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The Making of Plant Armor : The Periderm

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Title: The making of a plant armor: the periderm

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Running title: Periderm and cork/phellem development

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Abstract:

The periderm acts as armor protecting the plants inner tissues from biotic and abiotic stress. It forms during the radial thickening of plant organs such as stems and roots and replaces the function of primary protective tissues such as the epidermis and the endodermis. A wound periderm also forms to heal and protect injured tissues. The periderm constitutes of a meristematic tissue called the phellogen or cork cambium, and its derivatives: the ligno-suberized phellem and the phelloderm. Research on the periderm has mainly focused on chemical composition of the phellem due to its relevance as a raw material for industrial processes. Today, there is an increasing interest in the regulatory network underlying periderm development as a novel breeding trait to improve plant resilience and to sequester CO_2. Here, we discuss our current understanding on periderm formation, focusing on aspects of periderm evolution, mechanisms of periderm ontogenesis, regulatory networks underlying phellogen initiation and cork differentiation and future challenges of periderm research.

1. INTRODUCTION

To adapt to adverse conditions, plants have evolved a range of mechanical, molecular and physiological responses as well as protective tissues. Depending on the developmental stage, the physiological conditions and the plant organ, different barriers are formed to protect the organs from the environment (**Figure 1*a***). During embryogenesis and primary growth of the shoot tissues, when the aerial plant body is established and elongated, the cuticle is formed on the surface of the epidermis, the outermost cell type, to cover and protect embryos, leaves, stems and flowers. The cuticle is an extracellular lipophilic layer secreted by the epidermal cells. It is made of the polyester cutin, which is impregnated by waxes, and acts as protection against desiccation, extreme temperatures, UV radiation as well as from diverse biotic stresses (reviewed in 46, 72). During primary growth of roots, the major apoplastic barrier resides in

the endodermis rather than the epidermis. A common feature of endodermal cells is the presence of Casparian strips, which are localized lignin depositions that tightly connect adjacent cells blocking free diffusion of solutes from the soil into the vasculature. Later in development, another key plant polymer, suberin, is deposited in endodermal cell walls, thus further limiting nutrient uptake and hindering backflow of nutrients from the vasculature (for a more comprehensive overview see 2, 10, 35).

In many but not all angiosperms the endodermis is not the exclusive barrier present during root primary growth, and an additional protective layer, the exodermis (present in tomato, rice and maize roots but not in the model plant Arabidopsis), prevents water loss in dry and saline environments, and oxygen loss in waterlogged soils (67, 115, 134, 159). The exodermis or hypodermis, derived from the cortex-endodermis initials, is the layer below the epidermis, and often accumulates lignin and suberin in its cell walls, similarly to the endodermis (40, 74, 129, 134).

As tissues mature, a new protective armor called the periderm, is formed. Periderm replaces the endodermis and epidermis when they break or die due to root or shoot thickening (secondary/radial growth) (**Figure 1*a-b***). In addition, another type of periderm, wound periderm, is produced in response injuries and forms to repair and seal the wounded area during the healing process. Periderm formation is prevalent in trees but also occurs in many herbaceous plants that undergo secondary growth (41, 42). In many woody plants, when the first periderm is no longer functional, and thus cannot protect the growing tissue, it is replaced by a new periderm formed underneath, which in turn is replaced by (sub)sequent periderms formed over the years, leading to the formation of the rhytidome. Hence, the rhytidome comprises a succession of dead periderm layers alternated with non-functional secondary phloem plus the last active periderm (**Sidebar 1**) (**Figure 1*c***) (4, 41, 130). In contrast, in a few species such as cork oak, it appears that the same periderm grows over the years. In both cases,

the periderm or the rhytidome, are also commonly referred to as outer bark (**Sidebar 1**). Lenticels form a porous structure in the surface of the periderm to facilitate gas exchange between the atmosphere and inner tissues (**Sidebar 2**) (**Figure 1*d***).

The periderm is a complex system comprised of the phellogen meristem: the phellogen (also known as cork cambium) and the two tissue types it produces: the phellem (also commonly referred to as cork) and the phelloderm (**Sidebar1**) (**Figure 1*b***). The architecture of the periderm is reminiscent of the vasculature, and thus phellogen, similarly to the vascular cambium, constitutes a cylinder of meristematic cells, which divides bifacially producing on the outside the phellem and on the inside the phelloderm. The number of phellem and phelloderm layers is highly variable among species and phellogen activity/differentiation can be unbalanced resulting in preferential formation of one of the two tissues. For instance, tropical trees have usually a pronounced phelloderm, whereas cork oak (*Quercus suber*) displays many layers of phellem and some plants completely lacks a phelloderm (41, 42, 57, 131). Phelloderm cells are parenchyma (thin cell walls with no structural modifications) and their morphology is often very similar to underlying tissues such as phloem or cortical parenchyma, thus their identification relies mainly on their radial alignment with the phellogen. In contrast, phellem cells exhibit cell wall modifications such as ligno-suberization, rendering them easy to distinguish from the inner phellogen. The physiological functions of the phelloderm remain enigmatic, but its parenchymatic nature and the presence of plastids in some trees suggest that it may serve as a storage tissue. Research on the periderm has mainly focused on the phellem, which confers the barrier property to the periderm. Phellem thickness and chemical composition correlates with the ability to withstand pathogen penetration and protection against abiotic stress such as high salinity and resistance to wildfires (2, 38, 64, 84, 164). Moreover, in the case of potato (*Solanum tuberosum*), tuber conservation and storage abilities are deeply influenced by the chemical composition of the phellem (98).

Understanding the mechanisms of periderm development is thus not only relevant for breeding processes aiming to improve plant tolerance to stresses but also for industrial processes as the phellem is an excellent raw material for insulant, polymer and wine stopper production due to the combination of its special mechanical, chemical and morphological characteristics (89, 120, 144). Despite the agronomic and economic importance of the phellem and the fact that phellem cells from cork oak were the first cells observed with a microscope in 1665 (66), research on the molecular mechanisms underlying periderm development has lagged behind the research on other plant developmental processes such as root and shoot primary growth. However, with the advance of next generation sequencing and genome editing techniques, and the rising interest in suberin and bark as a possible source of CO_2 sequestration, periderm development has become a blooming field (62, 118).

2. PERIDERM EVOLUTION AND FOSSIL RECORDS

Studies of the fossil record provide a valuable insight onto the multiple evolutionary origins of the periderm. The earliest evidence of periderm in the fossil record occurs in the Early Devonian roughly 400 million years ago. Fossils from the Early Devonian preserve a diverse range of early vascular plants (50, 152), including the earliest evidence of plants developing wood (49) prior to the origin of the first trees and forests in the Mid Devonian (149). The earliest evidence of a periderm was described from *Psilophyton dawsonii* (7, 8), a basal extinct member of the euphyllophytes, the group today that includes all monilophytes and seed plants (75) (**Figure 2**). *P. dawsonii* lacked secondary growth and a native periderm forming a concentric ring but developed a tissue fitting the description of wound periderm in above ground axes (7-9). This tissue, consisting of radial rows of cells, was found to develop in distinct local areas of axes and was covered by a closing layer of necrosed cells (7, 8). The cells closest to the closing layer were characterised by thin cell walls and are predicted to

represent phellem, whereas thicker walled cells were found internally and may represent phelloderm (7, 8). *P. dawsonii* (7, 8) is therefore the earliest evidence of a periderm in the fossil record and suggests that wound periderm evolved before a native periderm and before the origin of secondary growth derived from a vascular cambium.

The earliest occurrence of a native periderm occurs in the mid-Devonian roughly 385 million years ago in a group called the Aneurophytalean progymnosperms (107, 132), which are extinct precursors of modern seed plants (**Figure 2**). During the Carboniferous period there is extensive evidence of a periderm in the major groups of vascular plants. A periderm has been described in many fossils of seed plants (33, 34, 152) as well as ferns and lycophytes including: arborescent Lycopsids (32), Cladoxylopsids (33), Equisetopsids (28, 33), Zygopteridales (33, 123), Marattiales (127) and Leptosporangiate ferns (124) (**Figure 2**). As each of these groups are phylogenetically distinct and separated by relatives lacking periderm, this suggests that the periderm had multiple origins and evolved in association with the origin of secondary growth in multiple but not all lineages. Despite fossils recording the presence of a periderm in multiple groups in the past, today, native periderm is almost entirely restricted to seed plants, with the exception of one group of living ferns, the Ophioglossaceae (150) (**Figure 2**). Therefore, the fossil record suggests native periderm was a common trait gained and lost multiple times during land plant evolution. In contrast to native periderm, wound periderm is present in many living species today ranging from lycophytes (Doyle, 2013), ferns (Holden, 1912) to seed plants (Esau, 1965). Although not present in all vascular plants (Holden, 1930; Kevan *et al.*, 1975), the widespread nature of wound periderm across multiple groups, and its early occurrence in *P. dawsonii* fossils, suggests it may be a conserved feature of vascular plants. Despite the different evolutionary trajectories between wound periderm and native periderm their similarities suggest they may share underlying similarities and evolutionary origins.

3. PERIDERM ONTOGENESIS

While periderm is formed in many seed plants: gymnosperms, woody dicots and numerous herbaceous flowering plants with extensive secondary growth, it does not develop in monocots. However, monocot trees such as palms produces a periderm-like structure, which comprises dividing and suberized cells that do not form a full organized ring and thus are not considered equivalent to the phellogen and periderm of dicots (41, 42). In species that do develop a periderm, the periderm develops mainly in stem, branches and roots, during plant secondary development, but it also formed in regions that are exposed to the environment due to organ abscission or in response to wounding. For example, when fruits are damaged their skin is replaced by a periderm or strips of periderm in a phenomenon known as fruit russeting (103).

