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Forum Review Article

Transcribe to Survive: Transcriptional Control of Antioxidant Defense Programs for Neuroprotection in Parkinson's Disease

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Abstract

Parkinson's disease (PD) is a progressive, primarily motor disorder that is characterized by loss of dopaminergic (DA) neurons within the substantia nigra (SN). Cell death in PD has been associated with impaired mitochondrial function and increased oxidative stress. Strategies to reduce the oxidative load in DA cells may be beneficial in slowing the progression of PD. The transcription factor nuclear factor-erythroid 2 (NF-E2) related factor 2 (NRF2) is emerging as a master regulator of antioxidant defense systems, which makes it an attractive target for manipulations that aim to increase cellular resistance to oxidative stress. Peroxisome proliferator-activated receptor gamma (PPARγ) coactivator-1 alpha (PGC1α) is a regulator of mitochondrial biogenesis genes that simultaneously upregulates many genes known to protect against oxidative stress. *Pgc-1α* knockout mice show enhanced susceptibility to SN neuronal loss following MPTP exposure, whilst overexpression of *Pgc-1α* appears to protect against oxidative stress *in vitro*. This makes PGC-1α a highly attractive target for neuroprotective therapies in PD. This review will explore the mechanisms behind the induction of NRF2 and PGC-1α in response to oxidative stress and identify common pathways that may provide targets for upregulating antioxidant defense programs. *Antioxid. Redox Signal*. 11, 509–528.

Oxidative Stress in Parkinson's Disease

PARKINSON'S DISEASE (PD) RESULTS FROM A PROGRESSIVE DEGENERATION of specific sets of neurons including (but not restricted to) dopaminergic (DA) neurons in the substantia nigra (SN). Although the cause of the neuronal loss is not known, mitochondrial dysfunction and the generation of reactive oxygen species (ROS) are considered to be critical and interrelated factors (Fig. 1) (8, 10, 168). Impaired mitochondrial CI (CI) activity was identified in platelets (56, 104, 139) and the SN (119, 167) of PD patients, and reduced levels of CI subunits have also been demonstrated (125). The possibility that mitochondrial CI dysfunction might not be merely a secondary effect of neurodegeneration, but rather might play a causal role in PD, came from the identification of 1-methyl-4-1,2,3,6-tetrahydropyridine (MPTP) (106, 170). This toxin induces rapid onset of parkinsonism with degeneration of DA neurons, and has been found to inhibit mitochondrial CI activity (106, 134). Confirmation that CI inhibition can lead to a Parkinson's-like syndrome subsequently came from the demonstration that rotenone, a potent and specific inhibitor of CI, can induce progressive degeneration of DA neurons when administered chronically (4 weeks) in rodents (11). This raised the possibility that mitochondrial CI dysfunction might play a role in idiopathic PD as well. These data implicate mitochondrial CI dysfunction as a key factor in the pathogenesis of PD.

Oxidative stress is a consequence of impaired mitochondrial CI activity. The majority of reactive oxygen species (ROS) are produced in the mitochondria as a by-product of oxidative phosphorylation. Inhibition of mitochondrial CI leads to an increase in the production of these potentially damaging free radicals, including superoxide anions (18, 23, 105, 171). Superoxide anions can react with nitric oxide to form peroxynitrite ($ONOO^-$), a potentially damaging free radical species (Fig. 2). Alternatively, the superoxide anion

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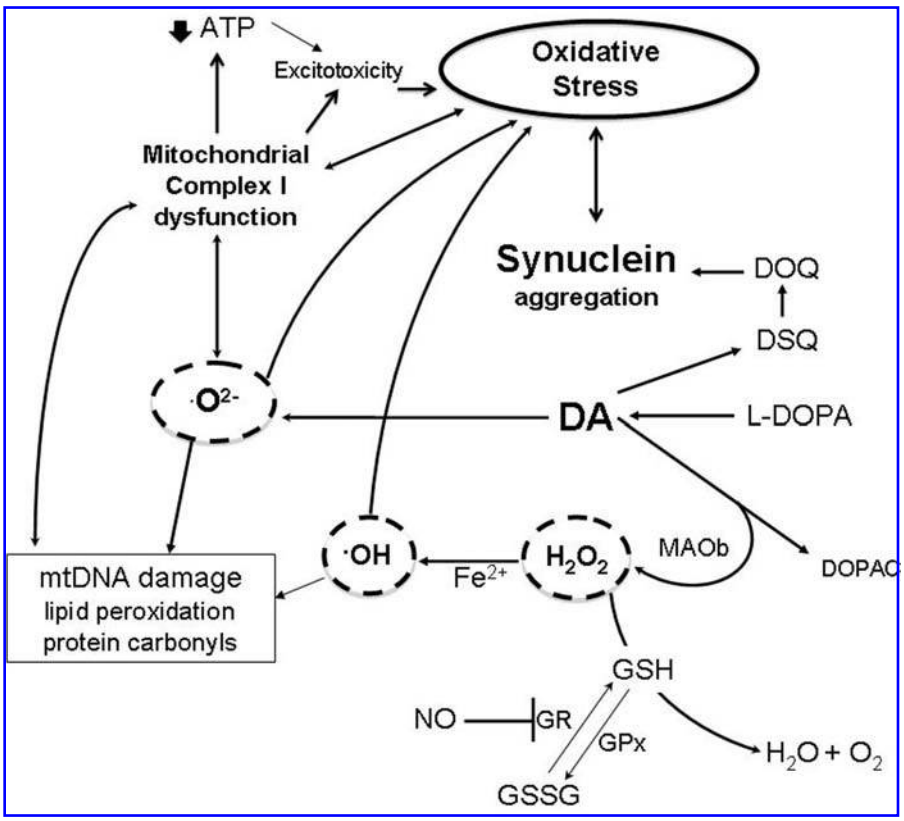

FIG. 1. Major sources of oxidative stress within dopaminergic cells. ATP, adenosine triphosphate; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; DOQ, dopamine orthoquinone; DSQ, dopamine semiquinone; Fe^{2+}, ferrous iron; GR, glutathione reductase; GSH, reduced glutathione; GSSG, glutathione disulfide; H_2O_2, hydrogen peroxide; L-DOPA, levodopa; MAOb, monoamine oxygenase B; NO, nitric oxide; $\cdot O_2^-$, superoxide; ·OH, hydroxyl radical.

can be converted by superoxide dismutase (SOD) to H_2O_2. H_2O_2 then can break down to form damaging hydroxyl radicals via Fenton chemistry. DA metabolism by monoamine oxygenase-b (MAOb) is another potential source of generation of H_2O_2 in these cells, and thus may contribute to the generation of oxidative stress in DA neurons.

Consistent with these data, markers of oxidative damage to lipids (35, 36), proteins (1, 52), and DNA (2, 163, 174, 223) are increased in the SN of PD patients (7, 78). Mitochondrial DNA (mtDNA) accumulates particularly high levels of potentially mutagenic oxidative damage (8-hydroxydeoxyguanosine; 8-OHdG) with age, 16-fold greater than levels in nuclear DNA (124), with even greater increases seen in PD (163, 174). This may result in part from the lack of protective histones on mtDNA and its proximity to free radical generation in the mitochondria (18). Oxidative damage to mtDNA may in turn cause further impairment of mitochondrial function. Some studies suggest that somatic mtDNA point mutations and deletions accumulate with age and may contribute to mitochondrial dysfunction in aging and in PD (9, 22, 55, 103, 186, 194, 200).

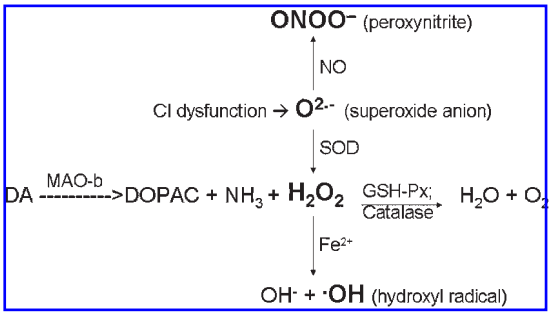

FIG. 2. Fenton chemistry and antioxidant enzymes required for ROS clearance in dopaminergic cells. CI, Complex I; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; Fe^{2+}, ferrous iron; GSH-PX, glutathione peroxidase; H_2O_2, hydrogen peroxide; NH_3, ammonia; NO, nitric oxide; SOD, superoxide dismutase.

Antioxidant Activities in PD

Cells have many antioxidant mechanisms to counteract the deleterious effects of ROS. L-γ-glutamyl-L-cysteinylglycine (GSH; glutathione) is an important endogenous antioxidant molecule, and depletion of glutathione likely contributes to the accumulation of high levels of oxidative damage to mtDNA in the SN (38, 77, 142, 169). GSH is the predominant intracellular thiol antioxidant (32) and GSH deficiency is a very early finding in the SN in PD (37). Induction of GSH deficiency in cell lines (79) and in mice (26) leads secondarily to a specific defect in CI activity. GSH depletion in rodents by pharmacologic or genetic methods potentiates MPTP toxicity (93, 214, 222). In the human SN, the density of glutathione peroxidase (GPX)-positive cells is higher in the vicinity of the DA cell groups known to be resistant to the pathological process of PD. In PD, an increased density of GPX-immunostained cells is found surrounding late-surviving DA neurons (33). These data support the hypothesis that GSH levels may play an important role in determining the vulnerability of DA neurons to oxidative stress.

