[Plant Signaling & Behavior 4:5, 467-469; May 2009]; ©2009 Landes Bioscience

Article Addendum

Role of nitric oxide in regulating stomatal apertures

Ian D. Wilson,[1,*] Dimas M. Ribeiro,[1,2] Jo Bright,[1] Ana Confraria,[1,3] Judith Harrison,[1] Raimundo S. Barros,[2] Radhika Desikan,[4] Steven J. Neill[1] and John T. Hancock[1]

[1]Centre for Research in Plant Science; Faculty of Health and Life Sciences; University of the West of England, Bristol; Bristol, UK; Coldharbour Lane; Bristol, UK; [2]Departamento de Biologia Vegetal; Universidade Federal de Viçosa; Viçosa, Minas Gerais Brazil; [3]Departamento de Botânica; Faculdade de Ciências; Universidade do Porto; Porto, Portugal; [4]Division of Biology; Faculty of Natural Sciences; Imperial College; London, UK

Key words: abscisic acid, nitrate reductase, nitric oxide, stomatal apertures, guard cells

During stomatal closure, nitric oxide (NO) operates as one of the key intermediates in the complex, abscisic acid (ABA)-mediated, guard cell signaling network that regulates this process. However, data concerning the role of NO in stomatal closure that occurs in turgid vs. dehydrated plants is limited. The data presented demonstrate that, while there is a requirement for NO during the ABA-induced stomatal closure of turgid leaves, such a requirement does not exist for ABA-enhanced stomatal closure observed to occur during conditions of rapid dehydration. The data also indicate that the ABA signaling pathway must be both functional and to some degree activated for guard cell NO signaling to occur. These observations are in line with the idea that the effects of NO in guard cells are mediated via a Ca^{2+}-dependent rather than a Ca^{2+}-independent ABA signaling pathway. It appears that there is a role for NO in the fine tuning of the stomatal apertures of turgid leaves that occurs in response to fluctuations in the prevailing environment.

Nitric oxide (NO) acts as a key signal that regulates many of the responses of plants to environmental stresses[1] and is an important intermediate in the abscisic acid (ABA) signal transduction pathway which controls stomatal apertures.[2] Prior studies have shown that ABA-induced stomatal closure in *Arabidopsis thaliana* is dependent on guard cell nitrate reductase (NR)-mediated NO synthesis.[3] More specifically, of the two NR isoforms encoded by the genes *NIA1* and *NIA2*, ABA-induced stomatal closure is dependent on the synthesis of NO by the *NIA1*-encoded isoform, NR1.[2] However, until now, there has been a lack of comparative data detailing the degree to which this is required as turgid plants subsequently dehydrate during periods of drought.

In our original paper[4] we showed that while the removal of guard cell NR1-associated NO prevented ABA-induced stomatal closure in turgid leaves, NO removal, either by scavenging with 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) or as a consequence of loss-of-function mutation of *NIA1* (*nia1*::Ds), did very little to reduce the ABA-enhanced stomatal closure observed in leaves undergoing rapid dehydration. Plants of the NR1-lacking mutant, *nia1*::Ds, were not obviously any more wilty than those of their wild-type *Landsberg* background when watering was withheld and must, therefore, be able to regulate the amount of water they lose through transpiration during periods of drought. It is, therefore, intriguing that the responses of turgid guard cells to NO require the ABA signal transduction pathway to be both functional and to some degree engaged.[4] The stomata of the turgid leaves of neither the ABA deficient, loss-of-function mutant, *aba1-1*, nor the ABA insensitive mutant, *abi1-1*, close in response to the application of either NO via the donor sodium nitroprusside (SNP) or the nitrite substrate of NR-mediated NO synthesis.[4] Thus, the inference is that stomatal closure is regulated by both NO-dependent and -independent mechanisms of ABA signaling that operate in a manner dependent on the prevailing environmental conditions. Indeed this observation aligns well with those concerning the involvement of Ca^{2+} in guard cell ABA signaling. For some time the ABA-dependent signaling of NO in guard cells has been thought to involve an elevation of intracellular Ca^{2+} which then alters the activities of Ca^{2+}-dependent K^+ and Cl^- ion channels.[5] Certainly, NO does affect cellular Ca^{2+} mobilisation[6] and a requirement for altered Ca^{2+} flux for normal guard cell responses to NO has been shown in *Vicia faba*.[7] In guard cells, ABA signaling is known to operate via both Ca^{2+}-dependent[8] and pH-dependent[9]/Ca^{2+}-independent pathways.[10-13] NO accumulates in ABA-treated guard cells of the *abi1-1* mutant, but this does not result in stomatal closure.[4] This is consistent with the concept that, in these cells, NO signals via the Ca^{2+}-dependent ABA signaling pathway which is impaired in this mutant.[14] Exactly how this occurs is still unclear, but may involve S-nitrosylation of thiol groups within Ca^{2+}-dependent K^+ ion channels.[15]

