Biochimica et Biophysica Acta 1822 (2012) 753–783

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Review

Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity ☆

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ARTICLE INFO

Article history:
Received 26 September 2011
Received in revised form 31 October 2011
Accepted 1 November 2011
Available online 6 November 2011

Keywords:
Hormesis
Antioxidant
Redox signaling
Cellular stress response
Mitochondria
Vitagene

ABSTRACT

Modulation of endogenous cellular defense mechanisms represents an innovative approach to therapeutic intervention in diseases causing chronic tissue damage, such as in neurodegeneration. This paper introduces the emerging role of exogenous molecules in hormetic-based neuroprotection and the mitochondrial redox signaling concept of hormesis and its applications to the field of neuroprotection and longevity. Maintenance of optimal long-term health conditions is accomplished by a complex network of longevity assurance processes that are controlled by vitagenes, a group of genes involved in preserving cellular homeostasis during stressful conditions. Vitagenes encode for heat shock proteins (Hsp) Hsp32, Hsp70, the thioredoxin and the sirtuin protein systems. Dietary antioxidants, such as polyphenols and L-carnitine/acetyl-L-carnitine, have recently been demonstrated to be neuroprotective through the activation of hormetic pathways, including vitagenes. Hormesis provides the central underpinning of neuroprotective responses, providing a framework for explaining the common quantitative features of their dose response relationships, their mechanistic foundations, their relationship to the concept of biological plasticity as well as providing a key insight for improving the accuracy of the therapeutic dose of pharmaceutical agents within the highly heterogeneous human population. This paper describes in mechanistic detail how hormetic dose responses are mediated for endogenous cellular defense pathways including sirtuin, Nrfs and related pathways that integrate adaptive stress responses in the prevention of neurodegenerative diseases. This article is part of a Special Issue entitled: Antioxidants and Antioxidant Treatment in Disease.

1. Introduction

In recent years, studies have shown that aging (more correctly defined as longevity) is due to a complex genetic and cellular process that seems to be partly regulated by sirtuins [1,2] the human and murine homologs of the *Saccharomyces cerevisiae* Sir2 that control both replicative and overall lifespan [3,4]. Findings have also shown that this gene family regulates longevity in *Caenorhabditis elegans* and *Drosophila melanogaster* [1,2], suggesting that there is an evolutionary need in many different complex species to preserve these proteins in the cells. Sirtuins, which are classified as class III histone deacetylases, differ from traditional class I and II histone deacetylases [5,6]. They differ from conventional HDACs in that their substrates range from metabolic enzymes to structural proteins and histones [7–9].

The activity, stability and intracellular location of nearly all proteins depend on post-translational modifications (PTMs) that include phosphor-ylations, acetylations, sumoylations, ubiquitinations, ADP-ribosylations and nitrations [10]. Most of these highly dynamic processes occur as a result of opposing enzyme activity (e.g. kinases vs. phosphatases, acetylases vs. deacetylases, etc.). Numerous studies have demonstrated that PTM signaling pathways play a significant role in crosstalk between the cell and its environment and in enabling cells to become flexible towards change [10]. For this reason, nicotinamide adeninedinucleotide (NAD) has aroused new interest as it is an

☆ This article is part of a Special Issue entitled: Antioxidants and Antioxidant Treatment in Disease.

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doi:10.1016/j.bbadis.2011.11.002

important substrate for a number of enzymes that catalyze a set of post-translational modifications, such as deacetylationorADP- ribosylation. The role of NAD in these regulatory processes differs from its involvement in energy metabolism since it is based on the ability to act as an ADP-ribose donor, thus requiring NAD re-synthesis to avoid depletion of the intracellular NAD pool [11]. The fundamental role of NAD in energy metabolism and protein modification has an important physiological influence on the control of cell metabolism, cell death and longevity [12,13].

Since transient post-translational modifications such as acetylation, phosphorylation and ubiquitination determine a rapid and efficient cell response to intra- and extracellular stimuli, they play a central role in cell signaling cascades. Recent research has identified a PTM involving the attachment of a SUMO peptide, a process frequently referred to as SUMOylation that occurs in signaling pathways both within the nucleus and other parts of the cell. SUMO attachment is thought to be involved in a number of cell processes, e.g. transcription, nuclear transport, DNA repair, mitochondrial activity, plasma membrane ion channels, cell cycle and chromatin structure. Although its function is as diverse as its substrates, modification of a protein substrate by SUMO generally alters its interactions with other protein and DNA molecules [14].

A prediction of oxidative stress theory of aging is that, among species, differential rates of aging may be apparent on the basis of intrinsic differences in oxidative damage accrual. Although widely accepted, exceptions to this theory, mostly related to the specificity of species, strain and even tissues of investigation, are occurring more frequently. Proteins are a principal target for oxidative damage and cysteine residues are highly sensitive to reversible and irreversible oxidation. To adapt and survive, cells and organisms need to sense proteotoxic insults and to protect themselves by coordinating cellular stress response pathways and chaperone networks related to protein quality control and stability. The mis-assembly or aggregation of proteins or peptides, in any cell type leads to toxic effects or proteotoxicity. Despite the abundance and apparent capacity of chaperones and other components of homeostasis to re-establish folding equilibrium, cells do not appear to adapt well to chronic proteotoxic stress which increases in cancer, metabolic and neurodegenerative diseases. The use of drugs to modulate cellular stress response pathways is emerging as a treatment for human diseases such as neurodegenerative disorders, cardiovascular disease and cancer. For medical intervention to be successful, the dose must be right, but it can be extremely difficult to achieve this goal on account of human inter-individual variation in age, gender, diet, exercise, genetic factors and health status. The past decade has witnessed considerable progress in the nature of the dose response in and adjacent to the therapeutic zones. Long-standing ideas about the nature of the dose–response in a low dose zone, have been challenged by the hormetic dose–response which could significantly influence the design of pre-clinical studies, clinical trials and optimal patient dosing strategies in the treatment of numerous diseases. The broad cytoprotective properties of the heat shock response have aroused strong interest in discovering and developing pharmacological agents that can induce stress responses, including carnitines. This paper illustrates the mechanisms by which hormetic dose responses are mediated for endogenous cellular defense pathways. These include the possible signaling mechanisms through which, by interplaying metabolism, mitochondrial energetics and activation of critical vitagenes, the carnitine system modulates signal transduction cascades that provide cytoprotection against chronic degenerative damage associated with aging and neurodegenerative disorders.

2. The mitochondrial theory of aging

Mitochondria are membrane-enclosed organelles found in eukaryotic cells where they generate ATP as a source of chemical energy. ATP synthesis occurs through the respiratory or electron transport chain (ETC) located at the inner mitochondrial membrane, and consists of five protein complexes (Complexes I–V) [15–18].

Besides supplying ATP, mitochondria are involved in many other cell functions including the biosynthesis of heme, cholesterol and phospholipids [16] and initiation of the apoptotic process [18]. According to the endosymbiosis theory, mitochondria are organelles that evolved from purpurbacteria approximately 1.5 billion years ago. Mitochondria have their own genome [17,19] and can replicate and transcribe their DNA semi-autonomously. Mitochondrial DNA (mtDNA), like nuclear DNA, is constantly exposed to DNA damaging agents. For many years it was thought, in mtDNA repair, that excessively damaged mtDNA molecules were simply degraded and replaced by newly-generated successors copied from undamaged genomes. However, findings now indicate that mitochondria possess the machinery needed to repair genome damage caused by endogenous or exogenous harmful agents. Harman [20,21] suggested that free radicals are involved in the aging process and that mitochondria-derived ROS may influence cellular aging [21]. The treatment of IMR-90 fibroblasts with N-tertbutyl hydroxylamine (an antioxidant recycled by the mitochondrial electron-transport chain), initially gave support to the theory of mitochondrial involvement in cellular senescence. N-tert-butyl hydroxylamine extends fibroblast replicative capacity and delays changes in mitochondrial function due to age by reducing ROS production, preservation of mitochondrial membrane potential and by increasing the cellular GSH/GSSG ratio [16]. A more recent study has demonstrated that mitochondria derived ROS play an important and direct role in the shortening of telomeres and the onset of senescence [16]. Other findings have proposed that mitochondrial dysfunction leads to mitochondrial biogenesis, thereby increasing the number of cell sites for the production of ROS that causes telomere shortening [15,18].

On account of its elevated mutagenic propensity, accumulation of mtDNA during life is thought to be a major cause of age-related disease. The lack of introns and protective histones, limited nucleotide excision and recombination DNA repair mechanisms, location in proximity of the inner mitochondrial membrane with exposure to an enriched free radical milieu are all factors contributing to a mutation rate that is 10-fold higher in the mtDNA than in the nuclear DNA (nDNA) [22]. Furthermore, considerable evidence suggests that mtDNA mutations increase as a function of age, reaching the highest levels in brain and muscle. It has been reported that more than twenty different types of deletions accumulate in aging human tissues. The first report on an age-related increase in a mtDNA deletion was found in elderly brain and in Parkinson's disease [22]. This deletion, called the “common deletion”, was observed between 13-bp sequence repeats beginning at nucleotides 8470 and 13447, removing almost a 5-kb region of mtDNA between ATPase 8 and the ND5 genes. The deletion takes place during replication of the mtDNA, the missing sequence encodes for six essential polypeptides of the respiratory chain and 5 tRNAs, and has been associated with several clinical diseases, such as chronic progressive external ophthalmoplegia and Kearns Sayre syndrome. In a comparison with age-matched controls, an association was found between numerous age-related disorders and higher levels of mtDNA mutations. In the central nervous system (CNS), patients with Parkinson's disease were found to have a 17 times higher level of the common deletion in the striatum, compared to age-matched controls. There is also evidence of higher levels of this deletion in patients with Alzheimer's disease, parallelling increased levels in the oxidized nucleotide 8-OH-dG [15]. More than 100 mutations of mtDNA have been associated with human diseas [23]. Phenotypic manifestation of mtDNA mutations is extremely broad ranging from oligosymptomatic patients with isolated deafness, diabetes, ophthalmoplegia, etc., to complex encephalomyopathic disorders that may include dementia, seizures, ataxia, stroke-like episode and so on. There is also a wide range of genotype variants, with

rearrangements (deletions, duplications) and point mutations affecting protein coding genes, tRNAs and rRNAs. Although there are some broad genotype/phenotype correlations, considerable overlap also occurs. Further research is needed to fully understand the pathogenetic mechanisms involved in the expression of mtDNA mutations. Recent studies have identified mutations of nuclear genes encoding subunits of the respiratory chain, particularly those of complex I. These principally involve infant onset disease with early death. Moreover recent research has shown that the function of the respiratory chain may be impaired by mutations affecting other mitochondrial proteins or as a secondary phenomenon to other intracellular biochemical derangements. An example is Friedreich ataxia where a mutation of a nuclear encoded protein (frataxin), probably involved in iron homeostasis in mitochondria, results in severe deficiency of the respiratory chain in a pattern indicative of free radical mediated damage [22,24–26].

Mutations of nuclear encoded proteins involved in cytochrome oxidase assembly and maintenance have been identified. As predicted, they are associated with severe deficiency of cytochrome oxidase and Leigh syndrome. Defects of intracellular metabolism, particularly with excess-free radical generation including nitric oxide or peroxynitrite, may cause secondary damage to the respiratory chain. This probably plays an important role in Huntington disease, motor neuron disease (amyotrophic lateral sclerosis) and Wilson disease. It is important to consider however that mutation and ROS induced DNA damage are different, and the diseases mentioned above are mostly dependent on genetics and not a causative factor of oxidative stress. Since a common pathway in the pathogenesis of these disorders appears to be defective oxidative phosphorylation, treatments designed to improve respiratory chain function may bring amelioration in the progression of these disorders [23]. These findings establish that a key feature of the aging process is the relationship between age-associated accumulation of mtDNA mutations and bioenergy dysfunction, at least in tissues predominantly composed of postmitotic cells, such as CNS and skeletal muscle.

With regard to mitochondrial bioenergetics, a significant decrease in state 3/state 4 ratio has been seen to occur in brain during aging [24]. Since this ratio relates to the coupling efficiency between electron flux through the electron transport chain and ATP production, an increase in state 4 would reduce mitochondrial complexes and consequently increase free radical species production. A decrease in state 3/state 4 respiration during aging has been associated with a significant decrease in cardiolipin content in brain mitochondria [27]. This loss may play a vital role in age-related reduction in mitochondrial function, and appears to be associated with both quantitative and qualitative region-specific protein changes such as decrease of the inner membrane surface, smaller and fewer cristae, decreased fluidity and increased fragility. It is known that functional changes in brain mitochondria are correlated with modifications in cardiolipin composition. Acetylcarnitine fed to old rats increased cardiolipin to levels found in young rats and restored protein synthesis both in the inner mitochondrial membrane and cellular oxidant/antioxidant balance, suggesting that cellular bioenergetics in aged rats may benefit from administration of this compound [28]. It is interesting to note that caloric restriction, a dietary regimen that increases life-span in rodents, maintains the levels of 18:2 acyl side chains and inhibits cardiolipin composition changes [29]. It has also been shown to retard aging associated changes in oxidative damage, mitochondrial oxidant generation and antioxidant defenses observed during aging [18,30].

Recent finding indicates that calorie restriction and specifically reduced glucose metabolism induce mitochondrial metabolism to extend life span in a number of model organisms including *S. cerevisiae*, *D. melanogaster*, *C. elegans* and possibly mice. In contrast with Harman's free radical theory of aging, these effects may be caused by an increase in ROS formation within the mitochondria leading to an adaptive response that culminates in increased stress resistance thought to ultimately cause a long-term reduction in oxidative stress [31]. This type of retrograde response, termed mitochondrial hormesis or mitohormesis, may be involved in the health-promoting effects of physical exercise in humans and, hypothetically, impaired insulin/IGF-1-signaling in model organisms. Abrogation of this mitochondrial ROS signal by antioxidants consistently impairs the lifespan-extending and health-promoting capabilities of glucose restriction and physical exercise, respectively [32]. In short, ROS are essential signaling molecules needed to promote health and longevity. The concept of mitohormesis therefore provides a common mechanistic denominator for the physiological effects of physical exercise, reduced calorie uptake and glucose restriction [33].

3. Redox regulation of mitochondrial dynamics fusion and fission: role in cell survival

In eukaryotic cells, mitochondria are the principal source of energy (ATP and NAD^+). Neuronal processes such as energy production, Ca^{2+} regulation, maintenance of plasma membrane potential, protein folding by chaperones, axonal and dendritic transport and the release and re-uptake of neurotransmitters at synapses all depend on mitochondrial function. Mitochondria help neurons to satisfy the high energy demand required for proper neuronal function since, unlike other cell types, they cannot switch to glycolysis when oxidative phosphorylation becomes limited. In response to changing energy demands, cells continually adjust the rate of mitochondrial fission and fusion to aid the distribution of mitochondria [18,30]. Mitochondria also produce large amounts of superoxide anion radical by abstracting an electron from oxygen in the so-called electron transport chain at the inner mitochondrial membrane [15]. Mitochondrial respiration accounts for about 90% of the oxygen consumed in the cell. Most of this is reduced to water through four consecutive one-electron reductions in which a small proportion of the oxygen molecules (1–2%) is converted to superoxide anion radicals. Complexes I and III are recognized the main sites of superoxide production in the respiratory chain [15] and recent studies have detected up to nine submitochondrial reactive oxygen species (ROS) generating sites [16]. The resulting superoxide then dismutates either spontaneously or enzymatically due to superoxide dismutase to form hydrogen peroxide [17] which may then diffuse through the cell and decompose to form harmful hydroxyl radicals, thus damaging the cell through interactions with macromolecules. These processes cause mitochondria to become a major source of physiological or endogenous ROS production [18]. Many studies have focused on the harmful effects of ROS, but it is now clear that mitochondrially generated ROS are also involved in regulating intracellular signal transduction pathways that result in cell activities such as proliferation [18]. Mitochondria can produce superoxide in relatively constant amounts, or elicit spontaneous or environmentally-induced “superoxide flashes” [18] and it is generally assumed that the main sites of superoxide production in the mitochondrial respiratory chain are complexes I and III [18]. Consequently any impairment in the electron transfer process causes a reduction in upstream carriers, thus filling Q cycle electron pool. The cytochrome (cyt)2 bc1 complex (EC 1.10.2.2) (cyt bc1 complex), situated in the inner membrane of mitochondria, couples the oxidation of a substrate quinol (QH2) with the generating proton motive force across the energy transducing membrane system, where the energy is ultimately stored in the form of ATP (Fig. 1). Cytochrome bc1 complexes contain four redox-active metal centers, arranged in two separate chains. The “high potential chain” consists of the Rieske iron-sulfur (2Fe2S) cluster and cyt c1. The “low potential chain,” which binds to the cyt b subunit of ubiquinol oxidizing complexes, consists of two b-type hemes, cyt bL and bH labeled for their relatively lower and higher electrochemical potentials [34]. Three enzymatic binding sites participate in catalysis on the cyt bc1 complex, the quinol oxidase (Qo) site, quinol reductase (Qi) site, and a docking site for soluble cyt c on cyt c1.

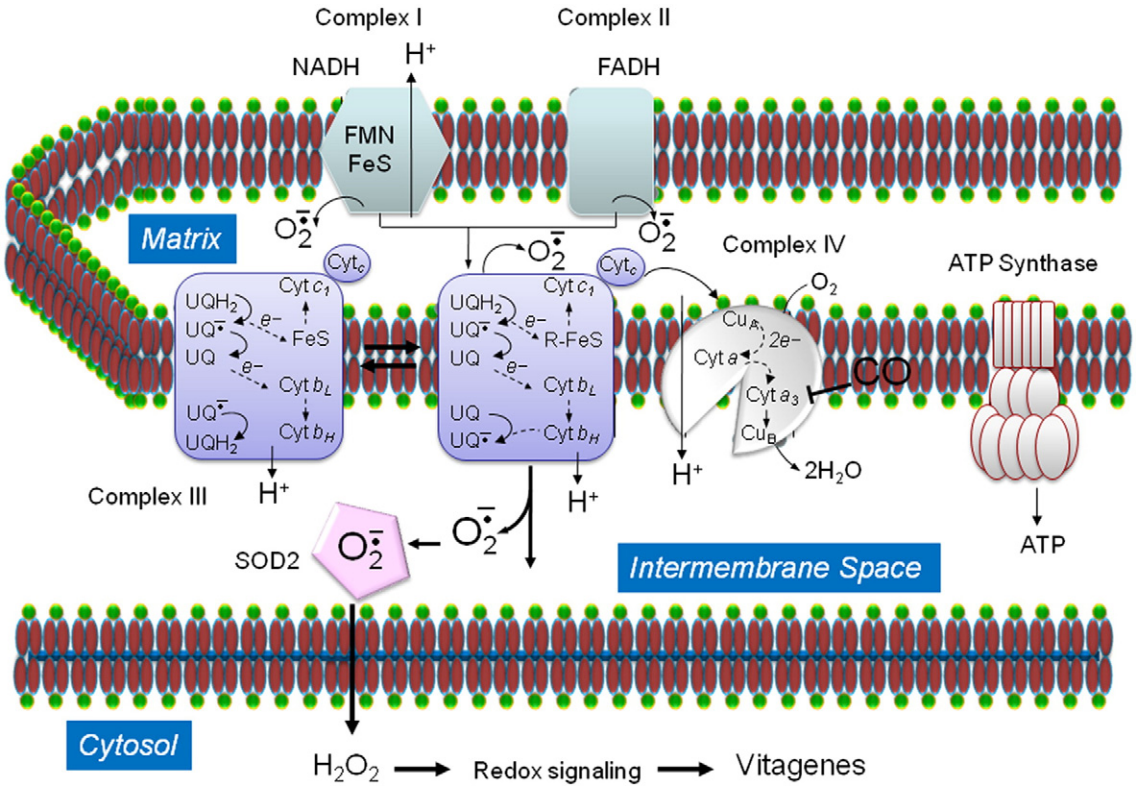

Fig. 1. The ubiquinone (Q) cycle. The ubiquinone (Q) cycle is initiated when one electron from ubiquinol (QH2) is donated to the Rieske-iron sulfur (R-FeS) protein and the second electron is donated to cytochrome b. The intermediate moiety is the free radical ubisemiquinone (Qo), which can donate electrons to molecular oxygen to generate superoxide. Mitochondrial electron transport chain generates superoxide at complexes I, II, and III. Complexes I and II generate superoxide within the mitochondrial matrix. Complex III can generate superoxide in both the intermembrane space and the matrix. Release of superoxide from complex III into the cytosol is followed by conversion to H_2O_2 and subsequent activation of oxidant-dependent (redox) signaling pathway of vitagene network, which results in preconditioning.

The Qo site is positioned toward the positively charged side of the membrane, where protons are released during enzyme turnover. The Qi site is located toward the negatively charged membrane surface, where protons are taken up during catalysis. The water-soluble cyt c docking site is located on the c-type cyt representing the terminal electron carrier within the cyt bc1 complex on the positive side of the membrane. Cyt bc1 complex catalysis is thought to occur by a "Q-cycle" mechanism. A key reaction in the Q-cycle may be the "bifurcated" electron transfer at the Qo site, but the exact mechanism has not yet been clarified [34]. In the bifurcated reaction, QH2 is oxidized at the Qo site, with one electron being transferred through the high potential chain to reduce cyt c, while the other electron is transferred through the low potential chain, to reduce a quinoid species (Q or SQ, depending on the state of the two-electron gate) at the Qi site. Two turnovers of the Qo site are required to reduce a Qi site Q to a QH2. Under some conditions, the Q-cycle can be short-circuited by various "bypass" reactions, some of which yield the superoxide that is physiologically harmful [18]. The bypass reactions are typically observed in vitro under "partially inhibited" conditions, e.g. in the presence of antimycin A, or under high proton motive force, where the (2Fe2S) cluster may oxidize QH2 to a semiquinone (SQ), but processing of electrons by the low potential chain is impeded, thus generating the accumulation of a SQ intermediate, which in turn can reduce O_2 to superoxide [18].

The electron transport chain consumes as much as 90% of the oxygen taken up by a cell, of which ~1% is transformed into superoxide under normal physiological conditions [35]. The rate of mitochondrial electron transfer from semi-reduced Q to molecular O2 to form superoxide is proportional to the product of the semi-reduced Q and O2 concentrations. Estimates indicate that under pathophysiological conditions, superoxide production occurs at 2–10% of the uninhibited rates. Superoxide dismutases, in turn, generate the more stable hydrogen peroxide before it is transformed into water by catalase or, if metals are present, into highly reactive hydroxyl radicals. Thus, SOD2, which directs H_2O_2 out of the mitochondrion, performs two functions: the classical scavenging of superoxide and its signalization as ROS-dependent signaling pathways. The binding of CO to cytochromes found in complex IV (i.e., cytochrome a3) enhances reduction in the respiratory carriers in the cytochrome bc1 region of complex III, associated with increased leakage of superoxide and H_2O_2 production.

Much of the superoxide produced is efficiently converted to hydrogen peroxide by the activity of superoxide dismutases that include the cytoplasmic (SOD1) and mitochondrial (SOD2) enzymatic forms. As the superoxide is a highly reactive free radical, it can damage molecules (DNA, proteins and lipids). Its conversion to hydrogen peroxide therefore protects cells, but if Fe^{2+} or Cu^{+} are present even in very low concentrations, this peroxide can generate hydroxyl radical that induces membrane lipid peroxidation [36]. Moreover, nitric oxide (generated in response to activation of the enzyme nitric oxide synthase by Ca^{2+}/calmodulin) can interact with mitochondrial superoxide to produce the highly reactive free radical peroxynitrite [37]. Antibodies that selectively recognize proteins modified by the lipid peroxidation product 4-hydroxynonenal and by nitration have been particularly useful instruments for detecting and quantifying oxidative damage to proteins, lipids and DNA [38]. In addition, researchers have made use of fluorescent probes for imaging relative levels of overall mitochondrial redox status and superoxide in order to define the role of mitochondrial ROS in a number of physiological and pathological processes [39]. An excessive ROS production is thought to contribute to damaging effects in a range of diseases such as cancers, cardiovascular disease and inflammatory conditions such as arthritis [40]. Neurons may be particularly vulnerable to mitochondrial ROS on account of their high energy demands, excitability, and the fact that they are postmitotic and therefore irreplaceable in most cases [64]. On the contrary, lower subtoxic

levels of mitochondrial ROS can activate signaling pathways that protect cells against injury and disease [18,38].

New findings suggest that modifications in mitochondrial activity can induce morphological adaptations of the mitochondrial network. Moreover, clinical studies indicate that molecular defects affecting mitochondrial dynamics bring about pathology. This suggests that there is a link between energy status and organellar network configuration. In this context, a balance between fusion and fission events, mediated by specific proteins that might be involved in the modulation of energy production, occurs controlling mitochondrial configuration [41]. Physiologically, mitochondria undergo continuous fission and fusion (known as mitochondrial dynamics) to generate smaller organelles or rather elongated structures, respectively (Fig. 2). During this process of mitochondrial biogenesis, in which new mitochondria are formed, defective mitochondrial DNA is repaired by redistributing mitochondria to high energy demand sites. In neurons, the fission/fusion proteins, regulated by a mechanism involving large dynamin-related GTPases, maintain mitochondrial integrity and ensure that they are present at critical sites such as the synapse where high concentrations of ATP are required [42]. These proteins include Drp1 and Fis1 that act as fission proteins, and Mitofusin (Mfn) and Opa1 that have the function of fusion proteins. Since mitochondria play a critical role in neurons, impaired mitochondrial dynamics is increasingly considered to be a factor in neurodegenerative disorders such as Parkinson's, Alzheimer's and Huntington's diseases [43].

Dysfunction in mitochondrial dynamics may be due either to genetic mutations in fission- or fusion-related genes or to post-translational changes in the fission or fusion proteins. Hereditary mutations in the mitochondrial fusion GTPases optic atrophy-1 (OPA1) and mitofusin-2 (Mfn2) occur in Charcot-Marie-Tooth Disease (CMT), a peripheral neuropathy characterized by muscle weakness and axonal degradation of sensory and motor neurons. It is also observed in hereditary motor and sensory neuropathy type VI, clinically similar to CMT, with the addition of optic atrophy and visual impairment, as well as in Autosomal Dominant Optic Atrophy. In addition, two proteins, PTEN-induced kinase 1 (PINK1) and Parkin, which are mutant in familial forms of PD have been reported in association with increased mitochondrial fission in Parkinson's disease (PD) models. Furthermore, mutant huntingtin, the disease-causing protein in Huntington's disease (HD), alters mitochondrial morphology and dynamics [43]. Rotenone, a pesticide and inducer of PD symptoms, and amyloid-β (Aβ) peptide, which has been causally linked to Alzheimer's disease (AD), are able to initiate mitochondrial fission [42].

Post-translational modifications due to nitrosative/oxidative stress may be the pathogenic cause of common sporadic cases of neurodegeneration, which seem to stem from intricate interactions of multiple genes and/or their products with environmental factors. According to Nakamura et al., three post-translational modifications – phosphorylation, sumoylation and ubiquitination – appear to regulate mitochondrial fission and fusion proteins [42]. Protein Kinase A (PKA, cAMP-dependent protein kinase) phosphorylates Drp1, which results in a significant decrease in GTPase activity and inhibition of mitochondrial fission. Interestingly, mitosis-promoting factor (MPF, Cdk1/cyclin B) phosphorylates Drp1 at Ser585 in rats during mitosis. Unlike phosphorylation by PKA, Cdk1/cyclin B phosphorylation seems to stimulate mitochondrial fission during mitosis. However, phosphorylation of Drp1 at different amino acids causes opposite effects [44,45]. Drp1 regulation also occurs through sumoylation. SUMO1 (small ubiquitin-related modifier-1) interacts with Drp1 and associates with mitochondria at fission sites before and after fission [45]. Over expression of SUMO1 results in mitochondrial fragmentation and apoptosis. This probably occurs because SUMO1 protects Drp1 from degradation, stabilizing Drp1 and promoting binding to mitochondria. Ubiquitination has also been associated with mitochondrial dynamics [45] since MARCH-V or MARCH5, a mitochondrial outer membrane protein, ubiquitinates Drp1, thus promoting fission. Finally, the functional link between mitochondrial dynamics and apoptosis is suggested by the fact that Bax and Bak, two pro-apoptotic regulators, interact with mitochondrial fission and fusion GTPases. Bax associates with Drp1 and Mfn2 on mitochondria during apoptosis. Furthermore, neurons under nitrosative stress exhibit Bax foci on mitochondria undergoing mitochondrial fission. Inhibition of Drp1 function delays mitochondrial fission and is related to Bax foci formation and neuronal cell death [45].

Another potentially harmful effect of increased ROS levels is chronic mitochondrial fission. In an animal model of stroke, nitrosative stress causes profound mitochondrial fission in neurons prior to the onset of neuronal loss and treatment with antioxidants has been shown to reduce mitochondrial fission [45]. Amyloid-beta peptide, possibly a key mediator of AD pathogenesis, engenders S-nitrosylation and thus hyperactivation of the mitochondrial fission protein Drp1. This activation results in excessive mitochondrial fragmentation, bioenergetic compromise, and synaptic damage in models of AD [42]. Expression of Mfn or dominant-negative Drp1 partially prevented mitochondrial fission and neuronal cell death by nitric oxide, which is known to be largely caspase independent. Another study demonstrated that mitochondrial fission in cerebellar granule neurons was enhanced by oxidative stress and that Mfn2 expression was protective [45]. Whether oxidative stress directly regulates the mitochondrial fission and fusion GTPases is not yet clear. However, since mitochondrial integrity is essential to cellular homeostasis, when the energetic demand is low, excess mitochondria are not needed and can generate excessive ROS [46]. Furthermore, if uncoupled, they can consume ATP, thus their elimination by autophagy is an efficient cytoprotective response. Uncoupled or unstable mitochondria may release ROS and a number of apoptosis-promoting factors, including cytochrome c, AIF, SMAC/DIABLO, thus promoting damage to neighboring mitochondria [47].

Mitochondrial biogenesis is another important event in mitochondrial biology. It is a phenomenon that relies on a space and time coordinated synthesis and the import of approx. 1000 nuclear-encoded proteins, some of which are assembled with mitochondrial-encoded proteins within newly synthesized inner and outer mitochondrial phospholipid membranes. Both replication of mitochondrial DNA and mitochondrial fusion and fission mechanisms must also be synchronized with these processes [48]. Oral acetylcarnitine supplementation increases mitochondrial content together with nuclear transcripts of factors involved in mitochondrial biogenesis (PGC-1α, NRF-1, TFAM). This event is associated with increased levels of mitochondrial transcripts for COX I, ATP6 and ND6, and also 16S rRNA. This is consistent with the notion that peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) positively modulates several genes involved in ROS detoxification and in turn PGC-1α is sensitive to ROS and/or RNS [49]. Acetylcarnitine also by stimulating the vitagene system, especially HO-1, leads to the production of carbon monoxide which, according to the model proposed by Piantadosi [50], can bind to the reduced heme a3 in the cytochrome c oxidase complex, thus promoting a parallel increase in superoxide formation from complex III. Superoxide then induces MnSOD overexpression with formation of hydrogen peroxide and signalization of so-called preconditioning. As signaling molecule H_2O_2 activates protein kinase B (Akt), which deactivates glycogen synthase kinase-3β, allowing the nuclear translocation of Nrf2. Nuclear Nrf2, which binds to antioxidant response elements in the HO-1, MnSOD, and NFR-1 gene promoters, amplifies the initial signal and drives the transcription of TFAM and other genes that have promoter binding sites for NRF-1 [18,37,38,51,52]. These genes play a role in the control of mitochondrial transcription, protein synthesis, mitochondrial protein import, and oxidative phosphorylation [52]. This was confirmed by research demonstrating that acetylcarnitine reversed the age-related decrease in complex III activity and oxidative phosphorylation through complexes III and IV, and increased the amount of

cytochrome b and aa3 hemes in mitochondria isolated from old rats [48]. Of note is the fact that both cytochrome b and aa3 proteins are encoded by the mitochondrial genome, suggesting that acetylcarnitine promotes either mtDNA transcription, the stability of mitochondrial mRNA, or mitochondrial protein synthesis [51].

4. Autophagy pathway

Autophagy is a lysosomal pathway for the degradation and recycling of long-lived proteins and organelles, i.e. a kind of 'self digestion' cell pathway. During autophagy, cytoplasmic constituents are taken into double-membraned autophagosomes and delivered to lysosomes to be degraded. This process generates nucleotides, amino acids and fatty acids, which are recycled to be used for ATP generation and macromolecular synthesis [53] (Fig. 3).

While the unfolding protein response (UPR) regulates the degradation of smaller units of unfolded or misfolded proteins through chaperone transcription, larger aggregates are detoxified via autophagy. Autophagy involves the encapsulation of cargo on a double-membrane vesicle to form the autophagosome. Autophagosomes then fuse with lysosomes to form the autolysosome where the cargo degradation occurs. Hybrid intermediates, known as amphisomes, are also formed when autophagosomes fuse with endosomes or MVEs, before fusing with lysosomes. In eukaryotic cells, the ubiquitin–proteasome and autophagy–lysosome pathways represent the two principal routes of protein clearance [53]. Proteasomes mainly degrade short-lived nuclear and cytosolic proteins. However, substrates must be unfolded in order to pass through the narrow pore of the proteasome barrel, so clearance of aggregated proteins observed in many neurological disorders is excluded [54]. On the contrary, lysosomal-related compartments are able to degrade substrates such as protein complexes and organelles. Recent evidence indicates that protein clearance via autophagy is linked to their propensity to aggregate. Since findings suggest that a number of different diseases may be caused by aggregate-prone proteins gaining function mechanisms, attempts have been made to find therapeutic strategies that reduce the levels of this type of proteins, at either the synthesis or degradation stage [54]. Recent studies suggest that autophagy has a crucial role in protein quality control, and a clear function in balancing homeostasis in the nervous system [55]. Notably, p62 (also termed sequestosome 1) interplays between polyubiquitination and the autophagy-degradative mechanisms, thus shuttling substrates of the proteasome system. Autophagy is less selective than the latter mechanism, but recent findings indicate that it is finely regulated and specific for diverse target organelles, such as mitochondria (mitophagy) endoplasmic reticulum (reticulophagy), peroxisomes (pexophagy), ribosomes (ribophagy), granules (crinophagy), and pathogens (xenophagy). Cytosol, cytoskeleton, nuclei (nucleophagy), and protein aggregates (aggrephagy) can also be removed by autophagy.

