Working with worms

A tenet of many drug discovery scientists is that human-derived targets should be used for the discovery of human pharmaceuticals. NemaPharm, Inc. (Cambridge, MA, USA) is challenging this axiom through its use of the humble nematode worm to identify new treatments for human diseases. Their success is a dramatic testimony to what Charles Darwin proposed more than 150 years ago: that all of nature is interconnected by a common heritage. It is clear that once successful molecular pathways evolved, they were used repeatedly with only slight variations between species. Consequently, the nematode can be used to explore molecular pathways relevant to human disease. This approach greatly simplifies the identification of new targets for drug discovery, and it has the potential to illuminate targets that can not be accessed through traditional research modalities with human cells and tissues.

NemaPharm, formerly a division of Cambridge NeuroSciences, began operations as a stand-alone company in January 1992 to exploit the rapidly expanding biological knowledge of the soil nematode *Caenorhabditis elegans*, which its aficionados claim to be the animal with the most completely understood anatomy, development, behavior and genome. According to Dr Carl K. Johnson, Founder and Director of Research, the approach used at NemaPharm "is an enabling technology that is a small, but sometimes very important, slice of what you need to do to get from a disease to a cure."

For many years NemaPharm existed with only four employees and financial support from Small Business Innovation Research (SBIR) grants and research collaborations with pharmaceutical partners. In October of 1996 NemaPharm was purchased by Sequana Therapeutics (La Jolla, CA, USA), a functional genomics company. NemaPharm now has 15 employees with plans to double in size soon. Currently, about 20% of NemaPharm's research projects are projects carried out in collaboration with Sequana aimed at the discovery of novel targets for drug discovery; the remaining programs were initiated by NemaPharm. Two major programs focus on an understanding of the function of genes that are involved in Alzheimer's disease and apoptosis.

The acquisition of NemaPharm by Sequana provided "access to the technologies needed to actually go from the genes that cause disease to treatments," says Johnson. NemaPharm's part of this process "comes after one figures out what [human] genes are mutant and cause the disease. Then we attempt to understand the function of those genes and the pathways they operate so as to identify the most appropriate sites of therapeutic intervention." Dr Timothy J. Harris, Sequana's Head of R&D, expresses enthusiasm for the collaboration; he believes that the acquisition will greatly enhance the capability to identify new therapeutic targets in Sequana's programs.

Model organisms

NemaPharm has the capability to create model organisms in which a nematode gene is replaced with either a human wild-type or mutant homologue gene. For example, the nematode has a copy of a gene, of which the function is unknown, that when present in man in mutated form is known to cause disease. The nematode version of the gene can be knocked out, and the wild-type human gene can then be inserted into the nematode genome. The human gene rescues the nematode by restoring the function of the lost gene, and the experimenter then observes the normal nematode phenotype. Likewise, the mutant version of the human gene can be inserted into the nematode genome, but in this case function is not restored, leading to a nematode phenotype that is lethal.

Screening assays

The nematode system provides an easy and very powerful screening assay enabling two different types of experiments to be performed. One experiment involves the generation of random mutations in the nematode containing the human disease gene and then screening for mutants that have reverted to the non-lethal phenotype. If such mutants are found, it is presumably because a nematode gene has been mutated that reverses the action of the disease gene. Identification of the mutated gene will lead to a molecular target that, when inhibited in the nematode, reverses the lethal phenotype. Importantly, inhibition of the same target in the human may reverse the human disease condition. The other experiment is to conduct high-throughput screening (HTS) of chemical libraries on the same nematode strains containing the human disease gene to search for compounds that will reverse the disease phenotype. In this case, instead of looking for mutations that give the phenotype of interest, the investigator is looking for chemical compounds that interact with a specific target to reverse the lethal phenotype. If such compounds are found, it suggests the existence of a pathway that can reverse the disease phenotype and is amenable to small-molecule intervention. The successful compounds are then used in other animal models or human cells or tissues to determine whether they have the same effect. If so, they may be useful candidates for drug discovery.

The assays for the nematode screens are conducted in standard 96-well microtitre plates. The nematodes eat bacteria, so the well contents initially appear cloudy because of the inclusion of bacteria in the assay. If the lethal phenotype is not reversed, the nematodes fail to develop and the wells remain cloudy. If the phenotype is reversed, the wells clear as the nematodes reproduce and

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consume the bacteria. Nematodes grow rapidly, with a two-day generation time. Each nematode produces approximately 200 progeny in a 48 h period, giving an effective doubling time of about 8 h.

