The Plant Cell, Vol. 5, 1089–1099, September 1993

Fungal Elicitor–Induced Bean Proline-Rich Protein mRNA Down-Regulation Is Due to Destabilization That Is Transcription and Translation Dependent

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In bean cells treated with fungal elicitor, the transcripts of *PvPRP1*, a gene encoding a proline-rich protein, decreased to ~6% of the original level within 4 hr. The apparent mRNA half-life during the period of rapid degradation was ~45 min. The rate of *PvPRP1* gene transcription remained constant over this period, as determined by nuclear run-off assays, indicating a decrease in mRNA stability. By using actinomycin D to block transcription, the half-life of *PvPRP1* mRNA in unelicited cells was estimated to be ~60 hr. In cells treated with actinomycin D followed by the addition of elicitor, the *PvPRP1* mRNA half-life was ~18 hr, whereas cells treated with these reagents in reciprocal order exhibited a half-life of ~6 hr. The protein synthesis inhibitors emetine and anisomycin also inhibited the rate of *PvPRP1* mRNA degradation in elicited cells. Based on these data, we concluded that the rapid decrease in the *PvPRP1* mRNA level in elicited cells is due to destabilization, which is dependent on new RNA and protein synthesis.

INTRODUCTION

One major determinant in the control of gene expression is the rate of mRNA degradation, which can vary widely among different mRNAs. Mammalian c-*fos* proto-oncogene mRNA has a half-life of only 8 to 30 min and is rapidly cleared from cells upon the cessation of gene transcription (Kruijer et al., 1984; Müller et al., 1984). In oat plants, the mRNA encoding the photoreceptor phytochrome has a short half-life of about 1 hr, which may be an important factor in light regulation of development (Seeley et al., 1992). In contrast, β-globin mRNA in erythroid cells has a half-life of greater than 24 hr, which contributes to sustained and high-level synthesis of the encoded protein (Ross and Pizarro, 1983).

The half-lives of many mRNAs vary in response to environmental or endogenous factors. In the presence of excess iron, the transferrin receptor mRNA in mammalian cells is destabilized (Casey et al., 1988; Müllner and Kühn, 1988). In gibberellin-treated barley aleurone tissues, heat shock destabilizes α-amylase mRNA (Belanger et al., 1986; Brodl and Ho, 1991). In soybean seedlings, the transcripts of the ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) genes are less stable in light-grown plants than in their etiolated counterparts (Shirley and Meagher, 1990). However, in mature soybean plants, *rbcS* mRNA is more stable in light than in darkness (Thompson and Meagher, 1990).

The occurrence of mRNAs with widely divergent decay rates indicates the interaction of specific *cis*-acting elements on the mRNAs and *trans*-acting factors. For unstable mRNAs, such as c-*myc* and c-*fos* mRNAs, portions of the coding regions and AU-rich sequences in the 3′ untranslated regions have been identified as two mRNA components conferring instability (Shyu et al., 1989; Wisdom and Lee, 1991; Laird-Offringa, 1992). The *trans*-acting factors affecting mRNA stability appear to be either labile or newly synthesized proteins in several cases, including a factor(s) affecting c-*fos* mRNA stability (Koeller et al., 1991; You et al., 1992). In plants, little is known concerning the mechanisms responsible for the alteration of mRNA stability in response to internal or external signals. The rapid degradation of specific *rbcS* mRNAs in potato plants placed in the dark appears to require a newly synthesized factor(s) (Fritz et al., 1991). The process of *rbcS* mRNA degradation involves loss of the poly(A)$^+$ tail and discrete RNA cleavage intermediates in soybean and petunia (Thompson et al., 1992).

Plant cells exposed to fungal elicitors exhibit the rapid induction of mRNAs encoding a variety of proteins, such as chitinases, glucanases, cell wall proteins, and enzymes involved in phytoalexin synthesis (Dixon and Harrison, 1990). In contrast, the levels of another group of mRNAs decrease within several hours of elicitor treatment. This phenomenon has been observed by in vitro translation of mRNAs and

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two-dimensional gel analysis of polypeptides in elicitor-treated bean and alfalfa cell suspensions (Cramer et al., 1985; Dalkin et al., 1990) as well as by cDNA cloning of two down-regulated bean mRNAs (Sauer et al., 1990; Sheng et al., 1991).

In this study, we have investigated the mechanism for the loss of one elicitor down-regulated bean transcript, *PvPRP1* (Sheng et al., 1991). The *PvPRP1* transcript encodes a proline-rich protein that is likely to be localized in the cell wall. We have hypothesized that the synthesis of the PvPRP1 protein is reduced during the defense response because of its low potential for wall strengthening by isodityrosine cross-linking (Sheng et al., 1991). In contrast, mRNAs encoding specific proline-rich proteins and hydroxyproline-rich glycoproteins with greater cross-linking potentials accumulate during the defense response (Lawton and Lamb, 1987; Tierney et al., 1988). The importance of cell wall structural protein regulation in plant defense is supported by recent work showing that elicitor-treated soybean cells undergo a very rapid oxidative cross-linking of cell wall proteins (Bradley et al., 1992).

The following mechanisms for the rapid decrease in *PvPRP1* mRNA abundance may be considered. First, elicitor might switch off *PvPRP1* transcription, and the mRNA steady state level would decline rapidly due to a constant, rapid mRNA turnover rate. A second possibility is that the *PvPRP1* transcription rate is unaffected by elicitor treatment and, instead, the *PvPRP1* mRNA is selectively destabilized. Third, both the transcription rate and mRNA stability decrease. Our results showed that transcription of the *PvPRP1* gene is unaffected by elicitor, as determined by run-off transcription assays. The application of actinomycin D, a transcription inhibitor, allowed us to directly demonstrate that the *PvPRP1* mRNA is destabilized by elicitor treatment. Furthermore, the rate of *PvPRP1* mRNA degradation in elicited cells is slowed in the presence of transcription or translation inhibitors. These results suggest that the rapid degradation process involves the production of a new protein(s) encoded by an elicitor-activated gene(s) or the participation of a labile protein(s).

RESULTS

Identification of a Gene-Specific Probe for the *PvPRP1* Gene

Based on sequence analysis of the PvPRP1 cDNA, the encoded protein can be divided into two major domains: the N-terminal half, which is proline-rich and resembles other proline-rich proteins in plants, and the C-terminal half, which is low in proline and unrelated to other characterized proteins (Sheng et al., 1991). To identify a gene-specific probe, an approximately 600-bp HincII-HincII cDNA fragment spanning the 5′ untranslated region and N-terminal domain sequences and a 527-bp HincII-EcoRI cDNA fragment spanning C-terminal domain sequences and the 3′ untranslated region were hybridized to gel blots of bean genomic DNA digested with several restriction enzymes. Figure 1A shows that the HincII-HincII probe hybridized predominantly to single fragments of 7.0, 4.2, 4.0, and 13 kb in EcoRI-, HindIII-, HincII-, and XbaI-digested DNA samples, respectively. In addition, 10 to 15 minor hybridizing bands were observed, indicating that this probe detects related genes within the genome. In contrast, the HincII-EcoRI probe hybridized to only 7.0-, 3.0-, and 13-kb fragments in the EcoRI, HincII, and XbaI digests, respectively, while 1.2- and 0.75-kb fragments were hybridized in the HindIII digest (Figure 1B). Longer exposure of the blot revealed no additional hybridizing bands (data not shown).

