Addendum

Vesicular secretion of auxin

Evidences and implications

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The plant hormone auxin is secreted in root apices via phospholipase Dζ2 (PLDζ2) activity which produces specific population of phosphatidic acid that stimulates secretion of vesicles enriched with auxin. These vesicles were reported to be localized at plant synapses which are active in auxin secretion, especially at the transition zone of the root apex. There are several implications of this vesicular secretion of auxin. In root apices, auxin emerges as plant neurotransmitter-like signal molecule which coordinates activities of adjacent cells via electric and chemical signaling. Putative quantal release of auxin after electrical stimulation, if confirmed, would be part of neuronal communication between plant cells. As auxin transport across plant synapses is tightly linked with integrated sensory perception of environment, especially of omnipresent gravity and light, this process is proposed to mediate the plant perception of environment. These neuronal features allow sessile plants to integrate multitude of sensory signals into the adaptive behavior of whole plants and the animal-like exploratory behavior of growing roots.

Auxin as Multipurpose and Mobile Signaling Molecule

In recent years, the classical plant hormone auxin emerges to act rather as mobile multipurpose signaling and communicator molecule orchestrating plant development and integrating it with the environmental abiotic factors, especially light and gravity. While the mainstream literature still considers auxin only as plant hormone, data are emerging suggesting that auxin is rather multipurpose signaling molecule having also morphogen-like and neurotransmitter-like properties.[1,2] The most characteristic feature of auxin, making it unique in the whole eukaryotic super-kingdom, is its polar cell-cell transport traversing the whole plant body.[3,4] In trees, this transport pathway spans up to several meters. This organismic auxin transport is sensitive to physical parameters of environment, especially light and gravity, allowing plastic shaping of plant bodies in response to these omnipresent physical forces.[5,6]

Auxin, being a weak organic acid, is theorized to be transported in the framework of the so-called chemiosmotic hypothesis which considers only two relevant compartments: neutral cytoplasm and acidic cell walls (reviewed in ref. 7). The Acidic trap aspect of this hypothesis implicates that auxin is easily accumulated within cells but need to be actively transported across plasma membrane to leave plant cell. Putative auxin efflux transporters have been postulated in the 80-ties and later identified in the 90-ties. In the last decade, we are witnessing dramatic breakthrough in nature of these transporters.[7] This new age of auxin efflux biology started, paradoxically, with the discovery of auxin influx transporter AUX1 in 1996, followed by the identification and characterization of several efflux transporters of the PIN family starting in 1998. More recently, ABC transporters enter the field of polar transport of auxin.[8-10]

Almost all papers published until now on this topic consider the plasma membrane as the membrane which needs to be traversed with aid of transporter activities in order to export auxin out of plant cells. Nevertheless, the second possibility is that auxin is also transported into recycling vesicles and then, after appropriate signal, secreted out of cells.[2,11] This model of signal-mediated secretion of auxin is very appealing as it would have far reaching biological implications, including explanation of several data which do not fit the classical model. Most importantly, the ability of plant cells to handle auxin via vesicular carriers and signal-mediated vesicular trafficking and secretion would allow them to gain finely-tuned control over this mobile multipurpose signaling and communicator molecule.

Evidences for Vesicular Secretion of Auxin

Our recently published paper provides genetic evidences for the vesicular secretion of auxin in root apices driven by phosphatidic acid which is generated specifically via phospholipase Dζ2.[12,13] Besides our recent study on the PLDζ2, there are several other data not compatible with the original version of the chemiosmotic theory and implicating, at least in addition, vesicular secretion of auxin. First of all, two inhibitors of secretion having different targets and mechanisms of action, brefeldin A and monensin, inhibit rapidly and effectively auxin export out of plant cells.[14,15] The rapidity of their action (less than ten minutes) precludes the popular interpretation of these data that these inhibitors simply prevent vesicular targeting

of efflux carriers to the plasma membrane. Moreover, classical inhibitors of polar auxin transport, such as NPA and TIBA, turned out to be inhibitors of endocytosis[16] which, similarly like brefeldin A and monensin, block vesicle-mediated membrane recycling.

Further evidences include proper localization of PIN1 to the plasma membrane of root apex cells in maize mutants having inhibited auxin transport.[2] Importantly, these mutant root cells do not show abundant auxin at these PIN1-enriched domains (inactive plant synapses) as it is the case of wild-type cells (active plant synapses). In wild-type root apices, auxin is enriched only at those cross-walls which are active in auxin export, being abundant not only at walls but also in adjacent endosomes. Similar evidence is provided by forcing roots to grow against the gravity vector due to placing them into thin glass capillaries. Such roots get progressively thinner and their root apices get depleted of dividing cells due to inhibited supply of auxin which can not be transported effectively against the gravity vector. Nevertheless, PIN1 in these root apices is localized properly at cross-walls (plant synapses) even if these do not accomplish rapid recycling of PIN1 as revealed by the exposure of such roots to brefeldin A (Markus Schlicht, Alina Schick, Dieter Volkmann, and František Baluška, data in preparation).

