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Differential growth regulation in plants — the acid growth balloon theory

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'To grow or not to grow' is a central question in developmental biology and is nowadays tackled wonderfully by cell-biological approaches in various species. The rigid plant cell wall is a neat evolutionary invention for sessile organisms, which require form stability in the face of an ever-changing natural environment. However, this cellular packaging places special constrains on mechanisms that guide cellular growth. Considering the largely non-reversible, man-made environmental changes and our dependency on plant products, further insights into plant-specific growth regulation are highly desirable. Here we provide our personal, current view on cellular growth regulation in plants, highlighting the mutual importance of extra- and intracellular processes.

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A scientific torch relay

Scientific progress can be viewed as a torch relay, where knowledge is passed on and current progress hinges on outstanding research achievements from the past. When in 1665 the scientist and architect Robert Hooke improved the performance of a light microscope, he also shed first light on a plant tissue [1]. He unearthed a completely new world, and, seeing structures that resembled the tiny, dark rooms that monks inhabit in monasteries, he called the observed forms 'cells'. Hooke may never have realized the importance of this particular discovery, but he was the first — though almost forgotten — ancestor in an increasingly long line of (plant) cell biologists. Looking today at his drawing, the cork tissue he sketched fairly resembles a brick building [Figure 1a–c]. This very concrete impression of a plant cell which remains today is also reflected in the terminology of its extracellular matrix — the so-called 'cell wall'. A central question in plant cell biology has always been how this very rigid cellular construction is able to grow. The cell wall not only provides plant cells with their shape, but it also keeps them in place, rendering them completely immobile. Hence, from this theoretical point of view, it is self-evident that a mobile growth regulator, rather than cellular migration (as seen for example in animals) controls plant patterning.

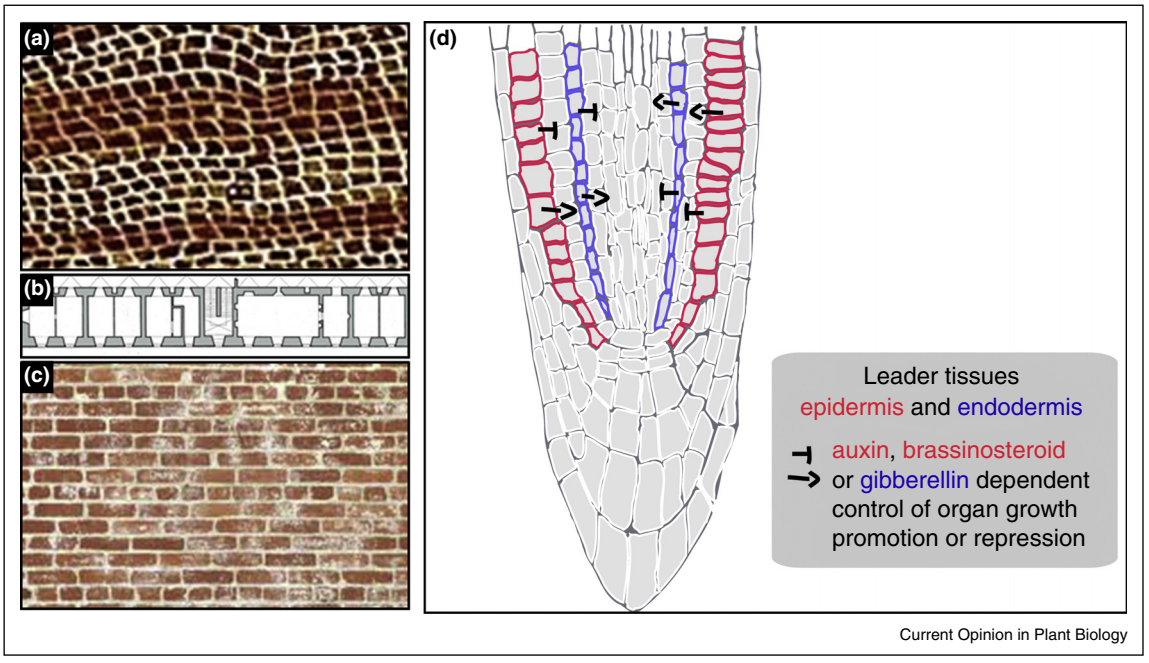
Historically, the mobile phytohormone auxin has aided plant growth researchers as a very beneficial tool, because it compels plant cells to grow or not to grow. In 1913, the Danish scientist Peter Boysen-Jensen confirmed Charles and Francis Darwin's hypothesis of a mobile growth regulator substance in plants. By observing the transport behaviour of what he named phototropic stimulus in coleoptile tips, he could show that a chemical signal (termed 'Wuchsstoff' at that time) prompts bending towards the light [2]. Approximately a decade later, the Dutch botanist Frits Warmolt Went proposed that the underlying substance is a growth-promoting hormone and the term 'auxin' (from the Greek 'auxein': to enlarge/grow) was coined [3]. Eventually, the chemical structure of auxin was identified as indole 3-acetic acid (IAA) in 1934 [4].

