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A molecular pinball machine of the plasma membrane regulates plant growth – a new paradigm

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Abstract

Novel molecular pinball machines of the plasma membrane control cytosolic Ca^{2+} levels that regulate plant metabolism. [https://youtu.be/zABg7LiBk88] Essential components involve: 1. an auxin-activated proton pump; 2. arabinogalactan glycoproteins (AGPs); 3. Ca^{2+} channels; 4. auxin-efflux "PIN" proteins.

Typical pinball machines release pinballs that trigger various sound and visual effects. However, in plants "proton pinballs" eject Ca^{2+} bound by paired glucuronic acid residues of numerous glycomodules in periplasmic AGP-Ca^{2+}. Freed Ca^{2+} ions flow down the electrostatic gradient through open Ca^{2+} channels into the cytosol thus activating numerous Ca^{2+}-dependent activities.

Clearly cytosolic Ca^{2+} levels depend on ***activity*** of the proton pump, the ***state*** of Ca^{2+} channels and ***size*** of the periplasmic AGP-Ca^{2+} capacitor: Proton pump activation is a major regulatory focal point tightly controlled by the supply of auxin: auxin efflux carriers conveniently known as "PIN" proteins [null mutants are pin-shaped!] pump auxin from cell to cell. Mechanosensitive Ca^{2+} channels and their activation by reactive oxygen species (ROS) are yet another factor regulating cytosolic Ca^{2+}.

Cell expansion also triggers proton pump/pinball activity by mechanotransduction of wall stress via Hechtian adhesion thus forming a Hechtian oscillator that underlies cycles of wall plasticity and oscillatory growth.

Finally, Ca^{2+} homeostasis of plants depends on cell surface ***external storage as source*** of dynamic Ca^{2+}, unlike the ***internal* ER storage *source*** of animals where the added regulatory complexities ranging from vitamin D to parathormone contrast with the elegant simplicity of plant life. This paper summarises a sixty year Odyssey.

Keywords: arabinogalactan proteins; proton pump; auxin; calcium signaling; Hechtian oscillator; PIN proteins; morphogenesis.

Introduction - brief historical perspective

Sixty years ago [1] the discovery of hydroxyproline (Hyp) firmly bound to cell walls was a "founder event" of a new field in plant biology. Proteins specific to the cell wall had not previously been considered as components of an otherwise polysaccharide structure, apart from occasional hints in the literature. It is therefore instructive to recall that this discovery originated in D.H. Northcote's lab adjacent to that of Fred Sanger [2]; his Nobel Prize for protein chemistry in 1958 inspired cell wall protein analyses. Those showed most of the hydroxyproline was localised in primary cell walls isolated and purified from the first cell suspension cultures generated from cambial explants of sycamore. Thus a tree reverts to its algal ancestors, provoking "Robin" Hill's remark "Wouldn't it be wonderful to turn an alga into a tree!" Indeed, the Hill reaction defined photolysis of water as the ancient source of atmospheric oxygen [3, 4]. On the evolutionary timescale an increased atmospheric oxygen level eventually led to its use as a terminal electron acceptor but also a direct source of the hydroxyproline hydroxyl. Biosynthesis of hydroxyproline first shown in sycamore

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cell suspensions involved direct incorporation of $^{18}O_2$ into the hydroxyproline hydroxyl group [5], hence the first step in understanding the structural and dynamic roles of the Hyp-rich proteins unique to plants. Quite remarkably, mammalian systems have recruited prolyhydroxylase as an oxygen sensor of the hypoxia inducible factor (HIF) that plays a crucial role in foetal development [6].
Fred Sanger's approach [2] exemplified molecular structure as an initial step towards biological function. That was effective for soluble proteins but not for insoluble hydroxyproline rich proteins of the cell wall that were only released as fragments [7]. Intensive efforts at solubilisation led to the search for soluble precursors. Candidates included soluble "arabinogalactan polysaccharides" isolated from sycamore cell suspension culture growth media and extracellular polysaccharides from numerous gymnosperms and angiosperm seeds; all contained hydroxyproline but alanine-rich and lacking tyrosine [8]. The search for a soluble cytoplasmic precursor of wall-bound hydroxyproline (Table 1.) yielded an acidic "protein polysaccharide complex" (AGPs) distinguished by its composition and solubility in 10% TCA that precipitates most proteins [9].

