Mol Neurobiol (2008) 38:178–198
DOI 10.1007/s12035-008-8041-0

Inclusion Body Myositis: A View from the *Caenorhabditis elegans* Muscle

Daniela L. Rebolledo · Alicia N. Minniti · Paula M. Grez · Ricardo Fadic · Rebecca Kohn · Nibaldo C. Inestrosa

Received: 29 July 2008 / Accepted: 16 August 2008 / Published online: 5 September 2008

Abstract Inclusion body myositis (IBM) is the most common myopathy in people over 50 years of age. It involves an inflammatory process that, paradoxically, does not respond to anti-inflammatory drugs. A key feature of IBM is the presence of amyloid-β-peptide aggregates called amyloid deposits, which are also characteristic of Alzheimer's disease. The use of animals that mimic at least some characteristics of a disease has become very important in the quest to elucidate the molecular mechanisms underlying this and other pathogeneses. Although there are some transgenic mouse strains that recreate some aspects of IBM, in this review, we hypothesize that the great degree of similarity between nematode and human genes known to be involved in IBM as well as the considerable conservation of biological mechanisms across species is an important feature that must be taken into consideration when deciding on the use of this nematode as a model. Straightforward laboratory techniques (culture, transformation, gene knockdown, genetic screenings, etc.) as well as anatomical, physiological, and behavioral characteristics add to the value of this model. In the present work, we review evidence that supports the use of *Caenorhabditis elegans* as a biological model for IBM.

Keywords IBM · Inclusion Body Myositis · Muscle · *C. elegans* · Aβ peptide · Myopathy · Invertebrate models

D. L. Rebolledo · A. N. Minniti · P. M. Grez · N. C. Inestrosa (✉)
Centro de Envejecimiento y Regeneración (CARE),
Centro de Regulación Celular y Patología "Joaquín V. Luco" (CRCP),
MIFAB, Facultad de Ciencias Biológicas,
P. Universidad Católica de Chile,
Alameda 340, Santiago, Chile
e-mail: ninestrosa@bio.puc.cl

R. Fadic
Departamento de Neurología, Facultad de Medicina,
Pontificia Universidad Católica de Chile,
Santiago, Chile

R. Kohn
Department of Biology, Ursinus College,
Collegeville, PA, USA

General Introduction

As we age, the chance of developing a series of illnesses increases. With the remarkable extension of life expectancy achieved in recent decades, certain neurodegenerative and muscular disorders, once rare, have become increasingly common. Among the many physiological disorders that are frequent in the elderly, we can mention Alzheimer's disease (AD) [1, 2] and inclusion body myositis (IBM) [3, 4]. While the first one is the most common form of senile dementia, the second one turns out to be the most common form of myopathy in people over 50 years of age. In spite of affecting different tissues (AD strikes the central nervous system while IBM affects the skeletal muscles), these two diseases have many features in common. Both disorders are characterized by amyloidosis, the formation of aggregates of the amyloid-β peptide (Aβ) derived from the proteolytic cleavage of the amyloid precursor protein, APP (Fig. 1), and by the presence of specific molecules with anomalous expression or molecular alterations.

In order to study these illnesses, several approaches are necessary. The analysis of human biopsies (IBM) and postmortem tissue samples (AD) is an essential step to characterize the disease and its effects in detail. However, these materials are scarce and also limited in their experimental potential. Therefore, biological models become necessary. A model is a specimen easy to manipulate

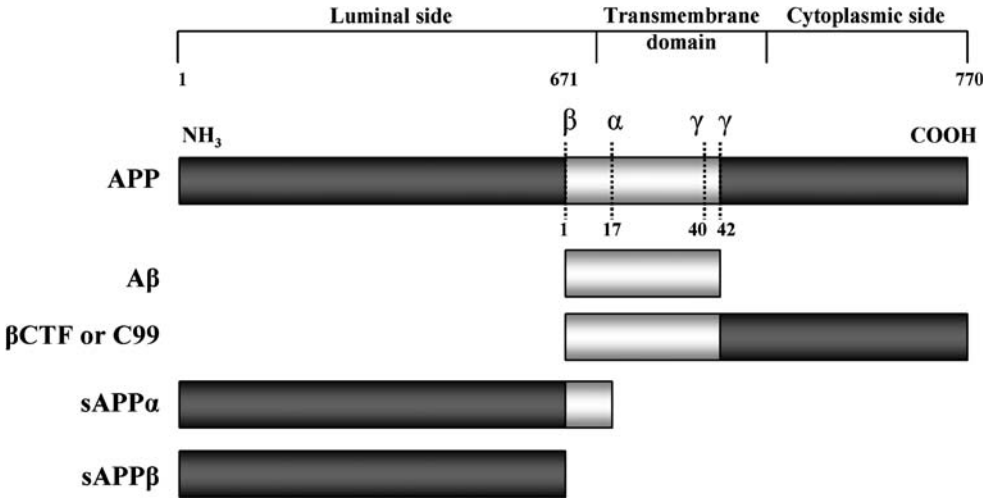

Fig. 1 The primary structure of APP is shown (770 amino acids) including the different domains. The Aβ peptide appears in *gray* and is originated by the proteolytic processing of β and γ-secretases. γ-secretase can cut either in the 40th or 42nd amino acid originating different Aβ peptide species

in the laboratory that approximates as closely as possible the phenomena to be studied and facilitates the investigation by providing large amounts of samples suitable for experimentation. While there has been an enormous amount of research done on AD (for a review, see [1, 5]), surely and understandably due to its devastating consequences, the work done on IBM has been much more limited. Therefore, in this review, we will focus specifically on IBM, presenting a brief description of the molecular characteristics of the illness and the biological models available today for its study. We will give special emphasis to the nematode *Caenorhabditis elegans* as a useful biological model for studying this disease.

What makes *C. elegans* a good biological model? *C. elegans* is a free-living, soil nematode introduced by Sydney Brenner in the 1960s as a suitable model for studying behavior and development [6, 7]. It has two sexes, hermaphrodites with a genotype XX (Fig. 2a) and males with a genotype X0 (Fig. 2b). This nematode has two modes of reproduction. Self-fertilization originates clones of the hermaphrodite, while crossing with males enhances variability, making this animal a useful genetic tool. One single hermaphrodite can produce 200–300 progeny. It has a very short life cycle (Fig. 2c), about 3 days (at 20°C) to grow from larval stage 1 (right after hatching) to adulthood; therefore, experimentation on many generations can be made in a short period of time [8, 9]. Its life span is around 3 weeks, allowing the study of longevity and other aging related processes [10–14]. *C. elegans* populations are easy to breed and do not require expensive culture media. They grow in regular agar plates containing *Escherichia coli* as a food source [8, 9]. Its small size, 1 mm during the adult stage, allows the culture of thousands of worms in a single Petri dish. This worm has a precise number of somatic cells, and its cell lineage is fully known [15]. Its fairly simple anatomy includes defined organs and complex behaviors, making it attractive to scientific research. It is a transparent organism. All organs can be easily observed *in vivo* directly and through the use of fluorescent proteins. In addition, there is a large collection of mutants; it is possible to perform RNAi gene knockdown and more complex genetic experiments (genetic screenings for new mutants, suppressor analysis to find interacting proteins, mosaic and epistasis analysis, etc.). It has a completely sequenced genome (which confirmed high conservation of biological mechanisms with humans) and it has relatively easy transformation techniques [to express proteins that are not normally present in this nematode, to confirm specific gene functions, to establish gene expression using green fluorescent protein (GFP) fusions, etc.]. These characteristics have facilitated its study and allowed scientists to carry out basic research to help elucidate fundamental cellular processes that relate to human diseases. Among the numerous studies done in this organism, investigations of apoptosis have led to a much better understanding of the molecules and pathways involved in programmed cell death. This knowledge was achieved due to powerful genetic techniques available in *C. elegans*, the knowledge of the lineage of each of its cells, and the fact that at the molecular level, there are striking similarities between nematodes and mammals [16–18]. The important advances in this field along with the contribution of this small nematode to scientific knowledge were recognized in 2002 with the award of the Nobel Price in Physiology or Medicine to *C. elegans* researchers Sydney Brenner, John Sulston, and Robert Horvitz.

Taken together, these characteristics, some of which will be discussed further later in this review, show that *C. elegans* can be an excellent model for researching many biological processes.

After examining what we have learned about IBM in this model, we will explore the possible role of metal homeostasis on IBM development as well as potential therapeutic procedures, as the current treatments of inflam-

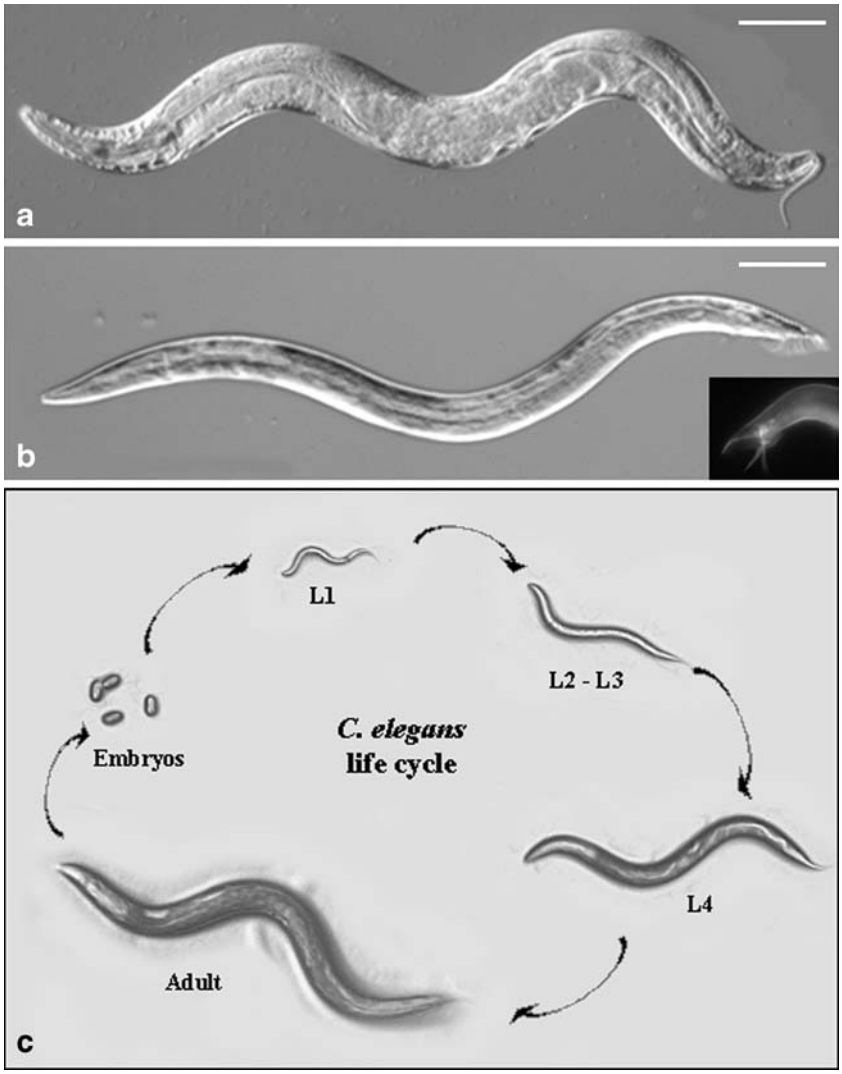

Fig. 2 Differential interference contrast (*DIC*) micrographs of *C. elegans* hermaphrodite (**a**) and male (**b**). The heads are to the *left* and the tails to the *right*. The male has a specialized tail that is essential for mating (*inset*). Bar, 0.1 mm. **c** *C. elegans* life cycle. It takes only 3.5 days at 20°C for a newly hatched larva (L1) to reach the adult reproductive stage. L2, L3, and L4 correspond to other larval stages

matory myopathies (specifically sporadic IBM) are only moderately successful.

Inclusion Body Myositis

Characteristics of IBM

IBM is the most common form of myopathy in people over 50 years of age, but can also occur in younger people [4, 19]. It was first described by Raymond Adams in 1965 [20] and is known to affect the skeletal muscles mainly in male patients [21]. It involves an inflammatory reaction similar to that seen in other inflammatory myopathies such as dermatomyositis and polymyositis [22]. It has been described as sporadic (s-IBM) since it does not have a hereditary component. Although familial IBM exists, it is an extremely rare physiological alteration with evidence that it is an autosomal dominant disorder [23]. There is another group of illnesses that share many characteristics with s-IBM but lack the inflammatory process [24]. These pathologies, caused by genetic alterations [25, 26], account for a very small fraction of the total IBM cases and were designated as hereditary inclusion body myopathies (h-IBM) [23, 27].

s-IBM is far more common than the h-IBMs and has a later onset: 50 years instead of 15 to 30 for h-IBM. s-IBM could also have an immunogenetic component, since human leukocyte antigen (HLA) class II has been found to mediate the autoimmune reaction responsible for this disease. However, no association has been found between HLA and h-IBM [28]. There are two inherited forms of h-IBM. The autosomal recessive form has been related to the GNE gene on chromosome 9. This gene encodes a bifunctional enzyme with epimerase and kinase domains, catalyzing the first two steps in *N*-acetilneuraminic (sialic) acid synthesis. Loss of function mutations in both of these domains have been

reported in h-IBM. In autosomal recessive h-IBM cases without GNE mutations, there is a laminin α2 chain deficiency due to mutations in the LAMA2 gene. The autosomal dominant form of the disease is linked to mutations in the myosin heavy chain IIa (MHCIIa) gene and in the valosin-containing protein (VCP) gene. The VCP is a member of a group of enzymatic chaperones involved in several cellular processes such as ubiquitin-proteosome-mediated degradation, cell cycle control, and apoptosis, among others [27, 29]. At least three heterozygous mutations in the VCP gene, with pathological consequences in human striated muscle, have been found in h-IBM associated with Panget disease and frontotemporal dementia [30].

In s-IBM, the inflammatory process has been related to factors such as activated $CD8^+$ T cells and their clonal expansion [31] and also to the MHC I-expressing (non-necrotic) muscle cells which are destroyed with the help of the inducible co-stimulatory molecule (ICOS) and its ligand (ICOS-L) [32]. It is important to mention that the clonal expression of the T cell receptors persists over time as well as the exposure of the antigen to these lymphocytes [33], thus inducing greater damage to the tissue, which will later cause the typical muscle weakness of this disease. In addition, myoblasts express molecules such as pro-inflammatory cytokines and chemokines (especially those induced by IFN-γ) which are important for generating an immune response and have been reported to be up-regulated in non-necrotic muscle fibers [34]. Despite these features, there is no response to anti-inflammatory drugs in this disease [22, 31]. Some studies carried out using intravenous immunoglobulin have shown an effect in some neuromuscular diseases, but its effects in IBM patients are not satisfactory [35].

