Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development

Miguel Angel Torres[1] and Jeffery L Dangl[1,2]

The production of reactive oxygen intermediates (ROI) is among the earliest temporal events following pathogen recognition in plants. Initially, ROI were thought to be cell-death executioners. Emerging evidence, however, suggests a broader role for ROI as signals that mediate responses to infection, the abiotic environment, developmental cues, and programmed cell death in different cell types. The *Respiratory burst oxidase homolog* (*Rboh*) gene family encodes the key enzymatic subunit of the plant NADPH oxidase. Rboh proteins are the source of ROI produced following pathogen recognition and in a variety of other processes.

Addresses
[1] Department of Biology, CB 3280, 108 Coker Hall, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA
[2] Department of Microbiology and Immunology and Curriculum in Genetics and Molecular Biology, Carolina Center for Genome Sciences, CB 3280, 108 Coker Hall, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA

Corresponding author: Torres, Miguel Angel (mtorres@email.unc.edu)

Current Opinion in Plant Biology 2005, **8**:397–403

This review comes from a themed issue on
Biotic interactions
Edited by Paul Schulze-Lefert and Edward Farmer

Available online 6th June 2005

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2005.05.014

Introduction

The bi-phasic production of apoplastic reactive oxygen intermediates (ROI), the so-called 'oxidative burst', is a hallmark of successful recognition of plant pathogens [1,2,3•]. Since Doke [4] first reported the generation of superoxide anions (O_2^-) during incompatible (plant resistant) interactions between potato and the late blight pathogen *Phytophthora infestans*, O_2^- and/or hydrogen peroxide (H_2O_2), its dismutation product, have repeatedly been detected in successful resistance responses [2,5]. Yet, the diverse functions of ROI, and the mechanism that regulates the initial generation of superoxide were elusive until recently.

ROI can be produced inside the plant cell, in chloroplasts, mitochondria and peroxisomes, as byproducts of metabolic processes such as photosynthesis and respiration [6••]. Several biochemical mechanisms have been proposed to explain ROI production. Apoplastic H_2O_2 production can be mediated by cell-wall peroxidases, germin-like oxalate oxidases or amino oxidases [6••,7••]. However, key lines of evidence implicated an NADPH oxidase, analogous to that which generates superoxide during the respiratory burst in mammalian phagocytes, as the source of the ROI detected in plants upon successful pathogen recognition. First, O_2^- is the primary radical produced in most systems [4,5,8]; second, the rate of ROI production per elicited cell is similar in mammals and plants [9]; and third, ROI generation is inhibited by diphenylene iodonium, a well-characterized inhibitor of the mammalian NADPH oxidase (and flavin-containing enzymes) [8,10].

The mammalian NADPH oxidase, also known as the respiratory burst oxidase (RBO), is a protein complex that is comprised of a membrane-bound NADPH-binding flavocytochrome b_{558} and cytosolic regulatory proteins. In phagocytes, these regulatory proteins include the $p47^{phox}$, $p67^{phox}$ and $p40^{phox}$ phosphoproteins and Rac2, a small GTP-binding protein [11,12]. The membrane cytochrome consists of the glycosylated transmembrane protein $gp91^{phox}$ and the non-glycosylated $p22^{phox}$ subunit. $gp91^{phox}$ contains the entire electron transport chain from NADPH to molecular oxygen to produce superoxide outside the plasma membrane. The NADPH oxidase exists in vesicles that are activated via Rac dissociation, phosphorylation of $p47^{phox}$ and $p67^{phox}$ and their subsequent recruitment to the membrane-bound complex [11,12]. In humans, chronic granulomatous disease (CGD) results from mutations that render the NADPH oxidase in phagocytes non-functional, and CGD patients suffer recurrent and prolonged microbial infections [13]. Mutations in *gp91^{phox}* account for all cases of X-linked CGD [13]. It was initially thought that direct toxicity of the ROI that are generated by the oxidative burst was responsible for the microbial killing activity of mammalian phagocytes. Surprisingly, superoxide that is generated via NADPH oxidase activity plays a signaling role in microbial killing, leading to K^+ flux that activates specific proteases [14,15]. Additional NADPH oxidases, different from those in phagocytes, have been identified in mammals [11,12]. These exhibit tissue-specific expression and mediate functions from cell proliferation to thyroid hormone biosynthesis. Thus, multiple isoforms of $gp91^{phox}$ act in NADPH oxidases from different cell types, and perform different functions in mammalian cells.