Studies have defined several upstream regulators of autophagy [53]. The class III PI3 kinase complex, including Beclin-1/Atg6, is involved in the generation of pre-autophagosome structures. The mammalian target of rapamycin (mTOR), a nutrient-sensing kinase complex that controls cell growth and survival, prevents autophagy during nutrient-rich conditions by inhibiting the Atg1 complex, which is present in the initiation stages of autophagic activity. Upstream activators of mTOR (NF-kB, class I PI3 kinase, Akt and NF-kB) block autophagy, whereas inhibitors of this pathway such as PTEN (phosphatase and tensin homologue) induce autophagy. For this reason, in vivo experiments have shown that rapamycin, a classical mTOR inhibitor, alleviates neurodegeneration in models of Huntington's disease, associated with reduced neuronal loss, improved survival and degradation of toxic protein aggregates [55]. Atg

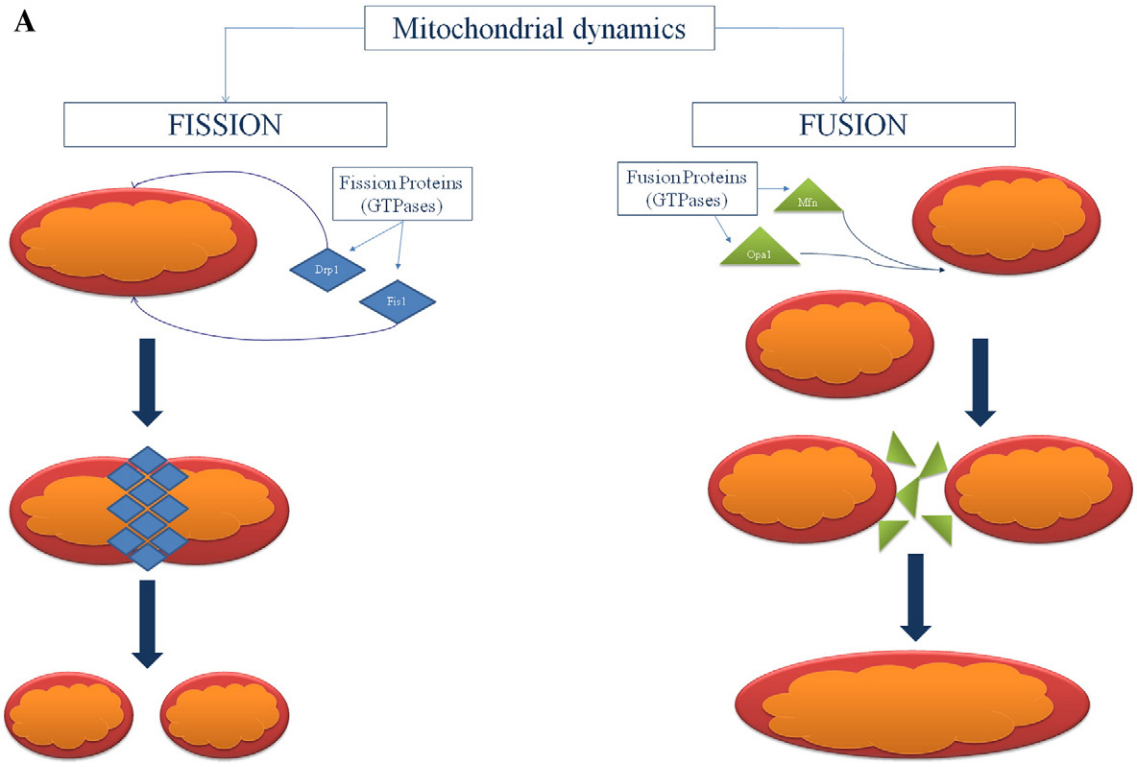

Fig. 2. Mitochondrial dynamics. Role of fissation (Drp1 and Fis1) and fusion (Mfn and Opa1) proteins in the mitochondrial dynamics (A). Post-translational modifications of fission and fusion proteins (B). The phosphorylation of dynamin-related protein (Drp1) by the Protein Kinase A (PKA) inhibits mitochondrial fission decreasing GTPase activity. On the contrary, the phosphorylation of Drp1 by mitosis-promoting factor seems to stimulate mitochondrial fission during mitosis. Moreover, the activity of Drp1 is regulated by the mechanism of sumoylation. The small ubiquitin-related modifier-1 (SUMO1) interacts with Drp1 at fission sites and its overexpression leads to mitochondrial fragmentation and apoptosis. Mitochondrial fission is promoted also by the ubiquitination of Drp1 by the human membrane-associated RING-CH (MARCH)-V. Finally, there is evidence of a functional link between mitochondrial dynamics and apoptosis. In fact, the Bcl-2-associated X protein (Bax) associates with Drp1 and mitofusin (Mfn2) on mitochondria during apoptosis and neurons under nitrosative stress exhibit Bax foci on mitochondria undergoing mitochondrial fission.

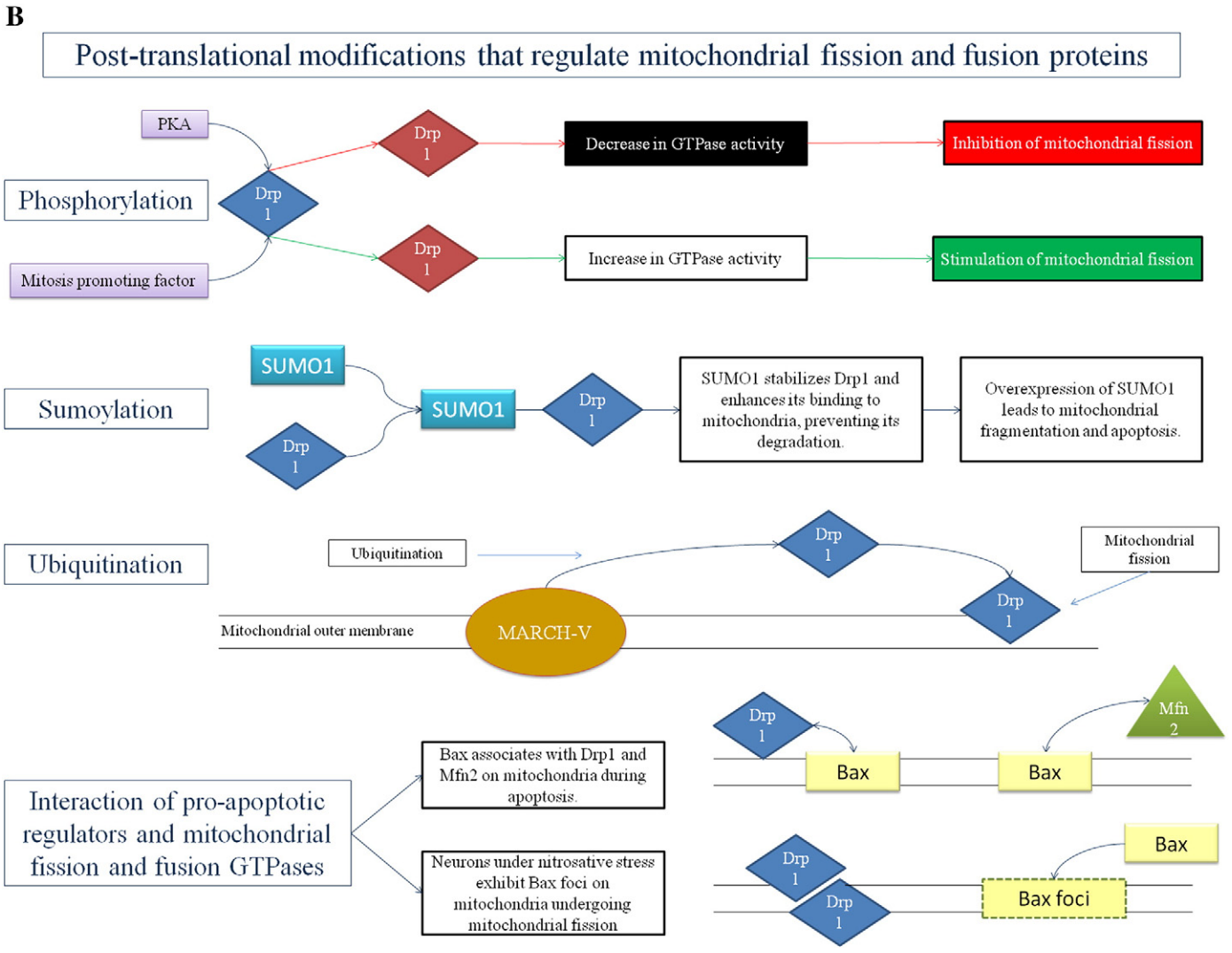

Fig. 2 (*continued*).

proteins regulate sequential steps in the autophagy process, centrally organized around two ubiquitin-like conjugation systems. When Atg12 is activated, it forms a covalent bond with Atg5, which then interacts with other components to form a multimeric complex that translocates to membranes of early autophagosomes. Alternatively, LC3 is cleaved and then conjugated to phosphatidylethanolamine (PE) via Atg7 and Atg3 (another ubiquitin carrier protein (E2)-like protein), generating LC3-II. The unconjugated LC3-I remains in the cytosol while the conjugated LC3-II form targets to the autophagosomal membrane after the formation of the active Atg12–Atg5 complex [56]. Following elongation of the autophagosomal membrane, cytoplasmic proteins and organelles are taken into double-membraned autophagosomes, delivered to lysosomes and degraded [53]. Autophagy is essential for maintaining neuronal homeostasis and plays a role in basal elimination of misfolded proteins in the nervous system. Brain specific ablation of the autophagy-related genes *atg5* and *atg7* results in spontaneous neurodegeneration typical of Alzheimer's and Parkinson's disease with pathological features such as neurological/motor dysfunction, accumulation of poly-ubiquitinated protein aggregates, neuronal loss and premature death [54]. As in UPR, autophagy is believed to play a dual role in cell survival or cell death. Autophagic degradation of cellular materials produces amino acids and fatty acids, which can be recycled for protein synthesis and ATP generation during stressful conditions such as starvation. It can also remove protein aggregates (which can trigger apoptosis) and damaged mitochondria (source of apoptotic proteins and toxic ROS), thus becoming a protective mechanism. On the contrary, prolonged autophagy can lead to cell death through excessive self-digestion or activation of apoptosis. In fact, there is evidence of prosurvival autophagy in response to starvation, growth factor withdrawal, ischemia/reperfusion injury, and various chemotherapeutic drugs [57,58], while autophagic cell death has been reported in response to hypoxia, oxidative stress, radiation, GF withdrawal, lipopolysaccharide, overexpression of smARF, and various chemotherapeutic drugs [57].

5. NFkB signal pathway: cell death vs cell survival

Nuclear factor-κB (NF-κB) is a family of transcription factors that have a crucial role in inflammation, immunity, cell proliferation, development, survival and apoptosis. Consequently, NF-κB dysregulation has been implicated in diverse human pathologies ranging from autoimmune diseases to cancers.

In mammalian cells, the NF-kB/Rel family contains five members: RelA (p65), c-Rel, RelB, NF-kB (p50; p105), and NF-kB2 (p52; p100) [58]. These proteins possess a structurally conserved 300 amino acid sequence called the REL region, which contains the dimerization, nuclear localization, and DNA-binding domains. Three of the family members, RelA, c-Rel, and RelB, have a transactivation domain at the C-terminus. NF-kB1/p105 and NF-kB2/p100 are the inactive precursors of the p50 and p52 proteins, respectively; in an unstimulated

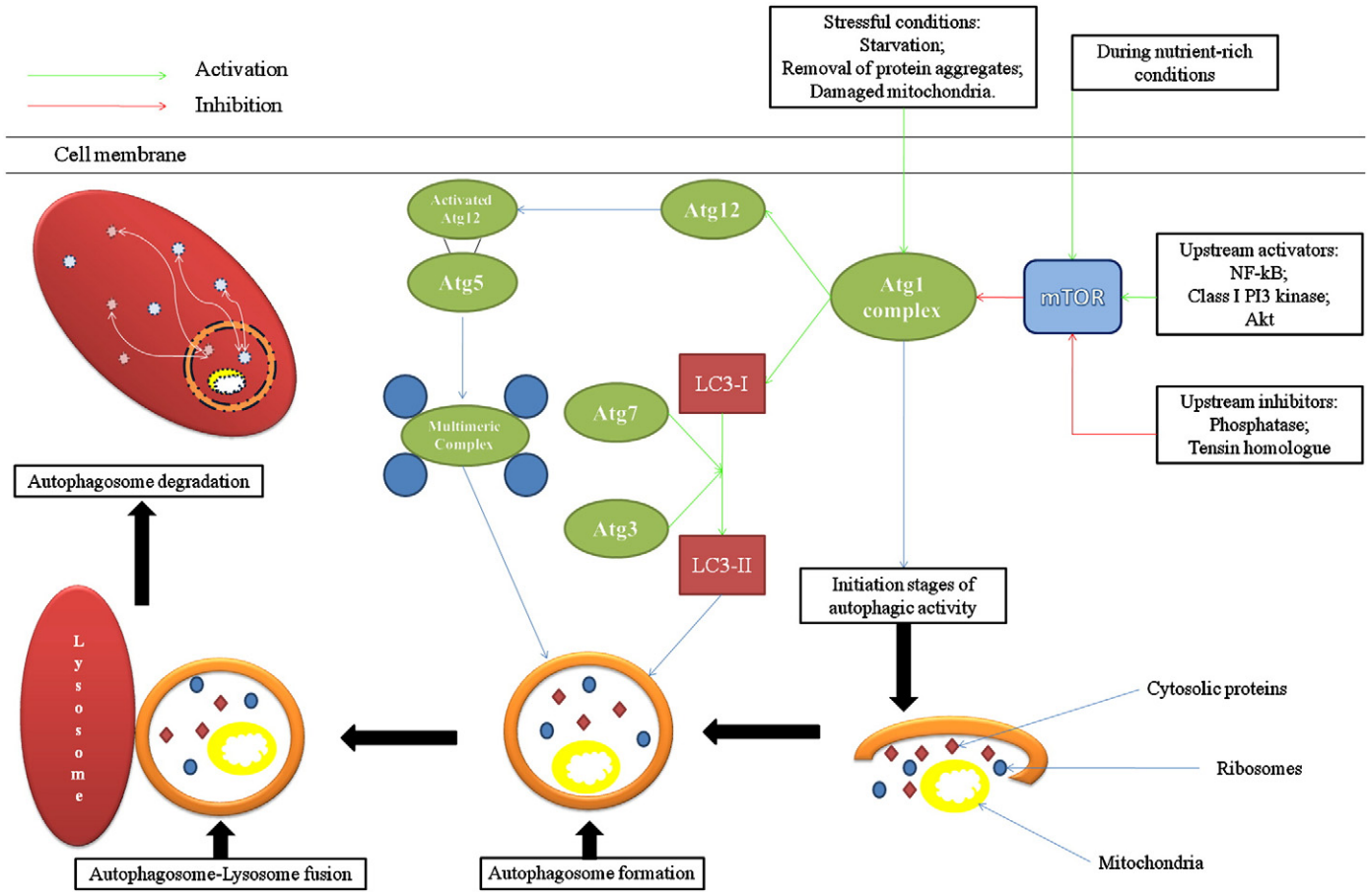

Fig. 3. Autophagy. Autophagy pathway: Role of the Atg proteins in the initiation stages of autophagic activity (Atg1 Complex) and autophagosome formation (Atg12 and LC3) and subsequent autophagosome-lysosome fusion and autophagosome degradation.

state, these proteins are localized to the cytoplasm. Proteolytic processing removes the C terminal inhibitory domains, allowing the resulting proteins to enter the nucleus [59].

NF-κB is induced by a variety of extra-cellular stimuli such as pro-inflammatory cytokines, bacterial lipopolysaccharides, viral RNA and DNA via the activation of membrane and cytosolic receptors [60,61]. It is also induced by low and high doses of ionizing radiation and numerous DNA damaging drugs [62,63]. Under normal conditions, NF-κB is present within the cytoplasm in an inactive state, bound to its inhibitory protein κB-α (IκB-α). This stimulation initiates an intracellular signaling cascade, resulting in the phosphorylation of IκB-α on serine residues 32 and 36 by IκB kinase (IKK). Phosphorylation and subsequent ubiquitination of these residues target IκB-α for degradation by the 26S-proteasome complex. Once liberated from its inhibitory protein, NF-κB translocates into the nucleus, where it orchestrates the transcription of a number of cytokines and chemokines, growth regulatory and survival genes [64,65].

NF-κB has been shown to activate, via transcription, the genes encoding pro-inflammatory cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-lβ and IL-12], cell adhesion molecules [vascular cell adhesion molecule (VCAM)-1 and intercellular cell adhesion molecule (ICAM)-1], inducible nitric oxide synthase (iNOS), cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2) [66,67]. These, together with nitric oxide (NO) derived from iNOS and prostaglandin E_2 (PGE_2) produced by both COX-1 and COX-2, play important roles in the pathogenesis of acute and chronic inflammation [68]. The production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide and hydroxyl radicals, as well as peroxynitrite, also contribute to the tissue injury observed during inflammation [68,69]. ROS and peroxynitrite also cause DNA damage [70,71], which results in the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP), depletion of NAD^+ and ATP and ultimately cell death [70].

It should be noted that pathogenic stimuli like excessive production of toxic reactive oxygen and nitrogen species, e.g., the oxidative stress and nitrosative stresses, and many genotoxic factors activate nuclear factor kappa beta (NF B) signaling pathways which in turn stimulates the activation of aging-related genes [72]. The NF B trigger genes that block the cell death (by apoptosis or necrosis) resulting in aging of the immune system or "immunosenescence", muscle atrophy, and inflammation [73].

Interestingly, the master of inflammation, NF-kB signaling, is also a key player in anti-apoptotic signaling [74,75]. For instance, NF-kB signaling activates the expression of c-FLIP, Bcl-xL, c-IAP1, c-IAP2, and XIAP proteins, all well-known inhibitors of apoptosis (IAPs). A few studies have focused on the effect of aging on the expression of IAP proteins, e.g.-chromosome-linked IAP (XIAP) level has been shown to increase in the brain during aging [76].

Additionally, there is considerable interest in the contribution of NF-κB–mediated chronic inflammation in aging. The age-related "constitutive activation of NF-kB" has been verified in various tissues during aging [77]. In mice and rats, NF-kB activity has consistently been shown to be increased with age and in a variety of tissues, including heart, liver, kidney, and brain [78]. In humans, NF-kB protein concentrations were found to be fourfold higher in elderly human muscles compared with those of young people [79].

Interestingly, the function of the NF-kB complex can be regulated by the acetylation and phosphorylation of the p65 component [80]. The acetylation of p65 transactivator protein especially increases the duration and efficiency of the NF-kB transcriptional response and

hence the abundance of target gene mRNA transcripts [81]. Histone deacetylases, such as HDAC3 and SIRT1, can regulate the acetylation level of p65 protein and reduce the efficiency of NF-kB-dependent signaling [82]. The observations of Adler et al. [83] indicate that this kind of posttranscriptional NF-kB activation occurs during aging.

SIRT1 is the most-widely studied of the Sirtuins and is known to regulate the function of several important transcription factors, such as p53, FoxOs and NF-kB, and is also a mammalian longevity gene [82]. Yeung et al. [82] were the first to show that SIRT1 can interact with RelA/p65 protein in the NF-kB complex and specifically deacetylates lysine 310, which has been earlier demonstrated to potentiate the transactivation capacity of the NF-kB complex [81]. Several studies have indicated that SIRT1 is a potent inhibitor of NF-kB transcription [82]. The signaling link between SIRT1 and NF-kB is especially interesting with respect to aging since according to the articles of Yeung et al. [82] and Adler et al. [83]. It seems that SIRT1 acts to extend the lifespan by inhibiting NF-kB signaling. Accordingly, it is proposed that the signaling cascades mediated via Sirtuins and FoxO represent the life-span extending, anti-aging type of regulation. Effects of sirtuin overexpression on aging, however, have been recently re-examined after standardization of genetic background and the use of appropriate controls both *C. elegans* and *Drosophila* suggesting that although a role for sirtuins in determination of metazoan lifespan cannot be rule out, but doubt on the robustness of the previously reported effects of sirtuins on lifespan in *C. elegans* and *Drosophila* are questioned [84,85].

6. Heat shock proteins and neuroprotection

Heat shock response (HSR), in which a set of heat shock proteins (Hsps) are induced and take a key role in cell repair and protective mechanisms, represents one possible type of cell stress response [86,87]. This response is regulated at the transcriptional, translational and post-translational levels by a family of heat shock transcription factors (HSFs) that are expressed and maintained in an inactive state under non-stress conditions. Of the three functionally different HSFs in humans, HSF type 1 (HSF1) is the principal regulator of the HSR genes. Its task is to mediate signaling of stress-induced stimuli, such as elevated temperatures, as well as those involved in development and many patho-physiological conditions such as cancer, ischemia-reperfusion injury, diabetes, and aging [88,89]. HSF1 is generally found in the cytoplasm as an inert monomer lacking transcriptional activity; both DNA-binding and transcriptional transactivation domains are repressed through intramolecular interactions and constitutive serine phosphorylation [90]. Upon exposure to heat shock and other types of stresses, which result in protein damage, HSF1 is derepressed in a stepwise process that involves oligomerization of HSF1 monomers to a trimeric state, localization to the nucleus, inducible phosphorylation and sumoylation, binding of nuclear-localized trimers to DNA, and transcription of HS genes (reviewed in 91). The main targets for HSF1 are specific promoter elements composed of repeats of the pentameric sequence nGAAn (heat shock elements, HSE) located upstream of HS genes. High rates of transcription are maintained only when HSF1 trimers remain bound to the HSE; when either the stress signal is removed or damaged proteins are no longer generated, the HSR attenuates rapidly, with subsequent conversion of HSF1 back to the monomeric state [92]. Recent studies have also shown that inducible acetylation negatively regulates DNA binding activity [86,93]. Mammalian genomes encode three homologues of HSF (HSF1, HSF2 and HSF4) regulating HSP expression. HSF1 is believed to be the paralog responsible for regulating the heat-induced transcriptional response [86,93], while HSF2 has also been reported to contribute to inducible expression of heat shock genes through interplay with HSF1 [87]. However, regulation of the mammalian HSR is a more complex phenomenon than was previously thought. Microarray and chromatin immunoprecipitation analysis, while confirming that many known heat-inducible genes have HSF1-binding sites in their promoters, have identified another class of genes that recruit HSF1 to their HSE promoter elements but are not induced by heat shock [88]. However, these studies have also proven that, in some cases, HSF1 can also control the expression of genes with non-chaperone function [94], such as superoxide dismutase, multiple-drug resistance genes, lactate dehydrogenase, and the T-cell death associated gene 51 [94]. Neurons appear to be deficient in the heat shock response while retaining the ability to express such HSF proteins [87]. Furthermore, activation of HSF1 does not occur in motor neurons even when microinjected with plasmids encoding an HSF1 expression vector, suggesting that the HSF1 signal transduction pathways are blocked in these cells [95]. Under non-stress conditions, HSF1 is repressed by a complex containing Hsp90 and other proteins. In this inactive state, HSF1 is a monomer that is unable to bind cis-acting heat shock elements (HSE) in the promoters of HSP genes [96]. During protein stress and the consequent generation of misfolded proteins which displace HSF1 from the inhibitory chaperone complex, HSF1 trimerizes, becomes phosphorylated and is translocated to the nucleus where it is acetylated and sumoylated before being able to bind to the heat shock element of Hsp genes [88,94]. Activation of HSF1 by heat shock is a multi-step process, involving multiple inducible phosphorylation, dephosphorylation, acetylation, deacetylation and sumoylation, which together result in the transcription of HSP genes. Extracellular signal input during heat shock involves tyrosine phosphorylation upstream of HSF1, involving the receptor tyrosine kinase HER2 and launching downstream signaling cascades through intracellular kinase Akt [96]. Akt regulates HSF1 at least in part through modulating its association with the phosphoserine binding scaffold protein [96].

The inducible expression of heat shock proteins is triggered not only by thermal stress, but also by environmental redox changes or exposure to electrophiles which cause trimerization and DNA binding of HSF1 [89,92], demonstrating that the cysteine redox state is critical for the activation of this transcription factor. Thus, an intermolecular disulfide bond formation between C36 and C103 within HSF1 induces trimerization and DNA binding, whereas an intramolecular disulfide bond formation (in which C153, C373 and C378 participate) inhibits the activity of the transcription factor [97]. Strategies for preventing damage to brain cells following cerebral ischemia or the progression of neurodegenerative diseases might be found in the manipulation of HSR [92]. Hsps are evolutionarily conserved and exist in all cell compartments [98,99]. Some of the major chaperones (Hsc70, Hsp90, small Hsps) are present at high concentrations in non-stressed cells reaching 1–5% of total cellular protein, indicating an important role for chaperones in cellular homeostasis. Hsps are classified according to their molecular weight [100]. Hsp70, the 70 kDa family of stress protein, has been extensively studied and includes Hsc70 (heat shock cognate, the constitutive form), Hsp72 (the inducible form, also referred to as Hsp72) and GRP-75 (a constitutively expressed glucose-regulated protein found in the endoplasmic reticulum) [101]. Hsp70s, which are involved in co- and post-translational folding and the quality control of misfolded proteins [102], participate in the folding and assembly of newly synthesized proteins into macromolecular complexes; aggregation prevention; dissolution and refolding of aggregated proteins; as well as protein degradation [103–106]. Recent findings demonstrate that the heat shock response decreases in aging cells and weakens when the life of organisms is prolonged beyond the mature adult stage [107]. Cells are no longer able to activate the transcriptional pathways leading to HSP synthesis. A decline in protein quality control in neuronal tissues has been strongly hypothesized, as the etiology of a number of diseases involve aggregation-prone proteins that form inclusion bodies known to be linked to pathology. Hsp70 has been extensively associated with the pathogenesis of misfolding disease [96]. The use of transgenic animals and gene transfer allowing over-expression of the gene encoding for

Hsp70, have demonstrated that overproduction of this protein leads to protection in several different models of nervous system pathology [22]. Overexpression of Hsp70 and/or its co-chaperones suppresses huntingtin aggregation and toxicity in yeast and mammalian cell models of misfolding disease [105]. Increased Hsp70 levels led respectively to reduced aggregation and toxicity of tau and A — two components associated with Alzheimer's disease [101]. Similarly, overexpression of Hsp70 reduces toxicity and accumulation of α-synuclein in high molecular weight and detergent-insoluble deposits [101]. Increased expression of Hsp70 has been linked to a reduction in apoptotic cell death, an increase in the expression of the antiapoptotic protein Bcl-2, a suppression of microglial/monocyte activation, and a reduction in matrix metalloproteinases. Up-regulation of Hsp70 likewise reduced apoptosis and the formation of co-aggregates of the prion disease protein, PrP [107]. In cases of focal cerebral ischemia, Hsp70 mRNA is synthesized in most ischemic cells except in areas blood flow is very low, due to scarce ATP levels. Hsp70 proteins are produced mainly in endothelial cells, in the core of infarcts in the cells that are most resistant to ischemia, in glial cells at the edges of infarcts and in neurons outside the areas of infarction [108–110]. This neuronal expression of Hsp70 outside an infarct might be used to define the ischemic penumbras, which means the zone of protein denaturation in the ischemic areas. Consistently, in in vivo transgenic mice overexpressing Hsp70, compared to wild-type mice in a middle cerebral artery occlusion model of permanent cerebral ischemia, it has been demonstrated that overexpression of Hsp70 reduces the overall lesion size and also limits the tissue damage within the lesion [110]. Numerous findings now point to a correlation between nitrosative stress mechanisms and Hsp induction. In vitro and in vivo experiments have shown that cytokine-induced nitrosative stress is associated with an increase in the synthesis of Hsp70 stress proteins in the brain [111–113]. The molecular mechanisms that control NO-induced activation of the heat-shock signal seem to be associated with cellular oxidant/antioxidant balance, maintained principally by the glutathione status and antioxidant enzymes [61,114–116].

7. Heme oxygenases

Heme oxygenases (HO) are known to be dynamic sensors of cell oxidative stress and modulators of redox homeostasis throughout the phylogenetic spectrum. Located within the endoplasmic reticulum, they act together with NADPH cytochrome P450 reductase to oxidize heme to biliverdin, free ferrous iron and carbon monoxide (CO). Biliverdin reductase further catabolizes biliverdin to the bile pigment, bilirubin [38,107,117,118], a linear tetrapyrrole which has been shown to effectively counteract nitrosative stress due to its interaction with NO and RNS [37,38,51,119]. Bilirubin is then conjugated with glucuronic acid and excreted [120]. In the past two decades, much experimental evidence has demonstrated that bilirubin is an endogenous cytoprotective molecule. In 1987, Stocker and colleagues were the first to report the antioxidant activity of bilirubin when they proposed bilirubin as a chain-breaker because it was able to scavenge peroxyl radicals, transforming into a stable carbon-centered radical [121]. Later, after observing that bilirubin, in the nanomolar range, may protect cortical neurons from the toxicity elicited by ~10,000 times higher levels of hydrogen peroxide, Snyder and colleagues [122], proposed a mechanism based on an amplification cycle whereby bilirubin is oxidized to bilirubin by reactive oxygen species and then recycled by biliverdin reductase back to bilirubin. However, the importance of this mechanism of action remains controversial since only a small fraction of the bile pigments undergo this redox cycle. Other studies have shown that bilirubin acts as an endogenous scavenger for both NO and RNS, which possibly alter the redox status of the cell and generate nitrosative stress [123]. Although bilirubin possesses these important antioxidant properties, if produced in excess (e.g. in haemolytic anemia or sepsis), unconjugated bilirubin becomes neurotoxic on account of multiple mechanisms involving disruption of the cell membrane structure, reduction of mitochondrial transmembrane potential and activation of the apoptotic cascade.

Although HO-1 and HO-2 have the same substrate and cofactor characteristics, the isoforms are encoded by distinct genes, which share only 43% amino acid sequence homology in humans, and differ substantially in molecular weight, electrophoretic mobility, tissue distribution, regulation, and antigenicity [120]. Several stimuli associated with oxidative and/or nitrosative stress, such as, dopamine analogs, H2O2, hyperoxia, UV light, heavy metals, heme, A prostaglandins, NO, peroxynitrite, Th1 cytokines, oxidized lipid products and lipopolysaccharide, as well as certain growth factors can induce HO-1 [120,124–127]. HO-1 gene expression, which is controlled by factors such as pro-oxidant states or inflammation [126,127], is regulated mainly by two upstream enhancers, E1 and E2 [118,119,128]. Both enhancer regions contain multiple stress (or antioxidant) responsive elements (StRE, also called ARE) that also conform to the sequence of the Maf recognition element (MARE) [120,128,129] with a consensus sequence (GCnnnGTA) similar to that of other antioxidant enzymes [51]. Polymorphisms in the lengths of GT repeats [11–40] within the HMOX1 promoter appear to be an important determinant of HO-1 expression and function in humans. Long GT sequences code for relatively unstable (Z-conformational) DNA with attenuated transcriptional activity and diminished baseline and stimulated HO-1 protein expression profiles. Short-GT polymorphisms which may protect against atherosclerosis-linked conditions (e.g. coronary artery disease) are accompanied by significantly higher HO-1 activity, whereas the malignant behavior of various neoplasms is fairly consistently enhanced by the short-GT form [120]. Interest has focused on the role of HO-1 in AD, a neurodegenerative disorder involving a chronic inflammatory response associated with oxidative brain injury and Aβ-associated pathology. Significant increases in HO-1 levels have been reported in AD brains associated with neurofibrillary tangles. An increase in HO-1 mRNA was also found in AD neocortex and cerebral vessels; the HO-1 increase was also found together with senile plaques and glial fibrillary acidic protein-positive astrocytes in AD brains [120]. The dramatic increase in HO-1 in AD could be the result of a direct response to an increase in free heme concentrations, associated with neurodegeneration, during which brain cells try to convert the highly toxic heme into the antioxidants CO and bilirubin [25]. The protective role played by HO-1 and its products in AD has focused attention on the possibility of using natural substances capable of increasing HO-1 levels, as potential drugs for the prevention and treatment of AD. Polyphenolic compounds found in some herbs and spices, e.g. curcumin, appear promising in this context [119,130–133]. Curcumin is the active anti-oxidant principle in *Curcuma longa*, a coloring agent and food additive commonly used in Indian dishes. This polyphenolic substance can not only inhibit lipid peroxidation and effectively intercept and neutralize ROS and RNS [124,132,133], but it can also significantly increase HO-1 in astrocytes and vascular endothelial cells [134,135]. Epidemiological studies suggest that curcumin, which is widely used by the Indian population in nutritional and medicinal compounds, is responsible for a much lower (4.4- fold) prevalence of AD in India compared to the United States [132,136,137]. Subsequently, Lim and colleagues have demonstrated that dietary curcumin given to an AD transgenic APPSw mouse model (Tg2576) for 6 months reduced indices of inflammation and oxidative damage in the brain of these mice [138]. Further studies indicate that by inhibiting NFkB activation, curcumin efficiently prevents cell death [139,140].

In the past few years, significant progress has been made in determining the diverse roles of HO-1 in brain senescence, aging-related human neurodegenerative disorders and other CNS pathologies. Although the acute induction of HO-1 in neural and other tissues has a predominantly cytoprotective outcome, protracted or repeated

up-regulation of the Hmox1 gene in astrocytes, oligodendroglia and possibly neurons may continue cellular dysfunction and demise in many chronic degenerative and neuroinflammatory conditions even when provocative stimuli have ceased [141,142]. In fact increasing evidence indicates that in normal brain aging, AD, PD, and other senescent neurodegenerations, iron sequestration in tissues, intracellular oxidative stress and mitochondrial dysfunction may produce pathogenic momentum that acts downstream from the sustained action of HO-1 within the astrocytic compartment. In AD and mild cognitive impairment, immunoreactive HO-1 protein is overexpressed in the cerebral cortex and hippocampus neurons and astrocytes compared to age-matched, cognitively intact controls and co-localizes to senile plaques, neurofibrillary tangles, and corpora amylacea. In PD, HO-1 is markedly over-expressed in astrocytes of the substantia nigra and Lewy bodies in affected dopaminergic neurons. HMOX1 is also reported to be up-regulated in glial cells located around human cerebral infarcts, hemorrhages and contusions, within multiple sclerosis plaques, and in other human CNS disorders involving oxidant degeneration and inflammatory conditions. Within this context, heme-derived free ferrous iron, CO, and biliverdin/bilirubin are all biologically active substances that can improve or exacerbate neural injury according to the specific disease conditions. For example, in 'stressed' astroglia, HO-1 hyperactivity promotes mitochondrial sequestration of non-transferrin iron and macroautophagy and may thereby contribute to the pathological iron deposition and bioenergetic failure found in most age-related oxidant neurodegenerative disorders. Glial HO-1 expression may also affect cell survival and neuroplasticity through modulation of brain sterol metabolism and proteosomal degradation of neurotoxic protein aggregates [143].

