ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 11 & 12, 2006

Comprehensive Invited Review

Redox Control of Neural Function: Background, Mechanisms, and Significance

PAMELA MAHER

Reviewing Editors: Narayan Bhat, Arthur Cooper, Hideo Kimura, George Perry, and Chandan K. Sen

ABSTRACT

The redox environment within neural cells is dependent on a series of redox couples. The glutathione disulfide/glutathione (GSSG/GSH) 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 also can modulate the activity of a variety of different proteins via S-glutathionylation of cysteine sulfhydryl groups. The thioredoxin system also makes a significant contribution to the redox environment by reducing inter- and intrachain protein disulfide bonds as well as maintaining the activity of important antioxidant enzymes such as peroxiredoxins and methionine sulfoxide reductases. The redox environment affects the activity and function of a number of different protein phosphatases, protein kinases, and transcription factors. The sum of these effects will determine how changes in the redox environment alter overall cellular function, thereby playing a fundamental role in regulating neural cell fate and physiology. *Antioxid. Redox Signal.* 8, 1941–1970.

INTRODUCTION

The field of redox regulation of cellular function is still in its infancy. This is particularly true with respect to nerve cells, as most studies have focused on the usually lethal effects of severe oxidative stress because of its potential relevance to a variety of neurologic disorders. However, it is clear that more subtle changes in the intracellular redox environment can have profound effects on the activities of a variety of phosphatases, kinases, and other enzymes, as well as on the function of transcription factors, and thereby alter cell function. In this review, I describe these effects and discuss how they can be modulated by changes in the oxidation/reduction state of critical cysteine and/or methionine residues in proteins. In addition, I present background information on the different enzyme systems within cells that play important roles in maintaining the redox environment. Finally, I describe the limited number of studies that show how changes in the redox environment can regulate neural cell fate. Although death is among the possible fates that result from a change in the intracellular redox environment, the main emphasis of this review is on other outcomes that also can affect CNS function. Whereas the main focus of this review is on nerve cells, I also describe studies with other CNS cells including astrocytes, oligodendrocytes, and microglia, because changes in the redox environment within these cells *in vivo* can alter both their function and the function of the neighboring nerve cells. I hope that this review will encourage other investigators to explore this exciting and challenging field.

Definitions

To begin a discussion of redox regulation of CNS cell function, it is first necessary to provide some definitions and background information. Although good evidence exists that many cellular activities depend on the redox state, this term, as observed by Schafer and Buettner (217), is not well defined. In the past, redox state was used to describe the ratio of the reduced-to-oxidized forms of a single, interconvertible pair of molecules such as NAD^+ and NADH. Because the

The Salk Institute for Biological Studies, La Jolla, California.

redox state of a cell is dependent on a number of these redox couples, Schafer and Buettner (217) suggested that it would be more accurate to talk about the redox environment of a cell. The redox environment is dependent on a linked set of redox couples and can be defined as "the summation of the products of the reduction potential and reducing capacity of the linked redox couples present." The redox environment, in turn, regulates the activity of a variety of different enzymes and transcription factors, thereby modulating cellular function.

Redox couples consist of pairs of compounds in which one compound can be converted to the other by adding one or more electrons plus one or more protons (93). A mixture containing 1.0 mole/L of each member of a redox pair will have a standard redox (oxidation/reduction) potential that is a measure of the affinity of the pair for electrons. Those pairs that have the most negative redox potential have the weakest affinity for electrons and therefore the strongest tendency to donate electrons. For example, the $NADP^+$/NADPH redox couple has a standard redox potential of ~−370 mV, and thus NADPH has a strong tendency to donate electrons to other molecules, whereas the O_2/H_2O redox couple has a standard redox potential of +820 mV, and thus O_2 has a strong tendency to accept electrons (*i.e.*, it is a good oxidizing agent). The standard redox potential (E°) is calculated based on equilibrium conditions relative to a standard hydrogen electrode. The Nernst equation, which was originally formulated to calculate the voltage of an electrochemical cell, can be used to estimate the reduction potential of a redox couple in a living cell:

$$E_{hc}\ (mV) = E°(mV) + RT/nF \log_{10}[\text{oxidized}]/[\text{reduced}]$$

In this equation, E_{hc} is the half cell (*i.e.*, with respect to a single electrode) redox potential for a redox couple at a defined pH, E° is the standard redox potential for the same redox couple, R is the gas constant (2 cal/mol°K), T is the absolute temperature (37°C = 310 K), F is Faraday's constant (2.3×10^4 cal/Vmol), and n is the number of electrons transferred. pH also plays a role in the actual redox potential of a redox couple in a cell, but unlike temperature, a correction for pH is not included in the Nernst equation, so the pH at which the E_{hc} was determined is usually noted.

Multiple, interrelated redox couples in the cell contribute to the intracellular redox environment. Among the most important of these are the $NADP^+$/NADPH, glutathione disulfide/glutathione (GSSG/GSH), oxidized thioredoxin/reduced thioredoxin [$TrxSS/Trx(SH)_2$], and CySS/Cys couples (120, 217). NADPH provides a major source of electrons for reductive biosynthesis. The NADPH/$NADP^+$ ratio in cells and tissues is ~100:1, resulting in a redox potential in liver of −380 mV for this couple. NADPH is also the main source of electrons for the maintenance of GSH, whose cellular concentrations are in the millimolar range. Because of its high concentration, a number of investigators have used the GSSG/GSH couple to estimate the redox state of a cell, tissue, or organelle. However, when the Nernst equation is used to estimate the redox potential for the GSSG/GSH redox couple in cells, the concentration of GSH must also be included in the calculation because each GSSG produces two molecules of GSH. Hence, unlike the $NADP^+$/NADPH couple, both the ratio of oxidized to reduced GSH and the concentration of reduced GSH will influence the redox environment. Normally, GSH is present in cells at ~100-fold excess over GSSG. However, the oxidation of only a small amount of GSH to GSSG can significantly change this ratio and thereby the redox environment of the cell. For example, in a cell with an intracellular GSH concentration of 1 m*M*, only 18 μ*M* must be oxidized to GSSG for the redox potential of the couple to change from −250 to −190 mV (217). This change is enough to switch the functional state of the cell (*e.g.*, from proliferation to differentiation; see Redox Regulation of Cell Function) (119, 217). The third major redox couple in cells is the $TrxSS/Trx(SH)_2$ couple. Along with the GSSG/GSH couple, it plays an important role in regulating the oxidation state of protein sulfhydryls. However, because the intracellular concentrations of $TrxSS/Trx(SH)_2$ are 100- to 1,000-fold less than those of GSH, its role is more limited. Recently, evidence was presented that the CysSS/Cys redox couple may also play a role in redox regulation by promoting the direct oxidation of protein sulfhydryl groups (120), particularly under conditions in which total GSH decreases and/or the GSSG/GSH redox couple becomes more oxidized.

Measurement

Because the overall redox environment is determined mainly by the GSSG/GSH redox couple, measurement of the redox environment usually relies on the determination of both GSH and GSSG levels in cells. This is not quite as straightforward as it sounds, because it can be difficult to measure GSSG accurately, as the levels of GSSG are very low relative to GSH (85). 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 (*e.g.*, 119, 205) or by a chemical assay (*e.g.*, 250). If the latter approach is used, then what is measured is actually total GSH and GSSG (after chemical treatment to remove GSH), and the levels of GSH are calculated from the difference. GSH levels can also be measured by using the fluorescent probe monochlorobimane (*e.g.*, 240). However, the reaction of GSH with monochlorobimane is dependent on the activity of glutathione transferases, so any treatment that affects transferase activity will alter this result, even if it does not affect GSH levels directly. A very recent study demonstrated that this approach could be used in combination with two-photon imaging to study GSH levels in the individual cells of the intact brain (241).

Compartmentalization of the GSSG/GSH redox couple

Although all cells contain millimolar levels of GSH, it is not distributed uniformly within the cell (217, 230). Furthermore, increasing evidence suggests that the GSH/GSSG ratio as well as the total GSH level in specific cellular compartments may be more important than the whole-cell ratio when looking at the effects of the redox environment on cell function. The majority of the total GSH in cells is found in the cytoplasm where it is synthesized. However, mitochondria have a separate pool of total GSH, which appears to be at least partially independent of the cytoplasmic pool in that its concentration does not always change in concert with that of the cy-

toplasmic pool (228, 230). In contrast, it is still a matter of debate whether the nucleus possesses a pool of total GSH that is independent of the cytoplasmic pool (95, 230). The endoplasmic reticulum (ER) also maintains a separate pool of GSH, but unlike the mitochondria and nucleus, the redox environment within the ER is quite oxidizing, with a reported GSH/GSSG ratio of (3:1–1:1). Recent studies suggest that the more-oxidizing environment in the ER both supports oxidative formation of disulfide bonds and is a consequence of disulfide bond formation (177). Recently, several alternative approaches to measuring the redox environment of specific organelles were described (95), and the use of these techniques may help to resolve questions surrounding the nuclear GSH pool.

GLUTATHIONE METABOLISM

GSH and GSH-associated metabolism provide the major line of defense for the protection of cells from oxidative and other forms of stress (for reviews, see 68, 97, 172). GSH can scavenge free radicals, reduce peroxides, and be conjugated with electrophilic compounds. It thereby provides cells with multiple defenses against both reactive oxygen species (ROS) and their toxic by-products. In addition, as discussed in Definitions, the GSSG/GSH pair forms the major redox couple in cells and as such plays a critical role in regulating redox-dependent cellular functions.

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 (172).

Substrate transport

Because glutamate and glycine occur at relatively high intracellular concentrations, cysteine is limiting for GSH biosynthesis in neural cells (72). 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. However, in the case of neuronal cells, this has proven to be something of a contentious point.

Cystine uptake in many cells occurs via the Na^+-independent x_c^- cystine/glutamate antiporter (for review, see 171). 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 a heavy chain (4F2hc) that is shared among a number of different amino acid transporters. It transports cystine into cells in a 1:1 exchange for glutamate and thus is inhibited by high concentrations of extracellular glutamate. 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 in which it is needed most (243). Although general agreement exists about an important role for system x_c^- in cystine uptake and GSH biosynthesis in astrocytes (7, 72) and oligodendrocytes (195), a controversy has been ongoing as to whether system x_c^- is relevant to neuronal cell function *in vivo*. An early report using *in situ* hybridization failed to detect xCT expression in neuronal cells in mouse brain, suggesting that system x_c^- is not present in these cells (215). More recent studies have provided functional (14, 15, 74) and morphologic (33, 74) evidence for the presence of system x_c^- in neuronal cells, where it also plays a role in regulating the levels of extrasynaptic glutamate.

Evidence also suggests that cysteine can be transported into neural cells mainly via excitatory amino acid transporters (224): EAAT3 in neuronal cells (40) and probably EAAT1 and/or EAAT2 in astrocytes. Cysteine transport is Na^+ dependent and is inhibited by glutamate, the classic substrate for these transporters. Because cysteine is readily oxidized outside of cells, it has been suggested that *in vivo*, the cysteine used for neuronal GSH biosynthesis comes from the breakdown of GSH that is released from nearby astrocytes (72, 73). This hypothesis is supported by studies showing that co-culture of astrocytes and neurons in transwells leads to an increase in neuronal GSH levels (73, 82), which is blocked if GSH biosynthesis in the astrocytes is inhibited (82). However, the relative contributions of cystine and cysteine uptake to neuronal GSH biosynthesis *in vivo* remain to be determined.

GSH biosynthesis

GSH is synthesized in cells by the consecutive action of two ATP-dependent enzymes (Fig. 1). 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 γGluCys, which is then combined with glycine to generate GSH in a reaction catalyzed by glutathione synthetase (GS). The synthesis of GSH

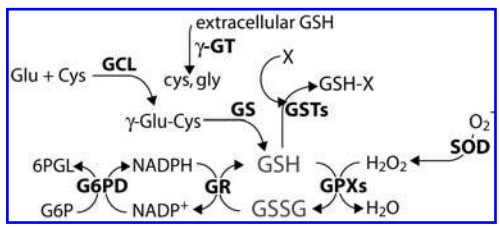

FIG. 1. Glutathione metabolism. GSH is synthesized from glutamate (glu), cysteine, (cys), and glycine (gly) by the sequential actions of glutamate cysteine ligase (GCL) and glutathione synthetase (GS). GSH is used to eliminate reactive oxygen species such as hydrogen peroxide (H_2O_2) in a reaction catalyzed 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). These conjugates, as well as GSH itself, can be transported outside the cell, where they can be acted on by γ-glutamyl transferase (γ-GT) to give γ-glutamyl amino acids and cys-gly.

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 posttranslational levels, and the complex interplay of these different levels of regulation is just beginning to be understood (for reviews, see 68, 231, 261). GCL is a heterodimer composed of a 73-kDa catalytic (GCLC) subunit and a 31-kDa regulatory (GCLM) subunit. GCLC has all of the catalytic activity and is the site of GSH feedback inhibition. Although neurons have significantly lower levels of GSH than astrocytes *in vitro* (*e.g.*, 99) and *in vivo* (241), both GCL subunits are present in neurons as well as astrocytes (270). Whereas it was originally thought that GCLC functions only in concert with GCLM, a recent study suggests that GCLC alone may be responsible for the constitutive synthesis of GSH (69). The association of GCLC with GCLM is needed 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 mice deficient in GCLM are viable but show a significantly enhanced sensitivity to stress (59). However, in primary cultures of rat neurons, knockdown of either subunit by 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 neuronal cells (67). In *Drosophila*, overexpression of GCLC but not GCLM predominantly in the nervous system increased mean and maximal life spans by ≤50% (196).

The two subunits of GCL are transcriptionally regulated by a wide variety of compounds (for reviews, see 69, 231, 261). 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 ARE (also known as EpRE, StRE; see Nrf2 and the Antioxidant Response Element). 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 posttranslational regulation of GCL activity, although evidence suggests that the activity of GCLC can be negatively regulated by phosphorylation (231). A number of kinases phosphorylate GCL, including protein kinase A (PKA), protein kinase C (PKC), and Ca^{2+}/calmodulin (CaM)-dependent kinase (231).

Glutathione synthetase (GS), the second enzyme required for GSH biosynthesis, is a 118-kDa homodimer (68). Very little is known about its role in regulating GSH biosynthesis, although in the rat, induction of GS can increase GSH synthetic capacity (150). Similar to the GCLC and GCLM promoters, the GS promoter contains potential binding sites for a number of transcription factors, including AP-1, AP-2, NF-κB, SP-1, and Nrf2 (150).

Glutathione disulfide reductase

Both enzymatic (via glutathione peroxidases) and nonenzymatic detoxification of ROS by GSH results in the production of GSSG. Because an increase in GSSG is harmful to cells, GSSG is often transported outside of cells, resulting in a depletion of GSH. The more economic 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 on 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, which also includes the thioredoxin reductases (see The Thioredoxin/Thioredoxin Reductase System). The active enzyme is composed of two identical subunits of 52,400 kDa, each of which contains binding sites for FAD, NADPH, and GSSG (162). The enzyme also contains a redox-active disulfide that plays a critical role in the flow of electrons from NADPH to GSSG. Recent evidence suggests that GR may be particularly susceptible to oxidative damage brought about by GSH depletion (19). Whether this damage is due to effects on the redox-active disulfide was not explored.

Glutathione peroxidases

Glutathione peroxidases (GPxs) catalyze the reduction of hydrogen peroxide and organic hydroperoxides at the expense of GSH (for reviews, see 11, 29). Although catalase and peroxiredoxins can also remove hydrogen peroxide, the relative levels of GPxs, Prxs, and catalase vary greatly from tissue to tissue (93). 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. Four different GPxs (GPx1–4) are found in mammals, all of which contain selenocysteine in the active site and therefore are dependent on an adequate supply of dietary selenium. The best characterized of the GPxs is GPx1, which is expressed in a variety of tissues with the highest levels in liver and kidney. It is found in both the cytoplasm and mitochondria and reduces mainly soluble inorganic and organic hydroperoxides. GPx1 is a tetrameric protein consisting of four identical 22- to 23-kDa subunits. GPx1 knockout mice show an increase in brain damage after exposure to a number of insults including ischemia–reperfusion injury (53), malonate, 3-nitropropionic acid, and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) (137). 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 (245), 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 disease (PD) (23, 246). In addition, treatment of hippocampal neurons with glucocorticoids results in a decrease in GPx1 activity and an increase in sensitivity to oxidative insults (200). 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 (117). GPx2 (gastrointestinal GPx) and GPx3 (plasma GPx) are expressed mainly in the gastrointestinal

tract and kidney, respectively. GPx4 (phospholipid hydroperoxide GPx) is a 20- to 22-kDa monomer that also can reduce hydroperoxides of complex lipids and can act on hydroperoxides embedded in membranes. GPx4 is expressed highly in the brain and testis (76).

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 from the biotransformation of foreign compounds by catalyzing their conjugation with GSH (for reviews, see 97, 209, 252). This is the first step in the pathway that leads to the elimination of toxic compounds from cells. More than 21 structurally diverse GSTs are found in humans, including both soluble and membrane-bound proteins. The GSTs are encoded by two separate multigene families, one comprising the soluble GSTs, and the other, the membrane-bound (microsomal) GSTs. The family of soluble GSTs contains six classes of transferases with different but overlapping substrate specificity. GSTs can also localize to mitochondria, where they may play a role in modulating the consequences of ROS production (24, 105). Many of the GST genes are polymorphic in humans, with certain alleles associated with impaired enzyme activity (253). 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 (253).

All of the GSTs use GSH to detoxify metabolites of xenobiotics as well as reactive α,β-unsaturated carbonyls, epoxides, and hydroperoxides. The GSH conjugates with these compounds are generally much more water soluble than the original compounds and are rapidly transported across cell membranes via one of the multidrug-resistance protein transporters (see later) 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 on further processing (209). For example, the neurotoxin methyl bromide caused severe neurologic symptoms in a patient with normal GST activity but not in a patient with reduced activity (81).

As with GCL and the light subunit of the cystine/glutamate antiporter, GSTs are phase II detoxification enzymes. Thus, their transcription is mediated by an ARE, which can be activated by the same compounds that activate the transcription of other genes involved in GSH metabolism. The coordinate upregulation of GSH biosynthesis along with the GSTs is necessary because 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 (209). Thus, their protective role is absolutely dependent on an adequate supply of GSH. Several of the cytosolic GSTs also play a regulatory role in the MAPK pathway (see Redox Regulation of Protein Kinases).

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 a carrier-dependent mechanism that is 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 16). ATP binding and hydrolysis provide the driving force for export through the MRPs. Although MRP1, MRP2, MRP4, MRP5, and the cystic fibrosis transmembrane conductance regulator have all been shown to export GSH and/or GSSG, the mechanisms underlying this export appear to be quite complex. Not only is GSH a substrate for export but export of other MRP substrates such as vincristine also require GSH co-transport. Other compounds require GSH for export, but the GSH is not co-transported. In addition, some compounds enhance GSH export without themselves being transported. The importance of the MRPs in mediating GSH and GSSG export in the CNS was highlighted by a recent study that showed that in astrocytes MRP1 was responsible for 100% of GSSG export and ~60% of GSH export (175).

In contrast to the MRP family of transporters, the OATP transporters function independent of ATP, instead relying on the large GSH electrochemical gradient across cell membranes to drive GSH export. Their substrate specificity is not yet known.

NRF2 AND THE ANTIOXIDANT RESPONSE ELEMENT

Recently, the role of the antioxidant response element (ARE; also called EpRE,) in regulating the transcription of genes involved in redox regulation has received a great deal of attention. These genes include the two subunits of GCL, GS, the light subunit of the cystine/glutamate antiporter (see Glutathione Metabolism/Substrate Transport), the GSTs (see Glutathione Metabolism/Glutathione Transferases), the peroxiredoxins (see Peroxiredoxins), and thioredoxin (see The Thioredoxin/Thioredoxin Reductase System). The transcriptional activation of the genes encoding these proteins is mediated at least in part by a *cis*-acting enhancer termed the ARE. Transcriptional activation through the ARE is dependent on the transcription factor NF-E2–related factor 2 (Nrf2), a member of the Cap'n'Collar family of bZIP proteins (for reviews, see 38, 69, 189). Currently, some controversy exists over the precise mechanisms underlying the activation of Nrf2. Very little Nrf2 is found in unstimulated cells. In the classic view (Fig. 2A), the Nrf2 that is present in unstimulated cells is held in the cytoplasm by the actin-bound protein Keap1, which also promotes the degradation of Nrf2 by the proteasome. On stimulation by agents that activate ARE-mediated gene transcription, Nrf2 is released from Keap1, which leads to both its accumulation and its translocation to the nucleus, where it can induce the expression of genes containing an ARE. Keap1 contains 25 cysteine residues. Current evidence suggests that many of the compounds that are able to activate the ARE do so by interacting with one or more of these thiols, thereby inducing a conformational change in Keap1 that results in the release of Nrf2 (70). However, sub-

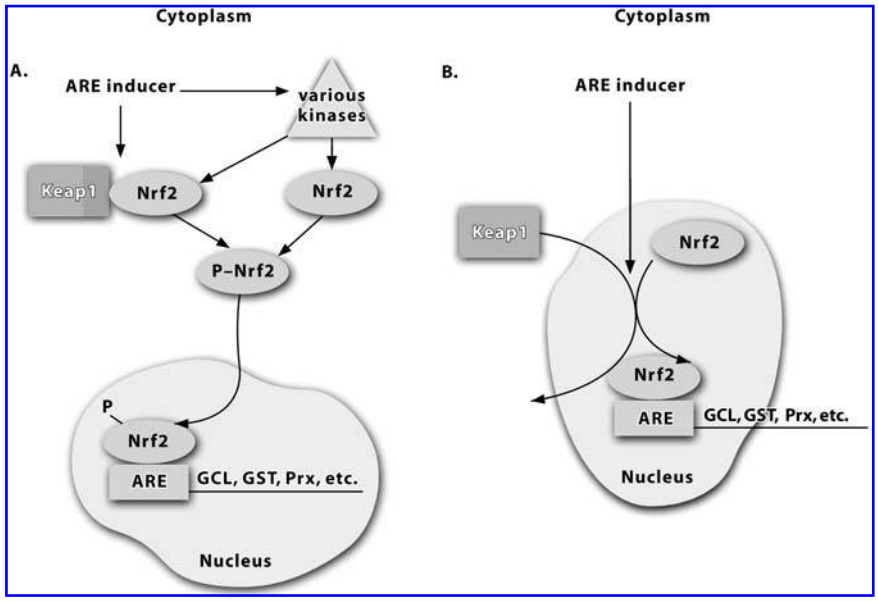

FIG. 2. Regulation of Nrf2 activity. Two models for the regulation of Nrf2 have been proposed. In the classic model (**A**), Nrf2 is maintained at low levels in the cytoplasm through its interaction with Keap1. Treatment with ARE inducers results in the activation of various kinases and the phosphorylation and release of Nrf2. Phospho-Nrf2 translocates to the nucleus, where it interacts with the ARE to induce the transcription of various antioxidant genes including glutamate cysteine ligase (GCL), glutathione-*S*-transferase (GST), and peroxiredoxins (Prx). In the alternative model (**B**), Nrf2 is a constitutively nuclear protein that is maintained in an inactive state in the nucleus through the transient localization of Keap1 to the nucleus. Treatment with ARE inducers results in the translocation of Keap1 to the cytoplasm, the subsequent interaction of Nrf2 with the ARE, and the induction of the transcription of various antioxidant genes, including glutamate cysteine ligase (GCL), glutathione-*S*-transferase (GST), and peroxiredoxins (Prx).

stantial evidence suggests that phosphorylation of Nrf2 also plays a role in promoting its nuclear accumulation (38, 189). Whether these modifications act in concert or are specific to distinct inducers of ARE-mediated gene transcription is not clear at this time.

