

# Modeling Oxidative Stress in the Central Nervous System

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**Abstract:** Oxidative stress is associated with the onset and pathogenesis of several prominent central nervous system disorders. Consequently, there is a pressing need for experimental methods for studying neuronal responses to oxidative stress. A number of techniques for modeling oxidative stress have been developed, including the use of inhibitors of the mitochondrial respiratory chain, depletion of endogenous antioxidants, application of products of lipid peroxidation, use of heavy metals, and models of ischemic brain injury. These experimental approaches can be applied from cell culture to *in vivo* animal models. Their use has provided insight into the molecular underpinnings of oxidative stress responses in the nervous system, including cell recovery and cell death. Reactive oxygen species contribute to conformational change-induced activation of signaling pathways, inactivation of enzymes through modification of catalytic cysteine residues, and subcellular redistribution of signaling molecules. In this review, we will discuss several methods for inducing oxidative stress in the nervous system and explore newly emerging concepts in oxidative stress signaling.

## OXIDATIVE STRESS IN THE CENTRAL NERVOUS SYSTEM

Aberrant generation of reactive oxygen species (ROS) is closely linked to the pathogenesis of a number of neurologic disorders, including stroke, Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis [1-4]. Oxidative stress arises from a homeostatic imbalance between the generation and scavenging of free radicals [5]. While ROS are found in all mammalian tissues, the brain is especially predisposed to oxidative stress [2, 6]. Neurons have high metabolic demand, and they express lower levels of endogenous antioxidants than other cells with high energy requirements [7-9]. In addition, the brain is rich in lipids and unsaturated fatty acids that serve as targets for lipid peroxidation. During aging the scavenging activities of enzymes are compromised, decreasing the ability of neurons to defend themselves from ROS [4, 5]. Thus, the discrepancy between metabolic demand and antioxidant resources renders neurons particularly vulnerable to oxidative damage.

Oxidative stress is reflected in the increase of free radicals and subsequent oxidation of lipids, DNA, and proteins [4]. Despite increasing evidence supporting the correlation of oxidative stress with neuronal pathology, it is unknown whether oxidative stress-induced death is a cause or consequence of neurodegenerative disease [3, 10]. Oxidative stress is known to engage an increasing number of candidate signaling molecules and networks that promote cell recovery or cell death [3, 4, 10]. Thus, a primary goal of oxidative stress research is to elucidate which signaling pathways are specifically

activated in response to ROS and to determine the roles of the ROS-induced signals in cell death or cell recovery.

To fully elucidate oxidative stress signaling pathways in neurons, it has been critical to develop a number of *in vitro* and *in vivo* experimental techniques that directly induce oxidative stress in the central nervous system (CNS). In the first part of this review, we will outline some of the more commonly used methods for inducing oxidative stress in the CNS, including exposure to peroxides or toxins, glutathione depletion, lipid peroxidation, heavy metals, and ischemia. These experimental approaches are of increasing complexity, and can be applied to dissociated neuronal cultures and slice cultures *in vitro* as well as *in vivo* in animal models of disease. Together, they provide a means for modeling acute and chronic oxidative stress in the nervous system. In the second part of the review, we will examine evidence that has emerged from the use of these experimental approaches towards elucidating some of the key signaling mechanisms involved in mediating oxidative stress responses in neurons.

## I. PARADIGMS AND STIMULI THAT TRIGGER OXIDATIVE STRESS IN THE NERVOUS SYSTEM

### Mitochondrial Reactive Oxygen Species

Reactive oxygen species (ROS) include superoxide, hydrogen peroxide, hydroxyl radicals, and intermediates of lipid peroxidation [4, 11]. While ROS can be generated by several means, including exposure to ionizing radiation, cytotoxic chemicals and phagocytes, the generation of superoxide by the mitochondrial respiratory chain is considered to be the primary source of endogenously occurring

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ROS [5, 11]. Thus, while mitochondria are essential for meeting cellular energy demands, they pose an inherent risk to cellular and organismal health.

The inner mitochondrial membrane of eukaryotes hosts the mitochondrial respiratory chain, whose principal role is to generate ATP to meet cellular energy demands. Through the process of oxidative phosphorylation, electrons are passed along the electron transport chain, generating a proton-motive force across the inner mitochondrial membrane that drives the production of ATP (for a review, see [11]). The respiratory chain consists of five enzyme complexes: (I) NADH-quinone oxidoreductase, (II) succinate-quinone oxidoreductase, (III) quinol-cytochrome c oxidoreductase, (IV) cytochrome c oxidase, and (V)  $F_1F_0$  ATPase. The production of superoxide at complexes I and III is believed to be the principal source of mitochondrially derived ROS Fig. (1) [11].

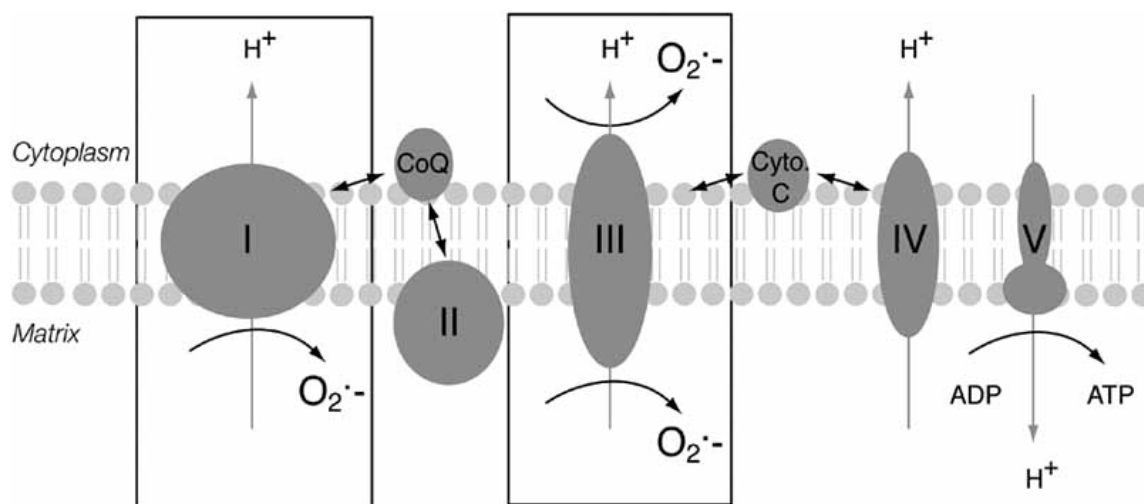
The generation of superoxide triggers a cascade of biochemical reactions that in turn generates additional ROS. Superoxide dismutase (SOD) catalyzes a reaction that dismutates superoxide to the less toxic hydrogen peroxide. Hydrogen peroxide itself is neutral and readily permeates cellular membranes. Once inside the cell, it serves as a substrate for several reactions, including (1) reduction by catalase to water and oxygen, (2) reduction by glutathione peroxidase to water, and (3) conversion by reduced metals *via* the Fenton reaction to the highly reactive hydroxyl radical Fig. (2) [12]. The hydroxyl radical reacts rapidly with biological macromolecules including proteins, lipids, nucleic acids, and carbohydrates, from which it abstracts hydrogen atoms, rendering stable free radicals with the potential to propagate chain reactions of radical formation.

Since hydrogen peroxide serves as a precursor to the hydroxyl radical, it has been widely used to induce oxidative stress *in vitro* in a number of cell culture systems [13-15]. However, due to its high reactivity and consequently short half-life, a number of additional methods are frequently used that allow for added experimental flexibility.

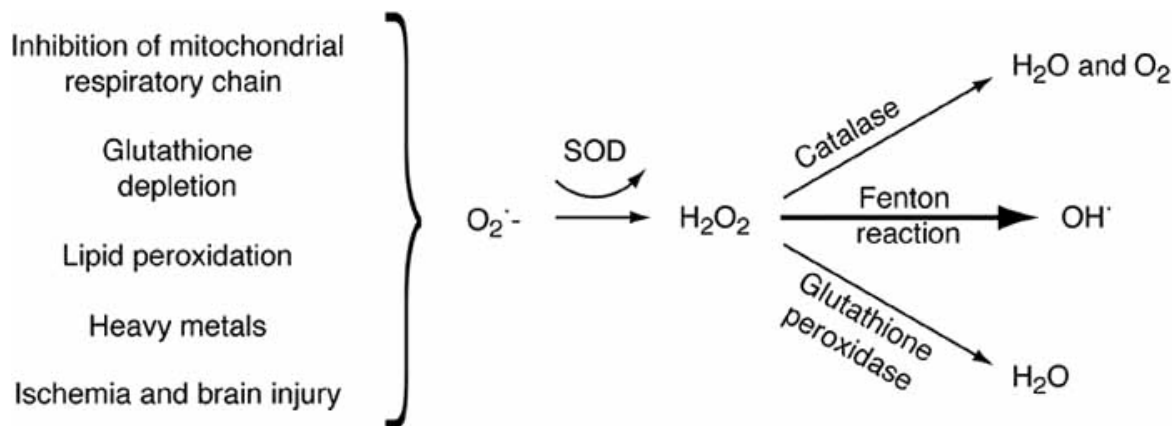
### Mitochondrial Respiratory Chain

A number of naturally occurring and synthetic reagents specifically target the mitochondrial respiratory chain and lead to the production of ROS *in vitro* and *in vivo*. While the exact mechanisms of action of these compounds are only beginning to be understood, many of these reagents can be used to selectively target subsets of neurons for oxidative stress-induced death. Among these, MPTP (*N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), rotenone, and paraquat (*N,N'*-dimethyl-4,4'-bipyridinium) are frequently used to induce oxidative stress in the CNS, and are thought to function by inhibition of the mitochondrial respiratory chain [16-18]. MPTP, the best characterized of these reagents, is a lipophilic compound that crosses the blood-brain-barrier. MPTP selectively destroys the nigrostriatal dopaminergic pathway and thus is widely used as a model for Parkinson's disease [19-21]. MPTP is taken up by astrocytes or serotonergic neurons, which metabolize MPTP to MPP<sup>+</sup> (1-methyl-4-pyridinium) by monoamine oxidase B [22, 23]. Subsequent uptake of MPP<sup>+</sup> *via* dopamine, serotonin, and norepinephrine transporters generates oxidative stress *via* inhibition of complex I of the respiratory chain [16, 23, 24].

