

## Auxin biology in roots

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Received on October 16, 2012; Accepted on June 12, 2013

**Abstract:** Auxin regulates almost every aspect of plant growth and development. Its intracellular concentration is controlled by biosynthesis and degradation. In addition, there is an “auxin pool” that consists of the conjugates with sugars, amino acids, and peptides. Some of the conjugates reversely release auxin, enabling alternative methods to regulate auxin concentrations. Auxin concentrations are also affected by transport. Besides the long distance delivery through the phloem, auxin is transported across the cell by influx and efflux carriers, from the shoot to root with maximum concentration at the root tip. At the root tip, the auxin flow reverses, and shootward auxin transport occurs. An auxin gradient formed this way is indispensable for proper development, maintenance of the meristem, and cell identity. The formation of root hairs is auxin-dependent. Auxin controls not only the initiation of root hairs but also regulates their elongation. In *Arabidopsis thaliana*, auxin accumulates in atrichoblasts and it is supplied to trichoblasts. Before hair initiation, randomization of cortical microtubule arrays is observed in lettuce seedlings. This action is promoted by auxin and is indispensable to hair formation. Furthermore, light promotes CMT randomization and root hair initiation via auxin signaling. Ethylene is another promoter of root hair formation. Ethylene affects auxin signaling and vice versa. Interactions between these hormones are synergistic for root growth inhibition but antagonistic for lateral root formation. Reactive oxygen species also regulate various responses in plants. They play an important role during root hair elongation, although their precise relationship with auxin is yet not clear.

**Keywords:** auxin, biosynthesis, metabolism, root growth, root hair, transport

**Abbreviations:** ACC, 1-aminocyclopropane-1-carboxylic acid; GUS,  $\beta$ -glucuronidase; IAA, indole-3-acetic acid; MS, mass spectrometry; NADPH, nicotinamide adenine dinucleotide phosphate

### Introduction

The life of a plant begins with seed germination and ends with death. Throughout their lifecycle, plants are always affected by various environmental stimuli such as gravity, temperature, and drought. In addition, they sometimes interact with other organisms. Because plants cannot move like animals, they have to critically monitor the surrounding environment and adapt to it by constantly changing their physiological conditions. Plant hormones play important roles in such processes.

Among the plant hormones, auxin was the first to be identified (Kögl and Kostermans 1934, Went and Thimann 1937). Its research history can be traced back more than 100 years. As early as 1880, Darwin and Darwin (1880) and von Sachs (1880) proposed the existence of a mobile substance which regulates plant growth. Today, auxin is known to play central roles in the regulation of growth and development of various plant organs. Auxin has crucial roles not only for shoot development but also in the development and patterning of the root (Sabatini et al. 1999, Galinha et al. 2007, Dinneny and Benfey 2008).

Even in the roots, many processes are highly dependent on auxin signaling: the establishment of the root pole in embryos (Friml et al. 2003b, Weijers et al. 2006), formation of the stem cell niche (Sabatini et al. 1999, Blilou et al. 2005, Vernoux and Benfey 2005, Sablowski 2007, Dinneny and Benfey 2008), maintenance of mitotic activity in the meristem (Beemster and Baskin 2000, Dello Ioio et al. 2007, Galinha et al. 2007, Stepanova et al. 2008), gravitropism (Chen et al. 1998, Rashotte et al. 2000, Sukumar et al. 2009), root hair formation (Masucci

and Schiefelbein 1996, Pitts et al. 1998), lateral root formation (Reed et al. 1998, Casimiro et al. 2001, Bhalerao et al. 2002), and elongation and differentiation of root cells (Rahman et al. 2007). The control of the auxin concentration in each cell is, therefore, important for proper root growth and development. For this, plants not only synthesize and degrade auxin but also have an “auxin pool,” which consists of auxin conjugates with sugars, amino acids, and peptides (Tanimoto 2005, Bajguz and Piotrowska 2009, Normanly 2010, Mano and Nemoto 2012, Zhao 2012).

Besides through metabolism, the auxin concentration is also controlled by its transport systems. One is a delivery system through the phloem, and the other involves the auxin influx and efflux carriers (Tanimoto 2005, Bohn-Courseau 2010, Overvoorde et al. 2010, Tromas and Perrot-Rechenmann 2010). Because the intracellular locations of these carrier proteins are critically restricted, plants enable the directional delivery of auxin, which is called “polar auxin transport.”

In the first half of this review, I have summarized auxin biology, namely, its biosynthesis, metabolism, and transport. In the second half, I have mentioned the role of auxin in root growth and development. However, the roles of auxin are too diverse, and auxin usually interacts with other plant hormones when exerting certain effects. Therefore, I have focused on and summarized the role of auxin in root hair formation and the interaction of auxin and ethylene in root growth and development.

### Auxin biosynthesis

The major form of natural auxin identified in plants is indole-3-acetic acid (IAA). Previously, it was considered that IAA was produced mainly in young leaves and transported throughout the plant body. However, it has been clarified that auxin can be synthesized in virtually all tissues, including roots (Müller et al. 1998b, Ljung et al. 2001, 2005, Marchant et al. 2002, Stepanova et al. 2005, 2008, Ikeda et al. 2009, Yamada et al. 2009). Similar to the other plant hormones, auxin, which is present in low concentrations, functions in a dose-dependent manner (Bhalerao and Bennett 2003). Therefore, the fine-tuning of auxin homeostasis in each cell is important for the control of physiological conditions, and thus, for proper growth and development.

