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Using *C. elegans* to Decipher the Cellular and Molecular Mechanisms Underlying Neurodevelopmental Disorders

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Abstract Neurodevelopmental disorders such as epilepsy, intellectual disability (ID), and autism spectrum disorders (ASDs) occur in over 2 % of the population, as the result of genetic mutations, environmental factors, or combination of both. In the last years, use of large-scale genomic techniques allowed important advances in the identification of genes/loci associated with these disorders. Nevertheless, following association of novel genes with a given disease, interpretation of findings is often difficult due to lack of information on gene function and effect of a given mutation in the corresponding protein. This brings the need to validate genetic associations from a functional perspective in model systems in a relatively fast but effective manner. In this context, the small nematode, *Caenorhabditis elegans*, presents a good compromise between the simplicity of cell models and the complexity of rodent nervous systems. In this article, we review the features that make *C. elegans* a good model for the study of neurodevelopmental diseases. We discuss its nervous system architecture and function as well as the molecular basis of behaviors that seem important in the context of different neurodevelopmental disorders. We review methodologies used to assess memory, learning, and social behavior as well as susceptibility to seizures in this organism. We will also discuss technological progresses applied in *C. elegans* neurobiology research, such as use of microfluidics and optogenetic tools. Finally, we will present some interesting examples of the functional analysis of genes associated with human neurodevelopmental disorders and how we can move from genes to therapies using this simple model organism.

Keywords Neurodevelopment · *C. elegans* · Autism · Epilepsy · Intellectual disability

Neurodevelopmental Disorders: Past, Present, and Future

Development of a fully functional nervous system comprises many cellular and molecular events, which need to occur in a precise and ordered manner. These include cell proliferation; migration; programmed cell death; cell differentiation (involving morphological and biochemical specializations); establishment of contacts between neurons, synapses, and pruning of less efficient ones; and also establishment of specialized relationships between neurons and other cell types. Disturbances in any of these steps will lead to loss of viability, if severe, or to neurodevelopmental disorders, if more subtle. Neurodevelopmental disorders as a group occur in over 2 % of the population and comprise intellectual disability (ID), epilepsy, autism spectrum disorders (ASDs), specific reading or writing impairments, hyperactivity, and attention deficit disorder, among others. Schizophrenia is also often seen as a neurodevelopmental disturbance manifesting only in adulthood. These disorders have an important impact in society, affecting not only the patients but whole families, especially when the care network is not well structured. They may result from genetic factors or from environmental interference with normal development process, as occurs, for instance, in the case of fetal alcoholic syndrome. Some of the effects of the environment may even be potentiated by a susceptible genetic background.

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Recently, important advances in our knowledge of genetic causes of neurodevelopmental diseases have emerged as a result of application of novel genomic analysis technologies (reviewed in [1]). To illustrate this, the genetic basis of disease can now be identified in up to 80 % of patients with ID, when applying array comparative genomic hybridization and whole exome sequencing techniques. Additionally, many gene variants putatively associated with more complex, multifactorial neurodevelopmental disturbances have also been identified in the last years using genetic linkage and association analyses. Nevertheless, following identification of novel gene variants potentially causing the disease of interest, difficulty is often the interpretation of findings, namely, lack of information on gene function and on the effect of a given mutation in the corresponding protein. This brings the need for model systems that can be used to study genes and mutations of interest in a relatively fast but effective manner.

Studies of Human Neurodevelopmental Genes in Lower Organisms

Geneticists have harnessed the power of model organisms for understanding of human gene function for many years now, with flies, yeast, and mouse leading the way. In a simpler perspective, human neuronal cell lines can be a very interesting model to study the function of genes identified as associated with human neurodevelopmental diseases, given presence of majority of molecular components. However, given their lack of integration in functional circuits; lack of interaction with other cell types, also relevant for function of the nervous system; and absence of a behavioral output that allows assessment of effectiveness of the circuits, for many studies, there is the need to use a whole organism approach. Mice have been used for this purpose with very encouraging results: globally, the structure of the human and murine nervous systems bears significant resemblance and even at the behavioral level, paradigms have been developed to analyze traits that are thought to be parallel between these two species. Rat models are even more advantageous (particularly in cognitive and social studies), but the tools for genetic manipulation have lagged behind. Disadvantages of the use of rodents are their relatively high maintenance costs and difficulty/cost/time consumption of their genetic manipulation. On the other hand, the complex structure of their nervous system, which is certainly advantageous for some studies, also presents serious constraints when trying to dissect molecular events leading to disease. In this perspective, organisms with simpler nervous systems and genetic amenability provide an elegant framework for the study of gene function and malfunction.

A simpler species in which neurobiology of memory has been widely studied, but in which genetic manipulation has not been so developed, is *Aplysia californica*. *Aplysia* has a relatively small number of neurons, and many of them are enormous, allowing electrophysiological studies, individual neuronal manipulation, and observation of their neuronal architecture. Moreover, its neurons are able to form and store memories, have plasticity, and for several of them, a functional role has been determined [2]. In contrast, *Drosophila melanogaster* is a model in which genetic tools are highly developed and which has been increasingly used in behavioral genetic studies, the advantage being that it has a brainlike structure and complex behaviors that can be analyzed. Moreover, identification of specific neuronal populations and neuron-to-behavior output has advanced greatly in recent years [3]. Zebrafish is also a simple model that has the main advantage of being a vertebrate with a high degree of genetic homology with mammals. Because it has a brain, zebrafish is often envisaged as the bridge between *Drosophila*/worms and murine models. This animal model has been widely used to study human neurological disorders because of its low maintenance cost, rapid life cycle, rapid external embryonic development, and optical clarity of embryos and larvae, which allows observation of the nervous system in vivo. In addition, both gain- (overexpression of mutated proteins) and loss-of-function (morpholinos; zinc finger nuclease deletions, etc.….) approaches can be considered to study gene function [4]. Finally, for very simple functional genomic studies, yeast and other fungi can also be used, with the advantage of simplicity and ease of genetic manipulation but with clear limitations when it comes to understanding function of a gene within the nervous system.

Caenorhabditis elegans as a Simple Model to Study Complex Neuronal Phenomena

C. elegans provides a good compromise between complexity of vertebrates like mouse and extreme simplicity of yeast and is a reference model in studying function and malfunction of the nervous system. This animal presents key advantages that make it unique in the field of neurosciences: first, the well-described neuronal lineage and interconnectivity provides an exceptional set up for the study of neuronal mechanisms. Second, amenability to genetic manipulation allows identification of genes important for neuronal formation, migration, and activity. Third, its transparency in combination with existence of specific transgenic reporter strains allows in vivo monitoring of particular neuronal events, with possibility of correlating temporal patterns of neuronal activity with behavioral outcomes. Herein, we will describe the model, tools available in the field, and some of the remarkable contributions of this nematode for the understanding of nervous system function and dysfunction, and its underlying genetics, with particular

focus on neurodevelopmental disorders such as epilepsy, ASDs, and ID.

C. elegans Nervous System

While neuronal wiring diagrams in higher species such as rodents often present ambiguities and misinterpretations inherent to their complexity, simplicity of the *C. elegans* nervous system and its well-described anatomy and interconnectivity make this model an attractive and complementary tool in the field of neuroscience. The hermaphrodite *C. elegans* has 302 neurons divided in surprising 118 distinct neuronal classes and 56 glial cells, altogether comprising 37 % of all the somatic cells in the worm [5, 6]. Neuronal classes include 39 classes of predicted sensory neurons, 27 of motor neurons, and the remainder as interneurons [7]. Lineage and morphology have been described in detail [6], and there are fluorescent reporter genes for almost every neuron with exquisite specificity (some examples are in Table 1 and Fig. 1) [5, 8, 9].

Worm synapses (around 7,000) occur en passant, i.e., synaptic boutons are formed along the axon shaft [10, 11]. The presynaptic site bears much resemblance to those of vertebrate nervous system, but the postsynaptic region appears to be simpler. The number of synapses between each partner can go up to 19 but normally is around five synapses [10, 11]. It is also possible to observe synapses in vivo by using fluorescent reporter molecules such as synaptobrevin (SNB-1) [12], an integral membrane protein of synaptic vesicles. Importantly, this marker not only allows determination of synaptic density because synaptobrevin puncta correlates with the number of synaptic vesicles in ultrastructural studies but also is a measure of steady-state rates of vesicles exocytosis and endocytosis (intensity of synaptobrevin in axons) [13, 14]. In an elegant RNA interference (RNAi) screening study that aimed to identify genes regulating GABA synapses, use of SNB-1::GFP marker allowed researchers to obtain insight on the nature of different neuronal defects [15]. By changing the promoter that controlled the marker, one could assess synaptic condition in either inhibitory (GABAergic) or excitatory (cholinergic) inputs of the neuromuscular junction (NMJ). Use of additional markers such as the postsynaptic UNC-49 GABAA receptor even allowed researchers to distinguish pre- from postsynaptic defects [15]. Moreover, specific markers of the active zone (specialized synaptic structures that mediate neurotransmitter release) were developed, such as SYD-2::GFP, which allows their direct visualization [16] and isolation of mutants with defective active zone morphology [17, 18].

