Auxin signaling

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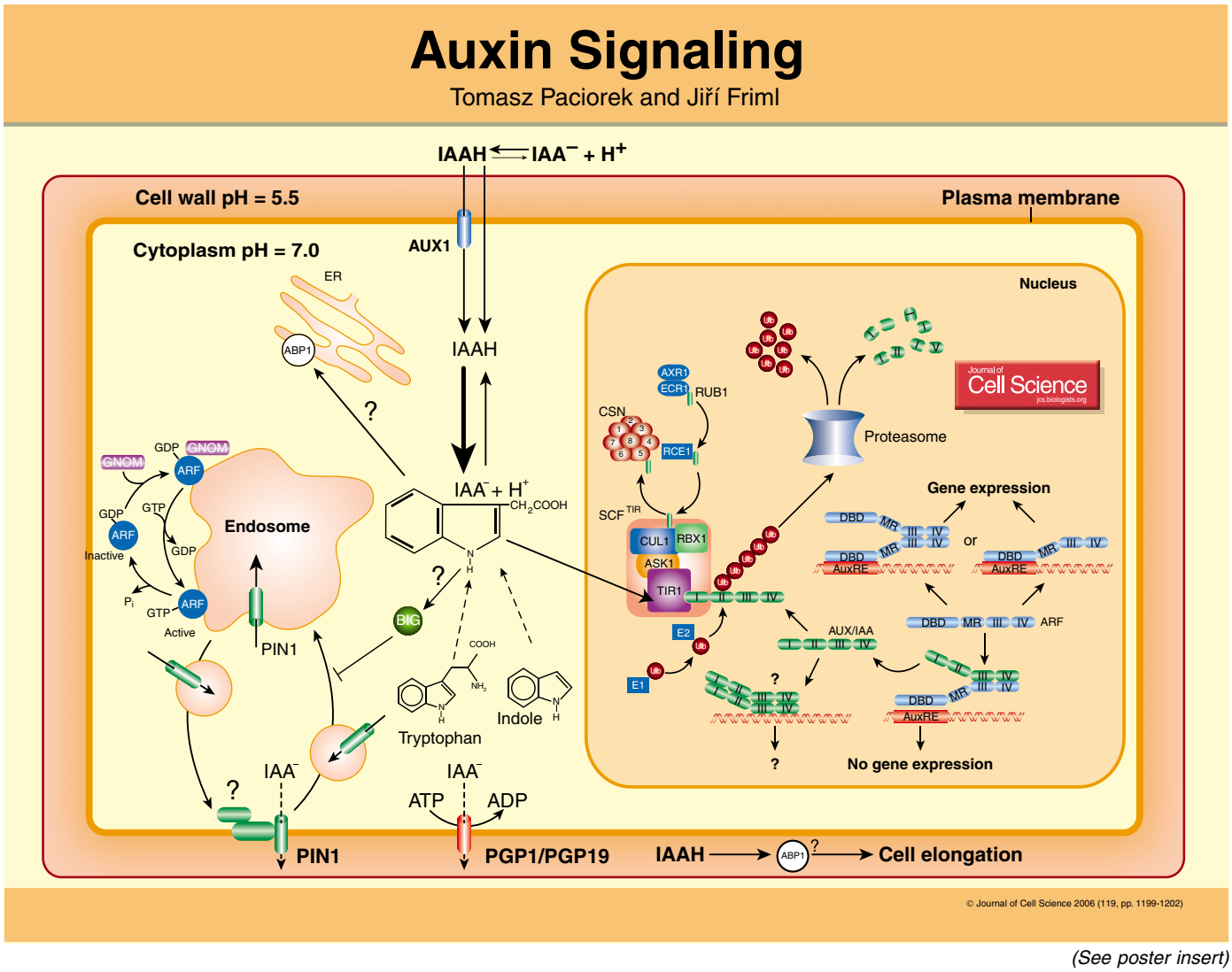
Charles Darwin, studying the response of canary grass coleoptiles to unilateral light, first demonstrated the existence of a moving signal that mediated plant phototropism (Darwin, 1880). This discovery of the first plant hormone, later termed auxin (from the Greek 'auxein', which means to grow), was followed several decades later by the identification of auxin as indole-3-acetic acid (IAA) (Koegl and Kostermans, 1934; Went and Thimann, 1937). Subsequent efforts have illuminated numerous physiological effects of auxin. There are many developmental processes in which auxin plays a role, including embryo and fruit development, organogenesis, vascular tissue differentiation, root patterning, elongation and tropistic growth, apical hook formation and apical dominance (for a review, see Kepinski and Leyser, 2005a). Some very rapid cellular responses, such as plasma membrane depolarization and apoplast acidification, have also been connected to auxin (Rück et al., 1993; Hager, 2003).

