Terminal Selectors of Neuronal Identity

Oliver Hobert
Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University, New York, USA
1Corresponding author: e-mail address: or38@columbia.edu

Contents

1. Historical Background 1
2. Coregulation of Terminal Effector Genes by Terminal Selectors 5
3. Terminal Selectors Initiate and Maintain the Terminally Differentiated State 9
4. Terminal Selectors Act in Reiteratively Used Combinations 10
5. Terminal Selectors Control Regulatory Subroutines 12
6. Parallel Regulatory Routines 13
7. Diversification of Neuronal Identity via Selective Repressibility 15
8. Generality of the Terminal Selector Concept 15
9. Regulation of Terminal Selector Genes 17
10. Conclusions 17
Acknowledgments 17
References 18

Abstract
The analysis of the developmental programs that define many different neuron types in the nematode Caenorhabditis elegans has revealed common themes in how distinct terminal differentiation programs are controlled. Rather than being controlled in a piece-meal manner, terminal identity features of a mature neuron are often coregulated by so-called terminal selector transcription factors. Here, I summarize the terminal selector concept and emphasize core features of this concept in the C. elegans system such as coregulation of terminal effector batteries, combinatorial control mechanisms, and the coupling of initiation and maintenance of neuronal identity.

1. HISTORICAL BACKGROUND
Nervous system development has traditionally been studied with a “top-down” focus, defining inductive events early in embryonic patterning and elucidating signaling events and transcriptional regulatory cascades that
progressively restrict developmental fates as embryogenesis proceeds (Edlund & Jessell, 1999; Hemmati-Brivanlou & Melton, 1997). This “top-down” approach is well illustrated by Waddington’s landscape model in which a ball travels down specific valleys, making several choices along the way (Waddington, 1957). An alternative perspective, the “bottom-up” angle, views the problem of neuronal development from the standpoint of the end product of neuronal development, asking how the terminal features of specific neuron types in a mature nervous system are genetically programmed. The bottom-up perspective has been extensively taken in the *Caenorhabditis elegans* field, not by design but as the result of the initial efforts of Sydney Brenner to identify *C. elegans* mutants that affect specific animal behaviors. Brenner’s first behavioral mutants displayed various types of uncoordinated (*unc*) locomotory behavior (Brenner, 1974). The ensuing establishment of various assays that measure the response of animals to specific sensory cues then lead to the identification of viable mutants that selectively affected very specific behaviors such as chemotaxis behavior (Lewis & Hodgkin, 1977), theromotaxis behavior (Hedgecock & Russell, 1975), mechanosensory behavior (Chalfie & Sulston, 1981), or odortaxis behavior (Sengupta, Colbert, & Bargmann, 1994). Strikingly, the molecular characterization of these behavioral mutants showed that many of them affect transcription factors that turned out to act at the terminal step of differentiation of postmitotic neurons (listed in Table 1; Baran et al., 1999; Finney, Ruvkun, & Horvitz, 1988; Hobert et al., 1997; Jin et al., 1994; Prasad et al., 1998; Satterlee et al., 2001; Sengupta et al., 1994; Uchida et al., 2003; Way & Chalfie, 1988).

