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The *C. elegans* epidermis as a model skin. I: development, patterning, and growth

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Abstract

The skin of the nematode *C. elegans* is composed of a simple epidermal epithelium and overlying cuticle. The skin encloses the animal and plays central roles in body morphology and physiology; its simplicity and accessibility make it a tractable genetic model for several aspects of skin biology. Epidermal precursors are specified by a hierarchy of transcriptional regulators. Epidermal cells form on the dorsal surface of the embryo and differentiate to form the epidermal primordium, which then spreads out in a process of epiboly to enclose internal tissues. Subsequent elongation of the embryo into a vermiform larva is driven by cell shape changes and cell fusions in the epidermis. Most epidermal cells fuse in mid-embryogenesis to form a small number of multinucleate syncytia. During mid-embryogenesis the epidermis also becomes intimately associated with underlying muscles, performing a tendon-like role in transmitting muscle force. Post-embryonic development of the epidermis involves growth by addition of new cells to the syncytia from stem cell-like epidermal seam cells and by an increase in cell size driven by endoreplication of the chromosomes in epidermal nuclei.

Introduction

All animals are encased in skin layers that play critical roles in development and survival [1]. The skin and its appendages (collectively the integument) form the outer protective layer of an animal, acting as a permeability and structural barrier. In addition to these well-known 'barrier' roles, skin layers have important physiological functions in innate immunity, endocrine and exocrine secretion, mechanosensation, and wound healing. We begin by outlining the basic structure and development of animal skin layers, emphasizing similarities among different animal groups and the main specializations specific to nematodes such as *C. elegans* (TABLE 1).

All epidermal layers are epithelial, with the apical surface of the epithelium facing the environment. In *C. elegans,* the epidermis (formerly termed the hypodermis; see Note 1) is a simple epithelium with an internal basal surface covered by a basal lamina and an apical

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Further Reading/Resources
For a complete account of adult epidermal anatomy, see the WormAtlas web site (www.wormatlas.org) or the *C. elegans* Atlas [9], Chapters 2 (epithelial system), 9 (cuticle), and 10 (pericellular structures). For general reviews of nematode epidermis and cuticle see The Biology of Nematodes [157], chapters 2 and 7.

surface that secretes a flexible collagenous cuticle. The form and function of the nematode epidermis and cuticle are so interdependent that they have been referred to as the 'epidermis-cuticle complex' [2]. In contrast, the epidermis of insects such as *Drosophila* is a simple cuboidal epithelium that secretes a rigid, chitinous cuticle [3]. Vertebrate skin layers are typically multilayered epithelia. In fish the stratified epidermis is composed entirely of living cells [4], whereas terrestrial vertebrates are covered by an outer layer of dead keratinized cells, the stratum corneum. In mammalian embryos the surface epidermis first generates a transient outer layer, the periderm. Subsequently the epidermis executes a stratification program, concomitant with development of barrier function, such that neonatal skin consists of four major cell layers [5].

The epidermis and the nervous system are derivatives of the ectoderm; a major early event in metazoan embryogenesis is the separation of epidermal and neuronal precursors in the ectoderm [6], and the subsequent internalization of neurons. *C. elegans* embryonic epidermal cells form on the dorsal side of the early embryo and enclose the ventral neurons by epiboly (see below). In *Drosophila* the epidermis forms laterally and encloses the embryo by epiboly at the dorsal midline. The mammalian epidermis begins as a single layer of multipotent cells that subsequently either stratifies or forms an appendage such as hair or nails; neurons develop at the dorsal midline and are internalized by neural tube closure. Growth of the skin is essential for animal growth. In *C. elegans* and *Drosophila*, which grow via larval molts, new epidermal cells are added by division of stem cells within the epidermis. The adult *Drosophila* skin forms from imaginal disks set aside in embryogenesis. After embryonic and postnatal growth, mammalian skin undergoes constant renewal from division of stem cells in the basal layer.

Although some aspects of the *C. elegans* epidermis and cuticle are clearly specialized aspects of the nematode body plan, others may reflect epidermal characters conserved among all metazoans [7]. For example, the *C. elegans* epidermis contains cytoplasmic intermediate filaments (cIFs) and hemidesmosomes (HDs) that provide mechanical strength, analogous to keratin cIFs and HDs in mammalian skin. Although the molecular composition of the nematode and *Drosophila* cuticle and the mammalian stratum corneum are very different, all require enzymatic crosslinking of substrate proteins for formation of a mechanically strong permeability barrier. An outer lipid-based waterproof layer (the epicuticle in *C. elegans*) also seems to be a common feature of skin layers. Findings that conserved families of transcription factors regulate *Drosophila* and mammalian epidermal development [8] suggest that additional homologies or functional analogies may be found between skin layers. Here we review the developmental biology of the *C. elegans* epidermis, considered as an integrated organ system. In the following review, the differentiation and physiological roles of the epidermis are discussed.

SPECIFICATION AND MORPHOGENESIS OF THE EMBRYONIC EPIDERMIS

The *C. elegans* epidermis is a simple epithelium (FIGURE 1), within which several subtypes of epidermal cell can be defined [9]. The specification of epidermal cell fates in development is highly invariant with respect to the cell lineage (FIGURE 2) [10]. Most epidermal cells, including all post-embryonic epidermal blast cells, derive from the major ectodermal blastomere AB; a few are made by the C blastomere. The epidermis is generated piecemeal

[1]The external epithelium of nematodes was historically known as the hypodermis or hypoderm [148]. This term persists in the literature (e.g. Worm Atlas) and in the nomenclature for *C. elegans* cells (hyp7, etc). However it has long been generally accepted, based on developmental and now molecular evidence, that the hypodermal epithelium is homologous to the epidermal epithelia of other animals. We follow Wright's view [149], namely that "The concept of phylogenetic origin of cuticles indicates that the term epidermis is more appropriate than hypodermis for the cell layer that forms the nematode cuticle". The term hypodermis is accurately applied to the sub-dermal connective tissue of vertebrates, or the subepidermal cell layers of plants.

from multiple descendants of AB and C; exclusively epidermal precursors do not form until approximately 3 hours post fertilization.

Varieties of epidermal cell types

The *C. elegans* epidermis is made up of a small number of cells, of several types, each with multiple distinct roles in epidermal development and physiology.

