Review

New Phytologist

Tansley review

The making of suberin

Authors for correspondence:
Olga Serra
Email: olga.serra@udg.edu

Niko Geldner
*Email: niko.geldner@unil.ch

Received: *23 February 2022*
Accepted: *15 April 2022*

Olga Serra[1]* and Niko Geldner[2]*

[1]Laboratori del Suro, Department of Biology, University of Girona, Campus Montilivi, Girona 17003, Spain; [2]Department of Plant Molecular Biology, University of Lausanne, UNIL-Sorge, Biophore Building, Lausanne 1015, Switzerland

Contents

New Phytologist (2022) **235:** 848–866
doi: 10.1111/nph.18202

Key words: apoplastic barrier, cell wall, fatty acyl metabolism, ferulic acid, lignin, lipid intracellular transport, suberin, suberin lamellae.

Summary

Outer protective barriers of animals use a variety of bio-polymers, based on either proteins (e.g. collagens), or modified sugars (e.g. chitin). Plants, however, have come up with a particular solution, based on the polymerisation of lipid-like precursors, giving rise to cutin and suberin. Suberin is a structural lipophilic polyester of fatty acids, glycerol and some aromatics found in cell walls of phellem, endodermis, exodermis, wound tissues, abscission zones, bundle sheath and other tissues. It deposits as a hydrophobic layer between the (ligno)cellulosic primary cell wall and plasma membrane. Suberin is highly protective against biotic and abiotic stresses, shows great developmental plasticity and its chemically recalcitrant nature might assist the sequestration of atmospheric carbon by plants. The aim of this review is to integrate the rapidly accelerating genetic and cell biological discoveries of recent years with the important chemical and structural contributions obtained from very diverse organisms and tissue layers. We critically discuss the order and localisation of the enzymatic machinery synthesising the presumed substrates for export and apoplastic polymerisation. We attempt to explain observed suberin linkages by diverse enzyme activities and discuss the spatiotemporal relationship of suberin with lignin and ferulates, necessary to produce a functional suberised cell wall.

I. Introduction

Terrestrial plants are one of life's great success stories. Whilst big city dwellers among our readers might not immediately be able to convince themselves of this fact by looking out of their windows, it is nonetheless estimated that their *c.* 300 000 species form close to 80% of Earth's biomass (Bar-On *et al.*, 2018). We therefore must conclude that plants are well protected as they dominate the biosphere in such a spectacular fashion. Much can be, and has been, written about the incredible diversity of defence metabolites that prevent heterotrophic organisms, from fungi to large herbivores, of benefiting unduly from this abundant source of biomass (Farmer, 2014). However, it is evident that significant protection is

*These authors contributed equally to this work.

conferred simply through the presence of highly resistant cell-wall polymers that have evolved in the land plant lineage.

Lignin, the defining constituent of wood, has been central in the evolution of vascular plants, allowing them grow to great heights. This is due to the efficient long-distance water transport systems that lignified cells enable to form. Moreover, lignified tissues provide great mechanical resistance, allowing the spectacular, gravity-defying, fractal architectures seen in modern trees. The mechanical resistance of lignin-impregnated cellulosic walls (wood) is matched by an equally impressive resistance to chemical degradation. An initial recalcitrance of lignin to efficient biogenic degradation is thought to be at the origin of the carboniferous era, in which unfathomable amounts of lignified plant biomass were fossilised and therefore withdrawn from the pool of rapidly cycling carbon (Floudas *et al.*, 2012).

Despite these impressive characteristics of lignin, the outer tissues, those that do the job of directly protecting plants from the environment, are not made of simple, ligno-cellulosic walls. In their stead, aerial young primary organs, with a unicellular protective layer, the epidermis, form a protective surface called the cuticle. Cuticles are defined by the biopolymer cutin, a poly-aliphatic polyester of fatty acids and fatty alcohols, with an additional presence of waxes, formed of unpolymerised alkanes, fatty alcohols and other compounds (Fich *et al.*, 2016). Older, radially thickened stems (including tubers) and roots, by contrast, form a multilayered protective tissue called the phellem (part of the periderm), or (outer) bark, that contains an even more complex arrangement of polymers in their walls (Serra *et al.*, 2022). Here, lignin-like polymers are found in close association with an additional cutin-like polymer, called suberin. While representing a lesser fraction of the biomass of extant plants, compared with the lignified wood, suberised tissues were found to be even more resistant to degradation than purely lignified tissues and might represent an important source of stable soil organic matter (Vane *et al.*, 2006; Harman-Ware *et al.*, 2021). This resistance possibly explains the occurrence of suberised tissues in many protective layers throughout the plant. In addition to phellem, suberisation in outer tissues is found in seed coats, abscission zones and fruit skins, and it generally occurs as a response to wounding. Additionally, inner cell layers, such as the endodermis and exodermis or leaf bundle sheaths, also suberise. Suberin, especially reported for phellem tissues, is often accompanied by suberin-associated waxes. As in the cuticle, they are unpolymerised compounds of variable composition including fatty alcohols, fatty acids, alkanes, monoacylglycerols and some other compounds, such as sterols. In general, these suberin deposits serve to block water, nutrient and gas exchange between the plasma membrane and the apoplast, as well as to protect against penetration by intruders.

It is thought that all three cell wall polymers mentioned above – lignin, cutin and suberin – might have had their origin in a common precursor, a cutin/suberin-containing cell-wall modification with high amounts of phenolics. This would have provided the crucial resistance to desiccation that had to evolve during the initial colonisation of terrestrial habitats. In this scenario, the predominantly aliphatic polyester cutin, as well as the purely poly-aromatic lignin would represent later specialisations that became associated with specific tissue types (Renault *et al.*, 2017; Philippe *et al.*, 2020). Suberin itself however, would have maintained the association with phenolic components and aromatic, lignin-like polymers. The great physiological and ecological importance of suberisation has been covered by excellent reviews over previous years (Barberon, 2017; Campilho *et al.*, 2020; Harman-Ware *et al.*, 2021; Holbein *et al.*, 2021; Shukla & Barberon, 2021; Serra *et al.*, 2022) and will not be the focus of this review. Instead, we will treat the many fundamental aspects of suberin formation that are still insufficiently understood: biosynthesis and transport, deposition and polymerisation, and native structure. We will propose models of suberisation that attempt to integrate very diverse data from a variety of technologies, plant models and tissue types used by researchers in their quest to understand this formidable protective polymer.

II. Suberin monomeric precursor formation and transport

Any review of suberin needs to start with a definition of the term itself, as there remain divergent uses of it within the scientific community. We will use the term suberin in its strict sense, as an insoluble poly(acylglycerol) polyester, whose compounds can be released in solution by ester-breaking depolymerisation treatments. This polyester molecule contains fatty acids and their oxidised derivatives, glycerol and ferulic acid, linked by ester bonds. Other authors prefer a more extensive terminology, defining suberin as a 'heteropolymer' consisting of a suberin poly(aliphatic) domain (SPAD) (equivalent to the polyester), accompanied by a suberin poly (phenolic) domain (SPPD) (Bernards, 2002). This polyphenolic domain is made of polyaromatics, comparable in composition and coupling with lignin polymers. In our opinion, the term suberin should be used exclusively for the aliphatic polyester, providing it with a clear chemical identity. The SPDD domain, by contrast, should be defined as a lignin, or lignin-like polymer that often accompanies suberin. In this way, both polymers have a definable structure and suberin can be described by a set of dedicated chemical and histological methods (Box 1). Whilst we define suberin as a polymer independent from lignin, we acknowledge that there are extensive, functionally important connections between both polymers in the suberised cell wall, as is the case for many other cell-wall polymers (please refer to Section III.2).

1. Biosynthesis of suberin monomeric precursors

Biosynthesis of the aliphatic precursors: fatty acid and derivatives Since the discovery and characterisation of the first suberin biosynthetic gene, *GPAT5*, in 2007, a large set of enzymes has been identified that produces the major suberin aliphatic monomers, providing a robust genetic framework of suberin biosynthesis (please refer to Table 1 for genes and references). Due to their similarity in chemical nature, suberin and cutin biosynthesis requires similar enzymatic functions. Biosynthesis of suberin monomers (Fig. 1) is initiated using long-chain (C16:0, C18:0 and C18:1) fatty acid (LCFA) precursors, biosynthesised in plastids, which are thought to be exported by a mechanism called vectorial acylation: the transport across the lipid bilayer is

Box 1 The many ways to look at suberin.

Like the prisoners in Plato's cave, we only have indirect and biased views on the actual, *in vivo* structure of suberin, due to the inherent limitations of the very different approaches used to visualise and analyse suberin. Already in Krömer's foundational work on suberised tissues more than a century ago, the view of the nature of suberin was based on a synthesis of data obtained from microscopical stains and 'micro-chemical' analyses (Krömer, 1903). At the time, suberin was defined as a cell-wall structure resisting oxidative and acidic treatments. However, it could be degraded by alkaline treatments, releasing 'fatty substances', despite the fact that the structure itself could not be dissolved in hydrophobic solvents before those treatments. When properly executed, these methods allowed the discrimination of suberised from lignified tissues, as the latter did not resist strong oxidative treatments, for example. The problem of clearly delineating lignified from suberised tissues with histological stains is topic of Krömer's entire first chapter, and is a problem that persists until now.

To date, a comprehensive view of suberin needs to integrate profoundly different perspectives, provided by highly diverse techniques. Whilst, microscopy techniques provide high spatial resolution, they offer little to no chemical information. By contrast, chemical analysis of suberin depolymerisation products provides detailed monomeric information, but offers little to no spatial resolution because it requires destruction of organ/tissue architecture in order to extract the suberin. Characterisation of suberin mutants in Arabidopsis combines microscopy techniques and depolymerisation analysis of suberised tissues. However, advanced chemical analyses, such as two-dimensional nuclear magnetic resonance (2D-NMR) or partial depolymerisation, have not been done in Arabidopsis. These analyses are biased toward phellem tissues in plant species where they can be purely isolated and provide high amounts of suberised material, however are either genetically more difficult to work with (i.e. potato) or completely intractable (i.e. cork oak).

In situ visualisation techniques

Today, Krömer's preferred Sudan dyes are still in use, but these colorimetric stains are now mostly replaced by fluorescent dyes, most notably by Fluorol Yellow (FY) (Brundrett *et al*., 1991; Lux *et al*., 2005). The reliability of FY as a suberin stain has been clearly established in works on Arabidopsis that demonstrated that FY sensitively reported increases or decreases of suberin, induced by hormonal treatments or genetic manipulations. It was also robustly correlated with the expression of reporters based on suberin biosynthetic genes (Naseer *et al*., 2012; Barberon *et al*., 2016; Andersen *et al*., 2021). Very recently, FY staining has been applied to previously fixed and ClearSee-treated samples, allowing confocal imaging of whole-mount sections while preserving the fluorescence of genetically encoded fluorophores, such as GFP, and co-staining with other compatible dyes such as Basic Fuchsin for lignin or Calcofluor White for polysaccharides (Kurihara *et al*., 2015; Ursache *et al*., 2018; Sexauer *et al*., 2021). Another fluorescent suberin dye, Nile Red, is also compatible with ClearSee, but appears to only stain older, more mature suberin in the Arabidopsis endodermis, compared with FY. Whilst the basis of these differences is not understood, they point to a suberin maturation processes that cannot currently be visualised by other methods. Due to the hydrophobic nature of suberin, true live staining will remain highly challenging and *in vivo* visualisation of suberin therefore relies on proxies, such as fluorescent marker lines of established suberin biosynthetic enzymes, like GPAT5, for example. Whilst generally reliable, the recent demonstrations of suberin degradation as part of developmental and physiological responses (Barberon *et al*., 2016; Ursache *et al*., 2021) illustrates that expression of suberin biosynthetic genes alone should not be taken as evidence that a given cell or tissue is indeed suberised.

Transmission electron microscopy (TEM) has established our idea of suberin as a secondary wall structure, tightly attached to, but distinct from, the primary wall below which it is formed, interposing itself between the plasma membrane and the primary cell wall. This particular apoplastic localisation is a crucial criterion to distinguish suberin from its closely related sister, cutin, which is deposited outside of the primary cell wall of epidermal cells, forming the cuticle. It is the particular deposition of suberin in the form of regular, successively deposited, electron dense and light layers, each of a thickness of a few nanometers, that has given rise to the term 'suberin lamellae'. What underlies this particular nanostructure has been much debated and some models have been proposed that have attempted to assign specific chemical structures to the electron light and dense layers (lamellae). However, it is important to note that, while lamellae often occur, suberin can also be deposited without an observable lamellar structure, as a homogenous layer of low-electron density below the primary cell wall (named nonlamellar suberin). However, this important feature of poly-aliphatic polyesters – to be often associated with a particular subcellular structure (suberin lamellae or cuticle) – should not lead us to state that suberin or cutin-like polymers must always be associated with such identifiable, subcellular structures. Indeed, current evidence does not exclude that low amounts of poly-aliphatic polyesters could form within primary or secondary cell walls, for example, rendering them more hydrophobic. Such impregnations could always occur to a certain degree in classical suberising tissue, but also in tissues where no suberin lamellae are formed and that would currently not be considered as being suberised. Such diffuse suberin has been observed with TEM as inconspicuous bands within the wall and has been postulated to occur in the epidermis of diverse plant roots, for example (Peterson *et al*., 1978). Although the composition was found to be similar to that of endodermal suberin, the amount was substantially lower (Thomas *et al*., 2007). Therefore, we think it is useful to distinguish three types of suberin: 'classical' lamellar suberin, nonlamellar suberin – that is nevertheless deposited as a distinct, secondary suberised wall – and diffuse suberin, in which suberin is not present as a distinct subcellular structure, but rather as an impregnation of pre-existing cell walls.

Chemical analysis of suberised tissues by transesterification provides another important view on the nature of suberin, which has been extensively used for cork or potato tuber phellem. Here, suberin is defined as the set of 'monomers' that are released upon a transesterification (depolymerization) reaction, in samples (i.e. 1–2 mg of dried roots) in which suberised cell walls were isolated and pre-treated with an organic solvent to extract the solvent-soluble lipids (nonpolar extractives or suberin-associated waxes (Schreiber *et al*., 2005; Kosma *et al*., 2012)). The monomers released are identified and quantified by gas chromatography–mass spectrometry (GC–MS) and gas chromatography-flame ionisation detector (GC–FID), respectively, and are of considerable variety, consisting of fatty acids and fatty alcohols, of variable chain lengths from C16–C30 (Franke *et al*., 2005; Molina *et al*., 2006). Many of these aliphatic compounds are bi- or multifunctional, meaning they often contain α- and ω-acid or alcohol groups, in addition to some monomers showing mid-chain hydroxylation/epoxidation in specific suberin types, such as that of cork oak. Unsaturated fatty acids and derivatives, especially those derived of oleic acid (C18:1), are very often major monomer constituents. As mentioned, beyond aliphatic compounds, suberin also contains significant amounts of glycerol, as well as ferulic acid. To understand suberin structure, the molar ratios (stoichiometry) of the different 'monomers' released upon complete transesterification need to be considered. However, although monomer composition is of great importance, it cannot provide the nature and rates of linkages between monomers, crucial for building an accurate model of the suberin structure. Partial depolymerisation circumvents this problem and allows a glimpse into the diversity of linkages used to build suberin (please refer to the following paragraphs). It is also

Box 1 (Continued)

important to consider that, during transesterification, not all ester bonds are necessarily cleaved. Additionally, not all monomers released in solution might be identified by the specific chromatographic conditions used. FHT(ASFT)-silenced potato tuber periderms, for example, have low ferulic acid in suberin and a lignin-like polymer enriched in guaiacyl units (Jin *et al*., 2018). However, the suberin depolymerisation residue still retains signals from aliphatic functional groups, indicating that a portion of aliphatic groups may be less accessible to transesterification reagents. Alternatively, these aliphatics could be part of a nonester-linked aliphatic fraction, described as 'suberan' (Serra *et al*., 2014). Indeed, equivalent samples, using different degradative techniques have shown discrepancies in the existence of this suberan fraction, suggesting differences in the efficiency of suberin depolymerisation techniques (Marques *et al*., 2016).

