The Endodermis

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Abstract

A Casparian strip–bearing endodermis is a feature that has been invariably present in the roots of ferns and angiosperms for approximately 400 million years. As the innermost cortical layer that surrounds the central vasculature of roots, the endodermis acts as a barrier to the free diffusion of solutes from the soil into the stele. Based on an enormous body of anatomical and physiological work, the protective endodermal diffusion barrier is thought to be of major importance for many aspects of root biology, reaching from efficient water and nutrient transport to defense against soil-borne pathogens. Until recently, however, we were ignorant about the genes and mechanisms that drive the differentiation of this intricately structured barrier. Recent work in *Arabidopsis* has now identified the first major players in Casparian strip formation. A mechanistic understanding of endodermal differentiation will finally allow us to specifically interfere with endodermal barrier function and study the effects on plant growth and survival under various stress conditions. Here, I critically review the major findings and models related to endodermal structure and function from other plant species and assess them in light of recent molecular data from *Arabidopsis*, pointing out where the older, descriptive work can provide a framework and inspiration for further molecular dissection.

Contents

INTRODUCTION

The functionality of the root as a selectively absorbing organ cannot be understood without a discussion of the barrier features of the endodermis. Accordingly, an explanation of the endodermis and its Casparian strips features prominently in every textbook that explains the working of roots (76, 91). In terms of function, the root can be likened to an "inverted gut," with the endodermis representing the plant version of an animal polarized gut epithelium. This analogy has been strengthened by recent studies revealing strict endodermal polarity and tight/adherens junction–like features of the Casparian strip and its associated plasma membrane. These parallels are discussed in two other recent reviews (2, 78) and are not a major focus of this review.

In the 150 years since Robert Caspary first described the endodermis and speculated on its function as a protective sheath, an impressive number of physiological and anatomical works have substantiated his speculation and demonstrated the unique position of the endodermis as a selective barrier within young roots. Both older and recent reviews have compiled and discussed these impressive efforts (for a more comprehensive overview of the twentieth-century literature, see 25, 61, 98). What I attempt to do in this review is selectively point out and discuss older data in light of recent molecular findings and from the perspective of an *Arabidopsis* cell and developmental biologist. What are the challenges and questions posed by earlier works for current researchers trying to understand the molecular basis of endodermal structure and function? What guidance can these works provide?

In light of the amazing conservation of the endodermis and its Casparian strips from ferns to angiosperms, investigating them in the best available plant model, *Arabidopsis*, should be an imperative for years to come. Experience tells us that the molecular mechanisms that underlie endodermal differentiation in *Arabidopsis* will provide a robust and invaluable framework for understanding this cell layer in the vast variety of other plant species. This is not to ignore the amazing variants, adaptations, and specific physiological roles that have been reported in the literature on the endodermis and Casparian strips. Casparian strips have been reported in other dermal tissues and in different organs, even in cell layers of nectar glands (34). The endodermis is a colonization barrier for mycorrhizal colonization (66), and a specialized nodule endodermis is thought to provide an oxygen diffusion barrier for rhizobia (12). These processes have in common that they cannot be investigated in *Arabidopsis*, yet it is safe to assume that research on them might

benefit enormously from an understanding of the basic molecular framework of endodermal differentiation.

ENDODERMAL SPECIFICATION

Endodermal cells become specified very close to the center of the root meristem. Plant meristems are highly dynamic structures that maintain an ordered arrangement of cells despite constant cell production, and in this respect they are comparable to standing waves—features that, on the basis of some underlying organizing principle, maintain a defined structure in spite of a constant flux of material through them.

The organizing principles that shape and maintain the meristem are beginning to be unraveled. They consist of polarly transported hormones, small peptides, mobile transcription factors, and other agents, which set up a system of self-sustaining positional cues that keeps an organized stem cell population at the tip of the developing root organ. Excellent reviews have been written on this topic (for example, see 71), and I only briefly sketch our current knowledge of endodermal specification, which is based almost exclusively on work in *Arabidopsis*.

Analysis of Endodermal Specification in the Model Plant

In the *Arabidopsis* model, the tight orchestration of formative divisions in the meristem allows the generation of organized cell files of different identities within a very small meristematic region. To generate the cell files surrounding the stele, two neighboring initial populations produce daughter cells that rapidly undergo a single tightly controlled formative (periclinal) division, generating four concentric rings of cells that each differentiate into a different cell type: lateral root cap, epidermis, cortex, and endodermis. The periclinal division of the daughter of the cortex/endodermis initial is often called asymmetric, although an unequal distribution of components before or during division, as is seen in many animal cells, has not been demonstrated (46). The division is nonetheless asymmetric in its effect, as it positions one of the daughters in the immediate vicinity of protostele cells, whereas the other will not be in direct contact.

This positioning exposes the inner daughter cell to SHORT-ROOT (SHR), a mobile transcription factor expressed exclusively in the stele (6, 39). The SHR protein moves between cells and accumulates in the nucleus of the inner derivative of the cortex/endodermis initial daughter (30, 64). This nuclear accumulation is caused by another transcription factor, SCARECROW (SCR), whose transcription is directly dependent on SHR and with which it interacts. The above is thought to lead to a positive-feedback loop that draws SHR into the nucleus, both restricting its further movement beyond this cell layer and initiating a transcriptional cascade that specifies the endodermal cell fate and drives its differentiation. This model has been borne out by SHR misexpression experiments, which lead to additional periclinal divisions and the specification of additional cell layers with endodermal characteristics (64).

Cells at the cortex and epidermis positions can also be induced to express endodermis-specific genes and form Casparian strip–like structures (52, 85). However, transformation of epidermal cells into endodermis is not complete, and epidermal cells continue to display epidermal differentiation features such as root hairs (85). This indicates either that SHR is not able to override competing epidermal specification signals or that additional positional signals, independent of SHR, might be required to execute the entire endodermal differentiation program. A complete override of epidermal cell fate seems to be possible, as can be seen by overexpression of the VASCULAR-RELATED NAC-DOMAIN 6 (VND6) or VND7 transcription factor, which leads to extensive formation of vascular cells in the epidermis position (49).

Variations on the Standard Model in Other Plants

The analysis of *Arabidopsis* mutants has been instrumental for our current understanding

of endodermal specification. *Arabidopsis*, however, represents a special case of a highly reduced cortex, initially consisting of only two cell types and files: the cortex and the endodermis. The cortex in many other plants consists of more cell types, some specialized into hypodermis/exodermis and sclerenchyma in addition to numerous parenchymatous cells. This complexity is reflected in a more elaborate division pattern of the initials than is seen in *Arabidopsis*. In rice, for example, one common epidermis/endodermis initial divides multiple times, successively generating the cell files that then differentiate into epidermis and the respective cortical cell types. Only the last formative (periclinal) division of the initial generates the cell file differentiating the endodermis (67).

The paradigm of endodermal specification occurring through a stele-derived short-range signal in the form of moving SHR can easily accommodate the situation in rice, and putative rice orthologs of SHR and SCR do indeed show expression patterns that are entirely consistent with a conserved patterning mechanism in rice (18). However, additional homologs of SHR and SCR exist in rice whose potential specific functions have not been investigated. Also, the existence of a common epidermis/endodermis initial requires a different spatiotemporal regulation between formative cell division and cell fate specification. An in-depth mutant analysis of the different rice homologs will allow an understanding of these differences and lead to models that accommodate the changes and adaptations that have occurred in the evolutionary history of vascular plants.

ENDODERMAL DIFFERENTIATION

Entering the Elongation Zone: Where Cells Start to Grow Up

After the formative division of the endodermis initial daughter, the inner derivative of this cell division is specified as proendodermis by the accumulation of SHR/SCR in the nucleus. It then continues to undergo transversal (generative) divisions, resulting in a growing group of cells that are slowly displaced apically (i.e., toward the tip of the plant, away from the meristem center). This displacement is due to the division activity of newly produced proendodermal cells situated closer to the meristem. Together, these groups of dividing cells constitute a transit-amplifying zone in which cells of a certain cell fate make more cells of their own type. The number of times that they divide is a crucial determinant of the cell production rate of the meristem (3). Displacement of proendodermal cells from the meristem center is initially very slow, driven by cytoplasmic (as opposed to vacuolar) cell growth of the initial and newly generated proendodermal cells below. Once a certain distance from the meristem is reached—perceived as changes in the relative concentrations of growth regulators, such as auxin, cytokinin, and gibberellic acid (20, 80, 94, 95)—cells cease division and begin to elongate rapidly, pushing their apical neighbors farther away from the meristem and into the position of the elongation zone. A recent study with *Arabidopsis* seedlings followed endodermal cell elongation and estimated that one proendodermal cell is pushed into elongation every 1–2 h (1) (**Figure 1**).

Elongation can be seen as a sort of preparatory phase of differentiation but can also be intermingled with it, as seen in epidermal cells that start to form root hairs while in the process of elongating. The question of when differentiation starts might be surprisingly hard to answer for many cell types. We often rely on some easily observable morphological alteration to assess differentiation (such as the Casparian strips of the endodermis). However, such morphological markers often score for the end rather than the start of differentiation. Molecular markers are certainly better suited to report the onset of differentiation—but what is a good differentiation marker? Transcription factors won't do, because they can only activate more genes, some of which must eventually do the job of determining the specific morphology and physiology of a cell. A good differentiation

marker should therefore be a gene whose product is directly involved in generating the distinguishing characteristics of the mature cell.

The Casparian Strip

For a long time, primary differentiation in the endodermis was assessed by scoring for the presence of the Casparian strip. The Casparian strip is a localized impregnation of the primary cell that surrounds the endodermal cell as a belt in the longitudinal direction. This belt is situated in the center of the transversal and anticlinal walls, initially taking up approximately a quarter to a third of the transversal/anticlinal cell wall (1, 25) (**Figure 2*a*,*b***). The nature of the Casparian strip as a primary wall impregnation—and not a secondary cell wall—is shown by electron micrographs (**Figure 2*b*,*c***) in which the Casparian strips can be seen to fill up the entire space between two adjacent endodermal cells (including the middle lamellae), appearing as a nonfibrous, highly homogenous feature of different electron density than the rest of the primary wall (10, 36, 42, 77).

