ACCUMULATION AND BINDING OF A POLYSOME-ASSOCIATED-PROTEIN IN MAIZE RESPONDING TO INFECTION BY *BIPOLARIS MAYDIS*[1]

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(Manuscript received 11 October 1990, revised version accepted 8 November 1990)

Abstract: Previous studies indicated that a 57 kDa polysome-associated-protein (p57) accumulated in cytoplasmic polysomes when W64A maize (*Zea mays* L.) seedlings were inoculated with *Bipolaris maydis*, heat shocked, or treated with the herbicide paraquat. Efforts were made to determine the binding of this protein to ribosomes, and to study whether this protein was synthesized *de novo* or mobilized from another cellular structure in leaf tissues responding to stress. Pulse-labeling of inoculated seedlings with H^3-leucine and treatments of seedings with the protein synthesis inhibitor, cycloheximide, indicated that p57 existed in cells before inoculation. Chloroplastic polysomes isolated from both inoculated and control leaves contained this protein. Presumably, p57 redistributed from chloroplasts to cytoplasmic polysomes and bound specifically to the 40S ribosomal subunit to inhibit translation in response to biotic and environmental stressses.

INTRODUCTION

Induction of proteins that are associated with pathogen infections have been shown in several compatible and incompatible plant-pathogen interactions. Yamamoto and Tani (1982) detected 11 proteins specific for incompatible oat-crown rust interactions. Hadwiger and Wagoner (1983) observed that a few specific proteins were synthesized in a compatible pea-*Fusarium solani* interaction at 24 to 96 h after inoculation but not in an incompatible interaction. Wagoner *et al.* (1982) found that 21 proteins increased and 4 decreased in quantity in pea within 6 h after inoculation with compatible and incompatible isolates of *Fusarium solani*.

Although the roles of these proteins in either active resistance mechanisms or susceptible responses have remained largely unknown, some proteins induced by pathogens are pathogenesis-related (PR) proteins, which may be involved in disease resistance (Van Loon, 1985; Bol, 1988; Carr and Klessig, 1989). The accumlation of PR proteins has been shown to correlate with disease severity (Camacho and Sanger, 1984) and with hypersensitive response or necrotrophy (de Wit and van der Meer, 1986). Certain PR proteins are hydrolytic enzymes, such as β-1,3-glucanases and chitinases, which inhibit fungal growth *in vitro* (Mauch *et al.*, 1988).

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In addition to induction by pathogens, PR proteins can be induced by plasmolysis, chemicals, high concentrations of plant hormones, culture filtrates from pathogenic fungi or bacteria, and at the onset of flowering and during aging (reviewed by van Loon, 1985). Alterations of protein synthesis are a reflection of both transcriptional and translational regulations (Key *et al.*, 1981; Matters and Scandalios, 1986; Sachs and Ho, 1986). Therefore, it has been suggested that the PR proteins might function to protect the plant from extensive damage (van Loon, 1985; Bowles, 1990).

Our recent studies indicated that SDS-polyacrylamide gel patterns of proteins isolated from polysomes in a compatible maize-*Bipolaris maydis* interaction at 6 to 48 h after inoculation showed an increase in the intensity of a 57 kDa protein (Wu *et al.*, 1988). This protein also accumulated in polysomes of maize seedlings responding to heat shock or paraquat treatments. Studies with *Tetrahymena thermophila* also indicated that a 22 kDa ribosome-associated protein was induced by heat shock and correlated with the decline in heat shock protein (hsp) synthesis and the resumption of non-hsp synthesis, suggesting that this 22 kDa protein might be associated with non-hsp mRNA usage (McMullin and Hallberg, 1986). Although the role of the 57 kDa polysome-associated-protein (p57) is not clear, this purified protein reassociated with polysomes isolated from healthy leaves and inhibited polysomal translation (Wu *et al.*, 1988).

As a first step to elucidate the role of p57, synthesis of this protein and its binding with ribosomal subunits in response to inoculation with *B. maydis* were determined.

MATERIALS AND METHODS

Plant Materials

Seeds of maize (*Zea mays* L.) inbred W64A with normal cytoplasm were germinated in moist vermiculite at 29±1°C with a 14 h photoperiod.

Seven-day-old seedlings were inoculated with a conidial suspension of *Bipolaris* (*Helminthosporium*) *maydis* race O. Conidial suspension was adjusted to a concentration of 5×10^5 conidia ml^{-1}. The conidial suspension was sprayed onto the foliage evenly with a Devilbis atomizer. Both inoculated and control seedlings were placed in a moist chamber up to 16 h after inoculation. Leaf tissues were harvested at 24 h after inoculation, frozen in liquid nitrogen, and stored at −70°C until polysome isolation.

