Molecular Communication in Host-Parasite Interactions: Hexosamine Polymers (Chitosan) as Regulator Compounds in Race-Specific and Other Interactions

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There is an impressive number of "gene-for-gene" interactions (10) in which disease resistance is conditioned by one or more genes, usually dominant genes, which reside at numerous loci within the plant genome. These resistance genes are believed to be expressed when matched with an appropriate gene in the pathogen, typically a dominant gene conditioning avirulence. When the function of an avirulence gene in the pathogen is mutated (9) or deleted, the fungal gene product-host gene product matchup does not materialize and susceptibility (the compatible reaction) prevails (10).

Resistance is often attributable to the presence of a single dominant gene, but a similar functional assignment can be provided by "minor gene resistance" (4). The individual contribution of minor genes to resistance is even more difficult to resolve. In concert these "minor" genes can provide the information adequate to produce a threshold response, somewhat comparable to that of the single gene factor, which tips the balance toward resistance. If a "minor gene-for-minor gene" relationship exists, one might also expect multiple "minor avirulence factors," which also act in concert.

There are reasons to suspect that a disease resistance response can be as precise a form of differentiation as any other genetically controlled process. As with other changes in differentiation, there are time-related changes in membranes, RNA synthesis, protein synthesis, and the production of new secondary products in the plant, which appear at predictable intervals following inoculations under a defined set of conditions. The precision of the entire process can be disrupted by many external factors. For example, resistance can be negated or reduced with inhibitors of protein synthesis (15, 22,37) or heat treatments (5). It can be demonstrated under standardized conditions that when a specific dominant gene is absent from the host (a gene required for matching the specific avirulence factor in the pathogen) the resistance response does not develop (10). On the other hand, plant cultivars that are considered highly susceptible are usually able to muster a basal level of resistance, which prevents the pathogen from penetrating all cells of the infected plant part. This basal resistance, which has no defined genetic factors, can be enhanced to a significant level by prior inoculation of the plant, sometimes either by the nonpathogen (25,29,36) or by the pathogen itself (30). Thus, it appears that most plants have the physiological machinery to resist the pathogen if it can be activated for a sustained period. Since the development of the resistance response within the plant under all of these circumstances is seldom immediate, it appears that resistance may truly be a developmental stage dependent on an orderly activation of genetic function.

The objective of this paper is to examine the role of one macromolecule, chitosan, which possess the potential to communicate regulatory changes in both the host and fungus. Chitosan is a polymer of β-1,4-linked glucosamine residues. Chitosan is present in the walls of many fungi, but there is little known about how it is assembled with other polymers.

EXPERIMENTAL EVIDENCE

Most of our biochemical investigations compare the compatible interaction between *Fusarium solani* f. sp. *pisi* and its normal pea host with the incompatible interaction between pea tissue and *F. solani* f. sp. *phaseoli*, a pathogen of beans. Additionally, chitosan has been implicated (20) in a gene-for-gene interaction between *Puccinia striiformis* and a wheat isoline (2) possessing a single dominant gene for resistance to Race 1.

Contact events in the compatible interaction between *Pisum sativum* endocarp tissue and *F. solani* f. sp. *pisi*. Macroconidia of compatible and incompatible *F. solani* become securely attached to the surface of the pea endocarp tissue within 2 hr (Fig. 1). This intimate contact between host and parasite is essential to initiate host-parasite developmental processes. That is, if various synthetic membrane barriers (which are water saturated and permeable to most macromolecules) are placed between the macroconidia and the plant cell, the normal induction of phytoalexin synthesis and the yellow-green discoloration (typical of the hypersensitivity response) are suppressed or totally prevented (28). Further, the incompatible fungus, if provided minimal nutrition, grows uninhibited on the upper side of such a barrier, even if disease resistance responses have been previously induced in the host tissue on the barrier's opposite side.

In the absence of a barrier there is no significant difference in the speed at which pathogenic or nonpathogenic macroconidia attach (Fig. 2) to the endocarp surface. When the sugars, glucose or mannose, which are capable of binding pea seed lectin-specific sites, are present there is no decrease in strength or speed of the attachment of the fungus to the endocarp. Thus, there is presently no reason to implicate lectin-specific agglutination in this contact phenomenon.

