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The Roco protein family: a functional perspective

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ABSTRACT **In this review, we discuss the evolutionary, biochemical, and functional data available for members of the Roco protein family. They are characterized by having a conserved supradomain that contains a Ras-like GTPase domain, called Roc, and a characteristic COR (C-terminal of Roc) domain. A kinase domain and diverse regulatory and protein–protein interaction domains are also often found in Roco proteins. First detected in the slime mold *Dictyostelium discoideum*, they have a broad phylogenetic range, being present in both prokaryotes and eukaryotes. The functions of these proteins are diverse. The best understood are *Dictyostelium* Rocos, which are involved in cell division, chemotaxis, and development. However, this family has received extensive attention because mutations in one of the human Roco genes (*LRRK2*) cause familial Parkinson disease. Other human Rocos are involved in epilepsy and cancer. Biochemical data suggest that Roc domains are capable of activating kinase domains intramolecularly. Interestingly, some of the dominant, disease-causing mutations in both the GTPase and kinase domains of *LRRK2* increase kinase activity. Thus, Roco proteins may act as stand-alone transduction units, performing roles that were thought so far to require multiple proteins, as occur in the Ras transduction pathway.—Marín, I., van Egmond, W. N., van Haastert, P. J. M. The Roco protein family: a functional perspective. *FASEB J.* 22, 3103–3110 (2008)**

Key Words: LRRK2 · GbpC · GTPase · kinase · signaling · Parkinson disease.

The discovery of the *Dictyostelium* cyclic guanosine monosphate (cGMP) -binding protein GbpC soon after the genome sequence of this model organism became available marked a new research area for cell biologists and biochemists. GbpC was the first described member of the Roco protein family, a group of complex proteins found in many prokaryotes and eukaryotes. Although the initial description of the Roco family of proteins did not draw much attention in the field, this rapidly changed when dominant mutations in the human Roco gene *LRRK2* were found to be involved in the development of Parkinson disease (PD). Extensive research was then started to elucidate the functions and biochemistry of LRRK2 and other Roco proteins. We review here all the available information for the genes and proteins of the Roco family. The focus of the review is on the functions and biochemistry of Roco proteins in *Dictyostelium* and humans. The fact that at least 2 human genes, *LRRK2* and *DAPK1*, are involved in neurodegenerative diseases (such as PD, Alzheimer disease, and epilepsy) and in cancer is also discussed in detail.

CHARACTERIZATION OF THE ROCO FAMILY

First discoveries of Roco family genes

Two animal Roco genes, *DAPK1* and *MFHAS1* (formerly called "MASL1"), were discovered long ago (1, 2). However, the first data suggesting that a family with a broad evolutionary range existed was obtained by Goldberg *et al.* (3) when they characterized the *gbpC* gene in the slime mold *Dictyostelium discoideum.* This gene was found in bioinformatic searches for cyclic nucleotide binding proteins. Goldberg *et al.* (3) found that human and *Drosophila* proteins similar to GbpC were present in the databases. Two related genes were found in *D. discoideum* within a few months of that first publication. Abysalh *et al.* (4) characterized the *Pats1* gene in a screen for genes involved in cytokinesis, and Abe *et al.* (5) detected the *QkgA* gene (known today also as *Roco2*) in a bioinformatic search for receptor tyrosine kinases. Both genes encoded proteins with the leucine-rich repeats (LRRs) and the GTPase and kinase domains detected in GbpC, although their structures were somewhat simpler, because GbpC has a unique regulatory C-terminal region.

Definition of the Roco family: the Roc + COR supradomain

These isolated findings were put in a broader context when the Roco protein family was formally described by Bosgraaf and Van Haastert (6). These researchers showed that it was possible to define by bioinformatic analyses a group of genes characterized by encoding products with 2 peculiar domains. The first domain, which they called

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Roc (Ras of complex proteins), was a GTPase domain with high sequence similarity to Ras and other related small GTPases. The second was a novel domain that they described and called COR (C-terminal of Roc) because of its characteristic position in all Roco proteins. By sequence comparisons and phylogenetic analyses, they showed that Roc was clearly distinct from the rest of the Ras-like GTPase domains and that the Roc and COR domains always appeared together. Thus, the Roco family can be defined as the group of proteins which contain the Roc and COR domains, and the Roc + COR structure can be considered a "supradomain" (7). The initial article in which the Roco family was described also contained a first characterization of the domain composition of multiple Roco proteins, which were shown to be considerably diverse. Moreover, it was demonstrated that genes of this family had a wide evolutionary range, being present in both prokaryotes and eukaryotes. Four genes were detected in vertebrates, which are now called *LRRK1*, *LRRK2*, *DAPK1*, and *MFHAS1*. Strikingly, bioinformatic searches in the *Dictyostelium* genome yielded 11 Roco genes (6).

