Bending to auxin: Fast acid growth for tropisms

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Abstract

The phytohormone auxin is the major growth regulator governing tropic responses including gravitropism. Auxin build-up at the lower side of stimulated shoots promotes cell expansion, whereas in roots it inhibits growth, leading to upward shoot bending and downward root bending, respectively. Yet it remains an enigma how the same signal can trigger such opposite cellular responses. In this review, we discuss several recent unexpected insights into the mechanisms underlying auxin regulation of growth, challenging several existing models. We focus on the divergent mechanisms of apoplastic pH regulation in shoots and roots revisiting the classical Acid Growth Theory and discuss coordinated involvement of multiple auxin signalling pathways. From this emerges a more comprehensive, updated picture how auxin regulates growth.

Directional growth as key mechanism for plant adaptive development

Plant cells do not migrate during tissue patterning and the whole body plan results from the orientated cell division and growth. This puts the regulation of cell expansion at the center of plant development and its adaptation to the environment [1] with tropisms being spectacular examples. During gravitropism, the phytohormone auxin is transported to the lower side of the stimulated organ, where the cell growth is promoted (in shoots) or inhibited (in roots). The resulting differential growth rate between the lower and upper side of the organ leads to upward or downward bending, respectively [2]. This is a prime example for the contribution of regulated cell expansion to general plant development and adaptive behavior. Despite the importance of auxin in cell signalling, how it regulates cell expansion oppositely in shoots and roots remained largely unknown. Several contemporary studies focusing on the mechanism of auxin-induced rapid root growth inhibition and shoot growth promotion, as well as novel auxin signalling pathways provide cutting-edge insights into this topic.

Main entry points for the regulation of cell expansion

To understand how the growth of plant cells is regulated, one must consider their special features. Distinct from animal cells, plant cells have a high turgor pressure ranging between 0.6 and 1 MPa [3] and are encased by a structural layer of the cell wall. Plant cell growth is the consequence of the balance between the driving force (turgor pressure) and the limiting force (cell wall). The turgor pressure increases by osmosis-driven water uptake driven by the membrane potential, which is built up by the difference in the ion concentrations across the plasma membrane (PM) resulting from the active H^+ pumping out of the cell. The vacuole which accumulates water and osmotic compounds possible also contribute to turgor-driven growth regulation [4]. The robust cell wall limits expansion of the pressurized cells. The cell wall rigidity depends not only on the composition and structural arrangements, which are regulated by cortical microtubules (CMTs), but also on the cell wall-based enzymes, whose activities are regulated by the apoplastic pH [5-7]. Hence, ion fluxes, apoplastic pH, CMTs, and vacuoles are all potentially contributing to the regulation of cell growth.

Auxin: one signal with manifold performances

Auxin is the main endogenous signal regulating cell growth across the plant with shoots and roots having distinct sensitivities. Exogenous auxin promotes the elongation of arabidopsis (*Arabidopsis thaliana*) hypocotyl segments even at 10 µM [8], whereas it already inhibits root growth at 5 nM [9]. Similarly, following gravistimulation, auxin accumulation stimulates cell expansion in shoots, whereas inhibiting it in roots [10, 11]. The timing of growth responses in the two organs is also different. Following gravistimulation, arabidopsis hypocotyl starts bending after 1-2 hours and it takes ca. 4-6 hours to reach the half-bending angle [12]. By comparison, the root starts bending visibly already 10 minutes after gravistimulation and it takes ca. 40-60 minutes to reach the half-bending angle [13, 14]. Similarly, exogenous auxin application promotes the growth of etiolated hypocotyl segments in about 20 minutes [15] whereas inhibits it in intact roots in less than 30 seconds [9, 16], despite that the organs transcription responds to auxin in a similar time scale of ca. 20 minutes as reported by DR5::LUC reporters [9, 15]. These differences in concentration and timing suggest that the mechanism of auxin-triggered cell growth regulation differs between shoots and roots.

To understand how auxin regulates cell growth in different tissues, we focus on: (i) auxin-triggered cellular responses and (ii) upstream auxin signalling. During auxin-induced

root growth regulation, auxin triggers a series of cellular responses, such as CMT reorientation, vacuole constriction, Ca^{2+} transient, apoplast alkalinization, membrane depolarization and K^+ efflux. We critically examine the involvement of those cellular responses and upstream signalling in growth regulation.

