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# Arabinogalactan proteins: Decoding the multifaceted roles in plant reproduction

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Arabinogalactan proteins (AGPs) are highly glycosylated cell wall proteins essential for plant growth and reproduction. AGPs are extensively decorated with arabinogalactan polysaccharides, composed primarily of arabinose and galactose, along with minor sugars such as glucuronic acid, fucose, and rhamnose. Their glycosylation patterns and glycosylphosphatidylinositol anchor enable interactions with receptors, modulating signal transduction pathways critical for reproduction. AGPs also associate with cell wall components like pectin and hemicellulose, impacting cellulose deposition and cell wall integrity. Recent research highlights AGPs' role as calcium ($Ca^{2+}$) capacitors, regulating $Ca^{2+}$ storage and release during crucial reproductive stages. Despite significant progress, their precise molecular mechanisms remain elusive. In this review, we explore the multifaceted roles of AGPs in plant reproduction, shedding light on the recent progress in their involvement in signalling pathways, cell wall interactions, and $Ca^{2+}$ homeostasis, while highlighting the ongoing research needed to fully understand their mechanisms in reproductive success.

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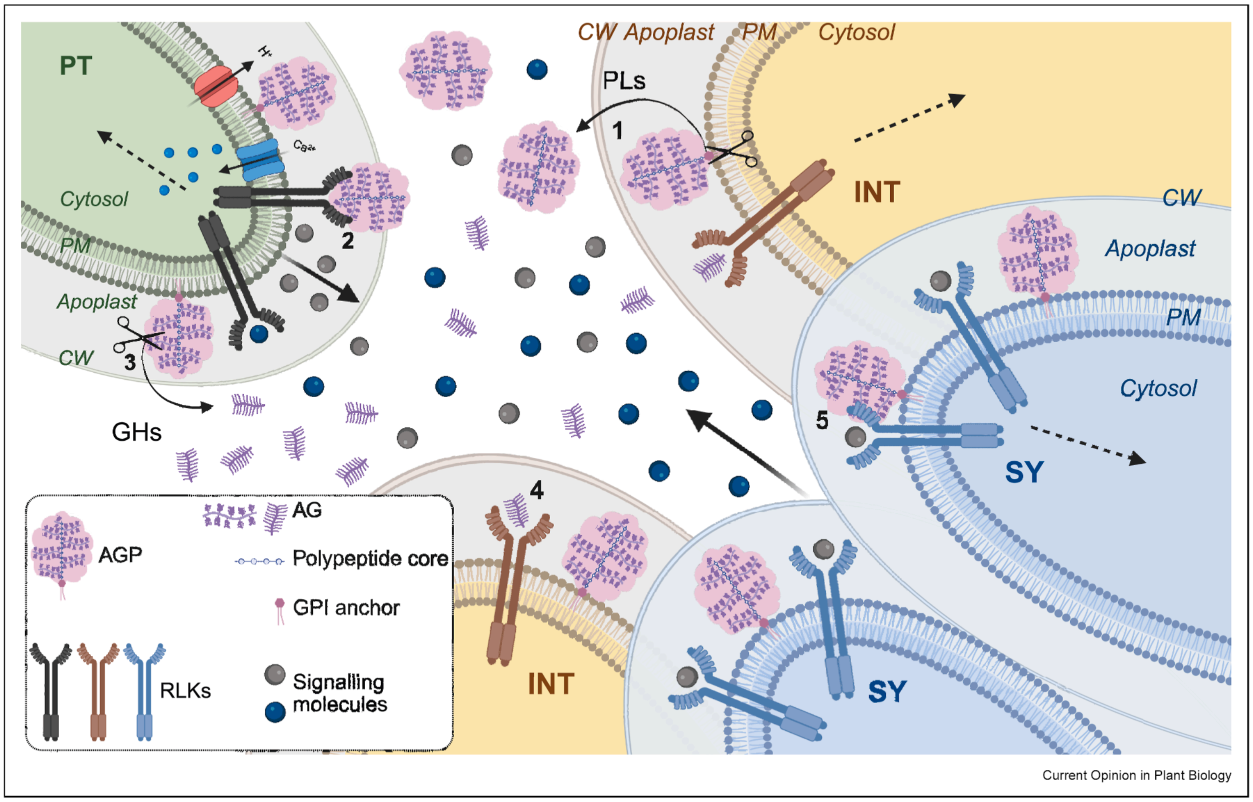
## Introduction

