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Hypoxic Stress Inhibits Multiple Aspects of the Potato Tuber Wound Response

William Butler, Linda Cook, and Michael E. Vayda*[1]

Department of Biochemistry, Plant Biotechnology Program, University of Maine, Orono, Maine 04469-0131

ABSTRACT

Potato (*Solanum tuberosum* L.) tubers subjected to wounding under hypoxic stress do not synthesize RNA species that are induced in response to wounding in aerobic conditions. Further, wound-response proteins fail to be synthesized when wounded tubers are transferred to hypoxic conditions although messenger RNAs which encode them persist for many hours after transfer. Hypoxic stress also prevents the incorporation of [^{3}H]thymidine by wounded tubers that occurs in aerobic conditions. In contrast, hypoxic tubers accumulate and translate transcripts of genes whose products are involved in anaerobic metabolism whether or not they are wounded. Both the hypoxic response and the aerobic wound response preclude the synthesis of proteins encoded by messenger RNAs which accumulated during the tuberization process and which can be translated *in vitro*. Finally, wounding elicits the degradation of a subset of these tuberization-associated transcripts. These data indicate a complex and precise regulation of gene expression at several levels of macromolecular synthesis.

The responses to two environmental stresses, wounding and hypoxia, impact the susceptibility and resistance of potato tubers to bacterial disease. Mechanical wounding induces the appearance of a set of polypeptides that are distinct from those produced during tuberization (29). Two of these have been identified as PAL[2] (29) and DAHP synthase (7). These enzymes are necessary for the synthesis of phenolic compounds which may limit bacterial growth or enzymatic activity, and are involved in the wound healing process (8, 16). Wound healing in other plants includes the induction of genes whose protein products are associated with the cell wall (6, 15, 28, 29) and may be involved in the formation of a wound periderm (1). Expression of both classes of genes is thought to be instrumental in limiting the access of a bacterial pathogen to the tuber parenchyma. By contrast, hypoxic stress promotes bacterial rot of tuber tissue, presumably by inhibition of the synthesis of potato wound-response proteins (29). Although genes induced by wounded plants or under hypoxic conditions have been identified previously (2–10, 12–18, 25–30), the interplay of these two responses in potato tubers has not been examined under environmental conditions where both stresses occur. In the present study, we report that there is a hierarchy to stress-mediated gene expression in potato tubers, and that the inhibition of the wound response is mediated at three distinct levels: RNA accumulation, protein synthesis, and DNA synthesis.

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[2] Abbreviations: PAL, phenylalanine ammonia-lyase; rDNA, DNA complementary to ribosomal RNA; Na_2HPO_4, dibasic sodium phosphate; dCTP, deoxyribo-CTP; kb, kilobases; GRP, glycine-rich protein; DAHP synthase, 3-deoxy-D-*arabino*-heptulosonate 7 phosphate synthase; S, Svedberg unit.

MATERIALS AND METHODS

Origin of Potato Tubers and Gene Probes

Potato tubers (*Solanum tuberosum* L. cv Russet Burbank) were obtained from the breeding facilities at the University of Maine Experiment Station. Prior to use, tubers were washed in tepid water, rinsed briefly in 0.5% sodium hypochlorite (v/v), then rinsed in deionized water, air dried, and incubated overnight at room temperature (29). The probe for bean phenylalanine ammonia-lyase (8) was kindly provided by C. Lamb and C. Cramer; the petunia glycine-rich protein (5, 6) and soybean actin (11) probes were the kind gift of R. Meagher and C. Condit; the carrot extensin (3) clone was provided by M. Tierney, A. Showalter, and J. Varner; the potato patatin (21) probe was the kind gift of W. Park; the tomato proteinase inhibitor I and II (9) probes were kindly provided by C. Ryan; the larch 18S ribosomal RNA probe was the generous gift of K. Hutchison; the maize aldolase (14) probe was kindly provided by P. Kelley; the petunia and tomato alcohol dehydrogenase probes were kindly provided by J. Strommer and V. Williamson, respectively.

Hypoxic Conditions

Whole tubers, or wounded tubers, were supported above a layer of water in a plastic pan covered with plastic wrap (Glad Cling Wrap, Union Carbide). Argon gas was bubbled through the water (80 to 150 mL/min) to displace oxygen and nitrogen by virtue of its greater density. Under these conditions, the oxygen concentration in the incubation chamber consistently measured less than 2.2% (v/v) using a portable gas monitor (EXOTOX 50, Neotronix, Inc.).

