

MINI-REVIEW

Plant chitinases

David B. Collinge^{1,*}, Karsten M. Kragh², Jørn D. Mikkelsen², Klaus K. Nielsen^{1,2}, Ulla Rasmussen^{1,3,†} and Knud Vad^{1,‡}

¹Section for Plant Pathology, Department of Plant Biology, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, 1871 Frederiksberg C, Copenhagen, Denmark,

²Maribo Seed Biotechnology, Danisco A/S, Langebrogade 1, 1001 Copenhagen K, Denmark, and

³Plant Biology Section, Environmental Science and Technology Department, Risø National Laboratory, P.O. Box 42, 4000 Roskilde, Denmark

Introduction

Plants respond to attack by pathogenic micro-organisms by the induction of expression of a large number of genes encoding diverse proteins, many of which are believed to have a role in defence (Bol *et al.*, 1990; Bowles, 1990; Collinge and Slusarenko, 1987; Dixon and Harrison, 1990; Linthorst, 1991). Outstanding amongst the induced genes are those encoding chitinases, since, until recently, no substrate for these enzymes had been identified in plants, whereas chitin is commonly a component of fungal cell walls and the exoskeleton of arthropods, organisms which include many important pathogens and pests. Furthermore, various chitinase preparations inhibit the growth of many fungi *in vitro* by causing lysis of hyphal tips, especially in combination with β -1,3-glucanase (Broekaert *et al.*, 1988; Broglie *et al.*, 1991; Mauch *et al.*, 1988b; Schlumbaum *et al.*, 1986), and chitinase has been shown to accumulate around fungal hyphal material *in planta* (Benhamou *et al.*, 1990; Wubben *et al.*, 1992). The combination of these factors has led to the proposal that the major natural role for chitinase is defence, primarily against fungal pathogens (Boller, 1987, 1988). Chitinase has therefore been the subject of intense study in recent years, and it has now been demonstrated that enhanced chitinase levels in transgenic plants can indeed reduce the damage caused by pathogens (e.g. Broglie *et al.*, 1991). However, chitinase expression is also under developmental control in certain organs and tissues and seems to have

an important function in early embryo development, indicating additional non-defensive roles (de Jong *et al.*, 1992; Lotan *et al.*, 1989; Neale *et al.*, 1990). Of particular interest, is the observation that chitinase can inactivate the lipo-oligosaccharide signal molecules produced by certain *Rhizobium* strains (Roche *et al.*, 1991a) which are responsible for the induction of root hair deformations, cortical cell divisions and nodule development in the roots of legume hosts (Truchet *et al.*, 1991).

Enzyme activities, substrates and biochemical properties

Chitinases catalyse the hydrolysis of chitin, a linear homopolymer of β -1,4-linked *N*-acetylglucosamine (GlcNAc) residues. The types of chitinase most extensively studied in plants are endochitinases (EC 3.2.1.14) which randomly hydrolyse internal β -1,4-linkages of chitin-releasing oligosaccharides of GlcNAc (Boller *et al.*, 1983; Molano *et al.*, 1979; Roberts and Selitrennikoff, 1988). In addition, exochitinase activity measured by the release of GlcNAc has been reported from a number of plants (Boller *et al.*, 1983; Roby and Esquerré-Tugayé, 1987; Wadsworth and Zikakis, 1984). Many purified plant endochitinases also show some degree of lysozyme (EC 3.2.1.17) activity, i.e. they can hydrolyse β -1,4-linkages between *N*-acetylmuramic acid and GlcNAc residues in peptidoglycan (Boller, 1988; Majeau *et al.*, 1990; Roberts and Selitrennikoff, 1988; Trudel *et al.*, 1989). Likewise, most plant lysozymes show a high level of chitinase activity (Audy *et al.*, 1988; Bernasconi *et al.*, 1987; Bernier *et al.*, 1971). Very little attention has been given to the possibility that plant chitinases may also be able to catalyse transglycosylation reactions as recently shown for two fungal chitinases and hen egg lysozyme (Usui *et al.*, 1987, 1990). However, wheat germ endochitinase did not show any transglycosylation activity (Molano *et al.*, 1979). Finally, a unique protein with both α -amylase inhibitor activity (against a locust enzyme) and endochitinase activity has been purified from the grass Job's tears (*Coix lachrymajobi*) (Ary *et al.*, 1989).

A number of assays can be applied to measure both endo- and exochitinase enzyme activity. These include colorimetric, radiochemical and gel electrophoresis-based assays (Boller *et al.*, 1983; Molano *et al.*, 1977; O'Brien and Colwell, 1987; Roberts and Selitrennikoff, 1988; Trudel and Asselin, 1989; Wirth and Wolf, 1990).

Chitin is a major component of the exoskeleton of

Received 1 May 1992; revised 12 August 1992; accepted 14 September 1992.

*For correspondence (fax +45 35 28 33 10).

†Present address: Department of Botany, Stockholm University, 10 691-Stockholm, Sweden.

‡Present address: Diabetes Research, Novo-Nordisk A/S, Novo Allé, 2880 Bagsværd, Denmark.

insects and of the cell wall of most fungi, i.e. the Ascomycetes, Basidiomycetes and Deuteromycetes. In fungi, chitin constitutes 3–60% of the cell wall (Bartnicki-Garcia, 1968; Wessels and Sietsma, 1981). Alternative substrates for plant chitinases are bacterial peptidoglycan, the soluble chitin derivative glycol chitin and in some cases chitosan (deacetylated chitin) (Bernasconi *et al.*, 1987; Boller *et al.*, 1983; Molano *et al.*, 1979). On the contrary, no endogenous substrates in plants have been identified until recently. Thus, when boiled homogenates of bean plants were treated with a bean endochitinase, no GlcNAc-containing fragments were detected indicating that chitin-like substrates are not present in the plant (Boller *et al.*, 1983). However, recent immunochemical studies using gold-labelled wheat germ agglutinin and bacterial chitinases revealed abundant GlcNAc residues in secondary walls of plants (Benhamou and Asselin, 1989). The data suggested that the GlcNAc residues may be present in the form of glycolipids whereas GlcNAc polymers analogous to chitin are absent. In this context, it is noteworthy that rhizobia (*Rhizobium*, *Bradyrhizobium* and *Azorhizobium*) secrete lipo-oligosaccharides (*nod* factors). These signal molecules are tetra- and pentamers of *N*-acetylglucosamine with an acyl group at the non-reducing residue (Lerouge *et al.*, 1990; Roche *et al.*, 1991b; Spaink *et al.*, 1991). These *nod* factors are a new class of signal molecules that, at very low concentrations (10^{-7} – 10^{-10} M), trigger root hair deformation, nodule primordium formation for establishing specific symbiotic interactions with leguminous plants (Lerouge *et al.*, 1990; Roche *et al.*, 1991b; Spaink *et al.*, 1991; Truchet *et al.*, 1991) and infection-related processes (Van Brussel *et al.*, 1992). It has been proposed that plant chitinases might interfere by hydrolising these bacterial lipo-oligosaccharides (Roche *et al.*, 1991a; Staehelin *et al.*, 1992). Interestingly, endogenous pea and bean chitinases have no influence on the pea or bean *nod* factors, but soybean chitinase can inactivate the soybean, pea and bean factors (Mellor, personal communication). Furthermore, it has been postulated that rhizobial *nod* factors resemble comparable glycolipid compounds of plant origin (Fisher and Long, 1992). If the endogenous glycolipids are indeed substrates for chitinases, then the products might act as plant signal molecules (see e.g. Fisher and Long, 1992; Spaink, 1992; Truchet *et al.*, 1991). Indeed, molecules which migrate similarly to rhizobial lipo-oligosaccharides in TLC have been observed following chitinase treatment of butanol extracts from *Lathyrus* flowers (Spaink *et al.*, 1992). In conclusion, alternative endogenous chitinase substrates including glycolipids need further examination.

