Revisiting transdominant-negative proteins in HIV gene therapy

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HIV remains a global public health issue and new therapies are actively being developed. Traditional treatments such as small-molecule inhibitors are being investigated; however, newer modalities are also being pursued, including the use of transdominant-negative proteins. A transdominant negative is a mutant of a protein designed to interfere with the normal activity of its wild-type counterpart. Transdominant negatives designed to block HIV replication are based on viral proteins; however, recent approaches show that transdominant negatives of cellular proteins have therapeutic potential. Recent discoveries have revealed that treatments based on transdominant negatives can greatly disrupt the replication cycle of the virus. This article aims to review viral and cellular protein-based transdominant negatives, the recent elucidation of their modes of action and their potential use in HIV gene therapy.

An estimated 2.5 million people worldwide were infected with HIV in 2011 [101]. Although the epidemic has not been controlled, considerable progress in HIV treatment has been made over the last 20 years. Current approaches to manage HIV/AIDS are based on combined antiretroviral therapy (cART), which is a cocktail of drugs designed to inhibit virus replication. It is clear that cART remains the best therapy against HIV since this treatment strategy became available in 1996. It can, however, have significant complications and side effects. Most importantly, cART does not cure patients of the infection because the virus becomes dormant in certain populations of lymphocytes and other immune cells, called reservoirs. Therefore, this therapy must be a lifelong engagement in order to suppress these reservoirs, which will periodically produce new virus and spread infection. Despite all the benefits of cART, new treatment modalities must be developed in order to protect immune cells from HIV and to target the latent viral reservoir with the aim of eradicating these last vestiges of HIV infection. Such future treatments may offer a functional cure for AIDS in terms of the permanent relief from daily antiretroviral therapy and from the risk of transmitting HIV.

Gene therapies designed to express proteins have potential as a therapeutic modality for HIV infection. One therapeutic approach makes use of transdominant-negative proteins, which are engineered mutants of pre-existing proteins that are capable of interfering with the normal activities of their wild-type counterparts. The engineering of transdominant negatives usually involves mutating or deleting specific critical amino acids in order to eliminate domains that the wild-type protein uses to execute its functions. In either case, transdominant negatives still retain the ability to interact with either the wild-type protein from which they are derived or another cellular target that the wild-type would usually bind to. Thus, transdominant negatives are still functional up to a certain point. However, due to the presence of their engineered mutations, they cannot functionally proceed past this point, in contrast to the wild-type protein. In this way, transdominant negatives block the molecular processes that the wild-type catalyzes. They are considered 'transdominant' because, once in their presence, the wild-type protein is incapable of overcoming the 'negative' block exerted by the transdominant negative.

Transdominant negatives are well suited for gene-based therapies. Being proteins, transdominant negatives are readily encoded by DNA sequences that may be conveyed by standard gene therapy delivery strategies, such as lentiviral particles. In the case of therapies against HIV/AIDS, researchers are developing transdominant negatives that can be classified into two broad categories: transdominant negatives derived from HIV proteins and those derived from human cell proteins. Here, we will review transdominant negatives from a brief historical perspective, highlight recent discoveries and developments of both viral and cellular transdominant negatives and offer a future perspective on their application in genetic therapies aimed at functionally curing AIDS.

Review
Future Virology

Keywords
■ AIDS ■ functional cure ■ gene therapy ■ HIV ■ transdominant-negative mutant

Future Medicine part of fsg

Viral transdominant-negative proteins

The concept of using mutated HIV proteins as viral inhibitors was first tested for HIV approximately 24 years ago, beginning with Rev and Tat of HIV [1–3]. These essential viral proteins remain attractive targets for antiviral approaches such as targeting with transdominant negatives (Figure 1). More recent transdominant inhibitors are based on the Vif [4,5] and Gag structural protein of HIV [6–11].

RevM10: a Rev transdominant-negative protein

Rev is typically a 116-amino acid viral protein that has several functional domains, including those for multimerization, nuclear localization, RNA binding and nuclear export [12,13]. In HIV replication, Rev binds to an RNA stem loop structure in the envelope-encoding region of viral mRNA transcripts called the Rev response element (RRE). By binding to the RRE, Rev can selectively transport RRE-containing unspliced and singly spliced viral mRNAs (but not RRE-deficient, multiply spliced viral mRNAs) from sites of their transcription in the nucleus to sites of their translation within the cytoplasm [14,15]. Rev can form homomultimers when bound to the RRE, and this multimerization is a prerequisite for its mRNA transport function [16]. Another prerequisite is the interaction between Rev and the nuclear export factor CRM1, which initiates the passage of RRE-containing viral mRNA through the nucleus for export into the cytoplasm [17,18]. It is unclear exactly when and where the Rev–CRM1 complex is formed in the nucleus.

