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Differential Activation of Potato 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Genes by Wounding and Pathogen Challenge

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Potato genes encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) were expressed in response to pathogen, elicitor, and wounding. HMGR catalyzes the rate-limiting step in isoprenoid biosynthesis leading to accumulation of phytoalexins and steroid glycoalkaloids. Wounding caused increases in HMGR mRNA levels. A rapid and transient peak occurred 30 minutes after wounding, followed by a slower peak at 14 hours; both were correlated with increased enzyme activity. Induction of HMGR mRNA by the soft rot pathogen *Erwinia carotovora* subsp *carotovora* or arachidonic acid began 8 hours after challenge and continued through 22 hours. Potato HMGR is encoded by a gene family. An HMGR gene-specific probe was used to demonstrate that one isogene of the HMGR family is pathogen activated and is distinct from isogene(s) that are wound activated. This provides evidence that defense-related increases in HMGR activity are due to mRNA level increases and that HMGR isogenes are activated differentially by wounding or pathogen challenge.

INTRODUCTION

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR; EC 1.1.1.34) catalyzes conversion of 3-hydroxy-3-methylglutaryl coenzyme A to mevalonic acid, precursor to all isoprenoid compounds. In plants, these include antimicrobial terpenoid phytoalexins, toxic steroid glycoalkaloids, sterols, plant growth regulators such as abscisic acid and gibberellins, electron transfer components such as plastoquinone and ubiquinone, carotenoid pigments, and natural rubber. In potato tubers, HMGR plays an important regulatory role in the synthesis of terpenoid phytoalexins (e.g., rishitin, phytuberin, and lubumin) and steroid glycoalkaloids, which are pathogen and wound response compounds, respectively (Shih and Kuc, 1973; Oba et al., 1985; Stermer and Bostock, 1987). Tuber HMGR activity increases in response to wounding and is enhanced further by inoculation with the pathogen *Phytophthora infestans* or treatment with arachidonic acid, an elicitor of sesquiterpenoid phytoalexins (Oba et al., 1985; Stermer and Bostock, 1987). These increases in enzyme activity are followed by accumulation of steroid glycoalkaloids or phytoalexins. Oba et al. (1985) showed that blasticidin S, a protein synthesis inhibitor, reduced both wound-induced and pathogen-induced HMGR activity as well as subsequent phytoalexin accumulation, suggesting that increases in enzyme activity are due to de novo HMGR synthesis. Because HMGR is induced by wounding, pathogen challenge, or elicitor and has a potential role in plant defenses that ameliorate biotic and abiotic stresses, it meets the criteria for a plant defense-related protein (see Collinge and Susarenko, 1987; Kuhlemeier, et al., 1987).

In the studies described here, we examined the response of potato tuber HMGR to wounding, arachidonic acid, and the plant pathogenic bacterium *Erwinia carotovora* subsp *carotovora* (Ecc), the causal agent of soft rot. Ecc is a broad host-range, opportunistic pathogen that does not elicit gene-for-gene resistance (reviewed in Gabriel et al., 1988; Lyon, 1989). Because Ecc is not a host-specific pathogen and requires a wound or physiologically compromised tissue for disease development, it has been suggested that host resistance is based on successful wound healing rather than pathogen-induced responses (reviewed in Gabriel et al., 1988). Pathogenicity is based on excretion of a variety of plant cell-degrading enzymes leading to tissue maceration (reviewed in Yang et al., 1989). Recently, we showed that Ecc inoculated onto potato tuber slices induces phenylalanine ammonia-lyase (PAL) mRNA and enzyme activity more rapidly and to greater levels than with wounding alone (Yang et al., 1989). Ecc also induces accumulation of sesquiterpenoid phytoalexins in potato tubers (Ghanekar et al., 1984). Resistance

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based on induction of proteins may be important to the host in restricting pathogenesis in nonstressed tubers; however, under hypoxic conditions that are especially conducive to soft rot, protein synthesis does not occur (Vayda and Schaeffer, 1988).

In the past, studies of HMGR regulation at the transcription level were limited because of a lack of cloned DNA sequences. Recently, several groups have isolated HMGR genes from plants including *Arabidopsis thaliana* (Caelles et al., 1989; Learned and Fink, 1989) and tomato (Narita and Gruissem, 1989; Park, 1990). In tomato, HMGR sequences isolated by Narita and Gruissem (1989) were expressed early in fruit development and associated apparently with membrane biogenesis rather than carotenogenesis. Park (1990) cloned a distinct tomato HMGR gene that shows defense-related activation. Because regulation of HMGR activity in defense responses is well documented in potato (Oba et al., 1985; Stermer and Bostock, 1987), we are interested in responses of HMGR mRNAs to pathogen challenge and wounding. We demonstrate here that isoprenoid biosynthesis in potato is correlated with resistance to tuber soft rot; that HMGR activity induced by wounding, elicitor, or pathogen challenge is correlated with increases in HMGR mRNA; and that wounding induces a biphasic response of HMGR, an early transient response followed by a slower response. In contrast to results reported for most plant defense-related genes, pathogen challenge by Ecc activates a potato HMGR isogene not activated by wounding.

