Turn to the worm!

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Caenorhabditis elegans will be the first multicellular animal to have its entire genome sequenced. This is not just good news for those currently working in the field, but also for those trying to understand the biology of more complex animals, including humans. *C. elegans* is a relatively simple animal that is amenable to studies of genetics and developmental processes that are common to all animals, making this an attractive model in which to study basic processes that are altered in human disease. Powerful forward and reverse genetics mean that virtually any gene of interest can be studied at the functional level.

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Current Opinion in Genetics & Development 1997, 7:410–415

http://biomednet.com/elecref/0959437X00700410

Abbreviations

BLAST	basic local alignment search tool
FGF	fibroblast growth factor
HLF	hepatic leukaemia factor
MDR	multidrug resistance
MRP	MDR-associated protein
PAR	proline- and acid-rich
TGF-β	transforming growth factor-β

Introduction

Human disease gene identification is increasing at a great rate. Many genes are now in hand but discovering the gene mutated in a disease is just the first step. What is its normal function? With what genes does it interact? What drugs might affect the biochemical pathway? Mouse models of human genetic disease are unbeatable at present for studying how diseases develop and for testing treatments but are not always best for studying basic gene functions. For such a study, the use of a simple animal model is ideal. The use of a simple animal model also allows one to identify new genes of related function and elucidate pathways within which such genes act; in this regard, *Caenorhabditis elegans* is exceptionally good [1••,2••,3].

The worm grows from an embryo to a 1 mm long adult in 3 days and is fully transparent at all stages of its life, allowing all cell divisions, migrations, and differentiation to be seen in live animals. Its anatomy is very simple but the 959 somatic cells of the adult represent most major differentiated tissue types, including muscles (111 cells), neurons (302 cells), intestine (34 cells), and epidermis (213 cells). In addition, it is the only animal for which the complete neuronal wiring pattern is known, having been reconstructed by serial section electron microscopy [4]. Maintenance in the laboratory is easy (they feed on *E. coli* on agar plates) and strains can be frozen for long-term storage. Both forward and reverse genetics are fast and at present there are mutants for ~2000 genetic loci. Producing transgenic lines is also easy and can be accomplished in a week, and the essentially complete physical map facilitates rapid gene cloning. Finally, *C. elegans* will be the first multicellular animal to have its entire genome sequenced: >65% of the 100 Mb genome is already finished and it is estimated that the project will be completed during 1998 [5].

These outstanding experimental features have attracted biologists from different scientific disciplines to the worm for detailed biological studies. Work in *C. elegans* has been important for understanding many conserved processes, such as axon guidance [6,7], olfaction [8–11], FGF signalling [12,13], and Ras signal transduction [14]. In many cases, progress made in *C. elegans* has been combined with results from research in other animals — such as *Drosophila* or vertebrate models — to build up a pathway of gene action. These successes, coupled with the large fraction of genes that are similar to those of more complex animals, are increasingly drawing researchers to the worm. In this review, I describe some recent studies in *C. elegans* that have relevance to human disease and discuss future prospects.

Over 50% of human gene sequences will have a significant match to a *C. elegans* gene

Table 1 shows the results of comparison of a set of 70 positionally cloned human disease genes (compiled by the authors of [15,16] and available on the World Wide Web at URL http://www.ncbi.nlm.nih.gov/Bassett/Yeast/) to 7299 predicted *C. elegans* genes (searching and retrieval of *C. elegans* sequence is available at the Sanger Centre and the Genome Sequencing Centre at Washington University; http://www.sanger.ac.uk/ and http://genome.wustl.edu/gsc/ respectively). 40% have at least one very strong match (probability $p<1.0\times10^{-45}$ according to BLASTp), and 65% at least a weak match ($p<1.0\times10^{-05}$), compared to 19% and 41%, respectively, in comparison with the complete yeast sequence ([15,16]; http://www.ncbi.nlm.nih.gov/Bassett/Yeast/). The best *C. elegans* match to a given human gene is shown. These assignments are not meant to indicate that the genes are orthologues — indeed in some cases the indicated *C. elegans* sequence has a better match to a different human gene — but to show that a large fraction of human disease genes will be significantly similar to *C. elegans* genes; most of these can be expected to have a similar biochemical activity. In this analysis, only half of the *C. elegans* genome was searched (7299 genes of 15 000 expected for the entire genome). One would anticipate that an equal number of new matches will be

found in the remaining sequence but many of these will be to human genes that already have a significant match. By extrapolation, >50% of human disease genes may be expected to have recognizable homologs in *C. elegans*.