DIVERSITY OF THE ROCO FAMILY

The initial description of the Roco family opened the door to precise analyses of the evolution and function of Roco family genes and proteins. We now have a clear picture of their patterns of diversification, which may be useful to trace their functional similarities. **Figure 1** shows the main subfamilies of the Roco family and typical structures of members of each subfamily.

Prokaryotic Roco genes

As mentioned before, Bosgraaf and Van Haastert (6) found both prokaryotic and eukaryotic Roco genes. Prokaryotic Rocos appear as a monophyletic group in phylogenetic reconstructions (8). It is unclear at present

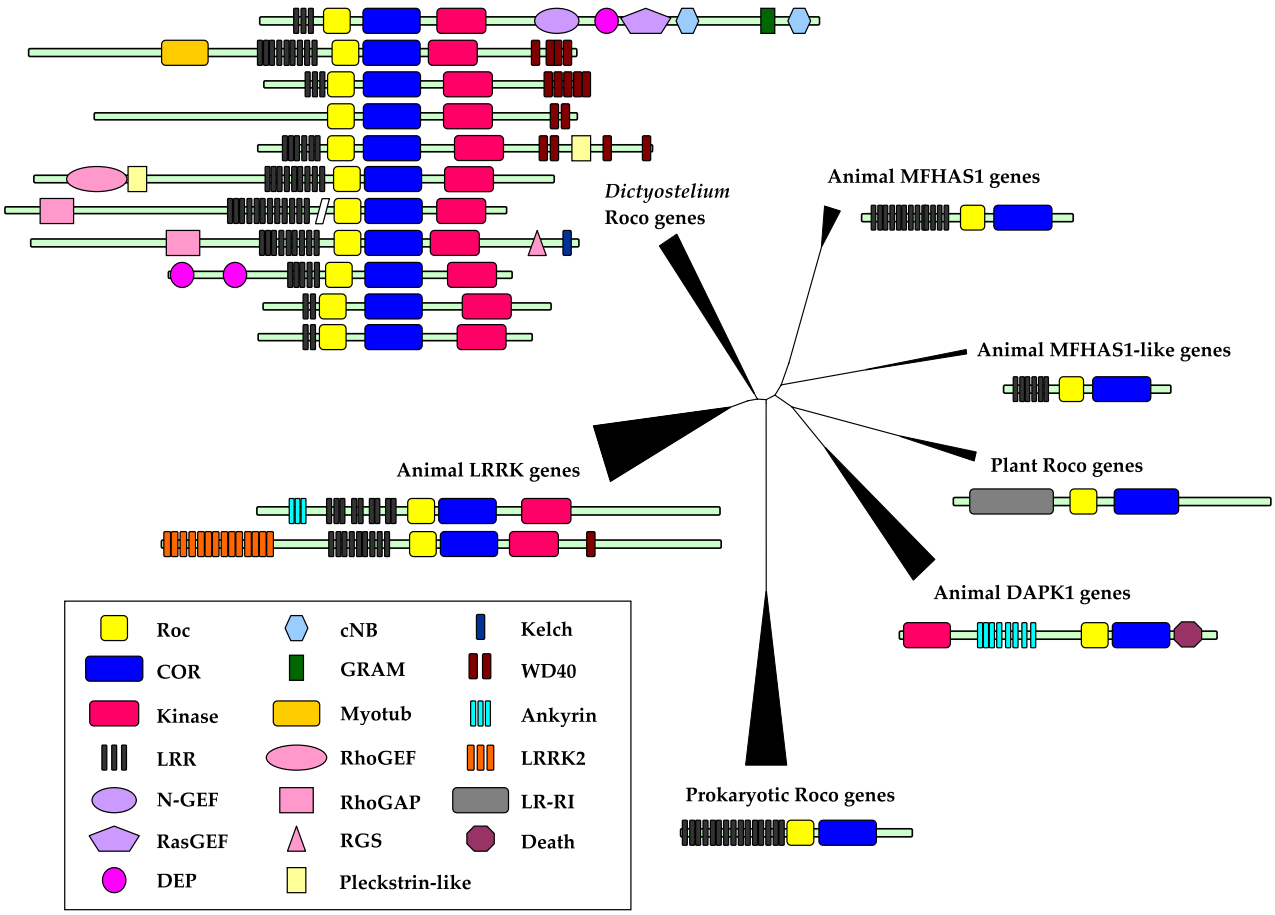

Figure 1. Structures of Roco proteins. The tree indicates the 7 main evolutionary branches of Roco genes (8). The 11 Roco proteins in *D. discoideum* are ordered according to their structural similarities, as deduced from InterProScan and comparative sequence analyses. From top to bottom: GbpC, Pats1, Roco4, Roco7, Roco6, Roco5, Roco9, Roco10, Roco8, Roco2/QkgA, and Roco11. To simplify the drawing, a small part of Roco9, without any domain, has been cut from its structure (white slash). Structures for all animal, plant, and prokaryotic orthologous proteins are very similar, so only a single example of each is shown. The animal proteins are all from *Homo sapiens* with the exception of MFHAS1-like, which is from *Gallus gallus*. Two LRRK proteins are shown, LRRK1 (top) and LRRK2 (bottom). The plant structure corresponds to *Arabidopsis thaliana* TORNADO1. Finally, the Roco prokaryotic protein shown is that of the cyanobacteria *Trichodesmium erythraeum*. Details for all domains but one can be found in structural databases such as Pfam; the LRRK2-specific domains (abbreviated as LRRK2) are described in ref. 8.

whether Roco genes originated in bacteria or if they were horizontally transferred to prokaryotes. However, the fact that they appear in both archaea and several distantly related eubacterial groups (cyanobacteria, proteobacteria, chlorobi, *etc.*) suggests an ancient origin. It is thus possible that eukaryotic Rocos have a symbiotic, mitochondrial origin (8). Some bacteria species have multiple Roco genes (*e.g.*, *Nostoc punctiforme*).