Asymmetric accumulation of auxin in specific cells (sometimes called auxin gradients) is a necessary prerequisite for various developmental roles of auxin (Sabatini et al., 1999; Friml et al., 2002a; Friml et al., 2002b; Friml et al., 2003; Benková et al., 2003). Cellular auxin levels results from complex interplay between auxin synthesis, degradation and (de)conjugation to/from sugars and amino acids (Ljung et al., 2005). The details of auxin biosynthesis and its regulation remain largely elusive, but it is known that both tryptophan and indole can serve as precursors (Cohen et al., 2003). The major determinant of asymmetric auxin accumulation is directional, intercellular auxin transport (Friml et al., 2002b; Weijers et al., 2005). The molecular components involved are the PIN and AUX1 auxin-efflux and -influx transporters, respectively (Gälweiler et al., 1998; Luschnig et al., 1998; Bennett et al., 1996). PIN proteins are thought to be part of a multicomponent complex, and their polar, subcellular localisation determines the direction of auxin flow



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(Wiśniewska et al., 2006). In addition, PGP1 and PGP19 P-glycoprotein transporters are involved in auxin efflux (Geisler et al., 2005). PIN proteins together with other components seem to form an auxin transport network that mediates local auxin distribution and triggers different cellular responses in various developmental contexts.

The hunt for an auxin receptor and downstream signaling components has used virtually all possible experimental approaches. The first cohesive data emerged from straightforward biochemical approaches aimed at isolating proteins that specifically bind to auxin with high affinity. A single protein termed auxin-binding protein 1 (ABP1) has repeatedly been identified in different plant species. including maize (Hertel et al., 1972) and *Arabidopsis* (Palme et al., 1992). Besides its strong ability to bind auxin, the physiological role of ABP1 remains unclear (Napier et al., 2002). Much of ABP1 is localized in the endoplasmic reticulum, where the pH is too high for efficient auxin binding (Henderson et al., 1997). Experiments using agonistic and antagonistic anti-ABP1 antibodies and peptides, as well as conditional ABP1 expression, have provided a functional connection between extracellularly localized ABP1 and some auxin-dependent cellular responses, especially cell expansion (Steffens et al., 2001). Since the *Arabidopsis abp1* loss-of-function mutant is lethal (Chen et al., 2001), genetic evidence for the involvement of ABP1 in auxin-dependent development is hard to obtain. Thus, the significance of its binding to auxin remains unclear.

Forward genetic screens identified a group of *Arabidopsis* mutants able to elongate roots despite the presence of inhibitory auxin levels [auxin-resistant (*axr*) mutants]. The molecular characterization of the *axr1* mutant revealed that AXR1 function is related to the ubiquitylation pathway, which can mark proteins for proteasome-mediated degradation (Leyser et al., 1993). Further strong support for this connection was obtained when the transport inhibitor response 1 (*tir1*) mutant was isolated in a similar screen for mutants resistant to auxin transport inhibitors (Ruegger et al., 1997). *TIR1* encodes a member of large family of F-box proteins involved in regulated proteasome-mediated protein degradation (Ruegger et al., 1998). The importance of TIR-based auxin signaling for plant development has been highlighted by the analysis of quadruple *tir1*-related mutants, which can display dramatic patterning defects in embryo development (Dharmasiri et al., 2005b).

