similar but produces a decrease in Ca, an increased leaching of A-site cations, and a relative increase in B-site cations. Both types of alteration are pervasive and affect physical properties (for example, lowering the refractive index and specific gravity). In some cases sperulitic recrystallization is initiated (*7*).

Alteration effects in metamict silicates are less well documented, but examination of selected specimens in the collection of the U.S. National Museum of Natural History (formerly the National Museum) (yttrialite, USNM 85070 and USNM 3780; gadolinite, USNM 5102; and allanite, USNM 96970) suggests that the effects are equally pervasive. Autoradiographs reveal clear evidence of uranium and thorium leaching along the numerous microfractures that are common in metamict minerals.

Detailed electron microprobe analyses of an altered zone in yttrialite [$(Y,Th)_2Si_2O_7$] from the Rode Ranch pegmatite in Texas are summarized in Table 1. The Rode Ranch pegmatite occurs in the Valley Springs gneiss of the Precambrian in the Central Mineral Region of Texas (*8*). The yttrialite is completely metamict and can only be identified by x-ray diffraction after recrystallization at 1000°C for 1 hour in air. The wet chemical and x-ray fluorescence analysis by Bruun and Jensen (*9*) was completed on unaltered hand-picked material. The unaltered areas of the yttrialite in a polished thin section were used as the electron microprobe standard for the analysis of altered zones. This allows direct comparison of the composition of the two zones. The results are similar to those noted for complex Nb-Ta-Ti oxides and indicate primary alteration. There is an increase in CaO and H_2O with a decrease in SiO_2, U_2O_3, ThO_2, and the REE's.

Although preliminary, the data suggest that a glass phase is susceptible to alteration and thus may be an inappropriate medium for radioactive waste disposal.

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4 March 1976

Tropomyosin Binding to F-Actin Induced by Myosin Heads

Abstract. *Tropomyosin is a regulatory protein associated with F-actin in many actomyosin contractile systems. If in vitro conditions are such that tropomyosin binds only slightly to F-actin, then the addition of myosin heads can induce stoichiometric binding between them. This suggests that formation of rigor bonds between actin and myosin heads may cause some change in the actin, stabilizing the appropriate binding site for tropomyosin.*

Tropomyosin was first isolated from skeletal muscle in 1948 (*1*) and shown to have a regulatory function on the actin-activated myosin (actomyosin) Mg-dependent adenosine triphosphatase (Mg-ATPase) (E.C. 3.6.1.3; ATP phosphohydrolase) in 1964 (*2*). Tropomyosin is also found in vertebrate cardiac and smooth muscles and in invertebrate muscles (*3*). There is evidence (*4*) for the existence of regulatory proteins similar to muscle tropomyosin in nonmuscle cells such as human blood platelets, the myxomycete *Physarum polycephalum*, embryonic chick brain cells, growing neurons, skin fibroblasts, and epithelial cells.

At the present time two basic types of models for skeletal muscle regulation are being seriously considered. I now report evidence in support of the type of model in which the regulatory proteins tropomyosin and troponin control the actomyosin Mg-ATPase activity by changing either (i) the conformation of the F-actin monomers (*5*, *6*), (ii) the charge distribution around them (*7*), or (iii) their relationship to each other in the polymer (*5*). The experiment is based on the earlier finding from this laboratory (*8*) that tropomyosin binds very poorly to F-actin in 30 m*M* KCl and less than 0.1 m*M* free Mg^{2+}. The two types of models make different predictions about the effect of myosin rigor bonds on this system.

For the type of model already described in which tropomyosin can increase the interaction between actin and myosin at low adenosine triphosphate (ATP) concentration by inducing a change in the actin, symmetry arguments predict that the binding of myosin to actin would induce the same change. This in turn would enhance the binding between tropomyosin and F-actin. This type of model thus predicts that the presence of myosin rigor bonds will induce binding between tropomyosin and F-actin when it otherwise would not occur.

