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# Forum Original Research Communication

# Cell Type-Specific Upregulation of Parkin in Response to ER Stress

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## ABSTRACT

***Parkin*** **is the gene responsible for a familial form of Parkinson's disease (PD) termed autosomal recessive juvenile parkinsonism (AR-JP)/PARK2. Parkin has been shown to protect cells from endoplasmic reticulum (ER) stress and oxidative stress, presumably due to its ubiquitin ligase (E3) activity that targets proteins for proteasomal degradation. Although the authors showed that parkin is upregulated in response to ER stress, subsequent reports suggest that it does not represent a universal unfolded protein response (UPR). Here the authors report different regulation of parkin in response to ER stress in different cell lines, demonstrating upregulation of parkin as a cell type-specific response to ER stress. 2-Mercaptoethanol (2-ME) and tunicamycin increased the expression of parkin in SH-SY5Y (H) cells, Neuro2a cells, Goto-P3 cells, but not in SH-SY5Y (J) cells and IMR32 cells. In parallel with these studies, similar upregulation of the** ***parkin*** **coregulated gene (PACRG)/gene adjacent to** ***parkin*** **(Glup) was also observed by ER stress. Luciferase assays failed to detect the transcriptional activation of 500 bp parkin/Glup promoter in response to ER stress. These results indicate that induction of parkin by ER stress represents a cell type-specific response.** *Antioxid. Redox Signal.* 9, 533–541.

## INTRODUCTION

PARKINSON'S DISEASE (PD) is the most common neurodegenerative movement disorder characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta. Most cases of PD are sporadic and the etiology of the disease remains unclear. However, recent identification of genes responsible for relatively rare familial forms of PD, which comprise 5–10% of PD, significantly advanced our understanding of the molecular mechanisms underlying PD (4, 22).

*Parkin* is the gene responsible for autosomal recessive juvenile parkinsonism (AR-JP)/PARK2, the most common form of familial PD (1, 16, 20). Since the identification of *parkin*, many studies focused on elucidating the function of this protein, and it has been shown it can function as an ubiquitin ligase (E3) (27). Emerging data suggest that parkin may protect cells from premature death by targeting misfolded or damaged proteins for degradation via the ubiquitin proteasome pathway (9, 11, 35). The majority of parkin mutations are found to either impair its binding to putative substrates or render its ligase activity defective, thus resulting in a decrease in its activity. Therefore, endoplasmic reticulum (ER) stress [also known as unfolded protein response (UPR) stress] derived from accumulation of misfolded or damaged proteins because of the absence of parkin might represent as a mechanism underlying the dopaminergic neurodegeneration (12, 29, 30). ER stress has also been implicated in α-synuclein- or PD toxins-induced neuronal cell death. Induction of A53T α-synuclein expression induced expression of CHOP

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and BiP, as well as increased phosphorylation of eIF2α and activation of caspase-12. Most importantly, suppression of ER stress partially protected PC12 cells from α-synuclein-mediated cell death, suggesting that ER stress at least in part, contributes to α-synuclein-induced cell death (28). In addition, numerous changes in genes associated with UPR were identified in toxins-induced PD cell models (6, 25, 34). Moreover, neurons, which generally have small capacity to deal with ER stress by inducing an appropriate UPR are more sensitive to PD mimicking toxins (25). Collectively, these researches raise the possibility of widespread involvement of ER stress in the pathogenesis of PD.

Several studies have implied that the physiological role of parkin may be directly related to unfolded protein stress and the UPR pathway (9, 11). The ER-associated E2 enzymes, Ubc6 and Ubc7, apparently function as collaborating partners of parkin, indicating that parkin acts as part of the ER-associated degradation (ERAD) machinery. We have reported that ER stress caused by accumulation of unfolded protein upregulates parkin mRNA and protein levels, and that overexpression of parkin prevents unfolded protein stress-induced dopaminergic cell death (5, 11). However, there is a discrepancy among these reports on the induction of parkin upon ER stress (5, 11, 18, 21, 32). To clarify this point, we investigated the alteration of parkin expression upon ER stress in a panel of cell lines. The present experiments confirm our previous results that parkin mRNA and protein levels are increased in a subset of cell lines, but not in the rest of the cell lines. Thus, response to ER stress by increasing the expression of parkin appears to be cell-type specific, and inability to upregulate parkin might result in a higher vulnerability to ER stress.

## MATERIALS AND METHODS

### *Plasmids, antibodies, and reagents*

The sequence between human *Parkin* and *Glup* genes was cloned by polymerase chain reaction (PCR) from genomic DNA of SH-SY5Y cells and subcloned into the reporter plasmid pGL4 (Promega, Madison, WI). Anti-Parkin, anti-Glup, and anti-Hdj2 antibodies were described elsewhere (8, 9, 11). Anti-BiP (N-20), anti-Hsp70 (K-20), and anti-Hsc-70 (K-19) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-actin (C4), anti-TCP1α (CTA-184), and anti-tyrosine hydroxylase (TH) (MAB318) antibodies were obtained from Roche Diagnostics (Mannheim, Germany), StressGen (San Diego, CA), and Chemicon (Billerica, MA), respectively. Tunicamycin, 2-mercaptoethanol (2-ME), retinoic acid, 12-*O*-tetradecanoylphorbol 13-acetate (TPA), and $N^6$, 2′-*O*-dibutyryladenosine-3′,5′-cyclic monophosphate (dcAMP) were obtained from Nakalai tesque (Kyoto, Japan). Nerve growth factor 2.5S and MG-132 were purchased from Sigma (St. Louis, MI) and the Peptide Institute (Osaka, Japan), respectively.

