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C. elegans GATA factors EGL-18 and ELT-6 function downstream of Wnt signaling to maintain the progenitor fate during larval asymmetric divisions of the seam cells

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SUMMARY

The *C. elegans* seam cells are lateral epithelial cells arrayed in a single line from anterior to posterior that divide in an asymmetric, stem cell-like manner during larval development. These asymmetric divisions are regulated by Wnt signaling; in most divisions, the posterior daughter in which the Wnt pathway is activated maintains the progenitor seam fate, while the anterior daughter in which the Wnt pathway is not activated adopts a differentiated hypodermal fate. Using mRNA tagging and microarray analysis, we identified the functionally redundant GATA factor genes *egl-18* and *elt-6* as Wnt pathway targets in the larval seam cells. EGL-18 and ELT-6 have previously been shown to be required for initial seam cell specification in the embryo. We show that in larval seam cell asymmetric divisions, EGL-18 is expressed strongly in the posterior seam-fated daughter. *egl-18* and *elt-6* are necessary for larval seam cell specification, and for hypodermal to seam cell fate transformations induced by ectopic Wnt pathway overactivation. The TCF homolog POP-1 binds a site in the *egl-18* promoter *in vitro*, and this site is necessary for robust seam cell expression *in vivo*. Finally, larval overexpression of EGL-18 is sufficient to drive expression of a seam marker in other hypodermal cells in wild-type animals, and in anterior hypodermal-fated daughters in a Wnt pathway-sensitized background. These data suggest that two GATA factors that are required for seam cell specification in the embryo independently of Wnt signaling are reused downstream of Wnt signaling to maintain the progenitor fate during stem cell-like divisions in larval development.

KEY WORDS: *C. elegans*, Wnt signaling, Asymmetric cell division

INTRODUCTION

During metazoan development, the highly conserved Wnt signaling pathway regulates the expression of target genes to direct cell fate specification, cell migration, cell proliferation and other processes (Clevers and Nusse, 2012). In *C. elegans*, there are two Wnt pathways that regulate these types of developmental events: the Wnt/BAR-1 canonical (WBC) pathway and Wnt/β-catenin asymmetry (WBA) pathway (Eisenmann, 2005; Jackson and Eisenmann, 2012; Van Hoffelen and Herman, 2008). The WBC pathway, which is similar to the well-studied canonical Wnt pathway of vertebrates, regulates gene expression in several postembryonic processes (see supplementary material Fig. S1A) (Eisenmann, 2005; Jackson and Eisenmann, 2012). The WBA pathway, which activates target gene expression by a different TCF-dependent mechanism, functions in many asymmetric cell divisions along the anterior-posterior axis during embryonic and postembryonic development (supplementary material Fig. S1B) (Jackson and Eisenmann, 2012; Mizumoto and Sawa, 2007b; Phillips and Kimble, 2009). One process controlled by Wnt signaling in *C. elegans* is the stem cell-like division of the hypodermal seam cells during larval development. The hypodermis of *C. elegans* is a single cell layer epithelium that surrounds the animal, provides structural support and secretes the protective cuticle covering (Page and Johnstone, 2007). Seam cells are specialized hypodermal cells that extend along the left and right sides of the animal in a single row from anterior to posterior. The seam cells divide in a stem cell-like manner in larval life and provide a model system with which to study the regulatory mechanisms controlling self-renewal and differentiation (Joshi et al., 2010).

In the early *C. elegans* embryo, the decision of cells to become epidermal is under control of the GATA transcription factor ELT-1; epidermal-fated cells adopt muscle or neural fates in *elt-1* mutant embryos (Page et al., 1997). These epidermal cells then adopt one of three identities: dorsal hypodermis, lateral seam cell or ventrolateral P cell (Herman, 2006). Adoption of the lateral seam cell fate requires two additional GATA factors, EGL-18 and ELT-6, which function downstream or in parallel to ELT-1 during embryogenesis (Koh and Rothman, 2001). Reduction of *egl-18* and *elt-6* activity causes seam cells to misexpress markers of the seam fate and fuse inappropriately, resulting in animals born with fewer seam cells that often arrest in early larval stages (Koh and Rothman, 2001). After embryogenesis, the newly hatched L1 larva has ten seam cells arrayed from anterior to posterior on the left and right sides (Fig. 1A, H0-H2, V1-V6 and T). During larval development, most seam cells perform one asymmetric, self-renewal division at each larval stage (L1-L4), generating a posterior daughter that retains the seam cell fate and can divide further, and an anterior daughter that terminally differentiates as a hypodermal cell, neuron or neuronal support cell (Joshi et al., 2010; Sulston and Horvitz, 1977). In addition, in the L2 stage, six of the seam cells (H1, V1-V4 and V6) undergo a symmetric proliferative division that increases the final seam cell number to 16. This proliferative division requires the function of the transcription factors *ceh-16/Engrailed* (Huang et al., 2009), *rnt-1/Runx* and *bro-1/CBFβ* (Kagoshima et al., 2005;

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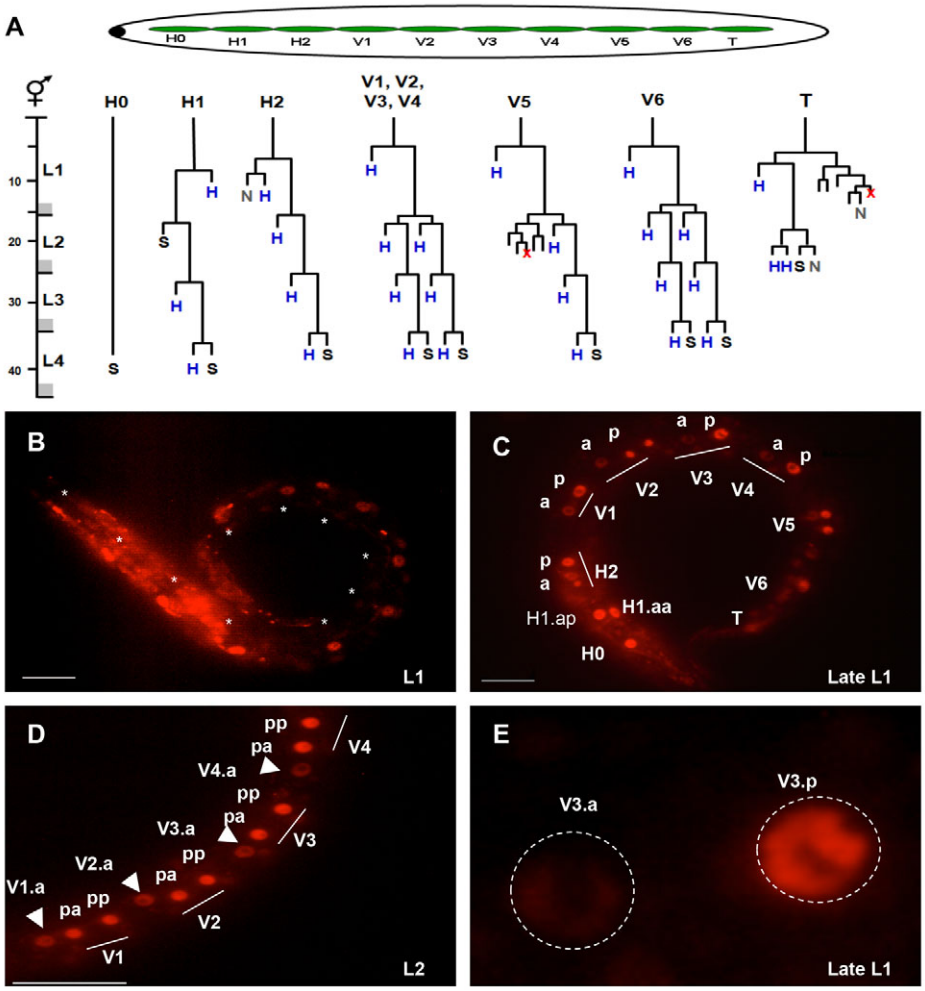

