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The UPR and the Anti-oxidant Response: Relevance to Sleep and Sleep Loss

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Abstract Oxidative stress has been linked to various physiological and pathological processes such as aging and neurological disorders. Recent evidence has now implicated a role for oxidative stress in sleep and sleep loss. Studies suggest that wakefulness results in an oxidative burden and sleep provides a protective mechanism against these harmful effects. Prolonged wakefulness/sleep deprivation activates an adaptive stress pathway termed the unfolded protein response (UPR), which temporarily guards against the deleterious consequences of reactive oxygen species. The UPR affects the function of the endoplasmic reticulum, which is the site for integral and secretory membrane processing and folding. Several downstream effectors of the UPR operate in an antioxidant capacity to reduce the load of these toxic species; a process that may be important in delaying the progression of neurodegenerative diseases. This review will highlight the molecular components of the UPR that ameliorate the accumulation of oxidative stress and may therefore provide potential therapeutic targets.

Keywords UPR · Sleep · Sleep loss · Oxidative stress · Anti-oxidant · Nrf2 · ATF4 · Aging

Introduction

Sleep is a universal, dynamic brain process that has been demonstrated in organisms ranging from invertebrates to mammals. Although the exact function of sleep has yet to be discovered, there are a multitude of molecular changes that occur within the sleep state, as well as wakefulness and extended wakefulness/sleep deprivation (SD) [1, 2].

There are several current working hypotheses on the functions of sleep. The synaptic homeostasis hypothesis postulates that a major role of sleep is to regulate synaptic weight in the brain. This hypothesis encompasses several specific aspects of sleep and claims that (1) wakefulness is associated with synaptic potentiation in several cortical circuits; (2) synaptic potentiation is tied to the homeostatic regulation of slow wave activity; (3) there is an affiliation between slow wave activity and synaptic downscaling; and (4) synaptic downscaling is linked to the beneficial effects of sleep on neural function and performance (for a more detailed review, see [3]).

The biosynthesis of key cellular components has also been postulated to be an essential function of sleep. Mackiewicz et al. identified approximately 3,988 genes in the cerebral cortex and 823 genes in the hypothalamus that alter their expression profiles in response to different behavioral states, i.e., periods of sleep and sleep deprivation [4]. Such a large change in the expression patterns of genes involved primarily in biosynthetic pathways gives credence to current models that implicate a role of sleep in restorative function(s) whose nature has yet to be uncovered. The concept that biosynthesis of cellular components, particularly protein synthesis, is a function of sleep is supported by studies that indicate that sleep homeostasis may be induced by an increased demand for brain protein synthesis [5]. One other possible function of sleep may include providing a protective role against oxidative stress [6]. Oxidative stress occurs when there is an imbalance between the reactive oxygen species (ROS) generated and clearance by the endogenous antioxidant defense system. Experiments

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aimed at testing the relationship between sleep and oxidative stress has primarily focused on sleep deprivation studies. Research has found that ROS and lipid peroxidation levels are increased in the hippocampus of rodents that have been sleep deprived [7]. Another study demonstrated that antioxidant capacity was decreased in peripheral tissues of animals that were deprived of sleep for 5 and 10 days [8]; parameters which were restored after recovery sleep. Studies such as these have led to the hypothesis that sleep functions to diminish ROS levels and/or prepare the antioxidant system to remove the elevated levels of ROS that are produced in wakefulness. However, the relationship between oxidative stress and SD remains unclear because of conflicting data generated from studies addressing this particular hypothesis. Gopalakrishnan et al. found no evidence of oxidative damage to neither lipids nor proteins in animals that had been sleep-deprived either in the brain or the peripheral tissues, which included the liver and skeletal muscle homogenates [9]. Animals were either sleep deprived short term (8 h) or long term (3-14 days). Although the authors did find an increase in total superoxide dismutase (SOD) activity in rats allowed to recover sleep after they had been selectively sleep deprived during REM sleep; this increase was absent in total sleep-deprived animals (3-14 days). Another group reported no increase in antioxidant enzymes SOD, catalase, glutathione peroxidase, or malondialdehyde activity following sleep deprivation in rats [10]. Other studies, however, infer that SD may cause conditions associated with oxidative stress, including a decrease in glutathione levels in whole rat brains [11] and significant reductions in the activity of SOD in the hippocampus and brain stem [12]. Chang et al. found a sharp increase in oxidative stress and lipid peroxidation in the hepatocytes of sleep-deprived rats [13]. Interestingly enough, studies have shown that sleep deprivation itself causes cellular stress, a condition that has been correlated with the interruption of various physiological processes [14], including the dysregulation of protein homeostasis in the endoplasmic reticulum (ER) [15].

