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A role for *Caenorhabditis elegans* in understanding the function and interactions of human disease genes

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A growing number of medical research teams have begun to explore the experimental advantages of using a genetic animal model, the nematode worm *Caenorhabditis elegans*, with a view to enhancing our understanding of genes underlying human congenital disorders. In this study, we have compared sequences of positionally cloned human disease genes with the *C.elegans* database of predicted genes. Drawing on examples from spinal muscular atrophy, polycystic kidney disease, muscular dystrophy and Alzheimer's disease, we illustrate how data from *C.elegans* can yield new insights into the function and interactions of human disease genes.

INTRODUCTION

In the early 1960s, Sydney Brenner selected the nematode worm *Caenorhabditis elegans* as an experimental model with which to begin a comprehensive study of animal development and the underlying principles of nervous system function in a simple metazoan (1). *Caenorhabditis elegans* is a transparent, free-living, soil nematode 1 mm in length with a maximum diameter of ~80 μm (adult hermaphrodite dimensions). This animal model offers several experimental advantages. It is very easy to grow in the laboratory on agar medium in Petri dishes, where it can be maintained on a diet of *Escherichia coli*. It has a short life cycle (3.5 days at 20°C) and adult hermaphrodites give birth to a large number of progeny (>300) by self-fertilization. There are only 959 somatic cells in the adult hermaphrodite of *C.elegans*, 302 of which are neurons. The number of cells is invariant, which has enabled the establishment of its complete cell lineage (2) as well as the complete connectivity of the nervous system and nerve–muscle synaptic connections (3).

In spite of its apparently rudimentary organization, many of the distinct cell types associated with complex functions in mammals such as muscle cells, neurons, gut and excretory cells can be recognized and identified uniquely in *C.elegans*. Another advantage of this model is the availability of a detailed and precise genetic map, containing >2000 loci. The construction of a physical map has been achieved by first subcloning into cosmids and yeast artificial chromosomes (YACs) almost all the *C.elegans* genomic DNA. Subsequently, these genomic clones have been ordered with respect to one another (4) and aligned to the genetic map. The molecular analysis of *C.elegans* genes is thereby greatly facilitated. It is a relatively simple matter to select a small number of genomic clones for use in the rescue–transformation of mutant animals by microinjection (5). Applying such approaches in *C.elegans* has resulted in the genetic determination of the signalling pathway involved in programmed cell death (for a review see ref. 6) and the discovery of substrate molecules, such as netrin, involved in axonal guidance (7). These are major discoveries each with an impact well beyond the confines of nematode biology. By the end of the 1980s, the physical map was complete, enabling the launch of a project to sequence the entire *C.elegans* genome. In addition to its intrinsic value, this undertaking was also envisaged as a pilot study for the human genome project. However, information derived from the *C.elegans* genome is, of itself, beginning to make an impact on our understanding of human disease genes.

Once a human disease gene has been identified and its sequence variants characterized, this information can be utilized as a diagnostic tool. Nevertheless, details of the biological role(s) of the newly cloned gene are often either sparse or entirely lacking. Exploration of the new sequence using bioinformatics may reveal the presence of functional domains of interest and thus provide clues to the role of the gene product (8,9). The genomes of several prokaryotes and eukaryotes are now either fully sequenced or close to completion. The functional analysis of orthologous genes in various genetic models is therefore possible and can result in rapid developments in the understanding of human disease mechanism(s), perhaps even suggesting new approaches to therapy.

A strategy of this kind has been applied to uncover the function of the Friedreich ataxia disease gene product, frataxin. A close homologue of frataxin has been identified in yeast (10–12). Knockdown of the yeast frataxin gene results in cells displaying hypersensitivity to iron stress, pointing to a role in iron homeostasis; mitochondrial dysfunction is also observed in this mutant yeast (13). In addition, cell culture studies on fibroblasts derived from Friedreich ataxia patients are found to be hypersensitive to iron and oxidative stress. This work has provided the basis for an experimental disease treatment using antioxidative molecules such as idebenone (14).

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In December 1998, the completion of the *C.elegans* genome sequence (97 Mb) was reported (15). An earlier analysis, based on a fraction of the *C.elegans* genome, had already resulted in the prediction that for a large fraction of human disease genes an orthologue would be found in *C.elegans* (16,17). Finding *C.elegans* orthologues of human disease genes can offer rapid and unforeseen insights into the functions of the human gene. For example, it may be possible to uncover genes which, when mutated, could either enhance or suppress a particular biochemical pathway and, as such, their gene products may represent novel candidate drug targets. Also, by screening for and analysing mutants that either suppress or enhance already characterized mutations, functionally interacting gene products can be identified. Both strategies can be deployed to identify the different components of a novel signalling pathway. For example, the tyrosine kinase receptor–RAS signalling pathway is involved in *C.elegans* vulval development (for a review see ref. 18). The corresponding human signalling cascade is involved in cell proliferation processes in general, and research in this area is proving to be of considerable interest in understanding the control of cell proliferation.

