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Identification of functional domains required for HearNPV P10 filament formation

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Abstract

Baculovirus encoded P10 form fibrillar structures in infected cells. We have tried to identify the functional domains for the P10 filament formation by green fluorescence protein (GFP) tag. The *p10* gene of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HearNPV) was the subject of these studies. Different lengths of HearNPV *p10* gene were constructed with GFP fused in frame to the C-terminus. The constructs were transfected into insect and mammalian cells and observed by confocal microscopy. The results indicated that the first N-terminal 66 amino acids, which form the complete coiled-coil domain, were necessary for the aggregation and formation of filament structures of HearNPV P10. The proline-rich region and the C-terminal positively charged amino acids were not necessary for the formation of fibrillar structure but had some impact on the shape of the fibrillar structures. No other baculoviral proteins were needed for the formation of P10 filament structures in transfected cells.

Keywords: HearNPV; P10; Green fluorescent protein; Functional domain; Filament structure

Introduction

The Baculoviridae is a large family of occluded, rod-shaped viruses with circular, double-stranded DNA genomes. It contains two genera: nucleopolyhedrovirus (NPV) and granulovirus (GVs) (Blissard et al., 2000). A characteristic of late infection of baculovirus is the production of large bundles of filaments in infected cells. Immunogold electron microscopy had revealed that these filaments were composed predominantly of viral encoded P10 proteins (Quant-Russell et al., 1987; van der Wilk et al., 1987). To date, the genomes of 23 NPVs have been completed sequenced and P10 has been found in all of these NPVs, except in NeleNPV (Lauzon et al., 2004), NeseNPV (Garcia-Maruniak et al., 2004), and CuniNPV (Afonso et al., 2001). Although it has been reported that P10 was involved in cell lysis, nuclear membrane disintegration, stabilizing polyhedra, and polyhedra liberation (Gross et al., 1994; Williams et al., 1989; van Oers et al., 1993), the exact function of P10 is still unclear.

Baculoviral P10 proteins share conserved secondary structures, even through their sequences exhibit low identity. Previous investigators have identified three structural and functional domains in P10 proteins (van Oers and Vlak, 1997). The amino-terminal part of P10 contains a coiled-coil domain made up of heptad repeats (HRs). This domain was implicated in the oligomerization of P10 (Alaoui-Ismaili and Richardson, 1996; Vlak et al., 1988; Wilson et al., 1995). Downstream from the coiled-coil domain is a proline-rich sequence which is probably involved in the liberation of polyhedra from infected cell nuclei (van Oers and Vlak, 1997). At the C-terminus is a positively charged sequence. It has been reported previously

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that C-terminus of P10 of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) was necessary for the alignment of P10 aggregation into fibrillar structures (van Oers et al., 1993).

The function of P10 structure was previous studied by constructing baculoviruses with deletion or mutation in the *p10* genes and observation of the effects by electron microscopy (Vlak et al., 1988; van Oers et al., 1993, 1994; Williams et al., 1989). Later, immunostaining was introduced into the studies of P10s and filament structure could be observed by using anti-P10 antibodies and immunofluorescent microscopy (Alaoui-ismaili and Richardson, 1998; Patmanidi et al., 2003). In this article we tagged the green fluorescent protein (GFP) to P10 and observed the filaments under the confocal microscope and attempted to use this technique to map the polypeptide regions important for filament formation.

The *p10* gene of *Helicoverpa armigera* single nucleopolyhedrovirus (HearNPV) (Chen et al., 2001) was the subject of these studies. HearNPV P10 contains 87 amino acids (aa) (Wang et al., 2001), the first 66 aa of which form a coiled-coil region which contains 8 complete heptad repeats. Following the coiled-coil region is a proline-rich region, which contains amino acids 67–80. The C-terminal 7 amino acids form a positively charged domain. Transient expression vectors were constructed fused with GFP to truncate C-terminus region, proline-rich region, or 1–4 HRs. The plasmids were transfected into HzAM1, a cell line permissive to HearNPV, and into Sf21 or into a mammalian cell line BHK. The latter two cell lines are not permissive to HearNPV. We were able to identify that the first N-terminal 66 amino acids were necessary for the formation of filament structures of HearNPV P10.

