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Mitochondrial pathway leading to programmed cell death induced by aluminum phytotoxicity in Arabidopsis

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Recent studies have reported some apoptosis-like characters under Al treatment including nucleus morphology changes and appearance of nucleus fragmentation in plant cells. Our recent report has suggested that mitochondrial oxidative burst, mitochondrial swelling and mitochondrial transmembrane potential (MTP) disrupt play crucial roles in Al-induced caspase-3-like activation and programmed cell death (PCD). And Complex I and III might be the sources of Al-induced mitochondrial reactive oxygen species (ROS) through interaction between Al and iron-sulfur (Fe-S) protein. Our study contributed to the understanding of mitochondria-dependent cellular signaling cascade of plant biological responses in Al-induced PCD. However, the mitochondria-dependent mechanism in Al-induced PCD needs further improvement, and the roles of mitochondria functional proteins are still poorly understood compared with the study of signaling pathways involved in animal cell apoptosis. By using the fluorescence techniques and the Arabidopsis mesophyll protoplasts as a reference model, the subsequent researches have been carried out to obtain comprehensive understanding of Al-induced plant PCD.

Key words: alternative oxidase, aluminum, Arabidopsis, caspase-3-like, cytochrome *c*, mitochondria, programmed cell death, reactive oxygen species, respiratory chain

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Aluminum (Al) is a non-essential metal widespread in environment, which is known to be toxic to both humans and plants. Its toxicity has been recognized as one of the major factors that limit crop production on acid soil, causing damage to not only the root that is always exposed to Al but also the aerial part of plants.[1-3] In plant cells, recent researches have reported some apoptosis-like characters under Al treatment including nucleus morphology disrupt and appearance of nucleus fragmentation.[1] Reactive oxygen species (ROS) burst and mitochondrial dysfunction are believed to be the crucial events in Al phytotoxicity.[1-5] Nowadays, more and more attentions have been paid to the mechanistic analysis of Al phytotoxicity, which is lacked in many previous studies. Our recent works have shown a potential cascade of cellular events during Al-induced programmed cell death (PCD): Al, after entering mitochondria, interacted with Fe-S protein of complex I and III, and inhibited the activity of electron transport chain, resulting in the excess electron and massive production of mitochondrial ROS; the ROS burst subsequently induced mitochondrial transmembrane potential (MTP) loss and mitochondrial swelling, and then caspase-3-like pathway was activated which resulted in the execution of PCD (**Fig. 1**).[6] We also found that the alternative oxidase 1a (AOX1a) was upregulated by Al stress in the transcript level, and overexpression of AOX1a in Arabidopsis protoplasts resulted in enhanced Al tolerance (**Fig. 1**).[6] These findings prompt us to carry out further investigations to determine the more comprehensive signaling network and to dig out the roles of mitochondria functional proteins including AOX in Al-induced PCD.

Mitochondria are recognized as crucial players in ROS-mediated PCD, and the collapse of MTP has also been reported to be an essential event in plant PCD.[7] The release of cytochrome *c* (cyt *c*), which is the result of mitochondrial oxidative burst and MTP loss, plays a vital role in the activation of caspase-like proteases. And inhibition of