People who suffer from s-IBM show progressive muscle weakness that affects the limbs, proximally at first and then progressing distally. IBM causes thinning of the forearms [36] and in later stages affects the respiratory muscles and may even cause dysphagia [4, 37, 38]. The loss of muscular strength does not show a specific pattern, but some symptoms predispose to others when they appear. Studies done on more than 64 patients that summarize the onset of symptoms after the age of 40 show preference for the quadriceps, finger flexors, and pharyngeal muscles, in that order. Weakness is progressive and the rates of progression are highly variable, but a latter age of onset associates with faster progression [19].

Skeletal muscles in these patients show cellular alterations such as vacuolation [39, 40]. These rimmed vacuoles are a distinct feature of several myopathies (they do not exist in normal muscle fibers) and share features with autophagic vacuoles. The affected myotubes also contain early endosomes as well as autolysosomes [41], and there are lymphocytic inflammation and filamentous inclusions in the cytoplasm and nuclei [24, 42], all of them causing degeneration of the fiber and the consequent muscular weakness. These fibers bind Congo Red, Thioflavin-S, and crystal violet, indicating the presence of amyloid deposits [36, 43, 44] which form senile plaque-like inclusions [24, 38, 42, 45].

Because IBM has an inflammatory pathogeny, several immunosupressor and immunomodulator therapies have been used, including corticosteroids, azatioprine, and ciclosporin A [31], metrotrexate [19], endovenose inmunoglobulins [35, 46], and etarnecept [47]. All of them have failed in changing the natural progress of the disease, although some patients respond to some degree or for short periods of time.

Molecules Involved in IBM and AD

Several studies have described different molecules involved in IBM. Most of them are also involved or present in AD. Below, we describe the main molecules reported in the literature and their relation to IBM and AD (summary is reviewed in Table 1).

- *Aβ peptide* is one of the most relevant molecules involved both in IBM and AD. It is originated from the proteolytic cleavage of its precursor protein APP, codified on chromosome 21. The APP glycosylation forms a glycoprotein, which is located at the plasma membrane with one extracellular N-terminal domain and an intracellular C-terminal domain. The Aβ segment corresponds to a part of the transmembrane portion that is cleaved by proteases called secretases [2, 48, 49] (Fig. 1). It can be found in different lengths, since the number of amino acids it contains can vary from 39 to 43, with $Aβ_{1–40}$ and $Aβ_{1–42}$ being the most common [44]. This peptide is the main component of the senile plaques (extracellular) in AD [49], and a series of findings link it with AD pathogenesis [2]. It is also part of the intracellular inclusions in the muscle fibers in IBM [24, 42, 50], specifically inside the rimmed vacuoles where its amyloid nature has been demonstrated using immunohistochemistry techniques with an anti-Aβ antibody (in particular, $Aβ_{1–42}$) [51]. In IBM, the N terminus as well as the C terminus of APP are found forming part of the muscle inclusions [38]. *In vitro* studies and clinical trials have demonstrated that the APP/Aβ peptide is a substrate for the autophagy pathway. There is co-localization between APP or Aβ and AtG8/LC3, an essential protein for autophagy. APP/Aβ containing autophagosomes are increased in s-IBM biopsies associated with inflammation-involved molecules (MHC I y II, $CD4^+$, and $CD8^+$ cells) [52].

  The above facts, together with the existence of a series of other molecules in the muscle of IBM patients that are also present in AD, reveal the similarities between these two diseases, which also include mis-

Table 1 Molecules involved in IBM and AD

Molecule	Disease	Characteristics	Refs.
Aβ	IBM	Present in intracellular inclusions	[42, 50]
	AD	Main component of SP	[1, 2]
Tau	IBM	In its phosphorylated status accumulates inside muscles cells	[36, 54]
	AD	Abnormal structure, function and phosphorylation status. Forms PHFs	[53]
ERK	IBM	Overexpressed and localized close to tau filaments	[40]
	AD	Co-localizes with Tau filaments	[55]
CKI	IBM	Alpha isoform is present in abnormal muscle fibers	[66]
	AD	Co-localizes with cytoplasmatic lesion including neurofibrillary tangles	[60, 61]
Presenilin-1	IBM	Enriched in PHFs inside the muscle fibers	[69]
	AD	Accumulates in NFT and SP. Missense mutation in familial form	[48, 67, 68]
BACE	IBM	Accumulates in inclusions of abnormal fibers	[72, 73]
	AD	Involved in APP processing	[70, 71]
NOGO	IBM	Increased in muscle biopsies	[75]
	AD	Involved in APP processing	[74]
ApoE	IBM	Co-localizes with Aβ, ubiquitin and p-Tau in amyloid deposits	[92]
	AD	Detected in NFT and SP. Interacts with Aβ *in vitro*	[74]
AChE	IBM	Used as indicator of normal NMJ	[24]
	AD	Present in SP with different properties	[77, 78]
Cholesterol	IBM	LDL and VLDL receptors co-localizes with Aβ and ApoE. LDL receptor co-localizes with PHFs	[91]
	AD	Possible variation in subcellular localization of secretasases. Hypocholesterolemia reduces the amount of Aβ in the brain	[38, 90]
Ubiquitin	IBM	Localized in the vacuolated muscle fibers together with amyloid and PHFs	[100, 101]
	AD	Present in NFT and SP	[102, 103]
Prion	IBM	Accumulates co-localizing with Aβ and Tau inside the vacuolated fibers	[113, 112]
	AD	Negatively regulates the β-secretase cleavage reducing the Aβ formation	[106]
α_1-Antichymio trypsin	IBM	Accumulates in vacuolated muscle fibers together with amyloid fibrils and ubiquitin.	[115]
	AD	Found in normal brain tissue and overexpressed in AD brains. Promotes the aggregation of the Aβ and colocalizes with it.	[115].
Cystatin C	IBM	Overexpressed in non-vacuolated muscle cells. Found close to PHFs	[116]
	AD	Mutations may be a risk factor for late onset AD	[117, 118]
Alpha β crystallin	IBM	Enhanced expression in both structurally abnormal and normal fibers	[124]
	AD	Closely related to neurons containing NFTs and associated with neuronal death. Binds Aβ preventing the formation of fibrils but generates a highly toxic αB-crystallin/Aβ peptide complex protein	[119, 123]
Myostatin	IBM	In cell culture model the precursor is increased. Accumulates in muscle fibers co-localizing with Aβ	[128, 129]
	AD	–	–

folding of proteins and the presence of specific post translational modifications.

- *Tau* is a microtubule-associated protein present primarily in brain tissue. It becomes abnormally phosphorylated and is structurally and functionally altered in AD [53]. Tau accumulates forming filaments inside muscle cells of patients with IBM [36, 54]. In neurons of AD, it is localized both inside and outside of the neurons in the form of PHFs. In both cases, there is co-localization with ubiquitin and the Aβ peptide [39].
- *Extracellular signal-regulated protein kinase* (ERK) is overexpressed and activated by phosphorylation in the cytoplasm of abnormal IBM muscle fibers and is also present in normal neuromuscular junctions. It is localized close to tau filaments but does not co-localize with them. It could be induced by oxidative stress related to the increased amount of APP [40]. In neurons of patients with AD, it colocalizes with tau filaments and could be involved upstream of tau phosphorylation through the MAPK pathway [55].
- *Casein kinase I* (CKI), a member of serine/threonne protein kinase family, is able to phosphorylate a great number of proteins. It is highly conserved from yeast to humans where there are several isoforms [56]. CKI isoforms have been shown to mediate protein hyperphosphorylation due to their ability to recognize sequences that contain phospho-amino acids [57]. The activity associates with brain microtubules [58], con-

tributes to tau phosphorylation in cell culture experiments [59] and, in brains from AD patients, co-localizes with cytoplasmatic lesions including neurofribrilary tangles [60, 61]. CKI has been associated with the Wnt signaling pathway [62]. This is relevant because there is evidence showing a participation of the Wnt signaling pathway in Aβ-induced toxicity [63–65]. The alpha isoform of CKI has been found in abnormal muscle fibers in IBM patients' biopsies, almost exclusively in phosphorylated tau-containing inclusions, similarly to AD [66].

- *Presenilin-1* is a transmembrane protein encoded on chromosome 14 with γ-secretase activity. In normal muscle, the protein is located at the neuromuscular junctions. It has been found to accumulate in the brain of patients with AD (familial and sporadic forms) in the neurofibrillary tangles and senile plaques. In the familial form, this protein has missense mutations, while in the sporadic form, it does not have alterations in its primary structure. Alterations in the amino acid sequence lead to a higher production of Aβ peptide *in vivo* and *in vitro* [48, 67, 68]. In IBM, this protein is also enriched in the PHFs inside the muscle fibers, but no mutations have been described for this disease [69].
- *BACE 1 and BACE 2* are secretase enzymes involved in APP processing [70, 71]. In normal muscle, they are expressed at the neuromuscular junction (NMJ) postsynaptic density and accumulate in inclusions in s-IBM as well as in h-IBM abnormal muscle fibers [72, 73].
- *NOGO*, also known as RTN4, is a member of the reticulon family. Proteins from this family are binding partners of BACE 1, which they negatively regulate. NOGO binds to BACE1 blocking access to APP, therefore decreasing Aβ production [74]. In IBM biopsies, NOGO-B is increased and co-localizes with BACE1; while in cell culture, APP overexpression increased NOGO. For this reason, it was proposed that a NOGO-B increase may represent a weak attempt to decrease Aβ production and that the manipulation of NOGO production can be a new therapeutic prospect [75].
- *Acetylcholinesterase* (AChE) is the enzyme responsible for the hydrolysis of acetylcholine (ACh), and it has been implicated in AD. AChE is found in plaques from AD patients brains [76, 77]. When present in Alzheimer plaques, this enzyme has different properties compared to the free enzyme [78, 79]. *In vitro* studies showed that AChE accelerates the assembly of the Aβ peptide into amyloid fibrils [80–83] through a specific structural motif [84]. On the other hand, *in vivo* studies demonstrated that increased AChE expression is able to induce plaque formation [85, 86] (for reviews, see [87, 88]). AChE is used as an indicator when analyzing human IBM biopsies in order to establish whether the neuromuscular junction has been formed [24], but it has not been described as forming part of the inclusions in the vacuolated muscle fibers.
- *Cholesterol*, together with its receptors, it has been related to Aβ production [38, 89]. The proposed mechanisms suggest an alteration in the membrane rigidity and a possible variation in the subcellular localization of the different secretases [90]. It has been described (in HEK 293 cells) that the depletion of cholesterol inhibits the proteolytic cut of APP by β-secretase, thus lowering the amount of the β-amyloid generated. Studies carried out in transgenic mice also show that hypocholesterolemia reduces the amount of Aβ in the brain [38]. The low-density lipoprotein (LDL) receptor and the very LDL receptor (VLDLR) have been found in the muscle fibers of IBM biopsies co-localizing with Aβ and apolipoprotein E (ApoE). Only LDL receptor co-localizes with PHFs. Free cholesterol can also be found in these tissues [91].
- *ApoE* is a component of lipoproteins such as LDL and high-density lipoproteins. It localizes in the amyloid deposits along with Aβ peptide, ubiquitin, and phosphorylated tau in muscle fibers of IBM patients [92]. Since its messenger RNA (mRNA) has not been found to be increased, it is thought that it may be transported into the cell with the help of LDL receptors located at the plasma membrane [93]. This fact could somehow be related to the inverse relationship existing between ApoE plasma levels and the amount of estrogen in blood, since this hormone induces the expression of LDL receptors. In the brain, its behavior is different. Its mRNA is abundant and several ApoE receptors are expressed [94]. In AD, it can be detected in neurofibrillary tangles and senile plaques and interacts with the Aβ peptide *in vitro*. In particular, its allele 4 isoform has been described as a risk factor for sporadic and familial AD, triggering the increase in the deposition of the Aβ peptide [95–97]. Several studies show discordant effects of this protein: while some of them suggest that ApoE4 has a neuroprotective effect against AD, others show that it inhibits growth of neurites in culture [94] and also inhibits the Wnt signaling pathway in PC12 cells [98]. It is also possible that ApoE participates in the regeneration of neurons, thus diminishing the effects of AD in the brain. Several ApoE polymorphisms have been described, but they do not influence the probability of having s-IBM nor the age at which it appears. Something similar has been observed with α1-antichymotrypsin [99].
- *Ubiquitin* is localized in the vacuolated muscle fibers in patients with IBM together with the amyloid and PHFs at the normal neuromuscular junctions [100, 101]. In AD, this protein forms part of the neurofibrillary tangles

and senile plaques, and it may be found in neurites that may or may not be associated with these fibrillar structures [102, 103]. The gene p97/VCP—member of ubiquitination machinery—has been shown to cause weakness and ubiquitinated protein inclusions when its mutant form is expressed in a transgenic mouse model [104]. Moreover, in s-IBM muscle fibers, there is a reduction in 26S proteasome activity, which was mimicked in cultured APP-overexpressing human muscle. It has been proposed that Aβ can induce proteasome dysfunction leading to the accumulation of misfolding proteins [105].