That the middle lamella is integrated into the strip effectively means that the two cell walls of the individual cells have become fused with each other. This is indeed beautifully illustrated in complete cell wall digestions, which leave only the resistant walls of the xylem and the Casparian strips intact (25) (**Figure 2*e***). In such digestions, the Casparian strips can be seen to have formed a supracellular network between endodermal cells, i.e., a fishnet-like structure that surrounds the xylem tissue of the stele (**Figure 2**). If the middle lamella had remained unmodified, then the Casparian strips of each cell would have become individualized into many distinct rings. Finally, the modification of the middle lamella follows from the observation that apoplastic tracer molecules are not able to cross the normally permissive middle lamella at the position of the Casparian strips (see below).

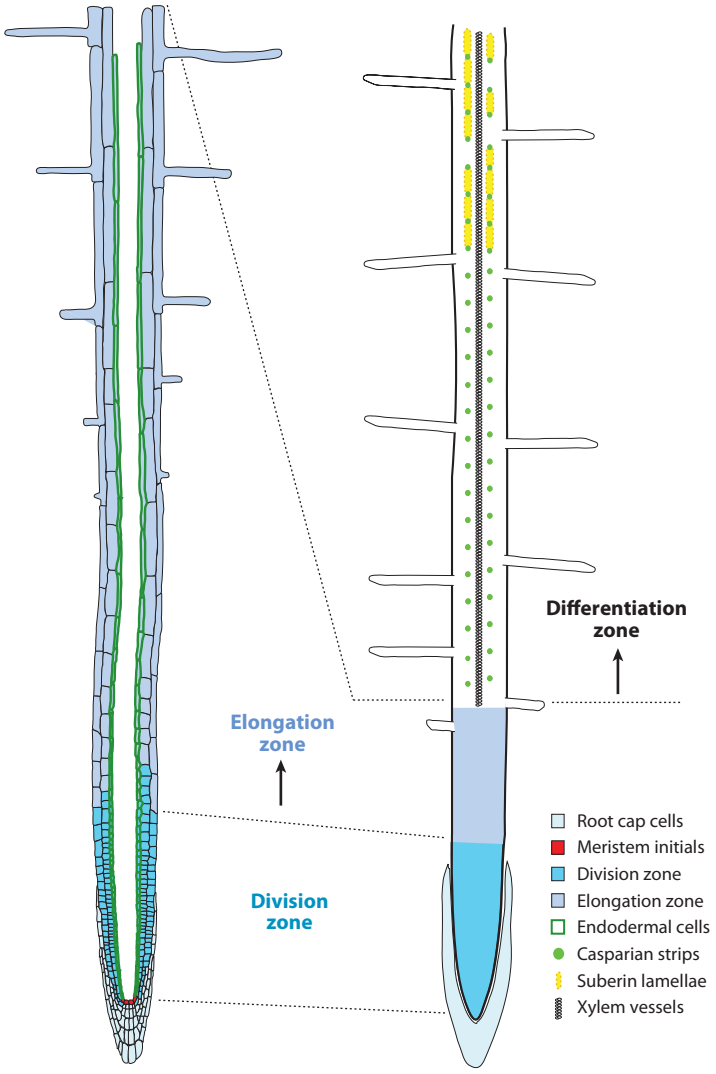

Figure 1

The path of an endodermal cell from the initial toward primary and secondary stages of differentiation. (*Left*) Detailed cellular schematic of the meristem tip, with endodermal lineage in green. (*Right*) Overview schematic of the roots, showing the appearance of Casparian strips (*green dots*) concomitant with xylem vessels and, later, the patchy appearance of suberin lamellae (*yellow*).

The Chemical Nature of the Casparian Strip

The chemical nature of this primary cell wall modification remained a matter of debate during the twentieth century. Caspary (14) pointed to the two main candidate polymers, *Holzstoff* (lignin) and *Korkstoff* (suberin), but was unable

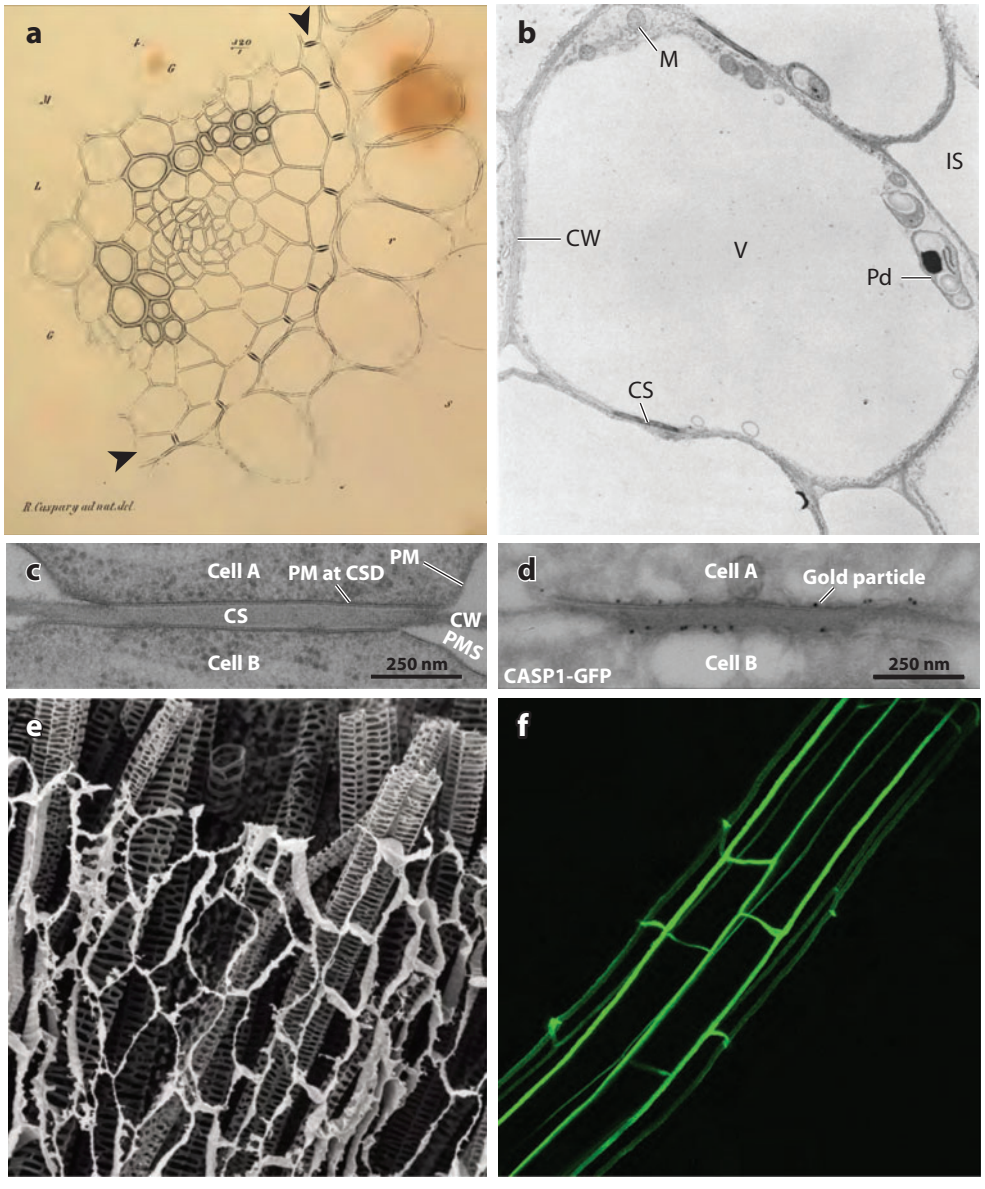

Figure 2

(*a*) Drawing by Robert Caspary in his description of the endodermis from 1865 (14). Black arrowheads indicate the endodermal cell layers with Casparian strips in radial walls. (*b*) Initial electron micrograph from Bonnett (10), showing an overview of an endodermal cell in a transversal cut, with the Casparian strip visible in the radial walls. The cortex is on the right; the pericycle is on the left. (*c*) Electron micrograph of Casparian strips in *Arabidopsis* (77). The primary cell wall, including the middle lamella, appears homogenous and slightly thickened. The plasma membrane adheres to the cell wall. (*d*) Immunogold localization of CASP1-GFP (77). Gold particles are visible at the plasma membranes on both sides of the Casparian strip. (*e*) Electron micrograph courtesy of Schreiber and colleagues (82), showing the netlike structure of the Casparian strip after complete digestion with cell wall–degrading enzymes. Nondigested vascular elements are shown in the background. (*f*) 3D maximum projection of CASP1-GFP in live roots of *Arabidopsis* (77), revealing a netlike arrangement of the CASP domain highly similar to that seen in panel *e*, except that the cells are more elongated and the vascular elements are not visible. Abbreviations: CS, Casparian strip; CSD, Casparian strip membrane domain; CW, cell wall; IS, intercellular space; M, mitochondria; Pd, plastid; PM, plasma membrane; PMS, plasmolysis-generated space; V, vacuole.

to decide between them. Krömer, in his epochal 1903 work (48), cited numerous authors who described the Casparian strips as suberized, but concluded from his own careful use of histological staining methods that Casparian strips stain in ways that are typical of lignin. A few years later, however, de Rufz de Lavison (21) continued to state that the Casparian strips are suberized (*les cadres subérisés*) without providing references. In his influential review of the endodermis in 1961, Van Fleet (98) still summarized the matter as undecided, but acknowledged that "phenolic material" must be present in Casparian strips and cited several publications that reported the lignin-like fluorescence and staining behavior of the Casparian strips. Esau, in her standard work on the anatomy of seed plants (26), described the Casparian strips as suberized (again without references). In the 1990s, Schreiber's group (82, 83, 110) conducted direct chemical analyses of isolated Casparian strip preparations of different plants and presented evidence that Casparian strips are made up in large part of a typical lignin polymer, although a minor, maybe functionally important, presence of suberin was always detected. Again, these findings did not change the prevailing view in many present-day textbooks that Casparian strips are a predominantly suberized structure (76, 91).

