CHAPTER 1

Introduction

None of their grace beauty is suggested by a name that carries the stigma 'worm.'

–B.G. Chitwood[1]

To most people[2], worms are associated with nasty medical conditions, decomposing matter and to sum it up in a word, "gross." I have to confess, I used to belong to this majority[3]. After spending the last 6.5 years of my life studying *Caenorhabditis elegans*, a worm species belonging to the Nematoda phylum, however, I have developed an affection for *C. elegans* and much more cordial feelings toward other worms. In their defense, they are much more aesthetically pleasing than you would imagine. *C. elegans* is a microscopic species that grows to about 1 mm long in adulthood. Under the microscope, they look and move like tiny little snakes but lie on either their left or right side instead of on their dorsal side (or "belly down" position). Beyond appearances, *C. elegans* has been and continues to be an excellent organism in furthering our understanding of biology. In 2002, the Nobel Prize in Physiology or Medicine was jointly awarded to Sydney Brenner, H. Robert Horvitz, and John E. Sulston for establishing and using *C. elegans* as a model organism to study organogenesis and programmed cell death. Thanks to evolution, we can study genes and their functions in this noble worm and gain some possible insight into how these genes function in humans.

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[1] One of the early pioneers of nematology

[2] In certain parts of the world, worms are considered delicacies although many are actually insect larvae. Here are examples of "true" worms and the dishes they end up in: sandworm (a type of sipinculid) jelly is eaten in Xiamen, China, while the sex organs of the palolo worm (or *eunice viridis*) baked into a loaf with coconut milk and onions are enjoyed in Samoa.

[3] In college, I sat behind my lab partner while he dissected an earthworm, our assignment for the day. I like to think I've come a long way since then.

Before we go any further, there are several important terms and concepts to keep in mind:

"Mutations" are changes to the coding and non-coding regions of a gene that cause the gene product to act abnormally or not at all. "Alleles" refer to the different gene mutations that exist. "Null" or "loss-of-function (lf) alleles" completely remove any gene product from being produced. "Reduction-of-function (rf) alleles" reduce the activity or level of the gene product. "Gain-of-function (gf) alleles" increase the level or activity of the gene product activated or causes the gene product to acquire a novel function.

"Fate specification" refers to the process by which a cell integrates extracellular signals with intracellular factors to select a developmental outcome. In the situation where an external cue is responsible for fate specification, it is termed "induction." Prior to specification, cells of a particular equivalence group have to be competent to respond appropriately, meaning that they must have the ability to acquire distinct cell fates associated with that equivalence group.

During development, previously unspecified cells acquire the correct fates by the interaction of extrinsic signals with intrinsic factors (Flores et al., 2000; Halfon et al., 2000; Xu et al., 2000). Groups of cells that have the same developmental potential are called equivalence groups (Cabrera et al., 1987; Kimble, 1981; Simpson and Carteret, 1990; Sulston and White, 1980). Within an organism, the same signaling pathways are often used multiple times during development to specify different fates. The invariant cell lineage of *C. elegans* provides us with a reproducible *in vivo* system of examining how signaling pathways interact at a single cell level.

Signaling pathways that instruct a cell to develop in a certain fashion often target "master control" genes such as Hox genes. Hox genes encode proteins, with a common 60 amino acid DNA-binding homeodomain, found in all metazoans[4] except sponges (Balavoine et al., 2002). In the late 1940s, Hox genes were first identified in *Drosophila melanogaster* mutant flies in which homeotic transformations had occurred, e.g., legs in place of antenna. Hox genes were found to pattern the anterior-posterior (AP) axis in most animals, and the genomic organization and expression pattern of Hox genes is conserved (Lemons and McGinnis, 2006; Lewis, 1978; Veraksa et al., 2000).

The Hox cluster in *C. elegans* is rudimentary and modified as compared to human and other vertebrate clusters, containing only six genes and an inversion between *ceh-13/labial/Hox1*[5] and *lin-39/Sexcombsreduced/Hox5* (Fig. 1) (Aboobaker and Blaxter, 2003). Although *C. elegans* Hox genes are highly divergent from *Drosophila* and vertebrate Hox genes, *Drosophila* Hox proteins can function in place of *C. elegans* Hox proteins to specify different cell fates (Hunter and Kenyon, 1995). Furthermore, Kuntz

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[4] AKA multicellular animals. Yes, that includes us humans.

[5] "Biologists would rather share their toothbrush than share a gene name" – Michael Ashburner, joint head of the European Bioinformatics Institute (Pearson, 2001).
Gene nomenclature can be confusing because each organism has it's own history and culture behind the naming of genes. One gene, *selectin L*, has 15 aliases. *Drosophila* geneticists have a penchant for more creative names that describe the mutant phenotype associated with a gene, such as *bazooka*, *comatose* and *mind-bomb* but do not give a clue about the gene product. Mouse scientists, on the other hand, have been more logical and guidelines for gene nomenclature state: A gene name should be specific and brief, conveying the character or function of the gene. Then, we have *C. elegans* nomenclature, which is mostly unhelpful, especially for the novice *elegans* grad student. *C. elegans* is similar to *Drosophila*, except the variety of mutant phenotypes in *C. elegans* is much smaller and the names aren't as fancy, explaining the 987 genes named *let(hal)-1* to *let-987* and 66 genes named *lin(eage defective)-1* to *lin-66*. Why is it important to know the names of the gene of interest in other organisms? Because as evolution would have it, knowing what your gene of interest does in other organisms helps you study the gene in your organism of interest. In my thesis, I will occasionally use the *C. elegans* name followed by the *Drosophila* name and finally the Human gene name (as I have here).

et. al (2008) identified regulatory elements in the *ceh-13-lin-39* intragenic region that were highly conserved between species and showed that the same elements from mouse drove the same expression pattern in *C. elegans* as the endogenous elements. This suggests that studying the regulation of Hox genes in C. *elegans* will likely shed light on Hox regulation in other species.

