1640, supplemented with 15% fetal bovine serum (FBS), were incubated for 2 to 4 hours with MOLT-4 cl.8 cells, and SIVagm(tyo-1) was incubated with rCD4 (5 μg/ml) before infection in 96-well plates, essentially as described in the legend to Fig. 1. Enhancement or inhibition was scored at 2 days after infection and was based on total inhibition of syncytium formation.
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19. For neutralization, heat-inactivated serum samples were incubated beginning at a dilution of 1:5 in complete medium with SIVagm for 2 hours at 25°C before the addition to MOLT-4 cl.8 cells. Cells were observed for cytopathic effects on a daily basis. Criterion for neutralization was a total loss in syncytium formation at 6 days.
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23. We thank G. Cianciolo and Genentech for providing rCD4 reagents, M. Hayami for providing SIVagm(tyo-1) and MOLT-4 cl.8 cell lines, R. Kennedy for helpful discussions, J. Eichberg for SIVmac serum samples, and M. Anthony for African green monkey serum samples. Supported by NIH grants 1RO1-AI28273 and 5UO1-AI26462.

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Calcium-Sensitive Cross-Bridge Transitions in Mammalian Fast and Slow Skeletal Muscle Fibers

JOSEPH M. METZGER* AND RICHARD L. MOSS

The fundamental mechanism underlying the differing rates of tension development in fast and slow mammalian skeletal muscle is still unknown. Now, in skinned (membrane-permeabilized) single fibers it has been shown that the rate of formation of the strongly bound, force-producing cross-bridge between actin and myosin is calcium-sensitive in both fast and slow fibers and that the rate is markedly greater in fast fibers. The transition rates obtained at high calcium concentrations correlated with myosin isoform content, whereas at low calcium concentrations the thin filament regulatory proteins appeared to modulate the rate of tension development, especially in fast fibers. Fiber type–dependent differences in rates of cross-bridge transitions may account for the characteristic rates of tension development in mammalian fast and slow skeletal muscles.

FAST AND SLOW MUSCLES ARE CHARacterized on the basis of differences in the duration of the isometric twitch and the maximum shortening velocity of unloaded shortening (*1*). During contraction in fast muscle, both the increase in myoplasmic Ca^{2+} concentration from $10^{-7}M$ to a peak of around $10^{-5}M$ (*2*) and the binding of Ca^{2+} to the low-affinity sites of troponin are too rapid to limit the rate of force development (*3, 4*). The Ca^{2+}-induced conformational changes in the thin filament are essentially complete before the initiation of tension development (*5*) and are thus unlikely to limit the rate of tension development in skeletal muscle.

We tested the hypothesis that the rate of formation of the strongly bound, force-producing cross-bridge state differs between fast and slow fibers from rat skeletal muscles, which could provide a basis for the difference in the rate of tension development between these fibers in vivo. We also determined whether the thin filament regulatory protein troponin is involved in conferring Ca^{2+} dependence to transition rates of attached cross-bridges.

We used a method that permits the measurement of the rate of the limiting step in the cross-bridge cycle leading to the production of force (*6*). Chemically skinned (sarcolemma permeabilized) single fibers are used so that the concentration of Ca^{2+} in the vicinity of the contractile proteins can be controlled directly. The measurement protocol involves a rapid release and subsequent reextension of fiber length during steady Ca^{2+} activation, resulting in the dissociation of cross-bridge connections to actin. Subsequently, tension redevelops to the initial value at a rate that is well fit by a single exponential curve. The observed rate constant of tension redevelopment (k_{tr}) reflects a rate-limiting step or steps in the transition of cross-bridges to the force-generating state.

Currently accepted models of the actomyosin kinetic scheme (*7*) consist of a series of reversible-state transitions, in which the rate-limiting steps are as yet unknown. In this scheme, k_{tr} is a measure of the rate of approach to steady-state isometric force. Thus, k_{tr} is determined by the arithmetic sum of the forward and reverse rate constants for those steps that are rate limiting in the process of force generation. The k_{tr} is Ca^{2+}-sensitive, increasing with Ca^{2+} concentration in the physiological range (*8, 9*). However, the influence of Ca^{2+} on k_{tr} is independent of the effect of Ca^{2+} to increase the number of cross-bridge attachments to the thin filament (*10*); thus, Ca^{2+} regulates k_{tr} via effects on specific rate constants that characterize cross-bridge transitions.