Effect of the incompatible macroconidia of *F. solani* f. sp. *phaseoli* on pea endocarp cells. The events crucial to the incompatible interaction between pea endocarp tissue and *F. solani* f. sp. *phaseoli* appear to occur very soon after inoculation since the growth of the germinating macroconidia stops within 4–6 hr (34) after inoculation (Fig. 1). Within 15–30 min fungal wall components and chitosan-containing compounds appear in the surface cells of the pea endocarp, (18) and structural changes occur within the nucleus of these cells (16). An enhanced incorporation of uridine into the RNA associated with dispersed nuclear components of the pea cells occurs within 20 min (16). The importance of RNA synthesis within the first 3 hr is indicated by the ability of RNA synthesis inhibitors such as 6-methyl purine to suppress the disease resistance response if applied within 3 hr, but not if applied 4 hr or more after inoculation (15). Within 2 hr the inoculated pod tissue produces sixfold increases in active mRNA specific for the synthesis of phenylalanine ammonia-lyase, a key enzyme in the synthesis of phenylpropanoid products including the phytoalexin pisatin (26). The de novo synthesis of this enzyme increases to levels more than 10-fold those in healthy uninoculated tissue (26).

Accompanying the specific increase in PAL are patterns of differential protein synthesis (within 5 hr after inoculation) that are detectable by autoradiographic analysis of electrophoretic separations of labeled proteins (W. Wagoner and L. A. Hadwiger, *unpublished*). Pisatin accumulations are first detected by extraction techniques at 5.5 hr after inoculation (34); concurrent accumulations of hexosamine polymers (chitosan) are readily detected histochemically by light microscopy both in the tip of the suppressed hypha and in plant cells (17,18).

The inhibition of fungal growth is usually complete within 6 hr and remains complete for 9 days or until the onset of visible senescence in the pod (34). The period beginning 6 hr after

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inoculation is characterized by further increases in chitosan in the fungus and pisatin accumulations in the plant. Also at 18 hr accumulations of a yellow-green compound(s) occur in plant cells adjacent to the fungal spore (34).

Resolution of early changes. Very early (within 15 min) ultrastructural changes in the plant nucleus (16) and in the cellular and nuclear membranes show up as physical changes in nuclear fractions. These changes may occur because of the deterioration of cellular compartmentalization. For example when lysine-^{3}H is applied to the tissue 4–6 hr after inoculation (Table 1), a greater proportion of the label is recovered in the chromatin from infected tissue than in that from the control tissue. The greater accumulation of label into chromatin from the compatible than from the incompatible reactions may be correlated with the more extensive alteration of the nuclear membrane (16) in the former. Thus, proteins newly synthesized in the cytoplasm may more easily traverse the membrane and contaminate the nucleus.

Effect of the pea endocarp tissue on basic processes within the cells of *F. solani*. Due to the rapid uniform reaction of the entire endocarp surface to incompatible Fusarium macroconidia as well as to the short delay in irreversible attachment of the spores to the surface cells, it has been possible both to label and recover fungal cells (with a camel's-hair brush) within the period crucial for the incompatibility response. The data in Table 2 support an effect of the plant cell on RNA and protein synthesis within the fungal cell as estimated by the rate of incorporation of uridine-^{3}H and mixed amino acid-^{3}H into TCA-insoluble material. The rate at which the fungus can incorporate precursors of RNA and protein is consistently reduced within the first 2 hr after the fungus is in contact with host tissue. Thus, it appears that spores of both pathogen and nonpathogen synthesize less RNA and protein within 2 hr after coming in physical contact with the host tissue, even though nutrition is not a limitation. This inhibitory effect was consistently noted with different harvests of pods and several lots of macroconidia.

Pea pod tissue contains enzymes that digest fungal wall components. Our recent investigation of glycolytic enzymes indicates a level of enzyme activity in pea pods that is capable of hydrolyzing carbohydrates possessing linkages similar to those in the Fusarium wall carbohydrates (28). If fungal wall carbohydrates are accessible to those hydrolytic enzymes, the degradation fragments of the fungal wall must be suspected as potential regulatory macromolecules influencing both host and fungal cell processes. Indeed fungal cell wall fragments are known to induce phytoalexin production in plant tissue (3,17). Phytoalexin inducers can be derived most easily from filtrates of pure fungal cultures near the end of or past log-phase growth when degradation

TABLE 1. Incorporation of lysine-^{3}H into the nuclear proteins of infected pea tissue

		Percent of lysine-^{3}H incorporated[a]				
Treatment	Host reaction	Nonidet-soluble protein	Total chromatin	"Histone protein"[b]	"Non-histone protein"[b]	Residual DNA[b]
H_2O		42.9	1.04	0.520	0.102	0.025
F. solani f. sp. *phaseoli*	Res	64.5	0.73	(.185)[c]	0.226	0.090
F. solani f. sp. *pisi*	Sus	49.2	2.34	0.363	0.728	0.331

