Phytoprotection

New insights in to ancient resistance: the molecular side of cell wall appositions

Nouveau regard sur la résistance ancienne : l'aspect moléculaire de l'apposition pariétale

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Article abstract

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New insights in to ancient resistance: the molecular side of cell wall appositions

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The epidermis lies at the interface between a plant and its environment. As such, the epidermis is crucial for protecting the plant against environmental insults. We focus primarily on cell wall reinforcement-mediated penetration resistance (papilla-resistance) against fungal pathogen attack. The epidermal cell layer of cereal leaves is the only tissue interacting with the powdery mildew fungus, *Blumeria graminis*, and papilla formation at sites of fungal penetration attempts provides a basal resistance, hampering fungal invasion irrespective of host specific compatibility or incompatibility. To elucidate the genetic scaffolding of penetration resistance mechanisms, we constructed a cDNA library from wheat leaf epidermis at 24-48 h post inoculation with *B. graminis* f. sp. *tritici*. We have sequenced 3,000 expressed sequence tags (ESTs) from this cDNA library. EST analysis revealed a large proportion of genes involved in plant defense/stress responses (1/3) and a low frequency of "house-keeping" genes. Enrichment of defense genes from this EST collection has allowed us to identify several defense and signaling pathways that have been hitherto poorly characterized, including cell wall biosynthesis, vesicle trafficking, redox regulation and metal homeostasis. Our results suggest that a global analysis of transcripts from this epidermis-specific cDNA library makes it feasible to define a full set of genes involved in early plant resistance associated with cell wall modifications.

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L'épiderme se situe à l'interface entre la plante et son environnement. L'épiderme est donc essentiel à la protection de la plante contre les assauts de l'environnement. Nous nous sommes concentrés sur la résistance à la pénétration par l'intermédiaire du renforcement de la paroi cellulaire (résistance papillaire) contre les attaques de champignons pathogènes. La couche de cellules épidermiques des feuilles des céréales est le seul tissu qui interagit avec le champignon de l'oïdium, le *Blumeria graminis*, et la formation de papilles aux sites des tentatives de pénétration du champignon fournit une résistance de base, empêchant l'invasion fongique peu importe que l'hôte soit compatible ou incompatible. Afin d'élucider l'échafaudage génétique des mécanismes de résistance à la pénétration, nous avons construit une bibliothèque génomique à partir d'épiderme de feuille de blé recueilli 24 à 48 h après inoculation avec le *B. graminis* f. sp. *tritici*. Nous avons séquencé 3000 séquences EST à partir de cette bibliothèque. L'analyse des séquences EST a montré qu'il y avait une proportion importante de gènes impliqués dans la défense de la plante ou les réponses aux stress (1/3) et une faible teneur en gènes « d'intendance ». L'enrichissement en gènes de défense de cette collection de séquences EST nous a permis d'identifier plusieurs voies de défense et de signalisation qui ont été peu caractérisées jusqu'à présent, y compris la biosynthèse de la paroi cellulaire, le transport au niveau des vésicules, la régulation de l'oxydoréduction et l'homéostasie des métaux. Nos résultats laissent penser qu'une analyse globale des produits de transcription provenant de cette bibliothèque génomique spécifique à l'épiderme pourrait permettre la description d'un ensemble complet de gènes impliqués dans la résistance précoce des plantes associée aux modifications de la paroi cellulaire.

INTRODUCTION

During pathogen attack, plants employ a complex of physical and chemical defence strategies to combat attempted invasions. Pathogen penetration attempts are often met with targeted cell wall fortification, and the formation of these localized cell wall appositions (CWAs) or papillae has long been recognized as a common plant response to fungal penetration attempts (de Bary 1863). Because the occurrence of CWAs often correlates to increased penetration failure, CWA formation is considered to be an important resistance mechanism. CWAs form in both susceptible and resistant hosts, as well as in nonhosts, and therefore constitute a basal or ancient form of resis-

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tance, halting the early stages of fungal ingress into host cells. Since commercial release 25 years ago, recessive mutations in the *Mlo* gene of barley have provided durable broad spectrum resistance to penetration by most powdery mildew races, based largely on strong oversized CWAs (reviewed by Lyngkjær *et al*. 2000). Although CWA formation has been recognized for over a century, the phenomenon continues to challenge researchers and many questions remain unanswered. The aim of the present review is to provide a brief synopsis of the state of CWA research, and to highlight future directions in understanding CWA genesis and function.