### *Cell culture and transfection*

Human SH-SY5Y cell lines were obtained from M. Nomura (Hokkaido University) and N. Hattori (Juntendo University) and termed SH-SY5Y (H) and (J) in this article, respectively. SH-SY5Y and mouse Neuro2a cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% bovine fetal calf serum (FCS). Human GOTO-P3 cells, obtained from RIKEN Cell Bank (Saitama, Japan), were maintained in RPM11640 plus 10% FCS. IMR32 cells, obtained from Cell Resource Center for Biomedical Research (Tohoku University), were maintained in Eagle's minimum essential medium plus 10% FCS and 1 m*M* sodium pyruvate. Transfection was performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. phRluc-TK synthetic renilla vector (Promega) expressing *Renilla* luciferase was also transfected into the cell cultures simultaneously to act as a control for transfection efficiency, in a molar ratio of 1:10 (phRluc-TK vs. pGL4). Twenty-four hours after transfection, the cells were treated with vehicle or ER stress inducers for the indicated time and the transcriptional activities were determined by measuring the luminescent signal produced by the firefly luciferase reporter.

### *Luciferase assay*

The luciferase activity was determined using the Dual-Luciferase ® Reporter Assay System (Promega), according to the manufacturer's user protocol. The activities of firefly (*Photinus pyralis*) and *Renilla (Renilla reniformis*) luciferases were measured sequentially from a single sample. Firefly luciferase activities were normalized by *Renilla* activities to correct for differences in transfection efficiencies. All transfection experiments were repeated at least three times in triplicate. Statistical significance was analyzed by Student's *t* test. Firefly luciferase activities normalized by *Renilla* activities are presented as fold induction relative to the normalized firefly luciferase activity in cells transfected with the pGL4 empty vector only, which was taken as 1.0.

### *Quantitative RT-PCR*

Taqman EZ RT-PCR was carried out as previously described (11). The reverse transcript (RT) parameters (initial step, 50°C 2 min; RT, 60°C 30 min; deactivation for the reverse transcriptase, 95°C 5 min) and thermal cycling parameters (denaturation, 94°C 20 sec; annealing/extension, 62°C 1 min; each 40 cycles) were used according to the manufacturer's protocol. The primers and probe sequences were as follows: human parkin forward primer, 5′-TACGTGCACAGACGTCAGGAG; human parkin priming and reverse primer, 5′-GACAGCCAGCCACACAAGGC; human parkin probe, 5′-CAACTCCCGCCACGTGATTTGCTTAGACTG; human TCP1α forward primer, 5′-AAACTATGCAACCAGCATGGG; human TCP1α priming and reverse primer, 5′-GGGCCTCATTATGAAAAGCTCTT; human TCP1α probe, 5′-TCTCGGGAACAGCTTGCGATTGC; human Glup forward primer, 5′-TCCTCCCTGTCCTGAACATCTT; human Glup priming and reverse primer, 5′-CTCGAAGGCCTCCAGTGTCT; human Glup probe, 5′-TCCGGAGACGGCATTGACTACAGCC; human glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) forward primer, 5′-GAAGGTGAAGGTCGGAGTC; human GAPDH priming and reverse primer, 5′-GAAGATGGTGATGGGATTC; human GAPDH probe, 5′-CAAGCTTCCCGTTCTCAGCC; mouse parkin forward primer, 5′-TGTGGAGCACACCCAACCT; mouse parkin priming and reverse

primer, 5′-GCGCCTGTTGCTGGTGAT; mouse parkin probe, 5′-AGACAAGGACACGTCGGTAGCTTTGAACCT; mouse Glup forward primer, 5′-GCTTTCTGAAATGACGTTTCCC; mouse Glup priming and reverse primer, 5′-CCGTGTTCCAGCATGTCG; mouse Glup probe, 5′- TGAGTTTTTTGCTCGGCGAGGAATCC. The primers and probe of mouse GAPDH were used from rodent GAPDH control reagent (Applied Biosystems, Warrington, UK). The validity of GAPDH as a housekeeping gene was confirmed by no significant change in mRNA during each stress treatment.

### *Western blot*

Each mouse tissue (C57Black/6, 4 weeks old) was dissected, and homogenized with a Teflon homogenizer in tissue lysis buffer (20 m*M* Tris-HCl, pH 7.5, containing 120 m*M* NaCl, 5 m*M* EDTA, 1% Triton-X 100) with Complete protease inhibitors cocktail (Roche, Mannheim, Germany). Twenty μg of soluble protein from each tissue was subjected to Western blot analysis. Cultured cells were lysed in cell lysis buffer (20 m*M* HEPES, pH 7.4, containing 120 m*M* NaCl, 5 m*M* EDTA, 10% glycerol, 1% Triton X-100) with Complete protease inhibitors cocktail. Soluble cell lysate was analyzed by Western blot analysis with ECL detection reagents (Amersham Bioscience, Buckinghamshire, UK).

