Sensory Organ Remodeling in Caenorhabditis elegans Requires the Zinc-Finger Protein ZTF-16

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ABSTRACT

Neurons and glia display remarkable morphological plasticity, and remodeling of glia may facilitate neuronal shape changes. The molecular basis and control of glial shape changes is not well understood. In response to environmental stress, the nematode Caenorhabditis elegans enters an alternative developmental state, called dauer, in which glia and neurons of the amphid sensory organ remodel. Here, we describe a genetic screen aimed at identifying genes required for amphid glia remodeling. We previously demonstrated that remodeling requires the Otx-type transcription factor TTX-1 and its direct target, the receptor tyrosine kinase gene ver-1. We now find that the hunchback/Ikaros-like C2H2 zinc-finger factor ztf-16 is also required. We show that ztf-16 mutants exhibit pronounced remodeling defects, which are explained, at least in part, by defects in the expression of ver-1. Expression and cell-specific rescue studies suggest that ztf-16, like ttx-1, functions within glia; however, promoter deletion studies show that ztf-16 acts through a site on the ver-1 promoter that is independent of ttx-1. Our studies identify an important component of glia remodeling and suggest that transcriptional changes may underlie glial morphological plasticity in the sensory organs of C. elegans.

The shapes of neuronal receptive structures such as dendritic spines and sensory receptive endings are plastic and can be remodeled by developmental, hormonal, and environmental signals. For example, the dendritic spines of Purkinje cells rapidly grow and retract during development of the mouse cerebellum (Dunaevsky et al. 1999), and estrogen levels affect the number and density of dendritic spines in the hippocampus during the estrous cycle in rats (Woolley et al. 1990). In the mouse barrel cortex, changes in somatosensory environmental input increase the turnover of pyramidal neuron dendritic spines (Trachtenberg et al. 2002). Likewise, reduced sensory input affects the shape of the AWB neuron sensory ending in the nematode Caenorhabditis elegans (Mukhopadhyay et al. 2008).

Glia, which are intimately associated with neurons, also exhibit complex shapes and morphological plasticity, and changes in glial shape often correlate with neuronal remodeling (Procko and Shaham 2010). For example, retraction of astrocyte processes in the hypothalamus of lactating rats correlates with synaptic changes in associated supraoptic nucleus neurons (Theodosiou and Poulain 1993); and perturbation of the glial ephrin-A3 cell-surface protein affects the shape of dendritic spines in the mouse hippocampus (Carmona et al. 2009). These observations, coupled with the close proximity of glia to neurons and the ability of glia to regulate and perceive their extracellular environment (Meyer-Franke et al. 1995; Porter and McCarthy 1996), suggest that glia are well positioned to facilitate or, more speculatively, to direct changes in neuronal receptive ending shapes. Although glial shape changes in vertebrate systems have been documented (Theodosiou and Poulain 1993; Lippman et al. 2008), the molecular basis for these changes is not well understood.

The nematode C. elegans has a relatively small number of glia and neurons with stereotyped shapes (Ward et al. 1975; White et al. 1986), and, unlike vertebrate glia, C. elegans glia can be ablated without affecting neuronal survival (Bacaj et al. 2008; Yoshimura et al. 2008). In response to environmental stressors, including starvation, crowding, and high temperature, C. elegans enters a developmentally arrested stress-resistant state termed “dauer” (Cassada and Russell 1975; Golden and Riddle 1984), in which the anterior bilateral amphid sensory organs are remodeled (Albert and Riddle 1983). Each amphid consists of sensory neurons that extend dendritic processes to the nose tip and there terminate in specialized sensory endings. Associated with these neurons are single amphid sheath...
(AMsh) glial cells. Each AMsh glia also extends a process toward the nose tip, and there ensheaths sensory neuron ciliated receptive endings (Figure 1A) (Ward et al. 1975). In dauer animals, the two AMsh glia expand at the nose tip and use the protein AFF-1 to fuse (Albert and Riddle 1983; Procko et al. 2011). Concomitantly, the ciliated sensory endings of the AWC amphid neurons expand within the new compartment defined by the glia, such that the left and right AWC cilia now extensively overlap (Figure 1, B and C). Importantly, AMsh glia remodel even in the absence of the AWC neurons, and blocking glial remodeling perturbs AWC neuron shape, suggesting that glial remodeling facilitates neuronal shape changes (Procko et al. 2011).

We previously found that glial remodeling depends on the Otx-type transcription factor txr-1 and its direct target gene, the receptor tyrosine kinase ver-1, both acting within AMsh glia (Procko et al. 2011). Expression of ver-1 is induced in AMsh glia following dauer entry and in non-dauer animals of all stages upon cultivation at high temperature (25°C), a dauer stimulus. Here, we report results of a genetic screen aimed at identifying mutants defective in ver-1 expression and identify the gene ztf-16 (zinc-finger putative transcription factor family) as a major regulator of ver-1 expression and AMsh glia remodeling. Our results suggest that transcription factors play important roles in AMsh glia morphological plasticity.

