Article

Parkin differently regulates presenilin-1 and presenilin-2 functions by direct control of their promoter transcription

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We previously established that besides its canonical function as E3-ubiquitin ligase, parkin also behaves as a transcriptional repressor of p53. Here we show that parkin differentially modulates presenilin-1 and presenilin-2 expression and functions at transcriptional level. Thus, parkin enhances/reduces the protein expression, promoter activity and mRNA levels of presenilin-1 and presenilin-2, respectively, in cells and in vivo. This parkin-associated function is independent of its ubiquitin-ligase activity and remains unrelated to its capacity to repress p53. Accordingly, physical interaction of endogenous or overexpressed parkin with presenilins promoters is demonstrated by chromatin immunoprecipitation assays (ChIP). Furthermore, we identify a consensus sequence, the deletion of which abolishes parkin-dependent modulation of presenilins-1/2 and p53 promoter activities. Interestingly, electrophoretic mobility shift assays (EMSA) revealed a physical interaction between this consensus sequence and wild-type but not mutated parkin. Finally, we demonstrate that the RING1-IBR-RING2 domain of parkin harbors parkin’s potential to modulate presenilins promoters. This transcriptional control impacts on presenilins-associated phenotypes, since parkin increases presenilin-1-associated γ-secretase activity and reduces presenilin-2-linked caspase-3 activation. Overall, our data delineate a promoter responsive element targeted by parkin that drives differential regulation of presenilin-1 and presenilin-2 transcription with functional consequences for γ-secretase activity and cell death.

Keywords: parkin, presenilins, transcription, γ-secretase activity, apoptosis

Introduction

Parkin is a pleiotropic protein responsible for most of autosomal recessive juvenile forms of Parkinson’s disease (PD) (Dawson and Dawson, 2010; Corti et al., 2011). This protein was first reported to behave as an ubiquitin ligase (Shimura et al., 2000), and therefore, to be involved in proteasome-mediated elimination of various substrates. To date, about two dozens of parkin substrates have been identified and it has been postulated that the control of their lifetimes underlies several dysfunctions taking place in PD, since most of parkin-associated mutations abolish its ubiquitin-ligase activity (Shimura et al., 2000). However, besides this canonical function, several studies have indicated that additional parkin-dependent cellular pathways could exist. Thus, we recently documented the fact that parkin could act, in an ubiquitin-ligase independent manner, as a direct transcriptional repressor of the tumor suppressor p53 promoter activity (da Costa et al., 2009).

We and others have documented the fact that p53 could regulate the expression and functions of presenilin-1 (PS1) and presenilin-2 (PS2) (Roperch et al., 1998; Pastorcic and Das, 2000; Checler et al., 2010), two proteins responsible for most of the familial cases of Alzheimer’s disease (AD) (Checler, 1999) that are mainly involved in the production of the amyloid β peptide (Aβ) and in caspase-3-mediated cell death (De Strooper et al., 1998; Wolfe, 2001; Alves da Costa et al., 2002, 2006). Interestingly, recent data also indicate that parkin could control the homeostasis of intracellular Aβ42 (Burns et al., 2009; Rosen et al., 2010). These observations underline the present study that aimed at examining whether parkin could control PS1 and/or PS2 and their related functions, and if so, whether
it was via a possible direct transcripional control of PS promoters or indirectly, via p53.

Our data strikingly show that parkin indeed acts as a direct transcripional activator of PS1 and as a direct transcripional repressor of PS2. Accordingly, this is accompanied by enhanced γ-secretase activity and lower caspase-3 activation. We establish that parkin-mediated control of PS promoters is independent of its ubiquitin-ligase activity and unrelated to its ability to modulate p53. Of most importance, in support of our description of a novel transcripional factor activity, we have delineated a conserved sequence on human PS1, PS2, and p53 promoters that could have served as parkin-targeted consensus sequence. Indeed, we document a direct physical interaction of parkin with this sequence, the deletion or mutation of which abolishes the ability of parkin to modulate the above-cited promoter activities. Our data indicate for the first time that PS1 and PS2 promoters could be controlled differently at transcriptional level and in an opposite manner and thereby identify another potential pathway to selectively interfere with PS1-mediated γ-secretase activity and PS2-associated cell death. Furthermore, the delineation of a parkin-targeted responsive element on presenilins and p53 promoters opens a new avenue to the identification of additional and yet unknown parkin transcriptional targets. This should avoid deleterious effects linked to pharmacological approaches that aim at interfering with parkin-mediated ubiquitination and subsequent proteasomal degradation of other proteins often involved in vital cellular functions.