Wound Induction

Whole tubers were wounded by repeated insertion of a plastic tip for the p200 pipetteman (USA Plastics) to a depth

of 1.0 to 1.5 cm (29). RNA was isolated from the same tuber prior to wounding, or at various times after wound induction as indicated in the text and figure legends. Proteins synthesized by wounded tubers were determined by presenting 0.25 mCi [^{35}S]methionine (>800 Ci/mM, New England Nuclear) to a wound for 1 h, and resolving the extracted, labeled proteins by subsequent SDS-PAGE, and fluorography as described previously (29). The stability of wound-response proteins was estimated by isolation of proteins synthesized during a 1 h label with [^{35}S]methionine at the times indicated below after removal of the label and presentation of unlabeled 10 mM L-methionine. Western blot analysis of unlabeled proteins was performed by electrophoretic transfer to nitrocellulose using a Hoeffer *trans*-blot apparatus as described by the manufacturer. [^{125}I]Protein-A (New England Nuclear) was used to detect binding (19) of anti-aldolase antibody and anti-alcohol dehydrogenase antibody, kindly provided by P. Kelley and J. Strommer, respectively.

RNA Analyses

RNA was isolated from fresh tissue that was frozen in liquid nitrogen, ground to a fine powder using a coffee grinder (Miracle Mill, Markson Scientific), and immediately extracted by the phenol method described previously (20). Poly(A$^+$) RNA was isolated by adsorption to mAP paper (Amersham) as described by the supplier. RNA species were identified by formaldehyde agarose gel electrophoresis and Northern blot analyses using the methods described previously (20). In all analyses, equal amounts (30 μg/lane) of total tuber RNA were loaded and confirmed by hybridization to an 18S rDNA probe. Probes used in the hybridization analysis of RNA were labeled by incorporation of [^{32}P]dCTP (New England Nuclear) using hexanucleotide Random Primers as described by the manufacturer (Bethesda Research Laboratories). Autoradiograms (Kodak XAR/5 film) were scanned with an RFT Scanning Densitometer (Transidyne General Corporation). The translational potential of these RNA samples was determined by translation *in vitro* using a rabbit reticulocyte lysate (New England Nuclear) in the presence of [^{35}S]methionine as described by the supplier.

Analysis of DNA Synthesis

Wounds were inflicted into a whole tuber by insertion of a plastic tip for the p200 micropipette (USA Plastics) to a depth of approximately 2.5 cm. Tubers were incubated for 1 h with 0.1 mCi [^{3}H]thymidine (>80 Ci/mM, New England Nuclear), added to the wound either immediately or at a specified time after wounding. A 1 cm diameter cylinder containing approximately 3 g of tissue immediately surrounding the wound site, and containing all except trace amounts of the added label, was removed using an apple corer. The sample was immediately frozen in liquid nitrogen, ground to a fine powder using a coffee grinder (Miracle Mill, Markson Scientific), and incubated at 50°C for 10 min in 10 mL of a buffer comprised of 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 0.1% SDS (w/v). Total nucleic acid was recovered by ethanol precipitation after extraction with phenol and chloroform. The pellet was resuspended in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA and incubated with 0.1 mg/mL RNAase A for 10 min at 20°C. Duplicate aliquots of each sample were spotted onto DE81 Ion-Exchange Paper (Whatman), dried under a heat lamp, and subsequently washed six times in 0.5 M Na_2HPO_4. Samples were assayed in a EconoFluor Scintillation Cocktail (New England Nuclear) using a Beckman LS 7500 Scintillation Counter. Data shown are the results of five independent time course experiments.

RESULTS

Markers of the Aerobic Wound Response

Cytoplasmic RNA species encoding PAL and other suspected wound-response proteins accumulate greatly in mature tubers in response to wounding (Fig. 1). The accumulation of these RNA species parallels the accumulation of newly synthesized tuber protein (29) and the reported induction of wound-response genes in other plant systems (2, 8, 15–18, 25, 28). For example, a 2.7 kb RNA species which hybridizes to the PAL probe appears within 1 h after a wounding event (Fig. 1A). This RNA remains at a constant level for many hours and begins to decrease 18 to 24 h after wounding. This accumulation is explained, at least in part, by an increased level of transcription (17, 18), but it is not yet known if the stability of these RNA species is also increased. Two RNA species of 1.0 and 0.8 kb also accumulate soon after wounding that hybridize to a probe encoding the cell wall-associated GRP (Fig. 1B) which has been shown to be induced in petunia upon wounding (5, 6, 13). We do not know whether these species are the products of different genes or are related as processing products of the same primary transcript. The presence of numerous restriction fragments that hybridize to the GRP probe in genomes of Solanaceous plants (5) leads us to suspect that these species may be transcripts of distinct genes.

A second class of tuber RNA species appears in response to wounding, but in a delayed time frame, appearing 12 to 24 h after the wound event. For example, three RNA species appear by 24 h after wounding that hybridize to the carrot extensin probe with apparent mobilities of 2.0, 1.6, and 1.3 kb, respectively (Fig. 1C). This delayed induction of extensin (the hydroxyproline-rich, cell wall-associated glycoprotein) is in accord with what has been reported previously in carrot and tomato (15, 28). Similarly, the 0.9 kb RNA species that hybridizes to a *Drosophila* histone H4 probe (Fig. 1D) also belongs to this class of genes that are induced 12 to 24 h after wounding. Histone H4 RNA is present in low but detectable amounts prior to wounding, and accumulates to high levels by 24 h after wounding. Histone gene expression is often linked to the S-phase of the cell cycle; the dramatic accumulation of this message 24 h after wounding suggests that cell division may be stimulated by wounding, perhaps necessary for the formation of wound periderm (1).

