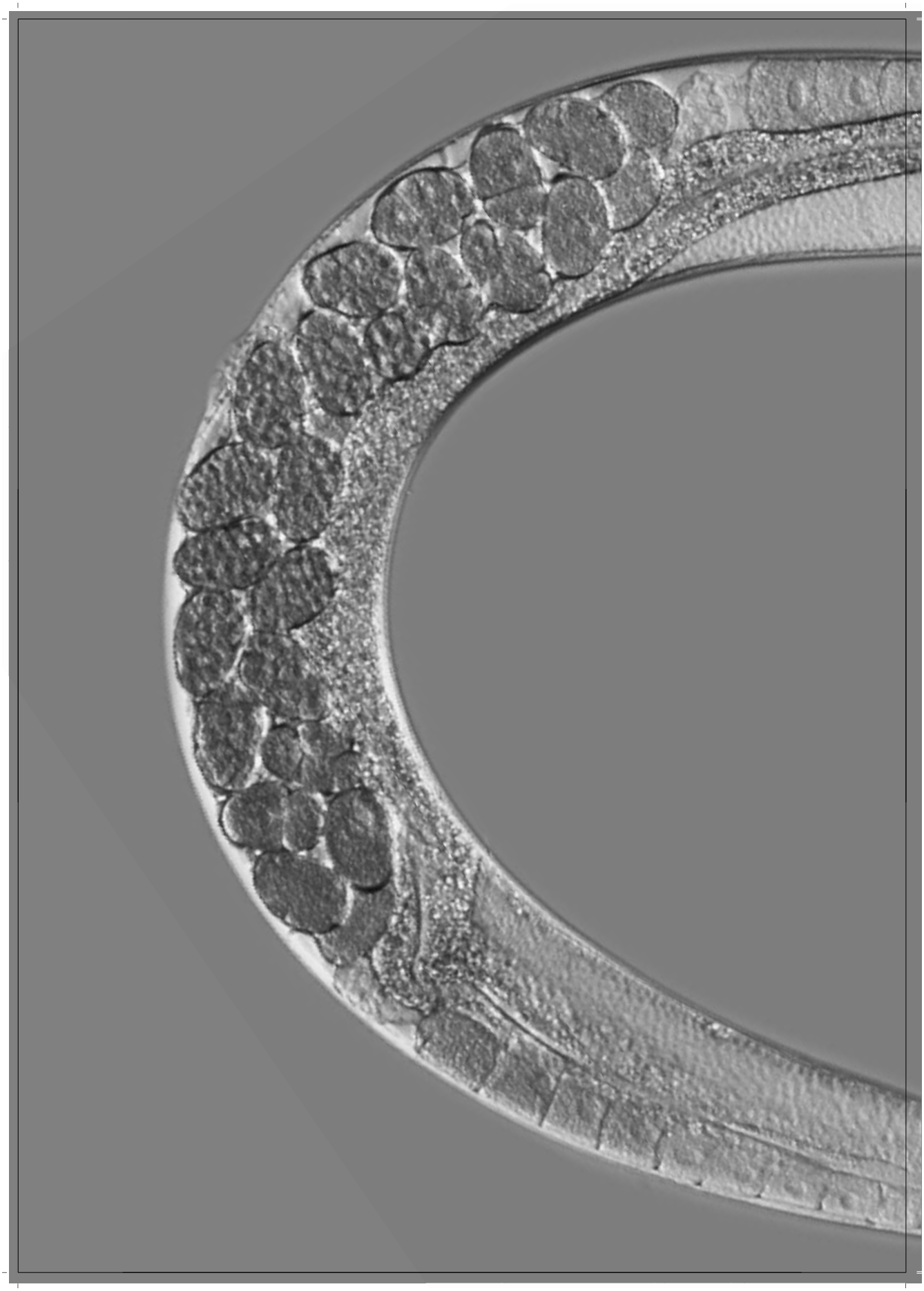
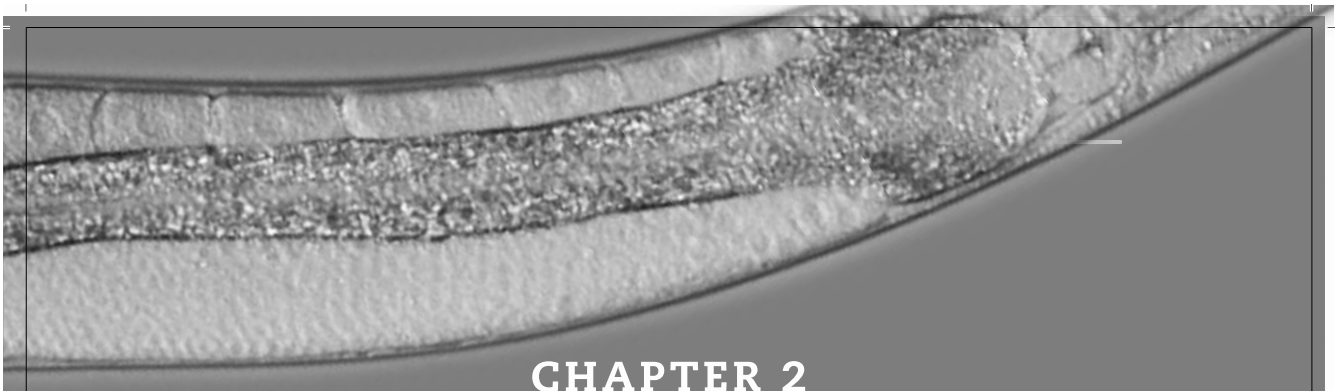
The cln-3 genes of Caenorhabditis elegans : making C. elegans models for Juvenile Neuronal Ceroid Lipofuscinosis.

Voer, G. de





CHAPTER 2

Caenorhabditis elegans as a model for Lysosomal Storage Disorders

Gert de Voer[1], Dorien J.M. Peters, Peter E.M. Taschner #

Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands
[1]Present address: The Cell Microscopy Center, Department of Cell Biology and Institute of Biomembranes, University Medical Center Utrecht, Utrecht, The Netherlands
Author responsible for correspondence: Peter E. Taschner
Department of Human Genetics
Center for Human and Clinical Genetics
Postzone S-04-P
P.O. Box 9600
2300 RC Leiden
The Netherlands
Telephone +31 (0)71-5269424
Fax +31 (0)71-5268285
e-mail: P.Taschner@lumc.nl
Subject index: *Caenorhabditis elegans*, lysosomal storage disease, lysosome

Running head title: *Caenorhabditis elegans* and lysosomal storage disorders

Abstract

The nematode *C. elegans* is the simplest animal model available to study human disease. In this review, the homologues of human genes involved in lysosomal storage disorders and normal lysosomal function have been listed. In addition, the phenotypes of mutants, in which these genes have been disrupted or knocked down, have been summarized and discussed. From the overview we can conclude that the phenotypic spectrum of worm models of lysosomal storage disorders varies from lethality to none obvious with a large variety of intermediate phenotypes. It is also clear that the genetic power of *C. elegans* provides a means to identify genes involved in lysosomal biogenesis and function, although genetic screens for loss or gain of easily distinguishable intermediate phenotypes are most successful.

Introduction

The lysosome

Christian De Duve discovered, in 1949, the cellular bodies in which digestion occurs and called them lysosomes, since they contained mixtures of lytic enzymes, reviewed in [1]. These organelles were identified due to the application of different homogenization procedures. Gentle homogenization allows the lysosomes to stay intact and therefore to contain the enzymatic activity inside, whereas drastic homogenization causes disruption of the lysosomal membranes permitting measurements of lysosomal enzyme activity. De Duve [1] drew a parallel between the digestive tract of a multicellular organism and the "digestive tract" of a cell, both having a resistant envelope with multiple functions, e.g., to protect the rest of the organism or cell from digestion, uptake and secretion of compounds, and to maintain the degradative environment. Moreover, he reasoned that defective functioning of lysosomes could possibly lead to incomplete breakdown and subsequent accumulation of the indigestible substance, eventually causing the cell that harbors the defective lysosomes to become inoperative or even go into apoptosis. Since De Duve's original discovery, significant progress has been made in elucidating the processes taking place in the lysosomes, although much is still unknown.

Lysosomes and degradation and transport

Most eukaryotic cells contain lysosomes, which exist in a variety of shapes and sizes. These acidic degradative organelles are surrounded by a single lysosomal membrane and have a pH of about 5, which is lower than the cytoplasmic pH of approximately 7.2. They contain 50-60 hydrolytic enzymes, collectively called the acid hydrolases that usually reside in the lysosomal lumen. In contrast, the lysosomal membrane proteins are functional in a variety of processes such as lysosome biogenesis, maintenance of endosomal transport, lysosomal enzyme targeting and autophagy [2]. Most of the hydrolytic enzymes are transported to the lysosomes via the 'direct' mannose 6-phosphate pathway, while lysosomal membrane proteins use both the 'direct' pathway and the 'indirect' pathway via the plasma membrane, to travel to the lysosome [3], [4].
Lysosomes obtain their constituents and material to be degraded through vesicular trafficking. For example, transport vesicles containing hydrolytic enzymes can combine with lysosomes or vesicles filled with endocytosed compounds called endosomes. After the endocytosed materials have been degraded, lysosomal proteins can be recycled, catabolized materials can be reused as building blocks for the anabolic processes

in the cell, and waste products may be excreted from the cell. Furthermore, similar processes of vesicular fission or fusion, and intralysosomal degradation may occur with autophagosomes in order to recycle old or obsolete organelles or other parts of the cell.

Lysosomal storage diseases

When any process discussed above is not working properly, this may lead to partly or completely dysfunctional lysosomes that are unable to degrade specific compounds causing their accumulation (See [5] for a detailed description of lysosomal storage diseases). The mass of stored materials can cause the lysosomes to become inflexible or enlarged cellular compartments that are disrupting other processes taking place in the cell, thus leading to a lysosomal storage disorder. Most of the lysosomal storage diseases are caused by defects in hydrolytic enzymes, such as the acid α-glucosidase deficiency in the first described lysosomal storage disorder, Pompe disease (MIM232300)[6]. Acid α-glucosidase deficiency causes storage of glycogen in lysosomes of numerous tissues. In the most severe form of this disease, patients suffer from prominent cardiomegaly, hypotonia, hepatomegaly and they finally die due to cardiorespiratory failure, usually before the age of two [7].
In addition to enzyme deficiencies, disturbed protein sorting or vesicular trafficking may also lead to lysosomal storage disorders, e.g., in mucolipidosis type II or I-cell disease (MIM252500). Cells of mesenchymal origin have reduced phosphotransferase activity in the Golgi apparatus and fail to add a phosphate group to the mannose residues already present on the lysosomal enzyme precursors. Lack of the proper modification leads to secretion of the lysosomal enzymes instead of normal transport to the lysosomes [8]. Affected cells contain dense inclusions of storage material, hence the name inclusion cell or I-cell disease. I-cell disease patients generally suffer from severe progressive psychomotor retardation and premature death in the first decade of life.
The severe pathologies of other lysosomal storage disorders has prompted research into the etiology of the more than 40 known lysosomal storage diseases [9], [10].
For most diseases causative mutations have been described and possible treatments are being developed. For instance, enzyme replacement therapy can be used to treat Gaucher disease, in which the gene encoding the enzyme acid β-glucosidase is mutated [11]. For most lysosomal storage diseases only symptomatic treatment of the patients is possible at the moment. Although much is known concerning lysosomal processes and the mechanisms required for a lysosome to be functional, some questions remain unanswered: whether and how undigested accumulated materials cause the symptoms observed in patients suffering from lysosomal storage diseases or whether the accumulation is a primary or merely a side effect [12]. With the innovative developments in technology for genome and protein analysis, e.g., completion of sequencing projects, microarray analysis techniques, the detailed examination and description of model organisms in which the processes involved in these diseases can be studied, and the development of other whole genome approaches, we may expect to get more insight in these processes in the future.

