Review Letter–Hypothesis

Phosphorylation of myosin in non-muscle and smooth muscle cells

Possible rules and evolutionary trends

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Reversible phosphorylation of myosin subunits is observed in almost all eukaryotic cells. The data concerning sites and effects of phosphorylation on actin-activated ATPase activity of myosin and on its filament formation are described. These observations are discussed in terms of possible evolutionary trends and rules which may govern the process of myosin phosphorylation.

Myosin phosphorylation *Muscle contraction* *Cell motility*

1. INTRODUCTION

Actin and myosin are responsible for muscle contraction and seem to be involved in different forms of cell motility such as amoeboid movement, cytoplasmic streaming, cytokinesis or endocytosis [1–3]. The structure and function of actin were recently described in several reviews [4,5]. In this article I will focus on some properties of the myosin molecule. The protein consists of six subunits: two heavy chains with a molecular mass about 200 kDa and two pairs of light chains with a molecular mass in the range 15–27 kDa (for recent reviews on myosin see [6,7]). Myosin is a highly asymmetric protein. Approx. 50% of the C-terminal parts of both heavy chains fold together to form a coiled-coil of α-helices referred to as a tail of myosin. This region is responsible for assembly of myosin into bipolar filaments. The remaining part of each heavy chain, together with two different light chains, forms a globular head containing the active site of the ATPase and the actin-binding site. The rate of ATP hydrolysis by myosin is low in the presence of Mg^{2+}, but is significantly enhanced upon interaction of myosin with actin. This actomyosin ATPase activity is an in vitro assay system analogous to interaction between filaments of actin and myosin in vivo.

The interaction of actin and myosin is controlled by regulatory systems which in most cases have been found to be calcium-sensitive (recent reviews [8–10]). A very widely distributed regulatory system is the reversible phosphorylation of myosin light chains and/or heavy chains (reviews [9–11]). The introduction of a charged phosphate moiety into a myosin molecule results in marked changes in its actin-activated ATPase activity and ability to form bipolar filaments. In the first part of the article the data concerning different sites and effects of phosphorylation on myosin properties are collated. The purpose of the present work was to search for order in all this complexity, and in the second part of the article possible evolutionary

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trends and rules which may govern the process of myosin phosphorylation are presented.

2. PHOSPHORYLATION OF MYOSIN FROM PROTISTA

Myosin isolated from amoebae and slime molds can be phosphorylated in vivo and in vitro. The sites and effects of such phosphorylation are summarized in table 1 and some details are given below for each individual myosin studied.

Acanthamoeba myosin II (*Acanthamoeba castellani* also contains myosins IA and IB. These two proteins are single headed and have no tail. Myosins IA and IB are not discussed in the article, although they can be phosphorylated.), a typical two-headed myosin, is phosphorylated at three serine residues located at the end of the tail [12,13]. Dephosphorylation of the heavy chains increases the ability of myosin II to form stable filaments and increases the maximum velocity of actomyosin ATPase activity with little, if any, effect on the affinity of myosin II for actin [14,15]. Maximally phosphorylated myosin II has no actin-activated ATPase activity and forms smaller and less stable filaments than dephosphorylated myosin [15,16]. It has been found that actin-activated ATPase activity and filament assembly are inhibited in parallel either by proteolytic removal of a region that contains the phosphorylation sites [17], or by monoclonal antibodies specific to this region [18]. It has also been shown that phosphorylated molecules inhibit the activity of dephosphorylated molecules when they are contained within the same filament [16]. In order to explain how a catalytic site of the myosin head is affected by regulatory

Table 1

Sites and effects of phosphorylation on myosin from muscle and non-muscle cells

Cell or tissue	Phosphorylatable subunit			In vitro effects of phosphorylation on	
	Heavy chain	Light chain		ATPase activity	Stability of filaments
Acanthamoeba[a]	+	−		inhibition	destabilization
Dictyostelium[a]	+	+	H	inhibition	destabilization
			L	?	?
Physarum	+	−		activation	stabilization
Leukemic myeloblasts	+	+	H	inhibition	?
			L	activation	
Lymphocytes	+	+	H	?	?
Macrophages[a]			L	activation	
Brain[a]					
Thyroid Platelets Thymus	−	+		activation	stabilization
Smooth muscle	−	+		activation	stabilization
Striated muscle	−	+		no effect[b]	no effect[c]

