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### ► To cite this version:

Cécile Polge, Roza Leulmi, Christiane Deval, Agnes Claustre, Lydie Combaret, et al.. Looking for E2s enzymes interacting with the E3 ubiquitin ligase MuRF1 during muscle wasting. EMBO workshop, Advances in protein–protein interaction analysis and modulation, Sep 2014, Hyères, France. , 2014, Advances in protein–protein interaction analysis and modulation. hal-02742389

**HAL Id: hal-02742389**

**<https://hal.inrae.fr/hal-02742389v1>**

Submitted on 3 Jun 2020

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## Looking for E2s enzymes interacting with the E3 ubiquitin ligase MuRF1 during muscle wasting

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As a result of an imbalance between protein synthesis and/or proteolysis, important protein losses occur during various physiological and pathological situations. The ubiquitin proteasome system (UPS) is recognized as one of the major actor for controlling muscle mass. This proteolytic system controls precisely the activity of numerous cellular proteins that are first tagged by a polyubiquitin chain on an internal lysine. Ubiquitination is based on the sequential action of an E1-activating enzyme, an E2 ubiquitin-conjugating enzyme and an E3 ligase. The E1 ubiquitin-activating enzyme activates the ubiquitin that it is transferred to an E2. E3 ligases recruit and bind specific substrates. However, E2s are generally the main determinants that select the lysine for building ubiquitin chains, and thereby directly control the fate of the substrate. A given E2 can work with different E3s so that combinations between E2s (tens) and E3 ligases (hundreds) allow the targeting of virtually all cellular proteins. MuRF1 (Muscle Ring Finger 1) is one of the two E3 enzymes involved in muscle atrophy process during various catabolic situations. MuRF1 targets major myofibrillar proteins (troponin I, actin, myosin heavy chains, regulatory myosin light chains 1 and 2) for breakdown by the 26S proteasome. Elaborating new strategies to prevent muscle wasting requires information about the precise mechanisms of contractile protein degradation, including the recognition and the ubiquitination steps.

Our main objective is then to identify the E2s working in concert with MuRF1 to target myofibrillar proteins in atrophying skeletal muscles. We focused on 13 E2 enzymes that are abundant in the skeletal muscle and/or up-regulated in atrophying skeletal muscles and determined the expression levels of these enzymes in catabolic C2C12 myotubes treated or not with dexamethasone (Dex). One  $\mu$ M Dex increased mRNA levels of five E2s, i.e. UBE2A, UBE2B, UBE2D1, UBE2D2 and UBE2G1. Classical biochemical approach such as pull-down did not allow identifying any MuRF1 interacting partner among these E2s, suggesting that E2/ MuRF1 interactions are weak and/ or transient. We then focused on more sensitive approaches such as Yeast-Two-Hybrid (Y2H) and Surface Plasmon Resonance (SPR). We obtained preliminary results that could lead to the identification of MuRF1 interacting E2s. The affinities between MuRF1 and the different E2s are currently determined using SPR technology. The functionality of the E2/E3 combinations identified will be then examined by *in vitro* ubiquitination assays.