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The *C. elegans* epidermis as a model skin. II: differentiation and physiological roles

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Abstract

The *C. elegans* epidermis forms one of the principal barrier epithelia of the animal. Differentiation of the epidermis begins in mid embryogenesis and involves apical-basal polarization of the cytoskeletal and secretory systems as well as cellular junction formation. Secretion of the external cuticle layers is one of the major developmental and physiological specializations of the epidermal epithelium. The four post-embryonic larval stages are separated by periodic moults, in which the epidermis generates a new cuticle with stage-specific characteristics. The differentiated epidermis also plays key roles in endocrine signaling, fat storage, and ionic homeostasis. The epidermis is intimately associated with the development and function of the nervous system, and may have glial-like roles in modulating neuronal function. The epidermis provides passive and active defenses against skin-penetrating pathogens and can repair small wounds. Finally, age-dependent deterioration of the epidermis is a prominent feature of aging and may affect organismal aging and lifespan.

Keywords

EPITHELIA; MORPHOGENESIS; PAR PROTEINS; barrier epithelia; wound healing

INTRODUCTION TO PART II

The first of these reviews addressed the anatomy and development of the *C. elegans* epidermis. Here we focus on the differentiation of the epidermis as a polarized barrier epithelium, its intimate relationship with the external cuticle, and its many roles in the physiology of the developing and mature worm.

THE EPIDERMIS AS A POLARIZED EPITHELIUM

The epidermis is one of the principal epithelia of the *C. elegans* body, and has provided important insights into epithelial differentiation. The epidermal cytoskeleton, junctional complexes, and apical basal polarity have been extensively reviewed^{1–3} and are summarized here (FIGURE 1).

Junctional complexes and polarity—Epidermal cells have provided an excellent model for understanding the development and function of junctions in epithelialization. Two major subapical junctional complexes play partly redundant functions in epidermal adhesion, the apical-most cadherin-catenin complex (CCC) and the more subapical DLG-1/AJM-1 complex (DAC)¹; together these complexes make up the so-called *C. elegans* apical junction (CeAJ) in mature epidermal cells. In early embryos the CCC is widely expressed and localized at all sites of cell-cell contact (FIGURE 1A); the CCC functions redundantly with the adhesion molecule SAX-7/LICAM in early blastomere adhesion⁴. The first signs of epithelial differentiation in epidermal precursors are the punctate expression of DAC components AJM-1 and DLG-1 in the dorsal epidermal cells (FIGURE 4A in Part I). Both the CCC and DAC are initially distributed along the lateral sides of epidermal cells. Polarization begins with the redistribution of the CCC and DAC components to subapical locations.

Epithelial polarization involves several pathways, including the basolateral protein LET-413/Scribble⁵ and the apical PAR-3/6 complex. LET-413 is uniformly expressed in early embryos; in differentiating epidermal cells it becomes basolaterally localized and serves to confine the AJ components to subapical domains. The PAR-3/6 complex is critical for polarization in many cell types, including the early embryo, complicating efforts to examine its role in the epidermis. Recent elegant experiments have revealed that neither PAR-6 nor PAR-3 are essential for epidermal polarity^{6,7}. PAR-6 is required for maturation of apical junctions, independent of PAR-3; PAR-3 has a more restricted role in epidermal development⁷. As epidermal cells are generated in an intrinsically asymmetric context, in that they contact other cells only on their basal and lateral sides, they may be less dependent on polarity mechanisms such as the PAR genes. In late gastrulation laminin deposition on the basal surface of the epidermis begins to establish the epidermal basal lamina⁸; the role of the BL in epidermal polarization has yet to be precisely established.

The CCC and DAC can assemble independently of one another^{9,10}, suggesting neither complex is individually essential for initial epidermal polarity. Embryos lacking the CCC generate a motile epidermal epithelium but fail to establish stable adhesion in late epidermal enclosure¹¹. Conversely, the DAC does not become essential until the 2-fold stage. Embryos lacking LET-413/Scribble display highly disorganized junctions yet develop to the 1.7-fold stage⁵. Embryos lacking components of both the CCC and DAC show synergistic effects on adhesion, suggesting partial redundancy between the functions of the two complexes. It is unclear whether in such embryos the epidermis fails to polarize, or whether it initiates but fails to maintain polarization.

Apical junctions play critical roles later in embryonic morphogenesis. For example, the CCC links the circumferential actin bundles, allowing force transmission during epidermal elongation¹². Other modulatory CCC components such as JAC-1 are not individually essential for elongation, but their loss can exacerbate defects due to partial loss of CCC function¹³. The CCC also localizes further junctional molecules such as the claudin VAB-9, itself required for normal epidermal elongation¹⁴. Junctions are also highly dynamic: during ventral enclosure new junctions rapidly form at newly established cell contacts at the ventral midline, and then in the case of the posterior leading cells appear to be almost immediately disassembled as the two cells fuse. Indeed, epidermal cells that fuse with syncytia tend to be connected by apical junctions, with a few exceptions¹⁵.

The epidermal cytoskeleton—The epidermis is richly endowed with cytoskeletal structures, many of which are critical for cell shape changes in the embryo. Indeed, the epidermis is one of the few tissues in which the interplay between actin microfilaments,

microtubules, and cytoplasmic intermediate filaments (cIFs) can be studied in depth; the cIF cytoskeleton is considered in the next section.

The differentiated epidermal actin cytoskeleton alternates between a disordered meshwork of filaments and a set of highly organized circumferential bundles that form hoop-like structures along the body¹⁶. Actomyosin-based contraction of circumferential actin bundles within seam cells is essential for the cell shape changes of epidermal elongation (see below). Within the dorsal and ventral epidermis, although not in the seam, actin bundles influence the position of secretory vesicles and correlate with the eventual formation of circumferential cuticle furrows¹⁷. The cortical actin cytoskeleton is also critical for epidermal cell shape changes; association of cortical actin with the apical membrane is mediated by α -spectrin SPC-1¹⁸ and β_H spectrin SMA-1^{19,20}. Anchoring of nuclei to stable positions within epidermal syncytia is mediated by the giant protein ANC-1, which tethers nuclei to the actin cytoskeleton²¹.

The epidermal microtubule cytoskeleton is important for dorsal intercalation movements²² and for the contralateral migrations of nuclei in the dorsal epidermis²³. Imaging of MT dynamics in the dorsal epidermis has established that during dorsal intercalation the epidermal MT arrays reorganize such that their plus ends grow to the new tip of the intercalated cell (FIGURE 1B). The plus-end directed motor kinesin-1 is required for nuclear migrations and is recruited to the nuclear envelope by the UNC-83 and UNC-84 protein complex²³. Pharmacological inhibition of MT assembly also disrupts epidermal elongation, suggesting a later role for MTs in distributing the tension generated by actomyosin contraction¹⁶.

Trans-epidermal attachments: the epidermis as tendon—A key function of the epidermis is to provide a mechanically strong connection between body wall muscles and the external cuticle^{24,25}. This tendon-like role is mediated by specific structures in the cIF cytoskeleton known as trans-epidermal attachments (TEAs) or fibrous organelles²⁶. Each TEA consists of two hemidesmosome-like junctional plaques connected by an array of intermediate filaments (pink lines in FIGURE 1B). The junctional plaques contain the spectraplakins VAB-10A²⁷. The VAB-10 spectraplakins also provide cross-links between the actin, microtubule and intermediate filament cytoskeletons. *C. elegans* encodes a large family of cytoskeletal intermediate filaments (cIFs)²⁸, several of which are required in the epidermis and appear functionally analogous to vertebrate skin keratins^{29,30}. cIF assembly and function is tightly regulated by several mechanisms, including post-translational modification by SUMO³¹. Intracellular components of TEAs also include the conserved ankyrin domain protein VAB-19³², its interacting partner, the PTB domain protein EPS-8³³, and the nematode-specific protein PAT-12³⁴. All these components are essential for later epidermal elongation and muscle attachment.

The junctional plaques of TEAs are themselves linked to transmembrane proteins at the apical and basal plasma membranes: at the basal plaque, Myotactin/LET-805 functions to link epidermal cells to the muscle-adjacent basal lamina³⁵; at the apical plaque, the filaggrin-related transmembrane proteins MUA-3³⁶ and MUP-4³⁷ link the epidermis to the cuticle. The recruitment and maturation of TEAs has been a tractable genetic model for hemidesmosome biogenesis³⁸.

Epidermal TEAs are essential for muscle attachment and function, and are also essential for epidermal elongation beyond the two-fold stage. Epidermal elongation beyond the two-fold stage has long been known to require the contractile activity of the body wall muscles that attach to TEAs³⁹. The mechanism by which muscle contraction promotes epidermal cell shape changes has recently been elucidated and involves a tension-sensing process mediated

by a p21-activated kinase pathway acting at TEAs⁴⁰. TEAs thus not only function as tendon-like attachments but also play mechanosensory roles.