In contrast to this type of model for muscle regulation is the steric blocking model (*9*) in which actomyosin Mg-ATPase inhibition during relaxation results from the position of tropomyosin rather than from changes in F-actin. In its inhibitory position tropomyosin simply covers the myosin-binding sites on the actin filament, thereby preventing actin activation of the myosin Mg-ATPase. This model is based on recent x-ray diffraction studies of contracting skeletal and smooth muscles (*9*, *10*), but it should be pointed out that the data from these studies are actually compatible with either model type. An indication of a small mass redistribution led to the proposal that tropomyosin moves from a relaxation position to a contraction and rigor position when relaxed muscle is activated or allowed to go into rigor. When tropomyosin is in the contraction and rigor position, the filament is described as being turned on—this is biochemically analogous to an uninhibited actomyosin Mg-ATPase. From this perspective the terms relaxation position and inhibitory position are synonymous, and when tropomyosin occupies this position the filament is often described as being turned off. The x-ray diffraction data, however, provide no information about the mechanism by which the filament is turned off.

The steric blocking model (*9*) assumes that when relaxed muscles go into Ca^{2+}-free rigor, the myosin heads actually push the tropomyosin molecules from the relaxation to the contraction and rigor position. If this is correct, then adding myosin heads under rigor conditions to regulated actin filaments would be expected to reduce the binding of tropomyosin to F-actin by sterically blocking its more favorable binding position. This prediction is the opposite of that made by the former model type.

^{125}I-Labeled tropomyosin was used to measure the binding between tropomyosin and F-actin (*8*). The labeled tropomyosin and F-actin were mixed in centrifuge tubes at 25°C. The differences between the radioactivity of a sample of each solution before sedimentation and the radioactivity of a sample of the corresponding supernatant gave a measure of the amount of tropomyosin bound to the F-actin in the pellet. Binding experiments in the presence of rigor bonds were performed in the same manner, except that soluble proteolytic digestion products of myosin (myosin heads), which retain both actin binding sites and Mg-ATPase activity, were added to the

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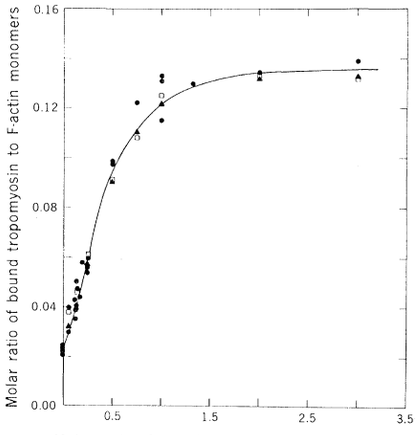

Fig. 1. Induction of binding between tropomyosin and F-actin by myosin heads. Conditions: 30 m*M* KCl, 1 m*M* EDTA, 0.2 mg of F-actin per milliliter, 0.046 mg of tropomyosin per milliliter, 1 m*M* imidazole buffer, *p*H 7.0, and increasing concentrations of (●) HMM, (▲) NEM-HMM, or (□) SF-1.

F-actin before it was mixed with the radioiodinated tropomyosin.

Figure 1 shows that myosin heads in the form of heavy meromyosin (HMM) and myosin subfragment-1 (SF-1) do induce binding between tropomyosin and F-actin. Even HMM modified by *N*-ethylmaleimide (NEM-HMM) induces binding between F-actin and tropomyosin. The extent of binding is a function of the number of myosin heads present and, as shown in Fig. 1, begins to level off as the ratio of myosin heads to F-actin monomers approaches unity. This suggests that it is necessary to saturate the actin filament with some form of myosin heads in order to induce stoichiometric binding between tropomyosin and F-actin and that it does not matter if the myosin heads are in the form of HMM, SF-1, or NEM-HMM.

This induction of binding between tropomyosin and F-actin by myosin rigor bonds could have been predicted from the well-documented observation that, at low ATP concentration, tropomyosin sometimes enhances the interaction between myosin heads and F-actin (*2, 8, 11, 12*). Bremel *et al.* (*12*) proposed a theory to explain this potentiation. They suggested that, when myosin heads are attached in rigor bonds to about half of the F-actin monomers, tropomyosin induces a cooperative change in the remaining monomers from a nonpotentiated state to a potentiated state. In other words, tropomyosin is expected to produce its maximum effect when F-actin is less than 60 percent saturated by rigor bonds. This theory would be reinforced if 60 percent saturation of the F-actin by rigor bonds had induced stoichiometric binding between tropomyosin and F-actin. Figure 1, however, shows a requirement for 100 percent saturation of the F-actin by rigor bonds before the amount of tropomyosin induced to bind reaches stoichiometry.

