Pea–*Fusarium solani* Interactions Contributions of a System Toward Understanding Disease Resistance

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ABSTRACT

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This mini-review points to the usefulness of the pea–*Fusarium solani* interaction in researching the biochemical and molecular aspects of the nonhost resistance components of peas. This interaction has been researched to evaluate the resistance roles of the phytoalexin, pisatin, the

cuticle barrier, and the activation of the nonhost resistance response. Concurrently, evaluations of associated signaling processes and the tools possessed by the pathogen to contend with host obstacles were included. The properties of some pathogenesis-related genes of pea and their regulation and contribution to resistance are discussed. A proposed action of two biotic elicitors on both chromatin conformation and the architectural transcription factor, HMG A, is presented and includes time lines of events within the host immune response.

There are various model systems of host–parasite interactions that have contributed to our knowledge of signaling. This mini-review will focus on disease resistance features, present and generated in the interactions between pea and *Fusarium solani*. Initially, both pea seedling and pea endocarp tissue systems were developed for studying the induction of the antifungal phytoalexin pisatin (17). Pisatin production is dependent on a series of secondary enzymes; some such as phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) are also regarded as pathogenesis-related (PR) proteins (67). Some enzymes in the pisatin pathway are yet to be characterized and this work is in progress by DiCenzo and VanEtten (24). Other PR proteins have the potential to be directly antifungal, such as the defensins. The seedling (15) and pod endocarp (18) tissues of the pea system each have some important uses for evaluating genetical, biochemical, and developmental changes. The seedling tissue is useful in following the gradual natural infection process in the region of stem proximal to the seed both in excised stem sections and in infections under natural conditions of the field or greenhouse. The endocarp tissue of the greenhouse-grown pod is useful in assessing a rapid defense response of its epidermal cells that (28) clearly distinguishes susceptibility to the pea pathogen from resistance to nonpathogens.

The communication between pathogen and the endocarp layer of immature pea pods (1 to 2 cm) is rapid because it is not obstructed by a well-developed cuticle layer (32,33). The endocarp epidermal cells are exposed by gently separating the two freshly harvested pod halves with a smooth spatula. The observation of cell viability in a UV microscope following treatment with fluorescein diacetate indicates that this separation is associated with little detectable cell damage. This endocarp tissue can be challenged with suspensions of macroconidia to initiate the “nonhost” resistance response against the bean pathogen, *Fusarium solani* f. sp. *phaseoli*. The germ tube growth of this fungus

is totally suppressed within 6 h. Alternately, the growth of the true pathogen of pea, *F. solani* f. sp. *pisi*, is partially suppressed at 6 h, but resumes growth within 12 h, eventually initiating a full-fledged infection. The rapidity of the interaction between the fungus spores and the individual surface cells of pea endocarp occurs because there are many individual fungal cells on individual host cells signaling the molecular and biological changes. Further, the challenging spores have not penetrated the plant's cells and can be quantitatively removed up to 6 h postinoculation. With this synchronization and sensitivity it is possible to detect early signaling events that initiate the resistance response. Cruickshank and Perrin (18), who introduced this system, typically detected pisatin accumulations in pea endocarp tissue in response to nonhost fungal spores at 40 h postinoculation. However, alterations such as changes in the density of nuclei of the upper endocarp cells occur within 20 min (31), which relate to the eventual outcome of the interaction. That is, the density of the nuclei, detected by sedimentation rates, is considerably less from surface pea cells altered by the pathogen and somewhat less dense from cells altered by the nonpathogen than the density of nuclei recovered from the water-treated tissue.

CONTRIBUTIONS OF THE PEA SEEDLING SYSTEM

The cuticle barrier. Enzymes released from fungi are potential enhancers of both the pathogenicity and virulence of the pathogen. It appears that the absence of any single enzyme-encoding gene researched to date is not sufficient to totally negate the pathogenicity of *F. solani* f. sp. *pisi*. However, enhanced expression of certain fungal-derived genes appears to increase virulence. One such enzyme from *F. solani* f. sp. *pisi* is cutinase. This enzyme was first purified in 1975 (68). Antibodies developed against this enzyme or specific inhibitors of serine hydrolases, both of which inhibit cutinase activity, reduced the capacity of the fungus to penetrate the cuticle of the pea in stem section assays (61). The results of these investigations and others suggest that cutinase enzyme activity was important for penetrating the cuticle barrier. Subsequently, an *F. solani* f. sp. *pisi* cutinase-deficient mutant of isolate T-8 was found to have an 80 to 90% reduction in enzyme activity and a 45% reduction in pathogenicity compared with the wild type (20).

