REVIEW ARTICLE

Cellular Stress Response: A Novel Target for Chemoprevention and Nutritional Neuroprotection in Aging, Neurodegenerative Disorders and Longevity

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Abstract The predominant molecular symptom of aging is the accumulation of altered gene products. Moreover, several conditions including protein, lipid or glucose oxidation disrupt redox homeostasis and lead to accumulation of unfolded or misfolded proteins in the aging brain. Alzheimer's and Parkinson's diseases or Friedreich ataxia

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are neurological diseases sharing, as a common denominator, production of abnormal proteins, mitochondrial dysfunction and oxidative stress, which contribute to the pathogenesis of these so called "protein conformational diseases". The central nervous system has evolved the conserved mechanism of unfolded protein response to cope with the accumulation of misfolded proteins. As one of the main intracellular redox systems involved in neuroprotection, the *vitagene* system is emerging as a neurohormetic potential target for novel cytoprotective interventions. Vitagenes encode for cytoprotective heat shock proteins (Hsp) Hsp70 and heme oxygenase-1, as well as thioredoxin reductase and sirtuins. Nutritional studies show that ageing in animals can be significantly influenced by dietary restriction. Thus, the impact of dietary factors on health and longevity is an increasingly appreciated area of research. Reducing energy intake by controlled caloric restriction or intermittent fasting increases lifespan and protects various tissues against disease. Genetics has revealed that ageing may be controlled by changes in intracellular NAD/NADH ratio regulating sirtuin, a group of proteins linked to aging, metabolism and stress tolerance in several organisms. Recent findings suggest that several phytochemicals exhibit biphasic dose responses on cells with low doses activating signaling pathways that result in increased expression of vitagenes encoding survival proteins, as in the case of the Keap1/Nrf2/ARE pathway activated by curcumin and NAD/NADH-sirtuin-1 activated by resveratrol. Consistently, the neuroprotective roles of dietary antioxidants including curcumin, acetyl-L-carnitine and carnosine have been demonstrated through the activation of these redox-sensitive intracellular pathways. Although the notion that stress proteins are neuroprotective is broadly accepted, still much work needs to be done in order to associate neuroprotection with specific pattern of

stress responses. In this review the importance of vitagenes in the cellular stress response and the potential use of dietary antioxidants in the prevention and treatment of neurodegenerative disorders is discussed.

Keywords   Cellular stress response · Redox homeostasis · Aging · Neurodegenerative disorders · Vitagenes · Antioxidants

Introduction

The brain has a large potential oxidative capacity but a limited ability to counteract oxidative stress [1–3]. Within the cell, reactive oxygen species (ROS) are physiologically present at minimal concentration as by-products of aerobic metabolism as well as second messengers in many signal transduction pathways and, in normal conditions, there is a steady-state balance between pro-oxidants and antioxidants which is necessary to ensure optimal efficiency of antioxidant defenses [4–7]. However, when the rate of free radical generation exceeds the capacity of antioxidant defenses, oxidative stress ensues with consequential severe damage to DNA, proteins and lipids [8–10] (Fig. 1). Oxidative stress has been implicated in mechanisms leading to neuronal cell injury in various pathological states of the brain, including neurodegenerative disorders such as Alzheimer's disease (AD) [11–15]. Recently the term "nitrosative stress" has been used to indicate the cellular damage elicited by nitric oxide and its congeners peroxynitrite, N_2O_3, nitroxyl anion and nitrosonium (all can be indicated as reactive nitrogen species or RNS) [16–18].

From a molecular point of view, in the central nervous system (CNS) cells are able to fight against oxidant stress using many resources, including vitamins (A, C and E), bioactive molecules (glutathione, thioredoxin, flavonoids), lipoic acid, enzymes (heat shock protein-32, superoxide dismutase, catalase, glutathione peroxidases, thioredoxin reductase, etc) and redox sensitive protein transcriptional factors (AP-1, NF-κB, Nrf2, HSF, etc) [19–21]. The heat shock proteins (Hsps) are one of the more studied defense system active against cellular damage (Fig. 1).

The idea of the pervasive nature of free radicals has been firmly entrenched in the minds of scientists ever since the group of Britton Chance [22] developed the basic biochemical techniques to show that in the resting state 2% of all oxygen consumed by cells is converted into ROS rather than water. McCord and Fridovich first described superoxide dismutase thus suggesting a physiological role of superoxide [23]. Although, there is now an appreciation that the physiological generation of ROS is likely to be an order of magnitude less, their impact on biomolecules has been amply documented. In response to this assault, the
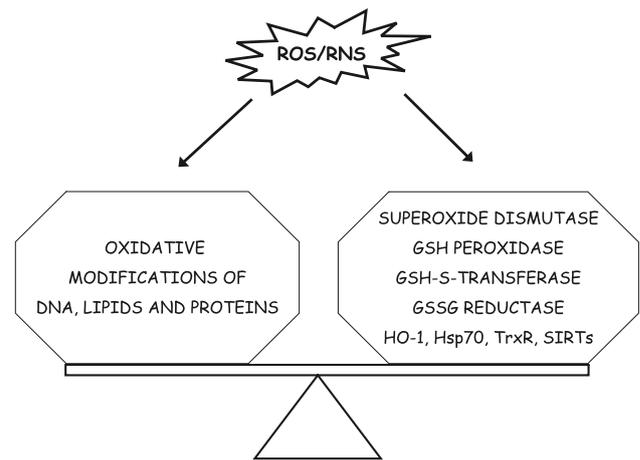
Fig. 1 Reactive oxygen species as second messengers. ROS at minimal concentration may act as second messengers in many signal transduction pathways and, under normal conditions, a steady-state balance between pro-oxidants and antioxidants exists. Conversely, when the rate of free radical generation exceeds the capacity of antioxidant defenses, oxidative and/or nitrosative stress ensues with consequential severe damage to DNA, protein and lipids. Among the cellular pathways involved in protection against oxidative and nitrosative stress, antioxidant enzymes such as superoxide dismutase, glutathione (GSH)-peroxidase, GSH-S-transferase, GSSG reductase and the vitagenes heat shock protein 70 (Hsp70), heme oxygenase-1 (HO-1), thioredoxin reductase (TrxR) and sirtuins (SIRTs) actively operate counteracting deleterious consequences of free radical damage. GSSG, oxidized glutathione

cell has developed a number of antioxidant defence systems such as superoxide dismutase, the peroxidases, the glutathione redox cycle with its associated constitutive enzymes as well as glutathione itself, whose concentration is higher in the cell than that of glucose [22]. Therefore, the cell has become well equipped to cope with the normal production of reactive species. There is growing evidence that the continuous presence of a small stimulus such as low concentrations of ROS is in fact able to induce the expression of antioxidant enzymes and other defence mechanisms. The basis for this phenomenon may be encompassed by the concept of hormesis [24], which can be characterized as a particular dose–response relationship in which a low dose of a substance is stimulatory and a high dose is inhibitory. In this context, radicals may be considered to be beneficial since they act as signals to enhance defences rather than deleterious as they are when cells are exposed to high levels of ROS. On the other hand when in excess can, over long term, disrupt redox homeostasis, impose oxidative stress and subsequently lead to a dramatic loss of molecular fidelity which is the major cause for accumulation of unfolded or misfolded proteins in brain cells. Alzheimer's (AD), Parkinson's (PD), and Huntington's diseases, but also amyotrophic lateral sclerosis and Friedreich ataxia belong to the so called "protein

conformational diseases" and affect several millions of aged people in all the world [21]. Cells have evolved mechanisms such as the unfolded protein response, where chaperones can rescue misfolded proteins by breaking up aggregates and assisting the refolding process, while proteins that cannot be rescued by refolding are delivered to the proteasome by other chaperones to be recycled [25]. In general, an unfolded protein response conformational diseases are conditions that arise from the dysfunctional aggregation of proteins in non-native conformations. This often is associated with multiple metabolic derangements that result in the excessive production of ROS and oxidative stress [25]. The ability of a cell to deal with ROS and RNS requires the activation of pro-survival pathways as well as the production of molecules endowed with antioxidant and anti-apoptotic activities. Among the cellular pathways involved in protection against oxidative and nitrosative stress, the heat shock proteins family plays a key role, in particular in brain cells. Hsp70, Hsp60, Hsp27 and ubiquitin are functional chaperones triggering a cascade of intracellular cytoprotective events [6, 7, 19–22, 26]. Heme oxygenase-1 (HO-1), also referred to as Hsp32, belongs to the Hsp family and protects brain cells from oxidative stress by degrading toxic heme into free iron, carbon monoxide and biliverdin [21, 26–28]. This latter is then reduced by biliverdin reductase (BVR) into bilirubin (BR), a linear tetrapyrrole with antioxidant properties; very recently, BR has been shown to effectively counteract also nitrosative stress, due to its ability to bind and inactivate NO and RNS [29–32].

In this paper we describe the recent discoveries about the biochemical changes occurring in the CNS when brain cells are exposed to chronic oxidative insult, as well as the function of members of the *vitagene* system, such as HO-1, thioredoxin and sirtuins in modulating the onset and progression of major neurodegenerative diseases such as, AD, PD or FRDA. In addition, the key role played by the heat shock response, particularly HO-1 and Hsp70 pathways, as well as sirtuins as potential target for nutritional interventions are discussed. Although the notion that stress proteins are neuroprotective is broadly accepted, still much work needs to be done in order to associate neuroprotection with specific patterns of stress responses. Emerging evidence underscores the high potential of the vitagene system as a target for new neuroprotective strategies, especially those aimed at minimizing deleterious consequences associated with oxidative stress, such as in neurodegenerative disorders and brain aging. We review here also the evidence for the role of some dietary antioxidants in modulating redox-dependent mechanisms leading to up-regulation of vitagenes in brain, and hence potentiate brain stress tolerance.