RESULTS

Isoprenoid Biosynthesis Is Correlated with Soft Rot Resistance

Initial experiments were designed to determine whether inducible host responses play a role in protection of potato tubers against Ecc and whether these responses are the same or different from wound healing (Bostock and Stermer, 1989). In one experiment, tuber slices were treated with water (control) or arachidonic acid (AA), an elicitor of sesquiterpenoid phytoalexin accumulation in potato (Bostock, et al., 1981; Stermer and Bostock, 1987), allowed to heal for 72 hr, rewounded, and inoculated with Ecc. Because healed tuber surfaces are resistant to Ecc colonization and soft rot, they were rewounded to obtain maceration. AA-treated and water-treated slices differed in appearance before rewounding; AA-treated slices developed dark brown surfaces and water-treated slices wound healed in a normal manner, developing a light tan surface.

As shown in Figure 1, samples pretreated with AA showed no signs of tissue maceration 48 hr after inoculation with Ecc although some weight loss (3.6%) was measured, presumably related to metabolic activity and drying due to the browning. In contrast, samples pretreated with water showed extensive rotting associated with 8.8% weight loss mostly due to removal of rotted tissue. Differences in weight loss were significant between treatments at the $P \leq 0.10$ level ($n = 7$). In a second experiment, slices treated with mevinolin, a specific competitive inhibitor of HMGR (Bach and Lichtenthaler, 1982, 1983), had increased weight loss (8.8%) compared with slices treated with AA (2.9%) or water (6.6%). The differences were significant at the $P \leq 0.10$ level ($n = 10$). These results support the hypothesis that HMGR is important in plant defense because AA stimulation of host defense responses, including terpenoid defense compounds, increased resistance to soft rot and specific inhibition of HMGR activity by mevinolin decreased disease resistance.

Figure 1. Effect of AA on Tuber Susceptibility to Soft Rot.

Tubers were sliced, treated with AA (left), an inducer of sesquiterpene phytoalexin biosynthesis, or water (right) and allowed to heal for 72 hr at 18°C, rewounded by furrowing with sewing needles, and challenged with Ecc as described in Methods. After healing, the AA-treated slice was darker and completely resistant to soft rot caused by Ecc; the water-treated slice was lighter in color and susceptible to soft rot. The slices shown here had any rotted tissue removed by washing before photography.

Pathogen Challenge and Induction of HMGR mRNA

To determine whether wounding and pathogen inoculation induce accumulation of HMGR mRNA in potato, RNA:DNA hybridizations were carried out using total RNA isolated from tubers that had been sliced or sliced and inoculated with Ecc. As shown in Figure 2, an RNA species, about 2.4 kb, hybridized to a 1.5-kb EcoRI fragment of tomato HMGR genomic sequences containing the region most

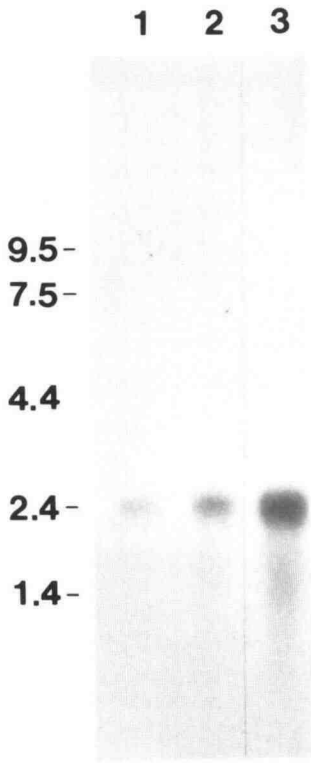

Figure 2. Wounding and Ecc Challenge Induces Tuber HMGR mRNA.

