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FORUM REVIEW ARTICLE

Control of Redox State and Redox Signaling by Neural Antioxidant Systems

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Abstract

The glutathione/glutathione disulfide (GSH/GSSG) redox pair forms the major redox couple in cells and as such plays a critical role in regulating redox-dependent cellular functions. Not only does GSH act as an antioxidant but it can also modulate the activity of a variety of different proteins. An impairment in GSH status is thought to be the precipitating event in a wide range of neurological disorders. Therefore, understanding how to maintain GSH in the CNS could provide a valuable therapeutic approach. Intracellular GSH levels are regulated by a complex series of pathways that include substrate transport and availability, rates of synthesis and regeneration, GSH utilization, and GSH efflux. To date, the most effective approaches for maintaining GSH levels in the CNS include enhancing cyst(e)ine uptake both directly and indirectly via transcriptional upregulation of system x_c^-, increasing GSH synthesis via transcriptional upregulation of the rate limiting enzyme in GSH biosynthesis, and decreasing GSH utilization. Among the transcription factors that play critical roles in GSH metabolism are NF-E2-related factor 2 (Nrf2) and activating transcription factor 4 (ATF4). Thus, compounds that can upregulate these transcription factors may be particularly useful in promoting the functional maintenance of the CNS through their effects on GSH metabolism. *Antioxid. Redox Signal.* 14, 1449–1465.

Introduction

THE REDOX ENVIRONMENT OF A CELL is dependent upon an independent but linked set of redox couples (89, 155) that regulate the activity of a variety of different enzymes, transcription factors, and other proteins, thereby modulating overall cellular function. Multiple redox couples in the cell contribute to the intracellular redox environment. Among the most important of these are the NADPH/$NADP^+$, glutathione/glutathione disulfide (GSH/GSSG), and the reduced thioredoxin/oxidized thioredoxin ($Trx(SH)_2$/TrxSS) couples (155). NADPH provides a major source of electrons for reductive biosynthesis. NADPH is also the main source of electrons for the maintenance of GSH whose cellular concentrations are in the millimolar range (155). Due to its high concentration relative to the other couples, the GSH/GSSG couple is often used to estimate the redox state of a cell, tissue, or organelle (155) and will be the focus of this review. However, since each molecule of GSSG produces two molecules of GSH, both the ratio of oxidized to reduced GSH as well as the concentration of reduced GSH will influence the redox environment. The third major redox couple in cells is the $Trx(SH)_2$/TrxSS couple. Along with the GSH/GSSG couple, it plays an important role in regulating the oxidation state of protein sulfhydryls. However, since the intracellular concentrations of $Trx(SH)_2$/TrxSS are 100–1000-fold less than GSH, its role is more limited. Interestingly, the $Trx(SH)_2$/TrxSS couple appears to mediate the reduction of a distinct set of substrates from the GSH/GSSG couple (89).

Since the overall redox environment is determined mainly by the GSH/GSSG redox couple, measurement of the redox environment usually relies upon the determination of both GSH and GSSG levels in cells. This is not quite as straightforward as it sounds since it can be difficult to measure GSSG accurately, as the levels of GSSG are very low relative to GSH (55). Furthermore, GSH can be oxidized during sample preparation, resulting in overestimates of the level of GSSG. GSH and GSSG levels can be measured either by HPLC (82, 146) or by a chemical assay (177). GSH levels can also be measured using the fluorescent probe monochlorobimane (MCB) (169). However, the reaction of GSH with MCB is dependent upon the activity of glutathione transferases, so any treatment that affects transferase activity will alter this result even if it does not impact GSH levels directly. Furthermore, GSH–MCB conjugates are rapidly transported out of neural cells (185). Alternatively, GSH levels can be assessed

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using anti-GSH antibodies (165). This approach allows the determination of both the cellular (using tissue sections) and subcellular (using cells) distributions of GSH. A recent study used a modification of this approach to address the question of whether GSH levels are higher in neurons or astrocytes (125). Since GSH is rapidly metabolized in postmortem tissue, adult mice were perfused with N-ethylmaleimide, a small, tissue permeable molecule that reacts with sulfhydryl groups, in order to tag GSH *in situ*. Following sectioning and staining of the brain with an antibody specific for NEM-tagged GSH, the highest levels of GSH were seen in neurons and white matter in all brain regions.

Approaches are also being developed to measure the intracellular GSH redox potential. Gutsher *et al.* (59) developed a new redox-sensitive protein consisting of a redox-sensitive GFP coupled to glutaredoxin-1 (Grx1-roGFP2). Transfection of cells with this fusion protein allowed dynamic, live imaging of the intracellular GSH redox potential in response to growth factor addition, changes in cell density, and induction of apoptosis. Thus, for cell-based studies, this probe could provide a useful, additional tool to the study of the role of redox potential in modulating neural function.

Glutathione Metabolism

GSH and GSH-associated metabolism provide the major line of defense for the protection of cells from oxidative and other forms of toxic stress (for reviews, see Refs. 43, 66, and 123). GSH can scavenge free radicals, reduce peroxides, and be conjugated with electrophilic compounds, thereby eliminating both reactive oxygen species (ROS) and their toxic byproducts. GSH also plays a role as a buffer of the important second messenger, NO (see below). In addition, GSH plays a critical role in modulating the activity of several key enzymes that can modulate neural cell function including glyoxylases and lipoxygenases (see below). Recently, GSH was shown to directly inhibit apoptotic cell death in nerve cells (182). Finally, as discussed above, the GSH/GSSG pair forms the major redox couple in cells and as such plays a critical role in regulating redox-dependent cellular functions (Fig. 1).

Intracellular GSH levels are regulated by a complex series of mechanisms that include substrate (mainly cyst(e)ine) transport and availability, rates of synthesis and regeneration, GSH utilization, and GSH efflux to extracellular compartments (117, 123).

Substrate transport

Since glutamate and glycine occur at relatively high intracellular concentrations, cysteine is limiting for GSH biosynthesis in neural cells (48). Therefore, treatments that stimulate cysteine or cystine uptake by neural cells can enhance GSH biosynthesis. In the extracellular environment, cysteine is readily oxidized to form cystine, so for most cell types cystine transport mechanisms are essential to provide them with the cysteine needed for GSH synthesis.

Cystine uptake in many types of cells is mediated by system x_c^-, a Na^+-independent cystine/glutamate exchanger (for review, see Ref. 2) (Fig. 2). System x_c^- is a member of the disulfide-linked heteromeric amino acid transporter family and consists of a light chain (xCT) that confers substrate specificity and appears to be rate limiting for system x_c^- activity and a heavy chain (4F2hc) that is shared among a number of different amino acid transporters (186). It transports cystine into cells in a 1:1 exchange with glutamate and is thus inhibited by high concentrations of extracellular glutamate (128). System x_c^- is expressed at the blood–brain barrier, as well as throughout the brain parenchyma (7, 25). The importance of system x_c^- for the maintenance of GSH levels in cells is demonstrated by the loss of GSH and subsequent cell death seen in nerve cells following exposure to millimolar concentrations of extracellular glutamate, a pathway termed oxidative glutamate toxicity or oxytosis (170). This can occur in a number of conditions, including after brain or spinal cord injury, thus leading to an inhibition of GSH synthesis under conditions where it is needed most (170). Importantly, overexpression of xCT in either nerve cells or in astrocytes in co-culture with nerve cells can prevent both the glutamate-mediated loss of GSH and the death of the nerve cells (160). Furthermore, mice that lack system x_c^- function, either via truncation (160) or deletion (154), show brain atrophy and redox imbalance, respectively.

