Identification of Tobacco Proteins Associated with the Stem-Loop 1 RNAs of *Potato virus X*

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***Potato virus X* (PVX) contains five viral proteins as well as *cis*-acting elements like stem-loop 1 (SL1) RNAs at the 5′ region. SL1 RNAs are involved in PVX RNA replication, encapsidation, translation, and cell-to-cell movement. In this study, we performed two-dimensional electrophoresis Northwestern blot analysis and matrix-assisted laser desorption ionization time of flight mass spectrometry and identified 24 tobacco proteins that interact with SL1 RNAs. Interestingly, one-third of the identified host proteins have been shown to interact with many plant viral proteins. In addition, we demonstrated that PVX capsid protein can bind to both SL1(+/-) RNAs. We further selected three *Nicotiana benthamiana* proteins including NbMPB2Cb, NbMBF1, and NbCPIP2a, to confirm results of Northwestern blot analysis. Electrophoretic mobility shift assay showed that NbMPB2Cb and NbMBF1 bind to both SL1(+/-) RNAs *in vitro*. In contrast, NbCPIP2a interacts only SL1(+) RNA. Taken together, we provide a list of host proteins interacting with PVX SL1 RNAs, which would be good candidates for the study of viral RNA-host protein interaction.**

INTRODUCTION

Potato virus X (PVX) is one of important plant RNA viruses in molecular plant pathology and causes very dramatic damage on crop yield. PVX is a flexuous rod-shaped virus and is a member of the genus *Potexvirus* in the family *Alphaflexiviridae* (Scholthof et al., 2011). Genome of PVX is a positive-sense single-stranded RNA and encodes a total of five viral proteins such as RNA-dependent RNA polymerase for viral RNA synthesis, triple gene block proteins for virus cell-to-cell movements, and capsid protein (CP) for viral assembly and cell-to-cell movement (Fig. 1A) (Miller et al., 1998). In addition, PVX specifically possesses *cis*-acting elements and structures at the 5′ end of its genome, which is defined as stem-loop 1 (SL1) RNAs (Fig. 1B). Previous studies demonstrated that the SL1 RNAs are involved in PVX translation, RNA replication, and initiation of PVX assembly (Kim and Hemenway, 1997; Kwon et al., 2005).

Until now, functional studies of plant host proteins are restricted to viral protein-host protein interaction rather than viral RNA-host protein. Numerous host proteins interacting with plant viral proteins have been identified. Little is known, however, for host proteins interacting with viral RNAs. A recent study has shown that PVX SL1 RNAs can efficiently bind to proteins extracted from tobacco protoplast as confirmed by a systematic evolution of ligands by exponential enrichment (SELEX) *in vitro* (Kwon and Kim, 2006).

In this study, we identified a total of 24 tobacco proteins which bind to PVX SL1 RNAs by Northwestern blot analysis and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). In addition, we further confirmed interaction of three selected proteins with SL1 RNAs by the electrophoretic mobility shift assay (EMSA).

MATERIALS AND METHODS

Preparation of SL1 RNA transcripts
The plasmid pSPVXp31 (agroinfiltration-based PVX replicon, Hemenway et al., 1990; Park and Kim, 2006) was used as a template to amplify SL1 RNAs. SL1 RNA probes containing (+) and (-) strand, respectively, were *in vitro* transcribed by T7 promoter. For the PCR, strand specific primers were used as described previously (Kang et al., 2010; Kim et al., 2002). Each PCR products were radioactively labeled in the presence of 50 μCi of α-^{32}P CTP (3,000 Ci/mmol) (PerkinElmer Life Science), 5 mM CTP, and 125 mM each of ATP, GTP, and UTP. Labeled RNAs were purified by NucAway Spin Columns (Ambion). Radioactivity of acquired RNAs was measured with liquid scintillation counter (LSC) and RNA probes with 20,000 counts per minute (cpm) were used for EMSA.