A mutant version of the Rev protein, called RevM10, was described by Malim and colleagues as a transdominant negative against Rev function and HIV replication [19]. RevM10 contains two amino acid substitutions within its nuclear export domain compared with wild-type Rev. The mutations do not prevent RevM10 from multimerizing with wild-type Rev, but do abrogate its interaction with CRM1 [17]. Thus, RevM10 does not export RRE-containing viral mRNA to the cytoplasm due to this lack of interaction with CRM1, even in the presence of wild-type Rev, leading to inhibition of synthesis of the viral proteins encoded by these mRNA. In order to exert a strong antiviral effect, however, RevM10 must be in molar excess relative to wild-type Rev [20].

RevM10 is the only viral transdominant negative to have been used thus far to treat cells in human clinical trials. In the mid-1990s, vectors conveying RevM10 were used to treat $CD4^+$ T lymphocytes apheresed from and then reinfused into patients [21]. Follow-up trials used RevM10 or a 'humanized' RevM10, called huM10, alone or in combination with other antiviral strategies, such as antisense RNA or treating autologous $CD34^+$ hematopoietic stem cells before engrafting back into patients [22–28]. Overall, the procedures proved to be safe [29], but did not result in a significant reduction in viral loads within patients. It is believed that more robust RevM10 expression and improved engraftment efficiency of the treated $CD34^+$ hematopoietic stem cells could improve therapeutic efficacy [30].

Tat transdominant-negative proteins

Tat is an essential viral regulatory protein of variable length (86–104 amino acids) encoded by two exons in the HIV genome. Tat's best-described function is during the activation of HIV gene expression, in which it upregulates RNA polymerase II-directed transcription by several orders of magnitude [31,32]. Two critical domains of Tat are required for viral gene expression: an activation domain (amino acids 1–48) and an RNA binding domain (amino acids 49–57). The RNA binding domain interacts with the transactivation response element RNA structure present on newly transcribed viral mRNA. This domain also contains a nuclear localization signal that retains Tat within the cell nucleus [33,34]. The activation domain of Tat interacts with the transcription elongation complex P-TEFb in order to hyperphosphorylate the C-terminal domain of RNA polymerase II, the result of which is the robust production of full-length viral mRNA [35,36]. Tat also has additional roles in HIV replication and pathogenesis. It can, for example, modulate HIV reverse transcription, inhibit apoptosis in cells, promote oxidative stress and elicit neurotoxicity, HIV-associated dementia and tumorigenesis [37].

Early studies showed that one exon-encoded Tat protein with either a truncated or deleted RNA binding domain or with alanine and glycine residues substituted for the RNA binding domain could inhibit the transactivation of HIV gene expression by wild-type Tat. In an early preclinical study, a stable human T-cell line expressing mutant Tat, where amino acids 50–57 were substituted with alanine and glycine residues (TatΔ50–57), was only marginally protected from HIV infection and replication compared with untreated T cells. However, the combined expression of TatΔ50–57 with RevM10 provided better protection from HIV than if they were expressed separately [38]. More recently, Meredith and colleagues created a two-exon-encoded

