Plant Pathology Bulletin 11:79-86, 2002

Interactions between Nuclear Inclusion Protein a (NIa) and Nuclear Inclusion Protein b (NIb) of *Zucchini yellow mosaic virus* and *Papaya ringspot virus*

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Accepted for publication: May 29, 2002.

ABSTRACT

Lee, K. C [1]., Lin, S. S [2]., Yeh, S. D [2]., and Wong, S. M [1,3]., 2002. Interactions between Nuclear Inclusion Protein a (NIa) and Nuclear Inclusion Protein b (NIb) of *Zucchini yellow mosaic virus* and *Papaya ringspot virus*. Plant Pathol. Bull. 11:79-86.

An inducible yeast two-hybrid system was used to test direct protein-protein interactions between the nuclear inclusion protein a (NIa) and nuclear inclusion protein b (NIb) protein of *Zucchini yellow mosaic virus* (ZYMV-S) and *Papaya ringspot virus* (PRSV-HA). Strong homologous and heterologous interactions of the NIb proteins were observed. The NIa and NIb proteins of ZYMV-S and PRSV-HA could interact with each other. This suggests that RNA replication domains of potyviruses are highly conserved and are not virus species-specific. For ZYMV-S, the NIb protein interacted with both the VPg and proteinase domain of the NIa protein. Up to eight-fold increase was displayed by NIb-protease as compared to that of NIb-VPg interactions, showing that NIb exhibits stronger interaction with the proteinase domain of the NIa protein. Our results indicate that NIb interacts with both VPg and proteinase to different levels. This is the first demonstration of NIa-NIb interaction between two different potyviruses.

Key words: NIa, VPg, proteinase, NIb, potyvirus, phylogenetic, two-hybrid

INTRODUCTION

The nuclear inclusion protein a (NIa) of potyviruses has a two-domain structure where the N-terminal domain is the VPg [30] and the C-terminal half has proteolytic activity [9]. The proteinase is involved in processing the C-terminal two-thirds of the potyvirus polyprotein [3,4,13]. NIb is believed to be the RNA-dependent RNA polymerase (RdRp) of potyviruses [8,9]. NIb is released by *cis*-cleavage from the C-terminal polyprotein by the NIa proteinase [19]. Together with cytoplasmic inclusion (CI) protein and VPg protein of NIa, the NIb forms a multicomponent, membrane-associated replication complex to direct the synthesis of viral RNA during replication [31].

Pairwise interactions between different viral-encoded proteins belonging to the same potyvirus have been studied using the yeast two-hybrid system [15,16,18,22]. Interactions of the *Tobacco vein mottling virus* (TVMV)-encoded proteins show that NIb interacts with both NIa and coat protein in yeast cells [18]. This interaction is diminished by mutations in the VPg domain of NIa. *In vitro* experiments show that TVMV VPg is retained on glutathione-Sepharose matrices when co-incubated with a glutathione S-transferase (GST)-NIb fusion protein, but not with GST alone [10], indicating that the VPg domain of NIa interacts with NIb. A specific interaction was also detected between NIa and NIb proteins encoded by *Tobacco etch virus* (TEV). However, NIb of TEV could only interact with the proteinase domain of NIa, but not with the VPg domain [22]. This suggests a difference between NIa-NIb interaction among potyviruses.

In this study, we describe interactions between NIa and NIb of two phylogenetically related members of the family *Potyviridae*, the Singapore isolate of *Zucchini yellow mosaic virus* (ZYMV-S) [20,36] and the HA-isolate of *Papaya ringspot virus* (PRSV-HA) [14]. ZYMV-S was isolated from cucumber in 1989. PRSV-HA is a severe strain infecting papaya and was first reported in Hawaii. Using the yeast two-hybrid system, we investigated the interactions of the VPg and proteinase domains of NIa to NIb of ZYMV-S and compared it with previous reports [18,22].

MATERIALS AND METHODS

Yeast strains and construction of plasmids

All yeast strains and plasmids for two-hybrid experiments (MATCHMAKER two-hybrid system) were purchased from Clontech Laboratories, Inc. *Saccharomyces cerevisiae* EGY48 (*MAT* α, *his3, trp1, ura3, LexA*$_{op(x6)}$*-LEU2*) was transformed with the p8op-lacZ reporter plasmid by the lithium acetate method [2] in order to utilize both the *lacZ* and *LEU2* reporter genes for assay of protein-protein interactions. The transformants (EGY48[p8op-lacZ]) were selected on minimal or synthetic dropout (SD) medium lacking uracil. The NIa and NIb genes of PRSV-HA (S46722) and ZYMV-S (AF014811) were PCR-amplified and cloned into two vectors: pLexA, which contains a LexA DNA-binding domain and pB42AD, which contains a GAL1 transcription activation domain. Similarly, the VPg and proteinase domain of the NIa gene of ZYMV-S was cloned into the *Eco*R I site of both the two-hybrid vectors. All constructs derived from PCR were verified for their correct orientation and presence of initiation and termination codons by automated sequencing (373A Applied Biosystems, Foster City, CA).