More recently, a somewhat different picture as to how Nrf2 is activated has emerged (Fig. 2B) (188). In this view, Nrf2 is a constitutively nuclear protein, whereas 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. Whether this is by modification of cysteine residues of Keap1, by phosphorylation of Nrf2, or by some combination of the two remains to be determined.

Although both neurons and astrocytes can respond to ARE inducers such as *t*-butyl hydroquinone in isolated cultures *in vitro* with an upregulation in the transcription of a variety of ARE-dependent genes (*e.g.*, 147, 148, 154), the response in astrocytes appears to be far more robust (226). In mixed cortical cultures, upregulation of ARE-dependent genes in astrocytes alone is sufficient to protect neurons from oxidative insults (140, 226), consistent with the idea that astrocytes provide neurons with GSH precursors (see Glutathione Metabolism/Substrate Transport).

THE THIOREDOXIN/THIOREDOXIN REDUCTASE SYSTEM

The thioredoxin/thioredoxin reductase system forms one of the main redox couples in mammalian cells (Fig. 3) (for reviews, see 9, 90, 192). This system consists of the redox-active protein thioredoxin (Trx), the enzyme thioredoxin reductase (TrxR), and NADPH. Trxs are small (~12 kDa), ubiquitously expressed proteins that are highly efficient at reducing disulfides in proteins and peptides. Trxs also modulate the activity of a variety of other proteins, including several antioxidant enzymes, through redox-regulated interactions (see Redox Regulation of Protein Kinases, Redox Regulation of Transcription Factors, Peroxiredoxins, Methionine Sulfoxide Reductases, and Glutaredoxin).

The three mammalian thioredoxins are Trx1, a cytoplasmic protein that can translocate to the nucleus under appropriate conditions; Trx2, a mitochondrial protein; and SpTrx, which is expressed mainly in spermatozoa. All Trxs contain the active-site sequence CGPC. The N-terminal of the two cysteines has a low pKa and therefore normally exists as the thiolate anion such that it can act as the attacking group in the reduction of protein disulfides. This results in the transient formation of a mixed disulfide between the target protein and thioredoxin followed by thiol-disulfide exchange, resulting in the reduction of the target protein and oxidation of Trx. Oxidized Trx is then reduced back to its original form by TrxR at the expense of NADPH. It is important to note that the nature of the reaction of Trx with target proteins depends on the

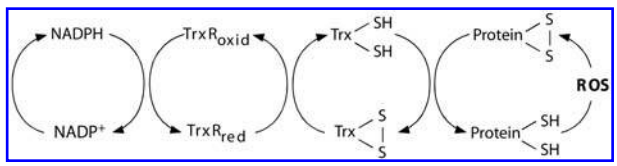

FIG. 3. The thioredoxin/thioredoxin reductase system. Thioredoxin (Trx) functions to reduce disulfide bonds in a variety of proteins, including methionine sulfoxide reductases (Msr) and peroxiredoxins (Prx). Oxidized Trx is reduced by thioredoxin reductases (TrxR) at the expense of NADPH.

redox potential of the target, so that in theory, Trx can both break and form disulfide bonds. The importance of the Trx system in maintaining normal cellular function is highlighted by the observation that animals lacking either Trx1 or Trx2 die during embryogenesis (183).

The mammalian TrxRs are proteins of ~55 kDa with a relatively broad substrate spectrum. They are homodimeric FAD-containing selenoproteins. The TrxRs contain two redox centers: a pair of cysteines next to the FAD binding site in the N-terminal portion of the protein, and a C-terminal selenocysteine–cysteine pair. The N-terminal cysteine pair is involved in the transfer of electrons from NADPH to the FAD group, whereas the selenocysteine–cysteine pair is required for the reduction of thioredoxin. Mutants in which selenocysteine is replaced by cysteine show only 1–10% of the activity of the wild-type enzyme (274). Thus, similar to that of the GPxs, the function of TrxR is intimately linked with the selenium status of cells. The three mammalian TrxRs include the following: TrxR1 is cytoplasmic, whereas TrxR2 is localized to mitochondria, and TGR, which contains both an N-terminal glutaredoxin domain and a C-terminal thioredoxin reductase domain, is expressed mainly in the testis (239). Mammalian TrxRs can reduce a number of small-molecule substrates in addition to their role in maintaining Trxs in their reduced forms (192). These substrates include ascorbic acid, lipid hydroperoxides, α-lipoic acid, and hydrogen peroxide. However, the K_m for the latter is very high (~2.5 m*M*) suggesting that this enzyme generally does not play an important role in removing hydrogen peroxide from cells.

Trxs have a number of important functions in cells. They are essential for maintaining the activity of peroxiredoxins (see Peroxiredoxins) and methionine sulfoxide reductases (see Methionine Sulfoxide Reductases). After translocating to the nucleus, Trx1 increases the activity of a variety of transcription factors such as AP-1, p53, NF-κB (see Redox Regulation of Transcription Factors). Trx1 acts as a negative regulator of the MAPKKK, ASK1 (see Redox Regulation of Protein Kinases). In addition, overexpression of Trx1 in mice was shown to increase median life span by 35% and maximal life span by 22% (272). It is likely that this effect is at least partially tied to its role in maintaining methionine sulfoxide reductase activity and perhaps peroxiredoxin activity as well. In mitochondria, Trx2 interacts with the tricarboxylic acid (TCA) cycle enzyme α-ketoglutarate dehydrogenase (KGDH) and protects it from self-inactivation during catalysis at low NAD^+ levels (32).

In the CNS, Trx1 and Trx2 are expressed throughout the brain but are most abundant in regions of high metabolic activity and ROS production (for review, see 201). A number of studies have reported a strong correlation between Trx1 upregulation and neuronal survival after a variety of insults to the brain (201). The promoter region of Trx1 contains several different elements, including an ARE; therefore its transcription is likely to be induced by many of the same treatments that upregulate peroxiredoxins and GSH metabolism. Less is known about Trx2 regulation in the brain, although certain insults such as ischemia–reperfusion upregulate both genes in concert. Overexpression of Trx1 in the brain (and other tissues) as well as systemic administration of recombinant Trx1 results in mice that are more resistant to focal ischemia (170), supporting the idea of an important role for Trx in maintaining redox homeostasis in the brain. Trx has also been implicated in nerve growth factor (NGF)-mediated neurite outgrowth in PC12 cells, suggesting that Trx1 plays a critical role in NGF signaling, perhaps by maintaining the activation of transcription factors (170). Whether this is true *in vivo* remains to be determined. Very little is known about TrxR expression in the brain (201).

Recently, several methods for measuring the TrxSS–$Trx(SH)_2$ redox couple in subcellular compartments were described, which should permit a better understanding of the role of this couple in regulating cellular functions (95). Both of these methods rely on a combination of tagging reduced Trx to alter its electrophoretic migration coupled with electrophoretic separation and Western blotting with an antibody that recognizes both oxidized and reduced Trx with equal affinity. By using these methods, it has been shown that different cellular treatments preferentially oxidize distinct pools of Trx.

In conclusion, the Trx–TrxR system plays a central role in the response of cells to changes in their redox status. Not only does it regulate the activity of several important enzymes that help protect cells from oxidative stress, but it also modulates the function of a variety of other proteins. It is likely that its importance in nerve cells will become even more evident with further studies on the redox regulation of nerve cell function.

PEROXIREDOXINS

The peroxiredoxins (Prxs) are a family of ~25-kDa thioredoxin-dependent peroxidases and thus are intimately tied to the Trx/TrxR system in cells (Fig. 4) (for reviews, see 111, 206, 268). The peroxiredoxins are thiol-specific antioxidant enzymes that are involved in the degradation of hydrogen peroxide, organic hydroperoxides, and peroxynitrite. They have also been proposed to regulate hydrogen peroxide–mediated signal transduction, playing a critical role in regulating redox-dependent changes in cellular functions. The peroxiredoxins are very abundant proteins, constituting 0.1–0.8% of the soluble protein in mammalian cells. The mammalian peroxiredoxin family contains six members in three subclasses defined on the basis of their catalytic mechanism: the typical 2 cysteine Prxs (Prxs1–4), the atypical 2-cysteine Prx (Prx5), and the 1-cysteine Prx (Prx6). As indicated by these definitions, the Prxs reduce peroxides via redox-active cysteine sulfhydryls in their catalytic sites. All three classes have a similar first catalytic step wherein the peroxidatic cysteine sulfhydryl attacks the peroxide substrate and is oxidized to a cysteine sulfenic acid. How the oxidized cysteine is returned to its reduced form is the distinguishing characteristic of each class. The typical Prxs are obligate homodimers with two identical active sites. In the second step of the catalytic reaction, the cysteine sulfenic acid (Cys-SOH) from one subunit is attacked by the resolving cysteine in the second subunit, resulting in a stable intersubunit disulfide bond. This bond is then reduced by thioredoxin. The atypical Prx functions in a similar manner except that both catalytic site cysteines are contained in a single protein. In contrast, the 1-cyteine Prx re-

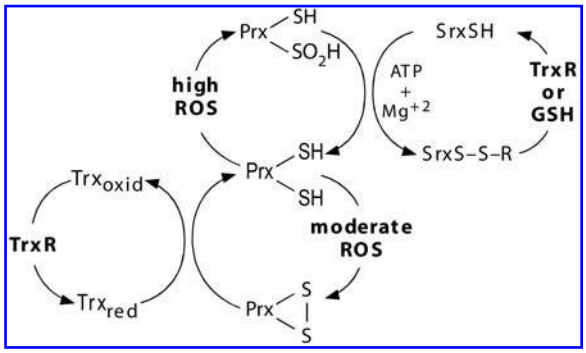

FIG. 4. The typical 2-cysteine peroxiredoxins. Peroxiredoxins 1–3 reduce hydrogen peroxide or other reactive oxygen species (ROS), yielding an intermolecular disulfide bond via a sulfenic acid intermediate. In the presence of moderate levels of ROS, this disulfide bond is reduced by the thioredoxin (Trx)/thioredoxin reductase (TrxR) system. In the presence of higher levels of ROS, the sulfenic acid intermediate is further oxidized to sulfinic acid (SO_2H). The sulfinic acid can be reduced via sulforedoxin (Srx) in an ATP-dependent reaction. Oxidized Srx is then reduced by either GSH or the thioredoxin (Trx)/thioredoxin reductase (TrxR) system.

lies on a thiol-containing redox partner to return the cysteine sulfenic acid to its reduced form. The nature of this redox partner proved difficult to identify, but recent evidence suggests that it is GSH working in conjunction with glutathione *S*-transferase π (GSTπ) (166). Oxidized Prx6 heterodimerizes with GSTπ followed by glutathionylation of the oxidized Prx6 cysteine sulfenic acid. Dissociation of GSTπ leaves glutathionylated Prx6, which, after reduction by Grx/GSH, results in the regeneration of the active enzyme.

The typical 2-Cys Prxs also undergo redox-regulated oligomerization (266). Current evidence suggests that the reduced form of these Prxs is a doughnut-shaped decamer of five homodimers. As the cysteine pairs in each homodimer form disulfide bonds, the decamer breaks down into lower-order oligomers and eventually into homodimers. Because the overoxidized form of 2-Cys Prxs (see later) also lacks intermolecular disulfide bonds, it too is a decamer.

The interaction of the resolving cysteine with the cysteine sulfenic acid in typical Prxs is a slow process, as the two cysteines are situated far apart. Because of this, in the continuous presence of an oxidant, the cysteine sulfenic acid can undergo further overoxidation to sulfinic acid (Cys-SO2H). For many years, it was thought that this would result in the irreversible inactivation of the enzyme, as no evidence existed that sulfinic acids could be reduced *in vivo*. However, recent studies pioneered in the laboratory of Sue Goo Rhee have shown that sulfinic acids can be reduced (263). This reduction is catalyzed by one or perhaps two distinct enzyme families, the sulfiredoxins and the sestrins (see later). Initial studies on the oxidation of Prxs relied on two-dimensional gel electrophoresis to distinguish the reduced from the overoxidized forms (263). However, at least in the case of Prx1, this approach is not without problems, as further analysis showed that the spot corresponding to the reduced form of Prx1 also contained a modified, oxidized form of Prx1 (41). The recent development of antibodies to the sulfinic/sulfonic forms of the different 2-Cys Prxs should allow more efficient and accurate analysis of this protein modification and its regulation in cells (265). Although overoxidized forms of all four of the typical 2-Cys Prxs can be rapidly reduced *in vitro* by purified sulfiredoxin (264) in a process termed retroreduction, the rate at which this occurs in cells is quite variable (41, 265) and may be cell-type specific. Furthermore, overoxidized Prx5 and Prx6 do not appear to be able to go undergo retroreduction (33, 223).

The enzyme responsible for the retroreduction of Prxs was first identified in yeast (27) and named sulfiredoxin (Srx). As initial studies suggested that this enzyme did not function in mammalian cells, a second, unrelated family of enzymes, named sestrins, which appeared to regenerate Prxs, were then identified in mammalian cells (31). However, it was subsequently shown that the mammalian homologues of yeast sulfiredoxin could indeed retroreduce mammalian Prxs (36). The reaction is relatively slow and is dependent on ATP hydrolysis and a thiol (either GSH or Trx) as an electron donor. In this novel two-step reaction, Srx first activates the sulfinic acid moiety of the overoxidized Prx by using ATP to generate the phosphoryl ester. This allows the catalytic cysteine of Srx to interact with the phosphoryl ester, resulting in the formation of a thiosulfinate bond. A thiol reductant (GSH, Trx) can then return both enzymes to their reduced states. Recent crystallization studies suggest that the enzyme is specific for the decameric, overoxidized form of the typical 2-Cys Prxs (121).

The combination of the inactivation of Prxs by cysteine oxidation coupled with the evidence for the role of Prxs in regulating cellular responses to hydrogen peroxide (207) led to the proposal that the typical Prxs act as "floodgates" for controlling the levels of hydrogen peroxide in cells while allowing hydrogen peroxide to function as a signaling molecule (267). Thus, in unstimulated cells, the high levels of typical Prxs keep ambient levels of peroxides very low, both to prevent damage to cellular organelles and to block inappropriate signaling. After a stimulus that results in a burst of hydrogen peroxide, the Prxs would be rapidly inactivated, allowing the hydrogen peroxide to activate one or more signaling cascades via oxidation of critical cysteine sulfhydryl groups in target proteins. Over time, the Prxs would get reactivated and be ready to function again. In the meantime, the signal would have been sent. Although this is an interesting hypothesis, it becomes somewhat problematic when the details are considered. Because the GPxs also react with hydrogen peroxide and are much more efficient (~1,000 times) at doing so than are the Prxs, it is not clear why the Prxs would be expected to form the first line of defense against peroxides in cells, although they are very abundant proteins. A second problem is that the reaction rate of Prxs with hydrogen peroxide is ~100,000 times faster than that of the cysteine sulfhydryl groups of known target proteins (236), suggesting that hydrogen peroxide would be eliminated before it had a chance to react with target proteins. These problems perhaps could be overcome if the peroxide generator as well as the target protein and the Prx were located together in a signaling complex so that the target protein was both protected from GPxs and transiently exposed to a very high concentration of hydrogen

peroxide, which would permit its oxidation as well as the overoxidation of Prx. Although currently no evidence is available for this type of signaling complex, this may reflect the lack of any effort to find one rather than its absence.

Prx activity can also be regulated by phosphorylation. Phosphorylation of a conserved cysteine residue in typical 2-Cys Prxs by cyclin-dependent kinases results in a decrease in peroxidase activity. The decrease in peroxidase activity is thought to result from the disruption of the decameric structure, because dimeric forms of Prx have lower levels of activity (268). To date, the phosphorylation of Prx1 has been observed only during the M phase of the cell cycle, suggesting that cyclin-dependent kinase phosphorylation of Prxs and the ensuing probable increase in hydrogen peroxide could play a role in cell division (206).

Prx1 gene expression is regulated mainly by an antioxidant response element (ARE) in its promoter region (111). Thus, the same stimuli that upregulate GCL expression will also increase Prx1 expression (see ARE section). Other transcription factors may also play a role in Prx expression. For example, AP-1 has been implicated in the upregulation of Prx1 expression in rat cells (111). Stress-dependent induction of Prx2 and Prx6 have also been noted.

Although Prxs are highly expressed in most cell types, their intracellular distributions differ (111). Prx1 and Prx2 are found in the cytosol, whereas Prx3 is localized to mitochondria, and Prx4 is a secreted protein that is also found in the ER. Prx5 is found as a long form in mitochondria and a short form in peroxisomes, whereas Prx6 is a cytosolic protein. Prx1, 2, and 6 can also be found in the nuclei of cells. Prx1 is the most ubiquitously distributed of the Prxs, including cells of the peripheral and central nervous system (201). Prx1 as well as Prx6 is expressed in astrocytes, whereas Prx2 is found exclusively in neurons. Prxs3, 4, and 5 have also been detected in the CNS, but their cell-type expression is not yet known. Several of these Prxs are induced in the brain in response to various types of oxidative stress (201). It is therefore likely that Prxs play important roles in the CNS, although the exact nature of these roles remains to be elucidated. Their enzymatic function as well as the regulation of their expression by an ARE indicates that they play important antioxidant roles. Whether they also have a role in intracellular signaling, as well as their contribution to mitochondrial redox homeostasis in neural cells, is not yet clear.

REDOX REGULATION OF PROTEIN CYSTEINE SULFHYDRYL GROUPS

The sulfhydryl groups on the vast majority of the cysteine residues (Cys-SH) in proteins have a pKa >8.0 and so remain protonated at physiologic pH in the reducing environment of most cellular organelles. However, a number of proteins possess cysteines that exist as thiolate anions ($Cys\text{-}S^-$) at physiological pH because of a lowering of their pKa values by charge interactions with neighboring amino acid residues (79). These sulfhydryl groups provide a redox-sensitive switch for regulating protein function (50, 217). The oxidation of cysteine sulfhydryl groups can lead to the formation of intra- and/or interchain disulfide bonds. Cysteine sulfhydryls can also be *S*-glutathionylated and/or *S*-nitrosylated. Theoretically, all of these reactions are reversible through the thioredoxin and glutaredoxin systems (26). In addition, cysteine sulfhydryls can be oxidized to yield sulfenic, sulfinic, and sulfonic acids. Although the formation of sulfenic acid is reversible and is often a first step in the generation of disulfide bonded or *S*-glutathionylated proteins, the further oxidation to the sulfinic acid generally appears to be irreversible (except see Peroxiredoxins). It is possible that different ROS/RNS can lead to distinct sulfhydryl modifications with varying effects on cellular function (79, 84). This alternative has been nicely demonstrated in studies with the bacterial transcription factor, OxyR (for review, see 194).

OxyR contains a single reactive cysteine sulfhydryl group, which can be modified *in vivo* to *S*-nitrosyl (SNO), sulfenic acid (SOH) or *S*-glutathionyl (SSG) forms in response to different inducers of oxidative stress. The differential effects of these sulfhydryl modifications were analyzed by circular-dichroism analysis of the isolated OxyR protein and correlated with their effects on its DNA binding and transcriptional activity. The reduced form of OxyR bound to a DNA fragment containing the OxyR binding site noncooperatively with high affinity, whereas the SNO and SOH forms bound the DNA fragment cooperatively but with somewhat lower affinities. The SSG form bound the DNA fragment noncooperatively but with very high affinity. The transcriptional activity of these different forms of OxyR spanned a 30-fold range, with SH being the least active and SSG being the most active. The possibility that these types of distinct modifications to a single protein residue occur in mammalian cells exposed to different forms of stress is quite likely but remains largely unexplored.

Outlined below are the cysteine sulfhydryl modifications that have been described in mammalian cells, the mechanisms underlying their formation, and their consequences on protein function, with a particular emphasis on proteins relevant to nerve cells. Later sections detail the effects of cysteine sulfhydryl modifications on specific classes of proteins that are known to be highly affected: protein kinases, protein phosphatases, and transcription factors.

Intra- and interchain disulfide bonds

Disulfide bonds have long been considered a rarity in the reducing environment present within the cytoplasm of cells, as well as within most cellular organelles, with the exception of the ER. Indeed, nonspecific formation of disulfide bonds in response to oxidative stress is thought to contribute to irreversible protein damage, including the formation of large, protein aggregates (247). However, cells do contain proteins such as the Trxs (see Thioredoxin/Thioredoxin Reductase System) that are able to reduce disulfide bonds, suggesting that disulfide bond formation could play a role in the regulation of protein function. Furthermore, disulfide bond formation is part of the mechanism of several antioxidant proteins including Prxs and methionine sulfoxide reductases (see sections on Peroxiredoxins and on Methionine Sulfoxide Reductases).

Additional support for a role for disulfide bonds in regulating protein function comes from studies using global ap-

proaches to look at protein disulfide bond formation. These studies showed that intermolecular disulfide bonding is not rare among cytoplasmic proteins and is regulated by oxidants. By using sequential nonreducing/reducing two-dimensional gel electrophoresis combined with mass spectrometry to identify disulfide-bonded proteins in the cytoplasm of a neuronal cell line, Cumming *et al*. (56) showed that disulfide bond formation occurs in unstressed as well as in oxidant-stressed cells. Proteins involved in glycolysis, protein translation, molecular chaperoning, cell growth, cytoskeletal structure, antioxidant activity, and signal transduction were found to contain intermolecular disulfide bonds in unstressed cells. Exposure to various oxidants resulted in both an increase in the amount of disulfide bonding in these proteins and disulfide bonding in additional proteins. These results suggest that disulfide bonding between proteins could be an important mechanism for regulating protein function and indicate that this is a research area that deserves significantly more attention.

Among the proteins shown to undergo redox-sensitive disulfide bonding is the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (57). GAPDH catalyzes the NAD-dependent conversion of glyceraldehyde 3-phosphate to 1,3-diphosphoglycerate. GAPDH readily undergoes disulfide bonding when cells are exposed to oxidants including the prooxidant amyloid β-peptide. Disulfide bonding leads to a reduction in GAPDH enzymatic activity as well as to the accumulation of detergent-insoluble GAPDH aggregates in the cytoplasm and nucleus of cells. Detergent-insoluble GAPDH aggregates were also observed in brain extracts from Alzheimer disease (AD) patients and transgenic AD mouse brain tissue but not in age-matched control tissues. Because evidence exists that allellic variations in GAPDH can influence the development of AD, these data suggest that these variations might function by influencing the ability of GAPDH to form disulfide bonds.

Protein S-glutathionylation

The addition of GSH to the cysteine sulfhydryl groups of proteins is defined as glutathionylation (also called glutathiolation). It can occur via several distinct mechanisms and appears to be regulated by the redox status of cells (Fig. 5) (52, 83, 87, 107, 135, 225, 248). The consequences of *S*-glutathionylation on protein function are protein dependent, so that some proteins are activated, others are inactivated, and still others are unaffected. Although protein *S*-glutathionylation does not appear to require enzyme activity, deglutathionylation generally does. Glutaredoxins (Grxs) are considered to be the main enzymes in cells for removing GSH from cysteine sulfhydryl groups (see Glutaredoxins). The details of the process of protein *S*-glutathionylation and what is known about it in nerve cells is described below. It should be noted that this is currently a very active area of research, and the mechanistic details are still being worked out.

