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The Plant Cuticle

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The plant cuticle is one of the key innovations that allowed plants to colonize terrestrial ecosystems. By limiting molecular diffusion, the cuticle provides an interface that ensures controlled interactions between plant surfaces and their environments. It confers diverse and sometimes astonishing properties upon plant surfaces at both the molecular level (from water and nutrient exchange capacities to almost complete impermeability), to the macroscopic level (from water repellence to iridescence). It takes the form of a continuous modification of the outer cell wall of the plant epidermis from early in plant development (surrounding the epidermis of the developing plant embryo) and is actively maintained and modified throughout the growth and development of most plant aerial organs including non-woody stems, flowers, leaves, and even on the root cap of emerging primary and lateral roots. The cuticle was first identified as a distinct structure in the early 19th century, and has since been the focus of intense research that, whilst revealing the fundamental role of the cuticle in the life of terrestrial plants, has also highlighted many unresolved mysteries regarding cuticle biogenesis and structure.

The function and properties of the plant cuticle

The fundamental function of the plant cuticle, in permitting controlled environmental interactions, is best illustrated in the leaves of tracheophytes. Leaves photosynthesise, converting environmental carbon-dioxide to sugars, and releasing oxygen. Leaves are also key drivers of transpiration. Evaporation of water vapor from the cells within leaves, and its subsequent loss from the leaf surface, drives the continuous movement of water and solutes critical for plant growth, from the roots to the aerial parts of the plant. However, uncontrolled water loss from the leaf surface would, in drying, terrestrial environments, be lethal. To prevent this, the leaf surface is covered by a continuous and relatively impermeable cuticle, and gas exchange is predominantly mediated by pores (stomata) whose apertures can be regulated in response to environmental and physiological cues (Figure 1A). Stomatal closure (for example under water stress) therefore permits the leaf to conserve water, due to the presence of the cuticle. Consistent with this, mutants showing defects in cuticle composition and integrity are often very sensitive to water stress.

Although of key importance, this fundamental function in leaves (and similar functions in other aerial organs of other tracheophytes), is only one of many, highly diverse roles played by cuticles in plant biology. The presence of a functional cuticle has been shown, or proposed, to affect multiple interactions, both abiotic and biotic. Cuticles are thought to limit the harmful effects of UV radiation at plant surfaces, either by reflecting light, or through the accumulation of UV-absorbing molecules, whilst the ingress of chemical aggressors such as ozone may also be attenuated by the cuticle (which has been proposed to act as a molecular “sponge”). Alterations in cuticle structure and composition also profoundly affect both plant-pathogen interactions, and the colonisation of organ surfaces with neutral and beneficial microorganisms. Furthermore, cuticle properties have been shown to affect critical reproductive processes, from reproductive organ fusion and the germination of pollen grains on female floral organs, to the attraction of pollinators. Importantly, the production of a functional cuticle is also critical for normal plant development. Defects in cuticle biogenesis

have been shown to strongly impact plant growth (from leaf expansion to the outgrowth of lateral roots). To what extent such growth defects are due to physiological changes, or to other factors such as changes in the mechanical properties of the cuticle itself, remains unclear, since disentangling these factors is technically extremely difficult. In extreme cases cuticle defects can lead to the inappropriate fusion of neighbouring organs as they develop, profoundly affecting organ function. **How the diverse properties and functions of plant cuticles are regulated at the level of organs, cells and molecules remains a major question in plant biology.**

A properly established cuticle forms a continuous hydrophobic modification, generally of uniform thickness, on the surface of plant organs. The physical integrity and diffusion limiting properties of the cuticle are essential for its functions. However, it is important to note that even in physically continuous cuticles biophysical, and in particular molecular diffusion regulating, properties are extremely variable depending on the molecules considered (lipophilic molecules will generally traverse cuticles more easily than charged or hydrophilic molecules), on the plant species studied, and on environmental and developmental contexts. Thus, the physically intact cuticle, present on the leaf-like phyllid of a moss gametophyte (Figure 2A) may be several orders of magnitude more permeable to water and ionic solutes than that on the surface of an ivy leaf (Figure 2B), while the cuticle of an Arabidopsis stem is highly impermeable compared to that of a developing Arabidopsis embryo.