The pesticide rotenone is a plant-derived toxin that is structurally similar to MPP<sup>+</sup>, is highly lipophilic, and permeates cellular membranes [25]. Continuous



**Fig. (1).** A schematic modeling the generation of mitochondrially-derived ROS. During oxidative phosphorylation, electrons are passed along the mitochondrial respiratory chain. This process ultimately leads to a potential energy across the mitochondrial membrane that drives the production of ATP. However, oxidative phosphorylation also allows for the generation of ROS. The production of superoxide at complexes I and III is thought to be the primary source of mitochondrially-derived ROS [11].



**Fig. (2).** A number of experimental techniques generate oxidative stress in the nervous system. Many experimental methods lead to the production of superoxide ( $O_2^{\cdot-}$ ) which can be dismutated by superoxide dismutase (SOD) to hydrogen peroxide ( $H_2O_2$ ). Hydrogen peroxide itself is neutral and can be converted to water ( $H_2O$ ) and oxygen ( $O_2$ ). However, hydrogen peroxide also reacts with reduced metals to produce the hydroxyl radical ( $OH^{\cdot}$ ) via the Fenton reaction. The hydroxyl radical in turn reacts with additional biological macromolecules, thus propagating a chain reaction of ROS production.

administration of rotenone to rats leads to a sustained inhibition of complex I and to a subsequent degeneration of the nigrostriatal dopaminergic pathway [17]. Another compound with structural similarity to MPP<sup>+</sup> that is widely used to generate oxidative stress is paraquat. Paraquat leads to increased superoxide levels and subsequent neuronal cell death [26-28]. However despite its similarity to MPP<sup>+</sup>, paraquat does not cross the blood-brain-barrier as easily [29], and may have a distinct mechanisms of action [30]. Taken together, MPTP, rotenone, and paraquat provide attractive means for chemically inducing oxidative stress both *in vitro* and *in vivo*.

The dopamine analog 6-hydroxydopamine (6-OHDA) is a commonly used neurotoxin that selectively induces the death of catecholaminergic neurons [31, 32]. Because 6-OHDA does not readily cross the blood-brain-barrier, for *in vivo* studies it must be injected directly into the brain. *In vitro*, cells can be treated directly with 6-OHDA. Once inside the cell, 6-OHDA inhibits both complex I and IV of the respiratory chain and leads to the generation of superoxide, hydrogen peroxide, and hydroxyl radicals [33-36]. Moreover, 6-OHDA leads to a ROS-related loss of mitochondrial membrane potential [37]. Thus, 6-OHDA represents an attractive paradigm for models of Parkinson's disease, in which oxidative stress is implicated in dopaminergic neuronal loss.

The plant-derived mycotoxin 3-nitropropionic acid (3-NP), is an important inducer of ROS that specifically targets striatal neurons *in vivo* [38, 39]. By irreversibly inhibiting the Krebs cycle and complex II of the respiratory chain, 3-NP promotes the generation of hydroxyl radicals leading to neuronal death. 3-NP can be administered intraperitoneally to model Huntington's disease [40, 41]. 3-NP has also been used to induce neuronal cell death in the

inferior olive to mimic the cerebellar ataxias associated with olivopontocerebellar atrophy [42].

Overall, several approaches have been developed that successfully induce ROS in the CNS through the use of reagents targeting the mitochondria. The use of these paradigms offers in some cases the possibility of targeting distinct neuronal populations, and thus the ability to model specific diseases.

### Glutathione Depletion

Glutathione depletion provides a unique approach for inducing oxidative stress by reducing levels of the endogenously occurring anti-oxidant glutathione. Glutathione depletion can be produced in primary cortical cultures by chronic exposure to glutamate or a glutamate analog, homocysteate quisqualate ibotenate [43-45]. These compounds induce oxidative stress *via* inhibition of glutathione synthesis, independently of ionotropic glutamate receptor-mediated excitotoxicity. Under basal conditions, cystine, the precursor of the essential amino acid cysteine, is taken up by cells *via* the cystine/glutamate transporter. In turn, cysteine is required for the synthesis of the antioxidant glutathione. Excess glutamate competitively inhibits cystine uptake by the cystine/glutamate transporter, and therefore leads to reduced glutathione synthesis [43]. While inhibitors of the rate-limiting enzyme of glutathione synthesis,  $\gamma$ -glutamylcysteine synthetase, are also available [46], glutathione depletion provides a means of inducing oxidative stress endogenously in neurons [44, 47-49].

### Lipid Peroxidation

Free radicals trigger the peroxidation of polyunsaturated fatty acids, including cholesterol

esters, phospholipids, and triglycerides. Thus, treatment of cells with products of lipid peroxidation provides an alternative method for monitoring the biological effects of oxidative stress [50, 51]. The aldehyde products of lipid peroxidation can be grouped into three major categories, 4-hydroxy-2-alkenals, 2-alkenals, and ketoaldehydes [50]. These aldehyde products diffuse freely and react with macromolecules, including proteins, phospholipids, and DNA [50, 52]. In this manner, they directly trigger oxidative damage within cells.

The aldehyde with the highest potential toxicity to cells, 4-hydroxy-2-nonenal (HNE), is a member of the class of 4-hydroxy-2-alkenals [53]. HNE treatment of primary neurons and tissues leads to the activation of cell repair or cell death programs [54-56]. Further, its adducts can be used as biomarkers of oxidative damage within cells [57]. Consistent with a role of lipid peroxidation in neurologic disease, HNE accumulation has been observed in models of ischemia/reperfusion [58] and in patients with neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis [9, 59-61].

### Heavy Metals

Many metals including cadmium, arsenic, and lead, cross the blood-brain-barrier, leading to oxidative stress and cell death [62-67]. The severity of the biological response to heavy metals is determined largely by the solubility, absorbency, transport, and reactivity of the metal in the formation of functional complexes with cellular proteins [64]. While the intracellular mechanisms regulating the effects of heavy metals are poorly understood, heavy metals contribute to multiple biological functions. For example, iron is required for normal cellular function; however iron also reacts with hydrogen peroxide, generating the hydroxyl radical [12, 68]. Heavy metal toxicity can lead to severe developmental disorders including open neural tubes, peripheral neuropathies, encephalitis, and cognitive dysfunction [65, 69-71]. Despite many differences in the properties of heavy metals, a unifying theme that has emerged suggests that toxic levels of heavy metals cause cellular damage through the generation of free radicals, and this process can be modeled experimentally both *in vitro* and *in vivo* [63, 64, 66, 67]. Excess metals promote the production of superoxide and hydrogen peroxide *via* depletion of glutathione and sulfhydryl groups and promote increased lipid peroxidation [64]. In this manner, heavy metals inactivate enzymes, disrupt cellular membrane integrity, damage cytoskeletal components, and compromise the integrity of DNA [63].

### Ischemia

Reduced blood flow and reperfusion during ischemia deprive neurons of oxygen, leading to an imbalance in cellular homeostasis and subsequent

cell death *via* apoptosis, necrosis, and/or necroptosis [72-76]. *In vivo* experimental models for ischemia have been designed by occlusion of arteries that supply the brain. These models include global ischemia, focal ischemia, and hypoxia/ischemia [77]. Reperfusion following ischemia leads to a rapid reactivation of the electron transport chain, leading to increases in intracellular calcium levels and excess glutamate [78]. Together, these fluctuations lead to long-term changes that are propagated by the generation of free radicals and peroxynitrites [77, 79-84]. Depending on the combinations of arteries ligated and the duration of the insult, gradients of injury will develop that closely parallel human pathology. Using these techniques, a cellular environment composed of heterogeneous neuronal subpopulations emerges and extends from the "core" of the injury where neurons die due to necrosis to the "penumbra" of the lesion where mild injury leads to apoptotic cell death [72, 77, 85-88].

