SI Text

Stiffness Measurements. *Stiffness changes during the stretch.* The change in stiffness of the half-sarcomere during the stretch, and thus the number of additional myosin motors that bind to actin, were calculated from the nonlinearity of the T_1 relation (Fig. 1*b*). During isometric contraction (T_0, 285 kPa) the half-sarcomere compliance (Y_0, estimated from the intercept of the T_1 relation with the abscissa; ref. 1) was 5.34 nm/T_0 (Fig. 1*b*, green line). The compliance at T_0 due to the myosin motors, calculated by subtracting the equivalent filament compliance (3.7 nm/T_0) from the half-sarcomere compliance, was 1.64 nm/T_0 (see also refs. 2-4). The number of additional myosin motors that bind to actin during each size of stretch can be calculated using the difference between the axial distortion of the myosin motors (Δz_o; obtained from the observed T_1 and the myofilament compliance as described in the main text in relation to Fig. 1 *a* and *c*), and the value (Δz_e) that would be expected if the compliance due to the myosin motors were the same as that at T_0. The 3.8-nm stretch produces an increase in force ΔT_1 of 0.75 T_0; Δz_o is 1.00 ($\pm$ 0.14) nm, whereas Δz_e is $(0.75 \times 1.64) = 1.23$ ($\pm$ 0.04) nm. The resulting increase in stiffness (23%) indicates that 20 new motors have attached from each myosin half-filament, in addition to the 88 attached during isometric contraction. Similarly, for the 5.3-nm stretch, which produces an increase in ΔT_1 of 1.08 T_0, Δz_o is $(5.34 - (1.08 \times 3.77)) = 1.34$ ($\pm$ 0.18) nm, whereas Δz_e is $(1.08 \times 1.64) = 1.77$ ($\pm$ 0.04) nm. In this case, the increase in stiffness (32%) indicates that 28 new motors have attached.

Stiffness changes after the stretch. Small length oscillations (peak to peak ≤2 nm per half-sarcomere) were imposed on the fiber at the plateau of the isometric tetanus, starting 4 ms before a stretch of 2, 4, or 5.7 nm, and lasting for 20 ms. The frequency of the oscillation, 4 kHz, was much faster than the quick force recovery due to the motor stroke, so the quadrature component in the force response was close to zero. The compliance of the half-sarcomere is the ratio of the synchronous 4-kHz half-sarcomere length and force changes. T_0 in this experiment was 240 kPa, Y_0 was 5.02 nm, so the compliance due to actin and myosin filaments is $(240 \times 0.013) = 3.13$ nm/T_0 and that due to myosin motors is (5.02 – 3.13) = 1.89 nm/T_0. At T_2, the stiffness of the half-sarcomere (the reciprocal of the compliance) increased by 6.5%, 19%, and 22% with respect to that at T_0 for the three stretch sizes, respectively, and the stiffness of the motors, calculated from these values after taking into account the contribution of myofilament compliance, increased by 19%, 71%, and 89%, indicating that 17, 62, and 78 additional motors attach to actin.

Structural Simulation. *Original model, with number of myosin motors attached to actin assumed to be constant during stretch.* The structural model used in the simulation of Fig. 2 is similar to that used previously to quantify the axial motion of the myosin motors induced by step reduction in sarcomere length (2) or load (3). In each myosin filament there are two arrays of myosin motors separated by a central bare zone at the M line. The resultant axial mass distribution has 49 repeats on each side of the M line. The catalytic domain (CD) of the myosin motors is assumed to bind to actin in the same conformation as that determined in isolated actin filaments decorated with myosin motor fragments in the absence of ATP (5, 6). The light-chain domain (LCD) tilts to accommodate the change in Δz associated with sliding between the myosin and actin filaments when the CD is attached to actin. In isometric contraction the long axis of the LCD of the myosin heavy chain, defined as the vector joining Cys 707 (in the converter region) and Lys 843 (the junction of the motor with the myosin tail), is at 63° to the filament axis, with residue 843 closer to the M line (2, 7). The model has the following constraints: (*i*) according to stiffness measurements only 30% of the myosin motors (i.e., 88 of the 294 motors present in each myosin half-filament) are attached to actin during isometric contraction (4, 8); (*ii*) the mass distribution of the attached motors, which is sensitive to filament sliding, contributes half of the intensity of the M3 reflection (2, 3); and (*iii*) the axial dispersion of the attached myosin motors must be large enough to be consistent with the small changes in the intensity of the second order of the M3 reflection that accompany step changes in length or load (3, 9, 10).

