Viewpoint.

Pollen tube growth and guidance:
Occam’s razor sharpened on a molecular AGP Rosetta Stone

Derek T. A. Lamport1 Li Tan2 Michael Held3 and Marcia J. Kieliszewski3

1. School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG
2. Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30602-4712
3. Department of Chemistry and Biochemistry, Ohio University, Athens, Ohio 45701

* For correspondence. E-mail derekt.t.a.lamport@googlemail.com

Running title: Molecular Roles of AGPs in Pollen Tube Growth and Guidance

Keywords: arabinogalactan proteins, pollen tube tip growth, cell extension, pectin, calcium signalling, Hechtian oscillator.

ABSTRACT 191 words
Occam’s Razor suggests a new model of pollen tube tip growth based on a novel Hechtian oscillator that integrates: (1) a periplasmic AGP-Ca²⁺ calcium capacitor with tip-localised arabinogalactan glycoproteins (AGPs); (2) tip-focussed cytosolic Ca²⁺ oscillations; (3) Hechtian strands evidence of adhesion between the plasma membrane and the cell wall of the growing tip. Thus Hechtian adhesion, as a piconewton force transducer, couples the internal stress of a rapidly growing wall to the plasma membrane. Such Hechtian transduction via stretch-activated Ca²⁺ channels and H⁺-ATPase proton efflux dissociating periplasmic AGP-Ca²⁺, creates a Ca²⁺ influx that activates exocytosis of wall precursors. In effect a highly simplified primary cell wall regulates its own synthesis and a Hechtian growth oscillator regulates overall tip growth. By analogy with the Rosetta Stone that translates trilingual inscriptions as a single identical proclamation, the Hechtian Hypothesis translates classical AGPs and their roles as a Ca²⁺ capacitor, pollen tube guide and wall plasticiser into a simple but widely applicable model of tip growth. Even wider ramifications of the Hechtian oscillator may implicate AGPs in osmosensing or gravising and other tropisms, leading us yet further towards the Holy Grail of plant growth.
Introduction

In 1682 Nehemiah Grew in “The Anatomy of Plants” (1682) described stamens as male organs and their pollen as necessary for fruit production. Somewhat later Amici (1824) observed pollen germinating on the stigma and suggested that the pollen tube carried sperm cells to the ovule. Over fifty years ago (Mascarenhas & Machlis, 1962) a chemotropic dependence on Ca\(^{2+}\) for pollen tube growth and guidance became evident. Such growth is of particular interest as the pollen tube tip has the simplest primary cell wall consisting largely of highly methyl esterified pectic polymers and shows the fastest known tip growth rate that is generally not continuous but pulsatile (Pierson et al., 1995) or oscillatory with associated ion fluxes, notably H\(^{+}\) and Ca\(^{2+}\) (Feijo et al., 1995) of similar periodicity. However these ion fluxes are not in phase with tip growth rates (Michard et al., 2009) so causal relationships are not obvious (Messerli & Robinson, 2003; Holdaway-Clarke et al., 1997). With Occam’s Razor as a guide we view tip growth as a biological oscillator that depends on two novel components, namely classical AGPs and Hechtian adhesion sites. Based on the pH-dependent reversible binding of Ca\(^{2+}\) by AGPs, the Hechtian oscillator accounts for pollen tube H\(^{+}\) and Ca\(^{2+}\) ion currents as follows: H\(^{+}\) dissociates periplasmic AGP-Ca\(^{2+}\) thus increasing free cytosolic Ca\(^{2+}\) that coordinates exocytosis of cell wall precursors. However, cytosolic Ca\(^{2+}\) also depends on opening stretch-sensitive Ca\(^{2+}\) channels by tension from the growing wall transmitted to the plasma membrane by Hechtian adhesion sites. Because the new model includes two components, notably classical AGPs and Hechtian adhesion sites (Fig. 1) not previously considered as essential to models of extension growth, we suggest that overall tip growth consisting of the above components is a Hechtian oscillator. This model (Fig. 2) may also illuminate the vexed problem of cell extension dating back to Heyn’s identification of cell wall plasticity and via its hormonal regulation, as the primary factor in controlling growth by cell extension.

AGPs, generally regarded as mysterious molecules (Pickard, 2013; Pereira et al., 2015) and ‘minor’ cell wall components are associated with many aspects of cell signalling (Seifert & Roberts, 2007) and cell expansion (Ding & Zhu, 1997). However, classical AGPs per se have not been viewed as essential regulatory components of pollen tube growth. Here we propose that classical AGPs based on...
their molecular properties and periplasmic location (Lamport et al. 2006), make a
threefold contribution: Firstly as a primary source of cytosolic Ca\(^{2+}\) waves, secondly,
as a pectic plasticizer and thirdly, as Ca\(^{2+}\) signposts to the ovule. These three
inscriptions on an allegorical AGP molecular Rosetta Stone translate into general
plant growth that depends on the remarkable chemical properties of AGPs
summarised in Table 1 of (Lamport et al. 2014) and briefly as follows:

(1) AGP amino acid composition, rich in hydroxyproline (Lamport, 1970); (2) AGP
O-Hyp glycosylation by small acidic arabinogalactan polysaccharides (Lamport,
1977) whose heterogeneity is generally exaggerated; (3) specific binding of Ca\(^{2+}\) by
these glycomodules (Lamport & Varnai, 2013) amounting to a typical AGP Ca\(^{2+}\)
content of ~1% w/w; (4) AGP location initially attached to the plasma membrane by
a GPI anchor (Oxley & Bacic, 1999), but cleaved to allow incorporation into the
growing wall; and finally (5) AGP molecular size of ~100kDa hence extrusion rather
than simple diffusion of AGPs through the wall matrix after GPI-anchor cleavage
(Lamport et al., 2006).

Several recent mathematical models predict oscillations in tip growth based on
Ca\(^{2+}\)-dependent vesicle recycling and tip plasticity (Zerzour et al., 2009; Hill et al.,
2012). However, none include AGPs and Hechtian feedback as essential components.

Our new model supplements these biophysical approaches with the evidence for a
central role of AGPs in a biochemical model of tip growth as follows.

**A Hechtian model of pollen tube tip growth**

Briefly, the new model (Fig. 2) proposes that a viscoplastic pectic cell wall
mechanically coupled to the plasma membrane by Hechtian adhesion (Hecht, 1912)
transmits wall strain to the PM and thus regulates H\(^{+}\), Ca\(^{2+}\) and other ion fluxes that
regulate the exocytosis of wall precursors. Together they constitute a Hechtian
oscillator (Fig. 2) consistent with Pickard’s pioneering work on mechanotransduction
(Pont-Lezica et al., 1993). This explains how the rapidly growing cell wall of the
pollen tube tip can act as a decision maker that regulates its own growth by Hechtian
feedback. Such dramatic oscillatory growth (0.1-0.5 \(\mu\)m s\(^{-1}\)) (Hepler et al., 2013)
associated with active ion fluxes predominantly Ca\(^{2+}\) (Miller et al., 1992) H\(^{+}\), and K\(^{+}\)
reflect Peter Mitchell’s chemiosmotic paradox (Mitchell, 1961): “*Not only can*
metabolism be the cause of transport, but also transport can be the cause of metabolism”. While there has been much effort to relate these ion fluxes to extension growth the Hechtian oscillator resolves the chemiosmotic paradox of the pollen tube by assigning specific roles to the Ca$^{2+}$ and H$^+$ ion currents essential for oscillations in tip growth (Hepler et al., 2006) driven by turgor pressure: Thus an initial acceleration of tip extension followed by exocytosis of wall precursors leads to deceleration, summarised in a simplified model (Fig. 2) partly based on Figure 3 of (Holdaway-Clarke & Hepler, 2003).

Proton flux

Ion fluxes drive all growth (Armstrong, 2015): Protons lead the way as the source of the chemiosmotic proton motive force (Mitchell, 1961) that generates ATP via mitochondrial F$_{1}$F$_{0}$ ATP synthase (Allegretti et al., 2015). However, in reverse the synthase pumps protons (Mazhab-Jafari et al., 2016). Thus plasma membrane H$^+$ ATPase proton efflux is essential for maintaining the membrane potential and ion transport (Hepler et al., 2013). However, in growing pollen tubes unevenly distributed membrane H$^+$-ATPases (Certal et al., 2008) result in a pronounced proton efflux at the tube shank with an apparent much smaller oscillatory influx at the tip (Feijo et al., 1999). A large proton efflux may explain the striking difference between the optimum extracellular pH of animal and plant cells (pH 7.4 and pH 5.5); this reflects the differing compositions of their extracellular matrix and their dynamic Ca$^{2+}$ storage that is largely peripheral in plant cells but mainly intracellular in animals. The lower external pH of plants reflects the low pK$_{a}$ of uronic acids enabling pH-dependent uptake and release of Ca$^{2+}$ from AGPs as a result of H$^+$ ATPase activity. That also accounts for the massive proton efflux at the tube shank and ensures Ca$^{2+}$ release from the abundant AGPs of stigmatic tissues hence a possible cooperative effect on the growth of other pollen tubes. (cf. (Lord, 2003)). The role of the less marked tip H$^+$ influx (Feijo et al., 1999) is less clear as direct measurement cannot detect a much smaller H$^+$ efflux into the nanometre dimensional domain of periplasmic AGP-Ca$^{2+}$.

AGP-Ca$^{2+}$ as a primary source of cytosolic Ca$^{2+}$

According to the prevailing view that ignores the largely methyl esterified status of tip pectin “Wall binding of Ca$^{2+}$ accounts for the extracellular influx”
(Hepler et al., 2013) via open Ca^{2+} channels. Here we correlate the tip-focused
cytosolic Ca^{2+} oscillations with the presence of tip-localised AGPs (Figure 3A in
(Mollet et al., 2002)) based on their pH-dependent dissociation (Lamport & Varnai,
2013) by plasma membrane H^{+}-ATPase (Koji et al., 2012). The nanometre
dimensions of periplasmic AGP-Ca^{2+} and its proximity to the proton source results in
a significantly lower pH than the pH in muro due to rapid dissipation of the proton
concentration by diffusion, dilution and buffering.