- *Prion* (PrP^c) is a protein that is normally found in the plasma membrane of neurons. It can suffer an alteration in its 3D organization originating a β-sheet enriched structure that causes Creutzfeld–Jacob disease in humans and bovine spongiform encephalopathy or "mad cow disease" in cattle. There is PrP inmunoreactivity in plaques from AD and was reported that PrP^c negatively regulates β-secretase clevage of APP, reducing Aβ formation [106], and a specific allele for Prion protein gene M129 was described as a risk factor for Alzheimer's disease [107]. This protein has copper-binding sites that are involved in copper brain homeostasis [108] and copper is able to induce the expression of PrP^c in neurons [109]. As with APP and Aβ peptide, there is controversy about the beneficial or deleterious role of copper [110]. It has been reported that the form of PrP^c found in normal muscle is different from that found in the brain [111]. Moreover, PrP^c has been found in the muscle cells of patients with s-IBM [112]. PrP^c accumulates in IBM where its mRNA is increased. The protein co-localizes with Aβ peptide and tau inside the vacuolated fibers. In contrast, the amount of mRNA of PrP^c in AD brains is normal [113]. Furthermore, overexpression of PrP^c in the muscles of mice is sufficient to induce a primary myopathy that correlates with accumulation of a truncated N-terminal PrP fragment [114].
- *α_1-Antichymiotrypsin* is a serine protease inhibitor which has been related to inflammatory processes and has been found to accumulate in the vacuolated muscle fibers of IBM patients together with amyloid fibrils and ubiquitin. It can be found in normal brain tissue and is overexpressed in AD brains and promotes the aggregation of the Aβ peptide. Just as in IBM, it co-localizes with the Aβ peptide [115].
- *Cystatin C* is a cysteine protease inhibitor that possesses amyloidogenic properties. This protein, just like prion, presenilin 1, and ubiquitin, can be found in normal neuromuscular junctions. In IBM, it is overexpressed compared to normal tissue and is located in the non-vacuolated cytoplasm of muscle cells. It interacts with the Aβ peptide as well as with APP. It can also be found close to the PHFs but not inside them. These characteristics have not been found in other muscle diseases [116]. Mutations in the amino acid sequence of cystatin C may be a risk factor for late onset AD [117, 118].
- *Alpha β crystallin* is a member of the small heat shock protein family with chaperone-like properties that interacts—in muscle cells—with desmin and actin filaments, preventing them from stress damage. It can also be found in tissues like glial cells of the central nervous system and eye lenses among others. It has been reported to be closely related to neurons containing neurofibrillary tangles and could be associated with neuronal death in AD patients [119]. This protein is overexpressed in astrocytes, microglia, and oligodendrocytes and has been linked to tauopathies [120] and many neurological disorders, while its mutations can cause cataract and myopathy [121, 122]. It has also been described to bind the Aβ peptide, preventing the formation of fibrils but generating a highly toxic αB-crystallin/Aβ peptide complex [123]. The protein expression is enhanced in muscle from IBM patients both in structurally abnormal fibers and normal fibers [124], and there is evidence that the Aβ peptide is a stressor that can cause αB-crystallin overexpression [125]. Other heat shock proteins and chaperones and their relation with Aβ have been reviewed (see [126]).
- *Myostatin* is a negative regulator of muscle mass and strength during development and adulthood [127]. In an IBM cell culture model, the myostatin precursor is increased [128]. On the other hand, it also accumulates within muscle fibers in s-IBM biopsies co-localizing with Aβ, suggesting a role in the disease [129].

Role of Mitochondria and Oxidative Stress

Oxidative stress is another important factor that plays a role in IBM as well as in AD, since there is an increased production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), nitric oxide, and superoxide radical (O_2^-), among others. The study of oxidative stress in AD has revealed a series of molecules that may take part in this process. One example is 4-hydroxynonenal (4-HNE), generated from membrane lipid peroxidation, which would be directly involved in the production of ROS [130].

Superoxide dismutase (SOD), an enzyme that eliminates O_2 from cells, is involved in AD because it prevents the damage produced by Aβ in endothelial cells [131]. It has also been shown that Aβ peptide produces H_2O_2 by reducing copper [132], while catalase, an enzyme that reduces hydrogen peroxide into water and oxygen, contributes to a decrease in Aβ toxicity [133, 134]. Another process that

could contribute to the production of ROS is the reduction of copper from Cu^{2+} to Cu^{+} by APP and its later oxidation (Cu^{+} to Cu^{2+}) in the presence of H_2O_2-generating free radicals [135]. For IBM, research has allowed scientists to propose a possible mechanism to relate oxidative stress and this disease: APP and Aβ accumulate, leading to increased oxidative stress, which would cause activation of the MAPK pathway [40]. This would have two consequences: phosphorylation of tau and overexpression of APP, leading to the development of the myopathy.

On the other hand, in muscle fibers from s-IBM patients, there are mitochondrial morphological alterations, abnormal mitochondria proliferation, and deficiencies in cytochrome c oxidase (COX) activity affecting segments of the muscle fiber. Deletions and point mutations on mitochondrial DNA are also present. Indeed, COX deficiency has been attributed to different and large-scale deletions in mtDNA in affected fibers [136]. The mitochondrial abnormalities may be affecting the normal oxidative status of muscle cells, increasing Aβ toxicity.

Mouse Model for Studying IBM

Before discussing the animal models used for the study of IBM, it is essential to mention that IBM is an exclusively human disease, as is the case of AD. Therefore, the researcher needs to have in mind that the models will only recreate some aspects of the disease. This point becomes particularly significant when exploring new therapies. Many treatments that were successful in laboratory animals failed to produce the same benefits in clinical trials.

In 1996, Fukuchi et al. [137] obtained a transgenic mouse strain that expressed the 99 C-terminal amino acids of the APP, which contains the Aβ peptide plus the cytoplasmic domain that has been described as the neurotoxic domain. They established the expression of β-amyloid peptide using Aβ-specific antibodies [42]. Further experiments using electron microscopy and immunocytochemistry led to the conclusion that the fragment observed was derived from APP proteolysis and caused amyloid fibril formation in skeletal muscle. This transgenic animal was proposed as a useful model for studying IBM, since the myopathy it develops has many similarities with the human pathology. It was later demonstrated that no extracellular deposits were formed in this transgenic mouse due to overexpression of the C-terminal of the APP [138].

Jin et al. [50] developed another transgenic mouse strain in 1998. It also expresses the 99 C-terminal amino acids of APP but included the mutation K612V, which prevents the proteolysis by the α-secretase. Therefore, the formation of Aβ peptide as well as the development of the inclusions is enhanced in an age-dependent manner, resembling IBM.

Another interesting transgenic mouse strain was developed in 2002 by Frank La Ferla and collaborators. The expression of the full-length APP under the control of the muscle creatine kinase (MCK) promoter resulted in an animal in which Aβ overexpression—contrary to what had been achieved by Fukuchi and Jin—occurs exclusively in muscle and not in every cell type, as happens when using the β-actin promoter. Several histological features can be observed in these mice that resemble those observed in the muscle biopsies from IBM patients that are used for diagnosis. These IBM-like pathological changes, such as intracellular depositions of Aβ peptide, central nuclei, neutrophil-mediated inflammatory response clustered around APP immunoreactive regions, deficits in motor system, among others, are age-dependent and were observed in mice older than 10 months of age [139]. Studies in this model were compared with biopsies from IBM patients, establishing that fast twitch muscle fibers may selectively accumulate and be more vulnerable to βAPP- and Aβ-mediated damage in IBM [140]. Then, in the parental MCK-APP line, the wild-type presenilin-1 (PS1) was replaced with the PS1 (M146V) allele, which is associated to an increment of the more amyloidogenic $Aβ_{1–42}$. APP transgene expression was unaltered, but Aβ levels, particularly $Aβ_{1–42}$, were elevated in skeletal muscle in this double transgenic. IBM-like features such as elevated phospho-tau accumulation and greater activation of GSK-3β and cdk5 were observed. In addition, there was an increment of inclusion bodies and inflammatory infiltrates. Motor coordination and balance were also more adversely affected and manifested at an earlier age. This observation suggests a critical role for $Aβ_{1–42}$ in the disease [141]. This double transgenic mouse was used to study the role of inflammation in IBM. The results identified a molecular mechanism by which pro-inflammatory stimuli affect tau pathology via the GSK-3β signaling pathway in skeletal muscle. Suppression of GSK-3β activity with specific inhibitors or LiCl significantly reduced tau phosphorylation and partially rescued motor deficiencies. In human IBM muscle, GSK-3β and phospho-tau were co-localized, further supporting the pathogenic role of GSK-3β in this disease and identifying a possible therapeutic tool [142].

Invertebrates as Models for Human Diseases

Although invertebrates may appear too distant from humans, at the molecular level the similarities are striking. In addition, they have several advantages when it comes to working in the laboratory, such as small size, short lifespan, and relatively simple culture techniques. When pathologies are studied in order to obtain new treatments or therapies, the molecular processes have to be fully understood. An invertebrate model may provide us with the possibility to

carry out sophisticated genetic analysis, such as suppressor screens, to identify other genes involved in the molecular processes being studied. It is possible to study the normal function of a protein homolog to a human protein that causes the disease, how the phenotypes change in mutant or knockout strains, as well as study the effects of the expression of a human protein in its wild-type or mutant forms. In addition, all these studies can be done in large populations. General reviews on this subject have been published and place *C. elegans* as a powerful genetic system that has been used to study human neurodegenerative diseases [143–145].

Special care has to be taken when deciding which disease to study in invertebrate models because when processes like immune response are involved, vast differences can exist in factors that turn out to be crucial to the development of the pathology and our understanding of it. Thus, it is important that in the model, the process resembles most aspects of the disease.

C. elegans as a Model for Studying Human Diseases

In 2000, Culetto and Sattelle [7] searched genome data bases for orthologs of human disease genes in *C. elegans.* One hundred genes were found in the nematode that encoded proteins related to neurological disorders (Wilson disease, Parkinson's disease, and AD) and muscular illnesses (myotubular myopathy, Duchenne muscular dystrophy) among other pathologies. Mutants in some of these 100 orthologs produce distinct phenotypes in the worms which can supply insights into the function and interactions that these molecules may have, thus helping us relate different structures and/or pathways involved in the disease. An example of what can be done with this tool comes from the work on the ortholog of the survival motor neuron protein (SMN) present in *C. elegans* (CeSMN) [146]. This is an RNA-binding protein normally present at the neuromuscular junction [39]. Under abnormal circumstances, it suffers mutations that cause spinal muscular atrophy (SMA), a disease characterized by motor neuron loss that results in muscle weakness and atrophy that when present in children can lead to a lethal outcome. When comparing organisms in different evolutionary levels such as mouse, rat, human, and *C. elegans*, an important percentage of homology and similarity is found, suggesting that its function is highly conserved. The fact that there is an ortholog gene in *C. elegans* allows scientists to analyze the protein's properties faster and more easily. This approach could open the way to a better understanding of the disease and eventually help elucidate the mechanism by which the SMN gene causes SMA [146].

Furthermore, mutants are very useful tools when it comes to studying illnesses, not only because of what has just been discussed above but also because temperature-inducible specimens can be obtained, allowing the timing of gene expression to be regulated by the researcher who can choose to grow the worms at a permissive or non-permissive temperature. The use of these strains has revealed interesting data about oxidative stress and β-amyloid deposition [7]. Furthermore, *C. elegans* gives us the possibility to study some features by generating mutations and fusions with fluorescent proteins [147] that can help to reveal properties that could be relevant for human diseases [146]. This property becomes particularly useful since the worm is transparent and it is not necessary to fix and process the animals to analyze under the fluorescent microscope (see Fig. 3).

As mentioned briefly in "General Introduction," another interesting technique that can be used in *C. elegans* is RNA interference (RNAi). This method consists of introducing a specific double-stranded RNA in the worm (by injection, soaking the worm in a dsRNA solution, or feeding them bacteria that produces the required dsRNA) that will block translation of the mRNA of the gene of interest. This approach will eventually create a pseudo knockout, since the protein will not be produced due to inactivation of its mRNA and will allow the observation of the loss-of-function phenotype. An example of the technique can be seen in Fig. 4 [148]. Use of RNAi has made reverse genetics a much more common technique for studying gene function in living organisms and has even enabled the screening of most of the *C. elegans* genome [149]. For the discovery of RNAi-gene silencing by double-stranded RNA in this model [150], the Nobel Prize in Physiology or Medicine (2006) was awarded to *C. elegans* investigators Andrew Fire and Craig C. Mello.

On the other hand, transgenic strains from *C. elegans* are easy to make, allowing the expression of a gene of interest despite its absence in the wild-type animal. This can be done by microinjection of the gene under the command of a strong tissue-specific promoter. The result is a new strain that contains a gene that the wild-type animal does not have [9]. Thus, a whole new series of diseases are beginning to be studied. This is the case of transgenic *C. elegans* expressing human Huntingtin, providing a model for studying Huntington's disease [151, 152]; *C. elegans* expressing *wt* or mutant forms of α-synuclein to study Parkinson's disease [153, 154]; or the Aβ peptide [155] resembling AD or IBM (Fig. 6a–d). In these cases, similar characteristics of the human illnesses can be obtained, making research easier.

C. elegans as a Model to Study IBM

Muscle Cells of *C. elegans*

Since we are interested in studying IBM and the muscular alterations it generates, it is particularly important to known

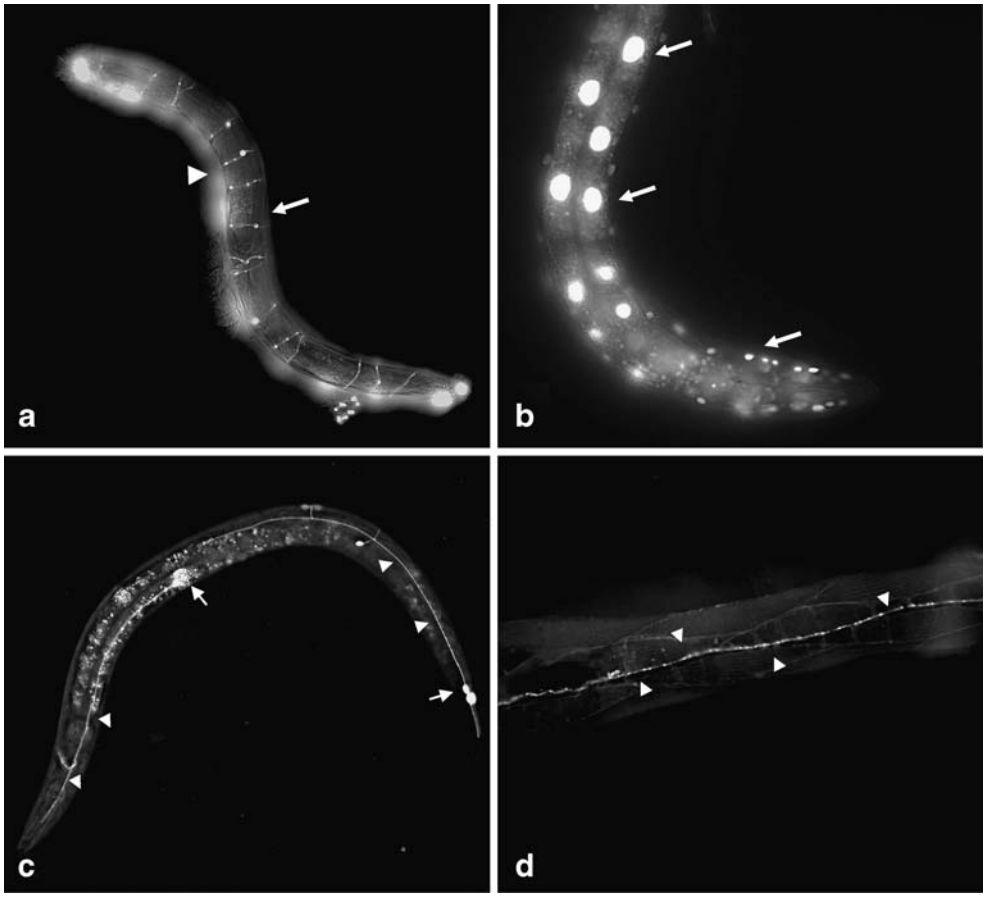

Fig. 3 Fluorescent micrographs of live *C. elegans* expressing GFP in different tissues and subcellular structures. **a** A *C. elegans* hermaphrodite that expresses GFP in all GABAergic neurons. The dorsal (*arrow*) and ventral (*arrowhead*) nerve cords are out of focus while the commissural processes that connect them are clearly seen. GFP expression is driven by the promoter of the *unc-47* gene that encodes a *C. elegans* GABA vesicular transporter. **b** Hermaphrodite that expresses GFP under the control of the *sur-5* promoter. *sur-5* is a gene that encodes an acetoacetyl-coenzyme synthetase localized primarily to the nuclei (*arrows*). **c** GFP is expressed in *C. elegans* touch neurons under the control of the promoter of the *mec-4* gene, which encodes an amiloride-sensitive Na^+ channel required to sense mechanical stimuli. *Arrows* indicate neuronal cell bodies and *arrowheads* show neuronal processes. **d** Neuromuscular junctions seen by expressing an UNC-49::GFP fusion protein. The *unc-49* gene encodes a $GABA_A$ receptor

