Appendix

Determining *ceh-13/labial/Hox1* function in γ

Because *ceh-13/labial/Hox1* was expressed in γ and EGF signaling regulates γ fate specification, we wanted to test whether *ceh-13* was required for γ fate specification. However, *ceh-13(null)* mutations cause embryonic lethality in the majority of animals and only a small percentage of sickly survivors manage to persist to adulthood. Of these survivors, males do not have any defects in mating, indicating that spicule formation is normal in these mutants (Stoyanov et al., 2003). Because the survivors are not healthy, it is difficult to perform lineage analysis.

To bypass the requirement for *ceh-13/labial* during embryonic development, I made a heat-shock inducible *ceh-13* exon 1 hairpin RNAi construct where the hairpin was cloned into the heat-shock vector pPD49.83. Heat-shock three to five hours before the first γ division had no effect on the axis of division: γ divided longitudinally in all heat-shocked HS::*ceh-13* RNAi animals and *ceh-13*:GFP expression was normal (n=16). Interestingly, I observed that there was ectopic expression of *ceh-13*::GFP in the other B.a progeny in these animals (Appendix Fig. 1): *ceh-13*::GFP was expressed in δ, ζ and α in about 20% of animals and in β and ε in about 50% of animals (n=15). My results suggest that *ceh-13* is present at very low levels to negatively autoregulate its own expression in the other B.a progeny. The effects of HS::*ceh-13* RNAi on α, β, δ, ε and ζ indicate that the construct is able to reduce *ceh-13* levels. However, it is still possible that *ceh-13* activity is not sufficiently lowered by the RNAi construct. Therefore, further analysis will be necessary to determine whether *ceh-13* is required to specify the γ fate.

mab-5/Antennapedia/Ultrabithorax/Hox6/8 expression in the Male Tail

mab-5 is expressed in P11.p in early-mid L3 males

I obtained males carrying translational *mab-5*::GFP extrachromosomal arrays from the Waterston lab in which the *mab-5*::GFP construct has GFP immediately inserted prior to the stop codon of *mab-5* within a fosmid clone. I observed *mab-5*::GFP expression in P11.p in all early-mid L3 males examined (n=25, Appendix Fig. 2A-B). I also found that *mab-5*::GFP was expressed in P10.p and P9.p in about half of the animals, although at much lower levels as compared to P11.p. Expression was not observed in any of the B progeny in these animals.

mab-5 expression is controlled by LIN-17/Frizzled

Since I had shown that Wnt signaling through the LIN-17/Fz receptor was required to specify the 1° HCG fate, we decided to test if Wnt signaling was required for *mab-5* expression in P11.p during the L3 stage. I found that only one of eight early L3 *lin-17(n671null)* males had wild-type expression of *mab-5*::GFP. In four males, there was no GFP expression in P11.p, while in the remaining three males, GFP expression in P11.p was very faint (Appendix Fig. 2C-D). Therefore, *lin-17Fz* is required to upregulate *mab-5*::GFP expression in P11.p (Fisher's Exact Test, p=0.0003).

lag-2/DSL Expression in the Male Tail

There are ten DSL coding genes in the *C. elegans* genome (Chen and Greenwald, 2004). I made a transcriptional *lag-2*::YFP reporter containing 6.2 kb of sequence upstream of the start site of the *lag-2* gene. The 6.2kb PCR fragment was designed with

a HindIII site on one end and a BamHI site on the other end and was cloned directionally into the L4643 vector that had been digested with BamH1 and HindIII. The extrachromosomal array carrying this *lag-2*::YFP transgene, *syEx971*, was generated. The integrated line *syIs209* was derived from *syEx971*. I examined *syIs209* males and found that the expression pattern of *lag-2* within the HCG is consistent with a role for Notch signaling in specifying the 2° HCG fate and the Bδ fate. Expression was also observed in ventral cord neurons.

