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Addendum

cGMP in ozone and NO dependent responses

Luisa Ederli,[1] Stuart Meier,[2] Andrea Borgogni,[1] Lara Reale,[1] Francesco Ferranti,[1] Chris Gehring[2] and Stefania Pasqualini[1,*]

[1]Department of Plant Biology and Agro-Environmental and Animal Biotechnology; University of Perugia; Perugia, Italy; [2]Department of Biotechnology; University of the Western Cape; Bellville, South Africa

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We have recently reported that ozone (O_3) can inhibit mitochondrial respiration and induce activation of the alternative oxidase (AOX) pathway and in particular *AOX1a* in tobacco. While O_3 causes mitochondrial H_2O_2, early leaf nitric oxide (NO) as well as transient ethylene (ET) accumulation, the levels of jasmonic acid and 12-oxo-phytodienoic acid remained unchanged. It was shown that both, NO and ET dependent pathways can induce *AOX1a* transcription by O_3. AOX plays a role in reducing reactive oxygen species (ROS) which in turn are linked to biotic and abiotic plant stresses, much like the second messengers guanosine 3', 5'-cyclic monophosphate (cGMP). The goal is to unravel specific cGMP signatures and induction pathways downstream from O_3 and NO, including transcription of *AOX1a*. Here we propose that some late (>3 h) responses to NO, e.g., the accumulation of phenylalanine lyase (PAL) transcripts, are critically cGMP dependent, while the early (<2 h) responses, including *AOX1a* induction are not.

A Role for Aox in Plant Stress Responses

It is conceivable that a function of the AOX pathway is to reduce the formation of ROS whereby increased activity of AOX could relieve the mitochondrial cytochrome pathway and prevent harmful hyper-reduction, reducing the formation of damaging radicals.[1,2] ROS generation is also involved in biotic and abiotic stresses responses in plants. While AOX abundance and AOX activity are low in unstressed plants, alternative respiration is enhanced after various developmental or environmental stimuli, e.g., under conditions such as wounding and plant disease[1] thus implicating AOX in stress alleviation. The nuclear gene that encodes AOX in tobacco (*Nicotiana tabacum; AOX1*) is rapidly induced in cultured cells when the cytochrome pathway is specifically inhibited by antimycin A.[3]

*Correspondence to: Stefania Pasqualini; Department of Plant Biology and Agro-Environmental and Animal Biotechnology; University of Perugia; Perugia I-06121 Italy; Fax: +39.075.5856404; Email: spas@unipg.it

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Addition of salicylic acid (SA) to tobacco cell suspensions or intact leaves also induces gene the expression of AOX associated genes[4] further linking AOX to plant stress responses. More recently NO has been identified as a key molecule that interacts with ROS in a number of ways, either as a crucial partner in determining cell fate or in signaling in response to developmental and stress-related conditions. NO appears to be involved in controlling or modulating various aspects of plant pathogen resistance, growth, development, and senescence, as well as stomatal movement.[5]

In NO treated Arabidopsis cell cultures expression is strongly induced, resulting in increased respiration through the alternative pathway.[6] Furthermore, *AOX1a* expression is affected in the Arabidopsis *ctr-1* mutant thus pointing to ethylene (ET) dependence.[7]

Different signaling molecules have been found to be involved in AOX induction, but their interactions during environmental stresses remain unresolved. Analyses of Arabidopsis mutants have produced a substantial body of information implicating SA, NO, ROS, jasmonic acid (JA), and ET as major endogenous signals in defense responses. However, much less information is available for other plant species. Our previous studies demonstrated that O_3 treatment induces SA and H_2O_2 accumulation in the O_3-sensitive tobacco BelW3 cultivar,[8] and the activation of a cell death program[9] as well as the induction of the AOX pathway by O_3 in tobacco.[10,11]

Early (1–1.5 h) production of NO in fumigated leaves was detected with a NO-specific fluorescence concomitant with H_2O_2 accumulation in mitochondria and ET evolution was recorded during O_3 exposure. In summary, while it is established that NO is the main upstream signaling molecule involved in *AOX1a* expression, which is coordinately activated by ET, the exact nature of the downstream signaling pathway remains to be elucidated.