	Sycamore	Tomato	Sphaerocarpos	Ginkgo	Tomato cell wall
Hyp	29	31	30	28	30
Pro	6	nd	nd	1	8
Asp	11	5	15	25	8
Thr	14	12	17	16	6
Ser	19	19	20	27	15
Glu	8	6	10	20	9
Gly	9	9	10	14	8
Ala	20	26	27	28	7
Val	6	7	15	9	8
Cys	6	4	4	5	0
Met	0	1	3	2	1
Ile	4	2	1	4	5
Leu	6	4	8	10	9
Tyr	1	0.5	1	2	3
Phe	3	0.5	2	3	3
Lys	7	4	3	6	11
His	1	0.5	1	2	2
Arg	2	1	1	2	4
Galactose	[++++]	740	[++++]	[++++]	150
Arabinose	[++++]	540	[++++]	[++++]	165

Table 1. Composition of the TCA-soluble cytoplasmic fraction.
First analyses of TCA-soluble cytoplasmic protein-polysaccharide complexes purified by preparative isoelectric focusing [9] subsequently identified as arabinogalactan proteins. Data from ref [9] show representative species across the plant kingdom included dicots, a gymnosperm and a bryophyte. These are the first AGP analyses notable for their high alanine and low tyrosine with a high galactose and arabinose content. Amino acid molar ratios normalised to 30 moles hydroxyproline compared with tomato cell wall.

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Such proteins later named arabinogalactan proteins (AGP) located mainly between the plasma membrane and cell wall were periplasmic analogous to Peter Mitchell's bacterial periplasm [10]. However, they were not precursors to wall bound protein based on composition and absence of turnover in ^{14}C-proline pulse-chase experiments [11].

Identification of the glycopeptide link between protein and polysaccharide depended again on a classical Sanger strategy exploiting the differing stability of various covalent bonds to chemical and enzymic attack. For example, both peptide and glycosidic linkages are acid labile. However peptide bonds are stable in cold anhydrous HF that cleaves glycosidic linkages. Thus HF-deglycosylation of highly glycosylated proteins [12] enabled peptide sequencing of difficult proteins like extensins, AGPs and mucins [13], with subsequent genomic sequencing [14]. Glycosidic linkages are generally stable in mild alkali alkali. That enabled discovery of the Hyp-O-glycosidic link [15] as Hyp-arabinosides in extensins and Hyp arabinogalactans (Hyp-AGs) in AGPs. Structural elucidation of Hyp-AGs [16] led to their essential role in Ca^{2+} homeostasis validated by recent direct evidence [17] and [18,19] as described in subsequent sections that show how the structure of Hyp-arabinogalactan glycomodules leads to Ca^{2+} homeostasis and plasma membrane Ca^{2+} ion influx that regulates plant growth.

The origin of ion gradients.

Wind and waves generate sea spray aerosols [20] that can act as prebiotic chemical reactors [21]. Prebiotic chemistry generated simple amphiphiles (typically with a hydrophilic headgroup and a hydrophilic tail) that provided a surface hydrophobic membrane that enabled evaporation, concentration of reactants and ion gradients that included proton gradients. Components of primordial energy transduction systems driven by light may have appeared first in protocells. For example in purple bacteria like Halobacterium bacteriorhodopsin absorbs photons to pump protons across the plasma membrane [22]. ***Protons initiate both cosmic evolution and biotic evolution, thus life based on proton gradients is universal!***

Plasma membrane dynamics!

Over the last billion years or so a simple lipid membrane evolved from a passive gatekeeper maintaining the "constancy of the interior milieu," to a dynamic control system with a plethora of elaborate import/export microtubule transport system kinesin/dynein motors that ferry clathrin coated vesicle cargo to the cell wall. Ultimately three critical ions H^+ Ca^{2+} and auxin (indole acetic acid) regulate plant growth. Of these, protons are preeminent players in energy transduction/ATP generation but also maintain the plasma membrane potential of -120 to -160 mV [23]. This creates the electrostatic gradient essential for influx of cations particularly K^+ osmolyte and the universal signalling ion Ca^{2+} that affects "every aspect of a cell's life and death. Ca^{2+} the most tightly regulated ion within all membrane-bound organisms, binds to thousands of proteins to effect changes in localization, association, and function. Hundreds of cellular proteins have been ***adapted to bind Ca^{2+} over a million-fold range of affinities*** (nM to mM), in some cases simply to buffer or lower Ca^{2+} levels, and in others to trigger cellular processes. The local nature of Ca^{2+} signalling is intimately tied to this large range of affinities" [24].