For heat shock treatment, 7-d-old seedlings of W64A were incubated in a growth chamber at 42°C for 2 h before harvest. For the herbicide treatment, seedlings were sprayed with 0.2% (v/v) paraquat (1, 1′-dimethyl-4, 4′-bipyridinium ion), a contact herbicide known to generate superoxide radicals and causing oxidative stress (Matters and Scandalios, 1986), and harvested 4 h after spraying. Harvested leaves were frozen in liquid nitrogen and stored at −70°C until polysome isolation.

Labeling and Extraction of Proteins Synthesized *In Vivo*

Healthy or *B. maydis*-inoculated maize seedlings were excised 1 cm below the primary leaf and placed singly into vials containing 30 μCi of H^3-leucine (131 Ci $mmole^{-1}$) in 100 μl water. The seedlings were incubated in a growth chamber at 32±2°C and with constant fluorescent light. After the solution had been taken up

by the seedling, 5 mM phosphate buffer (pH 6.5) was added to the vial. Seedlings were incubated for a total time of 1 h. After incubation, leaves of each seedling were removed and ground in a motar containing three volumes of extraction buffer (25 mM Tris-HCl, 200 mM glycine, pH 8.3, 2% SDS, 2% 2-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride). The extract was pipetted into an Eppendorf microcentrifuge tube and centrifuged at full speed on a table top centrifuge for 5 min. The supernatant was heated in a boiling water bath for 3 min, chilled, and centrifuged at 37,000 g for 30 min. The supernatant containing proteins was stored at −70°C before analysis by SDS-polyacrylamide gel electrophoresis.

Gel Electrophoresis and Fluorography of Proteins Synthesized *In Vivo*

Proteins synthesized *in vivo* were analyzed by one-dimensional SDS-polyacrylamide gel electrophoresis according to Laemmli (1970). Protein samples were mixed with five-fold concentrated sample buffer (final concentration was 25 mM Tris-HCl, 190 mM glycine, pH 8.3, 2% SDS, 2% 2-mercaptoethanol, and 10% glycerol), heated for 20 sec in a boiling bath, bromophenol blue added to 0.01%, and then loaded directly at 80,000 cpm into one of the sample slots of a slab gel (12.5% polyacrylamide). After electrophoresis, the gel was fixed in a solution containing 10% (w/v) TCA, 10% (v/v) glacial acetic acid, and 30% (v/v) methanol. Radioactive protein bands were detected by fluorography (Larskey and Mills, 1975) with EN^{3}Hance (New England Nuclear, Boston, MA, USA). The dried gel was exposed to Kodak XAR-5 film (Eastman-Kodak Rochester, NY, USA) at −70°C for 4 d.

Cycloheximide Treatments

Twenty-five 7-day-old seedlings were transferred into a 250 ml beaker containing 50 ml cycloheximide solution (25, 50, or 75 μg ml^{-1}) with aeration for 2 h before spraying with *B. maydis* conidia suspension. Control seedlings were also treated with cycloheximide but sprayed with water without conidia. Both inoculated and control seedlings were subsequently placed in a moist chamber up to 16 h with aeration. Leaf tissues were harvested 24 h after inoculation, frozen in liquid nitrogen, and stored at −70°C before polysome isolation.

Isolation of Cytoplasmic Polysomes

Cytoplasmic polysomes were isolated by a procedure described previously (Wu *et al.*, 1988). Leaf tissue (approximately 100 g) was ground in a mortar containing liquid nitrogen and homogenized subsequently with a Polytron in two volumes of extraction buffer (200 mM Tris-HCl, pH 9.0, 400 mM KCl, 200 mM sucrose, 1% Triton X-100, 35 mM $MgCl_2$, 25 mM EGTA, and 1% 2-mercaptoethanol). The homogenate was strained through four layers of cheesecloth and clarified by centrifugation at 29,000 g for 10 min. Total polysomes from the supernatant were subsequently pelleted through a 1.7 M sucrose pad (in 40 mM Tris-HCl, pH 9.0, 200 mM KCl, 30 mM $MgCl_2$, and 5 mM EGTA) by centrifugation at 260,000 g for 60 min at 4°C. The polysome pellet was suspended in sterile distilled water and stored at −70°C.

