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The Making of Plant Armor: The Periderm

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Keywords

phellem, bark, phelloderm, cork cambium, phellogen, periderm

Abstract

The periderm acts as armor protecting the plant's 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 comprises a meristematic tissue called the phellogen, or cork cambium, and its derivatives: the lignosuberized phellem and the phelloderm. Research on the periderm has mainly focused on the chemical composition of the phellem due to its relevance as a raw material for industrial processes. Today, there is 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 of 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.

Contents

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, and ultraviolet (UV) radiation as well as from diverse biotic stresses (reviewed in 47, 75). 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).

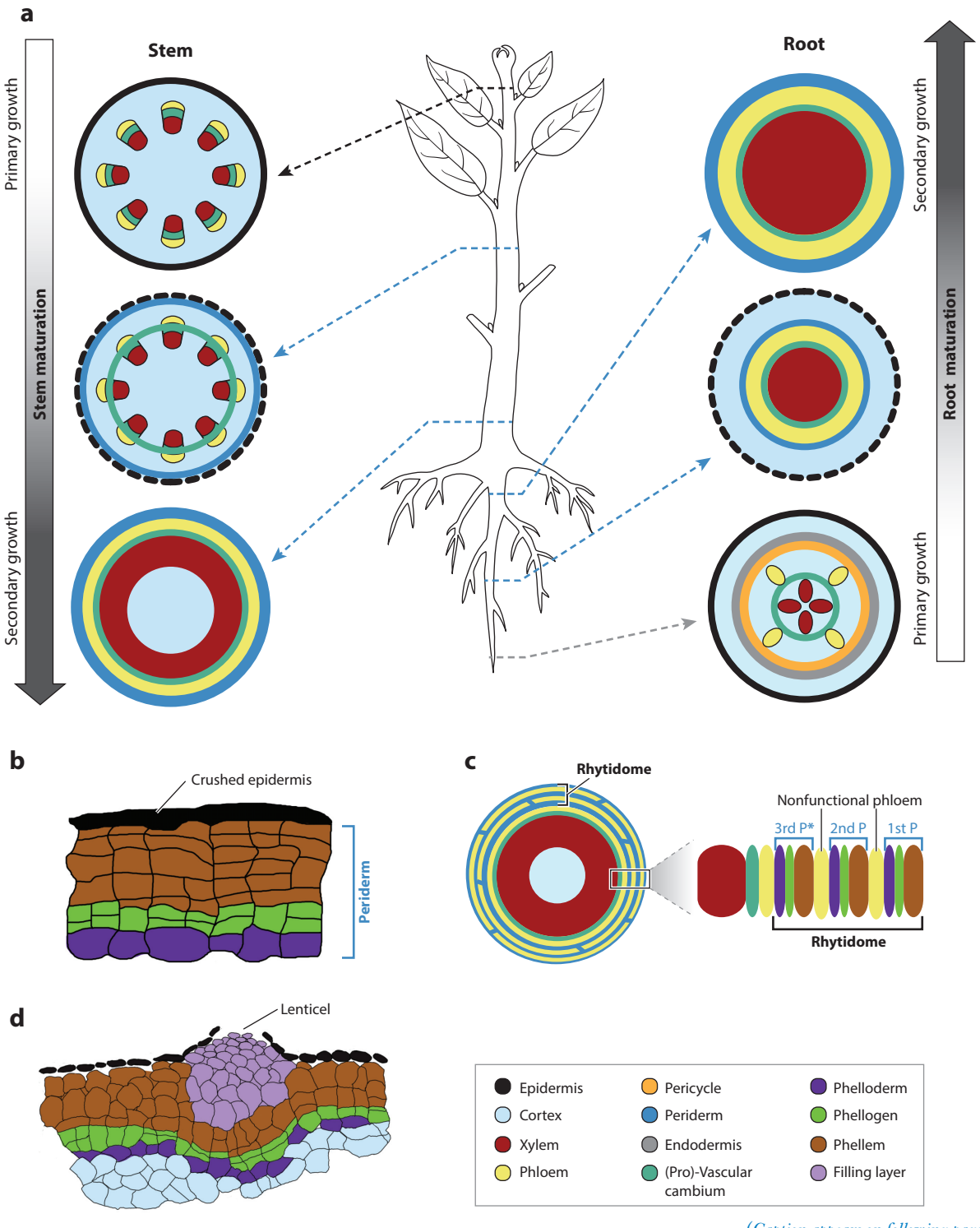
Waxes: solvent-soluble, lipophilic compounds deposited mainly on the surface of plant organs (e.g., roots and leaves)

Hypodermis: a layer of cells below the epidermis that is distinct from the ground tissue; in roots, also sometimes referred to as the exodermis

Wound periderm: periderm that arises in response to wounding

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 (70, 120, 139, 164). 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 (41, 77, 134, 139).

As tissues mature, a new protective armor, the periderm, is formed. The 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 to 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



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

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 (*gray*) regulates nutrient uptake and protects the root. The periderm (*blue*) replaces the epidermis and the endodermis during secondary growth. (*a*) Stem and root anatomy from primary to secondary growth. The color of each dashed arrow matches the color of the major barrier tissue at each phase. The discontinuous line in the epidermis indicates that primary tissues surrounding the periderm get crushed and detached to accommodate radial growth, except for the endodermis, which undergoes programmed cell death. (*b*) The periderm comprises the phellogen, phellem, and phelloderm. (*c*) The rhytidome is made of (sub)sequent periderms (P) (here from first to third) and the tissue enclosed in-between (asterisk refers to the last-formed periderm, which is active). (*d*) Lenticels are integrated into 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 the lenticular phellogen.

