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Ribosome biogenesis during skeletal muscle hypertrophy

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ABSTRACT

Muscle adaptation to chronic resistance exercise (RE) is the result of a cumulative effect on gene expression and protein content. Following a bout of RE, muscle protein synthesis increases and, if followed by consecutive bouts (training), protein accretion and muscle hypertrophy develops. The protein synthetic capacity of the muscle is dictated by ribosome content. Therefore, the general aim of this thesis is to investigate the regulation of ribosome biogenesis during skeletal muscle hypertrophy.

To begin addressing this question, we employed a prevalent rodent model of skeletal muscle hypertrophy, synergist ablation (SA) of plantar flexor muscles. SA resulted in muscle hypertrophy with a concomitant increase in total RNA content. We observed a marked re-induction of c-Myc in overloaded skeletal muscle correlating with the expression of Pol I specific factors and 45S pre-rRNA levels. UBF and WSTF were increased at the protein level in myonuclei and enriched at the rDNA promoter following mechanical loading. This was associated with increased Pol I loading and epigenetic marks of active, de-condensed chromatin at the rDNA promoter. Similarly, acute mechanical loading of human skeletal muscle resulted increased mTOR signaling, a re-induction of c-Myc and increased 45s pre-rRNA abundance. Once the hypertrophic phenotype was evident in both mouse and human, rDNA transcription had returned to baseline levels. A conditional, skeletal muscle specific c-Myc knockout model was generated to investigate the mechanistic importance of c-Myc in ribosome biogenesis and hypertrophy. Animals lacking c-Myc in skeletal muscle displayed normal post-natal development with respect to body weight, muscle size, rDNA transcription and RNA content. To further challenge the growth machinery in skeletal muscle lacking c-Myc, animals were subjected to SA-imposed overload. No difference with respect to RNA accumulation or hypertrophic response was detected, indicating that c-Myc is dispensable for cellular hypertrophy in terminally differentiated muscle cells. These results were verified in C_2C_{12} myotubes with compromised c-Myc function (Myra-A). On the contrary, c-Myc inactivation in proliferating C_2C_{12} myoblasts severely compromised rDNA transcription, DNA synthesis as well as cell proliferation. Thus, our data suggests cell stage-specific effects of ablated c-Myc function in cells of the myogenic lineage. Moreover, the importance of the mTOR network for rDNA gene regulation during skeletal muscle hypertrophy was investigated. mTOR inhibition using rapamycin prevented the development of hypertrophy, and decreased mTOR binding to rDNA correlated with decreased 45S pre-rRNA synthesis and perturbed rRNA accumulation. Selective inhibition of RNA Pol I with CX-5461 efficiently prevented skeletal muscle hypertrophy in a similar fashion.

Collectively, the data presented in this thesis proposes an important role for ribosome biogenesis at the onset of skeletal muscle hypertrophy, which, if blocked, prevents the development of the hypertrophic phenotype. During the time in which measurable hypertrophy is evident, rRNA synthesis rates have normalized. In addition, our results indicate that mTOR regulates this process via numerous different mechanisms, including direct binding to the rDNA promoter but likely not via p70S6K1-dependent functions. c-Myc proved dispensable for Pol I transcription and skeletal muscle hypertrophy in the differentiated state both in vivo and in vitro, instead controlling cell proliferation in myoblasts, likely via a Pol I-dependent mechanism.

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