Total potato RNA was isolated from sliced tubers frozen immediately (lane 1), sliced tubers incubated at 30°C for 9 hr (lane 2), or sliced tubers inoculated with Ecc and incubated at 30°C for 9 hr (lane 3). Total RNA (20 μg/lane) was glyoxalated, separated by electrophoresis on agarose gels, transferred to Nytran membranes, and hybridized to the ^{32}P-labeled 1.5-kb EcoRI fragment of the tomato HMGR gene that contains the sequences most conserved among yeast, plant, and mammalian HMGR genes. Indicated molecular weights were based on the Bethesda Research Laboratories' RNA ladder.

highly conserved among yeast, plant, and animal HMGRs (Park, 1990). Both wounding and Ecc inoculation resulted in accumulation of HMGR mRNA, although Ecc inoculation caused much more intensive accumulation than wounding. Accumulation of HMGR mRNA is consistent with reported increases in HMGR activity induced by wounding and pathogen challenge associated with sesquiterpene phytoalexin accumulation (Oba et al., 1985; Stermer and Bostock, 1987).

The kinetics of HMGR mRNA accumulation were compared with enzyme activity. Levels of HMGR mRNA accumulation were determined by slot blot RNA:DNA hybridization with the 1.5-kb EcoRI fragment of tomato HMGR gene as probe. Similar results were obtained from two experiments; representative data are presented in Figure 3. HMGR kinetics from microsomal and organellar fractions were consistently similar and the majority of the activity was microsomally associated; therefore, only data on microsomal activity are presented. HMGR mRNA levels and enzyme activity increased in response to wounding, arachidonic acid, and Ecc challenge. Wounding induced HMGR mRNA within 8 hr, reaching a maximum at 14 hr (Figure 3A), whereas induction by Ecc and arachidonic acid continued through 22 hr (Figures 3B and 3C). After 14 hr in Ecc-challenged tubers, when massive tissue maceration was observed, HMGR activity was not as high as predicted from mRNA accumulation (Figure 3C). This activity loss was due at least in part to HMGR inhibitors present in macerated tissue because extracts of macerated tissue were able to reduce HMGR activity in enzyme extracts of wounded tissue (Z. Yang, C.L. Cramer, and G.H. Lacy, unpublished results). Treatment of sliced tubers with cycloheximide, a protein synthesis inhibitor, blocked increases in HMGR activity (data not shown). This suggested that increases in HMGR activity that preceded increases in mRNA levels (Figures 3A and 3C) were probably not due to activation of the previously accumulated enzyme by post-translational modification. Examination of early events in the tuber wound response, shown in Figure 4, indicated a rapid increase in HMGR mRNA, peaking 30 min to 60 min after slicing, followed by the slower response observed previously. Changes in mRNA levels were followed closely by increases in HMGR activity during the early responses.

HMGR Is Encoded by a Gene Family in Potato

Bimodal induction kinetics of HMGR may be due to activation of multiple genes. To estimate the number of HMGR genes in potato, digested genomic DNA isolated from homozygous *Solanum phureja* was probed by DNA:DNA gel blot hybridization with a 1.5-kb EcoRI fragment of tomato HMGR genomic DNA containing regions highly conserved among yeast, plant, and mammalian HMGR genes (Park, 1990). As shown in Figure 5A, four EcoRI fragments (14.3 kb, 8.2 kb, 3.3 kb, and 2.3 kb) and four HindIII fragments (15.5 kb, 7.1 kb, 5.4 kb, and 4.2 kb) hybridized. Less intensively hybridizing fragments (5.2-kb EcoRI and 11.5-kb HindIII fragments) were observed with longer exposure. The 2.3-kb EcoRI and 15.5-kb HindIII fragments hybridized most intensively to the tomato HMGR probe sequences.

The amino terminus of HMGRs containing the membrane-spanning region is not highly conserved among species (Basson et al., 1988; Learned and Fink, 1989), and sequences from this region of HMGR may function as gene-specific probes. To test this possibility, a 0.7-kb AvaI-EcoRI fragment of the tomato HMGR clone which contains

