## A novel transactivation domain in parkin

Although Parkinson's disease (PD) is the second-most-common neurodegenerative disease, the causes of PD are still largely unknown. PD is a multifactorial syndrome that, in many cases, is not inherited[1]. Nevertheless, Polymeropoulos *et al.*[2] recently found mutations in the gene that encodes α-synuclein, a protein present in presynaptic vesicles, in autosomal dominant PD. In addition, Leroy and co-workers[3] have identified mutations in another gene that has a ubiquitin-like domain in familial PD.

Recently, Kitada and co-workers[4] reported a gene defect that causes Autosomal Recessive Juvenile Parkinsonism (AR-JP), a hereditary form of PD. The function of the respective gene product, parkin, is unknown, but the protein contains an N-terminal ubiquitin-like domain and a C-terminal ring finger[4]. In the course of our systematic study of disease-associated genes[5,6], we elucidated the modular architecture of parkin and several homologous proteins. We report the identification of a novel domain, the IBR (for in between ring figures) domain, and predict that parkin possesses DNA-binding and transcriptional activities.

Sequence-database searches with parkin (excluding the ubiquitin-like domain), using the BLAST tools[7], revealed a large family of mostly uncharacterized proteins that all share significant sequence similarity over 200 residues (see Figs 1 and 2; for some members, $E < 10^{-10}$). For only two of these proteins is functional information available: (1) the *Drosophila melanogaster* ariadne protein, which is involved in axonal path-finding in the

