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Detection and immunological analysis of a 112K protein of papaya ringspot virus produced *in vivo*

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Abstract. Using antiserum to the 51K amorphous inclusion protein (AIP) of papaya ringspot virus (PRV) as probes, a protein of 112K was detected in PRV-infected plants of *Cucumis metuliferus*. This 112K protein was purified by a procedure similar to that for AIP purification, with steps of extraction, differential centrifugation, and gel electrophoresis. Polyclonal and monoclonal antibodies (McAbs) against the purified 112K protein were produced and used to analyze the precursor-product relationship between 51K, 64K, and 112K proteins of PRV produced *in vivo*. Immunoblotting analyses on proteins from PRV-infected *C. metuliferus* showed that McAbs 112-51 reacted with both the 112K protein and the 51K AIP, whereas McAbs 112 reacted with proteins of 112K, 70K, 64K and 47K, but not with AIP. The results indicated that the 51K and the 64K proteins detectable by McAbs contained common epitopes as in the 112K protein, an evidence for the two proteins being processed from the precursor 112K protein produced *in vivo*.

Key words: Monoclonal antibody; Papaya ringspot virus; Potyviral gene products; Proteolytic processing.

Introduction

Papaya ringspot virus (PRV) is a member of the plant potyvirus group, with flexous particles of 780 × 12 nm. PRV particles contain a single-stranded RNA of positive polarity (De La Rosa and Lastra, 1983; Purcifull *et al.*, 1984) and a single type of capsid protein of 36,000 daltons (36K) (Purcifull and Hiebert, 1979; Gonsalves and Ishii, 1980). PRV induces both cylindrical (pinwheel) (Purcifull and Edwardson, 1967; Zettler *et al.*, 1968) and amorphous inclusions (Martelli and Russo, 1976; Christie and Edwardson, 1977) in the cytoplasm of infected host cells. The former consists of a virus-specific protein of 70K (Yeh and Gonsalves, 1984) and the latter a virus-specific protein of 51K (De Mejia *et al.*, 1985a). The capsid protein (CP), the cylindrical inclusion protein (CIP), and the amorphous inclusion protein (AIP) account for approximately half of the maximum coding capacity of the viral genome in one reading frame (Yeh and Gonsalves, 1985).

In vitro translation of PRV RNA in rabbit reticulocyte lysate system revealed that gene expression of the virus involves proteolytic processing. A polyprotein of 330K which reflects the maximal coding capacity of the viral genome was found to undergo proteolytic processing with the formation of such final products as CP, CIP, and AIP. Several intermediate polypeptides were also noticed (Yeh and Gonsalves, 1985). The elucidation of the complete sequences of other potyviruses confirmed that their genomes contain only one open reading frame and that viral proteins were generated by proteolytic processing (Domier *et al.*, 1986; Allison *et al.*, 1986).

When *in vitro* translation products of PRV RNA were immunoprecipitated with antiserum to PRV AIP, a 112K protein was the major precipitate and 51K, 65K, and 86K proteins were minor precipitates (Yeh and Gonsalves, 1985). The 112K protein was the major translation product in the rabbit reticulocyte lysate

system and was considered as an intermediate product processed from the 330K polyprotein (Yeh and Gonsalves, 1985). This coupled with the results from *in vitro* translation in the wheat germ system indicated that the 112K protein is generated from the 5' terminus of the PRV RNA and is the precursor for the production of a 64K protein and the 51K AIP (De Mejia *et al.*, 1985b; Quiot-Douine *et al.*, 1986; Yeh and Bih, 1989). Moreover, because the 64K protein was a major translation product produced in wheat germ system but not related to AIP, it was assigned as the first protein translated from the 5' terminus of PRV RNA (Quiot-Douine *et al.*, 1986; Yeh and Bih, 1989).

In this study, *in vivo* production of a 112K protein in PRV-infected *Cucumis metuliferus* (Naud.) Mey. was monitored by PRV AIP antiserum. A purification procedure for the 112K protein of PRV including steps of extraction, differential centrifugation, and gel electrophoresis was established. The McAbs against the purified 112K protein were produced by the hybridoma technique and used to analyze the precursor-product relationship of 51K, 64K, and 112K proteins of PRV produced *in vivo*.