Gene inhibition using antisense RNA

What can one do when one finds a *C. elegans* homolog of a human gene of interest? A useful first step is to inhibit the gene's function using antisense RNA [17]. Here,

Table 1

C. *elegans* matches to some positionally cloned human disease genes.

Human disease	Human gene (accession number)	*C. elegans* gene (accession number)	BLASTp prob. (#<1.0×10^{-45})	Description
Nevoid basal cell carcinoma syndrome	*PTC* (U59464)	*ZK675.1* (Q09614)	1.8×10^{-283} (2)	Patched membrane protein
Thomsen disease	*CLC1* (Z25884)	*E04F6.11* (Q19067)	1.1×10^{-215} (4)	Chloride channel
Glycerol kinase deficiency	*GK* (L13943)	*R11F4.1* (Q21944)	8.8×10^{-190} (1)	Glycerol kinase
Cystic fibrosis	*CFTR* (M28668)	*F57C12.4* (Q20943)	1.9×10^{-173} (5)	cAMP-dependent chloride channel
		mrp-2		
Adrenoleukodystrophy, X-linked	*ALD* (Z21876)	*C44B7.9* (Q18598)	8.5×10^{-163} (3)	Peroxismal transporter
Long QT syndrome, type 1	*KVLQT1* (U40990)	*C25B8.2* (Q18147)	6.6×10^{-135} (1)	Potassium channel
Diastrophic dysplasia	*DTD* (U14528)	*ZK287.2* (Q23454)	4.2×10^{-120} (3)	Sulfate permease
Ceroid lipopuscinosis, infantile	*INCL* (U44772)	*F44C4.5* (Q20390)	8.5×10^{-111} (1)	Palmitoyl-protein thioesterase
Neurofibromatosis, type 2	*NF2* (L11353)	*F42A10.2* (Q20307)	3.9×10^{-108} (1)	Ezrin/moesin/talin family
Aniridia	*PAX6* (M77844)	*F14F3.1* (Q19466)	4.0×10^{-108} (5)	Paired homeobox protein
		vab-3		
Pancreatic carcinoma	*DPC4* (U44378)	*R12B2.1* (P45897)	3.0×10^{-101} (3)	Putative TGF-β signal transducer
		sma-4		
Hyperekplexia	*GLRA2* (X52009)	*T21C12.1* (Q22637)	1.1×10^{-98} (18)	GABA receptor
Lissencephaly	*LIS1* (L13385)	*T03F6.F* (Z81113)	2.5×10^{-94} (3)	Platelet-activating factor acetylhydrolase
Fanconi syndrome, renal	*CLCN5* (X91906)	*R07B7.1* (Q21791)	1.3×10^{-88} (4)	Chloride channel
Bloom syndrome	*BLM* (U39817)	*K02F3.1* (P46064)	7.9×10^{-85} (2)	ReqQ DNA helicase
Werner syndrome	*WRN* (L76937)	*F18C5.2* (Q19546)	2.4×10^{-83} (3)	DNA helicase
Barth syndrome	*BTHS* (X92762)	*ZK809.2* (Q23598)	8.5×10^{-82} (1)	Novel protein
Marfan syndrome	*FBN1* (L13923)	*ZK783.1* (Q23587)	6.1×10^{-76} (5)	Fibrillin
Myotonic dystrophy	*DM* (L19268)	*R11G1.4* (Q21946)	2.2×10^{-72} (2)	Myotonin protein kinase
Aarskog–Scott syndrome	*FGD1* (U11690)	*C33D9.1* (Q18372)	6.7×10^{-64} (1)	Guanine nucleotide exchange factor
Hereditary multiple exostoses	*EXT1* (S79639)	*F12F6.4* (Q19369)	2.2×10^{-59} (1)	Novel protein
