

Negative regulation of *Caenorhabditis elegans* epidermal damage responses by death-associated protein kinase

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Wounding of epidermal layers triggers multiple coordinated responses to damage. We show here that the *Caenorhabditis elegans* ortholog of the tumor suppressor death-associated protein kinase, *dapk-1*, acts as a previously undescribed negative regulator of barrier repair and innate immune responses to wounding. Loss of DAPK-1 function results in constitutive formation of scar-like structures in the cuticle, and up-regulation of innate immune responses to damage. Overexpression of DAPK-1 represses innate immune responses to needle wounding. Up-regulation of innate immune responses in *dapk-1* requires the TIR-1/p38 signal transduction pathway; loss of function in this pathway synergizes with *dapk-1* to drastically reduce adult lifespan. Our results reveal a previously undescribed function for the DAPK tumor suppressor family in regulation of epithelial damage responses.

antimicrobial peptide | epidermis | innate immunity | wound repair | cuticle

The epidermis forms an outer protective barrier against environmental damage and pathogens for most animals. Epidermal responses to physical wounding have been extensively characterized in vertebrates and insects (1–3). Repair of a barrier epithelium such as the skin involves several coordinated processes: choreographed movement of cells at the wound edge, leading to reepithelialization (4); scab formation followed by synthesis of new external extracellular matrix (5, 6); and activation of local cutaneous innate immune defenses such as the expression of antimicrobial peptides (AMPs) that can defend against opportunistic infection at the wound site (7–9).

Despite the differing structures of epidermal layers in different animals, recent molecular genetic studies suggest that epidermal wound healing pathways are evolutionarily conserved. Activation of JNK and AP-1 transcription factors appears central to promoting cell motility at the leading edge of an epidermal wound (10). In both insects and vertebrates, transcription factors of the Grainyhead family are activated in response to wound signals via an ERK kinase pathway and promote transcription of extracellular matrix cross-linking enzymes (11, 12). Less is currently known about the pathways that induce innate immune responses to sterile wounding; in human skin, the EGFR pathway has been implicated in local activation of AMPs (13). As well as acting as antibiotics at the wound site, some AMPs may directly promote reepithelialization (14), linking these 2 arms of the wound response.

Like other immune responses, cutaneous responses to damage must be tightly regulated to prevent chronic responses to transient stimuli (15). Negative regulatory pathways prevent innate immune responses to infection or wounds from developing into pathological reactions (15); defects in such negative regulation can underlie chronic skin inflammatory diseases such as atopic

dermatitis (16). Loss of barrier repair functions can also result in chronic inflammation (e.g., loss of AP-1 function blocks reepithelialization; see ref. 17); and has been implicated in psoriasis (18), suggesting an intimate link between wound repair and regulation of innate immunity.

The *Caenorhabditis elegans* epidermis allows wound repair processes to be studied in the context of a simple epithelium that secretes an external collagenous cuticle. As in other animals, the nematode skin is likely to have an active role in preventing organismal damage from physical or biological challenges. We recently showed that laser or puncture wounding of *C. elegans* activates epidermal innate responses via the Toll-interleukin 1 receptor (TIR) domain protein TIR-1 and a p38 MAPK cascade (19). Here, we identify a new negative regulator of epidermal damage responses, the *C. elegans* ortholog of the tumor suppressor death-associated protein kinase, *dapk-1*. Loss of DAPK-1 function results in constitutive formation of scar-like structures in the cuticle, and up-regulation of antimicrobial gene expression. Transient overexpression of DAPK-1 represses the transcriptional response to puncture wounding. We show that up-regulation of innate immune responses, but not barrier repair, in *dapk-1* mutants requires the TIR-1/p38 MAPK pathway, and that *dapk-1* mutants depend on this innate immune pathway for adult survival. Our results reveal a previously undescribed role for the DAPK tumor suppressor in negative regulation of epithelial damage responses.

