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Epidermal Wound Healing in the Nematode *Caenorhabditis elegans*

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Significance: Healing of epidermal wounds is a fundamentally conserved process found in essentially all multicellular organisms. Studies of anatomically simple and genetically tractable model invertebrates can illuminate the roles of key genes and mechanisms in wound healing.

Recent Advances: The nematode skin is composed of a simple epithelium, the epidermis (also known as hypodermis), and an associated extracellular cuticle. Nematodes likely have a robust capacity for epidermal repair; yet until recently, relatively few studies have directly analyzed wound healing. Here we review epidermal wound responses and repair in the model nematode *Caenorhabditis elegans*.

Critical Issues: Wounding the epidermis triggers a cutaneous innate immune response and wound closure. The innate immune response involves upregulation of a suite of antimicrobial peptides. Wound closure involves a Ca^{2+} -triggered rearrangement of the actin cytoskeleton. These processes appear to be initiated independently, yet, their coordinated activity allows the animal to survive otherwise fatal skin wounds.

Future Directions: Unanswered questions include the nature of the damage-associated molecular patterns sensed by the epidermis, the signaling pathways relaying Ca^{2+} to the cytoskeleton, and the mechanisms of permeability barrier repair.

SCOPE AND SIGNIFICANCE

THIS REVIEW FOCUSES on epidermal wound repair in nematodes such as *Caenorhabditis elegans*. Two major branches of the wound repair process are discussed: cutaneous innate immune responses to damage and mechanisms that close the wound. Analysis of the genetic basis of these responses has revealed potentially conserved pathways involved in epidermal wound repair, including a G-protein-coupled receptor (GPCR)/mitogen-activated protein kinase (MAPK) cascade in innate immunity and a transient receptor potential M class (TRPM) channel/ Ca^{2+} pathway that regulates the actin cytoskeleton.

TRANSLATIONAL RELEVANCE

Despite the major differences in morphology and molecular composition of skin layers between different animal groups, increasing evidence points to the conservation of underlying molecular pathways in wound repair. Well-known examples are the Grainyhead/Grhl transcription factors involved in barrier repair in insects and in mammals. Studies of wound responses in *C. elegans* have pointed to conserved roles for plasma membrane TRPM channels in epithelial Ca^{2+} signaling. Reactive oxygen species (ROS) may also play ancient and conserved roles in promoting tissue repair. Additional

wound healing pathways discovered in simple models could provide new leads for therapies targeting wound healing pathologies.

CLINICAL RELEVANCE

Clinically, important wound healing pathologies range from excessive wound healing (keloid and hypertrophic scarring) to nonhealing chronic wounds and diabetic ulcers. The latter constitute a growing public health problem, costing an estimated \$5–\$10 billion annually, in the United States. Improved understanding of epidermal repair mechanisms in simple models will enhance our understanding of wound healing biology and should contribute to better approaches to wound care.

BACKGROUND AND OVERVIEW OF WOUND HEALING PROCESSES AND MODELS

Wound healing is required for organismal integrity and survival and an essential precursor to skin regeneration.¹ Wound healing in the mammalian skin involves a large number of tissues, cellular processes, and signaling pathways. In overview, three main branches of wound repair are the epidermal innate immune response, cell migration and wound closure, and barrier repair.^{2,3} Epidermal barrier epithelia of different animals display many differences in the structure and molecular composition, yet are increasingly recognized as being built on a common genetic ground plan. For example, transcription factors of the Grainyhead family play conserved roles in barrier epithelium formation and wound healing.^{4,5}

The complexity of mammalian skin wound repair has motivated examination of simpler models of wound healing. Studies of wound repair in the fruit fly *Drosophila* have identified signal transduction pathways, transcription factors, and cytoskeletal regulators involved in wound repair.^{6–9} Studies of repair in genetic model organisms such as *Drosophila* also allow the use of unbiased forward genetic screens to identify new and unexpected contributors to wound repair.^{8,10} Zebrafish are a tractable model for wound repair; studies in zebrafish revealed the role of ROS as wound-produced chemoattractants.^{11,12} Studies of scarless wound healing in embryos, mutants, or species that do not display scarring have highlighted the role of the inflammatory response in scar formation.^{13,14} Finally, single cell wound healing in models such as the *Xenopus* oocyte or the early *Drosophila* embryo reveals parallels between the single cell and epithelial repair processes.^{15,16} Here, we review wound healing in the nematode *C.*

elegans, a comparatively recent addition to the pantheon of wound healing models.

