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Neuronal specification in *C. elegans*: combining lineage inheritance with intercellular signaling

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Neuronal specification in *C. elegans*: combining lineage inheritance with intercellular signaling

Abstract

The nervous system is composed of a high diversity of neuronal types. How this diversity is generated during development is a key question in neurobiology. Addressing this question is one of the reasons that led Sydney Brenner to develop the nematode *C. elegans* as a model organism. While there was initially a debate on whether the neuronal specification follows a ‘European’ model (determined by ancestry) or an ‘American’ model (determined by intercellular communication), several decades of research have established that the truth lies somewhere in between. Neurons are specified by the combination of transcription factors inherited from the ancestor cells and signaling between neighboring cells (especially Wnt and Notch signaling). This converges to the activation in newly generated postmitotic neurons of a specific set of terminal selector transcription factors that initiate and maintain the differentiation of the neuron. In this review we also discuss the evolution of these specification mechanisms in other nematodes and beyond.

Keywords: neuronal specification, transcriptional regulation, Wnt signaling, *C. elegans*

Introduction

Bilaterian animals can display very sophisticated behaviors, mirrored by the cellular complexity of their nervous system that is composed by a high diversity of neuronal types. A major subject in neurobiology is to understand the origin of this diversity during development. This question was of great interest to Sydney Brenner and was one of the reasons why he chose to develop the nematode *C. elegans* as a model organism (Brenner, 1974). *C. elegans* indeed presents several advantages for the study of nervous system development. Its nervous system is relatively simple with only 302 neurons in the adult hermaphrodite (White, Southgate, Thomson, & Brenner, 1986). The animal is transparent, allowing researchers to follow nervous system development directly *in vivo* with single cell resolution. Finally, the life cycle is short (3 days at 25°C) and the brood size is large, making *C. elegans* a great system to identify genes involved in nervous system development via genetic screens.

The establishment of the lineage of *C. elegans* by John Sulston and colleagues showed that neurons are produced by an invariant series of cell divisions (Sulston and Horvitz, 1977; Sulston, Schierenberg, White, & Thomson, 1983). There was a debate among early *C. elegans* researchers as to whether the fate of a neuron is established in a ‘European’ way or ‘American’ way (Brown, 2003). In a ‘European’ model the identity of a neuron is determined by the ancestry of the cell in a vertical manner, while in an ‘American’ model it is determined by the cellular environment in an horizontal manner.

In this review, we discuss the molecular and cellular mechanisms at the basis of neuronal specification in *C. elegans* showing that neurons are generated via a combination of lineage inheritance (vertical) and intercellular signaling (horizontal). We then present how terminal selector transcription factors trigger neuronal differentiation and maintain the differentiated state of neurons following specification. Finally, we discuss to what extent these neuronal specification programs are conserved in other nematodes and beyond.

Neuronal specification: a combination of lineage inheritance and intercellular signaling

In *C. elegans*, the majority of neurons is generated during embryogenesis by series of asymmetric divisions oriented along the anteroposterior axis (Sulston, et al., 1983). Many transcription factors that affect neuronal cell fate specification have been identified, via

forward genetic screens or reverse RNAi screens (reviewed in (Hobert, 2010)). Analysis of their expression patterns suggests that, during development, a neuronal precursor goes through a series of transient regulatory states defined by the expression of different combinations of transcription factors (see for example (Bertrand and Hobert, 2009; Poole, Bashllari, Cochella, Flowers, & Hobert, 2011; Sarafi-Reinach, Melkman, Hobert, & Sengupta, 2001)). These observations have been recently generalized using single-cell RNA sequencing of most embryonic cells (Packer et al., 2019), showing that following each division of a neuronal progenitor, the two daughter cells acquire transcription profiles different from each other and from their mother cell.

This transition from one regulatory state to another is triggered by the asymmetric division process. In *C. elegans*, the Wnt/ β -catenin asymmetry pathway (a specialized Wnt/ β -catenin pathway) controls asymmetric divisions oriented along the anteroposterior axis (reviewed in (Bertrand, 2016; Phillips and Kimble, 2009; Sawa and Korswagen, 2013)). This pathway regulates many asymmetric divisions in the early embryo (Kaletta, Schnabel, & Schnabel, 1997; Lin, Hill, & Priess, 1998) and also controls the terminal asymmetric divisions of neuronal progenitors during neurulation (epidermal enclosure) (Figure 1A) (Bertrand and Hobert, 2009; P. M. Gordon and Hobert, 2015; Murgan et al., 2015). The Wnt/ β -catenin asymmetry pathway controls the expression of target genes in the daughter cells via the TCF transcription factor POP-1 and its transcriptional coactivator SYS-1, a β -catenin (Kidd, Miskowski, Siegfried, Sawa, & Kimble, 2005; Lin, et al., 1998; Lin, Thompson, & Priess, 1995; Phillips, Kidd, King, Hardin, & Kimble, 2007). The pathway modulates the activity of POP-1 and SYS-1 by regulating the nuclear export of POP-1 (Lo, Gay, Odom, Shi, & Lin, 2004; Rocheleau et al., 1999) and the degradation of SYS-1 (Huang, Shetty, Robertson, & Lin, 2007; Phillips, et al., 2007). Following asymmetric division, high levels of SYS-1 relative to POP-1 in the posterior nucleus lead to the formation of a POP-1:SYS-1 complex that activates transcription (Huang, et al., 2007; Kidd, et al., 2005; Phillips, et al., 2007). In the anterior nucleus, SYS-1 levels are low and POP-1 mostly free of SYS-1 represses transcription.

