doi:10.1017/S1431927620017626
Microsc. Microanal. 26 (Suppl 2), 2020

Actin Filaments in Flight Muscle Z-disks of *Lethocerus indicus* Show Screw Symmetry, Not Rotational Symmetry

Fatemeh Abbasi Yeganeh, Corrine Summerill, Dianne Taylor, Hamidreza Rahmani and Kenneth Taylor

Florida State University, Tallahassee, Florida, United States

The Z-Disc is an important structure that defines the ends of the sarcomere in striated muscle, which is composed of myosin (thick) and actin (thin) filaments and has many proteins involved in myofilament stability, signaling, and maintenance of the sarcomere. The flight muscle Z-disks of the large waterbug *Lethocerus indicus* as well as those of the honey bee *Apis mellifera* differ from those of vertebrate striated muscle in that actin filaments are arranged in a trigonal lattice rather than a quasi tetragonal lattice. There are three-dimensional models and simple diagrams of the Z-Disc available from vertebrate muscle samples, such as from fish fin muscle[1], rat soleus muscle[2]and human patients with nemaline myopathy[3]. Investigation into the structure of insect flight muscle (IFM) Z-Discs, on the other hand, has been relatively sparse, with most of the research done on *Apis* IFM[4][5] more than a quarter century ago. Therefore, the aim of this study was to generate a 3-Dreconstruction of an IFM Z-Disc from another species, *Lethocerus*, to compare with previous results and to determine the efficacy of extending those results using isolated Z-Discs imaged in the unstained, frozen hydrated state. In the IFM Z-Disc, the hexagonal lattice is composed of six thin filaments in the parallel orientation surrounding one thick filament, and these thin filaments will progress through the Z-Disc, overlapping with actin filaments from the adjacent sarcomere, and stopping just before entering the adjacent sarcomere.

In the A-band of *Lethocerus*, the thin filaments are arranged in a left-handed P64 lattice. Previous 3-D image reconstructions from *Apis* Z-disks [5][6] assumed 3-fold rotational symmetry in the Z-disk from which there is no clear path to generating the screw axis of actin filaments around the thick filaments in the A-band. There is ample evidence that the actin filaments in the overlap zone of both bee and waterbug flight muscle present a helical array of target zones around each thick filament. However, Squire [7] has pointed out that Cheng and Dethearage's 3D reconstruction of the honey bee Z-disk, when extrapolated into the A-band, implies rings of target zones rather than helices of target zones surrounding the thick filament. This discrepancy might be resolved if there were 3-fold screw axes at the lattice and trigonal positions of the of the insect Z-band instead of three-fold rotation axes; thus making the insect Z-band $P3_121$, instead of P321 assumed previously [5]. We aim to resolve the actin structure in Z-disc at sufficient resolution to find an answer to this problem. Here we have used cryoelectron tomography of frozen, isolated Z-disks from *Lethocerus* combined with subvolume averaging to determine the F-actin arrangement in the Z-disk. Tilt series are collected on a Titan Krios electron microscope, with a DE-20 camera and merged using PROTOMO. Fourier transforms of the Z-disks show spots from a hexagonal lattice with a spacing of 520Å extending to 87Å. We created a global average of F-actin by identifying each F-actin coordinate and aligning them using multireference alignment of class averages after reorienting the oppositely oriented F-actins from one side of the Z-disk. The global average showed the F-actin long pitch helices clearly with some indication of actin subunits, though the contrast there was not strong. The global average was then transformed back to the coordinates of the raw F-actins in the tomogram. We then searched for trimers of F-actin related by a quasi 3-fold axis, which define the location of the thick filaments. Surrounding the trimers are 6 F-actins of opposite orientation which correspond to the thin filaments in the Z-band. These 6 F-actins followed a P64 lattice which dictates that the oppositely oriented trimers in the Z-disk do not follow a simple rotational symmetry. In our Z-disk reconstruction, actin subunits supposed related by 3-fold symmetry occur at different Z-levels. This means the P312 arrangement assumed previously for the honey bee Z-disc in incorrect. Supported by NIH.