Verification of hybrid constructs

The DNA-binding and activation domain constructs were transformed separately into strain EGY48[p8op-lacZ]. Transformants were selected on SD media lacking histidine/uracil and tryptophan/uracil, respectively. X-gal (5-bromo-4-chloro-3-indolyl- β-D-galactopyranoside) was included in the media (20 mg/ml) to provide an *in vivo* whole-plate β-galactosidase assay.

Testing for protein interactions

EGY48[p8op-lacZ] was co-transformed with the DNA-binding and activation domain constructs using the small-scale yeast transformation method as mentioned above. The co-transformants were plated on SD medium lacking histidine/tryptophan/uracil to select for colonies containing both hybrid plasmids and the reporter plasmid. For positive control, cells were transformed with fusions of murine p53/DNA-binding domain and SV40 large T-antigen/activation domain. For negative control, an unrelated LexA/human lamin C fusion protein was used as the DNA-binding construct. The negative control fusion combinations are shown in Fig. 1, Plate A.

To test for activation of both reporter genes (*lacZ* and *LEU2*), 50 colonies from each pairwise interaction transformants were replica-plated on SD plates lacking histidine/leucine/tryptophan/uracil but containing 20 mg/ml X-gal and BU salts (0.026 M $Na_2HPO_4 \cdot 7H_2O$, 0.025 M NaH_2PO_4). Galactose (2%) and raffinose (1%) were added into the medium to induce expression of the activation domain fusion proteins. The plates were incubated at 30℃. Blue colonies were monitored at 12-h intervals up to 96 h. The results were compared with the positive and negative controls performed in parallel.

Quantifying strength of interactions

To reduce the variability in liquid β-galactosidase assays, 5 His^+ Trp^+ Ura^+ colonies from each plate were inoculated into SD liquid medium lacking histidine/tryptophan/uracil and incubated overnight at 30℃. The overnight cultures were diluted 5-fold into SD liquid medium lacking histidine/leucine/tryptophan/uracil but containing galactose (2%) and raffinose (1%) and grown to an OD_{600} of 0.5-0.8. Cell permeabilization was performed by repeated freeze/thaw cycles using liquid nitrogen to permit accurate quantification of β-galactosidase activity [28]. The assay was carried out in triplicate using o-nitrophenyl β-D-galactopyranoside (ONPG; Sigma #N-1127) as substrate and activity was calculated using Miller units [24] as follows:

$$\frac{1{,}000 \times OD_{420}}{\text{time (min)} \times \text{volume (ml)} \times OD_{600}}$$

Yeast mating

Yeast colonies bearing only the activation domain plasmids were isolated from the positive transformants by natural segregation. This was performed by culturing individual His^+ Leu^+ Trp^+ Ura^+ $LacZ^+$ transformants in 3 ml of SD medium lacking tryptophan and uracil but containing histidine and leucine for 2 days. A diluted sample of this liquid culture was plated on SD medium lacking tryptophan and uracil and incubated at 30℃ for 3 days. Thirty individual colonies from each plate were transferred to SD media lacking trytophan/uracil and histidine/trytophan/uracil. Only colonies that grew on SD medium containing histidine (histidine auxotrophs) were selected for replica plating on induction medium lacking tryptophan/uracil but containing X-gal and BU salts for β-galactosidase activity assay. Colonies that did not exhibit β-galactosidase activity were selected for yeast mating tests.

The yeast mating strain YM4271 (*MAT* α, *ura3-52*, *his3-200*, *lys2-801*, *ade2-101*, *ade5*, *trp1-901*, *leu2-3*, *112*, *tyr1-501*, *gal4-*$\triangle$*512*, *gal80-*$\triangle$*538*, *ade5::hisG*) was transformed separately with pLexA (DNA-binding vector only), DNA-binding constructs containing the NIa and NIb genes of PRSV-HA and ZYMV-S; and pLexA-Lam which represents control plasmid expressing a fusion of LexA and human lamin C. The transformants were selected on SD medium lacking histidine. The YM4271 transformants were mixed with the selected histidine auxotrophs colonies containing activation domain fusion constructs and incubated in YPD at 30℃ with shaking at 250 rpm for 18 h. An aliquot of the mating culture was plated on SD medium lacking

