Fungal sex receptors recalibrated to detect host plants

Yasin F. Dagdas[1] and Tolga O. Bozkurt[2,*]

[1]The Sainsbury Laboratory, Norwich Research Park, Norwich, UK
[2]Department of Life Sciences, Imperial College London, London, UK

*Correspondence: o.bozkurt@imperial.ac.uk

Summary: Secreted peroxidases are well-known components of damage-induced defense responses in plants. A recent study (Turrà et al., 2015) has revealed that these enzymes can inadvertently serve as reporters of wounded sites and constitute an 'Achilles heel', allowing adapted pathogens to track and enter host tissue.

The rhizosphere—the soil-root interface—constitutes one of the most complex terrestrial ecosystems (Bais et al., 2006). Roots associate with a wide array of bacteria and fungi, including mutualistic mycorrhizal fungi, endophytes and parasites. In the fungal realm, mycorrhizal fungi provide plants with mineral nutrients and increase their chances of survival under nutrient limiting environments (Bais et al., 2006). In contrast, pathogenic fungi such as *Fusarium oxysporum* cause wilting in crops that leads to yield losses (De Coninck et al., 2015). Hence, the survival of plants within this complex ecosystem depends on their ability to distinguish beneficial fungi from the parasites. Similarly, fungal survival also relies on identification of nearby suitable host plants, and an ability to overcome host defenses.

The microbial composition of the rhizosphere is influenced by the chemical environment established by plant roots. Plants deploy chemical attractants and repellents as cues for establishing beneficial relationships with microorganisms or keeping the parasites at bay (Bonfante and Genre, 2015; De Coninck et al., 2015). For example, plants secrete hormones known as strigolactones that attract beneficial mycorrhizal fungi to establish symbiosis. However, it is as yet unclear how mycorrhizal fungi perceive chemicals, such as strigolactones, that trigger fungal hyphal growth and facilitate root colonization (Bonfante and Genre, 2015).

Similarly, to combat pathogenic fungi, plants have evolved a complex underground warfare system. Root exudates contain a wide range of chemicals with broad-spectrum antimicrobial activities such as phytoalexins, phenolic compounds, and defense-related proteins (Bais et al., 2006). One such class of these proteins is a family of secreted, haem-containing class III peroxidases, which are conserved in all land plants. These enzymes catalyze the reduction of hydrogen peroxide to produce reactive oxygen species (ROS), the accumulation of which creates a toxic environment for the invading pathogens, for instance by causing DNA damage (Shigeto and Tsutsumi, 2015). Interestingly, a recent study by Turra *et al.* (2015) showed that *F. oxysporum* hijacks the catalytic activity of

these enzymes to detect and migrate towards nearby tomato roots. Upon sensing peroxidase activity, the fungus reorients its hyphae to attach to the tomato roots and subsequently colonize the plant. Using quantitative chemotropism assays, the authors elegantly showed that *F. oxysporum* displays a positive chemotropic growth towards root exudates that contain catalytically active class III peroxidases (Figure 1a, Turrà et al., 2015).

Similar to other eukaryotic organisms, fungi perceive environmental stimuli via surface receptors and integrate and transmit these signals through downstream mitogen-activated protein kinase (MAPK) signaling cascades (Rispail et al., 2009). For pathogenic fungi, the ability to perceive favorable hosts is crucial for parasitism. Hence, surface receptors and downstream signaling cascades are evolutionary customized to detect nearby host plants. To identify the signaling cascade responsible for peroxidase-triggered host perception, Turra *et al.* (2015) studied *F. oxysporum* mutants that lack conserved fungal MAP kinases. Functional characterization of these mutants revealed that *F. oxysporum* has different MAPK cascades specialized to detect nutrients versus peroxidase-elicited signals. MAPK mutants that are defective in the cell-wall-integrity pathway were defective in peroxidase-mediated chemotropism, whereas mutants defective in mating pathway were impaired in nutrient sensing. Strikingly, cell integrity pathway mutants were also impaired in pheromone response and moreover, pheromone receptor mutants were unable to reorient their hyphae towards tomato roots (Turrà et al., 2015). Since there is no known sexual stage in the life cycle of *F. oxysporum*, the existence of genes encoding putative pheromone precursors and their corresponding receptors in the *F. oxysporum* genome has been puzzling (Ma et al., 2010). However, the new study has now revealed that pheromone receptors and mating response pathway appear to have been neo-functionalized to detect favorable host roots in *F. oxysporum* (Figure 1b, Turrà et al., 2015). Nevertheless, it remains unclear whether these signals are directly or indirectly perceived by the pheromone receptor.

F. oxysporum, like several other pathogenic fungi, enters host tissue through the wounds in the root. Wounded plant tissue produce ROS within minutes after tissue damage. It is well known that the enhanced peroxidase activity at these wounded sites contributes to increased toxicity against invading pathogens (Shigeto and Tsutsumi, 2015). Thus, it was unexpected that apart from these defense related activities, secreted class III peroxidases can also inadvertently serve as reporters of wounded sites for adapted pathogens to track and enter host tissue (Figure 1a, Turrà et al., 2015). Whether this concept can be generalized remains to be determined. It would also be interesting to test whether other pathogenic fungi also rely on peroxidase activity to track host plants.

