α-Synuclein and LRRK2: Partners in Crime

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In this issue of *Neuron*, Lin et al. report that LRRK2 modulates age-related neurodegeneration caused by overexpression of α-synuclein in the forebrain of transgenic mice. Overexpression of LRRK2 accelerates the progression of α-synuclein-mediated neuropathological changes, whereas deletion of LRRK2 alleviates these alterations. The results reveal an interesting interaction between α-synuclein and LRRK2, two gene products linked to dominantly inherited Parkinson's disease.

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, affecting ~5 million people worldwide. PD is characterized clinically by resting tremor, rigidity, bradykinesia, and postural instability. The neuropathological hallmarks of PD are progressive degeneration of dopaminergic (DA) neurons and the presence of intraneuronal cytoplasmic inclusions known as Lewy bodies (LBs), though neuronal loss is also seen in other neuronal groups, such as noradrenergic neurons in the locus coeruleus. The clinical manifestations of PD are caused by degeneration of DA neurons in the substantia nigra pars compacta (SNpc), which results in dopamine depletion in the striatum, as shown by effective dopamine replacement therapy during early phases of the disease. The mechanisms underlying nigrostriatal dopaminergic degeneration, however, remain unresolved (Shen and Cookson, 2004).

During the last decade, mutations in at least five distinct genes (*α-synuclein*, *parkin*, *DJ-1*, *PINK1*, and *LRRK2*) have been linked to familial PD, permitting studies of the pathogenic mechanisms using genetic approaches. A dominantly inherited missense mutation (A53T) in α-synuclein was the first to be linked to early-onset familial PD. Subsequently, additional missense and gene multiplication mutations were identified (Singleton, 2005), and α-synuclein was shown to be a major constituent of LBs (Spillantini et al., 1998), highlighting the importance of α-synuclein in PD pathogenesis. α-Synuclein is expressed at high levels in the brain and is associated with presynaptic vesicles. While the normal function of α-synuclein is poorly understood, several lines of transgenic mice overexpressing wild-type or mutant α-synuclein have been reported to develop neuronal degeneration (Chandra et al., 2005; Giasson et al., 2002; Lee et al., 2002). However, none of them exhibits significant degeneration of DA neurons in the SNpc due to the predominant overexpression of α-synuclein in non-DA neurons in these transgenic mice.

More recently, several missense mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) gene have been linked to autosomal dominantly inherited PD (Singleton, 2005), which are collectively the most common genetic cause of late-onset PD. While α-synuclein is a small protein of 140 amino acid residues with few functional domains, LRRK2 is a large 2527 amino acid protein consisting of several functional domains, including a leucine-rich repeat domain, a Ras-like small GTPase domain, and a kinase domain with sequence homology to MAP kinase kinase kinases. The pathogenic mutations in LRRK2 are present in various functional domains of the protein, and all affect highly conserved amino acid residues. Most LRRK2 mutations cause clinically typical PD, but the neuropathological features vary, ranging from pure nigral degeneration without LBs to nigral degeneration with brainstem or widespread LBs, or neurofibrillary tangles. Extensive in vitro studies have shown increased kinase activity associated with some pathogenic mutations, such as the G2019S mutation (Cookson et al., 2007). In vivo studies using transgenic mice overexpressing the R1441G mutant form of LRRK2 or knockin mice expressing the R1441C mutant at endogenous levels revealed deficits in dopamine release and/or dopamine D2 receptor-mediated function but absence of neurodegeneration (Li et al., 2009; Tong et al., 2009). The mechanism underlying these DA functional defects is less clear.

How do mutations in five genes with seemingly divergent functions cause PD? While no common molecular pathways have emerged from studies of these five gene products, genetic analysis of recessive PD-linked gene products, Parkin, DJ-1, and PINK1, in mice and *Drosophila* has highlighted an interaction between Parkin and PINK1 and pointed to mitochondrial and dopaminergic dysfunction as possible converging mechanisms in PD. Thus, an intriguing question arises: do α-synuclein and LRRK2, the dominant PD-linked gene products, act together in processes relevant to PD pathogenesis?

In this issue of *Neuron*, an elegant genetic study by Lin et al. (2009) identifies an important functional interplay between α-synuclein and LRRK2 in the development of neurodegeneration and other neuropathological features caused by α-synuclein overexpression. Using the tetracycline-controlled transactivator system, the authors generated a series of inducible transgenic mice overexpressing the human A53T mutant form of α-synuclein or various forms of human LRRK2, including wild-type, G2019S, or kinase domain-deletion mutant in the adult forebrain. They found that 30-fold overproduction of A53T α-synuclein in the adult forebrain results in locomotor defects in the open field as early as 2 months of age and age-related neurodegeneration,

including increased apoptosis and gliosis as well as progressive loss of cortical and striatal neurons. 16-fold overproduction of wild-type or G2019S mutant LRRK2, however, does not cause neurodegeneration up to 20 months of age.