Eukaryotic Roco genes

Phylogenetic analyses indicated that Roco genes have independently diversified in animals, plants, and slime molds. In fact, although *D. discoideum* Rocos and LRRK vertebrate genes encode proteins with related kinase domains, these domains were probably acquired independently (8).

As we already indicated, 11 Roco genes can be identified in the *Dictyostelium* genome. Surprisingly, they are structurally more varied than the Rocos found in all the other species together (Fig. 1). Even so, they all contain closely related Roc, COR, and kinase domains (of the TKL group) and mostly also contain LRRs (Fig. 1) (6, 8, 9). There is good evidence for all *D. discoideum* genes to have evolved quite recently by gene duplications (8). Close inspection of the *Dictyostelium* Roco genes revealed that the kinase domain of Roco10 has most likely evolved into a catalytically inactive domain (9). The extended, peculiar C-terminal region of GbpC was probably acquired by the fusion of a Roco gene with a gene similar to *GbpD* (3).

Animals contain multiple Roco genes. Both protostomes (insects, nematodes) and deuterostomes (chordates, echinoderms) have *LRRK* and *DAPK1* genes, although *MFHAS1* genes were not found in protostomes (8). The diversification of Roco genes in animals is ancient: the cnidarian *Nematostella vectensis* contains 4 *LRRK* genes and a *MFHAS1* ortholog, although it apparently lacks a *DAPK1* ortholog (ref. 10 and unpublished results). It is significant that, although LRRK and DAPK1 proteins contain kinase domains (Fig. 1), they are clearly unrelated. LRRK kinase domains can be classified as belonging to the TKL group of kinases, although DAPK1 contains a domain typical of calcium/calmodulin-dependent kinases (6). MFHAS1 does not have a kinase domain. **Figure 2** shows the most parsimonious hypothesis deduced from the available data to explain the diversification of Roco genes in animals. Multiple duplications and losses have occurred.