Human lysosomal diseases and *C. elegans*

To investigate the mechanisms underlying lysosomal storage disorders and their relation with the disease phenotype, appropriate model organisms are required. These organisms preferably should be eukaryotic, multicellular organisms, but straightforward to use and genetically easily modifiable. Furthermore, to identify genes homologous to the genes involved in these heritable disorders, the genome of the model organism should be fully sequenced and the developmental life-course should be well characterized to study mutant phenotypes. Moreover, since neurological symptoms are common in lysosomal storage diseases, the presence of a well described nervous system would certainly be an advantage. Therefore, we have investigated the potential of the nematode *Caenorhabditis elegans* as a model organism for lysosomal storage disorders. This nematode is a convenient and nowadays widely used model organism, initially described by Sydney Brenner [13]. *C. elegans* has an entirely known cell lineage, a very well characterized and invariably wired nervous system, and this organism is amenable to large genetic screens. These worms exist in two sexes: hermaphrodites that self-fertilize to obtain homozygous mutants and males that can be used to perform crosses. Moreover, a comprehensive toolset for worm research on a genetic, cellular, and behavioral level is available and gene specific loss-of-function mutations can easily be phenocopied using RNAi by microinjection, soaking or feeding methods. Whole genome approaches have delivered a vast amount of data, even on previously unannotated sequences, providing clues to the function of the putative proteins [14]. We have compared all human protein sequences known to be involved in lysosomal storage disorders with *C. elegans* protein sequences to identify the putative worm homologues of these lysosomal disease proteins. Subsequently, we collected all phenotypes of nematodes with mutations in the putative homologous genes in order to get an overview of all possible lysosomal phenotypes present in the worm. Similar comparisons were performed with human proteins that were involved in lysosomal processes but that were still unassociated with disease. The homologues and their phenotypes were listed, as were those of the already known *C. elegans* loci involved in lysosomal function, encompassing all possible lysosomal phenotypes that are known in *C. elegans* at the moment. The feasibility of using these lysosomal phenotypes in genetic screens for modifying mutations was studied to discuss the possibilities of investigating the molecular genetic mechanisms the *C. elegans* homologue is involved in.

Results

C. elegans homologues to human lysosomal storage disease genes

We identified *C. elegans* homologues for almost all 58 human genes associated with lysosomal storage diseases (Table 1). Most of the human disease genes have only a single homologue, except *NPC1* (two homologues), *CLN3* and *SMPD1* (both three homologues). Conversely, a single worm homologue has been identified for each of three human gene pairs, *HEXA-HEXB*, *GLA-NAGA* and *GALNS-ARSA*. For all 42 *C. elegans* homologues, information on gene function can be found in literature and in the Wormbase database [15]. Ten of these *C. elegans* genes, which have been studied individually in the nematode, are homologous to genes involved in mucolipidosis type IV (MIM252650), Niemann-Pick type C (MIM257220), Danon disease (MIM300257), Hermansky Pudlak Syndrome-2 (MIM608233) and congenital, infantile, and juvenile forms of neuronal ceroid lipofuscinosis (NCL) (MIM610127, MIM256730, and MIM204200, respectively). Data on the other 32 genes were derived from sequence similarities or came from whole genome approaches, such as microarray analyses [16], and whole genome RNAi experiments [17]. After RNAi knockdown of 35 genes, worm phenotypes ranged from none (26 times) to (embryonic) lethality (twice) with seven subtle intermediate phenotypes (Table 1). Although mutants have been isolated for 19 of the genes, 7 out of the 11 mutants characterized had a mainly subtle phenotype. The available information about nematode homologues to human lysosomal storage disease genes is summarized below in order to get an overview of all possible phenotypes of worm models for lysosomal storage disorders.

Table 1 Human Lysosomal Storage Disorder Genes and their C. elegans Homologues

Lysosomal storage disorder	Human Gene	MIM number a)	C. elegans Homologue	% f)	Function of human protein	C. elegans Mutant c)	RNAi and phenotypes e)
Neuronal ceroid lipofuscinoses (NCL)							
Infantile NCL	PPT1 (CLN1)	600722	F44C4.5	54	Palmitoyl-protein thioesterase 1 precursor (EC 3.1.2.22)	ppt-1	M: delayed egg-laying, abnormal mitochondria; R: None observed
(Congenital ovine NCL)	CTSD	116840	R12H7.2b)	61	Cathepsin D precursor (EC 3.4.23.5)	asp-4 d)	R: Ced
Late infantile NCL	TPP1 (CLN2)	204500	None		Tripeptidyl-peptidase 1 precursor (EC 3.4.14.9)		
Juvenile NCL	CLN3	204200	F07B10.1	53	Unknown	cln-3.1	M: slightly reduced life span; R: None observed
			C01G8.2	49		cln-3.2	M: slightly reduced brood size; R: None observed
			ZC190.1	62		cln-3.3	R: None observed

Late infantile NCL, Finnish variant	CLN5	256731	None		Unknown		
Late infantile NCL, Indian variant	CLN6	601780	None		Unknown		
Northern epilepsy	CLN8	600143	None		Unknown		
Oligosaccharidoses							
Alpha-mannosidosis	MAN2B1	248500	F55D10.1	52	Lysosomal alpha-mannosidase precursor (EC 3.2.1.24)		R: None observed
Beta-mannosidosis	MANBA	248510	C33G3.4	53	Beta-mannosidase precursor (EC 3.2.1.25)		R: None observed
Fucosidosis	FUCA1	230000	W03G11.3	50	Tissue alpha-L-fucosidase precursor (EC 3.2.1.51)		R: None observed
Farber's disease	ASAH1	228000	K11D2.2	62	Acid ceramidase precursor (EC 3.5.1.23)	asah-1 d)	R: Age, Reduced lifespan
Aspartylglucosaminuria	AGA	208400	R04B3.2	56	N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase precursor (EC 3.5.1.26)		R: None observed
Galactosialidosis	PPGB	256540	F41C3.5	59	Lysosomal protective protein precursor (EC 3.4.16.5)		R: None observed
Sphingolipidoses							
Tay-Sachs disease, GM2 gangliosidosis I	HEXA	272800	T14F9.3	56	Beta-hexosaminidase alpha chain precursor (EC 3.2.1.52)	?	M: ND; R: None observed
Sandhoff disease, GM2 gangliosidosis II	HEXB	268800	T14F9.3	55	Beta-hexosaminidase beta chain precursor (EC 3.2.1.52)		
GM2-gangliosidosis type ab	GM2A	272750	None		Ganglioside GM2 activator precursor		
Krabbe disease	GALC	245200	C29E4.10	31	Galactocerebrosidase precursor (EC 3.2.1.46)		R: None observed
Gaucher disease	GBA	606463	F11E6.1	42	Glucosylceramidase precursor (EC 3.2.1.45)		R: None observed
Variant metachromatic leukodystrophy	PSAP	176801	C28C12.7	21	Proactivator polypeptide precursor, prosaposin	spp-10	M, R: None observed
Variant Gaucher disease	PSAP	176801	C28C12.7	21	Proactivator polypeptide precursor, prosaposin	spp-10	
Variant Tay-Sachs disease (gm2-gangliosidosis)	PSAP	176801	C28C12.7	21	Proactivator polypeptide precursor, prosaposin	spp-10	
Niemann-Pick disease A&B	SMPD1	257200	ZK455.4	55	Sphingomyelin phosphodiesterase precursor (EC 3.1.4.12)	asm-2 d)	R: None observed
			W03G1.7	53		asm-3	M: ND; R: None observed
			B0252.2	52		asm-1 d)	R: None observed
Niemann-Pick disease C1	NPC1	257220	F09G8.4	47	Niemann-Pick C1 protein precursor	ncr-2 (npc-2)	M: hypersensitive to cholesterol deprivation, hyperactive egg-layer, slow development, inappropriate dauer forming; R: Emb
			F02E8.6	46		ncr-1 (npc-1)	M: hypersensitive to cholesterol deprivation, hyperactive egg layer, slow development, inappropriate dauer forming; R: None observed

Fabry's disease, sphingolipidosis	GLA	301500	R07B7.11	54	Alpha-galactosidase A precursor (EC 3.2.1.22)	gana-1 d)	R: Reduced enzyme activity
Schindler disease	NAGA	104170	R07B7.11	53	Alpha-N-acetylgalactosaminidase precursor (EC 3.2.1.49)	gana-1 d)	R: Reduced enzyme activity
Mucopolysaccharidoses							
Mucopolysaccharidosis type I, Hurler/Scheie syndrome	IDUA	252800	None		Alpha-L-iduronidase precursor (EC 3.2.1.76)		
Mucopolysaccharidosis type II, Hunter syndrome	IDS	309900	None		Iduronate 2-sulfatase precursor (EC 3.1.6.13)		
Mucopolysaccharidosis type IIIA, Sanfilippo disease IIIA	SGSH	252900	F26H9.1	35	N-sulphoglucosamine sulphohydrolase precursor (EC 3.10.1.1)	chis-1	M: ND, R: None observed
Mucopolysaccharidosis type IIIB, Sanfilippo disease IIIB	NAGLU	252920	K09E4.4	42	Alpha-N-acetylglucosaminidase precursor (EC 3.2.1.50)		R: None observed
Mucopolysaccharidosis type IIIB, Sanfilippo disease IIIC	HGSNAT	252930	None		Heparan-alpha-glucosaminide N-acetyltransferase (E.C. 2.3.1.78)		
Mucopolysaccharidosis type IIID, Sanfilippo disease IIID	GNS	252940	K09C4.8	40	N-acetylglucosamine-6-sulfatase precursor (EC 3.1.6.14)	sul-1	M, R: None observed
Mucopolysaccharidosis type IVB, Morquio syndrome B	GLB1	230500	T19B10.3	41	Beta-galactosidase precursor (EC 3.2.1.23)		R: None observed
Mucopolysaccharidosis type IVA, Morquio syndrome A	GALNS	253000	D1014.1	41	N-acetylgalactosamine-6-sulfatase precursor (EC 3.1.6.4)	sul-2	M, R: None observed
Metachromatic leucodystrophy	ARSA	250100	D1014.1	40	Arylsulfatase A precursor (EC 3.1.6.8)	sul-2	
Mucopolysaccharidosis type VI, Maroteaux-Lamy	ARSB	253200	C54D2.4	33	Arylsulfatase B precursor (EC 3.1.6.12)	sul-3 d)	R: None observed
Mucopolysaccharidosis type VII, Sly syndrome	GUSB	253220	Y105E8B.9	39	Beta-glucuronidase precursor (EC 3.2.1.31)		R: None observed
Hyaluronidase deficiency (Mucopolysaccharidosis type IX)	HYAL1	601492	T22C8.2	31	Hyaluronidase-1 precursor (EC 3.2.1.35)		R: None observed
Lysosomal transporter defects							
Nephropathic cystinosis	CTNS	606272	C41C4.7	48	Cystinosin	ctns-1	M: ND, R: None observed
Infantile sialic acid storage disorder(ISSD) and Salla disease	SLC17A5	604322	C38C10.2	60	Sodium/sialic acid cotransporter, sialin		R: None observed
Lysosomal trafficking defects							
Mucolipidosis, type IV	MCOLN1	605248	R13A5.1	55	Mucolipin-1	cup-5	M: maternal-effect embryonic lethal; R: None observed
Hermansky-Pudlak syndrome	HPS1	604982	None		Biogenesis of lysosome-related organelles complex 3 component		
	AP3B1 (HPS2)	603401	R11A5.1	56	AP-3 complex subunit beta-1	apb-3 (apt-6)	M: ND; R: Emb, Let, Lva, Dpy, fat content reduced
	HPS3	606118	None		Biogenesis of lysosome-related organelles complex 2 component		