ER Stress and the Unfolded Protein Response

Recent evidence supports an important role for the adaptive ER stress response in injuries related to models of ischemia–reperfusion challenges [16–18]. The ER is an organelle that has an elaborate intracellular membranous network that expands throughout the cytoplasm. Its primary functions are to direct the synthesis, processing, folding, and post-translational modifications of all secretory and integral membrane proteins. Under normal physiological conditions, there is a system of ‘checks’ and ‘balances’, where nascent proteins are properly folded with the help of molecular chaperones (ER chaperones) and enzymes then transported to the Golgi apparatus. However, when newly synthesized proteins exceed the capacity of the folding machinery, it causes an accumulation of unfolded or misfolded proteins, which perturbs ER homeostasis and causes ER stress. Various physiological stress conditions, including disturbances in calcium homeostasis, glucose/energy deprivation, ischemia, expression of mutant proteins, and redox changes can evoke the ER stress response [19]. As a result to these insults, the cell has evolved an adaptive mechanism that diminishes the detrimental effects of accumulating unfolded/misfolded proteins that reach toxic concentrations in the ER. These signaling pathways are labeled the ER stress response or the unfolded protein response (UPR; for reviews, see [20–23]) and its primary goal is to return the ER to its normal functioning state. The UPR consist of three principal branches that include: (1) increasing transcription of resident chaperone proteins such as BiP/glucose-regulated protein 78 (GRP78), the master regulator in the ER stress response and a member of the heat shock protein 70 (HSP 70) family of molecular chaperones, (2) regulating the serine–threonine kinase PKR-like ER kinase (PERK), which phosphorylates the eukaryotic initiation factor 2α (eIF2α) and attenuates protein translation; and stimulates removal of misfolded proteins through a process called ER-associated degradation [24]. However, if ER stress is upregulated for an extended period of time, apoptotic pathways are activated (Fig. 1) [23, 25].

The Unfolded Protein Response and Sleep Deprivation

Studies have shown that sleep deprivation can also activate the UPR. Wakefulness and short periods of sleep deprivation are associated with upregulation of small heat shock proteins, molecular chaperones (HSP 70, HSP 60) and chaperones in the ER, which include BiP/GRP78 [2, 26–28]. Protein levels of BiP were upregulated in the cerebral cortex of mice sleep deprived between 6 and 12 h [15] and all elements of the UPR pathway occur within the sleep-deprived animals. In the fruit fly *Drosophila melanogaster*, BiP levels were shown to regulate the quantity of recovery sleep [29]. Transgenic animals that over-expressed BiP displayed a greater exaggeration in recovery sleep when compared to animals that had reduced BiP levels. Although increased BiP/GRP78 levels have yet to be identified in other brain regions, studies have shown upregulated protein levels in peripheral tissues of the AK mice [30] which suggest that an increase in BiP transcript levels is a strong possibility. It has been demonstrated that PERK is also upregulated by mild sleep deprivation [15] and its induction can protect cells from oxidative damage. Studies indicate

