Ion Channels Meet Auxin Action

I. Fuchs¹, K. Philippar¹,², and R. Hedrich¹
¹ Julius-von-Sachs-Institute, Molecular Plant Physiology and Biophysics, Biocenter Würzburg University, Julius-von-Sachs-Platz 2, 97082 Würzburg, Germany
² Present address: Department for Biology 1, Botany III, Ludwig-Maximilians-University Munich, Menzingerstraße 67, 80638 Munich, Germany

Received: November 17, 2005; Accepted: March 27, 2006

Abstract: The regulation of cell division and elongation in plants is accomplished by the action of different phytohormones. Auxin as one of these growth regulators is known to stimulate cell elongation growth in the aerial parts of the plant. Here, auxin enhances cell enlargement by increasing the extensibility of the cell wall and by facilitating the uptake of osmolytes such as potassium ions into the cell. Starting in the late 1990s, the auxin regulation of ion channels mediating K⁺ import into the cell has been studied in great detail. In this article we will focus on the molecular mechanisms underlying the modulation of K⁺ transport by auxin and present a model to explain how the regulation of K⁺ channels is involved in auxin-induced cell elongation growth.

Key words: Auxin, K⁺ channel, elongation growth, tropisms, maize, Arabidopsis thaliana.

Ion Channels Meet Auxin Action

Auxin History

More than one hundred years ago Charles Darwin and his son Francis initiated studies on auxin-regulated growth processes (Darwin, 1880). Investigating photostimulated grass coleoptiles, they hypothesized that “some influence” coming from the coleoptile tip moves downwards and causes phototropic bending of the seedlings. Different concentrations of this signaling component in the opposing flanks of the coleoptile were proposed to cause differential cell elongation growth and thus curvature. Almost fifty years later, Frits Went and Nicolai Cholodny discovered that this phytohormone, now termed auxin (derived from the greek word “auxein”, meaning “to grow”), is transported to the shaded flank of the photostimulated coleoptiles, where high auxin concentrations stimulate cell elongation and thus cause bending of the seedlings towards the light source (Cholodny, 1928; Went, 1928). Hence, differential growth and bending of both gravi- or photostimulated grass coleoptiles was identified as a consequence of the unequal distribution of the “growth stimulating substance” in the opposing flanks of the organ (Went and Thimmann, 1937). After the identification of IAA (indole-3-acetic acid) as this growth-promoting substance (Greenwood et al., 1972), this assumption known as the “Cholodny-Went Hypothesis” was confirmed by Parker and Briggs (1990) and Iino (1991).

The kinetics of auxin redistribution, however, varies in response to different tropistic stimuli. Moritoshi Iino demonstrated that, while in photostimulated plants endogenous auxin is redistributed only in the very tip, IAA is laterally translocated along the length of the entire coleoptile in gravistimulated plants (Iino, 1995). Measuring free endogenous IAA in gravi- or photostimulated maize coleoptiles, Fuchs et al. (2003) and Philippar et al. (1999) confirmed these findings. Already 5 min after the onset of gravistimulation, a significant increase in free IAA in the apical region was measured. This was most probably due to the release of IAA from its conjugated forms as an immediate response to the positional change of the seedling (Philippar et al., 1999). This pool of free auxin was redistributed in the coleoptile tip as early as 15 min after the beginning of gravistimulation and this relocation further spread over the entire length of the coleoptile during the course of the experiment. In photostimulated coleoptiles, however, a significant auxin gradient between the growing shaded half and the growth-restricted illuminated half could not be detected until 60 min after the onset of blue light irradiation (Fuchs et al., 2003). As expected, a pronounced translocation of free auxin was not observed in the basal part of the seedling. This translocation was found only in the coleoptile tip.

