Current Biology, Vol. 14, 2245–2251, December 29, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.12.030

Identification of Thermosensory and Olfactory Neuron-Specific Genes via Expression Profiling of Single Neuron Types

Marc E. Colosimo,[1,3,5] Adam Brown,[1,5] Saikat Mukhopadhyay,[1] Christopher Gabel,[2] Anne E. Lanjuin,[1,4] Aravinthan D.T. Samuel,[2] and Piali Sengupta[1,*]

[1]Department of Biology
Brandeis University
Waltham, Massachusetts 02454
[2]Department of Physics
Harvard University
Cambridge, Massachusetts 02138

Summary

Most *C. elegans* sensory neuron types consist of a single bilateral pair of neurons, and respond to a unique set of sensory stimuli. Although genes required for the development and function of individual sensory neuron types have been identified in forward genetic screens, these approaches are unlikely to identify genes that when mutated result in subtle or pleiotropic phenotypes. Here, we describe a complementary approach to identify sensory neuron type-specific genes via microarray analysis using RNA from sorted AWB olfactory and AFD thermosensory neurons. The expression patterns of subsets of these genes were further verified in vivo. Genes identified by this analysis encode 7-transmembrane receptors, kinases, and nuclear factors including *dac-1*, which encodes a homolog of the highly conserved Dachshund protein [1]. *dac-1* is expressed in a subset of sensory neurons including the AFD neurons and is regulated by the TTX-1 OTX homeodomain protein [2]. On thermal gradients, *dac-1* mutants fail to suppress a cryophilic drive but continue to track isotherms at the cultivation temperature, representing the first genetic separation of these AFD-mediated behaviors. Expression profiling of single neuron types provides a rapid, powerful, and unbiased method for identifying neuron-specific genes whose functions can then be investigated in vivo.

Results and Discussion

In principle, genes expressed in an individual sensory neuron type in *C. elegans* could be identified via comparison of the expression profiles of wild-type and mutant animals in which a specific neuron type fails to differentiate or is not generated. However, mutations in most developmental genes affect multiple cell types, and moreover, genes expressed in only two of approximately 950 cells in the adult *C. elegans* hermaphrodite cannot be detected via conventional microarray methods. Therefore, to identify sensory neuron-specific genes, we utilized a recently described method to culture and collect populations of single neuron types [3] (R. Fox et al., submitted).

We generated cultures of embryonic cells from transgenic *C. elegans* strains stably expressing the markers *gcy-8::gfp* and *str-1::gfp*. *gcy-8::gfp* is expressed specifically in the AFD thermosensory neurons [4], whereas *str-1::gfp* is expressed strongly and consistently in the AWB olfactory neurons and weakly in the AIN interneurons [5]. Cells expressing these markers were observed in culture at approximately the expected frequency (*gcy-8::gfp*: expected 0.36%, observed 0.38%; *str-1::gfp*: expected 0.36%, observed 0.41% for cells exhibiting strong *gfp* expression). Although the *gfp*-expressing cells appeared to adopt a neuronal morphology such that they frequently extended processes, most did not appear to be bipolar. Moreover, neuron-specific sensory structures [6] were observed only rarely, suggesting that these neurons may not have fully differentiated under these culture conditions.

To isolate enriched populations of single neuron types, cultures containing *gcy-8::gfp* or *str-1::gfp*-expressing cells were sorted with a fluorescence-activated cell sorter. In a typical run, we obtained cell populations in which approximately 50% were GFP^+, representing a 140-fold enrichment. For each experiment, mRNA was isolated from approximately 20,000–50,000 sorted cells and amplified linearly through two rounds of amplification. Affymetrix microarrays containing the majority of open reading frames predicted by the *C. elegans* genome were hybridized with mRNA isolated and amplified in three independent experiments. As controls for our analysis, arrays were further hybridized with amplified mRNA isolated from unsorted embryonic cells. Gene expression profiles of independent replicates were highly correlated (Figures S1 and S2 in the Supplemental Data available with this article online), indicating good overall reproducibility among independent experiments.

To identify neuron-specific genes, we selected genes with an arbitrary 2-fold or higher ratio of differential expression. Using this criterion, we identified 58 and 108 genes as being differentially expressed in the AWB and AFD neurons, respectively (the complete data set is shown in Table S1). As a first step in data validation, the expression of genes that were previously reported to be differentially expressed between these neuron types were examined. 6/11 genes previously reported to be expressed in the AWB but not the AFD neurons were identified as being differentially expressed in this analysis (Table 1). Conversely, 4/10 genes reported to be expressed in the AFD but not the AWB neurons were detected (Table 1). Failure to detect the remaining genes could be due to several reasons. Genes such as *str-1* were not identified due to misannotation of their sequences in the databases or failure to be included on the Affymetrix array (M.E.C. and P.S., unpublished data). Expression of a subset of genes was below the threshold of detection, suggesting that they may be embryonically expressed at low levels. It is also likely that the pre-

*Correspondence: sengupta@brandeis.edu
[3]Present address: The MITRE Corporation, Bedford, Massachusetts.
[4]Present address: Department of Molecular and Cell Biology, Harvard University, Cambridge, Massachusetts 02138.
[5]These authors contributed equally to this work.

