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ARTICLE ADDENDUM

Wound signaling: The missing link in plant regeneration

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ABSTRACT

Wounding is the first event that occurs in plant regeneration. However, wound signaling in plant regeneration is barely understood. Using a simple system of *de novo* root organogenesis from *Arabidopsis thaliana* leaf explants, we analyzed the genes downstream of wound signaling. Leaf explants may produce at least two kinds of wound signals to trigger short-term and long-term wound signaling. Short-term wound signaling is primarily involved in controlling auxin behavior and the fate transition of regeneration-competent cells, while long-term wound signaling mainly modulates the cellular environment at the wound site and maintains the auxin level in regeneration-competent cells. *YUCCA* (*YUC*) genes, which are involved in auxin biogenesis, are targets of short-term wound signaling in mesophyll cells and of long-term wound signaling in regeneration-competent cells. The expression patterns of *YUC*s provide important information about the molecular basis of wound signaling in plant regeneration.

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Plant cells are highly plastic and have powerful regenerative abilities.[1-4] Wounding is the first event in plant regeneration.[1, 5] Studies on wounding have suggested several candidates for wound signals, such as electrical pulses, hydraulic pressure, Ca^{2+}, reactive oxygen species, the oligopeptide systemin, oligosaccharides, jasmonic acid, salicylic acid, ethylene, abscisic acid, and changes in various metabolic processes.[6,7] Many studies have tried to clarify the effects of wounding on regeneration by analysis of the genes downstream of wound signaling.[8-27] However, our knowledge about whether and how the above physical and chemical signals serve as wound signal (s) in plant regeneration is still limited.

To study the role of wound signaling in *de novo* root organogenesis, we cultured leaf explants on B5 medium to regenerate adventitious roots.[28] Wounding has complex biological effects, and may have multiple roles in *de novo* root organogenesis from leaf explants. Our previous studies revealed that at least two pathways are triggered by wounding of leaf explants: short-term and long-term wound-signaling pathways.

Short-term wound signaling is required for auxin production and cell fate transition (indicated by the red flow path in Fig. 1A).[10,29] This pathway comprises at least three stages of signal delivery (Fig. 1A). In stage I, wounding first triggers short-term wound signaling that lasts from seconds to hours. This wound signal spreads very rapidly from the wound site to mesophyll cells and activates *YUCCA1* (*YUC1*) and *YUC4* expression in mesophyll cells within 4 hours. Stage II involves auxin behavior. Auxin begins to be produced by YUCs in mesophyll cells within 4 hours and then polar-transported to regeneration-competent cells in the vasculature near the wound site at around 12 hours after wounding. In stage III, the accumulation of auxin in regeneration-competent cells activates expression of *WUSCHEL RELATED HOMEOBOX11* (*WOX11*) and *WOX12*, which start the cell fate transition to form root founder cells at around 1 to 2 days d after wounding.

The long-term wound-signaling pathway functions for a relatively long time, for around 2 days after wounding (indicated by the blue flow path in Fig. 1A).[11] One event downstream of long-term wound signaling is the activation of a group of *NAC* (NAM, ATAF1,2, and CUC2) transcription factor genes including *NAC1* (Fig. 1A).[11] *NAC1* is expressed in many cells at the wound site around 1 to 2 days after detachment of the leaf explant. However, *NAC1* does not affect the fate transition of regeneration-competent cells because *WOX11* and *WOX12* expression are normally activated when *NAC1* function is blocked. The role of *NAC1* might be to control the cellular environment, including cell wall metabolism. On the other hand, the long-term wound-signaling pathway also activates *YUC4* expression in regeneration-competent cells near the wound site at around 2 days after wounding (Fig. 1A),[10] and this might contribute to maintaining a high auxin level in regeneration-competent cells. Here, we analyzed $YUC4_{pro}$:*GUS*[10] in leaf explants cultured on B5 medium containing naphthylphthalamic acid (NPA, a polar auxin transport inhibitor) to test whether *YUC4* is a direct target of long-term wound signaling or is activated by auxin accumulation in regeneration-competent cells. Treatment with NPA can prevent auxin accumulation in regeneration-competent cells in leaf explants.[29] We observed strong β-glucuronidase (GUS) signals in regeneration-competent cells near the wound in the leaf explant cultured on medium containing NPA at 2 days after culture