However, increased expression of system x_c^-, especially in parallel with downregulation of glutamate transporters, which normally keep extracellular glutamate concentrations in the brain low (87), might be a double-edged sword as system x_c^- activity is inevitably coupled to glutamate release, which might put nerve cells at risk for excitotoxicity by overstimulation of ionotropic glutamate receptors (50, 168). Indeed, glutamate transporters are known to be sensitive to oxidative stress (127). For example, 4-hydroxynonenal (HNE),

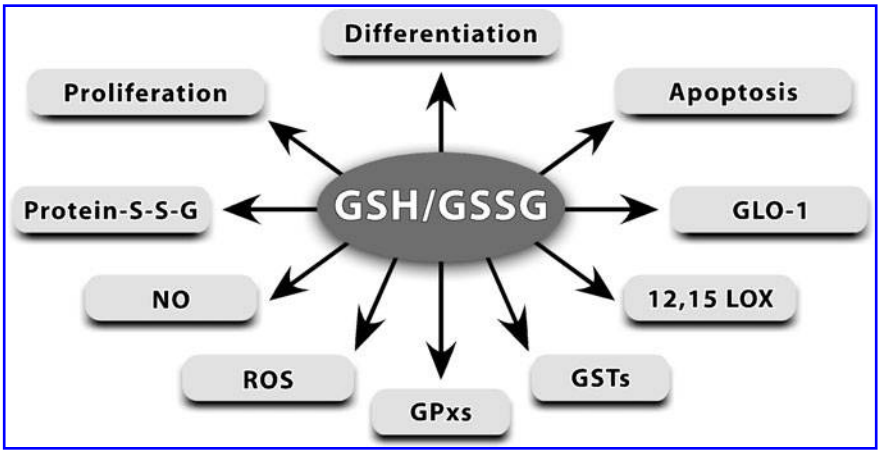

FIG. 1. GSH regulates many aspects of cell function. Not only does GSH, along with its oxidized form glutathione disulfide (GSSG), form the major redox couple in cells and thereby determine whether cells proliferate, differentiate, or die, but it also directly or indirectly regulates the activity of a number of key enzymes [glutathione transferases (GSTs), glutathione peroxidases (GPxs), 12,15 lipoxygenase (12,15 LOX) and glyoxylase 1 (GLO-1) as well as other proteins (glutathionylated proteins (protein-S-S-G) and small molecules (reactive oxygen species (ROS), nitric oxide (NO)].

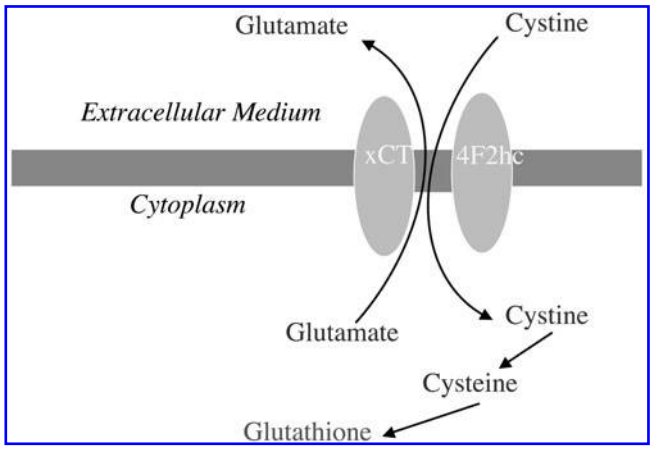

FIG. 2. The glutamate/cystine antiporter regulates intracellular GSH levels. The glutamate/cystine antiporter system x_c^- transports cystine into cells in exchange for glutamate at a ratio of 1:1. It is composed of a specific light chain, xCT, and a heavy chain, 4F2hc, linked by a disulfide bridge. Intracellularly, cysteine is the rate-limiting precursor of GSH.

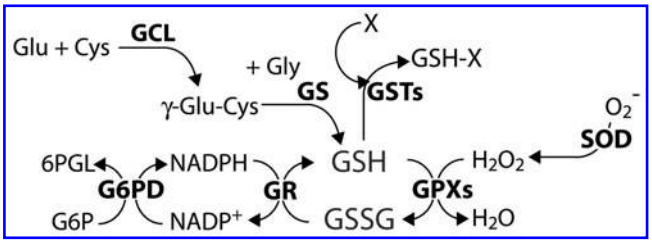

FIG. 3. Outline of GSH metabolism. GSH is synthesized from glutamate (glu), cysteine (cys), and glycine (gly) by the sequential actions of glutamate cysteine ligase (GCL) and glutathione synthetase (GS). GSH is used to eliminate reactive oxygen species such as hydrogen peroxide (H_2O_2) in a reaction catalyzed by the glutathione peroxidases (GPX). H_2O_2 is produced from superoxide (O_2^-) by superoxide dismutase (SOD). The glutathione disulfide (GSSG) produced in this reaction can be converted back to GSH through the action of glutathione disulfide reductase (GR). GR requires NADPH for its activity which is mainly supplied from the conversion of glucose 6-phosphate (G6P) to 6-phosphoglucono-δ-lactone (6PGL) by glucose-6-phosphate dehydrogenase (G6PD), the first enzyme of the pentose phosphate shunt. GSH can also be used to detoxify endogenous and exogenous electrophiles (X) through conjugation via the glutathione-S-transferases (GST).

a product of lipid peroxidation, has been shown to decrease EAAT levels and glutamate uptake in astrocytes (20). In contrast, in nerve cells, overexpression of EAATs supports cystine import via system x_c^-, probably by both decreasing extracellular glutamate, which inhibits cystine uptake, and increasing intracellular glutamate which increases the driving force for cystine import (107).

Interestingly, cystine availability for import by system x_c^- is regulated by extracellular pH, as one amino group of cystine is protonated in the presence of acidosis in the range that occurs in pathophysiological states in the brain, such as stroke, thereby inhibiting its recognition by the transporter (11, 106).

Alternative routes to provide intracellular cysteine to cells exist and consist of either the enzymatic breakdown of extracellular GSH by γ–glutamyl transpeptidase to cysteinylglycine by transferring the γ–glutamyl residue to other amino acids (48, 172) and subsequent production of cysteine by aminopeptidase N (47) or a disulfide exchange reaction between extracellular reduced GSH and cystine to produce cysteinyl-GSH and cysteine (188). Cysteine can be taken up by cells via a variety of different transporters (32). Importantly, however, in an oxidizing environment, cysteine is prone to auto-oxidation to cystine, and both pathways of extracellular cysteine production rely on the presence of GSH, for whose production cysteine is essential.

GSH biosynthesis

GSH is synthesized in cells by the consecutive action of two ATP-dependent enzymes (Fig. 3). Glutamate cysteine ligase (GCL), formerly called γ-glutamylcysteine synthetase (γ-GCS), catalyzes the first and rate-limiting step in GSH biosynthesis to form the dipeptide γ-GluCys, which is then combined with glycine to generate GSH in a reaction catalyzed by glutathione synthetase (GS). The synthesis of GSH in cells is normally regulated by feedback inhibition of GCL by GSH.

A variety of different compounds increase GSH levels in cells by increasing GCL activity. The activity of GCL is regulated at the transcriptional, translational, and post-translational levels and the complex interplay of these different levels of regulation is just beginning to be understood (43, 117, 167). GCL is a heterodimer composed of catalytic (GCLC) and regulatory (GCLM) subunits. GCLC has all of the catalytic activity and is the site of GSH feedback inhibition. Current evidence suggests that GCLC is responsible for the constitutive synthesis of GSH (44), while the association of GCLC with GCLM is needed in order to overcome feedback inhibition by GSH when a higher rate of synthesis is required such as during stress. In agreement with this conclusion, mice deficient in GCLC are early embryonic lethal, whereas GCLM knockout mice are viable but show a significantly enhanced sensitivity to stress (38). However, in primary cultures of rat neurons, knockdown of either subunit using a small hairpin RNA strategy resulted in significant cell death, in the absence of toxic stimuli, and enhanced cell death, in the presence of glutamate or nitric oxide, suggesting that GCLM may be particularly important for modulating GCL activity in nerve cells (42). Similarly, astrocytes from GCLM knockout mice had significantly lower basal levels of GCL activity and GSH (102). Furthermore, in contrast to astrocytes from wild-type mice, the astrocytes from GCLM knockout mice failed to upregulate GSH synthesis in response to several different Nrf2 inducers (102).