the muscular system in *C. elegans*. This nematode has two muscle types, striated and non-striated. The striated muscle is formed by multiple sarcomeres and forms the body wall muscles used for locomotion (Fig. 5a). In contrast to cross-striated muscle in vertebrates, the nematode muscle is obliquely striated. The non-striated muscle is composed of single-sarcomere muscles that form part of the pharynx (Fig. 5c), intestine, vulva (Fig. 5d), anal sphincter and anal depressor (Fig. 5b). Some of these muscles are present at birth, while others are added later depending on the sex of the specimen. Males will need muscles that allow mating, while hermaphrodites will need to develop a vulva that will allow the egg laying processes. Furthermore, in nematodes, there is no fusion of the muscle cells, so there is no formation of multinucleated myotubes. Instead, the individual cells anchor themselves to the neighboring cells, to the hypodermis, and to the cuticle (the external collagen structure that corresponds to the exoskeleton of the worm) in order to become a single structure that will allow movement. Unlike other organisms where neurons send processes to their target muscle cells, nematode muscles extend cytoplasmatic projections or arms (Fig. 5e) to the motor neurons to make synapses [156–158].

The structural unit of nematode muscle is analogous to the sarcomere of vertebrates [157]. The thick filaments (A-bands) are centrally placed and overlap with two sets of thin filaments, one set extending in from either end of the unit. The thin filaments are attached to dense bodies (rather than Z lines in vertebrates) which originate at the cell membrane adjacent to the hypodermis [159]. The thick filaments are stacked in columns extending into the cell on either side of an amorphous electrondense material analogous to the M-line of vertebrate striated muscle. Like the dense body, the M-line analog also appears to be anchored in the membrane; it probably functions to establish and maintain the alignment of the thick filaments. The thick and

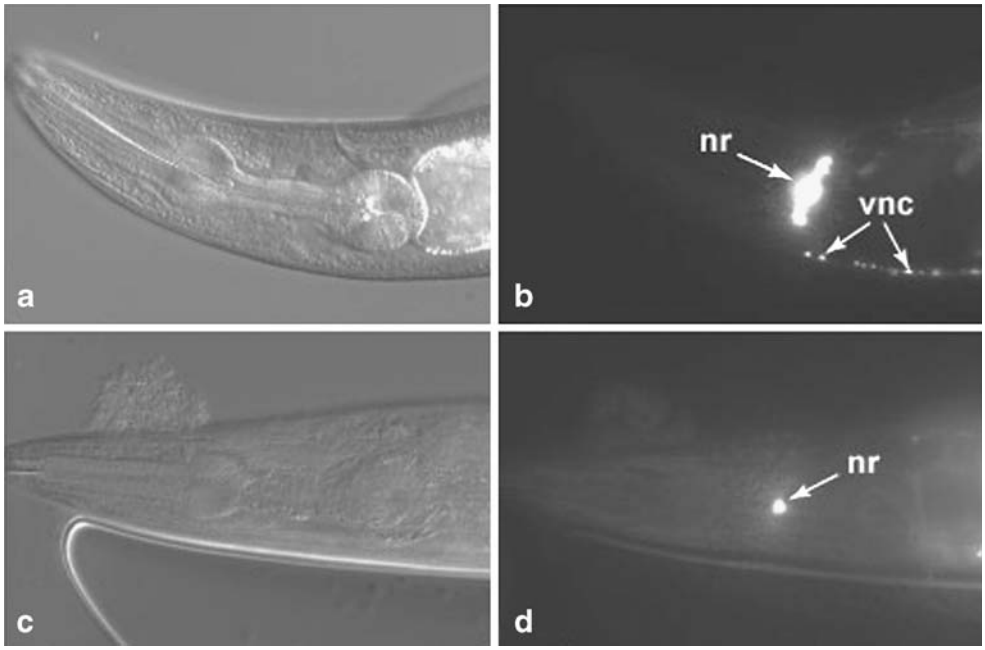

Fig. 4 RNAi gene knockdown in *C. elegans*. In *C. elegans*, RNAi is generally ineffective in the nervous system, but there are mutations that can enhance its efficiency. In this experiment, *C. elegans* strains expressing GFP in GABAergic neurons were fed bacteria producing double-stranded GFP RNA. **a** DIC image of an adult *C. elegans* head following RNAi feeding. **b** Fluorescence microscopy of the same animal in **a** shows there is strong GFP expression in GABAergic neuronal cell bodies in the nerve ring (*nr*) and ventral nerve cord (*vnc*) despite RNAi feeding. **c** DIC image of an adult *C. elegans* following RNAi feeding. This worm carries mutations in two genes (*unc-13* and *eri-1*) that enhance the effects of RNAi. **d** Fluorescence microscopy of the same animal in **c** shows GFP expression in only one GABAergic neuronal cell body in the nerve ring (*nr*)

thin filaments are different, in size and composition, from vertebrate muscles. The thick filaments are estimated to be about 10 μm in length with a diameter between 33.4 nm (centrally) and 14.0 nm (distally), in contrast with 1.6 μm in length and 12.0–14.0 nm in diameter in vertebrates, and are formed by both myosin and paramyosin [160] as in molluscs [161]. The thin filaments are estimated to be 6 μm in length (1 μm in vertebrates) and are composed of actin, tropomyosin, and troponin [156]. One important characteristic is that the assembly of the A-bands (containing thick filaments) and the I-bands (containing thin filaments) occurs in an independent way, and their interaction would only take place after they have been totally formed. This has been described in other models such as *Drosophila* [162] and in cultured cells [163]. Another interesting feature is that the cell membrane and the structures that interact with it during this assembly are very important for the final result, since mutations in the molecules involved in this process can lead to lethal phenotypes such as the Mup class (*mu*scle *p*ositioning abnormal) and Pat class (*p*aralyzed *a*rrest at embryonic *t*wofold stage) by affecting the animal's movement. Among the molecules that participate in this last process are α-actinin [164], α-integrin, β1-integrin [165], and Perlecan [158].

Nematodes Expressing Human Aβ Peptide in Muscle

The production of transgenic *C. elegans* strains is a rather simple process using microinjection. In this procedure, a plasmid containing the gene of interest is injected into the animal's gonad where the syncytial germ line is located. Because the nuclei are only partially surrounded by membrane, the new genetic material will be incorporated into the oocytes once the cell membrane is formed completely. Afterwards, this new DNA can be incorporated in the genome using x-rays or γ-rays in order to get 100% transmission of the transgene to future generations [9].

There is an APP-related protein in *C. elegans* encoded by the *apl-1* gene, but it does not have the sequence that corresponds to the Aβ peptide [166]. In order to obtain an invertebrate model for studying amyloidogenic diseases, Christopher Link built a transgenic *C. elegans* strain that expressed the human $A\beta_{1-42}$ peptide inside its body wall muscle cells [155]. The mini-gene encoding the $A\beta_{1-42}$ peptide is under the control of the promoter and enhancer sequences of the *unc-54* gene that encodes the major body wall myosin heavy chain, one of the most abundant proteins in this animal [157]. As a result, these animals show a significant amount of Aβ peptide inside their

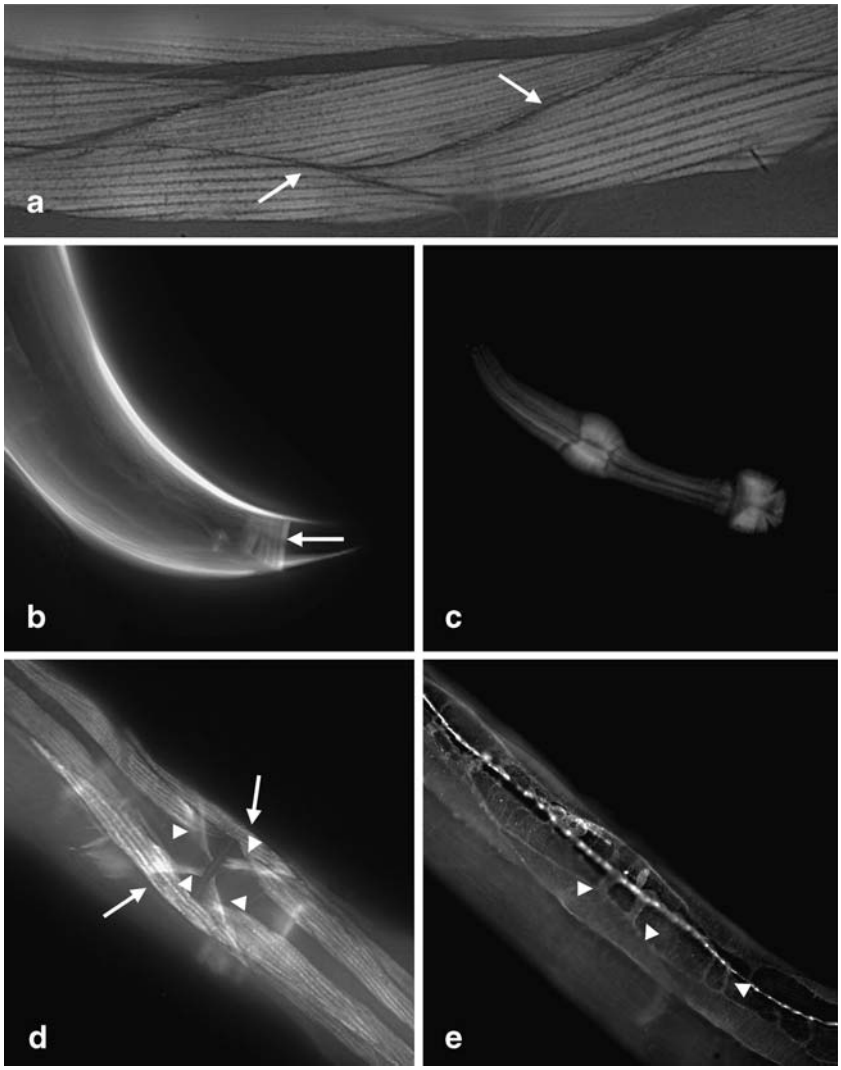

Fig. 5 The muscles of *C. elegans*. **a** Body wall muscle cells stained with phalloidin show dark and light bands that reflect the alternation of thick and thin filaments. These cells do not fuse to form multinucleated myofilaments as in mammals and the banding pattern is discontinued by the cell boundaries (*arrows*). **b** Anal depressor muscle (*arrow*) stained with phalloidin. **c** *C. elegans* muscular pharynx stained with phalloidin. **d** Four of the eight vulval cells (*arrowheads*) are clearly seen in this *C. elegans* hermaphrodite stained with phalloidin. Surrounding the vulval cells, there are body wall muscle cells (*arrows*). **e** Contrary to what happens in most organism where motor neurons send out axons to target muscles, the *C. elegans* muscle cells extend long processes (muscle arms) that reach to the motor neuron (*arrowheads*)

muscle cells (Fig. 6a,b). The peptide aggregates as occurs in AD and IBM, so it makes this worm a good model for studying both illnesses. These aggregates show Thioflavine-S (ThS) reactivity as well as immunoreactivity to anti-Aβ antibodies [155], indicating the presence of Aβ peptide. A compound derived from Congo Red called X-34 [167] has also proven to be effective in staining amyloid deposits *in vivo* [144] and has been successfully tested in our laboratory for studying the aggregates in *C. elegans*. The deposits in the worm are located intracellularly in the body wall muscle cells and thus can be seen all over the body and their structure analyzed (Fig. 6a–d). Despite the fact that mice models resemble humans more closely, *C. elegans*, as we described in "General Introduction", has many advantages when compared to mammalian models for the study of IBM and other diseases. Regarding the study of IBM in particular, there are several transgenic *C. elegans* strains available (constructed by Link and collaborators) that express the Aβ peptide directly without the need to express the full-length APP. We can then be sure that the worms will express the peptide we are interested in, eliminating the chance of expressing other protein forms due to incorrect proteolytic processing.

The use of this model is a powerful tool to study Aβ aggregation *in vivo*. The interaction with other proteins that could be involved in the metabolism of Aβ or lead to cell toxicity can also be examined. Analysis of gene expression has identified several genes that are differentially regulated in transgenic strains that express wild-type Aβ in an inducible way. Sixteen genes were found to be up-regulated and 240 down-regulated. Interestingly, this differential expression was found previous to the formation of the amyloid deposits, a short time after Aβ expression was triggered [168]. The value of this work lies in the possibility of identifying genes that could be involved in early stages of the disease, something impossible to do in AD postmortem tissue or IBM biopsis which represents a final or very advanced disease stage.

