Virology 385 (2009) 245–260

Contents lists available at ScienceDirect

Virology

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

Translation elongation factor 1A is a component of the tombusvirus replicase complex and affects the stability of the p33 replication co-factor

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ARTICLE INFO

Article history:
Received 6 October 2008
Returned to author for revision
1 November 2008
Accepted 25 November 2008
Available online 7 January 2009

Keywords:
Tomato bushy stunt virus
Replication
Protein array
Proteome array
Yeast as a model host
eEF1A
Host factor
Replicase
TEF1

ABSTRACT

Host RNA-binding proteins are likely to play multiple, integral roles during replication of plus-strand RNA viruses. To identify host proteins that bind to viral RNAs, we took a global approach based on the yeast proteome microarray, which contains 4080 purified yeast proteins. The biotin-labeled RNA probes included two distantly related RNA viruses, namely *Tomato bushy stunt virus* (TBSV) and *Brome mosaic virus* (BMV). Altogether, we have identified 57 yeast proteins that bound to TBSV RNA and/or BMV RNA. Among the identified host proteins, eleven bound to TBSV RNA and seven bound to BMV RNA with high selectivity, whereas the remaining 39 host proteins bound to both viral RNAs. The interaction between the TBSV replicon RNA and five of the identified host proteins was confirmed via gel-mobility shift and co-purification experiments from yeast. Over-expression of the host proteins in yeast, a model host for TBSV, revealed 4 host proteins that enhanced TBSV replication as well as 14 proteins that inhibited replication. Detailed analysis of one of the identified yeast proteins binding to TBSV RNA, namely translation elongation factor eEF1A, revealed that it is present in the highly purified tombusvirus replicase complex. We also demonstrate binding of eEF1A to the p33 replication protein and a known cis-acting element at the 3′ end of TBSV RNA. Using a functional mutant of eEF1A, we provide evidence on the involvement of eEF1A in TBSV replication.

Introduction

Plus-stranded (+)RNA viruses, the largest group among viruses, contain relatively small genomes and thus greatly depend on the infected hosts in many steps during their infection cycles. Indeed, viruses are known to recruit numerous host proteins to facilitate their replication and spread (Ahlquist et al., 2003; Nagy, 2008; Noueiry and Ahlquist, 2003). Several host RNA-binding proteins have been implicated in replication of (+)RNA viruses, including ribosomal proteins, translation factors and RNA-modifying enzymes (Ahlquist et al., 2003; Buck, 1996, 1999; Nagy, 2008; Noueiry and Ahlquist, 2003; Strauss and Strauss, 1999; Wang and Nagy, 2008). In addition, recent genome-wide screens of yeast genes conducted with two distantly related viruses, *Brome mosaic virus* (BMV) (Kushner et al., 2003) and *Tomato bushy stunt virus* (TBSV) (Jiang et al., 2006; Panavas et al., 2005b) revealed that their replication is affected by ~100 different host genes. The genome-wide screens with TBSV also identified ~30 host genes affecting TBSV RNA recombination (Cheng et al., 2006; Serviene et al., 2006, 2005). The identified host genes code for proteins involved in various cellular processes, such as translation, RNA metabolism, protein modifications and intracellular transport or membrane modifications (Jiang et al., 2006; Kushner et al., 2003; Panavas et al., 2005b). Additional genome-wide screens with *Drosophila* virus C and West Nile virus have also identified over 100 host genes (Cherry et al., 2005; Krishnan et al., 2008). However, these genome-wide screens likely missed the identification of host genes with overlapping functions. Therefore, additional screens are needed, which are less affected by gene redundancy, to identify the total number of host genes affecting virus replication.

One of the major groups of host factors that likely affect RNA virus replication is RNA-binding proteins that play essential roles in many cellular processes, such as transcription, splicing, translation, mRNA turnover, and antiviral mechanisms. These protein–RNA interactions affect the structures and functions of abundant ribonucleoprotein complexes, which contain many different proteins and RNAs. Thus, subverting some RNA-binding proteins could be beneficial for facilitating replication of RNA viruses.

