A direct interaction between MBNL and SFPQ drives alternative splicing regulation

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Background: Muscleblind-like proteins (MBNLs) are key regulators of RNA metabolism that drives tissue-specific alternative splicing (AS), generating multiple mRNA isoforms from a single gene and expanding proteomic diversity. Dysregulation of MBNL-dependent splicing is a hallmark of myotonic dystrophy type 1 (DM1), the most common adult muscular dystrophy. DM1 is an autosomal dominant neuromuscular disorder caused by CTG repeat expansion (>50) in the 3'UTR of DMPK gene, producing toxic RNAs. These RNAs sequester MBNL proteins, causing widespread splicing dysregulation. Despite many characterized MBNL-dependent events, protein interaction networks regulating MBNL activity remain poorly understood. Method: To gain mechanistic insight into MBNL activity, we aimed to identify the interactome of MBNL1, one of three MBNL paralogs, characterizing novel proteins regulating AS. We performed proteomic screening using BioID2 proximity labeling and mass spectrometry to identify MBNL1-interacting proteins. Candidate interactions were validated using an orthogonal GFP-trap pull-down, and domainmapping analyses were conducted to determine regions required for interaction. To investigate the functional relevance of identified interactors, we knocked down MBNLs, the candidate gene, or both in a cellular model and analysed transcriptomic changes using RNA-seq. Results: Proteomic screening identified Splicing Factor Proline and Glutamine Rich (SFPQ) as a previously unrecognized MBNL1 interaction partner. The MBNL1–SFPQ interaction, validated by GFPtrap pull-down, revealed partial RNA-dependence of complex formation. Domain-mapping showed that the N-terminus of MBNL1 is required for binding, while C-terminus deletion unexpectedly strengthened the interaction, suggesting a potential modulatory role of this region. Transcriptomewide RNA-seq revealed transcripts co-regulated by both MBNL1 and SFPQ, including several DM1-associated splicing events. Conclusion: Coregulation of disease-relevant targets suggests that SFPQ influences MBNL-dependent splicing defects, identifying it as a novel component of the MBNL1 regulatory network. Further investigation of the functional impact of the MBNL1–SFPQ interaction in pathological settings may reveal a novel modulatory pathway with potential therapeutic relevance.