Effect of small release on force during sarcomere-isometric tetani in frog muscle fibers

A. Horowitz, H. P. M. Wussling, and G. H. Pollack
Center for Bioengineering WD-12, University of Washington, Seattle, Washington 98195

ABSTRACT We investigated the effect of small shortening imposed on frog muscle fibers during sarcomere-isometric tetani. Sarcomere length was initially kept constant, then slightly shortened (1%–5% of initial length) and clamped again for the remainder of the tetanus. Force level after the shortening was higher than the force level preceding the release. The size of the increase was larger than that predicted by the descending limb of the linear force-length relation. The difference between measured and predicted force levels increased with sarcomere length. At a sarcomere length of 3.2 μm, the force level after the shortening was higher by 50% than the force level expected from the linear descending limb.

Dispersion of sarcomere-length within the sampled region was measured by two independent methods: striation imaging and analysis of the intensity profile of the first diffraction order. Sarcomere-length inhomogeneity in the sampled region was too small (standard deviation from the average sarcomere-length was ± 0.03 μm) to account for the size of the increase in force.

We studied the dependence of increase in tetanic force level after small sarcomere-length release on the size, velocity and timing of the release, as well as on initial sarcomere-length. Release size was the major determinant of the amount of increase in force. Release of 20 nm per half sarcomere was sufficient to produce an almost full force increase. Larger releases increased the force only moderately. Over the range studied, release velocity and timing had little or no effect.

INTRODUCTION

Force-length relations obtained by the segment or sarcomere-length clamp method have been reported by several investigators (Gordon et al., 1966; Edman and Reggiani, 1987; Bagni et al., 1988; Granzier and Pollack, 1990), and tend to be in close agreement with one another. The descending limbs of the force-length relation obtained in these experiments are linear and steep, in accordance with the predictions of the cross-bridge model (A. F. Huxley, 1957). However, numerous other investigators reported that under fixed-end conditions, the force-length relation was higher at all sarcomere-lengths than the linear descending limb (see review by Pollack, 1983). The differences between fixed-end tetani and sarcomere-isometric ones have been attributed mainly to sarcomeres creeping up the descending limb during the plateau phase of the tetanus (Gordon et al., 1966).

This explanation was challenged by ter Keurs et al. (1978), who found that sarcomere-length inhomogeneity in the fibers they used was less than 4% of the average sarcomere-length during the tetanic plateau. This inhomogeneity was too small to account for the high force levels they obtained in fixed-end tetani. The source of the elevated force level in fixed-end tetani, relative to sarcomere-isometric ones, could, therefore, lay somewhere else.

One possibility is that the extra force is caused by a small amount of sarcomere shortening. In pilot studies (Horowitz et al., 1989*a*), we found, as did Burton et al., (1989), that some sarcomeres between the end and the middle region of the fiber stay isometric after an initial small shortening (several percent of their initial length). We raised the question whether the slight shortening might induce a disproportionately higher force, larger than predicted by the linear descending limb of the force-length relation.

To test this possibility, we performed sarcomere-length clamps in which a small and controlled sarcomere release (1–5% of initial sarcomere length) was induced, and compared the force levels before and after the release. We found a significant increase in force level after the release. The increase became more pronounced at longer sarcomere-lengths; at 3.2 μm, the post-release force was as much as twice the total force predicted by the linear descending limb of the force-length relation.

A short account of these experiments has been published (Horowitz et al., 1989*a, b;* Horowitz and Pollack, 1990).

METHODS

Preparations

We studied single intact skeletal muscle fibers dissected from the semitendinosus muscle of the frog *Rana temporaria*. Slack length was ~10 mm, and cross-sectional area was typically 0.005 mm^2 (cross-sectional area was calculated by measuring the largest and shortest dimensions of the fiber width; assuming the cross-section was ellipsoidal, those values were used as the long and short axes of the ellipse, respectively). The fibers were isolated together with their tendons. Small holes were punched in the tendons at a distance of 0.5–1.0 mm from the fiber ends.

Fibers were placed in a transparent chamber and mounted between two metal hooks inserted in the holes in the tendons. The chamber,

Address correspondence to Dr. Horowitz.
Dr. Horowitz's current address is Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02254.

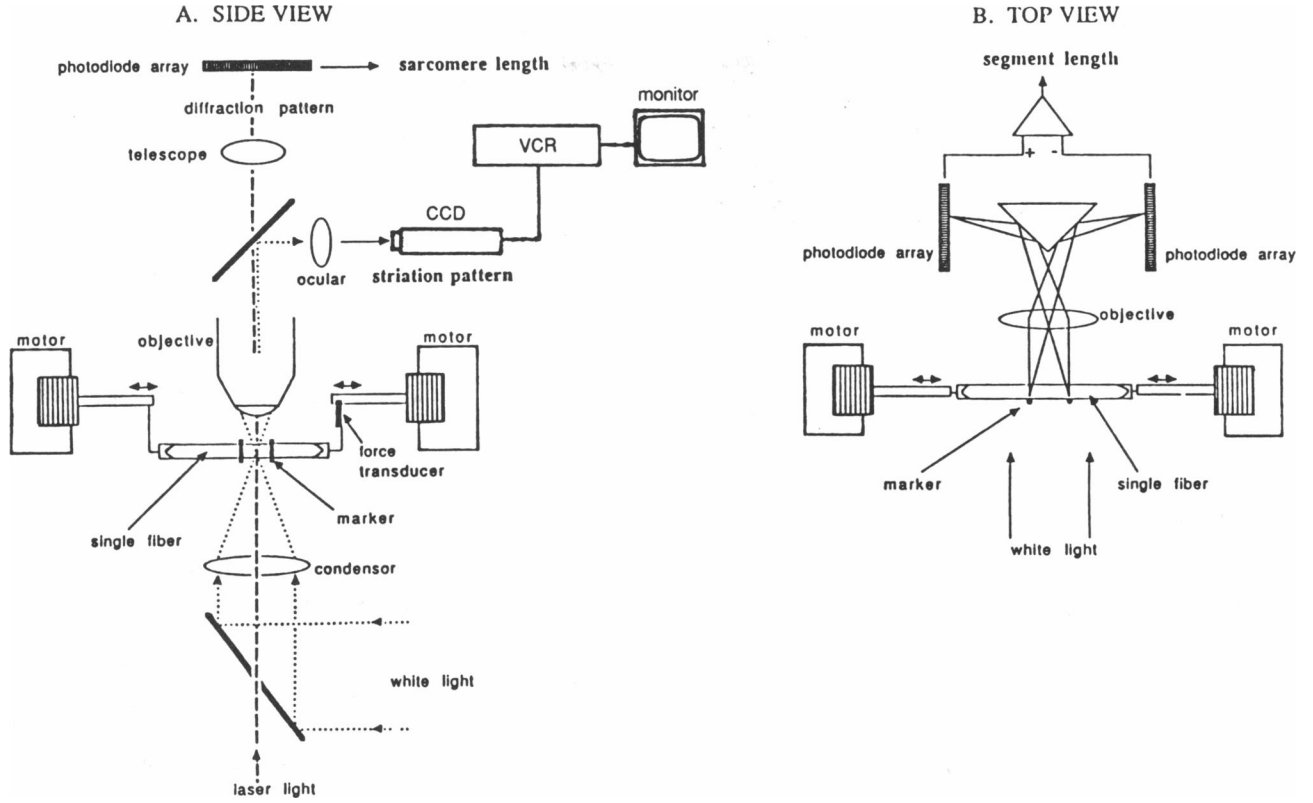

FIGURE 1 Experimental set-up. *A* shows the vertical optical axis, along which the laser beam and white light were projected. The laser beam passed through a 1-mm hole drilled in the condenser lens, and after being diffracted by the fiber, was collected by a bright field objective (model L32/0.6, E. Leitz, Inc., Rockleigh, NJ), passed through a telescope (model 464822-9902, Carl Zeiss, Inc., Thornwood, NY), compressed by a cylindrical lens, and projected onto a photodiode array (model RL256 C/17, EG & G Reticon, Sunnyvale, CA). Striation pattern was observed continuously by the CCD camera, and, when necessary, recorded on tape. *B* shows the horizontal optical axis, along which white light was projected for obtaining the images of the surface markers. The left motor controlled sarcomere or segment length, and the right motor controlled marker position.

made of glass strips, had a volume of ~600 μl. The transparency of both its walls and its bottom allowed the fiber to be illuminated along two perpendicular axes: vertically, with a laser beam for diffraction measurement and white light for striation imaging (Fig. 1); and horizontally, with another white light beam for acquisition of marker position.

Fibers were bathed in a Ringer solution (composition in mM: NaCl: 115.5; KCl: 2.0; $CaCl_2$: 1.8; $MgSO_4$: 1.0; Na_2HPO_4: 6.3; NaH_2PO_4: 1.0; glucose: 5.0; pH: 7.1). The solution was kept at 2–3°C, using a flow of cooled air directed along the sides and the top of the chamber.

Sarcomere-length measurement

Sarcomere length was principally measured by laser diffraction. In some of the experiments we also employed segment length as an experimental control method. For laser diffraction, we used a collimated He-Ne light beam (model LLS5R, Aerotech, Pittsburgh, PA). Incident beam diameter was reduced to 0.25 mm. After passing through the fiber, the beam was collected with a long working distance (×32) objective lens (N.A. 0.6) and a telescope lens. The diffraction pattern produced by the striations was finally compressed with a cylindrical lens and projected on a photodiode array (EG & G Reticon). The position of the first-order diffraction maximum was determined by locating the median position of the first-order intensity profile on the array. The voltage corresponding to the first-order position was recorded by a digital oscilloscope, and later converted to sarcomere length by calibration with gratings of known spacings. The calibration was repeated for each experiment. RMS sarcomere-length noise level was 0.41 ± 0.15 nm per sarcomere (Granzier and Pollack, 1989).