The phenotypic analysis of these mutants profited from some of the key defining features of the *C. elegans* system: (a) an exceptionally well-described cell lineage history (Sulston, 1983), (b) a most detailed anatomical description of all individual neuron types (White, Southgate, Thomson, & Brenner, 1986), (c) the knowledge of functional features of many individual neuron types based on laser ablation (e.g., Bargmann & Horvitz, 1991; Mori & Ohshima, 1995), and (d) the availability of a vast collection of molecular markers in the form of *gfp* reporter transgenes (Chalfie, Tu, Euskirchen, Ward, & Prasher, 1994). These molecular markers, built by the community over the past 20 years (curated at www.wormbase.org), monitor the neuron-type specific expression of hundreds of genes that define terminal properties of a neuron type (e.g., genes encoding neurotransmitter synthesis, receptors, ion channel, etc.). Together, these anatomical, functional, and molecular features assign precise and unique “phenotypic spaces” to
<table>
<thead>
<tr>
<th>Gene</th>
<th>Transcription Factor Type</th>
<th>Behavioral Phenotype Leading to Identification</th>
<th>Neuron Class Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>unc-3</em></td>
<td>EBF/COE</td>
<td>Uncoordinated locomotion&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cholinergic ventral cord motorneurons&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>unc-30</em></td>
<td>Pitx-type homeodomain</td>
<td>Uncoordinated locomotion&lt;sup&gt;a&lt;/sup&gt;</td>
<td>GABAergic ventral cord motorneurons&lt;sup&gt;c&lt;/sup&gt;, Cholinergic PVP interneurons&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>unc-42</em></td>
<td>Homeodomain</td>
<td>Uncoordinated locomotion&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Glutamatergic ASH sensory neurons&lt;sup&gt;e&lt;/sup&gt;, Glutamatergic AIB interneurons&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>unc-86</em></td>
<td>Brn3-type POU homeodomain</td>
<td>Meanosensory defects&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Meanoansory ALM/AVM/PLM/PVM neurons&lt;sup&gt;h&lt;/sup&gt;, Serotonergic HSN motor neurons&lt;sup&gt;i&lt;/sup&gt;, Serotonergic NSM neurons&lt;sup&gt;j&lt;/sup&gt;, Inner labial IL2 sensory-motor neurons&lt;sup&gt;k&lt;/sup&gt;, URA motor neurons&lt;sup&gt;l&lt;/sup&gt;, URB interneurons&lt;sup&gt;m&lt;/sup&gt;, Glutamatergic URX sensory neurons&lt;sup&gt;n&lt;/sup&gt;, Glutamatergic AQR/PQR sensory neurons&lt;sup&gt;n&lt;/sup&gt;, Glutamatergic PVR interneuron&lt;sup&gt;n&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>mec-3</em></td>
<td>Lhx1/5 LIM homeodomain</td>
<td>Meanosensory defects&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Meanoansory ALM/AVM/PLM/PVM neurons&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>che-1</em></td>
<td>C2H2 Zn finger</td>
<td>Chemotaxis defects&lt;sup&gt;m&lt;/sup&gt;</td>
<td>Gustatory ASE sensory neurons&lt;sup&gt;n&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>ttx-1</em></td>
<td>Otx homeodomain</td>
<td>Thermotaxis defects&lt;sup&gt;o&lt;/sup&gt;</td>
<td>Thermosensory AFD neuron&lt;sup&gt;p&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Continued*
individual neuron types which then permitted a detailed assessment of how the regulatory factors defined by the classic behavioral mutants (listed in Table 1) affect neuronal differentiation. Strikingly, all of the transcription factors listed in Table 1 were found to have very broad effects on the adoption of the correct terminal identity of very specific neuron types. They

Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Transcription Factor Type</th>
<th>Behavioral Phenotype Leading to Identification</th>
<th>Neuron Class Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>ttx-3</td>
<td>Lhx2/7 LIM homeodomain</td>
<td>Thermotaxis defects&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cholinergic AIY interneuron&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cholinergic AIA interneuron&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serotonergic NSM neuron&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Glutamatergic ASK sensory neuron&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>odr-7</td>
<td>C4 Zn finger</td>
<td>Odortaxis defects&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Olfactory AWA neuron&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Brenner (1974).
<sup>b</sup>Kratsios, Stolfi, Levine, and Hobert (2011), Kratsios et al. (2015), and Prasad et al. (1998). unc-3 locomotory defects match the effect expected from ablating of A- and B-type motor neurons (Chalfie et al., 1985).
<sup>c</sup>Jin, Hoskins, and Horvitz (1994). unc-30 locomotory defects match the defects observed upon losing signaling by GABAergic D-type motor neurons (McIntire, Jorgensen, & Horvitz, 1993).
<sup>d</sup>Pereira et al. (2015).
<sup>e</sup>Baran, Aronoff, and Garriga (1999) and Serrano-Saiz et al. (2013). unc-42 mutants phenocopy the sensory defects observed in ASH-ablated animals (Baran et al., 1999).
<sup>f</sup>E. Serrano-Saiz and O. Hobert (unpublished data).
<sup>g</sup>Chalfie and Sulston (1981).
<sup>h</sup>Duggan, Ma, and Chalfie (1998). unc-86 mutants phenocopy ablation of mecanosensory neurons (Chalfie et al., 1985).
<sup>i</sup>Desai, Garriga, McIntire, and Horvitz (1988). unc-86 mutants phenocopy loss of HSN neurons.
<sup>j</sup>Serrano-Saiz et al. (2013).
<sup>k</sup>Duggan et al. (1998), Way and Chalfie (1988), and Zhang et al. (2002). mec-3 mutants phenocopy ablation of mecanosensory neurons (Chalfie et al., 1985).
<sup>l</sup>Lewis and Hodgkin (1977).
<sup>m</sup>Etchberger et al. (2007) and Uchida, Nakano, Koga, and Ohshima (2003). dve-1 mutants phenocopy the ablation of the ASE neurons (Bargmann & Horvitz, 1991).
<sup>n</sup>Hedgecock and Russell (1975).
<sup>o</sup>Satterlee et al. (2001). ttx-1 mutants phenocopy ablation of the AFD neurons (Mori & Ohshima, 1995).
<sup>p</sup>Altun-Gultekin et al. (2001). ttx-3 mutants phenocopy ablation of the AFD neurons (Mori & Ohshima, 1995; Tsilik & Hobert, 2003).
<sup>q</sup>Sengupta et al. (1994).
<sup>r</sup>odr-7 mutants phenocopy ablation of the AWA neurons (Bargmann, Hartwieg, & Horvitz, 1993).