Under these constraints, the observed changes in R_{M3} and I_{M3} in response to a step reduction in load can be reproduced by a uniform distribution of LCD orientations of ± 17° for the attached motors, and a Gaussian distribution of LCD orientations for the detached motors centered at 70° with $\sigma = 34°$ (supplementary information in ref. 3). The ± 17° LCD orientation distribution for the attached motors corresponds to an axial dispersion of ± 2.7 nm, which would allow motors to bind to actin monomers with a 5.5-nm axial separation. The axial dispersion of the catalytic domains of the detached motors is ± 5.3 nm. Although two types of detached motors must be present, depending on whether their partner motor in the same myosin molecule is actin-attached, in this model both types of detached motors were assumed to have the same dispersion. This model fits the observed values of R_{M3} and I_{M3} at T_1 and T_2 for small releases and stretches, but not those for larger stretches (Fig. 2 *a* and *c*). In the latter case: (*i*) the calculated increase in R_{M3} is larger than observed; (*ii*) the calculated reduction in I_{M3} at T_1 is smaller than observed; and (*iii*) the observed saturation of I_{M3} reduction at T_2 for large stretches is not reproduced.

Revised Model with Additional Binding of Myosin Motors to Actin During a Stretch. Single population of detached motors. Our qualitative explanation for the failure of the original model to fit the observed values of R_{M3} and I_{M3} after a stretch is that the stretch not only shifts the catalytic domains of the attached motors Z-ward but also induces attachment of partner motors to the next actin monomer on the M-ward side (Fig. 3*a*). In this way the Z-ward shift of the attached motors is counteracted by the M-ward attachment of the additional motors, so that the net Z-ward mass shift is reduced. This reduces the increase in interference distance during the stretch, which is observed as a smaller increase in R_{M3} (Fig. 2*c*). Attachment of the partner motors also increases the overall axial dispersion, thus reducing I_{M3} (Fig. 2*a*). The width of the LCD orientation distribution of the newly attached motors was assumed to be the same as that of the originally attached motors, but the mean orientation differs by 34°, corresponding to attachment of the CD to the next actin monomer on the M-ward side. Values of R_{M3} and I_{M3} calculated from the revised model are shown in Fig. 5, with colored lines indicating the fraction of new attachments with respect to the number of isometric attachments. For each stretch size, the increase in R_{M3} is depressed in proportion to the fraction (f_s) of newly attached motors as expected, both at T_1 (Fig. 5*a*) and at T_2 (Fig. 5*b*). The experimental value of R_{M3} for the 3.8 nm stretch is reproduced by $f_s = 0.2$ (red line) at both T_1 and T_2. R_{M3} for the 5.5 nm stretch is fitted by $f_s = 0.4$ (green) at T_1 and $f_s = 0.6$ (blue) at T_2. However this model did not give a good fit to either the magnitude of the observed change in I_{M3} or its dependence on stretch size. The calculated values of I_{M3} at T_1 do not reproduce the progressive reduction of I_{M3} with stretch size (Fig. 5*c*). This is related to the greater axial dispersion of detached motors compared to that of attached motors; when partner motors attach, their axial dispersion decreases substantially, and this counteracts the reduction in I_{M3} produced by binding to an adjacent actin monomer (7, 11).

Two populations of detached motors. This discrepancy can be removed using the concept of two types of detached motors, i.e., those with an attached partner and those with a detached partner that was already introduced above, and proposing that these have different axial dispersions (10). Recruitment can occur only from the former population (the yellow motors in Fig. 3*a*), which have a Gaussian distribution of LCD orientations centered at 72° with $\sigma = 27°$, corresponding to an axial dispersion of CD positions of 4.3 nm. The axial mass distribution produced by this LCD angle distribution is shown in Fig. 6 (thick gray line). The attached motors during isometric contraction (thick black line) have a uniform ± 17° distribution of LCD orientations centered on 63° (thick black vertical line), as before. Detached motors with detached partners are represented by a Gaussian mass distribution (thin gray line) with $\sigma = 5.6$ nm, shifted M-ward by 2 nm with respect to that of the attached motors. The axial mass distribution of the detached-detached population has the same mean as that of the detached motors with attached partners (thick gray vertical line) but is substantially wider.

This model can reproduce the observed changes in both R_{M3} and I_{M3} at both T_1 at T_2 for each size of stretch (Fig. 7). The only adjustable parameter is the fraction of newly attaching motors (f_s), indicated as before by the colored lines. Both R_{M3} and I_{M3} at T_1 (SI Fig. 7 *a* and *c*, respectively) are fitted by $f_s = 0.2$ (red) for the 3.8 nm stretch; at T_2 the observed value of R_{M3} for this stretch size is best fitted by $f_s = 0.4$ (SI Fig. 7*b*, green), and this value of f_s also gives a good fit to I_{M3} (SI Fig. 7*d*).

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