The simplest way to control the intracellular auxin level is to regulate its biosynthesis. IAA is synthesized through two major pathways. The first is the tryptophan-dependent pathway, which consists of up to four distinct routes (Normanly 2010, Zhao 2010, Mano and Nemoto 2012): (i) indole-3-acetamide

pathway; (ii) indole-3-pyruvic acid pathway; (iii) tryptamine pathway; and (iv) indole-3-acetaldoxime pathway. Mutant analyses revealed that YUCCA (YUC) flavin monooxygenases (Zhao et al. 2001, Cheng et al. 2006, 2007a, Dharmasiri et al. 2005b) and TRYPTOPHAN AMINOTRANSFERASE OF *ARABIDOPSIS* (TAA) family of amino transferases (Stepanova et al. 2008, Tao et al. 2008) play important roles in auxin biosynthesis, because the inactivation of the members of either family caused severe developmental defects. Although YUCs and TAAs have been considered as a part of two independent pathways, several studies have suggested that they participate in the same auxin biosynthesis pathway because these mutants show similar phenotypes (Cheng et al. 2007b, 2008, Strader and Bartel 2008, Zhao 2010). Recently, TAA was found to convert tryptophan to indole-3-pyruvate, and YUCs were suggested to function in the conversion of indole-3-pyruvate to IAA (Mashiguchi et al. 2011, Stepanova et al. 2011, Won et al. 2011). Of the four above-mentioned routes, the one based on indole-3-pyruvate pathway has been speculated to be mainly involved in IAA biosynthesis (Mashiguchi et al. 2011, Won et al. 2011). However, whether the TAA-YUC pathway is widely distributed in the plant kingdom is yet unknown.

The second major pathway for IAA synthesis is the tryptophan-independent pathway. Although the molecular components and physiological functions of this pathway are yet to be elucidated, it surely exists because the *A. thaliana* mutants that are defective in tryptophan synthase  $\alpha$  or  $\beta$  can accumulate IAA conjugates (Last et al. 1991, Radwanski et al. 1996). For the details of each pathway, see reviews such as Mano and Nemoto (2012) and Zhao (2012).

### Auxin metabolism

Plants sometimes have to decrease, instead of increasing, auxin concentration to cease the effect of auxin. The oxidation of IAA plays a major role in the decrease in auxin concentration (Östin et al. 1998, Kowalczyk and Sandberg 2001). Besides this, plants have developed other ways to eliminate active auxin. Plants synthesize the auxin conjugates. Most of the endogenous IAA of plants is found not in the free and biologically active form, but as conjugates that can be divided into three main groups (Bajguz and Piotrowska 2009, Normanly 2010): (i) low-molecular-weight amide conjugates with amino acids, (ii) low-molecular-weight ester conjugates with sugars, and (iii) high-molecular-weight conjugates with peptides and proteins.

Of these three forms, the amide conjugate with amino acids is well studied. GH3 proteins catalyze

the formation of the conjugates *in vitro* (Staswick et al. 2005). Several types of auxin conjugates with amino acids are known to exist. Auxin conjugates with glutamate and aspartate are eventually degraded in a manner similar to that of IAA oxidation (Östin et al. 1998, Tam et al. 2000, Kowalczyk and Sandberg 2001).

On the other hand, auxin conjugates with amino acids such as alanine and leucine can provide free IAA through their hydrolysis. Formation of these conjugates is thought to store the excess IAA, and such reversible conversion processes might allow these conjugates to form a convenient intracellular “pool” of IAA. Because some *GH3* genes are auxin-inducible (Hagen and Guilfoyle 1985), elimination of active IAA as auxin conjugates might facilitate plants to avoid the over-function of auxin.

Further, Staswick (2009) found another type of IAA conjugates. Unlike other IAA conjugates, the IAA conjugate with tryptophan was found to be an IAA antagonist. It not only inhibited IAA-induced lateral root formation but also rendered agravitropic root growth and resistance to root growth inhibition by high concentration of auxin (Staswick 2009).

Interestingly, accumulating evidences have shown the involvement of auxin conjugates in the tolerance for biotic and abiotic stresses such as plant-pathogen interaction, salt, drought, high temperature, and freezing (Oetiker and Aeschbacher 1997, Junghans et al. 2006, Park et al. 2007, Zhang et al. 2007, Ding et al. 2008). In these responses, several auxin conjugate hydrolases and GH3 proteins play important roles to balance the effects of these stressors on auxin physiology (Ludwig-Müller et al. 1996, Staswick et al. 2005, Junghans et al. 2006, Park et al. 2007, Campanella et al. 2008, Ding et al. 2008).

The IAA conjugates function simultaneously in transport, compartmentalization, and protection against peroxidative degradation (Cohen and Bandurski 1982). The endoplasmic reticulum is predicted to be an important compartment for auxin conjugates (Mravec et al. 2009, Ludwig-Müller 2011). This prediction has been made on the basis of the observations that the auxin binding protein 1 (ABP1) and most of the auxin conjugate hydrolases have endoplasmic reticulum retention signals (Anai et al. 1997, Campanella et al. 2003), and that the IAA transporter PIN-FORMED 5 (PIN5) is localized to endoplasmic reticulum (Mravec et al. 2009).