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

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

secondary growth (42, 43). 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 that forms underneath, which in turn is replaced by (sub)sequent periderms forming over the years, leading to the formation of the rhytidome. Hence, the rhytidome comprises a succession of dead periderm layers alternating with layers of nonfunctional secondary phloem plus the last active periderm (4, 42, 135) (**Figure 1*c***; see also the sidebar titled Confusion and Heterogeneity in Periderm/Bark and Phellem/Cork Nomenclature). By contrast, in a few species such as cork oak, it appears that the same periderm grows over the years, known as the persistent periderm. In both cases, the periderm and the rhytidome are also commonly referred to as outer bark (see the sidebar titled Confusion and Heterogeneity in Periderm/Bark and Phellem/Cork Nomenclature). Lenticels form a porous channel within the periderm to facilitate gas exchange between the atmosphere and inner tissues (**Figure 1*d***; see also the sidebar titled Lenticels).

The periderm is a complex system composed of the phellogen meristem (also known as cork cambium) and the two tissue types it produces—the phellem (also commonly referred to as cork) and the phelloderm (see the sidebar titled Confusion and Heterogeneity in Periderm/Bark and Phellem/Cork Nomenclature) (**Figure 1*b***). The architecture of the periderm is reminiscent of the vasculature, and thus the phellogen, similar to the vascular cambium, constitutes a cylinder of meristematic cells that divide bifacially, producing the phellem on the outside and the phelloderm on the inside. The number of phellem and phelloderm layers is highly variable among species, and phellogen activity and differentiation can be unbalanced, resulting in preferential formation of one of the two tissues. For instance, tropical trees usually have a pronounced phelloderm, whereas

CONFUSION AND 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 for relevant information and comparisons among different studies. For instance, the term bark has been frequently used to refer to the periderm, and 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 are popular in bark literature to classify the periderms based on their putative role in protecting living tissues from adjacent dying tissues or from the 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 (163). In this review, we mainly use the terms as defined by the International Association of Wood Anatomists (IAWA) (4, 163). However, we avoid using the term cork as a synonym of phellem and reserve it to refer to commercial cork.

LENTICELS

Lenticels are protrusions (**Figure 1*d***), present in the periderms of most plants, that facilitate oxygen and water exchanges (61). 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 the lenticular phellogen and is very active, producing many complementary or filling cells. The high production rate of filling cells causes the periderm to bulge outward, resulting in fracture of the surface layer. Filling cells differ from phellem cells as they display a loose arrangement with many intercellular spaces, a feature that allows efficient gas exchange. Filling cells may be suberized (the simplest type of lenticel), may be unsuberized during the growing season, or may have layers of suberized and unsuberized cells (4, 43, 133). Lenticels can be formed prior to, during, or after phellogen establishment, depending on the site of phellogen initiation, plant organ, species, 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, 43, 133).

cork oak (*Quercus suber*) displays many layers of phellem, and some plants completely lack a phelloderm (42, 43, 58, 136). Phelloderm cells are parenchyma cells (surrounded by 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 lignosuberization, 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 correlate with the ability to withstand pathogen penetration and protection against abiotic stress such as high salinity and wildfires (3, 39, 65, 88, 169). Moreover, in the case of potato (*Solanum tuberosum*), tuber conservation and storage abilities are deeply influenced by the chemical composition of the phellem (103).

Understanding the mechanisms of periderm development is thus relevant not only for breeding processes aiming to improve plant tolerance to stresses but also for industrial processes as the phellem of cork oak (cork) is an excellent raw material for insulant, polymer, and wine stopper production due to the combination of its special mechanical, chemical, and morphological characteristics (93, 125, 149). 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 (69), research on the molecular mechanisms underlying periderm development has lagged behind that of other plant developmental processes such as root and shoot primary growth. However, with advancements in next-generation sequencing and genome editing techniques, and the rising interest in suberin and bark as possible sources of CO_2 sequestration, periderm development has become a blooming field (63, 123).

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

Phellogen: secondary meristem whose stem cells divide bifacially, forming phellem outside and phelloderm inside

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

Phelloderm: parenchyma tissue formed by the phellogen

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

Parenchyma cells: living cells, with a thin cell wall, that can have metabolic or storage functions

2. PERIDERM EVOLUTION AND FOSSIL RECORDS

Studies of the fossil record provide valuable insights into the multiple evolutionary origins of the periderm. The earliest evidence of periderm in the fossil record occurs in the Early Devonian period, roughly 400 million years ago. Fossils from the Early Devonian period preserve a diverse range of early vascular plants (51, 157), including the earliest evidence of plants developing wood (50) prior to the origin of the first trees and forests in the Mid-Devonian period (154). The earliest

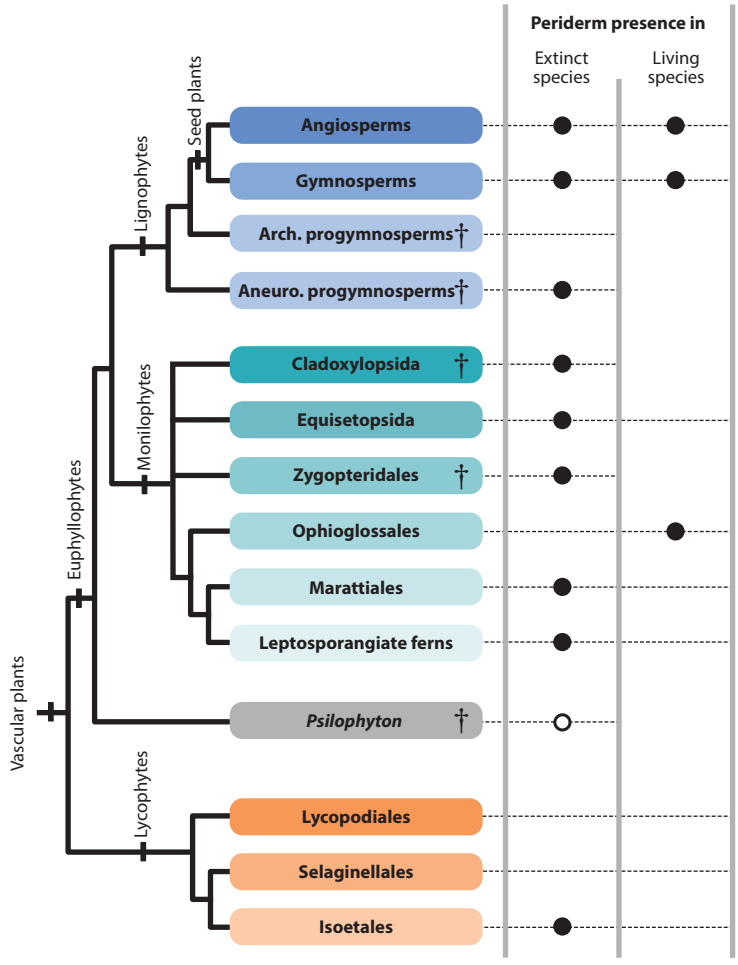


Figure 2

Occurrence of periderm across major groups of extinct and living vascular plants. The white circle indicates the presence of wound periderm in *Psilophyton*. Black circles indicate the presence of native periderm. Extinct groups are indicated by dagger symbols. Abbreviations: Aneuro., aneurophytalean; Arch., archaeopteridalean.

