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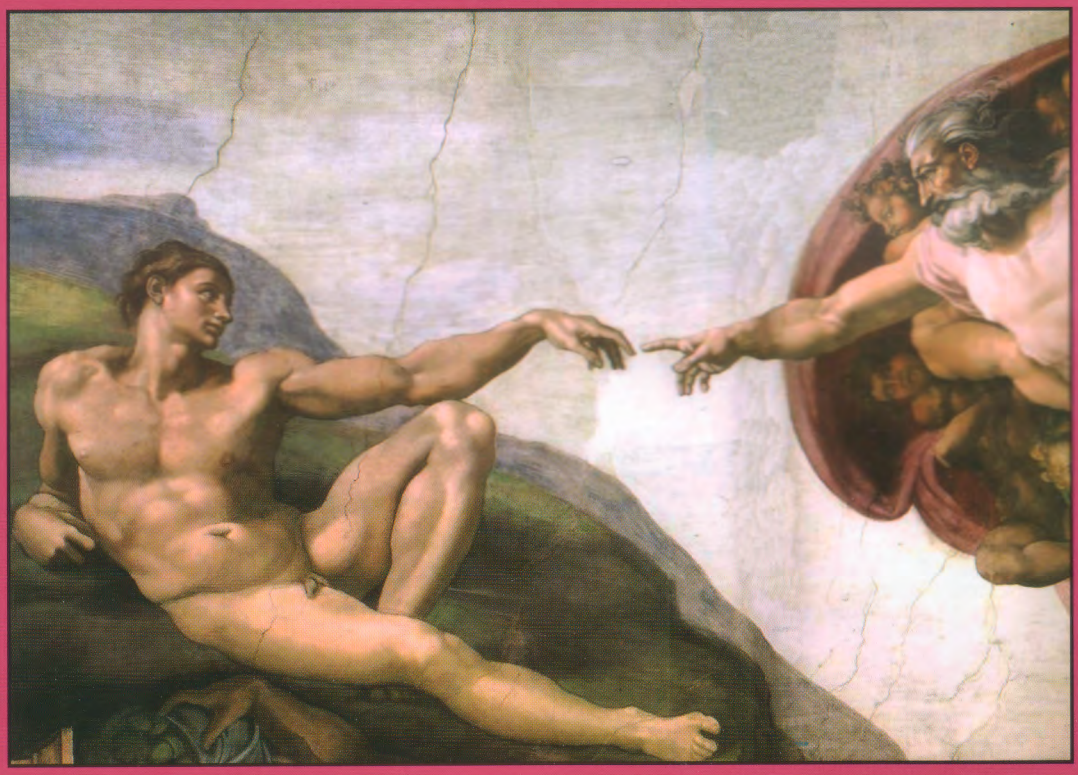

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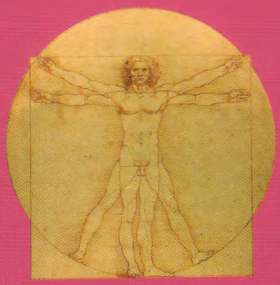

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REDOX PROTEOMICS STUDIES ON *C. ELEGANS* MODELS: A SIMPLE ORGANISM THAT PROVIDES INSIGHTS INTO COMPLEX NEURODEGENERATIVE DISORDERS

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The nematode *Caenorhabditis elegans (C. elegans)* offers an effective model to study the molecular pathways that might be involved in human neurodegenerative diseases, such as Alzheimer disease (AD) and Parkinson disease (PD). Despite a vast evolutionary distance between nematodes and humans, a cross-species translation of proteins was extensively demonstrated and this greatly facilitates the study of human diseases in such simple organisms. Proteomics, as well, is an emerging platform important for better understanding of onset and progression of neurodegenerative diseases. In addition to protein expression levels, proteomics can be applied to identification of post-translational modifications (i.e. acetylation, phosphorylation, etc.), which are key regulators of protein functions. Further, the recent development of redox proteomics approach as for the identification oxidatively modified protein in different animal models of neurodegenerative diseases has led to novel results that correlate with human findings. In the present review, redox proteomics studies performed on *C. elegans* models of AD and PD are summarized. The results demonstrated that several proteins involved in energy metabolism, protein degradation, mitochondrial function, and organismal structures, are common targets of oxidative modification and may play a crucial role in the neurodegenerative process. These findings provide a strong rationale for using C. elegans models for specific studies aimed to identify the molecular pathways perturbed in these diseases, to confirm their relevance in human disease and to eventually provide insight into therapeutic intervention.