Plant reproduction is a complex and multi-step process dependent on cellular and molecular interactions. In angiosperms, the process of reproduction begins when a pollen grain adheres to the stigma, germinates, and forms a pollen tube (PT) that delivers two sperm cells to the ovule. During double fertilisation, one sperm cell fertilises the egg cell, originating the embryo, and the other fertilises the diploid central cell, generating the endosperm. Among the various molecules involved, arabinogalactan proteins (AGPs) have emerged as essential players for reproductive success, contributing to gametophyte development, male–female interactions, and seed formation [1].

AGPs constitute a large family of highly glycosylated cell wall proteins with multifunctional roles in plant development [2], likely due to their heterogeneous structure. AGPs are characterised by a unique combination of features, including a protein domain rich in Pro/Hyp, Ala, Ser, and Thr, often forming dipeptide repeats like Ala-Hyp, Ser-Hyp, and Thr-Hyp; an N-terminal secretion signal sequence; extensive glycosylation, with 90 % of their structure made up of with type II arabinogalactan (AG) polysaccharides, which mainly contain arabinose (Ara) and galactose (Gal), along with minor sugars such as glucuronic acid (GlcA), fucose, and rhamnose (Rha), attached to Hyp residues, and enabling interaction with the β-Yariv reagent; additional functional domains; and a C-terminal glycosylphosphatidylinositol (GPI) anchor tethering them to the plasma membrane [3].

Uncovering the function of AGPs through genetic approaches is challenging due to the high gene redundancy within this family. The carbohydrate moiety of AGPs is crucial for their functional diversity, influencing interactions with other signalling molecules and cellular

**Figure 1**



Proposed model of AGPs in signal transduction during pollen tube-ovule interactions.
AGPs can be released from the plasma membrane into the apoplast and extracellular environment through the activity of phospholipases (PLs) (1). Released AGPs may serve as ligands, recognised by receptor-like kinases (RLKs) (2). Glycoside hydrolases (GHs) can cleave AGPs, releasing arabinogalactans (AGs) (3), which then can act as messengers recognised by RLKs (4). AGPs may interact with RLKs and function as co-receptors (5), facilitating the recognition of additional ligands and activating downstream signal transduction pathways (dashed arrow). This figure is intended as a conceptual model, and some mechanisms illustrated remain hypothetical for the specific cell types shown. Abbreviations: AG, arabinogalactan; AGP, arabinogalactan protein; CW, cell wall; GHs, glycoside hydrolases; GPI, glycosylphosphatidylinositol; INT, inner integument cell; PLs, phospholipases; PM, plasma membrane; PT, pollen tube; RLK, receptor-like kinase; SY, synergid cell. Created in https://BioRender.com.

structures [2]. AGPs are *O*-glycosylated on Hyp residues by galactosyltransferases (GALTs) that add the first Gal residue. Successive additions of Gal residue form the main β-1,3-galactan chain, further branched with β-1,6-galactan side chains, which are extended by several glycosyltransferases (GTs), including glucuronosyltransferases (GLCATs) responsible for adding GlcA [3].

Despite decades of research, the mode of action of AGPs remains unclear, particularly during plant reproduction; this review explores their signalling capabilities, involvement in calcium ($Ca^{2+}$) signalling, and impact on the cell wall.

## AGPs in signal transduction

During its journey inside the pistil, the PT grows through the extracellular matrix until it reaches the embryo sac, involving numerous interactions between the PT and female tissues. AGPs, either as integral cell wall proteins or extracellular matrix components, are likely implicated in this process, influencing intracellular signalling [4]. Their extensive carbohydrate composition and GPI anchor presence support their role in signal transduction by providing a large glycosylated surface for molecular interactions and enabling release from the plasma membrane into the apoplast through phospholipase action (Figure 1) [3]. Recent studies highlight the importance of AGP carbohydrates in reproductive functions [5], with an octuple GALT mutant showing severe reproductive defects [6,7].