Many additional terminal selectors were identified through forward and reverse genetic approaches and are not listed here. See an upcoming review (O. Hobert, WIREes Dev. Biol., in preparation) for a more comprehensive summary. As illustrated in Fig. 2, one terminal selector can control distinct neuronal differentiation programs through its pairing with distinct cofactors.
affect the functional properties of the respective neuron types in the sense that the behavioral phenotypes of these mutants match the phenotypic consequences of laser ablating these neuron types and they broadly affect many—if not all—known molecular markers normally expressed specifically in the respective neuron; however, they do not affect the generation of these neurons. Given the neuron identity-defining properties, I proposed to call these transcription factors “terminal selectors” (Hobert, 2008, 2011). This terminology is an extension of the selector gene concept, originally proposed by Garcia-Bellido (1975). “Classic” selector genes are genes that define the identity of specific domains of a developing organism and they act transiently during specific phases of development. In contrast, terminal selectors control the terminally differentiated state of a neuron, i.e., they initiate and maintain the stable identity of nondividing differentiated cells.

I will provide here a concise summary of the properties of terminal selectors as revealed primarily by studies in C. elegans, and I will organize these properties around specific themes that are also highlighted in Fig. 1. The description of these properties is based on the few original examples that led to the original terminal selector definition (Hobert, 2008, 2011) but also covers a plethora of recent examples that have allowed further generalization of this concept. The themes that I will discuss are

- Identity-defining terminal effector genes are coregulated.
- Terminal selectors initiate and maintain the terminally differentiated state.
- Terminal selectors act in reiteratively used combinations.
- Terminal selectors control regulatory subroutines.
- Parallel regulatory routines define specific aspects of neuronal identity.
- Diversification of neuronal identity is achieved via selective gene repression.
- Generality of the terminal selector concept.

2. COREGULATION OF TERMINAL EFFECTOR GENES BY TERMINAL SELECTORS

Every fully differentiated neuron type is uniquely defined by the expression of a unique combination of “nuts and bolts,” “terminal effector” genes that code for proteins that define the functional features of a mature neuron type, such as neurotransmitter receptors, neurotransmitter synthesizing enzymes, neuropeptides, ion channels, sensory receptors, and synaptic recognition molecules (see Table 2 for an exemplary list of terminal effector genes). As illustrated in Fig. 1, the identification of cis-regulatory elements
Continuous expression (autoregulation) Operate in combinations Hourglass topology Direct coregulation Parallel regulatory routines Subroutines Neuron-type specific identity Unique identity Shared with other neuron types Terminal effectors: (Neurotransmitter synthesis & transport, ion channels, receptors, synaptic connectivity neuropeptides, etc.) Pansensory identity Panneuronal identity Morphological features Modular organization of effectors

Figure 1 See legend on opposite page.
required for the coexpression of terminal effector genes in specific neuron types, and the analysis of the expression of many terminal effector genes in many different terminal selector mutant backgrounds demonstrated a simple common theme: neuron-type-specific terminal effector batteries are organized into regulons, i.e., they are all coregulated, and this coregulation is mediated by shared cis-regulatory motifs and their cognate transcription factors in the form of specific terminal selector transcription factors (or combination thereof; as discussed below) (Cinar et al., 2005; Eastman et al., 1999; Etchberger et al., 2007; Flames & Hobert, 2009; Gordon & Hobert, 2015; Kratsios et al., 2011; Wenick & Hobert, 2004; Zhang et al., 2002).