Intriguingly, co-overexpression of either wild-type or G2019S LRRK2 with A53T α-synuclein dramatically accelerated neurodegeneration in double-transgenic mice. At 1 month of age, when no neuropathology was detected in any of the single-transgenic brains, increased apoptosis and gliosis as well as loss of neurons were evident in the double-transgenic striatum. Furthermore, the effect of LRRK2 overexpression on neurodegenerative features in *A53T α-synuclein* transgenic mice is more dependent on the level of LRRK2 overexpression than the presence of the G2019S mutation. Surprisingly, LRRK2 lacking the kinase domain also accelerated A53T α-synuclein-mediated neuropathology to a similar extent as wild-type LRRK2 when expressed at similar levels, suggesting that the kinase domain of LRRK2 is not essential in the interplay of α-synuclein and LRRK2. Lin et al. further verified that this interplay is specific between α-synuclein and LRRK2 by showing that co-overexpression of A53T α-synuclein with a control protein, the green fluorescent protein (GFP), did not accelerate the progression of A53T α-synuclein-mediated neuropathology, and that co-overexpression of G2019S LRRK2 with a mutant version of the amyloid precursor protein (APP) did not accelerate APP-mediated astrocytosis and microgliosis.

LRRK2 overexpression also promoted abnormal somatic accumulation of α-synuclein in striatal neurons of *A53T α-synuclein* transgenic mice. While α-synuclein is normally localized in axonal terminals, overexpression of A53T α-synuclein results in its accumulation in cell bodies in an age-dependent manner. Overexpression of LRRK2 in *A53T α-synuclein* transgenic mice caused earlier and more widespread somatic accumulation of α-synuclein. LRRK2 overexpression also accelerated α-synuclein aggregation, which can be detected as high-molecular-weight species in brain extracts from older *A53T α-synuclein* or younger *A53T α-synuclein/G2019S LRRK2* transgenic mice.

To explore further how LRRK2 overexpression promotes α-synuclein-based neuropathology, including neurodegeneration and abnormal α-synuclein accumulation and/or aggregation, Lin et al. examined ER/Golgi trafficking, the ubiquitin-proteasome system, and mitochondria. Earlier studies have shown that overexpression of α-synuclein resulted in blocked ER/Golgi vesicular trafficking in yeast and Golgi fragmentation in cultured cells (Cooper et al., 2006; Gosavi et al., 2002). While the ER structure appeared normal, immunohistochemical analysis with specific markers showed that the Golgi complex was disrupted in striatal neurons of young *A53T α-synuclein/LRRK2* transgenic mice. Both *cis*- and medial/trans-Golgi networks were drastically altered in various single-transgenic mice, with the exception of *A53T α-synuclein* mice, in which the *cis*-Golgi system appeared normal, and were further disrupted in double transgenic mice. Additional investigation revealed a perturbed dynamics of microtubule assembly with a shift from free tubulin to polymerized tubulin in *LRRK2* but not *A53T α-synuclein* transgenic mice. This perturbation of the microtubule network was thought to underlie the disrupted structure of the Golgi apparatus. However, it is somewhat puzzling why overexpression of LRRK2 alone was sufficient to cause disruption of the microtubule network and Golgi apparatus but insufficient to cause neurodegeneration, whereas *A53T α-synuclein* transgenic mice exhibited much subtler microtubule and Golgi phenotypes but striking neuronal degeneration. Co-overexpression of A53T α-synuclein and G2019S LRRK2 also appeared to have caused increases in ubiquitin-positive aggregates and abnormal mitochondria.

The last piece of data provided perhaps the most important supporting evidence for the major conclusions of the paper, showing that genetic deletion of LRRK2 ameliorated neurodegeneration, gliosis, α-synuclein aggregation, and the Golgi phenotypes in *A53T α-synuclein* transgenic mice. Given the very high levels of the transgene expression (~30-fold for A53T α-synuclein, and ~16-fold for wild-type and G2019S LRRK2) and the lack of significant differences between wild-type and G2019S LRRK2 on their modulation of α-synuclein-mediated neuropathological changes, a valid concern was that the observed phenotypes could be simply due to overproduction of the proteins expressed from the transgenes. Inclusion of transgenic mice overexpressing GFP or APP as controls dispelled some of the concerns, though understandably these control mice were not analyzed as exhaustively. The fact that inactivation of LRRK2 rescues the loss of striatal neurons in *A53T α-synuclein* transgenic mice provides key support for a regulatory role of LRRK2 in α-synuclein-mediated neuropathology under overexpression conditions.