Isolation of Chloroplastic Polysomes

Chloroplasts were purified from leaf tissues 24 h after inoculation with *B. maydis* or from the healthy control according to the procedure of Schwartzbach *et al.* (1979). Leaf tissues (approximately 50 g) were ground in a motar containing two

volumes of Buffer I (250 mM sorbitol, 250 mM sucrose, 2.5% (w/v) Ficoll, 1 mM Mg-acetate, 0.01% (w/v) bovine serum albumin, 0.1% 2-mercaptoethanol, 0.5 mM spermidine trihydrochloride, and 5 mM HEPES, pH 7.6) before centrifugation at 700 g for 2 min. The pellet was suspended in Buffer II (150 mM sorbitol, 150 mM sucrose, 2.5% Ficoll, 1 mM Mg-acetate, 0.01% bovine serum albumin, 0.1% 2-mercaptoethanol, 0.5 mM spermidine trihydrochloride, and 5 mM HEPES, pH 7.6) and centrifuged at 500 g for 10 min to obtain the chloroplast pellet. The supernatant was centrifuged again at 29,000 g for 10 min and saved for the preparation of cytoplasmic polysomes as described above. The chloroplast pellet was suspended in Buffer III (10 mM Tris-HCl, pH 7.6, 60 mM KCl, 12 mM Mg-acetate, 0.5 mM spermidine trihydrochloride, and 0.1% 2-mercaptoethanol) and broken at 4000 psi in an Aminco French pressure cell before centrifugation at 15,000 g for 10 min. The supernatant containing chloroplastic polysomes was subsequently pelleted through a 1.75 M sucrose pad as described.

Purification of Ribosomal Subunits

A combination of high salt, puromycin, and EDTA was used to obtained the 40S and 60S subunits from cytoplasmic polysome preparations (Blobel and Sabatini, 1971). To 300 μl of the polysome preparation (containing approximately 40 A_{260} units), high salt buffer and puromycin were added to a final concentration of 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 500 mM KCl, and 1 mM puromycin. After incubation at 0°C for 15 min and 37°C for 10 min, the preparation was layered on a 5-20% linear-log sucrose gradient in buffer containing EDTA (50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 500 mM KCl, and 5 mM EDTA) and centrifuged at 170,000 g for 5 h. The gradients were prepared by layering 1.9 ml of sucrose at 200 mg ml^{-1} in nitrocellulose tubes, followed by 3.8 ml at 150 mg ml^{-1}, 3.8 ml at 100 mg ml^{-1}, and 1.9 ml at 50 mg ml^{-1}. After centrifugation, gradients were fractionated in an ISCO model UA5 absorbance monitor. The fractions containing the subunits, either 40S or 60S, were pooled before addition of Na-acetate, pH 6.0, to a final concentration of 300 mM. The ribosomal subunits were precipitated with two volumes of cold 95% ethanol and kept at −20°C overnight. Ribosomal pellets were collected by centrifugation and dissolved in sterile distilled water.

Agarose Gel Electrophoresis of RNAs

Cytoplasmic ribosome subunit samples containing 8 μg of RNA were added with dye to a final concentration of 0.05% (w/v) bromophenol blue and 5% (w/v) Ficoll before loading into one of the sample slots of a slab gel (1.5% agarose in TBE buffer, 90 mM Tris, 90 mM boric acid, and 2 mM EDTA at pH 8.3). Each gel was run in TBE buffer at 5 V cm^{-1} for 5 h on a horizontal gel apparatus. Gels were stained for 15 min in 1 μg ml^{-1} ethidium bromide, rinsed for 15 min in water, and the stained RNAs were visualized with ultraviolet light.

Electrophoretic Analysis of Polysome-Associated-Proteins

Polysome preparations and ribosomal subunits were analyzed for their protein composition by SDS polyacrylamide gel electrophoresis according to Laemmli (1970). Polysomes or ribosomal subunits containing 1.2 A_{260} unit were loaded onto each sample slot of a slab gel (percentage of polyacrylamide in indicated in the figure legend) after addition of sample buffer and heating for more than 20 sec unless otherwise indicated. The protein bands were detected by staining with Coomassie brilliant blue R.