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Until quite recently the mechanism of auxin-induced Ca^{2+} signalling in plants was unclear [25]. Now however, the subtlety of Ca^{2+} homeostasis and its dynamic storage is apparent: Paired glucuronic acid sidechains of AGP Hyp-AG glycomodules bind Ca^{2+} specifically on the outer surface of the plasma membrane and thus connect membrane components involved in Ca^{2+} signalling with auxin-regulated cell expansion: auxin-activated ATPase proton pumps dissociate AGP-Ca^{2+} supplying the Ca^{2+} channels that regulate cytosolic Ca^{2+}. The pinball hypothesis conveniently summarises this scenario of proton "pinballs" ejecting bound Ca^{2+} ions from AGP-Ca^{2+}as discussed in the following sections.

Proton pumps.

The plasma membrane regulates all aspects of plant growth by an array of mechanisms that include specific receptors, transporters, channels and ion pumps, chiefly proton pumps [26, 27]. The Arabidopsis genome encodes eleven proton pumps [23]. That significant redundancy underlies their essential role in maintaining the membrane potential and generating the low cell wall pH required by the "acid growth hypothesis." However, we re-interpret the "acid growth hypothesis" involving novel roles of the F_1F_o ATPase proton pump in Ca^{2+} homeostasis and auxin transport. This new paradigm unique to plants identifies the proton pump as the focal point of many activators and regulators (Figure 1.).

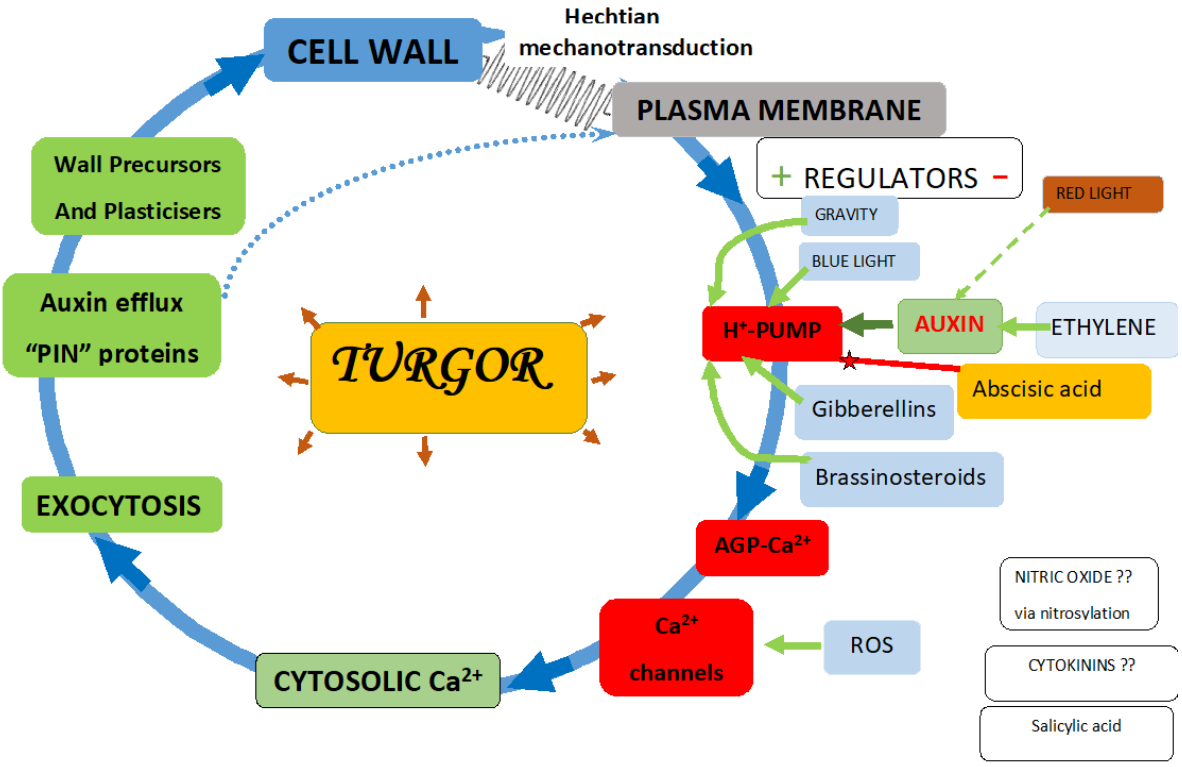

Figure 1.