Auxin-Dependent Membrane Polarization, Cell Wall Acidification, and Wall Loosening

Up to now, it is not fully understood how the auxin gradient as a signal in tropistically stimulated plants is converted into the bending response. One of the first steps to be identified was the change in apoplastic pH which is associated with the redistribution of auxin. The concentration of protons is increased in the apoplast of the fast-growing half of gravi- or photostimulated coleoptiles, characterized by an elevated IAA concentration (Mulkey et al., 1981), which is consistent with the “Acid Growth Theory” established by Hager in 1971 (Hager et al., 1971). He proposed an acidification of the apoplast accomplished by the enhanced activity of the PM-H⁺-ATPase as a prerequisite for cell elongation in response to increased auxin concentrations. In fact, in auxin-sensitive Vicia faba guard cells, treatment with the synthetic auxin 2,4-D activates the H⁺-ATPase and thus causes the plasma membrane to hyperpolar-
ize (Lohse and Hedrich, 1992). In maize coleoptiles IAA treatment does not only seem to increase the proton pump activity but also the density of the respective protein in the plasma membrane (Hager et al., 1991; Felle et al., 1991; Rück et al., 1993; Frias et al., 1996). It should be noted that Jahn et al. (1996), for example, could not confirm this finding, a controversy which might result from the fact that not all isoforms of the PM-H+-ATPase are induced by auxins. Thus, the induction of MHA2 might have been masked by unregulated ATPases (see also Hager, 2003).

In the context of his theory, Hager also proposed that the decrease in apoplastic pH leads to the activation of cell wall-loosening enzymes. Expansins, catalytic acid-activated proteins which loosen bonds between cellulose and hemicellulose fibrils in the cell wall, are potential candidates to perform this task (reviewed by Cosgrove, 2000). Interestingly, the expression of some expansins is also regulated by auxins (Hutchinson et al., 1995; Catala et al., 2000). Additionally, XTHs (xyloglucan endotransglycosylases/hydrolases, Rose et al., 2002), another class of acid-activated enzymes (Fry et al., 1992), may enhance the plasticity of the cell wall by regulating the xyloglucan turnover. It is interesting to note that one XTH expressed in tomato hypocotyls, LeEXT is transcriptionally induced by the synthetic auxin 2,4-D as well (Catala et al., 1997).

**Auxin Activation of K⁺ Channels**

Apart from cell wall loosening, the uptake of osmolytes provides a basic requirement for cell elongation growth. Moreover, to balance the charge of the secreted protons, cations have to be imported into the cell, a task easily fulfilled by the proton pump, K⁺ and anion channel activity have also been shown to be modulated by the auxins IAA and 1-NAA (Marten et al., 1991; Lohse and Hedrich, 1992). In maize coleoptiles, auxin-induced growth strongly depends on the availability of potassium ions in the bathing medium and their uptake via potassium-selective ion channels: auxin-induced growth is characterized by a depletion of IAA, whereas the amount of ZMK1 mRNA in the lower, fast-growing half remained constant (Philippar et al., 1999). This differential expression of the K⁺ channel gene led to 6 – 7-times higher ZMK1 mRNA levels in the auxin-enriched lower coleoptile half 90 min after the onset of gravitostimulation. In photo-stimulated maize seedlings, ZMK1 expression also followed the translation of endogenous auxin, but the difference in ZMK1 transcripts in the opposing flanks was not as pronounced as seen with the gravity response: 90 min after the onset of blue light illumination, the ZMK1 mRNA content was only 2 – 3-times higher in the faster-growing shaded coleoptile half (Fuchs et al., 2003). As photo-stimulated seedlings display a much smaller bending angle than gravistimulated plants, gravity seems to restrict further phototropic bending of the coleoptile. A clinostat equipped with blue LEDs (Blue Light Cyclor, BLC, see Fig. 1), however, allowed simultaneous rotation of the seedlings and illumination of the coleoptile tip unilaterally (Fuchs et al., 2003). Thereby gravitropism is uncoupled from the blue light response. As expected, the bending angle of plants photostimulated on the BLC was comparable to that of gravistimulated seedlings, but stronger bending could not be correlated to a more pronounced ZMK1 mRNA gradient 90 min after the beginning of the experiment (Fuchs et al., 2003). However, auxin redistribution appeared to remain differential under varying gravity conditions. Interestingly, plants photostimulated on the BLC were characterized by a massive rise in IAA in the shaded half of the coleoptile tip 60 min after illumination onset, whereas this peak was missing in seedlings exposed to unilateral blue light under normal gravity conditions. Taking into account that the activation of ZMK1 transcription was detectable 45 – 60 min after the application of auxin in earlier experiments (Philippar et al., 1999), it was hypothesized that the auxin peak in BLC-treated plants at 60 min might lead to changes in ZMK1 transcription at a later time, not previously investigated (Fuchs et al., 2003). By monitoring differential ZMK1 expression for up to 4 h, it could indeed be
shown that, in seedlings photostimulated on the BLC, ZMK1 transcription remains differential for at least 3 h, whereas a ZMK1 mRNA gradient is no longer visible in plants illuminated under normal gravity conditions (Fuchs et al., 2003). In our working model, prolonged differential expression and thus enhanced K⁺ uptake via ZMK1 is required for differential growth and stronger bending of BLC-treated plants. Therefore, the perception of two different stimuli, blue light illumination and a change in the gravity vector, merges into a common signalling pathway, leading to auxin redistribution, differential expression of ZMK1, and differential cell elongation growth (Fig. 2).