evidence of a periderm was described from *Psilophyton dawsonii* (7, 8), a basal, extinct member of the euphyllophytes, the group that today includes all monilophytes and seed plants (78) (**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 aboveground 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 characterized by thin cell walls and are thought to represent the phellem, whereas thicker-walled cells were found internally and may represent the 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 period, roughly 385 million years ago, in a group called the aneurophytalean progymnosperms (112, 137), which

are extinct precursors of modern seed plants (**Figure 2**). During the Carboniferous period, there is extensive evidence of periderm in the major groups of vascular plants. Examples of periderm have been described in many fossils of seed plants (33, 34, 157) as well as ferns and lycophytes, including arborescent lycopsids (32), cladoxylopsids (33), equisetopsids (28, 33), Zygopteridales (33, 128), Marattiales (132) and leptosporangiate ferns (129) (**Figure 2**). Each of these groups are phylogenetically distinct and separated by relatives lacking a periderm, suggesting 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 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 (155) (**Figure 2**). Therefore, the fossil record suggests that 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 (38) and ferns (67) to seed plants (42). Although not present in all vascular plants (68, 79), the widespread nature of wound periderm across multiple groups and its early occurrence in *P. dawsonii* fossils suggest that it may be a conserved feature of vascular plants. Despite the different evolutionary trajectories between wound periderm and native periderm, their similarities suggest that they may share underlying features and evolutionary origins.

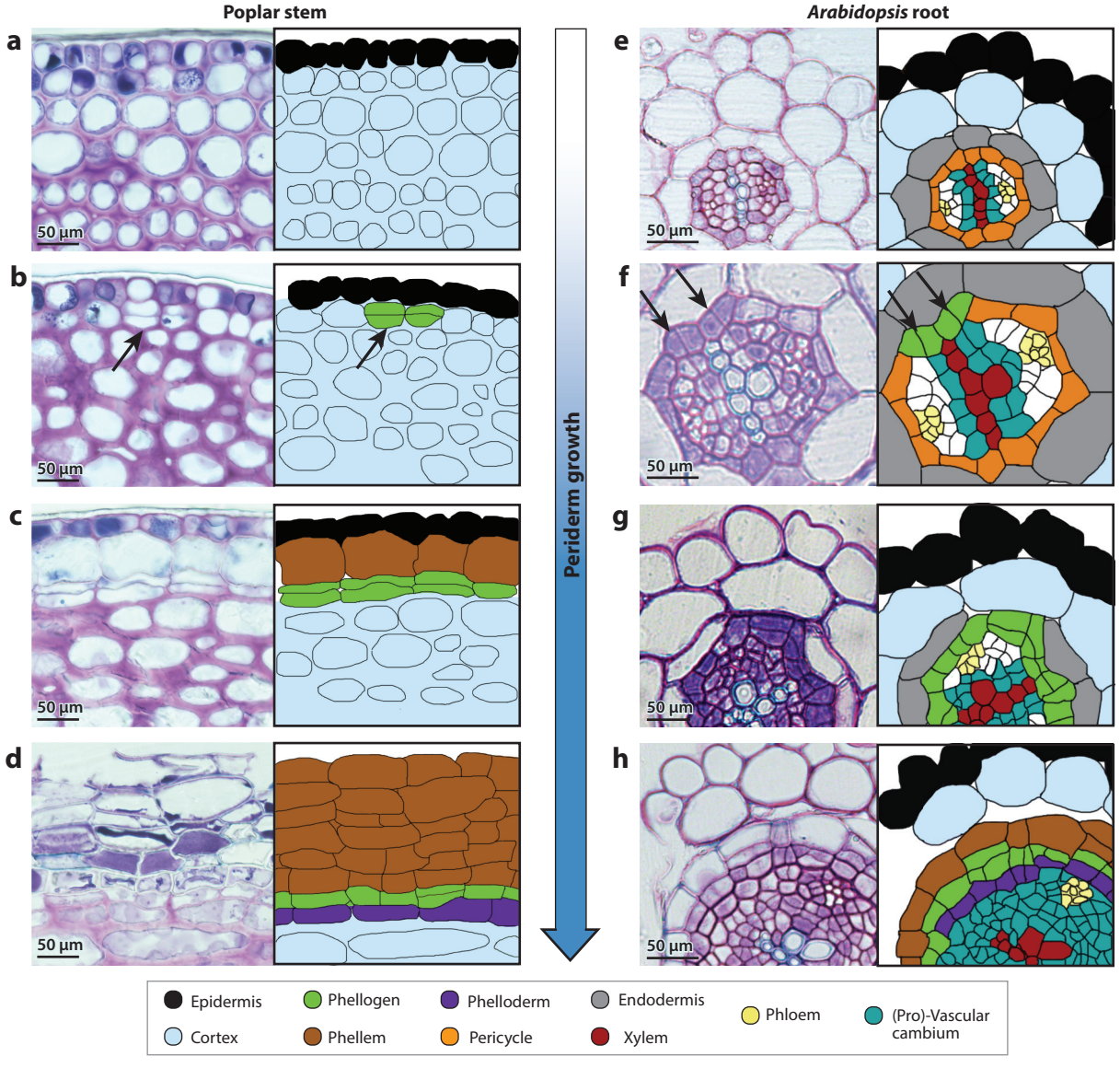
3. PERIDERM ONTOGENESIS

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 produce a periderm-like structure composed of 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 (42, 43). In species that do develop a periderm, it develops mainly in stem, branches, and roots during plant secondary development, but it also forms 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 (108).

