GENETIC ANALYSIS OF LET-23 MEDIATED IP_3 SIGNALING IN *CAENORHABDITIS ELEGANS*.

Thesis by

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Abstract

Our understanding of signal transduction has increased from the use of genetically tractable organisms combined with biochemical analysis in cell culture. An example is LET-23 receptor tyrosine kinase signaling in *Caenorhabditis elegans*. The epidermal growth factor receptor homolog, LET-23 RTK, mediates multiple functions: development of the male tale, vulva induction, viability, and fertility. One of the ways in which activation of the same receptor can generate a specific response is through distinct signaling pathways downstream. Fertility is mediated by a RAS-independent inositol 1,4,5- triphopshate (IP_3) signaling pathway downstream of LET-23 activation.

In this thesis, I take a genetic route to the analysis of IP_3 signaling in *C. elegans,* which mediates ovulation in the fertility pathway. Genetic screens for suppressor or enhancers of mutant phenotypes remain an imporant tool for dissecting signaling pathways. They can uncover new genes or new mutations in existing genes, which upon analysis, will help us understand more about how that particular protein functions. I describe a genetic screen to identify genes that act to suppress the ovulation/sterility defects associated with both a gain of function in the IP_3 receptor and loss of function in the IP_3 3-kinase. Initial characterization of four suppressors identified is reported. Disruption of genes identified by genome sequencing has allowed us to determine whether a protein is essential for a given response. The importance of regulating IP_3 levels is illustrated by the complexity of kinases and phosphatases that metabolize IP_3. Nomarksi video microscopy analysis shows the *C. elegans* IP_3 3-kinase defective mutant, *lfe-2,* has no ovulation phenotype. Using reverse genetics, I targeted a deletion in the *C. elegans* 5-phosphatase, *ipp-5,* and demonstrate that IPP-5 plays a

critical negative regulatory function for distal spermatheca contraction behavior. Evidence for levels of IP_3 signaling regulating spermatheca contractions which affect fertility, comes from my analysis of multiple mutants that perturb IP_3 signaling. The work presented in this thesis provide the most extensive genetic analysis of IP_3 signaling to date. These results imply thresholds are important for achieving an appropriate response. Finally, I present the genetic characterization of a novel phospholipase C, Ce PLC210, and implicate its critical function for regulating spermatheca-uturine valve contraction behavior. A multitude of proteins is involved in generating a precise biological response.

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