Ever since, research on the phytohormone auxin has been central to plant biology, not least because it is implicated in the vast majority of plant development processes, including embryogenesis and organogenesis, as well as phototropism and gravitropism [5]. In performing its versatile developmental effects, auxin impacts at single cell level, steering cell division, cell expansion, and cell differentiation [6]. Without doubt, the auxin-dependent regulation of cellular growth is among the most examined and best-studied processes in plant biology and has provided us with a mechanistic understanding of how plant cells grow.

The lead tissue concept

As the cell wall sticks plant cells together it appears obvious that the regulation of plant tissue or organ growth is above the level of single cells. The plant hormone auxin is central to this supra-cellular growth and its perception in particular cells or tissue types is sufficient to impact on the growth behaviour of entire organs. To characterize those tissue responses that can feedback on the growth of entire organs, we would like to introduce the term 'leader tissue'. Auxin is not alone in controlling plant growth; other hormones also appear to steer plant architecture in a lead tissue aspect. The epidermis, particularly, seems to control organ size

Figure 1



Cells and the lead tissue concept. **(a)** Detail of Robert Hooke's drawing of cork tissue. For him, the patterning resembled the tiny chambers monks inhabit in monasteries, consequently he termed the structure 'cells'. **(b)** The picture shows a cell section of the layout for the Disentis monastery (modified from http://www.sueddeutscher-barock.ch). **(c)** Hooke's drawings are reminiscent to a wall of bricks. The plant cell surrounding cell wall provides the shape stability and is also an important factor in cellular growth/enlargement processes. **(d)** Leader tissues, such as the root epidermis and endodermis, steer organ size and growth in a phytohormone-dependent fashion. The integration of auxin, brassinosteroid and gibberellin signals permits the regulation of growth promotion and repression.

in an auxin and brassinosteroid-dependent manner [7–10,11••], while the endodermis appears to determine organ growth in a gibberellin-dependent fashion [12–14] [Figure 1d], possibly allowing external (rhizosphere) and internal (stele) derived signals to steer organ growth.