[a] Phosphorylation sites on the heavy chains are located at the end of the tail
[b] Under certain ionic conditions a small difference in the actin-activated ATPase activity of skeletal muscle myosin is observed
[c] State of phosphorylation seems to affect conformation of filaments

H, heavy chain; L, light chain

sites located at the other end of a molecule the supramolecular mode of action of reversible phosphorylation was proposed [16,17,19]. According to this hypothesis activation of actomyosin ATPase activity by dephosphorylation of the heavy chains occurs as a result of intermolecular interactions within the filaments and not at the level of a single molecule. It was therefore suggested that the phosphorylation state defines whether filaments of myosin II may achieve the enzymatically active conformation.

Heavy chains of myosin from *Dictyostelium discoideum* are reversibly phosphorylated in vivo and in vitro [20,21]. Each heavy chain has one phosphorylation site located at the end of the tail [22,23]. *Dictyostelium* myosin with phosphorylated heavy chains has lower actin-activated ATPase activity and forms less stable filaments than a myosin with dephosphorylated heavy chains (table 1) [21]. A myosin heavy chain kinase isolated from *D. discoideum* seems to be inhibited by the calcium-calmodulin complex [24]. One pair of light chains of *Dictyostelium* myosin is phosphorylated by an endogenous kinase, but the effects of such phosphorylation on myosin's properties are not known [21]. Myosin filaments are present in the cortical regions of *Dictyostelium* cells [25,26]. In response to a chemotactic stimulus the translocation of these filaments seems to occur in coordination with the assembly/disassembly of the molecules [25]. A change in phosphorylation of myosin heavy chains was found to be induced by the same stimulus [27].

Myosin from *Physarum polycephalum* plasmodia is phosphorylated on its heavy chains, but the location of the phosphorylation site is not known [28,29]. It was shown that unphosphorylated myosin from *Physarum* has lower actin-activated ATPase activity than phosphorylated myosin (table 1). This is surprising, since phosphorylation of the heavy chains of myosin from *Acanthamoeba, Dictyostelium* and leukemic myeloblasts has the opposite effect on their actomyosin ATPase activity (table 1). Filaments of phosphorylated myosin from *Physarum* are stable in vitro under physiological conditions, but filaments of unphosphorylated myosin (enzymatically inactive) depolymerise to monomers and small oligomers with heads located at one end and tails on the other.

3. PHOSPHORYLATION OF MYOSIN FROM NON-MUSCLE CELLS

Two groups of vertebrate non-muscle myosins can be distinguished with respect to location of their phosphorylation sites (table 1). In the first group are myosins where both the light chains and heavy chains are phosphorylated. These are myosins from fibroblasts [30], macrophages [31–33], lymphocytes [34,35], brain tissue [36–39] and leukemic myeloblasts [40,41]. In the second group are myosins where only light chains are phosphorylated. These include myosins from platelets [42], astrocytes [43], myoblasts [44], baby hamster kidney cells [45], thyroid [46], thymus [47,48], myoepithelial cells [49], brush border [50,51] and adrenal medulla [52].

The effect of heavy chain phosphorylation on vertebrate non-muscle myosins is only known for leukemic myeloblasts: phosphorylation inhibits actin-activated ATPase activity of myosin from these cells (table 1). In myosins from brain and macrophages, phosphorylation sites on the heavy chains are located at the end of the tail [33,39], similar to the location of the phosphorylation sites of *Acanthamoeba* and *Dictyostelium* myosin.

