The conserved zinc finger protein VAB-23 is an essential regulator of epidermal morphogenesis in Caenorhabditis elegans

Pellegrino, M W; Gasser, R B; Sprenger, F; Stetak, A; Hajnal, A

Abstract: Caenorhabditis elegans is an excellent model to observe cell movements and shape changes during the morphogenesis of the egg-shaped embryo into an elongated tube-like larva. Although much is known about the structural determinants involved in epidermal morphogenesis, relatively little is known about the transcriptional and post-transcriptional regulatory networks involved. Here, we describe the identification and functional characterization of the novel nuclear protein VAB-23, which belongs to a conserved protein family found in all metazoans. C. elegans VAB-23 is essential for ventral closure and elongation of the embryo. Time-lapse analysis indicates that VAB-23 is required for the formation of proper cell contacts between contralateral pairs of ventral epidermal cells. Tissue-specific rescue experiments reveal a function of VAB-23 in ventral neuroblasts that control the enclosure of the embryo by the overlaying epidermal cells. Finally, we provide evidence suggesting a role of VAB-23 in post-transcriptional gene regulation. We thus propose that VAB-23 regulates the expression of multiple secreted guidance cues in ventral neuroblasts that direct the migration of the overlaying epidermal cells. Members of the VAB-23 family may perform similar functions during morphogenesis in other metazoans.

DOI: https://doi.org/10.1016/j.ydbio.2009.09.036

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-23852

Originally published at:
DOI: https://doi.org/10.1016/j.ydbio.2009.09.036
The conserved zinc finger protein VAB-23 is an essential regulator of epidermal morphogenesis in Caenorhabditis elegans

Mark W. Pellegrino, Robin B. Gasser, Frank Sprenger, Attila Stetak, Alex Hajnal

The University of Melbourne, Department of Veterinary Science, Werribee, Victoria 3030, Australia
University of Zurich, Institute of Zoology, Winterthurerstrasse 190, CH-8057, Switzerland

ABSTRACT

Caenorhabditis elegans is an excellent model to observe cell movements and shape changes during the morphogenesis of the egg-shaped embryo into an elongated tube-like larva. Although much is known about the structural determinants involved in epidermal morphogenesis, relatively little is known about the transcriptional and post-transcriptional regulatory networks involved. Here, we describe the identification and functional characterization of the novel nuclear protein VAB-23, which belongs to a conserved protein family found in all metazoans. C. elegans VAB-23 is essential for ventral closure and elongation of the embryo. Time-lapse analysis indicates that VAB-23 is required for the formation of proper cell contacts between contralateral pairs of ventral epidermal cells. Tissue-specific rescue experiments reveal a function of VAB-23 in ventral neuroblasts that control the enclosure of the embryo by the overlying epidermal cells. Finally, we provide evidence suggesting a role of VAB-23 in post-transcriptional gene regulation. We thus propose that VAB-23 regulates the expression of multiple secreted guidance cues in ventral neuroblasts that direct the migration of the overlying epidermal cells. Members of the VAB-23 family may perform similar functions during morphogenesis in other metazoans.

© 2009 Elsevier Inc. All rights reserved.

Introduction

Morphogenesis refers to a variety of processes that create the shape of an organ or an entire organism during development. The execution of a particular cell fate involves cell–cell recognition, cell migration, attachment or fusion in order to generate proper tissue architecture and eventually a functional organ. In most cases, these developmental processes proceed normally. However, factors such as age, environmental influences and heritable genetic anomalies can lead to aberrant cell fates, morphogenesis and subsequent onset of disease.

The Caenorhabditis elegans (C. elegans) embryo is a widely used model to understand the intricacies of tissue morphogenesis and organ formation (Chisholm and Hardin, 2005; Marston and Goldstein, 2006). Morphogenesis of the embryo begins approximately 4–5 hours following the first cell division with the birth of the epidermal precursor cells on the dorsal surface of the embryo (Sulston et al., 1983). One of the first steps of morphogenesis is known as dorsal intercalation, in which two rows of ten epidermal cells wedge in between themselves, resulting in the formation of an epidermal sheet (Heid et al., 2001). Morphogenesis proceeds with ventral closure, where contralateral rows of epidermal cells migrate and extend along the sides of the embryo towards the ventral surface (Sawa et al., 2003). Upon converging on the ventral side, contralateral pairs of epidermal cells attach to each other and thereby seal the internal contents of the embryo. The process of ventral closure is dependent on the underlying neuroblasts, which act as a substrate to guide the epidermal cells towards the ventral side (Chin-Sang et al., 1999; George et al., 1998). The Ephrin tyrosine kinase receptor VAB-1 and its ligand VAB-2 are required in the neuroblasts for the sealing of the gastrulation cleft, which is necessary for the correct positioning of the ventral neuroblasts and the subsequent migration of the overlying epidermal cells towards the ventral midline (Chin-Sang et al., 1999; George et al., 1998). In addition, MAB-20, the semaphorin-2A ortholog, prevents ectopic epidermal cell contacts during embryonic and post-embryonic development (Roy et al., 2000). Lastly, the embryo undergoes elongation via actin-mediated contractile forces shortly after the attachment of the epidermal cells on the ventral side has been completed (Diogon et al., 2007). During elongation, the embryo takes on the tube-like shape of the worm.