The soil nematode *Caenorhabditis elegans* (*C. elegans*) was established as a model organism in 1965 by Sydney Brenner (Brenner, 1974) and it gained immediate success for its simplicity compared to other multicellular organisms. *C. elegans* reproduce with a life cycle of about 2/3 days under optimal conditions during which it goes through four larval stages (L1 to L4) before reaching adulthood and reproductive maturity. The worm can be easily grown on agar plates seeded with a lawn of bacteria or in liquid culture in both 96- and 384-well microtiter plates, and brood sizes are approximately of 300 progenies (Schrimpf and Hengartner, 2010). Each worm has a fixed number of cells totaling 959 for hermaphrodites and 1031 for males. This small number of cells generates a surprisingly diverse set of organs and tissues, including a nervous system (which account for 302 neurons), muscles, a hypodermis, intestine, gonads, glands, and an excretory system. *C. elegans* exhibits all the features of a multicellular organism, such as a complex organ system, or social, sexual, and learning behaviors (Shim and Paik, 2010; Baumeister and Ge, 2002). Notwithstanding its simplicity, *C. elegans* organogenesis and even

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complex neuronally controlled behaviors can be studied and dysfunctions can be attributed to defects in individual cells. Another major advantage of using *C. elegans* as a model organism in research is the availability of its genomic sequence. Indeed in 1998, this nematode was the first multicellular organism of which the genome encoding about 19000 open reading frames, was sequenced completely (The *C. elegans* Genome Sequencing Consortium 1998). An astounding result of this approach was that ~65% of the human disease genes have a counterpart in the worm (Baumeister and Ge, 2002; Madi et al, 2003). Thus, many human disease genes present orthologs in the worm and several processes such as programmed cell death (Ellis and Horvitz, 1986), insulin signaling, formation, trafficking, and release of synaptic vesicles are highly conserved in *C. elegans*, employing many of the same proteins used in mammalian neurons (Bargmann and Kaplan, 1998; Chalfie et al, 1986). Moreover, thank to its transparency and ease of manipulation, *C. elegans* is often used as a model to elucidate several developmental and molecular processes, and recently GFP fusions have been extensively used to visualize specific neurons and synapses in living animals (Teschendorf and Link, 2009). Since *C. elegans* receptor pharmacology is remarkably similar to that of humans, drugs and side effects can be tested in large scale using *C. elegans* and its mutants as an *in vivo* model. Consequently, drug development using *C. elegans* knockout strains expressing the homologous human disease mutant together with the development of RNA interference approaches, increased the interest on *C. elegans* as a model of human disease (Fire et al, 1998).

Transgenic *C. elegans* models have been established for a number of (generally age-associated) neurodegenerative diseases, including AD (Link, 1995), PD (Kuwahara et al, 2006; Lakso et al, 2003; Ved et al, 2005), Huntington disease (HD) (Faber et al, 2002) and amyotropic lateral sclerosis (ALS) (Oeda et al, 2001). The worm short lifecycle/lifespan allow the construction of transgenes models by microinjection in only two weeks, which combined with the ability to readily freeze and recover transgenic strains, permit to generate large collections of strains expressing related transgenes. However, it is crucial to always account for the limitations of studying neurodegeneration in *C. elegans*. Indeed, *C. elegans* has neither an adaptive immune system nor a circulatory system, and lacks some characteristics of vertebrate cells (e.g., myelinated neurons) (Teschendorf and Link, 2009). In addition, worm neurons are small and difficult to handle in experimental setting. For these reasons, *C. elegans* cannot be used to model some human diseases (e.g., multiple sclerosis) or some likely important components of AD or other neurodegenerative diseases, such as microglial activation.

Proteomic analysis is a great complementary tool to genomics in analyzing the global distribution of gene products under specific experimental settings. Importantly, it was estimated that over 80% of the nematode proteome has human orthologous proteins (Lai et al, 2000). Proteome scale studies of protein structure, function, and interactions have become a new paradigm for both investigating basic biology and developing therapeutics, and *C. elegans* proteomics analysis is receiving increasing attention as proteomic techniques advance. Indeed, the continuous improvement of sensitivity and measurement speed of proteomics instrumental analysis will allow increasing the depth and quality of the *C. elegans* proteome studies. The analysis of PTMs associated with *C. elegans* aging is an open field because many different physiological states may be controlled by reversible or irreversible protein modification such as glycosylation, phosphorylation, acetylation or oxidative modifications (Hillier et al, 2005; Shim and Paik, 2010).

The worm's proteomics studies began 10 years after the introduction of *C. elegans* as a model organism (O'Farrell, 1975). Studies using MS after separation on high-resolution 2D gels covering broad pH-ranges or directly by LC-MS/MS yielded proteome datasets encompassing over a thousand proteins (Bini et al, 1997; Kaji et al, 2000; Mawuenyega et al, 2003; Schrimpf et al, 2001). The peptidome and subproteomes, like the mitochondrial proteome (Li et al, 2009), or other selected pathways were characterized in *C. elegans* (Choi et al, 2009; Paik et al, 2006). In a successive study using a shotgun proteomics approach in combination with MS/MS, Merrihew et al (2008) identified 6779 proteins, including 429 unknown coding sequences used for the first time as protein-based validation of genome

sequences to improve genome annotation. Recently, much effort was made on phosphoproteome studies showing that the *C. elegans* phosphoproteome encompassed about 4500 phosphorylation sites and that proteins with signaling-related functions and transcriptional and translational regulator activities were enriched in the phosphoproteome. However, the worm's phosphoproteome differs from other species regarding biological processes, kinase specificity and evolutionary conservation (Zielinska et al, 2009).

