Virology 385 (2009) 496–504

Contents lists available at ScienceDirect

Virology

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

AcMNPV EXON0 (AC141) which is required for the efficient egress of budded virus nucleocapsids interacts with β-tubulin

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ARTICLE INFO

Article history:
Received 28 October 2008
Returned to author for revision
15 November 2008
Accepted 8 December 2008
Available online 19 January 2009

Keywords:
AcMNPV
AC141
Exon0
Baculovirus
Viral egress
Microtubules

ABSTRACT

The *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) encoded protein, EXON0 (AC141), is required for the efficient transport of nucleocapsids out of the nucleus for the production of budded virus (BV). To further elucidate the molecular mechanisms by which EXON0 regulates BV production, EXON0 was tagged at the N-terminus with 3× FLAG–6× His. Protein complexes were isolated by tandem affinity purification and potential EXON0 specific interacting protein partners were gel purified and identified by LC–MS/MS. This analysis showed that the cellular protein, β-tubulin, co-purified with EXON0 which was confirmed by co-immunoprecipitation. In addition, immunofluorescence showed that EXON0 and β-tubulin co-localized during virus infection. The microtubule inhibitors colchicine and nocodazole were used to treat AcMNPV infected Sf9 cells and results showed that BV production was reduced by over 85%. These data suggest that the egress of AcMNPV budded virus may be facilitated by the interaction of EXON0 with β-tubulin and microtubules.

Introduction

Autographa californica multiple nucleopolyhedrovirus (AcMNPV), the archetype of the *Baculoviridae*, has a 133.9 kbp genome and encodes 154 predicted genes of 50 amino acids or greater. *Exon0* (*orf141*) is a conserved gene found in all lepidopteran baculoviruses of the genus *alphabaculovirus*. The deletion of *exon0* reduces the level of BV production up to 99% and the infection of Sf9 cells with an *exon0* knockout (KO) virus is restricted to a single cell or a few neighbouring cells. However, viral replication and polyhedra production are unaffected, suggesting that *exon0* plays a key role specific to BV production pathway (Dai et al., 2004). EXON0 is a structural component of both BV and ODV that localizes to the nucleocapsid fraction. Electron microscopy has also revealed that EXON0 is required for efficient transport of progeny nucleocapsids from the nucleus to the cytoplasm (Fang et al., 2007).

Baculovirus BVs enter insect and mammalian cells *via* adsorptive endocytosis (Hefferon et al., 1999; Volkman and Goldsmith, 1985). The acidification of late endosomes activates the fusogenicity of GP64 and results in the fusion of the viral and endosomal membranes (Blissard and Wenz, 1992; Leikina et al., 1992; Long et al., 2006). After the nucleocapsids are released from the endosomes, the incoming nucleocapsids induce the formation of thick transient actin bundles which appear to facilitate the transport of nucleocapsids to the nucleus (Charlton and Volkman, 1991, 1993; Lanier and Volkman, 1998). After the entry into the nucleus, viral transcription and DNA replication occur in a cascade-like fashion. At late times post infection, the progeny nucleocapsids are assembled in the virogenic stroma, transported out of the nucleus and bud at the cytoplasmic membrane to become BV. At very late times post infection, the progeny nucleocapsids are retained in the nucleus, enveloped by a virally induced intranuclear membrane and finally occluded into polyhedra to form ODV (Williams and Faulkner, 1997). The mechanism by which the nucleocapsids are shuttled so that they are destined to become BV or retained in the nucleus to become ODV is unknown. Also poorly understood is how nucleocapsids are transported from the nucleus to the plasma membrane but our results have shown that EXON0 is required for this process (Fang et al., 2007).