The epidermal basal lamina and interactions with internal tissues

The basal (inner) surface of the epidermis is lined by a basal lamina (BL) that maintains epithelial integrity, allows adhesion between epidermis and muscles, and provides a permissive and instructive substrate for cell and axon migrations (FIGURE 2A,B). In the absence of BL components such as laminins the epidermis differentiates as a polarized epithelium but intermingles and adheres to other internal epithelia⁸.

Biogenesis of the basal lamina—The epidermal BL separates ectodermal tissues (epidermis and neurons) from mesoderm, endoderm and the pseudocoelomic body cavity. As in other animals, laminins are the earliest components of the BL, and begin to be deposited during gastrulation. Interestingly, several BL components, including laminins, type IV collagens⁴¹, and F-spondin/SPON-1⁴², are expressed by muscles and not by the epidermis, and are required for later epidermal elongation. Recruitment of such proteins to the epidermal BL may involve epidermal cell-matrix receptors such as dystroglycan/DGN-1⁴³. However the mechanisms that target nascent BL to specific cell surfaces are not yet well understood in *C. elegans*. The BL that forms the tight mechanical linkage between body muscles and epidermis appears to be a fusion of the muscle and epidermal BL.

The epidermis itself makes a subset of BL components. The extracellular matrix peroxidasins PXN-1 and PXN-2 are expressed in the epidermis, and PXN-2 at least is likely to cross-link BL target proteins⁴⁴. Fibulin/FBL-1 is expressed both by muscles and the epidermis⁴⁵. UNC-52/Perlecan is expressed by the epidermis; multiple UNC-52 isoforms are generated by MEC-8-dependent alternative splicing of *unc-52*⁴⁶. *unc-52* mutants with stop codons in alternatively spliced exons display normal embryonic development but are synthetic-lethal with *mec-8*. *mec-8* itself is not essential for epidermal development but displays synthetic lethality with several *sym* genes⁴⁷, some of which are expressed and function in the epidermis⁴⁸. MEC-8-dependent alternative splicing appears to have an important yet cryptic role in epidermal differentiation.

The dynamics of the BL during embryogenesis remain incompletely understood. During epidermal elongation the BL is likely highly dynamic, accommodating the overall change in shape of the animal. Little is known of how the BL remodels during elongation while retaining tight mechanical coupling between muscles and epidermis.

The epidermis as a source of cues for patterning other tissues

The epidermis and development of the nervous system—The development of the epidermis and the nervous system are intimately connected. As discussed above, epidermal enclosure involves migration of epidermal epithelial cells over a largely neuronal substrate. Later, the epidermis provides a substrate for outgrowth and guidance of axons in the developing nervous system^{49, 50}. Almost all neurons extend axons in the compartment between the epidermis and the basal lamina (BL). The excretory cell also develops in this compartment (FIGURE 2A).

The dorsal and ventral midline regions of the epidermis form 'ridges' adjacent to the dorsal and ventral nerve cords^{51, 52}. Ridges are subcellular epidermal protrusions between regions of muscle attachment, and appear to express distinct molecular determinants⁵³. The dorsal epidermal ridge develops during embryogenesis whereas the ventral ridge only becomes prominent in larval development. Although initially symmetrical, both ridges become left-right asymmetric: the ventral ridge confines axons into a large right-hand bundle and a much

smaller left-hand bundle; a few axons normally cross the epidermal ridge at defined positions. Conversely the dorsal ridge constrains axons to the left-hand side. The asymmetry of the ventral nerve cord appears to arise from initially asymmetric outgrowth of pioneer axons, and is only later reinforced by the epidermal ridge. It is unknown if the left-right asymmetry of the ridges is intrinsic to the epidermis, which otherwise appears bilaterally symmetrical⁵⁴. In larval development axons maintain their positions on either side of the ventral epidermal ridge; screens for mutants with ectopic axonal crossovers have identified many factors involved in maintenance of axon position at the midline. Among these, heparan sulfated proteoglycans and the FGF receptor EGL-15 are expressed in the epidermis^{55,56}. The epidermal function of EGL-15 is kinase-dependent and involves the Ras pathway.

The epidermal BL appears to be permissive for the migrations of axonal growth cones and other migratory cells in the developing animal, and forms a matrix for instructive guidance cues⁵⁷. For example, ventral epidermal (P) cells are sources of netrin/UNC-6 that repels or attracts growth cones in the dorsoventral axis⁵⁸. Other proteins such as the ADAM UNC-71 may be secreted by the epidermis and act as short-range permissive factors for axon guidance⁵⁹. Although the BL is important for guidance, in many cases the growth substrate for developing axons appears to be the epidermis itself rather than its BL (FIGURE 2C; R. Durbin, Ph.D. thesis, WormAtlas). Motor neuron growth cones insert 'fingers' into the dorsal epidermal ridge (Durbin thesis, p. 69), suggesting that commissural growth cones stop at the dorsal midline not simply because they have reached the bottom of a concentration gradient but also because some attractive factor keeps them there.

Epidermal cells also provide localized cues for axonal branching and synaptogenesis, most intensively analyzed in the developing vulva. Vulval epidermal cells express the neurexin-like protein BAM-2, which locally represses branching of VC neurons⁶⁰. Another VC branch-inhibiting factor, the N-glycanase PNG-1 may also have an epidermal focus⁶¹. Vulval epidermal cells express the nephrin SYG-2, a local 'guidepost' cue that enhances branching and synaptogenesis by the HSN neuron⁶².

The epidermis and internal tissues—The epidermal BL provides substrate and guidance cues for migrations of a variety of mesodermal cells and tissues. In larval stages the somatic gonad and ventral epidermis undergo multiple reciprocal interactions during vulval development. Somatic gonadal cells first induce vulval differentiation in the ventral epidermis. Later the attachment of the uterine and vulval epithelia requires removal of the intervening BL⁶³. Epidermally expressed cues trigger the invasion of the BL by the gonadal anchor cell, and actively constrain the extent by which the BL is remodeled⁶⁴.

The epidermis is both source and recipient of Wnt signals throughout development. Wnt signals control polarity and patterning of seam cells and other dividing cells within the epidermal layer^{65,66}. Epidermally expressed Wnts instruct migrations of neurons and neuroblasts over the epidermis⁶⁷, and the guidance and polarity of axons along the anteroposterior axis⁶⁸. Wnts themselves are expressed by subsets of epidermal cells: EGL-20 is expressed in posterior ventral and rectal epidermal cells⁶⁹ and LIN-44 is expressed in tail epidermis⁷⁰.

CUTICLE STRUCTURE, SECRETION, AND MOLTING

Structure of the cuticle

The true external surfaces of animals are generally not skin cells but the extracellular matrices secreted by skin cells. These matrices can consist of dead skin cells encased in a lipid-rich matrix (stratum corneum of mammals), or rigid extracellular chitinous cuticles

(arthropods). In nematodes the extracellular layers consist of a flexible collagenous cuticle, a lipid rich epicuticle, and a glycoprotein-rich surface coat (FIGURE 3). All of these extracellular structures play important roles in the integrity of the animal and its interactions with the environment. The cuticle is not a rigid exoskeleton, but acts in concert with muscle tone and the hydrostatic skeleton to maintain the animal's shape (see BOX). However the cuticle has limits to its flexibility, and is shed four times to accommodate larval growth.

BOX 1

The epidermis and the 'hydrostatic skeleton'

C. elegans has few rigid skeletal structures. It has long been thought that forces from muscle contraction can be transmitted because internal fluids and tissues are under hydrostatic pressure, the 'hydrostatic skeleton'. The internal pressure of *Ascaris* was measured to be between 70–225 mm Hg²⁰⁵ and *C. elegans* may be similar. Thus, a sufficiently drastic puncture wound can lead to flaccidity and death. However *C. elegans* can seal and repair small wounds such as those caused by microinjection needles¹⁵⁹. Needle wounds have been found to cause a relatively modest (20%) decrease in body stiffness²⁰⁶, suggesting the shape of the nematode body mostly reflects cuticle stiffness and that hydrostatic pressure has a secondary role. *C. elegans* body stiffness thus results from a combination of cuticle mechanics, muscle tone, and internal pressure²⁰⁷. The relative importance of these mechanisms likely varies between species; for example, in some nematodes the epidermis itself has been proposed to act as a compartmentalized hydrostatic skeleton²⁰⁸.

The collagenous cuticle—The structure and composition of the cuticle have been reviewed extensively⁷¹ and are briefly summarized here. Collagens make up the bulk of the soluble protein component of the cuticle. *C. elegans* cuticle collagens form a multigene family with ~170 members. A small subset of collagen genes were identified from their mutant phenotypes (Dumpy, Blistered, Roller, Squat, Long, Ray morphology). RNAi of most collagen genes does not cause overt defects; collagens may have highly redundant roles in development. A distinct protein family, the cuticlins (see ^{Note}), makes up the insoluble fraction of the cuticle^{72–74}. Cuticlins are important for formation of patterned structures within the cuticle such as the lateral ridges or alae. In contrast to the external cuticle produced by the epidermis, the more rigid cuticle lining the pharyngeal lumen contains chitin⁷⁵.

