

Plant lipid transfer proteins: are we finally closing in on the roles of these enigmatic proteins?

Johan Edqvist^{1,4}, Kristina Blomqvist¹, Jeroen Nieuwland², Tiina A. Salminen³

¹IFM, Linköping University, 581 83 Linköping, Sweden

²Faculty of Computing, Engineering and Science, University of South Wales, CF37 1DL, Pontypridd, UK

³Structural Bioinformatics Laboratory, Biochemistry, Faculty of Science and Engineering, Åbo Akademi University, FI-20520, Turku, Finland

⁴Corresponding author (Johan.Edqvist@liu.se; +4613281288)

Abstract

The nonspecific lipid transfer proteins (LTPs) are small, compact proteins folded around a tunnel-like hydrophobic cavity, making them suitable for lipid binding and transport. LTPs are encoded by large gene families in all land plants, but they have not been identified in algae or any other organisms. Thus, LTPs are considered key proteins for plant survival on and colonization of land. LTPs are abundantly expressed in most plant tissues, both above and below ground. They are usually localized to extracellular spaces outside the plasma membrane. Although the *in vivo* functions of LTPs remain unclear, accumulating evidence suggests a role for LTPs in the transfer and deposition of monomers required for assembly of the water-proof lipid barriers—such as cutin and cuticular wax, suberin, and sporopollenin—formed on many plant surfaces. Some LTPs may be involved in other processes, such as signaling during pathogen attacks. Here, we present the current status of LTP research with a focus on the role of these proteins in lipid barrier deposition and cell expansion. We suggest that LTPs facilitate extracellular transfer of barrier materials and adhesion between barriers and extracellular materials. A growing body of research may uncover the true role of LTPs in plants.

Overview of plant non-specific lipid transfer proteins

The plant non-specific lipid transfer proteins (LTPs) are abundant, secreted, soluble, cysteine-rich and small proteins with a molecular size usually below 10 kDa (1, 2). In the LTPs four conserved disulfide bridges, formed by an eight-Cys motif (8CM) with the general form C-Xn-C-Xn-CC-Xn-CXC-Xn-C-Xn-C, stabilize the folding of four or five α -helices into a very compact 3D-structure (3, 4 and Fig. 1). The folding of the helices results in a central hydrophobic cleft suitable for the binding of hydrophobic ligands, such as fatty acids and other lipids (Fig. 2). The compact structure renders the LTPs very insensitive to heat and denaturing agents (5, 6). LTPs are expressed in all investigated land plants, but have not been detected in any other organisms (7). LTPs are encoded by large gene families in seed plants (2, 8-11). In bryophytes and ferns the gene families are significantly smaller (7, 12). LTPs are classified in five major types (LTP1, LTP2, LTPc, LTPd and LTPg) and four minor types (LTPe, LTPf, LTPh, LTPj and LTPk) (7). The classification is based on the spacing between the Cys residues in the 8CM, the polypeptide sequence identity and the position of evolutionary conserved introns. The classification also reflects post-translational modifications, e.g. LTPs with a GPI-anchor belong to LTPg. LTPd and LTPg are encoded in all land plants which suggests that these were possibly the first LTP types that evolved in land plants. The most well-studied LTP types in flowering plants LTP1 and LTP2 probably evolved later since these are not found in liverworts, mosses or other non-seed plants (7). The LTPs are translated with an N-terminal signal peptide that has a potential to localize the protein to the apoplastic space (1).

Several *in vitro* binding experiments show that LTPs bind both saturated and unsaturated fatty acyl chains, presented in various molecules such as in LPC, PG, acyl-CoA or as free fatty acids (13-17). Furthermore, many LTPs can fit two fatty acyl chains in the cavity (18 21). Some LTPs are reported to bind to hydroxylated acyl chains (12, 16, 19). The rice LTP2 OsLTP2.3 binds to dehydroergosterol (22), which is to our knowledge the only report of a LTP binding to a sterol. The dissociation constants (K_d) for LTP-ligand interactions are usually in the micromolar range suggesting that the LTPs are involved in low-affinity interactions. The preferred ligands are, in most cases, fatty acyl-chains with 14 to 18 carbons (19, 23). One of rather few LTPs shown *in vitro* to bind very-long-chain-fatty acids is Arabidopsis AtLTP1.4, which in a lipid-protein-overlay assay could bind to fatty acids from

C22 to C27 (24). There is still rather poor knowledge of the *in vivo* binding patterns for LTPs. A phytosphingosine bound camptothecin derivative was associated with purified peach LTP Pru p 3 and could hence represent an *in vivo* ligand (25). Nevertheless, the biological relevance of this observation is at present unclear.

The biological role of the LTPs remains rather obscure. However, there are data accumulating suggesting that these proteins are required for the deposition and function of wax and lipid-based polymers, such as suberin, sporopollenin and the cuticle that form water-proof barriers on plant surfaces (24, 26-32). Additionally, some LTPs with cell wall loosening activities could be important for plant growth (33). The roles of LTPs in lipid barrier deposition and cell wall loosening will be the primary focus of this review. A more encyclopedic review on plant LTPs was published rather recently (2). Nevertheless, it is important to also here point out that LTPs are suggested to be involved in many other processes in plants, such as signalling (34, 35), defence against biotic hazards (36-40) and tolerance to abiotic stresses just to mention a few examples (12, 41-45).

LTP – a major plant food allergen

The plant LTPs is the most frequent cause of food allergy in adults living in the Mediterranean area (46). Curiously, such LTP sensitized allergies are rare in Northern Europe and United States. Exposure to birch pollen may counteract the sensitivity to LTPs. This could explain the geographical distribution of the LTP sensitized allergies (47). The peach LTP Pru p 3 is the molecule that dominates the immune response to LTPs and is considered as a marker for severe systemic reactions to plant-derived food. However, since LTPs are abundantly expressed in most plants, LTP-sensitized patients may show reactions against a large number of plant foods. The role of LTPs in allergic reactions is only briefly mentioned here. We recommend reading other reviews for an introduction and update on this interesting and clinically relevant aspects of plant LTPs (47-50).

LTPs in lipid barrier polymer deposition

When plants conquered land more than 500 million years ago (51) they developed specialised tissues to survive the harsh climate outside the water. They developed new cell components like lignin which gave sturdier cell walls and waxes and hydrophobic lipid-based polyesters like suberin, cutin, and sporopollenin that form water proof barriers to protect the plants from abiotic and biotic stresses, such as water loss, radiation, pathogens and herbivores. Suberin is

a heteropolymer with polymeric aliphatic carbon chains associated with aromatic compounds. Long-chain oxygenated fatty acids provide the core of the suberin polyester. Suberin is usually found in seed coats and in periderms in roots and on stems where it accumulates in layers between the plasma membrane and the cell wall. The suberin layers contribute to the control of diffusion of water and solutes across seeds, roots, stems and other tissues (52). Cutin is the structural polymer of the epidermal cuticle, the waterproof layer covering the exterior surface of the cell wall of primary aerial organs. Cutin is a polyester of C16 and C18 hydroxy fatty acids and glycerol, which in the cuticle is interspersed with and covered by waxes (53-55). Sporopollenin is another very complex lipid-based biopolymer derived mainly from saturated precursors such as long-chain fatty acids and long aliphatic chains. Sporopollenin is found in the exine layer of spores and pollen walls. The exine protects spores and pollen in harsh environments and serves as a barrier against various physical and chemical factors and biological pathogens (56). The sporopollenin is chemically inert due to insolubility in both aqueous and organic solvents.