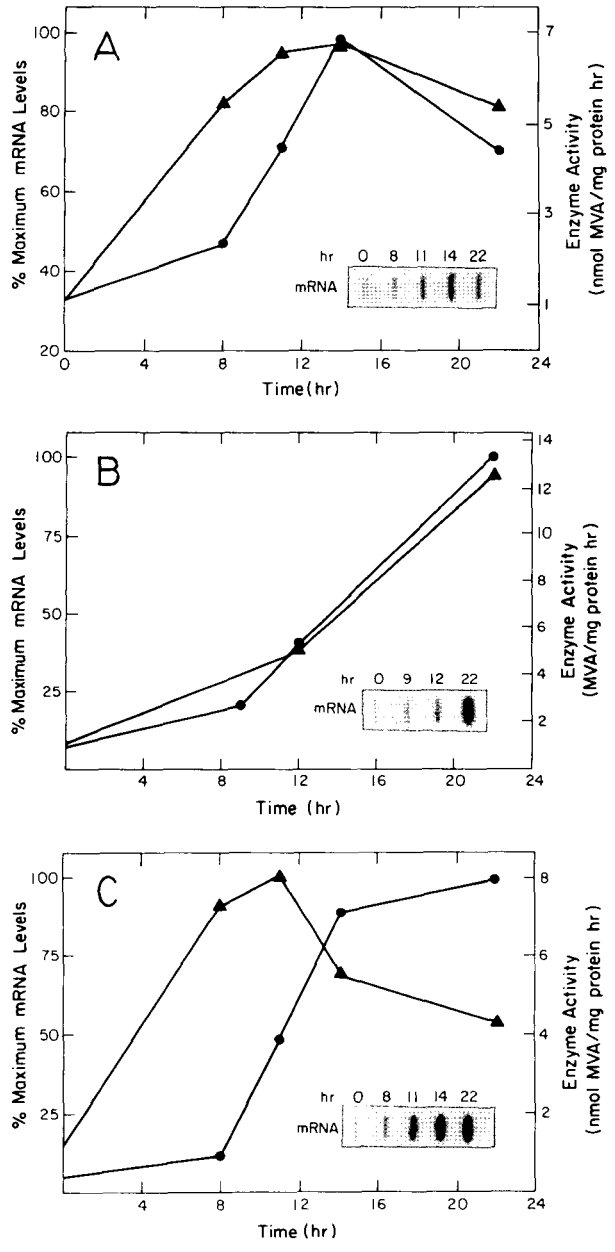

Figure 3. Defense-Related Induction of HMGR mRNA and Microsomal Enzyme Activity.

(A) Slices of potato tubers were mock treated with water.
(B) Slices of potato tubers were treated with AA.
(C) Slices of potato tubers were challenged with Ecc.
All slices were incubated at 30°C and harvested at various times after the treatment for analysis of HMGR mRNA levels (circles) and enzyme activity (triangles). HMGR mRNA levels were determined by slot blot analyses of total RNA (5 μg/well) probed with the ^{32}P-labeled 1.5-kb EcoRI fragment of tomato HMGR genomic sequences. Enzyme activity was assayed according to Stermer and Bostock (1987).

5′ nontranslated regions and 241 bp encoding the amino terminus of HMGR was also used as a probe. This fragment functions as a gene-specific probe in tomato (Park, 1990). When a duplicate blot was probed with the 0.7-kb AvaI-EcoRI fragment of tomato HMGR, only one EcoRI fragment (4.7 kb) and one HindIII fragment (15.5 kb) were detected (Figure 5B). Longer exposure did not reveal additional bands. These results indicated that potato HMGR is encoded by a small gene family containing at least two and probably more isogenes and that the 0.7-kb tomato HMGR fragment functions as a gene-specific probe in potato under the conditions described.

Distinct Isogenes Are Induced by Wounding Versus Ecc Challenge

The complex induction pattern of HMGR by wounding and bacterial challenge suggested the potential for differential regulation of HMGR isogenes. To test this possibility, we used the conserved 1.5-kb EcoRI fragment and the gene-specific 0.7-kb AvaI-EcoRI fragment of our tomato HMGR gene as probes for hybridization with potato RNA isolated from wounded slices or wounded tubers challenged with Ecc. As shown in Figure 6A, the conserved-region HMGR probe hybridized to HMGR mRNA of both rapid and slow wound responses as well as response to Ecc challenge. In contrast, the gene-specific probe hybridized to RNA isolated from Ecc-challenged tuber slices but not to mRNA isolated from wounded slices (Figure 6B). Background hybridization observed in the figure represents the constitutive level (time zero in Figures 6A and 6B) of gene

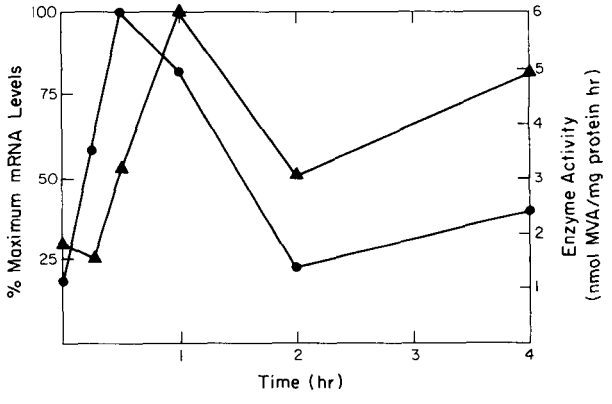

Figure 4. Early Induction of HMGR mRNA and Microsomal Enzyme Activity by Wounding.