Vitagenes

Vitagenes are 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-1/Hsp32, Hsp70, by the thioredoxin system [19–21] and by sirtuin proteins (Fig. 2). Among these genes HO-1 is receiving considerable attention because of its major role in counteracting both oxidative and nitrosative stress. In fact, HO-1 induction is one of the early events in the cell response to stress. Heme oxygenase-1 exerts protective role, by degrading the intracellular levels of pro-oxidant heme and by producing biliverdin, the precursor of bilirubin, this latter being an endogenous molecule with potent antioxidant and antinitrosative features [21, 26–32] (Fig. 3).
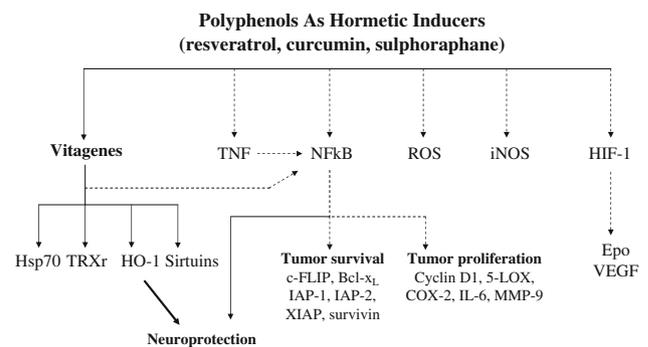
Fig. 2 The cellular stress response mediated by vitagenes and the concept of hormesis. Hormesis represents an evolutionary-based adaptive responses to environmentally induced disruptions in homeostasis. Cellular signaling pathways and molecular mechanisms that mediate hormetic responses typically involve antioxidant enzymes and transcription factors such as Nrf-2 and NF-κB. As a result, cells increase their production of cytoprotective and restorative proteins including growth factors, phase 2 and antioxidant enzymes, and protein chaperones. Under conditions of oxidative/nitrosative stress, there is an increased formation of ROS or RNS. They play a key role in the pathogenesis of free radical-induced diseases, such as neurodegenerative disorders. Polyphenols are strong inducers of vitagenes heme oxygenase-1 (HO-1), heat shock protein 70 (Hsp70), thioredoxin reductase and sirtuins. Vitagenes, either individually or by acting in concert, contribute to counteract the NF-κB-dependent ROS/RNS-mediated damage thus originating a neuroprotective response. NF-κB inhibition might also have profound impact on cancerogenesis, modulating tumor cell survival and proliferation. BV, biliverdin; BVR, biliverdin reductase; BR, bilirubin; c-FLIP: cellular FLICE-like inhibitory protein; IAP-1: inhibitor of apoptosis protein 1; IAP-2: inhibitor of apoptosis protein 2; XIAP: X-linked inhibitor of apoptosis protein; 5-LOX: 5-lipooxygenase; COX-2: cyclooxygenase-2; IL-6: interleukin-6; MMP-9: matrix metalloproteinase-9; Epo: erythropoietin; VEGF: Vascular Endothelial Growth Factor; ROS: Reactive oxygen species; iNOS: inducible nitric oxide synthase; TNF: tumor necrosis factor; HIF-1: Hypoxia Inducible Factor-1; NF-κB: nuclear factor κB; Hsp70: heat shock protein 70; TRXr: thioredoxin reductase; HO-1: heme oxygenase-1
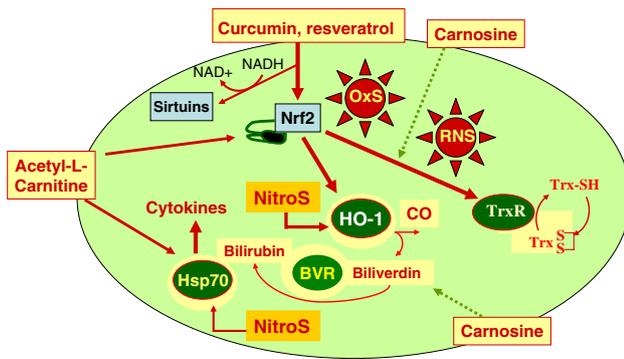
Fig. 3 Vitagenes and the pathway of cellular stress response. Environmental stressors (metabolic or physical stress, heavy metals, cytokines, etc.) or pathophysiological conditions of oxidant antioxidant balance perturbation (inflammation, neuronal damage, ischemia) are all situations associated with increased oxidative or nitrosative stress. Vitagenes, such as heme oxygenase, Hsp70, thioredoxin reductase and sirtuins represent an integrated system for cellular stress tolerance which can be activated by nutritional antioxidants including curcumin, resveratrol, acetyl-L-carnitine or carnosine. Acetyl-L-carnitine and polyphenols 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. In the same figure are indicated the redox cycling between bilirubin/ biliverdin as well as oxidized/reduced TrxR. OxS, oxidative stress; NitroS, nitrosative stress; HO-1, heme oxygenase-1; TrxR, thioredoxin reductase; Trx, Thioredoxin, SH: reduced form, S-S: oxidized form

Heme Oxygenase-1

The mechanisms responsible for neuronal death are not completely elucidated, even if many studies suggest that ROS are primarily involved in the genesis of neurodegenerative disorders [11, 12, 33–35]. Due to its strong antioxidant properties and wide distribution within the CNS, HO-1 has been proposed as a key enzyme in the prevention of brain damage [26–29, 36]. Panahian et al., using transgenic mice over-expressing HO-1 in neurons, demonstrated the neuroprotective effect of this enzyme in an experimental model of ischemic brain damage [37]. The neuroprotective effects of over-expressed HO-1 has been attributed to several factors such as: increased level of both cGMP and bcl-2 in neurons, inactivation of the pro-apoptotic transcription factor p53, increase in antioxidant sources in the iron sequestering protein, ferritin [37]. Particularly interesting is the role played by HO-1 in AD, a neurodegenerative disorder which involves a chronic inflammatory response associated with both oxidative brain injury and β-amyloid associated pathology. Significant increases in the levels of HO-1 have been observed in AD brains in association with neurofibrillary tangles and HO-1 mRNA was found to be increased in AD neocortex and cerebral vessels [38, 39]. HO-1 increase was not only

associated with neurofibrillary tangles, but also co-localized with senile plaques and glial fibrillary acidic protein-positive astrocytes in AD brains [40]. Therefore, it is plausible to conclude that the dramatic increase in HO-1 in AD may be a direct response to an increase in free heme concentrations, associated with neurodegeneration, and can be considered as an attempt of brain cells to convert the highly toxic heme into the antioxidant BR. The protective role played by HO-1 and its products in AD prompted investigators to propose natural substances, which are able to increase HO-1 levels, as potential drugs for the treatment of AD. In this light, several in vitro studies have been focused on polyphenolic compounds contained in some herbs and spices, e.g. curcumin [41]. Curcumin is the active anti-oxidant principle in *Curcuma longa*, a colouring agent and food additive commonly used in Indian culinary preparations. In vitro, this polyphenolic substance has the potential to inhibit lipid peroxidation and to effectively intercept and neutralize ROS and RNS [42]. In addition, curcumin has been shown to significantly increase HO-1 in brain cells [43]. This latter effect on HO-1 can explain, at least in part, the anti-oxidant properties of curcumin, in particular keeping in mind that HO-1-derived BR has the ability to scavenge both ROS and RNS [29–32]. A single epidemiological study suggested that curcumin, as one of the most prevalent nutritional and medicinal compounds used by the Indian population, is responsible for the reduced (4.4-fold) prevalence of AD in India compared to United States [44]. Based on these findings, Lim and colleagues have provided evidence that dietary curcumin given to an Alzheimer transgenic APPSw mouse model (Tg2576) for 6 months resulted in a suppression of indices of inflammation and oxidative damage in the brain of these mice [45]. Furthermore, in a human neuroblastoma cell line curcumin inhibits NF-κB activation, efficiently preventing neuronal cell death [42].

Carbon monoxide (CO) is the gaseous products of HO and it has been found to play a role in several biological phenomena, including hippocampal long-term potentiation, non-adrenergic non-cholinergic gastrointestinal relaxation and vasodilatation, and is currently regarded as a neuromodulator in the peripheral and central nervous system (for extensive reviews on CO and its functions in the nervous system, see [28, 46] (Fig. 3). Evidence from in vitro and in vivo studies suggests that the HO-CO pathway is involved in the modulation of the neuroendocrine mechanism of stress. Thus, increased CO generation is clearly associated with the inhibition of K^+ stimulated arginine vasopressin (AVP) and oxytocin release from rat hypothalamic explants, whereas the inhibition of HO activity significantly potentiates the LPS-induced increase in AVP circulating levels while reducing the hypothalamic content of this neuropeptide

[47–49]. With regards to corticotropin-releasing hormone (CRH), the effects of CO on the release of this hormone are contradictory, since increases in CO generation induced by two HO substrates, hematin and hemin, were associated with reduced or enhanced CRH release respectively, in two different in vitro models [50, 51]. As far as the intracellular mechanism(s) by which CO exerts its biological functions, it is generally agreed that this gas activates the cytosolic form of guanylyl cyclase (sGC) which in turn increases intracellular cGMP levels [27]. However during the last 10 years many studies demonstrated that CO signals through the activation of alternative intracellular signal transduction pathways. Studies from our laboratory suggested that the activation of another hemoprotein, cyclooxygenase (COX), plays a significant role in CO signaling in the rat hypothalamus. In these studies we demonstrated that hemin, the precursor of CO via HO, dose-dependently increases PGE_2 production from rat hypothalamus in vitro and this effect is specifically due to CO because it is counteracted by the HO inhibitor Sn-mesoporphyrin-IX and oxyhemoglobin, the latter being a well known scavenger for CO [52]. The direct evidence about the stimulatory role of CO on PGs production was obtained incubating hypothalami directly in CO saturated solutions and measuring significantly increased PGE_2 levels with respect to control tissue [53]. Jaggar and colleagues demonstrated that exogenous or endogenously produced CO dilates cerebral arterioles by directly activating large-conductance Ca^{2+}-activated K^+ (K_{Ca}) channels primarily by increasing the coupling ratio and amplitude relationship between Ca^{2+} sparks and K_{Ca} channels [54]. Although CO is a potent and effective activator of K_{Ca} channels, the gas does not dilate arterioles in the absence of Ca^{2+} sparks. Therefore, CO appears to act by priming K_{Ca} channels for activation by Ca^{2+} sparks, and this ultimately leads to arteriole dilation via membrane hyperpolarization [54]. Finally, Otterbein and colleagues have shown that in organs and tissues different from brain, CO exerts anti-inflammatory and anti-apoptotic effects dependent on the modulation of the p38 MAPK-signaling pathway [55]. By virtue of these effects, CO confers protection in oxidative lung injury models, and likely plays a role in HO-1 mediated tissue protection [56].

Heat Shock Protein 70

The 70 kDa family of stress proteins is one of the most extensively studied. Included in this family are Hsc70 (heat shock cognate, the constitutive form), Hsp70 (the inducible form, also referred to as Hsp72) and GRP-75 (a constitutively expressed glucose-regulated protein found in the endoplasmic reticulum) [26, 57].

Only recently, the availability of transgenic animals and gene transfer allowed us to over-express the gene encoding for Hsp70, thus demonstrating that overproduction of this protein leads to protection in several different models of nervous system injury [58–60]. Following focal cerebral ischemia, Hsp70 mRNA is synthesized in most ischemic cells except in areas of very low blood flow, 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 [61]. It has been suggested that this neuronal expression of Hsp70 outside an infarct can be used to define the ischemic penumbras, which means the zone of protein denaturation in the ischemic areas [61].

As mentioned above, Hsps are induced in many neurodegenerative disorders mainly in the view of its cytoprotective function. Hsp72 was overexpressed in postmortem cortical tissue of AD patients and an increase in Hsp70 mRNA was found in cerebellum hippocampus and cortex of AD patients during the agonal phase of the disease [62–64]. Recently Kakimura et al. demonstrated that Hsp70 induces IL-6 and TNF-α in microglial cells and this event is associated with an increased phagocytosis and clearance of Aβ peptides [65] (Figs. 2, 3). The same authors hypothesize that Hsps could activate microglial cells through NF-κB and p-38 MAPK-dependent pathways [65].

A large body of evidence now suggests a correlation between mechanisms of nitrosative stress and Hsp induction. We have demonstrated in astroglial cell cultures that cytokine-induced nitrosative stress is associated with an increased synthesis of Hsp70 stress proteins. The molecular mechanisms regulating the NO-induced activation of heat-shock signal seems to involve cellular oxidant/antioxidant balance, mainly represented by the glutathione status and the antioxidant enzymes [66, 67].