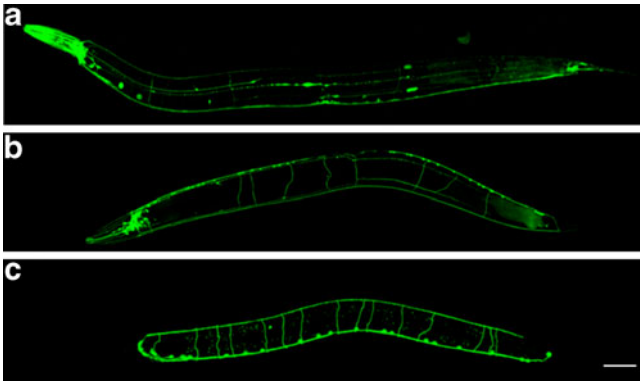

Fig. 1 Confocal pictures of commonly used *C. elegans* strains that express GFP in specific neurons. **a** Pan-neuronal expression of GFP observed in OH441 strain. This strain expresses GFP under the control of *unc-119* promoter. The function of UNC-119 is still unknown, but this protein is necessary for neuronal formation and migration, is expressed since early embryonic stages until adulthood, and is present in nearly all neurons. **b** Strain LX929 expressing GFP in all cholinergic neurons. The fluorescent protein is in frame with UNC-17, a synaptic vesicle acethylcholine transporter. **c** Expression of GFP in all GABAergic neurons in strain EG1285. The fluorescent marker is expressed under the control of *unc-47* promoter; UNC-47 is a transmembranar vesicular GABA transporter. Scale 50 μm

Table 1 Some examples of *C. elegans* strains expressing a fluorescent marker in a specific group of neurons. All referred strains are available at the Caenorhabditis Genetics Center (CGC)

Strain	Genotype	Description	Expression pattern
OH441	*otIs45 V*	*Integrated Ex[unc-119::GFP]*	Pan-neuronal marker
NM440	*unc-104(e1265); jsIs1*	*jsIs1[pSB120 (snb-1::GFP); pRF4 (rol-6(su1006))]*	Nerve ring, ventral cord, dorsal cord
SK4005	*zdIs5*	*zdIs5 [mec-4::GFP + lin-15(+) (pSK1)]*	Touch neurons
LX929	*vsIs48*	*vsIs48[unc-17::GFP]*	All cholinergic neurons
EG1285	*lin-15B(n765); oxIs12*	*oxIs12 [unc-47p::GFP + lin-15(+)]*	All GABAergic neurons
CZ333	*juIs1*	*juIs1 [unc-25p::snb-1::GFP + lin-15(+)]*	Presynaptic terminals of GABAergic DD and VD motor neurons and RME neurons
NM306	*jsIs1*	*jsIs1[pSB120(snb-1::GFP) + pRF4(rol-4(su1006))]*	Nerve ring, ventral cord and dorsal cord
OH7547	*otIs199*	*otIs199 [cat-2::GFP + rgef-1(F25B3.3)::dsRed + rol-6(su1006)]*	Dopaminergic neurons and dsRed expressed pan-neuronally
BZ555	*egIs1*	*egIs1[Pdat-1::GFP]*	Dopaminergic neuronal soma and processes

C. elegans presents a stereotyped synaptic positioning, both the number and type of synaptic connections formed being similar between individuals (75 % reproducibility) [6, 10, 11, 19]. Yet, recent studies demonstrate that, as in mammals, synaptic activity may play a decisive role in shaping synaptic patterns after the initial pattern is established. As an example, mutants with reduced cholinergic synaptic transmission present enhanced sprouting of cholinergic SAB neurons [20].

Considering its simplicity, invariant neuronal network, and all the markers available, it is fairly easy to score (neuro)developmental defects in *C. elegans*, making this model a powerful tool to identify genes involved in neuronal formation and maturation and axonal outgrowth and migration. Despite its simplicity, *C. elegans* neurons use an array of classical neurotransmitters similar to those of mammals such as acetylcholine, dopamine, serotonin, GABA, and glutamate, whereas histamine, epinephrine, and norepinephrine seem to be absent [5].

Acetylcholine is the major excitatory neurotransmitter at nematode NMJs, and more than a third of the cells release acetylcholine, which is important for locomotion, egg laying, feeding, and male mating [21]. Aldicarb inhibits acetylcholinesterase, the enzyme responsible for hydrolysis of acetylcholine, culminating in buildup of this neurotransmitter, causing paralysis. Thus, several genes involved in biosynthesis and metabolism of acetylcholine have been identified by presence of the "Ric" phenotype (for resistance to inhibitors of cholinesterase) in response to aldicarb or other similar compounds [22].

As in mammals, fast excitatory neurotransmission in *C. elegans* is mainly glutamatergic, and both excitatory and inhibitory ionotropic glutamate receptors (iGluR) exist [23–25]. Glutamate-gated chloride channels are also present, though less studied and well understood [26]. iGluR are important for locomotion, feeding, defecation, and recently, were shown to be a determinant for learning and memory formation. For example, *eat-4* encodes a vesicular glutamate transporter highly expressed in sensory neurons that respond to tapping [27–29], and deletion of this gene induces a more rapid habituation to tap [29, 30], suggesting a crucial role for glutamate in this type of learning. Interestingly, complementation with the human counterpart reverts the impairment, suggesting a common functional role [31]. Furthermore, in certain paradigms, worms can learn to associate paired stimuli and this is dependent on *glr-1* [32].

Bioamines, such as canonical dopamine and serotonin and the invertebrate-specific octopamine and tyramine, act in both neurons and muscles to affect egg laying, pharyngeal pumping, locomotion, and learning [33]. Such as in mammals, dopamine D1 and D2 receptors (*dop-1* and *dop-3*, respectively) can act antagonistically, and their balance in specific dopaminergic neurons tightly controls response to food [33, 34]. Similar to vertebrates and in further support of common neurotransmitter systems, in *C. elegans*, exposure to 6-OHDA induces programmed cell death in dopaminergic neurons [35–37].

GABA is an important inhibitory neurotransmitter in *C. elegans*, but in contrast to vertebrates where it acts at synapses of the central nervous system, in nematodes, GABA acts primarily at neuromuscular synapses, being important for locomotion, defecation, and foraging [38]. GABA is expressed in 26 of the 302 neurons present in *C. elegans*, and the proteins involved in GABA biosynthesis and transport are remarkably conserved (Fig. 2). Such as in mammals, there are two types of receptors, $GABA_A$ and $GABA_B$, based on sequence similarity [39–42].

In addition to conventional neurotransmitter molecules, to date, 113 genes encoding over 250 distinct neuropeptides have been identified in worms [43]. These neuropeptides are involved in a wide range of worm behaviors such as locomotion, egg laying, social behavior, and ethanol response [43] and are expressed in both nervous and non-nervous tissues. Of these, 40 encode insulinlike peptides (*ins* family), 31 encode FMRFamide-related peptides (FLPs), and 42 encode other types of peptides (neuropeptidelike peptides, NLPs). Neuropeptides are short amino acid sequences that act directly (as primary neurotransmitters) or indirectly to modulate synaptic function. Identification of neuropeptides and their receptors is a complicated task since peptides may functionally overlap and are able to bind to various receptors, depending on the physiological condition of the animal. Among the most studied neuropeptides are members of the insulinlike family, such as *ins-1*, highly expressed in neuronal tissues and that have been shown to regulate reproductive growth and longevity [44]. Under harsh environmental conditions, *C. elegans* undergoes an alternative life stage, called dauer, and this decision is dependent on the activity of an insulinlike receptor, *daf-2*, and *daf-28*, a beta-type insulin [45]. Involvement of this signaling pathway in longevity, discovered in *C. elegans*, has also been identified in *Drosophila* and mammals [46–48].

While it is undeniable that neurotransmitter systems and neuropeptides are significantly conserved in *C. elegans*, we cannot overlook the fact that worm findings do not always mimic the human picture nor are easily translatable. As an example, fluoxetine, a serotonin reuptake inhibitor, is an antidepressant in humans and other mammals, while in worms, it is a potent stimulator of egg-laying [49–51]; these two apparently unlike phenotypes are the result of similar neuronal control by serotonin. In mammals, cocaine primarily exerts its behavioral effects by inhibiting dopamine reuptake, leading to a stimulant effect. In contrast, in worms, cocaine leads to hypolocomotion and its effects are not dependent on dopamine, being mediated by the ionotropic serotonin receptor MOD-1 [52]. These pitfalls cannot be

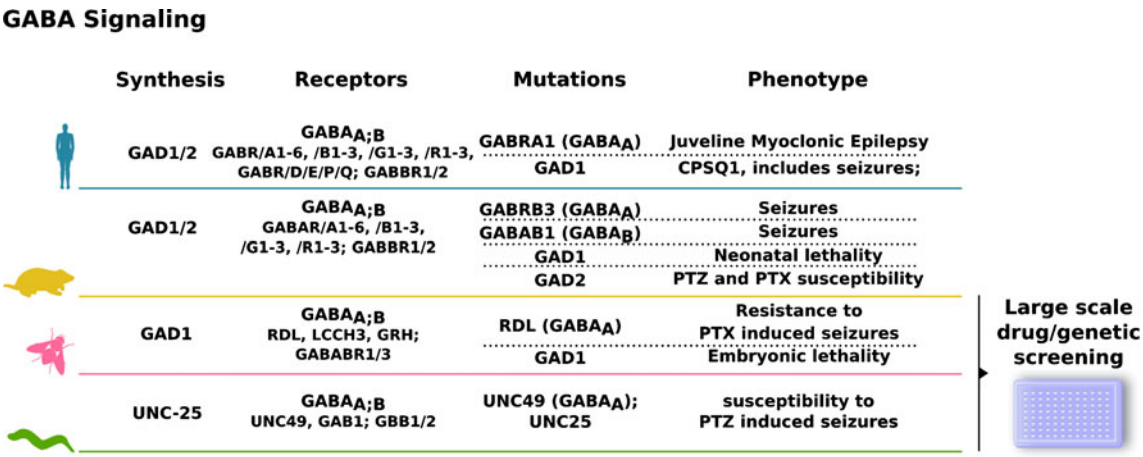

Fig. 2 Evolutionary conserved GABAergic signaling. The proteins involved in the metabolism of GABA and its receptors are remarkably conserved in humans, mice, *Drosophila*, and *C. elegans*. Mutations in *GAD1* have been associated with recessive cerebral palsy, a condition in which patients often present seizures. Murine knockout models for *Gad1* and *Gad2* also display seizures. Wild type worms are resistant to proconvulsing effects of pentylenetetrazol (PTZ); however, knockout animals for *unc-25* (*GAD1/2* ortholog) present PTZ-induced convulsions. *Drosophila* deletion of *Gad1* gene is lethal. Mutations in $GABA_A$ receptors have been associated with epilepsy in humans. In mice, deletion of both $GABA_A$ and $GABA_B$ increases seizure susceptibility. Worm mutants for $GABA_A$ receptor *unc-49* also present severe PTZ-induced convulsions. On the contrary, *Drosophila Rdl* knockouts are resistant to picrotoxin (PTX)-induced seizures. [137, 143, 277, 293–298]

neglected but this nematode is still an attractive and complementary model to study cellular and molecular mechanisms underlying neuronal phenomena. Furthermore, its tractability, genetic amenability, and feasibility of doing large-scale analysis have led to substantial use of this model in drug and/or genetic screenings. Among all the models, *C. elegans* is the most cost-effective to use in high-throughput analysis and still offers the advantage of being a multicellular organism in comparison with cell culture systems or yeast (reviewed in [53]).