Regulatory light chains of myosins isolated from non-muscle cells are phosphorylated by a kinase that is activated by calmodulin. This phosphorylation increases actin-activated ATPase activity of all myosins studied and affects the ability of myosins to assemble into filaments (table 1). Filaments of phosphorylated (enzymatically active) myosin are stable under physiological conditions, but those of unphosphorylated myosin depolymerise to monomers with a characteristic conformation [46–48]. In this conformation the tail of the molecule is folded twice to generate three segments of approximately equal length. The sedimentation coefficient of these monomers is 10–12 S, whereas the sedimentation coefficient of monomers that are simply extended rods without any folds is about 6 S (such monomers are typically observed in solutions containing higher than physiological salt concentrations). When folded monomers are phosphorylated by myosin light chain kinase they unfold and assemble into bipolar filaments [48].

The presence of myosin filaments in stress fibers of cultured cells [53] and in human glioma cells has been demonstrated recently [54].

4. PHOSPHORYLATION OF SMOOTH MUSCLE MYOSIN

Muscle myosins, with the exception of molluscan myosin, have a pair of light chains that are phosphorylated by a protein kinase which is activated by calmodulin (reviews [9–11]). There are no confirmed reports of myosin heavy chain phosphorylation in muscle. Much of the information regarding the effects of light chain phosphorylation has been obtained from studies of myosin from smooth muscle. Evidence from many experiments, in vivo and in vitro, indicates that phosphorylation of myosin plays a primary role in regulation of the contraction-relaxation cycle of smooth muscle (reviews [55,56]). It has been shown that contraction does not occur in the absence of phosphorylation, and that phosphorylation precedes tension development. It also appears that phosphorylation regulates the cross-bridge cycling rate. However, all these physiologically oriented studies indicate that phosphorylation alone may not be adequate to account fully for all the experimental observations and it is evident that other factors are implicated (reviews [57,58]).

It has been established that phosphorylated smooth muscle myosin exhibits high actin-activated ATPase activity in vitro whereas unphosphorylated myosin exhibits low activity [59,60]. The relationship between the extent of light chain phosphorylation and actomyosin ATPase activity depends on the aggregation state of myosin which may indicate that reactivity of myosin heads is different in filaments than in monomers [61–63]. Phosphorylation of regulatory light chains of smooth muscle heavy meromyosin (a soluble proteolytic fragment of myosin) regulates its actin-activated ATPase activity similarly to that of undigested myosin. Phosphorylated smooth muscle myosin has higher affinity for actin than unphosphorylated myosin, but the difference is too small to explain the difference in their maximal rates of ATP hydrolysis [64]. It was therefore suggested that light chain phosphorylation regulates some steps in the ATP hydrolysis cycle and that unphosphorylated light chain does not cause a steric blocking between actin and myosin [64]. The phosphorylation state of smooth muscle myosin influences its ability to assemble into bipolar filaments in vitro [48,65–68]. At physiological salt concentrations and in the presence of ATP only phosphorylated myosin is filamentous, and unphosphorylated myosin exists as a 10 S monomer. This 10 S conformation is identical to the folded conformation described for myosin from non-muscle cells [48]. As a consequence of folding and intramolecular interaction a 10 S molecule is unable to polymerize and has lower ATPase activities than myosin in the 6 S conformation [69]. Phosphorylation of 10 S monomers by myosin light chain kinase induces unfolding of tails and assembly of formed 6 S monomers into stable bipolar filaments [48]. There is no indication that the 10 S monomer exists in smooth muscle in situ. In contrast, it has been shown that myosin is filamentous even in relaxing smooth muscle [70,71]. It is possible that the 10 S conformation can be mimicked by intermolecular interactions between the hinge region of one myosin molecule and the tail of another in the same filament and that it generates analogous changes in the reactivity of myosin heads.

Phosphorylation of myosin light chains also takes place in skeletal and cardiac muscle, although its function appears to be markedly different from that in smooth muscle (reviews [3,8–11]). The contraction-relaxation cycle of striated muscle is regulated by the Ca^{2+}-sensitive complex of troponin and tropomyosin. However, some evidence indicates that light chain phosphorylation acts as a secondary mechanism of regulation in these muscles. Actin-activated ATPase activity of striated muscle myosin does not depend on the state of phosphorylation in vitro. Unphosphorylated as well as phosphorylated forms exhibit high actomyosin ATPase activity and form stable filaments.