Mechanisms. Two basic mechanisms underly protein *S*-glutathionylation (Fig. 5) (83, 107, 135, 225, 248). GSH addition can occur either by a thiol-disulfide exchange reaction wherein the thiolate anion of a protein sulfhydryl group reacts with an activated form of GSH (GSSG, GSOH, or GSNO) or by the reaction of a partially oxidized cysteine sulfhydryl group (thiyl radical, sulfenic acid intermediate, or *S*-nitroso intermediate) with GSH.

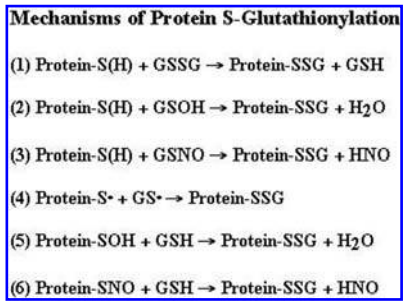

FIG. 5. Mechanisms for the *S*-glutathionylation of proteins. Two basic mechanisms underly protein glutathionylation. GSH addition can occur either by a thiol–disulfide exchange reaction (1–3), wherein the thiolate anion of a protein sulfhydryl group [S(H)] reacts with an activated form of GSH (GSSG, GSOH, or GSNO) or by the reaction of a partially oxidized cysteine sulfhydryl group [thiyl radical (4), sulfenic acid intermediate (5), or *S*-nitroso intermediate (6)] with GSH.

Thiol-disulfide exchange occurs when the thiolate anion of a cysteine sulfhydryl reacts with GSSG to form a mixed disulfide, releasing GSH in the process. The reactivity of specific protein cysteine sulfhydryls with GSSG will depend on both the pKa of the sulfhydryl (and therefore whether it will form a thiolate anion at neutral pH) and the accessibility of the sulfhydryl to GSSG. Thiol-disulfide exchange would be expected to occur most readily under conditions of oxidative or nitrosative stress in which the levels of GSSG or other reactive GSH derivatives are significantly increased in cells. Evidence exists from a variety of laboratories that this is indeed the case (135, 225). For example, treatment of the murine nerve cell line, HT22, with hydrogen peroxide results in a threefold increase in the level of GSSG and a 14-fold increase in the overall level of *S*-glutathionylated proteins (56). More specifically, it was shown that a critical cysteine in the DNA binding site of the transcription factor c-Jun becomes *S*-glutathionylated when the GSH/GSSG ratio falls from its normal level of ~100:1 to 13:1 (136).

Reaction of GSH with NO or other reactive nitrogen species can lead to the formation of GSNO, which can also react with cysteine sulfhydryl groups to yield mixed protein disulfides. Moreover, the reaction of protein sulfhydryl groups with GSNO can also lead to *S*-nitrosylation (86). Which modification predominates appears to be protein specific. Cysteine sulfhydryl groups that are targets for this reaction tend to have particularly low pKas (135).

S-glutathionylation of proteins is also seen in the absence of any increase in GSSG levels (225). This is thought to occur through the reaction of an oxidized protein sulfhydryl group with GSH to form the mixed disulfide. Cysteine sulfhydryl groups can be oxidized by reactive oxygen or nitrogen species. One electron oxidation yields a thiyl radical (Protein-S•). Two-electron oxidation by hydrogen peroxide or peroxynitrite produces sulfenic acid (Protein-SOH). Either group can then react with GSH to form the more stable mixed disulfide. The reaction of nitric oxide with cysteine sulf-

hydryls yields a reactive *S*-nitrosylated sulfhydryl, which can also react with GSH.

Depending on the specific intracellular conditions, both of these mechanisms can be very efficient at carrying out protein-*S*-glutathionylation. For example, in the HT22 nerve cell line, treatment with diamide, which causes oxidation of sulfhydryl groups but no increase in GSSG levels, results in a 19-fold increase in overall protein *S*-glutathionylation whereas, as noted earlier, treatment with hydrogen peroxide, which does increase GSSG levels, brings about a similar increase in overall protein *S*-glutathionylation (56).

Function. In considering possible functions for protein *S*-glutathionylation, it is important to remember that the reaction is reversible via a mechanism that requires Grx and is generally dependent on GSH (although see section on Glutaredoxin). A potential role for protein *S*-glutathionylation that is receiving increasing attention is as a signaling mechanism somewhat analogous to protein phosphorylation (26, 83, 87, 225). Although some evidence exists for this possibility, several critical questions remain to be addressed before it can be considered an important physiologic role for *S*-glutathionylation of proteins. Shelton *et al*. (225) proposed five criteria that must be met before protein *S*-glutathionylation can be viewed as *bona fide* regulatory mechanism for any protein. These criteria are as follows: (a) *S*-glutathionylation must change the function of the modified protein; (b) *S*-glutathionylation must occur within intact cells in response to a *physiologic* stimulus; (c) *S*-glutathionylation must occur at a relatively high (*i.e*., physiologic) GSH/GSSG ratio; (d) a rapid and efficient mechanism must exist for the formation of specific Pr-SSG; and (e) a rapid and efficient mechanism for reversing the *S*-glutathionylation must be present. No protein has yet met all of these criteria. In particular, very few have been shown to be *S*-glutathionylated under physiologic conditions. Furthermore, how *S*-glutathionylation could be regulated is unclear since regardless of which of the two mechanisms is responsible for the *S*-glutathionylation of a specific protein, the reactions do not appear to be enzyme mediated and thus may not have a high degree of specificity, although the specificity could lie in the microenvironment of the cysteine sulfhydryl group (83). In the test tube, Grx can specifically *S*-glutathionylate proteins, but whether it does so in cells remains to be determined (225).

Many investigators have suggested that *S*-glutathionylation serves a protective role, preventing the further oxidation of protein sulfhydryls to sulfinic or sulfonic acids or even the formation of disulfide bonds (107, 135, 248). Although recent studies suggest that some sulfinic acids can be reduced (see section on Peroxiredoxins), this does not appear to be a general phenomenon. Thus, the reaction of GSH with sulfenic acids would prevent their overoxidation and the resulting inactivation of the protein. This type of reaction would generally be expected to occur under conditions of oxidative stress. Although such a role for *S*-glutathionylation does not have to meet all of the criteria for a regulatory mechanism, it still should occur under physiologic forms of stress, and it should be reversible once that stress is removed. Another function for protein *S*-glutathionylation that pertains specifically to thiol-disulfide exchange is as a mechanism for the cell to deal temporarily with excess GSSG groups (83). Not all cysteine sulfhydryl groups are critical for protein function, and so the reaction of those groups with GSSG under conditions of oxidative stress could provide a means of preserving the intracellular redox potential without permanent loss of GSH, such as through its export in the form of GSSG.

Targets. Two general approaches have been taken to examine protein *S*-glutathionylation. One is to focus on specific proteins and ask whether they are *S*-glutathionylated under a condition of interest, such as oxidative stress. The second approach is to look at overall protein *S*-glutathionylation, again under a condition of interest. Several of these global proteomics approaches have been described (84, 87, 94, 156). The advantage of the first approach is that by focusing on a single protein of interest, it is generally possible to assess the functional role of *S*-glutathionylation. The advantage of the second approach is that it provides an overall picture of protein *S*-glutathionylation under a specific condition. The disadvantage is that it is difficult to determine much about function.

A variety of proteins have been shown to be *S*-glutathionylated in cells (84, 87, 222). Among these are protein chaperones, cytoskeletal proteins, cell-cycle regulators, and metabolic enzymes (156). Many of these are abundant proteins whose *S*-glutathionylation has been demonstrated only under quite severe conditions of oxidative stress, so that the functional significance of the *S*-glutathionylation is not always clear. However, a number of proteins have been shown to be *S*-glutathionylated either under basal conditions or in response to physiologically relevant stimuli, suggesting that *S*-glutathionylation could play a role in regulating the function of these proteins. Among these proteins are transcription factors and protein phosphatases, which are discussed later in their respective sections. A number of other proteins relevant to nerve cell function can also be regulated by *S*-glutathionylation, and these are discussed below.

An important signaling protein that is *S*-glutathionylated in a regulated manner is the small GTPase Ras. Ras modulates a variety of kinase-dependent signaling pathways (54) and has a reactive cysteine (Cys118) in its GTP-binding region (145). Several studies have shown that Ras is *S*-glutathionylated in response to stimuli such as angiotensin II treatment (2) and oxidized LDL (48). In both cases, *S*-glutathionylation of Cys118 of Ras results in Ras activation.

Another signaling molecule shown to be *S*-glutathionylated is the MAPKKK, MEKK1 (55). MEKK1, along with ASK1, phosphorylates the MAPKK MKK4, an activator of the MAPKs JNK and p38 MAPK. ASK1 activity is regulated by its interaction with Trx (see Redox Regulation of Protein Kinases) and is activated by oxidative stress. In contrast, MEKK1 is inactivated by oxidative stress–induced *S*-glutathionylation. This was shown both with purified MEKK1 and in intact cells and was confirmed by mass spectrometry. Curiously, activation of MEKK1 is associated with a prosurvival response, whereas activation of ASK1 is associated with cell death. Thus, the *S*-glutathionylation of MEKK1 in combination with the activation of ASK1 would result in a very clear-cut cell-death signal.

Several mitochondrial proteins can also undergo *S*-glutathionylation and may alter mitochondrial function (107). However, most of these studies were done with either purified proteins or isolated mitochondria, so whether *S*-glutathionylation of mitochondrial proteins occurs in intact cells and, if so, under what conditions, remains to be determined. Nevertheless, the existing evidence suggests that *S*-glutathionylation could regulate the activity of both TCA-cycle enzymes and the mitochondrial electron transport chain (ETC). Complex I of the ETC has been implicated in ROS production by mitochondria and is the target of chemicals implicated in PD, such as MPTP and rotenone (for review, see 3). Loss of complex I activity is also thought to contribute to idiopathic PD and to Huntington disease. Complex I contains ~45 different peptides, but only a few of these appear to contain redox active cysteine sulfhydryl groups (244). These sulfhydryls were shown to be *S*-glutathionylated after treatment of isolated complex I with GSSG and intact mitochondria with *t*-butyl hydroxide, which causes an increase in mitochondrial GSSG levels at the expense of GSH. The *S*-glutathionylation of these proteins correlated with an increase in the mitochondrial production of ROS, suggesting a mechanism whereby ROS production could be regulated by the cellular redox status. Hence, transient *S*-glutathionylation of complex I could be involved in mitochondrial signaling (39), whereas more-extended S-glutathionylation could play a role in the development of neurodegenerative diseases.

The activities of the TCA cycle enzymes α-ketoglutarate dehydrogenase (KGDH) and isocitrate dehydrogenase (ICDH) also appear to be regulated by *S*-glutathionylation (133, 193). In both cases, oxidizing conditions that favor *S*-glutathionylation resulted in a loss of enzyme activity, which was restored on removal of the oxidant or treatment with Grx. In the case of ICDH, similar changes were shown in both oxidant-treated cells and in the brains of mice treated with MPTP. Interestingly, *S*-glutathionylated ICDH appeared to be more resistant to proteases, suggesting a protective role for transient *S*-glutathionylation. However, ICDH can generate NADPH, which is essential for several enzymes involved in maintaining the redox status of cells, including GR and Trx. Therefore, the loss of ICDH activity itself could contribute to an increase in oxidative stress.

S-glutathionylation can also regulate the function of heat-shock cognate protein 70 (Hsc70) (101). Hsc70 is a constitutively expressed homologue of the heat-shock protein Hsp70 that acts as a protein chaperone. Although several previous screens for *S*-glutathionylated proteins in cells exposed to oxidative stress identified Hsc70 (84, 156), the effect of *S*-glutathionylation on protein function was not determined. Interestingly, *S*-glutathionylation significantly enhances the ability of Hsc70 to prevent protein aggregation. However, this enhancement is no longer apparent in the presence of ATP. These results suggest that *S*-glutathionylation of Hsc70 could play an important role in protecting cells from moderate levels of stress that lead to reduction of ATP levels, such as those that occur after ischemia.

Evidence for an interaction between the GSH and Trx systems was found in studies showing that Trx is *S*-glutathionylated in cells treated with diamide, resulting in a significant reduction in Trx activity (35). The glutathionylated sulfhydryl, Cys72, is also involved in the dimerization of Trx, consistent with the reactivity of this thiol. However, whether *S*-glutathionylation of Trx occurs in response to a physiologic stress or other stimuli is not known.

A recent study used immunoprecipitation and Western blotting with anti-GSH antibodies to identify *S*-glutathionylated proteins in the CNS under basal conditions and after oxidative stress (234). Actin, β-tubulin, and the light chain of neurofilaments were found to be constitutively *S*-glutathionylated, and this *S*-glutathionylation increased under oxidative stress. All three of these proteins are essential for nerve cell physiology, suggesting that reversible *S*-glutathionylation could play a role in modulating nerve cell functions both under basal conditions and after oxidative stress. This hypothesis is supported by studies showing that actin polymerization is regulated by *S*-glutathionylation both in the test tube and in cells (see later). The role of S-glutathionylation in regulating the polymerization of tubulin or neurofilaments remains to be determined.

Reversible *S*-glutathionylation of actin regulates actin polymerization (58, 71). *S*-glutathionylated actin is impaired in its ability to polymerize because of changes in its conformation induced by *S*-glutathionylation. *S*-glutathionylation may also destabilize F-actin. A physiologic role for actin *S*-glutathionylation was shown in EGF-treated A431 cells (256). EGF treatment resulted in the deglutathionylation of G-actin, probably by Grx, and an increase in F-actin at the cell periphery. In nerve cells, increases in actin *S*-glutathionylation may play a role in the neurodegeneration seen in the autosomal recessive disease, Friedreich ataxia (199).

Tyrosine hydroxylase, the initial and rate-limiting enzyme in the biosynthesis of dopamine, has also been found to undergo *S*-glutathionylation in response to treatment of PC12 cells with diamide, and this is correlated with a loss of enzyme activity (28). In the test tube, the loss of TH activity could be partially reversed by treatment with Grx2. However, whether a physiologic oxidative stress also results in *S*-glutathionylation of TH and loss of activity remains to be determined. Nevertheless, it is an intriguing possibility that the oxidative stress seen in PD leads directly to a decrease in TH activity through specific modification of TH sulfhydryl groups.

Although a number of other proteins are *S*-glutathionylated, many of these were shown to be so only in the test tube, and/or their relevance to nerve cell physiology is not clear. These proteins are not discussed further here, although the interested reader is advised to consult several reviews (84, 87, 135, 222) for comprehensive lists.

Protein S-nitrosylation

Protein cysteine sulfhydryl groups can also be modified by *S*-nitrosylation. This can occur through the interaction of the sulfhydryl either with an "activated" form of nitric oxide (N_2O_3, NO^+, $^{\bullet}NO$) or with a previously formed low-molecular-weight *S*-nitrosothiol such as *S*-nitrosoglutathione (GSNO) (for reviews, see 87, 168). *S*-nitrosylation is thought to be a very labile modification under physiologic conditions, generally leading either to the formation of disulfide bonds if a nearby cysteine sulfhydryl is available or to an *S*-

glutathionylated protein. Research on protein *S*-nitrosylation as it relates to neuronal function was pioneered by Stuart Lipton's laboratory, which showed that *S*-nitrosylation of the NMDA receptor results in its inhibition (157). The inhibition is due mainly to the *S*-nitrosylation of a cysteine sulfhydryl located in the linker region that separates the two domains of NR2A, thereby stabilizing the receptor in a "closed" conformation. Further studies on *S*-nitrosylation were stimulated by the development of a relatively simple method, the biotin-switch assay, for measuring protein *S*-nitrosylation in cell and tissue extracts (114). In this assay, nitrosylated cysteine sulfhydryls are converted to biotinylated cysteine sulfhydryls and then either analyzed with SDS–PAGE/immunoblotting with antibodies to biotin or isolated by immunoprecipitation/column chromatography with streptavidin and then analyzed with SDS-PAGE. Specifically, free cysteine sulfhydryls are first blocked with a thiol-specific methylating agent, and then nitrosothiol bonds are selectively decomposed to the free sulfhydryl by treatment with ascorbate. The newly formed free sulfhydryls are reacted with a biotinylating agent to tag the sites of *S*-nitrosylation. This assay has been used to demonstrate protein *S*-nitrosylation of a number of proteins under a variety of physiologic and pathophysiologic conditions (*e.g*., 46, 218). The assay relies on the assumption that ascorbate is a selective reductant of all protein *S*-nitrosothiols. However, a very recent article raises significant doubts about the validity of this assumption (146), thereby calling into question many of the observations made by using this technique. Landino *et al*. (146) showed that ascorbate can also reduce disulfide bonds, suggesting that, at least in some cases, the biotin-switch technique could pick up disulfide-bonded proteins rather than *S*-nitrosylated proteins. This conclusion is supported by the observation that treatment with NO can lead to disulfide bond formation (157) subsequent to *S*-nitrosylation. It will be important to clarify this point in future studies on protein *S*-nitrosylation.

In summary, modification of protein cysteine sulfhydryl groups can occur through multiple mechanisms and, depending on the protein, can result in either the activation or the inhibition of protein function. How the different mechanisms are regulated and whether specificity exists are key areas for future research. In addition, it will be important to evaluate these processes in response to physiologic stimuli, as most studies continue to be done on cells treated with high doses of various oxidants.

GLUTAREDOXINS

The glutaredoxins (Grxs) are the enzymes that are generally considered to be responsible for the removal of GSH from glutathionylated proteins (Fig. 6) (for reviews, see 77, 183). Of the two mammalian Grxs, Grx1 is localized to the cytoplasm, and Grx2 localizes to both mitochondria and nuclei. The structure and catalytic site of Grx are similar to those of Trx, but the mechanism of protein-GSH reduction is distinct from the Trx-dependent reduction of protein disulfides. The N-terminal cysteine of the redox active pair in the active site CXXC attacks the *S*-GSH link in an *S*-glutathionylated protein, yielding a GSH-Grx intermediate that is then reduced by GSH, regenerating Grx and producing GSSG (Fig. 6). Because GSH is required for the reduction of Grx, Grx activity is dependent on both GR and NADPH. In contrast, Grx2, which has only 34% sequence homology with Grx1, can by reduced by either GSH or Trx (118). It has been suggested (225) that Grxs can also glutathionylate proteins by using a GS-radical. Although *in vitro* evidence exists for this with specific proteins, it is not yet clear that it occurs *in vivo*.

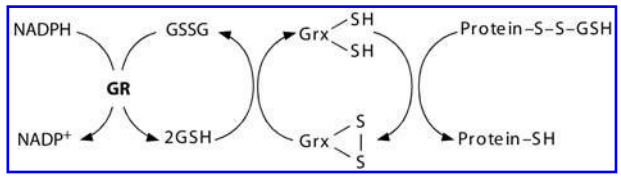

FIG. 6. Glutaredoxins. Glutaredoxins specifically reduce *S*-glutathionylated proteins. Oxidized glutaredoxin is then reduced by GSH. The GSSG produced in this reaction is reduced by the action of glutathione disulfide reductase (GR) at the expense of NADPH.

Although not a great deal is known about Grx in the CNS in terms of distribution, interesting data suggest that Grx can play an important role in maintaining nerve cell function in the presence of oxidative stress. Ravindrath *et al*. (130,131) have studied Grx1 in the context of specific insults to the CNS. For example, after treatment of mice with the excitotoxic amino acid BOAA, they found a time-dependent decrease in GSH levels and a concomitant increase in *S*-glutathionylated protein levels in both the motor cortex and the lumbosacral segment of the spinal cord (130). This correlated with a time-dependent decrease in the activity of the mitochondrial ETC complex I and a delayed increase in the expression and activity of Grx1. Over the time course of the experiment (4 hr), GSH levels and complex I activity recovered in the motor cortex but not in the lumbosacral segment of the spinal cord, and this recovery correlated with a decrease in protein *S*-glutathionylation to control levels. Recovery of complex I activity was prevented by treatment with antisense oligonucleotides directed against Grx1. In a similar set of experiments, the same group looked at the activity of complex I and Grx1 after treatment of mice with a single dose of the neurotoxin MPTP (131). Complex I activity in the striatum decreased shortly after treatment with MPTP but then rebounded by 4 hr in concert with an increase in Grx1 activity. The restoration of complex I activity was blocked if the mice were treated with antisense oligonucleotides against Grx1. Together these data support the hypothesis that Grx1 activity is critical for restoring complex I activity after protein *S*-glutathionylation as a consequence of mild oxidative stress. The data are also consistent with the idea that protein *S*-glutathionylation can modulate complex I activity (see Protein *S*-Glutathionylation) and may serve as a mechanism to protect proteins from irreversible inactivation by oxidative stress.

Unlike Trx, Grxs are enzymes whose study is in its infancy. Because they appear to play an important role in maintaining nerve cell function, further investigation of their distribution in the CNS, the regulation of their expression, and the modu-

lation of their activities under various pathophysiologic conditions seems warranted.

METHIONINE SULFOXIDE REDUCTASES AND THE REDOX MODIFICATION OF PROTEIN METHIONINE RESIDUES

The sulfur-containing amino acid methionine is very sensitive to oxidation. Similar to cysteine oxidation, the oxidation of methionine to methionine sulfoxide is reversible, although further oxidation to methionine sulfone is not. The reduction of methionine sulfoxide is dependent on a family of enzymes, the methionine sulfoxide reductases (Fig. 7) (for reviews, see 102, 153, 169, 259). Similar to the oxidation of cysteine, the oxidation of methionine can have profound effects on protein structure and function. The methionine side chain is long, flexible, and nonpolar, but when oxidized, it becomes stiffer and more polar. The hydrophobicity index of methionine sulfoxide was estimated to be similar to that of the positively charged amino acid lysine. Thus, as outlined later, the oxidation/reduction of methionine residues may provide another mechanism for the regulation of protein function. In addition, under conditions in which reduction is impaired, methionine oxidation could lead to loss of protein function. It has also been proposed (153) that the oxidation/reduction of noncritical, surface-exposed methionines could have an antioxidant function.

Oxidation of methionine leads to a diastereomeric mixture of methionine-*S*-sulfoxide and methionine-*R*-sulfoxide. Two distinct families of enzymes reduce these sulfoxides in mammals. The first of these families to be characterized contains a single, cytoplasmic enzyme of ~25 kDa called methionine-*S*-sulfoxide reductase (MsrA), which catalyzes the reduction of methionine-*S*-sulfoxide (259). The reduction of oxidized MsrA is carried out by thioredoxin reductase with NADPH as a co-factor, thus tying MsrA into several of the major redox couples in the cell. The more recently discovered MsrB family contains at least three members with molecular masses between 12 and 20 kDa. These enzymes catalyze the reduction of methionine-*R*-sulfoxide (134). MsrB1 (also known as SelR), the family member with the highest specific activity due to a selenocysteine group in the active site, localizes to both the cytoplasm and nucleus. MsrB2 (also known as Cbs-1) contains cysteine instead of selenocysteine in the active site and is found in mitochondria. MsrB3 can exist in two forms because of alternative splicing; MsrB3A is found mainly in the ER, whereas MsrB3B localizes to mitochondria. Although MsrB2 is detected in all human tissues examined, MsrB1 is not found in brain. The tissue distribution of MsrB3 is not known.

