Chapter V: Discussion

Hormonal networks play a critical role in commitment to reproductive maturity throughout the animal kingdom, yet the cellular and molecular network architecture of commitment is not well understood. Here we characterized a molecular mechanism connecting environmental signals to hormonal regulation during the commitment to reproductive development. These studies allow us to ascribe specific time windows and pinpoint levels of hormone required to drive an endocrine network over thresholds for adult maturation.

I have characterized a mechanism that guarantees the robust development of the *C. elegans* nematode in fluctuating conditions. The small amount of DA which crosses the dauer bypass DA threshold is amplified through the hypodermal *daf-9* DAF-12 positive feedback loop coordinates the decision over many tissue types. When the feedback loop is uncoupled, it reveals intermediate phenotypes that are similar to a reaction norm when adding in increasing amounts of DA. The comparison between the wild type positive feedback loop and the uncoupled *daf-9* mutants reflects the canalizing properties of the mechanism. Commitment to the adult fate via the positive feedback loop can be viewed as an evolutionary mechanism that constrains the reaction norm into a polyphenism thus increasing fitness of the nematode.

The goal of this study was to understand how the known components of the dauer regulatory network shape system level interactions. Time series analysis coupled with controlled changes of environmental conditions have helped elucidate mechanisms and interactions that are not possible with classical genetics methods. For example,

epistasis analysis placed *daf-9* downstream to *daf-7/*TGFβ ligand indicating that the phenotypes regulated by the steroid hormone pathway had already accounted for population density. Our approach of uncoupling the physiological contribution of low DA amounts and high DA amounts has revealed that DA initially has to cross the dauer bypass DA threshold and later during development as a coordinator of the decision. Population density can regulate the threshold of DA.

This analysis has not identified new components of the decision making, but has implicated the commitment points and experimental framework for discovery of target genes that may become markers for commitment to either fate. In a follow-up study, we are performing high throughput sequencing on *daf-9* mutants treated with DA in order to find genes that are transcribed at the point of commitment to L3 as well as dauer. Coupling these experiments to changes of environments can help identify genes that integrate the environmental inputs.

Once we identify markers for the dauer and L3 commitment, we will be able to perform experiments and dissect to greater depth several different classes of regulators mechanisms: (i) integrators of different physiochemical properties such as food availability, population density and temperature. (ii) Molecular characterization of the persistence detector mechanism. (iii) integrators of internal physiological states with real time environmental sensation. Combining these mechanisms together will shed light on the architecture of decision making mechanisms in *C. elegans* which can then be extended to other organisms.

Most of the experiments performed in this study offer conclusions based on population statistics. The synchronization methods developed in order to achieve sharp response curves of commitment (Figure 2.1-2.4) extend poorly to experiments elucidating the mechanisms that regulate of length of the persistence window. To this end, single animal analysis performed in smaller artificial environments will reveal much about individual decision making. Tracking the expression levels of L3 and dauer commitment genes in a Microfluidic device coupled to real-time microscopy will help elucidate the integration mechanisms that nematodes use to understand their environment and make decisions based on integration of input with internal information.

Characterizing the polyphenic dauer switch is by no means a finished story. There are many more questions to be answered. First, what are the molecular events that regulate the dauer phenotypes. Is this decision also made systematically reducing information complexity to a hormonal switch? An alternative mechanism may be orchestrating this branch of development. Several lines of evidence indicate this reasoning: (i) dauers that are formed in starved conditions with low population density have less fat granules in their gut, and dauers that developed when deprived of cholesterol are not SDS resistant indicating that individual environmental cues regulate not only the switch but also the morphology of the dauer. It will be interesting to understand the differences in mechanisms that coordinate reproductive development as opposed to the dauer.

Understanding the evolutionary forces that drive polyphenism has been a long lasting challenge, mostly, since polyphenisms are hard to reproduce in laboratory conditions. The majority of research has focused on the insect class which initially

proved to be a powerful tool due to the ease of extracting hormone titers and performing electrophysiological studies, thus linking environmental cues to molecular mechanism. However, these studies have revealed that much of the regulation is based on genetic interactions which happen on the cellular and molecular level requiring transgenic and genomic techniques. For example, queen selection in Hymenopterans has revealed that methylation of non-coding sequences by *Dnmt-3* explains 72% of the queen/soldier polyphenism. Hormonal regulation in *C. elegans* has only recently been technically possible, and along with well established methods in genetics, transgenics, genomics, and facile and reproducible environmental regulation make *C. elegans* a powerful tool in understanding polyphenism.