Available online at www.sciencedirect.com
SCIENCE DIRECT
Virology 352 (2006) 27–38
VIROLOGY
www.elsevier.com/locate/yviro

Inhibition of viral assembly in murine cells by HIV-1 matrix

Wolfgang Hübner, Benjamin K. Chen *

Department of Pharmacology and Biological Chemistry, Center for Immunobiology, Mount Sinai School of Medicine, One Gustave Levy Place Box 1215, New York, NY 10029, USA

Received 27 October 2005; returned to author for revision 28 November 2005; accepted 6 April 2006
Available online 5 June 2006

Abstract

In human cells, the N-terminal matrix (MA) domain of the human immunodeficiency virus type 1 (HIV-1) Gag targets assembly to specific membrane compartments. In murine fibroblasts, membrane targeting of Gag and assembly of HIV-1 are inefficient. These deficiencies are relieved by replacement of HIV-1 MA with murine leukemia virus (MLV) MA in chimeric proviruses. In this study, we examined chimeric HIV-1 carrying tandem MLV and HIV-1 MA domains and found that the addition of MLV MA to the N-terminus of HIV-1 Gag enhanced membrane binding in murine cells, but was not sufficient to stimulate virus production. Removal of HIV MA was required to observe more efficient Gag processing and increased virus production in murine cells. Deletion of the globular head of MA also alleviated the blocks to membrane binding and Gag processing in murine cells, yet did not lead to increased virus production. These MA-dependent, cell-type-specific phenotypes suggest that host factors interact with the globular head of MA to regulate membrane binding and additional membrane-independent step(s) required for assembly.

Keywords: Retrovirus; Human immunodeficiency virus type 1; Murine leukemia virus; Virus assembly; p55 Gag protein; p17 matrix protein

Introduction

The study of blocks to HIV-1 replication in murine cells has provided important insights into key virus–host interactions (Feng et al., 1996; Garber et al., 1998; Maddon et al., 1986). The addition of human CD4, chemokine receptor (CXCR4 or CCR5), and human cyclinT1 to murine fibroblasts allows them to be infected and to express the viral Gag protein (Garber et al., 1998). Although these cell lines support HIV-1 gene expression, they do not support efficient HIV-1 assembly (Bieniasz and Cullen, 2000; Chen et al., 2001; Garber et al., 1998; Koito et al., 2003; Mariani et al., 2001; Mariani et al., 2000). In murine cells, the HIV-1 Gag protein exhibits a severe processing defect that is characterized by an unusually high p55/p24 ratio, and virus production is impaired (Bieniasz and Cullen, 2000; Koito et al., 2003; Reed et al., 2002). Electron microscopy and deconvolution fluorescence microscopy demonstrate that the HIV-1 Gag protein does not traffic to the plasma membrane in murine cells and is instead localized diffusely within the cytoplasm (Chen et al., 2001; Mariani et al., 2000). Furthermore, membrane flotation experiments indicate that HIV-1 Gag does not associate efficiently with membranes in murine cells (Koito et al., 2003; Swanson et al., 2004).

Human mouse heterokaryons studies suggest that the poor assembly of HIV-1 in murine fibroblasts is due to the absence of a species-specific, or cell-type specific, factor(s) that promotes HIV-1 assembly (Bieniasz and Cullen, 2000; Mariani et al., 2001). The Rev cofactor, p32, can enhance assembly in murine cells by increasing RNA export and Gag expression (Zheng et al., 2003). Furthermore, the addition of a retroviral mRNA nuclear export element to a Gag-encoding mRNA can enhance the plasma membrane binding and assembly of HIV-1 Gag protein in murine cells (Swanson et al., 2004). These reports indicate that the nuclear export and cytoplasmic transport of the Gag mRNA can influence the efficiency of Gag assembly in murine cells.

* Corresponding author. Fax: +1 212 849 2525.
E-mail address: ben.chen@mssm.edu (B.K. Chen).

0042-6822/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2006.04.024

It is also clear that the membrane-targeting matrix (MA) domain of Gag plays an important role in limiting assembly in these cells (Chen et al., 2001; Reed et al., 2002). Retroviral MA domains are not essential for capsid formation but do regulate the location of capsid formation and its relationship to membrane binding. This relationship is best defined in Mason Pfizer monkey virus (MPMV), where point mutants in MA can convert the virus from a D-type morphology which assembles intracellularly, prior to membrane engagement, to a C-type or lentiviral morphology, like HIV-1, where the Gag protein assembles at the plasma membrane (Rhee and Hunter, 1990; Rhee and Hunter, 1991).