Partial depolymerisation protocols by transesterification have allowed crucial information to be obtained about monomer connections and revealed that much of suberin is structured around glycerol. Glycerol is mainly linked to diacids forming dimers, but also trimers of a glycerol-diacid-glycerol block. In partial depolymerisation ω-hydroxyacids are underrepresented when compared with total depolymerisation. This may be an indirect indication that the position of ω-hydroxyacids within the suberin structure is not accessible enough to the methanolysis reaction used in partial depolymerisation (Graça & Pereira, 2000). ω-Hydroxyacids were found as ferulate dimers, linear homodimers or heterodimers with diacids and also esterified to glycerol forming monoacylglycerols (Graça & Pereira, 2000).

Thermally assisted transmethylation (pyrolysis in the presence of tetramethylammonium hydroxide–mass spectrometry, Py-TMAH-GC–MS) is a thermally assisted hydrolysis and methylation compositional analysis that has been successfully applied to ligno-suberised cork oak phellem (cork) samples (Bento *et al*., 1998; Marques *et al*., 2016). In comparison with transesterification protocols for suberin chemical analysis, Py-TMAH is not only able to cleave the ester linkages, but also the ether linkages; this estimated an overaccumulation of ferulic acid in Py-TMAH analysis and indicate that ferulic acid may also be ether linked to the polymeric lignin matrix (Marques *et al*., 2016).

Solid-state nuclear magnetic resonance (ssNMR) techniques, applied to wound-healing, but also native suberin and cork, are useful to study the suberised cell-wall polymers without compromising its inherent molecular structure, by analysing the aliphatics, aromatics/phenolics and polysaccharides functional groups. Using advanced ssNMR techniques, sometimes in combination with exogenous application of labelled substrates or prior suberin depolymerisation, positional information and linkages within and between functional groups have been deduced (Serra *et al*., 2012). In suberin, two aliphatic CH_2 moieties co-exist, one restricted by polyester cross-links or cell-wall attachments and the other with motional freedom (Stark & Garbow, 1992; Gil *et al*., 1997; Yan & Stark, 2000). Suberin types with less bifunctional oxygenated aliphatics (*CYP86A33*-RNAi) or esterified ferulic acid (*FHT*-RNAi) are enriched in the mobile aliphatic fraction (Serra *et al*., 2014). Recently, 2D-NMR, able to determine proton-carbon bond correlations, has been applied to cork and potato suberised phellems, providing resolution on overlapping NMR signals of similar molecular moieties and revealing covalent linkages in suberin and lignin (Marques *et al*., 2016; Correia *et al*., 2020; Bento *et al*., 2021). These analyses may be highly informative for revealing the molecular structure of suberin but are still limited for the relatively high amount of purified ligno-suberised material needed (*c*. 50 mg).

mediated by the coupled function of a fatty acid transporter and a long-chain acyl-CoA synthetases (LACS) that produces the acyl-CoA thioesters for subsequent integration into suberin metabolism, while generating a passive gradient across this membrane (Li *et al*., 2016). To date, only LACS2 has been implicated in Arabidopsis seed and endodermis suberin polyester formation (Ayaz *et al*., 2021; Renard *et al*., 2021). Nevertheless, LACS2 is probably involved in the synthesis of ω-hydroxy fatty acyl-CoA intermediates, because (1) LACS2 is localised to the endoplasmic reticulum (ER) (Ayaz *et al*., 2021) and (2) *in vitro* activity showed higher affinity on ω-hydroxyacids than fatty acids (Schnurr *et al*., 2004). However, a LACS-mediated plastid fatty acid export and fatty acyl-activation by outer plastidial envelope localised LACS9 (Schnurr *et al*., 2002) remain a possibility. The acyl-CoA intermediates are elongated to very-long-chain fatty acids (VLCFA) (> C18) by the ER membrane-embedded fatty acid elongase (FAE) complex in which ketoacyl-CoA synthases (KCS) catalyse the rate-limiting step (Haslam & Kunst, 2013; Batsale *et al*., 2021) and are involved in producing suberin VLCFA-CoA products of different chain lengths (KCS2, KCS20, KCS6 and probably KCS1) (Todd *et al*., 1999; Franke *et al*., 2009; Lee *et al*., 2009; Serra *et al*., 2009a). To produce primary alcohols, fatty acyl reductases (FAR) reduce the α-carboxylic group of (L)VLCFA-CoA intermediates: FAR1, FAR4 and FAR5 are partially overlapping chain-length-specific FARs that produce the suberin primary alcohols (Domergue *et al*., 2010).

In contrast with unmodified fatty acids and primary alcohols, the suberin ω-hydroxyacids and α,ω-diacids have particular importance for suberin polyester extension, as they are α,ω-bifunctional fatty acids with oxidised groups at both ends. In suberin, cytochrome P450 monooxygenases from subfamilies CYP86A, CYP86B and CYP94B ω-hydroxylate fatty acids and produce the ω-hydroxyacids (Fig. 1) (Höfer *et al*., 2008; Compagnon *et al*., 2009; Molina *et al*., 2009; Serra *et al*., 2009b; Krishnamurthy *et al*., 2020, 2021). As other cutin-related cytochrome P450s – CYP86 and CYP94 – CYP86A1 (HORST) *in vitro* produces ω-hydroxyacids using free fatty acids as substrates (Benveniste *et al*., 1998). Assuming the presence of this activity *in planta*, we have to postulate a preceding thiosterase activity on (V)LCFA-CoA to release the CoA and then a second downstream CoA-activation of ω-oxidised products by some other LACSs, possibly LACS2 (please refer to the previous sentences), in order to be further glycerylated by GPATs (please refer to the following paragraph) (Fig. 1). In a simpler scenario, ω-hydroxylation could use (V)LCFA-CoA as substrates, as suggested (Beisson *et al*., 2012), obviating the need for a release and subsequent relinking to CoA. Although untested for suberin ω-hydroxylases, CYP86A22, producing ω-hydroxyacids in *Petunia* stigmas, displays such a substrate preference for fatty acyl-CoAs over free fatty acids (Han *et al*., 2010). α,ω-Diacids biosynthesis uses ω-hydroxyacids as precursors and can be produced by two sequential dehydrogenase activities on ω-hydroxyacid and ω-oxoacid groups (Agrawal & Kolattukudy,

Table 1 List of genes involved in suberin biosynthesis, transport and polymerization.

Gene abbreviation	Gene name description	Arabidopsis identifier	References
Fatty acyl-CoA formation			
LACS2	Long-chain acyl-CoA synthetase 2	AT1G49430	Ayaz *et al*. (2021); Renard *et al*. (2021)
Fatty acyl elongase (FAE) complex			
KCS1	3-Ketoacyl-CoA synthase 1	AT1G01120	Todd *et al*. (1999)
KCS2/DAISY	3-Ketoacyl-CoA synthase 2	AT1G04220	Franke *et al*. (2009); Lee *et al*. (2009)
KCS6	3-Ketoacyl-CoA synthase 6	AT1G68530	Serra *et al*. (2009a)
KCS20	3-Ketoacyl-CoA synthase 20	AT1G04220	Lee *et al*. (2009)
Fatty acid ω-oxidation			
CYP86A1 (Arabidopsis) *CYP86A33* (Potato)	Cytochrome P450, family 86, subfamily A, polypeptide 1	AT5G58860	Li *et al*. (2007); Höfer *et al*. (2008); Serra *et al*. (2009b)
CYP86B1	Cytochrome P450, family 86, subfamily B, polypeptide 1	AT5G23190	Compagnon *et al*. (2009); Molina *et al*. (2009); Krishnamurthy *et al*. (2021)
CYP94B1	Cytochrome P450, family 94, subfamily B, polypeptide 1	AT5G63450	Krishnamurthy *et al*. (2020)
CYP94B3	Cytochrome P450, family 94, subfamily B, polypeptide 3	AT3G48520	Krishnamurthy *et al*. (2021)
Fatty acyl reduction			
FAR1	Fatty acid reductase 1	AT5G22500	Domergue *et al*. (2010); Vishwanath *et al*. (2013)
FAR4	Fatty acid reductase 4	AT3G44540	Domergue *et al*. (2010); Vishwanath *et al*. (2013); Wang *et al*. (2021)
FAR5	Fatty acid reductase 5	AT3G44550	Domergue *et al*. (2010); Vishwanath *et al*. (2013)
Glycerylation			
GPAT5	Glycerol-3-phosphate acyltransferase 5	AT3G11430	Beisson *et al*. (2007); Li *et al*. (2007); Yang *et al*. (2010)
GPAT7	Glycerol-3-phosphate acyltransferase 7	AT5G06090	Yang *et al*. (2012)
Feruloylation and other aromatic compound esterification			
ASFT1/RWP (Arabidopsis) *FHT* (Potato)	Aliphatic suberin feruloyl transferase	AT5G41040	Gou *et al*. (2009); Molina *et al*. (2009); Serra *et al*. (2010); Boher *et al*. (2013, p. 20); Wei *et al*. (2020)
FACT	Alcohol:Caffeoyl-CoA transferase (suberin-associated waxes)	AT5G63560	Kosma *et al*. (2012)
GELP-mediated suberin polymerisation			
GELP22	GDSL-type esterase/lipase 22	AT1G54000	Ursache *et al*. (2021)
GELP38	GDSL-type esterase/lipase 38	At1G74460	Ursache *et al*. (2021)
GELP49	GDSL-type esterase/lipase 49	AT2G19050	Ursache *et al*. (2021)
GELP55	GDSL-type esterase/lipase 55	AT2G30310	Ursache *et al*. (2021)
GELP72	GDSL-type esterase/lipase 72	AT3G48460	Ursache *et al*. (2021)
GELP-mediated suberin degradation			
GELP12	GDSL-type esterase/lipase 12	AT1G28650	Ursache *et al*. (2021)
GELP55	GDSL-type esterase/lipase 55	AT2G30310	Ursache *et al*. (2021)
GELP72	GDSL-type esterase/lipase 72	AT3G48460	Ursache *et al*. (2021)
ABCG transporters			
ABCG1 (Arabidopsis) *SvABCG* (*Setaria viridis*) *StABCG1* (Potato)[1] *RCN1/OsABCG5* (Rice)[2]	ABCG transporter family protein 1	AT2G39350	Landgraf *et al*. (2014); Shiono *et al*. (2014); Yadav *et al*. (2014); Shanmugarajah *et al*. (2019); Danila *et al*. (2021)
ABCG2	ABCG transporter family protein 2	AT2G37360	Yadav *et al*. (2014)
ABCG6	ABCG transporter family protein 6	AT5G13580	Yadav *et al*. (2014)
ABCG20	ABCG transporter family protein 20	AT3G53510	Yadav *et al*. (2014); Fedi *et al*. (2017)
ABCG11/ABCG11/DSO/COF1	ABCG transporter family protein 11	AT1G17840	Panikashvili *et al*. (2010)
Apoplastic lipid transfer proteins			
LTPG15	Glycosylphosphatidylinositol-anchored lipid transfer protein 15	AT2G48130	Lee & Suh (2018)
LTPG6	Glycosylphosphatidylinositol-anchored lipid transfer protein 6	AT1G55260	Edstam & Edqvist (2014)
LTP2/AtLTPI.4	Lipid transfer protein I.4/Lipid transfer protein 2	AT2G38530	Deeken *et al*. (2016)

[1]StABCG1 putative orthologues are: ABCG1, ABCG6 and ABCG16.
[2]OsABCG5 and StABCG putative orthologues are: ABCG1, ABCG6, ABG16, ABCG2 and ABCG20.

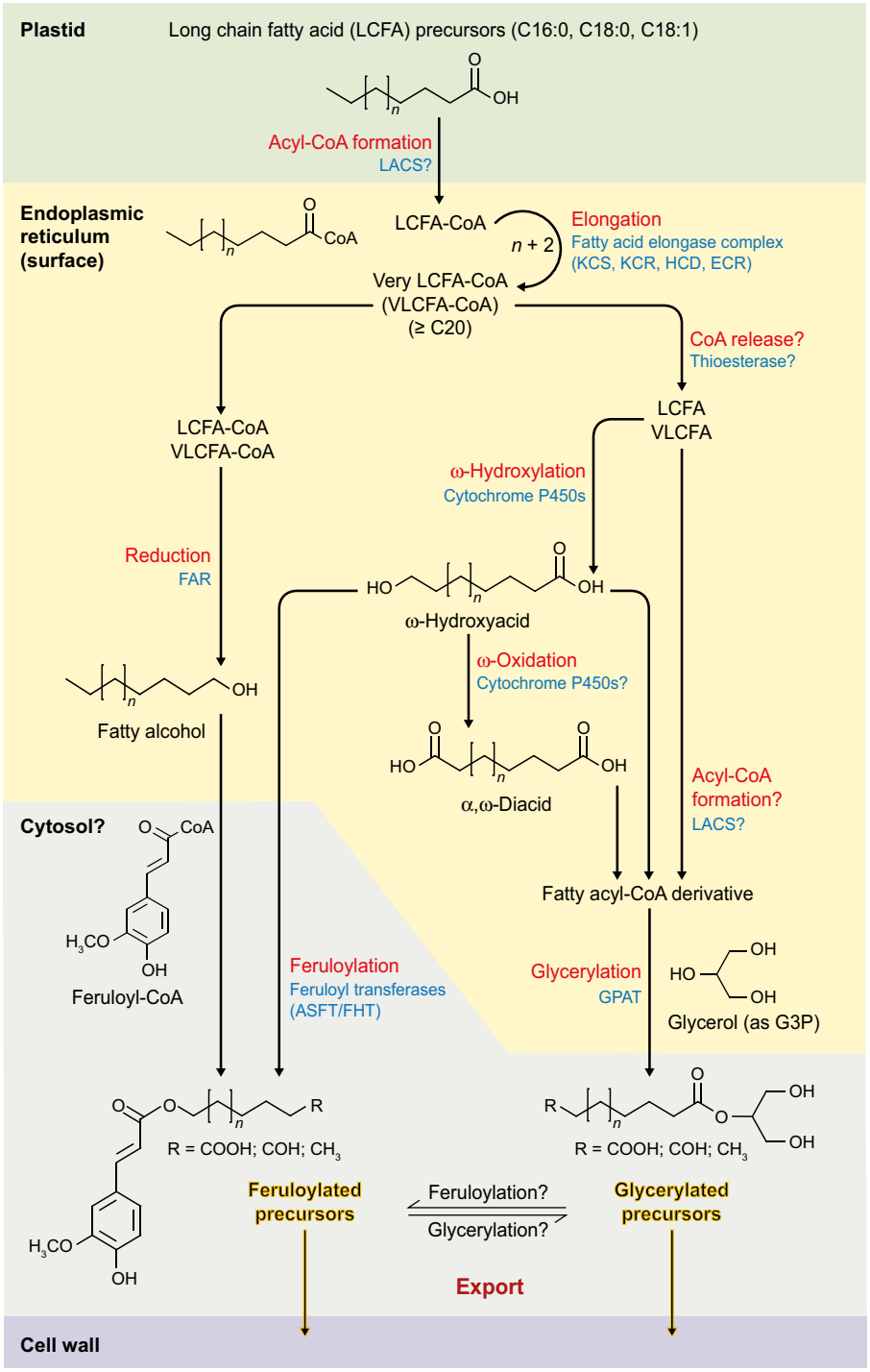


Fig. 1 Biosynthesis of suberin monomeric precursors. Different background colours indicate compartments in which reactions are thought to take place. Reaction steps indicated in red, enzyme classes catalysing these reactions are in blue. The putative end-products of biosynthesis, termed *suberin monomeric precursors* are highlighted in yellow. These are defined as the products that are subject to export into the cell wall and use as substrates for the polymerising enzymes. Although we know that all the above-mentioned reactions are necessary to accumulate suberin, the order of the reactions that produce these suberin monomeric precursors is not however certain in all cases. Biochemical and genetic data support the idea that ω-hydroxylation by CYP86s would precede the acyl transfer to glycerol at sn-2 position by GPAT5 (Yang *et al*., 2010, 2012), but the integration of feruloylation reaction and the acyl-CoA activation by long-chain acyl-CoA synthetases (LACS) is still unclear. (*n*) indicates a variable number of CH_2 groups. Possible mid-chain modifications of fatty acyl-derivatives, such as mid-chain hydroxylation, double bonds, etc., have been omitted for simplicity. Fatty acids and primary alcohols, as well as their feruloylated and glycerylated forms, respectively, are also identified as suberin-associated wax compounds. ω-Hydroxyacids and α,ω-diacids are found exclusively in suberin depolymerisation products. G3P, glycerol 3-phospate. Specific enzymes are identified in Table 1.