## RESULTS

### *Upregulation of parkin is a cell type-specific response to ER stress*

To confirm whether induction of parkin is specifically induced by ER stress, we evaluated the effect of a variety of cytotoxic agents on the transcription of parkin or Glup in SH-SY5Y (H) cells. Using primers specific for parkin, Glup, and GAPDH, mRNA levels of parkin in SH-SY5Y (H) cells cultured under various stress conditions for indicated time were measured by quantitative RT-PCR (Fig. 1). The PCR product for each mRNAs formed a single band (Fig. 1A). Consistent with our previous report, treating cells with the reducing agent 2-mercaptoethanol (2-ME; 7.5 m*M*) or the *N*-glycosylation inhibitor tunicamycin (Tunica; 10 μg/ml), both of which are known to cause ER stress, resulted in a significant increase of parkin mRNA levels at 12 and 24 h. In contrast, treatment with the proteasome inhibitor MG-132 (20 μ*M*) or short wavelength ultraviolet light (UV; 40 J/m$^2$) failed to alter the mRNA level of parkin. Concomitant with upregulation of parkin mRNA, Glup mRNA also increased upon 2-ME or tunicamycin treatment, consistent with the fact that these genes share a common promoter and are co-regulated. However, TCP1α mRNA, which increased upon proteasome inhibition, was unaltered upon treatment with 2-ME or tunicamycin, indicating that parkin or Glup upregulation is specifically increased in response to ER stress in SH-SY5Y (H) cells (Fig. 1B).

In addition to SH-SY5Y (H) cells, 2-ME and tunicamycin also increased parkin and Glup mRNAs in several other neuronal cell lines, including Neuro2a cells (Fig. 1C) and GOTO-P3 cells (Fig. 1D). However, no change was observed in IMR 32 cells (Fig. 1E). Given the different response by parkin alteration upon 2-ME or tunicamycin treatment, our results indicate that upregulation of parkin upon ER stress is cell-type specific.

We then performed Western blot analysis to determine if ER stress also altered parkin expression at the protein level. Consistent with the observation of increased parkin and Glup mRNAs, the parkin and Glup protein expression levels were increased during the ER stress tests in SH-SY5Y (H) (Fig. 2A). The parkin protein was also slightly increased upon MG-132 treatment (Fig. 2A). Given that parkin mRNA level is unaltered and parkin protein is degraded through proteasome, increased parkin protein might be due to decreased degradation of parkin by proteasome inhibition. Heat shock proteins Hsp70 and BiP were also increased upon treatment with ER stress inducers, whereas Hsc70 and HDJ-2 proteins showed no or minor changes (Fig. 2A). Although MG-132 treatment significantly induced TCP1α transcript (Fig. 1B), the protein level remains unaltered (Fig. 2A). Concomitant with ER stress-induced increases in parkin and Glup mRNAs, their protein levels were also increased in Neuro2a cells (Fig. 2B). Although both 2-ME and tunicamycin significantly induced parkin and Glup mRNAs in GOTO-P3 (Fig. 1D), increased parkin and Glup protein levels were only observed in 2-ME treatment (Fig. 2C). Unexpectedly, rather than increases, decreases in parkin and Glup proteins were observed upon tunicamycin treatment (Fig. 2C). Reduction of Glup protein was observed in tunicamycin-treated IMR32 cells, whereas an increase was observed upon 2-ME treatment (Fig. 2D), although both tunicamycin and 2-ME had no effect on Glup mRNA levels (Fig. 1E). An truncated band of parkin was observed in IMR32, and treatment with 2-ME for 24 h caused an increase in this truncated molecule (Fig. 2D), possibly derived from cleavage of full length parkin by activated caspases (14, 15).

### *Two kinds of SH-SY5Y cell lines demonstrate different characters*

Since parkin is differently regulated upon ER stress in a panel of cell lines studied, existence of conflicting data regarding the upregulation of parkin upon tunicamycin treatment in SH-SY5Y cells prompted us to investigate whether this difference is due to different features of SH-SY5Y, possibly derived from different manipulations (such as maintenance or passage conditions) between different laboratories. We therefore compared the effects of a variety of stress inducers on parkin expression in two SH-SY5Y cell lines, which we obtained from two different laboratories. Both 2-ME and tunicamycin treatment increased parkin mRNA level, whereas hydrogen peroxide ($H_2O_2$; 600 μ*M*), high osmolarity (0.3 *M* sorbitol), the DNA alkylating agent (methyl methanesulfonate (MMS); 100 μg/ml), UV radiation, and heat shock (HS; 42°C, 1 h) had little or no effect in SH-SY5Y(H) cells. However, SH-SY (J) cells responded differently: Dramatic reduction in the parkin mRNA level was observed under UV radiation, whereas 2-ME and tunicamycin had no obvious effect (Fig. 3A). We surmised the different responsiveness might be due to distinct features between these two kinds of SH-SY5Y cells. The effects of