	HPS4	606682	None		Biogenesis of lysosome-related organelles complex 3 component		
	HPS5	607521	W09G3.6	27	Biogenesis of lysosome-related organelles complex 2 component		R: fat content reduced
	HPS6	607522	None		Biogenesis of lysosome-related organelles complex 2 component		
	DTNBP1 (HPS7)	607145	None		Dystrobrevin-binding protein 1 (Dysbindin), BLOC1 subunit		
	BLOC1S3 (HPS8)	609762	None		Biogenesis of lysosome-related organelles complex-1 subunit 3		
	VPS33A	610034	B0303.9	25	Vacuolar protein sorting 33A	slp-1	M: ND, R: None observed
Mucolipidosis, types II and IIIA	GNPTAB	607840	None		N-acetylglucosamine-1-phosphotransferase subunits alpha/beta precursor (EC 2.7.8.17)		
Mucolipidosis, type IIIC	GNPTG	607838	ZK1307.8	38	N-acetylglucosamine-1-phosphotransferase subunit gamma precursor (EC 2.7.8.17)	?	M: ND, R: None observed
Others							
Glycogen storage disease type II, Pompe disease	GAA	232300	D2096.3	37	Lysosomal alpha-glucosidase precursor (EC 3.2.1.20)	?	M, R: ND
Chediak-Higashi syndrome	LYST (CHS1)	214500	VT23B5.2	32	Lysosomal-trafficking regulator	?	M: ND, R: None observed
			F10F2.1	34			R: response to contact abnormal
Wolman disease / cholesteryl ester storage disease	LIPA	278000	R11G11.14	59	Lysosomal acid lipase/ cholesteryl ester hydrolase precursor (EC 3.1.1.13)		R: Him, fat content reduced
Glycogen storage disease type Iib, Danon disease	LAMP2	300257	C03B1.12	25	Lysosome-associated membrane glycoprotein 2 precursor	lmp-1	M: gut lighter, one type of intestinal granule missing; R:Clr
Sialidosis	NEU1	256550	None		Sialidase-1 precursor (EC 3.2.1.18)		
Multiple sulfatase deficiency	SUMF1	272200	None		Sulfatase-modifying factor 1 precursor		
Lipoid proteinosis of Urbach and Wiethe	ECM1	602201	None		Extracellular matrix protein 1 precursor		
Dyggve-Melchior-Clausen dysplasia/Smith-McCort dysplasia	DYM	607461	C47D12.2	96	Dymeclin		R: Emb, Let, Muv
Cathepsin E deficiency	CTSE	116890	R12H7.2b)	59	Cathepsin E precursor (EC 3.4.23.34)	asp-4 d)	R: Ced

a) Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/Omim/searchomim.html)

b) Due to high similarity between cathepsin homologues, the highest unidirectional man-worm hit is shown

c) ? Deletion mutant, gene name unknown. Old gene designations are shown between parentheses

d) Gene name assigned, but no mutant available

e) M: Mutant, R: RNAi, ND: not determined

f) % Similarity to human gene

C. elegans cup-5, homologous to the Mucolipidosis type IV gene *MCOLN1*, and lysosome biogenesis

Mucolipidosis type IV, caused by mutations in the *MCOLN1* gene, is a neurodegenerative lysosomal storage disorder of which the main symptoms are psychomotor retardation and ophthalmologic abnormalities [18], [19], [20]. Ultrastructural analysis of patient tissues usually reveals many enlarged vacuoles, presumably lysosomes in which lipids are stored as well as water-soluble granulated substances. The *MCOLN1* gene encodes the lysosomal membrane protein h-mucolipin-1, which has six predicted transmembrane domains and functions as a non-selective cation channel of which the activity is modulated by pH [21]. The *C. elegans MCOLN1* homologue *cup-5* is a functional orthologue of the human *MCOLN1* gene, since the phenotype of *cup-5* mutants, heterogeneous enlarged vacuoles and embryonic lethality, can be rescued by expressing the human gene in these mutants [22]. Mutations in the *cup-5* gene were identified by screening for mutants with disrupted endocytosis [23], and in mutants with affected programmed cell death [22]. The *cup-5* gene is expressed in most tissues in adult worms, and subcellularly localized to nascent and mature lysosomes [24]. Similar to patient cells, cells from *cup-5* mutants have enlarged vacuoles and lysosomes, apparently due to defective lysosomal degradation processes. The CUP-5 protein was suggested to play a role in lysosome biogenesis or maturation [25], because h-mucolipin-1 was shown to be a Ca^{2+}-permeable channel [26], and Ca^{2+} transport is essential for fusion of late endosomes and lysosomes and for reformation of lysosomes [27]. Interestingly, through a screen for modifier alleles, a mutation in the *mrp-4* gene, encoding an endosomal-lysosomal ABC transporter, was shown to suppress *cup-5* lethality and rescue the lysosomal degradation and developmental defects of the *cup-5* mutants [28]. In the *cup-5* mutants, degradation of the ABC transporter was suggested to be delayed, causing an imbalance in the endosomal-lysosomal import of compounds, which probably interferes with normal degradation processes. The affected degradation is thought to lead to starvation of the cells and independently to developmental defects. Absence of the ABC transporter results in rescue of lysosomal function, thereby permitting the cells to survive. Whether similar events contribute to the cellular and neuronal degeneration in Mucolipidosis type IV patients is still unknown, but if this is the case reducing the activity of ABC transporters might provide for a therapy for the treatment of mucolipidosis type IV.

Nematode Niemann-Pick type C homologues involved in cholesterol trafficking

Multiple forms of Niemann-Pick disease exist, either caused by deficient acid sphingomyelinase activity leading to accumulation of sphingomyelin in Niemann-Pick type A and B [29], [30], or by defective cholesterol trafficking resulting in lysosomal storage of unesterified cholesterol in Niemann-Pick type C [31]. Symptoms caused by acid sphingomyelinase deficiency vary from hepatosplenomegaly and progressive neurodegenerative disease to pulmonary infiltration. Patients suffering from defective cholesterol trafficking also display progressive neurological disease and possibly

prominent hepatic damage. Three acid sphingomyelinase (ASM) homologues were identified in *C. elegans*, while in other organisms only one *ASM* could be identified [32]. Unfortunately, according to the Wormbase database, mutants for only one of the *C. elegans* ASM homologues are available, but their phenotypes were not described. No *ASM* RNAi phenotypes emerged from whole genome approaches [15], but RNAi targeted against multiple *ASM* genes perhaps could result in an interesting knock-down phenotype and provide a model for Niemann-Pick type A and B.

Niemann-Pick type C can be caused by mutations in two genes, *NPC1* and *NPC2*, encoding proteins with NPC and sterol sensing domains that are implicated in retrograde transport from sterols and other cargo from lysosomes [31], [33], [34]. Each has one worm homologue, *ncr-1* and *ncr-2*, respectively [35]. Worms without *ncr-1* are hypersensitive to lack of cholesterol, an essential substance for the nematode, and to exposure to progesterone, which can inhibit intracellular cholesterol trafficking in mammalian cells [35], [36]. In contrast, *ncr-2* single mutants appear superficially wild type. Whereas *ncr-2; ncr-1* double mutants display constitutive inappropriate dauer formation, which could be rescued by microinjection of NCR-2 or NCR-1wildtype genes, suggesting both proteins play redundant roles to prevent dauer formation under favorable conditions. The dauer is a relatively stress-resistant alternative larval life stage that the animal can form when it develops under stressful conditions. Furthermore, *ncr-2; ncr-1* double mutants have abnormal morphology of certain neurons during transient dauer stage. Other *ncr-2; ncr-1* double mutant phenotypes encompass developmental pleiotropic phenotypes similar to *daf-9* and *daf-12* mutants including reproductive defects, concordant reduced brood size and life span, vulval abnormalities and disruption of the alae cuticle. Epistasis analysis placed NCR-1 and NCR-2 upstream of DAF-9 in the dauer formation pathway [35], [36]. Therefore, the NCR-1 and NCR-2 proteins that were suggested to play a role in intracellular cholesterol trafficking may provide the substrate for DAF-9, the ER localized cytochrome P450 enzyme, which catalyzes a reaction to form a lipophilic hormone for the DAF-12 nuclear receptor [37], [38]. Under cholesterol-deprived conditions, *ncr-2*; *ncr-1* double mutant worms could be unable to efficiently traffic this hormone progenitor to the site of DAF-9 action and hence stimulate dauer formation due to absence of the signal to bypass the dauer stage. Further examination of how the mutations in *NCR-1* and *NCR-2* lead to the other phenotypes, and, most interestingly, the abnormal neuronal morphology, may elucidate other functional aspects of these proteins..

C. elegans LMP-1 protein, homologous to the Danon disease protein LAMP2, involved in lysosome biogenesis

Mutations in the lysosome-associated membrane protein-2 gene (*LAMP2*) cause glycogen storage disease type IIb or Danon disease, characterized by cardiomyopathy, myopathy and variable mental retardation [39]. Originally, Danon disease was described as a variant glycogen storage disease type II, since acid-maltase or alpha-glucosidase activity was normal [40]. LAMP2 and structurally similar LAMP1 are heavily glycosylated lysosomal membrane proteins with one transmembrane domain

and a major intralysosomal part, and are thought to be functional in lysosome stability and integrity [2]. LAMP-1 and LAMP-2 knock-out mice have been generated to investigate LAMP protein function [41], [42]. LAMP-2 knock-out mice suffered from cardiomyopathy and accumulation of autophagic vacuoles, similar to human Danon disease patients. LAMP-1 knock-out mice displayed no overt phenotype but overexpressed LAMP-2, suggesting functional redundancy. Additional functional and morphological studies of the heart of LAMP-2 knock-out mice showed that these mice suffered from contractile dysfunction that was suggested to be due to morphological changes [43]. However, how mutations in *LAMP-2* cause these morphological changes and why the autophagic vacuoles accumulate is still unknown. Kostich and coworkers [44] have searched the *C. elegans* genome for LAMP like sequences and identified a LAMP homologue *lmp-1*, which has a lysosomal targeting sequence (GYXXΦ, in which Φ is a large hydrophobic amino acid residue and X any amino acid) at its COOH terminus. A BLAST search with the human LAMP2 protein sequence against the *C. elegans* protein database Wormpep [15] results in two hits, LMP-1 and LMP-2, the latter of which has no GYXXΦ motif. Nematode *lmp-1* deletion mutants are viable and fertile, show alternative intestinal granule populations, and apparent loss of one type of granule, hence *LMP-1* is likely to be functional in lysosome biogenesis or maintenance [44]. How loss of the *lmp-1* gene causes this change in granule composition and whether autophagy is affected in these mutants remains to be elucidated. This could be done by investigating the genetic interactions of the nematode *lmp-1* gene by screening for mutations that modify the Lmp-1 phenotype. Additional players that are involved in LMP-1 function could be identified by isolation of other mutants with the Lmp-1 phenotype. The feasibility of these screens depends on the ease with which the *lmp-1* gut granule loss can be scored under a standard Differential Interference Contrast (DIC) microscope [45].

Hermansky Pudlak syndrome type 2 (HPS-2) and the AP 3a homologue in the worm implicated in development

The heterogenous group of diseases termed the Hermansky-Pudlak syndrome (HPS) are pathologically characterized by prolonged bleeding, albinism and lysosomal storage of ceroid, and presumably result from defects in multiple cytoplasmic organelles, such as melanosomes, platelet-dense granules, and lysosomes [46]. Mutations in eight genes, *HPS1 - HPS8*, have been shown to cause this disorder and the proteins encoded by these genes are thought to be involved in the biogenesis or transport of lysosomes or lysosome related organelles [47], [48]. The HPS-1 and HPS-4 proteins appear to form a complex that might play a role in the biogenesis of lysosomes [49], [50]. These proteins were suggested to function independently from the AP-3 complex which is involved in formation of carrier vesicles and cargo recruitment, for protein transport. We could not identify *C. elegans* sequences homologous to HPS-1, HPS-3, HPS-4, and HPS-6 by mere protein sequence comparison, suggesting that these proteins are simply not present in the nematode or their sequences have diverged beyond recognition. HPS-5, HPS-7 and HPS-8 have putative homologous proteins in the nematode, but these have not yet been investigated individually and the high throughput approaches did not elucidate

any functional aspects of these proteins [15]. HPS-2 is caused by mutations in the gene encoding the β3a subunit of the adaptor protein 3 (AP-3) complex [51]. Although AP-3 is involved in the sorting of transmembrane proteins from endosomes and the *trans*-Golgi network to lysosomes and endosome-lysosome related organelles, it is unknown how altered AP-3 function leads to HPS-2 [52], [53]. The *C. elegans* homologue for this protein, Apb-3, appears to be required for development as RNAi knock-down of the *apb-3* transcript causes embryonic and larval lethality [54]. Interestingly, worm knock-outs for two other AP-3 complex subunits, *apt-6* and *apt-7*, encoding β3 and μ3 subunits respectively, have an embryonic gut granule loss (Glo) phenotype and larvae and adult mutant worms have less autofluorescent gut granules [55]. The autofluoresent gut granules are presumed to be secondary lysosomes that may contain yolk or other nutrients [56], [44]. This raises the possibility that loss of gut granules due to affected Apb-3 function leads to nutrient deprivation and subsequent lethality in *apb-3* RNAi worms. Additional investigations, such as genetic screens for alleles modifying the larval arrest or the Glo phenotypes, could provide further insight into AP-3 dependent processes and the precise role of AP-3 in protein trafficking.

