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Host recognition by toxigenic plant pathogens

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Abstract. Certain fungal pathogens release host-selective (or host-specific) toxins (HST) as a host recognition factor during spore germination at the infection site on plants. Prior to penetration of the pathogen into its host, the released toxin specifically binds to a putative receptor on the host cells and initiates signaling mechanisms leading to pleiotropic effects on cells. Of these, the crucial one negates the general and inducible defense reactions of the cells. This is accomplished by a signal from the HST, which is transduced through a path way at or near the step of plasma membrane modulation, which is directly or indirectly triggered by the HST. This mechanism operates even though the toxin may affect mitochondria or chloroplasts as the primary target organelle. The fungal spore is able to penetrate the so-called 'narcotized cell' and completes the initial colonization of the host. The host recognition process may take place without necessitating host cell death, even in the case of perthophytic parasites. At the molecular level, HST-mediated recognition of the host by a pathogen requires strict stereochemical precision like a lock and key.
Key words. Host recognition; host specificity factors; host-specific toxins; host-selective toxins; *Alternaria*; *Cochliobolus*; plasma membrane; mitochondrion; chloroplast; toxin receptor.

Introduction

In spite of repeated and massive exposure to diverse fungal parasites, higher plant species actually suffer from only a very minute fraction of the potential plant pathogens they come in contact with. In general, most plants have evolved 'non-host' defense mechanisms which can act against all but a few specialized parasites[16]. Such 'specificity in parasitism' or 'host recognition' has been of great interest to plant pathologists for a long time. It is not an easy task, however, to define 'recognition' precisely. Sequeira's definition[53], proposed in 1978, is most

helpful for plant pathologists: 'host recognition' can be considered as 'an early specific event that triggers a rapid overt response by the host, either facilitating or impeding further growth of the pathogen.'

There are now two different theories about the problem. As pointed out by Nishimura[41,42], the question is, does 'specificity' result from resistance or susceptibility? Research has long been dominated by the former view, that the determination of 'specificity' arises from the activation of a mechanism for resistance, e.g. the release of specific or non-specific elicitors from fungal pathogens which stimulate phytoalexin production and/or the hypersensitive reaction of the host plant. The other view is that the susceptibility reaction is determined by a genotype-specific suppression of inducible and general resistance by specificity factors such as host-selective (or host-specific) toxins (HST) and specific suppressors[44]. Recent progress in studies of diseases involving HST strongly favors the second view of specificity[26,40,42,44,45]. A concept for a mechanism that determines specificity envisages at least three basic processes[40,41]: a) spores of a fungal parasite release, before penetration, a host recognition factor (for example a highly selective and potent HST) as a signal, b) the released signal factor selectively binds to receptor sites in host cells, and c) the signal is transduced in such a way as to immobilize the host's defense reactions and condition the cells to produce an 'accessible state' for fungal penetration, prior to cell death. This hypothetical scheme has been supported by concrete evidence during the last decade, in consequence of in-depth research on HSTs, with critical evaluation of their role in the penetration and initial colonization of plants by pathogens[24-26,44].

Our present knowledge of HSTs has mostly been obtained from the so-called saprophytic pathogens such as some species of *Alternaria* and *Cochliobolus*, acting on highly susceptible genotypes of crop plants. The structures of many HSTs have been elucidated in the last decade, with the help of modern analytical techniques particularly suitable for the purification and characterization of minute amounts of bioactive natural products. The growing view that HSTs are primary determinants of disease has received attention in symposia and workshops worldwide, and stimulated the publication of several books[14,24,44], chapters[25,26,41], and review articles[8,23,40,51,61]. These are milestones indicating a marked advance in research into the molecular basis of the parasitism mediated by HSTs. In this review we focus primarily on the roles played by HSTs in host recognition by toxigenic pathogens.

Definition of HST as the key determinant of disease

The term 'host-specific toxin' was coined in 1964 by Pringle and Scheffer[49,51]. As often noted in articles about diverse HSTs, there are certain diseases in which the host range of the pathogen coincides with the range of plants sensitive to the phytotoxic metabolite of the pathogen. All isolates of the pathogen that produce such a toxin are pathogenic to certain plant genotypes; all isolates that fail to produce the toxin are not pathogenic to these host plants. In addition, the toxin is released on germination of the pathogen at the site of infection (Nishimura and Scheffer)[43], with the consequence that toxin-exposed host cells allow penetration or initial colonization by the pathogen (Yoder and Scheffer)[62]. These classic requisites appear to be reasonable and persuasive even now. There is no doubt, that genetic analysis of the involvement of a toxin in pathogenesis offers the most critical approach[61], but this requires an extended knowledge of the pathosystem. As the second best approach at least during the beginning stage of research, we should carefully examine whether the toxin in question can satisfy the above definition before it is classified as an HST.

Structures and host-selective toxicity

About fourteen HSTs have been reported in the literature so far (table). Most of them are produced by species of *Alternaria* and *Cochliobolus*[44,51]. For a long time, limited results, requiring great efforts, were obtained in work directed towards the isolation and structure-determination of HSTs; nowadays, with the availability of advanced analytical methods, these studies have rapidly progressed. Figure 1 reports the structures of HSTs so far elucidated; they belong to various classes of chemical compounds and are often produced as a mixture of related substances.