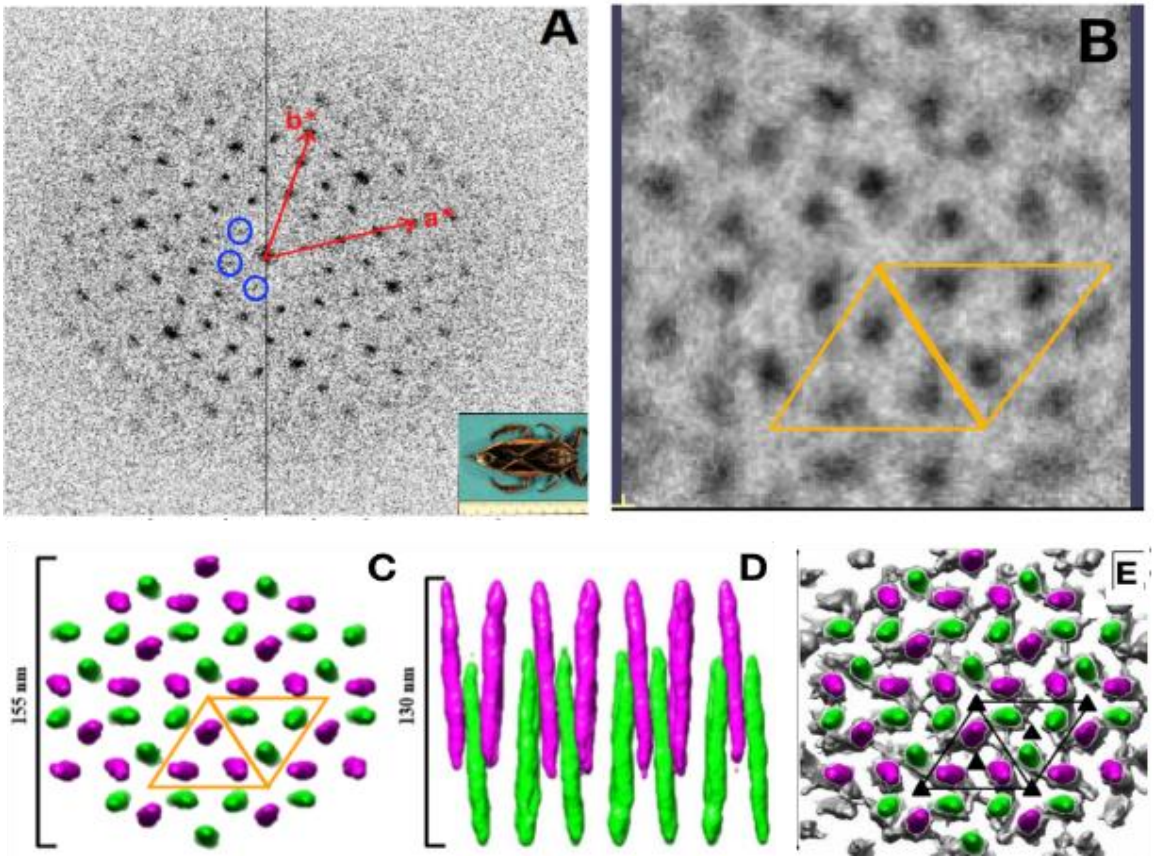

Figure 1. A. Fourier transform of cryo-EM micrograph of a Lethocerus Z-disc which extends to 6 orders. A repeating lattice is demonstrable in the Fourier transform. B. Raw subvolume of Lethocerus Z-disc tomogram showing Top view of IFM Zdisc (z=0) and Arrangement of Thin Filaments in Lethocerus Z-Disc. C. Density map of (B), thin filaments forming a hexagonal lattice arrangement. Each unit cell contain six thin filaments, three filaments each with opposite orientations. D. Side view of C, filaments of opposite orientation overlapped for a distance of ~87 nm, which is a value close to the ~80 nm overlap found in Apis Z-Discs [6]. E. structure of Lethocerus Z-disc showing Unique network of connecting densities that maintains the arrangement of the thin filaments of opposite and same orientations

https://doi.org/10.1017/S1431927620017626 Published online by Cambridge University Press