Cortical microtubule reorientation: a consequence not the cause

CMTs are microtubule arrays located close to the PM. In elongating cells, they co-localize with and are required for guiding the cellulose synthase complex, which produces cellulose fibrils building the main structure of the cell wall [17, 18]. The orientation of CMTs thus determines the anisotropy of the cell wall, to either restrict or allow cell expansion in a certain direction. Therefore, CMTs contribute to growth regulation and may be, potentially, part of the mechanism by which auxin regulates growth.

In response to auxin, CMTs reorient from longitudinal to transversal in respect to the growth axis in etiolated arabidopsis hypocotyls and oppositely in roots. In both organs, the CMT orientation correlates with the growth regulation. Nonetheless, the causal relationship has remained a matter of debate over the years [19, 20]. Recent pharmacological and genetic studies in arabidopsis hypocotyls consistently argued that CMT reorientation is not a crucial part of the auxin-triggered mechanism for growth regulation [8]. For example, auxin can promote growth normally, even when CMTs are depolymerized, confirming that intact CMTs are not essential. On the other hand, auxin treatment in hyperosmotic conditions that prevent growth, does not lead to CMT reorientation. This shows that in shoots CMT reorientation responds to the growth promotion but not to auxin itself [8]. Similarly in roots, kinetic analysis of CMTs after auxin treatment demonstrated that a significant CMT reorientation occurred later than growth inhibition [16]. Furthermore, the inhibition of auxin-triggered CMT reorientation by the MT stabilizer taxol does not influence the growth inhibition by auxin [16]. Collectively, in both shoots and roots, CMT reorientation is the indirect consequence rather than cause of the auxin-induced growth change (Figure 1).

Vacuolar constriction: too late for the show

Vacuoles are unique plant organelles. Their development is a dynamic combination of fusion and fragmentation of liquid pouches, the size of which can take up to 90% of a mature plant cell [21]. Due to its potential contribution to the osmotic properties of cells, vacuoles have been linked to the regulation of cell growth [4, 22].

During auxin-triggered root growth inhibition, a concomitant constriction of vacuoles has been observed [4]. Similar to CMT reorientation, the question remains whether the vacuolar constriction contributes to or is only the consequence of growth inhibition. The kinetics of vacuole morphology and cell length in roots after auxin treatment revealed that vacuole changes take place within 15-25 minutes, thus seemingly preceding cell length changes, which were visible in the late meristematic zone by the applied method only after about 45-55 minutes. All genetic and pharmacological manipulations however of auxin signalling and cellular processes were analysed only after 20 hours of the respective treatment [4, 22] not allowing for definite statements about time dynamics. Also, there was no obvious auxin-triggered change in the vacuole morphology in the elongating cells [16], which have the highest capacity of growth regulation by auxin [23, 24]. This puts the process of vacuolar morphology changes well outside the time scale of auxin-triggered root growth inhibition, which occurs faster than 30 seconds [9] arguing against its direct involvement in the mechanism for the immediate auxin-induced root growth inhibition (Figure 1).

Early auxin birds: Ca^{2+}, H^+ and K^+ fluxes across the PM

Unlike CMT reorientation and vacuole constriction, ion fluxes across the PM in root cells change practically immediately after auxin application. The most significant ones are Ca^{2+} and H^+ influxes (Figure 1). Specifically, a cytosolic Ca^{2+} transient and a rhizospheric pH increase occurred within 7-14 seconds and 15 seconds, respectively after auxin treatment [25]. Consistently, the apoplast pH was increased upon auxin in 30 seconds or earlier [16]. During gravitropism, both cytosolic Ca^{2+} levels and the rhizospheric pH changed in both upper (decreased Ca^{2+} and pH) and lower (increased Ca^{2+} and pH) flank 2-6 minutes after gravistimulation [25]. Therefore, the Ca^{2+} transient and external pH changes are very early responses to auxin and closely correlate with auxin-induced rapid root growth inhibition [16] (Figure 1).