Indeed, the wounding of tuber tissues triggers a period of DNA synthesis that corresponds with the appearance and maximal accumulation of histone RNA (Fig. 2, closed circles). Incorporation of [^{3}H]thymidine by tuber tissues commences between 10 to 16 h after wound induction, peaks 24 h after wounding, and decreases to a minimum between 33 to 34 h after wounding. A second, less distinct period of [^{3}H]thymi-

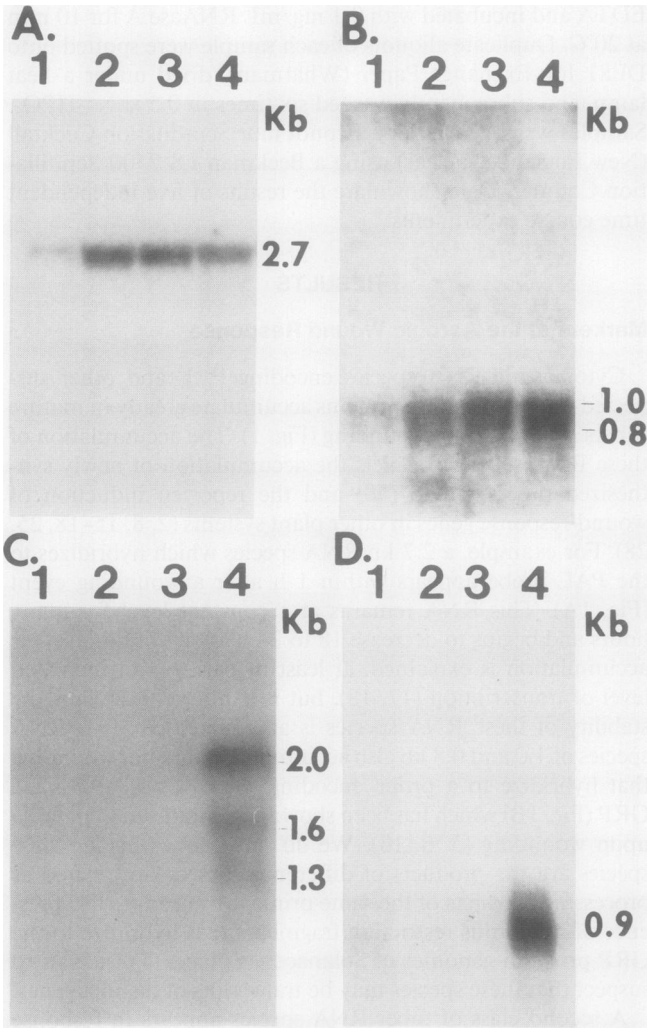

Figure 1. Accumulation of RNA species in response to wounding. RNA isolated from an unwounded Russet Burbank tuber (lanes 1) or the same tuber 1, 6, or 24 h after wounding (lanes 2–4, respectively), was resolved by electrophoresis through formaldehyde agarose gels (20), blotted to nylon filters (20, 30), hybridized with [^{32}P]labeled DNA probes and visualized by autoradiography using Kodak XAR/5 film and a tungsten intensifying screen. A, RNA hybridized to labeled bean PAL probe; B, to petunia GRP probe; C, to carrot extensin probe; D, to *Drosphila* histone H4 probe. Sizes of the RNA species indicated are estimated relative to the mobility of RNA standards (Bethesda Research Laboratories).

dine incorporation is apparent between 35 to 62 h after wounding, which may be interpreted as a less synchronous, second round of DNA replication. No DNA synthesis is observed after wounding under hypoxic conditions (Fig. 2, open circles).

Fate of Tuberization-Associated RNA Species Upon Wounding

The RNA species encoding the two most abundant tuber proteins are rapidly degraded upon wounding (Fig. 3, A and B). RNA species encoding patatin, the major tuber storage