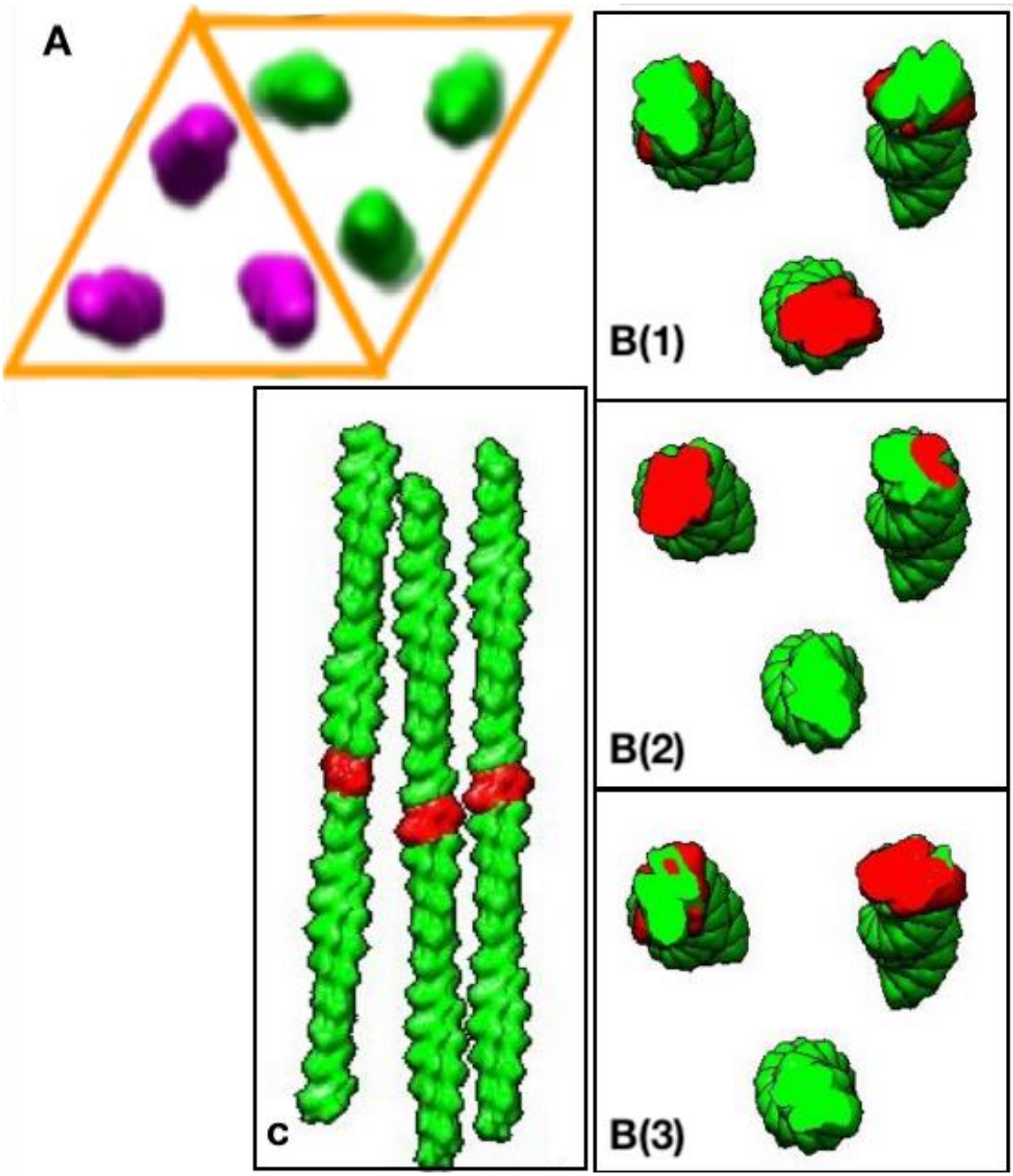

Figure 2. A. unit cell of hexagonal lattice containing six thin filaments, three filaments each with opposite orientations (green and puple show opposite orientations). B(1),(2) and (3). Top views of green trimer in different z-levels, showing that designated subunit starts to appear in different z-levels and not at the same level. C. Side view of trimer actin subunits supposed related by 3-fold symmetry occur at different Z-levels that shows the P312 arrangement assumed previously for the honey bee Z-disc in incorrect.

References

[1] Luther, P. K. (2000). Three-Dimensional Structure of a Vertebrate Muscle Z-band: Implications for Titin and α-Actinin Binding. *Journal of Structural Biology*, *129*(1), 1-16.

https://doi.org/10.1017/S1431927620017626 Published online by Cambridge University Press

[2] Yamaguchi, M., Izumimoto, M., Robson, R.M., & Stromer, M.H. (1985). Fine structure of wide and narrowvertebrate muscle Z-lines. A proposed model and computer simulation of Z-line architecture. *Journal ofMolecular Biology*, *184*(4), 621-643.
[3] Morris, E. P., Nneji, G., & Squire, J. (1990). The three-dimensional structure of the nemaline rod Z-band.*The Journal of Cell Biology*, *111*(6), 2961-2978.
[4] Saide, J. D., & Ullrick, W. C. (1973). Fine structure of the honeybee Z-disc. *Journal of Molecular Biology*,*79*(2), 329-337.
[5] Deatherage, J. F., Cheng, N., & Bullard, B. (1989). Arrangement of filaments and cross-links in the bee flight muscle Z disk by image analysis of oblique sections. *The Journal of Cell Biology*, *108*, 1775-1782.
[6] Rusu, M., Hu, Z., Taylor, K.A. *et al.* Structure of isolated Z-disks from honeybee flight muscle. *J Muscle Res Cell Motil* **38,** 241–250 (2017)
[7] SQUIRE, J. M. (1972) J. molec. Biol. 72, 125-38.

https://doi.org/10.1017/S1431927620017626 Published online by Cambridge University Press