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Re-programming of *C. elegans* male epidermal precursor fates by Wnt, Hox, and LIN-12/Notch activities

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Abstract

In *C. elegans* males, different subsets of ventral epidermal precursor (Pn.p) cells adopt distinct fates in a position-specific manner: three posterior cells, P(9–11).p, comprise the hook sensillum competence group (HCG) with three potential fates (1°, 2°, or 3°), while eight anterior cells, P(1–8).p, fuse with the hyp7 epidermal syncytium. Here we show that activation of the canonical BAR-1 β-catenin pathway of Wnt signaling alters the competence of P(3–8).p and specifies ectopic HCG-like fates. This fate transformation requires the Hox gene *mab-5*. In addition, misexpression of *mab-5* in P(1–8).p is sufficient to establish HCG competence among these cells, as well as to generate ectopic HCG fates in combination with LIN-12 or EGF signaling. While increased Wnt signaling induces predominantly 1° HCG fates, increased LIN-12 or EGF signaling in combination with MAB-5 overexpression promotes 2° HCG fates in anterior Pn.p cells, suggesting distinctive functions of Wnt, LIN-12, and EGF signaling in specification of HCG fates. Lastly, wild-type *mab-5* function is necessary for normal P(9–11).p fate specification, indicating that regulation of ectopic HCG fate formation revealed in anterior Pn.p cells reflect mechanisms of pattern formation during normal hook development.

Keywords

pattern formation; fate transformation; Axin; β-catenin; Hox; WNT; Notch

Introduction

Patterning of cell fates in a position-specific manner is a general feature of development, and ventral epidermal development in the nematode *Caenorhabditis elegans* has been a useful experimental model to study this fundamental problem. In particular, twelve *C. elegans* ventral ectodermal precursor cells, Pn.p cells (where n refers to the numbers 1–12), have sexual-dimorphic cell fusion patterns. Pn.p cells that do not fuse to the hyp7 syncytium have distinct developmental fates: six central Pn.p cells, P(3–8).p, become vulval precursor cells (VPCs) in hermaphrodites, and three posterior Pn.p cells, P(9–11).p, form the hook sensillum competence group (HCG) in males (Sulston and Horvitz, 1977; Sulston and White, 1980). Maintenance of Pn.p cells as individual epithelial cells allows them to remain

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competent to the inductive signals that specify the vulval and hook fates. The fates of both HCG and VPC cells depend on Wnt and EGF signaling; however, it appears that WNT plays the greater role in HCG induction while EGF plays the major role in VPC induction (Yu et al., 2009). LIN-12/Notch signaling promotes both the 2° HCG and VPC fate during development (Greenwald et al., 1983).

The sex-specific fusion pattern of Pn.p cells is established in part by region-specific activities of homeotic complex (HOM-C) genes (Salser et al., 1993). Three members of a HOM-C pseudo-cluster, *lin-39*, *mab-5*, and *egl-5*, are expressed in different subsets of Pn.p cells during early development. *lin-39* is expressed in the cells P(3–8).p (Clark et al., 1993; Wang et al., 1993), whereas *mab-5* is expressed in P(7–11).p (Salser et al., 1993; Wang et al., 1993). *egl-5* is expressed in P12.p and its daughter P12.pa (Jiang and Sternberg, 1998; Ferreira et al., 1999) and represses *mab-5* expression in these two cells (Salser et al., 1993). In hermaphrodites, *lin-39* activity in P(3–8).p keeps these six cells unfused and competent to form VPCs, while other Pn.p cells fuse with hyp7 in the L1 stage (Clark et al., 1993; Wang et al., 1993). Wnt signaling has been shown to be required for the expression of *lin-39* in P(3–6).p (Eisenmann et al., 1998). In males, either *lin-39* or *mab-5* activity alone prevents cell fusion, but co-expression of *lin-39* and *mab-5* in the same cell neutralizes each other's activity (Salser et al., 1993). As a consequence, P(1–2).p cells, which express none of these three HOM-C genes, and P(7–8).p cells, which express both *lin-39* and *mab-5*, fuse in the late L1 stage; while P(3–6).p and P(9–11).p remain unfused at the same stage. The HCG cells, P(9–11).p, correspond to a domain with only *mab-5* activity (Salser et al., 1993; Maloof and Kenyon, 1998). A *mab-5* loss-of-function mutation completely eliminates this equivalence group by causing the fusion of P(9–11).p cells with hyp7 during the late L1 stage (Kenyon, 1986).

Male P(9–11).p cells each adopt one of the three hook fates (Sulston and White, 1980). In wild-type males, P10.p and P11.p adopt the induced 2° fate and 1° fate, respectively, generating descendants with various epidermal and neuronal fates (Sulston et al., 1980). A 1° fate marker, *eat-4*::GFP, is expressed in one of the P11.p descendants, the PVV motor neuron (Yu et al., 2009). *osm-6*::GFP and *ceh-26*::GFP are two 2° lineage markers: *osm-6* is expressed in both hook neurons (HOA and HOB), while *ceh-26* is expressed only in HOB (Collet et al., 1998; Yu et al., 2003). Another 2° P10.p descendant generates the hook structure. The P9.p cell adopts the un-induced 3° fate, and usually fuses with the hyp7 epidermal syncytium during the late L2 stage. However, P9.p can express either induced HCG fate when a posterior P10.p and/or P11.p is missing or in the presence of increased EGF or Notch activity (Sulston et al., 1980; Yu et al., 2009). In contrast, male P(3–6).p cells fuse with the surrounding epidermis in the late L2 stage and P(7–8).p cells fuse in the late L1 stage. Unlike the P9.p cell, these anterior Pn.p cells adopt vulval-like fates in response to increased EGF or Notch signaling (Ferguson and Horvitz, 1985; Greenwald et al., 1983), suggesting that male P(3–8).p cells do not have the same developmental potential or competency as male P(9–11).p; in particular, they are competent to make vulval tissue but not hook tissue. In both the VPC and HCG equivalence groups, competence includes the blocking of fusion to the hyp7 epidermis prior to receiving strong EGF, WNT or NOTCH signals.

Here, we present evidence that activated Wnt signaling, as the result of eliminating PRY-1/Axin, not only changes the competence of P(3–8).p but also produces ectopic 1° HCG fates in these Pn.p cells. The Hox gene *mab-5* is the determinative element for HCG competence acting downstream of Wnt signaling. Increased MAB-5 activity, in combination with a proliferation signal from either EGF or Notch signaling, mimics the effect of Wnt signaling hyperactivity to induce HCG-like fate transformations in anterior Pn.p cells (P(1–8).p). The requirement of *mab-5/Hox* for HCG specification revealed in the ectopic conditions is also

true for the wild-type HCG, P(9–11).p. We also show that Wnt signaling regulates *mab-5* expression in the 1°-fated P11.p cell.

Results

Activated Wnt signaling promotes 1° HCG fates in anterior Pn.p cells

pry-1 encodes an ortholog of Axin, a negative regulator of canonical Wnt signaling (Korswagen et al., 2002). We found that the *mu38* reduction-of-function (rf) mutation in the *pry-1* gene causes abnormal ventral invaginations in the middle body region of L4 male larvae and multiple small protrusions at similar central positions of adult males. These invaginations and protrusions displayed morphological features resembling structures associated with HCG-like fates: an anchor-shaped structure that looks like the ectopic hooks generated in *lin-12* gain-of-function mutant males (Greenwald et al., 1983) was sometimes seen inside the invaginations, and almost all the protrusions had yellow autofluorescence under UV illumination regardless of the shape (Fig. 1A–B). By examining AJM-1–GFP, a marker of cell junctions (Gupta et al., 2003; Sharma-Kishore et al., 1999; Shemer et al., 2000), we noticed that the central Pn.p cells including P(3–6).p and P(7–8).p were often unfused in late L2 or L3 *pry-1* mutant males, and some of those Pn.p cells divided multiple times during the late L3 to early L4 stage. Therefore, additional ventral invaginations observed in *pry-1* mutant males resulted from inappropriate proliferation of the central Pn.p cells. About 70% of *pry-1(mu38)* males yielded such a morphological alteration in the P(3–6).p region and about 27% in the P(7–8).p region (Table 1).

It has been shown previously that the *pry-1(mu38)* mutation causes excessive vulval differentiation in hermaphrodites; specifically, three normally 3° VPCs, P3.p, P4.p, and P8.p, can adopt induced fates and form extra vulval invaginations (Gleason et al. 2002). As the shape of an ectopic hook-like invagination or protrusion can be quite irregular, it is not easily distinguishable from structures generated by a vulval-like fate in some cases. To clarify the nature of the abnormal morphological structures produced by P(3–8).p in *pry-1(mu38)* males, we examined the expression of vulval and hook lineage-specific markers in *pry-1* mutants. Expression of *egl-17*::CFP, a vulval fate marker, was never detected at the ventral protrusions of *pry-1* mutant males. However, *pry-1* male mutants displayed many *eat-4*::GFP-expressing ventral neurons with processes similar to PVV, a descendent of the 1° HCG lineage, each frequently associated with a ventral protrusion (Fig 1E–F; Table 1). The expression of a 1° HCG lineage marker as well as morphological features of ventral structures suggests that a *pry-1* mutation induces ectopic HCG fates, rather than abnormal vulval differentiation, among the central Pn.p cells in males. In wild-type males, none of the P(3–8).p cells is a part of the HCG.

