CAENORHABDITIS ELEGANS AS A MODEL ORGANISM FOR ER-RELATED DISEASES

SIMONA GHENEA*

Institute of Biochemistry of the Romanian Academy,
Splaiul Independenței 296, 060031 Bucharest 17, Romania

(Received 11 May, 2011)

Endoplasmic reticulum (ER) is the site where nascent polypeptides fold and assemble into proteins that are secreted, targeted for expression at the plasma membrane or directed to intracellular organelles. Mutations in the ER machineries that perform the quality control, assemble the protein complexes, export or send misfolded proteins for degradation could trigger ER-related diseases. In this review, I will summarize the recent contributions of the model organism *Caenorhabditis elegans* towards identification and characterization of factors involved in ER-related disease pathogenesis.

Key words: endoplasmic reticulum related diseases, Alzheimer, Parkinson, *Caenorhabditis elegans.*

INTRODUCTION

Nearly a third of the human genome products including secreted hormones, transmembrane proteins and catabolic and metabolic enzymes are processed through the ER (1), therefore defects in the folding and assembly of these proteins trigger ER stress response. Malfunction of the ER stress response could further be the cause of various diseases, including *diabetes mellitus*, inflammation and the neurodegenerative disorders known as "conformational diseases". The pathogenesis of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and prion disease, are causally linked to aggregation-prone proteins. These proteins do not share amino acids homology but lack a well defined 3D structure and are able to adopt a variety of conformations, some of which are prone to self-aggregation (2). Despite major advances in the past years, there is still a significant gap in understanding the risk factors and disease etiology that could lead to identification of efficient targets for treatments. In this review I will summarize the role of the nematode *C. elegans* in understanding the pathogenesis of ER-related diseases. Most of the studies in *C. elegans* have been focused on identification and

*Corresponding author (E-mail: gheneas@biochim.ro)

characterization of gene products associated with Alzheimer's and Parkinson's diseases pathology. Using *C. elegans* as a model for human diseases has a couple of advantages. First, *C. elegans* is a genetically tractable organism that shares many similarities at the molecular level to pathological processes found in humans; in addition, many mammalian genes are able to compensate for mutations in the worm homologous genes. Another advantage is the rapid construction of transgenic models and quick assessment of experimental interventions. In particular, *C. elegans* has an accessible and well characterized nervous system which confers advantage for investigation of neurodegenerative diseases.

ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common cause of progressive neurodegeneration. Pathologically, AD patients are characterized by accumulation of extracellular senile plaques and intracellular neurofibrillary tangles (NFT) in the brain. Whereas the major plaque component is the β-amyloid peptide (Aβ), which is the cleavage product of amyloid precursor protein (APP) (3), the NFTs are formed by aggregation of microtubule-associated protein tau (4). The Aβ peptides are 39–43 amino acids long, and among these forms, the Aβ 42 form is the primarily component of amyloid fibrils. However, the cellular and molecular mechanisms involved in AD pathogenesis are still not clear, recent studies suggesting that although Aβ has a central role, the small Aβ oligomers actually trigger toxicity in AD model organisms and humans (5).

β-AMYLOID PRECURSOR PROTEIN PROCESSING

The human APP belongs to a conserved family that includes APP-like protein 1 and 2 (APLP1, APLP2) and all three proteins have redundant and overlapping functions (6). *C. elegans* has only one APP protein, the amyloid precursor-like protein, APL–1 (7). All APP family members are type 1 integral membrane proteins with a single membrane-spaning domain, a large ectoplasmic N-terminal region and a short cytoplasmic C-terminal region. The extracellular domain contains a heparin-binding/growth-factor-like domain (HFBD/GFLD), a copper-binding domain (Cu) and a zinc-binding domain (Zn), followed by an acidic region. In the cytoplasmic region, a protein interaction motif is conserved in all APP proteins. The APP family proteins are posttranslationally modified by N-glycosylation, the N^{467}-glycosylation site being conserved in all APP homologues. In addition, processing of both APP and APLP2 is affected by O-linked glycosylation, sialylation, sumoylation, and phosphorylation (8–11). Inhibition of N-glycosylation by tunicamycin generates additional APLP1 fragments, whereas the processing of APLP2 and APP is not affected (12). The biological function of APP is not clear although there is evidence that it is implicated in cell adhesion, synaptogenesis, cell

migration, signaling, apoptosis and axonal transport (13). The *C. elegans* APL–1 plays an essential role in early larval development, including molting and morphogenesis (14) and has a role in synaptic transmission (15). As in mammals, the N-terminus of APL–1 has an important function since expression of a C-terminus truncated transgene is sufficient to rescue the molting and lethality phenotypes.

All APP family members undergo regulated proteolysis. First, they are cleaved by either the α- or β-secretase to generate a common secreted N-terminal ectodomain sAPPβ, and the cytoplasmic region. While the C83 cytoplasmic fragment generated by α-secretase does not lead to toxic Aβ species, the cytoplasmic C99 fragment resulted by the action of β-secretase BACE (beta amyloid cleaving enzyme) generates aggregation-prone peptides. The γ-Secretase cut subsequently the C99 fragment at multiple sites and produces Aβ peptides of different length. γ-Secretase is actually a protein complex that consists of anterior pharynx defective 1 (APH–1), APH–2/nicastrin, presenilin proteins, PS1 and PS2, and presenilin enhancer–2 (PEN–2). PS1 constitute the active site of the enzyme (reviewed in (16)). As APLP1 and APLP2, the *C. elegans* APL–1 does not contain the Aβ sequence.