Several structural components required for morphogenesis of the embryo have been described. For instance, the attachments between epidermal cells require the HMR-1/HMP-1/-2 cadherin–catenin complex that forms adherens junctions (Costa et al., 1998) and the APC homolog APR-1 (Hoier et al., 2000). Other proteins such as AJM-1, DLG-1 and LET-413 form a distinct, apical cell junction complex that acts redundantly with the cadherin–catenin complex (Bossinger et al., 2001; Firestein and Rongo, 2001; Koppen et al., 2001; Legouis et al., 2003). Upon converging on the ventral side, contralateral pairs of epidermal cells attach to each other and thereby seal the internal contents of the embryo. The process of ventral closure is dependent on the underlying neuroblasts, which act as a substrate to guide the epidermal cells towards the ventral side (Chin-Sang et al., 1999; George et al., 1998). The Ephrin tyrosine kinase receptor VAB-1 and its ligand VAB-2 are required in the neuroblasts for the sealing of the gastrulation cleft, which is necessary for the correct positioning of the ventral neuroblasts and the subsequent migration of the overlying epidermal cells towards the ventral midline (Chin-Sang et al., 1999; George et al., 1998). In addition, MAB-20, the semaphorin-2A ortholog, prevents ectopic epidermal cell contacts during embryonic and post-embryonic development (Roy et al., 2000). Lastly, the embryo undergoes elongation via actin-mediated contractile forces shortly after the attachment of the epidermal cells on the ventral side has been completed (Diogon et al., 2007). During elongation, the embryo takes on the tube-like shape of the worm.

Several structural components required for morphogenesis of the embryo have been described. For instance, the attachments between epidermal cells require the HMR-1/HMP-1/-2 cadherin–catenin complex that forms adherens junctions (Costa et al., 1998) and the APC homolog APR-1 (Hoier et al., 2000). Other proteins such as AJM-1, DLG-1 and LET-413 form a distinct, apical cell junction complex that acts redundantly with the cadherin–catenin complex (Bossinger et al., 2001; Firestein and Rongo, 2001; Koppen et al., 2001; Legouis et al., 2003). Upon converging on the ventral side, contralateral pairs of epidermal cells attach to each other and thereby seal the internal contents of the embryo. The process of ventral closure is dependent on the underlying neuroblasts, which act as a substrate to guide the epidermal cells towards the ventral side (Chin-Sang et al., 1999; George et al., 1998). The Ephrin tyrosine kinase receptor VAB-1 and its ligand VAB-2 are required in the neuroblasts for the sealing of the gastrulation cleft, which is necessary for the correct positioning of the ventral neuroblasts and the subsequent migration of the overlying epidermal cells towards the ventral midline (Chin-Sang et al., 1999; George et al., 1998). In addition, MAB-20, the semaphorin-2A ortholog, prevents ectopic epidermal cell contacts during embryonic and post-embryonic development (Roy et al., 2000). Lastly, the embryo undergoes elongation via actin-mediated contractile forces shortly after the attachment of the epidermal cells on the ventral side has been completed (Diogon et al., 2007). During elongation, the embryo takes on the tube-like shape of the worm.
In contrast to the relatively good knowledge of the structural determinants of embryonic morphogenesis, less is known about the transcriptional and post-transcriptional machinery involved. The C2H2 zinc finger transcription factor DIE-1 and the redundant T-box transcription factors TBX-8 and TBX-9 are necessary for dorsal intercalation and elongation (Heid et al., 2001; Pocock et al., 2004), while the homeodomain-containing proteins PAL-1 and UNC-62 are essential for proper morphogenesis of the posterior region (Edgar et al., 2001; Van Auken et al., 2002).

Here, we describe the functional characterization of the conserved nuclear zinc finger protein VAB-23. VAB-23 prevents the formation of ectopic cell contacts between adjacent, ipsilateral ventral epidermal cells and mediates contact formation between contralateral pairs of cells across the ventral midline. Tissue-specific rescue experiments indicate that VAB-23 acts in the underlying ventral neuroblasts that guide the epidermal cells to the midline. Lastly, we present evidence for a possible function of VAB-23 in post-transcriptional gene regulation through the C-terminal Zinc finger domain.