More and more details of the synthesis of the cuticle, suberin and sporopollenin polymers are elucidated (57). Basically, the synthesis of the lipid barriers requires *de novo* synthesis of precursors, which occurs mainly in the ER. This is followed by transfer of the precursors to extracellular spaces through the plasma membrane. This process is likely facilitated by ABC- or ABCG-transporters located to the plasma membrane (58-61). When the monomeric precursors have reached the extracellular apoplastic environment they need to diffuse or transfer to the site for polymer assembly (62).

It has been suggested that, LTPs are important for the last step described above, thus in facilitating the movement of lipid polymer and wax components to the sites of polymer accumulation on the extracellular side of the plasma membrane (26, 56, 62). This could involve passage of hydrophobic waxes or polymer precursors through the hydrophilic cell wall for cuticle accumulation, across the locule for pollen exine assembly, or in the apoplast for suberin deposition. From a mechanistic perspective, it is still rather unclear how the LTPs aid in the extracellular transport of the building blocks for lipid polymers. It is possible that the ABC transporters deliver the polymer building blocks to LTPgs, which are attached to the apoplastic side of the plasma membrane through their GPI-anchor. The cargo may then be transferred from an LTPg to an LTP of another type that may diffuse freely in the relevant

apoplastic environment. Proposed models for LTPs in the deposition of barrier lipids in different tissues are illustrated in Fig 3.

Although the details regarding the role of LTPs in lipid polymer biosynthesis remain somewhat mysterious, there are more and more data accumulating to support that the LTPs are involved and also important for this process. For instance, when gene expression data of LTPgs from rice and Arabidopsis were investigated for co-expression patterns the LTPgs could be arranged in three co-expressed LTPg clusters (63). For the first cluster (I), expression was observed in aerial parts of the plant. The second cluster (II), was the only one with expression in roots, while expression of the third cluster (III) was restricted to reproductive tissues. Thus, LTPs in seed plants, based on expression patterns, can be classified into root LTPs, green LTPs and reproductive LTPs. Further, gene ontology analyses of genes co-expressed with the three Arabidopsis LTPg-clusters showed an enrichment of genes involved with cuticular wax accumulation for cluster I, an enrichment of genes involved with suberin synthesis or deposition for cluster II and for cluster III an enrichment for genes acting in sporopollenin accumulation. Hence, the co-expression patterns suggest that the LTPgs in the three clusters are involved in the assembly of the cuticle, suberin and sporopollenin, respectively (63).

This division of LTPs in separate functional clusters is also supported from the analysis of knock-down, knock-out and overexpressing plant lines. In Arabidopsis the knock-down of *AtLTPg1* results in reduced wax load on stem surfaces (26) while in *Atltpg1* and *Atltpg2* knock-out single mutants there are a 4-20% reduction in stems and siliques of the C29 alkane (nonacosane) component of cuticular wax (27). In the *Atltpg1Atltpg2* double-mutant there are even stronger reductions of nonacosane and less total wax load in the stems and siliques (29). Curiously, overexpression of the *Brassica rapa BrLTPd1* gene in *Brassica napus* causes reduced wax deposition on leaves and morphological changes of leaves and flowers (63). Possibly, the overexpression of *BrLTPd1* may lead to disordered secretion of wax which is lost from the surface, or to the inhibition of other LTPs involved in wax deposition (63).

In rice, silencing of *OsC6* (later renamed to *OsLTPg25* (2)) results in reduced pollen fertility. In the silenced plants the anthers follow normal development until young microspores are prematurely released from the tetrad (28). The *OsLTPg25* silenced lines also show microspores and pollen walls with irregular shapes and structures. Further, ectopic expression of

OsLTPg25 results in granule-like droplets on the inner surface of the tapetal cells. These obtained phenotypes suggest that OsLTPg25 are facilitating the transport of exine precursors in the locule from the tapetal cells to the pollen grains (28).

In plants, crown gall tumors develop after infection of virulent *Agrobacterium tumefaciens* strains. These tumors are covered with a suberin containing periderm that protects against water loss and pathogen infections. In Arabidopsis, the LTP *AtLTP1.4* is expressed in the crown gall tumors (24). In an *Atltp1.4* T-DNA mutant the suberin composition in crown galls is altered, as there is a decrease in C18 alkanolic acids, as well as an increase in C18-C24 2-hydroxy alkanolic acids. The results indicate that *AtLTP1.4* enhances the extracellular delivery in the apoplast of fatty acyl precursors for suberin deposition. Interestingly, although *AtLTP1.4* is not normally expressed in epidermis, ectopic expression of the gene in epidermis resulted in altered wax composition in the cuticular wax (24).

Artemisin is a highly oxygenated sesquiterpene that is used for malaria treatment. Artemisin is produced by the plant *Artemisia annua* on the glandular trichomes on leaves and ovules. It is formed, non-enzymatically, from the precursors artemisinic acid and dihydroartemisinic acid (64). When artemisin was transiently produced in *Nicotiana benthamina*, it was found that the simultaneous expression of genes required for artemisin biosynthesis together with an *A. annua* LTP and the *A. annua* ABC-transporter Pleiotropic Drug Resistance (PDR) resulted in higher yield of artemisinic acid and dihydroartemisinic acid in the *N. benthamina* apoplast (65). The *A. annua* LTP seems to be able to retain artemisin precursors in the apoplast, and thereby preventing reflux back to the cell. The mechanism behind the retaining effect is unknown, although the authors speculated that the LTPs may deposit the artemisin precursors on the outer side of the cell wall, and once deposited reflux would be prevented (65). Possibly, such a retention mechanism that improves the yield for export of precursors from the cell, as suggested for artemisin biosynthesis, could explain the requirement for LTPs in lipid polymer deposition.

We suggest a classification of the LTPs involved in extracellular lipid polymer precursor transfer and secretion as Transporter LTPs (Fig. 3). This classification is relevant as there are also data that suggests that some other LTPs rather act by stabilizing the adhesion between lipid polymer barriers and cell walls, such as between the cuticle and the cell wall in green tissues, between the exine and intine in pollen, or between suberin and the cell wall in the

seed coat. We classify these LTPs as Adhesion LTPs (Fig. 3). Intriguingly, such a role as an Adhesion LTP was suggested for Arabidopsis AtLTP1.4 (previously named AtLTP2 but renamed to emphasize that it is of type LTP1 (2)) (32). An *Atltp1.4*-mutant has an increased cuticle permeability and structural defects at the cell wall–cuticle interphase. It was therefore proposed that AtLTP1.4 could play a major structural role by maintaining the integrity of the adhesion between the mainly hydrophobic cuticle and the underlying hydrophilic cell wall (32).

