Virology 384 (2009) 59–68

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

The impact of altered polyprotein ratios on the assembly and infectivity of Mason-Pfizer monkey virus

Zdena Kohoutová [a], Michaela Rumlová [b], Martin Andreánsky [c], Michael Sakalian [d], Eric Hunter [d], Iva Pichová [b], Tomáš Ruml [a,b,*]

[a] Department of Biochemistry and Microbiology and the Center for Integrated Genomics, Institute of Chemical Technology, Technická 3, 166 28 Prague, Czech Republic
[b] Department of Biochemistry, Institute of Organic Chemistry and Biochemistry, Academy of Sciences, Flemingovo nám. 2, 166 10 Prague, Czech Republic
[c] Department of Pediatrics, University of Arizona Health Sciences Center, 1501 N Campbell Ave., Tuscon, AZ 85724, USA
[d] Panacos Pharmaceuticals, 209 Perry Parkway, Gaithersburg, MD 20877, USA
[e] Emory Vaccine Center, Emory University, 954 Gatewood Road, Atlanta, GA 30329, USA

ARTICLE INFO

Article history:
Received 23 July 2008
Returned to author for revision
11 August 2008
Accepted 31 October 2008
Available online 4 December 2008

Keywords:
Ribosomal frameshift
Retrovirus
Assembly
Mason-Pfizer monkey virus
Capsid

ABSTRACT

Most retroviruses employ a frameshift mechanism during polyprotein synthesis to balance appropriate ratios of structural proteins and enzymes. To investigate the requirements for individual precursors in retrovirus assembly, we modified the polyprotein repertoire of Mason-Pfizer monkey virus (M-PMV) by mutating the frameshift sites to imitate the polyprotein organization of Rous sarcoma virus (Gag-Pro and Gag-Pro-Pol) or Human immunodeficiency virus (Gag and Gag-Pro-Pol). For the "Rous-like" virus, assembly was impaired with no incorporation of Gag-Pro-Pol into particles and for the "HIV-like" virus an altered morphogenesis was observed. A mutant expressing Gag and Gag-Pro polyproteins and lacking Gag-Pro-Pol assembled intracellular particles at a level similar to the wild-type. Gag-Pro-Pol polyprotein alone neither formed immature particles nor processed the precursor. All the mutants were non-infectious except the "HIV-like", which retained fractional infectivity.

Introduction

The formation of a retrovirus particle is initiated by the expression of polyprotein precursors Gag, Pro and Pol, comprising all structural proteins and enzymes. The expression strategy controls the proper ratio of viral components in the immature particles originating either at the plasma membrane (type-C) or within the cytoplasm (type D). During or shortly after budding, retroviral Gag polyprotein precursor is specifically processed by the viral protease and the immature viral particle is rearranged to form mature, fully infectious virus.

The proteolytic processing of Mason-Pfizer monkey virus (M-PMV) Gag (Pr78) yields matrix protein (MA, p10), phosphoprotein (pp24/pp16–18), p12, capsid protein (CA, p27), nucleocapsid protein (NC, p14), and p4 (Bradac and Hunter, 1984). In addition to the Gag derived structural proteins the processing of the Gag-Pro polyprotein (Pr95) results in the release of an NC-dUTPase fusion protein and protease (PR). The Gag-Pro-Pol polyprotein (Pr180) yields reverse transcriptase (RT) and integrase (IN).

The differential synthesis of two (Gag, Gag-Pol) or three (Gag, Gag-Pro, Gag-Pro-Pol) polyprotein precursors per virus is regulated by ribosomal frameshifting from one reading frame to the (−1) one (Jacks and Varmus, 1985). The exception is the gammaretrovirus genus that utilizes suppression of a termination codon during translation of *pro* and *pol* (Yoshinaka et al., 1985). Frameshifting requires two *cis*-acting signals in the retroviral mRNA, a slippery sequence for the shift of the ribosome and a structural element several nucleotides downstream of the slippery sequence, which enhances the efficiency of the slip (Jacks et al., 1988). A fraction of the ribosomes translating *gag* of the M-PMV from unspliced genomic transcript undergoes the (−1) frameshift (with frequency ~10–15%) upstream of the end of the *gag* gene allowing translation through the *pro* stop codon. A second (−1) frameshift (2–3%) yields the *pol* region in fusion with both the *gag* and *pro* products i.e. Gag-Pro-Pol precursor. In contrast to M-PMV, most retroviruses employ only one frameshift event to synthesize only Gag and Gag-Pol precursors.