Results

The vab-23 gene is essential for embryogenesis

The vab-23 gene (annotated by the C. elegans Genome Sequencing Consortium as ZK930.3) was selected during an RNAi screen for regulators of vulval morphogenesis (M.W.P. et al., unpublished results). We chose the gene name vab because of the variable abnormal phenotype observed in vab-23 mutants as shown below. Since we observed a strong protruding vulva (Pvl) phenotype in vab-23 RNAi-treated animals (Suppl. Fig. S1), we wished to further analyze the developmental function of vab-23. For this purpose, we used the vab-23 (tm1945) deletion strain (kindly provided from the Japan Knockout Consortium), which eliminates 606 bp of the genomic open reading frame (Fig. 1A). The tm1945 deletion produces a frameshift mutation and results in an early stop codon at the beginning of the coiled-coil region of VAB-23, indicating that this deletion likely represents a strong reduction-of-function or null allele (Fig. 1A). Using RT–PCR, we were able to amplify a cDNA corresponding to a truncated vab-23(tm1945) transcript (data not shown). The vab-23(tm1945) deletion resulted in a 100% penetrant zygotic lethal phenotype that could be rescued by germ line transformation of the entire vab-23 genomic locus (Figs. 1A and 8A) (e.g., among the progeny of vab-23(tm1945)/+ heterozygous mothers, no vab-23(tm1945) homozygous larvae that had developed past the early L1 stage were found, n = 1005). In the following, we concentrate our analysis on the functions of vab-23 during embryogenesis.

The gene prediction program Genefinder indicated the existence of two vab-23 transcripts: A long 762 bp vab-23a transcript, which includes five exons, and a shorter 600 bp vab-23b transcript produced from an alternative promoter within the first intron (Fig. 1A) (www.wormbase.org; Stein et al., 2001). We used 5′ and 3′ Rapid Amplification of cDNA Ends (RACE) to confirm the predicted splicing pattern and isoform-specific primers to detect the two predicted vab-23 transcripts. Reverse transcription (RT)–PCR experiments confirmed the existence of the major vab-23a transcript and of a less abundant vab-23b transcript starting at an alternative promoter in intron 1 and covering exons 2 to 5 (Suppl. Fig. S2). However, in transgenic animals carrying a rescuing translational GFP reporter construct containing both promoters, only one isoform corresponding to the predicted size of the VAB-23A::GFP fusion protein was detected by immunoblotting (Figs. 1A and B). Moreover, a translational vab-23B::gfp transgene lacking promoter sequences of vab-23a (Fig. 1C) was unable to rescue the vab-23

![Fig. 1.](image-url)
mutant phenotypes described below (data not shown). We thus conclude that the VAB-23A protein produced by the long *vab-23a* transcript is the major isoform, whereas the protein encoded by the shorter *vab-23b* transcript was below our detection limit. Expression of a *vab-23a* minigene was sufficient to rescue the *vab-23(0)* mutant phenotypes (Figs. 1C and 8A). Thus, the long *vab-23a* isoform (referred to as vab-23 henceforth) is both necessary and sufficient to carry out the essential functions of vab-23 during embryogenesis.

VAB-23 belongs to a novel family of coiled-coil proteins conserved in metazoans

The VAB-23 protein consists of 253 amino acids with a predicted molecular weight of 29 kDa (Fig. 1D). Protein sequence analysis of VAB-23 predicts two putative coiled-coil domains from amino acids 24 to 104 and 106 to 126 and a putative C4H2 zinc finger domain at the C-terminus (Fig. 2A). A potential nuclear localization signal (NLS)
is located between amino acids 245 and 251. The gene ZK930.2, which is located immediately adjacent to \textit{vab-23}, exhibits close similarity to VAB-23 at the protein level and is likely the result of a recent gene duplication event (data not shown). While similar at the protein level, the gene products of \textit{vab-23} and ZK930.2 are unlikely to be functionally redundant since loss of VAB-23 alone already leads to a completely penetrant lethal phenotype (see below).

\textit{C. elegans} VAB-23 shows strong homology to a human hepatocellular carcinoma antigen (HCA127) of unknown function (Fig. 2A) (Wang et al., 2002). Except for \textit{C. elegans}, all metazoan genomes analyzed encode a single VAB-23 ortholog (Fig. 2B). The coiled-coil domains of \textit{C. elegans} and human VAB-23 possess approximately 25% sequence identity and 35% similarity, while amino acids 212 to 253 at the carboxy-terminus display the highest degree of conservation (80% identity). This most conserved C-terminal domain includes the putative C4H2 zinc finger domain that is characteristic for all members of this protein family. Even though VAB-23 belongs to a conserved protein family existing probably in all metazoans, no functional analysis has been reported for any of the VAB-23 orthologs in other species.