[a] Fifteen grams of immature pea pods were split and inoculated with 5 μl of a dense (3×10^6 spores per milliliter) suspension of washed spore germlings. The inoculated pods were allowed to air-dry and incubate for 3.5 hr. Pods were then pulse labeled for 2 hr with 50 μCi lysine-^{3}H. The pods were washed in 500 ml of sterile water to remove excess label. The chromatin extraction procedure performed at 0–4 C was essentially that of Goff (11). Pods were blended 1 min in Goff's buffer (0.3 M sucrose, 0.04 M $NaHSO_3$, 0.025 M Tris-HCl, 0.01 M $MgSO_4$, 0.5 mM EDTA, and 0.3% Nonidet P-40, and the final pH was adjusted to 7.4. The homogenate was centrifuged 30 min at 5,000 rpm. The nuclear pellet was resuspended in Goff's buffer and recentrifuged at 10,000 rpm for 10 min. The pellet was dispersed with a tissue grinder in 0.01 M Tris, pH 8.0, containing sodium bisulfite and was layered on 20 ml of 1.7 M sucrose gradient, and centrifuged 90 min in a Spinco SW 25.2 rotor at 22,000 rpm. The nuclear pellet was recovered along with some of the starch layer and was repeatedly resuspended in 0.01 M Tris pH 8.0, and centrifuged at 10,000 rpm until the starch was removed. The "histone" and "nonhistone protein fractions" were extracted according to Elgin (8). Isotope incorporation was based on the percent label present in the homogenate, which was considered the total label taken up by the tissue.

[b] The percent of label incorporated was adjusted to the percent incorporated per milligram of DNA recovered to correct for differences encountered in DNA recovery.

[c] This incorporation rate was found to be higher in repeated analysis.

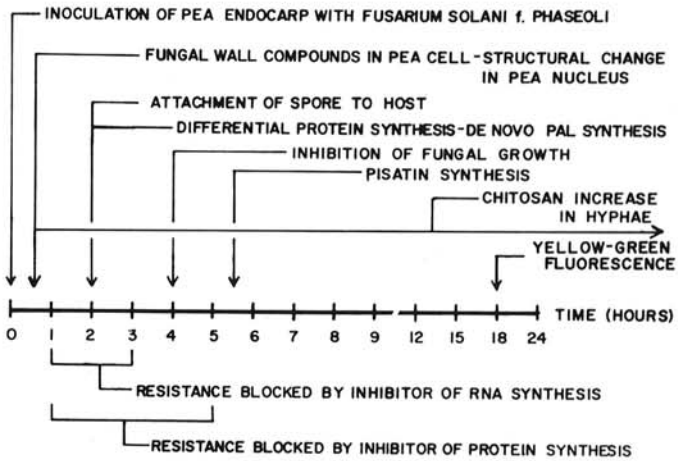

Fig. 1. Major events in the incompatible interaction between *Fusarium solani* f. sp. *phaseoli* and pea endocarp tissue.

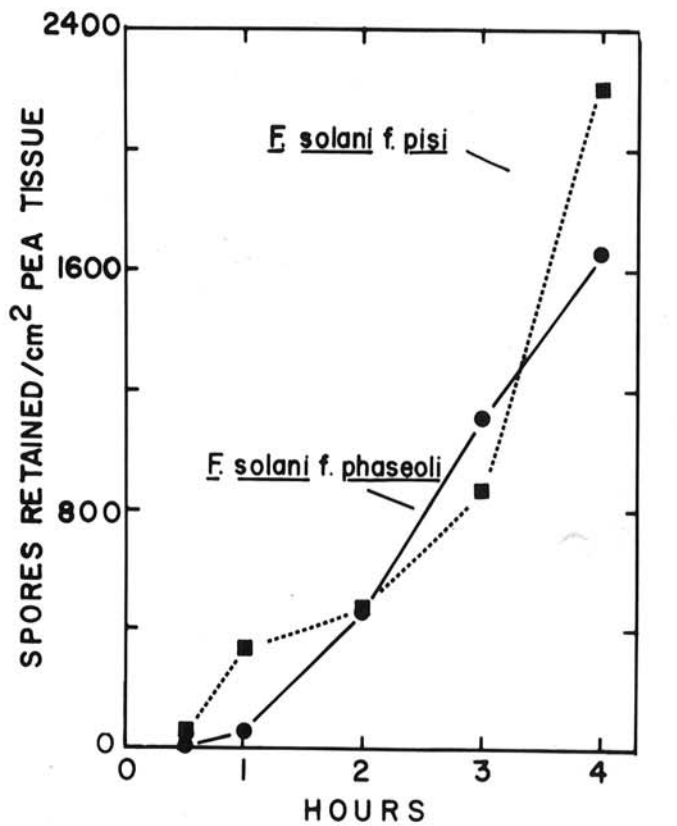

Fig. 2. Number of macroconidia of *Fusarium solani* f. sp. *pisi* or *phaseoli* that attach to pea endocarp tissue within 4 hr after inoculation. Macroconidial suspensions with uniform spore counts (1×10^6/ml) were prepared and applied to the exposed endocarp surface of each pea pod half (2 cm in length). At specific intervals after spore application the pod halves were added to 5 ml of sterile H_2O in conical centrifuge tubes and stirred on an omnimixer for 15 sec. Counts of spores retained on the endocarp were direct microscopic counts of multiple surface sections of the entire endocarp surface.

products are present. Since phytoalexin elicitors are released immediately after healthy fungal spores are introduced onto plant tissue, the potential of plant hydrolytic enzymes (28) to release these elicitors immediately and in abundance should not be overlooked in the total host-parasite interaction.