Proteasome-mediated degradation is common to many fundamental cellular processes in animals and plants (Gray and Estelle, 2000; Robinson and Ardley, 2004). First, ubiquitin (Ub) is activated by binding to a ubiquitin-activating enzyme (E1). Next, it is transferred onto a ubiquitin-conjugating enzyme (E2). Then, ubiquitin is covalently attached to the target protein, which is mediated by E2 in association with a ubiquitin-protein ligase (E3). The modified protein is then targeted to the 26S proteasome and subsequently degraded (Voges et al., 1999). A subunit of SCF-type E3 ligases is the F-box protein, which confers specificity by binding to the substrates (Ruegger et al., 1998). The activity of the TIR1-containing SCF-like complex (SCF^{TIR1}) is regulated by covalent conjugation of RUB1 (a protein related to ubiquitin and known as NEDD8 in mammals) to the cullin (Cul1) subunit of SCF (del Pozo and Estelle, 1999). This three-step process again requires the coordinated action of several enzymes; importantly, one of them is AXR1. Modification of Cul1 by RUB1 seems to be very important for SCF^{TIR1} activity and normal auxin responsiveness (del Pozo et al., 1998). Mutations in the *AXR1* gene, that decrease the number of RUB1-Cul1 complexes result in an auxin-insensitive phenotype (Leyser et al., 1993). Deconjugation of RUB1 from Cul1 also contributes to SCF^{TIR1} regulation. This is mediated by a multiprotein enzymatic complex present in most eukaryotic organisms: the COP9 signalosome (CSN) (Lyapina et al., 2001). CSN has eight subunits, most of which were identified in a screen for components of the light signaling pathway (Chamovitz at al., 1996). Knocking out any of these leads to seedling lethality, but a leaky loss-of-function mutation in subunit 5 leads to decreased degradation of SCF^{TIR1} substrates. Such plants resemble *axr1* mutants and are auxin resistant, demonstrating the importance of the RUB1 deconjugation process for proper auxin signaling (Schwechheimer et al., 2001). Forward genetics has thus firmly established ubiquitin-dependent protein degradation as an important part of auxin signaling.

It was initially shown that, in different plant species, auxin rapidly (in some cases within 3 minutes) regulates gene expression (Theologis et al., 1985). Subsequent analyses revealed that many of the target genes (e.g. the *AUX/IAA*, *SAUR* and *GH3* families) are primary auxin-responsive genes (Guilfoyle, 1998). An auxin-response element (AuxRE) with the sequence TGTCTC was identified in promoters of many auxin-responsive genes, which allowed the isolation of the transcription factor that binds to this sequence (Ulmasov et al., 1997a). This auxin-response factor (ARF) is part of a large family (with 23 members) of transcription factors encoded by the *Arabidopsis* genome (Ulmasov et al., 1999). All members of this family share a very similar protein architecture: the N-terminal end consists of a large DNA-binding domain (DBD), which interacts directly with the AuxRE; a middle region determines whether a particular ARF acts as a transcriptional activator or repressor; and the C-terminal part contains the conserved domains III and IV, which serve as a platform for homo- and hetero-dimerization with other ARFs (Tiwari et al., 2003; Ulmasov et al., 1999). Interestingly, the other interaction partners of ARFs are small, short-lived nuclear proteins of the AUX/IAA family (29 members), which can specifically inhibit ARFs (Ulmasov et al., 1997b). AUX/IAAs have an architecture similar to that of ARFs. They consist of four domains. Domains III and IV (related to domains III and IV of ARFs) play an important role in homodimerization and heterodimerization with other AUX/IAAs and ARFs. Although the function of AUX/IAA dimers is not clear, they may have a topology similar to prokaryotic transcriptional repressors that allows them to bind DNA directly (Morgan et al., 1999). AUX/IAA proteins thus might also directly regulate gene transcription. Domain II seems to be of particular importance. A single amino-acid substitution in this domain results in a gain-of-function mutation that stabilizes

the protein and confers severe auxin-related phenotypes, such as agravitropic roots, short hypocotyls, loss of root hairs or pronounced embryo defects (Timpte et al., 1994; Hamann et al., 1999; Tian et al., 2002). This suggests that domain II is crucial for AUX/IAA stability, which in turn is important for the auxin response (Worley et al., 2000; Ramos at al., 2001; Tian et al., 2002). A characteristic feature of AUX/IAA proteins is their very short half-life, ranging from ~10 to 80 minutes, which means that they are very rapidly degraded (Gray et al., 2001). Indeed, chemical inhibition of proteasome function interferes with AUX/IAA degradation, which suggests a connection between AUX/IAAs and the proteasome pathway (Ramos et al., 2001). In addition, genetic interference with the ubiquitylation pathway – for example, in *axr1* or *tir1* mutant backgrounds – leads to stabilization of AUX/IAA proteins. Importantly, AUX/IAAs can directly interact with the TIR1 subunit of the SCF^{TIR1} complex, and after auxin application this interaction is promoted and AUX/IAA degradation is accelerated. This suggested that auxin stimulates the degradation of AUX/IAAs by promoting the TIR1-AUX/IAA interaction (Gray et al., 2001). These exciting results largely clarified the involvement of the ubiquitin-dependent degradation machinery in auxin signaling and placed it upstream of AUX/IAA transcriptional repressors.