These differences are exquisitely tailored to physiological requirements. The surfaces of moss phyllids (photosynthetic leaf-like structures) for example, do not contain stomatal pores. The phyllid surface thus forms an interface with the terrestrial environment which must achieve two contradictory functions; allowing the exchange of gases necessary for photosynthesis, whilst limiting water loss (and in some contexts, in the absence of vascular tissues, likely allowing controlled water and mineral uptake). This functional balancing-act may be a key factor restricting the ecological range of many bryophytes. Similarly, angiosperm embryos develop within seeds, and absorb nutrients and water from surrounding tissues (notably the endosperm), across an epidermal surface that is also devoid of pores. Nonetheless embryos produce continuous (albeit highly permeable) cuticles from early on in their development, and cuticle establishment appears to be important for both embryo development and for survival upon germination. These examples, in which cuticles appear to act as “filters”, contrast with the example of the post-germination leaf cuticles of Angiosperms, which can be thought of more in terms of true diffusion barriers (Figure 1, Figure 2B), and which can attain huge thicknesses, particularly in plants adapted to arid environments. These profound differences underline the fact that cuticle properties, particularly in terms of permeability, must be exquisitely tailored to different contexts.

Cuticle composition and structure

Plant cuticle biosynthesis is a highly complex process involving the production of precursors in epidermal cells. These molecules, many of which are very hydrophobic, are exported from epidermal cells in a polar fashion (towards the plant surface), and must then cross the highly hydrophilic extracellular matrix before being assembled into a continuous structure at the plant surface. During cuticle biogenesis, two major classes of molecules, lipids and phenolics, are synthesised, exported and then either associate with, and/or are deposited outside the carbohydrate-rich plant cell wall. The principal structural components of cuticles are solvent-insoluble crosslinked lipid polymers, generally described as cutan (a polymer recalcitrant to chemical analysis, and of which the structure and composition remain unclear), and cutin. Cutin is composed principally of oxygenated and/or carboxylated fatty acids. However, the degree of crosslinking of these components, and to what extent they form a branched (reticulated) polymer can vary dramatically depending upon context and monomer composition. Cutin is integrated into the outer part of the epidermal cell wall, to which it is thought to be attached via covalent crosslinks, forming a continuous macromolecular matrix.

Phenolic components, which are intimately associated with the cutin matrix, although often minor in terms of quantities, appear to be important in determining both the physical organisation and biophysical properties of cuticles. However, the molecular basis for their apparently key structural role, remains poorly understood. Interestingly phenolics appear to be both particularly abundant in, and important for, the assembly of functional bryophyte cuticles. More detailed studies of the biology of the cuticles of these organisms, some of which are very genetically tractable, could thus provide key elements to bolster our understanding the role of phenolic components.

Although the cutin and cutan polymers do confer barrier functions, the main “waterproofing” agents of plant cuticles are usually waxes, which can either be intercalated with other cuticle polymers (inter-cuticular waxes), or can be deposited onto the cuticle surface often as crystals that can have a wide variety of shapes. Waxes can be readily extracted from cuticles using organic solvents, whereas the analysis of the composition of the cutin polymer requires the hydrolytic breakage of ester bonds. This dependence on hydrolytic “cracking” limits our current visions of the true composition and structure of the cuticle, due to the likely presence of non-hydrolysable linkages (as present in cutan).

As suggested above, the cuticle is highly complex (and variable), at the biochemical level, but also spatially heterogeneous. Cuticle organisation within the cell wall is often visualised using Transmission Electron Microscopy (TEM), and reveals dramatic differences between different tissues and species. Spatial heterogeneity is less apparent in extremely “young” cuticles (sometimes called “protocuticles”), such as those at developing embryo surfaces or root caps, which appear as thin, condensed bands, only a few nm in thickness. However, the more biochemically complex cuticles of mature organs can present multiple layers, as well as apparent gradations in the proportions of different lipid-derived components (schematised in Figure 1B). Some even show clear lamellae. These cuticles can reach thicknesses of 10s of microns. Nonetheless, due to the difficulty of standardising fixation and staining protocols, it remains challenging to compare and interpret TEM images from different sources and, more importantly, to attribute chemical signatures to specific zones. Other techniques, such as Raman spectroscopy can be useful in the second endeavour, but lack spatial resolution and are better adapted to thick cuticles. Lipophilic dyes have also been employed to visualise plant cuticles, but again are better adapted to the analysis of the thicker cuticles of mature plant organs.