A Simple Organism Presenting Complex Behaviors

In contrast to its simplicity, in terms of neuronal architecture, *C. elegans* presents a repertoire of relatively complex behaviors. Worms can sense hundreds of different odors even at a very low concentration, discriminate among them, and generate behavioral responses that are appropriate to the cue. Similarly, *C. elegans* is able to sense a variety of noxious stimuli, including low pH, heavy metals, detergents, and high osmolarity [54–58], using specific sensory neurons identified by laser ablation studies (reviewed in [7]). Simplicity of the neuronal circuit allowed identification of neurons (and genes) involved in sensing and discrimination of several of these compounds. Interestingly, worms present some degree of olfactory adaptation given that naïve animals will respond more than preexposed animals to a variety of signals. Moreover, *C. elegans* is capable of learning the odors of different bacteria and avoid strains that make them ill [59]; these learned olfactory behaviors are associated with neurochemical changes that induce behavioral (re) modeling. Curiously, *C. elegans* sensory perception is also able to regulate its longevity, suggesting that in nature, lifespan may be regulated by environmental cues rather than being determined solely genetically [60], a finding later confirmed in *Drosophila* [61].

Pioneering studies have proven that worms are able to learn and present both short- and long-term memory underlying the nonassociative form of learning—habituation [29, 30, 62, 63]. Later, evidence showed that worms also present classical conditioning/associative learning using different types of stimuli (chemosensory and thermosensory) [32, 64, 65]. As an example, worms chemotax to NaCl if previously associated with food [66, 67]. Similarly, in a temperature gradient plate, worms will migrate to the food-associated temperature with remarkable accuracy [64, 68]. Conversely, animals can also make a negative association if the attractant was previously associated with an aversive stimulus such as starvation [69]. In mammals, learning is strongly dependent on experience-dependent synaptic changes in glutamatergic synapses. Likewise, glutamatergic transmission is important for behavioral plasticity and learning in *C. elegans*. For example, *glr-1* (AMPA-type glutamate receptor) mutations block olfactory associative and nonassociative learning in *C. elegans* [30, 32, 70]. As mentioned before, *eat-4*, encoding a vesicular glutamate transporter, is crucial for tap habituation learning [30].

Similar to other species, distributed training (blocks of stimuli separated by longer resting periods) appears to be fundamental for long-term memory formation in *C. elegans*, in contrast with massed training (similar number of stimuli in just one block) [71]. *C. elegans* goes beyond simple learning and memory, presenting context

conditioning that is sensitive to latent inhibition and extinction (reviewed in [7]).

C. elegans also presents some degree of social interaction, and this is controlled by the neuropeptide Y (NPY) receptor (NPR-1). Some strains, upon encountering food/bacteria, reduce locomotion and disperse in the bacteria lawn and feed individually, whereas other strains move fast across the lawn and aggregate [72]. A single nucleotide substitution in the receptor was shown to be sufficient to transform the isolated strains to become social. In mammals, NPY and its receptors are involved in regulation of food consumption, anxiety, and stress resilience (reviewed in [73]), a somewhat different role from that in nematodes. Yet, recent work suggests that social isolation can induce expression changes in NPY in mammals [74] and that administration of an antagonist of NPY receptor subtype 2 (Y2R) can revert nicotine-induced social anxiety [75], suggesting that NPY can also play a key role in social behavior in higher species.

Cutting Edge Tools in the Field

Due to its size and easy and unexpensive maintenance and tractability, *C. elegans* is suitable to large genetic and drug screenings. This is a noteworthy benefit of using this model in the initial study of several disorders, including those of the nervous system. Generation of knockout and transgenic strains is a relatively straightforward process and certainly less time- and money-consuming than in other species. Apart from classical mutagenesis (chemically induced or by radiation), we can also take advantage of RNAi, a technique that is well established in worms and works for most genes. However, systemic delivery of RNAi (usually by feeding worms with bacteria expressing the interest dsRNA) occasionally masks pertinent neuronal phenotypes and commonly neurons are refractory to classic RNAi [76]. Since RNAi is a powerful tool to ascertain gene function, several groups have tried to overcome difficulty of achieving efficient neuronal RNAi silencing in *C. elegans* either by using specific RNAi-sensitive strains [77] or simply based on the expression under neuronal specific promoters of sense and antisense RNAs corresponding to the gene of interest [78]. Others have developed a knockdown technique based on the in vivo expression of heritable inverted-repeat genes. This approach allows effective gene inactivation in the nervous system in a time-specific manner using inducible promoters, for example. Moreover, stable lines harboring the transgene can be easily maintained [79]. Besides deletion/knockdown of specific genes, increasing evidence suggests that several neurodevelopmental disorders present a dosage defect rather than a loss-of-function mutation. In this perspective, *C. elegans* is still a very attractive model, since it is fairly easy to create transgenic animals and control expression of genes with temporal and cellular specificity by use of specific promoters.

In the last years, several technical improvements have been implemented in the study of *C. elegans* nervous system, some of them simple to set up and some other requiring a significant optimization process. Live imaging is particularly attractive and simple to use in *C. elegans* considering its transparency and well-described anatomy. In addition to fluorescent markers that tag specific neuronal populations, one can monitor neuronal excitability in vivo and in freely moving animals by live calcium imaging [80]. For example, calcium imaging studies determined that the AWC neuron responds to temperature changes and that response thresholds differ depending on previously experienced temperature [81]. Using the same technique, others have shown that the AFD neuron transmits both stimulatory and inhibitory temperature signals and that the activity of this neuron is compromised in animals depleted for CREB, a protein necessary for memory and learning [82]. This technique allows multiple neuronal recording and temporal correlation of neuronal activity but it is always dependent on imaging methods and is often inadequate to detect subthreshold membrane potential changes [83]. Other precise but drastically more invasive methods have been adopted, such as electrophysiological measurements, though several constraints exist considering the highly pressurized *C. elegans* body and the small size of its neuronal cell bodies (reviewed in [84]). Nevertheless, with careful dissection and some training, it is possible to obtain reliable data using this technique. Patch clamping was initially performed in the pharynx, in which the contraction (as in other fast muscles) is controlled by changes in membrane electrical potential because it was easier to identify and access. By recording pharyngeal activity, several studies have identified mutations in presynaptic proteins [85] and ion channels [86]. Later studies were performed in exposed neurons from dissected animals [87] and some were even able to record touch response currents from PLM mechanosensory neurons [88]. Electrophysiological recording of both endogenous excitatory and inhibitory postsynaptic currents of the NMJ was an excellent tool to identify important genes in the control of GABA or acetylcholine release [89]. An adaptation of this technique was also applied to record currents from head neurons with success [90–92].

Besides genetic manipulation of selected neuronal subtypes, it is possible to perform specific neuronal laser ablation in *C. elegans* in order to better dissect the function of a particular neuron or group of cells. This technique was used with success to scrutinize the neural circuit underlying habituation [93, 94], thermotaxis [95, 96], and head-touch-mediated backward movements [93, 97]. Manipulations of the timing of laser ablation during the training process of the

animals even allowed researchers to understand the kinetics of habituation [94]. Genetically induced cell death is also possible in worms through, for example, ectopic expression of a dominant version of the *mec-4* allele, which encodes a subunit of a candidate mechanotransducing channel. Overexpression of mec-4-dominant allele is thought to elevate ion influx through the channel, leading to vacuolation of several cell types, including neurons and muscular cells [98]. Another example is use of light-inducible and tissue-selective expression of mini singlet oxygen generator (miniSOG), a newly engineered protein that generates singlet oxygen upon blue light excitation, leading to cellular death without detectable damages to surrounding tissues [99]. More recently, laser ablation has emerged as an excellent tool to study the process of neuronal regeneration. Using high-energy pulses, it is possible to severe axons (axotomy) and then perform subsequent regeneration studies [100, 101].

Pioneering techniques such as optogenetics have also been employed in worms with great success [102–104]. First, by manipulating the release of acetylcholine or GABA at the NMJ using targeted expression of channel rhodopsin-2, researchers were capable of analyzing neurotransmission with high temporal precision [105, 106]. Later, researchers developed a new system that allows manipulation of neural activity with high spatial and temporal resolution, enabling control of locomotion in real time [107]. Further expansion of this technique by combination with microfluidics technology and computer automation was developed in order to reach higher throughput and improve standardization and consistency in data gathering. In addition, it is possible to infuse drugs during optogenetic manipulations using microfluidics, providing a significant contribution for the study of synaptic function, for example [106]. Other opsins, namely, archaerhodopsin-3, a neuronal silencer, were recently applied in the study of the *C. elegans* nervous system [108].

Recently, a high-throughput microfluidic approach has been used for automatic identification and sorting of *C. elegans* mutants with possible neurodevelopmental or neurodegenerative phenotypes by using a GFP marker for GABAergic motor neurons, with impressive speed and efficacy [109].

C. elegans in the Study of Human Disorders

Despite its evolutionary distance from mammals, *C. elegans* possesses thousands of genes orthologous to humans [110]. Worms have allowed insight into molecular mechanisms underlying neurodegenerative disorders such as tauopathies [111, 112], Alzheimer's disease [113–115], Parkinson's disease [116, 117], polyglutamine disorders [118–122], juvenile neurolipofuscinosis [123], and amyotrophic lateral sclerosis [124], among others [125]. Most of the models involve transgenic expression of the human protein containing the mutation in specific tissues/neurons. For example, overexpression of an expanded polyglutamine tract in *C. elegans* neurons induces protein aggregation in vivo, and selective neuronal toxicity and motility defects [118, 126], equivalent to humans and mouse models. Pan-neuronal expression of mutated tau caused progressive motor uncoordination and accumulation of insoluble hyperphosphorylated tau in *C. elegans*. These animals presented substantial neurodegeneration, with axonal disruption and presynaptic defects [112, 126]. This model was later used to mechanistically dissect tau-induced neurodegeneration and to identify drugs/genes that inhibit tau toxicity [111, 127–130].

Reverse genetics is another way of dissecting the biological role of a given gene and to better understand how loss of function mutations originates specific neuronal deficits. Mutations in presenilin genes cause one form of aggressive familial Alzheimer's disease. The two worm orthologous genes, *sel-12* and *hop-1*, are required for correct morphology and function of two cholinergic neurons involved in temperature memory formation [115]. Interestingly, insertion of the wild type human gene, but not a mutated form, is able to revert neuronal deficits and memory impairment, suggesting an overlapping function between worm and human counterparts [115].