8. Hormesis

In hormesis dose response is characterized by a low dose stimulation and a high dose inhibition. Graphically it can be represented by either an inverted U-shaped dose response or by a J- or U-shaped dose response (Fig. 4). The term hormesis first appeared in 1943 when Southam and Ehrlich reported that low doses of extracts from the Red Cider tree enhanced the proliferation of fungi with the overall shape of the dose response being biphasic. However, it was in fact Hugo Schulz who demonstrated the occurrence of hormesis experimentally in 1888 [144] by observing biphasic dose responses in yeast following exposure to a large number of toxic agents. The work of Schulz encouraged researchers in many different fields to ascertain whether such low dose effects were a general feature of biological systems. In fact, similar types of dose response were observed by numerous researchers assessing chemicals [145] and radiation [146–157]. Despite the bulk of historical literature on hormetic dose responses, this concept was incorporated into routine safety assessment and pharmacological investigations with difficulty, principally because (i) it required more accurate evaluation in the low dose zone, (ii) investigators failed to understand its clinical significance (iii) the quantitative features of the hormetic dose response were not appreciated (iv) the limitations of its implications for commercial applications in agriculture as well as medicine were not well understood (v) during most of the 20th century interest focused on responses at relatively high doses (vi) there was a continuing, yet inappropriate, tendency to associate the concept of hormesis with homeopathy [158–160]. However, after the late 1970s [161,162] biomedical sciences in general showed a growing interest in hormetic-like biphasic dose responses due to the possibility of measuring progressively lower doses of drugs and chemicals, the adoption of cell culture methods that allowed more efficient testing of numerous doses, the need to re-examine the validity of linearity at low dose modeling of cancer risks as well the astute observations of independent investigators and their capacity to generalize their findings across biological systems [162,163].

These research initiatives undertaken in extremely diverse biomedical areas demonstrated that hormetic dose responses are common and highly generalizable, irrespective of biological model, endpoints measured and chemical class and/or physical agent studied [146,154–157,164–166]. This finding caused surprise in mainstream branches of toxicology and pharmacology as hormetic responses were often considered to be paradoxical, not commonly expected and unreliable because of the lack of capacity for replication. During the mid decades of the last century, the hormesis concept was largely absent from the leading toxicological and biomedical textbooks, but this situation has radically changed as hormesis is now included into all leading toxicology textbooks (e.g. [166] encyclopedias [159,167] and other leading monographs. In fact, while the terms hormetic and hormesis appeared only about 160 times during the entire decade of the 1980s, in the Web of Science database, in 2010 alone, these terms were cited nearly 3200 times (Fig. 5).

Observations indicating that these broad ranging dose response relationships also shared the same general quantitative features were of further significance. More specifically, the low dose stimulation which is manifested immediately below the pharmacological and toxicological thresholds is modest in magnitude, being not more than 30-60% greater than the control group response. The width of the hormetic stimulation is usually about 10–20 fold starting immediately from the zero equivalent dose (i.e. estimated threshold). The hormetic dose response may result from either a direct stimulation or through an overcompensation stimulatory response following disruption in homeostasis [145,154]. The quantitative features of hormetic dose responses are similar, regardless of the way in which stimulation occurs as is confirmed by copious data in the literature ranging from plants to humans [160,165], and involving numerous receptor systems [158,168–171]. As a result of these findings, nearly 60 biomedical scientists have suggested that biological stress responses, including those of pre- and post-conditioning, should be

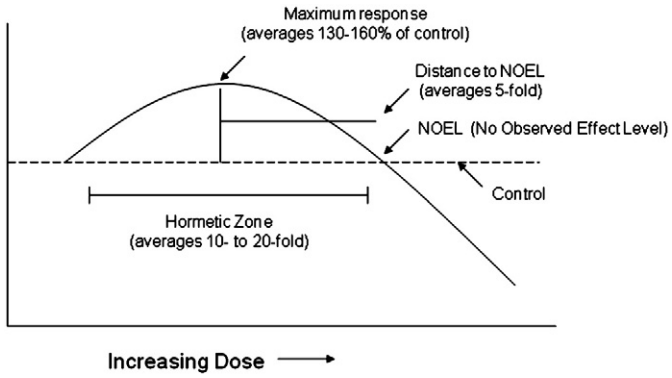

Fig. 4. Dose–response curve depicting the quantitative features of hormesis.

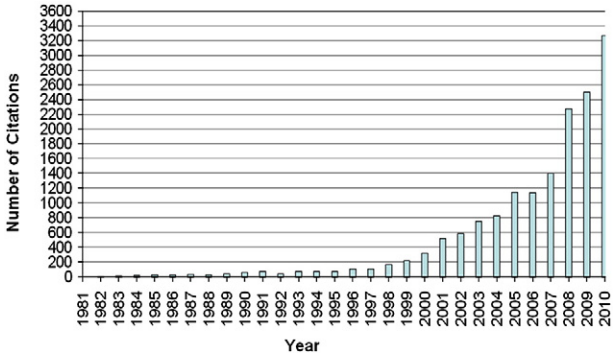

Fig. 5. Citations of Hormesis/Hormetic in Web of Science Database in the period 1981–2010.

integrated within a hormetic context, and a specific terminology should be based within an interdisciplinary framework [172].

The hormetic dose response requires a different set of interpretations for the dose response. At high doses within a toxicological setting, the typical endpoints measured indicate cellular damage. However, as the dose falls below the threshold, the low dose stimulation more likely represents a manifestation of an adaptive response that conforms to a measure of biological performance visible in modest increases in cognition, growth, longevity, bone density and other biomedical endpoints of interest.

Since hormetic findings display broad consistency, this strongly implies that this dose response may demonstrate the plasticity of biological systems. That nearly all biological models respond to imposed stress with the same quantitative features of the dose response is a relatively innovative and significant finding within the biological sciences. These findings suggest that the hormetic dose response would have been broadly selected for and highly conserved. This adaptive response not only enhances survival by providing resistance to environmental stress, but it also helps to regulate the allocation of biological resources in a manner that ensures cell and organismal stability.

These quantitative features of the hormetic dose response have important medical implications since constraint is imposed upon the magnitude of a drug to induce a desired effect. For example, if a drug increased cognitive performance in an elderly patient by approximately 25–30%, the hormetic model suggests that no further increase in this level could be obtained by using a new drug combination. Numerous studies on hormesis and drug interaction have corroborated this concept. Flood [173–176] demonstrated that the hormetic response for memory was limited to the 30–60% increase even when several drugs were used in combination to maximize memory outcome. Similar response magnitude constraint has also been reported for immune stimulation, bacterial growth, increases in hair growth, plant growth, decrease in anxiety, decreases in tumor incidence and many other endpoints [177].

This limitation in the magnitude of the stimulatory response, which is documented by extensive findings, is a critical feature of the hormesis dose response concept and defines what pharmaceutical companies can expect to achieve with drugs designed to enhance performance. However, the limitation in the magnitude of response is also potentially important as regards the possibility of detecting a desirable response. Although this may not be an important issue when using highly inbred animal models or cell cultures where experimental conditions can be highly controlled, measuring a low dose hormetic stimulation in clinical trials can be problematic. Since human response to a drug can vary considerably, responsiveness of the test population may be distributed over a range of responses that includes toxicity, optimal response and a group in which the dose is ineffective. The data from all subjects in such studies would normally be averaged together leading to a marked dilution of an overall positive treatment effect in the optimal response zone subgroup. This probably explains why drugs that were very successfully tested in preclinical studies with highly inbred strains of animals could and often have failed during the clinical trial. It is of note that investigators may have to modify doses according to the sensitivity or susceptibility of the subjects. Calabrese and Baldwin [178] have shown that the hormetic dose response is often expressed in a broad range of subjects regardless of their susceptibility. As expected, those individuals that are very resistant to the drug or chemical treatment would have their hormetic response shifted to the right on the dose response graph, whereas those individuals with above normal susceptibility would have their hormetic response shifted to the left. The hormetic dose response therefore presents considerable challenges for the biomedical community that develops drugs that aim to improve human performance.

The hormetic dose response can also have undesirable effects as may be most readily seen in the case of drugs designed to suppress growth or kill cells or organisms at higher doses. For example, considerable evidence now indicates that low doses of many antitumor drugs can stimulate the proliferation of such cells at lower concentrations [179]. This also occurs with antibiotics, including penicillin [180] and streptomycin [180,181]. The same phenomenon has also been observed with selected cardiac glycosides that have effects on non-target tissues such as the prostate where it can enhance smooth muscle cell proliferation by about 30% with clinically relevant doses [182,183]. However, this 30% increase in prostate smooth muscle was thought likely to impede urination in males. If the possibility of the hormetic response is not taken into consideration, this can lead not only to failure to recognize a desirable drug-induced response, but it can also result in failure to prevent an adverse effect of drug treatment.

Since hormetic dose responses have now been widely reported in biological systems, there is the desire to develop a new subfield of hormetic mimetics that can activate hormetic pathways in order to produce a desirable clinical effect without risks associated with exposure as in the case of certain chemical agents and radiation.

8.1. Hormesis and adaptive stress response

The ability to sense oxidative and proteotoxic insults and to coordinate defensive stress response are basic elements for cellular adaptation and survival [184]. Over the past decade, an increasing interest has emerged on the hormetic mechanisms leading to adaptive responses of cells and organisms to moderate stress [51,185,186]. Mild oxidative or nitrosative stress (characterized by environmental insults, such as metabolic or physical stress, heavy metals, or pathophysiological conditions of oxidant-antioxidant balance perturbation), is able to induce the activation of cellular defense signaling pathways. These are part of a programmed cell life survival setting and serve to balance more severe stress conditions [51]. Typically, stressor elicited molecular responses do not only protect the cell against higher doses of the same agent, but also cross react and function against other specific stressors including oxidative, metabolic, and thermal stress. According to the hormetic principles, low doses of drugs, toxins, and natural substances may elicit a positive response in terms of adaptation to or protection from the stressor, whereas at higher concentration the toxic effect prevails [25,154,187]. The hormetic dose–response can occur through different mechanisms: as a direct stimulatory response; after an initial disruption in homeostasis followed by the modest overcompensation response; or as a response to an “adapting” or “preconditioning” dose that is followed by a more massive challenging dose [51].

Recent findings have elucidated the cellular signaling pathways and molecular mechanisms that mediate hormetic adaptive response which typically involve the synthesis of various stress resistance proteins as the products of “vitagenes”, a group of genes strictly involved in preserving cellular homeostasis during stressful conditions. The vitagene family is composed of the heat shock proteins (Hsp) HO (Heme-oxygenase)-1/Hsp32, Hsp70, Hsp60, by the thioredoxin system [22,188,189] and by sirtuin proteins. In this context, as detailed in the subsequent parafraphs, there is growing evidence that low concentrations of ROS may be beneficial since they are able to induce the expression of antioxidant enzymes or other defense mechanisms, while higher doses may be dramatically deleterious for the cellular redox homeostasis. Moreover, as an interesting emerging point of scientific debate, is the role of exogenous small molecules, including resveratrol, carnosic acid, sulphoraphane, dymethyl fumarate in activating hormetic mechanisms in response to deleterious stress conditions, such as during neurodegeneration and cancer [190,191].

As hormetic paradigmatic cases, they may induce adaptive stress response at subtoxic doses while may be toxic to mammalian cells at high concentrations [18]. From a molecular point of view, they

are able to activate adaptive cellular stress response pathways, including kinases and transcription factors that induce the expression of genes that encode antioxidant enzymes, protein chaperones, phase 2 enzymes, neurotrophic factors and other cytoprotective proteins [22,25,37,190,192,193] (Fig. 6). They resulted also inducers of the Keap1/Nrf2/ARE pathway. Trascription factor Nrf2 has shown the ability, both in in vitro and in in vivo experiments, to activate a series of vitagenes, such as heme-oxigenase-1 (HO-1) [51,119,124,132,134,194–196], coordinately with other cytoprotective proteins [124,195,197–204]. Interestingly, from an hormetic perspective, HO-1 exerts protective role by degrading the intracellular levels of pro-oxidant heme, producing biliverdin, the precursor of bilirubin, and carbon monoxide (CO), molecules with potent antioxidant-anti-nitrosative and cytoprotective features at low concentrations [51,111,118,189,205–207]. However, upregulation of HO-1 (i.e., HO/BVR axis) is not always beneficial for cells: the heme depletion and accumulation of CO and bilirubin it causes are potentially toxic. Therefore, new pharmacological modulators of HO/BVR activity must act in a dose-dependent manner. This would allow dose titration to achieve a desired pharmacologic effect without producing toxicity. Unfortunately, this goal is more complicated than it seems because toxicity has to be defined in terms of each of the main products of heme metabolism. Furthermore, sensitivity to the therapeutic/toxic effects of these products is likely to be tissue- or cell-type specific. The solution may lie in the use of novel drug-delivery systems that allow targeted delivery of low doses of the HO/BVR modulator to selected tissues [208,209]. Consistent to this notion, biliverdin reductase-A (BVR-A) is emerging as a pleiotropic enzyme involved in cellular stress responses. It not only transforms biliverdin-IX alpha into the antioxidant bilirubin-IX alpha but through its serine/threonine/tyrosine kinase activity is able to modulate cell signaling networks. BVR-A's involvement in neurodegenerative disorders such as Alzheimer disease (AD) and amnestic mild cognitive impairment (MCI) was previously described. Statins significantly increased BVR-A protein levels, phosphorylation and activity in parietal cortex, which significantly correlated with β-secretase protein levels in the brain, suggesting a possible role for BVR-A in Aβ formation [210–212]. Thus, a better knowledge of the vitagene defense network and of the leading hormesis mechanisms may support the stimulation of various maintenance and repair pathways through exogenous interventions, such as mild stress or compounds targeting the vitagene network, as a novel approach to delay various alterations in cells, tissues and organs and potentially to prevent and treat many different diseases [25,186].

8.2. Mitostress vs mithormesis and neuroprotection

Recent findings have revolutionized the previous belief that mitochondrial ROS have only a negative impact on cell function and survival since it is now clear that mitochondrial superoxide and hydrogen peroxide play important roles in a range of cellular functions and are able to activate signaling pathways that promote cell survival and disease resistance. Exposure of hippocampal neurons to subtoxic levels of hydrogen peroxide triggers the release of Ca^{2+} from the endoplasmic reticulum by causing the opening of both IP_3 and ryanodine receptor channels [213]. Interestingly, superoxide enhances long-term potentiation of synaptic transmission in hippocampal CA1 neurons by a mechanism requiring activation of ryanodine receptors and extracellular regulated kinases [214]. Mitochondrial ROS may also be involved in recovery from injury as superoxide can stimulate neurite outgrowth by directly activating protein kinase C [215]. Moreover, peroxynitrite can promote the phosphorylation and nitration of regulatory sites within receptor tyrosine kinases thereby activating cell survival signaling pathways including those involving PI3 kinase — Akt and mitogen-activated protein (MAP) kinases [18]. Nitration of proteins involved in synaptic vesicle trafficking can enhance glutamate release, suggesting that superoxide and peroxynitrite have a potential role in regulating neurotransmission [18]. Mitochondrial superoxide production is known to contribute to neuron damage in pathological conditions such as chronic cerebral hypoxia or Alzheimer's disease [18]. Nevertheless, abundant evidence shows that transient neuron exposure to low levels of superoxide which are converted into hydrogen peroxide can protect them against a subsequent exposure to a level of stress that what would otherwise have been lethal. This neuroprotective effect due to a subtoxic increase in cellular oxidative stress is known as “preconditioning” and clearly falls under the paradigm of hormesis [18]. Although studies have revealed the involvement of oxidants in many signaling pathways, cell strategies for conferring pathway specificity to such reactive molecules still requires elucidation. Recent research suggests that cells may spatially restrict oxidant production to allow microdomain-specific signaling [216]. The specific molecular mechanisms by which mitochondrial ROS elicit hormetic responses in neurons are not fully understood, but recent findings suggest that important roles can be attributed to certain transcription factors with regulatory effects. For example, NF-kB is activated in neurons in response to oxidative stress and plays a critical part in the adaptive response that protects neurons against more severe oxidative stress,

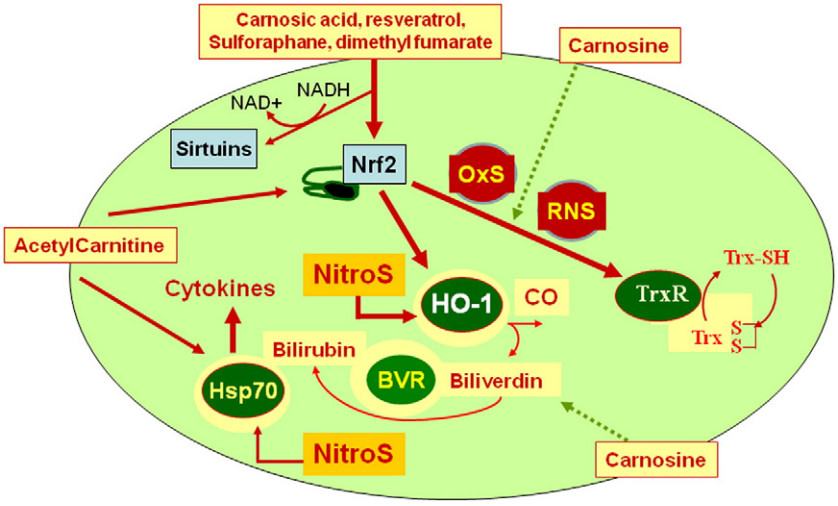

Fig. 6. Vitagenes and the pathway of cellular stress response. Nutritional antioxidants, including carnosic acid, resveratrol, sulforaphane, dimethyl fumarate, acetyl-L-carnitine or carnosine are able to activate vitagenes, such as heme oxygenase, Hsp70, thioredoxin reductase and sirtuins which represent an integrated system for cellular stress tolerance. Phytochemicals and Acetyl-L-carnitine act through the activation of transcription factor Nrf2, which after binding to the ARE (antioxidant responsive element) in the HO-1 gene, up-regulates both HO-1 and TrxR, thus counteracting pro-oxidant conditions. In addition, carnosine has been shown to inhibit the induction of both HO-1 and Hsp70 following strong nitrosative conditions.

by promoting the expression of protective genes including SOD2 and Bcl-2 [217], TNF also up-regulates the expression of Mn-SOD with an NF-kB-mediated mechanism, thereby protecting neurons against excitotoxic, ischemic and oxidative injuries [218]. While mitochondrial H_2O_2 may activate adaptive stress response pathways in neurons, they may also exert a neuroprotective effect by acting on other cell types in the nervous system. For example, microglial activation in response to increased oxidative stress can have either positive or detrimental effects on neurons and neural progenitor cells according to the type and amounts of cytokines and growth factors secreted by the microglia [219]. Oxidative stress can also promote angiogenesis in the brain [18], which is of vital importance in restoring normal neuronal perfusion during the days and weeks after a stroke. This latter effect is an example of "trans-cellular" hormesis mediated by ROS [220].

8.3. The neuroprotective effects of hormetic phytochemicals that activate the KEAP1/NRF2/ARE pathway

An elaborate network of protective mechanisms allows all eukaryotic organisms to defend themselves against the damaging effects of oxidants and electrophiles, the principal agents responsible for the pathogenesis of cancer, atherosclerosis, neurodegeneration, and aging [221]. Endogenously-formed and exogenously-encountered electrophiles and oxidants are counteracted by a group of functionally diverse cytoprotective proteins which are regulated by the Keap1/Nrf2/ARE pathway (Fig. 7). Based on numerous biochemical studies and global gene expression profiling, it is now evident that the family of cytoprotective proteins is characterized by extraordinary diversity and includes: (i) conjugating enzymes (e.g. glutathione *S*-transferases (GSTs) and UDP-glucuronosyltransferases); (ii) export pumps (e.g., multidrug resistance proteins (MRPs)), (iii) antioxidant enzymes (e.g., heme oxygenase 1 (HO-1), NAD(P)H:quinone oxidoredctase 1 (NQO1)); (iv) enzymes that participate in the synthesis and regeneration of glutathione, the principal small molecule antioxidant in the cells (e.g., γ-glutamatecysteine ligase, glutathione reductase); (v) enzymes that promote the synthesis of reducing equivalents, i.e., NADPH (e.g., glucose-6-phosphate dehydrogenase, malic enzyme); (vi) proteins that do not have enzymatic activities, but possess an enormous capacity to protect against metal overload (e.g., ferritin); (vii) enzymes that inhibit inflammation (e.g., leukotriene B_4 dehydrogenase); (viii) proteins that participate in the repair and removal of damaged proteins (e.g., Hsp40, Hsp70, p62 and subunits of the 26S proteosome) and DNA (e.g., O^6-methylguanine-DNA methyltransferase). The basis for grouping them into one "family" is the fact that they share common transcriptional regulation, are inducible, and catalyze reactions that, in almost all cases, collectively result in protection against electrophiles and oxidants, and in enhanced survival [222–225].

The upstream regulatory regions of the genes coding for cytoprotective proteins contain single or multiple copies of the antioxidant response element (ARE, also known as electrophile response element, EpRE) with the consensus sequence 5′-A/CTGAC/GNNNGCA/G-3′) [226–231] (Fig. 8). Nuclear factor erythroid 2-related factor 2 (Nrf2), a basic leucine zipper protein, is the major transcription factor that binds to the ARE [232]. Activation of transcription requires that Nrf2 binds to the ARE as a heterodimer with members of the small Maf family of transcription factors [233]. Under homeostatic conditions the expression of cytoprotective genes is low due to the low intracellular levels of Nrf2 which are maintained by Kelch-like ECH-associated protein 1 (Keap1) [234]. Keap1 binds to the E3 ubiquitin ligase Cullin3-RING box1 (Cul3-Rbx1) and presents Nrf2 for ubiquitination and subsequent proteosomal degradation [235–238]. However, the pathway is activated by various stress conditions (e.g., shear stress, hyperoxia), and also by many small-molecules (inducers). This activation renders the cell well-equipped to meet a subsequent challenge due to the transcriptional upregulation of a large number (more than 100) and extraordinary functional versatility of cytoprotective proteins.

Inducers of the Keap1/Nrf2/ARE pathway belong to ten distinct chemical classes: (i) oxidizable diphenols, phenylenediamines, and

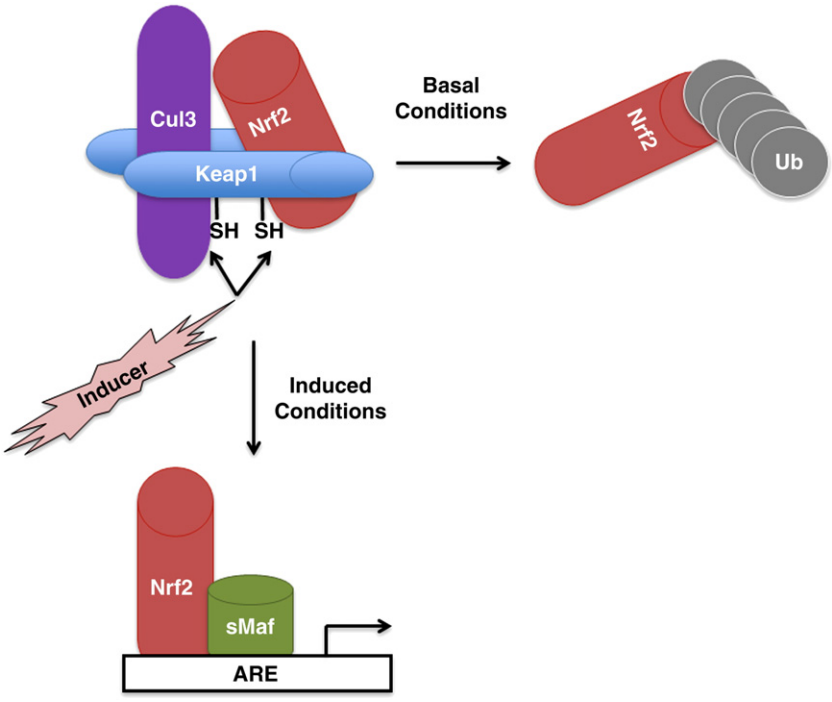

Fig. 7. The Keap1/Nrf2/ARE pathway. Under basal conditions, Keap1 binds and targets transcription factor Nrf2 for ubiquitination and proteasomal degradation via association with the Cullin 3 (Cul3)-based E3 ubiquitin ligase. Under induced conditions, small-molecule inducers chemically modify specific reactive cysteine residues (−SH) of Keap1 which consequently loses its substrate adaptor function. This leads to increased stabilization of Nrf2, its nuclear translocation, binding to the ARE (as a heterodimer with small Maf), and ultimately transcriptional activation of the expression of cytoprotective genes.

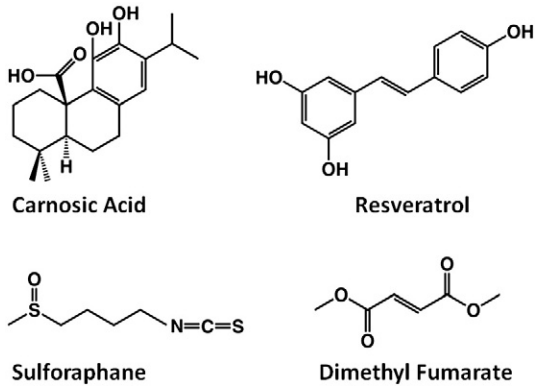

Fig. 8. Structures of some phytochemical inducers of the Keap1/Nrf2/ARE pathway.

quinones; (ii) Michael acceptors (olefins or acetylenes conjugated to electron-withdrawing groups); (iii) isothiocyanates; (iv) thiocarbamates; (v) trivalent arsenicals; (vi) dithiolethiones; (vii) hydroperoxides; (viii) vicinal dimercaptans; (ix) heavy metals; and (x) polyenes. They all have a common property: chemical reactivity with sulfhydryl groups by oxido-reduction, alkylation, or disulfide interchange [239–243]. It is now widely accepted that exogenous and endogenous inducers chemically modify specific reactive cysteine residues of Keap1, which in addition to serving as an adaptor protein for the ubiquitination and degradation of Nrf2, also functions as the intracellular sensor for inducers [224,244]. The cysteine modification(s) within Keap1 result in conformational changes that abrogate its substrate-adaptor function, ultimately resulting in Nrf2 stabilization, binding to the ARE and recruitment of the basal transcriptional machinery to activate transcription of cytoprotective genes (reviewed in 197,199,230,231,245,246).

Many inducers of the Keap1/Nrf2/ARE pathway are natural products. Plants have evolved a versatile array of molecules (phytochemicals) with potent activities (i.e., ultraviolet radiation-filtering, antimicrobial, antifungal, antioxidant) which allow their successful adaptation and survival in an environment full of challenges, such as solar radiation, toxins, pathogens and infectious agents. Some of these phytochemicals (e.g., the phytoalexins) are synthesized de novo in response to pathogen attack [247]; others (e.g., the isothiocyanates) exist as metabolic precursors which are "activated" by enzymes that normally are expressed in highly specialized cells and can only be in direct contact with their substrates following plant injury [248]; yet a third category (e.g., malondialdehyde) has the capacity to potently induce transcriptional upregulation of the plant's own defense mechanisms [249]. The now compelling evidence for biphasic dose response effects of environmental "toxins" in biological systems suggests the possibility that phytochemicals from edible plants might exert biphasic dose responses and could contribute to the well-recognized health benefits from plant-rich diets. There are currently multiple lines of evidence on the protective effects of sulforaphane, curcumin, and resveratrol, all present in edible plants. Although the antioxidant activity of certain phytochemicals might be partly responsible for their health benefits, most people do not consume fruits and vegetables at quantities that are sufficient to achieve the relatively high concentrations of the antioxidants that are required to scavenge free radicals directly. Furthermore, the clinical trials have not demonstrated benefits of dietary supplementation with antioxidants such as vitamin E and vitamin C. Emerging evidence suggests that activation of the Keap1/Nrf2/ARE pathway, rather than direct free radical scavenging activity, may better account for the health benefits of phytochemicals. Indeed, many phytochemicals have been shown to activate adaptive cellular stress response pathways that induce the expression of networks of genes encoding antioxidant enzymes, protein chaperones, neurotrophic factors and other cytoprotective proteins. In contrast to direct antioxidants (such as vitamin C) which have short half-lives, activators of the Keap1/Nrf2/ARE pathway have long-lasting effects because their action is based on induction of transcription-mediated signaling [225,250]. Here, we give examples of some of the most studied phytochemicals that activate the Keap1/Nrf2/ARE pathway, focusing on their neuroprotective activities.

8.3.1. Carnosic acid

Compounds with hydroquinone and catechol moieties that are abundant in plants, activate the Keap1/Nrf2/ARE pathway, and have been shown to be neuroprotective [251]. In order to activate the Keap1/Nrf2/ARE pathway, hydroquinones and catechols have to be oxidized to their corresponding electrophilic quinones [252–254]. As such they can be viewed as "pro-drugs" that are converted to the ultimate inducers "via the pathological activity that they are intended to combat" [255]. One catechol-containing phytochemical is carnosic acid (Fig. 8), a compound found in rosemary (*Rosmarinus officinalis*) which accounts for 5% of the dry weight of the leaves of this plant [256]. A recent investigation in rats has demonstrated good bioavailability of carnosic acid following oral administration [257]. Treatment of cells with carnosic acid was reported to cause chemical modification of cysteine residues of Keap1 and induce Nrf2-dependent genes, resulting in protection of neurons against oxidative stress and excitotoxicity [258]. The same study showed that carnosic acid crosses the blood–brain barrier, increases the level of reduced glutathione in the brain of C57BL/6 mice, and protects against middle cerebral artery ischemia/reperfusion. A subsequent study in murine hippocampal HT22 cells confirmed the role of increased glutathione levels in the neuroprotective mechanisms of carnosic acid [259]. This catechol was also shown to induce expression of the neurotrophin nerve growth factor (NGF) promoting neurite outgrowth and differentiation [260]. Most recently, it was reported that enhanced expression of nerve growth factor by carnosic acid also occurs in normal human astrocytes, and that this induction is dependent on activation of Nrf2 [261]. Increased brain neurotrophin levels were also observed following intranasal administration of carnosic acid to Sprague–Dawley rats [262].

8.3.2. Sulforaphane

The isothiocyanate sulforaphane (1-isothiocyanato-(4*R*)-methylsulfinyl)butane) (Fig. 7) is one of the most potent naturally occurring inducers of the Keap1/Nrf2/ARE pathway. Sulforaphane was isolated from extracts of the commonly consumed cruciferous vegetable broccoli (*Brassica oleracea*) based on its ability to induce the classical Nrf2-target gene NQO1 [263]. In the nearly 20 years subsequent to its discovery, the protective effects of sulforaphane have been demonstrated in various cell culture systems and animal models. Thus, sulforaphane treatment caused Nrf2 activation and upregulated Nrf2-dependent gene expression in astrocytes [264–267]. This activation resulted in protection against the damaging effects of transient exposure to O_2 and glucose deprivation [266], as well as of the toxicities of hydrogen peroxide and glutamate, and only in cell cultures isolated from wild-type, but not Nrf2-knockout animals [265]. Reintroduction of Nrf2 restored both the upregulation of Nrf2-dependent genes and the neuroprotection, implying activation of Nrf2 as the main mechanism of protection [265]. Importantly, the neuroprotective effects of this isothiocyanate are long-lasting: following sulforaphane exposure of astrocytes for 4 h the levels of NQO1 and HO-1 mRNA remained elevated for 24 h, and the protein levels for at least 48 h; repeated exposures resulted in even further accumulation of NQO1 and sustained protection against oxidative stress [267]. In dorsal root ganglion neurons, sulforaphane treatment caused Nrf2 nuclear translocation and upregulation of the activities of GST and NQO1 [268]. In rat organotypic nigrostriatal cocultures, sulforaphane protected against the toxicity of 6-hydroxydopamine [269]. Similarly,

neuroprotective effects by sub-micromolar concentrations of sulforaphane were observed when primary murine cortical neurons were treated with the endogenous neurotoxin S-S-cysteinyl dopamine; protection was dependent on induction of Nrf2-dependent genes, and also, on activation of ERK1/2 and PI3K/Akt signaling [270]. Cultured embryonic hippocampal neurons were also protected by sulforaphane against the toxicities of hemin or of exposure to oxygen in combination with glucose deprivation [271]. In motor neurons grown in organotypic cultures of rat spinal cord, sulforaphane reduced extracellular accumulation of glutamate and protected against glutamate-mediated excitotoxicity caused by threohydroxyaspartate [272]. Kainate-induced cell death was reduced upon treatment with sulforaphane in hippocampal slices of wild-type, but not Nrf2-knockout mice [273]. When mitochondria were isolated from brain of Fischer 344 rats that had been treated with sulforaphane (10 mg/kg, *i.p.*, 40 h prior to isolation of mitochondria), they were found to be more resistant to oxidative stress-induced Ca^{2+}-dependent permeability transition pore opening than brain mitochondria from control animals [274].

Administration of sulforaphane (5 mg/kg, *i.p.*) to Long–Evans rats, increased the levels of HO-1 mRNA and protein in brain; increases were detected in both neurons and astrocytes [194]. In a model of focal cerebral ischemia, when sulforaphane was administered 15 min after the onset of ischemia, the infarct volume (evaluated 3 days later) was reduced by ~50% [194]. In a model of traumatic brain injury in Sprague–Dawley rats, sulforaphane administered 6 h post-injury attenuated the loss of the water channel aquaporin-4 (AQP4) in the injury core and increased the levels of AQP4 in the penumbra region [275]. In a subsequent study, a similar treatment with sulforaphane reduced the blood–brain barrier permeability and the loss of endothelial cell markers and tight junction proteins, and preserved the blood–brain barrier function [195]. The protective effects of sulforaphane were also evident in C57BL/6 wild-type mice, but not in Nrf2-knockout animals, implicating activation of Nrf2 as the underlying mechanism of protection.