It is surprising that structurally related toxins are produced by three distinct pathotypes of *A. alternata*, i.e.: 1. the cause of the black spot disease of Japanese pear in Japan; 2. the cause of the black spot disease of strawberry in Japan; and 3. the cause of the brown spot disease of certain mandarin oranges and tangerines in Australia and the USA. The toxins have in common an 8-substituted-9,10-epoxy-9-methyldecatrienoic acid structure.

In the early 1980s Nakashima et al.[34] isolated two toxins in crystalline form from culture filtrates of *A. alternata*, the Japanese pear pathotype (called AK-toxins I and II), and determined their structures by chemical and spectral analysis, and by X-ray diffraction[35]. The structures were further confirmed by total synthesis by three independent research groups[5,6,20,36]. Out of 36 cultivars of Japanese pear examined, only 9, such as 'Nijisseiki' and related cultivars, are affected by AK-toxin I at a concentration as low as 10^{-9} M, whereas the others, in addition to non-host plants, are insensitive even at 10^{-4} M[47]. This all-or-nothing type of selectivity in toxic action completely matches the host-or-nonhost response to the pathogen in pear cultivars. AK-toxin II, a demethyl derivative, has the same selective toxicity as AK-toxin I but its activity is reduced to one-fifteenth.

In Japan, the strawberry pathotype of *A. alternata* affects only the susceptible strawberry cv. Morioka-16 in the

field. However, in laboratory tests, it also affects certain cultivars of Japanese pear, showing the same host range as the Japanese pear pathotype (AK-toxin producer) of *A. alternata*[31]. This host range coincides with the sensitivity spectrum of Japanese pear to AF-toxins of the strawberry pathotype. In 1986 Nakatsuka et al.[36] isolated AF-toxins I, II and III from culture filtrates of the pathogen and elucidated their structures by spectral analysis and total synthesis. AF-toxin I causes leaf necrosis of both strawberry and Japanese pear, while AF-toxin II causes necrosis of pear only[31]. AF-toxin III is toxic for strawberry and less toxic for pear[38]. The decatrienoic acid moiety in the major AF-toxins has a 2*E*,4*E*,6*Z* geometry, while that of the AK-toxins is 2*E*,4*Z*,6*E*. In addition, the strawberry pathogen produces small amounts of stereoisomers which are different with respect to the trienoic acid structure. Among the three isomers of AF-toxin I examined for toxicity, that with the 2*E*,4*E*,6*Z* configuration showed the highest toxicity to strawberry leaves. The three isomers of AF-toxin II, characterized by a free hydroxy group at the 2′-position of the 8-acyl moiety, showed no toxicity to strawberry but were still toxic to pear. Moreover, some 2′-*O*-acyl derivatives (acetyl, propionyl and isovaleryl) prepared from AF-toxin II by acylation were as toxic as AF-toxin I or III, on both strawberry and pear leaves. Nishimura et al.[42] suggested that the 2′-hydroxy group represents a feature of the molecule essential only for toxicity to pear. Its role may be the same as that played by the amide NH at the 2′-position of AK-toxin. Interestingly, the 2*E*,4*Z*,6*E* isomer of AF-toxin II isolated from culture filtrates exhibited high toxicity to pear, comparable to that of AK-toxin I, which has the same stereochemistry. The free ω-epoxy-decatrienoic acid itself had no toxicity for any of the plants tested.

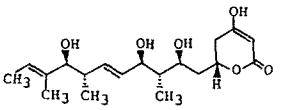
The tangerine pathotype of *A. alternata* produces multiple toxins that are highly active against citrus hosts such as 'Dancy' tangerine, 'Emperor' mandarin and 'Murcott' tangor[21,29]. Several selective toxins, named ACTG-toxins, were isolated and characterized by Kono et al.[28,29]. ACTG-toxins A and B are toxic to susceptible plants at a concentration of 0.1 μg/ml. Recently, we have found that this pathotype also produces a group of HSTs designated as ACT-toxins, which are quite different chemically from the ACTG-toxins and more closely related to AK- and AF-toxins (fig. 1)[21,37]. The ACT-toxins are produced during spore germination, while no information is as yet available about whether ACTG-toxins are released during this phase. This is an important point for the pathological evaluation of toxins, as noted in the preceding section. Among 65 citrus plants examined, the major HST, named ACT-toxin Ib, was toxic at a concentration of 10 ng/ml only to species, cultivars and hybrid lines susceptible to the *A. alternata* tangerine pathotype. Resistant citrus and non-host plants were not affected by the toxin even at a concentration of 20 μg/ml. There was a close correlation between disease susceptibility and toxin sensitivity. The ω-epoxydecatrienoic moiety of ACT-toxin Ib has the same stereochemistry (2*E*,4*Z*,6*E*) as that of AK-toxin. ACT-toxin Ic, which is produced in minor amounts, contains the 2*E*,4*E*,6*E* isomer and is less active. Surprisingly, in laboratory tests, ACT-toxin Ib is toxic to certain Japanese pears that are not known to be affected by the pathogen in the field.