Here we summarize how the *C.elegans* genome sequencing project and the experimental advantages of the nematode can assist in understanding some of the mechanisms underlying human diseases and in particular congenital disorders. First reviewed by Ahringer (16), the topic merits revisiting in the light of many new findings. We therefore provide an updated list of *C.elegans* genes which resemble human disease genes and also discuss some recent examples of the use of such *C.elegans* genes to explore biological function.

SIMILARITY SEARCH

We selected human disease gene sequences which have been positionally cloned. The sequences were selected by supplementing the compilation made by D.E. Bassett Jr and colleagues available on the website XREF (http://www.ncbi.nlm.nih.gov/XREFdb/) from the results of searching the OMIM database using the keywords: disease and positional cloning. We then compared the cloned human disease genes with a recent (18th) Wormpep version of the *C.elegans* genome (released November 1999) containing 18 576 proteins and 96 Mb. We used Wu BLAST and default parameters as set on the site at the Sanger Center with the filters in place (http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml). The results are summarized in Table 1.

There were no matches for 15 genes, whereas 100 genes did show a match to a *C.elegans* predicted gene with a *P* value of $<10^{-4}$. Of these 100 genes, 62 match with a *P* value of $<10^{-40}$ (62%) and 25 with a *P* value of $<10^{-10}$ (25%). We investigated whether or not each of the matching *C.elegans* genes constituted a genuine orthologue of the human disease gene. This was done by using the nematode candidate gene to search a human gene database using advanced BLAST at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/). We found that in 48 cases, the human disease gene is the closest human gene to the *C.elegans* gene. Based on this survey, we conclude that ~42% of human disease genes have an orthologue in *C.elegans*, a finding very close to the value of 50% predicted by Ahringer (16) and comparable to the value of 44% obtained with a random collection of human cDNA (19). Clearly the approach outlined has limitations, being insensitive to the ordering of domains. However, it does make the case that there are many putative *C.elegans* genes that could be explored in the context of contributing to an understanding of human diseases.

Early-onset Parkinson's disease (Parkinson disease juvenile 2), which results in neural degeneration, is an autosomal recessive disease caused by mutations in the protein parkin. The function of parkin, however, remains to be determined. As shown in Table 1, there is a *C.elegans* orthologue of parkin, which has been found in the Wormpep database under the open reading frame number K08E3.7. The percentage identity and similarity are 26 and 53%, respectively. An alignment is shown in Figure 1 and a detailed analysis of the human parkin gene and its *C.elegans* orthologue are proving to be instructive (see also ref. 20). The human gene shows a conserved N-terminal domain that is a member of the ubiquitin family of proteins (21). We can see from Figure 1 that this domain is also well conserved between the human parkin protein and the *C.elegans* orthologue (60% similarity). On the other hand, at the C-terminus, the motif similar to a RING finger motif is completely conserved between the *C.elegans* and human genes. However, point mutations, which give rise to a non-functional protein, have been discovered outside this conserved C-terminal domain (22,23). It is thought that the five mutated amino acids must all be important for the function of parkin, as three of them are found in conserved or semi-conserved positions when the human and *C.elegans* parkin genes are compared. These findings suggest that the important domains are not restricted to the N-terminal part of the protein. Interestingly, the Trp453stop mutation, which lies at the extreme C-terminus, also results in the disease (22).

Several of the orthologues of human disease genes listed in Table 1 currently are being studied in *C.elegans* laboratories and have the potential for yielding new insights into human disease mechanisms. There are also opportunities to investigate orthologues of genes not yet implicated in any disease but known to be triplicated in humans. It is the triplication of genes (trisomy) on human chromosome 21 that results in Down's syndrome.

In the remainder of this review, we highlight recent work on *C.elegans* that is contributing to our understanding of human disease genes underlying spinal muscular atrophy, polycystic kidney disease and muscular dystrophy.

SPINAL MUSCULAR ATROPHY

Spinal muscular atrophy (SMA) is a common human autosomal recessive disorder resulting in muscle weakness and wasting. With an affected frequency of 1 in 10 000 births, it is among the most common of the genetic neurological diseases. It is characterized by the loss of lower motor neurons in early stages of development. The aetiology of this genetic disorder is not fully understood (for a review see ref. 24). However, mutations in the survival motor neuron (SMN) gene, which lies on a complex duplicated region of human chromosome 5q13, are responsible for this disease. The SMN protein is ubiquitously expressed and seems to be involved in RNA processing and metabolism (25). The specific effect on motor neuron degeneration therefore remains to be explained. An orthologue of the human SMN gene has been identified on chromosome 1 of *C.elegans* (CeSMN) (26) and a full-length

Table 1. *Caenorhabditis elegans* homologues of positionally cloned genes mutated in human disease