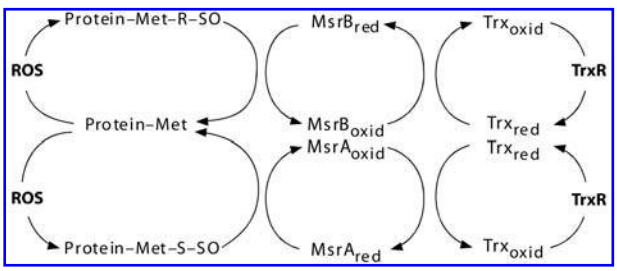

FIG. 7. Methionine sulfoxide reductases. Oxidation of methionine residues in proteins by reactive oxygen species (ROS) yields a diastereomeric mixture of methionine-*S*-sulfoxide (protein-Met-*S*-SO) and methionine-*R*-sulfoxide (protein-Met-*R*-SO). A single methionine sulfoxide reductase-A (MsrA) reduces Met-*S*-SO, and a family of methionine sulfoxide reductase-B enzymes (MsrB) reduces Met-*R*-SO. The Msrs are in turn reduced by the thioredoxin (Trx)/thioredoxin reductase (TrxR) system.

Because MsrA was the first methionine sulfoxide reductase to be identified, quite a bit is known about it. Although MsrA can be detected in all tissues that have been examined, the highest levels are found in liver, kidney, and brain. Within the brain, expression, as determined by immunostaining, is significantly higher in neurons than in glial cells (180). The highest levels were seen in the cell bodies and dendrites of neurons in the cerebellum, olfactory bulb, substantia nigra, CA1 region of the hippocampus, pontine reticular nucleus, medulla oblongata, and cerebral cortex, as well as in the large neurons of the spinal cord. An important role for MsrA in CNS function has been suggested by studies in which the enzyme was either overexpressed or knocked out. MsrA knockout mice develop a form of ataxia starting at ~6 months of age and also have a 40% decrease in maximal life span (179). Consistent with an antioxidant role for MsrA, its elimination led to an increase in protein carbonyl levels in brain and other tissues after exposure to hyperoxia, as well as a more rapid rate of death. Overexpression of MsrA predominantly in the nervous system of *Drosophila* led to an ~70% increase in median life span, as well as a delayed onset of the senescence-induced decrease in physical activity (211). The flies were also more resistant to oxidative stress generated by feeding them paraquat. In addition, MsrA overexpression in PC12 cells (271) protected the cells from ischemia. Curiously, whereas MsrA overexpression reduced ROS production in the PC12 cells, it had no effect on the overall levels of protein methionine sulfoxide. Together, these data suggest that methionine oxidation/reduction plays an important role in the maintenance of normal CNS function. However, it is not yet clear whether this is due predominantly to antioxidant activity or to specific roles in regulating protein function. Most likely, as suggested by the studies with PC12 cells as well as those described below, it is a combination of effects.

Methionine oxidation has been shown to play a role in regulating several proteins involved in nerve cell function. Among these proteins are specific voltage-dependent K^+ channels. These channels open in response to depolarization and are then inactivated. The kinetics of inactivation plays an important role in determining cellular excitability. Hoshi and co-workers (47) showed that the oxidation of a methionine in the N-terminal domain of the Shaker ShC/B voltage-dependent K^+ channel disrupts its inactivation. In contrast, methionine oxidation appears to enhance the inactivation of a different type of K^+ channel (102).

Another protein whose function is modified by methionine oxidation is the calcium-binding protein calmodulin (CaM) (25). Nine of the 148 amino acids in calmodulin are methion-

ines, a very high number compared with most other proteins of its size. The methionines are clustered in the N- and C-terminal domains, creating Met-rich hydrophobic patches that mediate productive interactions between Ca^{2+}–CaM and a large group of Ca^{2+}-regulated proteins. However, because the methionines are on the surface of CaM, they also are subject to oxidation. Oxidation leads to a decrease in the ability of CaM to activate specific proteins such as the plasma membrane Ca^{2+}–ATPase (238). Interestingly, CaM isolated from the brains of very old rats (27 months) shows a ~50% decrease in its ability to activate the plasma membrane Ca^{2+}–ATPase, and this activity can be restored by treatment with MsrA (25). These results are consistent with the decreases in MsrA activity seen in the brains of very old rats (202) and support the idea of a critical role for MsrA in maintaining brain function during aging.

In summary, much tantalizing evidence suggests that methionine oxidation and its regulation by the Msrs plays an important role in the maintenance of brain function. However, much work remains before the exact nature of this function is clearly established. The development of additional tools, such as antibodies specific to methionine sulfoxide, would prove very useful in stimulating this research.

REDOX REGULATION OF PROTEIN PHOSPHATASES

A major mechanism for the activation of kinase-dependent signaling pathways by changes in the intracellular redox state is through the inhibition of specific phosphatases. This is because kinases are activated by phosphorylation, but phosphatases generally have an ~10 times faster reaction rate than kinases, so normally the dephosphorylated and therefore inactive state of the kinase predominates. Three major classes of phosphatases are found in cells: tyrosine phosphatases (PTPs), dual-specificity (Thr/Tyr) phosphatases (DSPs), and serine/threonine phosphatases (PPs). The best-understood effects of redox state on phosphatase activity are on the PTPs. The ~40 different PTPs serve a variety of functions within cells (for reviews, see 42, 45, 62, 207, 213). These can be divided into three classes: cytoplasmic PTPs (*e.g.*, PTP1B, SHP-2); receptor-like PTPs (*e.g.*, CD45, LAR, RPTPα) and low-molecular-weight (LMW)-PTPs (*e.g.*, LMW-PTP). All of these PTPs have a conserved, catalytic cysteine that exists within the signature motif of Cys-Xaa_5-Arg. Because of the nature of this motif, the cysteine sulfhydryl has a pKa of ~5.0–6.7 and therefore exists as the thiolate anion at physiologic pH. This cysteine sulfhydryl group directly participates in the dephosphorylation reaction by acting as the attacking nucleophile; thus its modification renders the PTP inactive. Because of its low pKa, the sulfhydryl is also very sensitive to the redox status of the cell. Direct evidence that PTPs were major targets of oxidants first came from studies done in the laboratory of Denu (64), which showed that a variety of different PTPs could be directly and rapidly inactivated by treatment with low concentrations of hydrogen peroxide. This treatment resulted in the conversion of the essential catalytic cysteine to a sulfenic acid intermediate. Because sulfenic acids tend to undergo further oxidation to sulfinic and sulfonic acids, which are generally not reversible (but see Peroxiredoxins), it is important that they undergo some form of stabilization for the cysteine sulfhydryl to function as a redox-regulated switch. As described later, the oxidized forms of the different PTPs are stabilized by distinct mechanisms that are dependent on the structure of the specific PTP (Fig. 8).

PTP1B is the prototypic member of the PTP family, and the most is known about its redox regulation in cells. A number of studies have shown PTP1B can be reversibly inactivated by ROS (probably hydrogen peroxide) generated in cells in response to stimulation of the cells with growth factors such as EGF or insulin (for reviews, see 207, 213). Interestingly, treatment of cells with either EGF or insulin inhibits only a portion of the PTP1B, whereas treatment with hydrogen peroxide inhibits a much higher percentage of the phosphatase activity. These data suggest that growth factor treatment might target a specific pool of PTPs that are particularly involved in regulating signaling via the growth factor, perhaps as part of a larger complex. Although these studies were done with fibroblasts and epithelial cells, EGF can also interact with specific classes of nerve cells, where it has been implicated in both neurotrophic activity (113) and neurogenesis (75, 203), as well as signaling by G protein–coupled receptors (223). However, the role of redox regulation of EGF signaling in nerve cells is currently unknown. The stabilization of the sulfenic acid of the catalytic cysteine sulfhydryl of PTP1B is mediated by its conversion to a stable cyclic sulfenylamide (S-N-R) via its intramolecular interaction with the protein chain amide nitrogen of an adjacent Ser residue (213, 229). This novel modification triggers changes in the PTP1B catalytic site and is thought both to stabilize the cysteine sulfhydryl against overoxidation and to facilitate its reduction by thiols such as GSH. The catalytic cysteine sulfhydryl can also be glutathionylated. It is not yet clear whether the formation of the sulfenamide bond, glutathionylation, or a combination of the two is the relevant mechanism for preserving the catalytic cysteine sulfhydryl in cells.

The activity of the cytoplasmic PTP SHP-2 is also regulated by treatment of cells with growth factors, particularly PDGF and EGF (42, 213). Inhibition of PTP activity is confined to a small pool of SHP-2 that appears to be specifically associated with the growth-factor receptor. More recently, by using the biotin switch assay (see Protein *S*-Nitrosylation), SHP-1 and SHP-2 were shown to be *S*-nitrosylated in response to mild oxidative stress induced by low doses of ionizing radiation or low concentrations of hydrogen peroxide (21). As with any modification of the catalytic cysteine sulfhydryl, this resulted in inactivation of the PTPs.

Receptor-like PTPases (RPTs) play important signaling roles in axon outgrowth and guidance (for review, see 235). In addition, PTPα is a positive regulator of Src family tyrosine kinases (197), some of which modulate nerve cell function (124). In contrast to the cytoplasmic PTPs, the RPTPs contain two intracellular PTP domains (62). The domain closest to the plasma membrane (D1) is catalytically active, whereas the more distal PTP domain (D2) is catalytically inactive despite the presence of the signature PTP Cys-Xaa_5-Arg motif. Recent evidence suggests that the PTP motif of the D2 domain is sig-

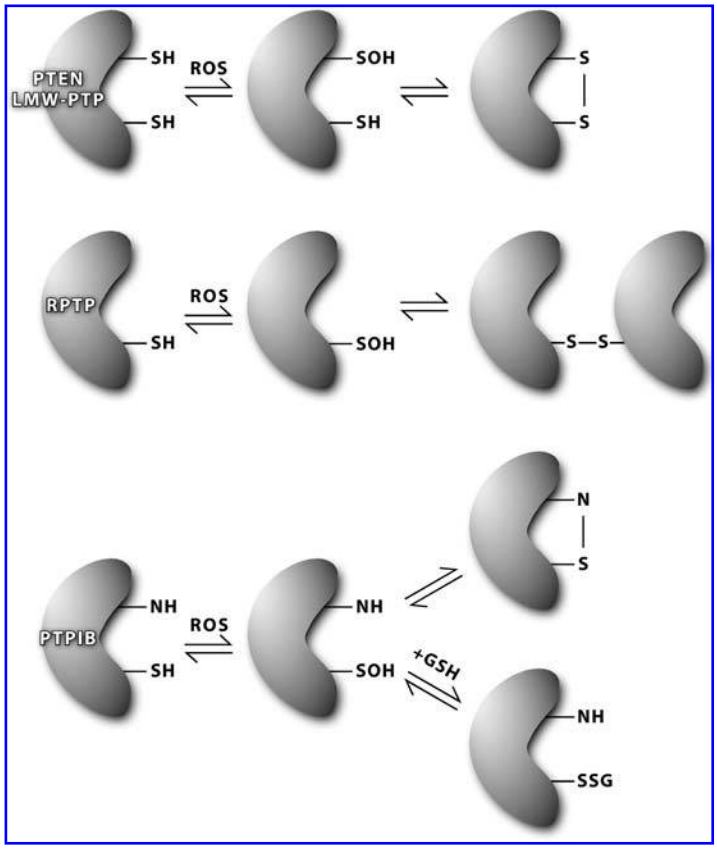

FIG. 8. Regulation of protein tyrosine phosphatases (PTPs) by oxidants. Oxidation of the catalytic cysteine in the active site of PTPs results in the formation of a cysteine sulfenic acid (SOH) intermediate that can be stabilized by several different mechanisms. In the case of PTEN and LMW-PTP, a disulfide bond is formed with a neighboring cysteine sulfhydryl group. In contrast, in RPTP and other receptor PTPs that contain two PTP domains, the cysteine sulfhydryl that tends to be oxidized is in the regulatory domain, and it forms a disulfide bond with a neighboring RPTP, resulting in dimer formation. Oxidation of the catalytic cysteine sulfhydryl in PTP1B leads to the formation of both a sulfenamide (S-N) and *S*-glutathionylation (SSG).

nificantly more sensitive to oxidation than the D1 domain and appears to act as a redox sensor to regulate the activity of this class of PTPs. Oxidation of the D2 domain results in the formation of inactive, RPTP dimers. Whether this mechanism actually regulates RPTP activity in cells in response to physiologic stimuli remains to be determined.

The LMW-PTPs are ~18-kDa PTPs that participate in regulating growth-factor signaling via the dephosphorylation of receptors for PDGF, insulin, and ephrins (45). They also play a role in regulating cell spreading and lamellipodia formation (43). Only a single LMW-PTP has so far been identified in humans. Oxidation of the catalytic cysteine sulfhydryl both *in vitro* and in cells leads to the formation of a readily reversible disulfide bond with a nearby cysteine sulfhydryl. The formation of this bond is essential for preserving enzyme activity because a mutant lacking the nearby cysteine does not regain activity after ROS-mediated inactivation, whereas the wild-type protein regains full activity within 40 min (213). Clearly, this PTP could play an important role in the redox regulation of nerve cell function.

PTEN functions as both a protein phosphatase and a lipid phosphatase, and it is probably best known for this latter role (for reviews, see 45, 152, 207). PTEN catalyzes the removal of phosphate attached to the 3′-hydroxyl group of phosphatidylinositol 3,4,5-trisphosphate, thereby negatively regulating the PI3K–Akt signaling pathway. Mutations in PTEN cause human tumors in a number of tissues. Similar to LMW-PTP, on oxidation, the catalytic cysteine sulfhydryl of PTEN forms a disulfide bond with a neighboring cysteine sulfhydryl. Evidence exists that this disulfide bond is reduced by thioredoxin. In PC12 cells, the redox-regulated inactivation of PTEN was shown to increase their susceptibility to oxidative stress (187).

The dual-specificity phosphatases (DSPs) can be divided into two major classes: those that act specifically on the terminal members of the MAPK family (*e.g.*, ERKs, JNKs, and p38 MAPKs) (for review, see 132) and the cell-cycle phosphatases of CDKs (*e.g.*, Cdc25). More than 60 different DSPs are found in mammalian cells, and, similar to the PTPs, all contain an essential cysteine in their active sites that has been shown to undergo the same type of regulation in response to hydrogen peroxide or other oxidants as PTPs (213). In general, the oxidized cysteine sulfhydryl in the DSPs is stabilized by disulfide bond formation, similar to LMW-PTP and PTEN (45).

Unlike the PTPs, PPs do not contain an essential cysteine residue at their active sites, so that the mechanisms underlying their regulation by oxidants are likely to be different. Indeed, even the oxidants that can and cannot inactivate these phosphatases are still unclear. The observation that superoxide dismutase could protect calcineurin (PP2B) from inactivation (257) suggested that the iron in the active site of calcineurin could be oxidized by superoxide. Similar to the other PPs, calcineurin contains a pair of metal ions (iron and zinc) in its active site that are important for both catalytic activity and the structural integrity of the phosphatase (63). More recent studies have shown that superoxide-mediated inactivation of calcineurin is Ca^{2+}/CaM dependent (254). Ca^{2+}/CaM

binding leads to the activation of calcineurin via the opening of the enzyme active site, allowing access both of substrates and superoxide. Superoxide causes oxidation of the ferrous iron group in the active site, yielding ferric iron and thereby rendering the phosphatase inactive. Calcineurin plays an important role in nerve cells, where it participates in the processes of synaptic plasticity, learning and memory, gene transcription, and apoptosis. Thus, redox regulation could alter these processes through effects on calcineurin. Because both PP1 and PP2A also have pairs of metal ions in their active sites that are important for catalytic activity, it is possible that they could be inactivated by superoxide by a mechanism similar to that described earlier for calcineurin. In addition, both PP1 and PP2A are inactivated by reaction with GSSG (186). Because GSSG interacts with cysteine sulfhydryl groups (see Redox Modification of Protein Cysteine Sulfhydryls), these data suggest that a cysteine outside the active site but within the catalytic domain of these phosphatases is critical for enzyme activity and can be reversibly modified in a manner dependent on the redox status of the cell. Thus, PPs may be redox regulated by mechanisms similar to those seen in PTPs, as well as through mechanisms specific to their metal ions.

More recently, the activation of Ca^{2+}/CaM-dependent protein kinases II and IV (CaMKII and CaMKIV) in cells by both hydrogen peroxide and the PKC activator, phorbol 12-myristate 13-acetate, was shown to be mediated by the oxidative inactivation of PP2A (104). Because CaMKII, CaMKIV, and PP2A play important roles in nerve cells and have been implicated in synaptic plasticity, learning, and memory, these studies provide additional support for redox regulation of these critical CNS functions (for reviews, see 100, 158, 167, 182).

Overall, these data indicate that phosphatases are important redox active proteins whose regulation by oxidants can be quite specific when it is coupled to growth-factor or cytokine signaling. In contrast, a broader spectrum of phosphatase inactivation would be expected in the presence of higher levels of oxidants and will likely depend on both the nature of the oxidizing agent and its interactions with the various detoxifying systems within the cell. The ultimate consequences of more global phosphatase inactivation on cellular function are not clear at this time but are likely to be cell-type specific because they will depend on the spectrum of kinase pathways that are affected. However, evidence exists that this inactivation could play a role in several neurodegenerative diseases and could even be involved in the more subtle cognitive defects that occur as part of the normal aging process.

REDOX REGULATION OF PROTEIN KINASES

Oxidants have been shown to increase the activity of both protein tyrosine kinases (PTKs) and serine/threonine kinases. However, in many cases, it is still not clear whether this is a direct effect on the kinase itself, an effect on an upstream activator, or an effect on downstream protein phosphatases (see Redox Regulation of Protein Phosphatases) (42). Both receptor and nonreceptor PTKs can be activated by oxidants. In both cases, the activation often appears to be due to an effect on downstream phosphatases. For example, the treatment of cells with a variety of agents, including oxidants such as hydrogen peroxide, induces tyrosine phosphorylation of the EGF receptor (EGFR) and PDGF receptor (PDGFR) by a mechanism involving the inhibition of dephosphorylation (138) rather than a direct effect on kinase activity.

A number of nonreceptor tyrosine kinases such as c-Src (65) and c-Lck (96, 184) are activated by the treatment of cells with hydrogen peroxide or other oxidants, as determined both by increases in protein tyrosine phosphorylation and *in vitro* kinase activity. However, in the case of c-Lck, this activation could not be mimicked by direct treatment of the kinase with hydrogen peroxide (96), suggesting that the effects were probably mediated via the inhibition of phosphatase activity. Similarly, treatment of cells with hydrogen peroxide as well as adhesion of cells to the extracellular matrix stimulated the tyrosine phosphorylation of the focal adhesion kinase, FAK, via inhibition of LMW-PTP (see Redox Regulation of Protein Phosphatases) (43). In contrast, a recent study showed that c-Src is oxidized in response to cell attachment to the extracellular matrix and that this oxidation leads to both an increase in tyrosine kinase activity and an activation of downstream signaling pathways (43). c-Src, as well as other Src family kinases, is highly expressed in nerve cells, where it has been implicated in proliferation and differentiation during development, as well as in regulating the activity of NMDA receptors in mature neurons (124). Thus, redox regulation of c-Src could have multiple effects on nerve cell function.

Another mode of PTK regulation by oxidants was shown in studies on the receptor PTK c-Ret (43). UV irradiation of cells expressing c-Ret resulted in the dimerization and activation of many of the membrane-bound c-Ret molecules. This dimerization was mediated by the formation of disulfide bonds between cysteine sulfhydryls in adjacent c-Ret monomers. Some evidence indicates that the activity of the PDGFR may be regulated by the redox status of the cell. Rigacci *et al.* (208) showed that the cellular GSH level specifically affected the tyrosine phosphorylation of PDGFR in fibroblasts. These effects of oxidants on PTK phosphorylation and/or activity may be cell-type dependent. Although the consequences to the cell of PTK activation by oxidants is still not entirely clear, in some cases, it may be part of a protective response. For example, UV irradiation of HeLa cells results in the oxidant-dependent activation of c-Src, and inhibition of c-Src activity potentiates cell killing by UV (65).

Oxidants also affect the activities of serine/threonine kinases. Perhaps the best known of these are members of the mitogen-activated protein kinase (MAP kinase) family. These serine-threonine kinases are activated by dual phosphorylation in response to a variety of extracellular stimuli. In mammalian cells, at least three distinct members of the MAP kinase family are expressed: ERKs (also known as MAPKs), stress-activated protein kinase [also known as c-Jun NH2-terminal kinase (JNK)], and p38 MAPK. The standard pathways for activation of these protein kinases consist of two upstream protein kinases: MAPKKKs, which phosphorylate and thereby activate MAPKKs, which phosphorylate the MAPKs on both a threonine and a tyrosine residue (for reviews, see

49, 143, 210, 216, 258). All three members of this family, including ERKs (12, 91), can be specifically activated by hydrogen peroxide and other oxidants, as determined both by dual phosphorylation and *in vitro* kinase assays. The specific oxidants that lead to ERK activation may be cell-type dependent. In some cells, activation is seen with hydrogen peroxide (91), whereas in others, it is seen with superoxide but not hydrogen peroxide (12). In some cases, ERK activation by hydrogen peroxide appears to be indirect because it can be blocked by a dominant negative Ras mutant, suggesting that ROS activate an upstream member of the prototypical Ras–ERK cascade. Indeed, a number of reports suggest that Ras is responsive to the redox status of the cell and can be directly activated by oxidants such as hydrogen peroxide (145) or nitric oxide (144) as well as by *S*-glutathionylation (see Protein *S*-Glutathionylation). In other cells, ERK activation appears to be mediated by inhibition of dual-specificity phosphatases (149).

Several alternative mechanisms have been identified for the oxidant-induced activation of the two other members of the MAPK family, JNK and p38 MAPK. JNK is generally thought to be activated through a pathway involving small GTP-binding proteins and a series of protein kinases terminating in the dual-specificity kinases, MKK4 and/or MKK7. However, certain stimuli, including oxidative stress induced by a variety of agents, activate JNK not through this cascade but via inactivation of the phosphatases, which generally maintain this kinase in a dephosphorylated and therefore inactive state (174).

A second mechanism for the activation of both JNK and p38 MAPK by oxidative stress has also been described. ASK1 is an MAPKKK that can stimulate pathways leading to both p38 and JNK activation (212) and is implicated in the promotion of programmed cell death (89, 212). ASK1 activity in turn is regulated by the redox status of the cell. A rather complicated mechanism underlies this regulation (for review, see 237). Trx associates with the N-terminus of ASK1 in a redox-sensitive fashion, resulting in inactivation of the kinase. Exposure of cells to oxidants results in the oxidation of Trx and its release from ASK1. This is followed by the homodimerization and phosphorylation of ASK1, thereby leading to the activation of JNK and p38 MAPK. Recent studies have identified two additional negative regulators of ASK1: Grx (232) and glutathione transferase M1 (GSTM1) (252). Grx interacts with the C-terminal domain of ASK1 (232), whereas GSTM1 interacts with the N-terminal domain (252). Both interactions appear to be redox sensitive. A third mechanism for the regulation of JNK activity involves another member of the glutathione transferase family. Glutathione transferase π (GSTπ) was identified as a specific inhibitor of JNK (4, 252). An increase in ROS levels in cells led to GSTπ oligomerization, dissociation of the GSTπ–JNK complex, and an increase in JNK activity. Furthermore, fibroblasts from GSTπ-null mice had high levels of basal JNK activity relative to their normal counterparts, which could be reduced by transfection with GSTπ.

