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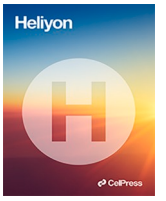

Review article

# Structural and physiological functions of *Caenorhabditis elegans* epidermis

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ABSTRACT

Research on the skin is continuously evolving, and it is imperative to select a streamlined and efficient research model. *Caenorhabditis elegans* is a free-leaving nematode whose epidermis serves as the primary barrier epithelium, composed of a collagen matrix. Differentiation of the epidermis begins in the middle of embryonic development, including polarization of the cytoskeleton and formation of cell junctions. Cuticle secretion is one of the main developmental and physiological features of the epidermis. Mutations in the collagen genes of individual worms lead to cuticle defects, thereby changing the shape of the animals. The complete genome sequence of *C. elegans* indicates that more than 170 different collagen genes may be related to this structure. Collagen is a structural protein that plays an important role in the development of extracellular matrix. Different collagen genes are expressed at different stages of matrix synthesis, which may help form specific interactions between different collagens. The differentiated epidermis also plays a key role in the transmission of hormonal signals, fat storage, and ion homeostasis and is closely related to the development and function of the nervous system. The epidermis also provides passive and active defenses against pathogens that penetrate the skin and can repair wounds. In addition, age-dependent epidermal degeneration is a prominent feature of aging and may affect aging and lifespan. This review we highlight recent findings of the structure and related physiological functions of the cuticle of *C. elegans*. In contrast to previous studies, we offer novel insights into the utilization of *C. elegans* as valuable models for skin-related investigations. It also encourages the use of *C. elegans* as a skin model, and its high-throughput screening properties facilitate the acceleration of fundamental research in skin-related diseases.

## 1. Introduction

The skin is the largest organ in the human body. Its main function is to provide a protective barrier against environmental factors, such as heat, ultraviolet (UV) radiation, infection, injury, and water loss. The skin is composed of a dense extracellular matrix (ECM) rich in collagen, which is the most abundant ECM protein, and constitutes the majority of the skin [1]. The skin undergoes natural aging over time [2]. The skin is constantly exposed to harmful environmental pressures and damages. Reduced collagen synthesis is a characteristic of aging skin in a time sequence [3]. In addition, the changes in aging skin not only affect wound healing but also make the skin more susceptible to damage [4]. The skin encases an animal and plays a core role in its form and physiological functions [5].

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The epidermis is the main barrier epithelium of animals, passively and actively defending against pathogenic organisms that penetrate the skin and promoting wound healing [5,6]. In addition to these well-known "barrier" functions, the skin has important physiological functions in immunity, endocrine and exocrine secretion, mechanical sensation, and age-dependent epidermal degeneration, which are prominent features of aging that may affect aging and lifespan [7,8].

*C. elegans* is a multicellular organism that is widely used in experiments. The epidermis is composed of a syncytium and cuticle. In *C. elegans*, the apical extracellular matrix (aECM) is the cuticle. The worm's cuticle is a complex multilayered structure consisting of an outer surface coat, a lipid-rich epicuticle, and a collagenous cuticle [5,9,10]. Recent studies have identified various components of the *C. elegans* aECM, including over 700 proteins [11]. The cuticle provides mechanical support and protection to the organism. This review examines recent research on the structure and physiological functions of the cuticle in *C. elegans*, as well as the properties of *C. elegans* skin, to provide insights into its potential use as a model for skin research.

We conducted an electronic search in PubMed database, using the following string:

*C.elegans* [text word] AND ("Epidermis" [text word] OR "Skin" [text word]. Only articles in English have detailed characterizations of the structure and physiological function of the epidermis in *C. elegans*. The reference lists of all articles were scanned to retrieve additional relevant research. In this search, we discussed only the most relevant papers in the review as personally evaluated by the authors.

## 2. Cuticle structure of *C. elegans*

### 2.1. The composition and changes in the C. elegans cuticle

The cuticle of *C. elegans* is synthesized by epidermal cells [12]. It is attached to muscles through hemidesmosome [13,14]. Collagen is the primary protein component. The cuticle is synthesized five times during development and sheds with each molt, first during embryogenesis, when the cuticle is formed in the L1 larva, and then again during the four larval stages before each molt. During synthesis, actin filaments are assembled in the epidermal cells and surround the cylindrical body of the worm. The alignment of these filaments corresponds to the furrows formed on the cuticle surface by membrane-bound actin filaments during cuticle synthesis (Fig. 1) [15–19]. At certain developmental stages, in addition to rings, there are longitudinal ridges called alae (Fig. 1) [20]. Although both structures can be observed optically, the cuticle is transparent, creating the illusion of true complexity. As observed under an electronic microscope, it may be composed of six distinct layers at the sub-microscopic level [21]. Recent studies have suggested that the arrangement and organization of collagen proteins play important roles in the development and maintenance of *C. elegans* morphology [22].