The plant *Rboh*-encoded NADPH oxidase

The first plant NADPH oxidase gene to be identified was the rice gene *OsrbohA*, which is related to mammalian

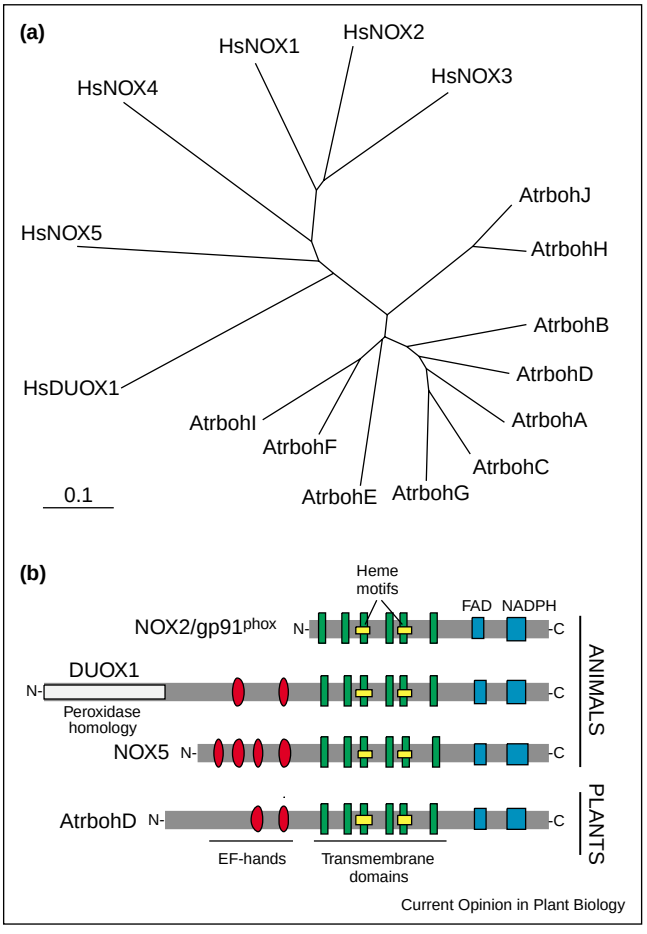
gp91^{phox} [16]. Subsequent studies documented different *Respiratory burst oxidase homolog* (*Rboh*) genes in other plant species, including *Arabidopsis*, tomato, tobacco and potato [17–19,20•,21,22]. Cell fractionation indicated that at least two Rboh proteins are located in the plasma membrane [18,22]. As in the mammalian NADPH oxidase, the rice homologs of human Rac2 can regulate ROI production and HR [23]. But simple comparisons of the regulation and assembly of NADPH oxidases between plants and animals end there. For example, no homologs of the mammalian p47^{phox} or p67^{phox}were found in the *Arabidopsis* genome [24]; and initial reports [25] that antibodies against the mammalian p47^{phox} and p67^{phox} proteins cross-reacted with plant proteins of similar size have been dismissed [26].

Plant species contain *Rboh* gene families that have homology to *gp91^{phox}*. *Arabidopsis* encodes ten *Atrboh* genes ([17,24]; Figure 1a). All plant *Rboh* genes carry a presumably cytosolic 300-amino-acid amino-terminal extension with two EF-hands that bind Ca^{2+} [18], which could account for the direct regulation of these oxidases by Ca^{2+}. This is relevant in several functional contexts in which Ca^{2+} signaling precedes ROI production. This domain is absent from the mammalian phagocyte gp91^{phox} but is present in other mammalian NADPH oxidase homologs (Figure 1b). For example, the mammalian NADPH oxidase 5 (NOX5) protein contains four EF-hands in its amino-terminal region and exhibits Ca^{2+}-dependent production of superoxide [27]. In contrast to the mammalian gp91^{phox}, plant Rboh proteins can produce O_2^- in the absence of additional cytosolic components, and are stimulated directly by Ca^{2+} [28]. Thus, a superoxide-generating NADPH oxidase exists in plants, although its precise subunit structure and the regulation of its activation are different than in mammalian phagocytes.

The Rboh-NADPH oxidase and biotic interactions

ROI were initially proposed to orchestrate the establishment of the plant defense response and the hypersensitive response (HR) cell death that often accompanies successful pathogen recognition [29,30]. Several groups have reported that *Rboh* genes are transcriptionally upregulated by pathogens or fungal elicitors [21,22,31]. Genetic proof of the function of *Rboh* in the pathogen-induced oxidative burst came from analysis of *Rboh* mutants and antisense lines. Infection of *Arabidopsis* mutants that lack functional *AtrbohD* and *AtrbohF* demonstrated that *AtrbohD* is responsible for nearly all of the ROI produced in response to avirulent bacterial or oomycete pathogens, whereas *AtrbohF* is important in the regulation of HR [32]. Antisense reduction of *NtrbohD* indicated that the Rboh encoded by this gene is responsible for the production of ROI after treatment of tobacco cells with the fungal elicitor cryptogein [22]. Silencing of

Figure 1



Relationship and domain structure of the NADPH oxidases.
(a) Phylogenetic tree comparing the mammalian NADPH oxidases with the *Arabidopsis* Atrboh proteins: AtrbohA (At5g07390), AtrbohB (At1g09090), AtrbohC (At5g51060), AtrbohD (At5g47910), AtrbohE (At1g19230), AtrbohF (At1g64060), AtrbohG (At4g25090), AtrbohH (At5g60010), AtrbohI (At4g11230), AtrbohJ (At3g45810). The rbohA described in [18] is identical to AtrbohF in [17,32] and is represented here as AtrbohF. Only the carboxy-terminal with homology to gp91^{phox}/NOX2 (excluding the EF hands) was used in the alignment.
(b) Schematic representation of the NADPH oxidase proteins with their functional domains. Mammalian NOX1, NOX3 and NOX4 are similar to gp91^{phox}/NOX2. The plant Rboh are similar to AtrbohD. FAD, flavin adenine dinucleotide, DUOX1, Dual oxidase1.

