New insights into the regulation of actin cytoskeleton dynamics via the Rho/ROCK/LIMK2/cofilin signalling pathway: a novel mechanism of regulation of cofilin by LIMK2

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LIM kinase 1 (LIMK1) and LIM kinase 2 (LIMK2) are serine/threonine and tyrosine kinases. They play a crucial role in cytoskeleton dynamics as they independently regulate actin filament and microtubule remodelling. By phosphorylating and thus inhibiting cofilin, an actin-depolymerizing factor, LIM kinases prevent actin filaments turnover. The molecular mechanism by which LIM kinases regulate microtubules remodelling is still unknown. Because of their key role in the regulation of cytoskeleton dynamics, LIM kinases have been shown to be involved in cancer development, metastasis, neurological diseases and viral infections, and have recently emerged as new and promising therapeutic targets. Three isoforms of LIMK2 are described in the literature: LIMK2a, LIMK2b and LIMK2-1. LIMK2-1 has a slightly shorter kinase domain and a supplementary Protein Phosphatase 1 (PP1) inhibitory domain in its C-terminal extremity compared to its two counterparts. Recently, we have shown that LIMK2-1 is unable to phosphorylate cofilin although it possesses the threonine 505, phosphorylated and activated by the upstream regulating kinase ROCK (Vallee et al., (2019), Biochemical Journal, 475:3745-3761). Our purpose is now to understand why LIMK2-1 does not phosphorylate cofilin, and therefore to determine the molecular requirements for cofilin phosphorylation by LIM kinases. We have shown that the full-length kinase domain is not sufficient for cofilin phosphorylation, while the C-terminal part of LIMK2a is indispensable for this process. Furthermore, the C-terminal part of LIMK2a is phosphorylated in its own. By site-directed mutagenesis, we pointed out a single amino acid of LIMK2a C-terminal extremity that is phosphorylated, and its phosphorylation is required for cofilin phosphorylation. We want to further characterise this process. Altogether, our data unravel the existence of a new mechanism of regulation of LIM kinases.