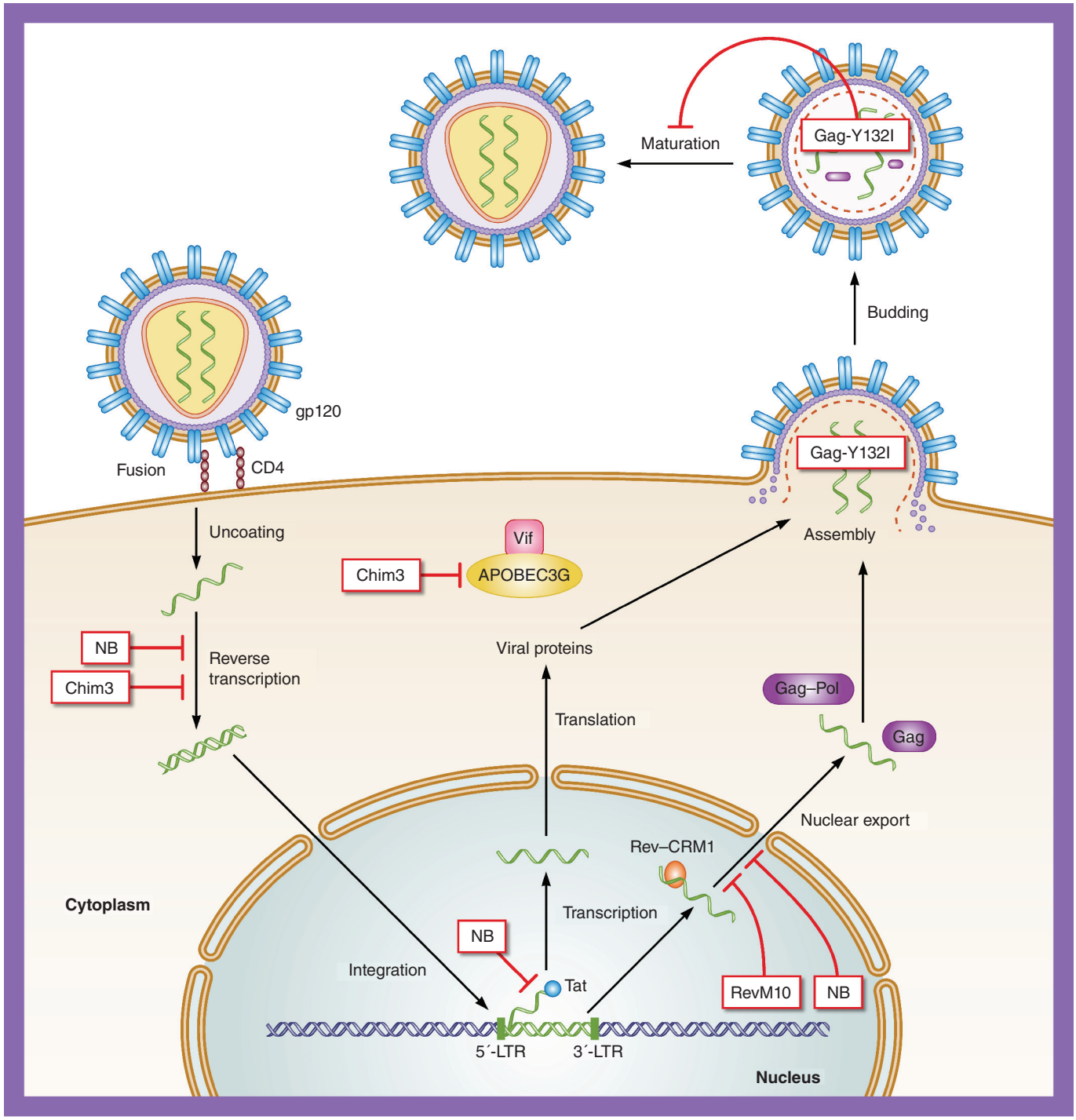

Figure 1. Modes of action of viral transdominant-negative proteins. A schematic diagram of the complex HIV life cycle is shown, with all major steps displayed and the modes of action of the viral transdominant-negative proteins indicated in red. The viral transdominant negatives NB and Chim3 are capable of inhibiting the reverse transcription of viral RNA to proviral dsDNA. Chim3 can also downregulate viral replication by forming nonfunctional dimers with HIV Vif, which renders it incapable of neutralizing the cellular antiviral factor APOBEC3G. Later in the replication cycle, NB downregulates Tat-mediated viral gene transcription, as well as viral mRNA nuclear export, which is mediated by HIV Rev and the cellular export factor CRM1. Rev-dependent viral mRNA export can also be downregulated by the transdominant negative RevM10. Maturation of the newly formed virion can be inhibited by the Gag transdominant mutant Gag-Y132I, which prevents protease from cleaving the capsid and matrix viral proteins from the Gag and Gag–Pol precursor polyproteins, resulting in an abnormal and noninfectious virus particle.
LTR: Long terminal repeat; NB: Nullbasic.

mutant of Tat, termed Nullbasic, where the RNA binding domain was similarly substituted with alanine and glycine residues [39]. Like other Tat transdominant mutants, Nullbasic disrupts Tat-dependent transactivation by presumably competing with wild-type Tat for interaction with P-TEFb. Unlike previous Tat mutants, however, Nullbasic was also described to have novel antiretroviral effects. When expressed in a HIV-infected cell, Nullbasic could suppress the expression of unspliced and singly spliced viral mRNA by a mechanism attributed to the inhibition and mistrafficking of Rev [39,40]. Nullbasic was also observed to inhibit reverse transcription within cell-free virus particles, although the mechanism by which this occurs remains to be defined. When introduced into cultured human T cells or primary $CD4^+$ T cells isolated from human blood, Nullbasic sharply inhibited HIV replication in a sustained manner and cytotoxic effects were not noted in cells expressing Nullbasic [41]. This appears to be the first transdominant negative that targets three independent steps of the HIV life cycle. Whether Nullbasic inhibits Rev and reverse transcription as a transdominant negative or as a consequence of the mutation imparting a new antiviral activity remains to be determined.

Vif transdominant-negative proteins

Vif is a HIV protein that plays critical functions during infection. One well-described role is preventing the action of the cellular anti-HIV restriction factor APOBEC3G (hA3G) by inducing its degradation [42]. In the absence of Vif, hA3G counteracts the viral replication cycle by inhibiting reverse transcription and proviral DNA integration [43], and by inducing a DNA damage response pathway that targets HIV-infected cells for cytolysis by NK cells [44]. Early systematic mutations of Vif have demonstrated antiviral potential by inhibiting the interaction between wild-type Vif and hA3G [5]. Another study by Porcellini and coworkers [4] has identified a mutant chimera of Vif from the F12 clone of HIV [45], called Chim3, which is able to inhibit HIV replication in a transdominant-negative fashion with regards to the antiviral action of hA3G. They observed that Chim3 formed nonfunctional heterodimers with wild-type Vif when coexpressed in HIV-producer cells, thereby preserving the anti-HIV activity of hA3G. While hA3G is a known inhibitor of reverse transcription, sole expression of Chim3 in human $CD4^+$ T lymphocytes and macrophages inhibited HIV reverse transcription in the absence of either Vif or hA3G, suggesting that Chim3 may inhibit reverse transcription in a hA3G-independent manner [4]. The precise mechanism by which Chim3 downregulates reverse transcription, however, remains to be elucidated. Interestingly, Chim3-treated human $CD4^+$ T lymphocytes had a survival advantage during HIV infection compared with untreated cells, an attribute that was due in part to Chim3-induced G2 cell cycle delay [46]. Hence, Chim3 has many properties that make it an excellent candidate for a cell-based therapy to protect human immune cells from HIV infection.