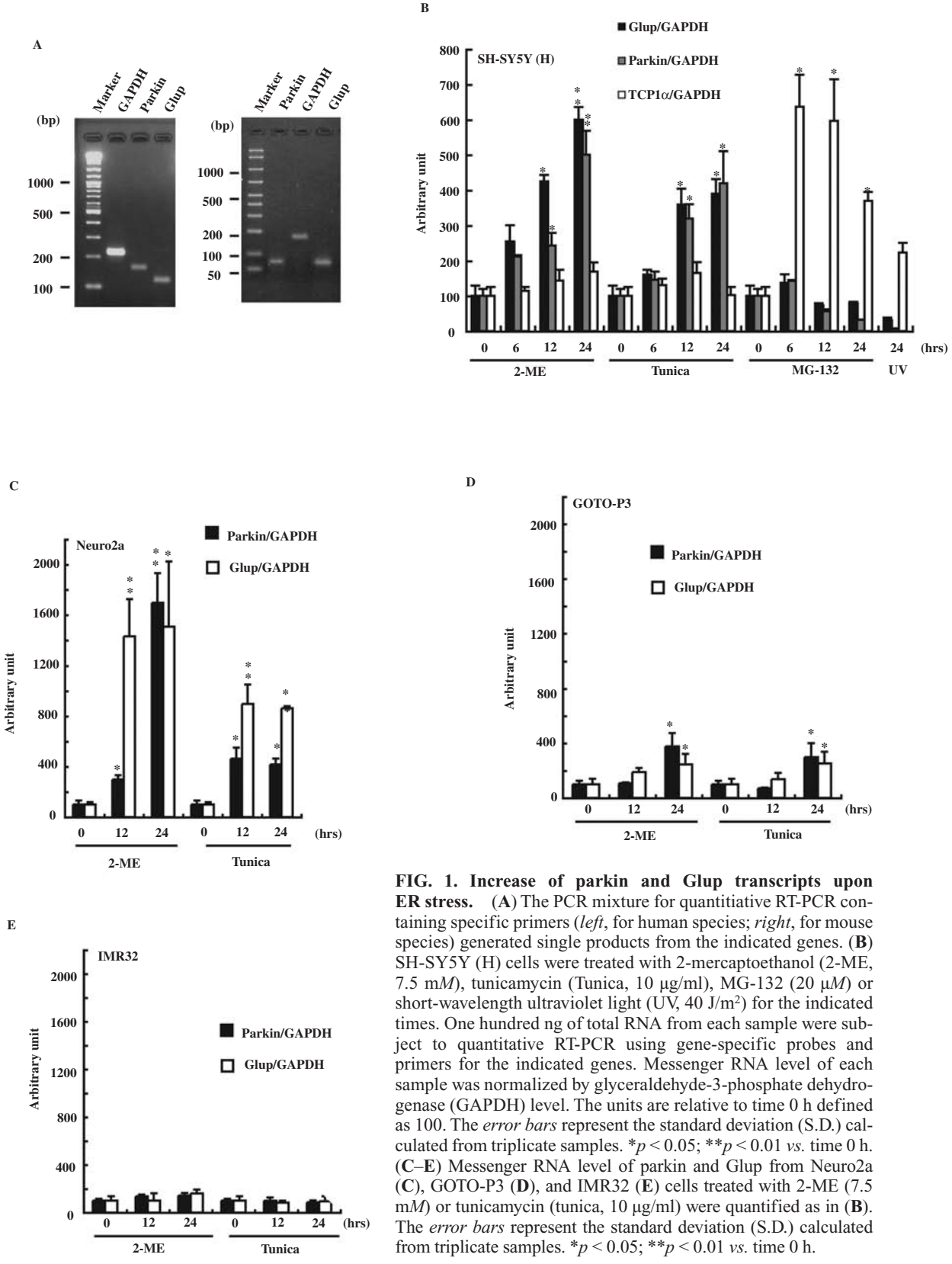


**FIG. 1. Increase of parkin and Glup transcripts upon ER stress.** (**A**) The PCR mixture for quantitiative RT-PCR containing specific primers (*left*, for human species; *right*, for mouse species) generated single products from the indicated genes. (**B**) SH-SY5Y (H) cells were treated with 2-mercaptoethanol (2-ME, 7.5 m*M*), tunicamycin (Tunica, 10 μg/ml), MG-132 (20 μ*M*) or short-wavelength ultraviolet light (UV, 40 J/m$^2$) for the indicated times. One hundred ng of total RNA from each sample were subject to quantitative RT-PCR using gene-specific probes and primers for the indicated genes. Messenger RNA level of each sample was normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) level. The units are relative to time 0 h defined as 100. The *error bars* represent the standard deviation (S.D.) calculated from triplicate samples. *$p < 0.05$; **$p < 0.01$ *vs.* time 0 h. (**C**–**E**) Messenger RNA level of parkin and Glup from Neuro2a (**C**), GOTO-P3 (**D**), and IMR32 (**E**) cells treated with 2-ME (7.5 m*M*) or tunicamycin (tunica, 10 μg/ml) were quantified as in (**B**). The *error bars* represent the standard deviation (S.D.) calculated from triplicate samples. *$p < 0.05$; **$p < 0.01$ *vs.* time 0 h.