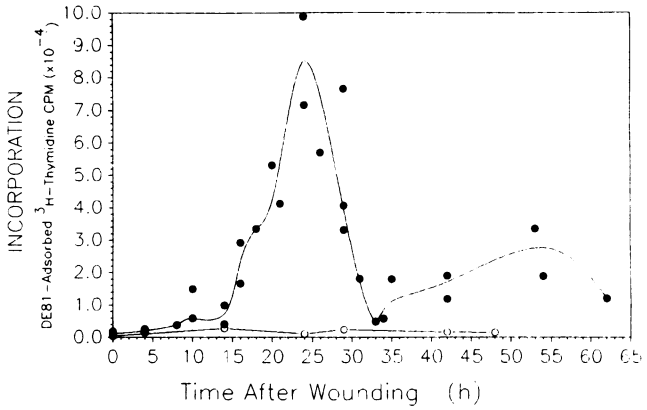

Figure 2. Induction of DNA synthesis in response to wounding. Wounds were inflicted into a tuber and 0.1 mCi [^{3}H]thymidine was presented to the wound site at the time of wounding or 4, 8, 10, 14, 16, 18, 20, 24, 26, 29, 31, 33, 34, 35, 42, 48, 53, 54, or 62 h after wounding. A 3 g sample surrounding the wound site was removed 1 h after presentation of label, and RNAase-resistant nucleic acid was extracted as described in "Materials and Methods." The relative amount of incorporated [^{3}H]thymidine in each sample was determined by adsorption to DE81 ion exchange paper (Whatman) and scintillation counting in EconoFluor (New England Nuclear). Counts per minute incorporated by aerobically incubated tubers (●——●), Counts per minute incorporated by tubers maintained under hypoxic conditions from the time of wounding (○——○).

protein (21, 22, 24) and the protease inhibitor II protein (9, 12, 27), are present in tubers for many months after harvest, but disappear within 6 h of the wounding event (Fig. 3, A and B). This degradation must be specific for a subset of RNA species, rather than for global destruction of cytoplasmic RNA species, because there is a large class of tuber RNA species whose steady state levels remain unchanged whether tubers are unwounded, wounded, or subjected to hypoxic stress. For example, RNA species that hybridize to a soybean actin probe are not induced upon wounding and remain at the same levels after wounding (Fig. 3C). Most tuber RNA species appear to comprise this class, because the products of total tuber RNA (Fig. 3E), or poly(A^{+}) RNA (Fig. 3F), translated *in vitro* using a rabbit reticulocyte lysate (New England Nuclear) are essentially identical whether tubers are unwounded or wounded prior to RNA isolation. This failure to detect a significant change in the translatable RNA pool indicates that most tuber RNA species are not degraded upon wounding, and that the wound-response RNA species known to accumulate (Figs. 1 and 3D) must either represent a small minority of the total tuber RNA, or are not translated efficiently *in vitro*. In contrast, polypeptides that are translatable *in vitro*, such as the 115, 95, and 40 kD species (Fig. 3, E and F) and which comigrate with abundant tuber proteins that are visible by staining with Coomassie blue (22, 29), are not synthesized efficiently by wounded tubers *in vivo* (29). However, polypeptides of at least two wound-induced RNA species, PAL and DAHP synthase, are synthesized *in vivo* by wounded tubers (7, 23, 29). In summary, potato appears to respond to mechanical trauma by stimulating DNA synthesis, accumulating transcripts of specific genes, and destabilizing transcripts of

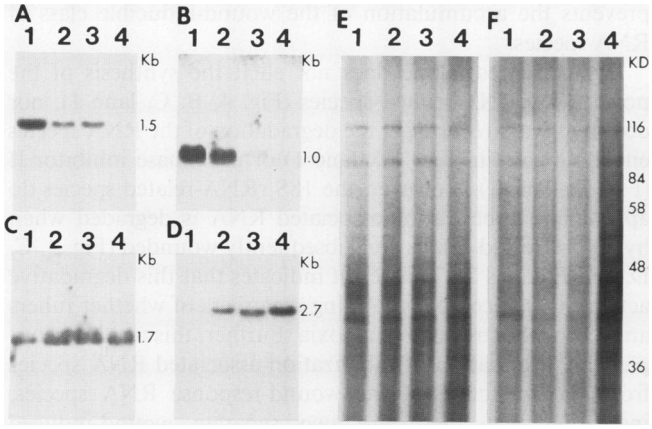

Figure 3. Fate of RNA species present in tubers prior to wounding. Panels A, B, C, and D, RNA isolated from an unwounded tuber (lanes 1) or the same tuber 1, 4, or 6 h after wounding (lanes 2–4, respectively), was resolved and blotted as described in the legend to Figure 1, hybridized to ^{32}P-labeled probes of potato patatin cDNA (A), or tomato proteinase inhibitor II cDNA (B), of soybean actin cDNA (C), or of bean PAL (D) and visualized by autoradiography. Sizes of the RNA species indicated are estimated relative to the mobility of known-length RNA standards (Bethesda Research Labs). E and F, Total RNA isolated from an unwounded tuber (lanes 1) or the same tuber 1, 6, or 24 h after wounding (lanes 2–4, respectively), was translated *in vitro* using a rabbit reticulocyte lysate in the presence of [^{35}S]methionine for 1 h. The polypeptide products of these reactions were resolved by electrophoresis through an 11% SDS-polyacrylamide gel (29) and subsequent fluorography using En^{3}Hance (New England Nuclear) and Kodak XAR/5 film (E). Poly(A$^+$) RNA was selected from the total RNA samples by adsorption to mAP paper (Amersham). The products of translation *in vitro* of poly(A$^+$) RNA are resolved in F. The mobility of polypeptide markers (Sigma) are indicated.