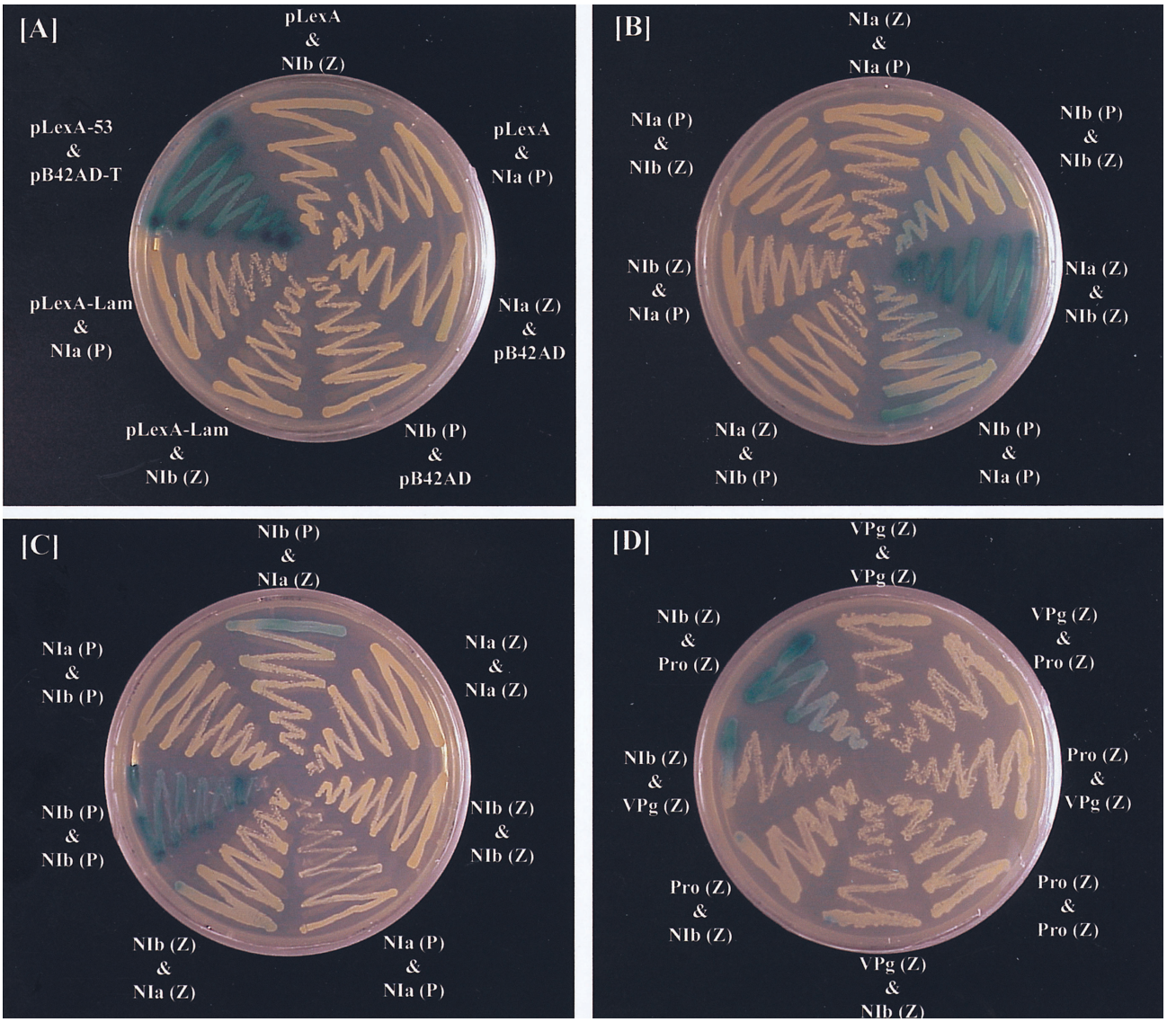

Fig. 1. *In vivo* whole plate beta-galactosidase assay of NIa and NIb proteins belonging to ZYMV-S and PRSV-HA. Colonies were streaked on SD plates lacking histidine, tryptophan and uracil. VPg and Pro indicates genome-linked protein and protease domains of NIa, respectively. (P) and (Z) indicates the HA isolate of PRSV and Singapore isolate of ZYMV, respectively. Plate [A] contained the positive and negative interaction controls. Plates [B], [C] and [D] represent the various two-hybrid fusion combinations.

histidine/tryptophan/uracil. The plates were incubated at 30℃ for 5 days to allow diploid cells to form visible colonies. To assay for both *lacZ* and *LEU2* expression, the transformants were replica-plated to induction medium lacking histidine/leucine/tryptophan/uracil but containing 20 mg/ml X-gal and BU salts.