*Correspondence to: Ian D. Wilson; Centre for Research in Plant Science; Faculty of Health and Life Sciences; University of the West of England, Bristol; Coldharbour Lane; Bristol BS16 1QY UK; Tel.: 0.117.3282799; Fax: 0.117.3282904; Email: ian2.wilson@uwe.ac.uk

Submitted: 03/20/09; Accepted: 03/24/09

Previously published online as a *Plant Signaling & Behavior* E-publication: http://www.landesbioscience.com/journals/psb/article/8545

Addendum to: Ribeiro DM, Desikan R, Bright J, Confraria A, Harrison J, Hancock JT, Barros RS, Neill SJ, Wilson ID. Differential requirement for NO during ABA-induced stomatal closure in turgid and wilted leaves. Plant Cell Environ 2008; 59:165–76; PMID: 19021879; DOI: 10.1111/j.1365-3040.2008.01906.x.

It is also interesting that the accumulation and signaling of NO in Arabidopsis guard cells is dependent on the prior synthesis of H_2O_2 by the NADPH oxidase isoforms AtrbohD/F[16] and that this generation of H_2O_2 is in turn dependent on the normal ethylene-sensing function of the ethylene receptor encoded by *ETR1*.[17] Neither the histidine kinase function of ETR1 nor the downstream components of the ethylene signaling pathway appear to be required for the guard cell accumulation of H_2O_2 to occur per se, but do appear to be required for the synthesised H_2O_2 to be effective. Thus, in turgid guard cells the production of H_2O_2 and the subsequent synthesis and signaling of NO may constitute points of convergence of the ethylene and ABA signaling pathways. Figure 1 illustrates our current thinking with regard to the integration of these different signals in guard cells.

One of the questions that remains is that if NO is not involved in stomatal closure during conditions of rapid dehydration, what exactly is its role in mediating guard cell movements? Turgid guard cells accumulate NO during the light to dark transition[18] and scavenging NO or preventing its synthesis, as in the *nia1*::Ds mutant, prevents the dark-induced closure from occurring.[4] Thus, it would seem that, in this context, NO has a physiological role in mediating guard cell movements in well hydrated plants. However, stomatal closure does not always occur in the dark and many other factors dictate whether or not this happens.[19] High levels of CO_2 applied in the form of the bicarbonate ion, for example, induce the sequential accumulation of H_2O_2 and NO in guard cells during stomatal closure.[20] Presumably, the degree of closure that occurs in the dark is dependent on the balance of multiple input signals. On reflection this makes sense considering the seasonal changes in the growing environment that occur in temperate regions. Currently, it seems as though NO acts to mediate this balance and does so by signaling via a Ca^{2+}-dependent ABA signal transduction pathway. The focus of future studies will then be to determine exactly how such signaling "crosstalk" operates and which proteins are specifically modified by NO during the stomatal closure that is thus regulated.