To complement the *in vivo* approach, ischemia can be induced *in vitro* by exposing dissociated cultures of neurons or organotypic slice cultures to hypoxia together with glucose deprivation [77, 89-92]. Exposure to hypoxia *in vitro* leads to a wide range of cellular damage [77, 90]. While these models are limited by how closely they mimic ischemic cell injury, their strength lies in providing a paradigm for investigating early cellular changes that take place during ischemia. Thus, models of ischemic injury allow for the examination of specific stages of oxidative stress-induced death in distinct neuronal populations *in vitro* and *in vivo*.

## II. SIGNALING PATHWAYS REGULATED BY OXIDATIVE STRESS

In addition to damaging macromolecules, oxidative stress is thought to trigger the activation of specific signaling pathways in cells that in turn lead to alterations in gene expression, ultimately coupling the oxidative stress signal to cell death or cell recovery [46, 93, 94]. Although the pathways that link oxidative stress signals to cellular responses remain largely to be elucidated, a number of proteins have been implicated in these responses. These proteins include the mitogen activated protein kinases (MAPKs: ERK1/2, JNK, p38MAPK, ERK5) [66, 95-98], the transcription factors cAMP response element-binding protein (CREB) [99, 100], Sp [49, 101], and Forkhead (FOXO) [102-104], as well as the oxidoreductase apoptosis inducing factor (AIF) [13, 105, 106]. Whether a neuron engages in repair and defense mechanisms against oxidative stress or succumbs to cell death results from the integration of signals from multiple signaling pathways.

### Protein Kinases

Oxidative stress activates a number of intracellular signaling pathways which mediate cellular effects on recovery, survival, and death. The MAPK family

consists of ERK1/2, the stress-activated kinases JNK and p38MAPK, and the big MAPK1 (BMK1 or ERK5). These serine/threonine kinases which are activated upon phosphorylation by upstream activating MAPK kinases, integrate cues to mediate cellular responses [107]. They respond to a wide range of biological stimuli including oxidative stress, and regulate cell survival and death through transcription dependent and transcription independent means in different cellular contexts [2, 96, 104, 108-114].

While ERK1/2 has been widely viewed as a pro-survival kinase, emerging evidence points to a dual function for ERK-mediated signaling in response to oxidative stress [115, 116]. In cultures of primary neurons and neuronal cell lines, oxidative stress leads to robust ERK1/2 activation and subsequent neuronal death [48, 95, 97, 116-118]. In addition, in models of acute oxidative stress induced by traumatic brain injury, regions of the hippocampus in which neurons stained positively for phosphorylated ERK1/2 were found to eventually undergo cell death [119]. Treatment of oxidatively stressed primary cortical neurons or the HT22 hippocampal neuronal cell line with the MEK inhibitor U0126 protected neurons from oxidative stress-induced death [120]. In addition, the neuronal death induced upon middle cerebral artery occlusion is reduced by more than 50% with intravenous treatment of animals with the MEK-1-specific antagonist PD98059 prior to focal ischemia [121]. Further, treatment of gerbils with the MEK inhibitor U0126 during reperfusion protected the hippocampus from reperfusion injury [122]. Interestingly, the use of these same pharmacological inhibitors in cultures of PC12 cells treated with hydrogen peroxide lead to reduced ERK5 activation and increased cell death [98]. These results suggest that oxidative stress-induced ERK signaling has differential effects on cell survival.

The stress-activated JNK is especially interesting as it has been shown to mediate oxidative stress signaling across species [104, 123-125]. Both JNK3 and p38MAPK are required for arsenite-induced neuronal death [66, 67]. In these experiments, sustained JNK and p38MAPK activity leads to caspase activation and apoptotic neuronal death [66, 67]. Together with these findings, evidence from *in vivo* models of oxidative stress including cerebral ischemia-reperfusion support the role of JNK in mediating neuronal death [126, 127]. In addition, JNK activity has been associated with activation of c-Jun, increased expression of FasL, and apoptotic neuronal death [126]. Loss of the brain-specific JNK3 in *jnk3* *-/-* mice protects neurons from injury produced by occlusion of the common carotid artery followed by hypoxia [96]. Together, these results indicate that the JNK signaling pathway plays an important role in oxidative stress-induced death signals in the nervous system.

Consistent with the model that MAPK pathways may serve multiple roles in stress responses,

oxidative stress has been shown to lead to biphasic activation of the MAPKs ERK1/2 and JNK [46, 115, 128]. The biphasic profile of MAPK activation may reconcile in part the seemingly opposite roles of MAPKs in survival and death signaling. The early rapid phase of MAPK activation due to an acute stress may promote a survival response and preemptively prepare neurons for a more severe exposure later on, as observed in ischemic pre-conditioning [116, 129]. In contrast, a sustained or delayed MAPK peak due to chronic stress promotes cell death possibly as a result of depleted endogenous antioxidant stores, such as glutathione [48, 115, 125, 130-132]. This view is consistent with the exciting possibility is that neurons contain a "stress-sensor" such that acute stimuli evoke a cellular response that promotes pre-conditioning against future insults.

Several mechanisms may account for oxidative stress-induced MAPK activation. In one model, oxidation of amino acid residues on membrane receptors is hypothesized to lead to receptor activation. For example, upon exposure of PC12 cells to arsenite, the epidermal growth factor receptor (EGFR) becomes phosphorylated on tyrosine residues, leading to increased binding of the Grb2 adapter protein and subsequent activation of Ras-dependent signaling [108, 133-135]. While the mechanisms for oxidative stress-mediated receptor activation are still not well understood, one possibility is that free radicals induce conformational changes in receptor dimerization domains. Beyond receptor activation, evidence for the activation of intracellular signaling pathways *via* oxidation of critical residues in adapter proteins and signaling molecules including the small GTPase Ras, support the model where oxidative stress triggers the activation of signal transduction cascades at multiple points along these cascades [135, 136].

The reversible modification of enzymatic activities by redox signaling provides another attractive mechanism for fine tuning on-going kinase activity in response to oxidative stress [136-138]. In addition to protein kinases and their upstream activators, proteins that inhibit protein kinase cascades including protein phosphatases are subject to regulation by stress [139]. Catalytic residues of phosphatases that have a low pKa and exist in the thiolate form at neutral pH including MAPK phosphatases (MPKs) can be oxidized to either sulfenic (S-OH), sulfinic (S-O<sub>2</sub>H), or sulfonic (S-O<sub>3</sub>H) acid [137, 138, 140-143]. Oxidation of the cysteine residue inactivates the phosphatase, and consequently the sustained activity of substrate kinases such as ERK1/2 and JNK promotes cell death [46, 94, 97, 137, 141]. The reactivation of oxidized phosphatases by glutathione or thioredoxins is contingent upon the degree of oxidation, and thus phosphatase regulation provides a powerful means for regulating the kinetics of kinase activation in response to stress [46, 141, 142, 144].

## Transcription Factors

Downstream of MAPK and other signaling molecules, a number of transcription factors including CREB, c-Jun, FOXO, Sp1/3, and others engage genetic programs that promote cell recovery or cell death [108, 145-147]. The transcription factor CREB is established to be important for neuronal survival signaling through the induction of prosurvival genes, such as *bcl-2* [109, 146, 148-150]. Surprisingly, although the ERK1/2 are activated in response to oxidative stress, the exact role of CREB in oxidatively stressed neurons is less clear. Some studies have found that in cultures of cerebellar granule neurons exposed to HNE, CREB appears to be inhibited, leading to a decrease in CREB and AP-1 binding to DNA [56]. CREB has also been suggested to be dephosphorylated and cleaved by a calpain-dependent mechanism in response to hydrogen peroxide treatment of cerebellar granule neurons [151]. However, treatment of primary midbrain neurons with 6-OHDA induces the robust CREB phosphorylation at serine 133 [99]. Interestingly, the subcellular localization of phosphorylated CREB varies in response to stress [99]. Oxidative stress leads to an increase in phosphorylation of the cytoplasmic pool of CREB, while the nuclear pool of phosphorylated CREB decreases [99]. The differences in localization are further reflected in the decreased induction of CREB target genes, such as *bcl-2* and *bdnf* [99]. In other studies, treatment of glutathione-deprived neurons with iron chelators, thought to decrease oxidative stress by sequestering redox active iron, induces ATF-1/CREB DNA binding and protects neurons from death [100, 152, 153]. Consistent with a role in oxidative stress and disease, increased cytoplasmic CREB has also been found in degenerating substantia nigra neurons of patients with Parkinson's disease or Lewy body disease [99].

