CHEMISTRY-TO-GENE SCREENS IN *CAENORHABDITIS ELEGANS*

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Abstract | The nematode worm *Caenorhabditis elegans* is a genetic model organism linked to an impressive portfolio of fundamental discoveries in biology. This free-living nematode, which can be easily and inexpensively grown in the laboratory, is also a natural vehicle for screening for drugs that are active against nematode parasites. Here, we show that chemistry-to-gene screens using this animal model can define targets of antiparasitic drugs, identify novel candidate drug targets and contribute to the discovery of new drugs for treating human diseases.

MODEL ORGANISMS

ANTHELMINTIC
A chemical that controls parasitic worms. An example is levamisole, which controls parasitic nematodes.

ENDECTOCIDE
An antiparasitic drug which controls both internal parasites (such as worms) and external parasites (such as ticks). An example is ivermectin.

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Nematode parasites cause substantial mortality and morbidity in the human population throughout the tropical and sub-tropical world. The main species involved — *Ascaris lumbricoides* (intestinal roundworms), *Trichuris trichiura* (whip worms) and several species of hookworms — each currently infects around a billion people worldwide[1]. Parasitic nematodes also cause considerable losses in livestock and domestic animals, and are responsible for more than US$100 billion in annual crop damage[2]. The cost of developing a new animal health drug that controls parasites in livestock is around US$40 million, and the cost for human drug discovery can exceed US$800 million. Novel targets and their associated new active drugs are identified only about once a decade, and so there is an urgent need to expedite both discovery and development of new compounds. In the case of animal health drugs, the increasing resistance of parasitic nematodes to currently used ANTHELMINTICS provides a further pressure to develop new drugs[3]. Broad-spectrum compounds, including ENDECTOCIDES such as ivermectin that control both ectoparasites (such as ticks) and endoparasites (nematode worms), are of particular importance. For each candidate antiparasitic agent, there are a number of steps to go through: identify a validated target, either one for which there is a precedent or a novel target; clone the target; set up an automated functional screen; and, finally, discover target-selective small molecules. Discovering a novel target can offer considerably higher commercial returns than developing a known target, but such discovery in the field of endoparasites is hindered by difficulties in culturing parasites outside a host. The free-living nematode worm *Caenorhabditis elegans*, whose genome has been fully sequenced, is well suited for mutagenesis studies, lends itself to high-throughput assays, and shares some physiological and pharmacological characteristics with parasitic nematodes[4]. This well-studied invertebrate is therefore of immense value in antiparasitic drug discovery and is also proving to be of utility in human drug discovery.

The rise of *C. elegans*

In 1963, Sydney Brenner stated that "the *new major problem* in molecular biology is the genetics and biochemistry of control mechanisms in cellular development." To address the problem, Brenner proposed that "the simplest differentiated organism" should be studied "which has a short life cycle, can be easily cultivated, and is small enough to be handled in large numbers. Furthermore, it should have relatively few cells, so that exhaustive studies of lineage and patterns can be made, and should be amenable to genetic analysis." *C. elegans* possesses all of these characteristics, and as a result moved quickly to centre stage in biological research[5]. Adult *C. elegans* are 1-mm long, which means that a large number of worms can be maintained in a small