The two subunits of GCL are transcriptionally regulated by a wide variety of compounds (44, 117). A number of *cis* elements are implicated in the transcriptional activation of both GCLM and GCLC mRNAs, including AP-1, AP-2, NF-κB, SP-1, and the antioxidant response element (ARE; also known as EpRE, StRE) (see below). However, the pathways for the transcriptional upregulation of the two subunits appear to be independent and vary with both inducing agent and cell type.

Not a lot is known about the post-translational regulation of GCL activity, although there is evidence that the activity of GCLC can be negatively regulated by phosphorylation (117). A number of kinases phosphorylate GCL, including protein kinase A, protein kinase C (PKC), and Ca^{+2}/calmodulin-dependent kinase (117). GCLC can also be negatively regulated by caspase 3-dependent cleavage during apoptosis (117). Moreover, GCL is prominently inhibited by intracellular acidosis (121). The importance of the regulation of GCL activity by acidosis is supported by the observation that in cells exposed to acidosis where intracellular cysteine delivery by system x_c^- is bypassed by the addition of N-acetylcysteine, GSH synthesis is still impaired (106).

The second enzyme required for GSH biosynthesis is glutathione synthetase (GS). Although GS is not considered rate limiting for GSH biosynthesis because its activity generally exceeds that of GCL by several-fold, this may not be the case in all tissues, especially under conditions of stress (117). This possibility deserves further examination in neural cells. Similar to the GCLC and GCLM promoters, the GS promoter contains potential binding sites for a number of transcription factors, including AP-1, NF-κB, Nrf1, and Nrf2 (117). Similar to GCL, GS is inhibited by acidosis (164) and GSH synthesis in acidotic cells is impaired even in the presence of an excess of the GS substrate, γ-GluCys (106).

Glutathione disulfide reductase

Both enzymatic (via glutathione peroxidases) and nonenzymatic detoxification of ROS by GSH results in the production of GSSG. Since an increase in GSSG is harmful to cells (see below), GSSG is often transported outside of cells, resulting in a depletion of GSH (136). The more economical way to remove GSSG is via the activity of glutathione disulfide reductase (GR), which regenerates GSH from GSSG in a reaction that is absolutely dependent upon NADPH. Increases in GR activity can be mediated by two distinct mechanisms: an increase in the level and/or activity of GR or an increase in the levels of NADPH by increasing the activity of the pentose phosphate shunt, the main source of NADPH in the cell. GR belongs to the family of FAD-containing pyridine nucleotide:disulfide oxidoreductases that also includes the thioredoxin reductases. Recent evidence suggests that GR may be particularly susceptible to oxidative damage brought about by GSH depletion (12).

In addition to increasing GSSG levels in cells, inhibition of GR increases protein glutathionylation (199). One target of this glutathionylation is the enzyme 12,15-lipoxygenase (12,15-LOX) whose catalytic activity is increased resulting in nerve cell death (136; Fig. 4). 12,15-LOX is a member of a family of enzymes that metabolizes 20-carbon unsaturated fatty acids, such as arachidonic acid, which are produced from membrane phospholipids by the action of phospholipases, to eicosanoids, including hydroxyperoxyeicosatetraenoic acids (HPETEs), hydroxyeicosatetraenoic acids (HETEs), and leukotienes (for review, see Ref. 141) The LOXs are dioxygenases that incorporate molecular oxygen into specific positions of arachidonic acid and can be distinguished on the basis of their site of oxygen insertion. Previously, we showed that 12,15-LOX plays a critical role in the production of mitochondrially-derived ROS in nerve cells exposed to high concentrations of glutamate (112).

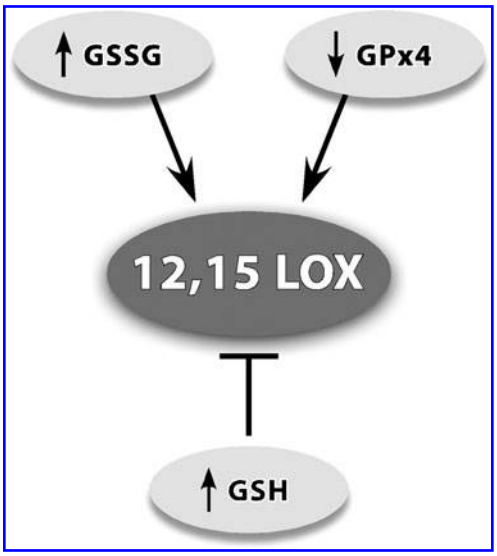

FIG. 4. GSH-dependent regulation of 12,15 LOX activity. 12,15 LOX is activated following increases in glutathione disulfide (GSSG) levels or decreases in glutathione peroxidase 4 (GPx4) activity. GPx4 normally removes the lipid peroxides that both activate 12,15 lipoxygenase (12,15 LOX) and are a product of its activity. Maintenance of GSH levels can block the activation of 12,15 LOX.

Glutathione peroxidases

Glutathione peroxidases (GPxs) catalyze the reduction of hydrogen peroxide and organic hydroperoxides at the expense of GSH (for reviews, see Refs. 4, 24). Although catalase and peroxiredoxins can also remove hydrogen peroxide, the relative levels of GPxs, Prxs, and catalase vary greatly from tissue to tissue (61). In particular, the brain has very low levels of catalase activity and relatively high levels of GPx activity. Furthermore, GPxs, but not catalase, are found in mitochondria. There are four different GPxs (GPx1–4) in mammals, all of which contain selenocysteine in the active site and therefore are dependent upon an adequate supply of dietary selenium. The best characterized of the GPxs is GPx1, which is expressed in a variety of tissues. It is found in both cytoplasm and mitochondria and reduces mainly soluble inorganic and organic hydroperoxides. GPx1 knockout mice show an increase in brain damage following exposure to a number of insults, including ischemia/reperfusion injury (35), malonate, 3-nitropropionic acid, and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) (92). The increased sensitivity of nerve cells in GPx1 knockout mice to ischemia/reperfusion injury correlates with reduced activation of the phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway (173), although how loss of GPx1 alters the activation of this pathway remains to be determined. Consistent with these data, overexpression of GPx1 protects dopaminergic neurons in two different models of Parkinson's disease (PD) (15, 175). In addition, treatment of hippocampal neurons with glucocorticoids results in a decrease in GPx1 activity and an increase in sensitivity to oxidative insults (138). In contrast, GPx1 knockout mice are more resistant to kainic acid-induced seizures and neurodegeneration, apparently due to NMDA receptor inactivation via oxidation of NMDA receptor subunit NR1 cysteine sulfhydryls (81). GPx4 (phospholipid

hydroperoxide GPx) is unique in its ability to reduce hydroperoxides of complex lipids including hydroperoxides embedded in membranes. GPx4 is expressed highly in the brain (49) and is the only GPx essential for life (192). Overexpression of GPx4 can protect nerve cells *in vitro* from a variety of oxidative insults (145), while knockdown of GPx4 both *in vitro* and *in vivo* promotes nerve cell death (159). Loss of GPx4 results in the activation of 12,15-LOX and the accumulation of its products (159). In addition, GPx4 can prevent the formation of the lipid peroxidation end-product HNE (145) which is an important mediator and marker of cellular dysfunction and death (for review, see Ref. 122). Since GPx4 is dependent on an adequate supply of GSH, GSH depletion also results in GPx4 inactivation (159), increases in 12,15 LOX activity and HNE, and subsequent nerve cell death (26, 112, 187; Fig. 4). Thus, GPx4 appears to play a key role in modulating nerve cell death.

