Caenorhabditis elegans ced-3 Caspase Is Required for Asymmetric Divisions That Generate Cells Programmed To Die

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ABSTRACT Caspases have functions other than in apoptosis. Here, we report that Caenorhabditis elegans CED-3 caspase regulates asymmetric cell division. Many of the 131 cells that are “programmed” to die during C. elegans development are the smaller daughter of a neuroblast that divides asymmetrically by size and fate. We have previously shown that CED-3 caspase is activated in such neuroblasts, and that before neuroblast division, a gradient of CED-3 caspase activity is formed in a ced-1 MEGF10 (multiple EGF-like domains 10)-dependent manner. This results in the nonrandom segregation of active CED-3 caspase or “apoptotic potential” into the smaller daughter. We now show that CED-3 caspase is necessary for the ability of neuroblasts to divide asymmetrically by size. In addition, we provide evidence that a pig-1 MELK (maternal embryonic leucine zipper kinase)-dependent reciprocal gradient of “mitotic potential” is formed in the QL.p neuroblast, and that CED-3 caspase antagonizes this mitotic potential. Based on these findings, we propose that CED-3 caspase plays a critical role in the asymmetric division by size and fate of neuroblasts, and that this contributes to the reproducibility and robustness with which the smaller daughter cell is produced and adopts the apoptotic fate. Finally, the function of CED-3 caspase in this context is dependent on its activation through the conserved egl-1 BH3-only, ced-9 Bcl-2, and ced-4 Apaf-1 pathway. In mammals, caspases affect various aspects of stem cell lineages. We speculate that the new nonapoptotic function of C. elegans CED-3 caspase in asymmetric neuroblast division is relevant to the function(s) of mammalian caspases in stem cells.

KEYWORDS caspase; nonapoptotic function; asymmetric cell division; neuroblasts; C. elegans; pig-1 MELK

During embryonic and postembryonic Caenorhabditis elegans development, 131 somatic cells reproducibly die (Sulston and Horvitz 1977; Sulston et al. 1983). Genetic screens resulted in the identification of four genes that can mutate to block most of these cell deaths and that define a conserved apoptotic cell death pathway (egl-1 BH3-only, ced-9 Bcl-2, ced-4 Apaf-1, and ced-3 caspase) (Horvitz 2003; Conradt et al. 2016). Interestingly, most of the cells that are programmed to die during development are generated through divisions that are asymmetric by fate and size, and that produce a smaller daughter that is programmed to die. The apoptotic death of the smaller daughter is triggered through the transcriptional upregulation (and, hence, increase in expression) in that cell of egl-1 BH3-only, which induces apoptosome formation, and the maturation and activation of the protease CED-3 caspase. Active CED-3 caspase cleaves specific substrates and thereby induces the killing, dismantling, and phagocytosis of the cell in a cell-autonomous manner. For example, CED-3 caspase cleaves and activates the lipid scramblase CED-8 Xkr8, which results in the exposure of the “eat-me signal” phosphatidylserine (PS) on the surface of the dying cell (Stanfield and Horvitz 2000; Suzuki et al. 2013). This signal is recognized by receptors on neighboring cells, namely CED-1 MEGF10 (multiple EGF-like domains 10), which leads to receptor clustering and the activation of two conserved parallel engulfment pathways in the engulfing cell (Zhou et al. 2001; Venegas and Zhou 2007). Recently, we demonstrated that active CED-3 caspase is already present in the mother of at least
one cell programmed to die, the embryonic neurosecretory motor neuron (NSM) neuroblast, which divides to give rise to the larger NSM, which survives and differentiates into a serotonergic motor neuron, and the smaller NSM sister cell (NSMsc), which dies (Chakraborty et al. 2015; Lambie and Conradt 2016). Furthermore, this active CED-3 caspase causes the clustering and activation (in a *ced-8* Xkr8- and PS-independent manner) of CED-1 MEGF10 and the two engulfment pathways in the two dorsal neighbors of the NSM neuroblast. This activation of the engulfment pathways in turn is necessary for the formation and/or maintenance of a gradient of CED-3 caspase activity in the NSM neuroblast, and the nonrandom segregation of active CED-3 caspase into the smaller NSMsc, where it promotes the robust and swift execution of apoptotic cell death (Chakraborty et al. 2015; Lambie and Conradt 2016).

The formation of a gradient of CED-3 caspase activity in the mother of a cell programmed to die has so far only been demonstrated in the embryonic NSM neuroblast lineage. For this reason, the generality of this phenomenon has so far been unclear. In addition, whether active CED-3 caspase plays a role in the mother other than promoting its own enrichment in one part of the cell, has been unknown. To address these questions, we examined the postembryonic QLp neuroblast lineage. Our results support the notion that the formation of a gradient of CED-3 caspase activity is a general phenomenon. Furthermore, we provide evidence that the *ced-3* caspase gene plays an active role in the asymmetric division of mothers. Specifically, we provide evidence that *ced-3* caspase is required for their ability to divide asymmetrically with size and fate, and, hence, to produce the smaller daughter, which is programmed to die.

Materials and Methods

**Strains and genetics**

All *C. elegans* strains analyzed were maintained at 25°C on Nematode Growth Medium, unless otherwise specified (Brenner 1974). The following mutations and transgenes were used in this study: LGI: *ced-1(e1725)* (Hedgecock et al. 1983); LGIII: *ced-4(n1162)* (Ellis and Horvitz 1986), *ced-6(n1813)* (Ellis et al. 1991), *unc-119(ed3)* (Maduro and Pilgrim 1995) and rdsvl1(*P*egl-1::mCherry::his-24; *P*egl-1::myristoylated mCherry; *P*ecl-1::mig-10::yfp) (Ou et al. 2010); LGIV: *ced-3(n2433)* (Ellis et al. 1991), *ced-3(n717)* (Ellis and Horvitz 1986), *ced-3(n2433)* (Shaham et al. 1999), *ced-3(n2427)* (Shaham et al. 1999), *pig-1(gm344)* (Cordes et al. 2006), and *bsl82 (*P*toe-2::*mKate2::tag-1) (this study); and LGV: *egl-1(n3330)* (Sherard et al. 2017), *bds190 (*P*unc-59::*gfp)* (M. Driscoll (Rutgers University), personal communication), *bsl44 (*P*unc-59::*mCherry::*::pRFP) (Audhya et al. 2005), and *ens1 (*P*ced-1::*GFP::zu) (Zhou et al. 2001).

Additional transgenes used in this study are: *bsl133 (*P*unc-2::*gfp)* (this study), *bcEx1277 (*P*unc-2::*ced-1::*::gfp) (this study), and *bcEx1334 (*P*unc-2::*mKate2)* (this study).

