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**How to cite**

SHUKLA, Vinay, BARBERON, Marie. Building and breaking of a barrier: Suberin plasticity and function in the endodermis. In: Current opinion in plant biology, 2021, vol. 64, p. 102153. doi: 10.1016/j.pbi.2021.102153

This publication URL: https://archive-ouverte.unige.ch/unige:168729
Publication DOI: 10.1016/j.pbi.2021.102153

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

# Building and breaking of a barrier: Suberin plasticity and function in the endodermis

Vinay Shukla and Marie Barberon

**Abstract**

Plant cells coated with hydrophobic compounds constitute a protective barrier to control movement of materials through plant tissues. In roots, the endodermis develops two barriers: the Casparian strips establish an apoplastic barrier and suberin lamellae prevent diffusion through the plasma membrane. Suberin is a complex biopolymer and its deposition is highly responsive to the environment. While the enzymatic framework involved in suberin biosynthesis is well characterized, subsequent steps in suberin formation and regulation remained elusive. Recent publications, studying suberin from a cell biological perspective, have enriched our knowledge on suberin transport and polymerization in the cell wall. These studies have also elucidated the molecular mechanisms controlling suberin biosynthesis and regulation as well as its physiological role in plant abiotic and biotic interactions.

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**Current Opinion in Plant Biology** 2021, **64:**102153

This review comes from a themed issue on **Cell Biology 2021**

Edited by **Siobhan Braybrook** and **David Mendoza-Cózatl**

For a complete overview see the Issue

Available online 30 November 2021

**https://doi.org/10.1016/j.pbi.2021.102153**



**Keywords**

Suberin, Endodermis, Transport, Secretion, Polymerization, MYB, Nutrients.

## Introduction

Similarly to the animal skin, plants require a first line of defense to face the environment. This can be achieved by hydrophobic biopolymers deposited at the periphery of cells, forming protective barriers to control the movement of water, solutes, gases and pathogens. Suberin is one of such biopolymers deposited below the primary cell wall and is found in both aboveground and underground plant tissues. Suberin is a complex heteropolymer made of a variety of monomers including aliphatic compounds such as long chain fatty acids (C16—C24) and their derivatives, glycerol and aromatic monomers such as ferulic acid [1,2]. Biosynthesis of these monomers involves a series of enzymatic steps including the fatty acid and phenylpropanoid pathways (Figure 1). These monomers are exported to the apoplastic space where ester bonds and oxidative coupling form a complex matrix of suberin polymer [3]. Beside the well-known suberin deposition in the bark of trees, suberin is also deposited in different plant tissues such as the endodermis (Figure 2) and exodermis of roots, the periderm in stems and roots, the seed coat and the bundle sheath cells in the Kranz anatomy. Studies of suberin in these different models have profoundly impacted our current biochemical understanding of this polymer.