A. Extracellular Signals

In general, a signaling pathway consists of the signal (or ligand) that is presented to a cell. If the cell has the appropriate receptor for the ligand, upon ligand binding to the part of the receptor that lies outside the cell, the receptor activates downstream components within the cell to specify a certain response.

The Epidermal Growth Factor (EGF) Pathway

Binding of the EGF ligand to the EGF receptor causes the receptors to dimerize with each other. Subsequently autophosphorylation between the receptors occurs which leads to the recruitment of signaling components including the Adaptor proteins Growth Factor Receptor-Bound Protein-2 (GRB2) and Nck Adaptor Protein (Nck), Phospholipase-C-Gamma (PLC-Gamma), SHC (Src Homology-2 Domain Containing Transforming Protein), STATs (Signal Transducer and Activator of Transcription). EGF signaling often leads to changes in gene expression downstream of these diverse signaling pathways.

In particular, downstream of Grb2 and Son of Sevenless (SOS), Ras is a GTP-ase that activates a Mitogen Activated Protein Kinase Kinase Kinase (MAPKKK), Raf, at the

plasma membrane. Activated Raf phosphorylates a MAPKK, which in turn phosphorylates a MAPK. The final kinase in the cascade, MAPK, phosphorylates a range of downstream targets that can affect gene transcription and the activity of other proteins.

There is only one EGF ligand, LIN-3, and one EGF receptor, LET-23, in *C. elegans*. Activation of the Ras/MAPK cascade is required for several developmental events, e.g., vulva development and male tail development. The gene names of the other signaling components are as follows: *let-60/Ras*, *sem-5/Grb-2*, *lin-45/Raf*, *mek-2/MAPKK* and *mpk-1/MAPK*.

The Wnt Pathway

Wnts are a large family of secreted, hydrophobic, glycosylated ligands that are involved in diverse processes during development (Mikels and Nusse, 2006). Wnts can interact with a number of receptors including Frizzled (seven-pass transmembrane receptor), Ryk/Derailed (characterized by a Wnt Inhibitory Factor (WIF) domain), LRP5 and-6 (single pass transmembrane receptors of the low-density lipoprotein family) and ROR (receptor orphan tyrosine kinase). Downstream of the receptors, there are three Wnt subpathways: Wnt/β-catenin, Wnt/Ca^{2+} and Wnt/planar cell polarity (PCP) (Nelson and Nusse, 2004).

Here, we will limit the discussion to canonical Wnt/β-catenin signaling. In the absence of Wnt signaling, phosphorylation of β-catenin by casein kinase I (CKI) and glycogen synthase-3β (GSK-3β), which are bound to the Axin and adenomatous polyposis coli (APC) scaffolding proteins, causes ubiquitination and subsequent

degradation of β-catenin in the cytosol. Wnt stimulation leads to the inhibition of the Axin degradation complex, and β-catenin accumulates in the nucleus, allowing it to interact with the T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factor to regulate gene transcription.

In *C. elegans*, there are five Wnt genes (*lin-44*, *egl-20*, *mom-2*, *cwn-1*, *cwn-2*), four Frizzled Wnt receptors (*lin-17, mom-5*, *cfz-1* and *cfz-2),* one Ryk (*lin-18*) and one Ror (*cam-1*). *bar-1* is the *C. elegans* β-catenin that participates in canonical Wnt signaling.

B. Fate Specification in *Drosophila* Eye and Wing Imaginal Discs

Since *Drosophila melanogaster* was the first model organism established about a century ago, some of the most well-studied examples of fate specification of equivalence groups are found in this species. Examining the patterning of eye and wing imaginal discs in *Drosophila* have led to general principles of how cell fate is determined using a limited toolbox of signaling pathways during deveolopment.

I. The Eye Imaginal Disc

The *Drosophila* eye imaginal disc consists of a field of undifferentiated cells that initially possess the same developmental potential but subsequently acquire different cell fates due to spatially and temporally controlled cell-cell signaling events (Tomlinson and Ready, 1987). The eye disc gives rise to a highly ordered compound eye in the adult constituting about 800 ommatidia, each containing 8 light-sensing photoreceptor neurons (R1-8) and a complement of non-neural support cells arranged in a hexagonal shape.

During development, cellular differentiation is initiated by a morphogenetic furrow (MF) that moves posterior-to-anterior across the eye disc (Ready et al., 1976). Immediately posterior to the MF, 5-cell preclusters emerge in which the first photoreceptor neuron to be specified is R8, the founder cell of each ommatidium. Shortly after, R2 and R5 then R3 and R4 are determined within the precluster. At this point, all undifferentiated cells divide once followed by the specification of the cone cells and pigment cells.

All the eye cell fates except R8 require either EGFR signaling or both EGFR and Notch signaling (Voas and Rebay, 2004). It has been shown that in addition to instructional information provided by these pathways to specify the different eye fates, a combinatorial code of transcription factors (including Atonal, Rough, Prospero, D-Pax2, Lozenge, Spalt and Tramtrack88) affects the response of undifferentiated cells over time.