Preparation of plant cell extracts and *Escherichia coli* expressed PVX CP
S100 protein extracts were prepared from tobacco BY-2 suspension cell as described previously (Kim and Hemenway, 1997). PVX CP gene was cloned and expressed in *E. coli* using pET vector system (Novagen). The purification of His-tagged CP fusion protein was purified as described previously (Kwon et al., 2005). The extracted protein contents were determined with a Bradford analysis and used as positive control for north-

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western analysis.

One- and two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis

The protein pellets of S100 were solubilized in 250 μl of rehydration buffer containing 8 M urea, 2% CHAPS, 0.5% IPG 4-7 buffer (GE healthcare), 18 mM DTT, and bromophenol blue. Isoelectric focusing (IEF) was conducted at 20°C using IPG strips (pH 4-7, 13 cm, GE healthcare) and an Ettan IPGphor II system (GE healthcare). Denatured proteins (50 μg) were loaded into the IEF tray, and passive rehydration was carried out overnight under a layer of mineral oil. IEF was performed as follows: 5 h at 30 V, 1 h at 100 V, 1 h at 500 V, 3 h gradient to 8000 V, and 7 h at 8000 V at 10 μA/strip (56,000 Vh) at 20°C. Focused strips were equilibrated according to the manufacturer's instruction and then subjected to 10% tricine acrylamide gel electrophoresis; proteins were silver stained. To detect SL1-binding proteins, proteins were transferred to nitrocellulose membranes and subjected to Northwestern blot analysis.

Northwestern blot analysis

Northwestern blot analysis was performed as previously described (Bichsel et al., 1997; Gustafson et al., 1998; Schiff et al., 1988) with slight modifications. In brief, highly purified S100 proteins and recombinant PVX CP were separated on 12% polyacrylamide (1-DE) and 10% tricine polyacrylamide (2-DE) and transferred electrophoretically to nitrocellulose membranes. To denature proteins, the membranes were incubated with 6 M guanidine hydrochloride (GuHCl) in northwestern buffer containing 10 mM Tris-HCl (pH 6.8), 25 mM NaCl, 1 mM EDTA, 0.04% BSA, 0.04% Ficoll 400, and 0.02% polyvinyl pyrrolidone-40 for 30 min. The membrane was then washed with 6, 3, 1.5, 0.75, 0.375, and 0.187 M GuHCl in northwestern buffer. The membranes were blocked with 0.5% fat-free dry milk and 10 μg/μl yeast tRNA in northwestern buffer for 30 min at room temperature. The blots were incubated with ^{32}P-labeled transcripts at room temperature for 2 h and then washed three times for 1 min each with northwestern buffer to remove unbound or non-specifically bound RNA. After they were air-dried for 10 min, the membranes were exposed to a BAS imaging plate (IP) for autoradiography (Fuji film).

Trypsin digestion and sample preparation for peptides analysis

For in-membrane digestion, spots were excised from the membrane based on analysis of Multi Gauge V2.2 (Fuji film). Membrane slices (~2 mm^2) were incubated in digestion buffer containing 1% hydrogenated Triton X-100 (RTX), 10% acetonitrile, and 100 mM Tris-Cl (pH 8.0), for 30 min and hydrated in 5 μl of trypsin (0.5 μg/μl) (Sequencing grade, Promega) for 24 h at 37°C. Membrane pieces were sonicated for 5 min and then centrifuged. Supernatants of extracts were dried to ~5 μl with a Speed Vac evaporator (Savant).

MALDI-TOF MS and database search based on peptide mass fingerprint spectra

The peptides were eluted with 2 μl of matrix solution containing α-cyano-4-hydroxycinnamic acid (HCCA) in acetonitrile/0.1% TFA 1:1 and transferred onto the MALDI target plate using Zip Tips (Millipore). The extracts were processed for MALDI on the ZipPlate (Millipore) according to the manufacturer's protocol. The spectra were collected using the Voyager-DE STR Biospectrometry Workstation MALDI-TOF Mass Spectrometer (Applied Biosystems) at the National Instrumentation Center for Environmental Management (NICEM), Seoul National Univer-

sity. The mono-isotopic masses were subjected to search in the NCBI protein database with the Protein Prospector algorithm (http://prospector.ucsf.edu) MS-Fit. For MS-Fit searches, masses derived from trypsin and keratin were excluded. To increase the specificity of protein identification, we restricted searches to include only proteins derived from *Nicotiana tabacum*. The searches were performed with fixed default parameters: 0-100 kDa molecular mass and tryptic digest with a maximum number of one missed cleavage. The mass tolerance was set to 100 ppm.