In skin composition, collagen genes are encoded by a polygenic family of approximately 177 members [23]. Through random mutagenesis of *C. elegans*, about 100 genes sites have been identified that affect the body shape of *C. elegans* [11,22,24]. At present, there are 17 known gene sequences, nine of which are cuticle collagen genes (*bli-1*、*bli-2*、*dpy-2*、*dpy-7*、*dpy-10*、*dpy-13*、*rol -6*、*sqt-1* and *sqt-3*) [25,26]. The phenotypes associated with collagen gene mutations fall into three basic types: (1) BLIster (Bli), where the detachment of the outer layer of the cuticle results in a blister appearance; (2) DumPY (Dpy), where the body is shorter than the wild type; and (3) ROLler (Rol), where the body moves in a spiral twist (Fig. 2) [27,28]. However, these phenotypes are not necessarily fixed or specific. The Bli phenotype is limited to the adult stage and recognizes cuticle structures unique to adults [22]. The Dpy phenotype can affect all stages of development after the embryonic stage, and mutations in different *dpy* genes can cause large or small effects at different stages. Mutations that cause *bli*, *dpy*, or a combination of *dpy* and *rol* are usually recessive. Mutations that cause *rol* but are not *dpy* are usually dominant. Recessive alleles result in inherited invalid genes, but most are glycine replacements within the collagen domain [29]. The dominant alleles include mutations in the "subtilisin protease" cleavage site, which is necessary for processing intact collagen [30,31]. Some of the mutations that lead to these phenotypes may be recessive and lead to a loss of function, suggesting that at