Results
Overexpressed and endogenous parkin control presenilin transcription

Figure 1A–F shows that the stable overexpression of parkin in TSM1 neurons increases PS1 protein expression (+72.7% ± 12.3%, n = 8, P < 0.001, Figure 1A), promoter activity (+45.2% ± 2.7%, n = 8, P < 0.001, Figure 1B), and mRNA levels (+108.5% ± 7.4%, n = 4, P < 0.01, Figure 1C), and decreases PS2 expression (−30.1% ± 19.7%, n = 6, P < 0.05, Figure 1D), promoter activity (−42.6% ± 12.4%, n = 6, P < 0.01, Figure 1E), and mRNA levels (−52.3% ± 12.4%, n = 6, P < 0.05, Figure 1F). Interestingly, the above data were fully confirmed in non-transformed primary cultured neurons where parkin transfection leads to increased PS1 expression (Supplementary Figure S1A), promoter transactivation (Supplementary Figure S1B), and mRNA levels (Supplementary Figure S1C). Conversely, parkin overexpression reduces PS2 expression (Supplementary Figure S1D), promoter activity (Supplementary Figure S1E), and mRNA levels (Supplementary Figure S1F). The above overexpression data were corroborated by the examination of the ability of endogenous parkin to modulate PS1 and PS2 in immortalized mouse embryonic fibroblasts (MEF). Thus, parkin depletion decreases PS1 expression (−56.0% ± 4.1%, n = 6, P < 0.01, Figure 1G, see NPS1 in left panel and grey bars), promoter activity (−45.2% ± 8.7%, n = 8, P < 0.001, Figure 1H, upper panel), and mRNA levels (−79.8% ± 8.7%, n = 4, P < 0.01, Figure 1I, grey bars), while parkin deficiency increases PS2 protein levels (+184.8% ± 38.4%, n = 6, P < 0.01, Figure 1J, see CPS2 in left panel and black bars), promoter activity (+590.8% ± 72.5%, n = 8, P < 0.001, Figure 1H, lower panel), and mRNA levels (+118.8% ± 20%, n = 8, P < 0.01, Figure 1I, black bars). These data were reinforced by our demonstration that the depletion of parkin in mouse brain reduces PS1 expression (−65.2% ± 22%, n = 4, P < 0.05, Figure 1J, see NPS1 in left panel and grey bars) and mRNA levels (−45.2% ± 8.7%, n = 8, P < 0.05, Figure 1K, grey bars) and enhances PS2 expression (+77.3% ± 23.7%, n = 4, P < 0.05, Figure 1L, see CPS2 in left panel and black bars) and mRNAs levels (+89.6% ± 43.6%, n = 6, P < 0.05, Figure 1K, black bars).

Impact of parkin on presenilin-1 and 2-associated functions

We have examined whether parkin-mediated modulation of PS1 and PS2 could induce functional consequences. In order to directly assess the influence of parkin on PS1-associated γ-secretase, we used an in vitro assay with reconstituted membranes that allows monitoring productions of Aβ and its C-terminal counterpart βAPP intracellular domain (AICD) (Pardossi-Piquard and Checler, 2011) from an exogenous recombinant substrate (C100, see Supplementary Figure S3, box Figure 2, panels A–F), thereby reflecting the activity/levels of γ-secretase only (Sevalle et al., 2009). Figure 2A and B shows that the overexpression of parkin in TSM1 neurons (see parkin expression in Figure 1A) increases Aβ (+59% ± 14%, n = 9, P < 0.001, Figure 2A) and AICD (+25% ± 4%, n = 6, P < 0.01, Figure 2B) productions, while parkin gene depletion in immortalized fibroblasts (see parkin expression in Figure 1G) decreases both Aβ (−63% ± 5.5%, n = 9, P < 0.001, Figure 2C) and AICD (−92% ± 14%, n = 6, P < 0.001, Figure 2D). In order to avoid any artifactual effect due to parkin depletion engineering or immortalization process, we confirmed this data in another cell system corresponding to primary MEFs (provided by Dr J. Shen) in which parkin had been deleted (see parkin expression in Supplementary Figure S3, box Figure 2). Indeed, we confirmed that endogenous parkin depletion triggered decreases of both Aβ (−64.2% ± 3.8%, n = 9, P < 0.001, Figure 2E) and AICD (−46.5% ± 3.8%, n = 9, P < 0.001, Figure 2F) productions. Finally, in order to examine whether parkin-linked modulation of PS could impact on their cell death associated functions, we analyzed the influence of parkin on thapsigargin (TPS)-induced reticular stress conditions by monitoring caspase-3 activity. Three lines of independent data indicate that this paradigm can be indeed considered a read out of PS function. First, both parkin and PS (see PS expressions in HEK293 and MEF in Supplementary Figure S3, box Figure 3, panels C, E and panel D, respectively) interfere with TPS-induced caspase-3 activation. Thus, the overexpression of parkin (Figure 3A, compare black and white + bars) or PS1 (Figure 3C, compare black and white bars labeled +) reduces TPS-stimulated caspase-3 activity, while PS2 increases this activity (Figure 3C, compare black and grey bars labeled +). Second, conversely, the depletion of parkin (Figure 3B, compare black and white bars labeled +) or PS1 (Figure 3D, compare black and white bars labeled +) increases TPS-induced caspase-3 activation, while PS2 deficiency reduces this activity (Figure 3D, compare black and light grey bars labeled +). Interestingly, the depletion of both PS1 and PS2 mimics the phenotype observed with PS2 depletion only (Figure 3D, compare black and dark grey bars labeled +).
indicating, in agreement with our previous study (Alves da Costa et al., 2002, 2006), that PS2 is dominant over PS1 for the control of ER-stress-associated cell death. Third, we observed that transient expression of HA-parkin (see expression in Supplementary Figure S3, box Figure 3, panel E) reverses PS2-associated enhancement of TPS-induced caspase-3 activation (−51.4% ± 18.9%, n = 6, P < 0.001, Figure 3E, compare EV and Pk grey bars), while it does not affect PS1-protective activity (Figure 3E, compare EV and Pk white bars). The above set of data clearly indicates that parkin-mediated control of PS1 and PS2 has functional consequences for both PS1-associated γ-secretase activity and PS2-linked modulation of caspase-3 activity.