Studies of *cis*-regulatory regions have established how the lineage history (transcription factors inherited from the mother cell) and the asymmetric division cue (Wnt/ β -catenin asymmetry pathway) are integrated to generate novel regulatory states in the daughter cells. For example, in the larval neuroectodermal T lineage, the Hox transcription factor NOB-1 and

the Pbx transcription factor CEH-20 are inherited from the mother cell and cooperate with the POP-1:SYS-1 complex in the posterior daughter to activate the expression of the Meis transcription factor gene *psa-3* by directly binding to its *cis*-regulatory regions (Arata et al., 2006). Similarly, in the AIY neuron lineage of the embryo, the LIM-homeodomain transcription factor TTX-3, inherited from the mother cell (SMDD/AIY mother), cooperates with POP-1:SYS-1 to induce the transcription of the homeodomain gene *ceh-10* only in the posterior daughter (AIY neuron), by binding to its *cis*-regulatory regions (Bertrand and Hobert, 2009) (Figure 1B, division generating the SMDD and AIY neurons). While direct target genes activated in the posterior daughter cell contain POP-1 binding sites and are activated by the POP-1:SYS-1 complex, genes activated in the anterior daughter are not regulated via POP-1 binding sites. For example, in the AIY neuron lineage of the embryo, it has been observed that POP-1, in the absence of SYS-1, activates the expression of an anterior target genes *ttx-3* by forming a complex with another transcription factor protein REF-2 (a Zic factor) and binding to a REF-2 binding site in the *ttx-3 cis*-regulatory regions (Murgan, et al., 2015) (Figure 1B, division generating the SMDD/AIY mother and the SIAD/SIBV mother). Whether POP-1 activates transcription in a similar manner in anterior daughters of other neuronal lineages remains to be established. These data illustrate how combining lineage ancestry (via inherited transcription factors) with intercellular signaling (via the asymmetric Wnt pathway) generates neuronal type diversity in the nervous system of *C. elegans* (Figure 1A). Secreted Wnt ligands can play an instructive role in the asymmetric divisions regulated by the Wnt/ β -catenin asymmetry pathway. For example, it has been established that for the endomesoderm precursor cell EMS or the larval blast cell T, Wnt ligands, secreted from a posterior source, control the division orientation and the asymmetry of daughter cell fates (Goldstein, Takeshita, Mizumoto, & Sawa, 2006). Interestingly, it has been recently observed that three Wnt ligands (CWN-1, CWN-2 and MOM-2), coming from the posterior of the embryo, regulate in an instructive manner the terminal asymmetric divisions of embryonic neuronal progenitors (Kaur et al., 2020). This illustrates the importance of intercellular signaling during neuronal development in *C. elegans*.

Another intercellular signaling pathway playing an important role in neuronal cell fate specification in *C. elegans* is the Notch pathway. This pathway is involved in the generation of left-right asymmetries in the nervous system. While the nervous system of *C. elegans* is essentially bilateral symmetric, some neuronal left-right pairs, which are symmetric at the morphological level, display some functional and molecular asymmetries (Hobert, Johnston,

& Chang, 2002). In the case of the pair of ASE taste neurons, the left and right cells express different sets of chemoreceptors that allow sensing different chemicals (Pierce-Shimomura, Faumont, Gaston, Pearson, & Lockery, 2001; Yu, Avery, Baude, & Garbers, 1997). The difference between left and right ASE is induced in the very early embryo (four-cell stage) by a Notch signal, coming from the posterior P lineage, which represses the expression of the T-box transcription factor genes *tbx-37* and *tbx-38* in the blastomere ABp, giving rise to the right ASE, but not in the blastomere ABa, giving rise to the left ASE (Poole and Hobert, 2006). Another example is the pairs of AIY interneurons and SMDD, SIAD and SIBV motor neurons, which act in a circuit controlling navigation behavior downstream of ASE. In these neurons, the bHLH gene *hlh-16* is expressed in a left-right asymmetric manner and is important for their correct axonal projections (Bertrand, Bisso, Poole, & Hobert, 2011). This asymmetry is established independently of the ASE asymmetry by a later Notch signal (during gastrulation) coming from the left mesoderm, which increases *hlh-16* expression on the left side. Another example of left-right asymmetry is the pair of AWC olfactory neurons: the odorant receptor gene *str-2* is activated in only one of the two AWC neurons in a stochastic manner with no left-right bias. Communication between the two AWC neurons, involving calcium signaling but not Notch, ensures that only one AWC expresses *str-2* (Troemel, Sagasti, & Bargmann, 1999).