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*The e-Xtra logo stands for “electronic extra” and indicates that Figure 1 appears in color online.

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Both the cloning of the cutinase gene that made possible mutation by gene replacement and the availability of the *Nectria haematococca*, mating population VI (sexual stage), *F. solani* f. sp. *pisi* (anamorph) that allowed the genetic crossing, enabled Stahl and Schafer (76) to investigate the role of cutinase directly by the construction of a cutinase-deficient mutant by gene replacement. A number of these transformants showed no difference in pathogenicity and virulence on pea compared with the wild type and a control transformant. This led to the suggestion that cutinase was not as important in pathogenicity as previously proposed. It was subsequently reported (56) that the apparent lack of dependence on cutinase was because the highly virulent strains of *F. solani* f. sp. *pisi* have multiple cutinase genes, enabling the transformants to retain virulence. This conclusion was based on inoculations with the *F. solani* f. sp. *pisi* isolate, 77-2-3, which has one cutinase gene, and T-8, which has multiple cutinase genes and is hypervirulent. Isolate 77-2-3 had 10 to 20% of the cutinase activity of isolate T-8. Furthermore, lesion formation was less frequent and less severe for the cutinase-gene-disrupted mutant of 77-2-3.

Additional work on the regulation of cutinase expression revealed that there are two cutinase gene promoters. The promoter of a gene termed *cut2* is constitutively expressed in *F. solani* f. sp. *pisi*, but its expression is higher under starvation conditions. The promoter for gene *cut1* is highly induced by cutin monomers. This differential expression was related to the palindrome binding protein (PBP) binding to the respective promoters. PBP is thought to interfere with the binding of a transcription factor (TF), CTF1, involved in induction of cutinase (58).

Because other nonpathogenic (e.g., saprophytic) fungi also possess cutinase-producing capabilities (45), this enzyme is clearly not the sole component determining pathogenicity in *F. solani* f. sp. *pisi*. However, the observation that cutinase expression in spores of *N. haematococca* can be induced by monomers of cutin (85) indicates that it is likely to be involved in penetration of the cuticle layer. This explanation for transgressing the cuticle in the pea-*Fusarium* interactions appears to be better documented than that for direct entrance via physical pressure from a penetration peg.

Pisatin degradation and *F. solani* f. sp. *pisi* virulence. Research on the pathogen's ability to degrade the phytoalexin, pisatin, has provided insight into the virulence of the *F. solani* f. sp. *pisi* pathogen. The VanEtten Laboratory investigated the capacity of the *F. solani* f. sp. *pisi* P450 enzyme, later termed pisatin demethylase (PDA), to degrade the phytoalexin, pisatin (23). Was this degradation potential the basis for *F. solani* f. sp. *pisi* being a pathogen of pea (73)? PDA assays performed with an array of isolates of *F. solani* f. sp. *pisi* suggested that the more virulent of these isolates could also more readily degrade pisatin (81). The ability to make crosses in *N. haematococca* mating population VI's (80) along with the cloning of the pisatin demethylase gene (84) made possible a genetic analysis of PDA in relation to virulence. The genetic analyses of a number of isolates of *Nectria haematococca* (*F. solani* f. sp. *pisi*) indicated that multiple genes control pathogenicity on pea (52,60,78). The gene considered particularly important was *Pda* coding for PDA. All naturally occurring isolates without the PDA gene (*Pda*-) were essentially nonpathogenic on peas. *Pda*- mutants of a virulent *F. solani* f. sp. *pisi* isolate, created by transformation-mediated gene disruptions, were modestly reduced in virulence (83). As a bonus, these disruptions allowed the detection of other pathogenicity genes that were also located on dispensable chromosomes of this fungus (43,51,79,83). The possibility was proposed that, analogous to bacterial plasmids, these dispensable DNA containing segments may control or influence the fungal host range.