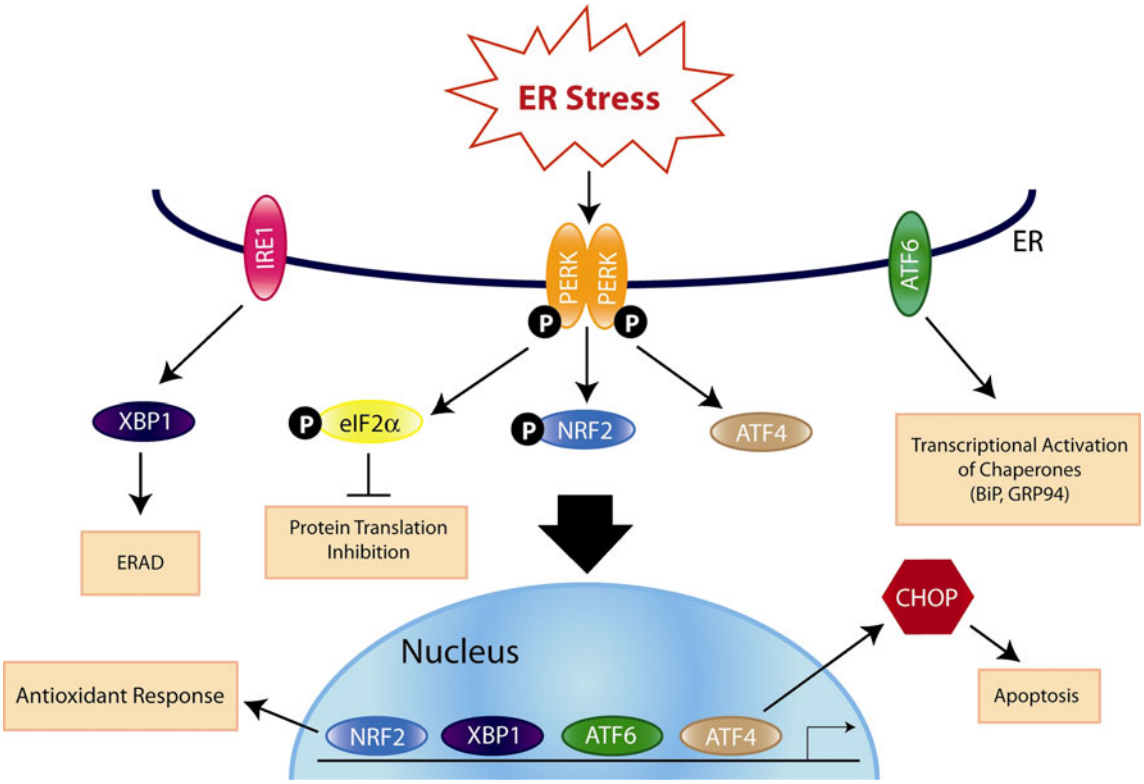

Fig. 1 Schematic displaying the three major transducers of the unfolded protein response (UPR). Accumulation of misfolded and/or unfolded proteins dissociates the major endoplasmic reticulum (ER) molecular chaperone BiP for the three transducers of ER stress: inositol requiring 1 (IRE1), PERK (PKR (RNA-dependent protein kinase)-like ER kinase), and activating transcription factor 6 (ATF6). IRE1 induction leads to x-box binding protein (XBP)-1 splicing, transcriptional activation of chaperones, and stimulation of protein degradation. PERK activation phosphorylates eIF2α to attenuate protein translation and induce some downstream antioxidant response genes. Cleaved activated ATF6 leads to induction of molecular chaperones such as BiP and glucose regulated protein 94 (GRP94). The various ER chaperones, such as BiP and GRP94 are protective and control protein folding and components of the UPR

that with 6 h of sleep deprivation BiP dissociates from PERK, a process that activates the UPR [15].

Linking the UPR and Oxidative Stress

A study by Malhotra et al. found that accumulation of misfolded proteins in the lumen of the ER generates ROS, and that both ROS and unfolded proteins collaborate to induce the UPR and apoptosis [31]. They also demonstrated that ROS production was attenuated and protein folding was improved by genetic or pharmacological interventions. UPR induction and activation of its adaptive and apoptotic downstream effectors was ameliorated in mice fed butylated hydroxyanisole, a lipid-soluble antioxidant [31]. Although the UPR and oxidative stress are closely correlated, the molecular signaling pathways that link these two processes are not clear. However, interactions between ROS and specific transducers in the UPR are being discovered. PERK is a transmembrane kinase that is responsible for repressing protein synthesis, which is negatively regulated by binding to BiP/GPR78, the ER-resident protein chaperone [19]. Under ER stress, PERK is activated by dissociation from BiP/GPR78 and by autophosphorylation. Following its release from BiP/GRP78, PERK is post-translationally modified to initiate the UPR [32, 33]. Activation of PERK also induces a signaling branch that is independent of protein translation. This pathway induces the expression of pro-survival factors that diminish reactive oxygen species. Harding et al. reported that *Caenorhabditis elegans* deficient in the PERK homologue *pek-1* display a significant reduction in their average life span, which is consistent with the role of PERK in oxidative stress and aging. Also, disruption of a major ER oxidizing enzyme ERO-1 by RNA interference in the *pek-1* mutants greatly diminished ROS levels in these animals [34]. Production of ROS, by inhibition of the mitochondrial transport chain, is a mechanism that is believed to contribute to neuronal cell death. 6-Hydroxydopamine (6-OHDA), a compound the mimics Parkinson's disease (PD) by selectively destroying dopaminergic neurons and as a consequence generates ROS, has been shown to induce ER stress and activate the UPR in cultured neuronal cells [35]. Several components of the UPR including elevation in BiP levels and the appearance of phospho-PERK and phospho-eIF2α upon

application of 6-OHDA, were observed. Later activation of downstream target genes involved in ameliorating oxidative stress were also reported [35]. These studies implicate PERK in the activation of downstream defense mechanisms against oxidative stress that have been induced by ER stress.