Some plants contain 1 or 2 Roco genes. All known plant Roco genes are quite similar, encoding proteins that lack kinase domains (Fig. 1) (6). As discussed later, the only Roco gene in plants that has been studied in detail is called *TORNADO1* (*TRN1*, also known as *lopped*) (11–13). Plant Rocos are ancient. A likely ortholog of *TRN1* is found in the moss *Physcomitrella patens* (unpublished results). In dendrograms, they form a monophyletic group without clear relationship to any of the animal or slime mold Roco genes (8).

FUNCTIONS OF ROCO PROTEINS

The fact that mutations in Roco genes cause diverse human pathologies has led to a considerable interest in understanding the functions of their proteins. The presence of both GTPase and kinase domains in *D. discoideum* and some animal Roco proteins suggested, given its obvious parallelism with the Ras signal transduction system, a role in intracellular signaling.

Functions of *D. discoideum* Roco proteins

From a functional point of view, *D. discoideum* Rocos are the best understood. In particular, intensive research on *D. discoideum* GbpC has provided the most significant functional data in this species. GbpC is particularly interesting given its involvement in chemotaxis, a process for which *Dictyostelium* serves as a useful model organism. Apart from typical Roc, COR, and kinase domains, GbpC has an N-terminal region that contains LRRs and a peculiar C-terminal region with a Ras guanine exchange factor (GEF) domain, as well as GRAM, DEP, and 2 cGMP-binding domains that bind the second messenger cGMP with high affinity (k_d~4 nM) (Fig. 1). Decades ago, scientists studied a cGMP-binding protein that now appears to be GbpC (14). Finally, soon after the *Dictyostelium* genome was sequenced, GbpC was identified, and a cell line was obtained in which the *gbpC* gene was disrupted (15). Analysis of this cell line confirmed earlier assumptions that cGMP is involved in the phosphorylation of myosin II and its assembly in the cytoskeleton, which is needed for proper chemotaxis. It was shown that the phosphorylation patterns of both myosin light chain (MLC) and myosin heavy chain (MHC) are affected after

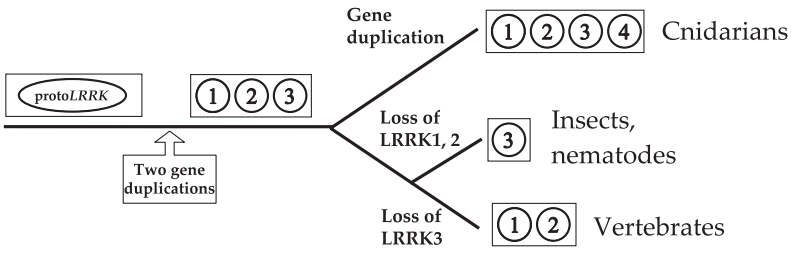

Figure 2. Origin of the LRRK genes in animals. The reconstruction of the evolutionary history of these genes demonstrates that 3 of them (*LRRK1*, *LRRK2*, and *LRRK3*; circles) existed before the split between cnidarians and the rest of the animals. The anemone *Nematostella vectensis* currently has 4 *LRRK* genes, those 3 and an additional gene (*LRRK4*), which seems to be cnidarian-specific. Protostome model animal species such as *Drosophila melanogaster* or *Caenorhabditis elegans* have lost *LRRK1* and *LRRK2*, whereas deuterostomes, including humans, have lost *LRRK3*. Data obtained from refs. 8, 10.

cAMP-stimulation of cells, a result confirmed in cells that lack cGMP (15). Chemotaxis toward a cAMP-source is severely affected because *gbpC*-null cells cannot polarize effectively and form an abnormal amount of lateral pseudopodia (16). Additional evidence for the involvement of GbpC in myosin regulation was provided by Goldberg *et al.* (17); they showed that the activation of MLCK-a, the protein kinase that phosphorylates MLC after cAMP-stimulation, depends on cGMP and GbpC. However, a direct interaction between GbpC and MLCK-a or other proteins has not yet been described, leaving the search for a direct target of GbpC still open. More recently, a crucial role for GbpC in chemotaxis was demonstrated: cells in which 2 other chemotaxis signaling pathways were inhibited (PI3K and PLA2) were solely dependent on the cGMP-pathway to chemotax toward a cAMP-source (18). Apart from myosin regulation during chemotaxis, GbpC might also be involved in protection against osmotic stress because some proteins have been shown to be regulated by both high osmotic levels and cGMP. For example, the phosphorylation state of the transcription factor StatC was highly elevated after treatment of cells with the membrane-permeable cGMP analog 8-Br-cGMP as well as by osmotic stress (19). Similar phosphorylation patterns were found for the protein kinase SAPKα (20). These results suggest that these proteins may lie downstream of GbpC. Notably, Kuwayama and Van Haastert (21) found a strong activation of guanylyl cyclases (GCs) when cells were treated with hyperosmotic compounds, suggesting that cGMP has a function in osmotic responses in the cell. This activation is G-protein independent, in contrast to cAMP-stimulated activation of GCs.