Role of hexosamine molecules in the interaction of pea and *F. solani*. An analysis of purified *F. solani* cell walls indicated that they contain only 1.5% chitosan (25). Phytoalexin induction by commercially prepared chitosan (177-μm [80-mesh]) surpassed that of cell walls prepared from cultures of *F. solani* f. sp. *phaseoli*. Furthermore, the effectiveness of chitosan increased as the particle size decreased. When a means for chemical cleavage of chitosan into small polymers (<2,000 mol wt) was devised (Fig. 3), pisatin synthesis was induced with cleaved chitosan at levels less than 7 ppm (17).

An unexpected regulatory property of chitosan was its inhibitory action on pathogenic fungi. Again the effective level of action is dependent on its molecular size. Chemically cleaved chitosan completely inhibits *F. solani* at 3–7 ppm. Finally, 2 ppm of chemically cleaved chitosan, when applied 24 hr in advance to pea tissue, can protect against *F. solani* f. sp. *pisi*.

Histochemical, immunochemical, and isotopic techniques (18) were devised to determine if and where chitosan was involved in the regulatory communication between host and parasite. Although chitosan is not a major constituent of the fungal cell wall, it soon became evident from these studies that chitosan or possibly smaller hexosamine polymers accumulate as the host-parasite interaction progresses (18). All three analyses indicate that chitosan (or hexosamine-containing polymers) is released from the fungal cell following contact with the plant tissue. These hexosamine-rich polymers can quickly enter the plant cell (15 min) (moving with the electrochemical gradient) and accumulate in the walls, cytoplasm, and nucleus. The substantial accumulation of the chitosan-specific label in the nucleus along with its high affinity for DNA (17,18) suggest that it might have a direct effect on regulation of plant responses.

Figure 4 summarizes diagrammatically the observed (25,26) migration and localization of chitosan-containing components released from the fungus. The major structures of the fungal cell specifically recognized by antichitosan antisera are within the outer edge of the fungal cell wall. Antichitosan antisera-specific, electron-dense stain accumulates mainly in regions of the outer edge of the fungal wall and eventually (24 hr) in organelles just inside the fungal cell. Stain also is found throughout the plant cell and conspicuously in the pea nucleus. Histochemical staining (17) of chitosan polymers indicates that chitosan accumulates in the germ tubes of both compatible and incompatible fungi. The compatible fungus, however, is often able to continue growth via secondary hyphal growth (escape hypha). These escape hypha have little detectable chitosan when growing freely on the endocarp tissue. The accumulation of chitosan in a restricted region (a few cells) around the Fusarium macroconidia appears initially more intense in the incompatible interaction but after 2 days many of the

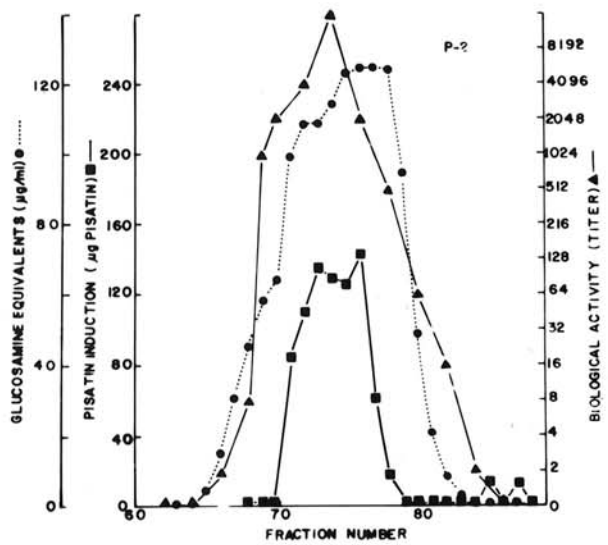

Fig. 3. Correlation of glucosamine content with activity against *Fusarium solani* and phytoalexin-inducing potential of water-soluble chitosan molecules (<2,000 mol wt). The soluble chitosan molecules were prepared and assayed as described previously (27) with nitrous acid cleavage. Chitosan was fractionated on a 1.5 × 120-cm column containing Bio-Gel P-2 (149–74 μm, 100–200 mesh). Biological activity (growth inhibition of *F. solani*) of the diluted fractions = triangles. Pisatin induction = squares. Hexosamine content = circles.