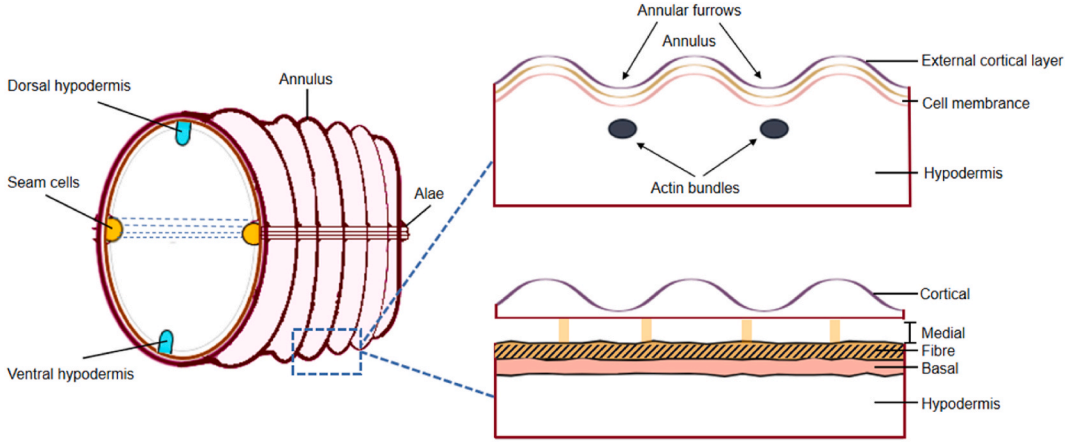


**Fig. 1.** Epidermal and subcutaneous structures of *C. elegans*.

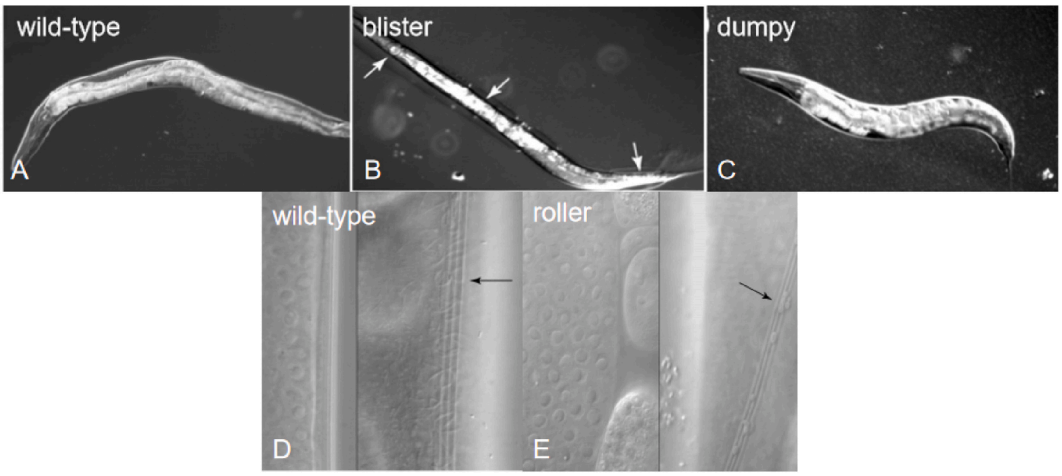


**Fig. 2.** Mutant phenotype of collagen gene in *C. elegans* [29].

least some collagens play a specific role in cuticle structure formation [15,32]. Therefore, changes in the body structure of *C. elegans* are closely related to collagen expression in the skin. Additionally, the researchers generated a single copy insertion transgenic *C. elegans* strain that expresses a GFP fused collagen protein in the epidermis, allowing them to monitor and quantify the protein mass of collagen through fluorescent intensity [33]. This enables the researchers to study collagen secretion and assembly in a high-throughput manner. Overall, the characteristics of *C. elegans*, such as its simplicity, transparency, and ability to express and monitor collagen proteins, make it a suitable model for screening compounds that promote collagen secretion.

### *2.2. Specificity and homology of the cuticle in C. elegans compared with that in other species*

In all animals, the epidermis plays a critical role in growth and survival. This section provides an overview of the basic structure and development of the epidermis, highlighting the similarities between different animal groups and the main specificity of *C. elegans* (Table 1) [34].

The epidermis of all animals is epithelial, with the surface facing the environment. The epidermis of *C. elegans* is a layer of epidermal cells. The upper surface of the epidermis secretes a flexible collagenous cuticle. The structure and function of the epidermis and cuticle are mutually dependent and thus are referred to as the "epidermis-cuticle complex" [35]. In contrast, the epidermis of insects, such as flies, is a simple cuboidal epithelium that secretes a hard chitin cuticle [36]. The epidermis of vertebrates is typically multilayered. Fish have a fully cellular epithelium, while land vertebrates have an epithelium covered by a layer of keratinized cells [37]. In mammals, the epidermis initially forms a brief outer layer known as the stratum corneum. Subsequently, the epidermis undergoes a stratification program, accompanied by the development of a barrier function, such that the newborn skin is composed of multiple major cell layers [38].

The epidermis and nervous system are derivatives of the ectoderm; one of the main early events in the development of the embryo is the separation of epidermal and neural precursor cells from the ectoderm, followed by their internalization [39]. The epidermal cells, which constitute the ventral surface of the worm upon hatching, undergo migration towards the ventral midline as they acquire a neuroblast fate [40]. In flies, the epidermis extends laterally and is surrounded by ectoderm at the midline of the back [41]. In mammals, the epidermis is initially a single-layer multipotent cell, which then stratifies or forms appendages, such as hair or nails.

**Table 1**
Comparison of *C. elegans, Drosophila,* and mammalian epidermis.

| | *C. elegans* | Drosophila | Mammalian |
|---|---|---|---|
| Epidermal epithelial cell | Syncytial; Simple epithelium | Cuboidal cellular epithelium | Stratified epithelium |
| Adult size | ~0.1 $mm^2$ | ~$10^6$-$10^7$ cells | ~$10^{11}$ cell (eg. human) |
| Growth period | Four molt stages | Larval molts and imaginal disks | Basal epidermal stem cells |
| Surface structure | collagenous cuticle; lipid; | chitinous cuticle; epicuticle; cuticulin | Stratum corneum |
| Major cytoskeletal elements | cIFs; actin; microtubules (MTs) | MT bundles; actin | Keratins; HDs |
| Fate pathways | ELT-1/GATA | Notch | BMP; p63; Grhl |
| Innate immune response pathways | TIR-1; p38MAPK: TGF-β | IMD | EGFR |
| Antimicrobial factors | p38 MAPK; nlp; cnc gene | Cecropin | Defensins and cathelicidins |
| Wound healing pathways | $Ca^{2+}$ signals | AP1、Rho、PDGFR | Epidermal self-regeneration |
| Sensory functions | Embedded touch neurons and Sensilla | Sensory hairs | Mechanoreceptors; Thermoreceptors; nociceptors |
| Attachment | Lateral alae | Trichomes, denticles | Hair, nails, teeth, glands |

Neurons develop in the midline and are enclosed by the neural tube and internalized. Skin growth is crucial to animal growth. In *C. elegans* and flies, new epidermal cells are formed through cell division within the stem cell epidermis. The skin of an adult fruit fly is formed from an adult disc that is left aside during embryonic development. During embryo growth and after birth, the skin of mammals undergoes constant division and renewal of basal stem cells [41].