The (first) periderm arises from different tissues depending on the organ and the plant species: even closely related species may show differences in the site of phellogen origin (**Figure 3**). Moreover, it is known that the developmental stage and age of an organ also influences the site of phellogen origin (41, 42). Based on anatomical and histological analyses, we can distinguish at least five types of periderm ontogenesis where the phellogen arises from either: 1) the epidermis, 2) the subepidermal layer, 3) deeper tissues within the cortex, 4) the phloem or 5) the pericycle in root and hypocotyl. In most woody genera, the first phellogen becomes inactive and (sub)sequent phellogens arise in progressively deeper tissues until they are initiated in the phloem (37, 41, 136).

3.1 Periderm initiation in stems

Periderm formation starts after the initiation of the vascular cambium. In the majority of plants, the first phellogen in stems originates from the subepidermal layer, whereas the formation of the first phellogen from the epidermis (genus *Malus*, *Pyrus and Oleander)* or the

phloem (genus *Vitis, Pinus mugo, Alnus glutinosa)* is rare (41, 42, 136) (**Figure 3*a,b***). For instance, in cork oak and poplar (*Populus trichocarpa*) the first formative division of the periderm occurs periclinally in the subepidermal layer and the inner daughter cell differentiates into phelloderm whereas the outer daughter cell, which constitutes the phellogen, divides periclinally forming the first phellem cell. This process spreads rapidly to all cells of the subepidermal layer, forming a continuous meristematic ring by the end of the first year of growth (57) (**Figure 3*c,d***). Examples of phellogen initiation from superficial and deeper cortical layers come from the families of Conifers. In Pinaceae (Gymnosperm, Pinophyta) the origin of the phellogen ranges from the 1st to the 5th layer beneath the epidermis, whereas in the closely related Cupressaceae (Gymnosperm, Pinophyta) the phellogen originates in both inner and outer cortical layers (90), highlighting heterogeneity in periderm ontogenesis among related conifers.

The genus *Cornus* (Eudicots, Asterids), which comprises shrubs and small trees cultivated for their flowers and foliage, has been reported as an interesting example of diversity and complexity of periderm ontogenesis: due to the hybrid origin of the phellogen from both the epidermis and subepidermis. In *C. mas*, *C. sanquinea* and *C. florida*, the first phellogen is usually initiated by periclinal divisions of the epidermis at the lower side of branches, which rapidly spread and form a continuous cylinder of meristematic cells by the second year of growth. However, sometimes, periclinal divisions start in the subepidermal layer and later spread to the epidermis resulting in a phellogen with hybrid origin (25-30% subepidermal and 70-75 epidermis) (114).

Finally, tubers, such as those from potato, originate from modified stems named stolons that during radial growth form a periderm. In potato the phellogen arises in the hypodermis (122).

3.2 Periderm establishment in roots

In most roots, the site of phellogen initiation is the pericycle, however in *Citrus sinensis* (orange) and other Rutaceae with limited secondary growth, the first phellogen may arise from the subepidermal layer, in a fashion more similar to development in stems, and only (sub)sequent periderms arise from the pericycle (26). The detailed progression of periderm development has been reported for Arabidopsis and cork oak roots. Briefly, in cork oak, the pericycle consisting of 2-3 cell layers, starts to divide periclinally to form the phellogen. The phellogen produces 2-3 phellem layers which as they divide and expand cause the surrounding endodermis, cortex and epidermis to rupture and eventually completely detach from the root (102). By contrast in Arabidopsis, the periclinal divisions that form the phellogen are preceded by anticlinal divisions in the pericycle (**Figure 3*e,f*)** and only 1 layer of phellem cells differentiate before the cortex and the epidermis break (170) (**Figure 3*g,h***). Periderm development has been recently characterized in Arabidopsis beyond classical histology descriptions, setting the Arabidopsis root as a model to study the mechanisms of phellogen initiation (146, 170). Lineage tracing analysis confirmed that the phellogen arises from the pericycle and revealed that pericycle cells located in correspondence to the xylem axis have a dual fate, giving rise to both phellogen and vascular cambium (146). Moreover, live imaging experiments showed that endodermal cells undergo programmed cell death after the phellogen is established. By contrast cortex and epidermis cells are detached after phellogen produces suberized and lignified phellem (170).

A special periderm: the polyderm is produced in roots of strawberry, eucalyptus and other plants belonging to the Rosaceae and Myrtaceae. In the polyderm, alternating layers of suberized (1 layer) and unsuberized cells (2-4 layers) differentiate from the phellogen (117, 156). Massive root periderms with more than 20 layers of phellem cells are also formed in

alpine species of *Saxifraga,* which grow at high altitudes (94) protecting the root from extreme temperature fluctuations.

3.3 Fruit russeting

Fleshy fruits commonly have smooth skin corresponding to a thick epidermal cuticular layer, which may present lenticels formed under non-functional stomata (**Figure 3*i,j*)**. Nevertheless, in a variety of fruits, such as apples (*Malus domestica*), pears (*Pyrus communis*), grapes (*Vitis vinifera*), mangos (*Mangifera indica*) and melons (*Cucumis melon*), a periderm, in the form of russeted areas or reticulation, is formed as a response to skin failure (6, 30, 69, 83, 166) (**Figure 3*i,j*)**. Russeting initiates when the growth stress applied to the skin surface exceeds a threshold, resulting in microcracks, which in apples often arise at lenticels (6, 103). The new periderm originates in the hypodermis just beneath the microcracked epidermis and the new multilayered phellem arises as a new surface with the typical brown, rough, dull and corky appearance of russeting (77). In agreement with phellem formation, the composition of the russeted patch is enriched in suberin and triterpenoid-derived compounds (**Figure 3*j*)**. In apples, these russeted areas seal microcracks and they confer plasticity in contrast to the stiffer, but more permeable epidermal regions. This provides a functional solution to compensate stress growth and avoid pathogen entrance while partially restoring the water barrier function (77, 78).

Skin russeting is often considered a fruit defect, although in some commercially appreciated varieties it is viewed as a fruit ornament (103). For its economic importance, fruit skin russeting is a target of many breeding programs (103) and many transcriptomics studies, aiming to understand the molecular mechanisms controlling microcracking and russeting, have been recently performed (*see* **Supplementary Table 1**).

4. REGULATION OF PHELLOGEN ACTIVITY

Plant meristems can be subdivided into two different cell types: stem cells and transit amplifying cells. Both cell types can divide, however, only stem cells, per definition, will retain a cell lineage that remain in the meristem. All transit amplifying cells and their daughters have the fate to differentiate (133). While the stem cell concepts has been well established in shoot and root meristems (59), and begins to be established in vascular cambium (20, 141, 146), the phellogen has remained unexplored. Phellogen is typically described as a single layer of dividing cells (41), so it appears that this lateral meristem may consist solely of stem cells. However, during regeneration after wounding, phellogen has been described as transiently comprising several cell layers (**Figure *4a***) (18, 79), suggesting that transit amplifying cells may appear in special conditions. Thus, molecular and lineage tracing studies are needed to identify stem cells of phellogen. Stem cell organizer or organizing center is another stem cell concept that has been identified in the primary meristems (133) and in vascular cambium (145). The function of the organizer is to define and maintain adjacent cells as stem cells through cell-to-cell signaling. It will thus be interesting to study whether the stem cell organizer concept applies also to the periderm. In the next sections, we will discuss the regulation of meristem activity in the phellogen during the whole plant life, including seasonal and annual changes and response to wounding.

4.1 Longevity and seasonal changes

The timing of first periderm formation varies between species and may be influenced by environmental conditions (41, 42, 128). In the majority of cases a continuous periderm is formed in the first year of growth, however periderm formation can also be delayed for several years. For example, carob (*Ceratonia siliqua*) (5) and box elder (*Acer negundo*) (163) form first continuous periderm when they are approximately 6 years old. Several studies suggest that

light intensity regulates the timing of periderm initiation: in fact, seedlings of red pine (*Pinus resinosa)*, green ash (*Fraxinus pennsylvanica)*, and black locust (*Robinia pseudoacacia)* maintained in the dark fail to form a phellogen, while exposure to light restore periderm initiation in a manner proportional to light intensity (19). Consistently, Wunderling and colleagues (170) reported that in the Arabidopsis root phellogen initiation occurs earlier in long day conditions than in short day conditions.

Also, periderm longevity varies extensively between species depending on how often given species form new periderm to replace the old one. Many species replace the old periderm every 6-12 years, thus forming a rhytidome (41, 42), while in cork oak (*Quercus suber*) the periderm may function for tens of years, and in European beech (*Fagus sylvatica*) even up to 200 years (41, 42). In these species, the periderm is referred to as persistent or long-lived as it is not replaced by (sub)sequent periderms. Persistent periderms result either from phellogen reactivation (similar to vascular cambium), or phellogen replacement (by a new phellogen layer formed from phelloderm) (128, 142).

In trees from temperate climates, the phellogen is not active all year-long but it displays rhythmic activity, which varies according to species and geographical location. For instance, in cork oak, the phellogen is active from March to October and in monarch birch (*Betula maximowicziana*) from May to July, whereas in stone (*Pinus pinea*) and aleppo pine (*Pinus halepensis)* only in June (44, 142, 165). Only a few studies have concomitantly addressed phellogen and vascular cambium seasonal activities and the emerging scenario is that some species such as stone and aleppo pine display independent rhythms (phellogen and vascular cambium activity peak maximum do not coincide), whereas in carob the period of activity coincides (5, 128, 165). Nonetheless, which scenario applies to the majority of plants and how this process is regulated at the molecular level remain to be investigated.