II. The Wing Imaginal Disc

The Drosophila wing imaginal disc is a single layered epithelium made up of about 50,000 cells (Crozatier et al., 2004). Very early on during development, the imaginal disc is divided into two groups of cells defined by their position — Anterior (A) or Posterior (P). Both groups of cells express different types of transcription factors (i.e. *engrailed*, *invected* and *cubitus interruptus*). Three secreted morphogens are used to pattern the wing imaginal disc: *wingless (wg)*, *decapentaplegic (dpp)* and *hedgehog (hh)*. The P cells secrete *hh*, which induces the A cells to express the growth factor *dpp*. *hh* and *dpp* control patterning of the A/P axis, while *wg* patterns the Dorsal/Ventral (D/V) axis. These three morphogens form concentration gradients and cells of the imaginal disc

are thought to detect their position within these gradients and generate the correct developmental fate accordingly (Gurdon and Bourillot, 2001).

C. Fate Specification in *C. elegans* Equivalence Groups

I. The P11/12 Equivalence Group

At hatch, the twelve P precursor cells form six bilateral pairs and each pair is named P1/2L, P1/2R, etc. (Sulston and Horvitz, 1977). During the mid-L1 stage, the P cells migrate into the ventral cord, line up along the anterior-posterior body axis and are subsequently renamed as Pn (n=1, 2, 3…, 12) cells (Fig. 2). The P11/12 pair exhibits a stereotypic migration where the left cell in the pair moves anteriorly to become P11, while the right cell moves posteriorly to become P12. If either cell of the P11/12 pair is killed prior to migration, the remaining cell always adopts the P12 fate (Sulston and White, 1980). Therefore, the P12 fate is the primary fate since it is the fate acquired by each cell in the equivalence pair if the other is absent. Two hours after migration into the ventral cord, both P11 and P12 divide once. In both hermaphrodites and males, the neuroblasts P11.a and P12.a subsequently generate several ventral cord neurons that are morphologically indistinguishable by Nomarski optics while P12.p divides once to generate P12.pa, which becomes the epidermal hyp12, and P12.pp, which undergoes cell death. P11.p fate, however, is sexually dimorphic: in hermaphrodites, P11.p does not divide and fuses to hyp7 in the late L1; in males, P11.p becomes part of the hook competence group (described in section III of this chapter).

EGF and Wnt signaling act synergistically to specify P12 fate (Jiang and Sternberg, 1998). Mutations in the EGF pathway components, *let-23/EGFR*, *sem-5/Grb2*

and *let-60/Ras*, that reduce EGF signaling activity cause P12-to-11 transformations. In contrast, excessive EGF signaling in *lin-15/lf* males results in P11-to-12 transformations. Although *lin-44/Wnt* and *lin-17/Fz* mutants exhibit similar defects to animals in which EGF activity is lowered, *lin-44/Wnt* overexpression does not have any effect on P12/11 fate. In addition, epistasis experiments indicate that Wnt signaling does not regulate *lin-3/EGF* activity to influence P12 fate. Therefore, the EGF and Wnt pathways appear to act in parallel to specify the P12 fate. EGF signaling is both necessary and sufficient to induce P12 fate, while Wnt signaling is necessary but not sufficient for P12 fate specification.

The Hox gene, *egl-5/AbdominalB/Hox9-13*, is upregulated by the EGF pathway (Jiang and Sternberg, 1998) and likely the Wnt pathway (Teng et al., 2004) during P12 fate specification (Fig. 3). *egl-5(null)* mutants exhibit P12-to-11 fate transformations (Chisholm, 1991; Kenyon, 1986). Furthermore, *egl-5* is sufficient to specify P12 fate in a reduced EGF signaling background, indicating that *egl-5* has an instructive as opposed to permissive role.

II. The hermaphrodite vulval precursor cells (VPCs)

The most well characterized equivalence group *in C. elegans* is the vulval precursor cells (VPCs) in hermaphrodites, which are also derived from the ventral P precursor cells (Sulston and White, 1980). After entering the ventral cord in the L1 stage, each Pn cell divides once to produce an anterior (Pn.a) and posterior (Pn.p) daughter. The VPCs, P(3-8).p, are located in the mid-body and each VPC has the potential to adopt either a 1°, 2° or 3° fate (Sulston and Horvitz, 1977). In about 50% of

hermaphrodites, however, P3.p fuses to the hyp7 epidermal syncytium without dividing, termed the "F" fate, prior to induction during the L2.

VPC Competence

VPCs must be maintained as individual epithelial cells to remain competent to respond to inductive signals. During the L1 stage, P(1-2).p and P(9-11).p are unable to bypass fusion in hermaphrodites because they do not express *lin-39/Sexcombsreduced/Hox5* (Salser et al., 1993). Expression of *lin-39/Scr*, however, prevents the VPCs from adopting the F fate. It is unknown what regulates *lin-39/Scr* expression at this stage. Later in development, *lin-39/Scr* activity is required again to prevent fusion to hyp7 in the VPCs. During the L2, Wnt signaling, through the downstream components *apr-1/APC* (Hoier et al., 2000) and *bar-1/β-catenin* (Eisenmann et al., 1998), and EGF signaling (Myers and Greenwald, 2007) act to establish VPC competence. Reduced Wnt signaling causes P(5-7).p to often adopt the 3° or F fate and P3.p, P4.p and P8.p to adopt the F fate, whereas the requirement for EGF signaling to maintain competence are only seen when Wnt activity is lower. Rf mutations of EGF pathway components enhance the F fate defects of Wnt signaling mutants. The Wnt pathway maintains *lin-39/Scr* expression to prevent fusion, while target genes of the EGF pathway are presently unknown (Eisenmann et al., 1998; Wagmaister et al., 2006). It is unknown which Wnt ligand(s) or receptor(s) act upstream of *bar-1* and *apr-1* in vulval competence.