To conclude, neuronal cell fate specification in *C. elegans* involves both lineage transmission (inherited transcription factors) and intercellular signaling (such as Wnt or Notch). It therefore follows neither a pure ‘European’ nor a pure ‘American’ model, but is instead a mix of both.

Neuronal differentiation: initiation and maintenance of terminal neuronal fate by terminal selectors

The function of a neuron relies on the specific set of terminal differentiation genes (or effector genes) that it expresses such as neurotransmitter receptors, ion channels or neurotransmitter synthesis pathway genes. In the *C. elegans* nervous system, the expression of many neuron type-specific terminal differentiation genes has been mapped. For example the cholinergic, GABAergic or glutamatergic neurons have been systematically identified (Gendrel, Atlas, & Hobert, 2016; Pereira et al., 2015; Serrano-Saiz et al., 2013). For several neuron types, the analysis of *cis*-regulatory regions of their specific terminal differentiation genes have revealed

that they are often regulated by a common set of transcription factors, called terminal selectors (Hobert, 2008). These transcription factors also frequently autoregulate their expression, therefore maintaining the type identity of the neuron throughout the life of the animal. One example is the cholinergic interneuron AIY where the homeodomain transcription factors TTX-3 and CEH-10 directly activate and maintain the expression of a large battery of terminal differentiation genes: the choline acetyltransferase *cha-1*, the acetylcholine vesicular transporter *unc-17*, the neurotransmitter receptors *ser-2*, *gar-2* and *mod-1*, etc. (Figure 2) (Wenick and Hobert, 2004). Other examples of terminal selectors include the two homeodomain transcription factors MEC-3 and UNC-86 in touch receptor neurons (Zhang et al., 2002), the zinc finger transcription factor CHE-1 in the taste neuron ASE (Etchberger et al., 2007) or the COE transcription factor UNC-3 in cholinergic motoneurons (Kratsios, Stolfi, Levine, & Hobert, 2011). In addition, many other transcription factors controlling the expression of terminal differentiation genes in various neuronal types have been identified (for a comprehensive review see (Hobert, 2016)).

The connection between these terminal differentiation programs and earlier cell fate specification events, such as asymmetric divisions, has been less characterized. The link has been elucidated in the case of the cholinergic interneuron AIY (Figure 2) (Bertrand and Hobert, 2009; Murgan, et al., 2015): the expression of the AIY terminal selector genes *ttx-3* and *ceh-10* is set up in the early postmitotic AIY neuron by two types of transient developmental inputs. One is the specific transcription factors inherited from the lineage history: proneural bHLH factors (HLH-3, HLH-16), their binding partner HLH-2 and the Zic transcription factor REF-2. The other input is the Wnt/ β -catenin asymmetry pathway, which regulates the terminal asymmetric divisions in the AIY lineage. These lineage specific transcription factors and the Wnt/ β -catenin asymmetry pathway are directly integrated at the level of the *cis*-regulatory regions of *ttx-3* and *ceh-10* to establish their coexpression specifically in the early AIY neurons. The lineage specific transcription factors (HLH-3, HLH-16, HLH-2 and REF-2) and the Wnt pathway effectors (POP-1 and SYS-1) subsequently disappear from the AIY neuron, and TTX-3 and CEH-10 expression is then maintained via a positive autoregulatory loop, where they directly bind their own *cis*-regulatory regions and positively regulate their expression. In the postmitotic AIY neuron, TTX-3 and CEH-10 directly activate and maintain the expression of a large battery of terminal differentiation genes, therefore determining the type-specific function of the AIY neuron. This illustrates how terminal selector transcription factors can connect early

specification events to the terminal differentiation of the neuron. Whether a similar regulatory logic applies for the activation of other terminal selectors remains to be established.