The second method measured sarcomere length indirectly, by determining the distance between two markers placed on the fiber surface. We used short pieces (of about the diameter of the fiber) of human hair. The distance between the markers was in the range of 0.5–1.0 mm. The length of the segment between the two markers was acquired on-line by projecting the images of the markers through a condensing lens and on two photodiode arrays identical to those used for laser diffraction. The segment-length values were converted to sarcomere length by correlating the slack length of the segment to the sarcomere-length calculated from the striation image of the segment. Change in segment length was considered to be proportional to change in sarcomere length, as verified by Granzier and Pollack (1989). RMS noise of the segment-length signal was 0.72 ± 0.02 nm per sarcomere (Granzier and Pollack, 1989).

Estimation of sarcomere-length inhomogeneity

Sarcomere-length inhomogeneity in the sampled region was deduced from the width of the intensity profile of the first diffraction order, and from analysis of striation images.

To obtain striation images, fibers were illuminated by strobe light (model 71B, Chadwick-Helmuth, Monrovia, CA) directed along the vertical optical axis (Fig. 1). Striation images were captured by a video camera (CCD model XC-37, Sony Corp., Japan) mounted on one of the oculars of a compound microscope, and recorded on a video cassette recorder (Model BV-100, Mitsubishi, Japan) for later analysis. Maximal acquisition frequency was 30 frames per second.

Intensity-profile records of the first diffraction order were recorded on a storage oscilloscope (model HP1201B, Hewlett-Packard, Palo

Alto, CA), and photographed for later analysis. Sarcomere-length dispersion was inferred from the width of the intensity profile at half of its height.

Force measurement

The right hook shown in Fig. 1 was connected to the arm of a linear positioning motor through a force transducer (AME 801E, Horten, Norway). The signal produced by the transducer was fed into the digital oscilloscope through an amplifier, and could be later converted to force units by using a calibration previously performed with a set of standard weights. The noise level of the combination of force transducer and amplifier was about 0.04% of the maximum force produced by a typical fiber (Granzier and Pollack, 1989)

Length and position control

Each hook on which the fiber was mounted was connected to an independent motor. The left motor was controlled by a feedback loop, through a microcomputer. Either sarcomere-length or segment-length could be initially kept constant, and then changed to a new value during the tetanus. The time, magnitude and velocity of release or stretch could be predetermined. Sarcomere or segment-length was controlled by feeding either the sarcomere-length or segment-length signal as input, and by minimizing the difference between the input signal and a predetermined reference signal (see Granzier and Pollack, 1989, for a more detailed description).

The right motor compensated for the translation of the sampled region by controlling marker displacement during the tetanus. The position of the marker image on the photodiode array served as a feedback signal, and the right motor moved so as to minimize the difference between the actual marker position and a predetermined one.

Experimental protocol

Fibers were placed in the chamber and mounted on the two hooks. Markers (oriented vertically) were attached to the surface of selected fibers. The fibers were carefully aligned relative to both optical axes, to allow optimal detection of diffraction and marker signals by the photodiode arrays. Calibration of the diffraction measurement was then carried out by using a grid with a known spacing.

Fibers were tetanized electrically by current pulses from two platinum electrodes which ran along both its sides. The amplitude of the current was gradually increased until a twitch occurred. Stimulation frequency of 100 Hz was used for obtaining a fused tetanus. The duration of the tetanus depended on the sarcomere length, and was increased at longer lengths so as to obtain a stable force. The rest interval between tetani was at least 200 times the duration of the tetanus (which was typically 2 s), to minimize fiber fatigue.

After measuring maximal tetanic force at slack length (2.0–2.2 μm), the fiber was stretched to the desired length and tetanized under either sarcomere or segment-length control. We interspersed the measurements with repeats of tetani at slack length to monitor deterioration during the course of the experiments. The experiment was discontinued if the fixed-end force level at slack length declined by more than 10% of the force measured at the beginning of the experiment.

Most of the releases were carried out at sarcomere lengths above 2.8 μm. At this degree of stretch, the passive force in the fiber begins to rise exponentially and is not negligible in comparison to the active force. The passive force-length relation of the fiber was measured at the beginning of each experiment (see Fig. 2). At a sarcomere-length of 3.2 μm, passive force was 7 ± 5% of active force (mean ± SD, n = 68). The active force after the release was calculated by subtracting the new lower passive force from the total force. In some of the releases, the decrease in passive force was measured by repeating the release without stimulating the fiber. There was no significant further relaxation of the post-release passive force (data not shown).

Most releases were imposed on sarcomere groups in the central third of the fibers. Sarcomere groups showing substantial creep during sarcomere-isometric contraction were not used.

RESULTS

The main finding is the force-enhancement effect of a small release that did not exceed 5% of initial sarcomere-length. We present evidence for this effect, followed by a series of controls addressed at potential artifacts, and then a systematic investigation of the effects of the release parameters: timing, velocity and size, on the magnitude of the force-increase.

The standard descending limb of the force-length relation, to which the post-release forces were compared, was constructed from the prerelease force levels. This descending limb (see Fig. 15) was similar to that of Gordon et al. (1966).

Effect of shortening on force production

The sample records in Fig. 2 *A* illustrate the central observation of these experiments. Force traces of sarcomeres that were released during the tetanus (lower three traces in each panel) are compared with those of sarcomeres that were kept at a constant length throughout contraction (upper three traces). The force levels of the two tetani match during the early part of the tetanus, when sarcomere length is still kept constant. However, the force trace of the released sarcomeres show a short dip, corresponding to the shortening phase, followed by recovery to a plateau that is higher by 10% than the total force predicted by the linear descending limb of the force-length relation based on the pooled prerelease force levels (Fig. 15). Note that force recovery started even before the release ended.

In the second set of records (Fig. 2 *B*), which corresponds to a larger initial sarcomere-length, the increase in force level after the release is larger by 62% than the total force predicted by the pooled prerelease force-length relation (Fig. 15). The difference between the force levels before and after the release generally grew as initial sarcomere length was increased (Fig. 3).

Controls

Analysis of sarcomere-length inhomogeneity

To test for possible artifactual effects of the existence of either excessively shortened or slightly stretched sarcomere sub-groups within the observed region, we examined the extent of sarcomere-length inhomogeneity. If the region were to contain some sarcomeres that shortened to a significantly smaller length than the one measured by diffraction or by segment-length (thereby stretching others in the field), the rise in force could be attributed to the higher force-production capability of those sarcomeres.

To assess the potential effect of sarcomere-length inhomogeneity on force production, quantitative criteria need to be established with regard to: (*a*) the size of the sarcomere population within the sampled region that