In general, plant endochitinases are proteins of 25–35 kDa molecular weight, which occur as monomers and have either high or low isoelectric points (for review see Boller, 1988). However, the bifunctional endochitinase

and α -amylase inhibitor from Job's tear consists of a dimer of 26 kDa subunits (Ary *et al.*, 1989). Chitinases usually have very broad pH optima around pH 6 and are stable at temperatures up to 50°C (Boller *et al.*, 1983; Molano *et al.*, 1979). Glycosylation has been reported for acidic chitinases from yam, bean and carrot (de Jong *et al.*, 1992; Margis-Pinheiro *et al.*, 1991; Tsukamoto *et al.*, 1984).

Classification and structure of chitinases

Three classes of plant chitinases have been proposed based on the primary structure (Shinshi *et al.*, 1990): class I chitinases are enzymes with a N-terminal cysteine-rich domain of approximately 40 amino acids and a highly conserved main structure, separated by a variable hinge region (Figure 1). Class II chitinases lack the N-terminal cysteine-rich domain but have a high amino acid sequence identity to the main structure of class I chitinases. Class III chitinases show no sequence similarity to enzymes in class I or II (see Figure 1).

We propose a chitinase class IV to include the basic sugar beet chitinase IV (Mikkelsen *et al.*, 1992), the basic rape chitinase ChB4 (Rasmussen *et al.*, 1992a, 1992b) as well as the acidic bean PR4 chitinase (Margis-Pinheiro *et al.*, 1991). These chitinases contain a cysteine-rich domain and a conserved main structure which resemble those of class I chitinases but are significantly smaller due to four deletions. Thus the three class IV chitinases contain only 241–255 amino acid residues in the mature protein compared with about 300 amino acid residues of class I chitinases (Figure 1). The amino acid sequence identity of class IV chitinases with class I chitinases is only 41–47% compared with 59–63% identity between the individual chitinases of class IV and more than 69% identity between the individual chitinases of class I (Brogie *et al.*, 1986; Rasmussen *et al.*, 1992a; Samac *et al.*, 1990; Shinshi *et al.*, 1987, 1990). Furthermore, class IV and class I chitinases are serologically distinguishable (Mikkelsen *et al.*, 1992). A list of cloned chitinase genes is presented in Table 1.

The N-terminal cysteine-rich domain is believed to be a chitin-binding domain and is also found in other proteins

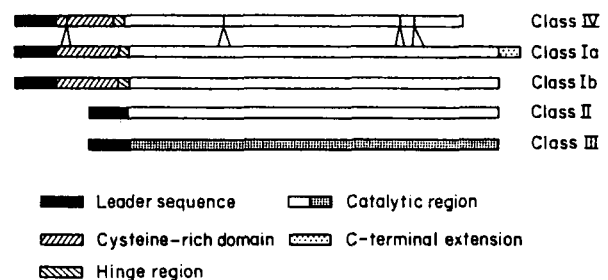


Figure 1. The structure of chitinase proteins.

Table 1. Cloned chitinases

Plant	Clone	Class	Accession no.	Reference
<i>Allium sativum</i>	cDNA		M94105, M94106	van Damme <i>et al.</i> (unpublished)
<i>Arabidopsis</i>	gene	II	M38240	Samac <i>et al.</i> (1990)
	gene	III	M34107	Samac <i>et al.</i> (1990)
Barley	cDNA	Ib	X15349	Swegle <i>et al.</i> (1989)
	cDNA	II	M36989, M62904	Leah <i>et al.</i> (1991)
Bean	cDNA	Ia	M13968	Broglie <i>et al.</i> (1986)
	cDNA	Ia	M19052	Hedrick <i>et al.</i> (1988)
	cDNA	IV	X57187	Margis-Pinheiro <i>et al.</i> (1991)
Cucumber	cDNA	III	M24365	Métraux <i>et al.</i> (1989)
Maize	cDNA		M84164, M84165	Huynh <i>et al.</i> (unpublished)
Pea	cDNA	Ib	X63899	Vad (1991)
Peanut	cDNA	Ia	—	Hergert <i>et al.</i> (1990)
<i>Petunia</i>	cDNA	II	X51427	Linthorst <i>et al.</i> (1990)
Poplar	cDNA	Ia	M25336–25344	Parsons <i>et al.</i> (1989)
	gene	Ia	X59995	Davis <i>et al.</i> (1991)
Potato	cDNA	Ia	X14133	Laflamme and Roxby (1989)
	cDNA, gene	Ia	X07130, X08011	Gaynor (1988)
			X15494	
Rape	cDNA	IV	X61488	Rasmussen <i>et al.</i> (1992a)
Rice	gene	Ia	X54367	Huang <i>et al.</i> (1991)
	cDNA	Ia	—	Nishizawa and Hibi (1991)
	cDNA, gene	Ia	—	Zhu and Lamb (1991)
Sugar-beet	cDNA, gene		—	Mikkelsen <i>et al.</i> (1992)
	gene, cDNA	III, IV	—	Mikkelsen <i>et al.</i> (1992)
Tobacco	cDNA	Ia	M15173	Shinshi <i>et al.</i> (1987)
<i>Nicotiana</i> spp.	gene	Ia	X64518	Van Buuren <i>et al.</i> (1992)
Tobacco	cDNA, gene	Ia	X16938, X16939	Shinshi <i>et al.</i> (1990)
	gene	Ia	X51599	Fukuda <i>et al.</i> (1991)
	gene, cDNA	II	X51425, X51426	Linthorst <i>et al.</i> (1990)
	cDNA	II	M29868, M29869	Payne <i>et al.</i> (1990)
	cDNAs	III	Z11563	Lawton <i>et al.</i> (1992)

such as the wound-induced WIN proteins, hevein, wheat germ agglutinin and nettle lectin (Broekaert *et al.*, 1989; Lucas *et al.*, 1985; Shinshi *et al.*, 1990; Stanford *et al.*, 1989; Wright *et al.*, 1984). The proteins containing this common cysteine-rich domain have possibly evolved as a result of a gene fusion of the chitin-binding domain with unrelated domains such as hevein, chitinase etc. (Chrispeels and Raikel, 1991; Shinshi *et al.*, 1990).

The active site in a chitinase from *Zea mays* has been tentatively localized by selective modification of an essential tyrosine residue (Verburg *et al.*, 1992). This residue corresponds to the first tyrosine (Y) in the sequence N Y N Y G, which is conserved in most basic chitinases. These results are further supported by evidence that a tyrosine residue also acts in the catalytic mechanism of β -galactosidase, which, like chitinase, acts on β -1,4-linked saccharides (Verburg *et al.*, 1992).

Class III chitinases show a weak similarity to bacterial chitinases, and a putative active site containing four aspartic or glutamic acid residues has been deduced by hydrophobic cluster analysis (Henrissat, 1990; Watanabe *et al.*, 1992).

Regulation of chitinases

Chitinase is induced by various factors including pathogen and pest attack as well as treatment with elicitors, abiotic factors such as heavy metals and plant hormones. Induction of chitinase is often co-ordinated with the induction of specific β -1,3-glucanases and other pathogenesis-related (PR) proteins (Bowles *et al.*, 1991; Brederode *et al.*, 1991; Conrads-Strauch *et al.*, 1990; Hedrick *et al.*, 1988; Joosten and De Wit, 1989; Kombrink *et al.*, 1988; Pegg and Young, 1982; Rasmussen *et al.*, 1992b; Shinshi *et al.*, 1987; Vad *et al.*, 1991; Vögeli *et al.*, 1988; Vögeli-Lange *et al.*, 1988). Differential regulation of individual chitinase isozymes has been reported from pea, barley, tobacco, turnip and cell cultures of peanut. In pea, at least two chitinases show differential regulation during development and in response to fungal infection (Mauch *et al.*, 1988a; Vad *et al.*, 1991). In barley, different chitinase isoforms are found in leaves and grain and only one out of five basic barley chitinases is induced in leaves infected with *Erysiphe graminis* (Kragh *et al.*, 1990, and unpublished data). In tobacco leaves, differential induction of basic and acidic class I and II chitinases has been demon-

strated in response to various forms of stress (Brederode *et al.*, 1991; Keefe *et al.*, 1990; Memelink *et al.*, 1990), whereas the acidic and basic class III chitinases appear to be co-ordinately regulated (Lawton *et al.*, 1992). Similarly, different chitinases are induced in peanut cell cultures: the mRNA representing one chitinase form is induced following elicitor treatment whereas other forms are unaffected (Herget *et al.*, 1990).