TRANSGENIC *C. ELEGANS* MODELS OF NEURODEGENERATIVE DISEASES

Despite vast evolutionary distances between nematodes and humans, human transgenes show a remarkable ability to function appropriately within the context of *C. elegans*. Human proteins, such as presenilin, LRRK2 and TDP-43, exhibit functional activities that are shared between *C. elegans* and humans despite sequence homologies that are often only 30 – 45% concordant (Caldwell and Caldwell, 2008).

Recently, considerable effort has been made to study *C. elegans* genes whose mutations in the human counterparts result in the onset of complex neurological disorders, such as AD, HD and PD, through transgenic expression of the respective human wild-type genes (Harrington et al, 2010; Link, 2006). Thus, studies of neurodegeneration in worms provide strong insight into mechanisms of degeneration that apply across species. Essentially all of the major age-associated neurodegenerative diseases have been linked to accumulation of specific proteins in the CNS. Any of the phases seen in the neurodegenerative process (accumulation of the toxic protein, toxic insult to neurons, and neuronal dysfunction and death) can be identified in worm models of neurodegenerative diseases, making this organism an ideal model system for the in vivo study of various biological and physiological processes relevant to humans. *C. elegans* dopaminergic neurons show vulnerabilities to toxins and aggregation of human proteins that are largely similar to the situation in dopaminergic neurons in the human brain.

Transgenic *C. elegans* strains can easily be obtained by microinjection of DNA hosting the gene of interest, together with promoter expressed in all or selective tissues and a marker to identify the transgenic progeny, making this nematode clearly a resourceful organism that holds high value in the studies of the biology of disease (Baumeister and Ge, 2002). *C. elegans*, even as described represents an important model for studies of human neurodegenerative processes, is less applicable to studies whose goal is to understand neuronal connectivity. Indeed, the synaptic connectivity in nematodes is entirely different than that of the human brain and moreover nematodes have no substantia nigra, striatum or cortex for modeling circuits present in the human brain (Wolozin et al, 2011). Thus, to consider *C. elegans* as a reliable model it is important to determine what aspects of the disease one is willing to investigate. Overall the simplicity of the nematode and the ability to genetically manipulate the organisms makes it a valuable tool to fill the gap between simple *in vitro* studies and the complex analysis of human brain.

REDOX PROTEOMICS APPROACHES

A characteristic feature of a disease state is the alteration of genome or/and proteome profiles. Growing interest to develop new research strategies that allow the identification of such alterations and how they translate into clinical, pathological features is apparent. Unlike genes, proteins are the functional entities of the cell and conduct most of the cellular and biochemical reactions, enzymatic processes, and signal transduction pathways. The amount of proteins is much greater than the number of their corresponding genes due to post-translational modifications (PTMs), conformational changes and alternative splicing process that accounts for the huge number of proteomics informations. Accordingly, proteins can be considered "dynamic" biomarkers as the expression of specific proteins is altered in disease conditions (Thambisetty and Lovestone, 2010).

Proteomics aims to describe all the proteins expressed in an organism by analyzing their sequence, localization, abundance, PTMs, and biomolecular interactions (Beck et al, 2011). The high complexity of the cellular proteome and the low abundance of various proteins require very sensitive analytical methods. To reach this goal, mass spectrometry

Table I. *Redox proteomics data tables in C. elegans models of neurodegenerative diseases. Carbonylated proteins in C. elegans models of AD*

PATHWAYS	PROTEINS	*C. ELEGANS* MODELS COMPARISONS
Energy metabolism	medium-chain acyl-CoA dehydrogenase; enoyl-CoA hydratase; 3-hydroxyacyl-CoA dehydrogenase; malate dehydrogenase; arginine kinase; adenosine kinase; transketolase	Aβ 1-42 vs. nontransgenic
	ATP synthase α chain; glutamate dehydrogenase	GFP::degron vs nontransgenic
Structural proteins	tropomyosin; regulatory light chain	Aβ 1-42 vs. nontransgenic
Protein degradation	proteasome alpha subunit 7; proteasome beta subunit-7	Aβ 1-42 vs. nontransgenic
	proteasome beta subunit 6; proteasome beta subunit 7	GFP::degron vs nontransgenic
	proteasome beta subunit-4	ypkA toxin vs. nontransgenic
Antioxidant	glutathione S-transferase	Aβ 1-42 vs. nontransgenic

Protein HNE modified in C. elegans models of PD.