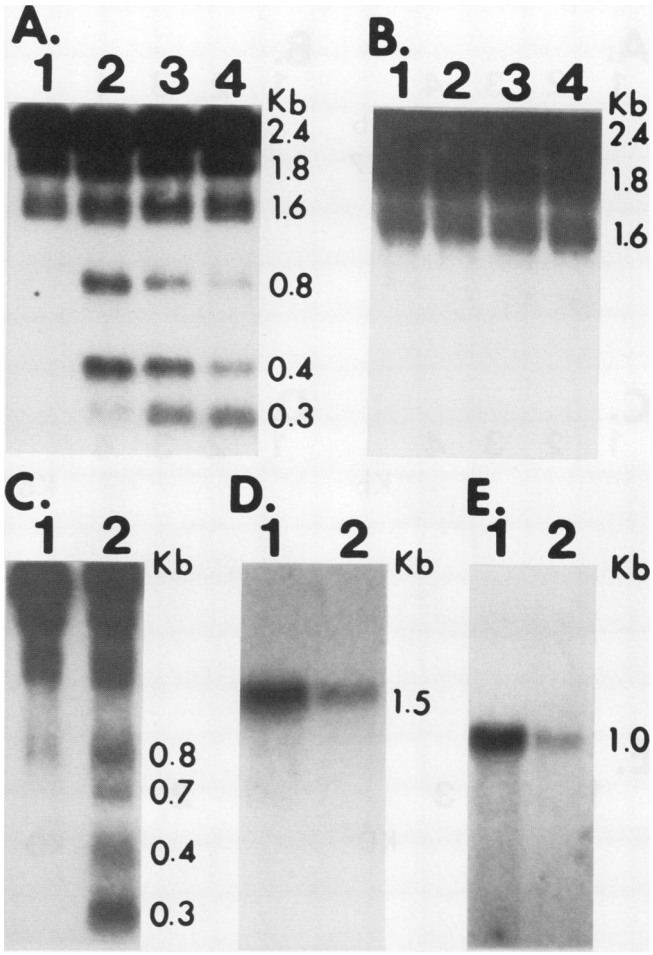

Figure 4. Fate of RNA species that hybridize with 18S ribosomal DNA, patatin or proteinase inhibitor II probes. A, RNA was isolated from a tuber prior to wounding (lane 1), or 1 h (lane 2), 6 h (lane 3), or 24 h (lane 4) after wounding, was resolved and blotted to a nylon filter (Amersham) as described in the legend to Figure 1, and hybridized to the 18S rDNA probe. B, RNA was isolated from an unwounded tuber incubated in air (lane 1), from the same tuber incubated under an oxygen-depleted, argon atmosphere for 1 h (lane 2), 6 h (lane 3), or 24 h (lane 4), resolved, blotted, and hybridized to the 18S rDNA probe. C, D, and E, RNA was isolated from a tuber incubated under hypoxic conditions for 6 h (lanes 1), and that same tuber 9 h after subsequent wounding in the argon atmosphere (lanes 2). The RNA was resolved by electrophoresis through 1.7% (w/v) agarose-formaldehyde gels, blotted to Hybond nylon filters (Amersham) and hybridized to ^{32}P-labeled probes of the 18S rDNA (C), patatin cDNA (D), or proteinase Inhibitor II cDNA (E). Sizes of the RNA species detected by autoradiography are estimated relative to the mobility of RNA standards (Bethesda Research Labs).

the major tuberization genes, while the bulk tuber RNA population remains unchanged.

Wounding also elicits the appearance of a novel set of small RNA species that hybridize to an 18S rDNA probe (Fig. 4A) but do not hybridize to a 28S rDNA probe. These small RNA species are likely to be cleavage products of 18S rRNA or 16S amyloplast rRNA, generated either by the induction of, or by the release of a nuclease upon wounding. For example, they could result from a conformational change in ribosome structure that renders specific sites susceptible to nuclease attack.

Effects of Hypoxic Stress

Hypoxic stress has profound effects on several aspects of the wound response. Wound-induced DNA synthesis is completely inhibited during hypoxic incubation (Fig. 2, open circles). This could result from direct inhibition of the replicative machinery, or indirectly by inhibition of hypothetical wound-induced messages that may be necessary to trigger DNA replication.