4.2 Regeneration upon wounding

In response to injury events that damage the outer protective tissue, including mechanical wounding and pathogen intruders, a wound periderm also known as necrophylactic periderm, is formed (42) (**Sidebar 1**). The sequential events and the cytological changes occurring during wound healing in the stems of gymnosperms and woody angiosperms are similar to those observed in potato tuber, which is extensively studied (13, 14, 42, 112, 155). During the healing process, two structures, which are spatially and temporally separated, are formed: the outer closing layer and the underneath wound periderm (100, 101) **(Figure *4a,b*)**. Closing layer formation is a rapid response, which involves first lignification and later suberization of the 1-2 layers of parenchyma cells neighboring the wound. This serves as a "temporary" protection from drought and pathogen entry (42, 96, 97, 155). Underneath the closing layer, parenchymatic tissue is dedifferentiated to form a wound phellogen, which through periclinal divisions, organizes a new periderm with an outer ligno-suberized multilayered phellem **(Figure *4a,b*)**. Since the wound periderm is adjacent to the closing layer and development of both barriers overlaps in time (13, 101), their physiological functions remain unclear.

4.3 Hormonal and transcriptional control of the phellogen

Plant meristems are commonly regulated by a sophisticated interplay between phytohormone and peptide signaling pathways. For instance, in the vascular cambium, auxin, which peaks on the xylem side of the cambium, acts as an organizing center activating WUSCHEL HOMEOBOX4 (WOX4), *PHLOEM INTERCALATED WITH XYLEM (PXY)/TDR* and HD-ZIPs (146). *WOX4* expression is also induced in the vascular cambium by the interaction of the peptide CLE41 with the receptor like kinase *PXY,* which is required for correct vascular cambium patterning and activity (reviewed in 125).

Only a few genes controlling phellogen initiation and activity are known so far, even though with the advent of next generation sequencing, many putative regulators have been identified in different species and the number is bound to rise (1, 44, 92, 153, 162) (*see also* **Supplementary Table 1 and 2**). A major challenge, in the functional characterization of phellogen regulators, is to prove their specific role, as impairing the function of the vascular cambium, indirectly affects phellogen activity (171). Thus, vascular cambium mutants are likely to show a periderm phenotype independently of their function in the phellogen.

Recently, Xiao and colleagues (171) showed that auxin peaks on the phelloderm side of the phellogen in the Arabidopsis root. Moreover, by inhibiting auxin signaling specifically in the periderm, they could demonstrate that auxin is required for the initiation and maintenance of the phellogen (171) (**Figure *4c***). Downstream of auxin in the periderm act two transcription factors: WOX4 and BREVIPEDICELLUS/KNAT1. Even though this is known only for the Arabidopsis root, the presence of WOX4 and BP also in transcript profiling of other species suggests a general function for these two TFs in phellogen regulation. Since WOX4 and BP have been previously characterized as key regulators of vascular cambial activity (174), it indicates that these two meristems likely share core signaling components (**Figure *4c-d***). In agreement with this idea, it has been reported that other known cambial regulators such as *AINTEGUMENTA* (*ANT*), which promotes stem cell proliferation downstream of cytokinins (CKs) (126), *SHOOT MERSTEMLESS* (*STM*), the closest homologues of BP (91), *MYB87*, which represses secondary growth (174) and *VASCULAR TISSUE SIZE* (160) are also expressed in the phellogen (1, 162) (**Figure *4d***). Intriguingly, available transcriptomic resources and studies on Arabidopsis root indicate that *PXY/TDR* is vascular cambium specific, suggesting that other so far unknown- receptors regulate phellogen activity (1, 44, 171).

It is reasonable to assume that CKs promote meristem cell proliferation in the phellogen as CKs accumulate during tuber healing process at the times that a wound phellogen is formed

(162) and components of the CK signaling, such *ARR5,* and downstream factors, such as *ANT,* are expressed in the wound or native phellogen (1, 44, 162). Another hint of the role of CKs during periderm formation came from a recent study on the Arabidopsis root. Ye and colleagues (173) showed that at the onset of periderm formation, CKs accumulate in the pericycle to activate secondary growth through LATERAL ORGAN BOUNDARIES DOMAIN3 (LBD3) and LBD4 transcription factors (173). Interestingly, LBD4 expression peaks in the outer bark of cork oak at the beginning of the growing season (in April), when many cells are dividing, whereas its expression decreases during cork differentiation (in June-July) (44). Finally, in poplar *PttSHR2B*, an ortholog of the transcription factor SHORT ROOT, which in the Arabidopsis root is required for endodermis and root stem cell niche specification (63), is expressed in the phellogen. Overexpression of *PttSHR2B* results in plants with an enhanced bark relative to wood and increased CKs content, suggesting that *PttSHR2B* may modulate phellogen activity by regulating CK homeostasis (109).

New evidences suggest that RNA metabolism, RNA processing and epigenetic control may influence periderm development via the RNA-binding protein RS2-INTERACTING KH PROTEIN (RIK), although the molecular insights remain unknown (17).

5. PHELLEM DIFFERENTIATION, FUNCTIONS AND REGULATION

Phellem cells, derived from the outer side of phellogen, confer protective properties to the periderm and are usually organized in compact layers with no intercellular spaces, except for the lenticular regions (**Figure *5a***). Exceptions are seen during flooding or in tropical trees, where phellem cells lose compactness and are more loosely arranged to enhance gas exchange (42). Typically, once formed, phellem cells radially expand to acquire their final dimensions, accumulate a set of different specialized polar and non-polar soluble compounds and modify

their cell walls by depositing lignin and suberin (**Figure *5b***). As chemical differentiation ends, phellem cells remodel chromatin, express developmental programmed cell death marker genes, degrade their cytoplasm and fragment their DNA, in agreement with programmed cell death (61, 70, 71, 170), and eventually autolyze and dehydrate. The empty space framed by cell walls becomes gas-filled(128).

Notably in tree barks, with the exception of lenticels, phellogen derivatives can differentiate into diverse types of phellem cells differing in their cell wall thickness (thin- and thick-walled phellem cells). Additionally, phellem cells vary in their composition: they may remain unsuberized (phelloids), differentiate into sclereids or become enriched in crystals (crystalliferous cells) or tannins (phlobaphene cells). These diverse types of phellem cells originate from the same phellogen mother cell, usually in different stages of the growing season and thus, form tangential layers of particular phellem cell populations (42, 128). The seasonal pattern of thin- and thick-walled phellem cells corresponds to growth increments (41, 42), and thus, in cork oak and birch outer phellem tissues anatomically constitute growth rings, which at some extent represent annual increments (120, 142) (**Figure *5c***). Moreover, the variety and the layered/stratified pattern of phellem cells, together with phloem anatomy in which sequential phellogens are formed, contribute to the compactness and the shedding of the bark, and eventually to outer bark visual appearance **(Figure *5d*)** (reviewed in 128).

In the next sections, we will focus on phellem cells that are suberized, reviewing mostly the research undertaken in two phellem models –the outer bark of cork oak and potato tuber skin. In both models, phellem cells are rich in suberin and can be easily separated as almost pure layers (some phellogen cells may be retained) in sufficient amounts for chemical and transcriptomic studies (**Supplementary Table 1**). In cork oak, each year the phellogen produces a 2-3 mm thick layer of heavily suberized phellem cells that adhere to that produced in previous year, constituting what is commercially known as cork (25). For its thickness and

also other exceptional physical properties such as biological inertness, durability and specific mechanical properties, cork oak phellem is exploited to produce wine-stoppers and other cork-derived products (144). Few other tree species are also able to produce pure suberized phellem barks or a rhytidome enriched in suberized phellem cells, all of them with an interesting potential source of chemicals and suberized-phellem-derived products (89). In potato, the native periderm protects and covers the tuber. Upon wounding the tuber, a wound periderm forms from flesh tissue. Both native and wound periderms have high economic importance for tuber protection and conservation (51, 95). Recently, the Arabidopsis root has emerged as a novel model for phellem biology, due to the abundance of genetic and molecular tools available and its rapid formation (less than 2 weeks, for the formation of first phellem cells) (170).

5.1 Major components of phellem cells: waxes, suberin and lignin

Phellem cells contain suberin, lignin and polysaccharides (cellulose and hemicellulose) as insoluble structural components and a set of soluble lipophilic (waxes) and phenolic substances, which can be extracted using solvents of different polarity (**Figure 5b**).

Suberin is an important phellem structural component, accounting for 38%, 30-50% and 20-25% of the total composition in potato skin, cork oak and birch outer barks, respectively (45, 108, 144). Suberization in phellem differentiating cells occurs very rapidly, consistently the suberin biosynthetic protein *FHT* (suberin feruloyl transferase) accumulates already in the phellogen (16). Suberin is a polyester of fatty acyl compounds containing hydroxyl and carboxyl groups in their terminal α- and ω-positions (α, ω-diacids and ω-hydroxyacids - bifunctional aliphatics- and fatty acids and primary alcohols) as well as some aromatics, mainly ferulic acid, and glycerol (reviewed in 58). Phellem transcriptomics (**Supplementary Table 1**) often highlight suberin-related genes, which have been characterized in both phellem and other suberized tissues such as the endodermis or the seed coat (e.g. *CYP86A1/HORST/CYP86A33*,

ASFT/AtHHT/FHT, *ABCG1/ABCG6*) (53, 65, 82, 110, 138, 140, 172), pointing out that the suberin enzymatic machinery may be partially conserved among different tissues. Nevertheless, the level of similarity and whether this extends to regulation and other concurrent process such as lignification, is unknown.