Further support for this hypothesis comes from data related to the function of DJ-1. Mutations in DJ-1 cause autosomal recessive early onset PD (17). Loss of DJ-1 function

renders cells more susceptible to oxidative stress (120, 218, 224). It has recently been demonstrated that overexpression of wild-type (but not mutant) *Dj-1* protects against oxidative stress by increasing GSH synthesis (224). Experimental GSH depletion in mice recently has been shown to result in a substantial increase in DNA deletion frequencies, an effect that is blocked by *N*-acetylcysteine (NAC) (156). Given that GSH is deficient at early stages in PD, these data potentially could account for the recent report of increased levels of large mtDNA deletions in cyclooxygenase (COX)-deficient SN neurons in PD (9). Thus, a convergence of data appears to implicate GSH as a key player in the pathogenesis of PD.

Connection to α-Synuclein

A growing body of evidence indicates a connection between α-synuclein (SNCA), oxidative stress, and mitochondrial dysfunction in PD. Selective and specific oxidative damage to both soluble and insoluble α-synuclein, which increases the tendency of α-synuclein to aggregate, has been demonstrated in the brains of PD patients as well as in other neurodegenerative diseases associated with α-synuclein-positive aggregates (43, 51, 62, 140, 180). CI inhibition by MPTP (100, 201) or rotenone (11, 172), which increases free radical production *in vitro* and *in vivo* (61, 105, 144, 146, 171, 176, 220), induces the accumulation of insoluble α-synuclein aggregates similar to Lewy bodies in DA neurons (Fig. 1). Other *in vitro* data implicate a connection between α-synuclein toxicity and this increase in free radicals caused by CI dysfunction. Free radicals lead to the stabilization of a toxic soluble "protofibril" form of α-synuclein, and this stabilization appears to depend on the oxidative ligation of α-synuclein to DA (27, 180, 214). For example, DA cells are susceptible to toxicities associated with *SNCA* overexpression, whereas nondopaminergic cells are not (214). Inhibition of DA synthesis and antioxidant agents block the enhanced susceptibility to oxidative stress associated with *SNCA* overexpression (214). Thus, the toxicity of α-synuclein requires DA synthesis and is mediated by free radicals. This may account for the fact that inhibition of mitochondrial CI, even if systemic, leads to the selective degeneration of DA neurons in the SN, as demonstrated by Betarbet and colleagues (11).

Transcriptional Regulation of Antioxidant Enzymes

These data, implicating oxidative stress and inadequate antioxidant activities in the pathogenesis of PD, highlight the importance of understanding the mechanisms of transcriptional regulation of antioxidant enzyme activities, both to advance our understanding of the pathogenesis of PD and to facilitate the search for neuroprotective strategies. Nuclear factor-erythroid 2 (NF-E2) related factor 2 (NRF2) is an important transcription factor that upregulates multiple antioxidant defense mechanisms in response to oxidative stress. Peroxisome proliferator activated receptor (PPAR) γ coactivator 1α (PGC-1α; previously known as PPARGC1a), is a transcriptional coactivator that coordinately regulates genes required for mitochondrial biogenesis, and is another key contributor to the upregulation of antioxidant activities in response to oxidative stress (Fig. 3). NRF2 and PGC-1α are, themselves, regulated through complex pathways that are only partially understood. In this review, we will outline the roles of NRF2 and PGC-1α in the response to oxidative stress, and summarize the regulatory mechanisms that may influence their transcriptional regulation of the antioxidant program. We will highlight where these mechanisms may be of relevance to the pathogenesis of PD and/or to the development of neuroprotective strategies.

A Brief Overview of Transcription

Transcription is a tightly regulated and coordinated process. Activation of a gene requires the coordinated action of a large number of proteins at various sites on the chromosome. The fundamental event to initiate transcription occurs when the core promoter binds RNA polymerase II and the basal transcription factors (TFIIA-TFIIH) that form the general transcription machinery. *In vivo* the binding of the general transcription machinery alone is sufficient to support only low levels of transcription (128); the coordination of ad-

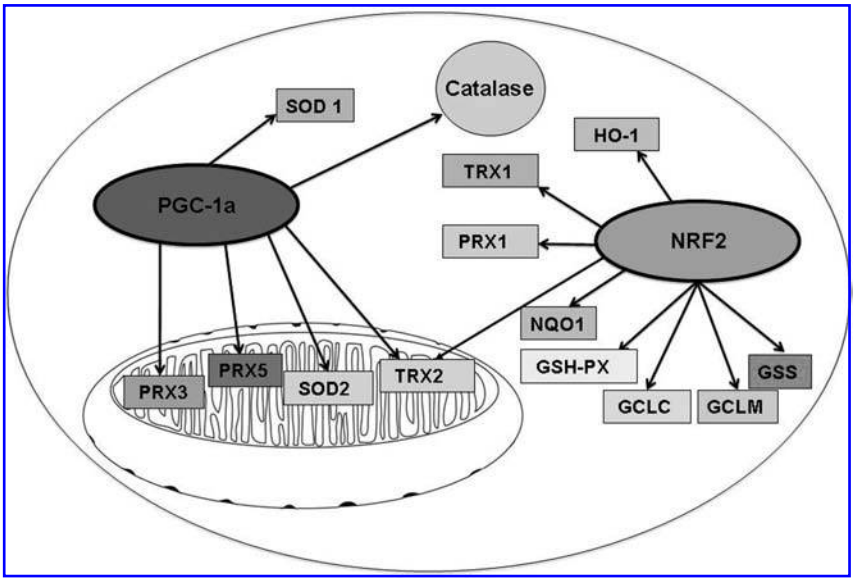

FIG. 3. PGC-1a or NRF2 regulated antioxidant enzymes. Catalase is shown within a peroxisome. PRX3, PRX5, SOD2, and TRX2 are mitochondrial. All other enzymes are cytosolic. GCLC, glutamyl-cysteine ligase catalytic subunit; GCLM, glutamyl-cysteine ligase regulatory subunit; GSH-PX, glutathione peroxidase; GSS, glutathione synthetase; HO-1, heme oxygenase-1; NQO1, NAD(P)H dehydrogenase quinone-1; PRX, peroxiredoxin; SOD, superoxide dismutase; TRX, thioredoxin.

ditional transcription factors and coactivators at the promoter is required for more active transcription of a gene.

Coactivators of transcription do not bind directly to DNA. Broadly speaking coactivators can function in three ways. Complexes such as Mediator function to bind transcription factors, recruit RNA polymerase II, and interact with general transcription factors and other co-factors (203). Other proteins such as cAMP-responsive element binding protein (CREB) binding protein (CBP) and its functional homologue p300 influence transcription through enzymatic activity to produce covalent modifications in histones; p300/CBP act as histone acetyl transferases (HAT) (122). Finally, ATP-dependent chromatin remodeling enzymes such as SNF2 (183) alter the contacts between DNA and the histone octamers, thereby decondensing the chromatin. NRF2 is a canonical DNA-binding transcription factor, whilst PGC-1α is a transcriptional coactivator with pleiotropic effects.

NRF2 and the Antioxidant Response Element

NRF2 belongs to the cap'n'collar (CNC) family of basic leucine zipper transcription factors, which includes NF-E2, NRF1, and NRF3 (24). These transcription factors share the CNC-like bZIP domain and require heterodimerization with small musculoaponeurotic fibrosarcoma oncogene (MAF) proteins to bind the MAF recognition element (MARE) and activate transcription of the target gene. MAF proteins may homodimerize or differentially bind CNC-family bZIP proteins or Bach family proteins; the presence of functionally distinct heterodimers between the CNC-family bZIP proteins and small MAF proteins confers specificity to transcription of target genes (215). The MARE is a long palindromic DNA sequence [TGCTGAC(G)TCAGCA] (87); known MAREs include the cyclic AMP-response element (CRE; TGACGTCA), to which the CREB/activating transcription factor (ATF) family of bZIP transcription factors bind (127), the 12-O-tetradecanoylphorbol-13-acetate (TPA) response-element (TRE; TGACTCA) to which the transcription factor activating protein-1 (AP-1) binds (15) and the antioxidant response element (ARE; TGACNNNGC). NRF2 binds to the ARE to regulate the transcription of phase II antioxidant enzymes (71, 159), including heme oxygenase-1 (HO-1) (46, 147), NAD(P)H quinone oxidoreductase-1 (NQO1) (46, 204), glutathione-*S*-transferases (GSTs) (160), the glutathione synthetic enzyme glutamate-cysteine ligase (GCL), GPX, glutathione reductase (GR) (48, 49, 129), and the thioredoxin (TRX) (65) and peroxiredoxin (PRX) families (70).