The tangerine pathotype of *A. alternata* produces another HST, ACT-toxin IIb, which is the 5″-deoxy derivative of ACT-toxin Ib. Interestingly, this subtle change in the structure of Ib causes a 2000-fold reduction in toxicity to tangerines while it increases the toxicity to Japanese pears up to 10-fold, thus reaching a potency comparable to that of AK-toxin. Moreover, the sensitivity spectrum in Japanese pear cultivars to toxins Ib and IIb is the same as to AK-toxin. AK-toxin, however, is not toxic to tangerines and mandarins. A comparative study of structure-activity relations among these three structurally related HST groups (AK, AF and ACT) suggests that the susceptible Japanese pear is less specific in recognizing signal molecules at putative receptor sites than the susceptible strawberry, tangerine and mandarin are.

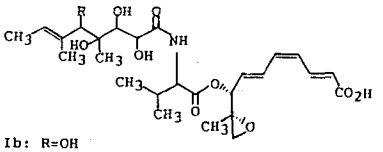
A. alternata pathogenic strains appear to have developed as highly specialized parasites with distinct pathotypes through elaboration of different HSTs via a common biosynthetic pathway[42]. It is of great interest that in countries very distant from one other, different strains of *A. alternata* produce toxic metabolites which are closely related in their structures, but differ in their specific toxicity towards plant genotypes which are hosts of the pathogen. Thus, a molecular basis is provided for the concept of a pathotype system[41] in which mutations result in the production of a new metabolite which makes a potentially saprophytic pathogen virulent for a new host: a new pathogenicity follows the acquisition of the ability to produce a toxin or a recognition factor which is genotype-specifically active in a potential host.

Another memorable achievement in the structural elucidation of HSTs has come from the transnational research conducted by Macko's team (Ithaca, N.Y., USA) and Arigoni's team in Zurich, Switzerland, on HV-toxin(victorin) produced by *Cochliobolus victoriae*[59,60]. The structure of this toxin was in fact determined nearly 40 years after it was first discovered in Victoria blight of oats in the USA. As shown in figure 1, the major toxin, victorin C, is composed of an acyclic combination of glyoxylic acid and five unusual amino acids, some of them containing chlorine. Structure-activity studies revealed that an essential component for the toxicity of victorin is the hydrated aldehyde group of the glyoxylic acid residue[59]. Removal of glyoxylic acid completely abolishes toxic activity; toxicity was also lost on reduction of the aldehyde group, but the reduced compoind prevents or reduces the effects of subsequent additions of native, biologically active victorin C[59]. Acylation of the ω-amino group of the β-hydroxylysine residue causes a nearly 100-fold reduction in the toxicity of victorin C,

ACR-toxin

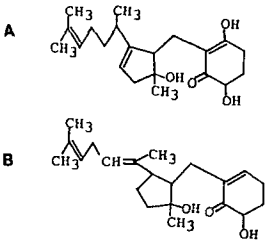


ACT-toxin

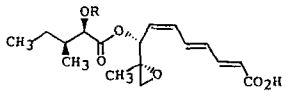


Ib: R=OH
IIb: R=H

ACTG-toxin

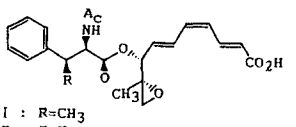


AF-toxin



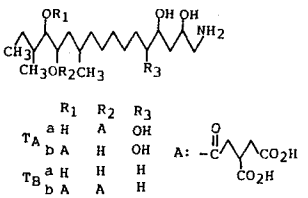
I : R=COCH(OH)C(CH$_3$)$_2$OH
II : R=H
III : R=COCH(OH)CH(CH$_3$)$_2$