RESULTS

Protein Synthesized *In Vivo*

Alterations of protein synthesis *in vivo* were observed in leaves inoculated with *B. maydis* followed by labeling with H^3-leucine. Increases in banding intensity were observed for three proteins with mol wts of 70, 75, and 81 kDa (Fig. 1). Heat

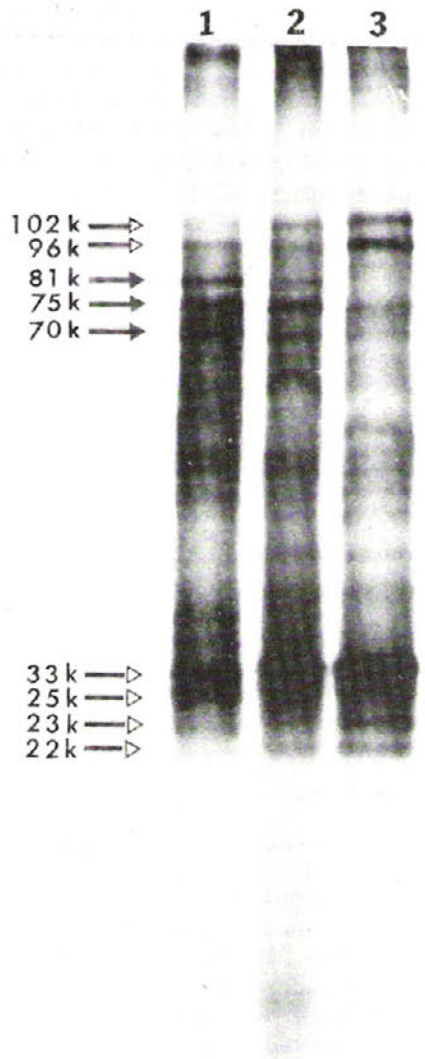

Fig. 1. Fluorograph of SDS-polyacrylamide gel of proteins synthesized *in vivo* by healthy and *B. maydis*-inoculated leaves. Proteins were synthesized *in vivo* during a 1 h pulse with H^3-leucine by healthy W64A leaves (lane 3) or leaves that had been inoculated with *B. maydis* for 12 and 24 h (lane 2 and 1, respectively). Gel concentration was 12.5%. Arrows indicate the protein bands with decreased (——▷) or increased (——▶) intensity.

shock treatment at 42°C for 2 h also induced the synthesis of the same three proteins (data not shown). Inoculation with *B. maydis*, while increasing the intensity of these three proteins, caused a reduction in the intensity of several proteins, i. e., the 22, 23, 25, 33, 96 and 102 kDa proteins (Fig. 1). The intensity of these protein bands diminished gradually with an increase in time after inoculation, and the decrease in intensity was disease-specific. No obvious change in the intensity of a protein band equivalent to the p57 was observed due to infection.

Aggregation of p57

When a polysome sample prepared from inoculated leaves was mixed with sample buffer and heated for more then 10 sec in a boiling bath before electrophoresis, a protein band of 57 kDa (p57) was observed (Fig. 2, lanes 5–8). On the other hand, a brief heating of less then 10 sec resulted in a protein band with mol wt of 92 kDa (p92) at the expense of the 57 kDa band (Fig. 2, lanes 2–3). Both p57 and p92 bands were observed when polysome samples were heated for approximately 10 sec (Fig. 2, lane 4). The p57 induced by heat shock and paraquat treatments also aggregated after a brief heating (Fig. 3).