NRF2 Functional and Regulatory Domains

The NRF2 protein has six functional domains, termed Nef2 ECH homology (Neh) domains (Fig. 4), which are highly conserved among vertebrates (98, 187). Neh1 is important for the transactivation activity of the protein and consists of the CNC basic DNA-binding domain and the bZIP domain. Neh2 is an important redox-sensitive regulatory domain containing ETGE and DLG amino acid motifs that are required for the binding of the NRF2 regulating protein Kelch-like erythroid cell derived protein with CNC homology (ECH)-associated protein 1 (KEAP1) (123, 192). The Neh2 domain also contains one of the three nuclear localization sequences that have been identified in NRF2 (189). In addition to Neh2, the Neh6 domain may also function as a degron (degradation signal), with important regulatory activity on levels of nuclear NRF2 under conditions of oxidative stress (123). Neh3, Neh4, and Neh5 are thought to constitute the transactivation domains of NRF2. Although these regions are not required for nuclear import of NRF2 or DNA-binding activity, they are critical for the ability of NRF2 to activate the transcription of target genes (88, 133). It is likely that these

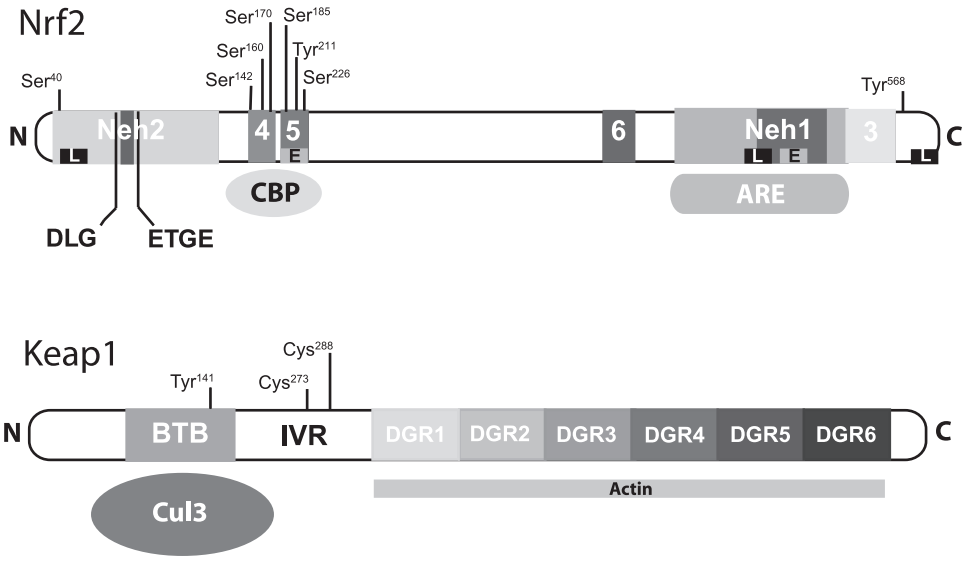

FIG. 4. Functional domains and phosphorylation sites of NRF2 and KEAP1. NRF2: The Neh2 domain of NRF2 contains the DLG and ETGE motifs required for binding to KEAP1 and a phosphorylation site (Ser^{40}) required for NRF2 release from KEAP1. Neh domains 4 and 5 constitute the transactivation domains and bind to CBP. Casein kinase 2 phosphorylation on sites located within these domains is important for NRF2 transactivation activity. Neh6 is a degradatory domain. Neh1 contains the CNC binding motif and leucine zipper (*darker portion*) and binds the ARE. Neh3 is another domain required for transactivation activity. The C-terminus contains a phosphorylation site (Tyr^{568}) required for nuclear export. 'L' and 'E' represent nuclear localization and nuclear export domains respectively. KEAP1: The BTB domain is required for homodimerization and binds Cul3 to mediate the proteasomal degradation of NRF2. The IVR contains cysteine residues required for conformational changes in response to oxidative stress. Not to scale. DGR domains 1–6 bind both NRF2 and F-actin. ARE, antioxidant response element; BTB, broad/tramtrack/bric-a-brac; CBP, Creb-binding protein; Cul3, Cullin 3; IVR, intervening region; DGR, double glycine repeat. Neh, NE-F2 ECH homology.

domains recruit other proteins of the transcription complex that are required for the activation of transcription at the ARE. Neh4 and Neh5 have been found to cooperatively bind the transcriptional coactivator CBP (88), which functions as a coactivator for hundreds of transcription factors and can form a molecular bridge between sequence specific transcription factors and the RNA polymerase II holoenzyme (91, 131, 202). The cooperative binding of CBP by two domains in the same protein may place a priority on the expression of NRF2 cytoprotective target genes at the expense of other genes (88). The C-terminus of NRF2 is thought to be important for NRF2 protein function and is known to contain a nuclear localization sequence (amino acids 587–593 of murine NRF2) and a tyrosine residue (Tyr^{568}) that, when phosphorylated by the nuclear kinase Fyn, is involved in the nuclear export of NRF2 (74).

Regulation of NRF2 by KEAP1

Oxidant molecule detection provides the stimulus for translocation of NRF2 from the cytoplasm to the nucleus and for the binding of NRF2 to the ARE. The relocation of NRF2 from the cytosol to nucleus is a de-repression event. The protein responsible for both oxygen sensing and the repression of NRF2 activity under basal conditions is termed KEAP1. KEAP1 acts as both an oxidant sensor and as a substrate adaptor protein for NRF2 degradation by Cul3-BTB^{KEAP1} E3 ligase (29, 47, 96, 221). Under basal conditions, KEAP1 represses the action of NRF2 by accelerating its turnover by the ubiquitin–proteasome system (72, 96). KEAP1 is also involved in shuttling NRF2 in and out of the nucleus (184, 199).

KEAP1 binds to NRF2 at Nhe2. The KEAP1 protein has five, distinct functional domains: (Fig. 4) an N-terminal region (NTR; amino acids 1–60), the broad complex, Tramtrack, Bric-a-brac domain (BTB; amino acids 61–179) which is highly conserved and indispensable for homodimerization, and KEAP1 function (225), an intervening region (IVR; amino acids 180–314) which is relatively rich in cysteine residues, a double-glycine repeat domain (DGR or Kelch domain; amino acids 315–598) which binds to both F-actin and NRF2 to sequester NRF2 in the cytoplasm (73, 86, 198), and a C-terminal region (CTR; amino acids 599–624) which is also thought to participate in the binding of KEAP1 to NRF2 (41, 192).

Under basal conditions, KEAP1 functions as part of the Cul3-BTB^{KEAP1} E3 ligase complex to promote the ubiquitination of lysine residues in the Neh2 domain of NRF2 (Fig. 5). Under conditions of oxidative stress, reactive cysteine residues within the IVR of KEAP1 may be differentially modified (40), resulting in a conformational change in the KEAP1

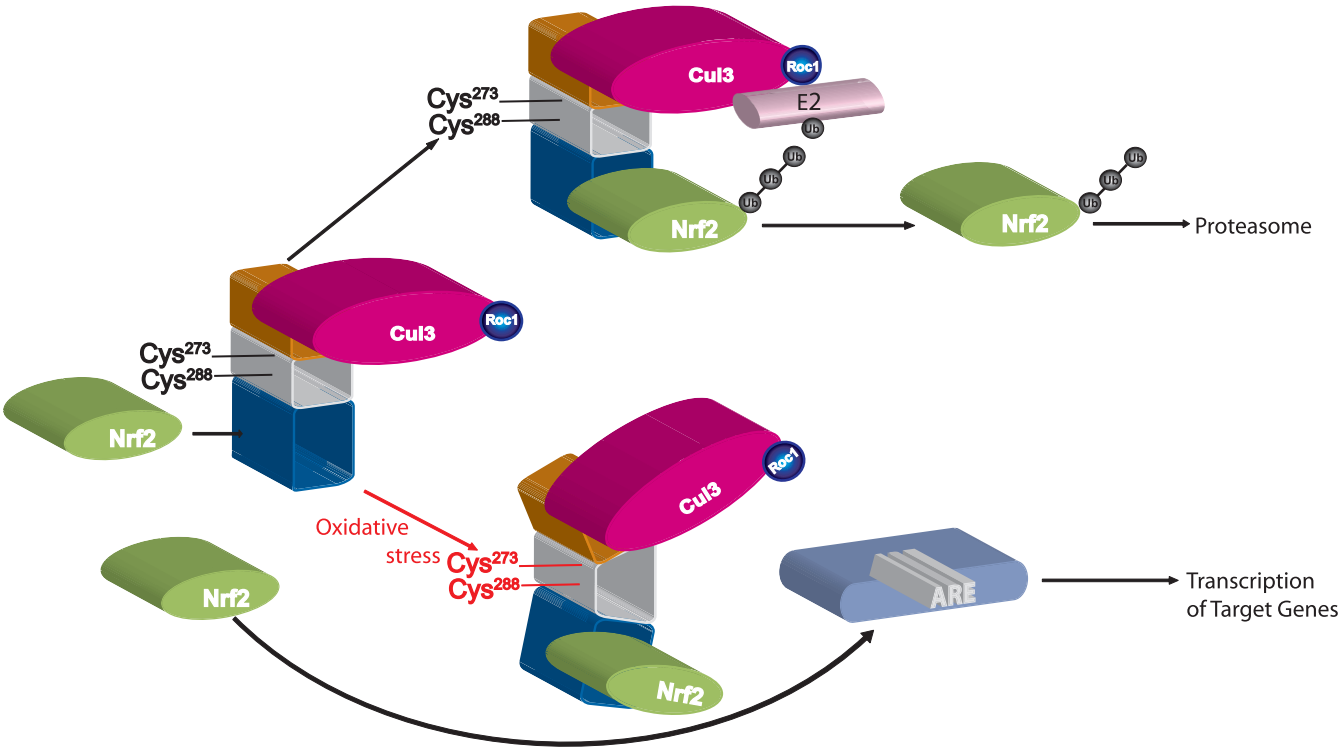

FIG. 5. Hinge–Latch mechanism for NRF2 release from KEAP1. Under basal conditions NRF2 is bound by the DRG domain (*blue*) and Cul3 is bound by the BTB (*orange*) domain of Keap1. The Cul3-BTB^{Keap1} complex mediates the addition of ubiquitin moieties from an E2 ubiquitin-conjugating enzyme to NRF2 and targets it for proteasome-mediated degradation. Alternatively, oxidative modification of Cys^{273} and Cys^{288} (*red arrow*) induces conformational change in KEAP1 and prevents interaction between Nrf2 and Cul3 whilst retaining NRF2 at the DRG domain. Newly synthesized NRF2 can bypass Cul3-BTB^{Keap1} mediated-degradation and can facilitate induction of target genes by binding to the ARE. Not to scale. After Tong *et al.*, 2006. ARE, antioxidant response element; Cul3, Cullin 3; DRG, double glycine repeat; Roc1, regulator of Cullins 1; Ub, ubiquitin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

protein that blocks ubiquitination of NRF2 but does not release the Neh2 domain of NRF2 from KEAP1 (97, 193). This retention of nonubiquitinated NRF2 by KEAP1 under conditions of oxidative stress seems paradoxical but may function to effectively inhibit KEAP1, allowing newly synthesized NRF2 proteins to escape degradation and bind to the ARE for transcription of target genes (Fig. 5). KEAP1 may also be modified by phosphorylation; recent work has identified that phosphorylation of tyrosine residue 141 in the BTB of KEAP1 is required for its function and stability (75).