But how does the auxin signal enter the pathway and promote the TIR1-AUX/IAA interaction? Surprisingly, the auxin-perception mechanism is remarkably simple. Auxin binds TIR1 directly and this is sufficient to promote interaction of TIR1 with the AUX/IAAs even in vitro (Dharmasiri et al., 2005a; Kepinski and Leyser 2005b). Thus, TIR1 and several related F-box proteins, such as AFB1, AFB2 and AFB3, are functional auxin receptors that mediate the effect of auxin on gene expression (Dharmasiri et al., 2005b). There are still many details of this mechanism that remain unclear, such as how ARF is inhibited by AUX/IAAs, the precise physiological roles of different AUX/IAAs and ARFs, and their interactions with each other. It is also not clear whether there are parallel arms or unknown branches of the pathway that mediate part of the effect of auxin on gene transcription.

Because of our striking success in elucidating the nuclear auxin pathway, other cellular auxin effects not so obviously connected to the regulation of gene expression have received less attention. These include auxin-dependent changes in membrane K^+ currents, activation of H^+ pumping, MAP kinase and phospholipase A (Hedrich and Jeromin, 1992; Hager, 2003; Mizoguchi et al., 1994; Scherer and Andre, 1989), as well as the recently discovered inhibition of the endocytic cycling of plasma membrane proteins (Paciorek et al., 2005). In plants, many plasma membrane proteins constantly recycle in vesicles between the plasma membrane and endosomal compartments. In *Arabidopsis*, the fungal toxin brefeldin A (BFA) (Peyroche et al., 1999) interferes specifically with the exocytosis step. One of its targets is the endosomal regulator of vesicle trafficking GNOM (Geldner et al., 2003), a guanide nucleotide exchange factor (GEF) for ARF family GTPases (Steinmann et al., 1999). By contrast trafficking of vesicles from the plasma membrane to the endosomes is BFA-insensitive.

BFA thus causes accumulation of recycling proteins, such as auxin transport components PIN1, in so-called BFA compartments (Geldner et al., 2001). Auxin instead inhibits the endocytic step of the recycling process. The auxin-mediated inhibition of endocytosis results in an increase in the number of PIN proteins at the plasma membrane and this, in turn, facilitates greater auxin transport out of cells (Paciorek et al., 2005). This provides a mechanism for a feedback regulation of auxin transport by auxin itself. The action of auxin on endocytosis requires a functional callosin-like protein, BIG, whose orthologue in *Drosophila* is also needed for coordinated, vesicle-dependent synaptic transmission at the neuromuscular junctions (Gil et al., 2001). The molecular function of BIG remains unknown, but it is likely to be involved in some more general aspect of vesicle trafficking or endocytosis because *big* mutations also modulate the effect of auxin transport inhibitors on PIN targeting (Gil et al., 2001). The inhibition of endocytosis and some other physiological auxin effects, such as membrane hyperpolarisation (Rück et al., 1993), occur very fast; thus it is likely that they do not involve regulation of gene expression. This suggests that, besides TIR1-dependent signaling, another pathway mediates non-transcriptional auxin effects. A plausible candidate for an auxin receptor acting in this pathway could be ABP1, which is known to be required for rapid auxin-dependent cellular responses (Steffens et al., 2001) and does not appear to be connected to the nuclear pathway (Dharmasiri et al., 2003).

We can thus now roughly track the auxin signal from its interaction with a receptor to gene expression, and it may seem that one chapter in auxin biology is close to its end. However, in the constant chase for a better understanding of the action of auxin, it might be that we are just beginning another exciting story, which at the end will tell us how auxin, by using several independent pathways, causes the whole repertoire of its diverse physiological effects.

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