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INSIGHTS FROM MODEL SYSTEMS
A Model of Elegance

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To start with we propose to identify every cell in the worm and trace lineages. We shall investigate the constancy of development and study its genetic control by looking for mutants. (Sidney Brenner)

Since Sidney Brenner wrote this statement in a visionary research proposal addressed to Max Perutz 35 years ago (Wood 1988, p. xiii), an enormous amount of information has been gathered on the biology of the nematode *Caenorhabditis elegans* ("the worm"), both fulfilling his predictions and exceeding his original expectations. Researchers have identified every cell in the worm and have described all the lineages by which these cells are formed (Horvitz and Sulston 1980; Sulston et al. 1983; Wood 1988). Adult hermaphrodites contain 959 somatic nuclei, which are organized into differentiated tissue types such as muscle, intestine, epidermis, and nervous system. The latter is composed of 302 neurons that are connected by ~7,500 synaptic junctions (Wood 1988). The function of many individual cells has been inferred from ablation experiments using laser-beam technology (e.g., see Bargmann and Avery 1995).

Genes involved in development and behavior have been identified through the analysis of mutations that affect these functions. The number of genes identified genetically has increased from the original 103 described by Brenner (1974) to the ~1,600 complementation groups known at present (Hodgkin 1997). A comprehensive genetic map has been compiled and continues to expand. A detailed physical map of overlapping cosmids and YACs covers the genome almost entirely (Coulson et al. 1995). Finally, the genome sequence is expected to be completed by the end of this year (Hodgkin et al. 1995).

The availability of the complete *C. elegans* genome sequence will not only benefit worm researchers but will also undoubtedly be useful for the elucidation of human gene function. Thus, in addition to providing fundamental information on how a small, free-living animal develops and responds to its environment, the worm field is now recognized as potentially useful in the understanding of the development and behavior of humans. Again as Brenner put it, "our field has prospered and has come of age; what was once a joke organism…has now become a major experimental system for the study of development" (Wood 1988, p. ix). Not only can the results obtained in *C. elegans* teach us about the mechanisms that underlie normal development, but they can also be very useful in the study of biochemical mechanisms underlying devastating human diseases. Moreover, the worm system has the potential to become a convenient tool for the design of therapeutic strategies required for the treatment of these diseases.

For these reasons, the readers of the *Journal* might be interested in an overview of *C. elegans* genetics and genomics and of how worm biology can benefit their research. Here, we place particular emphasis on the benefits of the study of worm gene functions and interactions to the understanding of human biology; on the importance of the genomic information that should soon become available for *C. elegans;* and on the promise of the worm as a model for drug discovery.

Searching for Elegance

Defining the mechanism of the action of human genes can be extremely difficult, because of the complexity of human biology and the lack of reliable tools to selectively alter gene function in vivo, not to mention obvious ethical constraints. Since entire protein complexes and biochemical pathways appear to be conserved between humans and relatively simple model organisms, many laboratories have turned to such organisms, with the goal of establishing functional models that can ultimately teach us about human biology. A number of excellent recent reviews describe the experimental features of the worm and detail its virtues as a model organism (Hodgkin et al. 1995; Plasterk 1996; Ahringer 1997;

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Kuwabara 1997). In addition, three books are available in which most of the current biological information and detailed protocols can be found (Wood 1988; Epstein and Shakes 1995; Riddle et al. 1997).

The worm model combines ease of genetic manipulations with the ability to address biological questions related to development, behavior, and cancer. *Caenorhabditis elegans* genetics is very convenient. For example, worm cultures can be grown easily in the lab, and strains of interest can be stored frozen (Epstein and Shakes 1995). Since most adult worms develop into self-fertilizing hermaphrodites, it is usually trivial to obtain F_2 animals homozygous for a mutation of interest. Moreover, males can also be obtained, which allows the exchange of genetic material by cross-fertilization. Transgenic animals can also be generated by injection of cloned DNA (a gene, a cDNA, a cosmid, or a YAC), along with a dominant marker, into the germ line. The resulting transgenes are transmitted in the form of extrachromosomal DNA arrays. Although this technique does not allow cloning of genes by complementation using cDNA libraries, it is very useful for determination of which of several candidate DNA fragments can rescue the phenotype of loss-of-function mutations (Mello et al. 1991; Epstein and Shakes 1995). Finally, as discussed below, gene function can be analyzed by relatively convenient antisense technologies.

