Virology 443 (2013) 363–374

Contents lists available at SciVerse ScienceDirect

Virology

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

The P6 protein of *Cauliflower mosaic virus* interacts with CHUP1, a plant protein which moves chloroplasts on actin microfilaments

Carlos A. Angel [a], Lindy Lutz [c], Xiaohua Yang [b], Andres Rodriguez [a], Adam Adair [a], Yu Zhang [a], Scott M. Leisner [c], Richard S. Nelson [b], James E. Schoelz [a,*]

[a] Division of Plant Sciences, University of Missouri, Columbia, MO 65211, USA
[b] The Division of Plant Biology, The Samuel Roberts Noble Foundation, Ardmore OK 73401, USA
[c] Department of Biological Sciences, University of Toledo, Toledo OH 43606, USA

ARTICLE INFO

Article history:
Received 11 February 2013
Returned to author for revisions
11 March 2013
Accepted 18 May 2013
Available online 12 June 2013

Keywords:
Intracellular movement
Subcellular localization
Inclusion bodies
CaMV

ABSTRACT

The gene VI product, protein 6 (P6), of *Cauliflower mosaic virus* (CaMV) assembles into large, amorphous inclusion bodies (IBs) that are considered sites for viral protein synthesis and viral genome replication and encapsidation. P6 IBs align with microfilaments and require them for intracellular trafficking, a result implying that P6 IBs function to move virus complexes or virions within the cell to support virus physiology. Through a yeast two-hybrid screen we determined that CHUP1, a plant protein allowing chloroplast transport through an interaction with chloroplast and microfilament, interacts with P6. The interaction between CHUP1 and P6 was confirmed through colocalization *in vivo* and co-immunoprecipitation assays. A truncated CHUP1 fused with enhanced cyan fluorescent protein, unable to transport chloroplasts, inhibited intracellular movement of P6–Venus inclusions. Silencing of CHUP1 in *N. edwardsonii* impaired the ability of CaMV to infect plants. The findings suggest that CHUP1 supports CaMV infection through an interaction with P6.

Introduction

Plant viruses have at least three distinct activities necessary to complete their disease cycle in a host: those required to replicate, encapsidate, and move the virus throughout the plant. Some plant viruses encode additional proteins with functions dedicated to vector transmission or defeating plant defenses, but it is generally accepted that the genomic capacity of plant viruses is small, with an upper limit of perhaps 15–20 proteins. To overcome their limited coding capacity, it is likely that each viral protein has multiple functions and contacts with host factors. Collectively these functions and interactions determine the outcome of the infection.

The P6 protein of *Cauliflower mosaic virus* (CaMV) is an example of a plant virus protein with multiple functions and interactions. P6 functions to support the synthesis of viral proteins from the 35S viral RNA, suppresses gene silencing, and inhibits SA-mediated plant defenses (Hohn and Fütterer, 1997; Love et al., 2007, 2012; Park et al., 2001). In addition, P6 functions as an avirulence determinant in some solanaceous and cruciferous species, and is a chlorosis symptom determinant in susceptible hosts (Baughman et al., 1988; Daubert et al., 1984; Hapiak et al., 2008; Schoelz et al., 1986). P6 is the most abundant protein component of the amorphous, electron dense inclusion bodies (IBs) present during virus infection (Odell and Howell, 1980; Shockey et al., 1980). P6-containing IBs induced during virus infection are likely "virion factories", as they are the primary site for CaMV protein synthesis, genome replication, and assembly of virions (Hohn and Fütterer, 1997). P6 physically interacts with the CaMV capsid (P4) and movement (P1) proteins, as well as the two viral proteins necessary for insect transmission (P2 and P3) (Hapiak et al., 2008; Himmelbach et al., 1996; Lutz et al., 2012; Ryabova et al., 2002).

Recently, a new function for P6 was suggested when it was shown that P6 IBs induced by ectopic expression of P6 associate with actin microfilaments, microtubules and the endoplasmic reticulum, and were capable of intracellular movement along microfilaments (Harries et al., 2009a). Furthermore, latrunculin B, a pharmacological agent that disrupts microfilaments by preventing polymerization of actin monomers, abolished CaMV local lesion formation in *N. edwardsonii*, an indication that intact microfilaments are essential for CaMV infections (Harries et al., 2009a). Collectively, these experiments suggested that P6 IBs might be responsible for intracellular trafficking of virions to plasmodesmata, in addition to the role of the P6 protein in translation of the 35S RNA and gene silencing suppression.

