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Plant Phototropic Growth

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Summary (200 words max)

Plants are photoautotrophic sessile organisms using environmental cues to optimize multiple facets of growth and development. A classical example is phototropism; in shoots it is typically positive leading to growth towards the light while roots frequently show negative phototropism triggering growth away from the light. Shoot phototropism optimizes light capture of leaves in low light environments and hence increases photosynthetic productivity. Phototropins are plasma-membrane-associated UV-A/blue-light activated kinases triggering phototropic growth. Light perception liberates their protein kinase domain from the inhibitory action of the amino-terminal photosensory portion of the photoreceptor. Following a series of still poorly understood events, phototropin activation leads to the formation of a gradient of the growth hormone auxin across the photo-stimulated stem. The greater auxin concentration on the shaded compared to the lit side of the stem enables growth reorientation towards the light. In this review we briefly summarize the signaling steps starting from photoreceptor activation until the establishment of a lateral auxin gradient ultimately leading to phototropic growth in shoots.

Introduction

"This problem has been around for many years and has generated far more heat than light." - W.R. Briggs

Plants are sedentary organisms and dependent on sunlight for photosynthesis. Consequently, they have evolved the ability to alter their growth to optimise light capture and increase photosynthetic productivity. Phototropism is a classical example of such adaptive growth responses. In flowering plants it is induced by UV/blue wavelengths (290-500 nm) and remarkably this phototropic response is evoked over a large range of light intensities ranging from minute amounts of light to the blue light intensity present on a sunny day. Hence plants have the ability to detect a very shallow light gradient irrespective of the light level [1, 2]. For centuries, phototropism has provided biologists with a

convenient experimental system to study how light shapes plant growth [1, 3] (**Fig. 1**). This mode of growth adaptation is familiar to most of us that grow plants on a windowsill and is widely used for teaching purposes to illustrate the developmental plasticity of plants.

In his seminal publication The Power of Movement in Plants, Darwin used mostly monocotyledonous grass seedlings to study phototropism [1]. These simple, but effective experiments still cement our current understanding of how economically important plants such as oat, rice and maize are established upon their emergence from the soil. Darwin's work inspired generations of plant physiologists to investigate the phototropic response of flowering plants. Subsequent theories were introduced to explain how light brings about this growth adaptation. One notable concept is the Cholodny-Went hypothesis [1, 3], which states that light from one direction drives lateral movement of the phytohormone auxin from the irradiated to the shaded side of the stem. It is this greater accumulation of auxin on the shaded side that stimulates the differential growth of the seedling towards light (**Fig. 1**). Although central to the Cholodny-Went hypothesis, a complete cellular assessment of these lateral auxin movements and how they are regulated is still lacking. However, much progress has been made in the last decade or so in defining key signalling events associated with phototropism using the dicotyledonous genetic model *Arabidopsis thaliana*. In this review we summarize recent advances in uncovering the molecular complexity underlying this emblematic plant growth phenomenon. Key steps including a timeline are graphically illustrated in (**Fig. 2**). For more extensive reviews we suggest the following publications [3-6].

Photoreceptors for Phototropism

Arabidopsis has proven instrumental in defining the molecular identity of light perception and auxin signalling components involved in phototropism [3, 4]. Both root and hypocotyl phototropism in Arabidopsis is regulated by phototropin blue light receptors [5]. Since their discovery, considerable progress has been made in understanding how these photoreceptors are activated by blue light. All flowering plants, including Arabidopsis contain two phototropins

(phot1 and phot2) [7]. Phot1 functions primarily as the photoreceptor for root phototropism [5] and hypocotyl phototropism over a broad range of blue light intensities [7]. By contrast, the action of phot2 in hypocotyl phototropism is restricted to high light intensities owing largely to light-mediated increases in protein abundance [7, 8]. Arabidopsis phototropins also elicit hypocotyl curvature to UV-B wavelengths [2]. Intriguingly, phototropin-deficient seedlings still exhibit phototropism towards UV-B [2]. This phototropin-independent response is mediated by the newly identified plant UV-B photoreceptor, UV RESISTANCE LOCUS 8 (UVR8) [2]. The growth mechanism underlying UVR8-dependent hypocotyl phototropism may be distinct from phototropin-mediated events triggering hypocotyl curvature towards blue light [9].