**Cloning**

*pBC1591* (*P*unc-7::*gfp)* and *pBC1681* (*P*unc-7::*ced-1::*::gfp): The *toe-2* promoter (*P*toe-2) (2117 bp immediately upstream of the *toe-2* start site) was amplified by PCR using N2 genomic DNA, and restriction sites for AgeI and SacI were introduced at the ends of the PCR product. Primers used to amplify *P*toe-2 were *SacI* *P*toe-2-F (5'--aaaaa GAGCTCTtatgtagcaacaatactc-3') and *AgeI* *P*toe-2-R (5'--aaaaa ACCGGTtgggaatcgagtaaagt-3'). The resulting PCR product and plasmid *pBC1408* (*pBC1408*: *unc-54 3* UTR in blue-script) were digested with *AgeI* and *SacI* (which drops out *P*toe-2 from *pBC1408*), and the PCR product cloned into the pBc1408 backbone using T4 ligation to obtain pBC1565 (*P*toe-2::*gfp)*.

*pBC1805* (*P*toe-2-ced-1::*mKate2)*: pBC1805 was cloned by Gibson cloning (Gibson et al. 2009). Primers pBSK Ptoe-2-F (5'--ATCCCACC CCGCTGCAAGAA TCGAGTAATTATCT GTACCACA AATCTCTTG-3') and ced-1 Ptoe-2-R (5'--GAATGAGACGCATT TTTGACCTGGGACATG-3') were used to amplify *P*toe-2 using restriction digest with *AgeI* and *SacI*, and T4 ligation to generate plasmid *pBC1591* (*P*unc-7::*gfp)*. A ced-1 minigene was amplified from plasmid pZ2210 (*pZ2210*: *ced-1::*::gfp, Zhou et al. 2001) by PCR using the primers *AgeI* ced-1-F (5'--aaaACCGGT Ttgggtagaaaaatcctcttgatgct-3') and ced-1-R (5'--tttttacccgg tactgtaaactct-3'). The resulting PCR product and pBC1591 were digested with *AgeI* and the ced-1 minigene was inserted by T4 ligation into the linearized pBC1591 to obtain plasmid pBC1681 (*P*unc-7::*ced-1::*::gfp)*.

*pBC1807* (*P*toe-2-ced-1::*mKate2)*: pBC1807 was generated by Gibson assembly (Gibson et al. 2009). Primers pBSK Ptoe-2-F (5'--ATCCCACC CCGCTGCAAGAA TCGAGTAATTATCT GTACCACA AATCTCTTG-3') and ced-1 Ptoe-2-R (5'--GAATGAGACGCATT TTTGACCTGGGACATG-3') were used to amplify *P*toe-2 using restriction digest with *AgeI* and *SacI*, and T4 ligation to generate plasmid *pBC1591* (*P*unc-7::*gfp)*. A ced-1 minigene was amplified from plasmid pZ2210 (*pZ2210*: *ced-1::*::gfp, Zhou et al. 2001) by PCR using the primers *AgeI* ced-1-F (5'--aaaACCGGT Ttgggtagaaaaatcctcttgatgct-3') and ced-1-R (5'--tttttacccgg tactgtaaactct-3'). The resulting PCR product and pBC1591 were digested with *AgeI* and the ced-1 minigene was inserted by T4 ligation into the linearized pBC1591 to obtain plasmid pBC1681 (*P*unc-7::*ced-1::*::gfp)*.
using the primers NM1 (5’-gagctctgctctcctctgtcaaggTTATC TGACCAAATTCCTTG-3’) and NM2 (5’-tgagctcggacattTT TGAGCTCTAGAGACATG-3’), and pBC1565 as a template. mKate2::tac-1 was obtained by PCR amplification using plasmid TMD34 (P_mec-4::mKate2::tac-1::ttb-2 3’UTR; T. Mikeladze-Dvali, (LMU Munich) personal communication) as a template, and the primers NM3 (5’-ctctcaggctaaaaAGTTCG AGTCATCAAG-3’) and NM4 (5’-aattcagtaaatTTATGC ATCCGTCAAATAAC-3’). Similarly, pBC1565 was used as a template to amplify the unc-54 3’UTR using primers NM5 (5’-gcggagctctagaATTGCCAGGACATG-3’) and NM6 (5’-agtcgtaaatcctctcattaaGAAAAGTTATGTTGTATAT TGG-3’). These fragments were introduced into Stul-digested pCFJ909 (Frøkjær-Jensen et al. 2014) using Gibson assembly to obtain pBC1807 (P_toe-2::mKate2::tac-1).

**Microinjection**

**bcIs133 (P_toe-2::gfp):** pBC1565 (20 ng/µl) + pRF4 (80 ng/µl) was injected into the gonads of young N2 adult hermaphrodites and extrachromosomal array was integrated by UV irradiation to obtain bcIs133, which was 5X backcrossed with N2.

**bcEx1277 (P_hyp-1::ced-1::gfp):** pBC1681 (51 ng/ml) + IR101 (10 ng/µl) (P_pe-4::HygR::gpd-2::gpd-3::mCherry::unc-54 3’UTR; Radman et al. 2013) was injected into the gonads of young unc-119(ed3); ced-3(n2427); bcIs133 adult hermaphrodites to obtain ced-1(e1735); ced-3(n2427); bcIs133; bcEx1277.

**bcEx1334 (P_toe-2::ced-1::mKate2):** bcEx1334 was generated by injecting pBC1805 (26 ng/µl) + pCFJ90 (P_mec-4::mCherry::unc-54 3’UTR; Frøkjær-Jensen et al. 2008) (2.6 ng/µl) + pBluescript II KS+(+) (60 ng/µl) into the gonads of young unc-119(ed3) animals (Frøkjær-Jensen et al. 2014). First, 10 ng/µl of pBC1807 was injected into the gonads of HT1593 animals along with pCFJ601 (50 ng/µl), pGH8 (10 ng/µl), pCFJ90 (2.5 ng/µl), and pCFJ104 (5 ng/µl) (Frøkjær-Jensen et al. 2014). Worms were allowed to starve for 1 week, after which wild-type movers were examined for integration.

**bcSi82 (P_toe-2::mKate2::tac-1):** bcSi82 was generated by miniMOS injection of pBC1807 (P_toe-2::mKate2::tac-1) into HT1593 [unc-119(ed3)] animals (Frøkjær-Jensen et al. 2014). First, 10 ng/µl of pBC1807 was injected into the gonads of HT1593 animals along with pCFJ601 (50 ng/µl), pGH8 (10 ng/µl), pCFJ90 (2.5 ng/µl), and pCFJ104 (5 ng/µl) (Frøkjær-Jensen et al. 2014). Worms were allowed to starve for 1 week, after which wild-type movers were examined for integration.

**Extra PVM neurons**

Posterior ventral mechanosensory (PVM) neurons were visualized using the transgene P_mec-4::gfp (bzIs190), which labels all mechanosensory neurons (Mitani et al. 1993). Fourth larval stage (L4) larvae of the desired genotype were anesthetized in a drop of sodium azide solution (30 mM in M9 buffer) on a 2% agarose pad on a glass slide. A 100X/1.3 NA oil-immersion objective lens on a Zeiss (Carl Zeiss), Thornwood, NY) ImagerM2 epifluorescence microscope was used to visualize PVM neurons.

