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Development or Disease: Caspases Balance Growth and Immunity in *C. elegans*

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Caspase proteases execute apoptosis but also function in development. In this issue of *Developmental Cell*, Weaver et al. report that *C. elegans* CED-3 caspase promotes animal growth through PMK-1/p38 kinase cleavage, and at the expense of pathogen and stress immunity, revealing an unexpected homeostatic relationship between development and disease.

Caspases are widely known as the enzymatic executioners of apoptosis. These cysteine proteases are synthesized as proenzymes with weak catalytic activities and are activated through self-cleavage or by other caspases. In mammals, it has been proposed that initiator caspases cleave and activate executioner caspases, which in turn proteolytically target cellular proteins, leading to cell death. A similar hierarchy has been proposed in *Drosophila*, while in *C. elegans*, which possesses four caspase-related genes, only CED-3 caspase is required for apoptosis (Fuchs and Steller, 2011). Why animals harbor multiple different caspase genes has been a long-standing mystery.

Over the past 15 years, an accumulation of studies reporting that caspases function independently of cell death has raised the possibility that caspase gene family diversity may reflect an underlying diversity in cellular roles (Abraham and Shaham, 2004). Beyond their cell-lethal activities, caspases can selectively eliminate subcellular compartments, such as the cystic bulge formed during spermatogenesis in *Drosophila* (Arama et al., 2003), and nuclei of red blood cells (Zhao et al., 2016). Caspases also appear to have roles entirely distinct from cellular degradation. Multiple studies show that caspases function to limit stemness and promote differentiation, as with caspase-mediated cleavage of Nanog, which promotes embryonic stem cells differentiation (Fujita et al., 2008). Caspases have also been suggested to participate in postsynaptic remodeling in the zebra finch auditory forebrain during song learning (Huesmann and Clayton, 2006) and were recently proposed to function in blocking epithelial cell migration (Gorelick-Ashkenazi et al., 2018). While many such examples have been documented, the molecular mechanisms and relevant targets are only known in a handful of cases. In this issue of *Developmental Cell*, Weaver et al. (2020) report that the *C. elegans* caspase CED-3 promotes larval development and growth by cleaving and inactivating PMK-1/p38 kinase in the epidermis. This cleavage blocks activation of a pathogen- and stress-response pathway that drives expression of hundreds of genes. Weaver et al. expand the functional repertoire of *C. elegans* caspases beyond cell-death control, demonstrate non-canonical effects of caspases on gene expression, and highlight a surprising interaction between development and immunity.

To determine whether *C. elegans* CED-3 caspase has roles other than cell death-control, Weaver et al. (2020) performed an RNAi screen seeking genes required for normal development when CED-3 caspase is compromised. They found that concurrent inactivation of VHP-1, a dual specificity phosphatase (DUSP), and CED-3 caspase results in synergistic growth arrest. Importantly, this defect is observed in a *ced-3* catalytic site mutant and also in a mutant in the gene *ced-4/Apaf1*, encoding a CED-3 activator, supporting a role for the enzymatic activity of CED-3 in driving development.

VHP-1 had been previously implicated in controlling a conserved immunity and stress-response pathway mediated by PMK-1/p38 MAP kinase (Mizuno et al., 2004). Remarkably, Weaver et al. (2020) show that *pmk-1* removal suppresses the synthetic developmental delay observed in *ced-3*(−); *vhp-1*(RNAi) double mutants. Thus, the growth arrest of *ced-3*(−); *vhp-1*(RNAi) animals is, at least in part, a result of PMK-1/p38 kinase activation. Consistent with this idea, loss of *pmk-1* speeds up developmental progression, suggesting that PMK-1/p38 can function as a rheostat to control growth rate.