Evolution of neuronal specification programs in nematodes

Neuronal determination mechanisms based on lineage and cell neighborhood could constrain neuronal development and affect its flexibility and evolvability. Indeed, there was, early on, an impression of very high conservation of the nervous system across the vast phylogenetic distances of nematodes. In one of the seminal papers by John Sulston and Sydney Brenner, it was noted that: ‘ The nervous system of the nematode *Ascaris lumbricoides* appears to be very similar to that of *C. elegans*. The homology of the catecholaminergic neurons lends support to the possibility that, notwithstanding their great disparity in size, there is a strong conservation of the properties of the nervous system between these two nematodes ’ (Sulston, Dew, & Brenner, 1975). It is indeed tempting to try to assign almost one-to-one correspondence of the 302 *C. elegans* neurons (Sulston and Horvitz, 1977; Sulston, et al., 1983) to the 298 neurons found in *Ascaris* (Stretton et al., 1992). But this apparent morphological conservation could underlie pervasive functional divergence. Due to the difficulty of duplicating genetic screens performed in *C. elegans* in other nematode species, there has been limited number of experiments analyzing the evolution of neuronal specification mechanisms. Overall, studies suggest that the neuroanatomy of nematodes is not as highly conserved as previously described (Han, Boas, & Schroeder, 2015) (Figure 3A), although the ability to assign single-cell homology across great evolutionary distances is a critical factor to assess conservation and variation in neuronal fates.

Variations in neuroanatomy and lineage

Gross neuroanatomical comparisons across many nematode species, most not amenable to manipulation, revealed extensive variability in number and time of birth of ventral nerve cord neurons and dye-filling sensory neurons across multiple clades (Han, et al., 2015). Finer analyses, comparing individual neurons between *C. elegans* and *Pristionchus pacificus* by serial electron microscopy, in the paryngeal (Bumbarger, Riebesell, Rodelsperger, & Sommer, 2013) and olfactory (Hong et al., 2019) nervous systems, found remarkable conservation of

neuron homology, as defined by cell body position and neurite anatomy, but also extensive rewiring and changes in fate, e.g. from interneuron to motor neuron.

Embryonic lineages were established for other species in the *Caenorhabditis* genus, namely *C. briggsae* (Zhao et al., 2008), *C. remanei* and *C. brenneri* (Memar et al., 2019). For these three species, embryonic development until the start of muscle contraction is identical, but for some small variations in the timing of division, and one cell escaping programmed cell death to become part of the pharynx in *C. briggsae* and *C. remanei* (Memar, et al., 2019). Accordingly, the number and identity of neurons appear to be perfectly conserved in the species investigated. However, once comparisons reach outside the *Caenorhabditis* genus, like with the marine nematode *Pellioditis marina* (Houthoofd et al., 2003), developmental differences become apparent: while cell divisions maintain a strong homology (95.5%), final fate is less well conserved; only 85% of cells acquiring a neuronal fate in *P. marina* are also neurons in *C. elegans*. The difference is made up by cells acquiring pharyngeal, epidermal or programmed cell death fates, for a final count of 194 neurons at muscle contraction compared with 244 in *C. elegans*. In the more distantly related nematode *Romanomermis culicivorax* (Schulze and Schierenberg, 2009), the lineage is more generally divergent, and early development proceeds in a more monoclonal fashion. Cell positions are also markedly different, with neuronal precursors arranged as rings around the embryo, suggesting possible changes in cell-cell interactions.

Variations in neurotransmitter identity patterns

One of the first tools used to compare the nervous system across species is the Falck-Hillarp method, where formaldehyde reacts with catecholamines to form fluorophores. Using this method, John Sulston (Sulston, et al., 1975) compared dopamine neurons between the yet to be described *C. elegans* nervous system and *Ascaris lumbricoides*, and identified strong similarities between the two species in the head and body, but noted the presence of a pair of dopaminergic neurons in the *A. lumbricoides* tail without equivalent in *C. elegans*. A later study, looking at dopaminergic neurons in rhabditid nematodes, including the *Caenorhabditis* genus, found perfect conservation of pattern and easy assignation of one-to-one orthology (Rivard et al., 2010). This constant pattern of dopaminergic neurons, although striking, might give an impression of almost perfect conservation. However, in the more distant specie *Haemonchus contortus*, dopamine was not detected in head neurons, but only in commissures

(Rao, Forrester, Keller, & Prichard, 2011) suggesting a diverging pattern of dopaminergic neurons.

Similar studies targeting serotonergic neurons reveal that they are also highly conserved within the *Caenorhabditis* genus; however, in more distantly related species, only the serotonergic NSM neurons were found to be consistently conserved (Rivard, et al., 2010), including with *Ascaris suum* (Johnson, Reinitz, Sithigorngul, & Stretton, 1996) and *H. contortus* (Rao, et al., 2011). Finally, some GABAergic neurons were found to be so well conserved between *C. elegans* and *A. suum*, that clear homologs were identified for the four RMEs and DVB (Guastella, Johnson, & Stretton, 1991), and for DDs and VDs in the ventral nerve cord (Johnson and Stretton, 1987); however, three pairs of head neurons found in *A. suum* have no clear homologs in *C. elegans* (Guastella, et al., 1991).

Taken together these data reveal that, while neuronal types seem relatively well conserved in the *Caenorhabditis* genus, there are important variations at greater evolutionary distances.