Recently, a study conducted in *Arabidopsis* finally introduced experimental manipulations of lignin and suberin production, combined with histological and chemical analysis and functional assays for the apoplastic barrier (65). This combination of methods demonstrated that suberin is neither present nor required in early Casparian strips of *Arabidopsis* and that these cell wall structures should be viewed as being made of a rather typical lignin polymer, which can provide an apoplastic barrier in young roots without the presence of suberin.

Why is suberin so persistently viewed as being the substance of Casparian strips? One of the main reasons is probably that it is present in large amounts in endodermal cells and can even be used as a convenient marker for endodermal cell identity, as it is exclusive to the endodermis if exodermal/hypodermal walls are not present. However, the bulk of suberin is deposited in the endodermis after Casparian strips are formed in what is called the secondary stage of endodermal differentiation (see below). This eventual deposition of suberin nevertheless led many authors to assume that Casparian strips are somehow the start of a suberization, which then encompasses the entire set of endodermal cells, though suberin formation has been shown to start in the tangential walls, away from the Casparian strips (36). In addition, the chemical nature of suberin as the main component of cork made it the perfect candidate polymer for an apoplastic diffusion barrier, and it was assumed that lignin, which is much more hydrophilic, would not be suitable as a barrier polymer for water and nutrients. To this argument it can be said that the conducting channels for water and nutrients in the plant—the vessel elements and tracheids—are walled with lignin, suggesting that lignin is at least able to provide some degree of apoplastic barrier. Everyday observation also tells us that wood is well able to contain or repel water when used as material for buckets or boats. Still, many researchers were tempted by the more parsimonious assumption that Casparian strips are the localized start of a more general suberization of the endodermis. Unfortunately, a convenient indicator of lignin, its blue-green autofluorescence, can also be observed to some degree in suberin/suberized tissues, which does not allow the use of autofluorescence alone as an indicator for the lignin-like nature of Casparian strips.

Finally, an influential and extensive definition of suberin postulated lignin-like polymers as an integral part of it, which certainly promoted the confusion of many researchers about the nature of Casparian strips (see sidebar Suberin: One Substance or Two?). It is important to note that claiming Casparian strips have a lignin-like nature does not mean that this lignin impregnation cannot be rendered more impermeable by the addition of some hydrophobic substance. This is certainly supported by numerous early histochemical

SUBERIN: ONE SUBSTANCE OR TWO?

Suberin is most easily defined as a mainly aliphatic polyester, consisting largely of long-chain fatty acids and fatty alcohols of different lengths and bearing additional alcohol or carboxy function in omega or midchain positions (31). Added to this are low levels of phenolic compounds, such as ferulic acid, and glycerol (27, 74). This composition allows for extensive polymerization and could lead to the formation of linear or branched, high-molecular-weight polymers. This "lipid polyester" is hydrophobic and highly resistant to chemical and enzymatic degradation.

The term suberin derives from the Latin *suber*, meaning cork—the material gained from the bark of cork oak, *Quercus suber*, which is widely used for its chemical resistance, hydrophobicity, and isolating properties. There can be no straightforward equation between cork (a complex material) and suberin (the chemical substance thought to confer its major distinctive properties). This also applies to lignin, a phenolic polymer that determines the characteristics of a material, wood, that is itself much more complex, containing large amounts of cellulose and other polymers. Another example is cutin, found in the cuticles of areal plant organs, after which it was named; again, cutin represents the defining polymer of the cuticle, but it is intimately connected with other polymers to form the complex compound material of the cuticle.

Cork also consists of highly complex cell wall material that originated from different cell types, and a large proportion of cork is made of polymers that resemble lignin in monomer composition and coupling mode (56). Whereas researchers on cork chose to give this additional polymer the straightforward designation of cork lignin, other researchers, working on different suberized tissues (such as wound-healing potato periderm), decided to define this lignin-like polymer as the "polyaromatic domain" of suberin, postulating an intimate connection between the two (for reviews, see 7, 8). According to this logic, suberin should be seen as a compound polymer with a polyaromatic and a polyaliphatic domain.

I feel that this domain nomenclature is more confusing than helpful. Applying its logic, one could say that there is a "cellulosic domain" of lignin or a "pectic domain" of cutin. This is not done, because it confuses a substance with a material in which that substance is found (think of a concrete wall, in which both concrete and steel are found) and would make it impossible to use terms like lignin or cutin in a chemically defined way. Following the use of the terms lignin or cutin as defined substances, I use the term suberin narrowly, as a mostly aliphatic polyester of fatty acids and alcohols. It can then be further stated that this substance is often found to be intimately associated with lignin-like substances in complex, suberized tissues such as cork and potato periderm.

and chemical analyses, from which it was concluded that some fatty substance, distinct from suberin, is associated with the lignified Casparian strips (48, 72, 84, 100). High-resolution expression maps, combined with molecular genetics and sensitive analytical techniques in *Arabidopsis*, could certainly identify the genes responsible for hydrophobic modifications of the Casparian strips. It would be interesting to genetically modify the strips' hydrophobicity and study the consequences. The recently reported early expression of *ALIPHATIC SUBERIN FERULOYL TRANSFERASE* (*ASFT*) (65) could provide a fatty acid–linked

ferulic acid, which, if integrated into the lignin during Casparian strip synthesis, would provide such a hydrophobic modification.

Localization of the Casparian Strip

If building a Casparian strip is about generating a localized impregnation of the primary cell wall, then how can we imagine this to be achieved by the cell? Clearly, some form of localization of lignin biosynthetic enzymes must occur, which would necessitate specific localization of proteins in the plasma membrane at the Casparian strips. It has been known for a long time that the plasma membrane at the Casparian strips has distinct characteristics compared with the rest of the plasma membrane, a discovery initially made when the protoplast of the endodermal cell was found to remain tightly attached to the cell wall upon plasmolysis (4, 48). The advent of electron microscopy enabled direct observation of the plasma membrane at the Casparian strips, which was found to be often more electron dense in appearance and indeed to be tightly adherent (10, 42) (**Figure 2*c*,*d***). This strongly suggested the localized presence of specific proteins in the plasma membrane that would mediate cell wall attachment, possibly even before formation of the strip itself (24).

An additional intriguing feature of this plasma membrane domain was uncovered in a recent study in *Arabidopsis* that investigated the timing of endodermal differentiation in live roots (1). Using newly developed plasma membrane markers with endodermis-specific expression, the authors revealed that plasma membrane proteins become excluded from the membrane region before the appearance of Casparian strips themselves. At the same time, membrane lipid tracers are not able to cross this membrane region by lateral diffusion. These findings suggested the presence of a highly scaffolded, protein-rich membrane domain in the endodermis that is able to act as a molecular fence, mediates cell wall attachment, and precedes formation of the Casparian strips themselves. The authors named this region the Casparian strip membrane domain (CSD).

Earlier ingenious experimental manipulations of the endodermis of pea epicotyls had investigated how this domain becomes established, but the limitations of the system only allowed the conclusion that brefeldin A–dependent secretory processes are required for establishment of the CSD and Casparian strip and that some early positional information must exist in the cell that determines the width of the Casparian strip later on (43, 109). Based on the tight attachment of this plasma membrane region to the cell wall, Karahara & Shibaoka (42) also attempted a purification of proteins associated with Casparian strip fractions from hand-dissected, digested pea hypocotyls. This impressive effort demonstrated the presence of some tightly attached proteins that could be eluted only by treatment with sodium dodecyl sulfate. However, techniques at the time did not allow the identification of those proteins.

Cell type–specific microarray data in *Arabidopsis* were recently used to systematically mine genes with endodermis-specific expression (77). This led to the identification of a family of small transmembrane proteins of unknown function that strongly accumulate at the site of Casparian strip formation and nicely fit the proteins found in pea in both size and biochemical characteristics. These proteins were called CASPARIAN STRIP DOMAIN PROTEINS 1–5 (CASP1–5) and were shown to possess all the features expected of the CSD's major structural proteins. CASPs accumulate specifically in endodermal cells at the end of elongation and quickly coalesce from an initially random distribution at the plasma membrane into a precisely aligned longitudinal ring (**Figure 2*d*,*f***). Once localized, CASPs are highly immobile, not undergoing measurable rates of endocytosis or lateral diffusion. They show the ability to self- and cross-interact, and precipitate together with the cell wall upon native extraction. The CASPs are the first proteins to localize to the enigmatic CSD, and mutant analysis demonstrated that they are important for the correct formation of Casparian strips themselves. This led to the

straightforward model that the small CASPs are organizers of a membrane platform that guides the assembly of lignin biosynthetic enzymes.

Indeed, it was recently found that the *enhanced suberin 1* (*esb1*) mutant has phenotypic similarities to *casp1 casp3* double mutants, including a destructured Casparian strip (D. Salt, personal communication). ESB1 is a secreted protein that localizes to the Casparian strips themselves and encodes for a dirigent protein. This class of proteins influences stereospecificity during lignin monomer coupling in vitro (19, 53), but its implication in lignin biosynthesis in vivo had remained to be demonstrated. The CSD may act to localize ESB1 to the correct location in the cell wall by either direct or indirect interaction with CASPs. CASP localization itself, however, is also affected in *esb1* mutants, hinting at an interdependency between CASP membrane domain formation and the assembly of a cell wall biosynthetic machinery. ESB1 itself can be only one of many factors necessary to drive localized lignin polymerization. Identification of CASP-interacting and coexpressed proteins, as well as factors necessary for their polymerization and localization, will provide us with a molecular understanding of how the endodermal cell manages to locally impregnate the primary cell wall with lignin.

