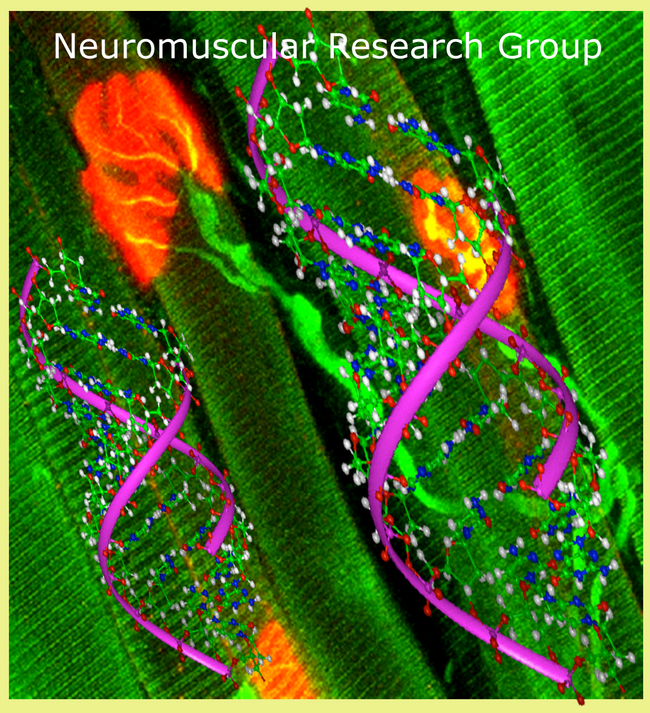
C2 Centre for Chronic
D2 Diseases & Disorders



A molecular link between the sarcomeric z-disc and skeletal muscle hypertrophy

Laura Faas, Xiang Li and Gonzalo Blanco

Department of Biology, University of York

Background:

- Muscle wasting is a known morbidity factor in many clinical or behavioural conditions involving chronic muscle paralysis and there are no effective countermeasures to prevent it. To be able to maintain size or grow, skeletal muscles must sense loads; therefore, a link between molecular sensors of mechanical stress and the mTOR pathway, which is necessary for muscle hypertrophy, must exist.
- How mechanical stress of the sarcomere triggers hypertrophy through the mTOR pathway is not yet established. An ideal venue for sensing mechanical stress is the z-disc, since it lies in series with the force-generating sarcomeres, experiences force directly and contains a structural scaffold that can modulate mechanical sensors.
- We have previously described that mice that lack KY protein in their muscles fail to respond to stimuli that would normally make muscles grow, such as chronic overload (Blanco et al HMG, 2001). The KY protein is, to our knowledge, the only example of a skeletal muscle z-disc protein underlying a blunted hypertrophic response in adult mice
- We have previously shown that KY is a novel component of the z-disc in in vitro culture (Baker et al, Exp Cell Res, 2010), thus establishing a link between hypertrophy and a z-disc defect in skeletal muscle.
- All identified partners of KY in the Yeast-Two-Hybrid system present a similar domain structure characterized by repeats of immunoglobulin and fibronectin-like domains (Beatham et al, HMG, 2004; see Figure 1). In skeletal muscle, some of these proteins act by cross-linking cytoskeletal structures.

Objectives. We aim at revealing the mechanotransduction mechanism involved in sensing loads and converting a mechanical input into the activation of cellular pathways culminating in bespoke muscle adaptation.

Methodology. Whole muscles are transiently electroporated *in vivo* with recombinant versions of the KY protein in order to characterize its subcellular localization dynamics and identify proteins that associate with it. The expression of previously identified Yeast-Two-Hybrid putative partners, in particular the actin crosslinker filaminC and Xin, is also analysed in muscles from *ky/ky* mice to reveal the effect that the absence of the KY protein has on these cytoskeleton crosslinking proteins.