Thioredoxin/Thioredoxin Reductase

The thioredoxin system, originally identified in *Escherichia coli*, in 1964, as a hydrogen donor for ribonucleotide reductase required for DNA synthesis, plays a key role in cell function by limiting oxidative stress directly via antioxidant effects and indirectly by protein–protein interactions [68]. It is well established that, in mammals, cellular redox regulation of many processes is provided by the cooperation between the Trx and glutathione systems [69]. In fact, Trx and GSH systems are involved in a variety of redox-dependent pathways such as supplying reducing equivalents for ribonucleotide reductase, and peptide methionine sulfoxide reductase, the latter being involved in antioxidant defence and regulation of the

cellular redox state [70]. Therefore, Trx and GSH form a powerful system controlling redox regulation of gene expression, signal transduction, cell proliferation, protection against oxidative stress, anti-apoptotic functions, growth factor and co-cytokine effects, as well as regulation of the redox state of the extracellular environment [71]. The promoter of the Trx gene contains a series of stress-responsive elements, various transcription factor binding sites, such as SP1, AP-1, NF-κB, and the antioxidant-response element (ARE) [72–74] (Fig. 3). Importantly, induction of thioredoxin reductase and glutathione has been demonstrated to occur in parallel with other ARE-dependent phase 2 cytoprotective genes in several experimental systems, e.g., in cortical astrocytes, in human hepatoma cells and in human keratinocytes [75–77]. Similarly to induction of HO-1 gene expression, the ARE-mediated Trx-1 induction involves transcription factor Nrf2 [78] (Figs. 3, 4).

Importantly, it has been reported that Trx is constitutively present as a surface-associated sulfhydryl protein in plasma membrane of a wide range of cells [79]. Many physicochemical stimuli, such as UV irradiation and hydrogen peroxide, have been shown to induce Trx expression and secretion, as a redox-sensitive molecule with cytokine-like and chemokine-like activities to prevent cell injury against oxidative stress [70]. In addition to UV irradiation, treatment of cells in culture with phorbol esters, hydrogen peroxide, hypoxia, the cancer drug cisplatin and hemin has been reported to cause the translocation of Trx from the cytoplasm to the nucleus, where it regulates the

Fig. 4 Mechanism of induction of cytoprotective phase 2 genes. Under basal conditions transcription factor Nrf2 is bound to the inducer sensor, the dimeric protein Keap1, and targeted for ubiquitination and proteasomal degradation via association with the Cullin 3-based E3 ubiquitin ligase complex. Inducers modify highly reactive cysteine residues of Keap1 which loses its ability to target Nrf2 for degradation. Consequently, Nrf2 is stabilized and migrates to the nucleus where it binds to the ARE (as a heterodimer with a small Maf transcription factor), and activated transcription of cytoprotective phase 2 genes

redox-activation and DNA binding activity of critical transcription factors (Jun, Fos, p53, CREB, PEBP2/CBF, Myb), all involved in fundamental processes, such as gene expression, cell growth and apoptosis [79]. Thioredoxin plasma levels in normal individuals vary between 20 and 30 ng/ml [80, 81] and increase in certain human diseases including HIV infection and cancer [80, 82]. Noteworthy, several studies reported increased Trx-1 expression in many human primary cancers and tumor cell lines, including astrocytic brain tumors [83, 84]. Elevated Trx levels may contribute to increased cancer cell proliferation and resistance to chemotherapy by several mechanisms as the stimulation of DNA synthesis and the activation of redox-modulated transcription factors [79, 85]. Recent work suggests that Trx-1 is involved in nerve growth factor (NGF) signaling pathways [86]. NGF, a neurotrophic factor regulating development, maintenance and function of the CNS, has been shown to activate Trx-1 expression via cyclic AMP (cAMP)-response elements (CREs) present in the Trx-1 gene promoter, and also to induce nuclear translocation of Trx1 [87]. Recent data suggest that, beyond its ability to regulate the function of proteins through thiol-disulfide exchange reactions, Trx and its substrates may also have beneficial effects during oxidative stress by upregulating HO-1, with important cytoprotective pleiotropic effects deriving from heme degradation and bilirubin formation [88, 89]. Besides the role as a source of reducing equivalents, Trx per se acts as antioxidant or ROS scavenger. In fact, Trx eliminates singlet oxygen, hydroxyl radical and hydrogen peroxide [90]. It has also been reported that some of the neuroprotective effects of GSNO on beta-amyloid- or ferrous citrate-induced toxicity in rat cortical neurons or in rat *substantia nigra* can be due to the activation of multiple signalling pathways including thioredoxin [91, 92]. Interestingly, the interaction between Trx and GSNO seems to involve both the activation of sGC and the following cGMP generation and a direct *S*-nitrosylation reaction [92]. Finally, the NO-dependent expression of Trx has been shown to be involved in the neuroprotection against oxidative stress mediated by estrogens [93].

Sirtuins

The sirtuins are a group of proteins linked to aging, metabolism and stress tolerance in several organisms. In mammalian cells seven sirtuins have been identified. SIRT1, 2, 3, 6 and possibly 5 are NAD-dependent deacetylases (Fig. 5), SIRT4 and 6 are ADP-ribosyltransferases (Fig. 5), and the activity of SIRT7 has not been defined [94]. The sirtuin family of histone deacetylases (HDACs) was named after their homology to the *Saccharomyces cerevisiae* gene silent information regulator 2 (Sir2). In the yeast, Sir2 has been shown to mediate the effects of caloric
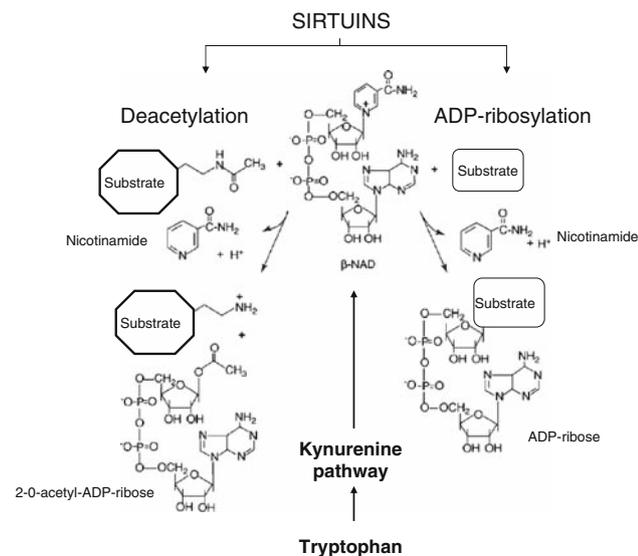
Fig. 5 Sirtuin biochemistry. Sirtuin-mediated deacetylation and ADP-ribosylation are related in that both cleave NAD as the initial chemical step of the reaction cycle. In deacetylation, the ADP-ribosyl transfer directly participates in the removal of the acetyl group from the protein substrate to generate 2,3-*O*-acetyl-ADP-ribose, whereas in ribosylation, the ADP ribosyl moiety is transferred to the protein substrate. Free tryptophan is used as the precursor of NAD via the kynurenine pathway

Fig. 6 Sirtuin activation and neuroprotection. Caloric restriction or nutritional interventions with SIRT activating compounds, such as polyphenols, induce up-regulation of SIRT1. Activated SIRT1 suppresses Nf-κB , UCP-2 (uncoupling protein 2) and PPARγ (peroxisome proliferator-activated receptor gamma), whereby modulating energy transduction processes, insulin secretion, gluconeogenesis and adipogenesis, with resulting neuroprotection and cardioprotection. SIRT inhibitors, nicotinamide, sirtinol and *O*-AA-ribose are also reported

restriction on the extension of life span, with high levels of Sir2 activity promoting longevity [95]. Like their yeast homologs, the mammalian sirtuins (SIRT1-7) are class III HDACs and require NAD+ as a cofactor to deacetylate substrates ranging from histones to transcriptional regulators. Through this activity, sirtuins are shown to regulate important biological processes, such as apoptosis, cell differentiation, energy transduction or glucose homeostasis (Fig. 6) [96]. In particular, the NAD+/NADH ratio can be considered as a "biochemical sensor" to evaluate the energetic status of the cell; in fact, among the several mechanisms through which dietary antioxidants may be useful for tissues, it is noteworthy to mention the improvement of metabolic conditions secondary to proinflammatory damage [96]. In this light, the interaction between NAD+/NADH and the members of the sirtuin family, puts in a single frame the cytoprotective activity of dietary antioxidants through the regulation of both cellular redox and metabolic state [96]. Since the Sir2 family of proteins exert their enzymatic activity not only on histones but also on numerous other proteins, including transcriptional factors, they are involved in many cellular processes, e.g., gene silencing, DNA repair, progression of the cell cycle, whereby controlling the mechanism of cellular ageing [96]. Sirtuin-mediated deacetylation and ADP-ribosylation are related in that both cleave NAD as the initial chemical step of the reaction cycle, as shown in

Fig. 5. In deacetylation, the ADP-ribosyl transfer directly participates in the removal of the acetyl group from the protein substrate to generate 2,3-*O*-acetyl-ADP-ribose, whereas in ribosylation, the ADP ribosyl moiety is transferred to the protein substrate. Deacetylation of sirtuin substrates can inhibit or induce their activities, whereas ADP-ribosylation has only been shown to be inhibitory. [94]. The connection between the biochemical activation of Sir2 orthologs through NAD-dependent protein deacetylases and the involvement of NAD/NADH in several metabolic reactions within cells prompted the hypothesis of a biochemical relationship between diet, metabolism and aging processes (Fig. 7). In fact, many studies in *Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (worms), *Drosophila melanogaster* (fly) and rodents have shown that caloric restriction (CR) extends lifespan, and sirtuins are considered to mediate, at least partly, this effect [97, 98]. Most interestingly, overexpression of the gene encoding for Sir2 protein leads to a decrease in histone acetylation and, correspondingly, to an increase in life span in yeast, in the nematode *C. elegans* and in metazoans. Similarly, Sir2-activating compounds (STACs), such as resveratrol, promote longevity in yeast and other organisms such as worm, drosophila or mouse. In addition, both mutations of the Sir2 gene and pharmacological inhibition of Sir2 protein by nicotinamide induces an acceleration of ageing in yeast, and also SIRT1 knockout mice fail to display a prominent phenotype of CR (i.e. increased physical activity). Sirtuins apparently mediate their life-extending effects in different organisms by targeting different
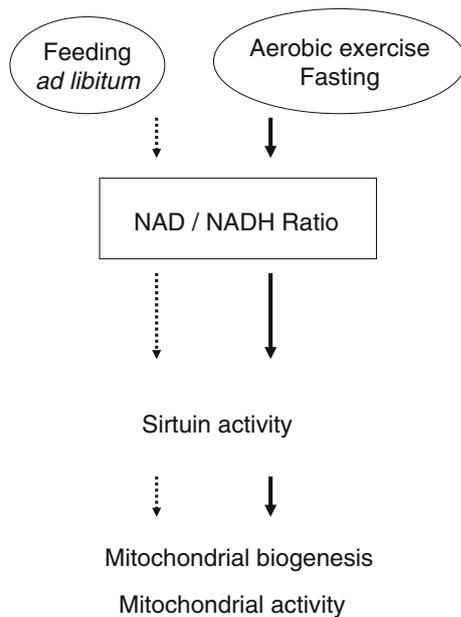
Fig. 7 Sirtuin activation as function of NAD/NADH ratio. Interrelationship and overlap between sirtuin regulation, generation of altered proteins and mitochondrial biogenesis and activity, exerted by metabolic effects on NAD and NADH levels