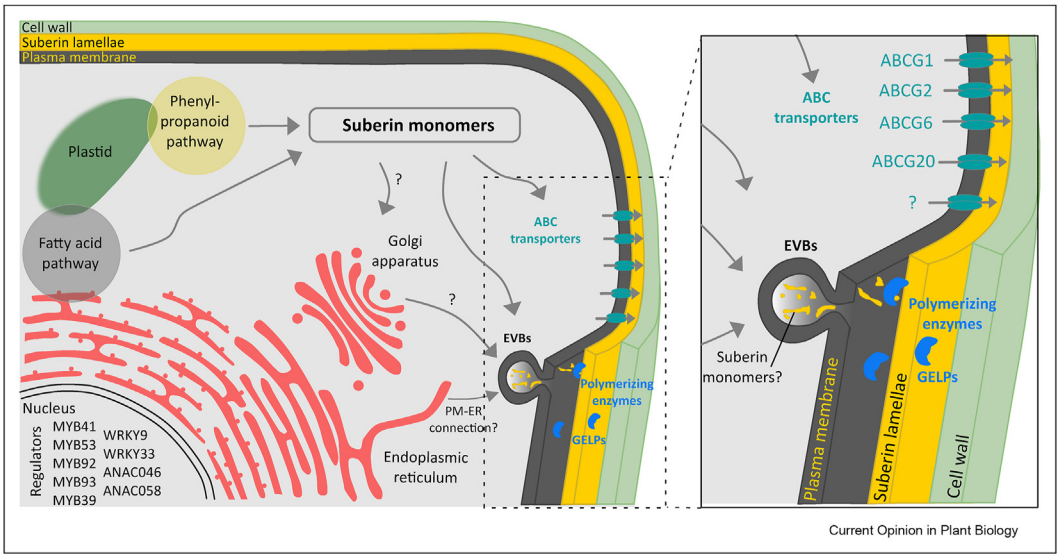
In the past decade, suberin has attracted an increasing interest and studies of the mechanisms controlling its deposition through a combination of genetics, cell biology and developmental approaches are becoming the state of the art. This has been particularly well characterized in the endodermis of the Arabidopsis root where mutants and reporter lines are easily generated and where the root with its simple organization is particularly well suited for microscopy (Figure 2).

These recent studies unraveled a complex regulation of suberin deposition in response to environmental and endogenous cues and provided tools to functionally test the role of suberin. In this review we will highlight recent advances in understanding the mechanisms controlling suberin deposition, its regulation as well as its functions in roots.

## New pieces to an old puzzle

Most of the enzymatic machinery for the biosynthesis of suberin monomers at the endoplasmic reticulum (ER) has been characterized in the past decade and already extensively reviewed [2—4]. The next steps for suberin deposition are the following: export of suberin monomers to the apoplastic space and their polymerization, two key aspects that were further characterized recently (Figure 1).