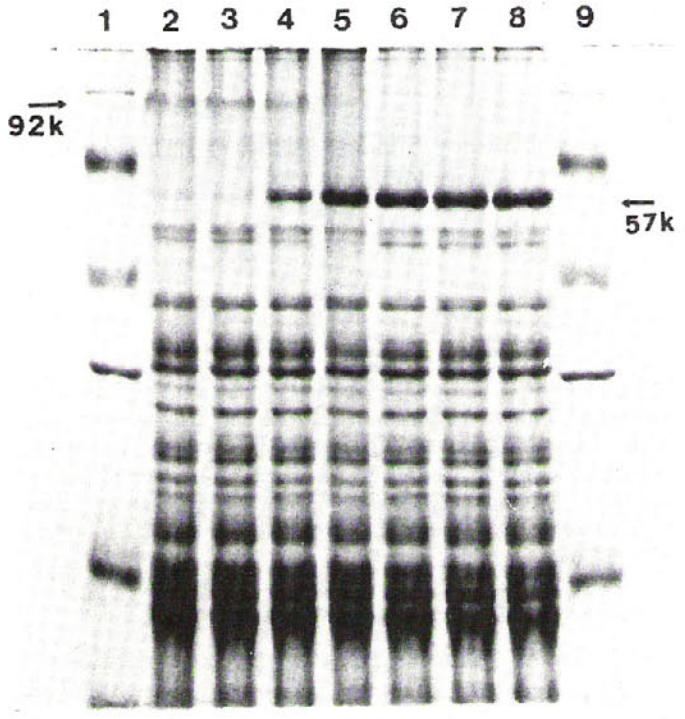

Fig. 2. Effects of heating on SDS-polyacrylamide gel electrophoretic pattern of polysome-associated-proteins isolated from leaves inoculated with *B. maydis*. Polysome samples were heated with sample buffer for 2 (lane 2), 5 (lane 3, 10 (lane 4), 20 (lane 5), 30 (lane 6), 60 (lane 7), and 120 sec (lane 8), respectively, before electrophoresis. Lanes 1 and 9 are mol wt standard in order of decreasing size: 97.4, 66, 45, 29, 20.1, and 14.2 kDa. Gel concentration was 9%. Arrows indicate p92 and p57.

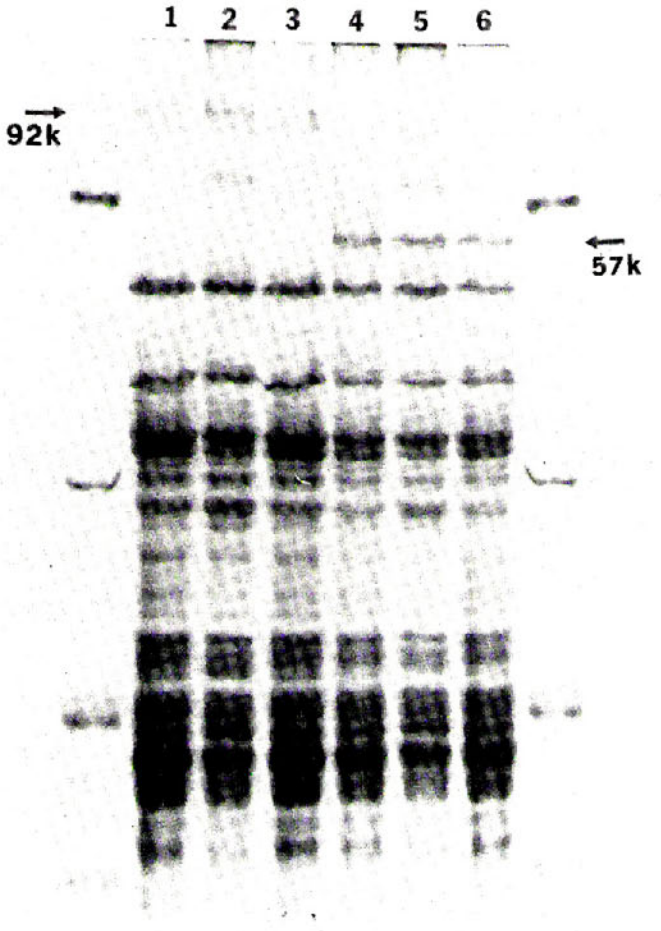

Fig. 3. Conversion of p92 to p57 by heating of polysomes prepared from leaves inoculated with *B. maydis*, heat-shocked, or treated with paraquat. Polysomes were isolated from *B. maydis*-inoculated leaves (lanes 1 and 4), heat-shocked at 42°C for 2 h (lanes 2 and 5), or treated with paraquat for 4 h (lanes 3 and 6) with a heating of 5 (lanes 1-3), or 20 seconds (lanes 4-6) before electrophoresis. Gel concentration was 12.5%. Arrows indicate p92 and p57.

Effects of Cycloheximide on the Accumulation of p57

Maize seedlings were incubated with varying concentrations of cycloheximide 2 h before inoculation with *B. maydis* in order to determine whether the p57 is synthesized *de novo* in response to infection. Cycloheximide at 25, 50, or 75 μg ml^{-1} did not inhibit the accumulation of p57 in the polysomes of infected leaves (data not shown).

Localization of p57 in Chloroplasts

Polysomes were isolated from chloroplasts and cytoplasm of inoculated and control leaves to compare their protein profile on SDS-polyacrylamide gels. The