Prior to the report by Harries et al. (2009a), the P6 protein of CaMV had not been considered to have a role in CaMV movement,

* Corresponding author. Fax: +1 573 882 0588.
E-mail address: schoelzj@missouri.edu (J.E. Schoelz).

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.05.028

but there was evidence suggesting its function in this activity. CaMV virions move from cell to cell through plasmodesmata modified into tubules through the function of its movement protein, P1 (Kasteel et al., 1996; Perbal et al., 1993). However, it is unlikely that the CaMV P1 protein transports the virions to the plasmodesmata since P1 does not appear to directly interact with the virion. The CaMV P3 protein does interact with virions through the formation of a tetrameric structure anchored into the virions (Leclerc et al., 1998, 2001). Electron microscopy studies have indicated that P1 and P3 colocalize with virions only within the plasmodesmata, and it has been suggested that the P3/virion complex travels to the plasmodesmata independently from P1 (Stavolone et al., 2005). Consequently, there is a need for a second CaMV protein to fulfill the role of intracellular transport. Since P6-containing IBs are the site for virion accumulation and they are capable of movement, they may be responsible for delivering virions to CaMV P1 located at the plasmodesmata (Harries et al., 2009a; Schoelz et al., 2011). At the very least, there must be a mechanism that would account for the transfer of CaMV virions from the P6 IBs to the plasmodesmata.

In this study we utilized a yeast two-hybrid assay to identify host proteins that interact with CaMV P6. We show here that P6 physically interacts with CHUP1 (Chloroplast Unusual Positioning 1), a protein which is encoded by a single gene in Arabidopsis and is localized to the outer membrane of chloroplasts (Oikawa et al., 2003, 2008). A CHUP1-ECFP (Enhanced Cyan Fluorescent Protein) fusion protein was observed to relocate a P6-Venus fusion protein to chloroplasts *in vivo*. An interaction between CHUP1 and P6 also was demonstrated through co-immunoprecipitation studies from plant extracts. Overexpression of a truncated CHUP1 in a transient expression assay in *N. benthamiana* blocked the movement of P6 IBs. This observation was correlated with a delayed rate of CaMV local lesion development when CHUP1 was silenced in *N. edwardsonii*. Our data provide an explanation for the subcellular localization of P6 IBs to, and movement along, microfilaments. In addition our data provide further evidence that a complex composed of P6 and virions may contribute to intracellular movement of CaMV particles necessary to infect the host.

Results

A yeast two-hybrid screen reveals that the CaMV P6 protein interacts with CHUP1

Previous studies showed that CaMV P6 interacts with the ribosomal proteins L13, L18, and L24, along with eukaryotic translation factor 3 subunit g (eIF3g) (Bureau et al., 2004; Leh et al., 2000; Park et al., 2001), consistent with the role of P6 in re-initiation of translation of the polycistronic 35S mRNA. P6 also interacts with the RNA silencing protein DRB4 (Haas et al., 2008), which is consistent with the role of P6 as a silencing suppressor. To identify additional proteins that interact with CaMV P6, a yeast two-hybrid screen of an *A. thaliana* cDNA library composed of transcripts representing one-week old seedlings was performed by Hybrigenics Services (Paris, France). The bait consisted of the full-length sequence of P6 of CaMV strain W260 (Wintermantel et al., 1993). Of the 85 Arabidopsis clones found in this screen, 17 were identified as eIF3g. None of the other proteins previously shown to interact with P6 (e.g. L13, L18, L24 or DRB4) appeared in this Y2H screen. Nonetheless, the result with eIF3g demonstrates the capacity of our screen to identify host proteins previously shown to interact with P6 through two-hybrid screens (Park et al., 2001).