Table 1. Genes Differentially Expressed between the AWB and AFD Neurons

Sequence Name	Gene Name	Description	Fold Change	P Value[a]	Real-Time PCR Ratio	Anatomical Expression[b]
AWB>AFD						
C42D4.9	*str-220*	7TM receptor	26.11	0.124		AWB
Y97E10B.9		7TM receptor	10.62	0.021	5.9 ± 0.8	AWB, AWC
B0240.3	*daf-11*	guanylate cyclase	8.26	<0.001		AWB +
R07B5.4	*sru-38*	7TM receptor	5.38	0.084		AWB, ASH
Y40H7A.5	*srd-23*	7TM receptor	5.00	0.016	91.3 ± 33.2	AWB, ASK
R01E6.1	*odr-1*	guanylate cyclase	2.72	0.069		AWB +
C53A5.9		KELCH protein	2.52	0.004		No neurons
C27H5.7b		TPR repeat	2.32	0.006		Variably in AWB
C37E2.5	*ceh-37*	homeobox domain	2.25	0.014		AWB +
C04E6.9	*srd-16*	7TM receptor	2.13	0.009		ASH, ASI, PHA, PHB
C34D1.3	*odr-3*	Gα subunit	2.08	0.009		AWB +
F48C11.3	*nlp-3*	neuropeptide	2.07	0.072		AWB +
AFD>AWB						
B0496.5		7TM receptor	−15.45	0.109	−49.1 ± 2.8	AFD, AWC
C49H3.1	*gcy-8*	guanylate cyclase	−13.39	0.002		AFD
C04H5.3		guanylate cyclase	−12.64	0.074		AWC, ASE +
B0412.1a	*dac-1*	SKI/SNO/DAC family	−9.9	0.148	−20.5 ± 4.9	AFD, AWC, ASE, ASK
Y113G7A.6b	*ttx-1*	homeobox domain	−7.38	0.031		AFD +
F54G8.2	*dgk-3*	diacylglycerol kinase	−3.54	0.054	−2.1 ± 0.1	AFD, AWB, AWC, ASE, ASK, ASI
F07C3.10		zinc finger, C4 type	−3.5	0.012		AFD, AWC, ASE, ADF +
R13H7.2c		7TM receptor	−3.26	0.197		Variably in AFD +
F18E9.2	*nlp-7*	neuropeptide	−3.03	0.102		AWC, ASE, ADF, ADL
C33B4.3		proline-rich and ankyrin domains	−2.77	0.034		AFD, AWC +
K01H12.3	*nhr-38*	nuclear hormone receptor	−2.18	0.161		AFD
Y48A6A.1	*zig-5*	C2 Ig domain, cell adhesion/signaling	−2	0.006		AFD +

Newly identified genes whose expression patterns were examined in this study are underlined. Additional genes are previously described genes whose differential expression was confirmed by this analysis. The AFD dataset was used as the control and AWB dataset as the experimental values.

In addition to the shown genes, expression ratios of the following genes were also examined.

Previously reported to be expressed in AWB but not AFD: lim-4, str-1, ace-2, tbx-2, str-219. These were not found to be differentially expressed at a ≥2.0-fold ratio between the AFD and AWB datasets.

Previously reported to be expressed in AFD but not AWB: ceh-23, egl-2, dbl-1, ceh-14, nlp-21, tax-6. These were not found to be differentially expressed at a ≥2.0-fold ratio between the AFD and AWB datasets.

Known to be expressed in both AFD and AWB (sensory genes): tax-4, osm-6, tax-2, osm-5. These were not found to be differentially expressed at a ≥2.0-fold ratio between the AFD and AWB datasets but were expressed at a ≥2.0-fold ratio in the (AFD +AWB) dataset when compared to the unsorted cell dataset (Table S2).

Likely expressed in both AFD and AWB (pan-neuronal or primarily neuronal genes): unc-14, snt-1, unc-104, slo-1, daf-19, syd-1, unc-13. These were not found to be differentially expressed at a ≥2.0-fold ratio between the AFD and AWB datasets but were expressed at a ≥2.0-fold ratio in the (AFD +AWB) dataset when compared to the unsorted cell dataset (Table S2).

Non-neuronal genes: hsp-16.2, unc-122, myo-3, act-5, elt-2, zig-7. These were not found to be differentially expressed at a ≥2.0-fold ratio between the AFD and AWB datasets but were expressed at a ≥2.0-fold ratio in the unsorted cell dataset when compared to the (AFD +AWB) dataset (Table S2).

The complete list of genes differentially expressed between AFD and AWB and between (AFD +AWB) and unsorted cells is shown in Tables S1 and S2, respectively.

[a]p Values were calculated by a paired two-tailed t-test.

[b]Relevant expressing cells are indicated. For genes whose expression patterns were examined in this study, identified cells are shown. + indicates additional neuronal and/or non-neuronal cells. None of the shown AWB>AFD genes are or have been shown to be expressed in the AFD neurons. Conversely, for the AFD>AWB genes, only *dgk-3* is expressed in both neuron types (see Figure 2).

viously reported expression patterns for a subset of these genes were incomplete. In particular, embryonic expression patterns have not been examined in detail for most genes. We also examined the expression of four genes shown to be expressed in and required for the sensory responses of both the AWB and AFD neurons, as well as seven genes encoding molecules required for general neuronal functions (see Table 1 legend). None of these genes were predicted to be differentially expressed between the AWB and AFD neuron types, although expression of these genes was upregulated when compared to the unsorted cell dataset (Table S2). In addition, several nonneuronal genes were not detected as being differentially expressed between the AWB and AFD datasets (see Table 1 legend).