The 4.0- and 4.2-kb fragments hybridized by the HincII-HincII probe in the HindIII and HincII digests of genomic DNA, respectively, correspond exactly to the fragments hybridized in similarly digested DNA of a *PvPRP1* genomic clone (S. Zhang, Y. Liu, and M. C. Mehdy, unpublished results). The sizes and

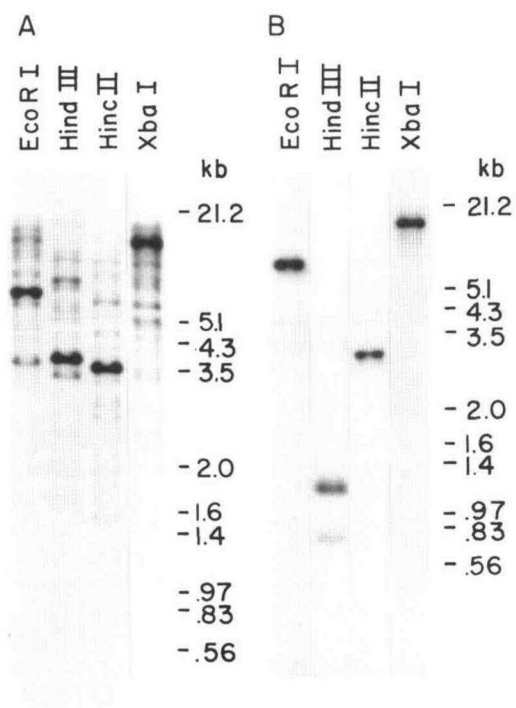

Figure 1. Identification of a *PvPRP1* Gene–Specific Probe by Genomic DNA Gel Blot Analysis.

(A) Genomic DNA was digested with the indicated restriction enzymes, size fractionated on a 0.8% agarose gel (10 μg per lane), and blotted onto a Zeta-probe membrane; the membrane was hybridized with the labeled HincII-HincII fragment of the *PvPRP1* cDNA. EcoRI-digested and HindIII-digested λ DNA was used as a length marker, and lengths are given in kilobases at the right of each gel.

(B) DNA samples from the same digestion reactions were run on a separate gel, and the blot was hybridized with the labeled HincII-EcoRI fragment of the *PvPRP1* cDNA. The lane containing HindIII-digested DNA was from a longer film exposure to show the weakly hybridized 0.75-kb band.

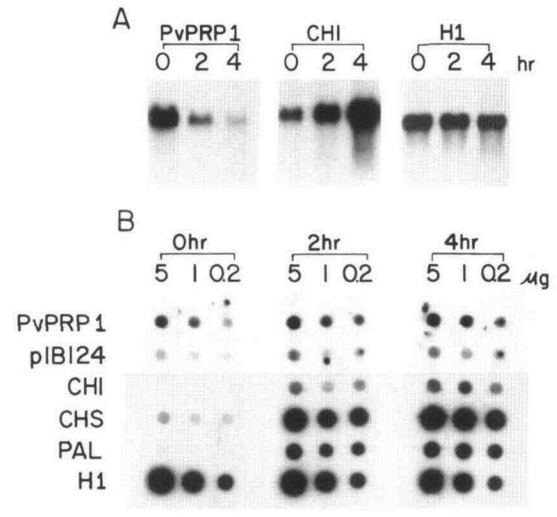

Figure 2. mRNA Steady State Levels and Transcription Rates of Different Genes in Bean Cells Treated with Fungal Elicitor.

(A) RNA gel blot analysis of *PvPRP1*, *CHI*, and *H1* mRNAs in control cells (0 hr) and cells treated with elicitor for 2 and 4 hr was performed. The same blot used for *PvPRP1* hybridization was washed and rehybridized with *CHI* and *H1* probes.

(B) Nuclei were isolated from the same batches of cells as shown in **(A)**, and transcriptional activity was determined by run-off assays. The membrane was dotted with 5, 1, and 0.2 μg of linearized recombinant plasmids, the HincII-EcoRI fragment subclone of the *PvPRP1* cDNA, *CHI1*, *CHS5*, *PAL5*, and *H1*. Vector pIBI24 DNA was also included as a measure of nonspecific hybridization. *PvPRP1* and pIBI24 signals were from an autoradiogram exposed for 14 hr, whereas other signals were from an autoradiogram exposed for 4 hr. The nonlinear increase in the amount of ^{32}P-labeled nuclear RNA that hybridized to the increasing amount of plasmid DNA was due to the excess amount of immobilized DNA relative to its corresponding transcripts in the hybridization solution.

numbers of fragments hybridized by the HincII-EcoRI probe in genomic DNA and the genomic clone DNA digested with HindIII or HincII are also identical. These results provide strong evidence that the HincII-EcoRI probe uniquely hybridizes to the homologous *PvPRP1* gene. The HincII-EcoRI fragment was employed as a gene-specific probe in all subsequent experiments. The genomic organization of the *PvPRP1* gene and related genes presented here is in agreement with our previous conclusions (Sheng et al., 1991). However, the HincII-EcoRI probe previously employed was later found to be contaminated with HincII-HincII sequences and, therefore, erroneously appeared not to be gene specific (Sheng et al., 1991).

Transcription Rate of the *PvPRP1* Gene Is Unaffected by Elicitor as Measured by Run-Off Transcription in Isolated Nuclei

Figure 2A shows that steady state *PvPRP1* mRNA levels decreased rapidly and substantially after the addition of elicitor. Quantitation of the RNA gel blot signals shows that ~6% of the original level remained 4 hr after the addition of elicitor. The same blot was washed and rehybridized with a chalcone isomerase (*CHI*) probe to show appropriate, positive regulation of a known elicitor-inducible mRNA (Figure 2A). (CHI is an enzyme in the phenylpropanoid pathway that is necessary for biosynthesis of antimicrobial phytoalexins [Dixon and Harrison, 1990].) The blot was washed again and rehybridized with the *H1* probe to verify equal RNA loading and transfer in each lane (Figure 2A). *H1* is a constitutive, abundant RNA (Lawton and Lamb, 1987).

To determine whether reduced transcription contributes to the decrease in the *PvPRP1* steady state level, nuclei were isolated from the same batch of cells used for the steady state mRNA determinations, and run-off transcription assays were performed. Specific ^{32}P-labeled transcripts were detected by hybridization to different amounts of immobilized DNA sequences. Figure 2B shows that there is little change in the level of nascent *PvPRP1* transcripts over a 4-hr period after the addition of elicitor. After subtraction of the background hybridization to the pIBI24 vector, the *PvPRP1* transcription rates in cells treated with elicitor for 2 and 4 hr were 1- and 1.1-fold, respectively, the rate in control cells at 0 hr, as shown in Table 1.

As controls for the run-off assay, the transcription rates of genes known to be transcriptionally activated by elicitor treatment were monitored. These genes encode enzymes that catalyze steps in phytoalexin biosynthesis: CHI, chalcone synthase (CHS), and phenylalanine ammonia-lyase (PAL). The increased transcription of *CHI*, *CHS*, and *PAL* genes measured after elicitation (Table 1) are comparable to the increased transcription rates of these genes reported previously using the same assay (Lawton and Lamb, 1987; Hedrick et al., 1988). Elicitor had no effect on *H1* gene transcription (Figure 2B and Table 1; Lawton and Lamb, 1987). These results indicate that the elicitor-induced decrease in the steady state level of *PvPRP1* mRNA is not due to reduced gene transcription but rather to destabilization of the mRNA.

Table 1. Relative Transcription Rates of *PvPRP1*, *CHI*, *CHS*, *PAL*, and *H1* Genes in Control and Elicitor-Treated Cells

	Time of Elicitor Treatment		
RNA Species	0 hr	2 hr	4 hr
PvPRP1	1.0	1.0	1.1
CHI	1.0	14.7	18.1
CHS	1.0	13.6	15.8
PAL	1.0	18.4	19.2
H1	1.0	1.0	1.0

The ^{32}P-labeled nuclear RNAs from run-off transcription hybridized to membranes with 5 μg of immobolized plasmid DNAs were quantified by scanning densitometry. The values representing hybridization to the vector pIBI24 were subtracted as background. The transcription rates at 0 hr were standardized to 1.0.