The findings in Lin et al. highlight an important interplay between two dominant PD gene products of α-synuclein and LRRK2. However, many questions remain. How does LRRK2 regulate the dynamics of the microtubule network? Are tubulins or microtubule-binding proteins physiological substrates of LRRK2's kinase activity? It will be important to determine by electron microscopy the ultrastructure of the Golgi apparatus and the subcellular localization of α-synuclein in the various single and double *A53T α-synuclein* and *LRRK2* mutant mice described in the current report. Such analysis may help to explain why *LRRK2* transgenic mice exhibit severe Golgi defects but no neurodegeneration, whereas *A53T α-synuclein* transgenic mice conversely show subtle Golgi phenotypes but striking neurodegeneration. In addition, it would be interesting to evaluate whether the ER/Golgi trafficking and microtubule network are disrupted in the recently reported *LRRK2* transgenic and knockin mice, which could contribute to the dopamine release defect in these mice (Li et al., 2009; Tong et al., 2009). Furthermore, it remains to be determined whether the microtubule network and the Golgi apparatus are affected in the absence of LRRK2. Lastly, it is important to establish whether the interplay between LRRK2 and α-synuclein is limited to overexpression conditions, and whether LRRK2 can interact with endogenous α-synuclein under physiological conditions.

As the authors rightly point out, while *A53T α-synuclein/LRRK2* transgenic mice provide very useful models to study the mechanisms of neurodegeneration and the genetic interaction between

α-synuclein and LRRK2, the occurrence of neurodegeneration in cortical and striatal neurons of the forebrain in these transgenic mice limits their relevance to PD pathogenesis. Thus, the generation of inducible transgenic mice targeting α-synuclein overexpression to DA neurons of the SNpc would enable investigation of the key mechanisms by which α-synuclein aggregation may lead to dysfunction and degeneration of DA neurons. In addition, inducible DA neuron-specific *α-synuclein* transgenic mice could then be crossed with *LRRK2* mutant mice carrying either pathogenic missense or null mutations to study how LRRK2 interacts with α-synuclein in the regulation of DA neuronal dysfunction and degeneration. Such innovative mouse models would provide a valuable platform for further mechanistic studies aimed at uncovering the molecular basis of DA neurodegeneration and developing improved therapeutic strategies.

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RETouching upon Mechanoreceptors

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The rapidly adapting (RA) low-threshold mechanoreceptors respond to movement of the skin and vibration and are critical for the perception of texture and shape. In this issue of *Neuron*, two papers (Bourane et al. and Luo et al.) demonstrate that early-born Ret$^+$ sensory neurons are RA mechanoreceptors, whose peripheral nerve terminals are associated with Meissner corpuscles, longitudinal lanceolate endings, and Pacinian corpuscles. The studies further show that Ret signaling is essential for the development of these mechanoreceptors.

In mammals, sensory neurons in the dorsal root ganglia (DRG) and trigeminal ganglia detect a wide variety of mechanical stimuli, and they are critical for the perception of touch, body positions, and pain. In this preview, we focus on low-threshold mechanoreceptors whose peripheral terminals are associated with specialized end-organs in the skin or the deep tissues (Rice and Albrecht, 2008; Lewin and Moshourab, 2004; Montaño et al., 2009; Tsunozaki and Bautista, 2009). Based on adaptation rates in response to sustained mechanical stimuli, these mechanoreceptors are divided into rapidly adapting (RA) and slowly adapting (SA) subtypes. In mice, RA mechanoreceptors terminate as Meissner corpuscles in the dermal papillae, longitudinal lanceolate endings in hair follicles, and Pacinian corpuscles in the joints and the periostea of bones; these neurons respond to movement of the skin, vibration, and the onset/offset of sustained indentation. SA mechanoreceptors innervate the Merkel discs and Ruffini corpuscles, and respond to skin movement and static indentation stretch. Collectively, these mechanoreceptors are critical for the discriminative perception of texture, shape, vibration, and pressure.

Despite extensive morphological and electrophysiological characterizations, developmental ontogeny and molecular identities of RA versus SA mechanoreceptors are still poorly studied. A few studies have implicated a crucial role for neurotrophic factors in the development of specific subtypes of RA or SA mechanoreceptors. For example, the neurotrophin NT3 is required to maintain Merkel cells and nerve terminals from SA mechanoreceptors (Airaksinen et al., 1996). In another example, BDNF-mediated TrkB signaling is critical for the formation of Meissner corpuscles but is dispensable