Functions of Stretch Activation in Heart Muscle

Kenneth B. Campbell and Murali Chandra

Department of Veterinary and Comparative Anatomy, Pharmacology, and Physiology, Washington State University, Pullman, WA 99164

Stretch activation is an intrinsic length-sensing mechanism that allows muscle to function with an autonomous regulation that reduces reliance on extrinsic regulatory systems. This autonomous regulation is most dramatic in asynchronous insect flight muscle and gives rise to wing beat frequencies that exceed the frequency capacity of neural motor control systems. Stretch activation in insect flight muscle allows the contractile features of the flight muscle to be matched and tuned to the wing-thorax-aerodynamic load to ensure proper muscle contraction frequency and effort for flight (Pringle, 1977); a role for which intrinsic autonomous regulation is especially suited. In stretch-sensitive insect flight muscles, neurally controlled intracellular calcium plays a permissive role (it needs to be present at adequate levels to allow the intrinsic stretch activation mechanisms to operate) but it is not the dominant player in force generation or in work production. That role belongs to stretch itself, which activates the myofilament system in such a way (i.e., with appropriate phase delay) to generate force and perform rhythmic work.

The function of stretch activation is less obvious in muscles, such as cardiac muscle, that rely heavily on rising and falling intracellular calcium to modulate force generation. Although stretch activation has been demonstrated in cardiac muscle (Steiger, 1971, 1977; Vemuri et. al., 1999), intracellular activator calcium is the primary determinant of force-generating capacity. However, two unique features of cardiac muscle function suggest that stretch activation could be important: (1) the rhythmic nature of cardiac muscle contraction begs a functional analogy for stretch activation in heart muscle with stretch activation in insect flight muscle; and (2) the steep length–tension relationship in cardiac muscle (relative to skeletal muscle, where stretch activation is much less pronounced) is necessary for the valuable function that the heart gains from the Frank-Starling relationship (Allen and Kentish, 1985). It is likely that stretch activation contributes significantly to this steepness. Thus, the relative contribution of stretch activation and calcium activation to cardiac muscle function is an important issue that remains largely unresolved.

In this issue, Stelzer et al. (p. 95) provide the first definitive study that dissects the relative contributions of calcium and stretch activation to cardiac muscle force generation. Stelzer et al. make use of the force response to a quick stretch in mouse myocardium where they separate the initial response (phase 1) and the rapid recovery from elastic distortion (phase 2) from the slower part of the response (phase 3), in which force rises to a higher steady-state level as a result of stretch-induced recruitment of new force-generating cross-bridges. Phase 3 is the expression of stretch activation and Stelzer et al. use both the amplitude of force rise and the apparent rate constant that governs the rate of force rise during phase 3 as measures of stretch activation. To explore the interaction between calcium activation and stretch activation, calcium activation levels were varied and the force response to quick stretch was evaluated. The authors demonstrate that stretch activation is most pronounced at low levels of calcium activation, where there are ample sites available on the thin filament for the formation of additional strong-binding cross-bridges, XBs. To test the hypothesis that strong-binding XBs cooperatively recruit more strong-binding XBs during stretch activation, they used a chemically modified myosin S1 subfragment (NEM-S1, which binds strongly to actin with no force generation) to occupy some fraction of thin-filament myosin binding sites. When the thin-filament binding sites are occupied with NEM-S1, stretch activation is much blunted, and they conclude that stretch activation involves strong-binding XBs cooperatively promoting further strong binding of XBs through an XB-based activation of the thin filament. This cooperative XB activation both increases the relative magnitude of the force response to stretch and slows the approach to the eventual steady state.