While some aspects of the epidermis and cuticle of *C. elegans* are clearly specialized aspects of the body structure of *C. elegans*, others may reflect epidermal features that are retained by all metazoans [42]. For example, the epidermis of *C. elegans* contains cytoplasmic intermediate filaments (cIFs) and hemidesmosomes (HDs) that provide mechanical strength similar to that of keratin cIFs and HDs in mammalian skin. Although the molecular compositions of the nematode, fruit fly cuticle, and mammalian aECM are very different, all require enzymatic cross-linking of the substrate proteins to form a mechanically strong osmotic barrier [43]. The cuticle is composed of multiple layers secreted by the epidermal cells, including the outermost lipid layer [44]. Findings from conserved families of transcription factors that regulate epidermal development in fruit flies and mammals suggest that there may be other homologies or functional similarities between layers of skin [43]. In summary, recent studies have significantly expanded our understanding of aECM biogenesis and function across various model organisms, shedding light on shared structural and functional principles. With a comprehensive inventory of aECM components at hand, the next crucial step will be to systematically analyze their expression patterns, akin to the recently generated basement membrane toolkit for *C. elegans* [45].

## 3. The functions of the epidermis of *C. elegans*

The differentiated epidermis plays many roles in the life of *C. elegans*. Similar to all skin layers, the main function of the epidermis is to act as a barrier. The epidermis not only acts as a passive barrier but also actively participates in wound healing, triggers the skin's innate immune response against pathogens, maintains ion balance, stores metabolic materials, and engulfs cellular debris through phagocytosis within the skin layer [46–48]. Finally, the epidermis mediates the communication between sensory neurons and the external environment. The focus of this section lies in exploring the physiological functions of the *C. elegans* epidermis, which have served as a source of inspiration for our investigation into skin-related mechanisms.

### 3.1. Barrier function of C. elegans epidermis

In the natural environment, whether in soil or humus, *C. elegans* often experiences alternating dry and humid conditions [49]. Therefore, the epidermis-cuticle composite of *C. elegans* can prevent internal solutes from leaking into the external environment and prevent substances in the external environment from entering the body through the epidermis. Pharmacological research has shown that the permeability of the cuticle of *C. elegans* to small molecules is low, and the effective concentration of drugs for *C. elegans* is usually several orders of magnitude higher than that used for cell culture. The epidermis also acts as a barrier. Tight junctions prevent solutes from leaking between adjacent epidermal cells [50]. *C. elegans* are protected by a robust cuticle, forming a barrier for the uptake of chemicals. This may pose obstacles to screening drug toxicity. In recent years, researchers have evaluated mutants with altered cuticle characteristics to identify sensitive strains suitable for toxicological testing. By assessing the trade-off between increased permeability and reduced adaptability, the *bus-5 (br19)* strain was determined as the most appropriate strain for chemical exposure [51]. In addition, recent research has discovered several epidermal gene mutations that affect *C. elegans* barrier function. These mutated genes involve various functions, including glycosylation, phosphoinositide phosphatase, Patched-related receptors, steroid dehydrogenase, and glucosyltransferase factors. It is worth noting that many of these mutants exhibit fragility in the stratum corneum, such as *srf-5*, *bus-10* and *bus-28* (D [52]). Specific types of collagen proteins are crucial for maintaining the permeability barrier of the cuticle in *C. elegans*. Loss of these collagen proteins leads to increased susceptibility to exogenous toxins and decreased survival rates.

