Visualizing the conformation of titin in live *C. elegans* body wall muscle

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Abstract

Titin is a giant, modular protein that connects the myosin – containing thick filament to the Z-line of the sarcomere (mammalian cells) or dense bodies (Z-line equivalent in the nematode worm *C. elegans*). In vertebrates, the assembly, organization, and mechanical properties of Titin have been studied extensively, but little is known about how the TTN-1 ortholog functions in *C. elegans*. To investigate the functional role of TTN-1 in *C. elegans* muscles, we used nested CRISPR gene editing to generate translational fusions of TTN-1 with a fluorescent tag (wrmScarlet) at either the N-terminus (exon 1) or in the middle (exon 25) of the molecule. By confocal microscopy, wrmScarlet appeared as narrow, continuous striations within body wall muscle (BWM) in all stages from late embryos to adults for both modifications. By super-resolution instant Structured Illumination Microscopy (iSIM), wrmScarlet appeared as non-uniform, yet relatively continuous striations, distinct from the punctate appearance of alpha-actinin or integrins located within dense bodies. Our data indicates that the N-terminus of TTN-1 is not restricted to the dense bodies and suggests that TTN-1 is organized into a semi-continuous structure that extends between dense bodies may help sarcomeres form oblique striations. Our gene edited TTN-1 strains will be useful for further investigating how BWM sarcomere lengths respond during movement and growth.

Figure 1

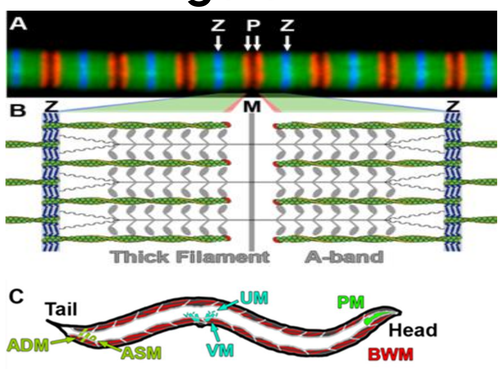

Figure 1.
A. Vertebrate striated myofibril. **B.** Vertebrate sarcomere structure **C**. Anal Depressor Muscle, Anal Sphincter Muscle, Vulval Muscle, Uterine Muscle, Pharyngeal Muscle, and the Body Wall Muscle

Introduction

Vertebrate striated myofibrils produce the contractile forces that power movement, consisting of repeating, contractile units (sarcomeres) composed of actin (thin) and myosin (thick) filaments (Fig. 1) that move past each other during myofibril shortening and lengthening. A flexible protein scaffold connects and aligns thin and thick filaments into uniform myofibrillar arrays (Benian and Qadota, 2005). In vertebrates, the elastic N-terminal (I-band) region of the giant (3 MDa) protein titin extends from Z-lines (similar to DBs) to the thick filament ends and is responsible for passive tension along the myofibril. The conserved C-terminal (A-band) region of titin spans the thick filament from the ends to the central M-line and specifies thick filament lengths (Benian and Qadota, 2005). Titin belongs to a super family of proteins that share structural similarities such as with the calcium stretch-activated Myosin light chain kinase (MLCK) (Bang et al., 2001). Invertebrates contain titin-like homologous elastic proteins such as projectin in Drosophila, twitchin and Ce-titin in *Caenorhabditis elegans* (*C. elegans*) indicating that these proteins may share an evolutionary relationship with vertebrate titin (Bullard et al., 2002). *C. elegans* nematodes are an ideal animal model to study striated muscle structure and function. For this project, Body wall muscle (BWM) is the primary focus because of its oblique striations. BWM sarcomeres are composed of dense bodies (DBs), a short I-band, and a long thick filament within the A-band. Compared to vertebrate titin, the titin isoform in *C. elegans* (Ce-TTN1) is shorter in length, extending only from the DBs to the thick filament ends (Benian et al., 2010).