Chitinase enzyme activity, protein and mRNA levels have been reported to be higher in resistant cultivars at early stages after inoculation than in susceptible cultivars in many, but not all, interactions. This has been demonstrated in tomato infected with *Cladosporium fulvum* (Joosten and de Wit, 1989), bean inoculated with *Colletotrichum lindemuthianum* (Daugrois *et al.*, 1990) and rape inoculated with *Phoma lingam* (Rasmussen *et al.*, 1992a, 1992b). Similar results have been found following inoculation of a single cultivar of bean following infection with virulent or avirulent races of *Pseudomonas syringae* (Voisey and Slusarenko, 1989). In turnip (*Brassica campestris*), two different isozymes (both 30 kDa) are found to be differentially induced in response to virulent and avirulent pathovars of *Xanthomonas campestris* (Conrads-Strauch *et al.*, 1990). Also in symbiotic interactions, represented by *Allium porrum* roots infected by mycorrhiza (Spanu *et al.*, 1989) and soybean nodules infected with *Bradyrhizobium japonicum* (Staehelin *et al.*, 1992), PR proteins including chitinase can be induced. This may reflect a need to protect the symbiotic host from external pathogenic invasion, or pathogenic development of the symbiont.

The induction of chitinase and β -1,3-glucanase by ethylene treatment has been studied in various plant species and tissues (Boller 1988; Boller *et al.*, 1983; Keefe *et al.*, 1990). In general, it can be concluded that ethylene treatment resulted in a co-ordinated increase in chitinase and β -1,3-glucanase activity. The implication of ethylene as an endogenous signal for chitinase induction has been proposed since increased biosynthesis of ethylene in the plant has been shown to accompany treatments with elicitors or pathogen infection, and an inhibitor of ethylene biosynthesis has been shown to reduce the accumulation of chitinase in French bean and melon (Broglie *et al.*, 1989; Roby *et al.*, 1985, 1986). However, the role of ethylene is unclear and may be different in specific species: ethylene was found to induce chitinase in pea and bean roots, but not in soybean roots (Vigniotelli, 1991) and ethylene-independent induction was demonstrated in tobacco and pea (Lotan and Fluhr, 1990; Mauch *et al.*, 1984).

Treatment of the plants with abiotic elicitors such as heavy metal or salt solutions induces chitinase activity (Fink *et al.*, 1990; Irving and Kúć, 1990; Nasser *et al.*, 1988). Systemic induction of chitinase activity was found in the leaves of barley and rape 2 days after the roots were

exposed to Cr(VI) (CrO_3), although not following Cr(III) (CrCl_3) treatment (Jacobsen *et al.*, 1992) and in cucumber plants sprayed with salt solutions (Irving and Kúć, 1990). It has been demonstrated that systemically induced resistance occurs following infection with pathogens or treatment with methyl-2,6-dichloroisonicotinic acid and salicylic acid (Métreaux and Boller, 1986; Tuzun *et al.*, 1989; Ward *et al.*, 1991). The latter is a well known abiotic elicitor which also acts as a systemic endogenous signal for induction of PR proteins in tobacco (Bol *et al.*, 1990; Ward *et al.*, 1991; Yalpani *et al.*, 1991).

Chitinase, like various other PR proteins, has been recorded to be developmentally regulated in several tissues including flowers, roots and lower leaves of tobacco and *Arabidopsis* (Lotan *et al.*, 1989; Memelink *et al.*, 1990; Neale *et al.*, 1990; Samac and Shah, 1991; Shinshi *et al.*, 1987), in pea pods (Mauch *et al.*, 1988a) and in cereal grain (Jacobsen *et al.*, 1990; Leah *et al.*, 1991; Molano *et al.*, 1979; Neucere *et al.*, 1991). Three chitinases isolated from barley grain also accumulate extracellularly in embryogenic cell suspension cultures of barley (Kragh *et al.*, 1991). Furthermore, chitinase accumulation in tobacco *in vitro* cultures is regulated by auxin and cytokinin (Grosset *et al.*, 1990; Shinshi *et al.*, 1987).

Analysis of regulatory elements has been initiated. In a bean chitinase promotor, regions necessary for ethylene-regulated expression have been identified (Broglie *et al.*, 1989). Furthermore, the bean chitinase promotor was investigated in response to fungal attack (Roby *et al.*, 1990). Similar studies have been reported for a class III chitinase gene from *Arabidopsis* (Samac and Shah, 1991) and a class I chitinase gene from tobacco (Fukuda *et al.*, 1991). Further investigations are necessary to provide a better understanding of the regulation of chitinases.

Subcellular localization

Immunocytochemical as well as cell fractionation studies have been used to localize different chitinase isozymes (Boller and Métraux, 1988; Boller and Vögeli, 1984; Fink *et al.*, 1988; Flemming *et al.*, 1991; Keefe *et al.*, 1990; Mauch and Staehelin, 1989; Nasser *et al.*, 1990; Wubben *et al.*, 1992). The general conclusion is that some class I chitinases are localized in vacuoles, whereas other chitinases are located apoplastically.

Recent studies have demonstrated that a short C-terminal extension of about six amino acids present in a basic class I chitinase of tobacco is both necessary and sufficient for vacuolar localization (Neuhaus *et al.*, 1991b). The significance of this short C-terminal vacuolar targeting sequence is supported by the presence of such sequences in basic vacuolar chitinases from tobacco, bean, potato, poplar and *Arabidopsis*, designated Ia in Figure 1 (Broglie *et al.*, 1986; Gaynor, 1988; Parsons *et al.*, 1989; Samac *et al.*, 1988).

al., 1990; Shinshi *et al.*, 1990). Class IV chitinases from sugar beet and rape, and some class I chitinases (designated Ib in Figure 1) from pea, rice and barley (Huang *et al.*, 1991; Mikkelsen *et al.*, 1992; Rasmussen *et al.*, 1992a; Swegle *et al.*, 1989; Vad, 1991) are synthesized without a C-terminal extension suggesting extracellular localization. The class IV chitinase from sugar beet is indeed deposited in the apoplast (Mikkelsen *et al.*, 1992).

Role of chitinase

The general induction of chitinases in plants exposed to various stress stimuli might indicate that chitinases are produced as a general stress response. However, this does not exclude their importance as an active component in the plant defence system as various differentially regulated isoforms present in the plant presumably may possess different roles.

Various *in vitro* studies have demonstrated a growth inhibitory effect of chitinases against fungi containing chitin in the cell wall (Broekaert *et al.*, 1988; Mauch *et al.*, 1988b; Roberts and Selitrennikoff, 1988; Schlumbaum *et al.*, 1986). During apical growth in filamentous fungi, chitin and β -1,3-glucan fibres are synthesized simultaneously in the apex of the growing hypha. In the mature cell wall distant from the apex, the polysaccharides are cross-linked to form mixed chitin-glucan fibres and may be overlaid by other polysaccharides and protein layers (Wessels, 1986, 1988). Thus at the apex, the exposed nascent chitin chains are accessible to hydrolysis by chitinase, whereas the chitin layer in the mature cell wall is inaccessible to degradation by the enzyme. This theory is supported by an autoradiographic study on *Cercospora beticola* showing that a purified sugar beet chitinase was able to degrade newly synthesized radioactively labelled chitin fibres in the hyphal apex, whereas it had no effect on the chitin layer in the mature cell wall (Nielsen and Mikkelsen, unpublished, see Figure 2). A destructive effect of chitinase treatment on immature fungal cell walls, indicated by apical swelling and/or lysis has been observed by others (Boller *et al.*, 1983; Broekaert *et al.*, 1988; Mauch *et al.*, 1988b; Ordentlich *et al.*, 1988). Nevertheless, most fungi tested in an *in vitro* assay were not inhibited by a pea chitinase alone (Mauch *et al.*, 1988b), thus the effectiveness of chitinases against fungi may depend on the simultaneous action of β -1,3-glucanase or other antifungal substances.