NbrbohA and *NbrbohB* in *Nicotiana benthamiana* plants led to less ROI production and reduced resistance to infection by *Phytophthora infestans* [20•]. There is also evidence of functional overlap between different Rboh proteins. For example, in *Arabidopsis*, the phenotypes of the individual *atrbohD* and *atrbohF* mutants are accentuated in the double mutant *atrbohD/atrbohF* [32,33••]. These data suggest a complex signaling network with interactions between different members of the *Rboh* family.

Interestingly, the downregulation or elimination of *Rboh* leads to variable effects on pathogen growth and HR. For

example, *Nbrboh*-silenced plants are more susceptible to normally avirulent *P. infestans* and HR is suppressed in these plants [20•]. By contrast, the *Arabidopsis atrbohF* mutant is more resistant to a weakly virulent strain of the oomycete *Peronospora parasitica*, and actually expresses enhanced HR [32]. Thus, although the Rboh proteins are required for pathogen-induced ROI production, these ROI might serve different signaling functions in disease resistance and HR.

Interactions with other plant defense regulators might account for the divergent outcomes of NADPH-dependent ROI signaling. Exogenously applied ROI can act synergistically in a signal-amplifying loop with salicylic acid (SA) to drive HR [34,35]. However, our recent studies indicate that ROI that are produced via AtrbohD actually antagonize SA to stop the spread of cell death beyond the site of HR (MA Torres, JDG Jones, JL Dangl, unpublished results). Also, coordinated levels of ROI and nitric oxide (NO) are required to produce HR [36], and both can mediate abscisic acid (ABA)-induced stomatal closure [37•]. These surprising results underscore how ROI that are produced via Atrboh proteins can mediate different functions in different contexts, and suggest that we have some way to go to explain the function of ROI in response to infection.

Characterization of plant Rac2 homologs (called Rop for Rho-like proteins [38•]) also tied plant NADPH oxidases to ROI production during defense signaling and cell death [23,39•]. These analyses also suggested that the combination of specific Rac isoforms with specific Rboh isoforms might have differential regulatory effects. For example, *Osrac1* is a positive regulator of ROI production and cell death [40], whereas *Ntrac5* acts as a negative regulator of *NtrbohD*-dependent ROI production after elicitation with cryptogein [41•]. Rac/Rop signaling is also involved in oxygen deprivation, potentially via an NADPH oxidase, and RopGAP4 acts as a rheostat to control ROI production in this context [42]. Comparisons among these datasets are complicated by the use of different plant species to study combinations of *Rac* and *Rboh* family members. These disparate datasets highlight the necessity of careful analysis of the functions of all *Rboh* family members in a single model system and in a variety of developmental and environmental contexts.

The Rboh-NADPH oxidase in abiotic stress and development

ROI that are generated by plant Rboh-NADPH oxidases also regulate abiotic stress responses and development. ROI function as intermediates in ABA signaling during stomatal closure [43]. H_2O_2 induces the activation of Ca^{2+} channels to mediate the increase in cytosolic Ca^{2+} concentration in intact guard cells [44]. *AtrbohD* and *AtrbohF* are expressed in guard cells and are transcriptionally induced in response to ABA. The *Arabidopsis atrbohD/atrbohF* double mutant is impaired in ABA activation of Ca^{2+} channels and stomatal closure, whereas exogenous application of H_2O_2 restored Ca^{2+} channel activation and partial stomatal closure in *atrbohD/atrbohF* [33••]. This study provided genetic evidence that Atrboh proteins mediate ABA signaling in stomata, and suggested that the same Atrboh isoforms might mediate different ROI-dependent functions in different cellular contexts.

In this light, it is important to note that a third family member, AtrbohC regulates cell expansion during root-hair formation. Analysis of the *atrbohC* mutant (also called *root hair defective 2* [*rhd2*]) revealed that ROI that are produced by AtrbohC activate hyper-polarization Ca^{2+} channels that are responsible for localized cell expansion during the root-hair formation [45••]. In this case, the very reactive hydroxyl radical (OH^-), which is generated from H_2O_2 in the presence of transition metals (such as Cu^{2+} or Fe^{2+}), is the specific ROI implicated.

