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Review

Caenorhabditis elegans: A versatile platform for drug discovery

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Drug discovery and drug target identification are two intimately linked facets of intervention strategies aimed at effectively combating pathological conditions in humans. Simple model organisms provide attractive platforms for devising and streamlining efficient drug discovery and drug target identification methodologies. The nematode worm *Caenorhabditis elegans* has emerged as a particularly convenient and versatile tool that can be exploited to achieve these goals. Although *C. elegans* is a relatively modern addition to the arsenal of model organisms, its biology has already been investigated to an exceptional level. This, coupled with effortless handling and a notable low cost of cultivation and maintenance, allows seamless implementation of high-throughput drug screening approaches as well as in-depth genetic and biochemical studies of the molecular pathways targeted by specific drugs. In this review, we introduce *C. elegans* as a model organism with significant advantages toward the identification of molecular drug targets. In addition, we discuss the value of the worm in the development of drug screening and drug evaluation protocols. The unique features of *C. elegans*, which greatly facilitate drug studies, hold promise for both deciphering disease pathogenesis and formulating educated and effective therapeutic interventions.

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1 Introduction: The nematode worm *Caenorhabditis elegans*

Early in the 1960s, Sydney Brenner introduced the soil nematode *C. elegans* as a model organism to study animal development and the nervous system. What led Brenner to choose the worm as a promising model? He reasoned that in order to understand development, one would need the simplest differentiated organism, small enough to be handled in large numbers, easy to cultivate, with a short life cycle and amenable to genetic analysis. Since then, *C. elegans* has proven instrumental in providing insights into the molecular mechanisms underlying numerous biological processes, such as cell death, cell cycle, sex determination, and neuronal function as well as in the genetic dissection of signaling pathways that were subsequently found to be conserved in mammals [1]. Similarly, *C. elegans* has contributed enormously to our understanding of human neurodegenerative disorders [2], cancer [3], and aging [4].

The adult *C. elegans* hermaphrodite is about 1 mm long and 80 μm in diameter. Animals can be grown on a diet of *Escherichia coli*, on agar plates or in liquid medium. The nematode life cycle is short (3.5 days at 20°C) and each hermaphrodite produces ~300 larvae by self-fertilization. Crossfertilization with occasional males arising at a frequency of about 0.1% is also possible, facilitating genetic analysis. The transparency of the animal body allows easy visualization and monitoring of cellular processes and has permitted recording and determination of the complete pattern of cell divisions that generate the 959 somatic cells of the adult (the cell lineage; ref. [5]). Despite its apparent simplicity, there is a high degree of differentiation; worms have muscle cells, hypodermis, a nervous system, intestine, gonads, glands, and an excretory system. Moreover, the complete wiring diagram of its 302

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Abbreviations: COPAS, complex object parametric analyzer and sorter; **DHP,** 1,4-dihydropyridine; **GPCR,** G-protein-coupled receptor; **G-αq,** G-protein α-subunit; **HTS,** high-throughput screening; **MOA,** mode-of-action; **SNP,** single-nucleotide polymorphism; **siRNAs,** small interfering RNAs; **UI,** urinary incontinence

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neurons and their connecting synapses has been elucidated from serial section electron micrographs [6]. The availability of a fully sequenced worm genome, in which 60–80% of the genes have a human counterpart [7], the wealth of information available at Wormbase (http://www.wormbase.org/; ref. [8]) regarding gene structures, mutant and RNAi phenotypes, microarray data and protein–protein interactions, and the availability of numerous mutant strains, have lead to the rapid adoption of the worm as a model organism. Importantly, *C. elegans* strains can be stored indefinitely in liquid nitrogen, making it feasible for any laboratory to posses an unlimited collection of mutants. *C. elegans* is the only multicellular organism for which routine cryo-conservation has been made possible.

In addition to these features, worms carrying mutations in a variety of highly conserved biochemical pathways display a wide variety of phenotypes that are easily scored. This makes *C. elegans* especially attractive for investigating the biological effects of drugs and identifying their molecular targets. The small size, the rapid life-cycle, and the high fecundity of *C. elegans* permit screening of thousands of animals over multiple generations in microtiter plates. Furthermore, the transparency of the worm allows appropriate fluorescent markers to be readily followed *in vivo*. In addition, the detailed description of the worm nervous system provides a unique setting for studying the action of many drugs that act on the central nervous system (CNS) in humans. Given that essential neuronal processes and functions (for example, neurotransmitters and their neuronal receptors) are highly conserved between *C. elegans* and vertebrates, such endeavors are expected to yield information relevant to humans [9].

C. elegans is becoming a popular platform that can be used to gain a deeper understanding of the mechanism of action of antiparisitic agents and various human drugs as well as for the discovery of new bioactive compounds. In this review, we discuss the potential of *C. elegans* as a tool for the identification of drug targets and the elucidation of the molecular mechanisms underlying drug action. In addition, we survey prospects of utilizing the nematode for screening compound libraries for new potential drugs.

2 Target identification and validation

The objective of drug discovery is to find synthetic compounds or natural products that will cure or ameliorate diseases. For this imperative task, drug discovery relies heavily on target identification and validation. At the same time, unraveling of the cellular pathways involved in a disease and identifying potential (druggable) target(s) remain a major challenge in drug discovery. Nutrition- and aging-related diseases, for example, are very complex, with multiple environmental as well as genetic factors influencing their development, requiring unconventional methods to identify valid targets for the development of therapies. The nematode *C. elegans* is a highly attractive model for the identification of novel targets for a number of reasons, in particular, the high conservation of biochemical pathways between worms and humans, the ease and speed of genetic methods designed to identify and characterize new mutants, and the relatively low cost associated with *C. elegans* research.

The strategies used for target identification in worms have been discussed in-depth recently [10]. Important steps and choices are summarized in Fig. 1. An example that underscores the importance and potential of worms for target identification is described by Ashrafi *et al.* [11], who used a genome-wide RNAi library [12] to screen for genes that regulate fat storage to ultimately identify potential targets for the treatment of obesity. Obesity is a rapidly increasing problem in the western world, dramatically increasing the risk for diseases such as diabetes and atherosclerosis. Fat storage is a highly complex process that involves many factors and although our understanding of it has improved dramatically over the years, obesity still remains difficult to treat due to the lack of global modulators of fat accumulation.

To identify the targets that affect fat accumulation in an intact and alive animal, wild-type worms were subjected to genome-wide RNAi and the total fat accumulation (regardless of the exact nature of the fat molecule) was measured by accumulation of the dye Nile Red (which is quenched in water but fluoresces in the absence of water, hence in fat). The genome-wide RNAi library is neatly organized in 96-well plates and typically one gene *per* well is tested, so the genes of interest can be identified immediately without any cloning. Out of 16 757 RNAi clones, corresponding to more than 80% of nematode genes, 305 caused reduced or distorted fat disposition, while silencing of 112 other genes resulted in increased fat content. The gene inactivations that alter fat content without affecting viability or fertility reflect a wide range of biochemical pathways. Some of the genes were already known to play an important role in mammalian fat metabolism. Interestingly, other *C. elegans* fat regulatory genes (over 50% of the total number of genes identified in this screen) have known counterparts in mammals but had not been previously implicated in fat metabolism or storage, and this could be a pool of potential novel targets.