As shown in Table 1, Table S1, and Figure S3, genes predicted to be differentially expressed between the AFD and AWB neuron types encode members of different protein families, including seven transmembrane domain receptors, transcription factors, kinases, and signal transduction molecules. To verify the predicted differential expression, we examined the in vivo expression patterns of subsets of these genes. We selected genes predicted to be differentially expressed at differ-

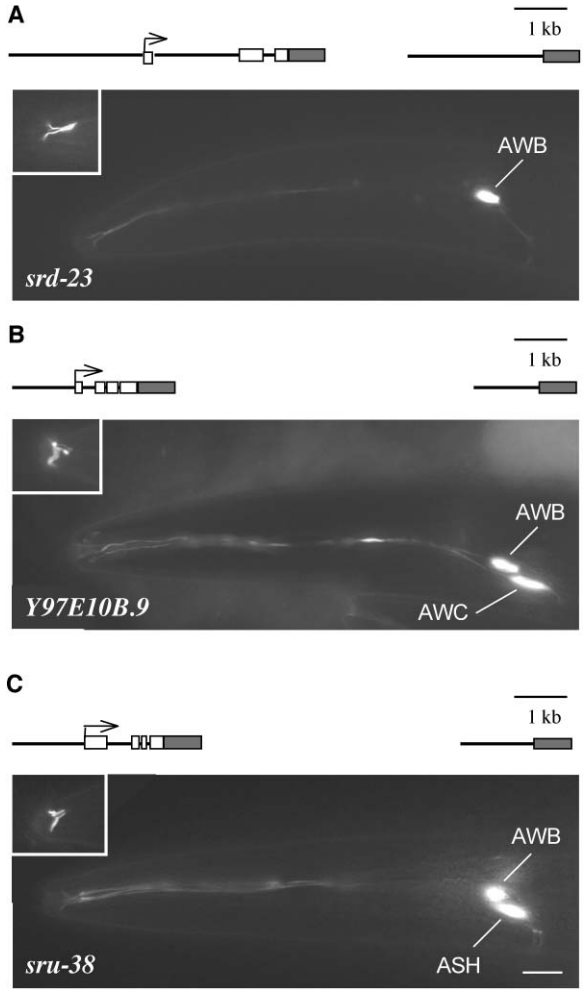

Figure 1. Expression Patterns of Three G Protein-Coupled Receptor Genes Predicted to Be Differentially Expressed in the AWB Neurons

Expression of promoter::*gfp* and full-length *gfp*-tagged (insets) *srd-23* (A), *Y97E10B.9* (B) and *sru-38* (C) fusion genes. Structures of the constructs are shown above each panel with shaded boxes representing GFP coding sequences. Localization of the GFP-tagged proteins to the characteristic forked cilia of the AWB neurons can be seen in A and C insets (the AWB cilia are out of the plane of focus in B inset). Cell bodies of expressing neurons are indicated. Anterior is at left. Scale – 10 μm.

ent ratios and that encode proteins with recognizable homologies to known protein domains. The results for each neuron type are summarized below.

AWB neuron type: We fused the promoters of selected genes to the *gfp* reporter and examined the expression of *gfp* in transgenic animals carrying the fusion genes on extrachromosomal arrays. Expression in the AWB neurons but not in the AFD neurons was observed for 4/6 fusion genes examined for which we could detect expression (Figure 1 and Table 1). None were expressed in the AFD neurons. We further quantitated and confirmed differential expression of two of these genes via quantitative real-time PCR (Table 1). Three of the AWB-expressed genes are predicted to encode candidate olfactory receptors, bringing the total number of olfactory receptors shown to be expressed in the AWB neurons to seven [5, 7] (this work). Because many *C. elegans* genes contain additional regulatory elements in their intronic sequences, we confirmed the spatial expression pattern by examining expression of *gfp*-tagged olfactory receptor genes. These fusion genes contain upstream regulatory sequences as well as all predicted introns. GFP-tagged proteins were localized to the specialized and unique ciliary endings of only those sensory neurons identified via expression of promoter::*gfp* fusion genes (Figure 1). Localization of these proteins to the cilia implicates these receptors in primary signal transduction events. Although a subset of these olfactory receptor genes are located in a cluster on LG IV, additional AWB-expressed receptor genes identified in this analysis are located elsewhere in the genome, indicating that these genes are not coregulated.

AFD neuron type: We examined the expression pattern of eight genes via promoter::*gfp* fusions. Six out of eight genes were expressed in the AFD neurons (Figure 2 and Table 1). With one exception, none of the examined genes were expressed in the AWB neurons. *dgk-3* encodes a predicted diacylglycerol kinase and was expressed in both the AFD and AWB neurons (Figure 2C). However, in four of four examined independent transgenic lines, >65% of transgenic animals expressed *dgk-3* more strongly in the AFD than in the AWB neurons (Figure 2D). Stronger expression in the AFD as opposed to the AWB neurons was further validated by real-time PCR (Table 1). It is possible that we did not detect expression of a subset of examined genes in the appropriate cell type because we failed to include all regulatory sequences in the fusion constructs. Alternatively, these genes may be expressed transiently in the AFD or AWB neurons at specific developmental stages or under specific environmental conditions. Taken together, the in vivo expression pattern analysis validates the microarray data, and suggests that we have identified new genes expressed in the AWB and the AFD neurons.