In the HCG

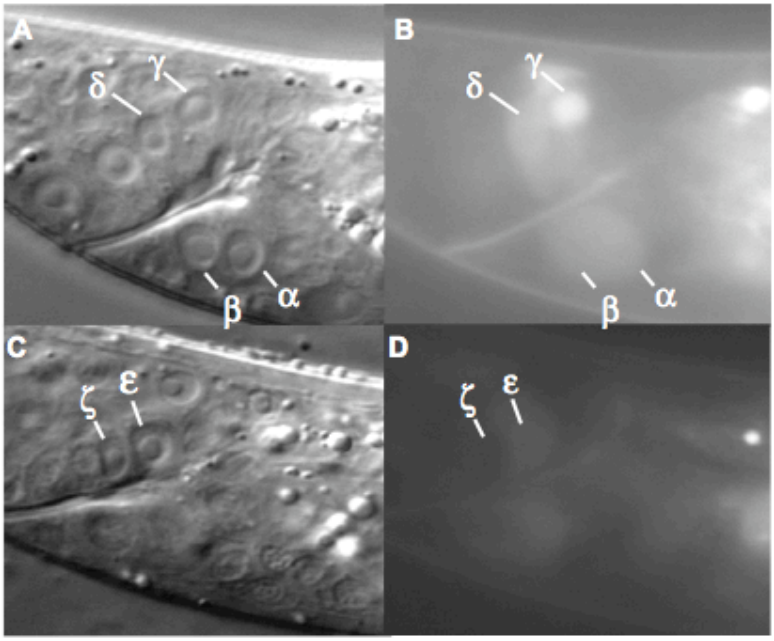
In Chapter 2, we showed that the 1° HCG fate is required to specify the 2° HCG fate, while previous work had indicated that LIN-12/Notch signaling specifies the 2° HCG fate (Greenwald et al., 1983). Consistent with these data, I found that *lag-2* is expressed in the 1°-fated cell P11.p in all early-mid L3 males examined (n=10, Appendix Fig. 3A-B) and in both P11.p daughters in all mid-L3 males examined (n=10). However, I also observed *lag-2::GFP* in P10.p, the presumptive 2° HCG cell, although expression is usually not as bright as in P11.p. One explanation may be that P10.p and P11.p both express *lag-2* prior to fate specification but *lag-2* expression becomes restricted to and upregulated in P11.p starting from the time of hook induction in the L2. The lower levels of expression in P10.p may be residual GFP.

In the B.a progeny

The signal from Y.p and LIN-12/Notch signaling are required to promote the δ fate (Chamberlin and Sternberg, 1994). Because Y.p is not present in *lin-12(null)*

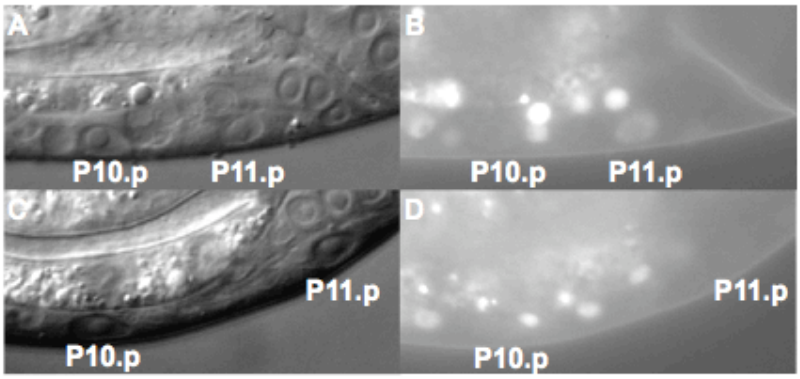
animals, it is not known if Y.p is sufficient to promote the δ fate when Notch signaling is absent. Consistent with a role for Y.p in promoting the δ fate, I found that *lag-2* was expressed in Y.p progeny as well as another cell slightly posterior to the γ/δ pair, likely DVB (Appendix Fig. 3A-B). This raises the possibility that Y.p acts as a major source of the Notch ligand to induce Notch signaling in δ. In addition, I observed *lag-2*::GFP expression in α and β in seven of ten early-mid L3 males.

Appendix Figures



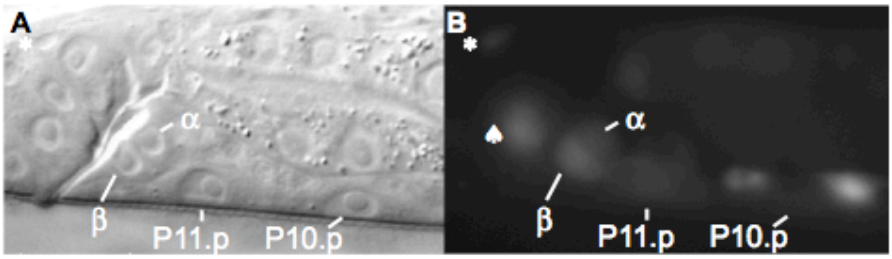
Appendix Fig. 1

Appendix Figure 1. Heat-shock inducible *ceh-13* RNAi caused ectopic *ceh-13*::GFP expression in B.a progeny. (A-B) Mid-L3 male. Ectopic expression was observed in α, β and δ. (C-D) Mid-L3 male. Ectopic expression was observed in ε. Left lateral views.



Appendix Fig. 2

Appendix Figure 2. *lin-17/Fz* is required for *mab-5*::GFP expression in P11.p. (A-B) Mid-L3 male. *mab-5*::GFP was expressed in P10.p at much lower levels than in P11.p. (C-D) Mid-L3 *lin-17(n671null)* male. *mab-5*::GFP was not observed in P11.p, indicating that *lin-17/Fz* upregulates *mab-5* expression. Left lateral views.



Appendix Fig. 3

Appendix Figure 3. *lag-2*::GFP expression in the male tail. (A-B) Mid-L3 male. *lag-2*::GFP was expressed in P11.p, P10.p and the B progeny, α and β. ∗ indicates *lag-2* expressing cell, probably DVB; ♠ indicates expression in Y.p progeny in lateral planes. Right lateral views.

References

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