STRUCTURAL ASPECTS OF FORCE GENERATION BY MYOSIN HEADS PROBED BY X-RAY INTERFERENCE IN FROG MUSCLE

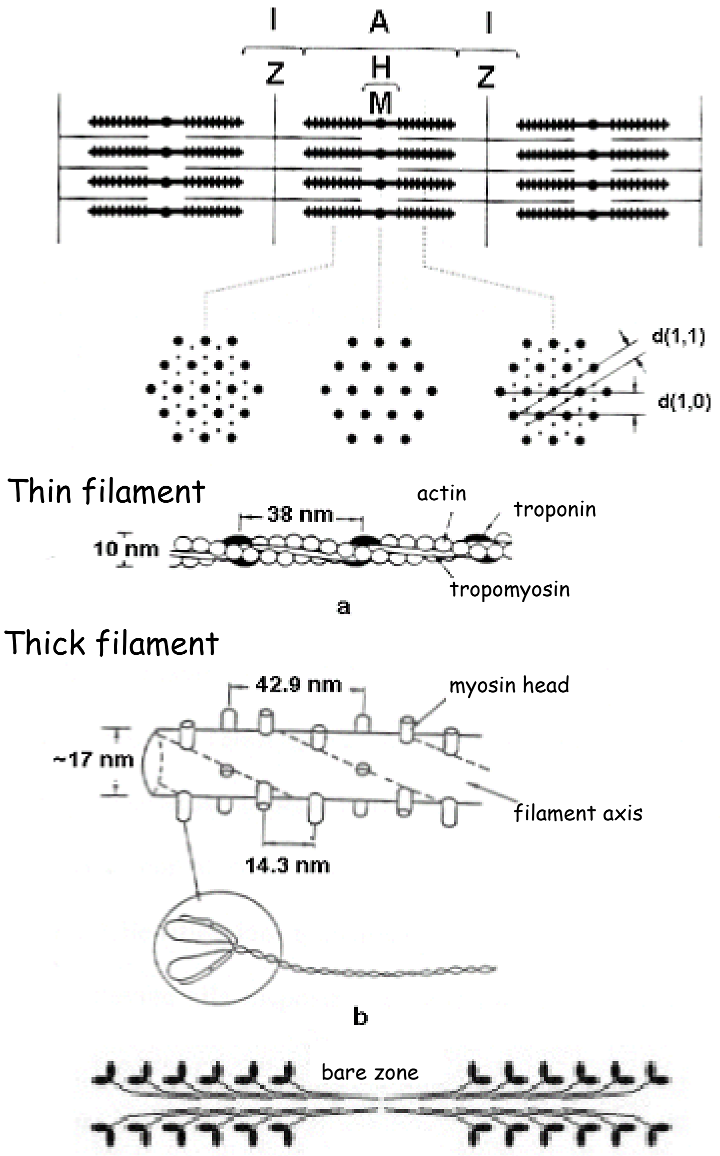
E. Brunello, M. Linari, M. Reconditi, Y.-B. Sun*,
T. Narayanan‡, P. Panine‡, G. Piazzesi, M. Irving*
and V. Lombardi