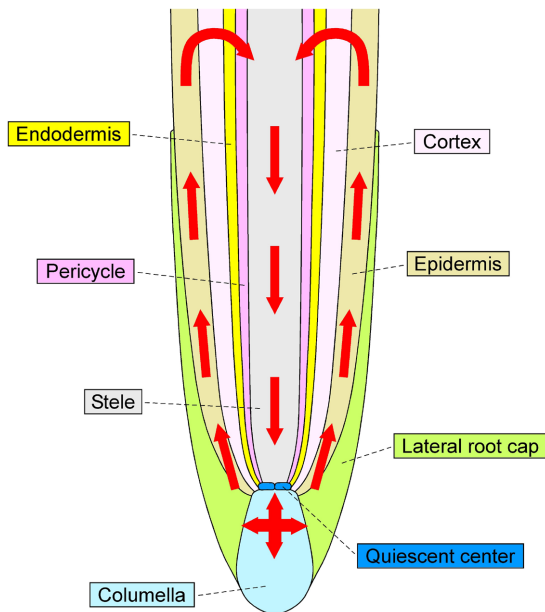
## Transport

When compared to other plant hormones, one of the outstanding features of auxin is its directional transport. Although auxin can be synthesized in most tissues (Ljung et al. 2001, 2005, Marchant et al.

2002), the main synthesizing portion is young leaves and cotyledons (Ljung et al. 2001). To deliver the synthesized auxin to the entire plant body, plants have developed two types of transporting systems. One is the non-specific auxin transport through the phloem with the sap flow (Morris and Kadir 1972). Most IAA is likely to be transported via the phloem (Tromas and Perrot-Rechenmann 2010). Since this IAA transportation occurs through membrane-less phloem channels, the auxin movement is rapid, reaching up to  $7 \text{ cm}\cdot\text{h}^{-1}$  in the roots of *Populus tremula* and *Vicia faba* (Eliasson 1972, Tsurumi and Wada 1980). Therefore, this system has the advantage of easily and quickly delivering auxin over long distances.

The other auxin transport system is a directional and active system from cell to cell using membrane-integrated transport proteins. Therefore, this system is more suitable to the fine-tuning of the auxin concentration in individual cells. The auxin movement controlled by this system is commonly referred to as “polar auxin transport” (Fig. 1). The flow begins from the shoot apex, moves toward the base of the plant, and finally reaches the root tip. There, the rootward transport through the central tissues allows a loop flow, and auxin is reversely transported in a shootward direction through the lateral root cap and epidermis (Blilou et al. 2005). Just below the beginning of the elongation zone, the auxin flow is recycled into the stele (Grieneisen et al. 2007, Doerner 2008). In these flows, the auxin shows the maximum concentration in the quiescent center, is less elevated in the endodermis and cortex, and is weak in the epidermis (Sabatini et al. 1999, Petersson et al. 2009). The rates of the polar auxin transport in roots of *Phaseolus vulgaris*, *Pisum sativum*, and *A. thaliana* reach  $1 \text{ cm}\cdot\text{h}^{-1}$ , which is significantly slower than the auxin flow through the phloem (Overvoorde et al. 2010).

Because auxin is a weak acid with  $\text{pK}_a = 4.75$ , it takes two chemical forms depending on the pH of the surroundings: a protonated form (IAAH) and anionic form (IAA<sup>-</sup>). Because the pH in the cell wall is relatively acidic around pH 5.5, about 15% of IAA is in its protonated form. The protonated form can diffuse to some extent into the cell across the cell membrane because auxin is a small molecule, and its protonated form is electrically neutral. Once in the cytosol, the pH is around 7.2. Thus, auxin favors the dissociation into the anionic form in the cytosol. Because the anionic form is electrically charged, it cannot be easily transported across the cell membrane into neighboring cells. Therefore, membrane-integrated transporter proteins are required for auxin exportation (Rubery and Sheldrake 1974, Davies and Sheldrake 1978, Bohn-Courseau 2010).



**Fig. 1.** Auxin flows around the root tip of *Arabidopsis*. Auxin, which comes from the shoot apex, passes the shoot–root junction, and it is further transported in a rootward direction in the stele in the root. At the root tip, auxin redistributes, and then it is transported in a shootward direction through the lateral root cap and epidermis. Immediately below the beginning of the elongation zone, the inverted auxin flow is recycled into the stele.

#### Auxin influx carriers

Although auxin can enter the cell by diffusion because of its chemical properties as mentioned above, plants also have influx carrier proteins for auxin (Imhoff et al. 2000, Parry et al. 2001a, 2001b, Tanaka et al. 2006, Teale et al. 2006). AUXIN RESISTANT 1 (AUX1) is the first influx carrier that was identified. AUX1 protein of *A. thaliana* comprises 485 amino acids with 11 transmembrane domains (Bennett et al. 1996). In later studies, three close relatives i.e. LIKE-AUX 1 (LAX1), LAX2, and LAX3 were found (Parry et al. 2001b, Swarup et al. 2008, Péret et al. 2012). The AUX1/LAX proteins act as symporters, which enable the entry of the anionic form of auxin with the help of the proton gradient (Yang et al. 2006).