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, the developmental stage and age of an organ also influence the site of phellogen origin (42, 43). Based on anatomical and histological analyses, we can distinguish at least five types of periderm ontogenesis where the phellogen arises from either (*a*) the epidermis, (*b*) the subepidermal layer, (*c*) deeper tissues within the cortex, (*d*) the phloem, or (*e*) 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, 42, 141).

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 (genera *Malus*, *Pyrus*, and *Oleander*) or the phloem (*Pinus mugo*, *Alnus glutinosa*, and genus *Vitis*) is rare (42, 43, 141) (**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 (**Figure 3*a*,*b***), 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,



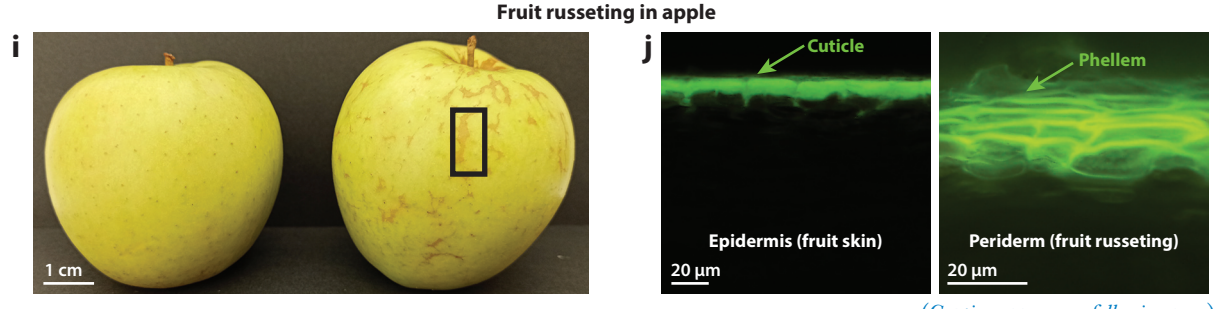


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Figure 3 (*Figure appears on preceding page*)

Examples of different types of phellogen ontogenesis and fruit russeting. (*a–d*) Periderm formation in the stem of poplar (*Populus tremula* × *Populus tremuloides*), illustrated by plastic cross sections stained with toluidine blue 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 constituting the phellogen, which divides and produces the first phellem cells (*brown*). (*d*) The phellogen keeps dividing and produces more phellem layers and the phelloderm (*purple*). (*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 two poles of phloem (*yellow*) embedded in the procambium (*aqua*), surrounded by the pericycle (*orange*), endodermis (*gray*), cortex (*pale blue*), and epidermis (*black*). (*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 keeps dividing and produces the phellem (*brown*) outward and the phelloderm (*purple*) inward. The outer tissues, the epidermis and cortex, are detached once the phellem is suberized. (*i–j*) Fruit russeting in apple. (*i*) Golden delicious apples show normal fruit skin (*left*) and fruit russeting (*right*, *black rectangle*). (*j*) Fluorol yellow staining highlights the cuticle in the epidermis (fruit skin) (*left panel*) and the suberized phellem in russeted areas (*right panel*).

forming a continuous meristematic ring by the end of the first year of growth (58) (**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 first to the fifth layer beneath the epidermis, whereas in the closely related Cupressaceae (gymnosperm, Pinophyta), the phellogen originates in both inner and outer cortical layers (94), 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, is 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 *Cornus mas*, *Cornus sanguinea*, and *Cornus 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 a hybrid origin (25–30% subepidermal layer and 70–75% epidermis) (119).

Finally, tubers, such as those from potato, originate from modified stems, stolons, that form a periderm during radial growth. In potato, the phellogen arises in the hypodermis (127).

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 two to three cell layers starts to divide periclinally to form the phellogen. The phellogen produces two to three phellem layers, which, as they divide and expand, cause the surrounding endodermis, cortex, and epidermis to rupture and eventually completely detach from the root (107). 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 one layer of phellem cells differentiates before the cortex and epidermis break (174) (**Figure 3*g,h***). Periderm development has recently been characterized in *Arabidopsis* beyond classical histology descriptions, establishing the *Arabidopsis* root as a model to study the mechanisms of phellogen initiation (151, 174). 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 the phellogen and vascular cambium (151). Moreover, live-imaging experiments showed that endodermal cells undergo programmed

cell death after the phellogen is established. By contrast, cortical and epidermal cells are detached after phellogen produces suberized and lignified phellem (174).

The polyderm, a special type of periderm, 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 (122, 161). Their state of lignification has not yet been described. Massive root periderms with more than 20 layers of phellem cells are also formed in alpine species of *Saxifraga*, which grow at high altitudes (99), 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 nonfunctional 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, 72, 87, 170) (**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, 108). 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 (81). 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 confer plasticity in contrast to the stiffer but more permeable epidermal regions. This provides a functional solution to compensate for stress growth and avoid pathogen entrance while partially restoring the water barrier function (81, 82).

Skin russeting is often considered a fruit defect, although in some commercially appreciated varieties it is viewed as a fruit ornament (108). For its economic importance, fruit skin russeting is a target of many breeding programs (108), and many transcriptomic studies, aiming to understand the molecular mechanisms controlling microcracking and russeting, have been recently performed (see **Supplemental Table 1**).

Supplemental Material >

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 remains in the meristem. All transit-amplifying cells and their daughters have the fate to differentiate (138). While the stem cell concepts have been well established in shoot and root meristems (60) and have begun to be established in the vascular cambium (20, 146, 151), the phellogen has remained unexplored. Phellogen is typically described as a single layer of dividing cells (42), thus 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 4*a***) (19, 83), suggesting that transit-amplifying cells may appear in special conditions. Thus, molecular and lineage-tracing studies are needed to identify stem cells of phellogen. A stem cell organizer or organizing center is a concept that has been identified in the primary meristems (138) and vascular cambium (151). 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 discuss the regulation of meristem activity in the phellogen during the whole plant life, including seasonal and annual changes and response to wounding.