TBSV and other tombusviruses are useful model viruses that infect a wide range of plants. The 4.8 kb TBSV genomic (g)RNA codes for two replication proteins, termed p33 and $p92^{pol}$, and three proteins involved in encapsidation, cell-to-cell movement and suppression of gene silencing (Nagy and Pogany, 2008; White and Nagy, 2004). Interestingly, yeast cells expressing p33 and $p92^{pol}$ replication proteins can efficiently replicate a short TBSV-derived replicon (rep)

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0042-6822/$ – see front matter © 2008 Published by Elsevier Inc.
doi:10.1016/j.virol.2008.11.041

RNA, termed defective interfering (DI) RNA (Panavas and Nagy, 2003; Panaviene et al., 2004). The tombusviral RNA plays several functions during infection, including serving as a template for replication and as an assembly platform for the viral replicase complex (Nagy and Pogany, 2008; Panaviene et al., 2005; Pogany et al., 2005). The viral or DI RNA also participate in RNA recombination (Serviene et al., 2005; White and Morris, 1994b; White and Nagy, 2004), which likely plays major role in virus evolution.

To identify host proteins that interact with viral RNA, we took a global approach based on the yeast proteome microarray (protein array) (Zhu et al., 2001, 2003). Previous studies using the yeast protein array have identified numerous yeast proteins involved in protein–protein interactions, lipid binding, DNA binding and small substrate binding, thus demonstrating the usefulness of the global analysis approach (Hall et al., 2004; Huang et al., 2004; Smith et al., 2005; Zhu et al., 2001, 2003). Also, the yeast protein array identified 58 yeast proteins interacting with the TBSV p33 and an additional 11 yeast proteins interacting with the readthrough portion of $p92^{pol}$ replication protein (Li et al., 2008). In addition, a yeast protein array approach was used to identify many host proteins interacting with a 3′ fragment of the BMV RNA (Zhu et al., 2007). Two of those host proteins were found to affect BMV infection in plants.

In the present work, we expanded the versatility of the yeast protein array by screening for host proteins that bind to viral RNA. Altogether, this work identified 57 host proteins that bound to either TBSV or to BMV RNA or both RNAs. Eleven of the identified host proteins that bound to the TBSV RNA included known helicases, translation factors, and RNA modifying enzymes. Host proteins binding to BMV RNA included tRNA binding proteins, and proteins that are part of large mRNP complexes. More detailed work with a TBSV RNA binding protein, namely eukaryotic translation elongation factor 1A (eEF1A), has revealed that eEF1A is a component of the purified replicase and binds to the 3′ end of the TBSV RNA as well as to TBSV p33 replication co-factor. An eEF1A mutant has provided evidence that eEF1A is important for TBSV replication by stabilizing the p33 replication protein.

Results

Yeast protein array-based identification of host proteins binding to TBSV and BMV RNAs

To probe the yeast protein array for RNA-binding proteins, we used biotinylated RNAs of two different RNA viruses, TBSV and BMV, which can replicate in yeast (Ishikawa et al., 1997; Panavas and Nagy, 2003). Because TBSV and BMV are only distantly related, they provided good substrates to identify virus-specific and nonspecific RNA-binding proteins. For example, the TBSV RNA is uncapped and