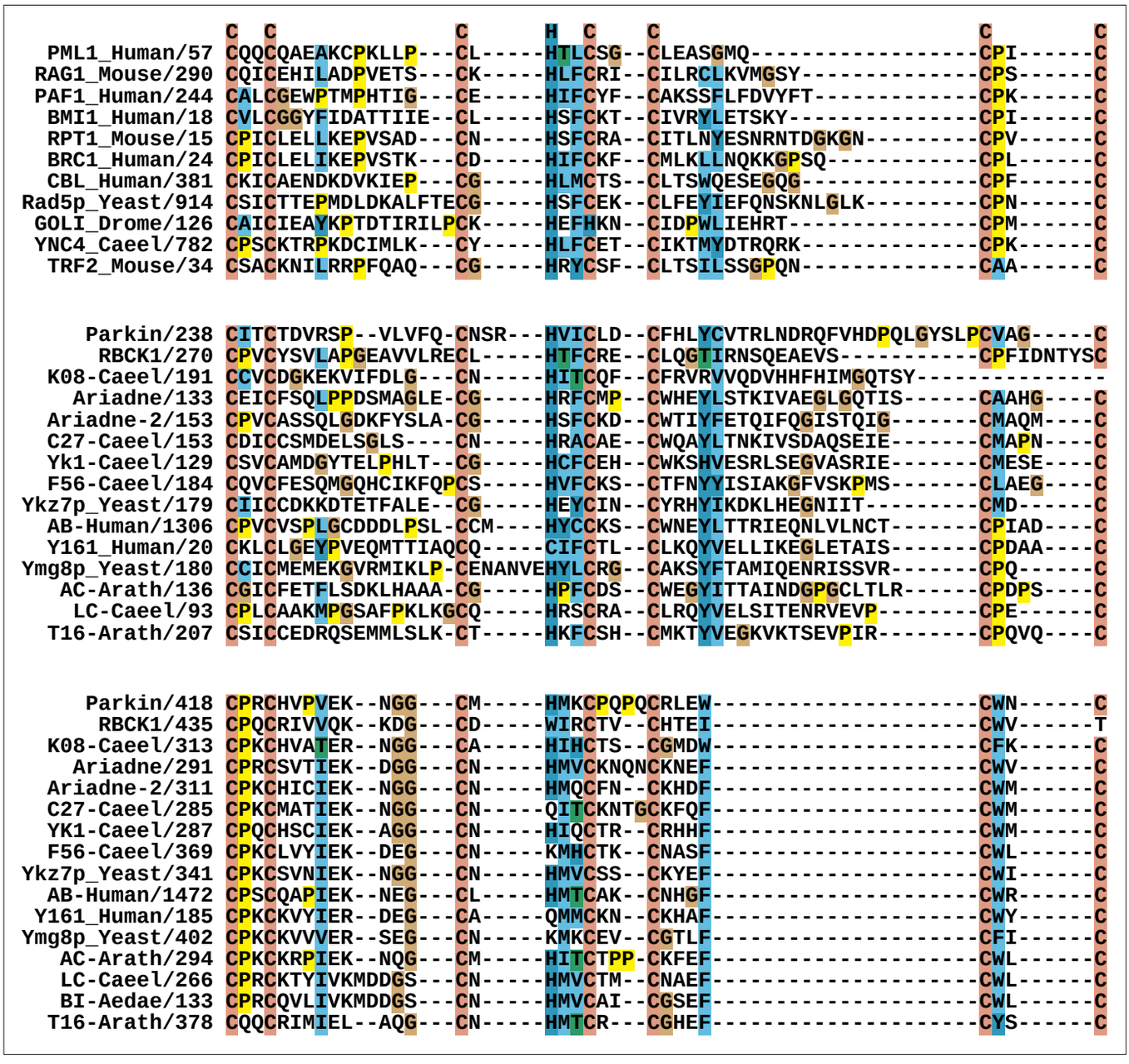


**Figure 1**

Alignment of selected ring-finger proteins (upper panel) with the first (central panel) and second (lower panel) ring fingers of IBR-family members. Numbers indicate the positions of the domains in the respective sequences. Amino acid residues are colored according to the Clustal X residue code[14]. PSI-BLAST searches[7] and profiles[11] derived from the first and the second ring fingers identify several classical ring-finger proteins. Furthermore, in reciprocal searches with classical ring fingers, parkin-related proteins scored above known ring-finger proteins. Aedae, *Aedes aegypti*; Arath, *Arabidopsis thaliana*; Caeel, *Caenorhabditis elegans*; Drome, *Drosophila melanogaster*.

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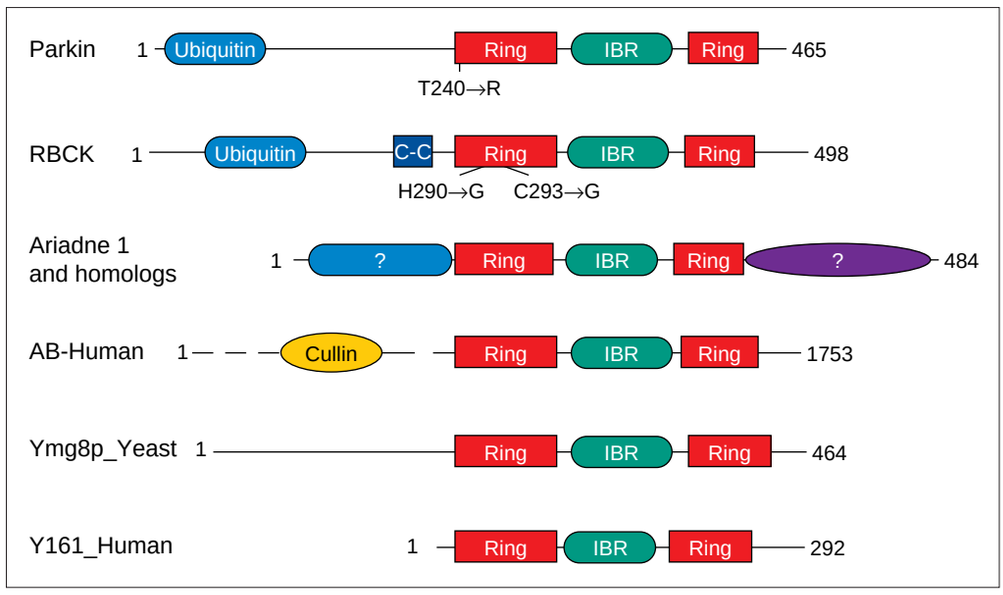


**Figure 2**

Modular architecture of parkin and selected homologs. Database accession numbers are given in parentheses. Parkin (AB009973), its close homolog the *Caenorhabditis elegans* (Caeel) protein K08-Caeel (Z81586) and RBCK1 (U48248) each contain a ubiquitin-like domain. Ariadne (X98309), ariadne 2 (AJ010169), Ykz7p_Yeast (Z28242), C27-Caeel (AF003137), an *Arabidopsis thaliana* (Arath) protein, AC-Arath (AC004512), and YK1-Caeel (U61944) have the same domain architecture. Other proteins of the family are AB-Human (AB014608), Ymg8p_Yeast (Z38114), Y161_Human (D79983), F56-Caeel (U13644), YK1-Caeel (U61944), LC-Caeel (U80847), BI-Aedae, (U84248) and T16-Arath (AL024486). Mutations in parkin that lead to Autosomal Recessive Juvenile Parkinsonism (AR-JP) and mutations in the RBCK1 gene that abolish transcriptional activation are indicated.

central nervous system (M. Aguilera *et al.*, unpublished; EMBL accession number X98309); and (2) mouse RBCK1 [for RBCC (Ring finger, B box, coiled coil) protein interacting with PKC1], which Tokunaga *et al.*[8] identified in a yeast two-hybrid screen, using protein kinase Cβ1 as bait.