Although AUX1/LAX family members have similarities to each other, they seem to have different roles. For example, the formation of lateral roots is one of the famous auxin-dependent responses in roots. Their initiation depends on local auxin accumulation and detection in the pericycle (Benková et al. 2003). AUX1 is required for the initiation of lateral roots

and is involved in the shootward auxin transport from the root tip toward the basal region through outer cell layers (Swarup et al. 2001, De Smet et al. 2007). On the other hand, LAX3 promotes lateral root emergence by affecting the auxin influx of the outer endodermis and cortex cells (Swarup et al. 2008). Member-specific developmental functions and regulatory mechanisms among AUX/LAX family are also reported in the recent study by Péret et al. (2012).

#### Auxin efflux carriers

Because auxin favors the electrically charged anionic form in the cytosol, efflux carrier proteins are essential for auxin to pass through the cell membrane. Thus far, two types of efflux carriers have been identified. The first one to be discovered is a member of the PIN family. The PIN1 protein of *A. thaliana* is composed of 622 amino acids with 8–12 putative transmembrane domains (Gälweiler et al. 1998). The phenotype of *pin1* mutants resembles that of the wild-type plants treated with the auxin efflux inhibitor, supporting an idea that PIN1 participates in auxin efflux. Now, 8 members (PIN1–PIN8) have been identified. However, their expression patterns differ, and each member seems to have specific roles (Friml and Palme 2002, Friml et al. 2002, 2003b, Ganguly et al. 2010) as the auxin influx carrier AUX1/LAX family members do.

For example, PIN1 and PIN7 establish the auxin gradient, which is needed to specify the basal root pole in the embryo (Friml et al. 2003b). During and after embryogenesis, PIN1, PIN3, PIN4, and PIN7 localize on the rootward side in the stele cells and direct the auxin flow toward the quiescent center (Friml et al. 2002, Blilou et al. 2005). In the root tip, PIN3 and PIN7 laterally localize and turn the auxin flow to the lateral root cap and the epidermis (Müller et al. 1998a, Friml et al. 2003a). PIN2 localizes on the shootward side in epidermal cells and redirects this auxin flow upward to the end of the meristematic zone where PIN1, PIN3, and PIN7 recycle auxin to the stele. Such PIN family member-specific distribution patterns form a loop of auxin flow at the root apex.

Several studies have demonstrated that the asymmetrical intracellular localizations of PIN proteins are regulated by auxin and the phosphorylation state of the PIN (Geldner et al. 2001, Friml et al. 2004, Paciorek et al. 2005, Michniewicz et al. 2007). One of the most characterized regulators is PINOID (PID). PID encodes a serine-threonine protein kinase, and its mutant shows a *pin*-like phenotype (Bennett et al. 1995, Christensen et al. 2000, Benjamins et al. 2001). Michniewicz et al. (2007) found that PIN is directly

phosphorylated by PID. This phosphorylation is important to the regulation of PIN localization and thus to the polar auxin transport (Woodward and Bartel 2005, Tanaka et al. 2006, Teale et al. 2006).

Besides the PIN family members, recent studies have revealed another auxin efflux carrier group. Members of the *A. thaliana* multidrug resistance (MDR)/P-glycoprotein (PGP) subfamily of ATP-binding cassette (ABC) transporters (ABCB/MDR/PGPs; Martinoia et al. 2002, Jasinski et al. 2003, Verrier et al. 2008) specifically bind the auxin efflux inhibitor NPA (Murphy et al. 2002). Their mutants or antisense plants show a pleiotropic auxin-related phenotype (Luschnig 2002). Auxin levels, expression of the auxin responsive DR5::GUS reporter gene, and rootward auxin transport are reduced in the mutants of ABCB/MDR/PGPs (Noh et al. 2001, Geisler et al. 2005, Lin and Wang 2005). Furthermore, Geisler et al. (2005) confirmed that AtPGP1 catalyzed the efflux of auxin and its oxidative products. All of these observations support the idea that ABCB/MDR/PGPs actually act as auxin efflux carriers (Luschnig 2002).

In contrast to PIN proteins, the localization of ABCB/MDR/PGPs at the plasma membrane is mostly nonpolar (Geisler et al. 2005, Petrášek et al. 2006, Cho et al. 2007, Mravec et al. 2008). ABCB/MDR/PGPs are thought to support auxin flow mediated by PINs. They may participate in the separation of auxin flows toward the root tip through the stele and those from the root tip to the epidermis (Wu et al. 2007, Mravec et al. 2008). Interestingly, mutations in *A. thaliana* ABCB/MDR/PGPs cause the mislocalization of PIN1, showing the interaction of PINs and ABCB/MDR/PGPs (Noh et al. 2003). Noh et al. (2003) found that ABCB/MDR/PGPs regulate the stability of PIN proteins in the plasma membrane. Therefore, ABCB/MDR/PGPs may function not only in the direct transport of auxin but also in the regulation of PIN localization (Luschnig 2002, Noh et al. 2003). These interactions appear to impart the specificity and directionality of auxin transport, thus establishing the polar auxin transport (Bandyopadhyay et al. 2007, Blakeslee et al. 2007).

