α-Synuclein Protects against Oxidative Stress via Inactivation of the c-Jun N-terminal Kinase Stress-signaling Pathway in Neuronal Cells*

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The expression of α-synuclein, a synaptic molecule implicated in the pathogenesis of neurodegenerative disorders such as Parkinson’s disease and Lewy body disease is increased upon injury to the nervous system, indicating that it might play a role in regeneration and plasticity; however, the mechanisms are unclear. Because c-Jun N-terminal kinase (JNK), a member of the mitogen-activated protein kinase family, plays an important role in stress response, the main objective of the present study was to better understand the involvement of this pathway in the signaling responses associated with resistance to injury in cells expressing α-synuclein. For this purpose, the JNK-signaling pathway was investigated in α-synuclein-transfected neuronal cell line glucose transporter (GT) 1–7 under oxidative stress conditions. Although hydrogen peroxide challenge resulted in JNK activation and cell death in cells transfected with vector control or β-synuclein, α-synuclein-transfected cells were resistant to hydrogen peroxide, and JNK was not activated. The inactivation of JNK in the α-synuclein-transfected cells was associated with increased expression and activity of JNK-interacting protein (JIP)-1b/islet-brain (IB)1, the scaffold protein for the JNK pathway. Similarly, cells transfected with JIP-1b/IB1 were resistant to hydrogen peroxide associated with inactivation of the JNK pathway. In these cells, expression of endogenous α-synuclein was significantly increased at the protein level. Furthermore, α-synuclein was co-localized with JIP-1b/IB1 in the growth cones. Taken together, these results suggest that increased α-synuclein expression might protect cells from oxidative stress by inactivation of JNK via increased expression of JIP-1b/IB1. Furthermore, interactions between α-synuclein and JIP-1b/IB1 may play a mutual role in the neuronal response to injury and neurodegeneration.

Neurodegenerative disorders such as Alzheimer’s disease and Parkinson’s disease (PD)† are characterized by selective neuronal and synaptic damage in cortical and subcortical regions resulting in cognitive and motor impairment (1). The mechanisms leading to neurodegeneration in these disorders are unclear; however, recent studies suggest that abnormal aggregation of misfolded neuronal proteins might play a central role (2, 3). Although in Alzheimer’s disease intracellular (4, 5) and extracellular aggregation of amyloid β-protein (5) has been implicated in PD, intraneuronal aggregation of misfolded α-synuclein (6–8) has been suggested to be involved in the pathogenesis of the neurodegenerative process. In supporting a role of misfolded α-synuclein in the pathogenesis of PD, previous studies show that missense mutations that accelerates α-synuclein aggregation (9) are associated with familial PD (10, 11). Furthermore, Lewy bodies that are intraneuronal inclusions found in patients with parkinsonism are primarily composed of misfolded α-synuclein (6–8), and overexpression of α-synuclein in transgenic mice (12) and Drosophila (13) resulted in Lewy body-like formation associated with degeneration of dopaminergic neurons.

α-Synuclein is an abundant synaptic protein that is a member of the synuclein family of peptides that includes α-, β-, and γ-synuclein as well as synoretin (14, 15). The physiological roles of these proteins are unclear, although they might play an important role in neuroplasticity and in response to neuronal cell damage (14, 16). In support of this possibility, recent studies show that α-synuclein expression is increased in models of developmental-targeted injury (17, 18). In this model, up-regulation of α-synuclein at the mRNA level is associated with an increase number of neurons expressing α-synuclein, and at the cellular level, α-synuclein is almost exclusively expressed in normal neurons rather than in apoptotic cells (18). Moreover, during development, α-synuclein expression is up-regulated (19), and the expression of this synaptic molecule is not further up-regulated during the period of natural cell death (17, 18). In neuronal cell lines, overexpression of wild type α-synuclein protects against oxidative stress (20). In contrast, neuronal cell lines expressing α-synuclein that contains mutations (A30P and A53T) associated with familial Parkinsonism and increased misfolding of α-synuclein (9) result in increased susceptibility to oxidative stress damage (20).