pathways. In mammals, SIRT1 deacetylates many key transcription factors and co-factors, such as p53, FOXO (forkhead) proteins, peroxisome proliferation activating receptor (PPAR)-gamma co-activator-1a (PGC-1a) and NF-κB, thereby affecting crucial cellular pathways involved in cellular stress resistance and metabolism (Figs. 5, 6), thus supporting the hypothesis that this class of conserved proteins are potential vitagenes [95] (Fig. 2). There are several factors supporting the action of sirtuins in mediating salutary physiological effects of CR in mammals. Oxidant/antioxidant balance perturbation and oxidative stress can induce sirtuin expression. It has been shown that hydrogen peroxide treatment increases SIRT2 expression. SIRT2 was found able to bind to FOXO3a and reduce its acetylation level, leading to an increase of FOXO DNA binding and to an elevation of the expression of FOXO target genes, such as manganese superoxide dismutase. Forkhead transcription factors of the FOXO subfamily are transactivators involved in growth control, differentiation and apoptosis [99]. SIRT1 targets FOXO3a and FOXO4 and represses their transcriptional activity in a deacetyla-tion-dependent manner. Consistently, SIRT1 reduces FOXO4-triggered apoptosis, and the expression levels of FOXO-activated genes are higher in Sirt1$^{-/-}$ mice [100, 101]. Additional work shows that SIRT1-dependent FOXO3 deacetylation reduces the expression of proapoptotic genes and the ensuing cell death, whereas it favours cell cycle arrest and the expression of genes involved in stress resistance in eukaryotic cells. In keeping with this,

deacetylation of FOXO1 by SIRT1 promotes the expression of p27kip1 and manganese superoxide dismutase (MnSOD). As a consequence, SIRT1 and SIRT2 upregulation is associated to a decrease in cellular levels of ROS [102]. Sirtuins have been shown to regulate important biological processes ranging from fat and glucose metabolism in mammals and cell survival [103]. Sirt1$^{+/-}$ mice did not mobilize as much fat to the blood as wild-type after overnight fasting showing a role of this sirtuin in fat mobilization [100, 101], as it has been demonstrated that the Sir2 homologue SIRT1, which modulates ageing in several species, controls the gluconeogenic/glycolytic pathways in liver in response to fasting signals through the transcriptional coactivator PGC-1alpha. They found that once SIRT1 is induced, it interacts with and deacetylates PGC-1alpha at specific lysine residues in an NAD(+)-dependent manner and induces gluconeogenic genes and hepatic glucose output, but does not regulate the effects of PGC-1alpha on mitochondrial genes [100, 101]. Several studies have determined a role for the human SIRT1 protein in cell survival. SIRT1 specifically associates with the p53 tumor suppressor protein and deacetylates it, resulting in negative regulation of p53-mediated transcriptional activation. Importantly, p53 deacetylation by SIRT1 also prevents cellular senescence and apoptosis induced by DNA damage and stress [104]. The identification of histones H3 and H4 as substrates for SIRT2 suggests a broader role in the cell, through transcription regulation. Additionally, Sir2 the yeast SIRT1 homologous, has been shown to mediate the effects of caloric restriction on the extension of life span, with high levels of Sir2 activity promoting longevity [96].

SIRT1 regulates important aspects of mitochondrial biology, e.g. it deacetylates the essential cofactor PGC-1α (PPAR-γ coactivator-1α) in mitochondrial biogenesis. An up regulation of the mitochondrial activity might be of therapeutic benefit for various diseases related to aging such as metabolic disorders (e.g. diabetes type 2) or mitochondrial disorders [105–107]. In active tissues, such as the muscle, the metabolic rate increases, activates the glucose metabolism resulting in improved insulin sensitivity. It is also known that the number of functional mitochondria decreases with aging. Thus an increase of the mitochondrial biogenesis might have an anti-aging effect. More tangible evidence that SIRT1 activation might have benefit via mitochondrial function comes from studies of the polyphenol, resveratrol, in mice. Resveratrol and other polyphenolic compounds are made by plants in response to stress. Resveratrol was recently shown to affect the activity of SIRT1 in vitro although its effects seem to depend on the nature of the substrate for deacetylation [108] However, in vivo, resveratrol has been shown to exert effects dependent on sirtuin orthologs—extension of lifespan in yeast, *C. elegans* and *Drosophila*, and metabolic effects on

mammalian cells [109]. Two recent studies show that deleterious effects of high fat, high caloric diets in mice were mitigated by resveratrol feeding. In one study, the shortening of lifespan by the high fat diet was reversed [110]. In a second study, resveratrol increased SIRT1 activation, PGC-1a deacetylation, and mitochondrial biogenesis in muscle [111]. These studies provide a powerful indication that SIRT1 activation offers a promising approach for treating metabolic disorders [108]. It has been suggested that metabolism of the redox couple NAD/ NADH provides a link between sirtuin activity and the control of cell senescence and organism life-span: NAD-dependent protein deacetylation helps maintain the juvenile phenotype, whereas inhibition of deacetylation activity by NADH or nicotinamide, or by NAD unavailability, promote the onset of cellular aging and decrease organism lifespan [105]. Raising NAD levels, or lowering NADH levels by increasing its oxidation, also promote sirtuin activation, with concomitant beneficial effects on cell survival [106, 107]. There exists an interrelationship and overlap between sirtuin regulation, generation of altered proteins and mitochondrial activity, exerted by metabolic effects on NAD and NADH level, as reported in (Fig. 7). In the Wallerian degeneration slow (Wlds) mouse model, SIRT1 activation protects axons against neuronal injury [96]. This Wlds mouse bears in fact a dominant mutation producing an overexpressed chimeric Wlds protein composed of the ubiquitin assembly protein Ufd2a and the NAD+ salvage pathway enzyme NMNAT1. Decreasing SIRT1 activity reduces the axonal protection originally observed, whereas SIRT1 activation by resveratrol decreases the axonal degeneration after neuronal injury [112]. This suggests that the neuroprotection in the Wlds mouse model is achieved by increasing the neuronal NAD+ reserve and/or SIRT1 activity [96]. Furthermore it has been reported that the SIRT1 agonist resveratrol protects *C. elegans* neurons expressing a fragment of the Huntington disease-associated protein huntingtin and mammalian neurons from mutant polyglutamine cytotoxicity in an HdhQ111 knock-in mouse model of Huntington disease [96]. There have been also identified potent inhibitor of sirtuin 2 (SIRT2), such as nicotinamide (NAM), *O*-acetyl-ADP-ribose (*O*-AA-ribose) or AGK, with interesting data showing that inhibition of SIRT2 rescued a-synuclein toxicity and modified inclusion morphology in a cellular model of Parkinson's disease [113]. Genetic inhibition of SIRT2 via small interfering RNA similarly rescued a-synuclein toxicity. Furthermore, the inhibitors protected against dopaminergic cell death both in vitro and in a *Drosophila* model of Parkinson's disease. The results suggest a link between neurodegeneration and aging [113]. In addition, SIRT1 activation significantly decreases neuronal cell death induced by amyloid-beta

(Aβ) peptides through inhibition of NF-κB signalling [96]. Specific brain hSIRT1 overexpression in transgenic animals induces a significant increase in the a-secretase activity, an enzyme that cleaves the amyloid precursor peptide (APP) within the Aβ peptide, favoring thereby the nonamyloidogenic pathway of the APP processing [96]. In addition, a recent study demonstrated the protective effect of CR against Alzheimer's disease-type brain amyloidosis in monkeys [114]. Monkeys maintained on CR diet had significantly reduced contents of Aβ peptides in the temporal cortex that correlated with enhanced SIRT1 concentrations as compared to monkeys under normal diet. From these studies, it became clear that pharmacological strategies aiming at activating SIRT1 would impeded Aβ peptide deposition in the brain and prevent the development of Alzheimer's disease [96, 114]. From the animal studies discussed above, it was suggested that SIRT1 could contribute to the pathogenesis of some complex diseases. In line with this hypothesis, genetic variants (SNPs) in the human SIRT1 gene have been shown to be tightly associated with energy expenditure [111]. Given their broad protective effects against oxidative stress, SIRT1 could hence be considered as a serious candidate target for therapeutic interventions in metabolic and neurodegenerative disorders.

Other Oxidative Stress-Related Neuroprotective Systems

Hsp90 chaperones are among the most abundant proteins in the cytosol of eukaryotic cells, and their abundance is increased further by condition of stress. Hsp90 chaperones not only have a role in the folding and assembly of cellular proteins, but they also regulate the activity of different signalling proteins, such as steroid hormone receptors and heme-dependent nitric oxide synthase [115].

Hsp60 family members are found in the mitochondria, where they interact with a cofactor of the HSP10 family and function in the folding and intracellular trafficking of many proteins [116].

Hsp40 proteins are important for protein translation, folding, unfolding, translocation, and degradation, primarily by stimulating the ATPase activity of chaperone proteins, Hsp70s [117].

Small heat shock proteins (sHSPs) belong to a family of 12- to 43-kDa proteins that are ubiquitous and are largely conserved in amino acid sequence among all organisms. The sHSPs (which include alpha crystallin) can form large multimeric structures and have a wide range of cellular functions, including endowing cells with thermotolerance in vivo and being able to act as molecular chaperones in vitro [118].

Natural Antioxidants, Vitagenes and Neurodegenerative Disorders

Curcumin

Curcumin (Figs. 2, 8a), the active principle of the turmeric *Curcuma longa*, has been proposed in the therapy of neurodegenerative disorders, but some problems may derive mainly due to its pharmacokinetic parameters. Although its stability at acidic pH, almost 40–80% of curcumin remains in the gastrointestinal tract upon ingestion [119]. Furthermore, studies in humans and rodents clearly demonstrated that a marked first-pass metabolism limits the systemic bioavailability of curcumin (∼60%) [120–122]. Interestingly, in order to increase its bioavailability, the co-administration of curcumin with piperine or its complexation with phospholipids have been proposed [120, 123, 124]. Preclinical studies have shown that administration of 1 g/kg of curcumin to the rat allows the polyphenol to reach plasma concentrations around 0.5 μg/ml; on the other hand, patients affected by malignant or pre-malignant conditions of the bladder, skin, cervix, stomach or oral mucosa, treated with high dose curcumin (0.5–8 g/day for 3 months) had a plasma concentration of this compound of 1.75 ± 0.8 μM [120, 125]. In the rat, the volume of distribution of curcumin is ∼190 l thus suggesting that this polyphenol may accumulate in many organs including colorectal tissue, and liver [120, 123, 126]. Evidence from rodents and humans have demonstrated that, after oral dosing, curcumin is transformed to curcumin glucuronide and curcumin sulfate as well as reduced into dihydrocurcumin (DHC), tetrahydrocurcumin (THC), hexahydrocurcumin, octahydrocurcumin and hexahydrocurcuminol [119, 127, 128]; curcumin, DHC and THC can be further converted in monoglucuronide conjugates [127, 129]. These metabolic changes do not occur only in the liver, the main organ deputed to biotransformation, but also in the intestinal tract [119, 128]. Interestingly, the metabolism of curcumin generates products such as THC endowed with anti-inflammatory activity comparable to that of the parental compound [119, 128]. In rodents and humans curcumin inhibits phase I and phase II enzymes such as cytochrome P450 enzymes, glutathione *S*-transferase and UDP-glucuronosyltransferases, therefore the ingestion of this spice may significantly alter the metabolism of drugs thus increasing their plasma concentrations and initiating potential toxic effects [130–133]. In the rat, curcumin is mainly excreted in the feces, only a small amount in the urine [121, 122] with a half-life of elimination of ∼1.5 h [123]. The urinary elimination of curcumin and its metabolites seems to increase if curcumin is administered at large doses (for example 3.6 g/day for up to 4 months) [120, 124]. With regard to the toxicity profile of curcumin, studies in rodents and primates have shown that doses of up to 3.5 g/kg body weight administered for up to 3 months were well tolerated by the animals [120]. In humans, curcumin at doses ranging from 2.1 and 8 g/day for up to 3 months did not originate main toxic effects [125, 134]. However, patients affected by advanced colorectal cancer treated with curcumin (3.6 g/day) developed diarrhea whereas a dose of 0.9 g/day was associated with nausea, which resolved by suspending the treatment. In the same patients, blood test abnormalities related to curcumin administration were a rise in serum alkaline phosphatase and lactate dehydrogenase, but the possibility that they resulted from the progression of cancer rather than curcumin toxicity should be carefully evaluated [120, 135].