In *Torenia fournieri*, a methyl-glucuronosyl AG called AMOR, is secreted by ovules and induces PT competency [8]. In fact, AMOR contains a terminal disaccharide structure [4-Me-GlcA-β-(1−6)-Gal] that naturally occurs at the end of AGP carbohydrate chains [9], and may be released by the action of glycoside hydrolases (Figure 1) [3]. Although not found in Arabidopsis, AMOR highlights the functional importance of AGPs, which are expressed along the PT growth pathway and have long been implicated in reproduction [10–13].

The predicted presence of a GPI anchor in many AGPs makes them ideal candidates to act in signalling pathways during pollen-pistil interactions. Plant GPI-anchored proteins are predicted to serve as molecular hubs that mediate interactions between the plasma membrane and cell wall, while also transducing signals into the interior of the cell to activate signal transduction pathways [14]. GPI-anchored proteins (GPI-APs) have been shown to be involved in plant reproduction, generally acting as chaperones and co-receptors for receptor-like kinases (RLKs). For example, two GPI-APs, LORELEI and LORELEI-like-GPI-anchored protein 1 (LLG1), can associate with the RLK FERONIA, to recognise extracellular ligands and regulate sperm cell release during double fertilisation [15]. Two other LLGs, LLG2 and LLG3, function as co-receptors in the PT surface-located receptor complex composed of ANXUR1/2 (ANX1/2) and Buddha's Paper Seal 1/2 (BUPS1/2) [16]. Among the GPI-anchored early nodulin-like AGP (ENODL) family, ENODL14 was shown to physically interact with the extracellular domain of FERONIA during PT reception at the ovule (Fig. 1) [17].

The functional importance of the GPI anchor was highlighted in recent work showing that truncated versions of the AGP4/JAGGER GPI anchor were unable to fully rescue the *jagger* reproductive phenotype [18]. Furthermore, Salt-overly Sensitive 5 (SOS5)/Fasciclin-like AGP 4 (FLA4) was suggested to function in a linear genetic pathway with two leucine-rich repeat RLK, FEI1 and FEI2, acting as a co-receptor in cell wall sensing to regulate seed coat mucilage production [19].

These findings highlight the crucial role of GPI-APs in regulating cell–cell communication for PT growth through pistil tissues. RLK signalling pathways, active throughout reproduction, are closely linked with AGP-mediated mechanisms, reinforcing the possible role of AGPs as co-receptors or modulators of RLKs.