Regulation of NRF2 activity by Post-Translational Modifications

The phosphorylation status of NRF2 affects its stability and subcellular localization. The major signaling pathways that are thought to impact upon phosphorylation of NRF2 in response to oxidative stress are the protein kinase C (PKC) pathway, the mitogen-activated protein kinase (MAPK) cascades, and the phosphotidylinositol 3-kinase/AKT (PI3K) pathway (14, 66, 82, 85, 110). PKR-like endoplasmic reticulum kinase (PERK) has also been shown to phosphorylate NRF2 and mediate its nuclear import (30). PERK is a key regulator of translation (28) and this finding also implicates NRF2 in cell survival via the unfolded-protein response, as well as the response to oxidative stress. Phosphorylation of NRF2 by PKC at Ser^{40} within the Nhe2 domain is important in releasing NRF2 from KEAP1 but is not sufficient for the nuclear accumulation or transactivation activity of NRF2 (14). Instead, transactivation activity may be determined by phosphorylation of the Neh4 and Neh5 domains by casein kinase 2 (CK2), as recently shown in neuroblastoma cells (6). There is likely to be coordinated modulation of NRF2 activity by the MAPK pathways; ERK2, ERK5, and JNK MAPK have been found to activate NRF2 (219), whilst p38 MAPK phosphorylation of NRF2 has been found to increase its association with KEAP1 and prevent translocation of NRF2 to the nucleus in human hepatoma cells (90).

Activation of the PI3K/AKT pathway is generally associated with cell survival (42, 152), and many inducers of NRF2, including *tert*-butylhydroquinone (tBHQ), hemin, and peroxynitrite upregulate PI3K/AKT signaling (12, 212, 217). This protective effect of PI3K/AKT activation may be due to inhibitory effects on glycogen-synthase 3-beta (GSK3β) (157, 162). GSK3β is a proline-directed serine/threonine kinase involved in energy metabolism, neuronal cell development, and midline development (145, 182). GSK3β is known to be pro-apoptotic in neurons (64, 113, 116). Rojo and colleagues (157) found that AKT (as detected by immunoblotting with an antibody specific for the Ser^{473} phosphorylated form of AKT) was rapidly but transiently activated following administration of H_2O_2 to N2a neuroblastoma cells, and that the inhibitory Ser^9 phosphorylation of GSK3β followed the same time-course. Transfection of N2a cells with EGFP-tagged NRF2 revealed that nuclear localization of NRF2 coincided with the appearance of the active form of AKT and the inactive form of GSK3β, suggesting a link between AKT activation and inhibition of GSK3β. Pre-incubation with the AKT inhibitor LY294002 increased the distribution of NRF2 in the cytoplasm of untreated cells and partially prevented H_2O_2 induced translocation of NRF2 to the nucleus, illustrating that AKT phosphorylation may have an important role in the nuclear translocation of NRF2 in response to oxidative stress. Transfection of an ETGE KEAP1-binding domain deleted mutant of NRF2 (ΔETGE-NRF2) targeted primarily to the nucleus; however, co-transfection with a Ser^9 deleted (and therefore constitutively active) GSK3β mutant resulted in a profound localization of ΔETGE-NRF2 to the cytosol. In addition, Rojo and colleagues (157) noted that co-transfection of the constitutively active GSK3β mutant prevented NRF2-mediated ROS clearance, as measured by flow cytometry for an oxidant sensitive fluorescent probe. Therefore, GSK3β may be involved in the cytoplasmic sequestration of NRF2 in the absence of ROS, a role associated with KEAP1 (199), and AKT inactivation of GSK3β may contribute to the release of NRF2 repression in response to oxidative stress. It may be that GSK3β acts in concert with KEAP1 to prevent localization of NRF2 to the nucleus, or this may represent a distinct mechanism of NRF2 negative regulation by GSK3β.

NRF2 and Parkinson's Disease

DJ-1 (PARK7) is a gene in which loss-of-function mutations have been found to be linked to 1–2% of early-onset PD cases (16, 118). DJ-1 is likely to be a multifunction protein that has been found to have neuroprotective capabilities in response to an oxidative insult (5, 117) and other functions highly relevant to oxidative stress and PD (68). It has been shown, *in vitro* and in *Dj-1*$^{-/-}$ mice, that DJ-1 functions to stabilize NRF2 by preventing the interaction between NRF2 and KEAP1 and subsequent degradation by the Cul3-BTB^{KEAP1} complex. Thus, NRF2 dysfunction may contribute to the pathogenesis of early-onset familial forms of PD linked to DJ-1 mutations.

NRF2 is ubiquitously expressed in most tissues. In human brain, NRF2 protein has been detected in substantia nigra neurons and there is some evidence from postmortem PD brain tissue that NRF2 is translocated to the nucleus in these cells; whereas in normal aged matched controls, the protein was distributed throughout the cytoplasm (155). This may represent a compensatory attempt to upregulate glutathione synthesis and other antioxidant mechanisms in response to the deficiency of glutathione that has been observed in postmortem SN in PD (35, 179). Studies of NRF2 target gene expression in postmortem PD brain tissues are scarce at the present time. However, in early-stage PD postmortem brain tissue, increased expression of the NRF2-ARE regulated gene *NQO1* has been detected in the nigral neurons of the SNpc and in astrocytes located to the same region (197), the increase in astrocytic NQO1 was absent in late-stage PD with advanced loss of DA neurons. The reason for the lack of *NQO1* expression at the later stage is unclear, but it seems that the upregulation of NQO1, at least in non-neuronal cells, in early PD may not be sufficient to protect the neuronal cells of the SN. Work with postmortem tissue from individuals with PD represents the 'aftermath' of the molecular events that have perpetrated the disease state, as PD is not usually diagnosed until there is already substantial neuronal loss. The PD nigral neurons observed in the study by Ramsey and colleagues (155) represent the late-surviving neurons. The status of NRF2 and NRF2-target genes in PD neurons that subsequently die during the course of the disease (*i.e.*, at very early stages of PD) remains unknown. Further study of the

regulation and activity of NRF2 in diseased states is required to ascertain the extent of NRF2 involvement in neurodegeneration in PD.

There is some evidence that DA itself can activate the NRF2 antioxidant program, especially in the non-neuronal cells of the nervous system (102, 173). Work by Shih and colleagues (173) illustrated the activation of NRF2 in cultured rat primary astrocytes and meningeal cells after transduction with an adenoviral construct expressing a reporter protein under the control of the ARE. This response was attributed to the production of H_2O_2 and DA-quinones (from excess DA), inducing the release of NRF2 from KEAP1. NRF2 induction was specific to oxidative stress produced by catecholaminergic neurotransmitters, as the monoaminergic neurotransmitter serotonin had no effect on NRF2 mediated expression from the ARE. The finding that low doses of DA (and 6-OHDA) can induce the antioxidant defense program has recently been corroborated in SH-SY5Y neuroblastoma cells and in human primary neuronal cultures (80, 81).

Further evidence that levels of NRF2 activity may be implicated in PD, or that NRF2 upregulation may be an effective strategy for treatment (190), comes from recent revelations that some drugs prescribed to treat the symptoms of PD may affect NRF2 activity. Subcutaneous infusion of apomorphine, a D_1/D_2 receptor agonist, is used for the treatment of certain motor complications that may arise during late-stage PD (67, 111). However, apomorphine is also thought to be a potent free radical scavenger and can prevent cell death *in vitro* in neuroblastoma cells (50, 59, 60). More recent work demonstrated that apomorphine activates the NRF2-ARE pathway to induce the expression of *HO-1* and protect cultured SH-SY5Y neuroblastoma cells (59). Interestingly, this protective effect persisted in the presence of DA_1 and DA_2 antagonists suggesting that, at least *in vitro*, the effects of apomorphine on NRF2 rather than D_1/D_2 may be critical for cell survival. The later study by Hara and colleagues (59) also illustrates the role of low levels of ROS as a signaling mechanism to activate the NRF2-ARE pathway. The ROS initially produced by the addition of apomorphine to the culture medium may provide the stimulus for KEAP1 release of NRF2 by oxidizing the reactive cysteine residues on KEAP1, as the induction of HO-1 mRNA in the presence of apomorphine was eliminated when the cells were treated with the antioxidant NAC.