**QL.pp survival**

QL.pp survival was determined using the transgene P_toe-2::gfp (bzIs133), which labels cells of the Q lineages (Gurling et al. 2014). In wild-type animals, QL.pp dies within ~17 hr post-egg laying during the first larval stage (L1 stage) (at 25°C) (Sulston and Horvitz 1977). To avoid false positives due to delayed cell death, we used larvae of the second larval stage (L2 larvae) (25–30 hr postegg laying) for analysis. Larvae were anesthetized in a drop of levamisole solution (10 mM in M9 buffer) on a 2% agarose pad on a glass slide. P_toe-2::gfp was visualized with the help of a 100X/1.3 NA oil-immersion objective lens on a Zeiss ImagerM2 epifluorescence microscope. We only considered those worms for assessing QL.pp survival in which QL.pp had divided and its daughters had formed visible neurite extensions at the time of counting. Thus, we ensured that slow-growing strains were not analyzed at an earlier developmental time point. Importantly, upon failure of death, QL.aa, which is produced in the vicinity of QL.p, migrates toward the tail, and therefore away from QL.pp. As a result, it does not interfere with our determination of QL.pp survival.

**Division of “undead” QL.pps**

The division of undead QL.pps was determined using the transgene P_toe-2::gfp (bzIs133). L2 larvae were prepared similarly as for QL.pp survival. Animals were assessed after QL.pps had divided to form PVM and SDQL neurons with visible neurite outgrowths.

**Live imaging of QL.p and QL.a divisions**

The transgene P_toe-2::gfp (bzIs133) was used to identify QL.p and QL.a, and to analyze their asymmetric divisions. L1 larvae were immobilized in 1 µl polybead microsphere suspension (0.1 µm diameter, 2.5% w/v, catalog number 00876; Polysciences, Warrington, PA) on a 10% agarose pad (agarose was dissolved in 67% M9 buffer). A glass coverslip was placed on the agar pad and the empty space around the agarose pad underneath the coverslip was filled with paraffin oil to prevent dehydration. Image Z-stacks were acquired every 3 min with a 63X/1.4 NA oil-immersion objective lens on the UltraVIEW VoX spinning disk microscope (Perkin Elmer [Perkin Elmer-Cetus], Norwalk, CT).

**QL.p and QL.a daughter cell sizes**

Q-lineage cells are relatively flat cells. As a result, single-plane cell areas provide a fair estimation of cell sizes (i.e., area is directly proportional to cell volume) (Cordes et al. 2006). Therefore, the image Z-stacks acquired of the daughters of QL.p and QL.a using the transgene P_toe-2::gfp (bzIs133) (see Live imaging of QL.p and QL.a divisions) were converged to obtain maximum-intensity Z-projections. Cell sizes were estimated by circumscribing the cells and measuring their areas with Fiji (Schindelin et al. 2012; Schneider et al. 2012).

**Determination of QL.p cleavage furrow position**

Images of QL.p undergoing cytokinesis were obtained from the movies generated to assess QL.p daughter cell sizes. The
position of the cleavage furrow was determined by measuring its distance from the anterior periphery of QL.p and dividing it by the total length of the cell (Figure 5D). Distances were measured using Fiji (Schindelin et al. 2012; Schneider et al. 2012).

Localization studies for CED-1ΔC::GFP

CED-1 localization was analyzed using the transgene Pced-1::ged-1ΔC::gfp (enls1) (Zhou et al. 2001). L1 larvae were anesthetized with levamisole (0.1 mM in M9 buffer), mounted on 3% agarose pads on glass slides, and examined using 63×/1.4 NA oil-immersion objectives on a Leica SP5 inverted confocal microscope. Q-lineage cells were identified with the help of the transgene Pegl-17::mCherry::his-24, Pcgl-1::myristoylated mCherry, Pegl-17::mig-10::yfp (rdvls1) (Ou et al. 2010).

TAC-1 ratio

The TAC-1 ratio was determined using the transgenes PIE::pie-2mKate2::::tac-1 (bcSi82) and PIE::pie-2::gfp (bcIs133). L1 larvae of the desired genotypes were grown at 20°C and immobilized in 1 μl polybead microsphere suspension, and slides prepared as described earlier for live imaging of QL.p and QL.a divisions. Image Z-stacks were acquired every 5 min with a 0.5-μm step size using a 100×/1.4 NA oil-immersion objective lens on the UltraVIEW VoX spinning disk microscope (Perkin Elmer). The time point at which QL.p was at metaphase was used to determine the amount of TAC-1 associated with the two centrosomes. Quantification of the amount of centrosome-associated TAC-1 was performed as described previously (Chakraborty et al. 2015). A region of the same size on the slide but outside the animal was considered as background noise, and its intensity was subtracted from the measured intensities of centrosome-associated TAC-1.

NSM neuroblast daughter cell sizes

The NSM and NSMsc were identified using the transgene Ppie-1::mCherry::phPLGB (lts44), and their sizes determined as described (Chakraborty et al. 2015; Wei et al. 2017). All images were acquired using a Leica TCS SP5 II confocal microscope. All strains were incubated at 20°C overnight before imaging.

Embryonic lethality

Five L4 larvae of each genotype were singled on seeded NGM plates. These were allowed to lay eggs for 30 hr at 20°C. Two-days later, the numbers of larvae and dead eggs were determined.

Statistical analysis

Proportions were compared using Fisher’s Exact Test, and the obtained P-values were adjusted using the Benjamini and Hochberg test for multiple comparisons (Fisher 1935; Yoav 1995). Wherever applicable, data were tested for normal distribution using the D’Agostino and Pearson normality test (D’Agostino and Pearson 1993). When data were found to be distributed normally, the Student’s t-test or parametric one-way ANOVA were performed to determine statistical significance between groups assuming that the groups had unequal SD, and Tukey’s or the Benjamini and Hochberg multiple comparisons test was applied (Student 1908; Fisher 1921; Tukey 1949). When comparing the amounts of TAC-1 on the anterior and posterior centrosomes (where all posterior TAC-1 levels were set to 1), we used the Wilcoxon signed-rank test to determine if the groups were statistically different (Wilcoxon 1945). The Mann–Whitney test was used to compare the ratios of QL.a daughter cell sizes in wild-type animals with those in ced-3(n717) animals (Mann 1947).

Data availability

All reagents and strains generated for this study are available from the authors upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article and figures. Supplemental material available at Figshare: https://doi.org/10.25386/genetics.7058609.