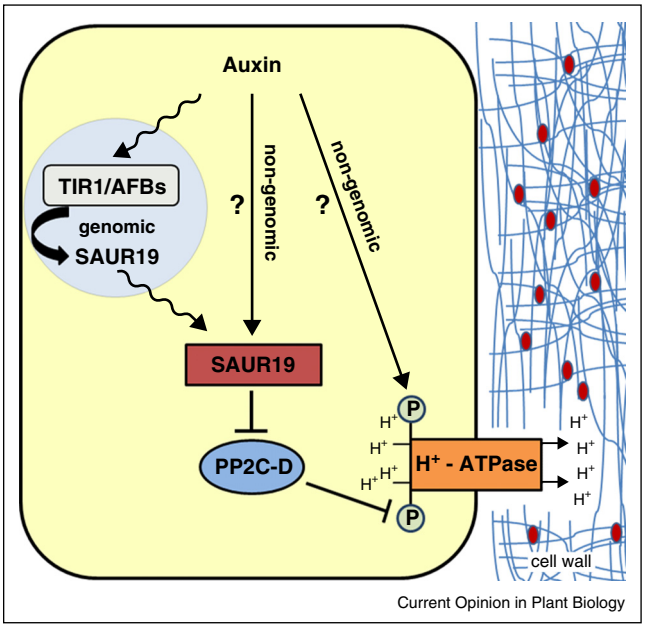
The acid growth theory

Since cellular shape is upheld by the cell wall, it has remained puzzling for a long time how plant cells can actually expand. Auxin research, roughly 45 years ago, paved the way for a better mechanistic understanding of this fundamental question. Ever since, the so-called acid growth theory has served as a model to explain cellular expansion [15-17] and largely stood the test of time. According to this hypothesis, auxin activates the plasma membrane (PM) H^+-ATPases. As a result, protons are extruded into the apoplast and consequently acidify the extracellular matrix. This acidification, in turn, activates expansins and other cell wall remodelling enzymes. Through their action, the network of cellulose and additional cell wall components is loosened, which together, with water uptake into the cell, providing the required turgor pressure, causes cells to enlarge [17,18,6] [Figure 2].

In principle, two distinct auxin receptors have been asserted as activating the acid growth pathway. On the one hand, the single copy gene AUXIN BINDING PROTEIN1 (ABP1) binds auxin and has been proposed to mediate fast, non-genomic (non-transcriptional) auxin responses as well as to contribute to the TIR1/AFBs-dependent genomic (transcriptional) responses in *Arabidopsis* [19,20]. Auxin rapidly induces growth and, hence, non-transcriptional, ABP1-dependent mechanisms were assumed to contribute to the acid growth theory [21]. By contrast to the assumed importance of ABP1 in this pathway, it has been recently shown that the full knock-out of *ABP1* does not affect *A. thaliana* development under standard conditions, currently questioning the developmental importance of ABP1 [22•]. Apoplastic acidification has not been experimentally addressed in the newly available *abp1* null alleles and it remains to be seen whether ABP1 has only a modulatory, but non-essential role. Alternatively, other still uncharacterised factors may act fully redundantly with ABP1.

Unlike the currently unclear situation for ABP1, the nuclear localized TRANSPORT INHIBITOR RESISTANT1/AUXIN SIGNALING F-BOX proteins (TIR1/AFBs) have unequivocally been shown to bind auxin and to function as genomic auxin receptors [23,24]. TIR1/AFBs act together with the transcriptional repressors AUXIN RESISTANT/AUXIN INDOLE 3-ACETIC

Figure 2



Molecular basis for the acid growth mechanism. Auxin promotes SAUR activity resulting in PP2C-D phosphatase inhibition. Thereby, PM H^+-ATPases are activated and extrude protons into the apoplast. The cell wall is consequently acidified and subsequently loosened by pH-dependent activity of expansins and other cell wall remodeling enzymes (depicted as red dots), thus enabling cellular enlargement. Non-genomic responses rely on fast non-transcriptional mechanisms, whereas genomic responses depend on TIR1/AFBs-reliant transcriptional output.

ACID INDUCIBLE (Aux/IAAs) as a co-receptor system. Auxin binding to TIR1/AFBs targets Aux/IAAs for degradation in the 26S proteasome, causing the release of AUXIN RESPONSE FACTORS (ARFs) transcription factors and thereby controlling auxin-dependent gene expression [25–27]. TIR1/AFBs emerged recently as prime candidate receptors for mediating auxin-dependent acid growth responses via the regulation of fast gene expression [28••]. Spartz and colleagues show that the rapidly auxin-induced SMALL AUXIN UP-RNA (SAUR) genes, particularly SAUR19, stimulate PM H^+-ATPase activity, thereby promoting cellular expansion. The proposed mechanism comprises the negative regulation of PP2C-D phosphatases by auxin-induced SAUR genes, thereby modulating PM H^+-ATPase phosphorylation status and subsequently its activity. This is a very elegant mode of action, but so far this model relies on the examination of SAUR gain-of-function mutants. The characterization of possibly multiple SAUR loss-of-function mutants will further define its developmental importance.