In addition to regulating the ratios of polyproteins, frameshifting provides a presence of common transport signal to all Gag related polyproteins for their targeting to the site of assembly (Farabaugh, 1996; Farabaugh, 2000). In contrast to betaretroviruses (B- and D-type retroviruses) that form immature particles within the cytoplasm, the C-type retroviruses concentrate their Gag and Gag-Pol polyproteins at the plasma membrane where assembly and budding occur simultaneously. M-PMV a prototype of D-type morphogenesis, transports its

* Corresponding author. Department of Biochemistry and Microbiology and the Center for Integrated Genomics, Institute of Chemical Technology, Technická 3, 166 28 Prague, Czech Republic. Fax: +420 220445140.
E-mail address: rumlt@vscht.cz (T. Ruml).

0042-6822/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2008.10.048

Gag-related polyproteins to the pericentriolar region, where they assemble into immature capsids that are transported via the vesicular transport system to the plasma membrane while acquiring *env*-gene product (Choi et al., 1999; Sfakianos et al., 2003; Sfakianos and Hunter, 2003; Vlach et al., 2008).

Retroviral protease activity levels vary inversely to their concentration in the virion, controlled by its gene position in the genome. For instance, in HIV the protease coding sequence is located at the 5′ end of the *pol* gene and it is thus expressed only in the *pol* reading frame. In avian retroviruses (ASLV, RSV), it is encoded at the 3′-end of the *gag* ORF, and it is thus present both in Gag and Gag-Pol molecules. The lower specific activity of the RSV protease compared to the HIV may be related to its higher concentration in RSV (Grinde et al., 1992). The active site of the RSV protease contains a serine in contrast to other retroviruses that contain threonine. Substitution of the threonine for the serine in the active site results in a 2-fold increase of catalytic activity of RSV protease (Ridky et al., 1996). In betaretroviruses (M-PMV and mouse mammary tumor virus) and deltaretroviruses (human T-cell lymphotropic virus), the protease is encoded in a separate reading frame between the *gag* and *pol* genes and intermediate protease concentrations are therefore incorporated into virions.

Alterations of a proper ratio of the polyprotein precursors dramatically impair the assembly, budding and processing (Hung et al., 1998;Karacostas et al., 1993;Park and Morrow, 1991;Shehu-Xhilaga et al., 2001). To further investigate the requirement for specific polyprotein precursors in assembly, maturation and virion infectivity, we mutated the frameshift sites in the M-PMV genome to produce only Gag and Gag-Pro-Pol or Gag-Pro and Gag-Pro-Pol polyproteins and to generate mutants mimicking HIV or RSV, respectively. Their properties were compared to the wild-type M-PMV and its D26N mutant with an inactive protease.

Results

Synthesis, processing, and release of M-PMV wild-type and mutant polyproteins

All wild-type M-PMV structural proteins and enzymes are synthesized as polyprotein precursors Gag (G), Gag-Pro (GP) and Gag-Pro-Pol (GPP). To study the role of polyprotein precursors ratios in assembly, maturation and virion infectivity, a series of vectors with mutated frameshift sites in the M-PMV genome was prepared (see Fig. 1). The insertion of a single A at the *gag-pro* frameshift site prevented the translation of sole Gag polyprotein. Resulting formation of Gag-Pro and Gag-Pro-Pol polyproteins (GP-GPP) mimics the organization of polyprotein precursors in Rous sarcoma virus (RSV) with protease at the 3′ end of Gag (Fig. 1). The insertion of T in *pro-pol* frameshift site yielded only the Gag and Gag-Pro-Pol in G-GPP mutant (Fig. 1). This imitates the strategy in HIV where protease is encoded in the 5′ terminus of *pol*. In addition two control constructs, GPP, expressing only the Gag-Pro-Pol precursor as a consequence of mutations in both frameshift sites, and G-GP, with termination codon at the end of *gag-pro* region were prepared (Fig. 1).