Results

A ced-1 MEGF10-dependent, but pig-1 MELK-independent, gradient of CED-3 caspase activity is present in the postembryonic QL.p neuroblast

During the L1 stage of postembryonic C. elegans development, the neuroblast QL.p divides asymmetrically by size and fate to produce a larger anterior daughter, QL.pa, which survives, and a smaller posterior daughter, QL.pp, which is programmed to die (Sulston and Horvitz 1977; Cordes et al. 2006) (Figure 1, wild-type). The observation that defects in the asymmetric division of QL.p by size can affect the fate of its daughters (especially the fate of QL.pp) indicates that daughter cell size and daughter cell fate are functionally coupled (Cordes et al. 2006; Singhvi et al. 2011; Gurling et al. 2014; Teuliere et al. 2014; Teuliere and Garriga 2017). To determine whether a gradient of CED-3 caspase activity forms in QL.p prior to its division, we used a reporter for the protein TAC-1 (Ptoe-2::mKate2::::tac-1). TAC-1 is a component of the pericentriolar material and a substrate of CED-3 caspase (Bellanger and Gonczy 2003; Le Bot et al. 2003; Srayko et al. 2003; Chakraborty et al. 2015). We have previously shown that in the embryonic NSM neuroblast, the amount of TAC-1 associated with the centrosome that is inherited by the larger daughter, which survives, is greater (by 1.30-fold) than the amount of TAC-1 associated with the centrosome that is inherited by the smaller daughter, which is programmed to die (Chakraborty et al. 2015). This “TAC-1 asymmetry” is dependent on a functional ced-3 caspase gene as well as a CED-3 caspase cleavage site in the TAC-1 protein and, hence, reflects a gradient of CED-3 caspase activity along the cleavage axis of the NSM neuroblast. Furthermore, the establishment and/or maintenance of this gradient of CED-3 caspase activity in the NSM neuroblast is dependent...
on the two conserved *C. elegans* engulfment pathways (Chakraborty et al. 2015). We found that at QL.p metaphase, the amount of TAC-1 associated with the anterior centrosome is 1.25-fold greater than that associated with the posterior centrosome (Figure 2, A and B). Furthermore, this asymmetry is lost in the background of a strong loss-of-function (lf) mutation of *ced-3* caspase, *n717*, or a strong lf mutation of the engulfment gene *ced-1* MEGF10, *e1735* (ratios of 0.97 and 0.99, respectively) (Figure 2, A and C). The gene *pig-1* encodes a PAR-1-like kinase orthologous to mammalian MELK (maternal embryonic leucine zipper kinase) and is important for the asymmetric division of QL.p. Specifically, the loss of *pig-1* MELK causes QL.p to divide symmetrically by size (Cordes et al. 2006) (see below). Interestingly, we found that *TAC-1* asymmetry at QL.p metaphase is not affected by a strong lf mutation of *pig-1* MELK, *gm344* (ratio of 1.28) (Figure 2, A and C). Therefore, a *ced-1* MEGF10-dependent gradient of CED-3 caspase activity is also formed in QL.p prior to its division. This gradient is along the anterior–posterior axis and presumably results in the nonrandom segregation of active CED-3 caspase into the smaller posterior daughter QL.pp, which is programmed to die. *pig-1* MELK is not required for the formation of this CED-3 caspase activity gradient. However, because of its role in the asymmetric division of QL.p by size, its loss nevertheless probably affects the concentration of active CED-3 caspase in QL.pp (the concentration is probably less than that in wild-type; see below).

Consistent with the notion that *ced-1* MEGF10 and the engulfment pathways play an instructive role in the establishment and/or maintenance of this gradient of CED-3 caspase activity, we found that an asymmetric contact exists between QL.p and a neighboring cell that exhibits detectable levels of CED-1 MEGF10 on its cell surface (Figure 3, Supplemental Material, Figure S1 and File S1). Specifically, QL.p is in contact with the syncytial cell hyp7, which is part of the hypodermis that covers large parts of the animal and which, after QL.p division, engulfs the QL.pp corpse (Sulston and Horvitz 1977). Using a CED-1 MEGF10 reporter (*P Ced-1CED-1ΔC::gfp*) (Zhou et al. 2001), we detected CED-1 MEGF10 on what appears to be the entire surface of hyp7 (Figure 3, Figure S1, and File S1). However, we found that while there is almost uniform contact between hyp7 and the lateral side of QL.p, there is an asymmetric contact between hyp7 and the medial side of QL.p. More specifically, the posterior, but not the anterior, part of QL.p’s medial side contacts hyp7. Therefore, a cell surface that contains detectable levels of CED-1

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**Figure 1** The *C. elegans* QL.p neuroblast lineage. (A) Schematic representation of the postembryonic QL.p neuroblast lineage in wild-type (left) and mutant (right) worms. Vertical and horizontal lines represent individual cells and cell divisions, respectively. “X” denotes a cell death. (B) Schematic representation of the cell sizes of the QL.p neuroblast and its daughter cells in wild-type (left) and mutant (right) worms. In wild-type worms, QL.pa is nearly three times as large as its sister, QL.pp. However, in mutants with defects in the asymmetric division by size of QL.p, QL.pa and QL.pp can be of similar sizes.
MEGF10 opposes the part of QL.p that will later form the smaller daughter, QL.pp, which is programmed to die, but not the part of QL.p that will later form the larger daughter, QL.pa, which survives (schematically represented in the drawing in the lower left hand corner of Figure 3; this drawing was compiled based on the images shown in Figure 3 and in Figure S1). We propose that this asymmetry in the presentation of CED-1 MEGF10 on apposing cell surfaces (and, hence, in the activation of CED-1 MEGF10 and the engulfment pathways) is critical for the establishment of a gradient of CED-3 caspase activity along the anterior–posterior axis of QL.p.

**The loss of ced-1 MEGF10 promotes the appearance of extra PVM neurons**

The larger daughter, QL.pa, not only survives but also divides to generate the neurons PVM and SDQL (Figure 1A, wild-type). To determine the possible function of the ced-1 MEGF10-dependent gradient of CED-3 caspase activity in QL.p, we analyzed the number of PVM neurons generated by this lineage. A block in apoptotic cell death prevents the death of QL.pp, and this can lead to the presence of two, rather than one, PVM neurons in L4 larvae (Figure 1A, Mutants). For example, using a PVM-specific reporter (Mitani et al. 1993), an extra PVM neuron is detected in 0% of wild-type animals but in 2% of ced-3(n717) animals (Chien et al. 2013) (Figure 4, A and B). In contrast, a compromised apoptotic cell death pathway, such as in animals homozygous for the weak ced-3 if mutation n2427, does not lead to the presence of an extra PVM neuron. We found that in a ced-3(n2427), but not wild-type, background, ced-1(e1735) causes the presence of an extra PVM neuron in 3% of the animals (Figure 4B). We also analyzed ced-1(e1735) in the background of pig-1(gm344). In an otherwise wild-type background, the loss of pig-1 MELK causes the presence of an extra PVM neuron in 30% of the animals (Cordes et al. 2006) (Figure 4B). We found that ced-1(e1735) as well as ced-3(n2427) significantly enhanced this phenotype to 58 or 70%, respectively. Furthermore, we found that 96% of the triple-mutant animals had an extra PVM neuron. Therefore, the loss of ced-1 MEGF10 (and, hence, the loss of the gradient of active CED-3 caspase in QL.p) promotes the presence of extra PVM neurons in a background in which the apoptotic cell death pathway is compromised as well as in a background in which QL.p divides symmetrically. Furthermore, in a background in which QL.p divides symmetrically [i.e., in pig-1(gm344)], the enhancements of the extra PVM neuron phenotype, either through compromising the apoptotic cell death pathway or through the loss of ced-1 MEGF10, are additive.

