210B Regulation of Cell Extrusion by Protein Ubiquitination in *C. elegans*.

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Cell extrusion, or shedding, is a cell elimination process that occurs in metazoans ranging from sponges to mammals. In humans, approximately 10^{11} cells are extruded from the small intestine epithelium each day. Dysregulation of cell extrusion is deleterious, as inadequate extrusion or excessive extrusion can lead to hyperplasia or a defective epithelial barrier, respectively. Despite the importance of cell extrusion in physiology and relevance to disease, little is known about how extrusion is regulated or what molecules are involved in the process.

We previously identified cells in *C. elegans* that are normally eliminated during embryogenesis by caspase-mediated apoptosis but are alternatively eliminated by cell extrusion in mutants defective in caspase-mediated apoptosis. When both caspase-mediated apoptosis and cell extrusion are disabled, these cells survive. For example, such cells survive in mutants with loss-of-function mutations in both the gene *ced-3*, which encodes the caspase required for programmed cell death, and the gene *pig-1*, which encodes a homolog of the protein kinase MELK (Denning *et al.,* Nature (2012)). One such cell, ABplpappap, when it survives in *pig-1 ced-3* double mutants divides to generate an extra excretory cell, resulting in the two excretory cell (Tex) phenotype.

We mutagenized *ced-3* mutants and screened for mutants with the Tex phenotype to identify additional genes involved in cell extrusion and identified the gene *C45G7.4*. *C45G7.4* encodes a RING/B-box protein with putative E3 ubiquitin ligase function. Consistent with a role of the ubiquitin proteasome system in extrusion, knockdown or knockout of *ubc-25*, which encodes an ubiquitin-conjugating enzyme and homolog of the mammalian UBE2Q1 and UBE2Q2 proteins, also produces the Tex phenotype in a *ced-3* mutant background.

We are currently performing rescue and epistasis experiments to confirm the role of *ubc-25* in cell extrusion and to determine whether it functions in the same pathway as *C45G7.4* to promote cell extrusion. With the assumption that C45G7.4 functions as an E3 ligase, we plan to use the UBAIT technique (O'Connor *et al*., EMBO Rep. (2015)) to covalently trap C45G7.4 targets and identify them by immunoprecipitation followed by mass spectrometry. We will subsequently test the identified targets for a role in cell extrusion.

Our study reveals that the ubiquitin proteasome system is involved in cell extrusion and suggests that ubiquitination of an unknown factor is a critical step in the mechanism of cell extrusion.