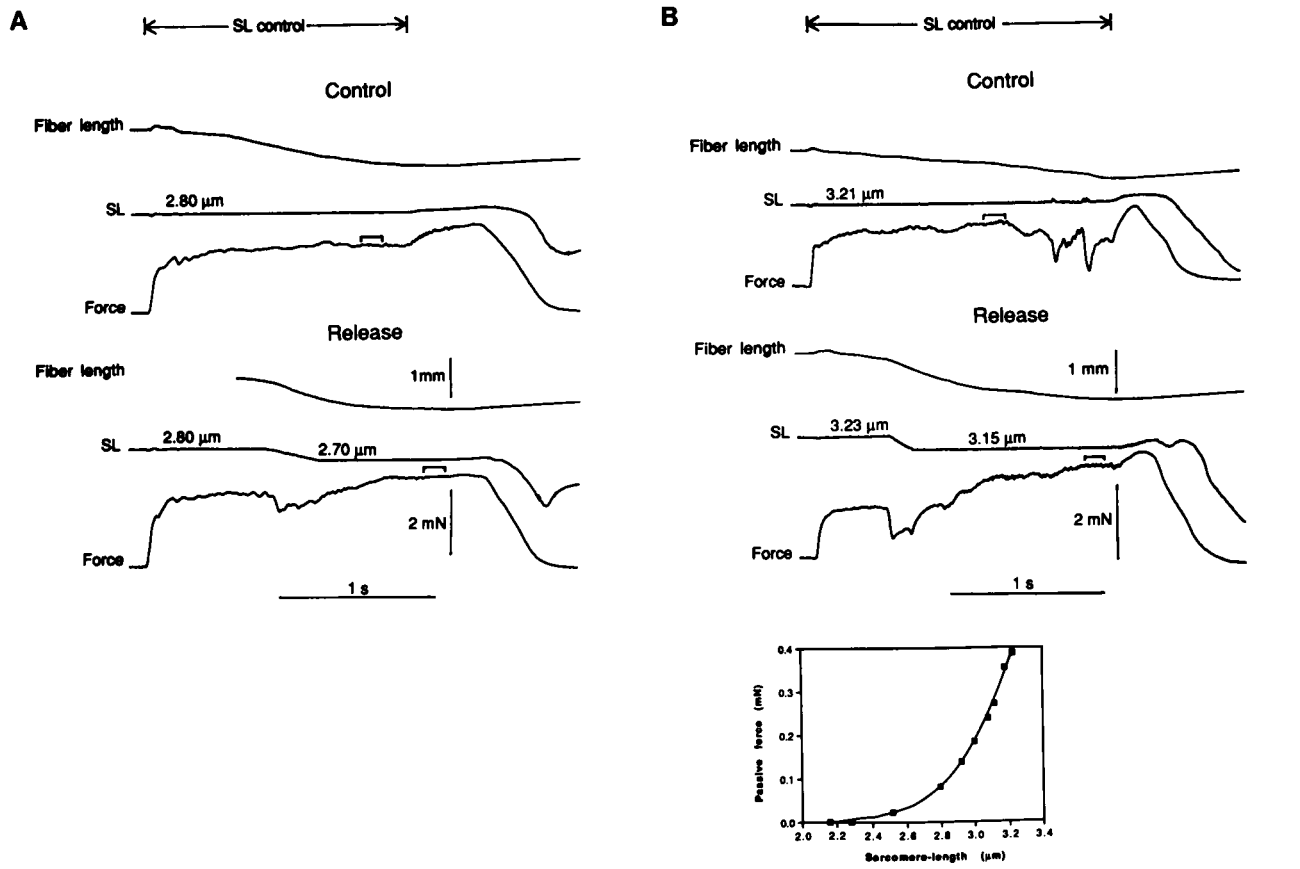

FIGURE 2 Representative records of control and release protocols. *A* shows, at the top, a set of fiber-length, sarcomere-length and force records of a fiber clamped at a sarcomere-length of 2.8 μm. The hump on the right side of the force record appeared after the length-clamp ended. The fiber-length, sarcomere-length and force records at the bottom correspond to a release of 0.10 μm imposed on the same fiber under identical conditions (the early part of the fiber length record is missing). *B* shows two similar sets from a different fiber tetanized at an initial sarcomere-length of 3.2 μm and released by 0.08 μm. The large fluctuations close to the end of the control force record were caused by noise in the sarcomere-length signal. Maximal active tensile stress developed by the fibers (at slack length) was 24 N/cm^2 (fiber *A*) and 34 N/cm^2 (fiber *B*); cross-sectional area, 9.4×10^{-3} mm^2 (fiber *A*) and 7.5×10^{-3} mm^2 (fiber *B*); temperature 3°C. The inset in *B* shows the passive force-length relation of sarcomeres in this fiber. The horizontal bars on top of the force records in this figure, as well as in the following ones, indicate the time intervals during which force peaked while sarcomere-length was still clamped (either before or after the release).

has to shorten below the apparent length to produce the measured increase in force; (*b*) the extent of shortening that this sarcomere population needs to undergo to produce the measured increase in force. These two parameters can be deduced from the following analysis. Assuming that the fiber cross-section contains two parallel sarcomere populations, one at the apparent (detected) length, and another one at a shorter undetected length, the total measured force produced by the fiber is:

$$F_m = F_1 s_1 + F_2 s_2,$$

where s_1 and s_2 are the fractions of the fiber cross section ($s_1 + s_2 = 1$) corresponding to the sarcomere populations with the measured and the shorter lengths, respectively; F_1 and F_2 are the forces that correspond to these two sarcomere populations; and F_m is the measured force.

Two limit cases can be calculated from the above equation: (*a*) the minimal fraction of the cross-section that is required to be at a shorter sarcomere-length, assuming it develops maximal force (and shortens to 2.2 μm); (*b*) if the whole cross-section contains shortened sarcomeres, the minimal required shortening. These limit cases reflect "parallel inhomogeneity" and "series inhomogeneity" respectively.

For releases from 3.2 μm to 3.1 μm, average post-release force level (F_m) was 55% of the tetanic force at slack length (see Fig. 15). The force level predicted from the linear descending limb at 3.1 μm (F_1) is 37% of the tetanic force at slack length. Substituting these values in the above equation, and substituting $1 - s_2$ for s_1 predicts that the minimal required size of a shortened sarcomere population (s_2) that develops maximal force (i.e., F_2 = 100%) is 30% of the fiber cross-section. Similarly, it can be calculated that the minimal required shortening of the whole cross-section (i.e., $s_1 = 0$, $s_2 = 1$ and F_2 is the unknown variable from which the sarcomere-length predicted by the linear descending limb is calculated) is 0.25 μm. These two estimates will be used to evaluate the

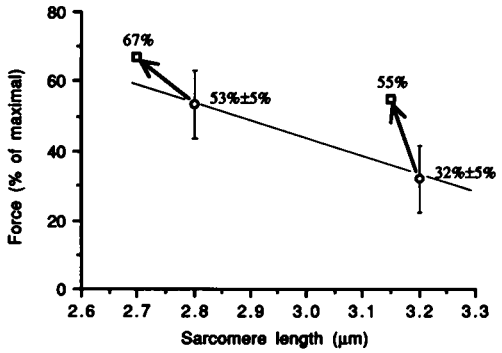

FIGURE 3 Comparison of the post-release total forces (*upper values*) shown in Fig. 2 (normalized by the maximal force level at slack length) and the total forces predicted by the linear descending limb of the force-length relation based on the pooled pre-release force levels (see Fig. 15). The difference between the force levels increases at longer sarcomere-lengths.

measurements of sarcomere-length inhomogeneity described below.

Inhomogeneity of sarcomere length was investigated by analyzing the intensity profile of the first order diffraction signal, as well as the striation image of the observed region, during the tetanus.

The intensity profile of the first diffraction order was acquired through the duration of the tetanus at a rate of four records per second (Fig. 4). Though the fine details of the profile changed during the tetanus, the overall shape, the height, and the width of the profile varied only to a small extent. Table 1 summarizes the change in sarcomere-length inhomogeneity in the region sampled by the laser beam as observed during tetani in five different fibers. Because the beam width was 0.25 mm, the sampled region contained ~100 sarcomeres. The average sarcomere-length inhomogeneity before the release was 0.046 μm, and increased to 0.066 μm after the release. The inhomogeneity is clearly too small to account for the observed increases in force, even in the limit case in which the whole cross-section contains sarcomeres shorter than the measured average length.

Inhomogeneity was also assessed by measuring the length of a string of 25–30 sarcomeres in series at each of three different locations in each image. The measurement was made directly from the screen of the monitor (Fig. 1). This procedure was repeated at three different focal planes.

Representative results are shown in Fig. 5. The first image in each row was taken before stimulation, the second during the first plateau (before the release), the third during the second plateau (after the release), and the fourth after relaxation. Sarcomere length was sampled by measuring the lengths of three strings of 25–30 sarcomeres in each focal plane, totalling nine strings for a series of three identical tetani. The three sarcomere strings to be measured in each image were selected from the top, center and bottom of each image. This method can reveal lateral sarcomere-length inhomogeneity in the sampled region, but does not rule out longitudinal inhomogeneity within each sarcomere string. Due to the thickness of the focal plane (~4 μm) the measurement of striation spacing also involves averaging in the direction parallel to the optical axis, which could reduce the apparent standard deviation of sarcomere length in the sampled population.

Inhomogeneity, taken as twice the standard deviation in sarcomere length, was ±0.048 μm after the release in this tetanus. The average sarcomere-length inhomogeneity in six different fibers was ±0.058 μm before the release, and ±0.054 μm after the release (Table 2). The post-release inhomogeneity is similar to that estimated by the previous method, and, likewise, is too small to account for the increase in force level.

Analysis of "hidden" slow stretch

Most of the releases were imposed on segments in the central two thirds of the fiber, at an initial sarcomere-

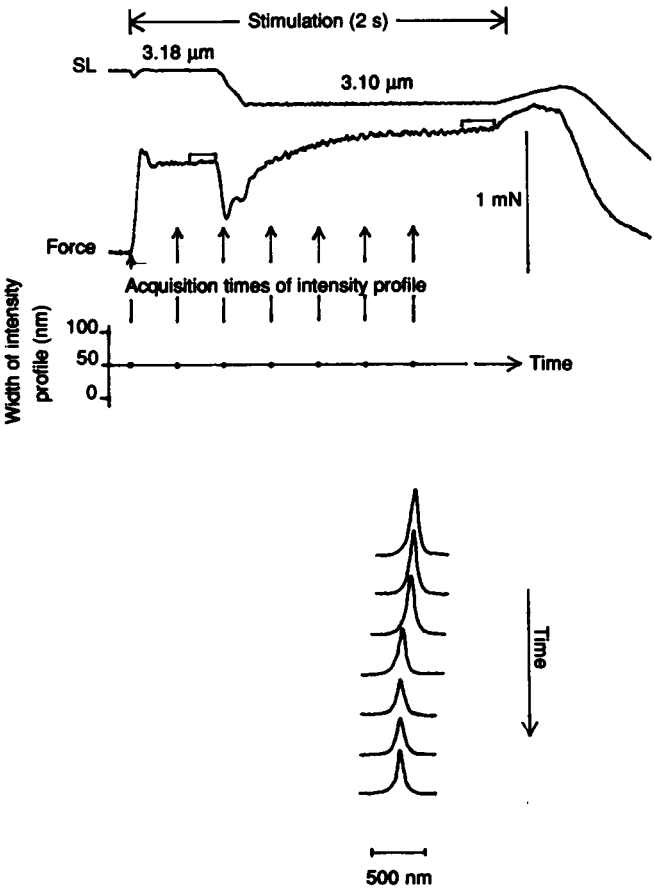

FIGURE 4 A series of intensity profiles of the first diffraction order acquired during a release protocol. The first profile was acquired at the onset of stimulation, and the time interval between consecutive profiles was 0.25 s. Intensity profiles were retraced from a photograph of the oscilloscope screen. Maximal active tensile stress developed by the fiber was 46 N/cm^2, cross-sectional area, 5.4×10^{-3} mm^2; temperature 3°C.