The information regarding phosphorylation of myosin from muscle of invertebrates is very limited. Actin-activated ATPase activity of myosin from *Limulus* increases when the light chains are phosphorylated by an endogenous kinase. The kinase is activated by calmodulin [72].

The striated muscle of scallop represents an example of a myosin that cannot be phosphorylated [73], but smooth muscle myosin from scallop seems to be phosphorylated, like other myosins [74].

5. POSSIBLE RULES AND EVOLUTIONARY TRENDS OF MYOSIN PHOSPHORYLATION

On the basis of the data presented above the effects of myosin phosphorylation are discussed in terms of possible rules and evolutionary trends.

(1) Reversible phosphorylation of myosin subunits seems to occur in all eukaryotic cells except striated muscle of some mollusca. In amoebae and slime molds the main subunit that is phosphorylated is a myosin heavy chain. On the other hand, in muscle and some vertebrate non-muscle cells only the light chains are phosphorylated. The change of location of the phosphorylation sites seems to be one of the major evolutionary events during specialization of regulatory mechanisms of contraction and motility. If this is true, non-muscle cells in which phosphorylation of both light chains and heavy chains is observed may represent the transitional step of these evolutionary changes. There is no direct evidence that such cells contain one type of myosin which is phosphorylated in two different ways. It is possible that these cells contain two myosin isoenzymes: one phosphorylated on the heavy chains and another phosphorylated on the light chains.

(2) Regulatory light chains of muscle and non-muscle myosins are phosphorylated by a specific protein kinase which is activated by Ca^{2+}-calmodulin. Heavy chains of myosin from *Dictyostelium* seem to be phosphorylated by a kinase that is inhibited by Ca^{2+}-calmodulin. It may be speculated that the heavy chain kinases responsible for inactivation of other myosins are also inhibited by calmodulin.

(3) Regulatory light chains of muscle myosins are located near the hinge region between the heads and the tails. It is likely that phosphorylatable light chains of non-muscle myosins have a similar location. Phosphorylation sites on the heavy chains of four myosins tested so far are located at the end of the myosin tail (table 1). It is probable that phosphorylation sites on the heavy chains of the other myosins are located at the homologous position.

(4) Actin-activated ATPase activity of smooth muscle and non-muscle myosins is regulated by the state of phosphorylation of either heavy chains and/or light chains. Phosphorylation of the light chains stimulates the actomyosin ATPase activity of all myosins tested. Phosphorylation of the heavy chains inhibits actomyosin ATPase activity in all cases except one. If light chains and heavy chains of the same myosin molecule are phosphorylated, then it would be reasonable to expect that only one of four forms of such a myosin will be enzymatically active, as shown by leukemic myeloblast myosin.

(5) The level of myosin phosphorylation seems to affect the V_{max} of the ATPase to a greater extent than the affinity of myosin for actin. This may indicate that regulation by reversible phosphorylation operates according to the kinetic model and not the steric blocking model. The new information suggests that other regulatory systems, such as troponin-tropomyosin in striated muscle [75] and Ca^{2+} binding to myosin from muscle of scallop also seem to operate according to the kinetic model [76]. It may be speculated, therefore, that the basic mechanisms of ATP hydrolysis by myosin and actomyosin have not changed during evolution and that all regulatory systems have had to adjust to these mechanisms.