Materials and Methods

Preparation of PRV AIP Antiserum

PRV HA, a typical strain of PRV type P originated from Hawaii (Gonsalves and Ishii, 1980), was propagated in *Cucumis metuliferus* (Yeh *et al.*, 1984). Fresh leaves of infected plants were harvested 10-14 days after inoculation. The 51K AIP was purified according to Yeh and Gonsalves (1985) by differential centrifugation and electrophoresis. The protein eluted from polyacrylamide gel was resuspended in 0.04 M Tris-acetate containing 0.002 M EDTA (pH 8.4) and used to immunize New Zealand white rabbits as described previously (Yeh and Gonsalves, 1985).

Detection of 112K Viral Protein Produced in vivo

Seedlings of *C. metuliferus* at 2-3 leaf stage were inoculated with PRV HA. The inoculated and the upper leaves (all leaves above the inoculated ones) were harvested at daily intervals through 12 days after inoculation. The leaf tissue was ground in extraction buffer (EB, 0.5% Na_2SO_3, 0.1 M Tris-HCl, pH 7.5) and filtered through 4 layers cheese cloth. Aliquots of 0.2 ml of the extract were treated with two volumes of degrading buffer (52.5 mM Tris-HCl, pH 6.8; 2% SDS; 5% 2-mercaptoethanol; 10% glycerol; 0.005% bromophenol blue) and incubated in boiling water for 3 min. Total proteins of different samples were electrophoresed in discontinuous minislab gels after dissociation (Laemmli, 1970). Separated proteins were then transblotted to nitrocellulose (NC) paper by the method of Gooderham (1984) using a Bio-Rad transblotting cell. Procedures of washing and blocking of the transblotted NC paper described in Bio-Rad instruction manual were followed. PRV AIP antiserum was used at a dilution of 1:2,000 and horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG at 1:2,000. The protein profiles reacting to PRV AIP antiserum were visualized by addition of the HRP color development reagent (1 volume of 3 mg/ml 4-chloro-1-naphthol in cold methanol mixed with 5 volume of 0.015% H_2O_2 in Tris-buffered saline, pH 7.5) according to Bio-Rad instruction.

The production of the 112K protein *in vivo* was also examined under different temperature conditions. The seedlings of *C. metuliferus* were incubated in the growth chamber starting from the fourth day after inoculation, with a daily light period of 12 h (5,000 lux) and humidity of 70-80%. Temperature was set at 17, 23, 29, 35, or 41°C. The leaves of the whole plants were harvested 8 days after inoculation and treated as described above.

Purification of 112K Protein Produced in vivo

For the purification of the 112K protein produced *in vivo*, the method of Yeh and Gonsalves (1985) for purifying AIP was followed with slight modification. PRV-infected tissues of *C. metuliferus* were harvested 8-10 days after inoculation and blended in two volumes of cold EB. The extracts were filtered through four layers of cheesecloth and the filtrates were centrifuged through a 30 ml 20% sucrose cushion (6,000 rpm, 5 min in Beckman JA-14 rotor). Pellets were resuspended in cold EB containing 5% Triton X-100 and stirred at 4°C for 1 h. The suspension was then centrifuged through a 15 ml 40% sucrose cushion at 7,000 rpm for 5 min (Beckman JA 14 rotor). Pellets were resuspended in EB, and the Triton X-100 treatment and centrifugation through 40% sucrose cushion centrifugation were repeated once. The pellets were then resuspended in the degrading buffer and subjected to SDS polyacrylamide gel electrophoresis (PAGE) (Yeh and Gonsalves, 1985). The 112K protein was visualized by cold KCl staining

(Hager and Burgess, 1980), eluted and concentrated by ISCO electrophoretic concentrator in 0.04 M Tris-acetate buffer containing 0.002 M EDTA (pH 8.4). Yield of the protein was estimated by spectrophotometry (assuming 1 mg/ml of $A_{280}=1.0$) and the purified protein was stored at -20°C.