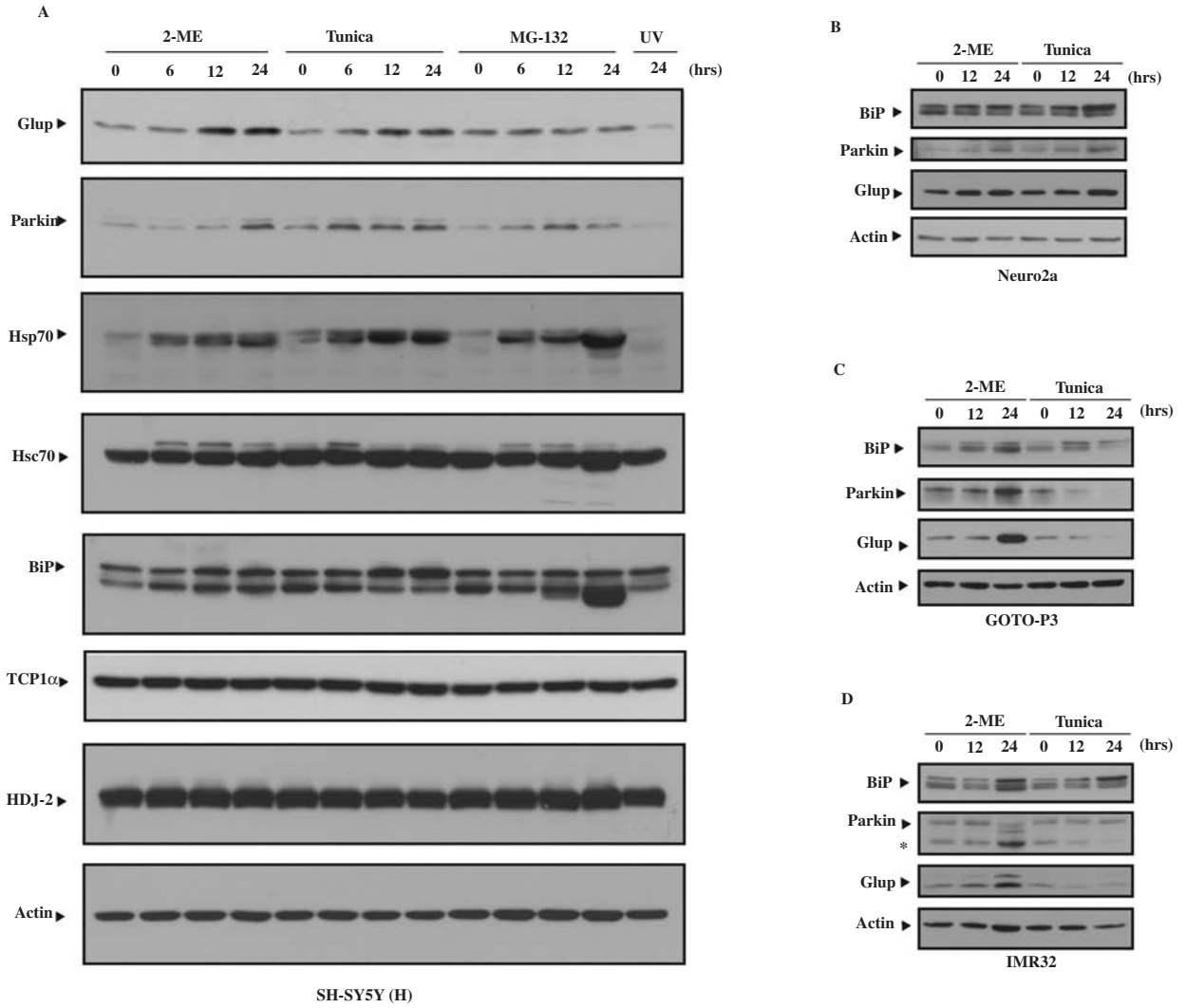


**FIG. 2. Protein levels of parkin and Glup upon ER stress.** (**A**) Total cell lysates from SH-SY5Y (H) cells in Fig. 1B were analyzed by Western blotting with antibodies against the indicated proteins. ER stress response was assessed by the induction of BiP. Total protein level of each sample was confirmed by the amount of actin. (**B**–**D**) Total cell lysate from various neural cell lines [(**B**) Neuro2a; (**C**) GOTO-P3; (**D**) IMR32 cells] loaded with ER stress (2-ME, 7.5 m*M*; Tunica, 10 μg/ml) for the indicated times, were analyzed by Western blotting as in (**A**). *An asterisk* in (**D**) indicates the putative processed molecules of parkin.

various differentiation reagents on these cells were therefore investigated. Tyrosine hydroxylase (TH), a dopaminergic neuronal differentiation marker of SH-SY5Y cells, was dramatically induced upon addition of 12-*O*-tetradecanoylphorbol 13-acetate (TPA, 160 n*M*) or $N^6$, 2′-*O*-dibutyryladenosine-3′:5′-cyclic monophosphate (dcAMP, 1 m*M*) in SH-SY5Y (J) cells. In contrast, SH-SY5Y (H) cells were quite refractory to differentiation into dopaminergic neuronal phenotype, only a slight increase of TH was observed under the same conditions (Fig. 3B).

## *Increased parkin and Glup mRNAs upon ER stress are not due to direct transcriptional activation*

Quantitative RT-PCR demonstrated increased parkin and Glup mRNA levels upon ER stress in SH-SY5Y (H) cells. However, it was not clear whether the increase in parkin mRNA was due to transcriptional or post-transcriptional regulation. We then measured the transcriptional activity of the 500 bp parkin core promoter using luciferase reporter gene assays. The parkin promoter has been found to function as a bidirectional promoter, regulating not only transcription of *parkin*, but also transcription of *Glup*, a gene located 204 bp upstream of and antisense to the *parkin* gene (Fig. 4A). Promoter constructs were transfected into SH-SY5Y (H) cells (Fig. 4B, left) or Neuro2a cells (Fig. 4B, right) and luciferase activity was assayed for basal levels and in response to 2-ME or tunicamycin. Both the parkin promoter vector and the Glup promoter vector failed to show any significant alteration in transcription levels relative to vehicle treated controls (Fig. 4B). This suggested that the increase in parkin and Glup mRNA levels observed in earlier experiments may not be due to direct transcriptional activation of the core promoter region of *parkin* or *Glup*.