Major postmitotic epidermal cells—Most of the larval and adult epidermis is composed of postmitotic syncytia, named hyp1 through hyp11, which form by the fusion of mononucleate epidermal precursors in mid-embryogenesis (see Box 1 and FIGURES 1,2). The larval *C. elegans* body is largely covered by a single epidermal cell, hyp7 (dark green in FIGURE 1,2). Additional cells fuse with hyp7 in each larval stage, so that in adults hyp7 contains 139 nuclei, over 80% of all epidermal nuclei. Although the 23 embryonic hyp7 precursors eventually fuse into a single syncytium, they can have distinct fates and roles: for example, fate transformations between hyp7 cells can lead to morphological defects, as in *vab-7* mutants [11]. The smaller epidermal cells hyp1 to hyp5 and hyp8 to hyp11 form the head and the tail respectively (FIGURE 1A,B); these are generated during embryogenesis and do not acquire additional nuclei by fusion during post-embryonic development.

BOX 1

A model for cell fusion: syncytial nature of the epidermis

The post-embryonic *C. elegans* epidermis consists almost entirely of multinucleate syncytia that form by fusion of epidermal precursors [64]. These epidermal syncytia range in size from 2 nuclei (e.g. hyp3) to 139 nuclei (adult hyp7). Genetic analysis led to the discovery of mutants defective in cell-cell fusion [150]. EFF-1 is a transmembrane fusogen protein necessary and sufficient for epidermal and other cell types to fuse [151]. Conversely, inappropriate fusions between epidermal cells are inhibited by the vacuolar ATPase [152]. Fusion-defective mutants such as *eff-1* are subviable and display aberrant epidermal elongation and subsequent morphogenesis, whereas mutants with excessive fusion are also inviable, suggesting that the precise pattern of syncytial fusion is critical for aspects of epidermal morphogenesis. Regulated cell fusions are also important in morphogenesis of post-embryonic epidermal structures such as the vulva [153].

Seam cells—Two chains of lateral epidermal cells known as ‘seam cells’ run the length of each side of the *C. elegans* body. The seam cells (H0–H2, V1–V6, and T) are born during embryogenesis (FIGURE 1B, 2A, dark blue) and, with the exception of H0, undergo stem cell-like divisions in post-embryonic development. After the seam cells divide in the L1 stage, their anterior daughters endoreduplicate their DNA then fuse with the surrounding hyp7 syncytium; their posterior daughters remain unfused (FIGURE 2B). In the second larval stage, the posterior daughters of the V cells undergo an additional ‘doubling division’ prior to the asymmetric seam division. An exception to this pattern is V5.p, whose anterior daughter is a neuroblast. In the third and fourth larval stages, seam cells again divide in a stem cell-like pattern as in the L1 stage. During the mid-L4 stage the posterior seam cells terminally differentiate and fuse to form a separate syncytium. Seam cells are responsible for the formation of cuticular specializations, the lateral alae, and play key roles in epidermal elongation and molting.

Ventral epidermal cells—Twelve ventral epidermal cells P1–12, along with adjacent hyp7 cells, form the ‘ventral pocket’ during epidermal enclosure (FIGURE 1B, C, red cells). The ventral epidermis secretes guidance cues such as netrins that play important roles in

emnbryonic patterning. The P cells comprise six bilaterally symmetrical pairs (denoted P1/2, P3/4, etc) that are developmentally equivalent until they migrate and intercalate at the ventral midline in the early L1 stage. During the L1 stage each P cell divides to generate an anterior daughter neuroblast (Pn.a cell) and a posterior daughter (Pn.p cell). Pn.p cells either fuse with the hyp7 syncytium or remain unfused until the L3 stage when they take on sexually dimorphic blast cell roles in vulva or male tail sdevelopment (see below) (FIGURE 1C).

Interfacial epidermal cells—The mouth, rectum and hermaphrodite vulva are orifices lined by rings of specialized epidermal cells (FIGURE 1B). The mouth is made by three small epidermal toroids (hyp1–3) and the epidermal-like arcade. The excretory pore forms a smaller opening and is lined by a single cell, the excretory socket. Numerous sensilla are embedded within the epidermis via specialized socket cells that form unicellular toroids around neuronal endings [12]. Socket cells are glial-like [13], yet partly resemble epidermal cells in that they secrete cuticle and are attached to surrounding epidermis via adherens and gap junctions [14]. Indeed, the seam cell T acts as the phasmid socket cell before it divides and transfers the socket function to its descendants.

Specification of epidermal fates

Epidermal fates are specified by a hierarchy of transcriptional regulators expressed in early embryogenesis (FIGURE 3). Many of these factors remain expressed in the post-embryonic epidermis and thus both initiate and maintain epidermal fates. The GATA factor ELT-1 is necessary and sufficient for specification of most epidermal fates and can be thought of as a master regulator of epidermal ‘organ identity’ [15–17]. In the C lineage ELT-1 itself is activated by blastomere-specific factors such as PAL-1 [18]; less is known about how ELT-1 is activated in the AB lineage.

At least three major targets of ELT-1 execute distinct aspects of epidermal differentiation: the GATA factor ELT-3 [19], the zinc finger protein LIN-26 [20], and the nuclear hormone receptor NHR-25 [21]. LIN-26 and NHR-25 are widely expressed in the embryonic epidermis and are essential for epidermal morphogenesis, whereas ELT-3 is restricted to non-seam epidermis and is not individually essential. A second nuclear hormone receptor, NHR-23, is also widely expressed in embryonic epidermis and is required for embryonic epidermal morphogenesis and post-embryonic molting [22]; NHR-23 and NHR-25 have closely related functions both in embryonic and post-embryonic epidermal development (see below); however their exact regulatory relationships have not yet been clearly elucidated. LIN-26 induces expression of genes involved in epithelial differentiation such as the junctional complex proteins AJM-1 and DLG-1 and the apical trafficking component CHE-14 (see below) [23].

Both the AB and C blastomeres have the autonomous potential to differentiate epidermal tissue [24]. In both lineages, epidermal potential is segregated asymmetrically at divisions of the AB grand-daughters and C daughters. Like other embryonic asymmetries the asymmetric distribution of epidermal potential requires POP-1 [25]. In the C lineage POP-1 asymmetry allows ELT-1 to remain active in anterior daughters, promoting epidermal organ identity. ELT-1 activates the three ‘differentiation’ factors, which together act as an integrated module to promote epidermal specification and repress muscle specification [26]. Epidermal specification in the AB lineage is more intricate, but can be viewed as a binary choice between epidermal versus neuronal potential. ELT-1 is not expressed in ‘minor’ epidermal cells, suggesting epidermal identity can be initiated independent of ELT-1.