Materials and Methods

Strains

Animals were cultivated at 20°C using standard methods (Brenner 1974), unless otherwise noted. The wild-type strain used was Bristol (N2). Mutant alleles used were the following: LGIII—daf-7(e1372) and lit-1(ns132, t1512); and LGV—txr-1 (p767, oy26). Mutant alleles isolated in this study were the following: LGV—tam-1(ns167, ns170, ns174, ns234, ns237, ns238, ns241, ns249, ns258, ns268) and txr-1(ns235, ns252, ns255, ns259, ns260, ns267); and LGX—ztf-16(ns169, ns171, ns178). Alleles not mapped to a chromosome include ns231 and ns257. ver-1 promoter::gfp was nsIs22. Other integrated transgenes and extrachromosomal arrays are as indicated elsewhere in the text.

Plasmid construction and isolation of cDNAs

The initial ver-1 promoter::gfp construct (~2 kb upstream promoter through +263 of the ver-1 gene fused to gfp) was a gift of R. Roubin and C. Popovici. Vector backbones are derived from the pPD vectors (gift of A. Fire). cDNA template was prepared from mixed-stage animals. The glia::ztf-16a and glia::ztf-16b constructs were made by replacing the heat-shock promoter of vector pPD49.78 with a 2-kb promoter from the F16F9.3 gene (Bacaj et al. 2008) at PstI/BamHI and then inserting either ztf-16a or ztf-16b cDNA at Xmal/NcoI. The glia::ztf-16 construct that was integrated to generate strain nsIs245 was made by inserting 2.5 kb of the T02B11.3 promoter (Bacaj et al. 2008) into pPD49.78 at PstI/BamHI and ztf-16b cDNA at Xmal/SacI. Embryonic glia::ztf-16b was made by inserting a lin-26 glia enhancer region and a myo-2 minimal promoter (Landmann et al. 2004) into pPD49.78 at Sphl/Xmal, followed by ztf-16b at Xmal/NcoI. The F16F9.3 promoter was inserted into the PstI site of pPD95.75 followed by ztf-16b cDNA in frame with gfp at Xmal/KpnI to generate glia promoter::ztf-16b::gfp. The ztf-16 promoter::gfp construct that gave gfp expression in the amphid and phasmid glia included the region ~4637 to ~2536 of the ztf-16 promoter relative to the +1 translation start site inserted into pPD95.75 at Sphl/KpnI. To generate the F58F9.10 promoter::gfp construct, 2 kb of upstream promoter was inserted into pPD95.75 at Sphl/BamHI. To make the F58F9.6 promoter::gfp, a 4-kb BamHI/Xmal promoter fragment was inserted at BamHI/AgeI.

Germline transformation and transgene integration

Germline transformations were carried out using standard protocols (Mello and Fire 1995). Co-injection markers used were either plasmid pRF4 (Mello et al. 1991) or unc-122 promoter::dsRed. pSL1180 (GE Healthcare) is an empty cloning vector used to increase the DNA concentration of injection mixtures. The glia promoter::ztf-16 rescue transgene (nsIs245) was integrated by treating animals carrying extrachromosomal arrays with UV/psoralen (Mello and Fire 1995). The generated strain was backcrossed to N2 three times.

Microscopy

ver-1 promoter::gfp (nsIs22) expression was assayed using a fluorescence dissecting microscope (Leica). Adult hermaphrodites were scored, except as noted. Compound microscope images were taken on an Axioplan II microscope using an AxioCam CCD camera (Zeiss) and analyzed using Axiosvision software (Zeiss). Additional images were taken on a DeltaVision Image Restoration Microscope (Applied Precision/Olympus) and analyzed using SoftWoRx software (Applied Precision). Dauer animals for electron microscopy
(EM) were grown at 25°C. These were prepared and sectioned using standard methods (Lundquist et al. 2001). Imaging was performed with an FEI Tecnai G2 Spirit BioTwin transmission electron microscope equipped with a Gatan 4K × 4K digital camera.

**Dauer selection**

Animals were starved and dauered selected by treatment with 1% SDS in M9 solution for 20 min. Alternatively, animals carrying the daf-7(e1372) mutation were induced to form dauers by incubation at 25°C.

**Mutagenesis and mapping**

L4 animals carrying the ver-1 promoter::gfp transgene (nsls22) in the N2 strain background were mutagenized with 30 mM ethyl methanesulfonate (EMS) for 4 hr. Mutagenized animals were picked to separate 9-cm NGM agar plates seeded with Escherichia coli OP50 and cultivated at 25°C. F2 animals were screened. Mapping was performed by crossing to the Hawaiian strain (CB4856), picking mutant F2 progeny, and observing linkage to single nucleotide polymorphisms (SNPs) (Wicks et al. 2001).

**Cytoplasmic mixing assay to score AMsh glia fusion**

Adult animals carrying an nsEx1391 (AMsh glia::gfp) array were picked to plates seeded with OP50 bacteria and cultivated at 25°C. From these plates, L1 and L2 progeny carrying the nsEx1391 array in one of the two AMsh glia were picked to freshly seeded plates. These mosaic animals were incubated for 48 hr at 25°C before scoring GFP presence in either one or both AMsh glia. Animals carrying a daf-7(e1372) mutation were scored only if they were dauer larvae by morphology at the end of the assay period. See Procko et al. (2011).

**Behavioral assays**

Thermotaxis and chemotaxis assays were performed as previously described (Procko et al. 2011).