Having a BLAST

Usually the first reaction of a human gene cloner in the seconds following the pile-up and translation of a long-awaited DNA sequence is to "BLAST" the predicted protein sequence against the available databases, with the hope of finding statistically significant homologies. With the complete genomic sequence in hand, it will be possible to determine, *in silico,* the existence of all potential orthologues in *C. elegans* ("comparative genomics"). The proportion of positionally cloned human disease genes that show significant homology ($P < 1 \times 10^{-10}$) to potential worm orthologues is currently 63% (Bassett et al. 1995, 1997; also see the XREFdb Website). This proportion drops to 38% for potential fly orthologues, probably because the genome-sequence project is less advanced in that organism. It is 35% for yeast proteins, since the evolutionary divergence is greater (in this case, despite the availability of its entire genome sequence). Until the complete genomic sequence of the worm is released into GenBank, it remains more advantageous to search for worm orthologues directly in the databases available from the two sequencing centers involved in the project (The Sanger Center and the Genome Sequencing Center, University of Washington School of Medicine).

Once a potential worm orthologue has been identified, ACeDB (A *C*. *elegans* Database) (Durbin and Thierry-Mieg 1994) can be used to fetch the information necessary to start work with the orthologue corresponding to the gene of choice. For simplicity, ACeDB can be thought of as an organized repository matrix composed of predicted open reading frames (ORFs) in one axis and the relevant information in the other. Just to name a few options, it is possible to find, for each locus, the available information on the genetic-map position and on neighboring genes, the different cosmids and YACs covering the region on the physical map, the genome sequence with the predicted ORFs and predicted intron/exon boundaries, and the availability and sequence of corresponding expressed sequence tags (ESTs) as a confirmation of the intron/exon structure. An excellent summarized version of a "guided tour" in ACeDB is available in a recent review (Kuwabara 1997).

1% Inspiration, 99% Perspiration

How can *C. elegans* be used to elucidate the function of a particular human disease protein (HDP)? One approach consists in finding a potential worm orthologue of HDP (WHD-1) *in silico* (see above) and analyzing the in vivo phenotype(s) resulting from the abrogation of its function. In addition to providing critical functional information, such phenotype(s) can eventually be used in genetic screens to identify interacting genes. Although convenient, such screens are not trivial and usually require the construction of strains that carry particular alleles; partial loss-of-function alleles are generally preferred, especially when the wild-type gene is essential for development or when one wishes to identify both enhancers and suppressors of an intermediate phenotype. The phenotypes that arise from loss of WHD-1 activity in the worm generally will not resemble the overt human disease phenotypes, but there will often be similarities at the subcellular or biochemical level, which afford insights into the molecular mechanisms of the disease. One example of this is the elucidation of some of the molecular mechanisms involved in the function of the human *ras* oncogene, by the analysis of the role of its *C. elegans* orthologue, *let-60,* in vulva development (Kayne and Sternberg 1995; Kornfeld 1997).

Finding a worm orthologue of *HDP* can be followed by a number of different approaches. In the most powerful scenario, *whd-1* has already been identified by classic forward genetics and subsequently has been cloned and sequenced. Previous identification of a gene by forward genetics means that a particular phenotype(s) has already been associated with the loss of its function (*whd-1*(*lf*)). An optimal disease model can be generated if HDP can functionally substitute for WHD-1. For example, the worm *sel-12* gene encodes a protein that shares 50% identity with two human presenilins in-

volved in Alzheimer disease. The egg-laying–defective phenotype of *sel-12(lf)* mutants is rescued by wild-type human presenilin but not by the mutant alleles found in Alzheimer patients (Levitan and Greenwald 1995; Levitan et al. 1996). It should be stressed that a negative result in such rescue experiments is not sufficient to rule out the possibility of a functional conservation between HDP and WDP.

In a second scenario, *whd-1* is already known through classic forward genetics, but the gene remains uncloned (of 1,600 complementation groups identified, ~500 have been cloned). In some cases, the physical position of *whd-1* might point to a few mutations located in the corresponding area of the genetic map and representing potential *whd-1* loci. The functional rescue, by wild-type *whd-1,* of one of these mutations then provides evidence that the *whd-1* gene corresponds to a particular complementation group. Although potentially powerful, this approach can be complicated in regions of the genetic map that are characterized by a high density of potential loci.