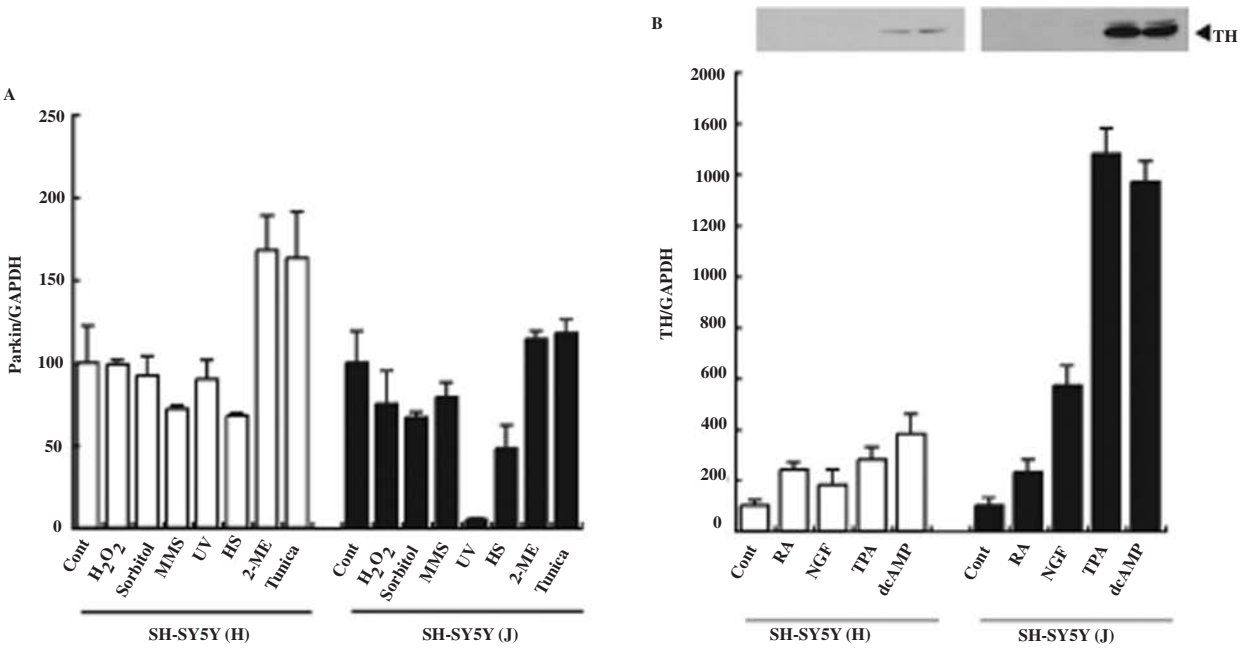


**FIG. 3. Characterization of two kinds of SH-SY5Y cell lines.** (**A**) SH-SY5Y (H) and (J) cells were treated with a variety of stresses [hydrogen peroxide ($H_2O_2$, 600 μ*M*), high osmolarity (sorbitol, 0.3 *M*), a DNA alkylating agent methyl methanesulfonate (MMS, 100 μg/ml), short-wavelength ultraviolet light (UV, 40 J/m$^2$), and heat shock (42°C for 1 h)]. After 5 h, total RNA was extracted and subject to quantitative RT-PCR as in Figure 1B. The *error bars* represent the standard deviation (S.D.) calculated from triplicate samples. (**B**) SH-SY5Y (H) and (J) cells were cultured with media with 5% horse serum and 5% bovine fetal calf serum containing retinoic acid (RA, $10^{-6}$ *M*), nerve growth factor-2.5S (NGF, 100 ng/ml), 12-*O*-tetradecanoylphorbol 13-acetate (TPA, 160 n*M*), or $N^6$, 2′-*O*-dibutyryladenosine-3′:5′-cyclic monophosphate (dcAMP, 1 m*M*). These media were exchanged every 2 days. After 7 days, mRNA level of parkin normalized with GAPDH (Graph) was assessed as in Fig. 1B, and protein level of tyrosine hydroxylase (TH) was analyzed by Western blotting. The *error bars* of the graph represent the standard deviation (S.D.) calculated from triplicate samples.

### *Tissue distribution of mouse parkin and Glup proteins*

Mouse parkin and Glup protein levels were measured in various tissues using Western blot analysis. Mouse Glup protein is mainly expressed in central nervous systems, with almost no protein expression in periphery tissues except for bladder and testis. On the other hand, parkin protein is rather ubiquitously expressed compared with Glup, abundant in central nervous systems, stomach, kidney, thymus, bladder, and testis (Fig. 5). These distinct tissue distribution patterns of parkin and Glup suggest that the protein expression levels of these proteins are regulated differently, although they share the same core promoter.

## DISCUSSION

Parkin is generally thought to be neuroprotective due to its role in the ubiquitin proteasome system. Parkin functions as an E3 ligase, targeting specific substrate proteins for degradation by the proteasome. It is conceivable that accumulation of some proteins, which cannot properly degraded because of the absence of parkin, causes ER dysfunction and ER stress. Several studies have focused on the regulation of endogenous parkin in the presence of ER stress. However, there is great disagreement in the literature regarding the expression of parkin upon ER stress. Our previous studies have shown that both parkin mRNA and protein levels were increased upon ER stress in SH-SY5Y cells (11). Higashi *et al*. (5) also demonstrated that parkin protein, as well as ER chaperones BiP and PDI, was upregulated in SH-SY5Y cells upon exposure to manganese (Mn) and overexpression of parkin protected cells from Mn-induced cell death. However, West *et al*. (32) demonstrated a lack of association between parkin and ER stress in SH-SY5Y cells. On the other hand, Mengesdorf *et al.* (21) reported that parkin mRNA was increased upon exposure to thapsigargin in rat cortical neuron cultures, whereas they failed to observe increased parkin protein level. Finally, Ledesma *et al*. (18) showed that when astrocytes and neurons were exposed to conditions associated with ER stress, a selective increase of parkin was observed in rat astrocytes but not hippocampal neurons. These apparent discrepancies prompted us to clarify the association between parkin and ER stress.