Sleep Loss/Recovery, the UPR and Antioxidant Response

The idea of the unfolded protein response has been suggested ever since microarray studies demonstrated increased cortical mRNA expression of the UPR-associated chaperones BiP and GRP94 in rats during wakefulness [36]. PERK cerebellar transcript levels were also shown to be elevated in wakefulness in comparison to the sleep state [36]. There are other UPR-specific transcripts that change with sleep deprivation. These include, but are not limited to: BiP, PERK, XBP-1, C/EBP homologous protein (CHOP), caspase-9, NF-E2-related factor 2 (Nrf2), and activating transcription factor 4 (ATF4; see Table 1). These recent studies illustrating the effects of sleep deprivation/sleep on the UPR substantiate earlier studies by Nakanishi et al. [37] and Ramm and Smith [38] that demonstrated enhancement of protein synthesis during sleep. Those earlier studies also aid in the understanding of recent findings that link sleep deprivation with attenuation in protein translation; with likely increases in protein synthesis during sleep rebound. A recent study utilizing surface-enhanced laser desorption/ionization mass spectrometry indicates that protein expression is reduced following sleep deprivation [39]. These results suggest that modifications in protein translation are an important consequence of sleep deprivation with recovery of this process taking place during sleep. Sleep has also been hypothesized to increase the efficiency of the antioxidant defense mechanisms in the brain [6]. Studies performed by Everson et al. demonstrated that recovery sleep was associated with restoration of antioxidant capacity in both the liver and the heart of rats [8]. Since it has been demonstrated that sleep deprivation activates the UPR, it has been suggested that one consequence of this cascade will be the upregulation of antioxidant mechanisms.

The Antioxidant Response-Nrf2

Antioxidant defense mechanisms are critical for maintaining health by providing protection against various insults to the system as a result of ROS. Healthy cells are equipped with fully functioning mechanisms to dispose of these toxic elements. Several transcription factors are known to bind the cis-acting enhancer sequence called the antioxidant response element (ARE) [40, 41], which partially regulates both basal and inducible protective activities [42, 43], such as phase II detoxification enzymes and antioxidant proteins. Nrf2 has been implied to have a potentially significant role in the adaptive stress response to oxidative stress [44–46] and xenobiotic detoxification [47]. Nrf2 belongs to the cap 'n' collar subfamily of basic leucine zipper transcription factors, and is one of the multiple substrates for PERK kinase activity. It is distributed ubiquitously throughout the cytoplasm through its association with Keap-1 (Kelch-like ECH associated protein 1), its specific repressor [45]. Nrf2 is activated by PERK independently of translational inhibition through eIF2α [48]. It requires heterodimeric binding to other bZiP transcription factors to activate gene expression (Fig. 2). Although its most prominent partner are small musculo-aponeurotic fibrosarcoma proteins [49, 50], it also heterodimerizes with Jun [46] and ATF4 [44]. Cells that have an Nrf2 deletion are more susceptible to

Table 1 ER stress response genes that are increased with sleep deprivation

Gene name	Function	Reference
BiP (immunoglobulin-binding protein)	ER (endoplasmic reticulum) chaperone, folding, ER (ER-associated degradation, ATPase activity, anti-apoptotic)	Shaw et al., 2000, [109] Cirelli et al., 2004, [36] Terao et al., 2003 [27], Mackiewicz et al., 2007 [4],
CHOP (C/EBP homologous protein)	Apoptotic signaling	Cirelli and Tononi, 2000 [26]
PERK (PKR [RNA dependent protein kinase]-like ER kinase)	Kinase, protein translation inhibition, antioxidant response	Cirelli et al., 2004 [36]
XBP-1 (X-box binding protein)	Transcription factor, ER chaperone induction	Mackiewicz et al., 2007 [4]
Caspase 9	Apoptotic factor	www.Sleepgene.org [110]
Nrf2 (NF-E2-related factor 2)	Transcription factor regulating antioxidant response	www.Sleepgene.org [110]
ATF4 (activating transcription factor 4)	Required for expression of genes involved in amino acid import, glutathione biosynthesis, and resistance to oxidative stress. ATF3 (activating transcription factor 3), CHOP,GADD34	www.Sleepgene.org [110]

