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The plant cell wall as a site for molecular contacts in fungal pathogenesis☆

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ABSTRACT

The plant cell wall, the most external layer of the plant surface, is the site where most pathogenic fungi first make contact with host cells. A plant–fungus interaction therefore commences at the interface between the plant and the spore. Our current research focusing on the plant cell wall has discovered an extracellular ecto-nucleoside triphosphate diphosphohydrolase (ecto-NTPDase/apyrase; EC3.6.1.15) as a key player in plant defense before the onset of PTI (PAMP-triggered immunity). This review focuses on our recent findings, especially the role of the plant cell wall in the extracellular defense against fungi as well as fungal strategies resulting in successful infection.

1. Introduction

Establishment of basic compatibility in plant–microbe interactions can be recognized as the result of a sophisticated process, in which an adapted pathogen(s) gained the ability to prevent preformed and/or induced defense mechanisms in a given plant species [1]. This form of resistance is referred to as non-host resistance that ensures plant protection from potential pathogens, and is currently known to be mounted upon perception of pathogen-associated molecular patterns (PAMPs) by cell surface pattern recognition receptors [2]. Therefore, successful infection and subsequent colonization by an adapted pathogen(s) can be achieved by suppression or circumvention of PAMP-triggered immunity (PTI) and/or by reprogramming of host cell physiology by secreted pathogen-derived effectors before or during invasion of a host cell [3].

In *Mycosphaerella pinodes* (Berk and Blox) Vestergren, which causes leaf spots (Mycosphaerella blight) on pea, two structurally-related glycopeptides named Supprescins A and B are secreted into the pycnospore germination fluid (Fig. 1a) [4,5]. The common moiety consisting of the *O*-glycosyl portion attached to the serine residue (GalNAc-Ser-Ser-Gly) is thought to be the important determinant for suppressing host defense (Fig. 1a) [5]. Pure suppprescins can inhibit the proton-pumping activity of the host plasma membrane ATPase [6–9] and the related signal transduction pathway [10–12], temporarily reducing the ability of the host cell

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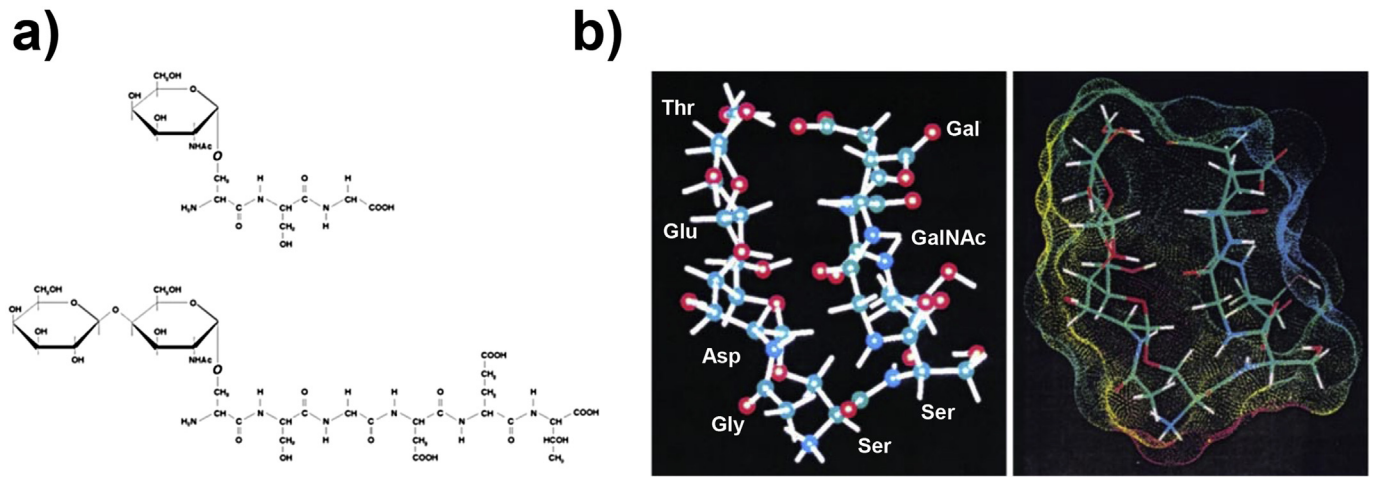

Fig. 1. Chemical structures of Supprescins A and B from *Mycosophaerella pinodes*. (**a**) Chemical structures of Suppprescin A (*upper*) and B (*lower*). The common moiety consisting of the *O*-glycosyl portion attached to the serine (GalNAc-Ser-Ser-Gly) is proposed to be essential for suppressor activity. (**b**) A predicted structure (*left*) of Suppprescin B, a major suppressor of *M. pinodes*, and the distribution of charge around this molecule (*right*).

to defend itself. In fact, suppprescin treatment renders host cells susceptible even to unrelated (non-pathogenic) pathogens [13]. Interestingly, Suppprescins A and B act as common elicitors when applied to non-host plants other than pea [14–17], indicating that suppressor production is essential for conditioning susceptibility of host cells.