The PKC family is a heterogeneous group of phospholipid-dependent serine/threonine kinases that are thought to be key elements in signaling pathways that regulate a wide range of cellular functions, including nerve cell survival (for reviews, see 106, 115, 176, 190). The PKC family contains 11 different members, which can be divided into three groups on the basis of structure and cofactor requirements. All PKCs contain an N-terminal, zinc finger–containing regulatory domain and a C-terminal catalytic domain that are separated by a flexible hinge. PKCs are maintained in an inactive state by the interaction of a pseudosubstrate sequence in the regulatory domain with the catalytic domain. Activation of PKCs is triggered by a conformational change that results in the release of the pseudosubstrate sequence from the catalytic domain. Phosphorylation of PKC also plays a role in its activation. Both the regulatory domains and catalytic domains contain cysteine-rich regions that have been shown to be targets of redox regulation (for review, see 88). Consistent with the mechanism of PKC activation, oxidative modification of the two pairs of cysteine-containing zinc fingers in the regulatory domain results in the release of zinc, loss of the zinc-finger conformation, and PKC activation. In contrast, oxidation of reactive cysteine sulfhydryl groups in the catalytic domain leads to inhibition of PKC activity. The regulatory domain appears to be more sensitive to redox regulation than the catalytic domain, so that low concentrations of oxidants cause PKC activation, whereas higher concentrations lead to inhibition of PKC activity (88, 155).

Short-term treatment of cells with hydrogen peroxide and other oxidants can also activate all members of the PKC family through tyrosine phosphorylation rather than oxidation (139). Although this effect of oxidants on PKCs is obviously indirect, it is still not clear whether it is mediated by activation of upstream PTKs or inhibition of downstream phosphatases, or both. Different members of the PKC family have been implicated in both protection and promotion of nerve cell death in response to a variety of oxidative insults (*e.g.*, 30, 51, 61, 66, 165). Whether different PKC isoforms are differentially activated/inhibited by oxidants remains to be determined but could affect whether a specific insult induces cell death. For example, PKCδ has been implicated in nerve cell death in a number of studies (30), whereas PKCγ and PKCε are generally thought to be involved in protection from death (66, 155). If a certain dose of a specific oxidant preferentially activated PKCδ but inhibited PKCγ, then it might be expected to promote cell death, whereas if the opposite were true, it could lead to protection. This type of process could be involved in preconditioning (*e.g.*, 204), although further research is needed to confirm this idea.

In conclusion, a number of protein kinases that play important roles in nerve cell physiology can be regulated by changes in the redox environment. How this affects the cell is likely to be dependent on the cell type, the specific stimulus, and the basal redox environment of the cell. Further studies that assess all of these factors will be needed to draw more specific conclusions regarding this aspect of redox regulation on nerve cell function.

REDOX REGULATION OF TRANSCRIPTION FACTORS

A number of different transcription factors can be regulated by the redox environment of the cell. Most of these transcription factors have cysteine sulfhydryl groups as the regu-

latory elements, but several additional mechanisms have been identified. These mechanisms include both direct effects on the transcription factors themselves and indirect effects mediated by either regulatory binding partners or upstream signaling pathways. Furthermore, at least in some cases, the regulation appears to be specific to distinct treatments that can perturb the cellular redox environment. The following paragraphs detail a number of these redox-dependent mechanisms and the transcription factors that they affect. A separate section describes research on the antioxidant response element and its transcription factor Nrf2. Not only is the activity of Nrf2 redox regulated, but the ARE is found in the promoters of a number of genes that are important in maintaining the redox balance of cells including GCL, Trx, and Prxs.

The regulation of the transcription factor AP1 by oxidants is quite complex and highly dependent on the context in which it is examined. However, the studies with this transcription factor provide an excellent example of some of the confusion in the field, as well as the way that the experimental paradigm affects the outcome. In addition, recent work on the regulation of AP1 by the intracellular redox environment points to a novel mechanism for transcription-factor regulation. More details on AP1 regulation by oxidants can be found in a number of reviews (*e.g.*, 5, 126, 159, 221, 242).

AP-1 is not a single transcription factor but rather a group of related, dimeric complexes composed predominantly of Jun homodimers and Jun-Fos heterodimers (for reviews, see 98, 151, 262). Both the Jun and Fos families contain multiple members, resulting in quite a complicated group. The differences in the various complexes are not well understood, although evidence exists that different complexes show distinct effects on transcriptional regulation. Dimerization of these proteins occurs via leucine zipper domains and results in the formation of a bipartite DNA binding site. Dimerization is necessary but not sufficient for DNA binding, which is mediated by amino acids in the adjacent basic region of the proteins. The dimers interact with the TPA-response elements in the promoter regions of a wide variety of genes implicated in cell proliferation, tumor promotion, and the cellular response to stress. Similar to the other transcription factors discussed here, the DNA-binding domains and the transcriptional activation domains of Jun and Fos reside in distinct regions of the protein. Curiously, the activation domains of these two proteins are controlled by different signaling pathways. The activation domain of Jun is regulated predominantly by the JNK kinases (see Redox Regulation of Protein Kinases), which phosphorylate Jun at serines 63 and 73. As discussed in the section on Redox Regulation of Protein Kinases, JNK kinase activity is regulated by oxidants. Fos activation domains are also regulated by phosphorylation. Some evidence indicates that the transcriptional activity of AP-1 complexes can be influenced by signaling through the ERK pathway, which is also regulated by oxidants (see Redox Regulation of Protein Kinases) (262), as well as by the expression of the Fos and Jun proteins. However, the relation between protein expression and transcriptional activation is not straightforward. Whereas hydrogen peroxide strongly induces Fos and Jun expression, it only weakly induces AP-1 activity, as defined by both DNA binding and transcriptional activation. In contrast, some antioxidants strongly induce both protein expression and AP-1 activity (10, 242).

It is clear from this description that oxidants can affect AP-1 activity at a number of different sites, ranging from induction of the synthesis of Fos or Jun to controlling protein phosphorylation to regulating DNA binding. In whole cells, treatment with hydrogen peroxide or agents that produce ROS can result in the activation of AP-1 (see 10, 126, 242). However, numerous studies have shown that in the test tube, oxidants inhibit DNA binding by AP-1. The redox regulation of DNA binding by AP-1 was first noted a number of years ago by Curran and co-workers (1). They showed that a conserved cysteine in the DNA-binding domain of Fos and Jun could be reversibly oxidized, resulting in a complex with little or no DNA-binding activity. Mutation of the cysteine to serine resulted in an increase in DNA-binding activity and a loss of redox regulation. Interestingly, the oncogenic homologue of c-Jun, v-Jun, already has this substitution, suggesting that part of its transforming potential may be due to a loss of redox regulation. More recent studies on the nature of the oxidized cysteine from Lamas and co-workers (136) uncovered a novel mode of redox regulation for AP-1. They set out to examine whether the DNA-binding activity of c-Jun could be regulated by the ratio of reduced-to-oxidized GSH. Half-maximal inhibition of AP-1 DNA-binding activity was found at a GSH/GSSG ratio of 13 and was shown to be due to the reversible *S*-glutathionylation of the conserved cysteine in the DNA-binding domain of c-Jun. Furthermore, de-*S*-glutathionylation of c-Jun was induced by shifting the GSH/GSSG ratio back up to higher values. These data suggest that GSSG can directly interact with the conserved cysteine in the DNA-binding domain of c-Jun, but whether this occurs in cells is not clear. Although *S*-glutathionylation will temporarily inhibit the DNA-binding activity of c-Jun, it could have the long-term effect of protecting the protein against oxidative damage by preventing the irreversible oxidation of the cysteine sulfhydryl.

The oxidation of AP-1 as well as other transcription factors such as NF-κB (see later), p53, activating transcription factor/cAMP-response element-binding protein (ATF/CREB), hypoxia-inducible factor (HIF)-1α, and HIF-like factor can be reversed by the bifunctional enzyme, Redox factor-1 (Ref-1) (for reviews, see 5, 159, 221). The C-terminal domain of Ref-1 functions as a DNA repair enzyme, whereas the N-terminal domain contains both a nuclear localization sequence and the redox regulatory domain. This regulatory domain has two critical cysteine residues that are involved in redox regulation, although the exact molecular mechanisms underlying transcription factor cysteine sulfhydryl reduction by Ref-1 are not yet clear. Furthermore, Ref-1 can also bind to Trx, and stimuli that activate AP-1 also cause the translocation of Trx to the nucleus. Thus, the most probable, but unproven, scenario is that Ref-1 reduces AP-1 as well as other transcription factors, and Trx acts to reduce oxidized Ref-1.

NF-κB was one of the first transcription factors shown to be regulated by ROS and is often considered to be a primary sensor of oxidative stress in cells (for reviews, see 78, 116, 173, 221). NF-κB is widely expressed in nerve cells and exists in both inducible and constitutively active forms (for review, see 125). The majority of the data on NF-κB suggest that it is important for both normal nerve cell survival and the survival of nerve cells exposed to oxidative stress (for review, see 125). NF-κB is a member of a family of proteins that can

homo- and heterodimerize to form a complex capable of binding to DNA. The p50/p65 dimer is the predominant form activated in nerve cells (125, 181). In unstimulated cells, NF-κB is found in the cytoplasm associated with the inhibitory protein IκB. Although a number of IκB proteins exist, the regulation of IκBα is the best understood. After treatment of cells with many different stimuli, IκB is phosphorylated by an IκB kinase (IKK) and subsequently degraded in a proteosome-dependent manner. The free NF-κB is then able to translocate to the nucleus where it can bind DNA and initiate transcription of a diverse array of target genes, including cytokines and growth factors, oxidative stress–related enzymes, antiapoptotic proteins, and cell-adhesion molecules. Many treatments that activate NF-κB increase the production of oxidants, including UV light and TNF-α. Anti-oxidants generally block NF-κB activation by these treatments. In addition, direct addition of hydrogen peroxide to some, but not all, cell lines activates NF-κB.

It is still not clear exactly how oxidants activate NF-κB. One activation pathway involves the degradation of IκB as described earlier, but it is not clear whether oxidants directly or indirectly increase the activity of an IKK or, instead, inhibit the activity of an IκB phosphatase. It is quite likely that different stimuli utilize somewhat distinct mechanisms and that is what has led to difficulties in identifying a single mechanism. A second pathway for NF-κB activation has also been described and may be the predominant pathway induced by some forms of oxidative stress. Tyrosine phosphorylation of IκB on specific residues can result in the release of IκB from NF-κB without the resulting degradation of IκB (110). This mechanism appears to be particularly prominent after reoxygenation after hypoxia (34, 110). Because oxidative stress can block PTP activity (see Redox Regulation of Phosphatases), it seems likely that this mechanism for NF-κB activation is due to the direct inactivation of a PTP that normally maintains IκB in a dephosphorylated state. Indeed, it was shown that in Jurkat cells, NF-κB activation required the activity of $p56^{Lck}$ (110), whose activity is known to be indirectly enhanced by ROS through the inhibition of PTPs (see Redox Regulation of Protein Phosphatases).

Similar to AP-1, NF-κB in the nucleus requires a reducing environment for DNA-binding activity. Cys62 in the p50 subunit of NF-κB is critical for DNA binding, and its oxidation or nitrosylation leads to a decrease in NF-κB DNA-binding activity (122). As with AP-1, Ref-1 and nuclear Trx restore the DNA-binding activity of oxidized NF-κB. Together, these data suggest that the agents that induce ROS production and NF-κB activation in the cytoplasm must do so locally, and that severe oxidative stress, which leads to oxidizing conditions within the nucleus as well as the cytoplasm, results in an inability of NF-κB to activate transcription.

Another transcription factor that is regulated by the redox state, but in a very different manner from the transcription factors discussed earlier, is hypoxia-inducible factor 1 (HIF-1) (for reviews, see 17, 220, 260). HIF-1 activates expression of genes involved in angiogenesis, oxygen transport, iron metabolism, glycolysis, glucose uptake, growth-factor signaling, apoptosis, invasion, and metastasis. HIF-1 is required for normal development of the brain (251) and is also involved in angiogenesis and neuroprotection (37, 233, 273). As its name suggests, HIF-1 is activated by hypoxia and hypoxia mimetics, but more recent research suggests that it can also be activated by growth factor–signaling pathways and oxidants (17). HIF-1 is a dimeric protein consisting of two basic helix–loop–helix Per/Arnt/Sim (bHLH-PAS) transcription factors, HIF-1α and HIF-1β (aka ARNT). The dimer is required for DNA binding and transcriptional activation. The regulation of HIF-1 activity occurs at the level of the stability of HIF-1α protein. The mRNAs for both HIF-1α and HIF-1β are constitutively expressed in cells. However, whereas HIF-1β protein is also constitutively expressed, in normoxia, HIF-1α protein is rapidly degraded by the proteasome. HIF-1α is targeted for degradation via the hydroxylation of two proline residues that promote its binding to the von Hippel–Lindau protein (VHL), the substrate-recognition component of an E3 ubiquitin ligase complex. Prolyl hydroxylation of HIF-1α is mediated by members of the egg-laying–defective nine (EGLN) hydroxylase family (123). The hydroxylation reaction requires molecular oxygen and iron as a cofactor so that the hydroxylases are inhibited both by hypoxia and by iron chelators. Hence HIF-1α acts as a link between the oxygen-sensing hydroxylases and gene transcription. However, HIF-1α activation can also occur in a normoxic environment in response to a variety of stimuli, including growth factors, ROS, and nitric oxide. The mechanisms underlying HIF-1α stabilization by these stimuli are under investigation and continue to be a matter for debate.

In addition to the transcription factors discussed earlier, the activity of a number of other transcription factors has been shown to be regulated by the redox status of the cell (for reviews, see 5, 10, 126, 178, 242). In almost all the cases that have been examined, this regulation involves direct effects on critical cysteine sulfhydryls in the DNA-binding or transcriptional activation domains of the proteins. A number of these transcription factors show the same conflict as seen with AP-1, wherein oxidants activate the transcription factor in whole cells, but test tube studies with the isolated factor show it to be inhibited by oxidation. In contrast, other transcription factors are inhibited by oxidation both in *in vitro* assays and in whole cells. Thus, the activity of a large number of transcription factors may be redox regulated in cells. Furthermore, the specific consequences of a change in the cellular redox status on a given transcription factor will depend not only on the nature of the transcription factor itself but also on the specific conditions within a given cell. This is most apparent for those transcription factors that appear to require an oxidizing cytoplasm but a reducing nucleus. Subtle changes in this balance could dramatically affect the activity of these transcription factors.

REDOX REGULATION OF CELL FUNCTION

As discussed in the preceding sections, changes in the redox environment of cells can have profound effects on the activity of a variety of enzymes including protein kinases and phosphatases, as well as transcription factors. Therefore, it is not unlikely that these changes in the activity of specific pro-

teins can be translated into overall changes in cellular physiology and behavior. In particular, it has been proposed that the overall redox status of cells plays a role in regulating cell fate (Fig. 9) (119, 217). Specifically, mounting evidence indicates that in normal cells, a more reducing environment is associated with cell proliferation, whereas a more oxidizing environment promotes differentiation. Further oxidation would be expected to induce cell death. Therefore, it is likely that, as the major redox couple in the cell, the GSSG/2GSH ratio plays a significant role in regulating these cell-fate decisions. However, as noted by Schafer and Buettner (217), the amount of GSH oxidation that would be required to shift cells from proliferation to death will be 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*.

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 (191). 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 studies, Mark Noble and colleagues (191) showed 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 whereas 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, whereas growth in the presence of endogenous factors that promote differentiation correlated with an increase in intracellular oxidation. Thus, these studies further suggest 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. Further studies will be needed to elucidate the specific molecular mechanisms underlying these effects.

In nerve cells, evidence that a more oxidizing environment promotes differentiation has come from studies with PC12 cells treated with NGF to induce differentiation (127). NGF-induced differentiation was enhanced by treatments that decreased the GSH/GSSG ratio and inhibited by treatments that increased the GSH/GSSG ratio. Further studies suggested that a more oxidizing environment led to a decrease in PTP activity, thereby potentiating the activation of the NGF receptor, TrkA. Although a more oxidizing environment may promote differentiation in nerve cells, it may adversely affect signaling through other neurotrophic factor receptors. For example, a recent study on signaling through the ciliary neurotrophic factor receptor (CNTF) demonstrated that oxidants as well as an inhibitor of glutathione disulfide reductase could block activation of the JAK/STAT pathway by CNTF in nerve cells but not in other cell types (129). Finally, other neuronal pathways are modulated by the intracellular redox environment, including neurotrophic factor secretion (255).

Together, these studies support the hypothesis that changes in the intracellular redox environment can have profound effects on cell function and fate. This may have important consequences in a number of areas including stem cell research and toxicology. For example, any treatment or exposure that promotes a more oxidizing environment in nerve cells could result in a reduction in the proliferation of progenitor cells and/or premature differentiation. This could have profound effects during development if pools of precursor cells failed to expand. Because a number of neurotoxins reduce the intracellular levels of GSH in nerve and glial cells (7, 60), further investigations of the possible effects of environmental toxins and toxins in our food supply are certainly warranted.

Another area in which evidence exists that a shift in the intracellular redox environment correlates with changes in cellular function is that of aging (92, 164) (Table 1). Good evidence is found for a decrease in the GSH/GSSG ratio in tissues, including the brain, taken from old rats (164). In addition, overall levels of GSH decrease (164), as well as the capacity to make GSH (109, 160, 214) and to reduce GSSG (109, 214). In contrast, GPx activity remains constant (109, 214) or increases in specific regions of the brain with age (18, 108), which may be a reflection of the response of neural cells to the shift in a more oxidizing intracellular redox environment. In the brains of very old rats (26 months), both the

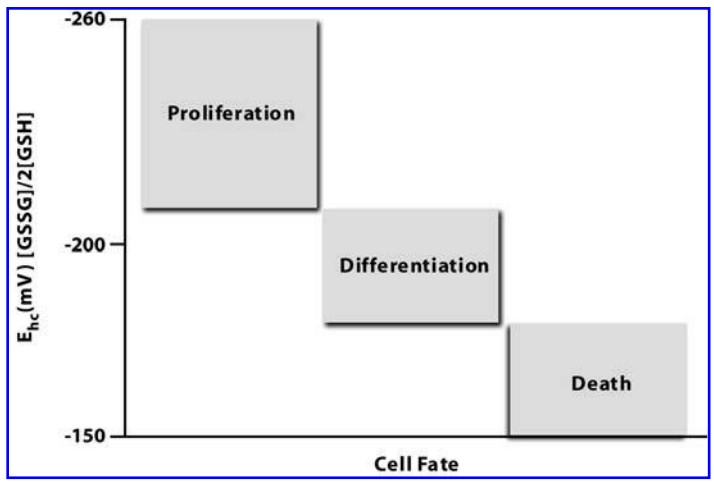

FIG. 9. Regulation of cell fate by the intracellular redox environment (after 119, 217). An electrochemical potential (E_{hc}) of the [GSSG]/2[GSH] redox couple between −260 and −210 mV is associated with cell proliferation, whereas an electrochemical potential above −210 mV promotes cell differentiation. A further increase in the electrochemical potential above −150 mV triggers cell death.

amount of MsrA and its specific activity are decreased as compared with both juvenile and adult rats (202). Prx2 and Prx3 protein levels were also found to be decreased in the aged brain (201). Thus, in older animals, CNS cells are more likely to be less able to tolerate oxidative insults, due to both a shifting of the redox environment and a loss of antioxidant capacity. Further studies are needed to determine if manipulation of the redox environment during aging can reduce the consequences of aging. Preliminary evidence suggests that this may be one of the mechanisms underlying the life span–promoting effects of calorie restriction (44, 205).

REDOX REGULATION IN NEURODEGENERATIVE DISEASES

The activity and/or expression levels of several of the enzymes described in this review that modulate the intracellular redox state or are affected by changes in the intracellular redox state have been studied in one or more neurodegenerative diseases including AD, PD, and amyotrophic lateral sclerosis (ALS). Although many gaps appear in this information, the major findings are described below and summarized in Table 1.

In brains from AD patients, both GPx and GR mRNA levels were found to be increased (6), as was TrxR activity (163). However, the levels of Trx protein were lower than in age-matched controls, suggesting that the complete redox system must be present for significant protection against oxidative stress. Whereas the level of the Trx-dependent enzyme Prx1 was shown to be similar in control and AD brains, the level of neuronal Prx2 was elevated in specific regions in AD brains although the level of Prx3 was decreased (141, 201). The significance of these findings is at present unclear, but they suggest that the loss of Prx3 could contribute to the mitochondrial damage seen in AD.

The activity of MsrA was found to be decreased in all regions of the brains of AD patients as compared with age-matched controls (80). The greatest losses in activity were in the superior and middle temporal gyri and the inferior parietal lobule and the hippocampus, all areas that show histopathologic changes in AD. The decreases in enzyme activity did not appear to be a result of specific defects in the transcription of the MsrA gene, because mRNA levels only decreased slightly and equally in all brain regions examined. Whether the loss of MsrA activity contributes to the nerve cell death seen in AD is not known. Several studies have shown that Met35 in the amyloid β peptide, a protein that is thought to play a central role in the development of AD, can be oxidized (20, 103, 185, 198). This oxidation results in both an alteration in the aggregation profile of the peptide, such that the formation of Aβ trimers and tetramers is reduced and fibril formation is inhibited, and a decrease in the ability of Aβ to interact with membranes. Together these changes result in an increase in the solubility of Aβ. Whether this is good or bad is not yet clear. Evidence suggests both that the Aβ aggregates cause nerve cell loss in AD and that the synaptic damage and cognitive impairment associated with AD correlate with soluble Aβ (249). If the latter turns out to be the case, then the combination of increased oxidation of Met35 in Aβ coupled with the decrease in MsrA activity in AD brain could play a critical role in the progression of AD. Further studies will be needed to determine if this is indeed the case, as well as whether changes are also seen in MsrB. However, it should be noted that amyloid proteins such as human amylin are as toxic as Aβ and contain no methionine (161), clearly demonstrating that methionine oxidation is not a requirement for amyloid toxicity.

In AD, a decrease also is found in PP2A activity (see Redox Regulation of Protein Phosphatases), which could be a result of the inactivation of this phosphatase due to the increase in oxidative stress associated with this disease. PP2A is responsible for the dephosphorylation of the microtubule-associated protein tau (112). Abnormal hyperphosphorylation of tau results in both the loss of normal function and the gain of a toxic function that causes the inhibition and disruption of microtubules. The hyperphosphorylation of tau is a result of both an imbalance between the activities of tau kinases and phosphatases and changes in the conformation of tau, which alter its interaction with these proteins.