Parkin-mediated control of PS1 and PS2 is independent of its ubiquitin-ligase activity

In order to assess whether parkin-mediated control of PS1 and PS2 required its ubiquitin-ligase activity, we transiently transfected various parkin cDNA-bearing mutations that either abolish [C418R (418), C441R (441)] or preserve [K161N (161), R256C (256)] parkin E3-ligase activity in dopaminergic SH-SYSY neuroblastoma cells (da Costa et al., 2009). We first confirmed that transient overexpression of wild-type parkin (Figure 4A) upregulates PS1 expression (+42.1% ± 4.5%, n = 7, P < 0.05, Figure 4B), mRNA levels (+151.9% ± 8.5%, n = 6, P < 0.001, Figure 4C), and PS1 promoter activity (+112% ± 28%, n = 8, P < 0.01, Figure 4D), and decreases PS2 expression (+26.1% ± 7.1%, n = 10, P < 0.05, Figure 4B), mRNA levels (−67.6% ± 16.6%, n = 6, P < 0.01, Figure 4D), and promoter activity (−57.1% ± 13.1%, n = 8, P < 0.01, Figure 4F). Interestingly, all mutations abolish parkin-associated effects on PS1 and PS2 proteins and mRNA levels and promoter activities (Figure 4A–F). This set of data was reinforced by rescue experiments in which parkin null fibroblasts were transiently transfected with either wild-type or mutated parkin cDNA. As expected, wild-type parkin increases or decreases PS1 or PS2 promoter activity, respectively, while mutant parkin constructs were totally inactive (Figure 4G and H). It is important to note that, in agreement with the above-described data, the same parkin mutations fully abolish caspase-3 activation and p53 repression in transiently

Figure 1 Parkin controls presenilins expression, promoter activity, and mRNA levels ex vivo and in mouse brains. (A–F) TSM neurons were stably transfected with pcDNA3 (Mock) or human parkin cDNA (Pk) and then PS1 and PS2 expression (A and D), promoter activity (B and E), and mRNA levels (C and F) were analyzed as described in Methods. The bars represent the means ± SEM of 3–4 independent experiments performed in duplicate and are expressed as percent of control mock-transfected cells. Actin was used as protein and mRNA loading control, while β-galactosidase measurement was used to normalize luciferase-based promoter assays as described in Methods. (G–I) Wild-type (Pk+) or parkin null (Pk−) immortalized mouse embryonic fibroblasts (MEF) were analyzed for PS1 and PS2 expressions (G), promoter activities (H), and mRNA levels (I) as described in Methods. The bars represent the means ± SEM of 3 or 4 independent experiments performed in duplicate and are expressed as percent of control wild-type fibroblasts. (J) and (K) PS1 and PS2 protein (J) and mRNA (K) levels normalized in function of actin protein and mRNA levels were determined in homogenates derived from wild-type (Pk+) and knockout (Pk−) mouse brains. The bars represent the means ± SEM of 4–8 brains. Expressions of proteins appearing in panels A, G, and J were measured and visualized as described in Methods.
transfected SH-SY5Y cells and fibroblasts and unlike wild-type parkin, failed to rescue the defect of parkin-associated function observed in parkin null fibroblasts (da Costa et al., 2009). Overall, these data demonstrate that parkin-associated control of PS1 and PS2 is not cell specific and is not artifactual related to cell transformation or engineering and, more importantly, that this function remains independent of its ubiquitin-ligase activity.

**Parkin-mediated control of PS1 and PS2 involves neither p53 nor PS1 and PS2 interplay**

We have examined whether parkin-associated control of PS transcription could be mediated by the tumor suppressor p53. This hypothesis was based on several considerations. First, several studies documented the ability of p53 to repress PS1 promoter transactivation (Pastoric and Das, 2000) and expression (Roperch et al., 1998). Second, our previous study indicated that parkin transcriptionally represses p53 (da Costa et al., 2009). Third, the present data show that parkin increases PS1 expression and promoter transactivation (see Figure 1A, B, G and H). Fourth, both parkin-mediated regulation of p53 and PS1 remain independent of its ubiquitin-ligase activity (see Figure 4A and B). It was, therefore, conceivable that parkin could indirectly activate PS1 transcription via p53 repression. This hypothesis was examined by comparing parkin cDNA transient transfection (parkin expression is shown in Supplementary Figure S3, box Figure 5, panels A, C and panels B, D) in two cellular models in which the p19Arf1 gene alone or in combination with

![Figure 2](https://academic.oup.com/jmcb/article-abstract/5/2/132/970695/2?12370695) 

**Figure 2** Parkin regulates PS-dependent γ-secretase activity. (A–F) In vitro γ-secretase-mediated production of Aβ (A, C, and E) and AICD (B, D, and F) by reconstituted membranes prepared from TSM1 neurons stably overexpressing parkin (A and B), immortalized (C and D) or primary (E and F) MEFs invalidated for the parkin gene were measured as described in Methods. Each panel contains a representative gel and a histogram in which bars correspond to the means ± SEM of 3 independent experiments performed in duplicate (B and D) and triplicate (A, C, E, and F). The absence of parkin in immortalized MEFs is illustrated in Figure 1G and the lack of parkin in primary MEFs is shown in Supplementary Figure S3 (see box Figure 2, panels E, F). The expression of recombinant substrate C100 is shown in Supplementary Figure S3 (see box Figure 2, panels A–F).