**Results**

**A genetic screen for mutants defective in ver-1 expression**

To study how dauer remodeling is initiated, we aimed to identify mutants defective in this process. However, existing methodologies for following the remodeling process—namely electron microscopy and assessment of glial fusion by tracking mosaic animals (Procko et al. 2011)—are low throughput. We turned, therefore, to a more indirect approach. The C. elegans tyrosine-kinase receptor gene ver-1 is expressed in the AMsh and phasmid sheath (PHsh) glia of the amphid and phasmid sensory organs upon dauer entry and at high temperature (25°C) and is important for amphid glia remodeling in dauers. ver-1 induction in dauers and at high temperature is, at least in part, similarly regulated as mutations in ttx-1, encoding a direct transcriptional activator of ver-1, blocks induction in both settings (Procko et al. 2011). Furthermore, ttx-1 mutations also block AMsh glia remodeling in dauers. To identify genes involved in the initiation of glia remodeling, we therefore sought mutants with defects in ver-1 expression at high temperature. Wild-type animals carrying a ver-1 promoter::gfp reporter transgene (nsls22) were mutagenized with EMS, and >35,000 F2 progeny grown at 25°C were screened for reduced GFP fluorescence in AMsh glia. A total of 21 independent mutant alleles were identified (Table 1). Two of these alleles, ns235 and ns252, failed to complement the ttx-1(p767) allele when scored for ver-1 promoter::gfp expression (Table 1), and both had the same G-to-A nucleotide change within the ttx-1-coding region, altering amino acid 230 from glutamate to lysine (Figure 2). The isolation of mutations in ttx-1, a known glial remodeling regulator, validated our screen strategy.

Four of the mutant lines that we identified, ns255, ns259, ns260, and ns267, had a strong dominant reduction in ver-1 promoter::gfp expression and could not be assayed in complementation studies (Table 1; data not shown). As shown in Figure 2 and in Supporting Information, Figure S1, all four dominant alleles also had sequence changes within the ttx-1-coding region. The allele ns260, which is predicted to lack sequences encoding the TTX-1 DNA-binding domain, is likely homozygous embryonic lethal, as viable progeny from ns260/+ parents either were homozygous for the wild-type ttx-1 allele and expressed wild-type levels of ver-1 promoter::gfp or were ns260/+ and had low GFP fluorescence (Figure S2A). Consistent with an essential role for ttx-1, we found that animals heterozygous for the dominant ttx-1 allele ns259 also gave rise to fewer than expected ns259 homozygous progeny. Only 7 of 68 progeny of an ns259/+ parent grown at 25°C were ns259 homozygotes (P = 0.02, χ² test), and all 7 homozygotes were sterile. Similarly, at 15°C, 6 of 65 progeny were ns259 homozygotes (P < 0.02), and all were sterile.

**ttx-1** is expressed in both AMsh glia and the AFD thermosensory neurons (Satterlee et al. 2001; Procko et al. 2011). However, we were unable to rescue ttx-1(ns260) lethality using either glial or AFD promoters (Figure S2). In addition, sterile ns259 homozygous animals possessed AMsh glia of normal morphology, as assayed by expression of a glial Fl1693.9 promoter::dsRed transgene (n = 16). Taken together, these results suggest that ttx-1 is likely to have essential developmental roles in cells other than AMsh glia and the AFD neuron.

Nine of the alleles that we isolated failed to complement the allele ns258, also identified in our screen (Table 1; data not shown). SNP mapping (Wicks et al. 2001) was used to map one of these alleles, ns268, to a ~160-kb interval on chromosome V (Figure 3A). Cosmid F26G5 within this interval rescued the ver-1 expression defect when injected into ns268 mutants (Figure 3B), and sequencing of coding regions spanned by this cosmid uncovered two mutations within the gene tam-1. Mutations in tam-1 were also identified in each of the other nine alleles of this complementation group (Figure 3C), confirming tam-1 as the relevant affected gene. tam-1 encodes a protein predicted to contain a C3HC4 zinc finger (RING finger) and a B-box motif and has been shown to
broadly and nonspecifically regulate gene expression of transgenes from simple DNA arrays (Hsieh et al. 1999). Therefore, the effects of \(\text{tam-1}\) mutations on \(\text{ver-1}\) promoter::\(\text{gfp}\) expression may not reflect a role in the control of endogenous \(\text{ver-1}\) expression, and we did not pursue further characterization of this gene.

Two additional alleles identified in our screen, \(\text{ns231}\) and \(\text{ns257}\), did not harbor mutations in \(\text{ttx-1}, \text{tam-1}, \text{or ztf-16}\) (see below), had only weak defects in \(\text{ver-1}\) expression, and were not further studied.

**C2H2 zinc-finger factor ztf-16 is required for \(\text{ver-1}\) expression**

The \(\text{ns169}\) and \(\text{ns178}\) alleles fail to complement \(\text{ns171}\), another allele isolated in our screen, and in all three alleles expression of a \(\text{ver-1}\) promoter::\(\text{gfp}\) transgene in the \(\text{AMsh}\) and \(\text{PHsh}\) glia of adult animals raised at 25\(^\circ\) is strongly attenuated (Table 1; Figure 4, A and B; data not shown). Furthermore, whereas 100% of wild-type dauers expressed \(\text{ver-1}\) promoter::\(\text{gfp}\) in the \(\text{AMsh}\) glia, 0% of \(\text{ns169}, \text{ns171}, \text{or ns178}\) mutant dauers induced by starvation at 15\(^\circ\) expressed the reporter (\(n = 50\) for each allele; Figure 4C). By contrast, mutations in this complementation group had little or no effect on an \(\text{AMsh}\) glia reporter that is expressed constitutively and independently of dauer entry (Figure 4D). Unlike mutations in \(\text{ttx-1}\), which also disrupt \(\text{AFD}\) sensory neuron-mediated thermotaxis behavior and \(\text{AFD}\) morphology (Hedgecock and Russell 1975; Satterlee et al. 2001; Procko et al. 2011), \(\text{ns171}\) mutants exhibited nearly normal thermotaxis behavior and \(\text{AFD}\) morphology (Figure 5, A–F; Figure S3). Together, these observations suggest that the gene defined by the \(\text{ns171}\) complementation group is specifically required for \(\text{ver-1}\) promoter::\(\text{gfp}\) expression in \(\text{AMsh}\) glia and does not affect glial cell fate, \(\text{AFD}\) cell fate, or general aspects of gene expression in these cells.

