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A New and Improved View of Force Production

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A central question in muscle contraction is "How is the energy release in ATP hydrolysis converted into mechanical force and work?" With the elucidation of the actomyosin ATPase reaction in the 1970s (1,2) and the fact that a large amount of free energy is produced during Pi release from an actomyosin-ADP-Pi species (3), the Pi release step from actomyosin-ADP-Pi must be associated with the generation of force and shortening. It was also known that increases in the [Pi] concentration in isometrically contracting skinned muscle fibers reduced isometric force (4). To probe the force-producing mechanism, one might quickly increase [Pi] in a skinned muscle fiber while monitoring the mechanical change in muscle force. The development of caged phosphate (5) allowed experiments to be performed in which 1 mM Pi increases were produced (<1 ms) in a contracting muscle fiber, and the subsequent force changes monitored. The photogeneration of inorganic phosphate (Pi) from caged Pi in an isometrically contracting skinned muscle fiber lattice produced a rapid exponential reduction of isometric force (k_{+Pi2}), as if the increase in [Pi] reversed the force generation mechanism. Kinetic analyses of the rate of force decline with variation of the final phosphate concentration were consistent with a hypothesis in which the Pi transient (k_{+Pi2}) behaved as if it were produced by a two-step process. This is one in which the first step Pi bound to a force-exerting actomyosin*-ADP state and formed a force-exerting actomyosin*-ADP-Pi state, followed by an isomerization in which the force step was reversed with some actomyosin-ADP-Pi dissociating to actin and myosin -ADP-Pi (6). There are however, two significant drawbacks associated with the use of the caged Pi in skinned fibers. The first is that one could only increase Pi (and reduce force) but one could not suddenly reduce the Pi and increase force. Second, only average sarcomere spacing can be monitored in skinned muscle fiber experiments (6).

In this issue of the *Biophysical Journal*, Stehle (7) reports results that produce a new and improved view of force production using measurements of the mechanical responses of single cardiac myofibrils to increases and decreases in Pi concentration.

In these studies, the [Pi] changes ranged from 0.16 to 20 mM phosphate, while simultaneously monitoring the sarcomere spacing of the 12 or so sarcomeres in the myofibril. This approach is superior to that using caged Pi in a single fiber because the solution bathing the fiber lattice can be increased or decreased in a millisecond and the sarcomere length of each sarcomere in the myofibril can be accurately monitored. A major finding in these experiments on isometrically contracting myofibrils is that following a sudden increase in [Pi] (as in the caged Pi experiments) there is a rapid force decline ($k_{+Pi(2)}$), the rate of which is associated with sarcomere "give" (lengthening of sarcomeres). The sarcomere give is rapidly propagated along the length of the fibers at a rate related to the Pi increase. This result shows that the rapid fall in force with Pi increases is unrelated to reversal of cross-bridge attachment, is produced by sarcomere dynamics, and invalidates the two-step hypothesis of force generation (6).

A second major finding is the elucidation of the asymmetric behavior exhibited by kinetics observed with respect to increases and decrease in phosphate changes. Stehle shows that before the rapid force change associated with sarcomere give after Pi increases, there is an initial slow force decay, ($k_{+Pi(1)}$) whose rate increases with [Pi] increases (but exhibits no sarcomere give), and is approximately fivefold slower than ($k_{+Pi(2)}$). With reductions in the [Pi] from 20 mM Pi to as low as 0.16 mM Pi, the rise in isometric force is a single exponential (with no sarcomere give) and whose rate (k_{-Pi}) is proportional to the final Pi. Stehle shows that these initial slow phase rates of force transients at given final levels of [Pi], ($k_{+Pi(1)}$ and K_{-Pi}), are the same as the rates of force changes produced by sudden rapid short releases and restretches (k_{TR}) in maximal isometric contractions and those produced by maximal step

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increases in Ca^{+2} (k_{ACT}) and thus correspond to the rate-limiting transitions in the cross-bridge cycle (8,9). Finally, in model calculations, Stehle shows that that Pi transients cannot directly provide information about steps in the cross-bridge cycle before or after the rate-limiting transition. This is a seminal article that will condition the experimentation and thought in muscle contraction.

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