VPC Induction

During the L3 stage, the major inductive signal, mediated by the EGF/Ras pathway, from the anchor cell (AC) causes the VPCs to divide during the L3 stage, generating a spatial pattern of 3°-3°-2°-1°-2°-3° (Kimble, 1981; Sommer, 2005; Sternberg, 2005; Sternberg and Horvitz, 1986). Rf mutations in *lin-3/EGF*, *let-23/EGFR*, *let-60/Ras*, *sem-5/Grb-2*, *mek-2/MEK* and *mpk-1/MAPK* as well as AC ablations cause a vulvaless (Vul) phenotype (Aroian et al., 1990; Beitel et al., 1990; Han and Sternberg, 1990; Hill and Sternberg, 1992; Kornfeld et al., 1995; Lackner et al., 1994; Sternberg and Horvitz, 1989; Wu and Han, 1994; Wu et al., 1995). Conversely, excessive EGF signaling results in a multivulva (Muv) phenotype. Mutations in the transcription factors *lin-1/ETS* and *lin-31/Forkhead*, which are phosphorylated by the EGF pathway, also cause vulval defects (Beitel et al., 1995; Miller et al., 1993; Tan et al., 1998).

In addition to EGF signaling, Wnt signaling has been shown to play a minor role during induction (Eisenmann et al., 1998). First, P(5-7).P in *bar-1/β-catenin* mutants occasionally adopt the 3° fate instead of 1° or 2° fates. Second, either overactivation of Wnt signaling in *pry-1/Axin* mutants or increased levels of a stabilized form of BAR-1/β-catenin causes an overinduction phenotype (Gleason et al., 2002).

Lateral signaling by the LIN-12/Notch pathway subsequently specifies the 2° fate and inhibits adjacent 1° fates (Ferguson et al., 1987; Greenwald et al., 1983; Sternberg and Horvitz, 1989). Consistent with Notch lateral signaling, the Delta/Serrate/Lag-2 (DSL) ligands, *lag-2*, *apx-1* and *dsl-1*, are upregulated by the EGF pathway in P6.p, which acquires the 1° fate (Chen and Greenwald, 2004).

lin-39/Scr also plays a role during vulval induction downstream of EGF signaling (Fig. 3) (Clandinin et al., 1997; Maloof and Kenyon, 1998). At the time of induction, EGF signaling upregulates *lin-39* expression, which is highest in P6.p (Wagmaister et al., 2006). Because Wnt signaling controls *lin-39* expression prior to induction, it is difficult to determine whether Wnt also is required for *lin-39* expression during the time of induction. Although loss of LIN-39 function can result in P(5-7).p adopting the 3° fate, increased levels of *lin-39* are not sufficient to induce vulval development when the AC (the source of the inductive signal) is removed. Therefore, EGF/Ras signaling probably has other targets besides *lin-39* which are required for vulval fate specification.

III. The male hook competence group (HCG)

The P descendants, P(9-11).p, in the male form the hook competence group (HCG) (Sulston and White, 1980). The hook is a reproductive structure that is required to locate the vulva and acts redundantly with the postcloacal sensillum. Sulston et al. (1980) demonstrated that P10.p gives rise to the major components of the hook sensillum, including a hook structural cell, two supporting cells (hook socket cell and sheath cell), and the hook sensory neurons HOA and HOB. Besides having similar developmental origins as the VPCs, the HCG also shares other similarities in terms of fate choices and use of LIN-12/Notch signaling (Greenwald et al., 1983). The adjacent anterior Pn.p (P10.p or P9.p) can substitute for the missing posterior cell if P11.p or P10.p is killed. This posterior-to-anterior direction of recruitment after cell killing designates P11.p as primary (1°), P10.p as secondary (2°), and P9.p as tertiary (3°). Each HCG cell fate has a distinct cell division pattern and produces different types of descendants. In addition, the

Notch pathway is required for 2° fate specification and inhibits adjacent 1° fates (Greenwald et al., 1983).

Hook Competence

Another similarity between vulval and hook development is that a Hox gene is required to prevent fusion of the HCG to hyp7 during the late L1 in males (Kenyon, 1986; Salser et al., 1993). *mab-5/Antennapedia/Hox6-8* is expressed in the HCG during the L1 (Fig. 3), and P(9-11).p fuses to hyp7 in *mab-5(lf)* mutants. Unlike in the VPCs, in which fusion must be prevented a second time in the L2 so that cells remain competent to be induced, no factors appear to be required to prevent fusion of the HCG to hyp7 during the L2. There is also evidence that induction occurs during the mid-L2, suggesting that maintenance of the HCG as independent epithelial cells only occurs once during the L1. In males, MAB-5 is probably required for more than just preventing fusion during hook development (discussed further in the next section) because *lin-39/Scr* is expressed in P(3-6).p, allowing them to bypass fusion in the L1, but they do not adopt hook fates.

Hook Induction

The EGF pathway, which is the major inductive signal during vulval development, does not appear to be required for HCG specification (H. Chamberlin, personal communication). However, excessive EGF signaling in *lin-15(lf)* mutants results in P9.p adopting 2°-like fate instead of a 3° fate, indicating that EGF signaling can influence hook fates. Ablations of cells and different combinations of cells has failed to identify the source of the inductive signal during hook development (Herman, 1991;

Sulston et al., 1980). However, 1° and 2° HCG lineage defects in *lin-17/Fz(null)* males (Sternberg and Horvitz, 1988) suggests that Wnt signaling is involved in patterning the HCG (Fig. 3). Furthermore, increased canonical Wnt signaling in *pry-1/Axin(lf)* mutants causes anterior Pn.p cells to express HCG fates (H. Yu, personal communication). However, none of the Wnt ligands have been implicated in HCG specification.