Direct evidence that the enzyme interacts with fungal cell walls *in vivo* comes from immunocytochemical techniques (Benhamou *et al.*, 1990; Wubben *et al.*, 1992). It was demonstrated that the enzyme accumulates around fungal cell walls *in planta*. Chitinase was mainly associated with altered fungal cell walls, suggesting that the enzyme activation is either preceded by the hydrolytic



Figure 2. Autoradiography.

A mycelial culture of *Cercospora beticola* was grown in medium containing tritium-labelled *N*-acetylglucosamine, the chitin monomer, for 20 min (a) and subsequently incubated with a purified sugar beet chitinase for 20 h (b). The intense radioactive labelling in the hyphal tips (a), showing an incorporation of the *N*-acetylglucosamine into nascent chitin chains during apical growth, was removed by the chitinase treatment, demonstrating a hydrolytic action of the enzyme on the exposed chitin layer.

action of other enzymes such as β -1,3-glucanase or coincides with these enzymes. This interpretation is supported by the observation that chitinase and β -1,3-glucanase act synergistically in the inhibition of fungal growth *in vitro* (Mauch *et al.*, 1988b). With respect to their localization and implication in the defence reaction, it has been proposed that extracellular chitinases are part of an early, induced response. They may act directly by blocking the growth of the hyphae invading the intercellular space and possibly indirectly as well by releasing fungal elicitors which then induce additional chitinase activity and other defence reactions in the host (Barber *et al.*, 1989; Kurosaki *et al.*, 1988; Roby *et al.*, 1987). According to this view, vacuolar chitinase functions late in the infection process when cell collapse releases the vacuolar content into the extracellular compartment (Mauch and Staehelin, 1989).

In contrast to the extensive data concerning the induction of chitinases in relation to pathogen attack, potential endogenous functions of plant chitinases have received little attention. Nevertheless, it has been shown recently

that an acidic endochitinase has an important function in early somatic embryo development of carrot, in that it allowed completion of embryo development in a temperature-sensitive carrot mutant cell line at non-permissive temperature (de Jong *et al.*, 1992). This may also explain the developmental and hormonal regulation of plant chitinases, discussed above, which has been proposed previously to be due to non-defensive functions during cell division and differentiation (Lotan *et al.*, 1989; Neale *et al.*, 1990; Nielsen, 1991). Whereas, it is possible that the constitutive presence of chitinase in certain organs reflects the vulnerability of, e.g. style and roots to pathogen attack, at least in certain organs, chitinases may participate in the generation of signal molecules which regulate plant development, e.g. organogenesis. If this is the case, then the newly discovered rhizobial lipo-oligosaccharides possibly mimic endogenous signals in order to trigger the formation of root nodules required for symbiotic interactions. Searches for endogenous chitinase substrates as well as substrate specificities and localizations are needed in order to clarify the roles of the many chitinases present in plants.

Perspectives

Despite the intensive research on plant chitinases, and with all our knowledge regarding properties of the enzyme, the actual role of the enzyme in defence reactions is still a matter of speculation. Nevertheless, the prospects for genetically engineering plants with improved resistance by transformation with chitinase genes seem good, and this approach is now being utilized in many laboratories all over the world. A few reports concerning transgenic plants with elevated expression levels of chitinase have been published recently (Broglie *et al.*, 1991; Jones *et al.*, 1988; Linthorst *et al.*, 1990; Neuhaus *et al.*, 1991a, 1991b), and other reports have been presented at international meetings. Transgenic tobacco and rape plants containing a bean chitinase gene with a constitutive promotor have been shown to exhibit higher basal levels of chitinase and concomitant increased resistance to *Rhizoctonia solani* when compared with control plants (Broglie *et al.*, 1991). In another report (Neuhaus *et al.*, 1991a) tobacco plants transformed with a basic chitinase from tobacco, under regulation of a constitutive promotor, accumulated up to 120-fold more active chitinase than non-transformed plants. However, the transformed plants were found to be no more resistant to the fungus *Cercospora nicotiana* than the controls. It is possible that the additional chitinase is located intracellularly in the transformed plants and does not come into contact with the intercellular developing fungus. It is also possible that this pathogen is resistant to the enzyme utilized in this particular study. The extra- or intercellular localization of the chitinases has to be evalu-

ated with respect to the growth pattern of the invading fungi.

It now seems likely that chitinase genes, perhaps in combination with other genes encoding antifungal products, will provide a means for improving resistance against at least some pathogens, thus reducing the need to spray with pesticides. An alternative approach might be to use chitinase, either from microbial or plant sources, as a means of increasing the effectivity of biological control agents (Ordentlich *et al.*, 1988; Shapira *et al.*, 1989). This approach is supported by results obtained with injected bacterial chitinase which inhibited the development of powdery mildew in barley coleoptiles (Toyoda *et al.*, 1991). In any case, although the results obtained to date are mixed, they are nevertheless promising, and justify the faith put into both these approaches.

Acknowledgements

We are grateful to Drs Ton Bisseling, Tomas Bryngelsson, Jean Dénarié, Max Dow, Rob Mellor, Herman Spaink, Sacco de Vries and Pierre J.G.M. de Wit for permission to quote unpublished data and their constructive criticism of the manuscript at various stages. Collaboration between our laboratories is supported by the Framework programme of the Center for Plant Biotechnology, Copenhagen.

References

- Ary, M.B., Richardson, M. and Shewry, P.R. (1989) Purification and characterization of an insect α -amylase inhibitor/endochitinase from seeds of Job's tears (*Coix lachryma-jobi*). *Biochim. Biophys. Acta*, **993**, 260–266.
- Audy, P., Trudel, J. and Asselin, A. (1988) Purification and characterization of a lysozyme from wheat germ. *Plant Sci.* **58**, 43–50.
- Barber, M.S., Bertram, R.E. and Ride, J.P. (1989) Chitin oligosaccharides elicit lignification in wounded wheat leaves. *Physiol. Mol. Plant Pathol.* **34**, 3–12.
- Bartnicki-Garcia, S. (1968) Cell wall chemistry, morphogenesis, and taxonomy of fungi. *Ann. Rev. Microbiol.* **22**, 87–108.
- Benhamou, N. and Asselin, A. (1989) Attempted localization of a substrate for chitinases in plant cells reveals abundant N-acetyl-D-glucosamine residues in secondary walls. *Biology Cell* **67**, 341–350.
- Benhamou, N., Joosten, M.H.A.J. and de Wit, P.J.G.M. (1990) Subcellular localization of chitinase and of its potential substrate in tomato root tissues infected by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. *Plant Physiol.* **92**, 1108–1120.
- Bernasconi, P., Locher, R., Pilet, P.E., Jollès, J. and Jollès, P. (1987) Purification and N-terminal amino-acid sequence of a basic lysozyme from *Parthenocissus quinifolia* cultured *in vitro*. *Biochim. Biophys. Acta*, **915**, 254–260.
- Bernier, J., Van Leemputten, E., Horisberger, M., Bush, D.A. and Jollès, P. (1971) The turnip lysozyme. *FEBS Lett.* **14**, 100–104.
- Bol, J.F., Linthorst, H.J. and Cornelissen, B.J. (1990) Plant pathogenesis-related proteins induced by virus infection. *Ann. Rev. Phytopathol.* **28**, 113–138.

