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Auxin biosynthesis site and polar transport in maize coleoptiles

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Since Darwins' pioneering experiments, monocot coleoptiles have been used to investigate indole-3-acetic acid (IAA) production and polar transport. In a recent study, using maize coleoptiles, we first showed that the asymmetric IAA flow from the tip in response to gravistimulus directly affects the TIR/AFBs-mediated auxin signaling pathway, which results in tropic curvature. In this work, we also showed that IAA is synthesized from tryptophan (Trp) in the apical 1 mm region, and from there the synthesized IAA moves to the basal part via polar transport by ZmPIN1(s). These results clearly show the importance of the tip region in perception of gravistimulus and in transmitting the perceived information to the lower region using IAA as a messenger signal. Thus, it is concluded that IAA production and transport from the tip are key factors controlling the cell elongation rate in the lower part of the coleoptiles, by making a regulated and dynamic IAA flow net work in the coleoptiles.

Key words: maize coleoptiles, IAA transport, IAA biosynthesis, ZmPIN1

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The plant hormone auxin plays an essential role in many aspects of plant physiological events. In recent studies, mainly using Arabidopsis, polar IAA transport and local IAA response have been identified as essential regulators during plant patterning in which the PIN protein, as an IAA transporter, functions to establish IAA concentration gradients in plant tissues.[1,2] In contrast, in monocot plants, such as oats, rice and maize, coleoptiles have long been used as a system for investigating IAA biosynthesis as well as polar and lateral transport,[3] since the pioneering work of Charles and Francis Darwin.[4] Using this system, it has been shown that IAA is synthesized in the tip region and is basipetally transported to the lower region.[5-12] Recently, *ZmPIN1a*, *1b* and *1c*, putative orthologs of *AtPIN1*, were identified in maize.[13,14] Immunolocalization studies revealed that ZmPIN1(s) was localized in the leaves, primary root and shoot apical meristem.[13,15,16] In contrast, analysis of the *ZmPIN1a* promoter-derived ZmPIN1a:YFP expression pattern showed a clear upregulation of ZmPIN1a in axial meristems and lateral organ primordia.[14,17] These findings suggested that polar IAA transport mechanism is necessary for the formation of axial meristems and lateral primordia in maize, as in Arabidopsis. However, the IAA transport proteins such as ZmPIN1(s), mediating the basipetal IAA transport, have not been characterized in maize coleoptiles.

In the present work, Nishimura et al.[12] utilizing a stable isotope labeling system using $^{13}C_{11}$,$^{15}N_2$-Trp, investigated the IAA biosynthesis site in maize coleoptiles, and showed the role of ZmPIN1 in basipetal transport of IAA from the site of synthesis, i.e., the apical 1 mm region of the coleoptile.

Auxin Biosynthesis in Maize Coleoptiles

Monocot coleoptile tips have long been recognized as a site of IAA production,[6,9-11] although there is evidence for some de novo IAA synthesis or hydrolysis of conjugated IAA.[18] In our previous studies, we showed that IAA is synthesized from Trp within the 0–2 mm region of the coleoptile tip.[9-11] In the present work, incorporation of the stable isotope from $^{13}C_{11}$,$^{15}N_2$-Trp into IAA mainly took place within the apical 0–1 mm region. In addition, when

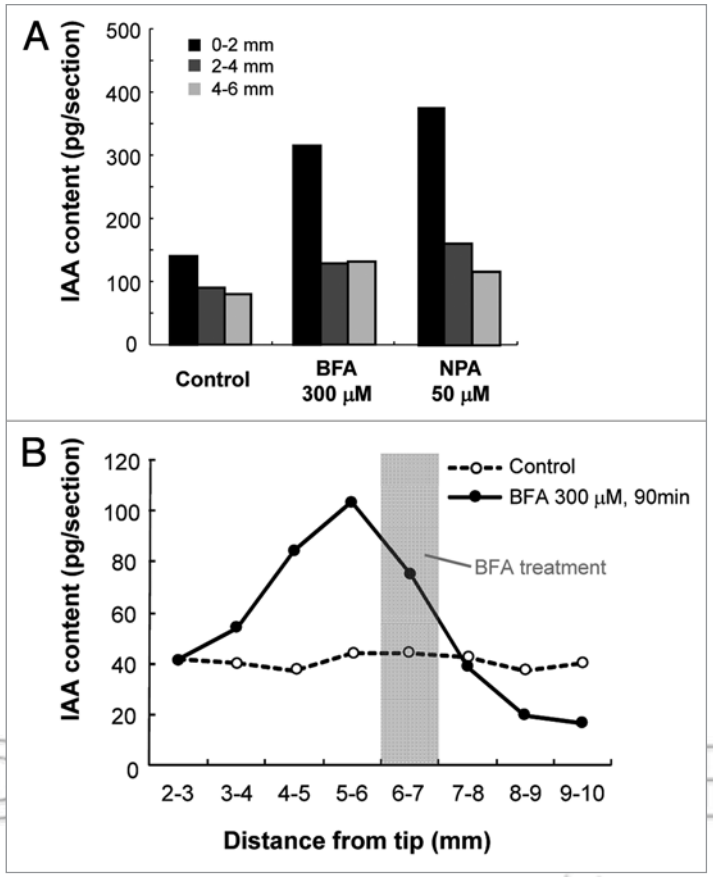

Figure 1. (A) Accumulation of IAA in the apical 0–6 mm region (inside) of maize coleoptiles after 300 μM BFA or 50 μM NPA treatment. BFA or NPA were applied within the 0–6 mm region. After 40 min incubation, the 0–2 mm, 2–4 mm and 4–6 mm sections of coleoptiles were harvested, and the IAA contents were determined using GC-SIM-MS. Data are the mean of two independent experiments. (B) Effect of 300 μM BFA on IAA transport in maize coleoptiles. BFA was applied within the 6–7 mm region (inside) from the tip. After 90 min incubation, the coleoptiles were cut into 1-mm sections from 2 mm from the tip towards the node. The IAA content was determined in each section. Data are the mean of three independent experiments.