PATHWAYS	PROTEINS	*C. ELEGANS* MODELS COMPARISONS
Energy metabolism	ATP synthase subunit beta, mitochondrial; Adenylate kinase	LRRK2 G2019S::Tau vs. nontransgenic;
	Enolase; arginine kinase	LRRK2 G2019S::Tau vs. Tau
	Fructose-bisphosphate aldolase 1	LRRK2 R1441C::Tau vs. non-transgenic
	Enolase	LRRK2 R1441C::Tau vs. Tau
Structural proteins	Disorganized muscle protein 1	LRRK2 G2019S::Tau vs. nontransgenic
	Actin-1/3	LRRK2 G2019S::Tau vs. Tau
	Actin-1/3; Tubulin beta-2 chain	LRRK2 R1441C::Tau vs. non-transgenic
	Actin-1/3; Translationally-controlled tumor protein	LRRK2 R1441C::Tau vs. Tau
Protein degradation	Proteasome subunit beta type-2; Aspartic protease	LRRK2 G2019S::Tau vs. nontransgenic
	Proteasome subunit beta type-2; lysosomal V-type proton ATPase subunit B; Aminopeptidase	LRRK2 G2019S::Tau vs. Tau
	Aspartic protease	LRRK2 R1441C::Tau vs. Tau
Protein biosynthesis	60S Ribosomal protein L6; Eukaryotic translation initiation factor;	LRRK2 G2019S::Tau vs. Tau
	Eukaryotic initiation factor 4A	LRRK2 R1441C::Tau vs. non-transgenic
	60S Ribosomal protein L7; 60S Ribosomal protein L6; Eukaryotic translation initiation factor; 40S ribosomal protein SA	LRRK2 R1441C::Tau vs. Tau
Chaperone proteins	Heat shock 70 kDa protein 6, mitochondrial; Heat shock 70 kDa protein A	LRRK2 G2019S::Tau vs. Tau

(MS) currently is the most powerful technology to analyze complex protein samples (Aebersold and Mann, 2003).

Proteomics utilizes different techniques to study both the structure and function of proteins including two-dimensional electrophoresis (2DE), liquid chromatography (LC/MS) and protein arrays. Two-dimensional gel polyacrylamide electrophoresis (2D-PAGE) separates proteins according to charge and migration rate (often related to size) in two individual steps: isoelectric focusing and sodium dodecyl sulphate polyacrylamide gel electrophoresis. By combining these separations, a pattern of protein spots is achieved and protein identification revealed by MS (Gorg et al, 2004). Despite the intrinsic limitations of 2D-PAGE, as for example the