Collagens and cuticlin genes are expressed either in the syncytial epidermis or in the lateral seam cells or in both, and undergo extensive post-translational processing in the epidermal secretory pathway. Collagens and noncollagenous proteins are extensively crosslinked by peroxidases secreted by the epidermis^{76,77}. The cuticle is built according to a common 'ground plan' that is modified in each stage by expression of stage-specific components. Some collagens are expressed in all larval stages, in cyclic patterns associated with cuticle synthesis at each molt, whereas others are highly stage-specific (e.g. *col-19* is only expressed in the L4 and adult). Collagen genes have been classified as early, intermediate, or late with respect to their expression peak in each stage⁷⁸. Early and intermediate collagens form distinct substructures in the cuticle⁷⁹, suggesting that tightly regulated transcription allows components of a common structure to assemble most efficiently. Along these lines, it

¹Cuticlins (non-collagenous proteins of the nematode cuticle) should not be confused with cuticulin, the outermost layer of the insect epicuticle. Nematode and insect epicuticle layers are both thought to be lipid-rich, are deposited early in synthesis of new cuticles, and likely have permeability barrier function. It is not known whether nematode and insect epicuticles share any other similarities in terms of biogenesis or molecular composition.

seems plausible that cuticle would be synthesized in an outside-in sequence, but this has not yet been established.

Some collagens such as *dpy-7* may be directly regulated by the epidermal GATA factors⁸⁰ and by NHRs⁸¹. Indeed, expression of NHR-23 and NHR-25 is also staggered within each intermolt⁸², although it is unclear if this is sufficient to explain the sequence of collagen expression. Other transcription factors implicated in collagen transcription include ELL-1⁸³ and LIN-29, which directly activates the L4-specific *col-17/19* loci^{84,85}. However a systematic understanding of collagen transcription within the epidermis remains lacking.

The epicuticle and surface coat—The epicuticle and surface coat are the two outermost layers of the cuticle (FIGURE 3A). In EM sections the epicuticle has a trilaminar structure resembling a thickened plasma membrane. The membrane-like properties of the epicuticle suggest the cuticle should be seen as a dynamic, homeostatic acellular compartment rather than an inert exoskeleton⁸⁶. The epicuticle itself is covered by an outer layer known as the surface coat (or glycocalyx), sometimes visible as a thin amorphous layer in electron micrographs. The molecular biology of the epicuticle and surface coat remain very poorly understood. Freeze fracture studies of the *C. elegans* epicuticle are consistent with a lipid bilayer in which glycoproteins are embedded⁸⁷. The surface coat is also thought to be composed of glycoproteins such as lectins and mucins; its composition varies during development, perhaps to allow evasion of pathogens⁸⁸. Mutants defective in the surface coat have been identified via aberrant expression of surface antigens⁸⁹, altered lectin binding⁹⁰, or defects in bacterial adhesion^{91,92}. Some of these genes (e.g. *bah-1*)⁹³ could encode components of the epicuticle or surface coat, whereas others encode carbohydrate biosynthetic enzymes likely required for post-translational modification of coat proteins⁹⁴. The surface coat and epicuticle pose a challenge to genetic analysis as individual components may be redundant or have subtle roles under lab conditions.

The epicuticle is synthesized by the epidermis, as the first step in formation of a new cuticle at a molt. The embryonic sheath resembles the epicuticle in ultrastructure and may be the precursor to the epicuticle of the L1. The cellular origin of the surface coat is less clear. Studies of other nematodes have suggested that excretory or pharyngeal gland cells secrete surface coat that spreads over the cuticle surface. On the other hand, several *bus* genes, likely involved in surface coat or epicuticle biosynthesis, are expressed in epidermal seam cells and not in the syncytial epidermis⁹⁴. Conversely many cuticular collagens are expressed by syncytial epidermis and not seam. These observations raise the conundrum of how the seam and non-seam epidermis coordinate the production of the various cuticle compartments. ‘Trans-cuticular’ transport mechanisms or channels for surface coat components have been reported in parasitic nematodes but not in *C. elegans*. Both mechanisms may apply: seam cells could be responsible for surface coat production during a molt whereas glands could maintain a flow of surface coat material in intermolt or adult periods.

Secretion of the embryonic sheath and first cuticle—Prior to formation of the skin the developing embryo is confined within a eggshell secreted by the zygote after fertilization. The developing epidermis generates an external extracellular matrix, the embryonic sheath, shortly after enclosure; this embryonic sheath is essential for epidermal elongation¹⁶. In its earliest state the sheath is a thin sheet that contacts the epidermal membrane close to the circumferential actin bundles discussed above. The composition of the early sheath is unknown; it later develops into a trilaminar lipid-rich layer¹⁷ that resembles the larval epicuticle. Lipid-binding proteins expressed by the embryonic epidermis could be important in formation of the sheath⁹⁵; a family of leucine-rich repeat proteins secreted by the embryonic epidermis may also play roles in epicuticle biogenesis⁹⁶.

The embryonic sheath, unlike the cuticle, must be able to accommodate rapid radial contraction and axial elongation. The circumferential actin bundles in the epidermis correlate with circumferential furrows in the sheath and in the later cuticle; secretion of the sheath and cuticle is somehow patterned by the epidermal cytoskeleton. The collagenous cuticle of the L1 stage is not deposited until late embryogenesis, after elongation, and is essential for the embryo to retain its fully elongated form. Mutants lacking cuticle collagens such as *sqt-3* undergo normal elongation to the threefold stage then retract to an almost un-elongated length. Loss of function in the secretory pathway blocks embryogenesis late in elongation, likely reflecting a failure in cuticle secretion⁹⁷.

The epidermal secretory apparatus—In keeping with its massive secretory needs, the epidermis is richly endowed with specialized secretory structures and organelles at its apical surface (FIGURE 3B). Golgi bodies are extremely abundant in epidermal cells, especially during cuticle synthesis at molts. Secretion of collagen and other cuticle components seems likely to be via the classical secretory pathway (ER-Golgi-post-Golgi vesicles); for example loss of function in *sec-23* (involved in ER-Golgi transport) blocks development due to failure of cuticle secretion. Collagen biosynthesis involves extensive post-translational modifications. By analogy with vertebrate fibrillar collagen biosynthesis, early modifications such as prolyl 4-hydroxylation and trimerization are likely to occur in the epidermal ER. Later steps in collagen processing and crosslinking presumably occur after secretion into the cuticle compartment⁷¹.

Vesicles with electron-dense cores are seen in the epidermis prior to molting⁹⁸; the molecular identity of these vesicles remains elusive. Genes critical for biosynthesis of neuronal dense-core vesicles, such as *unc-31/CAPS* or *unc-108/Rab2*, do not appear to act in the epidermis, suggesting the epidermal vesicles could represent a distinct secretory pathway. In contrast, secretion of hedgehog-related proteins uses an exosome-based mechanism. The epidermis contains numerous multivesicular bodies (MVBs)^{99, 100}, required for apical secretion of proteins such as WRT-2 and WRT-8¹⁰¹. The V0 ATPase component VHA-5 is involved in MVB secretion, possibly in fusion of MVBs with the apical membrane.

In addition to these better-known secretory structures, other epidermis-specific organelles have been observed, of uncertain function. The apical epidermal membrane is frequently infolded into stacked sheets up to 1 μm in diameter, associated with ribosome-like particles on their cytoplasmic faces. The resulting increased surface area might enhance apical secretion or uptake. Interestingly, the V0 ATPase subunit VHA-5, required for MVB secretion, is enriched on apical membrane stacks⁹⁹; perhaps membrane stacks boost recycling of secretory components that have fused with the apical membrane. Finally epidermal cells also contain membrane-bound ellipsoidal organelles containing stacked membrane-like sheets ('Ward bodies'; see Figure 2A in⁹⁸). The function of Ward bodies is unknown; speculatively, they could play roles in cuticle lipid (epicuticle) secretion, analogous to the lamellar bodies of vertebrate keratinocytes.

Molting

Molting, the periodic shedding and resynthesis of the cuticle to accommodate growth, is a central feature of the nematode life cycle¹⁰². Molecular analysis of *C. elegans* molting has begun to uncover mechanistic similarities with other kinds of molting such as those of arthropods.