However, this remarkable resemblance of the model with the human picture is not always obvious. In humans, mutations in either *PKD1* or *PKD2* genes cause almost indistinguishable clinical symptoms, leading to polycystic kidney disease (PKD). Mice PKD models develop cysts in the kidney and other organs, similarly to humans. Deletion of worm orthologous genes, *lov-1* and *pkd-2*, provided apparently discrepant and unrelated outcomes. No differences were found in the very rudimentary excretory system of *C. elegans* mutants; rather, *lov-1* mutation affected mating behavior in male worms [131]. This mating defect was due to dysfunctional cilia, and amazingly, later findings have shown that PKD proteins were expressed in cilia of kidney cells and that cilliar dysfunction could be responsible for the formation of cysts [132]. Whereas at first glance the findings in worms were odd, they certainly contributed to the understanding of the biological process underlying PKD. These results are quite interesting in the light of recent evidence suggesting that many human neurodevelopmental problems are linked to mutations in primary cilia formation (ciliopaties) [133], making *C. elegans* an appealing model to study the molecular basis of these disorders.

In fact and apart from being a great model to study the mechanisms of neurodegeneration, worms are very appealing in the study of neurodevelopment disorders such as

epilepsy, ID, and ASDs, considering the existing know-how of neuronal connectivity in this animal. Whereas environment can play a fundamental role in development of these disorders, numerous studies have shown that they have a strong genetic basis, with either monogenic or polygenic etiology. However, though various genetic studies pinpointed specific regions associated with these disorders, the functional validation of the findings has often been neglected. In fact, for a large proportion of the genes found to be associated with epilepsy, ID, and ASDs, nothing is known about their function or consequences of their mutation in the nervous system. *C. elegans* could be envisaged as an appealing biological platform, and the argument that *C. elegans* is too simple and limited in the behavioral repertoire to study these complex disorders is being abandoned in the light of evidence previously discussed. First, *C. elegans* displays complex behaviors such as learning and habit formation and even presents some degree of social interaction; second, the neurotransmitters/receptors and the basis of neuronal mechanisms are remarkably conserved, and thus, the neurobiological basis of human disease can be explored in detail in this model. In the next section, we will give some insights on emerging worm models in the study of the molecular mechanisms underlying epilepsy, ASDs, and ID.

C. elegans as a Model to Study Epilepsy

Epilepsy is estimated to affect 1–2 % of the population worldwide, and around 40 % of the cases are thought to have a genetic basis. Epilepsy is characterized by repeated seizures (or convulsions), which are episodes of disturbed brain activity, i.e., abnormal, excessive, or hypersynchronous neuronal activity in the brain. Mutations in several genes have been linked to different types of epilepsy, including many genes that code for protein subunits of either voltage-gated or ligand-gated ion channels [134–136]. Numerous genetically engineered mice/rats have been developed to study epilepsy and to better understand the contribution of specific genetic mutations for the development of the disease [137–142].

Other cases of idiopathic-generalized epilepsy are compatible with a multigenic mode of inheritance and are most likely the result of additive interaction of multiple susceptibility genes contributing to disease. However and although every year several genetic associations are reported, most lack biological/functional validation. This flaw is a consequence of the high cost, in terms of money and time, of creating novel genetically modifiable murine mutant models for each gene. In this context, simpler and genetically amenable animal models such as worms are essential tools in dissection of gene function and contribute to the understanding of phenotype(s)/genotype relationships (Table 2).

Seizures are caused by an unbalance in either the excitatory and/or inhibitory input. In this context, simplicity of the *C. elegans* locomotor circuit may be crucial in studying seizure susceptibility. Whereas cholinergic innervation excites muscles to contract alternately on the ventral or dorsal side, it simultaneously activates GABAergic inhibition to relax muscles on the opposite side. Wild type worms do not naturally display seizures, but null mutants for *unc-43*, a calcium/calmodulin-dependent serine/threonine kinase II (CaMKII) that regulates synaptic plasticity, were reported to present spontaneous convulsions [143].

Somehow contradictory to the evidence in rodents, researchers found that wild type animals are resistant to GABA(A) receptor antagonist Pentylenetetrazol (PTZ), a potent compound that induces seizures in mammals. However, in specific sensitized genetic backgrounds, PTZ can produce different types of convulsions, depending on molecules and circuits affected. For example, *unc-25* (GABA synthesis) mutants display repetitive contractions in the head, while *unc-43* mutants present full-body convulsions (Fig. 2) [143]. The definitive proof of concept for the use of *C. elegans* to study epilepsy is the confirmation that the epilepticlike phenotype was a result of the abnormal synchronous activity of specific neurons. Using calcium imaging, researchers found that *unc-43* animals displayed aberrant intestinal calcium oscillations that were reflected in abnormal defecation rhythm [144], raising the hypothesis that the same could occur in neurons, increasing susceptibility to seizures.

A mutation (gain of function) in a neuronal acetylcholine receptor, *acr-2*, causes spontaneous muscle convulsions in *C. elegans* due to cholinergic overexcitation accompanied with a decreased GABAergic inhibition in the locomotor circuit [145]. Mutations in human acetylcholine receptors have also been associated with epilepsy [146]. Additional studies have shown that this epilepsylike phenotype is dependent on the activity of the TRPM nonselective cation channel *gtl-2*, which plays a role in ion homeostasis. Researchers have suggested that the convulsions were the result of a local ionic imbalance [145] and that *glt-2* loss of function could counterweigh the excitation–inhibition imbalance caused by *acr-2* (rather than affecting basal synaptic transmission), probably through ion level modification. In further support of this hypothesis, they show that altering Zn^{2+} homeostasis (but no Mg^{2+}), had an anticonvulsant effect, analogously to *glt-2* loss of function. These promising and groundbreaking results may be translated into the human picture, since: (1) TRPM channels from other species also show permeability to divalent cations, including Zn^{2+} [147], and (2) manipulation of Zn^{2+} can activate acetylcholine receptors while inhibiting some GABA receptors [148, 149]. This study revealed a new role for ion homeostasis in seizure susceptibility and highlighted

t2.1 **Table 2** Epilepsy-related genes. Studies in *C. elegans* that added important value to our understanding of the function and malfunction of human genes associated with epilepsy

t2.2 Gene	Function	*C. elegans* findings	Disease association
t2.3 *CAMK2D*	Isoform delta 4 of calcium/calmodulin-dependent protein kinase type II; regulation of Ca^{2+} homeostasis; synaptic plasticity	*unc-43* is required for locomotion, neuronal cell fate specification and regulation of synaptic density, among others [275]	Polymorphism in *CAMK2D* gene is associated with seizure susceptibility of Sprague–Dawley rats [276]. No information about its association with human neurodevelopmental disorders
t2.4		*Unc-43* mutants present full-body convulsions [143]	
t2.5 *GAD1*	GABA neurotransmitter biosynthetic enzyme, glutamic acid decarboxylase (GAD)	*unc-25* encoder is required for GABA synthesis and GABA-mediated behaviors	Mutation in this gene associated with autosomal recessive spastic cerebral palsy-1, which includes seizures [277]
t2.6		*unc-25* mutants present head-bobbing convulsions [143]	
t2.7 *CHRNA7*	Nicotinic acetylcholine receptors (nAChRs); ligand-gated ion channels that mediate fast signal transmission at synapses	*acr-16* is required for the major fast cholinergic excitatory current at NMJ. ACR-16 localizes to postsynaptic regions and is regulated by a Wnt signaling pathway [275]	Is within the frequent 15q13.3 microdeletion that is associated with idiopathic generalized epilepsy [151, 152]
t2.8		*acr-16* mutants present reduced synaptic depression at the NMJ; imbalance in excitatory-inhibitory input [278]	
t2.9 *CHRNA3*	Nicotinic acetylcholine receptors (nAChR)	*acr-2* mutants present spontaneous muscle convulsions due to cholinergic excitation and decreased GABAergic inhibition [145]	Associated with lung cancer [279]. Genes encoding similar proteins have been linked with epilepsy (CHRNA2, CHRNA4, and CHRNB2) [153]. No information about its association with human neurodevelopmental disorders
t2.10 *STXBP1*	Syntaxin-binding protein; plays a role in release of neurotransmitters via regulation of syntaxin; regulation of synaptic vesicle docking and fusion	*unc-18* functions as a chaperone for UNC-64/syntaxin; it enables vesicle docking in synaptic regions before vesicle priming and fusion; it promotes synaptic vesicle exocytosis [275]	Associated with intellectual disability and epilepsy [161] and early infantile epileptic encephalopathy [280]
t2.11		*unc-18* mutants present reduced vesicle docking and are resistant to aldicarb (acetylcholinesterase inhibitor) [163]	
t2.12 *LIS1*	*LIS1:* microtubule association protein that interacts with dynein, doublecortin, and NudE (nuclear distribution E (NudE) family of proteins) members	*lis-1* mutants are sensitive to PTZ, displaying full-body convulsions [143]. Deletion of genes of the "*lis-1* pathway" also seems to increase susceptibility to seizures, eventually by decreasing GABA threshold [175]. Several of these genes are important for GABAergic synaptic vesicle location [175]	*LIS1* is a key gene underlying lissencephaly[168, 171]
t2.13 *DCX*	Doublecortin; directs neuronal migration by regulating the organization and stability of microtubules	*zyg-8* is a microtubule organizer in worm neurons; controls cell body shape/polarity and process outgrowth and morphology of the six touch receptor neurons and motor neurons as well as other neuronal and non-neuronal cells [281, 282]	Mutations in *doublecortin* cause abnormal migration of neurons during development leading to epilepsy, mental retardation, and lissencephaly in males [172, 283, 284]
t2.14 *NudE family NDE1, NDEL1*	*NDE1*: interacts with other centrosome components as part of a complex that regulates dynein function; essential role in microtubule organization, mitosis, and neuronal migration	NudE homologs—*nud-2* and *nud-1* mutants present PTZ-induced tonic–clonic convulsions [175]	Mutations in *NDE1* gene causes lissencephaly [285]
t2.15			No information about disease-associated mutations in *NDEL1*
t2.16	*NDEL1*: required for microtubule organization and anchoring at the centrosome; also positively regulates the activity of dynein and neurite outgrowth		

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Table 2 (continued)

Gene	Function	*C. elegans* findings	Disease association
DYNC1H1	Dynein heavy chain; microtubule-activated ATPases that have been implicated in a variety of intracellular motility, including retrograde axonal transport, among others	Dynein heavy chain homologs: *dhc-1* mutants display convulsions [175]	Mutations in *DYNC1H1* have been found in individuals suffering from severe intellectual disability and that present seizures [286]
CDK5 and *p35*	*CDK5*: phosphorylation of both high molecular weight neurofilaments and microtubule-associated protein tau *p35*: neuron-specific activator of CDK5. The complex p35/CDK5 is required for neurite outgrowth and cortical lamination; dendritic spine morphogenesis	*CDK5* and *p35* homologs: *cdk-5* and *cdka-1* mutants present PTZ-induced convulsions	*CDK5* is necessary for neuronal formation and differentiation [287] Mice knockout for *p35* present cortical defects and seizures [288] No information about its association with human neurodevelopmental disorders
RAC1	RAS superfamily of small GTP-binding proteins; regulation of diverse cellular events, including the control of cell growth and cytoskeletal reorganization	*RAC-1* homologs: *ced-10* and *mig-2* mutants present PTZ-induced convulsions [176]	No information about its association with human neurodevelopmental disorders
TRIO	Promotes the exchange of GDP by GTP	Worm homolog, unc-73, presents PTZ-induced seizures [176]	No information about its association with human neurodevelopmental disorders

TRPM channels as new players in this process, which can now be further explored in higher organisms and eventually used to develop novel pharmacological approaches.