Researchers assigned genes of interest (the 305 that reduced fat storage when inactivated) into specific signaling pathways by testing the effect of their inactivation on fat storage in "obese" strains, including the *daf-2* mutant (insulin signaling pathway), the *tph-1* mutant (serotonin pathway), and the *tub-1* mutant (tubby signaling). Inactivation of 32 genes caused a reduction in body fat in each of those strains. This group of genes is of particular interest, since it may represent the key fat regulatory genes that when inactivated, specifically and extensively

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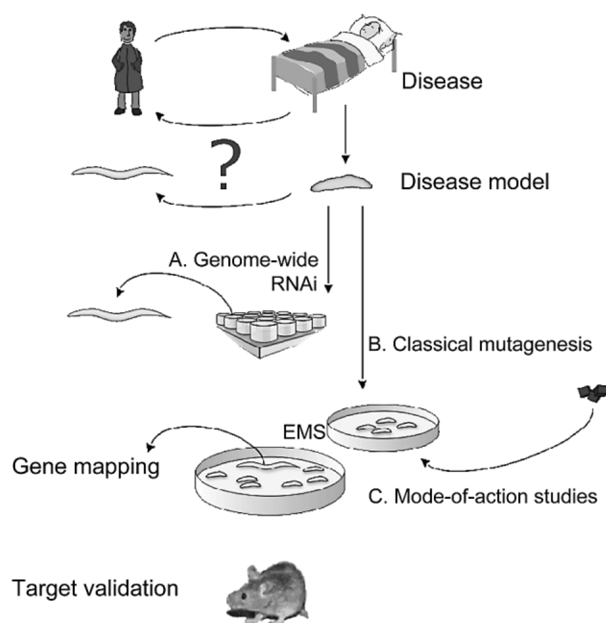

Figure 1. *C. elegans* as a model in target identification. An important step in target identification is the development of a relevant *C. elegans* disease model, designed to mimic molecular characteristics of the disease and to display a measurable phenotype suitable for high-throughput technologies. Next is to look for genetic modifications that have an effect on the severity of the phenotype (cure the "disease" in the worm). The mutated genes are likely to play a functional role in the disease and could therefore be potential drug targets. Two main genetic approaches are used to modulate the activity of most, if not all, *C. elegans* genes individually. (A) Genome-wide RNAi libraries have been developed, allowing large-scale knockdown of the majority of genes in 96-well plate format (one gene *per* well). Inactivation of genes that are involved in the disease pathway will result in modification of the phenotype of the model, and those genes can be readily identified, without any cloning and mapping. (B) Classical mutagenesis entails the treatment of the disease model ("sick" worm) with a mutagen (typically EMS), and the subsequent identification of mutants that are not sick anymore. The mutant genetic locus is mapped and the corresponding gene is indentified by genetic complementation and sequencing of mutant alleles. These alleles may harbor either suppressor or enhancer mutations. (C) MOA studies of known drugs can sometimes also lead to the identification of new targets (see text). Further analysis of the function of the potential target protein is done by classical worm genetics (see genome-wide RNAi analysis of fat regulatory genes in text). The target of choice is validated in a mammalian animal model to determine the relevance to human disease (for example, the Devgen diabetes program described in text).

affect fat storage without any obvious side effects, at least in worms [11]. Metabolic enzymes and other known fat homeostasis genes as well as receptors, signaling molecules, transporters, and vesicular transport molecules are represented in this group, emphasizing the complexity of body weight maintenance, even in a simple worm. Importantly, many of these genes code for potential drug targets based on expected structural similarities to known drug targets. This study demonstrates how typical and relatively straightforward *C. elegans* methods can uncover a wealth of information highly relevant to drug discovery.

In a commercial setting, Devgen in collaboration with Exelixis Inc. [13] (http://www.devgen.com/; http://www.exelixis.com/) created a genome-wide RNAi library that is used for various target identification screens. For example, a fat modulator screen was performed resulting in the discovery of a kinase involved in global modulation of fat accumulation. This potentially druggable target was designated the kinase-2 and the development of chemical modulators for this protein is ongoing [14].

The value of *C. elegans* as a novel tool for target identification was further demonstrated by researchers at Devgen, who have identified a potential target for the treatment of type 2 diabetes, a disorder which is characterized by insulin resistance. The insulin signaling pathway is central to the regulation of metabolism and is highly conserved among diverse species, including *C. elegans*. *daf-2* is the worm homolog of the insulin-like growth factor receptor and inactivation of the *daf-2* gene induces dramatic metabolic changes that resemble insulin resistance and cause worms to enter the easily observable dauer state. Under normal conditions, *C. elegans* develops to reproductive adulthood through four larval stages (L1-L4), in 3 days. Dauer is an alternative developmental stage induced by harsh environmental conditions such as starvation, high population density or high temperature. Dauers do not feed, are resistant to stress, can survive up to several months, and adopt a different morphology and posture making them easily recognizable among a mixed population of worms. The RNAi library was used to identify *C. elegans* suppressor mutations of the *daf-2* mutant phenotype.

These studies culminated with the identification of a novel kinase (designated kinase-1) as a potential drug target that upon inhibition was expected to enhance insulin sensitivity. To further validate this target, the kinase was knocked-out in mice that are prone to develop early signs of diabetes such as obesity and glucose intolerance when fed a rich diet. The mice in which kinase-1 was knocked-out were protected from diabetes when overweight, while there was no sign of side effects. This appears to be due to a combined positive effect on insulin secretion, which is restricted to feeding times, and slower clearance of insulin, avoiding hypoglycemia between meals, which is the most dangerous side effect of current insulin and insulin secretion therapies.

3 Mode-of-action (MOA) studies

To develop a safe drug and avoid unanticipated side effects, it is essential to obtain a complete picture of the mechanism of its action. A comprehensive knowledge of the biomolecules relevant to the activity of a given compound will greatly boost drug discovery by facilitating op-

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timization of the drug, *i.e.* increasing activity toward the desired targets and reducing effects on unwanted targets. In turn, this will result in an important decrease in the cost of development and in the risk associated with using the drug.

The elucidation of drug action mechanisms requires coupling of genetically tractable model systems and high-throughput technologies. While in theory MOA studies in mammalian animal models are most relevant to human pathologies, in reality the use of very expensive and elaborate mouse models for MOA studies or target identification is the major bottleneck in drug discovery. Among animal models, *C. elegans* is the most amenable to rapid genetic analysis and also more cost effective than any other animal model, while still offering the advantages of the whole-animal approach when compared to mammalian cell culture systems or the unicellular eukaryote *Saccharomyces cerevisiae*. The identification of phenotypes is easier, and the responses after experimental manipulations in the whole animal reflect better physiological responses. Therefore, *C. elegans* bridges the gap between *in vitro* assays and mammalian models since it allows high-throughput approaches, while still providing relevant physiological information. How MOA studies are performed in *C. elegans* is depicted in Fig. 2.

