Innovations

High-throughput worms

NemaPharm, Inc.

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By aesthetic and monetary criteria, whole-animal screening with row upon row of smelly mouse cages is no match for *in vitro* screening. Receptor-binding assays, enzyme-inhibition assays, and all of their cousins, can be done in single wells of a multiwell plate, with everything dispensed through robot-controlled fluidic systems. Now NemaPharm, Inc. (Cambridge, Massachusetts) has transformed the whole-animal screen into the same high-throughput format. "NemaPharm is establishing surrogates for human diseases, and then devising selections for drugs against those diseases," says Carl Johnson, a founder and Vice President of Research at NemaPharm. The key to this strategy is the humble worm *Caenorhabditis elegans*.

The wonders of worms

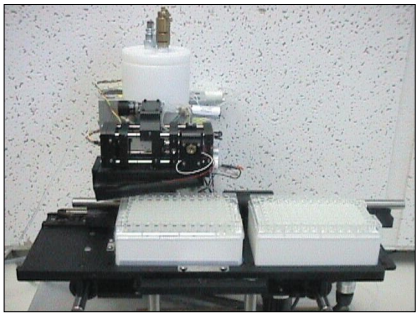
Johnson says that, with worms, he can make a thought experiment a reality. "What you want to do is to mutate a million people with Alzheimer's disease and look for those in whom the dementia goes away. Then the mutated gene is your drug target. Alternatively, you would like to give 100,000 different chemicals to humans with Alzheimer's and see which one works. We try to reconstruct the human disease in nematodes and then do the screens in nematodes."

The advantages of *C. elegans* are legion: They are small (a 9-cm-diameter plate can support the growth of 100,000 animals), fast-growing (in liquid culture the doubling time is as little as eight hours), and they can be frozen for storage. Their biology and behavior is well characterized, with the interconnections of all neurons catalogued, and the exact sequence of cell divisions from embryo to adult known. There is a good physical map of the genome, the genome sequence will be completed later this year, and the self-fertilization of the hermaphroditic worms makes both dominant and recessive genetics relatively simple. "Genetics in the end is the correlation between genotype and phenotype, so having all these tools on both sides is very powerful," says Geoffrey Duyk, Chief Scientific Officer of Exelixis, Inc. (South San Francisco, California). Exelixis uses *C. elegans* and a host of other model organisms to look for new components of disease-related pathways (see *Chem. Biol.* **4**, 703).

Knockout technologies

One thing lacking from the worm toolbox has been a method for making gene knockouts. In the solution devised by NemaPharm and others, mutated worms are divided into pools, and genomic DNA harvested from each pool is added to a separate PCR reaction. The gene of interest is amplified. Reaction conditions are such that a shorter reaction product, from a copy of the gene with a deletion, will be amplified many thousand-fold more efficiently. The store of live animals corresponding to the shorter PCR product is further subdivided until the individual clone with the deletion in the gene of interest is identified. As Johnson says, "it is not exactly rocket science," but it works. Although some details of the method are proprietary, the overall idea is not, and systematic application to all *C. elegans* genes will probably be taken on by a consortium of individual researchers, companies, and the Sanger Center in Cambridge, England. For now, NemaPharm has two full-time personnel, each generating one deletion strain per week. Johnson believes that a technological breakthrough to increase the speed of the method will be needed before it is reasonable to tackle the entire genome.

Figure 1



The ultimate worm dispenser. Image courtesy of NemaPharm.

High speed worm sorting

Knockouts are all very well, but what gets Johnson excited these days is high throughput phenotype analysis. "You need an organism compatible with 96-well plates, and *C. elegans* is, even with 1536-well plates," he says. "That is an advantage you won't find in the other model animals."

"The first step is to get the chemicals into the wells, but the pharmaceutical industry has solved that problem," he says. "The next step is to get the worms into the wells." The NemaPharm worm dispenser is modeled on a flow cytometer (Figure 1). Fast-moving fluid on the outside of the delivery cylinder straightens the worms out so a light-based detector can measure their length and therefore their age. Worms of the appropriate age are dispensed, filling a 96-well plate in a couple of minutes. Johnson prides himself on his speed in manually transferring worms. But he says that, based on a head-to-head match-up with the machine, "I've been made obsolete."

Picking your targets

"*C. elegans* is a molecular technology," says Johnson. "To do anything in *C. elegans* you need to know what molecules are involved in the disease. Schizophrenia, for example, has no molecular handle on which to start."

