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abl-1 Opposes the Engulfment of Apoptotic Cells. **Michael Hurwitz**[1], Pam Vanderzalm[2], Laird Bloom[1], Julia Den Uyl[1], Gian Garriga[2], Bob Horvitz[1]. 1) HHMI, Dept Biol, MIT, Cambridge, MA USA; 2) Dept. Molecular and Cellular Biology, UC Berkeley, CA 94720 USA.

In *C. elegans*, two parallel and partially redundant pathways comprising at least eight genes (*ced-1, ced-6, ced-7, dyn-1* and *ced-2, ced-5, ced-10, ced-12*) function in the engulfment of apoptotic cells. Loss-of-function (lf) mutations in these genes cause the persistence of unengulfed cell corpses. CED-7 is an ABC-type transporter that appears to signal through CED-1, a transmembrane receptor and CED-6, an adapter protein predicted to bind the phosphotyrosines of CED-1. The dynamin, DYN-1, which regulates membrane dynamics,acts downstream of CED-6. CED-2, a homolog of CrkII, is an adapter protein that activates the CED-5/CED-12 heterodimeric guanine nucleotide exchange factor (GEF). The CED-5/CED-12 GEF, in turn, activates CED-10, a small Rac GTPase. In mammals, CrkII functions in cell migration by activating the CED-10 homolog Rac1, which regulates cytoskeletal reorganization. This process is opposed by the oncoprotein Abl kinase, which phosphorylates CrkII. Abnormal Abl signaling plays a role in multiple human cancers. We tested whether mutations in the *C. elegans* Abl homolog *abl-1* can suppress the loss-of-function phenotype of engulfment pathway genes. *abl-1*(lf) suppresses the engulfment defects of strong loss-of-function mutations in the *ced-1/6/7* pathway and of *ced-2* but not of *ced-5* or *ced-12*, suggesting that *abl-1* opposes these pathways but not by directly blocking *ced-2* function. Instead, *abl-1* may act on a gene that is either downstream of *ced-2*, such as *ced-5*, *ced-12* or *ced-10*, or in a parallel pathway. To further investigate the role of *abl-1*, we determined the effects of *abl-1*(lf) on other phenotypes associated with engulfment mutants. Engulfment gene mutations enhance the cell-killing defects of mutants partially defective in programmed cell death, such as the *ced-3* caspase. Consistent with its effect on engulfment, *abl-1*(lf) suppresses the enhancement of a weak death defect by *ced-1*(lf) but not *ced-12*(lf) mutations. The *ced-2/5/10/12* genes are required for normal distal tip cell (DTC) migration and, hence, normal gonad morphology. By contrast to its effects in engulfment and enhancement of death defects, *abl-1*(lf) suppresses the DTC migration defects of strong loss-of-function alleles of *ced-5* and *ced-12* as well as of *ced-2*. We propose that *abl-1* suppresses a genetic pathway parallel to *ced-2/5/10/12* for DTC migration and, possibly, for apoptotic cell engulfment. Genetic screens are underway to identify components of this pathway.

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Characterizing of an apoptotic role for EGL-38 in germline cell in *C. elegans* . **Hongtao Jia**[1], DonHa Park[2], Helen Chamberlin[1,3]. 1) Molecular, Cellular and Developmental Biology Program, The Ohio State University, Columbus, OH; 2) Michael Smith Laboratories, University of British-Columbia, Vancouver, BC, Canada; 3) Department of Molecular Genetics, The Ohio State University, Columbus, OH.

In *C. elegans*, the core apoptotic pathway which influences both radiation-induced DNA damage cell death and physiological germ cell death has been well characterized. However, it is less well known what signals trigger apoptosis, and how these signals are transduced to the core apoptotic elements. In our previous studies, we have found that two *Pax2/5/8* genes, *egl-38* and *pax-2*, can regulate cell survival in *C. elegans*[2]. Using genetic and molecular analysis, we showed that the Pax2/5/8 proteins promote cell survival by regulating the *Bcl-2* gene *ced-9*. We are interested in understanding how these Pax2/5/8 proteins function with respect to other pathways that influence cell survival. Others have shown that germ cell apoptosis in *C. elegans* requires the Ras pathway [1]. Our genetic epistasis studies suggest that *egl-38/pax-2* act either downstream of or in parallel to the Ras pathway in this tissue. We are interested in testing the possibility that the Ras pathway may influence cell survival by regulating Pax2/5/8 protein function. We have found that *ced-9* mRNA abundance is increased in *mpk-1* mutants, suggesting that the Ras pathway may influence cell survival through the same mechanism as *egl-38* and *pax-2*. We have also found that the Pax2/5/8 protein DNA binding domain can be phosphorylated by *C. elegans* MAP kinase MPK-1 homolog ERK-1 *in vitro*. Mutating potential phosphorylation site in the Pax DNA binding domain can completely abolish its phosphorylation. The *in vivo* functional relationship between the Ras pathway and Pax2/5/8 proteins is being tested. References 1. Gumienny, T. L., Lambie, E., Hartwieg, E., Horvitz, R. H. and Hengartner, M. O. 1999. Genetic control of programmed cell death in the *Caenorhabditis elegans* hermaphrodite germline. Development 126: 1011-1022. 2. Park, D., Jia, H., Rajakumar, V. and Chamberlin, H.M. 2006. Pax/2/5/8 proteins promote cell survival in *C. elegans*. Development 133: 4193-4202.

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The role of the EGF pathway in programmed cell death. **Hang-Shiang Jiang**, Yi-Chun Wu. National Taiwan University , Molecular and Cellular Biology, Taipei, Taiwan.

The EGF-like growth factor LIN-3 stimulates a canonical RTK-Ras-MAPK cascade and promotes ovulation, vulval formation, male spicule development, and P12 neuroectoblast specification in C. elegans. During ovulation, Ras-MAPK pathway activation is required for germ cells to exit from pachytene arrest, which is a pre-requisite for germ cells to undergo programmed cell death (PCD). To examine if the Ras-MAPK pathway plays a role in somatic PCD, we counted cell corpses of mutants defective in this pathway. A significant decrease of cell corpse number was observed in the mutant embryos. Nevertheless, no extra cells were found in the anterior pharynx of these mutants. To understand the cause for the reduction of cell corpses in these mutants, we followed the lineage of the first 13 cells that die in the AB lineage using four-dimensional microscopy. In wild type, apoptotic cells are generated by asymmetric cell division, in which two descendent cells generated are of different sizes and fates. The small cell undergoes PCD, whereas the big one survives and differentiates or further divides. However, in an mpk-1 mutant we recorded, some asymmetric cell divisions were not observed, and, instead, two descendent cells of similar sizes were generated and both cells survive. In one case, the surviving daughter cell destined to die in the wild type underwent an extra round of cell division. We are in the process of analyzing more embryos and trying to identify the potential cell fate of the cells that survive in the mutants using various gfp-markers.