Lignin is also an abundant fraction of phellem; it consists in an aromatic polymer composed mainly of guaiacyl (G) and usually fewer syringyl (S) monolignol units, compared to the lignin found in xylem or phloem (36, 43, 93). In cork oak phellem, lignin is enriched in ferulic acid (93, 105) while in potato phellem, ferulic acid was also identified forming ether linked amides, such as feruloyltyramine (116).

TEM observations have revealed that the phellem cell wall is uniform around the cell and constituted by a primary layer of randomly oriented cellulose microfibrils, a suberized layer with a poly-lamella of alternating light and dark bands, and a tertiary layer proposed to contain waxes and/or polysaccharides, when present (42, 56, 128, 140) (**Figure 5e**). The components organizing the suberin ultrastructure as lamellae, as well as its linkage to lignin, are not fully clarified. Graça's model of suberin structure (56) proposes that the light lamellae accounts for the suberin polyester, structured by the bifunctional fatty acids and glycerol. In agreement, a genetic reduction of these specific suberin compounds (*CYP86A33-RNAi*, *cyp86a1*/*horst*) leads to a distorted lamellar structure (110, 140). The dark lamellae, in Graça's model, represents mainly polyaromatics and both lamellae are linked through the ferulic acid by ester bonds to aliphatics (light lamellae) and by forming lignin-like linkages with polyaromatics (dark lamellae). However, in phellem with a genetically reduced esterified ferulic acid (*FHT*-RNAi, *asft*), suberin lamellae are maintained, questioning dark lamellae composition and linkage to light lamellae (110, 138). Nevertheless, ferulic acid has been identified to be necessary to initiate the deposition of suberin in Arabidopsis phellem and endodermal cells (3).

Phellem cells also contain waxes, a group of lipophilic compounds extracted using an organic solvent. In outer bark, sterols and triterpenes, such as friedelin, betulin and lupeol and their derivatives, are the major wax components (1, 22, 27, 80). Recently, the cork oak oxidosqualene synthases likely to be responsible for producing several of these compounds have been biochemically characterized (22). Long chain and very long chain fatty acids and their fatty acid derivatives are also wax components, although fatty acids oxidized at both α- and ω-terminal positions are only found in suberin (1, 12, 27, 80). Waxes were suggested to interact with the suberin aliphatics in the light lamellae (56) or to be retained in the tertiary wall (128). Polyphenolics are another group of extractives, much less studied and with more heterogeneous composition among phellems, which include simple phenols and/or polymeric phenols such as tannins (23, 68). Several pieces of evidence support the crosstalk between the biosynthesis of precursors of phellem polymers and polar and non-polar compounds within extracts. For instance, changes in suberin biosynthesis impact wax composition, and specific blockage of suberin ferulic acid esterification affects the pool of soluble phenolics and the lignin composition (73, 137-139).

The specific role of polysaccharides such as cellulose, hemicellulose and pectins, as structural, functional and signaling elements of the cell wall in periderm, is to our knowledge, largely unknown. In potato, rhamnogalacturonan (RG)-I (pectin) accumulates preferentially in phellogen and phelloderm cell walls (98, 119) and the fragmentation of the RG-I backbone results in a random division plane and greater expansion of periderm cells, disorganizing the tissue structure in layers (119). Moreover, xylans are the major hemicelluloses in cork oak phellem (31, 121) and although their function in phellem and other suberized tissues is unknown, it may provide a structural anchor for ligno-suberin deposition through ferulic acid, as similarly described for lignin in grasses (47, 55).

5.2 Physiological function of phellem cells

The specific role of each structural and non-structural components of the phellem is very scarce and is mainly restricted to potato. A high number of phellem layers correlates with heat stress and resistance against tuber greening (52, 151), whereas, a 2-fold increase in suberin and waxes amounts corresponds to a decrease in water permeability (135). Beyond correlations, only a few functional genetic studies provide evidence of the protective role of the phellem and their components. In potato phellem, a shortening of 2 carbon-chain-length in suberin and wax, results in in 1.5-times higher permeability (*StKCS6*-RNAi) (139), suggesting that suberin and wax with longer chain-lengths confer higher protection. Phellems with reduced bifunctional fatty acids show a 60% reduction in suberin content (*CYP86A33-RNAi*) and disorganized suberin structure. Surprisingly, these dramatic changes in suberin only augment periderm permeability of 3.5-fold, indicating that the concomitant increase in wax content (2.4-fold) in *CYP86A33-RNAi* phellems might counteract suberin impairment (140). These findings indicate a partial role of suberin in establishing an efficient barrier and an active involvement of waxes. In agreement with an active role for waxes in water barrier function, dewaxed potato periderms increase their permeability 100-fold (135). Nevertheless, suberin and waxes are not the only components contributing to the barrier sealing properties, since a wound periderm, when compared to a native periderm accumulates 40% less suberin and waxes, and is surprisingly 100-times more permeable (135).

Interestingly, potato phellem that lacks ferulic acid-aliphatic esters and has altered lignin composition yields non-viable tubers that quickly dries due to high permeability (14-fold) (138). This suggests a disconnect between suberin-lignin polymers (or even polysaccharides), thus allowing water to pass through. In agreement, the Arabidopsis root phellem of plants in which the phenylpropanoid pathway is specifically inhibited in the periderm (*ELTP:MYB4)*, displays reduced suberin content, which highlights the importance of

aromatic compounds for correct suberin deposition. In these roots, phellem morphology is altered and a colored dye (Toluidine Blue) easily penetrates into the internal tissues in contrast to the impermeable phellem of wild type plants (**Figure 5b**). In addition, *ELTP:MYB4* plants are more sensitive to salt stress, emphasizing the protective role of the phellem against abiotic stresses (3). The development of universal permeability tests or quick methods to assess phellem barrier functionality are needed to further clarify the specific function of each phellem cell component.

The periderm, as an outer barrier, prevents pathogen penetration, while the development of wound (necrophylactic) periderm confines pathogen intruders within the infected area highlighting their protective role against biotic stresses (13, 29, 145). As such, cell wall hydrolyzing enzymes produced by most microorganisms are unable to degrade the suberized phellem tissue (135). However, several exceptions exist with respect to this generality: -pathogenic microorganisms like *Streptomyces scabies* cause corky lesions (scab) on the surface of potato tubers and root crops, similar to fruit russeting (76). Despite this important defense role of the periderm, the mechanisms triggering host resistance are not fully understood and are based on correlations with structural or chemical changes within phellem cells. For example, in potato tubers, the resistance to diverse invading pathogens correlates with lignin and suberin overaccumulation, and also with an increased number of phellem layers (21, 97, 111, 154). However, the resistance to tuber black dot, caused by a ubiquitous fungus with multiple host crop plants and weeds, positively correlates with the abundance of soluble compounds such as hydroxycinnamic acids, hydroxycinnamic acid amides and steroidal saponins (106).

In woody plants, pathogen attack and mechanical wounding may affect phellogen integrity rendering it non-functional (15) and trigger the development of closing (boundary or impervious) layers and wound periderms to reestablish phellogen functionality (15, 113). Host

resistance relies in the quick formation of such structures to confine the infected tissue (29, 145), whereas host susceptibility results from the ability of the pathogen to interfere with their formation (113). For instance, in hybrid poplars, resistance to the fungus *Septonia musiva* causing stem canker is associated with the development of a functional and unique thicker wound periderm, while susceptibility correlates with thinner periderms formed sequentially in response to the fungus penetration (169). In addition to structural defense, the production of terpenoids, phenolics or other bark compounds is also considered part of the resistance response (see for example 48). Overall, several pieces of evidence point out that the periderm participates in the plant defense system, and that defense mechanisms, rather specific for each host-pathogen interaction, correlate with its presence, structure and/or chemical composition.

5.3 Phellem regulatory network

Little is known regarding the regulatory network that determines how phellogen derivatives acquire phellem identity and progress toward phellem differentiation. Only two members of NAC (NAM/ATAF/CUC) family, the potato *St NAC103* transcriptional repressor and Arabidopsis *AtANAC46* transcriptional activator, have been characterized in the phellem by loss of function and overexpression lines, respectively (104, 148, 161). Transcripts from the MYELOBLASTOSIS (MYB) family are additionally induced in phellem tissues and are inferred to control suberin deposition as transcriptional activators in heterologous or *in vitro* systems (*QsMYB1, MdMYB93*) (24, 87) (**Supplementary Table 2**). The function of cork oak *QsMYB1* and apple *MdMYB93* have been inferred by the ability of QsMYB1 to bind to the promoters of suberin and lignin biosynthetic genes, and of *MdMYB93* to induce ectopic suberin deposition in tobacco (*Nicotiana benthamiana)* leaves (24, 87). Interestingly, all these transcription factors are highly expressed in other suberized tissues, and Arabidopsis *AtMYB93* and *ANAC46* are also able to promote suberization in the endodermis (104, 143). Additionally,

many other MYB suberin regulators (*MYB9*, *MYB32*, *MYB39/Suberman*, *MYB41*, *MYB53*, *MYB74*, *MYB92*, *MYB102*, *MYB107*) are enriched in phellems of different species and organs (24, 30, 44, 54, 81, 83, 85, 143, 157, 164, 167) suggesting that they also control phellem suberin deposition.

