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Antibodies and dyes: The anti-c-myc antibody, the β-actin antibody and the anti-VDAC/Porin antibody were obtained from Sigma-Aldrich (Steinheim, Germany), the rabbit anti-HA antibody was from BD Bioscience (Erembodegem, Belgium). The mouse anti-penta-His antibody was from Qiagen (Hilden, Germany). The anti-PINK1 rabbit polyclonal IgG antibody (Novus Biologicals, Littleton, CO, USA) was diluted 1:1000 for western blotting and 1:500 for immunofluorescence. Polyclonal antibodies against MARK1-4 (SA 4632 or SA 4633, both generated against aa 1-377 of MARK1) and MARK2 pT208 are described elsewhere (Timm et al, 2003). Antibody 12E8 against phosphorylated S262+S356 of tau was a gift of P. Seubert (Elan Pharma). The anti-phosphoserine and anti-phosphothreonine antibodies were from Millipore (Temecula, CA). All fluorescently (cyanine 5, tetramethylrhodamine isothiocyanate)-labeled secondary antibodies were from Dianova (Hamburg, Germany). For staining of mitochondria, the medium was removed and replaced with medium containing MitoTracker DeepRed 633 (Invitrogen, Karlsruhe, Germany) at a final concentration of 10 nM. Cells were incubated for 1h under growth conditions, then the MitoTracker solution was replaced with fresh prewarmed medium and mitochondria were observed.

Preparation of *in vitro* phosphorylated PINK1-peptides: GST-ΔN-PINK1 (20 µg) and $MARK2^{T208E}$ (3 µg) were incubated together for 3 h at 30°C in the kinase activity assay buffer containing 200 µM [^{32}P] ATP (1.85 x 10^7 MBq/mol). The proteins were precipitated by addition of TCA to a final volume of 10%, washed once with ice-cold 70% ethanol and resuspended in reduction buffer (0.4 M Tris-HCl pH 8.0, 2.6 mM DTT, 2% SDS, 10% glycerol, 0.01% NaN_3, 0.001% bromphenolblue, 0.003% EDTA). The samples were boiled at 95°C for 3 min and after cooling to room temperature 1/10 volume of 26 mM DTT was added. Alkylation was performed by addition of 1/10 volume of 20% (w/v) iodacetamide and incubation at room temperature for 30 min. The phosphorylated proteins were separated by SDS/PAGE. After staining with colloidal Coomassie Blue the ΔN-PINK1 band was cut out of the gel. To destain the protein band 50 mM ammonium bicarbonate in 50% acetonitrile was added for 30 min while shaking. In a second step 10 mM ammonium bicarbonate in 50% acetonitrile was added for another 30 min. The gel piece was lyophylized and rehydrated with 25 µl digestion buffer (10 mM ammonium bicarbonate pH 8.0, 1 mM $CaCl_2$) containing 1 µg of α-chymotrypsin (Sigma-Aldrich, Steinheim, Germany) for 10 min with mild agitation. The gel pieces were covered with additional buffer without proteinase and incubated overnight at 37°C. After the incubation, the liquid from the gel piece was removed and transferred to a new tube. This solution contained most of the extracted tryptic peptides. A second extraction step followed with 0.1% trifluoroacetic acid (TFA) in 60% acetonitrile for 1h and shaking at room temperature. The peptides were dried, resuspended in 0.1 % TFA and applied to a C18 column (Vydac, Western Analytical Products, Murrieta, CA; SMART-HPLC, Pharmacia, Uppsala, Sweden). The fractions containing the radioactively labeled peptides were then analyzed by MALDI-TOF MS. A small aliquot of the sample was subjected to phosphopeptide mapping.

Cell culture: Cell culture was performed with HEK293, CHO, PC12 and Sf9 cells following standard protocols at 37°C containing 5% CO_2. Briefly, HEK293 cells were grown in Dulbecco's modified Eagle's Medium (DMEM; PAA-Laboratories, Pasching, Austria) with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were transfected with Effectene-Transfection reagent (QIAGENE, Valencia, CA) according to the manufacturer's protocol. CHO cells were grown in Ham´s F-12 medium (PAA-Laboratories, Pasching, Austria) with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were transfected with Lipofectamine 2000 (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. For stabilizing the microtubules the cells were treated with 1 µM Paclitaxel (Taxol) (Sigma-Aldrich, Steinheim, Germany) during the transfection procedure for 4 h and observed after 18 h. For the protein stability assay cells were treated 16 h after transfection either with 10 µM Epoxomycin (Merck, Darmstadt, Germany) or 50 µM Cycloheximide (Sigma-Aldrich, Steinheim, Germany) and harvested after 1 h. PC12 cells were grown in Dulbecco´s modified Eagle´s Medium (DMEM) with 15% horse serum, 5% fetal bovine serum and 1% penicillin/streptomycin. Differentiation was triggered by differentiation medium consisting of DMEM with 0.1% serum (1:1 horse serum/fetal bovine serum) and 100 ng/ml NGF. Sf9 cells were cultured in Grace's Insect Medium containing 10% fetal bovine serum and 1% penicillin/streptomycin. Cortical neurons were isolated, cultured and transfected as described before (Stamer et al. 2002; Thies & Mandelkow, 2007). After 7 days cultures were transfected with Lipofectamine 2000 (Invitrogen, Karlsruhe, Germany) or were infected with adenoviruses of PINK1 (FL, ΔN or $ΔN^{T313M}$) and MARK2 (wt or T208A/S212A) for 24h. Retinal ganglion explants were prepared from white leghorn chicken eyes and subsequently cultured for 24h as described elsewhere (Stamer et al, 2002). For adenoviral transfection of

