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A shift in plant proteome profile for a *B*romodomain containing *R*NA binding *P*rotein (BRP1) in plants infected with Cucumber mosaic virus and its satellite RNA

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ABSTRACT

Host proteins are the integral part of a successful infection caused by a given RNA virus pathogenic to plants. Therefore, identification of crucial host proteins playing an important role in establishing the infection process is likely to help in devising approaches to curbing disease spread. Cucumber mosaic virus (Q-CMV) and its satellite RNA (QsatRNA) are important pathogens of many economically important crop plants worldwide. In a previous study, we demonstrated the biological significance of a Bromodomain containing RNA-binding Protein (BRP1) in the infection cycle of QsatRNA, making BRP1 an important host protein to study. To further shed a light on the mechanistic role of BRP1 in the replication of Q-CMV and QsatRNA, we analyzed the *Nicotiana benthamiana* host protein interactomes either for BRP1 alone or in the presence of Q-CMV or QsatRNA. Co-immunoprecipitation, followed by LC–MS/MS analysis of BRP1-FLAG on challenging with Q-CMV or QsatRNA has led us to observe a shift in the host protein interactome of BRP1. We discuss the significance of these results in relation to Q-CMV and its QsatRNA infection cycle.

Biological significance: Host proteins play an important role in replication and infection of eukaryotic cells by a wide-range of RNA viruses pathogenic to humans, animals and plants. Since a given eukaryotic cell typically contains ~30,000 different proteins, recent advances made in proteomics and bioinformatics approaches allowed the identification of host proteins critical for viral replication and pathogenesis. Although Cucumber mosaic virus (CMV) and its satRNA are well characterized at molecular level, information concerning the network of host factors involved in their replication and pathogenesis is still on its infancy. We have recently observed that a Bromodomain containing host protein (BRP1) is obligatory to transport satRNA to the nucleus. Consequently, it is imperative to apply proteomics and bioinformatics approaches in deciphering how host interactome network regulates the replication of CMV and its satRNA. In this study, first we established the importance of BRP1 in CMV replication. Then, application of co-immunoprecipitation in conjunction with LC–MS/MS allowed the identification of a wide range of host proteins that are associated with the replication of CMV and its satRNA. Interestingly, a shift in the plant proteome was observed when plants infected with CMV were challenged with its satRNA.

1. Introduction

Several studies on the replication of positive sense RNA viruses have shown that virus replication is intricately dependent on various host proteins and actively reprogram host cell metabolism for their survival [1]. Consequently, host factors interacting with viral RNAs play a critical role in different aspects of virus life cycle. Hence, identifying host proteins that interact with viral RNAs will not only help us in visualizing the viral RNA-protein interactome but also provide a foundation for developing novel antiviral strategies.

In 1992, a novel class of bromodomains, isolated from *Drosophila melanogaster* brahma protein, was identified as a primary amino acid sequence present in some proteins that have chromatin or transcription function [2]. Many bromodomain-containing proteins (BRP) since then are found in transcription complexes, where they perform scaffolding functions [3]. The bromodomain is a structural domain of 110 amino acids that is conserved through yeast through mammals. With regard to the implication of bromodomain containing proteins in viral pathology, they have been found to play an important role in the transcription of HIV [4], Epstein–Barr virus [5] and in the inhibition of E2 protein that is involved in the replication of human papillomavirus [6] and in *Potato spindle tuber viroid* (PSTVd), a subviral pathogen of plants [7]. Bromodomain containing RNA binding protein-1 (BRP1), present in different tissues of healthy plants, was the first bromodomain containing host protein isolated from tomato plants [8]. Orthologs of BRP-1 have been found in various *Solanaceae* species (*Lycopersicon esculentum*,

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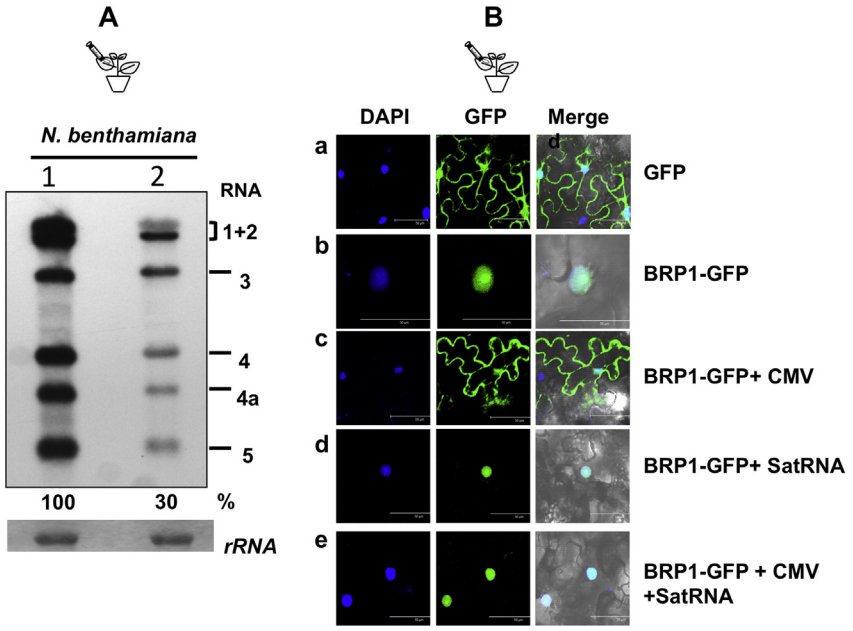