Potato tubers were sliced and incubated at 30°C for various times. After incubation, the top 1 mm of tuber tissues was harvested for analyses of mRNA (circles) and enzyme activity (triangles), as described in the legend to Figure 3.

expression, not the induced levels. Cross-hybridization of the 240-min AA sample to the conserved-region but not gene-specific probe reflects the "early" wound response and not induction due to AA. AA-induced mRNAs, occurring 12 hr to 22 hr after treatment (Figure 3B) have not been tested for hybridization with the 0.7-kb probe. Stripping and rehybridizing both blots A and B with each probe indicated that the mRNA was available for binding and in similar concentrations on both.

DISCUSSION

Plant responses correlated with pathogen challenge are characterized by de novo synthesis of plant defense-related compounds, including phytoalexin antibiotics, lytic enzymes such as chitinases and glucanases, plant cell wall components such as lignins and hydroxyproline-rich glycoproteins, protease inhibitors, and several other proteins of unknown function. In all cases in which responses have been characterized at the molecular level, they have been shown to involve activation of genes encoding these defense proteins or enzymes involved in their synthesis (reviewed in Ryder et al., 1986; Collinge and Slusarenko, 1987; Thornburg et al., 1987; Logemann et al., 1988; Templeton and Lamb, 1988; Matton and Brisson, 1989; Stanford et al., 1989).

Defense-Related Expression of Potato HMGR Genes

We demonstrated that accumulation of HMGR mRNA in potato tubers is induced by wounding, elicitor treatment, or pathogen challenge. Previous studies describe increases in tuber HMGR activity in response to wounding, elicitors, or pathogen challenge and show that these increases are required for production of steroid glycoalkaloids or sesquiterpenoid phytoalexins (Oba et al., 1985; Stermer and Bostock, 1987; Vögeli and Chappell, 1988). Our results with wounding, treatment with arachidonic acid, or challenge with Ecc, a plant pathogenic bacterium, showed that changes in HMGR activity were correlated with increases in HMGR mRNA levels and that cycloheximide inhibited stress-induced increases in HMGR activity. These results are consistent with the hypothesis that defense-related induction of HMGR activity is due to de novo synthesis resulting from increases in HMGR mRNA levels. Induction of many plant defense-related proteins is due to transcriptional activation (Lawton and Lamb, 1987). Thus, it is likely that the accumulation of HMGR mRNA in response to wounding, pathogen challenge, and elicitor described here is also due, at least in part, to increased gene transcription.

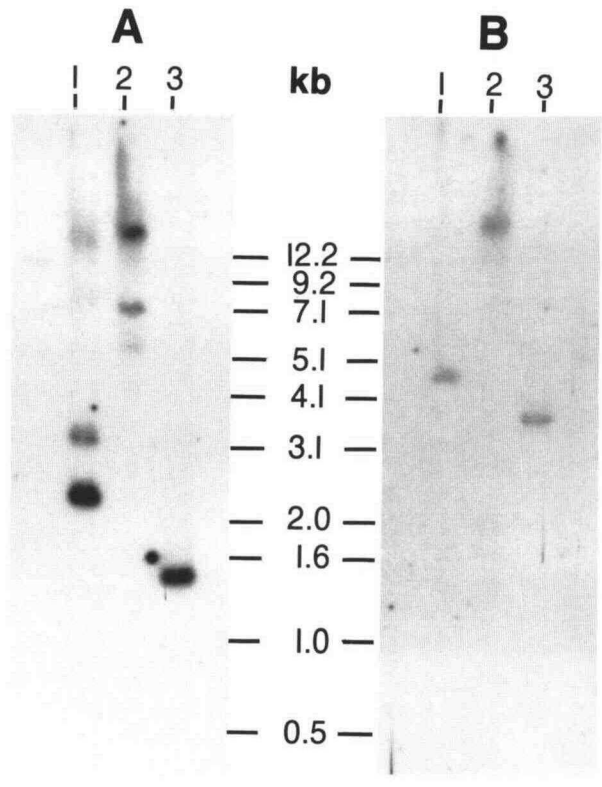

Figure 5. DNA:DNA Hybridization Analyses of Potato HMGR Genes.

Genomic DNA (15 μg/lane) isolated from leaves of homozygous diploid *S. phureja* was digested with EcoRI (lanes 1) or HindIII (lanes 2). Lanes 3 contained tomato HMGR clone pTH295 digested with EcoRI. Digested DNA was separated by electrophoresis on an 0.8% agarose gel, blotted to Nytran membrane by capillary transfer, and hybridized with the ^{32}P-labeled DNA probes.
(A) Hybridization probe was the ^{32}P-labeled conserved-region 1.5-kb EcoRI fragment of the tomato HMGR gene.
(B) Hybridization probe was the ^{32}P-labeled divergent-region 0.7-kb AvaI-EcoRI fragment of the tomato HMGR gene.
Indicated molecular weights were based on the Bethesda Research Laboratories' 1-kb DNA ladder.