### Auxin signaling

Mutant analyses have revealed a complicated but attractive mechanism of auxin signaling. Further, the most studied pathway is that which regulates the transcription of auxin-inducible genes. This pathway involves the nuclear auxin receptors TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX PROTEINs (TIR1/AFBs; Dharmasiri et al. 2005a, Kepinski and Leyser 2005) and the AUX/IAA family of transcriptional repressor proteins. When

auxin is absent, the AUX/IAA repressors form heterodimers with transcription factors named auxin response factor (ARF) and inhibit the transcription of auxin-inducible genes. When auxin is present, the SKP-Cullin-F-box<sup>TIR1/AFB</sup> (SCF<sup>TIR1/AFB</sup>) ubiquitin ligase complex catalyzes the ubiquitination and degradation of AUX/IAA proteins via the 26S proteasome system (Tan et al. 2007, Chapman and Estelle 2009). This change activates the ARF to induce auxin-inducible gene expression (Kepinski 2007).

Another auxin signaling pathway involves extra-cellular and cytoplasmic auxin receptors (Napier et al. 2002, Badescu and Napier 2006). The best characterized is auxin binding protein 1 (ABP1), which was first identified in maize (Löbner and Klämbt 1985, Venis and Napier 1995). Although most ABP1 is retained in the endoplasmic reticulum, it also exists at the plasma membrane or the extracellular matrix (Jones and Herman 1993, Diekmann et al. 1995, Leblanc et al. 1999). Tromas et al. (2009) revealed that ABP1 is essential for root growth. They proposed a model in which ABP1 is the major regulator for auxin action on the cell cycle, and it regulates auxin-mediated gene expression and cell elongation together with the TIR1/AFBs-mediated pathway. The mutant analysis suggests that ABP1 is implicated in the promotion of polar auxin transport (Effendi et al. 2011). The auxin binding to ABP1 reduces the clathrin-mediated endocytosis of PIN protein and enhances the auxin efflux transport (Geldner et al. 2001, Dhonukshe et al. 2007, Robert et al. 2010).

In addition to these auxin receptors, recent research identified a new receptor named S-phase kinase-associated protein 2A (SKP2A; Jurado et al. 2008, 2010). SKP2A is a nuclear F-box protein that can directly bind to auxin. It promotes the proteolysis of cell cycle transcription repressors and induces cell division (del Pozo et al. 2006, Jurado et al. 2008, 2010). On the other hand, auxin promotes the degradation of SKP2A through the 26S proteasome system. It is speculated that in the presence of auxin, SKP2A promotes the degradation of cell cycle repressors; subsequently, auxin enhances proteolysis of SKP2A to prevent its over-function (Jurado et al. 2008).

### Role of auxin in root hair formation

Root hairs are tubular protrusions from root epidermal cells that are formed by polarized tip growth. Root hairs increase the root surface area and enhance the uptake of water and nutrients from roots. They also play an important role in providing sites for interaction with a range of symbiotic microorganisms (Clarkson 1985, Dolan et al. 1994, Hofer 1996,

Peterson and Farquhar 1996, Ridge 1996). Because a root hair arises from a single epidermal cell and it is easily observed without dissecting the plant body, it has been a good model for studying the differentiation of a single cell.

The plant hormone auxin plays important roles in the formation of root hairs (Masucci and Schiefelbein 1996, Pitts et al. 1998). Auxin-resistant or auxin-insensitive mutants, such as *aux1*, *auxin resistant 1* (*axr1*), *axr2*, and *axr3*, are affected in root hair growth (Lincoln et al. 1990, Wilson et al. 1990, Okada and Shimura 1994, Masucci and Schiefelbein 1996, Leyser et al. 1996, Pitts et al. 1998, Rahman et al. 2002, Knox et al. 2003). Below, I have summarized how, when, and where auxin coordinates the formation of root hairs.

#### *Root hair initiation*

The root hair formation consists of two different stages. The first stage, called “root hair initiation,” is the formation of a small dome at the specific surface area of root epidermal cells. In *A. thaliana*, two types of root epidermal cells are determined in a position-dependent manner. Root hair cells (trichoblasts) arise over the crevice between two underlying cortical cells, whereas non-hair cells (atrachoblasts) develop over the position of a single cortical cell.

The patterning of the two epidermal cells is critically regulated by plural genes such as *TRANSPARENT TESTA GLABRA* (*TTG*), *GLABRA 2* (*GL2*), *GL3*, *ENHANCER OF GLABRA 3* (*EGL3*), *WEREWOLF* (*WER*), *CAPRICE* (*CPC*), *TRYPTICHON* (*TRY*), and *ENHANCER OF TRY AND CPC 1* (*ETC1*) (Grierson and Schiefelbein 2002, Pesch and Hülkamp 2004, Xu et al. 2005, Schellmann et al. 2007, Schiefelbein et al. 2009). Most of them encode transcription factors that influence the expression of genes involved in epidermal cell differentiation. Besides these regulatory gene products, auxin is assumed to modulate the final epidermal cell-type pattern in response to environmental factors.

