Virus Research 228 (2017) 79–89

Contents lists available at ScienceDirect

Virus Research

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

The 91-205 amino acid region of AcMNPV ORF34 (Ac34), which comprises a potential C3H zinc finger, is required for its nuclear localization and optimal virus multiplication

Jianxiang Qiu, Zhimin Tang, Meijin Yuan, Wenbi Wu*, Kai Yang

State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, China

ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form
18 November 2016
Accepted 19 November 2016
Available online 25 November 2016

Keywords:
Baculovirus
AcMNPV
ac34
Nuclear localization

ABSTRACT

During baculovirus infection, most viral proteins must be imported to the nucleus to support virus multiplication. *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) *orf34* (*ac34*) is an alphabaculovirus unique gene that is required for optimal virus production. Ac34 distributes in both the cytoplasm and the nuclei of virus-infected Sf9 cells, but contains no conventional nuclear localization signal (NLS). In this study, we investigated the nuclear targeting domains in Ac34. Transient expression assays showed that Ac34 localized in both the cytoplasm and the nuclei of Sf9 cells, indicating that no viral protein is required for Ac34 nuclear localization. Subcellular localization analysis of Ac34 truncations and internal deletions fused with green fluorescent protein in plasmid-transfected Sf9 cells identified that the 91-205 amino acid (aa) region is required for Ac34 nuclear localization. Mutations in a potential C3H zinc finger (aa 116-131) in Ac34 resulted in exclusive cytoplasmic distribution of GFP:Ac34, suggesting that the zinc finger is required for Ac34 nuclear localization. To assess the functional importance of Ac34 in the nucleus during virus replication, recombinant AcMNPV bacmids containing a series of Ac34 truncations, internal deletions, or site mutations fused with HA tags were constructed. Subcellular localization analysis showed that Ac34 with internal deletions in aa 91-205 or site mutations in the potential zinc finger was predominantly distributed in the cytoplasm. Viral plaque assays and virus growth curves indicated that disruption of Ac34 nuclear localization significantly impaired virus replication. Taken together, our findings demonstrated that the nuclear localization of Ac34 requires the 91-205 aa region and its nuclear localization is essential for optimal virus replication.

1. Introduction

The family *Baculoviridae* comprises a diverse group of viruses with double-stranded circular DNA genomes ranging from 80 to 180 kb in size (King et al., 2012). Baculoviruses are pathogenic to insects, mainly from the order *Lepidoptera* but also from the orders *Hymenoptera* and *Diptera* (King et al., 2012). Alphabaculoviruses consist of baculoviruses that specifically infect lepidopteran insects.

After entering host cells, alphabaculoviruses replicate in the nuclei. They initiate their replication with early gene expression, followed by DNA replication, late gene expression, and virion assembly (Rohrmann, 2013). A majority of virus-encoded proteins synthesized in the cytoplasm need to be imported to the nucleus to promote virus replication. Rapid and timely nuclear import of a protein generally requires a nuclear localization signal (NLS) and depends on importins, which bind to the NLS and translocate the protein to the nucleus through the nuclear pore complex (Cook et al., 2007). The best defined NLSs usually consist of a short cluster of positively changed residues and can be monopartite or bipartite. A monopartite NLS has one such element, while a bipartite has two, which are separated by a linker of 10 to 12 residues (Cook et al., 2007). Conventional NLSs are identified in many proteins, such as SV40 T antigen (Kalderon et al., 1984) and nucleoplasmin (Robbins et al., 1991). In baculovirus, some viral proteins contain one or more nuclear localization signals (NLSs) and can be transited to the nucleus through the NLSs, such as *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) LEF3 and DNApol (Au et al., 2009; Feng and Krell, 2014). Both of them are required for viral DNA replication in the nucleus. Some viral proteins do not contain any NLS and need to be translocated to the nucleus by other NLS-containing viral or cellular proteins. For example, AcMNPV PP78/83

* Corresponding author.
E-mail address: wuwenbi3@mail.sysu.edu.cn (W. Wu).

http://dx.doi.org/10.1016/j.virusres.2016.11.026
0168-1702/

is localized in the cytoplasm when transiently expressed in Sf9 cells (Wang et al., 2008), however, in virus-infected cells, it is translocated to the nucleus by another viral protein BV/ODV-C42, which contains a NLS; moreover, the nuclear localization of PP78/83 is essential for its function in promoting nuclear F-actin formation in virus-infected cells (Wang et al., 2008).