One possible mechanism that might lead to sequestering of nucleus-bound proteins, such as CREB, could be through oxidative stress-induced modification of shuttling and transport proteins. In fact, differences in the localization patterns of the MAPKs and CBP, has been observed in *in vitro* and *in vivo* models of ischemia and in neurons from Parkinson's disease patients and Huntington's Disease patients [48, 116, 130, 154-159]. Another possibility is that normal proteasomal function and phosphorylation-dependent degradation programs could be impaired under oxidative stress conditions. Thus oxidative stress could indirectly inhibit transcription and stress recovery. Consistent with this model, proteasomal malfunction has also been observed in a number of diseases in which oxidative stress is implicated [160-164]. Based on these findings, the specific localization of signaling molecules bears a strong impact on neuronal survival. This interpretation is consistent with growing evidence for the role of subcellular localization in the activation of distinct signaling pathways regulating neuronal survival [165-167].

Downstream of JNK signaling, several transcription factors including c-Jun and FOXO have been implicated in oxidative stress responses. In cortical neurons, JNK activity leads to the phosphorylation of c-Jun in neurons upon exposure to arsenite or ischemia [67, 126, 168]. The induction of c-Jun leads to the upregulation of proapoptotic genes, including *bim*, *Fas*, and *TNFRp55* [96, 169, 170]. JNK activation also leads to the activation of the FOXO family of transcription factors. The FOXO family of transcription factors consists of evolutionarily conserved proteins that exert pleiotropic effects on biological processes, including cell death, stress resistance, metabolism, and longevity [171]. In response to low levels of hydrogen peroxide treatment *via* activation of the small GTPase Ral, JNK phosphorylates mammalian FOXO4 at Thr447 and Thr451 and promotes both the nuclear translocation of FOXO and the induction of *MnSOD* [104]. Interestingly FOXO transcription factors also promote cell death [172]. Oxidative stress may also induce SIRT1-mediated FOXO deacetylation, and a subsequent shift from the induction of cell death genes including *bim* to the transcription of genes involved in stress resistance, such as *gadd45* [103]. In this manner, the FOXO transcription factors may mediate dual cellular responses to oxidative stress.

## Sp Transcription Factors

Sp1 and Sp3 (specificity protein 1) transcription factors are widely expressed including in the brain [147, 173]. Sp proteins bind GC-boxes through a zinc finger domain, and influence transcription of different classes of genes including those involved in differentiation, proliferation, and oxidative stress responses [147, 173]. Interestingly, Sp transcription factors induce the expression of a number of genes with diverse functions, such as pro-survival genes including *inhibitor of apoptosis*, *survivin* [174], and *MnSOD* [175], while also retaining the ability to activate pro-apoptotic genes such as *FasL* [176, 177] and *12-lypooxygenase* [178]. While Sp1 and Sp3 regulate distinct cellular functions, increasing evidence suggests that stimulus and cell type specificity determine their role in cell survival or cell death.

Studies carried out both *in vitro* and *in vivo* have identified a role for Sp1 and Sp3 in neuronal responses to oxidative stress [40, 101]. In primary cultures of cortical neurons, glutathione depletion-induced oxidative stress leads to both elevated protein levels of Sp1 and Sp3 and their increased binding to DNA target sequences [40, 49]. It has also been shown that mutant Huntingtin leads to oxidative stress *in vitro* and *in vivo* [179-183]. Consistent with these findings, mice harboring striatal lesions due to 3-NP-induced oxidative stress have increased Sp1 and Sp3 protein levels [49]. In addition, oxidative stress in cortical neurons promotes the acetylation of Sp1 both *in vitro* and *in*

*vivo*, enabling Sp1-dependent gene expression [40]. Further, by using 3-NP to induce oxidative stress and to model Huntington's disease, it has been found that inhibition of histone deacetylases prior to 3-NP injection leads to increased Sp1 acetylation that is coupled with protection from 3-NP-induced oxidative stress [40].

Consistent with the finding that Sp transcription factors play a role in oxidative stress, the R6/2 mouse model of Huntington's Disease has increased Sp1 and Sp3 protein levels [49, 184]. It has been shown that soluble mutant Huntingtin interacts with and sequesters Sp1 and its coactivator TAFII130, preventing Sp1 DNA binding to target promoters, including dopamine D2 receptor and the nerve growth factor promoter [101, 185]. Interestingly, the DNA binding of Sp1 has been found to be compromised in post-mortem brain tissue of both presymptomatic and patients of Huntington's Disease, supporting the model where Sp transcription factors respond to oxidative stress [101]. While the oxidative stress-regulated Sp1 and Sp3 targets remain to be elucidated, these findings point to a transcriptional mechanism that underlies Sp-regulated cellular responses to stress and disease.

### Oxidoreductase Enzymes

In addition to affecting the activity of protein kinases and transcription factors, oxidative stress impacts on the activity of other signaling molecules including oxidoreductases. The flavoprotein apoptosis inducing factor (AIF) has emerged as one such oxidoreductase which possesses a dual role in mediating neuronal survival and death [13, 105, 106, 186, 187]. Under basal conditions, AIF is located in the mitochondrial inner membrane space, and in response to stress stimuli, it translocates to the nucleus where it leads to neuronal death [105, 188, 189]. However evidence also suggests that AIF functions as a scavenger of free radicals that defends cerebellar granule neurons from oxidative stress [13].

Originally identified as an inducer of caspase-independent apoptosis, stimuli that promote the nuclear translocation of AIF also trigger hallmarks of apoptotic cell death: nuclear condensation and chromatin fragmentation [105]. In primary cultures of cortical, hippocampal, and cerebellar granule neurons, and in *in vivo* models of ischemia, AIF translocates from the mitochondria to the cytoplasm and nucleus nearly immediately following the insult [188, 190-192]. AIF is thought to act in both BAX-dependent (DNA damage induced by camptothecin treatment) and BAX-independent (NMDA or kainate-induced excitotoxicity) pathways to mediate cell death [193]. It is believed that AIF mediates death induced by poly (ADP-ribose) polymerase (PARP1), a nuclear enzyme that regulates DNA repair [188]. Although the exact mechanisms mediating the downstream signals from PARP1 remain to be

elucidated, it has been hypothesized that because PARP1 uses nicotinamide adenine dinucleotide (NAD<sup>+</sup>), the activation of PARP1 is sensed in the mitochondria by an as yet unidentified mechanism that triggers AIF release [188]. Subsequently, cytosolic AIF induces mitochondrial breakdown, promoting cytochrome c release, and caspase activation.

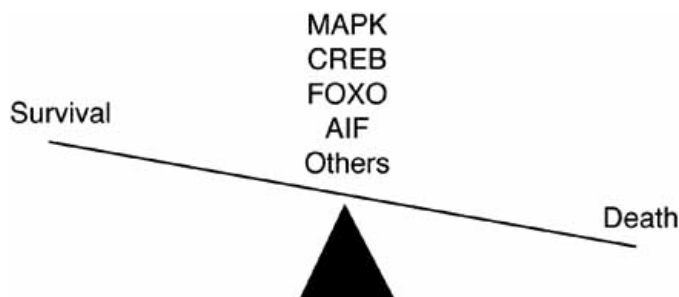
The dual role of AIF as a sentinel of cell death and survival is underscored in the naturally occurring mouse mutant Harlequin (Hq), which results from an insertion of a mouse leukemia virus in the AIF locus [13]. The Hq mouse has an 80% reduction in AIF levels, and develops ataxia, a condition which is coupled to a primary loss of cerebellar granule neurons [13]. AIF shares homology with the bacterial oxidoreductases, which endows AIF with NAD(P)H oxidase activity *in vitro* [194]. In line with its similarity to bacterial oxidoreductase, it was found that low levels of AIF in the Hq mouse impair free radical homeostasis and lead to subsequent oxidative stress through elevated lipid peroxidation [13]. Further, primary cerebellar granule neurons cultured from the Hq mice had increased sensitivity to hydrogen peroxide treatment that was rescued by the expression of AIF.

It will be interesting to identify the exact mechanisms that regulate AIF function in response to distinct stimuli. In addition, the elucidation of the downstream signals that are activated in response to AIF release will be critical in order to fully understand the role that AIF plays in the nervous system.

### Perspectives for the Future of Oxidative Stress Research

The development of experimental methods that generate ROS has enabled the modeling of oxidative stress in neurons. While certain techniques induce oxidative stress in distinct neuronal populations, other methods apply more broadly to nervous tissue. Together, these techniques will serve as a foundation for future advances in understanding the role of oxidative stress in neurologic disease. A major challenge in oxidative stress research is the accurate evaluation of the degree of oxidative damage incurred by cells and tissues due to the reactive nature of ROS. In the future, it will be critical to develop tools to standardize the assessment of oxidative stress across experimental systems. This may prove to be challenging, requiring technological advances that permit simple, reproducible, and accurate measurement of the load of ROS within living cells, ideally allowing for the evaluation of ROS levels within distinct subcellular locations.

ROS activate signaling pathways that regulate recovery and death signals in response to stress. However while many signaling molecules have been linked to oxidative stress signaling, we are just beginning to learn the specific roles of these



**Fig. (3). A growing number of signaling molecules are implicated in promoting both neuronal survival and death.** Dual functions of signaling molecules (e.g. protein kinases, transcription factors, and oxidoreductases) in neuronal survival and death are regulated in a cell- and stimulus-specific manner. Redox state, subcellular localization, and activation kinetics represent some of the properties of signaling molecules that may be modulated by ROS.

complex signaling networks. Further investigations will be required to unravel the intricate signaling mechanisms and chemistry of how ROS control specific signaling pathways.