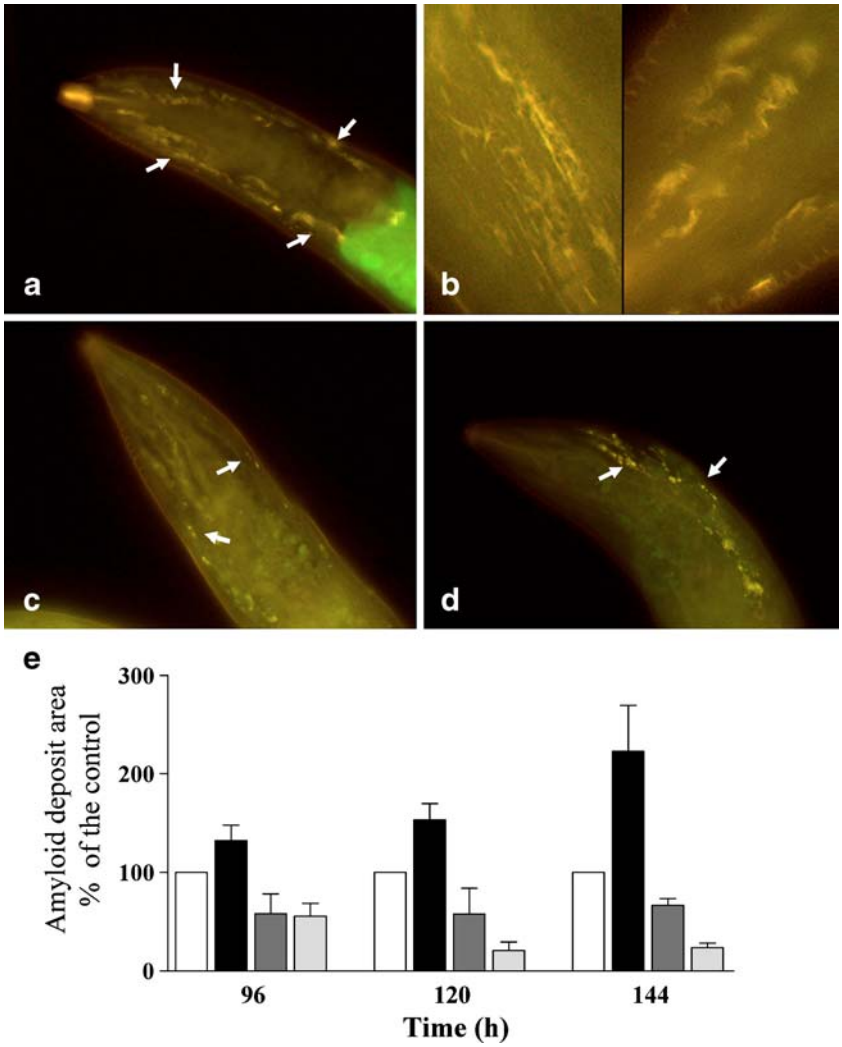

Fig. 6 Amyloid deposits in muscle cells of transgenic *C. elegans*. **a** Transgenic worms stained with Thioflavine-S (*ThS*) show the intracellular amyloid deposits (*arrows*) formed when the wild-type human Aβ is expressed in muscle cells. **b** Detail of amyloid deposits in *C.elegans* muscle cells. **c** The highly amyloidogenic Arctic Aβ variant (E22G) forms small intracellular deposits (*arrows*) in *C. elegans*. **d** The NIC variant (V18A), which favors the α-helical conformation over the β-sheet conformation and is therefore predicted to be non-aggregating, forms fewer and smaller intracellular deposits (*arrows*) in *C. elegans* muscle cells. **e** The graph shows the digital quantification of the ThS-positive amyloid deposits present anterior to the pharyngeal bulb in a transgenic strain expressing the wild-type Aβ peptide. The graph represents the percentage of total area of aggregates formed anterior to the pharyngeal bulb at different times (96–144 h) under control conditions (*white bars*) and when the animals were cultured in the presence of $CuCl_2$ (150 μM, *black bars*), histidine (150 μM, *dark gray bars*), or clioquinol (150 μM, *pale gray bars*)

Further analysis done by Link and collaborators identified different chaperone-like proteins that interact with intracellular Aβ in *C. elegans*, and their studies suggest that chaperone function can have a role in Aβ metabolism and toxicity [169]. The study of the family of heat-shock proteins (Hsp), small oxidative-response and heat-response proteins expressed in all eukaryotes including the wild-type *C. elegans*, has been carried out with the use of standard approaches such as reporter genes like β-galactosidase [170] or GFP [171]. The studies in *C. elegans* showed that Hsp-16 family members respond to the presence of Aβ peptide by increasing their expression levels in a similar way to their response to heat shock. There is also an induction in the expression of HSP-16 in response to oxidative stress induced by the production of superoxide as it happens in AD [171]. Furthermore, the overexpression of HSP-16.2 reduced Aβ toxicity, altering its oligomerization and interacting directly with the Aβ peptide [172]. There are no reports on Hsp-16 concerning IBM, but it should be looked at in muscle biopsies of patients affected by this disease. These studies show how relevant this model can be in order to contribute to our understanding of this muscle disorder.

Presenilins are proteins directly involved in the development of AD and possibly in IBM as well. Three presenilin homologs can be found in *C. elegans*: *hop-1*, *spe-4*, and *sel-12* [173]. Mutants in the *sel-12* gene have an egg laying defective phenotype [174] since it is a protein related to the organization of the vulval cells [175] including epidermal cells, neurons, and muscle cells [176]. *hop-1* and *sel-12* mutants have a loss of temperature memory and alterations in neuronal development and morphology [177]. SEL-12 has an important percentage of identity and is a functional homolog of human Presenilin 1 and 2. The heterologous expression of either one of the human presenilins in *C. elegans* can substitute for SEL-12 function. This feature makes it possible to use *C. elegans* as a model and get clues as to what takes place with the human protein [174].

AChE is another protein found in *C. elegans* that has also been implicated in AD. Four AChE genes are present

in the nematode: AChE classes A, B, C, and D encoded by genes *ace-1, 2, 3*, and *4*, respectively [178, 179]. Their expression is tissue-specific; thus, *ace-1* is expressed mainly in body wall and vulva muscles, while *ace-2* is mostly expressed in neuronal cells [180].

Other features in relation to Aβ and its toxicity, such as oxidative stress or degradation pathways, can be studied in this model. For example, an Aβ transgenic *C. elegans* strain presents high levels of oxidative stress even before the onset of Aβ aggregation [181]. This is another issue that makes this nematode suitable for studying IBM, since oxidative stress is an important factor in this disease and has been related to Aβ toxicity in AD. *In vitro* studies demonstrated that the methionine in position 35 and its oxidation status has a role in the Aβ peptide oligomerization and aggregation [182–184]. A mutation made in the Aβ encoding mini-gene (Met35Cys) has led to the notion that the methionine at position 35 is involved in amyloid formation because the substitution blocks the formation of ThS-reactive deposits [185]. Its change for cysteine leads to decreased Aβ-induced protein oxidation [186]. Protein oxidation is extensive in AD [187], and the role of Aβ as mediator of oxidative stress has also been studied in Aβ-expressing *C. elegans* strains. Several proteins that are significantly oxidized were identified in response to Aβ expression. These proteins are involved in energy metabolism, proteasome function, and scavenging of oxidants, which are consistent with functions found to be altered in the AD brain [188]. These studies offer an approach that points to proteins which could be studied with therapeutic aims both for AD and IBM.

Autophagy is a vital pathway for degrading normal and aggregated proteins. It is a constitutive mechanism activated under stress conditions that causes the turnover of cytoplasmic constituents, reduces cell size, and decreases the metabolic rate while generating new substrates for energy and cell remodeling, important for survival and longevity [189]. In AD, neuropathological markers are associated with abnormalities in the endosomal–lysosomal system, including accumulation of autophagic vacuoles (AVs). Furthermore, autophagy is increased in AD and in an AD mouse model where autophagosomes show impaired maturation and late AVs accumulate in dystrophic dendrites. AVs are active compartments for Aβ generation. In these vesicles, there is APP, βCTF-, the β-cleaved carboxyl-terminal domain of APP, and BACE, as well as increased β-secretase activity. Inmunoelectromicroscopy also shows the presence of Aβ peptide in AVs, suggesting that the abnormal accumulation of Aβ in AVs from affected neurons of AD brain contributes to β-amyloid deposition [190, 191]. Abnormal processes causing the accumulation of autophagy intermediates or vacuoles and a defective clearance of Aβ favoring its aggregation are reviewed by Nixon [192]. Studies in *C. elegans* show that the expression of Aβ causes autophagosome accumulation and that decreased insulin-receptor signaling can reduce it and decrease Aβ-induced paralysis [193]. These kinds of experiments done in *C. elegans* allow making connections among different pathways involved in human diseases and may point to possible new therapeutic approaches.

The transgenic *C. elegans* strains carrying Aβ in muscle can also be used to assay some compounds or drugs with potential protective effects. For example, in this model, the treatment with *Ginkgo biloba* extract EGb 761 and Ginkgolides are able to reduce the number of ThS-positive deposits, to change the status of Aβ species (lowering Aβ oligomers), to decrease ROS levels, and to suppress amyloid-induced pathological behaviors, including paralysis. These extracts also improve chemotaxis and serotonin sensitivity in another strain that expresses Aβ in neurons [194].

Variants of the Aβ Peptide Expressed in *C. elegans*

The existence of variants of the Aβ peptide among patients with AD represents a major concern among researchers, since the changes in the amino acid sequence cause alterations in the 3D structure of the peptide, thus changing the thermodynamics of the aggregate's formation. The Dutch variant for instance has a glutamine residue instead of a glutamate at position 22 of the peptide (or 693 of the APP). This change induces the deposition of amyloid in brain blood vessels causing cerebral amyloid angiopathy. For more information, see [195]. A second common variant of the Aβ peptide is the substitution of glutamate 22 for glycine. This mutation is called Arctic because it was first seen in a Swedish family. People carrying this variant show decreased plasma levels of Aβ, but there is faster aggregation than with the Aβ wild type [196]. Given that the ability to aggregate apparently relies on the β-sheet structure the peptide adopts, the induction of an α-helix-enriched structure should generate a peptide with a lower tendency to form aggregates. In 1995, Soto and collaborators designed an Aβ variant changing the valine at position 18 of the peptide (659 of the APP) for an alanine residue that would favor the α-helical structure. This variant is indeed less amyloidogenic in *in vitro* studies [44, 195]. We have named this variant NIC.

In our laboratory, we have been conducting experiments in order to obtain transgenic *C. elegans* strains expressing these three variants [197]. Bioballistic techniques have been adapted to transform worms carrying the *unc-119(ed3)* mutation [198]. This mutation causes an uncoordinated phenotype. The transformation consists of

introducing two different genes: one (*unc-119* wild type) is used as a co-transformation marker and will give the worm the ability to move normally (allowing us to see a clear phenotypic difference) and the other is the mini-gene for the corresponding Aβ variant. We have already obtained transformants containing the Arctic and NIC variants. Both of them show deposits reactive to Thioflavine-S, indicating the presence of aggregates but not as many as the worms containing the wild-type Aβ (Fig. 6c,d). The creation of these transgenic animals has particular relevance, since it will allow the characterization of the intracellular deposits formed by the peptides containing these mutations in muscle cells in a live animal.

Plasticity of Amyloid Deposits: Role of Metals

As it was previously mentioned, the Aβ peptide is an important constituent of the amyloid deposits in the vacuolated muscle fibers of IBM patients and in the senile plaques of AD patients. Several studies show that AD senile plaques are metal-enriched structures containing Cu^{2+}, Fe^{3+}, and Zn^{2+} [199, 200]. On the other hand, the presence of Al^{3+} in senile plaques and neurofibrillary tangles has been much more controversial [201–206] as is its involvement in AD development [207]. While some authors show that there is no influence of aluminum on Alzheimer-like pathology [208], others argue that the involvement of Al in the pathogenesis of AD should not be discarded [209]. Recent studies show that Al promotes Aβ aggregation which is able to induce toxic effects on neuroblastoma cells [210] and correlate chronic Al^{3+} exposure with Aβ deposition and decrease of the stress response protein GRP78, similar to that seen in congophilic amyloid angiopathy in humans [211].

It is possible that Cu^{2+}, Fe^{3+}, and Zn^{2+} may be responsible, at least in part, for plaque plasticity. For instance, copper and zinc accelerate aggregation of synthetic Aβ peptide *in vitro* [212, 213]. It has also been shown that clioquinol—a copper and zinc chelator—diminishes the size of the extracellular Aβ deposits present in an APP transgenic mouse model. This drug also improves the mice's overall health [214]. In a clinical trial in which 18 AD patients were treated with clioquinol during 36 weeks, there was a delay in the progression of the disease. It is hypothesized that the effect is due to an alteration in Aβ metabolism [215]. Nevertheless, there are conflicting reports that point either to a beneficial or detrimental role of copper in relation to Aβ. Transgenic mice that overexpress APP have diminished copper levels [216, 217], and when their diet is supplemented with bioavailable copper, they show decreased production of soluble Aβ and restored normal SOD-1 activity [218]. In addition, the *tx* mutation (metal ATPase7b transporter) in transgenic CRND8 mice that form amyloid deposits results in a decrease in plaque formation and Aβ plasma levels [219], which could be related to an increase in Aβ catabolism and/or Aβ clearance [220, 221]. On the other hand, copper ionophores like clioquinol and PBT2 show benefits in transgenic mouse models and phase 2 clinical trials of AD [214, 215, 222] (PRANABIO, personal communication)

In our laboratory, we are testing the hypothesis that copper levels modulate intracellular Aβ peptide aggregation in muscle cells of *C. elegans*. To test this hypothesis, we cultured the worms in agar plates supplemented with $CuCl_2$ or the copper chelators histidine and clioquinol to see whether or not there was an alteration in the Aβ peptide aggregation. Using ThS, we were able to visualize the amyloid deposits in 4- to 6-day-old worms. Quantification of the ThS-positive aggregates showed that the intramuscular amyloid deposits are indeed affected by copper levels (Minniti et al., in preparation; Fig. 6e). Taking into account the many similarities found between AD and IBM, we suggest that there is a possible role of copper and zinc in IBM too. It has been described that the isoform of SOD that is dependent on copper and zinc is involved in IBM [223], but no research has been done on these metals and their possible role in this myopathy.

Summary

In this review, we have discussed what is known about IBM, its symptoms, genetics, pathological landmarks, and molecules involved. We have also explained at length the features we think make the nematode *C. elegans* a suitable model for studying IBM at the molecular level. We showed that transgenic *C. elegans* strains have already made significant contributions to our knowledge of the changes that occur in muscle cells when amyloid aggregates form intracellularly (one of the features of IBM), for example, the onset of stress response in the affected muscle, the identification of genes that change their expression after Aβ expression in muscle cells, the role of autophagosomes, how Aβ oligomers and Aβ aggregates affect muscle function, and the possible role of metals in modulating intracellular Aβ in muscle cells. With this knowledge, it could be possible to go back to mammalian models, human biopsies, and cell culture models and test the hypothesis suggested by the work done in *C. elegans*. Finally, the experimental results obtained with the use of certain natural products and its derivatives that show behavioral improvement in the *C. elegans* models of IBM could point to new therapeutic alternatives for treating this disease.