**Figure 1**

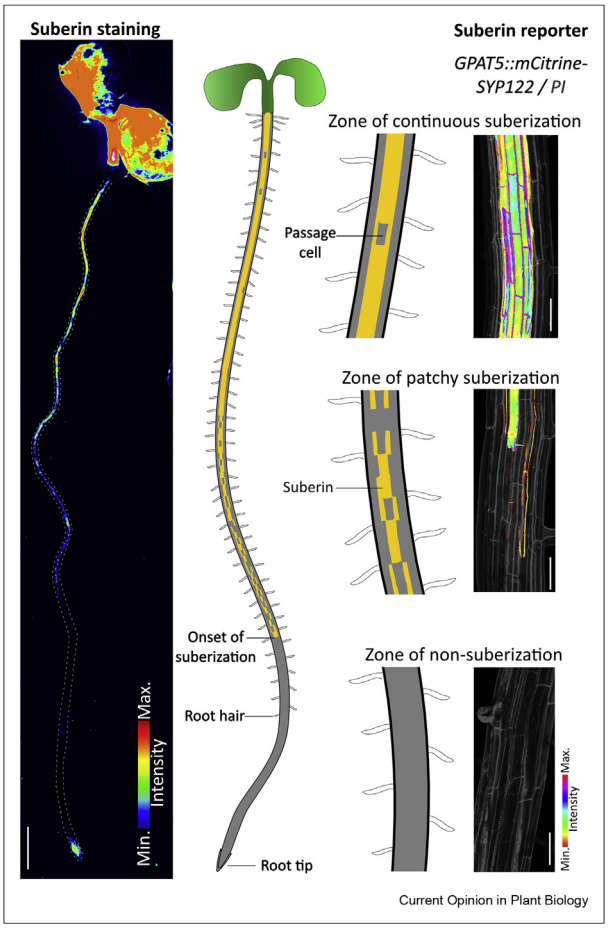



**Biosynthesis, transport and polymerization of suberin.** Several MYB, WRKY and NAC transcription factors induce the expression of suberin biosynthesis genes. Biosynthesis of suberin monomers is achieved through an enzymatic framework involving the fatty acid and the phenylpropanoid pathways. C16:0, C18:0 and C18:1 fatty acids are synthesized in plastids and exported to endoplasmic reticulum for subsequent chain elongation and modifications. Aromatic monomers such as ferulic acid and coumaric acid are synthesized by the phenylpropanoid pathway. Once synthesized, monomers are thought to be transported to the apoplastic space across the plasma membrane via ATP-binding cassette (ABC) transporters, and Extracellular Vesiculo-tubular containing Bodies (EVBs). Monomers are then polymerized by GDSL-type Esterase/Lipase protein family (GELP) to form the suberin polymeric structure. PM, Plasma membrane; ER, Endoplasmic reticulum.

Transport of suberin monomers to the apoplast has been proposed to be facilitated by lipid transfer proteins (LTPs) and ATP-binding cassette (ABC) transporters of the subfamily G. LTPs were suggested for their role in lipid transport [5] and some studies have supported this hypothesis with connections with suberin deposition in the seed coat and in crown gall periderm [6,7]. However, the direct role of LTPs in the export of suberin precursors remains to be fully demonstrated. Instead, clearer evidence of ABCG transporters' role in suberin monomer export has accumulated from recent studies. Three ABCG transporters, ABCG2, ABCG6 and ABCG20, have been suggested to be important in endodermal suberization. This is mainly supported by the co-expression of the three corresponding genes with suberin biosynthesis genes and by histological and chemical analysis in the root of the triple mutant *abcg2abcg6abcg20* [8–**10]. More recently, ABCG1 was also characterized for its role in the export of suberin monomers in roots: the protein displaying an ATPase activity stimulated *in vitro* by fatty alcohols and fatty acids, and the corresponding mutant displaying changes in root suberin composition [*11]. While no transport activity has been demonstrated for ABCG1,2,6 and 20, a recent study on cutin (chemically close to suberin) demonstrated an *in vivo* activity of the Arabidopsis ABCG32 and its tomato homologue SlABCG42 transporters in C16:0 fatty acid derivate's export [**12]. This important work reinforces our current models, providing evidence that ABCG transporters can transport fatty acids and could therefore play an important role in the export of suberin monomers and their deposition in the apoplast.

In addition, secretion through vesicles was postulated for the export of hydrophobic suberin monomers [1,3] (Figure 1). However, the role of secretion in suberin deposition has been largely understudied despite early observations with electron microscopy (EM) of large vesicles containing internal structures at the periphery of suberized endodermal and exodermal cells [13,14]. Recently, using EM on Arabidopsis suberizing root sections, vesiculo-tubular nanosized structures, contained inside larger bodies attached to the PM (Extracellular Vesiculo-tubular containing Bodies, EVBs) were observed [**15]. Developmental analysis, genetic manipulation or treatment inducing suberization strongly support a functional link between the accumulation of EVBs at the periphery of cells and suberin deposition. This was further demonstrated by using cortical cell suberization upon ABA treatment as an inducible system for suberization in combination with pharmaco-genetic interference with secretion. Interestingly, punctate structures of a size comparable to EVBs were stained by fluorol yellow (lipid dye staining suberin) in suberizing cells, suggesting that hydrophobic suberin precursors could be directly transported in EVBs and secreted to the apoplast [**15]. While these studies

**Figure 2**




**Suberin deposition along the root in Arabidopsis.** Pattern of suberin deposition in WT plants grown in unstressed conditions. Whole-mount root staining of 5-day-old seedling with fluorol yellow illustrating the pattern of suberin deposition in the endodermis (Left). Signal is presented as a Look Up Table (LUT); scale bar, 1000 μm. Schematic views highlighting the three distinct zones of suberization known as: non-suberized, patchy and continuous suberization (Middle). Highlight of the three zones with the suberin biosynthesis reporter *GPAT5::mCitrine-SYP122* (where the promoter of the suberin biosynthesis gene *GPAT5* controls the expression of a plasma membrane fluorescent reporter), in living roots (Right). Signal is presented as 3D projection with mCitrine as a LUT and propidium iodide (PI) used for counter staining in grey; scale bar, 50 μm.

suggest a role for secretion in suberin deposition, further work will be needed to clarify the content of EVBs and the compartment involved.

