Rod phosphorylation favors folding in a catch muscle myosin

(molecular conformations/ α -helical coiled coil)

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ABSTRACT Myosin from a molluscan catch muscle is unusual in being phosphorylated in the rod by an endogenous heavy chain kinase. The overall structure of the molecule resembles that of other muscle myosins, although the tail is somewhat longer (≈ 1700 Å). At low ionic strength the unphosphorylated molecules associate in filaments that display a striking axial repeat of 145 Å. Phosphorylation of the rod enhances myosin solubility in the range of NaCl between 0.05 and 0.15 M. Depending on the ionic strength and the counterions present, the soluble species corresponds to an antiparallel folded dimer (15 S) or to a folded monomer (10 S). Unphosphorylated myosin can also be partially solubilized into folded monomers by addition of ATP in 0.15 M NaCl. A similar molecular folding has also been observed in smooth muscle and nonmuscle myosins that depends, however, on the state of phosphorylation of the light chains in the myosin head. We discuss these results in relation to possible mechanisms for control of catch contraction.

Certain molluscan smooth muscles display a stretch-resistant state called "catch," characterized by slow cross-bridge cycling in the presence of ATP (1). Recent findings on the mechanical properties of one of these muscles, the anterior byssus retractor muscle (ABRM) of *Mytilus edulis*, have suggested that a phosphorylation step, mediated by cAMP, is related to the relaxation of catch (2–4). The overall organization of catch muscles is similar to that of vertebrate smooth muscles, but the structure of the thick filaments is distinctive: they consist of a large core of paramyosin surrounded by a layer of myosin (5–8). Unlike myosin from vertebrate smooth muscles, however, that from the ABRM is unusual in being phosphorylated by an endogenous kinase in the rod region of the molecule (4) but not in the light chains (4, 9). Phosphorylation of the myosin heavy chain has been described in a number of nonmuscle systems (see, for example, refs. 10–12). In *Acanthamoeba* and *Dictyostelium*, phosphorylation enhances myosin solubility and decreases actin-activated ATPase rates (10, 11, 13), but major conformational changes of these myosin molecules have not been found.

Here we report that phosphorylation of the ABRM myosin rod also increases myosin solubility, favoring as well a folding of the tail similar to that found in vertebrate smooth muscle and nonmuscle myosins. In these myosins, however, folding depends on the state of phosphorylation of the light chains in the head as well as ionic conditions (14–17). The special properties of ABRM myosin together with the architecture of catch muscle filaments suggest how changes in intermolecular interactions (myosin/myosin or myosin/paramyosin) might influence the rate of crossbridge cycling in the catch state.

METHODS

Protein Preparations. Myosin from the ABRM was prepared according to the method of Stafford *et al.* (18). Briefly, freshly dissected muscles were homogenized in 40 mM NaCl/1 mM MgCl_2 /0.1 mM EGTA/0.1 mM phenylmethylsulfonyl fluoride/10 mM NaPi , pH 7.0, with a Sorvall omnimixer and washed three times in the same buffer. All buffers used during purification contained leupeptin and pepstatin A at 1 $\mu\text{g}/\text{ml}$. Myosin extraction was carried out in high salt (0.6 M NaCl) in the presence of 5 mM MgATP. A 37.5% ammonium sulfate fractionation in the presence of ATP was performed to remove actin and paramyosin. The ammonium sulfate was increased to 60% and the precipitated myosin was dialyzed against the low salt buffer and washed three times to remove tropomyosin. This myosin may be stored for a few days on ice in a low salt buffer containing 0.5 mM dithiothreitol or for extended periods of time at -20°C in 0.5 M NaCl/1 mM MgCl_2 /0.1 mM EGTA/0.5 mM dithiothreitol/50% glycerol/10 mM NaPi , pH 7.0. Although the initial phosphate content of this preparation has not been determined, it will be referred to as "unphosphorylated" myosin. This myosin contains an endogenous heavy chain kinase.

