Plant Signaling & Behavior 7:11, 1493–1494; November 2012; © 2012 Landes Bioscience

# Sorting out the role of nitric oxide in cadmium-induced *Arabidopsis thaliana* programmed cell death

Yun Ye, Zhe Li and Da Xing*

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science; College of Biophotonics; South China Normal University; Guangzhou, China

**As a vital cell-signaling molecule, nitric oxide (NO) has been reported to regulate toxic metal responses in plants. Our recent report has suggested that caspase-3-like protease activation was detected in Arabidopsis (*Arabidopsis thaliana*) after $Cd^{2+}$ treatment. NO contributed caspase-3-like protease activation in $Cd^{2+}$ induced *Arabidopsis thaliana* programmed cell death (PCD), which was mediated by MPK6. It was first shown that NO promotes $Cd^{2+}$-induced Arabidopsis PCD by promoting MPK6-mediated caspase-3-like activation. Our study contributed to the understanding of NO signaling pathway in $Cd^{2+}$-induced *Arabidopsis thaliana* PCD. Although several studies have revealed that NO regulates plant PCD, compared with the study of signaling pathways involved in animal cell apoptosis, the mechanism of NO function still remains elusive and the molecular mechanisms of MAPK are far from clear in $Cd^{2+}$-induced PCD. By using the fluorescence techniques and the Arabidopsis seedlings as the reference model, the subsequent researches have been performed to obtain comprehensive understanding of $Cd^{2+}$-induced plant PCD.**

**Keywords:** Arabidopsis PCD, caspase-3-like protease, $Cd^{2+}$ stress, MAPK pathway, nitric oxide signal

Submitted: 08/19/12

Accepted: 08/20/12

http://dx.doi.org/10.4161/psb.21893

*Correspondence to: Da Xing;
Email: xingda@scnu.edu.cn

Addendum to: Ye Y, Li Z, Xing D. Nitric oxide promotes MPK6-mediated caspase-3-like activation in cadmium-induced Arabidopsis thaliana programmed cell death. Plant Cell Environ 2013;36: 1–15; PMID:22621159; http://dx.doi.org/10.1111/j.1365-3040.2012.02543.x.

Cadmium ($Cd^{2+}$) is a non-essential element that exhibits high levels of toxicity to humans, animals and plants.[1,2] Depending on the concentration, treatment with 50 μM $Cd^{2+}$ does not result in any differences vs. untreated cultures and treatments of 100 μM and 150 μM $Cd^{2+}$ in Arabidopsis suspension cultures led to a type of PCD that resembles an accelerated senescence process.[3] While well known as primary mechanism in animal apoptosis, caspase activity has also been identified in plants.[4] In our recent work, fluorescence resonance energy transfer (FRET) technique has been utilized to successfully detect real-time caspase-3-like activation in vivo during UV or Al-induced Arabidopsis protoplasts PCD.[4,5] But the mechanism of regulating caspase-3-like activation in plant PCD remains largely unknown. Nitric oxide (NO) is an important signaling molecule in plants PCD. Recently, some works provided evidence for the regulation of NO in $Cd^{2+}$ stress.[3,6] Our recent works have shown a potential NO signaling pathway in $Cd^{2+}$-induced Arabidopsis PCD: $Cd^{2+}$ stress resulted in NO production; the NO accumulation subsequently contributed the activation of 44 and 47 kDa MAPKs; and then caspase-3-like pathway was activated which resulted in the execution of PCD.[7] We also found that NO contributes to $Cd^{2+}$-induced PCD by facilitating caspase-3-like protease activation and NO promotes caspase-3-like activation via MPK6-mediation.[7] These findings prompt us to carry out further investigations to determine the much clearer NO signaling mechanism of $Cd^{2+}$-induced plant PCD.

Mitochondria play an essential role in nitric oxide (NO) signal transduction in plants.[8] In our study, we found that the induction of NO occurred first in the mitochondrial regions of protoplasts and subsequently in chloroplasts and even the whole cell as treatment time increased. Mitochondria have been thought to be the major NO producing compartment.[9] And chloroplasts have also been shown to be a source of NO in plants.[10] As DAF-FM

DA is a membrane-permeable fluorogenic reagent, there are two possibilities about the appearance of NO generation in chloroplasts and other organelles. First, it may be due to NO and DAF-FM DA diffusion from the mitochondria. Second, other organelles, such as chloroplasts, may participate in $Cd^{2+}$-induced NO production as the treatment time increased. It has been reported that changes in the distribution and mobility of mitochondria serve important functions in $Cd^{2+}$-induced cell death.[11] And we have previously found that pretreatment with the MPTP inhibitor CsA depresses the mitochondrial dysfunction and caspase-3-like activation induced by Al stress.[5] Hence, to realize a real-time in vivo subcellular localization of the NO release in living cells using fluorescence techniques will be a meaningful and interesting work, which will provide direct experimental evidences to determine the relation between mitochondrial dysfunction and caspase-like activation in plant cells.