Genetic studies showed that mutations causing familial early onset form of AD occur either in APP or in presenilin–1 or presenilin–2 (PS1 and PS2), most of these mutations being identified in the PS genes (17). *C. elegans* has two presenilin homologous genes, *sel–12* and *hop–1*, but it lacks an apparent BACE homolog. Presenilin is also required for intramembranous cleavage of LIN–12/GLP–1/Notch family of receptors (18), and the *C. elegans sel–12*/Presenilin has been identified in a screen for suppressors of a gain-of-function mutation in *C. elegans lin–12* (Notch) gene (19). Human PS1 rescue the *lin–12* mutant phenotype, confirming that *sel–12* is a PS. Because of dual function of γ-secretase in processing both Notch and APP, *aph–1* and *pen–2* were actually identified in a genetic screen for Notch pathway components. *aph–1* and *pen–2* are presenilin enhancer genes with phylogenetically conserved functions required for Notch pathway signaling, γ-secretase cleavage of APP and presenilin-dependant accumulation of γ-secretase complex (20). PS proteins are localized within the ER and early Golgi, compartments (20) and only a small fraction was found to the plasma membrane and endocytic compartments (21). APH–1 and PEN–2 were also found in ER/Golgi compartments, whereas APH–2 was primarily found at the plasma membrane (22). In *aph–1* or *hop–1; sel–12* mutants, the APH–2 localize to ER/Golgi compartments, suggesting that APH–1 and PEN–2 might interact directly with presenilin or presenilin/nicastrin complex to promote maturation and accumulation of the complex (20).

Interestingly, one of the earliest pathological changes observed in AD brain are endosomal abnormalities similar to Rab5-overexpressing defects, indicating that altered APP endocytosis might contribute to AD pathology (23). In *C. elegans*, knock-down of the small GTPase *rab–5* also leads to a dramatic decrease in the amount of *apl–1* expression in neurons.

TRANSGENIC *C. ELEGANS* AS A MODEL ORGANISM FOR AD

A more direct approach to understand molecular processes underlying AD and the cell responses to toxic Aβ accumulation used transgenic *C. elegans* with tissue-specific or inducible expression of human Aβ. Generation of *C elegans* AD models has been based on the "amyloid cascade hypothesis" which postulates that production and accumulation of Aβ 1–42 affect various cellular mechanisms (Fig. 1) and is critical for AD development. Thus, to investigate Aβ toxicity, Link laboratory engineered a couple of constructs that express the human Aβ 42 peptide in either body wall muscle or neurons (24, 25). The relevance of muscle expression of the Aβ was highlighted by the fact that accumulation of intracellular Aβ was also observed in the muscle of patients with inclusion body myositis (IBM), human brain neurons, and LaFerla 3 transgenic AD mouse model (26–28).

For muscle expression, a chimeric human Aβ 42 minigene designed to route Aβ into the secretory pathway was expressed under a strong muscle-specific promoter of *unc–54* or *myo–3* genes. The transgenic worms showed progressive paralysis due to extensive accumulation of amyloid deposits (24). Although the construct had a removable signal peptide to drive extracellular accumulation of Aβ as in the mouse model for AD, the Aβ was detected in the muscle cytoplasm. Interestingly, the Aβ co-immunoprecipitated with the ER chaperone HSP–3, the homologue of mammalian GRP78/BiP, which led to the hypothesis that the Aβ peptide is recognized by the ER quality control machinery as an abnormal protein and is retrotranslocated into the cytoplasm for proteosomal degradation (29). *aip–1*, which encodes for arsenite-inducible protein was found among the upregulated genes in a microarray analysis designed to identify the Aβ-induced genes in a *C. elegans* AD model (30). As its mammalian homologue AIRAPL, the AIP–1 associate and regulate the function of 26S proteasome. Overexpression of *aip–1* reduces Aβ accumulation and attenuates Aβ-induced paralysis, while AIP–1 depletion by RNAi exacerbates Aβ toxicity. AIRAPL can substitute for AIL–1 and protect against Aβ toxicity in this *C. elegans* AD model, suggesting that AIP–1 is a positive regulator of proteasomal function in response to toxic protein aggregation (30).

The *unc–54*::Ab 42 model was used for identification of proteins that directly interact with Aβ *in vivo*. Thus, co-immunoprecipitation studies coupled with mass spectrometry identified two HSP70 proteins (the cytoplasmic HSP70 and the ER GRP78/BiP homologues), 3 HSP–16 proteins homologous to B-crystallin, and a putative negative regulator of HSP70 as interacting partners (29). The HSP–16.2 is not involved per se in degradation of Aβ, because overexpression of HSP–16.2 does not reduce the amount of Aβ. However, the overexpression of HSP–16.2 suppressed the Aβ toxicity and significantly reduced paralysis of *unc–54*:: Aβ 42 animals (31). These findings suggest that interaction of chaperons with Aβ is part of a protective cellular response to alleviate Aβ toxicity. The protective response

could be achieved by promoting Aβ sequestration, degradation, multimerization or refolding.