To identify the gene corresponding to the \(\text{ns171}\) complementation group, we used SNP mapping (Wicks et al. 2001) to place the \(\text{ns171}\) mutation within an interval of \(~370\) kb on chromosome X (Figure 6A). Cosmids spanning the 5’ region of this interval were injected into \(\text{ns171}\) mutants and scored for rescue of \(\text{ver-1}\) promoter::\(\text{gfp}\) expression in adults raised at 25\(^\circ\). One of these cosmids, \(\text{R08E3}\), gave rescue (Figure 6B). Candidate coding regions were sequenced within this interval, and a single C-to-T substitution at codon 236 of the \(\text{ztf-16}\) open reading frame was identified. This mutation is predicted to cause a premature stop (Figure 6C). \(\text{ns178}\) mutants have the same base alteration as \(\text{ns171}\) animals, and \(\text{ns169}\) mutants harbor a different C-to-T mutation, at codon 131, which is also predicted to generate a premature stop (Figure 6C). Taken together, these studies demonstrate that \(\text{ztf-16}\) is the relevant gene affected in mutants of the \(\text{ns171}\) complementation group.

**ztf-16 encodes a protein predicted to contain up to eight C2H2 zinc-finger domains.** \(\text{C2H2}\) zinc-finger proteins are abundant transcriptional regulators in mammals, with >130 expressed in the brain alone (Iuchi 2001). On the basis of the pattern of \(\text{C2H2}\) zinc fingers, \(\text{ztf-16}\) has been classified as a \(\text{hunchback-}\) and \(\text{Ikaros-like}\) transcription factor (Large and Mathies 2010). In vertebrates, the \(\text{Ikaros}\) family of \(\text{C2H2}\) zinc-finger transcription factors have broad roles in the development of the hematopoietic system (Smale and Dorshkind 2006), while \(\text{hunchback}\) was identified as a factor regulating \(\text{Drosophila}\) embryo patterning (Tautz et al. 1987). \(\text{hunchback}\)- and \(\text{Ikaros-like}\) transcription factors have a unique arrangement of \(\text{C2H2}\) zinc fingers: four amino-terminal or middle \(\text{C2H2}\) zinc fingers bind DNA (Molnar and Georgopoulos 1994), while two carboxy-terminal \(\text{C2H2}\) zinc fingers mediate dimerization (McCarty et al. 2003). In \(\text{ztf-16}\), it is likely that zinc fingers 3–6 form the putative DNA-binding domain (Large and Mathies 2010).

On the basis of expressed sequence tag (EST) data available from WormBase (release WS225), we isolated two alternatively spliced cDNAs, \(\text{ztf-16a}\) and \(\text{ztf-16b}\), derived from the \(\text{ztf-16}\) locus. \(\text{ZTF-16a}\) and \(\text{ZTF-16b}\) proteins are predicted to differ at their carboxy-termini. \(\text{ZTF-16a}\) lacks zinc fingers.

### Table 1 Mutant strains that reduce \(\text{ver-1}\) promoter::\(\text{gfp}\) expression

| Allele | \(\%\) AMsh on | \(n\) |
|--------|---------------|------|
| Wild type | 98 | 60 |
| Complementation group 1 (see also dominant alleles below) | | |
| \(\text{ttx-1(p767)}\) (reference allele) | 0 | 40 |
| \(\text{ns235}\) | 0 | 64 |
| \(\text{ns252}\) | 0 | 52 |
| Complementation group 2 | | |
| \(\text{ns258}\) (reference allele) | 6 | 52 |
| \(\text{ns167}\) | 72 | 54 |
| \(\text{ns170}\) | 17 | 54 |
| \(\text{ns174}\) | 5 | 44 |
| \(\text{ns234}\) | 16 | 55 |
| \(\text{ns237}\) | 69 | 51 |
| \(\text{ns238}\) | 4 | 45 |
| \(\text{ns241}\) | 31 | 52 |
| \(\text{ns249}\) | 2 | 45 |
| \(\text{ns268}\) | 45 | 60 |
| Complementation group 3 | | |
| \(\text{ns171}\) (reference allele) | 4 | 55 |
| \(\text{ns169}\) | 14 | 51 |
| \(\text{ns178}\) | 2 | 52 |
| Alleles not falling into complementation groups 1–3 | | |
| \(\text{ns231}\) | 48 | 52 |
| \(\text{ns257}\) | 70 | 50 |
| Dominant alleles | | |
| \(\text{ns255}\) | 0 | 42 |
| \(\text{ns259}^a\) | 2 | 44 |
| \(\text{ns260}^a\) | 42 | 84 |
| \(\text{ns267}\) | 0 | 40 |