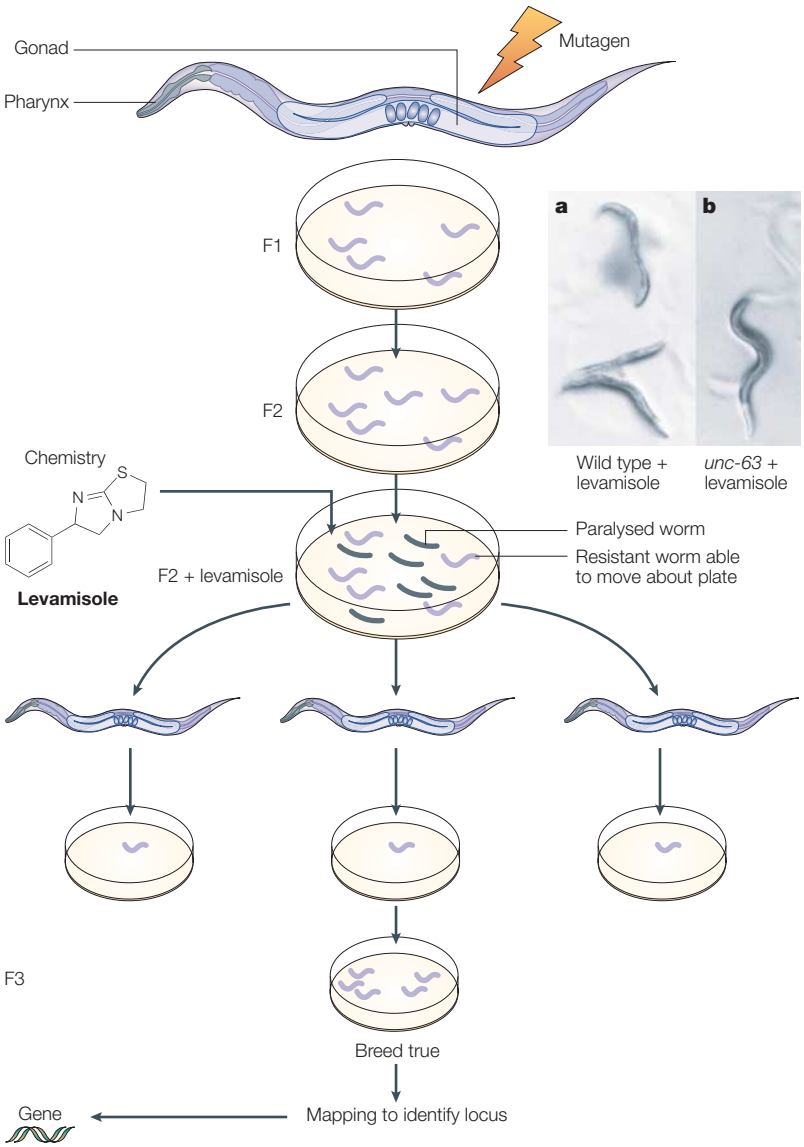

Figure 1 | **Chemistry-to-gene screening begins with mutagenesis of worms, usually by exposure to mutagens.** Mutants resistant to the chemical under investigation are selected on F2 culture plates containing the chemical (in this case 10^{-4} M levamisole). These mutants are then picked out individually and isolated on dishes in the continued presence of the active compound. The progeny that breed true will be homozygous and will therefore retain resistance to the compound and can be used to identify by genetic mapping the gene(s) in which mutations caused resistance. Mapping involves genetic crosses to determine recombination frequencies from known gene or DNA markers. **a** | Shows the paralysing effects of 10^{-4} M levamisole on *C. elegans*. Body-wall muscles first hypercontract and worms become immobilized and often straightened. **b** | Shows the resistance to levamisole conferred by mutation in the *unc*-63 gene. *unc*-63 worms retain motility as evidenced in the serpentine tracks left in the culture medium.

space, and are easy to cultivate in liquid media or on Petri dishes with bacteria as a food source. *C. elegans* has a hermaphroditic lifestyle and a life cycle of only 3 days, which facilitates the generation and maintenance of genetic strains that can be stored as frozen stocks. Their transparent bodies allow internal structures and biological processes to be viewed simply with a microscope. A complete description of cell lineage is available[6] and every neuron in its simple nervous system has been identified[7]. These features — together with the availability of a fully sequenced genome[8] in which 60% of the genes have vertebrate counterparts[9] — have led to the widespread adoption of *C. elegans* as a model organism. Genetic screens that identify mutations which disrupt specific biological processes[10] and genome-wide screens that systematically perturb gene function[11] have been highly effective in elucidating a number of cellular mechanisms. Indeed, studies using *C. elegans* have been crucial in providing new insights into factors underlying cell lineage[12], neurobiology[13], cell death[14] and human diseases ranging from neurodegenerative disorders[15,16] to cancer[17]. Conversely, genes particular to worms are also of interest not only from an evolutionary point of view, but also as potential targets of drugs that selectively control nematode parasites[18]. The wealth of information arising from studies of *C. elegans* — which includes genome sequence data, gene structures, mutant and RNAi phenotypes, gene-expression patterns, microarray-based gene-expression profiles, protein–protein interactions and additional information — is easily accessible at Wormbase[19].

Chemistry-to-gene screens

The principal aim of a typical CHEMISTRY-TO-GENE SCREEN is to discover the molecular target(s) of compounds that have an obvious effect on *C. elegans*, such as drugs that kill parasitic nematode worms. Such a screen involves chemically induced mutagenesis (FIG. 1), often using ethyl methane sulphonate (EMS)[20] to induce mutations in the DNA of sperm or oocytes of wild-type hermaphrodite worms. Mutagenized worms are grown on agar plates for two generations to produce homozygous mutant strains that are then placed on agar plates containing the compound of interest. Worm mutants that show resistance to the compound (that is, are able to move about the plate in which wild-type worms are paralysed or killed) are individually transferred to new plates to determine whether their resistance phenotype is transmitted to the next generation. In this way, homozygous mutant strains can be identified which are selected for further analysis to identify mutated genes underlying resistance. Such analysis involves identifying linked genes by determining their genetic distance on the basis of recombinant frequency from known gene markers (mutants possessing a distinguishable phenotype[20]) or DNA markers (such as single-nucleotide polymorphisms that can be detected by sequencing or restriction analysis[21]).

Transposons, stretches of DNA that can excise and reinsert themselves at other locations in the genome, can also be used to induce mutations[22,23]. In cases in which the *Drosophila melanogaster* mariner element transposon Mos*1* is used to induce mutations in *C. elegans*, genes mutated by insertion can be readily identified by inverse polymerase chain reaction, thereby circumventing the potentially lengthy process of genetic mapping[22]. However, the frequency of Mos*1*-mediated mutagenesis is considerably lower than that of EMS mutagenesis, and so there is a trade-off between time spent screening for mutants and time spent genetically mapping mutant alleles.

CHEMISTRY-TO-GENE SCREEN
Following mutagenesis of *C. elegans*, mutants are screened for resistance to a particular chemical and the resulting mutations are mapped and identified (useful for identifying hitherto unknown drug targets).