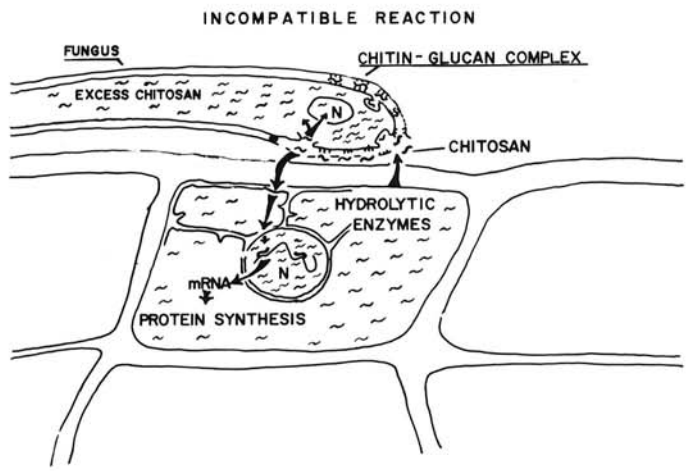

Fig. 4. A diagrammatic summary of the observed (18) migration and localization of chitosan-containing molecules and some proposed functional attributes.

TABLE 2. The comparative rate of uridine-^{3}H and mixed amino acid-^{3}H incorporation into the TCA-insoluble material of *Fusarium solani* f. sp. *phaseoli* (incompatible fungus) of *F. solani* f. sp. *pisi* (compatible fungus) while grown in Vogel's medium on pea tissue or in Vogel's medium only

	Incorporation of substrate dpm/10^6 spores[a]			
	Uridine-^{3}H		Amino Acid-^{3}H	
F. solani f. sp.	2 hr	4.5 hr	2 hr	4.5 hr
phaseoli in media on pea	13,732[b]	57,355	16,968	40,045
phaseoli in media only	26,847	120,762	19,028	130,000
pisi in media on pea	48,204	185,266	27,304	92,043
pisi in media only	95,791	38,235	39,540	65,900

[a] Two milliliters of freshly harvested macroconidia (3×10^6 spores per milliliter) of *F. solani* were washed in Vogel's medium (plus 500 mg casein hydrolysate per liter) and pelleted in a graduated centrifuge tube at 1,000 rpm. The pellet was resuspended in 0.5 ml of fresh medium and uniformly labeled with mixed amino acid-^{3}H, or uridine-^{3}H. The spore suspension was immediately dispersed on the exposed endocarp surfaces of 2 g of immature pea pods or on a comparable surface area of glass petri plate. Following the appropriate pulse label period, the macroconidia were quickly recovered with a camel's-hair brush in 10 ml of fresh media, then diluted 3× with sterile H_2O, pelleted, and resuspended in cold 5% trichloroacetic acid (TCA). The residual spores were collected on a Whatman 3 MM filter paper, washed 2× with cold TCA, and once with cold chloroform. The filter was added to scintillation cocktail and counted following a 24-hr holding period, which eliminated chemoluminescence.

[b] Values represent an average of two treatments; deviation from the mean was minimal.

infected pea cells of the compatible reaction also accumulate chitosan. We propose that the pea hydrolytic enzymes aid the release of chitosan molecules from the fungal cell and that chitosan influences mRNA and protein synthesis (Fig. 4). Chitosan, like *F. solani*, can enhance the activity and amounts of phenylalanine ammonia lyase and pisatin, the plant responses most studied in the pea-Fusarium interaction. These responses can be induced by several processes (14,19,31,32), many of which specifically alter the conformation or molecular makeup of DNA (19). Chitosan can also influence the physical properties of DNA (17).

It is important to reemphasize at this point that although the plant's response to DNA-specific compounds is reproducible for a given DNA-specific inducer, these compounds induce many and varied responses that often happen to include the synthesis of phenylalanine ammonia-lyase and pisatin. The associated alterations in protein synthesis indicate that each inducer can uniquely influence total protein synthesis (13,19). We have been able to demonstrate that phytoalexin induction occurs when the inducer alters only the DNA molecule within the pea cell (31). For example, the synthetic inducer 5-bromodeoxyuridine does not induce phytoalexins simply by entering the pea cell. In the lag phase this base analog must be incorporated into the DNA molecule for pisatin induction to occur.

LEADING QUESTIONS

Do hexosamine polymers function as a natural dormancy factor in the fungus? Hexosamine polymers have been detected both in mature urediospores of *P. striiformis* (20) and in the dormant chlamydospores of *F. solani* (17,18). The hexosamine content progressively increases as chlamydospores develop under stress (26) and as urediospores mature in the pustule (20). It is certainly possible that dormancy and hexosamine accumulation are simply concurrent phenomena and not related in a cause-and-effect manner. However, when linked to the above observations, the fact that a very low amount of exogenous chitosan is a powerful fungal growth inhibitor strongly enhances the possibility that hexosamine polymers play some type of repressive role in dormancy.