Glutathione transferases

Glutathione transferases (GSTs) play a critical role in defending cells against reactive chemicals formed both from the breakdown of endogenously produced compounds and the biotransformation of foreign compounds by catalyzing their conjugation with GSH (66, 147, 178). There are over 21 structurally diverse GSTs in humans, including both soluble and membrane-bound proteins. GSTs can also localize to mitochondria where they may play a role in modulating the consequences of ROS production (17, 73). Many of the GST genes are polymorphic in humans with certain alleles associated with impaired enzyme activity (179). For example, a polymorphism in GSTM2-2 which catalyzes the conjugation of GSH to aminochrome, a metabolite of dopamine, has been proposed to play a role in PD (179). All of the GSTs use GSH to detoxify metabolites of xenobiotics, as well as reactive α,β-unsaturated carbonyls, epoxides, and hydroperoxides. Among the endogenous substrates of GSTs is the lipid peroxidation end-product, HNE, which is rapidly converted to HNE–GSH-adducts and then usually exported from cells (18). The GSH conjugates produced by GSTs are generally much more water soluble than the original compounds and are rapidly transported across cell membranes via one of the multi-drug resistance protein transporters (see below) and ultimately excreted in the urine or feces. However, in some cases, the GSH conjugates can be more toxic to cells than the original compound, either because the conjugates are more reactive or because they form harmful metabolites upon further processing (147). For example, the neurotoxin methyl bromide caused severe neurological symptoms in a patient with normal GST activity but not in a patient with reduced activity (53). GST activity in neurons has been reported to be prominently inhibited by acidosis (193) that occurs in the brain during stroke and contributes to nerve cell loss (106).

Similar to GCL and the light subunit of the cystine/glutamate antiporter, GSTs are phase II detoxification enzymes. Thus, their transcription is mediated by an ARE that can be activated by the same compounds which activate the transcription of other genes involved in GSH metabolism. The coordinate upregulation of GSH biosynthesis along with the GSTs is necessary since the abundance and catalytic properties of GSTs indicate that they could empty the cellular GSH pool in a few seconds when a suitable substrate is present (147). Thus, their protective role is absolutely dependent upon an adequate supply of GSH.

Regulation of GSH/GSSG efflux

Regulation of intracellular GSH levels also can be mediated by controlling the efflux of GSH or GSSG. GSH and GSSG are transported out of cells by carrier-dependent mechanisms that are still not well characterized. Two different families of transport proteins have been implicated in GSH and GSSG export; members of the multidrug resistance-associated protein (MRP) family and members of the organic anion transporting polypeptide (OATP) family (for review, see Ref. 8). ATP binding and hydrolysis provide the driving force for export through the MRPs while the OATP transporters function independently of ATP, instead relying on the large GSH electrochemical gradient across cell membranes to drive GSH export. MRPs 1, 2, 4, 5, 6, 7, and 8, as well as CFTR, are expressed in the human brain (8). MRP1 is responsible for 60% of the GSH export and 100% of the GSSG export from astrocytes while MRP5 does not contribute to these processes (126).

Nrf2 and the Antioxidant Response Element

Recently, the role of the ARE in regulating the transcription of genes involved in redox regulation has received a great deal of attention. These genes encode multiple proteins involved in GSH metabolism (the two subunits of GCL, GS, the light subunit of the cystine/glutamate antiporter, GR (64), and the GSTs) as well as NAD(P)H:quinone reductase, peroxiredoxins, thioredoxin, multiple subunits of the proteasome, and the antioxidant protein heme oxygenase 1 (HO-1). Transcriptional activation through the ARE is dependent upon the ubiquitously expressed transcription factor NF-E2-related factor 2 (Nrf2), a member of the Cap'n'Collar family of bZIP proteins (for reviews, see Refs. 90, 111, 129, 197).

A large number of ARE inducers have been identified (for details, see Refs. 90, 94, 119) which cover a wide range of structures and reactivities. Based on this diversity, it is perhaps not surprising that there continues to be a good deal of controversy over the precise mechanisms underlying the activation of Nrf2 by these inducers. Generally low levels of Nrf2 are found in unstimulated cells. In the classical view (Fig. 5), the Nrf2 that is present in unstimulated cells is held in the cytoplasm by the actin-bound protein Keap1, which is a substrate adaptor for a Cul3-containing E3 ubiquitin ligase and thereby promotes the ubiquitination and degradation of Nrf2 by the 26S proteasome (197). Cullin-containing E3 ubiquitin ligases are responsible for the transfer of activated ubiquitin from an ubiquitin conjugation enzyme E2 to a lysine residue of a target protein and determine the substrate specificity of the ubiquitination reaction. Keap1 contains 25 cysteine residues. Initially, it was proposed that the interaction of an ARE inducer with one or more of these cysteine thiols caused a conformational change in Keap1 resulting in the release of Nrf2 (45). However, it now appears that rather than disrupting the Nrf2–Keap1 complex, ARE inducers promote the post-translational modification of specific cysteines in Keap1, thereby causing a decrease in the ability of Keap1 to target Nrf2 to the E3 ubiquitin ligase (197). The interaction of Nrf2 with Keap1 can also be altered by endogenous factors such as the Parkinson's disease-associated protein, DJ-1 (33).

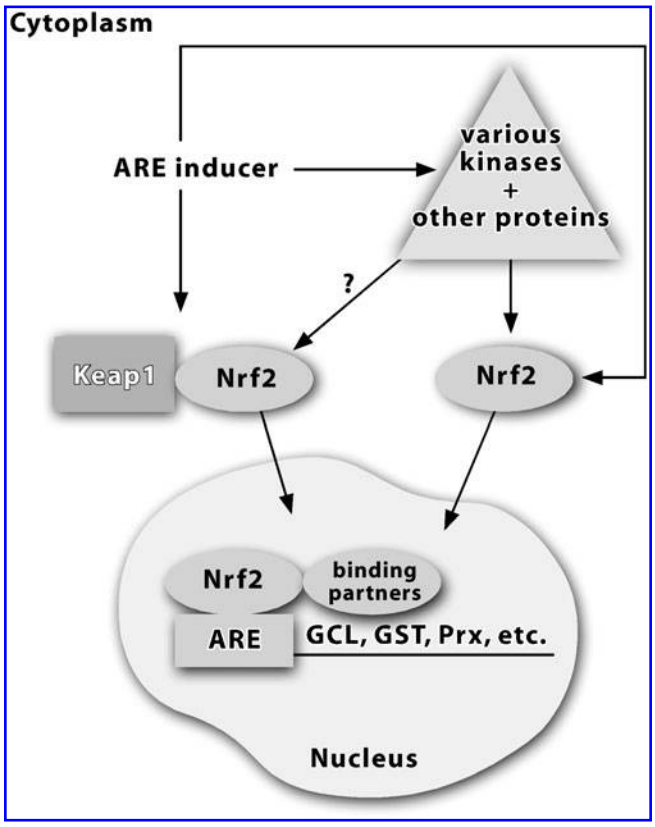

FIG. 5. Model of Nrf2 activation. Nrf2 is maintained at low levels in the cytoplasm through its interaction with Keap1. Treatment with ARE inducers results in either direct effects on the Nrf2-Keap1 interaction and/or the activation of various kinases and/or other proteins which either directly or indirectly promote the accumulation and nuclear translocation of Nrf2. Within the nucleus, Nrf2 dimerizes with one of its binding partners and interacts with the ARE to induce the transcription of various antioxidant genes, including glutamate cysteine ligase (GCL), glutathione-S-transferase (GST), and peroxiredoxins (Prx).

DJ-1 appears to stabilize Nrf2 by preventing its association with Keap1 by an as yet undetermined mechanism.

More recently, several alternative models as to how Nrf2 is activated have emerged (111, 129; Fig. 5). Both of these stem from the observation that Nrf2 controls the constitutive as well as the inducible expression of some ARE-dependent genes, indicating that at least some Nrf2 must be able to translocate to the nucleus in the absence of ARE inducers. Nguyen *et al.* (129) have proposed that Nrf2 is a constitutively nuclear protein while Keap1 transiently translocates to the nucleus where it promotes the degradation of Nrf2. Conditions that promote ARE-mediated gene transcription lead to Nrf2 stabilization in the nucleus by disrupting its nuclear interaction with Keap1 and leading to the export of Keap1 from the nucleus by an undefined mechanism. In contrast, in the model proposed by Li and Kong (111), there are two pools of Nrf2 in cells: a "free-floating" pool that can migrate between the cytoplasm and the nucleus and a Keap1-bound pool that is destined for ubiquitination and degradation. Conditions which promote ARE-mediated gene transcription increase the "free-floating" pool of Nrf2 and also enhance its accumulation in the nucleus by inactivating a redox-sensitive nuclear export signal present in Nrf2.