The KY protein interacts with proteins containing repeats of globular domains

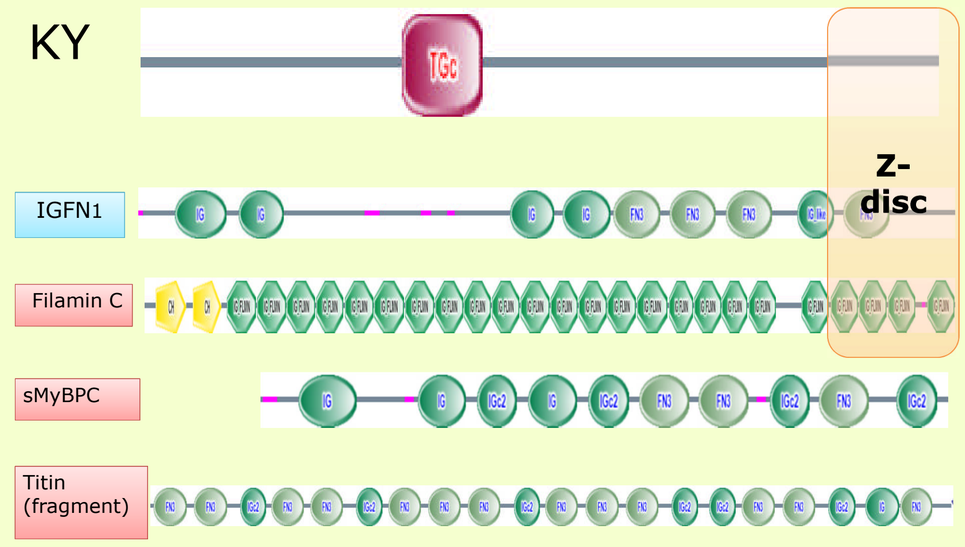


Figure 1. Y2H partners of KY identified using a human skeletal muscle cDNA library. All partners present repeats of globular domains regularly spaced. IGFN1 is a novel protein that was identified in this screen. KY, IGFN1 and FLNC locate to the z-disc.

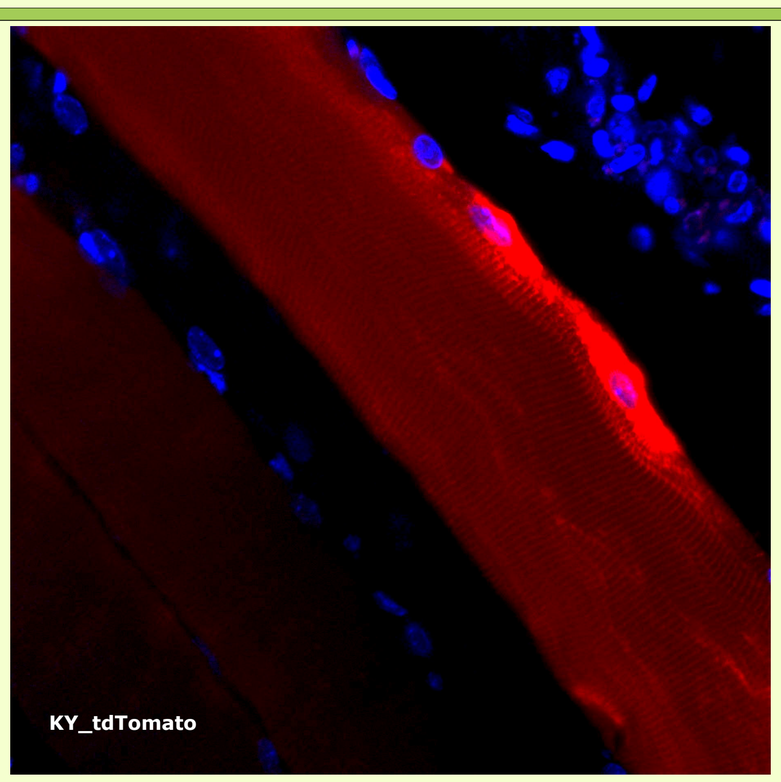


Figure 2. A confocal image of a whole mount from the EDL muscle electroporated with Ky_tdTomato. Note that, in addition to locating to z-disc striations, Ky_tdTomato appears to be weakly diffused throughout the sarcoplasm. The image shows also strong cytoplasmic staining of two apparently fusing satellite cells, most likely electroporated prior to fusion. Interestingly, the myofibril underlying these satellite cells shows distinctively higher expression of Ky_tdTomato. Note also that the large size of the electroporated fibre compared to the adjacent non-electroporated one.