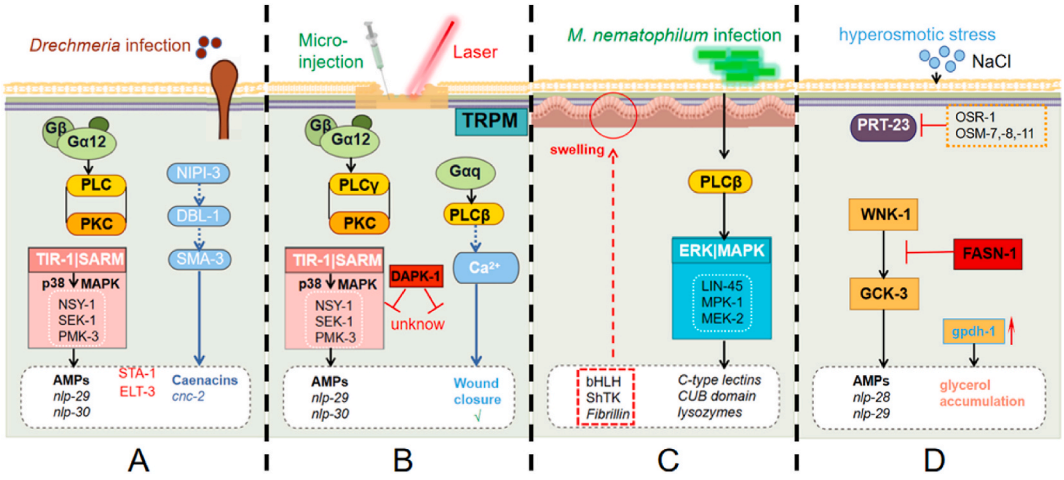


**Fig. 3.** Epidermal response to infection, injury, and stress.

The transcription factor BLMP-1 regulates the expression of these collagens, playing a role in maintaining the barrier function of the cuticle. It has been found that permeability determining collagens (DPY-7, DPY-8, DPY-9, DPY-10) are essential for maintaining the barrier resistance against exogenous toxins in the cuticle. Additionally, collagens required for groove formation (DPY-2 and DPY-3) have also been found to be important for the permeability barrier function of the cuticle [23,53]. This suggests that different collagen proteins may have distinct roles in maintaining permeability barriers and defending against exogenous molecules and toxins. These studies have enhanced the effectiveness of *C. elegans* in convenient toxicity assessments, contributing to a reduction in the use of vertebrates during critical early stages of product development. The strains identified in these studies will also enhance the sensitivity of *C. elegans* based drug discovery platforms.

### 3.2. Function of the epidermis in protecting C. elegans from pathogens and skin damage

In *C. elegans,* the epidermis is the first line of defense against external pathogenic organisms and environmental damage. A variety of pathogenic bacteria, fungi, and nematodes can penetrate the epidermis through the skin of *C. elegans* [47]. *C. elegans* can be infected by fungi, such as *D. coniosporia,* which penetrate the cuticle and have evolved specialized structures for the attachment or penetration of the cuticle (Fig. 3A) [47].

The response to cuticle penetration by the pathogenic organisms was closely related to the response to physical damage (Fig. 3B). Wound healing has been well studied in *C. elegans* [47]. Other nematodes, such as *Oncholaimus* exhibit "traumatic insemination," in which the male punctures the cuticle of the hermaphrodite during copulation by observing the wound created during copulation [54]. To survive in the wild, *C. elegans* must actively and passively protect itself from potentially lethal damage. Other pathogens, such as *Mycoplasma nematophilum* and *Yersinia,* do not penetrate the epidermis but persist on its surface which is perceived in some way by the worm and triggers swelling of the underlying epidermal cells (Fig. 3C) [55,56].

Although the skin structure of *C. elegans* is different from mammals, it still shares similarities in terms of wound response and repair. These similarities include the activation of the innate immune system and the remodeling of the permeability barrier. This suggests that studying wound response and repair in *C. elegans* can provide valuable insights into these processes in mammals. Triggering an immune response when the body is injured has a preventive effect and safeguards against the entry of potentially pathogenic microorganisms. As a host-pathogen interaction and skin innate immune model, the response of *C. elegans* to penetration of the cuticle by the fungus *Drechmeria coniospora* was studied. After infection with *Drechmeria*, *C. elegans* triggers the transcriptional upregulation of many small peptides that were initially labeled as neural peptide-like proteins (NLP), which have been proven to have antimicrobial activity [57]. The genes controlling these proteins include *nlp-29* clusters [58]. These genes are mainly regulated in an autonomous manner [59]. Non-infectious damage also induces the antimicrobial peptide (AMP) gene regulation of *nlp-29* transcription [60,61]. In general, peptides induced by injury are also induced by infection, indicating that the response to infection includes a response to non-specific damage. Other responses are infection-specific and require the tribolle-like kinase NIPI-3. The pathways mediating AMP induction after infection and injury are similar, but not identical, suggesting that the epidermis somehow distinguishes between sterile injury and pathogen infection, with different mechanisms acting on wound healing. In both cases, these changes in gene expression were caused by the activation of the GPCR DCAR-1, which acts upstream of the conserved p38 MAPK pathway [62]. The p38 MAPK PMK-1 acts upstream of the STAT transcription factor-like protein STA-2 [63]. Although the exact details are unknown, STA-2 activation is believed to require the SLC6 family protein SNF-12 [64]. Signal transduction in the epidermis also includes G protein signaling, toll-interleukin-like junction TIR-1, and transcription factors ELT-3 and STA-1 [53,60–62,65]. Part of the independent infection response involves the DBL-1 pathway, which triggers the expression of caenacin family peptides [66].