Another study has shown that increasing temperature in combination with exposure to higher levels of salts (NaCl and $MgCl_2$) triggers abnormal neuronal bursts in *C. elegans*. Baccoside A, a molecule found in extracts of the plant *Bacopa monnieri*, which has been shown to inhibit excitatory neurotransmission by blockade of calcium channels, significantly reduced seizure/convulsion at higher temperatures, eventually by modulating calcium entry in the cells [150]. Moreover, T-type Ca^{2+} channel mutant *cca-1* does not present seizures at any stage, suggesting that additional studies are required to dissect how this molecule works and the contribution of these channels for epilepsy.

The *CHRNA7* gene encodes the subunit alpha 7 of nicotinic acetylcholine receptors (nAChRs), members of a superfamily of ligand-gated ions mediating fast signal transmission at synapses. *CHRNA7* has been associated with several neurodevelopmental disorders, namely, epilepsy, ID, and schizophrenia [151, 152]. *CHRNA7* is a very strong candidate gene for epilepsy involvement, as genes encoding other subunits of nAChRs, e.g., *CHRNA2*, *CHRNA4*, and *CHRNB2*, are known to be associated with autosomal-dominant nocturnal frontal lobe epilepsy [153]. *C. elegans* possesses one of the largest nAChR families known for any organism and a combination of genetic, microarray, physiological, and reporter gene expression studies has added greatly to our understanding of the components of nematode muscle and neuronal nAChR subtype [154]. The *C. elegans* ortholog of *CHRNA7* is *acr-16* [155, 156], which encodes a similar subunit and works as a ligand-gated ion channel that is required for the major fast cholinergic excitatory current at *C. elegans* NMJ [157]. One elegant study has shown that in the NJM, one single stimulus is able to induce prominent long-lasting depression in acetylcholine motor neurons. This phenomenon is highly dependent on desensitization of the postsynaptic acetylcholine nicotinic receptor ACR-16 but not on its counterpart acetylcholine levamisole receptor UNC-38 [158]. *Acr-16* mutants presented slower synaptic depression in comparison with wild type animals, suggesting that *acr-16* plays a key role in the balance of excitatory and inhibitory inputs. Interestingly, similarities between worm and human nAChRs go beyond receptor function. The conserved Wnt pathway seems to be crucial for correct translocation of some types of nAChR into the pre- or postsynaptic membranes. In mammals, Wnt7a regulates presynpatic localization of α7-nAChRs [158]. Likewise, in worms, Wnt ligand CWN-2 binds to CAM-1/LIN-17 (Ror receptor tyrosine kinase/Frizzled) heteromeric receptors, activating downstream effector DSH-1 (disheveled), which regulates ACR-16 translocation into the postsynaptic membrane [159]. Mutants of all of these players present

accumulation of nonsynaptic ACR-16 and a significant reduction in synaptic current (Fig. 3b). However and despite this evidence suggesting altered excitatory–inhibitory synaptic balance, it remains to be determined if these mutants present enhanced seizure susceptibility.

STXBP1 (syntaxin-binding protein 1) encodes a neuronal specific syntaxin-binding protein, the mammalian homolog of the *C. elegans unc-18* gene [160]. Mutations in *STXBP1* have been found to lead to autosomal dominant epilepsy and ID [161]. The *C. elegans unc-18* gene was first identified as being required for maintenance of acetylcholine levels [162]. *Unc-18* is required for neurotransmitter release and regulation of vesicle exocytosis via SNARE interaction (Fig. 3a) [163, 164]. Accordingly, worms lacking functional *unc-18* show resistance to paralysis induced by aldicarb, an acetylcholinesterase inhibitor [15]. This mechanism is evolutionarily conserved, and as has been shown for *unc-18*, STXPB1 also binds to syntaxin-1, a SNARE protein involved in synaptic vesicle docking and fusion, and seems to act in the control of vesicle docking as well as the regulation of the vesicle fusion rate [165, 166]. In addition, it has previously been shown that mice lacking *Munc18-1* suffer from complete loss of neurotransmitter release from synaptic vesicles throughout development [167]. Aldicarb resistance can suggest a different neuronal excitability in these mutants, which would be interesting to explore in the context of seizure susceptibility.

Lissencephaly is a nervous system disorder characterized by a "smooth brain," lacking convolutions or gyri due to abnormal neuronal migration and poor survival of cortical neurons during development. Lissencephaly can be caused by mutations in the *TUBA1A*, *LIS1*, *ARX*, *DCX*, and *RELN* genes [168–174], among others, and several point mutations in these genes have been identified; importantly, patients

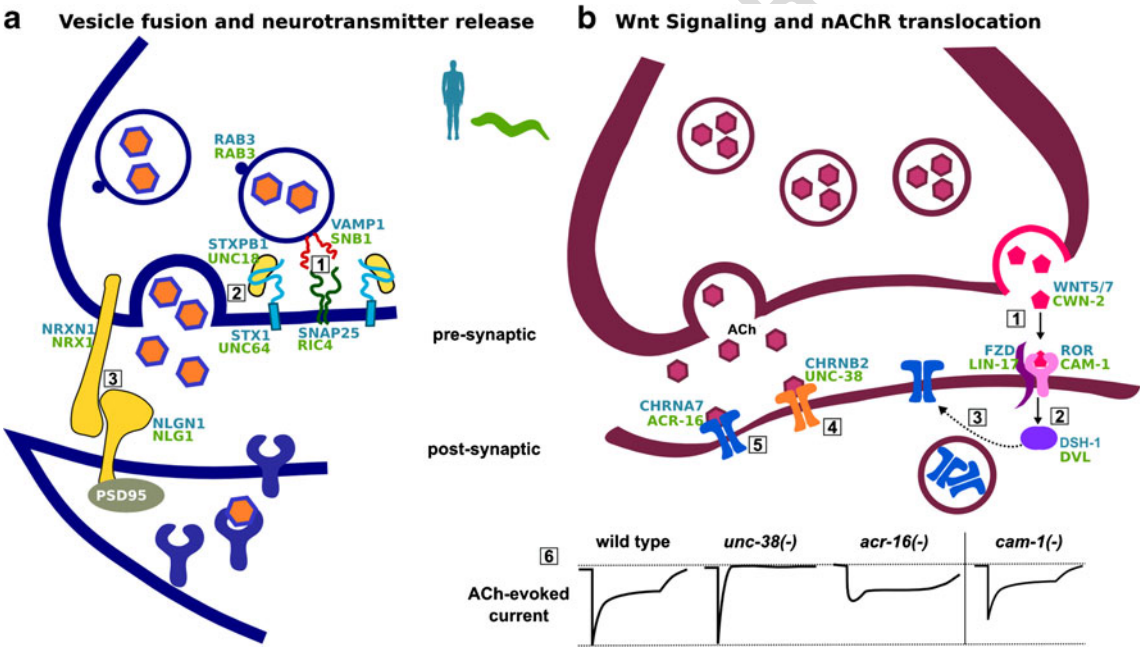

Fig. 3 Conserved neuronal pathways between *C. elegans* and humans, which are relevant in the context of different neurodevelopmental disorders. **a** At the presynaptic site, the conserved SNARE complex mediates vesicle fusion and neurotransmitter release to the synaptic cleft (*1*). STXPB1 (UNC-18 ortholog) binds to the SNARE protein STX1 (UNC-64 ortholog) (*2*) regulating this process. Mutations in STXPB1 are associated with epilepsy and intellectual disability. NLG-1 and NRX-1 are the *C. elegans* orthologs of neuroligins and neurexins, which are conserved cell adhesion proteins essential for synapse formation, maturation, and stability (*3*), and have been implicated in autism spectrum disorders. **b** Representative scheme of a cholinergic synapse between a motor neuron and a muscle cell in *C. elegans*. The translocation of nicotinic acetylcholine (ACh) receptors (nAChRs) may require conserved members of the Wnt signaling pathway in worms and humans. In *C. elegans*, CWN-2 (Wnt ligand; *Wnt5* ortholog) binds to CAM-1/LIN-17 heteromeric receptors (*1*) (CAM-1: Ror receptor tyrosine kinase ortholog; LIN-17: frizzled ortholog), which activate downstream signal transduction molecule DSH-1 (disheveled (DVL) ortholog) (*2*). This pathway is necessary for correct translocation of nAChR ACR-16 to the postsynaptic synapse (*3*). Mutants for all of these genes present a reduction in synaptic current (*6*) (an example of an ACh-evoked current in *cam-1*(−) is shown). This Wnt-dependent translocation pathway seems to be conserved in humans, since Wnt7 is required for presynaptic localization of nAChRs in hippocampal neurons. ACh binds to either levamisole-type receptor UNC-38 (*4*) (ortholog of CHRNB2) or to nicotinic-type receptor ACR-16 (*5*) (ortholog of CHRNA7). *6* ACh induces rapid and complete desensitization of nicotinic ACR-16 receptors (*unc-38(−); acr-16(+)*), whereas its effect in levamisole UNC-38 receptors is less pronounced (*acr-16(−);unc-38(+)*). This is translated into faster and slower synaptic depression in *unc-38* and *acr-16* mutants, respectively. This disturbance of excitatory–inhibitory balance may increase seizure susceptibility in worms. In humans, several mutations in different nAChRs have been associated with epilepsy. Human genes are depicted in *blue* and worm orthologs in *green*. [151–153, 158, 159, 161, 182–184, 186, 278, 280]

often suffer from intractable epilepsy. *C. elegans lis-1* mutants present more than 70 % of lethality, and the survivors present marked seizure susceptibility when exposed to PTZ [143], which works by lowering a threshold of GABAergic response, revealing sensitized neuronal states, which would otherwise not manifest in normal conditions. No major defects were observed in neuronal architecture, but severe presynaptic defects in GABAergic vesicle distribution were found in these mutants [143]. Later studies have analyzed mutants for other genes of the *lis-1* pathway and identified further "seizure-sensitive" genetic backgrounds [175].