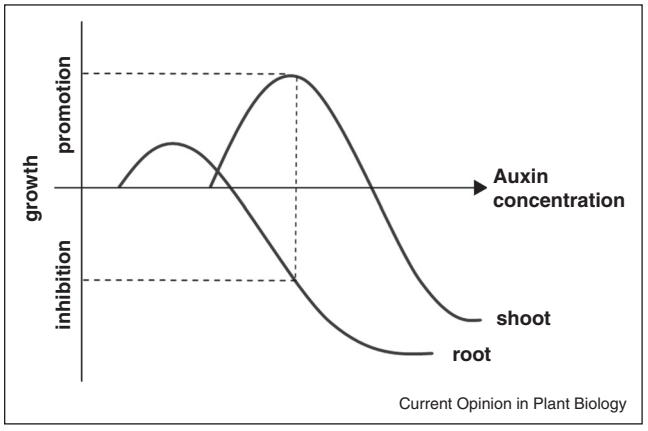
Nevertheless, a previous study suggests that the phosphorylation of PM H^+-ATPases does not require transcriptional and, hence, TIR1/AFBs-dependent auxin responses [29]. Their model, however, disregards a secondary, slower elongation phase requiring auxin-dependent gene expression and only accounts for rapid elongation occurring within a 10 minute time frame [28••]. Therefore, it is conceivable that auxin steers PM H^+-ATPase activity both directly via non-genomic (non-transcriptional) signalling, as well as indirectly, by inducing gene expression [Figure 2].

Growth induction and growth repression

Importantly, the alleged acid growth theory applies solely to tissues showing auxin-induced growth. Went, notably, was not entirely correct when stating that auxin is a growth-promoting hormone [3]. It turned out that auxin promotes and inhibits growth depending on its concentration as well as the underlying cell type [5]. It appears that low auxin concentrations induce cellular enlargements, whereas high concentrations impose growth repression. In the physiological concentration range, auxin preferentially induces growth in aerial, and represses growth in underground tissues [Figure 3].

In light of the supra-cellular and lead tissue growth mechanism, it appears obvious that auxin-dependent growth repression does not solely depend on mechanisms related to the acidification of the extracellular space. For instance, if a given lead tissue perceives a strong auxin-dependent growth repression signal, neighbouring cells possibly still continue to acidify the shared apoplastic space. Fast diffusing protons could, hence, still acidify the cell wall of the lead tissue and would consequently impose cellular expansion on them. An alternative model, featuring growth repression via intracellularly controlled regulatory switches, would overcome these shortcomings and would offer a mechanism for stable and neighbour-independent

Figure 3



Auxin promotes and inhibits growth in a tissue-dependent manner. At a given concentration (see dashed line), auxin induces growth in aerial parts of the plant (shoot) while it represses growth in hypogeal tissue (roots).

growth repression in leading tissues. Auxin-dependent growth inhibition, as largely applies for example for root cells, may require fundamentally different and yet to be defined mechanisms for the negative control of cell size.