The normal 3°-2°-1° pattern of P(9–11).p HCG cell fates is disrupted in *pry-1* mutant males as a consequence of P11-to-P12-like fate transformation during the L1 stage (Howard and Sundaram, 2002). In these mutants, the wild-type hook generated by a normal 2° P10.p lineage was absent at the cloacal region, and HCG-like invaginations and protrusions from P(9–10).p were observed at low frequency (Table 1).

We also observed that, based on both marker expression and morphology, ectopic 1° HCG fates were sometimes expressed by adjacent Pn.p cells in *pry-1(mu38)* males (Fig. 1E–F). In addition, since the 1° HCG fate is required to specify the 2° HCG fate (Yu et al., 2009), we expected that some of the induced Pn.p cells would adopt the 2 fate because of lateral signaling from a nearby 1° Pn.p cell. Indeed, the structures of some protrusions in those *pry-1(mu38*) males were similar to the 2° hook, and two 2° hook lineage markers, *ceh-26*::GFP and *osm-6*::GFP, were occasionally expressed in association with a ventral protrusion (Fig. 1C–D; Table 1). In general, presence of ectopic hook neurons, as indicated

by expression of the 2° markers, was less frequent than occurrence of the hook-like structure, indicating that differentiation of hook neurons perhaps requires more intricate interactions and ectopic 2° HCG fate formation is often incomplete in *pry-1* mutants. It is also possible that some 1° /2° hybrid fates were formed in the *pry-1(mu38)* genetic background.

Specification of ectopic HCG fates in *pry-1* mutants was fully suppressed by a *bar-1*/β-catenin mutation (Table 1). No anterior invaginations or protrusions were found in *pry-1; bar-1* double mutants. Furthermore, 2° hook structures and neurons were only derived from the normal HCG region (P(9–11).p and P12.p from P12-to-P11 transformation, n= 147). The hook sensillum was completely absent in 7% of double mutants, and was partially lost in another 11% (data not shown), indicating a weak defect in 2° lineage specification similar to what is seen in a *bar-1* single mutant (Yu et al., 2009). This suppression suggests that the constitutively activated canonical Wnt signaling pathway results in specification of ectopic 1° HCG fates in P(3–8).p cells of *pry-1* mutant males.

Co-activation of Wnt and Notch signaling induces ectopic 2° HCG fates in anterior Pn.p cells

Fusion of P(7–8).p with hyp7 in the late L1 larval stage determines the anterior boundary of the HCG. Although male P(3–6).p remain unfused in the L1 stage as P(9–11).p do, they cannot adopt HCG fates and normally fuse with hyp7 during late L2 (Sulston and White, 1980; Yu et al., 2009) (Fig. 2A–B). One possibility is that the fused P(7–8).p might serve as a physical barrier that prevents any possible inclusion of P(3–6).p into the HCG. In the *lin-22(n372)* mutant, defective in a hairy homolog, male P(3–8).p remain unfused in late L3, and in some cases divide (Fixsen, 1985; Wrischnik and Kenyon, 1997) (Fig. 2C–H). However, P9.p in *lin-22(n372)* males still fused with hyp7 normally in the middle-to-late L2 stage and only a single wild-type hook was formed at its normal position anterior to the cloaca, suggesting that just preventing P(7–8).p cell fusion has no effect on HCG patterning (Fig. 2C–D, F and H).

In response to excess LIN-12/Notch or EGF signaling, male P(3–8).p not only escape fusion with hyp7 but also proliferate (Greenwald et al., 1983; Ferguson et al., 1985). However, in both cases, these cells behave like hermaphrodite P(3–8).p and assume vulval-like fates to produce pseudo-vulvae at the ventral side (multivulva or Muv phenotype; WBPhenotype: 0000700). In particular, such animals are Muv in the middle of the body but also multi-hook at posterior since P(9–11).p in the same genetic background have inappropriate HCG fates (Ferguson and Horvitz, 1985; Greenwald et al. 1983; Yu et al. 2009). These observations suggest that male P(3–8).p and P(9–11).p have distinct developmental potentials and HCG competence is limited to P(9–11).p.

Introduction of a *pry-1* mutation into a *lin-12(gf)* mutant background changed the dimorphic sexual differentiation among different subsets of male Pn.p cells caused by activated Notch signaling. Additional ectopic hook neurons, visualized by *osm-6*::GFP in the L4 stage, were paired with well-formed ectopic hook invaginations among the central P(3–8).p region of *pry-1(mu38); lin-12(gf)/lin-12(null)* double mutant males, revealing increased production of 2° HCG fates (Fig. 3, Table 1). Apparently, activated Wnt signaling by a *pry-1* mutation causes P(3–8).p to adopt HCG fates, but distinct from the *pry-1* single mutants, in which ectopic 1° HCG fates are predominant, extra LIN-12 signaling in the *pry-1(mu38); lin-12(gf)/lin-12(null)* double mutants promoted 2° HCG fate formation (Fig. 1 and 3; Table 1). Surprisingly, we observed ectopic hook neurons and invaginations from P(1–2).p (Table 1), a phenotype not detected in either of the single mutants. One explanation is that increased Wnt signaling and LIN-12 signaling act together to inhibit P(1–2).p cell fusion:

Wnt signaling provides P(1–2).p with HCG competence, while excessive LIN-12 activity leads to the formation of ectopic 2° HCG fates in P(1–2).p.

The *pry-1* mutation acts through Hox genes to alter anterior Pn.p cell competence

Activities of two Hox genes *lin-39* and *mab-5* are required to establish the Pn.p fusion pattern in the L1 stage (Salser et al., 1993). The wild-type HCG domain corresponds to a region with only *mab-5* expression. Maloof and Kenyon (1998) speculated that generation of HCG competence depends on *mab-5* function. To determine whether *pry-1* regulates Hox genes to affect P(3–8).p fusion pattern and to alter cell competence, we examined cell fates of male Pn.p defective in both *pry-1* and each Hox gene used to determine Pn.p fusion. In most *pry-1(mu38); lin-39(n1760)* males, P(3–6).p fused with hyp7 and therefore greatly reduced the formation of HCG-like protrusions and ectopic expression of the 1° marker *eat-4*::GFP among these Pn.p cells. Apparently, in a *pry-1* mutant background, *lin-39* activity still plays a major role in determination of whether P(3–6).p fuse with the hyp7 epidermal syncytium. However, the occurrence of HCG fates in P(7–10).p or in occasional unfused P(3–6).p was not suppressed by a loss of *lin-39* activity (Table 2), suggesting that *lin-39* affects Pn.p cell fusion but has no effect on HCG competence.

The *mab-5(e1239lf)* mutation causes male P(7–8).p to stay unfused until the late L2 stage as P(3–6).p do, but causes P(9–11).p to fuse with hyp7 during the L1 stage (Kenyon, 1986). In *pry-1(mu38); mab-5(e1239)* double mutants, male P(9–10).p frequently fused during the L1 stage, while P(3–8).p often remained unfused until the L4 stage and in some cases divided. In contrast to a regional suppression of HCG specification in *pry-1; lin-39* double mutant males, the *mab-5(lf)* mutation fully abolished ectopic expression of HCG-like fates in *pry-1* mutants (Table 2). Thus, activated Wnt signaling in *pry-1* mutants requires *mab-5* activity to express HCG fates in Pn.p cells.

Using an integrated *mab-5::gfp* array *muIs16*, we found that expression of *mab-5::GFP* in *pry-1* mutants was not only extended to anterior part of the body but also elevated greatly in general. In such a bright green fluorescent background, we were not able to examine the details of *mab-5::GFP* expression in Pn.p cells, which could be at a relatively lower level compare to surroundings (data not shown). The *pry-1(mu38)* mutation probably causes *mab-5* misexpression in normally unfused P(3–6).p during the L1 and L2 stages, thereby establishing HCG competence in those cells. The additional *mab-5* expression may also shift the balance between antagonistic actions of the two Hox genes *lin-39* and *mab-5* to block P(7–8).p cell fusion and cause unfused P(7–8).p cells to be HCG competent. In summary, a Pn.p cell acquires HCG competence if it escapes L1 fusion and has MAB-5 activity.

Unfused P(3–6).p were observed in some *pry-1(mu38); lin-39(n1760)* males, and the fusion of P(9–10).p with the hyp7 syncytium in *pry-1(mu38); mab-5(e1239)* males was not observed in all mutants either (data not shown), suggesting that activities of *lin-39* and *mab-5* are likely both increased by the *pry-1* mutation and the requirement of one Hox gene by a subset of Pn.p cells to remain unfused could be partially remedied by extra activity of the other. However, up-regulation of *mab-5* expression should be favored in *pry-1* mutants, as indicated by unfused P(7–8).p, and only *mab-5* acts downstream of an activated canonical Wnt pathway to specify HCG competence in P(3–8).p.