Damage to the epidermis, through microinjection needles or laser irradiation, triggers nematode healing. To ensure long-term protection, injured tissues must be repaired to maintain tissue integrity. Thus, in addition to AMP gene induction, skin damage in *C. elegans* triggers a $Ca^{2+}$ -dependent signaling cascade that promotes wound repair through actin ring closure [67]. $Ca^{2+}$ in the epidermis promotes wound healing by triggering the formation of actin rings at wound sites. Actin regulatory and polymerization factors are required for the correct formation of actin rings. However, the loss of function of non-muscle myosin leads to faster ring closure, suggesting that myosin contractility may affect ring closure. Thus, wound repair in *C. elegans* is paralleled to the innate immune response that accompanies the injury [68,69]. Recent studies have shown that the *pals-25(Q293*)* mutant activates nuclear localization of the transcription factor ZIP-1 in epidermal cells. This activation leads to the induction of immune gene expression and promotes systemic resistance to pathogens [70]. The genetic tractability and live imaging opportunities available in *C. elegans* make it a powerful model organism for studying the fundamental mechanisms of wound detection, response, and membrane repair. The findings from *C. elegans* studies can reveal novel genes and signaling pathways involved in wound response and repair, which can be further tested in mammals. Additionally, the results of nematode studies are already influencing how we approach questions about wound healing in vertebrates. Therefore, *C. elegans* can be considered an important model for studying wound response, repair, and remodeling, complementing research carried out in other species.

### 3.3. Physiological function of the epidermis in C. elegans

#### 3.3.1. Resistance to osmotic shock and other pressure stimuli

The mature epidermis plays multiple roles in the animal body, including osmotic regulation, ion homeostasis, metabolic storage, and mechanical sensation. The activation of glycerol-3-phosphate dehydrogenase 1 expression by high osmotic shock increased the level of intracellular glycerol (Fig. 3D); however, high glycerol expression was insufficient to produce osmotic resistance, indicating the existence of other pathways [71]. GCK-3 and WNK-1 play a role in regulating osmotic stress response in the hypodermis and

excretory cells of *C. elegans*. Studies have shown that GCK-3 functions in the intestine and hypodermis to mediate acute volume recovery after hypertonic stress. Tissue-specific RNAi experiments have revealed that *rde-1(ne219)* worms, in which the gene was rescued in the hypodermis and excretory cells showed normal volume recovery and survival. However, when GCK-3 dsRNA-producing bacteria were fed to *rde-1(ne219)* worms with gene rescue in the hypodermis, their survival and volume recovery at high NaCl concentrations were significantly reduced. This suggests that GCK-3-regulated solute and water transport mechanisms in the intestine and hypodermis are important for systemic volume recovery and survival following hypertonicity-induced water loss and shrinkage [72,73]. The epidermis response to osmotic stress overlaps with its transcriptional response to infection. However, the response to pathogens and osmotic stress is different, indicating that the epidermis can distinguish stress [48,74].

In addition to these functions in osmotic resistance, the epidermis plays a key role in resistance to environmental toxins. For example, the MEK-1 MAP kinase cascade plays a role in conferring resistance to heavy metal stress [75]. Resistance to $H_2S$ or HCN toxicity involves the expression of epidermal oxidases such as SQRD-1 [76]. Finally, the expression of HRG-2, a transporter required for the uptake of hemoglobin in the epidermis, is upregulated by a deficiency of hemoglobin, indicating the existence of a regulatory pathway dedicated to the maintenance of hemoglobin homeostasis in the epidermis [77]. Physical contact between the aECM and epidermis is maintained through structures called hemidesmosomes and meisosomes. Meisosomes are present in the lateral epidermis and serve as connections between the epidermis and the cuticle. They are composed of stacked parallel folds of the epidermal plasma membrane alternately filled with a cuticle. They are believed to play a role in maintaining the structural integrity of the cuticle and epidermis [15]. Recent studies have shown that a damage sensor associated with furrows may be involved in coordinating three different stress defense pathways: osmolyte accumulation, antimicrobial response, and SKN-1 dependent detoxification genes. The disruption of annular furrows in the cuticle can lead to the accumulation of osmolytes, activation of the *nlp-29* gene, and activation of *skn-1* dependent detoxification genes [78]. These responses may be achieved through the detection and transmission of extracellular mechanical stimuli that generate ROS inside the cells. The disruption of annular furrows in the cuticle likely changes the mechanical strain on these structures, which is associated with the turgor pressure against the body wall. This mechanical strain may trigger the activation of osmolyte accumulation in response to hypertonicity-induced water loss, leading to the accumulation of osmolytes such as glycerol. This suggests that the signaling mechanism associated with furrow disruption may define a novel mode of signaling for the conserved family of stress and longevity factors. However, the specific details of this signaling mechanism and its implications for the SKN-1/Nrf pathway require further investigation [53]. The cuticle of *C. elegans* plays a role in coordinating three core environmental stress responses: detoxification, hyperosmotic, and antimicrobial responses. The activation of these stress responses is mediated by various transcription factors, including SKN-1/Nrf, ELT-3/GATA, and STA-2/Stat. These findings suggest the presence of a damage sensor associated with the furrows in the cuticle that coordinates these stress defense pathways.