Early studies have shown that curcumin and related products such as THC, have antioxidant activity. In fact, these compounds reduced free radical- or copper-induced lipid peroxidation in several experimental systems [136–138]. Furthermore, structure–activity studies clearly demonstrated the importance of the beta-diketone moiety, especially, the hydroxyl group, for the antioxidant activity of curcumin and its analogues [136, 139]. Very recently, many papers demonstrated that curcumin and its metabolites interact with NF-κB, inducible nitric oxide synthase (iNOS), hypoxia-inducible factor-1 (HIF-1) and members of the vitagene family (see below).

Reyes-Gordillo et al., have shown that curcumin reduced the CCl₄-induced liver toxicity in the rat; in particular, curcumin reduced the CCl₄-related increase in pro-inflammatory cytokines and blocked the nuclear translocation of NF-κB [140]. Similarly, curcumin prevented the dinitrochlorobenzene-induced colitis in the rat by down-regulating both NF-κB and iNOS [141]. In lung epithelial cells, curcumin exerted anticarcinogenic activity and prevented the cigarette smoke-induced NF-κB activation through inhibition of IkBα kinase activation, IkBα phosphorylation and degradation [142]. The inhibition of the NF-κB activation was paralleled by the suppression of many NF-κB-related genes, including cyclin D1, cyclooxygenase-2 and matrix metalloproteinase-9 [142]. Comparable results have been found in a macrophage cell line (RAW 264.7) challenged with lipopolysaccharide. In these cells, curcumin and its reduced metabolites blocked the activation of NF-κB, and the downstream activation of iNOS, via inhibition of the IkB kinases 1 and 2, thus providing further evidence about the importance of the effects on NF-κB in the anti-inflammatory and anticarcinogenic activity of this phenolic compound [143]. Through interaction with NF-κB, curcumin exerts protective function also in the regulation of T-cell-mediated immunity. In fact, overexpression of NF-κB in T cells confers protection against tumor-induced apoptosis, whereas when NF-κB is inhibited, the cell becomes much more vulnerable and undergoes apoptosis [144]. By so doing, NF-κB plays an
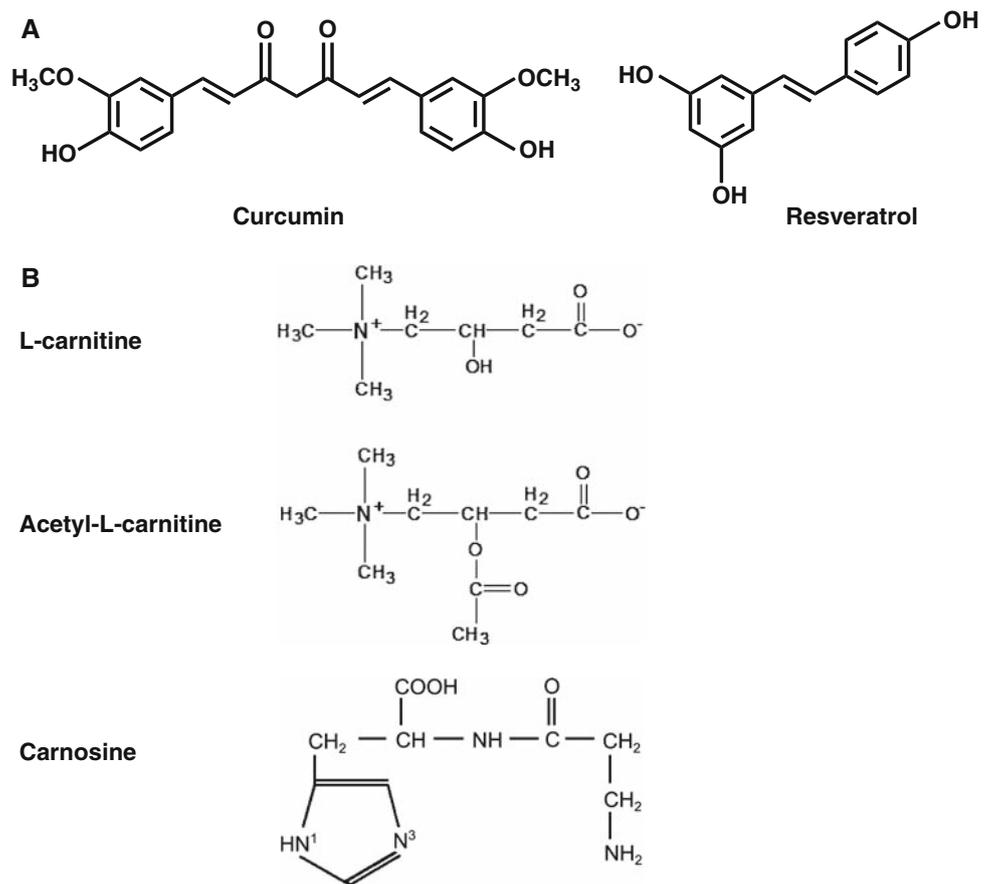
important role in the regulation of T cell apoptosis and the related thymic atrophy which occurs during carcinogenesis. In this experimental model, curcumin prevented the tumor-induced apoptosis and the following thymic atrophy by restoring the activity of NF-κB [144]. Another transcription factor involved in the anticarcinogenic effect of curcumin is HIF-1. Hypoxia-inducible factor-1 is composed of two proteins, HIF-1α and the aryl hydrocarbon receptor nuclear translocator (ARNT) and plays a major role in the development of hypoxic tumors [145]. Curcumin has been demonstrated to inactivate HIF-1 in several cell lines and this effect is related to its ability to promote ARNT degradation [145]. As a consequence of HIF-1 inactivation, several proteins downstream to HIF-1 were downregulated, such as erythropoietin and the vascular endothelial growth factor [145]. Particularly interesting is the interaction of curcumin with the vitagene system (Figs. 2–4). In particular, curcumin increased the expression of HO-1 in human cardiac myoblasts, hepatocytes, monocytes and endothelial cells, rat neurons and astrocytes as well as porcine endothelial cells [34, 146–150]. In rodents and human cells, curcumin-induced HO-1 overexpression was correlated with production of mitochondrial ROS, activation of transcription factors Nrf2 and NF-κB, induction of MAPK p38 and inhibition of phosphatase

activity [149, 151, 152]. Moreover, curcumin up-regulated Hsp70 in human colorectal carcinoma cells, proximal tubule cells and rat glioma cells [153–157]. Quite different is the effect of curcumin on TrxR, as it has been shown that curcumin irreversibly inhibits TrxR activity. As a consequence, there was increased NADPH oxidase activity which, in turn, produced an abundance of ROS [158]. This latter paradoxical effect may explain, at least in part, the cancer chemopreventive activity of curcumin [158]. Having two Michael acceptor groups on its molecule, curcumin is an activator of nuclear factor-erythroid 2-related factor 2 (Nrf2), a 66-kDa transcription factor [159] that is responsible for both basal and inducible expression of many cytoprotective genes, classically known as phase 2 genes [160]. The family of phase 2 genes is very diverse and includes NAD(P)H:quinone oxidoreductase-1, glutathione transferases, thioredoxin reductase, heme oxygenase-1, and many others that have single or multiple copies of common upstream regulatory sequences known as antioxidant response elements (ARE) [161–163]. Nrf2 is a cap'-n'-collar (CNC) transcription factor and shares with the other members of this family of transcription factors a highly conserved basic region-leucine zipper (bZIP) domain [164, 165]. It forms heterodimers with members of the small Maf family, the resultant dimeric complex binds to the ARE and

subsequently recruits the general transcriptional machinery to activate transcription of phase 2 genes [165–168]. Under normal conditions, Nrf2 has a very short half-life and is targeted for ubiquitination and proteasomal degradation via binding to a cytosolic repressor protein, Kelch-like ECH-associated protein 1 (Keap1) which in turn associates with Culin3 to form an E3 ubiquitin ligase complex [169]. Once in the cell, curcumin (and other Michael acceptor-containing molecules) modify specific highly reactive cysteine residues of Keap1 [170, 171]. As a result, Keap1 loses its ability to target Nrf2 for degradation, which then undergoes nuclear translocation. There is now overwhelming amount of experimental evidence that Nrf2 serves as a master regulator of the ARE-driven cellular defenses against various electrophiles and oxidants [172]. Indeed, *nrf2*-knockout mice exhibit enhanced sensitivity to many types of toxic chemicals, including carcinogens, allergens, and environmental pollutants [172–175]. Whereas inducers of Nrf2-dependent genes protect wild type mice against a variety of environmental challenges, they have diminished or no protective effects in *nrf2*-knockout mice. Figure 4 shows the current model for the mechanism of induction of cytoprotective phase 2 genes. In the absence of inducing stimuli Keap1 binds and targets transcription factor Nrf2 for ubiquitination and proteasomal degradation via association with the Culin 3 (Cul3)-based E3 ubiquitin ligase. Inducers (e.g., curcumin) react and chemically modify specific highly reactive cysteine residues of the sensor Keap1 rendering it unable to repress transcription factor Nrf2. Thus, Nrf2 is stabilized and undergoes nuclear translocation where it binds to the ARE (in heterodimeric combinations with a small Maf protein), and ultimately activated transcription of cytoprotective phase 2 genes.

Because both heme oxygenase 1 and the thioredoxin/thioredoxin reductase system can be upregulated in an Nrf2/ARE-dependent manner, the questions arise whether: (i) the third member of the vitagene family, Hsp70, is also inducible by other phase 2 inducers, and (ii) there could be a common regulatory mechanism. Indeed, in addition to curcumin, several other inducers of Nrf2-dependent genes have been shown to increase the protein levels of Hsp70. Among them are the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15dPGJ$_2$), and the vicinal dithiol reagent phenylarsine oxide [176, 177]. Importantly, all of these compounds react with sulfhydryl groups and the transcriptional activation of both Nrf2, the major transcription factor responsible for phase 2 gene expression, and heat shock factor 1 (HSF1), the major activator of Hsp70 gene expression, depend on cysteine modification either within the Nrf2 regulator Keap1 [167, 174], or within HSF1 itself [178].