Microtubules are key components in the cytoskeleton of eukaryotic cells and play an important role in organelle transport, cell shape maintenance, mitosis, motility, and cell division (Jordan and Wilson, 2004). Microtubules are highly dynamic polymers of heterodimers of α- and β-tubulin, arranged parallel to a cylindrical axis to form tubes of 24 nm diameter that may be many micrometers long. Microtubules have long been implicated in viral nucleocapsid movement since their close association was first observed by electron microscopy (Granados, 1978; Luftig and Weihing, 1975). In the last decade, the involvement of microtubules in virus transport has been reported for a number of viruses (Carter et al., 2003; Funk et al., 2004; Lakadamyali et al., 2003; McDonald et al., 2002; Pelkmans et al., 2001; Seisenberger et al., 2000;

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0042-6822/$ – see front matter.
doi:10.1016/j.virol.2008.12.023

Sodeik et al., 1997; Suomalainen et al., 1999). In addition, quite a few specific viral proteins that interact directly with microtubules or with microtubule motor proteins have also been identified (Alonso et al., 2001; Elliott and O'Hare, 1998; Martin et al., 2002; Nejmeddine et al., 2000; Ogino et al., 2003; Ward and Moss, 2004).

To further dissect how EXON0 plays its critical role in the viral egress part of the viral infection cycle the objective of this study was to identify additional proteins with which EXON0 interacts. Using 3× FLAG–6× His tandem affinity purification (TAP) and LC–MS/MS the cellular protein β-tubulin was shown to associate with EXON0. This interaction was confirmed using co-immunoprecipitation, confocal microscopy and microtubule inhibitor drugs. These results suggest that microtubules may play an important role in BV production and their association with EXON0 may facilitate the transport of nucleocapsids from the nucleus to the plasma membrane.

Results

Construction of virus expressing TAP tagged EXON0

EXON0 has been shown to be required for the high level production of BV and for the efficient egress of nucleocapsids from the nucleus to the cytoplasmic membrane (Dai et al., 2004; Fang et al., 2007). To investigate the molecular basis for these observations and to better characterize the function of EXON0, we attempted to identify cellular binding partners. We used tandem affinity purification (TAP) coupled with LC–MS/MS protein identification as it has been shown to be very successful in and highly sensitive in identifying protein–protein interactions (Puig et al., 2001; Rigaut et al., 1999).

The 3× FLAG–6× His TAP tag has been shown to be very efficient for purifying protein complexes in insect cells and was used in this study (Yang et al., 2006). The *exon0*KO bacmid bMON14272 *exon0*KO was repaired by a fusion construct of EXON0 in which the 3× FLAG–6× His TAP tag was fused at the N-terminus of EXON0 (Fig. 1A). The titer produced by *exon0*KO-3× FLAG–6× His-HA-EXON0 (3.3×10^8 $TCID_{50}$/ml) at 96 h post infection (hpi) was similar to *exon0*KO-HA-EXON0 indicating that the addition of the TAP tag did not affect the function of EXON0 and the fusion protein could fully rescue the KO bacmid. The fusion protein was detected at the correct size by western blot (Data not shown).

Identification of β-tubulin as an interacting partner of EXON0

Sf9 cells were infected with *exon0*KO-3× FLAG–6× His-HA-EXON0 or the control virus *exon0*KO-HA-EXON0 and protein complexes were isolated at 24 hpi using 3× FLAG–6× His TAP and the results are shown