Table 1 | **Genes conferring levamisole resistance**

Gene	Gene product	Function	References
lev-1	nAChR non-α subunit	Mediates ACh signalling	29,30
lev-8 or *acr-13*	nAChR α-subunit	Mediates ACh signalling	31
unc-29	nAChR non-α subunit	Mediates ACh signalling	29,34
unc-38	nAChR α-subunit	Mediates ACh signalling	29,34
unc-63	nAChR α-subunit	Mediates ACh signalling	30
lev-11	Tropomyosin	Muscle contraction	42
unc-22	Twitchin	Muscle contraction	41
unc-68	Ryanodine receptor	Intracellular Ca^{2+} regulation	39
unc-50	Inner nuclear membrane RNA-binding protein	nAChR assembly	37
lev-10	Transmembrane protein with CUB and LDL domains	Postsynaptic aggregation of nAChRs	38
lev-9	Unknown		
unc-74	Unknown	nAChR processing and assembly?	28

ACh, acetylcholine; LDL, low-density lipoprotein; nAChR, nicotinic acetylcholine receptor.

In this review, we discuss the use of chemistry-to-gene screens that employ *C. elegans* to discover targets of effective antiparasitic drugs. These screens have also identified proteins functionally linked to target molecules, thereby shedding light on new possible targets for the future. We will also discuss the application of chemistry-to-gene screens to the development of human drugs and review how such studies have furthered our understanding of the mechanisms of action of alcohol, nicotine and anaesthetics. Finally, we discuss the potential of recent developments incorporating RNAi and novel routes for drug discovery in the area of human health.

Defining molecular targets of antiparasitic drugs

Levamisole. Levamisole is a broad-spectrum anthelmintic drug widely used to eradicate roundworm (*Ascaris spp.*) and hookworm (for example, *Ancylostoma duodenale*) infestations in livestock[24] and humans. It causes hypercontraction of nematode body-wall muscles, leading to death. In the 1970s, Sydney Brenner isolated EMS-induced *C. elegans* mutant strains that were resistant to levamisole[20]: the mutant strains could migrate across an agar plate containing 10^{-4} M levamisole more quickly than sensitive wild-type worms. It was noted that the resistant worms had characteristic phenotypes, such as uncoordinated movement or twitching[20]. These phenotypes mimicked the effects of the acetylcholinesterase (AChE) inhibitor lannate, and so Brenner surmised that levamisole acted as an acetylcholine (ACh) agonist. Subsequent pharmacological studies on cut worms by Lewis and colleagues revealed that the levamisole-resistant mutants lack functional muscle NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs)[25] and electrophysiological studies on the muscles of *Ascaris suum*, a nematode parasite that infects pigs, showed that levamisole acts as an agonist on nAChRs[26,27]. These findings, together with the loss of high-affinity levamisole binding in two of the mutant strains (uncoordinated movement-50 (*unc-50*) and *unc-74*) led to the suggestion that the affected genes encode for proteins involved in the processing and assembly of the levamisole receptor[28].

During the past two decades, nearly all of the 12 genes that mediate levamisole resistance have been identified (TABLE 1). In accordance with previous suggestions made by Brenner and Lewis *et al.*[20,25,28], five of these genes encode nAChR subunits[29–31]. Nicotinic acetylcholine receptors are members of the CYS-LOOP SUPERFAMILY OF LIGAND-GATED ION CHANNELS that mediate fast cholinergic synaptic transmission and consist of five homologous subunits surrounding an ion channel[32]. Subunits possessing two adjacent cysteine residues within the ACh-binding site are referred to as α-subunits, whereas those lacking the cysteine doublet are referred to as non-α-subunits. *C. elegans* possesses one of the most extensive and diverse nAChR gene families known, consisting of at least 27 subunits that have been divided into subgroups on the basis of sequence similarity[33]. Of the five nAChR subunits mediating levamisole resistance, *unc-38*, *unc-63* and *lev-8* encode α-subunits, whereas *unc-29* and *lev-1* encode non-α-subunits. As anticipated from the hypercontraction effects of levamisole, all five of the nAChR subunits are expressed in body-wall muscle and several show overlapping expression patterns[29–31]. The stoichiometry of the muscle levamisole-sensitive receptor(s) remains to be resolved. However, electrophysiological studies on voltage-clamped *C. elegans* body-wall muscles show that inward currents elicited by levamisole are abolished, or substantially reduced, in mutants of all five nAChR subunits[30,31,34], which provides direct confirmation that these subunits are important components of receptor(s) targeted by the drug. Other electrophysiological studies on different strains of the pig parasitic nematode *Oesophagostomum dentatum* have shown that altered kinetic properties of nAChRs underlie resistance to levamisole and to another cholinergic anthelmintic, pyrantel[35,36], which highlights the importance of nAChRs in drug toxicity. Interestingly, the responses to ACh are abolished to a lesser degree than those of levamisole, indicating that body-wall muscles contain at least one other nAChR subtype that is insensitive to levamisole[34]. The subunit composition of the levamisole-insensitive nAChR(s) has yet to be determined, but they could represent important novel targets for parasitic nematode control.