The subtypes of epidermal cell are specified by the expression of distinct transcriptional regulators. Dorsal epidermal fates are specified by a redundant pair of T-box genes,

TBX-8/9 [27], which act upstream of VAB-7; in C-derived hyp7, TBX-8/9 are activated by PAL-1 [18]. The Zinc finger protein DIE-1 also functions to specify aspects of dorsal epidermal fates [28]. The seam cell subtype is specified by the *engrailed*-like homeobox gene CEH-16 [29], which activates two partly redundant GATA factors ELT-5 and ELT-6 [30]. ELT-5 and ELT-6 together repress ELT-3 in seam cells. ELT-1 itself is expressed in post-embryonic seam and is required for seam cell differentiation [31]. CEH-16 also promotes the L2-specific proliferative divisions of seam cells (Huang 2009). The RUNX complex RNT-1/BRO-1 acts in parallel to CEH-16 to control seam cell proliferative divisions [32, 33]. Factors specifying the ventral epidermal P cell fates have not yet been reported.

To what extent are these mechanisms of epidermal fate specification conserved? Many bilaterian phyla use ELT-1-like GATA factors to specify ectodermal fates [34]. Likewise, NHR-25 is related to the Ftz-F1 family of orphan nuclear receptors, implicated in epidermal cuticle formation in *Drosophila* [35]. Grainy head family transcription factors play conserved roles in epidermal development and repair in mammals and *Drosophila* [8, 36, 37]. The *C. elegans grainy head* ortholog *grh-1* is required for late aspects of epidermal differentiation [38] but its position in the epidermal transcriptional hierarchy remains to be determined. Conversely, LIN-26 is a divergent C2H2 Zinc finger protein with relatives identified only in other nematodes.

Morphogenesis of the embryonic epidermis

The major epidermal cells are generated on the dorsal surface of the gastrulation stage embryo and form an epithelial sheet, here referred to as the epidermal primordium (FIGURE 2,4A). Epidermal morphogenesis includes all the processes that shape the epidermal primordium into a skin layer covering the elongated embryo. Morphogenesis occurs over a period of ~3 hours in mid-embryogenesis and can be divided into three phases: dorsal intercalation, ventral enclosure, and elongation (FIGURE 4). These movements are driven by changes in cell shape or adhesion and do not involve cell proliferation; indeed morphogenetic processes such as enclosure are resistant to decreased epidermal proliferation [39].

Dorsal intercalation—The dorsal-most epidermal cells, hyp6 and hyp7 precursors, initially form two bilaterally symmetrical rows that intercalate into a single dorsal row (FIGURE 4B). Intercalation involves polarization and directed protrusions of the dorsal cells [40] and is regulated by DIE-1 [28]. A Wnt/β-catenin signaling pathway promotes cell fate determination of the dorsal epidermis [41, 42]. Following intercalation, the nuclei of dorsal cells undergo contralateral migrations, a process dependent on a polarized MT cytoskeleton [43] and the Arp2/3 complex [44]. Dorsal intercalation may play a convergent extension-like role to elongate the dorsal epidermis prior to enclosure. Intercalation may also help align the MT and actin cytoskeletons into circumferential arrays required in enclosure or elongation.

Ventral enclosure—Ventral enclosure or epidermal enclosure involves spreading of the epidermal sheet over lateral and ventral cells to meet at the ventral midline. Enclosure is an example of type of epithelial spreading movement known as epiboly; other examples include epiboly of fish embryos [45] and dorsal closure of the *Drosophila* epidermis [46]. Mechanisms of epiboly are of broad interest as they may be related to those underlying closure of epithelial wounds [47].

At least three partly independent processes have been defined in *C. elegans* epidermal enclosure [48] (FIGURE 4C-E): (1) four ‘leading cells’ in the midbody initiate enclosure by sending processes to meet at the ventral midline; (2) more posterior ‘ventral pocket’ cells

then enclose the posterior body, possibly via a 'supracellular purse-string' mechanism; (3) finally anterior epidermal cells (hyp1–5) enclose the head. Several processes contribute to epidermal motility during enclosure, including the epidermal actin cytoskeleton [49, 50] and IP_3/Calcium signaling [51, 52]. Cadherin-mediated adhesion is not required for motility of the spreading epidermis [53] but is necessary for stable adhesion of leading cells after they meet at the ventral midline [54].

Before enclosure the ventral surface of the embryo is covered by a set of neuroblasts and assorted non-neural cells that form the substrate for epidermal spreading. Correct development of the substrate is critical for epidermal enclosure [55]. Several partly redundant pathways required for normal epidermal morphogenesis are primarily involved in migration movements within the substrate. The first such neuronal pathway to be elucidated was the Eph receptor/VAB-1 and ephrin ligand signaling pathway [56]. Other pathways with partly overlapping functions include the LAR-like receptor tyrosine phosphatase PTP-3 [57], semaphorin MAB-20/ephrin EFN-4 signaling [58–60], and the KAL-1 (Kallmann syndrome/ Anosmin)/HSPG pathway [61, 62]. Loss of function in any one of these pathways results in variable and incompletely penetrant defects in neuronal substrate formation and subsequent epidermal enclosure; loss of function in any two pathways causes highly penetrant enclosure defects. These observations do not yet distinguish whether substrate cells are permissive or instructive for epidermal enclosure. Substrate cells undergo complex dynamic rearrangements during enclosure (C.A. Giurumescu and A.D.C., unpublished results) and form specific contacts with P cells during ventral pocket closure [63], consistent iwth the neuronal substrate playing an active role in epidermal movements.

Formation of epidermal syncytia by fusion—As noted above, the epidermis of the newly hatched larva consists of multinucleate syncytia in addition to the lateral and ventral blast cells (see BOX). The cell-cell fusions that generate these syncytia commence during epidermal enclosure and proceed in a stereotyped manner [64], although the exact order of fusion within hyp7 may not be completely invariant. The first epidermal cells to fuse are the posterior pair of ventral leading hyp7 cells (18 and 19 in FIGURE 1B), followed by cells 1 and 2 in dorsal hyp7. Subsequent fusions progress from anterior to posterior and are complete by the two-fold stage. As described below, post-embryonic seam-derived cells fuse with hyp7 within hours of their birth; in late L3 stage the hyp6 syncytium as a whole fuses with hyp7 [65].

Expression of the fusogen EFF-1 in many cases appears necessary and sufficient for adjacent cells to fuse; for example seam cells do not fuse with hyp7 because they express ELT-5, which transcriptionally represses EFF-1 [30]. These observations raise the question of how the epidermis can form multiple adjacent syncytia without the two syncytia fusing together. For example, EFF-1 is required for the fusions that form hyp6 and hyp7, yet hyp6 and hyp7 do not fuse until the L3 stage. At least two explanations may apply. First, EFF-1 expression or function in adjacent cells could be temporally separate [66]. Second, distinct fusogens may be involved in distinct sets of cells. The EFF-1-related fusogen AFF-1 is required for embryonic fusion of hyp5 and the late larval fusions of seam cells [67]. Alternating expression of non-homotypic fusogens might be sufficient to explain the ability of adjacent syncytia to form independently. Ephrin signaling has also been implicated in hyp6 fusion, although the mechanism remains unclear [68].

