X-RAY INTERFERENCE MEASUREMENTS OF THE MOTION OF THE MOLECULAR MOTOR IN MUSCLE WITH NANOMETER-MICROSECOND RESOLUTION

V. Lombardi [1*], M. Linari [1], M. Reconditi [1], E. Brunello [2], L. Fusi [1], T.C. Irving [3], T. Narayanan [4], G. Piazzesi [1], and M. Irving [2]

[1] *Laboratory of Physiology, Department of Evolutionary Biology, University of Florence, 50019 Sesto Fiorentino, Italy*

[2] *Randall Division of Cell and Molecular Biophysics, King's College London, London, UK*

[3] *BioCAT, Advanced Photon Source Argonne, IL, USA*

[4] *European Synchrotron Radiation Facility, Grenoble, France*

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**) e-mail: vincenzo.lombardi@unifi.it*

In a contracting muscle, arrays of the dimeric motor protein myosin II pull the actin filament towards the centre of the sarcomere (the structural unit of the striated muscle) during cyclical ATP driven interactions. When the external load is smaller than the array force, the sarcomere works as a motor, converting metabolic energy into mechanical work; when the external load is larger than the array force, the sarcomere acts as a brake resisting the load with reduced metabolic cost. Due to the regular arrangement of molecular motors in striated muscle, X-ray diffraction is the best structural technique for imaging muscle contraction at molecular level. The high spacial resolution of the third generation synchrotrons (European Synchrotron Radiation Facility, ESRF, Grenoble, France and Advanced Photon Source, APS, Argonne, IL, USA) allows to record the fine structure of the meridional reflection originating from the 14.5 nm axial repeat of myosin motors, showing that the reflection is split in two peaks by the X-ray interference between the two arrays of myosin motors in each sarcomere [1]. Using the interference effect, the motion of myosin motors can be directly measured with subnanometer precision [2, 3].

Here we use sarcomere-level mechanics and X-ray interferometry in intact single cells from the skeletal muscle of the frog (*Rana temporaria*), to investigate the molecular basis of the work production and the braking action of muscle. During isometric contraction, each motor bears a force of about 6 pN. During shortening against high and moderate loads, the number of myosin motors reduces in proportion to the external load, while individual motors maintain a force of 6 pN pulling the actin filament through a 6 nm stroke [4].

Rapid stretches of 1-5 nm between each overlapping set of myosin and actin filaments in a muscle sarcomere cause the stiffness of the array of myosin motors to double within 2 ms [5]. Simultaneous X-ray interference changes indicate a rapid attachment to actin of the second motor domain of the myosin molecules with the first domain already attached to actin [6]. This mechanism explains the ability of active muscle to efficiently resist a sudden increase in load.

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References

[1] M. Linari, G. Piazzesi, I. Dobbie, N. Koubassova, M. Reconditi, T. Narayanan, O. Diat, M. Irving, V. Lombardi, "Interference fine structure and sarcomere length dependence of the axial X-ray pattern from active single muscle fibres", *Proc. Natl. Acad. Sci. USA* **97 (**2000) 7226-7231.

[2] G. Piazzesi, M. Reconditi, M. Linari, L. Lucii. Y.-B. Sun, T. Narayanan, P. Boesecke, V. Lombardi, M. Irving, "The mechanism of force generation by myosin heads in skeletal muscle", *Nature* **415** (2002) 659-662.

[3] M. Reconditi, M. Linari, L. Lucii, A. Stewart, Y.-B Sun, P. Boesecke, T. Narayanan, R.F. Fischetti, T. Irving, G. Piazzesi, M. Irving. V. Lombardi, "The myosin motor in muscle generates a smaller and slower working stroke at higher loads", *Nature* **428 (**2004) 578-581.

[4] G. Piazzesi. M. Reconditi, M. Linari, L. Lucii, P. Bianco, E. Brunello, V. Decostre, A. Stewart, D. Gore, T. Irving, G. Piazzesi, M. Irving, V. Lombardi, "Skeletal muscle performance determined by modulation of number of myosin motors rather than motor force or stroke size", *Cell* **131** (2007) 784-795.

[5] L. Fusi, M. Reconditi, M. Linari, E. Brunello, R. Elangovan, V. Lombardi, G. Piazzesi, "The mechanism of the increase in resistance to stretch of isometrically contracting single muscle fibres", *J. Physiol.* **588** (2010) 495-510.

[6] E. Brunello, M. Reconditi, R. Elangovan, M. Linari, Y.-B. Sun‡, T. Narayanan, P. Panine, G. Piazzesi, M. Irving, V. Lombardi, "Skeletal muscle resists stretch by rapid binding of the second motor domain of myosin to actin", *Proc. Natl. Acad. Sci. USA* **104** (2007) 20114-20119.