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Downregulation of human immunodeficiency virus type 1 Gag expression by a gp41 cytoplasmic domain fusion protein

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Abstract

The cytoplasmic domain of human immunodeficiency virus type 1 (HIV-1) envelope (Env) transmembrane protein gp41 interacts with the viral matrix MA protein, which facilitates incorporation of the trimeric Env complex into the virus. It is thus feasible to design an anti-HIV strategy targeting this interaction. We herein describe that Gag expression can be downregulated by a cytoplasmic domain fusion protein of the Env transmembrane protein, β-galactosidase (β-gal)/706–856, which contains the cytoplasmic tail of gp41 fused at the C terminus of *Escherichia coli* β-gal. This mediator depleted intracellular Gag molecules in a dose-dependent manner. Sucrose gradient ultracentrifugation and confocal microscopy revealed that Gag and β-gal/706–856 had stable interactions and formed aggregated complexes in perinuclear, intracellular sites. Pulse-chase and cycloheximide chase analyses demonstrated that this mediator enhanced unmyristylated Gag degradation. The results demonstrate a novel mode of HIV-1 Gag downregulation by directing Gag to an intracellular site via the interaction of Gag with a gp41 cytoplasmic domain fusion protein.

Keywords: HIV-1; gp41; Cytoplasmic domain; Gag; Gag-cytoplasmic domain interaction; Anti-HIV-1 strategy; Gag downregulation; Degradation; Colocalization

Introduction

The assembly of human immunodeficiency virus type 1 (HIV-1), HIV-2, and simian immunodeficiency virus (SIV), all lentiviruses, is directed by the Gag protein, which contains all of the determinants necessary and sufficient for viral assembly and budding (for reviews see references Freed, 1998; Garnier et al., 1998; Sakalian and Hunter, 1998). Formation of infectious HIV and SIV particles involves incorporation of viral envelope (Env) glycoproteins as well as viral RNA into the nascent particles that bud from the cell surface. During virus assembly and morphogenesis, the Gag polyproteins assemble into a network of "ring-like" structures at the plasma membrane; following proteolytic cleavage of the Gag precursor, the mature capsid (CA) protein forms a "fullerene-like" shell of the viral core (Gelderblom et al., 1987; Nermut et al., 1994). Also, within the maturing viral particle, the matrix (MA) protein remains in the inner face of the virion envelope and is associated with the lipid bilayer by the myristic acid attached to its N-terminus. The basic multimeric unit of the MA protein is a trimer (Massiah et al., 1994; Matthews et al., 1994; Rao et al., 1995), and this model proposes that the cytoplasmic tail of the trimeric Env is localized to the cage hole present in the lattice-like MA structure formed upon MA trimerization (Hill et al., 1996).

During maturation, assembly, and budding processes, Pr55 Gag complexes are present as large, intermediate multimeric assembly complexes in the cytoplasm as well as membrane fractions of infected cells (Ono and Freed, 1999; Paillart and Gottlinger, 1999; Spearman et al., 1997). Further kinetic analyses of Gag synthesis and assembly demonstrated that newly synthesized Gag molecules rapidly form detergent-resistant, cytoplasmic Gag complexes and that a subpopulation of newly synthesized Gag can be chased into membrane-bound complexes of increasing size or density over the chase period (Tritel and Resh, 2000). Also, a major fraction of newly synthesized Gag may undergo proteasomal degradation, presumably due to misfolding of the Gag molecules (Schubert et al., 2000; Tritel and Resh, 2000).

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Extensive studies have revealed that the Env–MA interaction facilitates recruitment of the trimeric Env complex into buds where mature virions are released into the medium (for reviews see references Freed, 1998; Garoff et al., 1998). The intracellular targeting and localization properties of Gag and Env are often affected by alterations in their counterparts (Hermida-Matsumoto and Resh, 2000; Vincent et al., 1999), nevertheless, Salzwedel et al. (1998) showed that retention of the Env protein in the endoplasmic reticulum (ER) by adding an ER retention signal to the C terminus of the protein neither affects Gag intracellular localization nor alters Gag budding from the plasma membrane. Other studies have also substantiated the notion that interactions between the transmembrane gp41 protein and Gag are specific for the presence of the cytoplasmic tail of gp41 (Cosson, 1996; Vincent et al., 1999; Wyma et al., 2000).