Execution of molting: apolysis, cuticle synthesis, and ecdysis—Each molt involves three stages: separation of the old cuticle (apolysis), synthesis of new cuticle, and

shedding of the old cuticle (ecdysis) (FIGURE 4A). The overall timing and coordination of molting are likely under the control of as-yet unknown endocrine steroid hormones acting via epidermal nuclear hormone receptors that include NHR-23 and NHR-25¹⁰³. *C. elegans* requires dietary cholesterol¹⁰⁴, most likely for synthesis of steroid hormones involved in molting; epidermal uptake of cholesterol by the cholesterol receptor LRP-1 is essential for viability¹⁰⁵. The roles of other cholesterol trafficking proteins in molting^{106, 107} may be explained by the need for cholesterol in steroid hormone synthesis.

Within a given larval stage molting is tightly regulated to occur at the correct time relative to other developmental events. Insight into how the molting cycle is integrated with other developmental processes came from the finding that inappropriate activation of nicotinic acetylcholine receptors delays developmental events but does not affect onset of molting, leading to lethality¹⁰⁸. This uncoupling of developmental events and molting cycles requires activity of nAChRs, DAF-12 and the epidermally expressed cation transporter CATP-1¹⁰⁹.

The first phase in molting, apolysis, allows the new cuticle to be synthesised between the epidermis and the old cuticle. Separation of the old cuticle from the epidermis requires the elimination of linkages between the epidermis and the cuticle matrix. Interactions between epidermal receptors and the cuticle could be broken by several mechanisms, including downregulation of the receptors, alteration of cuticle composition, or degradation of the receptor-ligand complexes. Epidermally expressed thioredoxin and glutathione reductases are required for molting¹¹⁰. These enzymes have been proposed to generate an intracellular pool of GSH that is released to the extracellular milieu at apolysis. The resulting reduction of disulfide bonds within the cuticle could disrupt its attachment to the epidermis. Apolysis likely involves large-scale proteolysis of cuticle-epidermal attachments, and a variety of peptidases and proteases have been implicated in molting¹¹¹, including the cathepsin-like protease CPZ-1¹¹². Activity of such proteases must be under stringent control so as not to interfere with the synthesis of new cuticle; at least two serine protease inhibitors (serpins), BLI-5¹¹³ and MLT-11¹⁰², are required for molting.

The final stage in molting is ecdysis, the shedding and escape from old cuticle. *C. elegans* ecdysis involves a complex set of behaviors that include rotation, body contraction and thrusting, and regurgitation of pharyngeal cuticle. Together these result in mechanical disruption of any remaining linkage between old and new cuticle. The astacin metalloprotease NAS-37 is localized to the old cuticle and may be specifically involved in its degradation at the tip of the head during ecdysis¹¹⁴; the related protease NAS-36 is also involved in ecdysis¹¹⁵. Nematode-specific proteins such as MLT-10 and its relatives are important for cuticle synthesis and ecdysis although their biochemical functions are not yet known¹¹⁶.

Hedgehog-related signals appear to play multiple roles in epidermal development, including molting. The epidermis secretes many members of the Warthog, Groundhog families of Hedgehog-related secreted proteins^{117, 118}. Several of these, including the quahog gene *qua-1*, are expressed cyclically, peaking during molts¹¹⁹. QUA-1 is a candidate for a secreted cue to initiate ecdysis and is localized to the detaching cuticle; it may have other roles in cuticle development, as *qua-1* mutant L1 cuticles, which have not experienced a molt, are structurally abnormal¹¹⁹. Hh protein distribution can be influenced by HS proteoglycans, and indeed HSPG synthetic genes such as PST-1 are expressed in the epidermis and required for cuticle integrity¹²⁰. The epidermis expresses several Patched and Patched-related receptors¹²¹, raising the possibility of autocrine Hh signaling within the epidermis. Other signaling proteins such as the amyloid precursor protein APL-1 are expressed in the epidermis and required for molting^{122, 123}.

Genetic regulation of molting: the molting cycle and heterochronic genes—

Molting has been postulated to involve two distinct yet coordinated sets of regulatory controls (FIGURE 4B). First, a ‘molting oscillator’ has been proposed to initiate molts in a periodic fashion. Second, stage-specificity could be imposed by heterochronic genes, which ensure that each larval stage has the appropriate cuticle type. Certain heterochronic mutants lose stage-specific characters but continue to molt, indicating that stage progression can be uncoupled from the molt cycle. These inputs together trigger the processes that execute molting.

Several genes are known whose expression oscillates in a periodic fashion, peaking during each molt, including the nuclear hormone receptors NHR-23, NHR-25, and the primary transcript of the miRNA *let-7*¹²⁴. It remains unknown how the oscillations of these transcripts are related; although *let-7* has been shown to negatively regulate the NHRs at the larval to adult transition¹²⁵, mature *let-7* transcripts do not accumulate in earlier molts, suggesting other mechanisms must be responsible for periodic NHR transcript accumulation. The PERIOD protein LIN-42A has recently been found to be a critical component of a molting oscillator¹²⁶. *lin-42* mRNA levels were known to oscillate in larval cycles¹²⁷. The *lin-42* locus encodes multiple isoforms; loss of function in some of these isoforms results in heterochronic (but not molting) defects¹²⁷. Recent findings indicate that complete loss of function in the *lin-42* locus results in delayed or arrhythmic molts; conversely overexpression of the LIN-42A isoform triggers anachronistic molts¹²⁶, suggesting that LIN-42A is necessary and sufficient to initiate the molting program. The relationship of LIN-42A to the other cycling genes remains to be defined; NHR-25 appears to act partly in parallel to LIN-42A.

LIN-42 is a member of the PERIOD family of transcription factors, originally known from their roles in circadian rhythms in *Drosophila* and mammals. Although the *C. elegans* molting cycle is ultradian (8–10 h period), these results suggest the possibility of mechanistic similarities between the molting cycle and other biological clocks. Many biological oscillators are based around negative feedback loops, and indeed *let-7* and the DAF-12 steroid hormone receptor comprise a negative feedback loop^{128, 129}. The coupling of stage-specific phenotypes and the molting cycle could reflect coordinate regulation of molting genes by miRNAs also involved in heterochronic progression¹²⁵, although this remains to be shown. It seems likely that the mechanistic basis of the *C. elegans* molting oscillator and its interconnections with the heterochronic genes will be soon elucidated.

Cessation of molting—The L4-to-adult molt is the last in the normal life cycle. How is the molting oscillator switched off? A key upstream event in the larval-to-adult transition appears to be a massive upregulation of *let-7* and related miRNAs, resulting in strong inhibition of the molting factors such as NHR-23 and NHR-25¹²⁵. Loss of function in *let-7*, or in other factors required for the larval-to-adult transition, results in supernumerary molts that require NHR-23 and -25. These supernumerary molts may differ from normal molts; for example, NHR-23 and -25 are required for lethargus in *let-7* supernumerary molts but are not required for lethargus of regular molts¹³⁰. Other molting regulators such as *pqn-47*, have complex requirements: PQN-47 is required for ecdysis, but partial loss of *pqn-47* function results in a supernumerary adult molt¹³¹.

The systemic nature of molting—Where does the molting oscillator reside in the animal? LIN-42A expression in seam cells is sufficient to rescue *lin-42* molting defects, suggesting the seam is critical for molting cues¹²⁶. However the seam must synchronize with *hyp7* and other epidermal cells (FIGURE 4C). The seam is directly adjacent to *hyp7*, and is connected to it via gap junctions¹³², affording a variety of possibilities for regulation. Seam-derived cuticular structures such as alae are overtly stage-specific; however *hyp7* also

displays stage specific characteristics. For example, stage-specific collagens are expressed throughout the epidermis. As *hyp7* is post-mitotic it is interesting to imagine how it adopts these stage-specific features.

As well as cuticle synthesis and removal, molts involve highly stereotyped behaviors, implying the coordinated regulation of multiple tissues. During most of the molt animals enter a quiescent sleep-like phase known as lethargus, regulated by the EGF family protein LIN-3¹³³. LIN-3 activity may be induced by LIN-42A¹²⁶, and appears to act on a single neuron, ALA, which may play a neuroendocrine role in inducing lethargus. ALA neuronal processes are directly adjacent to seam cells. A second pathway regulating lethargus during molting involves OSM-7 and OSM-11, which are secreted by epidermal seam cells and act on neuronally expressed Notch family receptors to promote quiescence¹³⁴. Thus, at least two signals may originate in the epidermis to induce behavioral quiescence during molting.

In addition, muscle activity itself has been implicated in molting control. Loss of function in components of the muscle dense body or the BL results in upregulation of molting-related genes in the epidermis¹³⁵. Muscle contractions are inhibited during lethargus, perhaps to avoid rupturing the epidermis while it is unattached to cuticle; during molts muscles also autonomously downregulate expression of attachment site components¹³⁶. Such reduction in muscle-generated force could be sufficient to affect expression of molting related genes, but mechanisms underlying this non-autonomous regulation of epidermal gene expression are not yet known.