The broadness of the effect that terminal selectors exert on neuronal identity is illustrated by the way that these effects have been measured in many different neurons types: the repertoire of gfp reporter transgenes that the C. elegans community has built over the past two decades is large enough that for the majority of the 118 anatomically defined neuron types anything between ~3 and several dozen molecular markers have been described. These reporters most often mark biochemically unlinked molecular features of a neuron and provide a “random” snapshot of the identity of a neuron. In most cases examined, terminal selectors affect either all or most of the markers examined. In several instances many dozen markers were tested (Etchberger et al., 2007; Kratsios et al., 2011; Wenick & Hobert, 2004), in others just a handful of markers were tested (e.g., Serrano-Saiz et al.,

Figure 1 Key features of gene expression programs in mature neuron types. (A) Terminal selector regulons. Red (dark gray in the print version) squares highlight key concepts. Sustained expression of terminal selectors is often, but not always ensured by autoregulation. Small rectangles illustrate binding sites for transcription factors and indicate direct regulation of effector genes by terminal selectors. Terminal selectors shown on the left control neuron-type specific identity features that assign a unique identity to a neuron. See Table 2 for an exemplary list of effector genes. Parallel regulatory routines such as those that regulate pansensory features (via DAF-19) are controlled by factors that could also considered to be terminal selectors; but they do not assign unique identities. Morphology regulators control generic aspect of a neuron’s morphology, such as placement of axons/dendrites into specific fascicles or axo/dendritic polarity. TF, transcription factor. (B) Modular organization of terminal effector genes. X1 is a representative of any effector gene expressed in more than one neuron type (e.g., the vesicular transporter of glutamate, which is expressed in all glutamatergic neurons, but not in GABA or cholinergic neurons). This schematic also indicates the key principle of combinatorial “reusage” of the same terminal selector in different neuron types, as schematized in more detail in Fig. 2.
Ideally, one would want to be able to profile neurons in wild-type and terminal selector-deficient neurons to assess the full extent of the effects. While this has not been done yet, the random sampling of the markers examined, combined with the broad defects observed in terminal selector mutants, make the assessment of effects of terminal selector on neuronal identity only an extrapolation, albeit a reasonable one.

Coregulation of a multitude of identity features is not self-evident. A priori, one could easily envision that individual identity features could be independently regulated via distinct transcriptional regulatory factors. Transcriptome profiling studies of individual neuron types (in invertebrates or vertebrates) usually reveal dozens of transcription factors expressed in a mature neuron type (e.g., Etchberger et al., 2007), hence the job of initiating

### Table 2 Examples of Effector Genes of Terminal Selectors

<table>
<thead>
<tr>
<th>Categories</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurotransmitter pathway synthesis</td>
<td>GAD, TPH, TH, etc.</td>
</tr>
<tr>
<td>Vesicular neurotransmitter transporters</td>
<td>VGLUT, VGAT, VMAT, VACHT</td>
</tr>
<tr>
<td>Neurotransmitter receptors</td>
<td>ACh-gated, Glu-gated, GABA-gated ion channels, metabotropic ACh-, Glu-receptors, biogenic amine receptors</td>
</tr>
<tr>
<td>Ion channels</td>
<td>DEG/EnaC, TRP, TWK, Ca^{2+}, K^{+} channels</td>
</tr>
<tr>
<td>Gap junctions</td>
<td>Innexins</td>
</tr>
<tr>
<td>Sensory receptors</td>
<td>Olfactory GPCRs, receptor-type guanylyl cyclases, mechanosensory MEC channels</td>
</tr>
<tr>
<td>Neuropeptides</td>
<td>FMRFamides, NLPs</td>
</tr>
<tr>
<td>Neuropeptide receptors</td>
<td>FMRFamide-, tachykinin-, somatostatin-like receptors (and others)</td>
</tr>
<tr>
<td>Synaptic organizers</td>
<td>oig-1, madd-4, neuroligin</td>
</tr>
<tr>
<td>Signaling</td>
<td>TGFβ (dbl-1, unc-129), Slit/slt-1, G-proteins</td>
</tr>
<tr>
<td>Others</td>
<td>Tetraspanins, IgSF, etc.</td>
</tr>
</tbody>
</table>

The table is meant to illustrate the range of biochemical activities that are under terminal selector control. The examples shown were derived from the following studies: Cinar, Keles, and Jin (2005), Eastman, Horvitz, and Jin (1999), Etchberger et al. (2007), Flames and Hobert (2009), Gordon and Hobert (2015), Howell, White, and Hobert (2015), Kratsios et al. (2015, 2011), Serrano-Saiz et al. (2013), Wenick and Hobert (2004), and Zhang et al. (2002).
and maintaining the expression of terminal gene batteries could, in theory, be distributed over a number of distinct factors. However, as studies on the transcription factors shown in Table 1 (as well as ensuing analysis of additional terminal selectors) revealed, the expression of many, perhaps most terminal markers for neuronal identity is affected in terminal selector mutants, indicating that coregulation is achieved via a limited number of regulatory factors.