Recruitment of anterior Pn.p cells to the HCG by cooperative action of *mab-5* overexpression and a proliferation signal

We have shown that a *pry-1(rf)* mutation not only prevents fusion of the anterior P(3–8).p cells with hyp7 but also changes their competence and promotes ectopic 1° HCG fates among these cells. A *mab-5* gain-of-function (gf) promoter mutation *e1751* constitutively

expresses *mab-5* in all Pn.p cells and alters male Pn.p cell fusion pattern in the L1 stage: P(1–2).p remain unfused due to extra *mab-5* activity but P(3–8).p fuse with hyp7 because of a functional antagonism between *lin-39* and *mab-5* (Salser et al., 1993). However, no alteration in hook sensillum lineages was found in a *mab-5(e1751gf)* mutant (Table 3). Moreover, unlike suppression of the *pry-1* mutation by *bar-1(ga80lf)*, the loss-of-function *mab-5(e1239)* mutation suppressed ectopic HCG fate induction in *pry-1* mutants but did not change the scrawny appearance of *pry-1* mutant animals (see Materials and Methods), indicating that *mab-5* mediates only a part of Wnt signaling outcome. One possible role of Wnt signaling, in addition to establishing HCG competence, is that it can also act as a proliferation signal. To test this hypothesis, we tested whether the combined effect of *mab-5* overexpression and a non-Wnt proliferation signal is sufficient to generate ectopic hook fates among P(1–8).p – thereby mimicking the *pry-1* mutant phenotype.

First, we determined the effects of *mab-5(e1751gf)* mutation in a constitutively activated LIN-12/NOTCH background (see Materials and Methods). We found that 49% of *mab-5(e1751) +/+ lin-12(gf)* males had hook sensillum lineages in P(3–6).p and about 64% formed ectopic 2° HCG fates in P(1–2).p (Fig 4A–D; Table 3). Therefore, *mab-5* activity and excessive LIN-12 siganling cooperate to promote the 2° HCG fate in P(1–8).p. In addition, no ectopic 1° *eat-4*::GFP expression was detected in these anterior Pn.p cells in these double mutants (data not shown), reflecting a functional distinction between Wnt and LIN-12 signaling during HCG pattern formation.

The second combination we tested was the *mab-5(e1751)* mutation in a *lin-15*(*e1763lf)* background. Mutations in *lin-15* activate the LET-23(EGFR) pathway (Clark et al., 1994; Huang et al., 1994) likely by allowing inappropriate expression of LIN-3 in the hyp7 epidermis (Cui et al., 2006), the major signaling pathway inducing vulval development. Extra EGF signaling in *lin-15(e1763lf)* mutants causes P9.p to adopt a 2°-like fate, forming an ectopic rudimentary hook without additional hook neurons (Yu et al., 2009). These *lin-15(lf)* males also make pseudovulvae at P(3–8).p. We found that the male P(3–8).p Muv phenotype of a *lin-15* mutant was fully suppressed by the *mab-5(e1751gf)* mutation. About 10–20% of *mab-5(e1751gf); lin-15(e1763)* double mutants had HCG-like fates from male P(3–8).p, usually either P3.p or P8.p (Table 3). In addition, P(1–2).p cells in the majority of double mutants not only divided but also differentiated to make hook-like invaginations and protrusions (Fig. 4E–G; Table 3). Occasionally there was a complete 2° fate transformation as hook neurons were also detected based on ectopic *osm-6*::GFP expression (data not shown). The generation of P(1–2).p ectopic hooks in *mab-5(e1751gf); lin-15* double mutants suggests that additional EGF signaling is capable of inducing HCG fates in anterior Pn.p cells that have acquired HCG competence from *mab-5* misexpression.

It has been shown previously that *lin-22(+)* function inhibits *mab-5* expression in the anterior lateral epidermis (Wrischnik and Kenyon, 1997). Therefore, we also tested the effects of *lin-22(lf)* in an increased LIN-12/NOTCH signaling background. In general, proliferation of male P(3–8).p was enhanced in a *lin-12(gf)/lin-12(null); lin-22(n372)* strain compared to a *lin-12(gf)/lin-12(null)* genetic background (Fixsen, 1985; our observations). We observed ectopic hook formation in *lin-12(gf)/lin-12(null); lin-22(n372rf*) males: P(7–8).p in 27% of the double mutant males produced 2° hook invaginations and hook neurons. However, formation of pseudovulvae was predominant in P(3–6).p (Fig. 5B–C; Table 3). The *lin-22* mutation might cause a weak increase of *mab-5* expression in anterior Pn.p cells, which allows *mab-5* activity to out-compete *lin-39* activity in male P(7–8).p and results in unfused P(7–8).p cells in the *lin-12(gf)/lin-12(null); lin-22(n372)* double mutant males that are competent to adopt HCG fates. Intriguingly, about 5% of the double mutant males formed an ectopic hook and/or hook neurons in P(1–2).p (Fig. 5A; Table 3), although neither of the single mutants had unfused P(1–2).p cells. Unlike in *mab-5(e1751gf)* mutants,

male P(1–2).p in a *lin-22* single mutant still fuse normally during the L1 stage. Interaction between the *lin-12(gf)* and *lin-22* reduction-of-function mutations might keep P(1–2).p unfused and cause sufficient ectopic *mab-5* expression to provide these two Pn.p cells with HCG competence.

MAB-5 is required for wild-type HCG pattern formation and is regulated by the Wnt receptor LIN-17/Frizzled

Wild-type *mab-5* activity represses the fusion fate of male P(9–11).p in the L1 stage (Kenyon, 1986). However, early fusion of P(9–11).p to the hyp7 syncytium caused by *mab-5(lf)* mutations makes it unclear whether *mab-5* function is further required during normal HCG patterning at a later developmental stage. To address this question, we used an *eff-1* mutation to block P(9–11).p cell fusion in *mab-5(e1239lf)* mutants. *hy21* is a temperature-sensitive allele of *eff-1*, an integral membrane protein gene necessary for epithelial cell fusion (Mohler et al., 2002). When grown at 25° C, all hypodermal cells fail to fuse in *eff-1(hy21)* mutants. We found that *eff-1(hy21)* males exhibited a weak abnormality in hook morphology, while expression of *eat-4*::GFP in PVV was generally not affected (48/48), suggesting that HCG fate specification is not altered in these animals. In contrast, neither 1° expression of *eat-4*::GFP nor presence of the 2 hook structure was observed in *eff-1(hy21); mab-5(e1239)* males (n=78) even though P10.p and/or P11.p cells remained unfused. Therefore, in the absence of *mab-5* activity, the inhibition of cell fusion is insufficient for P(9–11).p to adopt HCG fates, indicating that HCG competence is not just a direct consequence of the prevention of cell fusion. *mab-5* activity is necessary for establishment of HCG competence in the normal HCG, P(9–11).p, as well as in anterior Pn.p cells during ectopic HCG fate formation (discussed in the previous sections).

Since we had shown that Wnt signaling is required to specify HCG fates, we investigated whether *mab-5/Hox* expression was regulated by Wnt signaling. We found that an extrachromosomal translational MAB-5 GFP reporter (Celniker et al., 2009) was expressed in P11.p in all 25 males examined (Fig. 6A–B). However, MAB-5 GFP expression was either abolished or greatly reduced in 7 of 8 *lin-17(n671lf)* males (Fig. 6C–D). Therefore, Wnt signaling is required for *mab-5/Hox* expression in P11.p. MAB-5 GFP expression in P10.p at the same stage is barely detectable (Fig. 6A–B). The effect of MAB-5 on 2° fate may be subtle (see below).

Overexpression of *mab-5* by the heat-shock treatment of a *hs-mab-5* transgenic line *muIs9* during the L2 to early L3 stage did not cause ectopic hook formation in P(9–11).p. Instead, we observed a high percentage of hook abnormalities (about 70%) in adult males, including missing, misshapen, or anteriorly-displaced hooks (Table S1). HOB seemed less affected by heat-shock-induced MAB-5 activity, indicated by *ceh-26::gfp* expression. About 16% animals had a second cell expressed *ceh-26::gfp* after the heat shock treatment. An extra *osm-6::gfp*-expressing cell, in addition to HOA and HOB, was also seen in *mab-5(e1751gf); lin-15(e1763)* and *mab-5(e1751)+/+lin-12(gf)* mutants occasionally. This cell is usually located posteriorly next to HOB and it is probably a HOB-like fate transformation within the 2° lineage due to excessive MAB-5 activity. In summary, MAB-5 activity needs to be limited in 2° P10.p; excessive *mab-5* activity at a later stage might have a negative effect on the 2° lineage and hook sensillum differentiation.

Discussion

MAB-5/Hox is required for HCG fates

Prior to HCG pattern formation, fusion of male Pn.p cells with the hyp7 epidermal syncytium in the late L1 stage has a direct impact on the existence of P(9–11).p precursor

cells. This pattern is regulated by the Hox genes *lin-39* and *mab-5* (Salser et al., 1993). A *mab-5* mutant lacks a hook structure because P(9–11).p fuse to hyp7 during the L1 larval stage. Our analysis of *mab-5; eff-1* double mutants indicates that *mab-5* is necessary for HCG fate specification in addition to preventing P(9–11).p cell fusion prior to fate specification.

Increased Wnt signaling in a *pry-1* mutant extends the boundary of the HCG to anterior Pn.p cells and this action depends on *mab-5* activity. Overexpression of *mab-5* by a *mab-5(e1751gf)* mutation makes anterior P(3–8).p cells in *lin-12(gf)* and *lin-15(null)* mutants switch from expressing vulval-like fates to HCG fates. Therefore, the Hox gene *mab-5* is a determinative component for HCG competence.