(6) It appears that the state of phosphorylation of heavy chains or light chains defines polymerising properties of myosin in vitro in such a way that only one species forms stable filaments in the presence of ATP and at physiological salt concentrations. Different stabilities of myosin filaments may reflect changes in intramolecular and intermolecular interactions within these structures. Dephosphorylation of light chains induces folding of myosin tails and consequently either depolymerises existing filaments or inhibits their formation. Folding of myosin tails has also been observed for myosins with phosphorylatable heavy chains, e.g. *Physarum* myosin. However, the mechanisms of regulation of filament assembly seem to be different from those that occur after light chain phosphorylation. The following observations indicate that heavy chain phosphorylation may inhibit primarily formation of antiparallel dimers. First, the phosphorylatable sites are located within the regions which take part in dimer formation. Second, the example of *Physarum* myosin which forms only one-polar oligomer in the dephosphorylated form and bipolar filaments in the phosphorylated form. Third, the example of

Acanthamoeba myosin II, which after proteolytic removal of the regulatory sites is unable to polymerise and forms only monomers and one-polar dimer.

(7) Two properties of myosin, actin-activated ATPase activity and stability of filaments, appear to be tightly coupled in vitro. Myosins from amoebae and slime molds form stable filaments in a form which has higher actomyosin ATPase activity (dephosphorylated heavy chains of *Acanthamoeba* and *Dictyostelium* myosin and phosphorylated heavy chains of *Physarum* myosin). Similarly, smooth muscle and non-muscle myosins form stable filaments when their light chains are phosphorylated, i.e. when their ATPase activity is actin-activatable. It appears, as postulated earlier by other authors, that only myosin forms which have high ATPase activity form stable filaments in vitro, irrespective of their origin.

(8) The coupling rule is not always supported when studies are carried out in vivo. For instance, actin-activated ATPase activity and filament formation of smooth muscle myosin are coupled only in vitro and not in vivo. This observation may be explained in at least two ways. One possibility is that interaction of dephosphorylated myosin with actin filaments stabilizes smooth muscle thick filaments. Another is that a hypothetical protein keeps filaments assembled in situ after myosin dephosphorylation.

Evidence from studies in vivo suggests that the transient assembly and disassembly of myosin filaments takes place in amoebae. It may be suggested therefore that in primitive motile systems the coupling of actomyosin ATPase activity and filament formation via heavy chain phosphorylation does exist. Whether the similar mode of action via light chain phosphorylation may operate in non-muscle cells in vivo remains to be established.

(9) The data from experiments in vitro may indicate that phosphorylation has a dual effect on myosin. The primary effect seems to be a regulation of myosin polymerisation. Assembly of bipolar filaments brings together a large number of ordered myosin heads into the proximity of actin filaments, which is important for efficient motility and contraction. It may be suggested that this primary effect of phosphorylation operates in the motile systems in which a transient assembly of myosin takes place.

Secondary effects of phosphorylation are probably based on the intermolecular and intramolecular interactions within the filaments between phosphorylation sites and hinge regions. The interactions define the conformation of myosin filaments and hence the level of actin-activated ATPase activity. The nature of the active or inactive configuration is not known in molecular detail, but is likely to involve different orientations and flexibility of myosin heads. In the case of heavy chain phosphorylation interaction between the hinge region and phosphorylation sites of aggregated myosin may take place only among different molecules. Therefore, maximal actomyosin ATPase activity is observed when myosin is filamentous, as shown for instance by *Acanthamoeba* myosin II. In the case of light chain phosphorylation interaction between regulatory sites and the hinge region may take place in two distinct ways: intramolecularly and intermolecularly. In the filaments, both types of interaction may occur and both may have relevance in situ. Intramolecular interactions seem to be sufficient in vitro for regulation of the soluble myosin fragments that contain a hinge region.

6. CONCLUSIONS

The rules which may govern the process of myosin phosphorylation in smooth muscle and non-muscle cells can be summarized in the form of the following hypothesis. During specialization of motile systems a change in location of phosphorylation sites took place: in the more primitive motile systems the regulatory sites are located on the heavy chains and in the more specialized systems on the light chains. It may be suggested that the change did not alter three major features of myosin phosphorylation.

First, Ca^{2+} regulation mediated by calmodulin remained, i.e. not only the light chain kinases, but also the heavy chain kinases are calmodulin-dependent.