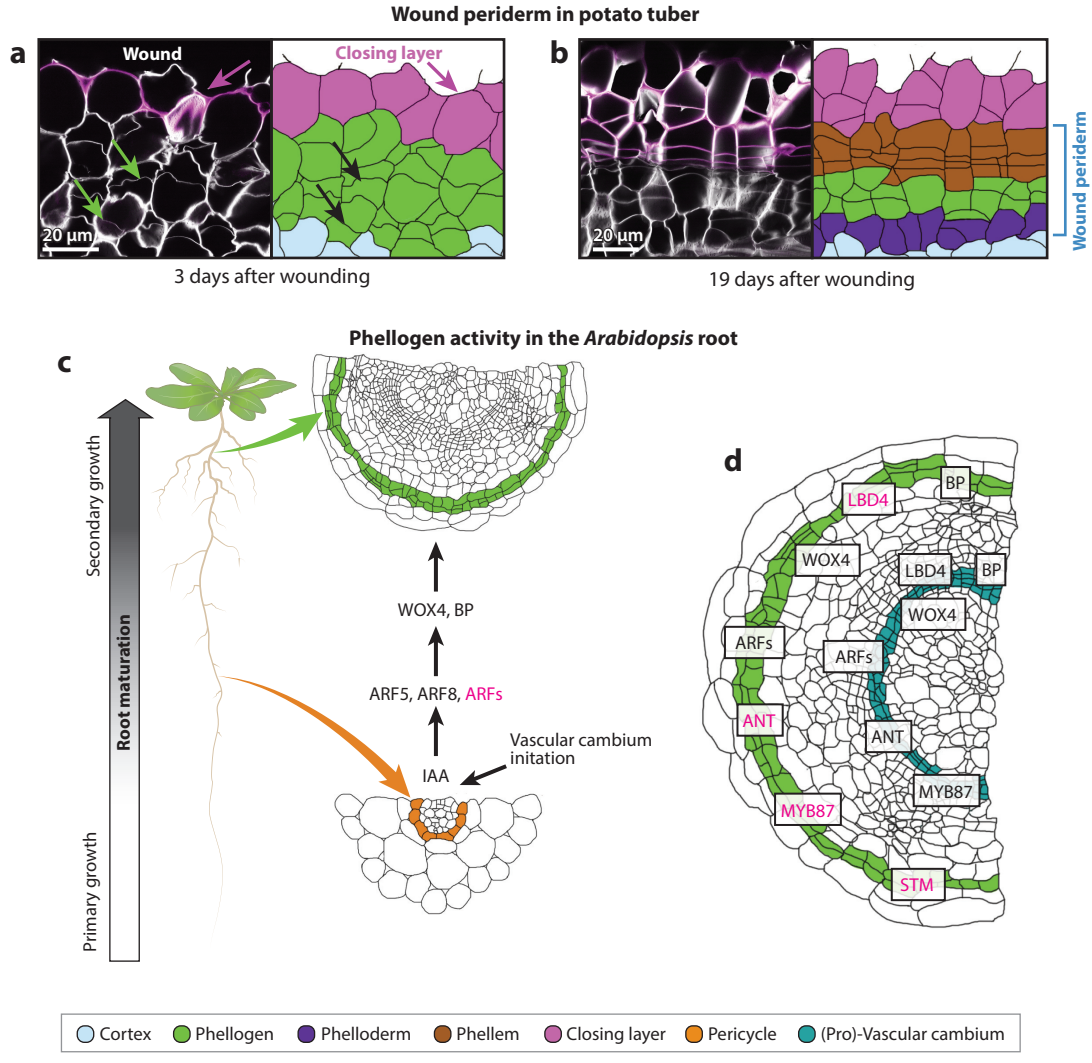

Figure 4

Wound periderm formation and phellogen regulatory network. (*a–b*) The formation of a wound periderm in potato tuber, illustrated by ClearSee cross sections, stained with basic fuchsin (lignin, *magenta*) and calcofluor white (polysaccharides, *white*), and diagrams. (*a*) After wounding, the first event is the formation of a closing layer (*pink*) with lignin (*pink arrow*) and suberin depositions, and at 3 days after wounding, cells beneath the closing layer divide periclinally (*green* and *black arrows*) and form a phellogen. (*b*) At 19 days after wounding, a wound periderm consisting of a multilayered phellem (*brown*), a phellogen (*green*), and a phelloderm (*purple*) is visible. (*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. IAA via 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. Putative regulators are listed in pink; known regulators are in black. Abbreviations: ANT, AINTEGUMENTA; ARF, AUXIN RESPONSE FACTOR; BP, BREVIPEDICELLUS; IAA, auxin (indole-3-acetic acid); LBD4, LATERAL ORGAN BOUNDARIES DOMAIN 4; STM, SHOOT MERISTEMLESS; WOX4, WUSCHEL-RELATED HOMEOBOX 4.

4.1. Longevity and Seasonal Changes

Persistent periderm/phellogen: a periderm/phellogen that is functional for multiple years and thus is not replaced by (sub)sequent periderms; also known as long-lived periderm/phellogen

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

The timing of the first periderm formation varies between species and may be influenced by environmental conditions (42, 43, 133). 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*) (168) form their first continuous periderm when they are approximately six 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 restores periderm initiation in a manner proportional to light intensity (18). Wunderling and colleagues (174) reported that in the *Arabidopsis* root, phellogen initiation consistently occurs earlier in long-day conditions than in short-day conditions.

Also, periderm longevity varies extensively between species, depending on how often a given species forms a new periderm to replace the old one. Many species replace the old periderm every 6–12 years, thus forming a rhytidome (42, 43), while in cork oak (*Q. suber*) the periderm may function for tens of years, and in European beech (*Fagus sylvatica*) even up to 200 years (42, 43). 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 from either phellogen reactivation (similar to vascular cambium) or phellogen replacement (by a new phellogen layer formed from phelloderm) (133, 147).