Phosphorylation Assay. Phosphorylation was performed at room temperature in 40 mM NaCl/1 mM MgCl_2 /0.1 mM EGTA/0.5 mM dithiothreitol/20 mM *N*-[tris(hydroxymethyl)methyl]aminoethanesulfonic acid, pH 7.5 (kinase assay buffer). The concentration of myosin, which contains an endogenous kinase, was 1 mg/ml. Reaction was started by addition of a mixture of ATP and [γ - ^{32}P]ATP to a final concentration of 0.5 mM. Aliquots of 100 μl were removed at different times and added to 0.5 mg of bovine serum albumin; the mixture was precipitated by the addition of 1 ml of ice-cold 10% trichloroacetic acid containing 2% pyrophosphate. Precipitates were washed twice with the trichloroacetic acid solution and resuspended in scintillation solution for assay of radioactivity. Blanks consisted of the labeled ATP mixture and bovine serum albumin. When this myosin is incubated with radiolabeled [γ - ^{32}P]ATP in kinase buffer, 1.3–1.5 mol of phosphate are incorporated per mol of myosin (four preparations examined). The kinase activity is inhibited about 85% by 0.2 M NaCl and about 70% by 10 mM MgCl_2 .

Solubility and Sedimentation Studies. Aliquots of unphosphorylated myosin at 3 mg/ml were incubated in kinase assay buffer at room temperature for 90 min in the presence or absence of 0.5 mM MgATP. Under these conditions, maximal phosphorylation is achieved in the presence of ATP. After addition of NaCl to 0.5 M to fully solubilize the myosin, the phosphorylated (with ATP) and unphosphorylated (without ATP) myosin samples were dialyzed extensively against 1 mM MgCl_2 /0.1 mM EGTA/0.5 mM dithiothreitol/20 mM *N*-[tris(hydroxymethyl)methyl]aminoethanesulfonic acid, pH 7.5, containing 0.050, 0.100, or 0.150 M NaCl. The

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Abbreviation: ABRM, anterior byssus retractor muscle.

dialyzed proteins with or without 0.5 mM MgATP were spun in a Beckman Airfuge ultracentrifuge at $\approx 85,000 \times g$ for 5 min. Under these conditions, only filamentous myosin sediments to the bottom of the tube. The total protein concentration and that of the supernatants were determined by the Bradford colorimetric method.

Sedimentation velocity measurements were carried out in a Beckman model E analytical ultracentrifuge at 20°C, using the Schlieren optical system. To study the effect of ATP on the solubility and sedimentation properties of unphosphorylated myosin, MgATP was added just before centrifugation to limit phosphorylation by the endogenous kinase.

Electron Microscopy. Negative staining. Myosin dissolved in 0.5 M NaCl was dialyzed against a buffer containing 0.1 M or 0.05 M NaCl, 1 mM MgCl₂, 0.5 mM EGTA, 0.5 mM dithiothreitol, 3 mM NaN₃, 5 mM NaP_i (pH 7.2) at 4°C. A drop of filament suspension was placed on a carbon-coated grid, washed with 0.1 M and 0.05 M ammonium acetate, respectively, and stained with 2% uranyl acetate.

Shadowing. Phosphorylated myosin in buffers at different ionic strengths (as described above) was diluted into the corresponding buffers containing 70% glycerol and sprayed at room temperature onto freshly cleaved mica, according to the method of Shotton *et al.* (19). The angle of the shadow was 6°. Electron micrographs were recorded in a Philips EM301 or Philips EM420 electron microscope, operated at 60 kV. The magnification was calibrated using negatively stained tropomyosin paracrystals.

RESULTS

The Myosin Molecule and Filaments. Myosin prepared from ABRM of *M. edulis* can be phosphorylated in the rod by an endogenous kinase (4). Regardless of the state of phosphorylation, ABRM myosin appears similar to other muscle myosins when visualized for electron microscopy by rotary shadowing in high salt (Fig. 1). The two heads of the molecule adopt a variety of orientations relative to the tail, and the rod appears to be quite flexible as indicated by the high frequency of bends. The average length of the tail, 1694 Å (SD = 63 Å), is somewhat longer than that of myosins from molluscan striated and various vertebrate striated and smooth muscles (20, 21).