Isolation of full-length ORF sequences for NbMPB2Cb, NbMBF1, and NbCPIP2a

To isolate the full-length cDNAs for *NbMPB2Cb*, *NbMBF1,* and *NbCPIP2a*, total RNAs were extracted from *N. benthamiana* plants, and cDNA was synthesized using an oligo (dT) 3′ primer. Known tobacco homologous genes were used to design following primers: MPB2Cb_F (5′-ATGGCGCTTGCATTT-3′) and MPB2Cb 3′ NTR_R (5′-ACAAACTATAGTAGTCAGTTTCCTA-3′) for NbMPB2Cb, MBF1 mRNA_F (5′-TCCCGAGAAGAAG AAGAA-3′) and MBF1 mRNA_R (5′-ACTGGAACGGAGATT TGAGA-3′) for NbMBF1, and CPIP2a_F (5′-ATGGGACTTG ATTACTATAA-3′) and CPIP2a_R (5′-TTAGTCAGCGCTCCT GCACA-3′) for NbCPIP2a.

EMSA

To construct a maltose binding protein (MBP)-fusion protein for EMSA, full-length fragments of individual gene were cloned into the pMAL-c2X vector (New England Biolabs Inc.) using *Eco*RI enzyme. EMSA was performed as described previously (Cho and Kim, 2012; Peal et al., 2011; Sriskanda et al., 1996).

RESULTS

Identification of 24 host proteins that bind to SL1 RNAs

We speculated that the 3′ end of genomic minus-strand RNA might have a similar RNA secondary structure. For that, we predicted the structure of SL1(-) (nt 6405-6299) *in silico* using the mfold program version 3.1 (http://mfold.rna.albany.edu/) (Fig. 1B). The predicted SL1(-) structure is quite similar to that of SL1(+). Both SL1 structures are composed of four stems, four loops, and one tetraloop. However, the stem in SL1(-), like stems C(-) and D(-), is slightly longer than that of SL1(+), and the loops of SL1(-), like loops C(-) and D(-), are relatively unstable.

To isolate host proteins that specifically bind to SL1(+) and SL1(-) of PVX RNA, we performed 1-DE Northwestern blot analysis. As a positive control, we used PVX CP, which was previously demonstrated to bind to SL1(+) RNA *in vitro* (Kwon et al., 2005). Coomassie Brilliant Blue staining of the gel revealed numerous bands (Fig. 2A). Several bands were detected after hybridizing with the ^{32}P-labeled SL1(+) and SL1(-) transcripts (Figs. 2B and 2C). Interestingly, 1-DE Northwestern blot analysis revealed that PVX CP binds not only to SL1(+) RNA transcripts but also to SL1(-) RNA transcripts. Although the same amounts of ^{32}P-labeled RNA transcripts were used for hybridization, the total signal intensity was weaker for SL1(+) than SL1(-).