AK-toxin

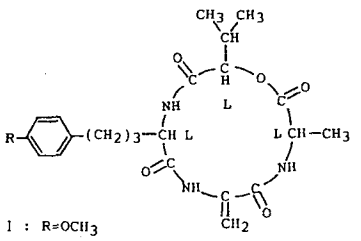


I : R=CH$_3$
II : R=H

AL-toxin

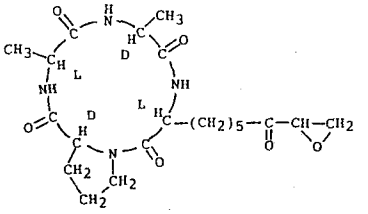


AM-toxin

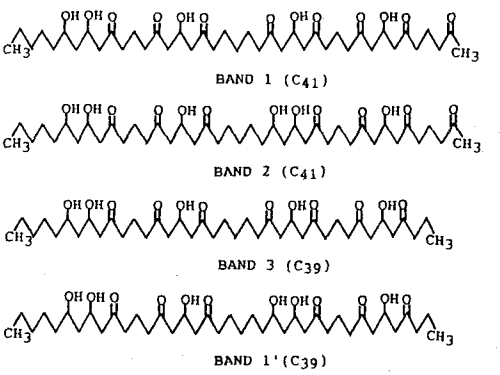


I : R=OCH$_3$
II : R=H
III : R=OH

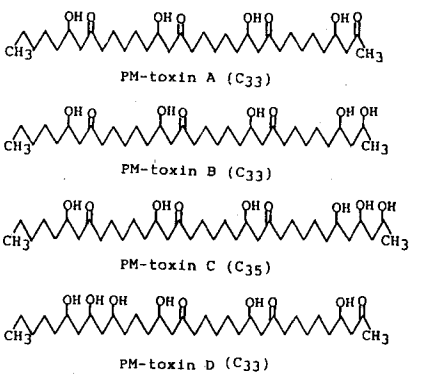
HC-toxin



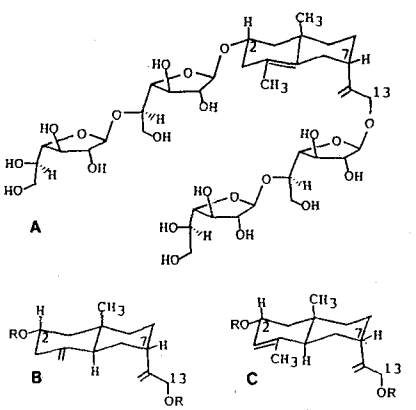
HMT-toxin



PM-toxin



HS-toxin



HV-toxin

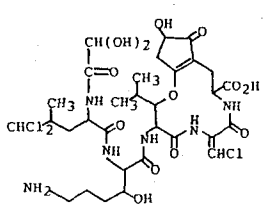


Figure 1. Chemical structures of host-selective toxins.

Host-specific toxins known to date (1990)

Disease	Pathogen Species/Pathotype (previous name)	Toxin (synonymous designation)	Host Host range (susceptible cultivar)	Genetic background (dominance)	Target site
Alternaria blotch of apple	*Alternaria alternata*/ Apple pathotype (*A. mali*)	AM-toxin I, II, & III	Apple (Red Gold, Starking)	Multiple genes (susceptible)	Chloroplast and plasma membrane
Alternaria leaf spot of pigeonpea	*A. tenuissima*	ATC-toxin	Pigeonpea	–	–
Alternaria stem canker of tomato	*A. alternata*/ Tomato pathotype (*A. alternata* f. sp. *lycopersici*)	AL (or AAL)-toxin (I & II or Ta & Tb)	Tomato (Earlypak 7, First)	Single (homo) gene (susceptible)	Mitochondrion? ACTase?
Black leaf spot of strawberry	*A. alternata*/ Strawberry pathotype	AF-toxin I, II, & III	Strawberry (Morioka-16)	Single (hetero) gene (susceptible)	Plasma membrane
Black spot of Japanese pear	*A. alternata*/ Japanese pear pathotype (*A. kikuchiana*)	AK-toxin I & II	Japanese pear (Nijisseiki)	Single (hetero) gene (susceptible)	Plasma membrane
Brown spot of rough lemon	*A. alternata*/ Rough lemon pathotype (*A. citri*)	ACR(L)-toxin I	Rough lemon	–	Mitochondrion
Brown spot of tangerine	*A. alternata*/ Tangerine pathotype (*A. citri*)	ACT-toxin I & II ACTG-toxin A & B	Tangerine (Dancy)	–	Plasma membrane
Brown spot of tobacco	*A. alternata*/ Tabacco pathotype (*A. longipes*)	AT-toxin	Tobacco	–	Mitochondrion
Eye spot of sugarcane	*Bipolaris sacchari* (*Helminthosporium sacchari*)	HS-toxin A, B, & C	Sugarcane (51-NG97)	–	Plasma membrane
Milo disease of sorghum	*Periconia circinata*	PC-toxin (Peritoxin A & B)	Grain sorghum (Giant milo)	Single gene (susceptible)	Plasma membrane
Northern leaf spot of maize	*Cochliobolus carbonum* (*B. carbonum*) race 1	HC-toxin I, II, & III	Maize (K-44, K-61)	Multiple genes (resistant)	Plasma membrane
Southern leaf spot of maize	*C. heterostrophus* (*B. maydis*) race T	HMT-toxin band 1, 2, 3, & 1'	Maize (Tms cytoplasm)	Cytoplasmic	Mitochondrion
Target leaf spot of tomato	*Corynespora cassiicola*	CC-toxin	Tomato (Ife no. 1)	–	–
Victoria blight of oats	*Cochliobolus victoriae* (*B. victoriae*)	HV-toxin (victorin C)	Oats (Victoria)	Single (hetero) gene (susceptible)	Plasma membrane
Yellow leaf blight of maize	*Phyllosticta maydis*	PM-toxin A, B, C, & D	Maize (Tms cytoplasm)	Cytoplasmic	Mitochondrion

but the derivative still retains host selectivity, indicating the possibility that the toxin could be radioactively labelled to a high specific activity using ^{125}I, as will be described later.

Multiple structural forms have been found for other HSTs such as ACR-[12], AL-[13], AM-[40], HC-[50], HS-[30], HMT-[28] and PM-toxins[9,28] (table). However, chemical multiplicity is not necessarily required for host recognition by the producing pathogen; it is only at the infection site on spore germination that 'released toxin species' can play a role in recognition, and others might be surplus for pathogenicity[40–42].