In a model of intracerebral hemorrhage in Sprague–Dawley rats, sulforaphane induced Nrf2-dependent genes and reduced markers of oxidative damage in the perihematoma area [202]. Three days following intracerebral hemorrhage, in the sulforaphane-treated animals, the perihematoma neutrophil count and the total hemorrhage-affected striatum neutrophil count were reduced compared to the same parameters in control animals. Importantly, 10 days after the hemorrhage, the neurologic deficits were substantially reduced in the sulforaphane-treated animals. In close agreement with the traumatic injury model, Nrf2-knockout animals had exacerbated neurologic deficits than wild-type mice, and the protective effects of sulforaphane were lost. Interestingly, sulforaphane was also shown to increase the levels of haptoglobin in oligodendroglia correlating with reduction in brain damage following intracerebral hemorrhage [276]. Haptoglobin which is synthesized in the brain is important for protecting neurons and oligodendrocytes against damage caused by intracerebral hemorrhage as it binds and neutralizes hemoglobin that could otherwise cause cytotoxicity. Sulforaphane treatment (5 mg/kg, *i.p.*, administered at 0.5, 12, and 36 h following blood injection) was recently reported to reduce brain edema, cortical apoptosis, blood brain barrier impairment, and motor deficits in a model of early brain injury after subarachnoid hemorrhage in Wistar rats [277].

In another study of traumatic brain injury, sulforaphane treatment caused activation of Nrf2 and upregulation of Nrf2-dependent genes, whereas there was reduction in oxidative damage, neuronal death, contusion volume, and neurological deficits [278]. Compared to wild-type, Nrf2-knockout mice showed greater severity of damage and were not protected by the sulforaphane treatment [279]. In addition to its immediate beneficial effects, sulforaphane treatment was also shown to improve long-term cognitive function following traumatic brain injury; however, this improvement only occurred when sulforaphane was administered 1 h, but not 6 h, post-injury [279]. In a rat model of neonatal hypoxic-ischemic injury evoked by carotid artery ligation and hypoxia, sulforaphane administration 30 min prior to injury activated Nrf2 and reduced oxidative stress markers and the infarct size in the brain [280].

Notably, sulforaphane, and inducers of the Keap1/Nrf2/ARE pathway in general, also have anti-inflammatory properties. Thus, in primary co-cultures of rat microglial and astroglial cells sulforaphane induces Nrf2-dependent genes and attenuates LPS-stimulated production of TNFα, IL-1β, IL-6 and NO [281]. In C57BL/6 mice that received endotoxin injection, sulforaphane treatment decreased microglial activation and the upregulation of inflammatory markers (iNOS, IL-6, and TNF-α) [282]. Compared to wild-type, Nrf2-knockout mice were hypersensitive to LPS-induced neuroinflammation, strongly suggesting that even the basal expression of Nrf2-dependent genes provides protection. In the methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson's disease, sulforaphane protected nigral dopaminergic neurons against cell death, reduced astrogliosis, microgliosis, and release of pro-inflammatory mediators in basal ganglia; in contrast to wild-type mice, no protection by sulforaphane was observed in Nrf2-knockout animals [283–285].

Similarly to the damaging effects of microgliosis to the brain, spinal cord microglia activation following peripheral nerve injury also causes tissue damage. Intrathecal administration of sulforaphane was shown to activate Nrf2 in the spinal cord, markedly reduce oxidative stress and pro-inflammatory cytokine expression caused by spinal nerve transection, and to inhibit the development of neuropathic pain [286]. Very recently, two studies reported the neuroprotective effects of post-injury treatment with sulforaphane in spinal cord injury models in Fischer rats and ICR mice; again, it is clear that both induction of Nrf2-dependent genes and anti-inflammatory activity play critical roles in the protective mechanism(s) [287,288].

Sulforaphane, through the Nrf2-dependent upregulation of cytoprotective proteins, also has the potential to accelerate the metabolism and elimination of neurotoxic chemicals. Thus, sulforaphane treatment before exposure to methylmercury decreased mercury accumulation in the brain and liver of wild-type, but not Nrf2-knockout mice most likely by facilitating the metabolism and excretion of methylmercury [289]. Indeed, studies in primary hepatocytes showed that exposure to sulforaphane led to activation of Nrf2, causing upregulation of γ-glutamatecysteine ligase (and consequently increased levels of GSH), GSTA1, and MRP2, thus reducing mercury accumulation and cytotoxicity [289].

8.3.3. Dimethyl fumarate

Fumaric acid is found in shepherd's purse (*Capsella bursa-pastoris*), a cruciferous weed which is used as a traditional herbal medicine. Before the discovery of Nrf2, it had been shown that inclusion of the methyl ester of fumaric acid, dimethyl fumarate (Fig. 8), in the diet of CD-1 mice and Sprague–Dawley rats elevated the activities of GSTs and NQO1 in many organs [204,290]. In primary co-cultures of rat microglial and astroglial cells, dimethyl fumarate induced Nrf2-dependent genes, and lowered the LPS-induced formation of TNF-α, IL-1β, IL-6 and NO [281]. In a model of chronic experimental autoimmune encephalomyelitis in C57BL/6 mice, dimethyl fumarate treatment increased blood levels of IL-10 and lowered macrophage infiltration in the spinal cord [291]. Orally administered dimethyl fumarate to mice increased Nrf2 positivity in neuronal subpopulations in the striatum and the motor cortex in models of Huntington's disease; Nrf2 activation correlated with preservation of morphologically intact neurons in these areas [292]. In a model of multiple sclerosis, dimethyl fumarate upregulated NQO1 activity in liver and cerebellum and had protective effects on oligodendrocytes, myelin, axons and neurons [293]. In a model of experimental autoimmune encephalomyelitis, dimethyl fumarate increased Nrf2 levels in oligodendrocytes, astrocytes, and in neurons of the

motor cortex and the brainstem [293]. In comparison to wild-type, Nrf2-knockout mice had exacerbated pathology, and the beneficial effects of dimethyl fumarate were lost [293]. Because fumaric acid salts and esters are already used as therapeutic agents [294–296], human studies have been recently conducted in patients with relapsing-remitting multiple sclerosis. In a pilot study, oral dimethyl fumarate reduced the formation of new inflammatory lesions [297]. These encouraging results were further confirmed and extended in a multicentre, randomized, double-blind, placebo-controlled, dose-escalation, Phase IIb clinical trial [298]. Currently, dimethyl fumarate is in a Phase III clinical trial [299].

8.3.4. Resveratrol

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) (Fig. 8) was identified as the active principle of an extract derived from a nonedible Peruvian legume (*Cassia quinquangulata* Rich.) in an activity-guided fractionation based on its ability to inhibit cyclooxygenase; in the same study, it was also found to induce the classical Nrf2-dependent enzyme NQO1 in Hepa1c1c7 cells, and, remarkably, to be able to inhibit the process of carcinogenesis at all of its stages [300]. Resveratrol is present in more than 70 plant species. Its quantity is especially high in grapes (50–100 μg of resveratrol per gram of fresh grape skin) and red wine [300,301]. There are currently more than 4000 publications on resveratrol, demonstrating the phenomenal amount of scientific interest fueled by the extraordinary broad spectrum of biological activities that have been attributed to this phytochemical [302].

One very widely-used experimental model demonstrating the neuroprotective effects of resveratrol is the rat pheochromocytoma cell line PC12. Thus, exposure of PC12 cells to resveratrol has been shown to cause activation of Nrf2 and upregulation of HO-1 [273], and to protect against the toxicities of hydrogen peroxide [196,303], β-amyloid [303,304], ethanol [305] oxidized lipoproteins [306,307], 4-hydroxy-2-nonenal [308], oxygen-glucose deprivation [309], and the combination of Fe^{2+} and *t*-butyl hydroperoxide [310]. Furthermore, in PC12 cells that express α-synuclein under the control of an inducible promoter, resveratrol activated the AMPK/SIRT1 pathway, induced autophagy and enhanced the degradation of α-synuclein [311]. Additionally, resveratrol was reported to inhibit the polymerization of β-amyloid in vitro [312]. In cultured dorsal root ganglion neurons, resveratrol treatment caused Nrf2 nuclear translocation and upregulation of the activities of GST and NQO1 [268]. Resveratrol increased glutamate uptake and intracellular glutathione levels, and protected against the toxicity of hydrogen peroxide in C6 astroglial cells, primary astrocytes and hippocampal slices [313–315]. In human neuroblastoma SH-SY5Y cells, resveratrol induced autophagy and protected against rotenone-induced apoptosis [311].

In a *Drosophila* model of Huntington's disease, resveratrol activated ERK [316] and suppressed neurodegeneration [317]. Neuroprotection by resveratrol has also been demonstrated in in vivo models of Alzheimer's disease and Parkinson's disease, with activation of SIRT1 being implicated as the protective mechanism (reviewed in 318). In a rat model of spinal cord injury, resveratrol improved functional recovery, reversed the spinal cord injury-induced decrease in activity of superoxide dismutase, and reduced the levels of markers of oxidative stress (malondialdehyde), inflammation (TNF-α, IL-1β, and myeloperoxidase), and apoptosis (caspase-3) [319]. In several other models of spinal cord injury, resveratrol protected against oxidative stress (as evidenced by lower malondialdehyde, nitric oxide, xanthine oxidase, and higher glutathione levels), reduced tissue injury, and improved neurological recovery [320–323]. Reduction in infarct volume and neuroprotective effects of resveratrol were also seen in rats subjected to focal cerebral ischemia injury [324,325]. Another study of cerebral ischemia injury reported that resveratrol treatment improved neurological scores and reduced infarct volume and brain water content [326]. The levels of malondialdehyde were lower in the resveratrol-treated animas, whereas the activity of superoxide dismutase was restored. These effects were accompanied by Nrf2 activation, upregulation of HO-1, and downregulation of caspase-3. A recent investigation using a rat model of fetal alcohol spectrum disorders also reported the Nrf2-dependent protective effects of resveratrol: the authors found that ethanol increased the levels of oxidative stress and inhibited the activation of Nrf2 in the cerebellum; administration of resveratrol prior to ethanol exposure restored the levels and the DNA-binding activity of Nrf2 and the expression of its downstream cytoprotective proteins NQO1 and SOD, increased the levels of reduced glutathione, reduced oxidative stress, and enhanced survival of cerebellar granule neurons [327].

It is becoming increasingly apparent that the neuroprotective activities that have been attributed to the phytochemicals discussed in this section in a great number of experimental systems are due to the fact that they are not *single-target* agents, but quite the opposite: all of these molecules have the ability to affect *numerous* cellular components and processes. It is also clear that many of these diverse protective effects can be attributed to the capacity to these molecules to mobilize endogenous defense mechanisms by providing two major triggers: (i) activation of transcription factor Nrf2-mediated gene expression, and (ii) suppression of pro-inflammatory responses. It is then the ability of the cell to mount and orchestrate its own versatile protective responses that determines its successful adaptation and survival.

8.4. A focus on the hormetic role of resveratrol

Due to its remarkably broad range of effects, including mostly antioxidant and anti-inflammatory activities, immunomodulation, chemoprevention, cardiovascular protection and longevity, resveratrol has attracted numerous researchers investigating the hormetic mechanisms underlining its biological responses in cells and organisms [168]. The emerging body of evidence indicates that the wide range of resveratrol induced end-points displayed a biphasic dose response with quantitative features consistent with the hormetic relationship.

Interestingly, many in vitro studies demonstrated the resveratrol ability to hormetically induce and inhibit cell proliferation in numerous human cancer cell lines, at low and high concentrations, respectively. Several cell lines were affected, such as breast [328–335], prostate [336–339], leukemia [340,341], colon [342,343], uterus [344], and lung [345] tumor cell lines. These results suggest that resveratrol may prevent or enhance tumor development, according to the extent of the dose applied. It is a crucial point of discussion in consideration of both the relevance given to resveratrol potential chemopreventive effects, to the implications that resveratrol dietary amount may have for human health and the potential therapeutic dose management [168,334].

Similarly a consistent hormetic biphasic dose–response relationship was detected by Gu et al. [346] for endothelial human cell proliferation, and also for cell migration, as well as cell adherence and eNOS expression/concentration in injured arteries. These in vitro findings were supported also in subsequent in vivo studies with a rat model. Such studies demonstrated that a low dose of resveratrol enhanced the mobilization of endothelial cells, facilitated re-endothelialization, reduced the occurrence of neointimal formation and up-regulated the expression of eNOS following an induced balloon injury. These findings were not only supported in subsequent research of Xia et al. [347] which corroborated the hormetic-like response of resveratrol on endothelial progenitor cell proliferation and cell migration, but further extended other observations by linking these responses to telomerase activity via AKT-dependent mechanisms. As in the case of the endothelial cell parameters measured, the resveratrol-induced alterations in telomerase activity were also indicative of hormetic responses.

Szende et al. [342] demonstrated that resveratrol biphasically affected proliferation with a low-concentration stimulation and a high-concentration inhibition in human endothelial cells. Using

bovine aortic endothelial cells (BAECs), In et al. [348] showed that the resveratrol biphasically affected endothelial cell migration. While the authors acknowledged the capacity of resveratrol to induce a biphasic concentration response, they emphasized that their data revealed that high concentrations of resveratrol might have utility as a potent anti-angiogenesis drug. However, at lower concentrations, the response could switch to angiogenic effects.

Resveratrol has anti-inflammatory properties such as shown with the inhibition of COX-1 [300] and COX-2 [349], down-regulation of prostaglandin biosynthesis and suppression of carrageenan-induced paw edema [300,350]. Since these collective findings suggested a possible effect on immune response, Falchetti et al. [351] explored, in detail, the effects of resveratrol on multiple immune functions of human T-cells in vitro. These included the development of cytokine-producing CD4+ and CD8+ T cells by stimulating peripheral blood mononuclear cells (PBMC) with anti-CD3/antiCD28, specific antigen-induced generation of cytotoxic T lymphocytes and natural killer (NK) activity of peripheral blood mononuclear cells. These authors reported that there was a hormetic-like biphasic dose response for each endpoint assessed. According to Falchetti et al. [351] these findings suggested a regulatory effect of resveratrol on the immune response. Gao et al. [352,353] revealed that resveratrol inhibited the Con A induced proliferation of the spleen cells but only at high concentrations. At lower concentrations, the proliferative response was significantly increased. These findings generally supported the conclusion that resveratrol has the capacity to suppress or upregulate immune response depending on the concentration.

Interestingly, a 2005 study by Juan et al. [354] provided such a mechanistic insight into how resveratrol affects a hormetic response in human aortic smooth muscle cells (HASMCs). Resveratrol induced HO-1 expression in a manner that conformed to the hormetic dose response, markedly enhancing HO-1 expression at low concentrations whereas at higher concentrations, this response was diminished. Resveratrol induction of the HO-1 gene was mediated via the NF-kB pathway. In fact, two separate NF-kB inhibitors abolished the capacity of resveratrol to induce HO-1 expression and the activity of the HO-1 promoter. Low concentrations of resveratrol enhanced NF-kB-binding activity based upon experiments assessing electrophoretic mobility shifts. At these low concentrations, the resveratrol trans-activated the NF-kB and this activation process enhanced the transmigration of NF-kB into the nucleus, which then led to the modulation of HO-1 gene expression.

It was demonstrated also that resveratrol had chemopreventive effects with respect to several conditions such as cardiovascular disease [355], Alzheimer's disease [304], osteoporosis [356] and gastric ulcers [357]. From a hormetic perspective, in these conditions, low doses of resveratrol were shown to confer protection whereas higher doses showed adverse health effects. However, in relation to digestive disorders, there are contradictory results that identified resveratrol as a beneficial agent at different doses, without detecting any biphasic dose responses [358].

Various parasitic diseases were also hormetically influenced by resveratrol. Animal models showed that elevated doses protected against Leishmaniasis and Trichinella, while low concentrations consistently enhanced their proliferation [359,360].

Globally viewing, low concentrations of resveratrol can be potentially beneficial or harmful, depending on the endpoint investigated. In this regard, low doses of resveratrol would have the capacity to increase the risk of tumor development of a number of organs and in contrast to be significantly cardio-protective. This type of conflict between beneficial or harmful effects is not uncommon and it is maybe related to the different biological system, tissue and chemical agent investigated.

Resveratrol is probably the most studied molecule of a series of phytochemicals, including sulforaphane, curcumin, fumaric acid and other chemicals (as previously detailed), that, as hormetic paradigmatic cases, may induce adaptive stress response at subtoxic doses while may be toxic to mammalian cells at high concentrations [18]. However, even if the evidence regarding the hormetic dose–response induced by resveratrol is quite strong, the molecular mechanisms underlining this phenomenon, at present, are not fully understood and deserve greater attention in future research. Moreover, future studies should be focused in putting the findings described into a realistic biological framework to determine whether or not these effects are relevant for human health [361].

8.5. The potential hormetic role of carnitine and carnosine

In the fight against oxidative and nitrosative stress, a great scientific interest has been attracted by the ability of the natural peptides L-carnitine (and its acyl-carnitine ester, Acetyl-L-carnitine (ALC) (Fig. 9), and carnosine (Fig. 9) to decrease cellular oxidative stress, associated with aging, improving mithocondrial bioenergetics, scavenging radicals or functioning as proton buffers and metal chelators [25,362,363].

Although, as previously described for the phytochemicals, the antioxidant activity of these molecules might be partly responsible for their health benefits, recent findings support the activation of a series of vitagene pathways such as networks of genes encoding antioxidant enzymes, protein chaperones, neurotrophic factors and also the activation of the Keap1/Nrf2/ARE pathway as leading cytoprotective mechanisms [225,250].

The emerging evidence for biphasic dose response effects of environmental "toxins" in biological systems and the analog mechanisms of action demonstrated for phytochemicals, carnitine and carnosine suggests the possibility that these latter exert their function according to a biphasic dose response relationship.

8.5.1. Carnitine

L-Carnitine (LC) is a nonessential nutrient first described in the beginnings of the 20th century [364]. Mammals can synthesize LC in the liver, kidney and brain from two essential amino acids, lysine and methionine [365,366]. In humans, 75% of the total body carnitine comes from dietary sources, particularly red meat and dairy products [367]. L-Carnitine main role is in cellular energy metabolism. It has two major functions: (1) transport of long-chain fatty acids in the form of acylcarnitines from the cytosol into the mitochondria for their subsequent use as a source of energy (via acetyl-CoA formation in the process known as β-oxidation) and (2) the removal of short- and medium-chain fatty acids formed as a consequence of normal metabolism, preventing a toxic accumulation of these compounds in the mitochondria and leading to an increase of free CoA. In this context, L-Carnitine is essential in the equilibrium between acetyl-CoA (plus carnitine) and CoA (plus acetylcarnitine) (acetyl-CoA/CoA ratio) which is crucial for mitochondrial metabolism.

Both a decline in mitochondrial energetics and an increase in oxidative stress are some of the effects of aging. Regarding the oxidative insult, in vitro experiments reported antioxidant properties of acetylcarnitine mediated by its iron chelating properties [368]. Comparatively, as a cellular antioxidant defense mechanisms, ALC decreased brain lipid peroxidation in old rats [369]. ALC may also prevent the age-associated changes to proteins. Oxidative modification to proteins appears to increase with age, as evidenced by increased levels of protein carbonyls, 3-nitrotyrosine and 4-hydroxynonenal [370]. Supplementing ALC to the older rats decreased many of these parameters in most of the brain regions investigated [370,371]. Pretreatment of cortical neurons with ALC significantly reduced the HNE-associated cytotoxicity, protein and lipid oxidation, and apoptosis in a dose-dependent manner as well as increased cellular reduced glutathione and Hsps as compared to controls [372,373]. ALC treatment also leads to the activation of phosphoinositol-3 kinase (PI3K), PKG, and ERK1/2 pathways that are important in neuronal cell

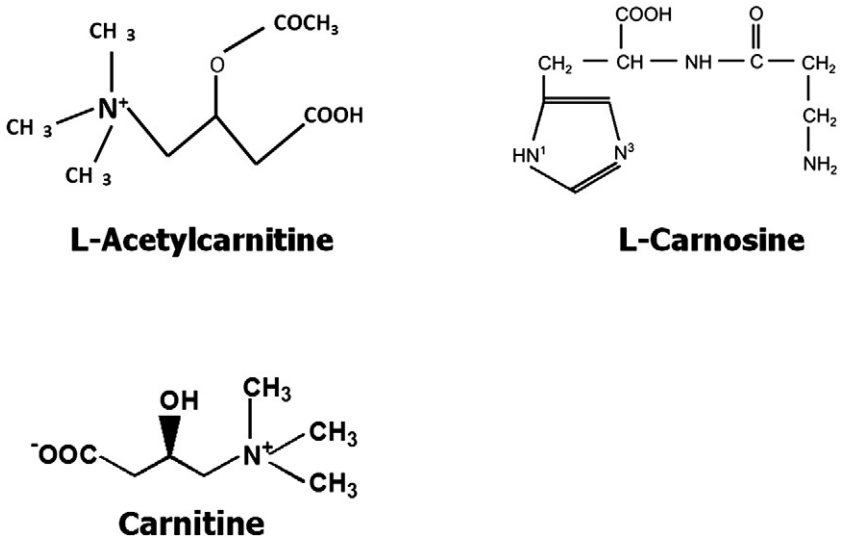

Fig. 9. Chemical structures of the L-carnitine, acetyl-L-carnitine and L-carnosine.

survival and differentiation [373]. ALC has also been found to protect against lipid peroxidation and membrane breakdown [374,375]. In streptozotocin-induced diabetic rats, treatment with ALC improved nerve conduction velocities (the speed of signal through the nerves), and this was associated with a reduction in elevated malonyldialdehyde (MDA) content, an indicator of lipid peroxidation [376]. When administered via the perfusate to ischemia-reperfused rat hearts, carnitine derivatives were able to scavenge peroxyl or superoxide radicals [377].

As an ulterior confirm of the ALC stimulated endogenous cellular antioxidant defense, treatment of astrocytes with acetylcarnitine increased the amount and activity of heme oxygenase-1 (HO-1) [378]. In addition, pre-incubation of astrocytes with acetylcarnitine before the initiation of a nitrosative stress with lipopolysaccharide and interferon, prevented the decrease in complex IV activity, protein nitration and restored the reduced glutathione/oxidized glutathione ratio [378].

One of the mechanisms responsible of the decrease in oxidative stress is the protective effect of ALC on mitochondrial structure and function. ALC has been considered as a mitochondrial nutrient that protect mitochondrial dysfunction by different mechanisms, such as preventing oxidant production or scavenging free radicals (processes that prevent oxidative stress in mitochondria); enhancing mitochondrial antioxidant defenses; enhancing mitochondrial metabolism to facilitate both the repair of less damaged and the degradation of more damaged mitochondria, and protecting mitochondrial enzymes and/or stimulating enzyme activity as enzyme substrates and cofactors [379]. In this regard, pretreatment of pancreatic β-cells with micromolar concentrations of acetylcarnitine was demonstrated to protect the cells from oleic acid-induced mitochondrial dysfunction and decrease ROS production [380]. ALC increased hepatocellular oxygen consumption and partially reversed the decrease in mitochondrial membrane potential associated with aging, while reducing MDA levels and restoring hepatocellular ascorbate levels [362]. A recent study showed that acetylcarnitine reversed the age-related decrease in the activity of complex III and oxidative phosphorylation through complexes III and IV and increased the amount of cytochrome b and aa3 hemes in cardiac mitochondria isolated from old rats [381]. Of interest, both cytochrome b and aa3 proteins are encoded by the mitochondrial genome, suggesting that acetylcarnitine enhances either mtDNA transcription, the stability of mitochondrial mRNA, and mitochondrial protein synthesis. ALC has been demonstrated to improve mitochondrial respiration in neurons and to be neuroprotective through a variety of other effects such as the increase in protein kinase-C activity [382]. Similarly to the phytochemicals above described, also ALC has been found to up-regulate HO-1 expression reducing the Aβ toxicity in primary cortical neuronal cultures [383] and to prevent age-related changes in mitochondrial respiration through the induction of HO-1, Hsp70, Hsp60 and Thioredoxin Reductase, and decreasing oxidative stress biomarkers in senescent rats [370–372,378,383–386]. The HO-1 up-regulation has been linked to activation of the trascriptor factor Nrf2 [378], implying the possibility that the ALC mediated acetylation of DNA-binding proteins, can induce post-translational modifications of critical target proteins involved in DNA competence and transactivating activity [37,51,371,378].

Interestingly, ALC and LC have been shown to reduce apoptosis through the mitochondrial pathway [388,390]. This anti-apoptotic effect has been observed in different cells, including neurons [390], myocytes [391], teratoma cells [392], hepatocytes [393,394] and lymphocytes [395]. Particularly, in neurons [396], cellular death induced by methamphetamine was reduced by high concentrations of acetylcarnitine. Acetylcarnitine protected also the dopaminergic system against the intraventricular injection of 1-methyl-4-phenylpyridinium (MPP+) in rats [397]. Less apoptosis was demonstrated in mouse fibroblasts treated with different concentrations of acetylcarnitine [389]. ALC and LC promoted neuronal survival and mitochondrial activity in addition to having anti-apoptotic effects in serum-deprived primary culture neurons [390]. In another tissue culture study, ALC treatment of TRX2-knockout DT40 cells suppressed oxidative stress in mitochondria, which prevented the mitochondrial signaling pathway leading to apoptosis [398]. The antiapoptotic effect of acetylcarnitine may be related to the overexpression and activation of HO-1, which increases the level of antiapoptotic bcl-2 protein and inactivates the pro-apoptotic transcription factor p53 in neurons [130]. Orally-supplemented acetylcarnitine in rats has been reported to decrease caspase activation by increasing the level of X-linked inhibitor of apoptosis protein (XIAP), thus limiting the mitochondrial-induced apoptosis in peripheral neurons [399]. Neither a protective effect on apoptosis induction nor a decrease in XIAP level was observed by these authors after carnitine administration, suggesting that the acetyl groups of acetylcarnitine have a fundamental role in protecting against mitochondrial-induced apoptosis.

ALC treatment has been found to produce several changes in gene expression [400–404]. An important role in the regulation of nucler DNA transcription was recently attributed to the acetylation and deacetylation of core histone tails at lysine residues in consideration of the activation and repression of the gene transcription induced by

the two reactions, respectively [405,406]. The limiting factor for histone acetylation is the acetyl-CoA availability in the nucleo-cytosolic pool [407]. From the analysis of the Poly(A)+RNAs isolated from brains of rats treated with ALC the expression of two genes resulted modified: the isoform c 14-3-3 protein, implicated in cell differentiation and growth [401,408,409] was up-regulated and the precursor mitochondrial P3 of ATP synthase lipid-binding protein, involved in transmembrane proton conduction, [410] was down-regulated. In addition, the gene coding for Hsp72 was up-regulated by treatment with ALC, which appears to help establish a cytoprotective state in inflammation, neurodegenerative disorders, and aging [385,401]. In addition to acetylation of histone proteins, site-specific acetylation of non-histone proteins plays an important role in transcriptional regulation. In particular, high mobility group (HMG)-box proteins are acetylated [411]. The mitochondrial transcription factor A (TFAM) required for mitochondrial DNA replication, contains two HMG-box-like domains. The total amount of TFAM increases in the liver, cerebellum and kidney with aging [412].

Acetylation can control the activity of mitochondrial enzymes, and possibly de novo synthesis of acetyl-CoA in mitochondria. Mitochondrial matrix- acetyl-Coa synthetase is reversibly acetylated at a lysine residue in the active site of the enzyme. The deacetylation of this site induced by the nicotinamide adenine nucleotide (NAD+)-dependent deacetylase silent information regulators (sirtuin, SIRT3) activates the enzyme [48,413]. It is conceivable that this modification can alter protein conformation and consequently its function. Moreover, glutamate dehydrogenase is another target for both SIRT3 [414] and SIRT4 [415] and is inhibited upon deacetylation. As a result, cytochrome b content increases, leading to increased activity of electron transport chain (ETC.) complexes, associated with stimulation of oxidative phosphorylation [416].

Oral acetylcarnitine supplementation in rats increases soleus muscle mitochondrial content, nuclear transcripts of factors involved in mitochondrial biogenesis (PGC-1α, NRF-1, TFAM), as well as the level of mitochondrial transcripts (COX I, ATP6, ND6, 16S rRNA), and prevents the unloading-induced downregulation of mRNA levels of kinases able to transduce metabolic (AMPK) and neuronal stimuli (CaMKIIβ) [400,417]. The activity and amount of HO-1 in cell culture increased by ALC treatment in a dose- and time-dependent manner [378]. Further, HO-1 was shown to increase mitochondrial biogenesis in cardiomyocytes via the transcriptional control of the nuclear respiratory factor-1 and 2 (NRF-1, 2) [52]. As a support of the ALC role in stabilizing mitochondrial transcripts or mitochondrial protein synthesis, recent data demonstrated that the decreased levels of a ribosomal RNA (12SrRNA) and a messenger RNA (mRNA for the subunit I of complex IV) were reversed in the brain and cardiac muscle of old rats 1 h after acetylcarnitine administration [418]. In addition, ALC treatment was able to contrast in heart mitochondria the reduction of cardiolipin content, the alteration of cholesterol/phospholipid ratio, the decrease of transport activity of adenylic nucleotides, pyruvate, phosphate and acylcarnitines [419–421]. Furthermore, the increase in the content of TFAM, which controls mitochondrial DNA transcription and translation, remains in skeletal muscle one month after the withdrawal from chronic acetylcarnitine supplementation, indicating the long-lasting effect of acetylcarnitine on mitochondria [422]. However, even if interesting findings are emergeng relative to the protective action of carnitine and ALC, particularly in relation to leading role of different vitagenes in many of these effects, further research is needed to fully clarify the ALC mechanisms in balance oxidative stress according to a hormetic dose–response relationship and its potential cytoprotective role. In this context, the interesting study carried out by Calabrese et al. [378] demonstrated the ability of ALC to increase the expression of OH-1 in astrocytes both in absence or presence of an oxidative stress stimulus and this does not allow to extrapolate a definite conclusion regarding the role of ALC as a defense mechanism after an external damage of the homeostatic equilibrium or as itself a preconditionating agent. Very importantly, the ALC capability to potentiate cellular stress response through the HO-1 pathway, appears to be a promising alternative therapeutic approach for those pathophysiological conditions where stimulation of this response is warranted [378].

8.5.2. Carnosine

Carnosine is a simple dipeptide, β-alanyl-L-histidine, that was isolated from Liebig's meat more than a century ago [423] and was subsequently classified as a histidine containing dipeptide by Krimberg [424,425], who demonstrated the hydrolysis of carnosine to its constituent amino acids (β-alanine and histidine) [426]. Carnosine is endogenously formed mainly in muscle and brain tissue, by bonding histidine and β-alanine in a reaction catalyzed by carnosine synthase [426]. It is present in vertebrate brain, cardiac muscle, kidney, stomach and high millimolar concentrations are found in the olfactory bulbs and in skeletal muscle [427–434]. Diet influences tissue carnosine concentrations [435–437]. Within the organism, carnosine breakdown is catalyzed by a specific carnosinase and a less specific homocarnosinase which are present in many tissues, with the exception of skeletal muscle [427,438,439]. The products of carnosine hydrolysis are metabolized and excreted in the urine. Many hypotheses have been made regarding the cellular action of carnosine as anti-oxidant and oxygen free radical scavenger, physiological buffer, histidine source, regulator of enzyme activity, metal-ion chelator, carbonyl scavenger, antiglycator, neurotransmitter, would healing agent and immunostimulant [430,440–442]. Among antioxidant mechanisms reported for carnosine, its ability to inactivate reactive oxygen species, scavenge free radicals, such as hydroxyl, superoxide radicals and singlet molecular oxygen, and chelate pro-oxidative metals, like copper and iron has been reported [441,443–447]. It has been shown to delay aging in cultured human fibroblasts [448] and to be neuroprotective because of its ability to counteract both oxidative and nitrosative stress related to several pathological conditions including ischemia [449–452], methamphetamine neurotoxicity [453] and neurodegenerative disorders [454,455]. Regarding the mechanisms leading to oxidative stress response, carnosine, administered to astrocytes prior to the LPS/INFγ stimulus, prevented the up-regulation of iNos and the induction of both HO-1 and Hsp70 [387]. These data are in line with recent findings demonstrating that HO-1 can be repressed following oxidant conditions. In contrast to the general agree that HO-1 overexpression was a common feature during oxidative stress, recent works demonstrated that exposure to hypoxia, thermal stress, and interferon-γ was able to induce a marked HO-1 repression in human cells [51,141,456–458]. The usefulness of the HO-1 repression relies on the possibility to maintain an efficient metabolic balance during stressful conditions, decreasing the energy costs necessary for heme degradation, reducing the accumulation of CO and biliverdin which can become toxic when in excess, and increasing the intracellular content of heme necessary for the preservation of vital functions [459]. Unfortunately, the limited number of studies and the lack of data regarding the role of carnosine alone on the HO-1 induction prevent to fully clarify the role of this protein in the stress response.

A rather unusual reported antioxidant property of carnosine was its ability to reduce concentrations of thiobarbituric acid reactive substances (TBARS) when added to previously oxidized lipids [441,460,461]. If carnosine could interact with aldehydic lipid oxidation products, this could potentially help protect biological tissues from oxidation, since aldehydes can form adducts with DNA, proteins, enzymes, and lipoproteins, causing alterations in their biological activity [441]. In this regard, the protective anti-peroxidation function of carnosine has been demonstrated on a variety of biomolecules including proteins [447,462–465], lipids [464,466,467], DNA [466] and DNA bases [443]. Initial studies demonstrated that carnosine was an efficient hydrogen ion (H+) buffer over the physiological pH range

[426,468]. In muscle, where its concentration is highest, carnosine makes an important contribution to the maintenance of intracellular pH, which is vital for normal muscle function during intense exercise [469,470]. At weakly alkaline pH, carnosine is easily able to suppress lipid peroxidation [435,428]. This allows carnosine to maintain its suppression of peroxidation. In addition, carnosine also exhibits heavy metal ion binding properties, which inhibit some enzymatic reactions [427].