Production of Polyclonal and Monoclonal Antibodies Against the 112K Protein

The production of monoclonal antibody against the 112K protein was performed according to the method of Kohler and Milstein (1975). Six to eight weeks BALB/c mice were first immunized intraperitoneally with 50 μg purified 112K protein in 500 μl PBS, emulsified with complete Freund's adjuvant. The second injection was conducted 7 days later with the same amount of antigen but emulsified with incomplete Freund's adjuvant. The boost injection was performed intravenously 10-14 days after the second injection with 30 μg antigen. Three days after the boost injection, antiserum with polyclonal antibodies was collected from eyepits of the mice. In addition, the spleen cells from the immunized animals were dissociated and allowed to fuse with SP2/0-Ag 14 myeloma cells (kindly provided by Dr. T. J. Chang, National Chung Hsing University) by PEG treatment (PEG-1500, from Boehringer Mannheim). Hybridoma cells were selected in HAT (hypoxanthine, aminopterin, thymidine) medium. Antibody-secreting cells were selected by indirect enzyme immunosorbent assay (ELISA) (Yeh and Gonsalves, 1984), using purified 112K protein or 51K AIP (4 μg/ml) as the coating antigen. Horseradish peroxidase (HRP) conjugate of goat anti-mouse IgG+IgM (H&L) (Jackson company) was used at a 1:2,000 dilution. ABST [2, 2'-Azino-bis (3-ethylbenzthiazoline sulfonic acid), Sigma] was used as enzyme substrate, and the absorbance was recorded by a Dynatech MR 700 reader at 405 nm.

Analysis of in vivo Viral Proteins

Polyclonal and monoclonal antibodies to the 112K protein were used to monitor the production of the 112K protein *in vivo* by immunoblotting. Five grams of PRV-infected leaf tissue of *C. metuliferus* 8 days after inoculation was collected, crushed in liquid nitrogen, and extracted with two volumes of EB. The extracts were filtered through cheese cloth, and centrifuged at 5,000 rpm (Beckman JA-20 rotor) for 10 min. Aliquots of 0.2 ml of the supernatant were mixed with two volumes of the degrading buffer, and incubated in boiling water for 3 min. The samples were electrophoresed in a discontinuous gel and transblotted to NC paper as described (Laemmli, 1970; Gooderham, 1984). Polyclonal antibody to AIP from rabbit, polyclonal antibody to the 112K protein from mouse, and monoclonal antibodies from culture fluid (McAbs 112-6, 112-9, 112-51-29, and 112-51-30) were used to detect the corresponding antigens. Goat anti-rabbit IgG-HRP conjugate (Bio-Rad) and goat anti-mouse IgG+IgM (H&L) HRP conjugate (Jackson company) were used as secondary antibody. The reactions were visualized by the addition of HRP color developing reagent as described above.

Results

Polyclonal Antibody to AIP

AIP was purified by steps of differential centrifugation, Triton X-100 treatment, and electrophoresis. The yields of 51K AIP were in the range of 3-4 mg per 100 grams of infected leaf tissue of *C. metuliferus*. Purified AIP was used to immunize New Zealand white rabbits. The titers of antisera collected at various bleedings were between 1-1/4 by immunodif-

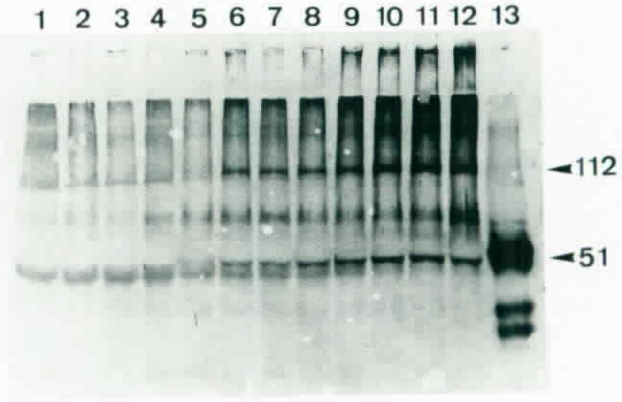

Fig. 1. Time course analysis of the production of PRV HA 112K protein in leaves of *Cucumis metuliferus* inoculated with PRV HA. Leaf tissues were collected at different time intervals after inoculation, total proteins were separated by electrophoresis and immunoblotted with PRV HA AIP antiserum. Lane 1, healthy control; lanes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12, inoculated leaf tissues collected at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 days after inoculation, respectively; lane 13, purified PRV HA AIP.

