The role of (auto)-phosphorylation in the complex activation mechanism of LRRK2

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Minireview

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The role of (auto)-phosphorylation in the complex activation mechanism of LRRK2

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Abstract: Mutations in human leucine-rich-repeat kinase 2 (LRRK2) have been found to be the most frequent cause of late-onset Parkinson's Disease (PD). LRRK2 is a large protein with two enzymatic domains, a GTPase and a kinase domain. A cluster of (auto)-phosphorylation sites within the N-terminus of LRRK2 have been shown to be crucial for the localization of LRRK2 and is important for PD pathogenesis. In addition, phosphorylation of sites within the G-domain of the protein affect GTPase activity. Here we discuss the role of these (auto)-phosphorylation sites of LRRK2 and their regulation by phosphatases and upstream kinases.

Keywords: GTPase; kinase; neuronal degeneration; Parkinson's disease; phosphatases.

Introduction

Human leucine-rich-repeat kinase 2 (LRRK2) has been identified to be the leading cause of late-onset hereditary Parkinson's disease (PD) (Gasser et al., 2011). PD is a common neurodegenerative disorder affecting 1–2% of the Western world's population (reviewed in Lees et al., 2009) and is characterized by the progressive death of dopaminergic neurons in the midbrain associated with the formation of fibrillar aggregates that consist mainly of α-synuclein and other proteins. LRRK2 mutations initiate an age dependent phenotype with complete clinical and neurochemical overlap with idiopathic disease (Healy et al., 2008; Alcalay et al., 2013). However, the penetrance of the LRRK2 mutations is even incomplete at later age and might partly depend on additional genes or environmental factors (Trinh et al., 2014). Mutations within LRRK2 have been identified in 5–6% of patients with familiar PD, but importantly have also been found in patients with sporadic PD (Lesage et al., 2007), while some LRRK2 PD patients can demonstrate quite a pleomorphic pathology (Zimprich et al., 2004). Despite a vast amount of research and the identification of several LRRK2 mediated pathways and interaction partners, the complete role of LRRK2 in the cell, and how different mutations of LRRK2 are contributing to the progression of the disease is still not well understood (Cookson and Bandmann, 2010). Importantly, LRRK2 interacts with synaptic vesicle proteins including synaptojanin-1 and Rab proteins were recently identified as the first physiological substrates of LRRK2, suggesting a role for LRRK2 in vesicle trafficking (Islam et al., 2016; Steger et al., 2016; Carrion et al., 2017; Pan et al., 2017).

LRRK2 is a large protein with two enzymatic domains, a GTPase and a kinase domain, and in addition several protein-protein interaction domains (Figure 1). Most of the PD mutations are located within the enzymatic core. Although it is generally accepted that the kinase activity of LRRK2 is essential for PD-mediated neuronal toxicity, conflicting data for the effect of the various PD mutations on LRRK2 kinase activity have been reported (reviewed by Greggio and Cookson, 2009). The main reason for these conflicting data was the lack of a physiological substrate. Data of recent studies that measured the phosphorylation of Rab proteins, the physiological LRRK2 substrates, strongly suggest that all common PD-mutations result in increased kinase activity (Steger et al., 2016). Interestingly, several of these mutations not only result in increased kinase activity, but also decreased GTPase activity (reviewed in (Terheyden et al., 2016), suggesting cross talk between the two enzymatic domains. Recent data suggest that LRRK2 kinase activity is regulated by the Roc domain, but that *vice versa* the kinase domain also regulates LRRK2 GTPase activity via auto-phosphorylation of the Roc domain (Figure 2) (reviewed in Terheyden et al., 2016). Other

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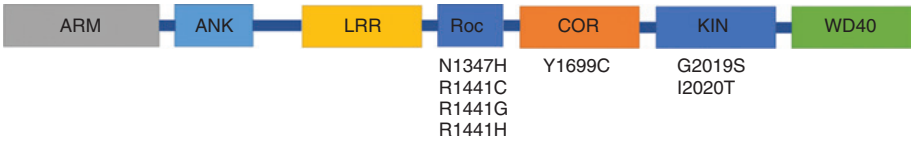

Figure 1: Schematic representation of LRRK2.
The domains of LRRK2 are shown with different colors. Below the most common PD associated mutations are indicated.

