Perspectives

Light-dependent control of redox balance and auxin biosynthesis in plants

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Abstract

Auxin, indole-3-acetic acid (IAA), plays a crucial role for morphogenesis, development, growth and tropisms in many plant species. Many studies have been investigating that auxin is biosynthesized via specific pathways depending on several enzymes started from amino acid, tryptophan. The particular region of auxin biosynthesis in maize was identified at the tip of coleoptile expressing abundant YUCCA (YUC) protein, which is essential for auxin synthesis. However, it is still mystery whether auxin biosynthesis is dependent on only such enzymes or not. *In vitro* experiment demonstrated that precursor of auxin molecule; indole-3-acetaldehyde (IAAld) was generated by light illumination of the mixture of tryptophan and flavin compounds in non-enzymatic manner. In addition, we have detected immediate production of reactive oxygen species (ROS) in illuminated Arabidopsis root cells. In this perspective, we are proposing the non-enzymatic regulation of redox homeostasis and auxin biosynthesis throughout the plant body under variable environmental light conditions.

Plant in light environment

Light is one of important environmental factors not only for photosynthesis producing sugars but also for plant germination, growth and morphogenesis. Since environmental light condition is not always uniform for a plant life cycle, plants have to cope with it using photo-responsive mechanisms based on several physiological signaling cascades. To date, many photoreceptors have been investigated which convert light information, especially specific wavelengths, into cellular signaling pathways. A growth toward or against light direction is mentioned as phototropism. Completing phototropic curvature, plants require asymmetrical growth modulation either at illuminated or at unilluminted side. In general, positive phototropism as aerial part of plants show is to maximize the efficiency of photosynthesis. Negative phototropism of roots allows them to escape from unfavorable light condition to go back into the dark soil. During photoresponses of plants, phytohormone auxin was shown to play an important role for the movements of plants by regulating differential cell growth. It has been considered that auxin molecule needs to be transported from one organ side to the another/opposite one in illuminated plant organs, which is referred to as Cholodony-Went model.[1] However this model remains controversial as although auxin might be involved in the growth regulation, it is not good enough to provide satisfactory explanations for plant tropic growth regulated only by auxin migration.[2-4] In following section, we are discussing that light might directly activate or influence photo-sensitive chemical compounds in plant cells that affect physiological condition resulting in phototropic plant movements.

Light-activated radical generation

Since light photons have physical energy, they can excite many chemical species with certain structures. Flavin compounds are ubiquitously present in cells such as riboflavin (Vitamin B_2). Both flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are present in flavoproteins. It was reported that flavins behave as photosensitizer to produce radical species in living cells. Hockberger et al. showed that hydrogen peroxide (H_2O_2) was produced in mammalian cells by blue light irradiation and as the ultimate source of H_2O_2 were proposed the photo-excited flavin-containing oxidases abundant in peroxisome.[5] Electron paramagnetic resonance (EPR) method revealed the illumination of flavin mononucleotide derived from cell fraction produced

reactive oxygen species (ROS).[6] It was also biochemically shown that superoxide anion radical, one kind of ROS, was generated by the reaction between reduced flavin and flavoproteins with molecular oxygen.[7,8] Interestingly, it has been recently reported that cryptochromes, flavin-biding photoreceptor found in animals and plants, are used as magnetoreceptor of bird, fish and insects.[9] Photo-excited flavin forms superoxide-flavin radical pair in the protein, which is probably important for detecting the magnetic field of the earth.[10] In plant cells, it was demonstrated that reactive oxygen species were generated in tobacco BY-2 cells during fluorescence microscopic observation even after 10 – 20 seconds of illumination of blue wavelength. This suggests that light for the excitation of fluorescent proteins such as GFP also contributes to endogenous ROS production, which causes potential cell damage during microscopy of living cells.[11] We have also reported that the blue light illumination for 10 seconds to Arabidopsis root elicited the generation of ROS in the cells of root apex region.[12,13] It is well known that ROS molecules in plant cells have many crucial physiological roles. ROS generated in the illuminated cells are likely to modulate cellular signaling resulting in the regulation of root photo-escaping growth.

Redox status affects auxin signaling

As aforementioned, auxin is an essential small molecule that controls plant phototropic growth. Several pathways for the biosynthesis of auxin molecule have been proposed. In the case of maize, it was shown that auxin can be produced from tryptophan by certain enzymes at the tip of coleoptile.[14] Koshiba and Matsuyama[15] demonstrated the *in vitro* formation of auxin molecule in the presence of tryptophan and crude extract derived from maize coleoptile. Cytosolic ascorbate peroxidase was identified from the coleoptile extract as the enzyme that helps the formation of auxin.[15] In Arabidopsis, it has been studied that cytosolic ascorbate peroxidase is a key player for controlling cellular redox balance in response to many stress conditions.[16,17] Besides auxin biosynthesis, it was also reported that ROS and auxin are closely connected in cellular signaling events.[18] Schopfer et al[19,20] demonstrated that auxin treatment of maize coleoptile increased the production of superoxide. This is a precursor of hydroxyl radical, one of the most active oxygen radicals, loosening cell wall by cleaving polysaccharide resulting in the promotion of cell growth. During root gravitropism, the

ROS-production by auxin in root cells was also reported.[21] Recently, it was shown that ROS molecule have an effect of attenuation of auxin signaling through the oxidation of IAA. As a result, oxIAA, 2-oxindole-3-acetic acid, is produced as an irreversible product and does not induce auxin-responsive gene, DR5, expression.[22] If environmental light is enough to provoke the generation of ROS in plant cells, then it is plausible that auxin function or distribution might be affected, to a greater or lesser extent, via illumination.