Several observations suggest that *mab-5/Ant* acts a second time during hook development to specify hook fates. First, excessive Notch signaling, which specifies both the 2° VPC and 2° HCG fates, in *lin-12(gf)* males causes P(3-8).p to acquire vulval fates and P(9-11).p to generate hook fates, implying that P(3-8).p and P(9-11).p have different tendencies to produce vulval and hook lineages, respectively (Greenwald et al., 1983). Second, overexpression of MAB-5 in *lin-39(rf)* hermaphrodites suggests that MAB-5 acts to specify hook versus vulval fates (Maloof and Kenyon, 1998). Third, the ectopic hook phenotypes in *pry-1/lf* males is suppressed by a *mab-5(lf)* mutation (H. Yu, personal communication). Current evidence suggests that Wnt signaling upregulates *mab-5* in the HCG to specify hook fates. However, this has not been demonstrated.

Competence and induction have been discussed previously as separate events because they were characterized in the VPCs where defects in competence and induction are distinct (i.e. F fate versus 3° VPC fate). Since the 3° HCG fate is to either fuse to hyp7 or to divide once and fuse to hyp7, insufficient inductive signaling in the hook can result in a phenotype that is associated with competence defects in the vulva. This suggests that competence and induction may not necessarily be separate events.

IV. The B cell equivalence groups (α/β, γ/δ, ε/ζ)

The B cell is a male-specific blast cell that generates all the cells of the spicule. B.a generates four pairs of cells: the ventral pair (aa), the dorsal pair (pp) and two identical lateral pairs (ap/pa) (Chamberlin and Sternberg, 1993). Each pair of cells has an anterior (α, γ or ε) and a posterior fate (β, δ or ζ), and each fate produces different cell lineages and cell types (Fig. 2). The male-specific blast cells, U and F, are required to specify the anterior fate of each equivalence pair. In addition, both the U and F cells express the EGF ligand, LIN-3, and reduced activity of several genes in the EGF pathway (*lin-3/EGF*, *let-23/EGFR*, *sem-5/Grb2*, *let-60/Ras*, *lin-45/Raf*) causes abnormal anterior cell lineages (Chamberlin and Sternberg, 1994). Using lineage analysis to assay fate, a γ-to-δ fate transformation is observed in these EGF pathway mutants. The posterior daughter of the male-specific blast cell, Y, as well as LIN-12/Notch is required to specify the posterior fate of the γ/δ pair. In males in which Y.p is killed or in *lin-12(null)* males, a δ-to-γ fate transformation occurs. It is not known if Y.p is the source of the Notch ligand because Y.p is absent in *lin-12(null)* mutants. The fate transformations that occur in the absence of EGF or Notch signaling or in the cell ablation experiment described indicate that lateral signaling between the γ/δ pair is not most likely not necessary for fate specification.

Similar to the other equivalence groups where a Hox gene is expressed in the cell fate specified by EGF signaling, expression of the Hox gene, *ceh-13/labial/Hox1*, was observed in γ (Stoyanov et al., 2003). The TGF-β pathway components, *dbl-1/ dpp/TGF-β*, *sma-2/R-Smad*, *sma-3/R-Smad* and *sma-4/Co-Smad*, were reported to upregulate *ceh-*

13 in γ, implying a role for TGF-β signaling in specifying the γ fate (Fig. 3). *ceh-13* function during γ fate specification has not been examined.

V. Using Hox genes to generate specific outcomes downstream of the same signals

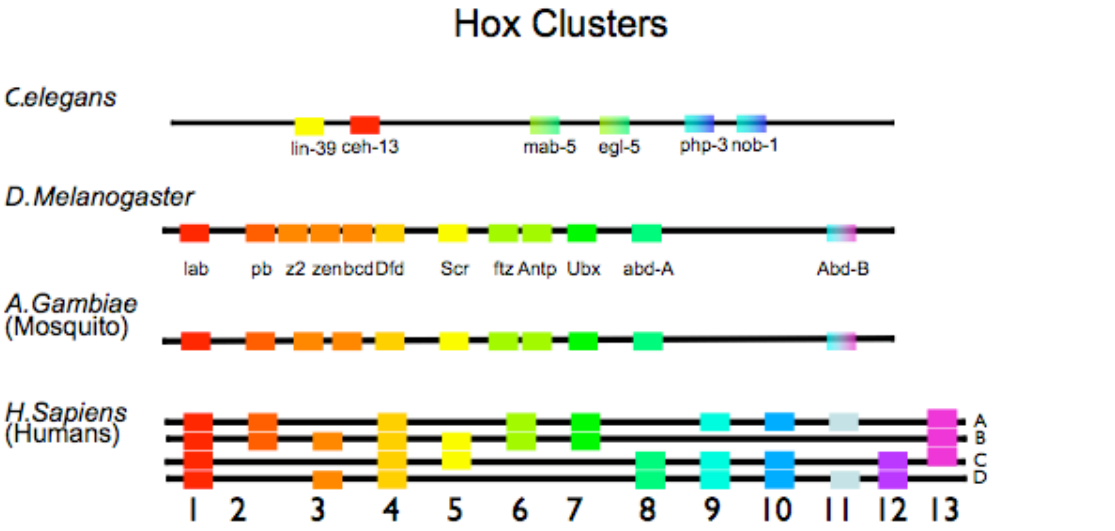
In the VPCs and the P11/12 equivalence group, EGF and Wnt pathways target different Hox genes to specify fate. As discussed above, there is evidence for the expression of a different Hox gene in each of the two equivalence groups, the HCG and the γ/δ pair, and the involvement of EGF and/or Wnt signaling to specify fate in the male B cell equivalence in these groups.