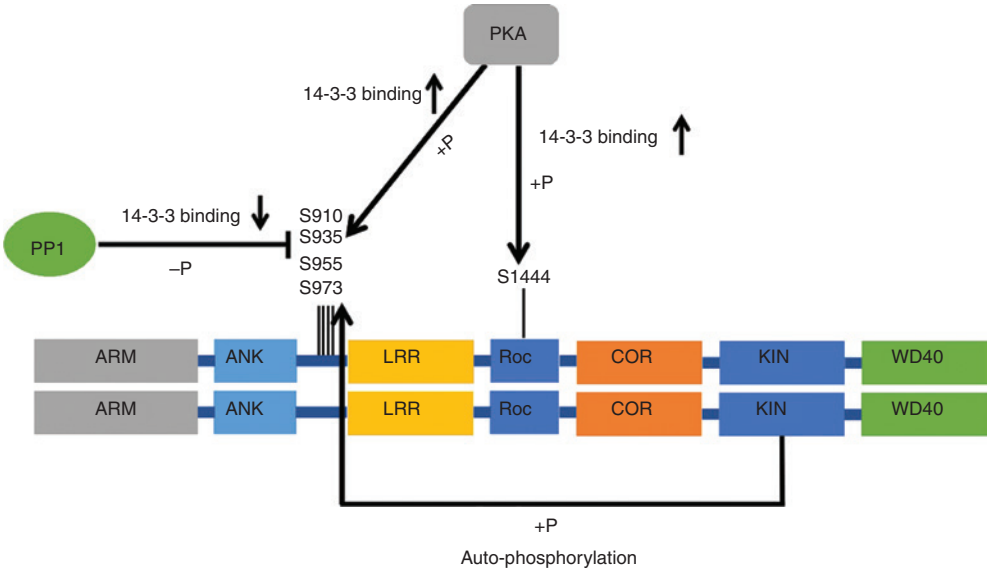

Figure 2: Regulation and function of N-terminal LRRK2 (auto)-phosphorylation sites.
Phosphorylation of S910, S935, S955 and S973 is induced by both auto-phosphorylation and upstream kinases such as PKA (S935). In addition, PKA is able to phosphorylate S1444 within the Roc domain. PP1 dephosphorylates the N-terminal sites. 14-3-3 binds in a phosphorylation dependent way to the N-terminal serine cluster and thereby regulates its localization.

(auto)-phosphorylation sites, within the N-terminus of LRRK2, are important for proper localization and binding to upstream and downstream regulators (Dzamko et al., 2010; Nichols et al., 2010; Doggett et al., 2012; Lobbestael et al., 2013). Here we highlight the important role of these LRRK2 (auto)-phosphorylation and discuss their regulation by upstream kinases and phosphatases.

Function and regulation of LRRK2 N-terminus phosphorylation

Phosphorylation of a cluster of serines (S910, S935, S955 and S973) within the N-terminal part of LRRK2 (West et al., 2007) has been shown to be essential for interaction with 14-3-3 (Dzamko et al., 2010; Nichols et al., 2010; Doggett et al., 2012; Lobbestael et al., 2013). Disruption of S935 phosphorylation results in impaired 14-3-3 binding and subsequently to the delocalization of LRRK2 protein to microtubules, instead of being transported from the cytosol to membranes (Nichols et al., 2010; Ramírez et al., 2017) (Figure 2). As addition of LRRK2 kinase inhibitors also resulted in the disruption of the 14-3-3/LRRK2 interaction, several studies postulated that these sites are primarily regulated by auto-phosphorylation (Dzamko et al., 2010; Nichols et al., 2010; Doggett et al., 2012; Lobbestael et al., 2013). However, it is now clear that PKA is the major regulator of S935 phosphorylation (West et al., 2007; Doggett et al., 2012). Dephosphorylation of S910/S935/S955 and S973 is facilitated by protein phosphatase 1 (PP1) and thereby stimulates the dissociation of 14-3-3 protein from LRRK2 (Lobbestael et al., 2013) (Figure 2). LRRK2 PD mutants showed enhanced PP1 binding compared to wild-type LRRK2 (Lobbestael et al., 2013) and consequently reduced S910 and S935 phosphorylation and reduced 14-3-3 binding (Nichols et al., 2010; Doggett et al., 2012). Consistently, increased 14-3-3 binding to LRRK2 seems to reduce kinase activity and to restore the reduced neurite

