33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

A Framework for Mapping Between "Living" Muscle Model Parameters and Systems Biology Data for Muscle Tissue

Jack M. Winters, *Member, IEEE*

Abstract—**Striated muscle represents a unique type of actuator in that it depends on living tissue. Models of muscle have historically focused on the role of actuator, using properties that do not adapt over time. This paper extends the foundation of Hill-based muscle models by considering muscle as a tissue composed of well-mixed composite materials (at the level of fascicle), and identifies three specific classes of protein families that occupy functional space functional space: excitation→activation; mechanical attachment/transmission, and myo-energy supply. Typically parameters describing nonlinear muscle properties have been estimated either directly or based on anthropometry and fiber composition. Here we develop a framework for augmenting such estimation through mapping to the up/down-regulation of specific proteins and their transcripts. While useful for establishing a framework for "living" muscle models that can evolve, it also provides an attractive approach for helping interpret high-throughput systems biology data, especially muscle tissue biopsies from studies that target interventional tasks and/or myo-disorders.**

I. Introduction

MODELS of striated muscle have focused on muscle as an actuator with specified properties that do not adapt over time. These models typically include a neuromuscular signal transmission component and a mechanical actuator component. For muscle mechanics, models can be roughly classified into two types:

1. "Systems" models that are rooted in controlled testing of isolated whole muscles. The mechanical properties are usually formulated using structural models rooted in the pioneering work of A.V. Hill [1] that consist of three classic lumped-parameter elements (contractile element, CE; series element, SE; parallel element, PE) that are driven by an "active state" [2] that is now usually associated with "calcium activation" of Troponin C sites on the actin-based thin filaments. These models aim to capture well-documented nonlinear phenomenological properties from animal and human studies [3], and are intended, ideally, to be reasonably task-independent [4] so that they can be used in larger musculoskeletal models to estimate muscle forces, and often other states such as calcium activation and end-point impedance [5].
2. "Reductionist" models that target underlying mechanisms such as swinging cross-bridges that cycle (e.g., Huxley-type models [6]). These models are narrower in scope, as the focus is on the behavior of one or a few proteins. Furthermore, there continue to be many challenges to core assumptions such as individual (vs collective) force generators [7], basic sources for force-velocity [8], and both the number of cycles per consumed ATP and of actins in contact with bridges [9].

There is a third "type" of model - by far the most common – is what we will call the "null" model. Here some aspects of experimental data (e.g., filtered EMGs, contact forces, instructions to subjects) are used to intuitively estimate muscle activity. The interpretive challenge is that we know that ***muscle force is a strong function of, in addition to activation, its ongoing velocity and length***. Thus without using a muscle model, intuitive estimates of muscle force can be misleading and can even provide disinformation [5].

It is in this context that the emerging field of high-throughput systems biology has rapidly "entered" into the world of muscle tissue evaluation. Reasons include the relative ease of extracting human and animal muscle tissue biopsies (especially from the vastus lateralis for humans), and the emerging focus on muscle tissue as an important window for studying diseases, disorders, and also the secondary consequences of dysfunction. Indeed, as of 2010, national repositories now house, just for human skeletal muscle using Affymetrix microarray chips alone, over 800 skeletal muscle tissue microarray datasets (nearly half sampling pathological muscle) contributed from over 30 studies. Many of these include both "control" and "experimental" data that are obtained both before and after controlled interventions (e.g., resistance training sessions). Others target various pathological conditions such as muscular dystrophy (MD), often with age-match young controls. Even in a genetic disorder involving a single gene product (e.g., dystrophin), we see changes in hundreds of transcripts that are consistent with observed morphology.

It would seem useful, both for systems biologists/ bioinformatists and for movement/rehabilitation scientists, to map strategic muscle properties (and underlying model parameters) to key protein/transcript levels from proteome/transcriptome data. Yet to date, I am not aware of any prior systematic effort to do so for muscle tissue.

In terms of the most appropriate type of model for such mapping, consider that the approaches of modern high-throughput systems biology tend to diverge from the narrow aims found in reductionist science studies. In contrast, lumped parameter "systems" models aim to capture all of the key properties and behavior of muscle actuators [3], which are composed of a muscle tissue that includes various types of cells as well as extracellular matrix. Such mapping has mutual benefits: proteome/transcriptome changes over time can help guide approaches for adapting parameters within

Manuscript received April 15, 2011.