The *PDA1* gene was also transferred to a pathogen of another host to determine if the transgenic pathogen would acquire a capability to infect pea (73). Reportedly, some virulence potential, based on lesion diameter, was donated to the recipient. Thus, it is

currently evident that the lack of the production of pisatin demethylase does not totally negate the *N. haematococca* virulence potential and its presence may not be the sole virulence determinant, but it does contribute to virulence (50).

MOLECULAR BIOLOGY OF THE PEA DISEASE RESISTANCE RESPONSE-PEA ENDOCARP SYSTEM

PR genes (proteins) and disease resistance. In pea, the single dominant gene resistance against races of *F. oxysporum* pv. *pisi* and *Pseudomonas syringae* pv. *pisi* as well as the nonhost resistance to *F. solani* f. sp. *phaseoli* involves some of the same PR genes (19,35,42). There are at least two lines of evidence that this disease resistance response (37) requires RNA and protein synthesis. First, by the use of specific inhibitors such as α -amanitin, which inhibits DNA-dependent RNA polymerase, cordycepin, which blocks the transfer of mRNA out of the nucleus, and cycloheximide, which inhibits protein synthesis, it is possible to determine the vital time following inoculation when the inhibition of each of these processes can no longer block the resistance response (36,37). Secondly, a striking loss of potential to generate the nonhost resistance via PR proteins was evident, monitored by mycelial growth and the mRNA-coded protein accumulation that occurs, following a 2-h heat treatment at 40°C. This loss of resistance is accompanied by the redirection, at the RNA level, of protein synthesis from normally increasing PR-specific message toward the increased synthesis of heat shock proteins (41). Following this heat shock, the potential to activate the synthesis of PR proteins is negated. After a 9-h recovery period, endocarp tissue recovers the potential to activate PR genes and simultaneously regains the potential to regenerate resistance. The PR gene products were identified using a process that is currently considered "proteomics." The pea mRNA extracted from each treatment was translated into protein in the presence of a labeled amino acid with an in vitro protein synthesizing system, and the induced proteins were detected as intense exposure spots with X-ray film following a two dimensional gel separation. This correlative loss and regeneration of disease resistance with PR protein synthesis is a strong indication of the need for the induction of PR proteins.

Nonhost resistance and R-gene resistance involve the same PR genes. In contrast to the initial communications of single-dominant-gene determinants of the gene-for-gene interactions, a considerable number of genes are induced at the PR gene level in nonhost resistance. Over 200 potential PR genes associated with the nonhost resistance response were first selected (on the basis of enhanced expression) from a pea cDNA library (72). Some of the same PR genes are activated in both gene-for-gene and nonhost resistance reactions (19). Nine genes whose induction best followed the actual expression of resistance were selected for further study. Assays for (i) the activation of these PR genes, (ii) cytological detection of the growth of the fungus, and (iii) the observation of the hypersensitive response provided a simple and direct means for monitoring the resistance response (44).

PEA PR GENES WITH KNOWN FUNCTIONS

PAL. The PAL gene family codes for the phenylalanine ammonia-lyase enzyme (48) which catalyzes the first step in the phenylpropanol pathway toward biosyntheses of lignin, pisatin, etc.

CHS. CHS codes for chalcone synthase (46), an enzyme required for isoflavonoids such as pisatin.

DRR206. DRR206 codes for an enzyme (22,27) in the lignin/lignan pathway.

DRR49, DRR276. These pea genes code for a protein homologous with RNase (65,72).

Chitinase gene codes for chitinase (11) an enzyme digesting chitin, a fungal cell wall component.

β -glucanase codes for an enzyme digesting β -glucans, fungal wall components (10).

pI39, pI230. These pea genes code for high cysteine content proteins (12,28) called defensins.