HMGR Regulation by Wounding Is Complex

Wounding increases expression of all known plant defense-related genes although their regulation may be complex. For instance, three bean transcripts (Hyp 4.1, Hyp 3.6, and Hyp 2.13) of hydroxyproline-rich glycoproteins accumulate in response to wounding (Corbin et al., 1987). Hyp 3.6 accumulation is rapid and transient with a maximum 1.5 hr after wounding, whereas Hyp 4.1 and Hyp 2.13 accumulate more slowly with maxima at 12 hr. This pattern is similar to the biphasic accumulation of HMGR mRNA—an early and transient accumulation peaking

around 30 min and a later one peaking around 14 hr after wounding. Gene-specific probes representing members of the HMGR gene family will be required to determine whether the early and late wound inductions of HMGR mRNAs are due to a single or more than one HMGR isogene.

The early HMGR response is the most rapid plant defense-related gene response heretofore described. For example, in our potato system, wound induction of PAL mRNA is not evident until 6 hr and peaks between 9 hr and 12 hr (Yang et al., 1989); these kinetics are similar to those reported for bean defense-related genes associated with isoflavonoid phytoalexin production (Liang et al., 1989). Other wound-inducible genes characterized in potato tubers (*wun*1, *wun*2, Logemann et al., 1988; *win*1, *win*2, Stanford et al., 1989) also show induction with mRNA first appearing after 2 hr and maximal accumulation at 10 hr to 20 hr, depending on the gene.

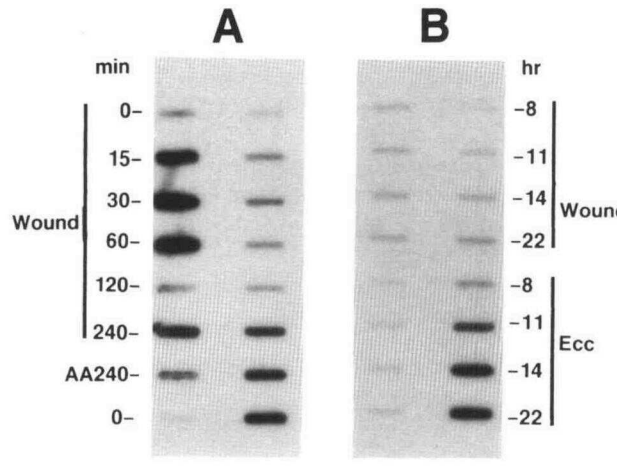

Figure 6. Differential Hybridization of Potato HMGR mRNA Induced by Wounding or Challenged with Ecc.

Total potato RNA was isolated from tubers wounded by slicing and incubated at 30°C for 0 min to 240 min (left lanes) and 8 hr to 22 hr (right lanes). Total RNA was also isolated from wounded slices that were inoculated with the pathogen (8 hr to 22 hr, right lanes) or isolated from wounded slices treated (see text) with AA at 240 min (AA240 in left lanes). Time 0 (left lanes, bottom) serves as control for wounded and challenged slices in the right lanes. Potato RNA (5 μg/well) was transferred to Nytran membranes using a slot blot apparatus.

(A) Blot was hybridized to the ^{32}P-labeled conserved-region probe described in the legend to Figure 5.

(B) Blot was hybridized to the ^{32}P-labeled divergent-region probe described in the legend to Figure 5.

Differential Regulation of HMGR Genes

Differential regulation by environmental and developmental stimuli is typical of many plant defense-related genes encoded by gene families (Templeton and Lamb, 1988). Although bean hydroxyproline-rich glycoprotein transcripts are all induced by wounding, Hyp 3.6 and Hyp 4.1 are expressed in the incompatible interaction with *Colletotrichum lindemuthianum*, but Hyp 2.13 is induced in the compatible interaction (Corbin et al., 1987). Also in bean, three PAL genes are induced by wounding but they are regulated differentially by several stimuli (Liang et al., 1989). We present evidence that HMGR is encoded by a small gene family in potato. DNA from a homozygous *S. phureja* line derived from diploidized anther-derived monoploid cultures was used to eliminate any allelic variations in our DNA:DNA hybridization analyses. The results of these and other experiments suggest that potato contains at least two HMGR genes. Moreover, our results show that a pathogen, Ecc, activates a distinct HMGR isogene that is not induced by wounding. In contrast, most defense-related genes appear to be activated both by pathogens and by wounding.