J. M. Winters is with the Department of Biomedical Engineering at Marquette University, Milwaukee WI, 53233 USA (phone: 414-288-6640; fax: 414-288-7938; e-mail: jack.winters@mu.edu).

"living" muscle models as a function of use history or other factors, while such models can help estimate strategic internal task-specific states (force, calcium activation) for key interventions that are presumed to drive signal-transduction and transcriptional networks.

In this paper we develop a framework for mapping key proteins-transcripts to muscle properties. In particular, we will partition muscle into three fundamental families, and then define a manageable set of ***"capacity parameters"*** that are ***naturally adaption-ready***, i.e. time-varying parameters that in a few cases adjust over short times (e.g., fatigue), but in all cases adapt over longer times (e.g., weeks) as tissues remodel as a function of use (including "disuse") history.

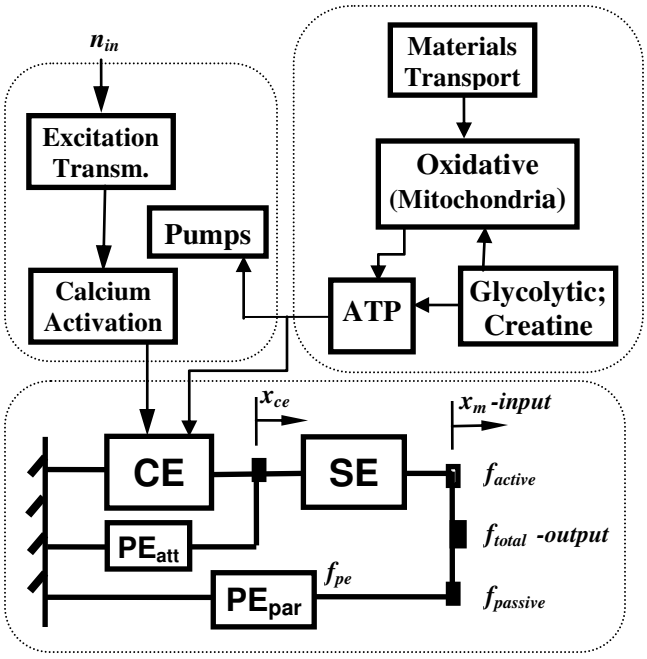

Fig 1: Model framework for "real-time" management of muscle activity and ongoing demands, based on a "mixed phase" muscle fascicle model: Excitation→Activation (top left, ~4-5% of space), Mechanical Attachment/ Transmission (bottom, ~60-80% of space), and the mostly slower-acting Myo-Energy Supply (top right, ~10-25% of space, very muscle and training specific). The remaining 5-25% of tissue space relates to other support functions (e.g., myo-nuclei, myo-production apparatus, cells such as fibroblasts and endothelial) and dysfunction such as fatty infiltration.

II. MAPPING FRAMEWORK

A. Overall Model Structure and Key Properties

We tie the duality of muscle as actuator and tissue by viewing muscle as a collection of muscle fascicles, each consisting of well-mixed components shown in Fig 1, similar to the mixed phases concept of Fung [10]. We also assume orderly recruitment of small-to-large motoneurons (MNs) that map to a well-mixed collection of muscle fibers within fascicles (recruited slow→intermediate→fast), and shear forces between fibers that enable a fascicle to be treated like a continuum with lumped properties.

The classic Hill-based model structure is provided in the bottom part of Fig 1, with the standard structures of the CE, SE and PE augmented by a second PE (PE_{at}) for reasons that will become evident. This model is transiently driven in real-time by Excitation→Activation signal transmission apparatus (top left). The Myo-Energy Supply (top right) apparatus, which transiently operates at a slower time scale of seconds to minutes, includes scattered mitochondria as well as distributed sarcoplasmic energy storage and metabolite transmission capacity.

For a given muscle actuator, inputs are the MN drive (top left) and the ongoing muscle length that is prescribed by the skeletal configuration. Muscle outputs an ongoing force (also impedance and power) that operates on the skeletal attachment sites. Many tasks are repetitive (e.g., walking, resistance training), with the ongoing performance a function of the capacity to supply ATP, manage sarcoplasmic calcium, and manage mechanical transmission. Each of these three "families" differ functionally in how they map between describing "capacity" parameters and strategic proteins/transcripts.