The remaining levamisole-resistance genes probably encode upstream and downstream molecular components associated with nAChR assembly and the events that follow receptor activation (FIG. 2). For instance, sequencing of UNC-50 revealed it to be a novel transmembrane protein, and subsequent characterization of its mammalian homologue, UNCL, showed that it is an inner nuclear membrane RNA-binding protein that increases cell-surface expression of vertebrate nAChRs (α4/β2) when expressed in *Xenopus laevis* oocytes and COS cells[37]. It has been suggested that UNCL regulates nAChR expression by associating with the RNA that codes for chaperone proteins required for nAChR

NICOTINIC ACETYLCHOLINE RECEPTORS (nAChRs). Prototypical members of the cys-loop ligand-gated ion channel superfamily classified by the ability of nicotine to activate them. On binding acetylcholine, the natural neurotransmitter activating these receptors, the ion channel is opened transiently resulting in an influx of ions into the cell.

CYS-LOOP LIGAND-GATED ION CHANNEL SUPERFAMILY An extended family of ligand-gated ion channels, each molecule composed of five subunits arranged around an ion permeable channel. Each subunit is characterised by an extracellular domain containing the cys-loop – two disulfide bond-forming cysteines separated by 13 amino acid residues. Members include nAChRs and ionotropic receptors for GABA, 5-HT3 and glycine.

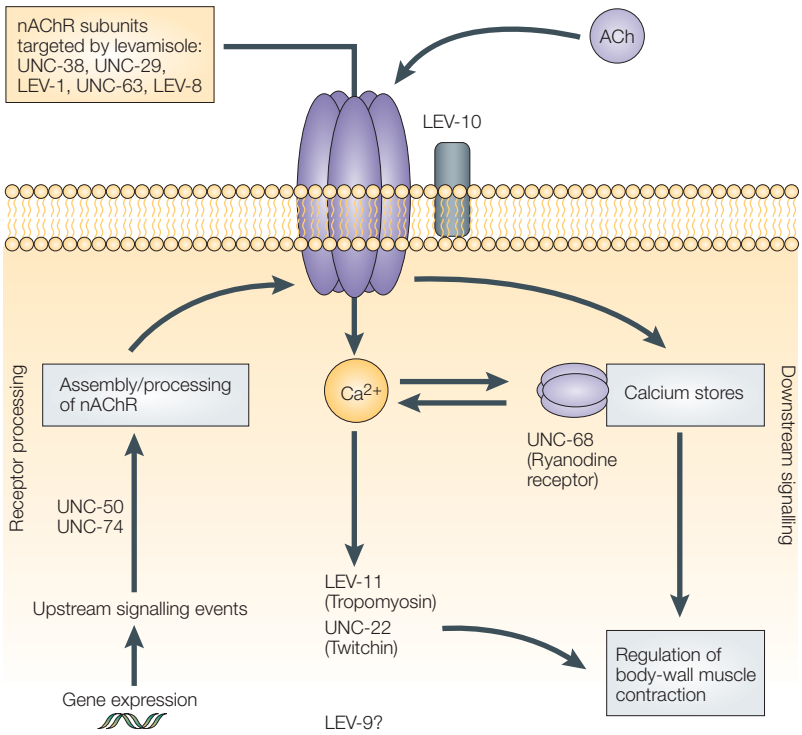

Figure 2 | **Chemistry-to-gene screens identify drug targets as well as functionally linked genes.** The screen for resistance to levamisole led to the discovery not only of the direct targets of levamisole (UNC-29, UNC-38, UNC-63, LEV-1 and LEV-8 — all nicotinic acetylcholine receptor (nAChR) subunits), but also of UNC-68 (a ryanodine receptor), UNC-22 (twitchin), LEV-11 (tropomyosin), LEV-10 (a membrane protein implicated in stabilizing the expressed nicotinic acetylcholine receptor) and two proteins — UNC-50 and UNC-74 — with roles in the synthesis of nAChR subunits. The role of LEV-9, another protein emerging from levamisole-resistance screens, remains to be determined.

assembly, a role similar to that postulated for UNC-50 by Lewis and colleagues more than a decade earlier[28]. Recently, screening of levamisole-resistant mutants created by Mos*1* insertion identified LEV-10 as a transmembrane protein required for postsynaptic aggregation of nAChRs at the neuromuscular junction[38]. This finding offers an explanation for why the density of levamisole-sensitive nAChRs in *lev-10* mutants is markedly reduced although the number of functional nAChRs remains unchanged[28]. Interestingly, LEV-10 functions through extracellular interactions with nAChRs or other nAChR-associated proteins through CUB (a family of domains originally identified in complement proteins and bone morphogenic protein 1) and low-density lipoprotein (LDL) domains. Other levamisole-resistance genes regulate signal transduction downstream of nAChR activation. For example, *unc-68* encodes a ryanodine receptor expressed in body-wall muscles that is necessary for normal locomotion and which plays a role in intracellular Ca^{2+} regulation[39]. Ryanodine, a natural product, has insecticidal activity and has been used commercially[40]. So, a protein functionally related to the primary target for levamisole, which is also a target for pesticides, has been identified. This is a direct validation of the notion that new molecular components highlighted in a chemistry-to-gene screen can include novel candidate targets.

The *unc-22* and *lev-11* genes are also both expressed in muscles: *unc-22* encodes twitchin[41], which contains fibronectin type III, immunoglobulin and protein kinase domains; *lev-11* encodes tropomyosin[42], an actin-binding contractile structural protein. The importance of both genes in muscle function is reflected by the observation that *lev-11* and *unc-22* mutants have strikingly uncoordinated and twitching phenotypes, respectively.

