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In a mouse model of Parkinson's disease, new evidence shows that L-DOPA, which is used to treat the symptoms of the disease but also causes dyskinesia, results in a persistent activation of the protein kinase mTOR (mammalian target of rapamycin) in a subset of striatal medium spiny neurons. Moreover, blockade of a specific type of mTOR signaling (mTORC1) prevents the development of dyskinesia, but not the antiakinetic benefits produced by L-DOPA. Thus, mTORC1 may be a viable therapeutic target for dyskinesia caused by L-DOPA treatment in patients with Parkinson's disease.

One of the difficulties in treating any disease, including those of the nervous system, is that even effective treatments can result in severe side effects. For example, currently the most effective treatment for Parkinson's disease is L-DOPA. Unfortunately, in addition to its antiakinetic properties, L-DOPA causes multiple motor alterations, including dyskinesia (unwanted spasmodic movements). Thus, it would be very desirable to prevent dyskinesia without negating the antiakinetic benefits provided by L-DOPA for Parkinson's disease patients. Santini *et al*. (*1*) describe a series of experiments that begin to address this problem.

Parkinson's disease is caused by the loss of dopaminergic neurons in the substantia nigra that project to the striatum. This loss of nigrostriatal neurons results in alterations in intracellular signaling in medium spiny neurons (MSNs) when they are exposed to dopaminergic drugs, including L-DOPA. One key signaling molecule that is activated in MSNs in response to L-DOPA is extracellular signal–regulated kinase (ERK) (*2*), an intensely studied protein kinase that is a core component required for multiple types of synaptic plasticity (*3*). L-DOPA triggers persistent activation of ERK in MSNs, which is required for dyskinesia (*4*). However, ERK is a "hub" molecule in signaling pathways, integrating diverse cellular signals and dispersing them to a multitude of downstream effectors, thereby making it a problematic drug target (*5*). Thus, targeting specific downstream effectors of ERK might be a more effective approach for preventing dyskinesia caused by L-DOPA.

In the hippocampus, long-lasting synaptic plasticity requires not only ERK but also the mammalian target of rapamycin (mTOR), a protein kinase that is intimately involved in the initiation of translation when in a complex with Raptor (regulatory associated protein of mTOR) (*6*, *7*); this complex is termed mTORC1. ERK is required for mTORC1 signaling in protein synthesis–dependent forms of long-term potentiation (*8*, *9*) and converges with mTORC1 signaling in regulating protein synthesis–dependent, metabotropic glutamate receptor–dependent long-term depression (*10*). Thus, ERK and mTORC1 are both required for long-lasting synaptic plasticity.

Because L-DOPA–induced dyskinesia has been associated with persistent ERK activation and enhancements in striatal synaptic plasticity (*11*), Santini *et al*. posited that mTORC1 might be one of the downstream effectors of ERK required for dyskinesia. The authors lesioned mice unilaterally with 6-hydroxydopamine (6-OHDA), a toxin often used to induce Parkinsonian symptoms in rodents. In biochemical experiments, the authors showed that administration of L-DOPA to 6-OHDA–lesioned mice increased striatal phosphorylation of two mTORC1 substrates, p70 S6 kinase (S6K) and initiation factor 4E–binding protein (4E-BP), both of which are involved in stimulating translation initiation (*6*, *7*), as well as ribosomal protein S6, a substrate of S6K. The L-DOPA–induced increase in the phosphorylation of mTORC1 substrates was blocked by inhibition of ERK signaling. In addition, the L-DOPA–induced increases in S6K and S6 phosphorylation in the striatum were blocked by a dopamine D1 receptor antagonist but not by a D2 receptor antagonist. Taken together, these findings indicate that D1 receptors and ERK are required for L-DOPA–induced activation of mTORC1 in a mouse model of Parkinson's disease (Fig. 1).

To identify the striatal MSNs in which the L-DOPA–induced increase mTORC1 signaling occurred, the authors performed an elegant set of experiments using immunocytochemistry with transgenic mice expressing enhanced green fluorescent protein (EGFP) under the control of the promoter for either the D1 receptor or the D2 receptor. They found that L-DOPA increased S6 phosphorylation in the MSNs that contain D1 receptors, but not those that contain D2 receptors. Moreover, increased phosphorylation of ERK, which is required for its activation, colocalized with increased phosphorylation of S6 after L-DOPA treatment. These findings suggest that mTORC1 signaling is increased in the same D1-containing MSNs that contain increased ERK in response to L-DOPA treatment.