PINK1^{FL}-YFP, ΔN-PINK1-YFP, CFP-MARK2^{wt} or CFP-MARK2$^{T208A/S212A}$, a 100-fold multiplicity of infection (MOI 100, 3 x 10^7 infectious particles) was applied to the cells. In the case of double transfections, 3 x 10^7 infectious particles of each recombinant adenovirus were added (Biernat et al, 2002). For staining of mitochondria in RGCs, medium containing MitoTracker DeepRed 633 (Invitrogen, Karlsruhe, Germany) at a final concentration of 10 nM were added and incubated for 1h under growth conditions. The expression of PINK1 and MARK2 and the movement of mitochondria were observed by confocal microscopy 24–48 h after transfection.

Phosphorylation of ΔN-PINK1 and PINK1^{FL} mediated by MARK2 in CHO cells: The observed phosphorylation and activation of ΔN-PINK1 by MARK2 (Fig. 1-4) raises the possibility that PINK1^{FL} may be also modified by MARK2. To examine this possibility, we performed phosphorylation assays in CHO cells. Fig. S1 illustrates that coexpression of active MARK2 in CHO cells promote phosphorylation of ΔN-PINK1-YFP and PINK1^{FL}-YFP (lane 5 and 6). In response to expression of MARK2^{T208E} ΔN-PINK1 becomes phosphorylated on threonine residues, whereas PINK1^{FL} shows phosphorylation on threonine as well as on serine residues. These data, together with the results of mitochondrial movement in axons and their dependence on PINK1^{FL}, ΔN-PINK1 and MARK2 (Fig. 7) confirms that PINK1^{FL} is indeed a cellular substrate of MARK2-signalling.

Localization of endogenous PINK1, MARK2 and mitochondria in differentiated PC12 cells: Primary cortical neurons show partial colocalization of endogenous active MARK2 with mitochondria, especially at the growth cone (Fig. 5A). Differentiating PC12 cells have nearly the same staining pattern of endogenous active MARK2, revealing a dotted but uniform distribution throughout the cell with high concentration at the tip of neurites (Fig. S2 panel 4). However, endogenous PINK1 shows also a vesicle-like distribution and is detectable particularly at the tip of single growth cones (Fig. S2, panel 2). The colocalization with mitochondria suggests a special function of the PINK1-MARK2 signaling pathway in differentiating neurites, e.g. in the supply of energy. Because the data described in Fig. 6 and 7 indicated an involvement of PINK1^{FL}, ΔN-PINK1 and MARK2 in the regulation of mitochondrial transport, we next asked whether kinesin participates in the assumed mitochondrial transport complex. The patterns of mitochondrial movement along MTs implicate motor proteins of the kinesin superfamily in anterograde organelle transport and those of the dynein family in retrograde transport (Hollenbeck & Saxton, 2005). The staining of endogenous kinesin in differentiating PC12 cells reveals nearly the same distribution as the endogenous PINK1 and active MARK2 (Fig. S2, panel 6). This result supports the previous conclusion that PINK1 and MARK2 are involved in the kinesin driven mitochondrial transport mechanism.

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Fig. S1: MARK2 induces phosphorylation of ΔN-PINK1 and PINK1^{FL}. CHO cells were transfected with plasmids encoding ΔN-PINK1-YFP, PINK1^{FL}-YFP or active HA-tagged MARK2 either singly (lane 2-4) or in combination (lane 5-6). Empty pShuttle vector (lane 1) was used to make the total amount of transfected DNA equivalent. Expression of the proteins were proved by Western Blotting using anti-PINK1 (row 1) or anti-HA antibody (row 2). Cell lysates were immunoprecipitated with anti-PINK1 antibody and phosphorylation was detected by immunoblotting with anti-phosphoserine (row 3) and anti-phosphothreonine antibody (row 4). After MARK2 expression, PINK1^{Fl} shows both serine and threonine phosphorylation (lane 6, row 3 and 4), whereas in ΔN-PINK1 phosphorylation affects mainly threonine residues (lane 5, row 4).

Fig. S2: Subcellular localization of endogenous PINK1 and endogenous active MARK in differentiating PC12 cells. Cells were differentiated with NGF for 15 h and stained for endogenous active

MARK2 using the phospho-specific pT208-antibody (cyan, 4), endogenous PINK1 (yellow, 2), mitochondria (green, 1, 3 and 5), and kinesin (red, 6). High amounts of all components were found at the tips of growth cones (see magnification). Scale bars: 10 µm.