Results

Construction of transient expression vectors containing truncated HearNPV P10 fused to GFP and detection of P10-GFPs expression in HzAM1 cells

To identify the functional domains needed for the formation of filament structures, we constructed seven pIZ/V5 plasmids containing different lengths of HearNPV P10 fused to GFP (Fig. 1). Plasmid pN87-GFP contains the full-length (87 aa) of HearNPV P10. pN80-GFP contained the N-terminal 80 amino acids with a deletion of the 7 amino acids from the C-terminal basic region. pN66-GFP contained the N-terminal 66 aa with a further deletion of the proline region. pN60-GFP, pN49-GFP, pN43-GFP, and pN36-GFP contained the N-terminal 60, 49, 43, and 36 aa, respectively, with further deletions of 1, 2, 3, and 4 HRs, respectively (Fig. 1). GFP was fused in frame to the C-terminal of the P10 polypeptides in the above plasmids. A control plasmid of pGFP was constructed where only the gene encoding GFP protein was cloned into pIZ/V5.

To investigate the expression of the different P10-GFP fusion proteins in HzAM1 cells, anti-P10 and anti-GFP antibodies were used to detect both proteins by Western blot analysis. The GFP antibody detected specific bands with molecular weights about of 39 kDa (pN87-GFP), 38 kDa (pN80-GFP), 36.5 kDa (pN66-GFP), 34.5 kDa (pN60-GFP), 33 kDa (pN49-GFP), 32 kDa (pN43-GFP), and 31 kDa (pN36-GFP) (Fig. 2A). The predicted sizes of the fusion proteins of N87-GFP, N80-GFP, N66-GFP, N60-GFP, N49-GFP, N43-GFP, and N36-GFP are 38.3 kDa, 37.5 kDa, 36.0 kDa, 35.3 kDa, 34.2 kDa, 33.6 kDa, and 33.0 kDa, respectively. Therefore, the sizes of the observed bands were

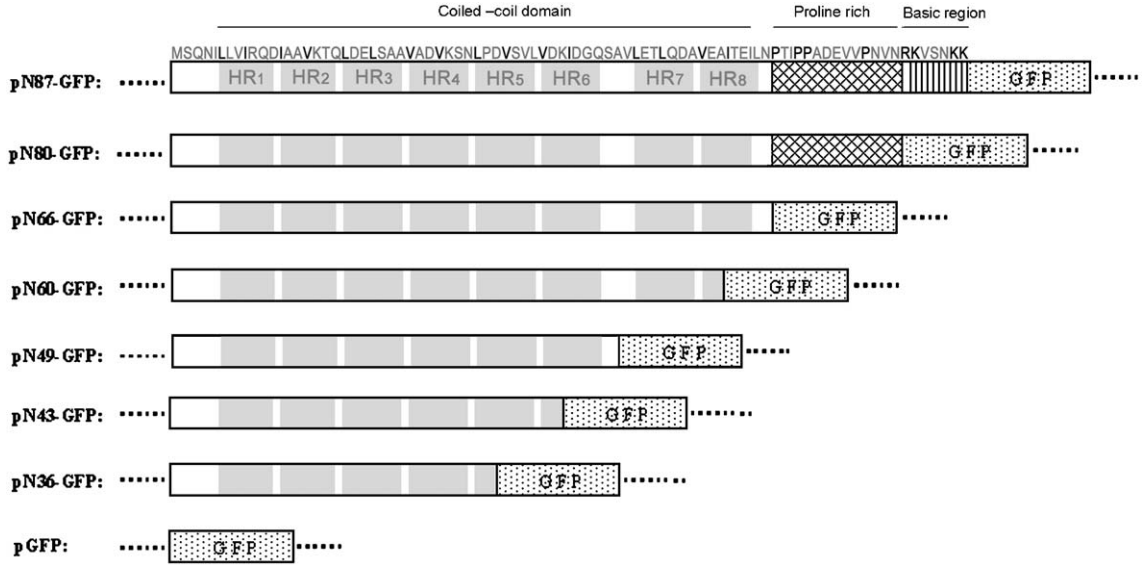

Fig. 1. Schematic representation of various P10 and GFP fusions in pIZ/V5 transient expression vector. Genes fused with GFP were under the control of the OpIE2 promoter. The coiled-coil domain, proline-rich and basic region were indicated above the amino acids sequence of HearNPV P10. The 1st and 4th hydrophobic amino acids in HRs, prolines in proline region, and basic amino acids in C-terminal basic region were blocked.