Another *Dictyostelium* Roco, Pats1, was found in a screen to find novel proteins involved in cytokinesis (4). Cells that lack the *pats1* gene are large and show a multinucleated phenotype, which becomes very evident in cultures that are grown in suspension. During division of these cells, MHC shows improper localization, suggesting a role for Pats1 in directing it to the cleavage furrow during cytokinesis. Interestingly, an interaction between the WD40 repeats of Pats1 and the actomyosin cytoskeleton was also found, supporting its involvement in myosin II localization during cell division. The protein encoded by a third *D. discoideum* Roco gene, *QkgA/Roco2*, was disrupted to show the validity of a new method to create knockout cell lines in *Dictyostelium* (5). Cells lacking this protein show a significant increase in growth rate, both in liquid medium and on bacteria. Moreover, this protein is involved in development; the mutants aggregate slowly and form aggregation centers that are larger than those formed by wild-type cells, a process that eventually leads to large slugs and culminants. From the remaining 8 *Dictyostelium* Roco proteins, only Roco5 has been mentioned recently in a large screen for mutants defective in the developmental cycle (22). The *roco5* null mutant was categorized as being important in the slug and culmination stage of development. Further studies should confirm this observation.

In summary, the current data on the described *Dictyostelium* Roco proteins suggest that they are involved in multiple cellular processes: they participate in cell division, chemotaxis, and development. However, the majority of *Dictyostelium* Rocos have not been described yet; most likely, future studies will elucidate more functions of these proteins.

Functions of mammalian Roco proteins

Dominant mutations in *LRRK2* are involved in both familial and sporadic PD (23–29) (See **Fig. 3** for a summary of mutations.) Mutations in *LRRK2* may also cause other neurodegenerative diseases (30). In humans, *LRRK2* is expressed in multiple tissues, including brain (24). Within the brain, expression appears in multiple areas, particularly in substantia nigra (31). This suggests a direct involvement in dopaminergic cell death, which could be the result of mitochondria-dependent apoptosis (32). LRRK2 interacts with the product of another gene involved in familial PD, *parkin* (33). Several other potential interactors have recently been described (34). LRRK2 efficiently phosphorylates moesin (35), but it is likely that its most interesting substrates are yet uncharacterized. Recently, an association of the LRRK2 protein with lipids has been demonstrated (36, 37). It has also been shown that LRRK2 regulates neurite morphology (38).

LRRK1 is the closest relative of *LRRK2* in vertebrates. It is also expressed in the human brain (39). As we will discuss in detail later, mutations in *LRRK1* seem to be less toxic than equivalent mutations in *LRRK2* in cell

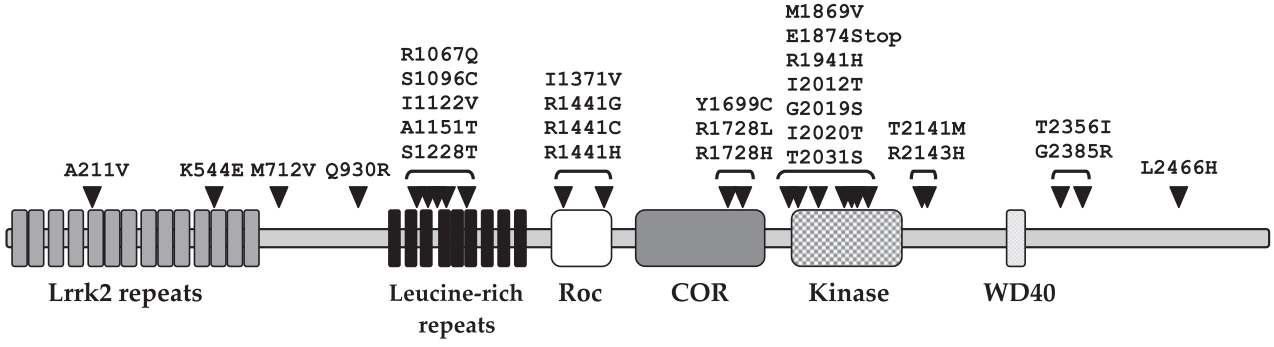

Figure 3. Missense mutations described in *LRRK2* that lead to PD. Data obtained from Paisán-Ruíz *et al.* (26).

systems (39). So far, *LRRK1* has not been found to be involved in PD or any other human disease (40, 41).