Abscisic acid (ABA) induces suberin biosynthesis, regulation and/or deposition in Arabidopsis endodermis (11, 86, 143), wound-healing tissues of potato tuber, tomato stem and kiwifruit (16, 88, 99, 167, 168). In agreement, the transient overexpression of the transcriptional activators in ABA signaling *AchnABF2* and *AchnMYC2* (*bHLH*) induces suberization in tobacco leaves (167, 168). In periderm-like structures of crown gall tumors induced by *Agrobacterium tumefaciens*, the suberization and protective function are ABA-dependent (39), suggesting a role of ABA as inducer of suberization in phellem, although the mechanisms involved in such regulation are still elusive.

6. CONCLUSIONS AND FUTURE CHALLENGES

Periderm formation is a complex process that integrates two major developmental steps, *de novo* formation of a meristem and the unique differentiation of its derivatives. The origin of the first phellogen differs among species, plant organs and growth stages adding another level of complexity to the system. The molecular mechanisms underlying periderm development are still largely unknown and only recently the first components have been elucidated. Auxin and most likely cytokinins have a positive role on phellogen initiation and/or proliferation. More players are likely to be added to the periderm regulatory network, as interesting candidates have already been identified in many species with the help of the next generation sequencing, phylogenetics and associated transcript profiling techniques. After gaining more insight into the core phellogen initiation and phellem differentiation regulatory modules, the next challenge will be to understand how different periderm variants (such as

wound periderm and rhytidome) are formed and have evolved through time, and how phellogen relates to the other lateral meristem, the vascular cambium. Finally, decades of work on barrier tissues have uncovered similarities in the chemistry between barrier tissues so it will now be interesting to compare if they share common regulatory networks for the development and differentiation.

SUMMARY POINTS

1. The periderm acts as an armor during secondary growth in woody and herbaceous plants, replacing the primary protective tissues such as the epidermis and endodermis. It protects the plant against pathogen penetration, abiotic stresses and seals the outer surface in case of traumatic events such as wounding and organ abscission.
2. The periderm comprises a meristematic layer: the phellogen, which divides bifacially forming the phellem outward and the phelloderm inward. In woody species, often the first periderm becomes inactive, and it is replaced by (sub)sequent periderms. New and old layers and the enclosed tissue form together a histologically recognized structure called the rhytidome.
3. A periderm has evolved multiple times independently during vascular plant evolution. In the fossil record, wound periderm occurs before native periderm suggesting that wound responses and periderm formation may share underlying similarities.
4. The phellogen arises from different tissues depending on the organs and/or species. It may remain active for several years or be replaced and its activity is modulated by seasonal changes.
5. Auxin is required for the establishment and maintenance of the phellogen and acts via two known cambial regulators WOX4 and BP, highlighting shared core components for lateral meristems.

6. Typical phellem cells are suberized, but diverse phellem cell types exist, some unsuberized and others differing in their cell wall thickness, or secondary metabolite content. During differentiation, phellem cells radially expand, accumulate polyphenolics and waxes, deposit lignin and suberin, and eventually die.
7. Wax and suberin deposits, and particularly ferulic acid-aliphatic esterification and/or lignin composition, are the factors influencing the protective function of phellem against dehydration.
8. The regulatory network triggering phellem differentiation, including suberization, includes transcriptional regulators from the NAC family and likely from the MYB family.

FUTURE ISSUES

1. Only a few regulators of phellogen proliferation and differentiation have been functionally characterized, and the whole regulatory network is far from being completed.
2. The vascular cambium and the phellogen share many regulators, raising the question on how specificity is achieved and whether phellogen and vascular cambium activities are coordinated.
3. In perennial plants, the mechanisms underlying the regulation of phellogen activity during seasonal changes, and the cessation of activity followed by the initiation of a new phellogen, remain to be studied.
4. Phellem cell wall modifications are known, but the spatio-temporal dynamics of suberin and lignin deposition as well as linkage at ultrastructural level require further investigations.

5. Phellem cells are remarkably similar in their cell wall composition to endodermal cells. This suggests the phellem may be equivalent functionally to the endodermis or may share the same regulatory networks.
6. The phellem components and associated mechanisms responsible for its protective function are not fully understood, but are central to understand how plants cope with abiotic and biotic stresses.
7. The phelloderm remains an enigmatic tissue, and its specific function should be further understood.
8. To advance periderm knowledge, new tools and methods needs to be developed: genetic tools for specific and conditional manipulation of periderm, single cell transcriptomics of periderm and methods to quantify its barrier function.

DISCLOSURE STATEMENT

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TERMS AND DEFINITIONS LIST:

Waxes: Solvent-soluble, lipophilic compounds deposited to the surface of plant organs (e.g. roots, leaves).

Lamella: a thin layer, membrane or plate of a tissue.

Bark: the group of tissues located outside the vascular cambium, which includes secondary phloem (inner bark), periderm or rhytidome (outer bark).

Rhytidome: the outer bark containing the innermost phellem, the older periderms and the tissue (cortex and secondary phloem) enclosed between them.

Parenchyma cells: living cells, with thin cell wall, which can have photosynthetic or storage functions.

Hypodermis: a layer of cells below the epidermis that is distinct from the ground tissue. Also sometime referred to as the exodermis.

Wound periderm: periderm that arises in response to wounding.

Phellogen: secondary meristem, whose stem cells divide bifacially forming phellem outward and inward the phelloderm.

Phellem: outer protective tissue, often suberized, that is produced by the phellogen.

Phelloderm: parenchyma tissue formed by the phellogen.

(Sub)sequent periderm/phellogen: the periderm/phellogen, often arising from secondary phloem, formed to replace the dead/inactive periderm/phellogen.

Persistent or long-lived periderm/phellogen: the periderm/phellogen that is functional over the years and thus it is not replaced by (sub)sequent periderms.

Vascular cambium: secondary meristem, whose stem cells divide bifacially forming wood inward and phloem outward.

Suberin bifunctional fatty acids: suberin monomers represented by α,ω-diacids and ω-hydroxyacids, both oxidized at α- and ω-terminal position and thus allowing polyester extension.

Phenylpropanoid pathway: the biosynthetic pathway that form hydroxycinnamic acids, monolignols, flavonoids and tannins.

Necrophylactic periderm: periderm that protects living tissues from adjacent dying tissues.

REFERENCE ANNOTATIONS

(171) This paper shows that auxin acts via WOX4 and BP to promote phellogen establishment and maintenance.

(146) This paper highlights partial common ontogenesis of the vascular cambium and phellogen.

(3) This paper illustrates that aromatic acids are essential for phellem function and morphology

(170) This paper presents the Arabidopsis root as the future model for studying periderm development.

(147) This was the first cork transcriptome analysis reporting candidate genes and processes for phellem formation.

(140) This paper highlights bifunctional fatty acids for suberin deposition and ultrastructure and periderm barrier function.

(138) This paper reports that ferulic acid esters in suberin are key for creating a functional periderm barrier.

(161) This paper functionally characterizes the phellem regulator StNAC103 acting as suberin repressor.

(109) This paper presents the functional characterization of poplar SHORT-ROOT, the first phellogen regulator described.

(1). This paper provides specific transcript profiles for all tissues of a birch stem and the metabolic analysis of birch phellem.

Sidebar1: Confusion/heterogeneity in periderm/bark and phellem/cork nomenclature

The terminology used to identify periderm, bark, and rhytidome in the literature is often ambiguous, which poses problems in the search of relevant information and comparison among different studies. For instance, the term bark has been frequently used to refer to the periderm, the word cork is employed as a synonym of phellem, to indicate a phellem cell that is suberized or as the phellem of cork oak. The rhytidome may or may not include the last living periderm and similarly the outer bark may comprise or not the last living periderm. The terms necrophylactic and exophylactic periderms become popular in bark literature to classify the periderms based on their putative role in protecting living tissues from adjacent dying tissues or from environment, respectively. Since this classification is based on the functional role of the different periderms, which is still unclear, we consider that the terms first and (sub)sequent periderms as well as wound periderm should be employed instead (158). In this review, we mainly use the terms as defined by International Association of Wood Anatomists (IAWA) (4, 158). However, we avoid using the term cork as a synonym of phellem and reserve the term cork with respect to commercial cork.

Sidebar2: Lenticels

Lenticels are protrusions, present in the periderms of most plants, which facilitate oxygen and water exchanges (60). Periderms that lack lenticels usually shed their outer layers of rhytidome annually, thus the living tissues are not far from the surface. The phellogen, which gives rise to lenticels, is known as lenticular phellogen and it is very active producing many complementary or filling cells. The high production rate of filling cells bulges the periderm outward resulting in fracture of the surface layer. Filling cells differ from phellem cells as they display a loose arrangement with many intercellular spaces, feature that allows efficient gas exchange. Filling cells may be suberized (simplest type of lenticel), unsuberized during the growing season or have layers of suberized and unsuberized cells (4, 42, 128). Lenticels can be formed prior, during or after phellogen establishment depending on the site of phellogen initiation, plant organ, specie and environmental conditions. Lenticels are usually initiated below stomata, even though they arise independently of stomata in plants in which the first phellogen is deep-seated (4, 42, 128).