In contrast to current progress in understanding disease resistance, little is known about the molecular mechanisms by which pathogens avoid or overcome host defense. However, our research focusing on the plant cell wall has revealed that it provides an additional layer of defense independent of PTI. In this article, several features of the plant cell wall as the outermost plant layer will be reviewed, especially in relation to the fungal strategy that suppresses cell surface immunity.

2. Mycosphaerella suppprescins: host-specific determinants for plant disease

M. pinodes is a hemibiotrophic pathogenic fungus causing leaf spots on pea. This disease is usually mediated by asexual spores (pycnospores), which normally germinate to form appressoria that directly penetrate into host cuticles, eventually forming infection hyphae to get nutrients from the host cells. However, at the early stage the hyphae grow only inside the host cell walls without killing epidermal cells [18]. At the late stage of infection, the hyphae grow entirely around mesophyll cells in pea [18] and the model host *Medicago truncatula* [19], eventually causing severe disease symptoms. In regard to the pathogenicity factor(s) produced by this fungus, we found that this fungus secrets both elicitors and suppressors of plant defenses into the germination fluid before actual penetration of the host tissue [4].

In 1992, Shiraishi and colleagues isolated and determined the chemical structures of two suppressor molecules, called Suppprescins A and B (Fig. 1a) [5]. Both are small mucin-type glycopeptides containing *N*-acetylgalactosamine attached to the serine residue in the peptide moiety. Fig. 1b shows the predicted structure of a major suppressor of *M. pinodes*, Suppprescin B, and the distribution of charge around this molecule. Interestingly, Suppprescin B exhibits a V-shaped structure with a strong positive charge, which would easily allow targeting of host protein(s) [4,5]. The suppprescins from *M. pinodes* inhibit elicitor-induced accumulation of mRNA encoding phenylalanine ammonia lyase (PAL), a key enzyme in the biosynthetic pathway for synthesis of the phytoalexin pisatin [20]. *PAL* mRNA is quickly induced in pea epicotyls within 1 h after elicitor treatment, whereas the concomitant presence of suppressor with elicitor results in a delay of at least 3 h in *PAL* mRNA accumulation. A similar result was also obtained with the model host *M. truncatula* [21]. Similarly, the elicitor induces *PAL*, *chalcone synthase* (*CHS*) and *isoflavone reductase* (*IFR*) mRNAs, followed by accumulation of a major phytoalexin of *M. truncatula*, medicarpin, whereas the suppressor significantly inhibits or delays the phytoalexin response. Consequently, a non-adapted *Mycosphaerella ligulicola* (a pathogen of chrysanthemum) could infect and cause severe disease symptoms when the spores were mixed with the suppressor from *M. pinodes* and then inoculated on *M. truncatula* leaves [21]. Other known components (targets) and cellular events associated with defense suppression in pea and *M. truncatula* are summarized in Table 1.

3. Signaling to promote disease susceptibility

In order to define the molecular mechanism(s) by which pathogens avoid host disease resistance, we carried out suppression subtractive hybridization (SSH) analysis to identify suppressor-responsive genes, using the model host *M. truncatula* [39]. The SSH analysis revealed that the suppressor induces transcriptional reprograming within 1 h after treatment [39]. On the basis of this result, it is possible that the suppressor *not only* interferes with expression of defense-related genes, *but also* alters the physiology of host cells through the newly activated genes. Actually, two genes encoding 13-lipoxygenase (LOX) and a peroxisome-localized multifunctional protein, MFP, which are presumably involved in jasmonic acid (JA) synthesis, are rapidly and transiently induced in *M. truncatula* leaves in response to the suppressor [39]. *MFP* encodes an enzyme that functions in the β-oxidation of fatty acids. Further detailed analyses showed that the suppressor coordinately induces accumulation of almost all mRNAs encoding enzymes involved in JA synthesis [39]. These results, consistent with our previous data that the suppressor induces *12-oxophytodienoic acid reductase* (*OPR*) in pea, which is likely involved in JA synthesis [36–38], indicate a possibility that JA-mediated process(es) are closely related to promoting susceptibility to *M. pinodes*. In fact, application of exogenous JA to pea epicotyls interfered with the elicitor-induced accumulation of *PAL* mRNA [36].