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The Paired-box protein PAX-3 regulates the choice between lateral and ventral epidermal cell fates in *C. elegans*

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ABSTRACT

The development of the single cell layer skin or hypodermis of *Caenorhabditis elegans* is an excellent model for understanding cell fate specification and differentiation. Early in *C. elegans* embryogenesis, six rows of hypodermal cells adopt dorsal, lateral or ventral fates that go on to display distinct behaviors during larval life. Several transcription factors are known that function in specifying these major hypodermal cell fates, but our knowledge of the specification of these cell types is sparse, particularly in the case of the ventral hypodermal cells, which become Vulval Precursor Cells and form the vulval opening in response to extracellular signals. Previously, the gene *pvl-4* was identified in a screen for mutants with defects in vulval development. We found by whole genome sequencing that *pvl-4* is the Paired-box gene *pax-3*, which encodes the sole PAX-3 transcription factor homolog in *C. elegans*. *pax-3* mutants show embryonic and larval lethality, and body morphology abnormalities indicative of hypodermal cell defects. We report that *pax-3* is expressed in ventral P cells and their descendants during embryogenesis and early larval stages, and that in *pax-3* reduction-of-function animals the ventral P cells undergo a cell fate transformation and express several markers of the lateral seam cell fate. Furthermore, forced expression of *pax-3* in the lateral hypodermal cells causes them to lose expression of seam cell markers. We propose that *pax-3* functions in the ventral hypodermal cells to prevent these cells from adopting the lateral seam cell fate. *pax-3* represents the first gene required for specification solely of the ventral hypodermal fate in *C. elegans* providing insights into cell type diversification.

1. Introduction

A major event in metazoan development is the separation of the epidermal layer from the ectoderm and the surrounding of the developing embryo by an epidermal epithelium. As development proceeds, epidermal precursors will become specified into different cell types that perform varied functions. In the nematode *C. elegans*, the epidermis is a single cell layer thick and is referred to as a hypodermis or hypodermal layer (for review, see Chisholm and Hardin (2005), Hall and Altun (2008), Chisholm and Hsiao (2012) and Chisholm and Xu (2012)). The majority of the *C. elegans* embryonic hypodermal cells are derived from the AB blastomere and are born after 240 min of embryogenesis. Most of the embryonic hypodermal precursors are initially present as a group of dorsal cells organized into six rows which will go on to surround the developing embryo by epiboly (Fig. S1A). These six rows of embryonic cells can be divided into three main hypodermal cell types found at hatching: most cells of the inner two rows will interdigitate and fuse together to form the syncytial dorsal hypodermis hyp 7 that eventually surrounds much of the animal; many of the cells in the outer two rows will become ventral hypodermal cells called P cells, while cells of the two middle rows will become the lateral hypodermal cells or seam cells located between the dorsal and ventral cell types (other minor hypodermal cells participate in formation of the head and tail hypodermis) (Fig. S1B).

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During larval development, the lateral and ventral hypodermal cells divide to generate over 100 cells that join the syncytial hypodermis surrounding the animal (hyp 7) as well as making other cells that form specialized epidermal structures (Sulston and Horvitz, 1977; Hall and Altun, 2008). The lateral hypodermal seam cells are present on the left and right sides of the newly hatched larva as a single row of cells extending from the nose to tail. Most seam cells divide once during each of the four larval stages in an asymmetric stem cell-like division to generate a daughter that joins the syncytial hypodermis and a daughter that retains the seam cell fate and the ability to divide further (Fig. S1D (reviewed in Hall and Altun (2008) and Joshi et al. (2010))). After the fourth larval stage, all of the seam cells terminally differentiate and fuse together to form a single lateral cell that secretes a specialized structure called alae. Conversely, at hatching a subset of the ventral hypodermal cells, called P cells, are found as two rows of six cells arranged on either side of the ventral midline (Fig. S1C; reviewed in Greenwald et al. (1997) and Sternberg (2005)). During the L1 stage the anterior daughters of seam cells send cellular protrusions between the P cells that separates the P cell pairs, which then rotate 90° to make a single row of 12 P cells (P1-P12) along the anterior-posterior axis. Toward the end of the L1 larval stage, the 12 P cells divide to produce anterior daughters that are neuroblasts (Pn.a cells) and posterior daughters that are hypodermoblasts (Pn.p cells) (Fig. S1D). In hermaphrodites, six of these cells (P1.p, P2.p, P9.p-P11.p, P12.pa) fuse with the hyp 7 syncytium in the L1. The remaining cells, P3.p-P8.p, do not fuse and constitute the Vulval Precursor Cells (VPCs); three of these cells are induced by extracellular signaling to form the vulva, which connects the uterus to the outside.

Several factors involved in the specification of these early hypodermal cell fates have been identified; however in comparison to our knowledge of other early embryonic cell types such as the germ line, endoderm or mesoderm, much less is known (Fig. S2; see Chisholm and Hsiao (2012)). Expression of two genes, *elt-1* and *lin-26,* is believed to confer a general hypodermal fate on cells. *elt-1* encodes a GATA-family transcription factor and is considered a 'master regulator' of the hypodermal cell fate; *elt-1* is expressed early in all hypodermal precursors and is necessary and sufficient for proper hypodermal cell fate specification (Spieth et al., 1991; Page et al., 1997; Gilleard and McGhee, 2001). The gene *lin-26* is a downstream target of ELT-1 that encodes a zinc-finger transcription factor. *lin-26* is also expressed in all hypodermal cells but is believed to function in the maintenance of epidermal cell fates, rather than cell fate specification (Labouesse et al., 1994, 1996). Finally, the nuclear hormone receptor gene *nhr-25* also functions downstream of *elt-1* and appears to play a role in early hypodermal fate specification, as well as functioning in other aspects of hypodermal function (Gissendanner and Sluder, 2000; Chen et al., 2004; Silhankova et al., 2005).