Phototropic responses in the gametophytes of the Japanese maidenhair fern *Adiantium capillis-veneris* are regulated by both red and blue light [10]. A dual red-blue light absorbing phytochrome-phototropin receptor chimera designated neochrome is responsible for this response in Adiantum (and other polypodiaceous ferns) and serves to provide enhanced light sensitivity in shaded habitats [10]. Recent phylogenetic studies indicate that ferns have acquired neochrome from non-vascular hornworts by horizontal gene transfer [11]. While mosses lack neochrome, phototropism to red and blue light in *Physcomitrella patens* is achieved through direct interactions between phytochrome and phototropin photoreceptors [12]. Neochromes have also been identified in the filamentous green algae *Mougeotia scalaris* but have arisen independently from those found in ferns and hornworts [11].

In contrast to ferns and mosses, hypocotyl phototropism in angiosperms is not induced by red light. However, red light sensing by the phytochromes is known to enhance this response [6]. Curvature enhancement is best observed when red light is given 1-2 hour prior to the directional blue light treatment [13] and is dependent on phytochrome A (phyA) [14]. Mutants lacking phyB phyC phyD and phyE appear to exhibit normal phototropic responsiveness suggesting that phyA is the primary phytochrome modulating phototropism in Arabidopsis [15]. Curvature enhancement by phyA has been attributed to an attenuation of phot1

internalization from the plasma membrane [16]. Yet, this enhancement response is still apparent in seedlings where phot1 is constitutively anchored to the plasma membrane [17]. Alternatively, phytochrome-regulated expression of key signaling components associated with auxin transport is likely to be involved [6, 18]. Blue light sensing by the cryptochromes is also reported to impact phototropism in Arabidopsis. Hypocotyl curvature is reduced in the *cry1cry2* double mutant implying that cryptochromes either indirectly control the process by modulating blue light-regulated growth or through more direct ways that still remain to be fully understood [6].

Activation of Phototropin by UV/Blue Light

As discussed, phototropins are the primary photoreceptors controlling hypocotyl phototropism in Arabidopsis to UV/blue wavelengths. Phototropins are plasma membrane-associated proteins comprising an N-terminal light-sensing domain and a C-terminal serine/threonine kinase domain **(Fig. 2)**. The N-terminal region contains two specialized Light, Oxygen or Voltage domains (designated as LOV1 and LOV2), which function as binding sites for the blue light absorbing chromophore flavin mononucleotide (FMN). In the absence of light, FMN is bound non-covalently within the LOV domain, absorbing strongly in the blue region (λ_{max} ~450 nm). Irradiation of the LOV domain results in the formation a covalent adduct between the C(4a) carbon of the FMN chromophore and the sulfur atom of a nearby, conserved cysteine residue [7]. Adduct formation occurs rapidly (within microseconds) absorbing maximally at 390 nm. This photochemical reaction is fully reversible in darkness and can be completely abolished by mutation of the cysteine involved [7]. Although both LOV domains can function as light sensors, phototropin kinase activity is regulated primarily by LOV2 [7, 19]. In darkness, LOV2 together with adjacent peptide sequences act to repress phototropin kinase activity **(Fig. 2)**. Photoactivation of LOV2 leads to protein conformational changes that relieve this repression activity resulting in receptor autophosphorylation and an initiation of phototropic signaling [7]. A detailed description of the photochemical and biochemical properties of phototropin blue light receptors is beyond the scope of this review and readers are directed to the recently published review article for further information [7].

Auxin and Phototropic Growth

Asymmetric growth between the shaded and irradiated sides of the hypocotyl enables a seedling undergoing phototropism to grow towards the light. In Arabidopsis and other species, phototropin-mediated curvature results from an increase in elongation at the shaded side combined with a decrease in elongation at the irradiated side [20]. In dark-grown (etiolated) Arabidopsis seedlings, the region of curvature (or elongation zone) and light sensing reside within the upper hypocotyl [21, 22], whereas elongation is initiated over a broader part of the hypocotyl in light-grown (de-etiolated) seedlings [23]. The modifications in growth rates during phototropism has been shown to correlate well with the redistribution of the principle auxin indole-3-acetic acid (IAA) between the shaded and the irradiated sides of *Brassica* hypocotyls – elongation being promoted by auxin accumulation on the shaded side [24]. Although this has not been measured directly in Arabidopsis hypocotyls, the use of the auxin response sensor DII-Venus has enabled the visualization of an auxin gradient across the hypocotyl prior to the onset of asymmetric growth [25]. The importance of auxin in establishing phototropism has been studied extensively by interfering, either genetically or pharmacologically, with its perception, transport and signaling [4]. In the following section, we will describe our current understanding of how phototropin activation leads to the establishment of this auxin gradient and point out remaining open questions **(Fig. 3)**.