Induction of the 1° fate is the key to establish a precise spatial pattern of 3°-2°-1° within the male HCG, given that specification of the 2° fate depends on presence of a 1° fate cell. Our previous work suggested that Wnt signaling acts together with the EGF pathway to induce the HCG fates (Yu et al., 2009). Furthermore, examination of *lin-17* and *bar-1* expression in P(9–11).p suggests a specific role of Wnt signaling in promoting the 1° HCG fate. Here, we provide further evidence for this function of Wnt signaling by showing that activation of a canonical Wnt pathway is sufficient to produce 1° HCG fates ectopically. We observed adjacent ectopic 1° HCG fate cells in a *pry-1* mutant. However, induction of the 2° HCG fate by these 1° fate cells is inefficient, suggesting that an activated canonical Wnt pathway is a potent 1° HCG fate inducer and 1° HCG fate specification of a cell antagonizes 2° fate specification in the same cell, much as is the case for VPCs (Sternberg, 1988; Sternberg, 2005). Supporting a role for *mab-5* in HCG specification, particularly 1° fate specification, we found that *lin-17*-mediated Wnt signaling regulates *mab-5* expression in the 1° HCG cell, P11.p.

Our *mab-5(gf); lin-12(gf)* double mutant analysis also indicates that the requirement for *lin-17* function cannot be bypassed during 1° HCG fate specification. In addition, MAB-5 activity seems fine-tuned in the 2° lineage, and unrestricted *mab-5* expression by a heat-shock transgene has a detrimental effect on hook formation.

Re-programming Pn.p cells: how to specify a hook

All Pn.p cells anterior to the wild-type HCG, P(9–11).p, can acquire HCG fates depend on the following factors: the type of ectopic signal that is received, which Hox gene is expressed in the cell in the wild type and whether *mab-5* activity is present (Fig. 7). The anterior Pn.p cells can be grouped into three categories based on the conditions necessary for ectopic HCG fates.

P(7–8).p—In wild-type males, antagonism between the Hox genes *lin-39* and *mab-5* prevents both P7.p and P8.p from responding to Wnt signals because they fuse early on in the L1 stage. However, P(7–8).p will express 1° and/or 2° HCG fates after the following genetic manipulations: 1) excessive Wnt signaling, 2) *mab-5* overexpression in combination with increased EGF signaling, 3) *mab-5* overexpression in combination with increased Notch signaling, or 4) increased Notch signaling together with reduced *mab-5* inhibition.

Male P(7–8).p cells are likely sensitive to the relative ratios of *lin-39* and *mab-5* activities for the L1 fusion (Salser et al., 1993). Unfused P(7–8).p cells in *pry-1(mu38)* males demonstrate that excess Wnt signaling breaks the antagonistic balance between Hox genes *lin-39* and *mab-5*. Two observations lead us to propose that *mab-5* is probably a preferred downstream target of Wnt signaling, as seen in specification of Q neuroblast lineages (Maloof et al., 1999; Korswagen et al., 2000). First, we found that *mab-5* is regulated in the HCG by *lin-17*-mediated Wnt signaling. Second, P7.p and P8.p do not acquire HCG fates in

pry-1(lf); mab-5(lf) mutants. Preferential upregulation of *mab-5* in P7.p and P8.p in *pry-1* mutants bypasses fusion in the L1 and causes the expression of ectopic HCG fates.

Either increased Notch or EGF signaling can also prevent P7.p and P8.p from fusing to hyp7 in the L1, possibly by favoring the upregulation of *lin-39/Hox*. However, unfused P(7–8).p behave like more anterior P(3–6).p and adopt vulval-like fates in the two mutant backgrounds, while posterior P(9–11).p have an overinduced HCG phenotype and form multi-hooks (Greenwald et al., 1983; Yu et al., 2009). Additional *mab-5* activity enables unfused P(7–8).p in the increased Notch or EGF signaling background to assume HCG fates.

P(3–6).p—Although central P(3–6).p only express *lin-39* in the wild type, they express HCG fates under similar conditions as P(7–8).p. Increased Wnt signaling in a *pry-1* mutant affects the L1 fusion decision of P(7–8).p cells but is not sufficient to significantly interfere with *lin-39* function to cause frequent abnormal fusion of P(3–6).p in the L1 stage as seen in *mab-5(e1751gf)* mutants. Unfused P(3–6).p become HCG competent in response to ectopic *mab-5* activity either by activation of Wnt signaling or a *mab-5(e1751gf)* mutation. The difference in *lin-39* and *mab-5* levels between P(7–8).p and P(3–6).p are reflected only in the penetrance of the expression of ectopic HCG fates by both groups as a consequence of escaping the L1 fusion and HCG induction.

P(1–2).p—Because both P1.p and P2.p express neither *lin-39/Hox* nor *mab-5/Hox* and fuse in the L1 stage in the wild type, we can identify what the minimum requirements for hook fate specification by examining the conditions under which P(1–2).p adopt HCG fates. Increased Wnt, EGF or Notch signaling alone do not change P(1–2).p cell fusion pattern; *mab-5* overexpression causes P(1–2).p to remain unfused but is not insufficient to generate ectopic HCG lineages. However, excessive Wnt signaling together with Notch signaling, excessive Notch or EGF signaling in a *mab-5(gf)* background, or increased Notch signaling in a *lin-22* mutant background result in P(1–2).p acquiring HCG fates, suggesting that both a proliferation signal and a threshold of *mab-5* activity are required to generate a HCG fate.

Stepwise specification of male HCG fates

Specification of cell fates in the male hook sensillum competence group (HCG) comprises multiple steps, a process similar to hermaphrodite vulval development. The first step is to distinguish HCG fates from non-HCG fates among male Pn.p cells; the second step is to choose between induced (1° or 2°) HCG fates and a non-induced (3°) HCG fate; the third step is to determine the 1° or 2° HCG fate. Mutation of the Axin homolog *pry-1* leads to pleiotropic effects on male Pn.p cells and the consequent formation of ectopic HCG fates in anterior Pn.p cells indicate multiple roles of Wnt signaling during HCG patterning. We propose the following scheme for male HCG fate specification (Fig. 8). Male P(9–11).p cells remain unfused in the late L1 stage due to *mab-5* activity, and thereby are potentially responsive to an inductive signal. A WNT signal from the posterior regulates *mab-5* activity thereby determining HCG competence. Since our reporter gene assay suggests that *mab-5* levels might be higher in P11.p than in P10.p, the levels of *mab-5* expression might help establish the pattern of cell fates among the P(9–11).p cells. In P11.p, activated LIN-17 receptor signals downstream via components such as BAR-1 to promote the 1 fate, which produces ligands for the LIN-12/Notch receptor to promote the 2° fate in the adjacent P10.p cell. The stepwise determination progressively restricts P(9–11).p cells into one of the three HCG fates. Once the cell fates are established, *mab-5* activity is further fine tuned among HCG sub-lineages to ensure precise differentiation of the male hook sensillum.

Comparing Competence and Fate specification in the VPCs and HCG

Overall, VPC and HCG patterning are quite similar: the precise cell fate is generated by progressive restriction through competence, induction, and lateral inhibition mediated by multiple signal integration at different steps, representing a general scenario of complex pattern formation.

Specifically, both VPC and HCG competence are established by Wnt signaling (Eisenmann et al., 1998; this work) and one of the two Hox genes, *lin-39* and *mab-5*, respectively (Clandinin et al., 1997; this work). Expression patterns of both Hox genes are the same in both hermaphrodite and male, with *lin-39* expression in P(3–8).p and *mab-5* expression in P(7–11).p (Clark et al., 1993; Salser et al., 1993; Wang et al., 1993). However, sex-specific utilization of these two Hox genes, *lin-39* and *mab-5*, determines whether a hermaphrodite vulva or a male hook, respectively, is formed (Maloof and Kenyon, 1998; this work). In hermaphrodites, *lin-39* function is favored in the central Pn.p cells, and the ability of *mab-5* to prevent P(9–11).p fusion with hyp7 is somehow blocked (Salser et al., 1993). As a transcription factor, *mab-5* regulates target gene expression. One possibility is that a negative regulator in hermaphrodites sequesters *mab-5* from its targets. Alternatively, *mab-5* may act with a co-regulator that is missing in hermaphrodites. The Hox genes appear to play a permissive role in VPC and HCG induction because neither multi-vulvae (Maloof and Kenyon, 1998) nor multi-hooks (this work) are observed when *lin-39* or *mab-5*, respectively, are overexpressed.