LIS-1 interacts with dynein, a well-characterized motor protein, regulator of microtubules and involved in vesicle and organelle transport. Considering the fact that integrity of neural cytoskeleton is essential for regulation of intrinsic neuronal activity, it is not so surprising that dynein mutants also present enhanced PTZ sensitivity [175]. Likewise, mutants for Rac GTPases, actin polymerization regulators, demonstrated a robust behavioral response to PTZ and also exhibited hypersensitivity to aldicarb (an acetylcholinesterase inhibitor), suggesting a deficit in inhibitory neurotransmission [176]. Aldicarb causes body paralysis, resulting from accumulation of acetylcholine at the NMJ; hence, mutations that reduce synaptic transmission cause resistance to aldicarb and vice versa. Another study has identified several endocrine molecules and kinases that regulate GABA transmission in worms, which inactivation increased activity of body muscles, which is directly controlled by GABAergic neurons [15]. Of the 90 positive candidate genes, 21 had previously been associated with seizures, reflecting the value of this model in the study of seizure susceptibility [15].

Treating seizure-susceptible strains with antiepileptic compounds would go in further support of the use of *C. elegans* in the study of epilepsy. However, pharmacological results in *C. elegans* in this regard are not so straightforward to interpret. Anticonvulsants such as valproic acid, ethosuximide, or trimethadione, significantly extend the life span of *C. elegans* [177, 178], a peculiar phenotype that is not easily translatable to the human context. Interestingly, combined treatment of animals with valproic acid and trimethadione produced an additive effect in longevity, suggesting different signaling pathways, and suggested that modulation of neuronal activity may control longevity signals [177]. Indeed, these compounds modulate neuronal activity in worms, since it was found that trimethadione treatment caused hypersensitivity to aldicarb, indicative of neuromuscular activity stimulation [178]. We believe that the studies about the effects of these drugs in *C. elegans* can go beyond behavioral evaluation. For example, valproic acid functions as a histone deacetylase inhibitor and has been exploited in the context of several pathologies, including cancer. By doing a cross-species functional genomic approach and in an attempt to improve therapeutic efficacy of this drug, Forthun et al. have identified novel conserved sensitizers and synthetic lethal interactors of valproic acid [179]. A similar approach could be employed to identify seizure susceptibility/resilience pathways.

Studies with proconvulsant drugs have originated findings that are more straightforward to analyze. PTZ is able to elicit seizures in genetically sensitive backgrounds. Moreover, levamisole, known to activate neuronal nAChRs, which is able to provoke seizures in mammals [180, 181], induces hypercontracted paralysis of wild type nematodes, usually followed by relaxation and death.

Worms and Social Behavior: Relevance for the Study of Autism Spectrum Disorders

ASDs comprise a range of conditions, sometimes classified as pervasive developmental disorders, which involve one or more of the following characteristics: (1) abnormal social behavior, (2) deficits in communication, and (3) presence of stereotyped and repetitive behaviors and obsession with routines (DSM-IV). Due to inherent complexity of ASD symptoms, the use of *C. elegans* as a model system to study this group of disorders is controversial. However, since altered neuronal migration/connectivity or deficits in synaptic transmission has been proposed to be at the basis of etiology of numerous cases of ASDs, even if *C. elegans* does not fully recapitulate core symptoms of ASDs, it can still be very useful to dissect neuronal events leading to these conditions (Table 3).

Mutations in genes encoding neuroligin, neurexin, and shank proteins alter synaptic function and have been reported to underpin ID and ASDs [182–186]. Neuroligins are postsynaptic cell adhesion proteins that bind specifically to presynaptic proteins called neurexins (Fig. 3a). Both are present in excitatory and inhibitory synapses and are crucial for correct neuronal network formation and synapse maturation, stability, and transmission. *C. elegans nrx-1* and *nlg-1* genes are orthologous to human *NRXN1* and *NLGN1* genes, respectively, with the corresponding proteins presenting similar functional domains [187, 188]. NGL-1 is expressed in a subset of neurons, and neuroligin-deficient mutants are viable, with no overt phenotype. However, these animals are defective in a subset of sensory behaviors and sensory processing and are hypersensitive to oxidative stress and mercury compounds [188–190]. Difficulties with processing and/or integration of sensory inputs are often part of the presentation of ASDs, though no sensory deficits have been recognized officially [191]. In this context, it is particularly interesting that *nlg-1* mutants have deficits in the processing of conflicting sensory inputs, as measured in an

t3.1 **Table 3** Intellectual disability (ID)-related genes. Studies in *C. elegans* that added important value to our understanding of the function and malfunction of human genes associated with ID

t3.2	Gene	Function	*C. elegans* findings	Disease association
t3.3	*ASPM*	Asp (abnormal spindle) homolog; may play a role in mitotic spindle regulation and coordination of mitosis	ASPM-1 binds to LIN-15 and is required for its correct localization in the spindle poles. *aspm-1* mutants present pleiotropic phenotypes suggesting that this gene is required for other cell types besides neurons [229]	Associated with autosomal recessive primary microcephaly [227]
t3.4	*PHF8*	Histone lysine demethylase; plays a key role in cell cycle progression, DNA transcription, and brain development	F29B9.2 has a similar function as the human counterpart and its knockdown leads to uncoordination [238]	Associated with X-linked ID and cleft lip/palate [235]
t3.5	*ARX*	Aristaless-related homeobox; transcription factor required for normal brain development and maintenance of specific neuronal subtypes in the cerebral cortex	*alr-1* mutants present deficits in the differentiation of a GABAergic neuron; *alr-1* acts through LIM1 homolog lin-11 pathway. It controls the expression of target genes such as mec-3 to ensure touch receptor neuron differentiation [240, 289, 290]	Associated with epilepsy and ID [239]
t3.6	*TUBA1A*	Tubulin A; major constituent of microtubules; crucial for microtubule formation and organization	*tba-1* mutants are viable: compensatory mechanism, since this mutation is lethal in combination with other tubulin gene mutations. Animals display neuronal synaptic deficits and axonal misguidance [242]	Associated with lissencephaly and polymicrogyria [173, 241]
t3.7	*DOPEY2*	May be involved in protein traffic between late Golgi and early endosomes	*pad-1* suppression showed embryonic lethality. Most of the tissues of the embryo failed to undergo proper patterning during gastrulation; incomplete morphogenesis did not occur [244]	Present in the Down syndrome critical region [243]
t3.8	*DYRK1A*	Dual-specificity tyrosine (Y) phosphorylation-regulated kinase 1A; suggested role in signaling pathways regulating cell proliferation and eventually brain development	*mbk-1* nulls are viable, contrary to mammals. However, overexpression of this gene leads to dose-dependent olfactory defects [250]. These defects were reverted by normalizing *mbk-1* expression, highlighting a possible therapeutic possibility	Present in the Down syndrome critical region [243]. Clinical trials with the aim of normalizing *DYRK1A* function are underway
t3.9	*DSCR1/RCAN1*	Regulator of calcineurin 1; inhibits calcineurin-dependent transcription by binding to the catalytic domain of calcineurin A. Could play a role during central nervous system development	RCN-1 has a similar function as the human counterpart. *rcn-1* deletion or overexpression leads to similar phenotype, including defects in growth, fertility, cuticle development, and egg laying. Importantly, normalization of *rcn-1* expression rescues the deficits [258]	Present in the Down syndrome critical region [243]
t3.10	*PQBP1*	Polyglutamine-binding protein 1; activation of transcription directly or via association with the transcription machinery	*pqbp-1.1* is necessary for lipid metabolism. No major neuronal phenotype [262]	Mutations in this gene have been found in patients with Renpenning's syndrome 1 and other syndromes with X-linked mental retardation [260, 261]. Patients present a lean body, which can be related to worm alterations in lipid metabolism
t3.11	*ATRX*	Alpha-thalassemia/mental retardation syndrome X-linked; contains an ATPase/helicase domain; SWI/SNF family of chromatin-remodeling proteins. Potential involvement in the gene regulation at interphase and chromosomal segregation in mitosis	No report regarding *xnp-1* mutant neurological phenotype. *xnp-1* is vital for gonadal development [267, 268]	Associated with alpha-thalassemia/mental retardation syndrome X-linked [264–266]. Patients present gonadal abnormalities, similar to worm mutant strains

approach–avoidance paradigm. *nlg-1* mutants respond normally to the volatile attractant diacetyl and the repellent cupric acetate; however, their response to simultaneous presentation of these two cues is clearly defective [190].

In further support of a similar role of mammalian and nematode proteins were the results showing that expression of human or rat neuroligin in *nlg-1* mutants rescues osmotic avoidance and gentle touch response phenotypes.

Remarkably, expression of mutant human proteins (with previously identified mutations in ASDs patients) did not revert behavioral impairments nor did expression of wild type NGL-1 under the control of muscular promoter [188], suggesting a key role for this protein in neuronal function.

One unexpected result in *C. elegans* was the fact that loss of neuroligin was not merely correlated with increased sensitivity to oxidative stress but actually caused oxidative stress [190]. Though there is no concluding evidence that oxidative stress may be involved in neurobiology of ASDs, some recent evidence shows that autistic patients present a significant elevation in oxidative stress biomarkers and reduced serum antioxidants such as transferrin and ceruloplasmin [192]. *C. elegans* NRX-1, ortholog of neurexin, is expressed in most of the neurons and localizes to presynaptic specializations [193]. Contrary to *ngl-1* mutants, *nrx-1* nulls do not present any major phenotype or deficits in osmotic avoidance, but interestingly, mutations in this gene suppress neuroligin deficits [187].

The shank gene family encodes postsynaptic proteins that function as part of the NMDA receptor-associated PSD-95 complex (Fig. 3a) [194]. In mammals, Shank cooperates with Homer protein to induce accumulation of inositol-1,4,5-trisphosphate (IP3) receptors in dendritic spines and formation of putative multisynapse spines [195]. Recently, mutations in *Shank* genes have been implicated in ASDs [185], suggesting an important role in normal cognitive development. Overexpression of *Shank1B* and *Homer1b* in hippocampal neurons induces spine maturation, including translocation of the intracellular Ca^{2+} channel inositol trisphosphate receptor (IP3R) [196]. The nematode *shn-1* gene is the ortholog of vertebrate *Shank1*. RNAi of *shn-1* did not cause lethality or major developmental abnormalities. However and in the same line of evidence of mammalian data, suppression of *shn-1* in a defective IP3R background resulted in animals with altered defecation rhythm [197], suggesting a possible role of this protein in affecting function of IP3R. Additional characterization of two different mutant alleles for *shn-1* revealed a crucial role for the ANK-repeat domain in Ca^{2+} signaling with IP3R [198]. It would be interesting to analyze these strains regarding Ca+ signaling and size and strength of synapses considering the fact that *Shank1* knockout mice present reduced size of dendritic spines and weaker basal synaptic transmission [199].