Interestingly, CASPs represent only a subfamily of a larger family of more than 30 CASP-like proteins in *Arabidopsis*. CASP-like proteins are found in all land plants and could represent a widespread protein module for the formation of membrane subdomains and localized cell wall formation. As alluded to above, the onset of cell differentiation is difficult to define. CASPs clearly are good molecular markers for this onset of differentiation in the endodermis. Identifying the transcriptional inputs that activate CASPs and their coexpressed genes would define an end point of the transcriptional cascade initiated by the SHR/SCR complex. A future aim should be to connect SHR/SCR activity to the initiation of CASP expression, thereby obtaining a complete chain of molecular events leading from endodermal specification to differentiation.

As is often the case, the importance of the discovery of CASPs lies not so much in the questions that it answers but in the questions that it allows us to ask. The two most fundamental of these questions are, first, how do CASPs manage to rapidly accumulate in a precisely positioned, central, longitudinal band? And, second, although polymerization of CASPs into a stable platform could explain their selective retention, what would trigger this polymerization in a precise subcellular position? Endodermal cells were recently shown to display an unequal distribution of proteins between the plasma membrane region facing the stele and the one facing the cortex. This central-peripheral polarity—also called inner-outer or proximal-distal polarity (50, 55)—is shared with other cell layers outside the stele and is already present in establishing meristems, long before formation of the CSD. This polarity could therefore be used as a system to determine the point of CASP polymerization (formally, in the minimum of the two overlapping polar domains).

It is also interesting to note the formal similarity between the problem of CASP positioning and the formation of a preprophase band during cytokinesis, both of which are about the establishment of a ring in the center of a cell (97). Could both processes use common modules to determine the position of the centered ring? CASP rings in individual cells are not only centered but also aligned between cells, even when slightly off-center. This allows Casparian strip formation in the cell wall space between two CASP domains (**Figure 2*d***). This alignment is crucial for the connection of Casparian strips into a supracellular network, without which a functional apoplastic diffusion barrier could not be formed. How is this fascinating crosstalk between cells achieved? Could there be a signal from polymerized patches in one cell favoring polymerization at closely apposed sites in the next? Some recent findings on Casparian strip formation in *scr* mutants could be seen to support such a mechanism (57), but what would be the molecular basis of such signals? Such a seemingly simple and well-described feature as

the Casparian strip turns out to depend on the complex interaction of many factors within the endodermis, and we are just beginning to unravel the first components in this fascinating example of coordinated subcellular patterning.

Secondary-Stage Endodermis and Formation of Passage Cells

With the formation of the Casparian strip, the endodermis has acquired its defining differentiation feature. As discussed above, the Casparian strip is intimately connected to the attributed function of the endodermis as a diffusion barrier, and it could be assumed that this stage represents a sort of developmental end point. Yet endodermal differentiation does not stop here; it merely enters a transient developmental plateau during which the endodermis does not undergo dramatic morphological changes. The endodermis then proceeds into a so-called secondary stage of differentiation (also called a state or phase, depending on the authors), which is characterized by the formation of suberin lamellae all around its surface, i.e., not restricted to the narrow band of the Casparian strip (25, 48, 98). Recent whole-mount suberin staining in *Arabidopsis* roots visualized this peculiar start of suberin lamella formation, which is turned on in individual interspersed cells rather than through a graded accumulation of suberin in all cells at the same time as—or at the same distance from—the meristem (65). Suberin staining eventually becomes homogenous within the endodermis, but only above a long zone exhibiting a patchy pattern in which strongly stained cells lie next to others that do not show any suberin accumulation (**Figure 1**). This peculiar onset of suberin staining is corroborated by the identical expression pattern of suberin biosynthetic genes, indicating that the staining method correctly reports suberin presence (65).

The accumulation of suberin in endodermal cell walls should profoundly affect the ability of endodermal cells to perceive and transport nutrients and signals, as discussed below. Therefore, the time between Casparian strip formation and the start of suberin lamella establishment defines a window during which the endodermis can actively participate in nutrient uptake. Multiple studies have investigated the onset of Casparian strips in relation to suberin lamella formation (48, 83, 84, 105). Either prolonging or shortening this window could be a powerful means of root adaptation to various environmental conditions. The small roots of *Arabidopsis* allow precise staging of the onset of Casparian strips and suberin lamellae, and it has been determined that suberin lamellae start to form at approximately 38 cells after the onset of elongation, 26 cells after the formation of Casparian strips (1, 65). This translates into a small zone of approximately 2 mm in which endodermal cells remain unsuberized (still a significant percentage of the total length of a five-day-old seedling) and a minimum time window of 26 h in which endodermal cells remain in their primary stage of differentiation.

Understanding the signals and transcriptional networks that determine the onset of suberin lamella formation will certainly be of great interest, as it might allow tuning of the opposing features of root uptake capacity and stress resistance. In this context, it will be important to identify the transcriptional modules that turn on suberin biosynthesis and to understand whether and (if so) how they are connected to the transcriptional network leading to the expression of CASPs and the associated proteins that execute Casparian strip formation during primary differentiation. As described above, the networks that lead from initial specification to differentiation are not well understood, and even less is known about the factors and signals that lead to secondary-stage differentiation. The recent discovery that the *esb1* mutant shows earlier and stronger onset of suberin synthesis and that the *casp1 casp3* double mutant shows a similar increase in suberin deposition (D. Salt, personal communication) allows the intriguing speculation that correct Casparian strip formation is necessary to suppress suberin lamella formation. Thus, perceived problems in Casparian strip integrity would lead to earlier and stronger deposition

of suberin, resembling the known cross-regulation between the inhibition of cellulose synthesis and the formation of lignin (37). Identifying the factors that monitor Casparian strip integrity and mediate such cross-regulation would be of great interest. In animals, the integrity of tight junctions is constantly under surveillance, and perceived defects lead to strong cellular responses (33, 86).

The chemical nature of suberin is well known, and great progress has been made in the past decade in identifying suberin biosynthetic enzymes, although many open questions remain about its biosynthesis, transport, monomeric building blocks, and macromolecular assembly in situ (27, 74, 81). It is generally agreed that suberin is laid down as a secondary wall (i.e., within the primary wall), although some degree of diffuse suberin has been reported as impregnations of the primary walls of epidermal cells (93). The question of whether suberin impregnates primary cell walls is significant, because only in this location could it act as an efficient apoplastic barrier. A polymer confined exclusively to a secondary cell wall should not be efficient in blocking the diffusion of substances through the outer, primary cell walls and middle lamellae of endodermal cells. (**Figure 3**). However, even the diffuse suberin in the primary cell walls of the epidermis cannot block tracer diffusion across this cell layer (69).

But what do we know about the degree to which suberin lamellae can serve as apoplastic diffusion barriers in the endodermis? The recent analysis of the *esb1* and *casp1 casp3* mutants indicates that endodermal suberin lamellae are indeed ineffective in generating an apoplastic (paracellular) diffusion barrier, because their stronger and earlier suberin accumulation does not compensate for the delayed formation of the Casparian strip diffusion barrier toward propidium iodide, which is observed in both mutants (P.S. Hosmani, T. Kamiya, J. Danku, S. Naseer, N. Geldner, M.L. Guerinot & D. Salt, manuscript submitted). The deposition of suberin as a secondary cell wall can readily account for these observations, which illustrates that one cannot equate the presence of suberin with the presence of an apoplastic barrier. Yet the suberized cell layers of the peridermis (the secondary dermal tissue replacing the epidermis), for example, are a highly effective apoplastic (paracellular) diffusion barrier. In this case, suberin might be deposited in the primary walls. Alternatively, a lignin-like polymer (intimately associated with suberized tissues—see sidebar Suberin: One Substance or Two?) could impregnate the primary cell walls and become connected to the suberin of the secondary walls, as seen in the endodermis with its Casparian strips. An intriguing recent report has indeed suggested that periderm tissue contains lignin-like primary cell wall modifications that resemble Casparian strips (60). That the apoplastic barrier properties of suberized cell layers are actually mediated by an associated lignification of their primary walls might turn out to be a general feature.

Clearly, a better appreciation of the subcellular organization and relationship between different cell wall polymers is needed. Advances in understanding subcellular deposition will require suberin stains or antibodies that can be used in electron microscopy, which are not currently available. Such stains or antibodies could also address the question of how the lignin of the Casparian strips is connected to the suberin lamellae when endodermal cells enter the secondary stage of endodermal differentiation. One interesting speculation is that ASFT-produced alkyl ferulates could provide covalent linkages between the two polymers.

If suberin is indeed inefficient in providing an effective diffusion barrier in young endodermal cells, then what purpose does it serve? Suberin lamellae in the endodermis might be produced not to provide a paracellular barrier—i.e., against diffusion between endodermal cells—but rather to block the access of water and nutrients (and possibly pathogens and symbionts) to individual endodermal cells. A hydrophobic polymer such as suberin laid down between the primary cell wall and the plasma membrane should effectively block, or at least strongly suppress, the access of small charged or polar molecules to the transporters residing