As already mentioned above, neurodegenerative disorders, such as, such as AD and Parkinson's disease (PD),

belong to the family of the "protein conformational diseases" and affect a large portion of our aging population [179]. In general, conformational diseases are conditions that arise from the dysfunctional aggregation of proteins in non-native conformations. It is known that the beta conformation in proteins is particularly susceptible to perturbations in the quality control system and that ROS play an important role in the development and/or pathogenetic progression in aging and neurodegenerative diseases [180–182]. Chaperones can rescue misfolded proteins by breaking up aggregates and assisting in the refolding process [181, 183]. Proteins that cannot be rescued by refolding can be delivered to the proteasome by chaperones to be recycled [183]. If the cell is not able to eliminate misfolded proteins multiple metabolic derangements resulting in the excessive production of ROS and RNS occur [184]. The ability of a cell to deal with oxidative and nitrosative stress requires functional chaperones, antioxidant production, protein degradation and a cascade of intracellular events collectively known as the "unfolded protein response", a form of cell stress response [185, 186]. As the cell's quality control system becomes overwhelmed, conformational changes occur to amyloid polypeptide intermediates, generating stable oligomers with an anti-parallel crossed beta-pleated sheet structure that eventually accumulate as space-occupying lesions within neurons [182]. Although it is clear why mutant proteins form amyloid, it is harder to rationalize why a wild-type protein adopts a native conformation in most individuals, but it misfolds in a minority of others, in what should be a common extracellular environment. This discrepancy suggests that another event likely triggers misfolding in sporadic amyloid disease. One possibility is that an abnormal metabolite, generated only in some individuals, covalently modifies the protein or peptide and causes it to misfold. Candidate metabolites are suggested by the recently recognized links between AD and atherosclerosis, in which known chronic inflammatory metabolites, may play a critical pathogenic role. If this holds true, then new targets are disclosed for a prevention strategy brought about through nutritional antioxidants.

Alzheimer disease is characterized by a subtly impaired cognitive function or a disturbance of behaviour. With time there is a gradual memory loss and disorientation which eventually progress into dementia. Although, most cases are sporadic, 5–10% or more are familial. Gross examination of the brain in AD shows a variable degree of cortical atrophy with narrowed gyri and widened *sulci* most apparent in the frontal, parietal and temporal lobes. Microscopically, the features include neurofibrillary tangles, neurite (senile) plaques, the central core of which is amyloid-beta peptide, derived from the transmembrane amyloid precursor protein (APP), amyloid angiopathy, granulovacuolar degeneration and Hirano

bodies. Importantly, all of these changes are present in the brains of nondemented older individuals but to a much lesser extent [187, 188]. Several lines of evidence now support a fundamental role for oxidative and nitrosative stress in the pathogenesis of this disease [20, 187, 189]. As said, the only evidence of a protective role of curcumin in the onset of AD was provided by Ganguli and colleagues demonstrated that Indian population, who have a curcumin-enriched diet, has a reduced prevalence of AD compared to United States [40]. Following this observation, many basic studies were conducted and the neuroprotective role of curcumin was corroborated. In vitro studies have shown that curcumin protects neuron-like PC12 cells from β-amyloid toxicity and, interestingly, the polyphenol displayed a neuroprotective effect greater than a well known antioxidant such as α-tocopherol [190]. By using an Alzheimer transgenic APPSw mouse model (Tg2576), Lim and colleagues have shown that dietary curcumin suppressed inflammation and oxidative damage in the brain of these mice [41]. More recently, Garcia-Alloza et al. in transgenic APPswe/PS1dE9 mice demonstrated that curcumin, given intravenously for 7 days, crosses the blood–brain barrier, binds to β-amyloid deposits in the brain and accelerates their rate of clearance [126]. These latter results are in good agreement with previous findings which demonstrated that curcumin disaggregates and inhibits β-amyloid aggregation [191, 192].

Parkinson's disease, whose cardinal features include tremor, slowness of movement, stiffness and poor balance, is attributed to a profound deficit in dopamine that follows the loss of dopaminergic neurons in the *substantia nigra pars compacta* and dopaminergic nerve terminals in the *striatum* [20, 193]. Although the mechanisms leading to PD are still uncertain, a large amount of experimental evidence implicates oxidative and nitrosative stress as one of the crucial factors in the pathogenesis of PD [194, 195]. Considerable insights into the pathogenesis of PD, indeed, have been achieved by use of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is commonly used to induce an experiment model of PD [194, 196]. Excessive free radical formation or antioxidant deficiency and the resulting oxidative stress are all mechanisms involved in MPTP neurotoxicity [197]. Rajeswari has shown that curcumin protects rat brain from MPTP-induced neurotoxicity by virtue of its scavenger activity [198]. On the other hand, curcumin has been shown to protect PC12 cells from MPP^+ (the active metabolite of MPTP) by inducing the antiapoptotic protein bcl-2, preventing the dissipation of mitochondrial membrane potential and reducing the intracellular iNOS levels [199]. The importance of mitochondria in the neuroprotective effect of curcumin has been also stressed by Mythri et al. [200] who demonstrated that curcumin prevents the

formation of peroxynitrite which is responsible for the complex I damage which is a common feature in PD.

Friedreich's ataxia (FRDA) is an autosomal recessive disease usually caused by a homozygous GAA expansion in the intron 1 of the frataxin encoding gene. This mutation results in a marked reduction in the amount of the FXN mRNA and subsequently of frataxin, a ubiquitously expressed mitochondrial protein [201–204]. Friedreich's ataxia is the most common inherited ataxia. Its estimated prevalence in European populations is 1 in 50,000 [201]. Friedreich's ataxia is characterized by onset before 20 years of age of ataxia of all four limbs, associated with cerebellar dysarthria, decreased/absent tendon reflexes, sensory loss and pyramidal signs. The progression of the disease is relentless. Skeletal deformities and cardiomyopathy are found in a majority of patients, who also have an increased frequency of impaired glucose tolerance and diabetes [201]. Generally within 15–20 years after the first appearance of symptoms, affected individuals are confined to a wheelchair. The neurological symptoms result from progressive degeneration of the dorsal root ganglia, posterior columns, corticospinal tracts and the dorsal spinocerebellar tracts of the spinal cord [205, 206]. How the GAA expansion leads to decreased FXN transcript levels remains not yet clarified [207, 208]. It could inhibit transcription initiation, block elongation, interfere with splicing, decrease mRNA stability or produce a combination of these effects. The absence of abnormally spliced frataxin mRNA species in FRDA cells suggests RNA splicing is not affected [204]. It has been suggested that the triplet repeats form a special structure, e.g. triplexes, which interferes with transcription elongation, however this was not confirmed in all studies [209, 210]. Transcription initiation could be inhibited by the formation of heterochromatin at the FXN locus in presence of the GAA expansion. Active promoters are associated with histone H3 and H4 acetylation (in particular H3/AcK9), with certain histone H3 methylations (in particular H3/(Me)3K4) and with the presence of the replacement histone H2A.Z; while inactive promoters are characterised by reduced histone acetylation, the presence of trimethylated H3/K27, K9 and K79 as well as trimethylated H4/K20. Highly repressed genes are also associated with DNA methylation [211]. Consistent with heterochromatin formation in presence of the GAA expansion, using a FRDA lymphoblast cell line decreased histones acetylation and increased trimethylated H3K9 levels were found in the FXN gene [212]. In an additional 3 FRDA lymphoblast lines the levels of H3/K9 methylation in FXN intron 1 and the methylation of 3 CpGs in intron 1 upstream of the GAA repeat were markedly increased [213]. In a recent study it was demonstrated that CpG sites in the region upstream the GAA repeat are consistently hypermethylated in FRDA

patient brain, heart and cerebellum [214]. Furthermore, in the same study it was shown an overall decrease of histone H3K9 acetylation together with increase of H3K9 methylation in FRDA brain tissue. Moreover HDAC inhibitors had been shown to increase frataxin expression from a FXN reporter construct using butyrate and in FRDA lymphocytes using BML-210 and its analogs, however the analysis of the relationship between increased FXN transcripts and gene specific histone modification in these cells was limited and the influence of trans acting factors was not excluded [212, 215].

The role of frataxin is controversial. At least five different hypotheses for its function have been proposed: (1) mitochondrial iron transport, (2) mitochondrial iron storage, (3) Fe–S cluster biosynthesis, (4) Fe–S cluster protection and/or repair and (5) protection from oxidative stress. An involvement of frataxin in iron homeostasis has been proposed since iron accumulation has been detected in yfh1 knockout models, in FRDA patients heart and liver mitochondrial [216–219]. Iron accumulation could increase the formation of OH radicals, through Fenton reaction, leading to oxidative damage of Fe–S cluster containing proteins, whose activity is reduced in FRDA yeast model and FRDA patient tissues [216, 220]. Frataxin may interfere with the activity of the Fe–S cluster-containing subunits of mitochondrial respiratory complexes I, II and III, as a reduction in activity of these complexes was found in FRDA yeast models, in FRDA mouse models and patient tissues. The activity of aconitase, an Fe–S protein involved in iron homeostasis, was found to be deficient as well [216, 220, 221]. Bulteau et al. [222] demonstrated that frataxin protects the aconitase [4Fe–4S]2+ cluster from disassembly and promotes enzyme reactivation, suggesting a role of frataxin in Fe–S cluster protection and/or repair. An increased oxidative stress has been demonstrated in individuals with FRDA. Elevated concentrations of oxidative stress markers were evidenced in patients with FRDA [223–226]. Urinary concentrations of 8-hydroxy-2′-deoxyguanosine (8OH2′dG), a marker of oxidative DNA damage, were found to be increased in patients with FRDA as compared with controls [224]. Plasma malondialdehyde (MDA) levels, a product of lipid peroxidation, were found to be raised in Friedreich's ataxia (FRDA) patients [225, 226]. These levels correlated with increasing age and disease duration, suggesting that lipid peroxidation increased with disease progression [226]. Piemonte et al. [223] evidenced a reduction of free glutathione levels in the blood of patients with Friedreich's ataxia, suggesting a relevant role of free radical cytotoxicity in the pathophysiology of the disease. Moreover an increased ROS production in frataxin deficient cells has been described [227].

Since the GAA repeat expansion leads to a reduction in the FXN transcript and frataxin protein, a number of studies have been conducted to identify compounds that increase frataxin expression. Sturm et al. [228] showed that the recombinant human erythropoietin (rhuEPO) significantly increases frataxin expression in primary lymphocytes from FRDA patients by a still unknown mechanism. In a recent open-label clinical pilot study treatment of 12 FRDA patients with recombinant human erythropoietin led to a persistent and significant increase in frataxin levels after 8 weeks ($P < 0.01$) [229]. HDAC inhibitors have been shown to increase frataxin expression. Using a FXN reporter construct Sarsero et al. [215] showed that butyrate is able to increase frataxin expression. Herman et al. [212] described an increase in FXN transcript and frataxin protein in FRDA lymphoblasts and lymphocytes after the treatment with BML-210 and its analogs. Given the evidence of oxidative damage in FRDA patients the treatment as been focused on antioxidant protection [230, 231]. FRDA patients have been treated with a variety of antioxidants including idebenone and vitamin E [231]. Lodi et al. [232], using in vivo phosphorus magnetic resonance spectroscopy (31P-MRS), showed that after only 3 months of treatment with coenzyme Q10 and vitamin E (Coenzyme Q10 400 mg/day, vitamin E 2100 IU/day), the cardiac phosphocreatine to ATP ratio increased to 178% ($P = 0.03$) and the maximum rate of skeletal muscle mitochondrial ATP production increased to 139% ($P = 0.01$) of their respective baseline values in the FRDA patients. These improvements were sustained after 6 months of therapy. The treatment with these agents of ten FRDA patients for 47 months resulted in a significant and sustained improvement in cardiac and skeletal muscle bioenergetics. Moreover echocardiographic data revealed significantly improved cardiac function, defined by increased fractional shortening at the 35- and 47-month time points [233]. Clinical trials with idebenone in FRDA patients showed a reduction of the left ventricular mass, left ventricle posterior wall and interventricular septal thickness [234–237]. In some of these studies an improvement of cardiac function was also detected [235, 236].

