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Effect of Heat Shock on the mRNA-Directed Disease Resistance Response of Peas[1]

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ABSTRACT

Pea tissue 'heat shocked' for 2 hours at 40°C accumulates mRNAs that code for a series of new proteins called heat shock proteins. A different messenger RNA population, which codes for a high level of 20 or more 'resistance proteins,' accumulates in pea tissue as it resists plant pathogenic fungi. Heat shock treatment applied prior to fungal inoculation prevents the high level accumulation of messenger RNA coding for the 20 resistance proteins and blocks disease resistance. If the resistance response is initiated before the heat shock treatment or after heat shock recovery, messenger RNA accumulates for the resistance proteins and disease resistance develops.

The survival of plants in nature in the presence of a wide variety of plant pathogenic fungi is assisted by inducible disease resistance responses of plants. One of the several biological, physical, and chemical factors which can alter or negate disease resistance in plants is heat shock (3). The effect of heat was first believed to interfere temporarily with the ability of the plant to produce phytoalexins, which are antifungal compounds produced by many plants in response to infection. More recently, it has been observed both that disease resistance can also be suppressed by RNA and protein synthesis inhibitors (2) and that heat shock can temporarily and dramatically change the mRNAs available to plant ribosomes (4). Thus, we have compared the mRNAs present in pea tissue resisting *Fusarium solani* f.sp. *phaseoli*, a fungus which normally causes an incompatible reaction with peas (6), with mRNAs in heat-treated (resistance-suppressed) tissue which is susceptible to the fungus. This temperature effect is a valuable tool in pursuing

Table I. *Effects of Heat Shock on the Resistance of Pea Endocarp Tissue Inoculated with F. solani f.sp. phaseoli*

Treatment Schedule[a]	Disease Symptom[b]	Hypersensitive Discoloration	Pisatin[c]
			μg/g
No treatment 2 h, then inoculation	R	+	176
No treatment 2 h, then H_2O		–	0
Heat shock 2 h, then inoculation	S	–	15
Heat shock 2 h, then H_2O		–	0
Heat shock 2 h, 9 h recovery, then inoculation	R	Trace	93
Heat shock 2 h, 9 h recovery, then H_2O		–	2
Inoculation and hold 6 h, then heat shocked 2 h	R	–	15

[a] The heat shock treatment was a 2-h incubation at 40°C. The inoculation treatment consisted of 1 500-μl suspension of 6.3×10^6 *F. solani* f.sp. *phaseoli* macroconidia in H_2O applied to the endocarp of split pods.

[b] Disease reaction R, no growth in excess of the spore length; S, extensive growth, more than 10X spore length.

[c] Pisatin content after 48 h.