Genes involved in the subsequent polymerization step of suberin monomers were recently characterized through an unexpected approach focusing on lateral roots (LR) (Figure 1). During LR initiation in pericycle cells, the overlying endodermal cells need to accommodate the expansion growth of newly formed primordia through remodeling of their shape, volume and cell wall properties including lignification and suberization [16–19]. Reconstituting the auxin-induced endodermal transcriptional changes in the context of LR development, a list of auxin regulated *GELPs* (*GDSL-type Esterase/Lipase Protein family*) were identified with five being induced (*GELP12-55-72-73-81*) and five being repressed (*GELP22-38-49-51-96*) during LR development [**18]. Some *GELPs* were previously characterized for their role in cutin polymerization/depolymerization [20–22] and their changes in expression could reflect a function of GELPs in suberin remodeling during LR emergence. In agreement with this hypothesis, *GELP12*, *55* and *72* were shown to be sufficient to induce suberin degradation while the quintuple mutant *gelp22-38-49-51-96* displayed a drastic reduction in endodermal suberin deposition with an overall 80% decrease in root suberin monomer content. Importantly, these results support a key role of GELPs in suberin polymerization and degradation not only in the context of LR emergence but in the whole endodermal layer.

## Suberin, a modular barrier

During root development, suberin deposition along the endodermis follows a specific pattern with three distinct zones: non-suberized, discontinuous/patchy (where only few endodermal cells are suberized) and continuous (where all endodermal cells are suberized except the so-called passage cells) (Figure 2) [23–27]. This well-defined pattern can be modified, in particular during LR emergence where local degradation and re-synthesis of suberin accommodate the newly formed primordium [**18]. In addition, this pattern of suberin deposition can be overruled in response to defects in Casparian strips through signaling involving the Leucine-Rich-Repeat Receptor-like Kinase SGN3/GSO1 (SCHENGEN3/GASSHO1) and its ligands CIF1/2 (CASPARIAN STRIP INTEGRITY FACTORS), leading to a continuous zone of suberization all along the differentiated endodermis [28–30]. Moreover, suberin deposition rapidly responds to external abiotic and biotic stress factors. This plasticity has been studied in particularly in the context of abiotic stresses where suberin induction in toxic environments, such as in the presence of salt or cadmium, in hypoxia or in drought conditions, has been described in different species [24,31–36]. More surprisingly, suberin plasticity was also observed in response to mineral deficiencies especially in Arabidopsis where potassium or sulfur deficiencies were shown to induce suberization while manganese, iron or zinc deficiencies were shown to reduce suberization and induce its degradation through abscisic acid (ABA) and ethylene signaling, respectively [24,26]. Suberin plasticity in response to mineral deficiencies has been also described in barley roots in response to manganese or nitrogen deficiencies [36–38]. In addition, suberin is also highly plastic in response to biotic interactions including nematodes, pathogenic and beneficial

microbes [**39—**42]. Importantly, even if we don't fully understand the interconnections between these different pathways, ABA signaling seems to play a central role in suberin regulation in the context of biotic interactions. This has been particularly well demonstrated with a large-scale approach where suberization was shown to be highly affected by the microbiota through a repression of ABA signaling in roots [**39]. In addition, it is now clear that ABA and SGN3/CIFs signaling induce suberization independently [**10,31].