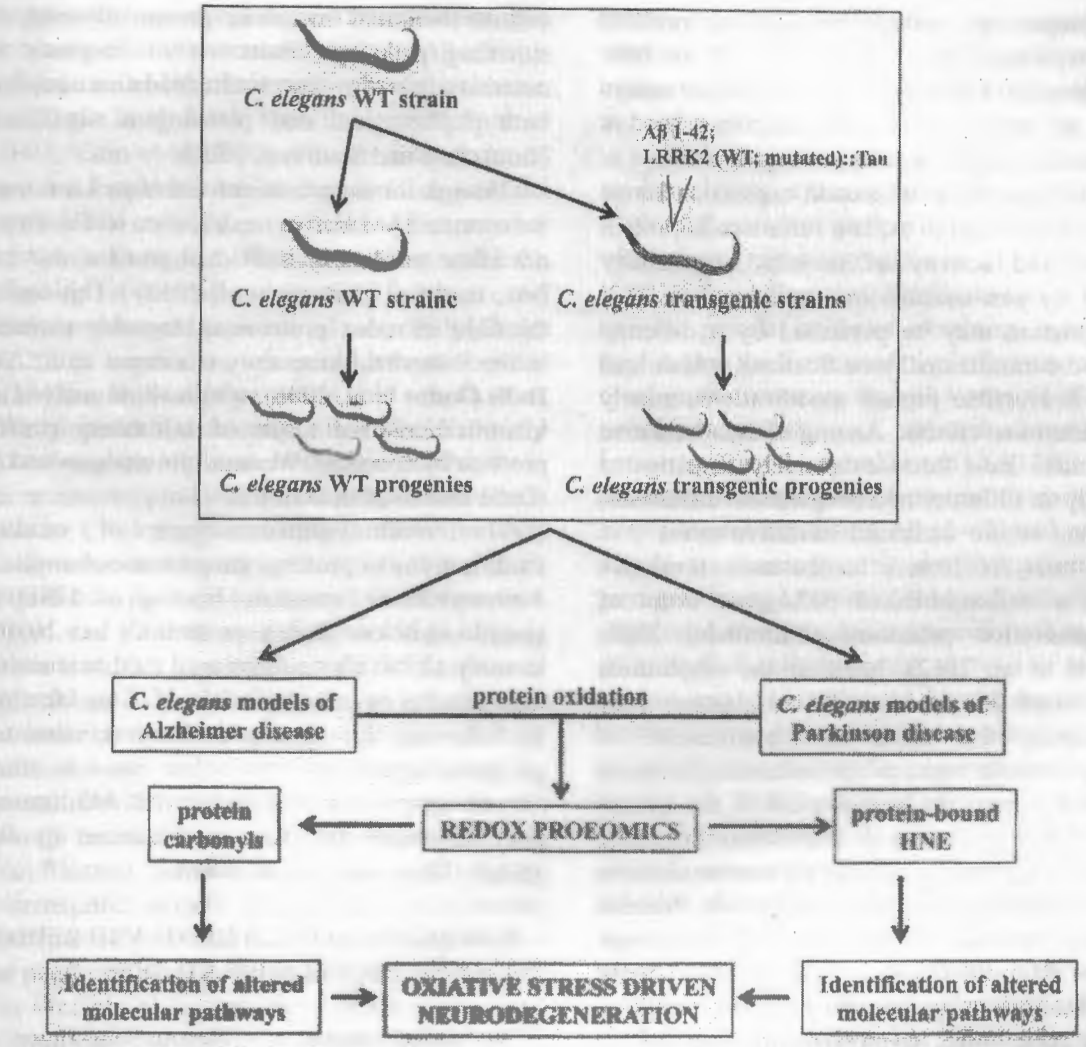

Fig. 1. *Experimental approach for the studies of neurological disorder by redox proteomics analyses of transgenic C. elegans models of AD/PD.*

challenges involved in studying membrane proteins, it is still the most common separation analysis when dealing with huge number of proteins.

LC processes allow for the fractionation of proteins by mass-transfer between a stationary and a liquid mobile phase. The combination of LC with MS or tandem mass spectrometry (MS/MS) (Domon and Aebersold, 2006; Mann et al, 2001) leads to the identification of peptides in mixtures in a single analysis and provides much more sentivity (Drabik et al, 2007). Surface enhanced laser desorption/ ionization time-of-flight (SELDI-TOF), introduced as a variation on the MALDI-TOF platform, utilizes protein chip arrays for selective capture of proteins (Issaq et al, 2002; Simonsen et al, 2012).

All these methods contributed to enhance the speed and accuracy of identifying and measuring proteins in different biological samples (tissues and body fluids). In addition, the recent development of quantitative proteomics platforms represents a step forward to reveal static or stress-induced variations of protein profile (Cox and Mann, 2011). Due the complexity of biological systems and nature of proteins, a multi-disciplinary approach to achieve the final goal of protein identification and quantitation is needed. Such a combined method usually comprises

several steps, i.e., sample preparation, protein/peptide separation, identification by MS or MS/MS, independent confirmation and validation assays (Rifai et al, 2006), as well as data processing via bioinformatics (Kall and Vitek, 2011).

However, alteration of protein expression levels alone is not sufficient to explain variations of protein functions. The activity of proteins is crucially regulated by post-translational modifications. This tight regulation may be perturbed by a different type of post-translational modifications, which lead to often irreversible protein modifications, mostly with deleterious effects. Among these, oxidative modifications have been extensively investigated commonly in different neurodegenerative diseases, a condition where increased oxidative stress is a constant treat for protein homeostasis. Oxidative stress is a well-established pathogenic event of neurodegenerative processes (Butterfield, 2006; Butterfield et al, 2012), however the mechanism that increased levels of oxidative damage play in mediating the onset and progression of neurodegeneration remains controversial. Oxidative stress results from the perturbation of the natural balance between pro- and anti-oxidant systems, which can affect the function of all macromolecules including proteins, lipids and nucleic acids. Proteins are ubiquitous in all organisms and represent major targets of ROS attack, which can elicit a variety of modifications in amino acid residues, including cysteine, methionine, tryptophan, arginine, lysine, proline, and histidine (Butterfield et al, 1997; Butterfield and Stadtman, 1997; Davies and Delsignore, 1987; Uchida and Stadtman, 1993). The most common markers of oxidative stress are: protein oxidation indexed by protein carbonyls, 3-nitrotyrosine and protein glutathionylation; lipid peroxidation indexed by thiobarbituric acid-reactive substances (TBARS), free fatty acid release, iso- and neuro-prostane formation, acrolein, and 4-hydroxy-2-trans-nonenal (HNE), DNA oxidation indexed by 8-hydroxy-2-deoxyguanosine and advanced glycation end products detection (AGEs) (Butterfield and Sultana, 2008; Di Domenico et al, 2009; Sultana and Butterfield, 2009; Suzuki et al, 2010).

The accumulation of oxidatively modified proteins is a characteristic hallmark of several neurodegenerative disorders and may affect multiple cellular functions including protein turnover, cell signaling pathways, induction of apoptosis and necrosis suggesting that protein oxidation could have both physiological and pathological significance (Butterfield and Stadtman, 1997).