As a further validation of this method, we wished to investigate the roles of the newly identified genes in sensory neuron development and function. As indicated in Table 1, one of the genes shown to be differentially expressed in the AFD neurons is *dac-1*. *dac-1* encodes the *C. elegans* homolog of the Dachshund nuclear factor, which has been implicated in the development of many organs including the eyes and mushroom bodies in *Drosophila* and muscle and limbs in vertebrates [1, 8–12]. In particular, in *Drosophila*, *Dac* is a member of a core set of genes necessary and sufficient for eye development [1, 13–15]. Dac has been shown to synergize with transcription factors and bind DNA, thereby regulating transcription [16, 17]. The expression pattern and functions of *dac-1* in *C. elegans* have not previously been examined. The approximately 100 amino-acid DD1/Dachbox-N domain is highly conserved across species and has been shown to be essential for all functions of *Drosophila* Dac [16, 18]. The DD1 domain of *C. elegans* DAC-1 is approximately 65% identical to *Drosophila* and mouse Dac homologs (Figures 3B and 3C), although additional domains are poorly conserved. A GFP-tagged DAC-1 fusion protein is expressed strongly in the AFD and weakly in the AWC, ASE, and ASK chemosensory neurons in the head and is nuclear localized

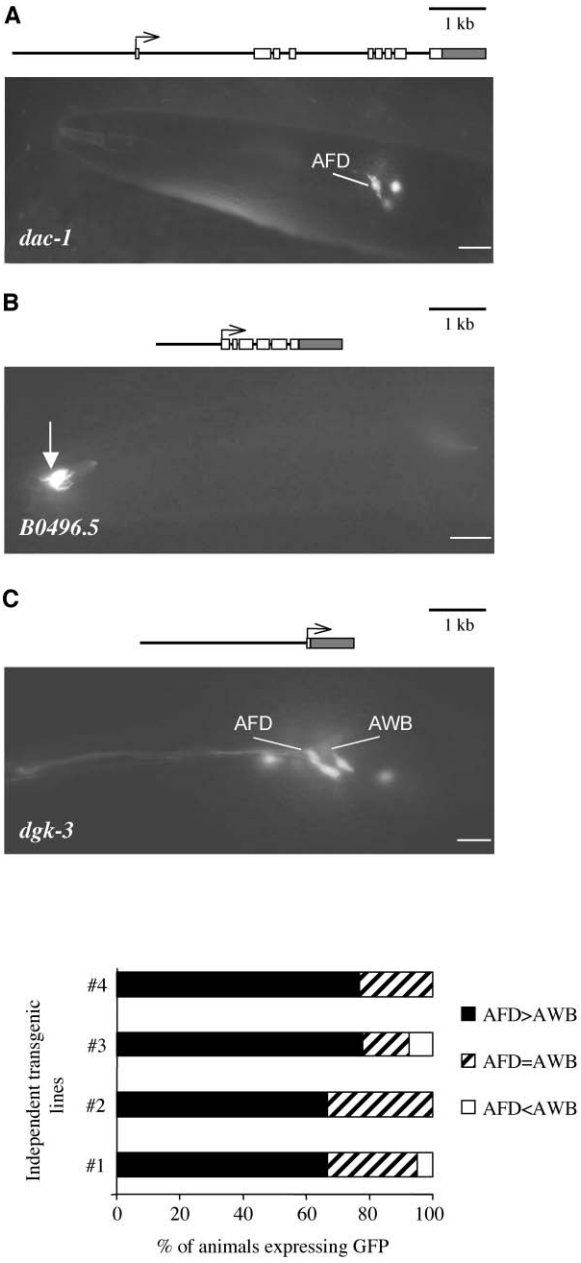

Figure 2. Expression Patterns of Genes Predicted to Be Differentially Expressed in the AFD Neurons

Expression of full-length *gfp*-tagged (A and B) or promoter::*gfp* (C) fusion genes. Structures of the constructs used are shown with GFP coding sequences indicated by shaded boxes. Cell bodies of relevant neurons are indicated. Arrow indicates GFP localization in the sensory endings of the AFD and AWC sensory neurons in (B). Scale – 10 μm. (D) Comparison of expression levels of a *dgk-3::gfp* fusion gene in the AFD and AWB neurons in transgenic animals from four independent lines. GFP expression levels were compared qualitatively in adult animals (n>100) examined at 400X magnification.

(Figure 2A). Expression was also observed in the alae, which arise from fusion of the hypodermal seam cells as well as additional unidentified cells in the tail (data not shown). Three *dac-1* alleles were available. Sequencing of the genomic region and analysis of cDNAs generated via RT-PCR indicated that *gk211* is a deletion that is predicted to encode a truncated protein lacking the DD1 domain (Figure 3A). Predicted alternate transcription/translation start sites are also deleted in *gk211*. In the *gk198* and *gk213* alleles, alternate start sites are retained, and transcripts predicted to encode proteins retaining most DD1 sequences were detected. Thus, *gk211* is likely a null allele.

We further examined the role of *dac-1* in the development and function of the AFD neurons. We have previously shown that differentiation of the AFD neurons requires the TTX-1 OTX-like homeodomain protein [2]. In *ttx-1* mutants, expression of genes such as the *gcy-8* guanylyl cyclase and *tax-2* cyclic nucleotide-gated channel subunit genes is abolished in the AFD neurons, and the structures of the specialized microvillar sensory endings of the AFD neurons are severely compromised [2, 19]. We found that although expression of a *ttx-1::gfp* transgene in the AFD neurons was unaffected in *dac-1* mutants, *dac-1* expression in the AFD neurons was regulated by TTX-1 (Table S3). However, unlike *ttx-1* mutants, the structures of the sensory endings of the AFD neurons was unaffected in *dac-1* mutants (data not shown). Moreover, the expression of *gcy-8::gfp* and *tax-2::gfp* fusion genes was also unaltered in *dac-1(gk211)* animals (Table S3). These results indicate that DAC-1 may act downstream of TTX-1 to regulate a distinct subset of AFD-mediated functions.