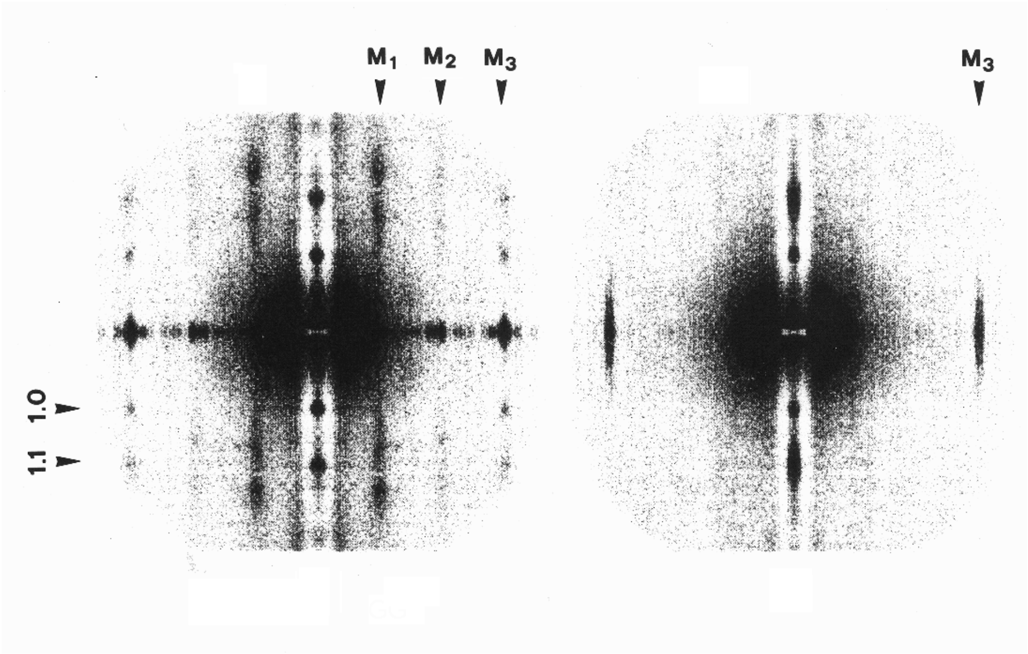
Laboratorio di Fisiologia DBAG, University of Florence, I-50134 Firenze, Italy
and Istituto Nazionale di Fisica della Materia, OGG, Grenoble, France;
*School of Biomedical Sciences, King's College London, SE1 1UL, UK; ‡ESRF, BP
220, 38043 Grenoble, France

INTRODUCTION

Force generation and filament sliding in muscle (Fig. 1) are thought to be due to an interdomain structural change in the globular part of myosin (the myosin head) that drives tilting of the light chain domain (LCD, blue in Fig. 2) about a pivot in the catalytic domain (CD, red in Fig. 2) firmly attached to actin (Rayment et *al. Science* **261**, 58-65, 1993). Force generation, but not filament sliding, is an endothermic process (Piazzesi *et al.*, *J. Physiol.* **549.1**, 93-106, 2003 and references therein), rising the question whether the same structural transition drives the two processes. The question is investigated here by using X-ray diffraction interference (Fig. 3) at ID2-SAXS (ESRF, Grenoble, France) to measure the changes in axial movement of the myosin heads associated to changes in isometric force with temperature (Fig. 4). We collected the intensity and fine structure of the meridional reflections (sensitive to the axial position of the myosin heads) as well as the intensity of the actin layer line reflections (sensitive to the fraction of stereospecifically attached heads) during isometric contractions of intact muscle fibres at four different temperatures between 0 and 17 °C.

