COMMENTARY

Pathway for Parkinson disease

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Parkinson disease (PD) is a common neurodegenerative disease with unknown etiology. PD is commonly referred to as a "motor disease," reflecting its clinical symptoms, including resting tremors of extremities, muscular rigidity, shuffling gait, stoop posture, and bradykinesia (1). The underlying pathology of PD is progressive neuronal loss, particularly in the substantia nigra pars compacta (SNc), and the presence of abnormal protein-rich aggregates—known as Lewy bodies—in the remaining neurons (2). The loss of dopaminergic neurons in the SNc, leading to dopamine deficiency, is believed to be responsible for the motor and nonmotor symptoms, including orthostatic hypotension, mood disorders, sleep disorder, and loss of sense of smell. Dopamine replacement therapy provides symptomatic relief, but disease progression continues unabated. Therefore, there is a need for understanding the mechanisms of PD to aid the development of more effective therapeutics. This endeavor is fueled by genetic discoveries in the past two decades that identified a number of genes associated with rare inheritable PD, including SNCA, Parkin/PRKN, DJ-1/Park7, PINK1, and LRRK2 (leucine-rich repeat kinase 2) (3). The push now is to understand the functions of these gene products and the biological pathways in which they operate. In PNAS, Beilina et al. identify a number of LRRK2 interactors whose jobs are to process endocytosed vesicles, thereby associating LRRK2 with the vesicle endocytosis pathways (4).

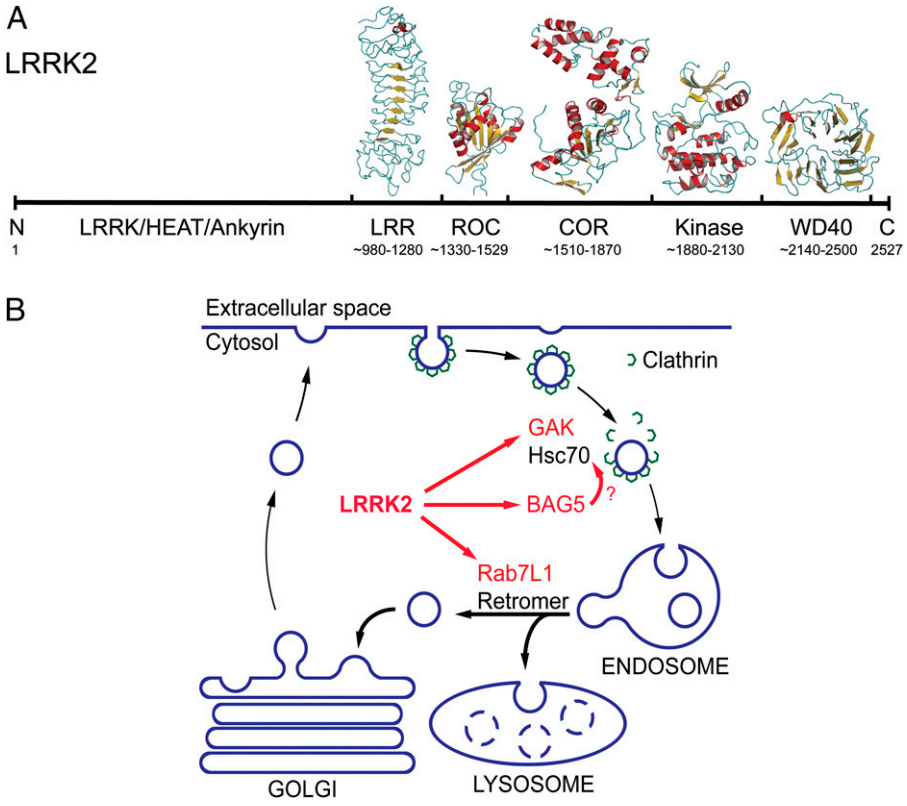

Fig. 1. (*A*) Cartoon presentation of LRRK2 showing multidomain structure. Termini and predicted domains labeled along approximate domain boundaries. Cartoon of domain structures were rendered using coordinates of homology models built based on other proteins with known structures. The N-terminus region preceding the LRR domain consists of sequence similarity to more than one additional protein–protein interaction domain, including HEAT [Huntingtin, elongation factor 3 (EF3), protein phosphatase 2A (PP2A), and the yeast kinase TOR1] repeats and Ankyrin repeats; however, their boundaries are more difficult to approximate. (*B*) A simplified drawing of the vesicular endocytosis pathway showing GAK/Hsc70 involvement in uncoating clathrin-coated vesicles. BAG5 might also be involved in the same process by interacting with Hsc70, and Rab7L1 functions in the retromer sorting process. LRRK2 interacts with all three proteins (GAK, BAG5, and Rab7L1); thus, the process of vesicular endocytosis, sorting, recycling, and degradation might be important in the pathogenesis of PD.

