Current Biology
Minireview

Revisiting Neuronal Cell Type Classification in *Caenorhabditis elegans*

Oliver Hobert[1,*], Lori Glenwinkel[1], and John White[2,*]
[1]Department of Biological Sciences, Columbia University, HHMI, New York, NY 10027, USA
[2]Laboratory for Optical and Computational Instrumentation, University of Wisconsin, Madison, WI 53706, USA
*Correspondence: or38@columbia.edu (O.H.), jwhite1@wisc.edu (J.W.)
http://dx.doi.org/10.1016/j.cub.2016.10.027

We revisit the classification of neuronal cell types in the nervous system of the nematode *Caenorhabditis elegans*. Based on anatomy and synaptic connectivity patterns, the 302 neurons of the nervous system of the hermaphrodite were categorized into 118 neuron classes more than 30 years ago. Analysis of all presently available neuronal gene expression patterns reveals a remarkable congruence of anatomical and molecular classification and further suggests subclassification schemes. Transcription factor expression profiles alone are sufficient to uniquely classify more than 90% of all neuron classes in the *C. elegans* nervous system. Neuron classification in *C. elegans* may be paradigmatic for neuron classification schemes in vertebrate nervous systems.

Introduction

Cataloguing cell types within a brain has been a central aim of neuroscience since the days of Ramon y Cajal. The overall logic behind such categorization is that the brain can be considered as a machine and to understand how a machine works, one needs to catalogue its component parts and their interrelations [1]. Technological advances over the past ten years, mainly in the fields of microscopy and molecular profiling, have led to an explosion of interest in defining neuronal types in the vertebrate brain. Historically, classification schemes that were exclusively based on morphology were extended by electrophysiological criteria (e.g., spiking patterns) [1]. More recently, in-depth profiling of the molecular composition of individual cell types has provided ample means to classify neurons into distinct types [2–6]. Moreover, the recent advent of high-throughput electron microscopy techniques has also led to synaptic connectivity being considered as a major criterion for neuron classification in the vertebrate central nervous system [7]. These recent developments in neuron classification warrant a revisit of the state of classification of the 302 neurons of the hermaphrodite nervous system of the nematode *C. elegans* (Figure 1A).

An Overview of Anatomy- and Connectivity-Based Neuron Classification in *C. elegans*

The electron micrographical reconstruction of the entire *C. elegans* nervous system revealed the precise morphology of all the component neurons, including their synaptic contacts, thereby providing a rich diversity of features that enabled robust cell type classifications to be made [8,9]. Based on their position, morphology, neurite projection patterns and synaptic connectivity, the 302 neurons were classified into 118 distinct classes [8,9]. As shown in Table 1, these 118 neuron classes can be ordered by the number of members per class, which range from one to 13 members per class. Anatomically defined neuronal classes can be summarized as follows:

- 26 classes defined by a single, unilateral neuron with distinctive morphology and connectivity. These neurons can be found in different ganglia and encompass sensory, inter- and motorneurons.
- 70 neuron classes each consisting of a bilaterally symmetric pair of neurons, again located in different ganglia and also encompassing sensory, inter- and motorneurons.
- One class of head motor neuron (SAB), containing 3 members whose processes are organized in a radially symmetric manner (see schematic drawing in Table 1).
- 10 distinct classes defined by 4 members which are organized in a radially symmetric manner. Nine of these are composed of one pair of sub-dorsally located neurons and one pair of sub-ventrally located neurons (see schematic drawing in Table 1). One class (RME) is composed of a lateral pair and a single dorsal and ventral member.
- Three classes (IL1, IL2, RMD), composed of six members each, which are also organized in a radially symmetric manner, but with 3 pairs of neurons each: a sub-dorsal, lateral and sub-ventral pair (see schematic drawing in Table 1).
- Lastly, ventral nerve cord motor neurons fall into 8 anatomically distinct classes (see schematic drawing in Table 1), with 6 to 13 members each. These neurons are aligned in a single row along the ventral midline of the worm.