When grown at a specific temperature in the presence of its bacterial food source, *C. elegans* forms a memory of the cultivation temperature (T_c). This memory modulates the behavior exhibited by worms when placed subsequently on a thermal gradient such that animals encountering ambient temperatures (T_a) $>$ T_c exhibit a cryophilic drive toward the T_c, although animals at $T_a <$ T_c do not exhibit an equally robust corresponding thermophilic drive [20–22]. When T_a is within 2°–3°C of T_c, animals track isotherms [20–22]. Animals lacking AFD neuronal function primarily exhibit a constitutive cryophilic drive regardless of T_c and fail to track isotherms at any temperature [2, 21]. These results suggest that at $T_a < T_c$, the AFD neurons may suppress a cryophilic drive whereas at $T_a = T_c$, the AFD neurons promote isothermal tracking. Whether these behavioral outputs are mediated by distinct or shared sets of genes is unknown. Unexpectedly, while *dac-1(gk211)* mutants exhibited a significant cryophilic drive both above and below T_c (Figures 4A and 4B), they tracked isotherms near T_c (Figure 4C). This behavior is in contrast to that of *ttx-1* mutants which exhibit constitutive cryophilic behavior and fail to track isotherms at any temperature (Figure 4) [21]. The cryophilic behavior of *dac-1(gk211)* mutants was rescued by expression of the *dac-1* transcript specifically in the AFD neurons under the *ttx-1* promoter (Figure 4B). Recent reports have suggested that migration down gradients and isothermal tracking are performed by distinct behavioral algorithms and that the AFD neurons are more active when $T_a >$ or $< T_c$ than when $T_a = T_c$ [20, 23, 24]. Thus, mutations in *dac-1* may specifically affect the expression of genes required for suppression of the cryophilic drive but not for promotion of isothermal tracking, suggesting a genetic separation of the pathways underlying these two behavioral paradigms.

