Insights into the role and regulation of the Translationally Controlled Tumor Protein in skeletal muscle

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The Translationally Controlled Tumor Protein (TCTP) is up regulated in tumor cells and is stimulated by mitogens/serum. Inhibition of the mechanistic target of rapamycin (mTOR) with rapamycin inhibits serum-stimulated increases in TCTP protein in cultured cells. Recent evidence suggests that TCTP may also indirectly stimulate mTOR by activating the small GTPase, Rheb. This has led to suggestion of the possibility of a positive feedback loop whereby mTOR-dependent increases in TCTP activates Rheb, leading to increased/prolonged mTOR signaling (Kobayashi *et al.*, 2014). In skeletal muscle, such a mechanism could help to explain how acute high-force contractions can promote a highly prolonged activation of mTOR signaling. To date, however, very little is known regarding the regulation and the role of TCTP in skeletal muscle. Therefore, the aims of this study were to determine: 1) whether TCTP protein is up-regulated, in an mTOR-dependent manner during skeletal muscle growth and 2) whether transient *in vivo* overexpression of TCTP is sufficient to activate mTORC1 signaling, increase protein synthesis and induce skeletal muscle fibre growth *in vivo*.

All methods were approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison. Female wild type (WT) and transgenic FVB/N mice with human skeletal actin promoter driven expression of a rapamycin-resistant (RR) or rapamycin-resistant kinase dead (RRKD) mutant of mTOR (8–10 wk) were used in this study. Before all surgical procedures, mice were anaesthetized with an intraperitoneal (IP) injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Vehicle (DMSO) or rapamycin (0.6 mg/kg) solutions were administered *via* IP injection. Synergist ablation (SA) surgeries, *in vivo* transfection *via* electroporation and the measurement of rates of protein synthesis using the SUnSET technique were performed as previously described (Goodman *et al.*, 2011a; Goodman *et al.*, 2011b; Goodman *et al.*, 2013).

Chronic mechanical overload-induced growth led to a 3.4-, 3.3- and 3.7-fold increase in TCTP protein in *plantaris* muscles from vehicle treated WT, RR and RRKD mice, respectively, compared to their Sham vehicle controls. TCTP protein in muscles from overloaded and rapamycin treated WT mice was ~33% lower than that in overloaded and vehicle treated muscles. Moreover, rapamycin's inhibitory effect was rescued in muscles from RR, but not RRKD, mice. Next, *tibialis anterior* (TA) muscles were co-transfected with GST-tagged p70 S6 kinase 1 (GST p70S6K1), and green fluorescent protein (GFP), HA-tagged Rheb or HA-tagged TCTP. After 3 d, muscles were collected and analyzed by western blot for changes in the phosphorylation of GST P-p70S6K1$^{(389)}$ as a marker of mTOR signaling. Rheb induced a ~2.6 fold increase in GST-p70S6K1$^{(389)}$ phosphorylation compared to the GFP control; however, TCTP reduced GST-p70S6K1$^{(389)}$ phosphorylation to ~35% of the GFP control. TA muscles were then transfected with plasmid DNA encoding WT HA-tagged TCTP or a HA-tagged E12V TCTP mutant, which is reported to abolish TCTP's ability to activate Rheb. Muscles were collected at 7 d post-transfection for measurements of muscle fibre cross-sectional area (CSA) and rates of protein synthesis using the SUnSET technique. The CSA of WT and E12V TCTP positive fibres were 22% and 17% larger, respectively, than non-transfected fibres from the same sections. Rates of protein synthesis in WT and E12V TCTP transfected fibres were 11.1% and 16.6% lower than LacZ transfected control fibres.

In summary, TCTP protein is up regulated during mechanically-induced muscle growth *via* a mechanism that is, in part, rapamycin-sensitive and mTOR kinase-dependent. TCTP is not sufficient to activate mTOR signaling and, thus, TCTP is unlikely to play a role in a positive feedback loop that involves the activation of the Rheb GTPase in skeletal muscle. Finally, the overexpression of TCTP is sufficient to induce muscle fibre growth that is independent of increases in global rates of protein synthesis.

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