Even in a nervous system as comparatively simple as *C. elegans*, only very few terminal effector genes are exclusively expressed in a single neuron type (e.g., olfactory receptor proteins). The much more prevalent scenario is that genes, like neurotransmitter receptor subunits, neurotransmitter synthesizing enzymes, neuropeptides, or various types of ion channels are expressed in several distinct neuron types. In each specific neuron type, these effector genes are under control of neuron-type specific terminal selectors. This effectively means that such effector genes harbor a modular array of response elements each activated by the different terminal selectors present in each neuron class where it is expressed (Fig. 1B). This quite intuitive concept has been experimentally validated in a number of cases, including the loci encoding the vesicular glutamate transporter (*Serrano-Saiz et al., 2013*) or the vesicular acetylcholine or choline transporter (*Kratsios et al., 2011; Wenick & Hobert, 2004*).

### 3. TERMINAL SELECTORS INITIATE AND MAINTAIN THE TERMINALLY DIFFERENTIATED STATE

A key defining feature of terminal selectors is that they are not only required to initiate terminal differentiation programs but are continuously expressed throughout the life of the neuron (and animal) to maintain the differentiated state of a neuron (*Doitsidou et al., 2013; Etchberger, Flowers, Poole, Bashllari, & Hobert, 2009; Flames & Hobert, 2009; Kratsios et al., 2011*). This has been amply illustrated through temporally controlled, late removal of terminal selectors not just in *C. elegans* but also in mice (*Deneris & Hobert, 2014; Holmberg & Perlmann, 2012; Laguna et al., 2015*). The continuous expression of terminal selectors is often ensured through autoregulatory feedback mechanisms (*Baran et al., 1999; Etchberger et al., 2007; Hobert et al., 1997; Sagasti, Hobert, Troemel, Ruvkun, & Bargmann, 1999; Way & Chalfie, 1989*).

This continuous role sets terminal selectors clearly apart from earlier and transiently acting transcription factors. For example, the *C. elegans* Zic gene
ortholog REF-2 is required for the terminal differentiation of the A1Y interneurons, but it is only transiently expressed in the progenitor of the A1Y interneurons, where it acts to initiate the expression of the two cooperatively acting terminal selectors of A1Y identity, ttx-3 and ceh-10 (Bertrand & Hobert, 2009). If the only role of REF-2 is to turn a terminal selector, REF-2 should not be considered a terminal selector because it is not required to maintain the differentiated state and it cannot be considered to be the “terminal” link of a regulatory cascade to a terminal effector gene (such as a neurotransmitter pathway gene). In principle, one could envision a scenario in which expression of a terminal effector is initiated by a transiently acting factor binding to some cis-regulatory element in the effector gene locus and its expression is then maintained via a separate cis-regulatory element that is controlled by a terminal selector. However, such a scenario has not yet been documented.

4. TERMINAL SELECTORS ACT IN REITERATIVELY USED COMBINATIONS

Terminal selectors are usually not single proteins that operate in complete isolation. Terminal selectors rather function in the context of combinations of cooperating factors (Fig. 2; Wenick & Hobert, 2004; Xue, Tu, & Chalfie, 1993; Zhang et al., 2014). In one recently published example, four terminal selectors cooperate in a given neuron type (Doitsidou et al., 2013). In an unpublished example, seven terminal selectors cooperate on the level of the cis-regulatory elements of the downstream effector genes (N. Flames and O. Hobert, unpublished data). In many cases, no partners for terminal selectors have been identified, but this should not be taken as evidence that terminal selectors can act completely alone.