The epidermis and cuticle of the dauer larva—The dauer larva is an alternate L3 stage formed when L1 or L2 animals are exposed to insufficient food or excessive crowding. The dauer larva is morphologically specialized to withstand stress or poor environmental conditions. The epidermis in particular undergoes major remodeling during the transition into the dauer state. The epidermal layer becomes thinner, leading to ‘radial shrinkage’ of the entire animal¹³⁷. Remodeling of the epidermis is dependent on autophagy pathways¹³⁸.

The cuticle of the dauer larva is highly impermeable; for example, dauer larvae are resistant to treatment in 1% SDS. The dauer cuticle is thicker than that of non-dauers, and has ultrastructurally distinct features¹³⁹, most striking of which is a highly ordered striated layer 80 nm thick that may act as an impermeable paracrystalline lattice. Two cuticlins, CUT-1 and CUT-6, play specific roles in the dauer cuticle^{73, 140}, but are not required for the striated layer itself. Little is otherwise known about the molecular composition of the dauer epidermis/cuticle.

The epidermis is important in generating and responding to the intercellular signals that coordinate dauer development. As the epidermis consists of gap-junction coupled syncytia that enclose the animal, it is well placed to act as an endocrine organ that regulates the entire organism. The nuclear hormone receptor DAF-12 and the cytochrome P450 DAF-9^{141, 142} act within the epidermis to promote normal development; in the absence of these signals, animals enter the dauer state and do not exit. DAF-9 may be important for production of steroidal hormones such as the dafachronic acids by the epidermis, suggesting the epidermis is a site of autocrine feedback and signal amplification in the dauer decision. The large number of NHRs expressed by the epidermis suggests the epidermis integrates multiple steroid hormone signals¹⁴³. Insulin signaling is also an important regulator of the dauer decision, and may involve the epidermally expressed insulin-like ligand INS-33¹⁴⁴.

FUNCTIONS OF THE DIFFERENTIATED EPIDERMIS

The differentiated epidermis plays many roles in the life of *C. elegans*. Like all skin layers, a primary function of the epidermis is as a barrier epithelium. In addition to this ‘passive barrier’ function, the epidermis actively repairs wounds and activates cutaneous innate immune responses to skin-penetrating pathogens. The epidermis functions in ionic homeostasis, metabolic storage, and phagocytosis of cellular debris. Finally, the epidermis mediates communication of sensory neurons to the external environment.

Barrier function of the epidermis-cuticle complex

In its natural environment, either soil or rotting fruit, *C. elegans* is subject to repeated desiccation and rehydration. The epidermis-cuticle complex prevents leakage of internal solutes to the environment (the inside-outside barrier), and blocks entry to molecules in the environment (the outside-inside barrier). The low permeability of the *C. elegans* cuticle to small molecules is well known to researchers attempting pharmacological manipulations; drug concentrations effective on *C. elegans* are often several orders of magnitude higher than those used for cultured cells (Rand and Johnson 1995). Permeability barrier function can be assayed by incubation in Hoechst 33258, which does not cross the plasma membrane or cuticle¹⁴⁵, or Hoechst 33342, which crosses membrane but not cuticle¹⁴⁶.

Analysis of mutants with increased permeability suggests one or more layers of the cuticle confer barrier function. Several genes required for adhesion to the pathogenic bacterium *M. nematophilum* are also required for cuticle integrity, including the glycosyltransferases BUS-8¹⁴⁷ and BUS-17^{91, 148}. The precise location of the barrier in the cuticle remains to be determined: it may reside in the cuticle proper, the lipid-rich epicuticle, the surface coat, or a combination of all three. In addition, the epidermis itself contributes to barrier function. Tight junctions prevent solute leakage between adjacent epidermal cells. The tetraspanin TSP-15 is important for the epidermal contribution to barrier function¹⁴⁵.

Cuticle integrity and the permeability barrier are also compromised in mutants defective in tetrahydrobiopterin (BH₄) synthesis such as the GTP cyclohydrolase *cat-4*^{149, 150}. Although BH₄ is essential for cuticle integrity in *Drosophila*, the requirement for BH₄ in *C. elegans* cuticle is mechanistically distinct. The rigidity of *Drosophila* cuticle is due to crosslinking of chitin by reactive quinones synthesized by Dopa decarboxylase (Ddc) or tyrosine hydroxylase (TH), both of which require BH₄ as cofactor. However *C. elegans* Ddc BAS-1 and TH (CAT-2) are not expressed in the epidermis, nor are they required for cuticle integrity. In contrast, the phenylalanine hydroxylase PAH-1 is expressed in the epidermis and is required for synthesis of cuticle melanin; *pah-1* mutants do not display overt cuticle defects but synergize with other cuticle mutants¹⁵⁰. At present the role of BH₄ in cuticle integrity remains mysterious, but appears distinct from the Bus genes. Fatty acid synthesis is also important for the permeability barrier, for unknown reasons¹⁴⁶.

The epidermis in defense: skin-penetrating pathogens and skin wounding

The epidermis and cuticle form the worm’s first line of defense against pathogens or mechanical damage by environmental insults. A wide variety of pathogenic bacteria, fungi, or protozoa can attack *C. elegans* or related nematodes via the epidermis. Many fungal pathogens of free-living and plant-parasitic nematodes (‘nematophagous’ fungi) enter via the cuticle/epidermis and have evolved specialized structures that adhere to or penetrate nematode cuticles (FIGURE 5A). The response to cuticle-penetrating pathogens is closely related to the response to physical wounding (FIGURE 5B). Although wound healing has only recently been investigated in *C. elegans*, other nematodes such as *Oncholaimus* exhibit ‘traumatic insemination’ in which the male punctures the hermaphrodite cuticle in mating;

observations of 'wound plugs' after mating support an endogenous wound healing capacity in nematodes¹⁵¹. To survive in the wild, nematodes must actively and passively defend themselves against such kinds of potentially fatal damage. Other pathogens such as *M. nematophilum* or *Yersinia* do not penetrate the cuticle but establish a stable infection on the cuticle surface^{92, 152}; infection is somehow sensed by the nematode, triggering swelling of underlying epidermal cells (FIGURE 5C).

The response of the epidermis to infection by the cuticle-penetrating fungus *Drechmeria coniospora* has been extensively studied as a model for host-pathogen interaction and cutaneous innate immunity. Infection of *C. elegans* by *Drechmeria* triggers transcriptional upregulation in the epidermis of numerous small peptides, initially annotated as neuropeptide-like (*nlp*) but now known to have antimicrobial activity^{153, 154}. The pathways transducing the innate immune response to infection have been analyzed in detail. Signal transduction in the epidermis involves G protein signaling¹⁵⁵, the Toll-interleukin like adaptor TIR-1, a p38 MAPK cascade and the transcription factors ELT-3 and STA-1¹⁵⁶. A partly independent infection response involves the DBL-1 pathway, which triggers epidermal expression of caenacin family peptides¹⁵⁷. The fate of epidermally synthesized AMPs remains unclear; they may be delivered to the cuticle by one of the secretory mechanisms described above.

Wounding of the epidermis, either by a microinjection needle or by laser irradiation, triggers responses that collectively allow the animal to survive and heal damage. Immediate responses to wounding include rapid and sustained elevation in epidermal Ca²⁺¹⁵⁸, membrane sealing, and formation of an autofluorescent scab¹⁵⁹. Wounding also induces transcription of AMPs such as *nlp-29*. In general the peptides induced by wounding are a subset of those induced by infection, suggesting the response to infection includes a core response to non-specific damage. Other responses are infection-specific and require the Tribbles-like kinase NIPI-3¹⁵⁹. The pathways mediating AMP induction after infection and wounding are similar but not identical, indicating that the epidermis somehow discriminates between sterile wounding and pathogen infection. Loss of function in the *C. elegans* ortholog of death associated protein kinase DAPK-1 results in constitutive upregulation of epidermal AMPs and cuticle thickening, suggesting DAPK-1 negatively regulates wound responses¹⁶⁰.

Epidermal innate immune responses to wounding appear to be independent of the Ca²⁺ response to wounding, which functions specifically in wound closure¹⁵⁸. Epidermal Ca²⁺ dynamics involve putative epidermal Ca²⁺ channels such as GTL-2, and ITR-1-dependent release from internal stores. A G protein pathway involving G α_q /EGL-30 and PLC β /EGL-8 is required for the epidermal Ca²⁺ response and may act in a common pathway with GTL-2. Epidermal Ca²⁺ signals promote wound closure by triggering formation of an actin ring at wound sites; this actin ring closes over a period of 1–2 h in the wild type. Several actin regulators and polymerization factors are required for proper formation of the actin ring. However, loss of function in non-muscle myosins results in faster ring closure, suggesting that actomyosin contractility may retard closure. Instead, wound closure may be driven by local polymerization of filopodia-like structures. Loss of DAPK-1 also causes faster wound closure, suggesting that DAPK-1 acts as a coordinate negative regulator of wound responses. Aspects of the *dapk-1* epidermal phenotype are suppressed by loss of function in the mRNA polyadenylation regulator *sydn-1*¹⁶¹. It will be illuminating to explore how DAPK-1 is regulated and how it limits wound responses.

