Thick Filament Regulation of the Simple Harmonic Motion of Tropomyosin and Active Tension in Cardiac and Skeletal Muscle

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Introduction

The simple harmonic (SH) theory was proposed to describe the regulatory mechanism of tropomyosin (Tm) in the length-dependent regulation of active tension, and the molecular basis for Starling's law of the heart[1]. As the amplitude of SH motion increases, Tm decreases in length, causing the activation-associated displacement of Tm into the actin groove, from OFF to ON[2], with an increase in active tension. The amplitude is regulated in a rectilinear manner by the thick filament electrostatic force of repulsion, F_{thck}, that increases in magnitude and direction as lattice spacing decreases with stretch or osmotic compression. More recent experimental evidence for the SH theory is described indicating the molecular mechanism involves more than lattice spacing-dependent regulation and plays a key role in the cooperative regulation of myosin crossbridge interactions through modulation of the Hill coefficient, n_H, and the slope of the force-pCa^{2+} relation.

The Simple Harmonic Theory of Tropomyosin

Scientific Question: What happens when tropomyosin - which exhibits large scale (5 – 8 Å RMS) random vibrational motion in crystals[3] - interacts with the highly repeating structure of thin filament as suggested in Figure 1?

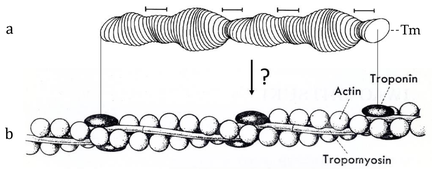

Figure 1. *Composite hypothetical diagram of Tm and thin filament.* ***a)*** *Anisotropic temperature factor probability ellipses (scaled 8x) represents RMS displacement along length of 2 molecules of Tm in crystals[3].* ***b)*** *diagram of thin filament.*

Hypothesis: As Tm interacts with thin filament, the random vibrational motion observed in crystals become less complex, vibrating with the actin repeat interval, approaching the ideal case of simple harmonic motion.

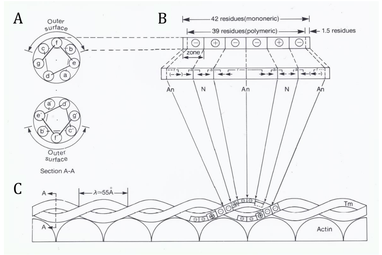

Figure 2. *Schematic of simple harmonic motion of Tm suggested by periodic distribution of charged amino acids in the outer surface positions b, c and f[4].* ***A)*** *Cross-sectional view of Tm chains illustrating heptapeptide repeat and outer surface positions, b, c and f, where* ***B)*** *the charge distribution of the 7 repeating units suggest anti-nodes (An) between adjacent zones of negative charge associated with* ***C)*** *the periodic bending of Tm with actin repeat interval[1].*

What is the Significance of SH Motion?

It provides a mechanism for regulating active tension. An increase in amplitude produces a decrease in length which requires the movement of Tm into the actin groove from OFF to ON[2], causing active tension to increase.

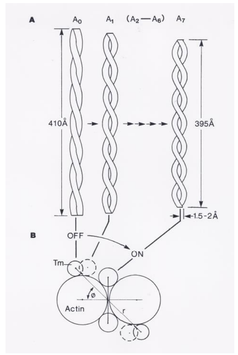

Figure 3. *Schematic to illustrate how* ***A)*** *an increase amplitude of SH motion causes* ***B)*** *the displacement of Tm from OFF to ON.*

Is There Support for SH Motion From Protein Secondary Structure?

Yes. Unique charge distribution (- + - - + -) on outer surface of Tm suggest bending of each chain with the actin repeat interval in alternating planes at right angles[1].