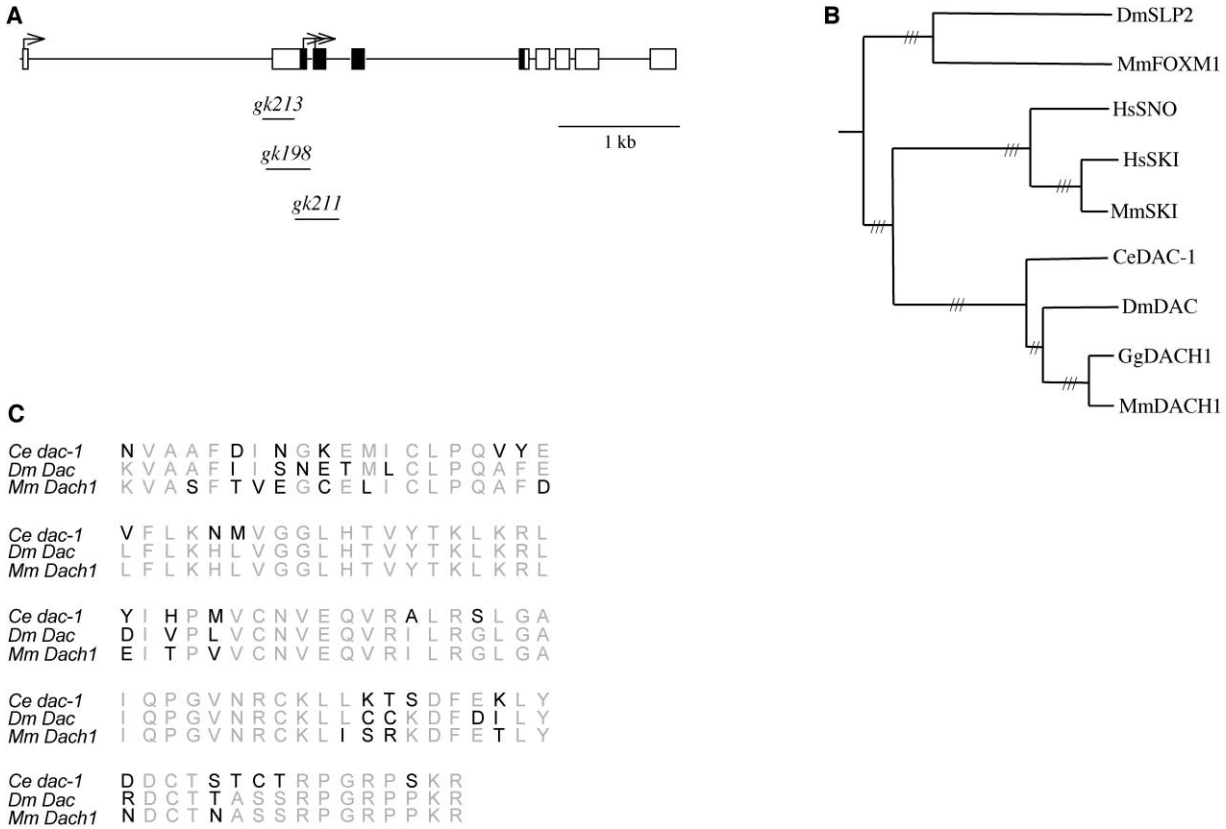

Figure 3. *dac-1* Encodes the *C. elegans* Dachshund Homolog

(A) Genomic structure of *dac-1*. Exons encoding the predicted DD1 domain are shaded. The extents of the deletions in the *gk198*, *gk211* and *gk213* alleles are indicated. Arrows mark the positions of putative translation start sites deduced from RT-PCR and sequencing of cDNAs and ESTs. (B) Phylogenetic tree of the DD1 domains of Dachshund homologs and the related SNO/SKI domain. The forkhead domains of DmSLP2 and MmFOXM1 were used as outliers. The tree was generated using Bonsai http://calliope.gs.washington.edu/software/index.html. 1000 bootstrap replicated were performed and bootstrap values are indicated by hatchmarks: (/) = <80%; (//) = 80%–89%; (///) = 90%–100%. Accession numbers: DmSLP2 (P32031), MmFOXMI (O08696), HsSNO (NP005405), HsSKI (P12755), MmSKI (AAH68305), DmDAC (AAC46510), GgDACH1 (AAL76234), MmDACH1 (Q9QYB2). (C) Alignment of the DD1 domain of DAC-1 with the DD1 domains of *Drosophila* DAC and mouse DACH1. Residues identical to DAC-1 are indicated in gray.

Since *dac-1* is also expressed in the AWC olfactory and the ASE chemosensory neurons, we examined the AWC-mediated olfactory behaviors of *dac-1* mutants. However, *dac-1(gk211)* mutants responded to all AWC-sensed odorants tested (data not shown). Moreover, the expression of AWC and ASE markers such as an *odr-1::dsRed* [25](expressed in the AWC and AWB olfactory neurons) and a *ceh-36::gfp* transgene [26–28](expressed in the AWC and ASE neurons) was unaffected in *dac-1* mutants (data not shown).

Conclusions

We have described the expression profiles of isolated embryonic populations of single sensory neuron types in order to identify subsets of genes expressed in these neurons. Similar methods have previously been used to identify genes expressed in the six mechanosensory neurons of *C. elegans* [29]. Caveats to this method include the fact that in the absence of functional physiological characterization, it is possible that the neurons in culture exhibit properties that differ from those in vivo. Moreover, genes may be expressed differentially at specific postembryonic developmental stages. As a consequence, we may have failed to identify all differentially expressed genes, or have identified a subset of genes that is not diffentially expressed in vivo. Nevertheless, this approach in combination with forward genetic screens should allow for the description of most, if not all genes required to confer neuron-specific functions.

Using this analysis, we have defined the role of the Dachshund nuclear factor DAC-1 in AFD neuron function. Since the behavioral defects of *dac-1* mutants are relatively subtle and thermosensory behavioral assays are non-trivial to perform, it is unlikely that *dac-1* mutations would have been easily identified in behavioral screens. Thermosensory behavioral analysis of *dac-1* mutants suggests that DAC-1 may function downstream of TTX-1 in the AFD neurons to regulate genes required for suppression of the cryophilic drive, but that additional DAC-1-independent pathways function to promote isothermal tracking. Dachshund proteins have been shown to function in a conserved genetic network with Pax-6/Eyeless, Sine Oculis/Six1 and Eyes Absent to regulate the development of diverse tissue types such

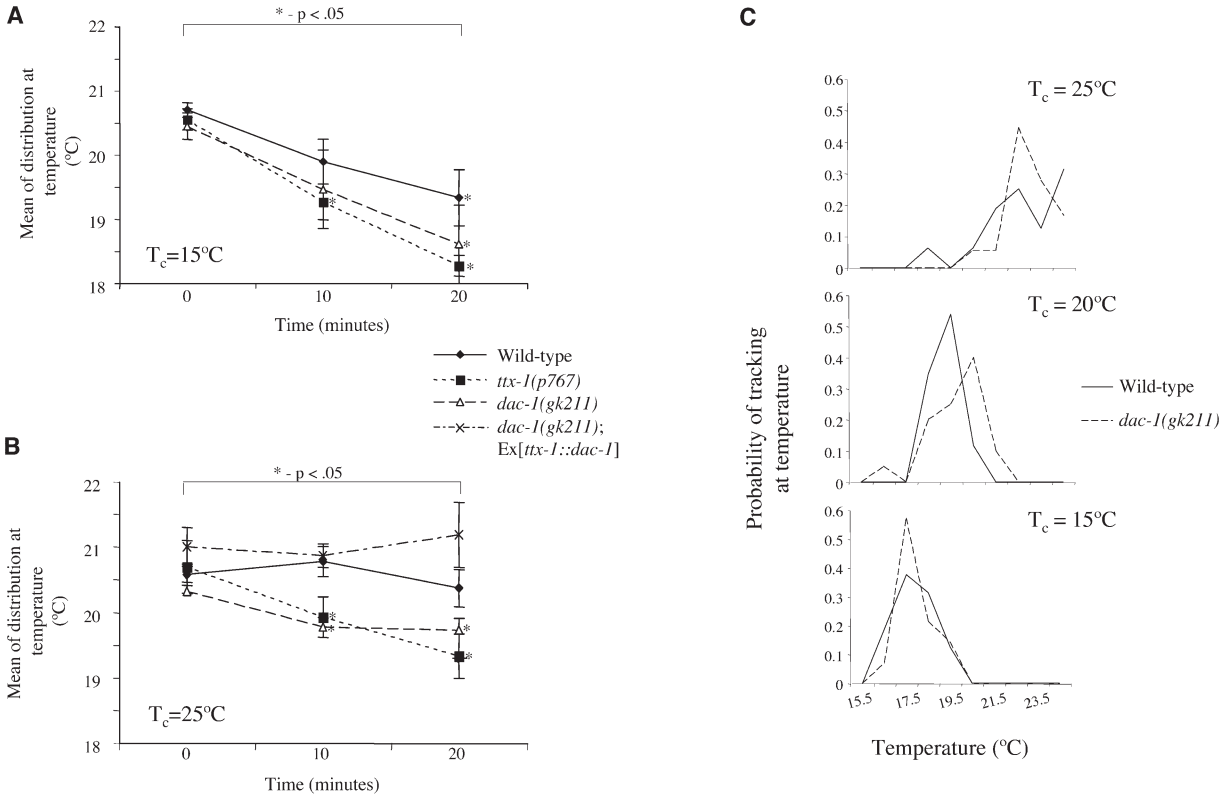

Figure 4. *dac-1(gk211)* Mutants Are Cryophilic but Track Isotherms at Their Cultivation Temperature