Human disease (OMIM accession no.)	Human gene (GenBank accession no.)	*C.elegans* ORF	BLASTp (*P* value)
Aarskog–Scott syndrome (305400)	FGD1 (U11690)	C33D9.1	3.2×10^{-56}
Achondroplasia (100800)	FGFR3 (M58051)	F58A3.2	1.7×10^{-89}
Adenomatous polyposis coli (175100)	APC (M74088)	K04G2.8A	3.0×10^{-33}
Adrenoleukodystrophy, X-linked (300100)	ALD (Z21876)	T02D1.5	1.5×10^{-196}
Alzheimer disease, type 3 (104311)	PS1 (L76517)	F35H12.3[a]	2.4×10^{-79}
Alzheimer disease, type 4 (600759)	PS2 (L44577)	F35H12.3	1.4×10^{-81}
Amyotrophic lateral sclerosis (105400)	SOD1 (K00065)	C15F1.7	3.2×10^{-45}
Aniridia (106210)	PAX6 (M77844)	F14F3.1	4.6×10^{-89}
Ataxia telangiectasia (208900)	ATM (U26455)	B0261.2	7.2×10^{-42}
Autoimmune polyglandular syndrome (240300)	AIRE (O43918)	F26F12.7	2.2×10^{-09}
Barth syndrome (302060)	BTHS (X92762)	ZK809.2	4.4×10^{-54}
Benign familial neonatal convulsions (121200)	KCNQ2 (AF033348)	C25B8.1	2.3×10^{-102}
Bloom syndrome (210900)	BLM (U39817)	T04A11.6	4.4×10^{-116}
Bor syndrome (600257)	EYA1 (Q99502)	C49A1.4	4.2×10^{-17}
Breast and ovarian cancer, early onset (113705)	BRCA1 (U14680)	C36A4.8	7.5×10^{-11}
Bruton agammaglobulinaemia (300300)	BTK (U78027)	M79.1C	3.6×10^{-82}
Carnitine deficiency, primary (212140)	OCTN2 (AB016625)	F52F12.1	4.8×10^{-74}
Ceroid lipofuscinosis, infantile neuronal (256730)	INCL (U44772)	F44C4.5	1.1×10^{-81}
Chediak–Higashi syndrome (214500)	CHS (U67615)	VT23B5.2	3.3×10^{-100}
Chondrodysplasia punctata (302950)	ARSE (X83573)	D1014.1	1.6×10^{-46}
Choroideraemia (303100)	CHM (X78121)	Y57G11C.1	5.2×10^{-42}
Chronic granulomatous disease (306400)	NCF1 (M55067)	Y116A8C.36	3.7×10^{-09}
Citrullinaemia, adult onset type II (603471)	SLC25A13 (AF118838)	K02F3.2	4.8×10^{-177}
Coffin–Lowry syndrome (303600)	RSK2 (P51812)	T01H8.1A	1.1×10^{-223}
Congenital adrenal hyperplasia (201910)	CYP21 (M26856)	F44C8.1	5.9×10^{-37}
Congenital nephrotic syndrome 1 (256300)	NPHS1 (AF035835)	C26G2.1	1.5×10^{-61}
Cyclic haematopoiesis (162800)	ELA2 (P08246)	C07G1.1	3.9×10^{-13}
Cystic fibrosis (219700)	CFTR (M28668)	F21G4.2	1.4×10^{-135}
Darier disease (124200)	ATPLA2 (P16615)	K11D9.2	0.0
Deafness 3, conductive (304400)	POU3F4 (X82324)	K02B12.1	1.1×10^{-60}
Deafness, autosomal recessive 9 (601071)	OTOF (AF107403)	F43G9.6	1.3×10^{-72}
Deafness, neurosensory, autosomal recessive 3 (600316)	MYO15 (AF053130)	T10H10.1	3.1×10^{-200}
Deficiency of coagulation factors V/VIII (227300)	ERGIC-53 (P49257)	K07A1.8	7.5×10^{-85}
Dent disease (300009)	CLCN5 (X91906)	C07H4.2	2.1×10^{-169}
Diastrophic dysplasia (222600)	DTD (U14528)	K12G11.2	1.8×10^{-76}
Duchenne muscular dystrophy (310200)	DMD (M18533)	F38B4.3	1.2×10^{-145}
Duncan disease (308240)	SH2D1A (O60880)	M79.1C	1.3×10^{-09}
Dyskeratosis congenita, X-linked (305000)	DKC1 (O60832)	K01G5.5	1.6×10^{-146}
Epidermolytic palmoplantar keratoderma (144200)	KRT9 (X75015)	W10G6.3	9.1×10^{-23}
Fragile histidine triad (601153)	FHIT (U46922)	Y56A3A.13	7.8×10^{-28}
Fragile site mental retardation, type 2 (309548)	FMR2 (U48436)	F35A5.1	1.6×10^{-05}
Friedreich ataxia (229300)	FRDA (U43747)	F59G1.7	3.5×10^{-23}
Glaucome primary open angle (137750)	GLC1A (Z97171)	C48E7.4	2.9×10^{-24}
Glycerol kinase deficiency (307030)	GK (L13943)	R11F4.1	3.6×10^{-133}
Gonadal dysgenesis (306100)	SRY (L08063)	K08A8.2	2.4×10^{-31}