Second, a role of phosphorylation in regulation of myosin contractile activity was preserved.

Third, a dual mode of action of phosphorylation was maintained. The state of phosphorylation regulates myosin assembly and, through interactions within the filaments, reactivity of myosin heads.

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REFERENCES

[1] Korn, E.D. (1978) Proc. Natl. Acad. Sci. USA 75, 588–599.
[2] Pollard, T.D. (1981) J. Cell Biol. 91, 156–165.
[3] Goody, R.S. and Holmes, K.C. (1983) Biochim. Biophys. Acta 726, 13–39.
[4] Korn, E.D. (1982) Physiol. Rev. 62, 672–737.
[5] Oosawa, F. (1983) in: Muscle and Nonmuscle Motility (Stracher, A. ed.) vol.1, pp.151–216, Academic Press, New York.
[6] Harrington, W.F. and Rodgers, M.E. (1984) Annu. Rev. Biochem. 53, 35–73.
[7] McLachlan, A.D. (1984) Annu. Rev. Biophys. Bioeng. 13, 167–189.
[8] Adelstein, R.S. and Eisenberg, E. (1980) Annu. Rev. Biochem. 49, 921–956.
[9] Kendrick-Jones, J. and Scholey, J.M. (1981) J. Muscle Res. Cell Motil. 2, 347–372.
[10] Scholey, J.M., Taylor, K.A. and Kendrick-Jones, J. (1981) Biochimie 63, 255–271.
[11] Cooke, R. and Stull, J.T. (1981) in: Cell and Muscle Motility (Dowben, R.M. and Shay, J.W. eds) vol.1, pp.99–133, Plenum, New York.
[12] Côté, G.P., Collins, J.H. and Korn, E.D. (1981) J. Biol. Chem. 256, 12811–12816.
[13] Collins, J.H., Côté, G.P. and Korn, E.D. (1982) J. Biol. Chem. 257, 4529–4534.
[14] Collins, J.H. and Korn, E.D. (1980) J. Biol. Chem. 255, 8011–8014.
[15] Collins, J.H., Kuźnicki, J., Bowers, B. and Korn, E.D. (1982) Biochemistry 21, 6910–6915.
[16] Kuźnicki, J., Albanesi, J.P., Côté, G.P. and Korn, E.D. (1983) J. Biol. Chem. 258, 6011–6014.
[17] Kuźnicki, J., Côté, G.P., Bowers, B. and Korn, E.D. (1985) J. Biol. Chem. 260, 1967–1972.
[18] Kiehart, D.P. and Pollard, T.D. (1984) Nature 308, 864–866.
[19] Kuźnicki, J., Atkinson, M.A.L. and Korn, E.D. (1984) J. Biol. Chem. 259, 9308–9313.
[20] Rahmsdorf, H.J., Malchow, D. and Gerisch, G. (1978) FEBS Lett. 88, 322–326.
[21] Kuczmarski, E.R. and Spudich, J.A. (1980) Proc. Natl. Acad. Sci. USA 77, 7292–7296.
[22] Peltz, G., Kuczmarski, E.R. and Spudich, J.A. (1981) J. Cell Biol. 89, 104–108.
[23] Pagh, K., Maruta, H., Claviez, M. and Gerisch, G. (1984) EMBO J. 3, 3271–3278.
[24] Maruta, H., Baltes, W., Dieter, P., Marme, D. and Gerisch, G. (1983) EMBO J. 2, 535–542.
[25] Yumura, S. and Fukui, Y. (1985) Nature 314, 194–196.
[26] Reines, D. and Clarke, M. (1985) J. Biol. Chem. 260, 14248–14254.
[27] Malchow, D., Bohme, R. and Rahmsdorf, H.J. (1981) Eur. J. Biochem. 117, 213–218.
[28] Ogihara, S., Ikebe, M., Takahashi, K. and Tonomura, Y. (1983) J. Biochem. 93, 205–223.
[29] Takahashi, K., Ogihara, S., Ikebe, M. and Tonomura, Y. (1983) J. Biochem. 93, 1175–1183.
[30] Muhlard, A. and Oplatka, A. (1977) FEBS Lett. 77, 37–40.
[31] Trotter, J.A. and Adelstein, R.S. (1979) J. Biol. Chem. 254, 8781–8785.
[32] Trotter, J.A. (1982) Biochem. Biophys. Res. Commun. 106, 1071–1077.
[33] Trotter, J.A., Nixon, C.S. and Johnson, M.A. (1985) J. Biol. Chem. 260, 14374–14378.
[34] Fechheimer, M. and Cebra, J.J. (1982) J. Cell. Biol. 93, 261–268.
[35] Fechheimer, M. and Zigmond, S.H. (1983) Cell Motil. 3, 349–361.
[36] Matsumura, S., Murakami, N., Yasuda, S. and Kumon, A. (1982) Biochem. Biophys. Res. Commun. 108, 1595–1600.
[37] Baryłko, B. and Sobieszek, A. (1983) EMBO J. 2, 369–374.
[38] Murakami, N., Matsumura, S. and Kumon, A. (1984) J. Biochem. 95, 651–660.
[39] Baryłko, B., Tooth, P. and Kendrick-Jones, J. (1986) Eur. J. Biochem., in press.
[40] Sagara, J., Nagata, K. and Ichikawa, Y. (1982) J. Biochem. 92, 1845–1851.
[41] Sagara, J., Nagata, K. and Ichikawa, Y. (1983) Biochem. J. 214, 839–843.
[42] Adelstein, R.S., Conti, M.A. and Anderson, W. (1973) Proc. Natl. Acad. Sci. USA 70, 3115–3119.
[43] Scordilis, S.P., Anderson, J.L., Pollack, R. and Adelstein, R.S. (1977) J. Cell Biol. 74, 940–949.
[44] Scordilis, S.P. and Adelstein, R.S. (1977) Nature 268, 558–560.
[45] Yerna, M.-J., Dąbrowska, R., Hartshorne, D.J. and Goldman, R.D. (1979) Proc. Natl. Acad. Sci. USA 76, 184–188.