Figure 1 Structure of striated muscle



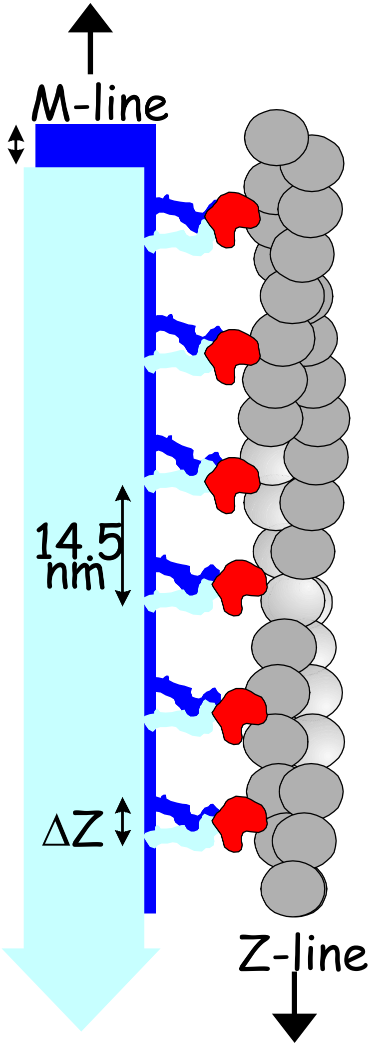

Resting

Isometric contraction

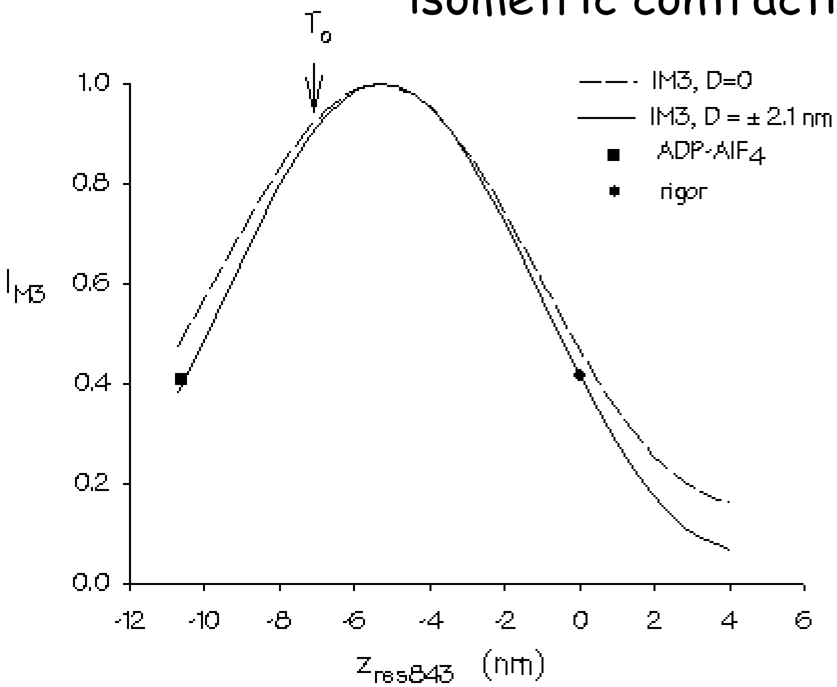
Axial and helicoidal periodicities in the 3D lattice of skeletal muscle generate axial and layer line reflections in the X-ray diffraction pattern

Figure 2

The intensity of the 3rd order myosin meridional reflection (I_{M3}) is sensitive to the axial motion of the myosin heads and can test the tilting LCD model of the working stroke.



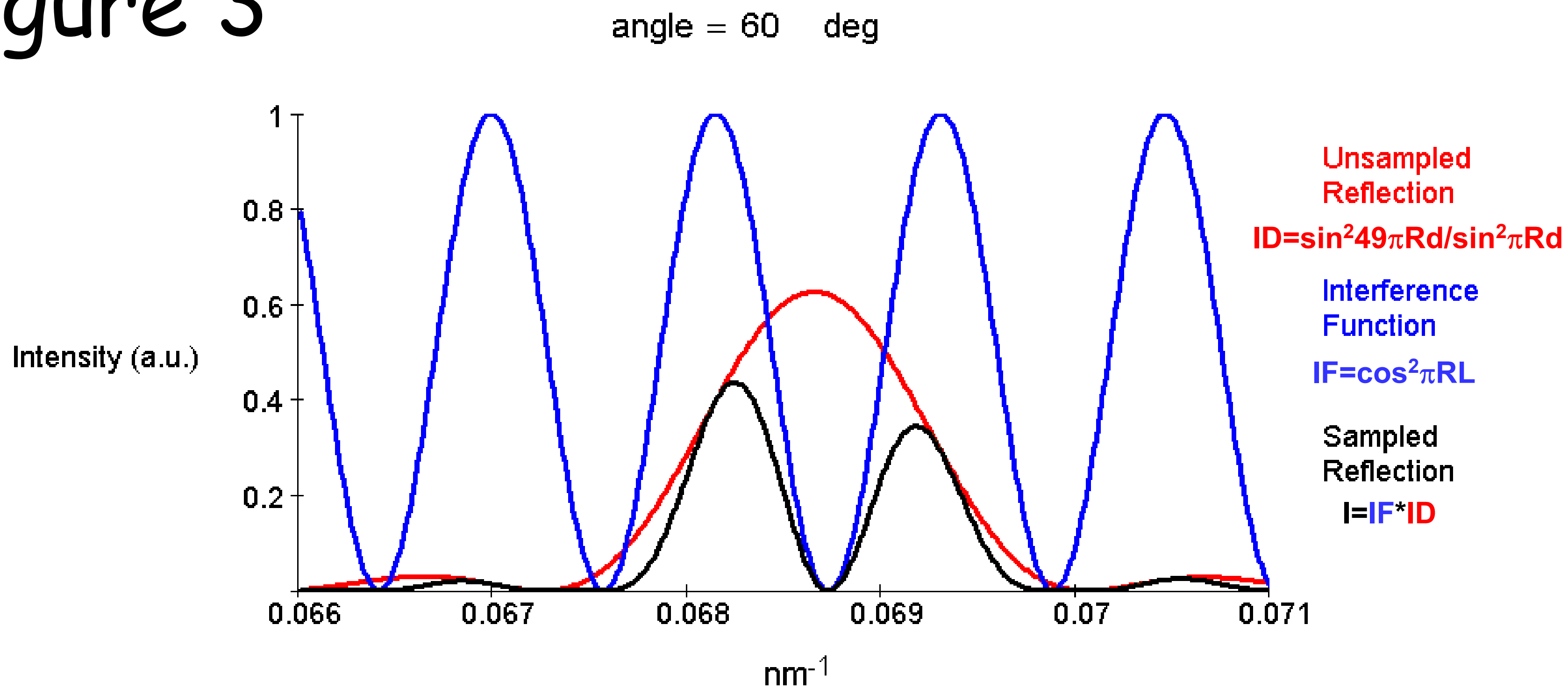
Length step experiments indicate that the LCD of the attached heads is at ~60° from the filament axis during isometric contraction (T_0, dark blue).