Fig. 1. Asymmetric expression of *egl-18::mCherry* between seam cell daughters corresponds to the activation of the Wnt pathway in those cells. (**A**) Ten seam cells in a newly hatched L1 hermaphrodite (top) and their lineages during the four larval stages (below). S, seam cell; H, hypodermal cell; X, cell death; N, neuron; unlabeled, neuronal support cells (V5 and T lineages). The seam cells H1, V1-V4 and V6 undergo a symmetric proliferative seam cell division at the start of the L2 stage, which generates two seam cell daughters. (B-E) Expression of *egl-18::mCherry* in the larval seam cells. (**B**) Expression in ten seam cells of newly hatched L1 (asterisks). (**C**) After the L1 seam cell division, the posterior daughters that adopt the seam cell fate show stronger expression [lines indicate the anterior (a) and posterior (p) daughters of the recently divided seam cells H2, V1-V4]. (**D**) In the early L2 stage, H1, V1-V4 and V6 undergo a symmetric division, and strong reporter expression is seen in both daughters (V1-V4 are shown). The hypodermal-fated daughters of the previous division (V1.a-V4.a) show fading reporter expression and have begun to move out of the seam line (arrowheads). (**E**) Higher magnification of V3.a and V3.p showing stronger expression in the posterior cell. The dotted circles indicate cell nuclei. Anterior is towards the left in all panels, ventral is downwards. Scale bars: 50 μm.

Kagoshima et al., 2007; Nimmo et al., 2005; Xia et al., 2007). After their final division in the L4, the 16 seam cells exit the cell cycle and differentiate, fusing to form a single cell running the length of the animal that secretes the specialized cuticular structures called alae (Joshi et al., 2010). The proper temporal regulation of seam cell behavior, including the timing of the L2 proliferative division and their terminal differentiation, is controlled by the heterochronic pathway of temporal developmental regulators (Nimmo and Slack, 2009; Resnick et al., 2010).

We have previously shown that the Wnt/β-catenin asymmetry (WBA) pathway functions in the larval self-renewal divisions of the seam cells to specify the seam cell fate in the posterior daughter of each division (Gleason and Eisenmann, 2010). We found that reductions in Wnt signaling result in symmetric divisions in which both daughters adopt the anterior hypodermal fate, while over-activation of Wnt signaling causes symmetric divisions in which both daughters adopt the posterior seam cell fate (Gleason and Eisenmann, 2010). However, it is not clear how the seam cell fate is reiteratively specified during each of the asymmetric larval divisions, and how the Wnt pathway acts to regulate this process. We have used a combination of seam cell transcript enrichment and microarray analysis on worms in which the Wnt pathway was activated or inhibited to identify Wnt pathway targets in the seam cells (L.G., W. Chen, T. Brodigan, M. Krause and D.M.E., unpublished). Here, we present evidence that the related GATA factors EGL-18 and ELT-6, which are necessary to specify the seam cell fate during embryogenesis, are reused downstream of Wnt signaling in the maintenance of seam cell fates during the larval asymmetric stem cell-like divisions.

MATERIALS AND METHODS

Strains and alleles

Bristol strain N2 of *C. elegans* was the wild-type strain. Experiments were performed at 20°C unless noted. The genes and alleles in this work are described in Wormbase (www.wormbase.org) (Harris et al., 2010; Yook et al., 2012) unless otherwise indicated.

LGIII: *wrm-1*(*ne1982*), *pha-1*(*e2123*) and *ceh-16* (*bp323*) (Huang et al., 2009)

LG IV: *egl-18*(*n162*, *ga97*, *ok290*), *elt-6*(*gk723*)

The following strains and transgenic arrays were used.

hsdelNTbar-1: *huIs7[mec-7::gfp; hsp-16.2::myc-delNT bar-1;dpy-20(+)]III; dpy-20*(*e1282*) *IV* (Gleason and Eisenmann, 2010) (a gift from H. Korswagen, Hubrecht Institute, Utrecht, The Netherlands).

dpy-7::yfp: *arIs99[dpy-7p::2Xnls::yfp; ceh-22::gfp]* (Myers and Greenwald, 2007).

scm::gfp: unc-119(e2498) III; wIs51[scmp::gfp; unc-119(+)] (Gleason and Eisenmann, 2010) strain JR667.

grd-10::gfp: *dpy-5*(*e907*)*I; sIs12963[F09D12.1p::gfp; pCeh361]* (McKay et al., 2003) strain BC13248.

egl-18::mCherry: *unc-119(ed3)III; Is[egl-18p::mCherry; unc-119(+)]* (Budovskaya et al., 2008).

hs::egl-18: *pha-1*(*e2123*)*III*; *grd-10::gfp; deEx111[pkk8;pkk9;pha-1(+)]*. *pkk8* and *pkk9* express *egl-18* from heat shock promoters (Koh et al., 2002). Strains used for mRNA tagging.

huIs1;deIs10: [*hsp-16.2::delNT bar-1; dpy-20*(+)]; [*bar-1e::flag$_{3x}$::pab-1; unc-30*(+)*; ajm-1::gfp*(+)]

deIs26; deIs10: [*hsp-16.2::delNTpop-1; dpy-20*(+)]; [*bar-1e::flag$_{3x}$::pab-1; unc-30*(+)*; ajm-1::gfp*(+)]

mRNA tagging and microarray profiling

A full description of the mRNA tagging, microarray analysis, complete target gene list and characterization of select Wnt targets will be provided elsewhere (L.G., W. Chen, T. Brodigan, M. Krause and D.M.E., unpublished). Briefly, we enriched for seam transcripts using the 'mRNA tagging' method of Roy et al. (Roy et al., 2002). We expressed FLAG-tagged polyA-binding protein (PAB-1) in the seam cells using a previously-described *bar-1* enhancer element (Natarajan et al., 2004). Synchronized L1 worms of control (N2) and experimental strains *huIs1;deIs10* (for activation of Wnt signaling) and *deIs26;deIs10* (for inhibition of Wnt signaling) were heat shocked at the L2/L3 molt as described previously (Gleason et al., 2002; Gleason and Eisenmann, 2010). mRNA enrichment by PAB-1 affinity purification was performed as described previously (Von Stetina et al., 2007). mRNA (50 ng) from three biological replicates of the three strains was linearly amplified using the NuGen WT-Ovation Pico System as per manufacturer's instructions and 3-6 μg of the sample was used for microarray analysis on Affymetrix *C. elegans* genome microarrays. Two-hundred and forty putative Wnt targets were identified as those with: (1) fold change in Wnt activated samples compared to N2 control ≥1.5; (2) ANOVA $P \leq 0.05$; and (3) ratio of fold change between Wnt-activated and Wnt-inhibited samples ≥1.5.