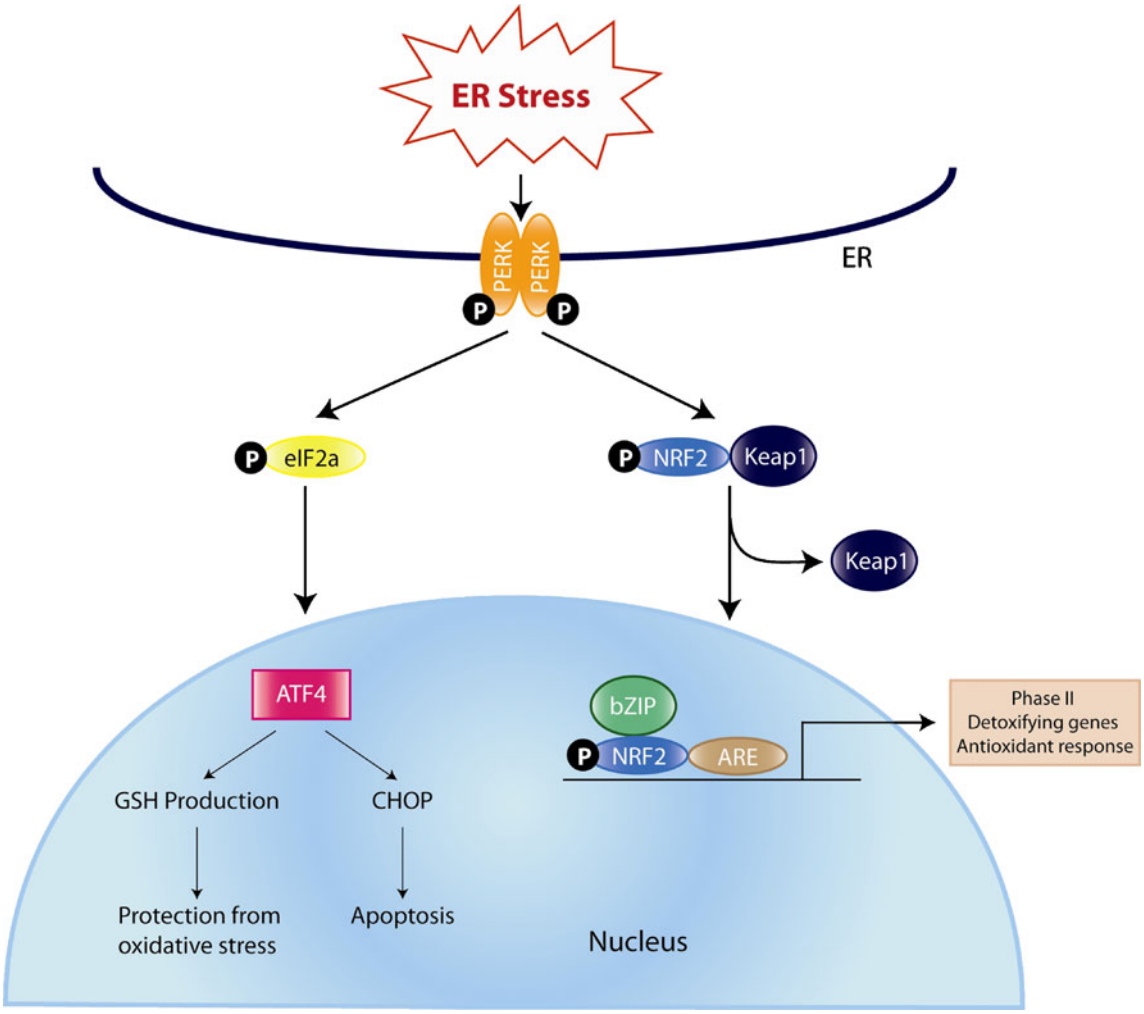

Fig. 2 Model depicting PERK signaling during stress conditions. ER stress and/or oxidative stress activate PERK kinase activity, which leads to the phosphorylation of eIF2α and Nrf2. The phosphorylation of Nrf2 promotes its disassociation from its specific repressor Keap-1, which is followed by its nuclear localization. Upon nuclear entry, Nrf2 binds to other bZIP transcription factors which results in the transcription of genes that aid in redox homeostasis. Although phosphorylation of eIF2α suppresses global protein translation, it selectively promotes the production of ATF4 and other transcription factors. Upregulation of ATF4 activity increases the expression of both pro-survival glutathione (GSH) and pro-apoptotic (CHOP) target genes. Although phosphorylation of eIF2α and Nrf2 occur independently, these two distinct pathways could interact to enhance PERK signaling