Physiological functions of the differentiated epidermis

Resistance to osmotic shock and other stresses—The mature epidermis plays a variety of roles in the animal, including osmoregulation and ionic homeostasis, metabolic

storage, and mechanosensory function. Hyperosmotic shock activates epidermal transcription of GPDH-1, elevating glycerol levels (FIGURE 5D)¹⁶². However high glycerol expression is not sufficient for osmotic resistance, suggesting the existence of additional pathways. The kinases GCK-3 and WNK-1 act in the epidermis to control fluid uptake in recovery from hypertonic stress¹⁶³. Loss of function in the epidermally expressed chloride channel *clh-1* results in increased epidermal size, a phenotype reversed by incubation in hyperosmotic media¹⁶⁴. Epidermal responses to osmotic stress overlap with transcriptional responses to infection¹⁶⁵. However the responses to pathogens and osmotic stress are not identical, indicating that the epidermis can distinguish between stresses.

How does the epidermis sense osmotic stress? Initial genetic screens for mutants resistant to osmotic stress (*Osr*) identified the nematode-specific gene *OSR-1*¹⁶⁶. Subsequent screens identified an unrelated family of genes that includes *OSM-7* and *OSM-11*¹⁶⁷. Both *OSR-1* and *OSM-7/11* are expressed in the epidermis and contain N-terminal secretion signals, hinting at a role for the cuticle in osmotic resistance. Interestingly, a subset of cuticle mutants display resistance to osmotic stress, including animals lacking the cuticle collagens *DPY-2/7/10*¹⁶⁶ and the mucin *OSM-8*¹⁶⁸. As *DPY-2/-7/-10* are localized to cuticular furrows between annuli, osmotic stress might be sensed via decreased tension between cuticle furrows and the epidermal plasma membrane. The epidermal patched-related transmembrane protein *PTR-23* is required for the upregulation of osmotic responses in *osm-8* mutants and could be involved in transduction of a cuticle tension signal¹⁶⁸; another epidermal patched family member *PTC-3* has also been implicated in osmosensation¹⁶⁹.

Most of the above *Osr* mutants display high basal levels of glycerol, contributing to their ability to withstand stress¹⁶⁷. The *NSY-1/SEK-1/p38* cascade is not required for acute osmotic stress resistance, but is required for the chronic *Osr* phenotype of *osr-1* mutants. In contrast, the *NSY-1* cascade is not required for the chronic *Osr* phenotype of *osm-7* mutants. Thus, the mechanisms of osmotic stress resistance may differ between these mutants. Although glycerol accumulation may be sufficient for chronic osmotic stress resistance, additional responses may be involved in acute stress resistance.

In addition to these roles in osmotic stress resistance, the epidermis has a critical role in resistance to environmental toxins. For example, the *MEK-1* MAP kinase cascade acts in the epidermis to confer resistance to heavy metal stress¹⁷⁰. Resistance to the toxic effects of H_2S or *HCN* involves epidermal expression of oxidizing enzymes such as *SQRD-1*, presumably limiting exposure of more internal tissues¹⁷¹. Finally, heme deficiency upregulates the expression of the *HRG-2* transporter required for epidermal heme uptake¹⁷², indicating the existence of a regulated pathway dedicated to epidermal heme homeostasis.

Lipid storage—While the intestine is the major adipose tissue of the worm, the epidermis is also a significant site of fat accumulation. The fat storage role of the epidermis may have been underestimated by the use of Nile red to detect fat, as Nile red does not efficiently stain the epidermis¹⁷³. Quantitative microscopy indicates that the epidermis accounts for ~10% of fat stores in the wild type and ~50% of the fat stores of dauer larvae¹⁷⁴. Indeed, L2 ‘predauer’ animals committed to enter the dauer stage can be recognized by the accumulation of fat in the epidermis¹⁷⁵. The epidermis is also the site of action of the AMP kinase (AMPK) pathway, which promotes the long-term survival of dauers by restraining lipid hydrolysis.

The epidermis, cell death, and cell damage—*C. elegans* lacks dedicated ‘professional’ phagocytic cells. Instead, cellular debris from dead cells is typically phagocytosed by adjacent tissue, in most cases the epidermis. Phagocytosis of apoptotic cell

corpses by the epidermis can be seen during epidermal enclosure (e.g. Movie 7 in Chisholm and Hardin 2005), indicating the epidermis expresses the phagocytic machinery early in its development. In post-embryonic development of the nervous system, dying apoptotic cells are engulfed by adjacent epidermis¹⁷⁶. The epidermis also phagocytoses necrotic or degenerating neurons^{177, 178}. Epidermal phagocytosis may be responsible for the degeneration and clearance of axonal fragments generated after laser axotomy¹⁷⁹ or in axon fragility mutants¹⁸⁰, analogous to the clearance function of glia in other organisms¹⁸¹.

Only a few programmed cell deaths occur in epidermal lineages in *C. elegans*, the exceptions including the epidermal-like tail spike cells¹⁸². In contrast, ventral epidermal blast cells in *P. pacificus* undergo apoptosis unless prevented from doing so by expression of LIN-39¹⁸³. Pathological cell death of *C. elegans* ventral epidermal blast cells occurs in *lin-24* and *lin-33* mutants¹⁸⁴; such 'cytotoxic' deaths do not resemble apoptotic or necrotic deaths. The cytotoxic deaths of Pn.p cells in these mutants are independent of the CED-3 caspase but partly dependent on the phagocytic machinery. *pvl-5* mutants display a distinct kind of CED-3-dependent non-apoptotic cell death¹⁸⁵. Epidermal cells may not express components of the apoptotic machinery and therefore (as in the case of the tail spike cells) rely on transcriptional activation of CED-3 to induce death.

The epidermis is also a major site of autophagy. Autophagosomes are prominent in the epidermis and as mentioned above, autophagy is important for the remodeling of the epidermis during dauer development. Decreased autophagy has been reported to result in decreased epidermal cell size and decreased body size¹⁸⁶. Autophagy and apoptosis may have redundant roles in epidermal morphogenesis¹⁸⁷.

The epidermis and neuronal function—Most nematode sensory neurons are closely associated with epidermis or cuticle, in a ways that reflect their sensory modality. Neurons sensing water-soluble chemicals such as amphid and phasmid neurons are exposed to the external environment via openings in the cuticle. Other sensory endings are embedded in specialized cuticle structures. In the case of the deirid, a putative mechanosensory neuron, the modified cuticle ('substructure') appears to be generated by the socket cell, as ectopic socket cells are sufficient to make ectopic substructures¹⁸⁸. The mechanisms by which sensilla or socket cells modify the cuticle remain unknown.

Other mechanosensory neurons do not contact the cuticle directly but are embedded within the epidermis (FIGURE 2D). The processes of ALM and PLM gradually become enveloped by the epidermis during larval development, and secrete a specialized ECM (the mantle) that attaches them to epidermal cells¹⁸⁹. Mechanosensory neurons recruit the ECM protein HIM-4, which then induces trans-epidermal attachments in the adjacent epidermis¹⁹⁰. Although HIM-4-dependent recruitment of trans-epidermal attachments is not essential for mechanosensation it might enhance sensory acuity under certain conditions. The PVD and FLP neurons form highly branched processes in close proximity to and in some cases partly embedded in the epidermis^{191, 192}. PVD and FLP neurons have nociceptive and proprioceptive roles that may depend on mechanical coupling to epidermal cells, but these cells do not recruit trans-epidermal attachments.

The dorsal and ventral nerve cords of nematodes are associated to various degrees with the adjacent epidermis. In *C. elegans* these axon bundles run alongside but are not enclosed by the epidermal ridges. At the other extreme, exemplified by *Ascaris*, nerve cords run almost entirely inside a channel termed the 'hypodermal chalice'^{193, 194}. Nematodes such as *Trichuris* display intermediate levels of association, with nerve cords partly embedded in epidermis¹⁹⁴. In *C. elegans* the epidermis frequently extends finger-like projections into

adjacent axon bundles [cf. Figure 18 in ¹⁹⁵]. It is unknown if these hypodermal protrusions play any role in neuronal function.

There is increasing evidence that the epidermis can affect neuronal function, either by controlling local ionic homeostasis adjacent to neurons or by providing other signals or trophic factors. Increased motor neuron excitability can be suppressed by loss of function in the epidermal TRPM channel *GTL-2* ¹⁹⁶. As mentioned above, *OSM-11* is secreted by epidermal seam cells and signals to neurons to regulate behavioral quiescence ¹³⁴. Conversely, synapse-like structures between neurons and the epidermis have been noted in serial section EM reconstructions, suggesting neuronal activity could feed back on the epidermis. Interactions between the epidermis and nervous system are ripe for future investigation.