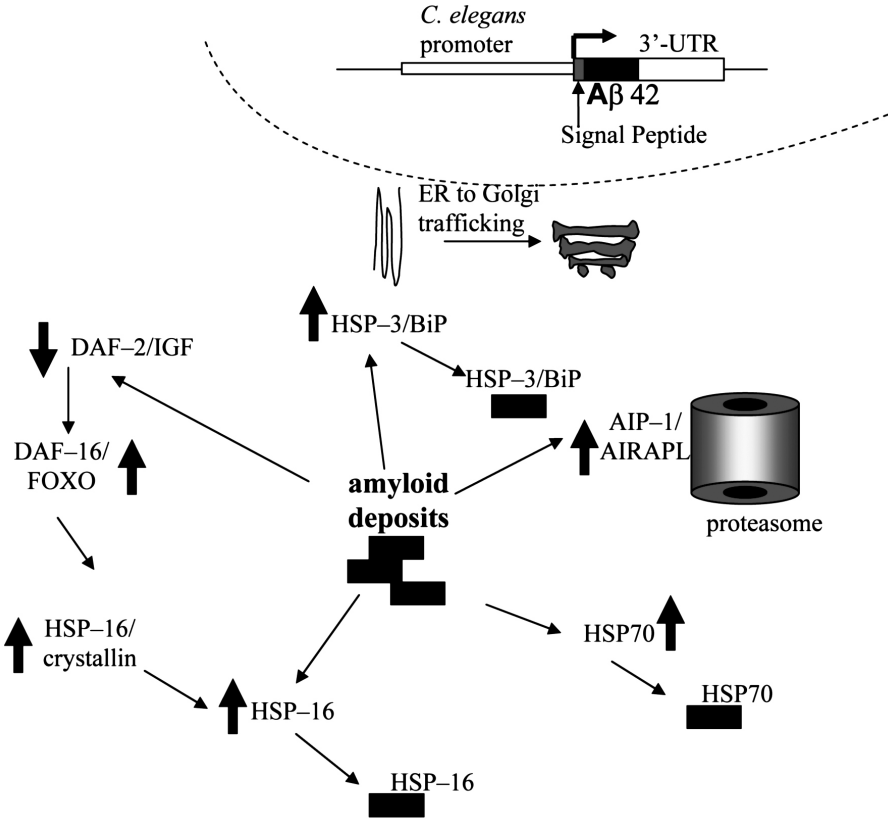

Fig. 1. – The Aβ accumulation in *C. elegans* re-program cellular responses.

Using *myo–3*:: Aβ42 model, Link group showed that Aβ expression in muscle cells induce autophagosome accumulation. Decreased insulin/insulin-like growth factor (IGF) signaling (IIS) due to a mutation in *daf–2* gene which encodes for insulin receptor, reduced both paralysis and autophagosome accumulation and also impairs the process of lysosome formation induced by Aβ. Complementarily, RNAi mediated knock down of lysosomal genes enhanced Aβ toxicity and autophagosome accumulation (32). This protection conferred by decreased insulin receptor signaling is dependent upon two transcription factors, heat shock factor 1 (HSF–1), which regulates Aβ disaggregation, and DAF–16/FOXO, which facilitates the formation of larger, less toxic Aβ aggregates (33). Subsequent observations in Alzheimer's model mouse that the decreased IIS signaling protects against Alzheimer's like disease symptoms validated the observations from *C. elegans* model. The protection was correlated with the hyperaggregation of Aβ

and promotes the hypothesis that reduced IGF signaling in mouse model promotes sequestration of soluble Aβ oligomers into dense aggregates of lower toxicity (33). This was an important result because ageing is a major risk factor for neurodegenerative diseases. Decreased IIS extends lifespan in various species including *C. elegans*. Recently, it was shown that the *C. elegans* IRE–1 and XBP–1 extend lifespan and increase resistance to ER stress of the *daf–2* mutant (34). IRE–1 senses accumulation of misfolded proteins in ER and has a key role in restoring ER homeostasis by activating unfolding protein response through XBP–1 splicing. In *daf–2* mutants, XBP–1 collaborates with DAF–16 to enhance ER stress resistance and to activate new genes that promote longevity (34). In addition, DAF–16 is essential for maximal *hsp–16* expression and for lifespan extension conferred by HSP–16 (35). All these results suggest that decreased insulin/IGF–1 signaling reprogram stress response regulators to promote longevity and resistance to various forms of stress.

PARKINSON'S DISEASE

Parkinson's disease (PD) is a disorder with the clinical hallmark represented by motor symptoms of tremor, bradykinesia, rigidity and postural imbalance due to progressive neurodegeneration, including loss of dopamine (DA) neurons from the substantia nigra (36). The neurohistological signature of PD is intracytoplasmic inclusions termed Lewy Bodies (LB) which contains the presynaptic protein α-synuclein (α-syn) in an aggregated, oligomeric or fibrillar form (37). Biochemically, PD is characterized by inhibition of complex I of the mitochondrial electron transport chain, production of reactive oxygen species, lipid droplet accumulation, and vesicle trafficking defects (38, 39). Ageing is a major risk factor for developing PD and recent studies showed that environmental toxins as diverse as pesticides, herbicides, manganese exposure or even a metabolite produced by a common soil bacterium, *S. venezuelae* may induce DA neurodegeneration (40, 41). Genetic causes may account for up to 10% of diagnosed PD patients, mutations in leucine-rich repeat kinase 2 (LRRK2) gene being the most frequent genetic cause. Dominant or recessive mutations in other genes, such as SNCA (α-syn), PARK2 (Parkin), PARK6 (PINK1), or ATP13A2 (PARK9) have also been associated with PD.