Protostomes such as *D. melanogaster* or *C. elegans* have single *LRRK* genes that are paralogs of human *LRRK2* (8, 10). Sakaguchi-Nakashima *et al.* (42) showed that mutations in the *C. elegans LRK-1* gene generate anomalies in the localization of synaptic vesicle proteins. Lee *et al.* (43) showed that loss of function of the *LRKK* gene of *D. melanogaster* leads to anomalies in locomotor activity and shrunken morphology as well as decreased tyrosine hydroxylase staining in dopaminergic neurons of the fly brain. However, contrary to what happens in humans, overexpression of the gene does not produce effects in the fly. Given that they are not true orthologs of *LRRK2*, extrapolations from these protostome models to humans in order to understand the parkinsonian phenotypes linked to *LRRK2* mutations are very problematic.

DAPK1 was first discovered as involved in a mechanism of cell death triggered by interferon in HeLa cells (1). Later, it was found that the product of this gene is a general positive regulator of cell death, both apoptotic and autophagic (44). *DAPK1* is implicated in cancer, given its function as a tumor suppressor by sensitizing cells to apoptotic signals. Therefore, its expression frequently is found diminished in tumors. In addition, and most interestingly, it has recently been shown that mutations that lead to reduced expression of *DAPK1* contribute to heritable predisposition to chronic lymphocytic leukemia (45). *DAPK1* may also be involved in several pathologies involving neuronal cell death, such as epilepsy (46) and Alzheimer disease (47). It may be significant that the Roc domain of DAPK1 has been shown to be a cytoskeleton-interacting domain (48).

Little is known about the function of the *MFHAS1* gene. It has been found to be amplified or translocated in different types of tumors, suggesting it has oncogenic potential (2, 49, 50).

Roco proteins in plants

The precise functions of Roco proteins in plants are still poorly understood. Mutations in an *Arabidopsis thaliana* Roco gene, *TRN1*, have been described (11–13, 51). These researchers, however, did not determine that *TRN1* belongs to the Roco family but only detected some similarity to the animal *DAPK1* gene (13). *TRN1* mutants have altered growth and morphogenesis and changes in auxin distribution. *TRN1* genetically interacts with *TRN2* (*Tornado2*), which encodes a tetraspanin (13). Tetraspanins are integral membrane proteins involved in many functions, including the regulation of intercellular signaling. This has led to the hypothesis that TRN1 and TRN2 proteins may be working together in an as yet unknown signaling pathway (13).

BIOCHEMISTRY OF ROCO PROTEINS

The large diversity found among Roco proteins demonstrates not only the importance of this family to understand diverse cellular functions but also that every member should be studied individually. However, since the Roc-COR-kinase structure is identical in LRRK2 and many other Roco proteins, biochemical data on other Rocos may contribute to elucidate the molecular mechanisms leading to *LRRK2*-dependent PD. So far, some details of the biochemical mechanisms involved in the activity and regulation of 3 Roco proteins (GbpC, LRRK1, and LRRK2) are available. In particular, research has focused on understanding the functions and activities of separate domains of these proteins.

Roc and kinase activities

Both GTPase and kinase activity have been demonstrated for LRRK2. There is good evidence that the loss of dopaminergic cells in patients with PD, resulting from mutations in either the kinase or GTPase domains, is caused by increased or constitutive kinase activity of the mutated LRRK2 protein (52–56). LRRK1 also binds GTP and has GTP-dependent kinase activity (39, 57).