Normally, only one hair arises from a single hair cell because the initiation position of root hair is critically controlled among the cells. In the case of *A. thaliana*, root hairs emerge close to the rootward ends of hair-forming cells (Masucci and Schiefelbein 1994, Grebe et al. 2002, Fischer et al. 2007). However, the exogenous application of auxin moves the hair position to the rootward end of the cells. In contrast, the auxin responsive mutant *axr2-1* or auxin influx mutant *aux1* causes a shootward shift of the hair position. Furthermore, the application of the auxin transport inhibitor also disturbs the hair positioning (Masucci and Schiefelbein 1994, Sabatini et al. 1999,

Grebe et al. 2002). These findings indicate that both appropriate response to auxin and auxin influx are needed to appropriately determine the root hair position.

Not only the influx of auxin but also its efflux is important in root hair positioning. Rigas et al. (2001) found that multiple root hairs are formed in *tiny root hair 1* (*trh1*) mutants. TRH1 encodes a potassium transporter, which interestingly enhances auxin efflux (Vicente-Agullo et al. 2004). Because the expression of TRH1 is restricted to the root tip, it is expected to function in the auxin transport from the root tip (Vicente-Agullo et al. 2004). Therefore, root hair positioning along epidermal cells is coordinated by the auxin gradient in the root, which is made by both influx and efflux of auxin (Sabatini et al. 1999, Grebe 2004, Fischer et al. 2006).

#### *Root hair elongation*

Besides an involvement in the process of root hair initiation (Masucci and Schiefelbein 1994, Grebe et al. 2002, Knox et al. 2003, Fischer et al. 2006), auxin also plays an important role in the elongation of root hairs (Pitts et al. 1998, Rahman et al. 2002, Lee and Cho 2006, Cho et al. 2007). Exogenous application of auxin to *A. thaliana* increases the length of root hairs, whereas inhibition of auxin signaling or disruption of auxin transport results in a decrease in their length (Pitts et al. 1998, Rahman et al. 2002). Furthermore, when the intracellular auxin concentration of developing hair cells is artificially changed, the length of the resulting root hairs shows a positive correlation with the auxin concentration (Lee and Cho 2006, Cho et al. 2007). The promoting effect of auxin in root hair elongation is also observed in lettuce seedlings (Takahashi and Inoue 2008). Because interesting findings have accumulated concerning lettuce root hair formation, I have summarized them in a later section.

#### *Auxin supply during root hair formation*

Although auxin plays important roles in root hair formation, the mechanism of auxin supply to trichoblasts was unclear until recently. Previous models assumed that auxin flows uniformly throughout the root epidermis (Swarup et al. 2005, Grieneisen et al. 2007). However, recent studies suggest a more complicated model for the auxin supply to trichoblasts.

As mentioned above, auxin flow is controlled by influx and efflux carriers. In wild-type *A. thaliana*, the efflux carrier PIN2 was detected in both trichoblasts and atrichoblasts. In contrast, although auxin is needed for root hair formation, the influx carrier

AUX1 was undetectable in trichoblasts. Instead, interestingly, it was highly expressed in atrichoblasts. In fact, higher auxin accumulation was observed in atrichoblasts than in trichoblasts in wild-type *A. thaliana* (Swarup et al. 2004, Abas et al. 2006). In contrast, auxin levels in these cells are comparable in the *aux1-22* mutant (Jones et al. 2009). These results suggest that atrichoblasts accumulate a higher concentration of auxin and auxin supply from atrichoblasts to trichoblasts sustains the development of root hairs.

The auxin gradient between trichoblasts and atrichoblasts contributes to maintain the elongation of root hairs (Knox et al. 2003). Although atrichoblasts are necessary to achieve root hair growth, an auxin response in the atrichoblasts is not required for it (Jones et al. 2009). Atrichoblasts may only supply auxin to developing hair cells in order to sustain root hair outgrowth. It is well known that fate determinant factors, such as CPC and GL3/EGL3, move and interact between trichoblasts and atrichoblasts to establish epidermal cell fate (Lee and Schiefelbein 2002, Bernhardt et al. 2005, Kurata et al. 2005, Guimil and Dunand 2006, Schiefelbein et al. 2009, Libault et al. 2010). The auxin supply from atrichoblasts to trichoblasts further strengthens the importance of atrichoblasts in root hair formation.

#### *Role of auxin in low pH-induced root hair formation in lettuce seedlings*

The distribution pattern of root hairs on the root varies among plant species. The position of hair cells and nonhair cells is determined by either an asymmetric division mechanism, a random mechanism, or a position-dependent mechanism (Clowes 2000, Dolan and Costa 2001, Kim et al. 2006). In roots of *A. thaliana*, the hair cells and nonhair cells are orderly aligned because their fates are determined by their position against the underlying cortical cells (Dolan et al. 1994, Galway et al. 1994). Although *A. thaliana* is undoubtedly a useful plant model for studies on plant physiology and molecular biology, the study of hair formation in other plant root types could be meaningful for such studies. Notably, all the root epidermal cells of lettuce (*Lactuca sativa* L.) have the potential to form root hairs (Inoue and Hirota 2000). Using lettuce seedlings, we revealed novel findings especially for the early stages of root hair formation. Here, I introduce only the findings that concern the role of auxin in lettuce root hair formation. For other findings, refer to a review by Takahashi (2012).