Tokunaga *et al.*[8] classified RBCK1 as an unusual member of the RBCC family[9]. In contrast to the coiled coil of RBCC proteins, the coiled-coil region of RBCK1 was predicted to precede the ring finger. In addition, Tokunaga *et al.*[8] proposed that a second B box is present in RBCK1.

Despite the highly significant sequence similarity shared by RBCK1 and parkin, the ring fingers predicted by Tokunaga *et al.*[8] and Kitada and co-workers[4] do not correspond to each other. Closer examination of the discrepancy revealed that RBCK1 has a modular architecture that is completely different from that proposed by Tokunaga *et al.*[8] and that relates it to parkin and several other homologous proteins (Fig. 2).

We were unable to confirm the existence of the two proposed B boxes in RBCK1. The conserved histidine residues typical of the B-box domain[10] are not present in either of the two cysteine-rich regions of RBCK1. Neither profile[11] nor PSI-BLAST[7] searches detected any of the family members when starting with B-box sequences. By contrast, our searches identified the proposed C-terminal B box as a ring finger, which confirms the existence of the reported ring finger in the equivalent position in parkin[4]. Thus, the ring finger in parkin corresponds to the proposed B box in RBCK1. The (first) ring finger of RBCK1 matches the ring consensus (Fig. 1), and PSI-BLAST searches with this sequence identified classical ring-finger proteins, yielding significant *E* values of $10^{-5}$. We

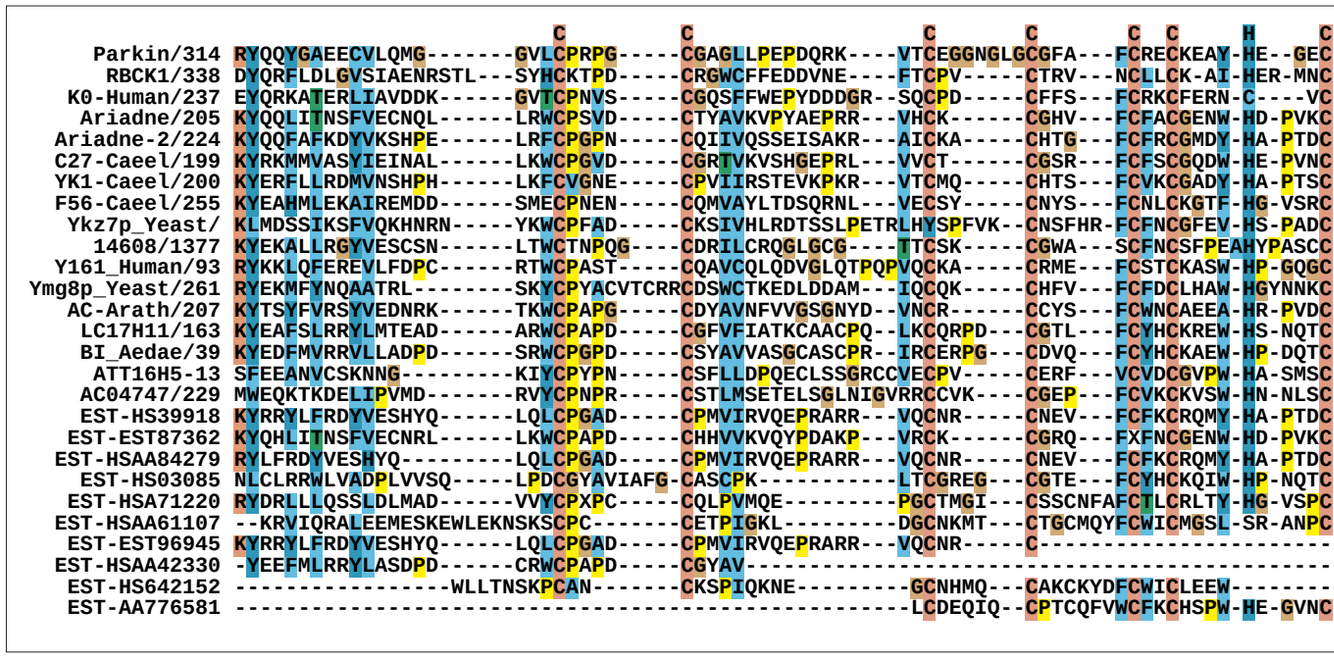


**Figure 3**

Alignment of selected IBR fingers. Twelve distinct human expressed-sequence tags (ESTs) that contain the IBR finger are also aligned and indicate that a large family of paralogs is present in humans. Amino acid residues are colored according to the Clustal X residue code[14]. Aedae, *Aedes aegypti*; Arath, *Arabidopsis thaliana*; Caeel, *Caenorhabditis elegans*; Drome, *Drosophila melanogaster*.

conclude that the entire family contains two C3HC4 ring fingers, which are separated by a new class of putative metal-binding (zinc-finger-like) cysteine-rich region of the type C6HC. We have named this region the IBR finger (Fig. 3).