Transient in-vivo overexpression of KY causes fibre hypertrophy

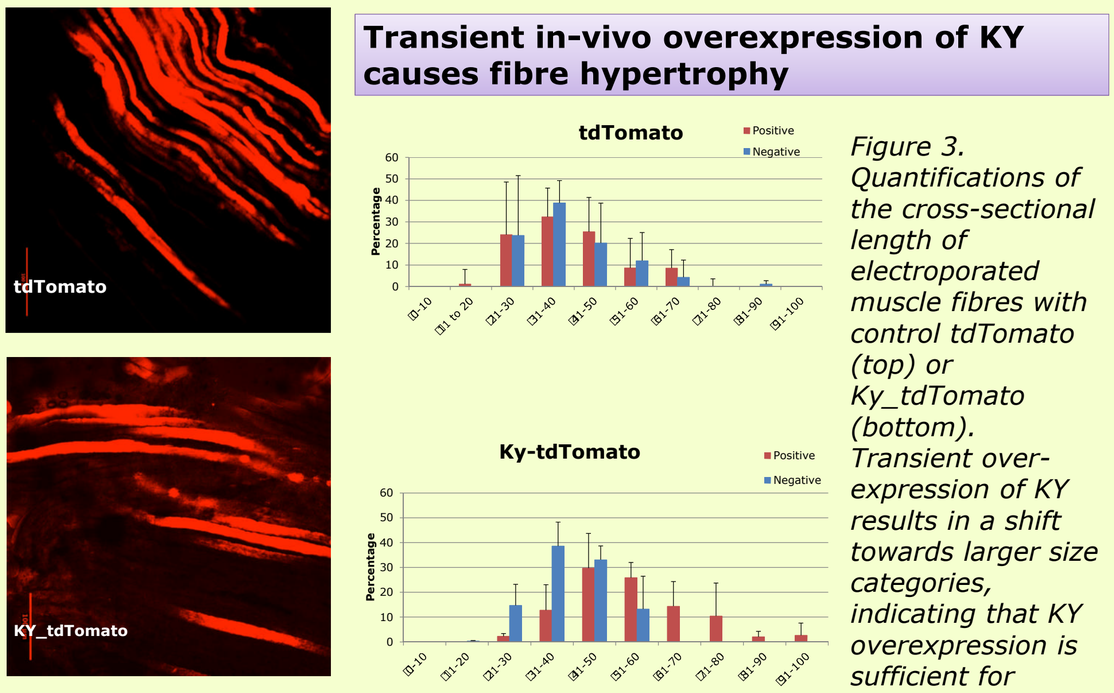


Figure 3. Quantifications of the cross-sectional length of electroporated muscle fibres with control tdTomato (top) or Ky_tdTomato (bottom). Transient over-expression of KY results in a shift towards larger size categories, indicating that KY overexpression is sufficient for muscle fibre hypertrophy.

The cytoskeletal crosslinkers *FLNC* and *Xin* aberrantly accumulate in *ky/ky* soleus muscle