Along with transgenic PD animal models there are several toxin-induced models that use various neurotoxins such as 6-hydroxydopamine (6-OHDA), rotenone, paraquat or 1-methyl–4-phenyl–1,2,3,6-tetrahydropyridine (MPTP), which selectively destroy DA neurons, producing a syndrome that phenocopy PD.

GENES ASSOCIATED WITH PD

α-syn is a small (14 kDa) protein that binds lipids. In its native form it is unfolded and has the propensity to form toxic oligomeric species; in a folded form it

associates with membranes (42). Point mutations, duplications or triplications of α-syn gene are cause of familial forms of PD. A schematic representation of the cellular processes affected by α-syn accumulation is represented in Fig. 2. Overproduction of α-syn leads to a cytotoxic blockage in intracellular vesicular trafficking. Unbiased genetic screen in yeasts identified a set of *rab* genes functioning in vesicular trafficking between ER and Golgi which alleviated the α-syn toxicity (43, 44). The Ypt1/Rab1, was further tested in neuronal models of PD and was able to prevent dopaminergic neuron loss (45). ER-Golgi transport blockage measured by carboxypeptidase Y maturation assay was also suppressed by Ypk9 (PARK9), although through a different mechanism than Ypt1/Rab1 (45). In transgenic *C. elegans* and neuronal PD models, dopaminergic neuron loss caused by human α-syn overexpression was rescued by co-expression with ATP13A2 (PARK9), a predicted lysosomal P-type transmembrane cation transporting ATPase, indicating that mutations in ATP13A2 contribute to PD due to abnormalities in lysosomal clearance of α-syn. The *C. elegans* TOR–2, a human torsin A-related protein with molecular chaperone-like activity, protects DA neurons against 6-OHDA injuries, excess intracellular DA production, α-syn overexpression, but also against polyglutamine repeat-induced protein aggregates, which suggest that TOR–2 is involved in regulation of protein folding and protein aggregation (45, 46). Torsin A localizes to ER (47) and the *C. elegans* TOR–2 probably acts upstream of PARK–9, because RNA interference (RNAi) inactivation of PARK–9 decreases the ability of TOR–2 to attenuate misfolding of α-syn protein (45).

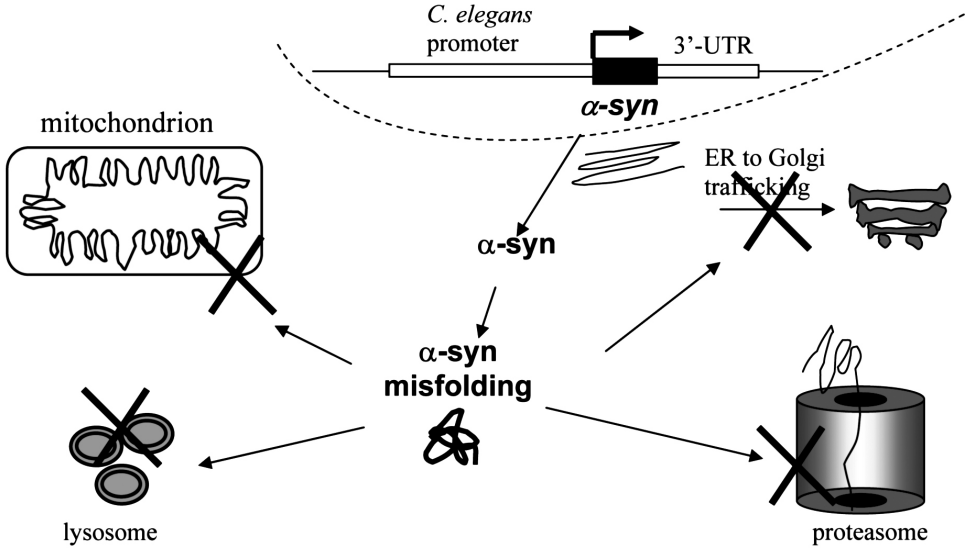

Fig. 2. – The α-syn misfolding in *C. elegans* affects various cellular mechanisms.