A major difference between VPC and HCG development is the major inductive signal used to specify the 1° fate: the EGF pathway induces the 1° VPC fate (Hill and Sternberg, 1992) while Wnt signaling promotes the 1° HCG fate (Yu et al., 2009; this work). However, both EGF and Wnt act to induce HCG as well as VPC fates, and it has been observed that excessive Wnt signaling can at least partially substitute for EGF signaling in VPC induction (Gleason et al., 2002), and vice versa in HCG specification (Yu et al., 2009). The local abundance of the signal could explain why different inductive signals are utilized in VPC and HCG patterning. The availability of the Wnt and EGF inductive signals differ spatially in hermaphrodites and males, contributing further to the sex-specific bias of Hox gene expression. Although Wnts are present in the central region of the body (Eisenmann et al., 1998; Inoue et al., 2004) and the EGF ligand is produced in the tail (Chamberlin and Sternberg, 1994; Jiang and Sternberg, 1998), the EGF signal emanates from a concentrated source, the gonadal anchor cell, only in the hermaphrodite, while Wnt signaling is more abundant in the tail region as elucidated by extensive tail defects caused by deficient Wnt signaling (Sternberg and Horvitz, 1988; Herman and Horvitz, 1994; Chamberlin and Sternberg, 1995; Herman et al., 1995; Jiang and Sternberg, 1998). As such, only the required Hox gene is promoted in each region in a sex-specific manner. E.g. *lin-39* activity in males is not reinforced due to lack of a strong extrinsic signal in the central region. Therefore, different signaling pathways may not be the direct cause of sexually dimorphic organogenesis. The specificity of signaling relies on Hox genes to direct sex-specific pattern formation among competent precursor cells.

Materials and Methods

General methods, nomenclature and strains

Methods for handling and culturing of *C. elegans* are described by Brenner (1974). Unless otherwise noted, all experiments were performed at 20°C. Alleles and transgenes that were used in this work are listed in Table S2. The detailed strain information is included in Table S3.

In the text, we refer to the *lin-12* gain-of-function mutation *lin-12(n137)* as "*lin-12(gf)*", and the null mutation *lin-12(n676 n909)* as "*lin-12(null)*". Unlinked double mutant strains were constructed according to standard methods (Huang and Sternberg, 1995). During some strain constructions, genetic markers within same linkage group of one allele were used in trans to that allele to facilitate selection.

The original *eat-4*::GFP transgene integrated on chromosome X is linked to *n765*, a temperature-sensitive mutant allele of *lin-15*, raising the possibility that additional PVV-like cells might be due to an interactions between the *pry-1* and *lin-15* mutations. To remove the *lin-15(n765)* allele linked to the *adIs1240 (eat-4*::GFP*)* transgene, *him-5 (e1490)* V*; adIs1240 lin-15(n765ts)* X males were crossed into *dpy-6(e14) unc-9(n101)* X hermaphrodites and F2 Unc-non-Dpy animals expressing *eat-4*::GFP were selected. We re-assayed *eat-4*::GFP expression in *pry-1* males grown at 15 C, a permissive temperature for the *lin-15(n765)* mutation, and in *pry-1* mutants after removal of the *lin-15(n765)* mutation from background by genetic recombination. In both conditions, *pry-1* mutants displayed significant ectopic *eat-4*::GFP expression, associated with ventral protrusions. These results suggest that *pry-1(mu38)* induces formation of ectopic PVVs from P(3–10).p cells, although we cannot rule out some minor effect from *lin-15*.

The strain carrying the *pry-1(mu38)* mutation and *syIs78 (*AJM-1–GFP*)* transgene, which are both located on linkage group I, was obtained by picking *pry-1(mu38)* homozygous hermaphrodites with AJM-1–GFP expression in a F2 population after cross. *pry-1(mu38); bar-1(ga80)* double mutant males had a Bar-1-like gross morphology, and were much healthier than *pry-1* single mutant males, suggesting a complete suppression of the *pry-1(mu38)* phenotype by the *bar-1(ga80)* mutation. In this double mutant, the presence of *pry-1(mu38)* allele in the strain was verified by strain deconstruction. By contrast, in *pry-1(mu38); mab-5(e1239)* double mutants, loss of *mab-5* function did not change the scrawny appearance of *pry-1* mutant animals, indicating that *mab-5* only participated in some aspects of Wnt signaling. Although small bumps were sporadically formed at the ventral side as a result of occasional proliferation of Pn.p cells, neither hook-like structure nor yellowish autofluorescence was seen in *pry-1(mu38); mab-5(e1239)* males. About one-third of *pry-1; mab-5* double mutant males had an additional neuronal cell expressing *eat-4*::GFP in the preanal ganglion region. By comparing them with *mab-5(e1239)* single mutants, we deduced that this neuron is not likely from the Pn.p lineages and is probably differentiated from a Pn.a progeny that fails to die in the *mab-5(e1239lf)* mutant background (Kenyon, 1986). Unlike what is seen in *mab-5* single mutants, the fusion of P(9–10).p to the hyp7 epidermal syncytium is not complete in *pry-1(mu38); mab-5(e1239)* males. This could be caused by additional *lin-39* activity, if excess Wnt signaling in *pry-1* mutants increased activities of both Hox genes, but with a preference for *mab-5*.

To generate a double mutant containing both *lin-12(n137gf)* and *mab-5(e1751gf)* alleles, we made a heterozygous strain since *mab-5* and *lin-12* are closely linked on linkage group III, with one copy of chromosome III containing a *mab-5(e1751)* allele and a wild-type *lin-12(+)* allele, and the other copy a *mab-5(+)* allele and a *lin-12(gf)* allele. In this compound heterozygous background, *mab-5(e1751)* single males can be distinguished by fused rays 1 and 2 with a wild-type hook. Males with more than one hook anterior to the cloaca, indicating the presence of *lin-12(gf)*, were examined for HCG-like fate formation in anterior Pn.p.

Despite their hookless phenotype and abnormal HCG lineages, *lin-17* mutations often do not completely abolish cell divisions of P10.p and P11.p, raising a question of whether *mab-5* overexpression is able to rescue the lineage defects in *lin-17* mutants. The *lin-17(n671); mab-5(e1751)* animals were very sick and the hermaphrodites usually rupture before

production of any progeny. In the double mutant males, the presence of *mab-5(e1751)* mutation was verified based on gaps in their lateral alae (cuticular ridges). The *lin-17(n671); mab-5(e1751gf)* double mutant males showed a Lin-17-like tail phenotype: 27/28 animals were hookless and only 1/28 had a rudimentary hook-like protrusion. It is unclear whether the *mab-5(e1751)* mutation causes extra MAB-5 activity in P(9–11).p and whether the extra MAB-5 activity, if any, is independent of *lin-17* regulation. However, production of ectopic 2° HCG fates in anterior Pn.p cells of *mab-5(e1751) +/+ lin-12(gf)* males was suppressed by introduction of a *lin-17(n671)* mutation to the background (n=30). Of the 30 mutants examined, at least two *lin-17 (n671); mab-5(e1751) +/+ lin-12(gf)* males displayed a Lin-17-like hookless phenotype at the tail but formed rudimentary hook-like protrusions among anterior Pn.p cells, indicating the presence of extra LIN-17-independent MAB-5 activity in anterior Pn.p cells in this triple mutant background. Therefore, additional MAB-5 activity is not sufficient to bypass the requirement of LIN-17-mediated Wnt signaling for HCG fate specification, consistent with a role of *lin-17* in the lineages generated by the HCG cells once their fates are specified.

Microscopy

Cell anatomy and lineages were observed in living animals using Nomarski optics as described (Sulston and Horvitz, 1977). We took advantage of B cell development as an internal reference for the developmental timing. The male-specific B blast cell divides in the late L1 stage, just after the generation of Pn.p cells. The second cell division of B lineage is in middle L2. By the late L2 stage, there are ten B progeny. These ten cells then adjust their positions and form a characteristic assembly around the proctoderm in the early L3 stage. Cells of the B lineage further divide just before P(10–11).p divisions in about the middle L3 stage. The appropriate position of the developing male gonad was another temporal indicator. For viewing GFP expression, a Chroma Technology High Q GFP long pass filter set [450 nm excitation, 505 nm emission] was used in conventional fluorescence microscopy (Zeiss Axioskop). The same filter was also used for visualizing autofluorescence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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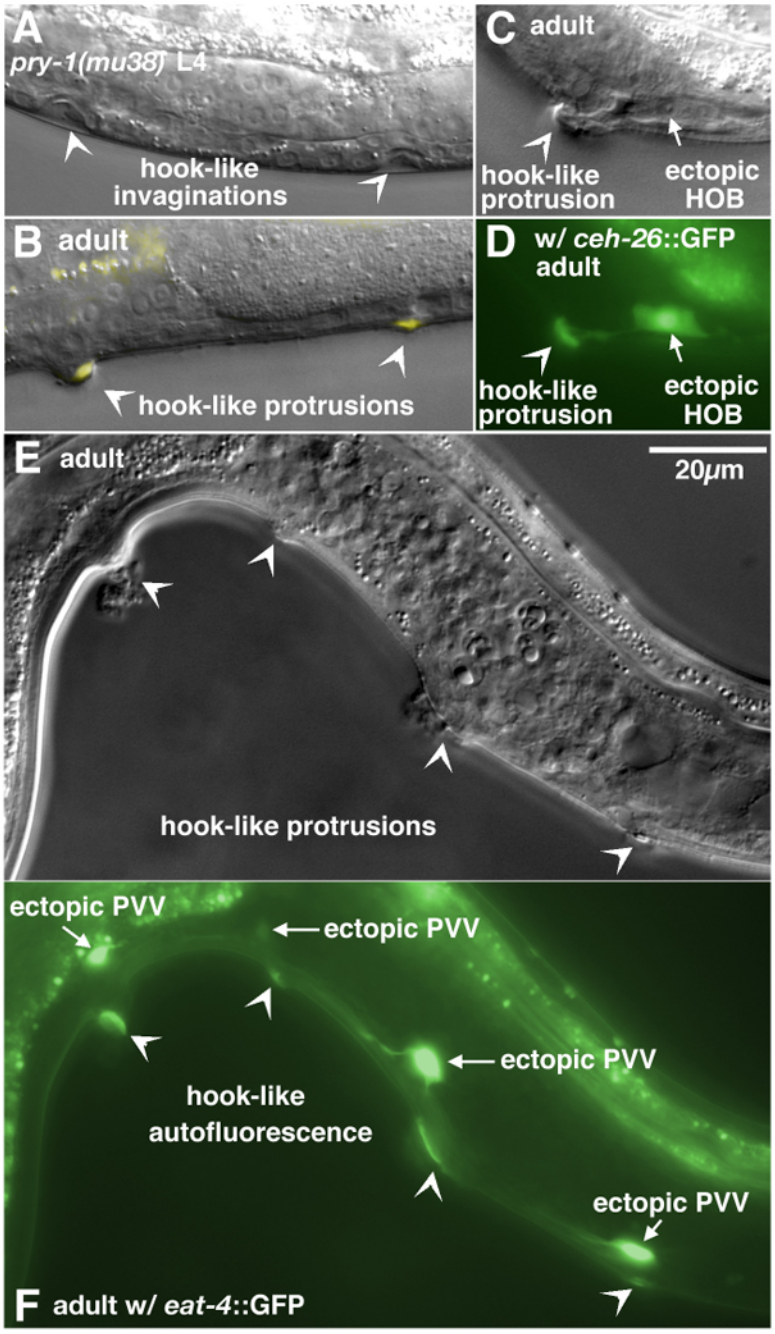