Though immunochemical techniques are useful for measuring oxidative modification levels, they do not allow to identify individual proteins that have been modified (Sultana et al, 2012) . This topic is the field of redox proteomics, arguably pioneered in the Butterfield laboratory (Castegna et al, 2002; Dalle Donne et al, 2006), which allow analysis and identification of a subset of oxidatively modified proteins by coupling, Western blot analyses and MS (Dalle Donne et al, 2006).

The most common types of oxidative modifications to proteins are protein carbonylation, 3-nitrotyrosine formation, binding of HNE and glutathionylation. Redox proteomics can be used to study all the above-mentioned modifications and many studies have been performed in our laboratory by following this approach. However, other non-gel based approaches that utilize liquid or affinity chromatography in combination with MS have also been developed for these modifications by other groups (Dalle Donne et al, 2006).

C. ELEGANS MODELS OF AD AND REDOX PROTEOMICS STUDIES

As noted above, *C. elegans* constitutes an excellent genetic system for studying molecular pathways involved in AD. The nematode has the gene *apl-1*, which encodes two almost identical isoforms orthologous to the human amyloid precursor protein (APP) involved in AD, although *C. elegans* does not produce Aβ peptides because it lacks β-secretase cleavage (Wentzell and Kretzschmar, 2010). As well as Aβ production the worm lacks tau protein expression. Consequently, β-amyloid and tau accumulation has been modeled in *C. elegans* by insertion of human genes that make the nematode express tau and Aβ human proteins. When Aβ42 peptide, derived from human amyloid precursor cDNA, is expressed with the muscle-specific unc-54 promoter/enhancer of *C. elegans*, the formation of inclusions containing amyloid is induced in the worm leading to a phenotype of paralysis (Link, 1995).

Transgenic *C. elegans* that express human Aβ (1–42) have been used as *in vivo* models to study Aβ toxicity and deposition and the link with increased oxidative stress (Drake et al, 2003; Yatin et al, 1999; Link et al, 2003; Yatin et al, 2000). This *in vivo* model of inducible Aβ production in transgenic *C. elegans* has been used. The temperature-inducible Aβ expression system in the *C. elegans* created a model in which the relationship between Aβ toxicity, fibril formation, and oxidative stress could be examined temporally (Drake et al, 2003). In addition, transgenic animals express Aβ (1–42) with Met35Cys amino acid substitutions were engineered to demonstrate that Met35 is a key residue for amyloid plaque formation. Indeed, these latter nematodes produced amyloid deposits at levels similar to *C. elegans* containing human Aβ (1-42) but showed reduced protein oxidation (Yatin et al, 1999). Wild-type tau and mutant protein forms, such as P301L and V337M, were expressed in *C. elegans* (Guthrie et al, 2009; Kraemer et al, 2003; Kraemer and Schellenberg, 2007). In all cases, the pan-neuronal expression of these tau proteins in the nematode caused uncoordinated locomotion. Tau pathology in the nematodes did not fully reflect that of the human AD brain because there was no evidence of paired helical filament formation. However, in *C. elegans* AD transgenic models, the presence of a strong phenotype and clear biochemical changes provide a good platform for cause-effect studies, which is one of the strengths of the nematode system

Though several studies demonstrated that Aβ is a key player in the pathogenesis and progression of AD, the precise mechanisms through which Aβ exerts neurotoxicity still remain unclear. According to the amyloid hypothesis, accumulation of Aβ in the brain is the primary influence driving AD pathogenesis. The rest of the disease process, including formation of neurofibrillary tangles containing tau protein, is proposed to result from an imbalance between Aβ production and Aβ clearance (Hardy and Selkoe, 2002). In this scenario, *C. elegans* expressing human Aβ (1–42) is a useful model to study specific intracellular target of Aβ-induced oxidative damage. However recent studies suggest that the oxidative damage theory of aging, applied to the short-lived nematode worm *C. elegans*, should be reconsidered (Gems and de la Guardia, 2012).