Results

In genetic screens for mutants displaying progressive defects in epidermal morphogenesis, we identified multiple alleles of *dapk-1*, which encodes the *C. elegans* member of the calcium-calmodulin activated DAPK family [supporting information (SI) Fig. S1]; *dapk-1* mutations form an allelic series (Table S1), in which the strongest allele, *ju4*, results in a missense alteration (S179L) in the peptide-binding ledge of the DAPK-1 kinase domain. RNA interference phenocopied these *dapk-1* epidermal phenotypes (data not shown), indicating that these mutations result in loss of DAPK-1 function.

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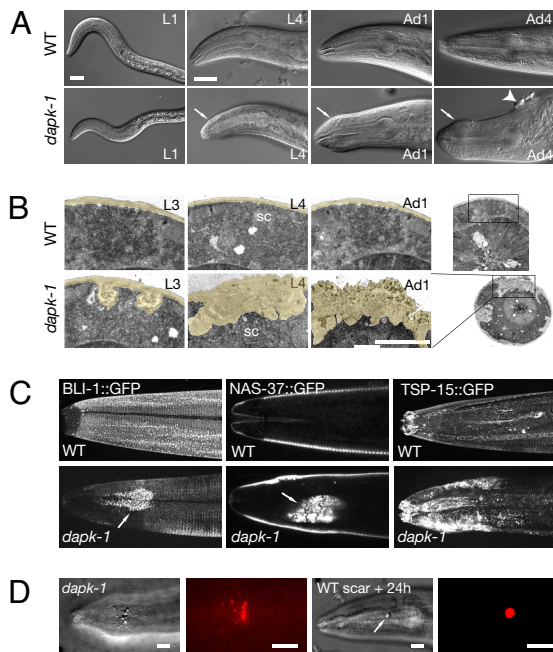


Fig. 1. Progressive hypertrophy of cuticle structure resembling the epidermal wound response displayed by *dapk-1* mutants. (A) Nomarski DIC micrographs showing single wild-type and *dapk-1(ju4)* mutant animals from L1 to adult. The *dapk-1* morphology defect (white arrow) becomes progressively more severe as the animals age; $\approx 30\%$ of *dapk-1* mutant adults also develop refractile encrustations or blisters at the dorsal midline (arrowhead); cuticle abnormalities also occur in the tail and vulval regions. (B) Electron micrographs of sections of L3, L4, and adult lateral epidermis and cuticle (head region, level of anterior pharyngeal bulb) in wild type and *dapk-1(ju4)*. The cuticle is colored yellow; sc, seam cell. Note expansion of cuticle layer in *dapk-1*. (C) *dapk-1* adults locally accumulate cuticle collagen markers BLI-1::GFP, a component of the strut layer, and COL-19::GFP (data not shown); other cuticle components (NAS-37::GFP) and apical epidermal membrane proteins (TSP-15::GFP) also accumulate in the head region. Images are confocal projections or sections (NAS-37::GFP) of lateral head. (D) Autofluorescence of cuticle in unwounded *dapk-1* and cuticle scar in wild type 24 h after needle wounding; Nomarski DIC and Rhodamine filters. (Scale bars, 5 μm .)

dapk-1 mutants appeared morphologically normal until mid larval development. Beginning in the L3 stage, *dapk-1(ju4)* mutants displayed striking and progressive defects in morphology of the epidermis and cuticle in specific body regions, especially in the nose, tail, vulva, and the dorsal midline in the region of the posterior pharyngeal bulb (Fig. 1A). Cuticle in these regions became up to 5–10 times thicker than the wild type, at the expense of underlying epidermis; this thickened cuticle appeared refractile under differential interference contrast (DIC) microscopy, and had aberrant ultrastructure with inclusions of electron-dense material (Fig. 1B). By using transgenic markers, we found that these regions of thickened cuticle accumulated collagens and other cuticle components (Fig. 1C). The thickened cuticle of *dapk-1* mutants also accumulated proteins such as TSP-15, a component of the epithelial apical membrane, suggesting a breakdown of epithelial-cuticle integrity. Other epidermal compartments such as subapical adherens junctions appeared normal (data not shown), suggesting *dapk-1* mutants have specific defects in synthesis or accumulation of apically secreted proteins. The *C. elegans* cuticle is not normally autofluorescent; in contrast, the regions of cuticle thickening in *dapk-1* contained autofluorescent aggregates (Fig. 1D).