A Gene Expression Atlas of the *C. elegans* Nervous System

How does this classic, anatomy-based classification scheme match with molecular data? The transcriptome of only a subset of individual neuron types have been relatively exhaustively profiled (e.g., [10–12]). However, over the past 20 years the *C. elegans* community, in the course of a variety of studies, has assembled an unsurpassed resource in the form of thousands of reporter transgenes that monitor gene expression with single cell resolution (see Figure 1B for one example). While these reporter genes may not necessarily capture the complete expression profile of the respective genetic locus, each reporter gene nevertheless provides a robust read-out of an active promoter

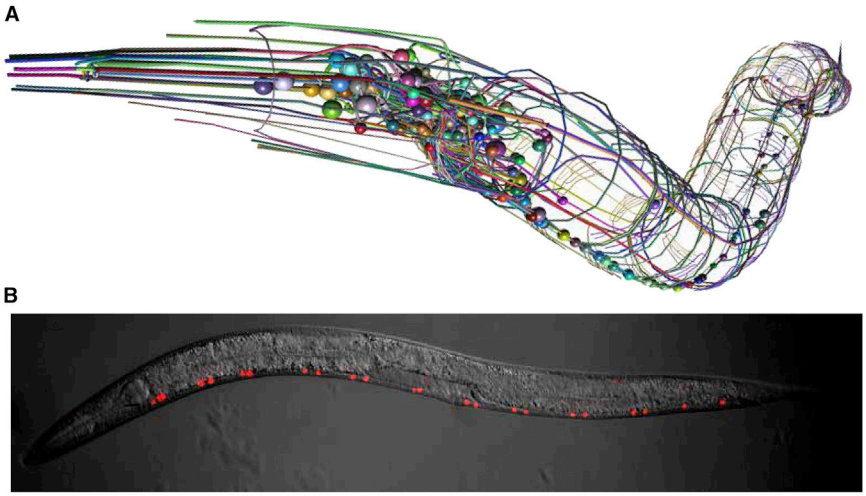


Figure 1. The *C. elegans* nervous system.
(A) Overview of the nervous system of the hermaphrodite. This image was shared by the OpenWorm project. (B) An example of a reporter gene that monitors gene expression in specific neuron types of the nervous system. In this example, a reporter for the *bnc-1* locus monitors expression in a subset of ventral nerve cord motor neurons (VA and VB classes) (Kerk *et al.*, unpublished observations).

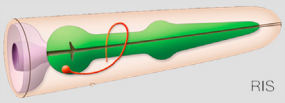
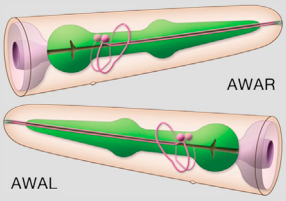
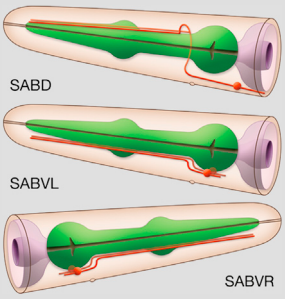
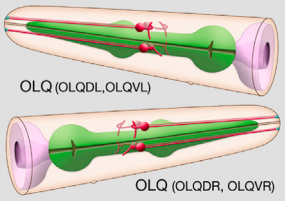
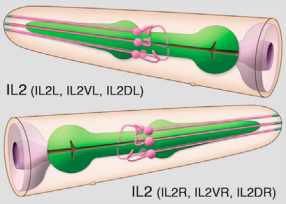
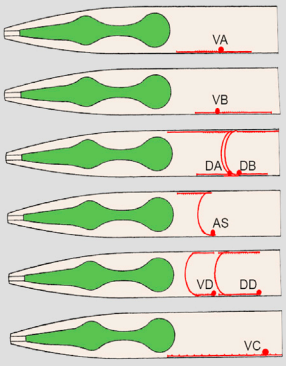
driven by differential transcription factor activity in individual neuron types. The expression of almost 1,000 reporter genes that show a neuron-type specific expression profile (i.e., are expressed in some, but not all cells of the nervous system) has been described with single cell resolution in the nervous system and can readily be extracted from www.wormbase.org. (Table S1 in Supplemental Information, published with this article online). On average, each neuron is associated with the expression of 32 reporter genes (range: 5 to 141 genes) and each reporter is expressed on average in 10 of the 302 neurons (range: 1 to 151 neurons). There are very few examples of genes exclusively expressed in a single neuron class and most of them are either GPCR-type sensory receptors or neuron-identity-specifying transcription factors (*hlh-4*, *che-1*, *odr-7*). Importantly, as expression patterns are relatively hard to determine in the developing embryo, this expression dataset is essentially derived entirely from mature neurons in the larval or adult nervous system. A caveat of this expression dataset are potential errors or omissions in cell identification of transgene expression. Nevertheless, the substantial number of overall data points warrants examination of the expression data.