B. Excitation-Activation

This subsystem addresses the design challenge of distributing the MN drive that crosses the neuromuscular junction (NMJ) throughout a ***spatial volume*** to reach troponin C sites on thin filaments, and to do so nearly simultaneously in time. Critical to subsystem design are: *i)* use of membranes to transmit the signal axially and internally, and *ii)* use of diffusion of small Ca^{++} ions to transmit within the local nano-space. Pumps and exchangers, requiring energy in the form of ATP, are used to continuously "reprime" this subsystem. Performance depends on an ***appropriate surface density*** of specialized transmembrane receptors and pumps, composed of protein complexes exhibiting ***high-quality operational parts***. While each is composed of 2-7 subparts that come from separate gene products, transcription data strongly suggests that often 1-2 subparts have higher turn-over rates (presumably reflecting the higher "wear" of subparts with moving parts). Table I summarizes key parameter-protein mapping.

1a. NMJ Time Delay. This will be about 1 ms with excellent synaptic transduction, ***assuming*** an appropriate high-quality NMJ structure maintained by specialized scaffolding proteins and an appropriate density of acytylcholine receptors on the post-synaptic surface (supported by a local collection of myonuclei). But performance can gradually diminish for various conditions (e.g., spinal cord injury, amyotrophic lateral sclerosis).

1b. Sarcolemma transmission. This should be about 2-5 mm/ms in either direction from the NMJ region, both axially along the sarcolemma shell (up to ~8 cm for long fibers) and inwardly via the honeycomb-like t-tubule meshwork (up to 50 μm), ***assuming*** an appropriate density of high-fidelity transient ion gate "receptor" structures composed of specialized protein subparts (e.g., for Na^+, K^+, Ca^{++}, Cl^-).

1c. Transduction causing Ca^{++} bolus from SR. This takes ~1 ms with a high Ca^{++} gain, ***assuming*** an appropriate density of DHPR voltage sensors and matching RYRs from the SR (for skeletal muscle there is DHPR-RYR mechanical latching), and adequate stored SR Ca^{++} (mostly depending on CALSQ) ready to be released as a bolus through RYR. There are also regulatory agents that can affect the gain.

TABLE I: Excitation→Activation mapping of key parameters to a finite "top 10" list of protein complexes plus 2 geometric factors *

Stage	*Parameter*	*Protein complex or geometric factor*
1. Forward signal transmission:		
a. NMJ:	$\uparrow TD_{nmj}$; $\downarrow G_{nmj}$	↓[AChR]
b.Sarcolemma:	$\uparrow TD_{mem}$ $\downarrow G_{mem}$	$\downarrow[Na^+]$;$\downarrow[K^+]$; $\downarrow[Ca^{++}]$; VOL_{fib}
c. Ca^{++} release:	$\downarrow G_{volt\text{-}ryr}$	↓[DHPR]; ↓[RYR]; $\downarrow[Na^+/K^+]$
2. Ca^{++} spatial activation:		
Diffusion:	$\uparrow\tau_{act}$; $\downarrow G_{act}$	$\uparrow DIA_{SR}$; ↓[CaM]
3. Ca^{++} deactivation:		
Ion pumps and exchangers:	$\uparrow\tau_{deact}$; $\downarrow G_{deact}$	$\uparrow[SERCA_{slow}]$; $\downarrow[SERCA_{fast}]$; ↓[CALSQ], [SLN]

* TD: time delays; G: quality of gain signal; DIA_{SR}: average myofibril diameter defining SR shell (range of 1-2 μm); VOL_{fib}: average volume of a fiber within a fascicle; Na: sodium; K: potassium; AChR: acetylcholine receptor DHPR: dihydropyridine receptor; RYR: ryanodine receptor; SERCA: sarco/endoplasmic reticulum Ca^{++}-ATPase; CALSQ: calsequestrin; [SLN]: sarcolipin; [CaM]: camodulin.