The Hechtian growth oscillator incorporates a molecular pinball machine.

The ATPase proton pump generates proton "pinballs" that release Ca^2 ions from AGP glycomodules which supply cytosolic Ca^{2+}. Turgor drives cell expansion and activates the pump by mechanotransduction of wall stress via Hechtian adhesion between the plasma membrane and the cell wall; this creates a Hechtian oscillator that regulates

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cycles of cell wall plasticity and oscillatory growth. Auxin is the other major proton pump activator transported by auxin efflux "PIN" proteins. ATPase proton pump regulation is central to plant growth and development [28].The figure depicts numerous additional inputs.
Green arrows indicate upregulation or red represent downregulation, with exceptions where the mechanism remains unknown.
High auxin levels generally enhance cell extension [29]; auxin driven morphogenetic patterns depend on unidirectional fluxes [30]; the evolution of auxin signalling and PIN proteins [31]; auxin biosynthesis occurs in meristems [32]; auxin activates the plasma membrane H+-ATPase via phosphorylation [28]. Negative regulation by abscisic acid decreases steady-state levels of phosphorylated H^+-ATPase possibly by promoting dephosphorylation via a protein phosphatase [33] and suppresses hypocotyl elongation in Arabidopsis [33]; abscisic acid stress signalling evolved in algal progenitors [34]. Blue light: the blue light photoreceptor pigment phototropin increases cytosolic Ca^{2+} [35]. Brassinosteroids: Increase cytosolic Ca^{2+} via increased auxin levels [36]. Cytokinins enhance cell division by unknown mechanisms.
Ethylene upregulates auxin biosynthesis in the Arabidopsis root apex and inhibits root cell expansion [37]; thus, anthranilate synthase mutants yield ethylene-insensitive root growth phenotypes. Ethylene specifically inhibits the most rapid growth phase of expanding cells—normally the root hair initiation zone but ethylene moves it much closer to the tip. Auxin and ethylene act synergistically to control root elongation, root hair formation, lateral root formation and hypocotyl elongation. Ethylene modulates root elongation through altering auxin transport [38].
Figure revised reprinted from [39] that contains more details.

ATPase H^+ pumps have two components, F_1 the ATPase catalytic site and F_0 a proton pore; they operate in two modes, "forward" as in mitochondria where a proton gradient drives the synthesis of ATP, and in "reverse" at the plasma membrane where ATP hydrolysis drives proton extrusion. In plant cells proton extrusion plays ***three major roles:*** **Firstly,** creation of a large membrane potential [~-120 to -160 mV] enhances cation uptake and anion extrusion through their respective channels. **Secondly,** the proton pump generates protons that eject Ca^{2+} from the plasma membrane AGP-Ca^{2+} glycomodules as the immediate source of cytosolic Ca^{2+}. **Thirdly,** auxin transport involves efflux of anionic auxin into the wall at low pH where it is protonated and thus becomes neutral. That allows it to diffuse through the plasma membrane into the cytosol of an adjacent cell at neutral pH where it re-ionises for efflux and polar transport of auxin against the concentration gradient [40, 41].
Here we connect an ***auxin-activated proton pump with AGPs and Ca^{2+} signalling.*** This reinterprets the "acid growth" hypothesis [42]. The ***hitherto unsuspected role of proton pumps in Ca^{2+} homeostasis is a new paradigm*** relevant throughout the Plant Kingdom from tropisms to phyllotaxis.