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Table 1. *Continued*

Human disease (OMIM accession no.)	Human gene (GenBank accession no.)	*C.elegans* ORF	BLASTp (*P* value)
Groenouw granular dystrophy, type 1 (122200)	BIGH3 (M77349)	F26E4.7	3.0×10^{-09}
Hereditary megaloblastic anaemia (261100)	CUBN (AF034611)	ZC116.3	2.0×10^{-225}
Hereditary multiple exostoses (133700)	EXT1 (U70539)	F12F6.3	5.2×10^{-90}
Hereditary non-polyposis colon cancer (120436)	MLH1 (U07418)	T28A8.7	2.5×10^{-107}
Hereditary non-polyposis colon cancer (120436)	MSH2 (U03911)	H26D21.2	1.1×10^{-99}
Hereditary pancreatitis (276000)	TRYP1 (U70137)	C07G1.1	7.6×10^{-05}
Hermansky–Pudlak syndrome (203300)	HPS (U65676)	F53H8.1	2.7×10^{-133}
Holt–Oram syndrome (142900)	TBX5 (Y09445)	F21H11.3	1.2×10^{-61}
Hyperekplexia (149400)	GLRA2 (X52009)	B0207.12	2.7×10^{-79}
Hypophosphataemic rickets, X-linked (307800)	XLH (U60475)	F18A12.8	2.6×10^{-91}
Kallmann syndrome (308700)	KAL (M97252)	K03D.10.1	7.1×10^{-34}
Lissencephaly (247200)	LIS1 (L13385)	T03F6.5[b]	9.5×10^{-127}
Long QT syndrome, type 1 (192500)	KVLQT1 (U40990)	Y54G9A.3	1.6×10^{-133}
Lowe syndrome (309000)	OCRL (M88162)	C16C2.3	2.4×10^{-57}
Marfan syndrome (154700)	FBN1 (L13923)	ZK783.1	4.6×10^{-121}
Maturity onset diabetes of the young (600496)	TCF1 (X59869)	W03D8.4	4.5×10^{-23}
Menkes syndrome (309400)	MNK (X69208)	Y76A2A.2[c]	1.9×10^{-38}
Miyoshi myopathy (254130)	dysferlin (AF075575)	F43G9.6[d]	1.4×10^{-82}
Mohr–Tranebjaerg syndrome (304700)	DDP (U66035)	Y39A3CR.E	2.5×10^{-13}
Multiple endocrine neoplasia 2A (171400)	RET (M57464)	F58A3.2	2.4×10^{-68}
Myotonic dystrophy (160900)	DM (L19268)	K08B12.5	4.8×10^{-121}
Myotubular myopathy (310400)	MTM1 (Q13496)	Y110A7A.5[e]	2.6×10^{-130}
Neurofibromatosis, type 1 (162200)	NF1 (M89914)	Z879.8	5.1×10^{-18}
Neurofibromatosis, type 2 (101000)	NF2 (L11353)	C01G8.5A	3.4×10^{-112}
Nevoid basal cell carcinoma syndrome (109400)	PTC (U59464)	ZK675.1	8.2×10^{-169}
Niemann–Pick C1 (257220)	NPC1 (NP000262)	F02E8.6[f]	1.6×10^{-133}
Nigmegen breakage syndrome (251260)	nibrin (AF051334)	B0041.7	1.3×10^{-05}
Non-syndromic deafness DFNA1 (124900)	DIAPH1 (O60610)	F11H8.2	1.1×10^{-42}
Opitz syndrome (300000)	MID1 (Y13667)	ZK1320.6	1.9×10^{-10}
Pallister–Hall syndrome (146510)	GLI3 (M57609)	Y47D3A.7	1.3×10^{-54}
Pancreatic carcinoma (260350)	DPC4 (U4437)	R12B2.1	7.7×10^{-77}
Parkinson disease juvenile 2 (600116)	parkin (AB009973)	K08E3.7	6.1×10^{-42}
Pendred symptom (274600)	PDS (O43511)	K12G11.2	2.1×10^{-68}
Polycystic kidney disease, type 1 (173900)	PKD1 (L33243)	ZK945	2.8×10^{-06}
Polycystic kidney disease, type 2 (173910)	PKD2 (U50928)	Y73F8A	2.5×10^{-54}
Retinitis pigmentosa 2, X-linked (312600)	RP2 (AJ007590)	C54G6.2	8.8×10^{-06}
Retinitis pigmentosa 3, X-linked (312610)	RP3 (X97668)	F07C3.4	2.0×10^{-17}
Retinoblastoma (180200)	RB1 (M15400)	C32F10.2	1.3×10^{-10}
Retinoschisis X-linked juvenile	XLRS1 (AF014459)	C25F6.4	1.1×10^{-11}
Rieger syndrome (180500)	RIEG (U69961)	B0564.10	1.3×10^{-25}
Simpson–Golabi–Behmel syndrome (312870)	GPC3 (L47125)	F59D12.4	2.7×10^{-22}
Spinal muscular atrophy (253300)	SMN (U18423)	C41G7.1	3.0×10^{-08}
Spinocerebellar ataxia 1 (164400)	SCA1 (X79204)	K04F10.1	2.6×10^{-20}
Spinocerebellar ataxia 2 (183090)	SCA2 (U70323)	D2045.1	1.8×10^{-13}
Stargardt disease (248200)	ABCR (U88667)	Y39D8C.1	8.3×10^{-168}

