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A cascade signal pathway occurs in self-incompatibility of *Pyrus pyrifolia*

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Pear (*Pyrus pyrifolia* L.) possesses an S-RNase-based gametophytic self-incompatibility (GSI) system and S-RNase, the self-incompatibility (SI) determinant in the pistil, has also been implicated in the rejection of self-pollen and genetically identical pollen. We have demonstrated that S-RNase depolymerises actin cytoskeleton, triggers mitochondrial alteration and DNA degradation in the incompatible pollen tube, which indicates programmed cell death (PCD) may occur in SI response of *Pyrus pyrifolia*. Recently, we have identified that S-RNase specifically disrupted tip-localized reactive oxygen species (ROS) of incompatible pollen tube via arrest of ROS formation in mitochondria and cell walls in *Pyrus pyrifolia*. Furthermore, tip-localized ROS disruption not only decreased the Ca^{2+} current and depolymerised the actin cytoskeleton, but it also induced nuclear DNA degradation in the pollen tube. The results mentioned above indicate that a cascade signal pathway may occur in SI of *Pyrus pyrifolia* and PCD is used to terminate the incompatible pollen tubes growth. In this addendum, we review the cascade signal pathway of *Pyrus pyrifolia* SI.

Key words: S-RNase, programmed cell death, reactive oxygen species, actin cytoskeleton, Ca^{2+} current, nuclear DNA

Abbreviations: GSI, gametophytic self-incompatibility; PCD, programmed cell death; ROS, reactive oxygen species; SI, self-incompatibility

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A Cascade Signal Pathway Occurs in Self-incompatibility of *Pyrus pyrifolia*

Pear (*Pyrus pyrifolia* L.), belonging to the family Rosaceae, has a S-RNase-based gametophytic self-incompatibility (GSI) mechanism, S-RNase has been implicated in the rejection of self-pollen and genetically identical pollen. The incompatible pollen tubes growth are arrested under S-RNase challenge in *Pyrus pyrifolia* in vitro.[1] During the inhibition process period, what happen to the pollen tube? It was known that pollen tubes possess a typical tip growth. There are many elements assembled to possess the process, for example, calcium ions, actin cytoskeleton, tip-localized reactive oxygen species (ROS), and so on. We are interesting in observing whether calcium ions, actin cytoskeleton or tip-localized ROS change during the growth inhibition process period. If the answer is yes, whether these changes are a consequence or the cause of pollen tube growth inhibition should be answered clearly. Luckily, we have demonstrated that S-RNase depolymerises actin cytoskeleton of incompatible pollen tube of *Pyrus pyrifolia* in vitro.[2] Recently, we published a paper[3] describing that tip-localized ROS disruption and Ca^{2+} currents decrease in incompatible pollen tube of *Pyrus pyrifolia* in vitro.[3] Otherwise, these changes happened before pollen tube viability compromise, which indicated these changes are the cause, not the consequence, of pollen tube growth inhibition. Moreover, we find that tip-localized ROS disruption decrease the Ca^{2+} current and depolymerises the actin cytoskeleton, finally degrading the nuclear DNA.[3] Actually, it is reported that ROS and Ca^{2+} have cross talk. On one hand, ROS can stimulate the activity of plasma membrane hyperpolarization-activated Ca^{2+} channels;[4] on the other hand, Ca^{2+} channel blockers can inhibit ROS formation in turn via inhibition activity of NADPH oxidise.[5] We speculate that, in pear SI response, Ca^{2+} current decrease

will also inhibit NADPH oxidise activity, and finally inhibit ROS formation in cell wall. So it is difficult to distinguish the order in time between tip-localized ROS disruption and Ca^{2+} current decrease. We think S-RNase decrease Ca^{2+} current and inhibit ROS formation simultaneously. Tip-localized ROS disruption and Ca^{2+} current decrease induce actin cytoskeleton depolymerization. It seems difficult to understand our results, because it was known that actin cytoskeleton depolymerise under high calcium condition.[6,7] But calcium was not the only regulator of actin cytoskeleton. H^+ (pH) can also be expected to regulate the actin cytoskeleton.[8] Actin cytoskeleton depolymerization should be an integrated response to early changes of pollen tube under SI challenge. It has been reported that either stabilization or depolymerization of the actin cytoskeleton is adequate to induce programmed cell death (PCD) in yeast and some animal cells.[9-12] Supporting this, the actin-depolymerizing agent, cytochalasin B (CB) or the actin-stabilization agent, phalloidin induces degradation of nuclear DNA of pollen tubes, a hallmark feature of apoptosis.[3,13] Moreover, we have demonstrated that nuclear DNA of incompatible pollen tube was degraded under SI challenge in vitro or in vivo.[3,13] Most importantly, we have demonstrated that nuclear DNA were degraded in advanced of pollen tube viability compromise,[13] which indicate that nuclear DNA degradation were the cause, not the result, of growth arrest of the pollen tubes.

Did PCD Occur in SI Response of *Pyrus pyrifolia*?

It is widely accepted that the growth of pollen tubs is inhibited by S-RNase degrading the RNA of incompatible pollen tubes. However, grafting incompatible pollen tubes on compatible styles,[14] in which the growth-arrest pollen reverted to normal growth, indicates RNA degradation may have only been the beginning of the SI response, not the end. S-RNase has been thought to function as a specific cytotoxin[15] and cytotoxins universally induce apoptosis in animal cells.[16] Theoretical support existed to suggest that S-RNase could trigger PCD in incompatible pollen tubes. In consistent with this, it has been demonstrated that tRNAs degradation can induce PCD in yeast.[17] We have also provided some preliminary evidence, including mitochondrial membrane potential collapse, cytochrome *c* leakage into cytosol, mitochondrial cristae reduction and nuclear DNA degradation, which indicate that PCD may occur in SI of *Pyrus pyrifolia*. Moreover, PCD is also triggered in incompatible pollen tube of *Papaver rhoeas* and *Olea europaea*.[18,19] It seems that the GSI systems use common mechanisms to reject incompatible pollens or pollen tubes, and incompatible pollens or pollen tubes are terminated by PCD. This is not mutually exclusive with RNA degradation of incompatible pollen tubes in S-RNase-based SI, as the degradation of RNA could be a results of PCD or these two events are independent of each other as has been shown in some cases.[17,20,21] It is not clear whether S-RNase directly or indirectly triggers the alteration of the pollen tube elements, in hence to inhibit the incompatible pollen tube growth. However, these results bring a new sight of GSI.

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