TABLE 1 **Width variation of diffraction intensity profile during tetanus**

Fiber #	Initial SL	Amount of release	Width of intensity profile		
	(μm)	(μm)	(μm)		
			Initial	Before release	After release
1	3.19	0.08	0.04	0.04	0.08
2	3.19	0.08	0.04	0.04	0.06
3	3.20	0.07	0.04	0.05	0.08
4	3.18	0.08	0.05	0.05	0.06
5	3.18	0.08	0.05	0.05	0.05
			Mean: 0.044	0.046	0.066
			SD: ±0.005	±0.005	±0.013

Width of the intensity profile of the first diffraction order before and after release, in five different fibers. Width was measured at half height of the profile.

length of 2.8 μm and above. Under fixed-end conditions at such sarcomere length, the tendency of the central part of the fiber is to undergo stretch. Even though the feedback loop strives to keep sarcomere length constant following the shortening, it could be argued that very slow stretch cannot be entirely avoided. Slow stretch might then bring about an artifactual increase in force. Based on the force-velocity relation for stretch measured by Granzier and Pollack (1990), the minimal stretch velocity required to produce the measured increase in force is 30 nm/s-sarc. Such a velocity is, however, large enough to have been detected by laser diffraction if it were to occur in a significant portion of the sampled sarcomeres, but it was not detected. Nevertheless, to ex-

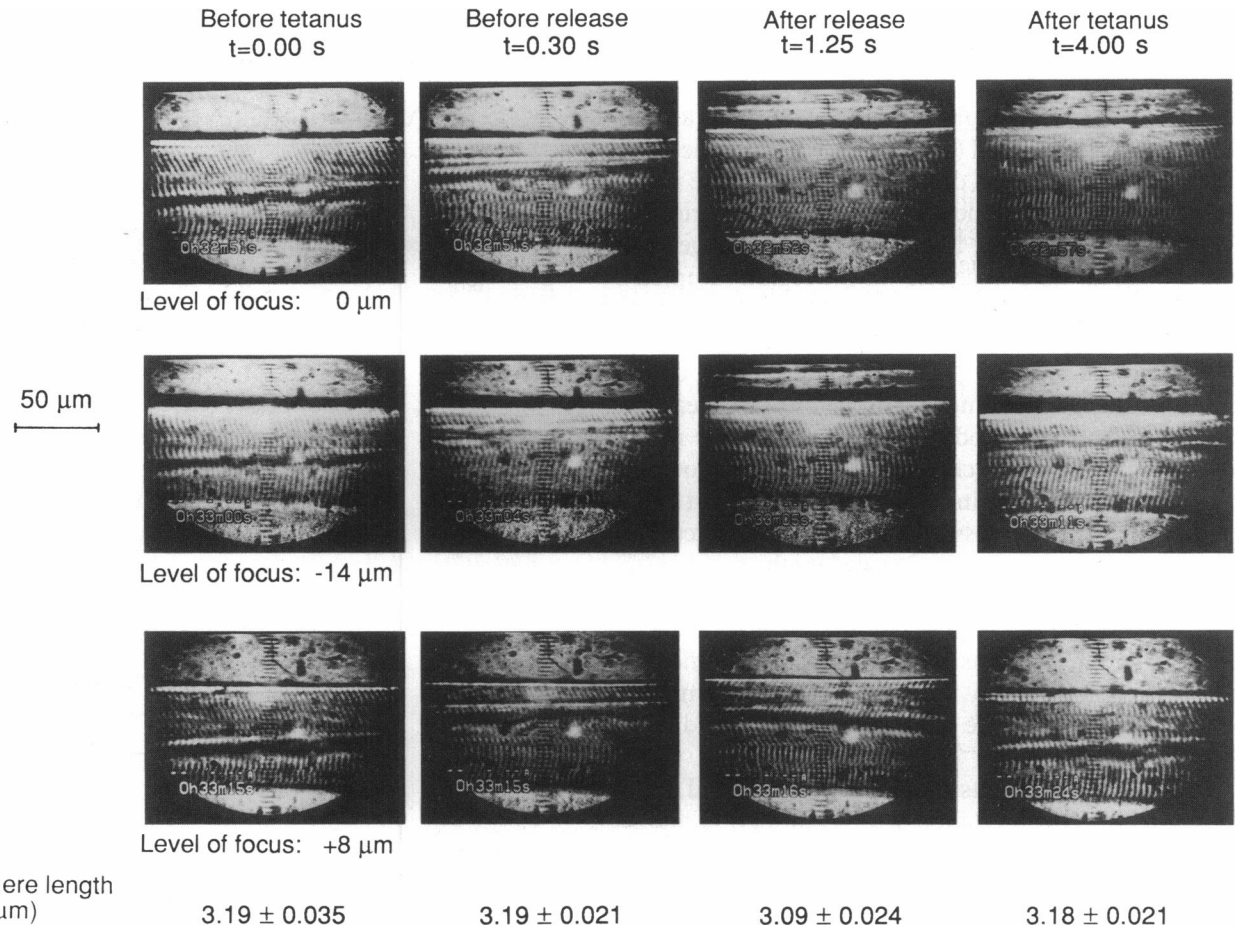

FIGURE 5 Series of striation images acquired during the release protocol. Each row of images corresponds to a different focal plane in the fiber, the focal plane of the top row serving as a reference. The images were acquired in three consecutive and identical tetani. Sarcomere-length was measured by sampling the length of three strings of 25–30 striations in series in each focal plane.

TABLE 2 **Variation of striation spacing during tetanus**

Fiber #	Initial	Amount of release	Standard deviation of striation spacing			
	(μm)	(μm)	(μm)			
			Before tetanus	Before release	After release	After tetanus
1	3.18	0.08	0.024	0.012	0.000	0.024
2	3.16	0.09	0.019	0.023	0.041	0.022
3	3.12	0.11	0.035	0.055	0.027	0.050
4	3.19	0.10	0.035	0.021	0.024	0.021
5	3.20	0.05	0.013	0.020	0.020	0.018
6	3.16	0.12	0.051	0.033	0.048	0.051
		Mean:	0.029	0.027	0.027	0.031
		SD:	±0.014	±0.015	±0.017	±0.015

Variation of striation spacing before and after the release, in six different fibers. Striation spacing was estimated at three different planes in the fiber, by measuring the length of a string of 25–30 sarcomeres in series at each of three different locations in each plane.

clude this possibility, we imposed a slow release during the second plateau, immediately following the experimental release. Such a slow release would have opposed a "hidden" slow stretch, thus decreasing its possible effect on the force. The magnitude of the resulting force increase was similar to that in tetani in which sarcomere length was kept constant after the initial release (Fig. 6), thus assuring that slow stretch does not explain the observation.

Comparison of force response after releases controlled with alternate first diffraction orders

When striations in a fiber are tilted at specific angles, erroneous sarcomere-length readings, known as Bragg-artifacts, can arise (Rüdel and Zite-Ferenczy, 1979). The Bragg-artifact affects each of the first diffraction orders differently. Consequently, significant differences between the force records corresponding to releases controlled by each of the diffraction first orders could arise. Therefore, we repeated several releases with the same fibers and sarcomere populations, using either the left-hand or right-hand first order, and examined the difference between the force levels after the release.

Fig. 7 shows two pairs of sarcomere length and force records obtained from the same fiber region, but controlled with either the left-hand or right-hand diffraction signals. Except for minor differences in records, there was no significant quantitative difference between force

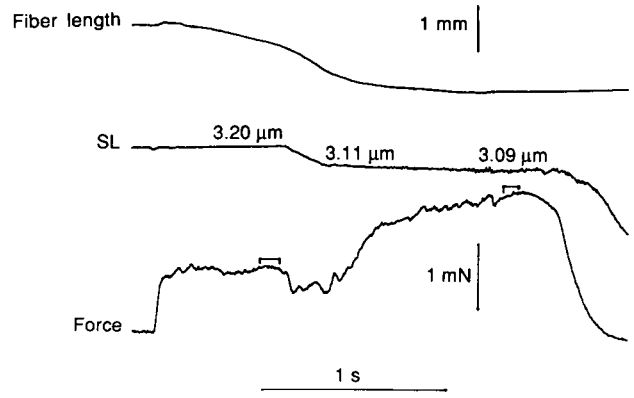

FIGURE 6 Fiber-length, sarcomere-length and force traces of a release followed by an additional slower release. The velocities of the releases were 0.45 μm/s-sarc and 0.02 μm/s-sarc, respectively. Maximal active tensile stress developed by the fiber was 44 N/cm^2; cross-sectional area, 8.8×10^{-3} mm^2; temperature 3°C.

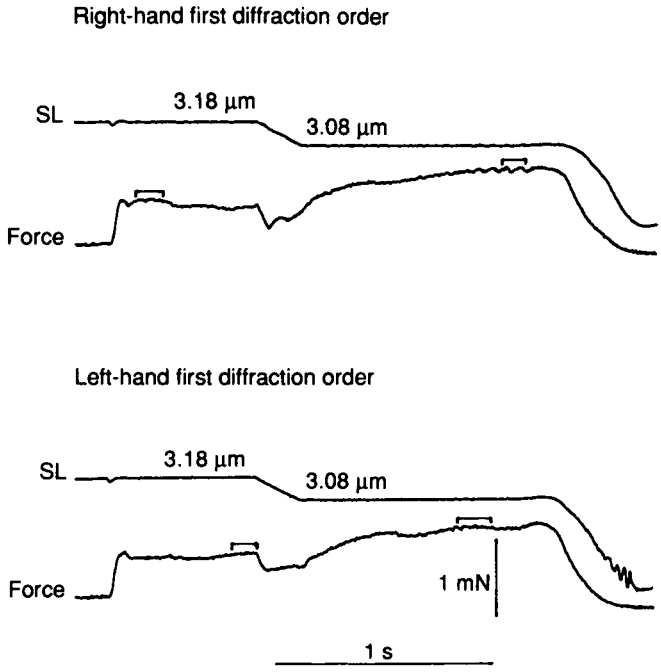

FIGURE 7 Comparison of sarcomere-length and force records of two releases performed on the same region, one (*top*) in which the right-hand first diffraction order was used as a control signal, and another in which the left-hand first diffraction order was used instead. All other parameters of the tetani were identical. Maximal active tensile stress developed by the fiber was 18 N/cm^2; cross-sectional area, 15.7×10^{-3} mm^2; temperature 3°C.