Neuronal ceroid lipofuscinoses (NCL) and the worm *ppt-1*, *asp-4* and *cln-3* homologues

The congenital, infantile and juvenile forms of NCL are caused by mutations in the *CTSD*, *PPT1* and *CLN3* genes respectively, which all lead to severe neurodegenerative disorders with similar disease progression but with different age of onset [57], [58], [59]. Initial symptoms include visual deterioration followed by epileptic seizures, progressing to a state of dementia and ending in premature death [10]. In addition to the differences between the age of onset and the genes affected, the NCLs can also be distinguished by the typical patterns of the lipopigment accumulations found in lysosomes of neurons and other cell types [60]. No direct links have been established between the causative mutations, the observed pathology and the accumulated material. The most severe form of the NCLs, congenital neuronal ceroid lipofuscinosis, is very rare and thus far only 10 patients have been described [57]. Patients suffering from this disease are microcephalic, may have seizures and die soon after birth. This disease was found to be caused by mutations in the *CTSD* gene encoding cathepsin D and strongly resembles the congenital ovine neuronal ceroid lipofuscinosis previously identified in sheep [61]. The nematode homologue of the *CTSD* gene *asp-4* is also the closest homologue of the human *CTSE* gene, which is mutated in CTSE deficiency, a disease distinct from NCL [62]. Asp-4 was shown to be involved in necrotic cell death as *asp-4* RNAi knockdown leads to decreased cell death, signifying its role as an executioner protease [63]. Thus, altered cell death may underlie the etiology of congenital neuronal ceroid lipofuscinosis.

PPT1 encodes the lysosomal enzyme palmitoyl protein thioesterase-1, which cleaves thioester linkages in S-acylated (palmitoylated) proteins and facilitates the removal of the palmitate residues [64]. In neurons however, PPT1 is also found in non-lysosomal compartments, synaptic vesicles and synaptosomes [65]. The *C. elegans* *CLN1* homologue is designated *ppt-1* and worms mutated in their *ppt-1* gene display

mitochondrial abnormalities at an ultrastructural level, and an egg laying defect or egl phenotype, where eggs hatch inside the parent [66]. This reproductive 'bagging' phenotype could be used in genetic screens to identify modifier genes, although this may be a rather laborious task. Alternatively, enhancement of the *ppt-1* mutant phenotype may result in a more robust phenotype that is more useful for genetic screens.

The juvenile NCL gene *CLN3* encodes a transmembrane protein, which is thought to be primarily localized to lysosomes and may be implicated in pH regulation or amino acid transport [60]. The nematode has three homologous genes, *cln-3.1*, *cln-3.2*, and *cln-3.3*, which when mutated and combined into one triple mutant strain causes a mild decrease in life span and brood size [67]. This phenotype is not suitable for genetic screens and requires an enhancement of the phenotype or an additional investigation of the *cln-3* mutants to establish a clear-cut difference between mutants and wild type as a basis for genetic screening to search for modifier genes.

C. elegans homologues to human genes associated with lysosomal function involved in other disorders

Apart from tissue-specific expression, several proteins with a clear lysosomal localization in certain cell types may exert their function at a different location in others. Depending on the importance of these proteins for the normal function of a specific cell type, mutations in genes encoding these proteins may impair the non-lysosomal function more than the lysosomal function. As a result, lysosomal storage is not observed in diseases caused by mutations in these genes. We have compiled a list of thirteen disease genes encoding proteins without lysosomal localization in the most affected cell types or organs and their worm homologues (Table 2). Three of these genes, *CTSC*, *MPO* and *TCIRG1* belong to gene families with multiple homologues in the worm. Remarkably, most of these genes seem to be associated with defects in polarized cells, such as renal tubular cells in renal acidosis or osteoclasts in osteopetrosis. About half of their proteins are localized in the plasma membrane. Most are subunits of the conserved vacuolar ATPase pumping protons through the plasma, lysosomal and other organellar membranes, but CLCN7 is a channel for chloride ions compensating the positive charges of the protons. Several proteins are involved in non-lysosomal degradation processes for which lysosomes provide hydrolytic enzymes, such as CTSC and CTSK. One of these processes is bone resorption, which is performed by specialized cells called osteoclasts [68]. On contact with bone, part of their membrane can form a ruffled border and create a resorptive pit, which is acidified and filled with hydrolytic enzymes by lysosomes to degrade bone. Defects in the acidification or the hydrolytic enzymes can prevent bone resorption leading to osteopetrosis. Bone defects similar to those in osteopetrosis have been observed in mucopolysaccharidosis type VII and have also been attributed to malfunctioning osteoclasts [69]. Thus, with the environment of the resorptive pit resembling that of a lysosome, one might look at osteopetrosis as a kind of extracellular lysosomal disorder. The remaining four genes,

Table 2 *C. elegans* Homologues to Human Genes associated with Lysosomal Function involved in other Disorders

Human disorders without lysosomal storage caused by lysosomal protein defects	Human Gene	MIM Number a)	C. elegans Homologue	% f)	Function/Name	C. elegans Mutant b)	RNAi and mutant phenotypes c)
Renal tubular acidosis with deafness	ATP6V1B2	606939	F20B6.2	92	ATPase, H+ transporting, lysosomal 56/58kDa V1 subunit B1	vha-12	M: ND; R: Ste Emb Let Adl Lvl Lva Prl Locomotion abnormal
			Y110A7A.12	87		spe-5?	M: Defective spermatogenesis; R: Emb Let Lvl Lva Adl Prl Locomotion abnormal Maternal sterile
Renal tubular acidosis with deafness	ATP6V1B1	192132	F20B6.2	92	ATPase, H+ transporting, lysosomal 56/58kDa V1 subunit B2	vha-12	
			Y110A7A.12	87		spe-5?	
Renal tubular acidosis, type I	ATP6V0A4	605239	F35H10.4	61	Vacuolar H+-ATPase V0 sector, subunit a	vha-5	M: homozygous lethal; R: Emb Let Lvl Lva Gro Prl Pvl Clr Bmd Sck Locomotion abnormal
Pyknodysostosis	CTSK	601105	T03E6.7	60	Cysteine proteinase Cathepsin K	cpl-1	M: ND; R: Gro Emb Let Locomotion abnormal
Haim-Munk syndrome	CTSC	602365	T10H4.12 d)	52	Cysteine proteinase Cathepsin C	cpr-3	M: ND; R: Emb
Papillon-Lefevre syndrome	CTSC	602365					
Juvenile periodontitis	CTSC	602365					
Myeloperoxidase deficiency	MPO	606989	ZK994.3 d)	55	Peroxidase catalyzing hypochlorous acid production	pxn-1	M:ND; R: None observed
Infantile malignant autosomal recessive osteopetrosis	TCIRG1	604592	ZK637.8 d)	57	ATPase, H+ transporting, lysosomal, V0 subunit A3	unc-32	M: severe coiler; R: Emb Let Gro Sck
	(OC116, TIRC7)						Locomotion abnormal Maternal sterile Pvl
Infantile malignant autosomal recessive osteopetrosis	CLCN7	602727	R07B7.1	91	Chloride channel 7	clh-6	M:ND; R: None observed
Autosomal dominant osteopetrosis	CLCN7	602727	R07B7.1	91		clh-6	
Infantile malignant autosomal recessive osteopetrosis	OSTM1	607649	F42A8.3	76	Osteopetrosis-associated transmembrane protein 1	?	M:ND; R: None observed
Lowe Syndrome	OCRL	309000	C16C2.3	86	phosphatidylinositol 4,5-bisphosphate-5-phosphatase	ocrl-1 e)	R: None observed
Corneal fleck dystrophy	PIP5K3	609414	VF11C1L.1	66	phosphatidylinositol 4-phosphate 5-kinase	ppk-3	M: Ste, Emb, enlarged lysosomes; R: None observed
Chorea acanthocytosis	VPS13A	605978	T08G11.1	95	Vacuolar protein sorting 13A		R: None observed
Arthrogryposis, renal dysfunction and cholestasis	VPS33B	608552	C56C10.1	46	Vacuolar protein sorting 33B		R: None observed

a) Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/Omim/searchomim.html)
b) ? Deletion mutant, gene name unknown
c) M: Mutant, R: RNAi, ND: not determined
d) Multiple hits with human protein. No reciprocal best hit due to higher similarity of worm protein to a different human protein. One-directional hit with highest similarity shown
e) Gene name assigned, but no mutant available
f) % Similarity to human gene

OCRL1, *PIP5K3*, *VPS13A*, and *VPS33B* play a role in lysosomal protein trafficking and lysosomal maturation. Mutant and/or RNAi knockdown phenotypes have been determined for all 13 genes, but no obvious lysosomal phenotype was observed apart from the enlarged lysosomes in the *ppk-3* mutants with their lysosomal maturation defect [70].

C. elegans homologues to human genes involved in lysosomal functioning

To complement the collection of potential lysosomal phenotypes of the worm, we have generated a list of 92 human lysosomal genes not yet implicated in disease and searched for their worm homologues (Table 3).
At least one worm homologue was found for 84 human lysosomal genes. In some cases, for example vacuolar ATPase subunit genes, the same worm homologue was found for several human genes or vice versa, indicating that these genes have been duplicated or lost in one organism compared to the other. In five other cases, the human gene is part of a gene family with many members, which is also represented in the worm, for instance, the cathepsin and lectin families.
For each of the 94 worm homologues listed, we have collected the available information about their mutant and RNAi knockdown phenotypes. Mutants for 40 homologues have been identified, but only half of them have been characterized phenotypically. Six of these have no obvious phenotype and seven genes lack a gene symbol. Most of the homologues have been included in genome-wide RNAi knockdown experiments, but for 57 of them no phenotype was observed. The most common phenotype observed in RNAi knockdown experiments for these genes was embryonic lethality (Emb: 28 times) followed by lethality (Let: 22 times) and larval arrest (Lva: 15 times). A selection of these homologues with interesting phenotypes is discussed below.

The *C. elegans* LRP-2/glycoprotein 330 homologue is essential for nematode life

LRP2/glycoprotein 330 is a very interesting member of the LDL receptor protein (LRP) family. This protein is involved in endocytosis, and its role in development appears to gain interest, due to investigation of its mouse, zebrafish and worm homologues [71]. The *C. elegans* homologue of this protein, *lrp-1* was shown to be essential for growth and development of the nematode, as *lrp-1* mutants arrest their development as larvae [72]. The most prominent morphological effect of mutations in *lrp-1* arose due to problems with shedding of the cuticle, which normally is renewed at each larval stage. Moreover, homozygote *lrp-1* mutants also are moderately dumpy and small, providing another indication for aberrant cuticle renewal, but cuticle synthesis appeared to be normal in these mutants. The dumpy phenotype has been observed previously in worms that had problems with cuticle synthesis [73]. Interestingly, wildtype worms that were starved for cholesterol phenocopied *lrp-1* mutants, supporting

a role for LRP-1 in sterol endocytosis. Recently, a genetic interaction was identified between LRP-1 and *hgrs-1*, which is involved in sorting of endocytosed proteins [74]. In *hgrs-1* mutants, the LRP-1 protein was mislocalized, indicating that *hgrs-1* is required for correct endocytic trafficking of the LRP-1 protein. The phenotype of *hgrs-1* mutants resembled that of *lrp-1* mutants and wildtype worms starved for cholesterol. Similarly, other genetic or biochemical interactions may be identified, through screening for *lrp-1*-like mutant phenotypes or modifiers of the RNAi phenotype.

The multifunctional role of cathepsin Z in nematode development

The cathepsin Z protein is a cystein protease that acts as a carboxymonopeptidase and is incorporated in the phagosome [75]. The protein was detected in cells of the immune system and in tumor cells and was suggested to also play a role in cell adhesion dependent on β2-integrin [76]. The nematode homologue of this protein, CPZ-1, was shown to be essential for development as RNAi knockdown and mutation of *cpz-1* both lead to embryonic or larval lethality in part of the worm population [77]. Whether this developmental arrest is caused by defective phagosome function or cell adhesion or has another cause, remains to be elucidated. The exact function of CPZ-1 could be further investigated in *cpz-1* mutants or worms depleted for CPZ-1 by RNAi knockdown.

A *C. elegans* chloride channel involved in endocytosis

The chloride channel protein CLC-3 may play a role in the stabilization of membrane potential also of intracellular organelles, transepithelial transport, cell volume regulation, and endocytosis [78]. Interestingly, deletion of the homologous mouse gene, *Clc3*, causes hippocampal neurodegeneration and retinal degeneration [79]. This was suggested to be the result of glutamate toxicity in synaptic vesicles or defective acidification in the endosomal or recycling pathway. Their ubiquitously expressed *C. elegans* homologue *CLH-5* was shown to be involved in receptor mediated endocytosis, because depletion of the protein by RNAi caused an endocytosis defect similar to the *cup-5* mutant [80]. The phenotype of *CLH-5* RNAi was somewhat milder than the *CUP-5* RNAi phenotype, which could be explained by redundancy of multiple other chloride channels.