fusion test against crude sap from infected *C. metuliferus*, using 5 mm diameter wells at the distance of 5 mm. The antiserum from a breeding made 42 days after the first injection had a titer of 1/4 and was used in this study.

Detection of 112K Protein Produced in ***C. metuliferus***

Since a 112K protein with serological relation to AIP was the major translation product *in vitro* (Yeh and Gonsalves, 1985), AIP antiserum was used to detect the 112K protein produced in PRV-infected *C. metuliferus*. In inoculated leaves, trace amount of AIP first appeared at the fourth day after inoculation and was more evident at the sixth day (Figs. 1). A protein with a relative molecular weight (Mr) of 112K was also detected at the fourth day after inoculation and became prominent after day six. The level of the 112K protein accumulated in the inoculated leaves was even higher than that of AIP 8–12 days after inoculation (Fig. 1, lane 8–12).

A 112K protein and the AIP were also detectable in the systemically infected upper leaves 4 days after inoculation, and the production of these two proteins increased thereafter (Fig. 2). The amount of the 112K protein in the upper leaves was slightly less than that of

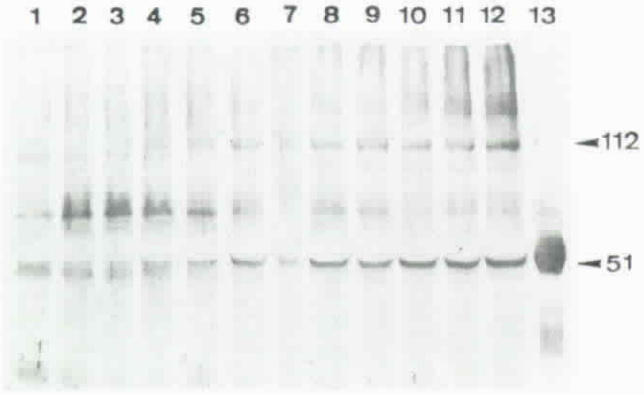

Fig. 2. Time course analysis of the production of PRV HA 112K protein in systemically infected *Cucumis metuliferus*. Leaf tissues of the plants were collected at different time intervals after inoculation with PRV HA, total proteins were separated by electrophoresis and immunoblotted with PRV HA AIP antiserum. Lane 1, healthy control; lanes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12, systemically infected leaf tissues collected at 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 days after inoculation, respectively; lane 13, purified PRV HA AIP.

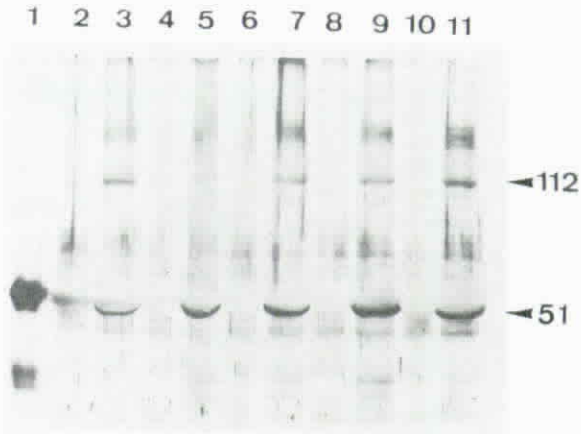

Fig. 3. Production of 112K protein in PRV-infected *Cucumis metuliferus* at various temperatures. Leaf tissues were collected 8 days after inoculation with PRV HA, and total proteins were extracted and immunoblotted with PRV AIP antiserum. Lane 1, purified PRV HA AIP; lanes 2, 4, 6, 8, and 10, crude saps from healthy plants incubated at 17, 23, 29, 35 and 41°C, respectively; lanes 3, 5, 7, 9, and 11, crude saps from PRV-infected plants incubated at 17, 23, 29, 35, and 41°C, respectively.

the AIP. In addition, high molecular weight (HMW) proteins of 200–300K were also detected in association with the appearance of the 112K protein and the AIP both in the inoculated and upper leaves (Figs. 1 and 2).