Achondroplasia	*FGFR3* (M58051)	*M79.1* (P03949)	2.8×10^{-58} (8)	Fibroblast growth factor receptor 3
Hypophosphatemic rickets, X-linked	*XLH* (U60475)	*ZK20.6* (Q23453)	6.7×10^{-58} (2)	Endopeptidase
Epidermolytic palmoplantar keratoderma	*KRT9* (X75015)	*T28C6.1* (Q22843)	1.6×10^{-57} (1)	Keratin
Multiple endocrine neoplasia 2a	*RET* (M57464)	*M79.1* (P03949)	2.7×10^{-57} (7)	RET tyrosine kinase
Chondrodysplasia punctata	*ARSE* (X83573)	*D1014.1* (Q18924)	1.8×10^{-55} (1)	Arylsulfatase
Amyotrophic lateral sclerosis	*SOD1* (K00065)	*F55H2.1* (P34461)	1.6×10^{-50} (1)	Superoxide dismutase
Hereditary non-polyposis colon cancer	*MSH2* (M84170)	*ZK1127.11* (Q23405)	3.8×10^{-49} (1)	MutS DNA repair protein
Gonadal dysgenesis	*SRY* (L08063)	*F40E10.2* (Q20201)	5.2×10^{-44}	HMG box protein
Duchenne muscular dystrophy	*DMD* (M18533)	*W04D2.1* (Q23158)	2.4×10^{-40}	Dystrophin
Ataxia telangiectasia	*ATM* (U26455)	*T06E4.3* (Q22258)	1.5×10^{-36}	PI3 kinase
Wilm's tumor	*WT1* (X51630)	*F54H5.4* (U80952)	5.8×10^{-35}	Zinc finger protein
Polycystic kidney disease, type 2	*PKD2* (U50928)	*ZK945.9* (Q09624)	1.9×10^{-34}	Novel membrane protein
Congenital adrenal hyperplasia	*CYP21* (M26856)	*F42A9.5* (Q27499)	1.1×10^{-33}	21-hydroxylase/cytochrome P450
Wiskott–Aldrich syndrome	*WASP* (U12707)	*C07G1.4* (Q17795)	6.8×10^{-33}	Proposed signal transduction adaptor
Adenomatous polyposis coli	*APC* (M74088)	*K04G2.8* (Q21227)	1.0×10^{-31}	Novel coiled-coil domain protein
Friedreich ataxia	*FRDA* (U43747)	*F59G1.5* (Q21055)	5.0×10^{-25}	PtdInsP 5-kinase
Waardenburg syndrome	*PAX3* (U02309)	*R08B4.2* (Q21836)	7.2×10^{-25}	Paired homeobox protein
Retinitis pigmentosa 3, X-linked	*RP3* (X97668)	*F07C3.4* (Q19149)	3.1×10^{-23}	Guanine nucleotide exchange factor
Neurofibromatosis, type 1	*NF1* (M89914)	*C07B5.1* (Q17774)	1.1×10^{-19}	GTPase activating protein
Lowe syndrome	*OCRL* (M88162)	*C50C3.7* (P34370)	1.3×10^{-16}	Inositol polyphosphate 5-phosphatase
Wilson disease	*WND* (U11700)	*W08D2.5* (Q27533)	7.5×10^{-15}	Copper transporter
Polycystic kidney disease, type 1	*PKD1* (L33243)	*ZK945.9* (Q09624)	9.0×10^{-15}	Polycystin/membrane glycoprotein
Chronic granulomatous disease	*NCF1* (M55067)	*C14F5.5* (P29355)	1.0×10^{-09}	Novel protein
Tuberous sclerosis 2	*TSC2* (X75621)	*T27F2.2* (Q22838)	1.4×10^{-07}	Tuberin/GTPase activating protein
Kallmann syndrome	*KAL* (M97252)	*ZK617.1A* (Q23550)	5.3×10^{-06}	Extracellular protein