## AGPs as cross-linkers in the cell wall

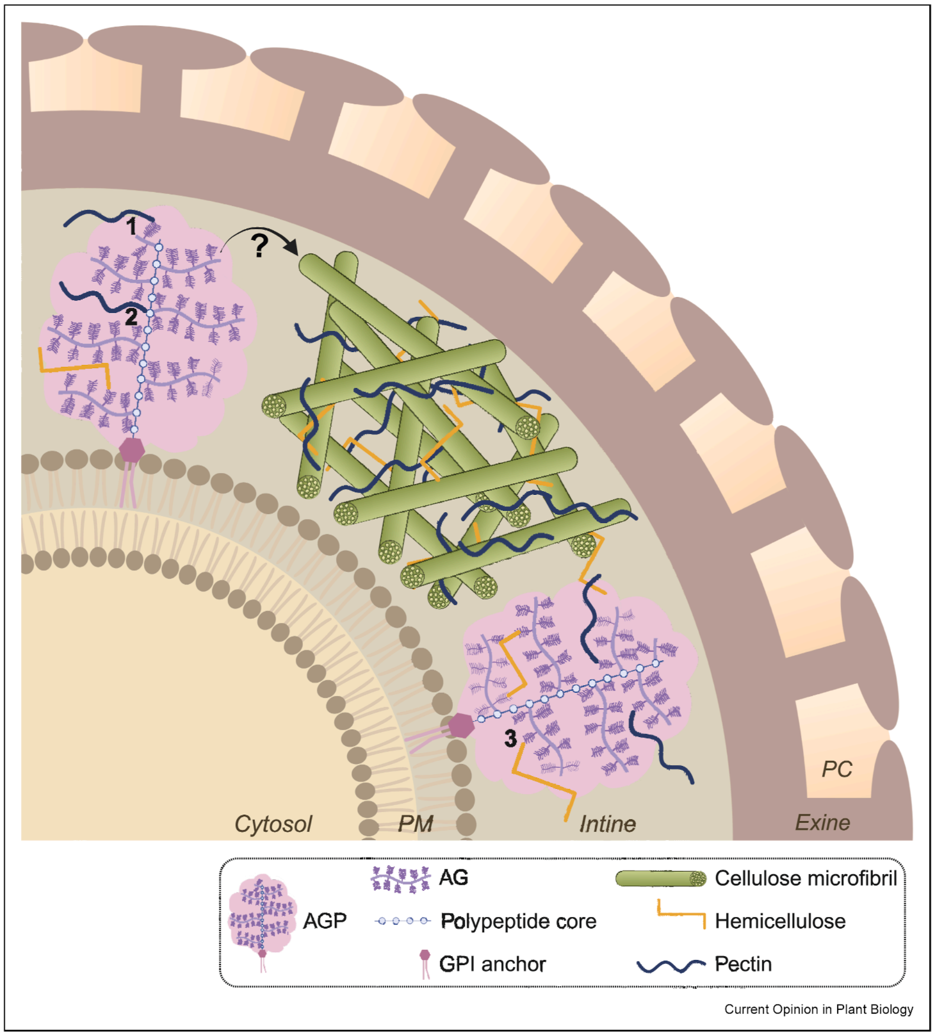
Plant cell walls are dynamic structures constantly remodelling to support cell growth, expansion, differentiation, and communication, all affecting PT growth [20]. Cell walls are primarily composed of cellulose, hemicellulose, pectin, and proteins, including AGPs [21]. AGPs play a crucial role in linking these structural components, with their unique glycosylation patterns enabling versatile interactions. One prominent example is the covalently linked pectic-AGP complex ARABINOXYLAN PECTIN ARABINOGALACTAN PROTEIN 1 (APAP1) isolated from Arabidopsis culture medium containing pectin polysaccharides, including rhamnogalacturonan I (RG-I) and homogalacturonan, linked to the Rha residue in the Rha-$\alpha$-(1 → 4)-GlcA side chain of the type II AGs of AGP57C (Figure 2) [22]. Pectic-AGP complexes were later identified in the cell walls of Arabidopsis suspension cultures [23] and tissues, such as flowers and siliques [24], where they were associated with larger pectin glycans compared to those secreted into the culture medium. AGPs also interact with hemicellulose, as demonstrated in the APAP1 complex, where arabinoxylan is attached either to a Rha residue in the RG-I domain or directly to an Ara residue in the AG glycan domain (Figure 2) [22].

AGPs contribute to cell adhesion and expansion [25,26], likely through covalent binding to pectin. For example, SOS5 has been proposed, based on mutant phenotypic analyses, to mediate seed coat mucilage adherence through interactions with pectin [25,27]. Similarly, AGP31 interacts with pectin through its PAC domain that binds to galactan branches of RG-I, and through its histidine stretch, which binds to methylesterified polygalacturonic acid (Figure 2) [28]. Recently, a study demonstrated that the recruitment and transport of AGP23 to the PT tip by Arabidopsis Formin 5 (AtFH5) influence pectin dynamics, and consequently, the flexibility and integrity of the cell wall [29]. Interestingly, pectic-AGPs extracted from various cell wall tissues exhibited variations in pectin components and glycosylation patterns, suggesting tissue-specific functions that may influence cell wall properties in response to developmental cues [24].

FLAs play a pivotal role in cellulose synthesis and deposition (reviewed in Ref. [30]). FLA11 and FLA12 ensure proper cellulose microfibril alignment and deposition, with mutants showing decreased cellulose content and altered microfibril angles, compromising structural integrity [31,32]. Similarly, FLA16 loss also reduced cellulose levels [33]. Pollen grains from FLA3 RNA interference and FLA14 overexpression lines exhibited abnormal cellulose distribution, as evidenced by the absence of fluorescence staining with calcofluor white [34,35], suggesting that both proteins may influence pollen wall development by modulating cellulose deposition (Figure 2). Additionally, the *sos5* mutant revealed reduced pectin content and disrupted cellulosic ray formation in seed mucilage [25,27]. SOS5 functions independently of cellulose biosynthesis and signalling pathways, forming complexes with other cell wall components like pectin and hemicellulose, which are hypothesised to mediate the deposition and organisation of cellulose microfibrils [25,36].