Destabilization of *PvPRP1* Occurs in Elicitor-Treated Cells and Is Dependent on Transcription

Figures 3A and 4A show that unelicited cells maintained the *PvPRP1* mRNA at a stable level throughout the sampling period. In elicited cells, the *PvPRP1* mRNA level remained stable for about 1 hr and then decreased dramatically. Within 3 hr, the mRNA level decreased to ~6% of the original level, indicating an apparent half-life of ~45 min during the rapid loss. Because transcription is ongoing in these elicited cells, the mRNA degradation kinetics provide an apparent half-life that represents the upper limit of the actual half-life (Belanger et al., 1986; Byrne et al., 1993).

To directly evaluate whether the half-life of *PvPRP1* mRNA decreases in elicited cells, cells were pretreated with actinomycin D to block transcription and then treated with water or elicitor; the decay kinetics of the *PvPRP1* mRNA were monitored by RNA gel blot analysis (Figures 3A and 4A). In cells treated with actinomycin D and water, the *PvPRP1* transcript had a half-life of about 60 hr, as calculated from the slope of

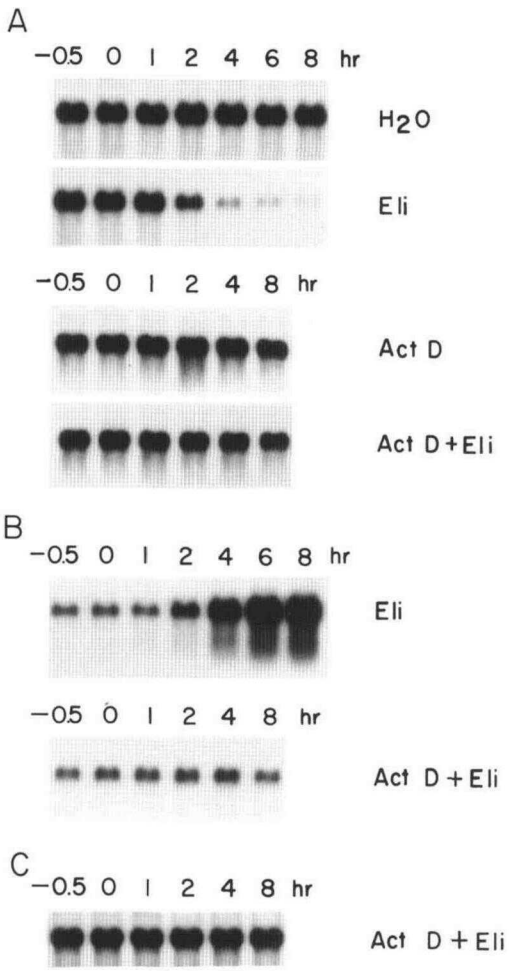

Figure 3. Effects of Actinomycin D Pretreatment on *PvPRP1*, *CHI*, and *H1* Transcript Levels in Control and Elicitor-Treated Cells.

(A) Total RNA from control cells (H_2O) and cells treated with elicitor (Eli), actinomycin D (Act D), and actinomycin D plus elicitor (Act D + Eli) was isolated at the indicated times and analyzed by RNA gel blot hybridization with the *PvPRP1* probe. Actinomycin D or water was added at −0.5 hr, and elicitor or water was added at 0 hr.

(B) The same blots were washed and rehybridized with the *CHI* probe. Only results from cells treated with elicitor and actinomycin D plus elicitor are shown.

(C) The same blots were washed again and rehybridized with the *H1* probe to show the equal RNA loading. Only the result from cells treated with actinomycin D plus elicitor is shown.

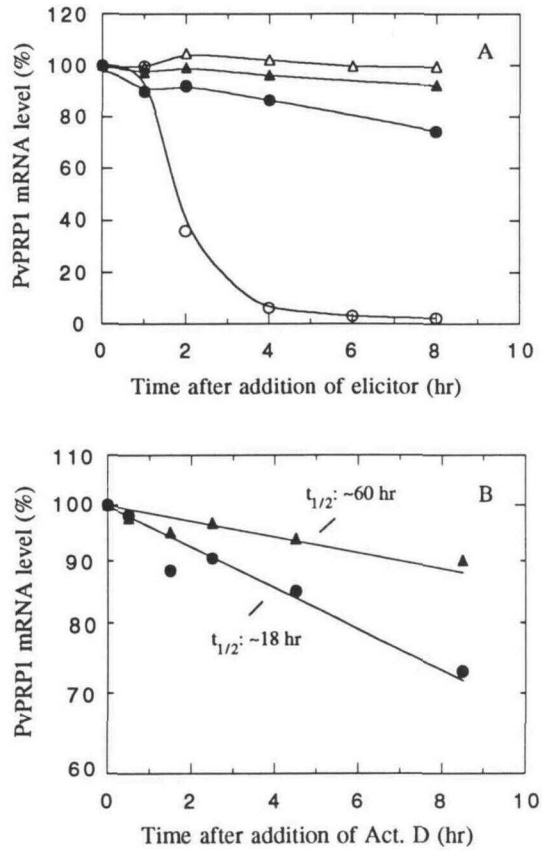

Figure 4. Quantitative Analysis of *PvPRP1* mRNA Levels.

(A) Time courses of *PvPRP1* mRNA levels, which are shown in Figure 3, in cells treated with water (△), elicitor (○), actinomycin D (▲), or actinomycin D plus elicitor (●). RNA levels are expressed as the percentage of 0 hr basal levels. Data shown represent one of two independent experiments that gave similar results.

(B) The semi-log plot of the data in **(A)** for cells treated with actinomycin D (▲; Act. D) or actinomycin D plus elicitor (●) from which half-lives ($t_{1/2}$) were calculated as described in Methods. mRNA levels at the time of actinomycin D addition were standardized to 100%, and 0 hr on the x-axis represents the time of actinomycin D addition.

the line in the semi-log plot of the data (Figure 4B). In cells treated with actinomycin D and elicitor, *PvPRP1* mRNA had a half-life of about 18 hr (Figure 4B). These data indicate that *PvPRP1* mRNA stability decreases in the presence of elicitor. However, the reduced degradation of the mRNA in cells pretreated with actinomycin D and then with elicitor compared to cells treated with elicitor alone suggests that the transcription of another gene(s) is required for maximal elicitor-induced destabilization of *PvPRP1* mRNA.

It is well documented that *CHI*, *CHS*, and *PAL* mRNAs increase in elicited cells due to activation of transcription (Lawton and Lamb, 1987; Hedrick et al., 1988). To assess the efficacy of transcription inhibition by actinomycin D, the same blots were rehybridized with ^{32}P-labeled probes to these genes. Figure 3B shows that the induction of *CHI* mRNA by elicitor was almost completely blocked by actinomycin D. Quantitative analysis showed that only about 5% of the elicitor-induced increase occurred in cells treated with actinomycin D and elicitor. Similar results were obtained for *CHS* and *PAL* mRNA (data not shown). These results indicate that the actinomycin D treatment effectively inhibited transcription in the bean cultures.

To investigate whether the observed reduction in *PvPRP1* mRNA decay in cells treated with actinomycin D and elicitor reflected a general phenomenon, we examined β-tubulin mRNA levels in the same samples. Figures 5A and 5B show that β-tubulin mRNA levels were stable in unelicited cells in the absence of actinomycin D, whereas the mRNA rapidly decreased with no apparent lag in cells treated with elicitor only. Because transcription was ongoing in these cells, only the apparent mRNA half-life of ~1 hr in elicited cells could be estimated. By using actinomycin D to block transcription, β-tubulin mRNA half-lives were calculated to be ~3 hr in unelicited cells and ~1 hr in elicited cells (Figure 5B). The close agreement between the half-life of β-tubulin in elicited cells measured by blocking transcription and the apparent half-life estimated from degradation kinetics in the absence of the inhibitor indicates that, in contrast to the *PvPRP1* mRNA, actinomycin D did not slow the elicitor-induced degradation of the β-tubulin mRNA.