Indirect evidence for the role of AGPs in Ca^{2+} homeostasis
Michael Jermyn (1975) demonstrated the ubiquity of AGPs (first known as beta lectins) in an impressive range of seed plants [8] suggesting a possible role in signalling *per se*. Over several decades a signalling role of AGPs was based intuitively on the complexity of their polysaccharide components and most significantly their cell surface location as shown by the remarkable series of anti-AGP

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"JIM" monoclonal antibodies, developed at the John Innes Institute [43], that recognise specific AGP carbohydrate epitopes. Thus recent reviews [44, 45] cover numerous papers that demonstrate specific AGP distribution particularly in metabolically active tissues involved in virtually all aspects of plant growth from Bryophytes onwards [46]. AGPs mark critical cell fate transitions [47] in both sporophyte and gametophyte generations. Thus, judging from extensive cytochemical studies, seed germination, vegetative growth, flowering, fertilisation [48], embryogenesis [49, 50, 51], phyllotaxis [52] and fruit ripening [53] all involve AGPs. However structural elucidation of Hyp glycosubstituents was lacking. That limited progress until detailed high-field NMR analyses of purified Hyp-oligosaccharides [54] showed an underlying simple repetitive consensus motif of 15 sugar residues consistent with earlier analyses of AGP carbohydrate. Most significantly the consensus 15-sugar glycomodule has a short β-1,3-linked galactan backbone with two short mobile sidechains terminated by glucuronic acid that together bind Ca^{2+} stoichiometrically [55]. This pH-dependent Ca^{2+}-binding enables peripheral cell surface AGPs to play a role in Ca^{2+}signalling that differs fundamentally from the endogenous Ca^{2+}storage of animal cells. Numerous previous observations of AGP localisation and chemical properties throughout the literature are consistent with the new paradigm that unifies AGPs and Ca^{2+} homeostasis with the regulation of plant growth.

An early indication of a connection between AGPs and Ca^{2+} appeared in 1991; the wound response of *Acacia Senegal* and its secretory product gum Arabic consists of polysaccharides and glycoproteins related to AGPs [56]. Significantly gum Arabic contains glucuronic acid (~10%) and binds approximately ~1% by weight Ca^{2+}. In the same year [57] analysis of isolated plasma membranes revealed a bound AGP content of (~10% w/w). These AGPs were hydroxyproline-rich with a significant glucuronic acid content (~10%). However, the crucial connection between Hyp-glycosubstituents and the pH-dependent Ca^{2+} binding property of AGPs with Ca^{2+} homeostasis only appeared quite recently when a molecular model depicted paired glucuronic acid residues of a Hyp-glycomodule that bound Ca^{2+} in a molecular dynamics simulation confirmed by in vitro assay [55]!

Direct evidence for AGP regulation of Ca^{2+} homeostasis – a new paradigm

The molecular pinball machine is a visual metaphor where "PIN" proteins trigger the machine by supplying auxin that activates the proton pump with release of proton "pinballs" that initiate Ca^{2+} influx. This falsifiable hypothesis makes several testable predictions: 1.) AGP glucuronic acid is essential for growth. 2.) AGP Glucuronic acid enables AGP-Ca^{2+} binding. 3.) AGP-Ca^{2+} binding is the source of cytosolic Ca^{2+}. 4.) Specific Ca^{2+} channels facilitate its influx. 5.) Auxin triggers rapid increase of cytosolic Ca^{2+}. 6.) Ca^{2+} waves are essential for root growth. 7.) AGP adaptation to salt stress also involves their upregulation.

Numerous other physiological predictions include tropisms and phyllotaxis [25]!

In vitro pH-dependent Ca^{2+}-binding by AGPs [55] implied in vivo significance by predicting that Ca^{2+}binding by paired glucuronic acid residues of Hyp-AG glycomodules is the major source of dynamic cytosolic Ca^{2+}. When theory predicts experiment decides! Therefore, plants should exhibit defective Ca^{2+} homeostasis if they lack AGP-glucuronic acid residues (i.e. if AGPs were not glucuronidated). However, generating AGPs that lack glucuronic acid was a considerable experimental challenge fraught by the redundancy of multiple glucuronosyl transferases (GTGlcA) in the Arabidopsis GT14 family. Both the Dupree [17] and Showalter [18] groups