AcMNPV *ac34* exists only in all sequenced alphabaculoviruses. Our previous study showed that *ac34* transcripts could be detected in both early and late stages of infection (Cai et al., 2012). Ac34, which is encoded by *ac34* with a length of 215 amino acids (aa), is not required for viral DNA replication and viral early gene transcription; however, it stimulates viral late gene transcription and promotes budded virion (BV) production; in addition, an *ac34*-deleted AcMNPV (vAc34KO) shows no virulence in vivo, which may due to its diminished rate and amount of BV production (Cai et al., 2012). Another study showed that deletion of *ac34* results in no infectious BV production, but enveloped nucleocapsids could be observed in the nucleus (Salem et al., 2013). During the studies of Ac34, we found that it was hard to detect infectious BVs by 50% tissue culture infective dose endpoint dilution assay when transfection efficiency of vAc34KO was low; moreover, according to Salem's deletion strategy, an *ac34* deleted-AcMNPV was constructed in our laboratory and studies showed that infectious BVs were still detected (unpublished data). Therefore, we suppose that the difference may result from low transfection efficiency in Salem's study. Ac34 is localized in both the cytoplasm and the nucleus of virus-infected Sf9 cells throughout the infection cycle (Cai et al., 2012). Since alphabaculovirus replicates in the nucleus, the nuclear localization of Ac34 may be critical for its function in promoting virus replication. However, no NLS was identified in Ac34 by bioinformatics analysis. To elucidate the molecular mechanism of Ac34 during virus infection, it is important to identify the functional regions in Ac34 that are required for optimal virus replication.

In the present study, we tried to find out the nuclear targeting regions in Ac34 by constructing a series of Ac34 mutants with truncations, internal deletions, and site mutations. The subcellular localization of these Ac34 mutants in virus-infected and uninfected cells were analyzed. Their effects on virus replication were also evaluated. Our study identified that the 91-205 aa region is required for infection-independent nuclear localization of Ac34 and that the nuclear localization of Ac34 is essential for optimal virus multiplication.

2. Materials and methods

2.1. Bioinformatics analysis

Sequence analysis of Ac34 was performed using the Phyre2 Server. The homologs of Ac34 were searched against the non-redundant protein sequences in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Multiple sequence alignments were performed using ClustalX 1.83 and were edited using GeneDoc.

2.2. Cell lines and viruses

Spodoptera frugiperda IPLB Sf21-AE clonal isolate 9 (Sf9) insect cells were cultured at 27 °C in Grace's medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 μg/ml penicillin, and 30 μg/ml streptomycin. The *ac34*-deleted AcMNPV bacmid (bAc34KO), the *ac34*-deleted AcMNPV with *polyhedrin* (*polh*) and *green fluorescent protein* (*gfp*) genes inserted (vAc34KO), the *ac34*-rescued AcMNPV on the basis of bAc34KO with an HA-tagged *ac34*, *polh* and *gfp* genes inserted (vHA:Ac34), and the wild type AcMNPV with *polh* and *gfp* genes inserted (vAcWT) were generated previously (Cai et al., 2012).

