

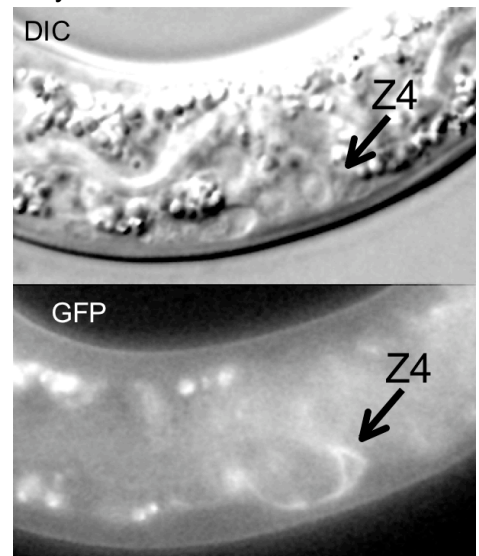


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Magnesium homeostasis in *C. elegans*.

Magnesium is the second most abundant intracellular cation and it plays an essential role in hundreds of different enzymatic reactions. Yet, the proteins that mediate uptake, storage, signal transduction and export of Mg^{2+} during animal development are only beginning to be identified, much less understood. We are using *C. elegans* as a model system to investigate the regulation of Mg^{2+} homeostasis. We began this line of investigation with a genetic characterization of the *gon-2* locus, which is required for the divisions of the gonadal precursors. Subsequently, we determined that *gon-2* encodes a Mg^{2+} -permeable TRPM channel that is orthologous to vertebrate proteins with comparable functions. To learn more about how GON-2 protein activity is regulated, and to identify other loci with important roles in Mg^{2+} homeostasis, we have used forward and reverse genetic methods to identify and characterize a small group of interacting loci.

First, we performed a series of large-scale mutageneses, screening for extragenic suppressors of the *gon-2* Gonadless phenotype. These screens identified five loci, *gem-1*, *gem-2*, *gem-3*, *gem-4* and *gem-5* (*gon-2* extragenic modifier). We have cloned and extensively characterized two of these genes, *gem-1* and *gem-4*. Through molecular and genetic analyses, we determined that *gem-4* encodes a copine family protein that may function as an inhibitor of *gon-2* by regulating the trafficking of channel proteins to the plasma membrane.

In the case of *gem-1*, all of the suppressor mutations that we identified are dominant gain-of-function alleles. *gem-1* encodes a multipass transmembrane protein that is most closely related to mammalian monocarboxylate transporters (MCTs). A GEM-1::GFP reporter is expressed within the gonadal precursor cells (e.g., Z4 at right) where it localizes to the plasma membrane. This would be the first example of a monocarboxylate family transporter that mediates Mg^{2+} uptake. We hypothesize that the *gem-1(gf)* mutations permit bypass of *gon-2* by increasing the abundance and/or activity level of the GEM-1 protein. We are currently using reverse genetic methods to test this hypothesis. GEM-1::GFP expression in the developing gonad.



gem-5 also encodes an MCT that mutates to dominant suppression. However, all of the suppressor alleles of *gem-5* that we have identified so far affect the same amino acid residue. This amino acid occupies a position within transmembrane domain 8 that has been shown to be critical for mammalian MCT function. Therefore, we suspect that this mutation results in a qualitative change in *gem-5* activity, such that it can now perform the same function as *gem-1*. We are currently performing structure function analyses of both *gem-1* and *gem-5* to determine how their relative activities are specified and regulated. Furthermore, we are using heterologous expression assays to test whether the mammalian MCTs can substitute for *gem-1* and/or *gem-5*.

We have also initiated second generation screens for interacting genes by taking advantage of the *gon-2; gem-1(gf)* double mutant background. For example, we screened for mutations that prevent *gem-1(gf)* from suppressing *gon-2* and this resulted in the identification of a handful of non-

complementing suppressors, all of which map to the same locus on chromosome IV. We have termed this locus *kos-1*, for *killer of suppression*. We recently cloned the *kos-1* gene and found that it encodes a transmembrane ATPase that has similarity to bacterial Mg²⁺ transporter proteins. We hypothesize that the KOS-1 and GEM-1 proteins interact directly to mediate Mg²⁺ uptake by the gonadal precursors. Human counterparts of *kos-1* have been found to be mutated in individuals with neurological disorders; however, their molecular function has not been determined. Therefore, we expect that the results of our characterization of *kos-1* are likely to be directly relevant to understanding the basis of these human disorders.

In a complementary avenue of investigation, we have begun a large-scale reverse genetic/RNAi screen to identifying genes that interact with *gon-2*. Thus far, we have screened most of the genes on chromosomes I and II for their ability to suppress a loss of *gon-2* function. We identified four candidate genes using this approach, one of which is likely to correspond to *gem-2*. These genes are predicted to mediate disparate biochemical functions within the cell (e.g., gap junction formation, transcriptional regulation, RNA degradation), so further analysis of their function with respect to GON-2 is likely to provide new insight into TRPM channel function and regulation.

Feel free to stop by 115 Gilman if you would like to talk with me about any of these projects.

Relevant lab publications:

Kemp, B.J., Church, D.L., Hatzold, J., Conradt, B. and E.J. Lambie. (2009) *gem-1* encodes an SLC16 monocarboxylate transporter-related protein that functions in parallel to the *gon-2* TRPM channel during gonad development in *Caenorhabditis elegans*. *Genetics* 181:581-591.

Teramoto, T., Lambie, E.J. and Iwasaki, K. (2005) Differential regulation of TRPM channels governs electrolyte homeostasis in the *C. elegans* intestine. *Cell Metabolism* 1:343-54.

Church, D.L. and Lambie, E.J. (2003) The promotion of gonadal cell divisions by the *C. elegans* TRPM cation channel GON-2 is antagonized by GEM-4 copine. *Genetics* 165:563-574..

E.J. Lambie (2002) Cell proliferation and growth in *C. elegans*. *BioEssays* 24: 38-53

West, R.J., Sun, A.Y., Church, D.L., and Lambie, E.J. (2001) The *C. elegans gon-2* gene encodes a putative TRP cation channel protein required for mitotic cell cycle progression. *Gene* 266:103-110.

Sun, A.Y. and Lambie, E.J. (1997) *gon-2*, a gene required for gonadogenesis in *Caenorhabditis elegans*. *Genetics* 147:1077-1089.