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CEH-34 is Required for M4 Sister Cell Death and Pharyngeal Development. **Takashi Hirose**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

In *C. elegans*, 131 somatic cells undergo programmed cell death during wild-type hermaphrodite development. While many genes involved in programmed cell death have been identified, it remains unclear how a particular cell is specified to survive or to die by programmed cell death. To identify genes involved in the specification of programmed cell death, we screened for mutants defective in the deaths of the sister of the pharyngeal M4 neuron. The M4 motor neuron is generated during embryonic development and survives to regulate muscle contraction in the pharynx, while the M4 sister undergoes programmed cell death. The reporter *Pceh-28::gfp* (kindly provided by Brian Harfe and Andrew Fire) is expressed in the M4 of wild-type animals and in both the M4 and the M4 sister of *ced-3* mutants, which are defective in all somatic programmed cell deaths.

Screens of 71,820 mutagenized haploid genomes yielded 75 independent mutations that result in at least one extra *Pceh-28::gfp*-expressing cell. One of the isolates, *n4780*, is mutated in *pig-1*, a *par-1-like gene* that encodes a serine/threonine kinase and affects asymmetric neuroblast divisions. We also identified two *egl-1* alleles, *n4820* and *n4827*, for which we could not find any mutations in a coding region. These results suggest that the processes of asymmetric cell division and the regulation of *egl-1* expression affect M4 sister cell death. One of the other isolates, *n4796*, is not an allele of any known cell-death gene and affects the M4 sister cell death but not the NSM sister, RIM sister, RIC sister or VC neuron cell death. Using SNP mapping and transformation rescue, we found that *n4796* is an allele of *ceh-34*, a Six class homeobox gene. CEH-34 is expressed in pharyngeal cells and anterior body wall muscles. A deletion mutant (kindly provided by Shohei Mitani) showed more severe pharyngeal morphological defects than *n4796* animals, suggesting that *ceh-34* controls a variety of pharyngeal cell fates, including the M4 sister cell death. In *Drosophila*, the Six family homeobox gene *sine oculis* is a component of a highly conserved transcriptional network that specifies eye development. This transcriptional network includes *eyeless/vab-3*, *eyes absent/eya-1* and *dachshund/dac-1*. We examined M4 sister cell death in *C. elegans* *vab-3*, *eya-1* and *dac-1* mutants and found that *eya-1* mutants also have an extra *Pceh-28::gfp*-expressing cell; *vab-3* and *dac-1* mutants do not. These results suggest that at least some of the components of this transcriptional network are required for M4 sister cell death in *C. elegans*.

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Wnt pathway components activate CED-10/Rac through the complex ced-2, ced-5, ced-12 to control spindle orientation, engulfment of apoptotic corpses and migration. **Juan Cabello¹**, Ufuk Gunesdogan², Lukas Neukomm³, Michael Hengartner³, Ralf Schnabel². 1) Centro Investigacion Cancer, Salamanca, Spain; 2) Institute of Genetic. TU Braunschweig, Germany; 3) Institute of Molecular Biology, University of Zurich, Switzerland.

Two parallel pathways achieve the recognition and engulfment of apoptotic corpses in *C. elegans*. Both converge to activate CED-10/Rac. The first one is defined by the proteins CED-6/GULP, CED-7/ABC A1 and contains CED-1/SREC as receptor to detect the apoptotic corpses. The second one include the protein complex CED-2; CED-5, CED-12 and its receptor was hitherto unknown. Here we show that the receptor MOM-5, a Frizzled homolog of the Wnt pathway, function upstream of the second pathway not only in the engulfment but also in the spindle orientation of early blastomeres during embryogenesis and gonadal migration. APR-1/APC transduce the signal from the Wnt pathway via direct interaction with CED-2. Therefore, in different developmental processes, CED-10/Rac links polar signals mediated by the Wnt pathway to the modulation of the cytoskeleton.

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C. elegans UNC-108 plays a novel and essential role in the removal of apoptotic cell corpses. **Paolo M. Mangahas¹**, Zheng Zhou^{1,2}, 1) Program in Developmental Biology, Baylor College of Medicine, Houston, Texas 77030 USA; 2) Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030 USA.

Programmed cell death or apoptosis is a common cell fate specified during development. After cells execute the death program, they are rapidly recognized by phagocytes and engulfed. These cell corpses are then sequestered within phagosomes where they are subsequently degraded. Genetic studies in the nematode *Caenorhabditis elegans* have identified at least eight genes required for the efficient clearance of apoptotic cells. These genes mediate the recognition of cell corpses and the rearrangement of the actin cytoskeleton during engulfment. Recent findings from our lab have shown that the recruitment of intracellular vesicles provides material required for cell corpse engulfment and degradation.

Rab GTPases regulate a variety of vesicular trafficking events. In work performed using mammalian tissue culture, Rab2 has been shown to function in the export of secretory proteins from the endoplasmic reticulum to the Golgi apparatus. Quite interestingly, proteomic studies in *Drosophila* and mammalian systems have identified Rab2 as a phagosomal component. However, the function of Rab2 during phagocytosis is unknown.

From a collection of mutants isolated based on their defects in the removal of cell corpses (Ced phenotype; Z. Z. and H. R. Horvitz, unpublished results), we identified *n3263*, a recessive mutation that results in both Ced and Unc (uncoordinated locomotion) phenotypes. We determined that *n3263* is a loss-of-function allele of *unc-108*, the gene which encodes the *C. elegans* homolog of the evolutionarily conserved small GTPase Rab2. Genetic analyses suggest that *unc-108* functions downstream of the engulfment process. Indeed, transmission electron microscopy of germline cell corpses reveals that while apoptotic cells in *unc-108* mutants are engulfed, they are retained within phagosomes. By following a variety of fluorescent reporters *in vivo*, we demonstrate that the degradation of apoptotic cell corpses is severely impaired in *unc-108* mutants. During the meeting, we will report our characterization of *unc-108* mutants and discuss potential mechanisms for the novel role that *unc-108* plays during cell corpse removal.