Several lines of evidence suggest that activation of the Roc GTPase domain leads to an increase in kinase activity, which is thought to be the output action of these proteins (39, 55–58). Some of these studies also showed that various common LRRK2 mutations cause an activated Roc domain and a subsequent increase in kinase activity *in vitro*. In contrast, conflicting data exists for the Parkinson-related mutations that affect the activation loop of the kinase domain of LRRK2. Recent studies showed a small but significant increase in kinase activity for the commonly found G2019S and I2020T mutations (52, 53), supporting the theory of a gain-of-function effect of these mutations. Korr *et al.* (57), however, found a decrease in kinase activity when they created the corresponding mutations in the paralog protein, LRRK1. In the same study, the researchers investigated the effect of some mutations in *LRRK1* corresponding to common Roc- and COR-domain mutations in *LRRK2*. These mutations did not abolish the ability to bind GTP, which is not surprising, because they affect residues that are located outside the GTP-binding pocket of the protein. In addition, Roc-stimulated kinase activity was still present, but at reduced levels, in the Roc mutant K745G (corresponding to LRRK2-R1441G). Interestingly, the COR-domain mutant was devoid of any kinase activity. These data are still to be confirmed for LRRK2.

Because the Roc domains of LRRK1 and GbpC are active as GTPases independently of other domains, it is very likely that Roc activation causes increased kinase activity instead of Roc activity being dependent on kinase activation (ref. 56 and unpublished results). A possible pitfall that one must keep in mind is that most phosphorylation data on LRRK2 mutations originate from *in vitro* assays, using immunoprecipitated proteins. Although these assays have yielded valuable information about the effect of mutations on kinase activities, it could be that the kinase activity is regulated

by proteins such as GEFs, GAPs (GTPase-activating proteins), or heat shock proteins, which may be inactive or absent in these assays, leading to misinterpretations of the effects of the mutations. Also, the use of artificial kinase substrates could mask the real nature of kinase activities.

Very recently, important structural information became available, as the crystal structure of the Roc domain of LRRK2 was solved (59). Previous biochemical observations suggesting that LRRK2 acts as a dimer were confirmed in this study. Moreover, 2 residues that suffer pathogenic mutations linked to PD (R1441 and I1371) were shown to be involved in stabilizing dimer formation. The PD-related mutations destabilize the dimer structure, resulting in decreased GTPase activity, and subsequently prolonged activation of the Roc domain. The likely result is an overactive kinase domain, possibly *via* the action of the COR domain, which may act as a molecular link between the Roc and kinase domains. These data fit very well with previous observations that indicated that dominant *LRRK2*-associated PD is the result of gain-of-function mutations.

Roles of other domains

It is currently not known whether the Roc domain of LRRK2 is regulated by GEFs and/or GAPs. This information could be essential to elucidate the effect of many mutations on *LRRK2*. A recent report from Lewis *et al.* (60) suggests that a mutation in LRRK2 causes reduced GTPase activity as a result of disruption of an interaction with a GAP. The GTPase activity of LRRK2 is in fact very low, supporting the existence of such an interaction (58). An interesting model for understanding the biochemistry of LRRK2 is provided by the *Dictyostelium* protein GbpC, which itself contains a GEF domain capable of activating its own Roc domain (unpublished results). This supports the hypothesis of an intramolecular GEF-Roc-kinase signaling cascade. Strikingly, this cascade may be even further extended, as cGMP binding to the cGMP-binding domains leads to an activated Roc domain *in vitro* (unpublished results). The importance of regulatory domains in Roco proteins is also reflected in the observation that GbpC proteins with inactivating mutations in various domains show mostly decreased or abolished activity *in vivo*. **Figure 4** summarizes these results, leading to a model for the intramolecular signaling cascade occurring in GbpC, which is likely to be related to that occurring in other Roco proteins. Each Roco protein, however, may have peculiarities. For example, for GbpC, not only are the GEF, Roc, and kinase domains essential for its chemotactic function, but also the GRAM domain-mediated localization to the membrane is crucial for protein function in the cell (unpublished results).