With our probes, we could not determine whether the pathogen induced one or more than one HMGR gene; however, hybridization patterns using the conserved or gene-specific sequences as probes suggested that a potato homolog to the cloned tomato isogene was induced by the pathogen. The tomato HMGR gene, the source of the hybridization probes used in this study, is also activated in tomato cells treated with fungal elicitors (Park, 1990) and is distinct from the gene isolated by Narita and Gruissem (1989) from tomato fruit cDNA. In *A. thaliana*, Caelles et al. (1989) observed two HMGR genes; HMG1 but not HMG2 was expressed in leaves and seedlings, suggesting differential regulation. In potato, further analyses using probes specific to other HMGR genes should provide greater understanding of differential regulation of HMGR genes by environmental and developmental stimuli in stems and leaves as well as in tubers.

The HMGR gene family in potato tubers may represent a unique defense-related gene system for study of plant defense responses and distinct signaling pathways involved in biotic versus abiotic stress responses. In addition, promoter isolation and characterization for the rapid-wound-response gene and the pathogen-specific gene may define useful promoters for genetic engineering of enhanced disease resistance.

That HMGR isogenes are induced in response to wounding versus Ecc challenge suggests differences in perception or signaling mechanisms for these two stresses. Our maceration studies also indicated fundamental differences between wound-induced and elicitor-induced tuber responses. Under our conditions, tubers sliced and healed were resistant to Ecc; however, these slices rotted if the healed surface was wounded and inoculated with Ecc, suggesting that wound healing is a localized response. In

contrast, slices treated with arachidonic acid and healed were resistant to soft rot even upon wounding and inoculation. Possible explanations are that elicitor (1) induces a response of greater magnitude, (2) triggers production of defense compounds not induced by wounding, or (3) induces host responses in cells further removed spatially from the sliced surface.

Analyses of stress-induced terpenoid compounds by Shih and Kuc (1973) suggest that wounding triggers accumulation of steroid glycoalkaloids whereas *P. infestans* challenge and elicitors induce rishitin and lubumin accumulation and suppress steroid glycoalkaloid production. It will be of interest to determine whether differential expression of HMGR isogene(s) by wounding versus Ecc determines the production of these stress compounds. The spatial expression patterns of two wound-inducible genes, *win2* and PAL, were analyzed histochemically in tubers using reporter gene fusions in transgenic potato (Bevan et al., 1989; Stanford et al., 1990). For both genes, wound-induced expression was localized at the wound site or in adjacent cell layers associated with wound periderm development. Similar studies of the spatial regulation of HMGR isogenes in wounded versus elicitor-treated or Ecc-treated tubers using reporter gene fusions or tissue-print hybridizations should address whether tuber responses to these stresses differ in magnitude or in distribution of the responding cells.

Plant-pathogen interactions are some of the most interesting phenomena in gene regulation because they involve mutual regulation between two interacting organisms. Contrary to the hypothesis that tuber resistance to soft rot erwinias is due to the wound response of healthy plants (reviewed in Gabriel et al., 1988), our results show that Ecc activates plant defense-related genes, including HMGR and PAL, differently than in wound responses (this report and Yang et al., 1989). Although defense activation has been described for a number of cultivar-specific pathogens and in nonhost plants challenged by pathogens (Lawton et al., 1983; Cramer et al., 1985; Dixon et al., 1986; Collinge and Slusarenko, 1987), our discovery is surprising because it is widely believed that Ecc, an omnivorous, wide host range pathogen, does not elicit host defense responses because (1) it does not incite hypersensitive reactions, (2) it is a facultative pathogen or even a saprophyte, and (3) it avoids host defenses by causing pathogenesis only on stressed plant tissues that show reduced defense-related gene response (Rumeau et al., 1990).

METHODS

Plant Materials, Bacterial Strains, and Their Preparation

Potato tubers (*Solanum tuberosum* cv Russet Burbank) purchased from local markets were surface-disinfested as described (Yang et al., 1989) and incubated in the dark for 24 hr at 18°C or 30°C, consistent with the experimental procedure used, before inoculation or treatment. Tubers were sliced, inoculated, and treated under a green photographic safe light to prevent inactivation of HMGR by light (Stermer and Bostock, 1987). Homozygous diploid plants (doubled monoploids from anther culture) of *S. phureja* were provided by R.E. Veilleux (Virginia Polytechnic Institute and State University, Blacksburg, VA). For DNA isolation, young leaves were harvested from plants grown in the glasshouse. The potato soft rot pathogen, Ecc strain EC14, described in previous studies (Roberts et al., 1986; Yang et al., 1989), was cultured in a minimal medium as described by Yang et al. (1989).