The advantage of AGP-Ca^{2+} as a source of cytosolic Ca^{2+} at the tip arises not
only from its cell surface location a prime area of signal perception, but also from the
**paired glucuronic acid sidechains** of AGP glycomodules (Fig. 3); these increase the
total Ca^{2+} binding capacity of AGPs compared with the lower binding capacity of
highly methyl esterified pectin that has largely unpaired galacturonic acid residues.
Because the AGP glucuronic acid pK_{a} is lower than that of pectin galacturonic acid
(Lamport & Varnai, 2013) AGPs bind Ca^{2+} more strongly at low pH. Thus
periplasmic AGPs can also act as a sink for less firmly bound hence more easily
released Ca^{2+} from pectin in the tip wall. Finally, the location of abundant periplasmic
AGPs **confers a large kinetic advantage to an AGP-Ca^{2+} capacitor** (Lamport &
Varnai, 2013) that can readily supply the stretch-activated Ca^{2+} channels regulated by
membrane tension (Dutta & Robinson, 2004) as follows:

**Hechtian transduction**

The significance of Hechtian adhesion evidenced by thread-like elastic extensions of
the plasma membrane physically connecting the membrane to the cell wall of
plasmolysed cells has remained obscure for more than a hundred years. (Fig. 1.)
(Hecht, 1912). Hechtian adhesion is particularly evident in rapidly growing cell
suspension cultures (Lamport, 1963) and during tip growth of root hairs (Volgger et
al., 2010) and pollen tubes (Fig. 4.) (Parton et al., 2001). However absence of
Hechtian adhesion from the pollen tube shank (Lord, 2003) confirms a significant role
during tip growth further emphasised by its presence during tip growth even in
chlorophycean algae like Closterium (Domozych et al., 2003). Such evolutionary
conservation also supports a fundamental biological role of Hechtian adhesion in
regulating plant growth inferred here: Stable Hechtian adhesion arises from strong
molecular anchoring forces, most likely of AGPs and formins. Arguably AGP GPI
lipids with an adhesion force of ~350 piconewtons (Cross et al. 2005), supplemented by formin transmembrane domains, enable the growing wall to transmit its stress/strain status at very low piconewton levels (cf. Buer et al. 2000) to the protoplast via multiple Ca$^{2+}$ channels and H+-ATPases of the plasma membrane. High sensitivity Hechtian stress transducers are thus consistent with much evidence of stretch-activated Ca$^{2+}$ channels (Dutta & Robinson, 2004) and a Hechtian “stress focussing” structure involving AGPs suggested earlier (Gens et al., 2000). Hypothetical wall-plasma membrane wall linkers proposed earlier (Pont-Lezica et al., 1993) involve specific candidates we identify here as AGP57C (At3g45230) and formin1 AtFH1; their well-defined molecular domains interact specifically with both plasma membrane and cell wall: AGP57C, an arabinoxylan-pectin-AGP glycoconjugate (APAP1) (Tan et al., 2013) has a C-terminal sequence directing GPI-anchor addition hence attachment to the plasma membrane, while the terminal rhamnose of an AGP glycomodule is attached to the reducing end of RGI a wall pectic polysaccharide. Formin1 has an N-terminal signal peptide followed by a transmembrane domain and a large extracellular domain anchored to the wall (Martiniere et al., 2011) most likely involving AGP glycomodules encoded by the Hyp glycosylation motif SPSALSPS. AGP57C and formin1 fulfil the criteria for bona fide crosslinks between a wall polysaccharide and the plasma membrane; at last providing tangible molecular evidence for Hechtian adhesion and its pivotal role in Hechtian signal transduction.

**Exocytosis of wall precursors**

Exocytosis, the final stage of the secretory pathway from Golgi to cell surface, involves actin guidance and transport of exocytotic Golgi vesicles, docking and fusing with the plasma membrane. Although treated here as a single “component” of an oscillator, exocytosis involves many proteins and elevated levels of cytosolic Ca$^{2+}$ at sites of pronounced exocytosis in growing pollen tubes (Camacho & Malho, 2003). Alteration of the Ca$^{2+}$ gradient alters the pattern of exocytosis (Ge et al., 2007) suggesting the role of cytosolic Ca$^{2+}$ as a coordinator of exocytosis consistent with the numerous Ca$^{2+}$-dependent membrane processes (Luckey, 2008) and greatly decreased exocytosis when the Ca$^{2+}$ chelator chlortetracycline decreased cytosolic Ca$^{2+}$ (Reiss
While Ca\textsuperscript{2+}-regulated exocytosis is a prime candidate for the regulation of oscillatory pollen tube growth, paradoxically some have concluded from the apparent lack of correlation between Ca\textsuperscript{2+} and secretion that although exocytosis may regulate oscillatory pollen tube growth, intracellular Ca\textsuperscript{2+} does not regulate oscillatory exocytosis (McKenna \textit{et al.}, 2009). Exocytosis, as a component of a Hechtian oscillator, connects H\textsuperscript{+} and Ca\textsuperscript{2+} ion gradients with cell wall tip growth (metabolism) and is thus a classic example of the Mitchell Paradox. Other models of tip growth based on ROP GTPases for example (Yan \textit{et al.}, 2009) did not include the cell wall or AGPs. However, due to technical difficulties the biochemical properties of the tip cell wall have received relatively little attention even though it is a major component of growth and its raison d'être.