\(^a\) All strains contained the \(\text{ver-1}\) promoter::\(\text{gfp}\) transgene (\(\text{ns222}\)).
\(^b\) The reference alleles were used to place other alleles into complementation groups.
\(^c\) These alleles mostly had weak, qualitative effects on \(\text{ver-1}\) promoter::\(\text{gfp}\) expression.
\(^d\) Dominant alleles were not scored for complementation. All dominant alleles were found to have a mutation in the \(\text{ttx-1}\) gene.
\(^e\) Animals homozygous for allele \(\text{ns255}\) were slow growing and unhealthy.
\(^f\) Alleles \(\text{ns259}\) and \(\text{ns260}\) were homozygous sterile and lethal, respectively. These strains were isolated from the screen as heterozygotes and are scored as such. Of the \(\text{ns260}\) animals that expressed \(\text{ver-1}\) promoter::\(\text{gfp}\), fluorescence was qualitatively reduced.
July 16 functions within glia to regulate ver-1 expression

*ztf-16* was previously suggested to play a minor role in somatic gonad development and was shown to be expressed in this tissue (Large and Mathies 2010). Our findings suggest that *ztf-16* also has roles in glia that may be independent of its gonadal functions. To test this, we generated animals carrying transgenes containing regions of the *ztf-16* promoter fused to *gfp*. A 2.5-kb region immediately adjacent to the *ztf-16* start codon is expressed in hypodermal and other cell types, but not in glia (data not shown). By contrast, a 2-kb region further upstream (Figure 7A) gives strong, specific expression in *AMS*h and *PSh* glia, in *AMSo* and *PSh* socket glia, and in an unidentified pair of neurons in the head (Figure 7B). Consistent with this expression pattern, cosmid F43C9, which includes all *ztf-16*-coding fragments but only 300 bp of upstream regulatory sequences, fails to rescue *ver-1* promoter::*gfp* expression in *ztf-16* (*ns171*) mutants (Figure 6, A and B). Furthermore, *ver-1* expression defects in *ztf-16*(*ns169*) and *ztf-16*(*ns171*) mutants are rescued by expression of either *ztf-16*a or *ztf-16*b cDNAs using the constitutive F16F9.3 glia-specific promoter (Bacaj et al. 2008) (Table 2). Finally, we found that a *ZTF-16::GFP* fusion protein tightly localizes to *AMsh* nuclei (Figure 7C; et al. 2008) (Table 2). Finally, we found that a ZTF-16::GFP using the constitutive C2H2 zinc finger and B-box domain factor *tam-1* reduce ver-1 promoter::*gfp* expression. (A) A schematic of the *tam-1* gene. Exons are represented by boxes; the start codon (+1) is indicated; and regions encoding the DNA-binding homeodomain are shaded. Mutant *tam-1* alleles isolated in our screen are shown, and the corresponding amino acid changes are indicated. The asterisk indicates a premature stop mutation. The region of the *ns260* deletion is represented by a solid horizontal line [includes insertion (T)7CA(T)14]. *ns255* likely represents a rearrangement of the gene, shown by a horizontal dashed line (precise ends unknown), as PCR products using primers covering exons 2–6 are absent, weak, or consist of multiple-sized fragments (data not shown). In addition, *ns255* has the same base substitution as *ns235* and *ns252*, causing amino acid change E230K. The previously described *txt-1* alleles *p767* and *oy26* are also shown (Hedgecock and Russell 1975; Satterlee et al. 2001). (B) Alignment of the DNA-binding homeodomains of Otx-type factors TTX-1 (A isoform), Drosophila OTD, and murine Otx1 and Otx2. Amino acid positions are shown in parentheses. *ns235* and *ns252* (and *ns255*) code for a glutamic acid to lysine change in a conserved residue.
residues +221 to +263 of the ver-1 gene are present. Specifically, GFP expression in animals carrying a transgene in which residues +221 to +263 of the ver-1 promoter are deleted is not altered in ztf-16 mutants (compare expression in dauers at 25° in wild-type and ztf-16 animals carrying either the top construct or the second construct from the bottom in Figure 8). Within the region of ver-1 regulated by ztf-16, we identified a potential ZTF-16-binding site, CATGAAAA, at positions +217 to +225 on the basis of homology to Drosophila Hunchback, which binds the consensus sequence (G/C)(C/A) TAAAAAA (Stanojevic et al. 1989). Mutating these residues to GGGCCCAAC resulted in reduced ver-1 promoter::gfp expression (compare expression from top and bottom constructs in wild-type adult animals at 25° in Figure 8), raising the possibility that ZTF-16 may bind directly to the ver-1 gene to regulate its expression. To test for direct binding in vitro, we initially attempted to purify soluble full-length GST::ZTF-16a or GST::ZTF-16b protein induced in E. coli, but were unable to do so. We were able to purify zinc fingers 2–6 of the protein, but these showed only weak, nonspecific binding to a 40-bp biotin-labeled probe from the ver-1 gene (data not shown). Thus, it remains unclear whether ZTF-16 directly binds ver-1.

Taken together, our promoter studies suggest that ZTF-16 regulates ver-1 expression directly or indirectly through a site in ver-1 that is distinct from that used by TTX-1. Our studies also suggest that ztf-16 does not confer dauer dependence on ver-1 expression: although ztf-16 mutants have reduced ver-1 expression, induction of expression in dauers at 25° is still evident (Figure 8).