The means of distribution of the indicated strains at time=0' (t_0), 10' (t_{10}) and 20' (t_{20}), on a linear thermal gradient are shown. Animals were cultivated at 15°C (A) or 25°C (B). Asterisks indicate means at t_{10} and t_{20} that are different from the mean at t_0 for a given genotype at $p < .05$. The means at t_0 were not significantly different among genotypes. Data shown are from at least five independent assays using 30-50 animals per assay. (C) Shown is the probability that wild-type or *dac-1(gk211)* animals cultivated at 15°C, 20°C and 25°C track isotherms at the indicated temperature. Probability is calculated as the number of worms tracking at a given temperature/total number of tracking worms. The mean tracking temperatures for wild-type and *dac-1* mutants were 17.8 ± 0.9°C and 18.0 ± 0.7°C for T_c = 15°C, 19.3 ± 0.6°C and 19.8 ± 1.2°C for T_c = 20°C, 22.9 ± 1.8°C and 22.9 ± 1.0°C for T_c = 25°C. *ttx-1(p767)* mutants did not track isotherms at any temperature. $n > 30$ animals for each assay.

as the eye in *Drosophila* and muscle in vertebrates [11, 14, 30, 31]. However, AFD development is unaffected by mutations in the Pax-6 homolog *vab-3* (M. Tsie and P.S., unpublished results), suggesting that *dac-1* may function in a different network.

Neuron-specific genes have also been identified on the basis of shared *cis*-regulatory motifs (e.g., [32]). However, since multiple transcriptional programs may function in a single cell type, subsets of genes regulated via different pathways and possessing alternate *cis*-regulatory architecture would not be identified by this method. The microarray approach described here provides an unbiased method by which multiple neuron-specific genes can be rapidly identified and their functions investigated. We expect that application of similar methods to identify the gene sets expressed differentially among different neuron types will allow us to gain an understanding of the developmental and functional basis of neuronal diversity.

Supplemental Data

Supplemental Data including detailed Experimental Procedures, an additional three tables, and three figures are available at http://www.current-biology.com/cgi/content/full/14/24/2245/DC1/.

Acknowledgments

We are extremely grateful to Rebecca Fox and David Miller for generously sharing their time, cell culture expertise and unpublished data, Maura Berkeley for technical assistance, Bill Nostrum for assistance with FACS analysis, Dae-Hwan Kim for assistance with *gfp* fusion plasmids, Ken Sugino for help with data analysis, Catherine Dulac for advice regarding nucleic acid amplification, and Mark Palfreyman and Miriam Goodman for initiating early versions of these experiments. We thank Oliver Hobert, Sacha Nelson, David Miller and the Sengupta lab for critical comments on the manuscript. M.E.C.'s affiliation with the MITRE Corporation is provided for identification purposes only and is not intended to convey or imply MITRE's concurrence with, or support for, the positions, opinions, or viewpoints expressed by the author. This work was partly funded by the National Institutes of Health (RO1 GM56223 and PO1 NS44232 – P.S. and T32 MH19929 – A.B.) and the National Science Foundation (IBN 021430 – P.S. and MRSEC DMR 0213805 – C.G.).

Received: September 9, 2004
Revised: October 18, 2004
Accepted: October 10, 2004
Published: December 29, 2004

References

1. Mardon, G., Solomon, N.M., and Rubin, G.M. (1994). dachshund encodes a nuclear protein required for normal eye and leg development in Drosophila. Development *120*, 3473–3486.

