Letters to the Editor

Modeling Thin Filament Cooperativity

We were very interested in the paper by Chen et al. (2001) on the modeling of the kinetic and equilibrium binding of myosin S1 to regulated actin filaments, containing actin, tropomyosin, and troponin (ATmTn). This is a formidable task, and the authors of the paper are to be commended on their considerable achievement. They have made a detailed comparison of the Hill et al. two-state model (1980) (referred to as the Hill model) and the McKillop and Geeves three-state model (1993) (referred to as the M and G model) and concluded that both can adequately describe the data. This could be interpreted, using Occam's razor, that a three-state model is not necessary. Although we would not wish to disagree with their calculations, we wish to point out that the authors: 1) have considered only some of the available data to test the two models; 2) have not compared the ability of the models to address fundamental issues in thin filament regulation; and 3) have not related the mathematical parameters of the models to the properties of the components.

We believe that the M and G model is a more useful model compared with the Hill model because: 1) the three states of ATmTn (Blocked-Closed-Open (M)) can be more readily related to three positions of Tm observed on actin, although the model was developed independently of structural information. 2) The two-step binding of myosin to actin can easily be integrated into the three states, including the coupling between the isomerization step and the C-O equilibrium. 3) It is more readily testable because the parameters that are used can be directly related to the properties of Tm, the regulatory component (such as strength of end-to-end interactions and flexibility which depend on amino acid sequence), and the modification of Tm function by Tn and Ca^{2+}, the allosteric components of the thin filament. 4) The M and G model is a complete biochemical model that involves equilibria between states that are affected by Ca^{2+} and myosin, rather than states that are defined by the absence or presence of Ca^{2+} or myosin. A given state, therefore, may not be fully occupied under a given set of experimental conditions (Table 1). 5) The M and G model can explain a much larger set of data, which were not considered in the Chen et al. (2001) paper.

These issues are expanded upon as follows:

The properties of the two- and three-states must be defined. Chen et al. described the Hill model as having two states, each with three substates (0, 1, and 2 Ca^{2+} bound for a total of six states). The M and G model on the other hand is described as a three-state model. Unless the meaning of the states is defined, the precise number of biochemical states will not be clear. If the states refer to the ATPase-activating potential of the thin filament then our model, like the Hill model, is a two-state model with the ATPase either *off* or *on*, and the ATPase *off* state consists of two substates, B and C (Table 1). The three-states of actinTmTn in our model, however, are defined in terms of three distinct myosin-binding properties of the actinTmTn complex. These binding states have more recently been associated with three distinct locations of Tm on the actin surface (Vibert et al., 1997; Holmes, 1995) as originally postulated by McKillop and Geeves (1993) and by Lehrer and Morris (1982).

The fundamental problem with any two-state model is that it does not readily take into account the major structural change occurring on removal of Ca^{2+} (fiber x-ray scattering (Holmes, 1995), electron microscopy (Vibert et al., 1997; Lehman et al., 2000), fluorescence probes (Bacchiocchi and Lehrer, 2000)), which has been interpreted as a large movement of Tm over the surface of actin away from a site where it blocks most of the myosin head-binding site on actin to a site where there is little direct interference. Chen et al. indicate that there are sufficient substates to account for the structural data. To make this argument the properties of the two fundamental states and the substates need to be defined. If the properties of the substates vary, then it is no longer a two-state equilibrium model. The assumed properties of the three states of the M and G model (*B*, *C*, and *M*) were carefully defined in 1993 and the evidence produced since that time has not required any change in these definitions. Furthermore, the model is readily compatible with the structural and spectroscopic data. The fundamental issue here is that all equilibrium studies require that actinTm and actinTmTn ($+Ca^{2+}$) exist (to ~80%) in a state which does not readily bind myosin (our *C*, or closed state). Removal of calcium turns the system more completely "off" and the issue is whether this simply changes the equilibrium between the two states (as in the original Hill et al. model) or indicates the presence of a new state, B, with different

TABLE 1 Properties and occupancy of the three thin filament states of McKillop and Geeves

Properties	B	C	O (M)	
Myosin-binding ability	none	weak (A)	strong (R)	
ATPase	OFF	OFF	ON	
Composition	Occupancy			
	B	C	O (M)	Tm position
−Tn	0	0.8	0.2	inner/outer
+Tn−Ca	0.7	0.25	0.05	outer domain
+Tn+Ca	0	0.8	0.2	inner/outer
+myosin	0	0	1.0	inner domain

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properties. Our data and the structural data point to a new state with different properties independent of whether the new state is called a state or substate.

The importance of the two-step myosin binding. In all our published work from the original version of the model (Geeves and Halsall, 1987) to the most recent comparison with Hill (Maytum et al, 1999) model we acknowledged that the two models were equivalent in describing equilibrium myosin-binding data. Our model was originally proposed (Geeves and Halsall, 1987) as an alternative to the Hill model because of the new insights that came from the multistep docking of myosin S1 to actin. The role of K_2, the equilibrium constant for the so-called A-to-R isomerization of myosin when bound to actin, is central to the underlying mechanism and can be defined independently of the model of regulation. This parameter is very powerful in predicting the ability of any myosin nucleotide complex to activate the thin filament. Chen et al. makes no use of this information.

Chen et al. did not use all the available data for the argument. The original evidence for the 3rd, *B* state, was supported by both kinetic and equilibrium myosin-binding data which included equilibrium titrations in which K_2 was varied from a value of 3 to 200. Kinetic binding data included measurements where the rates of S1 binding to actin were varied fourfold by varying the concentrations of the proteins and reduced sixfold by the use of a π analog bound to the nucleotide pocket. All the data can be well described by our model.