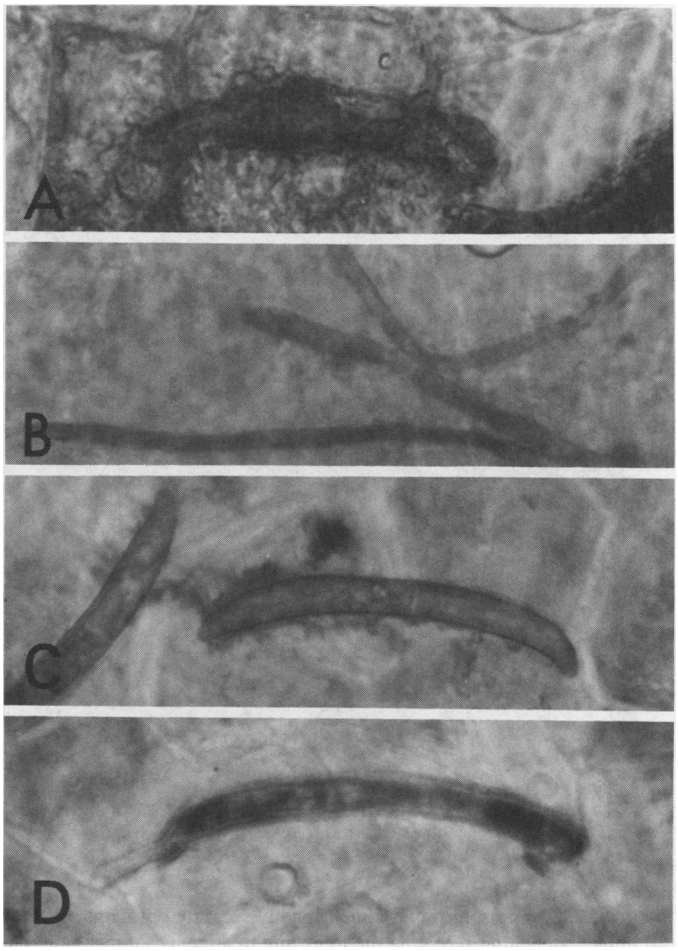

FIG. 1. Comparison of the development of macroconidia spores (*F. solani* f.sp. *phaseoli*) on pea pod tissue within 2 d after the following treatments: A, 2 h at 22°C, then inoculated; B, 2 h at 40°C, then inoculated; C, 2 h at 40°C, 9 h at 22°C (recovery period), then inoculated; D, inoculated 6 h prior to 2 h at 40°C.

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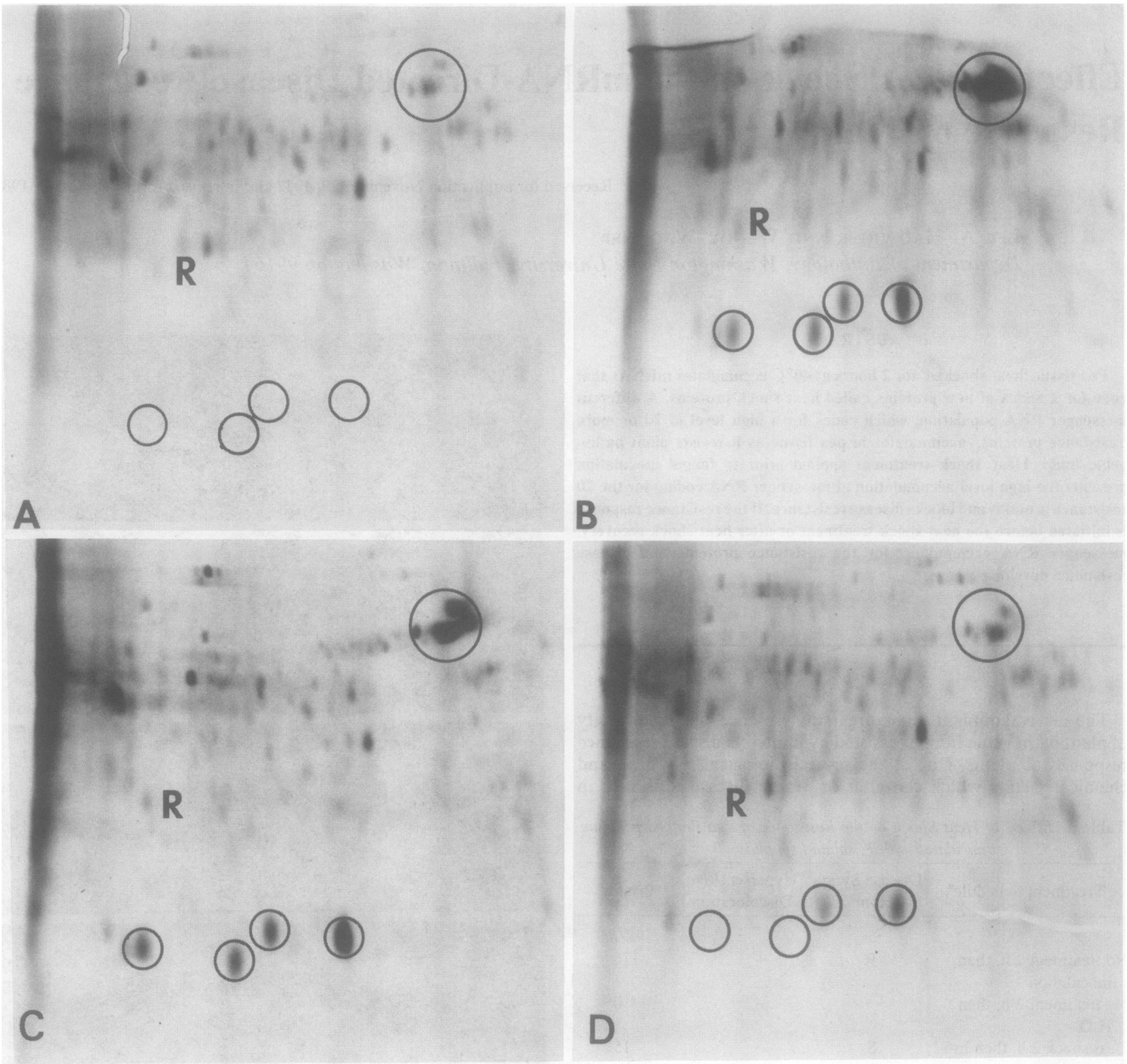