Gag-based transdominant-negative proteins

Pr55Gag (Gag) is a HIV structural precursor polyprotein that, along with Pr160Gag–Pol (Gag–Pol) and envelope proteins, assembles on the plasma membrane of cells to initiate virion formation and budding in conjunction with cellular proteins that include TSG101 and ALIX (reviewed in [47]). At this point, the nascent virus particles are immature and noninfectious and must be cleaved by HIV protease at distinct locations within Gag and Gag–Pol in order to form infectious virions [48]. Early studies established that HIV infectivity could be inhibited by targeting Gag processing with low-dose HIV protease inhibitors, suggesting that unprocessed Gag within the virion has an inhibitory effect on infectivity [49]. Similarly, virion infectivity could be inhibited by the coexpression of mutant Gag proteins in HIV-producer cells [6–11]. In particular, Lee and coworkers showed that incorporation of only 4% of Gag proteins possessing a tyrosine-132 to isoleucine mutation (Y132I) inhibited HIV infectivity by 50% [6]. Indeed, virions with a 20% ratio of mutant to wild-type Gag produced severely defective virus particles that were blocked at or before the reverse transcription step. Similarly, Müller and colleagues showed that when Gag was mutated at the protease cleavage site located within the CA-SP1 junction, the mutant had a transdominant-negative effect against protease-mediated cleavage of wild-type Gag, thus impairing maturation of the virus particle [8]. It is worth noting that the CA-SP1 junction is the target of the US FDA-unapproved maturation inhibitor bevirimat, treatment with which results in the formation of aberrant, noninfectious virion core structures (reviewed in [50]). Bevirimat has had limited success in human clinical trials due to the generation of drug-resistance mutations in HIV, as well as the occurrence of natural Gag polymorphisms that decreased virus susceptibility. Future progress

of Gag-based transdominant negatives awaits preclinical trials, such as testing in humanized mouse models of HIV infection, in order to determine whether they will fare better in the clinical setting compared with bevirimat.

Cellular transdominant-negative proteins

Similar to viral proteins, the proteins of the human cell may also have the potential to be developed into transdominant negatives against HIV (Figure 2). Such human cell proteins would normally be involved, either directly or indirectly, with the HIV replication cycle, so transdominant-negative versions of these proteins would therefore abrogate the usurped roles played by their wild-type counterparts during infection. Cellular transdominant negatives potentially expand and diversify the repertoire of antiviral factors available for a future HIV therapeutic regimen. Rigorous safety profiling of such cellular protein-based transdominant negatives will be necessary, however, in order to ensure that their expression in human cells does not alter the functionality of their wild-type counterpart in normal cell physiology. Here, we will review mutants of three cellular proteins – Sam68, INI1 and HDAC1 – which have demonstrated transdominant-negative activities against HIV.

C-terminal deletions of Sam68

Sam68 is an RNA binding protein involved in regulation of the cell cycle [51–53], neuronal activity-dependent alternative mRNA splicing [54], the TNF signaling pathway [55] and RNA metabolism during homeostasis [56] and tumorigenesis [57]. Reddy and colleagues discovered that Sam68 can enhance protein expression from RRE-containing HIV mRNA, a subset of transcripts expressed by HIV that normally depends on Rev for protein translation [58]. Sam68 was found to directly bind to the RRE structure, thereby partially substituting for Rev function during viral protein production. However, overexpression of both Rev and Sam68 synergistically enhanced Rev-dependent mRNA expression [58], suggesting that Rev and Sam68 function via different pathways to promote viral protein production. Furthermore, knockdown of Sam68 by RNAi in HIV-producer cells severely inhibits virus production, indicating the critical role of Sam68 in the HIV life cycle [59].

In their paper, Reddy and colleagues demonstrated that C-terminal-deleted Sam68 mutants, where amino acids 330–443 or 410–443 were removed from the protein (collectively called Sam68ΔC), were transdominant against both Rev activity and virus production [58]. A later investigation suggested that an Arg-429 to alanine substitution in Sam68 was sufficient to recapitulate this inhibitory effect [60]. This effect was dose-dependent, and Sam68ΔC mutants were as effective as RevM10 at inhibiting HIV production [58]. Reddy and colleagues also investigated the mechanism by which Sam68ΔC functions. *In vitro* gel-shift assays, which determine the quality of binding between proteins and nucleic acids, indicated that Sam68ΔC poorly bound RRE-containing RNA, despite retaining an RNA binding domain [58]. Instead, Reddy and coworkers discovered that Sam68ΔC was able to bind both Sam68 and Rev *in vitro*, and that the mutant was able to compete with or displace wild-type Sam68 bound to the RRE structure [58], implying that the mechanism by which the mutant exerts its effect is through the formation of nonfunctional complexes with Rev and Sam68 on the RRE.