In order to evaluate the expression and stability of the polyprotein precursors, transfected COS-1 cells were metabolically labeled and both the cell- and virus-associated proteins were immunoprecipitated after a pulse and 4 h chase with rabbit anti-M-PMV capsid protein serum (Fig. 2). All the anticipated precursors were found in the cells transfected with the mutant and the wild-type genomes (Fig. 2A). Despite the alteration of the frameshift sequences, a minor band corresponding approximately to the molecular weight of Gag-Pro (Pr95) was observed in the lysates of the cells transfected with mutants G-GPP or GPP (Fig. 2A). This suggests that an infrequent (−1) frameshift continued to occur resulting in a small amount of translation into the (−2) reading frame where there is a stop codon four amino acids downstream of the slippery sequence. The resulting polyprotein would therefore have a molecular mass of approximately 95 kDa.

Likewise, all the expected polyproteins for the wild-type and most of the mutants were found in the cells after the chase, indicating that there was no significant increase in activation of the viral protease or decrease in protein stability for most of the mutants (Fig. 2B). However, the Gag-related polyproteins of the GP-GPP and GPP mutants were significantly reduced in the cells during the chase period (Fig. 2B). This could be either due to nonspecific degradation within the cells or due to a higher level of protease produced by these mutants compared to the wild-type. To exclude this, a D26N protease

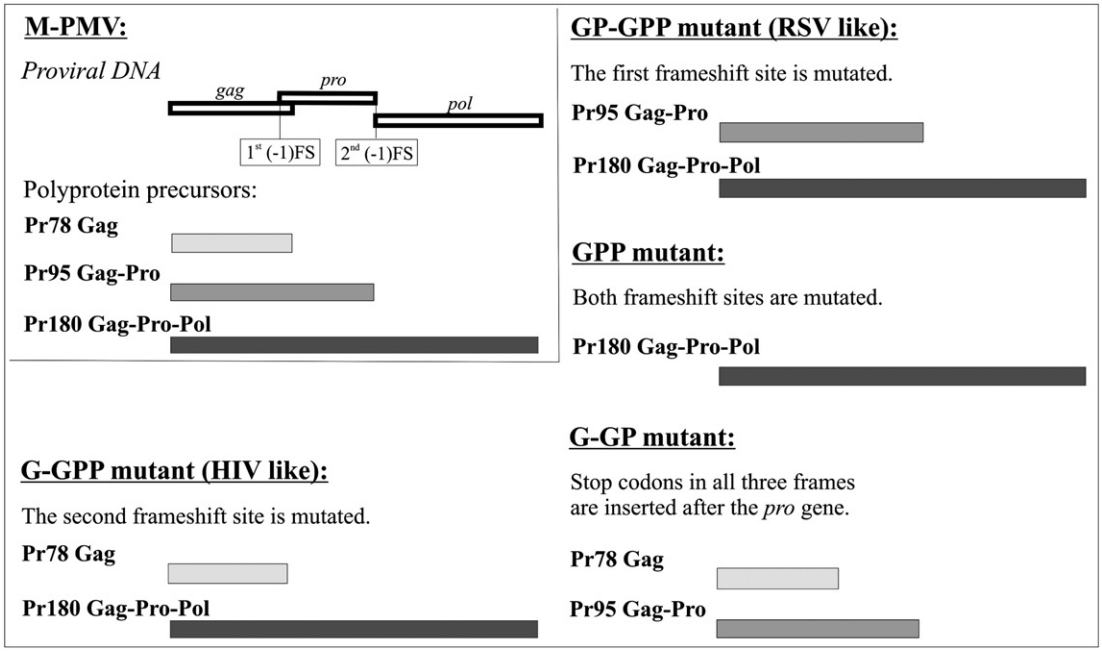

Fig. 1. Organization of the M-PMV *gag*, *pro*, and *pol* genes (upper left corner) and M-PMV frameshift mutants. The open boxes represent open reading frames in the proviral DNA; the filled boxes indicate the corresponding polyprotein precursors. (−1) FS indicates the frameshift site.