Variations in transcriptional regulation

The variations in neuronal cell fates observed between nematodes suggest that the transcriptional mechanisms that specify neuronal identity have changed. In addition, even if neuronal types are conserved, the detailed transcriptional regulation of their identity may have evolved. These variations can happen at the level of the *trans*-acting factors, such as transcription factors or miRNA, and at the level of *cis*-regulatory regions (Figure 3B). Strikingly, several *trans* regulators of neuronal fate were found to vary across nematode species. The miRNA *lxy-6*, triggering the left-right asymmetry of ASE neurons in *C. elegans*, is absent from the genome of *P. pacificus* (Ahmed et al., 2013). The transcription factor *che-1* involved in specifying ASE fate and restricted to that cell pair in *C. elegans*, is expressed in an additional pair of neurons, homolog of ASG, in *P. pacificus* (Hong, et al., 2019). For the nuclear receptor *odr-7*, involved in specifying AWA neuron cilia and expressed exclusively in that neuron in *C. elegans*, the *P. pacificus* ortholog is expressed in the homologs of AWC and ADF neurons, but not in the homolog of AWA (Hong, et al., 2019). The downstream terminal differentiation gene *odr-3* (a G-protein subunit) also diverged extensively in its expression compared with *C. elegans* (Hong, et al., 2019).

In addition to variations at the level of transcription factors, changes are also observed at the level of *cis*-regulatory elements. One tool of choice to study *cis*-regulatory changes between *C. elegans* and *C. briggsae*, which have one-to-one neuron homology, is to swap transcriptional reporters between species (K. L. Gordon and Ruvinsky, 2012) (Figure 3B). If the regulatory mechanisms are conserved, the expression pattern would stay the same; if changes happened in *cis*-regulatory elements or in *trans* factors, the expression patterns driven by the reporters would vary. For example, in *C. elegans*, the transcription factor CEH-6 regulates *lin-11* expression in the interneuron RIC by binding to an intronic enhancer; in *C. briggsae*, the endogenous *lin-11* enhancer fails to drive expression in RIC, while reciprocal promoter swaps do drive expression in RIC (Amon and Gupta, 2017). Interestingly, an independent comparative RNAi screen found evidence for functional divergence of the CEH-6 transcription factor between *C. elegans* and *C. briggsae* (Verster, Ramani, McKay, & Fraser, 2014). This illustrates one example of functional divergence both in *cis* and in *trans* in the transcriptional regulation of neuronal fate. Over the years, different teams have performed promoter swaps for multiple genes, including some genes involved in regulation of neuronal fates. While for some genes like the transcription factors *ceh-24* (Harfe and Fire, 1998), *pag-3* (Aamodt et al., 2000), *mec-3* (Xue, Finney, Ruvkun, & Chalfie, 1992), and *odr-7* (Colosimo, Tran, & Sengupta, 2003) the regulatory mechanisms appear to be conserved between *C. elegans* and *C. briggsae*, they diverged for others: regulation of the chemoreceptor *srsx-3* and of its putative transcription factor *lim-4* diverged between *C. elegans* and *C. briggsae* (Nokes et al., 2009); the *C. briggsae* promoter of *gcy-5*, a chemoreceptor, does not drive expression in ASE neurons while its *C. elegans* ortholog does (Etchberger, Flowers, Poole, Bashllari, & Hobert, 2009); and the *C. briggsae* promoter of another chemoreceptor *odr-10* lacks a repressive UNC-3 binding site present in its *C. elegans* ortholog, and drives expression in one extra neuron pair (Kim, Colosimo, Yeung, & Sengupta, 2005). A detailed study of the promoter of the vesicular GABA transporter *unc-47* also revealed compensatory coevolution of *cis* and *trans* regulatory factors between *C. elegans* and *C. briggsae* (Barriere, Gordon, & Ruvinsky, 2012). More generally, this trend of cryptic changes in transcriptional regulation was found to be pervasive, when testing multiple neuronal genes from several *Caenorhabditis* species in the *C. elegans* *trans*-regulatory context (Barriere and Ruvinsky, 2014). Even though the final expression pattern of many genes may be conserved within *Caenorhabditis* nematodes, in accordance with conservation of fine anatomy and neurotransmitter neuronal fates, these promoter swaps reveal extensive developmental systems drift (True and Haag, 2001): while purifying selection maintains the final phenotype, the molecular mechanisms

underlying its specification are less constrained and are able to diverge, provided that the output stays constant. *Cis*-regulatory elements diverged over time, but coevolved with their upstream transcription factors to maintain their outputs.

In conclusions, several scenarios of transcriptional evolution between *C. elegans* and *C. briggsae* are observed (Figure 3B): conservation of transcription factors and *cis*-regulatory elements (e.g. *ceh-24*); coevolution of *trans*-acting factors and *cis*-regulatory elements to maintain the final expression pattern (e.g. *unc-47*); and changes in regulatory mechanisms inducing variations in the final expression pattern (e.g. *lin-11*).