Recent studies have determined how the C-terminal deletion mutants of Sam68 inhibit Rev function within cells. RRE-containing HIV mRNA becomes sequestered within perinuclear regions of the cell upon expression of Sam68ΔC [61], but chemical release of mRNA from these perinuclear sites does not rescue viral protein translation [62]. Instead, Marsh and colleagues discovered that Sam68ΔC reduces the association of Rev-dependent viral mRNA with PABP1, a cellular protein involved in the translation of viral and cellular mRNA [62]. More thorough investigations revealed that Sam68ΔC can also suppress the expression of HIV Nef-encoding mRNA, which is not dependent on Rev function for translation, but not other Rev/RRE-independent viral mRNAs [63]. How Sam68ΔC can block the translation of both RRE-containing and only certain RRE-lacking HIV mRNAs remains to be fully explained, but may be due to differing compositions of the cytoplasmic ribonucleoprotein complexes between Sam68ΔC-sensitive and Sam68ΔC-resistant viral mRNA [64]. Overall, it is clear that C-terminal deletion mutants of Sam68 can potently block HIV mRNA translation in a transdominant-negative manner, which makes them as attractive for therapeutic application as RevM10.

S6: an inhibitory fragment of INI1

INI1, also known as hSNF5, is a tumor suppressor and core component of the SWI/SNF chromatin remodeling apparatus [65]. Integrase binds to INI1 through one of the two imperfect repeat regions that INI1 encodes [66,67]. INI1