An adhesive role for LTPs is also supported from the investigation of the Arabidopsis AtLTPc1, AtLTPc2 and AtLTPc3. These three LTPs all have an expression pattern restricted to the tapetum of developing anthers (66). Double RNAi silencing of AtLTPc1 and AtLTPc3 did not cause any abnormalities regarding pollen morphology or fertility. However, in the silenced plants the intine underneath the exine is impaired and uncharacteristically separated from the exine and the microspore plasma membrane (66). Further, several Arabidopsis *Atltpg* single mutants have deformed or collapsed pollen grains (31). The seeds of these *Atltpg* mutants are unable to restrict salt uptake and show other abnormal phenotypes, such as the protrusion of seed hairs, or a shrunken and irregular appearance. Lipid analysis of the seed coats from *Atltpg4-1*, *Atltpg4-2*, *Atltpg6-1* and *Atltpg6-2* revealed a large decrease in the ω -hydroxy fatty acid 24-hydroxytetracosanoic acid (C24 ω OH) and an increase in unsubstituted C20:0, C22:0 and C24:0 fatty acids (31). These ω -hydroxy fatty acid are all important constituents of the barrier polymer suberin (67). We speculate that the phenotypes shown for the *Atltpg*-mutants could result from defects in adhesion between the suberin layer and the cell wall.

Hence, there are rather different roles suggested for LTPs in the assembly and biosynthesis of the lipid polymer barriers. The Transporter LTPs may facilitate extracellular transport or diffusion of the hydrophobic polymer monomers and waxes and the Adhesion LTPs stabilize the interaction between lipid polymer barriers and cell walls, such as between the cuticle and the cell wall in green tissues, between the exine and intine in pollen, or between suberin and the cell wall in the seed coat. These different hypotheses on the function of LTPs are not necessarily contradicting each other. In the light of the variety of different LTP-types and the large number of members in the LTP family, it is possible that distinct members of the LTP family could participate as either a Transfer LTP or an Adhesion LTP. It is also possible that

both functions in transfer and adhesion could be fulfilled by singular LTPs, as already been suggested for AtLTP1.4 (24, 32). Further studies will hopefully reveal whether particular LTPs are involved in specific processes during lipid polymer deposition.

LTPs may facilitate cell expansion and plant growth

As described above, LTPs seems to have an important role in the deposition and adhesion of wax and lipid barrier polymers. However, there are also many other functions that have been linked to LTPs, such as signaling and tolerance to abiotic and biotic stress (2). Underpinning the plethora of proposed physiological functions are the more specific biochemical activities of LTPs. Nieuwland *et al* described a novel function of LTP as cell wall loosening proteins (33). In plant cells, growth by increase of volume is achieved by turgor driven expansion which is limited by the extensibility of the cell wall (for recent review see 68). The cell wall is a complex matrix consisting mainly of polysaccharide chains but also proteins. Cellulose microfibrils provide the load-bearing structure and this cellulose network is tethered together by branched polysaccharides. The structural scaffold provided by cellulose can be found throughout the plant kingdom but the cross-linking tethers vary (69). In dicotyledonous plants, the matrix in which cellulose is embedded consists of essentially xyloglucans and pectins. Although historically xyloglucan was thought to be the main cross-link involved in cell wall strength and extensibility, it has become clear that the pectin matrix plays also a critical role (70-73). Nevertheless, as turgor pressure remains constant, the expansion of the cell depends on the rigidity of the cell wall. Upon growth, cells actively decrease the rigidity by cell wall loosening which causes the wall polymers to slide along each other. The expansin protein family was shown to have cell wall loosening activity but it is important to note that cell wall loosening by expansins is not achieved by conventional enzymatic activity (74).

As a beta-expansin was expressed in the tobacco stigma, it was not a surprise to find that cell wall loosening activity was detected in stigma exudate. However, after purification of the protein with wall loosening activity it was identified as a LTP (33). Although expansins and LTPs do not share sequence similarity, it is possible that they have the same effect on the cell wall matrix. The availability of the hydrophobic cavity of this tobacco LTP was shown to be essential for cell wall loosening *in vitro* and the activity could be shown on an artificial matrix of cell wall material from bacterial cellulose and xyloglucan indicating it acts on the interface between those polysaccharides, similarly again to expansins (75). Further analysis and numerical simulation of cellulose-xyloglucan network extension with or without LTPs

revealed that this system shows slow glassy dynamics which depends on the weakening of xyloglucan-cellulose interactions (76). Besides the initial discovery of LTPs as cell wall loosening proteins, no further work directly on cell wall dynamics has since been published. However, indirect data does support a role of LTPs in plant growth. Proteomic and transcriptomic experiments have revealed LTPs spatially and temporally associated with plant growth. For example in a genome-wide transcriptome analysis of growth in *Populus trichocarpa*, LTP transcripts correlated with stem development (77). In maize transcripts of elongating internodes were compared to non-elongating internodes and LTPs were found to be expressed preferentially in the elongating internodes (78). In an over-expression experiment of BELLRINGER, a homeodomain transcription factor involved in development and growth, only the expression of genes associated with the cell wall were changed including several LTPs (79). Furthermore, the transcriptional regulator MED/PFT1 in Arabidopsis was shown to regulate cell wall gene expression including two LTPs (80). In a meta-analysis of cell wall proteomics data, LTPs were found to be a significant part of the protein population (81).

Although there is an overlap between LTP expression and plant growth which fits with a function of cell wall loosening, it could also fit the more traditional function of cuticle deposition. However, the cuticle does not represent the majority of the organs used for in cell wall proteomics approaches in Arabidopsis (81), although this is not true for other studies (to exemplify 82). Taken together and based on gene expression and phenotypic analyses, it is clear that LTPs are associated with plant growth. It is possible that LTPs could function in growth through both cell wall loosening and lipid deposition. It remains unclear if different LTPs have different or identical biochemical functions at the same time. Further experiments focusing on dissecting the two biochemical functions would be required to answer that question.

Structural plasticity of LTPs

The 3D-structure of a protein is a key to unlock the secrets and reveal their biological functions. In case of the LTPs, the first 3D structures of LTPs were presented in the early 1990s, still we are not entirely confident about their activities. Nevertheless, a large number of papers describing the structural or ligand binding properties of LTPs have been published thereafter (recently reviewed in 2). To exemplify, in wheat LTP1 TaLTP1.1 four helices are linked together by flexible loops (3). The helices are packed against the unstructured C-terminal part, which is

stabilized by a hydrogen bond to helix 3 (Fig. 1). Four disulfide bridges are formed by the eight Cys in the 8CM to stabilize the fold of the protein. Both the N-terminal end of helix 1 (H1) and the C-terminal part are linked to helix 3 (H3) by disulfide bridges (labeled 1 and 4 in Fig. 1), respectively. The position of helix 2 (H2) is stabilized by two disulfide bonds; one of them links the N-terminal part of H2 to the C-terminal part of H1 and the other one links H2 to helix 4 (H4) (bridges 2 and 3 in Fig.1). The central hydrophobic cleft is formed by the residues from H1 (Val10, Leu14), H2 (Val31, Leu34, Ala38), H3 (Ala47, Leu51, Ala54), and loop H3-H4 (Ile58), H4 (Ile69), and from the C-terminal part (Leu77, Tyr79 and Ile81) (Fig. 1).