The areas of thickened cuticle and autofluorescent aggregates in *dapk-1* mutants resemble the scars caused by needle or laser wounding of the *C. elegans* epidermis (Fig. 1D) (19). These

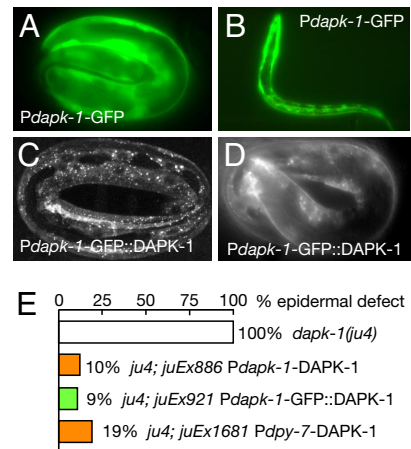


Fig. 2. DAPK-1 is expressed and functions within the epidermis to control epidermal development. (A and B) *dapk-1* transcriptional reporters (2.7-kb promoter) were expressed in epidermal cells from late embryonic (3-fold) stage onwards, as well as in muscles and neurons (data not shown). (C and D) Functional GFP::DAPK-1 localizes to cytoplasmic puncta in embryonic epidermis; images of *juEx921* (C) and rescued *ju4; juEx921* (D). (E) Quantitation of transgenic rescue of *dapk-1(ju4)* epidermal morphology phenotypes; equivalent rescue was observed for *dapk-1(gk219)* (Table S3).

similarities, and the progressive nature of the *dapk-1* epidermal defects, suggested that *dapk-1* mutants might have weakened epidermal layers that undergo breakage and scarring in response to mechanical stress caused by movement. However, inhibition of movement using the *unc-54* mutation (Table S2) or by levamisole (data not shown) did not suppress the epidermal defects of *dapk-1* mutants. Also, other mutants known to have fragile epidermal layers (intermediate filaments/*ifb-1*, plectin/*vab-10*; see refs. 20 and 21) do not display scar-like structures (data not shown). These findings suggest that the scar-like structures of *dapk-1* mutants are not a secondary result of a fragile epidermis. The scar-like areas appear to be structurally weak, because they occasionally rupture in *dapk-1* adults and in assays of cuticle fragility. Because DAPK has been implicated in regulation of endocytosis (22) and can phosphorylate syntaxin (23), we tested whether mutation or RNA interference of genes involved in cuticle secretion or endocytosis could enhance or suppress a weak *dapk-1* phenotype. Double mutants with the cuticle secretion gene *che-14* (24) showed additive interactions with *dapk-1* (Fig. S2A). Inhibition of genes involved in cuticle secretion (e.g., *sec-23*; see ref. 25) or endocytosis neither enhanced nor suppressed *dapk-1* defects (Fig. S2B). These results suggest that the cuticle hypertrophy in *dapk-1* mutants is not due to a primary defect in cuticle secretion or epidermal endocytosis.