Early Signaling Events

Light-mediated generation of a gradient of phototropin autophosphorylation across the seedling [26] has been proposed as the biochemical basis for establishing lateral auxin movements underlying phototropic growth. Given that protein kinase activity and phosphorylation within the activation loop of the phototropin kinase domain are essential for the phototropic response [27, 28], the current model is that phosphorylation of signaling components (directly by the phototropins or following a cascade of events) leads to the formation of an auxin gradient.

The importance of auxin redistribution in phototropism suggests that regulated auxin transport is a likely mechanism [29]. Young leaves (and cotyledons) are considered as a major source of auxin from where it is transported down towards the lower parts of the plant including stems (hypocotyls) and the roots [30]. Several well-known processes [31] control the directional flow of this so-called polar auxin transport. Under typical physiological conditions, a substantial fraction of the apoplastic auxin exists in the protonated form (IAAH) and can freely enter cells, a process that is further enhanced by members of the AUXIN (AUX)/LIKE AUX1 (AUX/LAX) family of auxin importers. Once in the cell where the pH is neutral the weak acid IAA^- is trapped and requires efflux carriers to pump it back out and hence allow transport from cell to cell. Two main families of transporters have been implicated in this process: long-PIN-FORMED efflux carriers (PIN1-4, PIN7 in Arabidopsis) and several multi-drug resistance transporters from the ATP-Binding Cassette B (ABCB) class, most notably ABCB19 [4, 23].

The discovery of polar PIN localization predicting auxin fluxes in several organs made them likely candidates regulating phototropic growth [29]. Indeed, a mutant lacking the three major PINs expressed in aerial plant parts are severely compromised in phototropism [32, 33]. However, the interpretation of this phenotype is not straightforward as compromised phototropism could result from reduced polar transport of auxin towards the elongation zone and/or reduced lateral redistribution of IAA within the elongation zone **(Fig. 3)**. Interestingly, PIN3 is laterally redistributed in the endodermal cells of etiolated seedlings following phototropic stimulation [34]. Moreover, PIN activity is regulated by phosphorylation by two protein kinase families from the AGCVIII class: PINOID (PID) and D6 PROTEIN KINASES (D6PKs) [35, 36]. Although phototropins also belong to the same AGCVIII protein kinase family [36], there is currently no evidence demonstrating direct phosphorylation of PINs by the phototropins [34]. In contrast, plants lacking D6PKs are severely defective in phototropism [33] suggesting that light-regulated PIN phosphorylation may represent an important regulatory mechanism. However, this remains conjecture at this time and how light-activated phototropin kinase activity would

lead to such an event remains unknown. Further, the importance of PID and members of this group of kinases in the regulation of phototropism appears to be restricted to rather specific conditions [18, 21].

A more direct regulation of auxin efflux by phot1 was established for ABCB19. In this case, phot1-mediated phosphorylation of ABCB19 inhibits its efflux activity, at least when expressed in HeLa cells [23]. This mode of regulation has been proposed to inhibit polar auxin transport and indirectly promote a lateral flux of auxin favoring phototropism in the upper region of the hypocotyl [23]. Phot1 also directly phosphorylates PHYTOCHROME KINASE SUBSTRATE 4 (PKS4), a post-translational modification that was suggested to inhibit the activity of this positive regulator of phototropism [37]. PKS4 and other members of this protein family directly interact with the phototropins and NON-PHOTOTROPIC HYPOCOTYL 3 (NPH3) at the plasma membrane. PKS proteins have been suggested to modulate auxin transport, however the mechanism of action of the phot1-PKS-NPH3 protein complex remains unknown [38]. NPH3, which is essential for phototropism, is rapidly dephosphorylated upon phot1 activation and acts early in the pathway upstream of auxin redistribution [39, 40]. NPH3 is part of a Cullin3-based E3 ligase complex, SCF^{NPH3} that has been proposed to regulate auxin redistribution through ubiquitin-mediated relocalization of auxin transporters [41].