We further fractionated the 1-DE by 2-DE analysis to separate such host proteins in high resolution. The silver-stained gel revealed many well-separated protein spots (Fig. 2D). Two-DE Northwestern blot analyses were conducted three times using ^{32}P-labeled SL1(+) and SL1(-) RNA transcripts and similar results were obtained (data not shown). The molecular mass of the separated proteins ranged from 6.5 to 55 kDa, and most of
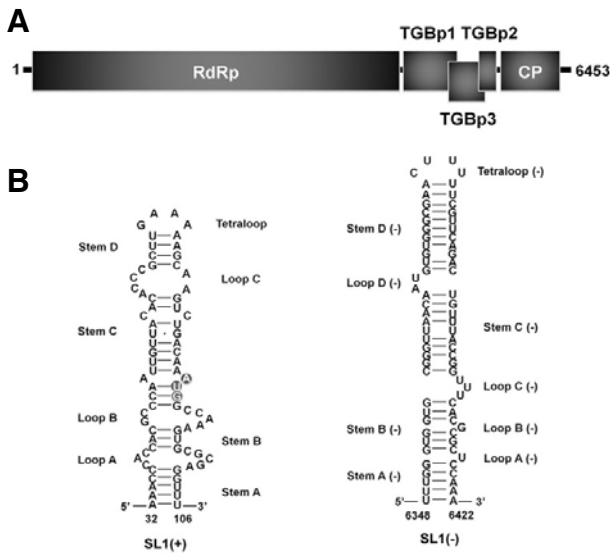
Fig. 1. Genome organization and secondary structures of PVX. (A) Schematic representation of PVX RNA genome. Single-strand PVX RNA contains five open reading frames (ORFs): RNA-dependent RNA polymerase (RdRp), triple gene block proteins (TGBp1, TGBp2, and TGBp3), and CP. (B) Secondary structure of the positive strand of SL1 RNA as determined by chemical and enzymatic probing (SL1+). The initiation codon of RdRp is indicated by grey circles. Secondary structure of the negative strand of SL1 RNA (SL1-).

them were rather acidic (Figs. 3A and 3B). The patterns of detected signals between SL1(+) and SL1(-) were different. However, some signals were common to both SL1 RNAs. Major spots were excised and were analyzed as described in the experimental procedures.

With the criteria that at least four peptides had to match and that sequence coverage had to be greater than 15%, we identi-

fied a total of 24 proteins that interacted with SL1(+) (12 proteins) and SL1(-) (17 proteins) RNAs (Figs. 3A and 3B; Table 1); five of these 24 bind to both SL1 RNAs. Moreover, we detected PVX CP used as a positive control indicating that our 2-DE Northwestern blot analysis was reliable. The molecular mass of the 24 proteins ranged from 15 to 53 kDa, and their isoelectric points (p*I*) were between 5 and 10 (Table 1). To obtain information about the function of the identified proteins, we searched orthologous *Arabidopsis thaliana* proteins. Blast search found several orthologous proteins with low E-values, indicating high conservancy of the 24 proteins in *N. tabacum* and *Arabidopsis*. According to their known functions and conserved domains in tobacco and *Arabidopsis*, the identified proteins can be divided into four functional groups: transcriptional factors, heat shock proteins, signaling proteins, and others including cytoskeletal protein and rRNA binding protein. Remarkably, many isolated host proteins in our study are homologous to other host proteins interacting with various viral proteins. They included *Tobacco mosaic virus* (TMV)-movement protein 30 (MP30) binding protein 2C (MPB2C) (Kragler et al., 2003), putative multiprotein bridging factor 1 (MBF1) (Matsushita et al., 2002), potyviral CP interacting protein 2a and 2b (CPIP2s), *Tobamovirus* multiplication 3 (TOM) (Yamanaka et al., 2000), *Cucumber mosaic virus* (CMV) 1a interacting protein 1 (Kim et al., 2008), and DnaJ-like protein (Shimizu et al., 2009). We further investigated the interaction of three *N. benthamiana* proteins including MPB2C, MBF1, and CPIP2a by EMSA.

Identification of NbMPB2Cb, NbMBF1, and NbCPIP2a homologous proteins from *Nicotiana benthamiana*