The cytoplasmic domain of gp41, encompassing residues 706 to 856, is characterized by the presence of three amphipathic α-helical segments, located at residues 828–856, 770–795, and 789–815, respectively, at its C-terminus. Due to their cytolytic effects on cell membranes, these three motifs are referred to as the lentivirus lytic peptide (LLP)-1, LLP-2, and LLP-3, respectively. The cytoplasmic tail has been shown to interact with various cellular components, such as proteins involved in the induction of apoptosis (Micoli et al., 2000; Pan et al., 1996), the AP-1 and AP-2 clathrin–adaptor complexes (Berlioz-Torrent et al., 1999; Boge et al., 1998; Ohno et al., 1997; Wyss et al., 2001), and TIP47, a protein required for the transport of mannose-6-phosphate receptors from endosomes to the *trans*-Golgi network (Blot et al., 2003). Our previous studies supported the proposal that the oligomerization and membrane-binding abilities of the cytoplasmic domain play crucial roles in Env assembly and virus infection (Chen et al., 1996, 1999, 2001; Lee et al., 2000). It is conceivable that Gag-cytoplasmic tail interaction can be used as a strategic target for the design of an anti-HIV strategy.

Since Gag is an attractive target for designing anti-HIV strategies, it is of interest to understand whether Gag expression can somehow be downregulated in Gag expression cells. We demonstrate herein that *Escherichia* (*E.*) *coli* β-galactosidase (β-gal) and a cytoplasmic tail fusion protein, β-gal/706–856, acts as a novel modulator to abrogate steady-state Gag expression via degradation. Also, the cytoplasmic tail fusion protein specifically interacts with Gag and directs Gag to an intracellular site. To our knowledge, this mode of down-regulation of HIV-1 Gag expression has never been noted in previously reported HIV dominant-negative mutants or anti-HIV strategies. Our study also has implications in devising a genetic anti-HIV approach to interfering with Gag maturation.

Results

Downregulation of HIV-1 Gag expression by a gp41 cytoplasmic tail fusion protein

To explore whether the interaction of the cytoplasmic tail of gp41 with Gag can serve as a strategic target for the development of an anti-HIV-1 approach, we examined whether a cytoplasmic domain fusion protein expressed in cells can affect steady-state Gag expression. 293T cells were cotransfected with equal amounts of pHIVgpt and pCDNA3-β-gal/706–856. pHIVgpt encodes an HIV genome in which the *env* gene is replaced by an simian virus (SV) 40 promoter-driven *gpt* gene (Page et al., 1990). pCDNA3-β-gal/706–856 encodes the entire cytoplasmic tail of the HXB2 strain Env fused to the C terminus of the *E. coli* reporter protein β-gal (Chen et al., 2001). Cotransfection with pCDNA3 or pCDNA3-β-gal was used as negative controls. Equal volumes of cell and virion lysates were resolved by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) followed by Western blotting using mouse monoclonal antibody (Mab) 183, which is specific the p24 CA protein. During or after virus budding, the Pr55 Gag precursor is cleaved by the virus-encoded protease into MA p17, CA p24, p2 (sp 1), nucleocapsid p7, p1 (sp 2), and the C-terminal p6 domain. With β-gal/706–856 coexpression, the intracellular levels of Pr55, an uncleaved MA and CA intermediate (p41), and p24, which are all recognized by Mab 183, were remarkably reduced compared both to the control, which did not express β-gal, and to β-gal coexpression (Fig. 1A, middle panel, compare lane 4 to lanes 2 and 3). The virion-associated levels of Pr55 and its cleavage products were also strikingly reduced in β-gal/706–856 coexpression (Fig. 1A, middle panel, lane 9). The effect of this mediator on reduced steady-state Gag expression was specific since β-gal/706–752, in which the sequence spanning residues 706 to 752 of Env was fused at the C terminus of β-gal, did not affect Gag expression or budding (Fig. 1A, middle panel, lanes 5 and 10). When samples were analyzed for expression of β-gal or the β-gal fusion proteins, a similar level of β-gal/706–856 was detected in cells compared to β-gal and β-gal/706–752 expression (Fig. 1A, top panel). β-gal/706–856 appeared to be prone to degradation since a smaller-molecular-weight species which co-migrated with β-gal could be detected with β-gal/706–856 coexpression (Fig. 1A, top panel, lane 4).

Effects of Gag structures on β-gal/706–856-modulated Gag expression

To examine whether Gag processing affects β-gal/706–856-modulated Gag expressions, 293T cells were cotransfected with a pHIVgpt-derived pHIVgpt*pro*$^-$ construct and each of the pCDNA3-β-gal plasmids at a 1:1 molar ratio. Since pHIVgpt-*pro*$^-$ encodes a dysfunctional protease in which the catalytic site at residue Asp-25 of the *pro* gene in pHIVgpt is mutated to Asn (Wang et al., 2000), the Pr55 precursor synthesized was not cleaved to produce p41 or p24. Similar levels of β-gal and β-gal/706–856 were expressed in cells (Fig. 1B). Again, β-gal/706–856 significantly reduced the intracellular and virion-associated Pr55 level compared to β-gal coexpression (Fig. 1B).

To study whether Gag targeting to the plasma membrane is crucial for the downregulating effect of β-gal/706–856 on Gag expressions, pHIVgpt*myr*$^-$, which encodes a Gly-to-Ala substitution at residue 2 of MA in the *env*-defective pHIVgpt backbone (Wang and Barklis, 1993), was examined.