Beyond nematodes

Cis-regulatory elements involved in neuronal specification are usually not conserved over greater evolutionary distances, between phyla (Ruvinsky and Ruvkun, 2003). However, some degree of functional conservation can be observed at the level of the transcription factors that regulate neuronal cell fate acquisition. For example, proneural bHLH transcription factors (such as Achaete-scute or NeuroD family members) that play an important role in the early steps of neuronal specification in *C. elegans* (Frank, Baum, & Garriga, 2003; Hallam, Singer, Waring, & Jin, 2000; Krause et al., 1997; Murgan, et al., 2015; Poole, et al., 2011), are also at the top of the hierarchy in the neuronal determination process in other animals (Baker and Brown, 2018). Conservation is also found at downstream steps. For example, in *C. elegans*, the dopamine pathway genes are activated by the Ets transcription factor AST-1 and the homeodomain transcription factor CEH-43 (Doitsidou et al., 2013; Flames and Hobert, 2009), a role conserved in mice where their orthologs, Etv1 and Dlx2, are activators of the dopaminergic fate, specifically in the olfactory bulb. In addition, it has been recently observed that the Zic transcription factor REF-2 represses dopaminergic fate in *C. elegans*, a function conserved in the mouse where Zic1 and Zic2 repress the dopaminergic phenotype in the olfactory bulb (Tiveron et al., 2017). Another interesting example is the role of COE transcription factors in cholinergic motor neuron differentiation. In *C. elegans*, the COE transcription factor UNC-3 regulates the expression of many terminal differentiation genes of cholinergic motor neurons including the cholinergic gene battery (Kratsios, et al., 2011). This role is conserved in the ascidian *Ciona intestinalis*, where the sole COE transcription factor controls the cholinergic fate of motor neurons (Kratsios, et al., 2011). However, while the COE transcription factor Ebf2 also regulates some aspects of axial motor neuron development

in mice, it does not control their cholinergic fate (Catela et al., 2019), suggesting both conservation and divergence in COE transcription factor functions. Some degree of conservation can also be observed over long evolutionary distances at cellular and signaling level. For example asymmetric divisions are widely used in diverse metazoans to generate neuronal diversity (Hartenstein and Stollewerk, 2015). In addition, the Wnt pathway, which controls neuronal progenitors asymmetric divisions in *C. elegans*, also plays a role in the regulation of neural stem cells asymmetric divisions in the mouse cortex (Chenn and Walsh, 2002; Delaunay, Cortay, Patti, Knoblauch, & Dehay, 2014; Kalani et al., 2008; Woodhead, Mutch, Olson, & Chenn, 2006).

Conclusions

Since the seminal works of Sydney Brenner and John Sulston, decades of studies of nervous system development in *C. elegans* have established that neuronal diversity is generated via the combinations of two types of information: lineage history (carried by inherited transcription factors) and intercellular signaling (especially Wnt and Notch signaling). Neuronal specification in *C. elegans* is therefore a mix between the lineage-based ‘European’ way and the interaction-based ‘American’ way. This information is integrated at the level of terminal selector transcription factors that activate and maintain the expression of large batteries of terminal differentiation genes in postmitotic neurons, and are therefore responsible for the acquisition and maintenance of neuronal type identities. While neuronal types are relatively well conserved within *Caenorhabditis* nematodes, there is more variability in distant nematodes. The evolution of neuronal specification involves changes at both the *cis*-regulatory element and *trans*-acting factor levels. However, some aspects of neuronal specification mechanisms are also conserved over large evolutionary distances. There is no doubt that, in the future, studies of nematodes, which offer an amazing level of cellular and molecular resolutions, will continue to deepen our knowledge of the mechanisms at the basis of nervous system development and evolution.

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Disclosure of interest

The authors report no conflict of interest.

Figure legends

Figure 1. Generation of neuronal diversity by the combination of lineage history and Wnt/ β -catenin asymmetry.

(A) Following each asymmetric division the Wnt/ β -catenin asymmetry pathway cooperates with transcription factors (TF) inherited from the mother cell to activate the expression of different transcription factors in the anterior and posterior daughter cells. (B) Example of the AIY neuron lineage (ABpl/rpapaa lineage). In the first cell division, the POP-1 protein interacts with the REF-2 protein to activate the transcription of the *ttx-3* gene in the anterior daughter (SMDD/AIY mother) via a REF-2 binding site present in the *ttx-3* promoter. This activation is blocked by SYS-1 in the posterior daughter (SIAD/SIBV mother). In the second cell division, the TTX-3 protein and the POP-1:SYS-1 complex activate the transcription of the *ceh-10* gene in the posterior daughter (AIY neuron) via TTX-3 and POP-1 binding sites present in the *ceh-10* promoter. In the anterior daughter (SMDD neuron), POP-1 without SYS-1 represses *ceh-10* expression.

Figure 2. Connection of early specification events to the terminal differentiation program in the AIY interneuron.