In our present studies, we have used 2-ME and tunicamycin to determine if they might result in any alterations in the transcription of parkin in a panel of cell lines. We show here that treatment with ER stress inducers results in upregulation of parkin and Glup at both mRNA and protein levels in a cell

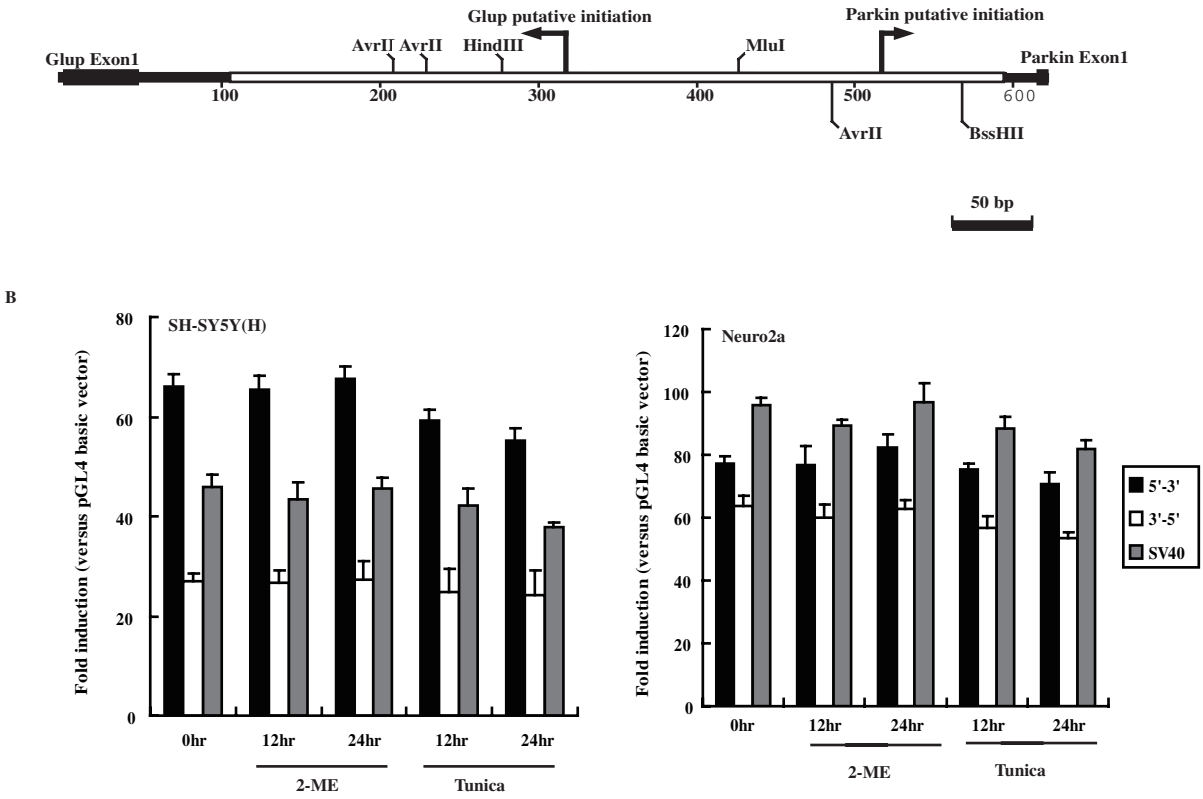


**FIG. 4. Parkin and Glup are co-regulated with a bi-derectional promoter in a reporter assay.** (**A**) A diagram of the human genomic region between the first exons of Parkin and Glup genes. The sequence used in this reporter assay is shown as an *open box*. (**B**) SH-SY5Y (H) (*left*) and Neuro2a (*right*) cells transfected with reporter plasmids for luciferase (pGL4) driven by the indicated promoters (5′-3′, the region as shown in **A** inserted in the sense direction to parkin gene; 3′-5′, the region as shown in **A** in the sense direction to Glup gene, SV40, SV40 promoter; Basic, without a promoter) and a reporter plasmid for *Renilla* luciferase driven with a TK promoter (phRluc-TK, Promega) were treated with various reagents (2-ME, 7.5 m*M*; Tunica, 10 μg/ml) for the indicated times. The luciferase activity normalized with transfection efficiency by *Renilla* activity was shown by fold induction *vs.* pGL4 basic vector. The *error bars* represent the standard deviation (S.D.) calculated from triplicate samples.

type-specific manner. The function of Glup is not clearly known, but there is some evidence that it may play a role in protection against stress-induced cell death (10, 33). It is noted that some novel components of the UPR that regulates the canonical UPR are expressed in a cell type-specific fashion, suggesting that different cells may have unique responses for adaptation to ER stress (17, 23, 36). The present study therefore suggests that upregulation of parkin and Glup might represent as cell type-specific adaptation to ER stress. Moreover, the parkin and Glup protein levels do not always reflect their mRNA levels, suggesting a complicated mechanism by which regulates their transcription, translation and/or degradation in different types of cells upon ER stress.