Studies of levamisole-resistant mutants therefore illustrate how a chemistry-to-gene screen can identify molecular components targeted by antiparasitic drugs (in this case a subset of nAChRs from a large gene family). This work also demonstrates how such screens can provide novel insights into cellular pathways linked with drug receptor function and thereby reveal other potential drug targets. Furthermore, as is evident from the findings for UNCL, such screens can uncover functions of human genes.

Aldicarb. A chemistry-to-gene screen for mutants resistant to the AChE inhibitor aldicarb also generated new insights into cholinergic synaptic transmission[43,44]. Aldicarb is a systemic insecticide, ACARICIDE and nematicide that causes hypercontraction and paralysis in worms, thereby preventing growth and development. Resistant mutants were identified because they could grow, reproduce and move in the presence of aldicarb at concentrations that would paralyse wild-type worms. Some of the resistant strains proved to be previously described uncoordinated mutants, and resistance was therefore shown to be attributable to mutations in known genes[43]. However, newly identified genes were also detected and designated '*ric*', for resistance to inhibitors of acetylcholinesterase. As shown in TABLE 2, a large number of the genes conferring resistance to aldicarb are involved in synaptic transmission, including those encoding pre- and postsynaptic proteins. Presynaptic gene products include CHA-1[45], which synthesizes the neurotransmitter ACh; UNC-17[46], which loads ACh into vesicles; and SNT-1[47], UNC-13[48] and UNC-41[49], which mediate neurotransmitter release. Postsynaptic proteins include the levamisole-sensitive nAChR subunit UNC-63[30] and RIC-3, which is involved in nAChR maturation[50]. The studies with aldicarb and levamisole therefore demonstrate how two chemistry-to-gene screens, each using a drug acting on the same pathway, can yield complementary findings, thereby enhancing our understanding of a signalling pathway.

Benzimidazoles. Benzimidazole drugs (BZs) are broad-spectrum anthelmintics that are effective against nematodes, cestodes (tapeworms) and trematodes (for example, liver flukes), and are used for the control of parasites infecting livestock, domesticated animals and humans[51,52]. They exert toxic effects because they bind to tubulin and inhibit the formation of microtubules[53], which are important for vital cellular processes including mitosis, nutrient absorption, intracellular transport, secretion and cell motility. To genetically confirm the identity of the BZ target in *C. elegans*, EMS-mutated worms were screened for resistance to

ACARICIDE
A chemical that controls mites.

movement defects caused by BZs[54]. It was found that BZ resistance arose from mutations in a single gene, benzimidazole-sensitivity 1 (*ben-1*), which encodes a β-tubulin. Studies investigating the mechanism of BZ resistance in the field showed that in the sheep parasitic nematode *Haemonchus contortus* drug sensitivity correlated with a β-tubulin allele[55,56] and that a Phe200 to Tyr200 mutation in the β-tubulin protein was likely to underlie BZ resistance[57]. To confirm the functional importance of the amino-acid mutation at residue 200, the *H. contortus* β-tubulin was stably expressed in *C. elegans ben-1* mutants[58]. *H. contortus* β-tubulin with Phe200 was found to confer BZ sensitivity to the *ben-1* mutants, whereas Tyr200 *ben-1* mutants remained BZ resistant, indicating a large degree of molecular conservation between a parasitic and a free-living nematode. This showed that *C. elegans* can be used as a valuable tool to study parasite genes and enhance our understanding of the mechanisms of drug action and drug resistance.

Ivermectin. In addition to discovering new targets, chemistry-to-gene screens have also been successful in enhancing our understanding of the action of a drug even when the target is already known. An excellent example is the discovery of the basis of sensitivity of *C. elegans* to ivermectin, a major veterinary endectocide widely used for treating livestock[59]. Ivermectin has also been used as a drug to treat river blindness in humans caused by the nematode *Onchocerca volvulus*[60]. Early studies showed that ivermectin causes an increase in chloride permeability in insect and crustacean

Table 2 | **Genes conferring aldicarb resistance**

Gene	Gene product	Function	References
cha-1	Choline acetyltransferase	ACh synthesis	45
ric-1	Unknown		
ric-3	Transmembrane protein localized to endoplasmic reticulum	Assembly or trafficking of nAChRs	50
ric-4	Unknown		
ric-8	Synembryn	Maintains proper activity of the Go to Gq G-protein signalling network, which regulates neurotransmitter secretion	101
snt-1	Synaptotagmin	Required for synaptic vesicle and secretory granule exocytosis	47
aex-3	Guanine nucleotide exchange factor	Required for intracellular vesicle trafficking and synaptic vesicle release	102
egl-10	G-protein signalling regulator	Inhibits Gα signalling, genetically interacts with *egl-30* signalling pathway	103
egl-30	Gqα protein	Involved in locomotion, egg laying and synaptic transmission	104
unc-2	Ca^{2+} channel α1 subunit	Required for desensitization to dopamine, regulates sensitivity to serotonin	105
unc-10	Protein with zinc-finger, Q/N-rich, PDZ and C2 domains	Regulates the priming step of presynaptic vesicle fusion by promoting conformational changes in syntaxin	106
unc-11	Clathrin-adaptor protein	Regulates neurotransmitter release by controlling vesicle trafficking and fusion	107
unc-13	Neurotransmitter release regulator	Regulates neurotransmitter release by altering the conformation of syntaxin	48
unc-17	Synaptic vesicle ACh transporter	Required for loading ACh into synaptic vesicles	46
unc-18	Vesicle trafficking protein Sec1	Synaptic vesicle docking	108
unc-26	Synaptojanin	Synaptic vesicle recycling	109
unc-31	Ca^{2+}-dependent activator protein	Neurosecretion	110
unc-32	Vacuolar-type H(+)-ATPase	Regulates acidification of intracellular compartments and is essential for locomotion and embryonic and larval development	111
unc-36	L-type voltage-dependent Ca^{2+} channel	Regulates sensitivity to serotonin	112
unc-41	UNC-41	Regulates ACh release	49
unc-63	nAChR α-subunit	Mediates ACh signalling	30
unc-64	Syntaxin	Essential component of the core synaptic vesicle fusion machinery necessary for neurotransmitter release	73
unc-65	Unknown		
unc-75	RNA-binding protein	Regulates GABA- and ACh-mediated neurotransmission	113
unc-104	Kinesin	Required for axonal transport of synaptic vesicles	114