Molecular Classification Reveals a Remarkable Congruence with Connectivity-Based Classification

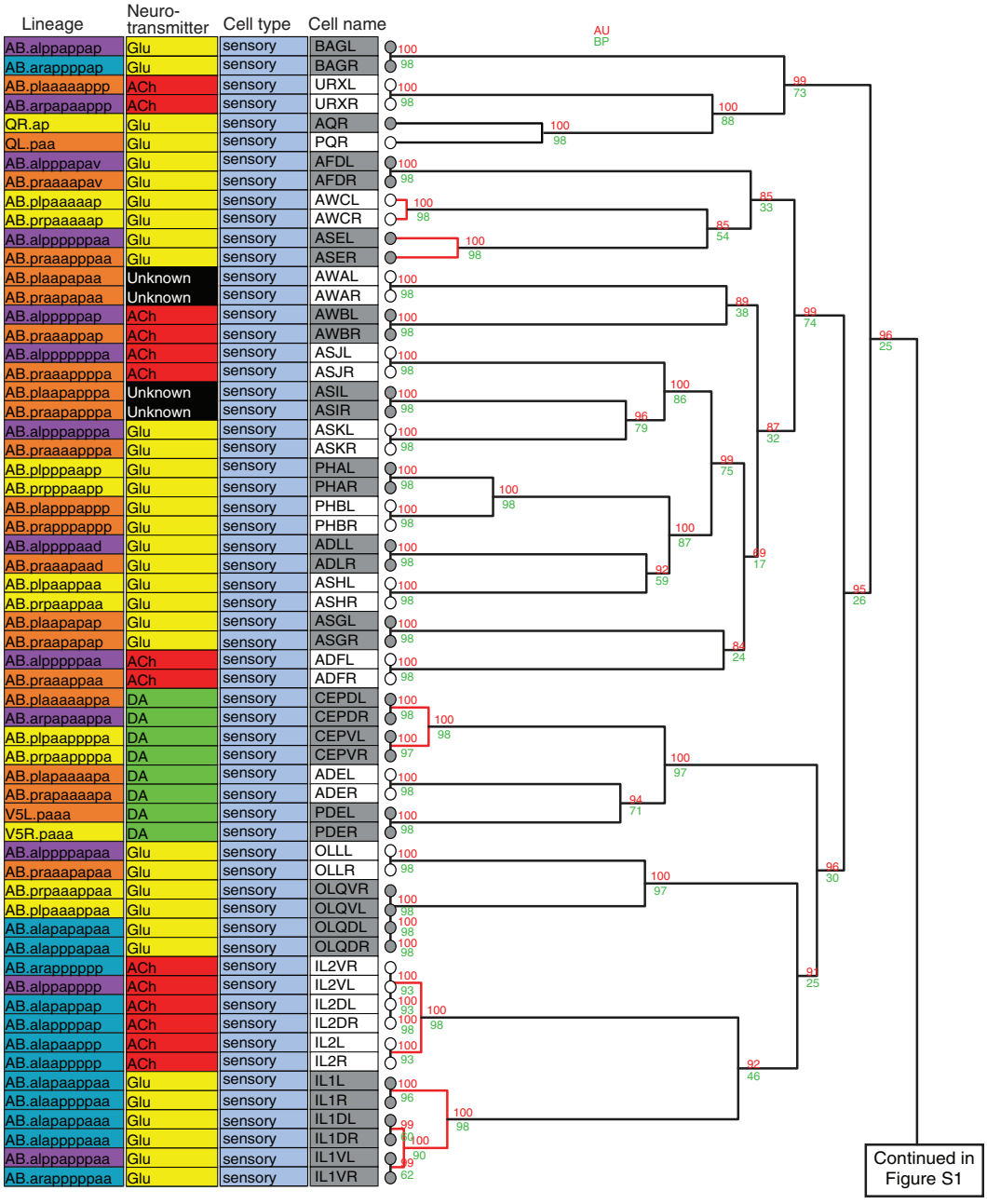
Unsupervised, hierarchical clustering of all neuronal expression patterns, as shown in Figure 2 and Figure S1, reveals a number of intriguing points. First, as they currently stand, the available molecular profiles strongly support the original anatomical classification scheme of 118 distinct classes revealed by electron microscope analysis [8,9]. Neurons that were placed in different classes based on distinct anatomy have very distinct expression profiles and members of the same anatomical class have very similar, if not identical, expression profiles (Figure 2, Figure S1 and Table S1). Moreover, neurons that were placed into distinct classes based on their anatomical features but noted to be quite similar (for example, the AQR and PQR neurons, or the AVM and PVM neurons) are molecularly similar, but nevertheless do express subtle but notable differences in gene expression. The complete agreement between molecular and anatomical classification can be quantified by unbiased support values from bootstrap analysis as described in the Supplemental Information.