In the past decades, small-molecule drug discovery has been mostly based on target-specific approaches, where an identified target protein is purified and used for *in vitro* screening of inhibitors of its biochemical activity using cell extracts or cultured cells. Once hits are identified they need to be tested in the context of the whole organism. In many cases, at this stage compounds may display toxicity or lack of specificity, which results in wasted time and funds. Therefore, the advantage of using a whole organism approach in early stages of drug discovery is that it allows chemical screening and animal testing simultaneously, providing information about permeability, toxicity, and effectiveness. Moreover, a target-based approach is biased toward the discovery of modifiers of a known molecular target and it is unlikely to reveal novel targets and provide insights into the molecular pathways affected by a given compound. Given that drugs are likely to interact with more than one target *in vivo*, a whole-animal approach increases the probability of identifying both, off-target and synergistic effects. An organism-based approach provides a more appropriate physiological context and allows tackling of biological processes for which molecular targets have not yet been identified.

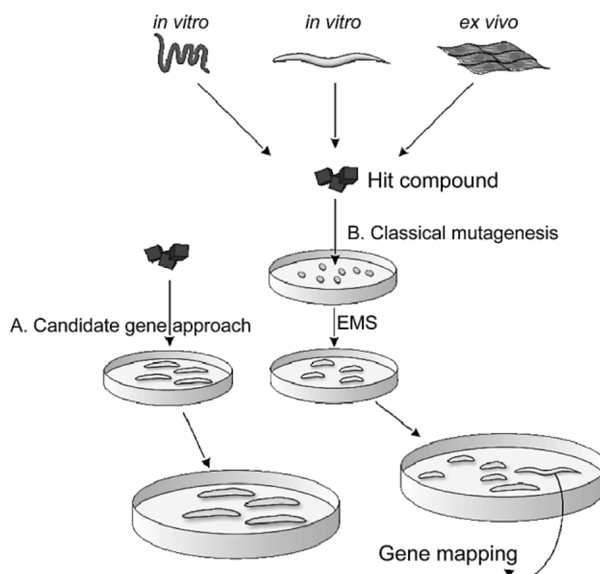

Figure 2. MOA studies in *C. elegans*. MOA studies in worms start with a potential drug candidate (found positive in a compound screen) that has a measurable effect in worms. The knowledge of the action of the molecule once it is identified as a hit depends entirely on the type of screen that was performed, for example, a hit from a target-based *in vitro* screen by definition has to hit the desired target, but nothing is known about potential off-target effects, while the exact target(s) of a positive from an *in vivo* end-point screen (for example in worms) is in principle unknown. However, in the case of *C. elegans*, extensive prior studies of biochemical pathways, including target identification, will allow at least a good guess of possible targets. In that case, MOA studies can be performed using the candidate gene approach (A), comprising the selection of a number of known mutants carrying mutations in genes that are part of pathways most likely to be affected by the hit compound. Undesired off-target effects will be uncovered relatively easily, since the screen is done on an intact organism and it will therefore bring about an additional effect (although the possibility that an undesirable off-target effect has no effect in worms cannot be excluded). Instead, or in parallel (see text for the development of UI treatments as an elegant example of MOA approaches in worms), one can perform an unbiased mutagenesis screen (B) to identify suppressor/enhancer mutations in genes affected by the compound. *C. elegans*-based MOA studies can also lead to the identification of interesting new targets for drug discovery.

4 Chemical genetics: Chemistry-to-gene screens

Classical forward genetic screens in *C. elegans* have provided important insights into many aspects of cell biology and in particular, in understanding the function of the nematode nervous system. In a forward genetic screen, hermaphrodites (P0) are randomly mutagenized, usually with ethylmethanesulfonate (EMS). Worms are then distributed in Petri dishes and allowed to reproduce for two generations to bring mutations to homozygosis. Phenotypes of interest are isolated and the responsible mutated genes can be readily mapped by using single-nucleotide polymorphisms (SNPs) that can be detected by sequencing or restriction analysis [15–17]. Hence, *C. elegans*, among the animal models, offers the most convenient and economically affordable high-throughput methodologies for genetic mapping. Efficient positional cloning in *C. elegans* has been made possible due to the availability of detailed genetic and physical maps. The strength of forward genetic screens in *C. elegans* is that it is possible to carry out relatively unbiased saturation mutagenesis, al-

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lowing the identification of genes/targets involved in a given molecular pathway or disease. Moreover, multiple mutations with specific phenotypes can be obtained within a single gene that can be utilized to outline functional domains in gene products such as the binding site of a chemical compound.

In a chemical genetic screen, or chemistry-to-gene screen, chemical compounds are used to produce an observable animal phenotype. Subsequently, worms are mutagenized and either resistant or hypersensitive mutants are isolated for further analysis. The mutations are likely to affect the cognate target(s) of the compound or any other gene acting in the same molecular pathway. Thus, in a chemical genetics approach small molecule compounds are screened for specific phenotypes in animals and their molecular targets are identified. Chemical genetics using simple animal models has the potential to accelerate drug target identification. Since genetic approaches are unbiased, they provide physiological information about the activity of the drug in the context of the organism.

Chemical genetics is also used to understand the *in vivo* action of an established drug for which the exact targets have not been identified properly. A classic example is the identification of targets of selective serotonin reuptake inhibitors (SSRIs). Fluoxetine (Prozac) is a widely prescribed antidepressant thought to act by increasing serotonin levels at the synaptic cleft and therefore, altering serotonin signaling. Fluoxetine administered to worms results in the stimulation of a number of behaviors such as feeding, egg-laying or locomotion which are modulated by serotonin (5-hydroxytryptamine, 5-HT) signaling. In a screen for fluoxetine resistance, loss-of-function mutations in MOD-5, the *C. elegans* serotonin reuptake transporter (SERT), were isolated, resulting in the identification of a candidate target for fluoxetine [18]. However, several lines of evidence indicated that fluoxetine can also act in a SERT and 5-HT independent manner. Indeed, chemical genetics studies of the effect of fluoxetine on egg-laying in *C. elegans* revealed that fluoxetine also acts on a G-protein-coupled receptor (GPCR) signaling pathway [19]. In addition, other genetic screens for fluoxetine-resistant *C. elegans* mutants have led to the identification of genes involved in the drug uptake process [20].

Forward classical as well as chemical genetic screens in *C. elegans* have been used successfully to identify the molecular targets and the mode of action of several drugs (Table 1). These include antiparasitic agents such as levamisole, aldicarb, ivermectin, and benzimidazoles [21, 22] and human drugs such as nicotine [23, 24], ethanol [25], anesthetics [26], antidepressants [18, 19], and cancer drugs [27]. These examples have been recently reviewed elsewhere ([10, 21] and references therein). Therefore, as illustrative examples we will discuss two other recent discoveries using *C. elegans*, which demonstrate the advantages of the nematode for target identification and drug optimization as well as for the discovery of new bioactive compounds. The first example discussed below presents proof-of-evidence that *C. elegans* can provide a platform for the discovery of new bioactive molecules, target identification, and MOA studies [28].