The crystal structure of a TaLTP1.1:lyso-myristoyl-phosphatidylcholine (LMPC) complex (15; PDB ID 2BWO) showed that TaLTP1.1 can accommodate two molecules of LMPC (Fig. 2) positioned head to tail. The aliphatic chains are positioned inside the cavity, while the polar head groups are directed towards the solvent areas, at each end of the tunnel. One of the bound LMPC ligands (in site 1) contacts wheat TaLTP1.1 via hydrophobic interactions and through a hydrogen bond with the side chain hydroxyl of Tyr79, whereas the other LMPC (in site 2) is only involved in few hydrophobic interactions. In the solution structure of a TaLTP1.1:Prostaglandin B2 (PGB₂) complex, the C-terminal part, which in the unliganded form makes contact with the H4 helix, moves outward (3, 83 (PDB ID 1CZ2)). The interaction induces a 100° rotation around the C β -C γ bond of the Tyr79 ring. This rotation facilitates the formation of a hydrogen bond between the carboxyl group of the ligand and the hydroxyl group of Tyr79 (Fig 2). In addition, several hydrophobic residues lining the internal cavity are pushed away by the ligand, which result in that the volume of the cavity increases from $300 \pm 50 \text{ \AA}^3$ in the unliganded protein to $786 \pm 43 \text{ \AA}^3$ in the TaLTP1.1:PGB₂ complex (83). Also in the TaLTP1.1:1,2-dimyristoyl phosphatidylglycerol (DMPG) complex, as assayed with ¹H NMR and fluorescence spectroscopy, both acyl chains are accommodated into the hydrophobic cavity. The only structural alteration induced by DMPG is seen in the C-terminal part of the structure where the aromatic ring of Tyr79 is moved outwards into the solvent, which exclude formation of hydrogen bonds between DMPG and TaLTP1.1. The volume of the cavity was estimated to increase to $750 \text{ \AA}^3 \pm 250 \text{ \AA}^3$ when occupied by the two acyl chains.

The swelling of the cavity is the result from conformational changes in the unstructured C-terminal end of the protein. In particular, Tyr79 located close to the opening of the cavity is a key residue that may act as a gate keeper controlling the shape, size and binding capacity of the hydrophobic cavity. Curiously, some ligands, as determined for DMPG, cause a shift in the

orientation that move the aromatic ring of Tyr79 outwards to the solvent to exclude the formation of hydrogen bonds between Tyr79 and the ligand, while other ligands, as demonstrated for PGB₂ seems to adjust the orientation of Tyr79 to allow for the formation of bonds to the ligand. Nevertheless, the properties of the ligand clearly influence the 3D structure of and around the cavity in the LTP. Similarly, in the maize LTP1 ZmLTP1.6, Tyr81 is often displaced from the cavity upon binding of a ligand to the protein (4, 16), as well as Tyr79 in rice OsLTP1.18 (17), and Tyr79 in NtLTP1.1 from *Nicotiana tabacum* (84), just to mention some additional examples.

Summary

The research on plant LTPs has now formed a solid ground with a wealth of information available regarding 3D-structures, lipid binding and expression patterns. More and more results are also accumulating from phenotypic investigations of knock-out or knock-down plants. When the current LTP research is reviewed and summarized, a role of many LTPs in the deposition lipid-based surface barriers seems more and more likely. However, this is not excluding that some LTPs also could have other biological functions to fulfill in plant, such as in signaling or pathogen defence. Still though, we cannot precisely describe the function and activity of the LTPs in lipid barrier formation. Here, we have suggested that LTPs are facilitating the extracellular transfer of barrier materials and/or are involved in enhancing the adhesion between the lipid based barriers and other extracellular materials, such as the cell wall or the intine layer in pollen grains (Fig. 3). Nevertheless, we need to learn more about the details surrounding the mechanisms of the LTP activities to truly pinpoint their different roles in the plants.

Thus, it is certainly a good time to advance forward with more exciting experiments on these intriguing and fascinating plant proteins. There are many challenges involved in LTP research, such as the complex gene families that potentially result in a high degree of gene redundancy, low sequence conservation, low specificity of the ligand:LTP interactions and the complex nature of the lipid polymer synthesis. Anyway, with systematic approaches it will definitely be possible to significantly advance our knowledge in a few years from now. Mosses and liverworts are emerging as models for studies on the assembly, function and evolution of the plant cuticle (31, 85, 86). Since the number of LTP genes is much lower in bryophytes compared to vascular plants (2, 7), suggesting less redundancy, it may be useful to apply bryophyte model systems to investigate the function of LTPs in lipid barrier biosynthesis. Another way forward

could be to use sophisticated microscopy to trace the movements of LTPs in living cells. There is also a need for identifying *in vivo* interaction partners, such as lipids, carbohydrates or proteins. Anyway, we look forward to enter a very productive and exciting period for the LTP research.

Acknowledgements

We are very grateful for the support of our research from Carl Tryggers Stiftelse för Vetenskaplig Forskning. Use of Biocenter Finland infrastructure at Åbo Akademi University (bioinformatics, structural biology, and translational activities) is acknowledged.

References

1. Kader, J. C. 1996. Lipid-transfer proteins in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**: 627-654.
2. Salminen, T. A., K. Blomqvist, and J. Edqvist. 2016. Lipid transfer proteins: classification, nomenclature, structure, and function. *Planta* **244**, 971-997.
3. Gincel, E., J. P. Simorre, A. Caille, D. Marion, M. Ptak, and F. Vovelle. 1994. Three-dimensional structure in solution of a wheat lipid-transfer protein from multidimensional ¹H-NMR data. A new folding for lipid carriers. *Eur. J. Biochem.* **226**: 413-422.
4. Shin, D. H., J. Y. Lee, K. Y. Hwang, K. K. Kim, and S. W. Suh. 1995. High-resolution crystal structure of the non-specific lipid-transfer protein from maize seedlings. *Structure* **3**:189-199.
5. Lindorff-Larsen, K., and J. R. Winther. 2001. Surprisingly high stability of barley lipid transfer protein, LTP1, towards denaturant, heat and proteases. *FEBS Lett.* **488**: 145-148.
6. Berecz, B., E. N. Mills, L. Tamás, F. Láng, P. R. Shewry, and A.R. Mackie. 2010. Structural stability and surface activity of sunflower 2S albumins and nonspecific lipid transfer protein. *J. Agric. Food Chem.* **58**: 6490-6497.
7. Edstam, M. M., L. Viitanen, L., T. A. Salminen, and J. Edqvist. 2011. Evolutionary history of the non-specific lipid transfer proteins. *Mol. Plant* **4**: 947-964.
8. Boutrot, F., N. Chantret, and M. F. Gautier. 2008. Genome-wide analysis of the rice and arabidopsis *non-specific lipid transfer protein (nsLtp)* gene families and identification of wheat *nsLtp* genes by EST data mining. *BMC Genomics* **9**: 86.
9. Li, J., G. Gao, K. Xu, B. Chen, G. Yan, F. Li, J. Qiao, T. Zhang, and X. Wu. 2014. Genome-wide survey and expression analysis of the putative non-specific lipid transfer proteins in *Brassica rapa* L. *PLoS One* **9**: e84556.
10. Wei, K., and X. Zhong. 2014. Non-specific lipid transfer proteins in maize. *BMC Plant Biol.* **14**: 281.
11. Li, F., K. Fan, F. Ma, E. Yue, N. Bibi, M. Wang, H. Shen, M. M. Hasan, and X. Wang. 2016. Genomic identification and comparative expansion analysis of the *non-specific lipid transfer protein* gene family in *Gossypium*. *Sci. Rep.* **6**: 38948.
12. Edstam, M. M., M. Laurila, A. Höglund, A. Raman, K. M. Dahlström, T. A. Salminen, J. Edqvist, and K. Blomqvist. 2014. Characterization of the GPI-anchored lipid transfer proteins in the moss *Physcomitrella patens*. *Plant Physiol. Biochem.* **75**: 55-69.