\textit{VAB-23 is necessary for ventral closure and elongation during morphogenesis of the embryo}

We next investigated the role of \textit{vab-23} during embryogenesis. Homozygous \textit{vab-23(tm1945)} mutants segregated by \textit{vab-23}(tm1945)/+ heterozygous mothers arrested with variable morphology defects either before embryonic elongation or shortly after hatching (Figs. 3C, G, and K). Epidermal morphogenesis in the wild-type embryo consists of three major events (Chisholm and Hardin, 2005; Marston and Goldstein, 2006). (1) During dorsal intercalation, the two contralateral rows of dorsal-most epidermal cells interdigitate and then fuse to form the dorsal epidermis. (2) During the subsequent ventral enclosure, the two contralateral rows of ventral epidermal cells migrate towards the ventral midline and form new cell junctions with their contralateral partner cells across the ventral midline. (3) Once the embryo is fully enclosed by the epidermal cells, actomyosin contractile forces in the middle row of epidermal cells (the seam cell precursors) drive the elongation of the oval embryo into a tube-like extended shape. Visualization of the epidermal cells in \textit{vab-23}(tm1945) mutant embryos using the AJM-1::GFP apical junctional marker demonstrated various defects during epidermal morphogenesis (Mohler and White, 1998). In around 30% \textit{vab-23}(tm1945) embryos (i.e., 5 out of 17 embryos, see below), ventral enclosure was defective, resulting in the extrusion of internal tissue (Figs. 3C and D). Embryos that were able to enclose arrested just prior to or during the elongation phase in around 50% of the cases (9 out of 17) or hatched with severe morphological deformities and arrested as L1 larvae in around 20% (3 out of 17) of the cases (Figs. 3G, H, K, and L). Interestingly, the morphological defects in \textit{vab-23}(tm1945) mutants that arrested as L1 larvae were restricted to the posterior region (Fig. 3L). We observed no changes in the number of epidermal cells in \textit{vab-23}(tm1945) embryos expressing AJM-1::GFP. In addition, although grossly malformed, \textit{vab-23}(tm1945) embryos were able to twitch, indicating proper muscle differentiation. Therefore, the lethality of \textit{vab-23}(tm1945) mutants likely results from defects in epidermal morphogenesis rather than cell fate changes.

\textit{VAB-23 is required for the formation of contacts between contralateral pairs of ventral epidermal cells}

We next employed 4D time-lapse microscopy using AJM-1::GFP as an epidermal marker to examine whether the \textit{vab-23}(tm1945) deletion affects cell adhesion \textit{per se} or whether a defect in the ventral

---

migration of the epidermal cells was the primary cause of the lethality. Dorsal intercalations appeared normal in all 17 vab-23 (tm1945) embryos observed (data not shown). Furthermore, no defects in the migration and attachment of the anterior leading cells were observed, and the initial migration of the posterior ventral epidermal cells (referred to as the ventral pocket cells) appeared superficially normal in the majority of embryos examined (Figs. 4A and B). However, ventral epidermal cells failed to form proper junctions with their contralateral partner cells across the midline in 5 out of 17 vab-23(tm1945) embryos resulting in an incomplete closure of the ventral cleft (Figs. 4B and C; Suppl. Movies 1 and 2). We consistently observed ventral epidermal cells making ectopic contacts with adjacent cells on the same side of the midline rather than making contact with their contralateral partner cells across the midline. Interestingly, the cell extensions made by the ventral epidermal cells that sought to make new contacts appeared misguided, and they often failed to cross the ventral midline (arrowheads in Fig. 4C and arrows in Suppl. Movie 2). In 9 out of 17 vab-23(tm1945) embryos, ventral closure appeared normal with only a few ectopic cell contacts made by the ventral pocket cells (Suppl. Movie 3). However, in these embryos, the contralateral pairs of epidermal cells in the mid-body region had not met at the midline by the time elongation started and the ventral cleft remained open, resulting in a developmental arrest later during elongation. Epidermal morphogenesis in the remaining three vab-23(tm1945) embryos observed by 4D microscopy appeared superficially normal, and they were able to elongate but arrested soon after hatching with posterior deformations (Fig. 3L).

We conclude that VAB-23 is required during ventral closure for the formation of proper cell contacts between contralateral pairs of ventral epidermal cells across the ventral midline.

VAB-23 function is required in neuroblasts to regulate epidermal morphogenesis

In order to examine the expression pattern of VAB-23 during embryogenesis, we used the vab-23::gfp translational reporter (Fig. 1A). This transgene rescued the morphogenesis defects observed in vab-23 (tm1945) embryos, demonstrating proper expression and functionality of the VAB-23::GFP fusion protein (Fig. 8A). VAB-23::GFP expression was observed at the onset of epidermal morphogenesis with predominant expression on the ventral posterior surface of the embryo (Figs. 5A–C). Expression continued throughout morphogenesis (Figs. 5D–F). Based on their position near the midline and the very similar expression pattern of another ventral neuroblast markers (see Fig. 6 and below), the majority of VAB-23::GFP expressing cells at the time of ventral closure are most probably ventral neuroblasts. In addition, at least two posterior ventral epidermal cells also expressed VAB-23::GFP (Figs. 5G–I, indicated with arrowheads).