Table 1. Examples of drugs studied in *C. elegans*

Drug	Description	Reference
Aldicarb	Antiparasitic drug	[63, 39]
Ivermectin	Antiparasitic drug	[64, 65]
Levamisole	Antiparasitic drug	[66, 67]
Halothane	Anaesthetic	[26]
Farnesyltransferase inhibitors (FTIs)	Anticancer drugs	[27, 51]
Resveratrol	Nutritional supplement	[68]
Ethosuximide	Anticonvulsant	[69]
Arimethadione	Anticonvulsant	[69]
Prednisone	Immunosuppressant	[53]
Nicotine	Alkaloid drug	[23]
Ethanol	Psychoactive drug	[25, 58]
Fluoxetine	Antidepressant	[18, 19]
Imipramine	Antidepressant	[19]
MPTP/MPP^+	Opioid toxins	[70]

With the aim of identifying new small-molecule tools for the biological analysis of *C. elegans*, four different chemical libraries (14 100 small membrane-permeable compounds) were screened for the induction of defects in wild-type worms [28]. Among these, 308 compounds induced a variety of phenotypes ranging from slow growth, lethality, uncoordinated movement, and morphological defects. This high hit rate, similar to that described for zebrafish [29], illustrates the suitability of *C. elegans* as a model system for the identification of new bioactive compounds. The usefulness of *C. elegans* for target identification was further explored by focusing on one compound named nemadipine-A. Nemadipine-A resembles prescribed antihypertension drugs such as 1,4-dihydropyridines (DHPs), which antagonize the α_1-subunit of L-type calcium channels. Nemadipine-A elicits different phenotypes in the worm, slow population growth defects (Gro phenotype), dramatic defects in morphology (called a Vab phenotype, for variable abnormal), and egg-laying defects (Egl phenotype). To identify the molecular targets of nemadipine-A, a chemical genetic suppressor screen was performed. 180 000 randomly mutagenized wild-type genomes were screened for dominant genetic suppressors of the Gro phenotype induced by nemadipine-A. Sixteen suppressors were isolated, of which five were further characterized. All five mutants suppressed the Gro, Vab, and Egl phenotypes. Snip-SNP mapping indicated that all five mutants were tightly liked to *egl-19*, which was further demonstrated by genetic crosses and sequencing. All five suppressors contained missense mutations in conserved regions of *egl-19* that form the pore of the chan-

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nel, strongly suggesting that EGL-19 is the primary target of nemadipine-A. *egl-19* encodes the only L-type calcium channel α_1-subunit in the worm genome and it received this name because reduction-of-function mutants are Egl. Additional genetic evidence, from phenotypic analysis of the mutants isolated and of other existing hypo- and hypermorphic alleles, also pointed to EGL-19 as the primary target for nemadipine-A. For example, wild-type animals grown on nemadipine-A phenocopy *egl-19* loss-of-function mutants and *egl-19* hypomorphs are hypersensitive to nemadipine-A. Moreover, *egl-19* hypermorphs are resistant to nemadipine-A, and nemadipine-A can rescue the defects resulting from EGL-19 hypermorphic activity. Combined with the fact that nemadipine-A is a DHP and EGL-19 an L-type calcium channel, these observations suggest that EGL-19 is the primary target of nemadipine-A. Not surprisingly, nemadipine-A antagonized EGL-19 better than any of the Food and Drug Administration (FDA)-approved DHPs, which were optimized for humans. Kwok *et al.* [28] investigated whether this was due to differences in the potency of the drugs to inhibit L-type calcium channels or due to differences in drug accumulation in worms. Their study revealed that the structure of nemadipine-A allows both, accumulation and channel antagonism. By studying structure-activity relationships, specific molecular substructures that enable nemadipine accumulation and channel inhibition were identified.

Taking advantage of the fact that nemadipine-A exclusively antagonizes EGL-19, possible redundant roles of EGL-19 in egg-laying were investigated by compromising EGL-19 function with nemadipine in different voltage-gated calcium channel mutants. *unc-2* encodes the α_1-subunit of N/P/Q-type calcium channel and is required to negatively regulate egg-laying. The only other voltage-gated calcium channel α_1-subunit encoded in the *C. elegans* genome is CCA-1, which is of the T-type and regulates pharyngeal pumping. Nemadipines resulted in a synthetic Egl phenotype in both mutants, which was suppressed by an *egl-19* hypermorph allele, demonstrating that the synthetic interactions depend on EGL-19 hypomorphic activity. This redundant function of the three calcium channels in the egg-laying circuit would not have been revealed without the specificity of nemadipines to regulate EGL-19, since the weakest *egl-19* hypomorphic allele is already egg-laying defective. Thus, a small-molecule screen in *C. elegans* revealed a very specific antagonist of a new L-type calcium channel, an achievement that will allow the genetic investigation of DHP-target interactions *in vivo*, and the identification of other loci that may modify the response to a drug widely used by humans.

Another example illustrating the significance of *C. elegans* in elucidating the molecular mechanism of action of a compound is the work of Fitzgerald *et al.* [30]. In an elegant combination of small-molecule screens, chemical genetics to identify molecular targets in *C. elegans*, genetic analysis in both *C. elegans* and yeast, and biochemical assays in mammalian systems, the molecular mechanism of action of a compound active in tissue models of urinary incontinence (UI) was unraveled [30]. UI disease has both, muscular and neuronal components, and current treatments for UI rely on antagonizing GPCRs of the muscarinic acetylcholine receptor class. Signaling pathways downstream of GPCRs are responsible for bladder muscle contraction and GPCR antagonists allow greater bladder filling. However, unwanted side effects occur due to the broad tissue expression of GPCR antagonist targets. GPCRs are targets for a wide range of diseases (*e.g.* hypertension, diabetes, obesity, depression, osteoporosis, and inflammation) and many of the currently marketed drugs (~45%) modulate GPCRs. Heterotrimeric G-proteins act downstream of GPCRs. Binding of acetylcholine to GPCRs results in the exchange of GDP for GTP in G-protein α-subunit (G-αq). This, results in dissociation of G-αq from the heterodimer G-β/γ and dissociated G-protein subunits mediate different cellular responses through interactions with different kinases, second messengers, and other enzymes. GTP hydrolysis on G-α induces reformation of the heterotrimeric complex and termination of signaling. G-proteins are controlled by RGS (regulators of G-protein signaling) proteins. RGS proteins bind directly to the G-α-subunit and enhance GTP hydrolysis, shortening GPCR signaling. More than 20 mammalian proteins contain a conserved RGS core domain, and because RGS have restricted expression patterns, they have the potential to allow tissue-specific control of G-protein signaling.

Several small-molecules were first tested in tissue models of UI and two active small-molecules were identified. In order to understand the mechanism of action of the compounds, small-molecules were tested on the nematode and phenotypes were scored. The two molecules active in the tissue model produced an egg-laying defective (Egl) phenotype in the worm, whereas related molecules that lack bladder-relaxing activity had no effect on *C. elegans*, an observation highlighting a conserved structure-activity relationship between *C. elegans* and mammals. The more potent of the two small-molecules was used for further analysis.