Hypoxic stress also prevents appearance of PAL RNA and of the other aerobic wound-induced RNA species upon subsequent wounding. PAL RNA is not induced by hypoxic incubation alone (Fig. 5A), nor does it appear in hypoxic tubers during the 19 h period after wounding (Fig. 5B). However, RNA species of at least two different genes do accumulate upon prolonged hypoxic stress. Both aldolase (Fig. 5C) and alcohol dehydrogenase (Fig. 5D) are associated with anaerobic metabolism in other plant species (10, 14, 26, 30), and the protein products of these genes are detected by Western blot analysis (19) in tubers incubated under hypoxic conditions for 24 h (Fig. 5, E and F). Thus, hypoxic stress induces the accumulation of one set of gene products but

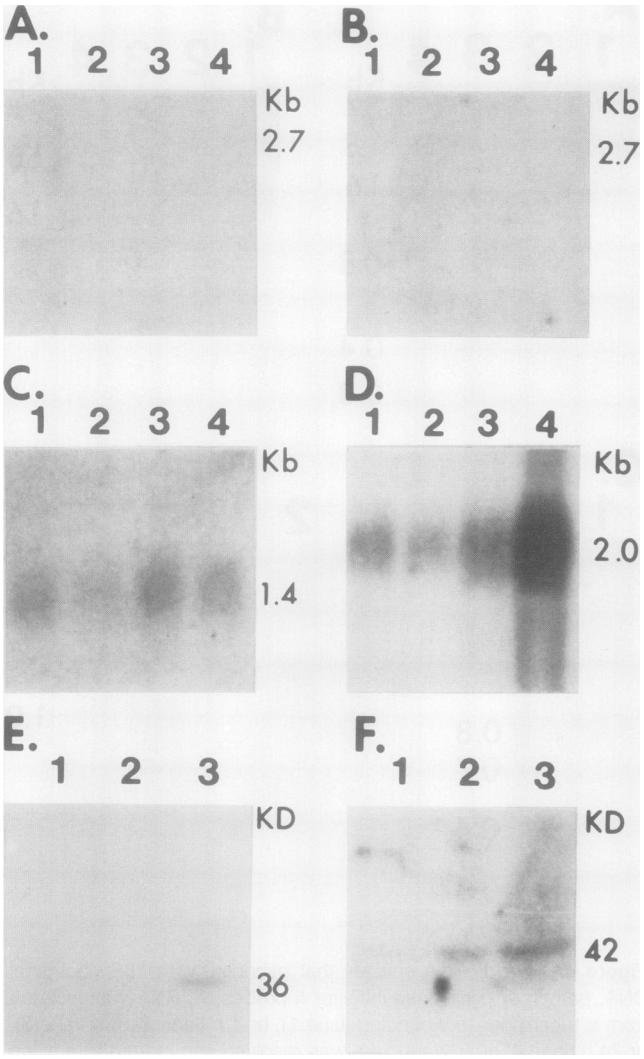

Figure 5. Effect of hypoxic stress on tuber RNA species. A, C, and D, RNA isolated from an aerobic tuber (lanes 1), or the same tuber incubated under an oxygen-depleted, argon atmosphere for 1 h (lanes 2), 6 h (lanes 3), or 24 h (lanes 4), were resolved by electrophoresis and blotted as described in the legend to Figure 1, and hybridized with ^{32}P-labeled cDNA probes for bean PAL (A), maize aldolase (C), or tomato alcohol dehydrogenase (D). B, RNA isolated from a 6 h hypoxic tuber (lane 1) and that same tuber 1 h (lane 2), 6 h (lane 3), or 19 h (lane 4) after subsequent wounding in the argon atmosphere, was hybridized to the bean PAL cDNA probe. Sizes of the RNA species indicated are estimated relative to the mobility of RNA standards (Bethesda Research Labs). E and F, Total proteins isolated from an unwounded tuber (lanes 1) maintained in an argon atmosphere for 4 h (lanes 2) or 24 h (lanes 3) were resolved by electrophoresis through an 11% SDS-polyacrylamide gel, and transferred electrophoretically to nitrocellulose in buffer comprised of 25 mM Tris (pH 8.3), 192 mM glycine, 20% methanol (v/v) and 0.1% SDS (w/v). The blots were incubated with either antibody raised against maize aldolase (E) or antibody raised against maize alcohol dehydrogenase (F). Bound antibody was detected by adsorption of [^{125}I]protein-A (New England Nuclear) and autoradiography with Kodak XAR/5 as described elsewhere (19).

prevents the accumulation of the wound-inducible class of RNA species.

Hypoxic stress alone does not elicit the synthesis of the novel, 18S rRNA-related species (Fig. 4, B; C, lane 1), nor does hypoxic stress trigger the degradation of the RNA species encoding patatin (Fig. 4D, lane 1) or proteinase inhibitor II (Fig. 4E, lane 1). However, the 18S rRNA-related species do appear and tuberization-associated RNA is degraded when hypoxia-stressed tubers are subsequently wounded (Fig. 4, C, D, and E, lanes 2). This result indicates that this degradative activity is induced by wounding regardless of whether tubers are aerobic or stressed by hypoxia. Further, this result uncouples the degradation of tuberization-associated RNA species from the induction of the wound-response RNA species, indicating that these are two separate wound-induced phenomena.