More recent studies straitened the connection between PD and ER by highlighting several genes that encode protein associated with ER unfolding protein response (UPR). For example, the ER stress reporter GRP78/BiP interacted with α-syn (48), and α-syn aggregation induced expression of both GRP78/BiP and UPR-related activating transcription factor ATF–4, suggesting that α-syn might also accumulate in ER, which would explain the post mortem activation of the UPR observed in nigral dopaminergic neurons of PD patients (48). In MPTP mice model, the MPTP neurotoxin induces loss of dopaminergic neurons more prominently in mice deficient in ATF6α than in wild-type mice, and oxidative stress activates both ATF6α and p38MAPK, which in turn, enhance ATF6α transcriptional activities (49). Furthermore, in a 6-OHDA mice model of PD, the ER E3 ubiquitin ligase Hrd1 was up-regulated in various encephalic neurons, suggesting that Hrd1 up-regulation may represent a protective response against neurodegeneration in PD (50). Yeast HRD1 was one of the genes identified in a genetic screen for α-syn modifiers. Overexpression of HRD–1 was able to suppress α-syn induced neurodegeneration in both *C. elegans* and neuronal models of PD (45). In another study, endogenous LRRK2 colocalized with ER markers in human dopaminergic neurons although in brain sections of PD LRRK2 co-localized with α-syn in Lewy bodies, the percentage of colocalization being increased by a LRRK2 mutation (51). Inactivation of the *C. elegans lrk–1*(the homologue of LRRK2)*, pink–1*, *parkin/pdr–1,* and *dj–1* disrupts mitochondrial function (52, 53). Thus, expression of human *LRRK2* in *C. elegans* protects against mitochondrial toxicity induced by rotenone (54). *C. elegans lrk–1* mutant animals displayed aberrant axon pathfinding, whereas mutation in the *C. elegans* homologue of *PINK–1* reduced mitochondrial cristae length in muscle and neuronal cells and produced defects in axonal outgrowth of canal-associated neurons. However, mutations in both genes suppressed the axonal defects observed in single mutants, suggesting an antagonistic effect between PINK–1 and LRK–1 (52). Furthermore, *pink–1* mutation reduced the hypersensitivity of *lrk–1* mutant animals to the ER stressor tunicamycin (52).

PARKIN/PDR–1 is an E3 ubiquitin ligase involved in protein degradation, which physically interacts with worm E2 enzymes, UBC–2, UBC–18, and UBC–15, as well as the E4 enzyme, CHN–1 (55). *parkin/pdr–1* deletion causes hypersensitivity to ER stress and PDR–1 is regulated by the UPR (55). *pdr–1* knockout enhanced vulnerability to mitochondrial complex I inhibitors (53). Coexpression of a truncated form of *C. elegans pdr–1* and α-syn variants in human cell cultures causes aggregation of α-syn, indicating a conserved role for *C. elegans* PDR–1 in α-syn clearance. DJ–1 (PARK7) also associates with PD but it may act as a neuroprotective sensor of oxidative stress (56). *C. elegans dj–1* loss of function mutation caused a decrease in oxygen consumption and survival after exposure to rotenone, effects that were reverted by antioxidants (53).

C. ELEGANS AS A MODEL ORGANISM FOR PD

C. elegans has a total of 302 neurons among which 8 are dopaminergic neurons (45). Loss of DA neurons in *C. elegans* is not lethal and DA is best known for mediating the slowing of movement when the animal encounters food. Exogenous DA inhibits locomotion and egg laying behavior (57).

With the exception of α-syn, *C. elegans* presents all the other homologues of the PD-related genes (58). However, worms expressing wild-type and mutant forms of human α-syn displayed age and dose-dependent dopaminergic neurodegeneration (45, 46, 59). Worms also exhibit apoptotic DA loss following treatments with the neurotoxins MPTP, 6-OHDA, as well as pesticides, rotenone and paraquat (46, 60). All these suggest that although *C. elegans* does not have the gene for α-syn, it is able to phenocopy the human α-syn overexpression and it is a suitable model organism for studying PD pathogenesis.

High-throughput RNAi-based screening for genetic modifiers identified suppressor and enhancer of α-syn aggregation. First, Hamamichi *et al.* took a hypothesis-driven approach and co-expressed α-syn and *tor–2* in muscle cells under the control of *unc–54* promoter. They chose muscle cells for expression because neuronal cells are generally resistant to RNAi; in addition, easy visual inspection of α-syn accumulation in muscle cells is possible since muscle cells are among the largest cells in *C. elegans*. TOR–2 maintains the overexpressed α-syn at a threshold and thus allows a fine tune of α-syn aggregates quantification. Screening of almost 900 candidates identified 20 genes whose downregulation by RNAi reproducibly enhanced α-syn aggregation. These genes included regulators of autophagy, lysosomal function, cellular trafficking, G-protein signaling and uncharacterized proteins (61). Using the same strategy based on α-syn expression in muscle cells, Van Ham *et al.* conducted an unbiased genome-wide RNAi screen to identify genetic factors that influence α-syn inclusion formation. They found that inactivation of 80 genes involved in a variety of processes such as detoxification of ER, quality control and vesicle-mediated trafficking increased the number of α-syn-containing inclusions, indicating a specific role for these genes in α-syn inclusion formation. Interestingly, ageing-associated genes such as *sir–2.1*, the homologue of human sirtuins 1 to 3, and *lagr–1*, the LASS2 sphingolipid synthase, were found to suppress α-syn inclusions formation (62). Finally, the third group used transgenic *C. elegans* that overexpressed human α-syn in neurons. Gene inactivation in neurons was achieved by using an RNAi-enhanced mutant *eri–1* strain that sensitizes neurons for RNAi uptake. Ten genes caused severe growth and motor abnormalities and, among them, four were related to endocytic pathway raising a novel pathogenic link between endocytic pathway and α-syn - induced neurotoxicity in synucleinopathy (63).