A second valuable contribution of the study by Stelzer et al. is that they give a lucid verbal account of the mechanisms by which XBs participate in stretch activation. The clarity of their account, in combination with an equally clear account given earlier by the same group (Moss et. al., 2004), allowed us, with very few additions

Correspondence to Kenneth B. Campbell:
cvselkbc@vetmed.wsu.edu

Abbreviations used in this paper: RU, regulatory unit; XB, cross-bridge.
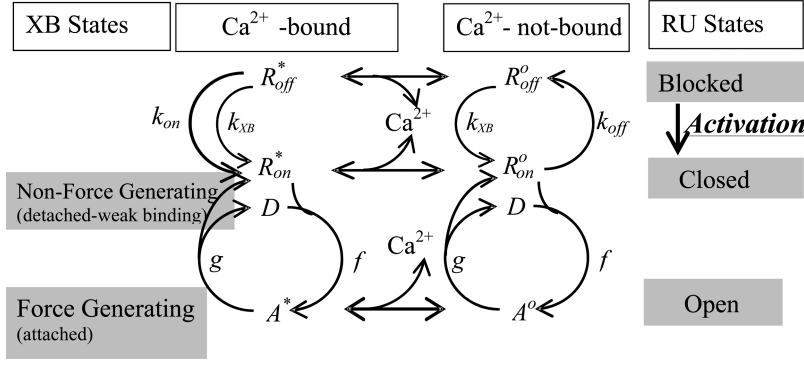
Figure 1. Kinetic scheme for model of myofilament activation and myosin cross-bridge cycling. Myofilament activation occurs by steps responsible for the transition between Blocked and Closed states. This scheme allows myofilament activation by both calcium binding and by cooperative cross-bridge mechanisms. See text for explanation and definition of symbols.

of our own, to formulate a simple mathematical model of the myofilament system, a model in which both calcium and strong-binding XBs each cause myofilament activation, and in which these two activation mechanisms could be treated separately in ways that could not be done experimentally.

The underlying kinetic scheme for the model is shown in Fig. 1. In brief, thin filament regulatory units (RUs) are distributed between "Blocked" (R^*_{off} and R^o_{off}), "Closed" (R^*_{on} and R^o_{on}), and "Open" (A^* and A^o) states. Each state has calcium bound, * (left Ca-bound column in Fig.1), or not, ^{o} (right Ca-not-bound column in Fig. 1). Myosin XBs cycle between a nonforce bearing state, D (Fig. 1, middle), and a force-bearing state, A (Fig. 1, bottom). Transition from the Blocked to the Closed RU states represents activation of the myofilament system, which is required for XBs to cycle between the D and A states. Activation occurs as a result of calcium binding: $R^o_{off} + Ca^{2+} \rightarrow R^*_{off} \rightarrow R^*_{on}$ with the last step representing the activation step, which is governed by the kinetic constant k_{on}. Calcium activation takes place only in the left Ca-bound column in Fig. 1. Alternatively, activation could result from the action of strong-binding XBs, numerically equal to A, causing allosteric change in neighboring RU and transition from Closed to Open states. This kinetic step, which is governed by the nonlinear rate coefficient k_{XB}, does not require calcium to be bound to the RUs and, thus, takes place in both the Ca-bound and Ca-not-bound columns in Fig. 1. Cross-bridge binding to the thin filament, which is governed by the kinetic rate constant f, occurs as long as the RU is in the Closed (R^o_{on} and R^*_{on}) states, independent of whether calcium is bound to the RU.

Both the RUs and the XBs obey conservation constraints with the total number of XBs (including detached and attached states) equal to three times the total number of RUs. Further, calcium binding to the RUs is considered to be in equilibrium with the binding constant, K_{eq}. As a result, R^o_{on} and R^*_{on} can be combined into a single R_{on} state and the A^o and A^* states can be combined into a single A state. The force, F, generated by this model myofilament system is equal to the number of XBs in state A times the average force generated by a single XB (Eq. 1). The average XB force is equal to XB stiffness, ε, times the average elastic distortion, x, among attached XBs. With this, the model myofilament kinetic system in Fig. 1 can be represented by the following set of four equations:

$$F = A(\varepsilon x) \tag{1}$$

$$
\begin{aligned}
\frac{dR_{on}}{dt} = &-\left[\frac{k_{off} + k_{XB} + K_{eq}Ca\ k_{on}}{1 + K_{eq}Ca} + fD\right]R_{on} \\
&+ \left[g - \frac{k_{XB} + K_{eq}Ca\ k_{on}}{1 + K_{eq}Ca}\right]A \\
&+ \left[\frac{k_{XB} + K_{eq}Ca\ k_{on}}{1 + K_{eq}Ca}\right]\left[\beta(L - L_0)\right]
\end{aligned}
\tag{2}
$$

$$\frac{dA}{dt} = [fD]R_{on} - [g]A \tag{3}$$

$$\frac{dx}{dt} = -[g][x - x_0] + \frac{dL}{dt} \tag{4}$$

Eq. 4 describes the dynamic changes in the XB elastic distortion when muscle length changes at a velocity, dL/dt. Eqs. 2 and 3 describe the dynamic processes of both RU state change and XB recruitment. The term $\beta(L - L_0)$ in Eq. 2 consists of muscle length, L, a reference muscle length, L_0, and a multiplying coefficient, β. This term represents the total number of RU in the filament overlap region of the sarcomere where structural features allow XB cycling. Because the focus is on the ascending limb of the length–tension relationship, the total RU is proportional to muscle length via the $\beta(L - L_0)$ term. This length effect on total RU is independent of the activation state of the muscle; passive stretch and active stretch had the same effect: bringing more RU into the overlap zone where XB cycling could occur. All other mechanisms for recruiting force-generating XBs reside in the terms contained within the square brackets that multiply each variable in Eqs. 2 and 3.
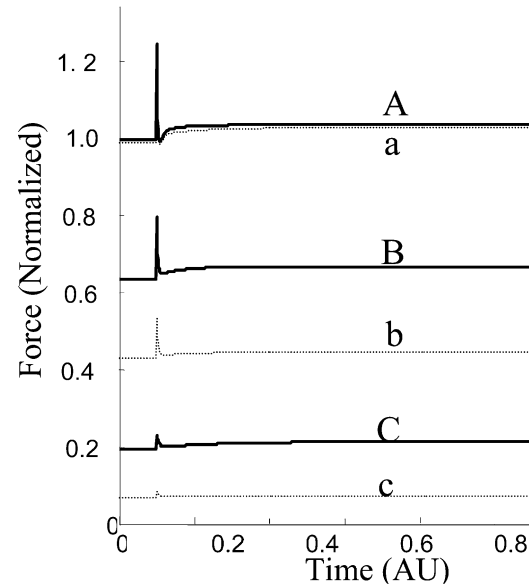
Of all possible XB recruitment mechanisms that could be implemented using terms within the square brackets, those of interest in this exercise are just Ca and k_{XB} because these are the only terms that bring about myofilament activation (Fig. 1). Ca and K_{eq} were combined into a single term, α. With a typical value of $K_{eq} = 10^{-6}$, α and pCa are related as follows: $\alpha = 0.1$, pCa $= 7$; $\alpha = 1$, pCa $= 6$; $\alpha = 100$, pCa $= 4$.

The manner in which the XB activating coefficient, k_{XB}, depends on the number of strong binding XB is important. To keep things simple, and to ensure that zero calcium resulted in zero force, while XB activation increases monotonically with A, we made the assignment:

$$k_{xb} = k_a A \qquad (5)$$

Thus, k_{XB} increases linearly with A. Although this assignment is not what normally is encountered in a cooperative process, we use the term "cooperative" because strong-binding XBs enhance myofilament activation by increasing k_{XB}, which enhances the formation of more strong-binding XBs. With this assignment for cooperative XB activation, it was possible to vary XB activation within the model by changing k_a.
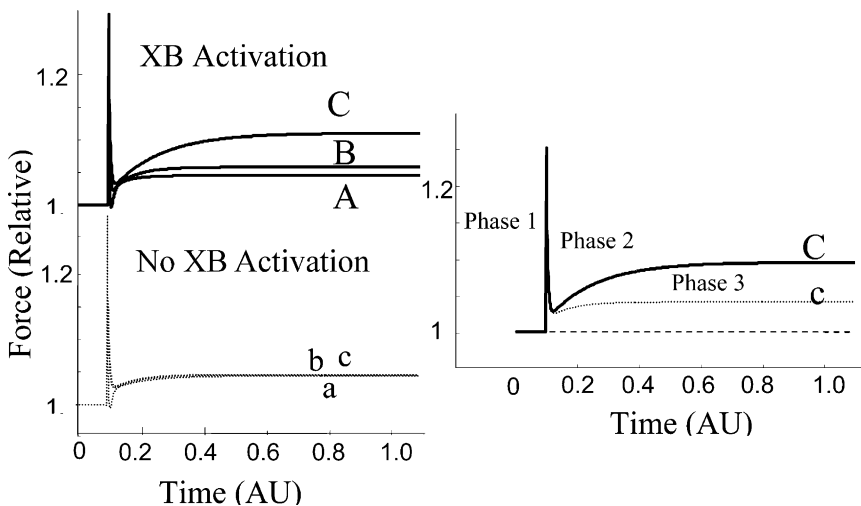
One difference between the Stelzer et al. dialogue and the model presented here is in the stoichiometric relationship between the RU Open states and attached force-generating XB. For simplicity, we set this stoichiometric relationship to 1, and, thus, A represents both the number of RUs in the open state and the number of force-generating XBs. In actuality, as Stelzer et al. discuss, the span of the RU over the thin filament dictates that there can be more force-generating XBs than RUs in the Open state. However, spatial considerations as required to represent an RU–XB stoichiometry different from 1 would quickly lead to a model that would be mathematically and computationally much more complicated. The qualitative and quantitative differences between the simple model presented here and a more

complicated but realistic model were judged unimportant to our argument.

To recreate the experimental results of Stelzer et. al., model muscle length, L, was set at a reference value and Ca, α, was varied among the values 0.1, 1.0, and 100 (corresponding to pCa's of 7, 6, and 4, respectively). After obtaining steady force for the given value of α, the muscle length was changed in the manner of a step equal to 1% of the initial length and the force response to this sudden 1% stretch was predicted. To roughly simulate the experiments of Stelzer et al. with NEM-S1, these varied levels of Ca activation were used to predict the force when k_a equaled zero (no XB activating effects


Figure 2. Model-predicted force response to sudden muscle stretch at varying levels of calcium activation. Model predictions were when cross-bridge activation was operative (A–C) and when it was not (a–c). Equal levels of calcium activation for the pairs: A-a, B-b, and C-c. Force was normalized to force at maximal activation.

Figure 3. Activation-dependent time course and amplitude of step response. Step responses shown in Fig. 2 normalized to prestretch force. Responses for conditions in which cross-bridge activation was operative (A–C) and when it was not (a–c). Effect of cross-bridge activation to slow the time course and to increase the amplitude of phase 3 at lowest level of calcium activation is shown in right panel.
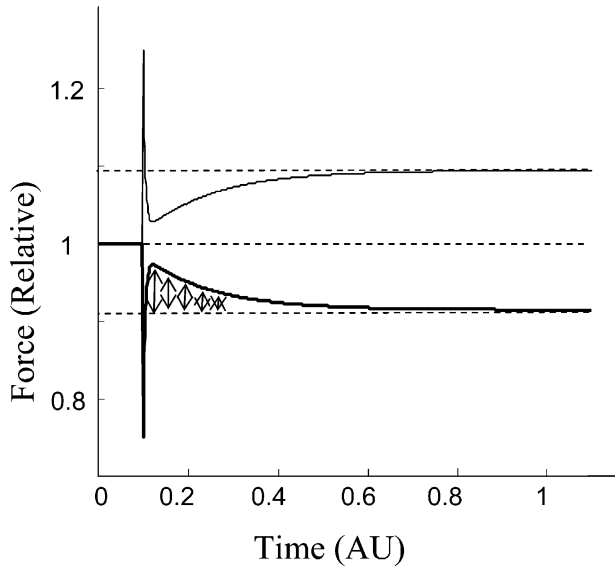
Figure 4. Stretch activation during quick release. Effect of stretch activation when muscle shortens with quick release (heavy solid line) compared with response to quick stretch (light solid line). Steady-state force before length change and eventual steady-state force after length change are shown as broken lines. Double-headed arrows between eventual steady-state force and the response transient after quick release demonstrate the slow loss of memory of the earlier higher force at each of the identified times.