Figure 1.

Formation of ectopic HCG fates in *pry-1* mutant males. (A) Late L4. Two ventral invaginations with anchor-like shapes inside (arrowheads) were formed in the central region of a *pry-1(mu38)* male. (B) Overlay of Nomarski and fluorescence images of an adult *pry-1(mu38)* male. Two ventral protrusions (arrowheads) were observed in the middle body. Autofluorescence could be seen at the tip of the protrusions. (C–D) Nomarski (C) and fluorescence (D) images of an adult *pry-1(mu38)* male. An autofluorescent ventral protrusion (arrowhead) was associated with an ectopic 2° HOB hook neuron cell (arrow), as indicated by *ceh-26*::GFP expression. (E–F) Correlation of ectopic 1° PVV-like *eat-4*::GFP-

expressing neurons (arrows) with ventral protrusions (arrowheads) in a *pry-1(mu38)* male (E, Nomarski; F, fluorescence). Scale bar, 20 μm. Left lateral views.

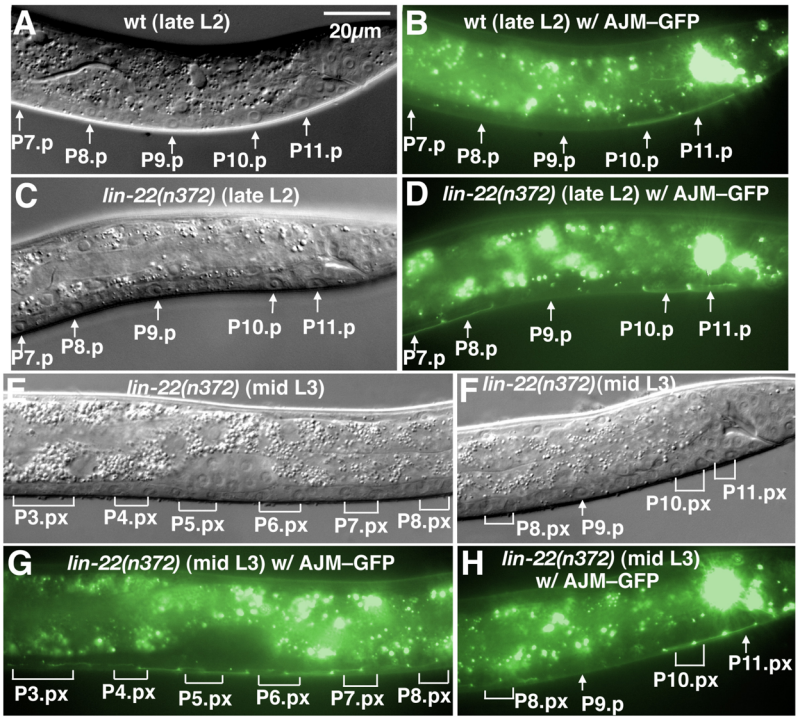

Figure 2.

Alteration of male Pn.p fusion pattern by a *lin-22* mutation. (A–D) Late L2. Nomarski (A) and fluorescence (B) images of a wild-type male. In the posterior, a line of AJM-1–GFP expression marked unfused P10.p and P11.p cells. P9.p and more anterior P(7–8).p were fused with hyp7 already. Nomarski (C) and fluorescence (D) images of a *lin-22(n371)* male in the same developmental stage. P9.p fused as indicated by the loss of AJM-1–GFP expression. However, P(7–8).p, as well as central P(3–6).p (not shown), remained unfused as do P(10–11).p. (E–H) Nomarski (E and F) and fluorescence (G and H) images of a *lin-22* male in the middle L3 stage. The unfused central P(3–8).p and posterior P(10–11).p all divided once. Expression of AJM-1–GFP was observed in all those Pn.p descendants. The P11.pa cell was located a little above and thus the AJM-1–GFP expression was not shown in this picture. Scale bar, 20 μm. Left lateral views.

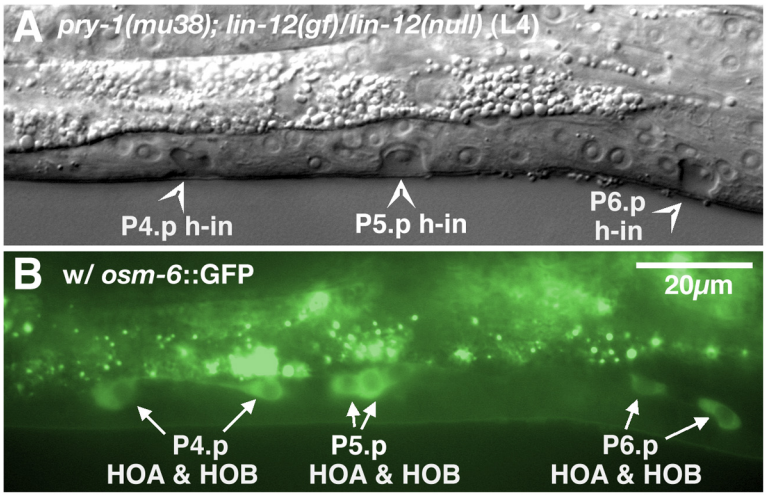

Figure 3.

Preferential 2° HCG fate transformation in anterior Pn.p cells by activated Wnt and LIN-12 signaling. Nomarski (A) and fluorescence (B) images of an L4 *pry-1; lin-12(gf)/lin-12(null)* male. Three hook invaginations (arrowheads) were each coupled with a pair of *osm-6*::GFP-expressing hook neurons (arrows) among the three middle Pn.p cells (P4.p-P6.p). h-in, hook invagination. Scale bar, 20 μm. Left lateral views.

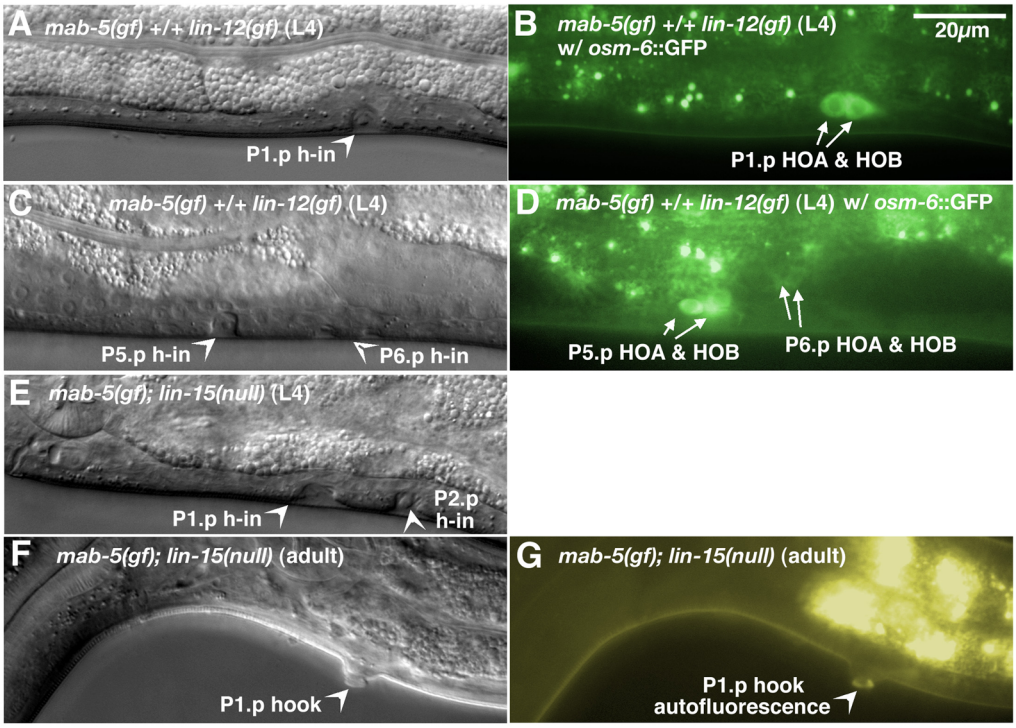

Figure 4.

