Published in final edited form as:

Sci Signal. ; 7(320): pe10. doi:10.1126/scisignal.2005273.

ROS Open Roads to Roundworm Infection

Baomin Feng¹ and Libo Shan^{2,*}

¹Department of Biochemistry and Biophysics and Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843, USA

²Department of Plant Pathology and Microbiology and Institute for Plant Genomics and Biotechnology, Texas A&M University, College Station, TX 77843, USA

Abstract

The rapid production of reactive oxygen species (ROS) upon pathogen attack is generally considered a defense mechanism for microbial killing and an initiation of host defense responses in plants and animals. In this issue, Siddique *et al.* show that nicotinamide adenine dinucleotide phosphate oxidase-derived ROS function as a pathogenicity factor to promote the roundworm nematode infection in *Arabidopsis thaliana*, revealing the complex action of ROS in host-pathogen interactions.

The amount of reactive oxygen species (ROS), consisting mainly of the superoxide anion and its dismutation product hydrogen peroxide, increases rapidly in response to developmental cues and biotic (for example, pathogen attack) and abiotic (for example, heat exposure) stimuli in plants and animals. The massive increase of ROS production can damage cells, yet on the other hand, ROS can serve as signaling molecules to orchestrate cellular events essential for cell growth, development, and stress responses (1, 2). The production of ROS in the apoplasts is one of the first measurable events ubiquitously observed in various plants' responses to pathogen attack (3). Plants evolved two detection systems to distinguish pathogens: (i) cell surface receptor-like kinases, which recognize evolutionarily conserved microbe-associated molecular patterns (MAMPs) for pattern-triggered immunity (PTI), and (ii) intracellular nucleotide-binding domain leucine-rich repeat proteins for effector-triggered immunity (ETI) (4). The magnitude and the amplitude of ROS production vary depending on the plant-pathogen interaction. A biphasic, sustained apoplastic ROS accumulation occurs during ETI responses, and a monophasic, transient ROS accumulation occurs during PTI responses (3).

Pathogen-stimulated ROS are primarily catalyzed by the plasma membrane-resident nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and cell wall-bound peroxidases (3, 5). NADPH oxidase is a multisubunit enzymatic complex in mammalian neutrophils mediating microbial killing. Homologs of the NADPH oxidase catalytic subunit gp91^{phox} are present in various plants and known as respiratory burst oxidase homologs (RBOHs) with 10 family members in *Arabidopsis thaliana* (6). Genetic analyses of *rboh*

mutants have revealed the essential roles of ROS and RBOHs in different plant pathosystems. In general, ROS production positively correlates with plant immunity by directly killing pathogens, strengthening cell walls, and activating defense genes (3). For example, *Arabidopsis rbohD/F* double mutants (*atrbohD/F*), which fail to produce pathogen-induced ROS, display compromised hypersensitive response (HR), a hallmark of plant ETI, in response to hemibiotrophic bacteria (6). In contrast, certain necrotrophic pathogens stimulate ROS production to induce cell death for the benefit of subsequent infection (7). In this issue, Siddique *et al.* report that NADPH oxidase-derived ROS function as a pathogenicity factor to facilitate infection of *Arabidopsis* by nematode, a biotrophic pathogen that establishes a long-term feeding relationship with host plants (8).

The cyst nematode *Heterodera schachtii* is a small plant-parasitic roundworm with unique and deliberated infection processes (Fig. 1). The juvenile nematodes invade plant roots and migrate toward the vascular cylinder. Once reaching the vascular cylinder, the worms start to search for the cell resisting collapse by subtle stylet piercing until an appropriate cell is found to serve as an initial syncytial cell (ISC). Subsequently, secretions released through the stylet into the ISC trigger the formation of feeding structures called syncytia. Syncytia, consisting of more than 200 multinucleate and metabolically active cells, serve as the sole nutrient source for nematodes and are essential for successful infection.

Siddique *et al.* performed a comprehensive analysis of nematode infection processes on *Arabidopsis rboh* mutants and found that the number of successful nematode infections was impaired in the *atrbohD* single mutant and *atrbohD/F* double mutant compared with that in wild-type (WT) plants. RBOHs were not required for the nematode invasion to the root. Rather, the *atrbohD/F* mutant showed impaired nematode migration rate toward vascular cylinder, ISC selection, and syncytial establishment, suggesting that ROS generated by RBOHs positively regulate these infection processes and promote nematode growth (8) (Fig. 1). ROS production was detected during the early stages of nematode infection, which largely depended on RBOHD (8), although the nematode-derived elicitors (MAMP or effectors) remain unknown. How then do ROS promote nematode parasitism? Cell death adjacent to the nematode infection site was enhanced in the *atrbohD/F* mutant, suggesting that nematode-stimulated ROS limited the activation of plant defense responses (8). This finding was unexpected because cell death, such as that which occurs during HR, is associated with ETI and prevents the spread of pathogen infection in plant-pathogen biotrophic interactions. ROS are required for HR-type cell death in plants' responses to an avirulent bacterium, *Pseudomonas syringae*, and an oomycete, *Phytophthora infestans* (6, 9). By contrast, the *atrbohF* mutant displays enhanced HR to an oomycete *Hyaloperonospora parasitica*. Notably, RBOHF plays a minor role in pathogen-induced ROS production, and *atrbohF* mutant plants are slightly growth retarded (6). Thus, plant NADPH oxidases have opposite effects on cell death depending on the biological context. Future studies are required to characterize the nature of nematode-induced cell death and the mechanism of its suppression by ROS. It is tempting to speculate that a plant resistance protein recognizes an effector protein secreted through the orifice of nematode and activates HR-type cell death. It is also possible that damage of root cells along the path of nematode migration releases unknown signals, which promote cell death.