13. Sodano, P., A. Caille, D. Sy, G. de Person, D. Marion, and M. Ptak. 1997. ^1H NMR and fluorescence studies of the complexation of DMPG by wheat non-specific lipid transfer protein. Global fold of the complex. *FEBS Lett.* **416**: 130-134.
14. Lerche, M. H., B. B. Kragelund, L. M. Bech, and F. M. Poulsen. 1997. Barley lipid-transfer protein complexed with palmitoyl CoA: the structure reveals a hydrophobic binding site that can expand to fit both large and small lipid-like ligands. *Structure* **5**: 291-306.
15. Charvolin, D., J. P. Douliez, D. Marion, C. Cohen-Addad, and E. Pebay-Peyroula. 1999. The crystal structure of a wheat nonspecific lipid transfer protein (ns-LTP1) complexed with two molecules of phospholipid at 2.1 Å resolution. *Eur J Biochem* **264**: 562-568.
16. Han, G. W., J. Y. Lee, H. K. Song, C. Chang, K. Min, J. Moon, D. H. Shin, M. L. Kopka, M. R. Sawaya, H. S. Yuan, T. D. Kim, J. Choe, D. Lim, H. J. Moon, and S. W. Suh. 2001. Structural basis of non-specific lipid binding in maize lipid-transfer protein complexes revealed by high-resolution X-ray crystallography. *J. Mol. Biol.* **308**: 263-278.
17. Cheng, H.C., P. T. Cheng, P. Peng, P. C. Lyu, and Y. J. Sun. 2004. Lipid binding in rice nonspecific lipid transfer protein-1 complexes from *Oryza sativa*. *Protein Sci.* **13**: 2304-2315.
18. Zachowski, A., F. Guerbette, M. Grosbois, A. Jolliot-Croquin, and J. C. Kader. 1998. Characterisation of acyl binding by a plant lipid-transfer protein. *Eur. J. Biochem.* **257**: 443-448.
19. Douliez, J. P., T. Michon, and D. Marion. 2000. Steady-state tyrosine fluorescence to study the lipid-binding properties of a wheat non-specific lipid-transfer protein (nsLTP1). *Biochim. Biophys. Acta* **1467**: 65-72.
20. Lascombe M. B., B. Bakan, N. Buhot, D. Marion, J. P. Blein, V. Larue, C. Lamb, and T. Prange. 2008. The structure of "defective in induced resistance" protein of *Arabidopsis thaliana*, DIR1, reveals a new type of lipid transfer protein. *Protein Sci.* **17**: 1522-1530.
21. Shenkarev, Z. O., D. N. Melnikova, E. I. Finkina, S. V. Sukhanov, I. A. Boldyrev, A. K. Gizatullina, K. S. Mineev, A. S. Arseniev, and T. V. Ovchinnikova. 2017. Ligand binding properties of the lentil lipid transfer protein: Molecular insight into the possible mechanism of lipid uptake. *Biochemistry* **56**: 1785-1796.
22. Cheng, C. S., D. Samuel, Y. J. Liu, J. C. Shyu, S. M. Lai, K. F. Lin, and P. C. Lyu. 2004. Binding mechanism of nonspecific lipid transfer proteins and their role in plant defense. *Biochemistry* **43**: 13628-13636.

23. Sawano, Y., K. Hatano, T. Miyakawa, H. Komagata, Y. Miyauchi, H. Yamazaki, and M. Tanokura. 2008. Proteinase inhibitor from ginkgo seeds is a member of the plant nonspecific lipid transfer protein gene family. *Plant Physiol.* **146**: 1909-1919.
24. Deeken, R., S. Saupe, J. Klinkenberg, M. Riedel, J. Leide, R. Hedrich, and T. D. Mueller. 2016. The nonspecific lipid transfer protein AtLtpI-4 is involved in suberin formation of *Arabidopsis thaliana* crown galls. *Plant Physiol.* **172**: 1911-1927.
25. Cubells-Baeza, N., C. Gómez-Casado, L. Tordesillas, C. Ramírez-Castillejo, M. Garrido-Arandia, P. González-Melendi, M. Herrero, L. F. Pacios, and A. Díaz-Perales. 2017. Identification of the ligand of Pru p 3, a peach LTP. *Plant Mol Biol.* **94**: 33-44.
26. Debono, A., T. H. Yeats, J. K. Rose, D. Bird, R. Jetter, L. Kunst, and L. Samuels. 2009. Arabidopsis LTPG is a glycosylphosphatidylinositol-anchored lipid transfer protein required for export of lipids to the plant surface. *Plant Cell* **21**, 1230-1238.
27. Lee, S. B.; Y. S. Go, H. J. Bae, J. H. Park, S. H. Cho, H. J. Cho, D. S. Lee, O. K. Park, I. Hwang, and M. Suh. 2009. Disruption of glycosylphosphatidylinositol-anchored lipid transfer protein gene altered cuticular lipid composition, increased plastoglobules, and enhanced susceptibility to infection by the fungal pathogen *Alternaria brassicicola*. *Plant Physiol.* **150**, 42-54.
28. Zhang, D., W. Liang, C. Yin, J. Zong, F. Gu, and D. Zhang. 2010. *OsC6*, encoding a lipid transfer protein, is required for postmeiotic anther development in rice. *Plant Physiol* **154**: 149-162.
29. Kim, H., S. B. Lee, H. J. Kim, M. K. Min, I. Hwang, and M. C. Suh. 2012. Characterization of glycosylphosphatidylinositol-anchored lipid transfer protein 2 (LTPG2) and overlapping function between LTPG/LTPG1 and LTPG2 in cuticular wax export or accumulation in *Arabidopsis thaliana*. *Plant Cell Physiol.* **53**, 1391-1403.
30. Edstam, M. M., K. Blomqvist, A. Eklöf, U. Wennergren, and J. Edqvist, 2013. Coexpression patterns indicate that GPI-anchored non-specific lipid transfer proteins are involved in accumulation of cuticular wax, suberin and sporopollenin. *Plant Mol. Biol.* **83**: 625-649.
31. Edstam, M. M., and J. Edqvist. 2014. Involvement of GPI-anchored lipid transfer proteins in the development of seed coats and pollen in *Arabidopsis thaliana*. *Physiol. Plant.* **152**: 32-42.
32. Jacq, A., C. Pernot, Y. Martinez, F. Domergue, B. Payré, E. Jamet, V. Burlat, and V. B. Pacquit. 2017. The *Arabidopsis* Lipid Transfer Protein 2 (AtLTP2) is involved in