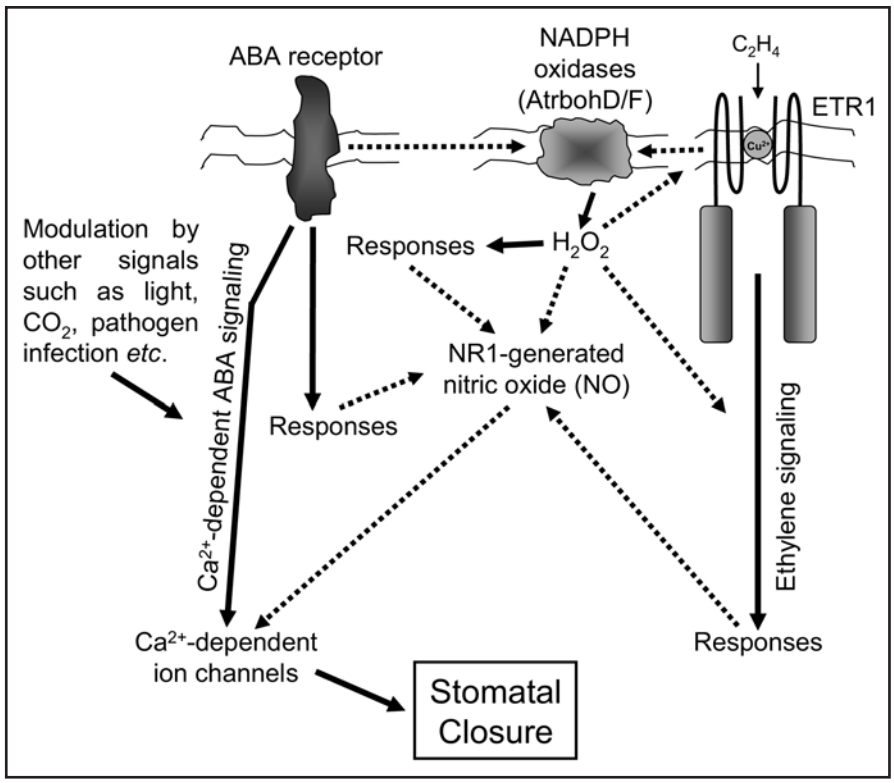

Figure 1. Signal crosstalk during NO-regulated stomatal closure in turgid leaves of *Arabidopsis thaliana*. In turgid leaves ABA-induced stomatal closure is dependent on the synthesis of H_2O_2 by various NADPH oxidases and the subsequent synthesis of nitric oxide (NO) by nitrate reductase 1 (NR1).[16] NO signals via a Ca^{2+}-dependent ABA signaling pathway,[4] possibly by the S-nitrosylation of Ca^{2+}-dependent ion channels.[15] Numerous environmental signals modulate this response and determine the actual stomatal apertures observed.[4,19] Interestingly the ABA-induced accumulation of H_2O_2 in turgid guard cells also appears to depend on the ability of the ethylene receptor, ETR1, to perceive ethylene and although not actually required for the ABA-induced accumulation of this reactive oxygen species, downstream components of the ethylene signal transduction pathway also seem to be required for H_2O_2 to signal its presence in these cells.[17,21] Thus, the synthesis and signaling of H_2O_2 and NO in turgid guard cells may constitute points of convergence of ABA and ethylene signaling. However, numerous question remain concerning how the activities of the NADPH oxidases and NR1 are regulated and whether or not this occurs via the direct regulation of the extant proteins or via increased transcription as a result of ABA and ethylene signaling. Additionally, questions remain as to exactly how H_2O_2 induces NR1-mediated NO synthesis and whether this is either by the direct modulation of protein activity or as a result of downstream signaling. For example, H_2O_2 may signal in its own right or may mediate the activity of components of the ethylene signal transduction chain leading to an increase in *NIA1* transcription. Future studies aim to clarify these questions.