Oxidative stress has been suggested to play a central role in the pathogenesis of PD (21–23), and chronic intracellular accumulation of misfolded α-synuclein promotes oxidative stress and mitochondrial alterations that could lead to dopaminergic neuronal death (24). Furthermore, oxidative stress promotes α-synuclein misfolding (25, 26). Thus, under some circumstances, increased expression of α-synuclein might protect against oxidative stress and promote regeneration, whereas under pathological conditions, misfolding of α-synuclein might lead to cell death. In this context the main objective of this study was to determine the signaling events involved in pro-
testing cells expressing wild type α-synuclein against oxidative stress. Neuronal response to oxidative stress involves regulation of multiple kinases including several members of the mitogen-activated protein kinase family (27). The mitogen-activated protein kinase family includes extracellular signal-regulated kinase, p38, and ε-Jun N-terminal kinase (JNK) (28). The extracellular signal-regulated kinases are activated by receptor tyrosine kinases and relay proliferation and differentiation signals, whereas p38 and JNK are activated predominantly by stress signaling and are involved in cell survival and cell death (28, 29). Finally, although activation of JNK stress-signaling pathways has been shown to play a role in cell death in PD (30), inactivation of JNK protects against cell death (31). For the present study we found that α-synuclein might protect neuronal cells against oxidative stress damage by down-regulating the JNK pathway.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—As previously described (24), experiments were performed with the glucose transporter (GT) 1–7 murine hypothalamic tumor cell line (kindly provided by Dr. Pam Mellon, University of California San Diego, Department of Neurosciences) maintained at 37 °C, 5% CO₂ in Dulbecco’s modified Eagle’s medium (high glucose) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and 1% v/v penicillin/antibody. In all experiments were viable. Dneurosecretory properties (32). Furthermore, express many neuronal markers and form synapses and because it has 10% fetal bovine serum (Hyclone, Logan, UT) and 1% v/v penicillin/antibody. In this cell line resulted in oxidative stress, mitochondrial alterations, and formation of intraneuronal inclusions (24).

**Expression Vectors and Stable Transfection of GT1–7 Cells—a-Synuclein overexpressing and vector-transfected GT1–7 cells were previously described (24). In addition, experiments were also performed with stable transfected GT1–7 cells overexpressing β-synuclein or JIP-1b/IB1. For this purpose, cDNA templates for murine α-synuclein, β-synuclein, and α-actin (mouse monoclonal, Chemical, expressed from total RNA extracted from the mouse brain by reverse transcriptase-PCR and inserted into pCR3.1 expression vector (Invitrogen). The murine β-synuclein sequence differs from the human sequence by 3 amino acid substitutions and one deletion (from human to mouse: R45S,E46S and a deletion, A66K, and P122S) (33). To create a stable cell line expressing β-synuclein, GT1–7 cells were transfected with LipofectAMINE (Invitrogen). For stable cell clones, selection was carried out for several weeks in growth medium containing 200 μg/ml G418 (Calbiochem) until ~20 resistant colonies were obtained. Clones were screened by immunoblotting and ribonuclease protection assay (RPA) to determine β-synuclein expression (34, 35). For JNK-interacting protein (JIP-1b)-islet-brain (IB)1 (JIP-1b/IB1), GT1–7 cells were transfected with pKB expression vector (CLONTECH, Palo Alto, CA) with or without the insertion of rat IB-1 (homologue to human JIP-1b) cDNA (kindly provided by Dr. Christophe Bonny, CHUV University Hospital, Lausanne, Switzerland) (36). Transfection and selection were performed as described above, and JIP-1b/IB1 expression was screened by RPA and immunoblot analysis.