RNA interference and phenotypic analysis

RNA interference by L1 feeding was performed as described (Ahringer, 2006) using RNAi constructs described previously (Gleason and Eisenmann, 2010) or obtained from a commercially available library (http://www.lifesciences.sourcebioscience.com). The RNAi constructs for *egl-18* and *elt-6* do not have significant homology to each other; however, as *egl-18* and *elt-6* are transcribed both separately and as a single transcript, RNAi of either gene could affect levels of the dicistronic message (Koh and Rothman, 2001). In all experiments, seam cell or hypodermal cell numbers on one side of young adult animals (48-52 hours post feeding of L1s) were counted. 'Control RNAi' indicates use of the L4440 'feeding vector' with no insert.

Heat-shock protocol

Synchronized L1s were subjected to a single heat shock as described previously (Gleason et al., 2002; Gleason and Eisenmann, 2010). For Wnt pathway activation, synchronized *huIs7; scmp::gfp, huIs7; scmp::gfp; elt-6*(*gk723*) or *huIs7; dpy-7p::yfp* L1 larvae grown at 20°C were treated with *egl-18* RNAi, heat shocked 37°C for 30 minutes at the L2/L3 molt (23.5 hours post feeding) and shifted back to 20°C. For overexpression of EGL-18 in *apr-1* and *wrm-1* backgrounds, RNAi by feeding for the appropriate gene or control was carried out on synchronized *deEx111* L1s grown at 25°C, heat shocked 33°C for 30 minutes at the L2/L3 molt and shifted back to 25°C.

Total RNA isolation and quantitative RT-PCR

Synchronized *hs::delNTbar-1; egl-18::mCherry* and *egl-18::mCherry* L1s were grown on plates for 23.5 hours, heat shocked at 33°C for 30 minutes and recovered for 1 hour at 20°C. Worms were lysed (gentleMAC Dissociator; Miltenyi Biotec) and total RNA was isolated (RNeasy mini kit; Qiagen). mRNA was converted to cDNA (iscript cDNA kit; Bio-Rad) and used in a qRT-PCR reaction (iQ SYBR Green Supermix; Bio-Rad) with *gpd-2* as control gene. Biological triplicates were performed. Fold change was calculated using the Pfaffl equation (Pfaffl, 2001). Primer sequences used were: *gpd-2*, 5′CCTCTGGAGCCGACTATGTC3′/5′TGGCATGATCGTACTTCTCG3′; *egl-18*, 5′TCTACTATCGATTGCACAAGGTTC3′/5′GCTCGAGTAAATTGAATGTGTTG3′; *elt-6*, 5′GTTCAACGGGAAATCACCATCA3′/5′CAATCCACACGCGTTACAAACA3′.

Construction of *promoter::YFP* and generation of extrachromosomal arrays

The 2.1 kb *egl-18* upstream region that drives reporter expression in the seam cells (Budovskaya et al., 2008; Koh and Rothman, 2001) was fused to the 2XNLS::YFP coding region by SOEing PCR using pLG3 [*egl-18p(2.1kb)::YFP*] as a template. A 5′ fragment and a 3′ fragment were separately amplified with primers containing either the wild-type or mutated POP-1 site, then these fragments were amplified together to make *egl-18p(WT)::YFP* and *egl-18p(MUT)::YFP*. These reporter plasmids (25 ng/μl) were injected into *pha-1(e2123)* with *pha-1(+)* (125 ng/μl) and *ajm-1::gfp* (25 ng/μl).

Purification of POP-1 protein

The DNA-binding domain (amino acids 180-280) from *pop-1* was amplified from a cDNA plasmid (Open Biosystems) and inserted into expression plasmid pRSETA (Invitrogen) in frame with six N-terminal His residues. The resulting plasmid (pHPDBD) was transformed into *E. coli* strain BL21(DE3) and purified on Nickel resin (Qiagen) following the manufacturer's instructions.

Electrophoretic mobility shift assay

Electrophoretic mobility shift assays were performed as previously described with slight modifications (Korswagen et al., 2000; Streit et al., 2002). Binding reactions (20 μl) [10 ng polyDIDC, 0.5 μg BSA, 10% glycerol, buffer (2 mM HEPES, 100 μM DTT, 5 mM KCl, 50 μM EDTA), 40,000 cpm ^{32}P-labeled probe (~1.5 ng) and POP-1 protein] were incubated at room temperature (20 minutes), resolved on 6% non-denaturing polyacrylamide gel and analyzed using a Storm phosphorimager (Molecular Dynamics). For competitions, cold competitor was added 5 minutes before ^{32}P-labeled probes. Labeled probes were made using standard techniques with the following sequences (mutation is underlined): 5′egl-18gs, ttctccactgcattcctttgttcggactcatcacggac; 3′egl-18gs, gtccgtgatgagtccgaacaaaggaatgcagtggagaa; 5′egl-18mugs, ttctccactgcattcc<u>gga</u>gttcggactcatcacggac; 3′egl-18mugs, gtccgtgatgagtccgaac<u>tcc</u>ggaatgcagtggagaa.

RESULTS

Identification of *egl-18* and *elt-6* as putative Wnt targets in the seam cells

The Wnt pathway functions in specifying the seam cell fate during the asymmetric larval self-renewal divisions of the seam cells; however, the mechanism by which this occurs is unknown (Gleason and Eisenmann, 2010). To address this issue, we used a combination of mRNA tagging and microarray analysis to identify genes transcriptionally regulated by activation of Wnt signaling in the seam cells (see L.G., W. Chen, T. Brodigan, M. Krause and D.M.E., unpublished). Briefly, at the L2/L3 molt, the Wnt signaling pathway was either globally activated in worms by overexpressing a heat shock-inducible, stable variant of BAR-1/β-catenin (Gleason et al., 2002), or was globally inhibited in worms by overexpressing a dominant-negative variant of POP-1/TCF (Korswagen et al., 2000). We have previously shown that seam cell fate specification is sensitive to alterations in Wnt signaling at this time (Gleason and Eisenmann, 2010). Transcripts expressed in the seam cells (and VPCs) were enriched by the mRNA-tagging method (Roy et al., 2002), in which we used a previously described *bar-1* enhancer (Natarajan et al., 2004**)** to drive tagged polyA binding protein (PAB-1) expression in those cells. Seam/VPC-enriched transcript pools from experimental and control strains were analyzed using Affymetrix microarrays, and a 240 genes that were upregulated in the Wnt-activated samples compared with the Wnt-inhibited samples were identified (L.G., W. Chen, T. Brodigan, M. Krause and D.M.E., unpublished). Among these potential Wnt pathway targets, were the GATA factor genes *egl-18* and *elt-6* [average fold change of 2.1 (P=0.06) and 2.8 (P=0.01), respectively]. We confirmed the microarray result by qRT-PCR on animals overexpressing stabilized β-catenin BAR-1 at the L2/L3 molt (supplementary material Fig. S2). This result suggests that EGL-18 and ELT-6, which are used to specify the seam cells initially during embryogenesis, may function downstream of Wnt signaling to maintain a population of dividing seam cells during larval asymmetric cell divisions.

elt-6 and *egl-18* function redundantly during larval seam cell development

Previous work studying these GATA factors in embryonic seam cell specification was carried out by injecting dsRNA against *egl-18* and *elt-6* into hermaphrodites and examining the effect on their progeny. We obtained mutant alleles for both genes, *elt-6*(*gk723*) (*C. elegans* Knockout Consortium; Vancouver KO Group) and *egl-18*(*ga97*, *n162* and *ok290*) (Eisenmann and Kim, 2000; Koh et al., 2002), and studied their effect on terminal seam cell number using the *scm::gfp* reporter, which is expressed in all seam cells from their specification in the embryo until early adulthood after their final larval division (Terns et al., 1997). Wild-type young adult animals have ~16 seam cells per side (SCs/side). Although *elt-6*(*gk723*) mutant animals have 15.8 SCs/side, all three *egl-18* mutants had fewer seam cells, with *egl-18*(*ga97*) having the strongest effect (8.9 SCs/side; Table 1; supplementary material Fig. S3).