Thus, ROI that are generated via the NADPH oxidase and the activation of Ca^{2+} channels might represent a signaling link that is common to many plant responses. In the defense response, the oxidative burst has also been implicated in activating Ca^{2+} influx [46]. Yet, Ca^{2+} is also required for ROI production both after pathogen infection and following ABA treatment [47,48,49•], and Ca^{2+} can activate an Rboh protein *in vitro* [28]. Therefore, Ca^{2+} fluxes appear to function both upstream and downstream of ROI production, indicating a complex spatio-temporal Ca^{2+} regulation of these signaling networks [46]. Fungal elicitors can induce both ROI and, through the activation of Ca^{2+} channels in guard cells, stomatal closure [50]. Thus, the NADPH-oxidase-dependent activation of Ca^{2+} channels might represent a cross-talk point between ABA and defense signaling.

Phosphorylation might also play important roles both upstream and downstream of Rboh function (see [6••] for review). Yoshioka *et al.* [20•] reported that MAPK-induced cell death might be mediated by ROI produced by NbrbohB, and that the same MAPK cascade acts to increase NbrbohB levels. Particularly relevant in this regard is the identification of Oxidative signal inducible 1 (OXI1)/AGC2-1 (AGC2-1 for protein related to cAMP-dependent and Cgmp-dependent protein kinases, and protein kinase C 2-1), a kinase involved in phospholipid signaling that is upregulated by stimuli that produce ROI [51••,52••]. Interestingly, the *oxi1* knockout displays alterations in root-hair formation, suggesting that the *OXI1* gene is linked to *AtrbohC* function.

ROI have been implicated in the regulation of cellular responses to stresses other than pathogen infection. For example, H_2O_2 acts as a second messenger for the induc-

tion of defense genes in response to systemin and jasmonate during wound responses [53]. Using anti-sense transgenics, Sagi *et al.* [54•] showed that ROI that are produced by an Rboh are required for the expression of certain wound-response genes. Reduced expression of *Rboh* additionally induced multiple pleiotropic developmental effects, resulting in the ectopic expression of flower-specific homeotic genes and an altered redox-related metabolism. These findings suggest that ROI that are produced by Rboh influence metabolic balances acting in several hormone signaling pathways. It is difficult to know, however, how many different isoforms of Rboh were affected in these anti-sense transgenics.

The response to ozone (O_3) also induces an oxidative burst that has similarities to the pathogen-induced oxidative burst [55•]. In *Arabidopsis*, ozone exposure drives both the spatial and the temporal progression of ROI signaling, commencing with the elevation of ROI levels in guard-cell chloroplasts and membranes, and spreading to neighboring cells [56••]. *AtrbohD* and *AtrbohF* have been implicated in the intercellular signaling and ultimate cell death that arises from O_3 exposure [56••]. Interestingly, heterotrimeric G proteins control this ROI production and sensitivity to O_3. Heterotrimeric G protein was also implicated in the regulation of ROI production through activation of extracellular calmodulin during stomatal closure [57••]. Also, heterotrimeric G protein functions upstream of *Osrac1* in the regulation of the defense response and cell death in rice [58]. Together, these studies suggest that heterotrimeric G proteins might be a common intermediate for ROI signaling in responses to different stimuli.

For ROI to exert a signaling function, the production and removal of ROI have to be tightly controlled and compartmentalized. In addition to the enzymatic production of ROI, chloroplasts (and also mitochondria and peroxisomes) can generate ROI during metabolic processes [6••]. Plant cells possess a battery of scavenging systems, including ascorbate peroxidases, glutathione, superoxide dismutases and catalases (see the excellent review by Mittler *et al.* [7••]), that maintain ROI homeostasis. The downregulation of scavenging systems in response to stress can lead to an oxidative burst and cell death that is phenotypically similar to HR [59•]. Davletova *et al.* [60••] showed that cytosolic ascorbate peroxidase APX1 provides cross-compartment protection to the chloroplast during light stress, and that *AtrbohD* might be required for an ROI-dependent signal that maintains high APX1 levels during light stress. Trienoic fatty acids from the chloroplast might mediate this activation [61•]. In tobacco, *NtrbohD* was upregulated in catalase-deficient plants in response to elevated levels of H_2O_2 that were produced under high light [59•], suggesting that ROI produced by the Rboh-NADPH oxidase also modulate the response to light stress.

Conclusions and future directions

Like mammals, plants have an *Rboh* gene family, and recent data suggest that specific *Rboh* genes function in different cellular contexts. The identification of the *Arabidopsis* RbohD-NADPH oxidase as the enzyme responsible for the production of apoplastic ROI in response to pathogens answered a question first posed 25 years ago [1,4]. The *Arabidopsis* Rboh protein family has a diversified functional portfolio. AtrbohC appears to have a very specific function in root-hair development, whereas AtrbohD and AtrbohF display pleiotropic functions following pathogen recognition and during ABA signaling. The accentuated phenotypes of the double mutant, compared to that of either single mutant, indicates functional overlap between AtrbohD and AtrbohF function, suggesting that interactions between different family members might help fine tune both ROI production and the response to that production. Further characterization of the other Rboh family members and the identification of knockout/knockdown lines for these genes should define additional functions for plant NADPH oxidases and the ROI that they produce.