The possible causal relationship between the auxin-induced Ca^{2+} transient, extracellular alkalinization, and root growth inhibition has been addressed pharmacologically and genetically. The Ca^{2+} channel inhibitor $LaCl_3$ interferes with auxin-induced rhizosphere alkalinization [25]. Similarly, mutation of the Ca^{2+} permeable cation channel Cyclic NUCLEOTIDE-GATED CHANNEL 14 (CNGC14) leads to a delay of apoplast alkalinization and growth inhibition of ca. 6 minutes after auxin application [16, 26]. Besides, depletion of Ca^{2+} in the medium results in a diminished Ca^{2+} transient as well as a delay of

pH and growth responses of ca. 4-6 minutes [16]. This suggests that CNGC14-mediated Ca^{2+} transients contribute to early auxin response by apoplast alkalinization and growth inhibition.

In contrast to influx of Ca^{2+} and H^+, K^+ is transported out of root cells after auxin [16]. The efflux of K^+ leads to less water uptake [27], in line with less cell expansion. Besides, the total net ion fluxes across the PM after auxin result in a rapid membrane depolarization [28, 29], contributing to the growth inhibition.

In the driver's seat: apoplastic pH changes and the Acid Growth Theory

Auxin application leads to rapid apoplastic pH changes simultaneously with the growth regulation in both shoots and roots. Not only the time scale, but also the trend of the change in the apoplastic pH and growth regulation coincide. In shoots, auxin leads to slower acidification and growth promotion [15, 30]; while in roots, it results in rapid alkalinization and growth inhibition [16, 25, 31]. The long-standing Acid Growth Theory suggests that the apoplastic pH directly regulates the cell growth. Acidification of the apoplast activates pH-dependent expansins that loosen the otherwise rigid cell wall allowing for cell expansion. Concomitantly, the H^+ efflux builds up a higher membrane potential that drives the secondary ion influx, leading to an increase in turgor pressure and water uptake [27]. In this theory, H^+ flux across the PM coordinates both the cell wall rigidity and turgor pressure to regulate cell growth [27].

The molecular mechanism of the Acid Growth Theory has been well established in the arabidopsis hypocotyl. Auxin transcriptionally upregulates the expression level of SMALL AUXIN Up-RNA 19 (SAUR19), which binds to and inhibits the TYPE 2C PROTEIN PHOSPHATASES (PP2C). PP2C normally de-phosphorylates and inhibits the activity of the PM H^+-ATPases [30, 32]. By inhibiting the PM H^+-ATPases inhibitor, this auxin-induced activation of the PM H^+-ATPases leads to apoplast acidification and thus promotes shoot growth [15, 33]. In addition, recent discoveries revealed that the PM H^+-ATPases can be directly phosphorylated and activated by the cell surface kinase TRANSMEMBRANE KINASE 1 (TMK1) in both shoots and roots [16, 34]. Particularly in shoots, auxin induces interaction between TMK1 and AHA1 in 10 seconds, and auxin-induced acidification require TMK1 and TMK4 [34]. This adds a missing mechanism in shoots for initial phosphorylation and activation of PM H^+-ATPases before the SAUR-mediated transcriptional mechanism hits in. Nonetheless, the relevance of this mechanism for shoot growth is not entirely clear, considering that auxin induces apoplast acidification and

growth in hypocotyl segments with delay of about 20 minutes and it strongly relies on transcriptional TIR1/AFB-mediated signalling [15].

In roots, the situation is more complex. Here, auxin induces apoplast alkalinization leading to growth inhibition, thus also following the main premise of the Acid Growth Theory. However, the auxin-triggered, TMK1-mediated activation of PM H^+-ATPases mediates apoplast acidification also in the root [16, 35]. This counteracts the observed more dominant apoplast alkalinization [16]. The physiological meaning of this antagonistic gas – brake growth regulations is unclear but it might be important to fine-tune the root growth during navigating a complex soil environment.

The mechanism underlying TIR1/AFB-mediated apoplast alkalinization remains unclear. Besides alkalinization of the apoplast, auxin triggers simultaneously acidification in the cytosol next to the PM and increases net proton influx, suggesting that auxin promotes H^+ influx to alkalinize the apoplast and depolarize the PM for rapid root growth inhibition [16, 28]. The question remains, how this is achieved. One possibility is that this inward H^+ flow is directly symported by the active auxin importer AUX1/LAX, with 2 H^+ per IAA molecule [29]. However, a conserved estimation does not favour it; the amount of auxin-induced H^+ influx measured in primary roots or root hairs is a magnitude higher than the maximum amount of H^+ symported by the overexpressed AUX1 in *Xenopus laevis* oocytes [16]. Additionally, bypassing auxin import by directly injecting auxin into root hair cytosol still led to a consistent membrane depolarization, though with a transient hyperpolarization [29]. This suggests that auxin-induced membrane depolarization or H^+ influx is not contributed significantly by auxin import itself.