Effect of different temperatures on the production of the 112K protein was investigated. PRV-infected plants of *C. metuliferus* were incubated at 17, 23, 29, 35, and 41°C. Leaves of the whole plant were collected 8 days after inoculation. The total proteins were extracted and immunoblotted with antiserum to AIP. The results showed that the 112K protein was produced in a greater amount at the temperature extremes (17, 29, 35, 41°C, especially at 41°C) than at a moderate temperature (23°C). At 23°C, it was barely detectable (Fig. 3). The amount of AIP increased when the temperature increased from 17 to 35°C (Fig. 3, lanes 3, 5, 7, and 9). A slight decrease occurred at the temperature of 41°C (Fig. 3, lane 11).

Purification of 112K Protein Produced in vivo

The study on the appearance of the 112K protein indicated that the production of the protein reached high levels 8–12 days after inoculation and accumulated more under higher temperature conditions. These combines the consideration of the severe effect of stunting on growth and distortion on leaves, PRV-in-

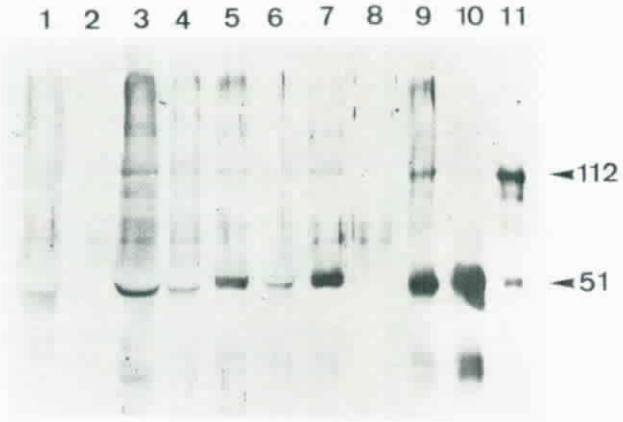

Fig. 4. Monitoring of PRV 112K protein by immunoblotting with antiserum to 51K AIP during the course of protein purification. Lane 1, crude sap from a healthy plant of *Cucumis metuliferus*; lane 2, crude sap from a PRV-infected papaya plant; lanes 3–9, fractions from different steps (Fraction 1–7) of purification; lane 10, purified 51K AIP; lane 11, purified 112K protein.