Some of the previously characterized regulators of epidermal morphogenesis such as vab-1 and vab-2 have been shown to be required in the underlying neuroblasts that form the substrate on which the epidermal cells migrate (Chin-Sang et al., 1999; George et al., 1998; Ghenea et al., 2005; Harrington et al., 2002). Since VAB-23 was predominantly expressed in the ventral neuroblasts during ventral closure, we determined the tissue(s) in which vab-23 acts during embryogenesis. Various promoters were used to drive the expression of the vab-23 cDNA in the epidermis (P unc-119::vab-23::gfp, P unc-2::vab-23::gfp), in neuroblasts (P unc-119::vab-23::gfp, P unc-33::vab-23::gfp, P par-31::vab-23::gfp, P ogrf-1::vab-23::gfp) or in muscle cells (P myo-3::vab-23::gfp). These transgenes were assayed for their ability to rescue the lethality of vab-23(tm1945) embryos (Table 1). Expression of vab-23 cDNA under the control of the unc-119 promoter that drives expression predominantly in neuroblasts and also some epidermal cells in the embryo (Maduro and Pilgrim, 1995; Hardin et al., 2008) or using the neuron-specific unc-33 promoter (Altun-Gultekin et al., 2001) rescued the vab-23(tm1945) lethality to produce viable and fertile adults. On the other hand, expression of VAB-23::GFP in the epidermis or in muscle cells failed to rescue (Table 1). Interestingly, expression of vab-23 under the rgef-1 promoter also failed to rescue the vab-23 (tm1945) lethality. Although rgef-1 expression is pan-neuronal, its expression does not precede embryonic epidermal morphogenesis but rather begins at the late comma stage of embryogenesis (Altun-Gultekin et al., 2001). Thus, VAB-23 acts in the ventral neuroblasts to guide the migration of the overlying epidermal cells prior to or at the time of epidermal ventral closure.
The ventral neuroblasts move after gastrulation towards the ventral midline to seal the gastrulation cleft and form a substrate for the migrating epidermal cells during the subsequent ventral epidermal closure (Chisholm and Hardin, 2005). Mispositioning of the ventral neuroblasts, as observed, for example, in kal-1 mutants prevents proper contact formation between the contralateral epidermal cells during the subsequent epidermal morphogenesis and results in a phenotype similar to the one observed in vab-23 (tm1945) mutants (Hudson et al., 2006). We therefore examined the positioning of the ventral neuroblasts using the pan-neuronal unc-119p::gfp reporter (Maduro and Pilgrim, 1995) or the kal-1::gfp reporter that is expressed predominantly in ventral neuroblasts at the time of ventral closure (Rugarli et al. 2002). We observed no obvious differences in the number or positioning of the unc-119p::gfp or kal-1::gfp positive ventral neuroblasts at the onset of epidermal enclosure in vab-23 (tm1945) embryos, although we cannot exclude the possibility of a slightly delayed enclosure of the gastrulation cleft in vab-23 (tm1945) mutants (Fig. 6 and data not shown).

VAB-23 localizes to nuclear speckles and requires the zinc finger domain for its function

The VAB-23::GFP fusion protein was localized almost exclusively to the cell nuclei. Interestingly, the subnuclear localization of VAB-23::GFP was not uniform but was rather enriched in discrete, rapidly moving punctate (Fig. 7A), which is characteristic of proteins involved in pre-mRNA processing or mRNA regulation (Lamond and Spector, 2003). One hallmark of proteins involved in mRNA biosynthesis is their localization to so-called nuclear speckles that are found in interchromatin regions. To further examine the subnuclear localization of VAB-23::GFP, embryos were co-stained with the DNA-binding dye DAPI. This experiment revealed that VAB-23::GFP-containing
Table 1

<table>
<thead>
<tr>
<th>Promoter used for vab-23 cDNA expression</th>
<th>Embryonic expression pattern</th>
<th>No. rescued lines/total lines analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>vab-23</td>
<td>Ventral neuroblasts, epidermis</td>
<td>3/3</td>
</tr>
<tr>
<td>ajm-1</td>
<td>Epidermis</td>
<td>0/5</td>
</tr>
<tr>
<td>dpy-7</td>
<td>Epidermis</td>
<td>0/12</td>
</tr>
<tr>
<td>unc-119</td>
<td>Early–late neuroblasts, epidermis</td>
<td>6/6</td>
</tr>
<tr>
<td>unc-33</td>
<td>Early–late neuroblasts</td>
<td>8/9</td>
</tr>
<tr>
<td>rgef-1</td>
<td>Late neuroblasts</td>
<td>0/4</td>
</tr>
<tr>
<td>myo-3</td>
<td>Muscle</td>
<td>0/4</td>
</tr>
</tbody>
</table>

The indicated promoters were used to express vab-23 cDNA with a C-terminal gfp tag and assayed for their ability to rescue the vab-23(tm1945) lethality (see Materials and Methods). The numbers of independent transgenic lines that showed rescue per total lines analyzed are indicated. *See Hardin et al. (2008).*

