CHAPTER 4

Concluding Remarks

Summary

I began my thesis work interested in how the same signaling pathways were used during development to specify different fates. EGF and Wnt signaling had been demonstrated to act together with different Hox genes during the specification of two equivalence groups in *C. elegans*, the VPCs and the P11/12 pair (Eisenmann et al., 1998; Jiang and Sternberg, 1998; Wagmaister et al., 2006). Both or one of the EGF and Wnt pathways as well as two other Hox genes, *mab-5/Hox6/8* and *ceh-13/labial/Hox1*, were also implicated in the development of two other equivalence groups, the HCG and the γ/δ pair (Chamberlin and Sternberg, 1994; Sternberg and Horvitz, 1988; Stoyanov et al., 2003) (H. Yu, personal communication). I wanted to investigate the conservation of Hox regulation by EGF and Wnt since one mechanism by which the same signaling pathways specify different fates may be through the regulation of such master control genes. The work I have presented on the patterning of the hook and γ/δ equivalence groups provides further support for the upregulation of Hox genes by EGF and/or Wnt pathway to specify fate in *C. elegans*.

Previous work had indicated that the Wnt receptor *lin-17/Fz* acted during HCG fate execution (H. Yu and P.W. Sternberg, personal communication), but it was unknown if it also acted during HCG fate specification and whether Wnts were involved as the hook inductive signal. In Chapter 2, I determined that Wnt signaling was the major hook inductive signal, and that the Wnts, in particular *lin-44* and *egl-20*, and *lin-17/Fz* are required to specify 1° and 2° HCG fates. A minor role for EGF signaling during hook specification was revealed only when Wnt activity was reduced. In addition, genetic analysis suggested that *mab-5/Hox6/8* functioned downstream of ectopic Wnt signaling to

specify hook fates outside the HCG (H. Yu, personal communication). I showed that Wnt signaling is required for *mab-5/Hox* expression in P11.p, providing the link between the two genes within the HCG. Since P11.p acquires the 1° hook fate and in turn specifies the 2° fate, my data provides further support that *mab-5/Hox6/8* is required to specify HCG fates.

In Chapter 3, I demonstrated that the EGF pathway is both necessary and sufficient to upregulate *ceh-13/labial/Hox1* expression in γ. In addition, I uncovered a role for the transcription factors *lin-1/Ets* and *lin-31/Forkhead* in regulating *ceh-13*::GFP expression and specifying the γ fate. TGF-β signaling had previously been shown to be absolutely required for *ceh-13*::GFP in γ, and I showed that the TGF- β ligand, *dbl-1*, does not act upstream of the EGF pathway to control *ceh-13*::GFP expression. My results indicated that TGF-β signaling either acts downstream or in parallel to EGF signaling during the regulation of *ceh-13*::GFP in γ and hence γ fate specification. I did not find evidence that Wnt signaling specifies the γ fate but found that the Wnts, *lin-44* and *mom-2*, and *lin-17/Fz* are required for γ division along the correct axis without significantly affecting POPTOP expression, suggesting that Wnt signaling orients the γ mitotic spindle probably by a transcription-independent mechanism. Such a function for Wnt signaling had not been observed in the other EGF-regulated *C. elegans* equivalence groups.

If Hox genes confer specificity downstream of EGF and Wnt signaling, how are different Hox genes upregulated in different equivalence groups? The characterization of the HCG and γ/δ equivalence pair provides some clues to how specific Hox genes are upregulated. First, HCG specification is similar to VPC and P12 specification in that

both EGF and Wnt signaling are required. However, Wnt signaling is the major hook inductive signal, and EGF signaling plays a minor role. By comparison, EGF signaling is the major inductive signal during vulval and γ specification. The EGF pathway also plays a more significant role during P12 specification as compared to hook induction. Therefore, the different relative importance of each signaling pathway during the development of each equivalence group might lead to the specificity of Hox gene expression. Second, another signaling pathway, TGF-β, upregulates *ceh-13/Hox* and appears to act only during γ fate specification. Therefore, TGF-β signaling may help to confer specificity to Hox expression in γ.

Where do we go from here?

In *Drosophila*, Hox expression patterns are known to be controlled by the gap and pair-rule genes (Veraksa et al., 2000). However, the upstream mechanisms that generate Hox expression in mammals remain poorly understood. Furthermore, regulation of Hox genes is of great interest because Hox gene expression is altered in a variety of cancers (Nunes et al., 2003). Although the Hox cluster in *C. elegans* does not exhibit spatial colinearity as neatly as in higher organisms, conserved regulatory cis-elements in the *lin-39/ceh-13* subcluster have been identified that drive the same expression pattern between species (Kuntz et al., 2008). Some of these elements are expected to regulate the transcription of both genes, and expression of these elements has not been characterized in the male. Since I have shown that EGF signaling controls the expression of *ceh-13*, in addition to *lin-39*, further analysis of the non-coding regions required for their expression and identification of elements which respond to EGF signaling will lead to a better

understanding of how the EGF pathway generates tissue-specific Hox expression. Similar analysis can be carried out with the *mab-5* and *egl-5* intragenic region.

Another direction to take would be to delve deeper into the mechanism of Hox specificity. There is growing evidence that Hox genes interact with a large variety of transcription factors to specify fate throughout development. Although candidate gene approaches using RNAi in *C. elegans* have proven to be a relatively easy and quick way to screen for factors of interest, RNAi does not seem to be effective in the VPCs, the most well-characterized equivalence group (J. Sanders, personal communication). Recently developed techniques such as single cell RNA sequencing may provide a better way to identify Hox co-factors and target genes in the different equivalence groups.

A third area to explore would be patterning of the other B cell equivalence groups, α/β and ε/ζ. The four Hox genes associated with the VPCs, HCG, P12 and γ fates are not expressed in these two B cell equivalence groups that are regulated by EGF signaling (data not shown). Conveniently, there are two remaining Hox genes in the *C. elegans* cluster that have not been carefully examined in terms of expression and function in the α/β and ε/ζ. These equivalence groups are closely related to the γ/δ pair and are positioned near by, yet they acquire different fates. If they are receiving similar signals, why is *ceh-13* not expressed in the anterior cell (α and ε) of the other two equivalence pairs? Lineage analysis of the B cell equivalence groups is time-consuming and difficult. Cell fate markers and other types of fate assays will be useful in further study of these groups.

To whoever made it to the end of this thesis

For what it's worth, some things I've learned along the way. Don't run to Paul the minute you see something exciting, take a day or at least an hour to think about it first. I still remember the day Cheryl found the two-headed worm…Find someone in lab who you can talk to about your project, besides Paul. If you're lucky, it may turn out to one or all of your labmates who share your room =) Don't believe everything you read. Expression patterns and mutant phenotypes are a few of my favorite things (not!) that have been reported incorrectly in published papers.

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