Implications of Auxin Secretion: Gravisensing Plant Synapses

One of the most characteristic feature of the polar transport of auxin, which still awaits for its biological explanation, is that the auxin transport is tightly linked to the gravity being all the time perpendicular to the gravity vector.[17,18] Apparently, the dynamic plant synapse, keeping its transversal position with respect of auxin stream, is able to 'feel' gravity and to reposition itself if the plant organ (for example the root apex) is positioned horizontally.[19] Within some 30 minutes, such gravistimulated root apex curve downward and its auxin-secreting synapses regain transversal position with respect to the gravity vector. In the root cap, PIN3-mediated auxin asymmetry starts to be visible within few minutes.[20] Obviously, auxin is secreted out of root cells all the time towards the physical bottom, irrespective of organ and plant polarity. In other words, internal genetic and epigenetic programmes, which are extremely robust and resist single or even double-gene mutations, are prone to be easily overridden by epigenetic environmental information. One can explain this gravity-dependent secretion of auxin via differential stretch-stress of the plasma membrane[6,19] which is expected, due to protoplast settling, to have the highest values at the physical bottom and the lowest values at the physical top of cells.[6,19] As the high tension stress of the plasma membrane can be relieved by exocytosis while the low tension stress stimulates endocytosis, the secretory model of auxin transport has power to explain the gravity dependent auxin transport. This unique feature of gravisensing plant synapses implies that they are inherently asymmetric not only molecularly but also mechanically. Importantly, epigenetic physical information, which underlies inherent mechanical asymmetry of plant synapse, is part of the plant synapse secreting auxin according to the gravity vector and shaping flexibly the plant body.[6,19]

Implications of Auxin Secretion: Unique Status of Root Apices

Growing roots exhibit several animal-like features. Having two growth zones which can curve independently,[21,22] but in highly coordinated manner,[6] growing roots show animal-like curiosity

in their exploratory growth.[23] Removing of root cap prevents this exploratory nature of root growth. Charles and Francis Darwins noted (pages 349–54 in ref. 24), by repeating original experiments of Theophil Ciesielski, this unique behavior of roots encompassing also some kind of plant memory. Darwins proposed that root apex, covered with the root cap, resembles in many respects brains of lower animals.[24,25] They noted that decapped roots still grow but do not show this exploratory behavior, but after regenerating root cap they express physiological memory from previous gravistimulation. Interestingly, Julius Sachs was not able to repeat these experiments as he was apparently removing also parts of the apical root meristem (pages 349–54 in ref. 24).

Besides gravity, root apices continuously monitor numerous other parameters from their environment to obtain sensory information which is integrated to allow adaptive behavior of exploratory root apices. Recent advances identified sensor molecules for hydrosensing[26] and sensing of low phosphate[27] in gravisensing root cap statocytes. Blue light sensor Phot1 is not expressed in root cap cells but localizes to plant synapses in the transition zone.[28] In addition, growing roots are sensitive to magnetic and electric fields. Physiological and adaptive relevances of electrotropism and magnetotropis are still obscure and should be studied intensively in future in order to understand roots in their whole complexity.

In accordance with the complex behavior, root apices show much more complex auxin transport when up to 5 PINs and 4 ABC channels are involved.[9,29] This high complexity of auxin transport pathways contrasts with morphological simplicity of root apices. In contrast, shoot apices initiate primordia of leaves and lateral shoot branches closely to the shoot apex[30] which is morphologically much more complex. The shoot apex represent complex organogenic surface which is devoted to morphogenesis and initiates new organs in a highly controlled manner. In contrast, the lateral root primordia are formed far away of the root tip, only after root cells are ceasing their rapid elongation. Obviously, the root apex represents some kind of sensory surface specialized for sensing of many parameters of environment. This feature allows roots to grow with the animal-like curiosity, to explore soil in searching for water and satisfying the mineral nutrition for the whole plant.

Quantal Secretion of Auxin: Synaptic Memory Based on Integration of Signals, Receptors and Flux Sensors?

Vesicular secretion of auxin can be expected to be under tight control allowing quantal release of auxin into the plant synaptic cleft after specific stimuli are received. It can be expected that this feature would serve not only for synchronization of cells in one cell file but also for propagation of plant action potentials. Besides eliciting electrical responses in adjacent cells (reviewed in ref. 31), extracellular auxin manipulates also levels of reactive oxygen species and nitric oxide.[32-34] Thus, quantal release of auxin after proper signals can have complex consequences for developmental biology. Among other aspects, vesicle recycling and secretion-based auxin efflux can be envisioned to act as the elusive flux sensor which is necessary for the canalization theory. This would then make redundant new hypotheses such as travelling-wave hypothesis proposed recently.[35] Moreover, these auxin-enriched vesicles recycling at plant synapses could also be involved in gravi- (reviewed in ref. 6) and electric-memory[36] phenomena. As root apices are well protected from predatory and

other environmental insults, it would be logical to expect location of the putative master clock of the circadian system, which is in brains of animals and, perhaps, in the root system. In accordance with this scenario, recent study revealed that auxin signaling is tightly linked with the plant circadian clock[37] and that the root growth, in contrast to the shoot growth, is not showing circadian rhythms but shows rather steady growth behavior.[38]

Outlook

Vesicular secretion of auxin at root apices has several implications and consequences. First of all, the auxin status changes from plant hormone to multipurpose mobile signaling molecule which, depending on developmental or environmental context, acts as plant hormone, morphogen, or plant neurotransmitter-like molecule. Interestingly in this respect, auxin is not unique for plants and it is used also in fungi and bacteria—often for organismic communication of plants, especially their roots, with these nonplant organisms. For instance, symbiosis with Rhizobium bacteria and mycorrhiza fungi is established via bacterial and fungal manipulation of auxin flows in roots.[39,40] Future studies will answer the critical questions if plant action potentials are related to vesicular secretion of auxin and if auxin secreted out of cells synchronizes cell activities within adjacent cells.[41] Should there be positive answers to these questions then this would place auxin also outside the plant physiology, reaching up to realms of plant neurobiology.

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