Analyses of Rboh function suggest that ROI act in complex signaling networks that operate in responses to developmental cues or to the environment. Particularly relevant are the links between Rboh-dependent ROI production and Ca^{2+}, and the regulation of Rboh function by Rac/Rop (in common with mammalian $gp91^{phox}$) by heterotrimeric G proteins or by phosphorylation. Also, SA and NO are important defense-response regulators that interact with ROI signaling in response to pathogens. Thus, ROI that are produced by the Rboh-containing NADPH oxidase are part of many signaling pathways and provide a crucial link in the cross-talk to difference responses.

Plants have to cope with excess ROI produced during metabolism, and have evolved sophisticated ways to use the reactive properties of these ROI to modulate cellular signals by tightly regulating ROI production and compartmentalization. We are just beginning to dissect the functions of plant Rboh proteins and to understand the varied functions of the ROI they ultimately produce.

Acknowledgements

We thank Drs Kirk Overmyer and Darby G Brown for critical reading and Dr Ben F Holt III for assistance with the sequence alignments. This research was funded by grants from the National Institutes of Health (NIGMS R01-GM057171) and National Science Foundation (IBN-0077887) to JLD.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lamb C, Dixon RA: **The oxidative burst in plant disease resistance**. *Annu Rev Plant Physiol Plant Mol Biol* 1997, **48**:251-275.

2. Grant M, Brown I, Adams S, Knight M, Ainslie A, Mansfield J: **The *RPM1* plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death**. *Plant J* 2000, **23**:441-450.

3. • Nurnberger T, Brunner F, Kemmerling B, Piater L: **Innate immunity in plants and animals: striking similarities and obvious differences**. *Immunol Rev* 2004, **198**:249-266.
An up-to-date review about signaling in plant defense that includes a comparison with mammalian immunity.

4. Doke N: **Generation of superoxide anion by potato tuber protoplasts during the hypersensitive response to hyphal cell wall components of *Phytophthora infestans* and specific inhibition of the reaction by suppressors of hypersensitivity**. *Physiol Plant Pathol* 1983, **23**:359-367.

5. Auh C-K, Murphy TM: **Plasma membrane redox enzyme is involved in the synthesis of O_2^- and H_2O_2 by *Phytophthora* elicitor-stimulated rose cells**. *Plant Physiol* 1995, **107**:1241-1247.

6. •• Apel K, Hirt H: **Reactive oxygen species: metabolism, oxidative stress, and signal transduction**. *Annu Rev Plant Biol* 2004, **55**:373-399.
An exhaustive recent review about ROI metabolism and signal transduction.

7. •• Mittler R, Vanderauwera S, Gollery M, Van Breusegem F: **Reactive oxygen gene network of plants**. *Trends Plant Sci* 2004, **9**:490-498.
A comprehensive review about the elements that mediate the production and scavenging of ROI to maintain ROI homeostasis in the plant cell.

8. Jabs T, Colling C, Tschöpe M, Hahlbrock K, Scheel D: **Elicitor-stimulated ion fluxes and reactive oxygen species from the oxidative burst signal defense gene activation and phytoalexin synthesis in parsley**. *Proc Natl Acad Sci USA* 1997, **94**:4800-4805.

9. Low PS, Merida JR: **The oxidative burst in plant defense: function and signal transduction**. *Physiol Plant* 1996, **96**:533-542.

10. Murphy TM, Auh C-K: **The superoxide synthases of plasma membrane preparations from cultured rose cells**. *Plant Physiol* 1996, **110**:621-629.

11. Lambeth JD: **NOX enzymes and the biology of reactive oxygen**. *Nat Rev Immunol* 2004, **4**:181-189.

12. Cross AR, Segal AW: **The NADPH oxidase of professional phagocytes-prototype of the NOX electron transport chain systems**. *Biochim Biophys Acta* 2004, **1657**:1-22.

13. Roos D, Deboer M, Kuribayashi F, Meischl C, Weening RS, Segal AW, Ahlin A, Nemet K, Hossle JP, Bernatowska-Matuszkiewicz E *et al.*: **Mutations in the X-linked and autosomal recessive forms of chronic granulomatous disease**. *Blood* 1996, **87**:1663-1681.

14. Reeves EP, Lu H, Jacobs HL, Messina CG, Bolsover S, Gabella G, Potma EO, Warley A, Roes J, Segal AW: **Killing activity of neutrophils is mediated through activation of proteases by K^+ flux**. *Nature* 2002, **416**:291-297.

15. Reeves EP, Nagl M, Godovac-Zimmermann J, Segal AW: **Reassessment of the microbicidal activity of reactive oxygen species and hypochlorous acid with reference to the phagocytic vacuole of the neutrophil granulocyte**. *J Med Microbiol* 2003, **52**:643-651.