as in the presence of NEM-S1) and when k_a equaled 20 (an arbitrary number giving modest XB activation). The model-predicted results, Fig. 2, emulate the experimental findings in the following essential respects.

The predicted force response to sudden stretch had three well-defined phases (compare our Figs. 2 and 3 with Figs. 1, 2, and 8 in Stelzer et al.). Phase 1 in the model response is due to the sudden stretch of attached XBs. The amplitude of phase 1 is directly proportional to the prestretch force; a relationship that can be derived from Eqs. 1 and 4. Phase 2 is a rapid recovery from the sudden XB stretch as the stretched XBs, which were distorted by the imposed length step, detach and are replaced by new attached XBs that had not been stretched by the imposed length change. In the model, as in Stelzer et al., the time course of phase 2 was only weakly related to activation or prestretch force. The phase of primary interest, phase 3, is due to the recruitment of more force-bearing XBs as a result of the increase in muscle length. Like the findings of Stelzer et al., the predicted amplitude and time course of phase 3 depend strongly on the prestretch force (or level of calcium activation). The relative amplitude was much higher, and the force approached a new steady state much slower, at low levels than at high levels of calcium activation. Furthermore, whereas phases 1 and 2 are largely independent of whether XB activation was operative, phase 3 depends strongly on XB activation. Without XB activation ($k_a = 0$), phase 3 is relatively rapid and independent of the degree of calcium activation, as is its relative amplitude (normalized to prestretch force). In contrast, with XB activation ($k_a = 20$), phase 3 is relatively slow and of large amplitude relative to the prestretch force at low activation, but much faster and of smaller relative amplitude at high activation. In fact, at saturating levels of calcium the prestretch force amplitude, the speed of phase 3, and the amplitude of phase 3 do not differ between conditions with vs. without XB activation. This is because at maximal activation all potential XBs are recruited into the cycling population and there is no opportunity to recruit more XBs with either calcium activation or cooperative XB activation. Our model results and mechanisms for obtaining these results agree with the explanations for experimental findings given by Stelzer et al. in terms of cooperative XB activation determining the characteristics of phase 3, i.e., the stretch activation response.

We used the model to extend the work of Stelzer et al. into functional domains they did not study by predicting the effects of XB activating mechanisms on the force–length relationship. We systematically varied muscle length before imposing a constant, midlevel ($\alpha = 1.0$) calcium activation signal and calculated the corresponding steady-state force both with and without XB activation. This relationship between muscle length and steady-state force was used to construct a force–length relationship. The force–length relationship was twofold steeper with XB activation ($k_a = 20$) than without ($k_a = 0$). Because the muscle force–length relationship is transformed, through the cardiac geometry, into the left ventricular chamber pressure–volume relationship, our finding means that stretch activation via XB activation is important in determining the steepness of the Frank-Starling relationship of the heart even though the stretch in question is applied before calcium activation, at a time when the muscle is relaxed. A steep Frank-Starling relationship enables the heart to respond sensitively to changes in venous return, i.e., it is an autonomous means for matching the heart with its preloading system.