**ced-1 MEGF10 is not required for the asymmetric division of QL.p by size**

The presence of an extra PVM neuron can be the result of a defect in the asymmetric division of QL.p by size, the asymmetric division of QL.p by fate, or a combination thereof. To determine which of these processes is affected by ced-1(e1735), we analyzed them individually. To analyze the asymmetric division of QL.p by size, we used a GFP reporter that is expressed in QL.p and its daughters (P

![Figure 2A](image-url)  A ced-1 MEGF10-dependent gradient of CED-3 caspase activity exists in QL.p at metaphase. (A) Representative images of centrosome-associated mKate2::TAC-1 in QL.p at metaphase in the indicated genotypes. Bar, 3 μm. QL.p was identified using P

![Figure 2B](image-url)  Bar graph showing the comparison of total mKate2::TAC-1 signals associated with the anterior and posterior centrosomes during QL.p metaphase in wild-type animals. Total “Anterior” TAC-1 signal was normalized to the total “Posterior” TAC-1 signal, which was set to 1 for each animal. Wilcoxon signed-rank test was used to determine statistical significance. **P < 0.01, n = 11 QL.ps. (C) Dot plot showing the spread of the TAC-1 ratios in QL.p (Anterior/Posterior) among animals of each genotype. The horizontal red lines indicate the mean for each genotype (also mentioned above the spread for each group). The dotted black line represents the mean for wild-type (+/+). The dotted green line represents the ratio 1. Each dot represents the TAC-1 ratio for one animal. Statistical significance was determined using the Student’s t-test and Benjamini and Hochberg multiple comparisons correction. * P < 0.05, n ≥ 10 QL.ps. n.s., not significant.

![Figure 2C](image-url)  A ced-1 MEGF10-dependent gradient of CED-3 caspase activity exists in QL.p at metaphase. (A) Representative images of centrosome-associated mKate2::TAC-1 in QL.p at metaphase in the indicated genotypes. Bar, 3 μm. QL.p was identified using P

![Figure 2D](image-url)  Bar graph showing the comparison of total mKate2::TAC-1 signals associated with the anterior and posterior centrosomes during QL.p metaphase in wild-type animals. Total “Anterior” TAC-1 signal was normalized to the total “Posterior” TAC-1 signal, which was set to 1 for each animal. Wilcoxon signed-rank test was used to determine statistical significance. **P < 0.01, n = 11 QL.ps. (C) Dot plot showing the spread of the TAC-1 ratios in QL.p (Anterior/Posterior) among animals of each genotype. The horizontal red lines indicate the mean for each genotype (also mentioned above the spread for each group). The dotted black line represents the mean for wild-type (+/+). The dotted green line represents the ratio 1. Each dot represents the TAC-1 ratio for one animal. Statistical significance was determined using the Student’s t-test and Benjamini and Hochberg multiple comparisons correction. * P < 0.05, n ≥ 10 QL.ps. n.s., not significant.
(Gurling et al. 2014), and acquired image stacks of QL.p in developing L1 larvae every 3 min. The first stack acquired after the completion of QL.p cytokinesis was then used to estimate the sizes of the two daughter cells (Figure 5A) (Q-lineage cells are relatively flat cells. Hence, measurements of their areas of maximum intensity projection images provide reliable approximations of their cell sizes (Cordes et al. 2006)). In wild-type animals, QL.p divides asymmetrically by size to produce the larger QL.pa and the smaller QL.pp with a size ratio of QL.pa to QL.pp of $C_{24}/3.0$ (Figure 1B, wild-type; Figure 5, B and C). As was reported previously (Cordes et al. 2006), in pig-1(gm344) animals, QL.p divides symmetrically to produce two cells of similar sizes with a size ratio of QL.pa to QL.pp of 1.1 (Figure 5, B and C). Using this assay, we did not observe a significant effect of ced-1(e1735) on the asymmetric division by size of QL.p. Therefore, the loss of ced-1 MEGF10 does not promote the presence of additional PVM neurons by affecting the asymmetric division by size of QL.p.

**ced-1 MEGF10 promotes the apoptotic fate of QL.pp**

QL.p divides asymmetrically by fate to produce QL.pa, which survives and divides to generate two neurons, PVM and SDQL (“mitotic fate”), and QL.pp, which is programmed to die by apoptosis (“apoptotic fate”) (Figure 1A). We tested whether the loss of ced-1 MEGF10 affects the ability of QL.pp to adopt the apoptotic fate. Using the $P_{egl-1:mCherry::his-24},P_{egl-1:myristoylated\ mCherry}, P_{egl-1:mig-10:yfp (rdvIs1)}$ and hyp7 with the transgene $P_{ced-1\Delta C::gfp \ (enIs1)}$ (see Materials and Methods for details). The diagram in the middle-right represents the orientation of the image planes, images for which have been shown at the bottom-right. In these images, white arrowheads indicate a contact between CED-1$\Delta C::GFP$ and QL.p, whereas blue arrowheads indicate the lack of contact. Bar, 2 $\mu$m. It can be seen in the images for slices 4–9 that CED-1$\Delta C::GFP$ is in contact with the posterior half of QL.p but not with its anterior part. The coronal section (bottom-left) has been created based on the fluorescence images (slices shown at the bottom-right) and the images of the electron microscopy sections. It can be seen in this diagram that while hyp7 almost entirely envelopes the posterior part of QL.p, it only forms contact with the left side of the anterior part.
n1813, respectively), two other engulfment genes, also enhance the ced-3(n2427) phenotype. To determine in which cell or cells the engulfment genes act to enhance QL.pp survival, we performed rescue experiments using promoters that drive transgene expression specifically in the Q lineage (toe-2 promoter) (Gurling et al. 2014) or in hyp7 (“hyp7 promoter”) (Hunt-Newbury et al. 2007). We found that the expression of a ced-1 minigene under the control of the hyp7 promoter, but not the toe-2 promoter, rescues the ced-1(e1735) phenotype (Figure 6B). Therefore, in a background in which apoptotic cell death is compromised, the loss of ced-1 MEGF10 or other engulfment genes increases the probability of QL.pp survival. Hence, the engulfment genes act to promote the apoptotic fate in QL.pp. Furthermore, in this context, ced-1 MEGF10, and most probably the other engulfment genes, act in hyp7 and therefore in a cell nonautonomous manner. Finally, based on our finding that a ced-1 MEGF10-dependent gradient of CED-3 caspase activity is established in QL.p before its division, we propose that ced-1 MEGF10 and the other engulfment genes promote the apoptotic fate of QL.pp by causing the nonrandom segregation of active CED-3 caspase into QL.pp.