Antifungal products of pea PR genes: Defensins. Defensins are antifungal products of some PR genes. Some of the first defensin genes cloned, pI39 and pI230, came from pea (7,12). These two genes, now named DRR39 and DRR230, are actively expressed in the pea endocarp during the resistance response against *F. solani* f. sp. *phaseoli* (28). The predicted amino acid sequence from DRR39 has 95% identity with a peptide *PsdI* isolated from pea seeds. The gene product of *PsdI* was shown to possess high antifungal activity at concentrations ranging from 0.04 to 22 μ g/ml (2,3). The defensins are usually lower molecular weight proteins (peptides) possessing high levels of the amino acid cysteine (3). The biological activity of the pea defensins corresponds to that of similar highly conserved antimicrobial peptides present in a broad range of biological organisms (75). Defensins can induce the membrane destabilization/permeabilization important for fungal growth (57). Some defensins also have been shown to inhibit protein synthesis, enzyme activity, and ion channels. Plant defensins form a characteristic structure known as the cysteine-stabilized α/β motif, a feature that is also shared by several toxins from insects, scorpions, honeybees, and spider venoms. The only residues that are absolutely conserved are the eight cysteines and a glycine residue (position 34). When pea defensin gene DRR230 is overexpressed in canola, extracts of these plants inhibited the in vitro germination of *Leptosphaeria maculans*, the causal agent of blackleg (82). Some intact transfor- mant adult plants were significantly more resistant than controls to blackleg. This pea defensin gene was also utilized in the biological control of blue mold of apple (47).

Other PR gene products. Direct antifungal action has been proposed for products of other PR genes such as those coding for chitinase and β -glucanase. Although increases in these enzymes in the pea endocarp system are evident beginning at 10 h after inoculation with *F. solani* f. sp. *phaseoli*, they are also initially present (62,63), albeit in lesser amounts, in healthy tissue. Because many fungi have substrates of both enzymes, chitin and β -glucan, within their cell walls, the proposed action of these enzymes in the interaction with the plant has been related to their combined potential to degrade the cell wall at the mycelial apex, exposing the fragile plasma membrane. Indeed, membranes do burst when exposed to high concentrations of β -glucanase and chitinase (64). Furthermore, there is a release of chitosan from *F. solani* f. sp. *phaseoli* when the walls are exposed to these enzymes (49). Chitinase attacks the *N*-acetylglucosamine polymers (within chitin) and possibly because native chitin is partially deacetylated, there is a release of deacetylated fragments (chitosan is 80% deacetylated chitin). The biologically active oligomers of chitosan possess seven or more residues and are released in greater quantity from *F. solani* f. sp. *phaseoli* than from *F. solani* f. sp. *pisi*. Chitosan is a potent inhibitor of *F. solani* spore germination and mycelial growth (49).

Analysis of pea PR gene contributions by transformation. Both the pea gene PR10 (formerly DRR49), which possesses homology with a plant RNase (65), and the pea chitinase gene were transformed into canola with little or no enhancement of resistance to blackleg (82). Transgenic 'Shepody' potatoes possessing the pea gene DRR49 displayed resistance to potato early dying disease (*Verticillium dahliae*) (8). However, the utility of this gene is negated by the close homology of its product to other plant allergens (6). Overexpression of the pea chitinase and β -glucanase genes in potato often reduced plant size without significantly benefiting disease resistance (9). Expression of pea gene DRR206, encoding an enzyme in the lignan pathway (22) in canola, conferred resistance to blackleg (82). In general, transfer of individual pea and other plant PR genes to different crop plant

species has only yielded incremental increases in resistance, rather than full immunity (67). Total immunity may require multiple PR genes.

Preformed antifungal compounds in peas. Pea tissues possess abundant saponins that possibly contribute to the antifungal properties of this tissue, again without accounting for full immunity (16).