**Cell wall plasticity**

At the tip of the Lily pollen tube maximum thickness of the wall coincides with a significantly decreased rate of tip growth (Figure 4c in (McKenna \textit{et al.}, 2009)) which \textit{precedes} a more rapid expansion (McKenna \textit{et al.}, 2009). As the wall thins its plastic extensibility increases with concomitant acceleration of the tip growth rate; further exocytosis restores wall thickness and thus decreases a growth rate that is inversely proportional to wall thickness (Kroeger \textit{et al.}, 2008). Evidently, wall plasticity plays a crucial role (Heyn, 1940) in determining rheology of the pollen tube tip primary cell wall. An explanation for the plasticity of primary walls in general is complicated by their wide range in composition with differing proportions of major components that include, cellulose, pectin, xyloglucan and structural protein (Fig. 5). However, the pollen tube tip is an ideal model of extension growth because it represents a simplified primary cell wall (originally defined by (Kerr & Bailey, 1934)) stripped down to a “single” major macromolecular pectin component. “Pectin is not just jelly” (Roberts, 1990) but forms a highly ordered composite of three major pectic polysaccharide domains, highly methylesterified polygalacturonide, RG-I, a highly methylesterified linear polygalacturonate and RG-II a complex rhamnogalacturonan with intermolecular borate crosslinked sidechains, unstable at low pH (Yapo, 2011). AGP α-L-arabinosyl sidechains may interact with pectin rhamnogalacturonan RG-II
by competing with the terminal α-L-Gal of RG-II sidechain-A (Yapo, 2011) with
concomitant disruption of its apiosyl borate ester intermolecular crosslink.

Anton Heyn’s great insight defined the essential role of cell wall plasticity in
cell extension (Heyn, 1940). The novel idea of the cell wall as a true plastic reflected
the plastics revolution and zeitgeist of the 1930s. Curiously the simple extrapolation
from plastics to plasticiser has been ignored due to the lack of candidates and a
general consensus requiring the cleavage of load-bearing bonds although these remain
unidentified. Not surprisingly the molecular basis of plasticity has remained
speculative with many competing hypotheses including: Insertion of pectin
polygalacturonate as a chelator of Ca\(^{2+}\) crosslinks (Proseus & Boyer, 2006; Hepler et
al., 2013) combined with fluctuations in apical stiffness ascribed to pectin
demethylesterification (Zerzour et al., 2009) (Bidhendi & Geitmann, 2016). An
alternative “acid growth hypothesis” (Kutschera, 1994) formulated almost fifty years
ago (Rayle & Cleland, 1970) involves proton secretion and concomitant cleavage of
putative acid labile polysaccharide crosslinks similar to the expansin hypothesis
(McQueen-Mason & Cosgrove, 1994). Auxin-induced proton secretion is a major
tenet of the acid growth hypothesis but it also increases cytosolic Ca\(^{2+}\) (Vanneste &
Friml, 2013); this is consistent with exocytosis of AGP wall plasticizers (Schopfer,
1990; Kutschera & Niklas, 2007) during pollen tube growth. Microscopically the
pollen tube tip wall appears as a single pectin layer ~100 nm in width equivalent to
>100 monomolecular layers of highly methylesterified pectin that are interspersed
with AGPs. The Yariv reagent shows AGPs concentrated at the pollen tube tip
(Mollet et al., 2002) (Jauh & Lord 1996) but also appearing as rings along the pollen
tube (Li et al., 1992). Classical AGPs are ideal pectic plasticizers. By analogy with
synthetic plasticisers that disrupt the orderly alignment of linear polymers, the
intercalation of the small bead-like Hyp-arabinogalactan glycosubstituents (Hyp-AGs)
(Lamport et al., 2014) likely disrupts linear pectin alignment. Indeed, direct
experimental evidence involves the Yariv reagent that inhibits tip growth with a
concomitant rapid accumulation of periplasmic AGPs (Mollet et al., 2002);
arguably a decreased level of AGPs in muro is causally connected with decreased
wall plasticity. We also infer that small arabinogalactan peptides may also increase
wall plasticity based on their dramatic upregulation during auxin induced root cell
elongation (Pacheco-Villalobos et al., 2016). Compared with the much larger classical AGPs (>100 kDa), the higher diffusibility of small (~20 kDa) AG-peptides (Van den Bulck et al., 2005) presumably enables them to plasticize thicker walls than at the pollen tube tip.