My overall aim was to characterize the roles of EGF and Wnt signaling in the HCG and γ/δ pair so as to understand better how signaling specificity is generated. I wanted to investigate signaling specificity at the pathway level and at the level of Hox regulation in the different *C. elegans* equivalence groups described above. In Chapter 2, I present my findings on EGF and Wnt signaling in the HCG. I provide evidence that Wnt signaling through the *lin-17/Fz* receptor specifies the 1° and 2° hook fates. Furthermore, I show that the role EGF signaling during 1° hook specification is only uncovered when Wnt signaling is reduced. Therefore, my data indicates that Wnt signaling is the major hook inductive signal and EGF signaling plays a minor role during hook development.

In Chapter 3, I examine EGF and Wnt signaling during γ fate specification. I demonstrate that the EGF pathway controls *ceh-13/labial* expression in γ. In addition, I show that certain Wnt signaling components are required to orient the γ mitotic spindle but do not appear to affect γ fate specification. Finally, I provide evidence that TGF-β

signaling does not appear to be required during vulval or P12 induction, suggesting that the TGF-β pathway may help provide specificity to the targets of EGF signaling during γ fate specification, such as *ceh-13*, as compared to the other targets required for vulval and P12 fate specification. Finally, I summarize my results in Chapter 4 and provide future directions for work in these areas I have worked on.

Figures



Based on Lemons and McGinnis, Science 2006 & Aboobaker and Blaxter, Current Biology 2003

Fig. 1

Fig. 1. Conservation of genomic organization of Hox genes. It is difficult to define precise homology relationships for *mab-5*, *egl-5*, *nob-1* and *php-3*.

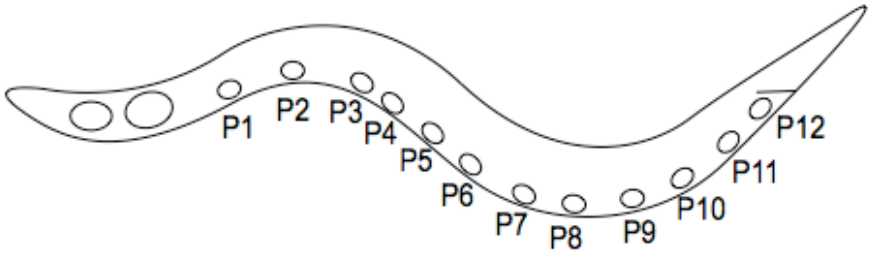


Fig. 2

Fig. 2. Arrangement of the twelve P cells in *C. elegans*.

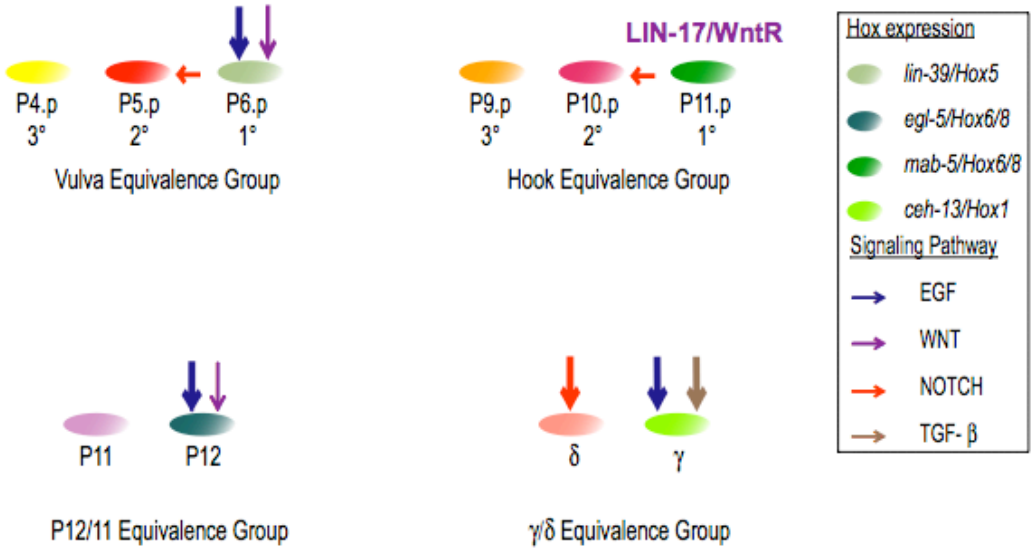

P4.p
3°
P5.p
2°
P6.p
1°
Vulva Equivalence Group
LIN-17/WntR
P9.p
3°
P10.p
2°
P11.p
1°
Hook Equivalence Group
Hox expression
lin-39/Hox5
egl-5/Hox6/8
mab-5/Hox6/8
ceh-13/Hox1
Signaling Pathway
EGF
WNT
NOTCH
TGF- β
P11
P12
P12/11 Equivalence Group
δ
γ
γ/δ Equivalence Group

Fig. 3

Fig. 3. Comparison of the signaling pathways involved in the patterning of different equivalence groups. *lin-39/Hox5* and *egl-5/Hox6/8* are upregulated by EGF and Wnt in the vulval and P11/12 equivalence groups, respectively. *mab-5/Hox6/8* is expressed in the hook equivalence group, while *ceh-13/Hox1* is expressed in γ. EGF signaling has been shown to specify γ fate but regulation of *ceh-13/Hox1* expression has not been examined.

References

Aboobaker, A. and Blaxter, M. (2003). Hox gene evolution in nematodes: novelty conserved. *Curr Opin Genet Dev* **13**, 593-8.