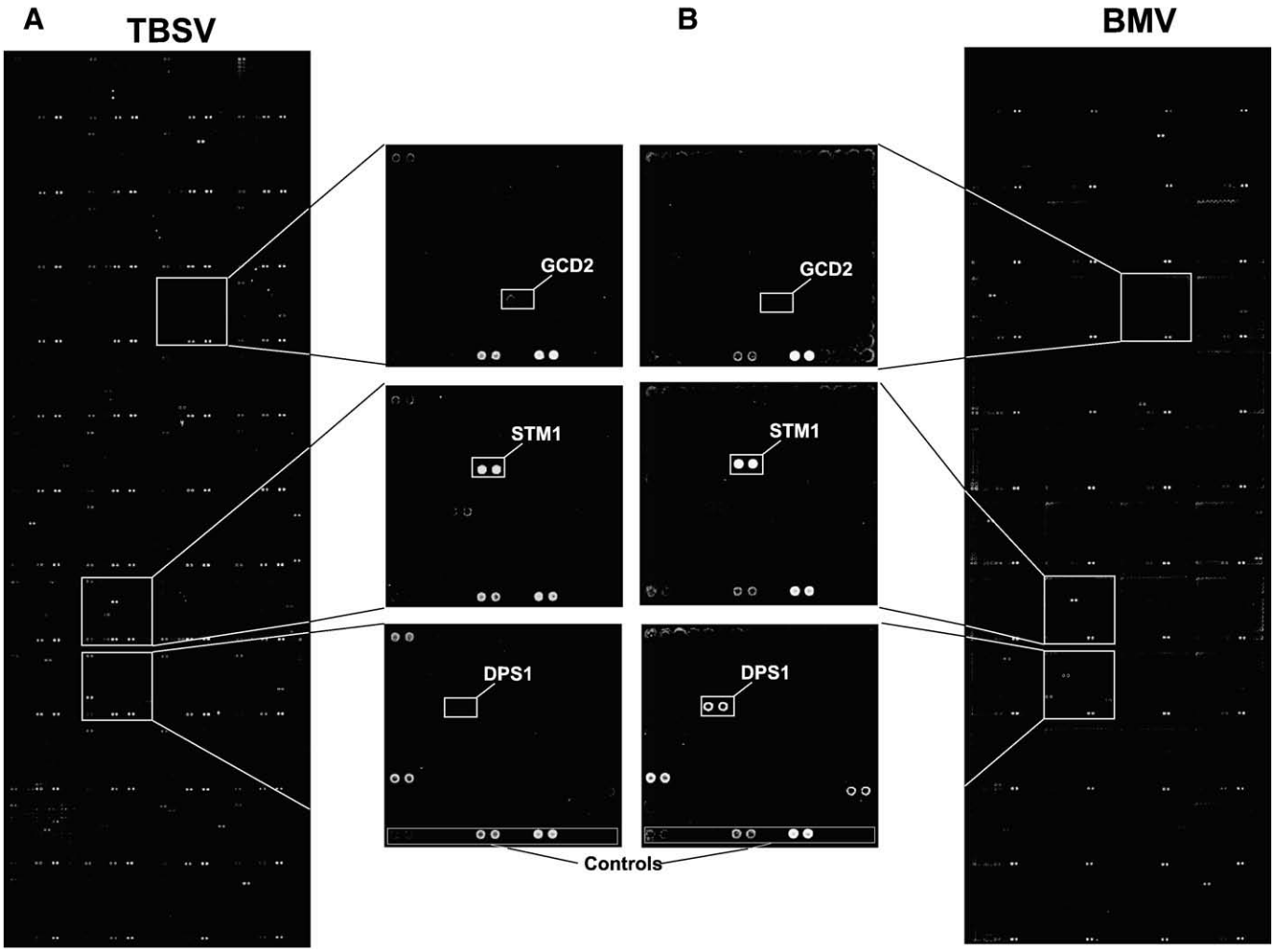

Fig. 1. Identification of viral RNA-binding proteins by the yeast proteome microarray. (A) Biotinylated TBSV gRNA (noncapped) or (B) BMV RNA1 (capped) probes were used as indicated. Three subarrays are shown at higher magnification to illustrate the binding of host proteins to TBSV (top), to BMV (bottom) or to both RNAs (middle). The array contains variable amounts of yeast proteins (supplied by Invitrogen) which were used to calculate the binding for each protein (see Table 1).