Less is known about the enzymes that regulate the intracellular redox environment in PD (Table 1), although of all the neurodegenerative diseases, evidence for a dysfunction in GSH metabolism is strongest in PD (for reviews, see 8, 13, 219). A number of studies have found that GSH levels are specifically decreased in the substantia nigra (SN) of PD patients and that a positive correlation exists between the severity of the disease and the extent of GSH loss. GSH levels are not reduced in other brain areas in PD patients or in patients with other neurodegenerative diseases that also affect dopaminergic neurons. In addition, GSH levels are decreased to almost the same degree in patients with incidental Lewy body disease, a preclinical and still asymptomatic form of PD. Thus, the decrease in GSH precedes other PD-associated changes in the SN, such as decreases in mitochondrial complex I activity and dopamine levels. Although GCL activity was found to be similar in the SN from control and PD patients (227), suggesting that GSH synthesis is not the cause of GSH loss, a near complete loss of GPx1 mRNA was observed (142). In contrast, Prx2 protein levels were significantly increased in the SN from PD patients (22)

Table 1. Changes in Key Proteins that Regulate the Cellular Redox Environment in Disease and Aging

AD (Brain)	*PD (SN)*	*ALS (spinal cord)*	*Aging (brain)*
Trx1 (p) ↓			
TrxR (a) ↑			
MsrA (a) ↓			MsrA (a, r, p) ↓
Prx1 (p) =			
Prx2 (p) ↑	Prx2 (p) ↑	Prx2 (p) ↓	Prx2 (p) ↓
Prx3 (p) ↓			Prx3 (p) ↓
GPx1 (r) ↑	GPx1 (r) ↓	GPx1 (p) ↓	GPx (a) ↑/=
GR (r) ↑			GR (a) ↓
	GCL (a) =		GCL (a, p) ↓

↑, increase; ↓, decrease; =, no change relative to age-matched control (AD, PD, ALS) or young animals (aging); a, activity; r, mRNA; p, protein. References are cited in the text.

Similar to the results with Aβ, methionine oxidation also prevents aggregation and fibril formation by α-synuclein, a protein implicated in PD (269). The formation of α-synuclein fibrils is thought to be a critical event in the development of PD so the inhibitory effect of methionine oxidation on fibril formation would appear to be beneficial. However, a recent study suggests that the presence of certain metals, such as lead, zinc, and aluminum, can overcome the inhibition of fibril formation resulting from methionine oxidation (269). This observation indicates that the beneficial effect of methionine oxidation can be overridden and is consistent with an environmental component to the development of PD. An interesting question that arises from these observations on α-synuclein is whether certain metals can override the antifibrillation effects of methionine oxidation in the Aβ peptide as well.

Even less is known about the enzymes that regulate the intracellular redox environment in ALS (Table 1). However, a recent study found that the number of motor neurons in the anterior horns of the spinal cord negative for both Prx2 and GPx1 increased with ALS disease progression in both sporadic ALS patients and superoxide dismutase 1 (SOD1)-mutated familial ALS patients (128). These results, along with the presence of motor neurons overexpressing both Prx2 and GPx1 early in the course of the disease, suggest that a breakdown in redox regulation contributes to the progression of the disease. The mutations in SOD1 that are associated with the familial forms of ALS also cause the oxidative inactivation of the redox-regulated phosphatase, calcineurin (254) (see Redox Regulation of Protein Phosphatases).

In summary, although clear evidence exists for changes in a number of the different proteins that play a role in the regulation of the intracellular redox environment in several neurodegenerative diseases, whether these changes contribute to the development and/or progression of the disease remains to be determined. In addition, although an upregulation of a subset of these proteins in some fraction of the affected brain regions is seen in all of these neurodegenerative diseases, this upregulation does not appear to be sufficient to prevent the neuronal cell loss that is the hallmark of these diseases. This observation is consistent with the idea that the maintenance of an intracellular redox environment that is conducive to normal cellular function is dependent on multiple redox couples.

CONCLUSIONS

Although convincing evidence indicates that increases in the intracellular level of oxidants resulting in changes in the intracellular redox environment can alter the activity of protein kinases, phosphatases, and transcription factors through effects on cysteine and methionine residues, the consequences of these changes on cellular function are only just beginning to be explored. Experimental approaches that allow the investigator to look at multiple changes rather than to focus on single proteins should in the future make evaluation of the global consequences of alterations in the redox environment much easier to evaluate. In addition, much greater emphasis must be placed on evaluating changes in the redox environment in the context of treatment with physiologic stimuli rather than high doses of exogenous oxidants. This is clearly an exciting time in this rapidly developing field, particularly as it relates to neural cell physiology, and the next few years are likely to bring some surprising discoveries.

ACKNOWLEDGMENTS

The author is supported by NIH grant AG025337. Thanks to David Schubert for reading the manuscript and to Elizabeth Grabowski for help with the figures.

ABBREVIATIONS

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; AP-1, activator protein 1 (transcription factor); ARE, antioxidant response element; ASK, apoptosis signal-regulating kinase; CaM, calmodulin; CDK, cyclin-dependent kinase; CNTF, ciliary neurotrophic factor; CNS, central nervous system; DSP, dual-specificity protein phosphatase; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; ETC, electron transport chain; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GCL, glutamate cysteine ligase; GPx, glutathione peroxidase; GR, glutathione disulfide reductase; Grx, glutaredoxin; GS, glutathione synthetase; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione *S*-transferase; HIF, hypoxia inducible factor; ICDH, isocitrate dehydrogenase; IκB, inhibitor of nuclear factor κB (NF-κB); IKK, inhibitor of nuclear factor κB (IκB) kinase; JAK, Janus kinase; JNK, c-jun N-terminal kinase; KDH, α-ketoglutarate dehydrogenase; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; MPTP, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine; MRP, multidrug-resistance protein; Msr, methionine sulfoxide reductase; NF-κB, nuclear factor κB (transcription factor); Nrf2, NF-E2–related factor 2; PD, Parkinson disease; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; PP, serine/threonine protein phosphatase; Prx, peroxiredoxin; PTP, protein tyrosine phosphatase; PTK, protein tyrosine kinase; Ref-1, redox factor-1; ROS, reactive oxygen species; SN, substantia nigra; SOD, superoxide dismutase; Srx, sulfiredoxin; STAT, signal transducer and activator of transcription; Trx, thioredoxin; TrxR, thioredoxin reductase; TrxSS, oxidized thioredoxin; $Trx(SH)_2$, reduced thioredoxin.

REFERENCES

1. Abate C, Patel L, Rauscher FJ, and Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. *Science* 249: 1157–1161, 1990.
2. Adachi T, Pimentel DR, Heibeck T, Hou X, Lee YJ. Jiang B, Ido Y, and Cohen RA. S-glutathiolation of ras mediates redox-sensitive signaling by angiotensin II in vascular smooth muscle cells. *J Biol Chem* 279: 29857–29862, 2004.

3. Adam-Vizi V. Production of reactive oxygen species in brain mitochondria: contribution by electron transport chain and non-electron transport chain sources. *Antioxid Redox Signal* 7: 1140–1149, 2005.
4. Adler V, Yin Z, Fuchs SY, Benezra M, Rosario L, Tew KD, Pincus MR, Sardana M, Henderson CJ, Wolf CR, Davis RJ, and Ronai ZE. Regulation of JNK signaling by GSTp. *EMBO J* 18: 1321–1334, 1999.
5. Adler V, Yin Z, ZKD, and Ronai Z. Role of redox potential and reactive oxygen species in stress signaling. *Oncogene* 18: 6104–6111, 1999.
6. Aksenov MY and Markesberry WR. Changes in the thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. *Neurosci Lett* 302: 141–145, 2001.
7. Allen JW, Shanker G, and Aschner M. Methylmercury inhibits the in vitro uptake of the glutathione precursor, cystine, in astrocytes but not in neurons. *Brain Res* 894: 131–140, 2001.
8. Anderson JK. Does neuronal loss in Parkinson's disease involve programmed cell death? *BioEssays* 23: 640–646, 2001.
9. Arner ESJ and Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. *Eur J Biochem* 267: 6102–6109, 2000.
10. Arrigo AP. Gene expression and the thiol redox state. *Free Radic Biol Med* 27: 936–944, 1999.
11. Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci* 57: 1825–1835, 2000.
12. Baas AS and Berk BC. Differential activation of mitogen-activated protein kinases by H_2O_2 and O_2^- in vascular smooth muscle cells. *Circ Res* 77: 29–36, 1995.
13. Bains JS and Shaw CA. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. *Brain Res Rev* 25: 335–358, 1997.
14. Baker DA, McFarland K, Lake RW, Shen H, Tang X-C, Toda S, and Kalivas PW. Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nat Neurosci* 6: 743–749, 2003.
15. Baker DA, Zheng-Xiong X, Shen H, Swanson CJ, and Kalivas PW. The origin and neuronal function of *in vivo* nonsynaptic glutamate. *J Neurosci* 22: 9134–9141, 2002.
16. Ballatori N, Hammond CL, Cunningham JB, Krance SM, and Marchan R. Molecular mechanisms of reduced glutathione transport: role of the MRP/CFTR/ABCC and OATP/SLC21A families of membrane proteins. *Toxicol Appl Pharmacol* 204: 238–255, 2005.
17. Bardos JI and Ashcroft M. Negative and positive regulation of HIF-1: a complex network. *Biochim Biophys Acta* 1755: 107–120, 2005.
18. Barja de Quiroga GR, Perez-Campo RC, and Lopez Torres M. Anti-oxidant defences and peroxidation in liver and heart of aged rats. *Biochem J* 272: 247–250, 1990.
19. Barker JE, Heales SJR, Cassidy A, Bolanos JP, Land JM, and Clark JB. Depletion of brain glutathione results in a decrease of glutathione reductase activity: an enzyme susceptible to oxidative damage. *Brain Res* 716: 118–122, 1996.
20. Barnham KJ, Ciccotosto GD, Tickler AK, Ali FE, Smith DG, Williamson NA, Lam Y-H, Carrington D, Tew D, Kocak G, Volitakis I, Separovic F, Barrow CJ, Wade JD, Masters CL, Cherny RA, Curtain CC, Bush AI, and Cappai R. Neurotoxic, redox-competent Alzheimer's β-amyloid is released from lipid membrane by methionine oxidation. *J Biol Chem* 278: 42959–42965, 2003.
21. Barrett DM, Black SM, Todor H, Schmidt-Ullrich RK, Dawson KS, and Mikkelsen RB. Inhibition of protein tyrosine phosphatases by mild oxidative stresses is dependent on S-nitrosylation. *J Biol Chem* 280: 14453–14461, 2005.
22. Basso M, Giraudo S, Corpillo D, Bergamasco B, Lopiano L, and Fasano M. Proteome analysis of human substantia nigra in Parkinson's disease. *Proteomics* 4: 3943–3952, 2004.
23. Bensadoun JC, Mirochnitchenko O, Inouye M, Aebischer P, and Zurn AD. Attenuation of 6-OHDA-induced neurotoxicity in glutathione peroxidase transgenic mice. *Eur J Neurosci* 10: 3231–3236, 1998.
24. Bhagwat SV, Mullick J, Avadhani NG, and Raza H. Differential response of cytosolic, microsomal, and mitochondrial glutathione S-transferases to xenobiotic inducers. *Int J Oncol* 13: 281–288, 1998.
25. Bigelow DJ and Squier TC. Redox modulation of cellular signaling and metabolism through reversible oxidation of methionine sensors in calcium regulatory proteins. *Biochim Biophys Acta* 1703: 121–134, 2005.
26. Biswas S, Chida AS, and Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. *Biochem Pharm* 71: 551–564, 2006.
27. Biteau B, Labarre J, and Toledano MB. ATP-dependent reduction of cysteine-sulphinic acid by *S. cerevisiae* sulphiredoxin. *Nature* 425: 980–984, 2003.
28. Borges CR, Geddes T, Watson JI, and Kuhn DM. Dopamine biosynthesis is regulated by S-glutathionylation. *J Biol Chem* 277: 48295–48302, 2002.
29. Brigelius-Flohe R. Tissue-specific functions of individual glutathione peroxidases. *Free Radic Biol Med* 27: 951–965, 1999.
30. Brodie C and Blumberg PM. Regulation of cell apoptosis by protein kinase Cδ. *Apoptosis* 8: 19–27, 2003.
31. Budanov AV, Sablina AA, Feinstein E, Koonin EV, and Chumakov PM. Regeneration of peroxiredoxins by p53-regulated sestrins, homologs of bacterial AhpD. *Science* 304: 596–600, 2004.
32. Bunik VI. 2-Oxo acid dehydrogenase complexes in redox regulation. *Eur J Biochem* 270: 1036–1042, 2003.
33. Burdo J, Dargusch R, and Schubert D. Distribution of the cystine/glutamate antiporter system X_c^- in the brain, kidney and duodenum. *J Histochem Cytochem* 54: 549–557, 2006.
34. Canty TG, Boyle EM, Farr A, Morgan EN, Verrier ED, and Pohlman TH. Oxidative stress induces NF-κB nuclear translocation without degradation of IκBα. *Circulation* 100: 361–364, 1999.
35. Casagrande S, Bonetto V, Fratelli M, Gianazza E, Eberini I, Massignan T, Salmona M, Chang G, Holmgren A, and Ghezzi P. Glutathionylation of human thioredoxin: a possible crosstalk between the glutathione and thioredoxin systems. *Proc Natl Acad Sci U S A* 99: 9745–9749, 2002.
36. Chang T-S, Jeong W, Woo HA, Lee SM, Park S, and Rhee SG. Characterization of mammalian sulfiredoxin and its reactivation of hyperoxidized peroxiredoxin through reduction of cysteine sulfinic acid in the active site to cysteine. *J Biol Chem* 279: 50994–51001, 2004.
37. Chavez JC and LaManna JC. Activation of hypoxia-inducible factor-1 in the rat cerebral cortex after transient global ischemia: potential role of insulin-like growth factor-1. *J Neurosci* 22: 8922–8931, 2002.
38. Chen C and Kong A-N. Dietary chemopreventive compounds and ARE/EpRE signaling. *Free Radic Biol Med* 36: 1505–1516, 2004.
39. Chen K, Thomas SR, Albano A, Murphy MP, and Keaney JF. Mitochondrial function is required for hydrogen peroxide-induced growth factor receptor transactivation and downstream signaling. *J Biol Chem* 279: 35079–35086, 2004.
40. Chen Y and Swanson RA. The glutamate transporters EAAT2 and EAAT3 mediate cysteine uptake in cortical neuron cultures. *J Neurochem* 84: 1332–1339, 2003.
41. Chevallet M, Wagner E, Luche S, van Dorsselaer A, Leize-Wagner E, and Rabilloud T. Regeneration of peroxiredoxins during recovery after oxidative stress. *J Biol Chem* 278: 37146–37153, 2003.
42. Chiarugi P and Cirri P. Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 28: 509–514, 2003.
43. Chiarugi P and Giannoni E. Anchorage-dependent cell growth: tyrosine kinases and phosphatases meet redox regulation. *Antioxid Redox Signal* 7: 578–592, 2005.
44. Cho CG, Kim HJ, Chung SW, Jung KJ, Shim KH, Yu BP,Yodoi J, and Chung HY. Modulation of glutathione and thioredoxin systems by calorie restriction during the aging process. *Exp Gerontol* 38: 539–548, 2003.
45. Cho S-H, Lee C-H, Ahn Y, Kim H, Kim H, Ahn CY, Yang KS, and Lee S-R. Redox regulation of PTEN and protein tyrosine phosphatases in H_2O_2-mediated cell signaling. *FEBS Lett* 560: 7–13, 2004.

46. Chung KK, Thomas KB, Li X, Pletnikova O, Troncoso JC, Marsh L, Dawson VL, and Dawson TM. S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. *Science* 304: 1328–1331, 2004.
47. Ciorba MA, Heinemann SH, Weissbach H, Brot N, and Hoshi T. Modulation of potassium channel function by methionine oxidation and reduction. *Proc Natl Acad Sci U S A* 94: 9932–9937, 1997.
48. Clavreul N, Adachi T, Pimental DR, Ido Y, Schoneich C, and Cohen RA. S-glutathiolation by peroxynitrite of p21ras at cysteine-118 mediates its direct activation and downstream signaling in endothelial cells. *FASEB J* 20: 518–520, 2006.
49. Cohen P. The search for physiological substrates of MAO and SAP kinases in mammalian cells. *Trends Cell Biol* 7: 353–361, 1998.
50. Cooper CE, Patel RP, Brookes PS, and Darley-Usmar VM. Nanotransducers in cellular redox signaling: modification of thiols by reactive oxygen and nitrogen species. *Trends Biochem Sci* 27: 489–492, 2002.
51. Cordey M and Pike CJ. Conventional protein kinase C isoforms mediate neuroprotection induced by phorbol ester and estrogen. *J Neurochem* 96: 204–217, 2006.
52. Cotgreave IA and Gerdes RG. Recent trends in glutathione biochemistry: glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? *Biochem Biophys Res Commun* 242: 1–9, 1998.
53. Crack PJ, Taylor JM, Flentjar NJ, de Haan J, Hertzog P, Iannello RC, and Kola I. Increased infarct size and exacerbated apoptosis in the glutathione peroxidase-1 (Gpx-1) knockout mouse brain in response to ischemia/reperfusion injury. *J Neurochem* 78: 1389– 1399, 2001.
54. Crespo P and Leon J. Ras proteins in the control of the cell cycle and cell differentiation. *Cell Mol Life Sci* 57: 1613–1636, 2000.
55. Cross JV and Templeton DJ. Oxidative stress inhibits MEKK1 by site-specific glutathionylation in the ATP binding domain. *Biochem J* 381: 675–683, 2004.
56. Cumming RC, Andon NL, Haynes PA, Park M, Fischer WH, and Schubert D. Protein disulfide bond formation in the cytoplasm during oxidative stress. *J Biol Chem* 279: 21749–21758, 2004.
57. Cumming RC and Schubert D. Amyloid-β induces disulfide bonding and aggregation of GAPDH in Alzheimer's disease. *FASEB J* 19: 2060–2062, 2005.
58. Dalle-Donne I, Giustarini D, Rossi R, Colombo R, and Milzani A. Reversible S-glutathionylation of Cys 374 regulates actin filament formation by inducing structural changes in the actin molecule. *Free Radic Biol Med* 34: 23–32, 2003.
59. Dalton TP, Chen Y, Schneider SN, Nebert DW, and Shertzer HG. Genetically altered mice to evaluate glutathione homeostasis in health and disease. *Free Radic Biol Med* 37: 1511–1526, 2004.
60. Dargusch R and Schubert D. Specificity of resistance to oxidative stress. *J Neurochem* 81: 1394–1400, 2002.
61. Deacon EM, Pongracz J, Griffiths G, and Lord JM. Isoenzymes of protein kinase C: differential involvement in apoptosis and pathogenesis. *J Clin Pathol* 50: 124–131, 1997.
62. den Hertog J, Groen A, and van der Wijk T. Redox regulation of protein-tyrosine phosphatases. *Arch Biochem Biophys* 434: 11–15, 2005.
63. Denu JM, Stuckey JA, Saper MA, and Dixon JE. Form and function in protein dephosphorylation. *Cell* 87: 361–364, 1996.
64. Denu JM and Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry* 37: 5633–5642, 1998.
65. Devary Y, Gottlieb RA, Smeal T, and Karin M. The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. *Cell* 71: 1081–1091, 1992.
66. Di-Capua N, Sperling O, and Zoref-Shani E. Protein kinase Cϵ is involved in the adenosine-activated signal transduction pathway conferring protection against ischemia-reperfusion injury in primary rat neuronal cultures. *J Neurochem* 84: 409–412, 2003.
67. Diaz-Hernandez JI, Almeida A, Delgado-Esteban M, Fernandez E, and Bolanos JP. Knockdown of glutamate-cysteine ligase by small hairpin RNA reveals that both catalytic and modulatory subunits are essential for the survival of primary neurons. *J Biol Chem* 280: 38992–39001, 2005.
68. Dickinson DA and Forman HJ. Glutathione in defense and signaling. *Ann N Y Acad Sci* 973: 488–504, 2002.
69. Dickinson DA, Levonen AL, Moellering DR, Arnold EK, Zhang H, Darley-Usmar VM, and Forman HJ. Human glutamate cysteine ligase gene regulation through the electrophile response element. *Free Radic Biol Med* 37: 1152–1159, 2004.
70. Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, and Talalay P. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci U S A* 99: 11908–11913, 2002.
71. Drewes G and Faulsticj H. Cooperative effects on filament stability in actin modified at the C-terminus by substitution of truncation. *Eur J Biochem* 212: 247–253, 1993.
72. Dringen R and Hirrlinger J. Glutathione pathways in the brain. *Biol Chem* 384: 505–516, 2003.
73. Dringen R, Pfeiffer B, and Hamprecht B. Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. *J Neurosci* 19: 562–569, 1999.
74. Dun Y, Mysona B, Van Ells T, Amarnath L, Shamsul M, Ola V, Ganapathy V, and Smith SB. Expression of the cystine-glutamate exchanger (xc-) in retinal ganglion cells and regulation by nitric oxide and oxidative stress. *Cell Tissue Res* 2005.
75. Enwere E, Shingo T, Gregg C, Fujikawa H, Ohta S, and Weiss S. Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. *J Neurosci* 22: 8354–8365, 2004.
76. Esposito LA, Kokoska JE, Waymire KG, Cottrell B, MacGregor GR, and Wallace DC. Mitochondrial oxidative stress in mice lacking the glutathione peroxidase-1 gene. *Free Radic Biol Med* 28: 754–766, 2000.
77. Fernandes AP and Holmgren A. Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. *Antioxid Redox Signal* 6: 63–74, 2004.
78. Flohe L, Brigelius-Flohe R, Saliou C, Traber MG, and Packer L. Redox regulation of NF-κB activation. *Free Radic Biol Med* 22: 1115–1126, 1997.
79. Forman HJ, Fukuto JM, and Torres M. Redox signaling: thiol chemistry defines which reactive oxygen and nitrogen species can act as second messengers. *Am J Physiol Cell Physiol* 287: C246– C256, 2004.
80. Gabbita SP, Aksenov MY, Lovell ML, and Markesbery WR. Decrease in peptide methionine sulfoxide reductase in Alzheimer's disease brain. *J Neurochem* 73: 1660–1666, 1999.
81. Garnier R, Rambourg-Schepens MO, Muller A, and Hallier E. Glutathione transferase activity and formation of macromolecular adducts in two cases of acute methyl bromide poisoning. *Occup Environ Med* 53: 211–215, 1996.
82. Gegg ME, Clark JB, and Heales SJR. Co-culture of neurones with glutathione deficient astrocytes leads to increased neuronal susceptibility to nitric oxide and increased glutamate-cysteine ligase activity. *Brain Res* 1036: 1–6, 2005.
83. Ghezzi P. Regulation of protein function by glutathionylation. *Free Radic Res* 39: 573–580, 2005.
84. Ghezzi P, Bonetto V, Fratelli M. Thiol-disulfide balance: from the concept of oxidative stress to that of redox regulation. *Antioxid Redox Signal* 7: 964–972, 2005.
85. Gilbert HF. Redox control of enzyme activities by thiol/disulfide exchange. *Methods Enzymol* 107: 330–351, 1984.
86. Giustarini D, Milzani A, Aldini G, Carini M, Rossi R, and Dalle-Donne I. S-Nitrosation versus S-glutathionylation of protein sulfhydryl groups by S-nitrosoglutathione. *Antioxid Redox Signal* 7: 930–939, 2005.
87. Giustarini D, Rossi R, Milzani A, Colombo R, and Dalle-Donne I. S-glutathionylation: from redox regulation of protein functions to human diseases. *J Cell Mol Med* 8: 201–212, 2004.
88. Gopalakrishna R and Jaken RS. Protein kinase C signaling and oxidative stress. *Free Radic Biol Med* 28: 1349–1361, 2000.
89. Gotoh Y and Cooper JA. Reactive oxygen species and dimerization-induced activation of apoptosis signal-regulating kinase 1 in