However, a steeper force–length relation, as a consequence of stretch activation, is a two-edged sword. On the one hand, it enhances force generation with increased muscle length favoring a steep Frank-Starling relationship, but this same property also means that muscle shortening, as during ejection, would strongly reduce the force; an undesirable outcome if the maintenance of force is required to overcome the afterload against which shortening occurs. Countering the reduction in force with muscle shortening is the second major effect of XB activation, which is to slow the attainment of a new force after a change in length. This is just as true for muscle shortening as it is for muscle lengthening. We used the model to demonstrate the slow reduction in force after a sudden 1% reduction
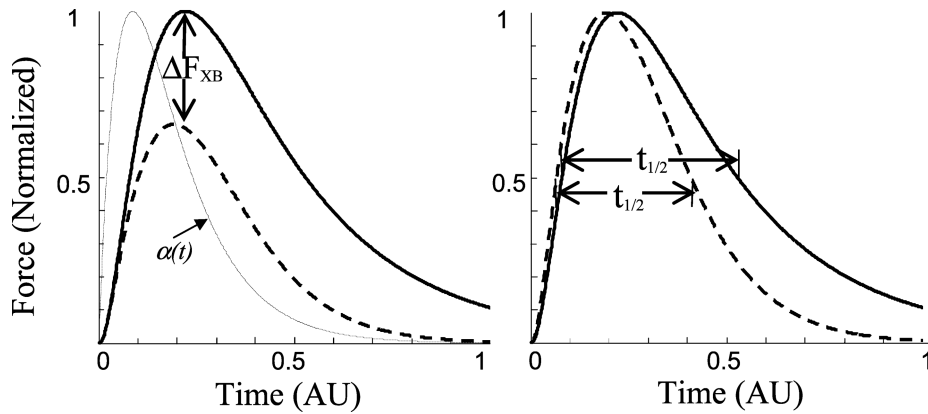
Figure 5. Effect of stretch activation mechanisms when there has been no muscle stretch or shortening. Calcium transient emulating that of a muscle twitch is shown as light dashed line in left panel and labeled $\alpha(t)$. Predicted force response to calcium transient when cross-bridge activation is operative (solid line) and when it is not (dashed line). Cross-bridge activation resulted in 35% increase in amplitude of force transient (left, force normalized to peak of twitch with cross-bridge activation) and a 30% increase in duration above 1/2 peak value, $t_{1/2}$ (right, force of both twitches normalized to each peak value).

in muscle length (Fig. 4). In this example, the velocity effects on force reduction are confined to just the onset of the response, allowing the slow actions of stretch activation to be observed without the obfuscations by velocity-related force reductions. Thus, although the muscle has shortened to a length that would sustain only a much reduced steady-state force, because XB activation dissipates slowly, force continues to be greater than the steady-state level for a considerable time. That is, the muscle effectively remembers the higher force associated with the longer length from which the shortening began. Hence, the dynamical aspects of stretch activation are functionally important even during muscle shortening and act to sustain force during shortening.

This effect, whereby initial length of muscle continues to exert positive effects on force generation during the shortening interval of cardiac ejection, has been observed previously by Hunter (1989) in left ventricular pressure–volume behavior during ejection . This effect gave rise to what Hunter called "positive effects of ejection." Hunter correctly described the underlying phenomena in terms of memory effects persisting throughout ejection. This seminal observation has gone unexplained for more than 15 years. Thanks to Stelzer et al., we now have a myofilament mechanism that explains this functionally important phenomenon in the whole heart.

Thus, stretch activation exerts a positive effect on function when muscle is stretched by increasing the force-producing capability as muscle is stretched to longer lengths and it exerts a second positive effect on function when muscle shortens by prolonging the force-generating capacity through a dynamic effect that remembers the greater force-producing capacity of previous longer lengths. We elucidated these effects