At low ionic strength (0.1 M NaCl), unphosphorylated ABRM myosin forms distinctive filaments of variable lengths up to $\approx 5 \mu\text{m}$ and of a diameter of $\approx 300 \text{ Å}$ in the center. Regions displaying a periodicity of 145 Å are often observed and a bare zone, lacking periodicity, can sometimes be identified (Fig. 2A). When the NaCl concentration is decreased to 0.05 M, the filaments, on average, seem to be thinner and show a tendency to aggregate into bundles. Many of these bundles show a pronounced set of axial striations with a repeat of 145 Å (Fig. 2B).

Effects of Phosphorylation on Solubility. The effect of phosphorylation on the ability of ABRM myosin to form filaments has been studied as a function of ionic strength. Sedimentation of phosphorylated myosin indicates that 50–65% of the protein is soluble in 0.05, 0.1, or 0.15 M NaCl in the presence or absence of 0.5 mM MgATP. In contrast, unphosphorylated myosin under the same ionic conditions is predominantly filamentous (only 5–10% of the total protein is soluble). Addition of MgATP to unphosphorylated myosin in 0.05–0.1 M NaCl has little effect on solubility; the soluble fraction of myosin increases by no more than 5–10%. At 0.15 M NaCl, however, the presence of ATP significantly increases the soluble portion of unphosphorylated myosin (up to 37% of the total protein). These results show that phosphorylation of the rod portion of the molecule by the endogenous myosin heavy chain kinase greatly diminishes filament formation at low ionic strength, whereas ATP has this effect only at 0.15 M NaCl.

Molecular Conformations. Sedimentation analysis together with electron microscopy was used to characterize the conformational states of the soluble fraction of phosphorylated ABRM myosin in different ionic strengths. In the analytical centrifuge at 0.04–0.05 M NaCl, ABRM myosin displays a major boundary corresponding to a sedimentation coefficient of 15 S and a minor one corresponding to that of 11 S (Fig. 3A). Note that in this and all subsequent experiments, the filamentous fraction of the myosin sediments rapidly at low speed. The presence of MgATP does not affect the velocity of sedimentation. Unphosphorylated myosin under the same ionic conditions sediments for the most part as large aggregates; only a small fraction of the protein is soluble and this portion sediments at 17 S. The sedimentation

FIG. 1. Field of ABRM myosin molecules (A) ($\times 100,000$) and histogram showing the distribution of lengths of myosin rods (B). Myosin is sprayed at room temperature in 0.45 M ammonium acetate containing 70% glycerol. Myosin tail lengths are measured by tracing enlarged images on a pad linked to a VAX 780. Measurements are corrected by an estimated metal thickness of 20 Å (see ref. 20).

FIG. 2. Negatively stained synthetic filaments of ABRM myosin formed in 0.1 M NaCl (A) and 0.05 M NaCl (B). ($\times 40,200$.)

coefficient is not affected by addition of 0.5 mM MgATP prior to centrifugation.

Visualization of phosphorylated myosin at 0.04 M salt by rotary shadowing shows filaments and solubilized molecules. The majority of the dispersed molecules are in a folded-antiparallel dimer conformation corresponding to the 15S species (Fig. 3B), similar to that observed in smooth muscle myosins (17). This conformation seems to be quite stable and is found when either chloride or acetate is used as the counterion in the spray buffer. Under these ionic conditions, molecules in a folded monomer conformation are also occasionally observed and may account for the 11S species seen in the analytical centrifuge. The folded structure appears to be maintained by the binding of a portion of the myosin tail (about two-thirds from the head-rod junction) to the neck region of the myosin molecule. The dimer is formed by the two folded monomers interacting in antiparallel fashion.

In 0.1 M NaCl, the soluble fraction of phosphorylated myosin shows a single symmetrical boundary, corresponding to a 10S sedimentation coefficient (Fig. 4A). Substitution of acetate for chloride shifts the major boundary to 15 S with a minor component at 7 S (Fig. 4B). This finding suggests that the 10S folded conformation is very sensitive to ionic conditions. Electron microscopic images of this preparation sprayed in chloride or acetate show the soluble myosin in a variety of conformations, the most common being that of the folded monomer (Fig. 4C) characteristically associated with the 10S sedimentation coefficient. In this conformation the tail loops back toward the neck region of the molecule and, in many cases, the end portion of the tail (≈ 700 Å) extends out. Molecules in a partial unfolded conformation are also observed. The apparent discrepancy between the relative sedimentation coefficients and the various conformations observed in the electron microscope may be due to the gradient of salt concentration produced during drying of the sample, together with some instability of the folded conformation. A similar effect has been reported for smooth muscle