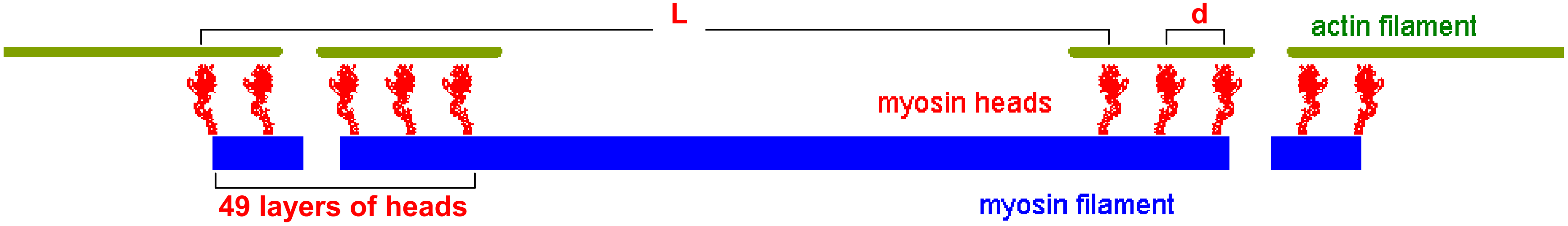


Irving et al.
Nature Str. Biol.
7, 482-485, 2000

Tilting of the LCD to the rigor conformation (light blue) shifts the axial position of the head-rod junction (Z_{res843}) attached to the myosin filament by 7 nm.

Figure 3





X-ray interference between the two bipolar arrays of myosin heads allows to measure axial motion of myosin heads with Å resolution

Figure 4

Increasing temperature

↓

Rise of isometric force

↙ ↘

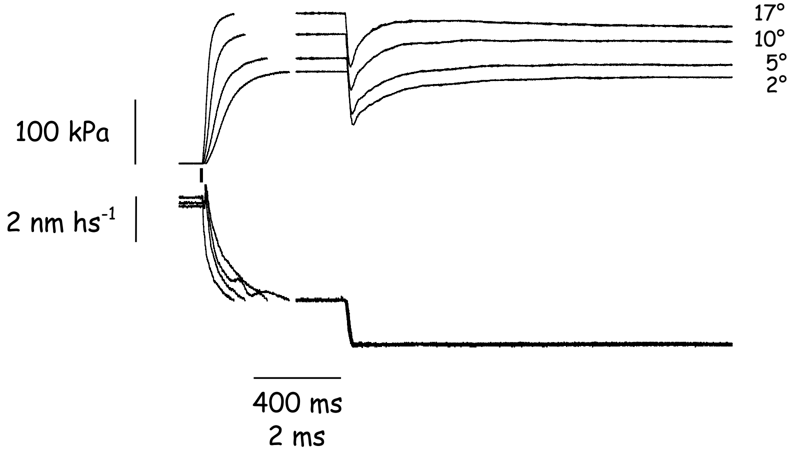
Rise of the force/head?