Primary action sites for HST

Investigations of the primary sites of action of HSTs have dominated the field of toxin studies for a long time, because information about the signalling mechanism initiated by the HST is fundamental to understanding host recognition. Three approaches have mostly been employed: physiological, biochemical, and ultrastructural. It has been suggested that the primary actions can be associated with three target organelles: plasma membrane, mitochondrion and chloroplast[25].

Dysfunction in the plasma membranes of susceptible genotypes results instantaneously from the application of most HSTs. A rapid and dose-dependent increase in electrolyte loss from the cells, and the concurrent invagination of their membranes, is a common syndrome caused by ACT-, AF-, AK-, AM-, HS-, HV-, and PC-toxins. An exception is HC-toxin, which enhances the uptake of ions. Electrophysiological studies[39,46,52] showed that AF-, AK-, HV-, and HS-toxins depolarized the membrane of susceptible genotypes but had little effect on the membrane potential of resistant genotypes, whereas HC-toxin hyperpolarized the membrane of susceptible maize. Decrease in polarization occurred mostly in the respiration-dependent component of the membrane potential, which is sustained by a H^+-pump; there was little effect on the diffusion potential component. Do HSTs directly affect the plasma membrane H^+-ATPase? In studies of HS-toxin[52], it appeared to be unlikely that the electrogenic H^+-pump of susceptible sugarcane is the primary site of action of the toxin, since this pump can be activat-

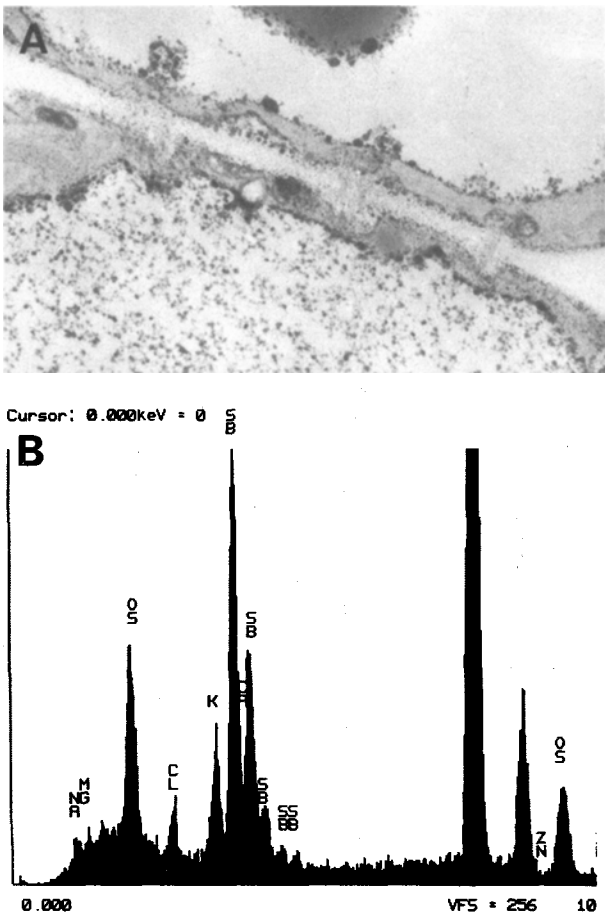


Figure 2. Susceptible Japanese pear leaves treated with AK-toxin for 15 min. The leaves were fixed with OsO_4-potassium antimonate fixative. *A* The precipitates of sodium antimonate and magnesium antimonate were located in the cell walls near plasma membrane invagination at plasmodesmata. × 22,000. *B* An X-ray spectrum from the precipitate-laden cell walls showing peaks for Na, Mg, Os, Cl, Sb, and Ni.

ed by light and fusicoccin. AK-toxin had no direct effect on ATPase activity in the isolated plasma membrane fraction of a susceptible pear genotype [24, 45]. No AK-toxin effect was observed with inside-out vesicles and solubilized membrane preparations. AF-toxin causes rapid and irreversible reduction of the light-dependent and independent components of the membrane potential [39]. Nevertheless, AF-toxin also did not affect ATPase activity in the plasma membrane fraction of a susceptible strawberry (Lee et al., unpublished data).

Recently Park et al. [48] have identified ultrastructural sites for leakage of Na^+ and Mg^{++} in susceptible Japanese pear leaves treated with AK-toxin by using a Na- and Mg-precipitation method and analytical electron microscopy. The earliest two effects of AK-toxin, namely plasma membrane modification and precipitation of sodium and magnesium antimonates, appear in the plasmodesmatal regions within 5 min after toxin exposure. The precipitates first occur at a site on the cell wall near the plasmodesmata. Shortly after, the plasma membrane becomes invaginated at both ends of the extended plasmodesmata (fig. 2). As the modification develops, the precipitate spreads from the original site to both neighboring and/or remote areas. There is no lag time between physiological and ultrastructural changes in toxin-treated susceptible tissues, which strongly supports the hypothesis that the primary site for AK-toxin action is at the plasma membranes of susceptible cells as previously suggested [25, 40, 41].