Human genes involved in lysosomal functioning without *C. elegans* homologues

Eight of the human genes listed in Table 3 have no worm counterpart based on protein sequence similarity. Four of them encode subunits of a complex involved in the biogenesis of lysosome-related organelles, BLOC1, of which eight subunits have been identified [81]. The worm has only homologues of one BLOC1 subunit involved in Hermansky-Pudlak syndrome, DTNBP1, and the BLOC1S1, BLOC1S2, and SNAPAP subunits, which are not associated with human disease. It is unclear whether these proteins are part of a conserved BLOC1 core complex which on its own can play a role in organelle biosynthesis, or whether they interact with unidentified partners, or have acquired a different function.

The other genes without homologues are involved in immunity or encode enzymes. One of these, the PCYOX1 gene encodes an enzyme involved in the degradation of prenylcysteines [82]. Although knockout mice accumulate prenylcysteines in brain cells, this does not seem to lead to lysosomal storage, brain pathology or histological features normally associated with lysosomal storage diseases. It is tempting to speculate that the accumulating prenylcysteines can be localized in (lysosomal) membranes without problems until their concentration starts to destabilize these membranes or the altered membrane composition interferes with the function of membrane proteins.

Table 3 Human genes encoding lysosomal proteins or proteins involved in lysosomal function unassociated with disease

Human Gene	Protein	MIM Number	C. elegans Homologue	% g)	Mutant / CGC Name a)	RNAi and mutant phenotypes b)	KO or spontaneous mouse mutants
ABCA2	ATP binding cassette transporter 2	600047	Y39D8C.1	53	abt-4	M, R: None observed	
ABCA3	ATP binding cassette transporter 3	601615	Y39D8C.1	54	abt-4	M, R: None observed	
ABCA5	ATP binding cassette transporter 5		Y39D8C.1	45	abt-4	M, R: None observed	
ABCB9	ATP binding cassette transporter B9	605453	W04C9.1	41	haf-4	M: Glo; R: Emb Gro Let	
			F43E2.4	40	haf-2	M, R: None observed	
ACP2	Lysosomal acid phosphatase 2	171650	T13B5.3	33		R: None observed	nax, acp2 null
			B0361.7	32		R: None observed	
			F52E1.8	32		R: None observed	
ACP5	Acid phosphatase 5	171640	F02E9.7	36		R: None observed	
ACPT	Acid phosphatase, testicular	606362	B0361.7	34		R: None observed	
			F52E1.8	32		R: None observed	
			T21B6.2	32		R: None observed	
ACPP	Acid phospatase, prostate	171790	T13B5.3	32		R: None observed	
			R13H4.3	50		R: None observed	
AP3B2	AP-3 adaptor complex, subunit Beta 2	602166	R11A5.1	69	apb-3	M: ND; R: Emb, Let, Lva, Dpy, fat content reduced	
AP3D1	AP-3 adaptor complex, subunit Delta 1	607246	W09G10.4B	65	apd-3 (apt-5) f)	R: Emb Let Lva Dpy	mocha
AP3M1	AP-3 adaptor complex, subunit Mu1	610366	F53H8.1	80	apm-3 (apt-7) f)	M: Glo; R: Emb Let Lva Dpy fat content reduced	
AP3M2	AP-3 adaptor complex, subunit Mu2		F53H8.1	79	apm-3 (apt-7) f)		
AP3S1	AP-3 adaptor complex, subunit Sigma 1	601507	Y48G8AL.14	86	aps-3 (apt-8) f)	R: Emb Let Lva Dpy maternal sterile	
AP3S2	AP-3 adaptor complex, subunit Sigma 2	602416	Y48G8AL.14	88	aps-3 (apt-8) f)		
ARL8B	ADP-ribosylation factor-like 8B		Y57G11C.13	95	arl-8	R: Emb Let	
ARSD	arylsulfatase D	300002	Arylsulfatase family c)	57			
ASAHL	N-acylsphingosine amidohydrolase (acid ceramidase)- like	607469	Y55D5A.3	29		R: None observed	
ATP6AP1	ATPase, H+ transporting, lysosomal accessory protein 1	300197	Y55H10A.1	30	vha-19	M: Let or Ste; R: Ste Sck	
ATP6AP2	ATPase, H+ transporting, lysosomal accessory protein 2	300556	R03E1.2	23		R: Lva Emb Let Unc Lvl	

ATP6V0A1	ATPase, H+ transporting, lysosomal V0 subunit a isoform 1	192130	ZK637.8f	57	unc-32	M: Unc; R: Ste Pvl Sck Emb Let Unc Gro	
ATP6V0A2	ATPase, H+ transporting, lysosomal V0 subunit a isoform 2		ZK637.8	47	unc-32	M: Unc; R: Ste Pvl Sck Emb Let Unc Gro	
ATP6V0B	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit b	603717	T01H3.1	64	vha-4	R: Stp Emb Unc Lvl Clr	
ATP6V0C	ATPase, H+ transporting, lysosomal 16kDa, V0 Subunit c	108745	R10E11.2	67	vha-2	M: ND; R: Ste Emb Let,	
ATP6V0D1	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d1	607028	C30F8.2	75	vha-16 f)	R: Let Lvl Lva Emb Maternal sterile	
ATP6V0D2	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2		C30F8.2	81			
ATP6V0E1	ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e1	603931	F49C12.13	71	vha-17	M: ND; R: Ste Sck Emb Lvl Lva	
ATP6V0E2	ATPase, H+ transporting, V0 subunit e2		F49C12.13	75			
ATP6V1A	ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A	607027	Y49A3A.2	82	vha-13 f)	R: Emb Let Gro Lva Prl maternal sterile	
ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 Subunit c1	603097	Y38F2AL.3A	56	vha-11 f)	R: Ste Emb Let Gro small Sck Lvl Lva Stp maternal sterile decreased broodsize	
ATP6V1C2	ATPase, H+ transporting, lysosomal 42kDa, V1 Subunit c2		Y38F2AL.3A	50	vha-11 f)	R: Ste Emb Let Gro small Sck Lvl Lva Stp maternal sterile decreased broodsize	
ATP6V1D	ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D		F55H2.2	68	vha-14 f)	R: Emb Let Lva	
ATP6V1E1	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E1	108746	C17H12.14	58	vha-8 (pes-6)	M: ND; R: Ste Emb Let Gro Lva decreased broodsize	
ATP6V1E2	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E2		C17H12.14	57	vha-8 (pes-6)	M: ND; R: Ste Emb Let Gro Lva decreased broodsize	
ATP6V1F	ATPase, H+ transporting, lysosomal 14kDa, V1 subunit F	607160	ZK970.4	70	vha-9 f)	R: Emb Let Lvl Unc Lva maternal sterile	
ATP6V1G1	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G1	607296	F46F11.5	53	vha-10 f)	R: Emb Lvl Ste Etv	
ATP6V1G2	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G2	606853	F46F11.5	35	vha-10 f)	R: Emb Lvl Ste Etv	
ATP6V1G3	ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G3		F46F11.5	42	vha-10 f)	R: Emb Lvl Ste Etv	
ATP6V1H	ATPase, H+ transporting, lysosomal 50/57 kDa, V1 subunit H	608861	T14F9.1	45	vha-15 f)	R: Emb Let Lva Prl Lvl Bli	
BLOC1S1	Biogenesis of lysosome-related organelles complex 1 (BLOC1) subunit 1	601444	T20G5.10	67		R: None observed	
BLOC1S2	Biogenesis of lysosome-related organelles complex 1 (BLOC1) subunit 2	609768	Y73B6BL.30	62		R: None observed	
BLOC1S3	Biogenesis of lysosome-related organelles complex 1 (BLOC1) subunit 3	609672	None d)				
CD222	IGF2R	147280	F23D12.2	34		R: None observed	
CD63	Lysosomal membrane glycoprotein CD63/GP53	155740	T23D8.2	34	tsp-7	R: None observed	
			T14G10.6	27	tsp-12	M, R: None observed	
CD68	CD68	153634	Lectin family c)				
CLCN3	Chloride channel	600580	C07H4.2	45	clh-5	M: Let, Ste; R: mild endocytosis defect (compared to cup-5)	clcn3
CLCN6	Chloride channel	602726	R07B7.1	60	clh-6	M: ND; R: None observed	clcn6
CNO	cappuccino, homolog of mouse, BLOC1 subunit	605695	None				cappuccino

CPVL	carboxypeptidase, vitellogenic-like	609780	Serine carboxypeptidase family c)	54			
CTBS	Chitobiase	600873	T01C4.1	25		R: None observed	
CTSB	Cathepsin B	116810	F57F5.1 e)	52	?	M: ND; R: Emb Unc Lva	ctsb
			W07B8.5	48	cpr-5 f)	R: None observed	
			C52E4.1	51	cpr-1 (gcp-1)	M: ND; R: None observed	
			F44C4.3	36	cpr-4	M: ND; R: None observed	
			C25B8.3	49	cpr-6	M, R: None observed	
			W07B8.4	49		R: None observed	
			W07B8.1	43		R: None observed	
			F36D3.9	45	cpr-2 f)	R: None observed	
			T10H4.12	45	cpr-3	M: ND: R: Emb Let	
			F32H5.1	40		R: Gro	
			Y65B4A.2	42		R: None observed	
CTSF	Cathepsin F	603539	F41E6.6	49	tag-196	M: mild Unc Egl; R: None observed	ctsf
			R09F10.1	35		R: None observed	
			R07E3.1	35	?	M: ND; R: None observed	
CTSH	Cathepsin H	116820	K02E7.10	37		R: None observed	
			C50F4.3	30	tag-329	M: Gro Lvl decreased broodsize; R: None observed	
			Y113G7B.15	33		R: None observed	
CTSL	Cathepsin L	116880	T03E6.7	49	cpl-1	M: ND; R: Emb Let Gro Unc	ctsl
			Y51A2D.8	32		R: None observed	
CTSL2	Cathepsin L2	603308	Y71H2AR.2	38		R: None observed	
CTSS	Cathepsin S	116845	Y40H7A.10	33	?	M: ND; R: None observed	
			F15D4.4	30		R: None observed	
CTSZ	Cathepsin Z	603169	F32B5.8	55	cpz-1	M: ND; R: Emb Let Lva Bmd Mlt maternal sterile	
			M04G12.2	50	cpz-2	M: ND, R: None observed	
DNASE2	Deoxyribonuclease II, lysosomal	126350	F09G8.2	34	tag-198	M, R: None observed	
ENTPD4	ectonucleoside triphosphate diphosphorylase 4	607577	C33H5.14 c)	32	ntp-1 f)	R: None observed	
GGH	gamma-glutamyl hydrolase	601509	None				
HLA-DMA	major histocompatibility complex, class II, DM alpha	142855	None				
HLA-DMB	major histocompatibility complex, class II, DM beta	142856	None				
HYAL2	Hyaluronidase 2	603551	T22C8.2	31		R: None observed	
IFI30	Interferon-inducible protein 30	604664	F37H8.5	51		R: None observed	
			C02D5.2	50		R: None observed	
LAMP1	Lysosomal-associated membrane protein 1	153330	C05D9.2	36	lmp-2 f)	R: None observed	
LAMP3	Lysosomal-associated membrane protein 3	605883	Lectin family c)				
LAPTM4A	lysosomal associated protein transmembrane 4A		C05E11.3	46		R: None observed	
			F23D12.1	50		R: None observed	
LAPTM4B	lysosomal associated protein transmembrane 4B		C05E11.3	45		R: None observed	
LAPTM5	Lys.-assoc. multispanning membrane protein-5	601476	C05E11.3	28		R: None observed	
LGMN/ PRSC1	legumain; hydrolysis of asparaginyl bonds	602620	T28H10.3	42		R: Emb Let	