Deprenyl (Selegeline), a selective MAOb inhibitor is a candidate drug for neuroprotection in PD (92, 166); however, it has been suggested that the protective effect of deprenyl is due to its effects on glyceraldehyde-3-phosphate dehydrogenase (GAPDH) rather than MAOb (188). In addition, deprenyl can induce the NRF2 antioxidant program via the activation of PI3K (130). Interestingly, this work by Nakaso and colleagues (130) seems to indicate that PI3K functions independently of its downstream effector AKT when activated by deprenyl. Bromocriptine, a D_2 receptor agonist and antioxidant used in PD therapy, has also recently been found to activate PI3K as well as AKT, and the subsequent protective effects *in vitro* were, again, found to be due to activation of the NRF2-ARE system and to be independent of effects on the D_2 receptor (114).

There is promising *in vitro* and *in vivo* data indicating that *NRF2* overexpression may be a potential protective therapy for PD. *Nrf2* overexpression has been found to protect against 6-OHDA toxicity and mitochondrial CI and CII inhibitors (20, 21, 76, 108, 109). Conversely, studies in $Nrf2^{-/-}$ mice have shown that these animals are more sensitive to CI inhibitors, such as MPTP, as evidenced by loss of striatal DAT (20). $Nrf2^{-/-}$ mice also exhibit increased sensitivity to striatal injection of 6-OHDA as measured by TH immunoreactivity in the SN and striatum compared to wild-type littermates (76). The same study also demonstrated that striatal injection of astrocytes expressing *Nrf2* from an adenoviral vector, 5 weeks before the administration of 6-OHDA, significantly reduced lesion volume in $Nrf2^{-/-}$ animals compared to an adeno-*GFP* control virus. These data together indicate the importance of investigating strategies to increase NRF2 activity as a strategy for neuroprotection in PD.

There may be caveats associated with upregulating NRF2 activity, at least with whole-organism overexpression. First, with the exception of luteolin, the known pharmacological inducers of NRF2 activity are not known to cross the blood-brain barrier to a significant degree (210). Therefore, it may be necessary to develop more potent inducers of NRF2 that can cross the blood-brain barrier before oral agents aimed at upregulating NRF2 can provide an effective neuroprotective strategy. There is also a more serious complication that may be associated with *NRF2* overexpression. Although NRF2 upregulation has been associated with cancer protection (141) and $Nrf2^{-/-}$ mice are more prone to develop tumors (154), recent work has implicated NRF2 in the development of certain cancers and in tumor resistance to chemotherapeutic drugs (135, 207). These problems of blood-brain barrier penetrance and deleterious effects with respect to certain cancers might be circumvented by specific strategies to restore or increase NRF2 function within the affected area (*e.g.*, SN), for example with gene therapy techniques or via stem cell transplants. Pharmacological agents may also be of use if their specificity can be dissected out. Characterizing the regulatory functions of MAF protein dimerization may enable strategies whereby NRF2-associated transcription can be targeted to specific gene promoters in certain tissues without affecting other ARE-containing promoter regions (13). Disruption of the repressive KEAP1-NRF2 complex may also be a further strategy to upregulate the NRF2-dependant antioxidant program in certain tissues (89).

PGC-1α and Transcriptional Co-Activation

Peroxisome proliferator activated receptor (PPAR) γ coactivator 1α (PGC-1α) is emerging as a key transcriptional regulator of antioxidant defense systems. PGC-1α is a multifunctional protein that activates most nuclear receptors and also functions as a coactivator to many transcription factors (151). PGC-1α is a large (~100 kD) protein with a number of functional domains that bind various protein complexes (Fig. 6). The N-terminal of PGC-1α contains an activation domain, which interacts with CBP/p300 (148). Neighboring this region is an inhibitory domain to which repressor proteins may bind. PGC-1α also contains a specific domain that binds the thyroid receptor-associated protein (TRAP)/vitamin D receptor-interacting protein (DRIP)/Mediator complex (203), illustrating a direct interaction of PGC-1α with the general transcription machinery. The C-terminus of PGC-1α contains a serine/arginine-rich domain (RS) and a putative RNA

recognition motif (RRM), that are both involved with RNA binding and splicing (53, 126). PGC-1α associates with the elongating form of RNA polymerase II and binds proteins that regulate RNA splicing (95), which contributes to PGC-1α's activity as a highly potent coactivator of transcription.

PGC-1α is a critical regulator of metabolism that links metabolic activity to relevant environmental stimuli in multiple pathways, including those responsible for adipogenesis, gluconeogenesis, myogenesis, and mitogenesis (58). In addition, PGC-1α can coordinate the expression of many antioxidant programs in response to oxidative stress (4, 143, 181, 196). Such a pleiotropic protein must be highly regulated in order to perform such diverse functions. The characterization of PGC-1α regulation is still underway, and only the mechanisms that contribute to antioxidant regulation are outlined here.

Regulation of PGC-1α Activity–Tissue Expression

PGC-1α has a highly defined pattern of tissue expression and is found at higher levels in tissues with high metabolic requirement such as brown fat, skeletal muscle, kidney, heart, and brain (44, 94, 212). In the rodent brain, *in situ* hybridization analysis has identified *Pgc-1α* expression in many areas, including cerebral cortex, striatum and globus pallidus, hippocampus, and SN (195). Expression of *Pgc-1α* in brain is not stimulated by physiological changes (such as caloric deficiency, leptin, obesity, or cold exposure) that induce *Pgc-1α* in other tissues. This may occur because the brain is buffered against low glucose and fluctuations in plasma levels of fats by physiological processes occurring in the periphery (some of which may involve the actions of PGC-1α in other tissues) and so there may be little requirement for a response to these classical inducers of *Pgc-1α*.

PGC-1α activity in neurons may be involved in regulating the cellular response to oxidative stress (181). ROS induce the expression of several genes coding for antioxidant enzymes in 10T1/2 cells, including superoxide dismutase 1 (*Sod1*), superoxide dismutase 2 (*Sod2*), and *Gpx1*, as well as the uncoupling proteins *Ucp1* and *Ucp2* (181). RNAi knockdown of *Pgc-1α* prevents the induction of these genes by ROS, indicating that PGC-1α mediates these protective responses. Catalase expression was also increased by ROS *in vitro*, but the increase was not blocked by *Pgc-1a* siRNAs, indicating that ROS-dependent induction of catalase may be otherwise regulated in this cell type. In the *Pgc-1$\alpha^{-/-}$* mouse baseline levels of catalase, as well as SOD1 and SOD2 (but not GPX1) appear decreased (115). Further studies are needed to clarify the precise role of PGC-1α in regulating catalase expression.

In addition, the *Pgc-1$\alpha^{-/-}$* mouse, which displays hyperactivity and neurodegenerative lesions, was found to be substantially more sensitive to low doses (10 mg/kg) of MPTP than wild-type littermates (181). This sensitivity was apparent through a reduced number of DA SN neurons, reduced TH immunoreactivity within the surviving TH positive SN neurons, and increased levels of nitrosylated proteins in the MPTP-treated *Pgc-1$\alpha^{-/-}$* mice compared to wild-type controls. The *Pgc-1$\alpha^{-/-}$* mice also display increased oxidative damage in the CA1 region of the hippocampus after excitotoxicity induced by the glutamate receptor agonist kainic acid (181). The CA1 neurons appeared hypereosinophilic with chromatin clumping and increased TUNEL-positive staining compared to wild-type kainic acid-treated controls. There was increased 8-OHdG staining in the CA1 region of *Pgc-1$\alpha^{-/-}$* mice compared to wild-type. The fact that both MPTP and kainic acid treatment resulted in increased oxidative stress in *Pgc-1$\alpha^{-/-}$* mice compared to wild-type mice indicates that levels of PGC-1α normally present in rodent brain play an important role in maintaining antioxidant defenses. This may have important ramifications for neurodegenerative diseases.

Regulation of PGC-1α Activity at the PGC-1α Promoter

The induction of PGC-1α in response to H_2O_2 is regulated by Creb binding to the conserved creb-response element (Cre) of the *Pgc-1α* promoter (181), which also accounts for

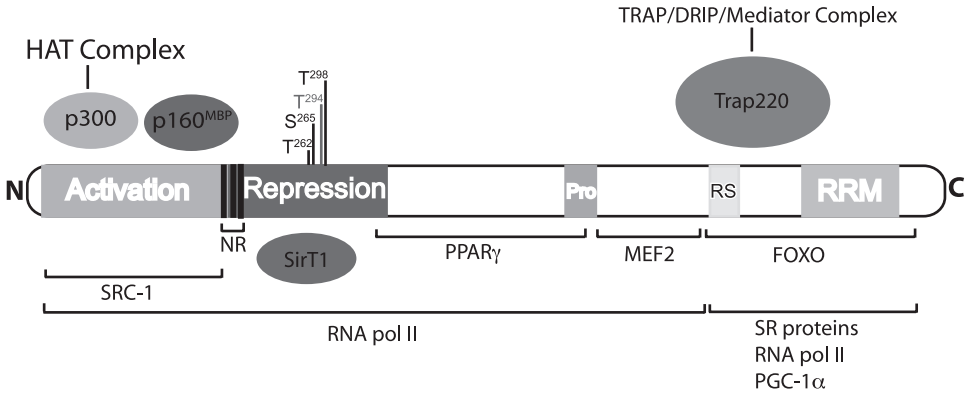

FIG. 6. Functional domains and phosphorylation sites of PGC-1α. The N-terminus contains an activation domain that binds p300 to facilitate transcriptional activation via the HAT complex. This activation domain may be regulated by the presence of an adjacent repression domain that can bind p160^{MBP}, which may prevent interaction with p300. Phosphorylation of serine and threonine residues in the repression domain by p38 MAPK prevents the PGC-1α-p160^{MBP} interaction and increases the activity of the protein. Pro denotes a proline rich domain. The RS domain is a serine/arginine-rich domain that may be required for RNA splicing. RRM binds RNA and is also required for the splicing activity of PGC-1a. The regions of PGC-1a that bind the main interacting proteins are outlined below the schematic of the protein. Not to scale. After Lin et al., 2005 (114a) and Knutti and Kralli, 2001 (95). FOXO, forkhead transcription factor; HAT, histone acetyl-transferase; MEF2, myocyte-enhancer factor 2; NR, nuclear receptors; SR, serine/arginine-rich RNA splicing proteins; SRC-1, steroid receptor coactivator 1.