A list of 70 positionally cloned human disease genes were obtained from http://www.ncbi.nlm.nih.gov/Bassett/Yeast/ and their sequences compared to a set of 7499 predicted *C. elegans* proteins (from http://www.sanger.ac.uk/ and http://genome.wustl.edu/gsc/) using BLASTp [42]. OMIM (Online Mendelian Inheritance in Man; http://www3.ncbi.nlm.nih.gov/Omim/) was used to find descriptions of the disease gene products. For each human disease gene, the highest scoring *C. elegans* protein is listed. *mrp-2*, *vab-3*, and *sma-4* are *C. elegans* genetic loci that correspond with the indicated predicted genes. Listed in the BLASTp probability column is the smallest sum probability score (p) and, in parenthesis, the number of *C. elegans* hits that had scores $<1.0\times10^{-45}$ (chosen as a reasonable measure of a strong match). The blank line divides scores below and above $p<1.0\times10^{-45}$. prob., probability.

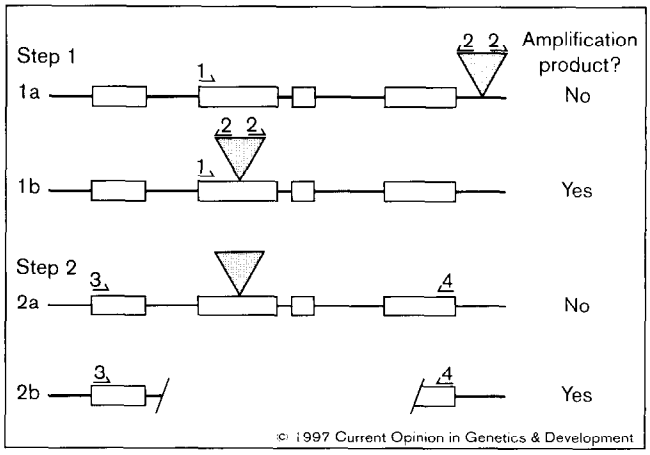
antisense RNA is injected into the syncitial germline of a wild-type adult hermaphrodite to inhibit gene function in her progeny. Although this was initially used for genes expressed in the embryo, it has become clear that the technique works generally; injection of antisense RNA has resulted in phenocopies of loss of function mutants in many genes, even ones that act late in development (C Mello, personal communication; P Kuwabara, personal communication). In practice, this means that one can easily examine the probable loss of function phenotype for any gene for which the sequence is available. This will be especially powerful for studying multigene families. Removing only one gene in a multigene family may cause no phenotype because of functional redundancy. When the *C. elegans* genome sequence is finished next year, it will be a simple matter to identify all members of any gene family by sequence searching. By injecting mixtures of antisense RNAs of members of such families, 'double' or 'multiple' mutants can be phenocopied and redundant functions examined.

Gene knockouts

Identifying a gene of interest is one thing but for many functional studies — and subsequent genetic analyses — a mutation is needed. How does one find a mutant of a gene of interest? There are two principal methods. First, the region of the genetic map can be screened for likely candidates; this is facilitated if one has carried out an antisense RNA injection and knows the phenotype expected of a mutant. Genomic DNA clones containing the gene can be tested for rescue of candidate mutants (see [18] for methods). If no mutant already exists, then one can be made using a two-step PCR-based method [19–21]. This involves first isolating a strain with a transposable element insertion in the gene of interest and then screening for excision of the element plus flanking DNA by PCR (Fig. 1). In a best-case scenario, a null mutation can be generated using this method in less than 2 months (R Plasterk, personal communication).