Cytoplasmic and cytoskeletal reorganization

One of the earliest recognizable changes in an epidermal cell responding to attempted pathogen penetration is cytoskeletal rearrangement. Penetration attempts cause microtubules and microfilaments in host cells to become radially arranged around the site of attack (Gross *et al*. 1993; Kobayashi *et al*. 1992). Treatment of barley, onion, *Arabidopsis* or tobacco with cytochalasin, an actin polymerisation inhibitor, partially compromises nonhost resistance at the penetration stage for fungal pathogens (Kobayashi and Hakuno 2003; Kobayashi *et al*. 1997; McLusky *et al*. 1999; Yun *et al*. 2003). Concurrent to cytoskeletal rearrangement, cytoplasmic streaming towards the pathogen contact site accelerates, and small volumes of cytoplasm termed cytoplasmic aggregates, which hold rough endoplasmic reticulum and vesicles presumably containing precursors necessary for CWA formation, begin to accumulate beneath the pathogen-plant contact site (Freytag *et al*. 1994; Gross *et al*. 1993; Kobayashi *et al*. 1992). As the cell becomes poised for pathogen attack, the cell nucleus migrates towards the pathogen contact site and the cell begins to produce a CWA (Skalamera and Heath 1998).

Vesicle traffic

Recently, the *Arabidopsis PEN1* (*PENETRATION1*) and barley *ROR2* (*REQUIRED FOR MLO RESISTANCE2*) genes we re isolated and characterised as homologous synaptome-associated protein receptors (SNAREs) (Collins *et al*. 2003). *Arabidopsis* or barley plants with mutations in their respective SNARE genes show increased penetration by barley powdery mildew. In addition to ROR2, a SNAP-25 homologue was also shown to be required for full resistance in barley, and ROR2 and SNAP-25 interacted in yeast two hybrid experiments. While the functions of PEN1, ROR2, and SNAP-25 are still not completely known, Collins *et al*. (2003) suggested that the proteins may be involved in the facilitation of vesicle exocytosis or the homotypic fusion of vesicles as they carry cargo towards the CWA.

Cell wall modification and reactive oxygen species

Although the structural rearrangements preceding CWA formation are now becoming clearer, the identity of the payload carried by CWA-destined vesicles remains somewhat mysterious. The structure and composition of CWAs show remarkably heterogeneity, but they commonly contain callose, pectic substances, phenolic derivatives, suberin, proteins, and some metal ions (reviewed by Smart 1991). The synthesis, deposition, and assembly of these materials are apparently accompanied by the localized release of reactive oxygen species (ROS), predominantly H_2O_2 (Thordal-Christensen *et al*. 1997; Fig. 1). Studies in our lab have shown that ROS production beneath the germinating conidium can be detected as early as 3 h after inoculation, before an appressorium is formed by the fungus (Wei *et al*., unpublished results). The oxidative burst, a rapid and transient ROS production in response to pathogen attack, has been prescribed several roles including direct toxic effects on the pathogen, the oxidative cross-linking of wall components, and local and systemic induced resistance signalling (reviewed by Hücklehoven and Kogel 2003). H_2O_2 has also been found in vesicles in transit to CWAs (Collins *et al*. 2003; Hücklehoven *et al*. 1999), suggesting that ROS may play a role upstream of CWA deposition, or that CWA materials are oxidatively cross-linked before arrival at front line. The origin of ROS in CWAs remains largely unknown, despite compelling evidence for specific ROS generating systems in several different pathosystems. For example, while pharmaceutical and genetic approaches indicate that H_2O_2 is generated from O_2^- produced by the NADPH oxidase complex in *Arabidopsis* (Torres *et al*. 2002) and tobacco (Yoshioka *et al*. 2003), ROS generation in barley (Hückelhoven and Kogel 1998) and wheat (Wei *et al*., unpublished) show low sensitivity to diphenyleneiodonium, an inhibitor of the NAD(P)H oxidase, suggesting an different unidentified ROS generating system in cereals attacked by pathogens.