Furthermore, there is increasing evidence that not all inducers work through Keap1. For example, a recent study from the Yamamoto laboratory using zebrafish showed that Nrf2 inducers can be divided into six classes, only four of which appear to function via modification of Keap1 (94). Interestingly, two of the four classes that worked through Keap1 also required an additional, as yet unidentified, factor for the induction of Nrf2.

There is also substantial evidence that phosphorylation of Nrf2 plays a role in modulating its nuclear accumulation (for reviews, see Refs. 29, 130). Both protein kinase C (22, 72) and the ER-stress-regulated kinase PKR-like kinase (PERK) (36) can directly phosphorylate Nrf2 and thereby increase its nuclear accumulation and transcriptional activity. The phosphorylation of Nrf2 by either PERK (36) or PKC (22, 72) promotes its dissociation from Keap1, thereby allowing its accumulation in the nucleus where it can activate gene transcription. Consistent with these results, PKC phosphorylates Nrf2 on Ser40 (22, 72), which is in the N-terminal, Keap1 binding domain of Nrf2.

In contrast to the positive effects of PKC and PERK on Nrf2 transcriptional activity, phosphorylation of Nrf2 by the Src family tyrosine kinase Fyn negatively regulates Nrf2 transcriptional activity by directly phosphorylating Nrf2 on Tyr568 and thereby promoting its Crm1-mediated export (79). Glycogen synthase kinase-3β (GSK-3β) can also block the nuclear accumulation of Nrf2 and the subsequent transcriptional activation of ARE-dependent genes (78, 152). Two distinct mechanisms have been proposed. Salazar *et al.* (152) showed that GSK-3β could directly phosphorylate Nrf2 and thereby prevent its accumulation in the nucleus, while Jain and Jaiswal (78) demonstrated that GSK-3β acts upstream of Fyn. In either case, the activity of GSK-3β can, in turn, be negatively regulated by PI3K and its substrate, the Ser/Thr kinase Akt so decreased PI3K pathway activity and/or increased GSK-3β activity, as occurs in some neurological disorders, could potentiate neuronal loss via a reduction in Nrf2 signaling (149). PI3K may also enhance the transcriptional activity of Nrf2 through additional pathways not dependent on Akt and GSK-3β (103).

The ERK, JNK, and p38 MAP kinase pathways have also been shown to regulate the transcriptional activity of Nrf2 but it is not yet clear whether this is via direct phosphorylation of Nrf2. In general, both the ERK and JNK pathways appear to positively regulate the transcriptional activity of Nrf2 following treatment of multiple types of cells with a variety of different ARE-inducing compounds (190, 194). In contrast, the effects of the p38 MAPK pathway on Nrf2 transcriptional activity are cell type and stimulus dependent with both positive (10) and negative (91, 195) effects reported.

Whether specific ARE inducers act in concert causing both modifications to Keap1 and phosphorylation of Nrf2 or whether the two mechanisms are specific to distinct inducers of ARE-mediated gene transcription is not known. Clearly, additional study is needed to resolve the basic mechanisms underlying the regulation of Nrf2 nuclear accumulation and transcriptional activation.

An important point that is generally not discussed is the fact that Nrf2 is unable to activate gene transcription by itself. Instead, once inside the nucleus, Nrf2 heterodimerizes with

another bZip protein to induce gene transcription. The most common partners for Nrf2 are members of the small Maf protein family, MafF, MafG, and MafK (21, 65), although there is evidence for heterodimerization with other transcription factors, including ATF4 (67) (see below). How different partners affect the specific array of genes whose transcription is induced by distinct inducers of Nrf2 activation remains to be determined but is a potentially very interesting area of research. A further level of complexity is introduced by the ability of the small Maf proteins to heterodimerize with the transcription factor Bach1 (41). This complex can also bind to the ARE but its binding results in the transcriptional repression of ARE-dependent genes. Thus, modulation of the Bach1–Maf interaction can also play a role in regulating ARE-dependent gene transcription. Moreover, two nuclear factors closely related to Nrf2, Nrf1 and Nrf3, were described to activate or inhibit ARE-dependent gene transcription, respectively (68, 153).

A number of studies have demonstrated that Nrf2 and the subsequent activation of ARE-dependent genes can protect neural cells from oxidative stress both *in vitro* and *in vivo* (39). This conclusion is based on studies using either ARE inducers such as tert-butylhydroquinone (tBHQ) (104, 109), genetic manipulation of Nrf2 levels (162), or a combination of the two (96, 161, 163). Studies in animals using either ARE inducers or Nrf2 knockout mice have supported and extended the cell culture data to animal models of neurological disorders. Nrf2 has been shown to enhance nerve cell survival in animal models of Huntington's disease (HD) (161), stroke (163), amyotrophic lateral sclerosis (ALS) (181), traumatic brain injury (198), and PD (30). In addition, a recent study suggested that an impairment in the nuclear accumulation of Nrf2 may underlie the progression of Friedreich ataxia (139). Moreover, at the age of 10 months, mice lacking Nrf2 develop vacuolar leukoencephalopathy with widespread astrogliosis, indicating an important role for Nrf2 in the maintenance of brain myelin (74). In summary, there is overwhelming evidence that enhancement of Nrf2 levels can protect neural cells from multiple forms of oxidative stress both *in vitro* and *in vivo*, and thereby may be an excellent therapeutic target for the treatment of neurological disorders.

ATF4

A second transcription factor that appears to play a key role in modulating GSH levels in nerve cells is activating transcription factor 4 (ATF4). ATF4 is a member of the ATF/CREB group of the bZIP transcription factor family (3). Via its binding to amino acid response elements (AAREs; also called NSREs), ATF4 is a key activator of gene expression in response to amino acid starvation and endoplasmic reticulum stress, and induces genes involved in amino acid import, GSH biosynthesis, and resistance against oxidative stress (19, 63, 76).

Although the formation of ATF4 homodimers has been described (88, 180), ATF4 is mainly thought to form heterodimers with members of the CCAAT/enhancer-binding protein (C/EBP) (5, 142, 180, 184) and AP1 families (60, 88). In addition, the formation of ATF4/Nrf2 heterodimers, which are thought to bind the antioxidant response element (ARE), have been described (67).

Increases in the cellular levels of ATF4 are regulated mainly at the translational level by eIF2α phosphorylation (Fig. 6) via

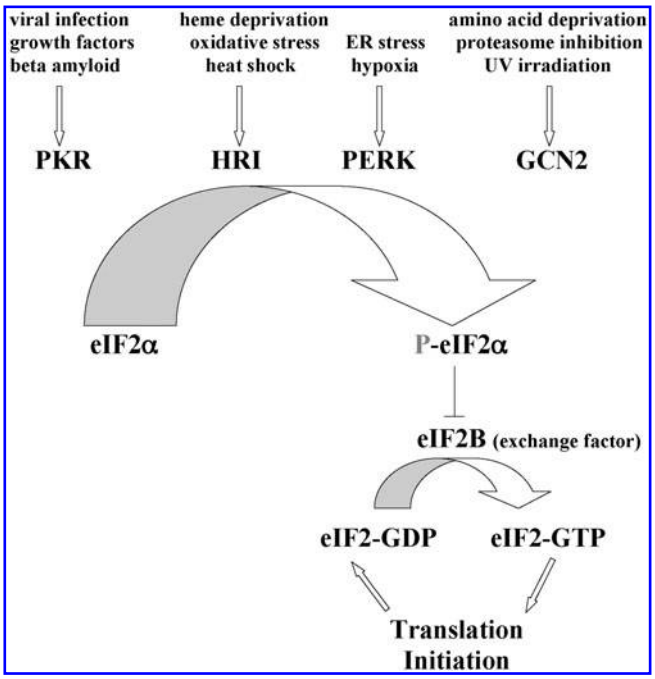

FIG. 6. Multiple protein kinases induce phosphorylation of eIF2α. Four different protein kinases [protein kinase R (PKR), heme-regulated eIF2α kinase (HRI), PERK and general control non-derepressible-2 (GCN2)] have been identified in mammalian cells that phosphorylate the α subunit of eIF2 on Ser51 in response to distinct forms of toxic stress. eIF2α is part of the multimeric eIF2 complex that is involved in the initiation of protein translation. The eIF2 complex brings the 40S ribosomal subunit together with the initiating $tRNA_{MET}$ when eIF2α is bound to GTP. Upon hydrolysis of GTP to GDP, the complex is no longer active and protein synthesis is not initiated. GDP/GTP exchange requires the activity of the guanine nucleotide exchange factor eIF2B. However, when eIF2α is phosphorylated on Ser51, it sequesters eIF2B, thus inhibiting GDP/GTP exchange and initiation of protein synthesis (after 189).