The effects of CED-3 caspase depletion on PMK-1/p38 activity could be direct or, alternatively, might reflect the presence of undead cells in *ced-3*(−) mutants. These persisting cells might constitute a stress signal, indirectly activating PMK-1/p38. A number of experiments support the former hypothesis. First, the authors demonstrate that CED-3 can proteolytically cleave wild-type PMK-1/p38 protein *in vitro*, but not a mutant PMK-1/p38 protein in which the caspase cleavage site aspartate is mutated to glutamate (D327E). Second, in an elegant experiment, the authors introduce the D327E-encoding substitution at the endogenous *pmk-1* locus using CRISPR-mediated gene modification, and show that this phenocopies the developmental delay of *ced-3*(−); *vhp-1*(RNAi) double mutants. Third, expression of the antimicrobial peptide-encoding gene *nlp-29*, which is strongly induced in *ced-3* mutants, is also induced, and to a similar extent, in *pmk-1*(D327E) mutants. Fourth, the authors demonstrate that apoptosis proceeds normally in *pmk-1*(D327E) mutants. While consistent with a specific role in PMK-1/p38 activity modulation, future experiments to identify cell-survival mutants

that do not induce a growth delay, such as perhaps mutations in the *egl-1*/BH3-only or *ced-9*/Bcl-2 genes, would strengthen the argument. Likewise, demonstrating cell-autonomous roles for CED-3 caspase in PMK-1/p38 control, and the control of downstream gene expression, would also support a non-apoptotic role for CED-3 caspase.

Importantly, Weaver et al. (2020) make the case that the effects of CED-3 caspase on PMK-1/p38 have consequences for *C. elegans* immune system activation. *nlp-29* expression is induced following exposure of *C. elegans* to the fungus *Drechmeria*. This induction is significantly heightened in both *ced-3*(−) and *pmk-1*(D327E) mutant animals. Taken together, the authors' results establish a surprising and unexpected antagonistic relationship between development and immune system activation, mediated by non-apoptotic caspase activity.

The GATA transcription factor ELT-3 is transiently expressed in the epidermis and was previously shown to function there downstream of PMK-1/p38 (Hu et al., 2017). Weaver et al. (2020) find that in *ced-3* mutant animals, ELT-3 protein levels are elevated, although mRNA levels remain unchanged, suggesting a post-translational effect. ELT-3 expression in the epidermis also persists significantly longer than in wild-type animals. Importantly, similar to *pmk-1* deletion, a loss-of-function mutation in *elt-3* rescues the developmental delay of *ced-3*(−); *vhp-1*(RNAi) mutants. These data show that CED-3 functions through ELT-3, a PMK-1/p38 effector, and that the growth effects of this pathway are mediated by the epidermis.

To assess the global impact of *ced-3* depletion, the authors determine whole-animal transcriptome and translatome profiles of synchronized larvae, identifying a set of 313 genes differentially regulated by CED-3 caspase and PMK-1/p38. Gene ontology analyses reveal that many of these are pathogen- and stress-responsive genes that function in the epidermis to confer innate immunity and structural integrity. For example, epidermal antimicrobial peptides NLP-29, NLP-30, and NLP-31 are negatively regulated by CED-3 and positively regulated by PMK-1. Thus, the effects of CED-3 on the immune transcriptome are pervasive.

The studies of Weaver et al. (2020) raise a number of intriguing questions. For example, if CED-3 caspase indeed functions cell autonomously in the epidermis to block PMK-1/p38 activity, how are epidermal cells protected from apoptosis and cleavage of substrates unrelated to PMK-1/p38? Is caspase activity in these cells below a minimal threshold needed to induce apoptosis? Is caspase activity restricted to a subcellular compartment and/or time? Are caspase moieties directed by adaptor proteins to specific targets?

More broadly, the results of Weaver et al. (2020) raise the exciting possibility that caspases may function as general regulators of large cellular programs by controlling the activity of specific regulators. The authors hint in their paper that their screen "revealed that *ced-3* has numerous other non-apoptotic functions during larval development." Thus, as with phosphorylation, or ubiquitylation, might modification of regulator genes by caspase cleavage provide a heretofore unappreciated mode of gene and protein expression control? Intriguingly, while some mice homozygous for a deletion in *Apaf-1*, a key apoptotic regulator and caspase activator, can survive to adulthood, suggesting that caspase-dependent cell death is not required for animal viability, perinatal animal death is the rule. A similar phenotype accompanies homozygous mutants of other apoptotic gene mutants. The results of Weaver et al. (2020), combined with other examples in the field, raise the possibility that this lethality may not be a consequence of apoptotic dysfunction. Rather, defects in non-apoptotic caspase functions in different cell types and tissues during development may be the culprit. Identification and molecular characterization of these non-apoptotic caspase functions would not only uncover a new field of developmental regulation, but also shed a different light on molecular events that take place during apoptotic cell demise.

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