Cuticle Biosynthesis

The biochemical pathways involved in the synthesis of cuticle components are relatively well understood and, as might be expected, mutants in many of the enzymes involved produce abnormal cuticles with altered chemical compositions and properties. Furthermore, several membrane-localized transporters involved in the export of cuticle components to the cell wall have been characterized. Defects in their function again lead to defects in both cuticle thickness and composition as well as, in some cases, to the intracellular accumulation of cuticle precursors. In contrast, the control, particularly at the subcellular level, of the transport and assembly of cuticle components within the cell wall remains poorly understood. Some evidence exists for the role of specialized molecules called Lipid Transfer Proteins in the transfer of cuticle components to the plant surface. However, exactly how these function, remains unclear. Another hypothesis that has been evoked in the literature proposes that the hydrophobic nature of cuticle components aids in their propulsion across the hydrophilic cell wall spaces and allows them to partition naturally via a phase-separation like process at the organ surface. It is possible that enzymes involved in the crosslinking of the cutin polymer components, such as CUTIN SYNTHASES co-localise with cutin components via a similar mechanism. However, phase separation alone seems a less likely explanation for the de-novo formation of cuticles, for example at the embryo-endosperm interface (see below), and it is

seems likely that a scaffold of crosslinked components must be established during such processes.

The processes of component transport and polymerisation, in addition to cuticle biosynthesis, are absolutely critical for the formation of a physically intact, uniform, and functional cuticle. As a result, they are likely to be key targets of monitoring mechanisms used by plants to assess cuticle properties.

Cuticle Quality Control: How thick is my skin?

The presence of cuticle quality control mechanisms has long been inferred from apparently compensatory changes in cuticle composition observed in mutants compromised in cutin monomer biosynthesis and assembly. These compensatory effects can make the interpretation of mutant phenotypes very challenging. The mechanisms involved are likely to overlap with those implicated in changes in cuticle composition triggered by environmental stimuli, but these also remain poorly understood (discussed below). Recently, the molecular details of an integrity monitoring pathway have been elucidated in the specific developmental context of the developing *Arabidopsis* embryo. Nonetheless, how cuticle properties are perceived by developing plants post-germination, and how this perception is translated into cellular and subcellular responses that can alter cuticle properties at the organ, cell and subcellular level, remains the subject of much speculation. How can a plant epidermal cell assess the integrity and properties of its cuticle?

The diffusion limiting properties of cuticles are determined by their molecular composition, their physical thickness, and their physical integrity. They can therefore be compromised in two main ways: either the cuticle is not completely sealed, or it is deposited irregularly, and presents physical discontinuities (at the subcellular or cellular level). Alternatively, the cuticle is continuous but shows abnormalities in diffusion limiting properties due to an altered monomer composition. It seems likely that mechanisms involved in the perception and rectification of both types of defects are similar.

In the model plant *Arabidopsis thaliana* (and probably in many other Eudicotyledons), the embryo cuticle is formed *de novo* during early stages of embryogenesis by the deposition of discontinuous patches of cuticle material within the cell wall that separates the embryo from the surrounding endosperm. These patches rapidly coalesce into a continuous and very thin cuticle. The coalescence of these patches and their condensation into a continuous structure has been shown to depend on a bidirectional peptide-mediated dialogue that informs the embryo of the physical status of its cuticle. This mechanism relies on the diffusion of a peptide across the cuticle from the embryo to the endosperm (Figure 3A), and back. In the presence of cuticular gaps, an inactive peptide precursor, synthesized by the embryo, can diffuse into the endosperm to be activated by an endosperm-specific protease (Figure 3B). The resulting, active, peptide can then bind and activate receptors located at the embryo surface, promoting gap coalescence, which in turn attenuates signalling when cuticle integrity is achieved. Cuticle integrity/permeability is then subjected to further quality control upon seed germination via a related pathway involving the release of active peptides from the endosperm, and their perception by the embryo. In both cases the “spatial information” informing the embryo of the integrity status of its cuticle is derived from surrounding endosperm tissues.