Regulation within the fungus. Although the morphological development, detected cytologically, of *F. solani* when exposed to pea endocarp tissue differs between the bean and pea pathogens, the growth of both is initially suppressed within 6 h. A percentage of mycelia of the true pea pathogen (*F. solani* f. sp. *pisi*) can resume growth within 18 h; however, the nuclei in the nongrowing hyphal tips of the bean pathogen (*F. solani* f. sp. *phaseoli*) are visibly fragmented (53). While it is logical to look for antifungal components generated from the plant defense side, it is entirely possible for the fungus to control, in part, its own destiny. For example, as the disease progresses, there is a thickening of fungal walls very reminiscent of the fungus's ability to acquire the dormancy and morphology of chlamydospores (77). These changes are similar to those that occur when spores are held in arid soil or when exposed to other stresses independent of the host response. Also, there is an accumulation of the DNase in *F. solani* f. sp. *phaseoli* beginning with germination (53). The production of DNase by the fungus could have evolved from a need to recover nutrients such as the nucleotides and bases of DNA from older mycelia. The premature accumulation of DNase in younger mycelia could account for the observed nuclear fragmentation, an attribute of programmed cell death, that likely suppresses the pathogen's growth. The plant's role in this scenario may reside in its ability to initially slow pathogen growth, allowing this premature DNase accumulation to occur. The generally better condition of the true pathogen's nuclei remains unexplained.

Summary statement of some plant mechanisms for slowing fungal growth. Some components with potential for slowing fungal growth include saponins, the cuticle barrier, cell wall components (e.g., callose, lignin, etc.), pisatin accumulation, enzymic digestion by chitinase and β -glucanase, chitosan oligomers, and defensins.

Techniques for monitoring pathogen effects in the presence of pharmaceuticals with specified modes of action. The condition of components of pea endocarp cells, primarily nuclear alterations, can readily be viewed with appropriate stains within the critical 6-h period required for resistance development (13, 53). Ultimately, the DNA sequences from selected PR genes have been used to assess gene activity by real-time PCR or northern analyses (28,44). Also, DNA damage assessments were assayed by TUNEL (TdT-mediated dUTP nick-end-labeling) assays and polyacrylamide gel electrophoresis separations of isolated DNA (53). In addition to analyzing changes by pathogens, the simplicity of these direct monitoring techniques has enabled the pharmaceutical evaluation of the effect of most known cell regulating compounds on the development of resistance over a broad range of concentrations (44). In the absence of a cuticle, most compounds are readily taken up by the endocarp cells. These sensitive assays also provided insight into how various chemical structures can initiate or affect the development of the defense responses. Earlier research examined many of the commercially available compounds as elicitors (38–40), whereas later evaluations utilized 40 known inhibitors that affect vital plant cell processes or contribute to the blockage or accumulation of the signaling initiators reported in *Arabidopsis* and other nonlegume systems (44). Remarkably, the resistance of pea legume tissue was not detectably altered by elicitors such as jasmonic acid, salicylic acid, nitric oxide, or superoxides. Additionally, these screenings found that compounds serving as precursors to these latter elicitors do not enhance induction significantly, and at high concentrations can negatively affect resistance.

The pea endocarp tissue can be, and has been, used to test many commercially available regulatory compounds for their ability to activate pisatin synthesis. Cruickshank and Perrin (18) determined early that low concentrations of heavy metals could efficiently elicit pisatin accumulations. Subsequently, it was observed that compounds that intercalate, alter, or helically distort DNA could also induce pisatin (34–39,66). Most importantly this simple system can provide an accurate assessment of any yet to be discovered regulatory compound or component with a potential to alter the disease resistance responses.

Mode of action of biotic elicitors. The natural defense response of pea is elicited by fungal-derived chitosan oligomers and/or a DNase enzyme, and both are known to be released from *F. solani* f. sp. *phaseoli*. These two components are categorized as “biotic inducers.” “Fsph DNase” (29,53) and chitosan (32,49) significantly stimulate both the pea defense response and total immunity to *F. solani* f. sp. *pisi*. Chitosan is a polycationic polymer of β -1,4-linked glucosamine residues (32). An explanation is warranted to explain how chitosan and Fsph DNase with such diverse characteristics may both manipulate similar nonhost resistance responses. Chitosan as a polycationic molecule requires a substantially higher concentration ($>20 \mu\text{g/ml}$) to elicit this response (32) than the Fsph DNase, which elicits at extremely low levels (53). There is evidence that both elicitors enter the pea cell and the pea nucleus (29,36). Fsph DNase is a single-strand nicking enzyme and can cause DNA degradation. This may explain the previously mentioned changes in nuclear density. Chitosan, in a manner similar to other basic polymers, associates with the negatively charged phosphates of nuclear DNA (36), possibly causing the exclusion or alteration of normal cellular proteins that are also basically charged (37) and consequently chromatin conformational changes.