Mitochondria in susceptible host cells are affected by ACR-, AL-, AT-, HMT-, and PM-toxins [1, 7, 27, 32]. Intensive studies have been focused on understanding the mechanisms of action of HMT-toxins. HMT-toxin at a concentration of 10^{-9} M immediately causes the following symptoms in mitochondria of Texas male-sterile (Tms) but not male-fertile (N) cytoplasm maize: a) swelling, reduction in numbers and vesiculation of the cristae, b) a decrease in electron density of the matrix, c) an increase in the rate of NADH oxidation, and d) an inhibition of malate oxidation. Matthews et al. [32] reported that the toxin increased the permeability to NAD^+ of the inner mitochondrial membrane in Tms, but not N, cytoplasm. This resulted in a depletion of the intramitochondrial pool of NAD^+ which caused inhibition in the oxidation of NAD^+-linked substrates such as malate. Additionally, Holden et al. [17, 18] examined direct effects of HMT- and PM-toxins on the membrane potential ($\delta\psi$) formed in isolated mitochondria, by continuously monitoring the absorbance change of a cationic dye. Both toxins dissipated $\Delta\psi$ very rapidly (within a few seconds) as did the protonophore CCCP, supporting the idea that these toxins increase H^+ permeability. The responses of susceptible rough lemon mitochondria to ACR-toxin appear to be similar to those of Tms mitochondria to HMT-toxin [1, 27], although each toxin retains its host-specificity.

In contrast to these HSTs, AT-toxin and AL-toxin induced ultrastructural damage to mitochondria in susceptible cultivars in 24 h. This delay in response does not necessarily rule out the mitochondrion as the target of the toxin. MTT-colorimetric assays indicated that the viability of root cells and cultured leaf cells of a susceptible tomato treated with AL-toxin was markedly decreased. However, no reduction in viability was observed in toxin-treated resistant tomato cultivars. Also, AL-toxin did not affect the respiration of isolated mitochondria (unpublished data), which might be a secondary target of the toxin. Our experiment showed that the toxin caused a significant increase of ethanolamine and phosphorylethanolamine in susceptible, but not in resistant, tomato leaves (Kawaguchi et al., unpublished data). On the other hand, the activity of aspartate carbamyltransferase from susceptible tomato was inhibited by the toxin under certain conditions [13]. Recently, Witsenboer et al. [56, 57] indicated that inhibition by the toxin was demonstrable at the cellular level in protoplasts, calli, leaves, shoots, roots and pollen of susceptible and resistant cultivars,

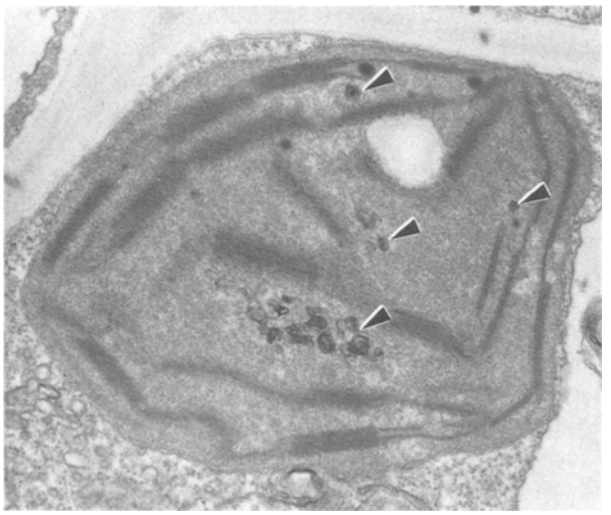


Figure 3. Susceptible apple leaves treated with AM-toxin I for 3 h. Marked fragmentation of grana lamellae (arrowheads) in the stroma of a chloroplast of a mesophyll cell. × 24,000.

but not in resistant leaves. These conflicting data imply that AL-toxin has multiple and non-specific targets in tomato plants, and that the susceptible genotypes may have high-affinity toxin receptors that are functionally expressed in leaves. Thus the primary action site of AL-toxin is still obscure.

Chloroplasts appear to contain additional action sites for AM-toxin, a cyclic depsipeptide, similar to tentoxin, from the leaf blotch pathogen of apple. A typical modification of chloroplasts in AM-toxin-treated susceptible apple leaves is the fragmentation and vesiculation of grana lamellae (fig. 3)[25]. This damage is comparable with the physiological alteration observed in susceptible leaf tissues: photosynthetic CO_2-fixation is inhibited by 10^{-8} M AM-toxin. Isolated chloroplasts also seem to have a site sensitive to AM-toxin. However, further biochemical studies of the mechanism of action using intact chloroplasts from apple leaves remain to be done.