using constant calcium activation. But, how do stretch activation and its XB activating mechanisms interact with calcium activation mechanisms when calcium is not constant? To approximate changing activator calcium as in a normal heartbeat, we created a time-varying $\alpha(t)$ signal that rose to peak level ($\alpha(t)_{max} = 1.0$) consistent with midlevel calcium activation (Fig. 5). We then predicted and compared the time course of force generation when the cooperative XB activating mechanisms of stretch activation were operative and when they were not. The first comparison is with the amplitude of force generation. When XB activating mechanisms were operative, the model predicted a force amplitude 35% higher than when they were not (Fig. 5, left). The second comparison is with respect to the time course of force production. When the XB activating mechanisms were operative, the model-predicted force transient is maintained above its half maximal value for a period ($t_{1/2}$) that was 30% longer than when they were not operative (Fig. 5, right). Thus, XB-based stretch activation interacts with calcium activation to significantly enhance force generation during the pulse of normal cardiac activation and, further, stretch activation interacts with calcium activation to prolong calcium-initiated force transients. Stretch activation acts to slow force transients, whether these transients are due to muscle shortening or due to calcium activation pulses. Both of these slowing actions help to sustain force during normal cardiac ejection.

We suggest that stretch activation in heart muscle functions as an intrinsic force-regulating mechanism to help match the heart with its preload by increasing the steepness of the Frank-Starling relationship and to help match the heart with its afterload by enhancing and sustaining force after a brief calcium activation pulse and during shortening. Thus, stretch activation in heart

muscle plays a role similar to that in insect flight muscle in that it functions to match the contractile organ with its mechanical load. The possible role of stretch activation in heart muscle in establishing optimal contraction frequencies (heart rates) has yet to be explored. Such explorations are likely to involve mathematical models much like the one we used here. Further, a myocardial model similar to the one used here will be useful when incorporated into a spatially distributed global heart model. For instance, a heart model constructed from modeled myocardium in counterhelically oriented epicardium and endocardium, each helix with different stretch activation features, will be useful in exploring the means whereby torsional motions and transmural inhomogeneity in stretch activation function to support ejection (Davis et. al., 2001). In summary, our understanding of the functional consequences of stretch activation in the heart will require representation of XB-based stretch activation mechanisms as described by Stelzer et al. in mathematical models of cardiac muscle and the whole heart.

REFERENCES

Allen, D.G., and J.C. Kentish. 1985. The cellular basis of the length-tension relation in cardiac muscle. *J. Mol. Cell. Cardiol.* 17:821–840.

Davis, J.S., S. Hassanzadeh, S. Winitsky, H. Lin, C. Sartorius, R. Vemuri, A.H. Aletras, H. Wen, and N.D. Epstein. 2001. The overall pattern of cardiac contraction depends on a spatial gradient of myosin regulatory light chain phosphorylation. *Cell.* 107:631–641.

Hunter, W.C. 1989. End-systolic pressure as a balance between opposing effects of ejection. *Circ. Res.* 64:265–275.

Moss, R.L., M. Razumova, and D.P. Fitzsimons. 2004. Myosin cross-bridge activation of cardiac thin filaments: implications for myocardial function in health and disease. *Circ. Res.* 94:1290–1300.

Pringle, J.W. 1978. The Croonian lecture, 1977. Stretch activation of muscle: function and mechanism. *Proc. R. Soc. Lond. B. Biol. Sci.* 201:107–130.

Steiger, G.J. 1971. Stretch activation and myogenic oscillation of isolated contractile structures of heart muscle. *Pflugers Arch.* 330:347–361.

Steiger, G.J. 1977. Tension transients in extracted rabbit heart muscle preparations. *J. Mol. Cell. Cardiol.* 9:671–685.

Stelzer, J.E., L. Larson, D.P. Fitzsimons, and R.L. Moss. 2006. Activation dependence of stretch activation in mouse skinned myocardium: implications for ventricular function. *J. Gen. Physiol.* 127:95–107

Vemuri, R., E.B. Lankford, K. Poetter, S. Hassanzadeh, K. Takeda, Z.-X. Yu, V.J. Ferrans, and N.D. Epstein. 1999. The stretch-activation response may be critical to the proper functioning of the mammalian heart. *Proc. Natl. Acad. Sci. USA.* 96:1048–1053.