Johnson is interested in pathways: fleshing them out, switching them on and off, and working out which components are most amenable to drug inhibition. Not all pathways are appropriate targets. For a substrate-dependent pathway, says Johnson, "if you have something knocked out up-front there is nothing you can alter downstream to restore it. You need something like gene therapy." The presenilin defect present in Alzheimer's disease may fall into this category. "We looked at approximately one million mutated genomes. There are no genes that can be knocked out to suppress mutated presenilins. You're not going to solve this by inhibiting another target." (Both NemaPharm and Exelixis have, however, isolated suppressors of a partial loss of presenilin function.)

"But in a switch pathway — the ras pathway, or apoptosis — the function of all the genes is reflected in whether the final part of the pathway is off or on," says Johnson. Although the disease may have put a positive regulator out of commission, if Johnson can inhibit a negative regulator he may be able to turn the pathway back on. "We are looking for other things that we can knock out that will correct the disease," he says. "It's like two wrongs make a right."

Defining drug targets

"This is really a target-identification technology not a drug-identification technology," says Johnson. Where there are at most a few existing targets, such as with the presenilins, NemaPharm looks for new pathway components based on mutants that increase or decrease the severity of the original mutant phenotype. But if there are already many known components of a pathway, as is the case with ras, then NemaPharm uses a chemical screen to define the targets most readily inhibited by drugs.

"Making a compound for a proof-of-concept in mammalian models is a lot of work," says Johnson. In one step, a *C. elegans* screen selects a chemical inhibitor and a target that can be inhibited. More inhibitors can then be found by *in vitro* screens against the new target.

Going from chemical to gene is difficult but not impossible. A mutant resistant to the chemical can be isolated. The mutant gene often encodes the drug's target, and it can be mapped by standard methods.

Death and depression

One NemaPharm interest is apoptosis, or programmed cell death. Selectively blocking cell death could decrease damage in degenerative diseases or after strokes. The treatment will have to be local so that apoptosis can function in the disposal of virally infected and cancerous cells.

The apoptosis screen uses *ced9*, the *C. elegans* homolog of human Bcl2. In the absence of *ced9* function all *C. elegans* cells die. Mutations in other genes — such as *ced3* and *ced4*, which encode components of the cell death machinery — will keep a *ced9* mutant animal alive. That can be detected automatically in a 96-well plate by measuring turbidity. Dead worms don't eat their bacteria and the well water remains cloudy. But in the presence of either a *ced3* mutation or a chemical identified by NemaPharm, the *ced9* worms eat up the bacteria and clear the water.

Other phenotypes are not so easy to detect automatically. So far NemaPharm has relied on visual inspection to find suppressors of a dominant ras mutation (which causes extra vulvae to appear on the females) or to screen chemicals that, like the anti-depressant Prozac, inhibit serotonin re-uptake and so induce egg laying in the absence of food. "The way the read-outs are done now is quite biological and requires an expert," says Johnson. "There has to be some easing of that requirement."

The company has several other interests, including proteins involved in pain control that are downstream of desensitization steps (so patients won't get opiate-like resistance), and inhibitors of the secretion of immune modulators implicated in allergies.

Just as egg laying is a good surrogate for anti-depressant action, for the newer targets any phenotype will do. "We are trying to use the same molecules and pathways in *C. elegans*, not to reproduce the same behaviors," says Johnson.

The test of the anti-depressants was used to confirm the accuracy of the *C. elegans* system, not as part of a search for new anti-depressants. The worm potency ranking actually outdid *in vitro* receptor-binding data as a predictor of potency in humans. This suggests that the worm system may mimic some *in vivo* factors like bioavailability. The human digestive tract and the skin of *C. elegans* are both largely impermeable to charged chemicals, although the worm's skin may exclude many more chemicals.

The similarity of worm and human detoxification ability is also hard to predict. "*C. elegans* has a tremendous potential to exclude chemicals," says Johnson. NemaPharm is therefore deleting genes for many of the worm's P-glycoproteins, the channels that pump foreign chemicals out of cells. Identifying the most important of the P450 detoxifying enzymes (there are ~70 in all) is also a priority.

Corporate takeover time

In January 1998, Arris Pharmaceuticals Corporation (South San Francisco) bought Sequana Therapeutics (La Jolla, California), which just over a year earlier had bought NemaPharm. The new entity is called AXYS Pharmaceuticals, and NemaPharm will move to South San Francisco. Sequana uses genomics to find genes, NemaPharm prioritizes new targets, and Arris uses combinatorial chemistry to make chemicals for screening. "Now we have the full gene-to-drug spectrum in one company," says Johnson, "and the chemicals are available without making deals. Over the next year, we are really going to start running screens."

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