LRRs are present in many Roco proteins, but the functions of these repeats are unknown. Since LRRs were originally described as domains that mediate protein–protein interactions, they may interact with partners of the Roco proteins. Another possibility is that the LRRs have a structural role for the protein itself, perhaps supporting proper folding of the conserved Roco core. Strikingly, a LRR-deletion mutant of GbpC still retains cGMP-stimulated Roc activation and proper localization, although the protein is not functioning *in vivo*. Thus, a role for the LRR in stabilization of the Roc or the C-terminal regulatory domains of GbpC is unlikely (unpublished results). Ankyrin, WD40, and other types of repeats (one of them LRRK2-specific) are also often present in Roco proteins. They all are probably involved in allowing protein–protein interactions (Fig. 1). Parkinson-related mutations affecting the LRRs and the LRRK2-specific repeats have been found (Fig. 3), demonstrating their importance for proper protein function. The effects at a molecular level of these mutations, however, are still completely unknown. It is very significant that no role has yet been

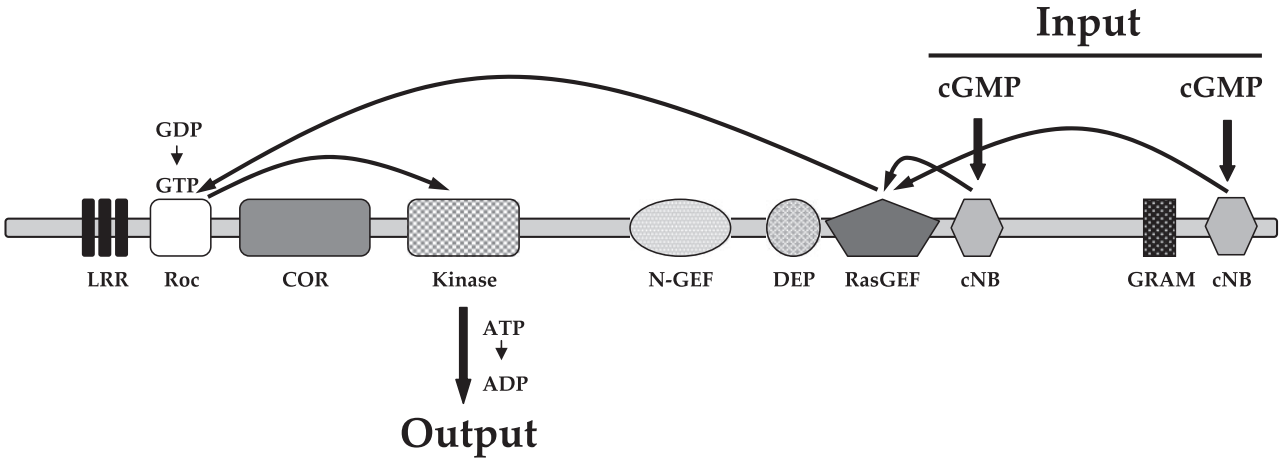

Figure 4. Intramolecular signaling cascade through GbpC. On binding of the second messenger cGMP to the cyclic nucleotide binding (cNB) domains, the GEF-domain is liberated to enhance GDP/GTP exchange in the Roc domain. GTP-binding to the Roc domain leads to an activated kinase domain, which is the output of GbpC. The GRAM domain is involved in membrane localization, and the function of the DEP domain and LRRs are unknown, although the latter are essential for protein function *in vivo*.

assigned to the characteristic COR domain, although some mutations in this domain of LRRK2 cause PD (Fig. 3).

FUTURE PROSPECTS

Our knowledge of Roco genes and proteins suggests several significant lines of research that will be explored in the next few years. A first aspect is the precise characterization of the biochemical functions of Roco proteins. This includes understanding the roles of their different protein domains and how these domains interact with each other. More crystalization data will become available, most likely from LRRK2 but also from other Roco proteins. They will help to elucidate common themes in how proteins of this family function and are regulated. Crystal structures will also be vital to explain how mutations in *LRRK2* lead to PD. These studies should also elucidate whether the COR domain indeed transmits Roc signals to the kinase domain, as has been suggested.

A second research line is the characterization of the diverse cellular functions of Roco proteins in different organisms, from bacteria to humans. Exploration of interaction partners, including GEFs, GAPs and proteins that are directly phosphorylated by the kinase domains, is needed. This research will lead to a better understanding of the signaling pathways to which Roco proteins belong and may explain why *Dictyostelium* uses many more Roco proteins than other Eukaryotes. Finally, the third expected line of research is the precise characterization of the roles of Roco proteins in humans and the understanding of their role in human diseases. Exciting prospects will be derived from these lines of research, which may contribute to our understanding of multiple cellular processes in very different organisms. FJ

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