ACh, acetylcholine; GABA, γ-amino butyric acid; nAChR, nicotinic acetylcholine receptor.

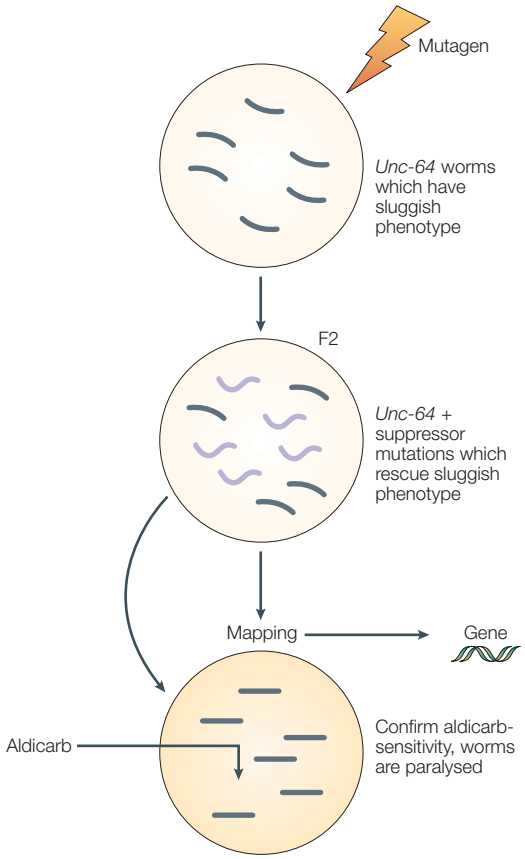

Figure 3 | **A novel use of chemistry-to-gene screening involves the discovery of phenotype-suppressor genes.** This is illustrated in a screen used to identify additional genes that are important for the action of volatile anaesthetics (VAs). *unc-64* worms, which show a lethargic locomotion phenotype, as well as aldicarb and VA resistance, are further mutagenized using ethyl methane sulphonate. F2 progeny are screened visually for suppressor mutations which result in improved locomotion when compared with *unc-64*. F3 progeny homozygous for the mutations were used to map the suppressor genes. Suppression of *unc-64* was also shown by conferred sensitivity to aldicarb.

muscles[61,62]. More recently, heterologous expression studies using *X. laevis* oocytes revealed that ivermectin acts on *C. elegans* glutamate-gated chloride channels (GluCls)[63–65], which are members of the ligand-gated ion channel superfamily that includes nAChRs. To clarify the role of GluCls in the antiparasitic actions of ivermectin, a screen designed to detect ivermectin-resistant *C. elegans* mutants[64,66] showed that loss of function in the GluCl subunits *glc-1* or *avr-15* did not confer resistance to ivermectin. However, further studies showed that simultaneous mutations in *glc-1*, *avr-15* and another GluCl subunit, *avr-14*, confer high-level ivermectin resistance, whereas double mutants only show either moderate or no resistance[67]. This not only provides unequivocal confirmation of GluCls as physiologically relevant ivermectin targets, but also led to the conclusion that *avr-14*, *avr-15* and *glc-1* constitute parallel genetic pathways, all of which must be disrupted by mutations to confer ivermectin resistance. This requirement for simultaneous mutations could help delay the development of drug resistance in the field and could account, in part, for the effectiveness of ivermectin as an antiparasitic agent.

C. elegans in screens for human drugs

Nicotine. Many human drugs are effective in *C. elegans*, which indicates a potentially useful role for the screening for mutant worms resistant to a particular drug to identify the molecular components targeted by these drugs. For instance, chronic exposure to nicotine, which is a pathogenic and addictive component of tobacco and also an agonist of nAChRs, leads to an altered abundance of nAChRs in vertebrates[68,69]. In *C. elegans*, nicotine stimulates egg-laying by acting on nAChRs expressed in vulval muscles. Prolonged exposure to nicotine leads to a long-lasting insensitivity to egg-laying stimulation by nicotine, indicating a decrease in nAChR activity and/or abundance[70]. Interestingly, mutant worms defective in the gene tetradecanoyl phorbol acetate resistance (*tpa-1*), which encodes a homologue of protein kinase C (PKC), were still sensitive to egg-laying stimulation after prolonged nicotine exposure. Phosphorylation of nAChRs regulates receptor function, and so it has been suggested that PKC phosphorylation might be linked to processes that affect nAChR abundance, such as protein stability, targeting or degradation.

