Virology 518 (2018) 163–171

Contents lists available at ScienceDirect

Virology

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

Networks of protein-protein interactions among structural proteins of budded virus of *Bombyx mori* nucleopolyhedrovirus

Jianjia Zhang, Min Feng, Ying Fan, Weifan Xu, Qin Zheng, Xiaofeng Wu*

College of Animal Sciences, Zhejiang University, Hangzhou 310058, China

A B S T R A C T

The structural proteins of baculovirus are well studied, but the interactions between them remain unclear. In order to reveal protein-protein interactions among viral structural proteins and their associated proteins of the budded virus of *Bombyx mori* nucleopolyhedrovirus (BmNPV), the yeast two hybrid (Y2H) system was used to evaluate the interactions of 27 viral genes products. Fifty-seven interactions were identified with 51 binary interactions and 6 self-associations. Among them, 10 interactions were further confirmed by co-immunoprecipitation assays. Five interaction networks were formed based on the direct-cross Y2H assays. VP39, 38 K, and FP were identified to interact with most of the viral proteins, and may form major structural elements of the viral architecture. In addition, each envelope protein was detected to interact with more than one capsid protein. These results suggest how viral structural and structural associated proteins may assemble to form a complete virus through interacting with each other.

1. Introduction

Baculoviruses are large rod-shaped enveloped viruses with double-stranded circular DNA genomes of 90–180 kb (Herniou et al., 2003), infecting insects of the orders Lepidoptera, Hymenoptera and Diptera. There are two virion phenotypes produced during the infection cycle, the occlusion-derived virus (ODV) and the budded virus (BV) (Theilmann et al., 2005). ODV establish horizontal insect-to-insect transmission, whereas BV are responsible for cell-to-cell spread within a susceptible host. During the early phase of infection, newly assembled nucleocapsids move out of the nucleus and obtain an envelope by budding through the cellular membrane modified by virally encoded proteins to form BV (Braunagel and Summers, 1994). In the late phase, nucleocapsids are retained in the nucleus and acquire a lipid bilayer membrane originating from a modified inner nuclear membrane to form ODV (Braunagel and Summers, 2007). Consequently, BV and ODV possess different envelopes, while their nucleocapsids appear to be structurally and genetically identical.

Many of the baculovirus proteins in the envelope and capsid have been determined, but their specific function and interactions are not completely clear. Recently, protein-protein interactions of the ODV-associated proteins of *Helicoverpa armigera* nucleopolyhedrovirus have been investigated (Peng et al., 2010b) and interactions of two or more structural proteins have been demonstrated for the *Autographa californica* MNPV (Danquah et al., 2012; Wu et al., 2008). However, these reports are mainly related to ODV and information on BV protein interactions is limited. In this study, we investigated the protein-protein interactions among structural proteins of BmNPV budded virus. For this purpose, 27 proteins reported to be associated with BmNPV BV, including viral envelope proteins, capsid proteins and other structural associated proteins, were employed for direct-cross Y2H assays. The interactions between capsid, envelope, and other structurally associated proteins were investigated. Based on our observations together with results of previous studies, a framework of protein interactions among BmNPV BV-associated proteins was constructed.

2. Results

2.1. Construction of yeast two-hybrid clones and self-activation verification

A total of 27 proteins of BmNPV BV implicated as being BV associated in other baculoviruses (Hou et al., 2013; Wang et al., 2010) were selected for this study. Specifically, there were 12 capsid proteins (VP39, 38 K, BV/ODV-C27, P24, ODV-C42, FP, LEF6, PP78/83,

Abbreviation: AcMNPV, *Autographa californica* multiple nucleopolyhedrovirus; *B.mori*, *Bombyx mori*; BmNPV, *Bombyx mori* nucleopolyhedrovirus; BV, budded virus; C42, BV/ODV-C42; Co-IP, co-immunoprecipitation; *E.coli*, *Escherichia coli*; E25, ODV-E25; E18, ODV-E18; EC27, ODV-EC27; FP, FP25K; HearNPV, *Helicoverpa armigera* nucleopolyhedrovirus; ODV, occlusion-derived virus; PIF, *per os* infectivity factor; PKIP, Protein kinase interacting protein; TM, trans-membrane; v-Ubi, v-Ubiquitin; v-Cath, v-Cathepsin; VS, Viral stroma; Y2H, yeast two hybrid

* Corresponding author.
E-mail address: wuxiaofeng@zju.edu.cn (X. Wu).

https://doi.org/10.1016/j.virol.2018.02.015
Received 1 October 2017; Received in revised form 15 February 2018; Accepted 16 February 2018

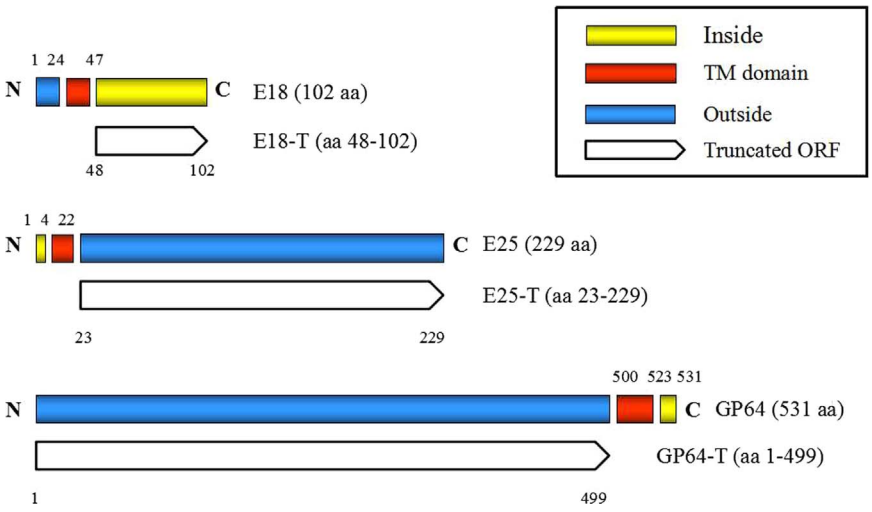

Fig. 1. Schematic of the truncated TM domains-containing proteins in this study. The potential TM domains of the envelope proteins was predicted by TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/), and the inside (towards the tegument) and outside (on the envelope surface) orientation were indicated by different colors. The letter "N" or "C" represented the N-termini or C-termini of the proteins. White arrows represented the truncated forms of these ORFs constructed for use in this study.