HST receptor model as a model for molecular recognition in parasitism

The most fascinating hypothesis for understanding the strict host-specificty of toxigenic fungal parasites of plants at the molecular level has been the HST-receptor model that Pringle and Scheffer first proposed in 1964[49]. Many lines of circumstantial evidence support the presence of HST-receptors in susceptible host tissues. For example, a study on the structure-activity relationships of AM-toxin revealed that no toxicity was exhibited by retroenantio-AM-toxin I, in which the peptide sequence is reversed and the configuration of each residue is inverted, and by enantio-AM-toxin I, an antipode of AM-toxin I, which has the same conformation as AM-toxin I[22, 33]. This implies that the initial interaction between AM-toxin I and its putative receptor site on the plasma membrane and/or chloroplast of susceptible apple cells most likely involves a biological reaction, such as ligand binding to a receptor that can recognize the chirality of the toxin, rather than a simple physicochemical reaction such as peptide-lipid interaction. A very strict precision in stereochemistry appears to be required for recognizing the signal carried by the toxin in cells. Agonist-antagonist relations were observed in the interactions between HS-toxin and sugarcane[30]. HV-toxin and oats[58], and AF-toxin and strawberry[38], where pretreatment of susceptible tissues with each toxoid prevented the toxicity of the corresponding native toxin.

The first attempt to demonstrate directly the existence of a putative receptor was focused on the HS-toxin binding protein in sugarcane leaves. For experiments on the binding of highly bio-active toxins to receptor sites, however, a labeled HST with a high specific radioactivity is usually required; as pointed out by Daly[7], a toxin with low specific radioactivity could hardly be measured in biological preparations at physiological concentrations. Recently Frantzen et al.[11] examined the binding to mitochondria of ^{3}H-labeled HMT- and PM-toxin analogs which had a high specific radioactivity and the same high selective toxicity as the native toxins. They found no significant difference in the binding to mitochondria of susceptible and normal Tms-cytoplasm maize. Furthermore, it was observed that HMT-toxin acts as an ionophore in an artificial membrane system. It was suggested that a possible interaction between the toxin and a membrane component(s) of normal mitochondria prevents the toxin from forming an ion channel. Tms-mitochondria would lack such a component(s).

In contrast, molecular genetic studies of HMT-toxin sensitivity in maize appear to disagree with this hypothesis. The mitochondrial gene, T-*urf13*, from the Texas cytoplasm is responsible for the sensitivity to HMT- and PM-toxins, because the gene product, a 13 kDa protein (T-URF13) was shown to confer toxin sensitivity on *Escherichia coli* cells: the toxins inhibited cellular respiration and caused an immediate ion leakage[2, 4, 10]. Does HMT-toxin ever bind to this polypeptide in vivo and in vitro? Or does the toxin, without binding directly to a site on this specific protein, interfere with electron transfer by insertion into the membrane in the vicinity of the protein?

These questions seem to be answered by experiments on the binding of ^{3}H-PM-toxin to the URF13 protein in maize mitochondria and *E. coli* expressing the T-*urf13* gene. Braun et al.[3] demonstrated in early 1990 that the toxin binds reversibly and competes with methomyl for the same, or overlapping, binding sites in *E. coli*, through competition and displacement studies.

Most recently, Haung et al.[19] constructed a chimeric gene coding for T-URF13 fused to the mitochondrial targeting peptide of the *Neurospora crassa* ATP synthase subunit 9 precursor. It was demonstrated that the expression of the gene in yeast (*Saccharomyces cerevisiae*) yields

a protein which is translocated into the mitochondrial membrane and processed to give a protein of the same size as maize T-URF13. This protein confers sensitivity to HMT- and PM-toxins; no sensitivity to the toxins is observed when T-URF13 protein is expressed without a targeting peptide. This strongly suggests that the mitochondrial localization of T-URF13 protein is crucial for the Tms-cytoplasm-specific toxins. This success in the introduction of the susceptibility gene into eukaryotic fungal cells will extensively promote the study of the expression of mitochondrial genes in heterologous higher plant systems.

More direct evidence for toxin binding has come from intensive studies by Macko's group on the structure-activity relations of HV-toxin[59]. Bolton-Hunter victorin C is a 100-fold less active derivative of the native toxin which nevertheless retains its host-specificity. It can be labeled with ^{125}I to a high specific activity. Electrophoretic analysis of leaf proteins from isogenic susceptible and resistant oat genotypes after treatment with the ^{125}I-labeled victorin derivative indicated that this binds in a covalent and genotype-specific manner to a 100 kDa protein only in susceptible leaves. This in vivo binding was competitively displaced by reduced victorin, a nontoxic protective compound, which indicated that the binding of the ligand was specific. However, in vitro binding required an exogenous reducing agent and was not genotype-specific. The hypothesis was proposed that the difference between susceptible and resistant genotypes is due to the presence of a reducing group, either in the 100 kDa protein or in associated molecules. The 100 kDa protein appears to be a probable candidate for the HV-toxin receptor. The search for toxin receptors involved in other toxigenic diseases is now under way: a putative receptor for AK-toxin seems to be a protein with SH-groups[24,45].

Process of HST action in cells and induction of accessibility

From the point of view of studying the pathology of HST action, more efforts should be centered upon determining the key event(s) necessary for successful penetration of the pathogen and initial colonization in host cells, even if each HST has a different, primary target organelle. Generally, an HST produces diverse physiological and biochemical effects on susceptible plants. Such effects have various timetables, and the reactions may develop as independent or coupled events. Consequently, the question can be posed as to how an HST triggers a series of reactions that finally leads to the induction of accessibility to fungal penetration and colonization. For dissecting such diverse effects of a toxin, and determining the essential component(s) among them, the use of metabolic inhibitors, modifying reagents, nitrogen gas to obtain anaerobic conditions, mild heat, and light may all be helpful, as already reported in several reviews and papers[18,25,26,45,55].