![Figure 3](https://academic.oup.com/jmcb/article-abstract/5/2/132/970695/2?12370695) 

**Figure 3** Parkin regulates presenilin-mediated control of cell death. (A–D) Determination of caspase-3 activity as described in Methods in TSM1 neurons stably overexpressing either an empty vector (Mock) or wild-type parkin (Pk) (A), in MEF cells invalidated (Pk–/–) or not (Pk+/+) for the parkin gene (B), in HEK293 overexpressing an empty vector (Mock), wild-type PS1 (PS1wt), or wild-type PS2 (PS2wt) (C), and in wild-type MEF cells (PS1+/+ PS2+/+) or MEF invalidated for the PSEN1 (PS1–/– PS2+/+), PSEN2 (PS1+/+ PS2–/–), or for both PSEN1 and PSEN2 (PS1–/– PS2–/–) genes (D). Cells were treated for 15 h with vehicle (DMSO, −) or with TPS (1 μM, +). The bars correspond to the means ± SEM of 3 independent experiments performed in duplicate. (E) HEK293 overexpressing either PS1 or PS2 was transiently transfected with a control empty vector (EV) or wild-type parkin (Pk) cDNA and analyzed for caspase-3 activity at 24 h after transfection as described in Methods. The bars express the percentage of control EV-transfected mock cells and are the means ± SEM of 3 independent experiments performed in triplicate.

*p* < 0.05, **p** < 0.01, ***p*** < 0.001. Expression of PS1 and PS2 in HEK293 cells used in C and E is shown in Supplementary Figure S3 (see box Figure 3, panels C, E). PS expression in cells invalidated for one or both PS appearing in D is shown in Supplementary Figure S3 (see box Figure 3, panel D). Expression of wild-type HA-parkin in E is shown in Supplementary Figure S3 (see box Figure 3, panel E).
bars) and PS$_2$ SEM of 3 transiently transfected with a control empty vector (EV, 2 EV-transfected p$_{19}^+$) or PS$_2$ (F) promoter/β-galactosidase constructs. Twenty-four hours after transfection, cells were harvested and examined for human PS$_2$ by da Costa et al. (2009). The bars represent the means ± SEM of 3–4 independent experiments performed in duplicate. (G and H) Parkin null fibroblasts were transiently transfected with the indicated wild-type or mutated parkin cDNA in combination with a mix of PS$_1$ (G) or PS$_2$ (H) promoter/β-galactosidase constructs and then luciferase activity was measured as described in Methods. *P < 0.05, **P < 0.01, ***P < 0.001.

![Figure 4](https://example.com/fig4.png)

**Figure 4** Parkin control presenilins independent of its ubiquitin-ligase activity. (A–D) SH-SY5Y human neuroblastoma cells were transiently transfected with the indicated cDNAs (EV: empty pRES2-EGFP vector, Pk: wild-type parkin, 441: C441R Pk mutation, 418: C418R Pk mutation, 161: K161N Pk mutation, and 256: R256C Pk mutation) alone (A–D) or in conjunction with a mix of PS$_1$ (E) or PS$_2$ (F) promoter/β-galactosidase constructs. We measured GFP expression in order to assess that similar transfection efficiencies were achieved. Note that parkin variation in expression is thus to be accounted for variable metabolic stability as already documented by da Costa et al. (2009) and not due to variable transfection efficiencies. The bars are expressed as percent of control EV-transfected cells and represent the means ± SEM of 3–4 independent experiments performed in duplicate. (G and H) Parkin null fibroblasts were transiently transfected with the indicated wild-type or mutated parkin cDNA in combination with a mix of PS$_1$ (G) or PS$_2$ (H) promoter/β-galactosidase constructs and then luciferase activity was measured as described in Methods. *P < 0.05, **P < 0.01, ***P < 0.001.

![Figure 5](https://example.com/fig5.png)

**Figure 5** Parkin-mediated control of presenilins is independent of p53 and is not affected by PS1 and PS2 functional interplay. (A–D) p19$^{Arf-/-}$ and p19$^{Arf-/-}$/p53$^{-/-}$ fibroblasts were transiently transfected with an empty vector (EV) or with wild-type parkin (Pk) cDNAs with (A and C) or without (B and D) corresponding PS-luciferase promoter construct. Twenty-four hours after transfection the cells were examined for PS$_1$ (A) and PS$_2$ (C) promoter activities and PS$_1$ (B) and PS$_2$ (D) mRNA levels as described in Methods. The bars are expressed as percent of control EV-transfected p19$^{Arf-/-}$/ cells and are the means ± SEM of 3 independent experiments performed in duplicate or triplicate. *P < 0.05, **P < 0.01, ***P < 0.001; ns, not statistically significant. (E–G) MEF invalidated for either PSEN2 (PS2$^{-/-}$) or PSEN1 (PS1$^{-/-}$) genes were transiently transfected with a control empty vector (EV, −) or wild-type parkin (Pk, +) and then analyzed for their endogenous PS$_1$ (grey bars) and PS$_2$ (black bars) protein expressions (E and F) and mRNA levels (G) as described in Methods. The bars represent the means ± SEM of 3 independent experiments performed in duplicate. *P < 0.05, **P < 0.01. (H) MEF cells invalidated for both PSEN2 and PSEN1 genes (PS1$^{-/-}$/PS2$^{-/-}$) were transiently co-transfected with a control empty vector (EV, −) or wild-type parkin (+) together with PS$_1$ (grey bars) or PS$_2$ (black bars) promoter/β-galactosidase cDNA constructs and analyzed for their luciferase activities as described in Methods. The bars represent the means ± SEM of 3 independent experiments performed in duplicate. **P < 0.01, ***P < 0.001. The characterization of p53 null cells used in A–D is shown in Supplementary Figure S3 (see box Figure 5, panels A–D). Parkin expression in promoter (A and C) or mRNA (B and D) analyses are shown in Supplementary Figure S3 (see box Figure 5, middle and lower panels).
the p53 gene p19<sup>Adv-1</sup>p53<sup>−/−</sup> (see p53 expression in Supplementary Figure S3, box Figure 5, panels A–D) had been invalidated (Kamijo et al., 1997). First, we confirmed that the absence of endogenous p53 increases PS1 promoter activity and mRNA levels (compare EV bars in p19<sup>Adv-1</sup>p53<sup>−/−</sup> and p19<sup>Adv-1</sup>p53<sup>+/−</sup> in Figure 5A and B). In p19<sup>Adv-1</sup>p53<sup>−/−</sup>, parkin clearly increases PS1 promoter activity and mRNA levels (Figure 5A and B, compare EV and Pk bars) and reduces PS2 transcription and mRNA levels (Figure 5C and D, compare EV and Pk bars). Interestingly, parkin still increases PS1 promoter transactivation (+63.8% ± 13.7%, n = 9, P < 0.001, Figure 5A) and mRNA levels (+135.8% ± 23%, n = 6, P < 0.001, Figure 5B) in p19<sup>Adv-1</sup>p53<sup>−/−</sup> fibroblasts, while parkin-mediated reduction of PS2 promoter activity (−52.9% ± 11.5%, n = 9, P < 0.01, Figure 5C) and mRNA levels (−55.7% ± 25.7%, n = 6, P < 0.05, Figure 5D) still occurs in p53-depleted fibroblasts. This set of data allowed us to conclude that parkin-mediated control of PS transactivation remains independent of p53.