DISCUSSION

C. elegans epidermal biology

Nematode worms are a large and successful animal phylum with representatives in almost every ecological niche. *C. elegans* is a small free-living nematode well known for its tractability for molecular and genetic manipulation: animals are small (1 mm long) and easily propagated in the laboratory on bacterial food sources. The generation time of *C. elegans* is 3 days and the lifespan ~3 weeks. *C. elegans* is also essentially transparent, facilitating imaging of epidermal cells and subcellular structures during wounding *in vivo*.

The skin of *C. elegans* like that of most nematodes is composed of a simple epithelium, the epidermis, or hypodermis, which secretes the outer extracellular layer or cuticle (Fig. 1); the biology of the nematode epidermis has recently been reviewed in depth.^{17,18} Nematodes lack a rigid exoskeleton; the organismal shape is maintained by internal pressure (the hydrostatic skeleton). The collagenous cuticle is flexible yet contributes to

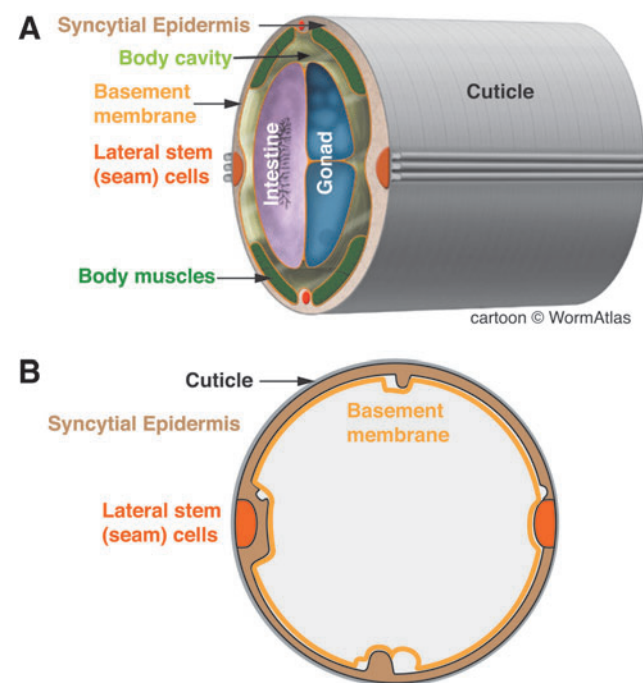


Figure 1. Anatomy of the *Caenorhabditis elegans* epidermis. (A) Stereoview of the adult hermaphrodite midbody, adapted from WormAtlas (www.wormatlas.org, Z. Altun and D.H. Hall, 2012). Major tissues are color coded. (B) Cross section, highlighting the epidermis and associated extracellular matrices (cuticle and basement membrane). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

the overall rigidity to the animal. In addition to the cuticle, additional less well-characterized extracellular layers (epicuticle and surface coat) are present and may contribute to the permeability barrier function of the skin.

The mature *C. elegans* epidermis consists of syncytial cells formed by cell–cell fusion as well as lateral stem cells (seam cells). In postembryonic development, the epidermis grows in size by addition of new cells from the seam; in adults, the epidermis is postmitotic and undergoes polyploidization to support continued growth. Thus, the adult epidermal syncytium combines the advantages of single-cell wound models (accessible imaging and cell biology) with the characteristics of a differentiated barrier epithelium.

Evidence for epidermal wound healing in nematodes

The literature on wound healing in nematodes is remarkably sparse. Abnormalities in tail morphology in nematodes collected from the wild were interpreted as resulting from imperfect wound healing or regeneration by Allgén.¹⁹ However, wound healing was not directly assayed, and there appears to have been little follow-up to these early observations. In 1988, Poinar concluded that “although the paucity of the information on the subject leaves one unable to draw any definite conclusions on wound healing and regeneration, the observations of Allgén and others indicate that some type of wound repair may exist in nematodes and that further investigation into this subject is warranted”.²⁰ Indeed, it has long been known that *C. elegans* survives epidermal puncture wounds caused by microinjection needles, initially used for electrophysiological recording²¹ and subsequently for introduction of macromolecules such as nucleic acids.²² Wounding by stabs with a microinjection needle is a simple and robust method to elicit epidermal wound responses, but may also damage internal tissues. More precise wounding of the epidermis using femtosecond laser irradiation induces a subset of wound responses (see next section).