The role of AUX/LAX auxin influx carriers in the control of phototropism is rather limited [3, 23, 25]. However, pH regulated auxin influx appears to be involved in this growth regulation [25]. Activation of plasma membrane H^+-ATPases acidifies apoplastic pH and thereby increases the fraction of IAAH that can freely permeate cells. Deregulating H^+-ATPase activity interferes both with IAA gradient formation and phototropism [25]. Moreover, light in a phototropin-dependent manner regulates phosphorylation of key residues required for H^+-ATPase activation [25]. An important prediction from this work that remains to be demonstrated is the detection of a rapid asymmetric apoplastic acidification across the Arabidopsis hypocotyl. In addition to the routes of auxin movement discussed above, auxin can also move symplastically through plasmodesmata

that connect cells. These symplastic connections work against the establishment of an auxin gradient across a tissue. A recent study has identified a mechanism that down-regulates plasmodesmata permeability thereby preventing dissipation of the auxin gradient that is established during phototropism [42].

Growth of elongating hypocotyl cells is highly anisotropic. Hence when considering growth coordination leading to phototropism we also need to take into account the mechanisms controlling growth in different directions. In elongating hypocotyl epidermal cells, cortical microtubules are organized perpendicularly to the major growth axis (transverse orientation) [43]. These cortical microtubules guide the deposition of cellulose microfibrils, which are determinants of growth anisotropy [43]. Application of exogenous IAA to maize coleoptiles induces growth and re-orientation of cortical microtubules from a longitudinal to a transverse orientation within about 15 minutes [44]. Asymmetric changes in microtubule orientation have also been observed during phototropism in maize coleoptiles and sunflower hypocotyls with an increase in transverse orientation occurring on the faster growing shaded side [45]. More recently, cortical microtubules are reported to reorientate upon phototropic stimulation of Arabidopsis hypocotyls in a phototropin-regulated manner [46]. Moreover, Arabidopsis mutants defective in microtubule reorganization are impaired in phototropism [46]. Taken together, these studies suggest that phototropin activation leads to auxin gradient formation and microtubule reorganization that are both required for regulated anisotropic growth. An important question is whether auxin regulates both the growth potential and its anisotropy or whether these two events simply coincide in time. A causal relationship was suggested based on the involvement of the auxin receptor AUXIN BINDING PROTEIN 1 (ABP1) for rapid microtubule reorganization [47]. However, this hypothesis has been questioned in the past and requires further evaluation following the recent discovery that ABP1 has no obvious function in auxin signaling [48].

Conclusion and Outlook

Extensive work with Arabidopsis has shed considerable light on the identity of key signaling events associated with hypocotyl phototropism. It is well established that photoactivation of phototropin blue light receptors is necessary to bring about this differential growth response. Yet the mechanisms that couple receptor activation to changes in auxin mobilization remain largely mysterious. Genetic analyses indicate the involvement of multiple auxin transporter proteins in addition to a number of important regulatory components, including D6PKs, PKS proteins, NPH3 and related proteins. Arabidopsis has been instrumental in providing some light at the end of the tunnel in understanding this complex growth phenomenon. However, deciphering how these factors are regulated at the biochemical level and how they are integrated to coordinate light-driven changes in auxin mobilization, pH and microtubule reorientation associated with phototropic growth still remains a major challenge for researchers in the field.

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Figure 1. Phototropism in a Brassica seedling. Note hypocotyl growth towards the light (positive phototropism) and root growth away from the light (negative phototropism).

Figure 2. Model (A) and timing (B) of early signaling steps leading to phototropism. (A) Phototropins have two light sensing LOV domains (L1 and L2) and a carboxyl-terminal protein kinase domain (KD). Upon light activation the FMN chromophore covalently binds to an invariant Cys residue in the LOV domains (symbolized by a change of color of the L1 and L2 domains); this is followed by conformational changes releasing the protein kinase domain from the inhibitory activity of LOV2. This leads to autophosphorylation of the phototropins and direct phosphorylation of the signaling components ABCB19 and PKS4 (symbolized by a single arrow) and activation of other signaling components through less clearly established mechanism (symbolized by two connecting arrows). Formation of a lateral auxin gradient and reorientation of cortical microtubules lead to oriented hypocotyl growth towards the light source. (B) Phototropin photochemistry occurs in the μs range; phot1 autophosphosphorylation and phosphorylation of PKS4 can be detected within 15-30 sec of blue light illumination; auxin gradient formation was not directly measured in Arabidopsis but is expected to start within less than one hour of

illumination; finally the speed of phototropic reorientation depends on the exact experimental conditions but typically starts about an hour after illumination.