When lettuce seedlings are pre-cultured at pH 6.0 and transferred to pH 4.0 medium, many root hairs formed in a highly synchronized manner whereas no root hairs formed on the seedlings cultured through-

out at pH 6.0 (Inoue et al. 2000). As in root hair formation in *A. thaliana*, auxin is needed for the induction of lettuce root hair formation. The addition of IAA induced root hairs at pH 6.0, while the auxin inhibitors prevented root hair formation at pH 4.0 (Takahashi et al. 2003c). Takahashi et al. (2003a) found perpendicular cortical microtubule arrays in root epidermal cells in cultures at pH 6.0. However, these cortical microtubule arrays were randomized before root hair initiation. Further, pharmacological and hormonal experiments showed that the cortical microtubule randomization is induced by auxin, and it is indispensable to the initiation of root hairs.

Besides the pH of the medium, light is also needed for the low pH-induced root hair formation in lettuce seedlings. Neither the cortical microtubule randomization nor root hair formation occurs in the dark even on the pH 4.0 medium (De Simone et al. 2000, Takahashi and Inoue 2008). However, the application of IAA to the seedlings induced root hair formation in the dark, suggesting that auxin also mediates the signal of light (Takahashi and Inoue 2008). Furthermore, it became clear that auxin was involved in the elongation process of lettuce root hairs as it was in *A. thaliana* (Takahashi and Inoue 2008).

#### **Interaction with ethylene**

Although auxin plays significant roles in root growth and development, it usually works through interactions with other plant hormones. Because the interactions are too diverse and complicated to summarize all of them here, I only introduce those of auxin and ethylene during root hair formation, root growth inhibition, and lateral root formation. For a discussion of the crosstalk between auxin and other plant hormones, as well as for interactions of auxin and ethylene in other phenomena occurring in the root, refer to a number of more comprehensive reviews such as Benková and Hejác̃ko (2009), Fukaki and Tasaka (2009), Perilli et al. (2010), and Muday et al. (2012).

As early as about a half century ago, it was already known that ethylene inhibited polar auxin transport (Morgan and Gausman 1966, Burg and Burg 1967). Now, it is clear that auxin and ethylene act synergistically or antagonistically depending on the phenomenon. Concerning the root hair formation, auxin and ethylene have similar effects. As mentioned above, root hair formation is promoted by auxin. Likewise, genetic and physiological studies have revealed that ethylene also promotes root hair formation (Dolan et al. 1994, Masucci and Schiefelbein 1994, Tanimoto et al. 1995, Pitts et al. 1998). However, if we look in more detail, the interactions between the two hormones show a little difference

depending on the stage of root hair formation.

The process of root hair initiation directly depends on the amount of auxin and its signaling. The effect of ethylene is less direct in this stage and seems to occur by enhancing the intracellular auxin level (Muday et al. 2012). This idea agrees with the case of lettuce root hair formation in which auxin is essential for cortical microtubule randomization and ethylene promotes the action of auxin (Takahashi et al. 2003c). On the other hand, auxin and ethylene have equivalent roles in the positive regulation of root hair positioning and root hair elongation (Muday et al. 2012).

The synergistic collaboration of auxin and ethylene is also observed in their effect on root growth (Rahman et al. 2001b, Swarup et al. 2002). Both auxin and ethylene inhibit root growth by reducing the length of the elongation zone and have little effect on elemental expansion (Rahman et al. 2007, Swarup et al. 2007). A mutation, which caused enhanced signaling or synthesis of auxin or ethylene, led to the formation of short roots (Guzmán and Ecker 1990, Kieber et al. 1993, Delarue et al. 1998, Zhao et al. 2001, Cheng et al. 2006, Rahman et al. 2007). In contrast, treatment with auxin or ethylene cannot severely inhibit root growth in auxin-resistant mutants or ethylene-insensitive mutants, respectively (Timpote et al. 1994, Leyser et al. 1996, Rahman et al. 2000, 2001a, Fukaki et al. 2002, Biswas et al. 2007, Růžička et al. 2007, Stepanova et al. 2007, Swarup et al. 2007).

Interestingly, mutations in auxin carriers, auxin signaling, or auxin biosynthesis confer ethylene insensitivity in root growth inhibition (Pickett et al. 1990, Roman et al. 1995, Leyser et al. 1996, Luschnig et al. 1998, Alonso et al. 2003, Dharmasiri et al. 2005a, Růžička et al. 2007, Stepanova et al. 2005, 2007, Swarup et al. 2007). It is known that ethylene stimulates auxin biosynthesis (Růžička et al. 2007, Swarup et al. 2007). These observations suggest that ethylene inhibits root growth through transport, responses, and biosynthesis of auxin (Roman et al. 1995, Luschnig et al. 1998, Müller et al. 1998b, Stepanova et al. 2005, 2007, Růžička et al. 2007, Swarup et al. 2007).