It has previously been suggested that *egl-18* and *elt*-6 function continuously in seam cell development based on defects in larval seam cells in animals that had been subjected to weak RNAi as embryos (Koh and Rothman, 2001). To distinguish unambiguously the role of these genes in larval seam cell specification from their role in the embryo, we performed RNAi treatment on synchronized newly hatched L1 larva and examined seam cell numbers in the same animals as adults (Ahringer, 2006). We found that L1 RNAi for *elt*-6 did not affect terminal seam cell number, but reduction of *egl-18* function during larval life caused a reduction to 12.7 SCs/side (Table 1), indicating a role for EGL-18 in the larval seam cells. As *egl-18* and *elt-6* are adjacent, we were unable to create a double mutant strain; however, we also used L1 RNAi to reduce function of one gene in the background of a mutation for the other. *elt-6*(*gk723*) *egl-18*(*L1 RNAi*) animals had 10.8 SCs/side, fewer than *egl-18*(*L1 RNAi*) alone (Table 1). Likewise, *egl-18*(*n162*) *elt-6*(*L1 RNAi*) animals had 5.2 SCs/side, compared with 10.6 in *egl-18*(*n162*) alone (Table 1). This result suggests that these genes, which encode GATA factors with similar DNA-binding domains (Koh and Rothman, 2001), may display functional redundancy in the larval seam cells, as seen with other processes (Koh et al., 2002; Nelson et al., 2011). We confirmed these results using two other seam cell markers: a *grd-10::GFP* transcriptional reporter that is expressed in the nucleus and cytoplasm of all seam cells from early larva to adult (McKay et al., 2003); and an *egl-18::mCherry* transcriptional reporter containing 2.0 kb upstream of the *egl-18* start codon, which is expressed in seam cells from embryo to adult (Budovskaya et al., 2008). *egl-18*(*L1 RNAi*) also reduced the number of cells expressing these seam reporters (Table 2). As RNAi treatment for *egl-18* and *elt*-6 after embryonic development is complete can reduce terminal seam cell numbers, these results indicate that these GATA factors continue to function in seam cell development during larval life beyond their initial function in seam cell generation (see also Koh and Rothman, 2001). This result is consistent with their identification as target genes acting downstream of Wnt signaling in larval seam cells.

Table 1. *egl-18* and *elt-6* function redundantly in larval seam cell development

Strain	*n*	Seam cells/side (average)	Range
WT; control(RNAi)	106	15.8	13-17
WT; egl-18(RNAi)	133	12.7*	6-16
WT; elt-6(RNAi)	113	15.4	7-18
elt-6(gk723); control(RNAi)	87	15.8	14-17
elt-6(gk723); egl-18(RNAi)	99	10.8*	2-13
egl-18(ok290); control(RNAi)	201	14.7	11-17
egl-18(ok290); elt-6(RNAi)	63	9.3*	4-16
egl-18(ok290); egl-18(RNAi)	70	0.9*	0-4
egl-18(n162); control(RNAi)	144	10.6*	2-16
egl-18(n162); elt-6(RNAi)	41	5.2*	0-11
egl-18(n162); egl-18(RNAi)	41	1.7*	0-5
egl-18(ga97); control(RNAi)	108	8.9*	1-16
egl-18(ga97); elt-6(RNAi)	51	8.9	1-15
egl-18(ga97); egl-18(RNAi)	76	4.9*	0-16

All strains contain *scm::gfp*. WT, wild type (*scm::gfp* alone). Newly hatched L1 animals were treated with RNAi against the indicated gene or control and the number of GFP$^+$ cells was scored in the same animals as young adults. The average number of GFP-expressing seam cells per side is shown, with the range and number of animals scored (*n*). *P<0.0001 (unpaired *t*-test) compared with the appropriate control animals.

Table 2. Expression of three seam cell markers is reduced in response to *egl-18* RNAi

Strain	*n*	Seam cells/side (average)	Range
scm::gfp; control(RNAi)	70	15.8	14-17
scm::gfp; egl-18(RNAi)	133	12.7*	6-16
grd-10::gfp; control(RNAi)	105	15.5	13-17
grd-10::gfp; egl-18(RNAi)	83	7.0*	2-14
egl-18::mCherry; control(RNAi)	56	15.8	15-17
egl-18::mCherry; egl-18(RNAi)	45	10.2*	3-14

scm::GFP, grd-10::gfp and *egl-18:mCherry* newly hatched L1 animals were treated with *egl-18* or control RNAi and the number of cells expressing the reporter was scored in the same animals as young adults, as in Table 1. *P<0.0001 (unpaired *t*-test) compared with the appropriate control animals.

Seam cell hyperplasia driven by Wnt pathway over-activation is dependent on *egl-18* and *elt-6*

Over-activation of the Wnt pathway results in an increase in seam cell number because anterior hypodermal-fated daughters of seam cell divisions adopt the posterior seam cell fate instead (Gleason and Eisenmann, 2010). If *egl-18* and/or *elt-6* are downstream targets of the Wnt pathway required to adopt or maintain the seam cell fate, then this increase in SC number should be dependent on *elt-6/egl-18*. We over-activated the Wnt pathway by two methods. First, we reduced function of *pop-1* by RNAi during larval life. In the Wnt/β-catenin asymmetry pathway, the level of POP-1 is lowered in the daughter cell in which the Wnt pathway is activated, so a reduction of *pop-1* levels by RNAi results in both daughters having lower POP-1 levels and adopting the seam fate (Gleason and Eisenmann, 2010). *pop-1*(*L1 RNAi*) *gfp* animals have 43.8 SCs/side (Table 3; supplementary material Fig. S4A). However, when *pop-1* L1 RNAi was carried out on *egl-18* (*ga97*) and (*n162*) mutant strains, the increase in seam cell number was strongly suppressed, with the terminal seam cell number reduced to ~3 SCs/side (Table 3; supplementary material Fig. S5A,B). We also activated the Wnt pathway using overexpression of the stable BAR-1 variant from the heat-shock promoter. When *hs::delNTbar-1; gfp* worms were given a single heat shock at the L2/L3 molt, they had 31.8 SCs/side (Table 3; supplementary material Fig. S4B). When this protocol was carried out on *egl-18*(*L1 RNAi*) animals, the terminal seam cell number was again drastically reduced (Table 3). Curiously, in both of these experiments the number of SCs/side was substantially lower when *egl-18* function was reduced in an activated Wnt background compared with wild type. Although we do not understand why activation of Wnt signaling sensitizes the animal to loss of *egl-18*, one possibility would be the upregulation of

Table 3. Increase in seam cell number upon Wnt pathway overactivation is dependent on *egl-18* but not *ceh-16*