Mutation in LRRK2 is a common cause of PD (5). LRRK2 is a large (2,527 amino acids) multidomain protein consisting of at least five putative domains (Fig. 1*A*) (6). The catalytic region consists of three of those domains: A Ras-like GTPase domain called Ras of complex proteins (ROC) followed by a domain called C-terminal of ROC (COR), which is in turn followed by a kinase domain (Kinase). The mechanism of LRRK2 in PD pathogenesis remains unclear; however, the most common disease-associated mutation in LRRK2, G2019S, shows higher kinase activity than wild-type. Therefore, overactivation of LRRK2 kinase activity might be associated with disease pathogenesis (7). The tandem ROC-COR-Kinase arrangement suggests that their activities might be coupled such that the GTPase activity of ROC might modulate the kinase activity. Indeed, several studies have shown that GTP binding to the ROC domain regulates the activity of the Kinase (8, 9). Moreover, PD-associated mutation in the ROC domain (R1441C) has been shown to have higher kinase activity (10), thus suggesting that mutations in the ROC domain also up-regulate LRRK2's kinase activity.

Flanking the catalytic tridomain region, including about 1,300 residues upstream of ROC and about 400 residues downstream of the Kinase domain, are predicted to encode protein–protein interaction domains, including a leucine-rich repeat domain (LRR), a seven–β-propeller structure called WD40, and likely other protein–protein domains upstream of the LRR domain. LRRK2 is equipped to perform multiple biochemical functions; in my group, we refer to it as the "Swiss Army Knife" protein. The presence of multiple protein–protein interaction domains in LRRK2

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See companion article on page 2626.

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suggests that it likely interacts with a number of different proteins and perhaps functions in a multiprotein complex. Therefore, knowing the identity of its interactors could reveal the cellular pathways in which it functions. LRRK2 has been proposed to interact with a number of different proteins, including the 14-3-3 family of proteins (11), microtubules (12), dishevelled proteins (13), carboxyl terminus of Hsp70-interacting protein (CHIP) (14), and mitogen-activated protein kinase kinase 6 (MKK6) (15). These proteins have broad functions; thus, whether they cooperate with LRRK2 in a common pathway is unclear.

To perform an unbiased screen for proteins that interact with LRRK2, Beilina et al. (4) used protein–protein arrays with immobilized LRRK2. The authors found a number of different interactors, including cyclin G-associated kinase (GAK), three members of the Bcl-2–associated anthanogene domain cochaperones (BAG), and RAB7 member RAS oncogene family-like1 (Rab7L1). An exciting aspect of Beilina et al.'s findings is that these LRRK2 interactors could function in the same cellular pathway, namely the vesicle trafficking process (Fig. 1*B*).

Among the interactors identified by Beilina et al. (4) are the following. GAK consists of multiple domains, including a clathrin-binding domain, a kinase domain, and a J-domain that interacts with heat-shock cognate 70 (Hsc70) (16). GAK and Hsc70 together power the uncoating of endocytosed clathrin-coated vesicles (17). Interestingly, GAK has been implicated as a risk factor for PD in genome-wide association studies (18). BAG5 is a member of the BAG-family of proteins known as cochaperones that interact with Hsc70/Hsp70 and modulate their functions. BAG proteins have been shown to interact with the ATPase domain of Hsc70 and accelerate the exchange of ATP for ADP, acting as a nucleotide-exchange factor. BAG5 might modulate the activity of Hsc70/GAK complex by binding to the ATPase domain of Hsc70. Interestingly, BAG5 has been previously shown to inhibit another PD-associated protein, Parkin, and enhances dopaminergic neuron degeneration (19). The third LRRK2 interactor identified by Beilina et al., Rab7L1, has been shown to be a risk factor for PD and was subsequently shown to interact with LRRK2 (20). PD-associated mutations in LRRK2 or Rab7L1 led to endolysomal and Golgi apparatus sorting defects and deficiency of the VPS35 component of the retromer complex (20). The retromer sorting process is downstream of clathrin-uncoating in clathrin-mediated endocytosis (Fig. 1*B*); thus, all three LRRK2 interactors identified by Beilina et al. (4) fit in this common pathway.

Beilina et al. identify a number of LRRK2 interactors whose jobs are to process endocytosed vesicles, thereby associating LRRK2 with the vesicle endocytosis pathways.

It remains to be determined whether this pathway plays a major role in the pathogenesis of PD; however, numerous lines of evidence that have emerged over the past two decades have implicated various areas of vesicular trafficking, retromer sorting, lysosomal degradation, and autophagy in PD, as well as other neurodegenerative diseases, including Alzheimer's disease, thus lending support for a common pathway leading to PD. An exciting aspect of Beilina et al.'s work (4) is that it identifies specific players that potentially unify these pathways. It was hoped that studying the mechanisms of rare familial PD might provide insights into the etiology of sporadic PD. The data by Beilina et al. support this notion in that the interactors of LRRK2, which is associated with familial PD, overlaps with genes identified in genome-wide association studies.

The mechanism of PD was virtually a "black-box" only two decades ago. We now know a handful of genes directly involved in pathogenesis and the cellular pathways in which they function are beginning to unravel. Much more work remains to be done to understand the disease at depths required for translation into therapeutic strategies. For example: How do LRRK2 and other PD-associated proteins perturb vesicular processing and other cellular processes? What is the molecular mechanisms of toxicity? What are the functions of these PD-associated proteins and how are they altered by the disease-associated point mutations? The report by Beilina et al. (4) paves the way for detailed molecular and quantitative investigations required to answer these questions.

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