LRP2	Glycoprotein 330	600073	F29D11.1	30	lrp-1	M: Lvl Mlt; R: Gro Prl Mlt Unc Lvl Bmd Dpy Lva Rup Mig	
LYPLA3	lysophospholipase 3 (lysosomal phospholipase A2)	609362	M05B5.4	46		R: fat content reduced	
MUTED	muted, homolog of mouse, BLOC1 subunit	607289	None				muted
NAGPA	N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase	607985	EGF-like domain family c)				
OSBP	Oxysterol binding protein	167040	Y47D3A.17	64	obr-1 f)	R: Emb Let	
PAQR8	progestin and adipoQ receptor family member VIII	607780	K11C4.2	26		R: None observed	
PCYOX1	prenylcysteine oxidase 1		None				
PLDN	pallidin, BLOC-1 subunit	604310	None				
PPT2	Palmitoyl-protein thioesterase 2 precursor	603298	F44C4.5	51	ppt-1	M: delayed egg-laying , abnormal mitochondria; R: None observed	
PRCP	prolylcarboxypeptidase	176785	ZK112.1	41	pcp-1 f)	R: None observed	
PRDX6	peroxiredoxin 6; Involved in redox regulation of the cell	602316	Y38C1AA.11	50	prdx-6 f)	R: ND	
PRSS16	protease, serine 16 (thymus)	607169	K12H4.7A	48		R: None observed	
			C26B9.5	44		R: None observed	
			F19C7.2	45		R: None observed	
			F19C7.4	43		R: None observed	
			F23B2.11	46	pcp-3 f)	R: None observed	
			F23B2.12	45	pcp-2	M: ND, R: None observed	
RAB7A	Ras oncogene family member RAB7A	602298	W03C9.3	86	rab-7	M: ND; R: Emb Let Bmd Dpy Prl Ced fat content reduced	
SCARB2	Lysosomal integral membrane protein II (CD36L2/LGP85/ LIMP II)	602257	Y49E10.20	28	?	M: ND; R: Pvl maternal sterile	scarb2
			Y76A2B.6	28	?	M: ND; R: None observed	
SLC30A2	solute carrier family 30 member 2, Zinc transporter	609617	T18D3.3	69	?	M: ND, R: None observed	
SLC36A1	solute carrier family 36 member 1	606561	T27A1.5	34		R: RIC	
SNAPAP	snapin, SNARE and BLOC-1 component	607007	C02B10.2	51	?	M: ND; R: Emb	
SNX1	sorting nexin 1	601272	C05D9.1	59	snx-1	M: Egl, slightly bloated, HSN migration defects; R: None observed	
STX7	syntaxin 7	603217	F36F2.4	58	syn-13	M: None observed; R: Emb Let	
STX12	syntaxin 12	606892	F36F2.4	58			
SYBL1/ VAMP7	synaptobrevin-like 1	300053	Y69A2AR.6	53		R: None observed	
SYT7	synaptotagmin 7	604146	C08G5.4	62	snt-6	R: None observed	
TMEM9	transmembrane protein 9. Involved in intracellular transport endosomes and lysosomes		R12C12.6	55		R: None observed	

a) ? Deletion mutant, gene name unknown
b) Phenotype abbrevations: Bli: blistered; Bmd: organism morphology abnormal, Ced: cell death abnormal; Clr: clear; Dpy: dumpy; Egl: egg laying defect; Emb: embryonal lethal; Glo: gut fluorescence loss; Gro: slow growth; Lva: larval arrest; Lvl: larval lethal;
Mig: cell migration abnormal; Mlt: molting defect; Prl: paralyzed; Pvl: protruding vulva; Ric: aldicarb resistant; Rup: exploded through vulva; Sck: sick; Ste: sterile; Stp: sterile progeny; Unc: uncoordinated
c) BLAST hit > 1 e-5, but no reciprocal BLAST hits; when given: similarity with highest hit
d) None: Blast hit < 1 e-5
e) Due to similarity of cathepsin protein sequences, different homologous pairs are shown
f) Gene name assigned, but no mutant available
g) % Similarity to human gene

Comparison of phenotypes related to *C. elegans* loci affecting lysosomal function

Mutation or RNAi knockdown phenotypes for each of the worm loci presumed to be involved in lysosomal function and missing from the tables presented above were mainly collected using the following criteria: changes should occur in either the gut autofluorescence caused by secondary lysosomes, or size, number or electron density of the lysosomal vesicles (Table 4). In addition, specific phenotypes, such as the cup phenotype, which are associated with at least one known lysosomal protein, were included. Following the initial screens, genetic mapping has led to the identification of several genes involved in the Cup and Glo phenotypes. The loci *cup-2*, *cup-4*, *glo-4* and *glo-5* were found to encode homologues to *DERL1*, *CHRNB1*, *HERC4* and *ABCB1*, respectively [80], [55], [83]. In addition, three existing *rme* mutants displayed the Cup phenotype [80], whereas five known genes were involved in the Glo phenotype [55] (Table 4). These genes encode proteins with functions in other locations within the cell. Many of these proteins probably play an important role in the processes discussed in the introduction: lysosome biogenesis, maintenance of endosomal transport and lysosomal enzyme targeting. The associated phenotypes vary in severity, but are relatively subtle and more similar to those in Table 1 than compared to those in Tables 2 and 3.

Table 4 *C. elegans* phenotypes associated with changes in lysosomal function

Mutant or RNAi phenotype	Primary gene Associated with Lysosomal Phenotype	C. elegans Locus Name	Gene Name	Human Homologue	% a)	Function of (human) protein	MIM Number	References
coelomocyte uptake defect,	cup-5		R13A5.1	MCOLN1	55	Mucolipin-1	605248	Nature Genet 28: 64 (2001)
accumulation of refractile cell corpses		cup-1, cup-3,	?	?				Genetics159: 133 (2001)
		cup-6 - cup-11						PNAS 99: 4355 (2002)
		cup-2	F25D7.1	DERL1	65	Misfolded protein degradation in ER	608813	
		cup-4	C02C2.3	CHRNB1	43	acetylcholine receptor	100710	
		rme-1	W06H8.1	EHD1	81	intracellular sorting	605888	Genetics159: 133 (2001)
		rme-6	F49E7.1	GAPVD1	52	Rab5-activating protein 6, endocytosis		Genetics159: 133 (2001)
		rme-8	F18C12.2	DNAJC13	64	DnaJ (Hsp40) homolog		Genetics159: 133 (2001)
gut granule biosynthesis defect	glo-1							
		glo-1	R07B1.12	RAB32	66	Ras oncogen family member RAB32		
		glo-3	?	?				
		glo-4	F07C3.4	HERC4	43	hect domain and RLD 4, ubiquitin ligase		
		pgp-2 (glo-5)	C34G6.4	ABCB1	66	ATP binding cassette transporter B1	171050	Mol Biol Cell 18: 995 (2007)
		haf-4	W04C9.1	ABCB9	41	ATP binding cassette transporter B9	605453	

		apt-6	R11A5.1	AP3B1 (HPS2)	56	AP-3 complex subunit beta-1	603401	
		apt-7	F53H8.1	AP3M1	80	AP-3 complex, subunit Mu1	610366	
		vps-16	C05D11.2	VPS16	44	Vacuolar protein sorting-associated protein	608550	
						16 homolog		
		vps-41	F32A6.3	VPS41	51	Vacuolar protein sorting-associated protein	605485	
						41 homolog		
loss of one type of intestinal granule	lmp-1		C03B1.12	LAMP1	57	Lysosomal-associated membrane protein	153330	J Cell Sci 113: 2595 (2000)
vacuolated intestinal cells, egg retention,	cad-1		?	?				Genetics 119: 355 (1988)
reduced aspartic protease activity,								J Biol Chem 275: 26359 (2000)
enzyme processing defect?								
slow development, premature egg laying	ncr-1		F02E8.6	NPC1	46	Niemann-Pick C1 protein precursor	257220	Curr Biol 10: 527 (2000)
dauer-constitutive (Daf-c) phenotype	ncr-1; ncr-2		F09G8.4	NPC1	47	Niemann-Pick C1 protein precursor	257220	Curr Biol 10: 527 (2000)
DNA in intestinal lumen not degraded	nuc-1		C07B5.5	DNASE2B	54	deoxyribonuclease II beta		Genetics 129: 79 (1991)
major endodeoxyribonuclease reduced >95%								Science 220: 1277 (1983)
condensed chromatin persists after apoptosis								
acc of enlarged lysosomes, paralysis during 1st larval	idi-1		K06H7.9	IDI1	56	isopentenyldiphosphate isomerase	604055	Mol Gen Genomics 273: 158 (2005)
stage, larval arrest, persistent apoptotic corpses								
coiler; very sluggish, moves poorly; slightly Egl	unc-101	unc-101	K11D2.3	AP1M1	88	AP-1 complex, subunit Mu1	603535	Genes Dev. 8: 60 (1994)
slightly short; defecation defects,								
abnormal FITC staining; subviable.								
Abnormal adult males tails and spicules								

a) % Similarity to human gene

Summary and conclusion

Most of the human genes involved in lysosomal storage disorders have single *C. elegans* homologues (Table 1). A few human genes have more than one worm counterpart and vice versa. The reason for the existence of multiple worm homologues of *SMPD1* and *CLN3* is unclear, although temporal and spatial expression patterns differ for the *asm* and *cln-3* paralogues [32], [67]. The representation of three human enzyme-encoding gene pairs, *HEXA-HEXB*, *GLA-NAGA* and *GALNS-ARSA*, by single worm homologues might be considered as an indication of simpler biochemical pathways in the worm. The worm GANA-1 homologue combines the GLA and NAGA enzyme activities into one multifunctional enzyme [84]. Still, from several genes (e.g., 6 *HPS* and 4 *CLN* genes) involved in human lysosomal storage disorders no worm counterparts could be identified using BLAST searches with protein sequences. It is tempting to speculate that this is associated in part with the lack of cell types with specialized lysosome-related cell organelles, such as melanosomes, in the worm. This may also explain why the worm lacks four subunits of the BLOC1 complex involved in the biogenesis of lysosome-related organelles (Table 3). Furthermore, due the complexity of the human brain, man may require a larger repertoire of CLN proteins, some of which have additional roles in synaptic vesicles. In general, however, the proteins necessary for normal lysosomal function seem to be conserved between man and worm.

Mutation or RNAi knockdown phenotypes were available for 147 of the 149 worm genes listed in Tables 1-3. The phenotypes ranged from embryonic lethality via subtle intermediate phenotypes to none observed. If observed, mutant and RNAi knockdown phenotypes of lysosomal storage disease related worm homologues (Table 1) or lysosomal worm mutants (Table 4) seem to be more subtle than those of homologues, which are not yet associated with lysosomal storage disease (Table 3).

The worm models of human lysosomal storage disorders have diverse phenotypes ranging from seemingly apparent lysosomal nature, such as the endocytosis defect in *cup-5* mutants or *lmp-1* mutants that have lost one type of intestinal granule presumed to be pre-lysosomes, to phenotypes that appear less directly related to lysosomes, such as the disturbed cholesterol trafficking of the *ncr* mutants, developmental arrest or decreased life span or brood size. Whether the latter phenotypes are directly caused by defective lysosomal processes in the worm remains to be determined. Alternatively, the developmental defects and embryonic lethality could also be caused by preceding defects, e.g., in endosomal transmembrane protein sorting in *apb-3* mutants [54].

Severe phenotypes, such as (embryonic) lethality and larval arrest, are observed for genes encoding parts of the vacuolar proton pump. An explanation might be that the latter genes play an important role in multiple processes and organelles and that their inactivation leads to pleiotropic effects, having larger impact on the worm and thus creating a more severe phenotype. In man, disruption of these genes might also have

severe pleiotropic effects. The existence of multiple copies of vacuolar proton pump genes, however, might result in sufficient redundancy to prevent severe effects unless multiple genes are hit.

Many other nematode homologues are on the other end of the spectrum and lack mutant and RNAi knockdown phenotypes. One explanation is that other proteins with a redundant function complement the effect of the genetic lesion or knockdown. Alternatively, most of the mutants were tested under standard laboratory conditions, which are presumed to be ideal growth conditions evading the possible stress that may be required for the manifestation of a phenotype. Furthermore, the life span of worms may be too short to reveal a phenotype. In addition, even when a knock-out mutation would cause a phenotype, RNAi knock-down of gene function will not always result in a phenotype [85]. Last but not least, much of the data was generated in regular genome-wide RNAi screens, which yield a mass of data potentially providing a starting point for the investigation of any individual gene, but present the researcher with the risk to overlook certain subtle phenotypes. Although coelomocyte uptake (Cup) and loss of gut autofluorescence (Glo) phenotypes are relatively easy to score using fluorescence microscopy [80], [55], these were not scored in the genome-wide RNAi screens. Determining a difference in number, size or electron density of the lysosomal vesicles (Lmp-1, Idi phenotypes) however, requires time-consuming ultrastructural investigations. Detection of such intricate phenotypes is usually not included in regular genome-wide RNAi screens.