Indirect experimental evidence also strongly correlates AGPs with pollen tube tip growth (Seifert & Roberts, 2007) and also with more general root epidermal cell expansion (Ding & Zhu, 1997). Thus, double null mutants of pollen-specific agp6 and agp11 yielded pollen grains highly defective in germination and growth (Coimbra et al., 2009). Anecdotal evidence also correlates the friability of cell suspension cultures with enhanced AGP secretion.

Interestingly, when the Yariv reagent inhibits normal growth and tip extension ceases the pollen tube does not rupture, presumably because further additions such as callose thicken the tip wall (Jauh & Lord, 1996).

Figure 2 combines wall biomechanics and biochemistry by integrating AGPs and Hechtian adhesion sites as essential components of a Hechtian oscillator. These components physically connect the wall with stretch-sensitive components of the plasma membrane that control the Ca\(^{2+}\) influx essential for coordinating the exocytosis of wall precursors. Thus, the wall regulates its own growth by Hechtian feedback.

### Pollen tube guidance from stigma to embryo sac

A plethora of guidance cues that may direct pollen tube growth include: K\(^+\) Cl\(^-\) Ca\(^{2+}\) (Hepler et al., 2006), glycoproteins (Sommer-Knudsen et al., 1998), reactive oxygen species (ROS) (Foreman et al., 2003), nitric oxide (Prado et al., 2016), peptides (Qu et al., 2015) and complex signalling networks that remain to be elucidated (Leydon et al., 2015). However other guidance cues can now be considered from the perspective of the female reproductive tract where AGP-rich regions visualised by anti-AGP monoclonals (Coimbra & Salema, 1997; Coimbra & Duarte, 2003) coincide with the pathway traversed from stigma to the egg cell. Indeed, the remarkable coincidence of AGPs and Ca\(^{2+}\) throughout the female reproductive tract (Coimbra & Duarte, 2003) is hardly fortuitous. Faced with competing hypotheses Occam’s Razor suggests a simple Ca\(^{2+}\) guidance cue: (Fig. 6) Pollen tubes grown in
vitro acidify their growth medium (Feijo et al., 1995); therefore in vivo they presumably dissociate AGP-Ca$^{2+}$ of the transmitting tissue thus enabling pollen tubes to blaze a Ca$^{2+}$ trail to the ovule. A dual source of cytosolic Ca$^{2+}$ seems likely: Firstly, at the tip derived from its periplasmic AGP-Ca$^{2+}$ capacitor that involves recycling or “reflux” of cytosolic Ca$^{2+}$. Secondly, Ca$^{2+}$ released from surrounding tissues by the marked proton efflux at the pollen tube shank rather than at the tip (Hepler et al., 2006). Due to the cooperative effect of multiple pollen tubes (Heslop-Harrison et al., 1985) enhanced H$^{+}$ efflux and release of Ca$^{2+}$ from the locally abundant AGP-Ca$^{2+}$ could contribute to reproductive success by ensuring fertilisation of multiple ovules. Evidence for the co-localisation of AGPs and Ca$^{2+}$ at each stage of the pollen tube pathway follows:

**Stigmatic tissue:**

Calcium antimonate histochemical detection of abundant Ca$^{2+}$ in stigmatic tissue of several species (Ge et al., 2007) parallels AGPs detected by the β-D-Yariv reagent and also by monoclonal antibodies JIM8 and JIM13. Significantly, in apple blossom, stigmatic receptivity was acquired concomitantly with the secretion of AGPs (Losada & Herrero, 2012).

**Stylar tissue**

Classic work (Mascarenhas & Machlis, 1962) showing Ca$^{2+}$-dependent pollen tube growth and an elevated calcium content of tissues from stigma and transmitting tract of the style to the ovule has been amply confirmed (Knox et al., 1976), including Ca$^{2+}$ chemotropism (Malho & Trewavas, 1996). As the distribution of AGPs and Ca$^{2+}$ in the style coincide, the role of AGPs as a primary source of cytosolic Ca$^{2+}$ seems likely.

**Ovule micropyle: synergids and filiform apparatus**

On its final path towards the egg cell the pollen tube makes a sharp turn from the transmitting tissue toward the micropyle (Coimbra & Duarte, 2003) (Fig. 6) guided by signals from the synergid cells and filiform apparatus both strongly expressing AGPs (Coimbra & Salema, 1997) and Ca$^{2+}$ (Chaubal & Reger, 1990) with highest levels of Ca$^{2+}$ in the synergid filiform apparatus (Ge et al., 2007).
Tip growth is a fine balance between cell wall thickening at high levels of Ca\(^{2+}\) (Picton & Steer, 1983) and tip bursting that occurs in low Ca\(^{2+}\) (Hill et al., 2012) and increased auxin levels (Zerzour et al., 2009). However, tip bursting at low oxygen levels is of physiological significance in the hypoxic ovary where failure of tip wall integrity (Linskens and Schrauwen 1966) releases gametes that fertilise the egg cell.