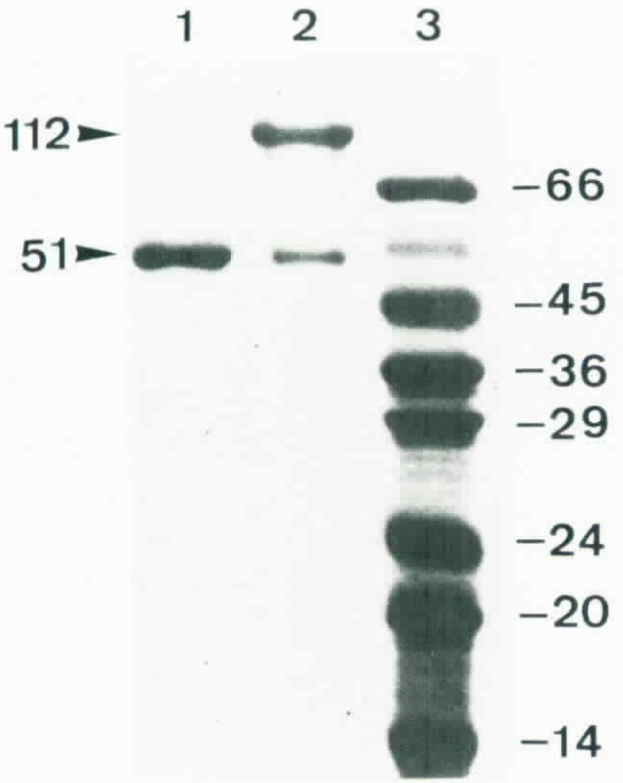

Fig. 5. Analysis of purified 51K and 112K proteins of PRV HA by SDS-polyacrylamide gel electrophoresis. Lane 1, PRV HA AIP; lane 2, PRV HA 112K protein; lane 3, molecular weight markers (14–66K).

fected plants of *C. metuliferus* were grown in greenhouse under summer conditions with temperatures between 26–43°C. The systemically infected leaves were harvested 8 days after inoculation to provide materials for the purification of virus specific proteins.

Monitored by antiserum to AIP, a protein of 112K was found in the crude sap (Fig. 4, lane 3) and in the pellets of sucrose cushion centrifugation (Fig. 4, lanes 5, 7, and 9). This protein was further purified by SDS-PAGE and concentrated in a ISCO concentrator. It strongly reacted with the AIP antiserum in immunoblotting reactions (Fig. 4, lane 11). Trace amount of AIP was found to be associated with the 112K protein even after SDS-PAGE (Fig. 5, lane 2). The yield of the 112K protein purified by the established procedure ranged from 0.3 to 0.4 mg per 100 g fresh leaf tissue.

When crude sap from PRV-infected papaya was analyzed by immunoblotting with AIP antiserum, no virus specific proteins could be detected (Fig. 4, lane 2).

Table 1. *Isotyping and reactivity of monoclonal antibodies prepared to the 112K protein of PRV*

Hybridoma line	McAb No.	Isotype	OD_{405}[a]	
			112K	51K AIP
2A7	112-51-1	IgG1	0.620	1.431
2C7	112-51-3	IgG1	0.357	1.279
2E7	112-51-18	IgG1	0.606	1.422
2E7	112-51-21	IgG1	0.479	1.337
3F7	112-51-29	IgG1	0.643	1.467
3F7	112-51-30	IgG1	0.878	1.793
3F10	112-3	IgM	0.313	0.093
3F10	112-4	IgM	0.306	0.069
3F10	112-5	IgM	0.280	0.074
3F10	112-6	IgM	0.474	0.088
3F10	112-7	IgM	0.307	0.093
3F10	112-8	IgM	0.367	0.112
3F10	112-9	IgM	0.412	0.108
3F10	112-10	IgM	0.398	0.062
3F10	112-11	IgM	0.234	0.096
	CK(−)[b]	–	0.063	0.082
	CK(+)[c]	–	1.314	1.752

[a]Antigens were diluted to 4 μg/ml in coating buffer, readings were recorded 15 min after addition of substrate.
[b]Culture fluid of SP_2 myeloma cells was used as negative control.
[c]Polyclonal antibody to the 112K protein was used at a dilution of 1:200 dilution as positive control.

This might be due to the interference of the strong protease papain present in the papaya plant.

Production of McAbs Against the 112K Protein

The hybridoma cells were screened by indirect ELISA using purified AIP or 112K protein as the coating antigens. The positive hybridoma cells were monoclonalized by two repeated limiting dilutions. The selected McAbs were classified into two major categories. Thirty one clones which reacted with both the 51K AIP and the 112K protein were designated as McAbs 112-51. Thirteen clones which reacted only with the 112K protein but not with the 51K AIP were designated as McAbs 112. Results of the isotyping of some typical clones are summarized in Table 1. The McAbs reacting with 112K protein and AIP were found to be IgG1, whereas the McAbs reacting with 112K alone belonged to the IgM type.