The regulation of PS1 and PS2 by parkin could have been complicated by the intimate cross-talk between PS1 and PS2. Thus, we and others (Alves da Costa et al., 2002, 2006; Kang et al., 2005) demonstrated that PS1 could control the expression of PS2 and vice versa as confirmed in Figure 5E (compare CPS1 and CPS2 expressions in PS2<sup>−/−</sup> and PS1<sup>−/−</sup> cells, respectively). This led us to compare the ability of parkin to control the transcription of PS1 or PS2 in cells lacking its congener (see PS expressions in Supplementary Figure S3, box Figure 3, panel D). Clearly, parkin overexpression (Figure 5E, bottom panel) still upregulates endogenous PS1 expression (+47.6% ± 16.4%, n = 6, P < 0.05, compare grey bars in Figure 5F) and mRNA levels (+56.2% ± 15.8%, n = 6, P < 0.01, compare grey bars in Figure 5G) in PS2<sup>−/−</sup> cells, while it decreases endogenous PS2 protein (−27.5% ± 7.7%, n = 6, P < 0.05, compare black bars in Figure 5F) and mRNA (−51.1% ± 8.2%, n = 6, P < 0.01, compare black bars in Figure 5G) levels in PS1<sup>−/−</sup> fibroblasts. Furthermore, transient co-transfection of PS1 or PS2 promoters with parkin cDNA (see parkin expression in Figure 5H, upper panel) still increases PS1 promoter activity (+169.4% ± 32.1%, n = 6, P < 0.01, Figure 5H, grey bars) and reduces PS2 promoter activity (−44.3% ± 5.3%, n = 6, P < 0.001, Figure 5H, black bars) in fibroblasts lacking both PS1 and PS2. The overall set of data indicates that the protective phenotype of parkin could indeed be mediated by a p53-independent control of PS1 and PS2, and does not involve PS1/PS2 functional interplay.

**PS1 and PS2 are parkin transcriptional targets**

Since parkin-mediated control of PS transcription was unrelated to its ubiquitin ligase activity and independent of p53, this led us to envision PS1 and PS2 as putative new parkin transcriptional targets. We have delineated the promoter region where parkin could have bound to PS promoters. The deletion of a −297/−11 nucleotide-long fragment in the 5′ region of the PS1 promoter abolishes parkin-mediated promoter activation (compare constructs 1 and 2 in Figure 6A), while the removal of the +353/+590 region of PS2 promoter fully abolishes the inhibitory effect of parkin on PS2 promoter transactivation (compare constructs 7 and 8 in Figure 6B). Chromatin immunoprecipitation assays (ChIP) performed with specific primers covering the promoter regions identified above demonstrate the physical interaction of parkin with PS promoters. Thus, the immunoprecipitation of parkin yields specific amplification products with both PS1 and PS2 primers that are drastically enhanced in parkin overexpression conditions (compare IP lanes in Pk and EV conditions in Figure 6C). Of most importance, ChIP analyses indicate that the PCR product of IPed material obtained with PS1 and PS2 primers in wild-type fibroblasts was not amplified in parkin null fibroblasts (Figure 6D). Altogether, this indicates that both endogenous and overexpressed parkin physically interact with PS1 and PS2 promoters.

**Identification of a parkin consensus binding sequence**

By means of an in silico approach, we have identified a common GCCGGAG heptamer motif in PS1, PS2, and p53 promoter sequences (Figure 7A). Interestingly, this putative consensus sequence was exactly located in the identified regions involved in parkin-induced promoter modulations (see Figure 6A and B and da Costa et al., 2009) and in the PS1, PS2, and p53 PCR products of amplification detected in ChIP experiments described in Figure 6C and D and in da Costa et al. (2009). In order to validate the functionality of this consensus sequence, we have deleted it from PS1, PS2, and p53 full-length promoter sequences. Of most interest, the ablation of this sequence in p53 (Figure 7B), PS1 (Figure 7C), and PS2 (Figure 7D) completely abolishes parkin-mediated transcriptional regulation of these promoters (Figure 7B–D) (see parkin expression in Supplementary Figure S3, box Figure 7, panels B–D).

