Point of VIEW

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Peptide Inhibitors of HIV-1 Egress

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ABSTRACT HIV-1 release requires a direct interaction between the p6 domain of the Gag protein and Tsg101, a component of the cellular endosomal sorting complex required for transport I (ESCRT-I). Disruption of the binding between Gag and Tsg101 is highly detrimental to particle release, making this viral−host cell interaction a potential target for the development of novel anti-HIV-1 agents. An article in this issue reports on the application of a bacterial reverse two-hybrid strategy to identify a cyclic peptide that disrupts Gag−Tsg101 binding and suppresses HIV-1 particle release.

HIV-1, the causative agent of AIDS, has led to >25 million deaths worldwide and infects 2−4 million additional people every year (www.unaids.org). Highly active antiretroviral therapy (HAART), which targets the viral enzymes protease and reverse transcriptase, has led to a sharp decline in AIDS deaths in developed nations. However, the emergence of drug-resistant strains has undermined the effectiveness of HAART therapy and has increased the need for novel drugs that target steps in the HIV-1 replication cycle that are not affected by currently available therapies. Research over the past decade has significantly increased our understanding of the molecular mechanisms involved in several key steps of the HIV-1 replication cycle, including the budding and release of virus particles from infected cells. On p 757 of this issue, Tavassoli *et al.* (*1*) report progress in the development of HIV-1 budding inhibitors.

The production of retroviral particles in infected cells is mediated by the Gag precursor protein, and distinct domains within Gag play well-defined roles in the assembly and release pathway. For example, the p6 domain, located at the C-terminus of the Gag precursor (Figure 1), promotes the release of virus particles from the cell (*2*). More than a dozen years ago, this virus release activity was mapped to a Pro-Thr/Ser-Ala-Pro (PT/SAP) motif in p6 (*3*).

The sorting of cargo proteins into vesicles that bud into late endosomes to generate multivesicular bodies requires the concerted action of a highly conserved machinery composed of three multiprotein endosomal sorting complexes required for transport (ESCRT-I, -II, and -III) and a variety of accessory molecules, including Hrs, Alix, and Vps4 (*4*–*6*). In one of many examples of viral pirating of host cell machinery, retroviruses hijack this endosomal budding machinery to promote their own budding event, which drives the release of nascent virions from the infected cell (Figure 1, panel a). The Gag proteins of most if not all retroviruses encode so-called "late" domains that interact directly with different components of the cellular endosomal sorting machinery. In the case of HIV-1, the PT/SAP motif of p6 recruits the Tsg101 component of ESCRT-I (*4*–*6*). HIV-1 p6 also bears a secondary late domain that interacts with Alix (*7*). A major physiological function of the PT/SAP binding site of Tsg101 is to interact with Hrs, a factor upstream of ESCRT-I in the sorting pathway (*8*).

To date, the Gag-Tsg101 interaction has not been developed as an antiviral target, though some success has been achieved in synthesizing PT/SAP-based peptide mimetics that display a high binding affinity for Tsg101 (*9*). With the goal of developing potent competitive inhibitors of Gag-Tsg101 binding, Tavassoli *et al.* (*1*) utilized an approach pioneered by the Benkovic laboratory for genetically selecting cyclic peptides that disrupt protein−protein interactions.

First, a large library of cyclic peptides was generated using the SICLOPPS (split intein-mediated circular ligation of peptides and proteins) method. Inteins are peptide sequences located within a larger precursor protein that are excised during a post-translational processing event known as

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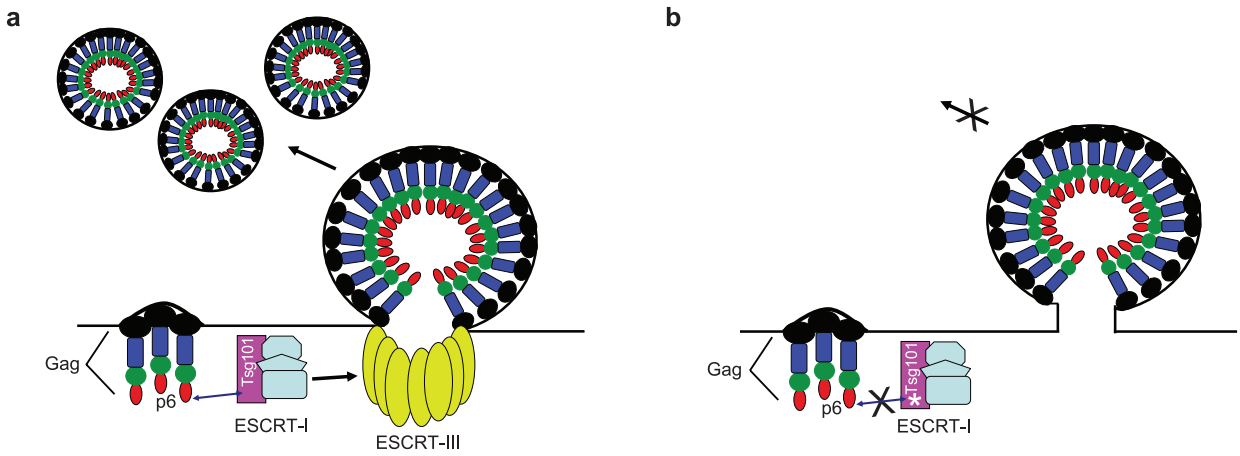