Continued opposite

Table 1. *Continued*

Human disease (OMIM accession no.)	Human gene (GenBank accession no.)	*C.elegans* ORF	BLASTp (*P* value)
Tangier disease (205400)	ABC1 (AF165306)	Y39D8C.1	3.6×10^{-180}
Thiamine-responsive megaloblastic anaemia (249270)	SLC19A2 (AJ238413)	F37B4.7	3.1×10^{-63}
Thomsen disease (160800)	CLC1 (Z25884)	C07H4.2	2.1×10^{-169}
Treacher–Collins syndrome (154500)	TCOF1 (U40847)	K06A9.1	1.6×10^{-07}
Tuberous sclerosis (191090)	TSC2 (X75621)	T27F2.2	3.1×10^{-13}
Waardenburg syndrome (193500)	PAX3 (U02309)	R08B4.2	1.0×10^{-23}
Werner syndrome (277700)	WRN (L76937)	F18C5.2[g]	1.6×10^{-72}
Wilms tumour (194070)	WT1 (X51630)	F53F8.1	2.9×10^{-27}
Wilson disease (277900)	WND (U11700)	Y76A2A.2	1.6×10^{-244}
Wiskott–Aldrich syndrome (301000)	WASP (U12707)	C07G1.4	1.0×10^{-11}

The names of diseases are given in alphabetical order, and in each case the OMIM database accession number is given in parentheses. In the second column, the abbreviation of the human protein is given together with its GenBank accession number. The closest *C.elegans* ORF (column 3) has been chosen based on the *P* value (column 4) and length of alignment. No BLAST hit has been obtained for genes responsible for the following human diseases: breast cancer, early onset (600185); congenital adrenal hypoplasia (300200); deafness (220290); Emery–Dreifuss muscular dystrophy (310300); fragile X syndrome (309550); hereditary haemochromatosis (235200); hereditary multi-infarct dementia (CADASIL) (125310); Huntington disease (143100); McLeod syndrome (314850); Norrie disease (310600); obesity (164160); ocular albinism (300500); Von Hippel–Lindau disease (193300); and progressive myoclonous epilepsy (254800). There are no BLAST hits for Mediterranean fever familiar (249100), although homologues with a different domain architecture can be detected.

[a]The *C.elegans* genes *hop-1* (50) and *spe-4* (51) are additional candidate orthologues of human presenilin genes.

[b]The *C.elegans* locus has been named *lis-1*.

[c]A *C.elegans* full-length cDNA has been cloned and is homologous to the Menkes and Wilson disease genes (52).

[d]This cosmid contains the *Fer-1* gene.

[e]Two other human MTM1 homologues have been characterized and named CeMTMH1 (located on cosmid F24A11 with a *P* value of 2×10^{-53}) and CeMTMH2 (located on cosmid F53A2) (53).

[f]This *C.elegans* locus has been called *npc-1*, and two other homologues have been found on cosmids F09G8 (*npc-2*) and C32E8.

[g]There are four members of the RecQ family in *C.elegans*.

cDNA has been isolated (27). A CeSMN gene–green fluorescent protein (GFP) fusion construct is expressed in the nuclei of neurons (Fig. 2), body wall and vulval muscle cells, hypodermal cells, gut cells and the excretory canal cell (27). GFP expression is also observed in the gonad (27). The endogenous CeSMN protein has been localized using a polyclonal antibody. The staining in embryos shows that CeSMN is mainly nuclear, is present at very early developmental stages and remains detectable in all blastomeres throughout embryonic development (27).