Hierarchy of Stress Responses

Hypoxic stress inhibits the synthesis of wound-response proteins whose messages are induced prior to the onset of hypoxic conditions. For example, the RNA species that are induced upon wounding, such as the 2.7 kb PAL message (Fig. 6A, lanes 1–2) and the 1.0 and 0.8 kb GRP messages (Fig. 6B, lanes 1–2), remain present in wounded tubers for many hours after tubers are transferred to hypoxic conditions (Fig. 6, A and B, lanes 3–5). Densitometer tracings of several autoradiograms indicate that 95 to 100% of the PAL transcript remains after 30 min, 50 to 70% after 6 h, 10 to 30% after 12 h, and less than 10% remains 20 h after transfer to hypoxic conditions, and up to 50% of the two GRP RNA species remain 20 h after transfer to hypoxic conditions. However, the detectable synthesis of wound-induced polypeptides in these tuber samples ceases within 30 min of transfer to hypoxic conditions (Fig. 7A; 29). The latter observation is unlikely to be the result of increased protein turnover, because the wound-induced proteins synthesized under aerobic conditions exhibit comparable stability when maintained in aerobic conditions (Fig. 7B) or when transferred to hypoxic conditions (Fig. 7C). Further, PAL enzymatic activity does not increase after wounding under hypoxic conditions as it does in air (M. Vayda, C. Cramer, W. Butler, G. Lacy, unpublished observations). Thus, we conclude that an aspect of stress-mediated gene regulation must reside at the translational level.

The 0.9 kb histone H4 RNA, which normally appears 12 to 24 h after wounding, does not accumulate when aerobically wounded tubers are subsequently transferred to hypoxic conditions (Fig. 6C), nor do the three extensin RNA species appear under hypoxic conditions (Fig. 6D). Alcohol dehydrogenase (Fig. 6E) and aldolase (Fig. 6F) RNA accumulate in tubers after prolonged hypoxic stress, regardless of whether tubers are wounded prior to hypoxia. We conclude from these data that wounding does not preclude accumulation of hypoxia-induced RNA species. In contrast, the hypoxic stress response inhibits the aerobic wound response by affecting translational specificity of wound-response messages as well as interfering with the accumulation of these species, but does not affect their turnover rate. Both stress responses appear to preclude expression of the preexisting tuber RNAs, and in

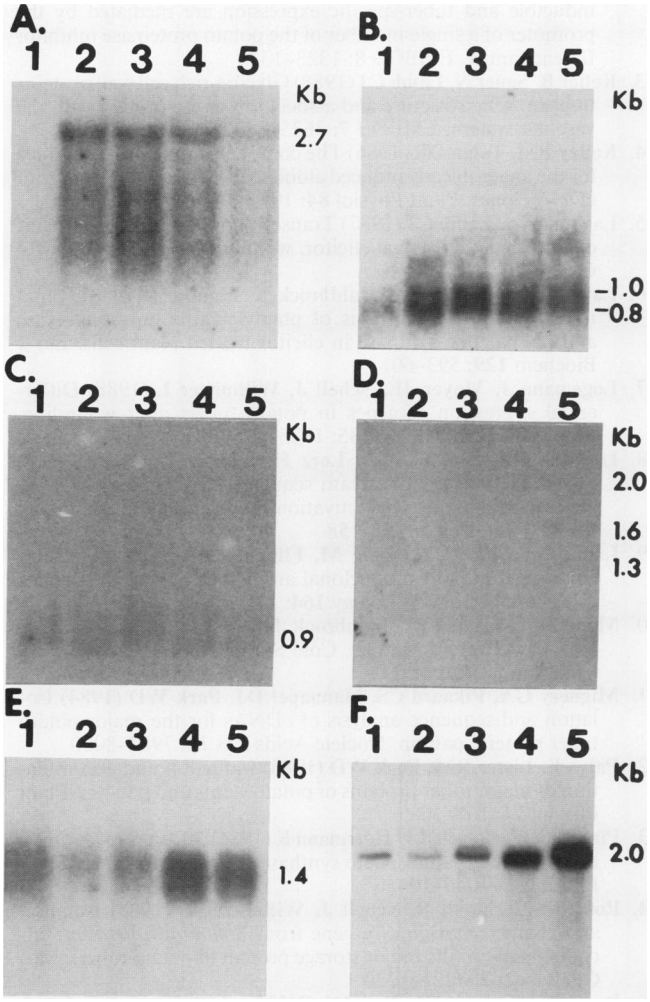

Figure 6. Fate of RNA species in tubers after wounding and subsequent hypoxic incubation. RNA was isolated from an unwounded tuber (lanes 1), the same tuber 2 h after wounding (lanes 2), and after subsequent incubation in hypoxic conditions for 0.5 h (lanes 3), 6 h (lanes 4), or 20 h (lanes 50). RNA was resolved by electrophoresis as described in the legend to Figure 1, blotted to nylon filters, hybridized to ^{32}P-labeled probes of bean PAL DNA (A), petunia GRP (B) *Drosophila* histone H4 DNA (C), carrot extensin (D), petunia alcohol dehydrogenase DNA (E), or maize aldolase DNA (F), and visualized by autoradiography. Sizes of the RNA species indicated are estimated relative to the mobility of RNA standards (Bethesda Research Labs).

addition, wounding elicits the degradation of at least two of these abundant tuberization-associated mRNA species.