Current research program on CWAs of powdery mildew attacked wheat

Recent work in our lab has focused on the *Triticum monococcum* L. epidermal transcriptome during attack by *Blumeria graminis* f. sp. *tritici*. The diploid wheat epidermis-powdery mildew system has proven to be an excellent platform for the study of cell wall mediated defence. On the plant side, we used *T. monococcum* because it has a simple wheat 'AA genome' and well defined resistance responses; we have lines that exhibit complete CWA resistance, late single cell hypersensitive response, and full susceptibility. One current project is to develop isogenic lines that differ only in CWA-mediated resistance and susceptibility. We used the epidermis specifically because it is the first line of defense against environmental insults, and because it is a relatively inert tissue that, when challenged, focuses the majority of its transcriptional effort on defence. On the pathogen side, we chose *B. graminis* f. sp. *tritici* because it only infects the epidermis, and because it has highly synchronous and well defined penetration, infection, and reproduction processes. We have sequenced 3000 expressed sequence tags (ESTs) from a powdery mildew infected *T. monococcum* epidermis cDNA library, and have found very few 'housekeeping genes' and over 1000 stress/defence related genes. Much of the transcriptional effort of the infected epidermis is devoted to ROS generation and scavenging. For example, 1.5% of all of the ESTs sequenced so far encode type III peroxidases (Liu *et al*., unpublished). We have found genes involved in many known aspects of CWA formation including cytoskeletal organisation and vesicle traffic (e.g. clathrin, COPI and COPII structural