RESULTS

NIa and NIb interactions between ZYMV-S and PRSV-HA

Yeast transformation of either the DNA-binding domain or activation domain constructs alone resulted in negative white colonies (results not shown). As the minimal media contained X-gal and BU salts, the absence of β-galactosidase activity indicated no autonomous activation of either types of hybrid constructs. This verified that the hybrid contructs alone did not activate the *lacZ* and *LEU2* reporter genes. Control experiments performed for the two-hybrid assay were compared with the test transformants (Fig. 1, Plate A).

The presence of β-galactosidase activity in yeast transformants was an indicator of interactions between the fusion protein constructs [11]. *In vivo* β-galactosidase assay showed that there was a strong interaction between the NIa and NIb proteins belonging to PRSV-HA and ZYMV-S (Fig. 1, Plates B, C). This supports previous observations made between NIa and NIb of TVMV and TEV using the yeast

two-hybrid system [18,22]. Moderately strong β-galactosidase activities were observed for homologous and heterologous interactions of NIb proteins of both PRSV-HA and ZYMV-S.

Quantitative β-galactosidase activity assay showed that interaction between NIa and NIb was stronger in ZYMV-S as compared to PRSV-HA (Table 1, expt. no. 9, 10, 19, 20). This could be due to lower expression of both the NIa and NIb hybrid proteins in the yeast nucleus for PRSV-HA. It was demonstrated that the NIa and NIb proteins of PRSV-HA and ZYMV-S were able to interact with each other. The strength of interaction varied from 6.7 to 235.1 Miller units (Table 1, expt. no. 11, 13, 14). This interaction was most apparent when the NIb protein of PRSV-HA and NIa protein of ZYMV-S were fused to the DNA-binding and activation domain, respectively. However, such an interaction was not observed in the combination of NIb (ZYMV-S) in DNA-binding domain and NIa (PRSV-HA) in activation domain. Failure to detect such interaction in yeast cells could be due to unfavourable folding of the proteins in the yeast nucleus or incorrect post-translational modification [33].

In the quantitative assay, the NIa and NIb proteins of both PRSV-HA and ZYMV-S exhibited homologous and heterologous interactions. A similar observation was also reported in the interaction of NIa and NIb proteins of TVMV [18]. With the exception of ZYMV-S NIb homologous interaction, NIb-NIb interactions were stronger as compared to NIa-NIa homologous and heterologous interactions of the two viruses in this study (Table 1, expt. no. 7, 8, 15, 17, 18). Although the values of NIa homotypic and heterotypic interactions were relatively low between 4.2-7.5 Miller units (Table 1, expt. no. 7, 15, 17), they were within the range of 3-187 Miller units observed for true interacting partners using the same yeast system [5]. Such low values may be attributed to improper folding or poor expression of the NIa and NIb hybrid proteins and thus influence the strength of the interactions. Another explanation is that the polarity of the two interacting fusion proteins may affect the protein conformation and thus the level of interactions.

Interactions of NIa domains with NIb in ZYMV-S and yeast mating assay

Quantitative assay was performed to determine which of the NIa domains of ZYMV-S interacts with NIb of ZYMV-S. Our results indicated that NIb interacted with both VPg and proteinase domains of NIa, albeit at different strengths of interaction (Table 2, expt. no. 5, 6, 7, 8). Proteinase domain NIb interactions were more than 8-fold greater as compared to VPg-NIb interactions, when NIb and NIa proteinase domain were fused to the DNA-binding and activation domains, respectively. A lack of interaction was observed between VPg and proteinase domains of NIa (Table 2, expt. no. 1, 2, 3, 4). This may indicate that in order for the weak homotypic NIa interaction to occur, the full-length NIa protein is required.