1977). It is still unclear whether these activities are performed by the same CYP ω-hydroxylases or by some oxidoreductases such as HOTHEAD, presumably involved in the oxidation of ω-hydroxyacid to α,ω-diacid in cutin (Kurdyukov *et al*., 2006). Enzymes for mid-chain hydroxylation and epoxidation to produce the mid-chain oxygenated fatty acids of specific suberins (such as that of cork oak; Graça & Santos, 2007) remain to be identified. This mid-chain oxidation of aliphatic monomeric precursors would provide an additional functional group that could contribute to a more cross-linked suberin, with potentially very different steric properties (Graça, 2015).

Glycerol esterification to create 'activated' polymerisation substrates? Glycerol has been identified as a major component of the suberin polyester (Schmutz *et al*., 1993; Graça & Pereira, 1997; Moire *et al*., 1999). The suberin glycerol phosphate acyltransferases (GPAT5 and putatively GPAT7) transfer the acyl-CoA to glycerol-3 phosphate (Beisson *et al*., 2007; Yang *et al*., 2012), preferentially

at the sn-2 position, yielding sn-2 lysophosphatidic acid that would be further processed to sn-2 monoacylglycerols by some unknown phosphatase (Yang *et al.*, 2010, 2012). GPAT5 has shown broad substrate specificity *in vitro* with the CoA-derivatives of C16–C22 fatty acids, ω-hydroxyacids and α,ω-diacids (Yang *et al.*, 2012). As mentioned previously, CoA-activated substrates for GPATs might either be directly provided by the CYP oxidases, or would require an additional step of CoA formation (Fig. 1). In agreement with GPAT5 *in vitro* activity, C22 and C24 fatty acids, ω-hydroxyacids and α,ω-diacids are greatly reduced in seed suberin of the *gpat5* mutant (Beisson *et al.*, 2007), indicating that the export and deposition of these compounds into the suberin polyester is dependent on prior, GPAT5-mediated, glycerol esterification. Similarly, the ectopic accumulation of suberin-like (C16–C24) ω-oxidised compounds in Arabidopsis cutin is not possible by simply overexpressing suberin CYP86A1/CYP86B1, but additionally requires GPAT5. This strongly supports the idea that glycerylation is required for export to the cell wall. Endodermal suberin showed practically no change in a *gpat5* knock-out, suggesting that other GPATs act redundantly to GPAT5 in the endodermis (Beisson *et al.*, 2007). In partial depolymerisation studies of suberin, in which intermolecular ester linkages are retained, glycerol has been preferentially esterified to α,ω-diacids, but also to ω-hydroxyacids, fatty acids and ferulic acid (Graça & Pereira, 1999, 2000). This points to a dual role of glycerol, serving both as a structural component of suberin, as well as a requisite modification for monomer transport to the apoplast and their subsequent transesterification by GDSL lipases (please refer to Section II.2 for more details). Acyl-glycerol-esters can therefore be considered as the final intracellular products of suberin biosynthesis, subject to transport into the apoplast and use as substrates for the polymerising GDSL/GELP enzymes. Therefore, as a monomer is strictly defined as the molecule that reacts with other molecules to form a large polymer (Jenkins *et al.*, 1996), we should consider terms such as 'suberin monomeric precursors', 'suberin monomeric substrates' or 'activated suberin monomers' for these intracellular products. This would allow continuation of use of the term 'monomer' for the individual aliphatic or aromatic molecules obtained after depolymerisation, but nevertheless indicates that the glycerylated GPAT products are the actual substrates to the polymerising enzymes (analogous to nucleotide tri-phosphates for nucleic acids, or acetyl/malonyl-CoA in fatty acid synthesis).

Ferulic acid esterification Ferulic acid is the main aromatic monomer of the suberin polyester (Graça *et al.*, 2015). Ferulic acid is produced by the phenylpropanoid pathway, which also produces other hydroxycinnamic acids, as well as the monolignols that polymerise into different types of lignins (Graça, 2010; Vanholme *et al.*, 2019). The importance of ferulic acid in suberin formation has been evidenced by genetically or chemically (using piperonylic acid) repressing the phenylpropanoid pathway in suberised endodermis and phellem. This leads to a block in suberin deposition that can be restored by exogenously applying ferulic acid in the endodermis (Andersen *et al.*, 2021). The Arabidopsis and potato feruloyl transferases (ASFT/RWP and FHT, respectively) can esterify feruloyl-CoA to ω-hydroxyacids and primary alcohols *in vitro* and its downregulation highly reduces the esterified ferulic acid in suberin and, to a varying extent, ω-hydroxyacids, but not α,ω-diacids (Gou *et al.*, 2009; Molina *et al.*, 2009; Serra *et al.*, 2010). This indicates that some ω-hydroxyacids require intracellular esterification to ferulic acid to be exported to the cell wall or for their integration into the polymer. This is also in agreement with the identification of ferulic acid esterified to ω-hydroxyacids and primary alcohols in partial suberin depolymerisation studies (Graça *et al.*, 2015), as well as soluble metabolites of potato periderms silenced for the *StABCG1*, a putative plasma membrane exporter of suberin monomers (Landgraf *et al.*, 2014). In this respect, ferulate addition to an aliphatic compound can be compared with glycerol addition – as a modification giving rise to an esterified suberin building block that will be transported to the apoplast for polymerisation. However, to add to the complexity, ferulate addition may also occur on glycerol-esters (or ferulic acid esters may become glycerylated) (Fig. 1), as ferulic acid – ω-hydroxyacid – glycerol products are detected in partial depolymerisation studies (Graça & Santos, 2007; Graça *et al.*, 2015) and also accumulate as soluble putative substrates in the potato periderm impaired in the cellular export of suberin (Landgraf *et al.*, 2014). It is therefore intriguing to postulate that suberin building blocks are largely, even exclusively, produced as esterified components, most probably in the form of an acid group esterified to glycerol alcohol (glycerylated precursors), but possibly also as an alcohol group esterified to ferulic acid (feruloylated precursors), even forms that are both glycerylated and feruloylated can be considered. We will further extend on this idea in Section III.1.

2. Endoplasmic reticulum as the hub of suberin monomeric precursors production

Although the suberin biosynthetic machinery has been largely elucidated, the subcellular localisation of these enzymes as well as the monomeric precursor exchange between the different compartments involved is still unclear. Fatty acids are synthesised in plastids and then transported to the ER for further modification and lipid assembly (Li *et al.*, 2016). Suberin monomeric precursors, as well as those of cutin, would then need to reach the plasma membrane and to be exported to the apoplast for polymerisation (Li-Beisson *et al.*, 2013). For the suberin enzymatic machinery, evidence of ER localisation include the Arabidopsis CYP86A1/HORST and CYP86B1 ω-hydroxylases, the *Avicennia officinalis* CYP86B1 and CYP94B3 ω-hydroxylases, and *Brachypodium distachyon* BdFAR4 using transient overexpression in *Nicotiana benthamiana* epidermal cells or Arabidopsis protoplasts (Höfer *et al.*, 2008; Compagnon *et al.*, 2009; Wang *et al.*, 2021). In addition, biochemical characterisation of GPAT5 was carried out in yeast using microsomal fractions, consistent with a localisation of this enzyme to the ER (Yang *et al.*, 2010). All current analyses demonstrate ER localisation using heterologous overexpression systems and the demonstration of enzymatic localisation at endogenous expression levels in actively suberising cells is still lacking. Despite this unsatisfying state of evidence, it can be expected that suberin biosynthetic enzymes are ER membrane embedded, acting presumably on the cytosolic side, as suggested for

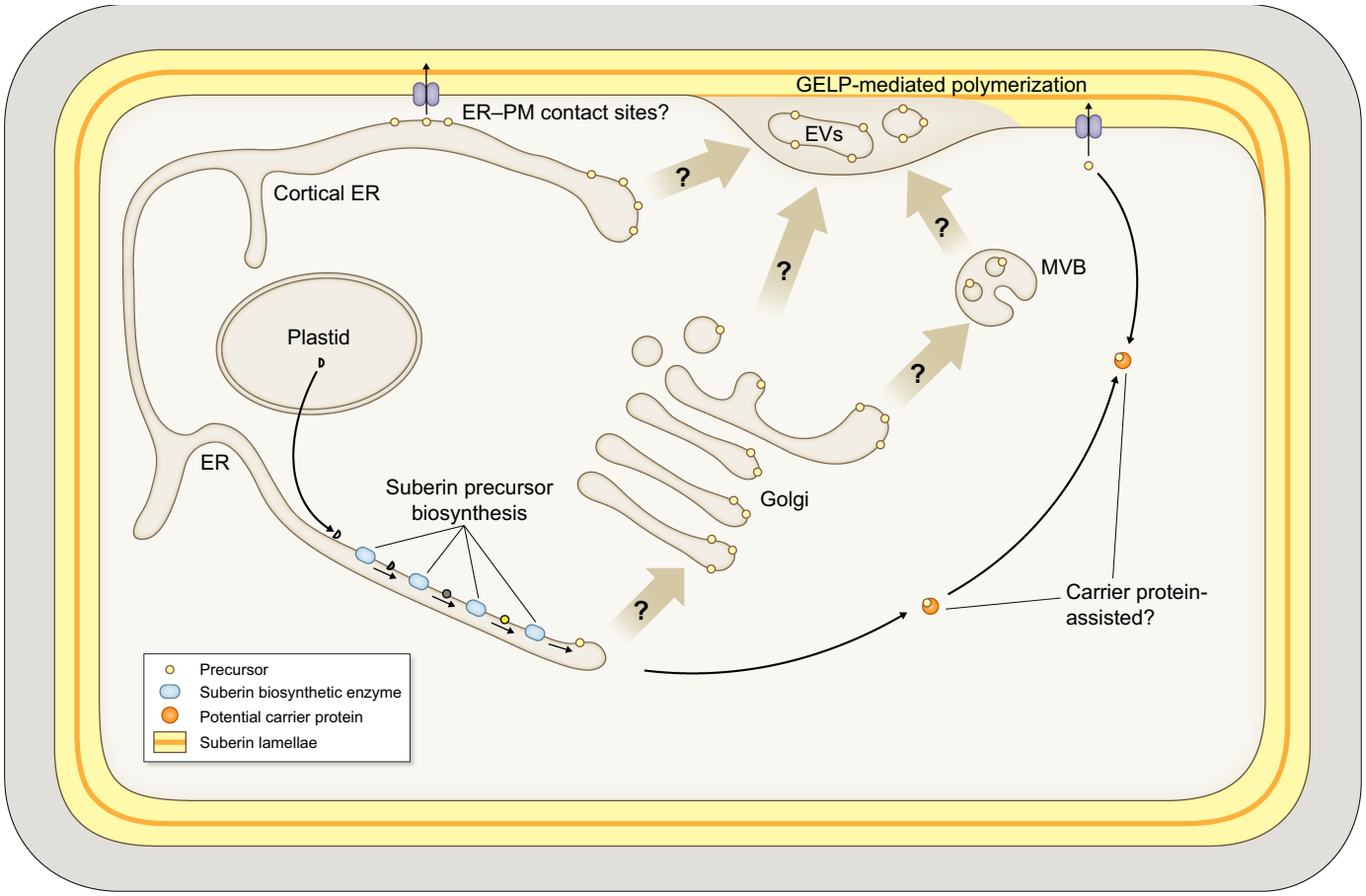


Fig. 2 Location and transport of suberin precursors in the cell. Two scenarios for transport of suberin monomeric precursors: ATP-binding cassette (ABC) transporter of the G-clade (ABCG) transporter-mediated (dark grey, rounded rectangles) or vesicle transport-mediated are shown. Precursors (yellow dots) are indicated as membrane resident molecules here, but they may be part of the lumen. The precise vesicle transport pathways of monomeric precursors from the endoplasmic reticulum (ER) to the apoplast are not known, possible pathways are indicated with question marks. Blue rounded rectangles, suberin biosynthetic enzymes described in Fig. 1. Orange circles, unknown, potential carrier proteins for suberin monomeric precursors. Yellow/orange border, suberin lamellae. EV, extracellular vesicles; MVB, multivesicular bodies; PM, plasma membrane.

the KCS complex (Haslam & Kunst, 2013) (Fig. 2). By contrast, the potato FHT (ASFT orthologue) was identified in the cytoplasmic, not microsomal, fraction of actively suberising phellem, using specific FHT antibodies and, in agreement the corresponding kiwi orthologue (AchnFHT) was localised in the cytosol of *N. benthamiana* epidermal cells when overexpressed (Boher *et al.*, 2013; Wei *et al.*, 2020). As substrates, FHT would need ω-hydroxyfatty acid/primary alcohols as well as feruloyl-CoA (Serra *et al.*, 2010) derived from the ER-associated phenylpropanoid pathway (Winkel, 2004). The multienzyme complex of phenylpropanoids is constituted by both cytoplasmic and ER-integral proteins, in which the latter would provide scaffolds for the self-assembly of the complex, forming metabolons (Winkel, 2004; Biała & Jasiński, 2018). In suberising cells, metabolon organisation would provide a way to control the metabolic channelling of phenylpropanoids to feed suberin or lignin biosynthesis, processes that are working concurrently in the same cell. It remains to be established whether suberin biosynthesis is also organised in a metabolon at the ER membrane, which may then also differ for suberin and wax production and whether this metabolon branches with the phenylpropanoid pathway for direct feruloyl-CoA substrate transfer to FHT. In order to assist movement of lipophilic molecules in an aqueous cellular environment, the intracellular trafficking of suberin monomeric precursors, during and after their synthesis should include fatty acyl transporters, as well as proteins that would allow delivery to, flip-transfer across, and movement out of membranes (for discussion please refer to Li *et al.*, 2016). In addition, in the actively suberising cell, the massive intracellular lipid production would need to be insulated from other fatty acid/lipid pathways (i.e. membrane lipids) to avoid distorting the fatty acid/lipid homeostasis, affect membrane bilayers and plant performance (Boutté & Jaillais, 2020). Therefore, further research is needed to address the funnelling of fatty acid precursors, their transport towards and across membranes and their distribution within cell compartments and the apoplast, processes that need to be tightly regulated in order to avoid lipid metabolic imbalance.