Table 1
Results of false positive tests. The experiment was repeated three times. False positive were indicated as " + ".

Gene	pGADT7-	pGBKT7-	Gene	pGADT7-	pGBKT7-
vp39		+	lef6	+	+
gp41		+	fgf	+	
vlf-1	+	+	vp1054		+
39K/pp31		+	pp78/83	+	

VP1054, 49 K, VP80, ME53), 7 envelope proteins (BV/ODV-E25, BV/ODV-E18, v-Cathepsin, v-ubiquitin, BV/ODV-E26, GP64, F), and 8 other structural associated proteins (P6.9, PP31, GP41, GP37, P48, PKIP, PP34, FGF). For the existence of putative trans-membrane domains (TM) in several envelope proteins may interfere with the detection of protein interactions in the yeast nucleus, TMHMM software was used to predict the potential TM domains of the 7 envelope proteins, and 3 were found to contain only one TM domain, *i.e.* ODV-E25, ODV-E18 and GP64. For accurate analysis, they were truncated by amplifying their open reading frames (ORFs) without the TM domains (Fig. 1). Therefore, 27 fragments of above related genes were cloned into pGADT7 and pGBKT7 vectors respectively, and then transformed into Y187 and Y2H yeast stains. To ensure the fidelity of hybridization results, false positive assays were performed before the Y2H analysis. Ten clones were identified to be auto-activating, including pGBKT7-VP39, pGBKT7-GP41, pGBKT7-PP31, pGBKT7-VP1054, pGADT7-VLF1 and pGBKT-VLF1, pGADT7-LEF6 and pGBKT7-LEF6, pGADT7-FGF, pGADT7-PP78/83 (Table 1). It indicated that these clones could not be analyzed by Y2H assays.

2.2. Yeast two-hybrid assays revealed 57 protein-protein interactions

To obtain a systematic protein-protein interaction network, the remaining 44 clones were analyzed, and each screening was repeated three times (Fig. 2). Positive and negative controls used were pGBKT7-P53 and pGBKT7-Lam with pGADT7-T-antigen, respectively. A total of 57 interactions were identified, including 51 reciprocal interactions of different proteins and 6 self-interactions comprising 38K-38K, EC27-EC27, FP-FP, PKIP-PKIP, P48-P48 and GP64-GP64. Among the 51 reciprocal interactions, 18 interactions were detected in only one direction. Among the other 33 reciprocal interactions, 3 interactions were reported before, including 38K-VP39 (Peng et al., 2010b), GP64-GP64 and FP-GP64 (Braunagel et al., 1999), and the other 30 interactions are described here for the first time.

2.3. Interactions between capsid proteins

Baculovirus have a number of nucleocapsid proteins that are present in both ODV and BV. It is unclear how do these proteins interact with one another with high specificity to form the capsid architecture, To answer this question, we analyzed direct-cross assays and detected 11 interactions among baculovirus capsid proteins (C42-38K, C42-FP, FP-FP, FP-38K, 38K-38K, 38K-EC27, EC27-EC27, EC27–49K, EC27-ME53, ME53-VP39, and VP39-EC27) (Table 2, Fig. 3). Among the 11 interactions, FP was found to self-associate and also interacted with C42 and with 38 K. Likewise, the same type of pattern also occurred to 38 K. It self-associated and interacted with EC27, which also self-associated and interacted with ME53.

2.4. Interactions between envelope proteins

Though interactions of envelope proteins play important roles in viral infection, details of the envelope structural arrangements remain unknown. Specifically, very little is known about the relationship between the structure and the mechanical properties of the viral envelope. In this study, 7 envelope proteins of BV were analyzed and 5 interactions were detected (Table 2, Fig. 3), *i.e.*, E25-E18, E18-v-Cath, v-Cath-v-Ubi, E18-GP64, GP64-GP64 (The F protein was not detected to interact with any envelope protein).

2.5. Interactions between capsid proteins and envelope proteins

16 interactions between capsid proteins and envelope proteins were identified (Table 2, Fig. 3), including E25–38K, E25-ME53, GP64-38K, GP64-VP39, GP64-FP, v-Ubi-VP39, v-Ubi-FP, v-Ubi-49K, E18–38K, E18-VP39, E18-FP, E18-ME53, E18-C42, v-Cath-38K, v-Cath-ME53, and v-Cath-EC27. These results suggested that each envelope protein interacts with more than one capsid protein. For instance, 38 K interacted with 4 envelope proteins, while the other three capsid proteins, VP39, FP and ME53 interacted with 3 envelope proteins, respectively. The structures assembled by these protein interactions may form a scaffold for the capsid-envelope interaction network since each of them was detected to interact with more than 3 envelope proteins.

2.6. Interactions between structural associated proteins and structural proteins

The process of virus assembly involves protein-protein interactions between viral structural and structural associated proteins. The structural associated proteins, including DNA binding protein P6.9, VS associated protein PP31, polyhedron envelope-associated phosphoprotein