Our finding that TTX-1 and ZTF-16 are each required for expression of ver-1 raised the possibility that these transcription factors require each other to promote AMsh glia-specific expression. To test this idea, we scanned the genome for TTX-1-binding sites similar to that found in ver-1 (Procko et al. 2011) and identified a highly similar sequence (GATTATCG GCCGCA) within a cluster of divergently transcribed genes encoding proteins with thrombospondin domains (Figure 8A). Other proteins with such domains had been previously implicated in glial function in C. elegans and in vertebrates (Christopherson et al. 2005; Bacaj et al. 2008). While a promoter::gfp reporter for one of these genes is expressed exclusively in the AFD neurons, which normally express TTX-1, a similar reporter for another of the divergently transcribed genes of the thrombospondin-domain gene cluster is expressed in AMsh glia (Figure S4A, B and C). Expression of the reporter is eliminated in tx-1 (p767) mutants (Figure S4D). Importantly, reporter expression was normal in ztf-16(ns171) mutants (n = 53), suggesting that tx-1 and ztf-16 need not function together to promote AMsh gene expression.

ztf-16 function is required in glia for AMsh glia remodeling

Our screen aimed to identify genes controlling the initiation of glia remodeling by identifying regulators of ver-1 expression. To examine whether ztf-16 is indeed required for glia remodeling, we used an assay that we previously developed.

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**Figure 4** Temperature- and dauer-induced expression of ver-1 is reduced in ns171 mutants. (A) Representative fluorescence images and DIC and fluorescence merged images of ver-1 promoter::gfp (nsIs22) expression in one of the two AMsh glial cells of a wild-type adult cultivated at 15° (left) and 25° (right). (B) As in A, except in a ns171 mutant animal. Exposure time for gfp (A and B), 800 msec. (C) Representative fluorescence images of ver-1 promoter::gfp (nsIs22) expression in one of the two AMsh glial cells of a wild-type dauer induced by starvation at 15° (left) and a ns171 mutant dauer animal (right). Exposure time, 200 msec. (D) Representative fluorescence images of vap-1 promoter::dsRed (nsIs53) expression in the AMsh glia of a wild-type adult (left) and a ns171 mutant adult animal (right). Exposure time, 600 msec. Bar in all images, 50 μm. Anterior is up.
to score fusion of the two AMsh glia in dauer animals (Procko et al. 2011). Briefly, young daf-7(e1372ts) mutant larvae cultivated at 25°C and harboring an AMsh glia-::gfp transgene on an unstable extrachromosomal array (nsEx1391) were selected for mosaic expression of GFP in one of the two AMsh glia. Mosaic animals were allowed to grow for an additional 48 hr, at which point nearly all became dauers as a result of the daf-7 mutation. These dauers were then examined for cytoplasmic mixing of GFP between the two glia, which occurs only if the cells have remodeled and fused (Procko et al. 2011).

Using this cytoplasmic mixing assay, we found that ztf-16 (ns169); daf-7(e1372) and ztf-16(ns171); daf-7(e1372) dauers had significantly reduced AMsh glia fusion compared to daf-7 (e1372) single-mutant dauers (Figure 9A). Consistent with this result, we found that three of three ztf-16(ns171); daf-7 (e1372) dauer animals examined by EM failed to exhibit AMsh glia extension and fusion (Figure 9, B and C; Figure S5). We could rescue the fusion defect by restoring ztf-16 function specifically in glia (Figure 9A). Rescue was more efficient for the ztf-16(ns171) allele, perhaps because it has a weaker defect than ztf-16(ns169). Together, these findings suggest that ztf-16 functions within glia to promote dauer-dependent remodeling.

Our findings that ver-1 mutants have reduced AMsh glia fusion in dauers (Procko et al. 2011) and that ver-1 expression is greatly reduced in ztf-16 mutants suggest that, like ttx-1, ztf-16 acts in part to effect remodeling by controlling ver-1 expression. We also previously demonstrated a role for the gene aff-1 in glia fusion (Procko et al. 2011). AFF-1 protein functions as a fusogen-promoting syncytium formation in C. elegans (Sapir et al. 2007). To test whether ztf-16 might also regulate aff-1 expression, we examined dauer animals carrying an aff-1 promoter::gfp reporter (hyEx167). We found that 95% of wild-type dauers expressed gfp in the AMsh glia (n = 44) and that 87% of ztf-16(ns171) mutants expressed gfp (n = 38). These observations suggest that ztf-16 is not required for aff-1 expression.

ztf-16 may function as a general regulator of AMsh glia morphology or may have specific roles in dauer remodeling. To distinguish between these possibilities, we examined non-dauer ztf-16 mutant adults carrying a glia-specific vap-1 promoter::dsRed reporter transgene (Figure 4D). We found no gross defects in AMsh glia morphology. Similarly, overall glial shape is normal in electron micrographs of ztf-16(ns171) mutants. However, in these micrographs, the amphid sensory channel stains abnormally darkly, as do pockets within the AMsh glia (two of three animals examined; Figure S6). Furthermore, some sensory neurons fail to traverse the amphid channel, instead becoming trapped within the AMsh glial cell (Figure S6). These results suggest that, while ztf-16 may have a general role in proper amphid channel morphology, its function in glial plasticity may be specific to dauer animals, consistent with our findings that ztf-16 appears to act postembryonically to regulate ver-1 expression.
We previously showed that AMsh glia morphological plasticity plays an important role in controlling shape changes of the associated AWC sensory neurons (Procko et al. 2011). Indeed, in the three ztf-16 mutant animals that we examined by EM, we found that the AWC wing-like cilia that are ensheathed by the AMsh glia failed to expand as they normally do in wild-type dauers (Figure 9, B and C). In non-dauer adult or fourth-stage (L4) animals, AWC wing morphology is only mildly affected in ztf-16 mutant adults (Figure 5I). These findings are consistent with the hypothesis that changes in AMsh glia influence shape changes of associated AWC neurons (Procko et al. 2011).