Phenotypic analyses of *galt2 galt5* and *glcat14a glcat14c* double mutants displayed similar reduced cellulose

**Figure 2**



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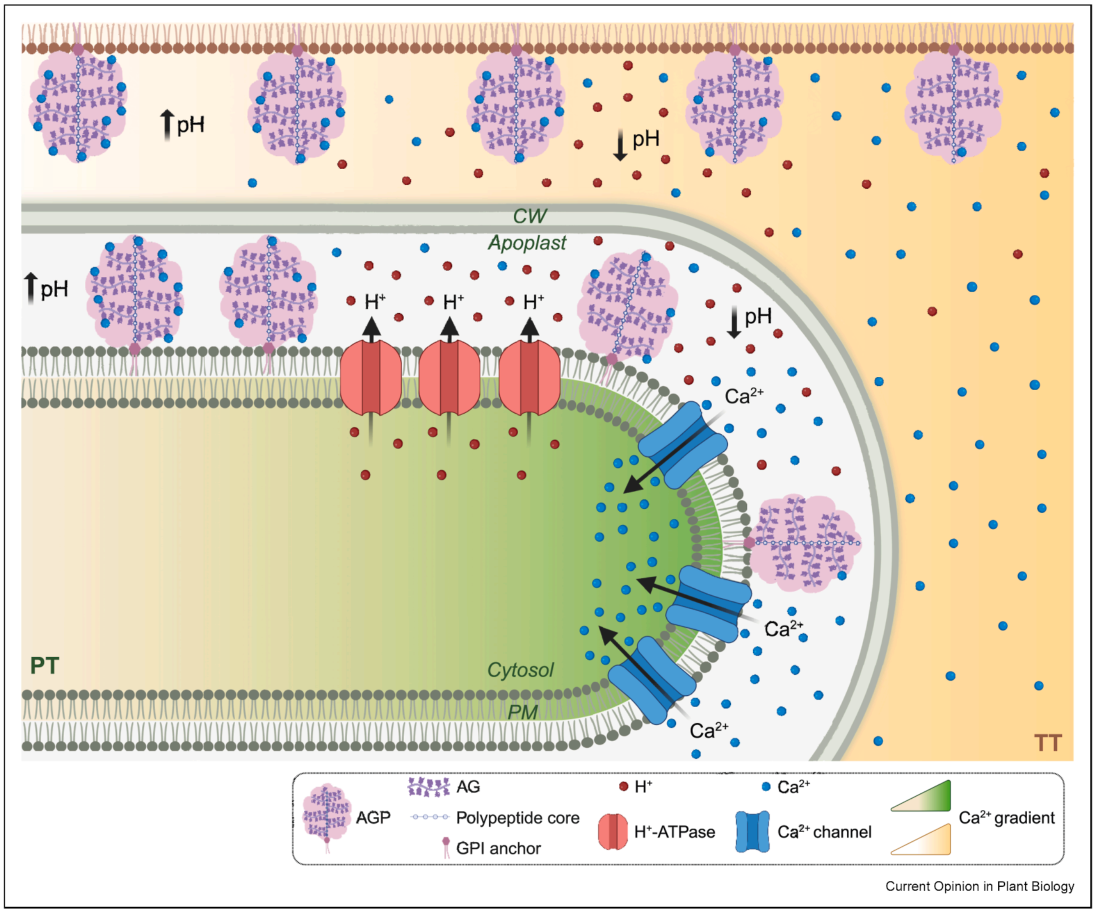
Model of AGPs as cross-linkers in the intine layer of the pollen cell wall.
The mature pollen wall consists of a pollen coat (PC), an outer exine layer made of sporopollenin, and an inner intine layer with a pectocellulosic composition. In this model, AGPs act as cross-linkers within the intine, contributing to the structural integrity and organisation of the cell wall components. Pectin binds to the rhamnose residue in the side chain of the type II arabinogalactan (AG) polysaccharides of AGPs, as suggested for the APAP1 complex (1). Other AGPs, such as AGP31, bind to pectin through their PAC domain and a histidine stretch (2). Hemicellulose interacts with AGPs directly with the arabinose residue in the AG glycan domain (3), as demonstrated in the APAP1 complex. Although a direct interaction between AGPs and cellulose has not yet been demonstrated (?), phenotypic analyses of mutants confirmed AGPs importance in mediating cellulose deposition within the intine layer. This figure is intended as a conceptual model to illustrate the cross-linking role of AGPs, using the intine as an example due to its defined composition of hemicelluloses, pectins, and cellulose. Similar cross-linking roles of AGPs are thought to occur in the cell walls of other tissues beyond pollen. Abbreviations: AG, arabinogalactan; AGP, arabinogalactan protein; GPI, glycosylphosphatidylinositol; PC, pollen coat; PM, plasma membrane. Created in https://BioRender.com.