From the compilation presented here, we may conclude that the nematode *C. elegans* has largely the same number of genes involved in lysosomal processes as man and that the worm is a suitable model organism to investigate lysosomal storage diseases. It became clear that some of the specific phenotypes associated with changes in lysosomal proteins can also be caused by defects in proteins, which do not localize to lysosomes, but exert their function in lysosome biogenesis, maintenance of endosomal transport and lysosomal protein targeting. Their human homologues might be good candidates for disorders with lysosomal storage or lysosomal dysfunction without known cause. The identification of additional worm mutants by genetic screens would help to identify new players in the different processes necessary for normal lysosomal function and potential new candidates for human hereditary disorders. The advantage of the most observed worm phenotypes in Table 3, developmental arrest or lethality in any life stage, is the relative ease with which genetic screens can be done for modifiers of these robust phenotypes. The nature of the mutant, genetic or RNAi knockdown, also determines the screenability of modifier genes as well as the penetrance of the phenotype. The subtle phenotypes observed in many of the lysosomal mutants of the worm, however, suggest that it may not be trivial to perform screens for new mutants.

Acknowledgments

This work was financially supported by the Center for Biomedical Genetics.

References

[1] C. De Duve, The lysosome, *Sci Am*. **208** (1963), pp. 64-72.

[2] E.L. Eskelinen, Y. Tanaka, P. Saftig, At the acidic edge: emerging functions for lysosomal membrane proteins, *Trends Cell Biol*. **3** (2003), pp. 137-45.

[3] C. Mullins and J.S. Bonifacino, The molecular machinery for lysosome biogenesis. *Bioessays.* **4** (2001), pp. 333-43.

[4] J.P. Luzio, V. Poupon, M.R. Lindsay, B.M. Mullock, R.C. Piper, P.R. Pryor, Membrane dynamics and the biogenesis of lysosomes. *Mol Membr Biol.* **2** (2003), pp. 141-54.

[5] C.R. Scriver and W.S. Sly WS (Eds.), *The metabolic and molecular basis of inherited disease*, 8th edition, McGraw-hill, Inc., New York, (2000).

[6] J.C. Pompe, Over idiopathische hypertrophie van het hart. Ned Tijdschr Geneeskd. **76** (1932), pp. 304-312.

[7] R. Hirschhorn and A.J.J. Reuser, Glycogen storage disease type II: Acid α-Glucosidase (acid maltase) deficiency, in: C.R. Scriver, W.S. Sly (Eds.), *The metabolic and molecular bases of inherited disease*, 8th edition, McGraw-Hill Inc., New York (2001), pp. 3389-3420.

[8] S. Kornfeld and W.S. Sly, I-cell disease and pseudo-Hurler polydystrophy: Disorders of lysosomal enzyme phosphorylation and localization, in: C.R. Scriver, W.S. Sly (Eds.), *The metabolic and molecular bases of inherited disease*, 8th edition, McGraw-Hill Inc., New York, (2001), pp. 3421-3452.

[9] A. Vellodi, Lysosomal storage disorders. *Br J Haematol.* **128** (2005), pp. 413-31.

[10] J. Rapola, Neuronal ceroid-lipofuscinoses in childhood. *Perspect Pediatr Pathol.***17** (1993), pp. 7-44

[11] R. Schiffmann and R.O. Brady, New prospects for the treatment of lysosomal storage diseases. *Drugs.* **62** (2002), pp. 733-42.

[12] A.H. Futerman and G. van Meer, The cell biology of lysosomal storage disorders. *Nat Rev Mol Cell Biol.* **7** (2004), pp. 554-65.

[13] S. Brenner, The genetics of Caenorhabditis elegans. *Genetics.* **77** (1974), pp. 71-94.

[14] P.E. Kuwabara and N. O'Neil, The use of functional genomics in *C. elegans* for studying human development and disease. *J Inherit Metab Disease.* **24** (2001), pp. 127-138.

[15] WormBase web site, http://www.wormbase.org, release WS173, date March 23, 2007

[16] S.L. Kim, J. Lund, M. Kiraly, K. Duke, M. Jiang, J.M. Stuart, A. Eizinger, B.N. Wylie, G.S. Davidson, A gene expression map for Caenorhabditis elegans. *Science.* **293** (2001), pp. 2087-92.

[17] R.S. Kamath, A.G. Fraser, Y. Dong, G. Poulin, R. Durbin, M. Gotta, A. Kanapin, N. Le Bot, S. Moreno, M. Sohrmann, D.P. Welchman, P. Zipperlen, J. Ahringer, Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. *Nature.* **421** (2003), pp. 231-7.

[18] R. Bargal, N. Avidan, E. Ben-Asher, Z. Olender, M. Zeigler, A. Frumkin, A. Raas-Rothschild, G. Glusman, D. Lancet, G. Bach, Identification of the gene causing mucolipidosis type IV. *Nat Genet.* **26** (2000), pp. 118-23.

[19] M.T. Bassi, M. Manzoni, E. Monti, M.T. Pizzo, A. Ballabio, G. Borsani, Cloning of the gene encoding a novel integral membrane protein, mucolipidin-and identification of the two major founder mutations causing mucolipidosis type IV. *Am J Hum Genet.* **67** (2000), pp. 1110-20.

[20] M. Sun, E. Goldin, S. Stahl, J.L. Falardeau, J.C. Kennedy, J.S. Acierno Jr, C. Bove, C.R. Kaneski, J. Nagle, M.C. Bromley, M. Colman, R. Schiffmann, S.A. Slaugenhaupt, Mucolipidosis type IV is caused by mutations in a gene encoding a novel transient receptor potential channel. *Hum Mol Genet.* **9** (2000), pp. 2471-8.

[21] M.K. Raychowdhury, S. Gonzalez-Perrett, N. Montalbetti, G.A. Timpanaro, B. Chasan, W.H. Goldmann, S. Stahl, A. Cooney, E. Goldin, H.F. Cantiello, Molecular pathophysiology of mucolipidosis type IV: pH dysregulation of the mucolipin-1 cation channel. *Hum Mol Genet.* **13** (2004), pp. 617-27.

[22] B.M. Hersh, E. Hartwieg, H.R. Horvitz, The Caenorhabditis elegans mucolipin-like gene cup-5 is essential for viability and regulates lysosomes in multiple cell types. *Proc Natl Acad Sci U S A.* **99** (2002), pp. 4355-60.

[23] H. Fares, I. Greenwald, Regulation of endocytosis by CUP-5, the Caenorhabditis elegans mucolipin-1 homolog. *Nat Genet.* **28** (2001), pp. 64-8.

[24] S. Treusch, S. Knuth, S.A. Slaugenhaupt, E. Goldin, B.D. Grant, H. Fares H, Caenorhabditis elegans functional orthologue of human protein h-mucolipin-1 is required for lysosome biogenesis. *Proc Natl Acad Sci U S A.* **101** (2004), pp. 4483-8.

[25] L. Schaheen, H. Dang, H. Fares,. Basis of lethality in C. elegans lacking CUP-5, the Mucolipidosis Type IV orthologue. *Dev Biol.* **293** (2006), pp. 382-91.

[26] J.M. LaPlante, J. Falardeau, M. Sun, M. Kanazirska, E.M. Brown, S.A. Slaugenhaupt, P.M. Vassilev, Identification and characterization of the single channel function of human mucolipin-1 implicated in mucolipidosis type IV, a disorder affecting the lysosomal pathway. *FEBS Lett.* **532** (2002), pp. 183-7.

[27] P.R. Pryor, B.M. Mullock, N.A. Bright, S.R. Gray, J.P. Luzio, The role of intraorganellar Ca(2+) in late endosome-lysosome heterotypic fusion and in the reformation of lysosomes from hybrid organelles. *J Cell Biol.* (2000), pp. 1053-62.

[28] L. Schaheen, G. Patton, H. Fares H, Suppression of the cup-5 mucolipidosis type IV-related lysosomal dysfunction by the inactivation of an ABC transporter in C. elegans. *Development.* **133** (2006), pp. 3939-48

[29] M.T. Vanier and K. Suzuki, Niemann–Pick disease, in: *Neurodystrophies and Neurolipidioses*, H.W. Moser (Eds.), vol. 66, Handbook of Clinical Neurology, Elsevier B.V., Amsterdam, 1996, pp. 133–162.

[30] E.H. Schuchman, R.J. Desnick Type A and B Niemann–Pick disease: deficiencies of acid sphingomyelinase activity, in: C.R. Scriver, W.S. Sly (Eds.), *The metabolic and molecular bases of inherited disease*, 8th edition, McGraw-Hill Inc., New York (2001), pp. 3589–3610.

[31] E.D. Carstea, J.A. Morris, K.G. Coleman, S.K. Loftus, D. Zhang, C. Cummings, J. Gu, M.A. Rosenfeld, W.J. Pavan, D.B. Krizman, J. Nagle, M.H. Polymeropoulos , S.L. Sturley, Y.A. Ioannou, M.E. Higgins, M. Comly, A. Cooney, A. Brown, C.R. Kaneski, E.J. Blanchette-Mackie, N.K. Dwyer, E.B. Neufeld, T.Y. Chang, L. Liscum, J.F. Strauss 3rd, K. Ohno, M. Zeigler, R. Carmi, J. Sokol, D. Markie, R.R. O'Neill, O.P. van Diggelen, M. Elleder, M.C. Patterson, R.O. Brady, M.T. Vanier, P.G. Pentchev, D.A. Tagle, Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. *Science.* **277** (1997), pp. 228-231.

[32] X. Lin, M.O. Hengartner, R. Kolesnick, Caenorhabditis elegans contains two distinct acid sphingomyelinases. *J Biol Chem.* **273** (1998), pp. 14374-9.

[33] S. Naureckiene, D.E. Sleat, H. Lackland, A. Fensom, M.T. Vanier, R. Wattiaux, M. Jadot, P. Lobel, Identification of HE1 as the second gene of Niemann-Pick C disease. *Science.* **290** (2000), pp. 2298-2301.

[34] E.N. Neufeld, M. Wastney, S. Patel, S. Suresh, A.M. Cooney, N.K. Dwyer, C.F. Roff, K. Ohno, J.A. Morris, E.D. Carstea, J.P. Incardona, J.F. Strauss 3rd, M.T. Vanier, M.C. Patterson, R.O. Brady, P.G. Pentchev, E.J. Blanchette-Mackie, The Niemann-Pick C1 protein resides in a vesicular compartment linked to retrograde transport of multiple lysosomal cargo. *J Biol Chem.* **274** (1999), pp. 9627-35.

[35] M. Sym, M. Basson, C. Johnson, A model for Niemann-Pick type C disease in the nematode Caenorhabditis elegans. *Curr Biol.* **10** (2000), pp. 527-30.

[36] J. Li, G. Brown, M. Ailion, S. Lee, J.H. Thomas NCR-1 and NCR-2, the C. elegans homologs of the human Niemann-Pick type C1 disease protein, function upstream of DAF-9 in the dauer formation pathways. *Development.* **131** (2004), pp. 5741-52.

[37] B. Gerisch and A. Antebi, Hormonal signals produced by DAF-9/cytochrome P450 regulate C. elegans dauer diapause in response to environmental cues. *Development.* **131** (2004), pp. 1765-76.

[38] H.Y. Mak and G. Ruvkun Intercellular signaling of reproductive development by the C. elegans DAF-9 cytochrome P450. *Development.* **131** (2004), pp. 1777-86.

[39] I. Nishino, J. Fu, K. Tanji, T. Yamada, S. Shimojo, T. Koori, M. Mora, J.E. Riggs, S.J. Oh, Y. Koga, C.M. Sue, A. Yamamoto, N. Murakami, S. Shanske, E. Byrne, E. Bonilla, I. Nonaka, S. DiMauro, M. Hirano, Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). *Nature.* **406** (2000), pp. 906-10.

[40] M.J. Danon, S.J. Oh, S. DiMauro, J.R. Manaligod, A. Eastwood, S. Naidu, L.H. Schliselfeld, Lysosomal glycogen storage disease with normal acid maltase. *Neurology.* **31** (1981), pp. 51-7.

[41] N. Andrejewski, E.L. Punnonen, G. Guhde, Y. Tanaka, R. Lullmann-Rauch, D. Hartmann, K. von Figura, P. Saftig, Normal lysosomal morphology and function in LAMP-1-deficient mice. *J Biol Chem.* **274** (1999), pp. 12692-701.

[42] Y. Tanaka, G. Guhde, A. Suter, E.L. Eskelinen, D. Hartmann, R. Lullmann-Rauch, P.M. Janssen, J. Blanz, K. von Figura, P. Saftig, Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature.* **406** (2000), pp. 902-6.

[43] J. Stypmann, P.M. Janssen, J. Prestle, M.A. Engelen, H. Kogler, R. Lullmann-Rauch, L. Eckardt, K. von Figura, J. Landgrebe, A. Mleczko, P. Saftig, LAMP-2 deficient mice show depressed cardiac contractile function without significant changes in calcium handling. *Basic Res Cardiol.* **101** (2006), pp. 281-91.