Figure Captions:

Figure 1

Different protective tissues act during primary and secondary growth of stem and roots. During primary growth, the epidermis (black) with its cuticle protects the stem while the endodermis (grey) regulates nutrient uptake and protects the root during primary root growth. The periderm (blue) replaces the epidermis and the endodermis during secondary growth. (*a*) Sketches showing stem and root anatomy from primary to secondary growth highlighting the main protective tissues (dashed arrows). The discontinuous line in the epidermis indicated that primary tissues surrounding the periderm get crushed and detached to accommodate radial growth, except for the endodermis, which undergoes programmed cell death. (*b*) Sketch of a periderm. The periderm constitutes of the phellogen, the phellem and the phelloderm. (*c*)

Sketch of a rhytidome. The rhytidome is made of (sub)sequent periderms (P) (from first to third) and the tissue enclosed in between (* refers to the last formed periderm, which is active). (*d*) Sketch of a lenticel. Lenticels are integrated in the periderm and facilitate oxygen and water exchanges, thanks to the loose arrangement of the filling/complementary cells. The phellogen forming the lenticel is known as lenticular phellogen.

Figure 2

Occurrence of a periderm across major groups of extinct and living vascular plants. White circle indicates the presence of wound periderm in *Psilophyton.* Black circles indicate the presence of a native periderm. Extinct groups are indicated by dagger symbols (†). Arch. and Aneuro. progymnosperms, abbreviations of, archaeopteridelean and aneurophytalean progymnosperm.

Figure 3

Examples of different types of phellogen ontogenesis and fruit russeting. (*a-d*) Periderm formation in the stem of hybrid aspen (T89) illustrated by plastic cross-sections and sketches. (*a*): the epidermis is the protective tissue during primary growth. (*b*): the first formative divisions that give rise to the phellogen (green) occur in the subepidermal layer (black arrow). (*c*): divisions propagate to the whole subepidermal layer and a phellogen is formed and then the first phellem cells (brown) are produced. (*d*): the phellogen keeps dividing and produces more phellem layers and the phelloderm (purple). Black scale bars are 50µm. (*e-h*) Periderm formation in the Arabidopsis root illustrated by plastic cross-sections and sketches. (*e*): the root prior secondary growth initiation, consists of a central xylem (red) and 2 poles of phloem (yellow) embedded in the procambium (aqua), surrounded by the pericycle, endodermis, cortex and epidermis. (*f*): the first formative divisions that give rise to the phellogen (green, black

arrow) occur adjacent to the xylem pole pericycle (orange). (*g*): divisions propagate to the whole pericycle and a phellogen is formed (green). (*h*): the phellogen keep dividing and produces the phellem (brown) outward and the phelloderm (purple) inward. The outer tissues, epidermis and cortex, are detached once the phellem is suberized. Black Scale bars are 20µm. (*i-j*) Fruit russeting in apple. (*i*) Golden delicious apples showing normal fruit skin (left) and fruit russeting (right, black rectangular), black scale bar is 1cm. (*j*) Fluorol yellow staining highlighting the cuticle in the epidermis (fruit skin) (right panel) and the suberized phellem in russeted areas (left panel). White scale bars are 20µm.

Figure 4

Wound periderm/phellogen formation and phellogen regulatory network. (*a-b*) The formation of a wound periderm in a potato tuber. (*a*): after wounding, the first event is the formation of a closing layer (in pink) with ligno-suberin depositions (pink arrow) and at 3-days after wounding, cells beneath the closing layer are dividing periclinally (green and black arrows) and form a phellogen. (*b*): at 19-day after wounding, a wound periderm consisting of a multilayered phellem (brown), a phellogen (green) and a phelloderm (purple), is visible. White scale bars are 20µm. (*c*) Current model explaining phellogen initiation in the Arabidopsis root. The initiation of the vascular cambium is required to trigger the auxin-induced-periderm program in the pericycle. Auxin (indole acetic acid, IAA) via AUXIN RESPONSIVE FACTOR 5/ARF5, ARF8 and probably other ARFs triggers phellogen initiation. Downstream of auxin, WOX4 and BP promote periderm formation. (*d*) Sketch of a root cross-section with indicated putative and known regulators of vascular cambium activity and/or phellogen activity. Orange: pericycle; pale green: phellogen, dark green: vascular cambium. Putative regulators: pink, known regulators: black.

Figure 5

Phellem morphology and cell wall modifications. (*a*) Scanning electron micrograph of potato tuber periderm showing 10-12 phellem cell layers. Phellem cells are compactly arranged and organized in radial files, as consequence of their formation by periclinal divisions of the lower mother phellogen cell (not reliably distinguished here). (*b*) Phellem cell wall modifications: the Arabidopsis root as an example. Left and middle left panels: lignin depositions detected by Basic Fuchsin staining and autofluorescence respectively. Middle right panel: suberin depositions detected by Fluorol yellow staining. Right panel: phellem cells act as an impermeable barrier, Toluidine Blue (a purple dye) cannot penetrate. White scale bar: 50 µm. (*c*) Phellem transverse section of a phellem plank (outer bark) extracted from cork oak. Each cork ring, usually corresponding to annual increments, includes the early cork formed massively at the beginning of the growing season (thin-walled phellem cells) and the late cork (less radially expanded thick-walled phellem cells, brownish) at the end of the growing season. Lenticels connect the inner tissue to the exterior. This cork oak bark tissue is commercially exploited to produce cork-derived products. (*d*) Diversity of outer bark surface appearance in trees of different species. (*e*) Transmission Electron Microscopy images of Arabidopsis phellem cells at different magnifications. The suberin lamellae (SL) are visible as alternating layers of light and dark lamellae. PW: primary wall.

Supplementary Table1: Periderm specific transcriptomic resources.

Supplementary Table 2: Periderm regulators.