DAPK regulates apoptosis and autophagy in mammalian cells (26), and *C. elegans dapk-1* mutants have defects in apoptotic cell death (R.-H. Chen, J.-Y. Chen, and Y.-C.W., unpublished work) and autophagy (27). If the epidermal defects of *dapk-1* mutants reflected defective apoptosis or autophagy, other mutants defective in these processes should show similar phenotypes. However, mutations that eliminate apoptosis (e.g., *ced-3*) or autophagy (*bec-1*) neither phenocopied nor suppressed the epidermal defects of *dapk-1* mutants, suggesting these defects are not caused by misregulation of cell death or autophagy (Fig. S3; Table S2).

dapk-1 transcriptional reporters were expressed in multiple tissues, including the epidermis, from late embryogenesis onwards (Fig. 2A and B). To determine whether DAPK-1 acts cell autonomously in epidermal development, we expressed DAPK-1 specifically in epidermal cells by using the promoter of the cuticle collagen *dpy-7* (28), and found that such transgenes rescued

dapk-1 epidermal defects (Fig. 2E and Table S3). Within epidermal cells, functional GFP::DAK-1 localized to cytoplasmic puncta of unknown identity (Fig. 2C and D). These results suggest that DAK-1 acts independently of cell death pathways and autonomously in the epidermis.

The similarities between the *dapk-1* phenotype and the effects of wounding led us to test whether loss of *dapk-1* also affected epidermal innate immune responses to wounding. We found that *dapk-1* mutants constitutively up-regulated transgenic reporters for several epidermal AMP genes, compared with unwounded controls (Fig. 3A and B, and Fig. S4A). These AMP genes are induced by infection by the epidermis-penetrating fungus *Drechmeria coniospora* and by sterile wounding (19, 29). By using oligonucleotide microarrays, we found that transcripts for multiple epidermal AMPs were up-regulated in *dapk-1* relative to the wild type (Fig. S4C; Table S4). The *dapk-1* mutant transcriptional profile overlapped strikingly with that of animals infected with *Drechmeria* (30) (19/303 genes in common with the top 419 *Drechmeria*-up-regulated genes; see Fig. S4C and Table S4), but not with those of animals infected with bacterial pathogens (0/303 were up-regulated in 2 or more bacterial infections) (31). Some epidermal AMPs, including *nlp-29* but not *nlp-30*, are also induced by osmotic stress and are constitutively up-regulated in osmotic stress response mutants such as *dpy-9* (30). However, *dapk-1* mutants did not inappropriately induce other transcriptional responses to osmotic stress such as *gpdh-1* (Fig. S4B) (32). This finding suggests that the constitutive induction of AMP expression in *dapk-1* mutants is not due to a defect in regulation of epidermal osmotic stress response. Epidermal specific expression of DAK-1 in *dapk-1(ju4)* mutants was sufficient to restore *Pnlp-29-GFP* expression to normal levels (data not shown), indicating *dapk-1* acts autonomously in the epidermis to repress innate immune responses. To address whether *nlp-29* up-regulation was a direct or indirect consequence of the loss of DAK-1 function, we tested whether transient overexpression of DAK-1 could suppress innate immune responses to needle wounding. We found that heat shock-induced overexpression of DAK-1 immediately after wounding strongly suppressed induction of *Pnlp-29-GFP* (Fig. 3C), suggesting DAK-1 can acutely repress innate immune responses to damage.

Fungal infection and sterile wounding stimulate transcription of *nlp-29* and *nlp-30* via the TIR domain adaptor protein TIR-1, which activates the NSY-1/SEK-1/PMK-1 p38 MAPK cascade in the epidermis (Fig. 3D) (19, 29). We tested whether the constitutive activation of AMP transcription in *dapk-1* also depended on the TIR-1/PMK-1 cascade. AMP transcription was significantly reduced in *dapk-1* double mutants with *tir-1* or with mutations in genes of the MAPK cascade (collectively *mapk*; see Fig. 3E and G); *tir-1* or *mapk* mutations did not suppress the morphological and cuticle fragility phenotypes of *dapk-1* mutants (Fig. 3F and Fig. S5C). To test whether AMP up-regulation in *dapk-1* mutants was caused by cuticle fragility of the scar-like areas, we took advantage of an extragenic suppressor of *dapk-1*, found in an unrelated screen. The *sydn-1(ju541)* mutation affects neuronal development (Y. Dai and Y. Jin, personal communication), and was fortuitously found to suppress *dapk-1(ju4)* epidermal and cuticle fragility phenotypes (Fig. S5A and C). However, such genetic suppression of *dapk-1* morphological defects did not block *Pnlp-29-GFP* up-regulation (Fig. S5B), suggesting the activation of innate immune responses in *dapk-1* mutants is not a consequence of morphological defects.