Santini *et al*. then addressed the critical question: Can inhibition of mTORC1 prevent L-DOPA–induced dyskinesia in mice that model Parkinson's disease? Mice were lesioned with 6-OHDA and treated with L-DOPA for 9 days along with either vehicle or the mTORC1 inhibitor rapamycin, which prevents mTOR from binding to Raptor (*12*). L-DOPA treatment increased mTORC1 signaling, which was prevented in the mice treated with rapamycin. Moreover, mice that were treated with L-DOPA and vehicle displayed robust dyskinesia, whereas mice that were treated with L-DOPA and rapamycin had a large reduction in these involuntary movements. Finally, to address whether rapamycin also inhibited the beneficial effects of L-DOPA treatment in the Parkinson's disease model mice, the authors conducted a cylinder test and found that rapamycin did not alter the ability of L-DOPA to prevent forelimb akinesia produced by the 6-OHDA lesion.

The array of approaches used by Santini *et al*. provided numerous independent lines of evidence consistent with the idea that mTORC1 signaling is necessary for L-DOPA–induced dyskinesia in Parkinson's disease model mice. First, they conducted the "measure" experiment (*13*) to directly test whether mTORC1 signaling occurred

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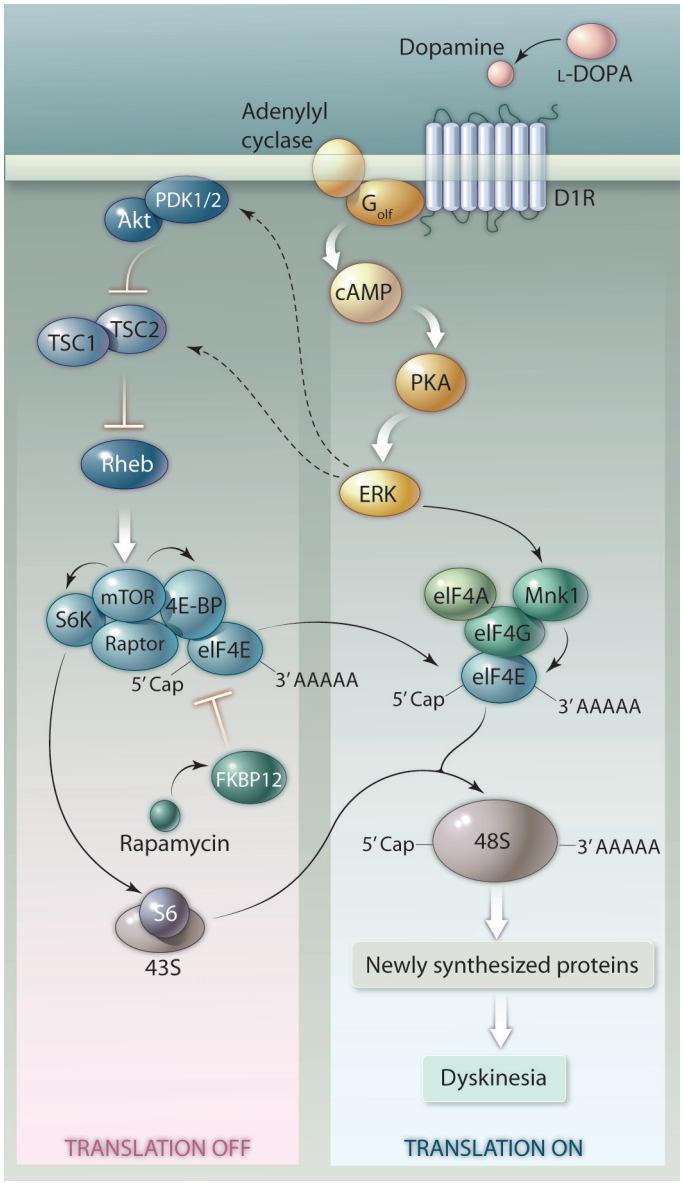