References

1. Selkoe DJ (2007) Developing preventive therapies for chronic diseases: lessons learned from Alzheimer's disease. Nutr Rev 65: S239–S243
2. Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81:741–766
3. Engel WK, Askanas V (2006) Inclusion-body myositis: clinical, diagnostic, and pathologic aspects. Neurology 66:S20–S29
4. Askanas V, Engel WK (2007) Inclusion-body myositis, a multifactorial muscle disease associated with aging: current concepts of pathogenesis. Curr Opin Rheumatol 19:550–559
5. Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 8:101–112
6. Brenner S (2003) Nature's gift to science (Nobel lecture). Chembiochem 4:683–687
7. Culetto E, Sattelle DB (2000) A role for *Caenorhabditis elegans* in understanding the function and interactions of human disease genes. Hum Mol Genet 9:869–877
8. Riddle DL, Blumenthal T, Meyer BJ, Priess JR (1997) *C. elegans* II. In: Riddle DL, Blumenthal T, Meyer BJ, Priess JR (eds) Cold Spring Harbor monograph series. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
9. Hope IA (1999) C. elegans: a practical approach. In: Hope IA (ed) The practical approach series. Oxford University Press, Oxford, pp 69–95
10. Crawford D, Libina N, Kenyon C (2007) *Caenorhabditis elegans* integrates food and reproductive signals in lifespan determination. Aging Cell 6:715–721
11. Ghazi A, Henis-Korenblit S, Kenyon C (2007) Regulation of *Caenorhabditis elegans* lifespan by a proteasomal E3 ligase complex. Proc Natl Acad Sci U S A 104:5947–5952
12. Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C (2008) A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. PLoS Genet 4:e24
13. Broue F, Liere P, Kenyon C, Baulieu EE (2007) A steroid hormone that extends the lifespan of *Caenorhabditis elegans*. Aging Cell 6:87–94
14. Hansen M, Hsu AL, Dillin A, Kenyon C (2005) New genes tied to endocrine, metabolic, and dietary regulation of lifespan from a *Caenorhabditis elegans* genomic RNAi screen. PLoS Genet 1:119–128
15. Sulston JE, Horvitz HR (1977) Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev Biol 56:110–156
16. Horvitz HR (2003) Worms, life, and death (Nobel lecture). Chembiochem 4:697–711
17. Metzstein MM, Stanfield GM, Horvitz HR (1998) Genetics of programmed cell death in *C. elegans*: past, present and future. Trends Genet 14:410–416
18. Sulston JE (2003) *Caenorhabditis elegans*: the cell lineage and beyond (Nobel lecture). Chembiochem 4:688–696
19. Badrising UA, Maat-Schieman ML, van Houwelingen JC et al (2005) Inclusion body myositis. Clinical features and clinical course of the disease in 64 patients. J Neurol 252:1448–1454
20. Adams RD, Kakulas BA, Samaha FA (1965) A myopathy with cellular inclusions. Trans Am Neurol Assoc 90:213–216
21. Lotz BP, Engel AG, Nishino H, Stevens JC, Litchy WJ (1989) Inclusion body myositis. Observations in 40 patients. Brain 112 (Pt 3):727–747
22. Figarella-Branger D, Civatte M, Bartoli C, Pellissier JF (2003) Cytokines, chemokines, and cell adhesion molecules in inflammatory myopathies. Muscle Nerve 28:659–682
23. Neville HE, Baumbach LL, Ringel SP, Russo LS Jr., Sujansky E, Garcia CA (1992) Familial inclusion body myositis: evidence for autosomal dominant inheritance. Neurology 42:897–902
24. McFerrin J, Engel WK, Askanas V (1998) Impaired innervation of cultured human muscle overexpressing betaAPP experimentally and genetically: relevance to inclusion-body myopathies. Neuroreport 9:3201–3205
25. Askanas V, Alvarez RB, Engel WK (1993) Beta-amyloid precursor epitopes in muscle fibers of inclusion body myositis. Ann Neurol 34:551–560
26. Askanas V, Engel WK (1993) New advances in inclusion-body myositis. Curr Opin Rheumatol 5:732–741
27. Needham M, Mastaglia FL, Garlepp MJ (2007) Genetics of inclusion-body myositis. Muscle Nerve 35:549–561
28. Koffman BM, Sivakumar K, Simonis T, Stroncek D, Dalakas MC (1998) HLA allele distribution distinguishes sporadic inclusion body myositis from hereditary inclusion body myopathies. J Neuroimmunol 84:139–142
29. Ranque-Francois B, Maisonobe T, Dion E et al (2005) Familial inflammatory inclusion body myositis. Ann Rheum Dis 64:634–637
30. Hubbers CU, Clemen CS, Kesper K et al (2007) Pathological consequences of VCP mutations on human striated muscle. Brain 130:381–393
31. Lindberg C, Trysberg E, Tarkowski A, Oldfors A (2003) Anti-T-lymphocyte globulin treatment in inclusion body myositis: a randomized pilot study. Neurology 61:260–262
32. Schmidt J, Rakocevic G, Raju R, Dalakas MC (2004) Upregulated inducible co-stimulator (ICOS) and ICOS-ligand in inclusion body myositis muscle: significance for CD8+ T cell cytotoxicity. Brain 127:1182–1190
33. Amemiya K, Granger RP, Dalakas MC (2000) Clonal restriction of T-cell receptor expression by infiltrating lymphocytes in inclusion body myositis persists over time. Studies in repeated muscle biopsies. Brain 123(Pt 10):2030–2039
34. Raju R, Vasconcelos O, Granger R, Dalakas MC (2003) Expression of IFN-gamma-inducible chemokines in inclusion body myositis. J Neuroimmunol 141:125–131
35. Dalakas MC (2004) Intravenous immunoglobulin in autoimmune neuromuscular diseases. JAMA 291:2367–2375
36. Askanas V, Engel WK, Bilak M, Alvarez RB, Selkoe DJ (1994) Twisted tubulofilaments of inclusion body myositis muscle resemble paired helical filaments of Alzheimer brain and contain hyperphosphorylated tau. Am J Pathol 144:177–187
37. Askanas V, Engel WK (2001) Inclusion-body myositis: newest concepts of pathogenesis and relation to aging and Alzheimer disease. J Neuropathol Exp Neurol 60:1–14
38. Askanas V, Engel WK (2002) Newest pathogenetic considerations in inclusion-body myositis: possible role of amyloid-beta, cholesterol, relation to aging and to Alzheimer's disease. Curr Rheumatol Rep 4:427–433
39. Broccolini A, Engel WK, Alvarez RB, Askanas V (2000) Paired helical filaments of inclusion-body myositis muscle contain RNA and survival motor neuron protein. Am J Pathol 156:1151–1155
40. Wilczynski GM, Engel WK, Askanas V (2000) Association of active extracellular signal-regulated protein kinase with paired helical filaments of inclusion-body myositis muscle suggests its role in inclusion-body myositis tau phosphorylation. Am J Pathol 156:1835–1840
41. Kumamoto T, Ueyama H, Tsumura H, Toyoshima I, Tsuda T (2004) Expression of lysosome-related proteins and genes in the skeletal muscles of inclusion body myositis. Acta Neuropathol 107:59–65
42. Fukuchi K, Pham D, Hart M, Li L, Lindsey JR (1998) Amyloid-beta deposition in skeletal muscle of transgenic mice: possible model of inclusion body myopathy. Am J Pathol 153:1687–1693
43. Morgan C, Colombres M, Nunez MT, Inestrosa NC (2004) Structure and function of amyloid in Alzheimer's disease. Prog Neurobiol 74:323–349

44. Soto C, Branes MC, Alvarez J, Inestrosa NC (1994) Structural determinants of the Alzheimer's amyloid beta-peptide. J Neurochem 63:1191–1198
45. Askanas V, Engel WK, Alvarez RB (1993) Enhanced detection of Congo-Red-positive amyloid deposits in muscle fibers of inclusion body myositis and brain of Alzheimer's disease using fluorescence technique. Neurology 43:1265–1267
46. Dalakas MC, Koffman B, Fujii M, Spector S, Sivakumar K, Cupler E (2001) A controlled study of intravenous immunoglobulin combined with prednisone in the treatment of IBM. Neurology 56:323–327
47. Barohn RJ, Herbelin L, Kissel JT et al (2006) Pilot trial of etanercept in the treatment of inclusion-body myositis. Neurology 66:S123–S124
48. Wolfe MS (2008) Gamma-secretase: structure, function, and modulation for Alzheimer's disease. Curr Top Med Chem 8:2–8
49. Zhang YW, Xu H (2007) Molecular and cellular mechanisms for Alzheimer's disease: understanding APP metabolism. Curr Mol Med 7:687–696
50. Jin LW, Hearn MG, Ogburn CE et al (1998) Transgenic mice over-expressing the C-99 fragment of betaPP with an alpha-secretase site mutation develop a myopathy similar to human inclusion body myositis. Am J Pathol 153:1679–1686
51. Tateyama M, Takeda A, Onodera Y et al (2003) Oxidative stress and predominant Abeta42(43) deposition in myopathies with rimmed vacuoles. Acta Neuropathol 105:581–585
52. Lunemann JD, Schmidt J, Schmid D et al (2007) Beta-amyloid is a substrate of autophagy in sporadic inclusion body myositis. Ann Neurol 61:476–483
53. Iqbal K, Alonso Adel C, Chen S et al (2005) Tau pathology in Alzheimer disease and other tauopathies. Biochim Biophys Acta 1739:198–210
54. Mirabella M, Alvarez RB, Bilak M, Engel WK, Askanas V (1996) Difference in expression of phosphorylated tau epitopes between sporadic inclusion-body myositis and hereditary inclusion-body myopathies. J Neuropathol Exp Neurol 55:774–786
55. Pei JJ, Braak H, An WL et al (2002) Up-regulation of mitogen-activated protein kinases ERK1/2 and MEK1/2 is associated with the progression of neurofibrillary degeneration in Alzheimer's disease. Brain Res Mol Brain Res 109:45–55
56. Gross SD, Anderson RA (1998) Casein kinase I: spatial organization and positioning of a multifunctional protein kinase family. Cell Signal 10:699–711
57. Flotow H, Graves PR, Wang AQ, Fiol CJ, Roeske RW, Roach PJ (1990) Phosphate groups as substrate determinants for casein kinase I action. J Biol Chem 265:14264–14269
58. Risnik VV, Adam G, Gusev NB, Friedrich P (1988) Casein kinases I and II bound to pig brain microtubules. Cell Mol Neurobiol 8:315–324
59. Li G, Yin H, Kuret J (2004) Casein kinase 1 delta phosphorylates tau and disrupts its binding to microtubules. J Biol Chem 279:15938–15945
60. Ghoshal N, Smiley JF, DeMaggio AJ et al (1999) A new molecular link between the fibrillar and granulovacuolar lesions of Alzheimer's disease. Am J Pathol 155:1163–1172
61. Kannanayakal TJ, Tao H, Vandre DD, Kuret J (2006) Casein kinase-1 isoforms differentially associate with neurofibrillary and granulovacuolar degeneration lesions. Acta Neuropathol 111:413–421
62. Price MA (2006) CKI, there's more than one: casein kinase I family members in Wnt and Hedgehog signaling. Genes Dev 20:399–410
63. Caricasole A, Copani A, Caraci F et al (2004) Induction of Dickkopf-1, a negative modulator of the Wnt pathway, is associated with neuronal degeneration in Alzheimer's brain. J Neurosci 24:6021–6027
64. Inestrosa N, De Ferrari GV, Garrido JL et al (2002) Wnt signaling involvement in beta-amyloid-dependent neurodegeneration. Neurochem Int 41:341–344
65. De Ferrari GV, Inestrosa NC (2000) Wnt signaling function in Alzheimer's disease. Brain Res Brain Res Rev 33:1–12
66. Kannanayakal TJ, Mendell JR, Kuret J (2008) Casein kinase 1 alpha associates with the tau-bearing lesions of inclusion body myositis. Neurosci Lett 431:141–145
67. Wolfe MS (2008) Gamma-secretase inhibition and modulation for Alzheimer's disease. Curr Alzheimer Res 5:158–164
68. Evin G, Sernee MF, Masters CL (2006) Inhibition of gamma-secretase as a therapeutic intervention for Alzheimer's disease: prospects, limitations and strategies. CNS Drugs 20:351–372
69. Askanas V, Engel WK, Yang CC, Alvarez RB, Lee VM, Wisniewski T (1998) Light and electron microscopic immunolocalization of presenilin 1 in abnormal muscle fibers of patients with sporadic inclusion-body myositis and autosomal-recessive inclusion-body myopathy. Am J Pathol 152:889–895
70. Vassar R (2004) BACE1: the beta-secretase enzyme in Alzheimer's disease. J Mol Neurosci 23:105–114
71. Cole SL, Vassar R (2007) The Alzheimer's disease beta-secretase enzyme, BACE1. Mol Neurodegener 2:22
72. Vattemi G, Engel WK, McFerrin J, Buxbaum JD, Pastorino L, Askanas V (2001) Presence of BACE1 and BACE2 in muscle fibres of patients with sporadic inclusion-body myositis. Lancet 358:1962–1964
73. Vattemi G, Engel WK, McFerrin J, Pastorino L, Buxbaum JD, Askanas V (2003) BACE1 and BACE2 in pathologic and normal human muscle. Exp Neurol 179:150–158
74. He W, Lu Y, Qahwash I, Hu XY, Chang A, Yan R (2004) Reticulon family members modulate BACE1 activity and amyloid-beta peptide generation. Nat Med 10:959–965
75. Wojcik S, Engel WK, Yan R, McFerrin J, Askanas V (2007) NOGO is increased and binds to BACE1 in sporadic inclusion-body myositis and in A beta PP-overexpressing cultured human muscle fibers. Acta Neuropathol 114:517–526
76. Carson KA, Geula C, Mesulam MM (1991) Electron microscopic localization of cholinesterase activity in Alzheimer brain tissue. Brain Res 540:204–208
77. Geula C, Greenberg BD, Mesulam MM (1994) Cholinesterase activity in the plaques, tangles and angiopathy of Alzheimer's disease does not emanate from amyloid. Brain Res 644:327–330
78. Inestrosa NC, Alarcon R (1998) Molecular interactions of acetylcholinesterase with senile plaques. J Physiol Paris 92:341–344
79. Alvarez A, Alarcon R, Opazo C et al (1998) Stable complexes involving acetylcholinesterase and amyloid-beta peptide change the biochemical properties of the enzyme and increase the neurotoxicity of Alzheimer's fibrils. J Neurosci 18:3213–3223
80. Inestrosa NC, Alvarez A, Calderon F (1996) Acetylcholinesterase is a senile plaque component that promotes assembly of amyloid beta-peptide into Alzheimer's filaments. Mol Psychiatry 1:359–361
81. Inestrosa NC, Alvarez A, Perez CA et al (1996) Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: possible role of the peripheral site of the enzyme. Neuron 16:881–891
82. Alvarez A, Bronfman F, Perez CA, Vicente M, Garrido J, Inestrosa NC (1995) Acetylcholinesterase, a senile plaque component, affects the fibrillogenesis of amyloid-beta-peptides. Neurosci Lett 201:49–52
83. Alvarez A, Opazo C, Alarcon R, Garrido J, Inestrosa NC (1997) Acetylcholinesterase promotes the aggregation of amyloid-beta-peptide fragments by forming a complex with the growing fibrils. J Mol Biol 272:348–361