FIG. 2. Comparison of two-dimensionally separated proteins synthesized *in vitro* in the presence of high mol wt RNA isolated from pea pod tissue (4) immediately after receiving the following treatments: A, incubation at 22°C for 2 h, then H_2O treatment 6 h at 22°C; B, incubation for 2 h at 40°C, then water-treated 6 h at 22°C; C, incubation for 2 h at 40°C, then inoculated with the incompatible fungus and incubated 6 h at 22°C; D, incubation at 40°C for 2 h, then a recovery incubation for 9 h at 22°C followed by water treatment and incubation for 6 h at 22°C; E, incubation for 2 h at 22°C, then inoculation and incubation for 6 h at 22°C; F, inoculation and incubation at 22°C for 6 h, then 40°C for 2 h; G, incubation for 2 h at 40°C, then a recovery incubation for 9 h at 22°C followed by inoculation and incubation at 22°C for 6 h. Arrows in E identify the major resistance proteins whose synthesis is enhanced. The positions of eight of the major heat shock proteins on the gels were mapped by circles. R is positioned to the right of one reference protein which appears on all gels and is a guide for comparisons. X-ray films were slightly underexposed to improve resolution of major proteins. The numbers on E indicate positions of mol wt markers: carbonic anhydrase, 30,000; ovalbumin, 46,000; BSA, 69,000; and phosphorylase *b*, 92,500.

the comparative molecular biology of disease resistance in plants, since the suppression of resistance is total, but temporary, and no alteration of the plant tissue can be detected visually. A preliminary report of this work has been published (7).

MATERIALS AND METHODS

Fusarium solani f.sp. *phaseoli*, strain W-8 (American Type Culture Collection 38135) was obtained from D. J. Burke, United States Department of Agriculture, Prosser, WA. The *Pisum sativum* pods were from Alaska variety peas. Rabbit reticulocyte translation kits and [^{35}S]methionine were purchased from New England Nuclear.

The heat shock treatment consisted of placing pods (0.5 g) in tightly sealed, plastic vials (8 ml) and submerging the vials in an incubator at 40°C for 2 h. Pea pod endocarps were inoculated with 0.5 ml suspension of *F. solani* f.sp. *phaseoli* macroconidia (6.3 $\times$ 10^6 spores/ml). Pods were held in the dark and at 22°C except