Figure 3. Model of auxin fluxes. Auxin fluxes (symbolized by red arrows) prior (on the left) and after (on the right) phototropic stimulation. Note the movement of auxin towards the shaded side that will grow faster to allow growth towards the light.

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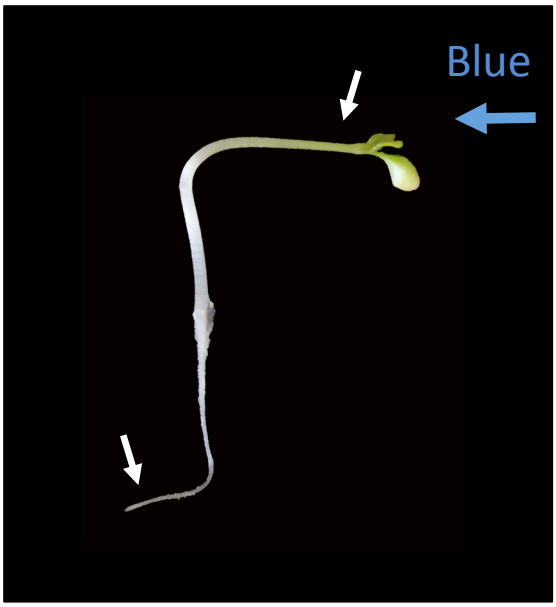

Figure 1. Phototropism in a Brassica seedling. Note hypocotyl growth towards the light (positive phototropism) and root growth away from the light (negative phototropism).