Pgc-1α upregulation by cAMP signaling in mouse liver and skeletal muscle (57, 63). Thus, cAMP signaling is important for upregulation of *Pgc-1α* transcript expression in a number of tissue types. The PGC-1α promoter also has forkhead transcription factor (FOXO), myocyte-enhancer factor 2 (MEF2), peroxisome proliferator-activated receptor α and γ (PPARα, PPARγ) and estrogen-related receptor α (ERRα) binding domains (31, 58, 205). These activators of PGC-1α are variably distributed across multiple tissues, and their specificity of tissue expression contributes to the pleiotropic effects of PGC-1α. However, each of these inducers is expressed in brain, which may contribute to a complex regulation of PGC-1α-mediated effects between neuronal cell types and glia and between different brain regions. In addition to being inducers of PGC-1α, FOXO, MEF2, PPARγ, and ERRα are also regulated by interactions with PGC-1α (Fig. 6). Therefore, there is an autoregulatory loop controlling the expression of *PGC-1α* and the activities of its downstream binding partners (31, 57, 149, 205).

There is some evidence that these binding partners of PGC-1α may be relevant to oxidative stress in PD. The FOXO protein family (FOXO1, FOXO3a, FOXO4, and FOXO6) is increasingly implicated in the transmission and regulation of oxidant signaling, in addition to roles in cell proliferation, differentiation, apoptosis, and DNA repair. FOXO3a has been found to coordinate the upregulation of SOD2 (99) and catalase (132) in response to oxidative stress; however, the role of PGC-1α in this response is not yet known. Inactivation of MEF2 may be implicated in PD, as phosphorylation on Ser^{444} of MEF2 has been identified as a critical factor for promoting DA cell death after MPTP treatment, and adenoviral delivery of a mutant MEF2 in which Ser^{444} was substituted to alanine was neuroprotective against MPTP toxicity in wild-type mice (178).

PPARγ agonists have been shown to protect against MPTP-induced cell death. Pioglitazone, which is clinically used to treat high glucose levels in diabetes, crosses the blood-brain barrier. Oral administration of Pioglitazone to mice prior to MPTP treatment (15 mg/kg) was found to protect SN neurons and reduce glial activation (19). Other work determined that administration of Pioglitazone prior to MPTP treatment of mice reduced markers of oxidative stress such as nitrotyrosine immunoreactivity, and also reduced iNOS induction in DA neurons and the number of glial fibrilliary acidic protein (GFAP) positive cells in both the SN and the striatum (34). However, further studies ascertained that some of the protective effect was caused by Pioglitazone blocking the action of MAO-b and thus preventing the conversion of MPTP to its metabolite MPP+ (153). Rosiglitazone has been found to have similar neuroprotective properties *in vitro* (83, 84). Concurrent treatment of neuroblastoma cells with Rosiglitazone prevented apoptotic cell death via acetaldehyde, a neurotoxin that also causes oxidative stress by inhibition of mitochondrial function. This protection is apparently mediated via induction of antioxidant enzymes and by differential regulation of *Bax* and *Bcl2* (83). The same group also found that Rosiglitazone protected neuroblastoma cells from MPP+ induced toxicity (84). Both Pioglitazone and Rosiglitazone are generally well tolerated, although recent reports have documented that these drugs may increase the risk of myocardical ischemic events in type II diabetes patients (158). Therefore, systemic administration of these drugs may not be desirable in all cases and targeted expression of *PGC-1α* in the SN through gene therapy or genetically modified stem cells may be a viable alternative that warrants further investigation.

Regulation of PGC-1α Activity By Post-Translational Modifications

Further regulation of PGC-1α activity occurs through a complex system of post-translational modifications, of which phosphorylation of PGC-1α by p38 MAPK is the most characterized. *In vitro* phosphorylation experiments, followed by mass spectrometry, identified the specific amino acid residues of PGC-1α that may be phosphorylated by p38 MAPK (Thr^{262}, Ser^{265}, and Thr^{298}). These are located within the repression domain of PGC-1α. Phosphorylation of PGC-1α by p38 MAPK lifts repression of PGC-1α activity and increases PGC-1α co-activation of target genes. Pulse-chase experiments with radiolabeled [^{35}Ser]-methionine in BOSC cells overexpressing either wild-type PGC-1α, or a mutant form in which the Thr^{262}, Ser^{265}, and Thr^{298} were mutated to alanine (PGC-1 3A) and experiments in BOSC cells treated with a constitutive activator of p38 MAPK (MKK6E), demonstrated that p38 MAPK phosphorylation of PGC-1α stabilizes the protein, and increases half-life of the protein from 2.28 h to 6.27 h. Mutation of the PGC-1α sites normally phosphorylated by p38 MAPK (Thr^{262}, Ser^{265}, and Thr^{298}) abolished this effect (150).

Further analysis of the repression domain of PGC-1α by affinity chromatography using GST-fusions of amino acids 200–400 against nuclear extracts of C2C12 myoblasts, identified an interaction with $p160^{MBP}$ (45), a protein with predominantly repressive effects on transcription (39, 137, 216). $p160^{MBP}$ and its splice variant $p67^{MBP}$ reduced the transcription activation activity of PGC-1α, in an *in vitro* transactivation assay, whereas the activity of a PGC-1α deletion mutant lacking amino acids 170–350 was not affected. The interaction between $p160^{MBP}$ and PGC-1α is specific to the Thr^{262}, Ser^{265}, and Thr^{298} residues, and phosphorylation of these by p38 MAPK disrupts the binding of $p160^{MBP}$ and its splice variant $p67^{MBP}$. Mutation of the Thr^{262}, Ser^{265}, and Thr^{298} phosphorylation sites also reduced PGC-1α co-immunoprecipitation with $p160^{MBP}$ or $p67^{MBP}$ (45). Repression by $p160^{MBP}$ or $p67^{MBP}$ does not require the removal of PGC-1α from chromatin, as PGC-1α was still recruited to the MEF2 site on the β-globin gene, therefore $p160^{MBP}$ and $p67^{MBP}$ must have some intrinsic repression activity. $p160^{MBP}$ represses the transcription activation activity of the redox-sensitive transcription factor NFκB by competing with p300 for the activation domain of NFκB (137). Given the close proximity of the PGC-1α p300 binding site (activation domain) and the p38 MAPK phosphorylation motif that prevents $p160^{MBP}$ repression of PGC-1α activity, it is possible that a similar competitive mechanism may be the cause of $p160^{MBP}$ mediated repression of PGC-1α (Fig. 7).

PGC-1α contains two putative cell division control protein 4 (Cdc4) phosphodegrons spanning the region phosphorylated by p38 MAPK (136). Cdc4 is the F-box component of the Skip/Cullin/F-box (SCF^{Cdc4}) ubiquitin ligase complex (161, 177). An *in vitro* ubiquitination assay in the presence of proteasome inhibitor revealed increased ubiquitination of PGC-1α in the presence of nuclear isoforms of Cdc4. In the