In the course of this analysis, we found that *dapk-1* double mutants with *tir-1* or other components of the PMK-1 MAPK pathway frequently died within early adulthood (Fig. 4A). Although *dapk-1* single mutants had normal lifespan (Fig. 4B), *dapk-1 tir-1* double mutants displayed synergistic reduction in lifespan (Fig. 4C). We hypothesized that a defective innate immune response might be particularly deleterious to *dapk-1* mutants, as such animals would be more vulnerable to bacterial

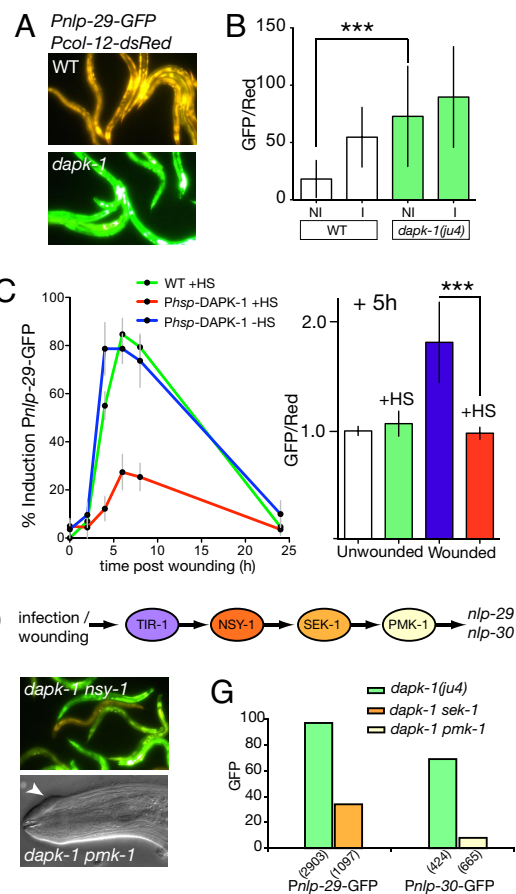


Fig. 3. DAK-1 negatively regulates epidermal innate immune responses via the TIR-1/p38 MAPK cascade and independently of epidermal integrity. (A) Photographs of *frls7 Pnlp-29-GFP/Pcol-12-dsRed* in young adult wild type and *dapk-1(ju4)*. (B) Biosort quantitation of *frls7* GFP/dsRed fluorescence ratios in L4 and adult wild-type and *dapk-1(ju4)*, uninfected (NI), and *Drechmeria* infected (I); mean \pm SD; $n > 100$, for each condition; *ju4* is significantly different from wild type ($P < 0.001$, Mann-Whitney test). (C) *Pnlp-29-GFP (frls7)* induction by needle wounding is suppressed by heat shock induced expression of DAK-1. *frls7; Phsp-16-DAK-1(juEx1933)* animals raised at 20 °C were wounded at 0 h and then transferred to 35 °C for 2 h. Mean and SEM for 3 experimental groups ($n > 9$, for each group) are shown. By using worm sorting at 5 h post wounding, we found significantly reduced *Pnlp-29-GFP* induction after heat shock ($P < 0.0001$, Mann-Whitney test); GFP/Red ratio is normalized to unwounded non-heat-shocked *frls7; juEx1933* ($n > 18$, per condition). (D) The p38 innate immune response pathway in *C. elegans* epidermis (19). (E) *nlp-29* induction in *dapk-1* mutants depends on the TIR-1/ MAPK pathway; *dapk-1 nsy-1* double mutants show reduced *nlp-29-GFP* expression, compared with *dapk-1*. (F) *pmk-1* does not suppress *dapk-1* morphogenetic defects (arrow; Table S2). (G) Biosort quantitation of *Pnlp-29-GFP* in *dapk-1 sek-1* and *Pnlp-30-GFP* in *dapk-1 pmk-1* shows suppression, compared with *dapk-1* single mutants; *frls7* expression in the double mutants is higher than that of *mapk* single mutants (data not shown), suggesting that *dapk-1* mutants may also activate AMP transcription in parallel to the TIR-1/ MAPK cascade.