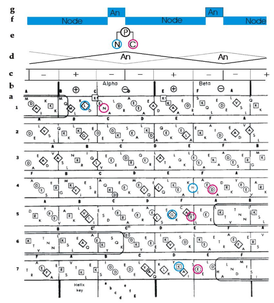

Figure 4. *Amino acid sequence of alpha-Tm on helical net[4] and location of bi-functional molecular probes[5] (before mutating to cysteine).* ***a, 1-7)*** *Seven-fold repeat of* ***b)*** *pair of Alpha and Beta zones of net positive and negative charge on outer surface residues, b, c and f [4] and* ***c)*** *the alternative charge distribution (- + - - + -) indicative of* ***d)*** *periodic bending of Tm between zones of net negative charge corresponding to anti-nodes (An) of SH motion.* ***e)*** *Schematic of bi-functional EPR molecular probe (P) with N and C ligation sites for 4 probes shown on helical net. Blue bars indicate corresponding region on helical net for location of N-terminal ligation site to b or f positions to detect* ***f)*** *nodes and* ***g)*** *antinodes. Note, all 4 probes are in nodal zones. No probe straddles an anti-node. Heptapepetide key at bottom. Symbols: (Circle) negative charge (E & D); (square) positive charge (R & K); (diamond) non-polar (A, G & Y)..*

Biophysical Evidence for the SH Motion of Tropomyosin

It is the presence of nodes (no bending) that distinguishes simple harmonic motion (Fig. 2c) from random vibrational motion (Fig. 1a). Four saturation transfer bi-functional EPR probes along the length of Tm were constructed to evaluate backbone motion[5].Upon binding to thin filament, motion of Tm at all 4 probes was reported to decrease > 1000-fold. As shown in Figure 5, Tm is further stabilized upon binding of troponin (Tn) and remains largely unchanged upon addition of Ca^{2+} and S-1. Data indicate regional differences in stabilization being most significant towards the C-terminal probe, 269/273. Location of probes are shown on helical net (Fig. 4). All probes are located in nodal zones where no bending and the stabilization of Tm structure was predicted to occur. No probe straddles an anti-node. The global flexibility and dynamic motion of tropomyosin is generally recognized. Consequently, detection of reduced motion or flexibility at or near predicted nodes provides biophysical evidence for the SH motion of tropomyosin in the regulation of muscle contraction.

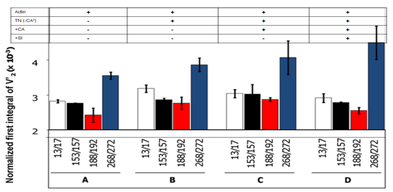

Figure 5. Four saturation transfer EPR bi-functional probes on the outer surface of Tm (positions b and f) report on Tm dynamics upon ***(A)*** *binding to F-actin on introduction into ghost muscle fibers,* ***(B)*** *addition of troponin complex,* ***(C)*** *addition of Ca^{2+} and* ***(D)*** *addition of myosin S-1[5]. EPR probes are designated by the location of amino acids for crosslinking after mutated to cysteines. Increasing the normalized first integral of V' corresponds to increased stability. Probe locations shown on helical net (Fig. 4).*

Rectilinear Regulation of the SH Motion of Tropomyosin

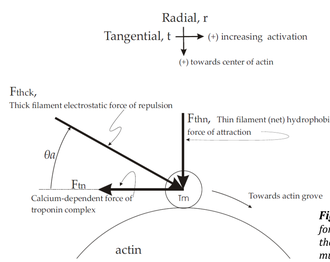

Figure 6. *Principle regulatory forces acting on Tm to regulate the SH motion in troponin-based muscle systems.*

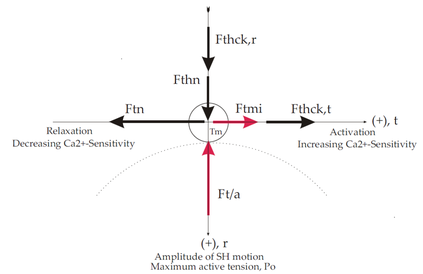

Figure 7. *Equilibrium - or free body - diagram of rectilinear (tangential, t and radial, r) components of the regulatory forces (black) and internal reaction forces (red) of Tm, F_{tmi}, and between Tm and actin, Ft/a.*

Radial Regulation and Maximum Active Tension, Po

The radial force (Figures 6 and 7) consist of thick and thin filament components. Only the thick filament component, $F_{thck,r}$ will be considered, but their effects are the same. The radial force determines the maximum amplitude of SH motion, the degree of activation and therefore maximum active tension, Po. (Fig. 2). Thus, changes in Po report on changes in the radial force, $F_{thck,r}$. As the radial force increases the amplitude increases along with Po. However, a point is eventually reached – typically beyond the normal range – where any further increase in the radial force dampens the SH motion, decreasing the amplitude and Po. This is referred to as the harmic transition. It is a necessary and decisive feature of the SH theory, occurring as $F_{thck,r}$ increases monotonically over an extended and tissue specific range and indicated by a biphasic variation in Po, increasing then decreasing.