Carnosine has also been demonstrated to be an effective anti-glycating agent, at least in model systems and cultured cells [432]. The structure of carnosine closely resembles that of preferred glycation sites in proteins, that is, a target amino group with proximal imidazole and carboxyl groups. Carnosine itself could, sacrificially, react with many potential glycating agents thereby protecting other potential targets against glycation [442,471]. The carnosine ability to react with toxic aldehydes (e.g. acetaldehyde, formaldehyde, malondialdehyde and methylglyoxal), and deoxyribose has been shown to protect cultured human fibroblasts and lymphocytes, rat brain endothelial cells and Chinese hamster ovary cells, by inhibiting the protein–protein and DNA–protein cross linking induced by those substances [442,463,471–474]. Interestingly, the dipeptide has been shown to inhibit formation of protein carbonyls generated by the actions of oxygen free radicals and related species (reactive oxygen species) as well as aldehydes or ketones on amino acid side chains [471,475]. Moreover, it resulted also able to react with (i.e. carnosinylate) protein carbonyls to form protein–carbosyl–carnosine adducts, inhibiting further cross-linking to normal proteins [476]. Finally, carnosine can suppress AGE formation [463] and AGE-induced protein modification [473]. Proteotoxic stress, characterized by an accumulation of altered proteins derived from biosynthetic errors and/or deleterious post-synthetic polypeptide modifications, is a typical feature of aging conditions [87,477–480]. Carnosine is able to exert anti-aging actions, induces rejuvenating effects [448,481], and protects against telomere shortening in cultured human fibroblasts [482].

The dipeptide also extended the life-span of senescence-accelerated mice [483]. There are many possible mechanisms by which carnosine exert these anti-aging actions [463]. Among these, the suppression of mRNA translation initiation [484] lowers production of error-proteins, reduces the formation of protein carbonyls and increases the relative availability of chaperone and proteolytic activities for the recognition and elimination of altered proteins arising from deleterious post-synthetic modification [108]. Carnosine has the potential to inhibit much deleterious posttranslational polypeptide modification caused by oxidative [443,485–487], nitroxidative [387,488] and glycoxidative agents [461,463,472–474,489–493]. Moreover, carnosine may improve cellular ability to deal with aberrant proteins through the nitric oxide mediated upregulation of certain proteolytic functions [494–501,487–494]. It was demonstrated in carnosine-treated cultured human fibroblasts [463], cardiomyoblasts [502] and in cell-free extracts of rat brain [503]. Stress/chaperone proteins participate in the recognition and proteolytic elimination of altered proteins and their upregulation is associated with increased organism longevity and suppression of some age-related diseases [25,87,504]. Carnosine-zinc complexes (polaprezinc) stimulate expression of certain stress (heat-shock) proteins [505–507], which could improve cellular ability to deal with altered proteins and may again contribute to the dipeptide's apparent anti-aging activity.

In consideration of the carnosine properties described, the therapeutic role of carnosine in managing secondary diabetes complications, such as cataractogenesis and atherosclerosis should be deeply investigated [463,508]. In fact, these complications result from protein glycation and oxidation [509,510] mediated by agents and processes against which carnosine may, theoretically, protect [473]. Some preliminary supportive evidence from animal studies has been obtained [432,511–513]. Glycoxidation management is also important in neurodegenerative conditions [514–520]. Chen et al. [521] suggest that carnosine could supplement glyoxalase's action, both by its aldehyde-scavenging action and its glyoxalase-mimetic activity. This capacity could also suppress aldehyde-mediated tau modification and consequent aggregation in a mouse model of Alzheimer's disease [522]. Carnosine treatment reduced inflammation and tissue damage in a mice animal model of spinal cord injuries [523]. Other experimental observation provided some support for the suggestion that carnosine could be useful in ameliorating aspects of Parkinson's, Alzheimer's diseases and also the consequences of dis-circulatory brain ischemia [455,523–532]. It has been suggested to be potentially useful for treating also autism [533], Down's syndrome [534], epilepsy [535] and aggregation processes. Further refinements have led to the synthesis of new carnosine derivatives able to survive to carnosinase hydrolytic activity and endowed with cytoprotective effects [536–538]. Interestingly, the different molecular behavior of carnitine and carnosine in response to similar stress stimuli, particularly in relation to the activation/repression of the Keap1/Nrf2/ARE pathway, should merit confirmation and deep future investigations. These differences, in fact, although not fully understood, may suggest that several factors could interplay in influencing the molecular mechanisms at the basis of the cellular defense response. Neverthless, the discoveries that molecules, which are toxic at high doses, play fundamental or protective roles in cellular signaling or metabolism at low concentrations, suggest that evolution triggered organisms to include environmental toxins for an advantageous use [51]. Thus, it seems important to plan future researches to deeply investigate the leading mechanisms induced by exogenous small molecules in response to stressfull conditions. It may be a valid tool to develop interventions able to activate hormetic signaling pathways in cells as new approaches for the prevention and treatment of a wide range of pathological disorders.

9. Conclusion and future directions

Due to considerable improvement in medical and environmental factors, in the past few hundred years, average human lifespan has increased substantially, bringing demographic change in most industrialized countries. Nevertheless, despite our achievements in average lifespan, the maximal lifespan of humans remains unchanged as the latter may be genetically programmed or determined by inevitable deleterious cell changes that occur with age. These changes are typical of the senescent phenotype and eventually lead to death. Many types of interventions, including genetic manipulations and caloric restriction, appear to increase the maximal life span in animal models, but it is not yet clear whether the results from these animal experiments will contribute to lengthening the human life span. Among the hypotheses put forward to explain the biological processes underlying senescence, one theory suggests that ROS formation is principally responsible for cell damage and senescence [539,540]. The deleterious effects of ROS could slowly accumulate over years and lead to dysfunction of a number of cell functions. The mitochondrion is the main organelle for the production of ROS. Although energy generation in the mitochondrion is extremely important for survival, mitochondrial ROS production also has some negative consequences for age-related intracellular changes. However, ROS is not only a waste of oxidative phosphorylation in the respiratory chain but is also highly regulated signal molecules involved in cellular stress response [541]. Cells and organisms, subjected to chronic proteotoxic stress associated with diseases, undergo a global decline in cellular function with negative consequences on viability. This increases the levels of damaged proteins, resulting in a decline in multiple biosynthetic and repair activities and, over time, negatively affects health and aging of the organism. Much evidence in the literature supports the theory that molecular determinants of longevity influence proteotoxicity, suggesting that the integrity of the

proteome is a vital mechanism for ensuring the efficient functioning of the cell and the healthy state of biological processes throughout the life span of the organism. This is achieved by a complex network of longevity assurance processes which are controlled by vitagenes, a group of genes involved in preserving cellular homeostasis during stressful conditions [542]. This network responds to the age related mitochondrial dysfunction and is modulated by mitochondrially produced ROS [51,543–545]. Thus, by sensing the nutritional status of the whole organism as well as the intracellular nutrient and energy status, functional state of mitochondria, and mitochondrial ROS concentration, the longevity network regulates life span across species by coordinating information flow along its signaling pathways. Given the unique roles of HSF1 and Nrf2/keap1 in stress biology and proteostasis, enhanced activity of these principal regulators during development and early adulthood is important for the stability of the proteome and the health of the cell. To this end, the crosstalk between distinct HSFs as well as between HSF1 and Nrf2/Keap1 pathway, only recently uncovered, raises obvious questions about the stoichiometry between the components in different complexes residing in different cellular compartments, and the mechanisms by which the factors interact with each other. Interaction between members of the vitagene system, HSFs and Nrf2/Keap1-related, could lead to new therapeutic approaches for protein-folding diseases, metabolic disorders and cancer, as well as healthy aging.

Acknowledgments

Work from the authors' laboratories was supported by grants from MIUR, FIRB RBRN07BMCT, FIRB RBNE08HWLZ (Programma MERIT), I.N.B.B., and by “Fondi Ateneo” 2008 and 2009. This paper is dedicated to the memory of Claudio Cavazza, who died on June 6th, while this manuscript was in preparation.

References

[1] B. Rogina, S.L. Helfand, Sir2 mediates longevity in the fly through a pathway related to calorie restriction, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 15998–16003.
[2] J.G. Wood, B. Rogina, S. Lavu, K. Howitz, S.L. Helfand, M. Tatar, D. Sinclair, Sirtuin activators mimic caloric restriction and delay ageing in metazoans, Nature 430 (2004) 686–689.
[3] W.C. Hallows, B.N. Albaugh, J.M. Denu, Where in the cell is SIRT3? Functional localization of an NAD+-dependent protein deacetylase, Biochem. J. 411 (2008) 11–13.
[4] S.D. Hursting, J.A. Lavigne, D. Berrigan, S.N. Perkins, J.C. Barrett, Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans, Annu. Rev. Med. 54 (2003) 131–152.
[5] L.R. Saunders, E. Verdin, Cell biology. Stress response and aging, Science 323 (2009) 1021–1022.
[6] G. Donmez, L. Guarente, Aging and disease: connections to sirtuins, Aging Cell 9 (2010) 285–290.
[7] L. Guarente, L. Partridge, D.C. Wallace, Molecular Biology of Aging, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2008.
[8] B. Schwer, E. Verdin, Conserved metabolic regulatory functions of sirtuins, Cell Metab. 7 (2008) 104–112.
[9] O. Ozden, S.H. Park, H.S. Kim, H. Jiang, M.C. Coleman, D.R. Spitz, D. Gius, Acetylation of MnSOD directs enzymatic activity responding to cellular nutrient status or oxidative stress, Aging 2011 (3) (2011) 102–107.
[10] Y.L. Deribe, T. Pawson, I. Dikic, Post-translational modifications in signal integration, Nat. Struct. Mol. Biol. 17 (2010) 666–672.
[11] A. Grahnert, A. Grahnert, C. Klein, E. Schilling, J. Wehrhahn, S. Hauschildt, NAD+: a modulator of immune functions, Innate Immun. 17 (2010) 212–233.
[12] S. Imai, Nicotinamide phosphoribosyltransferase (Nampt): a link between NAD biology, metabolism, and diseases, Curr. Pharm. Des. 15 (2009) 20–28.
[13] M. Gallí, F. Van Gool, O. Leo, Sirtuins and inflammation: friends or foes? Biochem. Pharmacol. 81 (2011) 569–576.
[14] A.M. Andreou, N. Tavernarakis, SUMOylation and cell signaling, Biotechnol. J. 4 (2009) 1740–1752.
[15] V. Calabrese, G. Scapagnini, A.M. Giuffrida Stella, T.E. Bates, J.B. Clark, Mitocondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disordes and longevity, Neurochem. Res. 26 (2001) 739–764.
[16] N.M. Druzhyna, G.L. Wilson, S.P. LeDoux, Mitochondrial DNA repair in aging and disease, Mech. Ageing Dev. 129 (2008) 383–390.
[17] S. Hamm-Alvarez, E. Cadenas, Mitochondrial medicine and mitochondrion-based therapeutics, Adv. Drug Deliv. Rev. 60 (2009) 1437–1438.
[18] V. Calabrese, C. Cornelius, A.T. Dinkova-Kostova, E.J. Calabrese, M.P. Mattson, Cellular stress responses, the hormesis paradigm and vitagenes: novel tragets for therapeutic intervention in neurodegenerative disorders, Antioxid. Redox Signal. 13 (2010) 1763–1811.
[19] L.P. Yap, J.V. Garcia, D. Han, E. Cadenas, The energy-redox axis in aging and age-related neurodegeneration, Adv. Drug Deliv. Rev. 61 (2009) 1283–1298.
[20] D. Harman, Aging: a theory based on free radical and radiation chemistry, J. Gerontol. 11 (1956) 298–300.
[21] D. Harman, The biologic clock: the mitochondria? J. Am. Geriatr. Soc. 20 (1972) 145–147.
[22] V. Calabrese, E. Guagliano, M. Sapienza, M. Panebianco, S. Calafato, E. Puleo, G. Pennisi, C. Mancuso, D.A. Butterfield, A.G. Stella, Redox regulation of cellular stress response in aging and neurodegenerative disorders: role of vitagenes, Neurochem. Res. 32 (2007) 757–773.
[23] A.H. Schapira, Primary and secondary defects of the mitochondrial respiratory chain, J. Inherit. Metab. Dis. 25 (2002) 207–214.
[24] V. Calabrese, G. Scapagnini, C. Colombrita, A. Ravagna, G. Pennisi, A.M. Giuffrida stella, F. Galli, D.A. Butterfield, Redox regulation of the heat shock protein expression in aging and neurodegenerative disorders associated with oxidative stress: a nutritional approach, Amino Acids 25 (2003) 437–444.
[25] V. Calabrese, C. Cornelius, C. Mancuso, G. Pennisi, S. Calafato, F. Bellia, T.E. Bates, A.M. Giuffrida Stella, T. Schapira, A.T. Dinkova Kostova, E. Rizzarelli, Cellular stress response: a novel target for chemoprevention and nutritional neuroprotection in aging, neurodegenerative disorders and longevity, Neurochem. Res. 33 (2008) 2444–2471.
[26] C. Tonon, R. Lodi, Idebenone in Friedreich's ataxia, Expert Opin. Pharmacother. 9 (2008) 2327–2337.
[27] G. Petrosillo, M. Matera, G. Casanova, F.M. Ruggiero, G. Paradies, Mitochondrial-dysfunction in rat brain with aging involvement of complex I, reactive oxygen species and cardiolipin, Neurochem. Int. 53 (2008) 126–131.
[28] B.N. Ames, J. Liu, Delaying the mitochondrial decay of aging with acetylcarnitine, Ann. N. Y. Acad. Sci. 1033 (2004) 108–116.
[29] S. Hempenstall, L. Picchio, S.E. Mitchell, J.R. Speakman, C. Selman, The impact of acute caloric restriction on the metabolic phenotype in male C57BL/6 and DBA/2 mice, Mech. Ageing Dev. 131 (2010) 111–118.
[30] M.P. Mattson, Dietary factors, hormesis and health, Ageing Res. Rev. 7 (2008) 43–48.
[31] M. Ristow, K. Zarse, How increased oxidative stress promotes longevity and metabolic health: the concept of mitochondrial hormesis (mitohormesis), Exp. Gerontol. 45 (2010) 410–418.
[32] M. Ristow, K. Zarse, A. Oberbach, N. Klöting, M. Birringer, M. Kiehntopf, M. Stumvoll, C.R. Kahn, M. Blüher, Antioxidants prevent health-promoting effects of physical exercise in humans, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 8665–8670.
[33] M. Ristow, S. Schmeisser, Extending life span by increasing oxidative stress, Free Radic. Biol. Med. 51 (2011) 327–336.
[34] I. Forquer, R. Covian, M.K. Bowman, B.L. Trumpower, D.M. Kramer, Similar transition states mediate the Q-cycle and superoxide production by the cytochrome bc1 complex, J. Biol. Chem. 281 (2006) 38459–38465.
[35] M. Bilban, A. Haschemi, B. Wegiel, B.Y. Chin, O. Wagner, L.E. Otterbein, Heme oxygenase and carbon monoxide initiate homeostatic signaling, J. Mol. Med. 86 (2008) 267–279.
[36] B. Halliwell, The wanderings of a free radical, Free Radic. Biol. Med. 46 (2009) 531–542.
[37] V. Calabrese, C. Mancuso, M. Calvani, E. Rizzarelli, D.A. Butterfield, A.M. Giuffrida Stella, Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity, Nat. Rev. Neurosci. 8 (2007) 766–775.
[38] V. Calabrese, C. Cornelius, E. Rizzarelli, J.B. Owen, A.T. Dinkova-Kostova, D.A. Butterfield, Nitric oxide in cell survival: a Janus molecule, Antioxid. Redox Signal. 11 (2009) 2717–2739.
[39] W. Wang, H. Fang, L. Groom, A. Cheng, W. Zhang, J. Liu, X. Wang, K. Li, P. Han, M. Zheng, J. Yin, W. Wang, M.P. Mattson, J.P. Kao, E.G. Lakatta, S.S. Sheu, K. Ouyang, J. Chen, R.T. Dirksen, H. Cheng, Superoxide flashes in single mitochondria, Cell 134 (2008) 279–290.
[40] J.M. McCord, Superoxide dismutase in aging and disease: an overview, Methods Enzymol. 349 (2002) 331–341.
[41] D.H. Cho, T. Nakamura, S.A. Lipton, Mitochondrial dynamics in cell death and neurodegeneration, Cell. Mol. Life Sci. 67 (2010) 3435–3447.
[42] T. Nakamura, P. Cieplak, D.H. Cho, A. Godzik, S.A. Lipton, S-Nitrosylation of Drp1 links excessive mitochondrial fission to neuronal injury in neurodegeneration, Mitochondrion 10 (2010) 573–578.
[43] Z. Gu, T. Nakamura, S.A. Lipton, Redox reactions induced by nitrosative stress mediate protein misfolding and mitochondrial dysfunction in neurodegenerative diseases, Mol. Neurobiol. 41 (2010) 55–72.
[44] H. Büeler, Mitochondrial dynamics, cell death and the pathogenesis of Parkinson's disease, Apoptosis 15 (2010) 1336–1353.
[45] A.B. Knott, G. Perkins, R. Schwarzenbacher, E. Bossy-Wetzel, Mitochondrial fragmentation in neurodegeneration, Nat. Rev. Neurosci. 9 (2008) 505–518.
[46] D.J. Bonda, X. Wang, G. Perry, M.A. Smith, X. Zhu, Mitochondrial dynamics in Alzheimer's disease: opportunities for future treatment strategies, Drugs Aging 27 (2010) 181–192.
[47] R.A. Gottlieb, R.S. Carreira, Autophagy in health and disease. 5. Mitophagy as a way of life, Am. J. Physiol. Cell Physiol. 299 (2010) C203–C210.
[48] M.G. Rosca, H. Lemieux, C.L. Hoppel, Mitochondria in the elderly: is acetylcarnitine a rejuvenator? Adv. Drug Deliv. Rev. 61 (2009) 1332–1342.
[49] S. Borniquel, N. García-Quintáns, I. Valle, Y. Olmos, B. Wild, F. Martínez-Granero, E. Soria, S. Lamas, M. Monsalve, Inactivation of Foxo3a and subsequent downregulation of PGC-1 alpha mediate nitric oxide-induced endothelial cell migration, Mol. Cell. Biol. 30 (Aug 2010) 4035–4044.

[50] C.A. Piantadosi, Carbon monoxide, reactive oxygen signaling, and oxidative stress, Free Radic. Biol. Med. 45 (2008) 562–569.
[51] V. Calabrese, C. Cornelius, A.T. Dinkova-Kostova, E.J. Calabrese, Vitagenes, cellular stress response, and acetylcarnitine: relevance to hormesis, Biofactors 35 (2009) 146–160.
[52] C.A. Piantadosi, M.S. Carraway, A. Babiker, H.B. Suliman, Heme oxygenase-1 regulates cardiac mitochondrial biogenesis via Nrf2-mediated transcriptional control of nuclear respiratory factor-1, Circ. Res. 103 (2008) 1232–1240.
[53] M.B. Azad, Y. Chen, S.B. Gibson, Regulation of autophagy by reactive oxygen species (ROS): implications for cancer progression and treatment, Antioxid. Redox Signal. 11 (2009) 777–790.
[54] M. Nassif, S. Matus, K. Castillo, C. Hetz, Amyotrophic lateral sclerosis pathogenesis: a journey through the secretory pathway, Antioxid. Redox Signal. 13 (2010) 1955–1989.
[55] F. Madeo, T. Eisenberg, G. Kroemer, Autophagy for the avoidance of neurodegeneration, Genes Dev. 23 (2009) 2253–2259.
[56] Y. Fujioka, N.N. Noda, H. Nakatogawa, Y. Ohsumi, F. Inagaki, Dimeric coiled-coil structure of *Saccharomyces cerevisiae* Atg16 and its functional significance in autophagy, J. Biol. Chem. 285 (2010) 1508–1515.
[57] J. Jakubowicz-Gil, E. Langner, I. Wertel, T. Piersiak, W. Rzeski, Temozolomide, quercetin and cell death in the MOGGCCM astrocytoma cell line, Chem. Biol. Interact. 188 (2010) 190–203.
[58] Q. Li, I.M. Verma, Nf-kappab regulation in the immune system, Nat. Rev. 2 (2002) 725–734.
[59] N. Silverman, T. Maniatis, Nf-kappab signaling pathways in mammalian and insect innate immunity, Genes Dev. 15 (2001) 2321–2342.
[60] S. Ghosh, M.S. Hayden, New regulators of nf-kappab in inflammation, Nat. Rev. 8 (2008) 837–848.
[61] M.S. Hayden, S. Ghosh, Shared principles in nf-kappab signaling, Cell 132 (2008) 344–362.
[62] T. Criswell, K. Leskov, S. Miyamoto, G. Luo, D.A. Boothman, Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiation, Oncogene 22 (2003) 5813–5827.
[63] S. Janssens, J. Tschopp, Signals from within: the DNA-damage-induced nf-kappab response, Cell Death Differ. 13 (2006) 773–784.
[64] R.T. Peters, S.M. Liao, T. Maniatis, Ikkepsilon is part of a novel pma-inducible ikappab kinase complex, Mol. Cell 5 (2000) 513–522.
[65] T. Shimada, T. Kawai, K. Takeda, M. Matsumoto, J. Inoue, Y. Tatsumi, A. Kanamaru, S. Akira, Ikk-i, a novel lipopolysaccharide-inducible kinase that is related to ikappab kinases, Int. Immunol. 11 (1999) 1357–1362.
[66] Y. Chen, L. Yang, T.J. Lee, Oroxylin a inhibition of lipopolysaccharide-induced inos and cox-2 gene expression via suppression of nuclear factor-kappab activation, Biochem. Pharmacol. 59 (2000) 1445–1457.
[67] K. Pahan, F.G. Sheikh, A.M. Namboodiri, I. Singh, Lovastatin and phenylacetate inhibit the induction of nitric oxide synthase and cytokines in rat primary astrocytes, microglia, and macrophages, J. Clin. Invest. 100 (1997) 2671–2679.
[68] D. Salvemini, E. Mazzon, L. Dugo, D.P. Riley, I. Serraino, A.P. Caputi, S. Cuzzocrea, Pharmacological manipulation of the inflammatory cascade by the superoxide dismutase mimetic m40403, Br. J. Pharmacol. 132 (2001) 815–827.
[69] G. Pistritto, C. Mancuso, G. Tringali, M. Perretti, P. Preziosi, P. Navarra, The relative contribution of constitutive and inducible cyclooxygenase activity to lipopolysaccharide-induced prostaglandin production by primary cultures of rat hypothalamic astrocytes, Neurosci. Lett. 246 (1998) 45–48.
[70] S. Cuzzocrea, A.P. Caputi, B. Zingarelli, Peroxynitrite-mediated DNA strand breakage activates poly (adp-ribose) synthetase and causes cellular energy depletion in carrageenan-induced pleurisy, Immunology 93 (1998) 96–101.
[71] M.G. Salgo, E. Bermudez, G.L. Squadrito, W.A. Pryor, Peroxynitrite causes DNA damage and oxidation of thiols in rat thymocytes [corrected], Arch. Biochem. Biophys. 322 (1995) 500–505.
[72] J.M. Gutteridge, B. Halliwell, Antioxidants: molecules, medicines, and myths, Biochem. Biophys. Res. Commun. 393 (2010) 561–564.
[73] A. Salminen, K. Kaarniranta, Nf-kappab signaling in the aging process, J. Clin. Immunol. 29 (2009) 397–405.
[74] J. Dutta, Y. Fan, N. Gupta, G. Fan, C. Gelinas, Current insights into the regulation of programmed cell death by nf-kappab, Oncogene 25 (2006) 6800–6816.
[75] H. Nakano, A. Nakajima, S. Sakon-Komazawa, J.H. Piao, X. Xue, K. Okumura, Reactive oxygen species mediate crosstalk between nf-kappab and jnk, Cell Death Differ. 13 (2006) 730–737.
[76] A. Hiona, C. Leeuwenburgh, Effects of age and caloric restriction on brain neuronal cell death/survival, Ann. N. Y. Acad. Sci. 1019 (2004) 96–105.
[77] H.J. Kim, K.W. Kim, B.P. Yu, H.Y. Chung, The effect of age on cyclooxygenase-2 gene expression: Nf-kappab activation and ikappabalpha degradation, Free Radic. Biol. Med. 28 (2000) 683–692.
[78] M. Helenius, M. Hanninen, S.K. Lehtinen, A. Salminen, Changes associated with aging and replicative senescence in the regulation of transcription factor nuclear factor-kappa b, Biochem. J. 318 (1996) 603–608.
[79] D. Cuthbertson, K. Smith, J. Babraj, G. Leese, T. Waddell, P. Atherton, H. Wackerhage, P.M. Taylor, M.J. Rennie, Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle, FASEB J. 19 (2005) 422–424.
[80] M.L. Schmitz, I. Mattioli, H. Buss, M. Kracht, Nf-kappab: a multifaceted transcription factor regulated at several levels, Chembiochem 5 (2004) 1348–1358.
[81] L.F. Chen, W.C. Greene, Regulation of distinct biological activities of the nf-kappab transcription factor complex by acetylation, J. Mol. Med. 81 (2003) 549–557.
[82] F. Yeung, J.E. Hoberg, C.S. Ramsey, M.D. Keller, D.R. Jones, R.A. Frye, M.W. Mayo, Modulation of nf-kappab-dependent transcription and cell survival by the sirt1 deacetylase, EMBO J. 23 (2004) 2369–2380.
[83] A.S. Adler, S. Sinha, T.L. Kawahara, J.Y. Zhang, E. Segal, H.Y. Chang, Motif module map reveals enforcement of aging by continual nf-kappab activity, Genes Dev. 21 (2007) 3244–3257.
[84] C. Burnett, S. Valentini, F. Cabreiro, M. Goss, M. Somogyvári, M.D. Piper, M. Hoddinott, G.L. Sutphin, V. Leko, J.J. McElwee, R.P. Vazquez-Manrique, A.M. Orfila, D. Ackerman, C. Au, G. Vinti, M. Riesen, K. Howard, C. Neri, A. Bedalov, M. Kaeberlein, C. Soti, L. Partridge, D. Gems, Absence of effects of Sir2 overexpression on lifespan in *C. elegans* and *Drosophila*, Nature 477 (2011) 482–485.
[85] O. Yogev, R. Goldberg, S. Anzi, O. Yogev, E. Shaulian, Jun proteins are starvation-regulated inhibitors of autophagy, Cancer Res. 70 (2010) 2318–2327.
[86] R.I. Morimoto, Stress, aging, and neurodegenerative disease, N. Engl. J. Med. 355 (2006) 2254–2255.
[87] R.I. Morimoto, Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging, Genes Dev. 22 (2008) 1427–1438.
[88] S.D. Westerheide, J. Anckar, S.M. Stevens, L. Sistonen Jr., R.I. Morimoto, Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1, Science 323 (2009) 1063–1066.
[89] A. Trott, J.D. West, L. Klaic, S.D. Westerheide, R.B. Silverman, R.I. Morimoto, K.A. Morano, Activation of heat shock and antioxidant responses by the natural product celastrol: transcriptional signatures of a thiol-targeted molecule, Mol. Biol. Cell 19 (2008) 1104–1112.
[90] P. Moi, K. Chan, I. Asunis, A. Cao, Y.W. Kan, Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 9926–9930.
[91] J. Anckar, L. Sistonen, Heat shock factor 1 as a coordinator of stress and developmental pathways, Adv. Exp. Med. Biol. 594 (2007) 78–88.
[92] R.I. Morimoto, Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators, Genes Dev. 12 (1998) 3788–3796.
[93] H.R. Brignull, J.F. Morley, R.I. Morimoto, The stress of misfolded proteins: *C. elegans* models for neurodegenerative disease and aging, Adv. Exp. Med. Biol. 594 (2007) 167–189.
[94] A. Rossi, E. Trotta, R. Brandi, I. Arisi, M. Coccia, M.G. Santoro, AIRAP: a new human Heat shock gene regulated by Heat shock factor 1, J. Biol. Chem. 285 (2010) 13607–13615.
[95] Z. Batulan, G.A. Shinder, S. Minotti, B.P. He, M.M. Doroudchi, J. Nalbantoglu, M.J. Strong, H.D. Durham, High threshold for induction of the stress response in motor neurons is associated with failure to activate HSF1, J. Neurosci. 23 (2003) 5789–5798.
[96] S.K. Calderwood, A. Murshid, T. Prince, The shock of aging: molecular chaperones and the heat shock response in longevity and aging—a mini-review, Gerontology 55 (2009) 550–558.
[97] M. Lu, H.E. Kim, C.R. Li, S. Kim, I.J. Kwak, Y.J. Lee, S.S. Kim, J.Y. Moon, C.H. Kim, D.K. Kim, H.S. Kang, J.S. Park, Two distinct disulfide bonds formed in human heat shock transcription factor 1 act in opposition to regulate its DNA binding activity, Biochemistry 47 (2008) 6007–6015.
[98] C. Söti, P. Csermely, Aging cellular networks: chaperones as major participants, Exp. Gerontol. 42 (2007) 113–119.
[99] C. Söti, P. Csermely, Protein stress and stress proteins: implications in aging and disease, J. Biosci. 32 (2007) 511–515.
[100] A.J. Macario, E. Conway de Macario, Molecular chaperones: multiple functions, pathologies, and potential applications, Front. Biosci. 12 (2007) 2588–2600.
[101] A.J. Macario, E. Conway de Macario, Sick chaperones, cellular stress, and disease, N. Engl. J. Med. 353 (2005) 1489–1501.
[102] P. Csermely, C. Söti, G.L. Blatch, Chaperones as parts of cellular networks, Adv. Exp. Med. Biol. 594 (2007) 55–63.
[103] S.A. Broadley, F.U. Hartl, The role of molecular chaperones in human misfolding diseases, FEBS Lett. 583 (2009) 2647–2653.
[104] P. Goloubinoff, P. De Los Rios, The mechanism of Hsp70 chaperones: (entropic) pulling the models together, Trends Biochem. Sci. 32 (2007) 372–380.
[105] H.R. Saibil, Chaperone machines in action, Curr. Opin. Struct. Biol. 18 (2008) 35–42.
[106] A.J. Macario, E. Conway de Macario, Chaperonopathies by defect, excess, or mistake, Ann. N. Y. Acad. Sci. 1113 (2007) 178–191.
[107] V. Calabrese, C. Cornelius, C. Mancuso, R. Lentile, A.M. Stella, D.A. Butterfield, Redox homeostasis and cellular stress response in aging and neurodegeneration, Methods Mol. Biol. 610 (2010) 285–308.
[108] A.R. Hipkiss, On why decreasing protein synthesis can increase lifespan, Mech. Ageing Dev. 128 (2007) 412–414.
[109] S.K. Sharma, P. Christen, P. Goloubinoff, Disaggregating chaperones: an unfolding story, Curr. Protein Pept. Sci. 10 (2009) 432–446.
[110] K. Zhang, T. Zhao, X. Huang, Z.H. Liu, L. Xiong, M.M. Li, L.Y. Wu, Y.Q. Zhao, L.L. Zhu, M. Fan, Preinduction of HSP70 promotes hypoxic tolerance and facilitates acclimatization to acute hypobaric hypoxia in mouse brain, Cell Stress Chaperones 14 (2009) 407–415.
[111] V. Calabrese, A.M. Giuffrida Stella, D.A. Butterfield, G. Scapagnini, Redox regulation in neurodegeneration and longevity: role of the heme oxygenase and HSP70 systems in brain stress tolerance, Antioxid. Redox Signal. 6 (2004) 895–913.
[112] M. Delgado, N. Varela, E. Gonzalez-Rey, Vasoactive intestinal peptide protects against beta-amyloid-induced neurodegeneration by inhibiting microglia activation at multiple levels, Glia 56 (2008) 1091–1103.