The clear wells of the screening assays are examined manually under a microscope to confirm the reversion of the nematodes to the desired phenotype. It is possible for one person to conduct about 1,000 assays per day, but this number is expected to increase significantly once the assay system is fully automated. The company is currently establishing an automated microscopy system to scan the wells and has developed an automated worm dispenser, similar to a flow cytometer, that will quickly and precisely place an exact number of worms in each well of a microtitre plate.

Making the case to big pharma

One major assumption of the nematode approach is that the regulation of the gene or gene products of interest in the human is the same, or at least very similar, to its regulation in the nematode, even if the gene may not be used for the same function by the two organisms. Johnson believes this is a reasonable assumption for many human gene products, especially for the constituents of signal transduction pathways. However, he recollects that when the nematode technology was first presented to pharmaceutical researchers, some 10 years ago, "They were extremely skeptical. They just didn't believe it at all, and they wanted complete proof ahead of time.... But that attitude has changed, and quite dramatically. They still want proof of the concept, but they don't require it ahead of time. They are willing to say you can come up with a putative target from a nematode screen, but we want progression criteria in mammalian cells and in mice models. And that's the part we do with Sequana since they have mammalian cell and transgenic mice capabilities."

One reason the attitude towards the nematode technology has changed has to do with the developments regarding the *ced 9* gene. In 1988, the *ced 9* gene had been discovered in the nematode and linked to apoptosis. Johnson attempted to convince pharmaceutical scientists that studies of the gene in nematodes would have relevance to humans, "but they were dismissive of it as being relevant only to nematodes". Today it is clear that the genes regulating apoptosis in nematodes parallel the function of similar human genes. "If they had only been willing to have some faith at that point [in 1988] they would have been 3–4 years ahead of the game", says Johnson. Companies are now willing to allow NemaPharm to look at the full nematode genome of 15,000 genes to come up with the 10 or 20 genes that appear to be good targets for their particular application. Those genes are then tested as possible targets in mammalian models.

Why worms?

So, why are nematode worms used instead of some other simple organisms? The answer is that the tools are available to do the experiments, although most of the biologists who developed such tools were not interested or did not foresee the drug discovery applications. In the early 1960s, when molecular biology pioneer Sydney Brenner was looking for an animal to use as a model system for understanding neurobiology, the nematode attracted his attention because of the seeming simplicity of its nervous system, which consists of only 302 neurons and about 5,000 interconnections. The nematode has additional attractions: it is transparent under the microscope, so it is easy to monitor visually all of its 959 cells, and it is easy to grow and propagate.

By the mid-1960s, Brenner had embarked on his mission of investigating the nematode as a new model biological system. By the mid-1970s he had thoroughly deciphered the genetics of the nematode, and it was clear that it would be an important model animal, just as *Drosophila* has been a key animal for understanding classical and molecular genetics. In the ensuing 20 years, much has been learned about the nematode, and the number of nematode biologists has grown substantially; nearly a thousand scientists met at the most recent meeting on nematode biology. One of the most interesting and useful aspects of the newly found knowledge of the nematode is that many human genes have nematode counterparts. Moreover, the techniques for transferring human genes into the nematode are well established. Understanding the neurobiology of the nematode, however, remains a challenge. Deciphering the wiring of the 5,000 neural connections has not provided as much insight into the neurobiology and behavior of the nematode as originally envisioned by Brenner.

The sequencing of the entire genome of the worm is under way and almost complete. With this sequence in hand, it will be possible to compare a human gene sequence with the 15,000 genes of the nematode to determine whether the worm has a homologue of the human gene. If a homologue is found, then the power of the nematode technology is available to understand the function of the gene or to discover novel molecular targets that regulate gene expression or function and that may serve as molecular targets for drug discovery.

Almost all of the biologists responsible for development of the nematode technology can trace their scientific ancestry back to Sydney Brenner's laboratory. Carl Johnson relates that he is the original grandson of the Brenner laboratory, having been the first graduate student of the first postdoctoral fellow to work with Brenner. Thus, he sees himself and NemaPharm as an integral part of the close-knit (mostly academic) nematode biology community.

"Although it is sometimes tricky to balance," relates Johnson, "the continued interaction with academics interested in fundamental questions of biology, as well as our existence as an integral part of a positional genomics company such as Sequana, is a powerful combination for finding novel molecular targets for drug discovery".

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