Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2012 | 121 | 327–328 doi: 10.1111/j.1471-4159.2012.07654.x

EDITORIAL HIGHLIGHT

New insights on regulation of LMTK2, a membrane kinase integrating pathways central to neurodegeneration

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Read the full article '*Cdk5/p35 phosphorylates lemur tyrosine kinase-2 to regulate protein phosphatase-1C phosphorylation and activity*' on page 343.

In a short communication in this issue (Manser *et al.* 2012), Christopher Miller's group at the Institute of Psychiatry, King's College London present an elegant and convincing set of experiments using molecular techniques to show that a brain-enriched membrane-associated protein kinase, lemur tyrosine kinase-2 (LMTK2), is directly phosphorylated by the cyclin-dependent kinase-5/p35 and this event is sufficient for LMTK2 to phosphorylate an abundant protein phosphatase, PP1C. LMTK2 has been little studied to date and, despite its name, is a kinase which phosphorylates serine or threonine residues of protein substrates. The paper adds to the evidence that this enzyme is a potentially important mediator positioned to integrate a number of intracellular signalling pathways relevant to neurodegeneration.

While it had been previously established that PP1C was a binding partner and substrate for LMTK2 (Wang and Brautigan 2002), in the current paper the authors have revealed, for the first time, the precise molecular interaction. They have done this by first characterising the cdk5/p35 phosphorylation site of LMTK2 using mass spectrometric techniques. Then they constructed a series of mutants of LMTK2, namely mutating ser^{1418} to alanine, to block cdk5/p35 phosphorylation ($LMTK2^{S1418A}$), by mutating ser^{1418} to aspartate to mimic a state of permanent phosphorylation, ($LMTK2^{S1418D}$) and also constructing a mutant lacking the PP1C-interacting domain. In co-expression experiments, they showed that LMTK2 allows cdk5/p35-dependent phosphorylation of PP1C, and that this is reduced by co-transfection of the $LMTK2^{S1418A}$ mutant incapable of being phosphorylated and enhanced by the $LMTK2^{S1418D}$ mutant which mimics the permanently phosphorylated enzyme.

Therefore, the group have established the biochemical mechanism linking this substrate of cdk5/p35 with a protein phosphatase PP1C (Figure 1). At the current time, the implications of cdk5/p35-dependent LMTK2 phosphorylation on CNS function are not known, and the molecular tools this group have developed should allow the functions to be more fully characterised. As cdk5 itself is a critical mediator of neuronal survival, its inhibition resulting in neuronal death (Hisanaga and Endo 2010), it is possible that some of the detrimental consequences of cdk5-inhibition are mediated

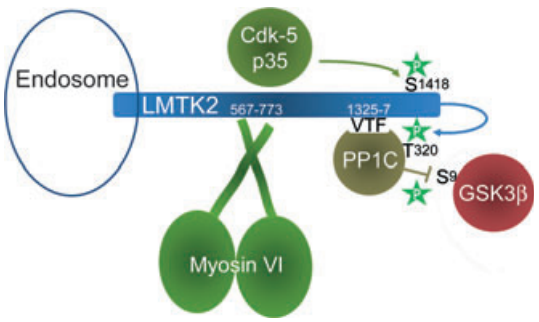

Fig. 1 Some molecular and functional interactions of Lemur tyrosine kinase-2 (LMTK2). The N-terminus of LMT2K contains transmembrane domains allowing interaction with intracellular vesicles, including early endosomes. The cdk5 activator, p35 and myosin VI tail region bind to a central region of LMTK2 (within amino acid residues 567–773). p35/cdk5 mediates phosphorylation of LMT2K at serine 1418. Phosphorylated LMTK2 induces phosphorylation of protein phosphatase 1C (PP1C) at threonine 320. PP1C binds to LMTK2 at a tripeptide motif (Valine Threonine Phenylalanine). Among CNS substrates for PP1C is glycogen synthase kinase-3β (GSK3β): activation of LMTK2 results in reduced phosphorylation of GSK3β at Serine 9. Diagram derived from data in Kesavapany *et al.* (2003), Chibalina *et al.*(2007) and Manser *et al.* (2011, 2012).

Received January 5, 2012; accepted January 5, 2012.

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Abbreviations used: GSK3β, glycogen synthase kinase-3β; LMTK2, lemur tyrosine kinase-2; PP1C, protein phosphatase 1C.

through loss of phosphorylation of LMTK2. In another article published late last year, the authors link the phosphorylation of PP1C by LMTK2 to further downstream signalling events (Manser *et al.* 2011). In that paper, they show that LMTK2, through PP1C, inhibits the phosphorylation of glycogen synthase kinase-3β (GSK3β) (Manser *et al.* 2011). GSK3β is a protein kinase with numerous functions, including an important role in neurodegenerative disease (Bhat *et al.* 2004; Hooper *et al.* 2008). Thus, it would be predicted that through inhibition of GSK3β, LMTK2 has neuroprotective functions, and that loss of function of LMTK2 may cause neurodegeneration. So far, however, there is no direct experimental data published to detail the role of LMTK2 in neurodegeneration. LMTK2 knockout mice are infertile caused by the inability to produce sperm (Kawa *et al.* 2006), and although these mice are reported to have no obvious histological abnormalities in the CNS, no detailed analysis yet exists.

The location of LMTK2 is of importance, and poses another mystery which is likely to be important in revealing its function and role in neurodegeneration. While it is a membrane protein, LMTK2 is not found highly expressed in the plasma membrane, but instead in intracellular compartments, particularly early endosomes (Kesavapany *et al.* 2003; Chibalina *et al.* 2007; Inoue *et al.* 2008). LMTK2 has a critical role in regulating conversion of early endosomes to recycling endosomes, a role dependent on its interaction with myosin VI (Chibalina *et al.* 2007). In this regard, it is of interest that one of the first functions ascribed to LMTK2 was nerve growth factor signalling (Kawa *et al.* 2004): retrograde transport of nerve growth factor signalling complexes is critically dependent on formation of endosomes, followed by transport of signalling complexes (Grimes *et al.* 1996; Harrington *et al.* 2011). In addition to a role in endosomal trafficking, LMTK2's functions suggest a role in axonal transport. The authors have shown that LMTK2-mediated dephosphorylation of GSK3β (via activation of PP1C) results in reduced phosphorylation (activation) of a kinesin-1 light chain, a protein which allows the transport of molecular cargoes, including signalling complexes, along microtubules (Manser *et al.* 2011). Therefore, LMTK2 may have a key role in endosomal and axonal trafficking in numerous processes critical to neuronal health.

Acknowledgements

The author has no conflict of interest to declare.

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