## The Normal Parkin Sequence

The recruitment of parkin to mitochondria in response to mitochondrial membrane depolarization[1] is an important aspect of parkin biology that has attracted extensive research.[2] We found that parkin with the sequence (AB009973.1) reported in the original discovery of the gene[3] was not recruited to mitochondria following carbonyl cyanide m-chlorophenylhydrazone (CCCP) treatment, while parkin with the current GenBank Reference Sequence (NM_004562.2) was robustly recruited to mitochondria under the same condition. The only difference between the 2 sequences is nucleotide 768, which is C in AB009973.1 (encoding for proline at amino acid position 223) and T in NM_004562.2 (for serine at 223). As shown in Figure 1, Hela cells were transfected with FLAG-tagged parkin (with either P or S at amino acid 223). Cells were treated without or with CCCP (10 μM) for 3 hours and immunostained for FLAG and Tom20. CCCP induced a strong mitochondrial recruitment of FLAG-parkin (223S), but no significant recruitment of FLAG-parkin (223P). This was confirmed using green fluorescent protein (GFP)-parkin with either P or S at position 223 (Fig. 1C). Similar results were also obtained in COS7 or HEK293 cells using FLAG-, Myc-, HA- or GFP-tagged human parkin with S or P at amino acid 223 (data not shown).

To test which version of parkin sequence is correct, we analyzed genomic DNA of 2102 individuals from Europe, China, and the United States. None of them had C at this position; all had T. Briefly, genomic DNA from 231 normal subjects and 294 Parkinson's disease patients in China, as well as genomic DNA from 19 normal subjects and 18 Parkinson's disease patients in the United States were amplified by polymerase chain reaction (PCR) using 2 primers in the introns flanking exon 6 of parkin (CTTGTCCAAAGAGATTGTTTACTGT GG and GGCTCGTGTGGCAGAACAATATTGGG). The PCR product (318 bp) should contain a single BsrI site (CCAGT) if AB009973.1 is correct. None of these samples could be cut by BsrI, while a positive control PCR product containing a BsrI site was cut at the same condition. Using a Basic Local Alignment Search Tool (BLAST) search of DNA sequences from 1540 individuals (1290 Parkinson's disease patients and 250 healthy controls), mostly of French origin (~70%), we found that all of them had T at position 768; none had C. Thus, the amino acid sequence reported in AB009973.1 (Pro223) is wrong; it should be serine instead, as reported in NM_004562.2. This important error needs to be corrected, as it may affect other aspects of parkin biology, in addition to mitochondrial recruitment.

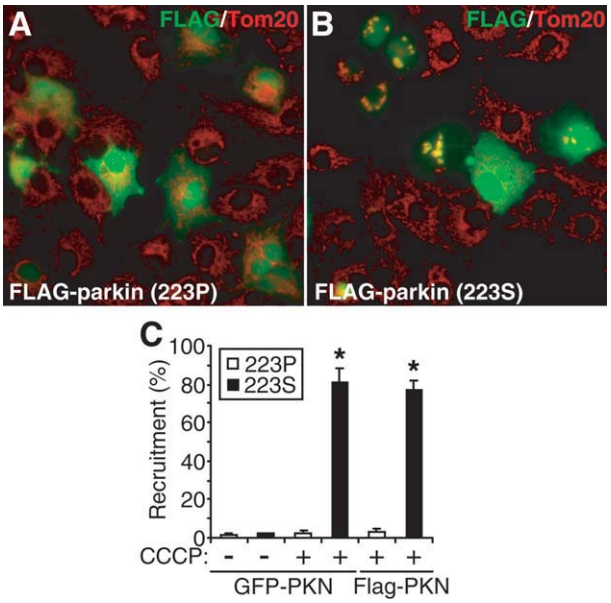


**FIG. 1.** Parkin sequence error has important functional consequence. Hela cells transfected with (**A**) FLAG-parkin (223P) or (**B**) FLAG-parkin (223S) were treated with CCCP (10 μM for 3 hours) and co-stained for FLAG and the mitochondria marker Tom20. **C**: Percentage of cells with mitochondrial recruitment of transfected GFP-parkin or FLAG-parkin with 223P or 223S. *$P < .001$, versus the preceding bar, n = 8–10 views from 4 independent experiments, with each view having 70 to 80 cells.

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