In the work reported by Boyd-Kimball et al (2006), proteomic techniques were used to identify proteins that were specifically oxidized in a transgenic *C. elegans* expressing human Aβ (1–42) in body wall muscle (CL4176). Moreover, in order to evaluate the role of protein aggregation per se in oxidative stress, a transgenic *C. elegans* expressing a green fluorescent protein (GFP) fusion protein, which forms rapid aggregates of GFP in the worms (CL2337) was also used. Finally, to control for non-specific protein oxidation resulting from muscle dysfunction itself, the oxidative effects in transgenic *C. elegans* expressing the ypkA subunit of *Yersinia pseudotuberculosis*, a serine/threonine kinase known to affect the cytoskeleton (XA1440) was also examined. Comparison of protein oxidation levels in *C. elegans* expressing Aβ (1–42) and control *C. elegans* was carried out by identifying carbonylated proteins via anti-DNP immunochemical development of proteins transferred to a nitrocellulose membrane (2D Oxyblot). However, the analysis of transgenic control strains demonstrated that the specific protein oxidation observed is not a byproduct of general cellular dysfunction or the accumulation of protein aggregates. Sixteen oxidized proteins were identified in *C. elegans* expressing human Aβ (1–42). These proteins are involved in a variety of cellular function including energy metabolism, proteasome functions, cellular structure, lipid transport, and signal transduction. A set of proteins associated with energy and metabolism were identified exclusively in the *C. elegans* expressing Aβ (1–42), a finding consistent with altered energy metabolism in AD. Acyl-CoA dehydrogenase, malate dehydrogenase, transketolase, arginine kinase, and adenosine kinase were all found to be oxidized by Aβ (1–42), but not in the other two *C. elegans* models, suggesting that oxidation of proteins involved in energy metabolism is a direct effect of exposure to Aβ (1–42) independent of protein aggregation and paralysis. All these proteins are involved directly or indirectly in production of ATP, which is essential for many cellular functions. This is consistent with studies from Butterfield's group reporting that several energy and metabolism-related proteins were oxidatively modified in AD brain (Butterfield et al, 2006; Perluigi et al, 2009; Sultana et al, 2006a; Sultana et al, 2006b).

The results obtained on *C. elegans* cannot address

whether or not protein oxidation results from direct interaction of Aβ with the specific protein, or from Aβ-induced secondary effects related to oxidative stress conditions. More importantly, these findings fit well with the Aβ hypothesis of AD and closely mimic what occurs in human brain. *C. elegans* is a robust and interesting model to study the mechanism of neurodegeneration and in addition to the role of Aβ has the potential to be used to study other putative mechanisms that contribute to the pathology of AD.

C. ELEGANS MODELS OF PD AND REDOX PROTEOMICS STUDIES

With the completion of the *C. elegans* genomic sequence, many worm orthologs of human genes have been identified including those that are linked to familial PD. Currently, six worm orthologs of human gene involved in familial PD have been identified (Table 2): parkin (*pdr-1*), PINK1 (*pink-1*), DJ-1 (*djr-1.1, 1.2*), LRRK2 (*lrk-1*), ATP13A2 (*catp-6*), and GBA (*F11E6.1, TO4A8.7* with the most notable exception being α-synuclein (PARK1) (Harrington et al, 2010; Caldwell and Caldwell, 2008). Knockout models of these orthologues have produced some important insights into their normal function and the effects of their mutation. In each case mutations that cause PD either cause direct degeneration of dopaminergic neurons, or sensitize the nematodes to mitochondrial toxins (Schmidt et al, 2007). *C. elegans* models of synucleinopathy have been established with human α-synuclein expression in either dopaminergic neurons or pan-neuronally. Lakso et al (2003) observed movement deficits in worms with pan-neuronal expression of wild type or A53T α- synuclein, while Kuwahara did not observe any phenotypic effects of pan-neuronal wild type, A30P, or A53T α- synuclein expression (Kuwahara et al, 2006; Lakso et al, 2003).

Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common known cause of familial PD. The overexpression of all active variants of LRRK2 including LRRK2 WT, R1441C and G2019S, but not the inactive form K1347A, is sufficient to induce neurodegeneration accompanied by behavioral deficits and depletion of dopamine. The pathologic phenotype is more severe in LRRK2 carrying PD-linked mutations R1441C and G2019S than that of LRRK2 WT (Yao et al, 2010; Saha et al, 2009; Liu et al, 2011; Yuan et al, 2011). A common feature in LRRK2 transgenic models is the presence of Tau alterations that are also present in a subset of mutated LRRK2 PD patients; indeed, mutations in LRRK2 generate PD that is sometimes associated with Tau pathology (Galpern and Lang, 2006). Based on this correlation, it is reasonable to hypothesize that LRRK2 dysfunction impacts Tau post-translational processing and compartmentalization and thus on its functional roles (Devine and Lewis, 2008). Therefore, Wolozin's group generated lines of *C. elegans* expressing human LRRK2 [wild-type (WT) or mutated (G2019S or R1441C)] with and without V337M Tau. Subsequently the investigation of Butterfield's group on the effect of oxidative stress caused by the co-expression of human mutated LRRK2 and human tau, in such transgenic lines, showed an increase in protein oxidation (Di Domenico et al, 2012). Initially this study employed the analysis of total levels of selected parameters of protein oxidation revealing significant differences between LRRK2 and Tau transgenic and non-transgenic strains, for protein-bound HNE marker of oxidation, while protein carbonyls and protein-bound 3NT levels were basically unaltered. This approach was then followed by the use of redox proteomics targeting specifically protein-bound HNE to identify proteins and molecular pathways prone to oxidation. The use of redox proteomics helped to delineate a sharper image of the entire phenomena providing insight about the proteins and the molecular pathways implicated and their possible involvement in increased oxidation.