We next tested the requirement of the conserved zinc finger domain at the C-terminus of VAB-23. This domain is highly conserved among most, if not all, metazoans. Interestingly, a H223Y substitution within the predicted C4H2 zinc finger domain is present only in C. elegans VAB-23. We therefore assayed the ability of a truncated version of VAB-23::GFP fusion protein that lacks 43 amino acids of the conserved C-terminus (referred to as VAB-23ΔC; Fig. 1D) to rescue the vab-23(tm1945) lethality. While the full-length VAB-23::GFP protein efficiently rescued the lethality of vab-23(tm1945) mutants, VAB-23ΔC::GFP was unable to restore VAB-23 activity (Fig. 8A). The inability of VAB-23ΔC::GFP to rescue could be due to a loss of nuclear localization, since a predicted NLS sequence resides in the conserved C-terminal region that was deleted. However, VAB-23ΔC::GFP was still localized to the nucleus similar to full-length VAB-23::GFP (Fig. 8B). Therefore, the highly conserved C-terminal zinc finger domain of VAB-23 is necessary for VAB-23 function, irrespective of its nuclear localization.

Discussion

We have identified the conserved VAB-23 protein as an essential regulator of epidermal morphogenesis in the C. elegans embryo. VAB-23 is necessary for the formation of proper cell contacts between contralateral pairs of epidermal cells across the ventral midline during ventral enclosure of the embryo. The observation of multiple ectopic cell contacts between ventral epidermal cells in vab-23 mutants suggests that VAB-23 functions mainly by preventing contact formation between adjacent, ipsilateral epidermal cells. Since vab-23 acts in the underlying neuroblasts and it encodes a nuclear protein, VAB-23 likely regulates the expression of specific target genes that act as secreted cues regulating different steps of morphogenesis. The localization to nuclear speckles and essential role of the zinc finger domain suggest that VAB-23 may function in the post-transcriptional regulation of target genes controlling epidermal morphogenesis, though we cannot exclude a role in transcriptional regulation. The direct downstream targets of VAB-23 are currently unknown, even though several secreted effectors of epidermal morphogenesis have been identified in C. elegans. For example, mutations in mab-20, which encodes a semaphorin-2A ortholog, cause similar ectopic cell contacts between epidermal cells, resulting in embryos with defective ventral enclosure and hatched larvae with body deformations (Roy et al., 2000). The two other semaphorin genes, smp-1 and smp-2, are necessary for proper epidermal cell positioning and adhesion during larval development, but they are not expressed in ventral neuroblasts during morphogenesis of the embryo (Ginzburg et al., 2002). Furthermore, the C. elegans Kallman syndrome homolog kal-1 acts in the underlying neuroblasts to regulate the formation of epidermal cell contacts during embryogenesis (Hudson et al., 2006). It is therefore tempting to speculate that VAB-23 might regulate mab-20 or kal-1 expression in ventral neuroblasts to prevent adhesion between adjacent ipsilateral cells. Even though we did not observe a regulation of a transcriptional kal-1 reporter by VAB-23, we cannot exclude the possibility that VAB-23 might regulate kal-1 at a post-transcriptional level. Nonetheless, since null mutations in mab-20 and kal-1 only result in partial embryonic lethality and cause milder phenotypes than vab-23 mutations, it is unlikely that misregulation of a single VAB-23 target is responsible for the completely penetrant lethality and strong morphogenesis defects seen in vab-23 mutants. Our results rather indicate that vab-23 controls the expression of multiple effector genes required for proper morphogenesis of the embryo.

The vab-1 and vab-2 genes, which encode an ephrin receptor tyrosine kinase and an ephrin ligand, respectively, also function in the ventral neuroblasts to regulate ventral enclosure of the embryo (Chin-Sang et al., 1999; George et al., 1998). It is unlikely that VAB-23 acts in an ephrin signaling pathway for the following two reasons: First, mutations in vab-1 or vab-2 rarely cause the formation of ectopic epidermal cell contacts as described here for vab-23 and reported...
previously for mab-20 mutants (Roy et al., 2000). Rather, VAB-1 and VAB-2 are required for the closure of the gastrulation cleft by the ventral neuroblasts. Second, vab-1 and vab-2 mutants that escape the embryonic lethality and arrest as L1 larvae display predominantly anterior morphology defects (i.e., a notched head phenotype) but do not exhibit strong posterior body defects as observed in arrested vab-23 L1 larvae. It thus appears that the ephrin pathway and vab-23 constitute two different systems regulating distinct aspects of epidermal morphogenesis in the anterior and posterior regions of the embryo, respectively.

The characteristic localization of VAB-23::GFP to dynamic nuclear speckles is reminiscent of two other C. elegans proteins that also localize to nuclear speckles and are important for neuronal development, the RRM-containing RNA binding protein UNC-75 and the C2H2 zinc finger protein SYD-9 (Loria et al., 2003; Wang et al., 2006). It is possible that VAB-23 functions similar to UNC-75 or SYD-9 as a post-transcriptional regulator that binds to specific mRNA targets with its conserved C-terminal zinc finger domain that is essential for VAB-23 function but not for nuclear localization. It will therefore be of interest to investigate whether VAB-23 does indeed act as a post-transcriptional regulator of morphogenesis and to identify mRNAs that might be bound by VAB-23.