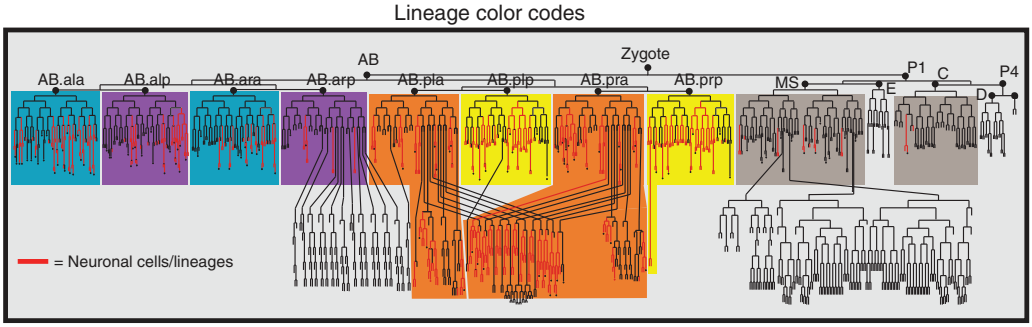
Second, members of the same class can often be subdivided into subclasses based on molecular, but also anatomical, features. This becomes immediately evident if one considers that there are 146 distinct expression profiles revealed by cluster analysis (recall that there are 118 anatomically distinct classes and 302 neurons total). Differences in expression profiles can be observed within distinct neuron classes based on the dendrogram branching patterns shown in color in Figure 2 and Figure S1. Specifically, for all but 1 of the 10 neuron classes composed of dorsal and ventral members, there are molecular differences between the dorsal and ventral cells, supporting their classification into two distinct subtypes (dorsal and ventral). In at least four of these cases (CEP, SAA, SIB, SMB), there are also subtle, but nevertheless clear, synaptic connectivity differences, corroborating this subclassification (the most recent connectivity data, collected in www.wormwiring.org was used for this analysis [13]). For example, the sub-dorsal CEP and sub-ventral CEP neuron pairs are extensively innervated by the same set of 8 neuron classes, yet only the sub-dorsal CEP neurons are also innervated by the URB neurons. A similar subclassification is warranted for the 3 neuron classes composed of 3 pairs, the sub-dorsal, lateral and sub-ventral pairs of the IL1, IL2 and RMD neurons. Although very similar in molecular and connectivity features, members of each class do display molecular and also some notable connectivity differences, the latter being particularly evident for the lateral left/right pair versus the sub-dorsal and sub-ventral pairs. For example, the sub-dorsal, sub-ventral and lateral IL2 neuron pairs are extensively innervated by more than 5 neuron classes, yet only the lateral pair is reciprocally connected to the ADE neurons. Moreover, most, and perhaps all, ventral cord motor neuron classes contain class members with distinct properties. For example, of the nine DA class members, DA8 and DA9 are notably distinct based on molecular features, but also connectivity features. Similarly, the VA2 to VA10 neurons are molecularly similar, while VA1, VA11 and VA12 are molecularly distinct in a subtle but notable manner. VA1 and VA12 indeed also display slight connectivity differences compared to other VA neurons. The genetic and anatomical subclassification of VA neurons is also exemplified by an analysis of *unc-4* mutants, in which VA2–VA10 have altered patterns of connectivity, but VA1, VA11 and VA12 do not [14].

Table 1. Anatomical classification of the 302 neurons of *C. elegans*.