The firsts interesting results was that LRRK2 or tau alone or in co-expression does not have a strong impact on expression levels and protein oxidation in *C. elegans*, while the co-expression of mutated LRRK2 (G2019S or R1441C) with Tau showed a large increase on protein oxidation for a number of proteins and molecular pathways involved. Moreover, the trend of increased oxidatively modified proteins was clearly shifted towards the mutated forms when compared with transgenic LRRK2 WT::tau or non transgenic worms. In that sense the redox proteomics data are consistent with the notion that the trend of oxidation is a result of the interaction of mutated LRRK2 and Tau.

Analyzing specific proteins alterations and pathways involved, the study suggest common mechanisms that lead to increased oxidative stress for G2019S::Tau and R1441C::TAU co-expression.

Indeed in both the transgenic models the oxidative modifications affect, mainly mitochondrial proteins, structural proteins and proteins involved in biosynthetic and degradative processes. Dysfunction of mitochondria is implicated in the pathophysiology of PD (Ved et al, 2005), and recent studies raised the possibility that LRRK2 modulates mitochondrial function and impairment (Saha et al, 2009). Our findings show the oxidation of several mitochondrial proteins, either energy-related or not suggest mitochondrial damage and strength a possible pathological correlation among LRRK2, tau and mitochondria. Alteration of the autophagic pathway in transgenic models represents another intriguing result. Indeed, the alteration of V-type proton ATPase in LRRK2 G2019S::Tau *C. elegans* strain, a vacuolar proton pump involved in lysosomal autophagy, is consistent with previous studies that showed the alteration of lysosomal membrane by oxidative stress and that lysosomal malfunction and mutations in ATP13A2, lead to a failure of autophagy execution in PD (Meredith et al, 2002; Yang and Mao, 2010). In addition to the autophagic pathway, we found oxidative modification of several proteasome subunits during co-expression of mutated (G2019S) LRRK2 and Tau suggesting the impairment of the ubiquitin/proteasome system that might lead to the formation of protein aggregates as seen for the mutations of parkin and UCH-L1 (Facheris et al, 2005; Maraganore et al, 2004) components of the system. Further, other very interesting results concern the oxidatively modification of proteins implicated in biosynthetic machinery and in cell structure. Accordingly, increased levels of protein-bound HNE for the eukaryotic translation initiation factor, eukaryotic initiation factor 4A, and several ribosomal subunits together with actin 1/3 and tubulin beta-2 chain in mutated LRRK2 and Tau models were demonstrated in both G2019S::Tau and R1441C:: *C. elegans* strains. Overall it was speculated that, in a mechanism of gain of function, mutated LRRK2 might increase its activity (West et al, 2007) triggering the activation of several downstream signals that lead to Tau hyperphosphorylation and increased oxidative stress, which in turn result in harmful effects for the above-mentioned pathways leading to a PD-like neurodegeneration.

CONCLUDING REMARKS

The identification of potential human disease-associated proteins and their related molecular pathways are of major importance. Model organisms have an essential role in this process and represent a first approach to analyze the alterations induced by different pathological conditions with the aim to develop novel diagnostic and therapeutic strategies. For many reasons, among which its simplicity and its aptitude to genetic manipulation, *C. elegans* provides the opportunity for conducting analysis aimed to identify factors, and certain pathways, putatively implicated in a variety of diseases. Thus, *C. elegans* represents a valuable link between the simplicity of *in vitro* studies and the complexity of the human organism. The crucial role of *C. elegans* transgenic models of neurodegenerative diseases (as described in this review) is supported by a great amount of studies and data collected on this model that often anticipate or parallel the results obtained in mammalian models and humans. Indeed, despite a vast evolutionary distance between nematodes and humans, a cross-species translation of proteins was extensively demonstrated and this greatly facilitates the study of human disease in such simple organisms. Proteomics, as well, is becoming increasingly indispensable for a through understanding of proteome alterations caused by onset and progression of diseases. Application of proteomics in *C. elegans* studies led to interesting results, which provided insights into the molecular pathways perturbed by genetic alterations. Further analysis of specific protein PTM (acetylation, phosphorylation etc.) and the recent use of redox proteomics approaches for the study of protein oxidation in *C. elegans* models of neurodegenerative diseases allowed the identification of altered protein and their alteration trend that represent an additional advantage in developing promising therapeutic strategies for the treatment of a diseased state. The redox proteomics studies performed on *C. elegans* models of AD and PD demonstrated that several proteins, involved in energy metabolism,

protein degradation, mitochondrial function, and organismal structure, are common target of oxidative modification in this disease model and may play a crucial role in neurodegenerative processes. The data presented are highly consistent with human studies and open new avenues in the knowledge of pathologic events involved in both AD and PD. These findings provide a strong rationale for using these models for specific studies aimed to target the molecular pathways, perturbed in these diseases, to confirm their relevance in human disease and develop therapeutic intervention.

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