References

1. Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, et al. Nitric oxide, stomatal closure and abiotic stress. J Exp Bot 2008; 59:165-76.
2. Wilson ID, Neill SJ, Hancock JT. Nitric oxide synthesis and signaling in plants. Plant Cell Environ 2008; 31:622-31.
3. Desikan R, Griffiths R, Hancock J, Neill SJ. A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 2002; 99:16314-8.
4. Ribeiro DM, Desikan R, Bright J, Confraria A, Harrison J, Hancock JT, et al. Differential requirement for NO during ABA-induced stomatal closure in turgid and wilted leaves. Plant Cell Environ 2008; 59:165-76.
5. Garcia-Mata C, Gay R, Sokolovski S, Hills A, Lamattina L, Blatt MR. Nitric oxide regulates K^+ and Cl^- channels in guard cells through a subset of abscisic acid-evoked signaling pathways. Proc Natl Acad Sci USA 2003; 100:1116-21.
6. Lamotte O, Courtois C, Dobrowolska G, Besson A, Pugin A, Wendehenne D. Mechanisms of nitric oxide induced increase of free cytosolic Ca^{2+} concentration in *Nicotiana plumbaginifolia* cells. Free Rad Biol Med 2006; 40:1369-76.
7. Sokolovski S, Hills A, Gay R, Garcia-Mata C, Lamattina L, Blatt MR. Protein phosphorylation is a prerequisite for intracellular Ca^{2+} release and ion channel control by nitric oxide and abscisic acid in guard cells. Plant J 2005; 43:520-9.
8. Grabov A, Blatt MR. A steep dependence of inward-rectifying potassium channels on cytosolic free calcium concentration increase evoked by hyperpolarization in guard cells. Plant Physiol 1999; 119:277-88.
9. Grabov A, Blatt MR. Parallel control of the inward-rectifier K^+ channel by cytosolic free Ca^{2+} and pH in Vicia guard cells. Planta 1997; 201:84-95.

10. Allan AC, Fricker MD, Ward JL, Beale MH, Trewavas AJ. Two transduction pathways mediate rapid effects of abscisic acid in *Commelina* guard cells. Plant Cell 1994; 6:1319-28.
11. Romano LA, Jacob T, Gilroy S, Assmann SM. Increases in cytosolic Ca^{2+} are not required for abscisic acid-inhibition of inward K^+ currents in guard cells of *Vicia faba* L. Planta 2000; 211:209-17.
12. Outlaw WH. Integration of cellular and physiological functions of guard cells. Crit Rev Plant Sci 2003; 22:503-29.
13. Israelsson M, Siegel RS, Young J, Hashimoto M, Iba K, Schroeder JI. Guard cell ABA and CO_2 signaling network updates and Ca^{2+} sensor priming hypothesis. Curr Opin Plant Biol 2006; 9:654-63.
14. Armstrong F, Leung J, Grabov A, Brearley J, Giraudat J, Blatt MR. Sensitivity to abscisic acid of guard-cell K^+ channels is suppressed by *abi1-1*, a mutant Arabidopsis gene encoding a putative protein phosphatase. Proc Natl Acad Sci USA 1995; 92:9520-4.
15. Sokolovski S, Blatt MR. Nitric oxide block of outward-rectifying K^+ channels indicates direct control by protein nitrosylation in guard cells. Plant Physiol 2004; 136:4275-84.
16. Bright J, Desikan R, Hancock JT, Neill SJ. ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis. Plant J 2006; 45:113-22.
17. Desikan R, Last K, Harret-Williams R, Tagliavia C, Harter K, Hooley R, et al. Ethylene-induced stomatal closure in Arabidopsis occurs via AtrbohF-mediated hydrogen peroxide synthesis. Plant J 2006; 47:907-16.
18. She XP, Song XG, He JM. Role and relationship of nitric oxide and hydrogen peroxide in light/dark-regulated stomatal movement in *Vicia faba*. Acta Bot Sinica 2004; 46:1292-300.
19. Caird MA, Richards JH, Donovan LA. Night-time stomatal conductance and transpiration in C_3 and C_4 plants. Plant Physiol 2007; 143:4-10.
20. Kolla VA, Raghavendra AS. Nitric oxide is a signaling intermediate during bicarbonate-induced stomatal closure in *Pisum sativum*. Physiol Plant 2007; 130:91-8.
21. Desikan R, Horák J, Chaban C, Mira-Rodado V, Witthöft J, Elgass K, et al. The histidine kinase AHK5 integrates endogenous and environmental signals in Arabidopsis guard cells. PLoS One 2008; 3:2491.