False positives were eliminated by using yeast mating to introduce two different plasmids into the same host cells. The mating assay showed that the activation domain fusion construct clones (Histidine auxotrophs) exhibited reporter gene expression. This occurred only when the activation domain fusion plasmids belonging to the Histidine auxotrophs

Table 1. Interactions of NIa and NIb of ZYMV-S and PRSV-HA in yeast two-hybrid system

Expt. no.	DNA-binding constructs	Activation domain constructs	β-galactosidase activity (Miller units)
1	pLexA-53 [1]	pB42AD-T	477.7 ±80.9
2	pLexA $^-$	NIb (ZYMV-S)	1.6 ±0.1
3	pLexA $^-$	NIa (PRSV-HA)	1.5 ±0.7
4	NIa (ZYMV-S) $^-$	pB42AD	1.4 ±0.9
5	pLexA-Lam $^-$	NIb (ZYMV-S)	1.1 ±0.3
6	pLexA-Lam $^-$	NIa (PRSV-HA)	1.2 ±0.4
7	NIa (ZYMV-S)	NIa (PRSV-HA)	6.2 ±0.4
8	NIb (PRSV-HA)	NIb (ZYMV-S)	327.8 ±51.4
9	NIa (ZYMV-S)	NIb (ZYMV-S)	332.8 ±50.7
10	NIb (PRSV-HA)	NIa (PRSV-HA)	262.7 ±51.3
11	NIa (ZYMV-S)	NIb (PRSV-HA)	12.0 ±1.1
12	NIb (ZYMV-S)	NIa (PRSV-HA)	1.0 ±0.1
13	NIa (PRSV-HA)	NIb (ZYMV-S)	6.7 ±0.6
14	NIb (PRSV-HA)	NIa (ZYMV-S)	235.1 ±26.6
15	NIa (ZYMV-S)	NIa (ZYMV-S)	7.5 ±0.5
16	NIb (ZYMV-S)	NIb (ZYMV-S)	1.2 ±0.2
17	NIa (PRSV-HA)	NIa (PRSV-HA)	4.2 ±1.4
18	NIb (PRSV-HA)	NIb (PRSV-HA)	267.7 ±41.7
19	NIb (ZYMV-S)	NIa (ZYMV-S)	67.6 ±10.4
20	NIa (PRSV-HA)	NIb (PRSV-HA)	6.0 ±0.9

[1.] Positive control; $^-$Negative control

Table 2. Interactions of NIa domains with NIb in ZYMV-S

Expt. no.	DNA-binding constructs	Activation domain constructs	β-galactosidase activity (Miller units)
1	VPg	VPg	2.9 + 1.7
2	VPg	Pro	4.4 + 2.0
3	Pro	VPg	3.1 + 1.0
4	Pro	Pro	3.1 + 1.0
5	VPg	NIb	17.2 + 1.6
6	Pro	NIb	46.7 + 3.2
7	NIb	VPg	17.3 + 6.0
8	NIb	Pro	142.2 + 27.9

were introduced by mating with the plasmids encoding the DNA-binding fusion construct. Expression of the *lacZ* reporter gene of the mating products on induction plates yielded the same results (Fig. 1, Plates B, C, D).

DISCUSSION

The two-hybrid assay has been used to map the interaction domains between proteins such as the helper component-proteinase of *Potato A virus* [15] and *Potato Y virus* [32]. Host plant proteins that interact with potyviral protein products were also identified using this technique. A positive interaction is found between the RdRp of ZYMV and poly-(A) binding protein (PABP) of cucumber, thus implying the role of host PABP in potyviral infection process [34]. In another study, an interaction between the VPg of TuMV and the translation eukaryotic initiation factor iso 4E (eIF4E) of *Arabidopsis thaliana* was also reported [35] and correlated with viral infection *in planta* [21]. The interaction of eIF4E with VPg of TEV was also demonstrated and shown to be strain-specific [27].

The DNA-binding target protein could activate reporter gene expression without an activation domain construct if it has a transcriptional activation domain. However, other proteins that are not normally involved in transcription are sometimes capable of activating transcription [23]. As it is not known if both NIa and NIb display such properties, autonomous activation was checked before using the DNA-binding constructs to test for interactions with the activation domain constructs. Furthermore, the NIa and NIb genes were also reciprocated in the activation domain vector.

In the yeast two-hybrid system, true-positive colonies exhibit reporter gene expression only when they contain the DNA-binding and a corresponding interactive activation domain. False-positives are Leu^+ or $lacZ^+$ cotransformants (or diploid colonies) that carry plasmids that do not encode hybrid proteins that interact directly. Such colonies may arise due to nonspecific interaction with the DNA-binding domain hybrid or from interaction with the DNA or DNA-bound proteins at a particular promoter [2]. Although the use of two different reporter genes (*lacZ* and *LEU2*) under the control of different promoters automatically eliminates many false-positive activation domain fusion constructs, putative true-positive clones should be tested further to determine that they activate reporter genes only in the presence of the DNA-binding fusion construct. Yeast mating is a convenient method of introducing two different plasmids into the same host cells to eliminate false positives [12,17]. Our results showed that His^+, Leu^+, Trp^+, Ura^+, $LacZ^+$ transformants obtained were true-positive clones, thus confirming the validity of the interactions observed for the two potyvirus gene products.

