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Mutant murine leukemia virus Gag proteins lacking proline at the N-terminus of the capsid domain block infectivity in virions containing wild-type Gag

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Abstract

We have investigated the properties of murine leukemia virus Gag mutants in which the p12-CA cleavage site is altered. In one mutant, the cleavage is blocked; in the other, the conserved proline at the N-terminus of CA has been replaced with glycine. No infectivity was detected in either mutant. Mutant particles cannot synthesize full-length DNA upon infecting permissive cells. Particles composed of a mixture of wild-type and mutant proteins have severely impaired infectivity. These mixed particles are defective in their ability to synthesize DNA upon infection, but this defect is less severe than the loss of infectivity. Thus, proteins lacking the correct N-terminus of CA inhibit DNA synthesis and also interfere with formation or integration of a full-length, normal provirus. The results imply that CA proteins function as part of a large, highly organized structure in reverse transcription and apparently at a later step as well.
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Introduction

The principal structural component of a retrovirus particle is the virus-coded Gag protein. After the particle is released from the virus-producing cell, Gag is cleaved by the viral protease (PR) into a series of cleavage products, always including (from N- to C-terminus) matrix (MA), capsid (CA), and nucleocapsid (NC). This series of cleavage events is termed "maturation" of the particle and is essential for infectivity (Swanstrom and Wills, 1997).

The structure of the CA protein of HIV-1 has been investigated in detail, using both nuclear magnetic resonance and X-ray crystallography. Retroviral CA proteins are composed of two domains connected by a flexible linker. Their N-terminal residue is always proline. Following the cleavage event that generates this N-terminus, the first ~50 amino acids of CA fold into a β-hairpin, and the proline forms a buried salt bridge with an internal aspartate residue (Gamble et al., 1996; Gitti et al., 1996; Tang et al., 2002).

Maturation entails a drastic change in the morphology of the virion. One of the hallmarks of this change is the appearance of the "mature core" of the particle, a densely staining body in the interior of the virion. Recent studies on structures formed in vitro by purified CA proteins of HIV-1 and murine leukemia virus (MLV) show that the mature core is composed of CA molecules. Within this core, CA proteins are evidently arranged in a lattice in which the N-terminal domains of groups of six CA molecules are in hexameric rings; each ring is joined to neighboring rings by dimeric contacts between C-terminal domains (Ganser et al., 2003; Li et al., 2000; Mortuza et al., 2004). The planar hexagonal lattice is closed by the presence of twelve pentameric "defects" (Ganser et al., 1999).

Several types of evidence suggest that the correct mature core structure is essential for infectivity, and that this structure depends in turn upon the presence of the proline at the N-

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terminus of CA. Thus, mutants in which the proline is replaced by another amino acid (Fitzon et al., 2000) (see below) or in which the release of CA from Gag is inhibited by a change in the cleavage site (Gottlinger et al., 1989; Oshima et al., 2004) are not infectious. Similar results are also observed when the aspartate partner in the salt bridge in HIV-1 CA is replaced by alanine (Tang et al., 2003).

In the present work, we have analyzed the properties of MLV mutants at the N-terminus of CA. We have also studied MLV particles containing a mixture of mutant and wild-type proteins. The mutants investigated here included one in which maturation cleavage at the N-terminus of CA is blocked by a change in the residue preceding proline in Gag and one in which the N-terminal proline in CA was replaced by glycine. We found that both of these mutants potently inhibit the functions of the wild-type, reducing the specific infectivity of the mixed particles. The data indicate that these mutant proteins interfere with the ability of the particle to synthesize viral DNA upon entering the new host cell. The results support the hypothesis that an organized assemblage of CA molecules in the mature particle plays a crucial role in facilitating reverse transcription; this functional complex is evidently disrupted by the presence of mutant proteins in the particle, even when wild-type proteins are in excess. However, the inhibition of DNA synthesis did not appear to be sufficient to explain the loss of infectivity in the mixed particles. Thus, the data further suggest that the correct structure of the mature core is also required for the correct formation of the final DNA product or for a subsequent step, such as integration of the DNA into the chromosome of the host cell.