One of the additional clones selected in the Hybrigenics screen was identified as CHUP1 (*At3G25690*), a unique Arabidopsis gene encoding a protein that localizes to the outer membrane of chloroplasts (Oikawa et al., 2003). Previously it was shown that CHUP1 has four important functional domains (Fig. 1A). The N-terminus contains a hydrophobic domain that targets CHUP1 to the chloroplast outer envelope. A second domain is a coiled-coil motif that interacts with the plasma membrane, permitting CHUP1 to serve as a bridge to anchor chloroplasts to plasma membranes along the cell wall, and also is important for homo-dimerization of the protein. A third domain binds to F-actin both *in vitro* and *in vivo*, and a fourth proline-rich domain interacts with profilin and actin. In addition, there are two embedded leucine-zipper motifs, one in the coiled-coil region and the other downstream from the proline-rich region, each of which may be important for intramolecular interactions. (Oikawa et al., 2003, 2008; Lehmann et al., 2011; Schmidt von Braun and Schleiff, 2008). In the Hybrigenics yeast two hybrid assay, CaMV P6 was shown to interact with the coiled-coil region of CHUP1 (Fig. 1A).

To identify the specific region(s) of P6 that interact with CHUP1, a second yeast two-hybrid assay was performed. Four domains of P6, previously investigated for their role in self-association (D1–D4; Fig. 1A) (Li and Leisner, 2002), were used as bait for CHUP1. Yeast cells co-transformed with the full length P6 fused to the LexA DNA-binding domain in the pEG202 plasmid and a portion of the coiled-coil domain of CHUP1 fused to the B42

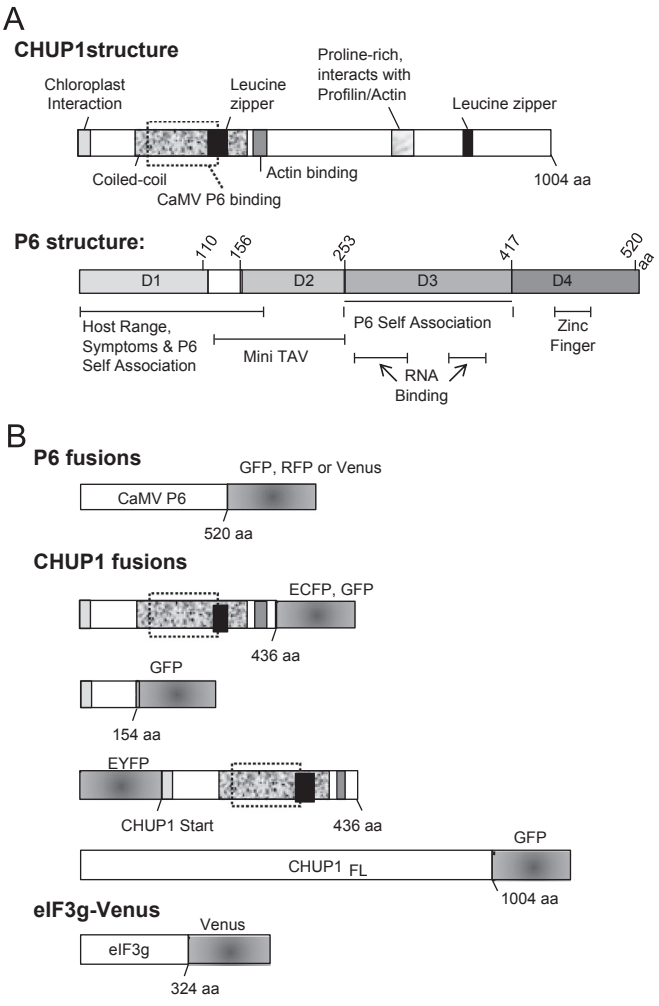

Fig. 1. CHUP1, P6 and eIF3g constructs used for confocal microscopy or co-immunoprecipitation. (A) Structure of CHUP1 and CaMV P6 proteins. The functions of P6 domains D1–D4 tested for self interaction (Li and Leisner, 2002) and interaction with a portion of CHUP1. The mini TAV is the minimal region for the translational transactivation function. The functions of CHUP1 domains are shown below. (B) Structure of P6, CHUP1, eIF3g fusions developed for confocal microscopy.