Figure 1. HIV-1 budding pathway and inhibition by peptides. a) The recruitment of cellular ESCRT machinery (ESCRT-I and -III) by HIV-1 Gag to the site of particle budding and release. The p6 domain of the Gag precursor is shown in red and the ESCRT-I component Tsg101 in pink. The double-headed dark-blue arrow represents the direct interaction between the PT/SAP motif of p6 and Tsg101. A ring of ESCRT-III complexes is shown at the budding site (yellow). b) The block in ESCRT-I recruitment imposed by the peptides (represented as ∗). The peptide is shown binding to Tsg101, though this remains to be formally established. "X" represents the block in p6—Tsg101 binding and the block in particle release.

protein splicing (*10*). During the splicing event, the inteins are removed and the flanking peptide sequences are ligated, producing a cyclic peptide. If the peptide sequence between the N- and C-terminal intein is encoded by random oligonucleotides, a large library of cyclic peptides is generated after protein splicing in bacteria. Tavassoli *et al.* (*1*) used the split intein derived from the DnaE protein of the cyanobacterium *Synechocystis* sp. strain PCC6803. The peptide library was then screened in a reverse two-hybrid system (RTHS) (*11*).

Unlike conventional yeast two-hybrid screens that select for the presence of a protein—protein interaction, the RTHS approach selects for disrupted interactions. One binding partner (in this case, the PT/SAP-binding fragment of Tsg101) is fused to the bacteriophage 434 DNA binding protein, while the other partner (here, the p6 domain of Gag, which bears the PTAP motif) is fused to the phage P22 DNA binding protein. The p6—Tsg101 interaction forms a repressor that prevents expression of downstream reporter genes. Disruption of the p6—Tsg101 interaction derepresses the promoter, leading to reporter gene expression. The type of SICLOPPS library used to encode cyclic peptides was SGWXXXXX, where X is any amino acid. The invariable residues serine, glycine, and tryptophan (SGW) are included to promote intein processing, to avoid racemization, and for HPLC purification, respectively. After several rounds of secondary screening, four cyclic peptides were identified that inhibited the p6—Tsg101 interaction.

Somewhat surprisingly, none of the peptides derived from SGWXXXXX contained a sequence resembling P(T/S)AP, suggesting that the tightest binding peptides are not simply mimicking PT/SAP. Nevertheless, some homology was observed among the three best inhibitors: IYWNVSGW, TNWYGSGW, and TLLVYSGW. Having identified cyclic peptides that disrupt the p6—Tsg101 interaction, Tavassoli *et al.* (*1*) tested the ability of the IYWNVSGW, TNWYGSGW, and TLLVYSGW peptides (linked to a cell-penetrating signal) to inhibit virus budding in Gag-expressing 293T cells. The authors observed that the peptide IYWNVSGW inhibited the release of virus-like particles by ~2—3-fold, with an IC_{50} of 7 μM (Figure 1, panel b). The TNWYGSGW and TLLVYSGW peptides displayed no inhibitory activity. In an important control for peptide specificity, the authors tested the ability of the IYWNVSGW peptide to inhibit the release of an HIV-1 Gag mutant in which PTAP was changed to LIRL. Although this mutation itself confers a marked defect in particle production (*3*), no further reduction in release efficiency was observed in the presence of the peptide. This result is consistent with the hypothesis that the inhibitory activity of the peptide is PT/SAP-dependent.

As mentioned above, a primary physiological Tsg101 partner is Hrs, which contains a PSAP motif that shares a common binding site with Gag on Tsg101 (*12, 13*). Indeed, Tavassoli *et al.* (*1*) observed that the IYWNVSGW peptide induced a ~ 2-fold reduction in Hrs—Tsg101 coprecipitation in 293T cell lysates. Despite the ability of the IYWNVSGW peptide to disrupt the Hrs—Tsg101 interaction, no effect was measured on one of the normal functions of Tsg101 and Hrs, namely, degradation of

the epidermal growth factor (EGF) receptor following EGF binding.

The elegant study of Tavassoli *et al.* (*1*) represents a significant step forward in developing inhibitors of HIV-1 budding that might eventually be used in the clinic to treat HIV-1-infected patients. However, a number of important questions remain to be answered: (i) Where is the binding site for the inhibitory peptides? The ability of the peptides to disrupt both p6—Tsg101 and Hrs—Tsg101 binding suggests that the binding site lies on Tsg101; however, the lack of peptide sequence homology with PT/SAP implies that the peptides do not simply competitively inhibit the interaction with Tsg101 by occupying the PT/SAP binding pocket. (ii) Is the 2- to 3-fold inhibition in virus release observed with peptide IYWNVSGW sufficient to block HIV-1 replication? This modest effect is in contrast to the more pronounced inhibition observed with PT/SAP mutations (*3*), and even PT/SAP mutants are able to replicate in some cell systems, including primary T cells (*2, 14*). One of the challenges in developing Tsg101-based inhibitors of HIV-1 replication is the presence of the above-mentioned, secondary late domain in p6 that recruits Alix. (iii) What effect would long-term peptide treatment have on cell viability? The lack of an effect of peptide IYWNVSGW on EGF receptor degradation is encouraging, and a recent report (*15*) documents long-term inhibition of feline immunodeficiency virus replication (which like HIV-1 depends on a PT/SAP motif) by stable expression of the PT/SAP-binding domain of Tsg101. (iv) Will resistance to inhibitors of the p6—Tsg101 interaction develop, and with what kinetics? (v) Can progress with peptide- or fragment-based inhibitors inform the development of small-molecule inhibitors that may be more viable from a therapeutic standpoint? The answers to these and related questions will emerge as research in this exciting field moves forward.

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