To isolate full-length cDNAs for *MPB2C*, *MBF1*, and *CPIP2a* homologous genes from *N. benthamiana*, RT-PCRs were performed using gene-specific primers based on known tobacco homologous gene sequence information. The isolated genes were referred as *NbMPB2Cb*, *NbMBF1*, and *NbCPIP2a*, respectively. Each host gene was cloned into a modified pMAL-c2X plasmid for expression as fusions to the C-terminus of non-secreted MBP. MBP-NbMPB2Cb, MBP-NbMBF1, and MBP-
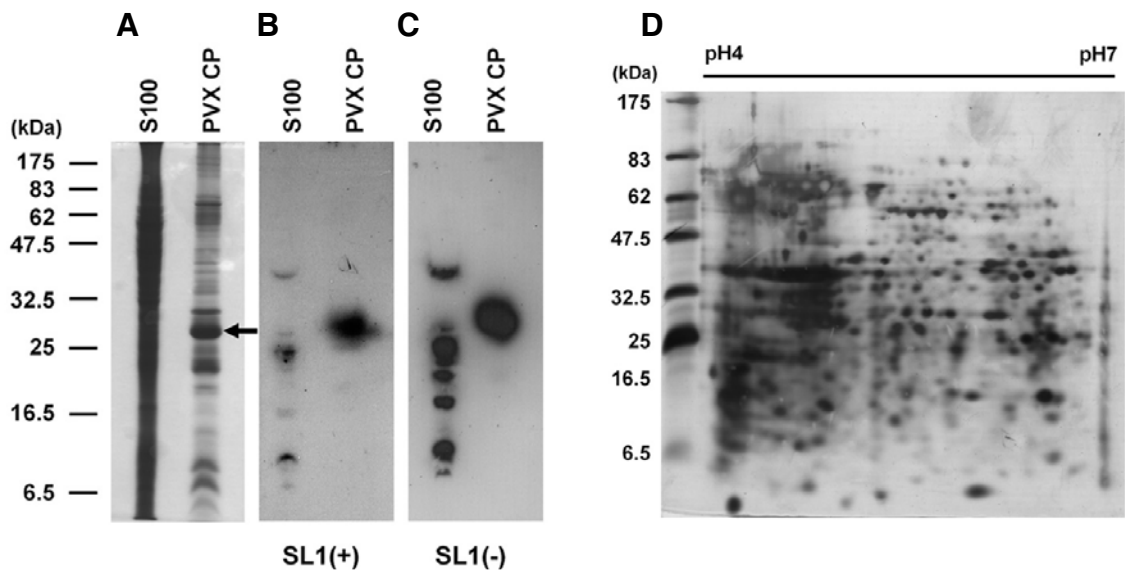

Fig. 2. Northwestern blot analysis to confirm interaction of PVX CP with SL1 RNAs. (A) One-DE analysis of extracted S100 and recombinant PVX CP. Proteins were stained with Coomassie Brilliant Blue after electrophoresis. The arrow indicates PVX CP recombinant protein (about 30 kDa). One-DE Northwestern blot analysis using ³²P-labeled SL1(+) RNA transcripts (B) and ³²P-labeled SL1(-) RNA transcripts (C). (D) Two-DE analysis of extracted S100 proteins visualized by silver staining.