Some discrepancies were observed in the results between *in vivo* and quantitative β-galactosidase activity assay. For example, quantitative assay indicates that interaction between NIb proteins of PRSV-HA and ZYMV-S (Table 1, expt. no. 8) was stronger as compared to interaction between the NIb and NIa proteins of PRSV-HA (Table 1, expt. no. 10). However, this was not reflected in the *in vivo* β-galactosidase assay (Fig. 1, Plate B). It is known that although whole plate *in vivo* assay is convenient for screening large-scale experiments, it represents the least sensitive of all types of β-galactosidase assay [37]. This may be attributed to uneven distribution of X-gal and thus localized variations in X-gal concentration on the plate. On the other hand, liquid cultures are assayed for β-galactosidase to verify and quantify two-hybrid interactions. Because of their quantitative nature, liquid assays using ONPG can be used to compare the relative strength of the protein-protein interactions observed in the transformants.

Both homologous and heterologous interactions of NIa and NIb of PRSV-HA and ZYMV-S were detected. Cells infected with some members of potyviruses show NIa and NIb accumulation in nuclear inclusions [9]. The interactions suggest that NIa and NIb may form multidimers in order to function. The possibility of having more than one NIb molecule recruited to the replication complex for enhanced genome replication by acting as a cooperative RNA-binding factor should be considered [25].

Two different observations are made with regards to the binding domain of NIa to NIb protein. Hong *et al*. have shown that the NIb protein of TVMV interacts with the VPg domain of the NIa in yeast cells [18]. This is subsequently supported by *in vitro* studies [10]. In contrast, Li *et al*. have documented an interaction that involved the proteinase domain of the NIa of TEV with the NIb protein, with the isolated VPg domain being unable to elicit a positive interaction in yeast cells [22]. This interaction is further supported with temperature-sensitive mutants that affect the proteinase domain [7]. Very recently, studies on interaction map for both PVA and PSbMV imply that the NIa-NIb interaction is mediated by a dimer formed by VPg and full-length NIa in which the NIa-proteinase domain is in contact with NIb [16].

Our results, however, showed that the NIb protein of ZYMV-S interacted with both the VPg and proteinase domains of the ZYMV-S NIa. A higher level of interaction

was observed for ZYMV-S NIb-proteinase domain as compared to ZYMV-S NIb-VPg domain. Previous interaction studies of PVA has also shown elevation of reporter gene activity occurred upon co-expression of NIa-proteinase and NIb, in contrast to co-expression of VPg and NIb [16]. Our data support the hypothesis that free NIb polymerase is recruited to membrane-bound initiation sites through interaction with the proteinase domain of the 6K-NIa polyprotein [6,22,26]. The higher affinity of the NIb to the proteinase may allow the RdRp region of NIb to come into closer contact, and subsequently interact with the VPg domain to stimulate polymerase activity [7].

At present, it is not known how the NIa-NIb complex is associated with the viral RNA. RNA-protein interactions are critical in diverse cellular processes, such as translation, early development, and infection by RNA viruses. It will be of interest to employ a system that can detect and analyze RNA-protein interactions *in vivo* so that various possible combinations of RNA and viral proteins can be analyzed. A three-hybrid system [29] which involves a hybrid RNA molecule bridges two fusion proteins, one containing a DNA-binding domain and the other containing a transcriptional activation domain may be used for such studies. The hybrid RNA will contain recognition sites for two RNA-binding domains. The interaction of this RNA with the two hybrid proteins will result in transcription of a reporter gene such as *lacZ*. Such a system has been employed to study binding of the *Human immunodeficiency virus* type 1 (HIV-1) Gag protein to the HIV-1 RNA encapsidation signal [1].

In summary, we have demonstrated that the ZYMV-S RNA polymerase (NIb) interacts with both the VPg and proteinase domain of ZYMV-S NIa in yeast cells. The NIa and NIb proteins of PRSV-HA and ZYMV-S could interact with each other. Strong homologous and heterologous interactions of the NIb proteins of PRSV-HA and ZYMV-S were also observed. These results indicate that interaction between proteins involved in replication of two different members of potyviruses can occur *in vivo*.