**Discussion**

Morphological changes are commonplace for both neurons and glia in the development and homeostasis of the vertebrate nervous system. How these structural changes in glia are controlled, and whether glial and neuronal shape changes are related, has been largely unexplored. We previously demonstrated that dauer-induced morphological remodeling of the two AMsh glial cells of C. elegans influences concomitant changes in the glia-ensheathed AWC sensory neurons (Procko et al. 2011), suggesting that this setting is appropriate for investigating mechanisms and functions of glia remodeling. We showed that glia remodeling depends on the transcription factor ttx-1 and its direct downstream target, the receptor...
tyrosine kinase \textit{ver-1}, whose transcription is induced by dauer entry and high temperature (Procko \textit{et al.} 2011). Here we demonstrate that, in addition to \textit{txl-1}, the transcription factor \textit{ztf-16} is required for both \textit{ver-1} expression and dauer-induced \textit{Amsh} glia remodeling. Furthermore, EM analysis of dauer animals suggests that the \textit{AWC} wing-like cilia fail to take on their expanded overlapping morphology in \textit{ztf-16} mutants, most likely as a result of a failure in glia remodeling. Our results are consistent with a model whereby the transcriptional regulators \textit{TTX-1} and \textit{ZTF-16} act independently through distinct binding sites to regulate \textit{ver-1} and perhaps other genes required for \textit{Amsh} glia remodeling.

How might \textit{ztf-16} function be regulated? \textit{ztf-16} was recently shown to interact with the Nemo-like kinase \textit{LIT-1} in a yeast two-hybrid assay (Oikonomou \textit{et al.} 2011). Intriguingly, \textit{lit-1} expression is strongly induced in dauers by the DAF-12 nuclear hormone receptor, which integrates dauer neuroendocrine signals to promote dauer entry (Shostak \textit{et al.} 2004). Furthermore, \textit{ztf-16} mutants possess similar defects in \textit{Amsh} glial compartment morphology to those of \textit{lit-1} mutants (Oikonomou \textit{et al.} 2011). These observations raise the possibility that \textit{LIT-1} kinase may control \textit{ZTF-16} function in am phid glia. However, we found that two different alleles of \textit{lit-1} had no effects in \textit{ver-1} promoter::\textit{gfp} expression (data not shown), suggesting that \textit{LIT-1} is unlikely to control \textit{ZTF-16} function in this context. Nonetheless, \textit{Amsh} glia remodeling requires membrane growth and is therefore likely mediated by extensive changes in the glial cytoskeleton. \textit{LIT-1} was proposed to regulate embryonic aspects of \textit{Amsh} morphogenesis through physical interactions with the Wiskott–Aldrich Syndrome Protein and actin (Oikonomou \textit{et al.} 2011). Thus, it is possible that \textit{LIT-1} and \textit{ZTF-16} function together in processes distinct from \textit{ver-1} expression to control glial remodeling.

If \textit{ZTF-16} does physically interact with other factors, it is possible that these interactions occur via the two amino-terminal or two carboxy-terminal C2H2 zinc-finger domains, which are unlikely to be required for DNA binding (Large and Mathies 2010). Indeed, the carboxy-terminal zinc fingers of the related Ikaros transcription factor enable dimerization of the protein (Sun \textit{et al.} 1996). However, in our \textit{ver-1} expression rescue studies, we found that the carboxy-terminal zinc fingers are dispensable for \textit{ztf-16} function. Nonetheless, different \textit{ZTF-16} isoforms may fine-tune \textit{ZTF-16} activity, as is the case for Ikaros, whose activity can be controlled by dimerization with nonfunctional isoforms of the protein (Sun \textit{et al.} 1996).

What other genes control glia remodeling? Most of the mutations that we identified in our screen were alleles of one of three different genes, \textit{ztf-16}, \textit{txl-1}, or \textit{tam-1}, suggesting that the screen was close to saturation. However, our screen selected for genes involved in controlling \textit{ver-1} expression at high temperatures, and not for genes specifically controlling glia remodeling.
required for dauer induction of ver-1. Indeed, it remains unclear how dauer signals that induce ver-1 transcription are perceived by the AMsh glia. These signals may be direct neuroendocrine signals from amphid sensory neurons [e.g., the TGF-β ligand DAF-7 (Ren et al. 1996)], secondary signals induced as a result of dauer entry (e.g., radial shrinkage of the body circumference), or environmental signals perceived directly by the glia. It is possible that mutant screens assessing ver-1 expression specifically in dauer animals, rather than in non-dauer adults, may uncover these signals. Direct assessment of glial remodeling, rather than reliance on ver-1 expression as a proxy, may reveal additional components functioning in parallel to or downstream of ver-1 to promote glial plasticity.