G-proteins are known players in the egg-laying process and the molecular pathways involved are well characterized. Twenty-seven available worm strains carrying mutations in pathways implicated in egg-laying were tested for either resistance or sensitivity to the compound. Three of them showed resistance, one strain carried a mutation in *eat-16* that leads to a truncated RGS protein, and the other two were gain-of-function mutations in *egl-19*. These, together with the lack of hypersensitivity or resistance in some other mutant strains already pointed to a target function in the area of the neurotransmitter/GPCR/G-protein complex. The candidate gene approach that was undertaken in this study underscores another advantage of using *C. elegans* as a model system,

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the availability of a vast amount of existing *C. elegans* mutant strains that allow epistatic analysis and rapid testing of putative molecular pathways mediating drug effects.

In a chemical genetics screen based on the drug-induced Egl phenotype, 4 resistant mutants were isolated out of 150 000 randomly mutagenized genomes. These mutants were egg-laying constitutive in the absence of treatment and were dominant or semidominant for both the Egl constitutive phenotype and drug resistance. Snip-SNP mapping, linkage tests to known alleles, and direct sequencing revealed that three of the four mutated genes were allelic to *egl-30*, *goa-1*, and *eat-16*. Strikingly, all the mutations affected members of the G-α/RGS complexes: *elg-30* encodes a G-αq subunit, *goa-1* a G-α0 subunit, and *eat-16* an RGS protein. These results together with the candidate gene analysis strongly indicate that the mechanism of action of the drugs involved calcium signaling through G-protein complexes. The involvement of G-proteins was confirmed in a mammalian system. Further, genetic tests in both *C. elegans* and yeast as well as chemical genetic and biochemical tests in mammalian cells were performed to differentiate between possible models of small-molecule action [30]. These analyses demonstrate that the new small molecules act downstream of the GPCR receptor, and that they limit G-α signaling resulting in reduced muscle contraction. Moreover, the novel isolated alleles of RGS and G-α proteins may help to develop new therapies to treat diseases related to inappropriate GPCR activation. This and the previous example highlight the value of using unbiased chemical genetics screens in *C. elegans* for the identification of drug targets and to increase our understanding of the mode of drug action.

5 RNAi screens: Gene-to-chemistry screens

Contrary to forward genetic approaches, which go from a phenotype of interest to the gene(s) responsible, reverse genetics approaches go from gene to phenotype. Thus, gene function is studied by knocking down genes with known sequences and observing the resulting phenotype. Reverse genetics has become widely applied after completion of the genome sequencing project and the discovery of RNA interference (RNAi) in *C. elegans* [31]. RNA-mediated gene knockdown can be accomplished by introducing dsRNA in the worm either by directly injecting dsRNA into the animal, by soaking animals in a dsRNA solution, or by feeding them bacteria engineered to produce dsRNA [31–33]. RNAi by feeding has become the technique of choice for high-throughput functional genomics because it does not require *in vitro* synthesis of dsRNA. Genome-wide RNAi libraries covering almost every gene in the *C. elegans* genome are available [12] and have proven powerful tools toward unraveling genes implicated in particular biological processes such as development [34, 35], fat storage [11], and longevity [36]. RNAi feeding libraries typically produce dsRNA fragments between 500–1500 bp. This is an advantage compared with the 22nt dsRNA molecules (siRNAs, for small interfering RNAs) required for the induction of RNAi in mammals. Not only because cloning of the fragments is easier, but also because the long dsRNA fragments are chopped up into many different siRNAs increasing the efficiency of RNAi. Another advantage is the possibility of interfering with gene function at any developmental stage, allowing the study of gene function at any time in the life cycle of the animal (*e.g.* RNAi of essential genes that result in embryonic lethality can be induced after egg hatching). The simplicity of RNAi experiments in *C. elegans* coupled with a well annotated genome has made genome-wide RNAi-screens possible. The whole *C. elegans* genome fits in 210 96-well microtiter plates, allowing the incorporation into high-throughput robotics. Bacteria producing dsRNA are transferred to the microtiter plates either in solid or liquid media and worms can be distributed and analyzed using the complex object parametric analyzer and sorter (COPAS; Union Biometrica; http://www.unionbiometrica.com; see below for details).

C. elegans is currently the only organism for which genome-wide RNAi screens are feasible, offering an obvious advantage when compared to other animal models. In particular, the potential of RNAi screens for target identification is enormous and has in fact been shown by several studies (see target identification and validation section). There are however some drawbacks associated with the use of RNAi for MOA studies. One of the limitations that may hamper introduction of RNAi screens in the drug discovery pipeline is the fact that genes expressed in the nervous system are often not efficiently targeted by RNAi, and many drugs act through the nervous system. Nevertheless, recent developments might change this landscape in the near future. Recently, mutant strains that allow RNAi in the nervous system have been isolated; a strain that contains a mutation in *eri-1*, encoding a ribonuclease enzyme that may suppress RNAi by degrading siRNAs [37], and a mutation in *lin-15B*, the *C. elegans* ortholog of the mammalian retinoblastoma tumor-suppressor gene (Rb). In *lin-15B* mutants, neurons are partly transformed into immature germline cells. Similarly to germline cells, these transformed neurons are sensitive to RNAi [38]. Seiburth *et al.* [39] used an *eri-1;lin-15B* strain to perform a large-scale RNAi screen that identified more than 100 novel genes involved in synaptic transmission. Investigators applied RNAi in combination with the drug aldicarb (an acetylcholinesterase inhibitor) in an *eri-1;lin-15B* genetic background and in an *eri-1;lin-15B* strain sensitized by another mutation in neurotransmission. They then looked for animals resistant to aldicarb. Remarkably, results could be verified with reference to existing mutations isolated with classical genetic screens (19 out of 21 known cholinesterase-resistance

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genes could be confirmed). This highlights the value of mutations that have been isolated by classical genetics in corroborating the outcome of high-throughput approaches in *C. elegans*.

RNAi can also be used to silence more that one gene simultaneously, which can allow identification of redundant genes, implicated in a given disease pathway that confer sensitivity or resistance to a specific compound. All these features will likely make genome-wide RNAi screens a useful tool in the discovery of new drug targets. Moreover, automated tracking and imaging systems are available for the analysis and quantification of behaviors such as locomotion or egg-laying which facilitate high-throughput screens. How a large-scale RNAi can be fitted into the drug discovery pipeline has been described elsewhere [10].

6 Compound screening using *C. elegans*-based assays

Over the past decade or so, the advancement of new medical technologies has been staggering; yet the pharmaceutical industry is experiencing an unexpected slowdown in the development of new and innovative medical products. This apparent paradox is often referred to as the innovation gap and reflected in the increasing burden of failing programs that drive up the cost of drug discovery tremendously. Indeed, rapid synthesis techniques have increased the number and diversity of compounds available for testing in state-of-the-art high and ultra-high-throughput *in vitro* screening assays, generating large amounts of preliminary hits. However, most "hits" turn out to be invalid after testing in very expensive and elaborate animal models, resulting in a gap in the discovery process and demonstrating the need for new and innovative assays to prevent this problem. It is now widely recognized that assays based on inexpensive *in vivo* models, amenable to high-throughput screening (HTS) such as *C. elegans*, may turn out to be invaluable in the development of novel screening methods to fill the gap.

