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The 5-phosphatase OCRL mediates retrograde transport of the mannose 6-phosphate receptor by regulating a Rac1-cofilin signalling module

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ABSTRACT: Mutations in the OCRL gene encoding the phosphatidylinositol 4,5-bisphosphate (PI(4,5)P(2)) 5-phosphatase OCRL cause Lowe syndrome, which is characterized by intellectual disability, cataracts, and selective proximal tubulopathy. OCRL localizes membrane bound compartments and is implicated in intracellular transport. Comprehensive analysis of clathrin-mediated endocytosis in fibroblasts of patients with LS did not reveal any difference in trafficking of epidermal growth factor, low density lipoprotein or transferrin, compared to normal fibroblasts. However, LS fibroblasts displayed reduced mannose 6-phosphate receptor (MPR)-mediated re-uptake of the lysosomal enzyme arylsulfatase B. In addition, endosome-to-trans Golgi network (TGN) transport of MPRs was decreased significantly, leading to higher levels of cell surface MPRs and their enrichment in enlarged, retromer-positive endosomes in OCRL-depleted HeLa cells. In line with the higher steady-state concentration of MPRs in the endosomal compartment in equilibrium with the cell surface, anterograde transport of the lysosomal enzyme, cathepsin D was impaired. Wild-type OCRL counteracted accumulation of MPR in endosomes in an activity-dependent manner, suggesting that PI(4,5)P(2) modulates the activity state of proteins regulated by this phosphoinositide. Indeed, we detected an increased amount of the inactive, phosphorylated form of cofilin and lower levels of the active form of PAK3 upon OCRL depletion. Levels of active Rac1 and RhoA were reduced or enhanced, respectively. Overexpression of Rac1 rescued both enhanced levels of phosphorylated cofilin and MPR accumulation in enlarged endosomes. Our data suggest that PI(4,5)P(2) dephosphorylation through OCRL regulates a Rac1-cofilin signaling cascade implicated in MPR trafficking from endosomes to the TGN.

STATEMENT: *"Our work describes a new regulatory mechanism of how deficiency of OCRL (oculocerebrorenal syndrome of Lowe) leads to abnormal trafficking processes in the cell. We identified a yet unappreciated connection between molecules implicated in intracellular transport processes and those required for reorganization of the actin cytoskeleton and demonstrate that the catalytic activity of OCRL regulates this pathway. Collectively, our data shed new light on the molecular basis underlying the rare X-linked oculocerebrorenal syndrome of Lowe.This was an interdisciplinary work between the Institute of Human Genetics, the Department of Biochemistry and Molecular Cell Biology and the Department of Biochemistry, Children's Hospital, at the University Medical Center Hamburg-Eppendorf and the Department of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA."*

BACKGROUND: This work was part of the PhD thesis of Vanessa A. van Rahden within the DFG research training group “Sorting and Interactions Between Proteins of Subcellular Compartments” (GRK 1459), headed by Professor Dr. Thomas Braulke. The work was performed at the Institute of Human Genetics in the group of Kerstin Kutsche who holds a professorship at UKE since 2004. The team of Kerstin Kutsche has strong research interests in uncovering the genetic basis of rare human diseases and understanding their underlying pathophysiological mechanisms.