a mechanism that is dependent on the unique 5′ untranslated region of the ATF4 mRNA (143, 189). eIF2α is part of the multimeric eIF2 complex which is involved in the initiation of protein translation (Fig. 6). The eIF2 complex brings the 40S ribosomal subunit together with the initiating $tRNA_{MET}$ when eIF2α is bound to GTP. Upon hydrolysis of GTP to GDP, the complex is no longer active and protein synthesis is not initiated. GDP/GTP exchange requires the activity of the guanine nucleotide exchange factor eIF2B. However, when eIF2α is phosphorylated on Ser51, it sequesters eIF2B, thus inhibiting GDP/GTP exchange and initiation of protein synthesis. As cells have considerably higher amounts of eIF2α compared to eIF2B, even modest increases in phospho-eIF2α can modulate eIF2B reactivation. The 5′ untranslated region of ATF4 contains two upstream open reading frames (uORFs) that contribute differentially to ATF4 expression (189). uORF1 positively regulates ATF4 expression by facilitating ribosome scanning and re-initiation at downstream coding regions in the ATF4 mRNA. In cells with low levels of phospho-eIF2α and therefore high levels of eIF2-GTP, ribosomes scanning downstream of uORF1 re-initiate at uORF2, an inhibitory ORF that blocks ATF4 expression. However, following

phosphorylation of eIF2α, the subsequent decrease in eIF2–GTP increases the time required for the scanning ribosomes to become competent to re-initiate translation. This delay allows for the ribosomes to scan through the inhibitory uORF2 and re-initiate at the ATF4 coding region, thereby increasing ATF4 levels in cells.

There are four known eIF2α kinases: protein kinase R (PKR), heme-regulated eIF2α kinase (HRI), PERK, and general control non-derepressible-2 (GCN2), (for reviews, see Refs. 40, 143, 189; Fig. 6), all of which are activated by distinct forms of stress. The interferon-inducible, double-stranded RNA-activated kinase, PKR, is activated not only by viral nucleotides but also by other compounds and is widely distributed in the brain. HRI coordinates globin synthesis with heme availability in erythroid cells, but it is also activated by oxidized GSH and heavy metals and is present in the brain. Both PERK and GCN2 phosphorylate eIF2α in cells responding to specific forms of toxic stress such as endoplasmic reticulum (ER) stress or hypoxia (PERK), and amino acid deprivation or UV irradiation (GCN2), respectively. Two different phosphatase complexes have been described which can mediate eIF2α dephosphorylation. During cell stress, eIF2α dephosphorylation is catalyzed by the serine/threonine phosphatase PP1 in conjunction with the nonenzymatic cofactor GADD34 (133), whereas GADD34 is replaced by its homolog, CReP, in the absence of stress (84). In general, the changes to the proteome induced by changes in the balance of eIF2α phosphorylation and dephosphorylation following activation of one of these kinases lead to adaptation of the cell's metabolism to stress with two possible, diametrically opposed consequences; survival or initiation of programmed cell death. The outcome seems to be determined by the duration of the insults, the interplay of different branches of the stress response and their time courses (114). In addition to a response to cellular stress, basal levels of phospho-eIF2α are present *in vitro* (157) and *in vivo* (54, 75), and eIF2α phosphorylation was shown to be involved in biochemical processes as diverse as cell cycle regulation (57), glucose homeostasis (156), and synaptic plasticity (34).

The importance of the phosphorylation of eIF2α and the subsequent increase in ATF4 levels in modulating the response of cells to stress is highlighted by studies in PERK$^{-/-}$ fibroblasts that are unable to phosphorylate eIF2α in response to ER stress and show a significant impairment in their ability to survive this stress (62). PERK also appears to be the kinase responsible for eIF2α phosphorylation in the early post-ischemic brain (98). While the role of post-ischemic eIF2α phosphorylation in the subsequent nerve cell death in the brain has yet to be resolved (40), there is evidence that a failure to activate the full ER stress response correlates with the induction of nerve cell death following exposure to oxidative stress in several models (99, 137). In support of this idea, a recent article showed that the loss of hippocampal neurons induced by treatment of mice with the glutamate receptor agonist kainate could be inhibited by a small molecule inhibitor of eIF2α dephosphorylation (166).

There is good evidence that eIF2α phosphorylation can modulate the resistance of nerve cells to oxidative stress. For example, in early studies from our laboratory (171), infection of the HT22 nerve cell line with a construct expressing the S51D mutant of eIF2α, which acts as a constitutively phosphorylated form of the protein, was shown to bring about an increase in the resistance of the cells to oxidative stress which correlated with an ability to maintain a high GSH concentration in the presence oxidative stress. Further studies from David Ron's laboratory using a different approach to generate constitutively phosphorylated eIF2α in the HT22 cells confirmed these results (116).

Our recent data (108) indicate that in nerve cells basal xCT expression is determined by ATF4, whereas Nrf2 appears to function mainly as an additional multiplier of basal expression levels. Furthermore, we found that ATF4 is sufficient to mediate increases in xCT expression, GSH content, and stress resistance in nerve cells. In addition, ATF4 was reported to regulate the expression of other proteins involved in GSH metabolism including both subunits of GCL and several different GSTs (76).

ATF4 levels and activity can be regulated by several additional mechanisms including phosphorylation and degradation. Phosphorylation of ATF4 by the growth factor-regulated and ERK-dependent kinase RSK2 significantly increases its transcriptional activity (191). In contrast, phosphorylation of ATF4 on Ser219 by an unknown kinase targets it for ubiquitination and degradation by the proteasome (101). However, degradation of ATF4 by the proteasome can be prevented by its interaction with the nuclear histone acetyltransferase and transcriptional co-activator, p300 (100). Although p300 can acetylate ATF4, the effect of p300 on ATF4 levels is independent of its acetyltransferase activity. Instead, the binding of p300 to the N-terminal domain of ATF4 inhibits its ubiquitination. In addition, under normal oxygen concentrations, prolines in the oxygen-dependent degradation domain of ATF4 are hydroxylated via interaction with the oxygen sensor prolyl-4-hydroxylase domain 3 (PHD3), which subsequently marks ATF4 for proteasomal degradation (95). By an unknown mechanism and independently of eIF2α phosphorylation, insulin upregulates and corticosteroids downregulate ATF4 protein levels as part of an anabolic and catabolic response, respectively (1, 120). How all these different mechanisms interact to regulate the levels and transcriptional activity of ATF4 remains to be determined. Furthermore, it is unclear how ATF4 and Nrf2 work together to upregulate GSH metabolism in response to various forms of stress as well in the CNS under physiological and pathophysiological conditions.

Other Roles for GSH

Regulation of glyoxylase activity by GSH

In addition to its well-known role in regulating protein glutathionylation which was thoroughly described in several recent reviews (37, 124), as well as its role described above in regulating 12,15 LOX activity, GSH plays a key role in regulating other aspects of cellular metabolism such as glyoxylase activity.