6.1 High-throughput methods

Besides being an excellent model for target identification and mechanism-of-action studies, the nematode *C. elegans* is being uncovered as a remarkably promising tool for compound screening assays, owing to the rapid development of worm-based HTS methods in recent years. A decisive innovation in this field has been the COPAS or worm sorter (Union Biometrica, MA, USA), which works like a conventional fluorescence-activated cell sorter (FACS), except that it has the capacity to sort living organisms ranging from 70 to 1300 μm long (for details see: http://www.unionbiometrica.com). The worm sorter allows automated analysis, sorting, and dispensing of living nematodes with incredible speed and accuracy, measuring the object size, OD, and the intensity of fluorescent markers. After analysis, nematodes are sorted according to criteria selected by the user and dispensed into multiwell microtiter plates for HTS. In this way, thousands of wells with the exact same amount of identical worms are produced allowing the screening of equally large numbers of compounds.

HTS in worms (depicted in Fig. 3) requires the cultivation of a large amount of staged wild-type, mutant or transgenic worms, which are dispensed in 96- or even 384-well plates using the worm sorter (1–100 worms *per* well, depending on the assay). Typically, each well contains one small molecule of a compound library of choice; the origin of the compounds may vary (combinatorial or medicinal chemistry, or natural products), but is, in principle, no different than those used in conventional *in vitro* assays. After the appropriate exposure time (depending on the assay), a nonvisual read-out of the end-point phenotype is measured using a multiwell plate reader. The fluorescent signal is usually derived from a specific dye or from fluorescent protein expressed by the worms. Traditionally, *C. elegans* phenotypes are visually analysed by scoring worms individually for a particular trait, but ever since the worm has been acknowledged as a valid drug discovery model, novel assays are being developed to monitor highly relevant phenotypes in a nonvisual and quantifiable manner suitable for HTS. Below, several examples of assay modernization are described to illustrate the versatility and potential of *C. elegans* in the discovery of novel therapeutic agents.

6.2 *C. elegans*-based assays for HTS

C. elegans is a particularly suitable drug discovery model for aging-related diseases, since various aging-related pathways relevant to human diseases have been identified in worms. To permit HTS for compounds that increase life span in worms, Gordon Lithgow and colleagues at the Buck Institute of Aging developed an alternative to lengthy aging studies called the "survival assay" [40] which uses stress resistance as a surrogate measure of life span. In order to rapidly and objectively score survival of nematodes exposed to a stressor (heat, oxygen, *etc.*), the fluorescent dye SYTOX, a nucleic acid stain that binds to DNA in damaged cells, is added to the worms. This molecule does not in itself affect the viability of the nematodes and upon binding to DNA, fluorescence can be measured using a fluorescence plate reader, allowing the quantification of the amount of dead worms. The survival assay has been optimized for HTS and can therefore, in principle, be used for the identification of pharmacological agents that extend nematode life span through enhanced resistance to stresses (oxygen radicals or other stressors).

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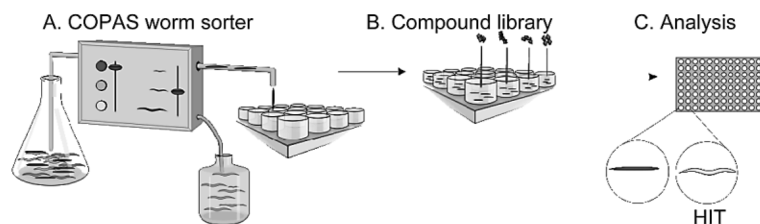

Figure 3. HTS in *C. elegans*. The development of the COPAS (worm sorter) combined with the modernization of worm assays has had a dramatic effect on the worm's amenability to HTS methods. (A) The principle of the worm sorter is shown using the example of the insulin-sensitivity screen described in the text. In this example, the user has set the machine to place medium-sized worms expressing red fluorescence in microtiter wells (for more information about the COPAS functionality, see: http://www.unionbiometrica.com/). Worms are sucked up by the machine from a large culture of more or less staged *daf-2* worms (flask), of which most (but not all) express red fluorescent marker driven by for example the *sod-3* promoter, which is particularly active in dauers (see text for insulin sensitivity screen). Subsequently, stretched worms pass the detectors and the ones identified as dauers (based on length, OD, and fluorescence) are dispensed in microtiter plates (usually 96 wells). Worms that are too small or too big and/or not red are discarded (bottle). (B) A different compound from a library is added to each well and worms that are incubated for a period of time in this case allow recovery from the dauer state in the presence of a potential hit compound. (C) Red fluorescence is analysed with a fluorescence plate reader, to identify wells containing worms that recover from the dauer state more easily (reduced fluorescence), due to the presence of compound. In order to test thousands of compounds reliably the COPAS dispensing method is absolutely critical; at the start of the experiment, the content (number of worms; fluorescence) of each well will be identical, thereby greatly reducing noise signal and allowing for the measurement of quantitative differences between wells. Coupling of the COPAS technology with a high-throughput microscope reader such as the one developed by MAIA scientific (MIAS-2; for more information see: http://www.maia-scientific.com/), further automates and streamlines the screening process.

Another example of a modernized *C. elegans* assay is the "chitinase assay" to measure egg-laying behavior, which is a highly relevant phenotype to study central nervous system (CNS)-related processes/diseases. Normally, egg-laying is measured by counting the eggs laid in a defined period of time, which is rather a time-consuming process. The chitinase assay measures egg-laying behavior indirectly *via* the chitinase activity that is released from the eggs by hatching larvae. Therefore, the total chitinase activity in a well reflects the amount of hatching larvae, which is proportional to the amount of eggs laid. The chitinase assay was developed by Pharmacia and Upjohn (now Pfizer; http://www.pfizer.com/) [41] to screen for molecules that inactivate SEL-12, the worm ortholog of presenilin, a protein involved in the development of Alzheimer's disease in humans. Worms that carry a loss-of-function mutation in *sel-12* are unable to produce eggs, and the chitinase assay enabled the researchers to screen a ~10 000 compound library for molecules that phenocopy a *sel-12* mutation. The screen remains to be validated.

Devgen Pharma is a drug discovery company specializing in nematode technology and focusing on metabolic syndrome and its consequences such as obesity, diabetes, and arrhythmia (http://www.devgen.com/). The complex and often obscure nature of these afflictions renders the use of target-specific *in vitro* assays for drug discovery unfavorable, while *in vivo* assays that measure a phenotypic end-point hold much more promise. The company has developed methods to measure both fat uptake and accumulation in living worms without bias toward a specific mechanism, by using specific fluorescent signals. In the lipid uptake assay, worms are exposed to a lipid molecule of choice that has attached to it a fluorescent probe as well as a quencher of the fluorescent signal. The quencher moiety is removed by intestinal enzymes, so that a fluorescent signal is produced only when the lipid molecule is really taken up by the gut [42]. Fat accumulation can be measured by exposing worms to the fluorescent probe Nile Red, which is fully quenched in water but not in lipids [11]. Such methods in principle make it possible to identify molecules that modulate lipid uptake and accumulation using standard *C. elegans* HTS methods, without prior knowledge about the target (see target identification section).