The inhibition of *PvPRP1* mRNA degradation in cells treated with actinomycin D prior to the addition of elicitor suggests that synthesis of an RNA(s) or protein(s) is necessary for maximal mRNA degradation. Therefore, cells treated first with elicitor followed by delayed additions of actinomycin D may exhibit half-lives that correspond more closely to the actual half-life in elicited cells. Figure 6A shows *PvPRP1* mRNA levels in cells treated with elicitor at 0 hr and then actinomycin D at 1 or 2 hr. Figure 6B shows the semi-log plots of the *PvPRP1* mRNA levels as a function of time after the addition of actinomycin D. The mRNA decay data are best fit by two components. The first components are characterized by more rapid mRNA decay and occur during the first 2 hr after the additions of actinomycin D. Analysis of *CHI* mRNA accumulation in the same experimental samples showed little inhibition of mRNA accumulation during the first 2 hr after the addition of actinomycin D and was followed by complete inhibition of mRNA accumulation by about 2.5 hr (data not shown). Thus, there was little inhibition of transcription during the first 2 hr, and the rates of *PvPRP1* mRNA decay of the first components were similar to the rate of mRNA degradation in cells treated only with elicitor. Therefore, the data represented by the first components' half-lives could not be used to determine the actual *PvPRP1* mRNA half-lives. The delayed effects of the additions of inhibitor were likely to be due to the time involved in uptake of actinomycin D to inhibitory levels; others have observed similar delays in the action of transcription inhibitors, including actinomycin D, with plant cells (Guerrero and Mullet, 1986). The second components have half-lives of ~5.5 and ~6.5 hr for *PvPRP1* in cells treated with actinomycin D at 1 and 2 hr

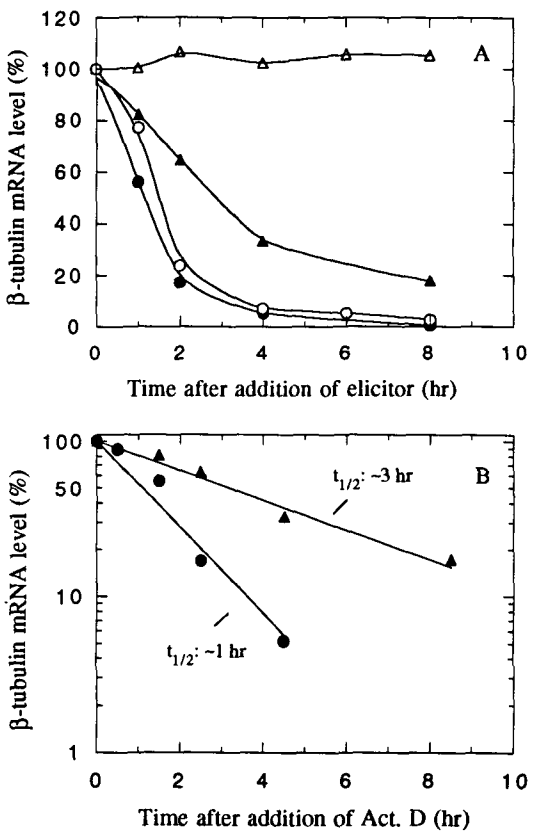

Figure 5. Effects of Actinomycin D Pretreatment on β-Tubulin mRNA Levels in Control and Elicitor-Treated Cells.

(A) Time courses of β-tubulin mRNA levels in the same cell cultures as described in Figure 3. RNA was analyzed in cells treated with water (△), elicitor (○), actinomycin D (▲), or actinomycin D plus elicitor (●). RNA levels are expressed as the percentage of 0 hr basal levels. **(B)** The semi-log plot of the data in **(A)** for cells treated with actinomycin D (▲; Act. D) or actinomycin D plus elicitor (●) from which half-lives ($t_{½}$) were calculated as described in Methods. mRNA levels at the time of actinomycin D addition were standardized to 100%, and 0 hr on the x-axis represents the time of actinomycin D addition.

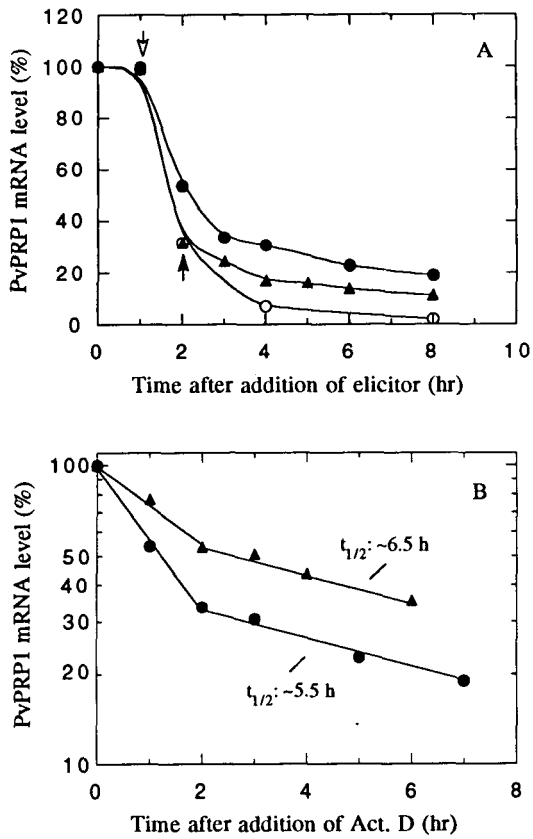

Figure 6. Effects of Elicitor Treatment Followed by Actinomycin D Treatments on *PvPRP1* mRNA Levels in Bean Cultures.

(A) Time courses of *PvPRP1* mRNA levels in cells treated with elicitor at 0 hr followed by the addition of water at 1 hr (○), actinomycin D at 1 hr (●), or actinomycin D at 2 hr (▲). RNA levels are expressed as the percentage of the basal levels at the time of elicitor addition. Arrows indicate the times when actinomycin D was added.

(B) Semi-log plots of the data in **(A)** for cells treated with actinomycin D (Act. D) at 1 hr (●) and 2 hr (▲) after the addition of elicitor. mRNA levels at the time of actinomycin D addition were standardized to 100%, and 0 hr on the x-axis represents the time of actinomycin D addition. $t_{1/2}$, half-life.

after elicitation, respectively. These half-lives are considerably longer than the apparent half-life of 45 min in cells treated with elicitor alone and suggest that ongoing transcription is required for maximal degradation of the *PvPRP1* mRNA even after elicitation.

Protein Synthesis Inhibitors Also Inhibit the Destabilization of *PvPRP1* mRNA Induced by Elicitor

To further investigate the *PvPRP1* mRNA destabilization process, we determined the effects of translational inhibitors on the *PvPRP1* mRNA degradation in elicited cells. If a newly synthesized protein factor(s) or labile protein factor(s) is involved in the destabilization of *PvPRP1* mRNA, then the inhibition of translation should also stabilize the *PvPRP1* mRNA in cells treated with elicitor. Table 2 shows that the uptake of ^{35}S-methionine was unaffected by the inhibitors under all experimental conditions. Higher concentrations of inhibitors gave no or little additional inhibition. Supplementing the inhibitors with 100 μg/mL of chloramphenicol, an organelle translation inhibitor, resulted in little additional inhibition, probably because the dark-grown bean cultures are nonphotosynthetic (data not shown). Cycloheximide, an inhibitor of translocase (Galling, 1982), had little effect on protein synthesis (Table 2). In independent experiments, cycloheximide inhibited protein synthesis by no more than 10%. Figures 7 and 8 show that cycloheximide had little effect on the destabilization of *PvPRP1* mRNA in elicitor-treated cells.

Emetine, also an inhibitor of translocase (Galling, 1982), blocked protein synthesis by ~58%. Cells treated with emetine exhibited a reduced rate of *PvPRP1* mRNA decay after elicitor treatment (Figures 7 and 8). While elicitor treatment alone resulted in ~98% decrease of *PvPRP1* mRNA over 8 hr, cells treated with emetine and elicitor exhibited only ~50% reduction over the same time period; i.e., the degradation was inhibited by ~50% (Figure 8).