The value of k_{tr} differed in fast and slow muscle at maximal activation by Ca^{2+} (that is, at *p*Ca 4.5, where *p*Ca is the $-\log Ca^{2+}$ concentration) (Fig. 1). Mean k_{tr} values (± SEM) at *p*Ca 4.5 were 22.9 ± 0.5 s^{-1} (n = 20) in rat fast-twitch (type II_b) superficial vastus lateralis (svl) skinned fibers and 3.0 ± 0.1 s^{-1} (n = 12) in rat slow-twitch (type I) soleus skinned fibers. In both svl and soleus fibers, k_{tr} was maximal at *p*Ca 4.5, since the rate constant was unaffected by increasing Ca^{2+} concentration to *p*Ca 4.0.

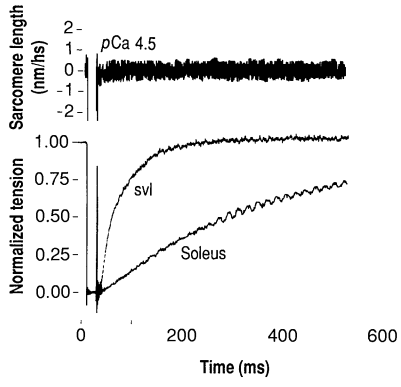


Fig. 1. Records of sarcomere length (upper traces) and tension (lower traces) obtained during the protocol to determine k_{tr}. Records were obtained during maximal Ca^{2+} activation of a fast svl skinned fiber and a slow soleus skinned fiber; sarcomere length traces from the svl and soleus are superimposed. The k_{tr} was 23.4 s^{-1} in the svl fiber and 3.0 s^{-1} in the soleus fiber. In both fibers, sarcomere length was clamped at 2.50 μm. Steady isometric tension was 55 mg in the svl fiber and 50 mg in the soleus fiber. Fiber type was determined on the basis of protein subunit composition by SDS–polyacrylamide gel electrophoresis (*14*). All soleus and svl fibers contained the slow and fast isoforms, respectively, of contractile (myosin heavy and light chains) and regulatory proteins (troponin). Records of tension redevelopment were best fit by a first-order exponential equation: $F_t = F_o(1 - e^{-kt})$, where F_t is force at time t, F_o is maximum force, and k is k_{tr}. Sarcomere length (obtained by laser diffraction) is held isometric to eliminate effects on k_{tr} caused by end compliance (*6*). Complete details of the experimental protocol, curve-fitting procedure, and mechanical set-up have been published (*9*). Relaxing and activating solutions consisted of 7 m*M* EGTA, 1 m*M* free Mg^{2+}, 4.42 m*M* total adenosine triphosphate, 14.5 m*M* creatine phosphate, 20 m*M* imidazole, and sufficient KCl to yield an ionic strength of 180 m*M*. The temperature was set at 15°C, and the solution *p*H was 7.00 (*9*); hs, half sarcomere.

Department of Physiology, School of Medicine, University of Wisconsin, Madison, WI 53706.

*To whom correspondence should be addressed.

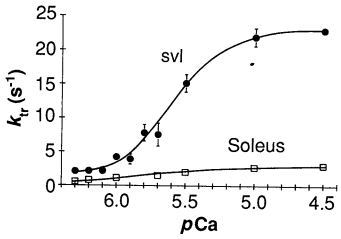
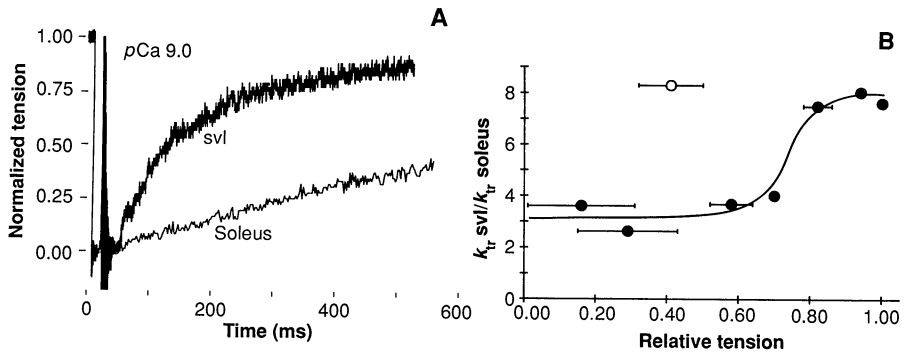


Fig. 2. The effect of Ca^{2+} on k_{tr} in svl and soleus muscle fibers. Values are mean ± SEM, with 7 to 20 observations per point. In some instances error bars were smaller than the symbol. The curves were fit to the data by eye. At each *p*Ca, the value for svl is significantly greater than the value for soleus.