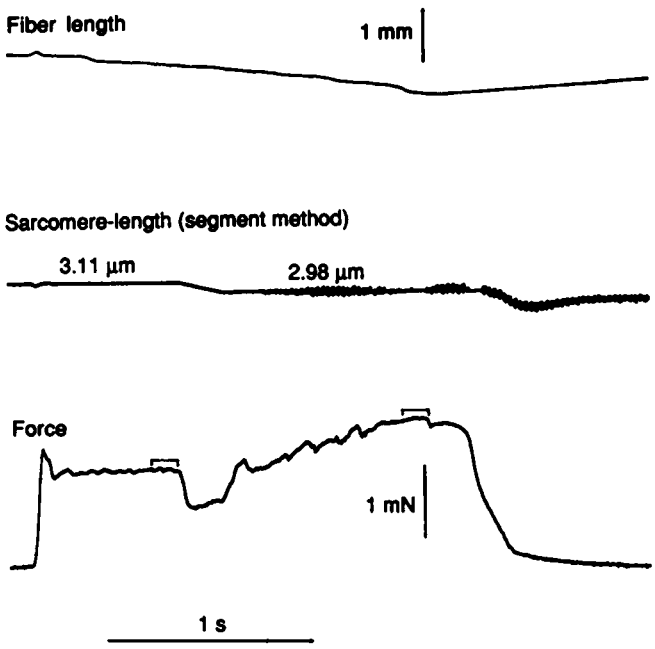

FIGURE 8 Fiber-length, segment-length and force traces of a release in which the controlled parameter was segment-length, and not diffraction-based sarcomere-length. Release size was 100 nm per sarcomere, and release velocity was 0.5 μm/s-sarc. Maximal active tensile stress developed by the fiber was 50 N/cm^2; cross-sectional area, 7.4 10^{-3} mm^2; temperature 3°C.

levels: the average difference between the two post-release maximal force levels in three fibers was 2.3%. Therefore, it is unlikely that Bragg-artifacts influenced the results.

Segment-length measurements

Segment-length was used in several tetani as an alternative feedback signal, instead of diffraction. The force records of the segment-controlled fibers showed an increase in the post-release level, similarly to diffraction-controlled releases (Fig. 8). The average post-release force level for five releases of 0.1 μm from an initial sarcomere length between 3.3 and 3.1 μm imposed on four fibers was 55 ± 4% (of force at 2.2 μm), the same as the post-release force level based on diffraction-controlled releases (see Fig. 15). The difference between the pre- and post-release force levels was smaller, though, than the one in the diffraction controlled releases (18% compared to 23%). Nevertheless, the increase is more than three times larger than the 5% predicted by the linear descending limb. Because the results of the segment method are based only on five releases (compared to 64 with the diffraction method) the difference might simply reflect the small size of the sample.

The similarity of the results with these two types of protocols significantly diminishes the possibility that the force increase is a diffraction-induced or translation-induced artifact.

Comparison of fiber-length and sarcomere-length releases

In addition to the sarcomere-length controlled releases, we have performed releases at the fiber level. The magnitude of the release at the fiber level was determined so as to produce sarcomere shortening similar in size to the shortening imposed in the sarcomere-length controlled releases (0.1 μm), assuming uniform sarcomere shortening along the fiber. Such releases did not produce, however, a parallel effect at the sarcomere level. Instead of shortening, sarcomeres in the central region of the fiber underwent stretch (data not shown). This confirms previous observations indicating that it is inaccurate to infer sarcomere-dynamics from fiber-length dynamics (Sugi and Tsuchiya, 1988; Granzier and Pollack, 1989).

Effect of release parameters

Timing of release

Fibers were released at varying time points during the tetanus, which typically lasted 2 s. The pooled results (n = 64, 12 fibers, release size between 20 and 160 nm per sarcomere) did not indicate any substantial time dependence as long as the release occurred at the onset of stimulation or later during the tetanus (Fig. 9). A release of 40 nm per sarcomere imposed 0.1 s before stimulation produced a force level identical to a strictly sarcomere-isometric tetanus with the same fiber at the post-release sarcomere-length (data not shown). Thus, the increase mechanism has no "memory" of length-change events that occur in the passive state before stimulation. The increase in force is dependent on the ability of the sarcomeres to undergo shortening only when activated.

Release velocity

The velocity of the release was varied between 0.2 and 1.2 μm per s per sarcomere. This range corresponded to

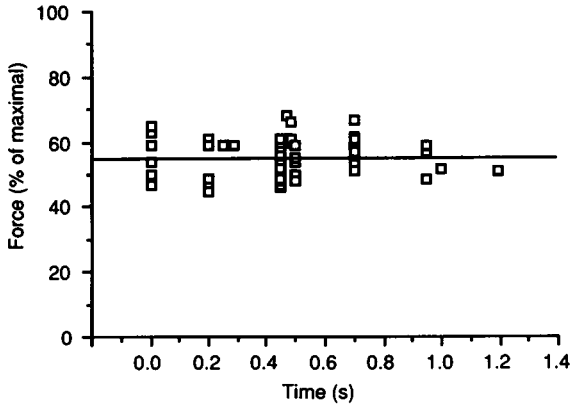

FIGURE 9 Dependence of post-release force level on release timing, based on the pooled results (n = 64, 12 fibers). Release sizes were between 0.02 and 0.16 μm per sarcomere, and release velocities were between 0.2 and 1.2 μm per sarcomere per second. Force was normalized relative to slack-length force.

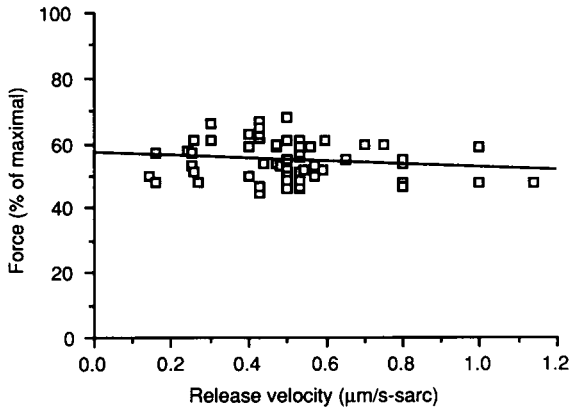

FIGURE 10 Dependence of post-release force level on release velocity, based on the pooled results (n = 64, 12 fibers). Release sizes were between 0.02 to 0.16 μm per sarcomere, and release timings were between 0.0 to 1.2 s after the onset of stimulation. Force was normalized relative to slack-length force.

the typical shortening velocities of sarcomere groups in the intermediate (between end and middle) regions of highly stretched, isometrically contracting fibers (Horowitz and Pollack, 1992).

The composite results (n = 64, 12 fibers) show no significant dependence of the increase in force on release velocity (Fig. 10). Based on the slope of the linear regression in Fig. 10, the fall in the relative force is only 2.5% for the whole variation range of release velocities.

Release size

Release size was varied from 10 to 80 nm per half sarcomere. This upper limit on release size avoided possible effects of shortening-induced deactivation (Edman, 1980).

A series of three progressively larger releases, imposed at the same time after stimulation, and at the same velocity, is shown in Fig. 11 *A*. The increase in force level became gradually larger as the size of the release was increased from 30 to 125 nm per sarcomere (from 1 to 4% of the initial sarcomere length, respectively). However, the range of increase between the smallest and largest releases in Fig. 11 *A* was only 7%. The composite results for releases between 20 and 160 nm (n = 64, 12 fibers, Fig. 11 *B*) show a linear dependence of force increase on release size for the larger releases. For smaller ones, the increment drops rapidly to zero. Data for releases less than 20 nm per sarcomere were difficult to obtain: the sarcomere-length diffraction signal contains some noise due to small irregularities in sarcomere alignment, connective tissue remnants on the sarcolemma, et cetera. Therefore, we felt it was unreliable to analyze releases smaller than 20 nm per sarcomere, even though the resolution limit of the diffraction sarcomere length measurement was 0.5 nm per sarcomere (Granzier and Pollack, 1989).

Location along fiber

We compared the effect of releases imposed on sarcomere groups at different locations along the fiber. Releases were imposed either on the end regions of the fibers, where sarcomeres ordinarily undergo shortening during fixed-end contraction, or on the central region, where sarcomeres ordinarily undergo stretch.

The occurrence of the force increase was indeed related to the dynamics of those sarcomeres under fixed-end conditions. Fig. 12 shows a set of records corresponding to sarcomeres at a distance of ~5% of total fiber-length from the tendon. These sarcomeres underwent shortening of 0.22 μm under fixed-end conditions. When released by 50 nm per sarcomere there was no increase in their force production. It seems, therefore, that releasing sarcomeres that would have shortened anyway under fixed-end conditions produces no force increase, unlike the results obtained in the central region of the fiber, as reported in the rest of this paper. The prerelease force produced by the end sarcomeres in the release described above was, however, higher than the average force produced by length-clamped sarcomeres in the center of the fiber. This difference could be related to the possibility that the end sarcomeres are intrinsically "stronger" than the central ones, due to different level of activation, or to distribution of myosin isoforms along the fiber (Gauthier and Lowey, 1979; Edman et al., 1988).

Prerelease history

To determine whether the post-release force is maximal, or whether additional release produces additional enhancement, we imposed two identical releases, one after the other, during a single tetanus. The released sarcomere groups were in the central region of the fiber. The first was triggered 0.7 s after the onset of stimulation, and the second one 1 s later (Fig. 13). The first release was accompanied by an average increase of 53% in force (n = 3, 3 fibers) relative to the force level before the release, whereas the second one produced only a 7% increase relative to the force level after the first release. This minor secondary increase is consistent with that expected from Fig. 11 *B*. The first release apparently exhausts most of the sarcomeres' potential for force enhancement.