3. Transport of suberin monomeric precursors to the apoplast

How the diverse suberin monomeric precursors are transported into the apoplast, for polymerisation at the plasma membrane–cell

wall interface, is an important and insufficiently understood aspect of suberin formation. As mentioned above, suberin monomer VLCFA-CoA elongation is believed to occur at the cytosolic face of the ER. Nevertheless, it is not described where in the ER the oxidation and glycerylation specifically occur. If fatty acyl modification takes place in the ER lumen, formally, monomeric precursors would need to cross the ER membrane through membrane-intrinsic FA/lipid transporter proteins, not described to date. From there, the suberin monomeric precursors would have to be transported through the secretory pathway and be secreted into the apoplast, much like the path used by extracellular proteins. However, most current models propose a cytoplasmic passage from the ER surface to the plasma membrane, where they would then be transported across the plasma membrane by ATP-binding cassette (ABC) transporter of the G-clade (ABCG) in order to be placed in the apoplast. Fig. 2 indicates that this passage could occur across very short distances at ER–plasma membrane (ER–PM) contact sites, alternatively or additionally it could be facilitated by cytoplasmic carrier proteins.

For suberin, the ABCG transporters involved in monomer transport include the half-size transporters of the Arabidopsis clade of AtABCG1/2/6/16/20 and their homologues, such as the potato StABCG1 and the rice OsABCG5/RCN1. Additionally, ABCG11, whose activity expands to cutinised tissues, has also been reported for suberin monomeric transport (Panikashvili *et al.*, 2010) (Table 1). Expression of the half-transporter *StABCG1* has been associated with suberising tissue and RNA interference was found to cause, among other defects, strong reductions of major monomers in suberin of potato periderm, with a concomitant increase in soluble feruloylated fatty acids and derivatives (Landgraf *et al.*, 2014). This suggests that StABCG1 might transport products of FHT (ASFT) (Landgraf *et al.*, 2014), instead of mono-acyl glycerols. One of its closest Arabidopsis homologues, AtABCG1, causes only minor reductions in specific suberin monomers in the root when knocked out, especially C26 fatty acids and alcohols and C22–C24 α,ω-diacids (Shanmugarajah *et al.*, 2019), which were also reduced in *StABCG1*-RNAi line, although at a much higher proportion. Clearly, if ABCG transporters were the major factors for transport of monomers to the apoplast, it would have to be postulated that there is a large set of transporters, each transporting subsets of monomeric precursors as substrate, with partially overlapping specificities. Indeed, numerous ABCG transporters have been found as being co-regulated with suberisation (Shukla *et al.*, 2021), suggesting that higher order transporter mutants will be needed for obtaining mutants with strong, broadly reduced levels of suberisation. Unfortunately, a triple mutant of the suberin transporter candidates, ABCG2, ABCG6 and ABCG20, presents confusing phenotypes, with overall suberin levels being increased in roots, and most monomer species found in suberin also being increased, rather than decreased (Yadav *et al.*, 2014). Additionally, the wax composition of root periderm in these mutants has less alkyl hydroxycinnamates but more primary alcohols. Conversely, the seeds of the triple mutant *abcg2 abcg6 abcg20* accumulate less suberin (Yadav *et al.*, 2014). Nevertheless, the change in suberin chemical composition is pretty similar to that of *awake1*, bearing a mutation in *ABCG20* (Fedi *et al.*, 2017), suggesting that the lack of *ABCG20* function alone might be responsible for the suberin seed phenotype of the *abcg* triple knockout. Indeed, the *abcg2* single mutant does not show altered seed permeability (Landgraf *et al.*, 2014; Fedi *et al.*, 2017). These results could clearly stem from further redundancy in suberin transporters and active compensatory responses in the mutants. The fact that half-transporters have the capacity to homo- and heterodimerise, potentially affecting their substrate preference and activity (McFarlane *et al.*, 2010), might further contribute to the currently confusing results. Mutating root-expressed AtABCG1, AtABCG16 and even AtABCG11 in this triple mutant background, potentially eliminating all suberin-associated half-transporters, could lead to a more clear-cut phenotype. Interestingly, a very clear phenotype already with a single mutant was obtained in rice for OsABCG5 (RCN1), a close homologue to the Arabidopsis ABCG1/2/6/16/20 clade (Shiono *et al.*, 2014) (Table 1). This mutant shows an inability to suberise its root exodermis in response to waterlogging, corroborating the idea that this clade of transporters mediates suberin precursor transport. Some evidence for precursor transport activity has been reported for AtABCG1, which has stimulated ATPase activity in the presence of C26–C30 fatty alcohols and fatty acids (Shanmugarajah *et al.*, 2019). Recently, direct transport assays, using plant protoplasts overexpressing cutin-associated AtABCG11 and AtABCG32 demonstrated the transport activity for both glycerol-esterified fatty acids (i.e. the products of GPATs) as well as ω-hydroxylated free fatty acid forms (Elejalde-Palmett *et al.*, 2021). However, it is believed that the glycerol-esterified forms are the biologically relevant substrates (please refer to Section II.1). In addition, it should at least be considered that a suberin formation defect observed in ABCG transporter mutants could be an indirect consequence of a defect in monolignol or hydroxycinnamic acid (i.e. ferulic acid) transport to the apoplast because: (1) ABC transporters in general, (Miao & Liu, 2010) and the ABCG transporter ABCG29, specifically (Alejandro *et al.*, 2012), have been shown to be involved in monolignol transport; and (2) ferulic acid is required for *de novo* formation of suberin (Andersen *et al.*, 2021).

Some electron microscopy (EM) studies, done mostly in the pre-molecular biology era of plant research have attempted to describe the cellular structures associated with suberisation. Due to the challenges of fixing and embedding differentiated suberised cells, images obtained from these studies are often hard to interpret (Wattendorff, 1974; Scott & Peterson, 1979; Oparka & Gates, 1982; Ma & Peterson, 2001). However, it was repeatedly noted in these works that suberin lamellae formation can be associated with PM invaginations that contain extracellular tubules or vesicles (EV). The origin of these vesicles has remained speculative, but, depending on species and tissue type investigated, accumulation of plasma membrane-adjacent ER cisternae or Golgi (dictyosomes), dilated or not, as well as multivesicular bodies, was described and proposed as sites from which these extracellular membrane structures could have originated. The possibility that these structures might represent transport intermediates of the lipid-like, monomeric precursors for suberin, was most clearly formulated by Peterson (Scott & Peterson, 1979; Ma & Peterson, 2001).

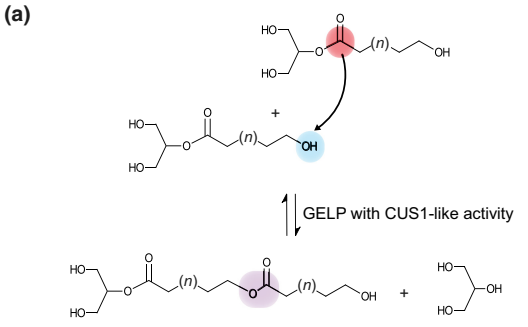
However, this idea has been largely forgotten and has, to our knowledge, not been explicitly discussed in any of the influential reviews of the last 2 decades (Ranathunge *et al.*, 2011; Vishwanath *et al.*, 2015). Recently, a study investigating different oversuberising mutants in the Arabidopsis root endodermis observed very similar structures, and quantified and described them using modern three-dimensional EM (3D EM) techniques (De Bellis *et al.*, 2021). Association of the extracellular vesiculotubular structures with suberisation was established by quantification of their co-occurrence with the developmental progression or stimuli-induced suberisation. Interestingly, when using superior cryofixation that enables a better conservation of cell turgidity in comparison with chemical fixation, these structures in the periplasmic space (the extracellular space close to the membrane) showed only slight invaginations and appeared flatter, rather lens shaped. In this lens-shaped space, fewer, but bigger and less reticulated extracellular membranes, gave the appearance of a branched ginger root in 3D reconstructions. Occurrence of such EV is not restricted to suberisation and is often observed in the context of biotic interactions, for example at penetration pegs during fungal infections (An *et al.*, 2006) and is associated with instances of strong secretory activity (Cui *et al.*, 2020). The high internal pressure of the plant protoplast raises the question how such structures can form in the first place and why they are not squeezed against the resisting cell wall. One explanation could be the presence of a matrix of low electron density in EM in this periplasmic space, which would resist compaction by the protoplast and into which these membrane tubules become embedded. It is tempting to speculate that these membrane tubules are a way of transporting suberin monomeric precursors into the apoplast and that these lipid-like precursors either form such tubules themselves or are carried within them (Fig. 2). The ontogeny of these tubules currently remains enigmatic, as there are no associated markers or intermediate forms that would indicate their origin. If suberin monomeric precursors would coalesce into membrane-like structures within the lumen of the ER, a fusion of the ER with the PM would directly place them into the apoplast. Indeed, an extensive ER network below the plasma membrane, as well as ER–PM contact sites have been well described and are important for lipid exchange (Molino *et al.*, 2017; Wang *et al.*, 2017), although there is very little evidence in the literature of direct fusion between ER and PM. Moreover, in the *abcg11* transporter mutant, lipid inclusions were observed that appeared to be derived from ER structures, suggesting a direct transport of cutin/wax precursors between ER and PM, circumventing Golgi/post-Golgi trafficking (Bird *et al.*, 2007; McFarlane *et al.*, 2010). Formation of endosomal, multivesicular bodies (MVBs) and their subsequent 're-direction' towards fusion with the PM (instead of vacuolar fusion) is another pathway proposed for the formation of extracellular membranes (Cui *et al.*, 2020). Finally, a direct evagination of PM domains into the apoplast would explain the identical contrast of PM and extracellular tubules observed in cryofixed samples. However, a machinery mediating such evaginations and fissions in plants is currently unknown and it is unclear how this would serve to place suberin precursors into the apoplast.

In summary, older and current electron microscopic studies suggest that suberin formation is, in the very least, associated with strong, focalised secretory activity, leading to the formation of extracellular membrane tubules. Whether these tubules are direct intermediates of suberin precursor transport – an attractive hypothesis in light of the lipid-like nature of the precursors – or whether they are simply a result of a high degree of membrane delivery, remains an open question. However, a model in which suberin precursors are solely transported by ABC transporters simply does not explain the presence of focalised formations of extracellular tubules. In light of the considerable structural diversity of suberin precursors, we think it is reasonable to postulate that some precursors could be subject to ABCG-mediated transport, while others could be placed in the apoplast through secretory processes, using extracellular membrane tubules as intermediates (Fig. 2).

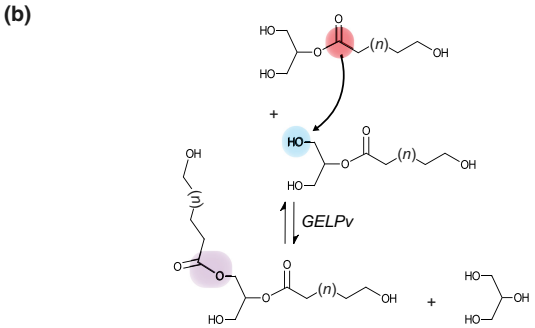
III. Suberin polymerisation and structure

1. Suberin polymer formation in the wall

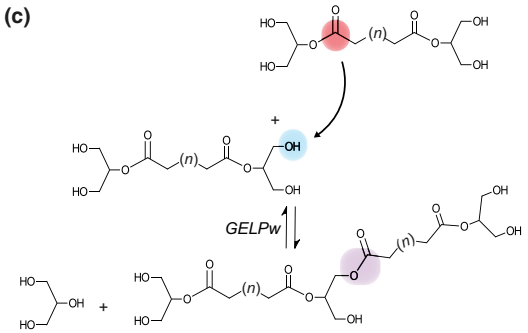
In light of this awe-inspiring complexity of possible linkages described for suberin, we think it is highly useful to try to sketch a 'generative' model of suberin (Fig. 3), asking what the monomeric substrates of suberin polymerising enzymes might be and what kind of reactions would have to occur in order to obtain the different linkages that have been experimentally observed. This model is based on the breakthrough identification of CUTIN SYNTHASE 1 (CUS1) as a critical component in cutin formation (Yeats *et al.*, 2012, 2014). In these studies, the authors demonstrated that CUS1 is an enzyme belonging to the large family of secreted lipases of the GDSL family (GELPs). CUS1 is able to generate linear chains of ω-hydroxyacids *in vitro* by catalysing a transesterification reaction, using a 2-mono-acylglycerol species as substrate and generating free glycerol as a co-product (Fig. 3a). That polymerisation of polyesters such as cutin or suberin proceeds by transesterification is chemically intuitive, as *de novo* ester-bond formation is a condensation reaction (releasing water), thermodynamically disfavoured in an aqueous environment. Indeed, biological ester-bond formation within the cell widely uses transesterifications, with CoA thio-esters as activated substrates, for example. We therefore think it is useful to make an inductive leap and to postulate that all, or a large part of, ester bonds observed in suberin are formed by analogous transesterification reactions. These would be mediated by different GDSL lipases in the apoplast, using glycerylated aliphatic esters (suberin monomeric precursors, please refer to Section II.1) as 'activated' substrates. A central role of GDSL lipases in suberin formation has recently been demonstrated by the strong reduction in suberin in quintuple mutants of endodermis-expressed GELPs (Ursache *et al.*, 2021). In this mutant, an 85% reduction of suberin levels is observed, together with a complete lack of suberin induction in response to ABA. This reduction is associated with the appearance of an unstructured layer of low electron density in the place of suberin lamellae, potentially due to the accumulation of unpolymerised suberin monomeric precursors in the apoplast. However, this



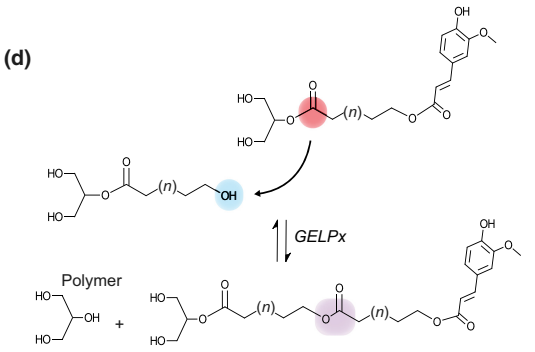
Linear omega-hydroxy acid ester: *'activated' acid group coupled to 'free' hydroxy group* (linkage type reported in Graça & Santos, 2006)



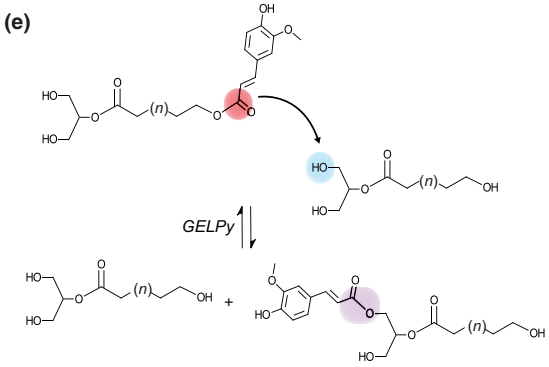
Glycerol branched ester: *'activated' acid group coupled to a 'free' 1, 3 hydroxy group of glycerol* (linkage type reported in Graça & Pereira, 2000)