phenotypes. While GALT mutants exhibited reduced cellulose ray and pectin staining in seed mucilage [37], GLCAT mutants showed alterations in cellulose ray formation and reduced crystalline cellulose content [38]. These phenotypes likely arise from altered AG conformation within AGPs, disrupting their interaction with pectin and, consequently, affecting cellulose deposition. Notably, while a direct link between AGPs and cellulose has yet to be demonstrated, their interactions with pectin and hemicellulose are crucial for cell wall structure and function.

## AGPs as calcium capacitors

$Ca^{2+}$ is an important second messenger in plant reproduction, with cytosolic $Ca^{2+}$ oscillations occurring during critical stages, such as pollen germination, PT

**Figure 3**



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AGP-$Ca^{2+}$ capacitor model during PT growth in the transmitting tract.
Proton ($H^+$) efflux by the action of $H^+$-ATPases in the plasma membrane (PM) of pollen tubes (PTs) causes acidification of the apoplast and extracellular medium (�pH). In the transiently low pH, calcium ($Ca^{2+}$) is dissociated from AGPs, resulting in $Ca^{2+}$ influx into the cytosol of PTs through $Ca^{2+}$ channels, thereby establishing a $Ca^{2+}$ gradient within the PT (green). The rise in cytosolic $Ca^{2+}$ levels drives $Ca^{2+}$-dependent processes. As the pH increases (�pH), the AGP-$Ca^{2+}$ capacitor is restored. As PTs grow through the transmitting tract, they acidify the surrounding environment, triggering the dissociation of $Ca^{2+}$ from AGPs in the tissue. This process creates a $Ca^{2+}$ gradient that guides the PTs towards the ovules. Abbreviations: AG, arabinogalactan; AGP, arabinogalactan protein; $Ca^{2+}$, calcium; CW, cell wall; GPI, glycosylphosphatidylinositol; $H^+$, proton; PM, plasma membrane; PT, pollen tube; TT, transmitting tissue. Created in https://BioRender.com.

growth and discharge, and gamete fusion [39–43]. AGPs have been proposed to be integral components of $Ca^{2+}$ signalling pathways, acting as $Ca^{2+}$ capacitors [44]. Like electrical capacitors that store charge, AGPs in the apoplast store $Ca^{2+}$ via GlcA residues, serving as an immediate source of cytosolic $Ca^{2+}$. AGPs bind $Ca^{2+}$ stoichiometrically at pH 5 (2:1 GlcA:$Ca^{2+}$) and release it as pH decreases. This hypothesis was supported by reduced GlcA levels in AGPs from GLCAT mutants, which diminished $Ca^{2+}$ binding capacity compared to wild-type plants [45–47]. Similarly, GALT mutants revealed reduced $Ca^{2+}$ binding, likely due to reduced Gal content, and consequently reduced GlcA linking to $Ca^{2+}$ [48]. GLCAT mutants also presented developmental phenotypes that were supressed by increasing $Ca^{2+}$ concentration in the medium, alongside altered intracellular $Ca^{2+}$ signatures [45]. Reduced AGP-$Ca^{2+}$ binding also impaired reproduction, with GLCAT and GALT mutants showing reduced fertility, collapsed pollen grains with wall defects, lower pollen germination rates [7,46–52], compromised ovule development [5,6], and polytubey block defects [52]. These defects phenocopy AGP-deficient mutants (reviewed in Refs. [11,53]), revealing the importance of glycosylation for AGP function.