Tangential Regulation and Ca^{2+}-sensitivity

A positively directed tangential force (Fig. 7) opposes the inhibitory force of troponin (F_{tn}). Thus, an increase in $F_{thck,t}$ causes an increase in Ca^{2+}-sensitivity, indicated by a leftward, horizontal shift of the force-pCa^{2+} relation. A change in Ca^{2+}-sensitivity, increasing or decreasing, reports on a change in $F_{thck,t}$, increasing or decreasing, as well.

Description of Regulatory and Internal Forces

F_{thck}, the thick filament electrostatic force of repulsion. This force may by represented by it's magnitude and direction (Fig. 6) or by the two rectilinear components, $F_{thck,r}$ and $F_{thck,t}$, (Fig. 7). The magnitude is determined by Coulomb's law and varies proportional to thick filament charge and inversely to the square of the distance between charges (i.e., thick filament and Tm). The apparent angle (θa) increases as lattice spacing decreases. F_{thck} would increase with 1) increased myosin electronegativity, for example due to myosin regulatory light chain phosphorylation, decreasing ionic strength, or increased pH and 2) as lattice spacing decreases as muscle is stretched, or by osmotic compression, typically with dextran T-500. Alterations to the structural protein titin affect lattice spacing[6,7] and represents an important regulatory mechanism as well.

F_{thn}, the thin-filament (net) hydrophobic force of attraction. This force is radially directed (a radial subscript is not shown here or for other forces with fixed direction). It corresponds to the well-established affinity of Tm for thin filament. It is modulated by factors that alter the Tm-actin affinity, such as pH, ionic strength, and ethanol as discussed[1]. In most experimental situations it's variation is considered small compared to changes in $F_{thck,r}$ with lattice spacing and charge, and is not considered here.

F_{tn}, the Ca^{2+}-dependent force of the troponin complex. This force is tangentially directed, opposing activation in agreement with Ebashi[8] who described troponin as a relaxing factor. In the absence of Ca^{2+}, F_{tn} acts to pull Tm out of the actin groove 'pinning' it to the periphery of thin filament. F_{tn} is a maximum in the absence of calcium (pCa^{2+} ~8.5), decreasing to zero at maximum Ca^{2+} activation (pCa^{2+} ~ 4.5).

F_{tmi}, the internal displacement force of tropomyosin. The SH theory is predicated on the existence of this force. It is tangentially directed and causes the activation-associated displacement of Tm, a consequence of the SH motion. It is convenient to make an analogue with an elastic rubber band and describe Fmi as an elastic restoring force. Consider a rubber band pinned at slack length on a horizontal surface a distance X from the left edge of the surface. Upon pulling the rubber band horizontally to the left edge, a restoring force should be self-evident. The left edge represents the limit that the rubber band can be pulled, analogues to the role of Tn as it pulls Tm to the periphery of thin. Here the elastic restoring force, F_{tmi}, is a maximum determined in large part by the distance X it was pulled. Upon releasing the rubber band, it will return to the initial position, X, determined by the maximum amplitude. Thus the tangential force, F_{tmi}, is a function of the radial force, $F_{thck,r}$, and therefore varies with Po.

$F_{t/a}$, the reaction force between Tm and thin filament. This force is equal and oppositely directed to the radial forces. Technically it causes the SH motion of Tm, as the earth causes us to have weight. As a reaction force it is not routinely shown in schematic diagrams for convenience. It's consideration, however, emphasizes that to a first approximation, the effect of increasing $F_{thck,r}$ is the same as increasing F_{thn}.

Cellular Evidence for the Harmonic Transition

Figure 8 illustrates how two components of an electrostatic force may vary as the distance and angle between point charges changes in an elementary manner. The distinctive and concurrent change in both components is sufficiently unique to provide a test of the SH theory, referred to as the dual biphasic (DBP) response. As $F_{thck,r}$ increases monotonically over an extended range the amplitude of the SH motion would vary biphasically, initially increasing then decreasing as it is dampened (i.e., harmonic transition). This would be reported on by the biphasic variation Po. Concurrently, the tangential force, $F_{thck,t}$, varies biphasically, in which case Ca^{2+}-sensitivity would vary biphasically as well, increasing and decreasing in phase.