**Protein Preparation and Immunoblot Analysis**—Total cell homogenates or cells homogenates fractionated into cytosolic and particulate fractions were prepared as previously described (34) and used for immunoblot analysis. For the JNK immunoblot analysis, cells were dissolved in extraction buffer (described in the immunocomplex kinase assay), and the Triton-soluble fractions were used. Protein concentrations were determined using BCA protein assay reagents (Pierce). Immunoblot analysis was performed essentially as previously described (34). Briefly, samples were separated on SDS-PAGE gels and transferred to nitrocellulose membranes (Schleicher & Schuell). Transfers were blocked with 3% bovine serum albumin in Tris-buffered saline (TBS: 20 mM Tris-HCl, pH 7.5, 150 mM NaCl) followed by incubation in primary antibodies diluted in 1% bovine serum albumin in TBS. Membranes were then incubated with either 125I-proteinA (ICN, Costa Mesa, CA) and analyzed with the PhosphorImager (Molecular Dynamics, Sunnyvale, CA) or subjected to detection using the enhanced chemiluminescence (ECL) system (Amersham Biosciences, Inc.). The primary antibodies used were as follows: JNK (C-17; rabbit polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA), JIP-1b/IB1 (E-19; goat polyclonal, Santa Cruz Biotechnology), C-terminal α-synuclein (amino acids 101–124; rabbit polyclonal) (16), phospho-JNK (pJNK) (G-7; mouse monoclonal, Santa Cruz), and α-actin (mouse monoclonal, Chemicon,
The kinase complexes were incubated for 20 min at 30°C in JNK assay buffer (20 mM HEPES, pH 7.6, 20 mM MgCl₂, 20 mM glycerophosphate, 20 mM p-nitrophenyl phosphate, 0.1 mM sodium orthovanadate, 0.1 mM phenylmethylsulfonyl fluoride, 10 mM sodium pyrophosphate, 0.1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 0.5 mM sodium orthovanadate, 0.1 mM phenylmethylsulfonyl fluoride, and 32P-oligonucleotides (30,000 cpm/sample) were end-labeled with [32P]-ATP. Samples were resolved on SDS-PAGE gels and exposed to film or phosphorimager (Molecular Dynamics) using the ImageQuant software and expressed as integrated pixel intensities over defined volumes. Final values were expressed as ratios of (specific signal background/actin signal background) to correct for differences in RNA content/loading across samples. Actin signals were used to correct for variations in mRNA content and loading.

To analyze the effects of a-synuclein on JIP-1b/IB1 DNA binding activity, nuclear extracts for EMSA were prepared by a mini-extraction protocol (37). The oligonucleotides were end-labeled with [γ-32P]dATP and T4 polynucleotide kinase (New England Biolabs, Beverly, MA). An assay was performed by a previously described method (38) with the some modifications. Briefly, nuclear extracts (5 µg) were incubated for 20 min at room temperature with 20 µl of poly(dI-dC) (Amersham Biosciences, Inc.), 10% glycerol, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 32P-oligonucleotides (30,000 counts/min). For competition studies, before the addition of the labeled probe, nuclear extracts were preincubated for 10 min at room temperature with a 100-fold excess of unlabeled (cold) oligonucleotides. DNA-protein complexes were resolved on a 6% non-denaturing polyacrylamide gel in 1× TGE (45 mM Tris borate, 1 mM EDTA) followed by vacuum-drying and autoradiography. The sequences of the sense strands of the oligonucleotides used in the EMSAs were as follows: GTT1, 5'-GTAAAGGGTTATGTGGAGTCCATCATGACACCT- TCT-3' mutant GTT1, 5'-GTAAAGGGTTACCAATTCGACAGCT- TGGCACCTACGTTCT-3'.

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buffer (5 mM Tris-HCl, pH 8.0, 20 mM EDTA) containing 0.5% v/v Triton X-100 for 20 min at 4 °C, and the high molecular weight DNA was removed by centrifugation at 14,000 rpm for 30 min in the presence of 0.1% SDS. The supernatants were then extracted with a mixture of phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform. After precipitation with ethanol, samples were resuspended in TE buffer containing 0.1 mg/ml DNase-free RNase A for 3 h at 30 °C and analyzed on 1.8% agarose gels.

