Available online at www.sciencedirect.com
ScienceDirect
Virology 365 (2007) 390–397
VIROLOGY
www.elsevier.com/locate/yviro

The heptad repeats region is essential for AcMNPV P10 filament formation and not the proline-rich or the C-terminus basic regions

Chunsheng Dong, Fei Deng, Dan Li, Hualin Wang, Zhihong Hu *

State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, PR China

Received 28 January 2007; returned to author for revision 14 February 2007; accepted 19 March 2007
Available online 3 May 2007

Abstract

Baculovirus P10 protein is a small conserved protein and is expressed as bundles of filaments in the host cell during the late phase of virus infection. So far the published results on the domain responsible for filament structural formation have been contradictory. Electron microscopy revealed that the C-terminus basic region was involved in filament structural formation in the *Autographa californica* multiple nucleocapsid nucleopolyhedrovirus (AcMNPV) [van Oers, M.M., Flipsen, J.T., Reusken, C.B., Sliwinsky, E.L., Vlak, J.M., 1993. Functional domains of the p10 protein of *Autographa californica* nuclear polyhedorsis virus. *J. Gen. Virol. 74*, 563–574.]. While in the *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV), the heptad repeats region but not the C-terminus domain was proven to be responsible for filament formation [Dong, C., Li, D., Long, G., Deng, F., Wang, H., Hu, Z., 2005. Identification of functional domains required for HearNPV P10 filament formation. Virology *338*, 112–120.]. In this manuscript, fluorescence confocal microscopy was applied to study AcMNPV P10 filament formation. A set of plasmids containing different P10 structural domains fused with a fluorescent protein were constructed and transfected into Sf-9 cells. The data indicated that the heptad repeats region, but not the proline-rich region or the C-terminus basic region, is essential for AcMNPV P10 filament formation. Co-transfection of P10s tagged with different fluorescent revealed that P10s with defective heptad repeats region could not interact with intact heptad repeats region or even full-length P10s to form filament structure. Within the heptad repeats region, deletion of the three amino acids spacing of AcMNPV P10 appeared to have no significant impact on the formation of filament structures, but the content of the heptad repeats region appeared to play a role in the morphology of the filaments.

Keywords: AcMNPV; HearNPV; P10; Heptad repeat; Filament formation; Green fluorescent protein

Introduction

A characteristic of the late infection stages of baculoviruses is the abundant production of a filamentous structure known as P10. It was identified by immunogold electron microscopy in *Autographa californica* nucleopolyhedrovirus (AcMNPV) infected cells as a viral encoded 10 kDa (Van der Wilk et al., 1987; Quant-Russell et al., 1987). The gene encoding P10 has been found in all the sequenced lepidopteran baculoviruses, but seems to be absent in the dipeteran and hemenopetran baculoviruses. For example, it was absent in NeleNPV (Lauzon et al., 2004), NeseNPV (Garcia-Maruniak et al., 2004) and CuniNPV (Afonso et al., 2001). Previously studies indicated that P10 was a multifunctional protein associated with cell lysis, nuclear membrane disintegration, stabilizing polyhedra, liberation of polyhedra and interaction with microtubules (Williams et al., 1989; van Oers et al., 1993; Gross et al., 1994; Patmanidi et al., 2003). But the exact function of P10 is still unclear and the deletion of p10 gene from AcMNPV did not affect the viral replication *in vitro* (Vlak et al., 1988).

Despite common low sequence identities, baculovirus P10s share a conserved secondary structure such as a heptad repeats region (also called coiled-coil domain) in the N-terminus half followed by a proline-rich domain and a positively charged basic C-terminus (van Oers and Vlak, 1997). These different domains were proposed to be responsible for different functions. The heptad repeats region was responsible for P10 aggregation, proline-rich domain was associated with cell lysis and the C-terminus region was responsible for filament structure formation (van Oers and Vlak, 1997).

* Corresponding author. Fax: +86 27 87197180.
E-mail address: huzh@wh.iov.cn (Z. Hu).

0042-6822/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.virol.2007.03.051

We have recently reported that confocal fluorescent microscopy, electron microscopy (EM), and immunogold electron microscopy (IEM) demonstrated that GFP-tag was a useful tool to study the filamentous structure of P10s (Dong et al., 2005). It was shown that in the *Helicoverpa armigera* NPV (HearNPV), the heptad repeats region, but not the proline-rich region or the C-terminus basic region was responsible for the formation of the filaments of P10 (Dong et al., 2005). However, this result contrasts previous findings with AcMNPV where the C-terminus region was appeared to be necessary for P10 filament structures (van Oers et al., 1993). In this paper, we used the same GFP-tagged p10 technique to study filament structure formation in AcMNPV. Our results revealed that, as in the case of HearNPV, the P10 filament structures of AcMNPV are also determined by the heptad repeats region and not by the C terminus region. We further studied the impact of the heptad repeats on the P10 filament structures by making mutants or chimeric heptad repeats and by using different fluorescent tags.

Results

Construction of P10-fluorescent protein fusion vectors and the expression of P10-GFPs in transfected cells

To investigate the role of different domains in AcMNPV P10 filament formation *in vitro*, we construct a set of plamids expressing mutant and chimeric P10s tagged with fluorescent proteins (Fig 1).