Aroian, R. V., Koga, M., Mendel, J. E., Ohshima, Y. and Sternberg, P. W. (1990). The *let-23* gene necessary for *Caenorhabditis elegans* vulval induction encodes a tyrosine kinase of the EGF receptor subfamily. *Nature* **348**, 693-699.

Balavoine, G., de Rosa, R. and Adoutte, A. (2002). Hox clusters and bilaterian phylogeny. *Mol Phylogenet Evol* **24**, 366-73.

Beitel, G. J., Clark, S. G. and Horvitz, H. R. (1990). *Caenorhabditis elegans* ras gene *let-60* acts as a switch in the pathway of vulval induction. *Nature* **348**, 503-9.

Beitel, G. J., Tuck, S., Greenwald, I. and Horvitz, H. R. (1995). The *Caenorhabditis elegans* gene *lin-1* encodes an ETS-domain protein and defines a branch of the vulval induction pathway. *Genes Dev* **9**, 3149-62.

Cabrera, C. V., Martinez-Arias, A. and Bate, M. (1987). The expression of three members of the achaete-scute gene complex correlates with neuroblast segregation in Drosophila. *Cell* **50**, 425-33.

Chamberlin, H. and Sternberg, P. (1993). Multiple cell interactions are required for fate specification during male spicule development in *Caenorhabditis elegans*. *Development* **118**, 297-324.

Chamberlin, H. M. and Sternberg, P. W. (1994). The *lin-3/let-23* pathway mediates inductive signalling during male spicule development in *Caenorhabditis elegans*. *Development* **120**, 2713-2721.

Chen, N. and Greenwald, I. (2004). The lateral signal for LIN-12/Notch in *C. elegans* vulval development comprises redundant secreted and transmembrane DSL proteins. *Dev Cell* **6**, 183-92.

Chisholm, A. (1991). Control of cell fate in the tail region of *C. elegans* by the gene *egl-5*. *Development* **111**, 921-32.

Clandinin, T. R., Katz, W. S. and Sternberg, P. W. (1997). *Caenorhabditis elegans* HOM-C genes regulate the response of vulval precursor cells to inductive signal. *Dev Biol* **182**, 150-161.

Crozatier, M., Glise, B. and Vincent, A. (2004). Patterns in evolution: veins of the *Drosophila* wing. *Trends Genet* **20**, 498-505.

Eisenmann, D. M., Maloof, J. N., Simske, J. S., Kenyon, C. and Kim, S. K. (1998). The b-catenin homolog BAR-1 and LET-60 Ras coordinately regulate the Hox gene *lin-39* during *Caenorhabditis elegans* vulval development. *Development* **125**, 3667-3680.

Ferguson, E. L., Sternberg, P. W. and Horvitz, H. R. (1987). A genetic pathway for the specification of the vulval cell lineages of *C. elegans*. *Nature* **326**, 259-267.

Flores, G. V., Duan, H., Yan, H., Nagaraj, R., Fu, W., Zou, Y., Noll, M. and Banerjee, U. (2000). Combinatorial signaling in the specification of unique cell fates. *Cell* **103**, 75-85.

Gleason, J. E., Korswagen, H. C. and Eisenmann, D. M. (2002). Activation of Wnt signaling bypasses the requirement for RTK/Ras signaling during *C. elegans* vulval induction. *Genes Dev* **16**, 1281-90.

Greenwald, I. S., Sternberg, P. W. and Horvitz, H. R. (1983). The *lin-12* locus specifies cell fates in *C. elegans*. *Cell* **34**, 435-444.

Gurdon, J. B. and Bourillot, P. Y. (2001). Morphogen gradient interpretation. *Nature* **413**, 797-803.

Halfon, M. S., Carmena, A., Gisselbrecht, S., Sackerson, C. M., Jimenez, F., Baylies, M. K. and Michelson, A. M. (2000). Ras pathway specificity is determined by the integration of multiple signal-activated and tissue-restricted transcription factors. *Cell* **103**, 63-74.

Han, M. and Sternberg, P. W. (1990). *let-60*, a gene that specifies cell fates during *C. elegans* vulval induction, encodes a ras protein. *Cell* **63**, 921-31.

Herman, M. A. (1991). Cell Interactions and the Polarity of Asymmetric Cell Division During *Caenorhabditis elegans* Development. In *Ph. D. Thesis, Massachusetts Institute of Technology*, (ed.

Hill, R. and Sternberg, P. (1992). The gene *lin-3* encodes an inductive signal for vulval development in *C. elegans*. *Nature* **358**, 470-476.

Hoier, E. F., Mohler, W. A., Kim, S. K. and Hajnal, A. F. (2000). The *Caenorhabditis elegans* APC-related gene *apr-1* is required for epithelial cell migration and Hox gene expression. *Genes Dev* **14**, 874-886.

Hunter, C. P. and Kenyon, C. (1995). Specification of anteroposterior cell fates in *Caenorhabditis elegans* by *Drosophila* Hox proteins. *Nature* **377**, 229-32.

Jiang, L. and Sternberg, P. W. (1998). Interactions of EGF, Wnt and Hom-C genes specify the P12 neuroectoblast fate in *C. elegans*. *Development* **125**, 2337-2347.

Kenyon, C. (1986). A gene involved in the development of the posterior body region of *C. elegans*. *Cell* **46**, 477-487.

Kimble, J. (1981). Alterations in cell lineage following laser ablation of cells in the somatic gonad of *C. elegans*. *Dev Biol* **87**, 286-300.