The knockdown of the CeSMN gene using the double-stranded RNA interference (dsRNAi) technique indicates a crucial role for CeSMN in embryonic viability, as its knockdown leads to a significant decrease in the progeny of injected worms. Surviving worms are uncoordinated, amorphous, lack muscle tonicity, have a protruding vulva and are sterile (27). These results suggest that SMN plays important roles both in locomotion and in maturation of the germ cell line. Results from yeast two-hybrid experiments suggest similar protein interaction properties to those of human SMN. These results demonstrate an evolutionary conservation of function of this important protein. Moreover, a careful inspection of the genetic loci adjacent on the physical map to the CeSMN gene has identified a good mutant candidate for the CeSMN gene, *egl-32*. This mutant shows a short genomic deletion in the promoter sequence of the CeSMN gene. The mutant phenotype has moderate bloating, is egg-laying defective, has abnormal germ lines and is partially temperature sensitive. It is of interest that *egl-32* displays part of the phenotype seen in CeSMN RNAi knockdown animals (27). Also, when mutated, genes *daf-3* and *daf-5* can suppress the *egl-32* mutant (28). The gene *daf-3* encodes a transcription factor that is epistatic to *daf-12*, a gene involved in regulation of stage-specific developmental programmes (29). However, further investigation will be required to establish whether or not *egl-32* is the structural gene for CeSMN (including attempted rescue transformation of the *egl-32* mutant using CeSMN).

Thus, *C.elegans* provides a good model organism with which to investigate the orthologue of the human SMN gene at the genetic, molecular and cellular levels. The down-regulation of the *C.elegans* orthologue is particularly informative in that it is not absolute (in contrast to the embryonic lethal knockout mouse), thereby enabling observations on differently affected individuals.

POLYCYSTIC KIDNEY DISEASE

Polycystic kidney disease is an autosomal, dominant disorder affecting 1 in 100 live births and is the most common single-gene disorder leading to kidney failure. Mutations in two proteins of unknown function, polycystin-1 (PKD1) and polycystin-2 (PKD2), result in clinical symptoms (30). Sequence analysis suggests the possibility of a role for these

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K08E3.7 MSDEISILIQDRKTGQRRNLTLNINITGNIEDLTKDVEKLTEIPSDELEVVFCGKKLSKSTIMRDLSL 68
        :     ...   . .. ... ....   .: .: . : :   .:.:.:.:.: ::.: ..  ... .:
Parkin  M----IVFV---RFNSSHGFPVEVDSDTSIFQLKEVVAKRQGVPADQLRVIFAGKELRNDWTVQNCDL 61

K08E3.7 TPATQIMLL-RP--KFNSHNENGATTAK---------------------------------ITTDS-- 98
           . . .. ::  : .  : .:.   .                                  . :::
Parkin  DQQSIVHIVQRPWRKGQEMNATGGDDPRNAAGGCEREPQSLTRVDLSSSVLPGDSVGLAVILHTDSRK 129

K08E3.7 -----------SILGSFYVWCKN-CDDVKRGKLRVYCQKCSSTSVLVKSEPQNWSDVLKSKRIPAVCE 154
                  :: .::::.::. :. :. ::::: :. : .... . . :. :.:::  .:. . :.
Parkin  DSPPAGSPAGRSIYNSFYVYCKGPCQRVQPGKLRVQCSTCRQATLTLTQGPSCWDDVLIPNRMSGECQ 197
                                    K161N

K08E3.7 ECCTPGLFAEFKFKCLAC----NDPAAALTHVRGNWQMTECCVCDGKEKVIFDLGCN--HITCQFCFR 216
            ::  ::: ::: :     ..  .::  .  : .   : .:   .. .. . ::  :. :  ::.
Parkin  SPHCPGTSAEFFFKCGAHPTSDKETPVALHLIATNSRNITCITCTDVRSPVLVFQCNSRHVICLDCFH 265
                                              T240R          R256C

K08E3.7 VR------------------------VVQDVHHFHIMGQTSYSEYQRKATERLIAVDDKGVTCPNVSC 260
        .                        .....:::.:.:. .:..::. ..:. . ..  :: ::  .:
Parkin  LYCVTRLNDRQFVHDPQLGYSLCPNSLIKELHHFRILGEEQYNRYQQYGAEECV-LQMGGVLCPRPGC 332
              R275W

K08E3.7 GQSFFWEPYDDDGRSQCPD-----CFFSFCRKC----FERNCVCQSEDDLTRT--------------- 304
        : ... ::  :. .  :       : :.:::.:     : .:    : . : :
Parkin  GAGLLPEP--DQRKVTCEGGNGLGCGFAFCRECKEAYHEGECSAVFEASGTTTQAYRVDERAAEQARW 398

K08E3.7 ------TIDATTRRCPKCHVATERNGGCAHIHCTS--CGMDWCFKCKTEWKEECQWDHWFN- 357
              ::  ::. ::.::: .:.:::: :..: .  : ..::..:  ::.. :. ::::.
Parkin  EAASKETIKKTTKPCPRCHVPVEKNGGCMHMKCPQPQCRLEWCWNCGCEWNRVCMGDHWFDV 460
              T415D *  *         * *  *   *    *  *  W453stop
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Figure 1. Alignment of the amino acid sequences derived from the human parkin gene (H. parkin) and its *C.elegans* orthologue (ORF K08E3.7). The sequence shown in italics is the ubiquitin-like domain (60% similarity). The conserved region surrounding the Lys48 residue (numbering based on the human sequence and shown in bold on both sequences) plays a role in the multi-ubiquitin chain formation. The five boxed amino acids denote mutations in human parkin that affect amino acids critical to the function of the protein, and below are indicated the amino acid substitutions. Two of these mutations involve amino acids that are conserved in *C.elegans*; another involves a semi-conserved amino acid. Residues forming the RING finger-like motif are all conserved in *C.elegans* and are indicated by the asterisks.