Fig. 1. Role of BRP1 in Q-CMV and its QsatRNA. (A) BRP1 is an essential host protein for Q-CMV replication. Northern blot analysis of total RNA recovered from (1) wild-type and (2) transgenic ph5.2nb (defective in BRP1) *N. benthamiana* plants following infiltration with agrotransformants of all three genomic RNAs of Q-CMV. The position of Q-CMV progeny is shown to the right: RNAs 1, 2 and 3 represent genomic RNAs while RNAs 4 and 4a are subgenomic; RNA5 is a mixture of 3′ 307 and 304 nucleotide regions of genomic RNAs 2 and 3 respectively and is generated independent of CMV replication [37]. Accumulation levels of Q-CMV progeny RNA shown were normalized against wild-type Q-CMV as 100%. rRNA represents loading control. (B) QsatRNA outcompetes Q-CMV for BRP1. *N. benthamiana* leaves were infiltrated with agrotransformants shown to the right. At 2 dpi, following DAPI staining to visualized the nucleus, leaves were subjected to confocal microscopy for green fluorescent signal detection. Bar = 50 μm.

Nicotiana tabaccum and *Nicotiana benthamiana*) [7] as well as in *Arabidopsis thaliana* [8]. Suppression of BRP-1 in *N. benthamiana* plants through RNA silencing failed to induce PSTVd infection, suggesting a role for BRP-1 in the PSTVd infection cycle [8].

Like PSTVd, satellite RNA (satRNA) is the smallest known infectious molecule that manipulates the cellular systems [9]. However, in contrast to self replicating PSTVd, satRNAs have been shown to be dependent on their helper viruses (HV) for replication [10–12]. Although satRNAs have no appreciable sequence homology with the HV genome, satRNAs not only utilize HV RNA-dependent RNA polymerase for their replication but also interfere with HV replication and thereby modify symptom expression on the host plants [13–16]. Recent application of molecular and cell-biology approaches showed that, when expressed with or without the HV, a satRNA of Q-strain of Cucumber mosaic virus (CMV) i.e. QsatRNA localized in the nucleus and was transcribed to generate multimers of genomic and anti-genomic strands [17,18].

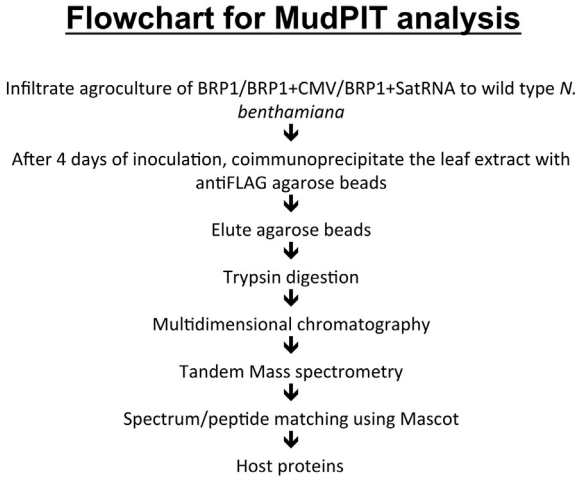

Fig. 2. Flow chart showing various steps involved in the MudPIT analysis.

Subsequent experiments showed that nuclear import of QsatRNA is mediated by BRP1, in a fashion similar to PSTVd [19]. However, we do not rule out the possible interaction of other host proteins with BRP1, QsatRNA and CMV during the regulation of the biological processes associated with the replication of CMV and its QsatRNA. In this study, we compare the host proteome profiles in plants challenged with CMV or its QsatRNA with an attempt to understand the mechanism of BRP1 regulated replication of CMV and its QsatRNA.

2. Materials and methods

2.1. Q-CMV strain, agroinfiltration and confocal microscopy

Throughout this study, we used Q strains of CMV(Q-CMV) and its QsatRNA (QsatRNA). The nature and characteristic features of *Agrobacterium*-based T-DNA constructs of the three genomic RNAs of Q-CMV and QQsatRNA are previously described [17,18]. BRP1 tagged with FLAG peptide (BRP1-FLAG) was kindly provided by Dr. Kriton Kalanditis [7]. Wild-type *N. benthamiana* leaves were infiltrated with agrocultures of GV 3101 strain containing the following inocula: BRP1-FLAG, or BRP1-FLAG + Q-CMV, or BRP1-FLAG + QsatRNA. For analyzing the shift in the localization of BRP1 in the presence of Q-CMV or QsatRNA, BRP1-GFP agrocultures (kindly obtained from Dr. Kalantidis) [7] were infiltrated to wild-type *N. benthamiana* leaves along with Q-CMV or QsatRNA or Q-CMV + QsatRNA, at 2 days post infiltration (dpi) leaves were stained with DAPI (5 μg/ml in PBS buffer), and were subjected to confocal microscopy. Each experiment was repeated at least three times.

2.2. Protein extraction, co-immunoprecipitation, and LC–MS/MS

After 4 dpi, agroinfiltrated leaves were ground in liquid nitrogen, and total protein was extracted in 3 volumes of extraction buffer (20 mM Tris–Cl [pH 7.5], 300 mM NaCl, 5 mM $MgCl_2$, 5 mM DTT, 1% plant protease inhibitor [Sigma, USA]). The liquid extract was centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatant was used