All the plasmids were transfected into Sf-9 insect cells and the fluorescence was observed by confocal microscopy. Western blots were performed to investigate the expression of different P10-GFPs in transfected cells. Anti-GFP antibody detected specific bands of approximately 39 kDa for pAcP10-GFP, pAcP10$_{\Delta 87-94}$-GFP, pAcP10$_{\Delta 3AA}$-GFP, pAcP10$_{mHR1}$-GFP, and pAcP10$_{\Delta HR1}$-GFP. Bands of 34 kDa were observed with pAcP10$_{\Delta 65-94}$-GFP, 32 kDa for pAcP10$_{\Delta 52-94}$-GFP, and 27 kDa for pGFP (Fig. 2A). The two chimeric P10s, pAcP10$_{HR(Ha-Ac)}$-GFP and pHaP10$_{HR(Ac-Ha)}$-GFP, were observed to yield peptides of 39 kDa and 36 kDa, respectively (Fig. 2B). The sizes of the observed bands were in close agreement with the predicted molecular weights suggesting that P10-GFP fusion proteins were expressed in the transfected cells.

The filament structure of AcMNPV P10 is determined by heptad repeats region, but not by the C-terminus basic region or the proline-rich region

When pAcP10-GFP was transfected into Sf-9 cells, a continuous net structure with spiral-like appearance was observed by confocal fluorescent microscopy (Fig. 3A). In cells transfected with pAcP10$_{\Delta 87-94}$-GFP (the C-terminus basic region was deleted) or with pAcP10$_{\Delta 65-94}$-GFP (both the C terminus

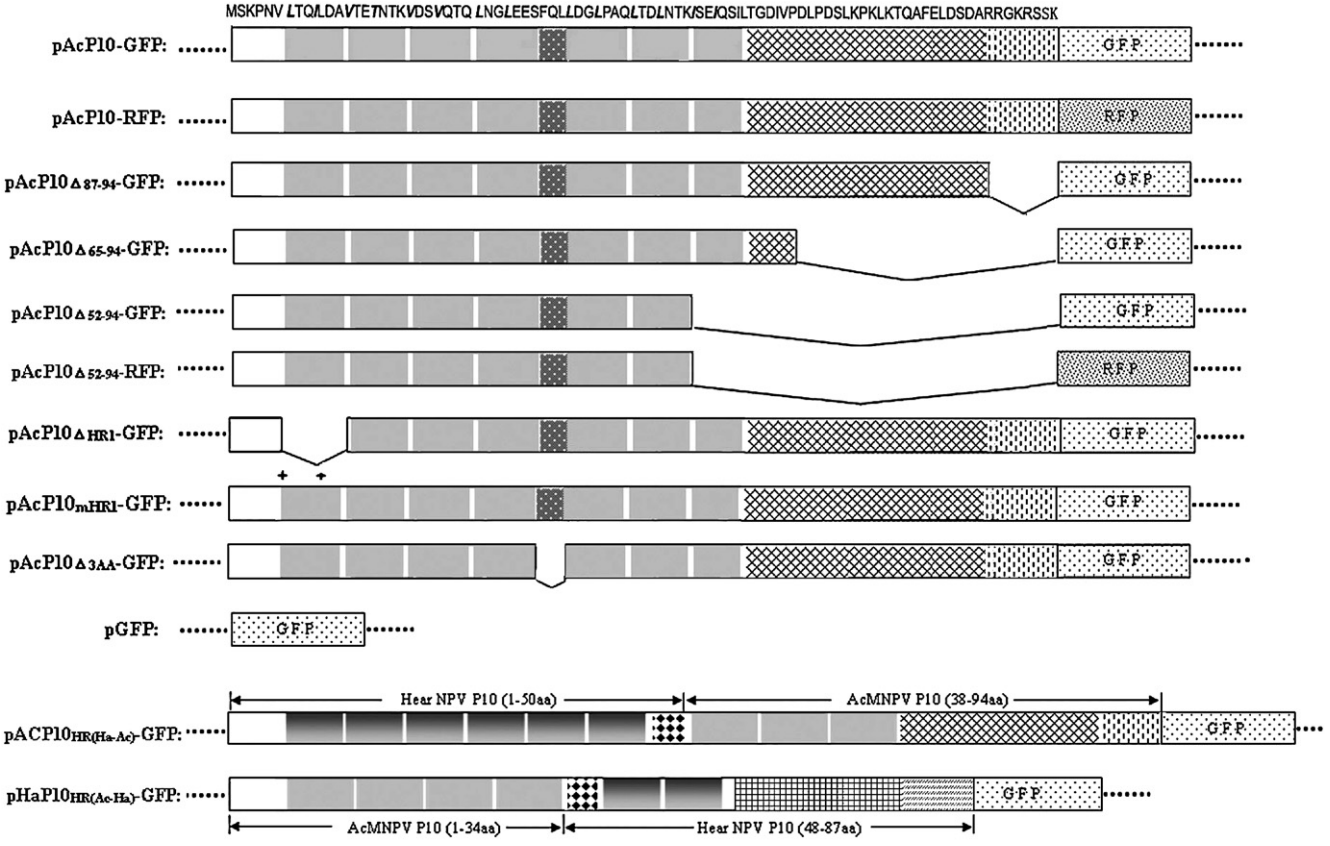

Fig. 1. Schematic representation of various P10 and GFP/RFP fusions in pIZ/V5 transient expression vector. The amino acid sequence of AcMNPV P10 was shown on the top. Different domains of P10 and GFP or RFP were represented by different blocks. The two mutation sites in pAcP10$_{mHR1}$-GFP were marked with *. In pAcP10$_{HR(Ha-Ac)}$-GFP and pHaP10$_{HR(Ac-Ha)}$-GFP, numbers indicated correspond to amino residue position. All the fusion proteins were under the OpIE2 promoter.