CONCLUDING REMARKS

Accumulation of misfolded proteins or protein aggregates disrupts cellular homeostasis and activates various stress responses as protective mechanisms for cell survival. Recently, convergent results lead toward a model in which the UPR has an important function in activating pathways that alleviate toxicity of various aggregates, and, therefore, molecules that regulate the ER stress response could be potential candidates for drug targets in various conformational diseases. Given newly developed experimental approaches and well-defined genetic systems, *C. elegans* models remain viable approaches for investigating the molecular processes underlying ER-related diseases.

Acknowledgements. The author acknowledges support from the Romanian Academy projects 1 of the Institute of Biochemistry, Romanian CNCSIS Grants ID 1171 - 1018/2008 and the postdoctoral program POSDRU/89/1.5/S/60746, from European Social Fund.

REFERENCES

1. Kelly JW, Balch WE, *The integration of cell and chemical biology in protein folding*, ***Nat. Chem. Biol.***, **2**, 224–7 (2006).
2. Fantini J, Yahi N, *Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases*, ***Expert Rev. Mol. Med.***, **12**, e27 (2010).
3. Glenner GG, Wong CW, *Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein*, ***Biochem. Biophys. Res. Commun.***, **120**, 885–90 (1984).
4. Kosik KS, Joachim CL, Selkoe DJ, *Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease*, ***Proc. Natl. Acad. Sci. USA***, **83**, 4044–8 (1986).
5. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E, *Alzheimer's disease*, ***Lancet***, **377**, 1019–31 (2011).
6. Sprecher CA, Grant FJ, Grimm G, O'Hara PJ, Norris F, Norris K, Foster DC, *Molecular cloning of the cDNA for a human amyloid precursor protein homolog: evidence for a multigene family*, ***Biochemistry***, **32**, 4481–6 (1993).
7. Daigle I, Li C, *apl–1, a Caenorhabditis elegans gene encoding a protein related to the human beta-amyloid protein precursor*, ***Proc. Natl. Acad. Sci. USA***, **90**, 12045–9 (1993).
8. Weidemann A, Konig G, Bunke D, Fischer P, Salbaum JM, Masters CL, Beyreuther K, *Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein*, ***Cell***, **57**, 115–26 (1989).
9. Lyckman AW, Confaloni AM, Thinakaran G, Sisodia SS, Moya KL, *Post-translational processing and turnover kinetics of presynaptically targeted amyloid precursor superfamily proteins in the central nervous system*, ***J. Biol. Chem.***, **273**, 11100–6 (1998).
10. Gandy S, Czernik AJ, Greengard P, *Phosphorylation of Alzheimer disease amyloid precursor peptide by protein kinase C and Ca2+/calmodulin-dependent protein kinase II*, ***Proc. Natl. Acad. Sci. USA***, **85**, 6218–21 (1988).
11. Nakagawa K, Kitazume S, Oka R, Maruyama K, Saido TC, Sato Y, Endo T, Hashimoto Y, *Sialylation enhances the secretion of neurotoxic amyloid-beta peptides.* ***J. Neurochem.***, **96**, 924–33 (2006).