apoptosis when exposed to ER stress compared to their wild-type controls [32] and Nrf2 mutant mice are sensitive to high levels of oxidative stress [32] and display a reduced life span [51], which suggests that there is a dysfunction in ROS detoxification. The Keap-1/Nrf2 complex has also been shown to regulate longevity in *Drosophila* [52]. Inducing ER stress causes PERK to phosphorylate Nrf2 and disassociate from Keap-1, leading to the nuclear recruitment of Nrf2 [45, 47]. PERK/Nrf2 then translocates into the nucleus to upregulate genes involved in redox maintenance [48]. Nrf2 regulates the inducible expression of ARE-containing target genes, such as enzymes involved in glutathione biosynthesis and chemical detoxification [53, 54], which are induced during the UPR. This implies that Nrf2 activation during ER stress conditions will be equivalent to activation during oxidative stress. Also, ROS was found to be generated before UPR markers were identified and antioxidants were able to vigorously suppress induction of the UPR [55]. These studies suggest that since Nrf2 can defend organisms against oxidative stress, it may also have a role in its subsequent consequences such as aging and neurodegenerative diseases [52].

The Divergent Effects of ATF4 Signaling

ATF4, a member of the cAMP response-element-binding (CREB) family of transcription factors, is potentially a key mediator in the inducible cellular response to oxidative stress [56, 57]. ATF4 is constitutively expressed at low

concentrations but under certain cell-stress conditions, it becomes rapidly induced [58]. It has a very short half-life, between 30 and 60 min, and compounds that suppress the proteasome system increase its stability [59]. Cells lacking ATF4 are unable to upregulate the expression of multiple genes that are required for redox homeostasis, demonstrating a protective role for ATF4 signaling (Fig. 2) [34]. Also, cells that were deficient in ATF4 were more susceptible to ER stress and oxidative stress [34]. ATF4 signaling lies downstream of PERK activation, since eIF2α is necessary for its translation [60]. When PERK is absent, upregulation of ER stress causes rapid accumulation of ROS, which is potentially due to the inability of PERK knockout cells to activate ATF4 and its downstream antioxidant effectors [61]. On the other hand, Lange et al. found that when ATF4 mRNA levels were induced by oxidative stress, glutathione (GSH) was depleted [62]. This same study further describes resistance to neuronal cell death with an ATF4 deletion [62]. There are various reasons why ATF induction produced opposite effects in these studies. ATF4 signaling promotes the transcription of two diametrically opposed programs: it increases the transcription of both pro-survival and pro-apoptotic target genes [60, 63, 64]. ATF4 can function as either a homodimer or as a heterodimer, [65] combining with other bZIP transcription factors, such as Nrf2 [44] to activate gene transcription.

The Relationship Between ATF4 and Nrf2 in the UPR

PERK activates two different regulators of redox homeostasis: Nrf2 and ATF4 [48]. Both Nrf2−/− and ATF4−/− cells are sensitive to ER stress and neither mutant cell line induces the necessary enzymes to synthesize GSH [56]. The primary role of glutathione is to balance redox reactions and prevent native disulphide bonds from forming [66]; actions which protect cells from oxidative stress. Nrf2 was shown to increase the viability of neuronal cells by regulating the synthesis and release of GSH in astrocytes [67]. ATF4 and Nrf2 couple ER stress to a general cellular response that increases the production of glutathione by increasing amino-acid metabolism [34, 48, 68]. Cells lacking ATF4 are growth restricted unless they are supplemented with a reducing agent because ER-generated ROS is unregulated. Conditions that lead to overproduction of ROS generated from ER stress require GSH production, suggesting a role for GSH in their elimination. He et al. found that when combined, both Nrf2 and ATF4 can induce ARE-dependent gene transcription [44]. Studies have shown that both arms of PERK signaling, Nrf2 and eIF2α, can modulate common targets, including the pro-apoptotic factor CHOP [32]. CHOP, also known as GADD153 is ubiquitously expressed at low levels [69]. However, when ER stress has not been mitigated, CHOP levels increase [70], which leads to activation of cell cycle arrest and apoptosis. Nrf2 negatively regulates both basal and ER stress-induced CHOP expression. In cells lacking Nrf2, CHOP expression levels are elevated as compared to their wild-type controls, and overexpression of Nrf2 ameliorates CHOP accumulation during the UPR [32]. On the other hand, ATF4, a major downstream target of eIF2α phosphorylation [60] promotes CHOP expression (Fig. 2) [56]. The expression of ATF4 and CHOP, which is induced by ER stress, is almost completely ameliorated in cells lacking PERK and eIF2α (S51A) cells [60, 71].