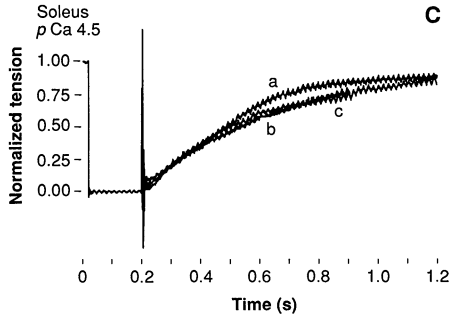


Fig. 3. Effect of protein extraction on k_{tr}. (**A**) Experimental records of tension redevelopment in the absence of Ca^{2+} (*p*Ca 9.0) after the partial removal of whole troponin complexes from a skinned svl fiber and a skinned soleus fiber. The k_{tr} was 7.8 s^{-1} in the svl fiber and 1.0 s^{-1} in the soleus fiber. During tension redevelopment, sarcomere length was clamped at 2.56 μm in the svl fiber and 2.60 μm in the soleus fiber. After the partial removal of whole troponin complexes, isometric tension in the absence of Ca^{2+} was 0.33 of the control value at *p*Ca 4.5 in the svl fiber and 0.44 of the control value at *p*Ca 4.5 in the soleus fiber. After troponin removal, k_{tr} values at *p*Ca 4.5 were 22.4 ± 0.7 s^{-1} (n = 4) for svl fibers and 3.2 ± 0.6 s^{-1} (n = 2) for soleus fibers, values that were not different from the control values at *p*Ca 4.5 in these fibers. To remove some of the whole troponin complexes from the thin filament, fibers were treated as described (*11*). The procedure to partially remove whole troponin does not damage the fiber because in the presence of saturating Ca^{2+}, maximum force and k_{tr} are unchanged from control values and Ca^{2+}-insensitive tension is completely reversed by adding purified troponin to the fiber (*11*). (**B**) Summary of the ratio of k_{tr} values (svl/soleus) as a function of relative steady-state isometric tension developed before the release step. Tensions were obtained at each *p*Ca and are scaled to the value in the same fiber at *p*Ca 4.5 before the removal of whole troponin (mean ± SEM). ●, Control data obtained by varying the *p*Ca of the activating solution; ○, mean value obtained from svl and soleus fibers activated in the absence of Ca^{2+} by partial removal of whole troponin. At *p*Ca 4.5, the k_{tr} ratio was identical to that in controls at *p*Ca 4.5. (**C**) Tension redevelopment in a soleus fiber during maximal Ca^{2+} activation. Trace a, control; trace b, after extraction of 50% of endogenous slow LC_2; trace c, readdition of purified LC_2 obtained from fast muscle. The k_{tr} was 2.5 s^{-1}, 1.9 s^{-1}, and 1.9 s^{-1} for records a, b, and c, respectively. The LC_2 extraction was performed as described (*12*). Sarcomere length was clamped at 2.52 μm.

There was also a marked difference between svl and soleus fibers in the dependence of k_{tr} on Ca^{2+} (Fig. 2). In svl fibers, k_{tr} increased 11-fold between *p*Ca 6.3 and 4.5. In soleus fibers, the increase in k_{tr} was fivefold over the same range of *p*Ca values. The *p*Ca at which k_{tr} was one-half maximal (that is, pCa_{50}) was 5.73 in soleus fibers and and 5.62 in svl fibers. The minimum measurable values of k_{tr} were obtained at approximately *p*Ca 6.3, with values of 2.1 s^{-1} in svl fibers and 0.6 s^{-1} in soleus fibers. In comparing values of k_{tr} from fast and slow fibers, it is evident that the magnitude of difference varies as a function of Ca^{2+} concentration; at submaximal Ca^{2+} concentrations, k_{tr} is threefold greater in svl than in soleus fibers, whereas at saturating Ca^{2+} concentrations the difference is eightfold.