Time course of post-release force rise

All releases provoked an instantaneous drop in force. However, force recovery generally began while the release was still in progress. The composite results, for eight records with intermediate release velocities (0.47–0.53 μm/s-sarc) at initial sarcomere length of 3.2 μm, indicate that force recovery starts 128 ± 35 ms (mean ± SD, n = 8, 6 fibers) after the onset of the release, and reaches half of its maximal level 317 ± 65 ms after the onset of the release (Fig. 14). Although force-recovery times in the entire pool of results were more variable

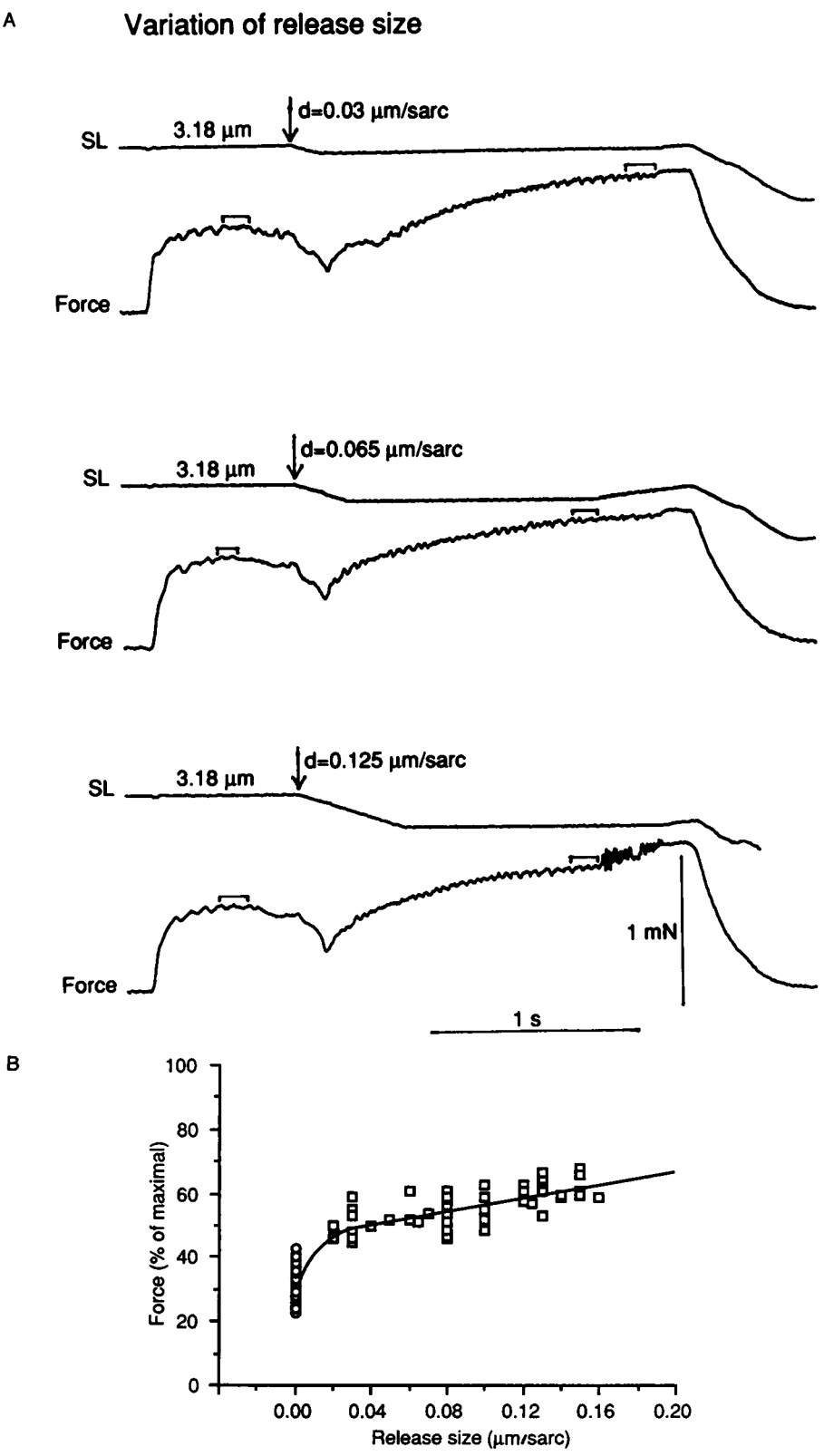

FIGURE 11 (*A*) Records of releases of three different sizes imposed on the same fiber during three consecutive tetani. Release timing was 0.70 s after the onset of stimulation, and release velocity was 0.25 μm per sarcomere per second in all three releases. Tetanus duration was 2 s, and initial sarcomere-length was 3.18 μm. Maximal active tensile stress developed by the fiber (at slack length) was 20 N/cm^2; cross-sectional area, 5.5×10^{-3} mm^2; temperature 3°C. (*B*) Dependence of post-release force level on release size, based on the pooled results (n = 64, 12 fibers). The circles indicate prerelease force levels. Release velocities ranged between 0.2 and 1.2 μm per sarcomere per second, and release timings were between 0.0 to 1.2 s after the onset of stimulation. Initial sarcomere-length was 3.2 μm. Force was normalized by slack-length force.

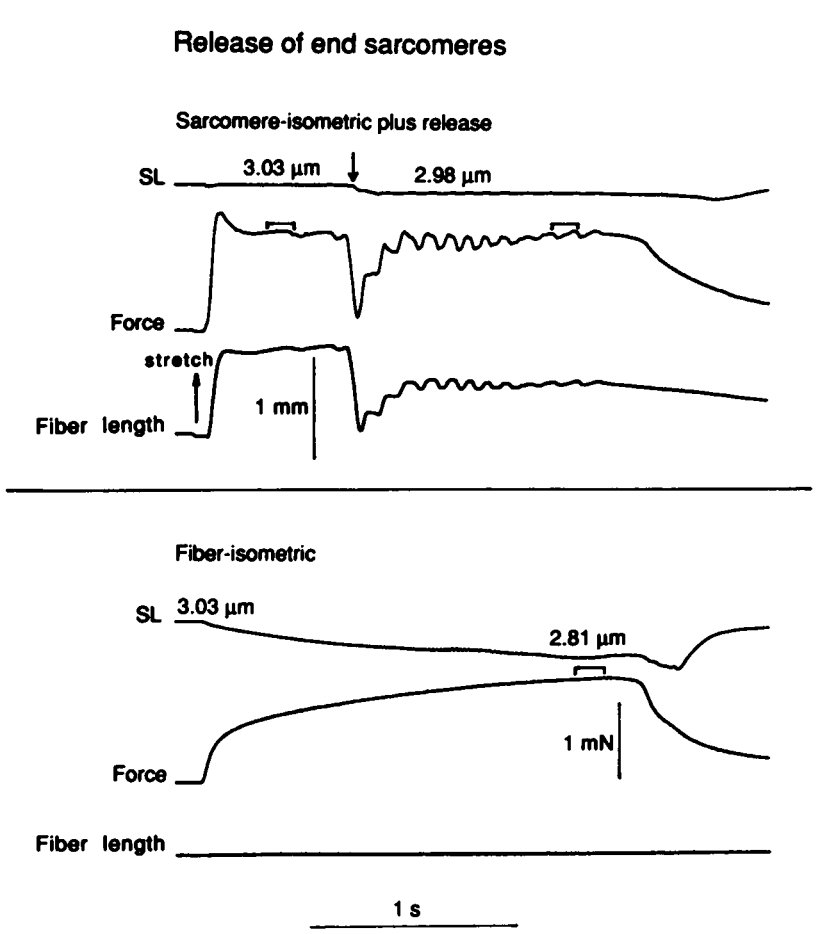

FIGURE 12 Records of a fixed-end tetanus and of a release imposed on the same end region of a fiber. Maximal active tensile stress developed by the fiber was 31 N/cm^2; cross-sectional area, 9.4 × 10^{-3} mm^2; temperature 3°C.

than in the above eight records, the recovery times were not drastically different even when initial sarcomere-lengths was much shorter. In three releases of similar velocity from 2.2 μm, force recovery started 185 ± 107 ms after the onset of the release, and reached half of its maximal level 487 ± 85 ms after the onset of the release.

Force-length relation

The measured force levels after the release were normalized and plotted as a function of the corresponding sarcomere lengths (Fig. 15). These data points represent force levels obtained with various sizes, speeds and times of release. In spite of the possible spread that could be caused by this variation, the average force-length curve of the pooled results is higher than the force levels predicted by the classical force-length relation. The difference is insignificant at intermediate sarcomere lengths, but becomes more pronounced above 2.8 μm. At a sarcomere length of 3.1 μm, the average force level after the release is 50% higher than the one predicted by the descending limb of the linear force-length relation. The difference was highly significant above sarcomere length of 2.8 μm. Based on a t test, the probability that both force levels belong to the same population was less than 0.01%.

Discussion

Comparison with previous results

Several studies have been performed on the effect of length changes on fiber force production. However, in none of these studies were small and relatively slow sarcomere releases carried out at long lengths, as done here. Ford et al. (1977) and Sugi and Kobayashi (1983) imposed very fast length changes (in the single millisecond range) at slack length. The releases performed by Edman (1980) in his studies of deactivation, and by Sugi and Tsuchiya (1988) and Granzier and Pollack (1989) were larger than those in the present study. Moreover, the parameter controlled by Granzier and Pollack (1989) was force, and not sarcomere length. Finally, the releases imposed by Güth and Kuhn (1978) and by Maréchal and Plaghki (1979) were done at the fiber level, not at the sarcomere level. As we have reported above, sarcomere dynamics in fiber-length controlled releases are very different from those in which sarcomere length is controlled directly.