Induction of ectopic 2° HCG fates by the combined action of constitutive MAB-5 activity and Notch or EGF signaling. (A–D) 2°-like HCG fate transformation in anterior Pn.p cells by increased MAB-5 activity and activated LIN-12 signaling. An L4 *mab-5(e1751gf) +/+ lin-12(gf)* male had an nice hook invagination and a pair of HOA and HOB hook neurons (marked by *osm-6*::GFP) derived from the P1.p lineage (A, Nomarski; B, fluorescence). Another L4 *mab-5(e1751gf) +/+ lin-12(gf)* male generated hook invaginations at the central P5.p and P6.p (arrowheads) (C, Nomarski). Each invagination was associated with a pair of hook neurons (arrows) (D, fluorescence). (EG) Ectopic hook formation in P(1–2).p by extra MAB-5 activity and EGF signaling. (E) An L4 *mab-5(e1751gf); lin-15(e1753)* male formed two hook-like invaginations at P(1–2).p. Nomarski (F) and fluorescence (G) images of a P1.p hook in an adult *mab-5(e1751gf); lin-15(e1753)* male. h-in, hook invagination. Scale bar, 20 μm. Left lateral views.

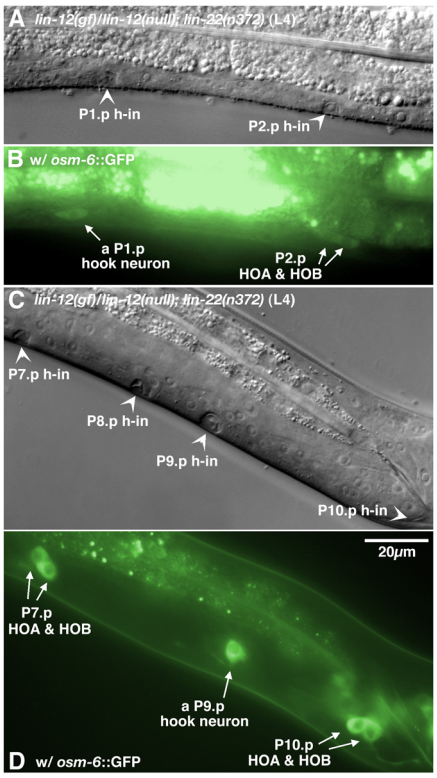

Figure 5.

2° HCG fate formation in male Pn.p cells caused by activated LIN-12 signaling in a *lin-22* mutant background. (A–B) A L4 *lin-12(gf)/lin-12(null); lin-22(n372)* male. Nomarski (A) and fluorescence (B) images of two hook-like invaginations at P1.p and P2.p (arrowheads in A) were observed. *osm-6*::GFP is expressed in both 2° hook neurons. A single *osm-6*::GFP-expressing hook neuron was seen near the P1.p hook invagination, and the P2.p lineage made a complete hook sensillum with the presence of two *osm-6*::GFP-expressing cells (arrows in B). (C–D) Nomarski (C) and fluorescence (D) images of (P7-10).p in another L4 *lin-12(gf)/lin-12(null); lin-22(n372)* male. A P9.p hook sensillum was produced in addition to a P10.p wild-type hook sensillum. Furthermore, P(7–8).p were also induced to form hook invaginations. The P7.p hook invagination was associated with a pair of hook neurons, indicating a complete 2° HCG fate transformation. P8.p only generated a hook invagination, indicating a partial 2° fate transformation. One of the P9.p hook neurons was located in a slightly right focal plane and cannot be seen in the picture. h-in, hook invagination. Scale bar, 20 μm. Left lateral views.

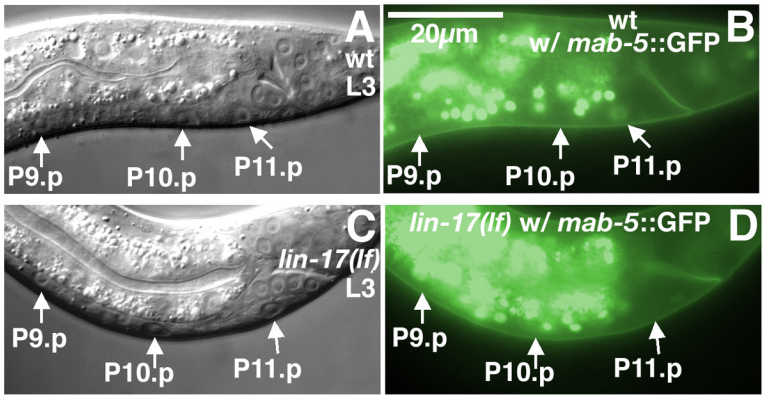

Figure 6.

Regulation of MAB-5–GFP expression in P(9–11).p by *lin-17*. (A–D) Early L3 wild-type male. Nomarski (A) and fluorescence (B) images of the HCG. P10.p and P11.p have moved slightly to the posterior. MAB-5–GFP was expressed in P11.p. Expression in P10.p was hardly detectable in this animal. (C-D) Early L3 *lin-17(n671lf)* male. MAB-5–GFP expression was absent in P11.p. However, this mutant male still retained some faint GFP expression in P10.p. Scale bar, 20 μm. Left lateral views.

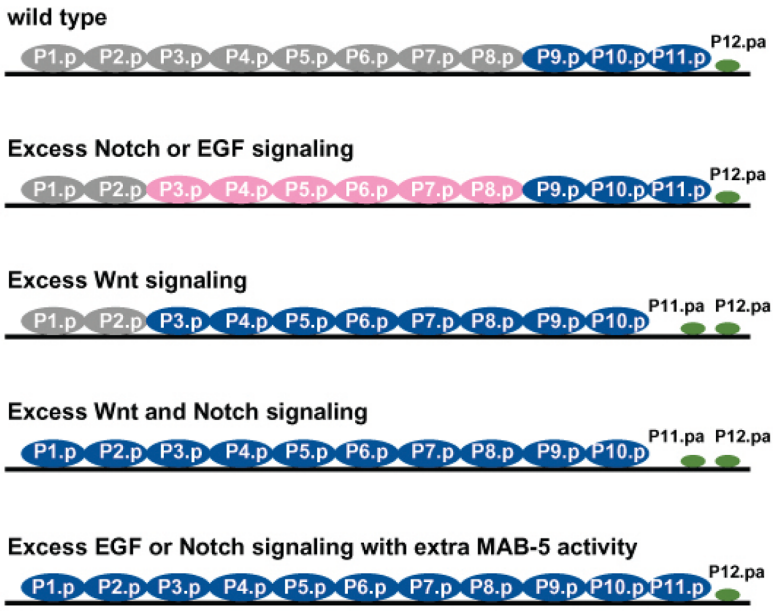

Figure 7.

Excess Wnt signaling or ectopic MAB-5 activity together with excess EGF or LIN-12/Notch signaling confers hook competence to male Pn.p cells anterior to the HCG. In wild-type males, only P(9–11).p possess HCG competence (blue). All anterior Pn.p cells (P(1–8).p) remain uninduced and fuse with hyp7 (grey). The most posterior cell P12.pa adopts a unique hypodermal fate (dark green). Increased Notch signaling due to a *lin-12(gf)* mutation or abnormal activation of EGF signaling by a *lin-15(null)* mutation causes the central Pn.p cells, P(3–8).p, to adopt a vulval-like fate (pink) but does not change the competence of the other Pn.p cells. Extra Wnt signaling caused by the *pry-1(mu38)* mutation confers HCG competence to P(3–8).p. Combined action of the *pry-1(mu38)* and *lin-12(gf)* mutations further extends the anterior boundary of the HCG to include P1.p and P2.p. A similar effect is also observed in conditions of excess MAB-5 activities combined with increased Notch (by a *lin-12(gf)* mutation) or EGF (by a *lin-15(null)* mutation) signaling whereby P(1–8).p is recruited into the HCG.

