Systematic analysis of myotubularins: heteromeric interactions, subcellular localization and endosome-related functions

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The myotubularins are a large family of lipid phosphatases with specificity towards PtdIns3P and PtdIns(3,5)P₂. Each of the 14 family members bears a signature phosphatase domain, which is inactive in six cases due to amino acid changes at the catalytic site. Fragmentary data have indicated heteromeric interactions between myotubularins, which have hitherto paired an active family member with an inactive one. In this study we have conducted a large-scale analysis of potential associations within the human myotubularin family, through directed two-hybrid screening and immunoprecipitation of epitope tagged proteins. We have confirmed all previously reported combinations and identified novel heteromeric interactions; MTMR8 with MTMR9 and MTMR3 with MTMR4, the first such combination of enzymatically active MTMs. We also report the capacity of several family members to self-associate, including MTMR3 and MTMR4. Sub-cellular localisation studies reveal a unique distribution of MTMR4 to endosomal structures, the major site of substrate lipid accumulation. All active MTMs we have tested (MTM1, MTMR2-4) reduce endosomal PtdIns3P levels upon over-expression. Amongst these, only MTMR4 induces a pronounced defect in the EGF receptor degradation pathway, which is independent of phosphatase activity, but requires an intact FYVE domain.