Anisomycin, an inhibitor of transpeptidase (Galling, 1982), resulted in the most effective inhibition of protein synthesis

Table 2. Efficacies of Protein Synthesis Inhibitors as Measured by ^{35}S-Methionine Incorporation

	^{35}S-Methionine Uptake		^{35}S-Methionine Incorporation	
Inhibitor	× 10^4 cpm/μg Protein	% of Control	× 10^3 cpm/μg Protein	% of Control
Control	1.86	100	3.05	100
Cycloheximide (15 μg/mL)	2.09	112	3.13	102
Emetine (150 μg/mL)	1.93	104	1.29	42
Anisomycin (80 μg/mL)	1.85	99	0.61	20

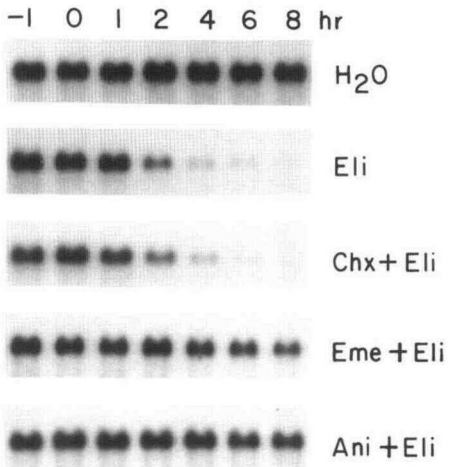

Figure 7. Effects of Protein Synthesis Inhibitors on *PvPRP1* mRNA Degradation Induced by Fungal Elicitor.

Total RNA from cells treated with water (H_2O), elicitor (Eli), cycloheximide plus elicitor (Chx + Eli), emetine plus elicitor (Eme + Eli), or anisomycin plus elicitor (Ani + Eli) for various times was analyzed by RNA gel blotting using the *PvPRP1* probe. Protein synthesis inhibitors or water was added at −1 hr, and elicitor or water was added at 0 hr. The same membrane was also washed and rehybridized with the *H1* probe to demonstrate equal RNA loading (data not shown).

(~80%) compared to the other two inhibitors (Table 2). The use of this inhibitor also resulted in the greatest inhibition (~75%) of elicitor-induced *PvPRP1* mRNA degradation (Figures 7 and 8). Neither emetine nor anisomycin detectably affected the *PvPRP1* mRNA abundance in unelicited cells (data not shown). These data further suggest that a de novo–synthesized or labile protein factor(s) is needed for the rapid degradation of *PvPRP1* mRNA in elicitor-treated cells.

DISCUSSION

In this study, we have investigated the mechanism of *PvPRP1* mRNA loss during the plant defense response to fungal elicitor treatment. We showed that the destabilization of *PvPRP1* mRNA is the principal factor affecting *PvPRP1* gene expression in cells treated with fungal elicitor. In unelicited cells, the *PvPRP1* mRNA half-life is ~60 hr. The rapid degradation of *PvPRP1* mRNA in elicitor-treated cells was slowed under conditions of blocked transcription and translation. Therefore, the *PvPRP1* mRNA half-life in elicited cells is best estimated from cells with ongoing transcription, which establishes an upper limit of 45 min for the half-life. Transcriptional regulation of the *PvPRP1* gene is not a factor in the rapid loss of the mRNA because the *PvPRP1* transcription rate remained constant for at least 4 hr after the addition of elicitor. This conclusion is based on the assumption that the nuclear run-off transcription assay accurately reflects in vivo transcriptional activity, an assumption that has been supported by results obtained for numerous plant and animal genes (Hofer et al., 1982; Walling et al., 1986).

Endogenous or exogenous signals change the half-lives of a number of mRNAs. The long half-life of the *PvPRP1* mRNA in unelicited cells is similar to the half-lives of many mRNAs in plant and animal cells during physiological states of the cells in which the encoded proteins are being actively synthesized. The average half-lives of one group of poly(A)$^+$ RNAs present in soybean cultured cells were estimated to be 30 hr (Silflow and Key, 1979). In the aleurone layers of gibberellin A_3–treated barley seeds, α-amylase mRNA has a half-life of greater than 100 hr. After heat shock treatment, the α-amylase mRNA half-life is reduced to less than 2 hr (Belanger et al., 1986; Brodl and Ho, 1991). Under conditions of iron starvation, the transferrin receptor mRNA in human cells is also very stable, with a half-life of ~30 hr. After the addition of iron, the transferrin receptor mRNA decays with a half-life of 1.5 hr, while transcription of the transferrin receptor gene remains constant (Müllner and Kühn, 1988). Because the *PvPRP1* mRNA is very likely to encode a structural protein in the cell wall, the long mRNA half-life would augment the transcription rate in supporting the synthesis of this protein for growing cells.

Recognition of a pathogen or pathogen-derived elicitors mobilizes massive biochemical changes in plant cells, including the selective transcriptional activation of many genes (Dixon and Harrison, 1990). Another valuable component of the defense response to pathogens appears to be the degradation of preexisting mRNAs whose products may interfere with the protective mechanisms against the pathogen. The reinforcement of the cell wall after infection or wounding involves the

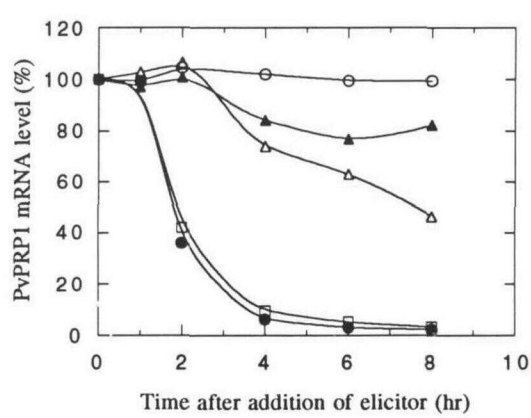

Figure 8. Quantitative Analysis of *PvPRP1* mRNA Levels.

Time courses of *PvPRP1* mRNA levels, which are shown in Figure 7, in cells treated with water (○), elicitor (●), cycloheximide plus elicitor (□), emetine plus elicitor (△), or anisomycin plus elicitor (▲) are given. RNA levels are expressed as the percentage of the 0 hr basal levels.

increased transcription of hydroxyproline-rich glycoprotein and proline-rich protein genes (Lawton and Lamb, 1987; Tierney et al., 1988). The encoded proteins are rich in tyrosine and are capable of insolubilization in the wall, presumably by isodityrosine cross-links. Insolubilization of these proteins has been correlated with a greater resistance to wall-degrading enzymes from phytopathogenic fungi (Bradley et al., 1992). A complementary mechanism to strengthen the cell wall may be to reduce the synthesis of low-tyrosine content structural proteins such as PvPRP1, which may form less cross-links. The destabilization of the *PvPRP1* mRNA is an efficient mechanism for clearing this mRNA from the cytoplasm, whereas cessation of gene transcription would have little effect for many hours. It is not known whether the other elicitor down-regulated mRNAs identified by cDNA cloning (Sauer et al., 1990) or in vitro translation (Cramer et al., 1985; Dalkin et al., 1990) are also regulated by differential mRNA stability. The response of plants to heat shock has also been shown to involve transcriptional activation of the heat shock protein genes and selective destabilization of existing mRNAs (Belanger et al., 1986; Nover, 1989; Brodl and Ho, 1991).