Neurobeachin (*NBEA*) has been identified as an autism candidate gene in a patient with a de novo chromosomal translocation [200]. This multidomain scaffolding protein has been suggested to be involved in neuronal post-Golgi membrane traffic with a role in neurotransmitter release and synaptic functioning [201–203]. Cellular knockdown of *NBEA* suggested that this protein is a negative regulator of secretion of large dense-core vesicles [203]. Study of the *C. elegans* ortholog, *sel-2*, further supported this role in vesicle transport. *Sel-2* was identified as a negative regulator of LIN-12/Notch activity [204], and members of the Notch pathway have also been shown to be modifiers of the *NBEA* homolog in *Drosophila* [205]. Deeper analysis of this interaction may contribute to better understanding of molecular events leading to a subset of ASDs due to deficits in vesicle formation.

L1CAMs are transmembranar cell adhesion receptors belonging to the immunoglobulin superfamily and are conserved in *C. elegans*. The mammalian L1CAM family is composed of four proteins: L1, CHL1, NrCAM, and neurofascin [206]. Mutations in *L1* can originate the X-linked neurological disorder, corpus callosum hypoplasia (CRASH, mental retardation, adducted thumbs, spastic paraplegia, and hydrocephalus) [207–209]. Latest evidence implicated a protein of this family, NrCAM, in autism [210]. *C. elegans* has two L1CAM homologs, *lad-2* and *lad-1* (or *sax-7*) [211–214], which have distinct biological roles. While *lad-2* expression is restricted to a few neurons, *sax-7* is widely expressed since embryonic stages [212, 213, 215]. LAD-2 is important for axon migration by anchoring MAB-20 (ortolog of semaphorin 2) to PLX-2 (ortolog of plexin) [215]. Concordantly, mammalian proteins also function as coreceptors for semaphorin-mediated axon pathfinding [216–218]. Despite involvement in the same pathway, *lad-2* mutants have significantly more axonal defects than *mab-20* or *plx-2* mutants [215], suggesting that *lad-2* may mediate axonal migration through another independent pathway, which could be interesting to ascertain in mammals. On the other hand, *sax-7* mutants present a "normal" development of the nervous system but display deficits in neuronal positioning [211–214], similarly to what is observed in *L1* and *CHL1* knockout mice [216, 218–221]. It is important to refer that L1CAMs are essential in mammals and flies but not in worms, providing a unique framework for the study of the biological role of these proteins.

Contrary to what was initially assumed, *C. elegans* exhibit a broad variety of social behaviors, including mutual attraction and aggregation, mating, population density sensing, and solitary- vs. group-feeding strategies. Variation in feeding strategy is solely due to a single amino acid substitution in NPY receptor, *npr-1* [72]. Solitary strains present high *npr-1* activity, whereas social strains display low activity. This receptor is particularly expressed in the RMG inter/motor neuron, the hub of a finely tuned pathway that controls aggregation and related behaviors [222]. No reports have directly implicated NPY receptors in ASDs, yet, there are some data that may corroborate this hypothesis. First, *Drosophila* NPY (dNFP) is involved in regulation of larval foraging and social behavior [223]. Second, NPY Y2 receptor-deficient male mice display an increase in social interaction [224]. Third, although not conclusive since several genes are within the affected region, there is at least one

reported case of an autistic child with a deletion leading to hemizygosity for genes encoding neuropeptide receptors NPY1R and NPY5R and for glutamine and glycine neurotransmitter receptor subunits (AMPA-2, GLRA3, and GLRB) [225]. Overall, these results seem to pinpoint NPY as an important modulator of social behavior in higher species as well, though more studies need to be performed to validate this theory.

Memory and Learning in *C. elegans*: Insights into Intellectual Disability

ID is one of the most frequent neurological impairments and is a very heterogeneous group of disorders. Increasing number of genes identified over the last years associated with ID suggests that this phenotype can emerge as the final common pathway of many different types of abnormal cellular processes. Overall, it is considered that ID can stem from two broad mechanistic themes: dysfunction of neurodevelopmental programs and alterations in synaptic organization and plasticity [226], both including cellular processes and molecular players present in *C. elegans* (Table 4).

Autosomal recessive primary microcephaly (MCPH) is characterized by a severe ID and is known to be associated with mutations in several genes, among which is the *ASPM* [227]. In MCPH patient cells, ASPM has been shown to be required for correct organization and orientation of the mitotic spindle and cytokinesis [228]. The *C. elegans* ortholog of this protein is ASPM-1, which binds to LIN-5 and is required for correct location of LIN-5 to meiotic and mitotic spindle poles [229]. LIN-5 is the ortholog of human *NuMA* and belongs to the conserved pathway controlling spindle position [230]. Large-scale *C. elegans* RNAi experiments also indicate that *aspm-1* is necessary for embryonic and larval viability, germline maintenance, vulval morphogenesis, and locomotion [231–233], which may indicate that *ASPM* may be relevant for other types of cells. Indeed, very recent work showed that lack of functional ASPM was associated with loss of germ cells, both in testis and ovaries [234].

The *PHF8* gene, which encodes a histone demethylase, has been found to be mutated in several patients with X-linked ID and cleft lip/palate [235, 236]. The zebrafish ortholog has been shown to regulate cell survival in the developing brain and to be involved in jaw development [237]. In *C. elegans*, the most closely related homolog is F29B9.2, which is expressed mainly in neuronal cells. Such as the human counterpart, F29B9.2 catalyzes demethylation of di- and monomethylated lysine 9 of histone H3 in vivo. F29B9.2 inactivation leads to a relatively mild phenotype in the form of uncoordinated locomotion [238], and reexpression of the gene in mutant background under a pan-neuronal promoter, but not under a muscle promoter, rescued the phenotype associated with loss of F29B9.2.

The aristaless-related homeodomain protein ARX has been shown to underlie multiple forms of X-linked ID [239]. *Arx* knockout mice exhibit thinner cerebral cortices because of decreased neural precursor proliferation and also exhibit defects in differentiation and migration of GABAergic interneurons [169]. *C. elegans* ortholog, *alr-1*, acts in a pathway with the LIM1 ortholog *lin-11* to regulate development of a subset of chemosensory neurons. Moreover, *alr-1* mutants present deficits in differentiation of a GABAergic motoneuron, suggesting parallels with *ARX* functions in vertebrates [240].

Mutations in *TUBA1A* gene have been associated with cortical dysgenesis such as lissencephaly and bilateral asymmetrical polymicrogyria [173, 241]. In *C. elegans*, null alleles of the orthologous gene, *tba-1*, do not present any major locomotor or neuronal defect, probably due to compensatory mechanisms, since this mutation is lethal in combination with other tubulin mutations [242]. However, interestingly, a gain-of-function mutation in *tba-1* leads to motor neuron synapse disruption and axonal defects [242], which is concordant with a role of this gene in the correct development of the nervous system. Analogously, (putatively) dominant mutations in human TUBA1A are associated with neuronal migration deficits and axonal malformation [170, 173].

Down syndrome is the most common form of ID worldwide, caused by a triplication of all or just a critical region of chromosome 21, which leads to a very specific and well-defined phenotype. *C21ORF5/DOPEY2* is one of the genes within the "Down Syndrome Critical Region," which are hypothesized to be responsible for majority of the phenotype [243]. Of notice, the first attempt to study Down syndrome-associated genes in *C. elegans* involved the *DOPEY2* ortholog *pad-1* [244]. *Pad-1* was found to be necessary for proper patterning during gastrulation and morphogenesis. In the same line of evidence, overexpression of human *DOPEY2* in mice leads to alterations in cortical layers together with behavioral impairment [245, 246].

Another gene putatively involved in Down syndrome is *DYRK1A*, a member of the dual-specificity tyrosine phosphorylation-regulated kinase [247]. *DYRK1A* involvement in critical neuronal processes such as neurogenesis and neuronal differentiation has been widely studied using mice but also simpler organisms such as *Drosophila* and *C. elegans* [248, 249]. The *C. elegans* ortholog is *mbk-1*, but in contrast to vertebrate *DYRK1A* orthologs and the fly minibrain ortholog, lack of *mbk-1* does not lead to any neuronal proliferation defects [250]. However, increased *mbk-1* expression was shown to lead to dose-sensible specific functional olfactory defects. Remarkably, these defects were reversible by normalizing *mbk-1* expression [250],

t4.1 **Table 4** Autism spectrum disorder (ASD)-related genes. Studies in *C. elegans* that added important value to our understanding of the function and malfunction of human genes associated with ASDs