Tuber Treatment with Arachidonic Acid and Mevinolin

Tubers were cut into 1-cm-thick cross-sectional slices and treated by spreading with 250 μL of sterile deionized water, arachidonic acid (Sigma, St. Louis, MO; 1 μg/μL), or mevinolin (Merck & Co., Rahway, NJ; 20 nM). Because phytoalexin accumulation is maximized at 18°C (Lyon, 1984), treated slices were allowed to heal at that temperature in the dark for 72 hr in a plastic box humidified by air bubbled through water (Yang et al., 1989). Healed slices were rewounded by furrowing the entire top surfaces about 0.5 mm deep with several sterile number 7 sewing needles embedded 0.5 mm apart in a rubber stopper. Rewounded slices were inoculated directly by spreading with 250 μL of an Ecc overnight culture adjusted to $OD_{550\,nm} = 1.0$ (about 6×10^6 colony-forming units); incubation at 18°C was continued. Maceration was estimated by weight loss (Yang et al., 1989). Seven or 10 slices were used per treatment in each experiment. Students' *t* test of means was used to estimate the probability that differences among treatment means were significant.

Challenge and Treatment of Tubers for RNA Isolation

Slices were treated by spreading their top surfaces with 300 μL of Ecc suspension ($OD_{550\,nm} = 1.0$; 8×10^6 colony-forming units) in minimal medium, 300 μL of minimal medium, 300 μL of arachidonic acid (1 μg/μL), or 300 μL of cycloheximide (Sigma; 50 μg/mL). Treated slices were incubated as described above but at 30°C. Soft rot developed by 10 hr under these conditions. At various incubation times, any macerated tissue was removed by washing and the top 1.0 mm of remaining tuber tissue was sliced off and frozen in liquid nitrogen for subsequent analysis of HMGR mRNA and enzyme activity.

DNA Isolation and DNA:DNA Hybridizations

Genomic DNA of homozygous diploid *S. phureja* was isolated from leaves according to Murray and Thompson (1980). DNA (15 μg) was digested to completion with appropriate restriction enzymes (Bethesda Research Laboratories, Gaithersburg, MD); restriction fragments were separated by electrophoresis on a 0.8% agarose gel and transferred to a membrane (Nytran, Schleicher & Schuell, Inc., Keene, NH) as described by Maniatis et al. (1982). The membrane was prehybridized overnight at 42°C in 50% formamide, 5 × Denhardt's solution, 5 × SSC, 0.25% SDS, 1 mM EDTA, and 100 μg/mL sheared and denatured salmon sperm

DNA (Sigma). Hybridization was performed under the same conditions but with the addition of ^{32}P-labeled tomato HMGR DNA sequences. Final wash conditions were 0.5 × SSC at 60°C for 30 min.

RNA Isolation and RNA:DNA Hybridizations

Total plant RNA was isolated by phenol extraction as described by Haffner et al. (1978). RNA concentrations were determined spectrophotometrically ($OD_{260\,nm}$) and monitored for consistency among treatments by fluorescence under UV light (304 nm) of ethidium bromide-stained rRNA in 1.2% agarose after electrophoretic separation. For slot blot RNA:DNA hybridization analyses, 5 μg of total RNA was denatured by glyoxalation and immobilized on membranes using a slot blot apparatus (Millipore Corp., Bedford, MA). Blotting,probe preparation, RNA gel blot hybridization, and subsequent washes followed the procedures of Yang et al. (1989), except that final wash conditions were 0.1 × SSC at 65°C for 30 min. RNA levels were quantitated by densitometer scans of autoradiograms.

Hybridization Probes

DNA probes used in this study were prepared from a tomato (*Lycopersicon esculentum* cv VFNT Cherry) DNA fragment cloned in plasmid pTH295, which encodes a full-length HMGR gene (GenBank Accession No. M63642; Park, 1990). Sequence comparisons with yeast, mammalian, and plant HMGRs indicate that the coding regions for the carboxy terminus of HMGR genes are highly conserved among species but that the amino termini show little or no sequence identity (Basson et al., 1988; Learned and Fink, 1989). For a conserved-region probe, we used a 1.5-kb EcoRI fragment containing sequence encoding the carboxy end of the tomato HMGR (as well as two introns). For a gene-specific probe, we used a 0.7-kb AvaI-EcoRI fragment containing 5′ untranslated sequences and 241 bases of the highly divergent amino terminus coding region. The probes were ^{32}P-labeled using random primer procedures defined by the manufacturers (Random Primer Kit, Bethesda Research Laboratories).

HMGR Activity Assay

Quantitation of HMGR enzyme activity and protein levels followed procedures of Stermer and Bostock (1987).

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