12. Eggert S, Paliga K, Soba P, Evin G, Masters CL, Weidemann A, Beyreuther K, *The proteolytic processing of the amyloid precursor protein gene family members APLP–1 and APLP–2 involves alpha-, beta-, gamma-, and epsilon-like cleavages: modulation of APLP–1 processing by n-glycosylation*, ***J. Biol. Chem.***, **279**, 18146–56 (2004).
13. Gralle M, Ferreira ST, *Structure and functions of the human amyloid precursor protein: the whole is more than the sum of its parts*, ***Prog. Neurobiol.***, **82**, 11–32 (2007).
14. Hornsten A, Lieberthal J, Fadia S, Malins R, Ha L, Xu X, *et al*, *APL–1, a Caenorhabditis elegans protein related to the human beta-amyloid precursor protein is essential for viability*, ***Proc. Natl. Acad. Sci. USA***, **104**, 1971–6 (2007).
15. Wiese M, Antebi A, Zheng H, *Intracellular trafficking and synaptic function of APL–1 in Caenorhabditis elegans*, ***PLoS One***, **5**, e12790 (2010).
16. Jacobsen KT, Iverfeldt K, *Amyloid precursor protein and its homologues: a family of proteolysis-dependent receptors*, ***Cell Mol. Life Sci.***, **66**, 2299–318 (2009).
17. Brunkan AL, Goate AM, *Presenilin function and gamma-secretase activity*, ***J. Neurochem.***, **93**, 769–92 (2005).
18. Petit A, Bihel F, Alves Da Costa C, Pourquie O, Checler F, Kraus JL, *New protease inhibitors prevent gamma-secretase-mediated production of Abeta40/42 without affecting Notch cleavage*, ***Nat. Cell Biol.***, **3**, 507–11 (2001).
19. Levitan D, Greenwald I, *Facilitation of lin–12-mediated signalling by sel–12, a Caenorhabditis elegans S182 Alzheimer's disease gene*, ***Nature***, **377**, 351–4 (1995).
20. Francis, R., Mcgrath, G., Zhang, J., Ruddy, D.A., Sym, M., Apfeld, J., *et al*, *aph–1 and pen–2 are required for Notch pathway signaling, gamma-secretase cleavage of betaAPP, and presenilin protein accumulation.* **Dev Cell**. **3**: p. 85–97 (2002).
21. Kim SH, Lah JJ, Thinakaran G, Levey A, Sisodia SS, *Subcellular localization of presenilins: association with a unique membrane pool in cultured cells*, ***Neurobiol. Dis.***, **7**, 99–117 (2000).
22. Goutte C, Hepler W, Mickey KM, Priess JR, *aph–2 encodes a novel extracellular protein required for GLP–1-mediated signaling*, ***Development***, **127**, 2481–92 (2000).
23. Schobel S, Neumann S, Hertweck M, Dislich B, Kuhn PH, Kremmer E, *et al*, *A novel sorting nexin modulates endocytic trafficking and alpha-secretase cleavage of the amyloid precursor protein*, ***J. Biol. Chem.***, **283**, 14257–68 (2008).
24. Link CD, *Expression of human beta-amyloid peptide in transgenic Caenorhabditis elegans*, ***Proc. Natl. Acad. Sci. USA***, **92**, 9368–72 (1995).
25. Link CD, *C. elegans models of age-associated neurodegenerative diseases: lessons from transgenic worm models of Alzheimer's disease*, ***Exp. Gerontol.***, **41**, 1007–13 (2006).
26. Askanas V, Engel WK, Alvarez RB, Glenner GG, *beta-Amyloid protein immunoreactivity in muscle of patients with inclusion-body myositis*, ***Lancet***, **339**, 560–1 (1992).
27. Gouras GK, Tsai J, Naslund J, Vincent B, Edgar M, Checler F, *et al*, *Intraneuronal Abeta42 accumulation in human brain*, ***Am. J. Pathol.***, **156**, 15–20 (2000).
28. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, *et al*, *Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction*, ***Neuron.***, **39**, 409–21 (2003).
29. Fonte V, Kapulkin V, Taft A, Fluet A, Friedman D, Link CD, *Interaction of intracellular beta amyloid peptide with chaperone proteins*, ***Proc. Natl. Acad. Sci. USA***, **99**, 9439–44 (2002).
30. Hassan WM, Merin DA, Fonte V, Link CD, *AIP–1 ameliorates beta-amyloid peptide toxicity in a Caenorhabditis elegans Alzheimer's disease model*, ***Hum. Mol. Genet.***, **18**, 2739–47 (2009).
31. Fonte V, Kipp DR, Yerg J 3rd, Merin D, Forrestal M, Wagner E, Roberts CM, Link CD, *Suppression of in vivo beta-amyloid peptide toxicity by overexpression of the HSP–16.2 small chaperone protein*, ***J. Biol. Chem.***, **283**, 784–91 (2008).
32. Florez-Mcclure ML, Hohsfield LA, Fonte G, Bealor MT, Link CD, *Decreased insulin-receptor signaling promotes the autophagic degradation of beta-amyloid peptide in C. elegans*, ***Autophagy***, **3**, 569–80 (2007).

33. Cohen E, Bieschke J, Perciavalle RM, Kelly JW, Dillin A, *Opposing activities protect against age-onset proteotoxicity*, ***Science***, **313**, 1604–10 (2006).
34. Henis-Korenblit S, Zhang P, Hansen M, Mccormick M, Lee SJ, Cary M, Kenyon C, *Insulin/IGF–1 signaling mutants reprogram ER stress response regulators to promote longevity*, ***Proc. Natl. Acad. Sci. USA***, **107**, 9730–5 (2010).
35. Walker GA, Lithgow GJ, *Lifespan extension in C. elegans by a molecular chaperone dependent upon insulin-like signals*, ***Aging Cell***, **2**, 131–9 (2003).
36. Braak H, Del Tredici K, *Neuroanatomy and pathology of sporadic Parkinson's disease*, ***Adv. Anat. Embryol. Cell Biol.***, **201**, 1–119 (2009).
37. Thomas B, Beal MF, *Parkinson's disease*, ***Hum. Mol. Genet.***, **16**, R183–94 (2007).
38. Malkus KA, Tsika E, Ischiropoulos H, *Oxidative modifications, mitochondrial dysfunction, and impaired protein degradation in Parkinson's disease: how neurons are lost in the Bermuda triangle*, ***Mol. Neurodegener.***, **4**, 24 (2009).
39. Outeiro TF, Lindquist S, *Yeast cells provide insight into alpha-synuclein biology and pathobiology*, ***Science***, **302**, 1772–5 (2003).
40. Shaw CA, Hoglinger, GU, *Neurodegenerative diseases: neurotoxins as sufficient etiologic agents?* ***Neuromolecular Med.***, **10**, 1–9 (2008).
41. Caldwell KA, Tucci ML, Armagost J, Hodges TW, Chen J, Memon SB, *et al*., *Investigating bacterial sources of toxicity as an environmental contributor to dopaminergic neurodegeneration*, ***PLoS One***, **4**, e7227 (2009).
42. Eliezer D, Kutluay E, Bussell R Jr, Browne G, *Conformational properties of alpha-synuclein in its free and lipid-associated states*, ***J. Mol. Biol.***, **307**, 1061–73 (2001).
43. Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, *et al*, *Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models*, ***Science***, **313**, 324–8 (2006).
44. Gitler AD, Bevis BJ, Shorter J, Strathearn KE, Hamamichi S, Su LJ, *et al*, *The Parkinson's disease protein alpha-synuclein disrupts cellular Rab homeostasis*, ***Proc. Natl. Acad. Sci. USA***, **105**, 145–50 (2008).
45. Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, *et al.*, *Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity*, ***Nat. Genet.***, **41**, 308–15 (2009).
46. Cao S, Gelwix CC, Caldwell KA, Caldwell GA, *Torsin-mediated protection from cellular stress in the dopaminergic neurons of Caenorhabditis elegans*, ***J. Neurosci.***, **25**, 3801–12 (2005).
47. Hewett J, Ziefer P, Bergeron D, Naismith T, Boston H, Slater D, *et al*, *TorsinA in PC12 cells: localization in the endoplasmic reticulum and response to stress*, ***J. Neurosci. Res.***, **72**, 158–68 (2003).
48. Bellucci A, Navarria L, Zaltieri M, Falarti E, Bodei S, Sigala S, *et al*, *Induction of the unfolded protein response by alpha-synuclein in experimental models of Parkinson's disease*, ***J. Neurochem.***, **116**, 588–605 (2011).
49. Egawa N, Yamamoto K, Inoue H, Hikawa R, Nishi K, Mori K, Takahashi R, *The endoplasmic reticulum stress sensor, ATF6alpha, protects against neurotoxin-induced dopaminergic neuronal death*, ***J. Biol. Chem.***, **286**, 7947–57 (2011).
50. Mei J, Niu C, *Alterations of Hrd1 expression in various encephalic regional neurons in 6-OHDA model of Parkinson's disease*, ***Neurosci. Lett.***, **474**, 63–8 (2010).
51. Vitte J, Traver S, Maues De Paula A, Lesage S, Rovelli G, Corti O, Duyckaerts C, Brice A, *Leucine-rich repeat kinase 2 is associated with the endoplasmic reticulum in dopaminergic neurons and accumulates in the core of Lewy bodies in Parkinson disease*, ***J. Neuropathol. Exp. Neurol.***, **69**, 959–72 (2010).
52. Samann J, Hegermann J, Von Gromoff E, Eimer S, Baumeister R, Schmidt E, *Caenorhabditis elegans LRK–1 and PINK–1 act antagonistically in stress response and neurite outgrowth*, ***J. Biol. Chem.***, **284**, 16482–91 (2009).