Immunocytochemical Analysis and Confocal Microscopy—Cells were seeded onto poly-L-lysine-coated glass coverslips, grown to 60% confluency, and fixed for 30 min in 4% paraformaldehyde. Fixed cells were first incubated overnight at 4 °C with the antibody against murine α-synuclein (rabbit polyclonal, 1:10,000) or the goat polyclonal anti-JIP-1b/IB1 (E-19, Santa Cruz) and detected with the Tyramide Signal Amplification™ Direct (Red) system (PerkinElmer Life Sciences) (19). Then, cells were incubated with the mouse monoclonal anti-microtubule-associated protein 2 (MAP2, dendritic and neuronal marker, 1:50) (Chemicon) or growth-associated protein 43 (growth cone marker, 1:1000) (Sigma) followed by detection with the fluorescein isothiocyanate-conjugated anti-mouse secondary antibody (Vector Laboratories, Burlingame, CA). Coverslips were air-dried overnight, mounted on slides with anti-fading media (Vector), and imaged with the laser-scanning confocal microscope (LSCM) (Bio-Rad, MRC1024) (19).

RESULTS

JNK Activity Is Decreased in Peroxide-challenged α-Synuclein-overexpressing GT1-7 Cells—To determine whether stress associated with α-synuclein accumulation activates the JNK pathway, levels of pJNK immunoreactivity by immunoblot (Fig. 1A) and immunocomplex kinase assay (Fig. 1B) was determined in α- or β-synuclein-overexpressing and vector-transfected cells (Fig. 2A). These cell lines have been previously characterized (19), and levels of synuclein expression were confirmed by RPA (Fig. 2, A and B). Under basal conditions no differences in pJNK immunoreactivity (Fig. 1A) or activity (Fig. 1B) were observed among α-synuclein, β-synuclein, or vector-alone-transfected cells. However, after hydrogen peroxide (~200 μM) treatment for 30 min, β-synuclein-overexpressing and vector-transfected cells showed a significant increase in pJNK immunoreactivity (Fig. 1A) and JNK activity (Fig. 1B), whereas in the α-synuclein-transfected cells peroxide challenge did not activate the JNK pathway. Because peroxide treatment activated the JNK pathway in the β-synuclein-overexpressing and vector-transfected cells but not in the α-synuclein-transfected cells and because JNK activation has been generally associated with cell death, both trypan blue exclusion and DNA fragmentation assays were performed to assess cell viability (Fig. 1, C and D). These studies showed that although α-synuclein-overexpressing cells were resistant to hydrogen peroxide treatment, both β-synuclein-overexpressing and vector-transfected cells were vulnerable to hydrogen peroxide treatment (Fig. 1, C and D). Taken together, these results indicate that JNK inactivation might confer resistance to peroxide and cell death in α-synuclein-overexpressing cells.

Up-regulation of JIP-1b/IB-1 Expression in α-Synuclein-overexpressing GT1-7 Cells Inactivates JNK—Decreased JNK activity in hydrogen peroxide-challenged α-synuclein-overexpressing cells could be attributed to alterations of various regulators of the stress response pathway (27, 28). Among them, the present study investigated the possibility that altered expression of JIP-1b/IB1 (scaffold proteins of JNK pathway) might play a role. The JIP-1b/IB1 is a 120-kDa molecule that has been shown to interact with JNK, and overexpression of JIP-1b/IB1 suppress JNK-induced apoptosis (39–41). In the α-synuclein-overexpressing cells, both RPA (Fig. 2, C and D) and immunoblot analysis (Fig. 3A) showed that JIP-1b/IB1 levels were significantly increased compared with both β-synuclein-overexpressing and vector-transfected cells. In the β-synuclein-overexpressing cells, the JIP-1b/IB1 mRNA expression levels were lower compared with vector controls (Fig. 2, C and D). Reverse transcriptase-PCR analysis confirmed that JIP-1b/IB1 was the only mRNA splicing isoform present in the synuclein-transfected cells (Fig. 2E). Treatment with hydrogen peroxide did not significantly alter further the levels of
JIP-1b/IB1 expression in the α-synuclein, β-synuclein, and vector-transfected cells (Fig. 3B). The JIP-1b/IB1 immunoreactive bands were confirmed in both mouse brain homogenates as well as cell extracts of PC12 cells as positive controls (41, 42) (Fig. 3C).