FIG. 3. Sedimentation pattern of phosphorylated and unphosphorylated ABRM myosin at 0.04 M NaCl and selected images of rotary shadowed phosphorylated myosin molecules in 0.04 M salt. (A) Sedimentation pattern in 0.04 M NaCl of phosphorylated ABRM myosin in the presence (upper pattern) or absence (middle pattern) of 0.5 mM MgATP and of unphosphorylated ABRM myosin in which 0.5 mM MgATP (lower pattern) was added just prior to centrifugation. The presence or absence of ATP does not affect the boundaries of either phosphorylated or unphosphorylated myosin. Myosin was at 2.7 mg/ml and the pictures were taken 8, 24, and 32 min after reaching a speed of 40,000 rpm. (B) Selected images of phosphorylated ABRM myosin in the absence (top row) and in the presence of MgATP (bottom row, first three images) diluted in 0.04 M ammonium acetate containing 70% glycerol. The majority of the molecules are in a folded-antiparallel dimer conformation. Molecules in a folded monomer conformation are occasionally observed (bottom row, last image). ($\times 120,000$.)

myosin where crosslinking has been used to stabilize the 10S monomer for electron microscopy (15, 17).

When the NaCl is increased from 0.1 M to 0.15 M, the soluble fraction of phosphorylated myosin displays a single asymmetric boundary of 9 S, suggesting the presence of a mixed population of molecular conformations. This sedimentation coefficient is slightly reduced to about 8 S by the presence of ATP (Fig. 5A). In the electron microscope this preparation shows myosin molecules with partially folded and extended tails (Fig. 5C) in equilibrium with the filaments.

Although ATP has little effect on the solubility of unphosphorylated myosin in salt concentrations up to 0.1 M, its solubility in 0.15 M NaCl is considerably increased by the addition of 0.5 mM ATP (see above). In the analytical centrifuge the soluble fraction of unphosphorylated myosin shows a single boundary corresponding to 10 S (Fig. 5B). In the electron microscope the soluble fraction of unphosphorylated myosin displays only bending of the tails (data not shown), suggesting that the folded conformation is destabilized by preparation for rotary shadowing.

DISCUSSION

Myosin from the ABRM resembles other muscle myosins in its overall conformation (20, 21) but is unusual since its solubility at low ionic strength can be modulated by phos-

FIG. 4. Sedimentation patterns of phosphorylated ABRM myosin in 0.1 M NaCl in the presence (upper pattern) or in the absence (lower pattern) of 0.5 mM MgATP (A) or in 0.1 M NaCl (upper pattern) or 0.1 M sodium acetate (lower pattern) in the absence of MgATP (B). Myosin was at 2.7 mg/ml and the pictures were taken 8, 24, and 32 min in A and 11, 35, and 51 min in B after reaching a speed of 40,000 rpm. Note that in A the boundaries do not lie under one another due to a small displacement of the meniscus in the two cells. (C) Selected images of phosphorylated ABRM myosin rotary shadowed in 0.1 M ammonium acetate containing 70% glycerol. ($\times 120,000$.)

phorylation in the tail portion of the molecule. A small nonhelical region in the carboxyl-terminal portion of the tail of a number of other myosins has been found to be critical in determining the solubility, and hence the assembly properties of the molecule (22, 23). Moreover, in some nonmuscle myosins the targets for phosphorylation also appear to be located in the carboxyl-terminal region of the molecule (24). In the case of *Acanthamoeba* myosin, for example, this region is a small proline-containing nonhelical domain at the tail end of the rod (25). Amino acid analysis of the phosphorylated peptides in ABRM myosin rod reveals that one of them contains proline (B. Elliott and L.C., unpublished data). In the case of ABRM myosin, we would therefore expect the site(s) of phosphorylation to be located near the region of the tail that may also control solubility.