In trees from temperate climates, the phellogen is not active all year, 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*) it is active only in June (45, 96, 147). 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 (the phellogen and vascular cambium activity peak maxima do not coincide), whereas in carob the period of activity coincides (5, 96, 133). 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 (43) (see the sidebar titled Confusion and Heterogeneity in Periderm/Bark and Phellem/Cork Nomenclature). The sequential events and the cytological changes that occur during wound healing in the stems of gymnosperms and woody angiosperms are similar to those observed in potato tuber, which has been extensively studied (13, 15, 43, 117, 160). During the healing process, two structures, which are spatially and temporally separated, are formed: the outer closing layer and the wound periderm underneath (101, 104) **(Figure 4*a*,*b*)**. Closing layer formation is a rapid response, which first involves lignification and later suberization of the one to two layers of parenchyma cells neighboring the wound. This serves as a temporary protection from drought and pathogen entry (43, 102, 105, 160). Underneath the closing layer, parenchymatic tissue is dedifferentiated to form a wound phellogen, which, through periclinal divisions, organizes a new periderm with an outer lignosuberized multilayered phellem **(Figure 4*a*,*b*)**. Since the wound periderm is adjacent to the closing layer and development of both barriers overlaps in time (13, 101), their specific 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-RELATED HOMEOBOX 4* (*WOX4*), *PHLOEM INTERCALATED WITH XYLEM* (*PXY*)/*TDR*, and HD-ZIPs (151). *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 130).

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, 45, 97, 158, 167) (see also **Supplemental Tables 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 (175). Thus, vascular cambium mutants are likely to show a periderm phenotype independently of their function in the phellogen.

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Recently, Xiao and colleagues (175) 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 (175) (**Figure 4*c***). Two transcription factors, WOX4 and BREVIPEDICELLUS (BP)/KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 1 (KNAT1), act downstream of auxin in the periderm. Even though their role is known only for the *Arabidopsis* root, the presence of WOX4 and BP in transcript profiling of other species suggests a general function for these two transcription factors in phellogen regulation. Since WOX4 and BP have previously been characterized as key regulators of vascular cambial activity (178), it indicates that these two meristems likely share core signaling components (**Figure 4*c*,*d***). In agreement with this idea, researchers have reported that other known cambial regulators such as *AINTEGUMENTA* (*ANT*), which promotes stem cell proliferation downstream of cytokinins (CKs) (131); *SHOOT MERSTEMLESS* (*STM*), the closest homologs of *BP* (95); *MYB87*, which represses secondary growth (178); and *VASCULAR TISSUE SIZE* (*VAS*) (165) are also expressed in the phellogen (1, 167) (**Figure 4*d***). Intriguingly, available transcriptomic resources and studies on *Arabidopsis* root indicate that *PXY/TDR* is vascular cambium–specific, suggesting that other as-yet-unknown receptors regulate phellogen activity (1, 45, 175).

It is reasonable to assume that CKs promote meristem cell proliferation in the phellogen because CKs accumulate during the tuber healing process when a wound phellogen is formed (167) and components of the CK signaling, such as *ARABIDOPSIS THALIANA RESPONSE REGULATOR 5* (*ARR5*), and downstream factors, such as *ANT*, are expressed in the wound or native phellogen (1, 45, 167). Another hint of the role of CKs during periderm formation comes from a recent study on the *Arabidopsis* root. Ye and colleagues (177) showed that at the onset of periderm formation, CKs accumulate in the pericycle to activate secondary growth through LATERAL ORGAN BOUNDARIES DOMAIN 3 (LBD3) and LBD4 transcription factors (177). 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 and July) (45). 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 (64), is expressed in the phellogen. Overexpression of *PttSHR2B* results in plants with enhanced bark relative to wood ratio and increased CK content, suggesting that *PttSHR2B* may modulate phellogen activity by regulating CK homeostasis (114).

New evidence suggests 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 5*a***). Exceptions are seen during flooding or in tropical trees, where phellem cells lose compactness and are more loosely arranged to enhance gas exchange (43). Typically, once formed, phellem cells radially expand to acquire their final dimensions; accumulate a set of different, specialized polar and nonpolar soluble compounds; and modify their cell walls by depositing lignin and suberin (**Figure 5*c***). As chemical differentiation ends, phellem cells remodel chromatin; express developmental programmed cell death marker genes; degrade their cytoplasm; fragment their DNA, in agreement with programmed cell death (62, 73, 74, 174); and eventually autolyze and dehydrate. The empty space framed by cell walls becomes filled with gas (133).

Notably, in tree bark, 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 whose nature is unknown) 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 (43, 133). The seasonal pattern of thin- and thick-walled phellem cells corresponds to growth increments (42, 43) and in cork oak and birch outer phellem tissues anatomically constitute growth rings, which to some extent represent annual increments (125, 147) (**Figure 5*b***). 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 (reviewed in 133) (**Figure 5*d***).