Epidermal elongation: role of the epidermal cytoskeleton—Immediately after enclosure the embryo undergoes dramatic elongation from a bean or comma-like shape into an elongated worm, folded over within the eggshell. Embryonic elongation involves rapid changes in shape of epidermal cells and of the entire embryo, ultimately driven by forces generated by the epidermal cytoskeleton. Classic embryological studies demonstrated that

the epidermal actin cytoskeleton was essential for elongation [69]. The epidermal MT cytoskeleton distributes forces generated by actomyosin based contraction and may be important for the epidermal response to contraction [70]. Although elongation appears as a single continuous process genetic analysis has revealed that elongation beyond the two-fold stage requires the contraction of underlying body wall muscles [71]. In the wild type, muscle contractions begin at the 1.75-fold stage of elongation. Muscle contractions influence epidermal cytoskeletal remodeling via a novel tension-sensing mechanism in trans-epidermal attachments (see part II). After elongation is complete the formation of the first cuticle maintains the elongated form of the larva.

Epidermal specification and morphogenesis in other nematodes

Epidermal development in *C. elegans* appears to be broadly representative of epidermal development in the nematode phylum. Embryonic development of a variety of nematode species has been examined, in some cases by timelapse microscopy and cell lineage analysis [72–74]. Although relatively few studies explicitly describe epidermal development, all nematode embryos examined pass through 'comma' or 'tadpole' morphogenetic stages in mid-embryogenesis, corresponding to the point at which embryonic cell divisions are essentially complete and the epidermis has enclosed the embryo. Late embryonic elongation and hatching are likewise conserved, and result in similar early larval forms. Development before the comma stage appears to be evolutionarily flexible, and in many cases does not closely resemble that of *C. elegans*.

Species in the class Chromadoria display epidermal development closely reminiscent of *C. elegans*: epidermal precursors are non-clonal descendants of AB and C, and divide to form of a dorsal epidermal primordium that encloses over a neuronal substrate [75, 76]. Other species, such as the Cephalobid *A. nanus*, display more regulative early development, yet arrive at a comma-like stage [77]. Gastrulation of a blastula with a prominent blastocoel occurs in *Tobrilus*, yet again this leads to a comma-like stage [78].

There are fewer studies of the embryology of the two more 'ancestral' or 'ancestrally diverged' nematode classes Enoplia and Dorylaimia, also termed clades I and II [79]. The Enoplid *Enoplus brevis* displays a variable early cell lineage in which fates may be allocated by position not ancestry [80]. Epidermal cells form dorsally, and might enclose the embryo by epiboly as in *C. elegans*. Epidermal cells in *Enoplus* do not fuse, and instead form a cellular epidermis in larval stages; it has been suggested that a syncytial epidermis is a derived character in nematodes. Among the Dorylaimids, *Trichinella spiralis* passes through a tadpole-like stage [72]. In *Romanomermis culicivorax*, epidermal cells originate from a single blastomere 'S2', possibly equivalent to the C blastomere in *C. elegans*. Epidermal cells form a series of rings at the posterior of the embryo before spreading anteriorly over a substrate of neurons [81].

In summary, nematode epidermal development illustrates well the concepts of the 'phylotypic stage' [72, 82] and the 'developmental hourglass' [83]. Specification of epidermal precursors, formation of the epidermal primordium, and epidermal enclosure can occur in a variety of ways. However all nematode species converge on a comma-like phylotypic stage, which then elongates into the typical nematode larva. Whether the comma and elongation stages are indeed the most conserved in nematode evolution needs to be substantiated by further comparative studies focusing on the epidermis. In subsequent larval development the epidermis and cuticle diverge in form according to the particular ecological niche of the species.

POST-EMBRYONIC GROWTH AND PATTERNING

The size and shape of the nematode body is principally determined by the post-embryonic growth of the epidermis and cuticle. The epidermis provides a model for understanding how the growth of a single tissue is integrated into organismal size control.

Growth: seam cell divisions and polyploidization

During larval development *C. elegans* grows six-fold in length and several-fold in diameter, an overall 32-fold increase in volume [84]. This increase in size is in large part a direct result of the increase in hyp7 nuclear number and DNA content. *C. elegans* continue to grow over the first 4 days of adult life, approximately doubling in volume [85]. Such post-L4 growth involves increased epidermal cell size, driven by polyploidization of epidermal nuclei.

Seam cells: stem cell-like epidermal blast cells—Epidermal seam cells have provided many insights into how self-renewing asymmetric stem cell-like divisions are controlled within a polarized epithelium. During each larval stage the seam cells divide asymmetrically to generate anterior daughters that fuse with the embryonically generated hyp7 syncytium; in total the seam cell divisions add a total of 98 hyp7 nuclei to the syncytium. Seam-derived hyp7 nuclei undergo round of DNA endoreduplication immediately before fusion. An additional 12 hyp7 nuclei are contributed by fusion of Pn.p cells or their daughters in the L1 and L3 stages. The hyp7 syncytium thus increases its nuclear DNA content about ten-fold from L1 to adult [86]. The higher ploidy of the seam-derived epidermal nuclei presumably increases the transcriptional capacity of the epidermis, allowing tissue growth. The DNA replication factor LIN-6/MCM-4 is required for endoreduplication cycles and is required in the epidermis for post-embryonic growth [87]. Nuclei that endoreduplicate in *C. elegans* divide in other nematode species such as *Panagrellus*, suggesting endoreduplication is an evolutionary abbreviation of cell division.

Wnt signaling plays multiple roles in seam development. Wnt signals directly or indirectly orient the polarity of asymmetric seam divisions. In at least some instances, such as the division of T, asymmetrically distributed Wnt signals play instructive roles in orienting the polarity of seam cells [88, 89]. In animals lacking all five *C. elegans* Wnts, seam cells generally divide asymmetrically but their polarity becomes more random [90]. These results suggest that the Wnts themselves are not essential for asymmetry per se, but function to orient asymmetry. In wild type seam cells the asymmetry of seam cell divisions is reflected in the asymmetric distribution of POP-1 activity in the anterior and posterior seam daughters: anterior daughters have high levels of nuclear POP-1 and fuse with hyp7, whereas posterior daughters have low nuclear POP-1 and remain seam cells [25, 91]. The intracellular components of Wnt pathways are involved in generation of asymmetry per se. Animals with reduced function in multiple Wnt receptors (Frizzleds) [90] or in the Wnt/β-catenin asymmetry pathway [92] display global loss of asymmetry in seam divisions, and consequent under- or over-proliferation of seam cells. Finally, Wnt signals act redundantly with other cues such as cell shape to constrain the orientation of seam divisions to the long axis of the larva [93].