The aging epidermis

Early investigations into nematode aging focused on the epidermis and cuticle. Older nematodes, of various species, display progressive deterioration in cuticle structure and in barrier function ¹⁹⁷, and possibly decreased surface coat thickness ¹⁹⁸. The epidermis adjacent to muscles expands and bulges, suggesting the cumulative effects of muscle contraction result in a local loss of epidermal integrity ¹⁹⁹. More recent studies have revealed major structural changes in the aging *C. elegans* epidermis ²⁰⁰, including subcellular deterioration, accumulation of lipid inclusions, and cuticle thickening. Older animals displayed ‘random but extreme local crises’ involving disruption of the plasma membrane, suggesting that a proximate cause of death of aged nematodes may be a decline in epidermal integrity leading to a loss of barrier function.

Genetic and genomic studies suggest the epidermis may actively regulate age-dependent changes. Age-related declines in oocyte quality in the germline are affected by an epidermally expressed *TGF β* pathway ²⁰¹, suggesting communication between the epidermis and the germline. Global age-dependent changes in gene expression are regulated by the epidermal GATA factors *ELT-3*, *-5* and *-6* ²⁰². *ELT-5* and *ELT-6* expression levels progressively increase with adult age, resulting in progressive repression of *ELT-3*. It is not yet known whether the increase in *ELT-5/6* expression reflects ‘selective drift’ in post-reproductive stages, or whether accumulated external stresses to the epidermis (mechanical trauma, oxidative damage) might underlie this regulatory shift.

Conclusions and Future Directions

Analysis of the differentiated *C. elegans* epidermis has begun to yield insights into its physiological roles, particularly in innate immunity, wound healing, and aging. Still, the analysis of the biology of the differentiated *C. elegans* epidermis remains in its early stages. Although the epidermis is a large and accessible organ, many of its functions are essential, potentially complicating genetic analysis. However the wild-type epidermis is generally sensitive to RNAi, so large scale RNAi screens for epidermal defects should be feasible. Epidermal-specific RNAi provides added specificity ²⁰³ and has been used to address epidermal-specific roles of essential genes ¹⁵⁸. Recent advances in transcriptional profiling of specific tissues ²⁰⁴ should stimulate more systems-level analyses of the molecular basis of epidermal biology.

The *C. elegans* cuticle retains an enduring fascination as an example of a self-assembling complex extracellular structure. The biomechanical properties of the cuticle may be of interest in bioengineering of impermeable layers. The epicuticle is a remarkable extracellular lipid bilayer, possibly analogous to extracellular lipid bilayers of the vertebrate stratum corneum, and deserves more attention from molecular genetics. Finally, the surface

coat plays a critical role in a nematode's interaction with its environment. A better understanding of these surface structures will inform efforts to control plant and animal parasitic nematodes.

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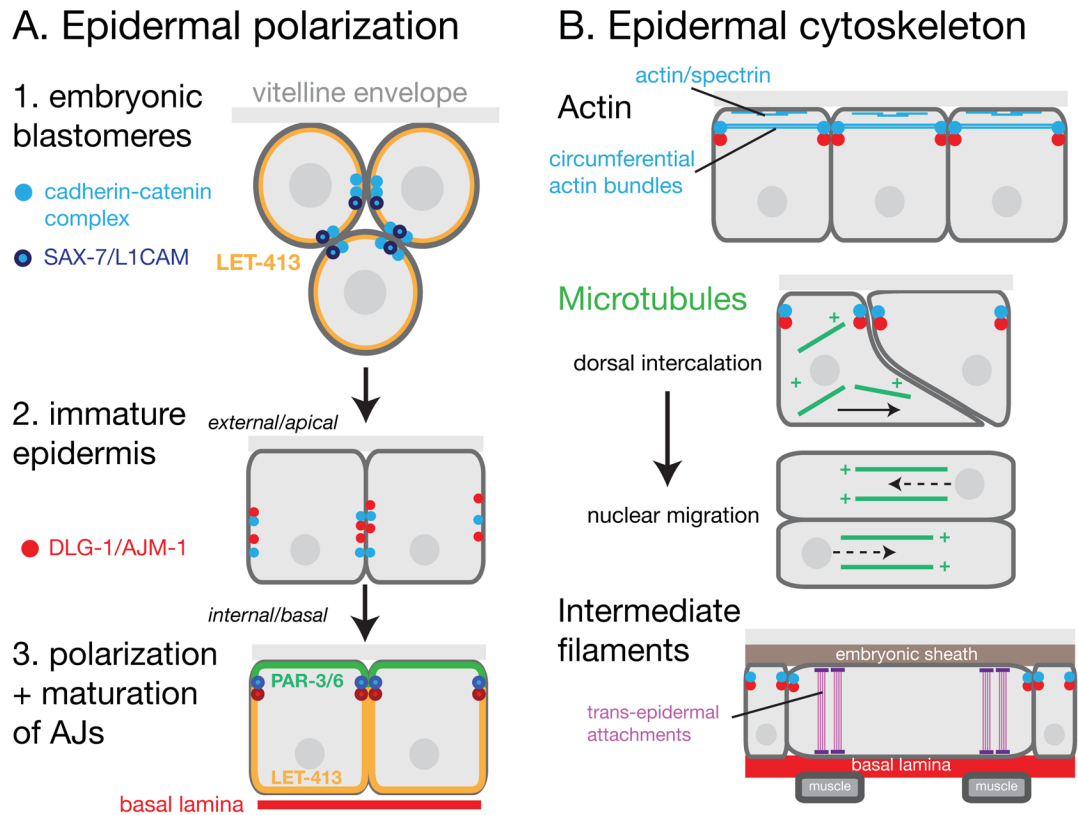


FIGURE 1. Development of epidermal polarity and cytoskeletal systems

(A) Overview of development of polarity and epidermal features, based on ¹. With a few exceptions, the order and dependency of the various polarization processes is not clearly established in the epidermis. All early embryonic blastomeres express classical cadherin/HMP-1 and LET-413/Scribble; outer blastomeres are contacted externally by the vitelline envelope. Epithelial differentiation begins with the onset of DLG-1 and AJM-1 expression in the premorphogenetic epidermis. In the early epidermis DLG-1 and AJM-1 are punctate and localized over the lateral surface. As the epidermis matures, DLG-1 and AJM-1 become confined to the subapical region to form adherens junctions (AJs), and LET-413 becomes basolaterally localized. PAR-3 and PAR-6 become apically localized in the epidermis, but are not essential for all aspects of apical-basal polarity. PAR-6 functions independently of PAR-3 to promote the assembly (but not localization) of the AJs.

(B) Epidermal cytoskeletal systems. Actin filaments (light blue lines) are found in the cortical spectrin cytoskeleton and in large circumferential bundles linking cadherin-catenin junctions. Microtubules (green lines) become polarized circumferentially in the dorsal epidermis during dorsal intercalation. Subsequent nuclear migrations are directed towards microtubule plus ends. Intermediate filaments (pink) such as IFB-1 are expressed in dorsal and ventral epidermal cells after enclosure and link apical and basal hemidesmosomes in muscle-adjacent regions of the epidermis.

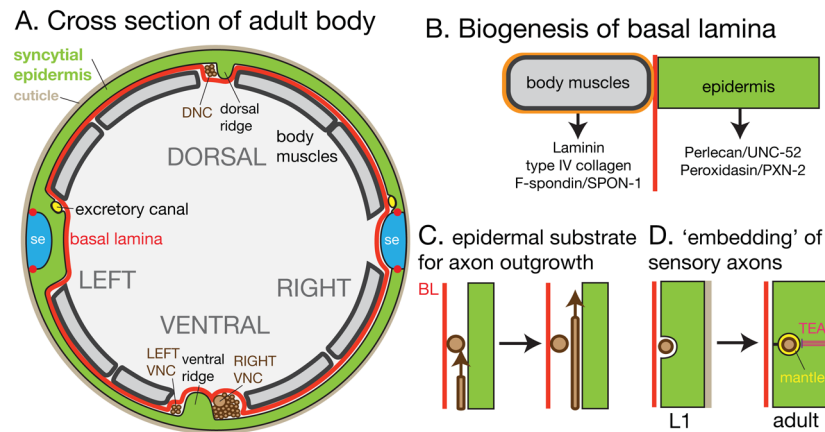
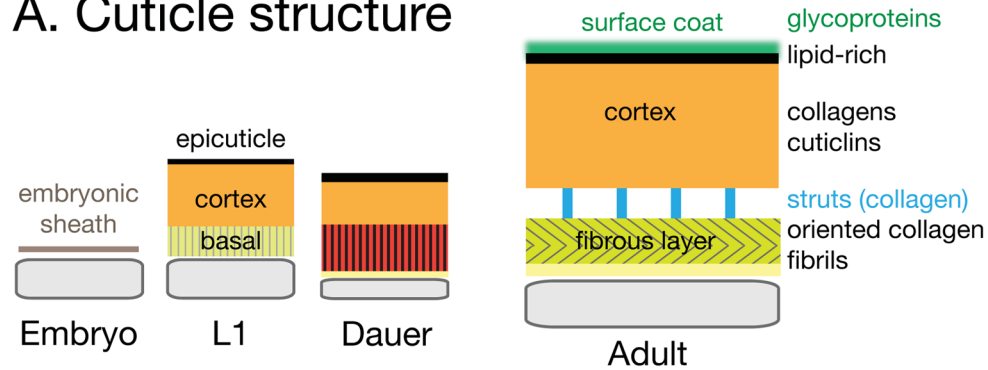