**pig-1 MELK, ced-1 MEGF10, and ced-3 caspase interact to promote the apoptotic fate of QL.pp**

We also determined the effect of ced-1(e1735) on the ability of QL.pp to adopt the apoptotic fate in a background in which QL.p divides symmetrically by size, i.e., in the pig-1(gm344) background. In pig-1(gm344) animals, 45% of QL.pps survive (Figure 6B). We found that the loss of ced-1 MEGF10 enhances QL.pp survival in this background to 86% (Figure 6B). Similarly, ced-3(n2427) enhances QL.pp survival in pig-1(gm344) animals from 45 to 94%. Finally, in the triple mutant, 100% of QL.pps inappropriately survive. The loss of pig-1 MELK does not affect the formation of the gradient of CED-3 caspase activity in QL.p (see above; Figure 2, A and C). However, since the size of QL.pp in pig-1(gm344) animals is increased by a factor of ~2.0, the loss of pig-1 MELK probably results in a significant reduction of the concentration of active CED-3 caspase in QL.pp after QL.pp division (since QL.p and its daughters are relatively flat cells, an increase by a factor of ~2.0 in cell size results in an increase by a factor of ~2.0 in cell volume as well; hence, the loss of pig-1 potentially could reduce the concentration of active CED-3 caspase by as much as twofold). Furthermore, the loss of pig-1 MELK may also affect the concentration in QL.pp of factors other than CED-3 caspase. For example, the loss of pig-1 MELK may cause a reduction in the concentration of other proapoptotic factors, such as transcriptional activators of egl-1 BH3-only. Combined with a reduction in the concentration of active CED-3 caspase, this may be sufficient to cause 45% of the QL.pps to inappropriately survive. Aboliishing the gradient of CED-3 caspase activity in QL.p [i.e., ced-1(e1735)] or compromising the apoptotic cell death pathway [i.e., ced-3(n2427)] is expected to reduce the concentration of active CED-3 caspase in QL.pp in pig-1(gm344) animals even further, thereby increasing the fraction of animals in which QL.pp inappropriately survives. Finally, in ced-1(e1735); pig-1(gm344) ced-3(n2427) triple mutants, the concentration of active CED-3 caspase in QL.pp is reduced below the threshold required to execute the apoptotic fate in 100% of the animals.

**pig-1 MELK, but not ced-1 MEGF10, is required to restrict mitotic potential to QL.p**

Next, we asked whether the loss of ced-1 MEGF10 affects the ability of an undead QL.pp to inappropriately adopt the mitotic fate, i.e., the fate normally adopted by QL.p. Specifically, using Pmec-2-gfp, we determined the fraction of undead QL.pps that divide (Figure 7A). Since in ced-1(e1735) animals QL.pp always dies, we addressed this question in the background of ced-3(n2427). In ced-3(n2427) or ced-1(e1735); ced-3(n2427) animals, 37 or 58% of QL.pps inappropriately survive, respectively. We found that none of the
undead QL.pps divide in either of these genetic backgrounds (Figure 7B). In contrast, in pig-1 (gm344) animals, in which 45% of QL.pps inappropriately survive, 56% of undead QL.pps divide. Therefore, the loss of pig-1 MELK, but not ced-1 MEGF10, causes undead QL.pps to inappropriately adopt the mitotic fate. Hence, pig-1 MELK, but not ced-1 MEGF10, is required to restrict the mitotic potential to QL.pa. Based on this observation, we propose that a pig-1 MELK-dependent, ced-1 MEGF10-independent gradient of mitotic potential exists in QL.p prior to its division. Furthermore, we propose that this gradient is along the anterior–posterior axis of QL.p reciprocal to the gradient of CED-3 caspase activity (with a higher concentration in the anterior rather than posterior part of QL.p) and that during QL.p division, this gradient results in the nonrandom segregation of mitotic potential into QL.pa, instead of QL.pp (Figure 9). According to this model, the loss of this gradient in pig-1 (gm344) animals should result in the inappropriate presence of mitotic potential in QL.pp after QL.p division, and, hence, the ability of undead QL.pps to adopt the mitotic fate and divide. In support of this model, Garriga and co-workers found that while the loss of pig-1 MELK or strd-1 STRD1 causes similar defects in the asymmetric division by size of QL.p and QR.p, pig-1 mutants have a more penetrant “extra neuron phenotype,” most likely as a result of the division of more undead QL.pps and QR.pps (Chien et al. 2013). Based on this observation, the authors proposed that pig-1 may act in a strd-1-independent manner to affect the segregation of “fate determinants” during QL.p and QR.p division. Alternatively, the mitotic potential could be distributed throughout QL.p, and inherited into QL.pa and QL.pp proportional to cell size, but the small size of QL.pp (~1/3 of QL.pa) may prevent its ability to divide. In that case, the loss of pig-1 MELK would increase by a factor of ~2.0 both the size of QL.pp
as well as the total amount of mitotic potential in QL.pp (with no change in its concentration), and this would lead to the inappropriate division of 56% of undead QL.pps. Finally, pig-1 MELK may also act in QL.pp to antagonize the activity of mitotic potential, and this could lead to the division of undead QL.pps in animals lacking pig-1.

Surprisingly, we found that ced-3(n2427) significantly increases the fraction of undead QL.pps that divide in pig-1(gm344) animals (from 56 to 80%; Figure 7B). In contrast, ced-1(e1735) fails to do so in a pig-1(gm344) (56 vs. 45%) or pig-1(gm344) ced-3(n2427) (80 vs. 87%) background. Based on this, we propose that CED-3 caspase antagonizes the activity of the mitotic potential. Furthermore, the finding that compromising the apoptotic cell death pathway (i.e., ced-3(n2427)) but not abolishing the gradient of CED-3 caspase activity in QL.p [i.e., ced-1(e1735)] increases the fraction of undead QL.pps that divide in pig-1(gm344) animals, indicates that it is CED-3 caspase activity per se rather than a gradient of CED-3 caspase activity that is capable of antagonizing the activity of the mitotic potential. Possibly, the functional interaction between CED-3 caspase and the mitotic potential could occur in QL.p (before the formation of the gradient of CED-3 Caspase activity) and not in QL.pp after QL.p division. Alternatively, the interaction could take place in QL.pp; in this case, ced-3(n2427) but not ced-1(e1735) would reduce the level of CED-3 caspase activity below the threshold necessary to antagonize the activity of the mitotic potential. Finally, the observation that ced-3(n2427) enhances the fraction of undead QL.pps that divide in pig-1(gm344), but not in wild-type animals, provides additional support for the notion that a gradient of mitotic potential exists in QL.p and that during QL.p division, little or no mitotic potential is normally segregated into QL.pp.

gel-1 BH3-only, ced-4 Apaf-1, and ced-3 caspase promote the ability of QL.p to divide asymmetrically by size