- Boller, T.** (1987) Hydrolytic enzymes in plant disease resistance. In *Plant-Microbe Interactions, Molecular and Genetic Perspectives*, Volume 3 (Koshuge, T. and Nester, E.W., eds). New York: Macmillan, pp. 385–411.
- Boller, T.** (1988) Ethylene and the regulation of antifungal hydrolases in plants. In *Surveys of Plant Molecular and Cell Biology*, Volume 5 (Miflin, B.J., ed.). Oxford: Oxford University Press, pp. 145–174.
- Boller, T. and Vögeli, U.** (1984) Vacuolar localization of ethylene-induced chitinase in bean leaves. *Plant Physiol.* **74**, 442–444.
- Boller, T. and Métraux, J.P.** (1988) Extracellular localization of chitinase in cucumber. *Physiol. Mol. Plant Pathol.* **33**, 11–16.
- Boller, T., Gehri, A., Mauch, F. and Vögeli, U.** (1983) Chitinase in bean leaves: induction by ethylene, purification, properties, and possible function. *Planta*, **157**, 22–31.
- Bowles, D.J.** (1990) Defense-related proteins in higher plants. *Ann. Rev. Biochem.* **59**, 873–907.
- Bowles, D.J., Gurr, S.J., Scollan, C., Atkinson, H.J. and Hammond-Kosack, K.E.** (1991) Local and systemic changes in plant gene expression following root infection by cyst nematodes. In *Biochemistry and Molecular Biology of Plant-Pathogen Interactions* (Smith, C.J., ed.). Oxford: Clarendon Press, pp. 225–236.
- Brederode, F.T., Linthorst, H.J.M. and Bol, J.F.** (1991) Differential induction of acquired resistance and PR gene expression in tobacco by virus infection, ethephon treatment, UV light and wounding. *Plant Mol. Biol.* **17**, 1117–1125.
- Broekaert, W.F., Parijs, J.V., Allen, A.K. and Peumans, W.J.** (1988) Comparison of some molecular, enzymatic and antifungal properties of chitinases from thorn-apple, tobacco and wheat. *Physiol. Mol. Plant Pathol.* **33**, 319–331.
- Broekaert, W.F., Parijs, J.V., Leyns, F., Joos, H. and Peumans, W.J.** (1989) A chitin-binding lectin from stinging nettle rhizomes with antifungal properties. *Science*, **245**, 1100–1102.
- Broglie, K.E., Gaynor, J.J. and Broglie, R.M.** (1986) Ethylene-regulated gene expression: molecular cloning of the genes encoding an endochitinase from *Phaseolus vulgaris*. *Proc. Natl Acad. Sci. USA*, **83**, 6820–6824.
- Broglie, K.E., Biddle, P., Cressman, R. and Broglie, R.** (1989) Functional analysis of DNA sequences responsible for ethylene regulation of a bean chitinase promoter in transgenic tobacco. *Plant Cell*, **1**, 599–607.
- Broglie, K., Chet, I., Holliday, M., Cressman, R., Biddle, P., Knowlton, S., Mauvais, C.J. and Broglie, R.** (1991) Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. *Science*, **254**, 1194–1197.
- Chrispeels, M.J. and Raikel, N.V.** (1991) Lectins, lectin genes, and their role in plant defence. *Plant Cell*, **3**, 1–9.
- Collinge, D.B. and Slusarenko, A.J.** (1987) Review: Plant gene expression in response to pathogens. *Plant Mol. Biol.* **9**, 389–410.
- Conrads-Strauch, J., Dow, M., Milligan, D.E., Parra, R. and Daniels, M.J.** (1990) Induction of hydrolytic enzymes in *Brassica campestris* in response to pathogens of *Xanthomonas campestris*. *Plant Physiol.* **93**, 238–243.
- Daugrois, J.H., Lafitte, C., Barthe, J.P. and Touze, A.** (1990) Induction of β -1,3-glucanase and chitinase activity in compatible and incompatible interactions between *Colletotrichum lindemuthianum* and bean cultivars. *J. Phytopath.* **130**, 225–234.
- Davis, J.M., Clarke, H.R.G., Bradshaw, H.D. and Gordon, M.P.** (1991) *Populus* chitinase genes: structure, organization, and similarity of translated sequences to herbaceous plant chitinases. *Plant Mol. Biol.* **17**, 631–639.
- Dixon, R.A. and Harrison, M.** (1990) Activation, structure and organization of genes involved in microbial defence in plants. *Adv. Genet.* **28**, 165–234.
- Fink, W., Liefland, M. and Mendgen, K.** (1988) Chitinases and β -1,3-glucanases in the apoplastic compartment of oat leaves (*Avena sativa* L.). *Plant Physiol.* **88**, 270–275.
- Fink, W., Liefland, M. and Mendgen, K.** (1990) Comparison of various stress responses in oat in compatible and nonhost resistant interactions with rust fungi. *Physiol. Mol. Plant Pathol.* **37**, 309–321.
- Fisher, R.F. and Long, S.R.** (1992) *Rhizobium*-plant signal exchange. *Nature*, **357**, 655–660.
- Flemming, T.M., McCarthy, D.A., White, R.F., Antoniwi, J.F. and Mikkelsen, J.D.** (1991) Induction and characterization of some of the pathogenesis-related proteins in sugar beet. *Physiol. Mol. Plant Pathol.* **39**, 147–160.
- Fukuda, Y., Ohme, M. and Shinshi, H.** (1991) Gene structure and expression of a tobacco endochitinase gene in suspension-cultured tobacco cells. *Plant Mol. Biol.* **16**, 1–10.
- Gaynor, J.J.** (1988) Primary structure of an endochitinase mRNA from *Solanum tuberosum*. *Nucl. Acids Res.* **16**, 5210.
- Grosset, J., Meyer, Y., Chartier, Y., Kauffmann, S., Legrand, M. and Fritig, B.** (1990) Tobacco mesophyll protoplasts synthesize 1,3- β -glucanase, chitinases, and "osmotins" during *in vitro* culture. *Plant Physiol.* **92**, 520–527.
- Hedrick, S.A., Bell, J.N., Boller, T. and Lamb, C.J.** (1988) Chitinase cDNA cloning and mRNA induction by fungal elicitor, wounding, and infection. *Plant Physiol.* **86**, 182–186.
- Henrissat, B.** (1990) Weak sequence homologies among chitinases detected by clustering analysis. *Protein Seq. Data. Anal.* **3**, 523–526.
- Herget, T., Schell, J. and Schreier, P.H.** (1990) Elicitor-specific induction of one member of the chitinase gene family in *Arachis hypogaea*. *Mol. Gen. Genet.* **224**, 469–476.
- Huang, J.-K., Wen, L., Swegle, M., Tran, H.-C., Thin, T.H., Naylor, H.M., Muthukrishnan, S. and Reeck, G.R.** (1991) Nucleotide sequence of a rice genomic clone that encodes a class I endochitinase. *Plant Mol. Biol.* **16**, 479–480.
- Irving, H.R. and Kuć, J.A.** (1990) Local and systemic inductions of peroxidase, chitinase and resistance in cucumber plants by K_2HPO_4 . *Physiol. Mol. Plant Pathol.* **37**, 355–366.
- Jacobsen, S., Mikkelsen, J.D. and Hejgaard, J.** (1990) Characterization of two antifungal endochitinases from barley grain. *Physiol. Plant.* **79**, 554–562.
- Jacobsen, S., Hauschild, M.Z. and Rasmussen, U.** (1992) Induction by chromium ions of chitinases and polyamines in barley (*Hordeum vulgare* L.) and rape (*Brassica napus* L. ssp. *oleifera*). *Plant Sci.* **84**, 119–128.
- Jones, J.D.G., Dean, C., Gidoni, D., Gilbert, D., Bonde-Nutter, D., Lee, R., Bedbrook, J. and Dunsmuir, P.** (1988) Expression of bacterial chitinase protein in tobacco leaves using two photosynthetic gene promoters. *Mol. Gen. Genet.* **212**, 536–542.
- de Jong, A., Cordewener, J., Lo Schiavo, F., Terzi, M., Vandeckerckhove, J., Van Kammen, A. and De Vries, S.C.** (1992) A carrot somatic embryo mutant is rescued by chitinase. *Plant Cell*, **4**, 425–433.
- Joosten, M.H.A.J. and de Wit, P.J.G.M.** (1989) Identification of several pathogenesis-related proteins in tomato leaves inoculated with *Cladosporium fulvum* (syn. *Fulvia fulva*) as 1,3- β -glucanases and chitinases. *Plant Physiol.* **89**, 945–951.
- Keefe, D., Hinz, U. and Meins, F. Jr** (1990) The effect of ethylene on the cell-type-specific and intracellular localization of β -1,3-glucanase and chitinase in tobacco leaves. *Planta*, **182**, 43–51.
- Kombrink, E., Schröder, M. and Hahlbrock, K.** (1988) Several "pathogenesis-related" proteins in potato are β -1,3-