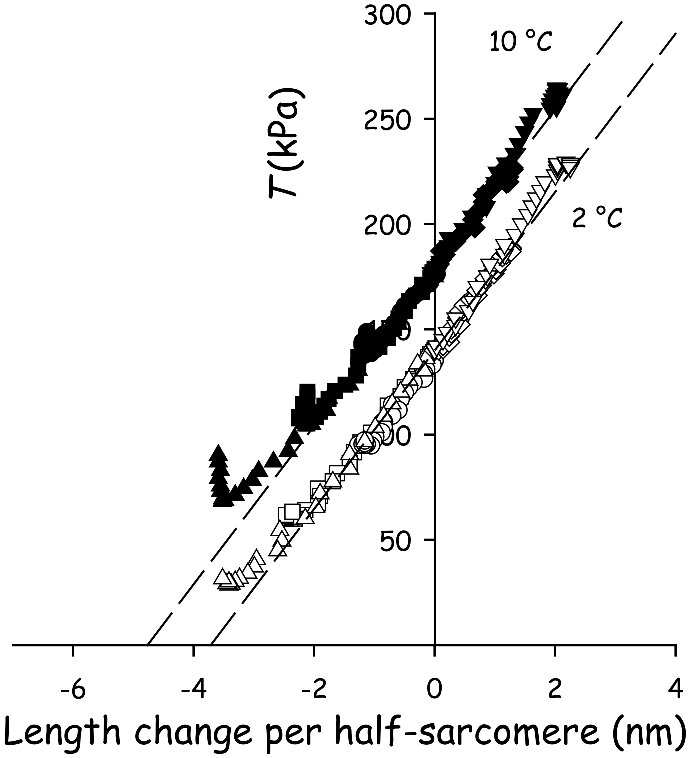
Rise of the number attached heads?

Stiffness (=ΔT/Δl) is constant at different temperatures

↓

The temperature-dependent increase in force is due to the increase in the average force per attached head (Piazzesi *et al.* 2003)