Like ced-1 MEGF10, the genes egl-1 BH3-only, ced-4 Apaf-1, and ced-3 caspase are required for the formation of the gradient of CED-3 caspase activity in the NSM neuroblast and presumably also in QL.p. However, egl-1 BH3-only, ced-4 Apaf-1, and ced-3 caspase, but not ced-1 MEGF10, are required for the initial maturation and full activation of CED-3 caspase. We found that unlike ced-1(e1735), egl-1(n3330), ced-4(n1162), and ced-3(n717) affect the ability of undead QL.pps to divide. Specifically, we found that in an otherwise wild-type background, these mutations cause between 2 and 11% of undead QL.pps to divide (Figure 7B). We also tested the ced-3 mutation n2433, which is a missense mutation in the coding region of the ced-3 gene and leads to the synthesis of CED-3 protein that lacks protease activity. We found that, similarly, ced-3(n2433) causes 4% of undead QL.pps to divide. Hence, the loss of CED-3 caspase activity per se rather than the loss of a gradient of CED-3 caspase activity can result in the inappropriate presence of mitotic potential in undead QL.pps after QL.p division. This suggests that it is the loss of ced-3 caspase in QL.p rather than QL.pp that causes the inappropriate presence of mitotic potential in QL.pp. Therefore, we tested whether the loss of egl-1 BH3-only, ced-4 Apaf-1, or ced-3 caspase affects the asymmetric division of QL.p by size and, hence, the partitioning of mitotic potential during QL.p division. We found that rather than exhibiting a cell size ratio of QL.pa to QL.pp of ~3.0, in these mutants, cell size ratios observed range from 2.4 to 2.0, indicating that the cleavage plane has shifted anteriorly resulting in larger QL.pps (Figure 5, B and C) [it has previously been reported that the loss of ced-4 affects the cell size ratio of QL.pa to QL.pp (Singhvi et al. 2011)]. To rule out
that the changes in cell size ratios observed are a result of
differences in the cell shape of undead QL.pps (and, hence,
our ability to estimate QL.pp size rather than the asymmetry of
the QL.p division), we determined the position of the cleavage
furrow in QL.p in the different mutant backgrounds. To that
e nd, we used the image stacks generated every 3 min of L1
larvae carrying the P_{toe-2gfp} reporter that we generated to
determine the cell size ratio of QL.pa to QL.pp (Figure 5A), and
used the last stack before QL.p division to determine the posi-
tion of the cleavage furrow as outlined in Figure 5D. Using this
approach, we found that in wild-type animals, the cleavage
furrow is positioned at 62.4% QL.p length and in pig-
1(gm344) animals at 51.7% QL.p length (Figure 5E). Consid-
ering that the cell size ratio of QL.pa to QL.pp is 2.9 in the wild-
type and 1.1 in pig-1(gm344) animals, it is likely that small
changes in cleavage furrow position may result in significant
changes in QL.pa to QL.pp cell size ratio. Next, we analyzed
animals lacking egl-1 BH3-only, ced-4 Apaf-1, or ced-3 caspase,
and found significant changes in cleavage furrow position in
ced-4(n1162) and ced-3(n717) animals (57 and 56.9% of
QL.p length, respectively). In addition, we found small changes
in cleavage furrow position in egl-1(n3330) and ced-3
(n2433), but not ced-1(e1735), animals. As mentioned above,
small changes in cleavage furrow position may result in sig-
nificant changes in QL.pa to QL.pp cell size ratio. The small
changes in cleavage furrow position detected in egl-1(n3330)
and ced-3(n2433) animals may therefore account for the signif-
cicant changes in QL.pa to QL.pp cell size ratios observed in these
animals (Figure 5, C and E).

Based on these observations, we conclude that the loss of
egl-1 BH3-only, ced-4 Apaf-1, or ced-3 caspase compromises
the ability of QL.p to divide asymmetrically by size. There-
fore, CED-3 caspase contributes to the asymmetric division by
size of QL.p. Furthermore, we propose that through the ob-
served shift in cleavage plane toward the anterior, mitotic
potential is segregated inappropriately into QL.pp during
QL.p division, and that this accounts for the small fraction
of undead QL.pps that divide in these mutants. Finally, the
ability of CED-3 caspase to contribute to the asymmetric di-
vision by size of QL.p depends on its activation through egl-1
BH3-only and ced-4 Apaf-1, and, hence, the apoptotic cell
d eath pathway.

The function of the apoptotic cell death pathway in
asymmetric cell division by size is not restricted to the
QL.p neuroblast

To determine whether the function of the apoptotic cell
d eath pathway in asymmetric cell division by size is specific
to the QL.p neuroblast, we analyzed the postembryonic QL.a
neuroblast and the embryonic NSM neuroblast, both of
which divide asymmetrically by size and fate to produce a
smaller daughter that is programmed to die (Sulston et al.
1983; Hatzold and Conradt 2008). In wild-type animals,
QL.a divides to generate a larger posterior daughter,
QL.ap, and a smaller anterior daughter, QL.aa, with a cell size
ratio of QL.ap to QL.aa of 2.67 (Ou et al. 2010) (Figure 8, A
and B) (cells of the QL.a lineages are also relatively flat
cells; therefore, cell size was determined as for QL.p daugh-
ters). We found that in ced-3(n717) animals, this ratio was
reduced to 2.13. Furthermore, we observed an effect of the
loss of ced-3 caspase on the asymmetric division by size of
the NSM neuroblast; however, only in the background of
pig-1(gm344). Specifically, the NSM neuroblast divides to
produce a larger ventral daughter, the NSM, and a smaller
dorsal daughter, the NSMsc, with a cell size ratio of NSM to
NSMsc of 1.57 (Hatzold and Conradt 2008) (Figure 8, C and
D) (since cells of the NSM neuroblast lineage are not flat
cells, cell size was estimated as described in Figure 8C and
in the Materials and Methods). As reported previously,
in pig-1(gm344) animals, the NSM neuroblast divides symmet-
rically by size and generates two daughter cells of similar
sizes, with a cell size ratio of NSM to NSMsc of 1.00 (Wei
et al. 2017). In ced-3(n717) or ced-4(n1162) animals, the cell
size ratio is not significantly different from that of wild-type
animals (1.47 and 1.51, respectively). However, in a pig-1(gm344) background, the cell size ratio of ced-3(n717) or ced-4(n1162) animals is significantly reduced compared to that of pig-1(gm344) animals (0.84 and 0.83, respectively). A reduction in the ratio below 1.0 is indicative of a reversal of polarity. Indeed, in these double-mutant animals, the NSM is smaller than the NSMsc. In summary, these results indicate that the function of the apoptotic cell death pathway in asymmetric neuroblast division, at least by size, is not restricted to the QL.p neuroblast.

Our finding that ced-4(n1162) and ced-3(n717) affect the asymmetric division of the NSM neuroblast in a pig-1(gm344), but not wild-type, background suggests that in certain lineages, the apoptotic cell death pathway may act in parallel to pig-1 MELK to affect cellular polarization. Therefore, we tested whether ced-3(n717) enhances embryonic lethality in pig-1(gm344) animals.

When grown at 20°C, we observed 0% embryonic lethality among ced-3(n717) animals (n = 1413) and 8% embryonic lethality among pig-1(gm344) animals (n = 1514). However, in pig-1(gm344) ced-3(n717) double mutants, 43% of the animals arrested during embryonic development (n = 397). Therefore, the apoptotic cell death pathway may play a more general role in cellular polarization and asymmetric cell division, and, in this context, act in parallel to pig-1 MELK.