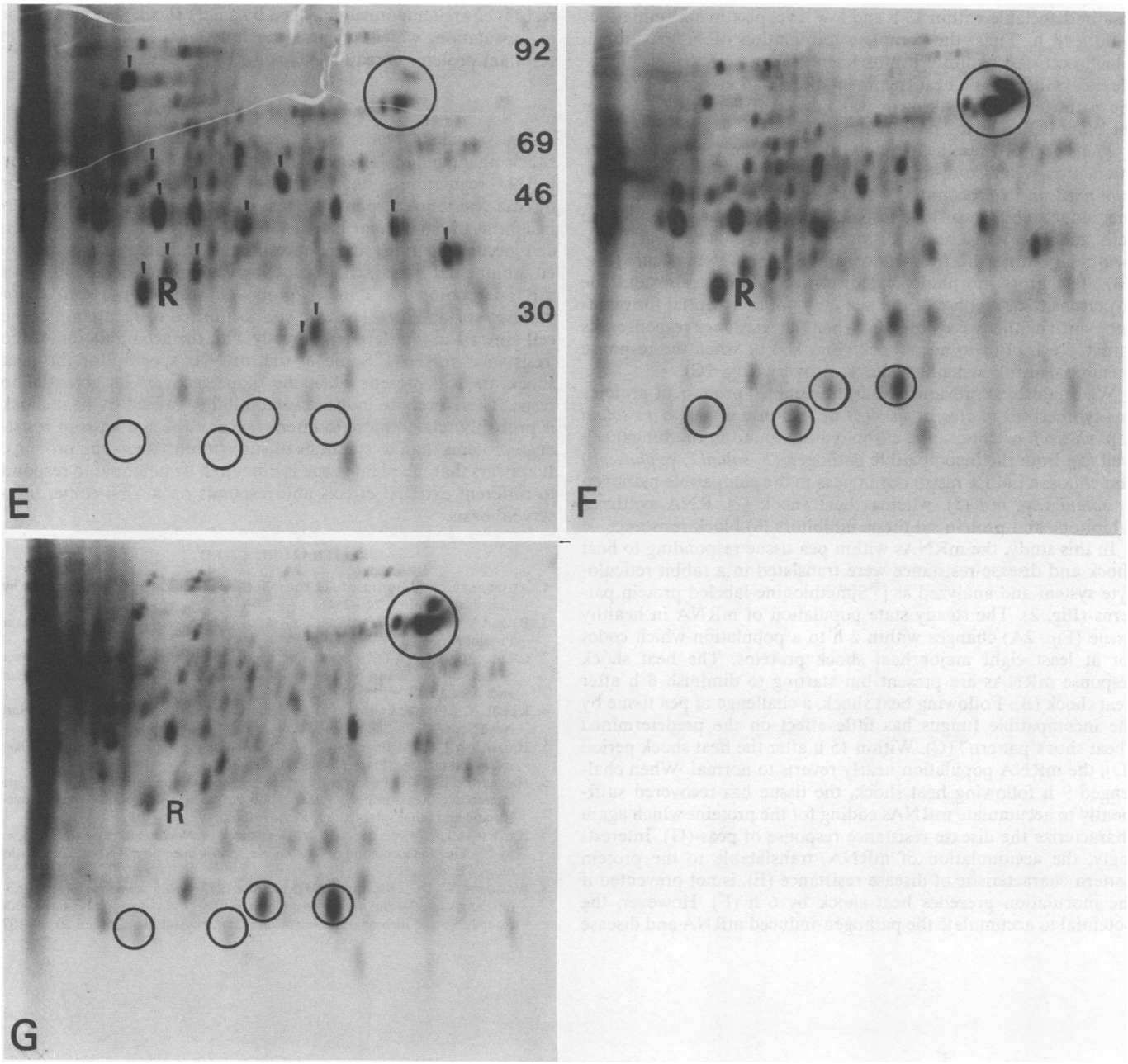

FIG. 2 *continued*

during heat shock. Pea seedlings treated for 2 h at 40°C recovered and grew normally, indicating that this heat shock treatment is not lethal to pea cells.

The techniques used to isolate high mol wt RNA, the *in vitro* translation synthesis of ^{35}S-labeled proteins, and the two-dimensional separation and autoradiography of proteins have been described (8). The positions of proteins in the second dimension differ from those published previously due to the use of a gradient gel. Uniform quantities of RNA (25 μg/assay) were translated with a rabbit reticulocyte translation kit from New England Nuclear and the two-dimensional gels were exposed ~21 d.

Pisatin was extracted by soaking the split pea pods (0.5 g) in 5 ml hexane overnight. The hexane was removed by evaporation. The residue was solubilized in 1 ml ethanol and quantitated spectrophotometrically at 309 nm (1.0 unit A_{309} = 43.8 μg pisatin). This preliminary quantitation of pisatin avoids much of the pisatin degradation by light and exposure on TLC plates; however, for all experiments, an aliquot of the hexane extract was also purified on silica gel G TLC developed in chloroform. Half of the plate was exposed to HCl fumes converting pisatin to anhydropisatin which fluoresces blue under long wave UV light. Pisatin on the opposite half was again quantitated and the UV spectra were compared with the authentic spectra of pisatin.