LITERATURE CITED

1. Bacharach, E., and Goff, S. P. 1998. Binding of the human immunodeficiency virus type 1 Gag protein to the viral RNA encapsidation signal in the yeast three-hybrid system. J. Virol. 72:6944-6949.
2. Bartel, P. L., Chien, C. T., Sternglanz, R. and Fields, S. 1993. Using the two-hybrid system to detect protein-protein interactions. Pages 153-179 in: Cellular Interactions in Development: A Practical Approach. D.A. Hartley ed. Oxford University Press, Oxford, England.
3. Carrington, J. C., and Dougherty, W. G. 1987. Small nuclear inclusion protein encoded by a plant potyvirus genome is a protease. J. Virol. 61:2540-2548.
4. Carrington, J. C., Cary, S. M., and Dougherty, W. G. 1988. Mutational analysis of tobacco etch virus polyprotein processing: cis and trans proteolytic activities of polyproteins containing the 49-kilodalton proteinase. J. Virol. 62:2313-2320.
5. Chen, C. M., Kraut, N., Groudine, M., and Weintraub, H. 1996. I-mf, a novel myogenic repressor, interacts with members of the MyoD family. Cell 86:731-741.
6. Daros, J. A., and Carrington, J. C. 1997. RNA-binding activity of NIa proteinase of tobacco etch potyvirus. Virology 237:327-336.
7. Daros, J. A., Schaad, M. C., and Carrington, J. C. 1999. Functional analysis of the interaction between VPg-proteinase (NIa) and RNA polymerase (NIb) of tobacco etch potyvirus, using conditional and suppressor mutants. J. Virol. 73:8732-8740.
8. Domier, L. L, Shaw, J. G., and Rhoades, E. 1987. Potyviral proteins share amino acid sequence homology with picorna-, como-, and caulimoviral proteins. Virology 158:20-27.
9. Dougherty, W. G., and Carrington, J. C. 1988. Expression and function of potyviral gene products. Annu. Rev. Phytopathol. 26:123-143.
10. Fellers, J., Wan, J., Hong, Y., Collins, G. B., and Hunt, A. G. 1998. In vitro interactions between a potyvirus-encoded, genome-linked protein and RNA-dependent RNA polymerase. J. Gen. Virol. 79:2043-2049.
11. Fields, S., and Song, O. 1989. A novel genetic system to detect protein-protein interactions. Nature 340:245-246.
12. Finley, Jr., R. L., and Brent, R. 1994. Interaction mating reveals binary and ternary connections between Drosophila cell cycle regulators. Proc. Natl. Acad. Sci. USA 91:12980-12984.
13. Garcia, J. A., Riechmann, J. L., and Lain, S. 1989. Proteolytic activity of the plum pox potyvirus NIa-like protein in Escherichia coli. Virology 170:362-369.
14. Gonsalves, D., and Ishii, M. 1980. Purification and serology of papaya ringspot virus. Phytopathology 70:1028-1032.
15. Guo, D., Merits, A., and Saarma, M. 1999. Self-association and mapping of interaction domains of helper component-proteinase of potato A potyvirus. J. Gen. Virol. 80:1127-1131.
16. Guo, D., Rajamaki, M. L., Saarma, M., and Valkonen, J. P. T. 2001. Towards a protein interaction map of potyviruses: protein interaction matrixes of two potyviruses based on the yeast two-hybrid system. J. Gen. Virol. 82:935-939.
17. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K. and Elledge, S. J. 1993. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 75:805-816.
18. Hong, Y., Levay, K., Murphy, J. F., Klein, P. G., Shaw, J. G., and Hunt, A. G. 1995. A potyvirus polymerase interacts with the viral coat protein and VPg in yeast cells.