Several studies have focused on how the seam switches between asymmetric self-renewal divisions and the L2-specific symmetric proliferative division. L2 developmental programs in general are regulated by heterochronic genes that include the HBL-1 transcription factor and microRNAs of the *let-7* family [94, 95]. The RNT-1/BRO-1 complex is necessary and sufficient for the L2 seam proliferative division, as is the engrailed-like gene CEH-16 [96, 97]. POP-1 remains asymmetric during the symmetrical proliferative divisions of the seam in the L2 stage, suggesting that POP-1 asymmetry can be uncoupled from seam fate asymmetry.

Genetic regulation of body size: TGFβ signaling and endoreduplication—The importance of TGFβ signals in regulation of body size was discovered through genetic analysis of Small body size (*sma*) mutants, most of which affect genes encoding TGFβ ligands, receptors, or downstream signaling components [98]. Animals lacking this pathway have normal body size and shape in the L1 stage and proceed through the normal number of molts, yet fail to grow to normal size. TGFβ signals are not required for divisions of seam cells that add nuclei to the epidermis. Animals lacking the TGFβ ligand DBL-1 display reduced endoreduplication of the seam-derived hyp7 nuclei. DBL-1 is expressed in the nervous system [99], suggesting signals from neurons might regulate epidermal polyploidization. However the precise source of DBL-1 is less critical than overall levels of signaling [100]. Conversely, loss of function in the epidermal glypican-like protein LON-2, a negative regulator of DBL-1, results in animals 25% longer than wild type [101]. The CRISP family protein LON-1 is a downstream target of DBL-1 that appears to repress polyploidization [102].

DBL-1 does not solely act via epidermal polyploidization. For example, overexpression of DBL-1 increases body length without increasing endoreduplication [103]. The cuticle collagen LON-3 is negatively regulated by TGFβ, in parallel to LON-1 [104]. Thus the DBl-1 pathway acts as a coordinate regulator of epidermal growth, influencing multiple targets in the epidermis that act together to regulate epidermal size [105]. Rictor signals also promote body size increase, in parallel to the DBL-1/polyploidization pathway [106].

Adult epidermal growth involves polyploidization and is sensitive to environmental conditions—Somatic cell or nuclear division has not yet been observed in adult *C. elegans.* Although germline proliferation continues for several days, animals lacking germlines are larger not smaller than wild type, suggesting the germline is a source of inhibitory growth signals. Instead, the post-L4 growth of the adult is at least partly due to continuing epidermal polyploidization [107]. Adult growth is also reduced by dietary restriction and sensory deprivation [108]. Sensory deprivation appears to act on the DBL-1 pathway via the cGMP kinase EGL-4 [109, 110]. Dietary restriction may also influence epidermal size independent of DBL-1, by increasing epidermal autophagy [111].

Spatial and temporal patterning of the post-embryonic epidermis

The epidermis has long been a tractable model for many aspects of spatial and temporal patterning in a single tissue. The post-embryonic seam and ventral blast cells, which show region-specific variations of serially reiterated lineages, provide excellent examples of spatial patterns. Larval stage-specific patterns of seam cell division exemplify temporal patterning of epidermal cell fates by the heterochronic gene system and are reviewed elsewhere. Finally, the epidermal components of the vulva and male tail are classic examples of inductive patterning.

Anteroposterior patterning of the epidermis: Hox clusters and Pax genes—The lateral epidermis in the L1 stage consists of the ten seam cells H0, H1, H2, V1–6, and T. Apart from V1–V4, each has its own distinct fate and lineage. Seam patterning in the body and tail is regulated by the *C. elegans* Hox cluster, a set of loosely clustered homeobox-containing genes whose chromosomal position is colinear with the anteroposterior location of the cells they affect (FIGURE 5) [112]. The fates of V5 and V6 are patterned by the Hox genes *mab-5* [113] and *egl-5* [114]. The *AbdB*-related posterior HOX gene NOB-1 is required for posterior epidermal morphogenesis [115] and functions with a related *AbdB*-like gene PHP-3 to specify the T fate [116]. The *labial*-like Hox gene *ceh-13* may be involved in specification of anterior seam cells V1 and H2 [117], although lineage transformations have not yet been described. The Hox cluster has not been clearly implicated in specification of the most

anterior seam cells; instead, patterning of H0 and H1 is mediated by isoforms of the VAB-3/PAX-6 paired domain protein [118, 119].

Anteroposterior pattern in the ventral epidermis is also defined by Hox gene activity [120, 121]. LIN-39, in concert with the homeobox cofactor CEH-20 (orthologous to *Drosophila* Exd/Pbx), specifies midbody P cell fates, including the vulval equivalence group [122]. LIN-39 appears to repress fusion of vulval precursor cells by activation of the seam GATA factors ELT-5 and ELT-6 [123]. LIN-39 and MAB-5 interact combinatorially in more posterior P cells, whereas EGL-5 acts in the most posterior P cell [114]. Studies of *C. elegans* epidermal patterning have thus elucidated how Hox gene clusters can pattern a tissue at the level of individual cell fates. How the *C. elegans* Hox genes become active in an anteroposterior sequence in mid embryogenesis remains to be addressed, as equivalents of the *Drosophila* segmentation gene hierarchy do not appear to be involved.

Post-embryonic organogenesis and sexual dimorphism in the epidermis—The epidermis contributes to two major sexually dimorphic organs, the hermaphrodite vulva and the male tail. The hermaphrodite vulva develops in the ventral midbody whereas the male tail develops in the lateral and ventral tail region. The vulva and male tail are integrated organs comprising neurons, muscles, and epidermis; here we focus on the epidermal contribution to these organs.

The vulva develops from three epidermal precursors P(5–7).p; three additional cells (P3.p, P4.p, P8.p) do not normally form the vulva but are developmentally equivalent, thus the vulval equivalence group of the midbody consists of P(3–8).p. The formation of the vulva is a dramatic example of orifice formation in an epithelium. Vulval precursors are induced by signals from gonadal cells, but their fates are also regulated by the surrounding syncytial epidermis. Loss of function in epidermally expressed genes such as *lin-15* or *lin-35*/Rb results in excessive vulval induction (the Multivulva or Muv phenotype). These genes act in the hyp7 syncytium to repress expression of the inductive signal LIN-3 [124, 125]. hyp7 is also one source of Wnt signals that act as competence factors for the vulva, preventing fusion of vulval precursors with hyp7 [126]. Vulval precursors interact extensively among themselves to ensure a highly invariant pattern of fates that then undergo morphogenetic movements to form the external opening to the uterus; genes such as VAB-23 act downstream of the midbody Hox gene LIN-39 to regulate vulval morphogenesis programs [127].