Potential role of HMG A. HMG A (formerly HMG-I/Y) has previously been shown in animal systems to have profound effects on chromatin structure and the activation of genes by associating with AT-rich regions in vital areas of the gene promoters (4, 69,70), some of which are present in PR gene promoters (55). HMG A has been described as an architectural TF. In the pea endocarp system, it has been shown to complex with AT-rich regions within the DRR206 promoter sequence (54), which are vital for induction of DRR206. Further, both the transcribed HMG A message and the detectable HMG A protein are diminished within 6 h after inoculation, but to a greater extent with *F. solani* f. sp. *pisi* than with *F. solani* f. sp. *phaseoli* inoculations. The pea HMG A protein is endowed with conserved amino acid motifs (PRGRP) that form “AT-hook” structures (55). In other eukaryotic systems, these AT-hooks are known to insert within the minor groove of double-stranded AT-rich DNA. This association can be altered by phosphorylation (88). That is, the more this TF is phosphorylated, the less secure the association. Because of the strong conservation of the vital HMG A amino acid sequences between plants and animals, much of what is learned about gene regulation in animal systems can be directly utilized in this pea system.

Phosphatase inhibition and the manipulation of resistance. In pea endocarp tissue it was observed that the following parameters could be enhanced or altered using specific inhibitors of phosphatases: pathogen growth, pisatin accumulation, hypersensitive discoloration, PR gene activation, and DNA damage (44). Calyculin A is a phosphatase 1 and 2A inhibitor, and at 0.15 and 10 $\mu\text{g/ml}$, it induces pisatin accumulations of 46 and 286%, respectively, compared with that induced by treatment with spores of the nonpathogen of pea, *F. solani* f. sp. *phaseoli*. Calyculin A at the higher concentration (10 $\mu\text{g/ml}$) can break the nonhost resistance to this fungus, even in the presence of accumulations of $>200 \mu\text{g}$ of pisatin per gram of tissue. Thus, the potential benefit of these high levels of pisatin is apparently negated by an interference with another component needed in resistance. On the

other hand, lower concentrations (0.03 to 0.07 $\mu\text{g/ml}$) of calyculin A can temporarily enhance the pea tissue's resistance to the true pathogen of pea, *F. solani* f. sp. *pisi*. Resistance can similarly be manipulated with okadaic acid, which also inhibits phosphatases 1 and 2A. Many legume proteins are selectively phosphorylated in response to pathogen challenge (26) or when tissues are subjected to these phosphatase inhibitors. Other legumes also experience enhanced production of flavonoid compounds and plant defenses (30,59) following treatment with phosphatase inhibitors.

Phosphorylation and the regulation of PR gene transcription. In pea, the low concentrations of both calyculin A (0.003 $\mu\text{g/ml}$) and okadaic acid (0.0028 $\mu\text{g/ml}$) enhanced the expression of pea PAL and CHS genes within 5 h (44). Preliminary results from P^{32}O_4 -labeled pea endocarp tissue indicated that the rate of HMG A phosphorylation was severely reduced within 4 h after inoculation with the pathogen, *F. solani* f. sp. *pisi*, and partially reduced by the nonpathogen, *F. solani* f. sp. *phaseoli* (S. Hartney and L. A. Hadwiger, unpublished data). If the plant scenario is analogous with that observed in animal cells, the reduced levels of HMG A phosphorylation should enable the HMG A protein's AT-hooks to better fit the AT-rich regions of PR gene promoters thereby increasing expression (25,88). The status-quo phosphorylation level of HMG A protein in nonchallenged pea tissue would be less able to bind the PR (and other promoters), possibly by preventing the chromatin remodeling required for the expression of certain PR genes. Alternatively, enhanced binding at another chromosomal location could likely give reverse effects.

The more nonspecific challenge of chromatin structure by the biotic elicitor, Fsph DNase, is likely to activate PR genes by a different mechanism. Because DNA is wrapped around nucleosomes, the single-stranded DNase cleaving action may directly affect nucleosome conformation (Fig. 1).