A number of interesting questions remain unanswered. How does *F. oxysporum* cope with the antimicrobial activities of these peroxidases? Is there a specific, fungal tolerance mechanism that enables *F. oxysporum* to survive in this toxic environment? Although there are no reports of such a system in root-infecting fungi, foliar pathogens have evolved diverse strategies to resist and suppress the

deadly host milieu. A common strategy deployed by pathogens is the secretion of molecules, enzymes and proteins known as effectors that serve to alter host processes, subvert host defenses and contribute to virulence (Hogenhout et al., 2009). For instance, a conserved glutathione peroxidase of the rice blast fungus *Magnaporthe oryzae* scavenges and degrades the plant generated ROS. Fungal mutants that lack this enzyme were shown to be more sensitive to exogenous ROS and were impaired in virulence (Huang et al., 2011). These enzymes are highly conserved in filamentous fungi and thus it will be interesting to determine if *F. oxysporum* harbors a similar detoxification system. Another detoxifying mechanism that is more specialized to peroxidase activity has evolved in the smut fungus *Ustilago maydis*. An effector protein secreted by *U. maydis* called Pep1 was shown to inhibit the activity of class III peroxidases and suppress the oxidative burst response during the initial immune response. Consistent with the presence of secreted peroxidases in various different plants, Pep1 appears to be conserved across all biotrophic smut fungi (Hemetsberger et al., 2015). Currently, there are no effectors shown to target peroxidases in soil-borne pathogens. Since horseradish peroxidases (HRP) are also inhibited by Pep1 (Hemetsberger et al., 2015) and induce a positive chemotropic response in *F. oxysporum* (Turrà et al., 2015), it would be easy to screen for effectors that target these peroxidases.

As a final note, dissecting the composition of the rhizosphere and how it functions is critical for sustainable intensification of agricultural yields. The new paper by Turra *et al.* (2015) also opens up potential for engineering disease resistance in plants against soil-borne pathogens, for instance by altering peroxidase activity through genetic engineering. This, and similar approaches could potentially reduce yield losses due to soil-borne pathogens.

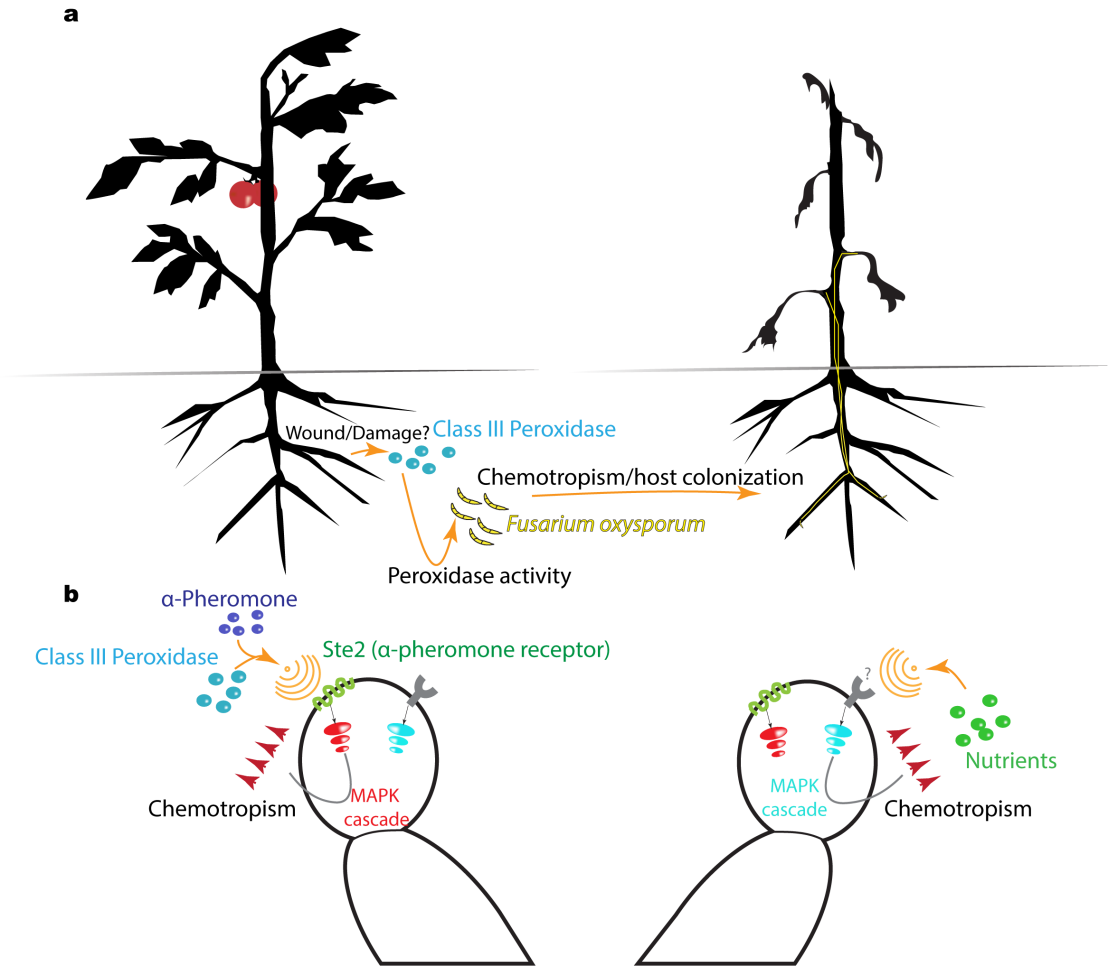


Figure 1. (a) *F. oxysporum* spores can sense and track host peroxidase activity. Damaged plant roots release peroxidases to soil. *F. oxysporum* re-polarizes its hyphae towards secreted peroxidases and subsequently enters through wounded root tissue and colonizes host plants. **(b) Fungal pheromone receptor Ste2 is essential for peroxidase-mediated hyphal chemotropism.** Ste2 and cell integrity MAPK pathway are required for recognition of peroxidase activity, whereas a different MAPK pathway regulates nutrient-mediated chemotropism.

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