**Discussion**

*The formation of reciprocal gradients of mitotic and apoptotic potential is critical for the asymmetric division of the QL.p neuroblast by fate*

Based on results presented here, we propose that prior to QL.p division, reciprocal gradients of mitotic and apoptotic potential form in QL.p, and that these gradients result in the non-random segregation of mitotic potential into QL.pa and of apoptotic potential into QL.pp (Figure 9). We speculate that the mitotic potential in QL.pa promotes its ability to divide, and we provide evidence that the apoptotic potential in QL.pp promotes its ability to execute apoptotic cell death. Furthermore, while we know that apoptotic potential encompasses at least active CED-3 caspase, we do not know the molecular nature of the mitotic potential. However, we speculate that it encompasses factors involved in cell cycle control, and/or molecules or organelles required for energy production. We also provide evidence that distinct genetic pathways are responsible for the establishment and/or
maintenance of these two gradients in QL.p. Specifically, the two conserved *C. elegans* engulfment pathways are necessary for the gradient of CED-3 caspase activity but not the gradient of mitotic potential. Conversely, a pig-1 MELK-dependent pathway is necessary for the gradient of mitotic potential but not the gradient of CED-3 caspase activity. How these distinct pathways control the two reciprocal gradients and how these gradients are generated in the first place, remains to be determined. Interestingly, in the case of the gradient of CED-3 caspase activity, we found that there is a mechanistic difference in the symmetry-breaking event that orients this gradient along the dorsal–ventral axis in the NSM neuroblast and along the anterior–posterior axis in the QL.p neuroblast. In the case of the NSM neuroblast, all neighboring cells appear to present CED-1 MEGF10 on their cell surface; however, CED-1 MEGF10 clustering and activation specifically occurs on the cell surface of the two dorsal neighbors (Chakraborty et al. 2015). In contrast, in the case of QL.p, at least on the medial side, CED-1 MEGF10 is asymmetrically presented and, for this reason, results in CED-1 MEGF10 activation only on the posterior side. Finally, the results presented here indicate that the formation of a gradient of CED-3 caspase activity in a mother of a cell programmed to die is not specific to the NSM neuroblast lineage. Based on this, we speculate that it represents a general phenomenon of cell death lineages in developing *C. elegans* animals.

*The apoptotic cell death pathway is required for the asymmetric division by size and fate of the QL.p neuroblast*

The asymmetric division of mothers of cells programmed to die is critical for the correct fate of their daughters and, in particular, for the correct fate of the daughters programmed to die. Hence, it has been postulated that asymmetric cell division regulates the apoptotic cell death pathway (Hatzold and Conradt 2008; Teuliere and Garriga 2017). We now demonstrate that the apoptotic cell death pathway contributes to the ability of mothers of cells programmed to die to divide asymmetrically. Specifically, we present evidence that the apoptotic cell death pathway is required for the asymmetric division of QL.p by size and, hence, the correct sizes of the daughter cells QL.pa and QL.pp. In addition, we present evidence that the apoptotic cell death pathway is also required for the asymmetric division of QL.p by fate. Specifically, by controlling the sizes of QL.pa and QL.pp, the apoptotic cell death pathway indirectly influences the relative amounts of mitotic potential or apoptotic potential that are segregated into either QL.pa or QL.pp during QL.p division. In addition, the apoptotic cell death pathway is required for the activation of CED-3 caspase in QL.p, which we propose antagonizes the mitotic potential. Hence, the apoptotic cell death pathway controls the total amount of apoptotic and mitotic potential present in QL.p, and, therefore, the amounts that can be segregated into QL.pa and QL.pp. Based on these new findings, we now postulate that not only does asymmetric cell division regulate the apoptotic cell death pathway, but that in the context of cell death lineages, the apoptotic cell death pathway regulates asymmetric cell division. Furthermore, this regulation of asymmetric cell division through the apoptotic cell death pathway is necessary for the production of smaller daughters that are programmed to die.

*A new nonapoptotic function of the C. elegans apoptotic cell death pathway in asymmetric neuroblast division*

The *C. elegans* apoptotic cell death pathway, and CED-3 caspase in particular, has been implicated in a number of nonapoptotic processes, which range from aging and neuronal regeneration to the control of the expression of specific genes, such as the heterochronic gene *lin-28* (Pinan-Lucarre et al. 2012; Weaver et al. 2014; Yee et al. 2014). Similarly, mammalian caspases have been shown to have various nonapoptotic functions during development (Nakajima and Kuranaga 2017). We now present evidence that the apoptotic cell death pathway is also involved in the asymmetric division of neuroblasts that generate a smaller daughter that is programmed to die. How CED-3 caspase affects the position of...
the cleavage plane in these neuroblasts remains to be determined. In *C. elegans*, the positioning of the cleavage plane is best understood in the one-cell embryo, which, like QL.p, divides asymmetrically by size and fate (Rose and Gonczy 2014; Wu and Griffin 2017). In general, the cleavage plane is perpendicular to and centered on the middle of the mitotic spindle. As a result of unequal dynein-mediated pulling forces emanating from the anterior and posterior poles (with more pulling forces emanating from the posterior pole), in one-cell embryos, the spindle is shifted posteriorly along the anterior–posterior axis. As a result, the cleavage plane is shifted posteriorly as well, generating a larger anterior cell, AB, and a smaller posterior cell, P1. It has been shown previously that the spindle is shifted posteriorly in QL.p as well (Ou et al. 2010), and we speculate that this is also caused by unequal dynein-mediated pulling forces. However, whereas the loss of *pig-1* MELK causes QL.p to divide symmetrically rather than asymmetrically, it fails to do so in the one-cell embryo (however, the loss of *pig-1* MELK does synergize with the loss of *ani-1*, which encodes one of two *C. elegans* anillin genes, to affect the position of the cleavage plane in one-cell embryos (Pacquelet et al. 2015)). This suggests that the function of *pig-1* MELK in the regulation of cleavage plane position differs between the one-cell embryo and the QL.p neuroblast lineage. Nevertheless, since the loss of *ced-3* caspase causes a partial defect in the posterior shift of the cleavage plane in QL.p, *ced-3* caspase may be necessary for the dynein-mediated pulling forces that emanate from the posterior pole. However, the finding that the loss of *ced-3* caspase also affects the asymmetric division by size of the QL.a neuroblast suggests that dynein-mediated pulling forces may not be the target of *CED-3* caspase activity in this context. Specifically, like QL.p, the neuroblast QL.a divides asymmetrically by size and fate to generate a larger daughter, which survives, and a smaller daughter, which dies (in contrast to QL.p, the smaller daughter of QL.a is the anterior rather than the posterior daughter). However, rather than through a shift of the mitotic spindle along the anterior–posterior axis, it has been suggested that two daughter cells of different sizes are generated from QL.a through asymmetric myosin-mediated contractile forces during QL.a division (Ou et al. 2010). Hence, *ced-3* caspase may be required for general cellular polarization of QL.p and QL.a, and affect the position of the cleavage plane or myosin-mediated contractile forces indirectly. Consistent with the notion that *ced-3* caspase may be required for general cellular polarization, we found that in the embryonic NSM neuroblast lineage, the loss of *ced-3* caspase in a *pig-1* MELK mutant background causes a reversal of polarity. Finally, the finding that the loss of *ced-3* caspase enhances embryonic lethality in animals lacking *pig-1* MELK function suggests that *ced-3* caspase (and possibly the entire apoptotic cell death pathway) also functions in asymmetric cell division and cellular polarization in lineages other than cell death lineages, and that this may be crucial for normal animal development.

Interestingly, there is increasing evidence that mammalian caspases have functions in different types of embryonic and adult stem cells (Baena-Lopez et al. 2017). Hence, our finding that *C. elegans* CED-3 caspase plays a role in cellular polarization, and the asymmetric division by size and fate of neuroblasts may very well be relevant to these nonapoptotic functions of mammalian caspases in stem cell lineages.

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