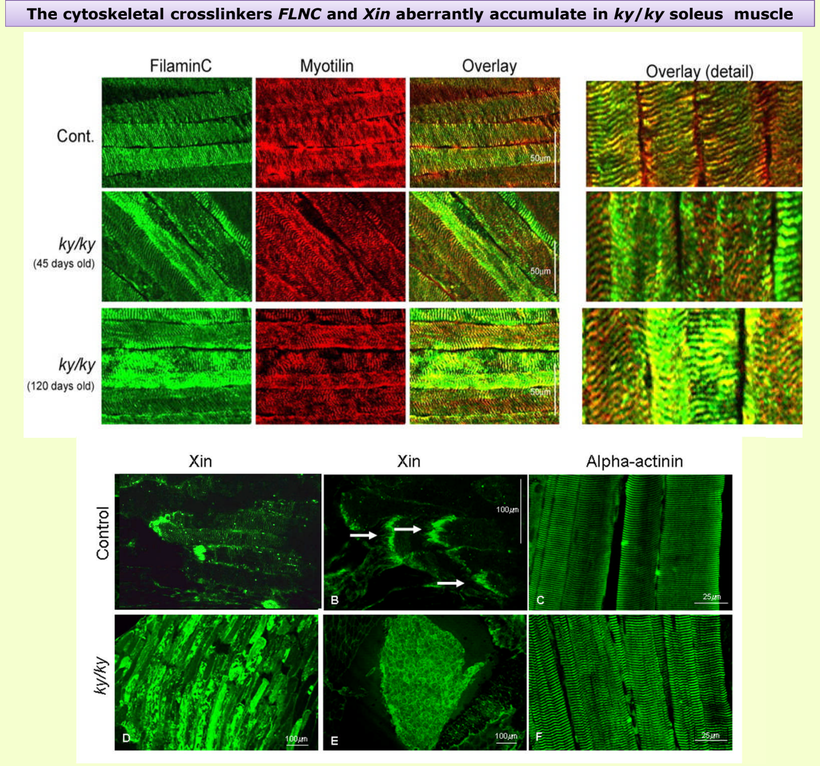
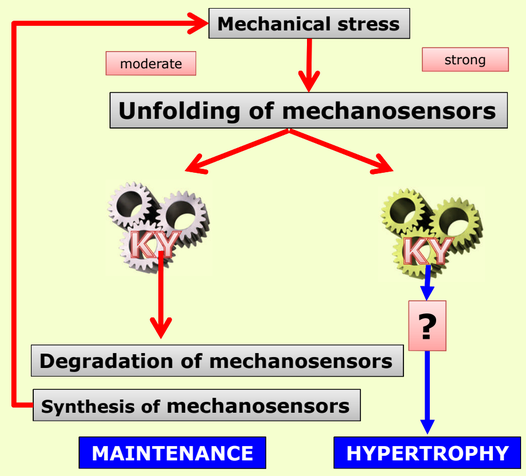


Figure 4. FlnC localizes in control muscle to the z-disc, but in soleus ky/ky muscle, FlnC (top panels) accumulates in smears, granules and thicker z-discs. Xin localizes to the myotendinous junction and the z-disc, but in soleus ky/ky muscle, Xin appears to accumulate aberrantly (D, E). Thus, the absence of KY appears to lead to the accumulation of specific cytoskeletal crooslinkers, suggesting that KY is required for the normal turnover of these proteins.

Is KY required for chaperone assisted authophagy *AND* hypertrophy?



A chaperone assisted autophagy mechanism termed CASA has recently been proposed as a mechanotransduction pathway essential for muscle maintenance (Current Biology, 2013 Vol 23 No 5). CASA involves coupling of the autophagic degradation of an unfolded cytoskeletal crosslinker, which would accumulate as a result of excessive cytoskeletal tension, to the upregulation of the same crosslinker gene. Since FlnC aberrantly accumulates in ky/ky muscles and has been shown to be a CASA target, we propose that KY is an essential component of this mechanotransduction pathway.

Moreover, since KY is required and sufficient for hypertrophy, KY is likely to be a mediator of a sarcomeric stress-dependent activation of the mTOR pathway. We suggest that hypertrophy, as opposed to muscle maintenance, could be triggered if a higher threshold of unfolding, which would occur under chronic overload conditions, is reached.

Soluble recombinant KY_V5 can be immunoprecipitated from electroporated muscles

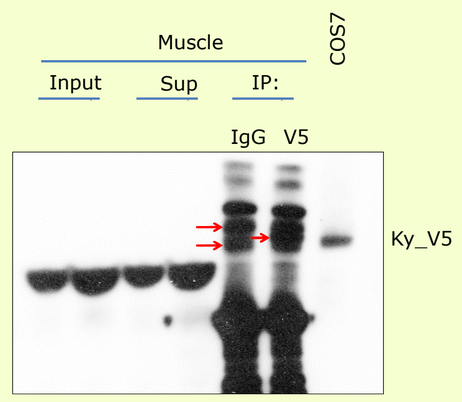


Figure 5. KY has not been previously detected in the soluble extracts from adult skeletal muscles, only the cytoskeletal fraction showed the corresponding band on WBs (not shown). Here, the soluble extracts from the electroporated EDL muscle (Input) also fails to show any recombinant KY. However, a band identical to the band from transfected COS7 cells with same construct and of the correct size is detected in the immunoprecipitated pellet (V5).

Successful immunoprecipitation brings the possibility of proteomic analysis upon appropriate scaling up of the experimental conditions. Future analysis of the immunoprecipitated proteins will allow us to detect endogenous partners of KY and test the hypothesis of KY being an essential component of the chaperone assisted autophagy pathway and a link to mTOR activation.

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