Involvement of ZMK1 in Auxin-Induced Cell Elongation Growth

Thus, the auxin regulation of a K⁺ channel gene provides a link between IAA redistribution and differential growth during gravi- and phototropism. This led to the following sequence of events for auxin-induced cell elongation growth during gravi- or phototropic bending of the maize coleoptile (Fig. 3): the direction of the incident light is perceived by the blue light photoreceptor PHOT1, which is equally distributed in the coleoptile tip (Hager and Brich, 1993; Kaldenhoff and lino, 1997; Briggs et al., 2001). In contrast, it is less clear how the change in the gravity vector is perceived, but it is most probably mediated by the displacement of statoliths in the root columella or shoot endodermal tissues (Kiss et al., 1989; Kiss et al., 1997; reviewed in Esmen et al., 2005). The perception of the two different stimuli, however, leads to the initiation of a common signalling pathway: Auxin is released from its conjugated forms in the shoot tip (Ljung et al., 2001) and translocated to one coleoptile half by the action of auxin transporters of the PIN and AUX family (Swarup et al., 2001; Friml, 2003). High auxin concentrations stimulate the activity of the PM-H⁺-ATPase, which results in the acidification of the apoplast and thus cell wall loosening. Additionally, K⁺ channels already existing in the plasma membrane might be activated by this decrease in extracellular pH, enabling K⁺ uptake during the initial growth response, which might also be needed for charge compensation. Furthermore, IAA is taken up into the cell and presumably perceived by the auxin receptor TIR1 or related F-Box proteins (Kepinski and Leyser, 2005; Dharmasiri et al., 2005a; Dharmasiri et al., 2005b). Binding of IAA to the receptor leads to the degradation of transcriptional repressors and thus to the transcription of auxin-regulated genes such as the PM-H⁺-ATPase or ZMK1. In the long term, enhanced K⁺ uptake via ZMK1 into the cells of one coleoptile half very likely is a prerequisite for sustained differential growth and thus bending of the organ.

To confirm the importance of ZMK1-mediated K⁺ uptake for auxin-stimulated growth, Philippar et al. isolated ZMK1-knockout mutants. Thereby plants carrying Mutator transposable element insertions inside the ZMK1 gene were identified (Philippar et al., 2006). Repeated backcrossing of the heterozygous mutant plants, however, resulted in wild-type or heterozygous plants only, leading to the speculation that deletion of this K⁺ channel might result in embryo or endosperm lethality. In fact, it was found that the expression of ZMK1 in wild-type embryos is massively induced around 40 days after pollination, indicating that K⁺ uptake via this channel might also take part in processes underlying the development of the maize embryo (Philippar et al., 2006). While maize seedlings are very well suited to study processes associated with tropistic bending of the coleoptile, genetic modification of maize plants raises some difficulties and is rather time-consuming, especially in comparison to other model systems. Thus, maize might no longer be a suitable system to study the involvement of K⁺ channel action in auxin-induced growth in greater detail. Importantly, almost all studies on auxin signal transduction in the past have been performed in Arabidopsis thaliana, and here most of the auxin-signalling mutants are available.