During embryonic development transient developmental inputs (lineage specific transcription factors and the Wnt/ β -catenin asymmetry pathway) initiate the expression of the terminal transcription factors TTX-3 and CEH-10 in the AIY lineage. TTX-3 and CEH-10 subsequently maintain their expression and activate a large battery of type specific terminal differentiation genes.

Figure 3. Conservation and divergence of neurodevelopment in nematodes.

(A) Phylogeny of species discussed in this review, showing conservation or divergence of cell lineage or neurotransmitter cellular patterns, compared with *C. elegans*, at different phylogenetic distances. (B) Possible scenarios of transcriptional evolution. If a transcription factor and its target *cis*-regulatory element are conserved between *C. elegans* and *C. briggsae*, there is no change in regulation and no difference in expression pattern is observed when the *C. briggsae cis*-regulatory element is placed in the *C. elegans trans*-regulatory environment. If there was coevolution between *trans*-regulatory factors and *cis*-regulatory elements, the *C. briggsae cis*-regulatory element placed in the *C. elegans trans*-regulatory environment will give an altered expression pattern. Finally, if there was a change in transcription factors binding to a *cis*-regulatory element, the regulation might be completely lost when the *C. briggsae cis*-regulatory element is placed in the *C. elegans trans*-regulatory environment.