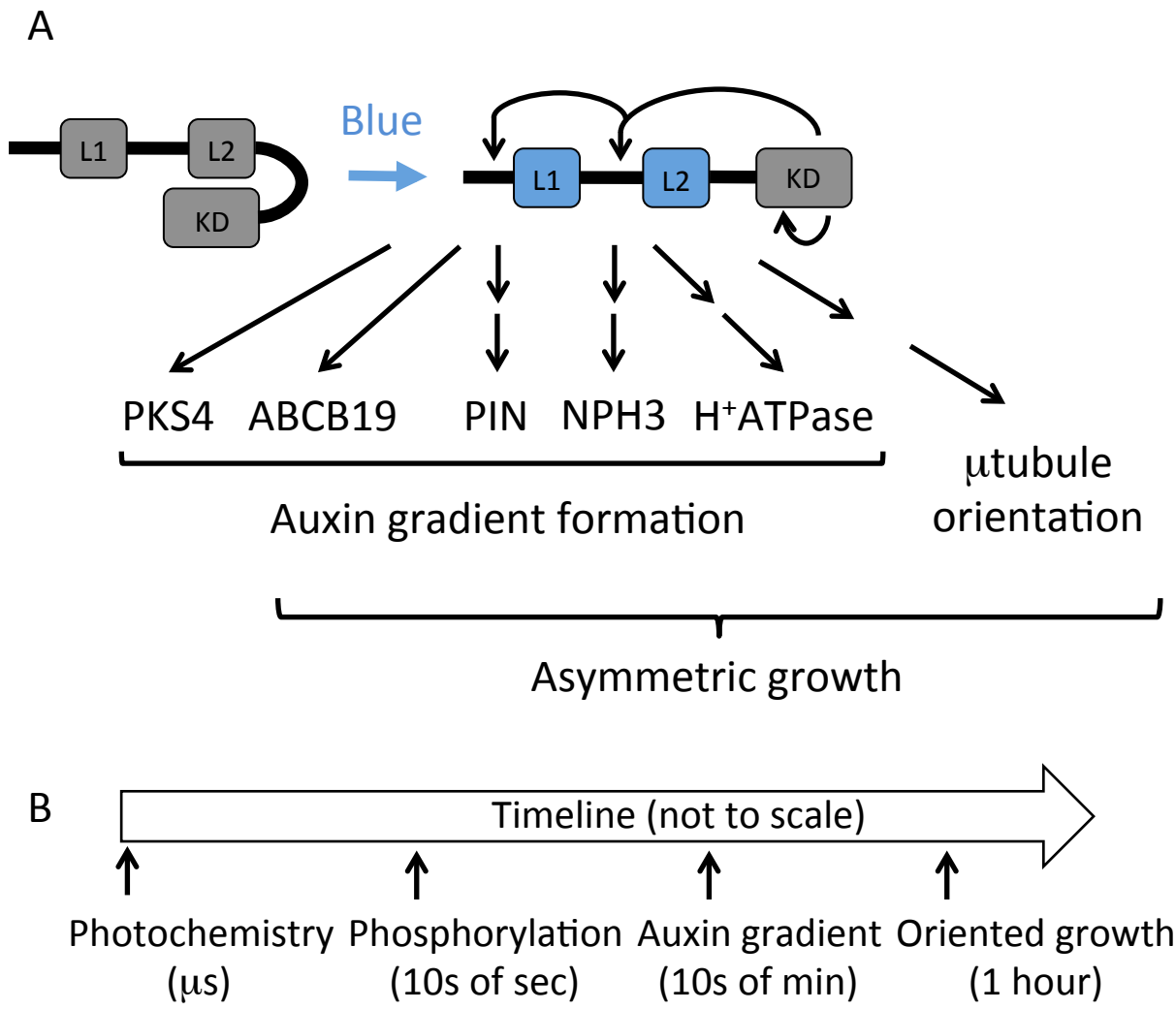

Figure 2. Model (A) and timing (B) of early signaling steps leading to phototropism.

(A) Phototropins have two light sensing LOV domains (L1 and L2) and a carboxyl-terminal protein kinase domain (KD). Upon light activation the FMN chromophore covalently binds to an invariant Cys residue in the LOV domains (symbolized by a change of color of the L1 and L2 domains); this is followed by conformational changes releasing the protein kinase domain from the inhibitory activity of LOV2. This leads to autophosphorylation of the phototropins and direct phosphorylation of the signaling components ABCB19 and PKS4 (symbolized by a single arrow) and activation of other signaling components through less clearly established mechanism (symbolized by two connecting arrows). Formation of a lateral auxin gradient and reorientation of cortical microtubules lead to oriented hypocotyl growth towards the light source.

(B) Phototropin photochemistry occurs in the μs range; phot1 autophosphosphorylation and phosphorylation of PKS4 can be detected within 15-30 sec of blue light illumination; auxin gradient formation was not directly measured in Arabidopsis but is expected to start within less than one hour of illumination; finally the speed of phototropic reorientation depends on the exact experimental conditions but typically starts about an hour after illumination.

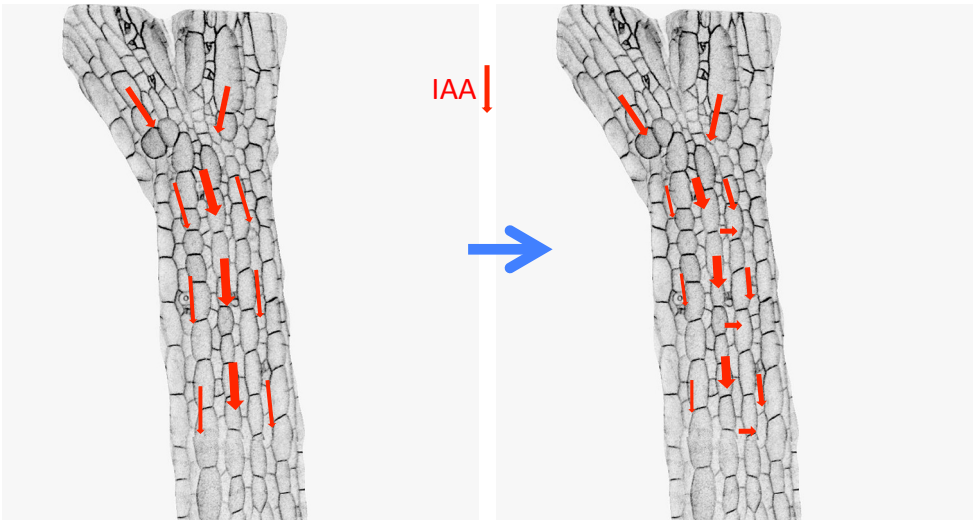

Figure 3. Model of auxin fluxes . Auxin fluxes (symbolized by red arrows) prior (on the left) and after (on the right) phototropic stimulation. Note the movement of auxin towards the shaded side that will grow faster to allow growth towards the light.