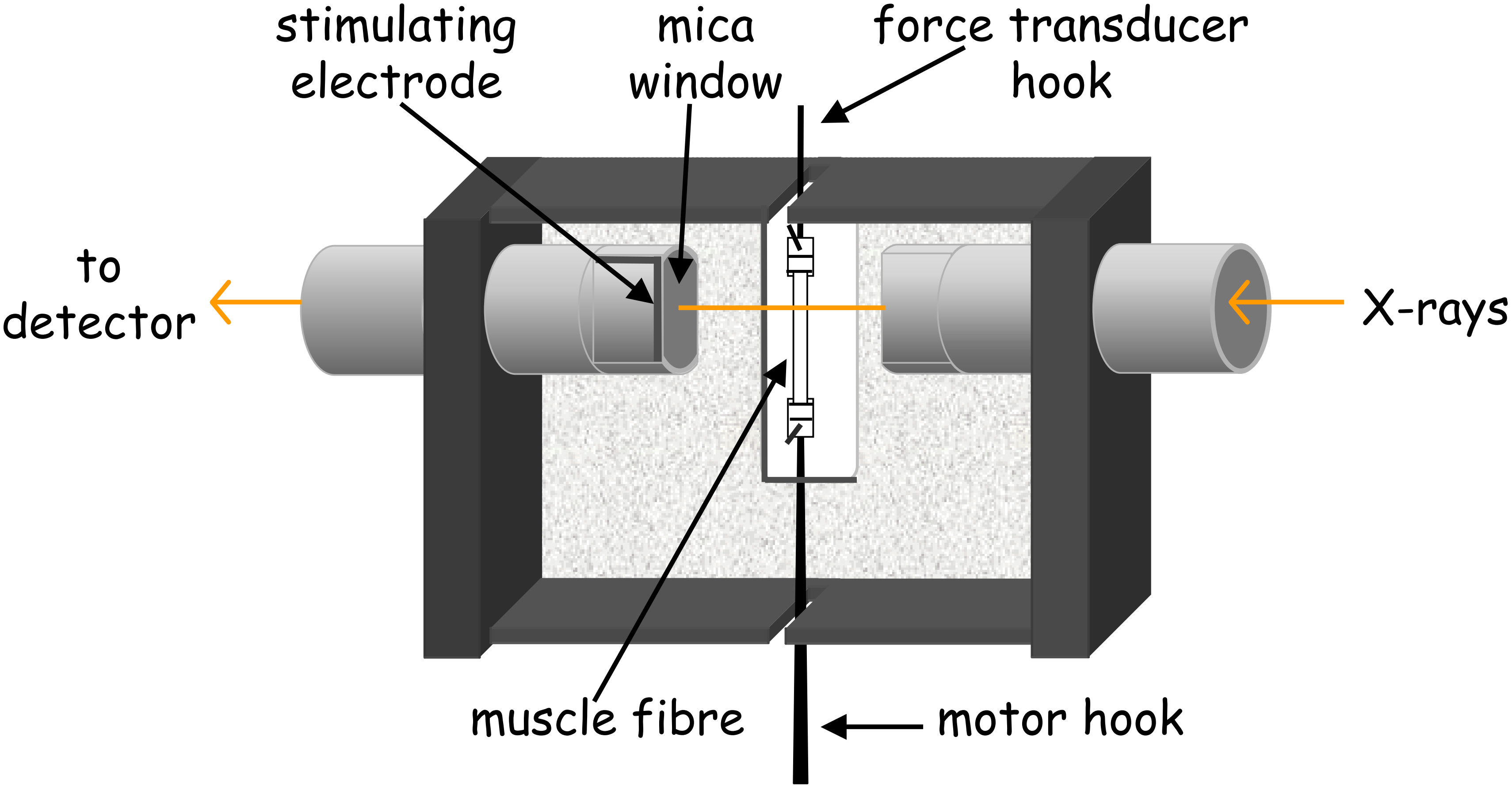


METHODS

Single fibres from the tibialis anterior muscle of *Rana temporaria* were vertically mounted in a trough (Fig. 5) containing physiological solution between a force transducer and a loudspeaker coil motor. Sarcomere length was set at ~2.2 μm. Two dimensional patterns were collected on the image intensified FReLoN CCD detector placed at either 10 m (to collect intensity and fine structure of the low order meridional reflections) or 3 m (to collect intensity of the higher order meridional reflections, up to M6, and of the actin layer lines) from the preparation. Data analysis was performed using Fit2D (by Dr A.P. Hammersley, ESRF) and Peakfit software package (SPSS Inc.). The radial integration limits were: ±40 nm for the M3 and M6 reflections and from 21 to 4.8 nm for the 1st actin layer line.