The gene knockout technique has been used to study many *C. elegans* genes identified by sequence. In a recent example, the functions of two classes of multidrug resistance genes (*MRP* and *MDR*) were studied [22,23•]. These genes are important medically, as multidrug resistance is a problem in cancer treatment. *C. elegans* genes of both classes were found to be involved in resistance to a variety of compounds [22,23•] and animals mutant for one gene of each class are hypersensitive to heavy metals. Additional work should lead to a better understanding of how these proteins work and possibly a way to avoid multidrug resistance in tumors. Furthermore, the MRP class is related in sequence to the cystic fibrosis gene *CFTR*, which encodes a cAMP-dependent chloride channel (reviewed in [24]). Though the *MRP* genes probably have a function different from CFTR, studying these related channel proteins could have implications for CFTR function.

Figure 1



Reverse genetic method for isolating a mutation in any *C. elegans* gene. Open boxes represent exons of a hypothetical gene; the grey triangle represents the transposable element *Tc1*; primers are indicated as half-arrows. Step 1: pooled samples of DNA from a frozen mutant bank are screened by PCR using primer 1 (to the gene) and primer 2 (to the inverted repeat ends of *Tc1*). PCR conditions are such that no product will be amplified (1a) unless a transposable element is near the gene-specific primer (1b). If a consistent band is amplified, it will point to an 'address' for the frozen tube of worms carrying the desired transposable element insertion. This is thawed and ~100 individual worms are tested to isolate the strain. As transposable elements are frequently spliced out of mRNAs, they often cause no phenotype [40••]. Step 2: to generate a deletion mutant, large cultures of the insertion mutant strain (1b) are grown and screened by PCR/sib selection using primer 3 and primer 4, gene-specific primers flanking the insertion site. Primers are chosen so that they will not amplify a fragment (2a) unless the transposable element plus some flanking DNA has been deleted (caused by improper repair after excision) bringing them near to each other (2b).

C. elegans and human disease genes

Two of the *C. elegans* homologs of human disease genes listed in Table 1 (*vab-3*/PAX-6, *sma-4*/DPC4) have been studied at the functional level, starting from forward genetic experimentation. In humans, mutation of the *PAX-6* gene (which encodes a paired box homeodomain protein) causes Aniridia, an autosomal dominant iris hypoplasia, which in the homozygous condition causes severe head defects and lack of eyes (reviewed in [25]). The gene *vab-3* is needed for proper development of the head and anterior sensory structures and was found to encode the *C. elegans* PAX-6 homolog [26]. In this striking case, the human and *C. elegans* genes both function in directing head and sensory structure development. Combined with the finding that the *Drosophila* eye development gene *eyeless* also encodes a PAX6 homolog [27], further study of these genes will give insights into eye evolution and development [28] as well as into Aniridia and perhaps other eye diseases.

The pancreatic carcinoma gene *DPC4* is similar to the sequence of *sma-4*, a member of a family of genes which includes *Drosophila* Mothers against decapenta-

plegic (Mad; reviewed in [29]). Work in *C. elegans*, *Drosophila*, and mammals suggests that these genes are involved in TGF-β signal transduction and the control of growth regulation [29,30]. Therefore, a mutation in *DPC4* might cause pancreatic cancer by an alteration in signalling by TGF-β or a related molecule. In both of these cases, it is very likely that further work in *C. elegans* will be directly relevant to understanding the functions of the human genes.

Genetic might

One of the most powerful forward genetic techniques is the use of enhancer and suppressor screens to quickly identify new genes that act within a pathway. Such a screen was carried out for genes affecting the *lin-12* signal transduction pathway; *lin-12* is a member of the Notch/*lin-12*/*glp-1* family of cell-fate-mediating receptors, which includes at least three human genes (see [31] for review). One group of suppressor mutations were in the gene *sel-12*, which was found to be homologous to presenilins PS1 and PS2 [32], two of the genes that can be mutated to cause early onset familial Alzheimer's disease [33]. In further work, human PS1 mutant proteins that cause Alzheimer's disease were expressed in *C. elegans* [34••]. It was found that wild-type human PS1 and PS2 proteins can substitute for *sel-12 in vivo* and that most of the mutant genes have reduced activity. Regions of the protein that were dispensable for function were also identified. Neither the role of presenilins in normal development nor in Alzheimer's disease are yet known but the *sel-12* results suggest that at least some function may be in signal transduction and provide a starting point for further progress.