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resolved that problem brilliantly by generating triple GTGlcA knockouts. The Dupree group generated *glcat14a/b/e triple mutant plants...* while the complementary approach by the Showalter group generated a *glcat14a glcat14b glcat14c triple glcat* CRISPR-Cas9 mutant line. Those benchmark papers describe how mutants generated AGPs that lacked glucuronic acid; their compelling evidence for the essential role of AGPs in Ca^{2+} homeostasis is as follows:

1. **AGP glucuronic acid is essential for growth.**
   Plants have a huge investment in AGPs represented by about 85 genes in the Arabidopsis genome. Two groups [17] and [18] reported that triple glucuronosyl transferase knockouts decreased AGP glucuronidation; the corresponding decrease in AGP Ca^{2+}-binding was associated with profound developmental defects: trichome branching; supressed growth phenotypes; decreased inflorescent stem length and a drastic decrease in progeny. Physiological defects included: sterility; abnormal Ca^{2+} transients; attenuation of Ca^{2+} wave propagation and decreased response to ROS activation of Ca^{2+} channels in roots.

2. **AGP Glucuronic acid enables AGP-Ca^{2+} binding**
   AGPs isolated from triple glucuronosyl transferase knockouts contained significantly less glucuronidation with a corresponding decrease in Ca^{2+} binding by AGPs in vitro.

3. **AGP-Ca^{2+} binding is the source of cytosolic Ca^{2+}.**
   Earlier work [55] showed enough periplasmic AGP for a significant increase in cytosolic $Ca^{2+}$and further corroborated by [17] and [18].

4. **Auxin increases cytosolic Ca^{2+}**
   A report that cellular Ca^{2+} signals generate defined pH signatures in plants [58] presents a conundrum of how to discriminate between stimulus and response. In this instance it was resolved by the observation [59] that auxin elicits a Ca^{2+} signal (aequorin blue fluorescence) ***"immediately"*** after addition of an auxin activating the proton pump, i.e. within seconds possibly involving enhanced diffusion of protons along the surface of the membrane hence their delayed escape from the membrane surface into the adjacent bulk phase [60].

5. **Ca^{2+} ATPase recycles cytosolic Ca^{2+}**
   While the pinball machine and Hechtian oscillator emphasise influx, Ca^{2+} homeostasis demands a finely balanced efflux evidenced by the fifteen Ca^{2+}-ATPase pumps located in both ER and plasma membrane of Arabidopsis [61].

6. **Ca^{2+} waves are essential for root growth.**
   The Dupree group also examined the dynamic aspects of Ca^{2+} homeostasis in roots. Using the fluorescent reporter R-GECO1 they observed organised cytosolic Ca^{2+} wave propagation at the inner and outer zones of wild-type roots. However, wave propagation was notably ***disorganized*** in *glcat14a/b/e* mutant roots. They extended these observations to the H_2O_2 activation of Ca^{2+} channels during growth that had been reported in a seminal paper [62]. The Dupree group confirmed that H_2O_2rapidly increased cytosolic Ca^{2+} particularly at the growing tip of wild-type roots. However, this increase was

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much less in *glcat14a/b/e* mutant roots (Figure 11 in ref [62]) which is consistent with the pinball hypothesis: However, the conclusion that H_2O_2-activated Ca^{2+} channels are the major regulator of cell expansion [62] needs to be re-evaluated: Decreased H_2O_2-induced Ca^{2+}influx of mutant roots shows that Ca^{2+} channels are ***necessary but not sufficient*** for complete cytosolic Ca^{2+} influx. Thus, we conclude that ***Ca^{2+} channels are essential components*** of the molecular pinball machine that needs ***both*** AGP-Ca^{2+} and activated Ca^{2+} channels for maximal activity. This also applies to other aspects of plant growth including tip growth of root hairs and pollen tubes.

7. **AGPs respond to salt stress**

Increasing sodium ion concentration competes with bound AGP-Ca^{2+} and so may account for salt-sensitive crop plants such as rice. On the other hand salt-resistant plants typified by salt-marsh flora and marine seagrass raises the question of mechanism. AGPs are involved in two well-documented instances:

Firstly, AGP upregulation is a direct response to increased salt levels in plant cell suspension cultures [63].