- cuticle-cell wall interface integrity and in etiolated hypocotyl permeability. *Front. Plant Sci.* **8**: 263.
33. Nieuwland, J., R. Feron, B. A. Huisman, A. Fasolino, C. W. Hilbers, J. Derksen, and C. Mariani. 2005. Lipid transfer proteins enhance cell wall extension in tobacco. *Plant Cell* **17**: 2009-2019.
34. Maldonado, A. M., P. Doerner, R. A. Dixon, C. J. Lamb, and R. K. Cameron. 2002. A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis*. *Nature* **419**: 399-403.
35. Champigny, M. J., M. Isaacs, P. Carella, J. Faubert, P. R. Fobert, and R. K. Cameron. 2013. Long distance movement of DIR1 and investigation of the role of DIR1-like during systemic acquired resistance in *Arabidopsis*. *Front Plant Sci* **4**: 230.
36. Jang, C. S., J. W. Johnson, and Y. W. Seo. 2005. Differential expression of *TaLTP3* and *TaCOMT1* induced by Hessian fly larval infestation in a wheat line possessing *H21* resistance gene. *Plant Sci.* **168**: 1319-1326.
37. Sun, J.Y., D. A. Gaudet, Z. X. Lu, M. Frick, B. Puchalski, and A. Laroche. 2008. Characterization and antifungal properties of wheat nonspecific lipid transfer proteins. *Mol. Plant Microbe Interact.* **21**: 346-360.
38. Zhu, X., Z. Li, H. Xu, M. Zhou, L. Du, and Z. Zhang. 2012. Overexpression of wheat lipid transfer protein gene *TaLTP5* increases resistances to *Cochliobolus sativus* and *Fusarium graminearum* in transgenic wheat. *Funct Integr Genomics* **12**: 481-488.
39. Schweiger, W., B. Steiner, C. Ametz, G. Siegwart, G. Wiesenberger, F. Berthiller, M. Lemmens, H. Jia, G. Adam, G. J. Muehlbauer, D. P. Kreil, and H. Buerstmayr. 2013. Transcriptomic characterization of two major *Fusarium* resistance quantitative trait loci (QTLs), *Fhb1* and *Qfhs.ifa-5A*, identifies novel candidate genes. *Mol. Plant Pathol.* **14**: 772-785.
40. Gangadhar, B. H., K. Sajeesh, J. Venkatesh, V. Baskar, K. Abhinandan, J. W. Yu, R. Prasad, and R. K. Mishra. 2017. Enhanced tolerance of transgenic potato plants over-expressing non-specific Lipid Transfer Protein-1 (*StmsLTP1*) against multiple abiotic stresses. *Front. Plant Sci.* **7**:1228.
41. Guo, C., X. Ge, and H. Ma. 2013. The rice *OsDIL* gene plays a role in drought tolerance at vegetative and reproductive stages. *Plant Mol. Biol.* **82**: 239-253.
42. Guo, L., H. Yang, X. Zhang, and S. Yang. 2013. *Lipid transfer protein 3* as a target of MYB96 mediates freezing and drought stress in *Arabidopsis*. *J. Exp. Bot.* **64**: 1755-1767.

43. Yu, G., W. Hou, X. Du, L. Wang, H. Wu, L. Zhao, L. Kong, and H. Wang. 2014. Identification of wheat non-specific lipid transfer proteins involved in chilling tolerance. *Plant Cell Rep.* **33**: 1757-1766.
44. Gonzalez, L.E., K. Keller, K. X. Chan, M. M. Gessel, and B. C. Thines. 2017. Transcriptome analysis uncovers *Arabidopsis F-BOX STRESS INDUCED 1* as a regulator of jasmonic acid and abscisic acid stress gene expression. *BMC Genomics* **18**: 533.
45. König, K., M. J. Vaseghi, A. Dreyer, A., and K. J. Dietz. 2017. The significance of glutathione and ascorbate in modulating the retrograde high light response in *Arabidopsis thaliana* leaves. *Physiol. Plant.* doi: 10.1111/ppl.12644.
46. Fernández-Rivas, M., E. González-Mancebo, R. Rodríguez-Pérez, C. Benito, R. Sánchez-Monge, G. Salcedo, M. D. Alonso, A. Rosado, M. A. Tejedor, C. Vila and M. L. Casas. 2003. Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *J. Allergy Clin. Immunol* **112**:789-795.
47. Asero, R., M. Piantanida, E. Pinter, and V. Pravettoni. 2018. The clinical relevance of lipid transfer protein. *Clin. Exp. Allergy* **48**: 6-12.
48. Salcedo, G., R. Sánchez-Monge, D. Barber, and A. Díaz-Perales. 2007. Plant non-specific lipid transfer proteins: an interface between plant defence and human allergy. *Biochim. Biophys. Acta* **1771**: 781-791.
49. Egger, M., M. Hauser, A. Mari, F. Ferreira, and G. Gadermaier. 2010. The role of lipid transfer proteins in allergic diseases. *Curr. Allergy Asthma Rep.* **10**: 326-335.
50. Van Winkle, R. C., and C. Chang. 2014. The biochemical basis and clinical evidence of food allergy due to lipid transfer proteins: a comprehensive review. *Clin. Rev. Allergy Immunol.* **46**: 211-224.
51. Wellman, C. H, P. L. Osterloff, and U. Mohiuddin, U. 2003. Fragments of the earliest land plants. *Nature* **425**: 282-285.
52. Ranathunge, K., L. Schreiber, and R. Franke. 2011. Suberin research in the genomics era-- new interest for an old polymer. *Plant Sci.* **180**: 399-413.
53. Samuels, L., L. Kunst, and R. Jetter. 2008. Sealing plant surfaces: cuticular wax formation by epidermal cells. *Annu. Rev. Plant Biol.* **59**: 683-707.
54. Javelle, M., V. Vernoud, P. M. Rogowsky, and G. C. Ingram. 2011. Epidermis: the formation and functions of a fundamental plant tissue. *New Phytol.* **189**: 17-39.
55. Fich, E. A., N. A. Segerson, and J. K. Rose. 2016. The plant polyester cutin: Biosynthesis, structure, and biological roles. *Annu. Rev. Plant Biol.* **67**: 207-233.

56. Ariizumi, T., and K. Toriyama. 2011. Genetic regulation of sporopollenin synthesis and pollen exine development. *Annu. Rev. Plant Biol.* **62**: 437-460.
57. Cohen, H., J. Szymanski, A. Aharoni, and E. Dominguez. 2017. Assimilation of 'omics' strategies to study the cuticle layer and suberin lamellae in plants. *J. Exp. Bot.* **68**: 5389-5400.
58. Pighin, J. A., H. Zheng, L. J. Balakshin, I. P. Goodman, T. L. Western, R. Jetter, L. Kunst, and A. L. Samuels. 2004. Plant cuticular lipid export requires an ABC transporter. *Science* **306**: 702-704.
59. Choi, H., J. Y. Jin, S. Choi, J. U. Hwang, Y. Y. Kim, M. C. Suh, and Y. Lee. 2011. An ABCG/WBC-type ABC transporter is essential for transport of sporopollenin precursors for exine formation in developing pollen. *Plant J.* **65**: 181-193.
60. Landgraf, R., U. Smolka, S. Altmann, L. Eschen-Lippold, M. Senning, S. Sonnewald, B. Weigel, N. Frolova, N. Strehmel, G. Hause, D. Scheelm, C. Böttcher, and S. Rosahl. 2014. The ABC transporter ABCG1 is required for suberin formation in potato tuber periderm. *Plant Cell* **26**: 3403-3415.
61. Fabre, G., I. Garroum, S. Mazurek, J. Daraspe, A. Mucciolo, M. Sankar, B. M. Humbel, and C. Nawrath. 2016. The ABCG transporter PEC1/ABCG32 is required for the formation of the developing leaf cuticle in Arabidopsis. *New Phytol.* **209**: 192-201.
62. Shepherd T., and D. Wynne Griffiths. 2006. The effects of stress on plant cuticular waxes. *New Phytol* **171**, 469-499.
63. Liu, F., X. Xiong, L. Wu, D. Fu, A. Hayward, X. Zeng, Y. Cao, Y. Wu, Y. Li, and G. Wu. 2014. *BraLTP1*, a lipid transfer protein gene involved in epicuticular wax deposition, cell proliferation and flower development in *Brassica napus*. *PLoS One* **9**: e110272.
64. Brown, G.D. 2010. The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao). *Molecules* **15**: 7603-7698.
65. Wang, B., A. B. Kashkooli, A. Sallets, H. M. Ting, N. C. A. de Ruijter, L. Olofsson, P. Brodelius, M. Pottier, M. Boutry, H. Bouwmeester, H., and A. R. van der Krol. 2016. Transient production of artemisinin in *Nicotiana benthamiana* is boosted by a specific lipid transfer protein from *A. annua*. *Metab. Eng.* **38**: 159-169.
66. Huang, M.D., T. L. Chen, and A. H. Huang. 2013. Abundant type III lipid transfer proteins in Arabidopsis tapetum are secreted to the locule and become a constituent of the pollen exine. *Plant Physiol.* **163**: 1218-1229.