β-Yariv reagent triggers intracellular $Ca^{2+}$ increases [54], inhibiting PT growth [55]. *In vitro*, PTs acidify their growth medium [56]; therefore, *in vivo*, PTs may dissociate $Ca^{2+}$ from AGPs, establishing a $Ca^{2+}$ gradient in the transmitting tissue to guide PTs towards the ovules (Figure 3) [57,58]. Remarkably, evidence suggests that $Ca^{2+}$ colocalises with AGPs along this pathway [57], and that AGP glycosylation increases from the stigma to the ovule [59].

PT growth has been explained through a novel Hechtian oscillator model involving an AGP-$Ca^{2+}$ capacitor and Hechtian adhesion sites [44,57]. Tension from the pectic growing wall, transmitted to the plasma membrane by Hechtian adhesion sites, activates $H^+$-ATPases, resulting in $H^+$ proton efflux, and apoplast acidification. Consequently, $Ca^{2+}$ is dissociated from tip-localised AGPs, and Hechtian transduction opens stretch-activated $Ca^{2+}$ channels, allowing $Ca^{2+}$ influx and increasing cytosolic $Ca^{2+}$, which activates exocytosis of cell wall precursors. The capacitor is then recharged by $Ca^{2+}$ recycled from the cytosol and possibly the wall matrix (Figure 3).

Auxin, a key hormone regulating plant reproduction [60,61], has been shown to induce $Ca^{2+}$ signals [62]. Recently, a molecular "pinball machine" in the plasma membrane was proposed [58,63,64], connecting $Ca^{2+}$ storage by AGPs with auxin transport by PIN proteins. Auxin efflux via PIN proteins activates a proton pump, releasing proton "pinballs" that dissociate $Ca^{2+}$ from AGPs, enabling $Ca^{2+}$ flow through open channels, and generating cytosolic $Ca^{2+}$ oscillations that regulate numerous $Ca^{2+}$-dependent processes.

The AGP-$Ca^{2+}$ capacitor in plant cells can be modulated by regulating the levels of surface AGPs and/or the size of AG polysaccharides [58]. As a key source of extracellular $Ca^{2+}$, AGPs play a crucial role in controlling the spatial and temporal release of $Ca^{2+}$ across different cells and tissues during various stages of reproduction.

## Concluding remarks

Nature guards her secrets well, but fifty years after their discovery, the molecular role of AGPs has begun to be understood. AGPs are essential for plant reproduction and fulfil triadic functions. First, AGPs participate in signal transduction, mediating communication between the PT and female tissues. Their glycosylation and GPI anchors enable interactions with receptors, essential for fertilisation. Second, AGPs interact with cell wall components like pectin, hemicellulose, and cellulose, contributing to cell growth, adhesion, and wall strength during reproductive events. Third, AGPs act as $Ca^{2+}$ capacitors, regulating $Ca^{2+}$ homeostasis to support intracellular signalling. GlcA residues, long regarded as minor components, now emerge as important players in AGP function, marking a significant breakthrough in understanding AGPs' role during PT growth through the female tissues. Future research should focus on characterising GTs and examining AGP sugar modifications in mutants to uncover the impact of specific changes on plant reproduction and cell wall properties. Additionally, uncovering glycan structures of individual AGPs and investigating tissue-specific functions will provide deeper insights into their role across different plant tissues and developmental stages. Finally, exploring AGP interactions with other cell wall components, particularly cellulose, will enhance our understanding of overall cellular communication.

## Author contributions

Conceptualisation: J.S.; Writing - Original Draft: J.S., D. M., M.J.F., A.M.P.; Writing - Review & Editing: J.S., D. M., M.J.F., A.M.P., L.G.P., S.C.; Visualisation: J.S., M.J.F; Supervision: S.C.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

No data was used for the research described in the article.

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