In order to substantiate the validity of this consensus sequence, we have produced recombinant wild-type and mutated parkin (see parkin expressions in Supplementary Figure S3, box Figure 7, panels E–I) and examined their ability to bind biotinylated probes corresponding to PS1 and PS2 sequences encompassing this consensus domain. Wild-type recombinant parkin interacts with both PS1 and PS2 probes (Figure 7E and F). This labeling was fully abolished by excess of specific probe (lanes s in Figure 7E and F) but was not competed by an excess of unspecific probe (lanes ns in Figure 7E and F). Interestingly, recombinant mutant parkins were unable to interact with both PS1 and PS2 sequences (Figure 7E and F). Finally, the interaction of recombinant parkin with PS1, PS2, and p53 probes in which the consensus sequence had been mutated was drastically reduced, though not fully abolished (Figure 7G–I, compare ct and ct mut lanes). Overall, these data clearly identify a putative consensus parkin-binding sequence that likely underlies its transcriptional activity. Parkin controls PS promoter modulation via its RING1-IBR-RING2 domain

We have previously established that parkin physically interacts with p53 via its RING1 domain (da Costa et al., 2009). We, therefore, assessed the contribution of the RING1-IBR-RING2 domain of parkin that has been suggested as a putative transactivation domain (Morett and Bork, 1999). Interestingly, these domains expressed alone or as multidomain constructs activate PS1 promoter and reduce PS2 promoter transactivation (Supplementary Figure S2A and B). Although these domains were all found active on PS promoters, it should be noted that the most potent constructs encode IBR and RING2 and that the IBR-RING2 construct appeared to be the most potent modulator of both PS1 and
PS2 promoter transactivation. Overall, this indicates that parkin-associated modulation of PS1 and PS2 promoters requires a multidomain centered around its RING1-IBR-RING2 as it was described for parkin-p53 interaction (da Costa et al., 2009).

**Discussion**

The canonical function of parkin has been established as a ubiquitin ligase and several studies supported the idea that a loss of function of parkin could be tightly linked to an accumulation of potentially toxic proteins due to a malfunction of the proteasomal machinery (Corti et al., 2011). Besides this degradation-oriented function, contributions of parkin in the control of various cell signaling pathways via protein mono-ubiquitylation have also been delineated (Corti et al., 2011). Thus, parkin can modulate the PI(3)-kinase-Akt-dependent EGF signaling via the mono-ubiquitylation of the endocytic adaptor protein Eps-15 (Fallon et al., 2006); parkin mediates Pick-1 mono-ubiquitylation, thereby abrogating Pick-1-linked control acid-sensing channels (Joeh et al., 2007) and parkin-associated multi mono-ubiquitylation of the chaperone protein Hsp70 accounts for the repression of c-Jun NH2-terminal kinase signaling cascade (Liu et al., 2008).

Besides its involvement in protein degradation and cell signaling, parkin could also contribute to additional functions. Thus, parkin regulates the gene expression of several proteins, including CHK, EIF4EBP1, GADD45A, and PTPN-5 (Unschuld et al., 2006), represses monoamine oxidases A and B expressions in dopaminergic neuroblastoma cells (Jiang et al., 2006), and enhances transcription and replication of mitochondrial DNA (Kuroda et al., 2006). Furthermore, drosophila parkin reduces JNK signaling via the decrease of bsk transcription (Hwang et al., 2010). However, the delineation of the mechanistic aspects accounting for parkin-mediated control of gene expression remained unclear, and one could postulate either a direct role of parkin as transcriptional modulator of promoter transactivation or alternatively, an indirect ligase-dependent or independent effect due to the protein fate control of cellular intermediate transcription factors regulating the expression of the above-cited genes.

We recently established a totally novel ubiquitin-ligase independent function of parkin as a transcriptional factor (da Costa et al., 2009). This study identified the pro-apoptotic tumor suppressor p53 as the first transcriptional target of parkin. Thus, we showed that parkin downregulates p53 protein levels.
Transcriptional control of presenilin 1 and 2 by parkin

and activity, and we established that wild-type but not mutants (either ligase-defective or not) of parkin physically interacts with p53 via its Ring1 domain and thereby, acted as a direct genuine transcriptional repressor of p53 (da Costa et al., 2009). These data highlighted the fact that the transcription factor function of parkin could be at the origin of several of its phenotypes yet to be mechanistically unraveled.

It had been described that parkin could interfere with Aβ regulation, the culprit of AD pathology, but the mechanism of such modulation remained unknown (Burns et al., 2009). Aβ derives from the proteolytic cleavage of a transmembrane precursor protein (β-amyloid precursor protein) by the two proteases β- and γ-secretases, which act sequentially (Checler, 1995). PS1 has been characterized as the catalytic core of a high-molecular-weight complex bearing γ-secretase activity responsible for productions of Aβ and its C-terminal counterpart AICD (Wolfe et al., 2002). Interestingly, it has been reported that PS1 transcriptional regulation indeed influences γ-secretase activity (Lee and Das, 2010).