[113] J. Kakimura, Y. Kitamura, K. Takata, M. Umeki, S. Suzuki, K. Shibagaki, T. Taniguchi, Y. Nomura, P.J. Gebicke-Haerter, M.A. Smith, G. Perry, S. Shimohama, Microglial activation and amyloid-beta clearance induced by exogenous heat-shock proteins, FASEB J. 16 (2002) 601–603.
[114] V. Calabrese, C. Mancuso, A. Ravagna, M. Perluigi, C. Cini, C. De Marco, D.A. Butterfield, A.M. Giuffrida Stella, In vivo induction of heat shock proteins in the substantia nigra following L-DOPA administration is associated with increased activity of mitochondrial complex I and nitrosative stress in rats: regulation by glutathione redox state, J. Neurochem. 101 (2007) 709–717.
[115] F. Bellia, V. Calabrese, F. Guarino, M. Cavallaro, C. Cornelius, V. De Pinto, E. Rizzarelli, Carnosinase levels in aging brain: redox state induction and cellular stress response, Antioxid. Redox Signal. 11 (2009) 2759–2775.
[116] I.R. Brown, Heat shock proteins and protection of the nervous system, Ann. N. Y. Acad. Sci. 1113 (2007) 147–158.
[117] V. Calabrese, S. Calafato, C. Cornelius, C. Mancuso, A. Dinkova-Kostova, Heme oxygenase: a master vitagene involved in cellular stress response, in: A.M. Eleuteri (Ed.), Enzymes and the Cellular Fight Against Oxidation, Research Signpost, Fort P.O., Trivandrum-695 023, Kerala, India, 2008, pp. 37–661, (2).
[118] M.D. Maines, The heme oxygenase system: a regulator of second messenger gases, Annu. Rev. Pharmacol. Toxicol. 37 (1997) 517–554.
[119] V. Calabrese, C. Cornelius, C. Mancuso, E. Barone, S. Calafato, T. Bates, E. Rizzarelli, A.T. Kostova, Vitagenes, dietary antioxidants and neuroprotection in neurodegenerative diseases, Front. Biosci. 14 (2009) 376–397.
[120] H.M. Schipper, Heme oxygenase-1: role in brain aging and neurodegeneration, Exp. Gerontol. 35 (2000) 821–830.
[121] R. Stocker, Y. Yamamoto, A.F. McDonagh, A.N. Glazer, B.N. Ames, Bilirubin is an antioxidant of possible physiological importance, Science 235 (1987) 1043–1046.
[122] S. Doré, M. Takahashi, C.D. Ferris, R. Zakhary, L.D. Hester, D. Guastella, S.H. Snyder, Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 2445–2450.
[123] C. Mancuso, G. Pani, V. Calabrese, Bilirubin: an endogenous scavenger of nitric oxide and reactive nitrogen species, Redox Rep. 11 (2006) 207–213.
[124] G. Scapagnini, C. Colombrita, M. Amadio, V. D'Agata, E. Arcelli, M. Sapienza, A. Quattrone, V. Calabrese, Curcumin activates defensive genes and protects neurons against oxidative stress, Antioxid. Redox Signal. 8 (2006) 395–403.
[125] P.A. Dennery, Regulation and role of heme oxygenase in oxidative injury, Curr. Top. Cell. Regul. 36 (2000) 181–199.
[126] M.D. Maines, P.E.M. Gibbs, 30 some years of heme oxygenase: from a "molecular wrecking ball" to a "mesmerizing" trigger of cellular events, Biochem. Biophys. Res. Commun. 338 (2005) 568–577.
[127] J. Kapitulnik, M.D. Maines, Pleiotropic functions of biliverdin reductase: cellular signaling and generation of cytoprotective and cytotoxic bilirubin, Trends Pharmacol. Sci. 30 (2009) 129–137.
[128] J. Alam, J.L. Cook, How many transcription factors does it take to turn on the heme oxygenase-1 gene? Am. J. Respir. Cell Mol. Biol. 36 (2007) 166–174.
[129] T. Nguyen, P.J. Sherratt, C.B. Pickett, Regulatory mechanism controlling gene expression mediated by the antioxidant response element, Annu. Rev. Pharmacol. Toxicol. 43 (2003) 233–260.
[130] N. Panahian, M. Yoshiura, M.D. Maines, Overexpression of heme oxygenase-1 is neuroprotective in a model of permanent middle cerebral artery occlusion in transgenic mice, J. Neurochem. 72 (1999) 1187–1203.
[131] W.K. McCoubrey, T.J. Huang, M.D. Maines, Heme oxygenase-2 is a hemoprotein and binds heme through heme regulatory motifs that are not involved in heme catalysis, J. Biol. Chem. 272 (1997) 12568–12574.
[132] V. Calabrese, T.E. Bates, C. Mancuso, C. Cornelius, B. Ventimiglia, M.T. Cambria, L. Di Renzo, A. De Lorenzo, A.T. Dinkova-Kostova, Curcumin and the cellular stress response in free radical-related diseases, Mol. Nutr. Food Res. 52 (2008) 1062–1073.
[133] V. Calabrese, M. Perluigi, C. Cornelius, R. Coccia, F. Di Domenico, C. Mancuso, G. Pennini, A.T. Dinkova-Kostova, Phenolics in aging and neurodegenerative disorders, in: C.G. Fraga (Ed.), Phenolic Compounds of Plant Origin and Health: The Biochemistry behind their Nutritional and Pharmacological Value, Wiley & Sons, New York, 2009, pp. 427–451.
[134] R. Motterlini, R. Foresti, R. Bassi, V. Calabrese, J.E. Clark, C.J. Green, Endothelial Heme oxygenase-1 induction by hypoxia: modulation by inducible nitric oxide synthase (iNOS) and S-nitrosothiols, J. Biol. Chem. 275 (2000) 13613–13620.
[135] R. Motterlini, R. Foresti, R. Bassi, C.J. Green, Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress, Free Radic. Biol. Med. 28 (2000) 1303–1312.
[136] G.P. Lim, F. Yang, T. Chu, P. Chen, W. Beech, B. Teter, T. Tran, O. Ubeda, K.H. Ashe, S.A. Frautschy, G.M. Cole, Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease, J. Neurosci. 20 (2000) 5709–5714.
[137] F. Yang, G.P. Lim, A.N. Begum, O.J. Ubeda, M.R. Simmons, S.S. Ambegaokar, P.P. Chen, R. Kayed, C.G. Glabe, S.A. Frautschy, G.M. Cole, Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo, J. Biol. Chem. 280 (2005) 5892–5901.
[138] Y. Li, W. Xu, M.W. McBurney, V.D. Longo, SirT1 inhibition reduces IGF-I/IRS-2/Ras/ERK1/2 signaling and ptotects neurons, Cell Metab. 8 (2008) 38–48.
[139] P. Anand, H.B. Nair, B. Sung, A.B. Kunnumakkara, V.R. Yadav, R.R. Tekmal, B.B. Aggarwal, Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity in vitro and superior bioavailability in vivo, Biochem. Pharmacol. 79 (2010) 330–338.
[140] S.K. Sandur, A. Deorukhkar, M.K. Pandey, A.M. Pabón, S. Shentu, S. Guha, B.B. Aggarwal, S. Krishnan, Curcumin modulates the radiosensitivity of colorectal cancer cells by suppressing constitutive and inducible NF-kappaB activity, Int. J. Radiat. Oncol. Biol. Phys. 75 (2009) 534–542.
[141] T. Kitamuro, K. Takahashi, K. Ogawa, R. Udono-Fujimori, K. Takeda, K. Furuyama, M. Nakayama, J. Sun, H. Fujita, W. Hida, T. Hattori, K. Shirato, K. Igarashi, S. Shibahara, Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells, J. Biol. Chem. 278 (2003) 9125–9133.
[142] Y.H. Chou, F.M. Ho, D.Z. Liu, S.Y. Lin, L.H. Tsai, C.H. Chen, Y.S. Ho, L.F. Hung, Y.C. Liang, The possible role of heat shock factor-1 in the negative regulation of heme oxygenase-1, Int. J. Biochem. Cell Biol. 37 (2005) 604–615.
[143] H.M. Schipper, W. Song, H. Zukor, J.R. Hascalovici, D. Zeligman, Heme oxygenase-1 and neurodegeneration: expanding frontiers of engagement, J. Neurochem. 110 (2009) 469–485.
[144] H. Schulz, Uber Hefegifte, Pflugers Arch. Ges. Physiol. 42 (1888) 517–541.
[145] E.J. Calabrese, Evidence that hormesis represents an "overcompensation" response to a disruption in homeostasis, Ecotoxicol. Environ. Saf. 42 (1999) 135–137.
[146] R.E.J. Mitchel, Low doses of radiation reduce risk in vivo, Dose Response 5 (2007) 1–10.
[147] A. Lacassagne, N.P. Buu-Hoi, G. Rudali, Induction of the carcinogenic action produced by a weakly carcinogenic hydrocarbon on a highly active carcinogenic hydrocarbon, Br. J. Exp. Pathol. 26 (1945) 5–12.
[148] P.J. Sykes, A.A. Morley, A.M. Hooker, The pKZ1 recombination mutation assay: a sensitive assay for low dose studies, Dose Response 4 (2006) 91–105.
[149] D.R. Boreham, J.A. Dolling, C. Somers, J. Quinn, R.E.J. Mitchel, The adaptive response and protection against heritable mutations and fetal malformation, Dose Response 4 (2006) 317–326.
[150] C. Mothersill, C.B. Seymour, Radiation-induced bystander effects and the DNA paradigm: an "out of field" perspective, Mutat. Res. 597 (2006) 5–10.
[151] K. Sakai, T. Nomura, Y. Ina, Enhancement of bio-protective functions by low dose/dose-rate radiation, Dose Response 4 (2006) 327–332.
[152] B.R. Scott, J. DiPalma, Sparsely ionizing diagnostic and natural background radiations are likely preventing cancer and other genomic instability-associated diseases, Dose Response 5 (2006) 230–255.
[153] J.L. Redpath, Suppression of neoplastic transformation in vitro by low doses of low LET radiation, Dose Response 4 (2006) 302–308.
[154] E.J. Calabrese, L.A. Baldwin, Chemical hormesis: its historical foundations as a biological hypothesis, Toxicol. Pathol. 27 (1999) 195–216.
[155] E.J. Calabrese, L.A. Baldwin, Radiation hormesis: its historical foundations as a biological hypothesis, Hum. Exp. Toxicol. 19 (2000) 41–75.
[156] E.J. Calabrese, L.A. Baldwin, Radiation hormesis: the demise of a legitimate hypothesis, Hum. Exp. Toxicol. 19 (2000) 76–84.
[157] E.J. Calabrese, L.A. Baldwin, The marginalization of hormesis, Hum. Exp. Toxicol. 19 (2000) 32–40.
[158] E.J. Calabrese, L.A. Baldwin, Ethanol and hormesis, Crit. Rev. Toxicol. 33 (2003) 407–424.
[159] E.J. Calabrese, Hormesis, in: E. Melnick, B. Everitt (Eds.), Encyclopedia of Quantitative Risk Assessment and Analysis, Wiley, Chichester, 2008, pp. 838–844.
[160] E.J. Calabrese, The road to linearity: shy linearity at low doses became the basis for carcinogen risk assessment, Arch. Toxicol. 83 (2009) 203–225.
[161] E. Szabadi, Model of 2 functionally antagonistic receptor populations activated by same agonist, J. Theor. Biol. 69 (1977) 101–112.
[162] A.R.D. Stebbing, Effects of low metal levels on a clonal hydroid, J. Mar. Biol. Assoc. U.K. 56 (1976) 977–994.
[163] T.D. Luckey, Radiation Hormesis, CRC, Boca Raton, 1992.
[164] E.J. Calabrese, L.A. Baldwiw, Tales of two similar hypothesis: the rise and fall of chemical and radiation hormesis, Hum. Exp. Toxicol. 19 (2000) 85–97.
[165] E.J. Calabrese, R. Blain, The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: an overview, Toxicol. Appl. Pharmacol. 202 (2005) 289–301.
[166] H.Y. Thong, H.I. Maibach, Hormesis (biological effects of low level exposure (BELLE)) and dermatology, Dose Response 6 (2008) 1–15.
[167] D.L. Eaton, C.D. Klaassen, Principles of toxicology, Casarett & Doull's Essentials of Toxicology, Chap 2, The McGraw-Hill Companies, Inc., 2003, pp. 6–20.
[168] E.J. Calabrese, Toxicology rewrites its history and rethinks its future: giving equal focus to both harmful and beneficial effects, Environ. Toxicol. Chem. 30 (2011) 2658–2673.
[169] E.J. Calabrese, L.A. Baldwin, Chemotherapeutics and hormesis, Crit. Rev. Toxicol. 33 (2003) 305–354.
[170] E.J. Calabrese, L.A. Baldwin, Inorganics and hormesis, Crit. Rev. Toxicol. 33 (2003) 215–304.
[171] E.J. Calabrese, L.A. Baldwin, Peptides and hormesis, Crit. Rev. Toxicol. 33 (2003) 355–406.
[172] E.J. Calabrese, K.A. Bachmann, A.J. Bailer, P.M. Bolger, J. Borak, L. Cai, N. Cedergreen, M.G. Cherian, C.C. Chiueh, T.W. Clarkson, R.R. Cook, D.M. Diamond, D.J. Doolittle, M.A. Dorato, S.O. Duke, L. Feinendegen, D.E. Gardner, R.W. Hart, K.L. Hastings, A.W. Hayes, G.R. Hoffmann, J.A. Ives, Z. Jaworowski, T.E. Johnson, W.B. Jonas, N.E. Kaminski, J.G. Keller, J.E. Klaunig, T.B. Knudsen, W.J. Kozumbo, T. Lettieri, S.Z. Liu, A. Maisseu, K.I. Maynard, E.J. Masoro, R.O. McClellan, H.M. Mehendale, C. Mothersill, D.B. Newlin, H.N. Nigg, F.W. Oehme, R.F. Phalen, M.A. Philbert, S.I. Rattan, J.E. Riviere, J. Rodricks, R.M. Sapolsky, B.R. Scott, C. Seymour, D.A. Sinclair, J. Smith-Sonneborn, E.T. Snow, L. Spear, D.E. Stevenson, Y. Thomas, M. Tubiana, G.M. Williams, M.P. Mattson, Biological stress response terminology: integrating the concepts of adaptive response and preconditioning stress within a hormetic dose–response framework, Toxicol. Appl. Pharmacol. 222 (2007) 122–128.

[173] J.F. Flood, G.E. Smith, A. Cherkin, Memory retention-enhancement by cholinergic drug combinations in mice, Gerontologist 22 (1982) 230–231.
[174] J.F. Flood, G.E. Smith, A. Cherkin, Memory retention-potentiation of cholinergic drug-combinations in mice, Neurobiol. Aging 4 (1983) 37–43.
[175] J.F. Flood, G.E. Smith, A. Cherkin, Memory retention-enhancement by synergistic oral cholinergic drug-combination in mice, Gerontologist 24 (1984) 149–158.
[176] J.F. Flood, G.E. Smith, A. Cherkin, Memory enhancement-supra-additive effect of subcutaneous chlolinergic drug-combinations in mice, Psychopharmacology 86 (1985) 61–67.
[177] E.J. Calabrese, Neuroscience and hormesis: overview and general findings, Crit. Rev. Toxicol. 38 (2008) 249–252.
[178] E.J. Calabrese, L.A. Baldwin, Hormesis and high risk groups, Regul. Toxicol. Pharmacol. 35 (2002) 14–428.
[179] E.J. Calabrese, Cancer biology and hormesis: human tumor cell lines commonly display hormetic (biphasic) dose responses, Crit. Rev. Toxicol. 35 (2005) 463–582.
[180] W.A. Randall, C.W. Price, H. Welch, Demonstration of hormesis (increase in fatality rate) by penicillin, Am. J. Public Health 37 (1947) 421–425.
[181] H. Welch, C.W. Price, W.A. Randall, Increase in fatality rate of E. Typhosa for white mice by streptomycin, J. Am. Pharm. 35 (1946) 155–158.
[182] J. Abramowitz, C. Dai, K.K. Hirschi, R.I. Dmitieva, P.A. Doris, L. Liu, J.C. Allen, Ouabain- and marinobufagenin-induced proliferation of human umbilical vein smooth muscle cells and a rat vascular smooth muscle cell lines, A7r5, Circulation 108 (2003) 3048–3053.
[183] S.C. Chueh, J.H. Guh, J. Chen, M.K. Lai, C.M. Teng, Dual effects of ouabain on the regulation of proliferation and apoptosis in human prostatic smooth muscle cells, J. Urol. 166 (2001) 347–353.
[184] B. Halliwell, Biochemistry of oxidative stress, Biochem. Soc. Trans. 35 (2007) 1147–1150.
[185] E.J. Calabrese, E.J. Stanek III, M.A. Nascarella, G.R. Hoffmann, Hormesis predicts low-dose responses better than threshold models, Int. J. Toxicol. 27 (2008) 369–378.
[186] E.J. Calabrese, Another California milestone: the first application of hormesis in litigation and regulation, Int. J. Toxicol. 27 (2008) 31–33.
[187] E.J. Calabrese, Hormesis and medicine, Br. J. Clin. Pharmacol. 66 (2008) 594–617.
[188] V. Calabrese, D. Boyd-Kimball, G. Scapagnini, D.A. Butterfield, Nitric oxide and cellular stress response in brain aging and neurodegenerative disorders: the role of vitagenes, In Vivo 18 (2004) 245–267.
[189] C. Mancuso, T.E. Bates, D.A. Butterfield, S. Calafato, C. Cornelius, A. De Lorenzo, A.T. Dinkova Kostova, V. Calabrese, Mitochondrial dysfunction, free radical generation and cellular stress response in neurodegenerative disorders, Front. Biosci. 12 (2007) 1107–1123.
[190] M.P. Mattson, Hormesis and disease resistance: activation of cellular stress response pathways, Hum. Exp. Toxicol. 27 (2008) 155–162.
[191] T.V. Arumugam, M. Gleichmann, S.C. Tang, M.P. Mattson, Hormesis/preconditioning mechanisms, the nervous system and aging, Ageing Res. Rev. 5 (2006) 165–178.
[192] E.J. Calabrese, Astrocytes: adaptive responses to low doses of neurotoxins, Crit. Rev. Toxicol. 38 (2008) 463–471.
[193] E.J. Calabrese, P-Glycoprotein efflux transporter activity often displays biphasic dose–response relationships, Crit. Rev. Toxicol. 38 (2008) 473–487.
[194] J. Zhao, N. Kobori, J. Aronowski, P.K. Dash, Sulforaphane reduces infarct volume following focal cerebral ischemia in rodents, Neurosci. Lett. 393 (2006) 108–112.
[195] J. Zhao, A.N. Moore, J.B. Redell, P.K. Dash, Enhancing expression of Nrf2-driven genes protects the blood brain barrier after brain injury, J. Neurosci. 27 (2007) 10240–10248.
[196] C.Y. Chen, J.H. Jang, M.H. Li, Y.J. Surh, Resveratrol upregulates heme oxygenase-1 expression via activation of NF-E2-related factor 2 in PC12 cells, Biochem. Biophys. Res. Commun. 331 (2005) 993–1000.
[197] J.D. Hayes, M. McMahon, NRF2 and KEAP1 mutations: permanent activation of an adaptive response in cancer, Trends Biochem. Sci. 34 (2009) 176–188.
[198] M. Kobayashi, M. Yamamoto, Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species, Adv. Enzyme Regul. 46 (2006) 113–140.
[199] H. Motohashi, M. Yamamoto, Nrf2-Keap1 defines a physiologically important stress response mechanism, Trends Mol. Med. 10 (2004) 549–557.
[200] A.T. Dinkova-Kostova, The isothiocyanate sulforaphane inducesthe phase 2 response by signaling of the Keap1-Nrf2-ARE pathway: implications for dietary protection against cancer, in: Y.J. Surh, Z. Dong, E. Cadenas, L. Packer (Eds.), Dietary Modulation of Cell Signaling Pathways, 2008, pp. 205–229.
[201] H.J. Prochaska, M.J. De Long, P. Talalay, On the mechanisms of induction of cancer-protective enzymes: a unifying proposal, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 8232–8236.
[202] H.J. Prochaska, A.B. Santamaria, P. Talalay, Rapid detection of inducers of enzymes that protect against carcinogens, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 2394–2398.
[203] X. Zhao, G. Sun, J. Zhang, R. Strong, P.K. Dash, Y.W. Kan, J.C. Grotta, J. Aronowski, Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage, Stroke 38 (2007) 3280–3286.
[204] S.R. Spencer, C.A. Wilczak, P. Talalay, Induction of glutathione transferases and NAD(P)H:quinone reductase by fumaric acid derivatives in rodent cells and tissues, Cancer Res. 50 (1990) 7871–7875.
[205] C. Mancuso, Heme oxygenase and its products in the nervous system, Antioxid. Redox Signal. 6 (2004) 878–887.
[206] V. Calabrese, C. Colombrita, G. Scapagnini, M. Calvani, A.M. Giuffrida Stella, D.A. Butterfield, Acetylcarnitine and cellular stress response: role in nutritional redox homeostasis and regulation of longevity genes, J. Nutr. Biochem. 17 (2006) 73–88.
[207] R. Stocker, Antioxidant activities of bile pigments, Antioxid. Redox Signal. 6 (2004) 841–849.
[208] C. Mancuso, E. Barone, The heme oxygenase/biliverdin reductase pathway in drug research and development, Curr. Drug Metab. 10 (2009) 579–594.
[209] C. Mancuso, P. Navarra, P. Preziosi, Roles of nitric oxide, carbon monoxide, and hydrogen sulfide in the regulation of the hypothalamic-pituitary-adrenal axis, J. Neurochem. 113 (2010) 563–575.
[210] E. Barone, C. Mancuso, F. Di Domenico, R. Sultana, M.P. Murphy, E. Head, D.A. Butterfield, Biliverdin reductase-A: a novel drug target for atorvastatin in a dog preclinical model of Alzheimer disease, J. Neurochem. (Oct 17, 2011, Electronic publication ahead of print).
[211] C. Mancuso, M. Perluigi, C. Cini, C. De Marco, A.M. Giuffrida Stella, V. Calabrese, Heme oxygenase and cyclooxygenase in the central nervous system: a functional interplay, J. Neurosci. Res. 84 (2006) 1385–1391.
[212] C. Mancuso, G. Scapagnini, D. Curro, A.M. Giuffrida Stella, C. De Marco, D.A. Butterfield, V. Calabrese, Mitochondrial dysfunction, free radical generation and cellular stress response in neurodegenerative disorders, Front. Biosci. 12 (2007) 1107–1123.
[213] F.J. Gerich, F. Funke, B. Hildebrandt, M. Faßhauer, M. Müller, H2O2-mediated modulation of cytosolic signaling and organelle function in rat hippocampus, Pflugers Arch. 458 (2009) 937–952.
[214] A.T. Huddleston, W. Tang, H. Takeshima, S.L. Hamilton, E. Klann, Superoxide-induced potentiation in the hippocampus requires activation of ryanodine receptor type 3 and ERK, J. Neurophysiol. 99 (2008) 1565–1571.
[215] R. Gopalakrishna, U. Gundimeda, J.E. Schiffman, T.H. McNeill, A direct redox regulation of protein kinase C isoenzymes mediates oxidant-induced neuritogenesis in PC12 cells, J. Biol. Chem. 283 (2008) 14430–14444.
[216] L.S. Terada, Specificity in reactive oxidant signaling: think globally, act locally, J. Cell Biol. 174 (2006) 615–623.
[217] M.P. Mattson, Y. Goodman, H. Luo, W. Fu, K. Furukawa, Activation of NF-kappaB protects hippocampal neurons against oxidative stress-induced apoptosis: evidence for induction of manganese superoxide dismutase and suppression of peroxynitrite production and protein tyrosine nitration, J. Neurosci. Res. 49 (1997) 681–697.
[218] A.J. Bruce-Keller, J.W. Geddes, P.E. Knapp, R.W. McFall, J.N. Keller, F.W. Holtsberg, S. Parthasarathy, S.M. Steiner, M.P. Mattson, Anti-death properties of TNF against metabolic poisoning: mitochondrial stabilization by MnSOD, J. Neuroimmunol. 93 (1999) 53–71.
[219] C.T. Ekdahl, Z. Kokaia, O. Lindvall, Brain inflammation and adult neurogenesis: the dual role of microglia, Neuroscience 158 (2009) 1021–1029.
[220] F. Jiang, Z.G. Zhang, M. Katakowski, A.M. Robin, M. Faber, F. Zhang, M. Chopp, Angiogenesis induced by photodynamic therapy in normal rat brains, Photochem. Photobiol. 79 (2004) 494–498.
[221] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, fourth ed. Oxford University Press, 2007.
[222] P. Talalay, Chemoprotection against cancer by induction of phase 2 enzymes, Biofactors 12 (2000) 5–11.
[223] X. Gao, A.T. Dinkova-Kostova, P. Talalay, Powerful and prolonged protection of human retinal pigment epithelial cells, keratinocytes, and mouse leukemia cells against oxidative damage: the indirect antioxidant effects of sulforaphane, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 15221–15226.
[224] A.T. Dinkova-Kostova, W.D. Holtzclaw, N. Wakabayashi, Keap1, the sensor for electrophiles and oxidants that regulates the phase 2 response, is a zinc metalloprotein, Biochemistry 44 (2005) 6889–6899.
[225] A.T. Dinkova-Kostova, P. Talalay, Direct and indirect antioxidant properties of inducers of cytoprotective proteins, Mol. Nutr. Food Res. 52 (2008) S128–S138.
[226] T.H. Rushmore, C.B. Pickett, Transcriptional regulation of the rat glutathione S-transferase Ya subunit gene. Characterization of a xenobiotic-responsive element controlling inducible expression by phenolic antioxidants, J. Biol. Chem. 265 (1990) 14648–14653.
[227] R.S. Friling, A. Bensimon, Y. Tichauer, V. Daniel, Xenobiotic-inducible expression of murine glutathione S-transferase Ya subunit gene is controlled by an electrophile-responsive element, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 6258–6262.
[228] T.H. Rushmore, M.R. Morton, C.B. Pickett, The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity, J. Biol. Chem. 266 (1991) 11632–11639.
[229] P. Nioi, M. McMahon, K. Itoh, M. Yamamoto, J.D. Hayes, Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD(P)H: quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence, Biochem. J. 374 (2003) 337–348.
[230] T. Nguyen, P. Nioi, C.B. Pickett, The nrf2-antioxidant response element signaling pathway and its activation by oxidative stress, J. Biol. Chem. 284 (2009) 13291–13295.
[231] J.D. Hayes, M. McMahon, S. Chowdhry, A.T. Dinkova-Kostova, Cancer chemoprevention mechanisms mediated through the Keap1–Nrf2 pathway, Antioxid. Redox Signal. 13 (2010) 1713–1748.
[232] K. Itoh, T. Chiba, S. Takahashi, T. Ishii, K. Igarashi, Y. Katoh, T. Oyake, N. Hayashi, K. Satoh, I. Hatayama, M. Yamamoto, Y. Nabeshima, An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements, Biochem. Biophys. Res. Commun. 236 (1997) 313–322.

[233] H. Motohashi, T. O'Connor, F. Katsuoka, J.D. Engel, M. Yamamoto, Integration and diversity of the regulatory network composed of Maf and CNC families of transcription factors, Gene 294 (2002) 1–12.
[234] K. Itoh, N. Wakabayashi, Y. Katoh, T. Ishii, K. Igarashi, J.D. Engel, M. Yamamoto, Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain, Genes Dev. 13 (1999) 76–86.
[235] S.B. Cullinan, J.D. Gordan, J. Jin, J.W. Harper, J.A. Diehl, The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase, Mol. Cell. Biol. 24 (2004) 8477–8486.
[236] A. Kobayashi, M.I. Kang, H. Okawa, M. Ohtsuji, Y. Zenke, T. Chiba, K. Igarashi, M. Yamamoto, Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2, Mol. Cell. Biol. 24 (2004) 7130–7139.
[237] D.D. Zhang, S.C. Lo, J.V. Cross, D.J. Templeton, M. Hannink, Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex, Mol. Cell. Biol. 24 (2004) 10941–10953.
[238] M. Furukawa, Y. Xiong, BTB protein Keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin 3-Roc1 ligase, Mol. Cell. Biol. 25 (2005) 162–171.
[239] P. Talalay, M.J. De Long, H.J. Prochaska, Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 8261–8265.
[240] S.R. Spencer, L.A. Xue, E.M. Klenz, P. Talalay, The potency of inducers of NAD(P)H:(quinone-acceptor) oxidoreductase parallels their efficiency as substrates for glutathione transferases. Structural and electronic correlations, Biochem. J. 273 (1991) 711–717.
[241] T. Prestera, Y. Zhang, S.R. Spencer, C.A. Wilczak, P. Talalay, The electrophile counterattack response: protection against neoplasia and toxicity, Adv. Enzyme Regul. 33 (1993) 281–296.
[242] A.T. Dinkova-Kostova, M.A. Massiah, R.E. Bozak, R.J. Hicks, P. Talalay, Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3404–3409.
[243] A.T. Dinkova-Kostova, J.W. Fahey, P. Talalay, Chemical structures of inducers of nicotinamide quinoneoxidoreductase 1 (NQO1), Methods Enzymol. 382 (2004) 423–448.
[244] A.T. Dinkova-Kostova, W.D. Holtzclaw, R.N. Cole, K. Itoh, N. Wakabayashi, Y. Katoh, M. Yamamoto, P. Talalay, Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 11908–11913.
[245] T.W. Kensler, N. Wakabayashi, S. Biswal, Cell survival responses to environmental stresses via the Keap1–Nrf2–ARE pathway, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 89–116.
[246] L. Baird, A.T. Dinkova-Kostova, The cytoprotective role of the Keap1–Nrf2 pathway, Arch. Toxicol. 85 (2011) 241–272.
[247] R.A. Dixon, Natural products and plant disease resistance, Nature 411 (2001) 843–847.
[248] B.A. Halkier, J. Gershenzon, Biology and biochemistry of glucosinolates, Annu. Rev. Plant Biol. 7 (2006) 303–333.
[249] H. Weber, A. Chételat, P. Reymond, E.E. Farmer, Selective and powerful stress gene expression in Arabidopsis in response to malondialdehyde, Plant J. 37 (2004) 877–888.
[250] T. Satoh, S.A. Lipton, Redox regulation of neuronal survival mediated by electrophilic compounds, Trends Neurosci. 30 (2007) 37–45.
[251] T. Satoh, S. Saitoh, M. Hosaka, K. Kosaka, Simple ortho- and para-hydroquinones as compounds neuroprotective against oxidative stress in a manner associated with specific transcriptional activation, Biochem. Biophys. Res. Commun. 379 (2009) 537–541.
[252] R.V. Bensasson, V. Zoete, A.T. Dinkova-Kostova, P. Talalay, Two-step mechanism of induction of the gene expression of a prototypic cancer-protective enzyme by diphenols, Chem. Res. Toxicol. 21 (2008) 805–812.
[253] X.J. Wang, J.D. Hayes, L.G. Higgins, C.R. Wolf, A.T. Dinkova-Kostova, Activation of the NRF2 signaling pathway by copper-mediated redox cycling of para- and ortho-hydroquinones, Chem. Biol. 17 (2010) 75–85.
[254] A.T. Dinkova-Kostova, X.J. Wang, Induction of the Keap1/Nrf2/ARE pathway by oxidizable diphenols, Chem. Biol. Interact. 192 (2011) 101–106.
[255] S.A. Lipton, Pathologically activated therapeutics for neuroprotection, Nat. Rev. Neurosci. 8 (2007) 803–808.
[256] K. Kosaka, T. Yokoi, Carnosic acid, a component of rosemary (*Rosmarinus officinalis* L.), promotes synthesis of nerve growth factor in T98G human glioblastoma cells, Biol. Pharm. Bull. 26 (2003) 1620–1622.
[257] E.H. Doolaege, K. Raes, F. De Vos, R. Verhé, S. De Smet, Absorption, distribution and elimination of carnosic acid, a natural antioxidant from *Rosmarinus officinalis*, in rats, Plant Foods Hum. Nutr. 66 (2011) 196–202.
[258] T. Satoh, K. Kosaka, K. Itoh, A. Kobayashi, M. Yamamoto, Y. Shimojo, C. Kitajima, J. Cui, J. Kamins, S. Okamoto, M. Izumi, T. Shirasawa, S.A. Lipton, Carnosic acid, a catechol-type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1, J. Neurochem. 104 (2008) 1116–1131.
[259] Y. Tamaki, T. Tabuchi, T. Takahashi, K. Kosaka, T. Satoh, Activated glutathione metabolism participates in protective effects of carnosic acid against oxidative stress in neuronal HT22 cells, Planta Med. 76 (2010) 683–688.
[260] K. Kosaka, J. Mimura, K. Itoh, T. Satoh, Y. Shimojo, C. Kitajima, A. Maruyama, M. Yamamoto, T. Shirasawa, Role of Nrf2 and p62/ZIP in the neurite outgrowth by carnosic acid in PC12h cells, J. Biochem. 147 (2010) 73–81.
[261] J. Mimura, K. Kosaka, A. Maruyama, T. Satoh, N. Harada, H. Yoshida, K. Satoh, M. Yamamoto, K. Itoh, Nrf2 regulates NGF mRNA induction by carnosic acid in T98G glioblastoma cells and normal human astrocytes, J. Biochem. 150 (2011) 209–217.
[262] S.R. Vaka, S.N. Murthy, M.A. Repka, T. Nagy, Upregulation of endogenous neurotrophin levels in the brain by intranasal administration of carnosic acid, J. Pharm. Sci. 100 (2011) 3139–3145.
[263] R. Luft, D. Ikkos, G. Palmieri, L. Ernster, A. Afzelius, A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical and morphological study, J. Clin. Invest. 41 (1962) 1776–1804.
[264] Y. Zhang, P. Talalay, C.G. Cho, G.H. Posner, A major inducer of anticarcinogenic protective enzymes from broccoli: isolation and elucidation of structure, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 2399–2403.
[265] A.D. Kraft, D.A. Johnson, J.A. Johnson, Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult, J. Neurosci. 24 (2004) 1101–1112.
[266] C.A. Danilov, K. Chandrasekaran, J. Racz, L. Soane, C. Zielke, G. Fiskum, Sulforaphane protects astrocytes against oxidative stress and delayed death caused by oxygen and glucose deprivation, Glia 57 (2009) 645–656.
[267] P. Bergström, H.C. Andersson, Y. Gao, J.O. Karlsson, C. Nodin, M.F. Anderson, M. Nilsson, O. Hammarsten, Repeated transient sulforaphane stimulation in astrocytes leads to prolonged Nrf2-mediated gene expression and protection from superoxide-induced damage, Neuropharmacology 60 (2011) 343–353.
[268] A.M. Vincent, K. Kato, L.L. McLean, M.E. Soules, E.L. Feldman, Sensory neurons and schwann cells respond to oxidative stress by increasing antioxidant defense mechanisms, Antioxid. Redox Signal. 11 (2009) 425–438.
[269] A. Siebert, V. Desai, K. Chandrasekaran, G. Fiskum, M.S. Jafri, Nrf2 activators provide neuroprotection against 6-hydroxydopamine toxicity in rat organotypic nigrostriatal cocultures, J. Neurosci. Res. 87 (2009) 1659–1669.
[270] D. Vauzour, M. Buonfiglio, G. Corona, J. Chirafisi, K. Vafeiadou, C. Angeloni, S. Hrelia, P. Hrelia, S.P. Spencer, Sulforaphane protects cortical neurons against 5-S-cysteinyl-dopamine-induced toxicity through the activation of ERK1/2, Nrf-2 and the upregulation of detoxification enzymes, Mol. Nutr. Food Res. 54 (2010) 532–542.
[271] L. Soane, W. Li Dai, G. Fiskum, L.L. Bambrick, Sulforaphane protects immature hippocampal neurons against death caused by exposure to hemin or to oxygen and glucose deprivation, J. Neurosci. Res. 88 (2010) 1355–1363.
[272] G. Chang, Y. Guo, Y. Jia, W. Duan, B. Li, J. Yu, C. Li, Protective effect of combination of sulforaphane and riluzole on glutamate-mediated excitotoxicity, Biol. Pharm. Bull. 33 (2010) 1477–1483.
[273] A.I. Rojo, P. Rada, J. Egea, A.O. Rosa, M.G. López, A. Cuadrado, Functional interference between glycogen synthase kinase-3 beta and the transcription factor Nrf2 in protection against kainate-induced hippocampal cell death, Mol. Cell. Neurosci. 39 (2008) 125–132.
[274] T. Greco, G. Fiskum, Brain mitochondria from rats treated with sulforaphane are resistant to redox-regulated permeability transition, J. Bioenerg. Biomembr. 42 (2010) 491–497.
[275] J. Zhao, A.N. Moore, G.L. Clifton, P.K. Dash, Sulforaphane enhances aquaporin-4 expression and decreases cerebral edema following traumatic brain injury, J. Neurosci. Res. 82 (2005) 499–506.
[276] X. Zhao, S. Song, G. Sun, R. Strong, J. Zhang, J.C. Grotta, J. Aronowski, Neuroprotective role of haptoglobin after intracerebral hemorrhage, J. Neurosci. 29 (2009) 15819–15827.
[277] G. Chen, Q. Fang, J. Zhang, D. Zhou, Z. Wang, Role of the Nrf2-ARE pathway in early brain injury after experimental subarachnoid hemorrhage, J. Neurosci. Res. 89 (2011) 515–523.
[278] Y. Hong, W. Yan, S. Chen, C.R. Sun, J.M. Zhang, The role of Nrf2 signaling in the regulation of antioxidants and detoxifying enzymes after traumatic brain injury in rats and mice, Acta Pharmacol. Sin. 31 (2010) 1421–1430.
[279] P.K. Dash, J. Zhao, S.A. Orsi, M. Zhang, A.N. Moore, Sulforaphane improves cognitive function administered following traumatic brain injury, Neurosci. Lett. 460 (2009) 103–107.
[280] Z. Ping, W. Liu, Z. Kang, J. Cai, Q. Wang, N. Cheng, S. Wang, S. Wang, J.H. Zhang, X. Sun, Sulforaphane protects brains against hypoxic–ischemic injury through induction of Nrf2-dependent phase 2 enzyme, Brain Res. 1343 (2010) 178–185.
[281] A. Wierinckx, J. Brevé, D. Mercier, M. Schultzberg, B. Drukarch, A.M. Van Dam, Detoxication enzyme inducers modify cytokine production in rat mixed glial cells, J. Neuroimmunol. 166 (2005) 132–143.
[282] N.G. Innamorato, A.I. Rojo, A.J. Garcìa-Yagüe, M. Yamamoto, M.L. de Ceballos, A. Cuadrado, The transcription factor Nrf2 is a therapeutic target against brain inflammation, J. Immunol. 181 (2008) 680–689.
[283] A.I. Rojo, N.G. Innamorato, A.M. Martín-Moreno, M.L. De Ceballos, M. Yamamoto, A. Cuadrado, Nrf2 regulates microglial dynamics and neuroinflammation in experimental Parkinson's disease, Glia 58 (2010) 588–598.
[284] N.G. Innamorato, A. Jazwa, A.I. Rojo, C. García, J. Fernández-Ruiz, A. Grochot-Przeczek, A. Stachurska, A. Jozkowicz, J. Dulak, A. Cuadrado, Different susceptibility to the Parkinson's toxin MPTP in mice lacking the redox master regulator Nrf2 or its target gene heme oxygenase-1, PLoS One 5 (2010) 11838.
[285] A. Jazwa, A.I. Rojo, N.G. Innamorato, M. Hesse, J. Fernández-Ruiz, A. Cuadrado, Pharmacological targeting of the transcription factor nrf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism, Antioxid. Redox Signal. 14 (2011) 2347–2360.
[286] D. Kim, B. You, E.K. Jo, S.K. Han, M.I. Simon, S.J. Lee, NADPH oxidase 2-derived reactive oxygen species in spinal cord microglia contribute to peripheral nerve