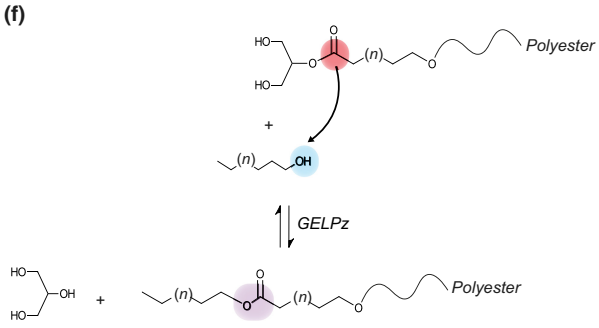
Glycerol branched di-acid-ester: *'activated' acid group coupled to a 'free' 1-hydroxyl group of glycerol* (linkage type reported in Graça & Pereira, 2000)



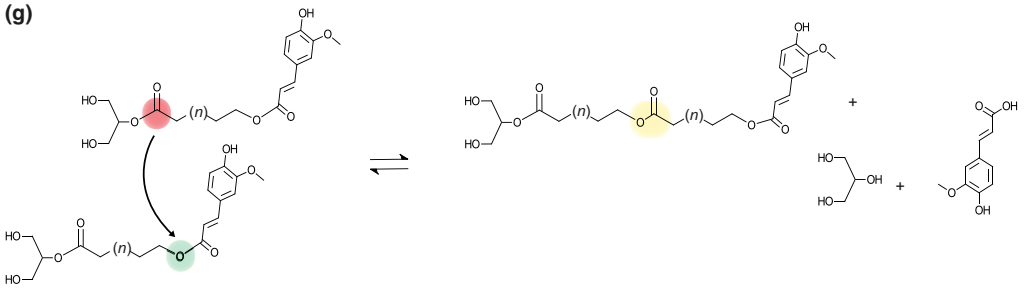
Variant of A, but using an omega-hydroxy acid that is esterified to both glycerol and ferulate as a substrate (linkage type reported in Graça & Pereira, 2000)



Integration of ferulic acid into the polymer by trans-esterification with 1-hydroxyl group of glycerol (linkage type reported in Graça & Pereira, 2000)



Incorporation of primary alcohols as polyester end groups by trans-esterification



Speculative alternative reaction model for A, postulating breaking of two ester linkages for the formation of one new bond

Fig. 3 Overview of possible transesterification reactions during suberisation. (a–f) Proposed suberin monomeric substrates and GELP-mediated transesterification reactions to produce ester-linked dimers/trimers detected in suberin partial depolymerisation data. Purple shade highlights the ester linkage of molecules found upon partial depolymerisation of suberin. Red shade indicates ester bonds of potential GELP enzyme substrate, whose breakage and transfer to an alcohol acceptor group (blue shaded), could explain the observed purple linkage types. GELPv-z is meant to indicate that each of these reactions might be mediated by a dedicated GELP. (g) Speculative 'double transesterification' model in which the second ester linkage is shaded in green and the newly formed linkage in the product is shaded in yellow.

accumulation of suberin monomers remains to be demonstrated by chemical analysis, as does the catalytic activity of these putative 'suberin polymerising GELPs' by *in vitro* assays. Nevertheless, these findings support a model in which several GELPs, with distinct or partially overlapping, substrate specificity mediate transesterification reactions, involving substrates with distinct functional groups (Fig. 3). It is interesting to note that polymerisation of other cell-wall polymers, such as cellulose, hemi-cellulose or pectins are all based on activated precursors in the form of nucleotide sugars. In these cases, however, monomer coupling takes place in the Golgi apparatus before secretion (pectins, hemicelluloses) or at the CESA rosette complexes at the PM, the cleaved activation groups (di-phospho nucleotides) never reaching the apoplast (Verbančič *et al.*, 2018). However, despite being more energetically favourable than direct esterification, transesterification of glycerylated substrates by CUS1 or homologous proteins was still shown to be rather inefficient with very high K_m and low turnover numbers of the enzymes (Yeats *et al.*, 2014). However, as pointed out before, it can be speculated that efficient polymerisation may be driven by the unique environment of a hydrophobic suberin phase and a hydrophilic phase in the proximity of the PM, which could favour polymerisation at their interface. In Fig. 3(g), we speculate on an additional way to render apoplastic ester formation more thermodynamically favourable: An enzymatic reaction that would couple formation of one new ester bond to the cleavage of two ester bonds in the substrates could use the negative free energy changes of their hydrolysis to drive a more efficient polymerisation reaction.

Evidently, the presence of glycerol moieties in the suberin polymer, much after full or partial depolymerisation, requires that we postulate that a significant fraction of glycerylated suberin precursors are not used as transesterification substrates, but are integrated as such into the suberin polymer (Graça, 2015). This can be easily understood if some GELP enzymes have a preference for the 1-alcohol groups of glycerol in the 2-monoacylglycerol units (instead of the ω-hydroxy group of the aliphatic chain as for CUS1) (compare Fig. 3a, b). This would generate the di- and tri-esters of glycerol, as found after partial depolymerisation reactions (please refer the previous sentences) (Graça *et al.*, 2015). Our 'all transesterification' model leads to further interesting predictions: The presence of free acid groups in diacids, for example, would not allow further polymer elongation by transesterification. This could be circumvented by the attachment of two glycerol groups to both α- and ω-moieties of the diacid. This could occur within the cell by the consecutive action of two GPATs and the export of a glycerol–diacid–glycerol trimer into the apoplast, which would allow GELP-mediated incorporation of the diacid–glycerol unit to the growing suberin polymer (Fig. 3c). The presence of ferulate is another feature of the suberin polyester that is interesting to consider in the context of transesterification. A consecutive action of FHT/ASFT and GPAT enzymes could generate the substrate unit glycerol – α, ω-hydroxyacid – ferulic acid shown in Fig. 3(d). A GELP enzyme using the α-acid group of hydroxyacid as acid donor could incorporate ferulic acid linked to α,ω-hydroxyacids into the suberin polymer (Fig. 3d) (generating the major structure found in Graça & Pereira, 2000). Alternatively, using the ferulate moiety as the acid donor could transfer ferulate groups onto free alcohol groups, such as a 1-glycerol group (Fig. 3e) (found in Graça & Pereira, 2000). Integration of primary alcohols into the suberin polyester by transesterification could be explained by GELPs using a glycerylated polymer as substrate (instead of monomers). This would integrate the primary alcohol as an end group into the polymer (Fig. 3f). The 'all transesterification' model sketched out here makes some testable predictions, namely that GELPs are the major enzymes for suberin polymerisation and that they either have very broad substrate specificity or that several enzymes with specialised substrate specificities work together in the generation of suberin. Studies combining GELP mutant analysis with partial depolymerisation approaches could address this, as well as studies of substrate specificity using different recombinant GELPs involved in suberisation.

Although it has recently been reported that ferulic (or sinapic) acid, ω-hydroxyacid and glycerol have the ability to spontaneously oligomerise *in vitro* in organic solvents (Kligman *et al.*, 2022), it is improbable that such a process is of relevance under *in vivo* conditions in the cell wall. Indeed, the inability to deposit suberin of *gelp* quintuple knock-out mutants (Ursache *et al.*, 2021) suggests that self-assembly cannot substitute the lack of enzyme activity for polymerisation *in planta*.

2. The suberised cell wall: a ménage à trois of suberin, lignin and ferulates

The plant cell wall appears as a nanostructured compound material, arising from the interaction and interconnection of distinct polymers, each complex and often heterogenous in nature. When trying to imagine the *in vivo*, three-dimensional structure that suberin would take in the cell wall, one has to consider how it might be embedded within, and connected to, the many other polymers that make up the cell wall, pectins, hemicelluloses, cellulose or lignin. It is therefore of great importance that cell-wall polymers are clearly distinguishable by their monomer composition and their type of coupling. As mentioned in the Introduction, suberin can be defined rather well as a largely poly-aliphatic polyester, with minor amounts of esterified phenolics, mostly in the form of ferulate. This represents a clear, chemical definition that distinguishes it from other wall polymers in a given cell, as opposed to the more extensive definitions of suberin (Kolattukudy, 1980; Bernards, 2002) as a 'heteropolymer' of poly-aromatic and poly-aliphatic domains, with esterified ferulic acid playing a role of connecting these different domains. Our stricter definition of suberin as a poly-aliphatic polyester (Geldner, 2013; Graça, 2015) does not dismiss an intimate pairing with lignin-like polymers in the same tissue, nor a possibly atypical nature of the lignin-like polymers found in suberised tissues (Marques & Pereira, 2013). A much discussed candidate connecting these two different polymers is ferulic acid, a nonreduced precursor of a major lignin monomer, coniferyl alcohol. The acid group of ferulic acid would allow the connection between the ester-based suberin polymer and the oxidatively coupled lignin polymer (Fig. 4d).

Evidence for lignin-like polymers have been found in all suberised tissues studied and, when quantified, it is generally found

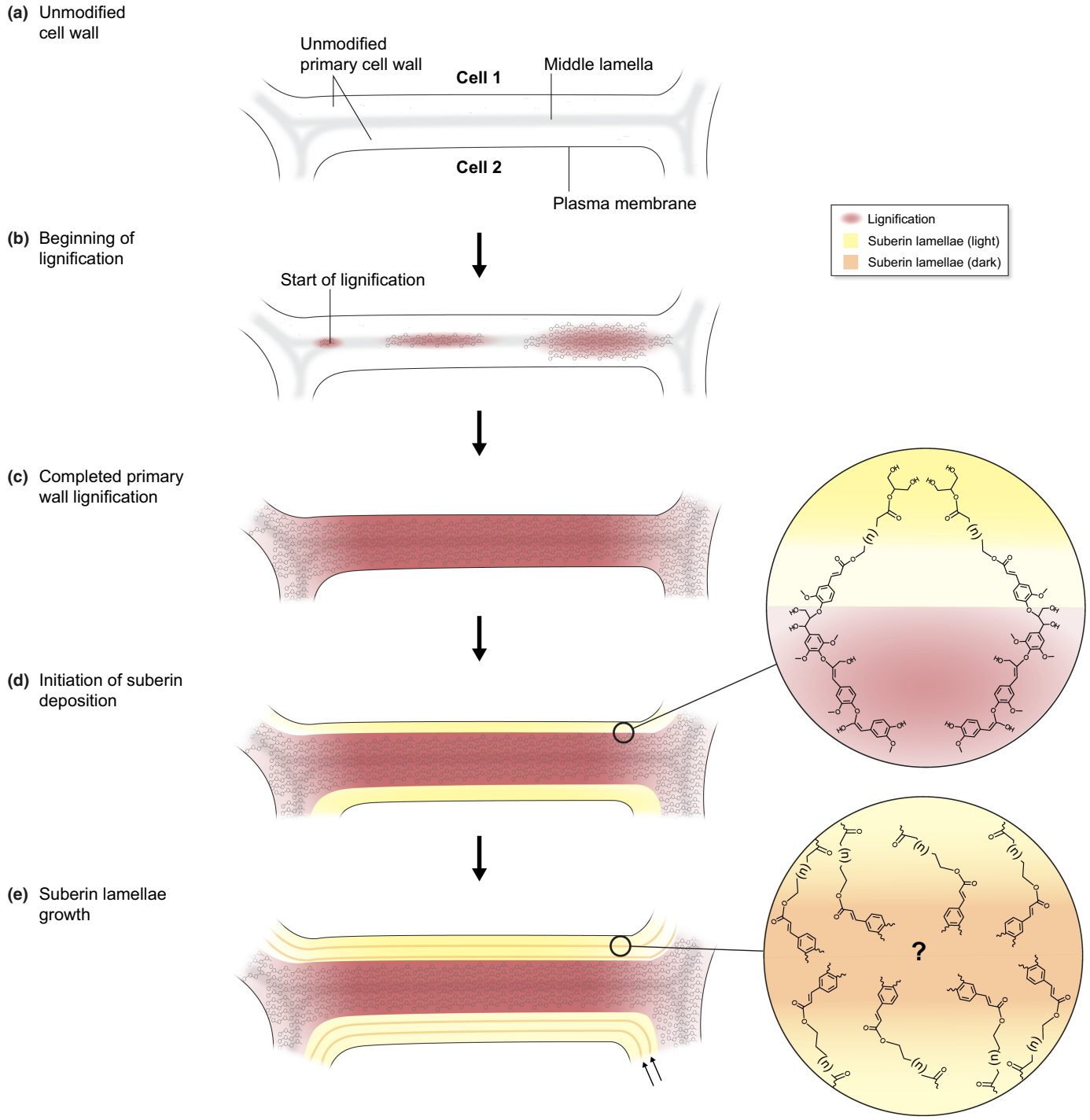


Fig. 4 Possible scenario for the spatiotemporal deposition and arrangement of suberin, lignin and ferulates within the suberised cell wall. Schematic, depicting the progression of cell-wall modifications in a nonspecified, suberising cell type (e.g. phellem). (a) Cell walls of undifferentiated cells, with unmodified primary walls and middle lamella (dark grey). (b) Lignification (red) of primary cell wall precedes suberisation and starts in the middle lamella. (c) Lignification eventually impregnates the entire primary wall. (d) Ferulates, possibly as aliphatic esters, become physically integrated into the lignin network through oxidative, lignin-like coupling. This would provide a 'priming surface' for suberin polyester growth by the transesterification of glycerylated aliphatic precursors to the ω-hydroxyacid carboxyl groups (Fig. 3) (ferulate group highlighted in bold in blow-up). (e) Formation of suberin lamellae (yellow and orange colours correspond to light and dark lamellae, respectively. Dark lamellae indicated by arrows. Enlarged image depicts current models, which propose that dark lamellae result from an increased presence of phenolics, predominantly or exclusively made of ferulates, but with a currently undetermined mode of coupling. Note that this would be a second role for ferulates in suberin formation, distinct from its priming/connectivity function to lignin, depicted in (d).

