DUX4 protein partners in muscle cells are linked to DNA repair, transcription and DUX4 post-translational regulation.

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Last year, we presented interactors of DUX4, a highly polarized protein, using a specific AP-MS measurements that reduce electrostatic artifacts. We now confirmed in muscle cells the previous interactors found using HEK293 cells. The C-terminal activation domain of DUX4 strongly interacts not only with p300/CBP, but also with the MED15 and MED25 subunits of the mediator complex, indicating that DUX4 can directly recruit the transcription machinery to the promoters of induced genes, further explaining its potency as transcription activator. DUX4 also interacts with PTOV1, a MED25 homologue, that is endogenously expressed in myoblasts, but of unknown function. The N-terminal DNA-binding region of DUX4 interacts most strongly and specifically with factors involved in DNA double-strand breaks (DSB) repair: C1qBP, XRCC5/6, PARP1 and H2AX. Their interaction with DUX4 may impact their functions and explain the DSB stress that follows DUX4 expression. Interestingly, the strongest interaction of DUX4 is with the RFPL4A protein, encoded by a DUX4-target genes. RFPL4A is the first protein we identified as an interactor of the DUX4 disordered region. As RFPL4A was suggested to be an E3-ubiquitin ligase, DUX4-RFPLA4 interaction might induce DUX4 degradation and co-immunofluorescence allowed their nuclear co-detection only in some dox-treated iLHCN-M2-iDUX4 cells. Overall, our findings extend the model of DUX4 activation in several important aspects and reveal new regulatory elements.