infection via the skin due to their epidermal defects. To test this model, we examined the effects of epidermal wounding on lifespan. Under our conditions, needle wounding of the wild type or of *dapk-1* resulted in an ≈ 1.8 -fold reduction of median lifespan (13 to 7 days; see Fig. 4B), whereas wounding of immunocompromised animals caused a 4-fold reduction of lifespan (8 to 2 days for *tir-1* and *pmk-1*; see Fig. 4C and D). This effect of wounding on immunocompromised strains was suppressed by inhibiting bacterial proliferation by using the DNA synthesis inhibitor 5-fluoro-2-deoxy uridine (FUDR) (Fig. 4D).

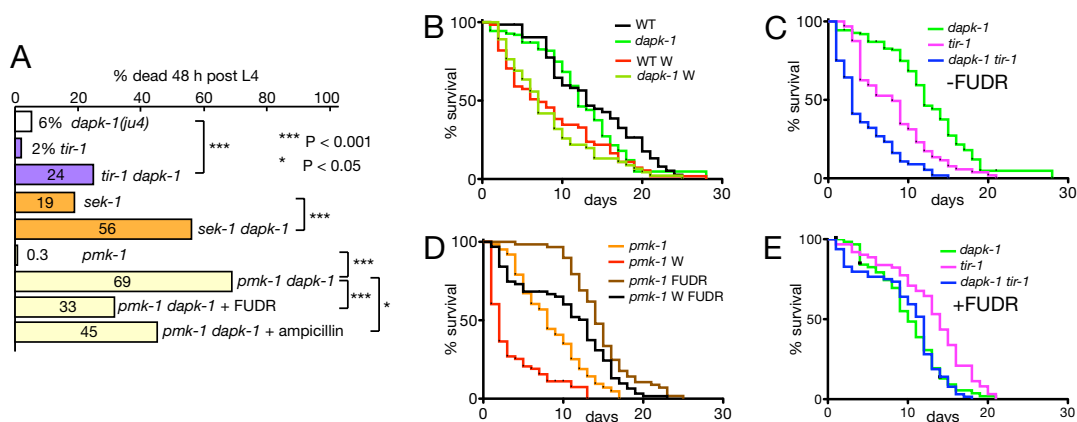


Fig. 4. Synergistic premature adult lethality of *dapk-1* with immunocompromised mutants. (A) *dapk-1* double mutants with *tir-1* or *mapk* mutants die within 48 h of L4 stage ($n > 200$, for each genotype); *dapk-1*; *pmk-1* premature lethality is partly suppressed by growth on ampicillin, carbenicillin (data not shown), or FUDR ($n > 50$, for each). Comparisons use Fisher exact test. (B) *dapk-1* lifespan is not significantly different from the wild type (median 12 days unwounded, 7 days wounded). (C) Synergism of *dapk-1* and *tir-1*: *dapk-1*; *tir-1* (median 3 days) is significantly different from *tir-1* (median 8 days) ($P < 0.001$). (D) The lifespan of *pmk-1* is reduced 4-fold by wounding ($P < 0.001$); wounding on FUDR reduces *pmk-1* lifespan by only 1.25-fold ($P = 0.06$ between *pmk-1* wounded and unwounded on FUDR). (E) The synergistic reduction in lifespan of *dapk-1*; *tir-1* double mutants is suppressed by FUDR; lifespan of *dapk-1 tir-1* FUDR+ is not different from *dapk-1* alone ($P = 0.9$).