Reactive carbonyl and dicarbonyl compounds such as methylglyoxal (MG) and glyoxal (GO) are generated by cell metabolism, glucose auto-oxidation, and lipid peroxidation (176). These compounds are involved in a number of toxic processes *in vivo*. They can react with amino and sulfhydryl groups on proteins as well as with nucleotides to form irreversible advanced glycation end products (AGEs). AGE formation has been shown to impair the function of a number of cellular proteins, including chaperones, kinases, and growth

factor receptors (27). AGE-modified circulating proteins can also interact with specific RAGE receptors triggering pro-inflammatory responses.

The glyoxylase system is the main pathway involved in removing MG and GO from cells and thereby suppressing the formation of AGEs (for review, see Ref. 176). The glyoxylase system is composed of two enzymes, glyoxylase I (GLO-1) and glyoxylase II, and a catalytic amount of GSH. GLO-1 is the rate-limiting enzyme for the system and is absolutely dependent on GSH. Indeed, in cells, experimental depletion of GSH can induce marked accumulation of MG and, to a lesser extent, GO. Glyoxylase I is differentially expressed in different cell types in the human brain (97). Interestingly, the levels of glyoxylase I expression and activity in the human brain show a biphasic age-dependent response with an increase up to approximately 55 years of age and a progressive decrease thereafter (97).

Interaction of GSH metabolism and nitric oxide signaling

Nitric oxide (NO) is an important small molecule second messenger in the nervous system that is produced by NO synthase (NOS). Three isoforms of NOS are present in the brain; endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). Excess production of NO by the calcium-activated nNOS is an import step in excitotoxic neuronal cell death in response to overactivation of ionotropic glutamate receptors. iNOS is induced by inflammatory stimuli.

Via S-nitrosylation of cysteines, NO modifies multiple proteins in the brain and thereby alters their function (for review, see Ref. 93). Protein S-nitrosylation can be reversed by intracellular GSH (150) giving rise to S-nitroso-GSH (GSNO). GSNO is also produced by the direct reaction of GSH with NO (70). Thus, GSH can act as an NO buffer system. GSNO can be metabolized by several different mechanisms. First, it can decompose to GSSG and NO in a spontaneous or a metal-catalyzed reaction (13). Second, thioredoxins can mediate the cleavage of GSNO into GSH and NO in a reaction that consumes NADPH (131). In addition, GSNO reductases can reduce GNSO. Carbonyl reductase 1, which uses NADPH as a co-substrate (13), and glutathione-dependent formaldehyde reductase, which uses NADH (80, 115), have been identified as GSNO reductases. Similar to GSH, extracellular GSNO is degraded by GGT to yield S-nitrosocysteinlyglycine, which is cleaved by dipeptidases into glycine and S-nitrosocysteine (CSNO) (71, 83). CSNO is taken up by cells by system L (110), a heterodimeric amino acid transporter that mediates bidirectional transport of large neutral and branched amino acids across the plasma membrane (183).

Redox Regulation of Cell Function

Changes in the redox environment of cells can have profound effects on the activity of a variety of enzymes and other proteins such as transcription factors (for review, see Ref. 118). Therefore, it is highly likely that these changes in the activity of specific proteins are translated into overall changes in cellular physiology and behavior. Indeed, it has been proposed that the overall redox status of cells plays a role in regulating cell fate (Fig. 7; 82, 155). Specifically, there is mounting evidence that in normal cells a more reducing environment is associated with cell proliferation, while a more oxidizing environment promotes differentiation. Further oxidation would be expected to induce cell death. As the major redox couple in the cell, the GSH/GSSG ratio plays a significant role in regulating these cell fate decisions. However, as noted (155), the amount of GSH oxidation that is required to shift cells from proliferation to death is highly dependent on the overall levels of GSH in the cell. Thus, much more oxidation must occur for cells with an intracellular GSH concentration of 10 m*M* to move from proliferation to differentiation than with cells whose intracellular GSH concentration is 1 m*M*. For example, in a cell with an intracellular GSH concentration of 1 m*M*, only 18 μM needs to be oxidized to GSSG in order for the redox potential of the couple to change from −250 mV to −190 mV (155) which is sufficient to switch the functional state of the cell from proliferation to differentiation (82, 155).

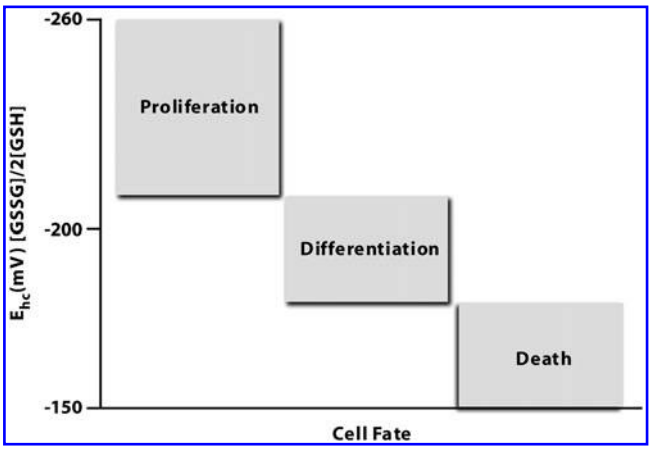

FIG. 7. Regulation of cell fate by the intracellular redox environment (after 82, 155). An electrochemical potential (E_{hc}) of the [GSSG]/2[GSH] redox couple between −260 and −210 mV is associated with cell proliferation, while an electrochemical potential above −210 mV promotes cell differentiation. A further increase in the electrochemical potential above −150 mV triggers cell death.

Perhaps the best evidence for redox regulation of cell function and, specifically, cell fate in the CNS comes from studies with oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells (132). These cells give rise to the myelin-forming oligodendrocytes of the CNS. Pure populations of these cells can be prepared and induced to undergo either proliferation or differentiation by growth in defined media. In a series of elegant studies, Mark Noble and colleagues first showed (132) that the balance between proliferation and differentiation in O-2A cells could be modulated by manipulation of the intracellular redox state such that a more reducing environment maintained proliferation while a more oxidizing environment promoted differentiation. Furthermore, growth of the cells in the presence of endogenous factors that stimulated proliferation was associated with a more reduced redox state while growth in the presence of endogenous factors that promote differentiation correlated with an increase in intracellular oxidation. Thus, these studies suggested that extracellular factors can promote a more oxidizing or reducing environment in cells and thereby play an important role in regulating the intracellular redox environment. In further studies, the same group showed that compounds that make the O-2A progenitor cells more oxidized promote differentiation by

reducing the level of receptors for growth factors that stimulate proliferation, such as PDGF (113). An oxidizing environment induces the activation of the tyrosine kinase Fyn that phosphorylates and thereby activates the ubiquitin ligase c-Cbl (174) which, in turn, promotes the degradation of target receptor tyrosine kinases such as the PDGF receptor. Whether the negative regulation of Nrf2 transcriptional activity by Fyn (79) also plays a role in promoting differentiation is unknown. Similar results were obtained with neural progenitor cells (NPCs) where an oxidizing environment was shown to both inhibit proliferation and stimulate differentiation into astrocytes (144). Evidence was presented that the latter effect of an oxidizing environment was mediated by the activation of the histone deacetylase Sirt1. However, much of this work relied on the use of the putative Sirt1 activator, resveratrol. Since it has now been demonstrated by multiple laboratories (14, 51, 58, 85, 134) that resveratrol is not a direct activator of Sirt1 but rather affects a large number of diverse targets (134), the role of Sirt1 in the oxidation-induced differentiation of NPCs into astrocytes should be viewed with caution, especially as a more recent study that did not use resveratrol found that Sirt1 activation enhanced the differentiation of NPCs into nerve cells (69).