16. Groom QJ, Torres MA, Fordham-Skelton AP, Hammond-Kosack KE, Robinson NJ, Jones JDG: ***rbohA*, a rice homologue of the mammalian *gp91phox* respiratory burst oxidase gene**. *Plant J* 1996, **10**:515-522.

17. Torres MA, Onouchi H, Hamada S, Machida C, Hammond-Kosack KE, Jones JDG: **Six *Arabidopsis thaliana* homologues of the human respiratory burst oxidase (*gp91-phox*)**. *Plant J* 1998, **14**:365-373.

18. Keller T, Damude HG, Werner D, Doerner P, Dixon RA, Lamb C: **A plant homolog of the neutrophil NADPH oxidase $gp91^{phox}$ subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs**. *Plant Cell* 1998, **10**:255-266.

19. Amicucci E, Gaschler K, Ward JM: **NADPH oxidase genes from tomato (*Lycopersicon esculentum*) and curly-leaf pondweed (*Potamogeton crispus*)**. *Plant Biol* 1999, **1**:524-528.

20. • 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 H_2O_2 accumulation and resistance to *Phytophthora infestans***. *Plant Cell* 2003, **15**:706-718.
Using virus-induced gene silencing, the authors show that the *Nicotiana benthamiana* genes *NbrbohA* and *NbrbohB* are required for the accumulation of H_2O_2 and for resistance to the fungus *Phytophthora infestans*. This work also provides genetic evidence of the involvement of a MAPK cascade in the regulation of *Rboh* genes.

21. Yoshioka H, Sugie K, Park HJ, Maeda H, Tsuda N, Kawakita K, Doke N: **Induction of plant gp91 *phox* homolog by fungal cell wall, arachidonic acid, and salicylic acid in potato**. *Mol Plant Microbe Interact* 2001, **14**:725-736.

22. Simon-Plas F, Elmayan T, Blein J-P: **The plasma membrane oxidase NtrbohD is responsible for AOS production in elicited tobacco cells**. *Plant J* 2002, **31**:137-148.

23. Kawasaki T, Henmi K, Ono E, Hataleuama S, Iwano M, Satoh H, Shimamoto K: **The small GTP-binding protein Rac is a regulator of cell death in plants**. *Proc Natl Acad Sci USA* 1999, **96**:10922-10926.

24. Dangl JL, Jones JDG: **Plant pathogens and integrated defence responses to infection**. *Nature* 2001, **411**:826-833.

25. Dwyer SC, Legendre L, Low PS, Leto TL: **Plant and human neutrophil oxidative burst complexes contain immunologically related proteins**. *Biochim Biophys Acta* 1996, **1289**:231-237.

26. Tenhaken R, Rubel C: **Cloning of putative subunits of the soybean plasma membrane NADPH oxidase involved in the oxidative burst by antibody expression screening**. *Protoplasma* 1998, **205**:21-28.

27. Banfi B, Tirone F, Durussel I, Knisz J, Moskowa P, Molnar GZ, Krause K-H, Cox JA: **Mechanism of Ca^{2+} activation of the NADPH oxidase 5 (NOX5)**. *J Biol Chem* 2005, **279**:18583-18591.

28. Sagi M, Fluhr R: **Superoxide production by plant homologues of the $gp91^{phox}$ NADPH oxidase. Modulation of activity by calcium and Tobacco Mosaic Virus infection**. *Plant Physiol* 2001, **126**:1281-1290.

29. Apostol I, Heinstein PF, Low PS: **Rapid stimulation of an oxidative burst during elicitation of cultured plant cells**. *Plant Physiol* 1989, **99**:109-116.

30. Levine A, Tenhaken R, Dixon R, Lamb CJ: **H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response**. *Cell* 1994, **79**:583-593.

31. Desikan R, Reynolds A, Hancock JT, Neill SJ: **Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on defence gene expression in *Arabidopsis* suspension cultures**. *Biochem J* 1998, **330**:115-120.

32. 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**:523-528.

33. •• Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JD, Schroeder JI: **NADPH oxidase AtrbohD and AtrbohF genes function in ROS-dependent ABA signaling in *Arabidopsis***. *EMBO J* 2003, **22**:2623-2633.
This work provides genetic evidence that ROI produced by an Rboh-NADPH oxidase mediates the activation of Ca^{2+} channels during ABA-induced stomatal closure. *AtrbohD* and *AtrbohF*, which are also involved in ROI production and cell death control following pathogen recognition, are highly expressed in guard cells. Both mRNAs are elevated in response to ABA. The double mutant *atrbohD/atrbohF* displays ABA insensitivity and impairment in the ABA induction of ROI and ABA activation of Ca^{2+} channels. Exogenous application of H_2O_2 restored Ca^{2+} channel activation and partial stomatal closure in *atrbohD/F*.

34. Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb CJ: **Salicylic acid potentiates an agonist-dependent gain control**

that amplifies pathogen signals in the activation of defense mechanisms. *Plant Cell* 1997, **9**:261-270.

