Responsible region of Parkin for mitochondrial targeting

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Abstract

We previously reported that Parkin shows different intracellular localization in cellular driving states. Parkin is strictly localized in the mitochondrial matrix in a proliferating state and is rapidly released to cytosol at differentiated or quiescent states. We also found that Parkin enhances mitochondrial transcription and replication in vitro and in vivo. We found a novel protein associated with Parkin[6]. In this study, we aimed to find the domain of Parkin that is essential for mitochondrial targeting using various deletion-mutants of Parkin. Most mutants were localized in mitochondria stained with CMXRos. Only two mutants, Parkin 295-415 and Parkin 77-310, lacking both C- and N- terminals were detected outside the mitochondria. This suggests the N-terminal region or the C-terminal region is responsible for mitochondrial localization.

Introduction

Parkinson's disease (PD) is a prevalent age-associated progressive neurodegenerative movement disorder primarily characterized by the death of nigrostriatal dopaminergic neurons and the presence of intracytoplasmic proteinaceous inclusions, termed Lewy bodies. Although PD is a sporadic disorder of unclear etiology, recent studies have demonstrated the importance of genetic contributions to PD that may provide insight into the mechanistic details of disease pathogenesis. Mutation of the gene encoding Parkin (PARK2) plays a major etiopathogenic role in autosomal recessive juvenile parkinsonism [1]. PARK2 contains RING finger motifs, and functions as a ubiquitin–protein ligase for protein degradation [2,3]. We previously reported that Parkin shows different intracellular localization in cellular driving states[4]. Parkin is strictly localized in the mitochondrial matrix in a proliferating state and is rapidly released to cytosol at differentiated or quiescent states. We also found that Parkin enhances mitochondrial transcription and replication in vitro and in vivo [4]. These findings include some problems to be resolved. Most mitochondrial proteins have a specific N-terminal pre-sequence that allows recognition by the mitochondrial import apparatus and subsequent import into mitochondria [5]. The fact that parkin lacks the mitochondrial targeting sequences encouraged us to hypothesize that an unidentified protein transports parkin to mitochondria, and we found a novel protein associated with Parkin [6]. In this study, we aimed to find the domain of Parkin that is essential for mitochondrial targeting using various deletion-mutants of Parkin.

Materials and Methods

Cell culture

COS-1 (a cell line derived from kidney cells) was cultured at 37°C (5% CO_2) in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) heat-incubated fetal bovine serum (FBS).

Mutagenesis and cell transfection

Full-length parkin cDNA and a deletion mutant lacking exons 3 and 4 were constructed as previously described [7]. In addition, we cloned mutants by using appropriate primer pairs and the QuikChange site-directed mutagenesis kit (Stratagene). In the present study, 12 mutants in total were constructed (Figure 1). Full length or deletion-mutant parkin cDNA was sub-cloned into pEGFP-C2 (Clontech, CA, USA), Cells were transfected using EffecteneTM Transfection Reagent (Qiagen, CA, USA) or LipofectamineTM 2000 (Invitrogen Corp.). Intracellular localization was analyzed using Mitotracker Red CMXRos (100 nM, for staining of mitochondria, Molecular Probes).

Results

We examined the intracellular expression of GFP-tagged parkin mutants to identify the responsible region for mitochondrial targeting. Surprisingly, most mutants were localized in mitochondria stained with CMXRos (Figure 2). Only two mutants, Parkin$^{295\text{-}415}$ and Parkin$^{77\text{-}310}$, lacking both C- and N- terminals were detected outside the mitochondria.

Discussion

We found that Parkin is expressed ubiquitously in a variety of cells, localized exclusively to mitochondria in a proliferating condition [8]. This was in sharp contrast to the results of previous studies, showing that parkin was not detected in mitochondria but in the cytosol, the trans-Golgi network and the synaptic vesicles in human and non-human brain tissues [9,10]. This discrepancy may be due to the difference in the cellular proliferative states because neural cells are completely differentiated in adult tissues, whereas the cells used in this study were grown under a proliferating condition. In cultured cells, intracellular localization of parkin has been controversial [11-13]. Parkin is abundant in the mitochondrial fraction and is located on the outer mitochondrial membrane [13]. In our previous results [8], Parkin was preferentially localized in the mitochondria of cultured cells. The mitochondria with large amounts of Parkin showed preserved membrane potentials even during treatment with carbonyl cyanide m-chlorophenylhydrazone. We found a novel protein named Klokin 1 that transports Parkin to the mitochondria. Klokin 1 was localized to the mitochondria and enhanced mitochondrial expression of Parkin. Klokin 1 enhanced cell viability in Parkin-silenced cells. Klokin 1 expression was enhanced in the brains of Parkin-deficient mice but not in an autopsied PARK2 brain. Our findings indicate that mitochondrial Parkin prevents mitochondrial depolarization and that Klokin 1 may compensate for Parkin deficiency.