In the previous studies, idebenone did not modify neurologic manifestations of the disease. However, a recent 6-month double-blind, placebo-controlled study conducted in 48 FRDA patients using three different doses of idebenone (approximately 5, 15, and 45 mg/kg) showed a significant improvement in ICARS and a dose-related response in ICARS, FARS, and ADL scores, when patients who required wheelchair assistance were excluded from the data analysis. The latter study suggests that higher doses may be necessary to have a beneficial effect on neurological function [238]. Since Friedreich Ataxia (FRDA) is characterized by an increased mitochondrial oxidative damage, antioxidants targeted to mitochondria should be particularly effective at slowing disease progression. Using FRDA

fibroblasts, Jauslin et al. [239] showed that the mitochondria-targeted antioxidant MitoQ was several hundred fold more potent than the untargeted analog idebenone and that the mitochondria-targeted antioxidant MitoVit E was 350-fold more potent than the water soluble analog Trolox. Since induction of HSPs synthesis results in tolerance against oxidative stress-induced damage, molecules activating this defense mechanism are possible candidates for treatment of neurodegenerative diseases, such as Friedreich's ataxia [240].

Transient forebrain ischemia is a common cause of stroke and occurs in people suffering from cardiovascular diseases [241]. As a consequence of ischemia and the following reperfusion, a cascade of events such as increased calcium release, the overexpression of COX-2 and iNOS both of which are important free-radical generators and trigger neuronal cell death in selected brain areas including the hippocampal cornu ammonis 1 (CA1) [1, 22, 52, 241]. Curcumin exerted a neuroprotective effect in rats who underwent ischemia/reperfusion injury and this effect has been related to the direct scavenger effect of curcumin as well as to a curcumin-induced interference with the apoptotic machinery, increase in antioxidant molecules (GSH) and enzymes (catalase, superoxide dismutase) [241–243].

L-Carnitine and Acetyl-L-Carnitine

L-Carnitine (LC) (Fig. 8b) is a natural compound and its biological role is to facilitate the transport of fatty acids to mitochondria. Dietary LC derives from the intake of red meats, but the endogenous synthesis of LC from the amino acid precursors lysine and methionine has been also documented [244]. The dietary intake of LC in humans ranges from 1 to 15 μmol/kg body weight/day, whereas the rate of biosynthesis is about 1–2 μmol/kg body weight/day [245]. Recently, exogenous LC, given by oral (p.o.) or intravenous (i.v.) routes, has been used for the treatment of cognitive disorders such as AD and dementia [244]. After oral ingestion, dietary LC is well absorbed by simple or carrier-mediated diffusion and its bioavailability is 54–86%; conversely, the bioavailability of exogenous LC is much lower, in the range 5–18% [244, 245]. This paradoxical effect can be explained considering that the absorption of LC decreases as the intake of LC increases, this to maintain the concentration of LC constant [244, 245]. The normal plasma concentration of LC in healthy adults with a mixed diet is 40–50 μM [244, 246]. When administered at doses 30–100 mg/kg p.o. in humans, LC peak plasma concentrations were 27–91 μM after 3 h, and returned to the baseline within 24 h [245, 247]. L-Carnitine undergoes acetylation in rodents and human intestine thus forming esterified compounds such as acetyl-L-carnitine

(ALC) (Fig. 8b) which is endowed with biological activity per se [244, 245]. Interestingly, ALC diffuses across membranes much better than LC and its efflux in the systemic circulation has been calculated to be four times greater than that of LC [245, 248]. Data from AD patients have shown that after supplementation with pharmacological doses of ALC (2 g/day) for 55 days, its plasma concentrations increased from 7.2 to 10.3 μM [245]. In the plasma, neither LC nor ALC are bound to proteins [244]. The volume of distribution of LC differs considering the dietary or exogenous source being approximately 3000 l and 20–50 l, respectively [244]. This great difference in the volume of distribution between dietary and supplemental LC depends on the different degree of absorption, slow accumulation in tissues such as the muscle and rate of kidney elimination (see below), and therefore these numbers should be considered purely indicative [244]. It is interesting to underlie that ALC is able to cross blood–brain barrier; as shown by Parnetti et al. AD patients treated with ALC i.v. or p.o. for 10–60 days have an increased concentration of ALC in the cerebrospinal fluid up to 3.55 nmol/ml [249]. In human subjects treated with LC i.v. its elimination half-life ranged from 3 to 12 h [244]. However due to the long-lasting release of LC by the muscle, the total time of turnover from the body has been estimated to be 66 days [245]. L-Carnitine is metabolised by the intestine to γ-butyrobetaine and trimethylamine, the former excreted by the feces and the latter in urine [244, 245]. Accordingly, the renal clearance of LC which is about 1–3 ml/min suggesting an extensive rate of tubular reabsorption, significantly increases at values close to the creatinine clearance with the increase in LC plasma concentrations indicating that tubular reabsorption approaches full saturation [244]. This last finding is very important and contributes to explain how exogenous LC is almost completely excreted during the first 12 h after administration whereas dietary LC is reabsorbed [244]. Due to its elimination mainly through the kidney, LC and should be administered very carefully to patients affected by renal impairment [250].

Acetyl-L-carnitine has been proposed to have beneficial effects in preventing the loss of brain function which typically occurs during aging and neurodegenerative disorders. The main mechanism of action of ALC is the improvement of mitochondrial respiration which allows the neuron to produce ATP necessary to maintain the normal membrane potential [251]. However, ALC has been shown to be neuroprotective through a variety of other effects such as the increase in PKC activity [251]. Interestingly ALC counteracted the loss of NMDA receptors in neuronal membrane and increased the production of neurotrophins, two effects strictly related to synaptic plasticity [251]. Recent studies have shown that ALC reduces Aβ toxicity

in primary cortical neuronal cultures by increasing both HO-1 and Hsp70 expression [252]. Studies in rats have shown that chronic ALC treatment increases life-span, improves cognitive behaviour in aged animals and guarantees long-term memory performance [251]. Furthermore, chronic ALC treatment has been shown to prevent age-related changes in mitochondrial respiration and decrease oxidative stress biomarkers through the up-regulation of HO-1, Hsp70 and superoxide dismutase-2 in senescent rat [253] (Fig. 3). Taken together, these pre-clinical studies suggested that ALC treatment could be beneficial for the treatment of age-related diseases and the potential use in humans has been encouraged. Patients affected by AD and treated with ALC at doses ranging from 1 to 2 g/day for 6–12 months, have shown an improved performance on several cognitive tests such as word recognition, name learning and world list recall with respect to placebo-treated patients, but none of these effects was significant [251, 254]. In two clinical studies, ALC 3 g/day for 1 year significantly reduced cognitive decline only in early-onset AD patients [255, 256], but this evidence was not confirmed in a later ad hoc designed study [257]. Consistently, we have demonstrated that ALC induces HO-1 in a dose and time dependent manner and that this effect was associated with up-regulation of other Hsps as well as high expression of the redox-sensitive transcription factor Nrf2. The results from this study show for the first time that acetylcarnitine induces heme oxygenase-1 and Hsp60 heat shock proteins, and that this effect may involve the transcription factor Nrf2, implying the conceivable possibility that acetylcarnitine, by promoting acetylation of DNA-binding proteins, can induce post-translational modifications of critical target proteins endowed with DNA competence and transactivating activity [258]. Very importantly, this new envisioned role of LAC as a molecule endowed with the capability of potentiating the cellular stress response pathways appear to be promising an alternative therapeutic approach for those pathophysiological conditions where stimulation of the HO pathway is warranted [258].

Carnosine

Carnosine is a natural dipeptide(β-alanyl-L-histidine) (Fig. 8b) present in long-lived mammalian tissues [259] and has numerous roles as proton buffer, metal chelator, antioxidant, antiglycating, immunostimulant, antitumoral and wound-healing agent [260, 261]. Since β-alanine is non-proteinogenic amino acid, it is obvious that carnosine is not product of protein catabolism: Instead it is synthesized enzymatically by carnosine synthetase, an enzyme present in brain and muscle that shows broad substrate specificity [262]. The hydrolysis of carnosine is catalyzed by two enzymes recently cloned and characterized [263]. Both enzymes belong to the M20 metalloprotease family. The enzyme named CN1 exhibits narrow specificity and the characteristics of the enzyme previously designated X-His dipeptidase or carnosinase [264]. The enzyme named CN2 displays broad substrate specificity and is ubiquitously expressed like the enzyme previously designated cytosol non-specific dipeptidase [265]. In the brain, carnosine has been found in glial cells and in some type of neurons [266]; uptake of carnosine has been found to be mediated by a high affinity, energy-dependent dipeptide transport system, identified as the peptide transporter PepT2 [267]. Carnosine has been shown to delay ageing in cultured human fibroblasts [268], male Drosophila [269] and senescence-accelerated mice [270]. Therapeutic potential has also been invoked in cataractogenesis [271] and diabetes [272–274]. The occurrence of carnosine and its analogue homocarnosine(γ-aminobutyryl-histidine) in brain, and homocarnosine in CSF, and their age-related alterations [275, 276] suggested a role for these peptides with respect to suppression of onset or progression of AD and other neurodegenerative diseases [277, 278]. In brain cells, carnosine has been shown to be neuroprotective because of its ability to counteract both oxidative and nitrosative stress related to several pathological conditions including ischemia [279–281], methamphetamine neuro-toxicity [282] and neurodegenerative disorders [283]. Importantly, carnosine may indirectly influence neuronal excitability by modulating the effect of zinc and copper [284, 285]. Furthermore, carnosine has been shown prevent β-amyloid aggregation and toxicity [286] and this effect can be due to the known ability of this peptide to inhibit protein misfolding and avoid the formation of advanced-glycation end-products [261]. More recently, it has been reported that the peptide is able to protect against Aβ-induced neurotoxicity in differentiated rat PC12 cells by regulation of glutamate release and glutamate release and NMDA receptor trafficking [287, 288]. Interestingly, carnosine plasma levels have been found to be lower in AD patients than in age-matched controls [289]. Furthermore, carnosine has been shown to counteract peroxynitrite-dependent protein alterations such as tyrosine nitration [290] and to inhibit the No-dependent activation of guanylate cyclase [291]. Recent evidence demonstrated that carnosine prevents the up-regulation of iNos and the induction of both HO-1 and Hsp-70 following strong nitrosative conditions [292]. In addition, a correlation has been found between cell protection and NO free-radical scavenging activity of carnosine that showed direct NO-trapping ability in cell-free experiments [293]. Taken together, these findings allow us to propose the metabolic pathway leading to carnosine formation as a potential target for the prevention and/or treatment of

neurodegenerative disorders. In addition, new carnosine, homocarnosine and anserine derivatives have been synthesized and characterized, showing chelating and antioxidant properties similar to those of the parent dipeptides [294–297]. In addition, these new compounds survive to attack by carnosinases [298] and should be explored for their ability to suppress age-related neurodegeneration where increased zinc and copper could play a role [299–301].