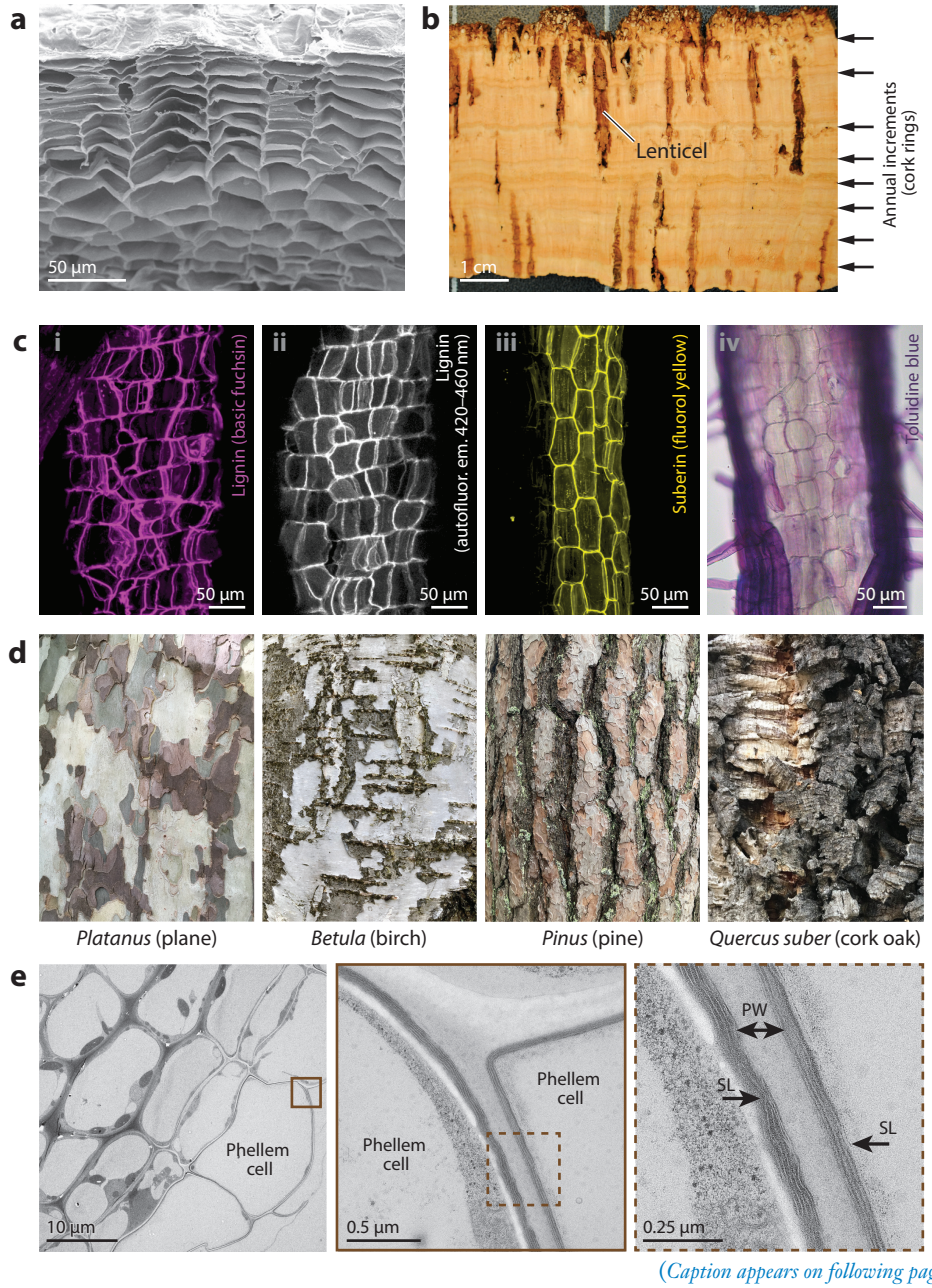
In the next sections, we focus on phellem cells that are suberized, reviewing mostly the research undertaken in two phellem models—the outer bark of cork oak and potato tuber skin. In both models, phellem cells are rich in suberin and can be easily separated as almost pure layers (some phellogen cells may be retained) in sufficient amounts for chemical and transcriptomic studies (**Supplemental 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 years, 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 (149). A few other tree species are also able to produce pure suberized phellem barks or a rhytidome enriched in suberized phellem cells, all of them providing an interesting potential source of chemicals and suberized-phellem-derived products (93). In potato, the native periderm protects and covers the tuber. When the tuber is wounded, a wound periderm forms from flesh tissue. Both native and wound periderms have high economic importance for tuber protection and conservation (52, 100). 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 two weeks for the formation of the first phellem cells) (174).

Supplemental Material >

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 5*b***).

Suberin is an important phellem structural component, accounting for 38%, 30–50%, and 20–25% of the total composition of potato skin and cork oak and birch outer bark, respectively



(*Caption appears on following page*)

Figure 5 (*Figure appears on preceding page*)

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 a consequence of their formation by periclinal divisions of the lower mother phellogen cell (not reliably distinguished here). (*b*) 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, brown coloration) 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. (*c*) Phellem cell wall modifications using the *Arabidopsis* root as an example. (*i*, *ii*) Lignin depositions are detected by basic fuchsin staining and autofluorescence, respectively. (*iii*) Suberin depositions are detected by fluorol yellow staining. (*iv*) Phellem cells act as an impermeable barrier; toluidine blue (a purple dye) cannot penetrate. (*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 SL are visible as alternating layers of light and dark lamellae. Abbreviations: autofluor., autofluorescence; em., emission; PW, primary wall; SL, suberin lamellae.

(46, 113, 149). Suberization in phellem-differentiating cells occurs very rapidly; the suberin biosynthetic protein suberin feruloyl transferase (FHT) consistently already accumulates 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 (suberin bifunctional fatty acids), fatty acids, and primary alcohols] as well as some aromatics, mainly ferulic acid, and glycerol (reviewed in 59). Phellem transcriptomics (**Supplemental Table 1**) often highlight suberin-related genes that 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*) (54, 66, 86, 115, 143, 145, 176), 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 processes such as lignification, is unknown.

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Lignin is also an abundant percentage of phellem; it consists of 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, 44, 98). In cork oak phellem, lignin is enriched in ferulic acid (98, 110), while in potato phellem, ferulic acid was also identified forming ether-linked amides, such as feruloyltyramine (121).

Transmission electron microscopy (TEM) observations have revealed that the phellem cell wall is uniform around the cell and composed of a primary layer of randomly oriented cellulose microfibrils, a suberized layer with a polylamella of alternating light and dark bands, and a tertiary layer proposed to contain waxes and/or polysaccharides when present (43, 57, 133, 145) (**Figure 5*e***). The components organizing the suberin ultrastructure as lamellae, as well as its linkage to lignin, are not fully clarified. Graça's (57) model of suberin structure proposes that the light lamellae account for the suberin polyester, structured by the bifunctional fatty acids and glycerol. In agreement, a genetic reduction of these specific suberin compounds [*CYP86A33*-RNA interference (RNAi), *cyp86a1/horst*] leads to a distorted lamellar structure (115, 145). The dark lamellae, in Graça's (57) model, represent 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, which raises questions about dark lamellae composition and linkage to light lamellae (115, 143). Nevertheless, ferulic acid has been identified as necessary to initiate the deposition of suberin in *Arabidopsis* phellem and endodermal cells (3).