The biochemical basis for cooperation of terminal selectors appears to be distinct in different cellular contexts. In some cases, terminal selectors exhibit cooperative DNA binding (Wenick & Hobert, 2004; Xue et al., 1993). For example, the UNC-86 and MEC-3 homeodomain proteins bind cooperatively to cis-regulatory elements required for the expression of effector genes that define touch neuron identity (Duggan et al., 1998; Xue et al., 1993). In other cases, terminal selectors may bind DNA in a noncooperative manner, but exert cooperative effects possibly through the cooperative recruitment of cofactors (Doitsidou et al., 2013; Gordon & Hobert, 2015). In yet other cases, the type and extent of cooperation is unclear. For example, the serotonergic NSM neurons fail to terminally differentiate in double mutant
animals lacking the POU homeobox gene \textit{unc-86} and the LIM homeobox gene \textit{ttx-3} (Zhang et al., 2014). However, in \textit{ttx-3} and \textit{unc-86} single mutants, some identity features are either not affected at all or are already affected as strongly as in the double mutant. There may be a spectrum of distinct types of cooperation between terminal selectors, depending on \textit{cis}-regulatory architecture of individual effector genes.

There are a number of key implications of the combinatorial activity of terminal selectors:

\textbf{(1)} It explains how one and the same terminal selector can control very distinct fates. For example, UNC-86 is required to define the terminal identity of vastly different neuron types (Table 1) and in several cases we understand how. UNC-86 works cooperatively with MEC-3 to control touch neuron effector genes via a well-defined UNC-86/MEC-3 binding site (Xue et al., 1993). In contrast, in the BDU neurons, UNC-86 binds to different sites and collaborates with another factor, the Zn finger transcription factor PAG-3 (Gordon & Hobert, 2015).

\textbf{(2)} Conversely, the combinatorial logic also explains why one factor can operate as a terminal selector in one cell type, while not having any discernible effect on the same set of target genes in another cell type.
For example, the *ttx-3* LIM homeobox genes, expressed in the three cholinergic interneuron types AIY, AIA, and AIN, controls the cholinergic properties of the AIY and AIA interneurons (as well as all other known properties of these neurons), but does not control the cholinergic identity of the AIN interneurons (Zhang et al., 2014).

(3) Combinatorial activities provide a solution to the “specificity problem” of transcription factors. Single transcription factors usually interact with only about 4–12 nucleotides. Because such small sites are found too commonly in the genome, the activation of very specific target genes is likely mediated by the much more restrictive co-occurrence of combinations of terminal selector binding sites.

(4) Combinatorial function can also provide a solution to the “sufficiency problem.” In many cases tested, terminal selectors found to be required to control a specific differentiation program are sufficient to induce this differentiation program in only some cellular contexts (e.g., Tursun, Patel, Kratsios, & Hobert, 2011; Wenick & Hobert, 2004). A broader effect may be achieved only upon misexpression of the complete set of combinatorially acting terminal selectors.

The examination of more and more *C. elegans* neuron types has uncovered another striking feature of transcription factor combinations. Even though the *C. elegans* genome has a repertoire of almost 1000 transcription factors, most terminal selectors are homeodomain transcription factors, which constitute only 10% of all *C. elegans* transcription factors. In those cases where non-homeodomain terminal selectors have been identified (e.g., the ETS domain factor AST-1), the non-homeodomain factor cooperates with a homeodomain transcription factor (Doitsidou et al., 2013; Serrano-Saiz et al., 2013). In cases where no homeodomain factor has been identified, it is conceivable that a homeodomain factor still awaits identification. The preponderance of homeodomain transcription factors may be a reflection of their very ancient use as drivers of neuronal identity.

5. TERMINAL SELECTORS CONTROL REGULATORY SUBROUTINES

A number of transcription factors have been identified that control only some, and perhaps in total very few genes in a terminally differentiated neuron. One example is the *ceh-23* homeobox gene which affects expression of a GPCR gene, but not other identity aspects of the cholinergic AIY interneuron (Altun-Gultekin et al., 2001); similarly, the *ceh-14* homeobox gene affects expression of neuropeptides in the BDU neurons, but does not affect
other BDU identity features (Gordon & Hobert, 2015). Notably, in all these cases, these transcriptional subroutines are under direct control of a terminal selector (schematized in Fig. 1A). That is, the ceh-23 gene, as well as its target, the GPCR gene sra-11, are under direct control of the terminal selectors of AIY identity, ttx-3 and ceh-10, which control scores of other terminal identity features of the AIY interneuron (Altun-Gultekin et al., 2001; Wenick & Hobert, 2004). Similarly, ceh-14 and its neuropeptide targets are controlled by the terminal selectors of BDU identity, unc-86 and pag-3 (Gordon & Hobert, 2015). Hence, in systems biology parlance, terminal selector frequently acts in feedforward loops (Alon, 2006) to control effector genes (Fig. 1A). It is easily conceivable that any terminal selector may regulate scores of regulatory subroutines. For example, the terminal selector che-1 directly controls not only scores of nuts- and bolts-type terminal identity features (e.g., chemoreceptors, neuropeptides, and channels) via well-defined CHE-1 binding sites but also controls the expression of dozens of transcription factors in the ASE gustatory neurons; each of these transcription factors may control specific regulatory subroutines (Etchberger et al., 2007). Indeed, some of these transcription factors ensure the left/right asymmetric expression of chemoreceptors in the left or right ASE neurons, yet these factors do not control other identity features of the ASE neurons (Chang, Johnston, & Hobert, 2003; Hobert, 2014).