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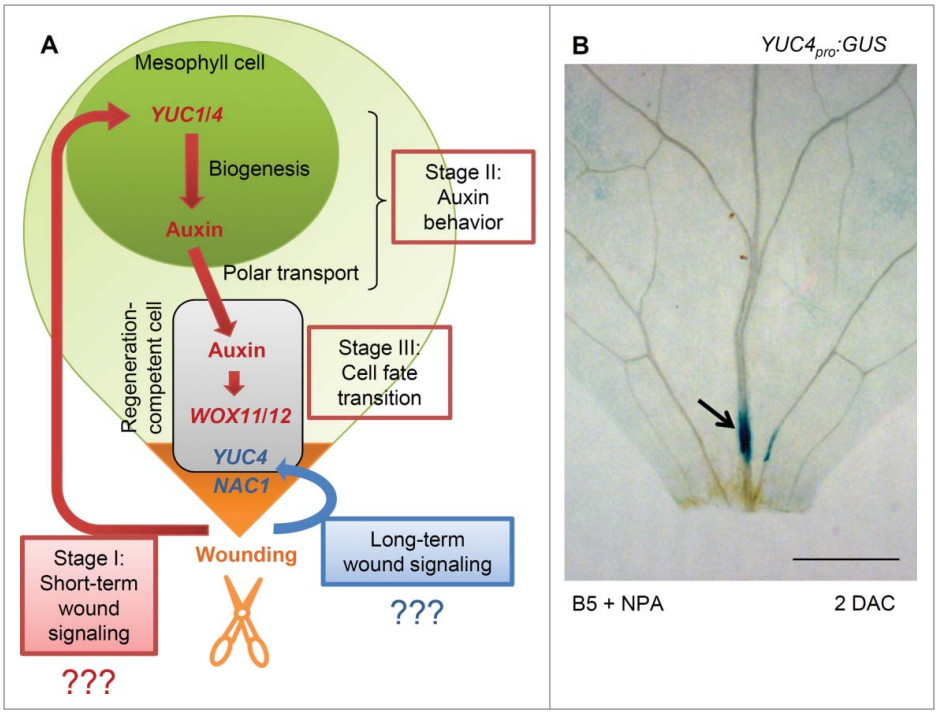

Figure 1. Wound signaling in *de novo* root organogenesis. (A) Model of short-term (red flow path) and long-term (blue flow path) wound signaling-mediated pathways in *de novo* root organogenesis. Orange region indicates wound site. (B) *YUC4* expression in leaf explant cultured on B5 medium containing 1 μM NPA. Arrow shows GUS signal in regeneration-competent cells at 2 DAC. Leaf explant was cultured in dark conditions,[28] and GUS staining was performed as described previously.[29,30] Scale bar, 500 μm in (B).

(DAC) (Fig. 1B), suggesting that *YUC4* might be a direct target of long-term wound signaling in regeneration-competent cells. Currently, it is unclear whether the activation of *NAC1* and *YUC4* is controlled dependently or independently by long-term wound signaling.

The short-term and long-term wound-signaling pathways act differently in leaf explants. First, the short-term wound signal is produced at the wound site and quickly moves to the mesophyll cells to activate *YUC1* and *YUC4* expression.[10] Therefore, this signal might be a moving signal that can spread from cell to cell. Long-term wound signaling functions at the wound site,[11] suggesting that it is a local signal. Second, short-term wound signaling lasts for seconds to hours,[10] while long-term signaling lasts for at least 2 days.[11] This suggests that the short-term wound signal has a short lifetime, while the long-term signal has a longer lifetime or is continuously produced in response to wounding. Third, short-term wound signaling seems to function in both the detached explant and in the wounded leaf residue on the source plant, because *YUC1* and *YUC4* expression are quickly activated in leaf explants as well as at the wound site on the source plant.[10] However, long-term wound signaling activates *NAC1* expression primarily at the wound site on the detached explant, and not in the leaf residue on the source plant.[11] This indicates that long-term wound signaling has a role in distinguishing different types of wounding. Overall, it seems that wounding produces complex molecules that serve as different types of wound signals in *de novo* root organogenesis from leaf explants.

Although many of the downstream effects of wounding have been identified, our knowledge about wound signals and wound signaling at the molecular level is still very limited. Recent studies have suggested that jasmonic acid, ethylene, and electrical pulses have effects on tissue repair,[9,15,17,25] raising the possibility that they may be candidates for wound signals to trigger regeneration. In our regeneration system of *de novo* root organogenesis, *YUC1* and *YUC4* serve as targets of short-term wound signaling in mesophyll cells, and *NAC1* and *YUC4* are targets of long-term wound signaling at the wound site. It will be interesting to test the upstream molecular events of *YUC*s and *NAC1* to study the molecular basis of wounding during *de novo* root organogenesis.[18]

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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