Cutaneous innate immune responses to wounding and infection

The first wound response to be characterized in detail in *C. elegans* is the epidermal innate immune response. Analysis of the epidermal innate immune response to damage began with pioneering studies of skin-penetrating pathogens.²³ Many nematophagous (nematode-eating) fungi attack their hosts through the skin.²⁴ Fungi such as *Drechmeria coniospora* generate spores that stick to the cuticle and extend hyphae through the underlying epidermis to eventually colonize the animal.

Fungal infection specifically induces epidermal expression of a large set of antimicrobial peptides (AMPs) and other proteins.^{25,26} The signal transduction pathways responsible for induction of AMP expression in response to infection have been extensively characterized and reviewed recently.^{27,28} In overview, at least two major pathways regulate epidermal AMP induction: a MAPK cascade required for induction of the neuropeptide-like (*nlp*) genes²³ (Fig. 2), and a TGF β cascade involved in induction of caenacin (*cnc*) peptide expression.²⁹ As the role of TGF β signaling in the response to wounding is not yet clear, we focus here on the pathway involved in *nlp* AMP induction.

The process of skin penetration by fungal hyphae resembles wounding, leading to the question of whether responses to infection are specific to the pathogen or are more general responses to skin damage. Using needles or lasers to wound the skin, Pujol *et al.* showed that physical damage was sufficient to induce some of the epidermal AMPs that are induced by infection, through the same MAPK cascade involved in AMP induction after infection.³⁰ The Tribbles-like kinase NIPI-3 is preferentially required for AMP induction after infection but not wounding, suggesting infection and wounding act through different upstream sensors that converge on common outputs to regulate AMP expression.³⁰ The chaperone HSP-3 is also specifically required for infection-induced but not for wound-induced AMP expression.³¹ Because *C. elegans* is constantly associated with its bacterial food source (*E. coli* in the laboratory), complete sterile wounding has not been performed. However, these experiments suggest that the innate immune response to infection overlaps with a transcriptional response to epidermal or cuticle damage. Although nematode AMPs do not resemble mammalian AMPs in primary sequence, sterile injury rather than infection or inflammation is a major inducer of the mammalian cutaneous innate immune response.³²

Upstream of the TIR-1/MAPK pathway, AMP induction by wounding is known to require a signaling cascade involving PKC δ /TPA-1, phospholipase C γ /PLC-3, the G α protein GPA-12, and the G β -like protein RACK-1.³³ The involvement of G protein signaling in the innate immune response to wounding suggests that one or more GPCRs sense tissue damage or a ligand generated by damage. Such host-derived damage-associated molecular patterns (DAMPs)³⁴ have been identified in some paradigms of injury³⁵ but have not yet been characterized in *C. elegans*.

Fungal infection and wounding also induce production of ROS through the Ca²⁺-activated

