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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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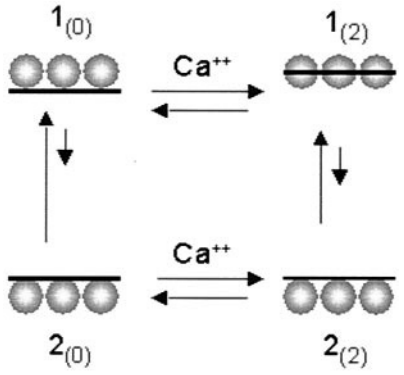

FIGURE 1 Relationship of key actin filament states of the Hill model with known structural states. Ca^{2+} and the occupancy of binding sites on actin with S1 control the state probability and the rate of ATP hydrolysis. The numbers refer to the major states of the actin filament and the subscripts define the number bound Ca^{2+}. In state $1_{(0)}$, the ATPase rate is low. S1-ATP can bind to actin in state $1_{(0)}$ whereas S1-ADP and rigor S1 bind weakly. The ATPase rate is $\sim$ 80-fold higher with saturating Ca^{2+}, mostly state $1_{(2)}$, and the ATPase rate of state 2 (both $2_{(0)}$ and $2_{(2)}$) is approximately eightfold higher than with Ca^{2+} alone. The population of state 2 is small in the absence of S1 binding both in the presence or absence of Ca^{2+} (*arrows*). In the presence of saturating Ca^{2+}, the transition to state 2 occurs at 1 S1 per tropomyosin unit, whereas in the absence of Ca^{2+}, >2 bound S1 per tropomyosin unit are required. The ATPase rate in state 2 is at its maximum. This maximum appears to be the same regardless of the number of bound Ca^{2+} (i.e., for $2_{(0)}$, $2_{(1)}$, and $2_{(2)}$). The relative ATPase activity of states $1_{(2)}$ and $2_{(n)}$ is unknown. The blocked, closed, and open states of the M and G model correspond to our states $1_{(0)}$, $1_{(2)}$, and $2_{(n)}$. In their model, these different structural states of actin do not correspond to different potential pathways of ATP hydrolysis. Rather, an actin unit in the closed state ($1_{(2)}$) with a bound myosin molecule must progress to the open state ($2_{(2)}$) to complete ATP hydrolysis. They also assume that the properties of the open state ($2_{(n)}$) are identical to those of actin in the absence of tropomyosin-troponin. Thus, it is not possible to obtain an ATPase rate that exceeds that of pure actin in their model. The intermediate states, $1_{(1)}$ and $2_{(1)}$, are omitted from the diagram, because they are assumed to have the same properties of $1_{(2)}$ and $2_{(2)}$, respectively. To model the regulation at nonsaturating Ca^{2+} concentrations, these states may have to be included in the diagram.

above. At a minimum it seems that an allosteric change in actin activity must be incorporated into the M and G model so that the effect of Ca^{2+} on the k_{cat} for ATP hydrolysis can be simulated. The evidence for actin allostery is growing (Miki and Hozumi, 1991; Egelman, 2001). Other models incorporating allosterism, such as that proposed by Tobacman and Butters (2000), are likely to be successful in simulating the regulation of ATPase activity.

Geeves and Lehrer imply that the Hill model is inconsistent with the known structural states of the regulated actin filament. We assert that there is no inconsistency (Fig. 1). The ability of actin to accelerate the ATPase activity of myosin and the ability of muscle to contract are dependent on whether each troponin is bound to 0, 1, or 2 calcium ions. Binding of rigor type S1 to regulated actin produces an even greater ATPase activity than seen with calcium alone, and greater than that seen with pure actin. This latter point is important in that it can not be explained by simply blocking/unblocking the binding of myosin to actin by the regulatory proteins. Several examples of this potentiation of ATPase activity exist (Eisenberg and Weihing, 1970; Murray et al., 1982; Williams et al., 1988; Fredricksen and Chalovich, 2001). The structural states that have been studied thus far correspond to the low Ca^{2+}-low S1 occupancy state, the high Ca^{2+}-low S1 occupancy state, and the low Ca^{2+}-high S1 occupancy state. These three states correspond to states $1_{(0)}$, $1_{(2)}$, and $2_{(0)}$ in the Hill model where the subscripts denote the number of Ca^{2+}-ions bound to troponin. It is not known how the structure of troponin and tropomyosin is changed when only 1 Ca^{2+} is bound to troponin.