6.3 *C. elegans*-based drug discovery

Although the potential of the above-described assays is obvious, their true value for the development of new drugs has not been established yet. In fact, only a handful of lead molecules that are in advanced stages of drug discovery originate from worm-based screening assays: insulin-sensitizers for the treatment of type 2 diabetes or insulin resistance, and voltage-gated ion channel modulators to treat arrhythmia, both classes developed by Devgen.

The *C. elegans* model of insulin resistance (type 2 diabetes) carries a mutation in the *daf-2* gene, the worm ortholog of the human insulin-like growth factor receptor. Normally, young nematodes that are exposed to adverse conditions such as overcrowding or lack of food develop into an alternative hibernation state, called the dauer stage. *daf-2* mutants become dauers even under favorable conditions and this phenotype can be cured by stimulation of the insulin signaling pathway, for example mutation of the novel kinase-1 described above (target identification section). Dauers are very distinct from proliferating worms and can be easily identified visually, but to

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Biotechnol. J. 2006, 1, 1405–1418

comply with HTS methods, genetically engineered *daf-2* worms producing a quantifiable fluorescent signal are used. The dauer-specific signal is produced by a fluorescent reporter gene under the control of a *C. elegans* promoter that displays substantially increased activity in *daf-2* dauer larvae such as *clt-1* or *sod-3*. A large compound library was screened for molecules that rescue the *daf-2* dauer phenotype and hits were identified that increase insulin signaling in worms by acting on the validated target kinase-1.

In contrast to the diabetes program, where compounds were in principle screened without bias toward a specific target, the arrhythmia program was set up to identify modulators of a specific target, namely a human voltage-gated ion channel of the Shal (Kv4) family that is involved in the development of arrhythmia [43]. Ion channels are notoriously difficult targets for conventional *in vitro* screening assays because their activity is not easily measured. More importantly, ion channels require a functional physiological environment to function properly making screening for ion channel modulators a costly and tricky affair. This is, in fact, an excellent example of a type of screening for which worms are uniquely suitable. In *C. elegans*, modulation of ion channel activity directly affects pharyngeal pumping rate, which can be monitored visually and very easily. This also holds for human voltage-gated channels expressed in worms ("humanized" worms), demonstrating that they are functional and that a change in activity can be observed very easily.

To allow HTS for compounds that modulate ion channel activity *in vivo*, Devgen developed the "drinking assay." The pharyngeal pumping efficiency can be conveniently measured by placing the nematodes in liquid containing a fluorescent marker molecule precursor such as calcein-AM. Calcein-AM present in the medium is taken up by the nematodes and the AM moiety is cleaved off by the action of esterases present in the *C. elegans* gut, resulting in the production of the fluorescent molecule calcein. As the quantity of calcein-AM that is delivered in the gut is dependent on the pumping rate of the pharynx, the fluorescence measured in a well is a measurement of ion channel activity. To be able to selectively identify compounds that act on the human voltage-gated ion channel, screening is performed on transgenic (expressing the human channel) as well as nontransgenic worms, which should be unaffected. After hit filtering in *C. elegans*, hits were confirmed in a Xenopus oocyte voltage-clamp electrophysiology [44].

By using the *C. elegans* drinking assay, Devgen has identified and developed a lead compound and proof-of-concept has been established in a rabbit model of arrhythmia. Importantly, this compound is much more selective than known arrhythmia therapies, which often display undesired off-target activity. This discovery demonstrates not only the relevance of the worm as a drug discovery model, but also the robustness of worm-based screening assays and secondary MOA studies to prevent off-target effects later on.

7 Clinical relevance

Numerous biological processes are conserved between *C. elegans* and humans to such an extent that *C. elegans* has provided key insights into the molecular mechanisms of many human diseases and is being used in the investigation of a vast number of human disorders. These include metabolic disorders such as obesity and diabetes [11, 45], neurodegenerative disorders, including Alzheimer's [46–48], Parkinson's [49] and Huntington's [50] diseases, depression [18, 20], cancer [51], and other genetic diseases such as autosomal dominant polycystic kidney disease (ADPKD; [52]), muscular dystrophy [53, 54], and arrhythmia [55]. Some of these disease models are being used by biotech companies having full access to HTS technologies. Devgen and Exelixis perform genome-wide RNAi screens using the dauer formation model for insulin signaling, and Devgen performs high-throughput compound screens in a *C. elegans* model of arrhythmia. Others, such as the Huntington's disease model, have been used in the academic world in genome-wide RNAi screens to identify regulators of polyglutamine aggregation [56].

For many disease genes a direct ortholog can be found in the *C. elegans* genome. In these cases, a *C. elegans* disease model can be generated and validated by knocking out, knocking down, or overexpressing the endogenous gene and looking for an observable phenotype. If known, other genes acting in the pathway can be tested for conservation of function in the nematode. Similarly, if possible, established drugs could be tested for conservation of MOA in the *C. elegans* model. In a *C. elegans* gene knock out model, the human gene can be expressed to check for functional replacement, and if the molecular lesion inducing the disease is known, the human mutant gene can be expressed to induce the worm phenotype that correlates with the disease. This was done, for example, expressing in worms a human ion channel associated with arrhythmia in humans [43]. Worms have no heart, but the activity of the human channel in worms directly affects pharyngeal pumping rate (see above; Section 6).

Remarkably, even in cases when the underlying disease gene does not have a recognizable ortholog in the worm genome, *C. elegans* can still be a valuable disease model if conserved interactions can be detected. This is exemplified by both, Parkinson's and Huntington's disease models. In the first case, overexpression of human α-synuclein causes neuronal and dendritic loss in *C. elegans* [49] and a pharmaceutical model has been validated [57]. Similarly, heterologous expression of a Huntington fragment with polyglutamine repeats causes neuronal de-

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fects and aggregate formation [50], and this model has proven useful in genome-wide RNAi screens [56].

But, how relevant can *C. elegans* be as a pharmaceutical model in the pipeline of drug discovery? It is often the case, that even expensive mammalian models are not reliable to predict the effect of a drug in humans. Therefore, invertebrate and nonmammalian models can only be useful at the early stages of research to provide quick answers and suggest putative new therapeutics, toward the identification of putative novel targets and drug leads. Obviously, any discovery will need to be further tested and validated in mammalian systems in order to increase the confidence to predict drug action and safety in humans.