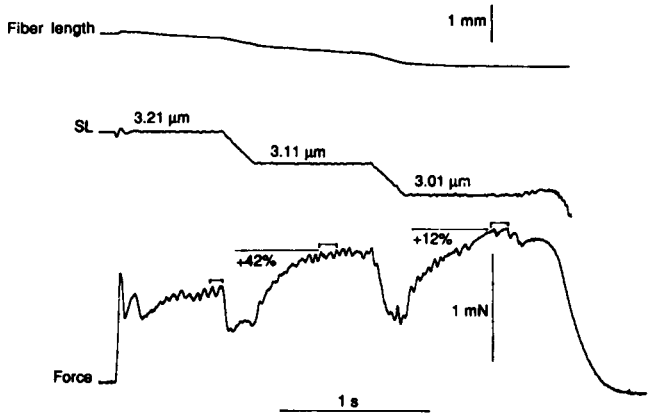

FIGURE 13 Two consecutive releases of the same size and velocity imposed during a single tetanus. The first release occurred 0.7 s, and the second release occurred 1.7 s after the onset of stimulation. Release size was 0.10 μm and release velocity was 0.50 μm/s-sarc. The force increase indicated after each release is relative to the force level before the release. Maximal active tensile stress developed by the fiber was 18 N/cm^2; cross-sectional area, 15.7×10^{-3} mm^2; temperature 3°C.

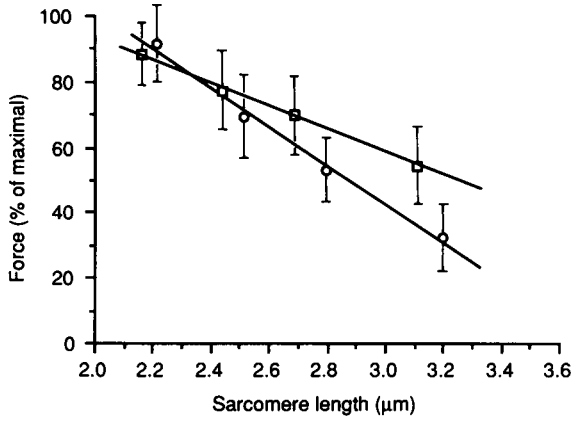

FIGURE 15 Relations between prerelease (*open circles*) and post release (*open squares*) sarcomere-length and force, based on the pooled results (n = 88, 20 fibers, with the following distribution: 4 releases at short (2.2–2.5 μm) sarcomere-length, 20 releases at medium (2.7–2.8 μm) sarcomere-length, and 64 releases at long (3.1–3.2 μm) sarcomere-length; the error bars indicate standard deviations). Force was normalized relative to the fiber tetanic force level at slack length, the latter given a value of 100%.

Effects that seem similar to those we found were observed by Bozler (1977, 1983), who imposed releases on frog heart and skeletal muscle, though not at the sarcomere level. He found that the rate of rise of tension in twitches or tetani was higher when release was imposed than when it was not. By such observations, Bozler concluded that there was a shortening augmentation phenomenon in striated muscle. The effect appears to parallel the effect of shortening on tension measured here.

Potential artifacts

Sarcomere-length inhomogeneity

Erroneous sarcomere-length measurement is the chief issue of concern. If the real length of some sarcomeres in the optical field were shorter than the measured length,

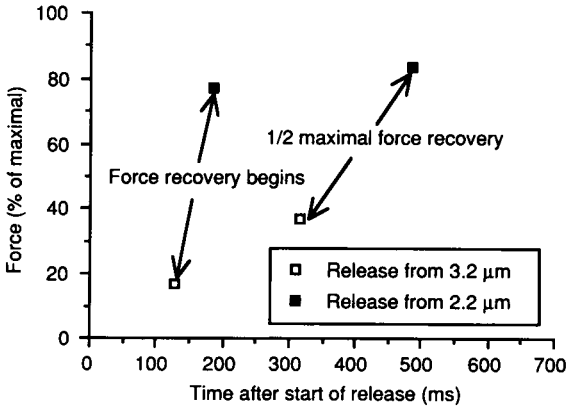

FIGURE 14 Mean beginning time of force recovery and average time of attainment of half maximal post-release force increase, based on eight records with similar release velocities (0.47–0.53 μm/s-sarc).

the difference between the post-release force level and the one predicted by the linear force-length relation would be diminished.

Errors in sarcomere-length measurement could arise mainly through length-inhomogeneity within the sampled sarcomere population. We used two different controls for measuring the dispersion of sarcomere-lengths in the sampled region: striation imaging and measurement of the width of the intensity profile of the first diffraction order. Both methods indicated a sarcomere-length inhomogeneity of ~ ±0.03 μm after release. Based on the quantitative analysis in the Results section, this value is too small to account for the increase in the force level, even if the whole fiber cross-section contains sarcomeres shorter than the apparent length. It should be pointed out, however, that both measurement methods contain several limitations. The intensity profile of the first diffraction order averages a large population (of ~100 sarcomeres), which could conceivably contain individual sarcomeres whose length lies beyond the range deduced from the width of the intensity profile. Striation imaging seems to be more direct, but it involves, in fact, averaging along each sampled sarcomere string, and through the depth of focus of the objective lens. This averaging could cause underestimation of the sarcomere-length inhomogeneity. Thus, the existence of sarcomere-length inhomogeneity cannot be absolutely ruled out.

Another source of uncertainty is sarcomere asymmetry. The amount of overlap between thick and thin filaments could be underestimated due to displacement of the thick filaments from the center of the sarcomeres, which can occur during fixed-end contractions (Horo-

wits et al., 1989). However, the release-induced force increase we observed was prominent particularly beyond sarcomere length of 2.8 μm. At such lengths, the connecting filaments, which anchor the thick filament to the Z-lines, are stretched and tend to prevent thick filament displacement (Horowits and Podolsky, 1987).

Finally, the increase in force could conceivably arise out of release-induced improvement in homogeneity (Brenner, 1983). Our results contradict this: though sarcomere-length inhomogeneity after release was small, it was usually equal to or larger than the inhomogeneity before the release (see Tables 1 and 2).

Axial translation

Another possible source of error is axial translation of the fiber during the tetanus. Because sarcomere length along the fiber may be inhomogeneous, translation could introduce either longer or shorter sarcomeres into the optical field. Sarcomere length is controlled by the feedback loop; therefore, introduction of slightly longer sarcomeres would result in further release of the fiber, and, consequently, in an artifactual change in force.

This problem was countered by using segment length as the feedback signal, instead of laser diffraction. Sampled sarcomere population remained consistent. Segment-length releases produced increases in force comparable to those obtained using optical diffraction. Translation-induced effects are therefore not sufficient to account for the force increase.

Force creep and fluctuations

Analysis of the force records was often encumbered by force creep and by force fluctuations, which appeared to varying degrees in most of the tetani. Under sarcomere or segment-isometric conditions, force creep and fluctuations are frequently observed (e.g., see Fig. 1. in Edman and Reggiani, 1987; at 3.0 μm, for example, creep reached $\sim$14%, and peak-to-peak force fluctuations reached $\sim$10% of force at the end of the fast-rise phase). The fluctuations may arise in part from noise in the sarcomere-length signal, which is then fed back into the left motor (e.g., see the "Control" records of Fig. 2 *B*). We systematically excluded records in which fluctuations were excessive, or in which prerelease force crept more than 5% of the force level at the end of the fast rise phase. This allowed us to distinguish between any creep-related force increase and a force increase associated specifically with the release. Generally, when the force plateau was preceded by a phase of creep, force magnitude was measured when the force trace levelled off, whether before the release or after it (e.g., see record of release in Fig. 2 *A*).

Bragg-effect

Another diffraction-related potential artifact is the Bragg effect, which could be caused by skewing of the striation pattern (Rüdel and Zite-Ferenczy, 1979). The skewing would affect each of the first diffraction orders differently. Therefore, we tested for Bragg effects by comparing releases in which alternate first orders were used as a control signal. There was no significant difference between the force traces in the two cases.

Dependence of force increase on release parameters

The results indicate little or no dependence of the size of the force increase on the timing or on the velocity of release. However, the force increase does depend on release size. Releases as small as 20 nm per sarcomere produced a substantial increase in force level. Beyond 40 nm per sarcomere, the incremental force increase became modest. The largest releases we imposed at a sarcomere length of 3.2 μm, 160 nm per sarcomere, produced only a limited amount of additional force increase (Fig. 11 *B*). It seems that merely imposing a release, even a minute one, makes the force production significantly different compared to the force production in a strictly sarcomere-isometric tetanus.

A second release imposed during the same tetanus had little additional augmentation effect on the force level. It seems that once a release occurs, most of the force-elevation potential of the fiber is exhausted.

Interestingly, the location of the sarcomeres upon which the release is imposed was also found to be a determinant of the response. Sarcomeres in the end region of the fiber, which ordinarily shorten under fixed-end conditions, exhibit little or no force increase, whereas the central sarcomeres, which ordinarily undergo stretch, show the force increase consistently. Thus, the response appears to be tied to some dynamic distinction among sarcomeres.

The force increase depends on the initial sarcomere length. The increase is insignificant at short sarcomere lengths, and becomes increasingly pronounced above 2.8 μm. This sarcomere-length dependent behavior resembles the sarcomere-length dependent difference between the (higher) fixed-end force-length relation and the (lower) linear sarcomere-isometric force-length relation (see Pollack, 1983, for review).

Mechanism

The source of the release-induced increase in force could be either in the activation process, or in the contractile mechanism itself. Gordon and Ridgway (1975), and Fabiato and Fabiato (1978) concluded that calcium-triggered calcium release in skeletal muscle fibers and in cardiac cells, respectively, could depend on length. Thus, shortening could provoke additional calcium release. However, sarcomere length in those studies was on the ascending limb of the force-length relation, and may not, therefore, be relevant here. Several other studies indicated possible dependence of the calcium sensitivity on length (Endo, 1972; Allen et al., 1974; Fabiato and Fabiato, 1976; Martyn and Gordon, 1988; Kuhn et al.,

1990). Activation conditions in those studies differed from those in the present one; fibers were skinned and partially activated by submaximal calcium concentrations. Hence, the relevance of these studies to ours also seems limited.