Resveratrol

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) (Fig. 8a) is a phytoalexin found in grapes, cranberries and peanuts [302, 303]. Several in vitro studies have shown that resveratrol is a powerful molecule endowed with antioxidative, anticancer, anti-inflammatory and estrogenic activities [302, 304]. Studies in rodents and humans have shown that after oral ingestion resveratrol is readily absorbed (at least 50% in the rat) reaching peak plasma concentration after 10–60 min up to 2 μM total resveratrol (i.e. genuine resveratrol *plus* resveratrol derived from the hydrolysis of its conjugated products) whereas the amount of unchanged resveratrol was in the low nanomolar range [302–306]. In hepatic cells, resveratrol has been absorbed by passive diffusion and carrier mediated processes [307]. Interestingly, the amount of resveratrol adsorbed did not change in the presence of ethanol [302]. Resveratrol binds to plasma proteins such as albumin and lipoproteins [307]. The plasma half-life was estimated to be 12–15 min in the rat and 9–12 h in humans [303, 305]. Resveratrol undergoes massive metabolism both in gastrointestinal cells and liver. The main metabolites detected in humans are resveratrol monosulphate, resveratrol monoglucuronide (two isomeric forms), dihydroresveratrol monosulphate and monoglucuronide [303, 304]. The serum half-life of these metabolites is about 9.2 h, i.e. significantly higher than that of unmodified resveratrol [304, 305]; both in rodents and humans, enteric recirculation of resveratrol metabolites has been proposed and may account for the increased half-life of these metabolites with respect to the unchanged form. Resveratrol has been shown to inhibit CYP3A4 irreversibly and to be a reversible inhibitor of CYP2E1 [304, 308]. Furthermore, in rat cardiomyocytes resveratrol increased the activity of glutathione transferases, well known phase 2 enzymes involved in the detoxification of drugs [309]. Resveratrol is mainly excreted by the kidney and only a small portion is recovered in feces [302]. It is noteworthy to mention that in rodents and rabbits the tissue concentrations of resveratrol are always below 1 nmol/g fresh tissue [302, 310], whereas in humans the organs in which resveratrol shows greater accumulation are intestinal

mucosa, stomach, liver, and kidney, but the exact concentrations have not been estimated [304].

The rationale to use resveratrol in the treatment of neurodegenerative disorders is based on the well-known antioxidant activity of this compound. Resveratrol has been shown to protect rat glioma cells from β-amyloid (Aβ) toxicity by reducing the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2), thus preventing the uncontrolled release of NO and prostaglandin E2 (PGE2) [311]. These effects, could be ascribed to the ability of resveratrol to prevent the Aβ-induced nuclear translocation of NF-κB [311]. Furthermore, in PC12 cells, resveratrol counteracted the Aβ$_{25-35}$-induced toxicity by the down-regulation of pro-apoptotic factors such as Bax and c-Jun N-terminal kinase (JNK) proteins [312, 313]. In rat hippocampal neuronal cell cultures, resveratrol protected cells from Aβ toxic effects by inducing protein kinase-C (PKC) [312, 314]. The neuroprotective effect of resveratrol against Aβ toxic effects could be also mediated by promoting the intracellular degradation of Aβ through the ubiquitin proteasome system (UPS) [315]. Interestingly, resveratrol reduced sodium nitroprusside (SNP)-released NO toxicity in an experimental system of rat hippocampal mixed neuronal/glial cultures [316]. In the rat, the chronic administration of resveratrol (21 days) resulted in a marked inhibition of the cognitive impairment secondary to the intracerebroventricular administration of streptozotocin and this effect has been related to the increase of brain GSH levels [317]. Still under investigation is the neuroprotective effect of resveratrol through the overexpression of sirtuins [312, 318]. In particular, Sirt1–3 play a main role in protecting neurons in AD patients at least by 3 independent mechanisms: (i) the repression of the pro-apoptotic protein p53 and forkhead transcription factor3 (FOXO3) expression, (ii) the reduction of the hyperphosphorylation of tau protein and (iii) the reduction of ROS formation through the interaction with the uncoupling protein 4 (UCP4) [319]. Of note is the interaction of resveratrol with the vitagenes. Very recently, resveratrol has been demonstrated to increase the expression of HO-1 in PC12 cells and primary neuronal cultures presumably through the activation of NF-E2-related factor 2 (Nrf2) in PC12 cells [320, 321]. Although not directly related to AD, these data are in good agreement with previous papers which demonstrate how the overexpression of HO-1, and the related increase in antioxidant capacity, is neuroprotective in several models of AD. Indeed, Takahashi et al. found that cortical neurons cultured from mice expressing the Swedish mutation of AD had defects in BR production with subsequent increase of hydrogen peroxide toxicity [322]. Furthermore, in transfected neuroblastoma cells overexpressing HO-1, the activity of this enzyme was increased, and conversely, the level of tau protein was

significantly decreased when compared with antisense HO-1 or vector transfected cells [38]. The suppression of tau protein expression was almost completely counteracted by zinc-deuteroporphyrin, a specific inhibitor of HO activity [38].

Conclusions and Perspectives

Protein conformational diseases, such as Alzheimer's, Parkinson's, Huntington's and ALS affect a large portion of our aging population. In general, conformational diseases are conditions that arise from the dysfunctional aggregation of proteins in non-native conformations. Cells have evolved mechanisms for rescuing and recycling misfolded proteins, but these systems sometimes fail. However, chaperones can rescue misfolded proteins by breaking up aggregates and assisting in the refolding process. Proteins that cannot be rescued by refolding can be delivered to the proteasome by chaperones to be recycled. This often is associated with multiple metabolic derangements that result in the excessive production of ROS and oxidative stress. These ROS set in motion a host of redox reactions which can result in unstable nitrogen and thiol species that contribute to additional redox stress which trigger neuroinflammation [323, 324].

Aging is characterized by a progressive deterioration of physiological functions and metabolic processes. Healthy aging remains one of the ideals of modern society. In aging and in diseases associated with the elderly, such as Alzheimer's or Parkinson's, the loss of cells in vital structures or organs may be related to several factors, among which the production of ROS and or RNS by mitochondria is a common denominator, leading to DNA damage, apoptosis and cell death. Although a diet rich in antioxidants seems to offer hope in delaying the onset of unhealthy disorders that accompany aging, no clinical treatment as such has yet been developed and anti-aging drugs are still unavailable. It is well established that reducing food intake (caloric restriction) extends the life-span in a wide range of species. The protein implicated in this protective process is the silent information regulator 2 (SIR2, SIRT1 in mammals), a vitagene enzyme that belongs to a nicotinamide adenine dinucleotide (NAD)+-dependent protein deacetylases [318]. SIRTs regulate gene silencing, DNA repair, rDNA recombination, and ageing, apart from regulating programmed cell death. In this context, increasing SIRT1 has been found to protect cells against amyloid-beta-induced ROS production and DNA damage, thereby reducing apoptotic death in vitro. Moreover, it has been demonstrated that Alzheimer's and Huntington's disease neurons are rescued by the over-expression of SIRT1, induced by either caloric restriction or administration of resveratrol, a

potential activator of this enzyme. The therapeutic use of polyphenols and other related compounds, which impact the vitagene network and, as such, acting as SIRT1 or Hsps pathway modulators, have been discussed in this review in the perspective of treating aging-related brain disorders. Although some beneficial phytochemicals might function solely as antioxidants, it is becoming clear that many of the beneficial chemicals in vegetables and fruits evolved as toxins (to dissuade insects and other predators) that, at subtoxic doses, activate adaptive cellular stress–response pathways, under control of genes defined by us as vitagenes in a variety of cells including neurons. Examples of such 'preconditioning' or 'neurohormesis' pathways include those involving cell-survival signaling kinases, the transcription factors NRF2 and histone deacetylases of the sirtuin family (Figs. 2, 3). In these ways, neurohormetic phytochemicals such as resveratrol, sulforaphanes and curcumin or other compounds such as acetyl-L-carnitine or carnosine might protect neurons against injury and disease by stimulating, through the vitagene system, the production of antioxidant enzymes, protein chaperones and other proteins that help cells to withstand stress.

The strong evidence that the vitagene network operates as a defense system in the brain during oxidative and nitrosative stress open new perspectives in the treatment of neurodegenerative disorders. Therefore, the nutritional manipulation of endogenous cellular defense mechanisms represents an innovative approach to therapeutic intervention in neurodegeneration, and propose potential novel therapeutic strategies relying upon the simultaneous activation of cytoprotective genes of the cell life program and down-regulation of proinflammatory and pro-oxidative genes involved in programmed cell death. Although the term vitagene was first proposed to indicate speculatively the existence of genes as opposed to gerontogenes, the first evidence based notion identifying vitagenes with stress responsive genes such as HO-1, Hsps, TrxR and sirtuins have been provided by our group (Fig. 3). Presented here is strong evidence that a functional interplay between stress response genes is important for cell stress tolerance, highlighting compelling reason for a renewed effort to understand the central role of this most extraordinary defense system in biology and medicine.

However, in the fight against oxidative and nitrosative stress, a major role is played by mitochondria which can be the target for cytoprotective effects of many dietary antioxidants. Acetyl-L-carnitine and lipoic acid (LA) has been shown to improve mitochondria bioenergetics and decrease oxidative stress associated with aging in many organs including rodent heart, liver and brain [325–329]. In particular, ALC ameliorates mitochondrial beta-oxidation whereas LA, due to its nature of thiol antioxidant as well as mitochondrial metabolite, increases the total antioxidant

status and counteract free radical-induced cell damage [327, 329].

In this respect the concept of hormesis has been receiving greater interest in the biomedical and toxicological research communities over the past decade [330]. In the fields of biology and medicine hormesis is defined as an adaptive response of cells and organisms to a moderate (usually intermittent) stress [330, 331]. The findings suggest that hormetic effects represent evolutionary-based adaptive responses to environmentally induced disruptions in homeostasis. Such adaptive responses, which are incorporated into organismal integrative physiological systems and now clarified at the mechanistic level for more than two dozen receptor systems, provide a cogent basis for the application of hormetic mechanisms in the elucidation of fundamental evolutionary-based biological processes and in the development of novel clinical modalities. Examples include ischemic preconditioning, exercise, dietary energy restriction and exposures to low doses of certain phytochemicals. Recent findings have elucidated the cellular signaling pathways and molecular mechanisms that mediate hormetic responses which typically involve enzymes such as kinases and deacetylases, and transcription factors such as Nrf-2 and NF-κB. As a result, cells increase their production of cytoprotective and restorative proteins including growth factors, phase 2 and antioxidant enzymes, and protein chaperones [330, 331]. A better understanding of hormesis mechanisms at the cellular and molecular levels is leading to and to novel approaches for the prevention and treatment of many different diseases [332].

The several lines of evidence listed above supports also the notion that stimulation of various maintenance and repair pathways through exogenous intervention, such as mild stress or compounds targeting the heat shock signal pathway, such as polyphenols, L-acetyl-carnitine and carnosine may have biological significance as a novel approach to delay the onset of various age-associated alterations in cells, tissues and organisms. Hence, by maintaining or recovering the activity of vitagenes can be possible to delay the aging process and decrease the occurrence of age-related diseases with resulting prolongation of a healthy life span.

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