This result is consistent with experiments in PC12 cells where NGF-induced differentiation was enhanced by treatments which decreased the GSH/GSSG ratio and inhibited by treatments which increased the GSH/GSSG ratio (86). A recent study (56) demonstrated that in PC12 cells, a more oxidizing environment promotes the activation of PKC. Among the different PKC isoforms activated, PKCε appeared to play a key role in differentiation via its ability to activate ERKs which are both necessary and sufficient for differentiation in PC12 cells (135).

Activation of certain receptor or cytoplasmic protein tyrosine kinases can also promote a more oxidizing environment and differentiation through the activation of nonphagocytic NADPH oxidases that produce superoxide (for review, see Ref. 31). As a consequence, protein tyrosine phosphatases, which normally counterbalance the action of the protein tyrosine kinases on diverse substrate proteins, are inhibited. A more oxidizing environment also inhibits the lipid phosphatase PTEN, thereby promoting the activation of the PI3K pathway. PTEN is the principal phosphatase that catalyzes the degradation of the lipid second messenger phosphatidylinositol 3,4,5-triphosphate that is generated by PI3K activity (for review, see Ref. 105).

An additional cell fate that is regulated by the cellular redox state is death. Recent studies in nerve cells have demonstrated that GSH can directly regulate apoptosis in nerve cells through two distinct mechanisms: its interaction with the pro-survival protein Bcl-2 (200), and its inhibition of cytosolic cytochrome C (182). Although Bcl-2 has long been known to suppress mitochondrial oxidative stress, the mechanisms underlying this effect have remained unclear since Bcl-2 has no intrinsic antioxidant scavenging activity. Studies by Zimmerman *et al.* (200) show that Bcl-2 interacts directly with GSH and thereby regulates an essential pool of mitochondrial GSH. Disruption of this interaction leads to mitochondrial oxidative stress and death in primary cultures of nerve cells. Vaughn and Deshmukh (182) showed that the redox environment and specifically GSH levels directly regulate the apoptosis-initiating function of cytosolic cytochrome C in nerve cells by maintaining it in a reduced and therefore less pro-apoptotic form. GSH depletion or inhibition of GR promotes both a more oxidized redox environment and increased nerve cell death. These results are consistent with a recent study which showed that oxidation of cytosolic cytochrome C stimulates caspase activation while reduction prevents caspase activation (23).

Redox regulation also plays an important role in regulating the function of microglia, the resident immune cells of the CNS (for reviews, see Refs. 46, 52, 148). They play important, protective roles in the CNS such as removing pathogens and promoting tissue regeneration after injury. Microglia are also implicated in the pathogenesis of a variety of acute and chronic neurological disorders including stroke, AD, PD, HIV-associated dementia, and multiple sclerosis. Activated microglia can produce a wide array of pro-inflammatory and cytotoxic factors including NO, cytokines, free radicals, excitatory neurotransmitters, and eicosanoids that can exert toxic effects on nerve cells and oligodendrocytes. ROS generated by NADPH oxidase play a key role in the activation of microglia and the downstream consequences, including NF-kB activation and induction of iNOS (140, 151). In both cases, addition of N-acetyl cysteine (NAC), a GSH precursor, prevented these changes. Furthermore, activation of Nrf2 both *in vitro* and *in vivo* can reduce the activation of microglia by the classical inducer bacterial lipopolysaccharide (LPS) (77). Among the products of Nrf2 activation, it was suggested that HO-1 may play a key role in modulating the response of microglia to activators since CO, one of the products of HO-1 activity, inhibits NADPH oxidase.

Together, all of these studies support the hypothesis that changes in the intracellular redox environment can have profound effects on cell function and fate.

Conclusions

GSH plays a key role in regulating the intracellular redox environment both as part of the major redox couple in cells

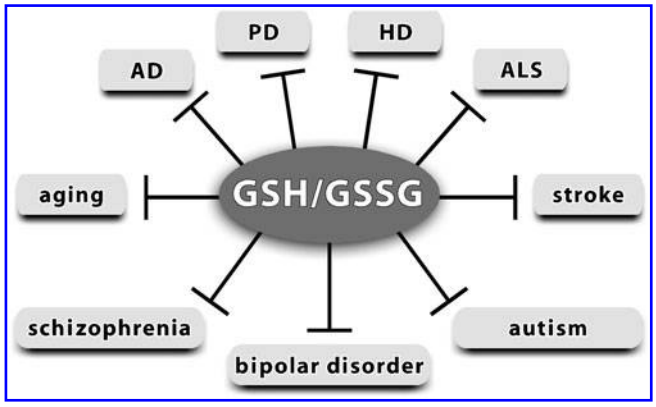

FIG. 8. Decreases in GSH or GSH/GSSG are seen in multiple neurological disorders, as well as normal aging, and may contribute to the neurological defects associated with these disorders. These disorders include neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), and psychiatric disorders such as schizophrenia, bipolar disorder, and autism, acute disorders such as stroke, as well as normal aging.

and also by modulating the activity of a variety of enzymes and other proteins. Consistent with the idea that higher GSH levels are associated with cell survival, GSH promotes the activity of proteins that maintain cell function such as GPxs, GLO-1, and Bcl-2, and inhibits the activity of proteins that can promote nerve cell death such as LOXs and cytosolic cytochrome C. Thus, maintenance of GSH levels is developing as an important therapeutic target not only for the treatment of neurodegenerative diseases such as AD and PD (6, 9, 158, 179, 196) but also the major psychiatric disorders including schizophrenia and bipolar disease (16) and childhood neurological disorders such as autism (28; Fig. 8). Indeed, the GSH precursor NAC has shown efficacy in the treatment of psychiatric disorders (24). A more complete understanding of the transcriptional regulation of GSH metabolism in neural cells is necessary to develop its potential fully as a therapeutic target, especially as it is now clear that Nrf2 is not the only player. Along with ATF4, the roles of Nrf2 homologues, Nrf1 and Nrf3, should also be explored. The next few years should prove to be an exciting time as redox regulation moves from cell- and animal-based studies to clinical applications.

Note Added in Proof

Two very recent papers describe additional endogenous regulators of Nrf2: p21 (30a) and p62 (95a).

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Abbreviations Used

AARE = amino acid response element
AD = Alzheimer's disease
AGE = advanced glycation end-product
ALS = amyotrophic lateral sclerosis
AP-1 = activator protein 1 (transcription factor)
ARE = antioxidant response element
ATF4 = activating transcription factor 4
CNS = central nervous system
ERK = extracellular signal regulated kinase
GCL = glutamate cysteine ligase
GCN2 = general control non-derepressible-2
GFP = green fluorescent protein
GLO = glyoxylase
GO = glyoxal
GPx = glutathione peroxidase
GR = glutathione disulfide reductase
Grx = glutaredoxin
GS = glutathione synthetase
GSH = glutathione
GSK-3β = glycogen synthase kinase 3-β
GSSG = glutathione disulfide
GST = glutathione S-transferase
HD = Huntington's disease
HNE = 4-hydroxynonenal
HO-1 = heme oxygenase-1
HRI = heme-regulated eIF2α kinase
JNK = c-jun N-terminal kinase
LOX = lipoxygenase
MCB = monochlorobimane
MG = methylglyoxal
MPTP = 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine
MRP = multidrug resistance protein
NF-κB = nuclear factor κB (transcription factor)
NO = nitric oxide
NOS = nitric oxide synthase
NPC = neural progenitor cell
Nrf2 = NF-E2-related factor 2
PD = Parkinson's disease
PDGF = platelet-derived growth factor
PDGFR = platelet-derived growth factor receptor
PERK = PKR-like kinase
PI3K = phosphatidylinositol 3-kinase
PKC = protein kinase C
PKR = protein kinase R
Prx = peroxiredoxin
PTK = protein tyrosine kinase
PTP = protein tyrosine phosphatase
ROS = reactive oxygen species
SOD = superoxide dismutase
tBHQ = t-butylhydroquinone
Trx = thioredoxin
TrxR = thioredoxin reductase
$Trx(SH)_2$ = reduced thioredoxin
TrxSS = oxidized thioredoxin
uORF = upstream open reading frame

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