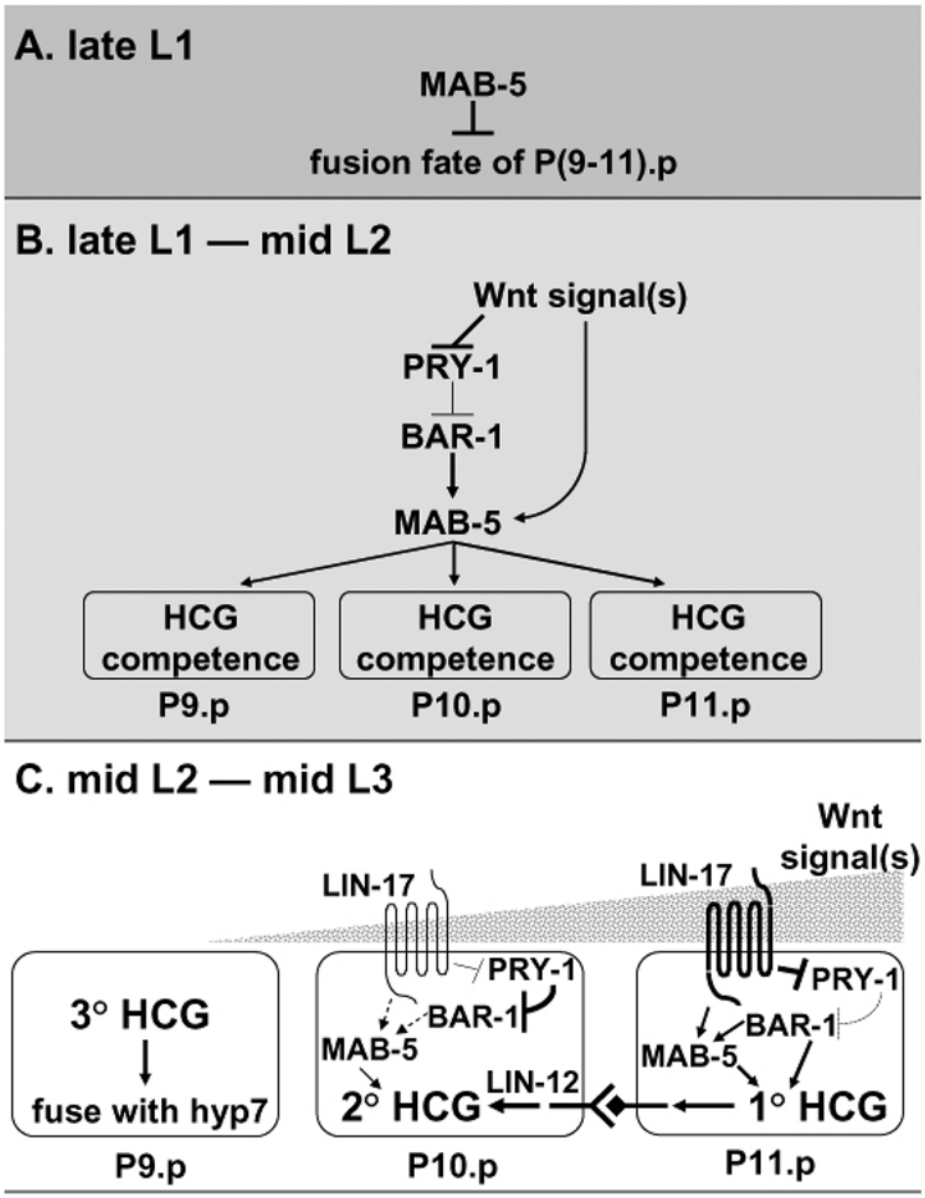

Figure 8.

Model for development of the male HCG. (A) Regional MAB-5 activity preset during embryonic development keeps P(9–11).p unfused in the late L1 stage. (B) During the late L1 to mid-L2 stage, continuing presence of *mab-5* activity in P(9–11).p, which is regulated by Wnt and probably other graded signals from the posterior tail region, makes these cells able to generate hook sensillum tissue (HCG competence). (C) During the mid-L2 to mid-L3 stage, a strong Wnt signal induces the 1° HCG fate in P11.p since it is closest to the posterior. The posterior signal(s) make P10.p assume an induced fate as well. With the presence of a 1° P11.p cell, the P10.p cell is locked into the 2° HCG fate by activated LIN-12 signaling. The P9.p cell, which has minimum *mab-5* activity and receives the lowest level of inductive signals, adopts the uninduced 3° fate and finally fuses with hyp7.

Table 1

Ectopic HCG induction in *pry-1* mutants

Genotype[a]		HCG-like fate formation[b] (%)(structure or marker expression)				avg. HCG-like structure[c] (1° or 2°)	avg. marker expression	n[d]
signaling	markers	P(1–2).p	P(3–6).p	P(7–8).p	P(9–11).p			
+	none	0	0	0	100	1.0	NA	many
pry-1	“	0	70	27	39	1.8	NA	79
+	*eat-4*::GFP (1°)	0	0	0	100	1.0	1.0 (1°)	117
pry-1	“	0	95	38	5	2.2	2.0 (1°)	39
+	*ceh-26*::GFP (2°)	0	0	0	100	1.0	1.0 (2°)	>2000 [e]
pry-1	“	0	83	17	13	1.9	0.2 (2°)	30
bar-1	“	0	0	0	86[f]	0.8	0.7 (2°)	71 [g]
pry-1; bar-1	“	0	0	0	93[f]	1.0	0.9 (2°)	147
+	*osm-6*::GFP (2°)	0	0	0	100	1.0	1.0 (2°)	>200[e]
pry-1	“	0	91	36	27	1.7	0.4 (2°)	22
lin-12(gf)/lin-12(lf)	“	0	0	0	100	2.3	2.3 (2°)	69
pry-1; lin-12(gf)/lin-12(lf)	“	26	100	22	4	2.8	1.4 (2°)	27

[a] Alleles used are: *pry-1(mu38)*, *lin-12(n137)* as *lin-12(gf)*, and *lin-12(n676n909)* as *lin-12(lf)*. Integrated arrays are *adIs1240 (eat-4*::GFP), *chIs1200 (ceh-26*::GFP), and *mnIs17 (osm-6*::GFP). All strains contain *him-5(e1490)* in the background.

[b] Percentage of animals in which Pn.p subgroups adopt HCG-like fates, determined by the presence of HCG-like structure (invagination if scored in L4 or ventral protrusion if scored in adult), and/or ectopic lineage marker expression.

[c] Including HCG-like invagination or ventral protrusion.

[d] Number of animals scored.

[e] Data are from a genetic screen described in Yu et al. (2003).

[f] Only the 2° hook and /or marker expression are scored. Actual HCG induction might be higher if a few animals had the 1° fate only.

[g] Data are also mentioned in Yu et al. (2009).

NA, not available.

Table 2

Hox genes are required for HCG fate transformation induced by Wnt signaling

genotype[a]			HCG-like fate formation[b] (%)(structure or marker expression)			avg. HCG-like structure[c] (1° or 2°)	avg. 1° marker expression	n[d]
Wnt signaling	Hox	markers	P(3–6).p	P(7–8).p	P(9–11).p			
+	+	none	0	0	100	1.0	NA	many
+	*lin-39*	“	0	0	100	1.0	NA	79
pry-1	+	“	70	27	39	1.8	NA	79
pry-1	*lin-39*	“	9	33	14	0.6	NA	43
+	+	*eat-4*::GFP (1°)	0	0	100	1.0	1.0	117
+	*lin-39*	“	0	0	100	1.0	1.0	63
+	*mab-5*	“	0	0	0	0	0	84
pry-1	+	“	95	38	5	2.2	2.0	39
pry-1	*lin-39*	“	4	56	25	0.8	0.9	48
pry-1	*mab-5*	“	0	0	0	0	0	83

[a] Alleles used are: *pry-1(mu38)*, *mab-5(e1239)*, *lin-39(n1760)*. Both Hox mutant alleles are probably null. All strains contain *him-5(e1490)*. The *eat-4*::GFP transgene is *adIs1240*.

[b] Some of the data in this table are present in Table 1.

[c] Including hook-like invaginations or ventral protrusions.

[d] Number of animals scored.

NA, not available.

Table 3

Ectopic HCG induction by increased MAB-5 activity and activated LIN-12 or EGF signaling

genotype[a]	markers	HCG-like fate formation (%)(structure or marker expression)				avg. hook-like structure (1° or 2°)	avg. 2° marker expression	n[b]
		P(1–2).p	P(3–6).p	P(7–8).p	P(9–11).p			
+	*osm-6*::GFP (2°)	0	0	0	100	1.0	1.0	>200[c]
mab-5(gf)	"	0	0	0	100	1.0	1.0	70
lin-12(gf)/lin-12(lf)	"	0	0	0	100	2.3	2.3	69
mab-5(gf) +/+ *lin-12(gf)*	"	64	49	5	100	3.7	3.4	61
mab-5(gf)	none	0	0	0	100	1.0	NA	70
lin-15	"	0	0	0	100	1.5	NA	68
mab-5(gf); lin-15		44	12	0	93	1.9	NA	41
lin-15	*osm-6*::GFP (2°)	0	0	0	100	1.4	1.0	41
mab-5(gf);lin-15	"	74	13	6	94	2.6	0.7	31
lin-22	*osm-6*::GFP (2°)	0	0	0	100	1.0	1.0	82
lin-12(gf)/lin-12(lf); lin-22	"	5	5	27	100	2.5	2.2	55

[a] Alleles used are: *mab-5(e1751)*, a gain-of-function allele; *lin-12(n137)* as *lin-12(gf)*, a gain-of-function allele; *lin-12(n676n909)* as *lin-12(null)*, a loss-of-function allele; *lin-15(e1763)*, a severe reduction-of-function allele; *lin-22(n372)*, a reduction-of-function allele. The *osm-6*::GFP transgene is *mnIs17*.

[b] Number of animals scored.

[c] Data from Yu et al. (2003).