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a

Stem

Primary growth

Stem Maturation

Secondary growth

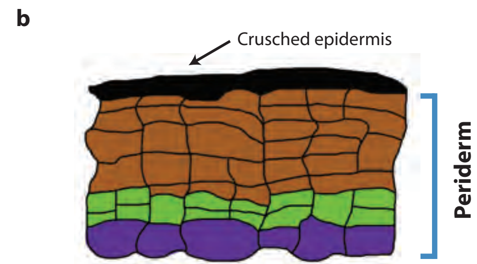
Root

Secondary growth

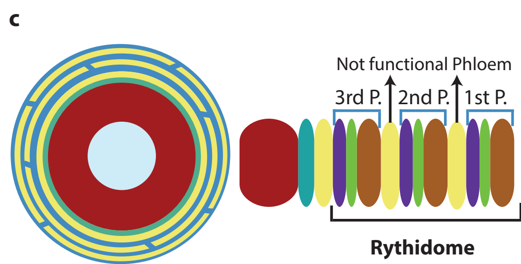
Root Maturation

Primary growth

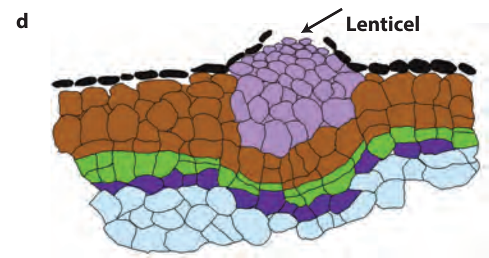
b



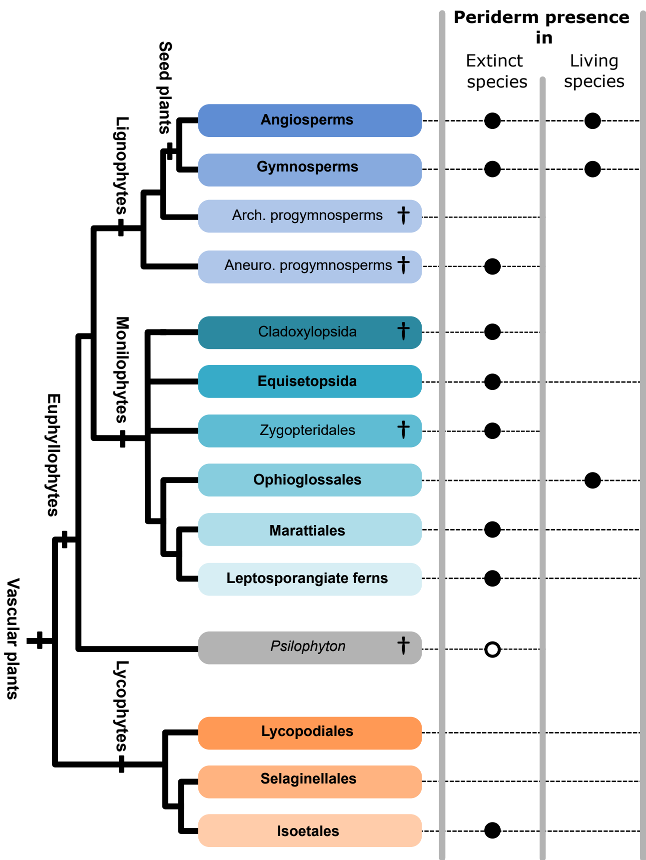
c



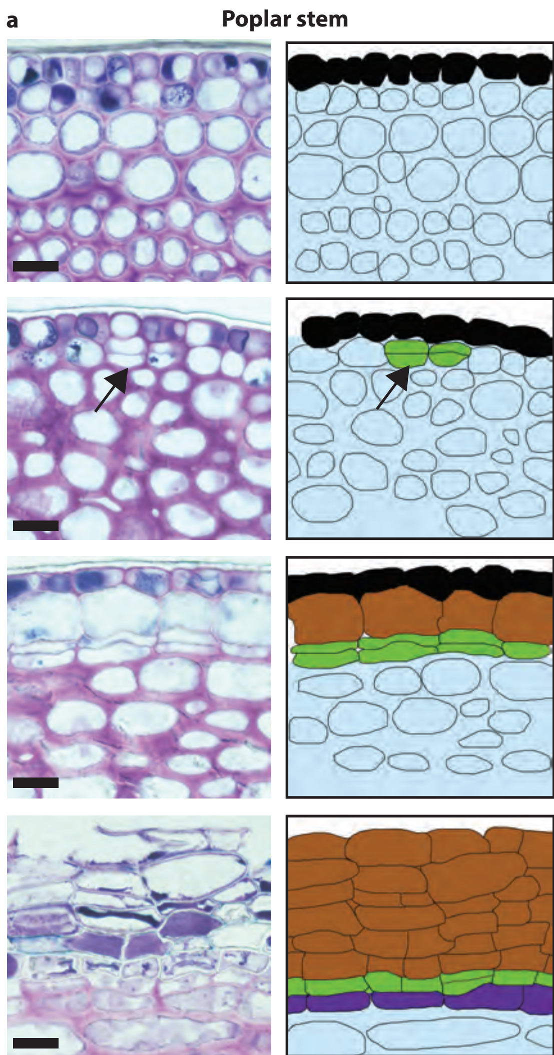
d

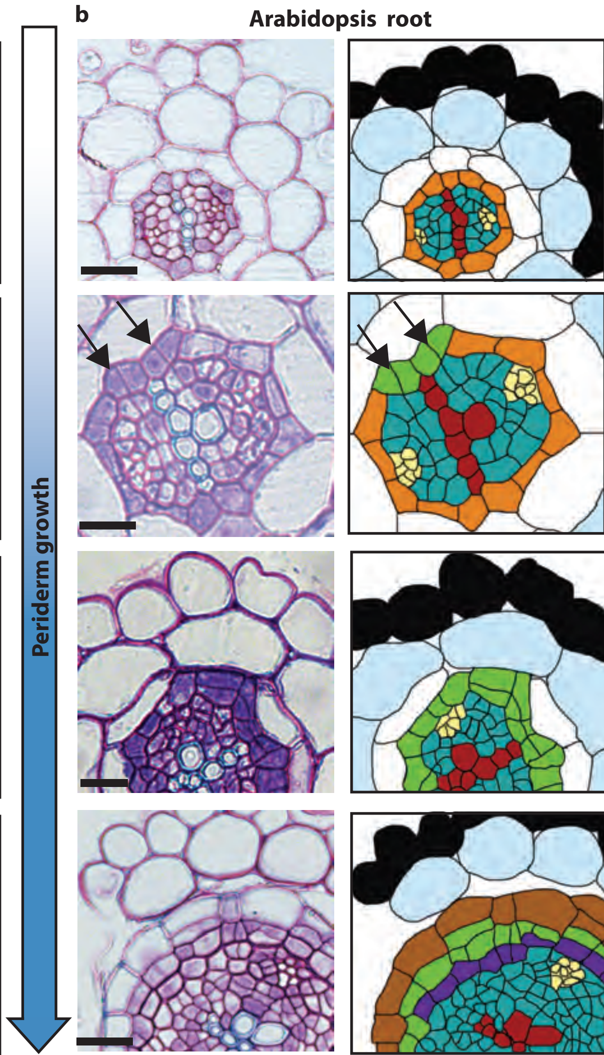


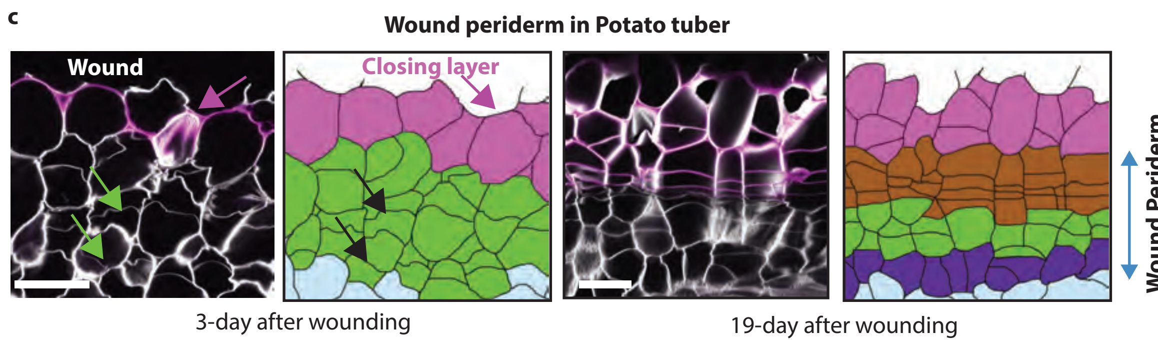
Epidermis	Pericycle	Phelloderm
Cortex	Periderm	Phellogen
Xylem	Endodermis	Phellem
Phloem	(Pro)-Vascular cambium	Filling layer

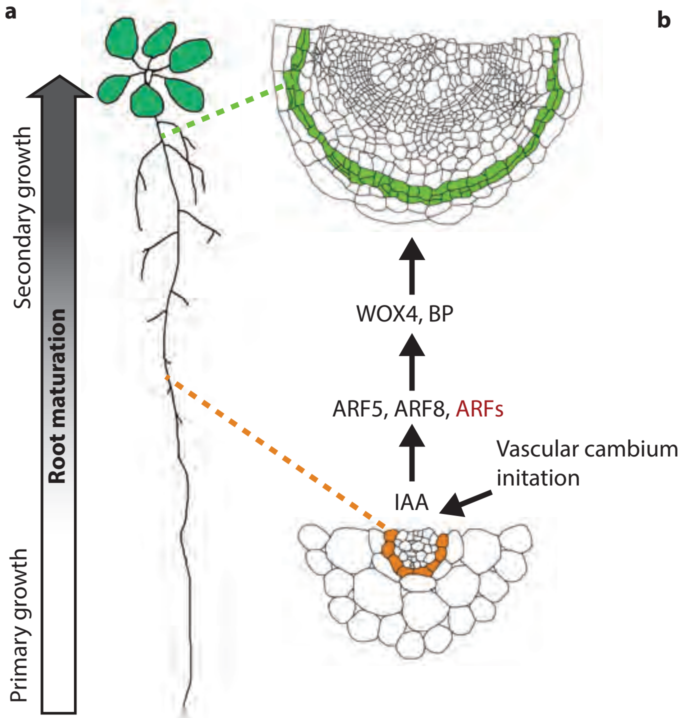


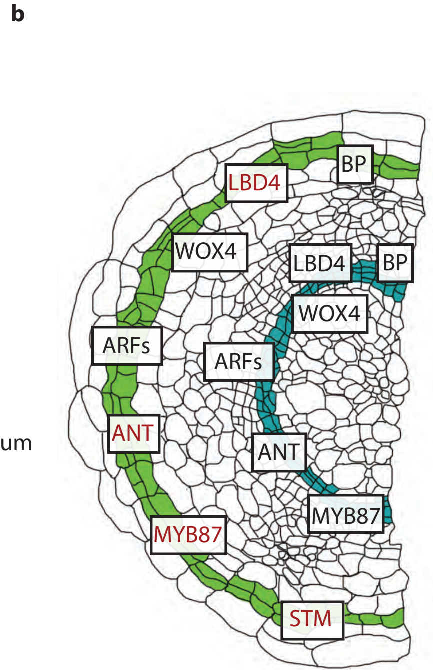
Periderm presence in
Extinct species
Living species
Seed plants
Lignophytes
Moniliophytes
Euphyllophytes
Vascular plants
Lycophytes
Angiosperms
Gymnosperms
Arch. progymnosperms †
Aneuro. progymnosperms †
Cladoxylopsida †
Equisetopsida
Zygopteridales †
Ophioglossales
Marattiales
Leptosporangiate ferns
Psilophyton †
Lycopodiales
Selaginellales
Isoetales

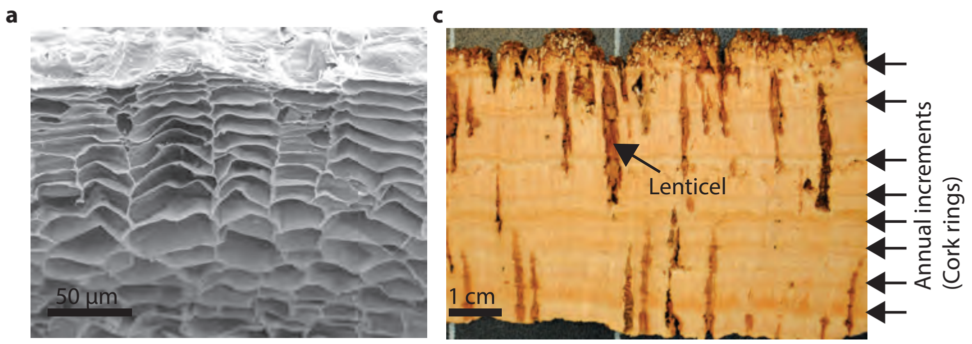

a
Poplar stem

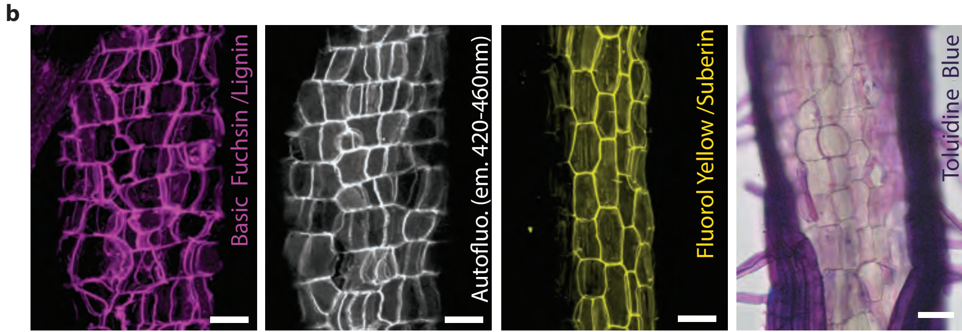

Periderm growth
b
Arabidopsis root


c
Wound periderm in Potato tuber
Wound
Closing layer
Wound Periderm
3-day after wounding
19-day after wounding