Other possibilities include that auxin regulates an ion transporter or channel that symports H^+, or actively opens a H^+ channel, or creates a H^+ leak in the membrane by some other mechanism. Considered that this process seems to be linked to cytosolic Ca^{2+} transients [36], the possible H^+ symporter might be a Ca^{2+} transporter or channel. Nonetheless, the Ca^{2+} transient and pH change displayed different kinetics following auxin treatment or gravistimulation [25] not supporting the hypothesis that Ca^{2+} and H^+ are symported. Therefore, it is likely that auxin actively opens an unknown H^+ channel that may be Ca^{2+}-dependent.

In summary, following the classical Acid Growth Theory, the auxin-induced apoplastic pH changes are the major cellular mechanism of the growth regulation in both shoots and roots. In shoots, auxin acidifies the apoplast via transcriptional activation [15, 30] and post-translationally maintaining the activation of PM H^+-ATPases [34]. In roots, though

this post-translational activation of PM H^+-ATPases also applies, a more dominant process is immediate, auxin-triggered apoplast alkalinization mediated by a non-transcriptional branch of the TIR1/AFB signalling (see next chapter), possibly occurring through non-transcriptional activation of a H^+ channel for a rapid H^+ influx [16].

Not so canonical: TIR1/AFB-mediated non-transcriptional responses

The canonical, nuclear auxin signalling pathway is well characterized and has been for decades thought, rather exclusively as the mechanism mediating auxin effect on gene transcription. It begins with the auxin perception facilitating the binding between the co-receptors, SCF-TIR1/AFB ubiquitin ligases and the Aux/IAA transcriptional repressors. This leads to the ubiquitination of the Aux/IAAs and their further degradation via the 26S proteasome. Consequently, the repression of AUXIN RESPONSE FACTOR (ARFs) is released and they are free to act on auxin response genes [37-39] **(**Figure 2**)**.

The exception has been discovered in roots, where auxin alkalinizes the apoplast and inhibits growth faster than 30 seconds. This response time is far too fast for the transcriptional regulation to be involved and, in addition, the rapid auxin effects are observed also when transcription is inhibited [9, 16], altogether suggesting a non-transcriptional signalling mechanism.

Nonetheless, several observations clearly show that this signalling is still dependent on TIR1/AFB receptors. For example, the *tir1* and *afb* mutants display less auxin sensitivity in terms of apoplast alkalinization, membrane depolarization, cytosolic Ca^{2+} increase and root growth inhibition [9, 16, 29]. Furthermore, using an engineered ccvTIR1 and cvxIAA pair system, which allows for specific and selective activation of TIR1/AFB signalling [35], the cvxIAA-mediated ccvTIR1 activation is sufficient to trigger apoplast alkalinization, cytosolic Ca^{2+} transients and root growth inhibition [9, 16]. These observations lead to the conclusion that TIR1/AFB signalling has a non-transcriptional branch mediating auxin effect on rapid responses including CNGC14-mediated Ca^{2+} transients, apoplast alkalinization and rapid root growth inhibition [40] **(**Figure 2**)**.

Recent observations provide initial insights into this novel branch of the TIR1/AFB pathway. First, the subcellular localization of all six TIR1/AFB proteins in arabidopsis was examined. In roots, AFB1 is most abundant in the cytosol while TIR1 is mainly found in the nucleus [41]. It has been proposed that the cytosolic fraction of TIR1/AFBs may contribute to the fast non-transcriptional regulation for the rapid growth response while the nuclear fraction is more responsible for the slower, transcriptional regulation **(**Figure 2**)**. Accordingly, the

afb1 mutant is less auxin-sensitive than WT and *tir1* in terms of root growth inhibition, membrane potential decrease or apoplast alkalinization [16, 28]; while *tir1* is more auxin-resistant to root growth inhibition than *afb1* in a longer term (>6h) [16].