35. Draper J: **Salicylate, superoxide synthesis and cell suicide in plant defense**. *Trends Plant Sci* 1997, **2**:162-165.

36. Delledonne M, Zeier J, Marocco A, Lamb C: **Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response**. *Proc Natl Acad Sci USA* 2001, **98**:13454-13459.

37. • Desikan R, Cheung M-K, Bright J, Henson D, Hancock JT, Neill SJ: **ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells**. *J Exp Bot* 2003, **55**:205-212.
A nice review describing new insights in ABA signaling, particularly the role of ROI and NO in ABA-induced stomatal changes.

38. • Agrawal GK, Iwahashi H, Rakwal R: **Small GTPase 'Rop': molecular switch for plant defense responses**. *FEBS Lett* 2003, **546**:173-180.
A review about Rac/Rop function in plants that focuses on defense responses.

39. • Moeder W, Yoshioka K, Klessig DF: **Involvement of the small GTPase Rac in the defense responses of tobacco to pathogens**. *Mol Plant Microbe Interact* 2005, **18**:116-124.
Overexpression of a dominant negative form of the rice *Osrac1* gene in tobacco significantly reduces HR-associated ROI production. In comparison with wildtype plants, these transgenic plants exhibit smaller tobacco mosaic virus (TMV) lesions, altered defense gene expression and suppressed SA accumulation. Interestingly, resistance to a non-host bacterium was also impaired.

40. Ono E, Wong HL, Kawasaki T, Hasegawa M, Kodama O, Shimamoto K: **Essential role of the small GTPase Rac in disease resistance of rice**. *Proc Natl Acad Sci USA* 2001, **98**:759-764.

41. • Morel J, Fromentin J, Blein JP, Simon-Plas F, Elmayan T: **Rac regulation of NtrbohD, the oxidase responsible for the oxidative burst in elicited tobacco cell**. *Plant J* 2004, **37**:282-293.
This article presents *Ntrac5* as a negative regulator of ROI production in tobacco cells after elicitation with the fungal elicitor cryptogein. The production of ROI in response to cryptogein was strongly inhibited in cell cultures that overproduced a dominant constitutively active *Ntrac5*. Expression of *NtrbohD* was also modified in these cultures.

42. Baxter-Burrell A, Yang Z, Springer PS, Bailey-Serres J: **RopGAP4-dependent Rop GTPase rheostat control of *Arabidopsis* oxygen deprivation tolerance**. *Science* 2002, **296**:2026-2028.

43. Zhang X, Zhang L, Dong F, Gao J, Galbraith DW, Song CP: **Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba***. *Plant Physiol* 2001, **126**:1438-1448.

44. Pei ZM, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ, Grill E, Schroeder JI: **Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells**. *Nature* 2000, **406**:731-734.

45. •• Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD *et al.*: **Reactive oxygen species produced by NADPH oxidase regulate plant cell growth**. *Nature* 2003, **422**:442-446.
This study provides genetic evidence that *AtrbohC* is required for cell elongation. Local production of ROI by AtrbohC mediates a Ca^{2+} gradient via activation of specific Ca^{2+} channels that are responsible for the elongation of tip growth in the root hair.

46. Levine A, Pennell R, Palmer R, Lamb CJ: **Calcium-mediated apoptosis in a plant hypersensitive response**. *Curr Biol* 1996, **6**:427-437.

47. Chandra S, Martin GB, Low PS: **The Pto kinase mediates a signaling pathway leading to the oxidative burst in tomato**. *Proc Natl Acad Sci USA* 1996, **93**:13393-13397.

48. Piedras P, Hammond-Kosack KE, Harrison K, Jones JDG: **Rapid, *Cf-9* and *Avr9* dependent, production of active oxygen species in tobacco suspension cultures**. *Mol Plant Microbe Interact* 1998, **11**:1155-1166.

49. • Jiang M, Zhang J: **Cross-talk between calcium and reactive oxygen species originated from NADPH oxidase in abscisic acid-induced antioxidant defence in leaves of maize seedlings**. *Plant Cell Environ* 2003, **26**:929-939.
The authors used a pharmacological approach to show that Ca^{2+} is required in the production of ROI by the NADPH oxidase in response to ABA, suggesting that Ca^{2+} functions both upstream and downstream of the NADPH oxidase.

50. Klusener B, Young JJ, Murata Y, Allen GJ, Mori IC, Hugouvieux V, Schroeder JI: **Convergence of calcium signaling pathways of pathogenic elicitors and abscisic acid in *Arabidopsis* guard cells**. *Plant Physiol* 2002, **130**:2152-2163.