84. De Ferrari GV, Canales MA, Shin I, Weiner LM, Silman I, Inestrosa NC (2001) A structural motif of acetylcholinesterase that promotes amyloid beta-peptide fibril formation. Biochemistry 40:10447–10457
85. Reyes AE, Chacon MA, Dinamarca MC, Cerpa W, Morgan C, Inestrosa NC (2004) Acetylcholinesterase–Abeta complexes are more toxic than Abeta fibrils in rat hippocampus: effect on rat beta-amyloid aggregation, laminin expression, reactive astrocytosis, and neuronal cell loss. Am J Pathol 164:2163–2174
86. Rees T, Hammond PI, Soreq H, Younkin S, Brimijoin S (2003) Acetylcholinesterase promotes beta-amyloid plaques in cerebral cortex. Neurobiol Aging 24:777–787
87. Inestrosa NC, Sagal JP, Colombres M (2005) Acetylcholinesterase interaction with Alzheimer amyloid beta. Subcell Biochem 38:299–317
88. Inestrosa NC, Dinamarca MC, Alvarez A (2008) Amyloid-cholinesterase interactions. Implications for Alzheimer's disease. Febs J 275:625–632
89. Askanas V, Engel WK (2003) Unfolding story of inclusion-body myositis and myopathies: role of misfolded proteins, amyloid-beta, cholesterol, and aging. J Child Neurol 18:185–190
90. Frears ER, Stephens DJ, Walters CE, Davies H, Austen BM (1999) The role of cholesterol in the biosynthesis of beta-amyloid. Neuroreport 10:1699–1705
91. Jaworska-Wilczynska M, Wilczynski GM, Engel WK, Strickland DK, Weisgraber KH, Askanas V (2002) Three lipoprotein receptors and cholesterol in inclusion-body myositis muscle. Neurology 58:438–445
92. Askanas V, Mirabella M, Engel WK, Alvarez RB, Weisgraber KH (1994) Apolipoprotein E immunoreactive deposits in inclusion-body muscle diseases. Lancet 343:364–365
93. Mirabella M, Alvarez RB, Engel WK, Weisgraber KH, Askanas V (1996) Apolipoprotein E and apolipoprotein E messenger RNA in muscle of inclusion body myositis and myopathies. Ann Neurol 40:864–872
94. Inestrosa NC, Marzolo MP, Bonnefont AB (1998) Cellular and molecular basis of estrogen's neuroprotection. Potential relevance for Alzheimer's disease. Mol Neurobiol 17:73–86
95. Roses AD, Saunders AM (1997) Apolipoprotein E genotyping as a diagnostic adjunct for Alzheimer's disease. Int Psychogeriatr 9 (Suppl 1):277–288 discussion 317–221
96. Roses AD (2006) On the discovery of the genetic association of Apolipoprotein E genotypes and common late-onset Alzheimer disease. J Alzheimers Dis 9:361–366
97. Roses AD (1996) Apolipoprotein E alleles as risk factors in Alzheimer's disease. Annu Rev Med 47:387–400
98. Caruso A, Motolese M, Iacovelli L et al (2006) Inhibition of the canonical Wnt signaling pathway by apolipoprotein E4 in PC12 cells. J Neurochem 98:364–371
99. Gossrau G, Gestrich B, Koch R et al (2004) Apolipoprotein E and alpha-1-antichymotrypsin polymorphisms in sporadic inclusion body myositis. Eur Neurol 51:215–220
100. Albrecht S, Bilbao JM (1993) Ubiquitin expression in inclusion body myositis. An immunohistochemical study. Arch Pathol Lab Med 117:789–793
101. Askanas V, Serdaroglu P, Engel WK, Alvarez RB (1991) Immunolocalization of ubiquitin in muscle biopsies of patients with inclusion body myositis and oculopharyngeal muscular dystrophy. Neurosci Lett 130:73–76
102. Mori H, Kondo J, Ihara Y (1987) Ubiquitin is a component of paired helical filaments in Alzheimer's disease. Science 235:1641–1644
103. Perry G, Friedman R, Shaw G, Chau V (1987) Ubiquitin is detected in neurofibrillary tangles and senile plaque neurites of Alzheimer disease brains. Proc Natl Acad Sci U S A 84:3033–3036
104. Weihl CC, Miller SE, Hanson PI, Pestronk A (2007) Transgenic expression of inclusion body myopathy associated mutant p97/VCP causes weakness and ubiquitinated protein inclusions in mice. Hum Mol Genet 16:919–928
105. Fratta P, Engel WK, McFerrin J, Davies KJ, Lin SW, Askanas V (2005) Proteasome inhibition and aggresome formation in sporadic inclusion-body myositis and in amyloid-beta precursor protein-overexpressing cultured human muscle fibers. Am J Pathol 167:517–526
106. Parkin ET, Watt NT, Hussain I et al (2007) Cellular prion protein regulates beta-secretase cleavage of the Alzheimer's amyloid precursor protein. Proc Natl Acad Sci U S A 104:11062–11067
107. Gacia M, Safranow K, Styczynska M et al (2006) Prion protein gene M129 allele is a risk factor for Alzheimer's disease. J Neural Transm 113:1747–1751
108. Inestrosa NC, Cerpa W, Varela-Nallar L (2005) Copper brain homeostasis: role of amyloid precursor protein and prion protein. IUBMB Life 57:645–650
109. Varela-Nallar L, Toledo EM, Larrondo LF, Cabral AL, Martins VR, Inestrosa NC (2006) Induction of cellular prion protein gene expression by copper in neurons. Am J Physiol Cell Physiol 290: C271–281
110. Varela-Nallar L, Gonzalez A, Inestrosa NC (2006) Role of copper in prion diseases: deleterious or beneficial?. Curr Pharm Des 12:2587–2595
111. Zanusso G, Vattemi G, Ferrari S et al (2001) Increased expression of the normal cellular isoform of prion protein in inclusion-body myositis, inflammatory myopathies and denervation atrophy. Brain Pathol 11:182–189
112. Kovacs GG, Lindeck-Pozza E, Chimelli L et al (2004) Creutzfeldt-Jakob disease and inclusion body myositis: abundant disease-associated prion protein in muscle. Ann Neurol 55: 121–125
113. Sarkozi E, Askanas V, Engel WK (1994) Abnormal accumulation of prion protein mRNA in muscle fibers of patients with sporadic inclusion-body myositis and hereditary inclusion-body myopathy. Am J Pathol 145:1280–1284
114. Huang S, Liang J, Zheng M et al (2007) Inducible overexpression of wild-type prion protein in the muscles leads to a primary myopathy in transgenic mice. Proc Natl Acad Sci U S A 104:6800–6805
115. Bilak M, Askanas V, Engel WK (1993) Strong immunoreactivity of alpha 1-antichymotrypsin co-localizes with beta-amyloid protein and ubiquitin in vacuolated muscle fibers of inclusion-body myositis. Acta Neuropathol 85:378–382
116. Vattemi G, Engel WK, McFerrin J, Askanas V (2003) Cystatin C colocalizes with amyloid-beta and coimmunoprecipitates with amyloid-beta precursor protein in sporadic inclusion-body myositis muscles. J Neurochem 85:1539–1546
117. Crawford FC, Freeman MJ, Schinka JA et al (2000) A polymorphism in the cystatin C gene is a novel risk factor for late-onset Alzheimer's disease. Neurology 55:763–768
118. Finckh U, von der Kammer H, Velden J et al (2000) Genetic association of a cystatin C gene polymorphism with late-onset Alzheimer disease. Arch Neurol 57:1579–1583
119. Mao JJ, Katayama S, Watanabe C et al (2001) The relationship between alphaB-crystallin and neurofibrillary tangles in Alzheimer's disease. Neuropathol Appl Neurobiol 27:180–188
120. Dabir DV, Trojanowski JQ, Richter-Landsberg C, Lee VM, Forman MS (2004) Expression of the small heat-shock protein alphaB-crystallin in tauopathies with glial pathology. Am J Pathol 164:155–166
121. Augusteyn RC (2004) alpha-crystallin: a review of its structure and function. Clin Exp Optom 87:356–366
122. Horwitz J (2003) Alpha-crystallin. Exp Eye Res 76:145–153

123. Stege GJ, Renkawek K, Overkamp PS et al (1999) The molecular chaperone alphaB-crystallin enhances amyloid beta neurotoxicity. Biochem Biophys Res Commun 262:152–156
124. Banwell BL, Engel AG (2000) AlphaB-crystallin immunolocalization yields new insights into inclusion body myositis. Neurology 54:1033–1041
125. Wojcik S, Engel WK, McFerrin J, Paciello O, Askanas V (2006) AbetaPP-overexpression and proteasome inhibition increase alphaB-crystallin in cultured human muscle: relevance to inclusion-body myositis. Neuromuscul Disord 16:839–844
126. Wilhelmus MM, de Waal RM, Verbeek MM (2007) Heat shock proteins and amateur chaperones in amyloid-Beta accumulation and clearance in Alzheimer's disease. Mol Neurobiol 35:203–216
127. Gonzalez-Cadavid NF, Bhasin S (2004) Role of myostatin in metabolism. Curr Opin Clin Nutr Metab Care 7:451–457
128. Wojcik S, Nogalska A, McFerrin J, Engel WK, Oledzka G, Askanas V (2007) Myostatin precursor protein is increased and associates with amyloid-beta precursor protein in inclusion-body myositis culture model. Neuropathol Appl Neurobiol 33:238–242
129. Wojcik S, Engel WK, McFerrin J, Askanas V (2005) Myostatin is increased and complexes with amyloid-beta within sporadic inclusion-body myositis muscle fibers. Acta Neuropathol 110:173–177
130. Miranda S, Opazo C, Larrondo LF et al (2000) The role of oxidative stress in the toxicity induced by amyloid beta-peptide in Alzheimer's disease. Prog Neurobiol 62:633–648
131. Thomas T, Thomas G, McLendon C, Sutton T, Mullan M (1996) Beta-amyloid-mediated vasoactivity and vascular endothelial damage. Nature 380:168–171
132. Opazo C, Huang X, Cherny RA et al (2002) Metalloenzyme-like activity of Alzheimer's disease beta-amyloid. Cu-dependent catalytic conversion of dopamine, cholesterol, and biological reducing agents to neurotoxic H(2)O(2). J Biol Chem 277:40302–40308
133. Behl C, Davis JB, Lesley R, Schubert D (1994) Hydrogen peroxide mediates amyloid beta protein toxicity. Cell 77:817–827
134. Santos MJ, Quintanilla RA, Toro A et al (2005) Peroxisomal proliferation protects from beta-amyloid neurodegeneration. J Biol Chem 280:41057–41068
135. Ruiz FH, Gonzalez M, Bodini M, Opazo C, Inestrosa NC (1999) Cysteine 144 is a key residue in the copper reduction by the beta-amyloid precursor protein. J Neurochem 73:1288–1292
136. Oldfors A, Moslemi AR, Jonasson L, Ohlsson M, Kollberg G, Lindberg C (2006) Mitochondrial abnormalities in inclusion-body myositis. Neurology 66:S49–S55
137. Fukuchi K, Ho L, Younkin SG et al (1996) High levels of circulating beta-amyloid peptide do not cause cerebral beta-amyloidosis in transgenic mice. Am J Pathol 149:219–227
138. Fukuchi K, Li L, Hart M, Lindsey JR (2000) Accumulation of amyloid-beta protein in exocrine glands of transgenic mice overexpressing a carboxyl terminal portion of amyloid protein precursor. Int J Exp Pathol 81:231–239
139. Sugarman MC, Yamasaki TR, Oddo S et al (2002) Inclusion body myositis-like phenotype induced by transgenic overexpression of beta APP in skeletal muscle. Proc Natl Acad Sci U S A 99:6334–6339
140. Sugarman MC, Kitazawa M, Baker M, Caiozzo VJ, Querfurth HW, LaFerla FM (2006) Pathogenic accumulation of APP in fast twitch muscle of IBM patients and a transgenic model. Neurobiol Aging 27:423–432
141. Kitazawa M, Green KN, Caccamo A, LaFerla FM (2006) Genetically augmenting Abeta42 levels in skeletal muscle exacerbates inclusion body myositis-like pathology and motor deficits in transgenic mice. Am J Pathol 168:1986–1997
142. Kitazawa M, Trinh DN, Laferla FM (2008) Inflammation induces tau pathology in inclusion body myositis model via glycogen synthase kinase-3beta. Ann Neurol 64:15–24
143. Feany MB (2000) Studying human neurodegenerative diseases in flies and worms. J Neuropathol Exp Neurol 59:847–856
144. Link CD (2001) Transgenic invertebrate models of age-associated neurodegenerative diseases. Mech Ageing Dev 122:1639–1649
145. Link CD (2005) Invertebrate models of Alzheimer's disease. Genes Brain Behav 4:147–156
146. Miguel-Aliaga I, Culetto E, Walker DS, Baylis HA, Sattelle DB, Davies KE (1999) The *Caenorhabditis elegans* orthologue of the human gene responsible for spinal muscular atrophy is a maternal product critical for germline maturation and embryonic viability. Hum Mol Genet 8:2133–2143
147. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC (1994) Green fluorescent protein as a marker for gene expression. Science 263:802–805
148. Chapin A, Correa P, Maguire M, Kohn R (2007) Synaptic neurotransmission protein UNC-13 affects RNA interference in neurons. Biochem Biophys Res Commun 354:1040–1044
149. Kamath RS, Ahringer J (2003) Genome-wide RNAi screening in *Caenorhabditis elegans*. Methods 30:313–321
150. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391:806–811
151. Faber PW, Alter JR, MacDonald ME, Hart AC (1999) Polyglutamine-mediated dysfunction and apoptotic death of a *Caenorhabditis elegans* sensory neuron. Proc Natl Acad Sci U S A 96:179–184
152. Satyal SH, Schmidt E, Kitagawa K et al (2000) Polyglutamine aggregates alter protein folding homeostasis in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 97:5750–5755
153. Lakso M, Vartiainen S, Moilanen AM et al (2003) Dopaminergic neuronal loss and motor deficits in *Caenorhabditis elegans* overexpressing human alpha-synuclein. J Neurochem 86:165–172
154. Kuwahara T, Koyama A, Gengyo-Ando K et al (2006) Familial Parkinson mutant alpha-synuclein causes dopamine neuron dysfunction in transgenic *Caenorhabditis elegans*. J Biol Chem 281:334–340
155. Link CD (1995) Expression of human beta-amyloid peptide in transgenic *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 92:9368–9372
156. Waterston RH (1998) The nematode *Caenorhabditis elegans*. In: Wood WB (ed) Cold Spring Harbor monograph series. Cold Spring Harbor Laboratory, NY, pp 281–335
157. Moerman DG, Williams BD (2006) Sarcomere assembly in *C. elegans* muscle. WormBook 16:1–16
158. Francis R, Waterston RH (1991) Muscle cell attachment in *Caenorhabditis elegans*. J Cell Biol 114:465–479
159. Lecroisey C, Segalat L, Gieseler K (2007) The *C. elegans* dense body: anchoring and signaling structure of the muscle. J Muscle Res Cell Motil 28:79–87
160. Epstein HF (1990) Genetic analysis of myosin assembly in *Caenorhabditis elegans*. Mol Neurobiol 4:1–25
161. Castellani L, Vibert P, Cohen C (1983) Structure of myosin/paramyosin filaments from a molluscan smooth muscle. J Mol Biol 167:853–872
162. Beall CJ, Sepanski MA, Fyrberg EA (1989) Genetic dissection of Drosophila myofibril formation: effects of actin and myosin heavy chain null alleles. Genes Dev 3:131–140
163. Lu MH, DiLullo C, Schultheiss T et al (1992) The vinculin/sarcomeric-alpha-actinin/alpha-actin nexus in cultured cardiac myocytes. J Cell Biol 117:1007–1022
164. Francis GR, Waterston RH (1985) Muscle organization in *Caenorhabditis elegans*: localization of proteins implicated in thin filament attachment and I-band organization. J Cell Biol 101:1532–1549