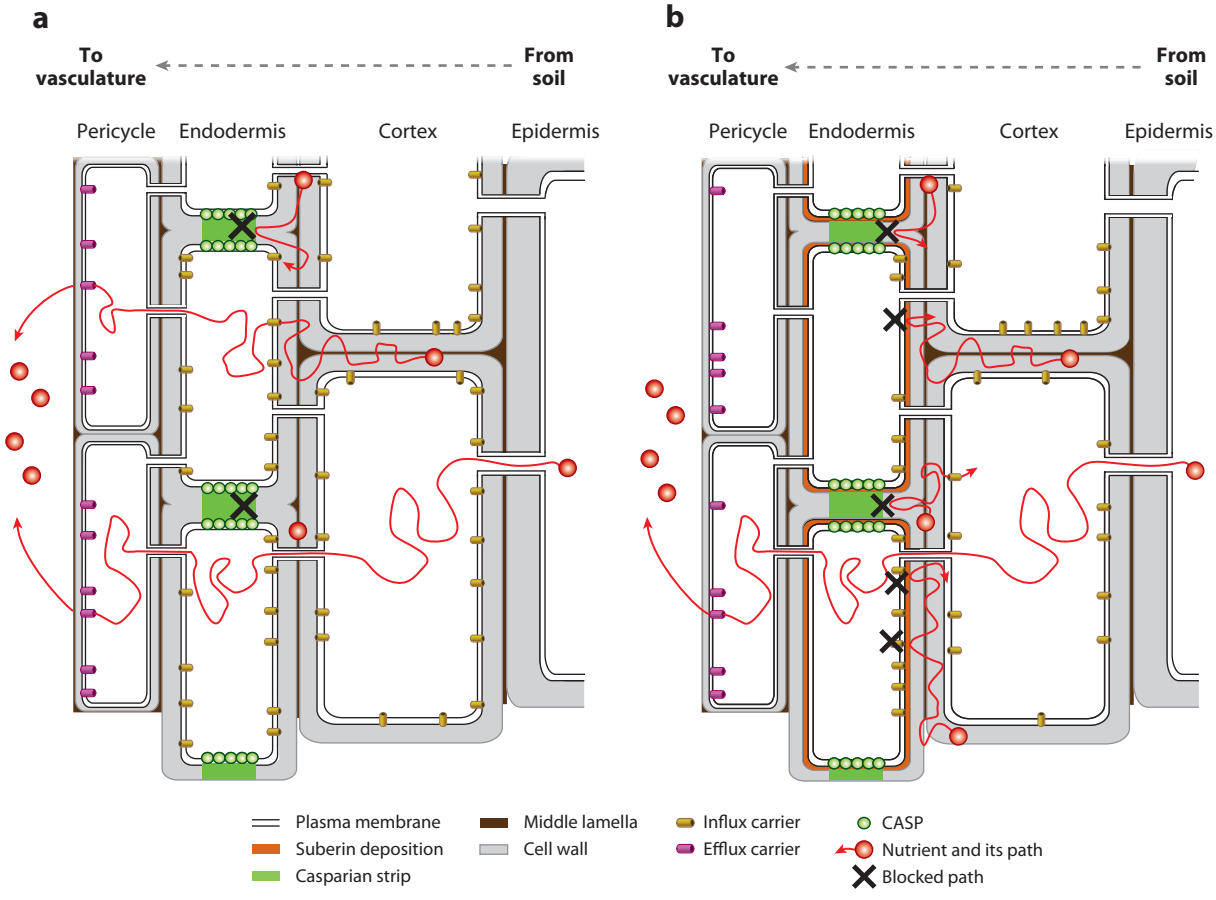

Figure 3

Schematic depicting the respective roles of Casparian strips and suberin lamellae in restricting ion transport across the endodermis. (*a*) Primary stage of endodermal differentiation with only Casparian strips. Nutrients can cross the endodermis either symplastically (after uptake into cortical cells) or directly across the endodermis through polarly localized influx and efflux carriers (transcellular transport). Only the direct apoplastic passage of nutrients into the stele is blocked by the Casparian strips. (*b*) Secondary stage of endodermal differentiation. Direct uptake into the endodermis is blocked by the presence of suberin lamellae between the plasma membranes and primary cell walls of endodermal cells, forcing the symplastic passage of nutrients.

in the endodermal plasma membrane. Suberin lamellae might therefore be less relevant for blocking paracellular (apoplastic) transport between endodermal cells, and would rather be responsible for blocking, or reducing, transport across the plasma membranes of suberized cells (transcellular transport) (**Figure 3*b***).

Classically, nutrient transport has been discussed mainly as following a symplastic or apoplastic pathway. However, the recent discovery of pairs of polarly localized influx and efflux carriers allows us to consider the importance of a third option: directional, coupled, transcellular transport in which nutrients are shuttled in and out of the cell in a directional fashion (for further discussion, see below). In such a context, blocking the access of nutrients to the endodermal plasma membrane through suberin lamella formation would break the cellular bucket brigade, forcing nutrients to undergo a longer symplastic passage through plasmodesmata, at a minimum from the innermost cortical layer to the pericycle, thus tunneling through the endodermal suberin

barrier (**Figure 3*b***). It is easy to imagine how this could profoundly affect radial nutrient flow through the root and cause the observed nutrient uptake and water transport phenotypes.

Closing off endodermal cells with suberin lamellae should generally make nutrient uptake more difficult, but it might be a highly efficient way of protecting cells against pathogen attacks. For effective colonization, many pathogens manipulate host cell function and immune response by injecting effector molecules into the cell (or otherwise transporting them across the plasma membrane). Suberin lamellae could be an efficient means to render endodermal cells refractory to manipulation by bacteria or fungi (**Figure 3*b***). Interestingly, the colonization of roots by mycorrhizal fungi is observed exclusively in cortical cells and never involves the colonization of endodermal cells, the reasons for which are unknown. In addition, fungi (9)—and also wilting bacteria like *Ralstonia solanacearum*—heavily colonize cortical spaces but are apparently unable to efficiently pass the endodermal cell layer (99), suggesting an inherent resistance feature of the endodermis that might be partially due to the formation of suberin lamellae.

The most intriguing aspect of secondary-stage endodermal differentiation may be that it does not occur in all endodermal cells. The endodermal cells that do not form suberin lamellae are always positioned at the xylem poles of the underlying vasculature (**Figure 4**). These cells have been intuitively termed passage cells because of the absence of secondary (and, later, tertiary) wall formation, which suggests a privileged function of those cells in the continued passage of material in an otherwise closed-off and protected endodermal layer. The problem with the classical anatomical definition of passage cells is that it defines them in the negative—as cells that do not undergo additional secondary wall formation. Passage cells can be viewed as simply resulting from a spatial bias in the onset of secondary differentiation. Indeed, their occurrence has been reported to decrease with age, going from longer continuous files to shorter files and then to single interspersed cells (48, 101, 106). Eventually, passage cells can no longer be observed in the older root parts of some species, although other species clearly maintain passage cells for the entire lifetime of the endodermis (48).

Krömer (48) described this developmental progression for a number of species, and a recent 3D anatomical analysis nicely visualized this progression in wheat roots (106). Krömer took the view that passage cells simply result from what he calls an *Intermediärzone*, an intermediary zone in which not all primary cells have acquired a secondary fate. Consequently, he did not name the cells but simply named the sites where primary endodermal cells were found within the secondary-stage endodermis, calling them *Durchgangsstellen* (passage sites). Later authors changed the term to passage cells without necessarily suggesting that they are different from primary endodermal cells.

Is there any evidence to suggest that there is more to passage cells than their ability to "stay young"? Or, formulated differently, is it possible to define passage cells in a positive way? Interestingly, a phosphate efflux transporter in *Arabidopsis*, PHO1, is reportedly expressed both in the stele and in single endodermal cells that were proposed to be passage cells (38). Other stele-expressed transporters seem to show a similar extended expression in single endodermal cells. Thus, analysis of transporter expression patterns may provide an entry point into defining endodermal passage cells in molecular terms and possibly distinguishing them from primary-stage endodermal cells in younger root parts. Although transporters such as PHO1 might define a difference between primary endodermal cells and passage cells, however, they are not exclusively expressed in these cells. Identification of genes specific to passage cells would be crucial to defining them as a root cell type in their own right.

Also interestingly, the hypodermis/exodermis, a peripheral "sister cell type" to the endodermis (see sidebar Exodermis and Endodermis: Twins Separated by Position?), shows a similar dimorphism within its layer, with some cells forming strongly thickened cell

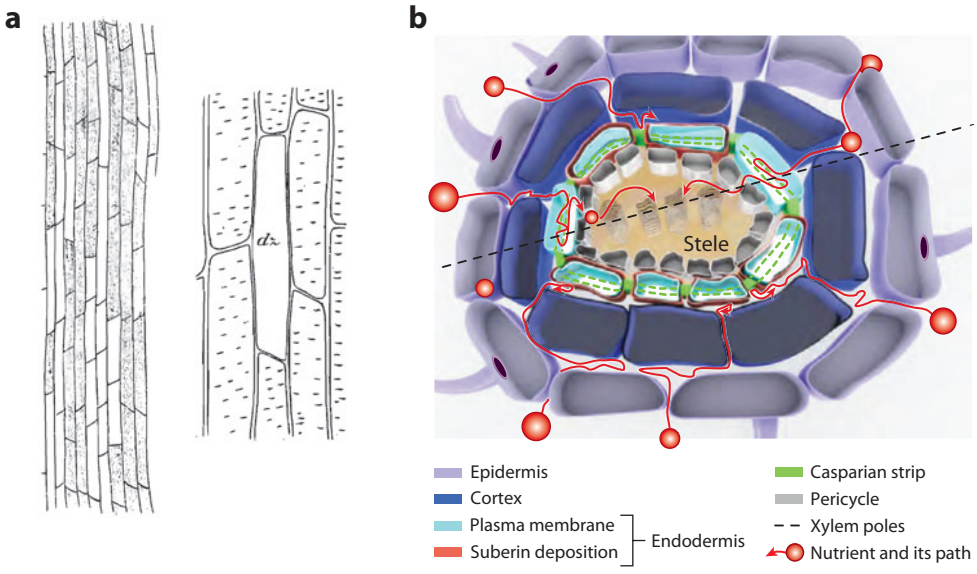

Figure 4

(*a*) (*Left*) Surface view of the endodermis in a longitudinal section of *Calla palustris* root. Shaded/stippled cells are suberized; white cells are those that remain in the primary stage (passage cells). Note the patchy occurrence of passage cells roughly organized as files. (*Right*) Surface view of the endodermis in a longitudinal section of *Hedychium gardnerianum* (Kahili ginger) root. Only single interspersed passage cells (*white*) can be observed in the otherwise completely suberized endodermis. Original drawings from Krömer (48). (*b*) Schematic depicting xylem pole–associated, nonsuberized passage cells that would still allow for direct, endodermis-mediated uptake of nutrients.

walls whereas others do not. These thin-walled exodermal cells are termed, in a parallel manner, exodermal passage cells. It has been known for a long time that these cells act as entry sites for both symbiotic and pathogenic mycorrhizal fungi (25). Again, however, it was not clear whether they are chosen simply because of the absence of a thickened cell wall or because specific, characteristic features of the cells actively attract the fungus.