Alcohol. Alcohol alters the behaviour of invertebrates and mammals at similar doses, indicating the existence of conserved ethanol targets in the nervous system. A chemistry-to-gene screen was set up to identify ethanol-resistant worms that were distinguished from non-resistant animals because they could either migrate in the presence of ethanol towards an attractant or resist the suppressing effect of ethanol on egg laying[71]. From this screen, a large number of loss-of-function mutations in the *slo-1* gene were found to confer high levels of ethanol resistance. *slo-1* encodes a Ca^{2+}-activated, large-conductance potassium channel (BK channel) that is known to regulate inhibition of neurotransmitter release, thereby playing an important role in determining the strength of neuronal signalling[72].

Anaesthetics. Other studies have investigated the molecular factors underlying the disruption of behaviour caused by volatile general anaesthetics (VAs). It had previously been shown that mutations in *unc-64*[73], which encodes syntaxin and was incidentally also shown to confer aldicarb resistance (TABLE 2), can also confer complete resistance to VAs at clinical concentrations[74]. To identify additional proteins that function synergistically with syntaxin to mediate neurotransmitter release and VA action, a variation of a chemistry-to-gene screen was performed (FIG. 3). In this screen, *unc-64* mutants were subjected to EMS mutagenesis and the resulting mutants screened for the suppression of aldicarb resistance[75]. Subsequent analysis of suppressor mutants showed that loss-of-function mutations in *slo-1* and a gain-of-function mutation in *egl-30* confer VA resistance, as

shown by their increased movement compared with wild-type when subjected to halothane. SLO-1 inhibits neurotransmitter release (TABLE 2)[72] and EGL-30 seems to act in a pathway that results in neurotransmitter release[76], and so VA resistance can be explained by increased neurotransmitter levels. Mutations in *slo-1* confer alcohol resistance, and so it seems that alcohol and anaesthetics act on the same cellular pathway. Chemistry-to-gene studies with nicotine, alcohol and anaesthetics therefore enhance our understanding of their actions and point the way to future research on mammals.

Future prospects

Gene-to-chemistry screens. From the 1970s onwards, forward genetics, which starts with a phenotype of interest and moves towards identifying genes associated with the phenotype, has been successfully used in *C. elegans* to determine the molecular components involved in various biological processes[10]. Reverse genetics, an approach that starts with knocking down genes with known sequences and observing the resulting phenotypes, has become widely applied to *C. elegans* since the advent of RNA INTERFERENCE (RNAi), which was characterized using *C. elegans*[77], to silence specific genes of interest. RNAi involves the introduction of double-stranded RNA either by injection[77], feeding[78] or soaking[79], and results in the specific silencing of the corresponding gene. RNAi feeding libraries are available, each designed to produce RNA to silence a particular gene. One example consists of 16,757 bacterial strains, which covers approximately 86% of the *C. elegans* genome[80]. Genome-wide screens using this and other RNAi libraries serve as powerful tools for uncovering new gene functions in a less labour-intensive manner than forward genetic approaches. Such screens have successfully implicated the involvement of genes in particular processes and diseases including storage of body fat[81], germline development[82], longevity[83], cancer[84], and neural and neuromuscular disorders[85]. Even though the roles of many genes have been successfully studied using RNAi, not every gene is inhibited by RNAi, particularly those involved in the functioning of the nervous system. This limitation has been offset by the generation of RNAi-hypersensitive strains of *C. elegans*, *rrf-3* and *eri-1*, which have mutations in a putative RNA-directed RNA polymerase[86] and a protein with nucleic-acid-binding and exonuclease domains[87], respectively. The improved penetrance of RNAi effects was highlighted by the finding that in a genome-wide RNAi screen using the *rrf-3* strain the number of genes giving a phenotype were significantly increased compared with a similar screen using wild-type N2 worms[88]. The use of RNAi to knockdown more than one gene simultaneously is being exploited to overcome functional redundancy between genes. In one example, the functional redundancy of two nucleoside transporters (CeENT1 and CeENT2) was demonstrated: the silencing of each transporter individually had no effect, whereas simultaneous silencing of both genes resulted in developmental intestinal and vulval defects[89].

Genome-wide RNAi screens are likely to accelerate the discovery of new drug targets simply by systematically silencing genes and producing resistance to a compound of interest. These screens can be facilitated by the use of automated phenotype selection, such as the recently developed complex object parametric analyser and sorter (COPAS) called Biosort (Union Biometrica). As shown by the levamisole screen, proteins associated with drug-targeted receptors can themselves represent potentially novel drug targets. RNAi screens that look for suppression or enhancement of drug resistance in a mutant background, or simultaneous RNAi on two genes, can therefore be used to identify proteins functionally linked with known drug targets. Recently, a large-scale TWO-HYBRID ANALYSIS of the *C. elegans* proteome generated a protein-interaction map of more than 4,000 interactions[90], which represents a useful resource for finding proteins that interact with drug targets. Efforts using hairpin RNAi and the Gateway cloning technology devised by Invitrogen are in progress to help establish a comprehensive phenome map for *C. elegans* that details RNAi silencing for every gene, as well as the effects of gene silencing in different environmental conditions, various genetic backgrounds or in specific cell types[91]. These studies will increase our understanding of biological processes and facilitate dissecting processes affected by drugs. An invaluable resource is the *C. elegans* Gene Knockout Consortium (see Online links), which aims to facilitate genetic research through the production of deletion alleles at specified gene targets that can be requested by investigators. For instance, if certain genes of interest are not silenced by RNAi, potential null mutants of the genes can be requested from the Knockout Consortium.