Auxin-Regulated K⁺ Channels in Arabidopsis thaliana

In Arabidopsis thaliana, however, the situation is more complex than in maize, as several K⁺ channel genes are expressed in tissues that exhibit auxin-induced cell elongation growth. In the Arabidopsis hypocotyl, the organ homologous to grass coleoptiles in dicots, the mRNA of all known inward-rectifying Shaker-type K⁺ channels, KAT1, KAT2, AKT1, AKT5, and AtKC1 (Schachtman et al., 1992; Basset et al., 1995; Mäser et al., 2001; Pilot et al., 2001; Reintanz et al., 2002), was detected. In flower stalks, where cell elongation growth can also be stimulated by auxins, however, KAT1 (K⁺ uptake channel Arabidopsis thaliana 1, see Schachtman et al., 1992) appeared to be the dominant K⁺ uptake channel (Philippar et al., 2004). Surprisingly, the expression of the ZMK1 ortholog in Arabidopsis thaliana, AKT1 (Arabidopsis thaliana K⁺ transporter 1, Basset et al.,

![Fig. 1 Blue Light Cycler (BLC): Experimental setup to uncouple phototropism and the gravity response. Maize seedlings can be illuminated with unilateral blue light during continuous rotation on a clinostat. (a) Side view. (b) Front view. Curvature of maize coleoptiles 24 h after the onset of clinostat rotation without illumination (right), or with simultaneous irradiation with blue light (left). (c) Magnified illustration of one BLC slot, showing a single plant bending towards the light source.](image-url)
was not induced by auxins. Similarly, the expression of one of the ZMK1 homologues in rice, OsAKT1, was also unaffected by the application of IAA. Rather than involvement in auxin action, OsAKT1, like AKT1, is involved in root K⁺ uptake from the soil and regulated in response to salt stress (Fuchs et al., 2005). However, two other K⁺ channel genes in Arabidopsis, KAT1 and KAT2 (a KAT1 homolog, see Pilot et al., 2001), were upregulated by the physiologically active auxins IAA and 1-NAA in a concentration-dependent manner (Philippar et al., 2004). Interestingly, the induction of KAT1 expression by 1-NAA was specific for organs that elongate in response to the application of auxin. In guard cells, however, where KAT1 also represents the dominating K⁺ inward rectifier, its transcription was not induced upon auxin treatment (Philippar et al., 2004). This might indicate that the regulation of KAT1 expression by auxins is associated with irreversible cell expansion during elongation growth rather than transient volume changes during stomatal movement. It should be noted that KAT1, like ZMK1, is activated upon acidification of the extracellular space (Hoth and Hedrich, 1999). This acid activation of both channels and their expression in highly auxin-sensitive tissues suggest a similar function in auxin-induced cell elongation growth.

Taking advantage of the availability of various knockout mutants in Arabidopsis, the auxin-induced increase in KAT1 transcripts was correlated with the abundance of the respective protein (Philippar et al., 2004). In patch-clamp experiments on protoplasts derived from hypocotyls of wild-type plants, inward K⁺ currents were recorded upon hyperpolarization of the plasma membrane. Surprisingly, similar results were obtained using hypocotyl protoplasts of kat1 knockout plants (kat1::En-1, Szyroki et al., 2001), although KAT1 transcripts were drastically reduced in the mutant (Philippar et al., 2004). This might be explained by the fact that Shaker K⁺ channels tend to form heterotetramers with other family members. As the assembly of KAT1 and AKT1 has already been demonstrated (Dreyer et al., 1997; Reintanz et al., 2002), it is very likely that AKT1 might represent the dominating subunit of heteromeric K⁺ uptake channels in the Arabidopsis hypocotyl. After the application of auxin, however, differences between protoplasts derived from wild type and kat1::En-1 could be
monitored (Philippar et al., 2004): The amplitude of K\textsuperscript{+} currents in kat1::En-1 was reduced two-fold in comparison to wild type, which might reflect the fact that, upon auxin treatment, the proportion of KAT1 subunits within the K\textsuperscript{+} channel complexes is increased in wild-type plants. Thus, auxin might regulate K\textsuperscript{+} uptake in Arabidopsis by shifting the subunit composition of the K\textsuperscript{+} channel heteromers in favour of KAT1.