Should those subroutine regulators also be considered as “terminal selectors” of these subroutines? The most striking difference is that in all known cases, loss of these subroutine regulators still leaves the affected neuron with a recognizable identity. For example, while loss of ceh-14 leads to loss of neuropeptide genes in BDU, the neuron is still recognizable as a BDU neuron in terms of intact expression of many other marker genes and overall morphology. In contrast, loss of pag-3, the terminal selector of BDU results in loss of all known molecular markers and alterations in neuron morphology. Even in cases where some terminal identity features are unaffected in a terminal selector mutant, these features are not sufficient to assign the neuron a specific identity. Therefore, it seems reasonable to propose that the terminal selector term should be restricted to regulatory factors whose loss results in a neuron not expressing a recognizable identity (or having switched to an alternative identity; Gordon & Hobert, 2015).

6. PARALLEL REGULATORY ROUTINES

Terminal selectors control the expression of many if not all terminal effector genes that are expressed in a neuron-type-specific manner and which, in
a combinatorial manner define the unique identity of a neuron type. Such effector genes include biosynthetic enzymes and transporters for specific neurotransmitter systems, neurotransmitter receptors, ion channel, synaptic adhesion molecules, neuropeptide, sensory receptors, and others (Table 2). However, neuron identity-determining terminal selectors do not control the expression of all genes in a terminally differentiated neuron type. Extensive phenotypic analysis has shown that features that are shared by many or all neuron types are not affected by removal of terminal selectors that control neuron-type-specific features. The most striking feature that are commonly unaffected by terminal selector are panneuronal genes (e.g., genes expressed in all neurons, such as those encoding for synaptic vesicle proteins) or pansensory genes (genes involved in building the ciliated structures of sensory neurons) (Altun-Gultekin et al., 2001; Etchberger et al., 2007; Flames & Hobert, 2009; Kratsios et al., 2011). This argues that regulatory routines exist that act in parallel to terminal selectors of neuron-type-specific identity (Fig. 1A). While regulators of panneuronal gene expression are presently ill defined (Stefanakis, Carrera, & Hobert, 2015), the parallel regulatory routine that controls pansensory identity features has been well documented. The RFX-type transcription factor daf-19 controls the coordinated expression of genes involved in building cilia (Burghoorn et al., 2012; Swoboda, Adler, & Thomas, 2000). daf-19 can therefore be considered to be a terminal selector of ciliated identity.

While terminal selectors have profound effects on synaptic connectivity of a neuron (Howell et al., 2015; Kratsios et al., 2015; Zhang et al., 2014; Pereira et al., 2015), some overt morphological features remain relatively intact in terminal selector mutants. For example, in unc-3 or unc-30 terminal selector mutants, ventral nerve cord motor neurons are miswired, motor axons still project in the ventral and dorsal nerve cord. Similarly, in che-1 terminal selector mutants, dendrites of the otherwise misspecified ASE chemosensory neurons are still present and its cell body is correctly placed (Uchida et al., 2003). Similarly, in unc-86 terminal selector mutants, the HSN motor neuron fails to adopt terminal identity features such as its neurotransmitter identity, but still migrates normally (Desai et al., 1988). Hence, there must be factors that act in parallel to terminal selectors to determine some basic morphological features. There are few examples of such regulators. In the case of the HSN neurons, the transiently acting Zn finger transcription factor egl-43 controls migration but not other features HSN identity (Baum, Guenther, Frank, Pham, & Garriga, 1999). A recent study in flies has shown that transcription factors called “morphology TF” (mTFs) define axonal/dendritic patterning features of motor neurons, but not other
terminal features, such as neurotransmitter identity (Enriquez et al., 2015). Transiently acting mTFs are selectors of terminal morphological features of a neuron, but their transient function in regulating effector genes that are likely just transiently expressed (e.g., genes involved in axon pathfinding or cell migration) clearly sets them apart from terminal selectors that are constitutively present in a neuron to initiate and maintain constitutively expressed terminal identity features of a neuron.