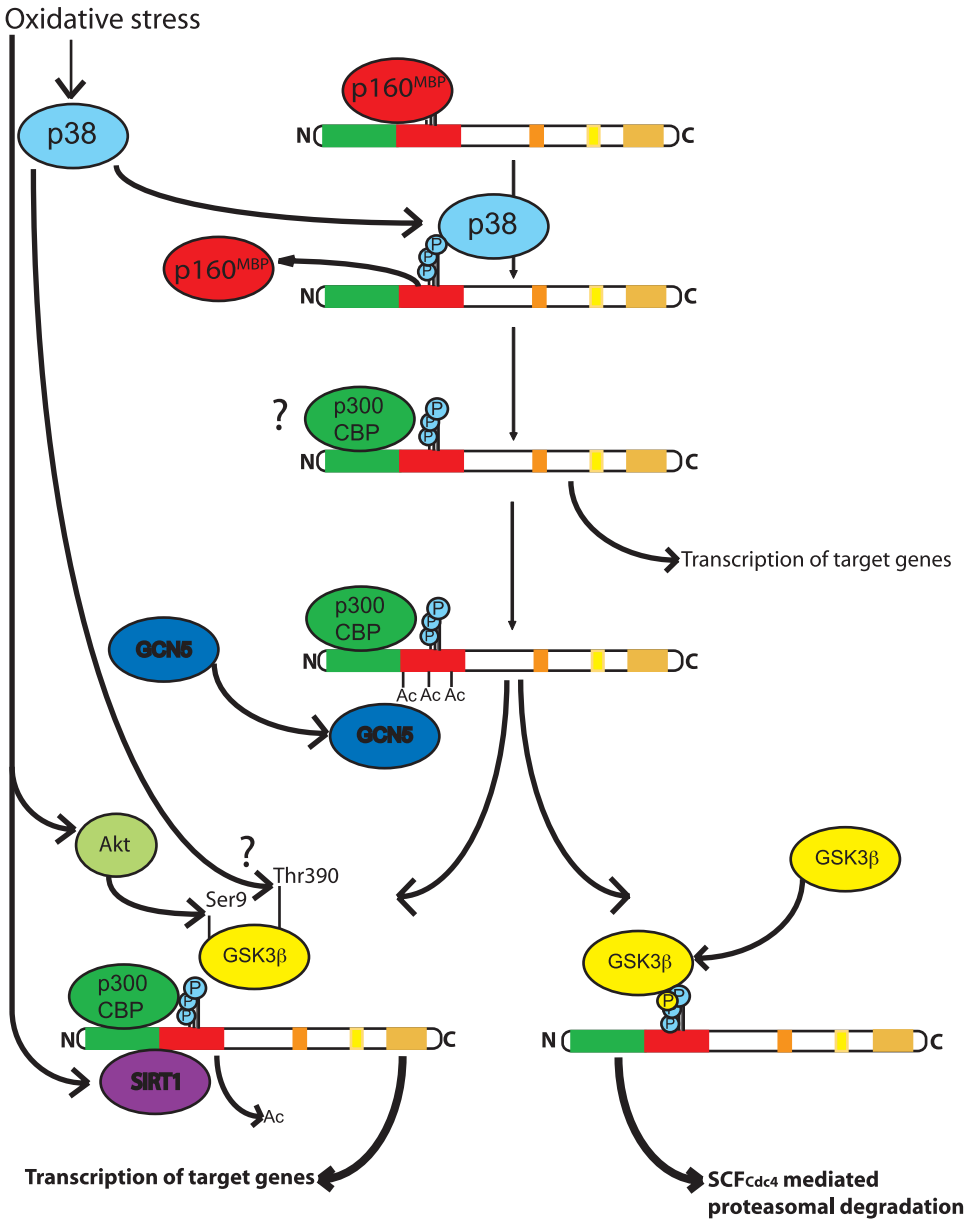

FIG. 7. Potential regulation of PGC-1a stability by p38 MAPK, GSK3b, and SIRT1 in response to oxidative stress. PGC-1α activity is repressed by binding of $p160^{MBP.}$ Phosphorylation of PGC-1a by p38 MAPK releases $p160^{MBP}$ from PGC-1α, facilitating transcriptional coactivation of target genes in response to oxidative stress (potentially via p300/CBP). Following coactivation of target genes, PGC-1α activity may be dialed down via acetylation by GCN5. *Left branch*: prolonged oxidative stress inhibits GSK3β, potentially through phosphorylation by AKT or p38 MAPK, which prevents proteasomal degradation of PGC-1α. SIRT1 is activated and removes GCN5-mediated inhibitory acetylation of PGC-1α. *Right branch*: GSK3β (*yellow*) may phosphorylate PGC1α and facilitate its proteasomal degradation via the SCF^{Cdc4} degradation complex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

absence of proteasome inhibition, this ubiquitination reduced the half-life of PGC-1α, demonstrating that PGC-1α is likely to be a target for SCF^{Cdc4} mediated proteasomal degradation after nuclear localization (136). Consistent with this, in murine embryonic primary neurons under conditions of oxidative stress, siRNA knockdown of Cdc4 increased the levels of PGC-1α protein and the transcription of the target gene TRX2.

GSK3β phosphorylation of the Cdc4 phosphodegron sequence is required for Cdc4 binding to target proteins (54, 185, 208, 209). GSK3β phosphorylates Thr^{295} within the Cdc4 phosphodegron sequence of human PGC-1α (136). In addition, phosphorylation of PGC-1α by p38 MAPK is absolutely required as a 'priming phosphorylation' for GSK3β phosphorylation of Thr^{295} and the degradation of PGC-1α by the SCF^{Cdc4} ubiquitin ligase complex. Therefore, phosphorylation of PGC-1α by GSK3β may represent a mechanism whereby activated PGC-1α protein is degraded via an autoregulatory loop. This may be especially important in light of the finding that the C-terminus of PGC-1α can be polyubiquitinated, and that this may lead to aggregations of PGC-1α that are not degraded by the proteasome (164). Interestingly, the activity of GSK3β in mouse embryonic primary neurons is reduced under conditions of oxidative stress via phosphorylation on Ser^{9} (136), which may be mediated by AKT, as previously discussed. Decreased GSK3β activity in response to oxidative stress increased PGC-1α protein levels and transcription of target genes. In addition, p38 MAPK is activated under conditions of oxidative stress (121) and recent research indicates that p38 MAPK can directly phosphorylate GSK3β on Thr^{390} which may cause an inhibition of GSK3β comparable to the inhibitory Ser^{9} phosphorylation by AKT (191). Therefore, the balance between GSK3β activity and p38 MAPK activity may be important in regulating

the activity of PGC-1α in response to oxidative stress (Fig. 7). Interestingly, a comparable mechanism has been elucidated for the regulation of skn-1, a *Caenorhabditis elegans* protein with similarity to NRF1 and NRF2 (3), raising the possibility that NRF2 may also be regulated by the coordinate action of p38 MAPK and GSK3β in under certain conditions.

Further work has implicated SIRT1 alongside GSK3β in the regulation of PGC-1α in response to oxidative stress (4). Treatment of PGC-1α overexpressing NIH3T3 cells with 350 μM H_2O_2 induced the co-localization of PGC-1α and SIRT1 in the nucleus, and increased cell survival after H_2O_2 in a PGC-1α dose-dependent manner. The inclusion of nicotinamide (a sirtuin inhibitor) decreased the resistance of wild-type cells to oxidative stress, a negative effect that was attenuated in cells overexpressing PGC-1α, which indicated that PGC-1α is downstream of SIRT1 in this system. The presence of nicotinamide prevented the accumulation of PGC-1α in the nucleus and also prevented a protective increase in mitochondrial membrane potential in response to oxidative stress. In accordance with the previously described work by Olson and colleagues (136), the nuclear degradation of PGC-1α was confirmed to be dependent on phosphorylation by GSK3β. However, Anderson and colleagues (4) noted that the activity of GSK3β was increased under conditions of oxidative stress, an apparent paradox that may be due to the different time-points investigated by both groups. GCN5 is currently the only acetyltransferase that has been found to acetylate PGC-1α and inhibit its activity (112). Anderson and colleagues demonstrated that deacetylation of PGC-1α by SIRT1 prevented proteasomal degradation of GSK3β phosphorylated PGC-1α, producing a sustained activation of target genes (4). Therefore, it is possible to hypothesize a regulatory system that includes the coordination of p38 MAPK, GSK3β, and SIRT1 functions to modulate the initial and sustained response of PGC-1α under conditions of oxidative stress (Fig. 7).

GSK3β in Parkinson's Disease

GSK3β is functionally implicated in the cytotoxicity observed in many common PD models. *In vitro*, GSK3β activity may facilitate 6-OHDA-mediated cell death and provoke ER stress in SH-SY5Y cells and cerebellar granule neurons (25). The addition of 6-OHDA was found to reduce the proportion of the Ser^9 phosphorylated GSK3β (inactivated) and increase the Tyr^{216} phosphorylated (active) form. Other work using novel GSK3β inhibitors illustrated that inhibition of GSK3β activity reduced the expression of endogenous *SNCA* resulting from MPP+ treatment in mesencephalic cells and in SH-SY5Y cells stably transfected to overexpress *SNCA* and the DA transporter (DAT). This reduction in the overall levels of α-synuclein correlated with an increase in the Ser^9 phosphorylated form of GSK3β (101). *In vivo* MPTP treatment (30 mg/kg) of wild-type C57BL/6 mice results in a dramatic loss of the inactive Ser^9 phosphorylated form of GSK3β that accompanies death of TH immunoreactive cells in the SN and the loss of striatal DA (206). Treatment of the animals with GSK3β inhibitors prior to MPTP administration prevented MPTP-induced loss of SN TH immunopositive neurons and striatal DA (206). Although Wang and colleagues (206) did not present data pertaining to total GSK3β or GSK3β Tyr^{216} levels in response to MPTP treatment, these data, alongside the previous work, suggest that GSK3β may play a role in DA cell death in PD and that this may be related, in part, to an apoptotic response to oxidative stress (165). Inhibition of GSK3β therefore represents a potential neuroprotective strategy in PD that requires further investigation.

Modulation of NRF2 and PGC-1α Activity as a Potential PD Therapeutic Strategy

In addition to regulating PGC-1α protein stability, GSK3β also seems to negatively regulate NRF2 in response to oxidative stress, although the precise mechanisms for each are still being characterized. Together, PGC-1α and NRF2 coordinate a large part of the enzymatic antioxidant defense system (Fig. 3). Therefore, GSK3β may be an important target for strategies aimed at the modulation of antioxidant defense programs. *GSK3β* has restricted tissue expression with relatively high expression in brain (107), which makes it a particularly attractive target for neurodegenerative disease therapy. Downregulation of *GSK3β* through the expression of dominant–negative forms or via small molecule inhibitors may have the potential to release inhibition of NRF2 and PGC1α and promote the transcription of antioxidant target genes that could potentially protect in areas of high oxidative load such as the SN in PD.