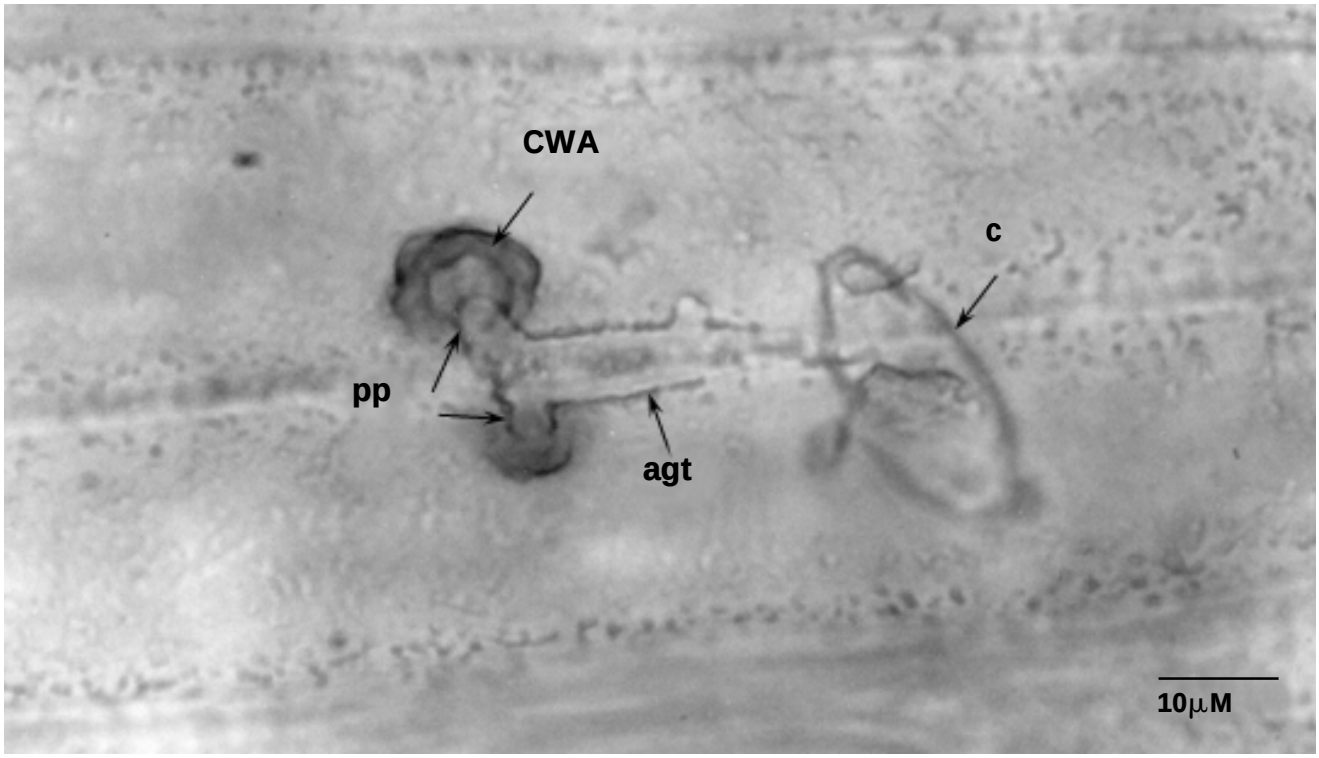

Figure 1. Penetration attempts of *Blumeria graminis* f. sp. *tritici* on wheat leaves are met with cell wall appositions and reactive oxygen species production. Inoculated leaves were subjected to 3,3-diaminobenzidine (DAB) uptake treatment overnight (Thordal-Christensen *et al.* 1997) and examined under a light microscope. The dark DAB staining indicates the presence of H_2O_2. c, condium; agt, apressorial germ tube; CWA, cell wall apposition; pp, penetration peg.

and regulatory elements), cell wall modification (e.g. enzymes involved in synthesis of cell wall polysaccharides and phenolic derivatives, and protein and lipid alterations), and generation and scavenging of ROS (e.g. NADPH oxidase, germin-like proteins, xanthine oxidases, ascorbate/glutathione cycle enzymes, superoxide dismutases). These ESTs provide seemingly endless angles from which to tackle the CWA phenomenon, and a major challenge now is to identify key components that regulate major steps leading up to CWA formation.

Challenges and future directions

Although CWAs are presumed to stop fungal penetration by reinforcing the cell wall at the point of attack, a recent paradox has emerged suggesting that there is more to CWAs than was first supposed. Two independent reports have shown that *Arabidopsis* plants with mutated callose synthase genes are in fact more resistant to attack by some pathogens (Jacobs *et al*. 2003; Nishimura *et al*. 2003). Nishimura *et al*. (2003) found that while callose synthase mutants were resistant to powdery mildew, double mutants with a blocked salicylic acid (SA) pathway had restored susceptibility, suggesting that callose could be involved in repressing other defence pathways, and an absence of callose allows for an enhanced SA response. Therefore, successful pathogenic fungi might have adapted to the plant surveillance system of cell wall integrity to establish their parasitism.

Recent studies have suggested that bacterial flagella are recognized by plants and induce cell wall-mediated defences, which can then be suppressed by secreted bacterial effectors (DebRoy *et al*. 2004; Hauck *et al*. 2003). The discovery of a common fungal signature that is able to induce CWA formation in the absence of a pathogen could be central to understanding early events in the CWA formation signal. Another long-standing question has been the origin of the ROS produced during plant responses to pathogen attack. Apparent differences between monocot and dicot systems (e.g. Hückelhoven and Kogel 1998 vs. Torres *et al*. 2002) have compounded this problem, and in monocot systems no clear ROS generating system has been defined. Significantly, recent work in our lab suggests that in cereals the oxidative burst may be generated primarily by redox active metals rather than by enzymatic means (Wei *et al*., unpublished). Although major strides have been made in understanding aspects of vesicle trafficking (Collins *et al*. 2003), the further identification of components involved in CWA-destined vesicle formation, fusion and exocytosis will surely provide important insights into the CWA formation process as a whole. Finally, although this review has focused primarily on basic research concerning CWA-mediated resistance, the barley *mlo* example illustrates the importance of this ancient form of resistance in combating 'real world' agricultural problems. The identification and eventual deployment of CWA regulators in other crops could provide durable broad spectrum resistance for years to come.

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