in significant amounts (Lapierre *et al.*, 1996; Graça, 2010; Lourenço *et al.*, 2016; Wunderling *et al.*, 2018). In systems that allow the induction of suberisation, such as wound-induced suberisation in potato tubers, it has been repeatedly observed that genes upregulated early after wounding are related to lignification, such as phenylalanine-ammonium lyase (PAL) or peroxidases and that suberin biosynthetic genes tend to be expressed at later stages (Lulai & Corsini, 1998; Bernards *et al.*, 2004; Lulai & Neubauer, 2014; Woolfson *et al.*, 2018). Indeed, despite lignin being described as a polymer associated with secondary cell walls, lignification is seen to start in pectin-rich cell corners and middle lamellae of primary cell walls (Fig. 4a–c). Typical suberin deposition forms inwardly of the primary cell wall (Sitte, 1962) and mostly after cells have stopped elongation. In this way, suberisation is a type of secondary wall formation, distinct from, and not requiring, cellulosic secondary wall formation. Nevertheless, suberin lamellae from neighbouring cells are separated by the primary cell walls of their cells (compare Fig. 4a, e). The functional importance of lignification in suberised tissues might therefore be to render the primary cell wall of suberised tissues more hydrophobic and resistant to chemical degradation, as well as to provide tissue cohesion by chemically bridging the suberin lamellae of individual cells with each other (Fig. 4c–e). Indeed, complete cell-wall digestion with polysaccharide-degrading enzymes leaves cells of suberised tissue connected to each other (Schreiber *et al.*, 2005) and the integrity of suberised tissues is compromised upon de-lignification (Pereira & Marques, 1988), supporting a role for lignin in providing tissue integrity. Connection of the primary wall lignin to the suberin lamellae is thought to be achieved by ferulic acid (Bernards, 2002; Graça, 2010). 2D-NMR combined with degradative techniques has been especially useful when describing the structure of the G-type lignin in cork cells and to demonstrate that it is rich in ferulic acid (Marques *et al.*, 2016). The presence of ferulic acid in both lignin (C–C and ether cross-linked) and suberin fractions (ester-linked to aliphatics) strongly supports a ferulate-mediated linking of suberin to the (lignified) primary cell wall (Fig. 4d, enlarged image). However, ferulates have also been shown to be covalently attached to nonlignin cell-wall components (Hatfield *et al.*, 1999) and could therefore also connect suberin to primary cell walls in cells with little, or locally restricted, lignification. An example would be the Arabidopsis endodermis, where there is the highly localised lignification of the Casparian strip, but little evidence for lignification outside the Casparian strip that would precede suberin formation (Naseer *et al.*, 2012; Lee *et al.*, 2013). Also in Arabidopsis, the relatively rapid induction of endodermal suberin closer to the root tip upon ABA treatment does not appear to be preceded by observable lignification (Barberon *et al.*, 2016). This led us to presume that suberin lamellae in the endodermis connect to a largely unlignified primary cell wall. However, it has been recently demonstrated that an endodermis with a suppressed phenylpropanoid pathway, lacking both monolignols and ferulic acids, was unable to correctly deposit new suberin, causing a droplet-like appearance in Fluorol Yellow stains. This could be complemented by feeding ferulic acid (but not caffeic acid or the mono-lignol *p*-coumaryl-alcohol) (Andersen *et al.*, 2021). Therefore, ferulic acid, but not lignin itself, appears to be required for the initiation of suberin deposition, possibly by providing connections to other primary cell-wall components and a nucleation point for aliphatic ester formation. The above findings seemingly conflict with the results obtained upon strong reduction of esterified ferulic acid of suberin (*asft* and FHT-RNAi) (Molina *et al.*, 2009; Serra *et al.*, 2010), for which no changes of suberin lamellae or their connection to the primary cell wall were observed with TEM, although suberin functionality was strongly affected. However, *ASFT* knockdowns (*FHT*-RNAi) displayed various additional changes, possibly originating from unpolymerised feruloyl-CoA and fatty acyl precursors, such as increases in soluble phenolics and primary alcohols, complex changes in esterified aliphatics, as well as effects on lignin composition (Serra *et al.*, 2014; Jin *et al.*, 2018). This indicates fascinating metabolic regulation and cross-talk between cell-wall components, but makes a straightforward interpretation of such biosynthetic mutants very difficult. Moreover, a similar genetic manipulation in maize, knocking out two *ASFT* paralogues, showed quite different phenotypes, such as suberin layer separation from the primary polysaccharide cell wall and more limited changes exclusively in aliphatics (Mertz *et al.*, 2020). This suggests that the specific role of ASFTs in suberin formation might very much depend on the cell type and species investigated. Nevertheless, the fact that ferulic acid showed cross-talk between suberin and lignin metabolism and that active phenylpropanoid production is needed to dynamically retain the suberin deposits in endodermis, as well as in phellem (Andersen *et al.*, 2021), all point to a tight interplay between lignin and suberin biosynthesis and deposition, with ferulic acid as a mediator between the two polymers.

3. Current models attempting to explain the lamellar structure of suberin

Different tentative models for suberin macromolecular structure have been proposed in an effort to integrate the results obtained using NMR, suberin full and partial depolymerisation and TEM observations of suberin as alternating light/dark lamellae (Kolattukudy, 1980, 2001; Bernards, 2002; Graça & Santos, 2007; Graça, 2015). The most recent model is the one proposed by Graça (2015), renamed as the 'integrated model' by Woolfson *et al.* (2022). In this model, the aliphatic portion of the suberin polyester is mostly restricted to the light lamellae structured by glycerol–α,ω-diacid–glycerol and also containing ω-hydroxyacid–glycerol units. The hydrocarbon chains of these aliphatics are thought to be 'stacked' or orderly arranged, accounting for the regular thickness of the light lamellae. These diacid and hydroxyacids would correspond to the dominant monomers in a given suberised tissue, which in potato are those derived from oleic acid (C18:1) and in suberised cotton fibres from docosanoic acid (C22). In cotton fibres, the treatment with an VLCFA elongase inhibitor showed thinner light lamellae, which would support the stacked distribution of the aliphatic compounds perpendicular to the lamellae plane (Schmutz *et al.*, 1996). In agreement with such a perpendicular arrangement, a mixture of ferulic acid, ω-hydroxyacid and glycerol was very recently shown to display nonenzymatic self-assembly and ester-bond formations, building

ferulic acid–ω-hydroxyacid–glycerol lamellar structures of *c.* 2.6 nm spacings (Kligman *et al.*, 2022), compatible with the light lamellae spacings reported previously (Schmutz *et al.*, 1996). The dark lamella would correspond to the lignin-like polymer rich in ferulic acid (termed polyaromatics) although locally crossed by aliphatic chains. Ferulic acid esterified to the ω-hydroxyacid–glycerol polyester unit would extensively and covalently link the light lamella (suberin) to the dark lamella (polyaromatics) through lignin-like linkages. When using this model as a framework, it is important to distinguish the postulated, lignin-like material of the dark lamellae from a, possibly more conventional, guaiacyl-rich lignin in the primary cell wall that we have described in the previous section. Unfortunately, it is exceedingly difficult to analyse, visualise or manipulate suberisation with a precision that would allow the separation of the primary cell-wall lignin from the postulated, 'dark lamella poly-aromatic'. The distortion of suberin lamellae observed in *CYP86A33*-RNAi potato suberin is correlated with a 70% and 90% reduction in C18:1 ω-hydroxyacid and α,ω-diacid compared with wild-type, a proportional reduction in the acylglycerols dimers and overrepresentation of ferulates (Serra *et al.*, 2009b; Graça *et al.*, 2015), that lends some support to this model. A different model has been proposed by Bernards (2002), named the 'two-domain model' in Woolfson *et al.* (2022), especially with respect to the nature of the dark lamella. Whilst in Graça's model the dark lamellae are made of lignin-like polymers, in Bernards' model they correspond to ferulic acid moieties forming part of the polyester. Both models concur in proposing ferulic acid as an important component of dark lamellae. The lignin-like nature of the dark lamellae is supported by the observed disappearance of electron opaque lamellae, replaced by empty spaces in suberised cotton fibres treated with phenylalanine ammonia lyase inhibitor (Schmutz *et al.*, 1993). But again, no changes in dark/light lamellation were observed in phellem suberin with highly reduced ferulic acid content (Molina *et al.*, 2009; Serra *et al.*, 2010). Therefore, the currently available data do not strongly support any of the proposed models concerning the lamellar structure of suberin, which should, for now, be taken as data-driven hypotheses rather than established facts. One prediction from Graça's model would be a requirement for ROS and peroxidases for the formation of dark lamellae, as this model postulates the presence of oxidatively coupled lignin-like polymers, while Bernard's model suggests exclusively esterified ferulates making up these dark lamellae, which would not require ROS or peroxidase activity for their formation. In our schematic (Fig. 4e, enlarged image), we have left open the putative nature of linkage of ferulates within the dark lamella (Fig. 4e, question mark).

4. Taking it back: suberin degradation in cell walls

One unexpected discovery of recent years is the large degree of plasticity of suberisation in endodermal cells of young roots, mediated by degradation of already formed suberin. This was first clearly demonstrated by studies on the effects of different nutrient deficiencies on suberisation in young Arabidopsis seedlings (Barberon *et al.*, 2016; Andersen *et al.*, 2018). In this case, deficiencies in metal ions, such as iron or manganese, led to a lower proportion of suberised endodermal cells in roots by inducing the degradation of pre-existing suberin in those cells. This disappearance of suberin requires a functional ethylene pathway and can be induced by ethylene treatments (Barberon *et al.*, 2016). How nutrient deficiency induces ethylene signalling and how this stress hormone causes degradation of suberin remains to be worked out and it is currently unclear how direct or indirect is the effect of ethylene. However, it had previously been found that endodermal suberin accumulation can be suppressed by overexpression of a single enzyme in the endodermis, demonstrating that induction of 'de-suberisation' by ethylene could, in principle, be very straightforward (Naseer *et al.*, 2012). Intriguingly, the enzyme originally found to induce de-suberisation belongs to the same family of enzymes as CUS1, the first cognate cutin synthase (Yeats *et al.*, 2014). This can be explained by the fact that a transesterification can be turned into a hydrolysis by simple switching the acceptor molecule from an aliphatic alcohol group to water, probably requiring only minor changes in enzyme structure.

More recently, additional members of this GELP family have been demonstrated to have the capacity for de-suberisation (Ursache *et al.*, 2021). Expression of these enzymes is clearly associated with another instance of de-suberisation, the emergence of lateral roots, which induces dramatic changes in endodermal structures, including a disappearance of suberin (Vermeer *et al.*, 2014). This new concept of reversible suberisation has been extended even further by recent findings that blocking suberin-promoting signals, such as ABA, can lead to the gradual disappearance of pre-existing suberin (Andersen *et al.*, 2018). Similar effects were obtained by chemically or genetically blocking the synthesis of ferulic acid (Andersen *et al.*, 2021). This suggests either a continuously ongoing de-suberisation – that would take over upon inhibition of suberisation – or that de-suberisation becomes induced upon blocking of suberisation. The surprisingly straightforward and widespread de-suberisation of Arabidopsis seedling roots seemingly contradicts the great chemical stability and recalcitrance of suberised tissue to degradation, mentioned in the Introduction. It is important to point out, however, that this phenomenon of de-suberisation might not occur in heavily lignosuberised tissues such as phellem, but may be restricted to the endodermis or, simply, to early stages of suberisation. It is indeed difficult to reconcile models of suberin that postulate an alternation of zones of oxidatively coupled polyaromatics with esterified polyaliphatics, with the observation that expression of a single esterase (Naseer *et al.*, 2012) appears to suffice for a complete degradation, or at least strong reduction, of suberin. The use of de-suberisation in response to environmental or developmental cues might also explain the conspicuous absence of evident cell-wall integrity or damage responses upon suberin degradation, responses that are often induced upon manipulations of cell-wall polymers such as cellulose or pectins.

IV. Outlook

Recent years have seen great strides in our understanding of the biosynthesis, transport, polymerisation, dynamic changes and *in vivo* structure of suberin, however major open questions remain for each of these areas. Indeed, as for any plant cell-wall structure,

the chemical complexity of the suberin polyester, its interconnection with other wall polymers and its nanoscale organisation are aspects that are extremely challenging to analyse. This is exacerbated by the insoluble, hydrophobic nature of suberin. These barriers to our understanding can only be overcome by novel or improved analytical technologies that are focused on the intact suberin polyester. Whilst mutant analysis cannot overcome these analytical limitations, it is powerful in challenging our current models. Better and quantitative assays for revealing suberin function in tissues will therefore be of great importance. Recent advances in genome editing will rapidly overcome the problems of genetic redundancy in suberin genes, especially in Arabidopsis, and provide new players and better phenotypes for suberin researchers. They will also hopefully extend genetic analyses to other organisms with suberised tissues not present in Arabidopsis, such as exodermis or suberised bundle sheath cells. Arabidopsis will nonetheless remain crucial for providing the rapid identification of novel players, providing a framework to guide work in other organisms. It will also allow the use of advanced genetic manipulations with spatial and temporal control, overcoming, or at least reducing, the protracted problem of pleiotropy that can confound interpretation of mutant phenotypes.

Suberin is gaining more and more attention as a polymer that provides crucial features to suberised tissues, allowing them to confer stress resistance and protection to various plant organs. Especially in roots, there is great hope that manipulating suberin levels and function in various tissues will enhance root function, potentially turning them into better carbon storage devices, improving their drought or salt resilience, and their resistance towards pathogen attacks, while modulating the interaction with beneficial microbiota. However, to fulfil these promises, we need to acknowledge that suberin itself still holds many mysteries and that more fundamental research into its formation, structure, stability and turnover is needed in order to understand this abundant and functionally important polymer of our biosphere.

Acknowledgements

We would like to thank Marie Barberon, Mercè Figueras, Inês Barbosa and Christiane Nawrath for critically reading the manuscript. We would also like to thank Tonni Andersen, Joop Vermeer, Robertas Ursache, Rochus Franke, Marisa Molinas, Mercè Figueras and many other colleagues for many years of stimulating discussions about suberin. Work in the laboratory of NG is currently funded by grants from the Swiss National Science Foundation and the European Research Council and work in the laboratory of OS from FEDER/Spanish Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación (PID2019-110330GB-C21 MCI/AEI/FEDER, UE). Open Access Funding provided by Université de Lausanne.

Author contributions

OS and NG both contributed equally to the conception, development and writing of this review. OS and NG contributed equally to this work.

ORCID

Niko Geldner https://orcid.org/0000-0002-2300-9644
Olga Serra https://orcid.org/0000-0002-1678-0932

References

Agrawal VP, Kolattukudy PE. 1977. Biochemistry of suberization: ω-hydroxyacid oxidation in enzyme preparations from suberizing potato tuber disks. *Plant Physiology* **59**: 667–672.

Alejandro S, Lee Y, Tohge T, Sudre D, Osorio S, Park J, Bovet L, Lee Y, Geldner N, Fernie A *et al.* 2012. AtABCG29 is a monolignol transporter involved in lignin biosynthesis. *Current Biology* **22**: 1207–1212.

An Q, Hückelhoven R, Kogel K-H, Bel AJEV. 2006. Multivesicular bodies participate in a cell wall-associated defence response in barley leaves attacked by the pathogenic powdery mildew fungus. *Cellular Microbiology* **8**: 1009–1019.

Andersen TG, Molina D, Kilian J, Franke RB, Ragni L, Geldner N. 2021. Tissue-autonomous phenylpropanoid production is essential for establishment of root barriers. *Current Biology* **31**: 965–977.

Andersen TG, Naseer S, Ursache R, Wybouw B, Smet W, De Rybel B, Vermeer JEM, Geldner N. 2018. Diffusible repression of cytokinin signalling produces endodermal symmetry and passage cells. *Nature* **555**: 529–533.

Ayaz A, Saqib S, Huang H, Zaman W, Lü S, Zhao H. 2021. Genome-wide comparative analysis of long-chain acyl-CoA synthetases (LACSs) gene family: a focus on identification, evolution and expression profiling related to lipid synthesis. *Plant Physiology and Biochemistry* **161**: 1–11.

Barberon M. 2017. The endodermis as a checkpoint for nutrients. *New Phytologist* **213**: 1604–1610.

Barberon M, Vermeer J, De Bellis D, Wang P, Naseer S, Andersen T, Humbel B, Nawrath C, Takano J, Salt D *et al.* 2016. Adaptation of root function by nutrient-induced plasticity of endodermal differentiation. *Cell* **164**: 1–13.