To test the involvement of thin filament regulatory proteins in conferring Ca^{2+} dependence to k_{tr} in fast and slow fibers, we partially removed the Ca^{2+} regulatory protein whole troponin (troponin-T, troponin-C, and troponin-I) from the thin filament. This resulted in a steady, submaximal tension in the absence of Ca^{2+} (*11*). Force development after partial removal of whole troponin presumably results from a shift of tropomyosin further into the groove between actin strands and subsequent cross-bridge attachment. Average values of k_{tr} at *p*Ca 9.0 were 12.0 ± 1.6 s^{-1} (n = 4) and 1.5 ± 0.5 s^{-1} (n = 2) for svl and soleus fibers, respectively (Fig. 3). These results indicate that the differences in k_{tr} between fiber types persist in fibers activated in the absence of Ca^{2+}. The observed eightfold difference in k_{tr} in troponin-deficient fast and slow fibers at *p*Ca 9.0 (Fig. 3) is similar to the difference between untreated control fibers during maximal Ca^{2+} activation (Fig. 1). These findings are summarized in Fig. 3B, and suggest that the basis for fiber-type differences in k_{tr} in maximally activated fibers resides in the myosin-containing thick filament rather than in the thin filament regulatory protein troponin. Indeed, in partially troponin-deficient fast fibers that were further treated to completely extract troponin-C, the effect of Ca^{2+} to increase k_{tr} still persisted (*10*). Thus, it is likely that the regulation of k_{tr} by Ca^{2+} involves effects on both the thin and thick filaments.

In striated muscles of certain invertebrate species, the effect of Ca^{2+} to regulate the contractile event is mediated by myosin light chain 2 (LC_2). In mammalian skeletal muscles, extraction of LC_2 has no effect on k_{tr} at maximal Ca^{2+} concentrations (*12*). Further, in soleus fibers in which the native slow isoform of LC_2 was replaced with the fast isoform, k_{tr} at *p*Ca 4.5 was only slightly reduced (Fig. 3C). We conclude, therefore, that fiber type–dependent variations in k_{tr} at maximal Ca^{2+} concentrations are functionally related to the different isoforms of the myosin heavy chain or myosin alkali light chains expressed in fast and slow muscles.

At low Ca^{2+} concentrations, the basis for fiber-type differences in k_{tr} is different from that at maximal Ca^{2+} concentrations, because the difference at low Ca^{2+} appears to involve an effect of Ca^{2+} on thin filament regulatory proteins. The fast/slow ratios of k_{tr} obtained from fibers activated to comparable submaximal tensions by either partial removal of whole troponin or by submaximal Ca^{2+} activation were compared (Fig. 3B). In fibers in which the thin filament is intact, the ratio of k_{tr} values from fast and slow fibers decreases below 8 as Ca^{2+} is lowered from *p*Ca 4.5, but in fibers activated by partial removal of whole troponin the ratio remains near 8 when the *p*Ca is changed from 4.5 to 9.0. Thus, partial Ca^{2+} activation of the regulated thin filament is somehow inhibitory to the full expression of differences in rates of tension development between fiber types.

Our findings show that the rate-limiting step, or steps, in the cross-bridge cycle leading to force development in fast fibers is three to eight times greater, depending on Ca^{2+} concentration, than in slow fibers. These fiber type–dependent variations in k_{tr} are sufficiently large to account for differences in the kinetics of the tension time course during tetanic contractions of these muscles in vivo. During a tetanus the maximum rate of approach to final tension is

about fourfold greater in fast- compared to slow-twitch muscle (*13*). If the steady myoplasmic Ca^{2+} is 3 to 7 μM during tetanus (*3*), then the fast/slow ratios of k_{tr} that we measured at similar concentrations of Ca^{2+} are comparable to those obtained in living fast- and slow-twitch muscles. Our results, however, do not exclude the possibility that the Ca^{2+} transient or subsequent thin filament activation steps are slower in slow- than in fast-twitch muscles.

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A Voltage-Dependent Chloride Current Linked to the Cell Cycle in Ascidian Embryos

MELODYE L. BLOCK* AND WILLIAM J. MOODY

A voltage-dependent chloride current has been found in early ascidian embryos that is a minor conductance in the oocyte and in interphase blastomeres but that increases transiently in amplitude by more than tenfold during each cell division. Repeated cycles in the density of this chloride current could be recorded for up to 6 hours (four cycles) in cleavage-arrested embryos, whether they were activated by sperm or calcium ionophore. These data suggest that there is a direct link between the cell cycle clock and the properties of this channel, a link that results in pronounced cyclical changes in the electrical properties of early blastomeres.