Figure 5

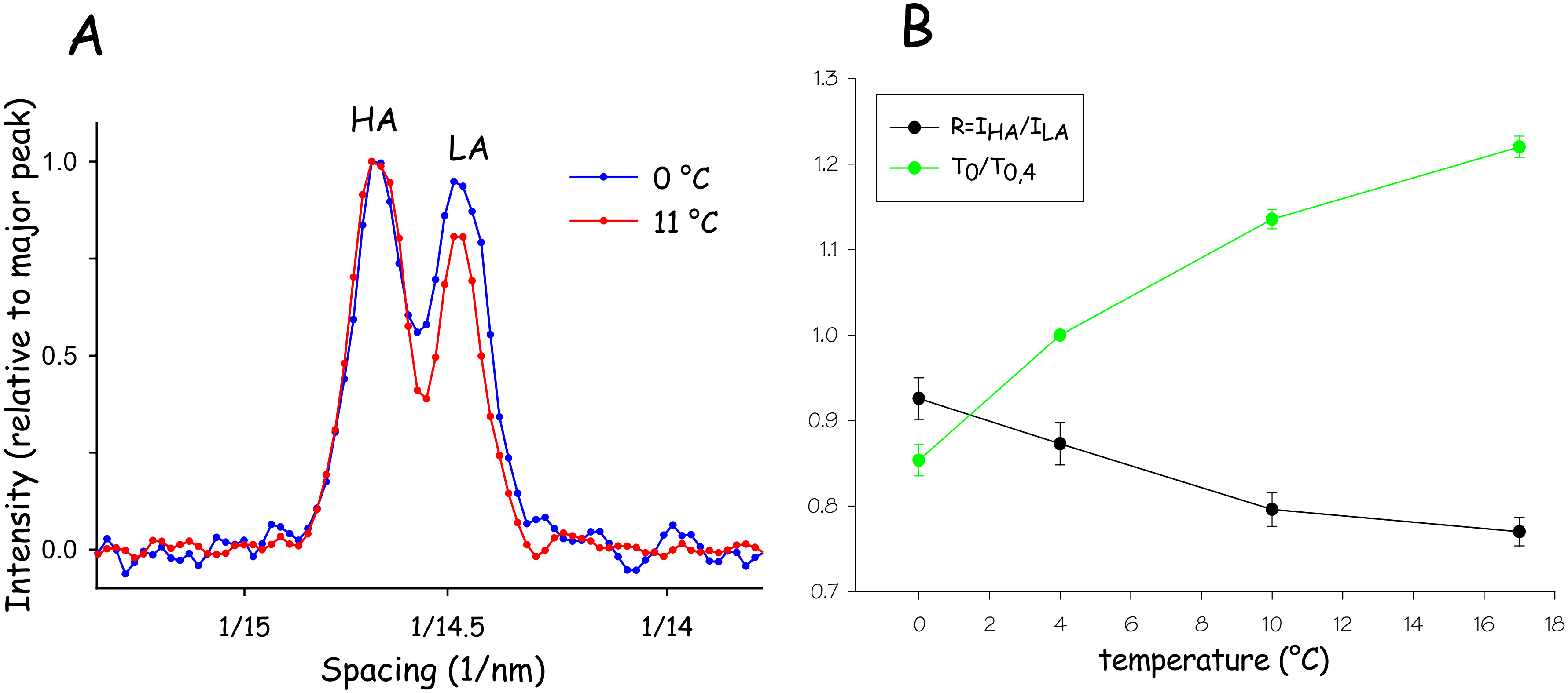
Physiological environment of the molecular motor is preserved in a single fibre, where it is possible to control mechanics of the half-sarcomere



RESULTS

X-ray interference between the two arrays of myosin heads in each myosin filament splits the M3 reflection from the 14.5 nm axial repeat of the heads into two main peaks (Fig. 3; Linari *et al. PNAS* **97**, 7226-31, 2000). Increasing the temperature from 0 °C to 17 °C increased the isometric tetanic force (T_0) by 44% (Fig. 6B and 7A) and decreased the relative intensity of the high angle peak versus the low angle peak of the M3 reflection from 0.93 ± 0.02 (mean ± SE, n= 5 fibres) to 0.77 ± 0.02 (Fig. 6B), indicating a shift of the centroid of the heads towards the centre of the sarcomere of 0.3 nm. The intensity of the 1st actin layer line, measured in the same range of temperatures, increased by 57 ± 18% (Fig. 7D, 5 fibres), while those of M3 and M6 did not vary significantly (Fig. 7 B and C).