An important consideration is whether drug uptake and the level of conservation between *C. elegans* and humans are good enough to improve the current gap between hit identification and drug candidates. Several examples indicate that, in many cases, this is indeed the case. Many known human drugs, including nicotine, ethanol, and anesthetics, have been tested in *C. elegans* showing high effectiveness and offering a model for chemistry-to-gene screens that resulted in the identification of potential new drug targets [23–26, 58]. Another example is the identification, in a blind compound screen, of prednisone as an active molecule against the effects of dystrophin deficiency in *C. elegans* [53]. Prednisone was already being used for the treatment of muscular dystrophy, highlighting conservation of the molecular mechanism of muscle survival between worms and humans.

However, caution has to be exercised when extrapolating these findings. As shown by Kwok *et al.* [28], DHP's that are known to act in humans are ineffective in worms, but in a worm-based screen for bioactive molecules researchers identified a new DHP, nemadipine-A, which appears to act on *egl-19* with high activity and specificity. The authors used the remarkable differences in activity of the closely related compounds to look for structural features that may be important for differences in uptake/activity in worms *versus* human and their results indicate that there might indeed be such disparity. These findings imply that in some cases considerable chemical optimization is required when a compound is identified in *C. elegans*, which might impact the initial specificity of the compound. On the other hand, this study clearly underlines the close relationship between man and worm in terms of important biochemical pathways.

C. elegans has also proven to be a particularly useful model system for testing new compounds. One clear advantage that an organism-based approach offers, as opposed to cultured cells, is the fact that drugs require uptake *via* the worm's intestine. This gives preliminary uptake and toxicity information, because only those compounds that are able to traverse gut membranes and do not adversely affect nematode physiology are likely to have *in vivo* activity and elicit an observable response. Several examples illustrate the power of *C. elegans* in screening for new drugs. As already mentioned, in a small-molecule screen of 14 100 compounds, 308 elicit a variety of observable phenotypes in *C. elegans*, which is a similar rate as that found in zebrafish [28]. *C. elegans* has also been used in a high-throughput screen to find new antimicrobials [59]. 6000 ChemBridge (http://chembridge.com/) synthetic compounds and 1136 natural product extracts from a National Cancer Institute (NCI) library (http://www.cancer.gov/) were tested for their capability of promoting the survival of *C. elegans* infected with the human opportunistic pathogen *Enterococcus faecalis*. Sixteen compounds and nine natural product extracts were identified. Interestingly, some of the identified compounds and extracts had significant activity only in the whole-animal assay and not *in vitro*, providing proof-of-principle for using whole-animal screens in drug discovery programs to identify novel antimicrobial compounds.

What are the compounds that have been identified in worms? Even though various assays have been validated, no drug exists yet that was identified explicitly in a worm-based screen. One obvious reason is that recently only relatively pharmaceutical and related companies have introduced *C. elegans* in their drug-discovery programs, and a comprehensive knowledge of both, specific and off-targets as well as proper validation in mammalian clinical trials is required before administering new drug leads to humans. One drug closest to clinical trials is that against arrhythmia (described above; http://www.devgen.com/human_therapeutics_technology.php; [44]) which was proven to be safe in various animal models in 2005, and does not induce the usual side effects. These are target-specific screens. However, screens based on pathways rather than specific targets are feasible in worms and particularly relevant for complex diseases such as diabetes type 2.

8 Outlook and future prospects

While definitive conclusions regarding the value of human therapeutics discovered in *C. elegans*-based screening assays cannot yet be made, the numerous developments and studies on the subject hold promise for exciting breakthroughs in the near future. What is the niche of the worm in drug discovery? The core objective of drug discovery is to find compounds of medical or otherwise economic interest. Toward this end, it is essential to identify every protein affected by a given drug in order to avoid unanticipated side effects. The identification and validation of every target affected by a given drug remains the major challenge in drug discovery. Whole-organism-based screens are unbiased toward potential targets, therefore allowing the identification of both, druggable- and off-targets. MOA studies, designed to identify the targets a compound acts on, require high-throughput ap-

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proaches that are not feasible in mammalian systems. *C. elegans* allows high-throughput approaches, while at the same time providing physiological information in the context of the whole organism, bridging the gap between *in vitro* approaches and mammalian models.

What are the potential pitfalls of the *C. elegans* model system? One potential limitation is the apparent, occasional absence of certain mammalian genes and molecular pathways in *C. elegans*. While the majority of human disease genes and pathways are present in *C. elegans*, and many studies have revealed surprisingly strong conservation in molecular and cellular pathways between worms and mammals, some molecular pathways relevant for human disease might just not exist in the worm. In addition, a particular *C. elegans* disease model might not completely recapitulate the pathophysiology of the human disease.

Conservation in drug uptake and activity needs to be good enough in order to improve the gap between the identification of hit compounds and their drug targets. As already mentioned, drugs or drug candidates optimized for humans may not necessarily act optimally in worms. Therefore, MOA studies in *C. elegans* are only possible if human-optimized drugs work in worms. Conversely, hits identified in worms may need considerable chemical optimization before being used in humans. Another potential drawback is related to protein therapeutics. Certain protein drugs may not be possible to test in *C. elegans* simply because they might not be taken up by worms. Nevertheless, mutants with increased drug uptake capacity are becoming available and may soon render this limitation obsolete.

A validated drug target should ideally produce the desired phenotype in several animal models that recapitulate at least some important aspects of the pertinent disease. Moreover, in order to validate a target molecule for a specific drug, it is important to know whether other gene products might be involved and be susceptible to toxic side effects. Among animal models, *C. elegans* is certainly the most cost-effective choice for a target validation scheme. The wealth of information available at Wormbase [8] regarding gene structures, mutant, and RNAi phenotypes, microarray data and protein–protein interactions should facilitate the prediction of putative additional targets of a drug. In addition, the availability of a plethora of mutant strains carrying mutations in highly conserved biochemical pathways from the *Caenorhabditis* Genetics Center and Gene Knockout consortia (http://www.cbs.umn.edu/CGC/; http://celeganskoconsortium.omrf.org/; http://www.grs.nig.ac.jp/c.elegans/index.jsp; http://elegans.imbb.forth.gr/nemagenetag/), tremendously helps to scrutinize the mode of action of a given drug, because it allows candidate gene approaches and epistatic analyses to be performed relatively fast.

Importantly, recent advances in *C. elegans*-based proteomics augur important new developments in genome-wide proteome analysis in a whole organism, including studies of protein structure, function, and interaction. The *C. elegans* ORFome project provides all predicted *C. elegans* ORFs as Gateway entry clones, which has allowed high-throughput expression of *C. elegans* proteins in bacteria [60] and high-throughput studies of solubility and structure determination [61]. Proteome-scale studies of protein 3-D structures should provide valuable information for developing therapeutics. Similarly, the ORFome collection allows rapid, in-frame cloning of recombinant proteins, which has already made a high-throughput yeast-two hybrid screen for the investigation of protein–protein interactions possible. The current Worm Interactome map (WI5) contains more than 5500 interactions connecting about 15% of all *C. elegans* genes [62].

Summarizing, numerous studies have already revealed the prowess of *C. elegans* for compound screening, drug target identification and MOA studies. These studies clearly demonstrate that once a *C. elegans* disease model has been evaluated, the addition of the worm in the drug discovery pipeline is likely to provide a significant boost to drug discovery.

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