How peptide perception in the epidermis is translated into targeted cuticle gap filling during early embryogenesis remains an open question. A pathway involving the same or related ligands and receptors, but monitoring different cell wall modifications, has been shown to cause both the local production of Reactive Oxygen Species (ROS) in the cell wall (leading to highly localised alterations in cell wall properties via molecular crosslinking) and the expression of genes involved in cell wall modifications (leading to global changes in the

production of components necessary for cell wall modification). It seems likely that both mechanisms could be implicated in the closure of gaps in the embryonic cuticle. However, in the absence of detailed information regarding embryo cuticle composition and molecular structure, how ROS accumulation in the cell wall might function in local gap filling remains unclear.

Once the cuticle has become established during embryogenesis, it is inherited through the anticlinal division of epidermal cells. At division, each daughter cell inherits its cuticle from its mother. Daughter cells subsequently expand and new material is continuously integrated into the cuticle, maintaining its thickness and barrier properties. How cuticle integrity and barrier function are monitored during organ expansion after germination, when the epidermis is in direct contact with the terrestrial atmosphere (Figure 3C), remains a mystery. In terms of cuticle component biosynthesis, it seems likely that combined tissue level transcriptional responses to developmental (growth/maturation-triggered and/or organ specific) and physiological (such as water loss-triggered) cues, are of key importance. The plant “stress” hormone Abscisic Acid has been proposed to play a key role as an intermediary in cuticle maintenance both under stress conditions (such as drought), and more recently, during growth under non-stress conditions (Figure 3D). However, although such mechanisms explain how cuticle load (thickness) can be maintained during growth and modified in response to environmental cues, they do not explain how cuticle integrity and uniformity at the subcellular level are achieved.

Intriguingly, defects in cuticle biogenesis that lead to increased permeability have been shown to lead to the release of ROS from epidermal surfaces. This may indicate that similar molecular mechanisms are involved in ensuring integrity and uniformity of the post germination cuticle at the subcellular level to those at play in the embryo (Figure 3D). Nonetheless the precise nature of the cues involved, which presumably originate in the environment, remains to be elucidated. A further important question revolves around how a potentially generic monitoring mechanism can be modulated, either in terms of sensitivity, or in terms of molecular output, to lead to different outcomes in terms of cuticle structure and permeability between plants and between different organs of the same plant.

In summary: the plant cuticle is critical for the survival of multicellular plants in drying, terrestrial environment. In Angiosperms, the cuticle has evolved a bewildering array of functions, achieved by dramatic variations in structure composition and biophysical properties, both within single species and between species. Understanding of how coherent cuticle deposition, and uniform biophysical properties are achieved in these diverse contexts lags well behind other fields in plant biology. Technological advances allowing the analysis of cuticle structure, composition and biophysical properties will be required. It is also possible that returning to the roots of cuticle evolution, by studying the biology of the cuticles formed by relatives of early emerging land plants (such as extant bryophytes), could provide valuable clues to advance understanding of these questions.

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Figure Legends

Figure 1

A) Leaf surface with open stomatal pore flanked by Guard Cells (GC). Gas exchange is indicated. Epidermal Cell (EC), Mesophyll Cell (MC). **B)** Expanded view of epidermal surface

showing cuticle (with cutin matrix indicated as grey strands), underlying cell wall (with cell wall polymers indicated a green strands). Phenolic monomers are indicated in red.

Figure 2

A) Gametophore of the model moss *Physcomitrium patens*. **B)** Ivy leaf with waxy water-repellent surface.