165. Gettner SN, Kenyon C, Reichardt LF (1995) Characterization of beta pat-3 heterodimers, a family of essential integrin receptors in *C. elegans*. J Cell Biol 129:1127–1141
166. Daigle I, Li C (1993) apl-1, a *Caenorhabditis elegans* gene encoding a protein related to the human beta-amyloid protein precursor. Proc Natl Acad Sci U S A 90:12045–12049
167. Styren SD, Hamilton RL, Styren GC, Klunk WE (2000) X-34, a fluorescent derivative of Congo Red: a novel histochemical stain for Alzheimer's disease pathology. J Histochem Cytochem 48:1223–1232
168. Link CD, Taft A, Kapulkin V et al (2003) Gene expression analysis in a transgenic *Caenorhabditis elegans* Alzheimer's disease model. Neurobiol Aging 24:397–413
169. Fonte V, Kapulkin V, Taft A, Fluet A, Friedman D, Link CD (2002) Interaction of intracellular beta amyloid peptide with chaperone proteins. Proc Natl Acad Sci U S A 99:9439–9444
170. Stringham EG, Jones D, Candido EP (1992) Expression of the polyubiquitin-encoding gene (ubq-1) in transgenic *Caenorhabditis elegans*. Gene 113:165–173
171. Link CD, Cypser JR, Johnson CJ, Johnson TE (1999) Direct observation of stress response in *Caenorhabditis elegans* using a reporter transgene. Cell Stress Chaperones 4:235–242
172. Fonte V, Kipp DR, Yerg J 3rd et al (2008) Suppression of in vivo beta-amyloid peptide toxicity by overexpression of the HSP-16.2 small chaperone protein. J Biol Chem 283:784–791
173. Westlund B, Parry D, Clover R, Basson M, Johnson CD (1999) Reverse genetic analysis of *Caenorhabditis elegans* presenilins reveals redundant but unequal roles for sel-12 and hop-1 in Notch-pathway signaling. Proc Natl Acad Sci U S A 96:2497–2502
174. Levitan D, Doyle TG, Brousseau D et al (1996) Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 93:14940–14944
175. Cinar HN, Sweet KL, Hosemann KE, Earley K, Newman AP (2001) The SEL-12 presenilin mediates induction of the *Caenorhabditis elegans* uterine pi cell fate. Dev Biol 237:173–182
176. Eimer S, Donhauser R, Baumeister R (2002) The *Caenorhabditis elegans* presenilin sel-12 is required for mesodermal patterning and muscle function. Dev Biol 251:178–192
177. Wittenburg N, Eimer S, Lakowski B, Rohrig S, Rudolph C, Baumeister R (2000) Presenilin is required for proper morphology and function of neurons in *C. elegans*. Nature 406:306–309
178. Arpagaus M, Combes D, Culetto E et al (1998) Four acetylcholinesterase genes in the nematode *Caenorhabditis elegans*. J Physiol Paris 92:363–367
179. Grauso M, Culetto E, Combes D, Fedon Y, Toutant JP, Arpagaus M (1998) Existence of four acetylcholinesterase genes in the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae*. FEBS Lett 424:279–284
180. Combes D, Fedon Y, Toutant JP, Arpagaus M (2003) Multiple ace genes encoding acetylcholinesterases of *Caenorhabditis elegans* have distinct tissue expression. Eur J Neurosci 18:497–512
181. Drake J, Link CD, Butterfield DA (2003) Oxidative stress precedes fibrillar deposition of Alzheimer's disease amyloid beta-peptide (1-42) in a transgenic *Caenorhabditis elegans model*. Neurobiol Aging 24:415–420
182. Triguero L, Singh R, Prabhakar R (2008) Molecular dynamics study to investigate the effect of chemical substitutions of methionine 35 on the secondary structure of the amyloid beta (Abeta(1-42)) monomer in aqueous solution. J Phys Chem B 112:2159–2167
183. Hou L, Shao H, Zhang Y et al (2004) Solution NMR studies of the A beta(1-40) and A beta(1-42) peptides establish that the Met35 oxidation state affects the mechanism of amyloid formation. J Am Chem Soc 126:1992–2005
184. Bitan G, Tarus B, Vollers SS et al (2003) A molecular switch in amyloid assembly: Met35 and amyloid beta-protein oligomerization. J Am Chem Soc 125:15359–15365
185. Fay DS, Fluet A, Johnson CJ, Link CD (1998) In vivo aggregation of beta-amyloid peptide variants. J Neurochem 71:1616–1625
186. Yatin SM, Varadarajan S, Link CD, Butterfield DA (1999) In vitro and in vivo oxidative stress associated with Alzheimer's amyloid beta-peptide (1-42). Neurobiol Aging 20:325–330 discussion 339–342
187. Hensley K, Hall N, Subramaniam R et al (1995) Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J Neurochem 65:2146–2156
188. Boyd-Kimball D, Poon HF, Lynn BC et al (2006) Proteomic identification of proteins specifically oxidized in *Caenorhabditis elegans* expressing human Abeta(1-42): implications for Alzheimer's disease. Neurobiol Aging 27:1239–1249
189. Mortimore GE, Schworer CM (1977) Induction of autophagy by amino-acid deprivation in perfused rat liver. Nature 270:174–176
190. Yu WH, Kumar A, Peterhoff C et al (2004) Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for beta-amyloid peptide over-production and localization in Alzheimer's disease. Int J Biochem Cell Biol 36:2531–2540
191. Yu WH, Cuervo AM, Kumar A et al (2005) Macroautophagy—a novel beta-amyloid peptide-generating pathway activated in Alzheimer's disease. J Cell Biol 171:87–98
192. Nixon RA (2007) Autophagy, amyloidogenesis and Alzheimer disease. J Cell Sci 120:4081–4091
193. Florez-McClure ML, Hohsfield LA, Fonte G, Bealor MT, Link CD (2007) Decreased insulin-receptor signaling promotes the autophagic degradation of beta-amyloid peptide in *C. elegans*. Autophagy 3:569–580
194. Wu Y, Wu Z, Butko P et al (2006) Amyloid-beta-induced pathological behaviors are suppressed by *Ginkgo biloba* extract EGb 761 and ginkgolides in transgenic *Caenorhabditis elegans*. J Neurosci 26:13102–13113
195. Soto C, Castano EM, Frangione B, Inestrosa NC (1995) The alpha-helical to beta-strand transition in the amino-terminal fragment of the amyloid beta-peptide modulates amyloid formation. J Biol Chem 270:3063–3067
196. Nilsberth C, Westlind-Danielsson A, Eckman CB et al (2001) The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation. Nat Neurosci 4:887–893
197. Grez PA (2005) Obtención y análisis de Cepas Transgénicas de *C. elegans.* Expresión de péptido Aβ wild-type y sus variantes NIC y Arctic *Facultad de Ciencias Químicas y Farmacéuticas*. Universidad de Chile, Santiago, Chile, p 56
198. Praitis V, Casey E, Collar D, Austin J (2001) Creation of low-copy integrated transgenic lines in *Caenorhabditis elegans*. Genetics 157:1217–1226
199. Deibel MA, Ehmann WD, Markesbery WR (1996) Copper, iron, and zinc imbalances in severely degenerated brain regions in Alzheimer's disease: possible relation to oxidative stress. J Neurol Sci 143:137–142
200. Lovell MA, Robertson JD, Teesdale WJ, Campbell JL, Markesbery WR (1998) Copper, iron and zinc in Alzheimer's disease senile plaques. J Neurol Sci 158:47–52
201. Candy JM, Oakley AE, Klinowski J et al (1986) Aluminosilicates and senile plaque formation in Alzheimer's disease. Lancet 1:354–357
202. Lovell MA, Ehmann WD, Markesbery WR (1993) Laser microprobe analysis of brain aluminum in Alzheimer's disease. Ann Neurol 33:36–42
203. Perl DP, Brody AR (1980) Alzheimer's disease: X-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. Science 208:297–299
204. Landsberg JP, McDonald B, Watt F (1992) Absence of aluminium in neuritic plaque cores in Alzheimer's disease. Nature 360:65–68

205. Chafi AH, Hauw JJ, Rancurel G, Berry JP, Galle C (1991) Absence of aluminium in Alzheimer's disease brain tissue: electron microprobe and ion microprobe studies. Neurosci Lett 123:61–64
206. Good PF, Perl DP, Bierer LM, Schmeidler J (1992) Selective accumulation of aluminum and iron in the neurofibrillary tangles of Alzheimer's disease: a laser microprobe (LAMMA) study. Ann Neurol 31:286–292
207. Ferreira PC, Piai Kde A, Takayanagui AM, Segura-Munoz SI (2008) Aluminum as a risk factor for Alzheimer's disease. Rev Lat Am Enfermagem 16:151–157
208. Sparks DL, Friedland R, Petanceska S et al (2006) Trace copper levels in the drinking water, but not zinc or aluminum influence CNS Alzheimer-like pathology. J Nutr Health Aging 10:247–254
209. Miu AC, Benga O (2006) Aluminum and Alzheimer's disease: a new look. J Alzheimers Dis 10:179–201
210. Drago D, Bettella M, Bolognin S et al (2008) Potential pathogenic role of beta-amyloid(1-42)-aluminum complex in Alzheimer's disease. Int J Biochem Cell Biol 40:731–746
211. Rodella LF, Ricci F, Borsani E et al (2008) Aluminium exposure induces Alzheimer's disease-like histopathological alterations in mouse brain. Histol Histopathol 23:433–439
212. Bush AI, Pettingell WH, Multhaup G et al (1994) Rapid induction of Alzheimer A beta amyloid formation by zinc. Science 265:1464–1467
213. Bush AI, Pettingell WH Jr., Paradis MD, Tanzi RE (1994) Modulation of A beta adhesiveness and secretase site cleavage by zinc. J Biol Chem 269:12152–12158
214. Cherny RA, Atwood CS, Xilinas ME et al (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 30:665–676
215. Ritchie CW, Bush AI, Mackinnon A et al (2003) Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. Arch Neurol 60:1685–1691
216. White AR, Zheng H, Galatis D et al (1998) Survival of cultured neurons from amyloid precursor protein knock-out mice against Alzheimer's amyloid-beta toxicity and oxidative stress. J Neurosci 18:6207–6217
217. Maynard CJ, Cappai R, Volitakis I et al (2002) Overexpression of Alzheimer's disease amyloid-beta opposes the age-dependent elevations of brain copper and iron. J Biol Chem 277:44670–44676
218. Bayer TA, Schafer S, Simons A et al (2003) Dietary Cu stabilizes brain superoxide dismutase 1 activity and reduces amyloid Abeta production in APP23 transgenic mice. Proc Natl Acad Sci U S A 100:14187–14192
219. Phinney AL, Drisaldi B, Schmidt SD et al (2003) In vivo reduction of amyloid-beta by a mutant copper transporter. Proc Natl Acad Sci U S A 100:14193–14198
220. Cerpa WF, Barria MI, Chacon MA et al (2004) The N-terminal copper-binding domain of the amyloid precursor protein protects against Cu^{2+} neurotoxicity in vivo. FASEB J 18:1701–1703
221. Cerpa W, Varela-Nallar L, Reyes AE, Minniti AN, Inestrosa NC (2005) Is there a role for copper in neurodegenerative diseases?. Mol Aspects Med 26:405–420
222. Bush AI, Tanzi RE (2008) Therapeutics for Alzheimer's disease based on the metal hypothesis. Neurotherapeutics 5:421–432
223. Tsuruta Y, Furuta A, Taniguchi N, Yamada T, Kira J, Iwaki T (2002) Increased expression of manganese superoxide dismutase is associated with that of nitrotyrosine in myopathies with rimmed vacuoles. Acta Neuropathol 103:59–65