injury-induced neuropathic pain, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 14851–14856.
[287] L. Mao, H. Wang, X. Wang, H. Liao, X. Zhao, Transcription factor nrf2 protects the spinal cord from inflammation produced by spinal cord injury, J. Surg. Res. 170 (2011) 105–115.
[288] X. Wang, J.P. De Rivero Vaccari, H. Wang, P. Diaz, R. German, A. Marcillo, R.W. Keane, Activation of the nuclear factor E2-related factor 2/antioxidant response element pathway is neuroprotective following spinal cord injury, J. Neurotrauma (Sep 21, 2011, Electronic publication ahead of print).
[289] T. Toyama, Y. Shinkai, A. Yasutake, K. Uchida, M. Yamamoto, Y. Kumagai, Isothiocyanates reduce mercury accumulation via an Nrf2-dependent mechanism during exposure of mice to methylmercury, Environ. Health Perspect. 119 (2011) 1117–1122.
[290] A. Begleiter, K. Sivananthan, T.J. Curphey, R.P. Bird, Induction of NAD(P)H quinone: oxidoreductase1 inhibits carcinogen-induced aberrant crypt foci in colons of Sprague–Dawley rats, Cancer Epidemiol. Biomarkers Prev. 12 (2003) 566–572.
[291] S. Schilling, S. Goelz, R. Linker, F. Luehder, R. Gold, Fumaric acid esters are effective in chronic experimental autoimmune encephalomyelitis and suppress macrophage infiltration, Clin. Exp. Immunol. 145 (2006) 101–107.
[292] G. Ellrichmann, E. Petrasch-Parwez, D.H. Lee, C. Reick, L. Arning, C. Saft, R. Gold, R.A. Linker, Efficacy of fumaric acid esters in the R6/2 and YAC128 models of Huntington's disease, PLoS One 6 (2011) 16172.
[293] R.A. Linker, D.H. Lee, S. Ryan, A.M. van Dam, R. Conrad, P. Bista, W. Zeng, X. Hronowsky, A. Buko, S. Chollate, G. Ellrichmann, W. Brück, K. Dawson, S. Goelz, S. Wiese, R.H. Scannevin, M. Lukashev, R. Gold, Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway, Brain 134 (2011) 678–692.
[294] C. Nieboer, D. de Hoop, P.N. Langendijk, A.C. van Loenen, J. Gubbels, Fumaric acid therapy in psoriasis: a double-blind comparison between fumaric acid compound therapy and monotherapy with dimethylfumaric acid ester, Dermatologica 181 (1990) 33–37.
[295] P.J. Altmeyer, U. Matthes, F. Pawlak, K. Hoffmann, P.J. Frosch, P. Ruppert, S.W. Wassilew, T. Horn, H.W. Kreysel, G. Lutz, Antipsoriatic effect of fumaric acid derivatives. Results of a multicenter double-blind study in 100 patients, J. Am. Acad. Dermatol. 30 (1994) 977–981.
[296] S. Zlotkin, A new approach to control of anemia in “at risk” infants and children around the world, Ryley-Jeffs memorial lecture, Can. J. Diet. Pract. Res. 65 (2004) 136–138.
[297] S. Schimrigk, N. Brune, K. Hellwig, C. Lukas, B. Bellenberg, M. Rieks, V. Hoffmann, D. Pöhlau, H. Przuntek, Oral fumaric acid esters for the treatment of active multiple sclerosis: an open-label, baseline-controlled pilot study, Eur. J. Neurol. 13 (2006) 604–610.
[298] L. Kappos, R. Gold, D.H. Miller, D.G. Macmanus, E. Havrdova, V. Limmroth, C.H. Polman, K. Schmierer, T.A. Yousry, M. Yang, M. Eraksoy, E. Meluzinova, I. Rektor, K.T. Dawson, A.W. Sandrock, G.N. O'Neill, BG-12 phase IIb study investigators. Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study, Lancet 372 (2008) 1463–1472.
[299] Trial watch: phase III success for Biogen's oral multiple sclerosis therapy, Nat. Rev. Drug Discov. 10 (2011) 404.
[300] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W. Beecher, H.H. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon, J.M. Pezzuto, Cancer chemopreventive activity of resveratrol, a natural product derived from grapes, Science 275 (1997) 218–220.
[301] G.J. Soleas, E.P. Diamandis, D.M. Goldberg, Resveratrol: a molecule whose time has come? And gone? Clin. Biochem. 30 (1997) 91–113.
[302] J.M. Pezzuto, The phenomenon of resveratrol: redefining the virtues of promiscuity, Ann. N. Y. Acad. Sci. 1215 (2011) 123–130.
[303] J.H. Jang, Y.J. Surh, Protective effects of resveratrol on hydrogen peroxide-induced apoptosis in rat pheochromocytoma (PC12) cells, Mutat. Res. 496 (2001) 181–190.
[304] A. Conte, S. Pellegrini, D. Tagliazucchi, Effect of resveratrol and catechin on PC12 tyrosine kinase activities and their synergistic protection from beta-amyloid toxicity, Drugs Exp. Clin. Res. 29 (2003) 243–255.
[305] A.Y. Sun, Y.M. Chen, M. James-Kracke, P. Wixom, Y. Cheng, Ethanol-induced cell death by lipid peroxidation in PC12 cells, Neurochem. Res. 22 (1997) 1187–1192.
[306] B. Draczynska-Lusiak, Y.M. Chen, A.Y. Sun, Oxidized lipoproteins activate NF-kappaB binding activity and apoptosis in PC12 cells, Neuroreport 9 (1998) 527–532.
[307] B. Draczynska-Lusiak, A. Doung, A.Y. Sun, Oxidized lipoproteins may play a role in neuronal cell death in Alzheimer disease, Mol. Chem. Neuropathol. 33 (1998) 139–148.
[308] M.A. Siddiqui, M.P. Kashyap, V. Kumar, A.A. Al-Khedhairy, J. Musarrat, A.B. Pant, Protective potential of trans-resveratrol against 4-hydroxynonenal induced damage in PC12 cells, Toxicol. In Vitro 24 (2010) 1592–1598.
[309] M. Agrawal, V. Kumar, M.P. Kashyap, V.K. Khanna, G.S. Randhawa, A.B. Pant, Ischemic insult induced apoptotic changes in PC12 cells: protection by trans resveratrol, Eur. J. Pharmacol. 666 (2011) 5–11.
[310] S. Chanvitayapongs, B. Draczynska-Lusiak, A.Y. Sun, Amelioration of oxidative stress by antioxidants and resveratrol in PC12 cells, Neuroreport 8 (1997) 1499–1502.
[311] Y. Wu, X. Li, J.X. Zhu, W. Xie, W. Le, Z. Fan, J. Jankovic, T. Pan, Resveratrol-activated AMPK/SIRT1/autophagy in cellular models of Parkinson's disease, Neurosignals 19 (2011) 163–174.
[312] C. Rivière, T. Richard, L. Quentin, S. Krisa, J.M. Mérillon, J.P. Monti, Inhibitory activity of stilbenes on Alzheimer's beta-amyloid fibrils in vitro, Bioorg. Med. Chem. 15 (2007) 1160–1167.
[313] A.Q. dos Santos, P. Nardin, C. Funchal, L.M. de Almeida, M.C. Jacques-Silva, S.T. Wofchuk, C.A. Gonçalves, C. Gottfried, Resveratrol increases glutamate uptake and glutamine synthetase activity in C6 glioma cells, Arch. Biochem. Biophys. 453 (2006) 161–167.
[314] L.M. de Almeida, C.C. Piñeiro, M.C. Leite, G. Brolese, F. Tramontina, A.M. Feoli, C. Gottfried, C.A. Gonçalves, Resveratrol increases glutamate uptake, glutathione content, and S100B secretion in cortical astrocyte cultures, Cell. Mol. Neurobiol. 27 (2007) 661–668.
[315] L.M. de Almeida, M.C. Leite, A.P. Thomazi, C. Battu, P. Nardin, L.S. Tortorelli, C. Zanotto, T. Posser, S.T. Wofchuk, R.B. Leal, C.A. Gonçalves, C. Gottfried, Resveratrol protects against oxidative injury induced by H2O2 in acute hippocampal slice preparations from Wistar rats, Arch. Biochem. Biophys. 480 (2008) 27–32.
[316] P. Maher, R. Dargusch, L. Bodai, P.E. Gerard, J.M. Purcell, J.L. Marsh, ERK activation by the polyphenols fisetin and resveratrol provides neuroprotection in multiple models of Huntington's disease, Hum. Mol. Genet. 20 (2011) 261–270.
[317] J. Pallos, L. Bodai, T. Lukacsovich, J.M. Purcell, J.S. Steffan, L.M. Thompson, J.L. Marsh, Inhibition of specific HDACs and sirtuins suppresses pathogenesis in a *Drosophila* model of Huntington's disease, Hum. Mol. Genet. 17 (2008) 3767–3775.
[318] D. Albani, L. Polito, A. Signorini, G. Forloni, Neuroprotective properties of resveratrol in different neurodegenerative disorders, Biofactors 36 (2010) 370–376.
[319] C. Liu, Z. Shi, L. Fan, C. Zhang, K. Wang, B. Wang, Resveratrol improves neuron protection and functional recovery in rat model of spinal cord injury, Brain Res. 1374 (2011) 100–109.
[320] Y.B. Yang, Y.J. Piao, Effects of resveratrol on secondary damages after acute spinal cord injury in rats, Acta Pharmacol. Sin. 24 (2003) 703–710.
[321] U. Kiziltepe, N.N. Turan, U. Han, A.T. Ulus, F. Akar, Resveratrol, a red wine polyphenol, protects spinal cord from ischemia–reperfusion injury, J. Vasc. Surg. 40 (2004) 138–145.
[322] S. Kaplan, G. Bisleri, J.A. Morgan, F.H. Cheema, M.C. Oz, Resveratrol, a natural red wine polyphenol, reduces ischemia–reperfusion-induced spinal cord injury, Ann. Thorac. Surg. 80 (2005) 2242–2249.
[323] O. Ates, S. Cayli, E. Altinoz, I. Gurses, N. Yucel, A. Kocak, S. Yologlu, Y. Turkoz, Effects of resveratrol and methylprednisolone on biochemical, neurobehavioral and histopathological recovery after experimental spinal cord injury, Acta Pharmacol. Sin. 27 (2006) 1317–1325.
[324] S.S. Huang, M.C. Tsai, C.L. Chih, L.M. Hung, S.K. Tsai, Resveratrol reduction of infarct size in Long–Evans rats subjected to focal cerebral ischemia, Life Sci. 69 (2001) 1057–1065.
[325] S.K. Tsai, L.M. Hung, Y.T. Fu, H. Cheng, M.W. Nien, H.Y. Liu, F.B. Zhang, S.S. Huang, Resveratrol neuroprotective effects during focal cerebral ischemia injury via nitric oxide mechanism in rats, J. Vasc. Surg. 46 (2007) 346–353.
[326] J. Ren, C. Fan, N. Chen, J. Huang, Q. Yang, Resveratrol pretreatment attenuates cerebral ischemic injury by upregulating expression of transcription factor Nrf2 and HO-1 in rats, Neurochem. Res. 36 (2011) 2352–2362.
[327] A. Kumar, C.K. Singh, H. Lavoie, D. Dipette, U. Singh, Resveratrol restores Nrf2 level and prevents ethanol-induced toxic effects in the cerebellum of a rodent model of Fetal Alcohol Spectrum Disorders, Mol. Pharmacol. 80 (2011) 446–457.
[328] H. Nakagawa, Y. Kiyozuka, Y. Uemura, H. Senzaki, N. Shikata, K. Hioki, A. Tsubura, Resveratrol inhibits human breast cancer cell growth and may mitigate the effect of linoleic acid, a potent breast cancer cell stimulator, J. Cancer Res. Clin. Oncol. 127 (2001) 258–264.
[329] J.P. Basly, F. Marre-Fournier, J.C. Le Bail, G. Habrioux, A.J. Chulia, Estrogenic/antiestrogenic and scavenging properties of (E)- and (Z)-resveratrol, Life Sci. 66 (1999) 769–777.
[330] E. Schmitt, L. Lehmann, M. Metzler, H. Stopper, Hormonal and genotoxic activity of resveratrol, Toxicol. Lett. 136 (2002) 133–142.
[331] R. Liu, G. Serrero, Resveratrol, a natural product derived from grape, exhibits antiestrogenic activity and inhibits the growth of human breast cancer cells, J. Cell. Physiol. 179 (1999) 297–304.
[332] B.D. Gehm, J.M. Mcandrews, P.Y. Chien, J.L. Jameson, Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 14138–14143.
[333] E. Pozo-Guisado, M.J. Lorenzo-Benayas, P.M. Fernandez-Salguero, Resveratrol modulates the phosphoinositide 3-kinase pathway through an estrogen receptor a-dependent mechanism: relevance in cell proliferation, Int. J. Cancer 109 (2004) 167–173.
[334] Y. Li, J. Liu, X. Liu, K. Xing, Y. Wang, F. Li, L. Yao, Resveratrol-induced cell inhibition of growth and apoptosis in MCF7 human breast cancer cells are associated with modulation of phosphorylated Akt and caspase-9, Appl. Biochem. Biotechnol. 135 (2006) 181–192.
[335] S. Vyas, Y. Asmerom, D.D. De Leon, Resveratrol regulates insulin-like growth factor-II in breast cancer cells, Endocrinology 146 (2005) 4224–4233.
[336] T.C. Hsieh, J.M. Wu, Differential effects on growth, cell cycle arrest, and induction of apoptosis by resveratrol in human prostate cancer cell lines, Exp. Cell Res. 249 (1999) 109–115.
[337] N. Kuwajerwala, E. Cifuentes, S. Gautam, M. Menon, E.R. Barrack, G.P. Veer Reddy, Resveratrol induces prostate cancer cell entry into S phase and inhibits DNA synthesis, Cancer Res. 62 (2002) 2488–2492.
[338] D.A. Benitez, E. Pozo-Guisado, A. Alvarez-Barrientos, P.M. Fernandez-Salguero, E.A. Castellón, Mechanisms involved in resveratrol-induced apoptosis and cell cycle arrest in prostate cancer-derived cell lines, J. Androl. 28 (2007) 282–293.

[339] T.T. Wang, T.S. Hudson, T.C. Wang, C.M. Remsberg, N.M. Davies, Y. Takahashi, Y.S. Kim, H. Seifried, B.T. Vinyard, S.N. Perkins, S.D. Hursting, Differential effects of resveratrol on androgen-responsive LNCaP human prostate cancer cells in vitro and in vivo, Carcinogenesis 29 (2008) 2001–2010.
[340] S.K. Lee, W. Zhang, B.J.S. Sanderson, Selective growth inhibition of human leukemia and human lymphoblastoid cells by resveratrol via cell cycle arrest and apoptosis induction, J. Agric. Food Chem. 56 (2008) 7572–7577.
[341] D. Bernhard, W. Schwaiger, R. Crazzolara, I. Tinhofer, R. Kofler, A. Csordas, Enhanced MTT-reducing activity under growth inhibition by resveratrol in CEM-C7H2 lymphocytic leukemia cells, Cancer Lett. 195 (2003) 193–199.
[342] B. Szende, E. Tyihak, Z. Kiraly-Veghely, Dose-dependent effect of resveratrol on proliferation and apoptosis in endothelial and tumor cell cultures, Exp. Mol. Med. 32 (2000) 88–92.
[343] C. Hope, K. Planutis, M. Planutiene, M.P. Moyer, K.S. Johal, J. Woo, C. Santoso, J.A. Hanson, R.F. Holcombe, Low concentrations of resveratrol inhibit Wnt signal throughput in colon-derived cells: implications for colon cancer prevention, Mol. Nutr. Food Res. 52 (2008) S52–S61.
[344] E. Sexton, C. Van Themsche, K. Leblanc, S. Parent, P. Lemoine, E. Asselin, Resveratrol interferes with AKT activity and triggers apoptosis in human uterine cancer cells, Mol. Cancer 5 (2006) 45–58.
[345] Y. Kimura, H. Okuda, Resveratrol isolated from *Polygonum cuspidatum* root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice, J. Nutr. 131 (2001) 1844–1849.
[346] J. Gu, C.Q. Wang, H.H. Fan, H.Y. Ding, X.L. Xie, Y.M. Xu, B.Y. Wang, D.J. Huang, Effects of resveratrol on endothelial progenitor cells and their contributions to reendothelialization in intima-injured rats, J. Cardiovasc. Pharmacol. 47 (2006) 711–721.
[347] L. Xia, X.X. Wang, X.S. Hu, x.g. Guo, Y.P. Shang, H.J. Chen, C.L. Zeng, F.R. Zhang, J.Z. Chen, Resveratrol reduces endothelial progenitor cells senescence through augmentation of telomerase activity by Akt-dependent mechanisms, Br. J. Pharmacol. 155 (2008) 387–394.
[348] K. In, J. Park, H. Park, Resveratrol at high doses acts as an apoptotic inducer in endothelial cells, Cancer Res. Treat. 38 (2006) 48–53.
[349] K. Subbaranmaiah, W.J. Chung, P. Michaluart, N. Telang, T. Tanabe, H. Inoue, M. Jang, J.M. Pezzuto, A.J. Dannenberg, Resveratrol inhibits cyclo-oxigenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells, J. Biol. Chem. 273 (1998) 21875–21882.
[350] J. Martinez, J.J. Moreno, Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production, Biochem. Pharmacol. 59 (2000) 865–870.
[351] R. Falchetti, M.P. Fuggetta, G. Lanzilli, M. Tricarico, G. Ravagnan, Effects of resveratrol on human immune cell function, Life Sci. 70 (2001) 81–96.
[352] X. Gao, Y.X. Xu, N. Janakiraman, R.A. Chapman, S.C. Gautam, Immunomodulatory activity of resveratrol: suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production, Biochem. Pharmacol. 62 (2001) 1299–1308.
[353] X. Gao, D. Deeb, J. Media, G. Divine, H. Jiang, R.A. Chapman, S.C. Gautam, Immunomodulatory activity of resveratrol: discrepant in vitro and in vivo immunological effects, Biochem. Pharmacol. 66 (2003) 2427–2435.
[354] S.H. Juan, T.H. Cheng, H.C. Lin, Y.L. Chu, W.S. Lee, Mechanism of concentration-dependent induction of heme oxygenase-1 by resveratrol in human aortic smooth muscle cells, Biochem. Pharmacol. 69 (2005) 41–48.
[355] J. Dudley, S. Das, S. Mukherjee, D.K. Das, Resveratrol, a unique phytoalexin present in red wine, delivers either survival signal or death signal to the ischemic myocardium depending on dose, J. Nutr. Biochem. 20 (2009) 443–452.
[356] Z. Dai, Y. Li, L.D. Quarles, T. Song, W. Pan, H. Zhou, Z. Xiao, Resveratrol enhances proliferation and osteoblastic differentiation in human mesenchymal stem cells via ER-dependent ERK1/2 activation, Phytomedicine 14 (2007) 806–814.
[357] A. Dey, P. Guha, S. Chattopadhyay, S.K. Bandyopadhyay, Biphasic activity of resveratrol on indomethacin-induced gastric ulcers, Biochem. Biophys. Res. Commun. 381 (2009) 90–95.
[358] C. Alarcón de la Lastra, Commentary on 'resveratrol commonly displays hormesis: occurrence and biomedical significance' by Calabrese et al, Hum. Exp. Toxicol. 29 (2010) 1021–1023.
[359] L. Kedzierski, J.M. Curtis, M. Kaminska, J. Jodynis-Liebert, M. Murias, In vitro antileishmanial activity of resveratrol and its hydroxylated analogues against Leishmania major promastigotes and amastigotes, Parasitol. Res. 102 (2007) 91–97.
[360] S. Ozkoc, S. Tuncay, S.B. Delibas, C. Akisu, In vitro effects of resveratrol on *Trichinella spiralis*, Parasitol. Res. 105 (2009) 1139–1143.
[361] D.G. Lindsay, Commentary on 'resveratrol commonly displays hormesis: occurrence and biomedical significance', Hum. Exp. Toxicol. 29 (2010) 1024–1025.
[362] T.M. Hagen, J.K. Liu, J. Lykkesfeldt, C.M. Wehr, R.T. Ingersoll, V. Vinarsky, J.C. Bartholomew, B.N. Ames, Feeding acetyl-L-carnitine and lipoic acid to old rats significantly improves metabolic function while decreasing oxidative stress, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 1870–1875.
[363] T.M. Hagen, R. Moreau, J.H. Suh, F. Visioli, Mitochondrial decay in the aging rat heart: evidence for improvement by dietary supplementation with acetyl-L-carnitine and/or lipoic acid, Ann. N. Y. Acad. Sci. 959 (2002) 491–507.
[364] V. Calabrese, C. Colombrita, R. Sultana, G. Scapagnini, M. Calvani, D.A. Butterfield, A.M. Giuffrida Stella, Redox modulation of heat shock protein expression by acetylcarnitine in aging brain: relationship to antioxidant status and mitochondrial function, Antioxid. Redox Signal. 8 (2006) 404–416.
[365] M. Krajcovicova-Kudlackova, R. Simonic, A. Bederova, K. Babinska, I. Béder, Correlation of carnitine levels to methionine and lysine intake, Physiol. Res. 49 (2000) 399–402.
[366] C. Hoppel, The role of carnitine in normal and alterated fatty acid metabolism, Am. J. Kidney Dis. 41 (2003) S4–S12.
[367] A. Steiber, J. Kerner, C.L. Hoppel, Carnitine: a nutritional, biosynthetic and functional perspective, Mol. Aspects Med. 25 (2004) 455–473.
[368] M.L. Schinetti, D. Rossini, R. Greco, A. Bertelli, Protective action of acetylcarnitine on NADPH induced lipid peroxidation of cardiac microsomes, Drugs Exp. Clin. Res. 13 (1987) 509–515.
[369] J. Liu, E. Head, H. Kuratsune, C.W. Cotman, B.N. Ames, Comparison of the effects of L-carnitine and acetyl-L-carnitine on carnitine levels, ambulatory activity, and oxidative stress biomarkers in the brain of old rats, Ann. N. Y. Acad. Sci. 1033 (2004) 117–131.
[370] H.F. Poon, V. Calabrese, M. Calvani, D.A. Butterfield, Proteomics analyses of specific protein oxidation and protein expression in aged rat brain and its modulation by L-acetylcarnitine: insights into the mechanisms of action of this proposed therapeutic agent for CNS disorders associated with oxidative stress, Antioxid. Redox Signal. 8 (2006) 381–394.
[371] V. Calabrese, A.M. Giuffrida Stella, M. Calvani, D.A. Butterfield, Acetylcarnitine and cellular stress response: roles in nutritional redox homeostasis and regulation of longevity genes, J. Nutr. Biochem. 17 (2006) 73–88.
[372] H.M. Abdul, V. Calabrese, M. Calvani, D.A. Butterfield, Acetyl-L-carnitine-induced up-regulation of heat shock proteins protects cortical neurons against amyloid-beta peptide 1-42-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease, J. Neurosci. Res. 84 (2006) 398–408.
[373] H.M. Abdul, D.A. Butterfield, Involvement of PI3K/PKG/ERK1/2 signaling pathways in cortical neurons to trigger protection by cotreatment of acetyl-L-carnitine and alpha-lipoic acid against HNE-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease, Free Radic. Biol. Med. 42 (2007) 371–384.
[374] M.A. Virmani, R. Biselli, A. Spadoni, S. Rossi, N. Corsico, M. Calvani, A. Fattorossi, C. De Simone, E. Arrigoni-Martelli, Protective actions of L-carnitine and acetyl-L-carnitine on the neurotoxicity evoked by mitochondrial uncoupling or inhibitors, Pharmacol. Res. 32 (1995) 383–389.
[375] M.A. Virmani, V. Caso, A. Spadoni, S. Rossi, F. Russo, F. Gaetani, The action of acetyl-L-carnitine on the neurotoxicity evoked by amyloid fragments and peroxide on primary rat cortical neurons, Neuroprotect. Agents 939 (2001) 162–178.
[376] S. Lowitt, J.I. MaloneI, A.F. Salem, J. Korthals, S. Benford, Acetyl-L-carnitine corrects the altered peripheral nerve function of experimental diabetes, Metabolism 44 (1995) 677–680.
[377] A.Z. Reznick, V.E. Kagan, R. Ramsey, M. Tsuchiya, S. Khwaja, E.A. Serbinova, L. Packer, Antiradical effects in L-propionyl carnitine protection of the heart against ischemia-reperfusion injury: the possible role of iron chelation, Arch. Biochem. Biophys. 296 (1992) 394–401.
[378] V. Calabrese, A. Ravagna, C. Colombrita, G. Scapagnini, E. Guagliano, M. Calvani, D.A. Butterfield, A.M. Giuffrida Stella, Acetylcarnitine induces heme oxygenase in rat astrocytes and protects against oxidative stress: involvement of the transcription factor Nrf2, J. Neurosci. Res. 79 (2005) 509–521.
[379] J. Liu, B.N. Ames, Reducing mitochondrial decay with mitochondrial nutrients to delay and treat cognitive dysfunction, Alzheimer's disease, and Parkinson's disease, Nutr. Neurosci. 8 (2005) 67–89.
[380] W. Shen, K. Liu, C. Tian, L. Yang, X. Li, J. Ren, L. Packer, E. Head, E. Sharman, J. Liu, Protective effects of R-alpha-lipoic acid and acetyl-L-carnitine in MIN6 and isolated rat islet cells chronically exposed to oleic acid, J. Cell. Biochem. 104 (2008) 1232–1243.
[381] E.J. Lesnefsky, D. He, S. Moghaddas, C.L. Hoppel, Reversal of mitochondrial defects before ischemia protects the aged heart, FASEB J. 20 (2006) 1543–1545.
[382] M.A. McDaniel, S.F. Maier, G.O. Einstein, "Brain-specific" nutrients: a memory cure? Nutrition 19 (2003) 957–975.
[383] V. Calabrese, M. Calvani, D.A. Butterfield, Increased formation of short-chain organic acids after chronic ethanol administration and its interaction with the carnitine pool in rat, Arch. Biochem. Biophys. 431 (2004) 271–278.
[384] V. Calabrese, D.A. Butterfield, G. Scapagnini, A.M. Stella, M.D. Maines, Redox regulation of heat shock protein expression by signaling involving nitric oxide and carbon monoxide: relevance to brain aging, neurodegenerative disorders, and longevity, Antioxid. Redox Signal. 8 (2006) 444–477.
[385] V. Calabrese, M. Calvani, D.A. Butterfield, Acetyl-L-carnitine-induced up-regulation of heat shock proteins protects cortical neurons against amyloid-beta peptide 1-42-mediated oxidative stress and neurotoxicity: implications for Alzheimer's disease, J. Neurosci. Res. 84 (2006) 398–408.
[386] V. Calabrese, G. Pennisi, M. Calvani, A.M. Giuffrida Stella, D.A. Butterfield, C. Mancuso, Heme oxygenase as therapeutic funnel in nutritional redox homeostasis and cellular stress response: role of acetylcarnitine, in: C. Ritcher-Landsberg (Ed.), Heat Shock Protein in Neural Cells, USA, Springer, N.Y., 2006, pp. 39–52.
[387] V. Calabrese, C. Colombrita, E. Guagliano, M. Sapienza, A. Ravagna, V. Cardile, G. Scapagnini, A.M. Santoro, A. Mangiameli, D.A. Butterfield, A.M. Giuffrida Stella, E. Rizzarelli, Protective effect of carnosine during nitrosative stress in astroglial cell cultures, Neurochem. Res. 30 (2005) 797–807.
[388] T. Furuno, T. Kanno, K. Arita, M. Asami, T. Utsumi, Y. Doi, M. Inoue, K. Utsumi, Roles of long chain fatty acids and carnitine in mitochondrial membrane permeability transition, Biochem. Pharmacol. 62 (2001) 1037–1046.
[389] R.T. Pillich, G. Scarsella, G. Risuleo, Reduction of apoptosis through the mitochondrial pathway by the administration of acetyl-L-carnitine to mouse fibroblasts in culture, Exp. Cell Res. 306 (2005) 1–8.
[390] T. Ishii, Y. Shimpo, Y. Matsuoka, K. Kinoshita, Anti-apoptotic effect of acetyl-L-carnitine and L-carnitine in primary cultured neurons, Jpn. J. Pharmacol. 83 (2000) 119–124.