Bar-On YM, Phillips R, Milo R. 2018. The biomass distribution on Earth. *Proceedings of the National Academy of Sciences, USA* **115**: 6506–6511.

Batsale M, Bahammou D, Fouillen L, Mongrand S, Joubès J, Domergue F. 2021. Biosynthesis and functions of very-long-chain fatty acids in the responses of plants to abiotic and biotic stresses. *Cells* **10**: 1284.

Beisson F, Li Y, Bonaventure G, Pollard M, Ohlrogge JB. 2007. The acyltransferase GPAT5 is required for the synthesis of suberin in seed coat and root of Arabidopsis. *Plant Cell* **19**: 351–368.

Beisson F, Li-Beisson Y, Pollard M. 2012. Solving the puzzles of cutin and suberin polymer biosynthesis. *Current Opinion in Plant Biology* **15**: 329–337.

Bento A, Moreira CJS, Correia VG, Escórcio R, Rodrigues R, Tomé AS, Geneix N, Petit J, Bakan B, Rothan C *et al.* 2021. Quantification of structure-property relationships for plant polyesters reveals suberin and cutin idiosyncrasies. *ACS Sustainable Chemistry & Engineering* **9**: 15780–15792.

Bento MF, Pereira H, Cunha MÁ, Moutinho AMC, van den Berg KJ, Boon JJ. 1998. Thermally assisted transmethylation gas chromatography–mass spectrometry of suberin components in cork from *Quercus suber* L. *Phytochemical Analysis* **9**: 75–87.

Benveniste I, Tijet N, Adas F, Philipps G, Salaün J-P, Durst F. 1998. CYP86A1 from *Arabidopsis thaliana* encodes a cytochrome P450-dependent fatty acid omega-hydroxylase. *Biochemical and Biophysical Research Communications* **243**: 688–693.

Bernards MA. 2002. Demystifying suberin. *Canadian Journal of Botany* **80**: 227–240.

Bernards MA, Summerhurst DK, Razem FA. 2004. Oxidases, peroxidases and hydrogen peroxide: the suberin connection. *Phytochemistry Reviews* **3**: 113–126.

Biała W, Jasiński M. 2018. The phenylpropanoid case – it is transport that matters. *Frontiers in Plant Science* **9**: 1610.

Bird D, Beisson F, Brigham A, Shin J, Greer S, Jetter R, Kunst L, Wu X, Yephremov A, Samuels L. 2007. Characterization of Arabidopsis ABCG11/WBC11, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. *The Plant Journal* **52**: 485–498.

Boher P, Serra O, Soler M, Molinas M, Figueras M. 2013. The potato suberin feruloyl transferase FHT which accumulates in the phellogen is induced by wounding and regulated by abscisic and salicylic acids. *Journal of Experimental Botany* **64**: 3225–3236.

Boutté Y, Jaillais Y. 2020. Metabolic cellular communications: feedback mechanisms between membrane lipid homeostasis and plant development. *Developmental Cell* **54**: 171–182.

Brundrett MC, Kendrick B, Peterson CA. 1991. Efficient lipid staining in plant material with sudan red 7b or fluoral yellow 088 in polyethylene glycol-glycerol. *Biotechnic & Histochemistry* **66**: 111–116.

Campilho A, Nieminen K, Ragni L. 2020. The development of the periderm: the final frontier between a plant and its environment. *Current Opinion in Plant Biology* **53**: 10–14.

Compagnon V, Diehl P, Benveniste I, Meyer D, Schaller H, Schreiber L, Franke R, Pinot F. 2009. CYP86B1 is required for very long chain ω-hydroxyacid and α, ω-dicarboxylic acid synthesis in root and seed suberin polyester. *Plant Physiology* **150**: 1831–1843.

Correia VG, Bento A, Pais J, Rodrigues R, Haliński ŁP, Frydrych M, Greenhalgh A, Stepnowski P, Vollrath F, King A *et al.* 2020. The molecular structure and multifunctionality of the cryptic plant polymer suberin. *Materials Today Bio* **5**: 100039.

Cui Y, Gao J, He Y, Jiang L. 2020. Plant extracellular vesicles. *Protoplasma* **257**: 3–12.

Danila FR, Thakur V, Chatterjee J, Bala S, Coe RA, Acebron K, Furbank RT, von Caemmerer S, Quick WP. 2021. Bundle sheath suberisation is required for C_4 photosynthesis in a *Setaria viridis* mutant. *Communications Biology* **4**: 254.

De Bellis D, Kalmbach L, Marhavý P, Daraspe J, Geldner N, Barberon M. 2021. Extracellular membrane tubules involved in suberin deposition in plant cell walls. *bioRxiv*. doi: 10.1101/2021.02.02.429332.

Deeken R, Saupe S, Klinkenberg J, Riedel M, Leide J, Hedrich R, Mueller TD. 2016. The nonspecific lipid transfer protein AtltpI-4 is involved in suberin formation of *Arabidopsis thaliana* crown galls. *Plant Physiology* **172**: 1911–1927.

Domergue F, Vishwanath SJ, Joubes J, Ono J, Lee JA, Bourdon M, Alhattab R, Lowe C, Pascal S, Lessire R *et al.* 2010. Three Arabidopsis fatty acyl-coenzyme A reductases, FAR1, FAR4, and FAR5, generate primary fatty alcohols associated with suberin deposition. *Plant Physiology* **153**: 1539–1554.

Edstam MM, Edqvist J. 2014. Involvement of GPI-anchored lipid transfer proteins in the development of seed coats and pollen in *Arabidopsis thaliana*. *Physiologia Plantarum* **152**: 32–42.

Elejalde-Palmett C, Martinez San Segundo I, Garroum I, Charrier L, De Bellis D, Mucciolo A, Guerault A, Liu J, Zeisler-Diehl V, Aharoni A *et al.* 2021. ABCG transporters export cutin precursors for the formation of the plant cuticle. *Current Biology* **24**: 2111–2123.

Farmer EE. 2014. *Leaf defence*. Oxford, UK: Oxford University Press.

Fedi F, O'Neill CM, Menard G, Trick M, Dechirico S, Corbineau F, Bailly C, Eastmond PJ, Penfield S. 2017. *Awake1*, an ABC-type transporter, reveals an essential role for suberin in the control of seed dormancy. *Plant Physiology* **174**: 276–283.

Fich EA, Segerson NA, Rose JKC. 2016. The plant polyester cutin: biosynthesis, structure, and biological roles. *Annual Review of Plant Biology* **67**: 207–233.

Floudas D, Binder M, Riley R, Barry K, Blanchette RA, Henrissat B, Martínez AT, Otillar R, Spatafora JW, Yadav JS *et al.* 2012. The paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science* **336**: 1715–1719.

Franke R, Briesen I, Wojciechowski T, Faust A, Yephremov A, Nawrath C, Schreiber L. 2005. Apoplastic polyesters in Arabidopsis surface tissues – a typical suberin and a particular cutin. *Phytochemistry* **66**: 2643–2658.

Franke R, Höfer R, Briesen I, Emsermann M, Efremova N, Yephremov A, Schreiber L. 2009. The DAISY gene from Arabidopsis encodes a fatty acid elongase condensing enzyme involved in the biosynthesis of aliphatic suberin in roots and the chalaza-micropyle region of seeds. *The Plant Journal* **57**: 80–95.

Geldner N. 2013. The endodermis. *Annual Review of Plant Biology* **64**: 531–558.

Gil AM, Lopes M, Rocha J, Neto CP. 1997. A ^{13}C solid state nuclear magnetic resonance spectroscopic study of cork cell wall structure: the effect of suberin removal. *International Journal of Biological Macromolecules* **20**: 293–305.

Gou J-Y, Yu X-H, Liu C-J. 2009. A hydroxycinnamoyltransferase responsible for synthesizing suberin aromatics in Arabidopsis. *Proceedings of the National Academy of Sciences, USA* **106**: 18855–18860.

Graça J. 2010. Hydroxycinnamates in suberin formation. *Phytochemistry Reviews* **9**: 85–91.

Graça J. 2015. Suberin: the biopolyester at the frontier of plants. *Frontiers in Chemistry* **3**: 1–11.

Graça J, Cabral V, Santos S, Lamosa P, Serra O, Molinas M, Schreiber L, Kauder F, Franke R. 2015. Partial depolymerization of genetically modified potato tuber periderm reveals intermolecular linkages in suberin polyester. *Phytochemistry* **117**: 209–219.

Graça J, Pereira H. 1997. Cork suberin: a glycerol based polyester. *Holzforschung* **51**: 225–234.

Graça J, Pereira H. 1999. Glyceryl-acyl and aryl-acyl dimers in *Pseudotsuga menziesii* bark suberin. *Holzforschung* **53**: 397–402.

Graça J, Pereira H. 2000. Suberin structure in potato periderm: glycerol, long-chain monomers, and glyceryl and feruloyl dimers. *Journal of Agricultural and Food Chemistry* **48**: 5476–5483.

Graça J, Santos S. 2007. Suberin: a biopolyester of plants' skin. *Macromolecular Bioscience* **7**: 128–135.

Han J, Clement JM, Li J, King A, Ng S, Jaworski JG. 2010. The cytochrome P450 CYP86A22 is a fatty acyl-CoA ω-hydroxylase essential for estolide synthesis in the stigma of *Petunia hybrida*. *The Journal of Biological Chemistry* **285**: 3986–3996.

Harman-Ware AE, Sparks S, Addison B, Kalluri UC. 2021. Importance of suberin biopolymer in plant function, contributions to soil organic carbon and in the production of bio-derived energy and materials. *Biotechnology for Biofuels* **14**: 75.

Haslam TM, Kunst L. 2013. Extending the story of very-long-chain fatty acid elongation. *Plant Science* **210**: 93–107.

Hatfield RD, Ralph J, Grabber JH. 1999. Cell wall cross-linking by ferulates and diferulates in grasses. *Journal of the Science of Food and Agriculture* **79**: 403–407.

Höfer R, Briesen I, Beck M, Pinot F, Schreiber L, Franke R. 2008. The Arabidopsis cytochrome P450 CYP86A1 encodes a fatty acid omega-hydroxylase involved in suberin monomer biosynthesis. *Journal of Experimental Botany* **59**: 2347–2360.

Holbein J, Shen D, Andersen TG. 2021. The endodermal passage cell – just another brick in the wall? *New Phytologist* **230**: 1321–1328.

Jenkins AD, Kratochvíl P, Stepto RFT, Suter UW. 1996. Glossary of basic terms in polymer science (IUPAC Recommendations 1996). *Pure and Applied Chemistry* **68**: 2287–2311.

Jin L, Cai Q, Huang W, Dastmalchi K, Rigau J, Molinas M, Figueras M, Serra O, Stark RE. 2018. Potato native and wound periderms are differently affected by down-regulation of FHT, a suberin feruloyl transferase. *Phytochemistry* **147**: 30–48.

Kligman A, Dastmalchi K, Smith S, John G, Stark RE. 2022. Building blocks of the protective suberin plant polymer self-assemble into lamellar structures with antibacterial potential. *ACS Omega* **7**: 3978–3989.

Kolattukudy PE. 1980. Biopolyester membranes of plants: cutin and suberin. *Science* **208**: 990–1000.

Kolattukudy PE. 2001. Polyesters in higher plants. In: Babel W, Steinbüchel A, eds. *Biopolyesters. Advances in biochemical engineering/biotechnology, vol. 71*. Berlin & Heidelberg, Germany: Springer.

Kosma DK, Molina I, Ohlrogge JB, Pollard M. 2012. Identification of an Arabidopsis fatty alcohol:caffeoyl-coenzyme A acyltransferase required for the synthesis of alkyl hydroxycinnamates in root waxes. *Plant Physiology* **160**: 237–248.

Krishnamurthy P, Vishal B, Bhal A, Kumar PP. 2021. WRKY9 transcription factor regulates cytochrome P450 genes *CYP94B3* and *CYP86B1*, leading to increased root suberin and salt tolerance in Arabidopsis. *Physiologia Plantarum* **172**: 1673–1687.

Krishnamurthy P, Vishal B, Ho WJ, Lok FCJ, Lee FSM, Kumar PP. 2020. Regulation of a cytochrome P450 gene *CYP94B1* by WRKY33 transcription factor controls apoplastic barrier formation in roots to confer salt tolerance. *Plant Physiology* **184**: 2199–2215.

Krömer K. 1903. Wurzelhaut, hypodermis und endodermis der angiospermenwurzel. In: Luerssen C, ed. *Bibliotheca botanica, vol. 59*. Stuttgart, Germany: Erwin Nägele Verlag.

Kurdyukov S, Faust A, Trenkamp S, Bär S, Franke R, Efremova N, Tietjen K, Schreiber L, Saedler H, Yephremov A. 2006. Genetic and biochemical evidence

for involvement of HOTHEAD in the biosynthesis of long-chain alpha-, omega-dicarboxylic fatty acids and formation of extracellular matrix. *Planta* **224**: 315–329.

Kurihara D, Mizuta Y, Sato Y, Higashiyama T. 2015. ClearSee: a rapid optical clearing reagent for whole-plant fluorescence imaging. *Development* **142**: 4168–4179.

Landgraf R, Smolka U, Altmann S, Eschen-Lippold L, Senning M, Sonnewald S, Weigel B, Frolova N, Strehmel N, Hause G *et al.* 2014. The ABC transporter ABCG1 is required for suberin formation in potato tuber periderm. *Plant Cell* **13**: 1–13.

Lapierre C, Pollet B, Négrel J. 1996. The phenolic domain of potato suberin: structural comparison with lignins. *Phytochemistry* **42**: 949–953.

Lee SB, Jung SJ, Go YS, Kim HU, Kim JK, Cho HJ, Park OK, Suh MC. 2009. Two Arabidopsis 3-ketoacyl CoA synthase genes, KCS20 and KCS2/DAISY, are functionally redundant in cuticular wax and root suberin biosynthesis, but differentially controlled by osmotic stress. *Plant Journal* **60**: 462–475.

Lee SB, Suh M-C. 2018. Disruption of glycosylphosphatidylinositol-anchored lipid transfer protein 15 affects seed coat permeability in Arabidopsis. *The Plant Journal* **96**: 1206–1217.

Lee Y, Rubio MC, Alassimone J, Geldner N. 2013. A mechanism for localized lignin deposition in the endodermis. *Cell* **153**: 402–412.

Li N, Xu C, Li-Beisson Y, Philippar K. 2016. Fatty acid and lipid transport in plant cells. *Trends in Plant Science* **21**: 145–158.

Li Y, Beisson F, Koo AJK, Molina I, Pollard M, Ohlrogge J. 2007. Identification of acyltransferases required for cutin biosynthesis and production of cutin with suberin-like monomers. *Proceedings of the National Academy of Sciences, USA* **104**: 18339–18344.

Li-Beisson Y, Shorrosh B, Beisson F, Andersson MX, Arondel V, Bates PD, Baud S, Bird D, DeBono A, Durrett TP *et al.* 2013. Acyl-lipid metabolism. *The Arabidopsis Book* **11**: e0161.

Lourenço A, Rencoret J, Chemetova C, Gominho J, Gutiérrez A, del Río JC, Pereira H. 2016. Lignin composition and structure differs between xylem, phloem and phellem in *Quercus suber* L. *Frontiers in Plant Science* **7**: 1612.

Lulai E, Corsini D. 1998. Differential deposition of suberin phenolic and aliphatic domains and their roles in resistance to infection during potato tuber (*Solanum tuberosum* L.) wound-healing. *Physiological and Molecular Plant Pathology* **53**: 209–222.