### *3.3.2. Lipid storage*

Although the gut is the main fat tissue in worms, the epidermis is also an important site for fat accumulation. Detection of fat using Nile red staining may underestimate the role of the epidermis in fat storage because Nile red dye is not effective in staining the epidermis [79]. Quantitative microscopy has shown that the epidermis accounts for approximately 10 % of fat storage in wild-type worms, whereas dauer larvae account for approximately 50 % of fat storage [80]. The epidermis is also an active site for the AMPK pathway, which inhibits lipid hydrolysis and promotes long-term cell survival. Studying lipid particles in *C. elegans* can contribute to our understanding of fat metabolism and regulation in both invertebrates and vertebrates.

### *3.3.3. Cell death and injury in the epidermis*

*C. elegans* lacks specialized "specific" phagocytic cells. Dead cell debris is typically phagocytosed by neighboring tissues, usually the epidermis. Phagocytosis of apoptotic cell debris by the epidermis can be observed during epidermal enclosure, indicating that the epidermis expresses phagocytic mechanisms during early development. During the postembryonic development of the nervous system, apoptotic cells are phagocytosed by adjacent epidermis, as well as necrotic or degenerating neurons [81]. This function is similar to the clearance of glial cells in other organisms [82].

Only a few cell death programmes occur in the epidermis of *C. elegans*, such as in the epidermal tentacles [83]. In *lin-24* and *lin-33* mutants, cells on the ventral side of the epidermis undergo pathological death [84]. This "cytotoxic" death is different from apoptosis or necrosis. These mutants do not depend on the CED-3 cysteine protease but partially depend on phagocytic mechanisms. *pvl-5* mutants exhibit unique CED-3-dependent non-apoptotic cell death [85,86]. Epidermal cells may not express the components of the apoptotic mechanism and may depend on CED-3 transcriptional activation to induce death.

The primary site of autophagy is the epidermis. Autophagosomes are prominent in the epidermis, and autophagy plays an important role in epidermal remodeling during cell development [87]. It has been reported that autophagy reduction leads to reduced cell and body sizes. Autophagy and apoptosis may play a common role in the shaping of the epidermis [88].

### *3.3.4. Function of neurons in the epidermis*

Most sensory neurons in *C. elegans* are closely associated with the epidermis, which reflects their sensing behavior. Sensory neurons that detect water-soluble chemicals, such as head and tail sensory neurons, are exposed to the external environment through openings in the cuticle, whereas other sensory terminals are embedded in specialized cuticle structures [89].

Other mechanical sensory neurons do not directly contact the cuticle but are embedded in the epidermis. During larval development, ALM and PLM are gradually encapsulated by the epidermis and secrete a special ECM membrane that adheres to epidermal cells [90]. Mechanical sensory neurons recruit the ECM protein HIM-4 and induce TEs in neighboring epidermal cells [91]. Although HIM-4-dependent TEA recruitment is not essential for mechanical sensing, it may enhance the sensory sensitivity under certain

conditions.

Increasing evidence suggests that the epidermis can affect neuronal function by controlling local ion homeostasis or providing other signals or nutrients near the neurons. The function of the TRPM channel GTL-2 in the epidermis is lost, which can inhibit the increase in the activity of motor neurons [92]. Electronic microscopic observations of continuous slices showed synaptic-like structures between neurons and the epidermis, indicating that the activity of neurons can be fed back to the epidermis. The interaction between the epidermis and nervous system requires further study [93]. To regulate sensory dendrites in *C. elegans*, studies have shown that epidermal cells play an active role. Epidermal cells instruct and stabilize dendritic growth by forming SAX-7/L1CAM stripes. L1CAM family proteins, including SAX-7, are required for neural development in various organisms [94]. It is possible that higher organisms also express L1CAM homologues to regulate dendritic growth. The study of epidermal neurons in *C. elegans* has provided valuable insights into the regulation of dendrite patterning and degeneration. These findings have implications for mammalian research, particularly in the field of sensory regeneration in patients with burns or physical lesions. The lessons learned from invertebrate studies, although with some variations, can potentially be extrapolated to mammalian systems.

The epidermis of *C. elegans* plays a role in endocrine signaling. The epidermis expresses nuclear hormone receptors and cytochrome P450 enzymes, such as DAF-12 and DAF-9, which are involved in promoting normal development and regulating the dauer state. DAF-9 is important for the production of steroidal hormones, suggesting that the epidermis is a site for autocrine feedback and signal amplification in the Dauer decision [95,96]. Additionally, insulin signaling, which is important for the dauer decision, may involve the epidermally expressed insulin-like ligand INS-33 [97]. Therefore, the differentiated epidermis of *C. elegans* is involved in the synthesis and secretion of steroidal hormones and insulin-like ligands involved in hormone signaling.