51. •• Rentel MC, Lecourieux D, Ouaked F, Usher SL, Petersen L, Okamoto H, Knight H, Peck SC, Grierson CS, Hirt H *et al.*: **OXI1 kinase is necessary for oxidative burst-mediated signalling in *Arabidopsis***. *Nature* 2004, **427**:858-861.
Arabidopsis OXI1 is a serine/threonine kinase that is transcriptionally upregulated by stimuli that are associated with generation of H_2O_2. *OXI1* is essential for basal disease resistance against a virulent pathogen and is also required for normal root-hair development during stress. OXI1 and AGC2-1 are the same protein.

52. •• Anthony RG, Henriques R, Helfer A, Meszaros T, Rios G, Testerink C, Munnik T, Deak M, Koncz C, Bogre L: **A protein kinase target of a PDK1 signalling pathway is involved in root hair growth in *Arabidopsis***. *EMBO J* 2004, **23**:572-581.
This article describes the identification of AGC2-1, a *Arabidopsis* protein kinase that is involved in lipid-signaling. Phosphatidic acid specifically binds AtPDK1 (3′-phosphoinositide-dependent kinase-1) and stimulates AGC2-1. Analysis of an *agc2-1* mutant suggested a role for this gene in root-hair growth and development. OXI1 and AGC2-1 are the same protein.

53. Orozco-Cardenas ML, Narvaez-Vasquez J, Ryan CA: **Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate**. *Plant Cell* 2001, **13**:179-191.

54. • Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratmann JW, Fluhr R: **Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in *Lycopersicon esculentum***. *Plant Cell* 2004, **16**:616-628.
The authors used an antisense construct to demonstrate that *Rboh* is required for the wound-inducible expression of certain tomato genes. Reduced levels of *Rboh* altered redox-related metabolism, induced multiple pleiotropic effects on plant development, and resulted in the ectopic expression of flower-specific homeotic genes.

55. • Overmyer K, Brosche M, Kangasjarvi J: **Reactive oxygen species and hormonal control of cell death**. *Trends Plant Sci* 2003, **8**:335-342.
A comprehensive review that compares cell-death signaling in response to pathogens and ozone.

56. •• Joo JH, Wang S, Chen JG, Jones AM, Fedoroff NV: **Different signaling and cell death roles of heterotrimeric G protein α and β subunits in the *Arabidopsis* oxidative stress response to ozone**. *Plant Cell* 2005, **17**:957-970.
This important article shows that mutations in genes that encode the α and β subunits of the heterotrimeric G protein have marked effects on ozone tolerance. The authors characterized the spatial and temporal progression of ROI signaling in response to ozone. Signaling commenced with the elevation of ROI concentrations in guard-cell chloroplasts and membranes, then spread to neighboring cells by means of extracellular ROI signals that are generated by the *AtrbohD* and *AtrbohF* NADPH oxidases.

57. •• Chen YL, Huang R, Xiao YM, Lu P, Chen J, Wang XC: **Extracellular calmodulin-induced stomatal closure is mediated by heterotrimeric G protein and H_2O_2**. *Plant Physiol* 2004, **136**:4096-4103.
The authors provide strong evidence that extracellular calmodulin stimulates stomatal closure through the activation of heterotrimeric G protein and the subsequent promotion of H_2O_2 and $[Ca^{2+}]_{cyt}$ elevation.

58. Suharsono U, Fujisawa Y, Kawasaki T, Iwasaki Y, Satoh H, Shimamoto K: **The heterotrimeric G protein alpha subunit acts upstream of the small GTPase Rac in disease resistance of rice**. *Proc Natl Acad Sci USA* 2002, **99**:13307-13312.

59. • Dat JF, Pellinen R, Beeckman T, Van De Cotte B, Langebartels C, Kangasjarvi J, Inzé D, Van Breusegem F: **Changes in hydrogen peroxide homeostasis trigger an active cell death process in tobacco**. *Plant J* 2003, **33**:621-632.

Under intense light, high concentrations of H_2O_2 accumulate in plants that have reduced catalase activity, inducing a cell death that has characteristics of the HR. Inhibition of this cell death by the NADPH oxidase inhibitor diphenylene iodonium (DPI), and the fact that *NtrbohD* is transcriptionally upregulated in response to intense light in *CAT*-deficient plants, suggests that one or more NADPH oxidases mediate cell death in this system.

60. Davletova S, Rizhsky L, Liang H, Shengqiang Z, Oliver DJ,
•• Coutu J, Shulaev V, Schlauch K, Mittler R: **Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of *Arabidopsis***. *Plant Cell* 2005, **17**:268-281.

The cytosolic ascorbate peroxidase APX1 plays an important role in modulating ROI concentrations, providing cross-compartment protection against light stress in the chloroplast. *AtrbohD* is involved in the signal transduction pathway for *APX1*, acting as a positive amplification factor that maintains the expression of the *APX1* transcript at high steady-state level.

61. Yaeno T, Matsuda O, Iba K: **Role of chloroplast trienoic fatty
• acids in plant disease defense responses**. *Plant J* 2004, **40**:931-941.

This interesting article shows that trienoic fatty acids from the chloroplast play an important role in the regulation of plant defense responses, among them the production of ROI by the NADPH oxidase.