In the combinations of AK-toxin/Japanese pear[25], AM-toxin/highly susceptible apple (unpublished data), and HV-toxin/oats[51], pretreatment of susceptible tissues with SH-alkylating reagents such as iodoacetamide or iodomethane protected the tissues from toxin action. But simultaneous or post-treatment did not. The pretreatment cancelled not only the induction of toxigenic tissue necrosis and successful infection of avirulent spores as the final events, but also the dysfunction of plasma membranes, i.e. very early electrolyte leakage and invagination of membranes. This type of protection agreed with the data showing that the binding of HV-toxin requires SH-groups close to the receptor site in vivo, and certain SH-substances in vitro[59].

Recently, light was also found to suppress the early stages of toxin action in AM-toxin/apple, ACR-toxin/rough lemon and HMT-toxin/maize systems[26,55]. In susceptible apple leaves, light inhibited leaf necrosis caused by AM-toxin, during a specified period from about 2 h (the time necessary for completing the first light-independent phase) through about 5 h after toxin application. In ACR-toxin/rough lemon and HMT-toxin/maize combinations, however, light inhibition seemed to occur shortly after toxin treatment. In apple leaves, light did not suppress an AM-toxin-induced increase in electrolyte loss[26], invagination of plasma membranes and vesiculation in chloroplasts[54].

Light had no effect on the formation of the infection hypha and development of the lesion induced by the pathogen, or by a saprophytic strain of *A. alternata* combined with a small amount of AM-toxin. These fungi were able to penetrate apple tissues exposed to the toxin, but necrosis was suppressed under continuous illumination; vital staining demonstrated that the toxin-treated cells were still alive at least for 4 days after the beginning of the experiment. This means that fungal penetration is successful, independently of toxin-induced plant cell death, even with necrotrophic or perthophytic parasites, which are believed to kill the host cells prior to invasion. By contrast with the situation with apple, light protected rough lemon and maize leaves from ACR- or HMT-toxin-induced dysfunction of plasma membranes and prevented the formation of infection hyphae and necrosis induced by these toxins[26]. However, light provided no protection against the first effect of these HST, which was on mitochondria. These results have led to the suggestion that there is a close association between early functional modification of plasma membranes, caused directly or indirectly by HST, and a predisposition of plant cells to be penetrated by the pathogen, or induction of susceptibility caused by HST. The 'HST signal' may be relayed by a signaling process which branches off at or around the step at which plasma membrane disorder occurs, and acts upon an initiation-point for the negation of those inducible general defense mechanisms[15,25,26] which would otherwise be effective at the penetration phase. A recent review discusses resistance to *A. alternata*

in Japanese pear leaves and its suppression by AK-toxin[45].

Interactions between fungi producing HMT-toxin and AL-toxin and their host plants present an intricate story in this respect; their specificity appears to be expressed not only during host penetration and subsequent initial colonization, but also during the later phases of pathogenesis. When avirulent spores of *A. alternata* or *Cochliobolus heterostrophus* were inoculated on leaves of Tms-cytoplasm maize along with a trace of HMT-toxin, the rate of formation of infection hyphae greatly increased and necrosis developed[26]. Race O and race T of *C. heterostrophus* could invade Tms-cytoplasm maize leaves and cause the development of symptoms: race O caused smaller, parallel-sided lesions, whereas race T caused long, spindle-shaped lesions. Both races caused small, parallel-sided lesions on leaves of normal cytoplasm maize. HMT- and AL-toxins may play at least two roles: 1. as an inducer for accessibility to fungal penetration, and 2. as a selective virulence factor. Such a virulence factor may have regulatory effects on a series of defense reactions against hyphal spreading in the host tissues after initial colonization, in addition to affecting the severity of the toxigenic symptom development.

Conclusions

There is growing evidence that certain fungal pathogens secrete a host recognition factor and/or a specific suppressor, such as an HST, during germination. This signal HST can predispose the cells of potential hosts to being in a susceptible state in advance of penetration of the pathogen, by specifically negating the plant's defense reactions. This scheme appears to be compatible with Sequeira's definition of host recognition.

As knowledge about the structures of HSTs accumulates, it will provide clues for elucidating the nature of the stereochemical interface between the recognition factor and its receptor. In the not-far-distant future, research should be aimed at completely understanding the modes of signal reception and transduction in plant cells at the molecular level: How does the HST signal interfere with the mobilization of the host plant's defense mechanisms? What is the key event in signaling which allows fungal penetration and initial colonization? Such studies will stimulate an extensive search for diverse types of pathogenicity determinants produced by fungal pathogens of plants.

Note added in proof:

After this review article was submitted for publication, a paper appeared reporting direct evidence with immunolabeling that T-URF13 is specifically localized in the mitochondrial inner membranes of Tms cells. [Hack, E., Lin, C., Yang, H., and Horner, H., T-URF13 protein from mitochondria of Texas male-sterile maize (*Zea mays* L.). Plant Physiol. *95* (1991) 861–870.]

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