At low ionic strength, ABRM myosin in the unphosphorylated state associates into filaments whose appearance is distinctive: in contrast to most *in vitro* myosin aggregates, an axial repeat of 145 Å is readily apparent. The strong tendency of the rod region of this myosin to assemble with a 145-Å intermolecular shift may be related to the unusual packing of this molecule on the paramyosin core in catch muscle thick filaments (see below). Periodicities of 28 and 196 (7×28) residues in the linear disposition of charged residues have been shown to account for the axial repeat of 145 Å in other myosins (26) and in paramyosin (27) on the basis of the optimization of intermolecular ionic interactions. It is there-

FIG. 5. Sedimentation patterns of phosphorylated ABRM myosin in 0.15 M NaCl in the presence (upper pattern) or in the absence (lower pattern) of 0.5 mM MgATP (A) or of unphosphorylated ABRM myosin in the absence (upper pattern) or in the presence (lower pattern) of 0.5 mM MgATP (B). Myosin was at 2.7 mg/ml and the pictures were taken 16, 32, and 40 min in A and 11, 27, and 43 min in B after reaching a speed of 40,000 rpm. (C) Selected images of phosphorylated ABRM myosin rotary shadowed in 0.15 M ammonium acetate containing 70% glycerol. ($\times 120,000$.)

fore likely that these periodicities will be highly significant in the ABRM myosin sequence.

The enhanced solubility of ABRM myosin at low ionic strength upon phosphorylation of the tail appears to be coupled with a folded conformation of the molecules. Depending on the ionic strength and the counterions present, the soluble species correspond in the main to an antiparallel folded dimer (15 S) or to a folded monomer (10 S). Although ATP does not affect the solubility or conformation of phosphorylated myosin, an increase in solubility is observed in unphosphorylated myosin upon addition of ATP at 0.15 M NaCl. The corresponding sedimentation coefficient of the soluble fraction (10 S) indicates that folding also occurs in unphosphorylated myosin in the presence of ATP, although this conformation is not preserved in rotary shadowing. We do not yet know whether the solubilizing effect of ATP is due to ionic interactions with the rod that disassemble the filaments (28) or to a specific binding to the head region that may also alter the myosin conformation (cf. ref. 17). These results show that phosphorylation of the tail at low ionic strength (≤ 0.15 M NaCl), or the addition of ATP at 0.15 M NaCl, promotes the solubilization of ABRM myosin in a folded conformation.

Folding of myosin molecules has been observed previously in vertebrate smooth and nonmuscle myosins, depending on the state of phosphorylation of the myosin light chains bound to the head region and on the ionic conditions (14–17). Binding of approximately stoichiometric amounts of MgATP to the head causes the disassembly of filaments of unphos-

phorylated smooth muscle and nonmuscle myosins into folded monomers, whereas phosphorylation of the regulatory light chains induces these myosins to reassemble into filaments (17). In contrast to vertebrate smooth muscle myosin, phosphorylation of ABRM myosin in the tail favors the disassembly of the filaments into folded molecules, whereas unphosphorylated myosin is primarily filamentous at these ionic strengths. Phosphorylation of the rod has thus far been associated with changes in molecular conformation only in this specialized myosin.

Taken together these results suggest several possible mechanisms for control of catch contraction in molluscan muscles. There is now considerable evidence showing that phosphorylation/dephosphorylation of contractile proteins is involved in catch. Paramyosin and myosin can be phosphorylated *in vitro* (4, 29) and are possible targets for this phosphorylation *in vivo*. A key structural aspect of thick filament design in catch muscles is that the myosin molecules are arrayed in a surface lattice surrounding a large paramyosin core and that myosin/paramyosin interactions are a critical feature of the assembly of the filament (30). In catch muscles it has been postulated that phosphorylation may influence the rate of crossbridge cycling by changes in the myosin/myosin or myosin/paramyosin interactions. Such a picture derives support from recent results on *Acanthamoeba* myosin: here phosphorylation or binding of antibody to the myosin tail results in filament disassembly and inhibition of the actin-activated ATPase activity of the heads (23, 31). We have now shown that *in vitro* phosphorylation of the myosin tail at low ionic strength increases myosin solubility favoring molecular folding in ABRM myosin. Phosphorylation of myosin in the native filament is unlikely to solubilize the molecule; part of the myosin rod could, however, detach from the thick filament upon changes in phosphorylation. Whatever the precise effect, it seems likely that some specific change in intermolecular interactions occurs upon phosphorylation. Identification of the residues that are phosphorylated and their location in the tail of the molecule, together with a satisfactory model of the packing of myosin on the paramyosin core, may reveal how phosphorylation-dependent changes in intermolecular interactions might control crossbridge cycling in the catch mechanism.