A number of transcription factor encoding genes are known to function in the specification of the three main embryonic hypodermal cell types. Adoption of the dorsal hypodermal fate is dependent on the function of the redundant T-box genes *tbx-8* and *tbx-9;* when function of both genes is compromised, severe defects in morphogenesis of the dorsal hypodermis result (Andachi, 2004; Pocock et al., 2004). The lateral hypodermal (seam) cell fate is regulated by the *ceh-16* gene, which encodes an engrailed homolog (Cassata et al., 2005), and the adjacent genes *egl-18* and *elt-6*, which encode functionally redundant GATA factors (Koh and Rothman, 2001). Expression of *egl-18* is dependent on *ceh-16*, so is likely to be a direct or indirect target (Cassata et al., 2005). Mutation of either *ceh-16* or *egl-18* results in misspecification of the lateral hypodermal (seam) cells; the lateral cells fuse with the syncytial hypodermis, perhaps indicating adoption of a dorsal hypodermal fate (Koh and Rothman, 2001; Cassata et al., 2005). *elt-3* is another GATA factor encoding gene that acts downstream of *elt-1* and is expressed early in development; however *elt-3* is expressed in only the non-lateral hypodermal cells (dorsal and ventral) (Gilleard et al., 1999). Unlike *elt-1*, *elt-3* is not necessary to specify epidermal fates, as *elt-3* mutants show normal development of the skin, however it is sufficient to drive adoption of epidermal cell fates in the absence of *elt-1* (Gilleard and McGhee, 2001). The repression of *elt-3* in the lateral hypodermal cells requires the activity of *egl-18*, although whether this is a direct repression or a consequence of *egl-18* mutants adopting an alternative fate is unclear (Koh and Rothman, 2001). Finally, little is known about the initial specification of the ventral hypodermal cell type (P cell fate); beyond the general hypodermal factors *elt-1* and *lin-26*, there are no genes currently known that are required to specify this hypodermal fate (Fig. S2; Chisholm and Hsiao, 2012).

We report here on the further characterization of a mutation, *pvl-4(ga96),* originally identified in a screen for mutants with defects in development of the vulva, a ventral hypodermal derivative arising from P cell progeny (Eisenmann and Kim, 2000). *ga96* mutants were previously found to have too few Pn.p nuclei in the ventral midline, resulting in vulval defects. We now find that *ga96* is a missense mutation in the gene *pax-3*, which encodes the only *C. elegans* member of the PAX3/7 Paired-box transcription factor family of vertebrates (Hobert and Ruvkun, 1999). We demonstrate that *pax-3* is expressed in the P cells in the embryo, and the P cells and their progeny (Pn.a and Pn.p cells) in early larvae. Consistent with this expression, *pax-3* reduction-of-function (rof) leads to defects in the P cells, the mothers of the Pn.p cells. At hatching the ventral P cells display defects in expression of the junctional marker *ajm-1::gfp*, display an abnormal shape, and make abnormal inter-P cell contacts that are associated with defects in body morphology. Further analysis showed that in *pax-3(rof)* animals, the P cells in the L1 and their lineal descendants in later larval stages misexpress three markers of the lateral hypodermal (seam) cell fate, including the seam cell specification gene *egl-18*. Ectopic expression of *pax-3* in the seam cells themselves inhibited the expression of seam markers in those cells. Together these results suggest that the function of *pax-3* is to inhibit expression of the lateral hypodermal fate in cells that will adopt the ventral hypodermal fate. Thus, we have identified the first factor that appears to be required for adoption of the ventral hypodermal fate, and the first factor involved in distinguishing the ventral hypodermal (P cell) and lateral hypodermal (seam cell) fates from one another.

2. Materials and methods

2.1. C. elegans strains and alleles

C. elegans genetic methods were performed as previously described (Brenner, 1974). N2 Bristol was used as the wild-type strain. Experiments were performed at 20 °C unless indicated otherwise. The following alleles used in this work are described in Wormbase (Harris et al., 2010; Yook et al., 2012):

LGII *dpy-10(e128)*, *pvl-5(ga87)*, *eff-1(hy21)*, *rol-6(e187)*, *pvl-4 (ga96)*, *bli-1(e768)*, *unc-52(e444)*, *mnDf29/mnC1*

LGIII *unc-119(e2498)*, *pha-1(e2123)*

LGIV *ced-3(n717)*

LGV *him-5(e1490)*.

The following integrated transgenic arrays were used:

jcIs1 [*ajm-1::gfp; unc-29(+); rol-6(su2006d)*] from strain SU93 (Köppen et al., 2001)

wIs51 [*scm::gfp; unc-119(+)*] from strain JR667 (Koh and Rothman 2001)

wdIs4 [*unc-4p::gfp; dpy-20(+)*] from strain NC197 (Pflugrad et al., 1997)