2. Satterlee, J.S., Sasakura, H., Kuhara, A., Berkeley, M., Mori, I., and Sengupta, P. (2001). Specification of thermosensory neuron fate in C. elegans requires ttx-1, a homolog of otd/Otx. Neuron *31*, 943–956.
3. Christensen, M., Estevez, A., Yin, X., Fox, R., Morrison, R., McDonnell, M., Gleason, C., Miller, D.M., 3rd, and Strange, K. (2002). A primary culture system for functional analysis of C. elegans neurons and muscle cells. Neuron *33*, 503–514.
4. Yu, S., Avery, L., Baude, E., and Garbers, D.A. (1997). Guanylyl cyclase expression in specific sensory neurons: A new family of chemosensory receptors. Proc. Natl. Acad. Sci. USA *94*, 3384–3387.
5. Troemel, E.R., Kimmel, B.E., and Bargmann, C.I. (1997). Reprogramming chemotaxis responses: Sensory neurons define olfactory preferences in C. elegans. Cell *91*, 161–169.
6. Ward, S., Thomson, N., White, J.G., and Brenner, S. (1975). Electron microscopical reconstruction of the anterior sensory anatomy of the nematode Caenorhabditis elegans. J. Comp. Neurol. *160*, 313–337.
7. Troemel, E.R. Chemosensory receptors in Caenorhabditis elegans. (1999). Ph.D. thesis. University of California, San Francisco: San Francisco.
8. Kurusu, M., Nagao, T., Walldorf, U., Flister, S., Gehring, W.J., and Furukubo-Tokunaga, K. (2000). Genetic control of development of the mushroom bodies, the associative learning centers in the Drosophila brain, by the eyeless, twin of eyeless, and Dachshund genes. Proc. Natl. Acad. Sci. USA *97*, 2140–2144.
9. Martini, S.R., Roman, G., Meuser, S., Mardon, G., and Davis, R.L. (2000). The retinal determination gene, dachshund, is required for mushroom body cell differentiation. Development *127*, 2663–2672.
10. Noveen, A., Daniel, A., and Hartenstein, V. (2000). Early development of the Drosophila mushroom body: the roles of eyeless and dachshund. Development *127*, 3475–3488.
11. Heanue, T.A., Reshef, R., Davis, R.J., Mardon, G., Oliver, G., Tomarev, S., Lassar, A.B., and Tabin, C.J. (1999). Synergistic regulation of vertebrate muscle development by Dach2, Eya2, and Six1, homologs of genes required for Drosophila eye formation. Genes Dev. *13*, 3231–3243.
12. Kida, Y., Maeda, Y., Shiraishi, T., Suzuki, T., and Ogura, T. (2004). Chick Dach1 interacts with the Smad complex and Sin3a to control AER formation and limb development along the proximodistal axis. Development *131*, 4179–4187.
13. Chen, R., Amoui, M., Zhang, Z., and Mardon, G. (1997). Dachshund and eyes absent proteins form a complex and function synergistically to induce ectopic eye development in Drosophila. Cell *91*, 893–903.
14. Hanson, I.M. (2001). Mammalian homologues of the Drosophila eye specification genes. Semin. Cell Dev. Biol. *12*, 475–484.
15. Shen, W., and Mardon, G. (1997). Ectopic eye development in Drosophila induced by directed dachshund expression. Development *124*, 45–52.
16. Ikeda, K., Watanabe, Y., Ohto, H., and Kawakami, K. (2002). Molecular interaction and synergistic activation of a promoter by Six, Eya, and Dach proteins mediated through CREB binding protein. Mol. Cell. Biol. *22*, 6759–6766.
17. Kim, S.S., Zhang, R.G., Braunstein, S.E., Joachimiak, A., Cvekl, A., and Hegde, R.S. (2002). Structure of the retinal determination protein Dachshund reveals a DNA binding motif. Structure *10*, 787–795.
18. Tavsanli, B.C., Ostrin, E.J., Burgess, H.K., Middlebrooks, B.W., Pham, T.A., and Mardon, G. (2004). Structure-function analysis of the Drosophila retinal determination protein Dachshund. Dev. Biol. *272*, 231–247.
19. Perkins, L.A., Hedgecock, E.M., Thomson, J.N., and Culotti, J.G. (1986). Mutant sensory cilia in the nematode Caenorhabditis elegans. Dev. Biol. *117*, 456–487.
20. Ryu, W.S., and Samuel, A.D. (2002). Thermotaxis in Caenorhabditis elegans analyzed by measuring responses to defined Thermal stimuli. J. Neurosci. *22*, 5727–5733.
21. Mori, I., and Ohshima, Y. (1995). Neural regulation of thermotaxis in Caenorhabditis elegans. Nature *376*, 344–348.
22. Hedgecock, E.M., and Russell, R.L. (1975). Normal and mutant thermotaxis in the nematode Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *72*, 4061–4065.
23. Samuel, A.D., Silva, R.A., and Murthy, V.N. (2003). Synaptic activity of the AFD neuron in Caenorhabditis elegans correlates with thermotactic memory. J. Neurosci. *23*, 373–376.
24. Kimura, K.D., Miyawaki, A., Matsumoto, K., and Mori, I. (2004). The C. elegans thermosensory neuron AFD responds to warming. Curr. Biol. *14*, 1291–1295.
25. L'Etoile, N.D., and Bargmann, C.I. (2000). Olfaction and odor discrimination are mediated by the C. elegans guanylyl cyclase ODR-1. Neuron *25*, 575–586.
26. Lanjuin, A., VanHoven, M.K., Bargmann, C.I., Thompson, J.K., and Sengupta, P. (2003). Otx/otd homeobox genes specify distinct sensory neuron identities in C. elegans. Dev. Cell *5*, 621–633.
27. Chang, S., Johnston, R.J., Jr., and Hobert, O. (2003). A transcriptional regulatory cascade that controls left/right asymmetry in chemosensory neurons of C. elegans. Genes Dev. *17*, 2123–2137.
28. Koga, M., and Ohshima, Y. (2004). The C. elegans ceh-36 gene encodes a putative homemodomain transcription factor involved in chemosensory functions of ASE and AWC neurons. J. Mol. Biol. *336*, 579–587.
29. Zhang, Y., Ma, C., Delohery, T., Nasipak, B., Foat, B.C., Bounoutas, A., Bussemaker, H.J., Kim, S.K., and Chalfie, M. (2002). Identification of genes expressed in C. elegans touch receptor neurons. Nature *418*, 331–335.
30. Treisman, J.E. (1999). A conserved blueprint for the eye? Bioessays *21*, 843–850.
31. Wawersik, S., and Maas, R.L. (2000). Vertebrate eye development as modeled in Drosophila. Hum. Mol. Genet. *9*, 917–925.
32. Wenick, A.S., and Hobert, O. (2004). Genomic cis-regulatory architecture and trans-acting regulators of a single interneuron-specific gene battery in C. elegans. Dev. Cell *6*, 757–770.