Here we document PS1 and PS2 as novel transcriptional targets of parkin. This demonstration stands in four lines of independent data. First, both endogenous and overexpressed parkin increase/reduce PS1/PS2 promoter transactivation and protein and mRNA expression in distinct cell types including neurons, ex vivo as well as in vivo, in mouse brains. Second, parkin-mediated control of PS1/2 transcription is abolished by mutations inactivating or not its ligase activity, a feature reminiscent of that observed for well-characterized parkin-mediated control of p53 transcription (da Costa et al., 2009). Third, ChIP analyses demonstrate that endogenous and overexpressed parkin physically interacts with PS1 and PS2 promoters. Fourth, electrophoretic mobility shift assays (EMSA) demonstrated that recombinant wild-type but not mutated parkin physically interacts with probes covering the promoter domains of PS1 and PS2 involved in parkin-mediated transcriptional modulation.

It is of importance to underline that we have shown that parkin-mediated control of PS1 and PS2 has functional consequences. Thus, parkin enhances PS1-associated γ-secretase-mediated productions of Aβ. Aβ production/secretion could be affected by several paradigms, including the cellular levels of its precursor βAPP (β-amyloid precursor protein), the regulation of its secretion within the constitutive secretory pathway, and the overall activity of the γ-secretase complex that is mainly driven by PS1 expression levels (Takasugi et al., 2003). It was, therefore, important to analyze the effect of parkin on PS1-mediated γ-secretase by means of an in vitro assay based on monitoring Aβ and AICD productions from an exogenous recombinant substrate. Our data show that parkin transcriptional regulation of PS1 directly impacts on γ-secretase activity and by consequence, on the production of Aβ and AICD. Our study also demonstrates that parkin-mediated control of PS2 promoter transactivation influences its caspase-3 associated control of cell death (Alves da Costa et al., 2002).

An important aspect of our study concerns the fact that parkin-associated control of both PS1 and PS2 promoter transactivation remains p53 independent. This is interesting because p53, which behaves as a parkin transcriptional target (da Costa et al., 2009), can modulate the expression of several members of the γ-secretase complex (Dunys et al., 2009; Checler et al., 2010) as well as caspase-3-dependent cell death (Cregan et al., 1999). Therefore, our study suggests that parkin can influence
Aβ production either by acting directly on the PS1 promoter or by indirectly controlling γ-secretase by a p53-dependent mechanism. On the other hand, parkin-mediated control of cell death can occur by directly repressing PS2 (present study) or by down-regulating p53 (Alves da Costa et al., 2002). The multiplicity of parkin-driven pathways likely allows cells to face distinct situations and adapt its response according to the cellular insults.

Is parkin-mediated modulation of PS1 potentially important in AD context? It has been documented that parkin-like immunoreactivity co-localizes with Aβ-related vascular lesions in AD-affected brains (Witte et al., 2009). Furthermore, in sporadic AD, there is little evidence of enhanced production of Aβ but it is rather admitted that Aβ degradation could be impaired at late stages of the disease. Accordingly, parkin can trigger ubiquitylation and degradation of intracellular Aβ42 (Burns et al., 2008; Belyaev et al., 2010). This could appear paradoxical with respect to our demonstration that parkin upregulates PS1-dependent γ-secretase activity. However, it should be kept in mind that AICD, the production of which derives from PS1-dependent γ-secretase activity (Passer et al., 2000; Pardossi-Piquard and Checler, 2011), could contribute to the pathology by enhancing the transcription of GSK3β (Ryan and Pimplikar, 2005), nephrilysin (Pardossi-Piquard et al., 2005; 2006; Belyaev et al., 2009), and p53 (Alves da Costa et al., 2006; Ozaki et al., 2006), thereby triggering Tau hyperphosphorylation (Sergeant et al., 2008; Iqlbal et al., 2010; Medina and Avila, 2010), augmentation of Aβ42/40 ratio (Kuperstein et al., 2010), and p53-dependent cell death (Kitamura et al., 1997; Garcia-Ospina et al., 2003; Ohyagi, 2008), which are all enhanced in AD pathology. Therefore, it is tempting to speculate that the increase of parkin expression in sporadic AD (Witte et al., 2009) and its mediated enhancement of γ-secretase activity (present study) could potentially contribute, at least in part, to AD neurodegenerative process. One should not, however, rule out the possibility that parkin-mediated enhancement of PS1 activity could influence the processing of many of its documented substrates (De Strooper and Annaert, 2010) that, besides βAPP, could contribute to either AD-related or unrelated functions.

Our study has two other fundamental implications besides the fact that it strengthens our initial description of a role of transcription factor for parkin (da Costa et al., 2009; da Costa and Checler, 2010) by identifying two novels targets. First, it shows that parkin could act either as a transcriptional activator or as a repressor. Such a duality has been previously reported for other transcription factors such as p53 (Rinn and Huarte, 2011) and might be due to motif context, competition with other actors of the transcriptional machinery, or recruitment of chromatin repressor and/or activator factors (Rinn and Huarte, 2011). Putative additional co-factors or above-described altered mechanisms have yet to be identified for parkin-associated transcriptional function. It remains that this differential effect as a repressor of two pro-apoptotic proteins (i.e. p53 and PS2) and as an activator of a protective effector (PS1) also suggests a coordinated function of parkin in the modulation of cellular cell death. This cellular mechanism of control could complete in an additive or synergistic manner, the ligase-dependent function of parkin in the control of the fate of other negative modulators of cell death. Second, in support of the previous point, we have delineated a common GCCGGAG heptamer motif in the promoters of PS1, PS2, and p53, the deletion of which abolishes parkin-mediated promoter transactivation.