An emerging theme in oxidative stress research is that signaling molecules, including protein kinases and transcription factors, possess dual roles in mediating survival and death in response to ROS, Fig. (3). The distinct functions of these signaling molecules may arise from differential localization, activation/inactivation kinetics, and/or a byproduct of the integration of several different signaling modules. The combined use of model systems such as *Drosophila* and/or *C. elegans* together with mammalian experimental systems provides a powerful means to extend the study of ROS to intact organisms. The advantage of genetics combined with a relatively fast developmental period and lifespan of these organisms allows for comprehensive, longitudinal modeling of oxidative stress.

Oxidative damage contributes to the progression of a growing number of neurologic diseases, including Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis. The increase in average human lifespan is leading to a rise in incidence of age-associated neurologic disease. Therefore, an improved understanding of oxidative stress signaling in the nervous system will be critical for the development of new therapies for the treatment of age-associated neurologic diseases.

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## ABBREVIATIONS

AIF	= Apoptosis inducing factor
ATP	= Adenosine triphosphate
CNS	= Central nervous system

CoQ	= Coenzyme Q
CREB	= cAMP-response element-binding protein
Cyto C	= Cytochrome C
EGFR	= Epidermal growth factor receptor
ERK1/2	= Extracellular signal-regulated kinase 1/2
ERK5/BMK1	= Extracellular signal-regulated kinase 5 or Big MAPK
FOXO	= Forkhead transcription factor
HNE	= 4-hydroxy-2-nonenal
Hq	= Harlequin
JNK	= c-Jun N-terminal kinase
MAPK	= Mitogen activated protein kinase
MPK	= MAPK phosphatase
MPTP	= N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP+	= 1-methyl-4-pyridinium
NAD+	= Nicotinamide adenine dinucleotide
3-NP	= 3-nitropropionic acid
6-OHDA	= 6-hydroxydopamine
PARP-1	= poly(ADP-ribose) polymerase
PC12	= Pheochromocytoma cell line
ROS	= Reactive oxygen species
SOD	= Superoxide dismutase
Sp1/3	= Specificity protein 1/3

## REFERENCES

- [1] Boland, B. and Campbell, V. (2003) *Neurobiol. Aging*, **24**, 179-86.
- [2] Chong, Z.Z., Li, F. and Maiese, K. (2005) *Prog. Neurobiol.*, **75**, 207-46.
- [3] Klein, J.A. and Ackerman, S.L. (2003) *J. Clin. Invest.*, **111**, 785-93.
- [4] Stadtman, E.R. (2001) *Ann. N. Y. Acad. Sci.*, **928**, 22-38.
- [5] Droge, W. (2003) *Adv. Exp. Med. Biol.*, **543**, 191-200.
- [6] Dringen, R. (2000) *Prog. Neurobiol.*, **62**, 649-71.