Linking cGMP to Stress Responses

It is becoming increasingly clear that the cyclic nucleotide cGMP has an important role in many diverse biological processes[12-14] including responses to both abiotic and biotic stresses,[15,16] and NO dependent signaling[5,17,18] as well as the regulation of transcription.[19]

It is interesting to note, that the time for cGMP increases does vary considerably from 15 min in response to NaCl and osmotica[15] to 2 h after application of an NO donor,[16] and 5 h in response to a gravitropic stimulus.[20] This can be taken as an indication for the complex and differentiated role of cGMP as signaling molecule. Our current experiments suggest that in Tobacco significant rises

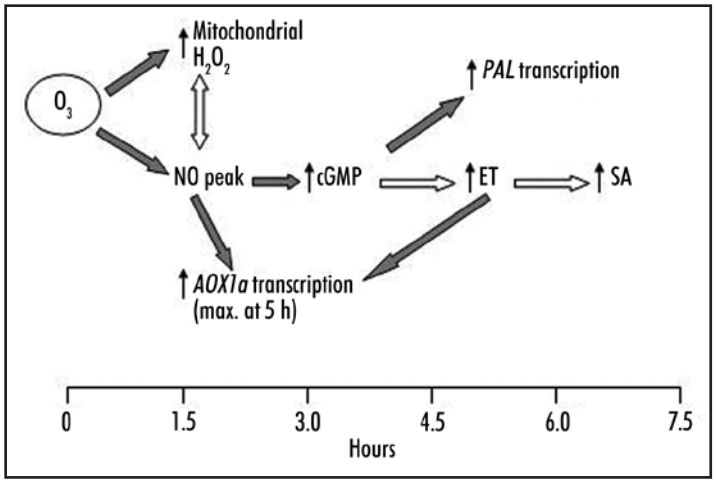

Figure 1. Time-course of NO, H_2O_2, ET, SA and cGMP accumulation after fumigation with O_3 in BelW3 tobacco. Gray arrows indicate established relationships between the signalling molecules, white arrows are proposed hypothetical links. The small arrows indicate the time of first significant accumulation of the respective molecules after fumigation with O_3. The response to O_3 includes a cascade of signal molecules leading to the expression of early and late defense genes. In this model early and NO responsive gene expression (*AOX1a*) does not depend on cGMP, while the late transcriptional response (e.g., of *PAL*) is at least in parts critically dependent on cGMP.

in cGMP levels occur only >3 h after NO fumigation. This would suggest that cGMP has no role in NO dependent *AOX1a* induction. Inspecting microarray data in the public domain we noted that in *Arabidopsis thaliana AOX1a* (At3g22370) is not significantly induced by cGMP neither after 2 nor 5 h[19] and this observation concurs with our own findings[21] whereas within the same timeframe *PAL* is (Fig. 1).

Outlook

We are currently performing an extended series of experiments that will resolve the temporal, spatial and stimulus specific induction patterns of O_3 and NO dependent cGMP transients and link them to the cGMP dependent transcriptome and proteome. Based on both the available microarray data and our preliminary results, we hypothesise, that early (<3 hours) O_3 and NO responsive gene expression including *AOX1a* does not depend on cGMP, while the late transcriptional response (>3 hours) is at least in parts critically dependent on cGMP. An example for a late response would be e.g., *PAL* (Fig. 1). In addition, we foresee that cGMP also has an important late role in responding to homeostatic disturbances caused by biotic and abiotic stresses. Such a role might require prolonged elevation of cytosolic cGMP levels—rather than short transients—which in turn can activate cGMP dependent ion channels. A function of cGMP in long-term ionic regulation after stress is also entirely compatible with the finding of cGMP dependent transcriptional activation of ion channel encoding genes.[19]