Male tail epidermal structures are a dramatic example of epidermal sculpting. Three posterior lateral epidermal seam cells (V5, V6, T) generate the sensory rays and acellular fan. The patterning of the male tail seam cells is under the control of Hox genes (MAB-5, EGL-5) [128] and the PAX-6 isoform MAB-18 [129], which activate expression of neurogenic genes such as LIN-32 to trigger ray formation. The positions of specific rays are determined by interactions with the surrounding epidermis, and are regulated by multiple signaling pathways, including Semaphorin, Ephrin [130, 131], and TGFβ [132–134]. Ray positioning is also dependent on myc family transcription factors expressed in epidermal cells [135]. The final stages of male tail morphogenesis involve extensive remodeling of the tail epidermis and cuticle and require specific cuticle collagens [136] and cell surface proteins [137, 138].

The sexual dimorphism of epidermal precursors to the vulva and male tail imply that epidermal fate specification intersects with the sex determination pathway. Somatic sex determination in *C. elegans* is regulated by the TRA-1 GLI family transcription factor, which is expressed in XX (hermaphrodite) animals and represses male developmental programs [139]. Few direct targets of TRA-1 in the sexually dimorphic epidermis have been characterized. Although vulval fates are hermaphrodite-specific, TRA-1 appears to inhibit rather than promote vulval differentiation, acting as a repressor of LIN-39 [140]. In the male

tail, the DM domain transcription factor MAB-3 acts downstream of MAB-5 in specification of male V5 and V6 fates [141, 142]. However MAB-3 is expressed throughout the lateral epidermis in both sexes [143], implying that sexual dimorphism results from regulation at other levels in the pathway.

Plasticity and regulation in the epidermis—Despite the high degree of invariance of wild type epidermal development, laser ablation experiments have revealed a limited capacity for regulation in the lateral epidermis of the male tail [144], suggesting cell signals can contribute to patterning of the lateral epidermis. Direct contact between seam cells is important in specifying lateral epidermal cell fates [145, 146]. The nature of the contact-dependent signal between seam cells is not yet known; it appears to counteract Wnt signals [147]. Semaphorin/Plexin signals are known to regulate seam cell contacts and may contribute to the 'stop' signal that prevents seam cells from extending past one another [132].

Conclusions and Future Directions

The *C. elegans* epidermis is a simple model for many aspects of skin developmental biology. Analysis of embryonic morphogenesis has illuminated our understanding of processes such as epiboly, hemidesmosome biogenesis, and tension-induced cell shape change. An important goal in the future will be to take a more quantitative approach to morphogenetic movements in the developing epidermis. Improvements in live imaging should allow the dynamics of epidermal cell shape changes to be described more accurately, permitting mathematical and physical models of force generation. The ability of the *C. elegans* embryo to execute complex morphogenetic movements with such high reproducibility suggests the existence of buffering systems that reduce variation; the variable phenotypes of morphogenesis mutants could reflect increased sensitivity to stochastic effects in these mutants.

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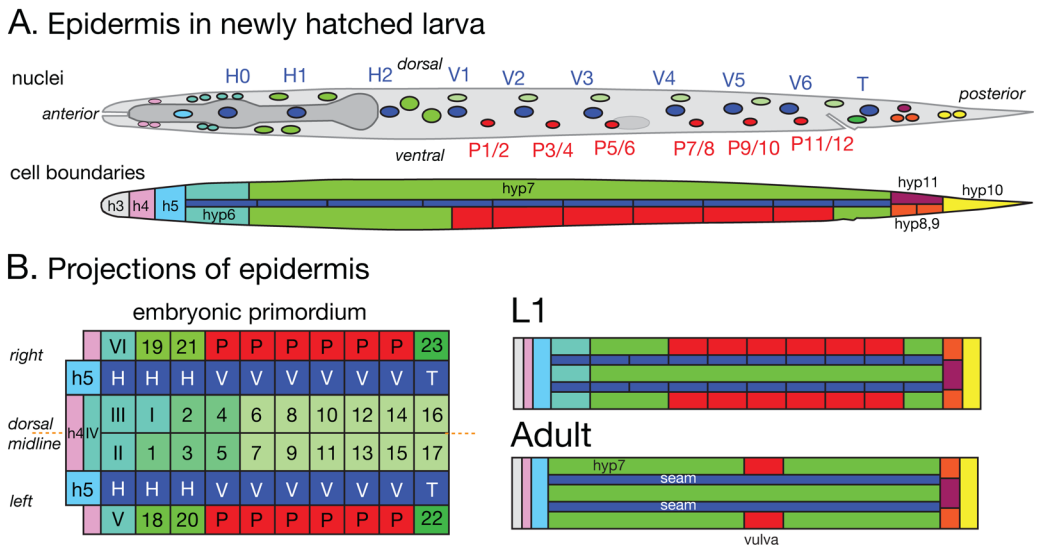

FIGURE 1. Anatomy, topology, and genealogy of the epidermis

(A) Anatomy of the *C. elegans* epidermis in L1 stage. Lateral views showing nuclei and cell boundaries, based on Sulston et al. (1983) and WormAtlas. In this and other figures, hyp7 is green, seam cells (H, V, T) are dark blue, ventral epidermal (P) cells are red; hyp4 is pink, hyp5 light blue, and hyp6 teal. (B) Cylindrical projections of the epidermis, separated at ventral midline and unrolled so that right is up and anterior to the left. The dorsal midline is indicated. Projections of the initial embryonic epithelium prior to cell fusion, L1 stage epidermis, and adult. The numbers and approximate disposition of cells are correct; the exact pattern of cell contacts is simplified. For more anatomically accurate projections, see Wormbook hypFIG2.