Transcriptional regulators controlling suberization were initially identified in different tissues. This includes the MYB (MYeloBlastosis) family homologues MYB9 and MYB107 involved in seed coat suberization [43]; MdMYB93 expressed in russeted skin of apple and inducing suberization in a heterologous system [44]; and the StNAC103 (NAM/ATAF/CUC protein 103, corresponding to ANAC058 in Arabidopsis) involved in potato tuber periderm formation [45]. Several Arabidopsis MYB transcription factors were shown in heterologous systems to be sufficient to induce suberin, such as MYB39, MYB41, MYB92 [*9,46,*47]. MYB41 can directly bind to the *LTP20* (*LIPID-TRANSFER-PROTEIN20*) promoter *in vitro*; MYB9, MYB39, MYB53, MYB92, MYB93 and MYB107 were demonstrated to bind to the *BCCP2* (*BIOTIN-CARBOXYL-CARRIER-PROTEIN2*, involved in fatty acid synthesis) promoter and to transactivate its expression in yeast one hybrid experiments; MYB92 can activate the expression of *ACP1* and *LPD1* (*ACYL-CARRIER-PROTEIN1*, *LIPOAMIDE-DEHYDROGENASE1*, involved in fatty acids biosynthesis); and MYB39 was shown to transactivate the expression of several genes involved in suberin biosynthesis such as *GPAT5* (*Glycerol-3-Phosphate-Acyl-Transferase5*), *ASFT* (*ALIPHATIC-SUBERIN-FERULOYL-TRANSFERASE*) or *CYP86B1* (*CYTOCHROME-P450-MONOOXYGENASE-86B1*) [*9,*47,48].

Transcription factors directly involved in the regulation of endodermal suberization were only recently characterized (Figure 1). Among them, ANAC046 is expressed in the endodermis and its overexpression induces root suberization [49]. The WRKY transcription factor 33 is expressed in the endodermis and induced by salt stress, while *wrky9 and wrky33* mutants displayed a reduced and delayed endodermal suberization [50,51]. Among all the MYBs shown to induce suberization in heterologous system, *MYB39* is expressed the endodermis, sufficient to induce suberization in all root layers upon overexpression, and its loss of function is associated with a slight delay in endodermal suberization [*9]. *MYB41*, *MYB53*, *MYB92* and *MYB93* are also expressed in the endodermis and are induced in response to ABA and SGN3-CIF signalling [**10]. Ectopic expression of any of these four *MYBs* in the early endodermis led to ectopic suberization close to the root tip. Among them only *MYB92* loss-of-function led to a strong delay in endodermal suberization in unstressed condition even though suberin induction by exogenous ABA or CIF was not affected [**10]. Simultaneous mutation of *MYB41*, *MYB53*, *MYB92* and *MYB93* (*quad-myb* mutant) led to an even more dramatic reduction of endodermal suberin deposition with an overall 78% decrease of suberin monomers in roots. Importantly, the response to ABA and CIF in this *quad-myb* mutant was nearly absent [**10] pointing towards a key role of these four MYBs in suberin regulation. However, considering the remaining induction of suberin in response to ABA and CIF observed in the *quadruple myb53-41-92-93* mutant and the plethora of signals modulating suberization we can predict an even more complex network of transcription factors involved in this regulation.

## Suberin, a multifunction barrier

Suberin function has been particularly well studied in the context of abiotic stresses especially in the last decade with the identification of the endodermal barrier mutants *esb1* (*enhanced-suberin 1*), *casp1casp3* (*casparian-strip-membrane-domain-protein*), *myb36* and *lotr1* (*lord-of-the-ring 1*), displaying moderate but specific changes in mineral accumulation including an increased potassium level and reduced manganese and calcium levels [25,31,52—55]. However, analysis of these mutants offered limited insight into the specific function of suberin, all these mutants displaying concomitantly several barrier defects with enhanced ectopic suberization and lignification in the endodermis, triggered by the SGN3/CIF signaling in response to Casparian strip defects [28,29,52—54,56]. For many years suberin-specific phenotypes were mainly studied through a synthetic construct expressing the CUTICLE-DESTRUCTING-FACTOR1 (CDEF1) specifically in the endodermis, resulting in endodermal suberin degradation without affecting Casparian strips [23,24,31,53]. The corresponding *CDEF1* plants accumulate higher amounts of boron, sodium, magnesium and calcium and lower amounts of potassium and are hypersensitive to salt [23—25]. The newly characterized *quintuple gelp22-38-49-51-96* and *quadruple myb53-41-92-93* mutants displaying respectively 80 and 78% reduction in suberin monomers, were both shown to be salt hypersensitive and represent *bona fide* mutants to study suberin function without requiring an artificial synthetic construct [**10,**18]. Interestingly ionomic analysis in *quad-myb* compared with *CDEF1* and specific suberin enhancer lines (endodermal *MYB41* overexpressor), suggest a more specific role of suberin than previously thought affecting mainly the acquisition of arsenic, boron, sodium and calcium [**10].