Lulai EC, Neubauer JD. 2014. Wound-induced suberization genes are differentially expressed, spatially and temporally, during closing layer and wound periderm formation. *Postharvest Biology and Technology* **90**: 24–33.

Lux A, Morita S, Abe J, Ito K. 2005. An improved method for clearing and staining free-hand sections and whole-mount samples. *Annals of Botany* **96**: 989–996.

Ma F, Peterson CA. 2001. Development of cell wall modifications in the endodermis and exodermis of *Allium cepa* roots. *Canadian Journal of Botany* **79**: 621–634.

Marques AV, Pereira H. 2013. Lignin monomeric composition of corks from the barks of *Betula pendula, Quercus suber* and *Quercus cerris* determined by Py–GC–MS/FID. *Journal of Analytical and Applied Pyrolysis* **100**: 88–94.

Marques AV, Rencoret J, Gutiérrez A, del Río JC, Pereira H. 2016. Ferulates and lignin structural composition in cork. *Holzforschung* **70**: 275–289.

McFarlane HE, Shin JJH, Bird D, Samuels L. 2010. Arabidopsis ABCG transporters, which are required for export of diverse cuticular lipids, dimerize in different combinations. *Plant Cell* **22**: 3066–3075.

Mertz RA, Ellsworth P, Ellsworth P, Tausta SL, von Caemmerer S, Berg RH, Nelson T, Carpita NC, Brutnell TP, Cousins AB. 2020. The maize Aliphatic Suberin Feruloyl Transferase genes affect leaf water movement but are dispensable for bundle sheath CO_2 concentration. *bioRxiv*. doi: 10.1101/2020.09.30.321265.

Miao Y-C, Liu C-J. 2010. ATP-binding cassette-like transporters are involved in the transport of lignin precursors across plasma and vacuolar membranes. *Proceedings of the National Academy of Sciences, USA* **107**: 22728–22733.

Moire L, Schmutz A, Buchala A, Yan B, Stark RE, Ryser U. 1999. Glycerol is a suberin monomer. New experimental evidence for an old hypothesis. *Plant Physiology* **119**: 1137–1146.

Molina I, Bonaventure G, Ohlrogge J, Pollard M. 2006. The lipid polyester composition of *Arabidopsis thaliana* and *Brassica napus* seeds. *Phytochemistry* **67**: 2597–2610.

Molina I, Li-Beisson Y, Beisson F, Ohlrogge JB, Pollard M. 2009. Identification of an Arabidopsis feruloyl-coenzyme a transferase required for suberin synthesis. *Plant Physiology* **151**: 1317–1328.

Molino D, Nascimbeni AC, Giordano F, Codogno P, Morel E. 2017. ER-driven membrane contact sites: evolutionary conserved machineries for stress response and autophagy regulation? *Communicative & Integrative Biology* **10**: e1401699.

Naseer S, Lee Y, Lapierre C, Franke R, Nawrath C, Geldner N. 2012. Casparian strip diffusion barrier in Arabidopsis is made of a lignin polymer without suberin. *Proceedings of the National Academy of Sciences, USA* **109**: 10101–10106.

Oparka KJ, Gates PJ. 1982. Ultrastructure of the developing pigment strand of rice (*Oryza sativa* L.) in relation to its role in solute transport. *Protoplasma* **113**: 33–43.

Panikashvili D, Shi JX, Bocobza S, Franke RB, Schreiber L, Aharoni A. 2010. The Arabidopsis DSO/ABCG11 transporter affects cutin metabolism in reproductive organs and suberin in roots. *Molecular Plant* **3**: 563–575.

Pereira H, Marques AV. 1988. The effect of chemical treatments on the cellular structure of cork. *IAWA Journal* **9**: 337–345.

Peterson CA, Peterson RL, Robards AW. 1978. A correlated histochemical and ultrastructural study of the epidermis and hypodermis of onion roots. *Protoplasma* **96**: 1–21.

Philippe G, Sørensen I, Jiao C, Sun X, Fei Z, Domozych DS, Rose JK. 2020. Cutin and suberin: assembly and origins of specialized lipidic cell wall scaffolds. *Current Opinion in Plant Biology* **55**: 11–20.

Ranathunge K, Schreiber L, Franke R. 2011. Suberin research in the genomics era —new interest for an old polymer. *Plant Science* **180**: 399–413.

Renard J, Martínez-Almonacid I, Queralta Castillo I, Sonntag A, Hashim A, Bissoli G, Campos L, Muñoz-Bertomeu J, Niñoles R, Roach T *et al.* 2021. Apoplastic lipid barriers regulated by conserved homeobox transcription factors extend seed longevity in multiple plant species. *New Phytologist* **23**: 679–694.

Renault H, Alber A, Horst NA, Basilio Lopes A, Fich EA, Kriegshauser L, Wiedemann G, Ullmann P, Herrgott L, Erhardt M *et al.* 2017. A phenol-enriched cuticle is ancestral to lignin evolution in land plants. *Nature Communications* **8**: 14713.

Schmutz A, Buchala AJ, Ryser U. 1996. Changing the dimensions of suberin lamellae of green cotton fibers with a specific inhibitor of the endoplasmic reticulum-associated fatty acid elongases. *Plant Physiology* **110**: 403–411.

Schmutz A, Jenny T, Amrhein N, Ryser U. 1993. Caffeic acid and glycerol are constituents of the suberin layers in green cotton fibres. *Planta* **189**: 453.

Schnurr J, Shockey J, Browse J. 2004. The acyl-CoA synthetase encoded by LACS2 is essential for normal cuticle development in Arabidopsis. *Plant Cell* **16**: 629–642.

Schnurr JA, Shockey JM, de Boer G-J, Browse JA. 2002. Fatty acid export from the chloroplast. Molecular characterization of a major plastidial acyl-coenzyme A synthetase from Arabidopsis. *Plant Physiology* **129**: 1700–1709.

Schreiber L, Franke R, Hartmann K. 2005. Wax and suberin development of native and wound periderm of potato (*Solanum tuberosum* L.) and its relation to peridermal transpiration. *Planta* **220**: 520–530.

Scott MG, Peterson RL. 1979. The root endodermis in *Ranunculus acris*. I. Structure and ontogeny. *Canadian Journal of Botany* **57**: 1040–1062.

Serra O, Chatterjee S, Figueras M, Molinas M, Stark RE. 2014. Deconstructing a plant macromolecular assembly: chemical architecture, molecular flexibility, and mechanical performance of natural and engineered potato suberins. *Biomacromolecules* **15**: 799–811.

Serra O, Chatterjee S, Huang W, Stark RE. 2012. Mini-review: what nuclear magnetic resonance can tell us about protective tissues. *Plant Science* **195**: 120–124.

Serra O, Hohn C, Franke R, Prat S, Molinas M, Figueras M. 2010. A feruloyl transferase involved in the biosynthesis of suberin and suberin-associated wax is required for maturation and sealing properties of potato periderm. *The Plant Journal* **62**: 277–290.

Serra O, Mähönen AP, Hetherington AJ, Ragni L. 2022. The making of plant armor: the periderm. *Annual Review of Plant Biology* **73**: 405–432.

Serra O, Soler M, Hohn C, Franke R, Schreiber L, Prat S, Molinas M, Figueras M. 2009a. Silencing of *StKCS6* in potato periderm leads to reduced chain lengths

of suberin and wax compounds and increased peridermal transpiration. *Journal of Experimental Botany* **60**: 697–707.

Serra O, Soler M, Hohn C, Sauveplane V, Pinot F, Franke R, Schreiber L, Prat S, Molinas M, Figueras M. 2009b. *CYP86A33*-targeted gene silencing in potato tuber alters suberin composition, distorts suberin lamellae, and impairs the periderm's water barrier function. *Plant Physiology* **149**: 1050–1060.

Sexauer M, Shen D, Schön M, Andersen TG, Markmann K. 2021. Visualizing polymeric components that define distinct root barriers across plant lineages. *Development* **148**: dev199820.

Shanmugarajah K, Linka N, Gräfe K, Smits SHJ, Weber APM, Zeier J, Schmitt L. 2019. ABCG1 contributes to suberin formation in *Arabidopsis thaliana* roots. *Scientific Reports* **9**: 1–12.

Shiono K, Ando M, Nishiuchi S, Takahashi H, Watanabe K, Nakamura M, Matsuo Y, Yasuno N, Yamanouchi U, Fujimoto M *et al.* 2014. RCN1/OsABCG5, an ATP-binding cassette (ABC) transporter, is required for hypodermal suberization of roots in rice (*Oryza sativa*). *The Plant Journal* **80**: 40–51.

Shukla V, Barberon M. 2021. Building and breaking of a barrier: suberin plasticity and function in the endodermis. *Current Opinion in Plant Biology* **64**: 102153.

Shukla V, Han J-P, Cléard F, Lefebvre-Legendre L, Gully K, Flis P, Berhin A, Andersen TG, Salt DE, Nawrath C *et al.* 2021. Suberin plasticity to developmental and exogenous cues is regulated by a set of MYB transcription factors. *Proceedings of the National Academy of Sciences, USA* **118**: e2101730118.

Sitte P. 1962. Zum feinbau der suberinschichten im flaschenkork. *Protoplasma* **54**: 555–559.

Stark RE, Garbow JR. 1992. Nuclear magnetic resonance relaxation studies of plant polyester dynamics. 2. Suberized potato cell wall. *Macromolecules* **25**: 149–154.

Thomas R, Fang X, Ranathunge K, Anderson TR, Peterson CA, Bernards MA. 2007. Soybean root suberin: anatomical distribution, chemical composition, and relationship to partial resistance to *Phytophthora sojae*. *Plant Physiology* **144**: 299–311.

Todd J, Post-Beittenmiller D, Jaworski JG. 1999. KCS1 encodes a fatty acid elongase 3-ketoacyl-CoA synthase affecting wax biosynthesis in *Arabidopsis thaliana*. *The Plant Journal* **17**: 119–130.

Ursache R, Andersen TG, Marhavý P, Geldner N. 2018. A protocol for combining fluorescent proteins with histological stains for diverse cell wall components. *The Plant Journal* **93**: 399–412.

Ursache R, De Jesus Vieira Teixeira C, Dénervaud Tendon V, Gully K, De Bellis D, Schmid-Siegert E, Grube Andersen T, Shekhar V, Calderon S, Pradervand S *et al.* 2021. GDSL-domain proteins have key roles in suberin polymerization and degradation. *Nature Plants* **7**: 353–364.

Vane CH, Drage TC, Snape CE. 2006. Bark decay by the white-rot fungus *Lentinula edodes*: polysaccharide loss, lignin resistance and the unmasking of suberin. *International Biodeterioration & Biodegradation* **57**: 14–23.

Vanholme R, De Meester B, Ralph J, Boerjan W. 2019. Lignin biosynthesis and its integration into metabolism. *Current Opinion in Biotechnology* **56**: 230–239.

Verbančič J, Lunn JE, Stitt M, Persson S. 2018. Carbon supply and the regulation of cell wall synthesis. *Molecular Plant* **11**: 75–94.

Vermeer JEM, von Wangenheim D, Barberon M, Lee Y, Stelzer EHK, Maizel A, Geldner N. 2014. A spatial accommodation by neighboring cells is required for organ initiation in Arabidopsis. *Science* **343**: 178–183.

Vishwanath SJ, Delude C, Domergue F, Rowland O. 2015. Suberin: biosynthesis, regulation, and polymer assembly of a protective extracellular barrier. *Plant Cell Reports* **34**: 573–586.

Vishwanath SJ, Kosma DK, Pulsifer IP, Scandola S, Pascal S, Joubès J, Dittrich-Domergue F, Lessire R, Rowland O, Domergue F. 2013. Suberin-associated fatty alcohols in Arabidopsis: distributions in roots and contributions to seed coat barrier properties. *Plant Physiology* **163**: 1118–1132.

Wang P, Hawes C, Hussey PJ. 2017. Plant endoplasmic reticulum-plasma membrane contact sites. *Trends in Plant Science* **22**: 289–297.

Wang Y, Xu J, He Z, Hu N, Luo W, Liu X, Shi X, Liu T, Jiang Q, An P *et al.* 2021. BdFAR4, a root-specific fatty acyl-coenzyme A reductase, is involved in fatty alcohol synthesis of root suberin polyester in *Brachypodium distachyon*. *The Plant Journal* **106**: 1468–1483.

Wattendorff J. 1974. The formation of cork cells in the periderm of *Acacia senegal* Willd. and their ultrastructure during suberin deposition. *Zeitschrift für Pflanzenphysiologie* **72**: 119–134.

Wei X, Lu W, Mao L, Han X, Wei X, Zhao X, Xia M, Xu C, Costa F. 2020. ABF2 and MYB transcription factors regulate feruloyl transferase FHT involved in ABA-mediated wound suberization of kiwifruit. *Journal of Experimental Botany* **71**: 305–317.

Winkel BSJ. 2004. Metabolic channeling in plants. *Annual Review of Plant Biology* **55**: 85–107.

Woolfson KN, Esfandiari M, Bernards MA. 2022. Suberin biosynthesis, assembly, and regulation. *Plants* **11**: 555.

Woolfson KN, Haggitt ML, Zhang Y, Kachura A, Bjelica A, Rey Rincon MA, Kaberi KM, Bernards MA. 2018. Differential induction of polar and non-polar metabolism during wound-induced suberization in potato (*Solanum tuberosum* L.) tubers. *The Plant Journal* **93**: 931–942.

Wunderling A, Ripper D, Barra-Jimenez A, Mahn S, Sajak K, Targem MB, Ragni L. 2018. A molecular framework to study periderm formation in Arabidopsis. *New Phytologist* **219**: 216–229.

Yadav V, Molina I, Ranathunge K, Castillo IQ, Rothstein SJ, Reed JW. 2014. ABCG transporters are required for suberin and pollen wall extracellular barriers in Arabidopsis. *Plant Cell* **26**: 3569–3588.

Yan B, Stark RE. 2000. Biosynthesis, molecular structure, and domain architecture of potato suberin: a ^{13}C NMR study using isotopically labeled precursors. *Journal of Agricultural and Food Chemistry* **48**: 3298–3304.

Yang W, Pollard M, Li-Beisson Y, Beisson F, Feig M, Ohlrogge J. 2010. A distinct type of glycerol-3-phosphate acyltransferase with sn-2 preference and phosphatase activity producing 2-monoacylglycerol. *Proceedings of the National Academy of Sciences, USA* **107**: 12040–12045.

Yang W, Simpson JP, Li-Beisson Y, Beisson F, Pollard M, Ohlrogge JB. 2012. A land-plant-specific glycerol-3-phosphate acyltransferase family in Arabidopsis: substrate specificity, sn-2 preference, and evolution. *Plant Physiology* **160**: 638–652.

Yeats TH, Huang W, Chatterjee S, Viart HM-F, Clausen MH, Stark RE, Rose JKC. 2014. Tomato cutin deficient 1 (CD1) and putative orthologs comprise an ancient family of cutin synthase-like (CUS) proteins that are conserved among land plants. *The Plant Journal* **77**: 667–675.

Yeats TH, Martin LBB, Viart HM-F, Isaacson T, He Y, Zhao L, Matas AJ, Buda GJ, Domozych DS, Clausen MH *et al.* 2012. The identification of cutin synthase: formation of the plant polyester cutin. *Nature Chemical Biology* **8**: 609–611.