THE RAPID CELL CYCLE OF EARLY embryos is driven by an endogenous clock that involves the synthesis and degradation of the protein cyclin, activation and inactivation of the kinase maturation-promoting factor, and release and reuptake of calcium ions from intracellular stores (*1, 2*). Changes in ion fluxes across the plasma membrane or in membrane electrical properties during embryonic cell divisions have been demonstrated in several preparations (*3–6*). In most cases, these are either permanent changes that are caused by the addition of new cleavage-furrow membrane to the cell (*4, 5*) or transient changes linked to cytokinesis that are caused, for example, by the mechanical strain of cleavage-furrow formation (*6*). It is not known whether the cell cycle oscillator can drive transient changes in the electrical or ionic properties of the plasma membrane that are unique to the time of cell division, but independent of the actual process of cytokinesis. We describe in this report a class of voltage-dependent Cl^- channels that is functional only at the time of embryonic cell divisions. Large oscillations in the functional density of these channels persist when cytokinesis is blocked, demonstrating that the cell cycle clock can exert a direct influence on the electrical properties of the plasma membrane.

Our experiments were done on cells from early embryos of the ascidian *Boltenia villosa*. Ascidians are primitive marine chordates (subphylum Urochordata) that have been used extensively in embryological and electrophysiological studies (*7–9*). Three major voltage-dependent currents are recorded from voltage-clamped *Boltenia* oocytes before fertilization (*8*): inward Na^+ and Ca^{2+} currents, activated by depolarizing voltage pulses from holding potentials more negative than −60 mV, and an inwardly rectifying K^+ current, activated at voltages more negative than −60 mV (Fig. 1). When the inwardly rectifying K^+ current was blocked by external Ba^{2+} (10 mM) (*10*), another inwardly rectifying current was revealed that had slower activation kinetics than the K^+ inward rectifier and was present at much lower density (Fig. 1). This Ba^{2+}-insensitive inward rectifier began to increase dramatically in amplitude about 100 min after fertilization (about 20 min before first cleavage at 12°C) (Fig. 2, A and B); by the time of cleavage the current density had increased by a factor of 12. In other cells, the current increased by factors of 10 to 30 as first cleavage approached.

Ion substitution experiments done near the time of cleavage, when the current was large enough to measure accurately, showed that the current was carried by Cl^- ions. Tail currents recorded in normal external Cl^- (530 mM) reversed at about −25 mV (Fig. 2C), a value significantly different from the equilibrium potentials of either K^+ (−70

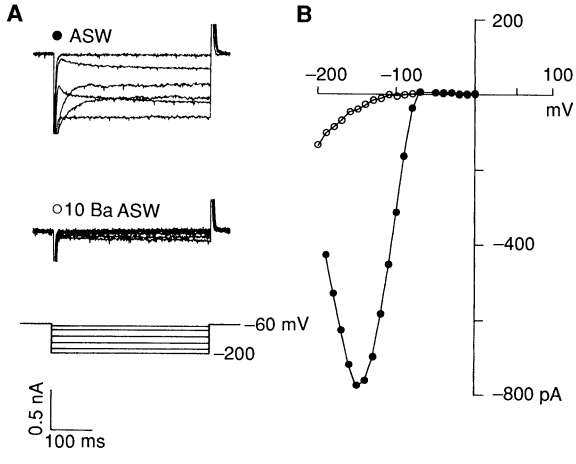

Fig. 1. Ba^{2+}-sensitive and insensitive components of the inward rectifier in the unfertilized *Boltenia* oocyte. (**A**) Current records during voltage-clamp pulses to −70, −90, −120, −150, −180, and −200 mV from a holding potential of −60 mV. Top records taken in ASW (*9*), bottom records in 10 Ba ASW. Top traces represent primarily the K^+ inward rectifier; the decline in current at the two most negative voltages is caused by a voltage-dependent block of the current by Na^+ ions (*20*). The bottom records represent the small inwardly rectifying Cl^- current. (**B**) Steady-state current-voltage (*I-V*) relations for the two currents in (A). Closed circles represent currents in ASW, predominantly the K^+ inward rectifier; open circles are currents in 10 Ba ASW, showing the Cl^- inward rectifier. In this cell the K^+ inward rectifier density was 5 pA/pF at −130 mV, and the Ba^{2+}-insensitive inward rectifier density was 0.2 pA/pF at −200 mV, values that were typical.

Department of Zoology, University of Washington, Seattle, WA 98195.

*Present address: Department of Physiology, University of California, San Francisco, CA 94143.