In the present study, most mutants were localized in mitochondria stained with CMXRos. Only two mutants, Parkin295-415 and Parkin77-310, lacking both C- and N-terminals were detected outside the mitochondria. This suggests the N-terminal region or C-terminal region is responsible for mitochondrial localization. Considering that Parkin lacks mitochondrial targeting sequence, the two terminal regions may be bound to associated proteins including Klokin 1.

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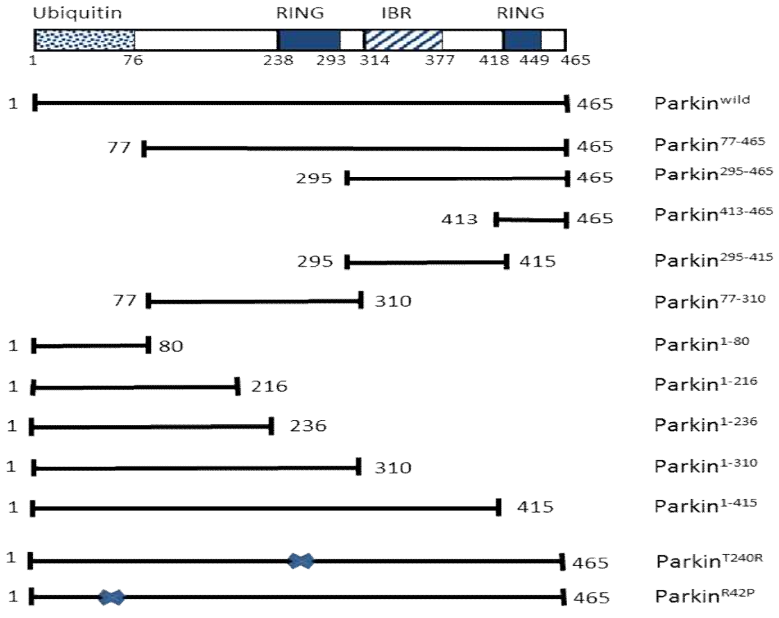

Figure 1. Constructs of Parkin deletion mutants.

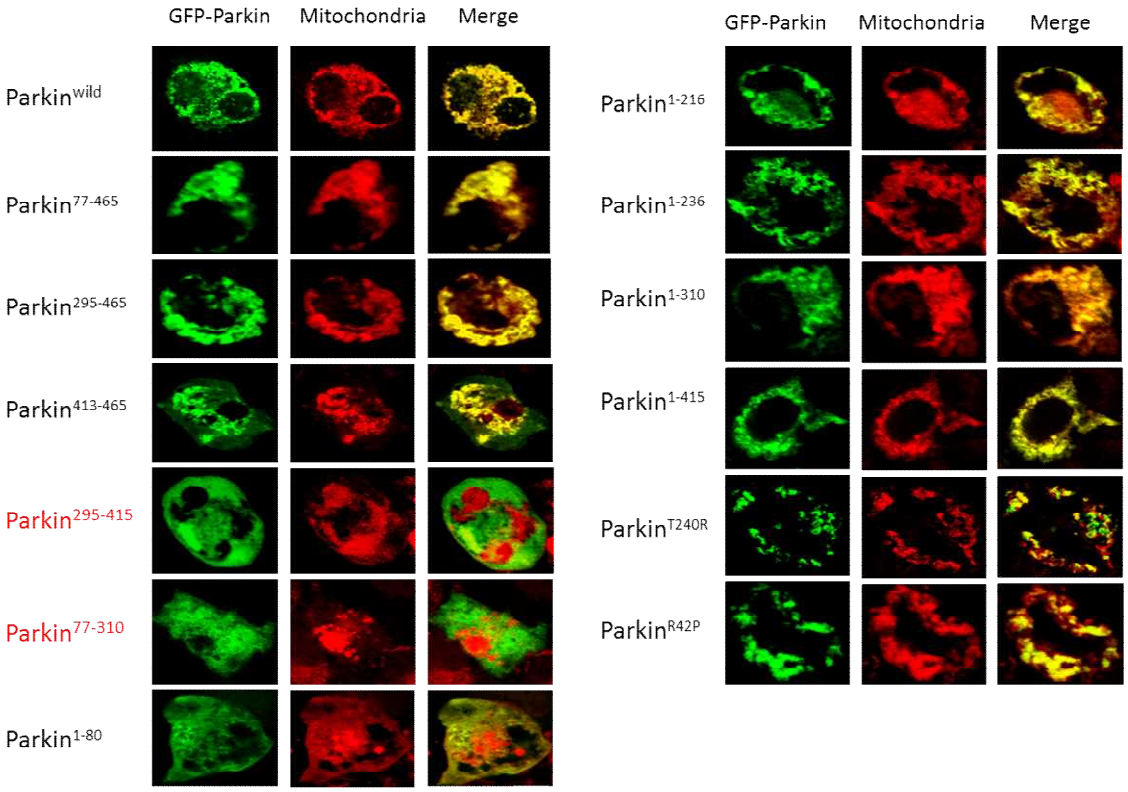

Figure 2. Intracellular localization of GFP-tagged Parkin wild type and various deletion mutants.