Experimental Plan

Determine the native range of Ce-TTN1 within sarcomeres using spinning disk confocal microscopy

- to measure the locations of fluorescently tagged Ce-TTN1 relative to dense bodies and thick filaments in *C. elegans* larvae and adults.
- We used Nested CRISPR gene editing (Fig. 2) to generate translational fusions of Ce-TTN1 and the red fluorescent protein wrmScarlet at exon 1 (N terminus) and exon 25 (middle)
- wrmSct-Ce-TTN1 N, M, and C strains will be crossed with a fiduciary marker strain :
  RSL 69 (DB and thick filaments tagged with GFP)
- Spinning disk confocal microscopy will be used to image the colocalization of the fiduciary marker strain crossed with Ce-TTN1n strains (wSctTTN1n-TTN1c) to confirm its expected functions such as linking the dense bodies to the thick filaments.
- To observe or confirm how Ce-TTN1 is involved in muscle assembly similarly to vertebrate TTN1, we will use confocal microscopy to visualize positions of Ce-TTN1 during early larval stages.

Methods

Figure 2

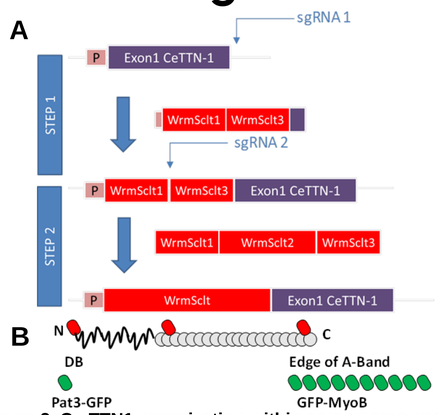

Figure 2. Ce-TTN1 organization within sarcomeres and Nested CRISPR procedure
A) Generation of fluorescent proteins by Nested CRISPR for Ce-Ttn1n **B)** The N-terminal end (N) of Ce-TTN1 is located at dense bodies (DB) and the C-terminal end (C) is located near the end of the A-band. Red ovals indicate where the wSct-tags are expected to be located. Green ovals indicate where sarcomere markers (Pat3-GFP and GFP-MyoB) are located. Flexible and non-flexible regions of titin are indicated with, lines and circles, respectively.

Figure 3

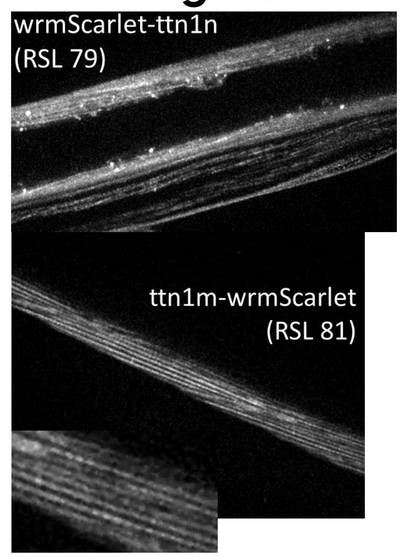

Figure 3. Spinning Disk Confocal image of RSL 79 and RSL 81 strains. A) Ce-TTN1 tagged with wrmScarlet at exon 1 (N-terminus) B) Ce-TTN1 tagged with wrmScarlet at Exon 25 (Middle) both show continuous striations with a dot-like appearance, but differ from the punctate appearance of the DBs

Preliminary Data

Table 1

Table 1. Strains generated

Strain	Phenotype	Source
RSL 58	GFP-unc-54 (green fluorescent protein expressed in Myosin B gene)	Littlefield Lab
RSL 60	pat-3-GFP (green fluorescent protein expressed in beta-integrin gene with red fluorescent nuclei)	Littlefield Lab
RSL 67-68	Ttn-1c-wrmscarlet1-3 insert (consists of partial wrmScarlet sequence inserted at exon 64 of Ce-TTN1)	This project
RSL 69	Cross between RSL 58 and RSL 60 (Fiduciary marker strain)	This project
RSL 71	wrmScarlet1-3-Ttn-1n (consists of partial wrmScarlet sequence inserted at exon 1 of Ce-TTN1)	This project
RSL 72-73	Ttn-1m-wrmScarlet1-3 (consists of partial wrmScarlet sequence inserted at exon 25 of Ce-TTN1)	This project
RSL 76-79	wrmScarlet-Ttn-1n (consists of full length wrmScarlet sequence inserted at exon 1 of Ce-TTN1)	This project
RSL80-82	Ttn-1m-wrmScarlet (consists of full length wrmScarlet sequence inserted at exon 25 of Ce-TTN1)	This project

Literature Cited

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Benian G.M., Forbes J.G., Flaherty D.B., Ma K., Qadota H., and Wang K. *Extensive and modular intrinsically disordered segments in C. elegansTTN-1 and implications in filament binding, elasticity and oblique striation* J Mol Biol. 2010 May 21; 398(5): 672–689

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