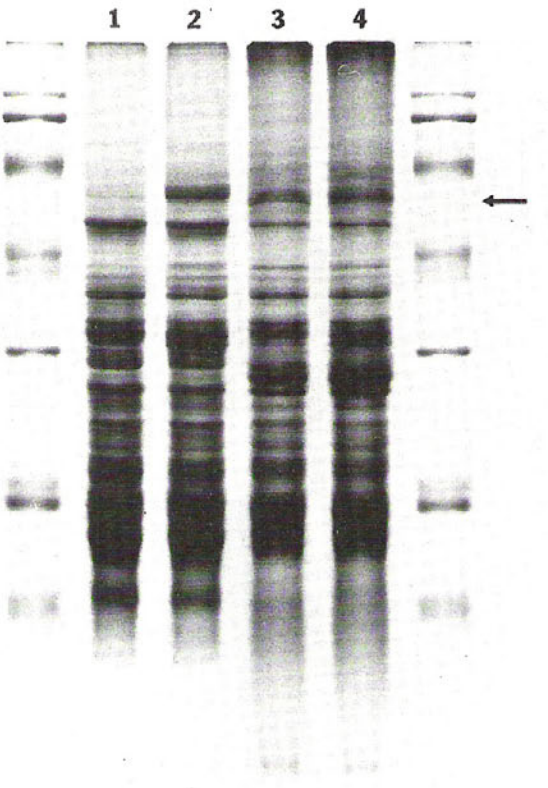

Fig. 4. SDS-polyacrylamide gel electrophoretic pattern of polysome-associated-proteins isolated from cytoplasm and chloroplasts of healthy or *B. maydis*-inoculated leaves. Polysomes were isolated from cytoplasm (lanes 1 and 2) and chloroplasts (lanes 3 and 4) of healthy leaves (lanes 1 and 3) or *B. maydis*-inoculated leaves (lanes 2 and 4). Gel concentration was 12.5%. Arrow indicates p57.

p57 accumulated in cytoplasmic polysomes of leaves 24 h after inoculation with *B. maydis* (Fig. 4, lane 2), and only a trace amount was found in the cytoplasmic polysomes of the controls (Fig. 4, lane 1). However, a protein band with mol wt of 57 kDa was present in the chloroplastic polysomes of both control and infected leaves (Fig. 4, lanes 3 and 4).

Binding of p57 to the 40S Ribosomal Subunit

Binding of p57 with the cytoplasmic ribosomal subunit was determined by analyzing the protein profile of subunits on SDS-polyacrylamide gel. Purity of the subunits was confirmed by analyzing rRNA on an agarose gel. Fraction I from sucrose gradient (Fig. 5A) contained only 18S rRNA (Fig. 5B, lane 1), indicating that the 40S subunit preparation was free of 60S contamination. Fraction II, which consisted of a portion of each subunit, contained about a 2:1 ratio of 18S and 28S rRNAs (Fig. 5B, lane 3). Fraction III collected from the 60S peak, had mostly 28S rRNA, but also contained a small amount of 18S rRNA (Fig. 5B, lane 2).

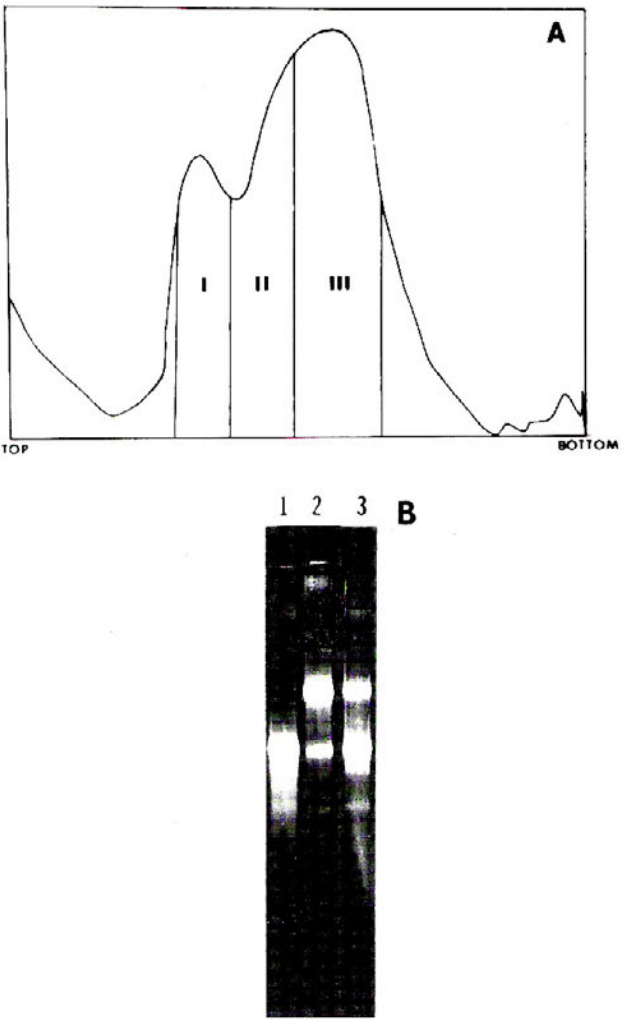

Fig. 5. Sucrose gradient profile and agarose gel electrophoretic patterns of ribosomal 40S and 60S subunits. A. Sucrose gradient profile of ribosomal 40S and 60S subunits released from polysomes disassembled by treatments with high salt, puromycin, and EDTA. Three fractions containing the subunits were collected as indicated. B. Agarose gel electrophoretic pattern of rRNAs from fraction I (lane 1), II (lane 3), and III (lane 2), respectively.