[391] E. Arrigoni-Martelli, V. Caso, Carnitine protects mitochondria and removes toxic acyls from xenobiotics, Drugs Exp. Clin. Res. 27 (2001) 27–49.
[392] G. Galli, M. Fratelli, Activation of apoptosis by serum deprivation in a teratocarcinoma cell line: inhibition by L-acetylcarnitine, Exp. Cell Res. 204 (1993) 54–60.
[393] R.P. Revoltella, B. Dal Canto, L. Caracciolo, C.M. D'urso, L-Carnitine and some of its analogs delay the onset of apoptotic cell death initiated in murine C2.8 hepatocytic cells after hepatocyte growth factor deprivation, Biochim. Biophys. Acta 1224 (1994) 333–341.
[394] J.W. Snyder, M.E. Kyle, T.N. Ferraro, L-carnitine delays the killing of cultured hepatocytes by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Arch. Biochem. Biophys. 276 (1990) 132–138.
[395] S. Moretti, G. Famularo, S. Marcellini, L-Carnitine reduces lymphocyte apoptosis and oxidant stress in HIV-1 infected subjects treated with zidovudine and didanosine, Antioxid. Redox Signal. 4 (2002) 391–403.
[396] A. Virmani, F. Gaetani, Z. Binienda, A. Xu, H. Duhart, S.F. Ali, Role of mitochondrial dysfunction in neurotoxicity of MPP+: partial protection of PC12 cells by acetyl-L-carnitine, Ann. N. Y. Acad. Sci. 1025 (2004) 267–273.
[397] V. Steffen, M. Santiago, C.P. de la Cruz, E. Revilla, A. Machado, J. Cano, Effect of intraventricular injection of 1-methyl-4-phenylpyridinium: protection by acetyl-L-carnitine, Hum. Exp. Toxicol. 14 (1995) 865–871.
[398] X.P. Zhu, E.F. Sato, Y. Wang, H. Nakamura, J. Yodoi, M. Inoue, Acetyl-L-carnitine suppresses apoptosis of thioredoxin 2-deficient DT40 cells, Arch. Biochem. Biophys. 478 (2008) 154–160.
[399] L. Di Cesare Mannelli, C. Ghelardini, M. Calvani, R. Nicolai, L. Mosconi, E. Vivoli, A. Pacini, A. Bartolini, Protective effect of acetyl-L-carnitine on the apoptotic pathway of peripheral neuropathy, Eur. J. Neurosci. 26 (2007) 820–827.
[400] P. Cassano, A.G. Sciancalepore, V. Pesce, M. Flück, H. Hoppeler, M. Calvani, L. Mosconi, P. Cantatore, M.N. Gadaleta, Acetyl-L-carnitine feeding to unloaded rats triggers in soleus muscle the coordinated expression of genes involved in mitochondrial biogenesis, Biochim. Biophys. Acta 1757 (2006) 1421–1428.
[401] G. Traina, S. Valleggi, R. Bernardi, M. Rizzo, M. Calvani, R. Nicolai, L. Mosconi, M. Durante, M. Brunelli, Identification of differentially expressed genes induced in the rat brain by acetyl-L-carnitine as evidenced by suppression subtractive hybridization, Brain Res. Mol. Brain Res. 132 (2004) 57–63.
[402] G. Traina, R. Bernardi, E. Cataldo, M. Macchi, M. Durante, M. Brunelli, In the rat brain acetyl-L-carnitine treatment modulates the expression of genes involved in neuronal ceroid lipofuscinosis, Mol. Neurobiol. 38 (2008) 146–152.
[403] G. Traina, G. Federighi, M. Brunelli, Up-regulation of kinesin light-chain 1 gene expression by acetyl-L-carnitine: therapeutic possibility in Alzheimer's disease, Neurochem. Int. 53 (2008) 244–247.
[404] G. Traina, G. Federighi, M. Brunelli, R. Scuri, Cytoprotective effect of acetyl-L-carnitine evidenced by analysis of gene expression in the rat brain, Mol. Neurobiol. 39 (2009) 101–106.
[405] T.I. Lee, H.C. Causton, F.C. Holstege, W.C. Shen, N. Hannett, E.G. Jennings, F. Winston, M.R. Green, R.A. Young, Redundant roles for the TFIID and SAGA complexes in global transcription, Nature 405 (2000) 701–704.
[406] D.H. Kim, M. Kim, H.J. Kwon, Histone deacetylase in carcinogenesis and its inhibitors as anti-cancer agents, J. Biochem. Mol. Biol. 36 (2003) 110–119.
[407] H. Takahashi, J.M. McCaffery, R.A. Irizarry, J.D. Boeke, Nucleocytosolic acetyl-coenzyme a synthetase is required for histone acetylation and global transcription, Mol. Cell 23 (2006) 207–217.
[408] J. Avruch, X.F. Zhang, J.M. Kyriakis, Raf meets Ras: completing the framework of a signal transduction pathway, Trends Biochem. Sci. 19 (1994) 279–283.
[409] J.A. Thorson, L.W. Yu, A.L. Hsu, N.Y. Shih, P.R. Graves, J.W. Tanner, P.M. Allen, H. Piwnica-Worms, A.S. Shaw, 14-3-3 proteins are required for maintenance of Raf-1 phosphorylation and kinase activity, Mol. Cell. Biol. 18 (1998) 5229–5238.
[410] W.L. Yan, T.J. Lerner, J.L. Haines, J.F. Gusella, Sequence analysis and mapping of a novel human mitochondrial ATP synthase subunit 9 cDNA (ATP5G3), Genomics 24 (1994) 375–377.
[411] M. Tanaka, F. Nakamura, S. Mizokawa, A. Matsumura, K. Matsumura, Y. Watanabe, Role of acetyl-L-carnitine in the brain: revealed by Bioradiography, Biochem. Biophys. Res. Commun. 306 (2003) 1064–1069.
[412] S.R. Bhaumik, E. Smith, A. Shilatifard, Covalent modifications of histones during development and disease pathogenesis, Nat. Struct. Mol. Biol. 14 (2007) 1008–1016.
[413] B. Schwer, J. Bunkenborg, R.O. Verdin, J.S. Andersen, E. Verdin, Reversible lysine acetylation controls the activity of the mitochondrial enzyme acetyl-CoA synthetase 2, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 10224–10229.
[414] D.B. Lombard, F.W. Alt, H.L. Cheng, J. Bunkenborg, R.S. Streeper, R. Mostoslavsky, J. Kim, G. Yancopoulos, D. Valenzuela, A. Murphy, Y. Yang, Y. Chen, M.D. Hirschey, R.T. Bronson, M. Haigis, L.P. Guarente Jr., R.V. Fareser, S. Weissman, E. Verdin, B. Schwer, Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation, Mol. Cell. Biol. 27 (2007) 8807–8814.
[415] M.C. Haigis, R. Mostoslavsky, K.M. Haigis, K. Fahie, D.C. Christodoulou, A.J. Murphy, D.M. Valenzuela, G.D. Yancopoulos, M. Karow, G. Blander, C. Wolberger, T.A. Prolla, R. Weindruch, F.W. Alt, L. Guarente, SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells, Cell 126 (2006) 941–954.
[416] B.H. Ahn, H.S. Kim, S. Song, I.H. Lee, J. Liu, A. Vassilopoulos, C.X. Deng, T. Finkel, A role for the mitochondrial deacetylase SIRT3 in regulating energy homeostasis, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 14447–14452.
[417] I. Mateo, J. Llorca, J. Infante, E. Rodríguez-Rodríguez, J. Berciano, O. Combarros, Gene-gene interaction between 14-3-3 zeta and butyrylcholinesterase modulates Alzheimer's disease risk, Eur. J. Neurol. 15 (2008) 219–222.
[418] M.N. Gadaleta, V. Petruzzella, M. Renis, F. Fracasso, P. Cantatore, Reduced transcription of mitochondrial DNA in the senescent rat. Tissue dependence and effect of L-carnitine, Eur. J. Biochem. 187 (1990) 501–506.
[419] G. Paradies, F.M. Ruggiero, M.N. Gadaleta, E. Quagliariello, The effect of aging and acetyl-L-carnitine on the activity of the phosphate carrier and on the phospholipid composition in rat heart mitochondria, Biochim. Biophys. Acta 1103 (1992) 324–326.
[420] G. Paradies, F.M. Ruggiero, G. Petrosillo, M.N. Gadaleta, E. Quagliariello, Effect of aging and acetyl-L-carnitine on the activity of cytochrome oxidase and adenine nucleotide translocase in rat heart mitochondria, FEBS Lett. 350 (1994) 213–215.
[421] G. Paradies, F.M. Ruggiero, G. Petrosillo, M.N. Gadaleta, E. Quagliariello, Carnitine-acylcarnitine translocase activity in cardiac mitochondria from aged rats: the effect of acetyl-L-carnitine, Mech. Ageing Dev. 84 (1995) 103–112.
[422] V. Pesce, F. Fracasso, C. Musicco, A.M. Lezza, P. Cantatore, M.N. Gadaleta, Acetyl-L-carnitine dietary supplementation to old rats increases mitochondrial transcription factor A content in rat hindlimb skeletal muscles, Ann. N. Y. Acad. Sci. 1019 (2004) 430–433.
[423] S. Gulewitsch, S. Amiradzibi, Ueber das carnosin, eine neue organische base des fleischextracts, Ber. Dtsch. Chem. Ges. 33 (1900) 344–379.
[424] R. Krimberg, Zur Kenntnis der Extraktivstoffe der muskelin. IV. Mutterlung. Uberdas vorkommen des carnosins, carnitins und methylguanidins im fleisch, Hoppe Seylers Z. Physiol. Chem. 48 (1906) 412.
[425] R. Krimberg, Zur Kenntnis der Extraktivstoffe der muskelin. X. Mitteilung. Uber die identitat des novsains mit dem carnitin, Hoppe Seylers Z. Physiol. Chem. 55 (1908) 466.
[426] C. Sale, B. Saunders, R.C. Harris, Effect of beta-alanine supplementation on muscle carnosine concentrations and exercise performance, Amino Acids 39 (2010) 321–333.
[427] A.A. Boldyrev, S.E. Severin, The histidine-containing dipeptides, carnosine and anserine: distribution, properties and biological significance, Adv. Enzyme Regul. 30 (1990) 175–194.
[428] S. De Marchis, C. Modena, P. Peretto, A. Migheli, F.L. Margolis, A. Fasolo, Carnosine-related dipeptides in neurons and glia, Biochemistry (Mosc.) 65 (2000) 824–833.
[429] L.M. Maynard, G.A. Boissonneault, C.K. Chow, G.G. Bruckner, High levels of dietary carnosine are associated with increased concentrations of carnosine and histidine in rat soleus muscle, J. Nutr. 131 (2001) 287–290.
[430] P.J. Quinn, A.A. Boldyrev, V.E. Formazuyk, Carnosine: its properties, functions and potential therapeutic applications, Mol. Aspects Med. 13 (1992) 379–444.
[431] L. Bonfanti, P. Peretto, S. De Marchis, A. Fasolo, Carnosine-related dipeptides in the mammalian brain, Prog. Neurobiol. 59 (1999) 333–353.
[432] A.R. Hipkiss, Glycation, ageing and carnosine: are carnivorous diets beneficial? Mech. Ageing Dev. 126 (2005) 1034–1039.
[433] M.S. Horning, L.J. Blakemore, P.Q. Trombley, Endogenous mechanisms of neuroprotection: role of zinc, copper, and carnosine, Brain Res. 852 (2000) 56–61.
[434] M. Sakai, M. Yoshida, N. Karasawa, M. Teramura, H. Ueda, I. Nagatsu, Carnosine-like immunoreactivity in the primary olfactory neuron of the rat, Experientia 43 (1987) 298–300.
[435] S.E. Gariballa, A.J. Sinclair, Carnosine: physiological properties and therapeutic potential, Age Ageing 29 (2000) 207–210.
[436] N. Tamaki, A. Funatsuka, S. Fujimoto, T. Hama, The utilization of carnosine in rats fed on a histidine-free diet and its effect on the levels of tissue histidine and carnosine, J. Nutr. Sci. Vitaminol. (Tokyo) 30 (1984) 541–551.
[437] W.K. Chan, E.A. Decker, C.K. Chow, G.A. Boissonneault, Effect of dietary carnosine on plasma and tissue antioxidant concentrations and on lipid oxidation in rat skeletal muscle, Lipids 29 (1994) 461–466.
[438] J.F. Lenney, S.C. Peppers, C.M. Kucera-Orallo, R.P. George, Characterization of human tissue carnosinase, Biochem. J. 228 (1985) 653–660.
[439] R.P. Ferraris, J. Diamond, W.W. Kwan, Dietary regulation of intestinal transport of the dipeptide carnosine, Am. J. Physiol. 255 (1988) 143–150.
[440] A.R. Hipkiss, C. Brownson, A possible new role for the anti-ageing peptide carnosine, Cell. Mol. Life Sci. 57 (2000) 747–753.
[441] E.A. Decker, S.A. Livisay, S. Zhou, A re-evaluation of the antioxidant activity of purified carnosine, Biochemistry (Mosc.) 65 (2000) 766–770.
[442] A.R. Hipkiss, C. Brownson, Carnosine reacts with protein carbonyl groups: another possible role for the anti-ageing peptide? Biogerontology 1 (2000) 217–223.
[443] R. Kohen, Y. Yamamoto, K.C. Cundy, B.N. Ames, Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain, Proc. Natl. Acad. Sci. U. S. A. 85 (1988) 3175–3179.
[444] A.A. Boldyrev, V.E. Formazyuk, V.I. Sergienko, Biological significance of histidine-containing dipeptides with special reference to carnosine: chemistry, distribution, metabolism and medical applications, Sov. Sci. Rev. D Physicochem. Biol. 264 (1994) 81–85.
[445] O.I. Aruoma, M.J. Laughton, B. Halliwell, Carnosine, homocarnosine and anserine: could they act as antioxidants in vivo? Biochem. J. 264 (1989) 863–869.
[446] A.A. Boldyrev, A.M. Dupin, E.V. Pindel, S.E. Severin, Antioxidative properties of histidine-containing dipeptides from skeletal muscles of vertebrates, Comp. Biochem. Physiol. B 89 (1988) 245–250.
[447] M. Mozdzan, J. Szemraj, J. Rysz, D. Nowak, Antioxidant properties of carnosine re-evaluated with oxidizing systems involving iron and copper ions, Basic Clin. Pharmacol. Toxicol. 96 (2005) 352–360.
[448] G.A. McFarland, R. Holliday, Retardation of the senescence of cultured human diploid fibroblasts by carnosine, Exp. Cell Res. 212 (1994) 167–175.

[449] A.R. Hipkiss, Could carnosine or related structures suppress Alzheimer's disease? J. Alzheimers Dis. 11 (2007) 229–240.
[450] D. Dobrota, T. Fedorova, S. Stvolinsky, E. Babusikova, K. Likavcanova, A. Drgova, A. Strapkova, A. Boldyrev, Carnosine protects the brain of rats and Mongolian gerbils against ischemic injury: after-stroke-effect, Neurochem. Res. 30 (2005) 1283–1288.
[451] S. Stvolinsky, M. Kukley, D. Dobrota, V. Mezesova, A. Boldyrev, Carnosine protects rats under global ischemia, Brain Res. Bull. 53 (2000) 445–448.
[452] S. Tang, T.V. Arumugam, R.G. Cutler, D. Jo, T. Magnus, S.L. Chan, M.R. Mughal, R.S. Telljohann, M. Nassar, X. Ouyang, A. Calderan, P. Ruzza, A. Guiotto, M.P. Mattson, Neuroprotective actions of a histidine analogue in models of ischemic stroke, J. Neurochem. 101 (2007) 729–736.
[453] D. Pubill, E. Verdaguer, F.X. Sureda, A. Camins, M. Pallas, J. Camarasa, E. Escubedo, Carnosine prevents methamphetamine-induced gliosis but not dopamine terminal loss in rats, Eur. J. Pharmacol. 448 (2002) 165–168.
[454] S. Dukic-Stefanovic, R. Schinzel, P. Riederer, G. Munch, AGES in brain ageing: AGE-inhibitors as neuroprotective and anti-dementia drugs? Biogerontology 2 (2001) 19–34.
[455] J.E. Preston, A.R. Hipkiss, D.T. Himsworth, I.A. Romero, J.N. Abbott, Toxic effects of beta-amyloid(25–35) on immortalised rat brain endothelial cell: protection by carnosine, homocarnosine and beta-alanine, Neurosci. Lett. 242 (1998) 105–108.
[456] M. Nakayama, K. Takahashi, T. Kitamuro, K. Yasumoto, D. Katayose, K. Shirato, Y. Fujii-Kuriyama, S. Shibahara, Repression of heme oxygenase-1 by hypoxia in vascular endothelial cells, Biochem. Biophys. Res. Commun. 271 (2000) 665–671.
[457] S. Okinaga, K. Takahashi, K. Takeda, M. Yoshizawa, H. Fujita, H. Sasaki, S. Shibahara, Regulation of human heme oxygenase-1 gene expression under thermal stress, Blood 87 (1996) 5074–5084.
[458] K. Takahashi, M. Nakayama, K. Takeda, H. Fujia, S. Shibahara, Suppression of heme oxygenase-1 mRNA expression by interferon-gamma in human glioblastoma cells, J. Neurochem. 72 (1999) 2356–2361.
[459] S. Shibahara, The heme oxygenase dilemma in cellular homeostasis: new insights for the feedback regulation of heme catabolism, Tohoku J. Exp. Med. 200 (2003) 167–186.
[460] G. Aldini, M. Carini, G. Beretta, S. Bradamante, R.M. Facino, Carnosine is a quencher of 4-hydroxy-nonenal: through what mechanism of reaction? Biochem. Biophys. Res. Commun. 298 (2002) 699–706.
[461] Y. Liu, G. Xu, L.M. Sayre, Carnosine inhibits (E)-4-hydroxy-2-nonenal-induced protein cross-linking: structural characterization of carnosine-HNE adducts, Chem. Res. Toxicol. 16 (2003) 1589–1597.
[462] V.P. Reddy, M.R. Garrett, G. Perry, M.A. Smith, Carnosine: a versatile antioxidant and antiglycating agent, Sci. Aging Knowledge Environ. 18 (2005) 12.
[463] A.R. Hipkiss, V.C. Worthington, D.T. Himsworth, W. Herwig, Protective effects of carnosine against protein modification mediated by malondialdehyde and hypochlorite, Biochim. Biophys. Acta 1380 (1998) 46–54.
[464] T. Nagasawa, T. Yonekura, N. Nishizawa, D.D. Kitts, In vitro and in vivo inhibition of muscle lipid and protein oxidation by carnosine, Mol. Cell. Biochem. 225 (2001) 29–34.
[465] J.H. Kang, K.S. Kim, Enhanced oligomerization of the alpha-synuclein mutant by the Cu, Zn-superoxide dismutase and hydrogen peroxide system, Mol. Cells 15 (2003) 87–93.
[466] J.H. Kang, W.S. Eum, Enhanced oxidative damage by the familial amyotrophic lateral sclerosis-associated Cu, Zn-superoxide dismutase mutants, Biochim. Biophys. Acta 1524 (2000) 162–170.
[467] E.A. Decker, V. Ivanov, B.Z. Zhu, B. Frei, Inhibition of low-density lipoprotein oxidation by carnosine histidine, J. Agric. Food Chem. 49 (2001) 511–516.
[468] E.C. Smith, The buffering of muscle in rigor; protein, phosphate and carnosine, J. Physiol. 92 (1938) 336–343.
[469] J.R. Stout, B.S. Graves, A.E. Smith, M.J. Hartman, J.T. Cramer, T.W. Beck, R.C. Harris, The effect of beta-alanine supplementation on neuromuscular fatigue in elderly (55–92 Years): a double-blind randomized study, J. Int. Soc. Sports Nutr. 5 (2008) 21.
[470] R.C. Harris, D.J. Marlin, M. Dunnett, D.H. Snow, E. Hultman, Muscle buffering capacity and dipeptide content in the thoroughbred horse, greyhound dog and man, Comp. Biochem. Physiol. A Comp. Physiol. 97 (1990) 249–251.
[471] A.R. Hipkiss, Carnosine and protein carbonyl groups: a possible relationship, Biochemistry (Mosc.) 65 (2000) 771–778.
[472] A.R. Hipkiss, J.E. Preston, D.T. Himsworth, V.C. Worthington, M. Keown, J. Michaelis, J. Lawrence, A. Mateen, L. Allende, P.A. Eagles, N.J. Abbott, Pluripotent protective effects of carnosine, a naturally occurring dipeptide, Ann. N. Y. Acad. Sci. 854 (1998) 37–53.
[473] A.R. Hipkiss, H. Chana, Carnosine protects proteins against methylglyoxal-mediated modifications, Biochem. Biophys. Res. Commun. 248 (1998) 28–32.
[474] A.R. Hipkiss, J. Michaelis, P. Syrris, Non-enzymatic glycosylation of the dipeptide L-carnosine, a potential anti-protein-cross-linking agent, FEBS Lett. 371 (1995) 81–85.
[475] E.R. Stadtman, Protein oxidation and aging, Science 257 (1992) 1220–1224.
[476] C. Brownson, A.R. Hipkiss, Carnosine reacts with a glycated protein, Free Radic. Biol. Med. 28 (2000) 1564–1570.
[477] R.F. Rosenberger, Senescence and the accumulation of abnormal proteins, Mutat. Res. 256 (1991) 255–262.
[478] A.R. Hipkiss, Accumulation of altered proteins and ageing: causes and effects, Exp. Gerontol. 41 (2006) 464–473.
[479] C. Schöneich, Protein modification in aging: an update, Exp. Gerontol. 41 (2006) 807–812.
[480] A.R. Hipkiss, On the enigma of carnosine's anti-ageing actions, Exp. Gerontol. 44 (2009) 237–242.
[481] G.A. McFarland, R. Holliday, Further evidence for the rejuvenating effects of the dipeptide L-carnosine on cultured human diploid fibroblasts, Exp. Gerontol. 34 (1999) 35–45.
[482] L. Shao, Q.H. Li, Z. Tan, L-carnosine reduces telomere damage and shortening rate in cultured normal fibroblasts, Biochem. Biophys. Res. Commun. 324 (2004) 931–936.
[483] M.O. Yuneva, E.R. Bulygina, S.C. Gallant, G.G. Kramarenko, S.L. Stvolinsky, M.L. Semyonova, A.A. Boldyrev, Effect of carnosine on age-induced changes in senescence- accelerated mice, J. Anti Aging Med. 2 (1999) 337–342.
[484] D.O. Son, H. Satsu, Y. Kiso, M. Totsuka, M. Shimizu, Inhibitory effect of carnosine on interleukin-8 production in intestinal epithelial cells through translational regulation, Cytokine 42 (2008) 265–276.
[485] A.A. Boldyrev, Protection of proteins from oxidative stress: a new illusion or a novel strategy? Ann. N. Y. Acad. Sci. 1057 (2005) 193–205.
[486] M.S. Alhamdani, H.F. Al-Azzawie, F.K. Abbas, Decreased formation of advanced glycation end products in peritoneal fluid by carnosine and related peptides, Perit. Dial. Int. 27 (2007) 86–89.
[487] M.S. Alhamdani, A.H. Al-Kassir, F.K. Abbas, N.A. Jaleel, M.F. Al-Taee, Antiglycation and antioxidant effect of carnosine against glucose degradation products in peritoneal mesothelial cells, Nephron Clin. Pract. 107 (2007) 26–34.
[488] M. Fontana, F. Pinnen, G. Lucente, L. Pecci, Prevention of peroxynitrite-dependent damage by carnosine and related sulphonamido pseudodipeptides, Cell. Mol. Life Sci. 59 (2002) 546–551.
[489] J.A. Vinson, T.B. Howard, Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients, Nutr. Biochem. 7 (1996) 659–663.
[490] N.W. Seidler, Carnosine prevents the glycation-induced changes in electrophoretic mobility of aspartate aminotransferase, J. Biochem. Mol. Toxicol. 14 (2000) 215–220.
[491] A.R. Hipkiss, J.E. Preston, D.T. Himswoth, V.C. Worthington, N.J. Abbot, Protective effects of carnosine against malondialdehyde-induced toxicity towards cultured rat brain endothelial cells, Neurosci. Lett. 238 (1997) 135–138.
[492] G. Aldini, R.M. Facino, G. Beretta, M. Carini, Carnosine and related dipeptides as quenchers of reactive carbonyl species: from structural studies to therapeutic perspectives, Biofactors 24 (2005) 77–87.
[493] M. Carini, G. Aldini, G. Beretta, E. Arlandini, R.M. Facino, Acrolein-sequestering ability of endogenous dipeptides: characterization of carnosine and homocarnosine/acrolein adducts by electrospray ionization tandem mass spectrometry, J. Mass Spectrom. 38 (2003) 996–1006.
[494] J.N. Min, R.A. Whaley, N.E. Sharpless, P. Lockyer, A.L. Portbury, C. Patterson, CHIP deficiency decreases longevity, with accelerated aging phenotypes accompanied by altered protein quality control, Mol. Cell. Biol. 28 (2008) 4018–4025.
[495] C. Yun, A. Stanhill, Y. Yang, Y. Zhang, C.M. Haynes, C.F. Xu, T.A. Neubert, A. Mor, M.R. Philips, D. Ron, Proteasomal adaptation to environmental stress links resistance to proteotoxicity with longevity in *Caenorhabditis elegans*, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 7094–7099.
[496] M.A. Bonelli, S. Desenzani, G. Cavallini, A. Donati, A.A. Romani, E. Bergamini, A.F. Borghetti, Low-level caloric restriction rescues proteasome activity and Hsc70 level in liver of aged rats, Biogerontology 9 (2008) 1–10.
[497] A.M. Cuervo, Calorie restriction and aging: the ultimate "cleansing diet", J. Gerontol. A Biol. Sci. Med. Sci. 63 (2008) 547–549.
[498] E. Bergamini, G. Cavallini, A. Donati, Z. Gori, The role of autophagy in aging: its essential part in the anti-aging mechanism of caloric restriction, Ann. N. Y. Acad. Sci. 1114 (2007) 69–78.
[499] V.G. Nicoletti, A.M. Santoro, G. Grasso, L.I. Vagliasindi, M.L. Giuffrida, C. Cuppari, V.S. Purrello, A.M. Stella, E. Rizzarelli, Carnosine interaction with nitric oxide and astroglial cell protection, J. Neurosci. Res. 85 (2007) 2239–2245.
[500] S. Tomonaga, T. Tachibana, H. Takahashi, M. Sato, D.M. Denbow, M. Furuse, Nitric oxide involves in carnosine-induced hyperactivity in chicks, Eur. J. Pharmacol. 524 (2005) 84–88.
[501] S. Thomas, S. Kotamraju, J. Zielonka, D.R. Harder, B. Kalyanaraman, Hydrogen peroxide induces nitric oxide and proteosome activity in endothelial cells: a bell-shaped signaling response, Free Radic. Biol. Med. 42 (2007) 1049–1061.
[502] L.A. Bharadwaj, G.F. Davies, I.J. Xavier, N. Ovsenek, L-carnosine and verapamil inhibit hypoxia-induced expression of hypoxia inducible factor (HIF-1 alpha) in H9c2 cardiomyoblasts, Pharmacol. Res. 45 (2002) 175–181.
[503] A.B. Bonner, M.E. Swann, J.S. Marway, L.C. Heap, V.R. Preedy, Lysosomal and non-lysosomal protease activities of the brain in response to ethanol feeding, Alcohol 12 (1995) 505–509.
[504] N. Erjavec, L. Larsson, J. Grantham, T. Nyström, Accelerated aging and failure to segregate damaged proteins in Sir2 mutants can be suppressed by overproducing the protein aggregation-remodeling factor Hsp104p, Genes Dev. 21 (2007) 2410–2421.
[505] M. Odashima, M. Otaka, M. Jin, N. Konishi, T. Sato, S. Kato, T. Matsuhashi, C. Nakamura, S. Watanabe, Induction of a 72-kDa heat-shock protein in cultured rat gastric mucosal cells and rat gastric mucosa by zinc L-carnosine, Dig. Dis. Sci. 47 (2002) 2799–2804.
[506] M. Odashima, M. Otaka, M. Jin, I. Wada, Y. Horikawa, T. Matsuhashi, R. Ohba, N. Hatakeyama, J. Oyake, S. Watanabe, Zinc L-carnosine protects colonic mucosal injury through induction of heat shock protein 72 and suppression of NF-kappaB activation, Life Sci. 79 (2006) 2245–2250.
[507] T. Ohkawara, J. Nishihira, R. Nagashima, H. Takeda, M. Asaka, Polaprezinc protects human colon cells from oxidative injury induced by hydrogen peroxide: relevant to cytoprotective heat shock proteins, World J. Gastroenterol. 12 (2006) 6178–6181.

[508] J. Michaelis, A.R. Hipkiss, S. Panagiotopoulos, Method for the treatment of the complications and pathology of diabetes, Approved United States Patent (5, 561,110) 1996.
[509] M. Brownlee, Biochemistry and molecular cell biology of diabetic complications, Nature 414 (2001) 813–820.
[510] N. Ahmed, Advanced glycation endproducts—role in pathology of diabetic complications, Diabetes Res. Clin. Pract. 67 (2005) 3–21.
[511] A.R. Hipkiss, C. Brownson, M.J. Carrier, Carnosine, the anti-ageing, anti-oxidant dipeptide, may react with protein carbonyl groups, Mech. Ageing Dev. 122 (2001) 1431–1445.
[512] M.A. Babizhayev, A.I. Deyev, V.N. Yermakova, I.V. Brikman, J. Bours, Lipid peroxidation and cataracts: N-acetylcarnosine as a therapeutic tool to manage age-related cataracts in human and in canine eyes, Drugs R&D 5 (2004) 125–139.
[513] M.A. Babizhayev, Rejuvenation of visual functions in older adult drivers and drivers with cataract during a short-term administration of N-acetylcarnosine lubricant eye drops, Rejuvenation Res. 7 (2004) 186–198.
[514] V.V. Shuvaev, I. Laffont, J.M. Serot, J. Fujii, N. Taniguchi, G. Siest, Increased protein glycation in cerebrospinal fluid of Alzheimer's disease, Neurobiol. Aging 22 (2001) 397–402.
[515] M.J. Picklo, T.J. Montine, V. Amarnath, M.D. Neely, Carbonyl toxicology and Alzheimer's disease, Toxicol. Appl. Pharmacol. 184 (2002) 187–197.
[516] V.P. Reddy, M.E. Obrenovich, C.S. Atwood, G. Perry, M.A. Smith, Involvement of Maillard reactions in Alzheimer disease, Neurotox. Res. 4 (2002) 191–209.
[517] A.R. Hipkiss, Could carnosine be a naturally-occurring scavenger for acrolein and other reactive aldehydes in the brain? Neurobiol. Aging 23 (2002) 645–646.
[518] S. Kikuchi, K. Shinpo, M. Takeuchi, S. Yamagishi, Z. Makita, N. Sasaki, K. Tashiro, Glycation—a sweet tempter for neuronal death, Brain Res. Brain Res. Rev. 41 (2003) 306–323.
[519] H.A. Ghanbari, K. Ghanbari, P.L. Harris, P.K. Jones, Z. Kubat, R.J. Castellani, B.L. Wolozin, M.A. Smith, G. Perry, Oxidative damage in cultured human olfactory neurons from Alzheimer's disease patients, Aging Cell 3 (2004) 41–44.
[520] N. Ahmed, U. Ahmed, P.J. Thornalley, K. Hager, G. Fleischer, G. Münch, Protein glycation, oxidation and nitration adduct residues and free adducts of cerebrospinal fluid in Alzheimer's disease and link to cognitive impairment, J. Neurochem. 92 (2005) 255–263.
[521] F. Chen, M.A. Wollmer, F. Hoerndli, G. Münch, B. Kuhla, E.I. Rogaev, M. Tsolaki, A. Papassotiropoulos, J. Götz, Role for glyoxalase I in Alzheimer's disease, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 7687–7692.
[522] S. Battah, N. Ahmed, P.J. Thornally, Novel anti-glycation therapeutic agents: glyoxylase-1-mimetics, Int. Congr. Ser. 1245 (2002) 107–111.
[523] R. Di Paola, D. Impellizzeri, A.T. Salinaro, E. Mazzon, F. Bellia, M. Cavallaro, C. Cornelius, G. Vecchio, V. Calabrese, E. Rizzarelli, S. Cuzzocrea, Administration of carnosine in the treatment of acute spinal cord injury, Biochem. Pharmacol. 82 (2011) 1478–1489.
[524] G. Münch, S. Mayer, J. Michaelis, A.R. Hipkiss, P. Riederer, R. Müller, A. Neumann, R. Schinzel, A.M. Cunningham, Influence of advanced glycation end-products and AGE-inhibitors on nucleation-dependent polymerization of beta-amyloid peptide, Biochim. Biophys. Acta 1360 (1997) 17–29.
[525] K.S. Kim, S.Y. Choi, H.Y. Kwon, M.H. Won, T.C. Kang, J.H. Kang, The ceruloplasmin and hydrogen peroxide system induces alpha-synuclein aggregation in vitro, Biochimie 84 (2002) 625–631.
[526] T. Miyata, C. van Ypersele de Strihou, Angiotensin II receptor blockers and angiotensin converting enzyme inhibitors: implication of radical scavenging and transition metal chelation in inhibition of advanced glycation end product formation, Arch. Biochem. Biophys. 419 (2003) 50–54.
[527] A.A. Boldyrev, S.L. Stvolinsky, O.V. Tyulina, V.B. Koshelev, N. Hori, D.O. Carpenter, Biochemical and physiological evidence that carnosine is an endogenous neuroprotector against free radicals, Cell. Mol. Neurobiol. 17 (1997) 259–271.
[528] A. Boldyrev, R. Song, D. Lawrence, D.O. Carpenter, Carnosine protects against excitotoxic cell death independently of effects on reactive oxygen species, Neuroscience 94 (1999) 571–577.
[529] A. Boldyrev, E. Bulygina, T. Leinsoo, I. Petrushanko, S. Tsubone, H. Abe, Protection of neuronal cells against reactive oxygen species by carnosine and related compounds, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 137 (2004) 81–88.
[530] A. Boldyrev, T. Fedorova, S. Stvolinsky, C. Borras, J. Sastre, J. Vina, Chemical intervention in senescence-accelerated mice metabolism for modelling neurodegenerative diseases: an overview, Int. Congr. Ser. 1260 (2004) 109–115.
[531] A.A. Boldyrev, S.L. Stvolinsky, T.N. Fedorova, Z.A. Suslina, Carnosine as a natural antioxidant and geroprotector: from molecular mechanisms to clinical trials, Rejuvenation Res. 13 (2010) 156–158.
[532] S. Gallant, M. Semyonova, M. Yuneva, Carnosine as a potential anti-senescence drug, Biochemistry (Mosc.) 65 (2000) 866–868.
[533] M.G. Chez, C.P. Buchanan, M.C. Aimonovitch, M. Becker, K. Schaefer, C. Black, J. Komen, Double-blind, placebo-controlled study of L-carnosine supplementation in children with autistic spectrum disorders, J. Child Neurol. 17 (2002) 833–837.
[534] R. Thiel, S.W. Fowkes, Can cognitive deterioration associated with Down syndrome be reduced? Med. Hypotheses 64 (2005) 524–532.
[535] O.A. Petroff, F. Hyder, D.L. Rothman, R.H. Mattson, Homocarnosine and seizure control in juvenile myoclonic epilepsy and complex partial seizures, Neurology 56 (2001) 709–715.
[536] A.M. Amorini, F. Bellia, V. Di Pietro, B. Giardina, D. La Mendola, G. Lazzarino, S. Sortino, B. Tavazzi, E. Rizzarelli, G. Vecchio, Synthesis and antioxidant activity of new homocarnosine beta-cyclodextrin conjugates, Eur. J. Med. Chem. 42 (2007) 910–920.
[537] F. Bellia, A.M. Amorini, D. La Mendola, G. Vecchio, B. Tavazzi, B. Giardina, V. Di Pietro, G. Lazzarino, E. Rizzarelli, New glycosidic derivatives of histidine-containing dipeptides with antioxidant properties and resistant to carnosinase activity, Eur. J. Med. Chem. 43 (2008) 373–380.
[538] V. Lanza, F. Bellia, R. D'Agata, G. Grasso, E. Rizzarelli, G. Vecchio, New glycoside derivatives of carnosine and analogs resistant to carnosinase hydrolysis: Synthesis and characterization of their copper(II) complexes, J. Inorg. Biochem. 105 (2011) 181–188.
[539] D. Harman, The biologic clock: the mitochondria? J. Am. Geriatr. Soc. 20 (1972) 145–147.
[540] A.W. Linnane, O. Takayuki, M. Sangkot, T. Masashi, Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases, Lancet 333 (1989) 642–664.
[541] M.H. Vendelbo, K.S. Nair, Mitochondrial longevity pathways, Biochim. Biophys. Acta 1813 (2011) 634–644.
[542] V. Calabrese, C. Cornelius, A.M. Giuffrida Stella, E.J. Calabrese, Cellular stress responses, mitostress and carnitine insufficiencies as critical determinants in aging and neurodegenerative disorders: role of hormesis and vitagenes, Neurochem. Res. 35 (2010) 1880–1915.
[543] J.R. Speakman, Body size, energy metabolism and lifespan, J. Exp. Biol. 208 (2005) 1717–1730.
[544] A. Bokov, A. Chaudhuri, A. Richardson, The role of oxidative damage and stress in aging, Mech. Ageing Dev. 125 (2004) 811–826.
[545] R. Sohal, R. Weindruch, Oxidative stress, caloric restriction, and aging, Science 273 (1996) 59–63.