1-*N*-naphthylphtalamic acid (NPA) or brefeldin A (BFA) was applied within the apical 0–6 mm region of the coleoptile, accumulation of endogenous IAA was observed only in the 0–2 mm region (**Fig. 1A**). These results indicate that the tip region is definitely the site of IAA biosynthesis from Trp. However, at present the relevant genes, enzymes and intermediates in the pathway are still undefined.

Polar IAA Transport in Maize Coleoptiles

Using the $^{13}C_{11}$,$^{15}N_2$-Trp stable isotope labeling method, we successfully traced movement of IAA synthesized in the coleoptile tip. $^{13}C_{10}$,$^{15}N_1$-IAA synthesized in the tip region was immediately transported towards the basal region at approximately 7 mm h^{-1}. When BFA was applied within the 6–7 mm region, IAA accumulated in tissues just above the site of BFA treatment and decreased in tissues below the treatment site (**Fig. 1B**). This result further indicated that IAA flows toward the basal region from the tip. Immunohistochemical analyses of ZmPIN1(s) showed a non-polar distribution in cells in the tip region, and a basal cellular localization in lower regions (**Fig. 2A and B**). This is consistent with the polar transport of IAA indicated by the isotope-labeling experiments, previously reported.[5,7] Thus, the IAA flow route is postulated as represented in **Figure 2C**. IAA could be transported in every direction in the tip where the IAA is synthesized, and IAA is basipetally transported from the tip toward basal region.

In this study, it was shown that tropic bending is visible within 60 min, and an asymmetric distribution of IAA occurs less than 30 min after exposure to a tropic stimulus. To investigate the redistribution of IAA in detail, IAA levels in the upper and lower halves of the coleoptile were determined after gravistimulus. After 30 min the stimulus, IAA was clearly redistributed throughout the coleoptile, and IAA levels on the lower side were greater than those on the upper side. Lateral movement of IAA appeared to occur between 10 and 30 min after gravistimulus in coleoptiles. Though IAA redistribution was observed within 30 min after the stimulus, intracellular distribution of ZmPIN1 did not change. The molecular components involved in lateral IAA transport have not been identified in grass coleoptiles. In Arabidopsis roots, AUX1, PIN2 and PIN3 are postulated to be involved in the IAA redistribution in response to gravistimulus. Furthermore, it is likely that not only the PIN family but also the PGP family controls the direction of IAA transport. Therefore, their role in the establishment of IAA redistribution in maize coleoptiles should be analyzed. More detailed analysis of IAA flow and localization, in relation to perception of gravistimulus, will be necessary to reveal the mechanisms by which the flow of IAA is regulated in response to gravistimulus.

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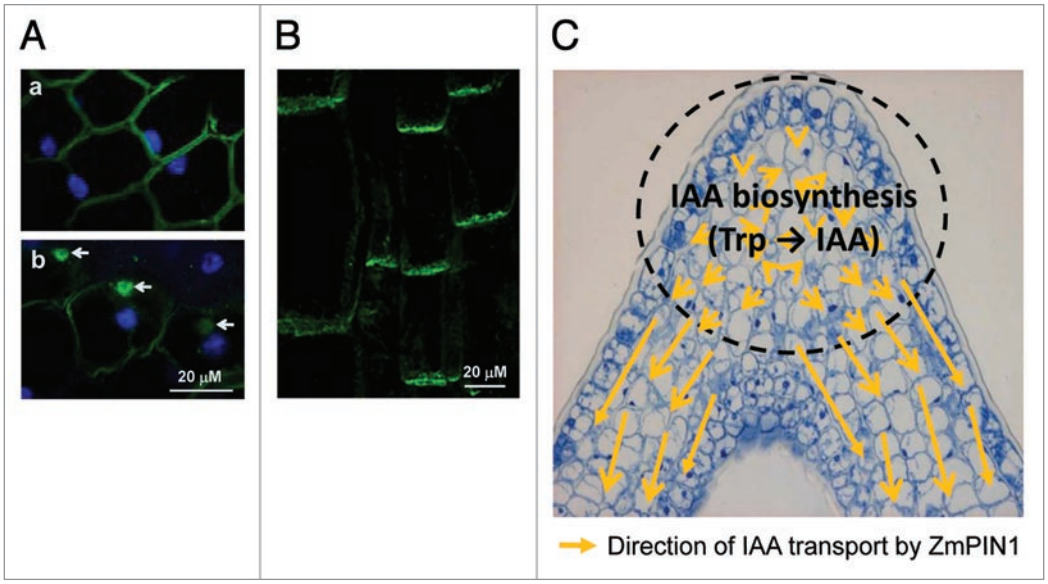

Figure 2. Immunolocalization of ZmPIN1(s). (A) Effect of BFA on intracellular localization of ZmPIN1(s) in maize coleoptile tips. (a) control (no BFA). (b) 300 μM BFA treatment. After 90 min incubation, BFA-induced aggregation of ZmPIN1(s) was observed in the cells in contrast to the control (arrows). (B) Localization of ZmPIN1(s) in the basal region of coleoptiles. ZmPIN1 was localized on basal membranes in the cells. (C) Hypothetical IAA flow direction from the tip to the basal region in maize coleoptiles, supposed by cellular ZmPIN1 localization in the tissue.

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