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1. Twarog, B. M. (1979) in *Motility in Cell Function*, eds. Pepe, F. A., Sanger, J. W. & Nachmias, V. T. (Academic, New York), pp. 231-241.
2. Cornelius, F. (1982) *J. Gen. Physiol.* **79**, 821-834.
3. Pfitzer, G. & Rüegg, J. C. (1982) *J. Comp. Physiol.* **147**, 137-142.
4. Castellani, L. & Cohen, C. (1987) *Science* **235**, 334-337.
5. Hanson, J. & Lowy, J. (1960) in *Structure and Function of Muscles*, ed. Bourne, G. H. (Academic, New York), Vol. 1, pp. 265-336.
6. Szent-Györgyi, A. G., Cohen, C. & Kendrick-Jones, J. (1971) *J. Mol. Biol.* **56**, 239-258.
7. Nonomura, Y. (1974) *J. Mol. Biol.* **88**, 445-455.
8. Bennett, P. M. & Elliott, A. (1981) *J. Muscle Res. Cell Motil.* **2**, 65-81.
9. Frearson, N., Farant, B. & Perry, S. V. (1976) *FEBS Lett.* **63**, 27-32.
10. Collins, J. H. & Korn, E. D. (1980) *J. Biol. Chem.* **255**, 8011-8014.
11. Kuczmarski, E. R. & Spudich, J. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7292-7296.
12. Matsumura, S., Murakami, N., Yasuda, S. & Kuman, A. (1982) *Biochem. Biophys. Res. Commun.* **108**, 1595-1600.
13. Collins, J. H., Kuznicki, J., Bowers, B. & Korn, E. D. (1982) *Biochemistry* **21**, 6910-6915.
14. Suzuki, H., Onishi, H., Takahashi, K. & Watanabe, S. (1978) *J. Biochem. (Tokyo)* **83**, 835-847.
15. Trybus, K. M., Huiatt, T. W. & Lowey, S. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6151-6155.
16. Craig, R., Smith, R. & Kendrick-Jones, J. (1983) *Nature (London)* **302**, 436-439.
17. Trybus, K. & Lowey, S. (1984) *J. Biol. Chem.* **259**, 8564-8571.
18. Stafford, W. F., Szentkiralyi, E. M. & Szent-Györgyi, A. G. (1979) *Biochemistry* **18**, 5273-5280.
19. Shotton, D. M., Burke, B. E. & Branton, D. (1979) *J. Mol. Biol.* **131**, 303-329.
20. Flicker, P. F., Wallimann, T. & Vibert, P. (1983) *J. Mol. Biol.* **169**, 723-741.
21. Elliott, A. & Offer, G. (1978) *J. Mol. Biol.* **123**, 505-519.
22. Nyitrai, L., Mocz, G., Szilagy, L., Balint, M., Lu, R. C., Wong, A. & Gergely, J. (1983) *J. Biol. Chem.* **258**, 13213-13220.
23. Kuznicki, J., Coté, G. P., Bowers, B. & Korn, E. D. (1985) *J. Biol. Chem.* **260**, 1967-1972.
24. Collins, J. H., Coté, G. P. & Korn, E. D. (1982) *J. Biol. Chem.* **257**, 4529-4534.
25. Coté, G. P., Robinson, E. A., Appella, E. & Korn, E. D. (1984) *J. Biol. Chem.* **259**, 12781-12787.
26. McLachlan, A. D. (1984) *Annu. Rev. Biophys. Bioeng.* **13**, 167-189.
27. Cohen, C., Lanar, D. & Parry, D. A. D. (1987) *Bioscience Rep.*, in press.
28. Harrington, W. F. & Himmelfarb, S. (1972) *Biochemistry* **11**, 2945-2952.
29. Cooley, L. B., Johnson, W. M. & Krause, S. (1979) *J. Biol. Chem.* **257**, 4529-4534.
30. Cohen, C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3176-3178.
31. Kiehart, D. P. & Pollard, T. D. (1984) *Nature (London)* **308**, 864-866.