, was isolated from *Phyllanthus niruri*, a plant widely used in Indian traditional medicine. This compound was shown to inhibit binding of Rev to the RRE at an IC_{50} of 3 μM, while the IC_{50} on an unrelated protein-RNA binding system (the R17 coat protein/operator RNA) was greater than 130 μM (109). However , this compound did not protect CEM-SS cells from acute HIV infection (109). Likewise, the two fungal metabolites, harziphilone and fleephilone, from the fungus *Trichoderma harzianum*, were found to inhibit Rev-RRE binding by 50 % at 2-8 μM, but had no anti-HIV activity as tested in the cytoprotection assay (110). Because of this lack of antiviral activity, these natural products are not considered useful. It is not clear why these compounds failed to inhibit HIV replication: since they were discovered in an in vitro assay, it is therefore possible that these metabolites fail to enter the cell, are metabolized by it, or have masking cytotoxic effects. These concerns were not addressed by these publications.

In contrast with Rev-RRE binding approaches, or cell-based assays measuring Rev function, a recent effort to discover inhibitors of Rev has focused on nuclear export (111). Rev acts in conjunction with the cellular nuclear export machinery, and to function it needs to translocate from the nucleus to the cytoplasm (see Sections 3.3 and 3.4). Four antibiotics of the leptomycin-kazusamycin family were found to inhibit the export of Rev to the cytoplasm at nanomolar concentrations, in Rev-expressing HeLa cells treated with actinomycin D. Leptomycin B was found to be specific in its inhibition of the nuclear export pathway, while it had no effect on nuclear import processes (111). This antibiotic was also found to inhibit HIV-1 replication in primary human monocytes, with an IC_{50} of 0.6 nM (111). However, because of its long-term toxicity in tissue culture, leptomycin cannot be used therapeutically. Although this drug was shown to affect only Rev-dependent gene expression, it is possible that the transport of other cellular molecules (proteins or ribosomal or small nuclear RNAs) is also inhibited. The inhibition of a cellular pathway used by Rev could explain the toxic effects of this drug, and it raises the possibility that all Rev inhibitors that affect this Rev function will prove unsuitable as therapeutic agents. A greater knowledge of the nuclear export pathway used by different cellular protein and mRNA species will be necessary to evaluate this hypothesis.

Although none of the compounds discovered to inhibit Rev function is currently being pursued as potential drugs, it is important to point out that drug discovery is a laborious and sometimes slow process, and that Rev has only recently become a target for discovery and development. At Oncogene Science, Inc., we are carrying out high throughput screening seeking Rev inhibitors, using a cell-based assay similar to the one used at Sterling-Winthrop (108). This program is in the early phases, and we hope to contribute new entities with new activities.

5. CONCLUSIONS AND PERSPECTIVE

The recent excitement generated by the combination therapies using reverse trancriptase inhibitors and protease inhibitors has produced renewed interest in the biotechnology/pharmaceutical industry to search for new therapies targeting other viral proteins, such as integrase. Because of its crucial function in HIV replication, Rev represents an attractive target. Gene therapy and nucleic acid-based approaches have been the primary focus of both academic and industry researchers in this area. Although these novel therapies are very promising, they are still in early stages. The encouraging results of these approaches in tissue culture systems validate Rev as a target for anti-HIV intervention. Using more classical approaches, some Rev inhibitors have been discovered, although none of them appears to be likely to be developed into a therapeutic agents. All of these approaches are in early phases, and these first attempts represent proof-of-principle experiments indicating that Rev-RRE interactions can be disrupted by small molecules, and that Rev interactions with the cellular machinery of nuclear export are also a valid molecular target for drug discovery.

6. ACKNOWLEDGMENTS

The author's drug discovery research is partly supported by an Small Business Innovative Research (SBIR) grant from NIAID, "Inhibition of the HIV-1 Rev transactivator", grant number 5 R44 AI34685-03

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