Chen et al. state "Nor has it been clearly demonstrated that the Geeves model is really able to predict the characteristic family of time courses of S1 binding for different S1 and actin concentration." We assume that this is referring to the kinetic data with S1 in excess, because all data with actin excess can be perfectly fitted without recourse to the complex modeling of Chen et al. We have also presented a range of data in 1995 for the kinetics of excess S1 binding to actin (Head et al., 1995). We used a very simple kinetic model and demonstrated that the lag phases observed were compatible with our model not simply for data with and without calcium but for a range of calcium concentrations.

Evidence that the B *to* C *states are in true equilibrium.* Head et al. (1995) tested the properties of the *B* state and the effect of several parameters on the equilibrium constant K_B were assessed. These included variations of actin concentration (fivefold), temperature (5–40°C), ionic strength (0.01–0.4 M), and calcium concentration (pCa 4–9). In all cases, the data were compatible with an equilibrium between the *B* and *C* conformations as predicted in the original model.

The most significant experiment involved the variation of Ca^{2+} concentration. The data showed that K_B was Ca^{2+}-sensitive and the calcium dependence of K_B showed a Hill coefficient of 1.8 and a midpoint at pCa 5.6; data very similar to the behavior of thin filaments in vitro and in muscle fibers (Potter and Gergely, 1975, Grabearek et al., 1983). Furthermore, similar results were produced using cardiac Tn with the predicted reduced Hill coefficient and a phosphorylation dependent shift in the midpoint of the curve (Reiffert et al., 1996; Zang et al., 1995). Significantly, the calcium dependence of K_B is the only factor required to produce a fit to the lag phase data mentioned above in addition to parameters which can be obtained using pure actin filaments.

Factors that determine the value of a model. The ability of any given model to mathematically fit experimental data is only one aspect of modeling. Another aspect is the predictive power of models and the new structural insights a model gives into underlying mechanisms. The Chen et al. analysis makes no attempt to use the structural and spectroscopic data of Ca^{2+} and myosin-induced changes of the thin filament to evaluate the models. These data integrate readily with the M and G model. The M and G model has introduced several concepts that have proved very helpful in understanding the nature of the cooperative process in the thin filament: 1) the importance of K_2, discussed above, in defining the dependence of the cooperative behavior of thin filaments on the nucleotide bound to myosin; 2) the use of the apparent cooperative unit size, *n*, to define the extent to which a single myosin head can activate the thin filament (Geeves and Lehrer, 1994); and the relation of the M and G model to the Monod et al. (1965) cooperative model in which actin catalyzes the breakdown of the myosin·ADP·Pi complex, Tm is the regulatory component, and Ca^{2+} and Tn are allosteric effectors of this process (Lehrer and Geeves, 1998).

We do not believe that the M and G model is the last word on thin filament models of regulation. Indeed, we have discussed in two recent papers the limitation of any model which relies on transitions of a single A_7TmTn unit, as both the M and G and the Hill models do. We firmly believe that we need to consider Tm forming a continuous cable over the surface of actin with a finite probability of being displaced from its most favorable position into other similar energy states. The key property of Tm is the strength of the head-to-tail interactions along the cable and the flexibility/persistence length of the Tm cable (Maytum et al., 1999; Lehrer et al., 1997). This is not the place to present such ideas in detail but the work of Smith and Geeves (Smith and Geeves, 2001; Smith, 2001) shows how this might be developed.

Many other problems remain to be addressed. The problem of binding Tm to actin discussed by Tobacman and Butters (2000) is not part of the M and G model. Nor has the model been used to assess ATPase or muscle fiber regulation in any detail, as these systems remain underdefined. However, the model does provide a useful framework within which to ask mechanistic questions and to devise tests of the underlying assumptions. Despite these reservations, we believe that our three-state model will remain viable for the immediate future.

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Response to the Letter by Geeves and Lehrer

The regulation of muscle contraction is a complex process that involves changes in both the organization of the troponin subunits and the orientation of tropomyosin on actin. The changes in tropomyosin may alter the manner in which myosin binds to actin, but, in our view, the more important change is an allosteric alteration of the ability of actin to participate in the catalysis of ATP hydrolysis. Because the ATPase activity of the system is closely coupled to muscle contraction, we have used the prediction of ATPase activity as our guide to successful modeling. At the same time we recognize that it is important to be consistent with the known structural changes of the components and other data, including the manner in which myosin binds to actin. The roots of the Hill model (the model that we support), similar to that of the M and G model (McKillop and Geeves, 1993) came from an explanation of the binding of myosin to actin. The Hill model began as a description of the equilibrium binding, whereas the M and G model was fashioned around the kinetics of binding.

The following observations are our primary benchmarks: 1) inhibition of ATPase activity by tropomyosin-troponin occurs without displacement of the S1-ATP and S1-ADP-Pi complexes from actin. (2) Inhibition is characterized by a large change in the k_{cat} for ATP hydrolysis over a wide range of conditions. (3) Under conditions of high occupancy of actin sites with nucleotide-free S1, the ATPase activity is enhanced beyond that in the absence of regulatory proteins. These observations have been reviewed earlier (Chalovich, 1992). The model of Hill et al. (1980) is consistent with all of these observations (Hill et al., 1981).

The M and G model does describe the binding of myosin to actin, but it is not known if that model can predict the features of regulation of ATPase activity that were outlined

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