To test the role of KAT1 for auxin-regulated growth, IAA-induced elongation of Arabidopsis hypocotyls in wild-type and kat1::En-1 plants was investigated (Philippar et al., 2004). Surprisingly, a significant difference in auxin-induced growth kinetics of both genotypes was not detected, indicating that the role of KAT1 might be taken over by residual K\textsuperscript{+} channels. As already mentioned, several other K\textsuperscript{+} channel and carrier genes (e.g., AtKUP2, Quintero and Blatt, 1997) are also expressed in the hypocotyl of Arabidopsis thaliana, probably compensating for the loss of KAT1 in the respective knockout. In this context, it is interesting to note that shy3-1 mutant plants defective in AtKUP2 are characterized by reduced hypocotyl and leaf cell expansion growth, pointing to the importance of K\textsuperscript{+} uptake via this carrier for elongation growth (Elumalai et al., 2002). Further studies investigating the phenotype of double or triple K\textsuperscript{+} transport mutants will have to be performed to reveal the individual contribution of each transport module to K\textsuperscript{+} uptake during auxin-induced cell elongation growth in Arabidopsis.

**Conclusions**

In summary, the involvement of K\textsuperscript{+} channel activation in auxin-induced cell elongation growth has been described in two model plant systems. Furthermore, evidence has been provided that the spatial regulation of K\textsuperscript{+} uptake via potassium-selective channels might represent a prerequisite for differential growth during tropistic bending. In the future, the regulation of K\textsuperscript{+} channel action in response to an auxin stimulus will have to be investigated in more detail. For example, it remains to be elucidated if the transcriptional regulation of ZMK1 and KAT1/KAT2 depends on a signalling pathway involving the action of TIR1 or related F-box proteins (Dharmasiri et al., 2005a; Dharmasiri et al., 2005b). The analysis of mutants defective in TIR1-associated signalling components identified in the future will presumably help to shed light on this topic. Another question to be answered is if K\textsuperscript{+} channel regulation by IAA is strictly restricted to auxin-sensitive tissues such as the epidermal and cortical layers of maize coleoptiles or the epidermis of Arabidopsis hypocotyls and roots (Cleland, 1991; Peters et al., 1992; Swarup et al., 2005). Therefore, methods to detect K\textsuperscript{+} channel transcript or protein abundance, even on a subcellular level, will have to be developed. In this context, one could suspect that, in response to a tropistic stimulus, K\textsuperscript{+} channel proteins might be relocated within auxin-responding cells, as was dem-

---

**Fig. 3** Model to describe the involvement of K\textsuperscript{+} uptake via ZMK1 in auxin-induced cell elongation growth. The perception of the gravitropic or photostimulus by different receptors merges into a common signalling pathway. Redistribution of IAA leads to the activation of the PM-H\textsuperscript{+}-ATPase and thus acidification of the extracellular space, which stimulates cell wall loosening enzymes and ZMK1 channels already existing in the plasma membrane. Additionally, IAA signalling results in enhanced transcription of both the PM-H\textsuperscript{+}-ATPase and ZMK1. In the long term, enhanced K\textsuperscript{+} uptake via ZMK1 leads to cell expansion and elongation growth.
onstrated for the IAA efflux carrier PIN3 (Friml et al., 2002). Here, it should be mentioned that an asymmetric intracellular distribution of a poplar K⁺ channel was recently discovered (Arend et al., 2005).

Ion channels seem to play a role in the control of processes such as cell cycle regulation, differential growth, stomatal movement, plant-pathogen interactions, long-distance transport, nutrient uptake, and plant movement (see Dietrich et al., 2001; Very and Sentenac, 2002; Becker and Hedrich, 2002; Very and Sentenac, 2003; Sano et al., 2006) and in the future are expected to control many more cellular processes: “Ion channels meet cell biology!”

References


Fuchs, I., Stölzle, S., Iwashikina, N., and Hedrich, R. (2005) Rice K⁺ uptake channel OsAKT1 is sensitive to salt stress. Plant 221, 212 – 221.


R. Hedrich
Julius-von-Sachs-Institute
Molecular Plant Physiology and Biophysics
Biocenter Würzburg University
Julius-von-Sachs-Platz 2
97082 Würzburg
Germany
E-mail: hedrich@botanik.uni-wuerzburg.de

Guest Editor: R. Reski