Thus, an unknown branch of auxin signalling pathway starting presumably with cytosolic TIR1/AFB receptors mediates rapid apoplast alkalinization, membrane depolarization and growth inhibition in roots. It remains unclear, at which point the branching occurs and whether the known downstream components such as Aux/IAAs and ARFs are involved. The key question is, however, the mechanism, by which this pathway promotes influx of H^+ into the cell leading to collapse of the H^+ gradient across the PM, apoplast alkalinization and membrane depolarization. It remains a challenge for future investigations to establish what this molecular mechanism of apopolast alkalinization may be and how it is activated by the fast TIR1/AFB signalling.

TMKs: Receptors or receptor-likes?

Four leucine-rich receptor-like kinases, which form the TMK family have been proposed as components of a largely elusive auxin signalling initiated at the cell surface. TMKs act in general growth regulation and downstream of auxin [42, 43]. At the concave side of the apical hook, TMK1 in response to auxin has its C-terminal kinase domain cleaved and translocated to the nucleus, where it phosphorylates and stabilizes non-canonical Aux/IAAs, resulting in gene transcription regulation [44] (Figure 2). This provides a mechanism, by which TMK1 and TIR1/AFB-Aux/IAA signalling mechanisms converge on transcriptional regulation.

On the other hand, TMKs contribute also to non-transcriptional regulation of cell growth. TMKs are required for the auxin-induced rapid activation (within 30 seconds) of RHO-RELATED PROTEIN FROM PLANTS 2 (ROP2) and ROP6 GTPases during pavement cell expansion [45-47]. A similar mechanism may act during root gravitropism, where TMK1 is important for ROP6 activation, which regulates PIN-FORMED 2 (PIN2) localization to affect root gravitropic response [48, 49] (Figure 2). Notably, both TMK and ROPs have been shown to localize into nanocluster structures presumably dependent on lipid membrane composition [50, 51] but physiological relevance of this localization remains unclear.

A mechanism emerges, by which the TMK pathway regulates apoplastic pH and cell growth via activating H^+ export. As mentioned before, TMK1 activation of PM H^+-ATPases [16, 34] in shoots maintains the initial phosphorylation of PM H^+-ATPases presumably

aiding the TIR1/AFB-mediated transcriptional regulation for a slow apoplast acidification and growth promotion [34]. On the other hand, in roots, the TMK1-AHA2 mechanism acts antagonistically to the rapid, non-transcriptional branch of the TIR1/AFB pathway, fine-tuning the root growth regulation [16] (Figure 2).

Another TMK family member, TMK4, was identified to have a distinctive role in regulating auxin biosynthesis. In response to auxin, TMK4 phosphorylates the TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS (TAA1), a key enzyme in the auxin biosynthesis pathway, leading to a suppression of auxin biosynthesis [52]. Therefore, downstream of the auxin pathway, TMK4 acts as negative feedback in the regulation of root meristem size and root hair development.

Taken together, TMKs regulate the general and the auxin-regulated cell expansion by multiple ways (Figure 2), however, the details of the downstream mechanisms are largely unknown. For example, whether auxin-triggered cleavage of TMKs' C terminus occurs and regulates other processes besides the apical hook, or how the downstream ROP activation participates in auxin-induced growth regulation, stays to be investigated.

The main open question concerns how auxin activates the TMK pathway. One possibility would be that auxin binds directly to extracellular domain of TMKs and activates them but there are no observations supporting this scenario. More plausible possibility is that another protein that binds auxin interacts with TMKs and activates them. The candidate for such "co-receptor" is AUXIN BINDING PROTEIN 1 (ABP1), which has been shown to interact with TMK1 [45]. ABP1 has been considered since decades as a possible auxin receptor, based on the ability of the maize ABP1 to bind to auxin [53, 54]. Any function of ABP1 however was put into doubt due to lack of obvious phenotypic defects in the verified knock-out mutants [55]. A systematic analysis confirmed only minor defects in the *abp1* loss-of-function mutants, whereas gain-of-function alleles showed a broad spectrum of growth and developmental aberrations [56]. This discrepancy might be caused by functional gene redundancy, presumably from the germin superfamily, to which ABP1 belongs [53, 57]. Nonetheless, until these potentially redundant proteins will be identified and/or involvement of both ABP1 and TMK in some process(es) will be genetically verified, the role of ABP1 as auxin receptor for the TMK-mediated auxin signalling remains hypothetical (Figure 2).