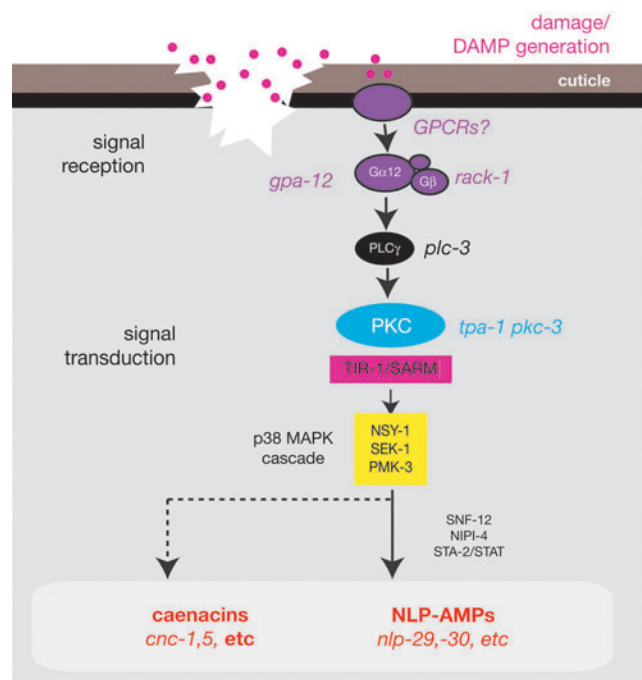


Figure 2. Signal transduction cascade inducing antimicrobial peptide expression after epidermal wounding, based on Engelmann and Pujol.²⁷ For details, see section titled “Cutaneous innate immune responses to wounding and infection.” Not shown are genes such as *nipi-3* or *hsp-3*, which are preferentially involved in the response to fungal infection. Induction of caenacins, such as *cnc-1* or *cnc-5* after wounding is partly dependent on the PMK-1 pathway. The role of TGF β signaling in wound responses is not yet known. SNF-12 and STA-2/STAT may act downstream of the p38 MAPK cascade; NIP1-4 acts downstream of GPA-12 but has not been further positioned in the pathway. MAPK, mitogen-activated protein kinase. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

enzyme Duox/BLI-3.³⁶ ROS appear to act through the DAF-16/FOXO pathway to promote survival after infection or wounding; the transcriptional targets of DAF-16 in the epidermal innate immune response have not yet been elucidated. Interestingly, FOXO transcription factors have recently been implicated in mammalian wound healing.³⁷

Wound closure: epidermal calcium and cytoskeletal rearrangement

How does the epidermis physically close wounds? Recent findings indicate that wounding triggers a rapid and sustained elevation of epidermal Ca²⁺ that is required for actin to polymerize into rings surrounding the wound site. Closure of these actin rings is required for survival of wounding (Fig. 3).

Calcium signals have long been known to be central to epidermal homeostasis and wound repair.^{38–40} Elevation of intracellular Ca²⁺ is seen in numerous models of single cell and tissue damage and appears to be a near-universal response to cellular injury. The advent of genetically encoded

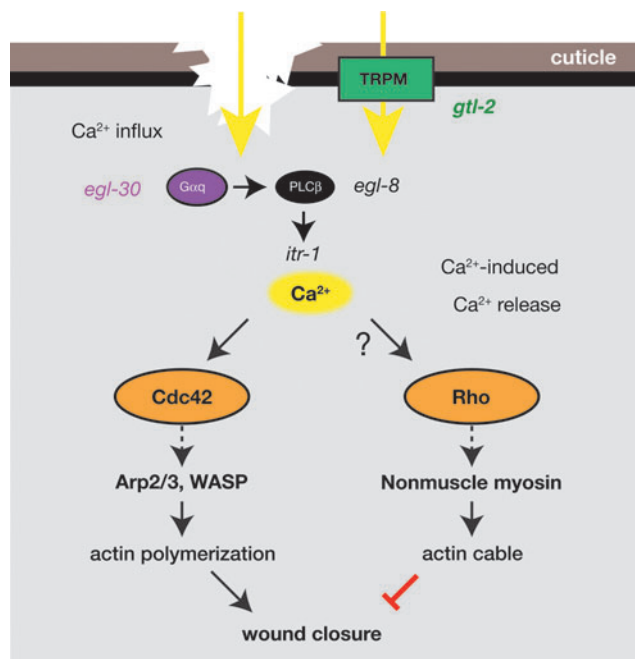


Figure 3. Signals and processes involved in epidermal wound closure, based on Xu and Chisholm.⁴² Wounding triggers a sustained rise in epidermal cytosolic Ca²⁺, initially due to influx from extracellular pools and subsequently from calcium-induced Ca²⁺ release. The TRPM channel GTL-2 is involved in the initial Ca²⁺ influx. Ca²⁺ is required for formation of actin rings that close wounds and may act through the antagonistic small GTPases CDC-42, which is required for actin ring formation, and RHO-1, which inhibits ring formation. TRPM, transient receptor potential, M class. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Ca²⁺ sensors such as the GCaMPs⁴¹ has greatly simplified imaging of Ca²⁺ dynamics *in vivo*. In *C. elegans*, wounding triggers elevation of Ca²⁺ at the wound site within less than a second⁴²; the elevated intracellular Ca²⁺ spreads out in a wave-like manner through the epidermal syncytium, eventually extending several hundred microns. The elevation in epidermal Ca²⁺ persists for 1–2 h after injury before returning to baseline levels.