Age-associated Changes in Antioxidant Responses

The aging process has been reported to diminish the effectiveness of the UPR [72]. This decreased efficacy, as well as unregulated brain oxidative stress, seems to play important roles in sleep. These dysfunctions could consequently aid in the development of neurodegenerative pathologies and a variety of other ‘conformational disorders’ that result from improper and/or malformed proteins.

Nrf2 and Aging

Nrf2-mediated gene expression is perturbed during the aging process. Transcriptional activity of Nrf2 was shown to be greatly diminished in the liver of aged mice [73], which suggests a potential mechanism that may be elemental to the loss of the antioxidant tripeptide, GSH in old animals, since *nrf2*−/− fibroblast cells have diminished GSH levels [74]. Nrf2 deficiency and aging synergistically diminish renal and hepatic reducing activity, seen as a delay in half-life of the compound Carbamoyl-PROXYL in Nrf2 mutant mice [75]. Aging also leads to reduced binding of Nrf2 to the antioxidant response element. This decrease in antioxidant activity is exacerbated in aged female Nrf2 knockout mice [76]. Loss of Nrf2 mimics several properties of aging including; declines in GSH levels [77], an increase in susceptibility to oxidative stress [74, 78], and a decrease in tolerance to toxic compounds such as acetaminophen [79]. These effects have been attributed to the reduced activity of the drug-metabolizing enzymes and antioxidant defense systems. However, the pathways that lead to Nrf2 activation remain somewhat intact in aged animals since they are responsive to exogenous antioxidant treatment [73].

Declines in Stress Resistance with Age

A universal decline in stress resistance generally accompanies aging. Research has shown that both basal and inducible CHOP expression levels are elevated during the

aging process [80, 81]. Expression of CHOP has also been shown to promote apoptosis in conditions of prolonged ER stress [80]. Other studies have indicated that aging hepatocytes are more vulnerable to ER stress, which leads to subsequent perturbations in the UPR signaling cascade [82]. The expression of CHOP and another pro-apoptotic molecule, caspase-12, was induced in aged rats that were stressed, but not in the young stressed animals [83], lending support to the idea that aged animals are more vulnerable to apoptosis. Although CHOP levels remain stable in young mice, aged animals have increased levels of CHOP [84], which can be further exaggerated with sleep deprivation [15].

Obstructive Sleep Apnea and Oxidative Stress

There is extensive literature that links oxidative stress to obstructive sleep apnea (OSA), a highly prevalent breathing disorder that occurs during sleep, which is characterized by intermittent and recurrent obstruction of the upper airway and sleep fragmentation. Evidence has implicated untreated OSA in the pathogenesis of various disorders, such as hypertension, ischemic stroke, and cardiovascular disease [85–87]. One major mechanism by which OSA induces detrimental repercussions is intermittent hypoxia, the process by which individuals undergo repeated periods of brief oxygen desaturation in the blood, followed by reoxygenation [88]. These cyclic episodes of hypoxia/reoxygenation leads to the production ROS and oxidative stress (for a more detailed review, see [89]), which can be characterized by markers commonly used to access oxidative damage such as lipid peroxidation, oxidized proteins and DNA fragmentation/degradation. Modulation of cellular antioxidant defense mechanisms in response to oxidation may also serve as a marker of oxidative stress. It has been reported that individuals with severe OSA [90] and end-stage renal disease displayed diminished antioxidant capacity [91], which was measured by serum total antioxidant status. Another study found that thioredoxin, an antioxidant protein induced by oxidative stress, was found to be elevated in patients with OSA [92], independent of age, body mass index, and current smoking habit. Neurological deficits have also been associated with chronic intermittent hypoxia such as that encountered in OSA. Row et al. reported an association between intermittent hypoxia, elevated lipid peroxidation, and oxidative stress in rat brain tissue [93]. This same study also demonstrated that impairments in spatial learning induced by redox alterations were attenuated by antioxidant treatment. Veasey et al. showed that mice placed under conditions of long-term intermittent hypoxia (LTIH) sustained oxidative injury to wake-promoting regions of the brainstem and basal forebrain [94]. This group later found that wake impairments in aged adult male mice were irreversible after exposure to LTIH and that these impairments were linked to selective neuronal degradation in the NADPH oxidase-containing dopaminergic and noradrenergic neurons located in the ventral peri-aqueductal gray and locus ceruleus, respectively [95] and that prolonged ER stress during LTIH was the major mechanism for the observed neuronal injury [96]. Ramanathan et al. found that hypoxia induces antioxidant responses in the cerebellum and pons of rats, which was measured by increased levels of SOD and glutathione reductase [97]. Upregulation of these enzymes are indicative of oxidative stress. The primary therapeutic strategy in the treatment of OSA is the application of nasal continuous positive airway pressure, which prevents the repetitive hypoxia/reoxygenation cycles and therefore the subsequent deleterious consequences, such as elevated levels of oxidative stress [98].