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Sensory Organ Remodeling in *Caenorhabditis elegans* Requires the Zinc-Finger Protein ZTF-16

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**Figure S1**  PCR assay for genotyping *ttx-1(ns260)* animals. (A) Schematic of the *ttx-1* gene, showing only exons 5 and 6 (boxes). The *ns260* deletion is marked. The location of the three oligonucleotide primers used to genotype individual animals (B) are indicated by arrows (5' to 3'). (B) Genotyping of four individual progeny from an *ns260/+* heterozygous parent grown at 25° and carrying a *ver-1* promoter::*gfp* transgene (*nsIs22*). Two of these animals had wild-type levels of *gfp* expression, and had a wild-type genotype (left), while the other two had low levels of *gfp* and were *ns260/+* heterozygous (right). Primers used in the PCR assay are shown in A. Wild-type animals exhibit a single amplified DNA fragment of ~450 bp. *ns260* homozygous animals are predicted to produce only a single DNA band of size ~600 bp.
A

$25^\circ C$

|       | 25°C | 15°C |
|-------|------|------|
| $n$   | 110  | 116  |

$gfp$ expression $25^\circ C$

- **high**
- **low**
- **not determined**

B

|        | F16F9.3 nsEx1923 | F16F9.3 nsEx1924 | lin-26 nsEx1915 | lin-26 nsEx1916 |
|--------|------------------|------------------|-----------------|-----------------|
| $n$    | 58               | 50               | 53              | 57              |

C

|        | gcy-8 nsEx1913   | gcy-8 nsEx1914   | ttx-1(AFD) nsEx1948 | ttx-1(AFD) nsEx1953 |
|--------|------------------|------------------|---------------------|---------------------|
| $n$    | 51               | 57               | 58                  | 58                  |
Glial or AFD-specific expression of \textit{ttx-1} cDNA fails to rescue \textit{ns260} lethality. (A) Genotyping of viable progeny from \textit{ns260/+} heterozygous parents grown at either 15° or 25°. Wild type, +/-; \textit{ns260} heterozygous, +/-; and \textit{ns260} homozygous, –/-\. All animals also carry a \textit{ver-1} promoter::\textit{gfp} transgene (nsIs22), and levels of \textit{gfp} expression in the sheath glia in each individual animal were scored as either high, low, or not determined. The number of progeny examined (n) is indicated. (B) Same as A, except the progeny also carry extrachromosomal arrays restoring \textit{ttx-1} expression in the AMsh glia. Cell-specific promoters driving \textit{ttx-1} cDNA include the \textit{F16F9.3} promoter (late embryo to adult expression (Bacaj et al. 2008)) and the \textit{lin-26} promoter (embryonic expression only (Landmann et al. 2004)). Two different rescuing arrays using each promoter were scored, and are indicated. The qualitative expression level of \textit{ver-1} promoter::\textit{gfp} at 25° is shown (see legend part A). (C) Same as B, except using AFD-specific \textit{gcy-8} and \textit{ttx-1} promoters. In B and C, the \textit{ttx-1a} splice form was used. In all lines shown in A, B, and C, viable \textit{ns260} homozygous animals (–/-) were never observed.
**Figure S3** *ns171* mutants have wild-type AFD sensory ending morphology. Electron micrograph (EM) showing a cross-section through an amphid sensory organ of an *ns171* mutant adult animal. AFD microvillar projections, red shading. See also Figure 5, A and B. Scale bar, 1 μm. For comparison, see (Ward et al. 1975).
Figure S4 TTX-1 directly regulates glial and AFD genes. (A) A schematic showing part of the F58F9 cosmid sequence, which includes a cluster of five thrombospondin (TSP)-domain containing genes (boxes). The gene numbers are designated by WormBase. The putative TTX-1 binding site, based on conservation with the ver-1 promoter, is indicated (conserved residues between ver-1 and F58F9 are 5′ GATTCGATTCAG 3′, with core TTX-1 binding residues underlined). Also shown are the F58F9.10 and F58F9.6 promoter regions used in expression studies. (B and C) Fluorescence images (left), and DIC and fluorescence merged images (right) showing gfp expression in the AFD neurons of an adult wild-type animal carrying an F58F9.10 promoter::gfp transgene (nsEx2284) (B), or in the AMsh glia of a wild-type animal carrying an F58F9.6 promoter::gfp transgene (nsEx2330) (C). GFP expression in AFD is indicated by arrowheads, and in AMsh glia by arrows. Expression of F58F9.6 promoter::gfp in AMsh glia was rare (1/13 lines). (D) As in (C), except in a ttx-1(p767) mutant. Exposure (C and D), 500 ms. Scale bar (B-D), 50 μm. Anterior is up. All animals grown at 25°C.
Figure S5 High resolution image of Figure 9C.
**Figure S6** Electron micrograph (EM) showing a cross-section through the amphid sensory channel of a ztf-16(ns171) mutant adult animal. In 2/3 animals examined, the amphid sensory channel (arrow, blue shading) stained abnormally darkly, as did pockets within the AMsh glia (asterisks). Some sensory neurons (red shading) failed to traverse the channel, and were trapped inside the AMsh glial cell (arrowheads). Scale bar, 600 nm. For comparison, see (Ward et al. 1975).