DISCUSSION

The interaction among programmed stress-responses of potato tubers are complex. There are several events that occur in response to wounding including the induction of DNA synthesis, the induction of a specific set of genes, the destabilization of other RNA species, and the appearance of small RNA species which hybridize to 18S rDNA. Although the latter two events can be elicited by wounding under hypoxic conditions, the expression of wound-response genes, and the

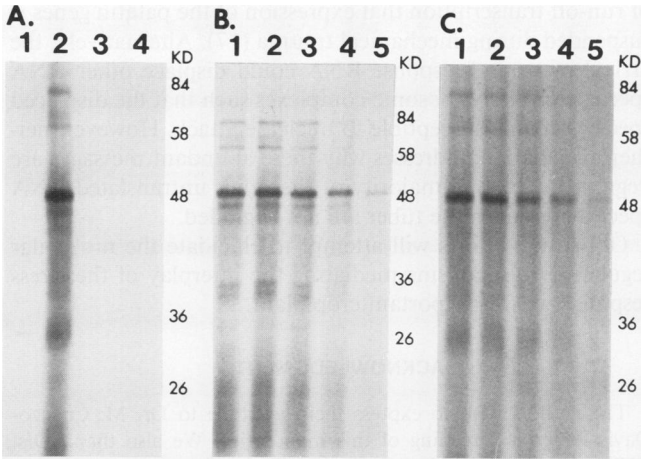

Figure 7. Fate of wound-response proteins during subsequent hypoxic incubation. A, Proteins synthesized by tubers were detected by a 1 h incubation with 0.25 mCi [^{35}S]methionine presented either at the time of wounding (lane 1), 2 h after wounding (lane 2), 2.5 h after wounding and 0.5 h after transfer to an argon atmosphere (lane 3), or 8 h after wounding and 6 h after transfer to an argon atmosphere. Proteins were resolved by SDS-PAGE and fluorography as described previously (29). B and C, Tuber proteins were labeled aerobically for 1 h with 0.25 mCi [^{35}S]methionine presented 4 h after wounding (lanes 1). Tuber wounds were rinsed with dH_2O and 10 mM L-methionine was added to deter further incorporation of label. Labeled proteins remaining after 1 h (lanes 2), 3 h (lanes 3), 6 h (lanes 4), or 12 h (lanes 5) in an aerobically incubated tuber (B) and a hypoxically incubated tuber (C) were resolved by SDS-PAGE and fluorography. The mobility of polypeptide markers (Sigma) are indicated.

perhaps consequential synthesis of DNA, is completely inhibited during periods of hypoxic stress. During this same period, other genes such as alcohol dehydrogenase and aldolase that are apparently necessary for anaerobic metabolism are expressed. Further, the hypoxic response appears to override the wound response at two distinct levels of gene expression: it inhibits the appearance of RNA species encoding the wound response proteins (Figs. 5 and 6), and it inhibits the translation of wound-response messages that are induced quickly, such as PAL and GRP. The dramatic response to hypoxic stress that precludes the wound response is sufficient to explain why periods of hypoxia compromise the plant's defenses against pathogen attack.

The wound response triggers the degradation of tuberization-associated RNA species, such as those which encode patatin and the proteinase inhibitors (Figs. 3 and 4; 17). The expression of the proteinase inhibitor genes is particularly interesting because it is organ specific: the same proteinase inhibitor genes which are induced by wounding in potato leaves and stems (4, 27) and which accumulate during the tuberization process (12), are not expressed in wounded tubers (12). Indeed, the proteinase inhibitor RNA which is present in mature tubers is rapidly degraded within 6 h of wounding (Figs. 3B and 4E). Why the tuberization-associated RNA species are degraded upon wounding is a matter of speculation; perhaps RNA species that have not been transcribed recently are targeted for degradation. It is known from assay

of run-off transcription that expression of the patatin genes is suspended during mechanical trauma (17). Alternatively, the arrival of wound-response RNA could displace other RNA species from polyribosome complexes such that the displaced species become susceptible to nuclease attack. However, neither explanation addresses why these abundant messages are degraded while the majority of the other untranslated RNA species present in the tuber are not degraded.

Our future efforts will attempt to elucidate the molecular regulatory mechanisms mediating the interplay of the stress responses in this important crop plant.

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