	Examples	Neuron classes *	Subclasses **
Unpaired (single neuron per class)		26 classes (26 neurons): ALA, AQR, AVG, AVL, AVM, DVA, DVB, DVC, PDA, PDB, PQR, PVM, PVR, PVT, RID, RIH, RIR, RIS, I3, I4, I5, I6, M1, M4, M5, MI	None
2-fold symmetry (Left/right pair per class)		70 classes (140 neurons): AWA, AIA, AIB, PHA, PHB, RMG and others	2/70 classes (AWC and ASE) display L/R asymmetric gene expression & functions
3-fold symmetry (3 neurons per class)		1 class (3 neurons): SAB	Two subclasses based on molecular markers and anatomy (dorsal SAB vs. ventral SAB pair)
4-fold symmetry (4 neurons per class: dorsal left/right pair + ventral left/right pair)		10 classes (40 neurons): CEP, OLQ, RME, SAA, SIA, SIB, SMB, SMD, URA, URY	At least 2 subclasses per class: in each class, dorsal and ventral pairs display differences (in all cases molecular differences, in some cases also synaptic connectivity)
6-fold symmetry (6 neurons per class: dorsal left/right pair + lateral left/right pair + ventral left/right pair)		3 classes (18 neurons): IL1, IL2, RMD	At least 2 subclasses per class (similar dorsal/ventral pair versus different lateral left/right pair, based on molecular and synaptic connectivity differences)
VNC motor neurons (each class with 6 to 13 individual, rostrocaudally aligned members)		8 classes (66 neurons total):	
		DA (9 neurons)	At least 4 subclasses based on molecules and/or connectivity
		DB (7 neurons)	
		DD (6 neurons)	
		VA (12 neurons)	At least 4 subclasses based on molecules and/or connectivity
		VB (11 neurons)	
		VC (6 neurons)	At least 3 subclasses based on molecules and/or connectivity
		VD (13 neurons)	
		AS (11 neurons)	At least 2 subclasses based on molecules and/or connectivity
		TOTAL: 118 classes (302 neurons)	**TOTAL:** 146 distinct molecular profiles

Neurons are ordered by number of neurons per class. Individual neuron images are courtesy of Wormatlas.org. Classification is from [8,9]. *Red = motor neuron, blue = sensory neuron, green = interneuron. **As discussed in the text.





(legend on next page)

Intriguingly, the molecular distinctions between subclass members are in some cases as strong as the differences between different classes. In the three most notable cases (SABD vs. SABVL/VR, RMED/V vs. RMEL/R or VC4/5 vs. other VC neurons), distinct subclass members have pronounced anatomical differences in the form of distinct projection patterns. These observations corroborate how closely even a limited number of molecular features track with anatomical features.

Lastly, at least 2 of the 70 neuron classes that are composed of two seemingly bilaterally symmetric neurons can also be subclassified. Specifically, the left and right AWC and the left and right ASE sensory neurons are similar in connectivity and in most expressed genes, but they each express distinct chemosensory receptors and respond differently to chemosensory cues [15,16]. Whether other seemingly bilaterally symmetric neuron pairs also display left/right asymmetric molecular and functional features is currently not known.

While the above conclusions are already apparent by a visual inspection of the tabular expression data, the third set of conclusions only becomes apparent by the hierarchical clustering of gene expression. Specifically, clustering reveals interrelatedness of individual neuron classes, a notion that we emphasize to be preliminary given the nature of the dataset. Some of these tentative molecular similarities mirror similarities in anatomy and connectivity, for example the relationship of phasmid neurons (PHA and PHB) or labial sensory neurons (OLL and IL classes) (Figure 2) and other similarities track well with functional similarities (e.g., the relationship of the nociceptive sensory neurons ADL and ASH). At a higher level it is interesting to see clustering of most pharyngeal neurons, even though support for this cluster is limited (Figure S1).