Strain	Single heat shock L2/L3 molt	*n*	Seam cells/side (average)	Range
WT; control(RNAi)	No	143	15.8	12-22
WT; pop-1(RNAi)	No	63	43.8	20-65
egl-18(n162); pop-1(RNAi)	No	92	3.2	0-13
egl-18(ga97); pop-1(RNAi)	No	100	2.8	0-8
hs::delNTbar-1; control(RNAi)	No	51	15.7	14-17
hs::delNTbar-1; control(RNAi)	Yes	42	31.8	21-48
hs::delNTbar-1; egl-18(RNAi)	Yes	50	2.6	0-15
hs::delNTbar-1;elt-6(gk723); control(RNAi)	Yes	49	32.0	22-48
hs::delNTbar-1;elt-6(gk723); egl-18(RNAi)	Yes	66	1.7	0-14
hs::delNTbar-1; ceh-16(RNAi)	No	52	15.5	14-17
hs::delNTbar-1; ceh-16(RNAi)	Yes	40	33.6	22-63
ceh-16(bp323); control(RNAi) 25°C	No	51	12.9	11-16
ceh-16(bp323); pop-1(RNAi) 25°C	No	50	29.7	14-46

All strains contain *scm::gfp*. Newly hatched L1 larvae were treated with RNAi and the number of GFP$^+$ cells was counted in the same animals as young adults, as in Table 1. The second column indicates whether a single heat shock was given at the L2/L3 molt to induce overexpression of stabilized BAR-1 and activate Wnt signaling. All experimental results are significant at $P<0.0001$ (unpaired *t*-test) compared with appropriate control strains.

negative regulators of the pathway. In fact we have seen that *pry-1*, which encodes a negative regulator of the Wnt pathway, is a target gene activated by hyperactive Wnt signaling (B. Jackson and D.M.E., unpublished; L.G., W. Chen, T. Brodigan, M. Krause and D.M.E., unpublished).

We repeated this result using a reporter for the hypodermal cell fate, *dpy-7::yfp*, which is expressed in the anterior hypodermal daughters of the seam cell divisions (Myers and Greenwald, 2007). Wnt pathway overactivation results in a decrease in *dpy-7::yfp*-expressing cells owing to hypodermal to seam fate transformations (Gleason and Eisenmann, 2010). Activation of the Wnt pathway via *pop-1* L1 RNAi resulted in a decrease in *dpy-7::yfp*-expressing cells from 57.1 to 41.9; however, loss of *egl-18* in this background reversed this effect (Table 4; supplementary material Fig. S5C,D). A similar reversal was seen when the Wnt pathway was activated using *hs::delNTbar-1* and *egl-18* or *elt-6* L1 RNAi was performed (Table 4). This suggests that when *egl-18* function is compromised in a hyperactivated Wnt background, cells that would become seam cells remain hypodermal cells. These results indicate that *egl-18* is necessary for the increase in cells adopting the seam cell fate when the Wnt pathway is ectopically activated during larval life.

The engrailed homolog *ceh-16* functions upstream of *egl-18* in the initial specification of the seam cells in the embryo (Cassata et al., 2005). Huang et al. have previously characterized a temperature-sensitive hypomorphic allele of *ceh-16*, *bp323ts*, that causes a loss of the L2 stage proliferative division when animals are shifted to restrictive temperature in the L1, indicating *ceh-16* also functions in the larval seam cells (Huang et al., 2009). We tested whether the decrease in seam cell number that we observed upon reduction of *egl-18* function in a Wnt pathway activated background also occurs when *ceh-16* function is reduced. As reported (Huang et al., 2009), we saw that *ceh-16*(*bp323ts*) raised at restrictive temperature as larvae have slightly fewer SCs as adults (Table 3). However, when we simultaneously shifted L1 *ceh-16*(*bp323ts*) animals to the restrictive temperature and performed *pop-1* L1 RNAi to activate the Wnt pathway, we found that the animals still displayed a strong increase in terminal seam cell number, unlike with *egl-18* (Table 3). Based on these results, we hypothesized that *egl-18* and *elt-6* function downstream of the Wnt pathway to specify the seam cell fate during asymmetric larval seam cell divisions, but that this occurs independently of their upstream regulator in the embryo *ceh-16*.

egl-18::mCherry expression is asymmetric in seam cell daughters during larval life

The Wnt pathway is activated in only one daughter during larval seam cell divisions, and the known Wnt-regulated genes *tlp-1* and

Table 4. Seam to hypodermal cell fate changes occur upon loss of *egl-18* and *elt-6* in a hyperactive Wnt pathway background

Strain	Single heat shock L2/L3 molt	*n*	Hypodermal cells/side (average)	Range
WT; control(RNAi)	No	60	57.1	46-65
egl-18(ga97); control(RNAi)	No	103	60.6	51-78
egl-18(n162); control(RNAi)	No	101	64.0	51-74
egl-18(ok290); control(RNAi)	No	73	61.0	50-73
WT; pop-1(RNAi)	No	30	41.9*	35-50
egl-18(ga97); pop-1(RNAi)	No	91	60.3*	51-74
egl-18(n162); pop-1(RNAi)	No	83	68.5*	41-86
egl-18 (ok290); pop-1(RNAi)	No	74	74.2*	51-96
hs::delNTbar-1; control(RNAi)	No	63	57.5	51-66
hs::delNTbar-1; elt-6(RNAi)	No	91	59.0	53-64
hs::delNTbar-1; egl-18(RNAi)	No	92	59.5	53-68
hs::delNTbar-1; control(RNAi)	Yes	111	37.1*	20-59
hs::delNTbar-1; elt-6(RNAi)	Yes	26	45.0*	30-60
hs::delNTbar-1; egl-18(RNAi)	Yes	97	60.5*	33-89

All strains contain *dpy-7::yfp*, which is expressed in cells adopting the syncytial hypodermal fate (Myers and Greenwald, 2007). Newly hatched L1 larvae of the indicated genotype were treated with RNAi and YFP$^+$ cell numbers were counted in the same animals as young adults. The average number of YFP-expressing hypodermal cells per side is shown, with the range and number of animals scored (*n*). The second column indicates whether a single heat shock was given at the L2/L3 molt to induce overexpression of stabilized BAR-1. *$P<0.0001$ (unpaired *t*-test) compared with appropriate control animals.

Table 5. *egl-18* is strongly expressed in the posterior seam division daughters

Seam cell L1 division	*egl-18::mCherry* distribution			
	n	%*P*>A	%*P*=A	%A>P
V1	54	69	31	0
V2	54	96	4	0
V3	54	100	0	0
V4	54	100	0	0
V6	54	100	0	0

The distribution of *egl-18::mCherry* in seam cells V1-V4 and V6 is stronger in the posterior seam daughter after the L1 division. The percentage of animals with stronger expression in the posterior daughter (*P*>A), with equal expression in both daughters (*P*=A) and with stronger expression in anterior daughter (A>P) is indicated.

psa-3 are expressed asymmetrically in seam cell daughters (Arata et al., 2006; Herman and Horvitz, 1994; Zhao et al., 2002). Strong expression of EGL-18 in the embryonic seam cells has been shown previously (Koh and Rothman, 2001). To determine whether EGL-18 is expressed asymmetrically after larval seam cell divisions, we used the *egl-18::mCherry* transcriptional reporter (described above) (Budovskaya et al., 2008; Koh et al., 2002; Koh and Rothman, 2001). All ten seam cells in newly hatched L1 larvae showed strong expression (Fig. 1B). After their first division, *egl-18::mCherry* expression was asymmetric between the daughters, with stronger expression in the posterior daughters that maintain the seam cell fate (Fig. 1C,E; Table 5). Expression in the anterior daughter faded after division, before the hypodermal daughters moved out of the seam cell line (data not shown). During the L2 stage, several seam cells undergo a symmetric expansion division, generating two seam daughters and increasing the seam cell number from 10 to 16 (Fig. 1A) (Sulston and Horvitz, 1977). In the early L2, we saw strong symmetric expression of *egl-18::mCherry* in both seam-fated daughters of these divisions (Fig. 1D). When these seam-fated daughters underwent their subsequent L2 and L3 stage asymmetric divisions, reporter expression faded in their anterior, hypodermal-fated daughters as observed in the L1 stage (data not shown). In the adult, differentiated seam cells continue to show strong expression of the *egl-18::mCherry* reporter as described previously (Budovskaya et al., 2008).