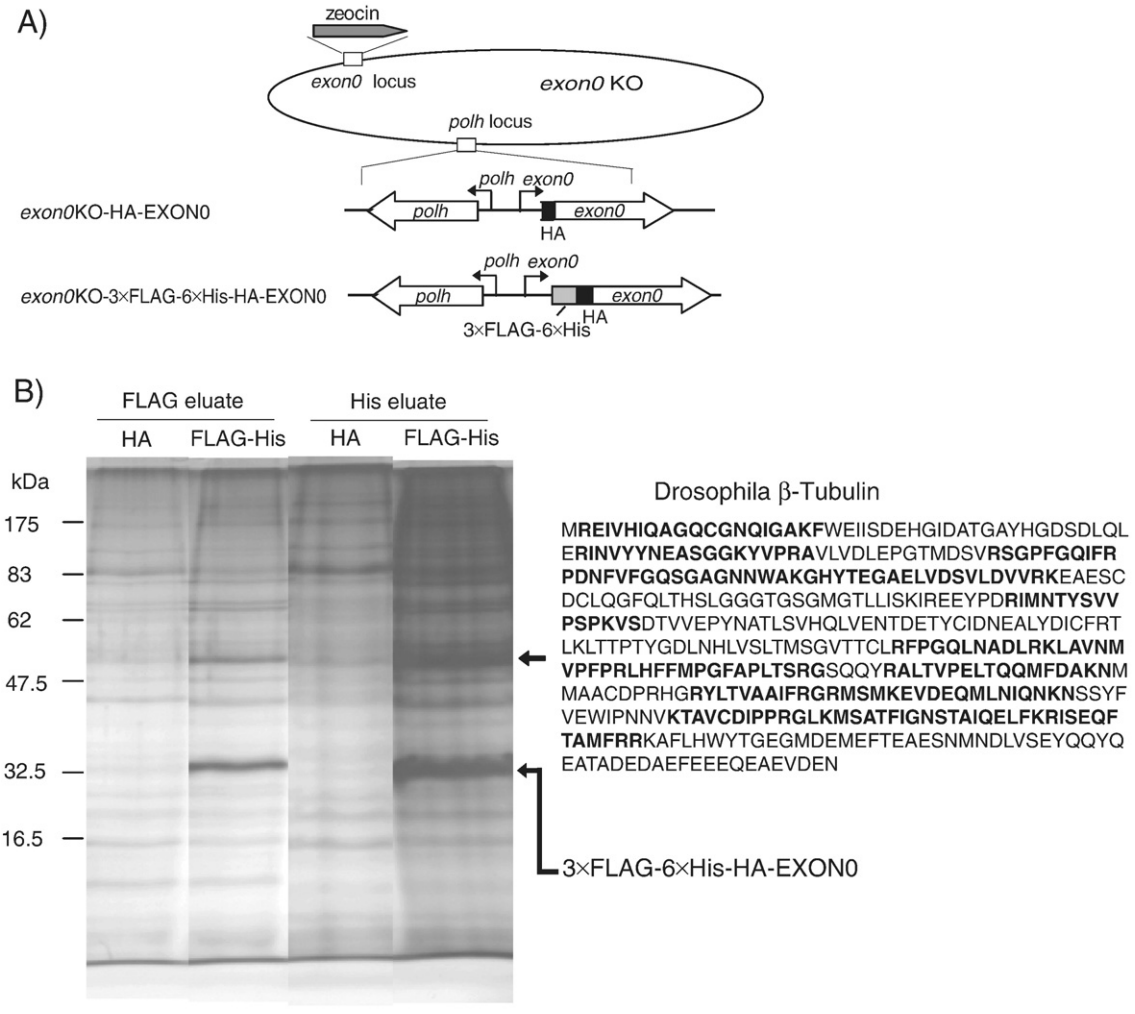

Fig. 1. Identification of β-tubulin as an interacting partner of EXON0. (A). Schematic diagrams of bacmid viruses that express EXON0 fusion proteins, *exon0*KO-3× FLAG–6× HIS-HA-EXON0 and *exon0*KO-HA-EXON0 that fused the TAP tag 3× FLAG–6× His-HA or just the HA epitope tag at the N-terminal of EXON0. (B). TAP complexes associated tagged EXON0 proteins. Sf9 cells were infected with the *exon0*KO-3× FLAG–6× His-HA-EXON0 (FLAG-His) or *exon0*KO-HA-EXON0 (HA) and harvested at 24 hpi and subjected to affinity purification. Complexes were separated by SDS-PAGE after the single affinity purification (FLAG eluate) and double affinity purification (His eluate) followed by silver staining (see Materials and methods). The β-tubulin specific band (top arrow) was identified by LC–MS/MS. The *Drosophila* β-tubulin sequence is shown on the right and the bold residues of β-tubulin represent the peptide fragments of β-tubulin identified by LC–MS/MS.