This result suggests that wounding can reduce lifespan in 2 ways: physical damage, and (in immunocompromised strains) opportunistic infection via the wound site by *Escherichia coli* OP50 or other environmental microorganisms. *E. coli* OP50 is pathogenic to older *C. elegans* (33, 34), and can be pathogenic if introduced across the epidermis by injection (35); *dapk-1* mutants appear to have normal wound responses; however, their epidermal defects render them susceptible to infection and, thus, dependent on the epidermal innate immune pathway for adult viability. In support of this hypothesis, growth on FUDR suppressed the synergistic reduction in lifespan of *dapk-1 tir-1* double mutants (Fig. 4E).

Discussion

Loss of function in the *C. elegans* DAP kinase ortholog DAPK-1 results in an unusual progressive epidermal defect that appears to reflect constitutive activation of cutaneous responses to damage. Several lines of evidence suggest that *dapk-1* mutants are not merely defective in epidermal integrity or cuticle secretion. First, other epidermal fragility mutants do not resemble *dapk-1* in phenotype, and RNAi of cuticle secretion or endocytosis genes failed to enhance or suppress *dapk-1* defects. Suppression of movement fails to suppress *dapk-1* phenotypes, whereas genetic suppression of *dapk-1* morphological and cuticle fragility defects by the *sydn-1* mutation does not abrogate its up-regulation of innate immune pathways. Last, transient overexpression of DAPK-1 blocked a transcriptional response to wounding. These findings suggest that DAPK-1 directly represses epidermal responses to damage.

DAPK was initially identified in functional screens for genes acting in IFN-mediated cell death (36). Numerous studies have placed DAPK in a network of pathways regulating apoptosis and autophagy (26). Unexpectedly, although *dapk-1* mutants display defects in autophagy (27) and apoptosis (R.-H. Chen, J.-Y. Chen, and Y.-C.W., unpublished work), we find no evidence that these processes are involved in the epidermal function of DAPK. These observations raise the possibility that DAPK family members have additional roles beyond their functions in cell death. Indeed, DAPK has recently been shown to function as a negative regulator of T cell activation via NF- κ B (37) and as a negative regulator of inflammatory gene expression in monocytes (38), suggesting a conserved role for DAPK as a negative regulator of various immune responses.

Although the upstream triggers of damage responses in *C. elegans* epidermis are not known, calcium signals often have this role (39). As a member of the calcium-calmodulin regulated kinase family, DAPK-1 could be directly activated by such signals; if the action of DAPK-1 is to limit wound healing responses, its activity must presumably be delayed relative to other pathways that promote acute responses to epidermal damage. We speculate that an initial calcium transient caused by wounding or infection activates the TIR-1/MAPK cascade via as yet unknown signaling pathways. Because the increased *nlp-29* expression of *dapk-1* mutants depends on TIR-1, DAPK-1 likely inhibits some step upstream of TIR-1 in this cascade. Last, the identification of a role for a well-known tumor suppressor in epidermal damage responses is highly intriguing, given the long-noted similarities between wound healing and tumorigenesis (40). Further analysis of the mechanism of DAPK function in *C. elegans* could shed light on both these processes.

Materials and Methods

Genetics and Phenotypic Quantitation. The wild-type strain used is Bristol N2 (41). All strains were maintained at 25 °C on NGM agar plates under standard conditions, unless stated. The following mutations were used: *atgr-18(gk378)*, *bec-1(ok700)*, *ced-2(e1752)*, *ced-3(n717)*, *che-14(e1960)*, *nsy-1(ky397)*, *pmk-1(km25)*, *sek-1(km4)*, *unc-51(e369)*, *unc-54(e190)*, *sydn-1(ju541)*, and *tir-1(tm3036)* (19). We used the following transgenes: *cgEx198* [BLI-1::GFP] (J. Crew and J. Kramer, personal communication), *kals12* [COL-19::GFP] (42), *oxEx587* [NAS-37::GFP] (43), *imEx1* [TSP-15::GFP] (44), *kbls5* [Pgpdh-1-GFP] (32), *frls7* [Pnlp-29-GFP + Pcol-12-dsRed] (19), and *juEx1384* [Pnlp-30-GFP + Pcol-12-dsRed].