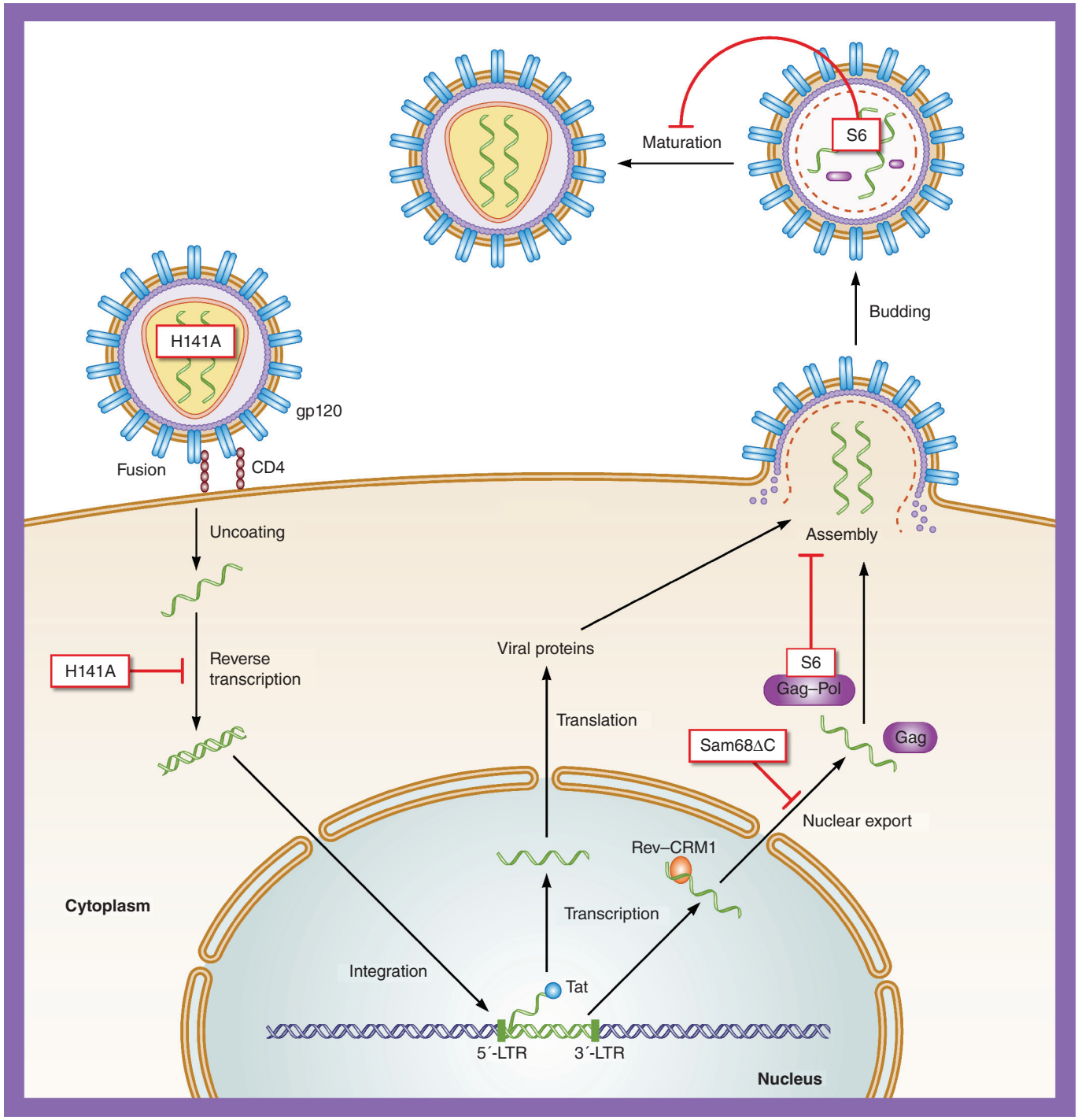

Figure 2. Modes of action of cellular transdominant-negative proteins. A schematic diagram of the complex HIV life cycle is shown, with all major steps displayed and the modes of action of the cellular transdominant-negative proteins indicated in red. The presence of H141A, a transdominant-negative mutant of the histone deacetylase HDAC1, within virus particles leads to a dramatic decrease in virion deacetylase activity that blocks HIV infectivity at a stage after cell entry, perhaps during reverse transcription. Carboxy-terminal deletions of Sam68 (Sam68ΔC) block the translation of key HIV proteins from Rev-dependent viral mRNA, as well as Nef-encoding, Rev-independent viral mRNA. The mechanism by which this occurs appears to be through inhibiting the cytoplasmic binding of PABP1 to these viral transcripts. S6, an inhibitory fragment of the integrase-interacting INI1 protein, interferes with HIV Gag and Gag–Pol trafficking to sites of virus budding. S6 can also disrupt viral particle maturation through its interaction with Gag–Pol, leading to the production of defective virions that are no longer infectious.
LTR: Long terminal repeat.

appears to be important for the replication cycle of HIV and is found in both the reverse transcription and integration complexes of infecting viruses [68]. Yung and colleagues screened deletion mutants of INI1 for inhibitory effects on integrase and HIV production [69]. They discovered that one mutant (called S6), encompassing INI1 amino acids 183–294, was strongly antagonistic of HIV particle assembly, production and replication. Mutation of amino acids within S6 that normally mediate the interaction between integrase and INI1 abrogated the transdominant-negative effect [69]. Furthermore, Das and colleagues determined by careful mutagenic analyses that multimerization, integrase binding and cytoplasmic localization are all important for S6 transdominant-negative activity [70].

As part of their investigation into the mechanism of S6 transdominance, Yung and coworkers found that fusion of integrase with the HIV Vpr protein (which independently traffics to assembling virus particles) and *trans*-complementation of integrase-deleted mutants of HIV with this integrase–Vpr fusion restored normal virus production [69]. This fusion of integrase with Vpr that rendered HIV resistant to S6 allowed Yung and colleagues to surmise that S6 acts on integrase within the context of Gag–Pol, one of the precursor polyproteins contained within immature virus particles. INI1 is normally a nuclear protein, but expression of S6 within cells showed that it was largely cytoplasmic [69–71]. This afforded speculation that the close vicinity of S6 to sites of virus budding from the plasma membrane allowed the incorporation of S6 into virus particles. However, competent interaction between S6 and Gag–Pol is still required because mutants of S6 that were unable to interact with integrase, but were still strongly cytoplasmic, could not inhibit HIV production [69]. Speculation on the incorporation of S6 into virus particles was confirmed when highly purified virions produced in the presence of S6 were analyzed by western blot. Indeed, S6 was discovered to be present within these virus particles [69,72].

A mechanism of transdominant action for S6 was hypothesized in which S6 incorporates into budding virions by binding to integrase within the context of Gag-Pol. This presents steric hindrance that interferes with Gag–Pol multimerization or blocks the interaction with Gag–Pol of cellular proteins that are necessary for proper virus maturation or correct subcellular localization following infection [69]. Cano and Kalpana, however, discovered that expression of S6 within cells producing HIV affected the processing and subcellular localization of Gag and Gag–Pol [71]. They observed that these HIV polyproteins altered their normally plasma membrane localization to a diffuse accumulation within the cytoplasm upon S6 coexpression. Thus, the expression of fragments of INI1 in cells producing HIV can interfere with the late stage of virus replication, from Gag and Gag–Pol trafficking to virus particle maturation, leading to defective virions that can no longer spread the infection.

The H141A mutant of HDAC1

Sorin and colleagues, in a yeast two-hybrid screen for INI1-interacting proteins, discovered that SAP18 interacts with both INI1 and HIV integrase *in vitro* [73]. Coimmunoprecipitations performed in human cells allowed the team to determine that INI1 could interact with the SAP18-containing Sin3a–HDAC1 complex. This complex is normally involved in epigenetic regulation of cellular gene transcription. They incidentally observed that coexpression of a HIV vector enriched INI1–SAP18–HDAC1 complexes in cells, which suggested to the authors that such complexes were enhanced by the presence of HIV-expressed integrase [73].