Kornfeld, K., Guan, K. L. and Horvitz, H. R. (1995). The *Caenorhabditis elegans* gene *mek-2* is required for vulval induction and encodes a protein similar to the protein kinase MEK. *Genes Dev* **9**, 756-68.

Lackner, M. R., Kornfeld, K., Miller, L. M., Horvitz, H. R. and Kim, S. K. (1994). A MAP kinase homolog, *mpk-1*, is involved in ras-mediated induction of vulval cell fates in *Caenorhabditis elegans*. *Genes Dev* **8**, 160-73.

Lemons, D. and McGinnis, W. (2006). Genomic evolution of Hox gene clusters. *Science* **313**, 1918-22.

Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565-70.

Maloof, J. N. and Kenyon, C. (1998). The Hox gene *lin-39* is required during *C. elegans* vulval induction to select the outcome of Ras signaling. *Development* **125**, 181-190.

Mikels, A. J. and Nusse, R. (2006). Wnts as ligands: processing, secretion and reception. *Oncogene* **25**, 7461-8.

Miller, L. M., Gallegos, M. E., Morisseau, B. A. and Kim, S. K. (1993). *lin-31*, a *Caenorhabditis elegans* HNF-3/fork head transcription factor homolog, specifies three alternative cell fates in vulval development. *Genes Dev* **7**, 933-47.

Myers, T. R. and Greenwald, I. (2007). Wnt signal from multiple tissues and *lin-3*/EGF signal from the gonad maintain vulval precursor cell competence in *Caenorhabditis elegans*. *Proc Natl Acad Sci U.S.A.* **104**, 20368-73.

Nelson, W. J. and Nusse, R. (2004). Convergence of Wnt, beta-catenin, and cadherin pathways. *Science* **303**, 1483-7.

Ready, D. F., Hanson, T. E. and Benzer, S. (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev Biol* **53**, 217-40.

Salser, S. J., Loer, C. M. and Kenyon, C. (1993). Multiple HOM-C gene interactions specify cell fates in the nematode central nervous system. *Genes Dev* **7**, 1714-1724.

Simpson, P. and Carteret, C. (1990). Proneural clusters: equivalence groups in the epithelium of *Drosophila*. *Development* **110**, 927-32.

Sommer, R. J. (2005). Evolution of development in nematodes related to *C. elegans*. *WormBook*, 1-17.

Sternberg, P. W. (2005). Vulval development. *WormBook*, 1-28.

Sternberg, P. W. and Horvitz, H. R. (1986). Pattern formation during vulval development in *C. elegans*. *Cell* **44**, 761-772.

Sternberg, P. W. and Horvitz, H. R. (1988). *lin-17* mutations of *Caenorhabditis elegans* disrupt certain asymmetric cell divisions. *Dev Biol* **130**, 67-73.

Sternberg, P. W. and Horvitz, H. R. (1989). The combined action of two intercellular signaling pathways specifies three cell fates during vulval induction in *C. elegans*. *Cell* **58**, 679-693.

Stoyanov, C. N., Fleischmann, M., Suzuki, Y., Tapparel, N., Gautron, F., Streit, A., Wood, W. B. and Muller, F. (2003). Expression of the *C. elegans labial* orthologue *ceh-13* during male tail morphogenesis. *Dev Biol* **259**, 137-49.

Sulston, J. E., Albertson, D. G. and Thomson, J. N. (1980). The *C. elegans* male: Postembryonic development of nongonadal structures. *Dev Biol* **78**, 542-576.

Sulston, J. E. and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev Biol* **56**, 110-156.

Sulston, J. E. and White, J. G. (1980). Regulation and cell autonomy during postembryonic development of *C. elegans*. *Dev Biol* **78**, 577-597.

Tan, P. B., Lackner, M. R. and Kim, S. K. (1998). MAP kinase signaling specificity mediated by the LIN-1 Ets/LIN-31 WH transcription factor complex during *C. elegans* vulval induction. *Cell* **93**, 569-80.

Teng, Y., Girard, L., Ferreira, H. B., Sternberg, P. W. and Emmons, S. W. (2004). Dissection of cis-regulatory elements in the *C. elegans* Hox gene *egl-5* promoter. *Dev Biol* **276**, 476-92.

Tomlinson, A. and Ready, D. F. (1987). Neuronal differentiation in *Drosophila* ommatidium. *Dev Biol* **120**, 366-76.

Veraksa, A., Del Campo, M. and McGinnis, W. (2000). Developmental patterning genes and their conserved functions: from model organisms to humans. *Mol Genet Metab* **69**, 85-100.

Voas, M. G. and Rebay, I. (2004). Signal integration during development: insights from the *Drosophila* eye. *Dev Dyn* **229**, 162-75.

Wagmaister, J. A., Gleason, J. E. and Eisenmann, D. M. (2006). Transcriptional upregulation of the *C. elegans* Hox gene *lin-39* during vulval cell fate specification. *Mech Dev* **123**, 135-50.

Wu, Y. and Han, M. (1994). Suppression of activated *Let-60* ras protein defines a role of *Caenorhabditis elegans Sur-1* MAP kinase in vulval differentiation. *Genes Dev* **8**, 147-59.

Wu, Y., Han, M. and Guan, K. L. (1995). MEK-2, a *Caenorhabditis elegans* MAP kinase kinase, functions in Ras-mediated vulval induction and other developmental events. *Genes Dev* **9**, 742-55.

Xu, C., Kauffmann, R. C., Zhang, J., Kladny, S. and Carthew, R. W. (2000). Overlapping activators and repressors delimit transcriptional response to receptor tyrosine kinase signals in the *Drosophila* eye. *Cell* **103**, 87-97.