PGC-1α and NRF2 are complementary and overlapping regulators of the antioxidant defense system. Thus far, the majority of antioxidant enzymes found to be regulated by PGC-1α function within the mitochondria. A decrease in mitochondrial ROS may break the 'vicious cycle' of CI deficit and ROS generation in PD (Fig. 8), thus reducing the impact of mitochondrially generated ROS throughout the cell and potentially maintaining the integrity of CI function in newly synthesized mitochondria. NRF2 is a regulator of both cytosolic and mitochondrial antioxidant programs, including the antioxidant enzyme NQO1 which may be important in detoxifying potentially electrophilic DA quinones. Therefore, in DA cells NRF2 function may be invaluable for the clearing of ROS produced through the auto-oxidation of DA and also for the detoxification of quinone end-products (80) to maintain the redox balance of this vulnerable cell type.

A molecular interaction between PGC-1α and NRF2 has not yet been characterized. As discussed previously, PGC-1α is often regulated by its target transcription factors; the PGC-1α promoter contains an ARE (181), although it is not known whether this is functional. Even without a direct interaction between NRF2 and PGC-1α (whereby PGC-1α forms part of a coactivator complex to upregulate *NRF2* transcription, or NRF2 binds to the *PGC-1α* promoter), it seems plausible that the expression of one gene may regulate the expression of the other via redox signaling. Both genes are induced by oxidative stress and upregulate downstream antioxidant enzymes, which in turn reduce levels of free radicals and ROS. So, although PGC-1α and NRF2 have overlapping functions as regulators of antioxidant defense systems, it is possible that the activity of one protein may decrease the expression of the other and affect the regulation of other proteins in the cell. Therefore, any PD therapeutic strategy that utilizes NRF2 or PGC-1α must be able to reduce oxidative stress produced by free radicals and ROS, but only to levels that restore redox homeostasis to the cell.

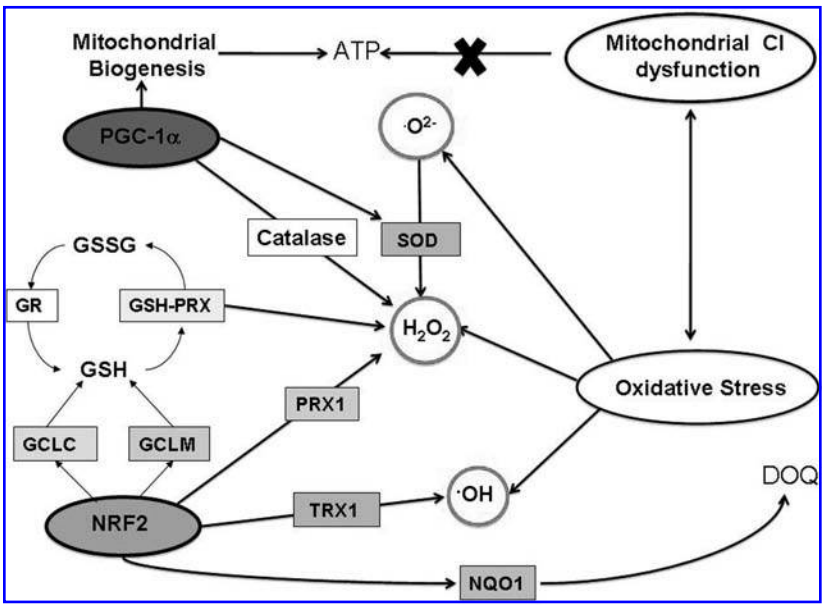

FIG. 8. Potentially decreased oxidative stress within dopaminergic cells as a result of PGC-1α and NRF2 upregulation. ATP, adenosine triphosphate; DOQ, dopamine ortho-quinone; GCLC, glutamyl-cysteine ligase catalytic subunit; GCLM, glutamyl-cysteine ligase regulatory subunit; GSH-Px, glutathione peroxidase; GSS, glutathione synthetase; HO-1, heme oxygenase-1; NQO1, NAD(P)H dehydrogenase quinone-1; PRX, peroxiredoxin; SOD, superoxide dismutase; Trx, thioredoxin.

Gene therapy using viral vectors is an emerging treatment for PD. As previously discussed, there may be an increased risk of cancer associated with the constitutive systemic overexpression of antioxidant defense master regulators such as *NRF2* (138, 175, 207). However, *Nrf2*$^{-/-}$ mice are more susceptible to carcinogens than their wild-type counterparts (154), illustrating that low NRF2 levels may also promote tumor formation. Apparently, striking the correct balance of NRF2 activity is crucial. One way to circumvent this issue may be to use a cell-type specific promoter, for instance, expression of *NRF2* or *PGC-1α* from a tyrosine hydroxylase promoter should ensure that the gene is expressed in DA neurons. An additional strategy is to use an inducible promoter that is 'switched on' under conditions of oxidative stress. This has been accomplished using a double ARE to overexpress HO-1 in cells positively transduced by a lentiviral vector (69). Applying a similar strategy to *NRF2* or *PGC-1α* expression provides a strategy to upregulate multiple antioxidant activities specifically where they are most needed in tissues or cells that are subjected to oxidative stress.

Many of the direct and higher-order regulatory mechanisms of antioxidant defense have been discussed in this review. Although some of these mechanisms have been linked to neurodegeneration in models of PD, many have not yet been directly linked to PD pathogenesis. However, all of the NRF2 or PGC-1α regulatory mechanisms outlined here relate to oxidative stress, which appears to play a major role in PD. Thus, further studies of the mechanisms of transcriptional control of antioxidant defenses may yield clues to the pathogenesis of PD and other neurodegenerative diseases, and the ability to manipulate the activity of transcriptional regulators of the antioxidant defense system, such as NRF2 and PGC-1α, may provide promising strategies for neuroprotective therapies.

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Abbreviations

AKT, V-akt murine thymoma viral oncogene homolog 1; ATF, activating transcription factor; ATP, adenosine triphosphate; Bach, BTB and CNC homology; Bax, BCL2-associated X protein; Bcl2, B cell lymphoma 2; BDNF, brain-derived neurotrophic factor; BTB, broad-tramtrack-bric-a-brac; bZIP, basic leucine zipper; cAMP, cyclic adenosine monophosphate; CBP, cyclic AMP-responsive element binding protein-binding protein; Cdc4, cell division control protein 4; CI, mitochondrial complex 1; CII, mitochondrial complex 2; CK2, casein kinase 2; CNC, cap'n'collar; COX, cytochrome c oxidase; CRE, cyclic AMP-responsive element; CREB, cyclic AMP-responsive element binding protein; CTR, C-terminus region; Cul3, Cullin 3, D_1, dopamine receptor 1 (excitatory); D_2, dopamine receptor 2 (inhibitory), DA, dopamine; DAT, dopamine transporter; DGR, double glycine repeat; DNA, deoxyribonucleic acid; DRIP, vitamin D receptor-interacting protein; EGFP, enhanced green fluorescent protein; ERK, extracellular signal-regulated kinases; ERRα, estrogen related receptor alpha; FOXO, forkhead transcription factor O; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase mechanistic subunit; GCN5, general control of amino acid synthesis 5; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione (reduced form); GSK3β, glycogen kinase 3 beta; GSSG, glutathione disulfide (oxidized form); GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide; HAT, histone acetyl transferase; HO-1, heme oxygenase 1, IL-1, interleukin 1; iNOS, nitric oxide synthase (inducible), IVR, intervening region; JNK, Hun N-terminal kinase; Keap1, Kelch-like erythroid cell-derived protein with CNC homol-

ogy (ECH)-associated protein 1; L-DOPA, 3,4-dihydroxy-L-phenylalanine; MAF, musculoaponeurotic fibrosarcoma oncogenic protein; MAO-B, monoamine oxygenase-beta; MAPK, mitogen-associated protein kinase; MAPT, microtubule-associated protein tau; MARE, musculoaponeurotic fibrosarcoma oncogenic protein recognition element; MEF2, myocyte-enhancer factor 2; MPP+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4,1,2,3,6-tetrahydropyridine; mtDNA, mitochondrial DNA; NAC, N-acetyl cysteine; Neh, Nef2 ECH homology; NF-E2, nuclear factor-erythroid 2; NF-κB, nuclear factor kappa beta; NQO1, NAD(P)H quinone oxidoreductase 1; NRF1, nuclear factor erythroid 2-related factor 1; NRF2, nuclear factor erythroid 2-related factor 2; NRF3, nuclear factor erythroid 2-related factor 3; 6-OHDA, 6-hydroxydopamine; 8-OHdG, 8-hydroxy-deoxyguanosine; ONOO, peroxynitrite; PD, Parkinson's disease; PERK, PKR-like endoplasmic reticulum kinase; PGC-1α, peroxisome proliferator-activated receptor gamma-coactivator 1-alpha; PI3K, phosphatidyl-inositol-3-kinase; PRC, protein kinase C; PKR, protein kinase R; PPARα, peroxisome proliferator-activated receptor alpha; PPARγ, peroxisome proliferator-activated receptor gamma; PRX, peroxiredoxin; RNA, ribonucleic acid; RNA pol II, RNA polymerase II; RNAi, RNA-interference; ROS, reactive oxygen species; RRM, RNA recognition motif; RS, arginine/serine-rich protein; SCF, Skip/Cullin/F-box; Ser, serine; siRNA, small interfering RNA; SIRT1, silent mating type information regulation 2 homolog; SN, substantia nigra; SNF2, sucrose nonfermenting 2; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; tBHQ, tert-butyl hydoroquinone; TFH, basal transcription factors; TH, tyrosine hydroxylase; Thr, threonine; Tnfα, tumor necrosis factor alpha; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRAP, thyroid receptor-associated protein; TRE, 12-O-tetradecanoylphorbol-13-acetate response element; TrkB, tyrosine receptor kinase beta; TRX, thioredoxin; Tyr, tyrosine.

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