Table 1. Host proteins that bind to PVX SL1 RNAs as determined by proteomics

Spot #	Accession #	Protein name	MW Da/p*I*	SL1 strand	No. of peptide	Seq. Cov. %	MOWSE score	Accession # of Arabidopsis	E-value	Function
1	20086364	Putative multiprotein bridging factor 1	15327/10.0	(-)	6	30.7	1856	AT3G58680	7.00E-45	TF
2	1841462	Elongation factor 2	19875/5.3	(-)	5	38	193	AT1G56070	7.00E-97	SP
3	90654964	DnaJ heat shock protein	20774/9.3	(-)	6	31.6	788	AT1G77930	4.00E-63	HSP
4	5932420	LIM domain protein WLIM2	20812/5.6	(+)(-)	10	35	2389	AT3G55770	2.00E-75	TF
5	5932413	LIM domain protein WLIM1	21521/9.1	(+)(-)	6	30.6	745	AT1G10200	8.00E-83	TF
6	3256375	Heat shock protein 26 (Type I)	22574/5.2	(+)(-)	5	33.3	381	AT4G27670	2.00E-37	HSP
7	15778156	14-3-3 protein	22981/5.6	(+)(-)	7	29.4	1149	AT4G09000	2.00E-90	SP
8	417063	Floral homeotic protein GLOBOSA	24691/8.9	(+)	5	29.7	231	AT5G20240	5.00E-51	TF
9	6552359	Myb-related transcription factor LBM1	31985/5.1	(-)	6	30.2	1141	AT3G23250	6.00E-71	TF
10	1732247	Transcription factor Myb1	32005/5.5	(-)	4	22.3	258	AT3G23250	9.00E-68	TF
11	5821136	Heat shock factor	32106/6.0	(+)	4	15.1	84.9	AT4G36990	2.00E-65	HSP
12	34811738	Potyviral CP interacting protein 2a	33872/9.3	(+)	5	23	592	AT1G10350	1.00E-101	HSP
13	34811740	Potyviral CP interacting protein 2b	33930/9.3	(+)	5	16.4	94	AT1G59725	1.00E-104	HSP
14	74038607	Tobamovirus multiplication 3	34023/9.8	(-)	4	28.9	315	AT2G02180	1.00E-114	TF
15	4760596	DNA-binding protein NtWRKY3	35940/9.6	(+)	6	15.5	3101	AT4G31550	3.00E-75	TF
16	19716176	TMV-MP30 binding protein 2C	35998/5.0	(+)(-)	5	21.2	102	AT5G08120	7.00E-34	CSP
17	57014014	Ribosomal protein L2	36644/10.9	(+)	11	28.1	756	ATMG00560	1.00E-129	RBP
18	6179940	DnaJ-like protein	37117/9.1	(-)	4	27.5	188	AT2G20560	1.00E-105	HSP
19	39598908	CMV 1a interacting protein 1	41538/6.4	(+)	7	27.9	5836	AT1G23360	2.00E-05	SP
20	78096654	Mitogen-activated protein kinase	42884/6.1	(-)	6	24.3	167	AT4G01370	0	SP
21	18143321	Wound induced protein kinase	42898/5.2	(-)	7	28	795	AT3G45640	0	SP
22	51458330	Trehalose-phosphate phosphatase	42945/7.6	(-)	10	31.4	2612	AT5G51460	1.00E-150	SP
23	11133532	Shaggy-related protein kinase NtK-1	46309/8.6	(-)	7	18.3	377	AT5G26751	0	SP
24	1617200	NSK6; Shaggy-like kinase6	53434/7.9	(-)	10	20.8	6019	AT4G00720	0	SP
25	70994254	PVX CP	25105/7.7	(+)(-)	5	16.5	269			

[1]Table headings: Spot #, protein spot number from Fig. 3; Accession #, accession number of *N. tabacum* proteins from NCBI; MW Da/p*I*, theoretical Daltons/isoelectric point; No. of peptide, number of peptides matched to the identified protein sequences; Seq. Cov. %, the percent coverage for the protein based upon the matched peptide sequences; MOWSE score, peptide identification score; E-value, expectation value between candidate proteins of *N. tabacum* and Arabidopsis

[2]TF, transcriptional factor; HSP, heat shock protein; SP, signaling protein; CSP, cytoskeletal protein; RBP, rRNA binding protein
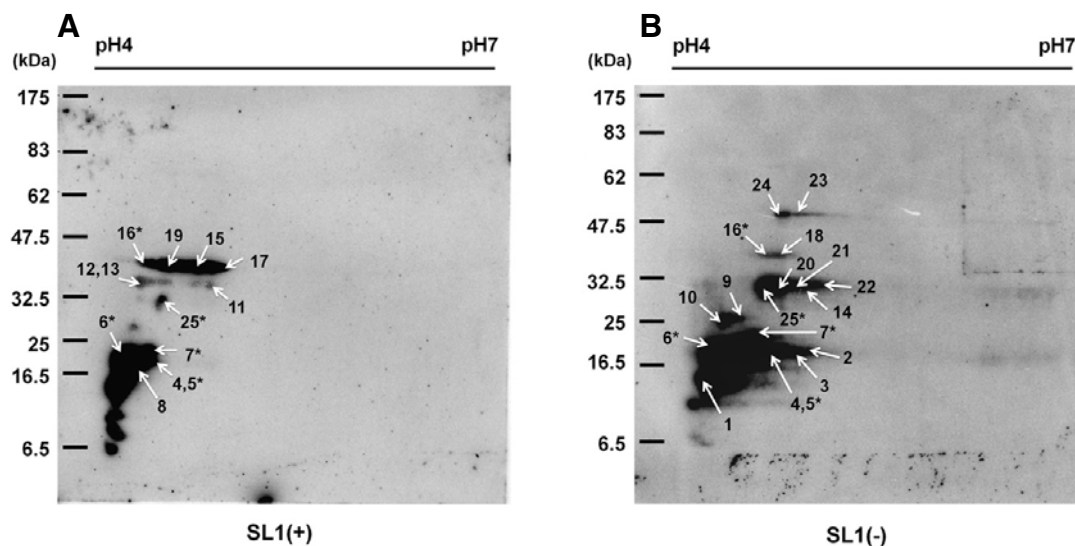