Up-regulation of JIP1-b/IB-1 DNA Binding Activity in α-Synuclein-overexpressing GT1-7 Cells—JIP-1b was independently isolated as IB1, a putative transcription factor for the glucose transporter (GT) II gene, in rat pancreatic β-insulin-secreting cells (36). JIP-1b/IB1 has a helix loop helix DNA binding domain that is spliced out in JIP-1a (39). Because JIP-1b/IB1 was elevated in α-synuclein-overexpressing cells and because this scaffold protein has been shown to contain a putative DNA binding sequence, then further confirmation of JIP-1b/IB1 activity was performed by EMSA using a sequence of promoters located in the glucose transporter II gene, GTII (36). This study showed that nuclear extracts from α-synuclein-overexpressing cellsdisplayed increased DNA binding activity when compared with both β-synuclein-overexpressing and vector-transfected cells (Fig. 4A). The DNA-protein complex was specifically inhibited by an excess amount of unlabeled wild type GTII but not by that of mutated GTII, indicating the binding is specific to GTII sequence (Fig. 4B).

JIP-1/IB1 Overexpression Results in Decreased JNK Activity in GT1-7 Cells—Because overexpression of α-synuclein in GT1-7 cells resulted in increased JIP-1b/IB1 expression, it is possible that these two molecules might interact or converge in stress response pathways that determine cell fate. To investigate this possibility, wild type GT1-7 cells were transfected with JIP-1b/IB1 cDNA (Fig. 2, C and D). Three cell lines were employed for these experiments, a low JIP-1b/IB1 expresser (JIB-1), a high expresser (JIB-4), and a vector control. Levels of expression in these cell lines was confirmed both by RPA (Fig. 2, C and D) and immunoblot analysis (Fig. 5A). Similarly to the results in the α-synuclein-overexpressed cells (Fig. 1), overexpression of JIP-1b/IB1 in GT1-7 cells resulted in decreased JNK activity in response to challenge with hydrogen peroxide (Fig. 5B) and increased DNA binding activity (Fig. 5C). Consistent with these results, overexpression of JIP-1b/IB1 in GT1-7 cells increased resistance to challenge with hydrogen peroxide (Fig. 5D). These effects were dose-dependent in that the cells expressing higher levels of JIP-1b/IB1 were more resistant than those expressing lower levels of JIP-1b/IB1 (Fig. 5, A–D).

JIP-1-transfected GT1-7 Cells Display Increased Accumulation and Redistribution of α-Synuclein to the Growth Cones—To determine whether increased expression of JIP-1b/IB1 affects endogenous α-synuclein expression in GT1-7 cells, RPA, immunoblot, and immunocytochemical analysis was performed. Although the RPA did not show significant differences in the levels of α-synuclein expression among the JIP-1b/IB1- and vector-transfected cell lines (Fig. 2, A and B) but the immunoblot showed that in JIP-1b/IB1-overexpressing cells there was increased α-synuclein immunoreactivity (Fig. 6A), then increased α-synuclein immunoreactivity could be attributed to post-transcriptional modifications or redistribution of α-synuclein in the subcellular compartment. To further investigate this possibility, homogenates were divided into cytosolic and particulate fractions. In the vector-transfected cells, most of the α-synuclein immunoreactivity was observed in the cytosolic compartment, and a small proportion was observed in the particulates (Fig. 6B). In contrast, in the JIP-1b/IB1-transfected cells α-synuclein immunoreactivity was observed only in the cytosolic fractions but not in the particulates (Fig. 6B), indicating that α-synuclein might be redistributed in the subcellular compartments.

