Structure-function relation of the myosin motor in striated muscle

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INTRODUCTION

The aim of our research is to elucidate the mechanism of energy transduction by the molecular motor in muscle (**Fig. 1**).
The working stroke responsible for force generation and interfilamentary sliding is due to a structural change in the globular part of the myosin molecule (the myosin head) cross-linking the myosin and the actin filaments. Both the size of the working stroke and its biochemical, energetic and kinetic features remain controversial.
We use single muscle fibre mechanics and time-resolved interference X-ray diffraction to study the myosin motor *in situ* with sub-nanometer resolution. Our present activity is dedicated to clarify the following problems:

1) the size of the myosin working stroke and its dependence on the load;
2) the conformational change in the myosin head leading to isometric force generation;
3) structural events in the myofilaments during activation of contraction.

The answers to these questions are essential for relating molecular and cellular studies of myosin motors, and for elucidating the mechanism of efficient mechano-chemical coupling.

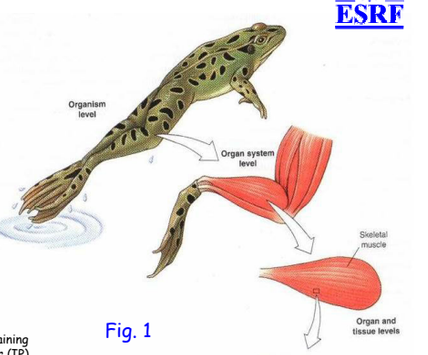


Fig. 1

METHODS

Single fibres from the tibialis anterior muscle of *Rana temporaria* at ~ 2.2 μm sarcomere length are vertically mounted in a trough containing physiological solution between a force transducer and a loudspeaker coil motor (**Fig. 2**). Patterns are collected on the imaging plate detector (IP) or on the image intensified FReLoN CCD detector placed at either 10 m or 3 m. The intensity and low divergence of the beam at ID02 beamline allow to rise the resolution of structural studies of the muscle motor by exploiting X-ray interference from the two arrays of myosin heads in each thick filament (**Fig. 3**).

Fig. 2 Scheme of the setup at the beamline

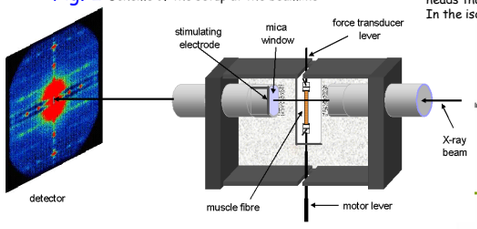
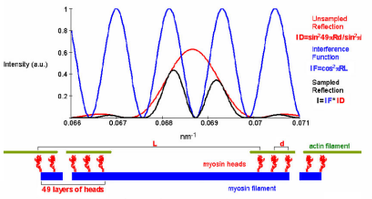
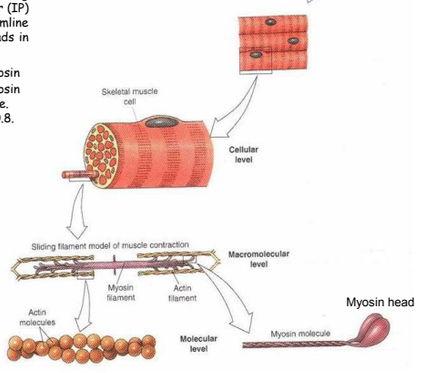


Fig. 3 X-ray interference between the two bipolar arrays of myosin heads allows to measure with Å-scale sensitivity the axial motion of myosin heads that drives the actin filaments toward the centre of the sarcomere. In the isometric contraction (T_0) the peak intensity ratio ($R=I_{HA}/I_{LA}$) is 0.8.



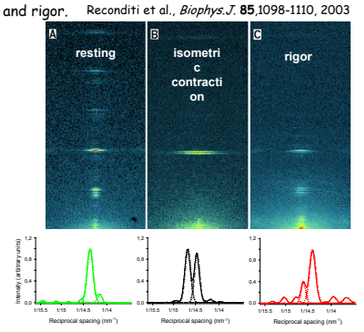
Linari et al., *Proc. Natl. Acad. Sci. USA*, **97**, 7226-7231, 2000.