53. Ved R, Saha S, Westlund B, Perier C, Burnam L, Sluder A, *et al.*, *Similar patterns of mitochondrial vulnerability and rescue induced by genetic modification of alpha-synuclein, parkin, and DJ–1 in Caenorhabditis elegans*, ***J. Biol. Chem.***, **280**, 42655–68 (2005).
54. Wolozin B, Saha S, Guillily M, Ferree A, Riley M, *Investigating convergent actions of genes linked to familial Parkinson's disease*, ***Neurodegener. Dis.***, **5**, 182–5 (2008).
55. Springer W, Hoppe T, Schmidt E, Baumeister R, *A Caenorhabditis elegans Parkin mutant with altered solubility couples alpha-synuclein aggregation to proteotoxic stress*, ***Hum. Mol. Genet.***, **14**, 3407–23 (2005).
56. Yanagida T, Kitamura Y, Yamane K, Takahashi K, Takata K, Yanagisawa D, *et al.*, *Protection against oxidative stress-induced neurodegeneration by a modulator for DJ–1, the wild-type of familial Parkinson's disease-linked PARK7*, ***J. Pharmacol. Sci.***, **109**, 463–8 (2009).
57. Schafer WR, Kenyon CJ, *A calcium-channel homologue required for adaptation to dopamine and serotonin in Caenorhabditis elegans*, ***Nature***, **375**, 73–8 (1995).
58. Harrington AJ, Hamamichi S, Caldwell GA, Caldwell KA, *C. elegans as a model organism to investigate molecular pathways involved with Parkinson's disease*, ***Dev. Dyn.***, **239**, 1282–95 (2010).
59. Lakso M, Vartiainen S, Moilanen AM, Sirvio J, Thomas JH, Nass R, Blakely RD, Wong G, *Dopaminergic neuronal loss and motor deficits in Caenorhabditis elegans overexpressing human alpha-synuclein*, ***J. Neurochem.***, **86**, 165–72 (2003).
60. Nass R, Hall DH, Miller DM 3rd, Blakely RD, *Neurotoxin-induced degeneration of dopamine neurons in Caenorhabditis elegans*, ***Proc. Natl. Acad. Sci. USA***, **99**, 3264–9 (2002).
61. Hamamichi S, Rivas RN, Knight AL, Cao S, Caldwell KA, Caldwell GA, *Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model*, ***Proc. Natl. Acad. Sci. USA***, **105**, 728–33 (2008).
62. Van Ham TJ, Thijssen KL, Breitling R, Hofstra RM, Plasterk RH, Nollen EA, *C. elegans model identifies genetic modifiers of alpha-synuclein inclusion formation during aging*, ***PLoS Genet.***, **4**, e1000027 (2008).
63. Kuwahara T, Koyama A, Koyama S, Yoshina S, Ren CH, Kato T, Mitani S, Iwatsubo T, *A systematic RNAi screen reveals involvement of endocytic pathway in neuronal dysfunction in alpha-synuclein transgenic C. elegans*, ***Hum. Mol. Genet.***, **17**, 2997–3009 (2008).