Transcription Factor Expression Profiles Predict Neuronal Classes

The abundance and diversity of the available nervous system-wide expression data allow specific subsets of genes to be sought that are sufficient to maintain the overall clustering pattern. Indeed, we found that the vast majority of neuron classes (>90%) are uniquely defined by the combinatorial expression of transcription factor-encoding genes (Table S2). Out of the 118 neuron classes, only 4 have not yet been associated with the expression of any transcription factor (RIA, RIM, RIF and RMF) and for three sets of distinct neuron classes the transcription factor combinations are presently the same (RIP, PLN: *ahr-1* + *unc-86*; AVF and VA: *unc-4* + *pag-3*; RMG and ADA: *unc-86* alone). Almost all of the transcription factors that define these neuron class-specific codes are conserved in vertebrates and are used reiteratively in multiple different combinations. Even though only making up ~10% of all transcription factors in the genome, homeodomain-type transcription factors constitute the most frequently occurring type of neuron class-defining transcription factors (see quantification in Table S2) [17].

Strikingly, even subclassifications of neuron classes based on connectivity and molecular profile can, in several cases, be deduced by distinct transcription factor profiles. For example, distinct RME motor neuron subclasses express unique transcription factor combinations (Table S2). While further analysis may reveal other gene families (e.g., ligand-gated ion channels) to provide similar power for neuron classification, profiling of transcription factor expression in the adult nervous system appears to currently provide the most straightforward path for neuron classification in other, more complex nervous systems.

Implications of Neuron Classification in *C. elegans*

The combination of anatomical data and molecular data serves to sharpen the contrast between the often very loosely used terms 'neuron class' (or the often interchangeably used term 'neuron type') and 'neuron subclass' (or the often interchangeably used term 'subtype'). In *C. elegans*, it appears most evident that classes are sets of neurons that share anatomical and molecular features that set them clearly and unambiguously apart from other classes, while subclasses show extensive similarity of expression profiles and synaptic connectivity patterns, yet express a small number of gene products and a small subset of synaptic partner choices that are distinct. This view of subclass diversification makes predictions about the nature of their genetic specification mechanisms. Members of the same class may be specified by the same regulatory factors (class selector genes) that define the differentiated properties of members of a neuron class. Multiple examples of such selector genes have indeed been identified [17,18]. Subclass-specific regulators may in turn act to refine (i.e., either selectively promote or repress) the activity of class selector genes in individual class members. For example, the two bilaterally symmetric ASE neurons are specified by the *che-1* selector while the left and right subclass (ASEL vs. ASER) are specified by additional regulatory factors that restrict the activity of *che-1* on certain target genes [16]. Similarly, all four RME motor neuron class members require the *nhr-67* transcription factor to be properly specified as GABAergic motor neurons, but *nhr-67* interacts with additional subclass-specific transcription factors to drive features that are unique to RME subclasses [19].

C. elegans neuron classification makes additional points about neuronal specification and the genetic mechanisms involved in these specification events. Most strikingly, although stereotyped, lineage has a surprisingly indeterminate impact on neuron

Figure 2. Hierarchical clustering of neurons by reporter expression.
Shown here is a part of the complete molecular clustering diagram shown in Figure S1.
Binary gene expression data are mostly based on reporter gene analysis extracted from Wormbase.org [26]. See supplemental methods for a description of the clustering analysis methodology. To indicate the distinct lineage history of neurons that cluster molecularly, the first column indicates lineage history, with the color code explained in the lineage inset (red lines in the lineage inset indicate neuronal cells/lineages). Note that left/right homologs (derived from an early, single cleavage in the AB lineage along the left/right axis [22]) are labeled with the same color. The second column indicates neurotransmitter identity (acetylcholine, red; glutamate, yellow; GABA, blue; aminergic, different shades of green, depending on type of monoamine; orphan, black), the next column indicates neuron types (sensory neuron, blue; interneuron, grey; motor neuron, orange; pharyngeal neurons, light green) and the last column indicates neuron name. Neuron class shadings are reiterated grey and white to visualize whether neurons belong to the same class. Branching patterns colored red indicate neuronal classes that can be further subdivided into subclasses based on molecular features and, in most cases, also synaptic connectivity features (as discussed in the text). Red numbers, approximately unbiased (AU) support value as percent; green numbers, bootstrap probability (BP) as percent.