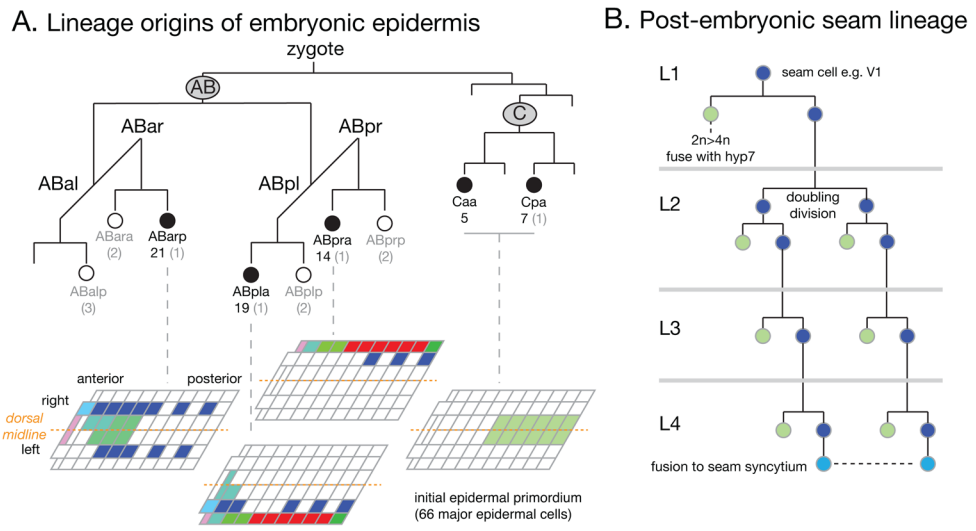

FIGURE 2. Lineage origins of the embryonic and post-embryonic epidermis

(A) Abbreviated embryonic cell lineage showing the origin of epidermal precursors from the AB and C lineages [10]. Cells are named according to standard *C. elegans* lineage nomenclature: AB and C are early embryonic blastomeres; a/p indicates anterior or posterior daughter and l/r indicates left or right daughter. Epidermal potential is intrinsic to AB and C and segregates at asymmetric divisions at the AB^8 and C^4 stages (i.e., at the division of ABar to ABara and ABarp, and so on) to five major epidermal precursors (filled circles). Epidermal cells are born in two successive rounds of cell division. 66 *major* epidermal cells (hyp4–7, seam, and P cells, shown in the projections) are born at ~240 minutes and comprise the initial epidermal primordium that forms dorsally and encloses the embryo. The remaining 12 *minor* epidermal cells of the head and tail (hyp1–3 and 8–11) are born in the next round of AB divisions (270–300 min) from 'minor epidermal precursors' (open circles) and are not shown in the cylindrical projections; 16 other epidermal-like cells (arcade, XXX, rectal epithelial cells, tail spike) are also born in this round of divisions and are not shown here. Numbers under cell names indicate the number of major and minor (gray) cells derived from each precursor. Projections show only the 66 major epidermal cells; note that except for V3 and V5, lineally related cells are adjacent in the epithelium. Color code as in FIGURE 1. (B) Representative lineage of a post-embryonic epidermal seam cell such as V1, illustrating asymmetric divisions in each larval stage and the L2-specific doubling division.

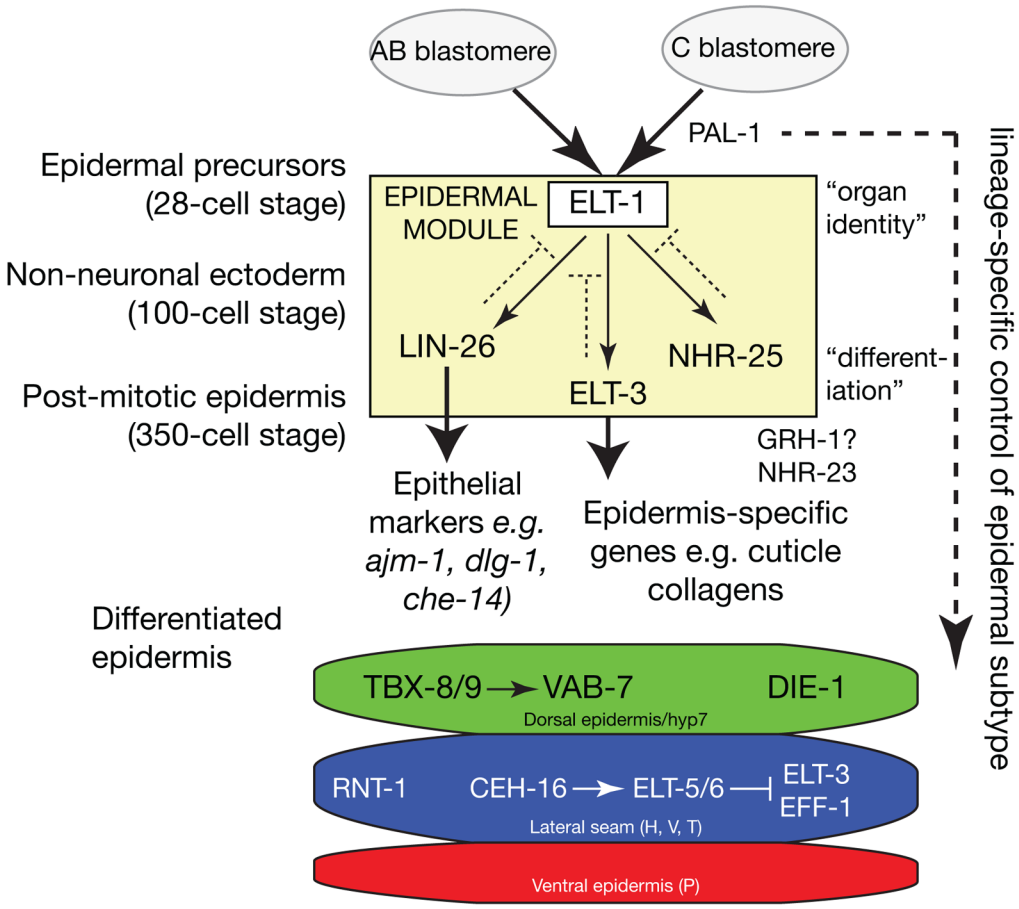

FIGURE 3. Transcriptional hierarchy regulating epidermal identity and differentiation

Regulatory hierarchy in embryonic epidermal specification, based on [16, 26 and 29]. Lineage-specific regulators activate the 'epidermal organ identity' gene ELT-1 in descendants of the AB and C blastomeres. ELT-1 initiates epidermal identity by inhibiting other organ identity modules and by directly activating three epidermal 'differentiation' factors: LIN-26, ELT-1, and NHR-25. LIN-26 confers generic epithelial characteristics [23]. ELT-3 promotes epidermal-specific aspects of terminal differentiation (e.g. cuticle collagen expression) but is not itself essential for epidermal development. The three differentiation factors appear to indirectly repress one another via negative feedback on ELT-1. NHR-23 and Grainyhead/GRH-1 promote other aspects of epidermal differentiation, but their location in the hierarchy has not been defined. In parallel, lineage-specific regulators specify subtypes of epidermal cell. Dorsal epidermal cells require the T-box genes TBX-8, 9; in C-derived hyp7, PAL-1 likely activates TBX-8/9 directly. One target of TBX-8/9 in C-derived hyp7 is the even-skipped ortholog VAB-7 [27]. DIE-1 is expressed in dorsal hyp7 in response to unknown signals. Lateral epidermal cells are specified by CEH-16 and ELT-5/6, which in turn repress the differentiation factor ELT-3. Post-embryonic seam cells are maintained in a stem-cell like state by the action of RNT-1/Runx and BRO-1/CBFβ; BRO-1 is directly activated by ELT-1 [155]. Dorsal and ventral cells (all non-seam epidermis) express ELT-3.