2. Ca^{++} ions then diffusion to Troponin C sites. As diffusion time is strongly length-dependent, certain regulatory and structural proteins maintain 1-2 μm dia range of myofibrils during hypertrophy/atrophy processes (e.g., ankryins). For most ***skeletal muscle*** where functional demands imply small time constants ($\tau_{activation}$) on the order of 5-25 ms, there are 2 t-tubules per sarcomere for a given myofibril, shortening the distance to troponin C sites. As the chances of binding decreases if there are fewer available troponin sites without Ca^{++}, the τ is commonly considered to be variable (e.g., Michaelis-Menten, or M-M, kinetics [5]). For ***cardiac muscle*** τ ~15-40 ms since Ca^{++} bolus demands are less (at most 3/sec), there is only one t-tubule per sarcomere and a lower density of cardiac DHPR-RYRs. In Table 1 CaM is one example of a Ca^{++}-managing protein.

3. Energy-consuming pumps/exchangers "re-priming" gradients. Functional demands strongly influence pumps/exchanger density and, in the case of SERCA pumps, isoforms. The most rate-limiting, under healthy normal operation, are the workhorse Ca^{++} pumps are located near the SR (SERCA). The rate of these ATP-consuming pumps depends on a number of factors: ***i)*** type of pump (there are different pump isoforms, with "fast muscle" pumps being faster but less efficient), ***ii)*** average local density of pumps, which varies dramatically (highest in highly-conditioned athletes), ***iii)*** availability of ATP in the local region of the pump; and ***iv)*** local availability of Ca^{++} to be pumped (implying M-M kinetics). The end result is that $\tau_{deactivation}$ (~25-80 ms, ~40-60 ms most) is more than double $\tau_{activation}$ and rate-limited more by the capacity of the pump than by diffusion; it is lowest for a high density of $SERCA_{fast}$.

C. Mechanical Attachment and Transmission Mapping

Each of the lumped elements has properties that depend on the relevant protein-based composite material and geometry (mostly "set" by up/down regulation of key proteins), and can be viewed as mixtures that span the fascicle space [10]. But in terms of interpreting proteome/transcriptome data, certain components span most of the volume (e.g., actin and myosin, and their supporting members), while others span smaller subsets (e.g., circular slices for z-disc cross-linking members, surface area shells for transmembrane scaffolding proteins, a mesh for extracellular matrix that envelopes fibers and fascicles.

1. CE properties. This includes CE force-length (part of activation→ attachment mapping) and CE force-velocity. A critical parameter is the maximal isometric force (F_{max}):

$$F_{max} = \sigma_{max} A_{physiological-cross-sectional-area} \quad (1)$$

where the peak stress capacity σ_{max} is a function of many proteins and factors (Table II). Two other key capacity parameters are included in Fig 2:

- *CE optimum length for peak force production ($L_{o\text{-}max}$)*, which is mostly a function of the number of sarcomeres in series, but at the local sarcomere level depends in part on nebulin, which spans the thin filament that adds strength; muscles operating under sustained shortening/lengthening will gain/lose series sarcomeres, causing changes in $L_{o\text{-}max}$.
- *Unloaded maximum shortening velocity (V_{max})*, as reflected in the following form of the Hill equation for shortening velocities [3]:

$$f_{ce} = f_{isometric} - \left[\frac{F_{isometric} + a_f}{v_{ce} + a_f V_{\max}}\right] v_{ce} \quad (2)$$

where here $f_{ce} < f_{isometric}$ during shortening of v_{ce}, and f_{ce} =0 at V_{max}. V_{max} can vary from ~2 L_o/sec for purely slow muscle to ~8-10 L_o/sec for purely fast [3]; most fascicles are a mix. V_{max} behavior is most commonly associated with the classic 3 isoforms of myosin heavy chains (MHC, e.g., MHC1 defines the Type IIx) whose estimates dominate the literature. However, as seen in Fig 2, there are also 2-3 functional isoforms for each of myosin light chain (MLC), and thin filament regulatory troponin subunits C, T and I and tropomyosin. Of note is that low activation rates recruit mostly slow muscle fibers, and assuming shear between fibers, for simulations $V_{max\text{-}instant}$ scales between $V_{max\text{-}slow}$ and V_{max} [4]. There is not always a perfect mapping between the MHCs and troponin isoforms, nor with oxidative vs glycolytic staining [11].

TABLE II. Factors decreasing σ_{max} (material stress production capacity).