Forward genetic studies in *C. elegans* can also contribute to the understanding of conserved developmental processes that are altered in disease. For example, *C. elegans* has been well exploited in the study of programmed cell death or apoptosis. Inhibition of the apoptotic pathway can be oncogenic [35], therefore this process has obvious implications for human cancer. In *C. elegans*, recent work has identified *ces-2* as the first known cell-type-specific cell death regulator [36••]. *ces-2* encodes a bZIP transcription factor of the PAR (proline- and acid-rich) type which includes the human gene *hepatic leukaemia factor* (*HLF*), an oncogenic form of which inhibits cell death in mammalian cells [37]. *ces-2* is thought to promote cell death by negatively regulating a survival gene. This suggests that the normal activity of *HLF* might be like that of *ces-2* but the oncogenic fusion protein is a positive rather than a negative regulator of a survival gene. Genetic screens have also identified the gene *ced-4*, which was recently found to produce two transcripts by alternative splicing. One *ced-4* product is death-promoting and the other is death-inhibiting [38•]; no mammalian *ced-4* homolog has yet been reported but the conservation of the cell death pathway suggests that it might be an important missing player.

Drug action and screening

If a drug has an obvious effect on *C. elegans*, then it should be possible to screen or select for mutants with altered sensitivity to the drug to identify genes involved in its effect. Successful screens of this type have been carried out for a range of compounds (see [39] for review and methods). In a recent study, mutants with resistance to a cholinesterase inhibitor were selected [40••]. A large number (165) of mutations were identified representing 21 genes. By using pharmacological and behavioural assays, the authors were able to separate the mutants into three groups which affected different steps of acetylcholine function: genes that are required generally for neurotransmitter release, those involved specifically in acetylcholine production, and one gene apparently needed for sensitive acetylcholine reception [41]. Some of these genes have been cloned; those in the first class encode genes involved in synaptic vesicle production or function, whereas those in the second class are involved in the production or transport of acetylcholine. These findings illustrate the power of drug screens, as genes at various steps in acetylcholine action were identified; cloning and study of these and the remaining genes will yield further insights into synaptic function and regulation. Working out the pathway within which a drug acts might also suggest potential alternative disease treatments, as a different step in a pathway could be targeted.

In principle, *C. elegans* could also be useful for therapeutic drug screening. For example, if the target of a particular drug is known—it could either be a *C. elegans* protein or a human protein expressed in *C. elegans*—and it has an assayable effect, then a large battery of drugs can be tested to look for sensitivity or resistance. This would give an indication whether the new drugs are more or less powerful than the original one and positive candidates could be tested further in a mammalian system. It remains to be seen how well this would work in practice but it is possible that *C. elegans* could be used as an inexpensive way to initially screen a large number of candidate drugs for those with the desired effect.

Conclusions

C. elegans is an advantageous system for studying the biochemical functions of genes identified in mammals. Their study is greatly simplified by the ease of experimental manipulation of *C. elegans* and the large body of knowledge of its development at the cellular level. Genetic or biochemical screens can often be designed to identify new genes involved in a pathway; subsequent gene cloning or knockout can be done quickly because of the complete physical map and fast reverse genetic methods. After finding new genes, one can then go back to seek and study human homologs. In the future, it will become increasingly commonplace to move back and forth between different organisms to take advantage of their specific features; for *C. elegans*, these are many.

Acknowledgements

I thank P Kuwabara and C Mello for sharing unpublished results and R Durbin for comments on the manuscript. J Ahringer is supported by a Wellcome Trust Fellowship (045515/Z/95/Z/PMG/AH).

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