[44] M. Kostich, A. Fire, D.M. Fambrough, Identification and molecular-genetic characterization of a LAMP/CD68-like protein from Caenorhabditis elegans. *J Cell Sci.* **113** (2000), pp. 2595-606.

[45] E.M. Jorgensen, S.E. Mango, The art and design of genetic screens: Caenorhabditis elegans. *Nat Rev Genet.* **3** (2002), pp. 356-69.

[46] J. Oh, L. Ho, S. Ala-Mello, D. Amato, L. Armstrong, S. Bellucci, G. Carakushansky, J.P. Ellis, C.T. Fong, J.S. Green, E. Heon, E. Legius, A.V. Levin, H.K. Nieuwenhuis, A. Pinckers, N. Tamura, M.L. Whiteford, H. Yamasaki, R.A. Spritz, Mutation analysis of patients with Hermansky-Pudlak syndrome: a frameshift hot spot in the HPS gene and apparent locus heterogeneity. *Am J Hum Genet.* **62** (1998), pp. 593-8.

[47] N.V. Morgan, S. Pasha, C.A. Johnson, J.R. Ainsworth, R.A. Eady, B. Dawood, C. McKeown, R.C. Trembath, J. Wilde, S.P. Watson, E.R. Maher, A germline mutation in BLOC1S3/reduced pigmentation causes a novel variant of Hermansky-Pudlak syndrome (HPS8). *Am J Hum Genet.* **78** (2006), pp. 160-6.

[48] S.M. Di Pietro and E.C. Dell'Angelica, The cell biology of Hermansky-Pudlak syndrome: recent advances. *Traffic.* **6** (2005), pp. 525-33.

[49] R. Nazarian, J.M. Falcon-Perez, E.C. Dell'Angelica, Biogenesis of lysosome-related organelles complex 3 (BLOC-3): a complex containing the Hermansky-Pudlak syndrome (HPS) proteins HPS1 and HPS4. *Proc Natl Acad Sci U S A.* **100** (2003), pp. 8770-5.

[50] J.A. Martina, K. Moriyama, J.S. Bonifacino, BLOC-3, a protein complex containing the Hermansky-Pudlak syndrome gene products HPS1 and HPS4. *J Biol Chem.* **278** (2003), pp. 29376-84.

[51] E.C. Dell'Angelica, V. Shotelersuk, R.C. Aguilar, W.A. Gahl, J.S. Bonifacino, Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta-3A subunit of the AP-3 adaptor. *Molec. Cell.* **3** (1999), pp. 11-21.

[52] A.A. Peden, V. Oorschot, B.A. Hesser, C.D. Austin, R.H. Scheller RH, J. Klumperman, Localization of the AP-3 adaptor complex defines a novel endosomal exit site for lysosomal membrane proteins. *J Cell Biol.* **164** (2004), pp. 1065-76.

[53] S. Fontana, S. Parolini, W. Vermi, S. Booth, F. Gallo, M. Donini, M. Benassi, F. Gentili, D. Ferrari, L.D. Notarangelo, P. Cavadini, E. Marcenaro, S. Dusi, M. Cassatella, F. Facchetti, G.M. Griffiths, A. Moretta, L.D. Notarangelo, R. Badolato, Innate immunity defects in Hermansky-Pudlak type 2 syndrome. *Blood.* **107** (2006), pp. 4857-64

[54] J. Shim and J. Lee, The AP-3 clathrin-associated complex is essential for embryonic and larval development in Caenorhabditis elegans. *Mol Cells.* **19** (2005), pp. 452-7.

[55] G.J. Hermann, L.K. Schroeder, C.A. Hieb, A.M. Kershner, B.M. Rabbitts, P. Fonarev, B.D. Grant, J.R. Priess, Genetic analysis of lysosomal trafficking in Caenorhabditis elegans. *Mol Biol Cell.* **16** (2005), pp. 3273-88.

[56] G.V. Clokey and L.A. Jacobson, The autofluorescent "lipofuscin granules" in the intestinal cells of Caenorhabditis elegans are secondary lysosomes. *Mech Ageing Dev.* **35** (1986), pp. 79-94.

[57] E. Siintola, S. Partanen, P. Stromme, A. Haapanen, M. Haltia, J. Maehlen, A.E. Lehesjoki, J. Tyynela, Cathepsin D deficiency underlies congenital human neuronal ceroid-lipofuscinosis. *Brain.* **129** (2006), pp. 1438-1445.

[58] J. Vesa, E. Hellsten, L.A. Verkruyse, L.A. Camp, J. Rapola, P. Santavuori, S.L. Hofmann, L. Peltonen, Mutations in the palmitoyl protein thioesterase gene causing infantile neuronal ceroid lipofuscinosis. *Nature.* **376** (1995), pp. 584–587.

[59] IBDC, Isolation of a novel gene underlying Batten disease, CLN3. The International Batten Disease Consortium. *Cell.* **82** (1995), pp. 949-57.

[60] S.E. Mole, R.E. Williams, H.H. Goebel, Correlations between genotype, ultrastructural morphology and clinical phenotype in the neuronal ceroid lipofuscinoses. *Neurogenetics.* **6** (2005), pp. 107-26.

[61] J. Tyynela, I. Sohar, D.E. Sleat, R.M. Gin, R.J. Donnelly, M. Baumann, M. Haltia, P. Lobel, A mutation in the ovine cathepsin D gene causes a congenital lysosomal storage disease with profound neurodegeneration. *EMBO J.* **19** (2000), pp. 2786-92.

[62] M. Yanagawa, T. Tsukuba, T. Nishioku, Y. Okamoto, K. Okamoto, R. Takii, Y. Terada, K.I. Nakayama, T. Kadowaki, K. Yamamoto, Cathepsin E deficiency induces a novel form of lysosomal storage disorder showing the accumulation of lysosomal membrane sialoglycoproteins and the elevation of lysosomal pH in macrophages. *J Biol Chem.* **282** (2007), pp. 1851-62.

[63] P. Syntichaki, K. Xu, M. Driscoll, N. Tavernarakis, Specific aspartyl and calpain proteases are required for neurodegeneration in C. elegans. *Nature.* **419** (2002), pp. 939-44.

[64] S.J. Kim, Z. Zhang, E. Hitomi, Y.C. Lee, A.B. Mukherjee, Endoplasmic reticulum stress-induced caspase-4 activation mediates apoptosis and neurodegeneration in INCL. *Hum Mol Genet.* **15** (2006), pp. 1826-34.

[65] M. Lehtovirta, A. Kyttälä, E.L. Eskelinen, M. Hess, O. Heinonen, A. Jalanko, Palmitoyl protein thioesterase (PPT) localizes into synaptosomes and synaptic vesicles: implications for infantile neuronal ceroid lipofuscinosis (INCL). *Hum Mol Gen.* **10** (2001), pp. 69–75.

[66] M.Y. Porter, M. Turmaine, S.E. Mole, Identification and characterization of Caenorhabditis elegans palmitoyl protein thioesterase1. *J Neurosci Res.* **79** (2005), pp. 836-48.

[67] G. De Voer, P. van der Bent, A.J. Rodrigues, G.J. van Ommen, D.J. Peters, P.E. Taschner, Deletion of the Caenorhabditis elegans homologues of the CLN3 gene, involved in human juvenile neuronal ceroid lipofuscinosis, causes a mild progeric phenotype. *J Inherit Metab Dis.* **28** (2005) pp. 1065-80.

[68] S.L. Teitelbaum, Y. Abu-Amer, F.P. Ross Molecular mechanisms of bone resorption. *J Cell Biochem.* **59** (1995), pp. 1–10.

[69] M.A. Monroy, F.P. Ross, S.L. Teitelbaum, M.S. Sands, Abnormal osteoclast morphology and bone remodeling in a murine model of a lysosomal storage disease. *Bone.* **30** (2002), pp. 352-9.

[70] A.S. Nicot, H. Fares, B. Payrastre, A.D. Chisholm, M. Labouesse, J. Laporte, The Phosphoinositide Kinase PIKfyve/Fab1p Regulates Terminal Lysosome Maturation in Caenorhabditis elegans. *Mol Biol Cell.* **17** (2006), pp. 3062-3074.

[71] C.E. Fisherand S.E. Howie, The role of megalin (LRP-2/Gp330) during development. *Dev Biol.* **296** (2006), pp. 279-297.

[72] J. Yochem, S. Tuck, I. Greenwald, M. Han, A gp330/megalin-related protein is required in the major epidermis of Caenorhabditis elegans for completion of molting. *Development.* **126** (1999), pp. 597-606.

[73] J.M. Kramer, Extracellular matrix, in: C. elegans II, D.L. Riddle, T. Blumenthal, B.J. Meyer, J.R. Priess (Eds.) Cold Spring Harbor Laboratory Press, New York, (1997), pp. 471-500.

[74] N. Roudier, C. Lefebvre, R. Legouis. CeVPS-27 is an endosomal protein required for the molting and the endocytic trafficking of the low-density lipoprotein receptor-related protein 1 in Caenorhabditis elegans. *Traffic.* **6** (2005), pp. 695-705.

[75] A.M. Lennon-Dumenil, A.H. Bakker, R. Maehr, E. Fiebiger, H.S. Overkleeft, M. Rosemblatt, H.L. Ploegh , C. Lagaudriere-Gesbert, Analysis of protease activity in live antigen-presenting cells shows regulation of the phagosomal proteolytic contents during dendritic cell activation. *J Exp Med.* **196** (2002), pp. 529-40.

[76] N. Obermajer, A. Premzl, T. Zavasnik Bergant, B. Turk, J. Kos, Carboxypeptidase cathepsin X mediates beta2-integrin-dependent adhesion of differentiated U-937 cells. *Exp Cell Res.* **312** (2006), pp. :2515-27.

[77] S.Hashmi J. Zhang, Y. Oksov, S. Lustigman, The Caenorhabditis elegans cathepsin Z-like cysteine protease, Ce-CPZ-1, has a multifunctional role during the worms' development. *J Biol Chem.* **279** (2004), pp. 6035-45

[78] A.M. Schriever, T. Friedrich, M. Pusch, T.J. Jentsch, CLC chloride channels in Caenorhabditis elegans. *J Biol Chem.* **274** (1999), pp. 34238-44.

[79] S.M. Stobrawa, T. Breiderhoff, S. Takamori, D. Engel, M. Schweizer, A.A. Zdebik, M.R. Bosl, K. Ruether, H. Jahn, A. Draguhn, R. Jahn, T.J. Jentsch, Disruption of ClC-3, a chloride channel expressed on synaptic vesicles, leads to a loss of the hippocampus. *Neuron.* **29** (2001), pp. 185-96.

[80] H. Fares and I. Greenwald, Genetic analysis of endocytosis in Caenorhabditis elegans: coelomocyte uptake defective mutants. *Genetics.* **159** (2001), pp. 133-45.

[81] M. Starcevic and E.C. Dell'Angelica, Identification of snapin and three novel proteins (BLOS1, BLOS2, and BLOS3/reduced pigmentation) as subunits of biogenesis of lysosome-related organelles complex-1 (BLOC-1). *J Biol Chem.* **279** (2004), pp. 28393-401.

[82] A. Beigneux, S.K. Withycombe, J.A. Digits, W.R. Tschantz, C.A. Weinbaum, S.M. Griffey, M. Bergo, P.J. Casey, S.G. Young , Prenylcysteine lyase deficiency in mice results in the accumulation of farnesylcysteine and geranylgeranylcysteine in brain and liver. *J Biol Chem* **277** (2002) pp. 38358-63.

[83] L.D. Schroeder, S. Kremer, M.J. Kramer, E. Currie, E. Kwan, J.L. Watts, A.L. Lawrenson, G.J. Hermann, Function of the Caenorhabditis elegans ABC Transporter PGP-2 in the Biogenesis of a Lysosome-related Fat Storage Organelle. *Mol Biol Cell.* **18** (2007). pp. 995-1008.

[84] J. Hujova, J. Sikora, R. Dobrovolny, H. Poupetova, J. Ledvinova, M. Kostrouchova, M. Hrebicek, Characterization of gana-1, a Caenorhabditis elegans gene encoding a single ortholog of vertebrate alpha-galactosidase and alpha-N-acetylgalactosaminidase. *BMC Cell Biol.* **6** (2005), pp. 5.

[85] C.I. Bargmann High-throughput reverse genetics: RNAi screens in Caenorhabditis elegans. *Genome Biol.* **2** (2001), REVIEWS1005.