FIGURE 2. The epidermal basal lamina, and neuronal development

(A) Cross-section of adult body showing epidermis and adjacent tissues and structures. The external surface is covered by the cuticle; the internal face is covered by basal lamina (BL, red). Neurons (brown) reside on the epidermal side of the BL; the positions of the major nerve tracts relative to the dorsal and ventral epidermal ridges are shown. (B) Biogenesis of BL. The majority of known BL components are synthesized in muscles; the composition of the muscle-adjacent BL is likely distinct from that of non-muscle adjacent BL. (C) Developing axons appear to use the epidermis rather than the BL as substrate, as they are seen to pass on the epidermal side of other axons (R. Durbin, Ph.D thesis). (D). Sensory axons have a close relationship with the epidermis. Mechanosensory processes become progressively confined within the epidermis and secrete a specialized ECM, the mantle.

A. Cuticle structure



B. Polarized secretion and endocytosis

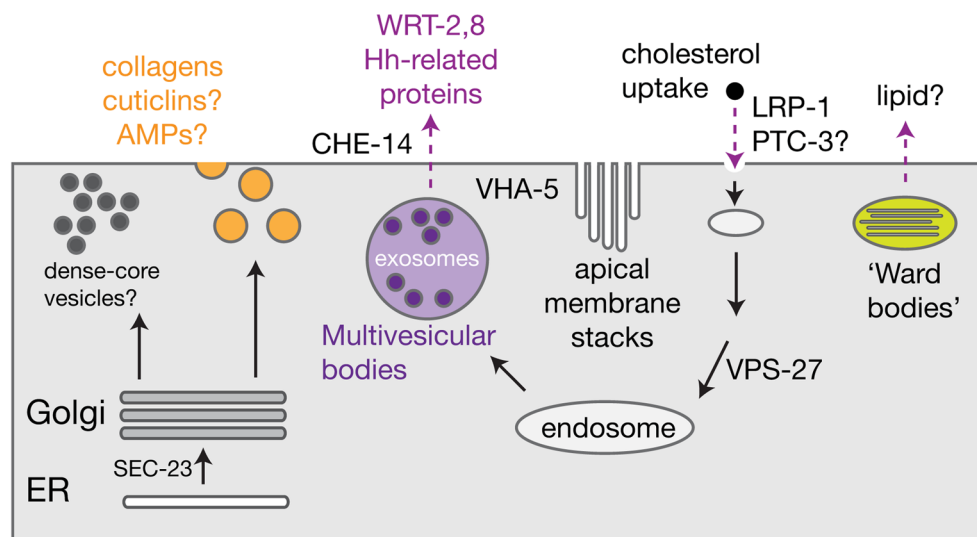


FIGURE 3. Cuticle structure and secretory apparatus

(A) Schematic cross-sections of cuticle in different larval stages and Dauer; the surface coat is likely present in all stages but may differ in composition. (B) Aspects of polarized secretion and endocytic pathways in the epidermis. Cuticle collagens are thought to be secreted through classical apical secretory pathway. Large dense-cored vesicles have been reported in the epidermis prior to molting, but may differ from the dense-core vesicles characterized in neurons. The epidermis is abundant in MVBs, involved in exosome-based secretion of hedgehog family proteins such as the WRTs¹⁰¹. The V0 ATPase subunit VHA-5 may function in MVB fusion with the apical membrane; VHA-5 is also localized to the prominent apical membrane stacks, of unknown function. Ellipsoidal organelles dubbed 'Ward bodies' contain membranous stacks; they have been observed in electron micrographs but are of unknown function.

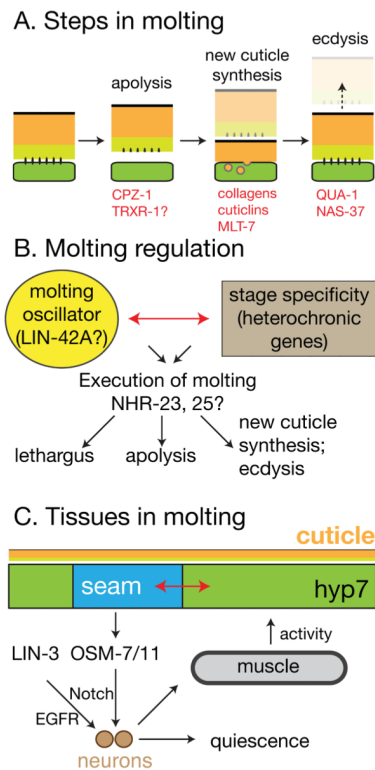


FIGURE 4. Stages, pathways, and tissues involved in molting

(A) Molts have been divided into three main stages: apolysis, cuticle synthesis, and ecdysis. Some genes may be involved in more than one stage. Proteases such as CPZ-1 are involved in apolysis. Ecdysis (separation of old cuticle) also involves a complex set of motor behaviors not depicted here. (B) Genetic pathways regulating molting. Highly speculative outline of possible regulatory relationships, partly based on ¹²⁶. Not all regulatory interactions may apply to all molts. (C) Tissue coordination during molts. The focus of LIN-42A function in molting appears to be in the lateral seam. LIN-42A might directly or indirectly regulate epidermal quiescence signals such as LIN-3 and OSM-7/11, which act on neurons. Quiescence involves decreased muscle contractions, which in turn may affect expression of epidermal molting genes ¹³⁵.

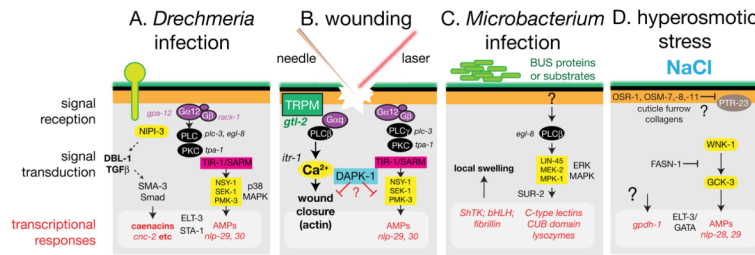


FIGURE 5. Epidermal responses to infection, wounding, and stress

(A) Pathways implicated in responses to infection by *Drechmeria coniospora*. *Drechmeria* spores adhere to the cuticle and extend hyphae that penetrate the epidermis²⁰⁹. A signal transduction pathway involving PKC, the SARM ortholog TIR-1, and the p38 MAPK cascade is involved in upregulation of transcription of the *nlp*-class AMPs^{153, 155}. A G-protein coupled receptor may be involved early in recognition of damage, as the same pathway is activated by sterile wounding; conversely, the Tribbles-like kinase is required for the response to infection but not to wounding. The GATA factor ELT-3 appears to be permissive for *nlp* upregulation¹⁵⁴. *Drechmeria* infection also triggers transcription of caenacin AMPs; *cnc* induction is partly independent of the p38 MAPK cascade and involves a non-cell-autonomous TGF β signal, possibly from neurons¹⁵⁷. (B) Epidermal wounding by microinjection needle or laser irradiation triggers expression of the NLP-type AMPs, via a pathway similar to that involved in *Drechmeria* responses, but does not induce the *cnc* genes¹⁵⁹. A G α_q -PLC β -Ca²⁺ signalling pathway is involved in wound closure after puncture wounding¹⁵⁸; DAPK-1 can negatively regulate both the innate immune response pathway and the wound closure pathway. (C) Infection by the bacterium *M. nematophilum* induces swelling of rectal epidermal cells. Genes required for bacterial adhesion are likely involved in biogenesis of the surface coat and/or epicuticle. *M. nematophilum* infection triggers expression of a variety of genes, some of which are involved in the epidermal swelling response²¹⁰. Swelling requires phospholipase C β (EGL-8)¹⁴⁸ and the ERK MAPK cascade²¹¹; it is not known if all transcriptional responses are ERK-dependent. (D) Osmotic stress may be sensed by structures at cuticle furrows coupled to epidermal cell surface receptors. Epidermal responses to hyperosmotic shock include elevated transcription of *gpdh-1* (leading to glycerol accumulation) and expression of a subset of AMPs¹⁶⁵.