Moreover, suberin affects the colonization of roots by pathogens and parasites such as nematodes [40,**42,57]. For example, the pathogenetic oomycete *Verticillium longisporum* root colonization was shown to be

accompanied by suberin reduction and restricted by ABA-dependent suberization in the endodermis [**42]. A recent study indicates a role for suberin in beneficial biotic interactions and selectivity to pathogens and mutualists. The microbiomes of 19 different genotypes affected in Casparian strip and/or suberin were analyzed and indicated the crucial role of these barriers in plant microbiome assembly [**39]. Studying in parallel the microbiome and ionome of these plants uncovered a complex interplay between the microbiota and endodermal barriers in turn affecting mineral homeostasis [**39].

Suberin also plays a crucial role during LR formation where the endodermis needs to accommodate the emerging LR primordium through a remodeling of endodermal barriers including lignified Casparian strips [17] but also suberin [**18]. This was well demonstrated with EM analysis on developing LR, showing a degradation of suberin in endodermal cells overlying the primordium concomitant with the deposition of a root cap cuticle in the newly formed primordium [**18,*58]. The identification of *GELPs*, expressed in endodermal cells overlying the forming primordium, regulated by auxin, and involved in suberin polymerization/degradation reinforce further a key role of suberin during LR formation [**18]. Consistent with a role of barriers in LR formation *esb1*, *casp1casp3* and *myb36* displayed a delay in LR emergence and their LR primordia a flattened shape [16,59]. However, these backgrounds do not distinguish between suberin and lignin, since both barriers are reinforced at the stage where LR are formed [52,54]. Hinting towards a specific role of suberin, *MYB93* was shown to be involved in LR formation, being expressed specifically in endodermal cells overlying the forming primordium and primordia emerging faster in *myb93* mutant [60]. Moreover, plants with enhanced suberin due to endodermal *MYB41* overexpression displayed less LR while the suberin deficient *CDEF1* line displayed more LR [**10]. Finally, loss of function for *GELP72* (induced by auxin and involved in suberin degradation), lead to a delayed LR emergence suggesting further a role of suberin remodelling in LR emergence [**18].

## Conclusion

The last few years of research have greatly extended our understanding on the mechanisms controlling endodermal suberin deposition in the apoplast, its polymerization/depolymerization and its regulation at the molecular level. Moreover, the increasing number of research where the pattern of suberin deposition was carefully studied with microscopy unraveled an even higher and more complex degree of suberin plasticity than previously thought, not only in response to abiotic stresses but also in response to biotic interactions and during root development. Understanding how these different pathways are coordinated to fine-tune development, nutrient homeostasis and microbial interactions will be particularly interesting for the future. Finally, research on the molecular mechanisms controlling suberin identified novel mutants and lines specifically affected in endodermal suberization and provide exciting tools to characterize in greater detail the many roles of suberin in roots.

## Declaration of competing interest

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

## Acknowledgements

We apologize to authors whose relevant work on suberin have not been cited, either inadvertently or because of length constraints. We would like to thank Lothar Kalmbach and Olga Serra for critical input. This work was supported by the Sandoz Family Monique De Meuron philanthropic foundation's program for academic promotion and the SNSF (project number PCEGP3_187007) to M.B.

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