Given the strong similarity between the VAB-23 orthologs in other species, it seems likely that this protein family plays an essential and conserved role during metazoan development. Consistent with this notion, a transposon insertion in the zebrafish vab-23 ortholog has been identified in a genetic screen for genes essential for early development (Amsterdam et al., 2004). Our study provides the first insight into the function of this conserved and essential protein family and a framework for future investigation.

Materials and methods

General methods and strains

C. elegans strains were maintained at 20 °C on standard nematode growth media as described previously (Brenner, 1974). The wild-type strain of C. elegans used was Bristol N2. Other strains used are as follows: LGII: vab-23(tm1945)/mIn1[mIs14 dpy-10(e128)] (this study), Extrachromosomal arrays (this study) and integrated arrays: jcIs1[ajm-1::gfp], otIs33[kal-1::gfp], zhEx229[pgib-1::gfp, plin-48::gfp], zhEx271[punc-119::vab-23::gfp, plin-48::gfp], zhEx275[pgib-1::gfp, pRF4(rol-6d)], zhEx280[pmyo-3::vab-23::gfp, plin-48::gfp, zhEx295[14kbvab-23, pTGred(sur-5::DsRed)], zhEx297[pajm-1.vab-23::gfp, plin-48::gfp], zhEx298[prgef-1.vab-23::gfp, plin-48::gfp], zhEx299[pvab-23::vab-23::gfp, plin-48::gfp, zhEx295[14kbvab-23, pTGred(sur-5::DsRed)], zhEx297[pajm-1.vab-23::gfp, plin-48::gfp, zhEx299[prgef-1.vab-23::gfp, plin-48::gfp], zhEx313[pvab-23B::vab-23::gfp, plin-48::gfp], zhEx321[punc-33.vab-23::gfp, plin-48::gfp], zhEx322[pdpy-7.vab-23::gfp, plin-48::gfp], zhEx329[pvab-23::vab-23 ΔC::gfp, sur-5::dsRed].

All constructs were microinjected into the gonad arms of adult worms at varying concentrations to generate stable transgenic lines (Mello et al., 1991). Injected solutions contained the desired plasmid to be tested along with a coinjection marker. Concentrations of each test construct was between 10 and 30 ng/μl along with the coinjection markers plin-48::gfp or sur-5::dsRed at 50–75 ng/μl and pBluescript added to a final concentration of 150–200 ng/μl.

Please cite this article as: Pellegrino, M.W., et al., The conserved zinc finger protein VAB-23 is an essential regulator of epidermal morphogenesis in Caenorhabditis elegans, Dev. Biol. (2009), doi:10.1016/j.ydbio.2009.09.036
Molecular analysis of the vab-23 locus

5′ and 3′ rapid amplification of cDNA ends (RACE) were performed using the BD SMART™ RACE cDNA Amplification Kit (BD Biosciences Clontech) according to the manufacturer’s instructions. Primers isb-1, RACE.R2 and isb-1.RACE.F were used for 5′ and 3′ RACE, respectively (primer sequences available upon request). RNA isolation from mixed-stage wild-type worms was performed using standard methods, and cDNA was synthesized using the Superscript™ III kit according to the manufacturer’s instructions (Invitrogen). Standard reverse transcription–polymerase chain reaction (RT–PCR) was also used to confirm the predicted splicing patterns of vab-23, using wild-type N2 cDNA as a template and gene-specific primers to detect the predicted transcripts of vab-23. Primers OMP25 and OMP26 were used to amplify the predicted vab-23a cDNA and bind to predicted exons 1 and 5, respectively. Primers OMP118 and OMP119 were used to amplify the predicted vab-23b cDNA and bind to predicted intron 1 (containing the start of the predicted coding sequence of vab-23b) and intron 4, respectively. RACE and RT–PCR products were sequenced to confirm their identity. Primers OMP93 and OMP94 were used to amplify the full vab-23 locus. The fosmid WRM-06-38-H17 (Geneservice) was prepared by lysing 100 L4s in Laemmli buffer and boiling for 5 minutes. Worm lysates for Western Blot analysis were prepared by lysing 100 L4s in Laemmli buffer and boiling for 5 minutes. Immunoblot analysis was performed using 1:1000 affinity-purified anti-GFP antibody (Roche) and standard methods.