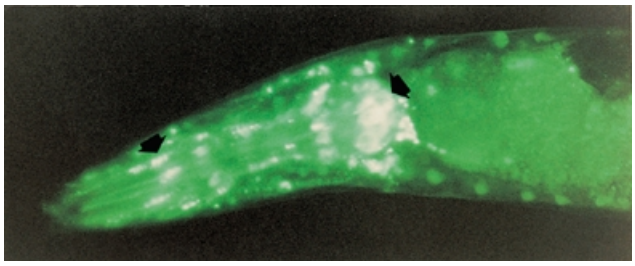

Figure 2. Expression of CeSMN–GFP in head neurons of *Caenorhabditis elegans*. The upstream region and the coding sequence of the CeSMN gene were fused to GFP and this CeSMN–GFP construct was microinjected into hermaphrodite adults to generate transgenic animals. This fluorescence photomicrograph shows the expression of GFP (white) in the anterior part of the nematode in certain neurons of the head ganglia (black arrows).

proteins in ion channel function (30). The *C.elegans lov-1* gene has been identified in a genetic screen for males defective in mating behaviour. The *lov-1* gene is in fact the orthologue of the gene encoding human PKD1 (31), although it should be noted that it shares only two of three conserved domains with the human gene (32). Moreover, a careful inspection of the *C.elegans* genome has led to the discovery of a close homologue of the human PKD2 gene (26) (Table 1), which is thought to be either functionally associated with *lov-1*, or at least located in the same signalling cascade. The authors have co-localized both gene products in a subset of male-specific tail neurons, suggesting that the two products interact with each other, but it appears that the genes *lov-1* and the *C.elegans* PKD2-like gene are not expressed in the excretory canal of the male. Undoubtedly, the signalling pathway responsible for male mating behaviour will be explored further using suppressor and/or enhancer screens to find new interacting protein partners. A pharmacological screen might also be feasible, since the *lov-1* mutant could serve as a screen for drugs which either enhance or suppress the mutant phenotype. A combination of these two approaches could discriminate rapidly between the functional roles of these two genes.

MUSCULAR DYSTROPHY

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are allelic progressive myopathies that affect ~1 in 3500 male births (33). Dystrophin is a 3685 amino acid protein found in skeletal muscle, cardiac muscle and the nervous system, and is the product of the gene mutated in DMD and BMD. The aetiology of the diseases and details of the function of dystrophin remain to be resolved.

Loss of function of a *C.elegans* dystrophin-like gene (*dys-1*) results in hyperactive worms that are slightly contracted (34). Reporter gene analysis and heterologous promoter expression point to a site of action for *dys-1* (cx40) in *C.elegans* muscle cells. The pharyngeal muscles of *dys-1* mutants are hypersensitive to acetylcholine and to the acetylcholinesterase (EC 3.1.1.7 AChE) inhibitor aldicarb (34). Studies of the nematode

have identified many genes involved in cholinergic synaptic transmission (35,36). For example, in *C.elegans* there are ~30 nicotinic acetylcholine receptor subunit genes currently known (37,38) and there are four acetylcholinesterase genes (39). Recently, Giugia *et al.* (40) have found a significantly reduced level of AChE activity (the products of the four *ace* genes) in the *dys-1* mutant. It will be of interest to know which of the four AChE genes are down-regulated. It may be that other genes expressed at cholinergic synapses are also affected. Thus, studies on *C.elegans* have provided direct evidence for a link between dystrophin function and cholinergic synaptic transmission. This genetic model is also being used to explore the interactions of the *C.elegans dys-1* gene product with other candidate members of the dystrophin complex, such as dystrobrevin and syntrophin (41).

USE OF *C.ELEGANS* AS AN ANIMAL MODEL TO INVESTIGATE HUMAN DISEASE GENES

Some *C.elegans* mutants may mimic morphological and/or functional defects, which are encountered in some aspects of human disease. Recently, several classes of *C.elegans* mutants defective in the morphology and function of the excretory system have been isolated by Buechner *et al*. (42) and may represent a new animal model with which to investigate the aetiology of human polycystic kidney disease. The *C.elegans* excretory system is made up of an excretory cell and accessory cells. The excretory cell undergoes cytoplasmic extension of two arms. Buechner *et al.* (42) have isolated 23 mutants and classified them into 10 complementation groups, each displaying different defects, ranging from a few focal cysts distributed anywhere along the lateral canals to a canal highly enlarged all along its entire length and a canal split into multiple convoluted channels, with or without disruption of its exoskeleton. One of the mutants prevents nearly all canal outgrowth. Extending this investigation may uncover novel morphoregulatory genes and potential new drug targets.