Previous work (*8*) had shown that the inhibitory subunit of troponin can induce binding between tropomyosin and F-actin in 30 m*M* KCl but not in 12 m*M* KCl. For this reason the experiment was repeated in the presence of HMM and 12 m*M* KCl. Binding between tropomyosin and F-actin is induced by HMM even at this very low salt concentration, as shown in Fig. 2. Under these conditions the binding curve is somewhat sigmoidal, suggesting that this phenomenon may be cooperative.

The main conclusion from these experiments is that the formation of rigor bonds between myosin heads and F-actin probably causes a change in either (i) the conformation of the F-actin monomers, (ii) the charge distribution around them, or (iii) their relationship to each other in the polymer, and that this change induces binding between F-actin and tropomyosin.

Biochemical data (*8*) in combination with structural data (*13, 14*) also appear to be inconsistent with the steric blocking model. Wakabayashi *et al.* (*13*) reconstructed a three-dimensional image from electron micrographs of F-actin paracrystals containing tropomyosin in the presence of two troponin subunits, and found tropomyosin in the relaxation position. When they analyzed micrographs of F-actin paracrystals containing tropomyosin in the absence of the troponin subunits, they found that the position of tropomyosin had shifted. They interpreted this as a movement to the contraction and rigor position. This interpretation is consistent with the steric blocking model only if tropomyosin under these conditions does not inhibit the actomyosin Mg-ATPase. Biochemical data (*8*) demonstrate that tropomyosin under similar conditions can inhibit the acto-HMM Mg-ATPase up to 60 percent. Others (*2, 11*) using myosin in place of HMM have found similar inhibition by tropomyosin. If one accepts the implicit assumption that F-actin paracrystals are comparable to F-actin in muscle and in solution (*13*), the steric blocking model is not reconcilable with these two sets of data, since tropomyosin in the contraction and rigor position (supposedly analogous to the uninhibited state) is capable of inhibiting the Mg-ATPase by 60 percent.

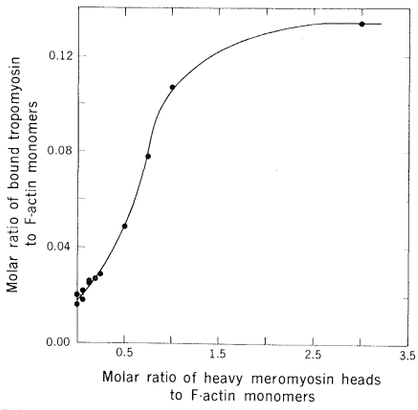

Fig. 2. Induction of binding between tropomyosin and F-actin by HMM at very low salt. Conditions: 30 m*M* KCl, 1 m*M* EDTA, 0.045 mg of tropomyosin per milliliter, 0.2 mg of F-actin per milliliter, 1 m*M* imidazole buffer, *p*H 7.0, and increasing concentrations of HMM.

Poo and Hartshorne (*14*) have developed an alternative method for measuring such structural changes. They attach two different fluorescent probes to tropomyosin and F-actin, respectively, and measure the energy transfer between them as an indication of the relative positions of the two chromophores. In contrast to the F-actin paracrystal results, Poo and Hartshorne's report (*14*) suggests a common position for tropomyosin on F-actin in solution, both in the presence of troponin under conditions that inhibit the Mg-ATPase almost completely and in the absence of troponin, a condition allowing only about 60 percent inhibition. The steric blocking model is not entirely compatible with these data either, because they imply that the inhibition of the Mg-ATPase can increase from a maximum of about 60 percent to almost 100 percent with no apparent change in the position of tropomyosin.

One additional difficulty with the steric blocking model is that it does not adequately explain the well-documented biochemical observation (*2, 8, 11, 12*) that the presence of tropomyosin alone can potentiate the actin activation of the Mg-ATPase of myosin heads at low ATP concentration. In terms of the steric blocking model, this observation implies that, when tropomyosin moves from the relaxation to the contraction and rigor position, it not only releases the blocked actin binding site for myosin but also enhances the actomyosin interaction in a way that increases the Mg-ATPase activity above that of actomyosin alone. Since the steric blocking model assumes that no conformational changes occur in the F-actin, such a potentiation requires some direct interaction between tropomyosin

and the myosin heads. Tropomysin does not bind to myosin in the absence of actin (*15*), however; hence there is no reason to expect tropomyosin to interact directly with myosin heads in the presence of actin.