Concluding Remarks

Auxin regulates cell expansion and triggers various short and long-term cellular responses. Some are direct parts of the mechanism for auxin-induced growth regulation, others the

indirect consequences of the growth regulation *per se*. Auxin-induced CMT reorientation and vacuole fragmentation belong to the latter case. Still, they regulate the capacity of cell growth and contribute to the control of the eventual cell size. In contrast, the auxin-induced Ca^{2+} transient is an instant response, which may be linked to auxin-triggered H^+ flux across the PM and the apoplastic pH change. The auxin-induced apoplastic pH change regulates cell growth following the Acid Growth Theory with acidification promoting and alkalinization inhibiting growth. However, the mechanisms how auxin regulates apoplastic pH varies between shoots and roots.

In shoots, auxin acidifies the apoplast through PM H^+-ATPase activation, the process mediated by both (i) the nuclear TIR1/AFB transcriptional pathway via inhibiting of PP2C phosphatase acting on PM H^+-ATPases and (ii) direct phosphorylation and activation by the cell surface-based TMK1 receptor-like kinase. In contrast, in roots, auxin alkalinizes the apoplast via rapid activation of H^+ influx, the process, which is mediated through an unknown, non-transcriptional branch of the cytosolic TIR1/AFB auxin pathway. While the nuclear fraction of TIR1/AFB presumably mediates the sustained and long-term effect of root growth inhibition. On the other hand, the cell surface-based TMK1 directly binds and activates PM H^+-ATPase also in roots; there functioning antagonistically to the apoplast alkalinization, fine-tuning the root growth regulation. A future challenge will be to unravel the mechanism of rapid H^+ influx and better characterize all various auxin signalling mechanisms (see Outstanding Questions).

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Figure 1. The time scale of auxin-triggered fast cellular responses in arabidopsis roots. In response to increased auxin levels, root cells show a rapid H^+ influx. This is contributed by CNGC14-mediated Ca^{2+} transient, but not by PM H^+-ATPases. The resulting apoplastic alkalinization causes root growth inhibition within seconds. Responding to the growth inhibition, the cortical microtubules (CMTs, green lines) are then reoriented from transversal to longitudinal/oblique. The vacuoles are constricted at later time points; not consistent with their direct involvement in rapid auxin-induced growth inhibition. Abbreviations: PM, plasma membrane; CNGC, Cyclic Nucleotide-Gated Channel; AHA, PM H^+-ATPase.

Figure 2. Auxin signalling pathways in arabidopsis. ① Non-transcriptional branch of the TIR1/AFB pathway in roots. Intracellular auxin perceived by the cytosolic fraction of TIR1/AFB triggers a rapid CNGC14-mediated Ca^{2+} influx and an unknown channel or transporter-mediated H^+ influx across the PM. The H^+ influx, contributed by the Ca^{2+} transient, leads to apoplast alkalinization and thus rapid root growth inhibition. ② The canonical, transcriptional TIR1/AFB pathway. Intracellular auxin perceived by the nuclear fraction of TIR1/AFB and Aux/IAAs leads to ubiquitination and 26S proteasome-mediated degradation of Aux/IAAs. Consequently, the inhibition of Aux/IAAs on the ARF-regulated downstream gene transcription is released including SAUR19, which inhibits PP2C that normally dephosphorylates and thus deactivates AHA. Thereby, AHA becomes activated. ③ The PM-localized TMK1, directly phosphorylates and activates AHA in both shoots and roots. ④ The PM-localized TMK1, which might perceive external auxin through ABP1, activates ROPs for pavement cell expansion and regulates PIN2 during root gravitropic response. ⑤ The PM-localized TMK1, in response to auxin, has its C-terminal kinase domain cleaved and translocated to the nucleus for phosphorylating and stabilizing non-canonical Aux/IAAs, regulating gene transcription in the apical hook. Abbreviations: PM, plasma membrane;

CNGC14, Cyclic NUCLEOTIDE-GATED CHANNEL 14; AHA, PM H^+-ATPase; TMK1, TRANSMEMBRANE KINASE 1; ABP1, AUXIN BINDING PROTEIN 1; ROP, RHO-RELATED PROTEIN FROM PLANTS; PIN2, PIN-FORMED 2; TIR1/AFB, TRANSPORT INHIBITOR RESPONSE1/AUXIN-SIGNALLING F-BOX protein; ARF, AUXIN RESPONSE FACTOR; SAUR19, SMALL AUXIN Up-RNA 19; PP2C, type 2C protein phosphatases.

