Interaction between LRRK2 ROC GTPase and COR domains as a regulatory mechanism of LRRK2 catalytic functions

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Abstract

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most prevalent known cause of autosomal dominant Parkinson's disease (PD). The LRRK2 gene encodes a ROCO protein featuring a ROC GTPase domain and a kinase domain linked by the C-terminal of ROC (COR) domain. Since increased kinase activity has been linked to neurotoxicity, gaining insight into the regulation of LRRK2 kinase activity is crucial to the development of new therapies for LRRK2 PD. The presence of two enzymatic domains in LRRK2 has been suggested to be predictive of an intrinsic regulation mechanism between the ROC domain and the kinase domain, in analogy to other G-protein to effector regulatory interactions.
In this study we use co-immunoprecipitation and GST pulldown to explore the interactions in the catalytic core of LRRK2. We show that there is only a weak interaction between ROC and kinase domains but there is a strong interaction between ROC and COR domains, suggesting that the ROC-COR interaction may transmit regulatory signals from the ROC to the kinase. We also tested the influence of pathogenic mutations and ROC domain activation status on the ROC-COR interaction. Our findings show that incorporation of the Y1699C pathogenic mutation in the COR domain significantly strengthens the ROC-COR interaction. Loading of the ROC domain with GTPγS also tends to enhance the ROC-COR interaction. To gain more insight into the interpretation of these results, we created a 3D model of the ROC-COR tandem of LRRK2 based on the model of the ROC-COR tandem of the bacterium *C. tepidum* (Gotthardt et al., EMBO J, 27(16), 2008). Our model confirms the presence of the Y1699C mutation on the ROC-COR interface. We show that the catalytic core of LRRK2 is the minimal fragment displaying substantial kinase activity, using autophosphorylation as a readout. Moreover incorporation of the Y1699C mutation significantly increases the kinase activity of the catalytic core, although this increase is not significant in full length LRRK2 Y1699C. The kinase activity of both the catalytic core and the full length protein is stimulated after a loading procedure with GTPγS. Finally we show that LRRK2 wt possesses weak GTPase activity which is reduced to background level in LRRK2 Y1699C.
All together our data are consistent with the theory that the ROC-COR interaction is a mechanism by which the ROC-GTPase transmits regulatory signals to the kinase domain thereby modulating LRRK2 kinase activity.