↑↑ myo-volume of stored energy	2-5% lipids and glycogen is normal, but above this reduces (e.g., fatty muscle can be >20%)
↑ mal-aligned filaments	If not well-packed and aligned lattice of f-actin & myosin myo-filaments, lower capacity
↓ muscle actin, myosin, titin	Poor quality and capacity, transition to "hybrid" tissue (e.g., embryonic, smooth musc. isoforms)
↓ desmin	Without, stress capacity only ~80%
↓ dystrophin	Without, suboptimal transmembrane force transmission, gradually reducing capacity

2. PE Properties. This includes the usual path through the collagen mesh (PE_{par}) that provides cavities contractile material (endomyosium encompasses fibers; perimysium defines fascicles; epimysium envelops collection of fascicles), which also involves passive pathways through the muscle fiber cytoskeleton (here represented by integrin focal adhesions and non-muscle actins). PE_{par} compliance in highest for skeletal muscle, then cardiac, then smooth; but with many diseases, skeletal muscles become less compliant, with transcripts for collagen and scaffolding proteins providing excellent markers for parameter adjustment.

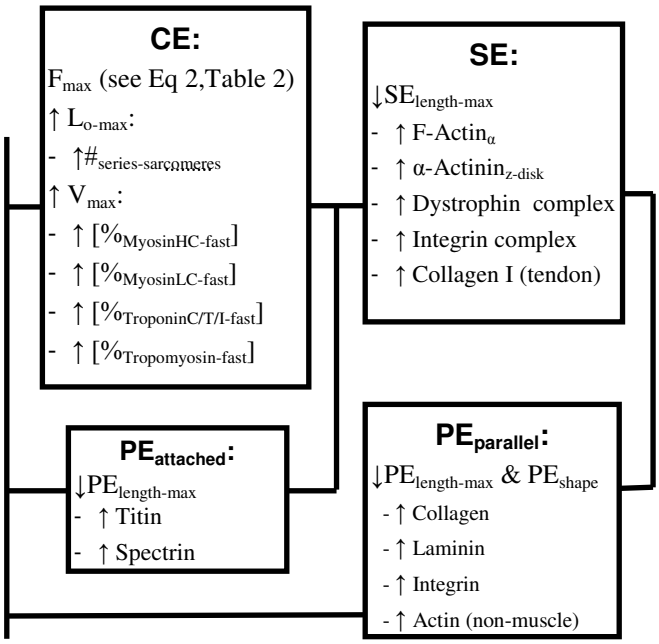

Fig 2. Summary of mapping between key capacity parameters and various proteins/transcripts or proportions, for each lumped element. See text.

The lumped $PE_{attachment}$ refers to the passive PE pathway that transmits through the thick filaments, via connection with very compliant titin macromolecules to z-disks; while recently viewed as a major passive force pathway by many muscle biologists, at least at shorter muscle lengths, the importance of the primary collagen-based PE_{par} path remains undeniable. A common approach for fitting the overall PE includes assuming a low-stiffness component (k_{sl}; think $PE_{attachment}$ and titin) plus the standard exponent shape (think PE_p and collagen), with PE_{sh} being a dimensionless shape factor [2], that dominates at longer lengths (with $PE_{l\text{-}max}$ typically defined as the extension at F_{max}):

$$f_{PE} = k_{sl}(l - l_{o1}) + \left[\frac{PE_{F-max}}{e^{PE_{sh}}-1}\right]\left(e^{\left(\frac{PE_{sh}}{PE_{l-max}}\right)l} - l_{o2}\right);\ l_{o2} > l_{o1} \quad (3)$$

where the key capacity parameters (Fig 2) are k_{sl} and $PE_{l\text{-}max}$.

$$\Delta L_{SE} = \sum_{i=1}^{n} \varepsilon_i L_{o_i} : \quad \Delta L_{musc}(att) + \Delta L_{musc}(f) + \Delta L_{tendon}(f)$$

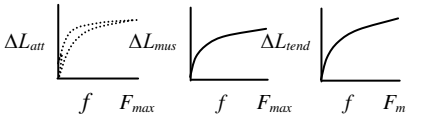

Fig. 3. SE contributions include all proteins in the serial mechanical transmission pathway since all exhibit strain (ε_i) as a function of attachment (*att*) and/or force (*f*), from thick-thin filament attachment through transmembrane skeletal insertion sites, here shown for 3 elements (right).