If *egl-18* and/or *elt-6* are downstream targets of Wnt signaling in the larval seam cell divisions, then their expression should be altered when Wnt signaling is perturbed. Activation of Wnt signaling by *pop-1* L1 RNAi causes a substantial increase in terminal seam cell numbers owing to anterior daughters undergoing a hypodermal to seam cell fate transformation (Gleason and Eisenmann, 2010). *pop-1* L1 RNAi also caused a large increase in the number of lateral midline cells strongly expressing the *egl-18::mCherry* reporter, from 15.5 cells/side in control animals to 50.5 cells/side for *pop-1*(*L1 RNAi*) (Fig. 2; Table 6). Conversely, diminishing Wnt pathway activation by reducing function of the variant β-catenin WRM-1 leads to a decrease in terminal seam cell number owing to posterior seam division daughters undergoing a seam to hypodermal fate change (Gleason and Eisenmann, 2010). We found the number of cells expressing *egl-18::mCherry* decreased to 8.8 per side in *wrm-1*(*ts*) animals shifted to the restrictive temperature during larval life (Fig. 2; Table 6). In summary, the expression pattern of this *egl-18* transcriptional reporter during normal larval seam cell divisions, as well as its behavior in animals with perturbations in Wnt signaling, is consistent with our identification of *egl-18* as a target gene of the Wnt pathway in the larval SCs.

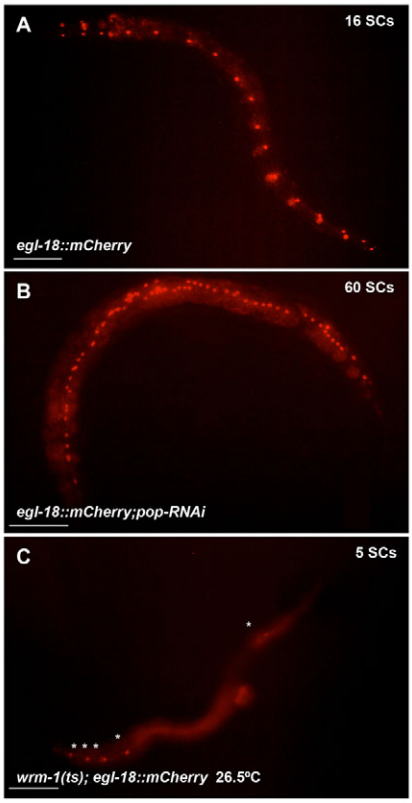

Fig. 2. *egl-18::mCherry* transcriptional reporter responds to activation or reduction of Wnt signaling. All worms express *egl-18::mCherry*. (**A**) Wild-type worms with 16 seam cells. The seam cells on the other side of the animal appear as blurred spots. (**B**) *pop-1* RNAi treatment results in increased number of seam cells strongly expressing *egl-18::mCherry*. (**C**) At the restrictive temperature, *wrm-1*(*ne1982ts*) worms show fewer seam cells that are weakly expressing *egl-18::mCherry* (asterisks). Anterior is towards the left and ventral is downwards in all panels. Scale bars: 50 μm.

POP-1 binds a site in the *egl-18* promoter *in vitro* that is necessary for robust seam cell expression *in vivo*

TCF/LEF DNA-binding proteins interact with β-catenin downstream of Wnt signaling pathways to activate target gene expression, and POP-1 is the sole TCF protein in *C. elegans* (Jackson and Eisenmann, 2012). The *egl-18::mCherry* transcriptional reporter that expresses in the seam cells contains 2.0 kb of DNA upstream of the *egl-18* start codon (Budovskaya et al., 2008; Koh et al., 2002; Koh and Rothman, 2001), and this region contains six sites matching a POP-1 binding site consensus sequence, C/T-T-T-T-G-A/T-A/T-G/C (Jackson and Eisenmann, 2012; supplementary material Fig. S6A,B). The first of these sites is within a 320 bp region strongly conserved in four other *Caenorhabditis* species (supplementary material Fig. S6C). We found that the bacterially expressed POP-1 DNA-binding domain bound to a 38 bp oligonucleotide containing this site *in vitro*, but did not bind when the invariant T residues in the consensus site were altered (CTTTGTTC to CGGAGTTC; Fig. 3A). The mutated site also did not compete effectively for binding of POP-1 to the wild-type probe (Fig. 3B). To address the relevance of this site *in vivo*, we placed the 2.0 kb *egl-18* promoter fragment upstream of YFP-coding sequences: 93% of animals carrying this reporter showed robust expression in the seam cells of young adult animals (*n*=2

Table 6. *egl-18::mCherry* transcriptional reporter responds to activation or reduction of Wnt signaling

Strain	*n*	Seam cells/side (average)	Range
egl-18::mCherry; control(RNAi)	57	15.5	15-17
egl-18::mCherry; pop-1(RNAi)	27	50.5*	33-70
egl-18::mCherry; wrm-1(ts) 15°C	35	15.8	14-17
egl-18::mCherry; wrm-1(ts) 26.5°C	30	8.8*	1-15

Newly hatched L1 larvae were treated with RNAi and mCherry-expressing cells were counted in the same animals as young adults. The average number of mCherry-expressing seam cells per side is shown, with the range and number of animals scored (*n*). *P<0.0001 compared with the appropriate control animals.

lines, range 88-97%; average of 14.5 cells expressing/animal; Fig. 3C; supplementary material Fig. S6D). However, when the POP-1 binding site mutation was introduced into this YFP reporter, only 51% of animals showed seam cell expression, fewer seam cells per animal showed YFP expression and the expression was considerably weaker than with the wild type reporter (*n*=4 lines, range 29-72%, average of 6.1 cells expressing/animal; Fig. 3D; supplementary material Fig. S6D). YFP expression in the seam cells and other cells of the embryo was not altered by the POP-1 binding site mutation (assayed 4-5 hours post egg lay; data not shown). A promoter fragment containing the remaining five POP-1 sites did not drive expression in the seam cells (supplementary material Fig. S6D). Therefore, POP-1 binds to an evolutionarily conserved site in a region of the *egl-18* promoter that directs seam cell expression, and mutation of this site reduces the penetrance and expressivity of seam cell expression driven by this fragment: these results strongly suggest that *egl-18* is a direct target of Wnt signaling in the larval seam cells.