In the third, and most likely, scenario, *whd-1* has not been identified by forward genetics, and there is virtually no functional information available. In this case, phenotypes associated with the loss of *whd-1* will have to be uncovered. A technique called "RNA-mediated interference" (RNAi) is very powerful in this respect. Antisense, sense, or double-stranded *whd-1* RNA injected into the germ-line syncytium of an adult hermaphrodite is often found to impede *whd-1* function in the progeny (Guo and Kemphues 1995; Rocheleau et al. 1997; Fire et al. 1998). The effect is likely specific, since the RNAi phenotype often resembles that of loss-of-function mutations. The effect of RNAi is not limited to embryos but is transmitted through larval development and adulthood, for several generations. In general, however, the effect of RNAi cannot be propagated for many generations, so subsequent genetic analyses, such as enhancer and suppressor screens, are not possible. Such screens depend on the availability of *whd-1* null or partial loss-of-function alleles.

Two methods are available to create *whd-1* null alleles. The first method is based on the ability to isolate a worm strain with the Tc1 transposable element inserted within *whd-1* and subsequent screening by PCR for imprecise excision leading to a deletion in *whd-1* (Zwaal et al. 1993). Although this method was very promising, it is now being replaced by a more efficient method, based on the use of a chemical mutagen to create libraries of deletion alleles (Jansen et al. 1997). Again in this case, strains containing deletions in *whd-1* are identified by a PCR reaction. The number of genes that have been deleted this way increases rapidly.

If neither RNAi nor the deletion of *whd-1* confers a detectable phenotype, then a few more tools based on the localization of expression of *whd-1* are available to aide in the formulation of hypotheses about the function of the gene. Whole-mount techniques are available for RNA in situ hybridization that are useful for the localization of expression down to single cells. More conveniently, transgenic animals can be generated that express β-galactosidase or green fluorescent protein (GFP) under the control of a *whd-1* promoter, or as a fusion with the *whd-1* gene product. Gene expression, as detected by the reporter construct, can then be used to screen for phenotypes in a more subtle way (Chalfie et al. 1994; Troemel et al. 1995)

If the human disease is caused by a gain-of-function mutation in *HDP,* one or more worm orthologues might also be identified and tested as described above. However, a more direct approach is to generate transgenic animals that overexpress the mutant dominant gain-of-function version of HDP and to analyze the resulting phenotype. In principle, these animals can also be used in screens to identify genetic interactors and to elucidate HDP function.

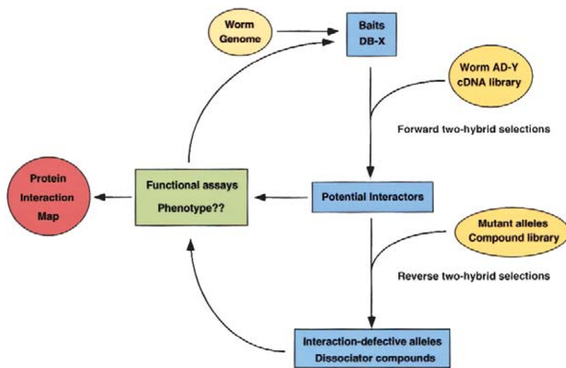
The Worm Available at the Click of a Mouse

Despite the power of genetics and cell biology available in model organisms such as the worm, the number of genes with a function assigned is still relatively small, compared with the invested efforts. The ~500 cloned *C. elegans* genes that correspond to genetically identified complementation groups represent ~4% of the expected 13,000 genes. Hence, it seems crucial to design complementary approaches to increase the speed of gene-function discovery. The current explosion of complete genome sequences should facilitate such global approaches (Lander 1996; Fields 1997).

With this in mind, several labs, including our own, are specializing in the generation of resources and functional information on a genomewide scale (a field that is often referred to as "functional genomics"). Supporting evidence for the necessity of such projects can be found in the genomic sequencing projects of several model organisms, which have taught us several lessons. First, it is very cost-efficient to centralize the efforts of repetitive experiments such as sequencing (Hodgkin et al. 1995). Since functional studies can also be somewhat repetitive (see below), the same principle should apply to functional genomics projects. Second, novel interesting and somewhat unexpected aspects of biology have emerged from genomewide projects. For example, the complete sequencing of the yeast genome has revealed that it derives from a duplication (Wolfe and Shields 1997). It is possible that new principles might similarly emerge from large-scale functional genomics analyses. Finally, standardized, or even automated, methods and computer tools that generate, analyze, or access sequence

The Two-Hybrid System: Forward and Reverse

The two-hybrid system makes use of the fact that regulatory transcription factors are generally composed of two separable domains: a sequence-specific DNA-binding domain (DB) and a transactivation domain (AD) (Keegan et al. 1986). Neither DB nor AD activates transcription unless they are held together (Triezenberg et al. 1988), so, when two proteins (X and Y) are expressed as hybrid proteins, one of which is fused to DB (DB-X) and the other of which is fused to AD (AD-Y), a physical interaction between X and Y can be identified through the reconstitution of a functional transcription factor (Fields and Song 1989). In "forward" two-hybrid screens, one selects *for* protein-protein interactions, using a positive selectable reporter gene which must be activated to allow cell growth under specific conditions. When applied to a large-scale project, such as the generation of a comprehensive protein-interaction map, high rates of false-positives, as observed in many conventional two-hybrid screens, can obscure genuine interactions. However, common causes of false positives have been eliminated by optimizing the assay in advance (see, e.g., Vidal and Legrain 1998).