3. SE compliance. The key distinction of the ***SE*** is that extensions and series compliances add (Fig 3): if all of the mechanical transmission components could be treated as rigid pipes, there would be no SE. But it turns out that all of the proteins within the series pathway exhibit notable extension at loads of F_{max}. A few have an attachment-dependent (vs force-dependent) compliance (Fig 3). The overall average series strain at F_{max} ($SE_{e\text{-}max}$) is ~3-5%, with relative contributions depending on relative lengths (e.g., some muscles have long tendons, or different tendon "factor of safety" to self-injury, as tendons fail at about 6%) [12]. Note that muscle biopsies typically sample the muscle SE contributions, with tendon not part of the extracted sample.

D. Myo-Energy Supply

This family, only briefly summarized here, addresses real-time myo-energy consumption demands for ATP. This involves metabolic processes, with temporary short-term demands met mostly via anaerobic (glycolytic) pathways and sustained demands via aerobic (oxidative) pathways. The latter use specialized "muscle" mitochondria that often take the shape of a lattice that includes the muscle-specific COX7A1 protein, as well as a various transporter proteins that operate on materials such as oxygen and fuels. Many protein members have very high transcripts, suggesting high turnover. Of special note is that dynamic ATP supply capacity, as measured via mitochondria volume, can change dramatically based on changing demands ("use history"), even within one week. Capacity is higher for slower "oxidative" muscles, often transitioning in tandem with slower isoforms for SERCA and mechanics (e.g., MHC).

ACKNOWLEDGMENT

This perspective was enriched by my recent sabbatical to the University of California at San Diego (UCSD), and I thank UCSD bioengineering colleagues S. Subramaniam and Y. Wang for helping embed me in the emerging field of systems biology, especially as applied to muscle tissue.

REFERENCES

[1] A.V. Hill, The heat of shortening and the dynamics constants of muscle, *Proc. Roy. Soc*, vol. 126B, pp. 136-195, 1938.

[2] A.V. Hill, "The abrupt transition from rest to activity in muscle," *Proc. Roy. Soc. B*, vol. 126, pp. 136-195, 1949.

[3] J.M. Winters and L. Stark, "Analysis of fundamental movement patterns through the use of in depth antagonistic muscle models," *IEEE Trans. Biomed. Engng*., vol. BME 32, pp. 826 839, 1985.

[4] J.M. Winters and L. Stark, "Muscle models: what is gained and what is lost by varying model complexity", *Biol. Cybern.*, vol. 55, pp. 403-420, 1987.

[5] J.M. Winters, "How detailed should muscle models be to understand multi-joint movement coordination?" *Human Movem. Sci.*, vol. 14, pp. 401-442, 1995.

[6] A.F. Huxley, Muscle structure and theories of contraction, Progr. Biophys. Biophys. Chem, vol. 7, pp. 255-318, 1957.

[7] J.E. Baker and D.D. Thomas, "A thermodynamic muscle model and a chemical basis for A.V. Hill's muscle aquation," *J. Muscle Res. & Cell Motil*., 21:335-344, 2000.

[8] A. Landesberg, L. Livshitz, H.E.D.J. ter Keurs, The effect of sarcomere shortening velocity on force generation, analysis, and verification of models for cross-bridge dynamics, "Iin *Skeletal Muscle Mechanics*, W. Herzog, Ed, Chichester: Wiley, 2000, pp. 155-178.

[9] T. Mitsui and H. Ohshima, "Remarks on muscle contraction mechanism," *Int. J. Mol. Sci*., vol. 9: pp. 873-904, 2008.

[10] Y.C. Fung, *Biomechanics: Mechanical Properties of Living Tissues*, 2nd-ed, SPeinger-Verlag, New York, 1993, ch. 9.

[11] N.A. Rubinstein and A.M. Kelly, "The diversity of muscle fiber types and its origin during development," in *Myology*, A.G. Engle, C. Franzini-Armstrong, Ed, pp. 87-103, New York: McGraw-Hill, 2004.

[12] R. McN. Alexander and R.F. Ker, The architecture of leg muscles. In *Multiple Muscle Systems*, J.M. Winters and S.L-Y. Woo, Ed, Chap. 36, pp. 568-577, Springer-Verlag, New York, 1990.