References

1. Purvis A, Shewfelt R. Does the alternative pathway ameliorate chilling injury in sensitive plant tissue? Physiol Plant 1993; 88:712-8.
2. Wagner A, Krab K. The alternative respiration pathway in plants - Role and regulation. Physiol Plant 1995; 95:318-25.
3. Vanlerberghe G, McIntosh L. Mitochondrial electron transport regulation of nuclear gene expression: Studies with the alternative oxidase gene of tobacco. Plant Physiol 1994; 105:867-74.
4. Rhoads D, McIntosh L. Cytochrome and alternative pathway respiration in tobacco: Effects of salicylic acid. Plant Physiol 1993; 103:877-83.
5. Neill SJ, Desikan R, Hancock JT. Nitric oxide signalling in plants. New Phytol 2003; 159:11-35.
6. Huang X, von Rad U, Durner J. Nitric oxide induces transcriptional activation of the nitric oxide-tolerant alternative oxidase in Arabidopsis suspension cells. Planta 2002; 215:914-23.
7. Simons B, Millenaar F, Mulder L, Van Loon L, Lambers H. Enhanced expression and activation of the alternative oxidase during infection of Arabidopsis with *Pseudomonas syringae* pv tomato. Plant Physiol 1999; 120:529-38.
8. Pasqualini S, Della Torre G, Ferranti F, Ederli L, Piccioni C, Reale L, Antonielli M. Salicylic acid modulates ozone-induced hypersensitive cell death in tobacco plants. Plant Physiol 2002; 115:204-12.
9. asqualini S, Piccioni C, Reale L, Ederli L, Della Torre G, Ferranti F. Ozone-induced cell death in tobacco cultivar BelW3 plants: The role of programmed cell death in lesion formation. Plant Physiol 2003; 133:1122-34.
10. Ederli L, Morettini R, Borgogni A, Wasternack C, Miersch O, Reale L, Ferranti F, Tosti N, Pasqualini S. Interaction between nitric oxide and ethylene in the induction of alternative oxidase in ozon-treated Tobacco plants. Plant Physiol 2006; 142:595-608.
11. Pasqualini S, Paolocci F, Borgogni A, Morettini R, Ederli L. The overexpression of an alternative oxidase gene triggers ozone sensitivity in tobacco plants. Plant Cell Environ 2007; 12:1545-56
12. Newton R, Roef L, Witters E, Van Onckelen H. Tansley review no. 106 - Cyclic nucleotides in higher plants: The enduring paradox. New Phytol 1999; 143:427-55.
13. Schaap P. Guanylyl cyclases across the tree of life. Front Biosci 2005; 10:1485-98.
14. Meier S, Gehring C. Emerging roles in plant biotechnology for the second messenger cGMP - Guanosine 3', 5'-cyclic monophosphate. African Journal of Biotechnology 2006; 5:1687-92.
15. Donaldson L, Ludidi N, Knight MR, Gehring C, Denby K. Salt and osmotic stress cause rapid increases in *Arabidopsis thaliana* cGMP levels. FEBS Lett 2004; 569:317-20.
16. Durner J, Wendehenne D, Klessig D. Defense gene induction in Tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci USA 1998; 95:10328-33.
17. Prado AM, Porterfield DM, Feijo JA. Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. Development 2004; 131:2707-14.
18. Delledonne M. NO news is good news for plants. Curr Opin Plant Biol 2005; 8:390-6.
19. Maathuis FJM. cGMP modulates gene transcription and cation transport in Arabidopsis roots. Plant J 2006; 45:700-11.
20. Hu X, Neill SJ, Tang Z, Cai W. Nitric oxide mediates gravitropic bending in soybean roots. Plant Physiol 2005; 137:663-70.
21. Tosti N, Pasqualini S, Borgogni A, Ederli L, Falistocco E, Crispi S, Paolocci F. Gene expression profiles of O_3-treated Arabidopsis plants. Plant Cell Envion 2006; 29:1686-702.