- glucanases and chitinases. *Proc. Natl Acad. Sci. USA*, **85**, 782–786.
- Kragh, K.M., Jacobsen, S. and Mikkelsen, J.D. (1990) Induction, purification and characterization of barley leaf chitinases. *Plant Sci.* **71**, 55–68.
- Kragh, K.M., Jacobsen, S., Mikkelsen, J.D. and Nielsen, K.A. (1991) Purification and characterization of three chitinases and one β -1,3-glucanase accumulating in the medium of cell suspension cultures of barley (*Hordeum vulgare* L.). *Plant Sci.* **76**, 65–77.
- Kurosaki, F., Tashiro, N. and Nishi, A. (1988) Role of chitinase and chitin oligosaccharides in lignification response of cultured carrot cells treated with mycelial walls. *Plant Cell Physiol.* **29**, 527–531.
- Laffamme, D. and Roxby, R. (1989) Isolation and nucleotide sequence of cDNA clones encoding potato chitinase genes. *Plant Mol. Biol.* **13**, 249–250.
- Lawton, K., Ward E., Payne, G., Moyer, M. and Ryals, J. (1992) Acidic and basic class III chitinase mRNA accumulation in response to TMV infection of tobacco. *Plant Mol. Biol.* **19**, 735–743.
- Leah, R., Tommerup, H., Svendsen, I. and Mundy, J. (1991) Biochemical and molecular characterization of three barley seed proteins with antifungal properties. *J. Biol. Chem.* **266**, 1564–1573.
- Lerouge, P., Roche, P., Faucher, C., Maillet, F., Truchet, G., Promé, J.C. and Dénarié, J. (1990) Symbiotic host-specificity of *Rhizobium meliloti* is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature*, **344**, 781–784.
- Linthorst, H.J.M. (1991) Pathogenesis-related proteins of plants. *Crit. Rev. Plant Sci.* **10**, 123–150.
- Linthorst, H.J.M., van Loon, L.C., van Rossum, C.M.A., Mayer, A., Bol, J.F., van Roekel, J.S.C., Meulenhoff, E.J.S. and Cornelissen, B.J.C. (1990) Analysis of acidic and basic chitinases from tobacco and petunia and their constitutive expression in transgenic tobacco. *Mol. Plant-Microbe Interactions*, **3**, 252–258.
- Lotan, T. and Fluhr, R. (1990) Xylanase, a novel elicitor of pathogenesis-related proteins in tobacco, uses a non-ethylene pathway for induction. *Plant Physiol.* **93**, 811–817.
- Lotan, T., Ori, N. and Fluhr, R. (1989) Pathogenesis-related proteins are developmentally regulated in tobacco flowers. *Plant Cell*, **1**, 881–887.
- Lucas, J., Henschen, A., Lottspeich, F., Voegeli, U. and Boller, T. (1985) Amino-terminal sequence of ethylene-induced bean leaf chitinase reveals similarities to sugar-binding domains of wheat germ agglutinin. *FEBS Lett.* **193**, 208–210.
- Majeau, N., Trudel, J. and Asselin, A. (1990) Diversity of cucumber chitinase isoforms and characterization of one seed basic chitinase with lysozyme activity. *Plant Sci.* **68**, 9–16.
- Margis-Pinheiro, M., Metz-Boutigue, M.H., Awade, A., Tapia, de M., Ret, le M. and Burkard, G. (1991) Isolation of a complementary DNA encoding the bean PR4 chitinase: an acidic enzyme with an amino-terminus cysteine-rich domain. *Plant Mol. Biol.* **17**, 243–253.
- Mauch, F. and Staehelin, L.A. (1989) Functional implications of the subcellular localization of ethylene-induced chitinase and β -1,3-glucanase in bean leaves. *Plant Cell*, **1**, 447–457.
- Mauch, F., Hadwiger, L.A. and Boller, T. (1984) Ethylene: symptom, not signal for the induction of chitinase and β -1,3-glucanase in pea pods by pathogens and elicitors. *Plant Physiol.* **76**, 607–611.
- Mauch, F., Hadwiger, L.A. and Boller, T. (1988a) Antifungal hydrolases in pea tissue. I. Purification and characterization of two chitinases and two β -1,3-glucanases differentially regulated during development and in response to fungal infection. *Plant Physiol.* **87**, 325–333.
- Mauch, F., Mauch-Mani, B. and Boller, T. (1988b) Antifungal hydrolases in pea tissue. II. Inhibition of fungal growth by combinations of chitinase and β -1,3-glucanase. *Plant Physiol.* **88**, 936–942.
- Memelink, J., Linthorst, H.J.M., Schilperroot, R.A. and Hoge, J.H.C. (1990) Tobacco genes encoding acidic and basic isoforms of pathogenesis-related proteins display different expression patterns. *Plant Mol. Biol.* **14**, 119–126.
- Métraux, J.P. and Boller, T. (1986) Local and systemic induction of chitinase in cucumber plants in response to viral, bacterial and fungal infections. *Physiol. Mol. Plant Pathol.* **28**, 161–169.
- Métraux, J.P., Burkhardt, W., Moyer, M., Dincher, S., Middlesteadt, W., Williams, S., Payne, G., Carnes, M. and Ryals, J. (1989) Isolation of a complementary DNA encoding a chitinase with structural homology to a bifunctional lysozyme/chitinase. *Proc. Natl Acad. Sci. USA*, **86**, 896–900.
- Mikkelsen, J.D., Berglund, L., Nielsen, K.K., Christiansen, H. and Bojsen, K. (1992) Structure of endochitinase genes from sugar beets. In *Advances in Chitin and Chitosan* (Brine, C.J., Sandford, P.A. and Zikakis, J.P., eds). Amsterdam: Elsevier, in press.
- Molano, J., Durán, A. and Cabib, E. (1977) A rapid and sensitive assay for chitinase using tritiated chitin. *Anal. Biochem.* **83**, 648–656.
- Molano, J., Polacheck, I., Durán, A. and Cabib, E. (1979) An endochitinase from wheat germ. *J. Biol. Chem.* **254**, 4901–4907.
- Nasser, W., De Tapia, M., Kaufmann, S., Montasser-Kouhsari, S. and Burkard, G. (1988) Identification and characterization of a maize pathogenesis-related proteins. Four maize PR proteins are chitinases. *Plant Mol. Biol.* **11**, 529–538.
- Nasser, W., De Tapia, M. and Burkard, G. (1990) Maize pathogenesis-related proteins: characterization and cellular distribution of 1,3- β -glucanases and chitinases induced by brown mosaic virus infection or mercuric chloride treatment. *Physiol. Mol. Plant Pathol.* **36**, 1–14.
- Neale, A.D., Wahleithner, J.A., Lund, M., Bonnett, H.T., Kelly, A., Meeks-Wagner, D.R., Peacock, W.J. and Dennis, E.S. (1990) Chitinase, β -1,3-glucanase, osmotin, and extensin are expressed in tobacco explants during flower formation. *Plant Cell*, **2**, 673–684.
- Neucere, J.N., Cleveland, T.E. and Dischinger, C. (1991) Existence of chitinase activity in mature corn kernels. *J. Agric. Food Sci.* **39**, 1326–1328.
- Neuhaus, J.-M., Ahl-Goy, P., Hinz, U., Flores, S. and Meins, F. (1991a) High-level expression of a tobacco chitinase gene in *Nicotiana glauca*. Susceptibility of transgenic plants to *Cercospora nicotianae* infection. *Plant Mol. Biol.* **16**, 141–151.
- Neuhaus, J.-M., Sticher, L., Meins, F., Jr and Boller, T. (1991b) A short C-terminal sequence is necessary and sufficient for the targeting of chitinases to the plant vacuole. *Proc. Natl Acad. Sci. USA*, **88**, 10362–10366.
- Nielsen, K.A. (1991) *Establishment and biochemical characterization of embryogenic cell suspensions of barley (Hordeum vulgare L.) Analysis of extracellular proteins secreted by cells in culture*. Ph.D. Thesis. Denmark: Aarhus University.
- Nishizawa, Y. and Hibi, T. (1991) Rice chitinase gene: cDNA cloning and stress-induced expression. *Plant Sci.* **76**, 211–218.
- O'Brien, M. and Colwell, R.R. (1987) A rapid test for chitinase activity that uses 4-methylumbelliferyl-N-acetyl- β -D-glucosaminide. *Appl. Environ. Microbiol.* **53**, 1718–1720.
- Ordentlich, A., Elad, T. and Chet, I. (1988) The role of chitinase of

- Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. *Phytopathology*, **78**, 84–88.
- Parsons, T.J., Bradshaw, H.D. and Gordon, M.P. (1989) Systemic accumulation of specific mRNAs in response to wounding in poplar trees. *Proc. Natl Acad. Sci. USA*, **86**, 7895–7899.
- Payne, G., Ahl, P., Moger, M., Harper, A., Beck, J., Meins, F. and Ryals, J. (1990) Isolation of complementary DNA clones encoding pathogenesis-related proteins P and Q two acidic chitinases from tobacco. *Proc. Natl Acad. Sci. USA*, **87**, 98–102.
- Pegg, G.F. and Young, D.H. (1982) Purification and characterization of chitinase enzymes from healthy and *Verticillium albo-atrum*-infected tomato plants and from *V. albo-atrum*. *Physiol. Plant Pathol.* **21**, 389–409.
- Rasmussen, U., Boisen, K. and Collinge, D.B. (1992a) Cloning and characterization of a pathogen-induced chitinase in *Brassica napus*. *Plant Mol. Biol.* **20**, 277–287.
- Rasmussen, U., Giese, H. and Mikkelsen, J.D. (1992b) Induction and purification of chitinase in *Brassica napus* L. spp. *oleifera* infected with *Phoma lingam*. *Planta*, **187**, 328–334.
- Roberts, W.K. and Selitrennikoff, C.P. (1988) Plant and bacterial chitinases differ in antifungal activity. *J. Gen. Microbiol.* **134**, 169–176.
- Roby, D. and Esquerré-Tugayé, M.T. (1987) Purification and some properties of chitinases from melon plants infected by *Colletotrichum lagenarium*. *Carbohydr. Res.* **165**, 93–104.
- Roby, D., Toppan, A. and Esquerré-Tugayé, M.T. (1985) Cell surfaces in plant-microorganism interactions. V. Elicitors of fungal and of plant origin trigger the synthesis of ethylene and of cell wall hydroxyproline-rich glycoprotein in plants. *Plant Physiol.* **77**, 700–704.
- Roby, D., Toppan, A. and Esquerré-Tugayé, M.T. (1986) Cell surfaces in plant-microorganism interactions. VI. Elicitors of ethylene from *Colletotrichum lagenarium* trigger chitinase activity in melon plants. *Plant Physiol.* **81**, 228–233.
- Roby, D., Gadelle, A. and Toppan, A. (1987) Chitin oligosaccharides as elicitors of chitinase activity in melon plants. *Biochem. Biophys. Res. Commun.* **143**, 885–892.
- Roby, D., Broglie, K., Cressman, R., Biddle, P., Chet, I. and Broglie, R. (1990) Activation of a bean chitinase promoter in transgenic tobacco plants by phytopathogenic fungi. *Plant Cell*, **2**, 999–1007.
- Roche, P., Lerouge, P., Ponthus, C. and Promé, J.C. (1991a) Structural determination of bacterial nodulation factors involved in the *Rhizobium meliloti*-alfalfa symbiosis. *J. Biol. Chem.* **266**, 10933–10940.
- Roche, P., Debellé, F., Maillet, F., Lerouge, P., Faucher, C., Truchet, G., Dénarié, J. and Promé, J.C. (1991b) Molecular basis of symbiotic host specificity in *Rhizobium meliloti*: *nodH* and *nodPQ* genes encode the sulfation of lipo-oligosaccharide signals. *Cell*, **67**, 1131–1143.
- Samac, D.A. and Shah, D.M. (1991) Developmental and pathogen-induced activation of the Arabidopsis acidic chitinase promoter. *Plant Cell*, **3**, 1063–1072.
- Samac, D.A., Hironaka, C.M., Yallaly, P.E. and Shah, D.M. (1990) Isolation and characterization of the genes encoding basic and acidic chitinase in *Arabidopsis thaliana*. *Plant Physiol.* **93**, 907–914.
- Schlumbaum, A., Mauch, F., Vögeli, U. and Boller, T. (1986) Plant chitinases are potent inhibitors of fungal growth. *Nature*, **324**, 365–367.
- Shapira, R., Ordentlich, A., Chet, I. and Oppenheim, A.B. (1989) Control of plant diseases by chitinases expressed from cloned DNA in *Escherichia coli*. *Phytopathology*, **79**, 1246–1249.
- Shinshi, H., Mohnen, D. and Meins, F. (1987) Regulation of plant pathogenesis-related enzyme: inhibition of chitinase and chitinase mRNA accumulation in cultured tobacco tissues by auxin and cytokinin. *Proc. Natl Acad. Sci. USA*, **84**, 89–93.
- Shinshi, H., Neuhaus, J.-M., Ryals, J. and Meins, F. (1990) Structure of a tobacco endochitinase gene: evidence that different chitinase genes can arise by transposition of sequences encoding a cysteine-rich domain. *Plant Mol. Biol.* **14**, 357–368.
- Spaink, H.P. (1992) Rhizobial lipo-oligosaccharides: answers and questions. *Plant Mol. Biol.* in press.
- Spaink, H.P., Sheeley, D.M., van Brussel, A.A.N., Glushka, J., York, W.S., Tak, T., Geiger, O., Kennedy, E.P., Reinhold, V.N. and Lugtenberg, B.J.J. (1991) A novel highly unsaturated fatty acid moiety of lipo-oligosaccharide signals determines host specificity of *Rhizobium*. *Nature*, **354**, 125–130.
- Spaink, H.P., Aarts, A., Bloemberg, G.V., Folch, J., Geiger, O., Schlaman, H.R.M., Thomas-Oates, J.E., van Brussel, A.A.N., Van de Sande, K., Van Spronsen, P., Wijffjes, A.H.M. and Lugtenberg, B.J.J. (1992) Rhizobial lipo-oligosaccharide signals: Their biosynthesis and their role in the plant. In *Advances in Molecular Genetics of Plant-Microbe Interactions*. Volume 2 (Nester, E., ed.), in press.
- Spanu, P., Boller, T., Ludwig, A., Wiemken, A., Faccio, A. and Bonfante-Fasolo, P. (1989) Chitinase in roots of mycorrhizal *Allium porrum*: regulation and localization. *Planta*, **177**, 447–455.
- Staehelin, C., Müller, J., Mellor, R.B., Wiemken, A. and Boller, T. (1992) Chitinase and peroxidase in effective (fix⁺) and ineffective (fix⁻) soybean nodules. *Planta*, **187**, 295–300.
- Stanford, A., Bevan, M. and Northcote, D. (1989) Differential expression within a family of novel wound-induced genes in potato. *Mol. Gen. Genet.* **215**, 200–208.
- Swegle, M., Huang, J.-K., Lee, G. and Muthukrishnan, S. (1989) Identification of an endochitinase cDNA clone from barley aleurone cells. *Plant Mol. Biol.* **12**, 403–412.
- Toyoda, H., Matsuda, Y., Yamaga, T., Ikeda, S., Morita, M., Tamai, T. and Ouchi, S. (1991) Suppression of the powdery mildew pathogen by chitinase microinjected into barley coleoptile epidermal cells. *Plant Cell Rep.* **10**, 217–220.
- Truchet, G., Roche, P., Lerouge, P., Vasse, J., Camut, S., de Billy, F., Promé, J.C. and Dénarié, J. (1991) Sulphated lipo-oligosaccharide signals of *Rhizobium meliloti* elicit root nodule organogenesis in alfalfa. *Nature*, **351**, 670–673.
- Trudel, J. and Asselin, A. (1989) Detection of chitinase activity after polyacrylamide gel electrophoresis. *Anal. Biochem.* **178**, 362–366.
- Trudel, J., Audy, P. and Asselin, A. (1989) Electrophoretic forms of chitinase activity in Xanthi-nc tobacco, healthy and infected with tobacco mosaic virus. *Mol. Plant-Microbe Interactions*, **2**, 315–324.
- Tsukamoto, T., Koga, D., Ide, A., Ishibashi, T., Horino-Matsushige, M., Yagashita, K. and Imoto, T. (1984) Purification and some properties of chitinases from yam, *Dioscorea opposita* Thumb. *Agric. Biol. Chem.* **48**, 931–939.
- Tuzun, S., Rao, M.N., Vögeli, U., Schardl, C.L. and Kuć, J. (1989) Induced systemic resistance to Blue Mold: Early induction and accumulation of β -1,3-glucanases, chitinases, and other pathogenesis-related proteins (b-proteins) in immunized tobacco. *Phytopathology*, **79**, 979–983.
- Usui, T., Hayashi, Y., Nanjo, F., Sakai, K. and Ishido, Y. (1987) Transglycosylation reaction of a chitinase purified from *Nocardia orientalis*. *Biochim. Biophys. Acta*, **923**, 302–309.
- Usui, T., Matsui, H. and Isobe, K. (1990) Enzymatic synthesis of useful chito-oligosaccharides utilizing transglycosylation by chitinolytic enzymes in a buffer containing ammonium sulfate.