Fig. 3. Northwestern blot analysis to identify tobacco host proteins that interact with PVX SL1 RNAs. Two-DE Northwestern blot analysis using ^{32}P-labeled SL1(+) RNA transcripts (A) and ^{32}P-labeled SL1(-) RNA transcripts (B). Arrows indicate the excised spots for MALDI TOF MS analysis; the spot number adjacent to each arrow indicates each of the 24 identified proteins (see Table 1). The five locations where host proteins were commonly found by both RNA probes are indicated by asterisks.
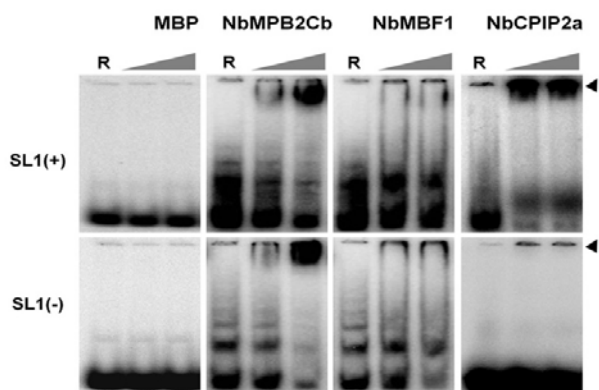
Fig. 4. EMSA to confirm the interactions of three *N. benthamiana* proteins and SL1 RNAs. In each image, only ^{32}P-labeled RNAs were loaded in the first lane indicated by 'R' whereas 0.5 μg and 2 μg of each purified protein were loaded in lanes 2 and 3, respectively. The top arrow indicates RNA-protein complexes while the bottom arrow indicates labeled SL1 RNAs.

NbCPIP2a productions of the expected sizes were detected by SDS-PAGE (data not shown). To confirm the result of the 2-DE Northwestern blot analysis, EMSA was performed using ^{32}P-labeled SL1 RNA and individual purified fusion protein. EMSA with only MBP used as a negative control showed that MBP does not interact with any SL1 RNAs. EMSA displayed reduced mobility of the NbMPB2Cb-RNA complex in both SL1(+/-) RNAs (Fig. 4 and Table 1). These data are consistent with results of Northwestern blot analysis. In case of NbMBF1, the Northwestern blot analysis showed that NbMBF1 can specifically interact with SL1(-) RNA, however, the EMSA results displayed weak interaction of NbMBF1 with both SL1 RNAs. NbCPIP2a can only bind to SL1(+) RNA, which is confirmed by both EMSA and Northwestern blot analysis.

DISCUSSION

In this study, we tried to identify host proteins interacting with PVX SL1 RNAs by proteomic approach since only a limited number of host proteins have been shown to interact with viral RNAs (Fujisaki and Ishikawa, 2008; Nicholson et al., 2010). Although we identified 24 host proteins by selecting only the best-matched protein from individual spots on the gels, it follows that the real number of host proteins interacting with viral RNAs is probably more than 24. Blotting results showed strong hybridization signals in many 2-DE areas. This seems to be caused by many proteins or protein complexes, which have similar p*I* and molecular mass. Of the 24 identified proteins, 14 out of 24 proteins have basic p*I* values ranging from 7.6 to 10.9. In addition to different molecular masses, proteins present in multiple spots migrated at different isoelectric points. Some of these differences may occur because of changes in primary sequences as a consequence of alternative splicing or the result of post-translational modifications, such as phosphorylation, sulfation, and acetylation.