Protein profiles of the cytoplasmic ribosomal subunits separated on a 10-16% linear gradient SDS-polyacrylamide gels are shown in Fig. 6. The harsh treatment (i.e., with a combination of high salt, puromycin, and EDTA) used to obtain the ribosomal subunits removed most of the p57 which was present originally in the polysomes isolated from the infected leaf tissues. However, a small amount of p57 and p92 could still be detected in the 40S subunit (Fig. 6, lane 5). Because the subunit samples were heated for only about 10 sec before electrophoresis, both p57 and p92 were observed. A trace amount of these two proteins was detectable in the fraction II containing a mixture of 40S and 60S (Fig. 6, lane 4), but p57 and

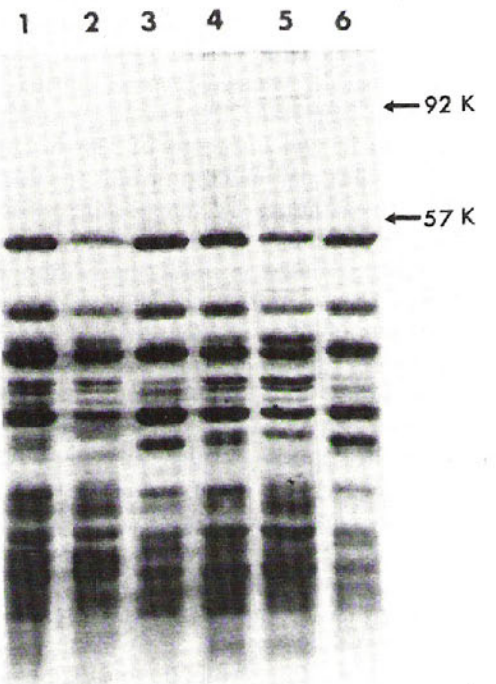

Fig. 6. SDS-polyacrylamide gel electrophoretic pattern of polysome-associated-proteins isolated from ribosomal subunits. The subunits were isolated from healthy leaves (lanes 1–3) and leaves inoculated with *B. maydis* for 24 h (lanes 4–6). The subunits are: 40S from fraction I (lanes 2 and 5), a mixture of 40 and 60S from fraction II (lanes 1 and 4), and 60S from fraction III (lanes 3 and 6). Gel concentration was a 10–16% linear gradient. Arrows indicate p92 and p57.

p92 were only barely detectable in the fraction III containing predominantly 60S subunit (Fig. 6, lane 6). These two proteins were not detected in either ribosomal subunit obtained from healthy leaf tissues (Fig. 6, lane 1–3).

DISCUSSION

Several disease-induced alterations in gene expression may be detected at an early stage of disease development, before symptom expression. Three proteins, i. e., 70, 75, and 81 kDa, were found to increase in their banding intensities (Fig. 1). However, the induction of these proteins was not disease-specific; heat shock also induced the synthesis of these three proteins (data not shown). These proteins are probably the same or similar to the 73, 76, and 84 kDa (Baszczynski *et al.*, 1983) or the 70 to 85 kDa heat shock proteins (Cooper and Ho, 1983) previously reported

in maize. However, the decrease in intensity of six proteins (22, 23, 25, 33, 96, and 102 kDa) was disease-specific (Fig. 1). The specific diminution of these six proteins was not observed with heat shock (data not shown). Because these proteins decreased specifically in a susceptible maize-*Bipolaris maydis* interaction, but they were not observed to change in resistant reactions, suggest that these proteins may also play an important role in the outcome of the host-pathogen interaction.