The team surmised that since INI1 is specifically incorporated into HIV particles [69], HDAC1 may similarly be encapsulated. Indeed, INI1, SAP18, Sin3a and HDAC1 were all found in HIV infectious particles, but not in other non-HIV retroviruses [73]. Importantly, not all of the members of the SWI/SNF family of complexes to which the Sin3a–HDAC1 complex belongs were found in HIV particles, indicating that Sin3a–HDAC1 is specifically enriched in virions. Further investigations using integrase-deficient HIV determined that this enrichment is integrase-dependent [73].

Discovery of HDAC1 in HIV particles enabled Sorin and colleagues to investigate the effect on HIV of a catalytically inactive mutant of HDAC1, called H141A, in which His-141 within the catalytic domain is mutated to alanine [73]. H141A is a transdominant-negative mutant of HDAC1 since, despite lacking catalytic activity, the mutation does not alter the protein's structure, stability or incorporation into Sin3a–HDAC1 complexes [74]. Expression of the H141A mutant in HIV-producer cells surprisingly led to a five- to ten-fold increase in virus particle production, but when these particles were assessed for infectivity, they were found to be three- to ten-fold less infectious than control virus [73]. Furthermore, when the investigators collected virions from H141A-expressing cells to assess their endogenous

deacetylase activity, they discovered a three- to ten-fold decrease in activity compared with virus produced in the absence of the H141A mutant. This suggested that HDAC1 accounts for the majority of deacetylase activity in the HIV particle, and that expression of a transdominant negative can counteract this endogenous activity.

Sorin and colleagues attempted to determine the mechanism of action of the H141A transdominant mutant [73]. They looked at early events of the HIV replication cycle of virus particles coexpressed with either wild-type HDAC1 or the H141A mutant. While not affecting the entry of virus into cells, the team discovered that reverse transcription (both early and late phases) was significantly inhibited in viruses that were coexpressed with the H141A mutant [73]. The effect of the transdominant negative on virion infectivity was similar in nature to the effect of silencing HDAC1 expression by siRNA in virus-producer cells [73]. Thus, it could be speculated that the mechanism of transdominance of the H141A mutant is to sequester wild-type HDAC1 in budding viruses, which leads to suppression of virion deacetylase activity that negatively impacts on virion infection at a stage after entry but before or during reverse transcription.

Transdominant-negative stacking as a genetic therapy

One exciting approach to gene therapy is the so-called 'stacking' of transdominant negatives with engineered HIV restriction factors. Restriction factors are components of a cell's innate immunity that have evolved to protect the cell from pathogen infection. Certain restriction factors, however, have been circumvented by HIV due to coevolution of viral resistance factors. Voit and coworkers generated HIV-resistant human T cells by stacking RevM10 with engineered versions of the restriction factors TRIM5α and APOBEC3G in cells specifically deleted for the HIV coreceptor CCR5 [75]. They first introduced nucleotide deletions in *CCR5* alleles using zinc finger nucleases, which specifically recognize customizable sequences in order to digest a few nucleotides in the genome at predictable locations. This digestion leads to frameshift mutations in the targeted gene, resulting in prematurely truncated or nonfunctional proteins.

Both the human endogenous versions of TRIM5α and APOBEC3G do not effectively restrict HIV replication. The authors, however, utilized a chimera of TRIM5α containing human and rhesus macaque sequences [76], and an Asp-128 to lysine (D128K) mutant of APOBEC3G [77], both of which have been shown to be active against HIV. Voit and colleagues were able to insert these engineered restriction factors by zinc finger nuclease-mediated homologous recombination, along with the RevM10 sequence, into the deleted *CCR5* loci. These restriction factor-stacked cells could resist infection by HIV that relies on the CCR5 coreceptor for cell entry by over 1000-fold compared with untreated cells. Interestingly, the same cells could resist CXCR4 tropic HIV, which does not require CCR5 to enter cells [75]. This was most likely due to the effects of the TRIM5α and APOBEC3G D128K factors, which restrict HIV after cell entry and are therefore coreceptor-independent in their modes of action. Early studies combined transdominant-negative Tat and Rev proteins that inhibited HIV replication in human T cells with at least additive antiviral effects [38]. This new stacking strategy combines transdominant negatives with engineered restriction factors and provides potent resistance against HIV by using what amounts to a genetic version of cART.

Conclusion

Since the discovery of RevM10 nearly 25 years ago, transdominant negatives have been slowly but surely recognized as a viable means to inhibit HIV replication within human cells. Over this time, the repertoire of transdominant negatives has greatly expanded to include both virus- and cell-based derivatives that show potent ability to block spreading HIV infections. Moreover, the range of mechanisms by which some transdominant negatives elicit their antiviral properties is much broader than those of traditional small-molecule antiretroviral therapies, thereby offering future clinicians diverse treatment options for their patients. As the molecular details on the modes of action of individual transdominant negatives continue to be resolved, we will soon find transdominant negatives poised to enter clinical trials, just as RevM10 was two decades ago.