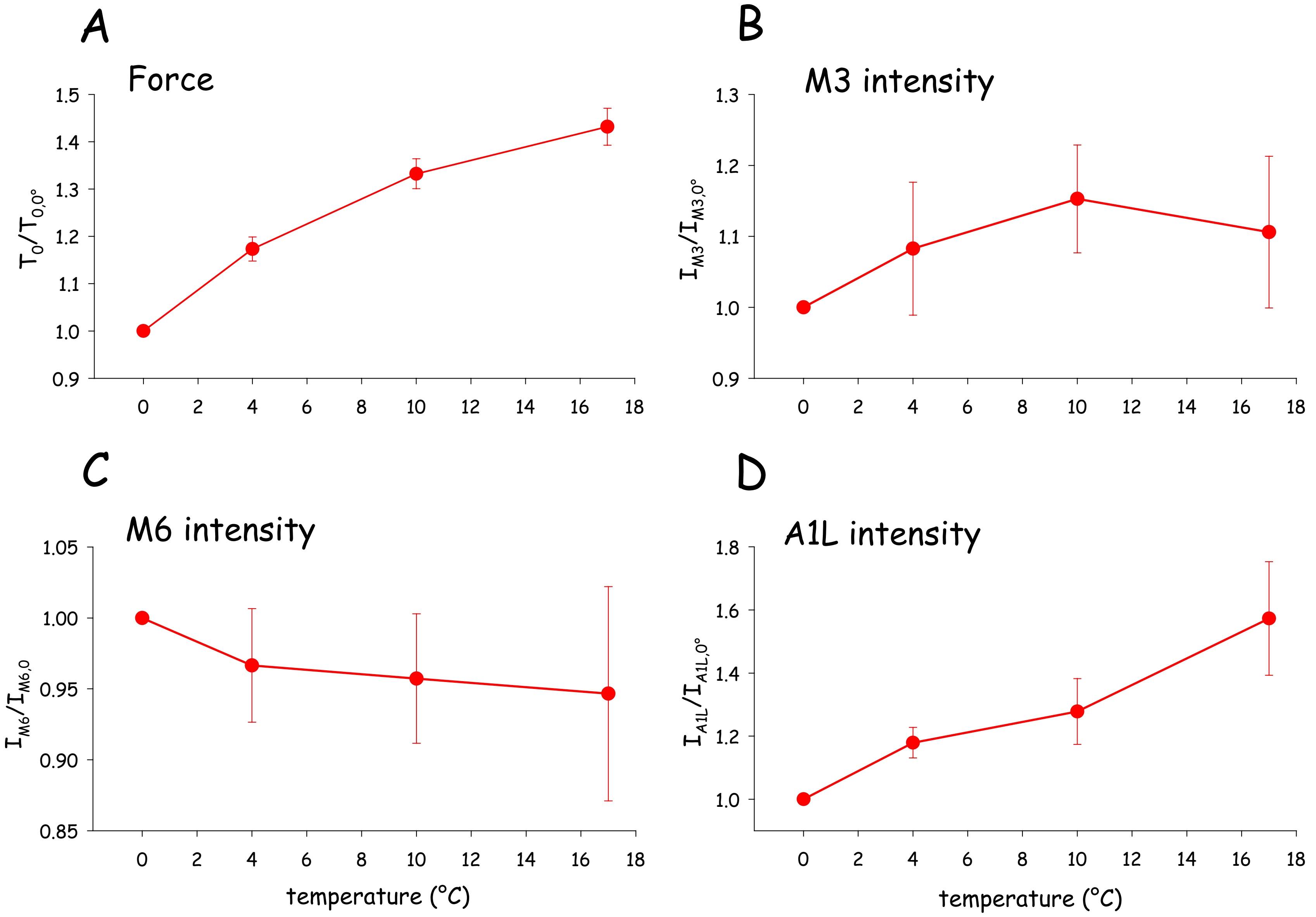
Figure 6



Meridional intensity distribution

R and force versus temperature

Figure 7


A
Force
$T_0/T_{0,0°}$
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0
2
4
6
8
10
12
14
16
18
B
M3 intensity
$I_{M3}/I_{M3,0°}$
1.3
1.2
1.1
1.0
0.9
0
2
4
6
8
10
12
14
16
18
C
M6 intensity
$I_{M6}/I_{M6,0}$
1.05
1.00
0.95
0.90
0.85
0
2
4
6
8
10
12
14
16
18
temperature (°C)
D
A1L intensity
$I_{A1L}/I_{A1L,0°}$
1.8
1.6
1.4
1.2
1.0
0
2
4
6
8
10
12
14
16
18
temperature (°C)

CONCLUSIONS

1. Interference splitting of the M3 reflection indicates that the increase in isometric force by temperature shifts the center of mass of the myosin heads towards the center of the sarcomere (Fig. 8). This result implies that a step forward in the working stroke can be entropically driven.
2. The increase in intensity of the 1st actin layer line reflection indicates that attached heads become more ordered on the actin helix as they progress in the working stroke.

Figure 8

Changes in the fine structure of M3 are interpreted using the tilting LCD model of the myosin head and a structural model of the sarcomere.