Figure 3

A) Developing Arabidopsis embryo with surrounding seed tissues indicated. **B)** Integrity monitoring and gap-filling in the nascent embryo cuticle. Peptides in the cell wall are shown as blue bars, with inhibitory domains shown in red. The endosperm-localised peptide-activating protease is shown as an orange oval. Membrane-localised peptide-receptors are shown in black, the nascent cuticle in yellow and the cell wall in green. **C)** Fresh hand-section of a mature ivy leaf. **D)** Proposed mechanisms involved in the reinforcement of leaves during their development. Colours as in B). ABA, Abscisic Acid. ROS, Reactive Oxygen Species.

Suggestions for further reading:

(1–15)

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Figure 1

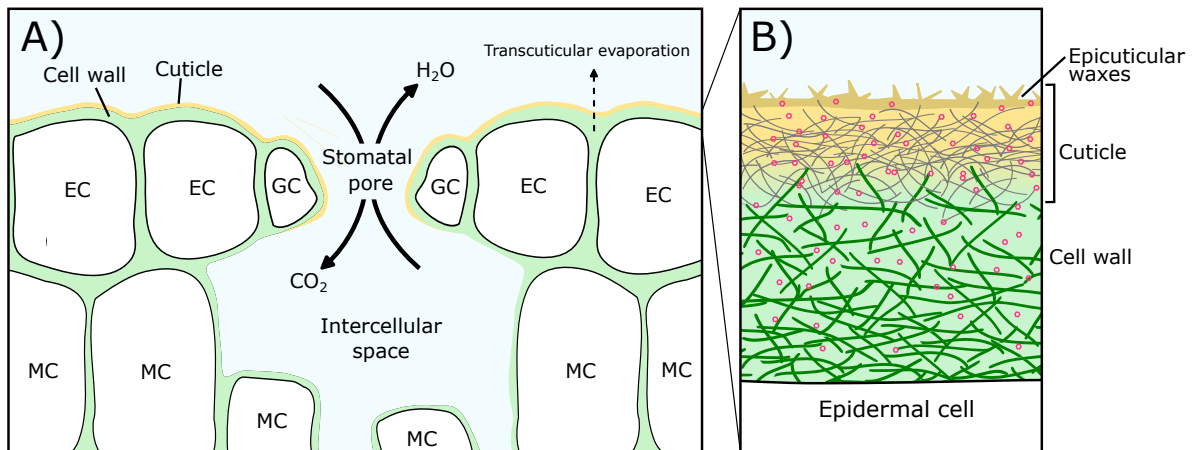


Figure 2

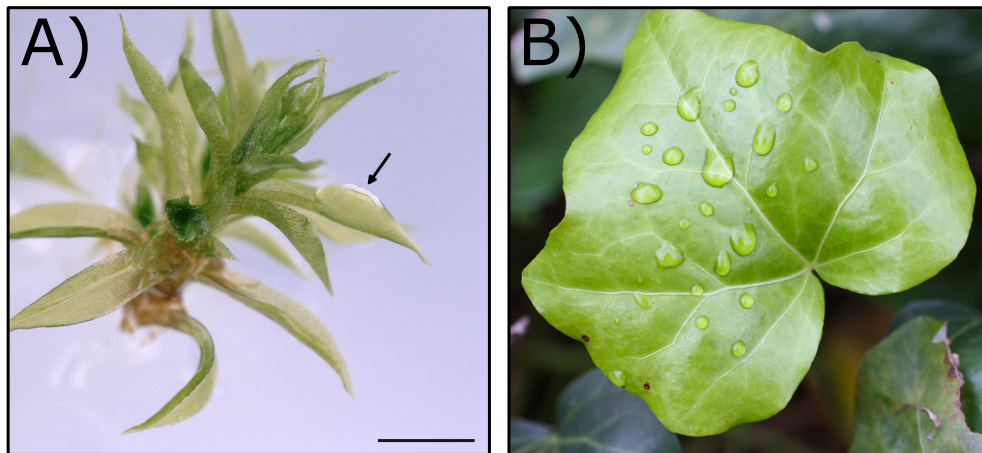


Figure 3