In conclusion, evidence that is incompatible with a simple steric blocking model of muscle regulation has been presented. It appears that the mechanism of inhibition of the Mg-ATPase during relaxation of skeletal muscle (and perhaps other actomyosin contractile systems as well) cannot be accurately portrayed merely as the movement of tropomyosin from a contraction and rigor position to a relaxation position in which it physically blocks the myosin from interacting with F-actin by covering F-actin's binding site for myosin. Consideration must also be given to the compelling evidence that changes do occur in the F-actin moiety.

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16. I thank Dr. D. R. Kominz who made the initial observations that prompted this study and who provided me with encouragement and laboratory space. I also thank Dr. S. Mulhern for preparing the NEM-HMM and Dr. E. Eisenberg for supplying most of the other proteins and for helpful discussions during the course of this work.

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Fertilization of Sea Urchins Needs Magnesium Ions in Seawater

Abstract. *When sea urchin eggs were inseminated in seawater free of magnesium, the fertilization rate was very low. Spermatozoa that had been treated with egg jelly to induce the acrosome reaction also failed to fertilize eggs in seawater free of magnesium. These results indicate that magnesium is indispensable for some process or processes at fertilization, such as membrane fusion or sperm penetration.*

Seawater contains calcium and magnesium as the main divalent cations. It has been shown that calcium ions are required for inducing the acrosome reaction, that is, the formation of a process from the apical part of the spermatozoon on contact with egg jelly in marine invertebrates, and is thus indispensable for their fertilization (*1*). Little attention has been paid, however, to the significance of magnesium ions in fertilization. Below, we present evidence for the necessity of magnesium ions for fertilization in sea urchins.

Gametes of the sea urchins *Hemicentrotus pulcherrimus, Strongylocentrotus intermedius*, and *Anthocidaris crassispina* were used as materials.

Eggs of *H. pulcherrimus* were washed four times with artificial seawater free of calcium and magnesium (Ca, Mg-free ASW) (*2*). The eggs were then transferred to artificial seawater (ASW) (*2*) containing different amounts of calcium and magnesium. Insemination was made by mixing each of such egg suspensions (50 ml) with a sperm suspension diluted with Ca, Mg-free ASW (0.1 ml), to give a final dilution of 1 : 50,000 of the original semen. After insemination, the suspensions of egg and sperm were stirred for 15 minutes. The spermatozoa were then killed according to either the method of Rothschild (*3*) or that of Hagström (*4*), with some modifications. The eggs were transferred to normal seawater and the percentage of formation of fertilization membrane was counted. Several hours later, the percentage of dividing cells was also counted. The two sets of values coincided well with each other.

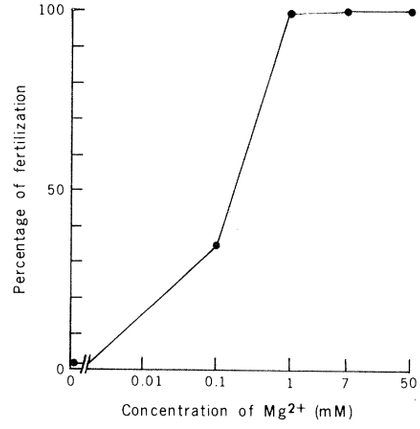

Fig. 1. Fertilization rate of *H. pulcherrimus* as a function of magnesium ion concentration in the presence of 10 m*M* calcium. The jelly layer of the eggs was removed by acidic seawater (*p*H 5) before washing with Ca, Mg-free ASW.

As shown in Fig. 1, the fertilization rate decreased markedly as the magnesium concentration of the ASW was reduced, even though 10 m*M* calcium chloride was included. When the eggs were suspended in ASW containing 10 m*M* calcium chloride and 50 m*M* magnesium chloride, all of them were fertilized. Similar results were obtained with *A. crassispina*.

When the concentration of inseminating spermatozoa was increased considerably (up to a final dilution of 1 : 5000), the fertilization percentage reached almost 100 percent in magnesium-free ASW that contained 10 m*M* calcium chloride. However, if the spermatozoa were washed once with Ca, Mg-free ASW prior to insemination, the fertilization rate became very low (less than 9 percent). In normal ASW, the washed spermatozoa could fertilize almost 100 percent of the eggs. These data are interpreted as suggesting that the high fertilization rates obtained with high sperm concentrations were due to magnesium ions either contained in the seminal plasma or associated with the sperm surface.

These results indicate that magnesium ions are also required, in addition to calcium ions, for fertilization. However, Loeb (*5*) reported that sea urchin eggs were fertilized in magnesium-free ASW that contained calcium, in contrast to our