We isolated *dapk-1* alleles *ju4* and *ju469* in screens for epidermal-defective mutants induced by ethyl methanesulfonate (EMS) or diepoxyoctane, respectively; *ju557* was isolated in a screen for EMS-induced mutations that failed to complement *ju4*; *gk219* was isolated by the *C. elegans* knockout consortium. Epidermal morphology defects were quantitated in complete broods of 2–6 animals raised at 15, 20, and 25 °C and scored as 1–2 day-old adults.

***dapk-1* Molecular Biology and Transgenes.** The *dapk-1* transcriptional GFP reporter was generated by subcloning a 2.7-kb genomic fragment into vector pPD95.75, yielding pCZ764 (transgenic lines *juEx927* and *juEx928*). Isolation of the *dapk-1* cDNA is described in *SI Methods*. To express full-length DAPK-1 under control of the 2.7-kb promoter, we digested the DAPK-1 cDNA clone pCZ763 with PstI and inserted the 2.7-kb *dapk-1* promoter to produce the *Pdapk-1*-DAPK-1 construct pCZ767; pCZ767 was injected at 2 ng/ μ L into *ju4* and *gk219* with SUR-5::GFP as coinjection marker to generate arrays *juEx886–889* and *juEx890–892*, respectively. To generate GFP-tagged DAPK-1, we partially digested pCZ767 with EcoRI and inserted GFP (amplified from pPD95.75) into an EcoRI site in the 5' UTR to create pCZ770

Pdapk-1-GFP::DAPK-1; pCZ770 was injected at 1.5 ng/ μ L into *ju4* and *gk219* with Pttx-3-RFP as coinjection marker to generate transgenes *juEx921–923* and *juEx924–926*, respectively. To express DAPK-1 in the epidermis, we amplified the 305 bp *dpy-7* promoter (28), and cloned it into pCZ763 to create *Pdpy-7*-DAPK-1 (pCZ766); pCZ766 was injected at 1 ng/ μ L into wild-type animals with Pttx-3-RFP as coinjection marker to generate transgenes *juEx1681* and *juEx1682*. *dapk-1* cDNA was cloned into the *hsp-16* vector pPD49.78 (Fire lab vector kit) to create pCZ763; pCZ763 was injected at 3 ng/ μ L with the coinjection marker Pttx-3-RFP to create *juEx1933*.

Electron Microscopy. *dapk-1* (*ju4*) animals were grown at 25 °C and prepared for electron microscopy as previously described (20). We sectioned 1 L3, 1 L4, and 2 adults from the tip of the nose to the anterior end of the gonad. Images in Fig. 1 are from the region of the anterior pharyngeal bulb.

Pnlp-29-GFP Scoring and Worm Sorting. Fluorescent worm sorting by using the Copas Biosort was performed as described (19), on synchronized populations grown at 20 °C. For quantitative analysis, we selected events with TOF 250–800 and GFP 15–250. To score *frls7* induction in the heat-shock time course (Fig. 3C), we wounded young adults and scored fluorescence by using a Leica MZFLIII fluorescence dissection microscope and long-pass GFP filter. Each animal was scored as uninduced (orange or yellow-orange) or induced (yellow, yellow-green, and green) at each time point to estimate the percentage induction in a population. Photomicrographs in Fig. 3 and Fig. S4 were taken by using a Zeiss Axioplan imaging microscope and Axiocam color camera, 40 \times objective, Zeiss GFP long-pass filters and 250 ms exposure time. Images were not processed further.

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