Supplementary Material

Cell culture and transfection

HEK293 cells were grown in Dulbecco's Modified Eagle Medium (Sigma) containing 10% fetal bovine serum under 5% CO_2 atmosphere. The cells were transiently transfected with the expression vector by the FuGENE6 transfection reagent according to the instructions provided by the manufacturer (Roche, Mannheim, Germany).

Plasmids

For pcDNA3.1(+)Myc and FLAG vector, Oligo DNAs encoding N-terminal Myc and the FLAG epitope were ligated into the *Kpn*I/*BamH*I site of pcDNA3.1(+) (Invitrogen, San Diego, CA). We amplified the cDNA of wild-type (WT), N-terminal, C-terminal and both terminal deletion mutants of parkin and wild-type α-SN by polymerase chain reaction (PCR) with appropriate primers and ligated them into *EcoR*I/*Not*I site for parkin and *Not*I/*Xho*I site for α-SN of the vectors. We prepared missense mutants of parkin and α-SN by mutating each wild type plasmids using the Quik Change site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the protocol provided by the manufacturer. pcDNA3.1(+)Myc14-3-3η was kindly provided by H. Ichinase, pGEX-3X14-3-3η by T. Ichimura and pcDNA3.1(+)V5-synphilin-1 by S. Ishigaki. The expression vectors of FLAG-UbcH7 and Ubc7 were described previously (Shimura *et al*., 2000).

Surface plasmon resonance (SPR) analysis

The SPR study was performed on a Biacore 2000 instrument (Biacore AB, Uppsala, Sweden). 14-3-3η and α-SN were expressed in *E. coli* while parkin was expressed in Sf9 cells. For studies of parkin binding to 14-3-3η, 14-3-3η was covalently linked to the surface of a CM5 Chip (Biacore) using amino-coupling via 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide HCl and *N*-hydroxysuccinimide at final densities of 5000 resonance unit (RU) and parkin was used for analysis. For studies of α-SN binding to 14-3-3η, α-SN was linked to CM5 sensor chip at a final density of 3000 RU and 14-3-3η was used for analyte. Blank surface treated without ligand was used as a control in each individual affinity analysis. Binding experiments were performed in PBS and 1 mM dithiothreitol at 25°C. Five different concentrations were used for each analysis. Analysis of the binding experiments was performed using the BIAevaluation v3.0 software package (Biacore).