Do plant enzymes release hexosamine polymers from *F. solani* spores? An in vitro simulation of the pea-Fusarium interaction was devised by combining the plant enzymes (in the form of pea acetone powders) with acetone-extracted fungal tissue (grown 48 hr in shake culture with complete medium). One-gram amounts of each of these dried tissues were incubated (37 C in 15 ml H_2O) in all combinations to determine if there were different potentials to solubilize hexosamine polymers in specific host-pathogen combinations. The difference between the compatible and incompatible host-parasite mixture was substantial within the first hour of digestion (Fig. 5). Only trace amounts of hexosamine-containing compounds were detected in the controls, which consisted of self-digests of pea acetone powders and acetone-washed mycelium of *F. solani* f. sp. *pisi* or f. sp. *phaseoli*. The host-parasite digest demonstrated the potential for the enzymatic release of hexosamine-containing compounds that are diverse in quantity and size. Of greatest interest was the fact that the digest of the incompatible interaction was capable of generating more antifungal compound(s) (as assayed in vitro) than the digest of the compatible reaction. It has not been established that these antifungal compounds contain hexosamine.

Do hexosamine polymers (chitosan) have a role in "gene-for-gene" interactions? All fungi are certainly not sensitive to hexosamine polymers (1) and it is likely that the diversity of disease resistance mechanisms in plants will prove to be immense. However, hexosamine polymers do accumulate in the growth-retarded hyphal tips of both *P. striiformis* (20) and *Puccinia recondita* (L. A. Hadwiger, *unpublished*) following inoculation on wheat leaves, and chitosan can inhibit the germination and growth of these fungi. In each of these wheat systems, resistance is controlled by major gene factors for disease resistance. We proposed earlier (21) that "in all cases where the specific gene for resistance in the host and the specific gene for avirulence in the pathogen are dominant, the gene in the pathogen may direct the synthesis of compounds that activate (derepress) the genes in the host." It has been possible to demonstrate in some interactions that resistance is dependent on RNA (15) or protein synthesis (22,34,38) as would be expected for any cellular differentiation that involves new or supplemental transcription and translation of genetic codes.

PROPOSALS

Proposed functional aspects of single gene factors for disease resistance and a speculative model mechanism involving hexosamine polymers. In studies of isolines containing various specific genes for disease resistance, it was observed that the presence of a single dominant gene for resistance was responsible more for a pleiotropic alteration in the synthesis of proteins (35) or enhanced enzyme activities (12) than for an enhanced synthesis of a single protein product. It is possible that a single gene-mediated product (RNA or protein) acting in a regulatory manner may be responsible for the observed range of effects. However, since it has not yet been possible either to isolate or attribute single functions to the individual products of genes controlling disease resistance, we are departing from the "one resistance gene-one protein product" dogma to raise the possibility that genes controlling disease resistance may themselves be genetic segments of the chromosome, which function in a *regulatory* fashion without transcription. To promote discussion, we offer this speculative view as one alternative.

Our model proposes that disease resistance draws together many factors that function to regulate adjacent structural genes the same way heterochromatin regions of the chromosome function to regulate certain "position effect" genes in *Drosophila* (33), corn, and other eucaryotes.

The phenomenon termed the "position effect variegation" in *Drosophila* is an example of how one (apparently nontranscribed) portion of the eucaryotic genome can control the expression of structural genes. An example of one such gene is a genetic locus w^+ controlling red eye color in *Drosophila*. A white mottled effect is caused by placing the w^+ locus near a heterochromatin region (Fig.