t4.2 Gene	Function	*C. elegans* findings	Disease association
t4.3 *Neuroligins NLGN3 NLGN4*	Neuroligin family; cell adhesion molecules present at the postsynaptic side of the synapse and may be essential for the formation of functional synapses	*nlg-1* mutants have sensory processing deficits; hypersensitive to oxidative stress and mercury. These animals also present osmotic avoidance deficits and touch response phenotype [188, 190]. Recent evidence suggests that *nlg-1* and *nrx-1* mediate a retrograde synaptic signal that inhibits neurotransmitter release at NMJ	Mutations in these genes have been associated with ASDs [182–184]
t4.4 *Neurexins NRX1 NRX2 NRX3*	Bind neuroligins and form complex that is required for efficient neurotransmission; involved in the formation of synaptic contacts	*nrx-1* mutants do not present observable phenotype; however, mutations in this gene suppress neuroligin mutations [187]	Mutations in neurexin genes have been associated with ASDs [186]
t4.5 *SHANK1*	Adapter protein in the postsynaptic density (PSD) of excitatory synapses; interconnects receptors including NMDA-type and metabotropic glutamate receptors via complexes with PSD-95 and Homer. Plays a role in the structural and functional organizations of the dendritic spine and synaptic junction	*shn-1* strain presents no overt phenotype; however, suppression of *shn-1* in a defective inositol-1,4,5-trisphosphate (IP3) receptor background alters defecation rhythm [197]. A key role for ANK repeat domain and PDZ in regulating Ca^{2+}-signaling with the IP3 receptor [198]	Mutations in *SHANK* have been associated with ASDs [185]
t4.6 *NBEA*	Neurobeachin; binds to type II regulatory subunits of protein kinase A and anchors/targets them to the membrane	*sel-2* is a negative regulator of LIN-12/Notch activity; involved in vesicle secretion (?) [204]	Mutations associated with autism [200]
t4.7 *L1CAM*	Transmembrane cell adhesion molecule with an important role in the development of the nervous system; involved in neuron–neuron adhesion, neurite fasciculation, and outgrowth of neurites	LAD-2 is required for axonal migration, since it anchors MAB-20 (semaphorin) to PLX-2 (plexin) [215]. *lad-2* mutants present severe axonal defects, which can partially be independent on the sempahorin/plexin pathway.	Mutations associated with CRASH [207–209]
t4.8		*lad-1* (*sax-7*) strain presents pleiotropic phenotypes that include uncoordination, embryonic lethality, and deficits in neuronal positioning and axonal-misguided trajectories [212]	
t4.9 *NPY1R NPY2R*	Neuropeptide Y receptor; family of Gi/o-protein-coupled receptors that mediate food intake, anxiety and stress response, and control of pituitary hormone release	Activity of *npr-1* is correlated with the degree of "socialization" [72, 291, 292]. Social and solitary strains differ naturally in the levels of NPR-1 protein	No information about its association with human neurodevelopmental disorders. Yet, *Drosophila* NPY is involved in social behavior as well [223], and NPY2 receptor knockout mice present social abnormalities [224]

which provided the first hint that *DYRK1* induced deficits could be reversed in fully differentiated neurons. This possibility has been confirmed later in higher model organisms [251–253] and has set off several therapeutic approaches that are now being evaluated in human clinical trials (ClinicalTrials.gov identifier: NCT01394796; NCT01699711).

DSCR1 is another gene residing in the Down Syndrome Critical Region [254]. DSCR1 is a known inhibitor of calcineurin-mediated signaling pathways [255], which are involved in multiple processes including neuronal plasticity [256] and neuronal development via NFAT signaling [257]. DSCR1, along with DYRK1A, is thought to downregulate NFAT-mediated gene activation [247]. Calcineurin regulators seem to be evolutionarily conserved, and *C. elegans* possesses a sole *DSCR1* homolog, *rcn-1* [254, 258]. Analogous to *DSCR1*, *rcn-1* also inhibits calcineurin phosphatase activity via calcineurin A interaction. Moreover, worms overexpressing *rcn-1* could reproduce multiple phenotypes of calcineurin loss-of-function mutants [258] providing an in vivo proof of *rcn-1*/calcineurin regulation and giving further support as to the relation between *DSCR1* overdosage and the phenotypes observed in DS.

Often, simple organisms do not replicate the complete phenotype spectra of human disorders. Still, they may allow studying and focusing on specific pathological features and

better understanding of protein function. This is the case of *PQBP1* gene that when mutated is associated with a complex X-linked disorder, Renpenning's syndrome [259–261], characterized by ID and lean body build (OMIM #309500). The *C. elegans* ortholog is *pqbp-1.1* that, such as the human counterpart, encodes a protein with a polyglutamine-binding region in polar amino acid-rich domain, a WW domain also involved in regulation of transcription activity, and a C-terminal domain involved in the interaction with a spliceosome component [262]. Although *pqbp-1.1* is expressed in few neurons, no neuronal phenotype was observed in *pqbp-1.1*-functional mutants. However, it was observed that lack of *pqbp-1.1* leads to alterations in lipid metabolism shown by a reduction of triglycerides [262], which could be somehow related to lean body observed in human patients. Considering that the lipidic metabolic pathways are fundamentally conserved between species, *C. elegans* could be a good model to study *PQBP1*-induced lipidic dysfunction and its effects in neurons.

Another example is the *ATRX* gene, which is associated with a complex X-linked ID syndrome, alpha-thalassemia mental retardation, X linked [263]. Patients exhibit severe ID and genital abnormalities, among other clinical features (reviewed in [264]). The *ATRX* gene encodes a member of a transcription regulator family of proteins, Swi2/Snf2 [265]. In mice, ATRX is suggested to interact with MeCP2 and cohesin (also involved in ID) to regulate gene expression during brain development [266]. Worm ortholog is *xnp-1*, and although no neurological phenotype has been reported, *xnp-1* has been shown to be required for correct embryogenesis. In parallel with what has been observed in humans, *xnp-1* is also necessary for normal gonad development [267, 268]. As at least the gonad development-related function of *xnp-1/ATRX* seems to be conserved, *C. elegans* could be a good model to identify additional interacting partners and developmental signaling pathways involved in the disorder and perhaps phenotype-modifying compounds.

Insights on Other Neurodevelopmental Disorders

Disrupted-in-Schizophrenia 1 (*DISC1*) is a very well-established susceptibility gene for schizophrenia that also seems to be involved in other disorders such as ASD, depression, and bipolar disorder [269]. DISC1 protein has been thoroughly studied and is known to act as a scaffold protein, with multiple and diverse interacting partners, involved in neurodevelopmental and neurosignaling processes [270]. The *C. elegans* genome does not contain a *DISC1* ortholog. However, a heterologous strain expressing *mDISC1* was useful to dissect the pathway by which DISC1 may regulate axonal connections. Studies using this model showed that in motor neurons, DISC1 interacts with UNC-73/TRIO and activates RAC–PAK signaling to regulate axon guidance [271]. Interestingly, these pathways are conserved, and in mammals, it is known that TRIO regulates axon growth and guidance via RAC [272]. Furthermore, this heterologous *C. elegans* model may represent a good tool to identify new small molecules with therapeutic effects in modulating the TRIO–RAC pathway such as those that regulate axonal connectivity.

From Genes to Therapies

C. elegans represents a powerful tool to dissect cellular and molecular processes of human disorders and has emerged as an attractive platform in the context of large drug or genetic screenings due to its simplicity, low cost of cultivation, and small size that allows their growth on microtiter plates. Moreover, ease of genetic manipulation and commonality of several biological processes are both valuable in the gene-to-drug and drug-to-gene discovery (nicely reviewed in [53]). If on one side, random mutagenesis can help in identification of novel gene targets conferring susceptibility or resistance to a specific group of drugs, large-scale drug screenings in specific genetic backgrounds may help dissect the mechanisms of drug action in normal and pathological conditions.

An elegant example is the identification of 185 aldicarb-resistant mutants, among which were 132 genes that had not be previously associated with synaptic transmission. Of these, 24 encoded proteins that were localized to presynaptic specializations, and loss-of-function mutations in 12 genes caused defects in presynaptic structure [22].

Others have used transgenic worm models expressing the mutated human protein to perform both genetic and drug screenings. For example, Kraemer's lab has used a worm model of tauopathy to screen a drug library containing 1,120 molecules. They identified azaperone, a typical antipsychotic drug, as a robust modifier of motor deficits and levels of insoluble tau [128]. Suggesting common drug-acting pathways in worms and humans, azaperone was also effective in reducing tau aggregation in a human cell line. Remarkably, other drugs acting on dopamine receptor D2 such as flupenthixol, perphenazine, and zotepine were also effective in ameliorating tau-induced dysfunction in both models, suggesting D2 antagonism as a promising therapeutic strategy for tau neurotoxicity [128], a pathway that without *C. elegans* contribution would be unlikely to be discovered.

In another study, four different chemical libraries comprising 14,100 small membrane-permeable compounds were screened for induction of behavioral/morphological defects in wild type worms [273]. Three hundred eight molecules led to a variety of phenotypes, from simple motility deficits to severe morphological problems. However,

despite this high-hit result for bioactivity of new drugs in *C. elegans*, an important consideration is the gap between worm and human mechanisms of drug absorption, distribution, metabolism, excretion, or toxicity. Nevertheless, in this screen, researchers also discovered that a novel compound, which they named nemadipine-A, resembling a class of antihypertension drugs called the 1,4-dihydropyridines that antagonize the alpha 1-subunit of L-type calcium channels, induced robust defects in morphology and egg laying. They identified *egl-19*, the only L-type calcium channel alpha 1-subunit in *C. elegans*, as the target gene in a genetic suppressor screening. Interestingly, the compound could also antagonize vertebrate L-type calcium channels, demonstrating that worms and mammals share a common target, despite originating divergent phenotypical outcomes.

Another example is the "hypothesis-free approach" screening of 900,000 small molecules that allowed identification of new classes of proteostasis regulators important in treatment of several conformational diseases such as polyglutamine disorders and Alzheimer's and Parkinson's diseases. Though some of these molecules acted via "canonical pathways" such as via HSF-1, FOXO, and NRF-2 and the chaperone machinery, the underlying mechanisms were distinct from previously identified small-molecule activators of the heat shock response [274].

Not much has been done in the context of neurodevelopmental disorders regarding large-scale genetic and/or drug screening approaches. Several factors may contribute to this: first, neurodevelopmental disorders frequently encompass complex and difficult "scorable" phenotypes (e.g., neuronal migration defects or abnormal synaptic transmission) that restrain large-scale analysis methodology. Second, for several neurodevelopmental disorders, there is no unique drug or gene that modifies the phenotype satisfactorily due to their inherent complexity. Nevertheless, considering all pros and cons of using *C. elegans* in this type of screenings, we still believe that the strategy of using this model as the first line of research may lead to identification of novel and implausible drugs and/or cellular/molecular pathways of drug action that otherwise would be difficult to pinpoint. Yet, once a drug (gene?) is identified as potentially relevant in the context of a specific disorder in worms, additional studies need to be performed in higher organisms to fully validate it and exclude all side effects that it may have in the context of a more complex organism.

Final Remarks

The transparent worm *C. elegans* is one of the most powerful and versatile model organisms, enabling elucidation of several cellular and molecular mechanisms underlying neuronal function and dysfunction. Due to easiness of genetic manipulation and similarity with vertebrate neuronal molecular pathways, this organism can be used to functionally validate genetic associations identified in neurodevelopmental disorders. Moreover, since *C. elegans* is amenable to high-throughput genetic and drug screenings, it is an excellent biological platform for drug identification and clarification of signaling pathways involved in novel therapeutic interventions.

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Q1. References 152 and 279 based on original manuscript we received were identical. Hence, the latter was deleted and reference list and citations were adjusted. Please check if appropriate.
Q2. Please check provided bibauthorname if correct.