A breakthrough in the understanding of fungus-plant interaction was recently achieved by the identification of an efflux transporter for strigolactone, a plant hormone that plays a crucial role in eliciting hyphal branching of mycorrhizal fungi and colonization of the plant (47). Intriguingly, this study demonstrated transporter expression specifically in exodermal passage cells. This finding now provides a molecular marker for this cell type, suggesting that the absence of cell wall thickening is associated with a specific developmental expression profile that allows these cells to fulfill unique physiological functions. It will be important to determine whether endodermal cells have similar specific expression profiles that would endow them with properties distinct from those of primary endodermal cells and their suberized neighbors. It is intuitive to view the existence of passage cells in the secondary-stage endodermis as the result of a tug-of-war between the two opposing needs of continued uptake and protection against abiotic stresses and pathogens. In this case, passage cells could be kept open or be closed off depending on the environmental conditions, strongly impacting the plant's ability to take up nutrients or resist various stresses. A better description of passage cell occurrence and a molecular analysis of their differentiation will be highly relevant for improving our understanding of root physiology. Passage cells should also be considered when attempting to model various aspects of root function.

EXODERMIS AND ENDODERMIS: TWINS SEPARATED BY POSITION?

Excellent reviews have been written about the exodermis and endodermis and the relationship between them (25, 61). The most straightforward definition of the exodermis is that it is a Casparian strip–bearing hypodermis—a hypodermis being a cell layer underlying the epidermis with thickened suberized/lignified cell walls, which acts as an additional outer diffusion barrier in many but not all angiosperms (*Arabidopsis* does not have it). The similarities between the two cell layers are extensive: Both have a Casparian strip, act as apoplastic diffusion barriers, enter secondary stages of cell wall deposition, and display a dimorphism in the form of passage/nonpassage cells (25, 61). Exodermis differentiation, however, occurs farther from the meristem and is often dependent on physiological conditions. In addition, Casparian strips are broader in the exodermis and formed when suberin lamellae are also deposited.

All this suggests that the exodermis might act mainly as an additional apoplastic barrier and to a lesser degree in selective uptake compared with the endodermis. Because *Arabidopsis* lacks the exodermis, how it is specified in molecular terms is unknown. It would certainly be intriguing if a similar transcription factor–based short-range signal from the epidermis specifies the outermost cortical cell layer to become the exodermis, as is seen for the stele and endodermis. Studies of root development in rice are interesting in this respect (67). Homologs of the recently discovered CASPs would be good candidates for molecular markers of exodermal differentiation, as they have been identified as important for Casparian strip formation in the endodermis (77). Another intriguing parallel is the specific expression of silicon transporters in rice in both the endodermis and exodermis, demonstrating similarities between the two cell layers with respect to nutrient transport.

The Tertiary Stage and Death of the Endodermis

Many plant species develop yet another distinct cell wall modification in endodermal cells, described as the tertiary stage of endodermal development, in which they form pronounced cellulosic cell wall thickenings that are often lignified. Even at this stage, passage cells can remain as thin-walled cells above the xylem poles and are then very easily recognized. The tertiary thickened cell walls are often described as U-shaped because of their appearance in sections: The inner periclinal and the radial wall appear thickened, whereas the outer periclinal wall remains thin. This probably amounts to a cup-shaped cell wall thickening, when considering the third dimension.

To my knowledge, there is no evidence for or compelling idea about the biological role of this specific tertiary wall shape. A cell layer with U-shaped wall thickenings might respond differently to mechanical stresses, but what type of mechanical stresses it might be advantageous for is entirely up for speculation. U-shaped endodermal cells could also remain more active in uptake from the cortical apoplast through their thin-walled surfaces, which would indeed be of some advantage under certain conditions; however, the earlier formation of suberin lamellae during secondary-stage differentiation occurs equally on all cell sides and would already provide an efficient block for uptake through the outer periclinal membrane of the endodermal cell. For this reason, I also do not find this hypothesis particularly appealing. A preferential, stele-oriented wall formation could be due to a preferential source of lignin monomers from stelar cell types, which could lead to lignification exclusively on inner and radial walls of the endodermis. This would explain the localized thickening not as a useful evolutionary adaptation but rather as a result of localized access to biosynthetic precursors. However, it does not explain the preferential formation of cellulosic walls, which often precedes the formation of lignin (as in xylem vessel formation, for example) (68). Whatever the case, in many plant roots tertiary thickening is not observed, and for a number of species the reason for this might lie simply in the restricted life span of an endodermal cell.

What process would cause endodermal cells to die before the organ itself dies? As with their shoots, the roots of many dicotyledonous plants undergo secondary growth; the onset and extent of this growth, however, are extremely variable between species. Secondary growth in roots is driven by cell layers forming within

the stele, leading to the formation of two radial meristems—the cambium and the phellogen—whose activities cause large increases in root diameter and circumference (26). For a certain period, outer cell layers such as the endodermis can adapt to this growth in girth by cell division and circumferential widening of cells, but at some point they inevitably break and slough off. Thus, continued secondary growth eventually eliminates the epidermis, cortex, and endodermis. Their protective role is then replaced by a suberized, multilayered, secondary dermal tissue, the periderm, formed by the phellogen. However, it is puzzling that before it sloughs off, the endodermis of many species is able to increase its circumference, both by cell divisions and by widening of individual cells. Even more strikingly, during cell division and growth the apoplastic barrier apparently remains intact (102).

As discussed above, the Casparian strip is made of a lignin polymer. Lignification is generally considered to interfere with the ability of cells to elongate—so how can endodermal cells accommodate the often many-fold increases in endodermal size reported for some species? Does a special type of lignin structure in Casparian strips allow for elongation growth, or does the lignin become modified such that it does not interfere with the stretching of the wall? Could there be a local severing and resealing of Casparian strips in a way that does not affect barrier function? The endodermis eventually breaks, a process that has been documented in multiple species, including *Arabidopsis* (23). Again, this process is intriguing when considering the Casparian strip as a lignified modification of the primary cell wall. Is the endodermis broken by sheer mechanical force, even though the lignified network of the strips should be able to withstand considerable mechanical force? Or is there some active modification of the lignin polymer—maybe some local weakening—that facilitates breaking and cell separation? A process reminiscent of this decortication or cortical shedding during secondary growth is seen earlier, during lateral root emergence, where endodermal cells also have to split. Again, this process has been described repeatedly (for example, see 5, 11, 44), and recent publications indicate that the overlying cortical and epidermal layers perceive and respond to the emerging primordia by expression of cell wall–degrading enzymes (90). However, how the critical lignified Casparian strip network is dealt with during this emergence process remains entirely obscure. The process of endodermal breakage during secondary growth as well as lateral root emergence could be molecularly dissected using *Arabidopsis* and might lead to important and novel insights into how plants modify lignified cell walls.

ENDODERMAL FUNCTION

The Endodermis: A Plant Variant of a Polarized Epithelium

The question of endodermal function is inextricably linked to the Casparian strip and its recognized role as a major apoplastic diffusion barrier in the root. Although very different in structure and origin, the strip and its associated membrane (the CSD) are functionally equivalent to the tight and adherens junctions of animal epithelia. These functional parallels have been extended by the recently uncovered strict polarity in the distribution of silicon and boron influx and efflux carriers, which is maintained by the molecular fence properties of the CSD (1, 55, 92) (see above). Surprisingly, the judicious view of the endodermis as a polarized epithelium has been promoted by only a few authors, mostly by Clarkson (for example, see 17). One reason for this might be that the endodermis—in contrast to the gut epithelium, for example—is an inner cell layer and not in immediate contact with the soil environment. The outermost epidermis is in immediate contact, and is consequently seen as the layer that mediates most of the selective nutrient uptake. The two epithelial functions—selective uptake and diffusion barrier—thus appear split between epidermis and endodermis in plants, which might have promoted the view of the endodermis as a naive diffusion barrier rather than a selective epithelium.