Interestingly, the functions of most host proteins identified in our study have been previously characterized. Moreover, one-third of the identified host proteins have been shown to interact with many plant viral proteins. For instance, MBF1-like protein binds to *Tomato mosaic virus* (ToMV) MP and this interaction could modulate host gene expression to suppress the general defense response in plants (Matsushita et al., 2002). MPB2C

and NtMPIP1 are involved in TMV cell-to-cell spread by interacting with TMV MP (Curin et al., 2007; Kragler et al., 2003). NtCPIP2a and NtCPIP2b act as potyviral susceptibility factors during *Potato virus Y* infection and along with heat shock protein 70, they act as plant chaperones and are essential for virus movement (Hofius et al., 2007). TOM1 and TOM3 proteins in *Arabidopsis* and *N. tabacum* are associated with the multiplication of TMV and tobamoviruses (Asano et al., 2005; Yamanaka et al., 2000). Based on those results, we suspect that various host proteins can bind to viral RNAs and/or proteins of multiple plant viruses. Although our data only revealed interactions between PVX viral RNA and host proteins, the host proteins identified in our study might be good candidates for the study of viral protein-host protein interactions with other viruses and hosts.

Similarly, many host proteins interacting with the 5′ end of the plus-strand of RNA or the 3′ end of the minus-strand were identified for *Rubella virus* and *Mouse hepatitis virus* (Furuya and Lai, 1993; Nakhasi et al., 1990). Our Northwestern blot analyses revealed that the list of host proteins interacting with each SL1 RNA is different from each other, although five proteins bind to both SL1 RNAs. These data support that viral RNA-host protein interactions are highly variable and depend on sequences and/or structures. It would also be of interest to survey functional differences between SL1(+) and SL1(-) interacting proteins in near future.

To confirm results of Northwestern blot analysis, we performed EMSA confirming interaction of NbMPB2Cb and NbCPIP2a with SL1 RNAs. However, we failed to get consistent results for NbMBF1, which showed weak interaction with both SL1 RNAs by EMSA. NbMBF1 is a member of transcription factor family and its protein abundance might be relatively lower to other proteins. Therefore, it will be difficult to detect it by the proteomic analysis. In contrast, we can use high amount of MBP-NbMBF1 fusion protein produced from *E. coli*. Thus, we suppose that results of EMSA could be more precise.

Previous studies have demonstrated that the sequences of upper region and C:C mismatch in SL structures of PVX and *Bamboo mosaic virus* (BaMV) play important roles in accumulation of genomic RNA (Chen et al., 2010; Miller et al., 1998). It has been shown that SL1(+) RNA binds to PVX CP and several tobacco proteins including the *N. tabacum* WRKY1 transcription factor (NtWRKY1) (Kim et al., 2002; Part and Kim, 2011). Silencing of NtWRKY1 in *N. benthamiana* caused plants to exhibit lethal apical necrosis by increasing PVX accumulation, suggesting that NtWRKY1 functions to regulate multiple defense response genes (Park and Kim, 2012). Recently, we identified NbDnaJ which interacts with SL1(-) RNA and showed that it plays a role in the early stages of viral infection by acting as a negative regulator of PVX replication and movement (Cho et al., 2011). In this regard it is worth noting that the 5′ or 3′ end of RNA behaves as *cis*-acting elements and can affect cell-to-cell movement of PVX (Lough et al., 2006). These results suggest that these interactions are necessary for PVX replication and assembly. Altogether, it is quite sure that the identified host proteins will play roles in PVX replication, assembly, and cell-to-cell movement. However, it will be our next studies to elucidate the identity and function of the host proteins associated with PVX infection cycles.

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