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

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

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, 85). 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, 85). Researchers suggest that waxes interact with the suberin aliphatics in the light lamellae (57) or are retained in the tertiary wall (133). 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, 71). Several pieces of evidence support the crosstalk between the biosynthesis of precursors of phellem polymers and polar and nonpolar compounds within extracts. For instance, changes in suberin biosynthesis impact wax composition, and the specific blockage of suberin ferulic acid esterification affects the pool of soluble phenolics and lignin composition (76, 142–144).

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

The specific role of polysaccharides such as cellulose, hemicellulose, and pectins, as structural, functional, and signaling elements of the cell wall in periderm, is largely unknown. In potato, rhamnogalacturonan (RG)-I (pectin) accumulates preferentially in phellogen and phelloderm cell walls (103, 124), 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 (124). Moreover, xylans are the major hemicelluloses in cork oak phellem (31, 126), and although their function in phellem and other suberized tissues is unknown, they may provide a structural anchor for lignosuberin deposition through ferulic acid, as similarly described for lignin in grasses (48, 56).

5.2. Physiological Function of Phellem Cells

The specific physiological role of each structural and nonstructural component of the phellem is not well understood and our knowledge of these roles is mainly restricted to potato. A high number of phellem layers correlates with heat stress and resistance against tuber greening (53, 156), whereas a twofold increase in the amounts of suberin and waxes corresponds to a decrease in water permeability (140). Beyond correlations, only a few functional genetic studies provide evidence of the protective role of the phellem and its components. In potato phellem, a shortening of 2 carbon chain lengths in suberin and wax results in 1.5-times-higher permeability (*StKCS6*-RNAi) (144), suggesting that suberin and wax with longer chain lengths confer higher protection. Phellems with reduced suberin 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 3.5-fold, indicating that the concomitant increase in wax content (2.4-fold) in *CYP86A33*-RNAi phellems might counteract suberin impairment (145). These findings indicate a partial role of suberin and an active involvement of waxes in establishing an efficient barrier. In agreement with an active role for waxes in water barrier function, dewaxed potato periderms increase their permeability 100-fold (140). 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 (140).

Interestingly, potato phellem that lacks esterified ferulic acid in suberin and has altered lignin composition yields nonviable tubers that quickly dry due to high permeability (14-fold) (143). 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 with the impermeable phellem of wild-type plants (**Figure 5*b***). 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 is 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, 150). As such, cell wall–hydrolyzing enzymes produced by most microorganisms are unable to degrade the suberized phellem tissue (140). However, several exceptions exist with respect to this generality: Pathogenic microorganisms such as *Streptomyces scabies* cause corky lesions (scab) on the surface of potato tubers and root crops, similar to fruit russeting (80). Despite this important defensive 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, 102, 116, 159). 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 (111).

In woody plants, pathogen attack and mechanical wounding may affect phellogen integrity, rendering it nonfunctional (14), and trigger the development of closing (boundary or impervious) layers and wound periderms to reestablish phellogen functionality (14, 118). Host resistance relies on the quick formation of such structures to confine the infected tissue (29, 150), whereas host susceptibility results from the ability of the pathogen to interfere with their formation (118). For instance, in hybrid poplars, resistance to the fungus *Septoria musiva*, which causes 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 (173). 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, 49). 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 the NAC (NAM/ATAF/CUC) family, the potato *StNAC103* transcriptional repressor and *Arabidopsis AtANAC46* transcriptional activator, have been characterized in the phellem by loss-of-function and overexpression lines, respectively (109, 152, 153, 166). 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, 91, 152) (**Supplemental Table 2**). The functions 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, 91). 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 (109, 148). Additionally, many other MYB suberin regulators (*MYB9*, *MYB32*, *MYB39/Suberman*, *MYB41*, *MYB53*, *MYB74*, *MYB92*, *MYB102*, *MYB107*) are enriched

Supplemental Material >

in phellems of different species and organs (24, 30, 30a, 45, 55, 84, 87, 89, 148, 162, 169, 172), suggesting that they also control phellem suberin deposition.

Abscisic acid (ABA) induces suberin biosynthesis, regulation, and/or deposition in *Arabidopsis* endodermis (11, 90, 148) and in wound-healing tissues of potato tuber, tomato stem, and kiwifruit (16, 92, 106, 171, 172). In agreement, the transient overexpression of the transcriptional activators in ABA signaling, *AchnABF2* and *AchnMYC2* (*bHLH*), induces suberization in tobacco leaves (171, 172). In periderm-like structures of crown gall tumors induced by *Agrobacterium tumefaciens*, the suberization and protective functions are ABA-dependent (40), suggesting a role of ABA as an 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 the first components have only recently been elucidated. Auxin and most likely CKs have a positive role in 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 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 have uncovered similarities in the chemistry between barrier tissues, so it will now be interesting to determine if they share common regulatory networks for development and differentiation.

SUMMARY POINTS

1. The periderm acts as 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 and 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, the first periderm often becomes inactive, and it is replaced by (sub)sequent periderms. New and old layers and the enclosed tissue together form 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, involves 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 completely identified.
2. The vascular cambium and the phellogen share many regulators, raising the questions of 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 spatiotemporal dynamics of suberin and lignin deposition as well as linkage at the ultrastructural level require further investigation.
5. Phellem cells are remarkably similar in their cell wall composition to endodermal cells. This questions whether 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 understanding 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 need to be developed: genetic tools for specific and conditional manipulation of periderm, single cell transcriptomics of periderm, and methods to quantify its barrier function.

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175. This article shows that auxin acts via WOX4 and BP to promote phellogen establishment and maintenance.