Results

Properties of "S2G"

The MLV Gag protein, Pr65, is cleaved into MA, p12, CA, and NC during virus maturation, as indicated in Fig. 1A. We have previously described mutants at cleavage site 2 ("S2"), the p12-CA cleavage site (Oshima et al., 2004). We found that replacement of the tyrosine at the C-terminus of p12 (the P1 position of S2) with a charged residue, such as aspartate, completely blocked cleavage at this site. This mutant was designated S2D. The location of this mutant in the Gag protein is illustrated schematically in Fig. 1A. We also observed that cleavage at the other two sites of maturation cleavage, particularly the CA-NC cleavage site, was somewhat inefficient in S2D particles (Oshima et al., 2004). In the present work, we have also investigated a mutant in which the proline at the N-terminus of CA (i.e., the P1′ position of site 2) was replaced by glycine; we refer to this mutant as "S2G" for convenience.

Fig. 1B shows an analysis of S2G particles, using immunoblotting with anti-CA antiserum. Also included in the figure, for comparison, are particles of S2D (in which there is no free CA, but only the 42-kDa p12-CA fusion protein (Oshima et al., 2004)), as well as wild-type and PR$^-$ particles. It can be seen that the S2G profile contains a substantial amount of CA protein. There is also a significant level of a protein migrating just slightly faster than the 42-kDa protein of S2D; this is p40, the CA-NC fusion protein, since it reacts with anti-NC as well as anti-CA antisera (data not shown). p40 was previously observed in S3R particles, in which cleavage between CA and NC was blocked (Oshima et al., 2004) (see Fig. 1A). Thus, despite the fact that this mutant is altered at the N-terminus of CA, it exhibits efficient cleavage at the N-terminus, but only partial cleavage at the C-terminus, of CA. Both S2G and S2D particles also contain two larger intermediates, presumably MA-p12-CA and p12-CA-NC.

The morphology of S2G particles was also examined. Released virions were collected for thin-section electron microscopy by immunoprecipitation with anti-SU antibody as described (Oshima et al., 2004). We found (Fig. 2) that they are similar to wild-type MLV particles, but that their structure is considerably more irregular. For example, many particles contain condensed material in their interiors but lack a clearly defined core. Other particles resemble immature particles, but

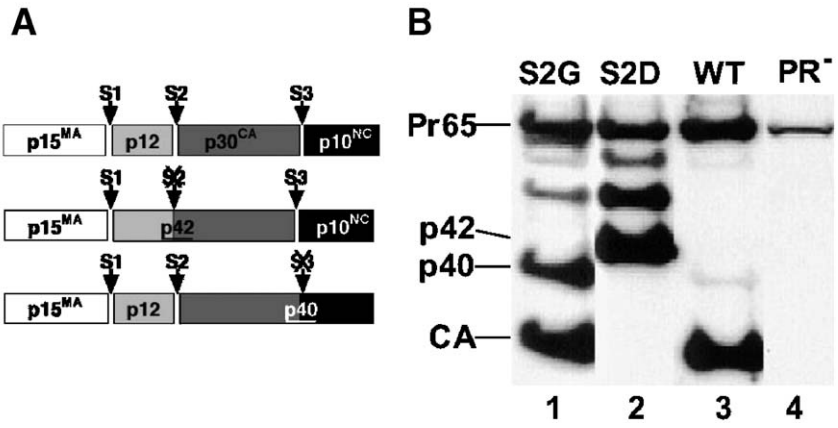

Fig. 1. Cleavage of MLV Gag and CA-containing protein species in S2G virions. (A) (top row) Scheme showing normal cleavage of MLV Gag into MA, p12, CA, and NC; (middle row) production of p42 in mutants such as S2D which block cleavage at site 2 (between p12 and CA); (bottom row) production of p40 when cleavage at site 3 is blocked. (B) Particles of S2G (lane 1), S2D (lane 2), wild-type MLV (lane 3), and PR$^-$ MLV (lane 4) were collected from culture fluid and analyzed by immunoblotting with anti-CA antiserum. Mobilities of Pr65^{Gag}, p30^{CA}, and the p42 and p40 cleavage intermediates are indicated.