Future perspective

HIV is arguably the most studied human pathogen in science and over 30 years of research has provided us with valuable insights and directions towards a possible cure for AIDS. Indeed, HIV researchers may be close to a breakthrough clinical trial that demonstrates a safe and powerful gene therapy that may greatly reduce or even eliminate the need for antiretroviral drugs. Development of the concept of transdominant-negative stacking shows that researchers and clinicians are learning

from past experiences with small-molecule antiretrovirals and are anticipating HIV resistance. Just as the virus can adapt to circumvent individual anti-HIV drugs, it is not inconceivable that the virus will do the same for individual transdominant negative-based therapies. Thus, similar to the development of cART, transdominant-negative stacking will play a central role in future clinical trials and treatment regimens. The rationale is simple: the more transdominant negatives that an infecting virus faces, the more steps of its replication cycle will be attacked, and the harder it will be for HIV to evolve the genetic changes required to develop resistance. The diverse modes of action of both viral and cellular transdominant negatives will be to their advantage in offering a robust barrier to HIV resistance. The same diversity, however, necessitates very careful scrutiny for toxic side effects and unwanted immune reactivity against any transdominant negative administered to patients. It is interesting to note that the RevM10 clinical trials did not report immune responses in patients against cells expressing RevM10 [78]. It is possible that transdominant negatives with multiple modes of action, such as Nullbasic and Chim3, may prove to be useful by offering multistage protection against HIV with as few antiviral agents as possible.

A concerted effort is required to turn transdominant negatives into treatments and cures for HIV/AIDS. Better and more specific gene therapy delivery systems will be needed to limit unnecessary expression in irrelevant cells, to reduce tumorigenic potential and, most importantly, to effectively target those latent reservoirs where HIV lies dormant. In this age when eradication of HIV is in sight [79], the latent viral reservoirs have become the challenge. Some transdominant negatives, such as Nullbasic and RevM10, which oppose HIV-1 gene expression, may be utilized in this regard if they can be targeted to the appropriate cells – a task that may someday be feasible using viral vectors targeting specific cell types [80–82]. Transdominant negatives are developing into worthy treatments that complement and enhance our existing strategies against the virus. Specific targeting and precise delivery will elevate transdominant negatives into being key components of any future prescription to functionally cure HIV/AIDS.

Executive summary

Viral transdominant-negative proteins

- RevM10 was the first viral transdominant-negative protein to be discovered. Since its discovery in 1989, RevM10 has been tested in human trials. It inhibits viral mRNA nuclear export in a CRM1-dependent manner.
- Tat transdominant-negative mutants interfere with HIV gene expression following integration of the virus into a cell. Nullbasic is a two-exon transdominant-negative mutant of HIV Tat that potently inhibits three distinct steps of the viral life cycle.
- Chim3, a mutant of HIV Vif, blocks the ability of wild-type Vif to neutralize the restriction factor APOBEC3G. Chim3 can also downregulate reverse transcription by an unknown mechanism.
- HIV Gag with a Y132I mutation or mutations in the CA-SP1 junction site are transdominant against viral particle production and maturation. Only 20% incorporation of Gag transdominant-negatives is required to severely inhibit HIV infectivity.

Cellular transdominant-negative proteins

- Carboxy-terminal deletions of the cellular Sam68 protein inhibit translation of HIV Rev-dependent mRNA. They can also inhibit HIV Nef-encoding mRNA, which is Rev-independent. Sam68 mutants appear to inhibit the binding of PABP1 to viral transcripts in the cytoplasm.
- S6, a fragment of INI1 spanning amino acids 183–294, inhibits HIV particle formation by mistrafficking Gag and Gag–Pol away from sites of virus budding. S6 can also block virus maturation by blocking Gag–Pol multimerization within the virion.
- A H141A mutation in HDAC1 allows S6 to abrogate wild-type HDAC1-mediated deacetylase activity in HIV particles, thereby inhibiting infection at a step after viral entry and before or during reverse transcription.

Transdominant-negative stacking

- In what amounts to a genetic version of combined antiretroviral therapy, multiple transdominant negatives may be 'stacked' into human immune cells to provide multiple, independent blockades to HIV infection. This approach strongly protects cells from HIV infection.

Conclusion

- The repertoire of transdominant negatives has grown significantly over the past 25 years. The broad range of antiviral modes of action elicited by transdominant negatives offers future clinicians diverse treatment options for patients.

Future perspective

- A concerted effort is required to progress transdominant negatives into human clinical trials.
- In parallel, gene therapy delivery systems will need to be improved, especially in their ability to target the latent HIV reservoirs.
- In the future, transdominant negatives will play a key role in therapeutic regimens aimed at functionally curing HIV/AIDS.

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