Although actinomycin D pretreatment reduced the degradation of the *PvPRP1* mRNA in elicitor-treated cells, there was no detectable effect of actinomycin D on β-tubulin mRNA degradation in these cells. These data show that the actinomycin D–treated cells were not generally inhibited for degradation of cellular mRNAs and indicate the existence of multiple mRNA degrading pathways. We showed that elicitor treatment decreased the β-tubulin mRNA half-life from ~3 to ~1 hr. The β-tubulin mRNA half-life in etiolated oat leaves was shown to be about 1.5 hr (Byrne et al., 1993). In a number of mammalian cell cultures, α-tubulin and β-tubulin mRNA half-lives are 1 to 2 hr, and these mRNAs are destabilized by high levels of tubulin heterodimers (Cleveland et al., 1981; Pachter et al., 1987; Gay et al., 1989). It is unknown whether fungal elicitor treatment of plant cells triggers β-tubulin mRNA decay by a similar mechanism.

A strong positive correlation was obtained between efficacy of inhibition of protein synthesis and inhibition of *PvPRP1* mRNA destabilization, indicating that the destabilization of *PvPRP1* mRNA is also dependent on translation. The different protein synthesis inhibitors varied considerably in their efficacies in blocking translation. This is presumably due to their variable uptake by the bean cells because all of these compounds are potent inhibitors of in vitro translation (Galling, 1982). The efficacies of protein synthesis inhibitors may differ among plant species. Emetine and anisomycin treatment of wheat seedlings provided 0 and 40% inhibition, respectively (Lam et al., 1989). In contrast, both inhibitors blocked protein synthesis in pea seedlings by 85% (Theologis et al., 1985).

The kinetics of the *PvPRP1* mRNA decay in elicited cells and its transcription and translation dependence are consistent with several models, but we will briefly consider only two. The simplest model is that elicitor induces the expression of a gene encoding a protein that is involved in the degradation of the *PvPRP1* mRNA. The observed 1-hr lag period before mRNA decay could be due to the time needed for transcription and the intervening steps prior to sufficient accumulation of the protein to participate in the decay process. A second model is that elicitor causes an alteration in the activity of an existing protein that is necessary for the rapid degradation of *PvPRP1* mRNA. In this model, either the activated protein or another protein involved in the degradation process must be labile and be encoded by a short-lived mRNA to account for the inhibitor results. In either model, the predicted protein(s) may be a sequence-specific ribonuclease or an RNA binding protein which targets the mRNA for degradation by a general ribonuclease.

Additional features of the *PvPRP1* mRNA degradation process in elicitor-treated cells are suggested by the experiments involving prior or delayed addition of actinomycin D. In cells pretreated with the inhibitor, the *PvPRP1* mRNA half-life is ~18 hr, which is approximately threefold less than the half-life in unelicited cells. Possible explanations are as follows: (1) the low residual transcription activity in actinomycin D–treated cells can still supply some de novo–synthesized *trans*-acting factor(s) required for the rapid degradation; (2) elicitor activates *PvPRP1* mRNA degradation to an intermediate rate by a transcription-independent process, but the maximal rate of degradation requires transcription. In elicitor-treated cells, the *PvPRP1* mRNA half-life was shorter in cells that received delayed additions of actinomycin D (~6 hr) compared to cells pretreated with the inhibitor (~18 hr) but longer than the apparent half-life in cells treated with elicitor alone. These observations suggest that cells given later additions of actinomycin D accumulated more of a critical protein(s) involved in the mRNA degradation and therefore sustained a higher rate of *PvPRP1* mRNA degradation relative to the pretreated cells. Furthermore, these results indicate that ongoing transcription is necessary even several hours after elicitor treatment for maximal *PvPRP1* mRNA degradation.

The transcription and translation dependence of *PvPRP1* mRNA rapid degradation is similar to the features of degradation pathways for a number of mRNAs. In plants, potato *rbcS* mRNA was degraded more slowly in the dark in the presence of cordycepin, a transcription inhibitor (Fritz et al., 1991). The rapid turnover of phytochrome mRNA in etiolated oat seedlings was inhibited by both cordycepin and cycloheximide (Colbert et al., 1991; Seeley et al., 1992). The activities of different proteins binding to c-*fos* and lymphokine mRNAs were reduced in extracts from cells treated with transcription and translation inhibitors (Bohjanen et al., 1991; You et al., 1992). The transferrin receptor and c-*myc* mRNAs are stabilized in the presence of protein synthesis inhibitors (Koeller et al., 1991; Wisdom and Lee, 1991). Our findings together with these previous studies suggest that the rapid degradation of many mRNAs in both plant and animal cells involves new RNA and protein synthesis.

In mammalian proto-oncogene mRNAs, the occurrence of AUUUA motifs in the 3′ untranslated regions has been implicated as one determinant resulting in the instability of these mRNAs (Jones and Cole, 1987; Wilson and Treisman, 1988).

There are two AUUUA motifs in the 3′ untranslated region of *PvPRP1* mRNA located 27 and 121 nucleotides downstream from the termination of the coding region. We are currently exploring the molecular mechanisms governing *PvPRP1* mRNA decay by examining the interactions of *cis*-determinants, such as the AUUUA motif, and RNA binding proteins.

METHODS

Plant Cell Culture and Treatment with Elicitor and Inhibitors

Suspension cultures of bean (*Phaseolus vulgaris* cv Immuna) cells were maintained in darkness at 23°C and subcultured every 2 weeks as described previously (Dixon and Bendall, 1978). Experiments were conducted with flasks of cells 8 to 10 days after subculturing from cells at equivalent stages in the growth cycle. The growth media of the subcultures exhibited conductivities between 2500 and 2800 Ω^{-1}/cm. Elicitor was prepared from the heat-released cell wall fraction of fungal pathogen *Colletotrichum lindemuthianum* as described previously (Anderson-Prouty and Albersheim, 1975) and was used at the final concentration of 60 μg of glucose equivalents per mL. Actinomycin D (Sigma) was applied at the final concentration of 100 μg/mL. Cycloheximide, emetine, and anisomycin (Sigma) were used at final concentrations of 15, 150, and 80 μg/mL, respectively, and were added 1 hr before elicitor or water treatment. Plant cells were harvested by filtration through Miracloth (Calbiochem), frozen in liquid nitrogen, and stored at −70°C.

Genomic DNA Isolation and DNA Gel Blot Analysis

Total genomic DNA was isolated from bean leaves (cv Tendergreen), digested, blotted onto Zeta-probe membranes (Bio-Rad Laboratories), and hybridized essentially as described previously (Sheng et al., 1991). The blots were hybridized with the indicated ^{32}P-labeled probes in 0.5 M NaH_2PO_4, pH 7.2, 7% SDS, and 1 mM EDTA at 65°C for 24 hr. The HincII-Hinc II probe (~600 bp) contains the 5′ end of the *PvPRP1* cDNA and terminates at the HincII site at position 588 in the *PvPRP1* cDNA (Sheng et al., 1991). The HincII-EcoRI probe is bound by the HincII site at position 588 and the downstream EcoRI site in the linker used during cDNA cloning. The blots were washed twice in 40 mM NaH_2PO_4, pH 7.2, 5% SDS, and 1 mM EDTA at 65°C and twice in 40 mM NaH_2PO_4, pH 7.2, 1% SDS, and 1 mM EDTA at 65°C for 30 min each and exposed to x-ray film at −70°C.