Consistent with the immunoblot findings, immunocytochemical and confocal analysis showed that, compared with the vector-transfected cells (Fig. 7A), in JIP-1b/IB1-transfected cells α-synuclein immunoreactivity was more prominent in the cytoplasm (Fig. 7B), with considerable redistribution to the neuritic processes (Fig. 7B), where both α-synuclein (Fig. 7C) and JIP-1b/IB1 (Fig. 7D) were co-localized in the growth-cones (Fig. 7E). In addition, JIP-1b/IB1-transfected GT1-7 cells had a tendency to cluster (Fig. 7F) and displayed increased formation of growth cones and cellular process (Fig. 7, G–I). Moreover, and consistent with the EMMA results (Fig. 5C), JIP-1b/IB1-transfected GT1-7 cells showed an increased JIP-1b/IB1 immunoreactivity in the nucleus (Fig. 7J), suggesting that this molecule not only concentrates in the growth cones but it is also translocated to the nucleus.
FIG. 5. Overexpression of JIP-1b/IB1 results in decreased JNK activity in GT1-7 cells. A, immunoblot analysis of JIP-1b/IB1 expression. The cytoplasmic fraction (100 μg) of cell homogenates derived from each cell line (JIB-1 (low expresser), JIB-4 (high expresser), and mock-transfected GT1-7 cells) were analyzed. B, immunocomplex kinase assay of JNK. Each cell line was treated with hydrogen peroxide (0, 100, 200 μM) for 30 min, and the 1% Triton X-100 soluble fractions (200 μg) were analyzed. A representative figure of three experiments is shown. In A and B, the positions of molecular mass markers are indicated on the left side (kDa). C, EMSA was performed with GTII-specific primers using nuclear extracts derived from each cell line. The arrow denotes specific (spe.) binding complex. D, cell viability estimated by the trypan blue exclusion assay (n = 4). Each cell line was treated with hydrogen peroxide for 24 h.

FIG. 6. α-Synuclein expression in the JIP-1b/IB1-transfected GT1-7 cells. A, immunoblot analysis for α-synuclein immunoreactivity in total cell homogenates (100 μg) from low expresser (JIB-1) and high expresser (JIB-4) JIP-1b/IB1 and vector-transfected GT1-7 cells analyzed using an antibody against anti-C-terminal α-synuclein. B, immunoblot analysis was performed with cells that were fractioned into cytosolic and particulate components (100 μg per lane). In A, the first lane to the left is recombinant α-synuclein, used as a control.

DISCUSSION

The present study showed that overexpression of α-synuclein conferred resistance to peroxide challenge via inactivation of JNK. Neither JNK phosphorylation nor JNK-mediated cJUN phosphorylation was stimulated by hydrogen peroxide in the α-synuclein-overexpressing cells, whereas both were significantly up-regulated in the β-synuclein-overexpressing as well as in the vector-transfected cells. JNK activation in the β-synuclein-overexpressing and vector-transfected cells was associated with cell death; in contrast, α-synuclein-overexpressing cells were resistant to hydrogen peroxide. This is consistent with recent studies that have shown that increased expression of α-synuclein might be protective in models of neuronal injury (17, 18). Furthermore, previous studies show that neuronal cell lines transfected with wild type α-synuclein were resistant to hydrogen peroxide treatment (20, 43). Moreover, although inactivation of the JNK pathway is associated with resistance to peroxide, activation of this signaling pathway by oxidative stress promotes cell death (44). Taken together, these studies suggest that by inactivating JNK, increased α-synuclein expression under stress injury might promote regeneration or might be a compensatory response to cell damage.

These results might seem paradoxical considering that previous studies show that α-synuclein-overexpressing GT1-7 cells display decreased mitochondrial function and neurotrans-
mitter release and evidence of oxidative stress (45). However, one possible interpretation is that clonal cell lines might become resistant against chronic oxidative effects caused by α-synuclein. Supporting this possibility, previous studies show that overexpression of α-synuclein results in oxidative stress accompanied by up-regulation of cellular glutathione, the primary cellular anti-oxidant response to free radical production (45). Then, although some reports have indicated that overexpression of α-synuclein resulted in enhanced vulnerability and cell death (46–50), others have shown that α-synuclein-transfected cells are resistant (20, 43, 51). Cell lines transfected with α-synuclein bearing mutations associated with familial PD showed a gain of neurotoxic function (46, 47, 49, 50), whereas in other cases, mutations were shown to cause a loss of anti-apoptotic functions (20, 51). Such diverse effects of α-synuclein in various cell lines might be consistent with the process of neurodegeneration and neuronal resistance to injury in vivo, where the damage to specific neuronal populations is heterogeneous. For example, although some neurons develop Lewy bodies, others from the same lineage do not, or where some neurons display neurodegenerative changes, adjacent cells are resistant to damage (52, 53).