class specification (Figure 2 and S1). While similar conclusions have been reached in the context of specific parts of other invertebrate or vertebrate nervous systems [20,21], the comprehensive lineage analysis of the entire *C. elegans* nervous system illustrates this point over and over again throughout all parts of the nervous system. Specifically, the *C. elegans* cell lineage analysis shows that members of the same neuron class can have very diverse lineage histories (Figure 2 and S1) [22]. The corollary of this observation is that neurons that share the same lineage history (i.e., sister cells) almost always belong to very different neuron classes [22]. The complex interplay of lineage and anatomical classification is further emphasized by molecular clustering. The 'molecular' tree shown in Figure 2 and Figure S1 bears essentially no resemblance to the lineage tree, also shown in Figure 2 (see Table S3 and supplemental methods for explicit calculation of lack of correlation). How can these observations be interpreted? It may well be that local inductive interactions may be more significant than cell lineage mechanisms in determining neuron class. Alternatively, distinct lineages may be able to instruct similar cellular identities via shared selector genes that have the capacity to integrate distinct lineage histories. For example, the six lineally distinct IL1 neurons are all specified by the *sox-2* terminal selector transcription factor [23], the six lineally distinct RMD neurons are all specified by the *unc-42* terminal selector [24], the terminal selectors *ast-1* and *ceh-43* specify the lineally distinct CEP neurons [25] and the terminal selector *nhr-67* specifies the four lineally distinct RME neurons [19]. Perhaps the *cis*-regulatory control regions of these terminal selector genes are capable of integrating very distinct lineal inputs.

Vertebrate nervous systems have many orders of magnitude more neurons than *C. elegans*, which makes the determination of neuron connectivity (the connectome) in vertebrates extremely challenging. Nevertheless, clearly defined classes have been described by anatomical and physiological studies in areas such as the cerebellum and retina [1,6]. Although the exact number of vertebrate neuron classes is unknown, it may be no more than an order of magnitude different from the 118 classes described in *C. elegans*. The implication of these comparisons is that the vastly increased capabilities of the vertebrate nervous system compared to that of *C. elegans* is derived from the enormous expansion of neuron numbers rather than neuron classes during evolution.

The recent development of single cell RNA sequencing has allowed the identification of scores of putative cell transcriptomic classes in distinct parts of the mammalian brain [2–4]. The observation that the *C. elegans* connectome is congruent with neuron-specific expression patterns suggests that, by analogy, neuron classes defined by transcriptomics in vertebrates will have members which share the same synaptic specificity and hence connectivity. This opens up the exciting possibility that predictions can be made in vertebrates of the synaptic connectivity of large ensembles of neurons whose individual identities have been determined by gene expression profiles.

Conclusions

In conclusion, we have revisited here the neuron classification scheme of *C. elegans* in the light of new molecular data. The mining of available molecular data corroborates anatomical classification schemes, supports the existence of additional subclasses, reveals possible layers of relatedness of neuron classes and points to transcription factor codes as being succinct 'classifiers' of molecular identity. Transcription factor expression profiles also make predictions about genetic specification mechanisms, including neuron subclass diversification. Lessons learned from *C. elegans* may help to solve the problem of neuron classification and specification in more complex nervous systems.

SUPPLEMENTAL INFORMATION

Supplemental Information contains methods, one figure, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.10.027.

ACKNOWLEDGEMENTS

We thank Sydney Brenner whose vision inspired these studies. We thank Daniela Raciti, Wen Chen and Juancarlos Chan at WormBase for curating and extracting data, Scott Emmons and his group for the WormWiring.org data, WormAtlas and OpenWorm for providing images, Gord Fishell, Raju Tomer and the Hobert lab for comments on the manuscript and the Howard Hughes Medical Institute for funding.

