[Plant Signaling & Behavior 3:6, 381-382; June 2008]; ©2008 Landes Bioscience

Article Addendum

Carbon monoxide

A novel and pivotal signal molecule in plants?

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Key words: auxin; carbon monoxide; nitric oxide; plants; reactive oxygen species; signal molecule

Carbon monoxide (CO), a by-product released during the degradation of heme by heme oxygenases (HOS EC 1.14.99.3) in animals, plays a major role as neurotransmitter, regulator of sinusoidal tone, inhibitor of platelet aggregation and suppressor of acute hypertensive response, and most of above effects are similar to or mediated by nitric oxide (NO), another signal molecule in both the animal and plant kingdoms. Previous result demonstrated that NO could act as a promoter of plant cell elongation, acting similarly to IAA, inducing morphogenetic responses leading to expansion in plant tissues. Recent observations revealed that CO is an inducer of cell expansion in wheat root segments, acting similarly to IAA and NO. Evidence also indicated that IAA could result in either the potent induction of *HO-1* transcript or endogenous CO releasing in wheat root segments. Additionally, our results suggested that above CO signaling might be related to NO/cGMP, Ca^{2+} and even ROS-dependent pathways. In this addendum, combined with other previous results, we further proposed a possible hypothesis for CO signaling role in regulation of plant root development induced by auxin.

Carbon monoxide (CO) is a low molecular weight diatomic gas that occurs ubiquitously in nature as an air pollutant. In plants, although several laboratories[1,2] have reported the direct emission of CO by living plants since 1959, little information is known about CO's physiological roles in the whole plant, except that exogenous CO was able to affect the plant seed germination, induce the adventitious rooting process.[3,4] While in animals, CO which was mainly produced by heme oxygenase (HO EC 1.14.99.3), has recently seen an explosion of research interest due to its newly discovered physiological and signaling effects. In animals, there are three forms of HOs. HO-1 is inducible, while constitutively expressed HO-2 and HO-3 display very low activity. Research in CO now embraces the entire field of medicine where reactive oxygen species (ROS), reactive nitrogen species (RNS), inflammation, growth control and apoptosis represent important pathophysiological mechanisms.[5-7] Meanwhile, downstream signaling events regulated by CO in animals have been described before.[7,8] Among the second messengers reported to be involved in CO signaling, there are at least cyclic GMP (cGMP), nitric oxide (NO) and ROS, but in plants this study is also in its infancy.

It is well known that the plant hormone auxin is involved in the regulation of most aspects of plant growth and development processes, including cell division, elongation and differentiation. It has been demonstrated that endogenous NO is involved in IAA-mediated root organogenesis.[9-11] The use of CO gas, or heme molecules hematin and hemin, also termed as HO inducer to yield CO in animals, has shown that exogenous CO is involved in adventitious rooting and lateral root formation.[12,13] In our study, we report that administration of hematin and hemin, exactly induced the significant increase in wheat root elongation as well as the actions of IAA and NO donor sodium nitroprusside (SNP) in a dose-dependent manner.[14] These responses were mimicked by the application of aqueous solution of CO with different saturation. Additionally, above heme molecule-induced effect is specific for CO produced by HO since the potent inhibitor of HO-1, zinc protoporphyrin-IX (ZnPPIX) or CO/NO scavenger hemoglobin (Hb) blocked the action of hematin and hemin, respectively. Our further results also confirmed that hematin could result in either the potent induction of *HO-1* transcript or endogenous CO releasing in the wheat root tip segments. In contrast, when ZnPPIX was added together, the increase of *HO-1* transcript or CO content was reversed.

Exogenously applied CO may not replicate the function of endogenous CO and may have side effects in plants. More recently, we also discovered[15] that under 100 and 200 μM cadmium treatments, CO releasing was increased and approximately consistent with the changes of HO activity and *HO-1* transcript, an important CO synthetic enzyme both in animals and plants.[16] In *Vicia faba* leaves, CO production and HO activity were firstly reported to increase in response to ABA treatment, which could result in stomatal closure. Interestingly, ABA-induced stomatal closure in *Vicia faba* guard cells was differentially blocked when ZnPPIX or Hb was added.[17] In our further experiments, our data presented that treatment with IAA could result in either the potent induction of *HO-1*

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Submitted: 11/30/07; Accepted: 12/04/07

Previously published online as a *Plant Signaling & Behavior* E-publication: http://www.landesbioscience.com/journals/psb/article/5374

Addendum to: Xuan W, Huang L, Li M, Huang B, Xu S, Liu H, Gao Y, Shen W. Induction of growth elongation in wheat root segments by heme molecules: A regulatory role of carbon monoxide in plants? Plant Growth Regul 2007; 52:41–51; DOI: DOI: 10.1007/s10725-007-9175-1.

transcript or CO releasing in wheat root segments. ZnPPIX with lower concentration could prevent the elongation induced by IAA, while in the SNP-treatment the prevention of root growth occurred solely at higher concentrations. By using specific histochemical assay combined with the inhibitor investigation, we also suggested that endogenous CO generated by HO might mediate the induction of growth elongation of wheat root segments elicited by IAA, which might be also related to NO/cGMP, Ca^{2+} and even ROS-dependent pathways.[14]

Furthermore, we advanced a simple possible signaling pathway accounting for findings showed in this paper (Fig. 1). In this model, endogenous CO, generated from HO acting as a potent signal molecule defines an underlying link between the upstream hormone IAA and the downstream signal NO, thus leading to wheat root elongation. Additionally, cGMP and ROS might also be related to above signal transduction. Considering that CO has been postulated as a possible signal molecule during development and adaptive plant response against some abiotic stresses,[15,17] our results further suggest that CO might be as a versatile molecule with different functions in plants, which was proven in animal systems recently. Additionally, functional studies will be directed to evaluate the participation of CO on auxin-mediated processes using mutants unpaired in CO production, thus leading to full understanding the role of auxin-induced and CO-mediated signaling pathways in plants.

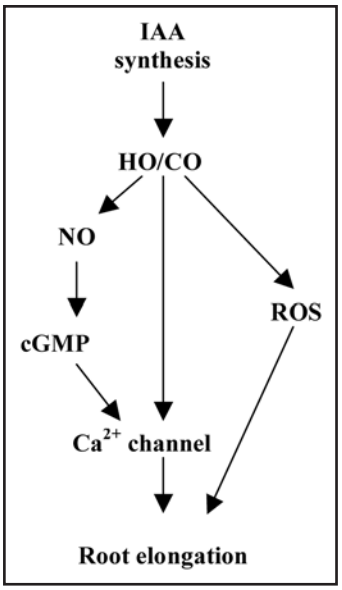

Figure 1. Schematic illustration of a proposed model for HO/CO signaling pathway of root elongation in wheat. IAA triggers a transient CO accumulation, which activates an NO or ROS-dependent pathway, thus leading to root elongation.

Acknowledgements

This work was supported by the Program for New Century Excellent Talents in University, the National Natural Science Foundation of China (grant no. 30671248), the Natural Science Foundation of Jiangsu Province of China (grant no. BK2007157), the 111 Project (grant no. B0703), and Student Research Training (SRT) Project (grant no. 0506B07) of Nanjing Agricultural University, China.

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