RNA Isolation and RNA Gel Blot Analysis

Total RNA was isolated from 1.5 to 2 g of cells for each sample by the phenol-SDS method with cetyltrimethyl-ammonium bromide treatment (Mehdy and Lamb, 1987; Cox and Goldberg, 1988). For RNA gel blot analysis, 7.5 μg of total RNA was denatured, size fractionated on 1.2% formaldehyde agarose gels, and transferred onto Zeta-probe membranes with 10 × SSC (1 × SSC is 0.15 M NaCl, 0.15 M sodium citrate); the RNA was UV cross-linked to the membrane. ^{32}P-labeled probes were phenylalanine ammonia-lyase (PAL) cDNA *PAL5* (Edwards et al., 1985), chalcone synthase (CHS) cDNA *CHS5* (Ryder et al., 1984), chalcone isomerase (CHI) cDNA *CHI1* (Mehdy and Lamb, 1987), the HincII-EcoRI fragment of the *PvPRP1* cDNA (Sheng et al., 1991), the *H1* cDNA (Lawton and Lamb, 1987), and soybean tubulin *SB2X3* (Han et al., 1991). Hybridization and washing condition were the same as described for DNA gel blot analysis, except that *SB2X3* was hybridized as previously described (Lambais and Mehdy, 1993). After autoradiography, specific transcripts were quantitated by scanning densitometry in the linear range of the film response using an absorbance/fluorescence detector (model UA-5; ISCO, Lincoln, NE). The linear range of film response was established by scanning densitometry of signals from a dilution series of radioactivity. When necessary, multiple exposures were made to obtain signals in the linear range of film sensitivity and normalized for the time of exposure to compare different signals on the same blot.

Nuclear Run-Off Experiment

Nuclei were isolated according to the method of Cox and Goldberg (1988) from 5 g of bean cells and stored at −70°C. Runoff transcription reactions (100 μL) contained nuclei equivalent to 100 μg of DNA and 125 μCi of α-^{32}P UTP (800 Ci/mmol) as described by Cox and Goldberg (1988). After RQ1 DNase and proteinase K digestions, 50 μg of tRNA was added. The mixtures were extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1), back-extracted, and extracted once with chloroform/isoamyl alcohol (24:1). RNA was precipitated by adding one-tenth volume of 3 M sodium acetate, pH 6.0, and 2.5 volume of ethanol. The pellet was dissolved in 200 μL of diethylpyrocarbonate-treated H_2O and then passed through a 1-mL Sephadex G-50 spin column (Pharmacia). ^{32}P-labeled nuclear RNAs (4×10^7 cpm) from each sample were hybridized to nitrocellulose membranes dotted with 5, 1, and 0.2 μg of linearized, denatured recombinant plasmid DNAs as described by Cox and Goldberg (1988) as Method 1, except that the hybridization time was extended to 48 hr. The filters were rinsed with 2 × SSC and washed three times with 0.2 × SSC, 0.01% SDS at 68°C for 1.5 hr. The amounts of linearized plasmid DNAs were determined by spectrophotometric quantitation. Hybridized transcripts were detected by autoradiography of the filters and quantified by scanning densitometry of films as detailed above.

Determination of mRNA Half-Life

PvPRP1 mRNA and β-tubulin mRNA half-lives were determined by monitoring mRNA decay after actinomycin D treatment. RNA levels were analyzed by RNA gel blotting and quantitated as described above. Because mRNA decay follows first-order kinetics, half-life was calculated using the equation: $t_{1/2} = 0.693 / (2.303 \times$ the slope of the line in the semi-log plot of $\log_{10}$ mRNA versus time). The slope is $\log_{10}(N_o/N)/t$, where N_o is the initial amount of mRNA, N is the amount of mRNA at time t, and t is the elapsed time. Under conditions of ongoing transcription, the apparent $t_{1/2}$ was estimated from mRNA degradation kinetics that defines the upper limit of the turnover rate (Belanger et al., 1986; Byrne et al., 1993).

Determination of Effectiveness of Protein Synthesis Inhibitors

The effect of protein synthesis inhibitors on global protein synthesis was assessed by measuring ^{35}S-methionine incorporation into trichloroacetic acid–insoluble material according to the method of Theologis

et al. (1985) with the following modifications. Cells were preincubated with an inhibitor or H_2O for 1 hr before adding ^{35}S-methionine to 10 μCi/mL (1000 Ci/mmol, Amersham Corp.). Unlabeled L-methionine was also added to a 50-μM final concentration to facilitate the uptake of labeled methionine. After a 3-hr incubation, cells were washed extensively with 20 mM Hepes, pH 7.5, and then disrupted in 1 mL of 50 mM Hepes, pH 7.9, 0.1 M methionine buffer by sonication. The homogenate was centrifuged at 13,000 rpm at 4°C for 10 min. ^{35}S-methionine uptake was determined by counting 10 μL of the supernatant. The incorporation of ^{35}S-methionine into trichloroacetic acid–precipitable protein was determined by using 100-μL aliquots. Protein concentrations were determined using the Bio-Rad Protein Assay method.

ACKNOWLEDGMENTS

We thank Christopher Lamb for providing the *CHS*, *PAL*, and *H1* clones and Don Fosket for providing the *SB2X3* clone. This work was supported by National Science Foundation Grant No. DCB-8810549 to M.C.M.

Received June 17, 1993; accepted July 1, 1993.

REFERENCES

Anderson-Prouty, A.J., and Albersheim, P. (1975). Host-pathogen interactions, VIII: Isolation of a pathogen-synthesized glucan that elicits a defense response in the pathogen's host. Plant Physiol. **56,** 286–291.

Belanger, F.C., Brodl, M.R., and Ho, T.-H.D. (1986). Heat shock causes destabilization of specific mRNAs and destruction of endoplasmic reticulum in barley aleurone cells. Proc. Natl. Acad. Sci. USA **83,** 1354–1358.

Bohjanen, P.R., Petryniak, B., June, C.H., Thompson, C.B., and Lindsten, T. (1991). An inducible cytoplasmic factor (AU-B) binds selectively to AUUUA multimers in the 3′ untranslated region of lymphokine mRNA. Mol. Cell. Biol. **11,** 3288–3295.

Bradley, D.J., Kjellbom, P., and Lamb, C.J. (1992). Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response. Cell **70,** 21–30.

Brodl, M.R., and Ho, T-h.D. (1991). Heat shock causes selective destabilization of secretory protein mRNAs in barley aleurone cells. Plant Physiol. **96,** 1048–1052.

Byrne, D.H., Seeley, K.A., and Colbert, J.T. (1993). Half-lives of oat mRNAs in vivo and in a polysome-based in-vitro system. Planta **189,** 249–256.

Casey, J.L., Hentze, M.W., Koeller, D.M., Caughman, S.W., Rouault, T.A., Klausner, R.D., and Harford, J.B. (1988). Iron-responsive elements: Regulatory RNA sequences that control mRNA levels and translation. Science **240,** 924–928.

Cleveland, D.W., Lopata, M.A., Sherline, P., and Kirschner, M.W. (1981). Unpolymerized tubulin modulates the level of tubulin mRNAs. Cell **25,** 537–546.

Colbert, J.T., Costigan, S.A., Avissar, P., and Zhao, Z. (1991). Regulation of phytochrome gene expression. J. Iowa Acad. Sci. **98,** 63–67.

Cox, K.H., and Goldberg, R.B. (1988). Analysis of plant gene expression. In Plant Molecular Biology: A Practical Approach, C.H. Shaw, ed (Oxford: IRL Press), pp. 1–35.

Cramer, C.L., Ryder, T.B., Bell, J.N., and Lamb, C.J. (1985). Rapid switching of plant gene expression induced by fungal elicitor. Science **227,** 1240–1243.

Dalkin, K., Jorrin, J., and Dixon, R.A. (1990). Stress responses in alfalfa (*Medicago sativa* L.), VII: Induction of defense related mRNAs in elicitor-treated cell suspension cultures. Physiol. Mol. Plant Pathol. **37,** 293–307.

Dixon, R.A., and Bendall, D.B. (1978). Changes in the levels of enzymes of phenylpropanoid and flavonoid synthesis during phaseolin production in cell suspension cultures of *Phaseolus vulgaris*. Physiol. Plant Pathol. **13,** 295–306.

Dixon, R.A., and Harrison, M.J. (1990). Activation, structure, and organization of genes involved in microbial defense in plants. Adv. Genet. **28,** 165–234.

Edwards, K., Cramer, C.L., Bolwell, G.P., Dixon, R.A., Schuch, W., and Lamb, C.J. (1985). Rapid transient induction of phenylalanine ammonia-lyase mRNA in elicitor-treated bean cells. Proc. Natl. Acad. Sci. USA **82,** 6731–6735.