Alternatively, the release-induced force increase could originate in the contractile mechanism. Long-term history dependence of the sort found here is not accounted for by the cross-bridge model in its most basic form. However, the release could conceivably shift all attached cross-bridges in the same direction. Subsequent strokes might, therefore, be more synchronized than those before the release, and force production might then be higher. Such a mechanism seems to be in agreement with some of the characteristics of the release: it would have no dependence on the release time and velocity.

More difficult to account for on this basis are other aspects of the phenomenon. Based on a cross-bridge detachment rate constant of 400 s^{-1} (Siemankowski et al., 1985), the cross-bridge synchronization induced by the release would be expected to decay during the post-release part of the tetanus in less than 2.5 ms. Our results, however, indicate that the contractile mechanism is history dependent over time intervals in the range of seconds: the force does not decay but tends to reach a sustained plateau (see, for example, Fig. 2). Decay of cross-bridge synchronization would also imply that a second release triggered at least 0.5 s after first one should produce an additional increase in force. However, releases imposed a second later had only minimal effect. Finally, it is difficult to explain why the absolute increase becomes smaller at increasing degrees of overlap: at 2.2 μm the increase vanishes.

On the other hand, the phenomenon is explicitly predicted by an alternative contraction model (Pollack, 1990, p. 258). In this model, a distinction is made between sarcomeres that are "generators" and those that are "sustainers". A generator is a sarcomere that is actively generating tension. A sustainer merely sustains the generated tension. Thus, sarcomeres that are being stretched, or that are held isometrically to prevent stretch, are sustainers. Sarcomeres that shorten are generators: by shortening a small amount, around 20 nm per half sarcomere according to the theory, these sarcomeres' thick filaments are themselves able to shorten, to undergo go a conformational transition, and to generate active tension. Thus, the small shortening imposed in these experiments converts sustainers to generators, and thereby adds active tension.

The model also accounts for the distinction between end and middle sarcomeres. Middle sarcomeres are ordinarily stretched during fixed-end contraction, and are, therefore, sustainers; imposed shortening converts them to generators and increases tension as above. End sarcomeres, on the other hand, ordinarily shorten during fixed-end contraction. When held isometrically at moderate to long length, these sarcomeres do not shorten, but limited overlap allows thick filaments to shorten by slipping past thin filaments. End sarcomeres held isometrically are therefore generators. Imposed shortening does not further increase their tension. Thus, the new theory accounts not only for the phenomenon's essential features, but also for the fact that it is observed only in certain sarcomeres.

We thank Mr. John Myers for his excellent support with instrumentation, and Mr. Wen Jun Sun and Mr. Kin Ng for their devoted dissection work.

Received for publication 15 July 1991 and in final form 19 March 1992.

REFERENCES

Allen, D. G., B. R. Jewell, and J. W. Murray. 1974. The contribution of activation processes to the length-tension relation of cardiac muscle. *Nature (Lond.).* 248:606.

Bagni, M. A., G. Cecchi, F. Colomo, and C. Tesi. 1988. Plateau and descending limb of the sarcomere length-tension relation in short length-clamped segments of frog muscle fibers. *J. Physiol. (Lond.).* 277:291–323.

Brenner, B. 1983. Technique for stabilizing the striation pattern in maximally calcium activated rabbit psoas fibers. *Biophys. J.* 41:99–102.

Bozler, E. 1977. Mechanical control of the rising phase of frog skeletal and cardiac muscle. *J. Gen. Physiol.* 70:695–705.

Bozler, E. 1983. Intrinsic control of activity of striated muscle by length changes. *Biomed. Res.* 4:347–362.

Burton, K., W. N. Zagotta, and R. J. Baskin. 1989. Sarcomere length behavior along single muscle fibers at different lengths during isometric tetani. *J. Muscle Res. Cell Motil.* 10:67–84.

Edman, K. A. P. 1980. Depression of mechanical performance by active shortening during twitch and tetanus of vertebrate muscle fibers. *Acta Physiol. Scandinavica.* 109:15–26.

Edman, K. A. P., and C. Reggiani. 1987. The sarcomere length-tension relation determined in short segments of intact muscle fibers of the frog. *J. Physiol. (Lond.).* 385:709–732.

Edman, K. A. P., C. Reggiani, S. Schiaffino, and G. te Kronie. 1988. Maximum velocity of shortening related to myosin isoform composition in frog skeletal muscle fibers. *J. Physiol. (Lond.).* 395:679–692.

Endo, M. 1972. Stretch-induced increase in activation of skinned muscle fibers by calcium. *Nature New Biol.* 237:211–213.

Fabiato, A., and F. Fabiato. 1976. Dependence of calcium release, tension generation and restoring forces on sarcomere length in skinned cardiac cells. *Eur. J. Cardiol.* 4:13–27. (Suppl.)

Fabiato, A., and F. Fabiato. 1978. Myofilament-generated tension oscillations during partial calcium activation and activation dependence of the sarcomere length-tension relation of skinned cardiac cells. *J. Gen. Physiol.* 72:667–699.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1977. Tension response to sudden length change in stimulated frog muscle fibers near slack length. *J. Physiol. (Lond.).* 269:441–515.

Gauthier, G. F., and S. Lowey. 1979. Distribution of myosin isoenzymes among skeletal muscle fiber types. *J. Cell Biol.* 81:10–25.

Gordon, A. M., A. F. Huxley, and F. J. Julian. 1966. The variation in isometric tension with sarcomere length in vertebrate muscle fibers. *J. Physiol. (Lond.).* 184:170–192.

Gordon, A. M., and E. B. Ridgway. 1975. Muscle activation: effects of small length changes on calcium release in single fibers. *Science (Wash. DC).* 189:881–884.

Granzier, H. L. M., and G. H. Pollack. 1989. Effect of active pre-shortening on isometric and isotonic performance of single frog muscle fibers. *J. Physiol. (Lond.).* 415:299–327.

Granzier, H. L. M., and G. H. Pollack. 1990. The descending limb of the force-sarcomere length relation of the frog revisited. *J. Physiol. (Lond.).* 421:595–615.

Güth, K., and H. J. Kuhn. 1978. Stiffness and tension during and after sudden length changes of glycerinated rabbit psoas muscle fibers. *Biophys. Struct. Mech.* 4:223–236.

Horowits, R., and R. J. Podolsky. 1987. The positional stability of thick filaments in activated skeletal muscle depends on sarcomere length: evidence for the role of titin filaments. *J. Cell Biol.* 105:2217–2223.

Horowits, R., K. Maruyama, and R. J. Podolsky. 1989. Elastic behavior of connectin filaments during thick filament movement in activated skeletal muscle. *J. Cell Biol.* 109:2169–2176.

Horowitz, A., C. J. Caljouw, and G. H. Pollack. 1989*a*. Force-length relation of "almost isometric" sarcomeres. *Biophys. J.* 55:409*a*. (Abstr.)

Horowitz, A., C. J. Caljouw, and G. H. Pollack. 1989*b*. Length-tension relation for slightly shortened sarcomeres (1–3%) is higher than for isometric sarcomeres. *Proc. 31st Int. Cong. Physiol. Sci.* 876a. (Abstr.)

Horowitz, A., and G. H. Pollack. 1990. Sarcomeres shortened by 1–3% produce 75% more force than isometric sarcomeres. *Biophys. J.* 57:547*a*. (Abstr.)

Horowitz, A., and G. H. Pollack. 1992. Force-length relation of isometric sarcomeres in fixed-end tetani. Submitted.

Huxley, A. F. 1957. Muscle structures and theories of contraction. *Prog. in Biophys. Biophys. Chem.* 7:257–318.

Huxley, A. F., and R. M. Simmons. 1971. Proposed mechanism of force generation in striated muscle. *Nature (Lond.).* 233:533–538.

ter Keurs, H. E. D. J., T. Iwazumi, and G. H. Pollack. 1978. The sarcomere length-tension relation in skeletal muscle. *J. Gen. Physiol.* 72:565–592.

Kuhn, H. J., C. Bletz, and J. C. Rüegg. 1990. Stretch induced increase in the Ca^{2+} sensitivity of myofibrillar ATPase activity in skinned fibers from pig ventricles. *Pflugers Arch. Eur. J. Physiol.* 415:741–746.

Maréchal, G., and L. Plaghki. 1979. The deficit of the isometric tetanic tension redeveloped after a release of frog muscle at constant velocity. *J. Gen. Physiol.* 73:453–467.

Martyn, D. A., and A. M. Gordon. 1988. Length and myofilament spacing-dependent changes in calcium sensitivity of skeletal fibers: effects of pH and ionic strength. *J. Muscle Res. Cell Motil.* 9:424–445.

Pollack, G. H. 1983. The cross-bridge theory. *Physiol. Rev.* 63:1049–1113.

Pollack, G. H. 1990. Muscles and Molecules: Uncovering the Principles of Biological Motion. Ebner and Sons, Seattle, WA.

Rüdel, R., and F. Zite-Ferenczy. 1979. Do laser diffraction studies of striated muscle indicate stepwise sarcomere shortening? *Nature (Lond.).* 278:573–574.

Siemankowski, R. F., M. O. Wiseman, and H. D. White. 1985. ADP dissociation from actomyosin subfragment 1 is sufficiently slow to limit the unloaded shortening velocity in vertebrate muscle. *Proc. Natl. Acad. Sci. USA.* 82:658–662.

Sugi, H., and T. Kobayashi. 1983. Sarcomere length and tension changes in tetanized frog muscle fibers after quick stretches and releases. *Proc. Natl. Acad. Sci. USA.* 80:6422–6425.

Sugi, H., and T. Tsuchiya. 1988. Stiffness changes during enhancement and deficit of isometric force by slow length changes in frog skeletal muscle fibers. *J. Physiol. (Lond.).* 407:215–229.