- [7] Floyd, R.A. and Carney, J.M. (1992) *Ann. Neurol.*, **32** Suppl, S22-7.
- [8] Margail, I., Plotkine, M. and Lerouet, D. (2005) *Free Radic. Biol. Med.*, **39**, 429-43.
- [9] Zarkovic, K. (2003) *Mol. Aspects Med.*, **24**, 293-303.
- [10] Andersen, J.K. (2004) *Nat. Med.*, **10** Suppl, S18-25.
- [11] Balaban, R.S., Nemoto, S. and Finkel, T. (2005) *Cell*, **120**, 483-95.
- [12] Winterbourn, C.C. (1995) *Toxicol. Lett.*, **82-83**, 969-74.
- [13] Klein, J.A., Longo-Guess, C.M., Rossmann, M.P., Seburn, K.L., Hurd, R.E., Frankel, W.N., Bronson, R.T., Ackerman, S.L. (2002) *Nature*, **419**, 367-74.
- [14] Behl, C., Davis, J.B., Lesley, R. and Schubert, D. (1994) *Cell*, **77**, 817-27.
- [15] Finkel, T. (2003) *Curr. Opin. Cell Biol.*, **15**, 247-54.
- [16] Nicklas, W.J., Vyas, I. and Heikkila, R.E. (1985) *Life Sci.*, **36**, 2503-8.
- [17] Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V. and Greenamyre, J.T. (2000) *Nat. Neurosci.*, **3**, 1301-6.
- [18] Dauer, W. and Przedborski, S. (2003) *Neuron*, **39**, 889-909.
- [19] Markey, S.P., Johannessen, J.N., Chiu, C.C., Burns, R.S. and Herkenham, M.A. (1984) *Nature*, **311**, 464-7.
- [20] Langston, J.W., Ballard, P., Tetrud, J.W. and Irwin, I. (1983) *Science*, **219**, 979-80.
- [21] Muthane, U., Ramsay, K.A., Jiang, H., Jackson-Lewis, V., Donaldson, D., Fernando, S., Ferreira, M. and Przedborski, S. (1994) *Exp. Neurol.*, **126**, 195-204.
- [22] Heikkila, R.E., Manzino, L., Cabbat, F.S. and Duvoisin, R.C. (1984) *Nature*, **311**, 467-9.
- [23] Javitch, J.A., D'Amato, R.J., Strittmatter, S.M. and Snyder, S.H. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 2173-7.
- [24] Tipton, K.F. and Singer, T.P. (1993) *J. Neurochem.*, **61**, 1191-206.
- [25] Talpade, D.J., Greene, J.G., Higgins, D.S., Jr. and Greenamyre, J.T. (2000) *J. Neurochem.*, **75**, 2611-21.
- [26] Day, B.J., Patel, M., Calavetta, L., Chang, L.Y. and Stamler, J.S. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 12760-5.
- [27] Manning-Bog, A.B., McCormack, A.L., Li, J., Uversky, V.N., Fink, A.L. and Di Monte, D.A. (2002) *J. Biol. Chem.*, **277**, 1641-4.
- [28] McCormack, A.L., Thiruchelvam, M., Manning-Bog, A.B., Thiffault, C., Langston, J.W., Cory-Slechta, D.A. and Di Monte, D.A. (2002) *Neurobiol. Dis.*, **10**, 119-27.
- [29] Shimizu, K., Ohtaki, K., Matsubara, K., Aoyama, K., Uezono, T., Saito, O., Suno, M., Ogawa, K., Hayase, N., Kimura, K. and Shiono, H. (2001) *Brain Res.*, **906**, 135-42.
- [30] Richardson, J.R., Quan, Y., Sherer, T.B., Greenamyre, J.T. and Miller, G.W. (2005) *Toxicol. Sci.*, **88**, 193-201.
- [31] Luthman, J., Fredriksson, A., Sundstrom, E., Jonsson, G. and Archer, T. (1989) *Behav. Brain Res.*, **33**, 267-77.
- [32] Ungerstedt, U. (1968) *Eur. J. Pharmacol.*, **5**, 107-10.
- [33] Javoy, F., Sotelo, C., Herbet, A. and Agid, Y. (1976) *Brain Res.*, **102**, 201-15.
- [34] Cohen, G., Heikkila, R.E., Allis, B., Cabbat, F., Dembiec, D., MacNamee, D., Mytilineou, C. and Winston, B. (1976) *J. Pharmacol. Exp. Ther.*, **199**, 336-52.
- [35] Asanuma, M., Hirata, H. and Cadet, J.L. (1998) *Neuroscience*, **85**, 907-17.
- [36] Barkats, M., Millemcamps, S., Bilang-Bleuel, A. and Mallet, J. (2002) *J. Neurochem.*, **82**, 101-9.
- [37] Glinka, Y., Tipton, K.F. and Youdim, M.B. (1996) *J. Neurochem.*, **66**, 2004-10.
- [38] Schulz, J.B., Henshaw, D.R., MacGarvey, U. and Beal, M.F. (1996) *Neurochem. Int.*, **29**, 167-71.
- [39] Kim, G.W. and Chan, P.H. (2002) *J. Cereb. Blood Flow Metab.*, **22**, 798-809.
- [40] Ryu, H., Lee, J., Olofsson, B.A., Mwidau, A., Dedeoglu, A., Escudero, M., Flemington, E., Azizkhan-Clifford, J., Ferrante, R.J. and Ratan, R.R. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 4281-6.
- [41] Beal, M.F. (1996) *Curr. Opin. Neurobiol.*, **6**, 661-6.
- [42] Stefanova, N., Reindl, M., Neumann, M., Haass, C., Poewe, W., Kahle, P.J. and Wenning, G.K. (2005) *Am. J. Pathol.*, **166**, 869-76.
- [43] Ratan, R.R., Ryu, H., Lee, J., Mwidau, A. and Neve, R.L. (2002) *Methods Enzymol.*, **352**, 183-90.
- [44] Murphy, T.H., Miyamoto, M., Sastre, A., Schnaar, R.L. and Coyle, J.T. (1989) *Neuron*, **2**, 1547-58.
- [45] Murphy, T.H., Schnaar, R.L. and Coyle, J.T. (1990) *FASEB J.*, **4**, 1624-33.
- [46] Kamata, H., Honda, S., Maeda, S., Chang, L., Hirata, H. and Karin, M. (2005) *Cell*, **120**, 649-61.
- [47] Ratan, R.R., Murphy, T.H. and Baraban, J.M. (1994) *J. Neurosci.*, **14**, 4385-92.
- [48] Stanciu, M. and DeFranco, D.B. (2002) *J. Biol. Chem.*, **277**, 4010-7.
- [49] Ryu, H., Lee, J., Zaman, K., Kubilis, J., Ferrante, R.J., Ross, B.D., Neve, R. and Ratan, R.R. (2003) *J. Neurosci.*, **23**, 3597-606.
- [50] Uchida, K. (2003) *Prog. Lipid Res.*, **42**, 318-43.
- [51] Esterbauer, H., Schaur, R.J. and Zollner, H. (1991) *Free Radic. Biol. Med.*, **11**, 81-128.
- [52] Schaur, R.J. (2003) *Mol. Aspects Med.*, **24**, 149-59.
- [53] Benedetti, A., Comporti, M. and Esterbauer, H. (1980) *Biochem. Biophys. Acta*, **620**, 281-96.
- [54] Parola, M., Bellomo, G., Robino, G., Barrera, G. and Dianzani, M.U. (1999) *Antioxid. Redox. Signal.*, **1**, 255-84.
- [55] Kruman, I., Bruce-Keller, A.J., Bredesen, D., Waeg, G. and Mattson, M.P. (1997) *J. Neurosci.*, **17**, 5089-100.
- [56] Ito, Y., Arakawa, M., Ishige, K. and Fukuda, H. (1999) *Neurosci. Res.*, **35**, 321-7.
- [57] Dianzani, M.U. (1998) *Free Radic. Res.*, **28**, 553-60.
- [58] Yoshino, H., Hattori, N., Urabe, T., Uchida, K., Tanaka, M. and Mizuno, Y. (1997) *Brain Res.*, **767**, 81-6.
- [59] Shibata, N., Nagai, R., Uchida, K., Horiuchi, S., Yamada, S., Hirano, A., Kawaguchi, M., Yamamoto, T., Sasaki, S. and Kobayashi, M. (2001) *Brain Res.*, **917**, 97-104.
- [60] Yoritaka, A., Hattori, N., Uchida, K., Tanaka, M., Stadtman, E.R. and Mizuno, Y. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 2696-701.
- [61] Ando, Y., Brannstrom, T., Uchida, K., Nyhlin, N., Nasman, B., Suhr, O., Yamashita, T., Olsson, T., El Salhy, M., Uchino, M. and Ando, M. (1998) *J. Neurol. Sci.*, **156**, 172-6.
- [62] Oberto, A., Marks, N., Evans, H.L. and Guidotti, A. (1996) *J. Pharmacol. Exp. Ther.*, **279**, 435-42.
- [63] Bernstam, L. and Nriagu, J. (2000) *J. Toxicol. Environ. Health B. Crit. Rev.*, **3**, 293-322.
- [64] Stohs, S.J. and Bagchi, D. (1995) *Free Radic. Biol. Med.*, **18**, 321-36.
- [65] Clarkson, T.W. (1987) *Environ. Health Perspect.*, **75**, 59-64.
- [66] Namgung, U. and Xia, Z. (2001) *Toxicol. Appl. Pharmacol.*, **174**, 130-8.
- [67] Namgung, U. and Xia, Z. (2000) *J. Neurosci.*, **20**, 6442-51.
- [68] Welch, K.D., Davis, T.Z., Van Eden, M.E. and Aust, S.D. (2002) *Free Radic. Biol. Med.*, **32**, 577-83.
- [69] Tabocova, S., Hunter, E.S., 3rd and Gladen, B.C. (1996) *Toxicol. Appl. Pharmacol.*, **138**, 298-307.
- [70] Snow, E.T. (1992) *Pharmacol. Ther.*, **53**, 31-65.
- [71] Domingo, J.L., Colomina, M.T., Llobet, J.M., Jones, M.M., Singh, P.K. and Campbell, R.A. (1992) *Fund. Appl. Toxicol.*, **19**, 350-7.
- [72] Benchoua, A., Guegan, C., Couriaud, C., Hosseini, H., Sampaio, N., Morin, D. and Oteniente, B. (2001) *J. Neurosci.*, **21**, 7127-34.
- [73] Johnson, E.M., Jr. and Deckwerth, T.L. (1993) *Annu. Rev. Neurosci.*, **16**, 31-46.
- [74] Gwag, B.J., Koh, J.Y., DeMaro, J.A., Ying, H.S., Jacquin, M. and Choi, D.W. (1997) *Neuroscience*, **77**, 393-401.
- [75] Friedlander, R.M. (2003) *N. Engl. J. Med.*, **348**, 1365-75.
- [76] Degterev, A., Huang, Z., Boyce, M., Li, Y., Jagtap, P., Mizushima, N., Cuny, G.D., Mitchison, T.J., Moskowitz, M.A. and Yuan, J. (2005) *Nat. Chem. Biol.*, **1**, 112-119.
- [77] Lipton, P. (1999) *Physiol. Rev.*, **79**, 1431-568.
- [78] Birnbaum, Y., Leor, J. and Kloner, R.A. (1997) *J. Thromb. Thrombolysis*, **4**, 185-195.
- [79] Zheng, Z., Lee, J.E. and Yenari, M.A. (2003) *Curr. Mol. Med.*, **3**, 361-72.
- [80] Chan, P.H. (2001) *J. Cereb. Blood Flow Metab.*, **21**, 2-14.
- [81] Lancelot, E., Callebort, J., Revaud, M.L., Boulu, R.G. and Plotkine, M. (1995) *Neurosci. Lett.*, **197**, 85-8.
- [82] Keller, J.N., Kindy, M.S., Holtsberg, F.W., St Clair, D.K., Yen, H.C., Germeyer, A., Steiner, S.M., Bruce-Keller, A.J., Hutchins, J.B. and Mattson, M.P. (1998) *J. Neurosci.*, **18**, 687-97.