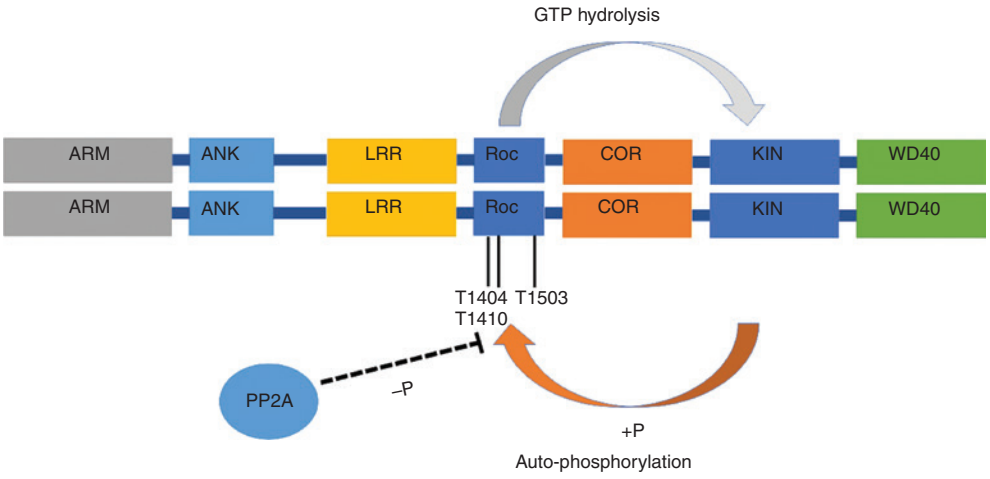

Figure 3: Intramolecular interplay between the LRRK2 Roc and kinase domain.
The Roc domain regulates the kinase domain, while the GTPase activity is regulated by the auto-phosphorylation of the Roc domain. T1404, T1410 and T1503 are some of the potential important auto-phosphorylation sites identified within the Roc domain.

length of G2019S neuronal cultures (Lavalley et al., 2016). These results show that N-terminal LRRK2 phosphorylation is important for PD pathogenesis and that it is thus important to further investigate the PP1/LRRK2 interaction and completely characterize the pathways and signals that regulate N-terminal LRRK2 phosphorylation.

Function and regulation of Roc phosphorylation

Also within the Roc domain a 14-3-3 binding site has been identified (Muda et al., 2014). PKA mediated phosphorylation of LRRK2 S1444 stimulates binding to 14-3-3 (Figure 2). In addition, several potential auto-phosphorylation sites have been identified within the Roc domain, however, both the regulation and biological relevance of these sites remains largely unknown [reviewed in (Terheyden et al., 2016)]. Conflicting results have been published about the impact of auto-phosphorylation of the Roc domain on LRRK2 GTPase activity. Taymans et al. reported that auto-phosphorylation of the Roc domain results in increased GTP binding and GTPase activity (Taymans et al., 2011). Consistently, Webber et al. showed that upon auto-phosphorylation of the Roc domain, the GTP bound state is stabilized (Webber et al., 2011). However, in contrast it also has been reported that mutants with reduced kinase activity have increased GTPase activity (Greggio et al., 2009). Consistently mutants with increased kinase activity have normal GTP binding affinity, but reduced GTPase activity (West et al., 2005; Greggio et al., 2006; Jaleel et al., 2007; Anand et al., 2009). Together this suggests that auto-phosphorylation of the Roc domain plays an important role in the regulation of the LRRK2 G-protein cycle, but that different phosphorylation sites might have a different impact on the GTPase activity. It is therefore crucial to better understand the kinetics and regulation of LRRK2 Roc phosphorylation.

So far it is unclear which phosphatases are regulating the phosphorylation state of the Roc domain. However, we recently identified protein phosphatase 2A (PP2A) as an interacting partner of the LRRK2 Roc domain (Athanasopoulos et al., 2016). PP2A interaction with LRRK2 is mediated by the Roc domain and takes place in the perinuclear region of Hela cells. Although we could not identify the phosphorylation sites within LRRK2 yet, it is tempting to speculate that PP2A regulates dephosphorylation of sites within the Roc domain (Figure 3). Importantly, expression of PP2A partially protects SH-SY5Y cells expressing LRRK2 R1441C and primary cortical neurons expressing LRRK2 G2019S from LRRK2 PD-induced neurotoxicity. Consistently, silencing the catalytic subunit of PP2A (PP2Ac) by shRNA in the same cell systems, resulted in increased mutant LRRK2-induced cell death. Thus, this shows that PP2A is important for the survival of cells expressing mutant forms of LRRK2. To explain the neuroprotective properties of PP2A's enzymatic activity in LRRK2-induced parkinsonism, it will be crucial to identify the PP2A target sites and understand how the phosphorylation of these sites impacts GTPase and kinase activity.

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Bionotes

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