RESULTS

A 2-h heat shock at 40°C breaks the resistance of pea pod tissue which is normally expressed against the incompatible pathogen, *F. solani* f.sp. *phaseoli* (Table I). A recovery time of 9 h at 22°C after heat shock is sufficient to allow the pea tissue to regain its ability to resist this pathogen and to produce pisatin (a phytoalexin). When the leaf tissue was challenged by *F. solani* f.sp. *phaseoli* 6 h prior to heat shock, total disease resistance developed, indicating that the processes which develop host resistance are initiated within 6 h. Although the resistance developed in this latter treatment is complete, there are only trace amounts of

pisatin detectable within 18 h and low level pisatin accumulations within 48 h. Thus, the complete termination of fungal growth which occurred within 6 h must entail factors other than phytoalexin synthesis. The heat treatment alone had no direct effect on the pathogen since its growth was normal if kept separate from the plant tissue during heat treatment.

The effect of these heat shock treatments on the host-parasite interaction was also distinguished by cytology (Fig. 1). *F. solani* f.sp. *phaseoli* macroconidia, while infecting nonheat-shocked tissue, induce a hypersensitive response in some of the pea endocarp cells and become severely distorted (Fig. 1A). The heat shock treatment reduces host resistance, and fungal growth occurs (Fig. 1B). The gross morphological distortion of the spore and the hypersensitive response of the pea may not be essential for resistance since neither were observed when the resistance response was initiated 6 h prior to heat shock (Fig. 1D) or when the response was initiated following heat shock recovery (Fig. 1C).

We established previously that the same 20 prominent proteins are synthesized *in vivo* or *in vitro* in response either to *F. solani* f.sp. *phaseoli* or chitosan, a carbohydrate found in the fungal cell wall (5). Both the incompatible pathogen (*F. solani* f.sp. *phaseoli*) and chitosan induce resistance in peas to the compatible pathogen *F. solani* f.sp. *pisi* (5), whereas heat shock (7), RNA synthesis inhibitors, and protein synthesis inhibitors (6) block resistance.

In this study, the mRNAs within pea tissue responding to heat shock and disease resistance were translated in a rabbit reticulocyte system and analyzed as [^{35}S]methionine-labeled protein patterns (Fig. 2). The steady state population of mRNA in healthy tissue (Fig. 2A) changes within 2 h to a population which codes for at least eight major heat shock proteins. The heat shock response mRNAs are present but starting to diminish 6 h after heat shock (B). Following heat shock, a challenge of pea tissue by the incompatible fungus has little effect on the predetermined "heat shock pattern" (C). Within 15 h after the heat shock period (D), the mRNA population nearly reverts to normal. When challenged 9 h following heat shock, the tissue has recovered sufficiently to accumulate mRNAs coding for the proteins which again characterize the disease resistance response of peas (G). Interestingly, the accumulation of mRNA, translatable to the protein pattern characteristic of disease resistance (E), is not prevented if the inoculation precedes heat shock by 6 h (F). However, the potential to accumulate the pathogen-induced mRNA and disease resistance are temporarily blocked by a heat shock treatment prior to inoculation, which suggests the functionality of some of the resultant proteins is required for disease resistance.

DISCUSSION

Heat shock in plants is comparable to the well studied heat shock phenomenon of *Drosophila* (1, 4). In peas, the site of the mRNA regulation is not yet known for either the heat shock or disease resistance response of peas; however, this study provides insight at the molecular level as to the changes in mRNA which can occur when the disease resistance response is altered by environmental stress. It seems, however, that availability of mRNAs coding for a major group of proteins is required for disease resistance (9). The heat shock-directed re-regulation of the cell appears to interfere temporarily with the accumulation of the 'resistance' mRNAs. Some of the mRNAs specific for the heat shock are still present when the tissue recovers its potential to resist. Therefore, the induced susceptibility caused by heat shock is probably related more to interference of the synthesis of resistance proteins than to synthesis of susceptibility-assisting proteins. It appears that the plant tissue is limited in its potential to respond to different external effects and responds on a 'first-come, first-served' basis.

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