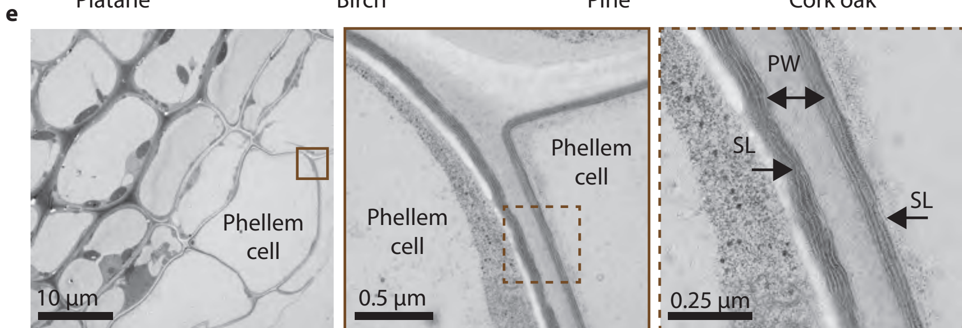

a
Secondary growth
Root maturation
Primary growth
WOX4, BP
ARF5, ARF8, ARFs
Vascular cambium
initation
IAA


b
BP
LBD4
WOX4
LBD4
BP
WOX4
ARFs
ARFs
ANT
ANT
MYB87
MYB87
STM


a
50 µm
c
Lenticel
1 cm
Annual increments
(Cork rings)


b
Basic Fuchsin /Lignin
Autofluo. (em. 420-460nm)
Fluorol Yellow /Suberin
Toluidine Blue

d
Platane
Birch
Pine
Cork oak


e
Phellem
cell
10 µm
Phellem
cell
Phellem
cell
0.5 µm
PW
SL
SL
0.25 µm

Supplementary Table1: Periderm specific transcriptomic resources.

Organism		Tissue	Type	Ref.
Cork oak	*Quercus suber*	Phellem obtained by scratching the inside of the bark from stems of 15-20-year-old trees (Virgin cork).	SSH library/ Microarray	[1]
Cork oak	*Quercus suber*	Phellem obtained by scratching the inside of the phellem plank from stems of mature trees producing good and bad quality cork (Reproduction cork).	RNA-seq	[2]
Cork oak	*Quercus suber*	Phellem obtained by scratching the inside of the phellem plank from stems of adult trees.	Small RNA-seq	[3]
Cork oak	*Quercus suber*	Phellogen obtained from 1-year-old branches from trees producing good and bad quality cork, using laser capture microdissection.	RNA-seq	[4]
Cork oak	*Quercus suber*	Phellem obtained by scratching the inside of the phellem plank from stems of 15-20-year-old trees (Virgin cork).	RNA-seq	[5]
Cork oak	*Quercus suber*	Phellem obtained by scratching the inside of the phellem plank from stems (Virgin and amadia cork (a type of reproduction cork, with high technical quality)).	RNA-seq	[6]
Cork oak	*Quercus suber*	Phellem and phellogen obtained by scratching the inside of the phellem plank from stems during the growing season: April (enriched in phellogen), June and July (enriched in differentiating phellogen).	RNA-seq	[7]
Holm oak	*Quercus ilex*	Outer bark (rhytidome) obtained from stems of 15-20-year-old trees.	RNA-seq	[5]
Hybrid of Turkey oak and Cork oak	*Quercus cerris x Quercus suber*	Annual stems from hybrid trees belonging to the same open-pollinated family (half-sibs), and displaying contrasting phenotypes for the typical phellem formation.	RNA-seq	[8]
Potato	*Solanum tuberosum*	Tuber phellem collected from field grown potatoes.	SSH library/ Microarray	[9]
Potato	*Solanum tuberosum*	Tuber phellem collected at 8 week-post-sprout emergence, considered immature periderm. Heat stress was applied for 1 week.	Microarray	[10, 11]
Potato	*Solanum tuberosum*	Tuber phellogen isolated using laser capture microdissection, collected from 3 up to 8 week-post-sprout emergence.	RNA-seq	[12]
Potato	*Solanum tuberosum*	Tuber phellem collected from 3 month-old-plants from wild type and two lines downregulating the StNAC103 transcription factor (*StNAC103*-RNAi and *StNAC103*-RNAi-c).	RNA-seq	[13]
Grey poplar	*Populus tremula* x *Populus alba*	Outer bark collected from stems at 2 developmental stages (4.0–5.5 mm and 6.0–8.0 mm diameter).	RNA-seq	[14]
Birch	*Betula pendula*	Cryo-sections of phellem or phellogen and phelloderm from 13-year-old stems.	RNA-seq	[15]
Arabidopsis	*Arabidopsis thaliana*	pGPAT5 (expressed in phellem) translatome of the upper region of 8 day-old roots.	TRAP-seq	[16]
Apple	*Malus domestica*	Fruit skin of three cultivars with fully-russeted phenotype and three cultivars with fully-waxy phenotype.	RNA-seq	[17]
Apple	*Malus domestica*	Fruit skin of the normal skinned ‘Reinders’ and the russeted ‘Rugiada’. 5 developmental stages.	RNA-seq	[18]
Apple	*Malus domestica*	Fruit skin of russeted (non-bagging) and non-russeted (bagging) phenotype. 3 developmental stages.	RNA-seq	[19]
Apple	*Malus domestica*	Fruit skin with waxy, partially russeted and fully russeted phenotype. 3 developmental stages.	RNA-seq	[20]
Melon	*Cucumis melo*	Fruit skin of 3 cultivars with reticulated phenotype and 3 cultivars with smooth phenotype.	RNA-seq	[21]
Sand pear	*Pyrus pyrifolia*	Skins of fruits with russeted and green phenotype from F1 offspring from ‘Qingxiang’ x ‘Cuiguan’.	RNA-seq	[22]
Sand pear	*Pyrus pyrifolia*	Skins of fruits with russeted (‘Nitikata’) and green (‘Suisho’) phenotype.	RNA-seq	[23, 24]
Pear	*Pyrus pyrifolia*	Skins of semi-russeted and non-russeted (bagged) ‘Cuiguan’ fruits.	RNA-seq	[25]
Grape	*Vitis vinifera*	‘Sunshine muscat’ russeted and non-russeted grape peels. 5 developmental stages pooled together.	RNA-seq	[26]
Tomato	*Solanum lycopersicum*	Skins of fruits with russet (SlDCR-RNAi) and smooth (wild type) phenotype. Mature green stage.	RNA-seq	[18]

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Supplementary Table 2: Periderm regulators.

Gene	Organism	Type	Functional and experimental evidences	Ref.
ARF5 and ***ARF8*** **(***At1g19850* and *At5g37020***)**	Arabidopsis *(Arabidopsis thaliana)*	ARF TF	**Promotion of periderm development.** Expressed in the periderm. Loss of function mutants show delay in periderm growth	[1]
WOX4 *(At1g46480)*	Arabidopsis *(Arabidopsis thaliana)*	WOX TF	**Promotion of phellogen proliferation.** Expressed in the phellogen and phelloderm. Specific induction in the periderm promotes phellogen proliferation and loss of function mutant shows delay in periderm growth	[1]
BP/KNAT1 *(At4g08150)*	Arabidopsis *(Arabidopsis thaliana)*	KNOX TF	**Promotion of phellogen proliferation** Expressed in the phellogen and phelloderm. Specific induction in the periderm promotes phellogen proliferation and loss of function mutant shows delay in periderm growth.	[1]
PtSHR2B *(related to SHR/At4g37650)*	Poplar (*Populs thricocarpa*)	GRAS TF	**Promotion of bark growth.** Expressed in the phellogen, when overexpressed, it increases bark thickness	[2]
QsMYB1 *(putative ortholog of AtMYB68/ At5g65790 and AtMYB84/ At3g49690)*	Cork oak *(Quercus suber)*	MYB TF	**Suberin transcriptional activator.** Highly expressed in phellem. It targets genes related to suberin and lignin biosynthesis and transport.	[3]
MdMYB93 *(putative ortholog of AtMYB93/ At1g34670)*	Apple *(Malus domestica)*	MYB TF	**Suberin transcriptional activator.** Identified as differentially expressed in russeted vs non-russeted apple skins. It activates suberin and lignin related genes and suberin deposition when ectopically expressed in *Nicotiana benthamiana* leaves.	[4]
StNAC103 *(putative ortholog of ANAC58/ At2g18400)*	Potato *(Solanum tuberosum)*	ANAC TF	**Suberin and wax transcriptional repressor.** When silenced, suberin related genes are upregulated an overproduction of suberin and suberin-associated waxes is observed in the phellem of tubers.	[5, 6]
ANAC46 *(At3g04060)*	Arabidopsis *(Arabidopsis thaliana)*	ANAC TF	**Suberin transcriptional activator.** Expressed in phellem root. When overexpressed, it induces the expression of suberin-related genes and suberin deposition in the cork.	[7]

References Supplementary Table2

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