Outstanding Questions:

- What is the molecular mechanism of auxin-triggered H^+ influx for root growth inhibition?

- How does the non-transcriptional AFBs/TIR1 signaling branch look like?

  Does it involve AUX/IAAs' ubiquitination and degradation?

- Do cytosolic and nuclear fractions of TIR1/AFBs mediate distinct functions?

- How does the TMK pathway perceive auxin?

Figure 1

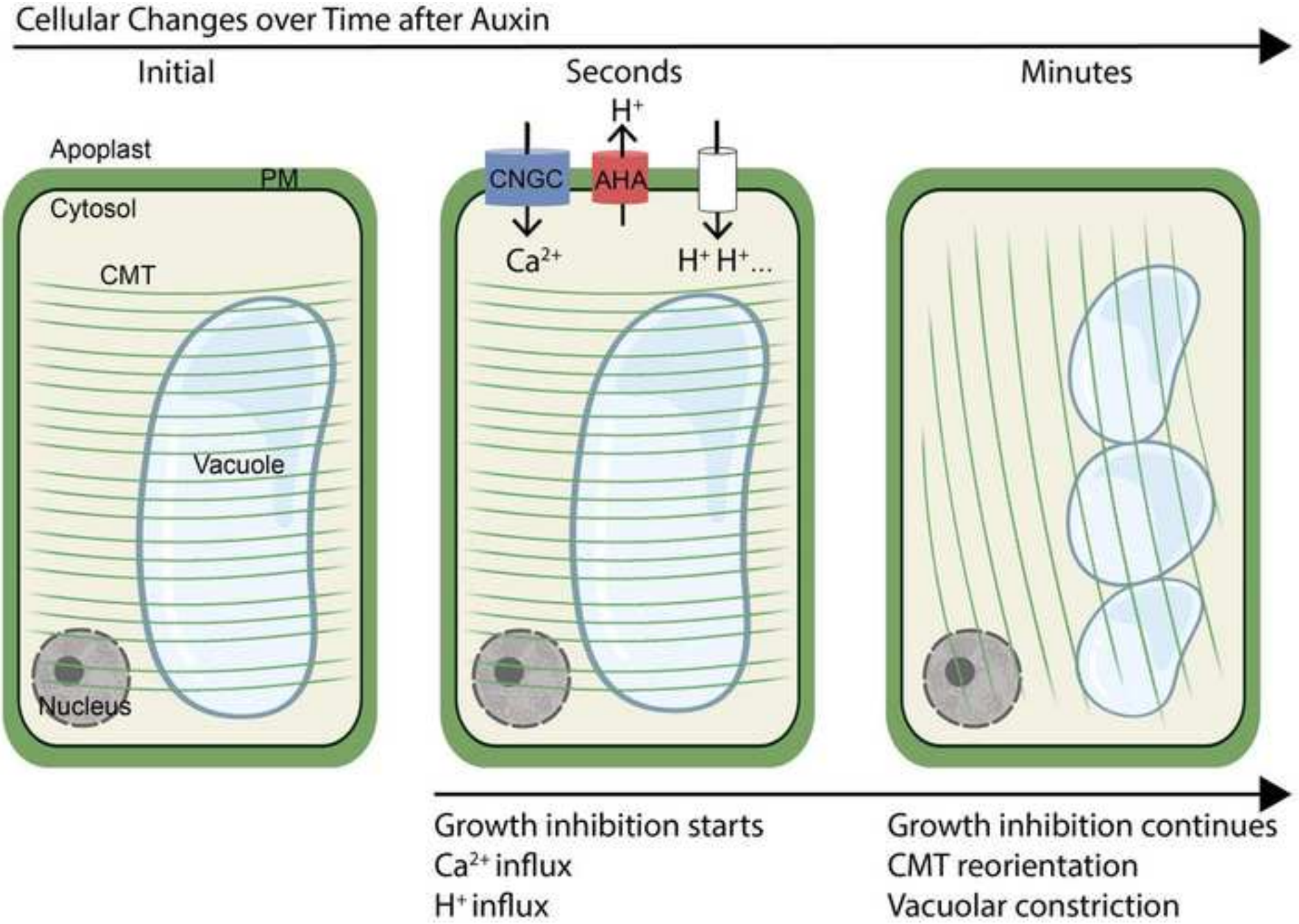

Figure 2