Beyond the need to optimize the forward two-hybrid assay is the fact that the system inherently lacks direct connections with the biological processes for validation of potential interactions. One approach for validation consists in observing the effect of disrupting a potential interaction in vivo in the relevant model organism. To this end, we have developed a "reverse" two-hybrid system that can select for interaction-defective alleles (IDAs) and for *trans*-acting molecules that specifically *dissociate* protein-protein interactions (Vidal 1997). Here, the inducible reporter gene encodes a protein that is *toxic* under specific conditions, allowing *selection against* protein-protein interactions (Vidal et al. 1996*a, b*). Our current challenge is to examine whether IDAs and/or dissociating molecules will be useful to validate worm protein-protein interactions. For example, IDAs of WHD-1 might be compared to the wild type for their ability to rescue the phenotype(s) conferred by *whd-1*(*lf*). In addition, dissociating peptides or compounds could be tested for their ability to "phenocopy" the *whd-1*(*lf*) phenotype.

data might be applicable to large-scale projects aimed at the elucidation of gene function. Importantly, functional genomics projects in model organisms will make available both materials and information of great potential biological or clinical interest. Much of the information revealed by time-consuming experiments in the worm might soon be retrieved by no more than a click of a computer mouse.

The complete genome sequence of all six chromosomes in *C. elegans* will lead to ~13,000 ORFs, predicted by a program called Genefinder. It has been estimated that <3,000 of these ORFs are essential (Hodgkin et al. 1995). Although the genome sequence itself is very accurate (>99.9%), Genefinder correctly predicts the intron/exon junctions in ~50% of the cases. However, a large-scale project of EST sequencing is expected to lead to definitive answers on the genomic structure and expression of many predicted ORFs. For example, it is possible that computer-predicted ORFs might not be expressed in vivo and therefore might not represent genuine genes, whereas other sequences that could not be recognized by Genefinder might indeed be expressed. In addition, genes may have an intron/exon structure different than that predicted by the program. The EST information currently available can be accessed in ACeDB.

The yeast model system, with the first eukaryotic genome sequenced (Goffeau et al. 1996), already provides a great example for the development of functional genomics projects: (i) genomewide expression analysis using DNA arrays representing every single predicted yeast ORF is possible (Schena et al. 1995); (ii) a consortium is making progress in deleting every single predicted ORF (e.g., see Smith et al. 1996); and (iii) a protein-interaction map is underway (Evangelista et al. 1997; Fromont-Racine et al. 1997).

Similar projects are now underway in *C. elegans*. Projects to delete every single predicted ORF or aimed at the identification of every ORF essential for worm development and using RNAi are in the planning stage (A. Coulson, R. Plasterk, and A. Hyman, personal communication), and a *C. elegans* cDNA array, which should be useful for the creation of expression maps, is also under development (S. Kim, personal communication). Other projects are underway to determine in what cell and at what stage of development each ORF is expressed (e.g., see Tabara et al. 1996).

Because protein-protein interactions are important for most biological functions, we have initiated a large-scale project to generate a protein-interaction map for *C. elegans*. Protein-interaction maps are defined here as publicly available databases in which information on potential protein-protein interactions can be found. Like other functional genomics resources, protein-interaction maps could produce functional information available at the click of a mouse. Together with additional information such as cellular localization or time of expression, they would lead to the formulation of hypotheses that could be tested in the relevant biological system. The two-hybrid system has been proved useful for the identification of protein-protein interactions (for a recent

review, see Vidal and Legrain 1998). However, the system can also be complicated by high numbers of false positives and can be limited by the lack of direct connections with biology. As shown in the sidebar we are addressing these two issues by integrating "forward" and "reverse" versions of the two-hybrid strategy with classical genetics available in the nematode. A protein-interaction map for *C. elegans* should nicely complement the information generated by a yeast protein-interaction mapping project. Although valuable in itself—and, in some ways, more tractable—the yeast protein-interaction project cannot be expected to elucidate interactions among proteins that arose during animal evolution. Furthermore, the technical challenges posed both by the worm's larger genome and by the greater prevalence of introns in *C. elegans* genes compared with yeast genes make the worm project a closer model for some future endeavor, to map human protein interactions.