The DPB response was previously described for skinned skeletal muscle[1]. More recent studies have now demonstrated the DBP response in cardiac muscle[9] and confirmed the DBP response in skeletal muscle[10] from the same lab, with osmotic compression to 15% dextran T-500. As shown in Figures 9A and 9B, the peak in both biphasic responses occur nearly in phase at about 5-7% dextran T-500. At longer sarcomere lengths (Fig 9B, 2.7 um) the response peaks between 0% and 5% and is broadened. This is consistent with results that the relative fiber widths were the same at 2.7 um with 0% dextran and 2.0 um with 5% dextran, and that the rate of decrease in lattice spacing with osmotic compression is greatly reduced at longer sarcomere lengths[10]. Together, these and previous observations provide cellular evidence for the harmonic transition and the overarching rectilinear regulation of the SH motion of Tm..

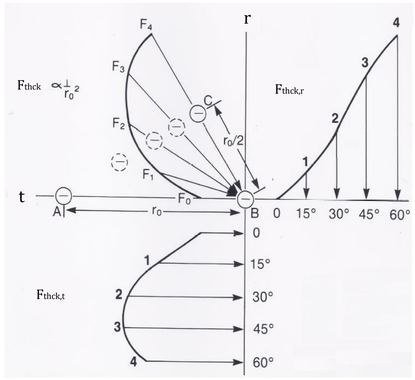

Figure 8. *Illustrates that as an electrostatic force increases exponentially as distance between point charges decreases while their orientation changes in an elementary manner, the radial component increases monotonically while the tangential component varies biphasically.*

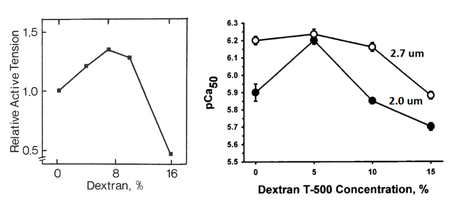

Figure 9. *Experimental support for dual biphasic (DBP) response in skinned skeletal muscle as lattice spacing decreases with osmotic compression and the radial and tangential components of thick filament electrostatic force vary as shown in Fig 8.* ***A)*** *Biphasic variation in Po with osmotic compression[11] reports on the harmonic transition as $F_{thck,r}$ increases monotonically.* ***B)*** *Biphasic variation in Ca^{2+}-sensitivity with osmotic compression[10] reports on biphasic variation of $F_{thck,t}$, at long and short sarcomere lengths.*

Beyond Lattice Spacing-dependent Regulation

The biphasic variation in Po and Ca^{2+}-sensitivity provides a critical test of the SH theory with changes in lattice spacing. To extend the scope of the theory, changes to a third activation parameter can be proposed while taking into consideration the role of myofilament charge.

An increase in Ca^{2+}-sensitivity is attributed to a uniform increase in the tangential force, typically $F_{thck,t}$. However, if the force increases non-uniformly, the slope of the force-pCa^{2+} curve would vary. In the case where the force increases at low [Ca^{2+}], yet remains unchanged at high [Ca^{2+}], the slope of the force-pCa^{2+} curve would increase, becoming more Ca^{2+} sensitive at lower [Ca^{2+}]. F_{tmi} is such a force. It is always zero at high [Ca^{2+}] and maximum at low [Ca^{2+}]. Yet the maximum increases the further Tm is pulled to thin filament periphery by troponin. This distance increases with the maximum amplitude which is regulated by $F_{thck,r}$ and reported on by Po. Thus, in a simple setting, it would be expected that the slope, n_H, or the Hill coefficient, decreases as Po increases, regardless of how Po increases (see Table 1).