Fritz, C.C., Herget, T., Wolter, F.P., Schell, J., and Schreier, P.H. (1991). Reduced steady-state levels of rbcS mRNA in plants kept in the dark are due to differential degradation. Proc. Natl. Acad. Sci. USA **88,** 4458–4462.

Galling, G. (1982). Use (and misuse) of inhibitors in gene expression. Encycl. Plant Physiol. New Ser. **B14,** 663–677.

Gay, D.A., Sisodia, S.S., and Cleveland, D.W. (1989). Autoregulatory control of β-tubulin mRNA stability is linked to translation elongation. Proc. Natl. Acad. Sci. USA **86,** 5763–5767.

Guerrero, F., and Mullet, J.E. (1986). Increased abscisic acid biosynthesis requires transcription. Plant Physiol. **80,** 588–591.

Han, I.-S., Jongewaard, I., and Fosket, D.E. (1991). Limited expression of a divergent β-tubulin gene during soybean (*Glycine max* (L.) Merr.) development. Plant Mol. Biol. **16,** 225–234.

Hedrick, S.A., Bell, J.N., Boller, T., and Lamb, C.J. (1988). Chitinase cDNA cloning and mRNA induction by fungal elicitor, wounding, and infection. Plant Physiol. **86,** 182–186.

Hofer, E., Hofer-Warbinek, R., and Darnell, J.E., Jr. (1982). Globin RNA transcription: A possible termination site and demonstration of transcriptional control correlated with altered chromatin structure. Cell **29,** 887–893.

Jones, T.R., and Cole, M.D. (1987). Rapid cytoplasmic turnover of c-*myc* mRNA: Requirement of 3′-untranslated sequences. Mol. Cell. Biol. **7,** 4513–4521.

Koeller, D.M., Horowitz, J.A., Casey, J.L., Klausner, R.D., and Harford, J.B. (1991). Translation and stability of mRNAs encoding the transferrin receptor and *c-fos*. Proc. Natl. Acad. Sci. USA **88,** 7778–7782.

Kruijer, W., Cooper, J.A., Hunter, T., and Verma, I.M. (1984). Platelet-derived growth factor induces rapid but transient expression of the c-*fos* gene and protein. Nature **312,** 711–716.

Laird-Offringa, I.A. (1992) What determines the instability of c-*myc* proto-oncogene mRNA? BioEssays **12,** 119–124.

Lam, E., Green, P.R., Wong, M., and Chua, N.-H. (1989). Phytochrome activation of two nuclear genes requires cytoplasmic protein synthesis. EMBO J. **8,** 2777–2783.

Lambais, M.R., and Mehdy, M.C. (1993). Suppression of endochitinase, β-1,3-endoglucanase, and chalcone isomerase expression in bean vesicular-arbuscular mycorrhizal roots under different soil phosphate conditions. Mol. Plant-Microbe Interact. **6,** 75–83.

Lawton, M.A., and Lamb, C.J. (1987). Transcriptional activation of plant defense genes by fungal elicitor, wounding and infection. Mol. Cell. Biol. **7,** 335–341.

Mehdy, M., and Lamb, C.J. (1987). Chalcone isomerase cDNA cloning and mRNA induction by fungal elicitor, wounding and infection. EMBO J. **6,** 1527–1533.

Müller, R., Bravo, R., Burckhardt, J., and Curran, T. (1984). Induction of c-*fos* gene and protein by growth factors precedes activation of *c-myc.* Nature **312,** 716–720.

Müllner, E.W., and Kühn, L.C. (1988). A stem-loop in the 3′-untranslated region mediates iron dependent regulation of transferrin receptor mRNA stability in the cytoplasm. Cell **53,** 815–825.

Nover, L. (1989). Control of gene expression. In Heat Shock and Other Stress Response Systems of Plants, L. Nover, D. Neumann, and K.-D. Scharf, eds (Heidelberg: Springer-Verlag), pp. 30–41.

Pachter, J.S., Yen, T.J., and Cleveland, D.W. (1987). Autoregulation of tubulin expression is achieved through specific degradation of polysomal tubulin mRNAs. Cell **51,** 283–292.

Ross, J., and Pizarro, A. (1983). Human beta and delta globin messenger RNAs turnover at different rates. J. Mol. Biol. **167,** 607–617.

Ryder, T.B., Cramer, C.L., Bell, J.N., Robbins, M.P., Dixon, R.A., and Lamb, C.J. (1984). Elicitor rapidly induces chalcone synthase mRNA in *Phaseolus vulgaris* cells at the onset of the phytoalexin response. Proc. Natl. Acad. Sci. USA **81,** 5724–5728.

Sauer, N., Corbin, D.R., Keller, B., and Lamb, C.J. (1990). Cloning and characterization of a wound-specific hydroxyproline-rich glycoprotein in *Phaseolus vulgaris.* Plant Cell Environ. **13,** 257–266.

Seeley, K.A., Byrne, D.H., and Colbert, J.T. (1992). Red light–independent instability of oat phytochrome mRNA in vivo. Plant Cell **4,** 29–38.

Sheng, J., D'Ovidio, R., and Mehdy, M.C. (1991). Negative and positive regulation of a novel proline-rich protein mRNA by fungal elicitor and wounding. Plant J. **1,** 345–354.

Shirley, B.W., and Meagher, R.B. (1990). A potential role for RNA turnover in the light regulation of plant gene expression: Ribulose-1,5-bisphosphate carboxylase small subunit in soybean. Nucl. Acids Res. **18,** 3377–3385.

Shyu, A-B., Greenberg, M.E., and Belasco, J.G. (1989). The c-*fos* transcript is targeted for rapid decay by two distinct mRNA degradation pathways. Genes Dev. **3,** 60–72.

Silflow, C.D., and Key, J.L. (1979). Stability of polysome-associated polyadenylated RNA from soybean suspension culture cells. Biochemistry **18,** 1013–1018.

Theologis, A., Huynh, T.V., and Davis, R.W. (1985). Rapid induction of specific mRNAs by auxin in pea epicotyl tissue. J. Mol. Biol. **183,** 53–68.

Thompson, D.M., and Meagher, R.B. (1990). Transcriptional and post-transcriptional processes regulate expression of RNA encoding the small subunit of ribulose-1,5-bisphosphate carboxylase differently in petunia and in soybean. Nucl. Acids Res. **18,** 3621–3629.

Thompson, D.M., Tanzer, M.M., and Meagher, R.B. (1992). Degradation products of the mRNA encoding the small subunit of ribulose-1,5-bisphosphate carboxylase in soybean and transgenic petunia. Plant Cell **4,** 47–58.

Tierney, M.L., Wiechert, J., and Pluymers, D. (1988). Analysis of the expression of extensin and p33-related cell wall proteins in carrot and soybean. Mol. Gen. Genet. **211,** 393–399.

Walling, L., Drews, G.N., and Goldberg, R.B. (1986). Transcriptional and post-transcriptional regulation of soybean seed protein mRNA levels. Proc. Natl. Acad. Sci. USA **83,** 2123–2127.

Wilson, T., and Treisman, R. (1988). Removal of poly(A) and consequent degradation of c-*fos* mRNA facilitated by 3′ AU-rich sequences. Nature **336,** 396–399.

Wisdom, R., and Lee, W. (1991). The protein-coding region of c-*myc* mRNA contains a sequence that specifies rapid mRNA turnover and induction by protein synthesis inhibitors. Genes Dev. **5,** 232–243.

You, Y., Chen, C.-Y.A., and Shyu, A.-B. (1992). U-rich sequence-binding proteins (URBPs) interacting with a 20-nucleotide U-rich sequence in the 3′ untranslated region of c-*fos* mRNA may be involved in the first step of c-*fos* mRNA degradation. Mol. Cell. Biol. **12,** 2931–2940.