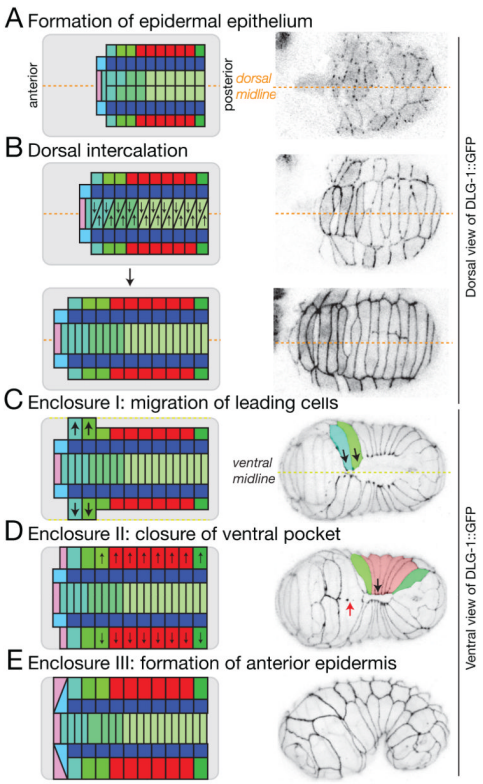

FIGURE 4. Embryonic epidermal morphogenesis

Early stages of epidermal morphogenesis, illustrated as projections and in frames from movies of the epidermal junctional marker DLG-1::GFP (*xnIs17*). (A) Initial formation of the dorsal epidermal epithelium, ~230–250 minutes post first cleavage. The epidermis occupies the posterior 2/3 of the dorsal part of the embryo. (B) Dorsal intercalation (~250–390 minutes), early and late stages. Intercalation is not as synchronous or as consistent as in the cartoon. (C) Ventral enclosure: migrations of four leading cells to the ventral midline, 365–375 min. (D) Ventral enclosure II: closure of the ventral pocket by P cells and hyp7 ventral cells, 370–385 min. (E) Enclosure III: formation of the anterior epidermis, 380–395 min. The leading cells move anteriorly; hyp4 and hyp5 cells enclose the head in short-range movements. Dorsal hyp7 cells begin to fuse. DLG-1::GFP images in A, B are dorsal views, from Movie 2 in Chisholm and Hardin 2005, WormBook. Frames in C-E are ventral views (C.A. Giurumescu and A.D.C., unpublished).

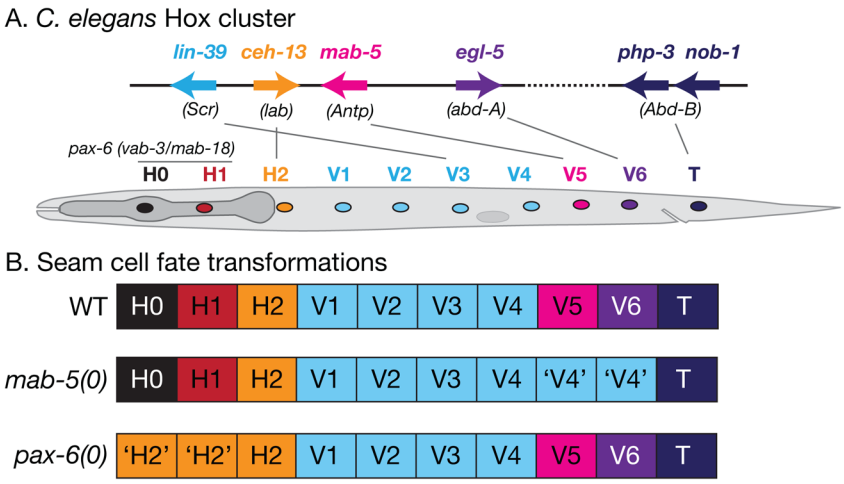

FIGURE 5. Anteroposterior pattern in the larval epidermis

Patterning of the seam in the trunk and tail is specified by genes in the *C. elegans* Hox gene cluster. Schematic showing Hox cluster genes on part of chromosome III; putative *Drosophila* homologs are indicated [156]. Examples of cell fate transformations: in *mab-5* mutants the posterior seam cells V5 and V6 adopt V1–V4-like fates ('V4') [113]. Patterning of the head seam cells is specified by the PAX-6 locus *vab-3/mab-18*. [118, 119]. In *pax-6* null mutants the anterior-most seam cells H0 and H1 are transformed to H2-like fates. Hox cluster genes also regulate anteroposterior pattern in the ventral epidermal (P) cells.

TABLE 1

Comparison of epidermal layers in *C. elegans*, *Drosophila*, and mammals

	C. elegans	***Drosophila***	**Mammal**
Epidermal epithelia	Simple epithelium; largely syncytial	Simple cuboidal cellular epithelium	Stratified epithelium
Dimensions, in adult	~0.1 mm^2, ~230 nuclei	10^6–10^7 cells?	2 m^2, 10^{11} cells (human)
Embryonic origin	AB, C blastomeres	lateral ectoderm	Surface ectoderm
Major morphogenetic processes	ventral enclosure; elongation	Dorsal closure	?
Post-embryonic growth	4 molts; Seam cells; polyploidization	larval molts; imaginal disks	Basal epidermal stem cells
Apical surface matrices	Flexible collagenous cuticle; Epicuticle (lipid); Surface coat	Rigid chitinous cuticle; epicuticle; cuticulin	Stratum corneum
Major cytoskeletal elements	cytoplasmic IFs, actin, MTs	Microtubule bundles; actin	Keratins, hemidesmosomes
Junctional complexes	hemidesmosomes; CCC, DAC	Adherens, septate junctions	desmosomes, focal adhesions, hemidesmosomes
Permeability barrier	cuticle or epicuticle	epicuticle	stratum corneum
Fate specification pathways	ELT-1/GATA	Notch; *grainy head*	BMP signaling; p63; Grhl
Innate immune response pathways	TIR-1, p38 MAPK pathway, TGFβ pathway	IMD pathway	EGFR transactivation;
Epidermal antimicrobial factors	p38 MAPK pathway; *nlp*, *cnc* genes	Cecropin	Defensins, Cathelicidins
Wound closure pathways	calcium signals	*grainy head*, AP1, Rho, PDGFR	Re-epithelialization
Sensory functions	embedded touch neurons and sensilla	sensory hairs	mechanoreceptors, thermoreceptors, nociceptors
Appendages	lateral alae	trichomes, denticles	hair, nails, teeth, glands

References: [154] (*Drosophila* innate immunity); [1] (mammals).