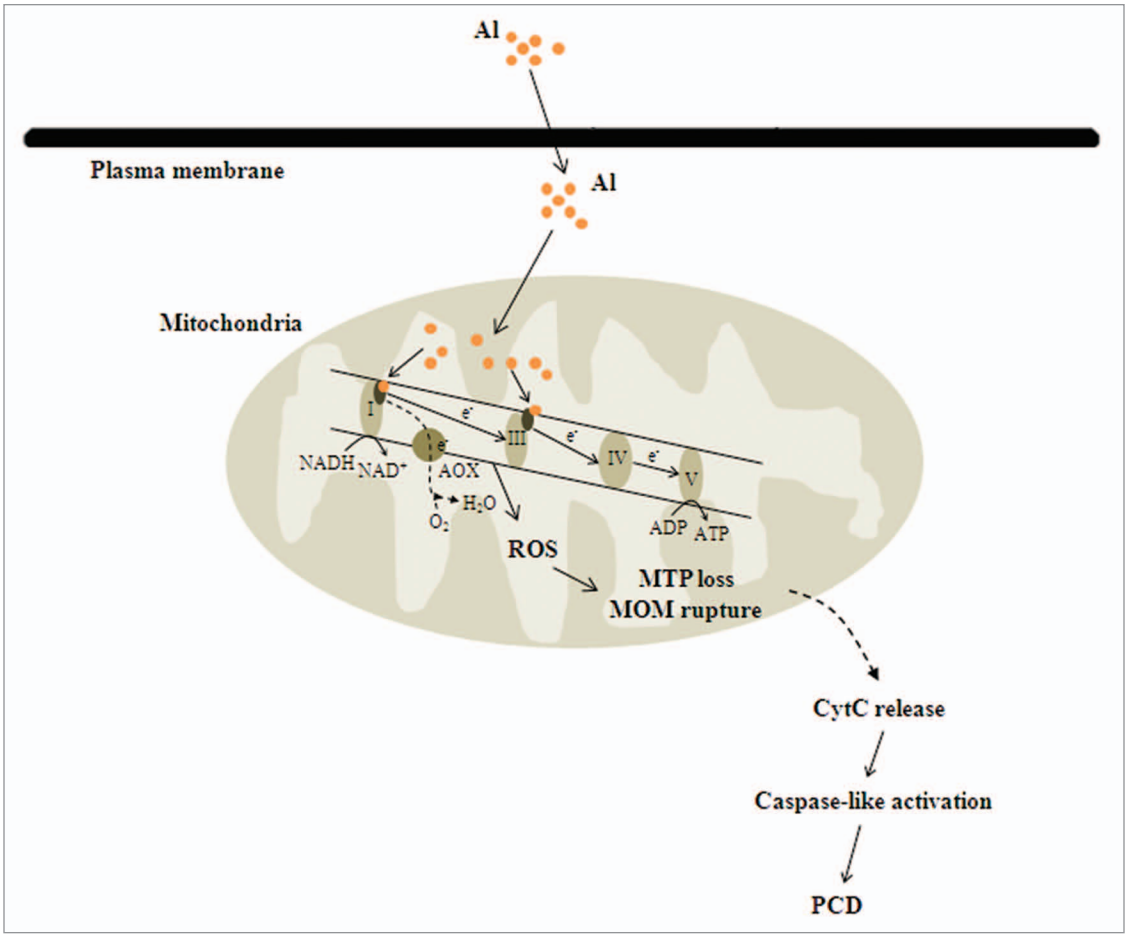

Figure 1. Proposed working model for mitochondria-dependent PCD induced by Al. AOX, alternative oxidase; Cyt *C*, cytochrome *C*; MOM, mitochondrial outer membrane; PCD, programmed cell death. For detailed explanation, see the text.

cyt *c* release can rescues plant cells from cell death.[8,9] The common method in plant cells to detect cyt *c* release is the western blot analysis, which cannot reflect the real-time process. Hence, to realize a real-time in vivo detection of the cyt *c* release from mitochondria 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.

ROS can be generated from the mitochondrial electron transport system through electron leaks when substrates are metabolized and the electron transport chain protein complex I and complex III are recognized as the most sensitive sites for ROS production.[10,11] Our data has determined that Al-induced mitochondrial oxidative burst was due to the damage of complex I and III caused by the interaction between Al and iron-sulfur (Fe-S) protein.[6] In response to adverse environments, plant cells establish a number of antioxidant regulatory mechanisms, including the alternative NADH dehydrogenases and AOX, to minimize the mitochondrial ROS production.[12,13] AOX, the unique respiratory terminal oxidase in plant, catalyzes the energy wasteful alternative respiration pathway. Under normal situation and various biotic or abiotic stresses, it helps to maintain the electron transport, prevent the electron excess, and reduce mitochondrial oxidative burst.[14,15] In our paper, AOX1a was upregulated by Al stress in transcript level, and played the protective roles against Al toxicity.[6] But the mechanisms how the AOX protein is regulated, and the ways in which AOX alleviates Al-induced PCD are still poor understood. Our preliminary results showed that overexpression of AOX1a alleviated the MTP loss and caspase-like activation under Al treatment. Our future work will focus on the upstream regulation and downstream function cascades of AOX protein under Al stress. These subsequent researches will certainly contribute to the signal transduction pathways in Al-induced Arabidopsis PCD.

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