Gene transcription and nucleosome condition. Recent work with nucleosomes (1,21) indicates that following nucleosome disassembly, certain genes therein can be actively transcribed without the requirement of TFs. However, this TF specificity returns after the nucleosome is re-assembled. The complexity of chromatin modeling and transcription is likely to remain mysterious in the foreseeable future; however, the pea endocarp system provides a degree of synchronization useful in detecting changes in individual nuclear proteins. The pea system can be up-scaled sufficiently to enable the purification of the nuclear proteins for analyses by mass spectrophotometry of any modifications, analyses that are difficult using genetic approaches. The disadvantage of this system remains the difficulty of cell culture and regeneration of pea tissue, which discourages permanent gene transfers or replacements (87). The use of transient transformation techniques can partially compensate for this deficiency.

Outlook for alternative approaches to manipulate resistance. Understanding how to manage the concerted induction of multiple PR genes may have more promise than overexpressing individual PR genes in manipulating immunity or high levels of resistance. Each of the two elicitors released by *F. solani* f. sp. *phaseoli*, Fsph DNase and chitosan, have the capability of inducing complete resistance to the true pathogen *F. solani* f. sp. *pisi* (49,53). The possibility of obtaining resistance, via the engineering within the plant of the multi-step biosynthetic pathway of chitosan, is problematic (9). However, a single fungal gene codes for Fsph DNase (53). The cloned Fsph DNase gene was then used in transformation experiments to assess if overexpression would lead to disease resistance. Here, a region of the promoter from pea gene DRR206, which responds to many pathogen challenges (14), was used. This construct was able to provide a high level of immunity in tobacco to moderate inoculum levels of *Pseudomonas syringae* pv. *tabaci* (14). However, the same construct transferred to potato provided little protection against *Phytophthora infestans*, probably because the potato tissue had a greater sensitivity to DNase. If the resistance that results in complete

immunity is due to an assembly of multiple PR gene contributions, it will be important to determine how this array of enhanced gene expression occurs.

Researchers are now reexamining the potential of chromatin changes to alter gene activation (69,71). HMG A proteins, previously mentioned, become a part of chromatin, which mainly consists of the nucleosomes that are repeating units along the DNA. The nucleosome has a central H3 histone/H4 histone tetramer flanked on both sides by H2A histone/H2B histone dimers (86) (Fig. 1). When transcription levels of the gene-containing DNA wrapped around the nucleosome are very high, histones can be transiently displaced. However, most of the transcribed genes retain a nucleosomal organization that the polymerase II (Pol II) of the polymerase complex must confront (74). During nucleo-

some traversal by Pol II, a single H2A/H2B dimer is released. A recent report indicates that there is a considerable DNA sequence and orientation-dependent variation in both the overall height and location of the nucleosome transcription barrier (5). Thus, nucleosomes are not uniform in the strength of these barriers to elongation by Pol II. Also, other mechanisms are needed to inflict the transient loss of the H2A/H2B dimer (75). The enhancement of transcription that has been associated with total nucleosome disassembly (1) may also be related to PR gene induction. Additionally, if future studies in plants also detect weaker nucleosome barriers in the vicinity of the PR genes, this may help explain why the PR genes are induced preferentially over the many other host genes following inoculation with the fungus. The preliminary observations on changes in HMG A in pea and the accumulating