Like MPMV MA, HIV-1 MA is thought to regulate the location of assembly and the timing of membrane binding. Mutations in HIV-1 MA or total replacement of MA with a constitutive membrane anchor results in increased assembly at inappropriate intracellular compartments (Cannon et al., 1997; Facke et al., 1993; Lee and Linial, 1994; Ono and Freed, 2004; Ono et al., 2000; Reil et al., 1998; Spearman et al., 1994; Yuan et al., 1993). The globular head of HIV-1 MA controls the exposure of its N-terminal myristoyl moiety to serve as a membrane-binding switch (Zhou et al., 1994). This switch is thought to be activated during HIV-1 assembly by oligomerization of Gag (Paillart and Gottlinger, 1999; Resh, 2004; Tang et al., 2004). A current model suggests that the MA myristoyl switch regulates membrane binding in a concentration dependent manner during infection (Perez-Caballero et al., 2004). The evidence in support of this model is compelling, yet it is still unclear how the regulation of membrane binding enforces the spatial regulation of assembly.

Some mutants of HIV-1 MA that assemble at intracellular compartments exhibit increased membrane binding (Lindwasser and Resh, 2001; Reil et al., 1998), while others do not (Ono et al., 2000; Yuan et al., 1993). These differences may indicate that the regulation of membrane binding and the location of assembly are distinct activities of HIV-1 MA as is the case for MPMV MA. Thus, the ability of MA to target assembly to specific membrane compartments may be governed by interactions of MA with specific, spatially restricted cellular factors (Ono and Freed, 2004). Supporting this hypothesis, a recent study has found that the delta subunit of the adapter protein complex 3, AP-3δ, interacts with MA and plays a key role in protein sorting mechanisms that target Gag to late endosomal compartments (Dong et al., 2005).

To better understand how the interaction of MA with cellular factors regulates assembly, we have been investigating the phenotype of poor assembly in murine cells where the assembly of HIV-1 is limited in a cell type-dependent and MA-dependent manner. Although HIV-1 and MLV MA share minimal sequence homology, they are likely to play similar functional roles in assembly (Deminie and Emerman, 1994). We and others found that replacement of the HIV-1 MA with the MLV MA in an infectious HIV-1 molecular clone enhances assembly in murine cells (Chen et al., 2001; Reed et al., 2002). Possible explanations for this outcome are that the MLV MA domain is able to interact with specific murine cofactor(s) that activate membrane binding of MA or that inhibitory interactions between host factors and HIV-1 MA are alleviated by removal of HIV-1 MA.

Here, we study MA-chimeric and MA-mutant Gag proteins to test how MA sequences regulate membrane binding, subcellular localization, and assembly in a murine versus human cell line. We examined chimeric HIV-1 carrying two MA domains in tandem, or MA deletion mutants, to determine how the presence of HIV-1 MA on Gag regulates the efficiency of assembly. We show that an N-terminal MLV MA domain provided a much more effective membrane-binding signal than HIV-1 MA in murine cells. Surprisingly, the presence of the HIV-1 MA domain on dual-MA viruses inhibited virus production even when an N-terminal MLV MA enhanced binding of Gag to membranes. We also observed that the removal of the globular head of HIV-1 MA domain effectively promoted Gag localization into punctate, membrane-associated structures, and resulted in increased processing of the Gag precursor in murine cell lysates. These data suggest that HIV-1 MA can inhibit assembly through a mechanism that does not involve its ability to regulate membrane binding. Thus, inhibitory interactions of HIV-1 MA with murine cell factors may regulate assembly at multiple steps.

Results

Previous studies have found that HIV-1 chimeras carrying MLV MA in place of HIV-1 MA assemble with greater efficiency than native HIV-1 in murine cells (Chen et al., 2001; Reed et al., 2002). In this study, we have examined how this replacement enhances virus assembly in murine cells. It is possible that the addition of MLV MA simply improves the ability of the chimeric Gag protein to interact with membranes allowing assembly to proceed. It is also possible that the removal of HIV-1 MA from the Gag facilitates assembly by removing inhibitory interactions with murine factors that inhibit assembly.

We constructed two HIV-1(NL4-3)-derived proviral constructs (Adachi et al., 1986) that encode chimeric Gag proteins with tandem MA domains, with one from HIV-1 and the other from MLV. These constructs allow us to examine whether the membrane targeting and assembly signals of HIV-1 or MLV MA are functional when placed together in *cis* (Fig. 1). They also test whether a combination of two MA domains is compatible with assembly in murine cells. This experimental design was encouraged by studies that indicate that HIV-1 Gag can tolerate insertions of additional MA domains or green fluorescent protein into the C-terminus of MA, without adversely affecting assembly (Muller et al., 2004; Wang et al., 2000). The dual-MA virus carrying MLV MA followed by HIV-1 MA was named MHIV(DMA-MH) and the dual-MA virus carrying HIV-1 MA followed by MLV MA was called MHIV(DMA-HM) (Fig. 1). Each of these dual-MA constructs also carried an additional HIV-1 protease cleavage site between the tandem MA domains to allow processing into individual MA proteins within virus particles. A theoretical advantage to this design is the addition of a full MA domain into a flexible