- [83] Murakami, K., Kondo, T., Kawase, M., Li, Y., Sato, S., Chen, S.F. and Chan, P.H. (1998) *J. Neurosci.*, **18**, 205-13.
- [84] Fabian, R.H., DeWitt, D.S. and Kent, T.A. (1995) *J. Cereb. Blood Flow Metab.*, **15**, 242-7.
- [85] Hakim, A.M. (1998) *Neurology*, **51**, S44-6.
- [86] Linnik, M.D., Zobrist, R.H. and Hatfield, M.D. (1993) *Stroke*, **24**, 2002-8; discussion 2008-9.
- [87] Li, Y., Chopp, M., Jiang, N., Yao, F. and Zaloga, C. (1995) *J. Cereb. Blood. Flow Metab.*, **15**, 389-97.
- [88] Charriaut-Marlangue, C., Aggoun-Zouaoui, D., Represa, A. and Ben-Ari, Y. (1996) *Trends Neurosci.*, **19**, 109-14.
- [89] Goldberg, M.P. and Choi, D.W. (1993) *J. Neurosci.*, **13**, 3510-24.
- [90] Kass, I.S. and Lipton, P. (1982) *J. Physiol.*, **332**, 459-72.
- [91] Lobner, D. and Lipton, P. (1993) *J. Neurosci.*, **13**, 4861-71.
- [92] Strasser, U. and Fischer, G. (1995) *J. Neurosci. Methods*, **57**, 177-86.
- [93] Finkel, T. (2000) *FEBS Lett.*, **476**, 52-4.
- [94] Meng, T.C., Fukada, T. and Tonks, N.K. (2002) *Mol. Cell*, **9**, 387-99.
- [95] Hu, B.R. and Wieloch, T. (1994) *J. Neurochem.*, **62**, 1357-67.
- [96] Kuan, C.Y., Whitmarsh, A.J., Yang, D.D., Liao, G., Schloemer, A.J., Dong, C., Bao, J., Banasiak, K.J., Haddad, G.G., Flavell, R.A., Davis, R.J. and Rakic, P. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 15184-9.
- [97] Guyton, K.Z., Liu, Y., Gorospe, M., Xu, Q. and Holbrook, N.J. (1996) *J. Biol. Chem.*, **271**, 4138-42.
- [98] Suzuki, Y., Yoshizumi, M., Kagami, S., Koyama, A.H., Taketani, Y., Houchi, H., Tsuchiya, K., Takeda, E. and Tamaki, T. (2002) *J. Biol. Chem.*, **277**, 9614-21.
- [99] Chalovich, E.M., Zhu, J.H., Caltagarone, J., Bowser, R. and Chu, C.T. (2006) *J. Biol. Chem.*, **281**, 17870-81.
- [100] Zaman, K., Ryu, H., Hall, D., O'Donovan, K., Lin, K.I., Miller, M.P., Marquis, J.C., Baraban, J.M., Semenza, G.L. and Ratan, R.R. (1999) *J. Neurosci.*, **19**, 9821-30.
- [101] Dunah, A.W., Jeong, H., Griffin, A., Kim, Y.M., Standaert, D.G., Hersch, S.M., Mouradian, M.M., Young, A.B., Tanese, N. and Krainc, D. (2002) *Science*, **296**, 2238-43.
- [102] Van Der Heide, L.P., Hoekman, M.F. and Smidt, M.P. (2004) *Biochem. J.*, **380**, 297-309.
- [103] Brunet, A., Sweeney, L.B., Sturgill, J.F., Chua, K.F., Greer, P.L., Lin, Y., Tran, H., Ross, S.E., Mostoslavsky, R., Cohen, H.Y., Hu, L.S., Cheng, H.L., Jedrychowski, M.P., Gygi, S.P., Sinclair, D.A., Alt, F.W. and Greenberg, M.E. (2004) *Science*, **303**, 2011-5.
- [104] Essers, M.A., Weijzen, S., de Vries-Smits, A.M., Saarloos, I., de Ruiter, N.D., Bos, J.L. and Burgering, B.M. (2004) *EMBO J.*, **23**, 4802-12.
- [105] Susin, S.A., Lorenzo, H.K., Zamzami, N., Marzo, I., Snow, B.E., Brothers, G.M., Mangion, J., Jacotot, E., Costantini, P., Loeffler, M., Larochette, N., Goodlett, D.R., Aebersold, R., Siderovski, D.P., Penninger, J.M. and Kroemer, G. (1999) *Nature*, **397**, 441-6.
- [106] Vahsen, N., Cande, C., Briere, J.J., Benit, P., Joza, N., Larochette, N., Mastroberardino, P.G., Pequignot, M.O., Casares, N., Lazar, V., Feraud, O., Debili, N., Wissing, S., Engelhardt, S., Madeo, F., Piacentini, M., Penninger, J.M., Schagger, H., Rustin, P. and Kroemer, G. (2004) *EMBO J.*, **23**, 4679-89.
- [107] Kaplan, D.R. and Miller, F.D. (2000) *Curr. Opin. Neurobiol.*, **10**, 381-91.
- [108] Martindale, J.L. and Holbrook, N.J. (2002) *J. Cell Physiol.*, **192**, 1-15.
- [109] Bonni, A., Brunet, A., West, A.E., Datta, S.R., Takasu, M.A. and Greenberg, M.E. (1999) *Science*, **286**, 1358-62.
- [110] Becker, E.B., Howell, J., Kodama, Y., Barker, P.A. and Bonni, A. (2004) *J. Neurosci.*, **24**, 8762-70.
- [111] Shimamura, A., Ballif, B.A., Richards, S.A. and Blenis, J. (2000) *Curr. Biol.*, **10**, 127-35.
- [112] Xia, Z., Dickens, M., Raingeaud, J., Davis, R.J. and Greenberg, M.E. (1995) *Science*, **270**, 1326-31.
- [113] Tamagno, E., Robino, G., Obbili, A., Bardini, P., Aragno, M., Parola, M. and Danni, O. (2003) *Exp. Neurol.*, **180**, 144-55.
- [114] Kyriakis, J.M. and Avruch, J. (2001) *Physiol. Rev.*, **81**, 807-69.
- [115] Luo, Y. and DeFranco, D.B. (2006) *J. Biol. Chem.*, **281**, 16436-42.
- [116] Chu, C.T., Levinthal, D.J., Kulich, S.M., Chalovich, E.M. and DeFranco, D.B. (2004) *Eur. J. Biochem.*, **271**, 2060-6.
- [117] Campos-Gonzalez, R. and Kindy, M.S. (1992) *J. Neurochem.*, **59**, 1955-8.
- [118] Stanciu, M., Wang, Y., Kentor, R., Burke, N., Watkins, S., Kress, G., Reynolds, I., Klann, E., Angiolieri, M.R., Johnson, J.W. and DeFranco, D.B. (2000) *J. Biol. Chem.*, **275**, 12200-6.
- [119] Otani, N., Nawashiro, H., Fukui, S., Nomura, N., Yano, A., Miyazawa, T. and Shima, K. (2002) *J. Cereb. Blood Flow Metab.*, **22**, 327-34.
- [120] Satoh, T., Nakatsuka, D., Watanabe, Y., Nagata, I., Kikuchi, H. and Namura, S. (2000) *Neurosci. Lett.*, **288**, 163-6.
- [121] Alessandrini, A., Namura, S., Moskowitz, M.A. and Bonventre, J.V. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 12866-9.
- [122] Namura, S., Iihara, K., Takami, S., Nagata, I., Kikuchi, H., Matsushita, K., Moskowitz, M.A., Bonventre, J.V. and Alessandrini, A. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 11569-74.
- [123] Oh, S.W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R.J. and Tissenbaum, H.A. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 4494-9.
- [124] Wang, M.C., Bohmann, D. and Jasper, H. (2005) *Cell*, **121**, 115-25.
- [125] Davis, R.J. (2000) *Cell*, **103**, 239-52.
- [126] Herdegen, T., Claret, F.X., Kallunki, T., Martin-Villalba, A., Winter, C., Hunter, T. and Karin, M. (1998) *J. Neurosci.*, **18**, 5124-35.
- [127] Ozawa, H., Shioda, S., Dohi, K., Matsumoto, H., Mizushima, H., Zhou, C.J., Funahashi, H., Nakai, Y., Nakajo, S. and Matsumoto, K. (1999) *Neurosci. Lett.*, **262**, 57-60.
- [128] Choi, W.S., Yoon, S.Y., Oh, T.H., Choi, E.J., O'Malley, K.L. and Oh, Y.J. (1999) *J. Neurosci. Res.*, **57**, 86-94.
- [129] Dirnagl, U., Simon, R.P. and Hallenbeck, J.M. (2003) *Trends Neurosci.*, **26**, 248-54.
- [130] Kulich, S.M. and Chu, C.T. (2001) *J. Neurochem.*, **77**, 1058-66.
- [131] Liu, Z.G., Hsu, H., Goeddel, D.V. and Karin, M. (1996) *Cell*, **87**, 565-76.
- [132] Colucci-D'Amato, L., Perrone-Capano, C. and di Porzio, U. (2003) *Bioessays*, **25**, 1085-95.
- [133] Chen, W., Martindale, J.L., Holbrook, N.J. and Liu, Y. (1998) *Mol. Cell Biol.*, **18**, 5178-88.
- [134] Knebel, A., Rahmsdorf, H.J., Ullrich, A. and Herrlich, P. (1996) *EMBO J.*, **15**, 5314-25.
- [135] Zhang, P., Wang, Y.Z., Kagan, E. and Bonner, J.C. (2000) *J. Biol. Chem.*, **275**, 22479-86.
- [136] Kamata, H. and Hirata, H. (1999) *Cell Signal.*, **11**, 1-14.
- [137] Levinthal, D.J. and DeFranco, D.B. (2005) *J. Biol. Chem.*, **280**, 5875-83.
- [138] Lee, S.R., Kwon, K.S., Kim, S.R. and Rhee, S.G. (1998) *J. Biol. Chem.*, **273**, 15366-72.
- [139] Takano, S., Fukuyama, H., Fukumoto, M., Hirashimizu, K., Higuchi, T., Takenawa, J., Nakayama, H., Kimura, J. and Fujita, J. (1995) *J. Cereb. Blood Flow Metab.*, **15**, 33-41.
- [140] Denu, J.M. and Tanner, K.G. (1998) *Biochemistry*, **37**, 5633-42.
- [141] Tonks, N.K. (2005) *Cell*, **121**, 667-70.
- [142] Claiborne, A., Yeh, J.I., Mallett, T.C., Luba, J., Crane, E.J., 3rd, Charrier, V. and Parsonage, D. (1999) *Biochemistry*, **38**, 15407-16.
- [143] Stamler, J.S. and Hausladen, A. (1998) *Nat. Struct. Biol.*, **5**, 247-9.
- [144] Singh, D.K., Kumar, D., Siddiqui, Z., Basu, S.K., Kumar, V. and Rao, K.V. (2005) *Cell*, **121**, 281-93.
- [145] Tran, H., Brunet, A., Griffith, E.C. and Greenberg, M.E. (2003) *Sci STKE*, RE5.
- [146] Lonze, B.E. and Ginty, D.D. (2002) *Neuron*, **35**, 605-23.
- [147] Chu, S. and Ferro, T.J. (2005) *Gene*, **348**, 1-11.
- [148] Riccio, A., Ahn, S., Davenport, C.M., Blendy, J.A. and Ginty, D.D. (1999) *Science*, **286**, 2358-61.
- [149] Shaywitz, A.J. and Greenberg, M.E. (1999) *Annu. Rev. Biochem.*, **68**, 821-61.
- [150] Chrivia, J.C., Kwok, R.P., Lamb, N., Hagiwara, M., Montminy, M.R. and Goodman, R.H. (1993) *Nature*, **365**, 855-9.
- [151] See, V. and Loeffler, J.P. (2001) *J. Biol. Chem.*, **276**, 35049-59.
- [152] Yonezawa, M., Back, S.A., Gan, X., Rosenberg, P.A. and Volpe, J.J. (1996) *J. Neurochem.*, **67**, 566-73.
- [153] Troy, C.M., Derossi, D., Prochiantz, A., Greene, L.A. and Shelanski, M.L. (1996) *J. Neurosci.*, **16**, 253-61.
- [154] Irving, E.A. and Bamford, M. (2002) *J. Cereb. Blood Flow Metab.*, **22**, 631-47.
- [155] Wang, X., Zhu, C., Qiu, L., Hagberg, H., Sandberg, M. and Blomgren, K. (2003) *J. Neurochem.*, **86**, 351-62.