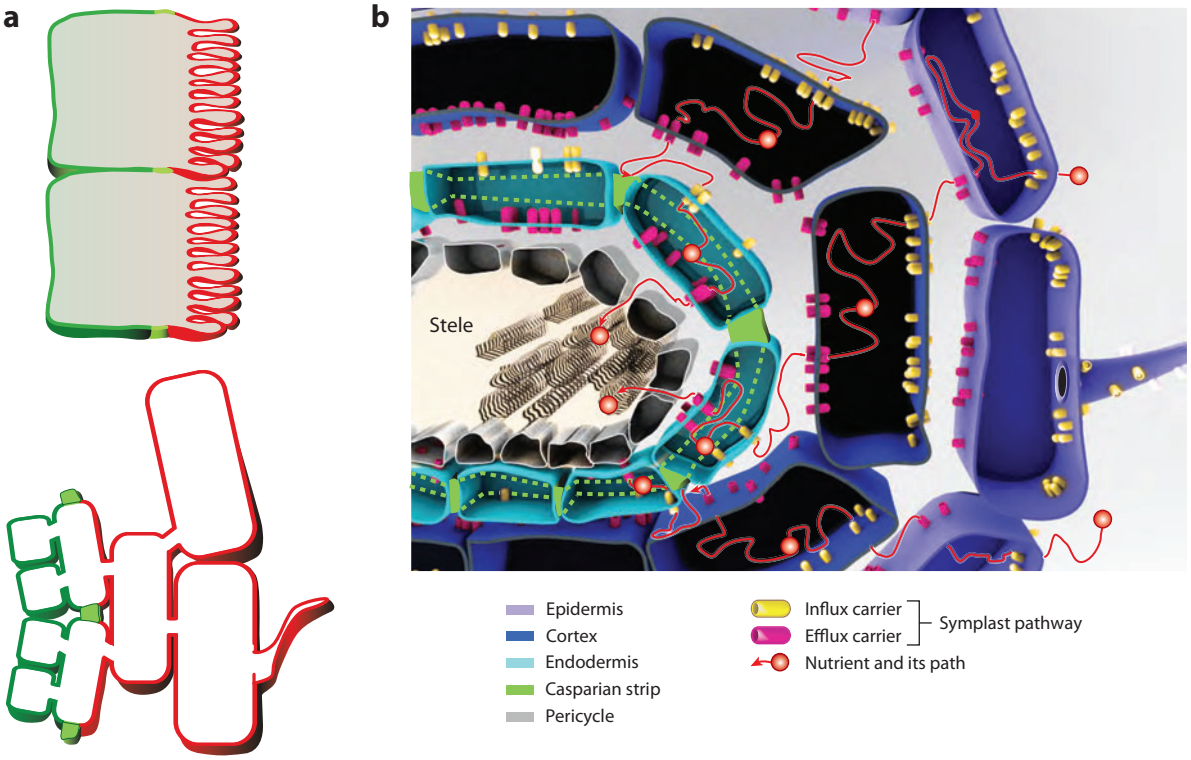

Figure 5

(*a*) Comparison of an animal gut epithelium cell (*top*) with the plant endodermis and its surrounding cell layers (*bottom*). The symplastic nature of the root organ allows viewing the peripheral domain of the endodermis plus the entire plasma membranes of cortical and epidermal cells as a single extensive plasma membrane surface that is functionally equivalent to the apical domain of a gut epithelial cell (*red*). The same applies to the central domain of the endodermis plus the plasma membranes of stelar cells (*green*, the "basolateral" domain in animals). In the bottom diagram, blue represents cortical (outer) apoplastic space, and gray represents vascular (inner) apoplastic space. (*b*) Schematic transversal view of a root depicting how the coordinated polar distribution of influx and efflux carriers in the epidermis, cortex, and endodermis could mediate a coupled transcellular transport of nutrients. This alternative mechanism could drive a directional movement of nutrients towards the stele.

Indeed, the symplastic connection between the different root cell layers allows a radical yet instructive comparison with a gut epithelium, as depicted in **Figure 5**. In this comparison, the entire root symplast constitutes a single supracellular epithelium centered on the endodermis. The combined plasma membrane surfaces of the epidermis, cortex, and peripheral plasma membrane domains of the endodermis could be seen as a huge "apical" membrane domain (the animal terminology for the membrane domain facing the gut lumen), and the plasma membranes of stelar cell layers (such as the pericycle, xylem parenchyma, and central domain of the endodermis) could be viewed as one hugely extended "basolateral" domain (the animal terminology for the domain facing the bloodstream). In this view, the cell layers peripheral to the endodermis would provide a greatly increased membrane surface, allowing efficient nutrient uptake, with the root hairs being just the most peripheral extensions of a functionally connected membrane network that extends deep into the root until it reaches the endodermis. The elegant feature of such a design is that the peripheral and central membrane networks would consist of independent transcriptional units and

could localize transporters to the peripheral membrane network simply by expressing them in the epidermis or cortex, and localization to the central network would be achieved by exclusive expression in the xylem parenchyma or pericycle. In this model, there is no need for cellular polarity within a layer; the restriction of transporter activities to one domain would be achieved simply by expressing them in cells either within or outside the endodermal barrier. Transporter localization could be nonpolar within a given cell layer and still mediate vectorial uptake across the root. The plasma membrane domains of the endodermis itself should then be devoid of carriers, which can be considered of minor importance, because the endodermal membrane surface represents only a fraction of the total membrane surfaces of the extended central and peripheral domains.

Such a supracellular division of labor—uptake in epidermis, barrier in endodermis, and export in xylem parenchyma, for example—probably occurs for some mineral nutrients. This division does require high nutrient mobility within the symplast, as the nutrient needs to cross all the cell layers from the epidermis to the stele by diffusion through the cytoplasm and plasmodesmata. However, many nutrients quickly become complexed with proteins, influencing their mobility and possibly their ability to cross plasmodesmata. In addition, the overall concentration of some nutrients in the cytoplasm needs to be kept very low because of their potential toxicity or their involvement in signaling processes, as in the case of calcium. In all these cases, it would be preferable to shorten the cytoplasmic path in order to place nutrients in the apoplast of the vasculature.

The shortest possible path would be an apoplastic passage of nutrients until they reach the endodermis, direct uptake through the peripheral domain, and export through the central domain (**Figure 5**). In this case, cellular polarity is absolutely required and the endodermis would be actively involved in uptake, completely analogous to a gut epithelium. The first scenario described above is generally called the symplastic path of nutrient uptake, whereas the second is termed the transcellular path. It should be pointed out, however, that both pathways require symplastic passage, although passage only through the cytoplasm of the endodermis does not involve plasmodesmata. More important, both scenarios should be seen as extreme ends in a continuum, in which nutrients can take a longer or shorter path through the symplast, and the region where uptake occurs can be more or less restricted (epidermis only, endodermis only, or epidermis, cortex, and endodermis together) (**Figure 5**; also see above). Plant roots probably selectively employ all of these options depending on the specific nutrient, its availability, and various other physiological conditions (water status, transpiration rates, etc.).

As mentioned above, an interesting variant that has not been considered much in the classical literature could be termed coupled transcellular transport. In this scenario, not only the endodermis but also cortical and epidermal cells have a polarized distribution of transporters. Nutrients would then be transported in a bucket brigade fashion, passing repeatedly from symplast to apoplast, reminiscent of the mechanism by which the plant hormone auxin is transported (45) (**Figure 5*b***). Such a scenario might seem energetically wasteful and inefficient, but it would have the advantage of providing a directionality of transport toward the stele over many cellular distances, which could be important if there is no mass flow of dissolved nutrients toward the stele and radial transport in the apoplast must rely entirely on diffusion. The possibility of such a coupled transcellular transport has been substantiated by the recent findings that polar distribution is indeed present in all peripheral cell layers (endodermis, cortex, and epidermis) (1, 50, 92) and that nutrient efflux carriers are not necessarily restricted to cell layers of the endodermis and stele (a requirement for coupled transcellular transport) (92). A clear case for such transport is seen in silicon/arsenite and probably manganese in rice, in which the polar distribution of transporters has been demonstrated in both the endodermis and exodermis (54, 55, 62,

79). In rice, the situation is complicated by the presence of an additional Casparian strip diffusion barrier in the exodermis, leading to the formation of three separate apoplastic spaces (see sidebar Exodermis and Endodermis: Twins Separated by Position?).

The Casparian Strips as an Apoplastic Barrier to Nutrients

All discussed scenarios of radial nutrient transport rely on the endodermis to provide a strict diffusion barrier within the apoplast, preventing both apoplastic bypass of nutrients (which would interfere with the plant's ability to actively control the nutrient composition in the xylem) and backflow of nutrients accumulated in the xylem into the cortical apoplast and soil. In the absence of this barrier, it is thought that transporter action would be compromised by constant apoplastic bypass and that the plasma membrane transporters would be ineffectual, like hammers working without an anvil.

A significant amount of correlative evidence supports this idea, and the ability of Casparian strips to act as this barrier has been established beyond doubt (see below). It is important to note, however, that we still lack strong, specific mutants of Casparian strips that would allow us to directly test the relevance and actual importance of the strips for the selective radial transport of individual nutrients. De Rufz de Lavison (21) provided the first good evidence for the role of Casparian strips in blocking the apoplastic path of nutrients (**Figure 6*a***). Later, electron microscopy studies using electron-dense tracers such as lanthanum showed a block of tracer penetration precisely at the Casparian strips (63) (**Figure 6*b***). Fluorescent tracers have also been repeatedly used to highlight the endodermal diffusion barrier, and it has been recently demonstrated that positively charged propidium iodide is an extremely convenient reporter for barrier formation in *Arabidopsis* (1, 65) (**Figure 6*c***).

It is generally accepted that the Casparian strip is essentially impermeable for charged mineral nutrients and that exclusively apoplastic transport from soil to stele is not used by plants, with the possible exception of calcium (16, 17, 103, 104). In the few cases in which apoplastic transport of a charged element such as calcium or sodium has been demonstrated, it can presently be assumed that this is due not to permeability of the Casparian strip but either to some degree of apoplastic passage through the root tips or to transient breaks in the Casparian strips, as is seen during lateral root formation (for an alternative view, see 75). Whether any ion is taken up in significant amounts entirely through apoplastic passage remains a matter of debate, but it has been proposed in the case of calcium (15, 104).

The Endodermis: A Barrier to Water Uptake?

If we consider the Casparian strip to be essentially impermeable to ions and other charged molecules, then what about the merely polar water molecule itself? Many studies have tried to define the contribution of apoplastic versus symplastic water passage, and exhaustive reviews have been written on this subject (see 41, 58, 87, 88). Water transport cannot be traced directly, and it is very difficult to untangle the contributions of the different pathways by using pressure probes to measure root hydraulic conductance. It is even more difficult to ascertain the contribution of Casparian strips or the presence of endodermal suberin lamellae as resistors in radial water transport. Based on hydraulic measurements after puncturing the endodermis, some reports have suggested that Casparian strips do not contribute significantly to water transport (70, 88). However, such mechanical manipulations are evidently traumatizing and prone to induce secondary confounding responses. Roots show abundant expression of different aquaporin isoforms, which mediate transcellular and/or symplastic water passage (58, 59, 108). The activity and expression of aquaporins are highly regulated, and the challenge of assessing the endodermal contribution to apoplastic water passage is to untangle it