Light-regulated auxin biosynthesis

Koshiba et al[23] demonstrated that light-dependent *in vitro* indole-3-acetaldehyde (IAAld) synthesis in the presence of tryptophan and flavins such as riboflavin, flavin FMN and FAD. It is very intriguing that the generation of IAAld as a precursor of IAA is promoted only by photo-excited flavin compound. IAAld can be oxidized by aldehyde oxidase (AO; EC 1.2.3.1) to produce IAA.[24,25] As summarized in figure 1, this reaction is likely to occur in the specific region receiving light (photon) energy. Additionally, as aforementioned, flavins itself produce ROS by light excitation, and ROS might be related to *de novo* auxin synthesis by attacking tryptophan molecule as proposed in figure 1. Flavins in living cells are found not only as free molecule e.g., riboflavin, but also in the protein-bound form, so-called flavoproteins. It is well documented that there are large numbers of flavoproteins, which use flavin as coenzyme in cellular signaling or metabolism. Interestingly, both YUCCA protein (flavin monooxygenase)[25] and AO protein[26] are important for auxin biosynthesis harboring FAD as a cofactor. FAD and FMN bound to proteins basically play a role in helping electron transfer while catalytic reaction. However, it functions also as chromophore and can be activated into photo-excited state by perceiving photon energy. Recently Nishimura et al[27] reported that YUCCA protein is abundantly expressed at the tip of maize coleoptile.[27] Besides, it was reported OsYUCCA1-GUS expression was observed only at rice root tip region but not in other part.[28] Since the sensitivity to light in a tip region of both coleoptile and root apex have been well documented[29-31], it might be relevant to a contents of flavo-protein like YUCCA or any other light-responsive factors/compounds compared to other region of plants. It implies that auxin synthetic reaction seems to be affected by light condition. Since light conditions of illuminated

plant body are not always uniform, different auxin levels can be produced by light at illuminated and shaded side of plants.

Light is a crucial environmental factor for many land plants. Living organisms on the ground have always been exposed by light from the sun for a long evolutionary process. Although many efforts of research work for investigating light perception of plant have been made, there are still unknown mechanisms that are not dependent on specific pathways. In other words, an initial light response of photoreceptors can be assumed as a certain physicochemical reaction. As discussed in this perspective, we propose that local auxin synthesis in plant cells might be promoted solely by illuminating these cells, resulting in prompt responses to light in form of positive or negative phototropism.

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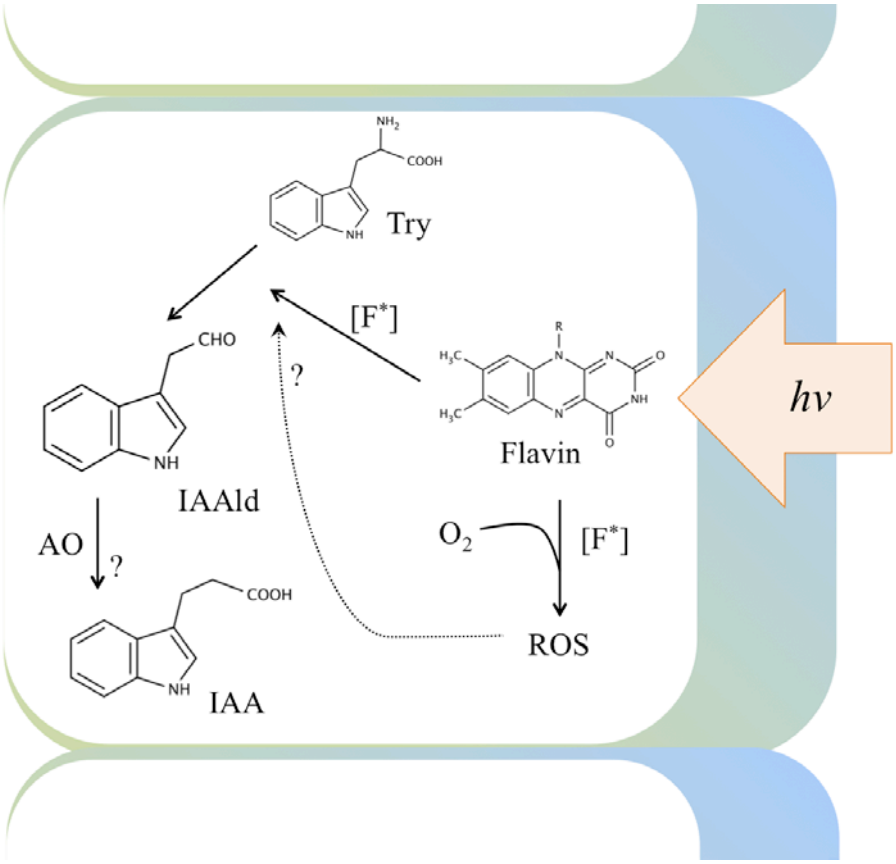
Figure



Local auxin biosynthesis via light-excited flavin compounds in the presence of tryptophan in plant cells. Indole-3-acetaldehyde (IAAld) is likely to be converted into auxin by endogenous aldehyde oxidase (AO). Excited flavin also generates reactive oxygen species.[8] This figure is adapted from the reference 23.