Molecular basis of behavioral plasticity in *C. elegans*: Dissecting intra- and extracellular signaling mechanisms

Andreina Rueda Pfister

Master thesis in Biology

To increase their chances of surviving in their natural environment, animals must constantly sense information from their environment and generate specific behavioral responses. Plasticity is an essential feature in sensory systems allowing to adapt these responses to the changes in the environment like temperature changes, food availability, harmful stimuli, dangerous situations, etc. This Master's thesis focuses on different intracellular and extracellular aspects of behavioral plasticity.

The first project focused on the intracellular aspect of behavioral plasticity and was related to nociception, the feature by which animals can detect and encode noxious stimuli via specialized sensory neurons. Nociception is coupled with avoidance behaviors to avoid or minimize potential damages to the organism. *C. elegans* is used for the study of nociception. Indeed, it can respond to noxious stimuli via avoidance behaviors and can adapt to heat stimuli after prolonged exposure by decreasing its avoidance behavior. Previous studies in the lab showed that protein kinase A (PKA) might be involved in this thermo-nociceptive plasticity. The goal of this project was to adapt the SPARK reporter to *C. elegans* to monitor PKA activity in real time *in situ* before and after adaptation to heat stimuli. SPARK is a recently developed reversible reporter consisting in a set of proteins that aggregate and form fluorescent droplets after being phosphorylated by PKA. We started by cloning an optimized version for *C. elegans.* Then, to validate our method, we expressed SPARK in N2 worms and incubated them with H89 PKA inhibitor to verify if the aggregation was dependent on PKA. Another validation step consisted in expressing SPARK in mutants with overactive PKA (*kin-2(ce179)*) and in N2 worms and comparing the aggregation in both situations.

The second project focused on the extracellular signaling part of plasticity and was part of a larger project carried out in the lab. The general goal was to understand the impact of temperature on behavior in *C. elegans.* In previous experiments, the speed of worms was measured in different conditions (15° on food, 25° on food, 15° starved, 25° starved) and it was found that starved worms at 25°C had higher speed. Further experiments showed that FLP-5 neuropeptide expressed in FLP neurons is required for this speed increase. *In vitro* screens were performed and three candidate GPCRs bound by FLP-5 were found: EGL-6, DMSR-1, and DMSR-7. To better understand how this pathway works, we cloned all the genes coding for receptors and *flp-5*, as well as their respective promoters. Then, using a reporter, we compared the expression levels of *flp-5* in FLP neurons among the four different conditions but did not find any significant quantitative difference among cells with detectable expression. However, we found a higher fraction of worms expressing *flp-5* reporter at 25°C, but this effect did not seem to be specific to this reporter.

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