A switch in metabolism underlies the initiation of MyoD transcription in satellite cells

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Adult skeletal muscle stem cells, termed satellite cells (SCs), are derived from the mesoderm during early development and are responsible for the highly regenerative capacity of skeletal muscle. Adult SCs primarily exist in a quiescent state and express the paired homeobox protein Pax7. In response to injury, SCs leave the quiescent state and undergo activation and myogenic specification (determined *via* the expression of MyoD), proliferation and eventually differentiation, fusion and maturation (Brack & Rando, 2012). The proximity of SCs to blood vessels, and the energetic demands imposed during the activation and proliferation of SCs suggest a potential regulatory role of metabolism in SC biology during these early processes. Among others, the NAD^+-dependent histone deacetylase SIRT1 is exquisitely sensitive to modifications in cellular metabolism, and presents as an attractive link to the regulation of transcription. Thus, we hypothesized that altered cellular metabolism may play an important role in SC activation *via* SirT1 mediated changes in transcription.

Compared to FACS-isolated quiescent SCs, SCs plated in growth media for 40 h ("active" SCs) exhibited a decrease in expression of genes that regulate fatty-acid oxidation, and an upregulation of genes that regulate glycolysis. These changes in gene expression in active SCs were accompanied by a 2.5-fold increase in basal glycolytic activity (P<0.05). Interestingly, while there was no change in SIRT1 protein expression in active SCs, we observed a dramatic decrease in activity - measured *via* a 17-fold increase in the global acetylation of the SirT1 histone target H4 lysine 16 (H4k16ac). Further, we determined that the decrease in SirT1 activity was likely a result of a 10-fold decrease in NAD levels in active SCs. Importantly, chromatin immunoprecipitation identified that H4k16ac was enriched on the promoter and gene body of MyoD in active SCs, indicating that SirT1, *via* H4k16ac may be linked to MyoD transcription.

To evaluate the role of SIRT1 *in vivo*, we generated Pax7-Cre:$SIRT1^{fl/fl}$ (scKO) mice wherein SirT1 is ablated in a Pax7 dependent manner. Adult scKO mice had a 10-20% reduction in skeletal muscle mass, with a concomitant decrease in fiber CSA, compared to littermate control mice (P<0.05). Interestingly, SCs isolated from scKO mice had elevated global H4k16ac and were prone to spontaneous activation, as revealed by aberrant MyoD staining. ChIP experiments confirmed a specific enrichment of H4k16ac on the MyoD promoter and gene body in scKO SCs.

Finally, we used the myogenic C2C12 cell line to confirm a link between metabolism, SirT1 activity and MyoD transcription. Actively proliferating control and SirT1 shRNA C2C12 cells were cultured for 3 hours in either a glucose (25mM) growth media or a galactose (10mM) growth media. Incubation of C2C12s with galactose resulted in a dramatic shift from glycolysis to oxidative phosphorylation for ATP generation, which led to increased levels of NAD^+. Control C2C12 cells incubated with galactose exhibited a significant reduction in both global H4k16ac and MyoD protein, and a 2-fold decrease of H4k16ac on the MyoD promoter and gene body. In contrast, SirT1 shRNA C2C12 cells incubated with galactose did not exhibit any change in either global H4k16ac or MyoD protein, and no difference in H4k16ac could be detected on the MyoD promoter or gene body.

These exciting results demonstrate for the first time that SC activation can be controlled *via* changes in metabolism, and that this process is regulated, at least in part, *via* reduced SirT1 deactylation of H4k16 on the MyoD promoter and gene body. A better understanding of the link between metabolism and transcription in SCs may lead to more efficient transfer therapies for numerous muscular pathologies.

Brack AS & Rando TA (2012). Tissue-specific stem-cells: lessons from the skeletal muscle satellite cell. *Cell Stem Cell* **10**, 504-514.

JGR is supported by an Overseas Biomedical Research fellowship from the NH&MRC