Detection of Viral Proteins Produced in vivo by McAbs

Polyclonal antibody to the 51K AIP from rabbit, polyclonal antibody to the 112K protein from mouse, and McAbs from culture fluids were used to detect viral antigens produced in PRV-infected *C. metuliferus* by immunoblotting techniques. Polyclonal antibody to

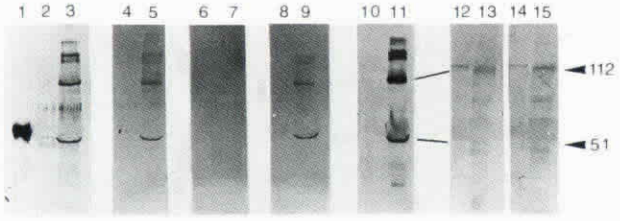

Fig. 6. Detection of the 112K protein *in vivo* by immunoblotting with polyclonal or monoclonal antibodies (McAbs) prepared against PRV 112K protein. Leaves of *Cucumis metuliferus* were collected 10 days after inoculation with PRV HA and extracted for total proteins which were then electrophoresed and immunoblotted. Lane 1, purified PRV AIP reacting with polyclonal antibody to AIP; lanes 2, 4, 6, 8, 10, 12, and 14, total proteins from a healthy plant reacting with polyclonal antibody to AIP, polyclonal antibody to 112K protein, mouse normal serum, McAb 112-51-29, McAb 112-51-30, McAb 112-6, and McAb 112-9, respectively; lanes 3, 5, 7, 9, 11, 13, and 15, total proteins from a PRV-infected plant reacting with polyclonal antibody to AIP, polyclonal antibody to 112K protein, mouse normal serum, McAb 112-51-29, McAb 112-51-30, McAb 112-6, and McAb 112-9, respectively.

AIP and the polyclonal antibody to the 112K protein recognized the 51K AIP and a protein of 112K. In addition, some proteins with molecular weights above 200K were also recognized (Fig. 6, lanes 3 & 5). The reaction patterns of McAbs 112-51-29 and 112-51-30 were similar to the patterns of polyclonal antibodies (Fig. 6, lanes 9 & 11). The reaction of McAbs 112-51-30 (Fig. 6, lane 11) was much stronger than that of polyclonal antibodies. McAbs 112-6 and 112-9 recognized a protein of 112K and three other proteins of 70K, 64K, and 47K, but these McAbs did not react with the 51K AIP (Fig. 6, lanes 13 & 15). The reaction patterns of McAbs 112-6 and 112-9 were clearly different from those of McAbs 112-51-29 and 112-51-30. Nonspecific reactions were not observed except in the reactions of the McAbs 112-6 and 112-9, where a 115K protein from the healthy control was found to react with the McAbs tested (Fig. 6, lanes 12 & 14).

Discussion

Previous studies of *in vitro* translation of PRV RNA revealed that its gene expression involves proteolytic processing. Immunoprecipitation analysis with antiserum to the 51K AIP revealed that a 112K protein was the major protein reacted in addition to the homologous 51K AIP (Yeh and Gonsalves, 1985). This study was directed to detect the possible presence of the 112K protein in PRV-infected *Cucumis metuliferus* plants and to establish a procedure for its purification. Moreover, monoclonal antibodies were produced against purified 112K protein and used as probes for exploration into the question whether such a protein plays a precursor role for the 51K AIP and other proteins of low molecular weight.

A 112K protein was first detected in *C. metuliferus* at day 4 after inoculation with PRV HA. Accumulation of this protein increased constantly thereafter and peaked at 8-10 days after inoculation. The time course of its appearance coincided with the appearance of the 51K protein. The accumulation level of the former was even higher than that of the latter in inoculated leaves. Since the two proteins constantly entered into same fractions during the purification steps and they both reacted with antiserum to AIP, one may ask whether the 112K protein is a dimer aggregate of the 51K AIP. This seems unlikely because we always employed a strong denaturing condition for the dissociation of sam-

ple proteins prior to PAGE and immunoblotting. However, the possibility can not be completely ruled out until peptide mapping and a direct N-terminus sequence analysis show to the contrary.