On the other hand, auxin affects ethylene production (Yang and Hoffman 1984, Liang et al. 1992, Bleecker and Kende 2000). Increased ethylene production by auxin treatment has already been reported a half century ago (Morgan and Hall 1962). In many cases, increased ethylene production is brought by upregulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase genes, whose products catalyze the rate-limiting process in the ethylene production. It is known that auxin treatment enhances the expression of ACC synthase genes in

the root meristem (Abel et al. 1995, Tsuchisaka and Theologis 2004, Stepanova et al. 2007). In lettuce seedlings, not only the root hair formation but also the inhibition of root growth is observed upon treatment with auxin or ethylene. These treatments upregulate ACC synthase genes and ACC oxidase genes in lettuce (Takahashi et al. 2003b, 2010).

In contrast to the cases of root hair formation and root growth inhibition, auxin and ethylene act antagonistically in lateral root formation, with a promoting effect from auxin and an inhibitory effect from ethylene. It is thought that the auxin concentration locally increases at the mature region of the root, and this increase promotes the emergence of lateral roots from the pericycle (Dubrovsky et al. 2008, Laskowski et al. 2008). In fact, when auxin transport is reduced, the number of lateral roots is also reduced (Reed et al. 1998, Casimiro et al. 2001, De Smet et al. 2007, Péret et al. 2009). The local auxin accumulation is brought by an inhibition of auxin movement from the mature region, which is due to a depletion of auxin efflux proteins PIN3 and PIN7 (Muday et al. 2012). Lateral root formation is inhibited in the ethylene- or ACC-treated wild-type *A. thaliana* plants and in *ctr1* or *eto1* mutants (Ivanchenko et al. 2008, Negi et al. 2008, Strader et al. 2010). In contrast, lateral roots increase in dominant negative ethylene receptor mutants or the ethylene-insensitive mutants of *A. thaliana* (Negi et al. 2008). Similar results are also obtained in tomato (Negi et al. 2010). The inhibitory effect of ethylene in lateral root formation is due to the stimulation of PIN3 and PIN7 expression, which promotes the rootward auxin transport away from the mature region (Lewis et al. 2011) and thereby weakens the promoting effect of auxin in lateral root formation.

### Reactive oxygen species

Plant growth and development are not solely regulated by plant hormones. Recent studies have shown a novel role of reactive oxygen species as signaling molecules. Reactive oxygen species were associated not only with responses to physiological stress and defense mechanisms but also with gravitropism, hormonal signaling, stomata opening, and ion channel regulation (Mittler and Berkowitz 2001). Because reactive oxygen species plays an additional key role in root hair formation, I have summarized it here.

In the *root hair defective 2* (*rhd2*) mutant of *A. thaliana*, root hairs initiate correctly, but the hairs cannot elongate. The *RHD2* gene encodes the respiratory burst oxidase homologue C (AtRBOH C), an NADPH oxidase. Thus, the production of reactive oxygen species in the *rhd2* mutant root is only half



that in the wild-type root (Foreman et al. 2003). These findings suggest an important role of reactive oxygen species during root hair elongation. In fact, reactive oxygen species accumulate at the hair tip but not at the surrounding areas of the hair upon initiation (Foreman et al. 2003, Carol et al. 2005). Monshausen et al. (2007) found an oscillatory growth of *A. thaliana* root hairs, which was followed by oscillations in the extracellular concentration of reactive oxygen species and pH. Increases in the extracellular reactive oxygen species and pH are thought to act in a coordinated and complementary manner to locally strengthen the cell wall and restrict tip growth (Monshausen et al. 2007).

The precise relationship between reactive oxygen species and auxin in root hair formation has not yet been elucidated. However, it is known that auxin induced the production of reactive oxygen species in the gravitropic response of maize and *A. thaliana* roots (Joo et al. 2001, 2005). Moreover, although it is not a phenomenon occurring in the root, auxin promotes the production of reactive oxygen species in the outer epidermis of maize coleoptiles (Schopfer 2001). These observations allow us to imagine a plausible interaction between auxin and reactive oxygen species during root hair formation. Further work is awaited to clarify this point.

## Perspective

The first identified plant hormone, auxin, controls various phenomena in growth and development throughout the plant's life. Thanks to the advances in research techniques, such as the mutant analyses, GFP fusion proteins or a GUS reporter gene, microarray and proteomic analyses, and various types of MS technology, our understanding of auxin biology is progressing at a fast speed. Currently, we can apply novel approaches, which utilize mathematical and computer simulating models. For example, the auxin transport and its flow were simulated using these methods (Jones et al. 2009, Kramer 2009, Krupinski and Jönsson 2010).

As mentioned above, interactions between auxin and other plant hormones are commonly observed. Recently, new substances that influence root growth through interactions with auxin have attracted attention. I have mentioned reactive oxygen species in the previous section. Besides reactive oxygen species, for example, serotonin, a tryptophan-derived signal common to plants and animals, was revealed to affect the root system architecture as a natural auxin inhibitor (Pelagio-Flores et al. 2011). Kapulnik et al. (2011a, 2011b) found that strigolactones interact with auxin and ethylene, and they regulate lateral root formation and root hair elongation. The discovery of

such new regulatory substances will further facilitate our understanding of auxin.

The contents that I have summarized in this review are only a handful of the concepts in auxin biology. There are many other attractive research areas. I believe that steady research in each area will help connect the scattered knowledge on auxin and aid in understanding auxin completely.

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