Previous evidence has demonstrated that $Cd^{2+}$ activates Arabidopsis MPK3 and MPK6 via the accumulation of ROS.[12] In our study, we found that NO is also involved in the $Cd^{2+}$-induced activation of Arabidopsis MAPKs.[7] In Arabidopsis suspension cultures, $Cd^{2+}$-induced $H_2O_2$ production occurred after NO production because $Cd^{2+}$-induced NO production negatively affected the CAT and APX capacity.[3] Meanwhile, it has been reported that NO promoted ROS accumulation by modulating the activity of NOX and antioxidative enzymes.[13] We could supposes that NO and ROS somewhat act synergistically to regulate the MAPK signaling pathway. Recently, our group found that MPK6 contributed the activation of Arabidopsis γVPE in HS-induced Arabidopsis PCD, which supported that MPK6 acts as an important regulator in plant PCD.[14] Wang et al. (2010) proved a function of MPK6 in modulating NO production and signal transduction in response to $H_2O_2$.[15] Consequently, the cross-talk between NO and MPK6 suggested that maybe there was an amplification loop in the NO signaling pathway during $Cd^{2+}$-induced Arabidopsis PCD, which would be interesting to investigate further in the future.


### Acknowledgments

This work is supported by the Program for Chang Jiang Scholars and Innovative Research Team in University (IRT0829), the Key Program of NSFC-Guangdong Joint Funds of China (U0931005) and the National High Technology Research and Development Program of China (863 Program) (2007AA10Z204).


### References


1. Pinto AP, Mota AM, de Varennes A, Pinto FC. Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. Sci Total Environ 2004; 326:239-47; PMID:15142779; http://dx.doi.org/10.1016/j.scitotenv.2004.01.004.
2. Järup L, Akesson A. Current status of cadmium as an environmental health problem. Toxicol Appl Pharmacol 2009; 238:201-8; PMID:19409405; http://dx.doi.org/10.1016/j.taap.2009.04.020.
3. De Michele R, Vurro E, Rigo C, Costa A, Elviri L, Di Valentin M, et al. Nitric oxide is involved in cadmium-induced programmed cell death in *Arabidopsis* suspension cultures. Plant Physiol 2009; 150:217-28; PMID:19261736; http://dx.doi.org/10.1104/pp.108.133397.
4. Zhang LR, Xu QX, Xing D, Gao CJ, Xiong HW. Real-time detection of caspase-3-like protease activation in vivo using fluorescence resonance energy transfer during plant programmed cell death induced by ultraviolet C overexposure. Plant Physiol 2009; 150:1773-83; PMID:19535476; http://dx.doi.org/10.1104/pp.108.125625.
5. Li Z, Xing D. Mechanistic study of mitochondria-dependent programmed cell death induced by aluminium phytotoxicity using fluorescence techniques. J Exp Bot 2011; 62:331-43; PMID:20937730; http://dx.doi.org/10.1093/jxb/erq279.
6. Besson-Bard A, Gravot A, Richaud P, Auroy P, Duc C, Gaymard F, et al. Nitric oxide contributes to cadmium toxicity in *Arabidopsis* by promoting cadmium accumulation in roots and by up-regulating genes related to iron uptake. Plant Physiol 2009; 149:1302-15; PMID:19168643; http://dx.doi.org/10.1104/pp.108.133348.
7. Ye Y, Li Z, Xing D. Nitric oxide promotes MPK6-mediated caspase-3-like activation in cadmium-induced *Arabidopsis thaliana* programmed cell death. Plant Cell Environ 2013; 36:1-15 PMID:22621159; http://dx.doi.org/10.1111/j.1365-3040.2012.02543.x.
8. Palmieri MC, Lindermayr C, Bauwe H, Steinhauser C, Durner J. Regulation of plant glycine decarboxylase by s-nitrosylation and glutathionylation. Plant Physiol 2010; 152:1514-28; PMID:20089767; http://dx.doi.org/10.1104/pp.109.152579.
9. Gupta KJ, Kaiser WM. Production and scavenging of nitric oxide by barley root mitochondria. Plant Cell Physiol 2010; 51:576-84; PMID:20185408; http://dx.doi.org/10.1093/pcp/pcq022.
10. Jasid S, Simontacchi M, Bartoli CG, Puntarulo S. Chloroplasts as a nitric oxide cellular source. Effect of reactive nitrogen species on chloroplastic lipids and proteins. Plant Physiol 2006; 142:1246-55; PMID:16980561; http://dx.doi.org/10.1104/pp.106.086918.
11. Bi YH, Chen WL, Zhang WN, Zhou Q, Yun LJ, Xing D. Production of reactive oxygen species, impairment of photosynthetic function and dynamic changes in mitochondria are early events in cadmium-induced cell death in *Arabidopsis thaliana*. Biol Cell 2009; 101:629-43; PMID:19453296; http://dx.doi.org/10.1042/BC20090015.
12. Liu XM, Kim KE, Kim KC, Nguyen XC, Han HJ, Jung MS, et al. Cadmium activates Arabidopsis MPK3 and MPK6 via accumulation of reactive oxygen species. Phytochemistry 2010; 71:614-8; PMID:20116811; http://dx.doi.org/10.1016/j.phytochem.2010.01.005.
13. Xu J, Yin H, Li Y, Liu X. Nitric oxide is associated with long-term zinc tolerance in Solanum nigrum. Plant Physiol 2010; 154:1319-34; PMID:20855519; http://dx.doi.org/10.1104/pp.110.162982.
14. Li Z, Yue H, Xing D. MAP Kinase 6-mediated activation of vacuolar processing enzyme modulates heat shock-induced programmed cell death in Arabidopsis. New Phytol 2012; 195:85-96; PMID:22497243; http://dx.doi.org/10.1111/j.1469-8137.2012.04131.x.
15. Wang P, Du Y, Li Y, Ren D, Song CP. Hydrogen peroxide-mediated activation of MAP kinase 6 modulates nitric oxide biosynthesis and signal transduction in *Arabidopsis*. Plant Cell 2010; 22:2981-98; PMID:20870959; http://dx.doi.org/10.1105/tpc.109.072959.