Screening drug candidates for human diseases. Although there are important physiological differences between nematodes and mammals, there a number of factors that make *C. elegans* an attractive organism to be used in screens of huge numbers of drugs to identify compounds that could ameliorate human diseases: the ease of maintaining *C. elegans* in the laboratory; the conservation of many genes and fundamental cellular processes between nematodes and mammals; the availability of resources for generating mutants; and the low cost, both in time and money, of running large-scale screens. Screens of a similar scale using mammals would take considerably more time (compare the 9-week generation time of mice with 3 days of *C. elegans*) and would require an enormous budget. A recent study applied gene-to-chemistry screens in *C. elegans* to search for

Box 1 | Companies using *C. elegans*

The following are examples of companies using *C. elegans* for drug discovery:

- Axys Pharmaceuticals
- Cambria Biosciences, LLC
- CyberGenome Technologies, LLC
- Devgen
- Divergence LLC
- Exelixis Pharmaceuticals, Inc.
- The Nef Lab, Hoffmann-La Roche, Basel

RNA INTERFERENCE (RNAi). The deployment of double-stranded RNA, which results in specific silencing of the corresponding gene through degradation of endogenous RNA.

COPAS
An automated high throughput system that can sort *C. elegans* (up to 100,000 worms per hour) based on physical and optical parameters.

TWO-HYBRID ANALYSIS
A large-scale yeast-two hybrid screen to identify protein– protein (or 'interactome') networks.

molecules active against the effects of dystrophin deficiency[92]. Mutations in the gene encoding dystrophin give rise to Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy, both of which are characterized by progressive weakening and degeneration of skeletal muscles[93]. *C. elegans* possesses a dystrophin-like gene, *dys-1*, in which mutations cause degeneration of body-wall muscles that causes impaired locomotion[94]. In a pilot screen, approximately 100 compounds were tested in a blind assay to determine whether any of them reduced muscular degeneration in *dys-1* mutants[92]. It was found that one compound, the steroid prednisone, reduced the number of degenerating cells in *dys-1* mutants by 40%. Prednisone is already used as palliative treatment for DMD, and so the screen demonstrates that the mechanisms underlying muscle survival are conserved between worms and humans. Furthermore, this study provides proof of principle for the use of *C. elegans* in searching for potential drugs to treat DMD, because the worms can be used in mass screens to select a few dozen compounds out of thousands for eventual testing in significantly more expensive vertebrate models.

Compounds have also been used on *C. elegans* to study mechanisms underlying non-disease processes, an example of which is an investigation into factors underlying aging. Reactive oxygen species (ROS) arising from metabolism are thought to contribute to the ageing process. Mimetics showing superoxide dismutase and catalase activities, which neutralize ROS, were found to significantly increase the mean and maximum lifespan of *C. elegans*[95]. The mimetics also restored a normal lifespan to abnormal methyl viologen sensitivity (*mev-1*) mutant worms, which age at an accelerated rate. *Mev-1* worms have a mutation in the cytochrome b subunit of succinate dehydrogenase (complex II) of the electron-transport chain, which results in an elevated accumulation of oxidative damage during aging and increased sensitivity to oxygen, and so the study suggests that endogenous oxidative stress is a major factor determining the rate of aging. Interestingly, the mimetics used successfully ameliorate disorders related to oxidative stress in mammals[96,97], raising the possibility of using *C. elegans* in screening for drugs to treat diseases arising from ROS action.

There is an increasing number of examples in which human genes associated with disease are heterologously expressed in *C. elegans*. For example, the human β-amyloid peptide (Aβ1–42), which is associated with the pathology of Alzheimer's disease, has been successfully expressed in *C. elegans*[98]. In the future, such models could be screened using chemistry and RNAi to identify drug candidates and new drug targets.

The facility with which *C. elegans* can be used in high-throughput screens has attracted interest from the pharmaceutical and related industries. Many biotechnology companies, as well as drug and chemical companies, now deploy *C. elegans* in their discovery processes (BOX 1).

Parasitic nematode genomes. Analysis of expressed-sequence tags (ESTs) from 30 species (28 of them parasitic) within the phylum Nematoda revealed that more than half the putative genes studied were unique to the phylum and therefore could represent nematode-specific drug/vaccine targets[99]. Interestingly, 23% of the genes were unique to the species from which they were derived, which indicates that *C. elegans* might not be a comprehensive model for other nematodes. This study did not exhaustively cover the transcriptomes of the nematodes analysed, and so other genes unique to certain nematode species will probably be identified. Genome projects of other nematodes, such as that for *Brugia malayi*[100], which causes elephantiasis, will be instructive in revealing genetic and genomic diversity within the phylum Nematoda and indicate the usefulness of *C. elegans* as a model for parasitic nematodes when studying a particular gene. Nevertheless, while the culture of nematode parasites remains difficult, *C. elegans* seems likely to remain an important experimental tool for drug discovery.

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Competing interests statement
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