Secondly, some higher plants, exemplified by *Zostera marina,* have returned to a marine habitat [64]. Zostera architects of the seagrass meadow "blue carbon" ecosystem provide an exquisite test of the pinball hypothesis. How do plants growing in such high salt cope with the serious competition of Na^+ with Ca^{2+} ions? Some plants such as Zostera have adapted by increasing their glucuronic acid content [65] hence increasing their ability to bind Ca^{2+}. With recent advances in genetic engineering in mind, the possibility of re-engineering AGPs to enhance salt-sensitive crop plants is apparent.

The quest for key regulators of plant growth

Oscillatory growth implies that a biochemical oscillator regulates the process with protons and Ca^{2+} ions as key players [66]. It also includes AGPs, auxin and auxin efflux "PIN" proteins. However the idea of a Ca^{2+} signature that encodes specific developmental cues has become quite attractive. Some [67] suggest that specificity *"involves one messenger with many translators*" and is encoded in Ca^{2+}-signalling systems by Ca^{2+}-binding proteins with different Ca^{2+} affinities. This may enable cells to decode an initial Ca^{2+} signal to yield graded or nuanced responses. They also noted [67] the apparent evolutionary loss of Ca^{2+}-influx systems in plants but suggested that "Ca^{2+}-influx mechanisms and components were still to be discovered." The molecular pinball machine fills the gap as an essential component of Ca^{2+} signalling and homeostasis; in quiescent cells auxin and Ca^{2+} trigger pinball activity. Thus "PIN" proteins supply auxin that activates the proton pump to promote expansion growth. However, the Ca^{2+}signal depends not only on AGP-Ca^{2+} but also on Ca^{2+} channels activated by reactive oxygen species (ROS) 62 and stress transduction from the cell wall to mechanosensitive ion channels [68, 69, 70] of the plasma membrane. Transmission of the stress vector most likely involves an arabinogalactan protein APAP1 [71] covalently attached to wall pectin and anchored to the plasma membrane by a C-terminal GPI lipid. That provides a physical basis for Hechtian adhesion and mechanotransduction. Hence turgor pressure powers a Hechtian oscillator and components of the pinball machine that regulate cell expansion. This raises the great question of cell wall plasticity that remains unanswered over sixty years since James

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Bonner and his colleagues [72] began the quest for "Haftpunkte" load-bearing bonds that allow cell wall loosening when broken. Although expansins [73] soften the wall the precise biochemical mechanisms remain unknown even after thirty years trying to "Round up the usual suspects" like hydrogen bonds [74]! Underlying assumptions must always be questioned. Specific load-bearing covalent bonds in the wall may not exist in a wall stretched by viscoplastic flow or "creep". If we view the primary cell wall as analogous to a plastic then a plasticizer like expansin may control wall rheology [75]. However the primary cell wall with its multiple interactions is a complex multicomponent structure rather like a molecular chess board which accounts for the slow progress in solving the problem. Pectin is the major component of the wall with its direct involvement likely although its subtle chemistry suggests unknown mechanisms. Our current hypothesis invokes the sycamore cell suspension culture [76] which is of historical interest [77] not only as the first primary cell wall to be isolated [1] but also as the initial source of AGPs as polysacharides [78] and hydroxyproline-rich glycoproteins [9]. Sycamore cell cultures characterised as finely divided pipettable suspensions [77] were always accompanied by soluble macromolecules including AGPs released into the culture medium during growth [78, 9]. AGPs released by phospholipase cleavage of their GPI anchor by may act as wall plasticizers by disrupting the alignment of linear pectin macromolecules. Another possibility highlights the versatility of AGPs that depends on their high affinity for Ca^{2+} [55]. Thus, pectin methyl esterase deesterifies pectin resulting in free carboxyls crosslinked by Ca^{2+} and a more rigid wall. However, AGPs in muro with a higher affinity for Ca^{2+} than pectic carboxyls effectively scavenge pectate Ca^{2+}. This results in negatively charged carboxyl ions so that pectin macromolecules now repel each other [75] and thus loosen the wall. The small 22 kDa diffusible AGP peptides [79] may also be effective scavengers of Ca^{2+}. This may account for the effect of low pH increasing wall plasticity by dissociating calcium pectate Ca^{2+} ions that are then captured by AGPs. That resolves the acid growth paradox!

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.
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Dedication: We dedicate this paper to all those research workers who have contributed to the AGP field of endeavour during the past fifty years.

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