Accumulation of the 112K protein was at a higher level at the extreme temperatures (29, 35, 41 and 17°C) than at the moderate temperature of 23°C. The high molecular weight (HMW) proteins in the range of 200 -300K were also reduced in amount at 23°C. But, the amount of the 51K AIP protein remained quite constant at different temperatures. These observations can be explained by assuming that proteolytic processing *in vivo* is optimal at a moderate temperature, 23°C, but hampered by either low (17°C) or high temperatures (29-41°C), resulting in higher levels of accumulation at the temperature extremes for the large, uncleaved proteins such as the protein of 112K.

Extracted from PRV-infected *C. metuliferus*, HMW proteins were found to react specifically with polyclonal antibody to AIP, polyclonal antibody to 112K protein and also the monoclonal antibodies produced against the 112K protein. Because these HMW proteins were not found in the healthy control plants of *C. metuliferus*, they must be the PRV gene products and could function as the precursor for proteins of lower molecular wights. A precursor role of the 112K protein for the 51K AIP and a 64K protein was demonstrated in our immunoblotting tests in which the monoclonal antibodies McAbs 112-51 reacted specifically with both the 51K AIP and the 112K protein, whereas the monoclonal antibodies McAbs 112 did not react with AIP but did with proteins of 112K, 70K, 64K and 47K. The implication of these observations is that McAbs 112-51 recognized a common epitope in the AIP and the 112K protein and that McAbs 112 recognized a different epitope which is present in the 64K and 112K proteins. We conclude from these analyses that the 51K AIP and the 64K protein are both the cleavage products of the 112K protein produced *in vivo*.

Analysis of *in vitro* translation products of PRV RNA by immunoprecipitation with polyclonal antibodies to the 112K protein prepared from mouse shared the same pattern as using the polyclonal antibody to the 51K AIP (Yeh and Gonsalves, 1985), with 112K protein as the major precipitate (data not shown). The identity in serological relationship and in relative molecular weight suggests that the 112K protein detected *in vivo* is the same protein as the 112K produce of *in vitro* translation. Using McAbs 112-51 to immunoprecipitate the *in vitro* translation products of PRV revealed the same pattern as polyclonal antibodies to the 51K AIP (data not shown). However, we were not able to detect any proteins reacting to McAbs 112, probably because they were IgM type and not reacting to protein A of *Staphylococcus* cells. Thus, several lines of evidence in previous and this studies strongly suggest that the 112K protein is the precursor for the production of 51K AIP and the 64K proteins in proteolytic processing both *in vitro* and *in vivo*. Further studies by peptide mapping and analysis of N-terminal amino residues of the 51K, 64K, and 112K proteins produced both *in vitro* and *in vivo* are needed to confirm their identity.

The monoclonal antibodies prepared in this experiment provide a useful tool for diagnostic, cytological, and serological studies of PRV, and could be used as probes for cDNA cloning and function analysis of PRV -specific protein genes, particularly those corresponding to the 51K, 64K and 112K proteins.

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木瓜輪點病毒 112 K 蛋白在生體內之偵測與免疫分析

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利用木瓜輪點病毒 (papaya ringspot virus, PRV) 51K 不定形內含體蛋白 (AIP) 抗血清作爲探針，於感染木瓜輪點病毒之刺角瓜 (*Cucumis metuliferus*) 植株內，偵測到一種 112K 蛋白。經萃取、高低速離心、電泳及濃縮步驟，將此 112K 蛋白純化，並製備其多元及單元抗體，以分析 112K 、51K 及 64K 蛋白之關係。感染木瓜輪點病毒的刺角瓜葉片萃取後，經免疫轉漬分析發現：單元抗體 McAbs 112-51 可與 112K 蛋白及 51K 不定形內含體蛋白反應，而單元抗體 McAbs 112 則可與 112K 、70K 、64K 及 47K 蛋白反應。本研究之結果顯示 51K 及 64K 蛋白分別含有與 112K 蛋白相同之抗原決定基，此結果說明這兩種蛋白在生體內是由 112K 裂解而產生的。因此，在生體內所產生之 112K 蛋白，顯然亦擔任裂解產生 51K 及 64K 蛋白之前驅物的角色，此印證了前人在生體外試管轉譯作用中之發現。