Looking for Drugs

Conventional drug-screening programs may lead to the identification of compounds that prove to be impractical as therapeutics, often because in vitro assays are inadequate to predict activity in a physiological setting. Some compounds score very well in terms of selectivity and sensitivity in vitro but are unstable or only poorly available to target tissues. Furthermore, screens performed in vitro can be costly and time-consuming. For these reasons, screens for compounds increasingly rely on the use of in vivo, cell-based assays.

It has been suggested that *C. elegans* could be a powerful system for in vivo drug-screening assays (Rand and Johnson 1995). For example, if either loss-of-function of *whd-1* or overexpression of HDP confers detectable phenotypes, libraries of compounds could be screened for molecules that partially rescue these phenotypes. Thereby, a "worm disease model" might assist in the development of therapeutic strategies and might represent a rather inexpensive and convenient way to screen hundreds of thousands of compounds. Moreover, unlike conventional in vitro screening programs, the resulting molecules would have been prescreened for their in vivo stability, ability to diffuse through a multicellular organism, and competence to penetrate cells. A drug that fulfills all of these criteria in worms should then be tested in a rodent or primate system before being tested on human patients.

A potential problem of nematode-based drug-screening systems is that the worm, with its relatively thick cuticle, is not sufficiently permeable to certain drugs. However, a few published test cases indicate that, at least at relatively high concentrations, some drugs will penetrate the animal. For example, a Ras farnesyltransferase inhibitor is capable of suppressing the multivulva phenotype caused by expression of *let-60*(*gf*), an activated *ras* allele that is analogous to those found in many human cancers (Hara and Han 1995). In other experiments, mutations conferring resistance and/or hypersensitivity to certain drugs have been identified, and the corresponding genes have been characterized. For example, the analysis of mutants resistant to a cholinesterase inhibitor has uncovered genes involved in synaptic vesicle function and in metabolism of acetylcholine (Miller et al. 1996). Other examples of drugs for which the approach has been tested include Prozac (Weinshenker et al. 1995), halothane, and other volatile anesthetics (van Swinderen et al. 1997). These studies support the potential use of the worm as a drug-screening tool.

Conclusion

In addition to currently available *in silico* techniques to reveal WHD-1 as a potential orthologue of an important HDP, functional data will eventually be available to gene cloners, for use in the prediction of the pathway in which WHD-1 acts in nematodes. They will also learn, with little effort, the cell types, the stage of development in which it is expressed, and the phenotype conferred by a *whd-1* loss-of-function mutation (fig. 1). Although this information will not reveal the precise function of HDP, it will surely provide a basis for the generation of hypotheses that can then be tested in the relevant human

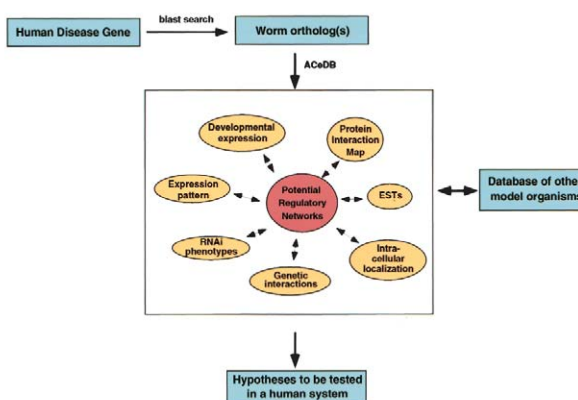


Figure 1 Anticipated *in silico* approaches, on finding a worm orthologue of *HDP*. A BLAST search with the translated HDP protein sequence will, in many cases, reveal at least one worm orthologue (*whd-1*). In an ideal worm functional genomic database, information will be readily available on several aspects of *whd-1*, such as its expression during development and in specific cell types, the phenotypes associated with loss of *whd-1* function, genetic interactions of *whd-1*, EST clones available, intracellular localization of the WHD-1 protein, and potential WHD-1 interacting proteins. In addition, this information can be compared with data available from other model organisms and should guide biologists in the forming of new and testable hypotheses on the molecular basis of a relevant human trait or disease.

biological systems. In addition, it will be interesting to see whether the worm model will be used widely to design novel therapeutic strategies.

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Electronic-Database Information

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank
Genome Sequencing Center, University of Washington School of Medicine, http://genome.wustl.edu/gsc/gschmpg.html
The Sanger Center, http://www.sanger.ac.uk
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