REFERENCES

1. Masland, R.H. (2004). Neuronal cell types. Curr. Biol. *14*, R497–R500.
2. Tasic, B., Menon, V., Nguyen, T.N., Kim, T.K., Jarsky, T., Yao, Z., Levi, B., Gray, L.T., Sorensen, S.A., Dolbeare, T., *et al*. (2016). Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. Nat. Neurosci. *19*, 335–346.
3. Zeisel, A., Munoz-Manchado, A.B., Codeluppi, S., Lonnerberg, P., La Manno, G., Jureus, A., Marques, S., Munguba, H., He, L., Betsholtz, C., *et al*. (2015). Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science *347*, 1138–1142.
4. Macosko, E.Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A.R., Kamitaki, N., Martersteck, E.M., *et al*. (2015). Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. Cell *161*, 1202–1214.
5. Nelson, S.B., Hempel, C., and Sugino, K. (2006). Probing the transcriptome of neuronal cell types. Curr. Opin. Neurobiol. *16*, 571–576.
6. Poulin, J.F., Tasic, B., Hjerling-Leffler, J., Trimarchi, J.M., and Awatramani, R. (2016). Disentangling neural cell diversity using single-cell transcriptomics. Nat. Neurosci. *19*, 1131–1141.
7. Seung, H.S., and Sumbul, U. (2014). Neuronal cell types and connectivity: lessons from the retina. Neuron *83*, 1262–1272.
8. White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. *314*, 1–340.
9. Albertson, D.G., and Thomson, J.N. (1976). The pharynx of Caenorhabditis elegans. Philos. Trans. R. Soc. Lond. B Biol. Sci. *275*, 299–325.
10. Spencer, W.C., McWhirter, R., Miller, T., Strasbourger, P., Thompson, O., Hillier, L.W., Waterston, R.H., and Miller, D.M., 3rd. (2014). Isolation of specific neurons from C. elegans larvae for gene expression profiling. PLoS One *9*, e112102.
11. 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.
12. Von Stetina, S.E., Watson, J.D., Fox, R.M., Olszewski, K.L., Spencer, W.C., Roy, P.J., and Miller, D.M., 3rd. (2007). Cell-specific microarray profiling experiments reveal a comprehensive picture of gene expression in the C. elegans nervous system. Genome Biol. *8*, R135.

13. Varshney, L.R., Chen, B.L., Paniagua, E., Hall, D.H., and Chklovskii, D.B. (2011). Structural properties of the Caenorhabditis elegans neuronal network. PLoS Comput. Biol. *7*, e1001066.

14. White, J.G., Southgate, E., and Thomson, J.N. (1992). Mutations in the Caenorhabditis elegans unc-4 gene alter the synaptic input to ventral cord motor neurons. Nature *355*, 838–841.

15. Hsieh, Y.W., Alqadah, A., and Chuang, C.F. (2014). Asymmetric neural development in the Caenorhabditis elegans olfactory system. Genesis *52*, 544–554.

16. Hobert, O. (2014). Development of left/right asymmetry in the Caenorhabditis elegans nervous system: from zygote to postmitotic neuron. Genesis *52*, 528–543.

17. Hobert, O. (2016). A map of terminal regulators of neuronal identity in Caenorhabditis elegans. Dev. Biol. *5*, 474–498.

18. Hobert, O. (2016). Terminal selectors of neuronal identity. Curr. Top. Dev. Biol. *116*, 455–475.

19. Gendrel, M., Atlas, E.G., and Hobert, O. (2016). A cellular and regulatory map of the GABAergic nervous system of C. elegans. eLife *5*, e17686.

20. Fishell, G., and Heintz, N. (2013). The neuron identity problem: form meets function. Neuron *80*, 602–612.

21. Ready, D.F., Hanson, T.E., and Benzer, S. (1976). Development of the Drosophila retina, a neurocrystalline lattice. Dev. Biol. *53*, 217–240.

22. Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode Caenorhabditis elegans. Dev. Biol. *100*, 64–119.

23. Vidal, B., Santella, A., Serrano-Saiz, E., Bao, Z., Chuang, C.F., and Hobert, O. (2015). C. elegans SoxB genes are dispensable for embryonic neurogenesis but required for terminal differentiation of specific neuron types. Development *142*, 2464–2477.

24. Pereira, L., Kratsios, P., Serrano-Saiz, E., Sheftel, H., Mayo, A.E., Hall, D.H., White, J.G., LeBoeuf, B., Garcia, L.R., Alon, U., *et al*. (2015). A cellular and regulatory map of the cholinergic nervous system of C. elegans. eLife *4*, e12432.

25. Doitsidou, M., Flames, N., Topalidou, I., Abe, N., Felton, T., Remesal, L., Popovitchenko, T., Mann, R., Chalfie, M., and Hobert, O. (2013). A combinatorial regulatory signature controls terminal differentiation of the dopaminergic nervous system in C. elegans. Genes Dev. *27*, 1391–1405.

26. Harris, T.W., Baran, J., Bieri, T., Cabunoc, A., Chan, J., Chen, W.J., Davis, P., Done, J., Grove, C., Howe, K., *et al*. (2014). WormBase 2014: new views of curated biology. Nucleic Acids Res. *42*, D789–D793.