The elevated epidermal Ca²⁺ appears to be derived from multiple sources. Ca²⁺ influx through the breach in the plasma membrane might account for some of the initial increase in cytosolic Ca²⁺; the external Ca²⁺ reservoir could reside in the cuticle or pseudocoelom. A plasma membrane TRPM channel GTL-2 is also required for Ca²⁺ influx in *C. elegans* wounding and might mediate Ca²⁺ influx directly.⁴² Interestingly, a TRPM channel is also required in *Drosophila* wound healing, acting upstream of the actin cytoskeleton.⁴³ In zebrafish, TRPV1 functions in keratinocyte migration, mechanistically relevant to wound healing.⁴⁴ Numerous TRP channels are expressed in the mammalian skin,⁴⁵ and some are implicated in epidermal barrier repair.⁴⁶ Whereas

the exact role of TRP channels in epidermal Ca^{2+} homeostasis is likely to differ between species, these results suggest an important conserved role for Ca^{2+} channels in wound repair.

Downstream of GTL-2, $\text{G}\alpha\text{q}/\text{EGL-30}$ and $\text{PLC}\beta/\text{EGL-8}$ act within the epidermis to control Ca^{2+} levels through the inositol trisphosphate receptor ITR-1.⁴² The sustained rise in epidermal Ca^{2+} involves ITR-1-dependent Ca^{2+} -induced calcium release from internal stores, as Ca^{2+} levels are reduced by expression of dominant-negative ITR-1. Wound-triggered Ca^{2+} signaling is not required for AMP induction,⁴² although as mentioned above it may play a role in ROS production. The epidermal Ca^{2+} signal appears to be specifically involved in the formation of actin rings at the wound site. After wounding, F-actin polymerization (visualized using GFP-moesin) begins within minutes, forming a complex ring structure that diminishes in radius over the next 1–2 h, corresponding to closure of the wound. Actin ring formation is blocked by Ca^{2+} chelation and in *gtl-2* mutants; the *gtl-2* defect is partly suppressed by incubation in buffers with high external Ca^{2+} .

Exactly how Ca^{2+} triggers local actin polymerization at wound sites remains to be deciphered. Actin ring formation is dependent on the small GTPase CDC-42 and actin nucleation factors such as WASP and the Arp2/3 complex. Unexpectedly, the loss of function in nonmuscle myosin leads to accelerated closure of the actin ring, suggesting that ring closure is driven by actin polymerization rather than by a purse-string mechanism and that nonmuscle myosin-based contractility restrains wound closure.

Negative regulation of wound responses by DAPK-1

Wound repair pathways appear to be under negative control in many systems. Such negative regulation may allow rapid modulation of repair processes, such that they are only invoked after damage above a certain threshold and then are repressed as soon as the damage has been repaired. Some insights into the mechanisms of negative control of wound repair have come from analysis of the *C. elegans* death-associated protein kinase, DAPK-1. DAPK-1 is the *C. elegans* member of a conserved family of serine–threonine kinases that include human DAPK and mouse Dapk1.⁴⁷ Mutations in *dapk-1* were isolated as displaying late-onset hypertrophic cuticle growth and were subsequently found to display constitutively elevated levels of epidermal AMPs.⁴⁸ Furthermore, *dapk-1* mutants display accelerated wound closure

compared with the wild type and suppress the wound closure defects of mutants such as *gtl-2*.⁴² Thus, in the absence of wounding, reduction in DAPK-1 activity results in inappropriate activation of wound responses (cuticle secretion and AMP expression); after wounding, lack of DAPK-1 accelerates wound closure. DAPK-1 therefore appears to act as a coordinate negative regulator of the multiple facets of the wound response. Although the DAPK family has been linked to apoptosis or autophagy in mammals,⁴⁹ DAPK-1 does not appear to regulate wound responses through known cell death pathways. Mammalian DAPK has not yet been tested for roles in wound healing, but is a negative regulator of inflammatory responses.⁵⁰ Identification of DAPK-1 interactors could shed light on how DAPK-1 regulates diverse wound response pathways.⁴⁷

Special aspects of the *C. elegans* wound model

Whereas *C. elegans* exemplifies many general features of wound repair, the nematode skin also exhibits some distinctive biological traits. These serve to illustrate the diversity of contexts in which wound healing can occur.

Most notably, the adult *C. elegans* epidermis is a postmitotic epithelium composed almost entirely of syncytia formed by cell–cell fusions. Stem cell (seam cell) divisions are completed in the fourth larval stage. Epidermal nuclei undergo polyploidization in the course of adult growth. As the adult epidermis is composed of postmitotic syncytia, wounding does not (apparently) induce a proliferative response as in other models. In *Drosophila*, wounding can induce epidermal polyploidization⁵¹; this has not yet been investigated in *C. elegans*.