Taken together, gene expression programs in terminally differentiated neurons may be controlled by parallel acting “regulons” that define distinct identity aspects; some of these identity aspects define the unique identity of a neuron, while others define more generic aspects of a neuron identity, such as pansensory features or some overt morphological features (Fig. 1A).

7. DIVERSIFICATION OF NEURONAL IDENTITY VIA SELECTIVE REPRESSIBILITY

Not every single neuron type appears to have its own, unique complement of terminal selector(s). Rather, distinct but related neuronal subtypes can be diversified by modulating the activity of a terminal selector on specific subsets of targets. This has been illustrated in two different contexts: the left and right ASE neurons are two neuronal subtypes that are very similar to one another but differ in their complement of chemoreceptor expression. Their identity is not controlled by distinct terminal selectors, but is controlled by the same terminal selector, che-1 (Etchberger et al., 2007). However, the ability of che-1 to activate some target genes (that contain CHE-1 binding sites) is prevented by the presence of repressor proteins in one of the two ASE subtypes (Etchberger et al., 2009; Hobert, 2014). This selective repression results in the ASEL- and ASER-expressing distinct subsets of genes, while simultaneously also expressing a vast amount of similar genes (under control of che-1). Similarly, the terminal selector unc-3 controls the expression of multiple genes that are expressed differentially in distinct motor neuron subtypes. The ability of UNC-3 to activate some genes in some subtypes is restrained by subtype-specific repressor proteins (Kratsios et al., 2011; Winnier et al., 1999; our unpublished data).

8. GENERALITY OF THE TERMINAL SELECTOR CONCEPT

At the time of writing the original essay on the terminal selector concept, only a handful of examples of such selector genes has been described (Hobert, 2008). By now, transcriptional regulators that control terminal
identity features of more than two-thirds of the 118 neuron classes have been identified (Fig. 3). Obviously, there is no other nervous system that is even close in the extent to which neuronal differentiation programs have been described throughout the nervous system. A concise summary of all these will be provided in an upcoming comprehensive review that is currently in preparation. Not all the regulators have been described in the same depth.
as others. In contrast to the original, almost “all or nothing” examples in which a mutation in a terminal selector affects the expression of dozens of terminal markers, two “50/50 examples” have now been described in which some terminal markers are affected, while others are not (Pereira et al., 2015; Zhang et al., 2014). It is conceivable that in these apparently exceptional cases, the respective neuron may harbor several terminal selectors that can partially compensate for the loss of each other, as proposed for the case of the NSM neurons (Zhang et al., 2014).

9. REGULATION OF TERMINAL SELECTOR GENES

As exemplified by the case of the terminal selector *ttx-3*, the induction of terminal selector gene expression is complex. Regulatory control regions of *ttx-3* integrate a number of distinct autonomous and nonautonomous inputs (Bertrand & Hobert, 2009; Murgan et al., 2015). Terminal selectors could therefore perhaps be viewed as critical junctions in a regulatory hourglass topology, sampling a diversity of upstream inputs and then branching out again to control a multitude of targets (Fig. 1A). The regulation of more terminal selectors needs to be studied to assess how broadly applicable this concept is.

10. CONCLUSIONS

The purpose of this chapter has been to provide a description of the general features of gene regulatory programs that operate in terminally differentiated neuron types throughout the *C. elegans* nervous system. I have focused here entirely on the *C. elegans* system. I will leave it to researchers of other model systems to assess the extent of similarity between gene expression programs in *C. elegans* in their favorite system and I hope that some of the findings made in *C. elegans* may provide experimental testable hypotheses for researchers in other systems.

ACKNOWLEDGMENTS

I thank former and current lab members whose work has been instrumental in building the terminal selector concept. I thank lab members as well as Nuria Flames, Evan Deneris, Richard Mann, Doug Allan and Stefan Thor for comments on the manuscript. Work in my laboratory is funded by the National Institutes of Health and the Howard Hughes Medical Institute.
REFERENCES