tumor necrosis factor-α signal transduction. *J Biol Chem* 273: 17477–17482, 1998.
90. Gromer S, Urig S, and Becker K. The thioredoxin system: from science to clinic. *Med Res Rev* 24: 40–89, 2004.
91. Guyton KZ, Liu M, Gorospe Xy Q, and Holbrook NJ. Activation of mitogen-activated protein kinase by H_2O_2. *J Biol Chem* 271: 4138–4142, 1996.
92. Hagen TM. Oxidative stress, redox imbalance, and the aging process. *Antioxid Redox Signal* 5: 503–506, 2003.
93. Halliwell B and Gutteridge JMC. *Free Radicals in Biology and Medicine*. Oxford, New York, 1993.
94. Hamnell-Pamment Y, Lind C, Palmberg C, Bergman T, and Cotgreave IA. Determination of site-specificity of S-glutathionylated cellular proteins. *Biochem Biophys Res Commun* 332: 362–369, 2005.
95. Hansen JM, Go Y-M, and Jones DP. Nuclear and mitochondrial compartmentation of oxidative stress and redox signaling. *Annu Rev Pharmacol Toxicol* 46: 215–234, 2006.
96. Hardwick JS and Sefton BM. Activation of the Lck tyrosine protein kinase by hydrogen peroxide requires the phosphorylation of Tyr-394. *Proc Natl Acad Sci U S A* 92: 4527–4531, 1995.
97. Hayes JD and McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic Res* 31: 273–300, 1999.
98. Herdegen T, Skene P, Bahr M. The c-Jun transcription factor: bipotential mediator of neuronal death, survival and regeneration. *Trends Neurosci* 20: 227–231, 1997.
99. Hirrlinger J, Resch A, Gutterer JM, and Dringen R. Oligodendroglial cells in culture effectively dispose of exogenous hydrogen peroxide: comparison with cultured neurones, astroglial and microglial cells. *J Neurochem* 82: 635–644, 2002.
100. Hook SS and Means RA. Ca2+/CaM-dependent kinases: from activation to function. *Annu Rev Pharmacol Toxicol* 41: 471–505, 2001.
101. Hoppe G, Chai Y-C. Crabb JW, and Sears J. Protein S-glutathionylation in retinal pigment epithelium converts heat shock protein 70 to an active chaperone. *Exp Eye Res* 78: 1085–1092, 2004.
102. Hoshi T and Heinemann SH. Regulation of cell function by methionine oxidation and reduction. *J Physiol* 531: 1–11, 2001.
103. Hou L, Kang I, Marchant RE, and Zagorski MG. Methionine 35 oxidation reduces fibril assembly of the amyloid Ab-(1–42) peptide of Alzheimer's disease. *J Biol Chem* 277: 40173–40176, 2002.
104. Howe CJ, LaHair MM, McCubrey JA, and Franklin RA. Redox regulation of the calcium/calmodulin-dependent protein kinases. *J Biol Chem* 279: 44573–44581, 2004.
105. Huang J and Philbert MM. Distribution of glutathione and glutathione-related enzyme systems in mitochondria and cytosol of cultured cerebellar astrocytes and granule cells. *Brain Res* 680: 16–22, 1995.
106. Hug H and Sarre TF. Protein kinase C isoenzymes: divergence in signal transduction? *Biochem J* 291: 329–343, 1993.
107. Hurd TR, Costa NJ, Dahm CC, Beer SM, Brown SE, Filipovska A, and Murphy MP. Glutathionylation of mitochondrial proteins. *Antioxid Redox Signal* 7: 999–1010, 2005.
108. Hussain S, Slikker W, and Ali SF. Age-related changes in antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione in different regions of the mouse brain. *Int J Dev Neurosci* 13: 811–817, 1995.
109. Iantomasi T, Favilli F, Marraccini P, Stio M, Treves C, Quatrone A, Capaccioli S, and Vincenzini MT. Age and GSH metabolism in rat cerebral cortex, as related to oxidative and energy parameters. *Mech Age Dev* 70: 65–82, 1993.
110. Imbert V, Rupec RA, Livolsi A, Pahl HL, Traenckner EBM, Mueller-Kieckmann C, Farahifar D, Rossi B, Auberger P, Baeuerle PA, and Peyron JF. Tyrosine phosphorylation of IκB-α activates NF-κB without proteolytic degradation of IκB-α. *Cell* 86: 787–798, 1996.
111. Immenschuh S and Baumgart-Vogt E. Peroxiredoxins, oxidative stress, and cell proliferation. *Antioxid Redox Signal* 7: 768–777, 2005.
112. Iqbal K, Alonso AC, Chen S, Chohan MO, El-Akkad E, Gong CX, Khatoon S, Li B, liu F, Rahman A, Tanimukai H, and Grundke-Iqbal I. Tau pathology in Alzheimer disease and other tauopathies. *Biochim Biophys Acta* 1739: 198–210, 2005.
113. Iwakura Y, Piao YS, Mizuno M, Takei N, Kakita A, Takahashi H, and Nawa H. Influences of dopaminergic lesion on epidermal growth factor-ErbB signals in Parkinson's disease and its model: neurotrophic factor implication in nigrostriatal neurons. *J Neurochem* 93: 974–983, 2005.
114. Jaffrey SR, Erdjument-Bromage H, Ferris CD, Temps P, and Snyder SH. Protein S-nitrosylation: a physiological signal for nitric oxide. *Nat Cell Biol* 3: 193–197, 2001.
115. Jaken S. Protein kinase C isozymes and substrates. *Curr Opin Cell Biol* 8: 168–178, 1996.
116. Janssen-Heininger YMW, Poynter ME, and Baeuerle PA. Recent advances towards understanding redox mechanisms in the activation of nuclear factor κB. *Free Radic Biol Med* 28: 1317–1327, 2000.
117. Jiang D, Akopian G, Ho Y-S, Walsh JP, and Andersen JK. Chronic brain oxidation in a glutathione peroxidase knockout mouse model results in increased resistance to induced epileptic seizures. *Exp Neurol* 164: 257–268, 2000.
118. Johansson C, Lillig CH, and Holmgren A. Human mitochondrial glutaredoxin reduces S-glutathionylated proteins with high affinity accepting electrons from either glutathione or thioredoxin reductase. *J Biol Chem* 279: 7537–7543, 2004.
119. Jones DP. Redox potential of GSH/GSSG couple: assay and biological significance. *Methods Enzymol* 348: 93–112, 2002.
120. Jones DP, Go Y-M, Anderson CL, Ziegler TR, Kinkade JM, and Kirlin WG. Cysteine/cystine couple is a newly recognized node in the circuitry for biologic redox signaling and control. *FASEB J* 18: 1246–1248, 2004.
121. Jonsson TJ, Murray MS, Johnson LC, Poole LB, and Lowther WT. Structural basis for the retroreduction of inactivated peroxiredoxins by human sulfiredoxin. *Biochemistry* 44: 8634–8642, 2005.
122. Kabe YK, Ando S. Hirao M, Yoshida, and Handa H. Redox regulation of NF-κB activation: distinct redox regulation between the cytoplasm and nucleus. *Antioxid Redox Signal* 7: 395–403, 2005.
123. Kaelin WG. Proline hydroxylation and gene expression. *Annu Rev Biochem* 74: 115–128, 2005.
124. Kalia LV, Gingrich JR, and Salter MW. Src in synaptic transmission and plasticity. *Oncogene* 23: 8007–8016, 2004.
125. Kaltschmidt B, Widera D, and Kaltschmidt C. Signaling via NF-κB in the nervous system. *Biochim Biophys Acta* 1745: 287–299, 2005.
126. Kamata H and Hirata H. Redox regulation of cellular signalling. *Cell Signal* 11: 1–14, 1999.
127. Kamata H, Oka S-I, Shibukawa Y, Kakuta J, and Hirata H. Redox regulation of nerve growth factor-induced neuronal differentiation of PC12 cells through modulation of the nerve growth factor receptor, TrkA. *Arch Biochem Biophys* 434: 16–25, 2005.
128. Kato S, Kato M, Abe Y, Matsumura T, Nishino T, Aoki M, Itoyama Y, Asayama K, Awaya A, Hirano A, and Ohama E. Redox system expression in the motor neurons in amyotrophic lateral sclerosis (ALS): immunohistochemical studies on sporadic ALS, superoxide dismutase 1 (SOD1)-mutated familial ALS, and SOD1-mutated ALS animal models. *Acta Neuropathol (Berl)* 110: 101–112, 2005.
129. Kaur N, Lu B, Monroe RK, Ward SM, and Halvorsen SW. Inducers of oxidative stress block ciliary neurotrophic factor activation of Jak/STAT signaling in neurons. *J Neurochem* 92: 1521–1530, 2005.
130. Kenchappa R, Diwakar L, Boyd MR, and Ravindranath V. Thioltransferase (glutaredoxin) mediates recovery of motor neurons from excitotoxic mitochondrial injury. *J Neurosci* 22: 8402, 2002.
131. Kenchappa RS and Ravindranath V. Glutaredoxin is essential for maintenance of brain mitochondrial complex I: studies with MPTP. *FASEB J* 17: 717–719, 2003.
132. Keyse SM. The role of protein phosphatases in the regulation of mitogen and stress-activated protein kinases. Free Radic Res 31: 341–349, 1999.
133. Kil IS and Park J-W. Regulation of mitochondrial NADP+-dependent isocitrate dehydrogenase activity by glutathionylation. *J Biol Chem* 280: 10846–10854, 2005.

134. Kim H-Y and Gladyshev VN. Methionine sulfoxide reduction in mammals: characterization of methionine-R-sulfoxide reductases. *Mol Biol Cell* 15: 1055–1064, 2004.
135. Klatt P and Lamas S. Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. *Eur J Biochem* 267: 4928–4944, 2000.
136. Klatt P, Molina EP, Garcia de Lacoba M, Padilla CA, Martinez-Galisteo E, Barcena JA, and Lamas S. Redox regulation of c-Jun DNA binding by reversible S-glutathiolation. *FASEB J* 13: 1481–1490, 1999.
137. Klivenyi P, Andreassen OA, Ferrante RJ, Dedeoglu A, Mueller G, Lancelot E, Bogdanov M, Andersen JK, Jiang D, and Beal MF. Mice deficient in cellular glutathione peroxidase show increased vulnerability to malonate, 3-nitropropionic acid and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *J Neurosci* 20: 1–7, 2000.
138. Knebel A, Rahmsdorf HJ, Ullrich A, and Herrlich P. Dephosphorylation of receptor tyrosine kinases as target of regulation by radiation, oxidants or alkylating agents. *EMBO J* 15: 5314–5325, 1996.
139. Konishi H, Tanaka M, Takemura Y, Matsuzaki H, Ono Y, Kikkawa U, and Nishizuka Y. Activation of protein kinase C by tyrosine phosphorylation in response to H_2O_2. *Proc Natl Acad Sci U S A* 94: 11233–11237, 1997.
140. Kraft AD, Johnson DA, and Johnson JA. Nuclear factor E2-related factor 2-dependent antioxidant response element activation by *tert*-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult. *J Neurosci* 24: 1101–1112, 2004.
141. Krapfenbauer KE, Engidawork N, Cairns M, Fountoulakis E, and Lubec G. Aberrant expression of peroxiredoxin subtypes in neurodegenerative disorders. *Brain Res* 967: 152–160, 2003.
142. Kunikowska G and Jenner P. Alterations in mRNA expression for Cu, Zn-superoxide dismutase and glutathione peroxidase in the basal ganglia of MPTP-treated marmosets and patients with Parkinson's disease. *Brain Res* 968: 206–218, 2003.
143. Kyriakis JM and Avruch J. Protein kinase cascades activated by stress and inflammatory cytokines. *BioEssays* 18: 567–577, 1996.
144. Lander HM, Milbank AJ, Tauras JM, Hajjar DP, Hempstead BL, Schwartz GD, Kraemer RT, Mirza UA, Chait BT, Burk SC. and Quillam LA. Redox regulation of cell signaling. *Nature* 381: 380–381, 1996.
145. Lander HM, Ogiste JS, Teng KK, and Novogroksky A. $p21^{ras}$ as a common signaling target of reactive free radicals and cellular redox stress. *J Biol Chem* 270: 21195–21198, 1995.
146. Landino LM, Koumas MT, Mason CE, and Alston JA. Ascorbic acid reduction of microtubule protein disulfides and its relevance to protein S-nitrosylation. *Biochem Biophys Res Commun* 340: 347–352, 2006.
147. Lee J.-M, Calkins MJ, Chan K, Kan YW, and Johnson JA. Identification of the NF-E2-related factor-2-depedent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. *J Biol Chem* 278: 12029–12038, 2003.
148. Lee J.-M, Shih AY, Murphy TH, and Johnson TH. NF-E2-related factor-2 mediates neuroprotection against mitochondrial complex I inhibitors and increased concentrations of intracellular calcium in primary cortical neurons. *J Biol Chem* 278: 37948–37956, 2003.
149. Lee K and Esselman WJ. Inhibition of PTPs by H_2O_2 regulates the activation of distinct MAPK pathways. *Free Rad Biol Med* 33: 1121–1132, 2002.
150. Lee TD, Yang H, Whang J. and Lu SC. Cloning and characterization of the human glutathionine synthetase 5′-flanking region. *Biochem J* 390: 521–528, 2005.
151. Leppa S and Bohmann D. Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. *Oncogene* 18: 6158–6162, 1999.
152. Leslie NR, Lindsay Y, Ross SH, and Downes CP. Redox regulation of phosphatase function. *Biochem Soc Trans* 32: 1018–1020, 2004.
153. Levine RL, Moskovitz and Stadtman ER. Oxidation of methionine in proteins: roles in antioxidant defense and cellular regulation. *IUBMB Life* 50: 301–307, 2000.
154. Li J, Lee J-M, and Johnson JA. Microarray analysis reveals an antioxidant responsive element-driven gene set involved in conferring protection from an oxidative stress-induced apoptosis in IMR-32 cells. *J Biol Chem* 277: 388–394, 2002.
155. Lin D and Takemoto DJ. Oxidative activation of protein kinase Cγ through the C1 domain. *J Biol Chem* 280: 13682–13693, 2005.
156. Lind C, Gerdes R, Hamnell Y, Schuppe-Koistinen I, Brockenhuus von Lowenhielm H. Holmgren A, and Cotgreave IA. Identification of S-glutathionylated cellular proteins during oxidative stress and constitutive metabolism by affinity purification and proteome analysis. *Arch Biochem Biophys* 406: 229–240, 2002.
157. Lipton SA, Choi Y-B, Takahashi H, Zhang D, Li W, Godzik A, and Bankston LA. Cysteine regulation of protein function, as exemplified by NMDA-receptor modulation. *Trends Neurosci* 25: 474–480, 2002.
158. Lisman J, Schulman H, and Cline H. The molecular basis of CaMKII function in synaptic and behavioral memory. *Nat Rev Neurosci* 3: 175–190, 2002.
159. Liu H.R, Colavitti I. Rovira I, and Finkel T. Redox-dependent transcriptional regulation. *Circ Res* 97: 967–974, 2005.
160. Liu R.-M. Down-regulation of γ-glutamylcysteine synthetase regulatory subunit gene expression in rat brain tissue during aging. *J Neurosci Res* 68: 344–351, 2002.
161. Liu Y and Schubert D. Cytotoxic amyloid peptides inhibit cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction by enhancing MTT formazan exocytosis. *J Neurochem* 69: 2285–2293, 1997.
162. Lopez-Barea J, Barcena JA, Bocanegra JA, Florindo J, Garcia-Alfonso C, Lopez-Ruiz A, Martinez-Galisteo E, and Peinado J. Structure, mechanism, functions and regulatory properties of glutathione reductase. In: *Glutathione: Metabolism and Physiological Functions*, edited by Vina J. CRC, Boca Raton, FL, 1990, pp 105–116.
163. Lovell MA, Xie C, Gabbita SP, and Markesbery WR, Decreased thioredoxin and increased thioredoxin reductase levels in Alzheimer's disease brain. *Free Radic Biol Med* 28: 418–427, 2000.
164. Maher P. The effects of stress and aging on glutathione metabolism. *Ageing Res Rev* 4: 288–314, 2005.
165. Maher P. How protein kinase C activation protects nerve cells from oxidative stress-induced cell death. *J Neurosci* 21: 2929–2938, 2001.
166. Manevich Y and Fisher AB. Peroxiredoxin 6, a 1-Cys peroxiredoxin, functions in antioxidant defense and lung phospholipid metabolism. *Free Radic Biol Med* 38: 1422–1432, 2005.
167. Mansuy IM. Calcineurin in memory and bidirectional plasticity. *Biochem Biophys Res Commun* 311: 1195–1208, 2003.
168. Martinez-Ruiz A and Lamas S. S-nitrosylation: a potential new paradigm in signal transduction. *Cardiovasc Res* 62: 43–52, 2004.
169. Mary J, Vougier S, Picot CR, Perichon M, Petropoulos I, and Friguet B. Enzymatic reactions involved in the repair of oxidized proteins. *Exp Gernontol* 39: 1117–1123, 2004.
170. Masutani H, Bai J, Kim Y-C, and Yodoi J. Thioredoxin as a neurotrophic cofactor and an important regulator of neuroprotection. *Mol Neurobiol* 29: 229–242, 2004.
171. McBean GJ. Cerebral cystine uptake: a tale of two transporters. *Trends Pharmacol Sci* 23: 299–302, 2002.
172. Meister A and Anderson ME. Glutathione. *Annu Rev Biochem* 52: 711–760, 1983.
173. Mercurio F and Manning AM. NF-κB as a primary regulator of the stress response. *Oncogene* 18: 6163–6171, 1999.
174. Meriin AB, Yaglom JA, Gabai VL, Mosser DD, Zon L, and Sherman MY. Protein-damaging stresses activate c-Jun N-terminal kinase via inhibition of its dephosphorylation: a novel pathway controlled by HSP72. *Mol Cell Biol* 19: 2547–2555, 1999.
175. Minich T, Riemer JB, Schulz P, Wielinga J, Wijnholds J, and Dringen R. The multidrug resistance protein 1 (Mrp1), but not Mrp5, mediates export of glutathione and glutathione disulfide from brain astrocytes. *J Neurochem* 97: 373–384, 2006.
176. Mochly-Rosen D and Kauvar M. Modulating protein kinase C signal transduction. *Adv Neurol* 44: 91–145, 1998.
177. Moldovan L and Moldovan NL. Oxygen free radicals and redox biology of organelles. *Histochem Cell Biol* 122: 395–412, 2004.

178. Morel Y and Barouki R. Repression of gene expression by oxidative stress. *Biochem J* 342: 481–496, 1999.
179. Moskovitz J, Bar-Noy S, Williams WM, Requena J, Berlett BS, and Stadtman ER. Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc Natl Acad Sci U S A* 98: 12920–12925, 2001.
180. Moskovitz J, Jenkins NA, Gilbert DJ, Copeland NG, Jursky F, Weissback H, and Brot N. Chromosomal localization of the mammalian peptide-methionine sulfoxide reductase gene and its differential expression in various tissues. *Proc Natl Acad Sci U S A* 93: 3205–3208, 1996.
181. Moynaugh PN. The NF-κB pathway. *J Cell Sci* 118: 4589–4592, 2005.
182. Munton RP, Vizi S, and Mansuy IM. The role of protein phosphatase-1 in the modulation of synaptic and structural plasticity. *FEBS Lett* 567: 121–128, 2004.
183. Nakamura H. Thioredoxin and its related molecules: update 2005. *Antioxid Redox Signal* 7: 823–828, 2005.
184. Nakamura K, Hori T, Sato N, Sugie K, Kawakami T, and Yodoi J. Redox regulation of a *src* family protein tyrosine kinase $p56^{lck}$ in T cells. *Oncogene* 8: 3133–3139, 1993.
185. Naslund J, Schierhorn A, Hellman U, Lannfelt L, Roses AD, Tjernberg LO, Silberring J, Gandy SE, Winblad B, Greengard P, Nordstedt C, and Terenius L. Relative abundance of Alzheimer Aβ amyloid peptide variants in Alzheimer disease and normal aging. *Proc Natl Acad Sci U S A* 91: 8378–8382, 1994.
186. Nemani R and Lee EYC. Reactivity of sulfhydryl groups of the catalytic subunits of rabbit skeletal muscle protein phosphatases 1 and 2A. *Arch Biochem Biophys* 300: 24–29, 1993.
187. Nemoto S and Finkel T. Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. *Science* 295: 2450–2452, 2002.
188. Nguyen T, Sherratt PJ, Nioi P, Yang CS, and Pickett CB. Nrf2 controls constitutive and inducible expression of ARE-driven genes through a dynamic pathway involving nucleocytoplasmic shuttling by Keap1. *J Biol Chem* 280: 32485–32492, 2005.
189. Nguyen T, Sherratt PJ, and Pickett CB. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu Rev Pharmacol Toxicol* 43: 233–260, 2003.
190. Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. *FASEB J* 9: 484–496, 1995.
191. Noble M, Smith J, Power J, and Mayer-Proschel M. Redox state as a central modulator of precursor cell function. *Ann N Y Acad Sci* 991: 251–271, 2003.
192. Nordberg J and Arner ESJ. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med* 31: 1287–1312, 2001.
193. Nulton-Persson A, Starke DW, Mieyal JJ, and Szweda LI. Reversible inactivation of the α-ketoglutarate dehydrogenase in response to alterations in the mitochondrial glutathione status. *Biochemistry* 42: 4235–4242, 2003.
194. O'Brian CA, and Chu F. Post-translational disulfide modifications in cell signaling-role of inter-protein, intra-protein, S-glutathionyl, and S-cysteaminyl disulfide modifications in signal transmission. *Free Radic Res* 39: 471–480, 2005.
195. Oka A, Belliveau MJ, Rosenberg PA, and Volpe JJ. Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms and prevention. *J Neurosci* 13: 1441–1453, 1993.
196. Orr WC, Radyuk SN, Prabhudesai L, Toroser D, Benes JJ, Luchak JM, Mockett RJ, Rebrin I, Hubbard JG, and Sohal RS. Overexpression of glutamate-cysteine ligase extends life span in *Drosophila melanogaster*. *J Biol Chem* 280: 37331–37338, 2005.
197. Pallen CJ. Protein tyrosine phosphatase alpha (PTPalpha): a Src family kinase activator and mediator of multiple biological effects. *Curr Top Med Chem* 3: 821–835, 2003.
198. Palmblad M, Westlind-Danielsson A, and Bergquist A. Oxidation of methionine 35 attenuates formation of amyloid β-peptide 1–40 oligomers. *J Biol Chem* 277: 19506–19510, 2002.
199. Pastore A, Tozzi G, Gaeta LM, Bertini E, Serafini V, Di Cesare S, Bonetto V, Casoni F, Carrozzo R, Federici G, and Piemonte F. Actin glutathionylation increases in fibroblasts of patients with Friedreich's ataxia: a potential role in the pathogenesis of the disease. *J Biol Chem* 278: 42588–42595, 2003.
200. Patel R, McIntosh L McLaughlin J, Brooke S, Nimon V, and Sapolsky R. Disruptive effect of glucocorticoids on glutathione peroxidase biochemistry in hippocampal cultures. *J Neurochem* 82: 118–125, 2002.
201. Patenaude A, Murthy MRV, and Mirault M-E. Emerging roles of thioredoxin cycle enzymes in the central nervous system. *Cell Mol Life Sci* 62: 1063–1080, 2005.
202. Petropoulos I, Mary J, Perichon M, and Friguet B. Rat peptide methionine sulfoxide reductase: cloning of the cDNA, and down-regulation of gene expression and enzyme activity during aging. *Biochem J* 355: 819–825, 2001.
203. Poulsen FR, Blaabjerg M, Montero M, and Zimmer J. Glutamate receptor antagonists and growth factors modulate dentate granule cell neurogenesis in organotypoc, rat hippocampal slice cultures. *Brain Res* 1051: 35–49, 2005.
204. Raval AP, Dave KR, Mochly-Rosen D, Sick TJ, and Perez-Pinzon MA. ePKC is required for the induction of tolerance by ischemic and NMDA-mediated preconditioning in the organotypic hippocampal slice. *J Neurosci* 23: 384–391, 2003.
205. Rebrin I, Kamzalov S, and Sohal RS. Effects of age and caloric restriction on glutathione redox status in mice. *Free Radic Biol Med* 35: 626–635, 2003.
206. Rhee SG, Chae HZ, and Kim K. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med* 38: 1543–1552, 2005.
207. Rhee SG, Kang SW, Jeong W, Chang T-S, Yang K-S, and Woo HA. Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr Opin Cell Biol* 17: 183–189, 2005.
208. Rigacci S, Iantomasi T, Marraccini P, Berti A, Vincenzini MT, and Ramponi G. Evidence for glutathione involvement in platelet-derived growth-factor-mediated signal transduction. *Biochem J* 324: 792–796, 1997.
209. Rinaldi R, Eliasson E, Swedmark S, and Morgenstern R. Reactive intermediates and the dynamics of glutathione transferases. *Drug Metab Dispos* 30: 1053–1058, 2002.
210. Robinson MJ and Cobb MH. Mitogen-activated protein kinase pathways. *Curr Opin Cell Biol* 9: 180–186, 1997.
211. Ruan H, Tang XD, Chen M-L, Joiner A, Sun G, Brot N, Weissbach Heinemann CH, Wu C-F, and Hoshi T. High-quality life extension by the enzyme peptide methionine sulfoxide reductase. *Proc Natl Acad Sci U S A* 99: 2748–2753, 2002.
212. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata MK, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17, 1998.
213. Salmeen A and Barford D. Functions and mechanisms of redox regulation of cysteine-based phosphatases. *Antioxid Redox Signal* 7: 560–577, 2005.
214. Sandhu SK and Kaur J. Alterations in oxidative stress scavenger system in aging rat brain and lymphocytes. *Biogerontology* 3: 161–173, 2002.
215. Sato H, Tamba M, Okuno S, Sato K, Keino-Masu K, Masu M, and Bannai S. Distribution of cystine/glutamate exchange transporter, system x_c^-, in the mouse brain. *J Neurosci* 22: 8028–8033, 2002.
216. Schaeffer HJ and Weber MJ. Mitogen-activated protein kinases: specific messages from ubiquitous messengers. *Mol Cell Biol* 19: 2435–2444, 1999.
217. Schafer FQ and Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 30: 1191–1212, 2001.
218. Schonhoff CM, Matsuoka M. Tummala H, Johnson MA, Estevez AG, Wu R, Kamaid A, Ricart KC, Hashimoto Y, Gaston B, Macdonald TL, Xu Z, and Mannick JB, S-nitrosothiol depletion in amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 103: 2404–2409, 2006.
219. Schulz JB, Lindenau J, Seyfried J, and Dichgans J. Glutathione, oxidative stress and neurodegeneration. *Eur J Biochem* 267: 4904–4911, 2000.