Ectopic *egl-18* expression is sufficient to cause hypodermal cells to express a marker of the seam cell fate

Our results indicate that *egl-18* is necessary for correct adoption of larval seam cell fates. We next tested whether *egl-18* and/or *elt-6* expression is sufficient to drive cells to adopt the seam fate that would not normally do so. Ectopic expression of specific GATA factors in very early embryos is sufficient to drive additional embryonic blastomeres to adopt epidermal (ELT-1, ELT-3) or intestinal (ELT-2 and END-1) fates (Fukushige et al., 1998; Gilleard and McGhee, 2001; Zhu et al., 1998). To examine whether overexpression of EGL-18 during larval life is sufficient to increase terminal seam cell number, we overexpressed EGL-18 from the heat shock promoter at the L2/L3 molt, a time when hyperactivation of the Wnt pathway using *hs::delNTbar-1* generates fate transformations in the seam cell asymmetric divisions (Gleason and Eisenmann, 2010). Overexpression of EGL-18 alone, or in combination with ELT-6 (data not shown), did not increase terminal seam cell number (Table 7 and data not shown). However, we did observe that 40% (*n*=43) of EGL-18-overexpressing animals showed ectopic expression of the seam cell marker *grd-10::gfp* in ventral hypodermal cells (non-vulval Pn.p cells), and in head hypodermal cells (in the position of hyp 6 cells), which never express this reporter normally (supplementary material Fig. S7). This result indicates that although ectopic expression of EGL-18 in a wild-type background is not sufficient to cause anterior seam division daughters to adopt the seam fate, it is sufficient to cause other larval hypodermal cells to express a seam specific marker.

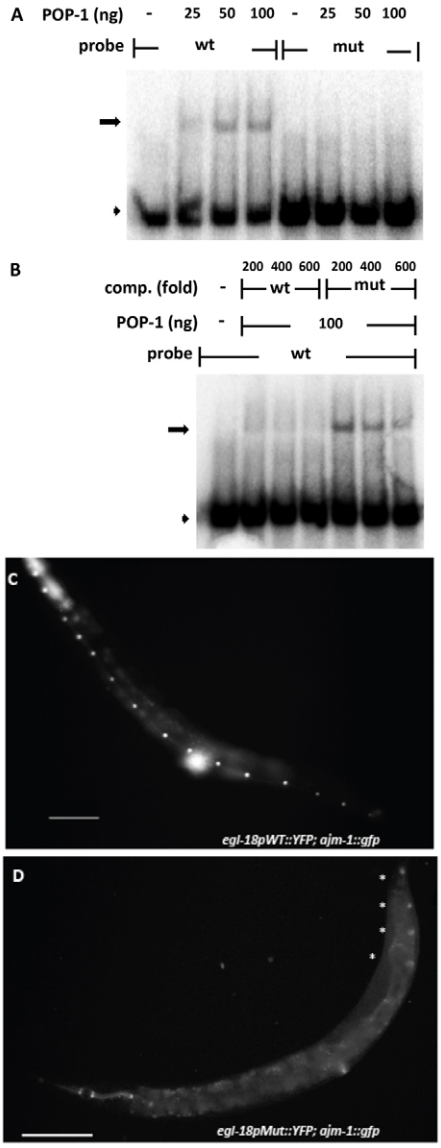

Fig. 3. A POP-1-binding site in the *egl-18* promoter is necessary for robust seam cell expression. (**A**,**B**) Electrophoretic mobility shift assays using bacterially expressed, purified POP-1 DNA-binding domain (DBD). Arrowhead indicates free probe and arrow indicates protein:DNA complex. (A) Indicated amounts of POP-1 DNA-binding domain were incubated with a probe containing either the wild-type *egl-18* promoter site (CTTTGTTC, lanes 1-4) or a mutated site (CGGAGTTC, lanes 5-8). Lanes 1 and 5 contain no protein. (B) POP-1 DNA-binding domain (100 ng) was incubated with labeled wild-type site probe in the absence of competitor (lane 1) or in the presence of increasing amounts of cold competitor oligonucleotide containing the wild-type POP-1 site (lanes 2-4) or the mutated site (lanes 5-7). Lane 1 contains no protein. (**C**,**D**) Fluorescence image of transgenic young adult animals carrying an *egl-18p::YFP* reporter containing the 2.0 kb *egl-18* promoter fragment. Anterior is leftwards and ventral is downwards. (C) Reporter contains the wild-type POP-1-binding site. Strong expression is seen in all seam cells. (D) Reporter contains a mutated POP-1-binding site. Weak expression is seen in only four seam cells in the tail (asterisks). Scale bars: 50 μm.

It is possible that the *hsp::egl-18* constructs do not provide sufficient levels of EGL-18 to phenocopy activation of the Wnt pathway in the seam daughters. It is also possible that the Wnt pathway may have additional downstream targets that are necessary

Table 7. Ectopic expression of *egl-18* can alter seam cell fates in a Wnt pathway sensitized background

Strain	Single heat shock L2/L3 molt	*n*	Seam cells/side (average)	Range
hs::egl-18; control(RNAi)	No	75	15.0	13-18
hs::egl-18; control(RNAi)	Yes	86	15.5	13-18
hs::egl-18; wrm-1(RNAi)	No	82	7.4	2-16
hs::egl-18; wrm-1(RNAi)	Yes	51	10.9*	5-16
hs::egl-18; apr-1(RNAi)	No	61	21.9	15-28
hs::egl-18; apr-1(RNAi)	Yes	40	26.1*	18-31

All strains contain *grd-10::gfp*, which is expressed in all larval seam cells (McKay et al., 2003). Newly hatched L1 animals were treated with RNAi and subjected to a single heat shock at the L2/L3 molt, and the number of GFP$^+$ cells was counted in the same animals as young adults. The average number of GFP-expressing seam cells per side is shown, with the range and number of animals scored (*n*). *P<0.0001 compared with the appropriate control animals.

along with EGL-18 to cause anterior daughter fate transformations. To address this possibility, we overexpressed EGL-18 in 'sensitized' backgrounds in which the Wnt pathway is weakly under-activated or over-activated. To do this, *hs::egl-18* L1 larvae were treated with RNAi to reduce function of either *wrm-1* [which reduces Wnt pathway activity (Gleason and Eisenmann, 2010)] or *apr-1* [which weakly increases Wnt pathway activity (Gleason and Eisenmann, 2010)], and these animals were then given a 30-minute heat shock at L2/L3 molt to overexpress EGL-18. Although *wrm-1*(*L1 RNAi*) animals had 7.4 SCs/side as adults, overexpression of EGL-18 in that background increased the terminal SC number to 10.9 per side (Table 7). Conversely, whereas *apr-1*(*L1RNAi*) animals had 21.9 SCs/side as adults, overexpression of EGL-18 in these animals enhanced the phenotype, with terminal seam cell number increasing to 26.1 SCs/side (Table 7). These results indicate that in a Wnt-sensitized background, increased expression of EGL-18 is sufficient to cause anterior daughters of seam cell divisions that would have adopted the hypodermal fate to express a marker of the seam cell fate instead.

DISCUSSION

At hatching, the *C. elegans* epithelial system consists of three major cell types: dorsal hypodermis, lateral seam cells and ventral P cells (Herman, 2006). These hypodermal cell fates are specified in the embryo by the action of GATA and other transcription factors (Chisholm and Hardin, 2005; Herman, 2006). First, the GATA factor ELT-1 is required for embryonic blastomeres to adopt hypodermal fates and not neural or muscle fates (Page et al., 1997). The dorsal hypodermal cells, which contribute to the hyp 7 syncytium, are specified by the action of ELT-1, the GATA factor ELT-3 (Gilleard and McGhee, 2001) and the zinc-finger protein LIN-26 (Labouesse et al., 1994). In addition to ELT-1, the lateral seam cell fate requires the Engrailed homolog CEH-16 acting upstream of two adjacent and redundant GATA factors, EGL-18 and ELT-6 (Koh and Rothman, 2001; Cassata et al., 2005). One function of EGL-18 and ELT-6 appears to be the repression of *elt-3,* allowing the seam cells to diverge from the dorsal hypodermal fate (Koh and Rothman, 2001).