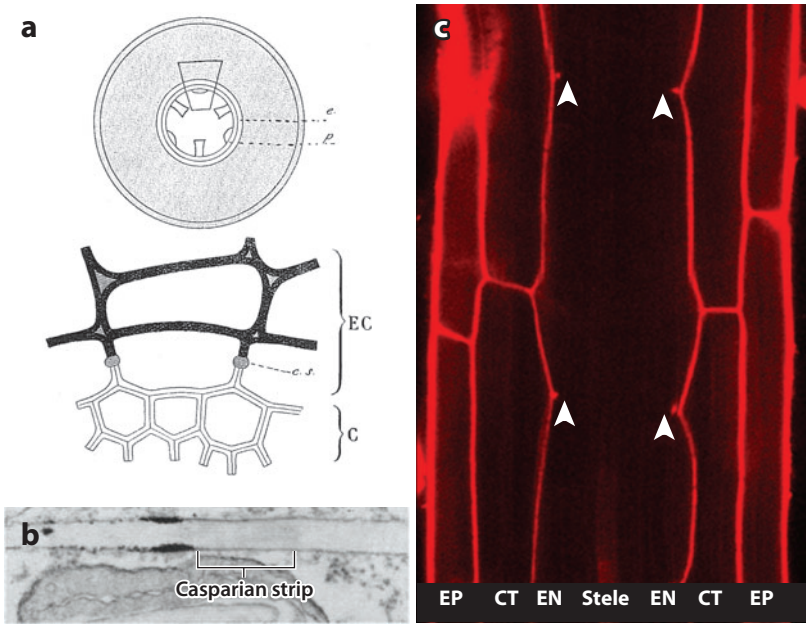

Figure 6

The endodermis as an apoplastic diffusion barrier. (*a*) Drawings of the original observation of de Rufz de Lavison (21) in 1910. The upper drawing is a root overview; shaded areas are those in which $FeSO_4$ uptake was observed after 24 h of treatment, and white areas are those without staining (e, endodermis; p, pericycle). The lower drawing is a magnification of the upper drawing, depicting $FeSO_4$ penetration exclusively in the apoplast of the cortex (*black areas*; EC, *écorce*) and the block at the Casparian strips (c.s., *cadres subérisés*). The stele (*white areas*; C, *cylindre central*) is unstained. (*b*) Electron micrograph after treatment of roots with lanthanum salt, showing lanthanum deposition as a dark staining precisely at the cortical (*left*) side of the Casparian strip, illustrating the strip's ability to block further uptake of the salt (63). (*c*) Recent live-imaging method in an *Arabidopsis* root, visualizing the endodermal barrier as a block in the uptake of propidium iodide (*red*), used as a fluorescent apoplastic tracer (1). Arrowheads mark the positions in the endodermal transversal wall at which uptake is blocked, precisely coinciding with the position of the Casparian strip. Abbreviations: EP, epidermis; CT, cortex; EN, endodermis.

from the significant contribution of aquaporin-mediated water flow.

As in the case of nutrient uptake, it is important to keep in mind that Casparian strips and the later-formed suberin lamellae affect endodermal function in different ways. Only the Casparian strips could be effective in blocking apoplastic water passage (if it is sufficiently hydrophobic), as they are a primary cell wall modification. The suberin lamellae, by contrast, are secondary wall formations that can only be effective in blocking water access to the endodermal plasma membrane. This, in turn, could greatly affect transcellular or symplastic (rather than apoplastic) water passage, depending on the contribution of endodermis-expressed aquaporins to root water passage. Natural variation in root hydraulic conductivity was recently measured in different *Arabidopsis* accessions, and no correlation was found between patterns and degrees of suberization and hydraulic conductivity (89). By contrast, *horst*—a mutant with lower root suberin content (40) and delayed formation of suberin lamellae in the endodermis (65)—was shown to have a higher hydraulic conductivity.

To dissect the contribution of the endodermis to water transport and nutrient uptake, it will be important to obtain new mutants that affect Casparian strip formation as well as

THE ENDODERMIS: ONE TERM, MANY MEANINGS?

It is surprisingly difficult to provide a precise definition of the term endodermis (51). It is agreed that the Casparian strip–bearing, innermost cortical cell layer of roots should be called an endodermis, but there is less consensus regarding how the term should be applied to cell layers in aerial organs that do not display Casparian strips.

The term was originally defined anatomically and included only Casparian strip–bearing cells (14, 26, 48, 100). Casparian strips can also be found in cell layers surrounding the vascular tissues of stems and leaves, although their occurrence is rare in angiosperms (51, 107). This nevertheless indicates that the innermost cortical layers surrounding vascular bundles in shoots and roots have fundamental commonalities. These innermost cortical cell layers are indeed often morphologically and functionally distinct from other cortical cells, and are then designated with specialized names such as starch sheath or bundle sheath. Thus, a very inclusive functional/topological definition of the endodermis would be that it is the innermost cortical layer surrounding the vascular bundle, or entire bundle systems, having acquired distinct characteristics from other cortical cells.

The SHR/SCR transcription factors have been identified as a module that specifies endodermal cell fate based on short-range signals from the stele (71). This finding provides molecular support for a topological definition based on proximity to the stele and adds a molecular definition to the endodermis as a cell layer showing SCR expression. A cell layer that is similar to the root endodermis in two of the three criteria—anatomical (Casparian strips), topological (innermost cortical cell layer surrounding the vasculature), and molecular (expression of SCR or its recognized ortholog)—can reasonably be defined as an endodermis. The use of a combinatorial term like bundle sheath endodermis or shoot endodermis would indicate that the term endodermis is used because of the (reasonable) assumption of a fundamental homology between often very different cortical cell layers in various organs.

suberin lamella deposition. Just as relevant will be obtaining a better description of existing mutants. Overall root suberin content, for example, is an insufficient criterion to judge the functionality of suberin mutants. Without knowledge of the developmental progression, tissue specificity, subcellular distribution, and ultrastructure of suberization or Casparian strip formation, it will be difficult to interpret the changes in water and nutrient uptake of mutants in a meaningful and coherent way.

Casparian Strip–Independent Functions of the Endodermis

I explained above how the formation of the Casparian strip, suberin lamellae, and even tertiary cell wall thickenings endow the endodermis with unique properties within the root, allowing it to act as a selective gateway for nutrients and water. But can we subsume the endodermis into "a cortex with Casparian strips"? Are these specific cell wall modifications all we need to know to understand endodermal function, possibly with the addition of some specific transporter expressions and localization? Clearly the answer is no—there are many more endodermis-specific functions that have no immediate connection to the Casparian strips. One well-known example is the shoot gravitropic response, mediated by starch granules within the shoot endodermis, which led to the specific term starch sheath for this tissue layer (see sidebar The Endodermis: One Term, Many Meanings?). The identification of *shr* and *scr* in a screen for shoot gravitropic mutants nicely illustrates the importance of a correctly differentiated endodermis in this process (29). The starch sheath endodermis is devoid of Casparian strips.

The endodermis may also hold a special role in phototropism, a process dependent on polar auxin transport and the unequal distribution of auxin in the stem. The auxin efflux carrier PIN-FORMED 3 (PIN3) is expressed in the endodermis and is necessary for a full phototropic response (28). Endodermal PIN3 relocalizes upon unilateral blue light irradiation (22), and a similar endodermal PIN3 response in shoot gravitropism has also been reported (73). This indicates that the endodermis might be a control point for the lateral redistribution of auxin in the stem. Root meristems maintain a highly localized auxin-response peak around their center, which is crucial to maintain their organization and activity. Auxin tissue

localization is thought to depend largely on the combined activities of different PIN auxin efflux carriers in many cell layers (32). Intriguingly, localization of the auxin efflux carriers indicates a complex, cyclic transport route of auxin—apical in epidermal/lateral root cap cell layers and basal in stelar cells (45). The amount of auxin recycling has been proposed as an important determinant of meristem size. Communication between these two opposing transport streams necessarily passes through the endodermis, which might play an important role in regulating the degree of cyclic auxin flow.

The endodermis also appears to have a specific function in mediating gibberellin-dependent root growth. When repressors of gibberellic acid perception were specifically expressed in individual root cell layers, only endodermal expression was able to severely inhibit primary root growth (96). Moreover, SCARECROW-LIKE 3 (SCL3), an endodermis-specific transcription factor and direct target of SHR/SCR, acts to attenuate gibberellin repressor action, thereby promoting gibberellin response specifically in the endodermis. Finally, a novel, functional fluorescent version of gibberellin was found to accumulate strongly and specifically in elongating endodermal cells (M. Estelle, personal communication). It therefore appears that the endodermis is the primary receiver of the growth-stimulating gibberellin input, which must then be conveyed to the other cell layers.

How could we rationalize this central role of the endodermis in root growth control? Perhaps its central position in defining the border between the stele and the cortex makes it well suited to transmit growth information to both root compartments. The formation of a longitudinal lignified band in the form of the Casparian strip may also be a more or less irreversible decision to stop elongation. If this were the case, it would make sense to let the endodermis determine when to stop elongating and enter differentiation. In development, borders between two compartments are often used as a reference point for the further elaboration of a pattern, as exemplified by the production of decapentaplegic (dpp) from a narrow cell stripe at the anterior-posterior compartment boundary that then spreads across the imaginal wing disc in *Drosophila* (35). The endodermis was also recently demonstrated to act as such a source of morphogenetic information by producing a microRNA that signals back into the stele, allowing protoxylem formation in the stele periphery by interfering with expression of class III homeodomain–leucine zipper transcription factors (13). It is intriguing to speculate on the possible evolutionary relationships between the endodermis as a central selective border between the stele and the cortex and its central regulatory functions in root growth and development.

CONCLUSIONS

The endodermis is a fascinating, intricately structured cell type, which can be used as a model for many fundamental biological questions about cell coordination, subcellular patterning, polarity, localization of cell wall biosynthesis, and the regulation and mechanisms of lignin and suberin formation. In addition, because of its central position in models of root function, the endodermis can be seen as a sort of Archimedean fulcrum that we might use to move many problems related to the inner workings of the root. To do so, it will be crucial to identify more of the molecular players of endodermal differentiation, so that we will be able to specifically interfere with endodermal structure and function and study the consequences of these manipulations on the plethora of processes that the endodermis is believed to be involved in.

NOTE ADDED IN PROOF

In a paper in press in *Cell*, Lee et al. (50a) propose a model whereby CASPs assemble an NADPH oxidase and peroxidases in order to promote Casparian strip formation.

DISCLOSURE STATEMENT

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Errata

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