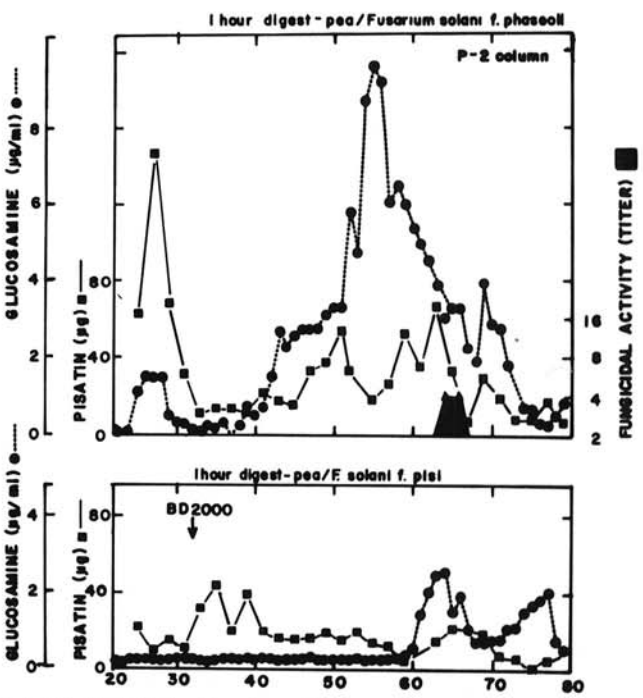

Fig. 5. Bio-Gel P-2 fractionations of enzyme digests of the combined plant enzymes and acetone-washed fungi representing compatible and incompatible interactions. One gram of acetone powders of pea pod tissue was incubated at 37 C in 15 ml of H_2O with 1 g of acetone-washed mycelium (from 2-day-old culture) of either *Fusarium solani* **A,** f. sp. *phaseoli* or **B,** f. sp. *pisi*. The soluble digest (7.5 ml) was separated on a 1.5 × 120 cm Bio-Gel P-2 column and assayed as described previously (17).

6). Thus, position effect variegation occurs when a chromosomal rearrangement juxtaposes a genetically normal eucaryotic gene to a heterochromatic region of a chromosome. When this structurally altered chromosome is heterozygous with a normal chromosome carrying a mutant allele of the rearranged locus, the resulting organism is a mosaic of mutant and wild-type tissue. The variegating phenotype results from polarized *inactivation* of normal euchromatic loci such that loci close to the heterochromatic break point are inactivated more frequently than those further away. Virtually every gene is susceptible to variegation, but the physical distance over which variegation occurs varies from region to region.

The extent to which the variegated phenotype is expressed can be modified by factors such as temperature or by extra heterochromatic elements elsewhere in the genome. A more recent proposal is that the nonacetylated histones, which are preferentially associated with inactive chromatin, play a role in the phenomenon of position effect variegation (27). Processes that reduce the deacetylation of histones reduce the inactivation of the w^v locus. In the example cited, the w^v locus again expressed red eye color. Thus, alterations in chromatin structure affect gene expression.

How might this phenomenon relate to the host-parasite interaction? The possibility that a major portion of the plant genome is involved in regulatory functions analogous to those of the heterochromatin "position effect" phenomenon may be viewed through improved techniques for estimating single-copy DNA sequences in eucaryotic organisms. Although the function of the repetitive sequence DNA is not known, hybridization experiments with mRNA populations reveal that expressed structural genes of plants are contained in the lesser amount of unique-sequence DNA. For example it has been estimated that there are 60,000 diverse structural genes expressed during the dominant phase of the life cycle of tobacco plants. These genes are identified by an ability of DNA to hybridize with polysomal mRNA collected throughout the life cycle and hybridized in an RNA-driven reaction. This amount of genetic information constitutes only 4.6% of the tobacco genome (23). Even assuming that the experimental technique was unable to detect many of the structural gene sequences, a very large proportion of the host plant genome appears to be available for regulation of the sequences coding for mRNAs. It has been established in studies of other eucaryotic organisms that repeat sequences are interspersed with unique-sequence DNA throughout most of the genome. In contrast, the unique sequences in fungi such as *Neurospora crassa* and *Aspergillus nidulans* constitute a much larger portion (97–98%) of the genome (only 3–4% are repeated sequences) (7).

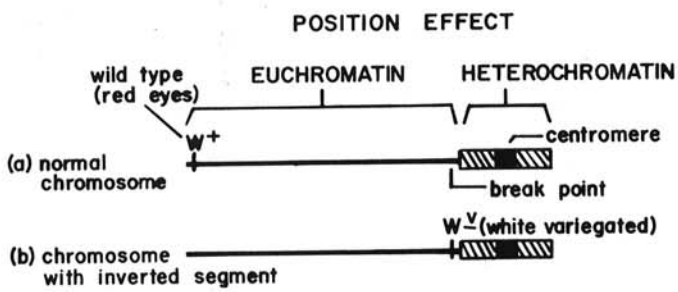

Fig. 6. A diagrammatic description of a chromosomal rearrangement that positions the wild-type gene for red eyes close to a heterochromatin region. The gene for red eye expression is suppressed on chromosome b due to the "position effect."