220. Semenza GL. Signal transduction to hypoxia-inducible factor 1. *Biochem Pharmacol* 64: 993–998, 2002.
221. Sen CK and Packer L. Antioxidant and redox regulation of gene transcription. *FASEB J* 10: 709–720, 1996.
222. Shackelford RE, Heinloth AN, Heard SC, and Paules RS. Cellular and molecular targets of protein S-glutathiolation. *Antioxid Redox Signal* 7: 940–950, 2005.
223. Shah BH, Neithardt A, Chu DB, Shah FB, and Catt KJ. Role of EGF receptor transactivation in phosphoinositide 3-kinase-dependent activation of MAP kinase by GPCRs. *J Cell Physiol* 206: 47–57, 2006.
224. Shanker G, Allen JW, Mutkus LA, and Aschner M. The uptake of cysteine in cultured primary astrocytes and neurons. *Brain Res* 902: 156–163, 2001.
225. Shelton MD, Chock PB, and Mieyal JJ. Glutaredoxin: role in reversible protein S-glutathionylation and regulation of redox signal transduction and protein translocation. *Antioxid Redox Signal* 7: 348–366, 2005.
226. Shih AY, Johnson DA, Wong G, Kraft AD, Jiang L, Erb H, Johnson JA, and Murphy TH. Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. *J Neurosci* 23: 3394–3406, 2003.
227. Sian J, Dexter DT, Lees AJ, Daniel S, Jenner P, and Marsden CD. Glutathione-related enzymes in brain in Parkinson's disease. *Ann Neurol* 36: 356–361, 1994.
228. Sims NR, Nilsson M, and Muyderman H. Mitochondrial glutathione: a modulator of brain cell death. *J Bioenerg Biomembr* 36: 329–333, 2004.
229. Sivaramakrishnan S, Keerthi K, and Gates KS. A chemical model for redox regulation of protein tyrosine phosphatase 1B (PTP1B) activity. *J Am Chem Soc* 127: 10830–10831, 2005.
230. Soderdahl T, Enoksson M, Lundberg M, Holmgren A, Ottersen OP, Orrenius S, Bolcsfoldi G, and Cotgreave IA. Visualization of the compartmentalization of glutathione and protein-glutathione mixed disulfides in cultured cells. *FASEB J* 17: 124–126, 2003.
231. Soltaninassab SR, Sekhar KR, Meredith MJ, and Freeman ML. Multi-faceted regulation of γ-glutamylcystein synthetase. *J Cell Physiol* 183: 163–170, 2000.
232. Song JJ, Rhee JG, Suntharalingam M, Walsh SA, Spitz DR, and Lee YJ. Role of glutaredoxin in metabolic oxidative stress. *J Biol Chem* 277: 46566–46575, 2002.
233. Soucek T, Cumming R, Dargusch R, Maher P, and Schubert D. The regulation of glucose metabolism by HIF-1 mediates a neuroprotective response to amyloid beta peptide. *Neuron* 39: 43–56, 2003.
234. Sparaco M, Gaeta LM, Tozzi G, Bertini E, Pastore A, Simonati A, Santorelli FM, and Piemonte F. Protein glutathionylation in human central nervous system: potential role in redox regulation of neuronal defense against free radicals. *J Neurosci Res* 83: 256–263, 2006.
235. Stoker AW. Receptor tyrosine phosphatases in axon growth and guidance. *Curr Opin Neurobiol* 11: 95–102, 2001.
236. Stone JR. An assessment of proposed mechanisms for sensing hydrogen peroxide in mammalian cells. *Arch Biochem Biophys* 422: 119–124, 2004.
237. Sumbayev VV and Yasinka IM. Regulation of MAP kinase-dependent apoptotic pathway: implication of reactive oxygen and nitrogen species. *Arch Biochem Biophys* 436: 406–412, 2005.
238. Sun H, Gao J, Ferrington DA, Biesiada H, Williams TD, and Squier TC. Repair of oxidized calmodulin by methionine sulfoxide reductase restores ability to activate the plasma membrane Ca-ATPase. *Biochemistry* 38: 105–112, 1999.
239. Sun Q-A, Su D, Novoselov SV, Carlson BA, Hatfield DL, and Gladyshev VN. Reaction mechanism and regulation of mammalian thioredoxin/glutaredoxin reductase. *Biochemistry* 44: 14528–14537, 2005.
240. Sun X, Erb H, and Murphy TH. Coordinate regulation of glutathione metabolism in actrocytes by Nrf2. *Biochem Biophys Res Commun* 326: 371–377, 2005.
241. Sun X, Shih AY, Johannsen HC, Erb H, Li P, and Murphy TH. Two-photon imaging of glutathione levels in intact brain indicates enhanced redox buffering in developing neurons and cells at the cerebrospinal fluid and blood-brain interface. *J Biol Chem* (in press), 2006.
242. Sun Y and Oberley LW. Redox regulation of transcriptional activators. *Free Radic Biol Med* 21: 335–348, 1996.
243. Tan S, Schubert D, and Maher P. Oxytosis: a novel form of programmed cell death. *Curr Top Med Chem* 1: 497–506, 2001.
244. Taylor ER, Hurrell F, Shannon RJ, Lin TK, Hirst J, and Murphy MP. Reversible glutathionylation of complex I increases mitochondrial superoxide formation. *J Biol Chem* 278: 19603–19610, 2003.
245. Taylor JM, Ali U, Iannello RC, Hertzog P, and Crack PJ. Diminished Akt phosphorylation in neurons lacking glutathione peroxidase-1 (Gpx1) leads to increased susceptibility to oxidative stress-induced death. *J Neurochem* 92: 283–293, 2005.
246. Thiruchelvam M, Prokopenko O, Cory-Slechta DA, Richfield EK, Buckley B, and Mirochnitchenko O. Overexpression of superoxide dismutase or glutathione peroxidase protects against the paraquat + maneb-induced Parkinson's disease phenotype. *J Biol Chem* 280: 22530–22539, 2005.
247. Thomas JA and Mallis RJ. Aging and oxidation of reactive protein sulfhydryls. *Exp Gerontol* 36: 1519–1526, 2001.
248. Thomas JA, Poland B, and Honzatko R. Protein sulfhydryls and their role in the antioxidant function of protein S-thiolation. *Arch Biochem Biophys* 319: 1–9, 1995.
249. Tickler AK, Wade JD, and Separovic F. The role of Abeta peptides in Alzheimer's disease. *Protein Pept Lett* 12: 513–519, 2005.
250. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem* 106: 207–212, 1969.
251. Tomita S, Ueno M, Sakamoto M, Kitahama Y, Ueki M, Maekawa N, Sakamoto Gassman M, Kageyama R, Ueda N, Gonzalez FJ, and Takahama Y. Defective brain development in mice lacking the *Hif-1*α gene in neural cells. *Mol Cell Biol* 23: 6739–6749, 2003.
252. Townsend DM and Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. *Oncogene* 22: 7369–7375, 2003.
253. Townsend DM, Tew KD, and Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother* 57: 145–155, 2003.
254. Ullrich V, Namgaladze D, and Frein D. Superoxide as inhibitor of calcineurin and mediator of redox regulation. *Toxicol Lett* 139: 107–110, 2003.
255. Wang H, Yuan G, Prabhakar NR, Boswell M, and Katz DM. Secretion of brain-derived neurotrophic factor from PC12 cells in response to oxidative stress requires autocrine dopamine signaling. *J Neurochem* 96: 694–705, 2006.
256. Wang J, Boja ES, Tan W, Tekle E, Fales HM, English S, Mieyal JJ, and Chock PB. Reversible glutathionylation regulates actin polymerization in A431 cells. *J Biol Chem* 276: 47763–47766, 2001.
257. Wang W, Culotta VC, and Klee CB. Superoxide dismutase protects calcineurin from inactivation. *Nature* 383: 434–437, 1996.
258. Wang X and Tournier C. Regulation of cellular functions by the ERK5 signaling pathways. *Cell Signal* 18: 753–760, 2006.
259. Weissbach H, Resnick L, and Brot N. Methionine sulfoxide reductases: history and cellular role in protecting against oxidative damage. *Biochim Biophys Acta* 1703: 203–212, 2005.
260. Wenger RH. Cellular adaptation to hypoxia: O_2 sensing protein hydroxylases, hypoxia-inducible transcription factors, and O_2-regulated gene expression. *FASEB J* 16: 1151–1162, 2002.
261. Wild AC and Mulcahy RT. Regulation of γ-glutamylcysteine synthetase subunit gene expression: insights into transcriptional control of antioxidant defenses. *Free Radic Res* 32: 281–301, 2000.
262. Wisdom R. AP-1: one switch for many signals. *Exp Cell Res* 253: 180–185, 1999.
263. Woo HA, Chae HZ, Hwang SC, Yang K-S, Kang SW, Kim K, and Rhee SG. Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation. *Science* 300: 653–656, 2003.
264. Woo HA, Jeong W, Chang T-S, Park KJ, Park SJ, Yang JS, and Rhee SG. Reduction of cysteine sulfinic acid by sulfiredoxin is

specific to 2-cys peroxiredoxins. *J Biol Chem* 280: 3125–3128, 2005.
265. Woo HA, Kang SW, Kim HK, Yang KS, Chae HZ, and Rhee SG. Reversible oxidation of the active site cysteine of peroxiredoxins to cysteine sulfinic acid. *J Biol Chem* 278: 47361–47364, 2003.
266. Wood ZA, Poole LB, Hantgan RR, and Karplus PA. Dimers to doughnuts: redox sensitive oligomerization of 2-cysteine peroxiredoxins. *Biochemistry* 41: 5493–5504, 2002.
267. Wood ZA, Poole LB, and Karplus PA. Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* 300: 650–653, 2003.
268. Wood ZA, Schroder E, Harris JR, and Poole LB. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* 28: 32–40, 2003.
269. Yamin G, Glaser CB, Uversky VN, and Fink AL. Certain metals trigger fibrillation of methionine-oxidized α-synuclein. *J Biol Chem* 278: 27630–27635, 2003.
270. Yantiri F, Gasparyan A, and Andersen JK. Glutamyl cysteine synthetase catalytic and regulatory subunits localize to dopaminergic nigral neurons as well as astrocytes. *J Neurosci Res* 64: 203–206, 2001.
271. Yermolaieva O, Xu R, Schinstock C, Brot N, Weissbach H, Heinemann SH, and Hoshi T. Methionine sulfoxide reductase A protects neuronal cells against brief hypoxia/reoxygenation. *Proc Natl Acad Sci U S A* 101: 1159–1164, 2004.
272. Yoshida T, Oka S-I, Masutani M, Nakamura H, and Yodoi J. The role of thioredoxin in the aging process: involvement of oxidative stress. *Antioxid Redox Signal* 5: 563–570, 2003.
273. Zhang B, Tanaka J, Yang L, Yang L, Sakanaka M, Hata R, Maeda N, and Mitsuda N. Protective effect of vitamin E against focal brain ischemia and neuronal death through induction of target genes of hypoxia-inducible factor-1. *Neuroscience* 126: 433–440, 2004.
274. Zhong L and Holmgren M. Essential role of selenium in the catalytic activities of mammalian thioredoxin reductase revealed by characterization of recombinant enzymes with selenocysteine mutations. *J Biol Chem* 275: 18121–18128, 2000.

Address reprint requests to:
Pamela Maher
The Salk Institute for Biological Studies
10010 N. Torrey Pines Rd.
La Jolla, CA 92037

E-mail: pmaher@salk.edu

First submission to ARS Central, April 24, 2006; date of acceptance, May 31, 2006.

This article has been cited by:

1. Fei Yin , Harsh Sancheti , Enrique Cadenas . Mitochondrial Thiols in the Regulation of Cell Death Pathways. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Kelsey H. Fisher-Wellman, P. Darrell Neufer. 2012. Linking mitochondrial bioenergetics to insulin resistance via redox biology. *Trends in Endocrinology & Metabolism* . [CrossRef]
3. Fang Liu, Lei Guo, Jie Zhang, Shuo W. Rainosek, Leming Shi, Tucker A. Patterson, Quan-Zhen Li, Natalya Sadovova, Joseph P. Hanig, Merle G. Paule, William Slikker, Cheng Wang. 2012. Inhalation Anesthesia-Induced Neuronal Damage and Gene Expression Changes in Developing Rat Brain. *Systems Pharmacology* **1**:-1, 1-9. [CrossRef]
4. Rajindar S. Sohal, William C. Orr. 2011. The redox stress hypothesis of aging. *Free Radical Biology and Medicine* . [CrossRef]
5. Arthur JL Cooper, John T Pinto, Patrick S Callery. 2011. Reversible and irreversible protein glutathionylation: biological and clinical aspects. *Expert Opinion on Drug Metabolism & Toxicology* **7**:7, 891-910. [CrossRef]
6. Katherine Bowers, Qing Li, Joseph Bressler, Dimitrios Avramopoulos, Craig Newschaffer, M. Daniele Fallin. 2011. Glutathione pathway gene variation and risk of autism spectrum disorders. *Journal of Neurodevelopmental Disorders* **3**:2, 132-143. [CrossRef]
7. Tae-Hoon Kim, Seong-Jin Yoon, Woo-Cheol Lee, Joon-Ki Kim, Joonshik Shin, Sangho Lee, Sun-Mee Lee. 2011. Protective effect of GCSB-5, an herbal preparation, against peripheral nerve injury in rats. *Journal of Ethnopharmacology* **136**:2, 297-304. [CrossRef]
8. Danilo Grünig Humberto Silva, Edis Belini Junior, Lidiane de Souza Torres, Octávio Ricci Júnior, Clarisse de Castro Lobo, Claudia Regina Bonini-Domingos, Eduardo Alves de Almeida. 2011. Relationship between oxidative stress, glutathione S-transferase polymorphisms and hydroxyurea treatment in sickle cell anemia. *Blood Cells, Molecules, and Diseases* **47**:1, 23-28. [CrossRef]
9. B. Carletti, C. Passarelli, M. Sparaco, G. Tozzi, A. Pastore, E. Bertini, F. Piemonte. 2011. Effect of protein glutathionylation on neuronal cytoskeleton: a potential link to neurodegeneration. *Neuroscience* . [CrossRef]
10. Jan Lewerenz , Pamela Maher . 2011. Control of Redox State and Redox Signaling by Neural Antioxidant Systems. *Antioxidants & Redox Signaling* **14**:8, 1449-1465. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
11. Marc Gleichmann , Mark P. Mattson . 2011. Neuronal Calcium Homeostasis and Dysregulation. *Antioxidants & Redox Signaling* **14**:7, 1261-1273. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
12. Torrie T. Jones, Gregory J. Brewer. 2010. Age-related deficiencies in complex I endogenous substrate availability and reserve capacity of complex IV in cortical neuron electron transport. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1797**:2, 167-176. [CrossRef]
13. Oxidative Stress and Movement Disorders 339-349. [CrossRef]
14. Isuzu Hama, Saya Nakagomi, Hiroyuki Konishi, Hiroshi Kiyama. 2010. Simultaneous expression of glutathione, thioredoxin-1, and their reductases in nerve transected hypoglossal motor neurons of rat. *Brain Research* **1306**, 1-7. [CrossRef]
15. Renata Padilha Guedes, Lidiane Dal Bosco, Alex Sander da Rosa Araújo, Adriane Belló-Klein, Maria Flávia Marques Ribeiro, Wania Aparecida Partata. 2009. Sciatic nerve transection increases gluthatione antioxidant system activity and neuronal nitric oxide synthase expression in the spinal cord. *Brain Research Bulletin* **80**:6, 422-427. [CrossRef]
16. Makoto Naoi, Hong Yi, Wakako Maruyama, Keiko Inaba, Masayo Shamoto-Nagai, Yukihiro Akao, Manfred Gerlach, Peter Riederer. 2009. Glutathione redox status in mitochondria and cytoplasm differentially and sequentially activates apoptosis cascade in dopamine-melanin-treated SH-SY5Y cells. *Neuroscience Letters* **465**:2, 118-122. [CrossRef]
17. Makoto Naoi, Wakako Maruyama, Hong Yi, Keiko Inaba, Yukihiro Akao, Masayo Shamoto-Nagai. 2009. Mitochondria in neurodegenerative disorders: regulation of the redox state and death signaling leading to neuronal death and survival. *Journal of Neural Transmission* **116**:11, 1371-1381. [CrossRef]
18. Jianyong Wang, Mohammed F. Rahman, Helen M. Duhart, Glenn D. Newport, Tucker A. Patterson, Richard C. Murdock, Saber M. Hussain, John J. Schlager, Syed F. Ali. 2009. Expression changes of dopaminergic system-related genes in PC12 cells induced by manganese, silver, or copper nanoparticles. *NeuroToxicology* **30**:6, 926-933. [CrossRef]
19. T. N. Pitlik, P. M. Bulai, A. A. Denisov, D. S. Afanasenkov, S. N. Cherenkevich. 2009. Redox regulation of ionic homeostasis in neurons. *Neurochemical Journal* **3**:2, 87-92. [CrossRef]

20. Pamela Maher, Jan Lewerenz, Carles Lozano, Josep Lluís Torres. 2008. A novel approach to enhancing cellular glutathione levels. *Journal of Neurochemistry* **107**:3, 690-700. [CrossRef]
21. V KARAMYAN, F GEMBARDT, F RABEY, T WALTHER, R SPETH. 2008. Characterization of the brain-specific non-AT1, non-AT2 angiotensin binding site in the mouse. *European Journal of Pharmacology* **590**:1-3, 87-92. [CrossRef]
22. Le Zhang, Tongle Deng, Yiguo Sun, Kezhou Liu, Yi Yang, Xiaoxiang Zheng. 2008. Role for nitric oxide in permeability of hippocampal neuronal hemichannels during oxygen glucose deprivation. *Journal of Neuroscience Research* **86**:10, 2281-2291. [CrossRef]
23. Robert C. Speth, Vardan T. Karamyan. 2008. Brain angiotensin receptors and binding proteins. *Naunyn-Schmiedeberg's Archives of Pharmacology* **377**:4-6, 283-293. [CrossRef]
24. Makoto Naoi, Wakako Maruyama, Hong Yi, Yuriko Yamaoka, Masayo Shamoto-Nagai, Yukihiro Akao, Manfred Gerlach, Masashi Tanaka, Peter Riederer. 2008. Neuromelanin selectively induces apoptosis in dopaminergic SH-SY5Y cells by deglutathionylation in mitochondria: involvement of the protein and melanin component. *Journal of Neurochemistry* **105**:6, 2489-2500. [CrossRef]
25. Yuanbin Liu, Richard Dargusch, Pamela Maher, David Schubert. 2008. A broadly neuroprotective derivative of curcumin. *Journal of Neurochemistry* **105**:4, 1336-1345. [CrossRef]
26. Isabella Dalle–Donne , Aldo Milzani , Nicoletta Gagliano , Roberto Colombo , Daniela Giustarini , Ranieri Rossi . 2008. Molecular Mechanisms and Potential Clinical Significance of S-Glutathionylation. *Antioxidants & Redox Signaling* **10**:3, 445-474. [Abstract] [Full Text PDF] [Full Text PDF with Links]
27. Hung-Ming Chang, Yi-Lun Huang, Chyn-Tair Lan, Un-In Wu, Ming-E Hu, Su-Chung Youn. 2008. Melatonin preserves superoxide dismutase activity in hypoglossal motoneurons of adult rats following peripheral nerve injury. *Journal of Pineal Research* **44**:2, 172-180. [CrossRef]
28. Ashok K. Singh, Shveta Gupta, Yin Jiang. 2008. Oxidative stress and protein oxidation in the brain of water drinking and alcohol drinking rats administered the HIV envelope protein, gp120. *Journal of Neurochemistry* **104**:6, 1478-1493. [CrossRef]
29. D. Allan Butterfield, Tanea Reed, Shelley F. Newman, Rukhsana Sultana. 2007. Roles of amyloid #-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radical Biology and Medicine* **43**:5, 658-677. [CrossRef]