It should be noted here that the heptamer sequence described above likely does not account for the whole structural features underlying parkin-associated transcriptional function. Thus, although wild-type but not mutated recombinant parkin physically interact with probes bearing this heptamer domain, wild-type (but not mutated) parkin also interacts, although with a drastically reduced efficiency, with identical probes in which the heptamer sequence had been mutated. This could mean that either the identified heptamer is part, but not all, of the consensus sequence or additional co-factors present in cells and organisms contribute to the modulation of the affinity of parkin for this sequence. The latter hypothesis is likely, since it would be difficult to envision that both activation and repression effects of parkin are due to the sole interaction with this unique domain without associated modulators. Accordingly, it should be emphasized that the RING1-IBR-RING2 domain of parkin responsible for PS transcriptional modulation is apparently wider than the sole RING1 domain sufficient to mediate parkin-associated repression of p53 (da Costa et al., 2009). The above set of data underlies the potential ‘plasticity’ in the structural requirements for parkin transcriptional function and that such a duality may depend upon the cellular context and molecular environment ultimately conditioning its phenotype. This also likely explains why mutations located outside the RING1-IBR-RING2 domain abolish parkin-mediated control of PS promoter transactivation. Overall, our work should allows the identification of novel transcriptional targets of parkin that would explain some of its known functions mechanistically yet unsolved or unravel additional putative cell-specific functions.

Materials and methods
Promoter activity assays and mapping of parkin functional domain
p53, PS1, and PS2 promoter-luciferase constructs have been previously described (Bienz-Tadmor et al., 1985; Mitsuda et al., 1997; Ounallah-Saad et al., 2009). Promoter constructs deleted of the parkin potential binding site as well as PS1 promoter truncation constructs have been obtained as described above. Promoter-luciferase activities were measured after co-transfection of 0.5–1 μg of the above cDNAs and 0.2–0.5 μg of β-galactosidase cDNA in order to normalize transfection efficiencies. For the experiences concerning the mapping of parkin domain involved in presenilins promoter regulation, we have co-transfected SH-SY5Y cells with 1 μg of the full-length PS promoters-luciferase constructs, 0.5 μg of the β-galactosidase, and 1 μg of either full-length or distinct HA-tagged parkin deleted constructs corresponding to its previously described Ring 1, IBR, and Ring 2 domains. The truncated HA-tagged parkin constructs have been graciously provided by Dr T. Dawson and have been described in Chung et al. (2001).

Caspase-3 activity measurements
Cells were grown in 6-well plates and incubated without or with TPS (1 μM) for 15 h. Caspase-3-like enzymatic activity was
measured fluorimetrically by means of a microtiter plate reader (Labsystems, Fisher Bioblock Scientific) as extensively detailed (Alves da Costa et al., 2000).

**In vitro γ-secretase assay**

The production of the C100-Flag fragment, the preparation of the 'solubilized' membranes fraction, and the in vitro γ-secretase assay were described in detail in a previous study (Sevalle et al., 2009). Briefly, 'solubilized membranes' (5 μl) from TSM1 neurons expressing empty vector or wild-type HA-tagged parkin or from wild-type and parkin-deleted fibroblasts, diluted in sodium citrate buffer, were incubated with 10 μl of reaction buffer containing recombinant C100-Flag (50 μg/ml). The 20 μl resulting reaction mixes were incubated over constant agitation for 16 h at 37°C. Samples were then supplemented with 2× Tris–Tricine loading buffer, boiled for 5 min, and subjected to western blot using 16% SDS-PAGE Tris–Tricine gel and then Aβ and AICD were analyzed with antibodies described below.

**Western blot analysis**

Parkin knockout murine brains have been recently described (Goldberg et al., 2003). Cellular and mouse brain extracted proteins (50 μg) were separated on 12% SDS-PAGE gels and wet-transferred to Hybond-C (Amer sham Life Science) membranes. Immunoblotting was performed by means of either anti-N-terminal PS1 and anti-PS2 loop antibodies (a gracious gift of Dr G. Thinakaran) or anti-PS1 (Ab111) and anti-PS2 (Ab333) antibodies (provided by Drs Araki and Tabira), mouse monoclonal anti-parkin (MAB5512, Chemicon), and anti-actin antibodies (Sigma). For Aβ detection, we used the 6E10 monoclonal antibody (AbCam) at a 1/1000 dilution. AICD immunoreactivity was analyzed by using the anti-FLAG M2 monoclonal antibody (Sigma) at a 1/1000 dilution. Protein immunoreactivities were revealed with either an anti-rabbit peroxidase or an anti-mouse peroxidase (Jackson Immunoresearch) by the electrochemiluminescence method (Roche Diagnostics S.A.S). Chemiluminescence was recorded using a luminescence image analyzer LAS-3000 (Raytest), and quantification of images was performed using the FUJI FILM Multi Gauge/AIDA image analyzer software.

**Primary cultured neurons**

Primary neuron cultures were prepared from gestational day 14 fetal of C57BL/6 fetuses (Charles Rivers) by a slightly modified procedure of Vincent et al. (1996). Briefly, brain hemispheres were placed in the plating Neurobasal media (Life Technologies) supplemented with 2% B-27, 0.5 mM L-glutamine, and 50 U/ml penicillin/streptomycin and dissociated by mechanical trituration. Cells were centrifuged at 200 × g for 5 min and resuspended in the plating media. Three million cells were plated on 6-well plates coated with poly-L-ornithine and were maintained in the plating media at 37°C. Three million cells were plated on 6200 cm² flasks and centrifuged at 1000 g for 5 min, and subjected to western blot using 16% SDS-PAGE Tris–Tricine gel and then Aβ and AICD were analyzed with antibodies described below.

**Supplementary material**

Supplementary material is available at Journal of Molecular Cell Biology online.

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