### *3.4. Aging of the epidermis in C. elegans*

Studies on aging in *C. elegans* have mainly focused on the epidermis and cuticle. Different species of aging *C. elegans* show progressive degeneration of cuticle structure and barrier function, and the thickness of the surface layer may decrease [98,99]. The expansion and protrusion of the epidermis adjacent to muscles indicate that the cumulative effect of muscle contraction leads to local loss of epidermal integrity [100]. Previous studies have revealed that the main structural changes in the aging epidermis of *C. elegans* include cellular degeneration, lipid vesicle accumulation, and thickening of the cuticle [101]. The epidermis of aging *C. elegans* showed membrane damage, indicating that the direct cause of death in aging *C. elegans* may be the destruction of epidermal integrity leading to the loss of barrier function.

Genetic and genomic studies have suggested that the epidermis actively regulates age-dependent changes. The quality of oocytes in the reproductive system is affected by the expression of the TGFβ signaling pathway in the epidermis, indicating a connection between the epidermis and the reproductive system [102]. Age-dependent changes in gene expression are regulated by epidermal GATA factors ELT-3, -5, and -6. The expression levels of *elt-5* and *-6* increases with age, leading to the progressive inhibition of *elt-3* [103]. These findings indicate that the aging and lifespan of *C. elegans* are regulated by several evolutionarily conserved signaling pathways. The regulation of transcription in the epidermis of *C. elegans* is closely related to the aging process. Studies have shown that transcription and regulatory factors in epidermal cells can regulate the expression of a series of aging-related genes. These genes encode antioxidant enzymes, DNA repair enzymes, and heat shock proteins, which play important roles in maintaining cellular homeostasis and delaying the aging process. Some transcription factors, such as DAF-16, SKN-1, and HIF-1, have altered expression levels in the epidermal cells during aging of the *C. elegans*, thereby affecting the worm's lifespan [104]. (1) Inhibition of the insulin/insulin-like growth factor-1 signaling pathway (IIS) extends the lifespan of *C. elegans* by activating the process of diapause associated with DAF-16/Fork head box stress tolerance [105,106]. (2) Inhibition of IIS also extends the lifespan of *C. elegans* through the Nrf2 homologues SKN-1, which is genetically different from DAF-16 [107]. (3) SKN-1 regulates the expression of genes involved in ECM formation, such as collagen. In *C. elegans*, the expression of collagen decreases with age, and the lifespan of long-lived *C. elegans* mutants delays the progressive decrease in collagen quality, ECM suppression of aging, and ECM degradation [108]. By prolonging the synthesis of collagen during the lifespan of *C. elegans*, the aging process can be slowed down, which is crucial for the health of *C. elegans*. Aging of nematodes is related to the changes in epidermal collagen content. Therefore, a precise evaluation of collagen deposition can unveil the mechanisms underlying maintenance and remodeling of the extracellular matrix during the aging process. In addition to studying the gradual decline in extracellular matrix integrity with age in mice, *C. elegans* provides another way to explore this aging phenomenon: the transparent outer skin of *C. elegans* can be used to label extracellular matrix components with fluorescent proteins, thereby directly monitoring the homeostasis and integrity of the extracellular matrix non-invasively [109]. Similar to mammals, collagen synthesis declines during aging in *C. elegans*. Studying *C. elegans* mutants that delay this progressive decrease in collagen mass has provided insights into the importance of proper collagen deposition and remodeling for healthy aging and longevity.

## 4. Conclusion

Compared to mouse models or other mammals, histological methods are still not mature for application in *C. elegans*, and the biological analysis of epidermal differentiation in *C. elegans* is still in its early stages. However, because of the simplicity of the epidermis in *C. elegans*, which is composed of a simple epithelial layer and a cuticle, its complete genome sequence is known and its capacity is small, making it a powerful model for studying the biology of skin development and capable of high-throughput drug screening. The cuticle of *C. elegans* plays an important role as a self-assembling cellular extracellular structure in skin-related studies. The extracellular lipid bilayer of the epidermis in *C. elegans* is a crucial cell-extracellular lipid bilayer, similar to the one found in vertebrate keratin, and warrants further investigation in molecular genetics. The epidermis plays a critical role in the interaction

between *C. elegans* and its environment. A better understanding of these surface structures would be beneficial for studying more complex skin structures. This study has potential value for research on innate immunity, wound healing, and aging in *C. elegans* based on the epidermis. Advanced methods for studying the skin of *C. elegans* are expected to apply to more complex skin systems.

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## Data availability

No data were used for the research described in the article.

## CRediT authorship contribution statement

**Enhui Wang:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Conceptualization. **Yanfei Jiang:** Writing – review & editing, Supervision, Conceptualization. **Chunyue Zhao:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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