67. Vishwanath, S. J., C. Delude, F. Domergue and O. Rowland. 2015. Suberin: biosynthesis, regulation, and polymer assembly of a protective extracellular barrier. *Plant Cell Rep.* **34**: 573-586
68. Chebli, Y., and A. Geitmann. 2017. Cellular growth in plants requires regulation of cell wall biochemistry Introduction: plant cell growth. *Curr Opin Cell Biol* **44**: 28–35.
69. Doblin, M. S., F. Pettolino, and A. Bacic. 2010. Evans Review: Plant cell walls: The skeleton of the plant world. *Funct. Plant Biol.* **37**: 357–381.
70. Cavalier, D. M., O. Lerouxel, L. Neumetzler, K. Yamauchi, A. Reinecke, G. Freshour, O. A. Zabolina, M. G. Hahn, I. Burgert, M. Pauly, N. V. Raikhel, and K. Keegstra. 2008. Disrupting two *Arabidopsis thaliana* xylosyltransferase genes results in plants deficient in xyloglucan, a major primary cell wall component. *Plant Cell* **20**: 1519–1537.
71. Park, Y. B., and D. J. Cosgrove. 2012. A revised architecture of primary cell walls based on biomechanical changes induced by substrate-specific endoglucanases. *Plant Physiol.* **158**: 1933–1943.
72. Park, Y. B., and D.J: Cosgrove. 2012. Changes in cell wall biomechanical properties in the xyloglucan-deficient *xxt1/xxt2* mutant of *Arabidopsis*. *Plant Physiol.* **158**: 465–475.
73. Tan, L., S. Eberhard, S. Pattathil, C. Warder, J. Glushka, C. Yuan, Z. Hao, X Zhu, U Avci, J. S. Miller, D. Baldwin, C. Pham, R. Orlando, A: Darvill, M. G. Hahn, M. J. Kieliszewski MJ, and D. Mohnen. 2013. An *Arabidopsis* cell wall proteoglycan consists of pectin and arabinoxylan covalently linked to an arabinogalactan protein. *Plant Cell* **25**: 270–287.
74. Cosgrove D. J. 2000. Loosening of plant cell walls bx expansins. *Nature* **407**: 321–326.
75. Whitney, S. E. C., M. .J. Gidley and S.J. McQueen-Mason. 2000. Probing expansin action using cellulose / hemicellulose composites. *Plant J.* **22**: 327-34
76. Manyuhina, O. V., A. Fasolino, and M. I. Katsnelson. 2007. Slow dynamics in a model of the cellulose network. *Polymer* **48**: 4849–4854.
77. Dharmawardhana, P., A. M. Brunner, and S. H. Strauss. 2010. Genome-wide transcriptome analysis of the transition from primary to secondary stem development in *Populus trichocarpa*. *BMC Genomics* **11**: 150.
78. Bosch, M., C. D. Mayer, A. Cookson and I. S. Donnison. 2011. Identification of genes involved in cell wall biogenesis in grasses by differential gene expression profiling of elongating and non-elongating maize internodes. *J. Exp. Bot.* **62**: 3545–3561.

79. Etchells, J. P., L. Moore, W. Z. Jiang, H. Prescott, R. Capper, N. J. Saunders, A. M. Bhatt, and H. G. Dickinson. 2012. A role for *BELLRINGER* in cell wall development is supported by loss-of-function phenotypes. *BMC Plant Biol.* **12**: 212.
80. Seguela-Arnaud, M., C. Smith, M. C. Uribe, S. May, H. Fischl, N. McKenzie, and M. W. Bevan. 2015. The Mediator complex subunits MED25/PFT1 and MED8 are required for transcriptional responses to changes in cell wall arabinose composition and glucose treatment in *Arabidopsis thaliana*. *BMC Plant Biol.* **15**: 215.
81. Albenne, C., H. Canut, and E. Jamet. 2013. Plant cell wall proteomics: the leadership of *Arabidopsis thaliana*. *Front. Plant Sci.* **4**: 111.
82. Calderan-Rodrigues, M. J., E. Jamet, T. Douché, M. B. R. Bonassi, T. R. Cataldi, J.G. Fonseca, H. San Clemente, R. Pont-Lezica, and C.A. Labate 2016. Cell wall proteome of sugarcane stems: comparison of a destructive and a non-destructive extraction method showed differences in glycoside hydrolases and peroxidases. *BMC Plant Biol* **16**: 14.
83. Tassin-Moindrot, S., A. Caille, J. P. Douliez, D. Marion, and F. Vovelle. 2000. The wide binding properties of a wheat nonspecific lipid transfer protein. Solution structure of a complex with prostaglandin B2. *Eur. J. Biochem.* **267**: 1117-1124.
84. Da Silva, P., C. Landon, B. Industri, A. Marais, D. Marion, M. Ponchet, and F. Vovelle. (2005) Solution structure of a tobacco lipid transfer protein exhibiting new biophysical and biological features. *Proteins* **59**: 356-367
85. Buda, G. J., W. J. Barnes, E. A. Fich, S. Park, T. H. Yeats, L. Zhao, D. S. Domozych, and J. K. Rose. 2013. An ATP binding cassette transporter is required for cuticular wax deposition and desiccation tolerance in the moss *Physcomitrella patens*. *Plant Cell* **25**: 4000-4013.
86. Renault, H, A. Alber, N. A. Horst, A. Basilio Lopes; E. A. Fich, L. Kriegshauser, G. Wiedemann, P. Ullmann, L. Herrgott, M. Erhardt, E. Pineau, J. Ehlting, M. Schmitt, M., J. K. Rose, R. Reski, R., and D. Werck-Reichhart. 2017. A phenol-enriched cuticle is ancestral to lignin evolution in land plants. *Nat. Commun.* **8**: 14713.