Previous studies indicated that p57 accumulated in polysomes of maize leaf tissues in response to stress treatments by pathogen infection, heat shock, or herbicide paraquat (Wu *et al.*, 1988). The binding of this prothin to cytoplasmic polysomes responding to various stresses is most likely duo to a subcellular redistribution of a pre-existing protein rather than originating from *de novo* protein synthesis. Pulse-labeling with H^3-leucine *in vivo* did not indicate an accumulation of a radioactive 57 kDa protein in the inoculated leaf tissues when compared with the control (Fig. 1). Furthermore, treatments with cycloheximide 2 h prior to inoculation with *B. maydis* did not prevent accumulation of p57 in polysomes. Preliminary study by Western blot analysis also indicated that p57 preexisted in cells before stress treatments (Wu *et al.*, unpublished data). A similar observation was made indicating that a ribosome-associated protein in *Tetrahymena thermophila* induced by heat shock was not synthesized *de novo* (McMullin and Hallberg, 1986). Although localization of p57 in cells of healthy leaves remains unknown, chloroplastic polysomes isolated from inoculated and control leaf tissues both contained a 57 kDa polysome-associated protein (Fig. 4). The nature of chloroplastic p57 is being investigated. Because this protein from both cytoplasmic and chloroplastic polysome preparations showed an identical mol wt, it is possible that the p57 may be compartmentalized in the chloroplast, and transported to cytoplasmic polysomes in response to stress.

The p57 purified from the polysomes of inoculated leaves reassociated with polysomes isolatd from healthy leaves and inhibited translation *in vitro* (Wu *et al.*, 1988). The observation that this protein was detected in the 40S, but not the 60S, suggests that the 40S subunit is probably the site on the ribosome where p57 is associated. This raises the possibility that p57 may play a role in regulating protein synthesis during initiation. A number of initiation factors have been shown to function at the level of mRNA attachment (Maitra *et al.*, 1982). Several of them are known to cause a differential rate of initiation of translation of mRNA and result in selective translation of certain messages (Marcus, 1982). Thus, like other ribosome-associated initiation factors (Weissbach and Ochoa, 1976), the p57 is induced by stress to interact with the 40S to affect translation. The role of this protein in regulating translation, whether on the over-all rate of translation or specific effect toward different messages, remains to be determined.

The fact that p57 reassociates with polysomes *in vitro* (Wu *et al.*, 1988) indicates a hydrophobicity of this protein for binding with other proteins. As shown in Figure 2, when polysome preparations obtained from inoculated leaf tissues were mixed with a sample buffer and heated briefly for less than 10 sec before electrophoresis, a 92 kDa band was observed at the expense of the 57 kDa. Prolonging the heating for more than 10 sec converted the 92 kDa component into the 57 kDa protein. Because the conversion did not involve other proteins, the 92 kDa component observed under a brief heating was presumably the result of dimerization of the 57 kDa protein. This dimer could be broken up under a more drastic treatment by heating for a longer period of time in the presence of 2% SDS.

The p57 induced by heat shock and paraquat treatment behaved similarly to that of the disease-induced protein in that a brief heating all led to the formation of dimer aggregates (Fig. 3). This is not surprising, since the Western blot indicated that the p57 induced by pathogen infection, heat shock, and paraquat treatment are identical (Wu *et al.*, unpublished).

These studies suggest that the p57 is hydrophobic in nature and capable of binding tightly to polysomes. This protein was not synthesized *de novo*, but is the result of a sub-cellular redistribution in response to stress. The p57 appears to bind specifically to the 40S ribosomal subunit to regulate translation.

ACKNOWLEDGEMENT

This research was supported in part by a grant from Dow Chemical U. S. A.

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玉米幼苗經 *Bipolaris maydis* 感染後的反應：一種多核糖體結合性蛋白質的累積及其結合

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摘　　要

由以前的研究結果得知：當 W64A 玉米 (*Zea mays* L.) 幼苗在接種 *Bipolaris maydis* 或經熱休克或除草劑 Paraquat 處理過後，在細胞質內的多核糖體會累積一種 57KD 多核糖體結合的蛋白質。爲了要進一步瞭解此蛋白質是如何結合於核糖體，並研究此蛋白質是否新合成或是在逆境下，在葉部組織之細胞內不同胞器轉移的反應。已接種細菌的幼苗，先用 ^{3}H-leucine 標誌，再用蛋白質合成抑制劑 cycloheximide 處理，由結果顯示 P57 蛋白質在接種前已存在於細胞之內。從已接種菌種及對照組（不接菌種）的葉片所分離出綠體多核糖體 部含存此蛋白質 。由本研究結果推測 P57 是由葉綠體合成，再轉移到細胞質後重新分佈，特別與 40S 核糖體次單元結合。在生物及環境壓迫時用以抑制蛋白質的轉譯作用。尤其是會產生毒素之品種。在過去幾年間之數量及分佈情形。文中並比較以間歇性破壞溫層及去除水中磷量以達減少藍綠藻數量之效果。