VAB-23 translational GFP reporter constructions

A 3.4 kb genomic fragment covering the region upstream of the vab-23 ATG was amplified by PCR and cloned into the SalI and BamHI sites of pPD95.75 yielding pZK930.3::GFP (details available upon request). The complete genomic open reading frame (ORF) of vab-23 was then amplified and cloned into the BamHI site of pZK930.3::GFP to produce the VAB-23 translational GFP reporter plasmid VAB-23::GFP. To create the vab-23 minigene GFP plasmid P_vab-23::VAB-23::GFP, the full-length cDNA of vab-23 was PCR amplified and cloned into the BamHI and SmaI sites of pZK930.3::GFP (details available upon request). For P_vab-23::VAB-23::GFP, a truncated vab-23 cDNA (i.e., deletion of the C-terminal 43 aa) was cloned into the BamHI and SmaI sites of pZK930.3::GFP. P_vab-23::VAB-23::GFP was obtained by amplifying the complete genomic ORF of vab-23 fused to GFP and unc-54 UTR from VAB-23::GFP.

Immunostaining and microscopy

Immunostaining of C. elegans embryos was performed as described previously (Miller and Shakes, 1995). Embryos laid from gravid hermaphrodite adults were mounted on poly-l-lysine coated slides in ~6–10 μl of M9 buffer (22 mM KH2PO4, 22 mM Na2HPO4, 85 mM NaCl, 1 mM MgSO4). After covering the dissected worms with a glass coverslip, slides were frozen on dry ice for 5–7 minutes. Eggs were permeabilized using the freeze-crack method (Miller and Shakes, 1995) and immediately fixed in absolute methanol at −20 °C. Air-dried slides were blocked using 3% bovine serum albumin (BSA) (in PBS: 0.2 M phosphate, 1.5 M NaCl, pH 7.4) for 30 minutes and specimens were then incubated with primary antibody (1:25 MH27) for 2 h at room temperature. Slides were washed five times with PBS-T (0.1% Tween 20 in 1x PBS) and incubated with secondary antibody (1:100 anti-mouse TRITC) for 1 h at room temperature. Specimens were then stained with 1:1000 4,6-diamidino-2-phenylindole (DAPI) and washed five times with PBS-T before mounting with Mowiol solution.

Fluorescent images were obtained using a Leica DMRA wide-field microscope, equipped with a cooled CCD camera (Hamamatsu ORCA-ER). Images were analyzed using Openlab 3.0 software package (Improvision). Four-dimensional (4D) GFP microscopy of living embryos was analyzed using an Olympus FV1000 confocal microscope. Images were captured every 5 minutes using stacks of 1 μm. Images were processed using the Imairis program.

Tissue-specific rescue experiments

To express vab-23 in the epidermis, the regulatory sequence from omd-1 (Koppen et al., 2001) was amplified and fused to a PCR fragment containing the vab-23 3′UTR, GFP and unc-54 UTR from P_vab-23::VAB-23::GFP using the fusion PCR technique (Hubert, 2002). For P_omp-1::vab-23::GFP, the vab-23 cDNA was first amplified and cloned into the BamHI and SmaI sites of pPD95.75 creating pPD95.75.ZK930.3.cDNA. 424 bp of dpy-7 promoter (Gillardeau et al., 1997) was then amplified and cloned into the SalI and BamHI sites of pPD95.75.ZK930.3.cDNA. To construct P_omp-1::VAB-23::GFP, approximately 3 kb of unc-119 promoter were amplified and cloned into the SalI and BamHI sites of pPD95.75.ZK930.3.cDNA yielding P_omp-1::VAB-23::GFP. For P_omp-1::vab-23::GFP, 2.9 kb of promoter sequence corresponding to unc-33 was amplified and cloned into the SalI and BamHI sites of pPD95.75.ZK930.3.cDNA. A fusion PCR approach was used to construct P_gpef-1::vab-23::GFP, using 2.1 kb of promoter sequence from rgef-1. To construct P_mpv-3::VAB-23::GFP the vab-23 cDNA was amplified and cloned into the KpnI and BamHI sites of pPD136.64. All transgenic strains were examined for expression of GFP using standard epifluorescent microscopy to confirm the expression of the transgenes. To quantify the rescuing capability of the vab-23 transgenes, vab-23(tm1945)/mIn1 transgenic parents were allowed to lay eggs for a period of 4 days. The rescuing capability is expressed as a percent ratio of the number of transgenic vab-23(tm1945) homozygous animals to the total number of transgenic progeny. Note that full rescue corresponds to a 25% ratio of vab-23(tm1945) homozygotes. All rescued transgenic lines shown in Table 1 and Fig. 8A segregated between 8% and 20% vab-23(tm1945) homozygotes, while nonrescued lines never segregated any vab-23(tm1945) homozygotes.

Acknowledgments

We wish to thank members of our group for critical discussion and comments relating to this manuscript. We are also grateful to the C. elegans genetics centre and S. Mitani (Japan Knockout Consortium) for providing strains and to Andrew Fire for GFP vectors. M.W.P. was the recipient of Postgraduate scholarships from the University of Melbourne, the Dr. Sue Newton Research Award, and a travel grant from the Australian National Health and Medical Research Council (NHMRC) Parasitology Research Network. This research was supported by grants from the Australian Research Council, Genetic Technologies Limited and Meat and Livestock Australia to R.B.G. and the Swiss National Science Foundation to A.H.

Appendix A. Supplementary data


References