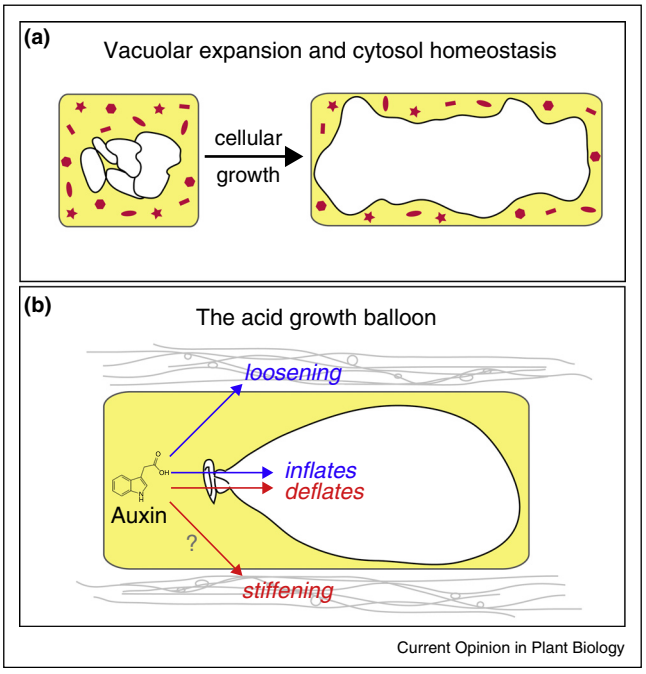
The acid growth balloon theory

Our recent findings suggest that auxin impacts on the appearance of the biggest plant cell organelle, the vacuole [11••]. We could show that the auxin effect on vacuolar morphology — leading to smaller luminal vacuolar structures — correlates, and is required for the auxin-dependent restriction of cell size in the root epidermis, ultimately contributing to root organ growth [11••]. This finding, therefore, proposes that the vacuole plays a substantial role in regulating auxin-dependent growth repression. On the contrary, it has been postulated that the vacuole may also drive cell elongation via turgor pressure [30], but this assumption remains to be addressed experimentally. The plasmodesmata (cell to cell bridges) are very likely to equalize the pressure within plant tissues. It has been shown that symplastic tissues, such as the root meristem, are capable of rapidly balancing small solute and water levels [31]. Accordingly, a distinct regulation of the turgor pressure required for a tissue-specific growth mechanism appears questionable. On the contrary, auxin responses may initiate callose-dependent closure of these cytosol bridges, at least in some instances [32]. Hence, auxin could exert distinct conditions in some tissues [33]. However, it is currently debatable whether the turgor pressure is a prerequisite or indeed a mechanism to control growth.

Notably, the genetic or pharmacological induction of bigger vacuoles (compared to wild type or mock treatment) did not increase cell size [11••]. This unidirectional limitation (smaller luminal structures restrict cell size, whereas bigger vacuoles do not increase cell size) might be due to the fact that the cell wall remains rigid in this experimental set-up, ultimately restricting cellular enlargements. We hypothesize that vacuolar morphology and cell wall composition/constitution are jointly controlled, in an auxin-dependent manner, to cooperatively allow or restrict cellular expansion.

It remains to be seen how the vacuolar shape ultimately restricts cellular growth. In speculating on this matter, we would like to volunteer the 'acid growth balloon theory'. It has been shown that vacuolar volume, but not the cytosol's dimensions, correlate with cell size in plant cell cultures [34]. We therefore hypothesize that the increase in vacuolar volume could actually be a mechanism for cytosol homeostasis, allowing a plant cell to grow without *de novo* production of cytosolic components [Figure 4a]. Accordingly, the vacuole may be viewed as a balloon that becomes inflated with water inside the cell, occupying cellular space and preventing the dilution of the cytosol. Such a mechanism would allow for rapid growth by using pre-existing resources. Further, any interference with this balloon function would slow down growth, as the cytosolic components would become a limiting factor.

Figure 4



Potential functions of the vacuole in cellular growth processes. **(a)** Vacuolar expansion and cytosol homeostasis. Cell volume occupancy by the vacuole is a putative mechanism for cytosol homeostasis during cellular growth. Such a system would allow for the rapid cell expansion without *de novo* synthesis of cytosolic constituents. **(b)** The acid growth balloon theory. Lead tissues possibly utilize both the auxin-dependent vacuolar balloon function and auxin-dependent alterations of cell wall rigidity in order to restrict (depicted in red) or promote (depicted in blue) cellular growth.

According to our theory, plant growth is an interplay between the intracellular space-filling 'vacuolar balloon' and the required extracellular cell wall acidification/loosening [Figure 4b]. Leading tissues could possibly employ the vacuolar balloon function to limit growth fully independent of their walls and, hence, their neighbouring cells. On the other hand, cellular expansion would require the coordinated 'inflation' of the vacuole and the loosening of the cell wall. Such a tuneable growth mechanism would allow plant cells to rapidly grow and to integrate possibly conflicting internal and external signals into their developmental growth program.

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