- Carbohydr. Res. 203, 65–77.
- Vad, K. (1991) *Molecular studies of the interaction between pea and the pathogenic fungus Ascochyta pisi*. Ph.D. Thesis. Copenhagen: Royal Veterinary and Agricultural University.
- Vad, K., Mikkelsen, J.D. and Collinge, D.B. (1991) Induction, purification and characterization of chitinase isolated from pea leaves inoculated with *Ascochyta pisi*. *Planta*, 184, 24–29.
- Van Brussel, A.A.N., Bakhuizen, R., Van Spronsen, P.C., Spaik, H.P., Tak, T., Lugtenberg, B.J.J. and Keijne, J.W. (1992) Induction of pre-infection thread structures in the host plant by lipo-oligosaccharides of *Rhizobium*. *Science*, 257, 70–72.
- Van Buuren, M., Neuhaus, J.M., Shinshi, H., Ryals, J. and Meins, F. (1992) The structure of regulation of homeologous tobacco endochitinase genes of *Nicotiana sylvestris* and *N. tomentosiformis* origin. *Mol. Gen. Genet.* 232, 460–469.
- Verbarg, J.G., Smith, C.E., Lisek, C.A. and Khai Huynh, Q. (1992) Identification of an essential tyrosine residue in the catalytic site of a chitinase isolated from *Zea mays* that is selectively modified during inactivation with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide. *J. Biol. Chem.* 267, 3886–3893.
- Vigniotelli, A. (1991) *Der Einfluss von Ethylen und Elizitoren auf die Chitinaseaktivität und symbiotische Stickstoff-Fixierung von Glycine max, Phaseolus vulgaris und Pisum sativum*. Diploma thesis. Basel: University of Basel.
- Voisey, C.R. and Slusarenko, A.J. (1989) Chitinase mRNA and enzyme activity in *Phaseolus vulgaris* (L.) increase more rapidly in response to avirulent than to virulent cells of *Pseudomonas syringae* pv. *phaseolicola*. *Physiol. Mol. Plant Pathol.* 35, 403–412.
- Vögeli, U., Meins, F. and Boller, T. (1988) Co-ordinated regulation of chitinase and β -1,3-glucanase in bean leaves. *Planta*, 174, 364–372.
- Vögeli-Lange, R., Hansen-Gehri, A., Boller, T. and Meins, F. (1988) Induction of the defence-related glucanohydrolases, β -1,3-glucanase and chitinase, by tobacco mosaic virus infection of tobacco leaves. *Plant Sci.* 54, 171–176.
- Wadsworth, S.A. and Zikakis, J.P. (1984) Chitinase from soybean seeds: Purification and some properties of the enzyme system. *J. Agric. Food Chem.* 32, 1284–1288.
- Wantanabe, T., Oyanagi, W., Suzuki, K., Ohnishi, K. and Tanaka, H. (1992) Structure of the gene encoding chitinase D of *Bacillus circulans* WL-12 and possible homology of the enzyme to other prokaryotic chitinases and class III plant chitinases. *J. Bacteriol.* 174, 408–414.
- Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Métraux, J.P. and Ryals, J.A. (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell*, 3, 1085–1094.
- Wessels, J.G.H. (1986) Cell wall synthesis in apical hyphal growth. *Int. Rev. Cytology*, 104, 37–79.
- Wessels, J.G.H. (1988) A steady state model for apical wall growth in fungi. *Acta Bot. Neerl.* 37, 3–16.
- Wessels, J.G.H. and Sietsma, J.H. (1981) Fungal cell walls: A survey. In *Encyclopedia of Plant Physiology*, New Series, Volume 13B, *Plant Carbohydrates II* (Tanner, W. and Loewus, F.A., eds). Berlin: Springer-Verlag, pp. 352–394.
- Wirth, S.J. and Wolf, G.A. (1990) Dye-labelled substrates for the assay and detection of chitinase and lysozyme activity. *J. Microbiol. Methods*, 12, 197–205.
- Wright, C.S., Gavilanes, F. and Peterson, D.L. (1984) Primary structure of wheat germ agglutinin isolectin 2. Peptide order deduced from X-ray structure. *Biochem.* 23, 280–287.
- Wubben, J.P., Joosten, M.H.A.J., Van Kan, J.A.L. and de Wit, P.J.G.M. (1992) Subcellular localization of plant chitinases and 1-3- β -glucanases in *Cladosporium fulvum* (syn. *Fulvia fulva*)-infected tomato leaves. *Physiol. Mol. Plant Pathol.* in press.
- Yalpani, N., Silverman, P., Wilson, T.M.A., Kleer, D.A. and Raskin, I. (1991) Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. *Plant Cell*, 3, 809–818.
- Zhu, Q. and Lamb, C.J. (1991) Isolation and characterization of a rice gene encoding a basic chitinase. *Mol. Gen. Genet.* 226, 289–296.