Significantly AGP expression is absent from the rudimentary ovules of male flowers of *Actinidia deliciosa* (Coimbra & Duarte, 2003) while laser ablation showed that only a single synergid cell in the isolated embryo sac of *Torenia fornieri* was necessary and sufficient for pollen tube attraction to the female gametophyte (Higashiyama et al., 2001). However, additional signals likely involve an AGP sidechain fragment designated AMOR or “Activation Molecule for Response” by the pollen tube to LURE guidance peptides from the synergids (Okuda et al., 2009).

However, AMOR was identified as methyl-O-glucuronosyl-\(\beta\)-D-galactose (Mizukami et al., 2016) that is a key component of the AGP Ca\(^{2+}\)-binding motif and thus suggests its possible role as a Ca\(^{2+}\) carrier. This complements the invariable coincidence of AGP and Ca\(^{2+}\). Clearly Occam is a guide not a guarantor!

**Future research pathways guided by Occam and Darwin**

In 1799 Napoleon’s troops entered the Egyptian village of Rosetta (Rashid) and discovered an ancient basalt slab with a trilingual inscription in praise of King Ptolemy V (205-180 BC) that finally enabled Champollion to decipher Egyptian hieroglyphics in 1822. Rosetta is a metaphor for AGPs whose primary function remained unknown for fifty years until their structural hieroglyphics (Fig. 3) were deciphered to reveal the molecular function as an AGP-Ca\(^{2+}\) capacitor at the cell surface. This leads to a unified role for tip-localised AGPs and tip-focussed Ca\(^{2+}\) in cell extension as proposed here. However, viewed as a true plastic the control of wall plasticity at the molecular level in particular appears to be an intractable problem with widely differing views and assumptions. Nevertheless, the properties of AGPs combined with Hechtian adhesion offer a solution based on a Hechtian oscillator that generates cytosolic Ca\(^{2+}\) oscillations. This role for classical AGPs depends on their precise cell surface location and their unique chemistry: an exquisitely designed
glycomotif with paired glucuronic acid residues that bind $\text{Ca}^{2+}$ but released on demand. Such elegance would have pleased Paley but puzzled Darwin. Many questions remain. They include evolutionary origins (Verret et al., 2010). A vital clue lies embedded in the chalk cliffs of the South Downs National Park built over two hundred million years of fossilised phytoplankton; the calcified cell walls of coccolithophores, typically *Emiliania huxleyi*, may be the evolutionary precursor to dynamic $\text{Ca}^{2+}$ storage at the cell surface of higher plants possibly with even wider implications of the Hechtian oscillator as an osmosensor (Haswell and Versluis 2015) or a gravisensor (Schnabl 2002) both in search of their identity as nanoscale molecular devices.

Finally, the reason for the wide range in classical AGP structure that includes the complex glycosylation of its protein core. Clearly the tiny minority of AGPs characterised biochemically only hints at their true diversity and versatility emphasised by recent bioinformatics that show higher plants have heavily invested in AGPs (Ma et al. 2017). Thus diverse stimuli might generate specific $\text{Ca}^{2+}$ signatures (Rudd and Franklin-Tong 2001) based on the distribution, size and composition of AGP glycomodules. Because AGP glycosylation is only indirectly coded by the genome, precise structural oligosaccharide details cannot be predicted by bioinformatics. Hence the technical problem of rapid polysaccharide/oligosaccharide structural analysis and the determination of $\text{Ca}^{2+}$ binding constants. Future *ab initio* computer simulations (cf. Fig. 3.) will enable the design of novel AGP glycomodules with properties optimised for a given environment in the perpetual quest for the Holy Grail of plant growth.

**Acknowledgements.**

Professor Marcia Kieliszewski, Ohio University, for advice and encouragement. The University of Sussex, School of life sciences, for past laboratory facilities. Pembroke College, University of Cambridge, academic home of DTAL (1955-1961) and also William Turner (first English Herbal) and Nehemiah Grew (Father of plant anatomy). DTAL is the corresponding author.

LT's structural work made this paper possible and contributed to paper preparation.
MAH contributed to earlier structural work and paper preparation.

MJK has contributed many years work to this project and has provided invaluable advice.

Figure Captions and legends.

**Fig. 1. Hechtian strands of plasmolysed onion epidermis**

These plasmolysed cells depict the founder event. Reprinted from (Hecht, 1912).