Another approach, using *C.elegans* to understand the mechanism of human disease, has been to engineer worm models of some human disorders by microinjection of ectopic genes. One such example relates to a gene linked to Alzheimer's disease, which claims >100 000 lives a year in the USA alone. Symptoms include dementia and memory loss. The genetic defect in the early-onset form of the disease is an altered β amyloid precursor protein. Transgenic *C.elegans* have been generated to express the human β amyloid peptide (43). The peptide is secreted specifically from body wall muscle cells. A characteristic peptide deposit is therefore observed at the surface of the muscles. Moreover, animals with such deposits show some phenotypic defect such as paralysis and vacuole formation linking the deposit to putative alteration of body wall muscle cell physiology. This animal model has been studied further to examine *in vivo* the effects of point mutations in the amyloid peptide β-sheet regions on the formation of amyloid deposits (44). Results confirm a previous study emphasizing the importance of Leu17 and Ala30. Moreover, the transition Met35Cys abolishes deposits and worms no longer show paralysis and toxic effects. Since the deposit is visible in 3-day-old animals, it is evident that such an animal model could be used in drug screening for the removal of amyloid deposit.

Polyglutamine-mediated gene dysfunction has been examined by Faber *et al*. (45) using *C.elegans*. The expansion of the CAG repeat [encoding the poly(Q) tract] is involved in eight human neurodegenerative disorders, and the mechanism underlying such diseases is not understood. However, in the case of one of these diseases, Huntington's chorea, there have been major advances. The CAG repeat is in the gene encoding the protein huntingtin and it confers a new deleterious property (toxic gain of function) without interfering with the gene's normal function. The N-terminal part of the human huntingtin gene fused to different lengths of CAG repeats has been expressed in *C.elegans* under the control of the *osm-10* gene promoter. The transgene is therefore expressed in eight sensory neurons, some of which are involved in the animal's response to nose touch. The authors have concentrated on a particular cell type, the paired ASH neurons. These neurons have their sensory processes in contact with the animal's outer surface and can therefore be visualized by dye filling. The effect of this ectopic expression in ASH neurons has therefore been addressed by means of a dye-filling test, GFP reporter gene expression and a nematode behavioural test. A mild ASH dye-filling defect is observed when the transgene is expressed alone. When the transgene is co-expressed with a subthreshold level of OSM-10 protein fused to GFP, the severity of the toxicity is clearly enhanced, as ASH cell death is observed. Moreover, this effect is dependent on the poly(Q) tract length; the higher the number of CAG repeats, the more pronounced is the number of transgenic worms showing ASH cell death. The mechanism is progressive since cell death occurs 8 days after the worm is adult. Symptoms of cell degeneration are tractable by monitoring worm chemosensitivity, which depends directly on ASH functionality, and dye-filling capacity. Transgenic worms become touch-defective before dye filling and cell death appear. On the other hand, aggregation of the expanded huntingtin protein is seen not in the nucleus, but in the cytoplasm. In many ways, *C.elegans* mimics certain aspects of the human disease. The *C.elegans* genetic system lends itself to future studies designed to locate other genes in that particular degeneration pathway and may shed light on possible targets for drug therapy. It is noteworthy that *ced-3*, which belongs to the apoptotic signalling pathway in *C.elegans*, is required for ASH cell death mediated by human huntingtin CAG repeats.

CONCLUSIONS AND PROSPECTS

The deployment of *C.elegans* in the study of human disease genes introduces a very powerful genetic model with more true orthologues than yeast and a powerful, well-tried genetic toolkit. Double-stranded RNA interference (dsRNAi) permits rapid knockdown of single genes or gene pools (46). Heritable and controllable interference by dsRNA can be used for the targeted disruption of a gene by combining RNAi and heat shock-induced expression (47). The first reports of *C.elegans* primary cell cultures (42) offer rapid developments in physiological analysis of *C.elegans* mutants, as does the application of electrophysiology (48) and ion imaging (49). All these technical developments will impact on the functional analysis of *C.elegans* human disease gene orthologues. Thus, many *C.elegans* genes related to human disease genes

currently are under investigation and we can anticipate new insights into their biological functions.

Caenorhabditis elegans can also be used to investigate human genes further even after the biological function has been identified. Candidate modifier genes can be identified using *C.elegans* and the nature of their actions explored. The nematode model could therefore be explored for testing the effects of gene therapy candidates, using, for example, gene overexpression studies. The controlled overexpression at various doses of the wild-type copy of the human *SMN* gene could perhaps be explored as part of the initial research studies aimed at assessing this approach to the treatment of SMA.

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