Geeves and Lehrer believe that the positions of tropomyosin are more readily explained in terms of a multiple-step binding of myosin to actin. We do not think that there are scientific grounds for making this distinction. It should be noted that incorporation of multiple-step binding into the M and G model requires some assumptions. Data supporting multiple-step binding of rigor S1 and S1-ADP to actin are strong (Trybus and Taylor, 1980; Geeves and Halsall, 1987). However, the idea that the equilibrium constant for the first process, K_1, is the same for all nucleotide states is an approximation (Taylor, 1991). Also, the M and G model incorporates a blocked state to which no myosin can bind. Yet, there are many data showing binding of S1-ATP–like states to actin in the absence of Ca^{2+}. Furthermore, in the current structural view of the regulated actin filament, none of the positions of tropomyosin overlap the putative site of electrostatic (low affinity) binding of the S1-ATP and S1-ADP-Pi states (Vibert et al., 1997). The Hill model does not assume that all myosin nucleotide complexes bind along the same two-step binding pathway and so is consistent with these and other data that show a difference between S1-ATP–like and S1-ADP–like states (Brenner et al., 1999).

It is worth reiterating that we do not take exception to two-step binding of myosin to actin. The question is: what is the relationship of this two-step binding to regulation of muscle contraction? It is not necessary to incorporate two-step binding to explain the regulation of ATPase activity (Hill et al., 1981). The Hill model was criticized because it was thought that the Hill model could not explain the kinetics of binding of myosin to actin unless multiple-step binding was included. We showed recently that the Hill model could simulate the binding kinetics with either actin or S1 in excess (Chen et al., 2001). We did additional simulations since the publication of that paper. It is also possible to simulate the data in Figure 4 in the presence of Ca^{2+} with $k_1 = 2\ \mu M^{-1}s^{-1}$ and $k_{1'} = 10\ s^{-1}$ (see Table 3 of the original paper). That is, the value of K_1 in the Hill model need not change with Ca^{2+}. *Incidentally, while responding to this letter we noticed a typographical error in Figure 1; L should be written as* β_0/α_0.

Geeves and Lehrer stated correctly that we have not modeled all of their data. It is possible that in future studies

we may find cases where it is necessary to include multiple-step binding. This can be included into our model just as any additional intermediate nucleotide state in the cycle of ATP hydrolysis can be included should we wish to simulate a particular event. The inclusion of an additional binding step is not the only difference between our models. The differences are summarized in the legend to Figure 1.

The point was made that tropomyosin should be treated as a continuous cable, but the Hill model assumed that a single tropomyosin covering seven actin monomers acts as a unit. In the M and G model, the size of the cooperative unit changes with conditions. Tobacman and Butters (2000) have incorporated a very large degree of flexibility into their model by allowing each actin monomer to be treated independently. In the Hill model, the cooperativity is altered by the strength of the interaction between adjacent tropomyosin molecules (the parameter *Y*). It is also possible to make the size of the cooperative unit variable in the Hill model while still preserving the more fundamental differences with the M and G model. It is mathematically nontrivial to rigorously incorporate this flexibility into either the Hill model or the M and G model. Because this level of detail was not necessary to simulate the regulation of ATPase activity, it was not incorporated into our model. We must not lose sight of the fact that this is a model.

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On the Potential Functions used in Molecular Dynamics Simulations of Ion Channels

The determination of the structure of the KcsA K^+ channel represents an extraordinary opportunity for understanding biological ion channels at the atomic level. In principle, molecular dynamics (MD) simulations based on detailed atomic models can complement the experimental data and help to characterize the microscopic factors that ultimately determine the permeation of ions through KcsA. A number of MD studies, broadly aimed at analyzing the dynamical motions of water molecules and ions in the KcsA channel, have now been reported (Guidoni et al., 1999; Allen et al., 1999; Shrivastava and Sansom, 2000; Åqvist and Luzhkov, 2000; Bernèche and Roux, 2000; Biggin et al., 2001; Luzhkov and Åqvist, 2001; Crouzy et al., 2001). The potential functions that were used to calculate the microscopic interatomic forces and generate the dynamical trajectory are

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