A. Incipient plasmolysis of onion epidermal cells in 3% KNO₃

B. Plasmolysis in 7% KNO₃

**Fig. 2. The pollen tube as a Hechtian oscillator**

A. The Hechtian oscillator commemorates the contributions of Karl Hecht. Nominally the three simple terms R, L and C form the pollen tube tip growth oscillator that shows how the wall regulates its own growth by coupling H⁺ and Ca²⁺ ion currents:

Turgor pressure charges the battery i.e. stretches the wall that transmits the resulting internal stress via Hechtian adhesion to the plasma membrane stretch-activated H⁺-ATPase and Ca²⁺-channels (resistance R). Together they work in parallel where H⁺ releases bound Ca²⁺ (capacitance C) to the Ca²⁺ channels hence a major source of cytosolic Ca²⁺ that activates exocytosis (inductance L) of wall precursors. Arguably these largely determine the frequency of tip growth oscillations; both auxin (Zerzour et al., 2009) and fusicoccin (Fricker et al., 1997) enhance oscillatory growth consistent with their activation of plasma membrane H⁺ ATPase.

Hechtian strands are prominent features of many cells on plasmolysis including root hairs, pollen tubes, stomatal guard cells and green algae like Closterium (Domozych et al., 2003). This implies a global role for Hechtian adhesion as a stress-strain gauge that can also act as a sensor and regulator of turgor pressure.

B. illustrates a single cycle of the oscillator based essentially on (Chebli & Geitmann, 2007; Hepler et al., 2013) arbitrarily divided into 14 stages beginning conveniently
with rapid tip growth and transduction of wall stress. Although depicted as a simple
cycle, most stages comprise critical control points with multiple inputs. Some stages
remain to be defined such as cleavage of GPI-anchored AGPs via PLC activity (Dowd
et al., 2006) that is possibly also stretch activated. Others such as PM ATPase activity
(Certal et al., 2008) at the tip itself are surmised although activity is strongest just
behind the tip at the pollen tube shank (Hepler et al., 2006). While ion fluxes are not
precisely in phase with growth their synchronicity suggests a close relationship as
inferred here:

1. Wall thins
2. Tube extension rate is inversely proportional to wall thickness (Kroeger et al.,
2008)
3. Hechtian transduction transmits wall stress (Pont-Lezica et al., 1993)
4. Increases tension in plasma membrane tethered to cell wall: (Hecht, 1912).
5. Proton efflux via PM H^+ATPase (Certal et al., 2008)
6. Decreases periplasmic pH
7. Dissociates periplasmic AGP-Ca^{2+} (Lamport et al., 2014; Lamport & Varnai,
2013)
8. Stretch-activated Ca^{2+} channels open (Ding & Pickard, 1993; Feijo et al., 1995;
Dutta & Robinson, 2004)
9. Ca^{2+} influx via open Ca^{2+} channels
10. Cytosolic Ca^{2+} increases (Miller et al., 1992)
11. Activates exocytosis of wall precursors. (Camacho & Malho, 2003)
12. Wall thickness at tip increases (Picton & Steer, 1983; McKenna et al., 2009)
13. PM tension decreases
14. Minimum rate of tip extension (McKenna et al., 2009)
Return to step 1.

15. Turgor drives tip extension (Hill et al., 2012)

Note: Cytosolic Ca$^{2+}$ recycles via Ca$^{2+}$-ATPase mediated efflux (Schiott et al., 2004; Frey et al., 2015) or sequestration by golgi exocytotic vesicles.

Fig. 3. Repetitive AGP glycomodule

The 15-residue glycomodule structure that binds Ca$^{2+}$ as depicted above corresponds to known highly expressed AGPs (Tan et al., 2010).

(A) The linkage connectivity of sugars involved in the repetitive 15-residue consensus Hyp–arabinogalactan, a conserved structure based on a β-linked galactosyl trisaccharide with paired sidechains that bind Ca$^{2+}$.

Galactose sidechain residues; R1 and R2: rhamnose sidechain residues; GlcA: glucuronic acid sidechain residues; Hyp: hydroxyproline.

(B) Three-dimensional molecular model simulating a Hyp–AG with bound Ca$^{2+}$ Hyp–AG interferon Hyp-polysaccharide-1 (IFNHP1) with Ca$^{2+}$ ions (green) bound by two glucuronic acid (GlcA) sidechains (red); the galactan backbone is in dark blue and sidechains in light blue. [Reprinted from (Lamport & Varnai, 2013)]

See Supplementary Movie S1 in (Lamport & Varnai, 2013). Molecular dynamics simulation of Hyp-AG IFNHP1 (Gal backbone in blue) showing the conformational change on Ca$^{2+}$ (green) binding by two GlcAs (red) over 500 ns.

Fig. 4. Plasmolysed pollen tube tip showing Hechtian strands.

Plasmolysis of a Lilium longiflorum pollen tube loaded with the fluorescent dye FM4-64 five minutes after transfer to medium containing 0.3 M sorbitol and 2 µM FM4-64.

(A) arrow head indicates fluorescent Hechtian strands. (B) Brightfield image. Bar = 15 µm. With permission from (Parton et al., 2001).