2.3. Construction of recombinant plasmids containing truncated, internal deleted, or site-mutated Ac34

DNA sequence encoding Ac34Δ(2-57) or Ac34Δ(2-84) was PCR-amplified from bMON14272 (Invitrogen) using primer pairs 34N575/343 (all primer sequences used in this study are shown in Table 1) and 34N845/343, respectively. Both PCR products were digested with *Kpn*I and *Bam*HI and individually ligated to pUC18-P_{ac34}-SV40 (Cai et al., 2012) to generate pUC18-Ac34Δ(2-57) and pUC18-Ac34Δ(2-84). Site-directed mutagenesis method (Chiu et al., 2004) was employed to generate *ac34* with internal deletions or site mutations using pUC18-ac34HA-SV40 (Cai et al., 2012) as the template. Specifically, primer pairs H4Ft/H4Rs and H4Fs/H4Rt were used to delete aa 111-136; primer pairs H5Ft/H5Rs and H5Fs/H5Rt were used to delete aa 144-169; primer pairs H6Ft/H6Rs and H6Fs/H6Rt were used to delete aa 175-196. The resulting plasmids were named pUC18-Ac34Δ(111-136), pUC18-Ac34Δ(144-169), and pUC18-Ac34Δ(175-196), respectively. In addition, primer pairs C128Ft/M1Rs and M1Fs/C128Rt were used to substitute Cys^{128} with Ala^{128} to generate pUC18-Ac34(C128A). Primer pairs H131Ft/M1Rs and M1Fs/H131Rt were used to substitute His^{131} with Ala^{128} to generate pUC18-Ac34(H131A). DNA sequence encoding Ac34Δ(206-215) were PCR-amplified from bMON14272 using primers 345 and 34ΔH73. The PCR product was successively ligated to pEASY-Blunt (TranGen) and pUC18-P_{ac34}-SV40 to generate pUC18-Ac34Δ(206-215). All Ac34 mutants were confirmed by DNA sequencing.

2.4. Construction of plasmids transiently expressing HA- or GFP-fused Ac34 and GFP-fused truncated, internal deleted, or site-mutated Ac34

pUC18-ac34HA-SV40 was digested with *Kpn*I and *Bam*HI and ligated to pIB/V5-His (Invitrogen), where the exogenous gene is cloned between the promoter and polyadenylation signal of Orgyia pseudotsugata multicapsid nucleopolyhedrovirus *ie2* gene, to generate pIB-HA:Ac34. All recombinant pUC18 plasmids containing full-length, truncated, internal deleted, or site-mutated Ac34 (generated above) were digested with *Kpn*I and *Bam*HI and ligated to pIB-GFP (generated on the basis of pIB/V5-His) (Wei et al., 2014), individually, to generate pIB-GFP:Ac34, pIB-GFP:Ac34Δ(2-57), pIB-GFP:Ac34Δ(2-84), pIB-GFP:Ac34Δ(111-136), pIB-GFP:Ac34Δ(144-169), pIB-GFP:Ac34Δ(175-196), pIB-GFP:Ac34Δ(206-215), pIB-GFP:Ac34(C128A), and pIB-GFP:Ac34(H131A). Meanwhile, five more plasmids were constructed as follows. pIB-GFP:Ac34Δ(62-84) containing Ac34Δ(62-84) was generated using site-directed mutagenesis (Chiu et al., 2004) with pIB-GFP:Ac34 as the template and H3Ft/H3Rs and H3Fs/H3Rt as primer pairs. With the same mutagenesis method, pIB-GFP:Ac34Δ(85-90) was generated with primer pairs L3Ft/L3Rs and L3Fs/L3Rt; pIB-GFP:Ac34(C116A) was generated with primer pairs C116Ft/M2Rs and M2Fs/C116Rt; and pIB-GFP:Ac34(C119A) was generated with primer pairs C119Ft/M2Rs and M2Fs/C119Rt. DNA sequence encoding Ac34Δ(197-215) was PCR-amplified from bMON14272 using primers 3451 and 341963. The PCR product was digested with *Kpn*I and *Bam*HI and ligated to pIB-GFP to generate the plasmid pIB-GFP:Ac34Δ(197-215).