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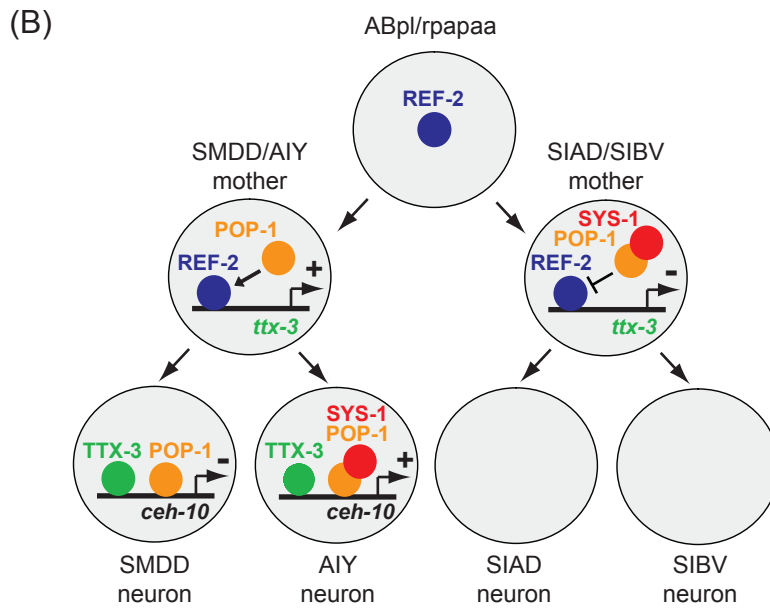
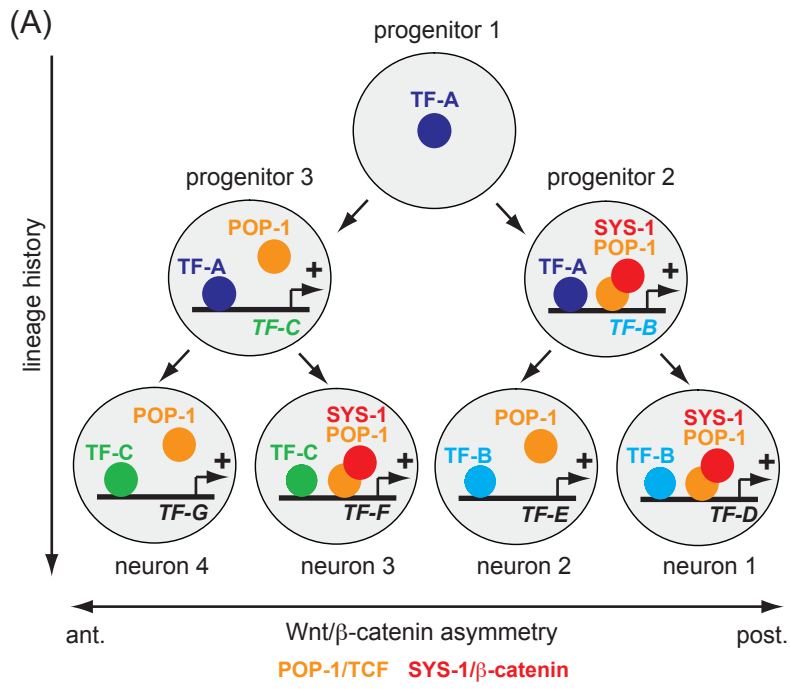
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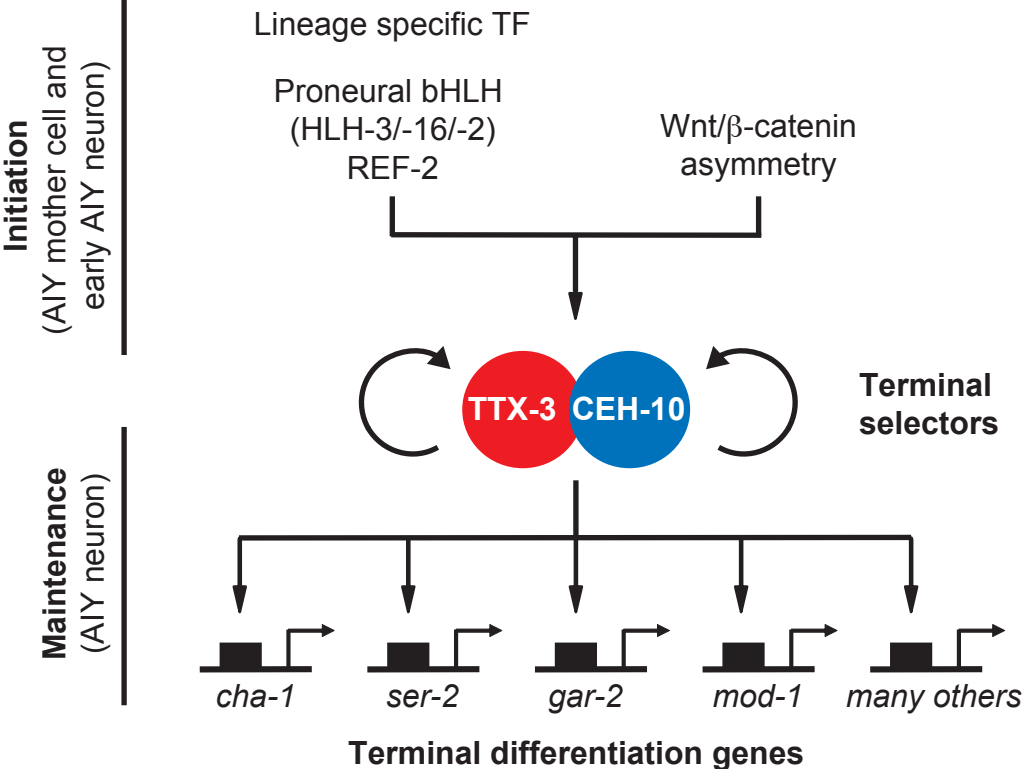
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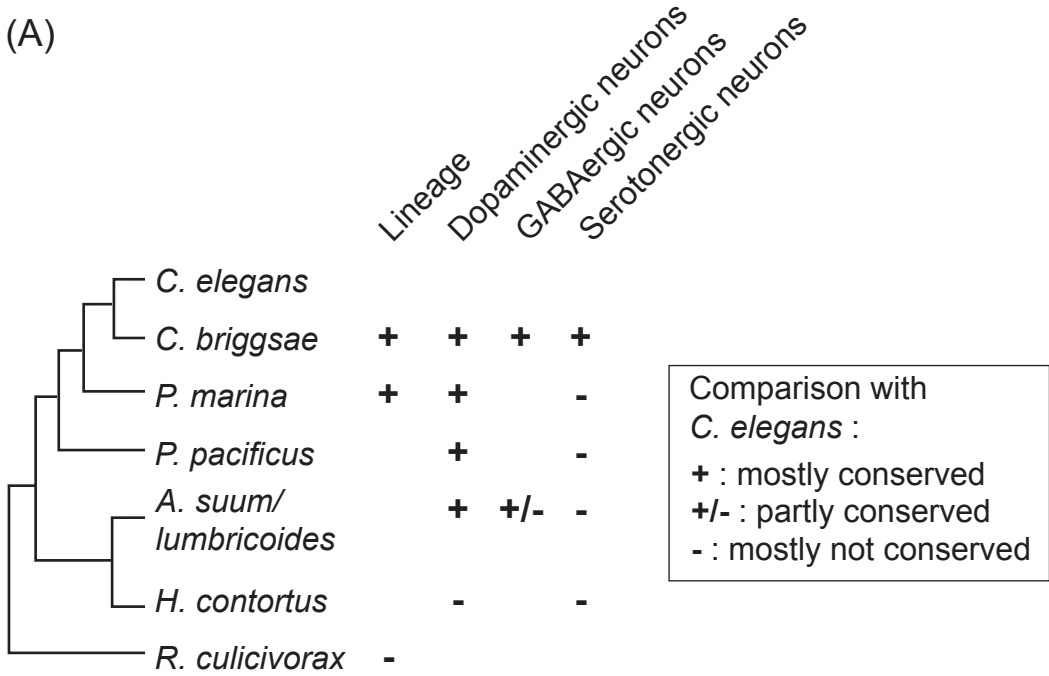
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Transient developmental inputs



(A)



(B)