To our knowledge, this is the first report of two ring fingers being present within one protein[9]. We also identified a ubiquitin-like domain in the N-terminal part of RBCK1 (Fig. 1; residues 50–120; *E* values for ubiquitins were $<10^{-4}$ in BLAST searches with the N-terminal part of RBCK1). Given that RBCK1 and parkin have the same overall modular architecture, we anticipate that the proteins share functional similarities.

Gel-mobility-shift experiments have shown that RBCK1 is a DNA-binding protein[8]. Moreover, *in vivo* transcription assays that employed constructs in which RBCK1 was fused to the DNA-binding domain of Gal4 showed that the ring–IBR–ring region alone is able to induce gene expression[12]. Mutations in two conserved residues of the first ring finger abolish transcriptional activation[12] (see Fig. 1). The importance of the first ring finger of parkin is supported by recent studies that identified a single Thr240→Arg point mutation in patients who had AR-JP (Ref. 13). This mutation maps to a region adjacent to the conserved second cysteine residue of the ring finger (Fig. 1) and places a positively charged residue in a position in which only hydrophobic residues are normally found (Fig. 2). Other disease-causing mutations also affect the ring–IBR–ring arrangement[4,13]. The considerable number, and species distribution, of proteins that contain this modular architecture [it is present in plants, animals and fungi, and there are at least 12 distinct human paralogs (Fig. 3)] suggests that the ring–IBR–ring arrangement is widely used to regulate gene expression.

## Acknowledgements

E. M. thanks CONACYT and DGAPA for support, and UNAM for a sabbatical leave. E. M. is an Alexander von Humboldt Fellow. P. B. is supported by the BMBF and DFG.

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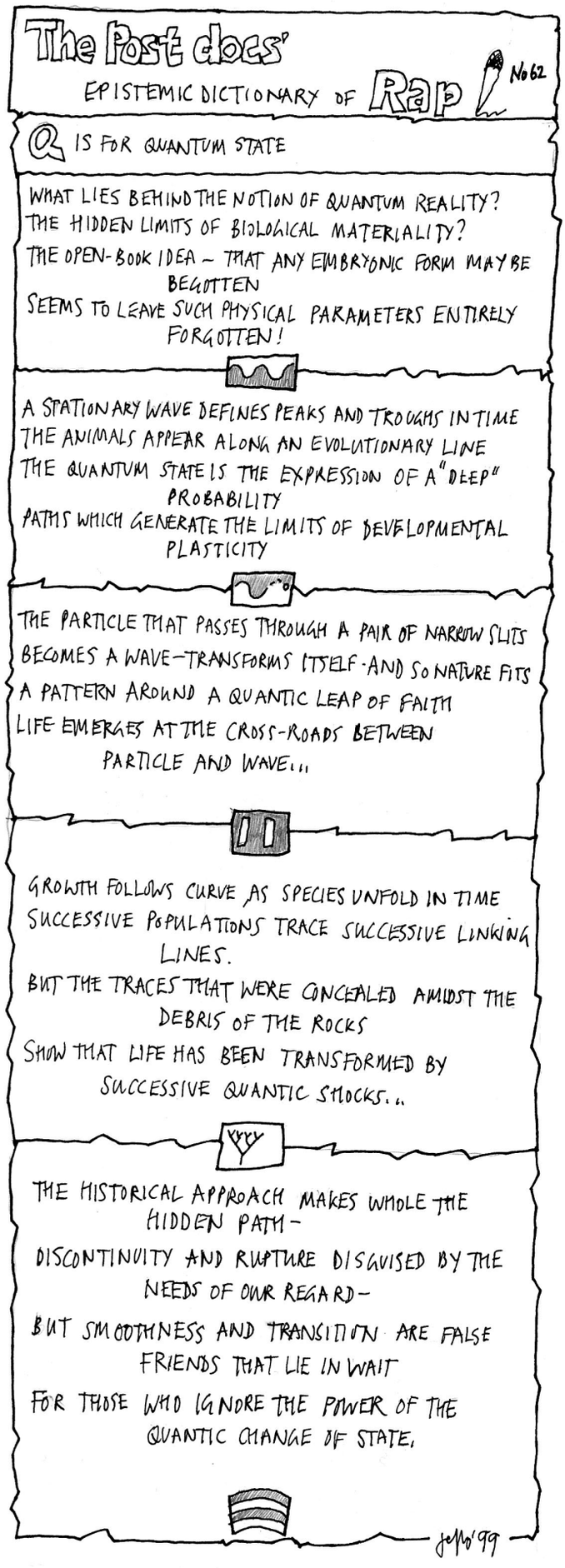


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