In many animals, injury activates coagulation systems leading to clotting of the blood (vertebrates) or hemolymph (insects, other invertebrates). Although vertebrate and invertebrate clotting factors are generally divergent, in both cases they are produced by circulating blood cells or hemocytes or are present as inactive precursors in the circulating hemolymph. As part of the inflammatory response to injury, neutrophils or macrophages (or their equivalents) are recruited to wound sites. In adult *Drosophila* and zebrafish, circulating blood cells are attracted to sites of injury^{52,53}; in *Drosophila* embryos, hemocytes migrate toward wounds even in the absence of a developed circulatory system.⁵⁴ In contrast, *C. elegans* lacks a defined circulatory system or migratory blood cells; a fluid-filled body cavity or pseudocoelom distributes nutrients and other molecules within the animal. Induction of the AMP *cnc-2* in the epidermis after fungal infection

involves neuronal expression of a TGF β signal²⁹; it is not yet known if wounding triggers neuroimmune or systemic wound responses analogous to those described in other organisms.

C. elegans lacks orthologs of known invertebrate coagulation or melanization factors such as transglutaminase or phenoloxidase.⁵⁵ However, *C. elegans* encodes several tyrosinases capable of generating melanin, and melanin has been detected in the *C. elegans* cuticle.⁵⁶ Melanization has been observed as a reaction to UV damage in the parasitic nematode *Teladorsagia circumcincta*.⁵⁷ It would be interesting to explore whether wounding triggers a melanization reaction in *C. elegans*.

SUMMARY AND REMAINING QUESTIONS

C. elegans has a robust and sophisticated set of responses that repair epidermal damage and defend against pathogenic attack at wounds. It seems likely that other nematodes would exhibit similar wound healing responses. At present, the innate immune response and wound closure are the best characterized of the *C. elegans* wound healing processes, yet many areas remain poorly understood, foremost of which are the identities of the initiating triggers (DAMPs) of the innate immune response. The involvement of distinct G protein subunits in the innate immune response and in wound closure argues that GPCRs may mediate initial damage sensing. Although the large number of GPCRs in the *C. elegans* genome (~1500) makes searching for such receptors challenging, identification of wound-triggered GPCRs could greatly elucidate the mechanisms by which epithelia sense damage.

Actin rings form locally at wounds yet require a Ca²⁺ signal that is delocalized throughout the syncytial epidermis, raising the question of how such a widespread rise in epidermal Ca²⁺ can have a local effect at the wound site. Additional triggers such as compartment mixing might locally regulate actin polymerization. Alternatively, cytosolic Ca²⁺ may not be the relevant Ca²⁺ pool. The TRPM channel GTL-2 is critical for the wound-induced rise in epidermal Ca²⁺, yet it is not understood whether or how GTL-2 is gated by tissue damage.

Many other aspects of nematode wound healing remain to be explored. The mechanisms responsible for plasma membrane and cuticle resealing immediately after damage are not yet known nor is the precise mechanism leading to cuticle scarring after wounding.³⁰ The restoration of the epidermal

TAKE-HOME MESSAGES

- The nematode *C. elegans* is a genetically tractable model organism that is able to heal and survive puncture or laser wounds to the skin.
- Skin wounding induces a cutaneous innate immune response involving transcriptional upregulation of AMPs.
- Independent of the innate immune response, a wound-triggered Ca²⁺ transient is required for actin cytoskeleton-mediated wound closure.
- Unresolved questions concern the nature of the DAMPs sensed by the epidermis.

permeability barrier after wounding likely involves the synthesis of new cuticle and other extracellular layers,³⁰ but mechanisms have not been characterized. Current studies have focused on relatively small needle or laser wounds that are efficiently repaired by wild-type animals; there has been little analysis of more drastic wounds such as severing of the tail, despite indications that animals may be capable of repairing such wounds. Finally, wound healing has mostly been studied in young adult animals; the effects of adult age on wound repair remain to be examined.

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Abbreviations and Acronyms

AMP = antimicrobial peptide
DAMP = damage-associated molecular pattern
GPCR = G-protein-coupled receptor
MAPK = mitogen-activated protein kinase
ROS = reactive oxygen species
TRPM = transient receptor potential, M class
WASP = Wiskott-Aldrich syndrome protein