Most seam cells divide in a stem cell-like manner in each larval stage, with one daughter adopting a differentiated (usually hypodermal) fate and the other daughter maintaining the seam cell fate and the ability to divide further; this asymmetric division allows for self-renewal of the seam cell population (Herman, 2006; Joshi et al., 2010) (Fig. 1A). The Wnt pathway was first implicated in specifying fates of descendants of the seam cells T and V5.p (Eisenmann, 2005; Mizumoto and Sawa, 2007b; Van Hoffelen and Herman, 2008) and later was shown to act in most or all seam cells during the larval stages to specify the seam cell fate in the posterior daughter of asymmetric seam cell divisions (Gleason and Eisenmann, 2010; Huang et al., 2009; Mizumoto and Sawa, 2007a; Takeshita and Sawa, 2005). The differential activation of Wnt signaling in the two daughters may be achieved in part by the asymmetric localization of some components of the Wnt/β-catenin asymmetry (WBA) pathway in the mother seam cell (Herman, 2002; Kanamori et al., 2008; Lin et al., 1998; Mizumoto and Sawa, 2007a; Sugioka et al., 2011; Sugioka and Sawa, 2010; Takeshita and Sawa, 2005). The WBA pathway also functions in establishing the polarity of the L1 seam cells (Yamamoto et al., 2011), participates in determining the axis of seam cell divisions (Wildwater et al., 2011) and may interact with the heterochronic pathway (Banerjee et al., 2010; Ren and Zhang, 2010).

We asked what factors function downstream of Wnt signaling to specify and maintain the seam/progenitor fate during the asymmetric seam cell larval divisions. To identify potential Wnt pathway targets, we used a combination of mRNA tagging (Roy et al., 2002) and microarray analysis, and identified the GATA factor genes *egl-18* and *elt-6* as upregulated in response to activated Wnt signaling (L.G., W. Chen, T. Brodigan, M. Krause and D.M.E., unpublished). Our analysis here shows that: (1) *egl-18* and *elt*-6 expression is increased in response to ectopic activation of the Wnt pathway; (2) EGL-18 and ELT-6 are necessary for adoption of the seam cell fate postembryonically in both the wild-type and Wnt pathway-activated backgrounds; (3) *egl-18* is asymmetrically expressed in larval seam division daughters with stronger expression in the posterior seam-fated cells in which the Wnt pathway is activated; (4) POP-1 binds a site in the *egl-18* promoter necessary for robust expression in the larval seam cells; and (5) overexpression of EGL-18 at the L2/L3 molt in a Wnt-sensitized background is sufficient to drive a seam cell marker in the anterior hypodermal daughters of seam cell divisions. Collectively, these results suggest a model that activation of the Wnt/β-catenin asymmetry pathway specifies the seam cell fate in the posterior daughter of seam cell larval divisions through the maintenance or upregulation of *egl-18* and *elt-6* expression (Fig. 4). This is the first report of a link between the Wnt/β-catenin asymmetry pathway and *egl-18/elt-6*, two crucial regulators of seam cell development (Koh and Rothman, 2001).

As noted above, these same GATA factor genes are required embryonically for the initial specification of the seam cell fate. Interestingly, two other GATA factor genes, *end-1* and *end-3*, function redundantly in the specification of the *C. elegans* embryonic endoderm and are directly regulated by the Wnt/β-catenin asymmetry pathway (Maduro et al., 2005; Maduro et al., 2002; Shetty et al., 2005). However, there is currently no evidence that Wnt signaling functions upstream of *egl-18* and *elt-6* in the initial specification of the seam fate in the embryo. Furthermore, the embryonic factor *ceh-16*, which acts upstream of *egl-18* in the embryo does not appear to function downstream of Wnt signaling

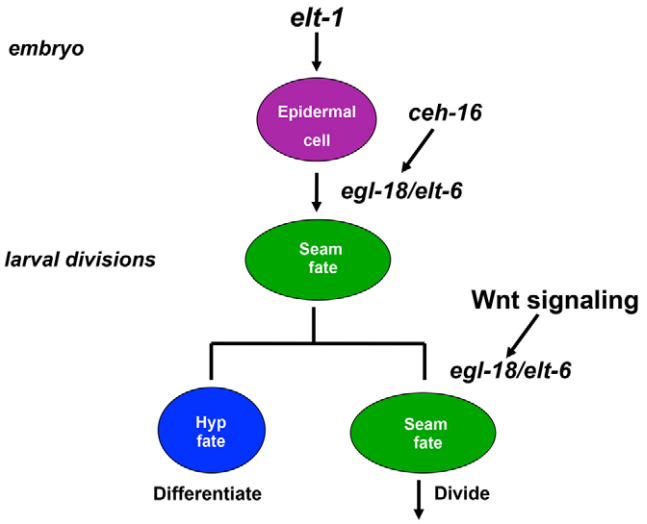

Fig. 4. Model of Wnt pathway regulation of *egl-18/elt-6* in larval seam cell fate specification. Specification of seam cells in the embryo requires the function of three GATA factor genes: *elt-1*, *egl-18* and *elt-6*. During the larval asymmetric self-renewal divisions of the seam cells, the Wnt pathway is activated in the posterior daughter to regulate expression of *egl-18/elt-6* (and other targets) and maintain the seam cell fate in that daughter cell.

in the larval seam cell asymmetric divisions. Therefore, it appears that the embryonic GATA factors have been co-opted or reused downstream of Wnt signaling to reiteratively specify the seam/progenitor cell fate during the asymmetric stem cell-like divisions of the seam cells in larval life (Fig. 4).

We show that *egl-18* and *elt-6* are necessary for proper adoption of larval seam cell fates, and EGL-18 overexpression is sufficient to induce ectopic seam fates in a Wnt-sensitized background. Interestingly, EGL-18 overexpression during the L2/L3 molt also drove seam marker expression in ventral and head hypodermal cells. We hypothesize that some factor(s) may normally function in these cells to keep e*gl-18* levels low and prevent them from adopting the seam cell fate. Consistent with this, reduction of function of the transcription factor PAX-3 results in ectopic expression of *egl-18::mCherry* and seam cell markers in the ventral P cell lineage (K.W.T. and D.M.E., unpublished), and this ectopic seam marker expression is dependent on EGL-18 (L.G. and D.M.E., unpublished). Therefore, larval overexpression of EGL-18 may overcome repression by factors such as PAX-3 in the ventral hypodermal cells and drive expression of seam cell markers in cells that would normally adopt other hypodermal cell fates.

Wnt signaling and GATA factors also regulate the choice between differentiated and progenitor fates in vertebrate systems. In *Xenopus*, GATA4 and GATA6 are involved in differentiation of cardiac progenitors and are regulated by a non-canonical Wnt pathway (Afouda and Hoppler, 2011). During erythropoiesis in mice, GATA1 is required for the differentiation of hematopoietic progenitors to erythrocytes, although Wnt signaling and GATA2 are required to maintain the stem cell niche in the bone marrow to ensure a steady supply of hematopoietic stem cells (Tsiftsoglou et al., 2009). Although the short-lived nematode *C. elegans* does not have a need for adult stem cells, the linking of Wnt signaling and GATA factors in the stem cell-like division of the larval seam cells shows this developmental module is found in invertebrates, and further study of seam cell development and differentiation in *C. elegans* may provide further insights relevant to progenitor cell biology in all systems.

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Competing interests statement
The authors declare no competing financial interests.

Supplementary material
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