Virology 214:159-166.
19. Joseph, J., and Savithri, H. S. 2000. Mutational analysis of the NIa protease from pepper vein banding potyvirus. Arch. Virol. 145:2493-2502.
20. Lee, K. C., and Wong, S. M. 1998. Variability of P1 protein of zucchini yellow mosaic virus for strain differentiation and phylogenetic analysis with other potyviruses. DNA Seq. 9:275-293.
21. Leonard, S., Plante, D., Wittmann, S., Daigneault, N., Fortin, M.G., and Laliberte, J.F. 2000. Complex formation between potyvirus VPg and translation eukaryotic initiation factor 4E correlates with virus infectivity. J. Virol. 74:7730-7737.
22. Li, X. H., Valdez, P., Olvera, R.E., and Carrington, J. C. 1997. Functions of the tobacco etch virus RNA polymerase (NIb): subcellular transport and protein-protein interaction with VPg/Proteinase (NIa). J. Virol. 71:1598-1607.
23. Ma, J., and Ptashne, M. 1987. A new class of yeast transcriptional activators. Cell 51:113-119.
24. Miller, J.H. 1972. "Experiments in Molecular Genetics." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
25. Pata, J. D., Schultz, S. C., and Kirkegaard, K. 1995. Functional oligomerization of poliovirus RNA-dependent RNA polymerase. RNA 1:466-477.
26. Schaad, M. C., Jensen, P., and Carrington, J. C. 1997. Formation of plant RNA virus replication complexes on membranes: role of an endoplasmic reticulum-targeted viral protein. EMBO J. 16:4049-4059.
27. Schaad, M. C., Anderberg, R. J., and Carrington, J. C. 2000. Strain-specific interaction of the tobacco etch virus NIa protein with the translation initiation factor eIfF4E in the yeast two-hybrid system. Virology 273:300-306.
28. Schneider, S., Buchert, M., and Hovens, C. M. 1996. An *in vitro* assay of β -galactosidase from yeast. BioTechniques 20:960-962.
29. SenGupta, D. J., Zhang, B., Kraemer, B., Pochart, P., Fields, S., and Wickens, M. 1996. A three-hybrid system to detect RNA-protein interactions in vivo. Proc. Natl. Acad. Sci. USA 93:8496-8501.
30. Shahabuddin, M., Shaw, J. G., and Rhoads, R. E. 1988. Mapping of the tobacco vein mottling virus VPg cistron. Virology 163:635-637.
31. Shukla, D. D., Ward, C.W., and Brunt, A. A. 1994. "The Potyviridae." CAB International, Wallington, UK.
32. Urcuqui-Inchima, S., Walter, J., Drugeon, G., German-Retana, S., Haenni, A.-L., Candresse, T., Bernardi, F., and Gall, O. L. 1999. Potyvirus helper component-proteinase self-interaction in the yeast two-hybrid system and delineation of the interaction domain involved. Virology 258:95-99.
33. van Aelst, L., Barr, M., Marcus, S., Polverino, A., and Wigler, M. 1993. Complex formation between RAS and RAF and other protein kinases. Proc. Natl. Acad. Sci. USA 90:6213-6217.
34. Wang, X., Ullah, Z., and Grumet, R. 2000. Interaction between zucchini yellow mosaic potyvirus RNA-dependent RNA polymerase and host poly-(A) binding protein. Virology 275:433-443.
35. Wittmann, S., Chatel, H., Fortin, M. G., and Laliberte, J. F. 1997. Interaction of the viral protein genome linked of turnip mosaic potyvirus with the translational eukaryotic initiation factor (iso) 4E of Arabidopsis thaliana using the yeast two-hybrid system. Virology 234:84-92.
36. Wong, S. M., Lee, S. C., and Loi, J. S. 1992. First report of zucchini yellow mosaic virus in Singapore. Plant Dis. 76:972.
37. Yeast Protocols Handbook. Clontech Laboratories, Inc. Palo Alto, California, USA.

摘 要

李建中[1]、林詩舜[2]、葉錫東[2]、王錫民[1,3] 矮南瓜黃化嵌紋病毒和木瓜輪點病毒之核內含體蛋白a 及b 間之交互反應. 植物病理學會刊 11:79-86. ([1.]國立新加坡大學生物科學系；[2.]國立中興大學植物病理學系；[3.] 連絡作者：)

本研究以酵母菌雙雜合系統來檢測矮南瓜黃化嵌紋病毒新加坡株系 (ZYMV-S) 和木瓜輪點病毒夏威夷株系 (PRSV-HA) 兩種病毒的核內含體蛋白 a (NIa) 及 核內含體蛋白b (NIb) 間的交互反應，結果顯示不論同一病毒或此二病毒間的NIa 及NIb 蛋白間均有強烈的交互反應，亦即是ZYMV-S 的 NIa 及NIb 蛋白 與PRSV-HA 的NIa 及 NIb 蛋白彼此間均有反應。這結果顯示馬鈴薯Y 群病毒RNA 的複製相關區域具有高度保留之共同性，不因病毒不同而有所差異。對ZYMV-S 而言，其NIb 蛋白和NIa 蛋白內的VPg 及 蛋白酵素二區域均有反應，其中對蛋白酵素區域的反應強度可達與 VPg 區域反應強度的八倍之多，顯示 NIb 蛋白和 NIa 蛋白的 VPg 區域或蛋白酵素區域有不同程度的相互反應。本研究之結果首次顯示不同馬鈴薯Y 群病毒的兩種病毒間的NIa 蛋白和NIb 蛋白有交互反應。

關鍵詞：核內含體蛋白a、核內含體蛋白b、蛋白酵素、遺傳多樣性、酵母菌雜合系統