Figure 1

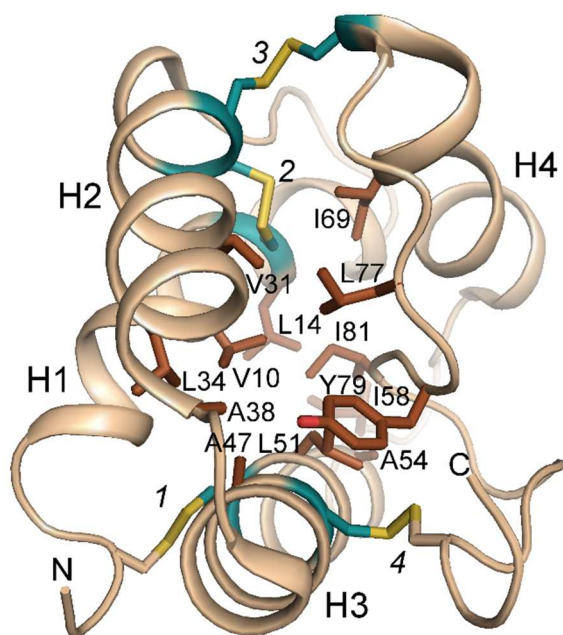


Figure 1. The 3D fold of wheat LTP1 (renamed TaLTP1.1 in 2) structure (PDB ID 1GH1; 3). The four helices are stabilized by four disulfide bridges (green sticks) of which the 1st and 4th bond connect the N- and C-terminal parts to helix 3 (H3), respectively. The 2nd and 3rd bond link helix 2 (H2) to helix 1 (H1) and helix 4 (H4). The hydrophobic central cavity of the LTP1 structure is formed by residues (brown sticks) from each helix and the unstructured C-terminal part.

Figure 2

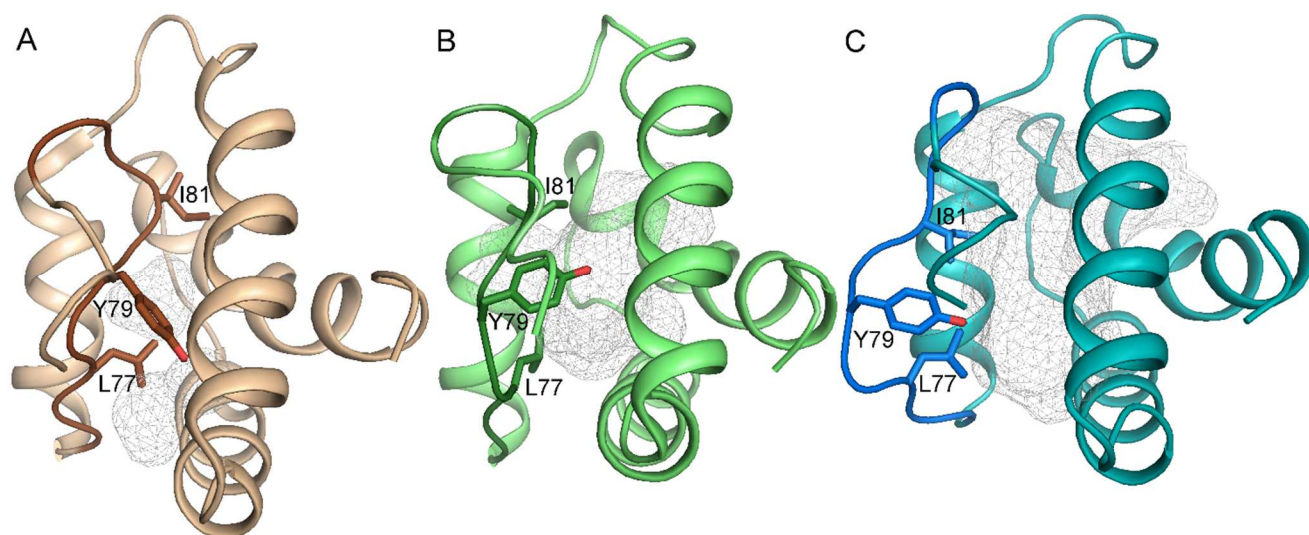


Figure 2. The size and shape of the ligand binding cavity of wheat LTP1 (renamed TaLTP1.1 in 2) without a ligand (PDB ID 1GH1; 3) (A), complexed with prostaglandin B2 (PGB₂) (PDB ID 1CZ2; 83) (B) and complexed with two lyso-myristoyl-phosphatidylcholine (LMPC) molecules (PDB ID 2BWO; 15) (C). The size of the cavity (shown as white wireframe) is adjusted by the size of the bound ligand by the movement of the C-terminal residues shown as sticks.

Figure 3

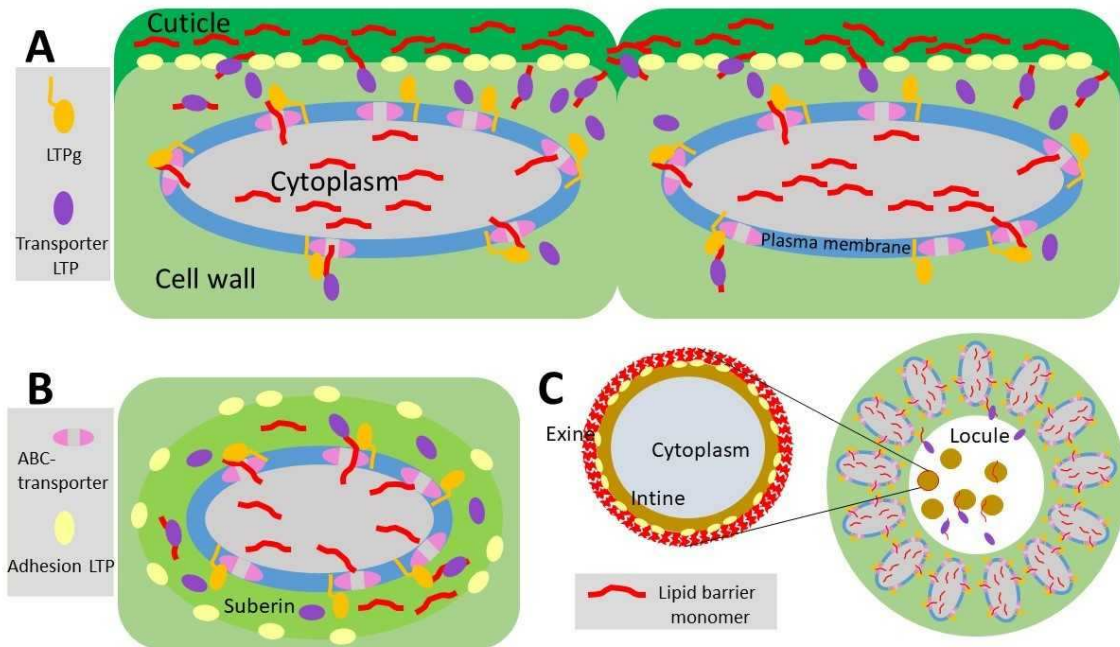


Figure 3. Putative roles for different LTPs during lipid barrier assembly in green tissues (A), in roots (B) and in pollen (C). Three different roles for LTPs in lipid barrier assembly are indicated. LTPg (orange) is attached to the plasma membrane through its GPI-anchor and dock the lipid barrier monomers when they leave the plasma membrane-localized ABC transporters. The Transporter LTPs (purple) facilitate the transfer of the lipid barrier monomers from the LTPg to the site of barrier deposition. The Adhesion LTPs (yellow) have a structural role adhering the hydrophobic barrier to the hydrophilic cell wall (A and B) or intine (C).