The Interactome of Dux4 Reveals an Inherent Feedback Mechanism by RFPL4

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The ectopic expression of DUX4 in skeletal muscle cells results in extensive gene induction that is harmful to the cells. In order to trace those early toxicity events, several studies have strived to characterize the proteins that directly interact with DUX4. However, these studies reported large sets of putative protein partners, from which it is difficult to identify the few most functionally relevant interactions. We believe that those unspecific interactions are caused by the highly polarized sequence of the DUX4. Here, we have taken several steps to reduce these electrostatic artifacts in AP-MS measurements, and thus provide a rigorous analysis of the DUX4 interactome. Surprisingly, we find that the strongest interaction of DUX4 is with members of the RFPL4 family, a set of genes strongly induced by DUX4. We have localized the DUX4-RFPL4 interaction using cross-linking mass-spectrometry (XL-MS) and deletion studies, and found that RFPL4A binds to the disordered region of DUX4. Although the RFPL4 family is poorly-characterized functionally, its sequence homology strongly suggests it to belong to the E3-ubiquitin ligase class, and thus it may be involved in DUX4 ubiquitin-dependent degradation. Hence, these results suggest that DUX4 may be inhibited by its own activation products, explaining its pulse-like expression profiles. Our findings reveal a novel regulatory pathway of DUX4 that may be employed in the future to inhibit the toxicity of DUX4.

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