Fig. 1. Proposed mechanism for mTORC1-dependent dyskinesia after L-DOPA treatment in Parkinson's disease. Treatment with L-DOPA triggers the D1 receptor (D1R)–mediated activation of ERK via G_{olf}–adnenylyl cyclase–cAMP–protein kinase A (PKA) signaling in MSNs of the nigrostriatal direct pathway. ERK activates mTORC1 either by phosphorylating and activating PDK1/2 (3-phosphoinositide–dependent protein kinase 1 or 2) (*9*) or by phosphorylating and inactivating TSC2 (tuberous sclerosis 2) (*20*). Deactivation of the TSC1-TSC2 complex results in the activation of Rheb and mTORC1, resulting in the phosphorylation of 4E-BP and S6K. The phosphorylation of 4E-BP results in the release of sequestered eIF4E (eukaryotic translation initiation factor 4E), permitting it to bind to eIF4G, which also binds to eIF4A, to form active eIF4F, which promotes mRNA binding to the 43S preinitiation complex to form the 48S initiation complex. ERK also phosphorylates and activates Mnk1 (mitogen-activated protein kinase–interacting kinase 1), which phosphorylates eIF4E. Phosphorylation of eIF4E and phosphorylation of S6 by S6K are correlated with enhanced translation initiation. Newly synthesized proteins, as yet unidentified, then cause dyskinesia. Rapamycin bound to FKBP12 disrupts mTORC1, thereby preventing the synthesis of new proteins and, subsequently, dyskinesia caused by L-DOPA.

in association with the dyskinesia. Using biochemical and immunocytochemical approaches, they found enhanced phosphorylation of mTORC1 substrates and their effectors. Next, using an elegant approach combining D1 EGFP transgenic mice and immunocytochemistry, the authors showed that the increased mTORC1 signaling occurred in D1 receptor–containing MSNs of the direct pathway. The authors proceeded to conduct the critical "block" experiment (*13*) and found that rapamycin blocked L-DOPA–induced dyskinesia, demonstrating that the activation of mTORC1 was functionally relevant for this behavior.

It would be of great interest if a "mimic" experiment (*13*) could be done to demonstrate that increasing mTORC1 activity in D1-containing MSNs could induce dyskinesia in the 6-OHDA model of Parkinson's disease. One potential way to conduct this type of mimic experiment would be with floxed FK506-binding protein 12 (FKBP12) mice. It has been shown that brain-specific deletion of FKBP12, a putative endogenous inhibitor of mTORC1, results in increased mTORC1 signaling (*14*). Strikingly, the FKBP12 mutant mice displayed an increase in repetitive behavior, which suggests that increased mTORC1 signaling might alter striatal plasticity and behavior. Thus, the specific deletion of FKBP12 in D1-containing MSNs might mimic the effect of L-DOPA in 6-OHDA–lesioned mice.

Rapamycin and its derivatives are currently being tested as a treatment for patients with cancer (*15*), and other classes of mTORC1 inhibitors are in development (*16*). This is of particular interest because rapamycin did not reduce the L-DOPA–induced increases in the phosphorylation of the individual mTORC1 substrates and their effectors to the same extent. Moreover, mTOR can also exist in a complex with Rictor (rapamycin-insensitive companion of mTOR) termed mTORC2, which regulates the cytoskeleton and phosphorylates Akt (*17*, *18*). Although mTORC2 was originally reported to be rapamycin-insensitive (*17*), subsequent findings indicated that prolonged rapamycin treatment can inhibit mTORC2 formation and Akt (*19*). Thus, it is possible that the ability of rapamycin to prevent L-DOPA–induced dyskinesia in 6-OHDA–lesioned mice is through inhibition of mTORC2 and Akt. Nonetheless, mTORC1 inhibitors such as rapamycin have the potential to be a viable therapy to treat Parkinson's disease patients with dyskinesia caused by long-term L-DOPA treatment.

Using an impressive multidisciplinary experimental approach, Santini *et al*. provide a strong argument that mTORC1 signaling in D1-containing MSNs in the striatum is a critical component of L-DOPA–induced dyskinesia in Parkinson's disease. These results demonstrate for the first time that translational control in the striatum is altered in response to L-DOPA treatment and further confirm the general role of mTORC1 in long-lasting changes in behavior.

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