RESULTS

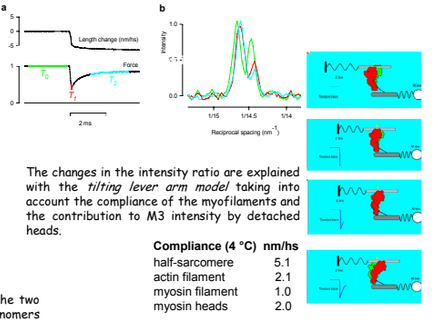
1. The size of the working stroke and its dependence on the load

a) Changes in the fine structure of myosin-based meridional reflections between isometric contraction and rigor. Reconditi et al., *Biophys.J.* **85**,1098-1110, 2003



In rigor (the state at the end of the working stroke) the two heads of each myosin molecule attach to consecutive monomers of the actin filament and their light chain domain is on average tilted by ca 40° (6 nm) with respect to the isometric contraction

b) Changes in the interference fine structure of the M3 X-ray reflection produced by a shortening step of 5nm/hs. Piazzesi et al., *Nature*, **415**, 659-662, 2002

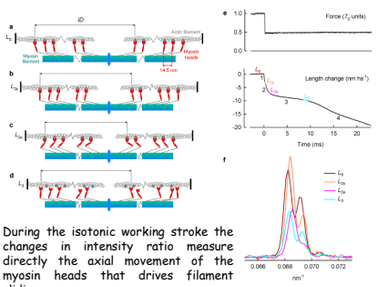


The changes in the intensity ratio are explained with the *tilting lever arm model* taking into account the compliance of the myofilaments and the contribution to M3 intensity by detached heads.

Compliance (4 °C)	nm/hs
half-sarcomere	5.1
actin filament	2.1
myosin filament	1.0
myosin heads	2.0

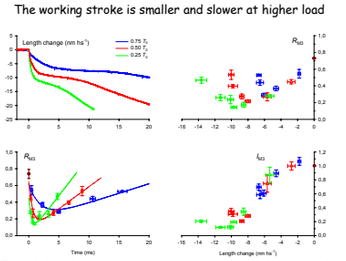
c) With a fast force clamp method we could determine the working stroke elicited by the drop in force to different fractions of the isometric force (Experiments conducted both at ID02, ESRF, and at BioCAT, APS.)

Changes in the interference fine structure of the M3 reflection produced by a force step to ½ the isometric force



During the isotonic working stroke the changes in intensity ratio measure directly the axial movement of the myosin heads that drives filament sliding.

The working stroke is smaller and slower at higher load



The sliding distance accounted for by the working heads is:

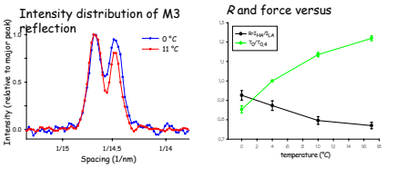
- ca 12 nm under a low load (¼ of the isometric force)
- ca 6 nm at high load (¾ of isometric force).

The smaller stroke at high loads shows that in muscle myosin the stroke size is not set by structural constraints, but rather by kinetics and energetic constraints (Reconditi et al., *Nature*, **428**, 578-581, 2004.)

2. The structural change leading to isometric force

Force generation is an endothermic process (Piazzesi *et al., J. Physiol,* **549**, 93-106, 2003), filament sliding is exothermic.

Are the two processes driven by the same structural transition?
We record the axial movement of the myosin heads associated to changes in the isometric force with changes in temperature in the range 0-17 °C.

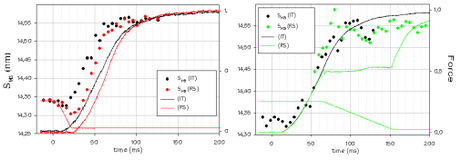


A step forward in the working stroke can be entropically driven.

3. Changes in the thick filament at the start of contraction

i) Time course of attachment of force generating myosin heads during the tetanus rise.

Changes in spacing of the M3 reflection during the rise of the isometric tetanus (IT) and the effect of superposing a ramp shortening (RS) at a velocity that prevents the force rise (5 ms time frames collected with the RAPID detector).



- Shortening at V_0 (but not at 1/4 V_0) prevents the formation of strong myosin-actin bonds.
- Keeping the force at zero after the end of latency relaxation reveals that the abrupt rise of an elasticity in parallel to myosin-actin bonds induce shortening of the thick filament.

ii) Structural changes in the myosin heads during the development of the isometric tetanus

The X-ray interference technique will be applied to determine the structural transition in the myosin heads that is coupled to the rise in filament strain during the development of the tetanus.