- [156] Zhu, J.H., Kulich, S.M., Oury, T.D. and Chu, C.T. (2002) *Am. J. Pathol.*, **161**, 2087-98.
- [157] Zhu, J.H., Guo, F., Shelburne, J., Watkins, S. and Chu, C.T. (2003) *Brain Pathol.*, **13**, 473-81.
- [158] Pei, J.J., Braak, H., An, W.L., Winblad, B., Cowburn, R.F., Iqbal, K. and Grundke-Iqbal, I. (2002) *Brain Res. Mol. Brain Res.*, **109**, 45-55.
- [159] Nucifora, F.C., Jr., Sasaki, M., Peters, M.F., Huang, H., Cooper, J.K., Yamada, M., Takahashi, H., Tsuji, S., Troncoso, J., Dawson, V.L., Dawson, T.M. and Ross, C.A. (2001) *Science*, **291**, 2423-8.
- [160] Lam, Y.A., Pickart, C.M., Alban, A., Landon, M., Jamieson, C., Ramage, R., Mayer, R.J. and Layfield, R. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 9902-6.
- [161] Moore, D.J., Dawson, V.L. and Dawson, T.M. (2003) *Neuromolecular Med.*, **4**, 95-108.
- [162] McNaught, K.S., Belizaire, R., Isacson, O., Jenner, P. and Olanow, C.W. (2003) *Exp. Neurol.*, **179**, 38-46.
- [163] Ding, Q., Reinacker, K., Dimayuga, E., Nukala, V., Drake, J., Butterfield, D.A., Dunn, J.C., Martin, S., Bruce-Keller, A.J. and Keller, J.N. (2003) *FEBS Lett.*, **546**, 228-32.
- [164] Keller, J.N., Dimayuga, E., Chen, Q., Thorpe, J., Gee, J. and Ding, Q. (2004) *Int. J. Biochem. Cell Biol.*, **36**, 2376-91.
- [165] Heerssen, H.M., Pazyra, M.F. and Segal, R.A. (2004) *Nat. Neurosci.*, **7**, 596-604.
- [166] Watson, F.L., Heerssen, H.M., Bhattacharyya, A., Klesse, L., Lin, M.Z. and Segal, R.A. (2001) *Nat. Neurosci.*, **4**, 981-8.
- [167] Shalizi, A., Lehtinen, M., Gaudilliere, B., Donovan, N., Han, J., Konishi, Y. and Bonni, A. (2003) *J. Neurosci.*, **23**, 7326-36.
- [168] Le-Niculescu, H., Bonfoco, E., Kasuya, Y., Claret, F.X., Green, D.R. and Karin, M. (1999) *Mol. Cell Biol.*, **19**, 751-63.
- [169] Matsuyama, T., Hata, R., Yamamoto, Y., Tagaya, M., Akita, H., Uno, H., Wanaka, A., Furuyama, J. and Sugita, M. (1995) *Brain Res. Mol. Brain Res.*, **34**, 166-72.
- [170] Matsushita, K., Wu, Y., Qiu, J., Lang-Lazdunski, L., Hirt, L., Waeber, C., Hyman, B.T., Yuan, J. and Moskowitz, M.A. (2000) *J. Neurosci.*, **20**, 6879-87.
- [171] Accili, D. and Arden, K.C. (2004) *Cell*, **117**, 421-6.
- [172] Brunet, A., Bonni, A., Zigmond, M.J., Lin, M.Z., Juo, P., Hu, L.S., Anderson, M.J., Arden, K.C., Blenis, J. and Greenberg, M.E. (1999) *Cell*, **96**, 857-68.
- [173] Suske, G. (1999) *Gene*, **238**, 291-300.
- [174] Li, F., Ackermann, E.J., Bennett, C.F., Rothermel, A.L., Plescia, J., Tognin, S., Villa, A., Marchisio, P.C. and Altieri, D.C. (1999) *Nat. Cell Biol.*, **1**, 461-6.
- [175] Tanaka, T., Kurabayashi, M., Aihara, Y., Ohshima, Y. and Nagai, R. (2000) *Arterioscler. Thromb. Vasc. Biol.*, **20**, 392-401.
- [176] Xiao, S., Matsui, K., Fine, A., Zhu, B., Marshak-Rothstein, A., Widom, R.L. and Ju, S.T. (1999) *Eur. J. Immunol.*, **29**, 3456-65.
- [177] Chou, C.F., Peng, H.W., Wang, C.Y., Yang, Y.T. and Han, S.H. (2000) *J. Biomed. Sci.*, **7**, 136-43.
- [178] Liu, Y.W., Arakawa, T., Yamamoto, S. and Chang, W.C. (1997) *Biochem. J.*, **324** (Pt 1), 133-40.
- [179] Davies, S.W., Turmaine, M., Cozens, B.A., DiFiglia, M., Sharp, A.H., Ross, C.A., Scherzinger, E., Wanker, E.E., Mangiarini, L. and Bates, G.P. (1997) *Cell*, **90**, 537-48.
- [180] Scherzinger, E., Lurz, R., Turmaine, M., Mangiarini, L., Hollenbach, B., Hasenbank, R., Bates, G.P., Davies, S.W., Leirach, H. and Wanker, E.E. (1997) *Cell*, **90**, 549-58.
- [181] Wyttenbach, A., Sauvageot, O., Carmichael, J., Diaz-Latoud, C., Arigo, A.P. and Rubinsztein, D.C. (2002) *Hum. Mol. Genet.*, **11**, 1137-51.
- [182] Browne, S.E., Ferrante, R.J. and Beal, M.F. (1999) *Brain Pathol.*, **9**, 147-63.
- [183] Beal, M.F. (1994) *Curr. Opin. Neurol.*, **7**, 542-7.
- [184] Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trotter, Y., Leirach, H., Davies, S.W. and Bates, G.P. (1996) *Cell*, **87**, 493-506.
- [185] Li, S.H., Cheng, A.L., Zhou, H., Lam, S., Rao, M., Li, H. and Li, X.J. (2002) *Mol Cell Biol.*, **22**, 1277-87.
- [186] Bonni, A. (2003) *Curr. Biol.*, **13**, R19-21.
- [187] Joza, N., Susin, S.A., Daugas, E., Stanford, W.L., Cho, S.K., Li, C.Y., Sasaki, T., Elia, A.J., Cheng, H.Y., Ravagnan, L., Ferri, K.F., Zamzami, N., Wakeham, A., Hakem, R., Yoshida, H., Kong, Y.Y., Mak, T.W., Zuniga-Pflucker, J.C., Kroemer, G. and Penninger, J.M. (2001) *Nature*, **410**, 549-54.
- [188] Yu, S.W., Wang, H., Poitras, M.F., Coombs, C., Bowers, W.J., Federoff, H.J., Poirier, G.G., Dawson, T.M. and Dawson, V.L. (2002) *Science*, **297**, 259-63.
- [189] Otera, H., Ohsakaya, S., Nagaura, Z., Ishihara, N. and Mihara, K. (2005) *EMBO J.*, **24**, 1375-86.
- [190] Zhu, C., Qiu, L., Wang, X., Hallin, U., Cande, C., Kroemer, G., Hagberg, H. and Blomgren, K. (2003) *J. Neurochem.*, **86**, 306-17.
- [191] Cao, G., Clark, R.S., Pei, W., Yin, W., Zhang, F., Sun, F.Y., Graham, S.H. and Chen, J. (2003) *J. Cereb. Blood Flow Metab.*, **23**, 1137-50.
- [192] Plesnila, N., Zhu, C., Culmsee, C., Groger, M., Moskowitz, M.A. and Blomgren, K. (2004) *J. Cereb. Blood Flow Metab.*, **24**, 458-66.
- [193] Cheung, E.C., Melanson-Drapeau, L., Cregan, S.P., Vanderluit, J.L., Ferguson, K.L., McIntosh, W.C., Park, D.S., Bennett, S.A. and Slack, R.S. (2005) *J. Neurosci.*, **25**, 1324-34.
- [194] Miramar, M.D., Costantini, P., Ravagnan, L., Saraiva, L.M., Haouzi, D., Brothers, G., Penninger, J.M., Peleato, M.L., Kroemer, G. and Susin, S.A. (2001) *J. Biol. Chem.*, **276**, 16391-8.