Fig. 5. Comparison of cell wall compositions
Ternary graph showing a simplified cell wall composition based on their content of pectin, cellulose and Hyp-rich glycoprotein with extremes ranging from the pollen tube tip (~100% pectin), secondary cell walls (nominally 100% cellulose) to Chlamydomonas (~100% Hyp-rich glycoprotein) with the primary cell wall of higher plants and the Charophycean alga Coleochaete representing intermediate values.

Fig. 6. Pollen tube pathway to the embryo sac
Redrawn from (Qu et al., 2015) to show pollen tubes (yellow) growing through transmitting tissue lined with AGP-Ca\(^{2+}\) (red ellipsoids with green Ca\(^{2+}\))

Pollen tubes signal to the transmitting tissue to supplement their endogenous Ca\(^{2+}\) levels by analogy with marathon runners who signal for a water bottle at stations along the track.

References


A

Stress/strain via Hechtian adhesion

PM ATPase 

H^+

Ca^{2+} channels

H^+

AGP-Ca^{2+}

C

EXOCYTOSIS

Cell Wall

Precursors

Cell Wall

Ca^{2+}

B

Wall thins

PM tension minimum

Wall thickens

EXOCYTOSIS

Cytosolic Ca^{2+} increases

Ca^{2+} influx

Ca^{2+} channels OPEN transiently

AGP-Ca^{2+} dissociates

PM H^+-ATPase

Periplasmic pH decreases

PM tension MAX

Hechtian transduction

TURGOR
Fig. 2. The pollen tube as a Hechtian oscillator

A. The Hechtian oscillator commemorates Karl Hecht and Hechtian adhesion between the plasma membrane and cell wall that we consider integral to a tip growth oscillator. Modelled as a simple electronic circuit it consists of three terms R, L and C that enable the wall to regulate its own growth by coupling H⁺ and Ca²⁺ ion currents: Turgor pressure charges the cell wall “battery” i.e. stretches the wall that transmits the resulting internal stress to the plasma membrane stretch-activated H⁺-ATPase and Ca²⁺-channels (resistance R). Together they work in parallel where H⁺ dissociates AGP-Ca²⁺ (capacitance C) releasing bound Ca²⁺ to the Ca²⁺ channels hence a major source of cytosolic Ca²⁺ that activates exocytosis (inductance L) of wall precursors. Arguably these largely determine the frequency of tip growth oscillations; both auxin (Zerzour et al., 2009) and fusicoccin (Fricker et al., 1997) enhance oscillatory growth consistent with their activation of plasma membrane H⁺ ATPase.

Hechtian strands are prominent features of many cells on plasmolysis including root hairs, pollen tubes and stomatal guard cells. This implies a global role for Hechtian adhesion as a stress-strain gauge that can also act as a sensor and regulator of turgor pressure.

B. illustrates a single cycle of the oscillator based essentially on (Chebli & Geitmann, 2007; Hepler et al., 2013) arbitrarily divided into 14 stages beginning conveniently with rapid tip growth and transduction of wall stress. Although depicted as a simple cycle, most stages comprise critical control points with multiple inputs. Some stages remain to be defined such as cleavage of GPI-anchored AGPs via PLC activity (Dowd et al., 2006) that is possibly also stretch activated. Others such as PM ATPase activity (Certal et al., 2008) at the tip itself are surmised although activity is strongest just behind the tip at the pollen tube shank (Hepler et al., 2006). Although ion fluxes are not in phase with growth their similar periodicity suggests a close relationship as inferred here.

1. Wall thins
2. Tube extension rate is inversely proportional to wall thickness(Kroeger et al., 2008)
3. Hechtian transduction transmits wall stress (Pont-Lezica et al., 1993)
4. Increases tension in plasma membrane tethered to cell wall: (Hecht, 1912).
5. Proton efflux via PM H⁺ATPase (Certal et al., 2008)

6. Decreases periplasmic pH

7. Dissociates periplasmic AGP-Ca²⁺ (Lamport et al., 2014; Lamport & Varnai, 2013)

8. Stretch-activated Ca²⁺ channels open (Ding & Pickard, 1993; Feijo et al., 1995; Dutta & Robinson, 2004)

9. Ca²⁺ influx via open Ca²⁺ channels

10. Cytosolic Ca²⁺ increases (Miller et al., 1992)

11. Activates exocytosis of wall precursors. (Camacho & Malho, 2003)

12. Wall thickness at tip increases (Picton & Steer, 1983; McKenna et al., 2009)

13. PM tension decreases

14. Minimum rate of tip extension (McKenna et al., 2009)

Return to step 1.

15. Turgor drives tip extension (Hill et al., 2012)

Note: Cytosolic Ca²⁺ recycles via Ca²⁺-ATPase mediated efflux (Schiot et al., 2004; Frey et al., 2015) or sequestration in golgi vesicles.
Fig. 6. Pollen tube pathway to embryo sac
Fig. 5. Comparison of cell wall composition
Fig. 3. Repetitive AGP glycomodule
Fig. 4. Plasmolysed pollen tube showing Hechtian strands.