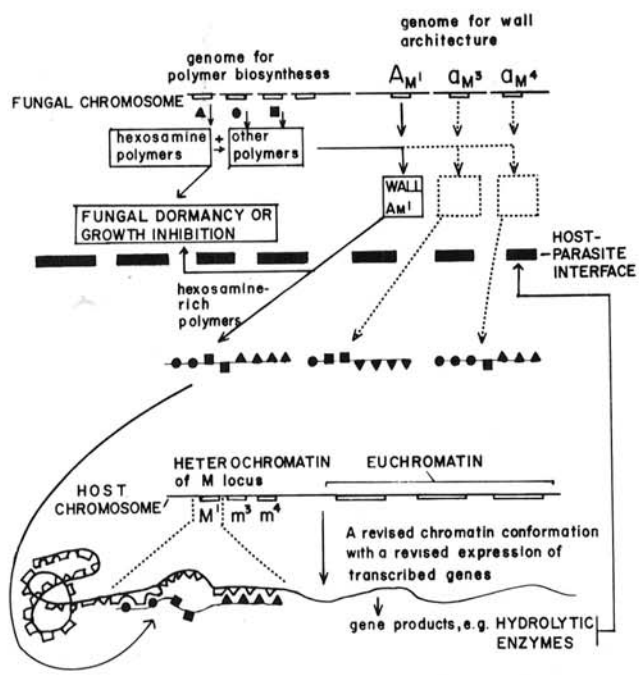

Fig. 7. Proposed roles for gene loci in host and pathogen, which determine the infection type in gene-for-gene interactions. Resistance occurs when a host with dominant gene for resistance (M^1) interacts with a race of the pathogen that contains the corresponding dominant gene for avirulence (A_{M^1}).

Host genes for disease resistance. What has been called a particular gene regulating the resistance to a particular pathogen or race of the pathogen may be instead a physical arrangement of DNA adjacent to authentic structural genes that are awaiting the appropriate stage of plant development for expression. A healthy plant may have a limited role for these structural gene functions and their inherent regulatory compounds may only solicit a basal quantity of gene product at the appropriate stage of differentiation. In the presence of a given race of the pathogen, however, the nucleus of the plant cell may become associated with the molecular debris specifically derived from the fungal cell by plant hydrolytic enzymes. These fragments may indeed interact with and influence many chromosome segments (18). If some portion of this macromolecular debris could influence the structure of one or more heterochromatin regions, the altered transcription of the pertinent structural genes in neighboring loci might enhance a general resistance response. One could also then visualize other closely linked resistance genes (10) as being within the same heterochromatin region but as responding to different molecular stimuli. Thus, the number of *structural* genes functioning in these disease resistance responses could be few or many. In any event, there would be no need for the resistance response products to be numerically equivalent to the dominant genes conditioning resistance.

In the absence of the specific dominant genes for disease resistance the fungal debris could still influence conformational properties of many heterochromatic sequents and generate responses, but possibly be inadequate in content, intensity, or response time to reach the threshold level required for successful resistance.

This concept definitely is not intended to explain all eucaryotic gene regulation and does not exclude other models such as that recently proposed by Davidson and Britten (6) in which repetitive DNA plays a very different role. Rather, we conceive the conformational control mechanism to be a superimposed and perhaps even a rather crude form of regulation that has arisen from selective pressure to respond to attacking pathogens.

A proposed basis of resistance. We have observed that hexosamine polymers similar in size to those that inhibit the growth of some pathogens can be solubilized following the incubation of fungal cells with crude plant enzymes. Thus, as one

example, resistance may take the form of sustained production of plant enzyme proteins capable of releasing antifungal compounds from fungi, which eventually totally suppress fungal growth in the incompatible reaction. The less intense host protein synthesis of the compatible response may well generate some resistance; however, if the release of antifungal compounds is initially too slow it would allow for the success of hyphae that escape.

Although the cytogenetic evidence is lacking to equate the disease resistance loci with heterochromatin regions, there is evidence (35) that single genetic factors at a given locus are responsible for characteristic, pleiotropic effects in the inoculated tissue. It has been proposed that hexosamine-containing polysaccharides have a role in pretranslational control of protein synthesis in eucaryotes because they induce loosening of chromatin structure and because nuclei from different kinds of cells contain glycosaminoglycans in significant amounts (24).

Proposed specificity in the fungus. The specificity of genes for virulence or avirulence in the fungal genome may be expressed in relationship to the architecture of the spore from which this host-regulating fungal cellular debris is derived (Fig. 7). The characteristic macromolecular organization of the fungal debris fragments would insure or limit their influence on the pertinent segment of the chromosome. The fungal debris in this particular interaction plays two roles, that of host regulator and that of fungal inhibitor. Although attention is focused on hexosamine-containing polymers, other fungal cell-derived compounds may inhibit fungal growth following plant-fungus contact.

SUMMARY STATEMENT

When the presence or production of antifungal compounds *by the plant* does not provide a convincing explanation for the plant's ability to resist fungal pathogens, alternative sources of antifungal compounds (eg, from the fungus itself) should be pursued. Additionally, when the disease resistance controlled by a single gene factor is not readily attributable to a single structural gene product, the basic premise that this single gene codes for a single protein may not always be correct. One hypothetical explanation is that some resistance genes might reside in segments of heterochromatin and that their base sequences influence the chromatin composition and conformation. Structural properties of the locus and subsequent expression of neighboring genes would be influenced by some of those components of the fungal hyphae that enter the plant cell. The resultant pleiotropic response associated with resistance may occur via the changes in the expression of the structural genes regulated by such heterochromatin regions.

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