Fig. 1. Summary of changes detectable following inoculations of the “cuticle-less” pea endocarp system. Events of the nonhost resistance response following the inoculation with the bean pathogen, *Fusarium solani* f. sp. *phaseoli* (F.s. phas), areas follow. The elicitors, DNase and chitosan, are produced by the fungi. The chitosan heptamer from deacetylated chitin is directly antifungal (49) and fungal DNase (Fsp DNase) is associated with nuclear fragmentation (53) in the plant nucleus and possibly in nuclei of the fungal germ tube. Elicitors enter plant cells and plant nuclei (29,36), affecting chromatin changes, DNA strand breakage (53), histone displacement and/or HMG A phosphorylation/dephosphorylation and degradation (54) (S. Hartney and L. A. Hadwiger, unpublished data). These changes may enhance the accumulation of pathogenesis-related (PR)-specific RNA 2 to 6 h postinoculation. The rapid induction of PR genes following elicitation by the chitosan heptamer and the fungal DNase is instrumental in suppressing fungal growth through PR genes (e.g., defensins). The increased synthesis of chitinase and β-glucanase genes 10 h postinoculation may contribute to the stabilization of fungal growth suppression. Events associated with the inoculation of the pea endocarp with the pea pathogen, *F. solani* f. sp. *pisi* (F.s. pisi), are as follow. Most of the same processes described above are involved, except they appear at a slightly slower rate. The chitosan elicitors released have a greater percentage of pentamers, which have a documented lower eliciting and inhibiting capacity (49). A percentage of the *F. solani* f. sp. *pisi* hyphae are temporarily suppressed; however, a percentage of mycelial tips with visibly intact nuclei appear capable of continued growth at 10 h (in contrast, no further growth of *F. solani* f. sp. *phaseoli* occurs beyond 6 h). This model involves three proposed mechanisms for enhancing PR gene transcription. (i) Chitosan heptamers alter chromatin via competition with basic nuclear proteins for DNA attachment sites, potentially displacing H2A/H2B histones. (ii) Fungal infection leads to reduced HMG A phosphorylation and HMG A levels (54) (S. Hartney and L. A. Hadwiger, unpublished data) altering the presence at or fit into crucial AT-rich regions in PR gene promoters. (iii) Fungal DNase (single-strand nicking) attacks plant DNA (53) potentially opening sites for transcription initiation. Red = HMG A (architectural transcription factor). Yellow = DNA-dependent RNA polymerase complex. Blue = Histones of the nucleosome units.

data on histone modifications observed in other eukaryotic systems warrant further investigations that may help determine if and how the two are related in plant defense responses.

In summary, the PR gene elicitors chitosan and FspH DNase may affect chromatin modeling in the following manners (Fig. 1): (i) chitosan as a polycationic molecule may compete with histones and other nuclear proteins for sites on the DNA; (ii) FspH DNase as a single-strand DNA nicking enzyme may dramatically loosen the helical condensation of nuclear DNA. Other fungal-initiated signals may affect PR gene transcription by altering state of phosphorylation, methylation, or ubiquitination of HMG A, histones and/or other proteins. As indicated, the phosphorylation of HMG A has been shown to suppress its association with AT-rich sites on promoter DNA (25). The challenges of pea tissue with *F. solani* f. sp. *phaseoli* or *F. solani* f. sp. *pisi* both promote a lower rate of phosphorylation of some nuclear proteins and the eventual disappearance of HMG A, a development that could also change promoter functions (54).

The microarray and proteomic techniques currently utilized to determine the differential expression of genes and their protein products often identify, as did previous library screenings (72), multiple hundreds of genes associated with disease resistance and other stress responses. Because it appears that overexpressed single PR genes can rarely lead to engineering complete immunity to pathogen infection, it may be more productive to strive for manipulative control over multiple genes at the chromatin level rather than in engineering single genetic traits.

Hopefully this review will encourage the continued examination of the biochemistry and molecular cell biology of nuclear proteins, and to the use and development of intact tissue models such as the pea endocarp, to further our understanding of the gene activation associated with disease resistance. Tools that will favor the pea endocarp system include the cloned PR genes, cloned fungal genes, defined biotic elicitors, cytological and biochemical monitoring capabilities of intact tissue, and synchronized resistance development. The completed sequence of the *N. haematococca* (*F. solani* f. sp. *pisi*) will also be a very useful tool for this system.

As an update on the genome sequencing, the following communication is from H. VanEtten at the University of Arizona: *N. haematococca* mating population VI has been sequenced by the DOE Joint Genome Institute (JGI) and is available online by JGI.

Nectria sp. has one of the largest genome (~60 Mb) of any sequenced ascomycete. The isolate sequenced is pathogenic on pea and having this sequence should help to identify those genes specifically involved in pathogenesis on pea as well as identifying the genes that allow this ubiquitously distributed fungus to colonize a wide variety of habitats.

A diagrammed summation of some points presented in this review is presented in Figure 1.

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