The mechanisms by which α-synuclein might confer resistance or sensitivity to oxidative stress are not completely clear; however, activation of selective signaling molecules might play a central role. In this regard, the present study showed that down-regulation of the JNK pathway in the α-synuclein-overexpressing GT1–7 cells could be at least in some parts accounted for the up-regulation of JIP-1b/IB1. Because JIPs act as scaffold proteins for the JNK pathway in the cytoplasm (39, 40, 55), high expression of JIP-1b/IB1 might lead to inhibiting the signal cascades of the JNK pathway in these cells, thus conferring resistance to peroxide damage in cells overexpressing wild type α-synuclein. This is consistent with previous studies showing that overexpression of JIP-1b/IB1 resulted in suppression of the JNK pathway, rendering cells refractory to various apoptotic stimuli (39, 40, 55). In addition, a recent study showed that increased JIP-1b/IB1 expression protected dopaminergic cells from the Parkinson’s associated neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (31). Interestingly, MPTP promotes cell death via JNK activation (30); therefore, inhibition of the JNK pathway by JIP-1b/IB1 might provide the basis for novel strategies for protecting neurons in patients with PD. More importantly, it was shown that missense mutation of JIP-1b/IB1 was segregated in a familial type II diabetes mellitus (DM) (56), indicating that deregulation of JNK pathway through missense mutation of JIP-1b/IB1 may underlie the pathogenesis of DM. Because DM is highly associated with amylin amyloidogenesis in pancreatic β cells, one intriguing possibility is that deregulation of JNK pathway through JIP-1b/IB1 might be a common mechanism shared by various amyloid-related disorders, including DM- and α-synuclein-related neurodegeneration. In this context, it also is noteworthy that JIP-1b/IB1 was independently identified as a transcription factor for the glucose transporter II gene in pancreatic β cells (36).

The result of EMSA using a sequence of the glucose transporter II gene promoters showed that the DNA binding activity of the nuclear extracts derived from the α-synuclein-overexpressing GT1–7 cells were significantly up-regulated compared with those derived from both β-synuclein-overexpressing and mock-transfected cells (Fig. 3). Although the role of JIP-1b/IB1 as a transcription factor is still controversial (28), further studies might reveal a possibility that direct gene regulation by JIP-1b/IB1 may be important in the pathogenesis of both DM- and α-synuclein-related neurodegeneration.

Interestingly, overexpression of JIP-1b/IB1 in GT1–7 cells resulted in the redistribution of α-synuclein to the growth cones, in the clustering of neurons, and in the formation of abundant neuritic processes. This is consistent with previous studies showing that JIP-1b/IB1 was localized in the tip of neurites in differentiated neuronal cells, suggesting that JIP-1b/IB1 may play an important role in neurite formation (42). Furthermore, recent evidence suggests that JIP-1b/IB1 plays a role in axonal transport and guidance (57) because JIP-1b/IB1 links kinesin (57) and various signaling molecules in the membrane of vesicles, such as apolipoprotein E receptor 2 (apoER2) (58) and amyloid precursor protein (APP) (59), that are important for neuronal migration and neuritic outgrowth. The role of α-synuclein in neuritic outgrowth is unclear; however, previous studies show that α-synuclein is associated with synaptic vesicles (16) and is transported through the axon (60), and overexpression of α-synuclein in B103 neuroblastoma cells results in decreased adhesion and neuritic length (34). Collectively, these findings suggest that JIP-1b/IB1 overexpression might interfere with α-synuclein distribution and axonal transport that in turn might affect neuritic outgrowth. However, future co-immunoprecipitation studies are necessary to better characterize the interaction of these molecules.

In conclusion, these results suggest that increased α-synuclein expression might protect cells from oxidative stress by inactivation of JNK via increased expression of JIP-1b/IB1. Furthermore, interactions between α-synuclein and JIP-1b/IB1 may play a mutual role in the neuronal response to injury and neurodegeneration in PD.

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