Upregulation of parkin or Glup transcripts upon ER stress is not ascribable to direct transcriptional activation since the 500 bp promoter region shared by parkin and Glup fails to confer transcriptional activation upon ER stress as assessed by luciferase assays. A previous report showed that the parkin promoter region containing 4,500 bp sequence 5′ to parkin exon 1 was unresponsive to ER stress, consistent with our results. The increased parkin or Glup mRNA levels might be derived from the result of post-transcriptional events, such as increased mRNA stability and/or decreased mRNA turnover. Alternatively, given the bulk of introns in the *parkin* gene, some regulatory elements outside our luciferase constructs might affect parkin transcription upon ER stress, which should be clarified in the future study.

The cell lines used in this study are all derived from neuroblastomas with neuronal properties. IMR 32 is a neuroblastoma cell line derived from human. The culture of IMR 32 is a mixture of two morphologically distinct cell types: the pre-

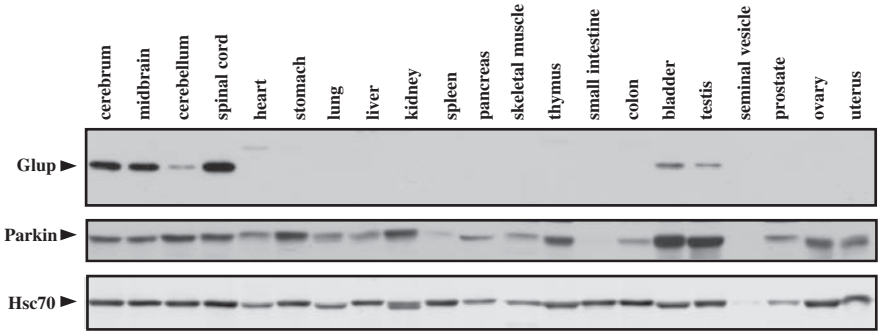


**FIG. 5. Tissue distribution of parkin and Glup protein.** Mouse various tissue samples were analyzed with Western blot for parkin, Glup, and Hsc70.

dominant one is a small neuroblast-like cell and the other is a large hyaline fibroblast-like cell (31). GOTO-P3 has been established in tissue culture from a human neuroblastoma arising from the adrenal grand. The cells of GOTO-P3 are small and fibroblast-like (26). SH-SY5Y is a thrice-cloned subline of bone marrow biopsy-derived line SK-N-SH, both epithelial-like and neuroblast-like morphologies have been reported (2, 3, 13). In our hands, SH-SY5Y (H) cells are epithelial-like, whereas SH-SY5Y (J) cells demonstrate small neuroblast-like. Neuro2a is a mouse neuroblastoma line established from the spontaneous tumor of *albino* mouse strain. The cells are neuronal and ameboid-like (24).

The presence of conflicting data regarding different parkin alteration upon ER stress in the "same" SH-SY5Y cells prompted us to further characterize two SH-SY5Y cell lines, which we obtained from two different laboratories. Actually, these two cell lines demonstrated different properties among manipulation. SH-SY5Y (H) but not SH-SY5Y (J) responded to ER stress by upregulation of parkin. On the other hand, SH-SY5Y (H) was more refractory to induced differentiation compared with SH-SY5Y (J), strongly suggesting that SH-SY5Y (H) and SH-SY5Y (J) are different in their properties. It is thus conceivable that conflicting data observed previously in SH-SY5Y cells might be due to different properties of SH-SY5Y cells, possibly acquired in the process of maintenance in individual laboratories.

It remains unclear how ER stress causes upregulation of parkin in a subset of these neuroblastomas with neuronal properties. It is noted that upregulation of parkin was not observed in IMR32 as well as in SH-SY5Y (J) cells. Like SH-SY5Y (J) cells, IMR 32 has been demonstrated readily to differentiate into dopaminergic neuronal phenotype, since TH proteins were already detectable upon addition of TPA for only 3 days (19). Morphologically, parkin appeared to be upregulated in predominantly fibroblast-like SH-SY5Y (H), GOTO-P3, and Neuro2a cells, but not in predominantly neuroblast-like SH-SY5Y (J) and IMR32 cells. Consistent with the data from Ledesma *et al.* (18), our current experiments suggest that upregulation of parkin may not occur in response to ER stress in neurons.

It is likely that certain cell-type specific ER stress related molecules affect parkin expression. Recently it has been reported that OASIS (old astrocyte specifically induced substance) is a novel ER stress transducer that specifically regulates UPR signaling in astrocytes (7, 17). Although we examined whether OASIS is responsible for the cell type-specific upregulation of parkin upon ER stress, only negative results were obtained (data not shown). Although parkin and Glup share the same promoter, the tissue distribution patterns of these proteins are different, suggesting the presence of cell type- or tissue-specific regulators for parkin and Glup protein expression (Fig. 5). Further work is necessary to examine the mechanisms whereby the upregulation of parkin occurs specifically in certain types of cells upon ER stress.

## ACKNOWLEDGMENTS

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## ABBREVIATIONS

AR-JP, autosomal recessive juvenile parkinsonism; DMEM, Dulbecco's modified Eagle's medium; ER, endoplasmic reticulum; ERAD, ER associated degradation; FCS, fetal calf serum; Glup, gene adjacent to parkin; 2-ME, 2-mercaptoethanol; Mn, manganese; OASIS, old astrocyte specifically induced substance; PACRG, parkin coregulated gene; PCR, polymerase chain reaction; PD, Parkinson's disease; RT, reverse transcript; TH, tyrosine hydroxylase; TPA, 12-*O*-tetradecanoylphorbol 13-acetate**;** UPR, unfolded protein response.

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