Point of VIEW

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Drug Discovery: Here Comes the Worm

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ABSTRACT Identification of bioactive molecules and their targets impedes the process of drug development. In a recent paper, a genetically tractable organism, the *Caenorhabditis elegans* worm, is shown to be a viable screening system in which the drug target and the pathway it activates can be readily identified.

In a landmark paper, Roy and colleagues (*1*) have used the nematode *Caenorhabditis elegans* to identify new compounds of therapeutic interest by screening a large library of small-molecule chemical candidates. They called the compounds nemadipines. Nemadipines show a structural homology with the well-known antihypertension drugs called dihydropyridines. By taking advantage of the powerful *C. elegans* genetic tools, they have established that nemadipines block the activity of *C. elegans* L-type calcium channels. More interestingly, nemadipines also antagonize vertebrate L-type calcium channels. This study is a proof of concept for the use of this invertebrate system in molecule screens and subsequent target identification.

The tiny, free-living *C. elegans* has been a model organism in biology for 40 years and can now be found in ~1000 laboratories worldwide. *C. elegans* is harmless, ~1 mm long, composed of ~1000 cells, and has a life cycle of only ~3 d. It can be grown in liquid media as well as on agar plates. Although quite a primitive organism, this animal is endowed with many of the basic organs, muscles, and systems common to most members of the animal kingdom (neurons, muscles, intestine, epidermis, and a detoxification and excretory system). During the first few decades of its laboratory career, *C. elegans* was considered primarily as a model organism for tackling developmental biology issues, to the delight of developmental biologists who appreciated its many unique qualities. In recent years, *C. elegans* has been used for two additional purposes. First, it has become widely used as a model for human diseases, because it provides a cheap and fast alternative to traditional mouse and rat models. Second, it is used in drug screens to identify new compounds of potential medical interest.

Although 5 years ago the idea that this tiny animal could provide a useful means of drug identification was almost heretical, it has recently gained momentum. Drug screens on *C. elegans* can be performed in two ways. First, drugs can be searched that will correct a phenotype that has been induced in *C. elegans* as a result of a mutation or by transgenesis (*2*, *3*). In such cases, *C. elegans* is an alternative to *in vitro* and cell-based screening methods preceding preclinical trials. Because such methods do not exist for numerous genetic diseases, *C. elegans* is of particular interest. In a slightly different perspective, drug screens can also be performed on healthy *C. elegans* nematodes to assay the effects of molecules under development and/or to identify targets for such molecules. The study by Roy and colleagues falls into the latter category (*1*).

A long-standing bottleneck in drug development has been the identification of bioactive molecules and their targets. *C. elegans* can be added to the existing arsenal, but like any system of this kind, it has pros and cons (*4*). Supporters appreciate that *C. elegans* is compatible with high-throughput requirements because it can be grown in multiwell plates. Proponents also welcome the fact that, although *C. elegans* is quite a simple animal, it has a level of complexity and regulatory processes higher than that of

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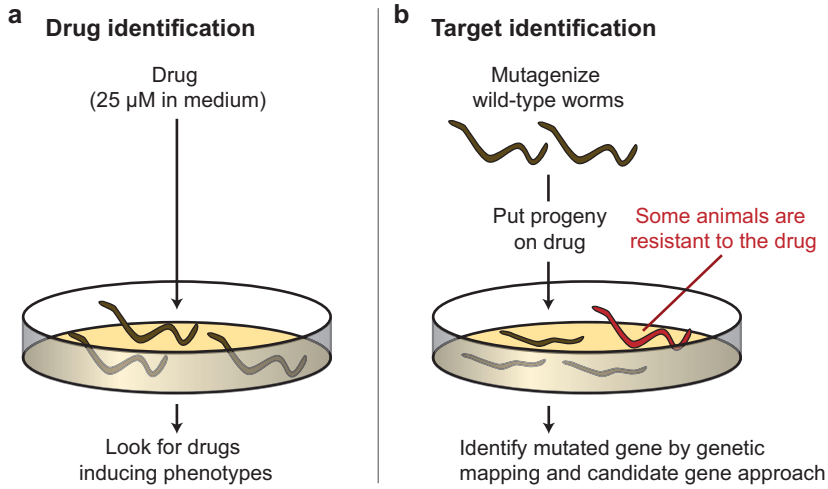

Figure 1. Drug and target identification using the nematode *C. elegans* is performed in two steps. a) Bioactive drugs are identified because they induce visible phenotypes (lethality, slow growth, or morphological defects) on wild-type *C. elegans*. b) Target identification is obtained by isolating mutants resistant to the drug and identifying the genes mutated.

cells in culture. Therefore, it may allow the exploration of pharmacological domains that could not be investigated with *in vitro* assays. This is particularly true for drugs acting on neurons and muscles: bacteria and yeast, widely used for drug identification, do not have any neurons and muscles and cultured neurons and myotubes have a physiology far different from that of their natural counterparts.

C. elegans also has some cons. First, it is surrounded by a thick cuticle that acts as a barrier to many molecules. In a compound screen, a significant fraction of the molecules tested will show no effects, because they will just not make it into the animals. Second, another fraction of possible hits may be missed: although they make it into the animal, these compounds will not recognize the nematode orthologous target. Protein divergence between nematodes and vertebrates is high. Third, measuring the drug concentration inside the animals is quite challenging: tests are mostly qualitative. Last, but not least, as far as high-throughput screens are concerned, there are not that many phenotypes to look at in worms.

Despite these limitations, among the 14,000 small membrane-permeable molecules screened by Kwok *et al.* (*1*), 308 (2%) induced a clearly visible phenotype on *C. elegans* (lethality, slow growth, paralysis, and abnormal morphology). Although comparing the hit rates obtained with very different systems is difficult, 2% seems reasonable for a wide-spectrum screen of structurally diverse molecules. Not surprisingly, structurally similar molecules were found to have similar effects on the worms.

Next comes the question of target identification. Remember that *C. elegans* has been the workhorse of hard-core geneticists who have developed elaborate genetic tools. Once a drug produces a specific phenotype in *C. elegans*, such as slow growth and morphological defects in the case of nemadipine, genetics can be used to identify the drug target as well as the pathway it activates. This strategy is similar to the one used to identify the bacterial targets of antibiotics. After a random mutagenesis on thousands of animals, in which virtually all the genes are mutated at least once, one looks for mutant animals that have become insensitive (resistant) to the drug (Figure 1). In most cases, the mutations will affect either the drug receptor or downstream elements mediating its action. In the case of Nemadipine-A, after a preliminary mapping of 5 independent mutations on the *C. elegans* genetic map, Kwok *et al.* (*1*) found that 5/5 mutants were located near the gene *egl-19*, which encoded an L-type calcium channel and was one of the candidate targets. This finding was confirmed when the mutants were shown to carry alterations in the *egl-19* DNA sequence.

In conclusion, the worm system is a new addition in the toolbox of therapeutic-drug identification. This system has its limitations and will obviously miss potentially interesting drugs. However, we should regard the glass as half-full rather than half-empty. Despite its drawbacks, *C. elegans* will catch a few drugs that would not have been identified otherwise. This is a good enough reason to justify its use.

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