As summarized in Table 1, numerous studies have demonstrated a coordinated and statistically significant increase in Po and Ca^{2+}-sensitivity and a decreased slope (n_H) in cardiac and skeletal muscle, upon increased myosin regulatory light chain phosphorylation (pMLC), decreasing ionic strength (IS), increasing pH, increased stretch, increased osmotic compression, and alterations of titin. When viewed in the context of the of Coulomb's law, collectively they indicate the existence of a prominent thick filament electrostatic force as a final common biophysical pathway acting on the activation process that can most sensibly be explained by the rectilinear regulation of the SH motion of Tm by the thick filament electrostatic force of repulsion, F_{thck}. Moreover, the associated decrease in the Hill coefficient provides strong support the existence of F_{tmi}. The force that causes the activation-associated displacement of Tm.

$$\text{Causes} \rightarrow \uparrow F_{thck} \propto \frac{\uparrow \text{Myofilament Charge}}{\downarrow (\text{Lattice Spacing})^2}$$

Coordinated Activation Parameters	↑Myofilament Charge: ↑pMLC C	↑pMLC S	↓ IS C	↓ IS S	↑ pH C	↑ pH S	↓ Lattice Spacing: ↑Stretch C	↑Stretch S	↑OC C	↑OC S	Titin (1S→2O, 2S→2O) C	Titin S	Rectilinear Components
↑ Po	12	13	14	16a	17	16b	19	21	20b	22	7		$F_{thck,r}$
↑ Ca^{2+}-sen	12	13	14	16a	17	16b	19 20a	16c 21	20b	16d	7	23	$F_{thck,t}$
↓ n	12	13	14	16a	17	16b	19 20a	16c 21	20b	16d	7	23	F_{tmi} ($F_{thck,r}$)

Table 1. Selected references that report a coordinated change in 2 to 3 activation parameters in skinned cardiac (C) and skeletal (S) muscle for 6 experimental conditions associated with an increase in myofilament charge or decrease lattice spacing, corresponding to the numerator or denominator of Coulomb's law for what would be an increasing electrostatic force, F_{thck}, which causes the coordinate changes through the rectilinear regulation of the SH motion of tropomyosin. Po, maximum active tension; Ca^{2+}-sen, Ca^{2+}-sensitivity; n, Hill coefficient; pMLC, myosin light chain phosphorylation; IS, ionic strength; OC, osmotic compression. Reference color statistical significance: Blue, P < 0.01; magenta, P < 0.05; orange, P = 0.57; black, data presented graphically and likely statistically significant, but P not reported explicitly.

For specific experimental conditions see supplemental data sheet available below or online at www.bostonbiomedicalresearch.com.

Conclusion

Biophysical and cellular experiments have now confirmed crucial and fundamental features of the SH theory indicating the presence of predicted nodes and the harmonic transition, respectively.

The coordinated change in the activation parameters, Po, Ca^{2+}-sensitivity, and the Hill coefficient, n_H, in cardiac and skeletal muscle, provides further support for a thick filament electrostatic force of repulsion, F_{thck}, acting on Tm, regulating the simple harmonic motion in a rectilinear manner, and for extending the scope of the SH theory as follows:

1. Alterations in the thick filament electrostatic force now includes alterations in myofilament charge, in addition to alterations in lattice spacing, as they correspond to the numerator and denominator for Coulomb's laws, respectively. The governing equation for the magnitude of an electrostatic force.
2. It was previously proposed that radial and tangential components of the thick filament electrostatic force regulate Po and Ca^{2+}-sensitivity, respectively. In addition, it is now proposed that a non-uniform change in the tangential force alters the slope, n_H, of the force-pCa^{2+}- relation, where a decreased slope was associated with a non-uniform increase in the tangentially directed internal 'elastic' restoring force, F_{tmi}.
3. Regulation of the activation parameters by changes in lattice spacing is not limited to more direct mechanical changes associated with stretch or osmotic compression, but now includes any mechanism that alters lattice spacing, including, but not limited to, alterations in the sarcomeric structural protein, titin, by mutations, phosphorylation and enzymatic digestion, for example.
4. Myofilament charge was not previously considered. Now included, it provides a molecular mechanism for regulating the activation process with phosphorylation of myosin regulatory light chain, changes in ionic strength and pH, by what is fundamentally the same mechanism for regulating active tension with lattice spacing and Starlings' Law of the heart.

Future reports will consider X-ray diffraction evidence for the SH motion of tropomyosin and its role in thick filament regulation of muscle relaxation.

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