Oxidative stress, the UPR, and Neurodegenerative Diseases

Increased ROS production and oxidative stress contributes to ER stress, protein misfolding, and upregulation of the UPR [99], all of which subsequently play a role in the pathogenesis of neurodegenerative diseases (see reviews [100, 101]). These diseases which include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease, normally appear later in life and are thus associated with the aging process. UPR markers such as BiP/GRP78 and p-PERK were increased in the neurons of AD patients versus the non-demented controlled individuals [102]. PERK signaling independent of protein translation initiates nuclear localization of pro-survival transcription factor Nrf2 and upregulation of antioxidant response elements. It has been reported that Nrf2 is involved in the regulation of life span [52] and oxidative stress tolerance. It has also been shown to increase resistance to apoptotic signals by maintaining redox homeostasis [47, 103]. Since aging leads to a less effective UPR [72, 104], the accumulation of unfolded/misfolded proteins is a major cause of oxidative injuries to the cell. It has been demonstrated that Nrf2-deficient neurons were sensitive to drug induced mitochondria-mediated toxicity [105]. Shih et al. discovered that increasing Nrf2 signaling in a cell model, conferred protection when the cells were later exposed to neurotoxins [67]. It has been suggested that initial participation of the UPR in neurodegenerative disorders is probably cytoprotective; however, when activation of the UPR is sustained over an extended period of time, apoptotic pathways are upregulated. ATF4, which has

dual roles in the UPR induces the transcription factor CHOP, a well-known pro-apoptotic factor [20, 70], after prolonged activation. ATF4 also confers resistance against oxidative glutamate toxicity in hippocampal and cortical neurons [106], through eIF2α signaling. Sleep disturbances are prevalent in age-related neurological disorders [97, 107], and it has been suggested that increased oxidative damage could be a mechanism by which aging affects sleep [108]. The contradictory role of oxidative stress in relation to sleep may result from reports that measure markers of oxidative damage. Evidence insinuates that earlier events, such as the UPR, may be more beneficial in elucidating a correlation between the burden of oxidative stress and a restorative function of sleep. Because of these various relationships, some reports propose that compounds activating the Nrf2 signaling pathway may be efficacious in blocking neuronal cell death and may provide a novel therapeutic intervention in alleviating some of the pathological changes seen in neurodegenerative diseases.

Conclusions/Future Directions

The ER coordinates a variety of cellular processes and responses. This organelle is responsible for proper protein folding, modifications, packaging, and transport. Disruptions to these processes cause proteins to misfold and aggregate leading to ER stress. This ER stress is relieved by a series of coordinated cell protective responses known as the UPR, which shields the cell from the detrimental effects of these misfolded/unfolded proteins. Long-term stress can overwhelm this protective response and if the cumulative burden is not alleviated, then pro-apoptotic signaling is induced. Short-term sleep deprivation has been shown to induce the UPR and antioxidant defense mechanisms. Although antioxidant capacity has been shown to be upregulated during sleep loss; data that points to a role of oxidative stress in the function of sleep remains contradictory and thus requires more work. The literature abounds, however, on the subject of oxidative stress and aging. Evidence from invertebrates to mammals, has linked reactive oxygen species, aging, and life span. Research has shown that with age, key elements of the UPR, such as the transcription factor Nrf2, diminish with age. Also, deletion of Nrf2 mimics many of the characteristics associated with the aging process. Studies have also shown that in older animals, there is an increase in pro-apoptotic factors, such as CHOP. Although the antioxidant defense mechanisms have been vastly attenuated due to aging, some parts of the pathways still remain intact since aged animals were mildly responsive to exogenous antioxidant treatment. This could possibly represent the start of pharmacological interventions that could be used to modulate this particular pathway to counteract the effects of sleep deprivation and is thus, an area that needs further investigation.

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