Understanding the functions of ROS in plant defense is further complicated by their interaction with other defense signals, such as salicylic acid (SA) (3). Evidence suggests both synergistic and antagonistic actions between ROS and SA in plant defense (3). ROS inhibits unrestricted cell death in the lesion mimic mutant *lsd1*, in which SA promotes cell death (10). Siddique *et al.* addressed the relationship of ROS and SA in the context of nematode infection. By using a mutant deficient in SA biosynthesis (*sid2*) crossed to *atrbohD/F* mutant plants, they concluded that the initial failure of establishing ISC was independent of SA, whereas the retarded growth of female nematodes was SA-dependent. Thus, SA-mediated defense was required for plants to limit nematode infection, but the cell death controlled by ROS did not require SA (8).

Compared with WT plants, nematodes in *atrbohD/F* plants had defective ISC selection and reduced size of syncytia (8). It remains unknown whether ROS play a role in promoting differentiation of syncytia in addition to suppressing cell death. Syncytia consist of greater than 200 cells, only one end of which is attached to the nematode orifice. After the ISC is established, how the signal of differentiation spreads to distal cells remains unknown. ROS play critical roles as signaling molecules in plant cell expansion, including growth of root hair and pollen tube tips (11). It will be interesting to examine whether and how ROS contribute to cell differentiation during syncytial formation upon nematode infection.

The apparent paradox is that NADPH oxidase-derived ROS serve as a double-edged sword and exert opposite effects on cell death and disease susceptibility in different plant-pathogen interactions. Nevertheless plant RBOH-derived ROS seem to open roads to the roundworm nematode infection and act as a key pathogenicity factor to promote nematode parasitism and feeding site development.

Acknowledgments

The work in the L.S. laboratory was supported by NIH (1R01GM097247).

References and Notes

1. Marino D, Dunand C, Puppo A, Pauly N. A burst of plant NADPH oxidases. *Trends Plant Sci.* 2012; 17:9–15. [PubMed: 22037416]
2. Suzuki N, Miller G, Morales J, Shulaev V, Torres MA, Mittler R. Respiratory burst oxidases: The engines of ROS signaling. *Curr Opin Plant Biol.* 2011; 14:691–699. [PubMed: 21862390]
3. Torres MA, Jones JD, Dangl JL. Reactive oxygen species signaling in response to pathogens. *Plant Physiol.* 2006; 141:373–378. [PubMed: 16760490]
4. Jones JD, Dangl JL. The plant immune system. *Nature.* 2006; 444:323–329. [PubMed: 17108957]
5. Daudi A, Cheng Z, O'Brien JA, Mammarella N, Khan S, Ausubel FM, Bolwell GP. The apoplastic oxidative burst peroxidase in *Arabidopsis* is a major component of pattern-triggered immunity. *Plant Cell.* 2012; 24:275–287. [PubMed: 22247251]
6. Torres MA, Dangl JL, Jones JD. *Arabidopsis* gp91^{phox} homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proc Natl Acad Sci USA.* 2002; 99:517–522. [PubMed: 11756663]
7. Govrin EM, Levine A. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Curr Biol.* 2000; 10:751–757. [PubMed: 10898976]
8. Siddique S, Matera C, Radakovic ZS, Hasan MS, Gutbrod P, Rozanska E, Sobczak M, Torres MA, Grundler FMW. Parasitic worms stimulate host NADPH oxidases to produce reactive oxygen

- species that limit plant cell death and promote infection. *Sci Signal*. 2014; 7:ra33. [PubMed: 24714570]
9. Yoshioka H, Numata N, Nakajima K, Katou S, Kawakita K, Rowland O, Jones JD, Doke N. *Nicotiana benthamiana* gp91^{phox} homologs *NbrbohA* and *NbrbohB* participate in H₂O₂ accumulation and resistance to *Phytophthora infestans*. *Plant Cell*. 2003; 15:706–718. [PubMed: 12615943]
10. Torres MA, Jones JD, Dangl JL. Pathogen- induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in *Arabidopsis thaliana*. *Nat Genet*. 2005; 37:1130–1134. [PubMed: 16170317]
11. Takeda S, Gapper C, Kaya H, Bell E, Kuchitsu K, Dolan L. Local positive feedback regulation determines cell shape in root hair cells. *Science*. 2008; 319:1241–1244. [PubMed: 18309082]

Fig. 1. ROS promote nematode infection on *Arabidopsis*

Illustration of the early processes during nematode infection of wild-type (susceptible) and *atrbohD/F* mutant (resistant) roots. Nematodes invade (i) root tips and migrate (ii) toward vascular cylinder (dark blue). By subtle stylet piercing, nematodes select an ISC (pink) (iii). This process is defective in *atrbohD/F* plants, where the absence of ROS in ROS-producing cells (dotted yellow) causes the SA-independent expansion of cell death (brown). Syncytia formation (iv) and the growth of nematodes are defective in *atrbohD/F* plants in a SA-dependent manner.