[46] Martin, F., Gabrion, J. and Cavadore, J.-C. (1981) FEBS Lett. 131, 235–238.
[47] Scholey, J.M., Smith, R.C., Drenckhahn, D., Gröschel-Stewart, U. and Kendrick-Jones, J. (1982) J. Biol. Chem. 257, 7737–7745.
[48] Craig, R., Smith, R. and Kendrick-Jones, J. (1983) Nature 302, 436–439.
[49] Olins, G.M. and Bremel, R.D. (1982) Endocrinology 110, 1933–1938.
[50] Keller, T.C.S. and Moosaker, M.S. (1982) J. Cell Biol. 95, 943–959.
[51] Broschat, K.O., Stidwill, R.P. and Burgess, D.R. (1983) Cell 35, 561–571.
[52] Kanda, K., Sobue, K. and Kakiuchi, S. (1985) J. Biochem. 97, 961–964.
[53] Langanger, G., Moeremans, M., Daneels, G., Sobieszek, A., De Brabander, M. and De Mey, J. (1986) J. Cell Biol. 102, 200–209.
[54] Karlsson, R. and Lindberg, U. (1985) Exp. Cell Res. 157, 95–115.
[55] Walsh, M.P. and Hartshorne, D.J. (1982) in: Calcium and Cell Function (Cheung, W.Y. ed.) vol.3, pp.223–269, Academic Press, New York.
[56] Ikebe, M. and Hartshorne, D.J. (1985) Experientia 41, 1006–1010.
[57] Hartshorne, D.J. (1982) in: Cell and Muscle Motility (Dowben, R.M. and Shay, J.W. eds) vol.2, pp.185–220, Plenum, New York.
[58] Murphy, R.A. (1982) Hypertension (suppl.II), 4, II-3–II-7.
[59] Sobieszek, A. (1977) in: The Biochemistry of Smooth Muscle (Stephens, N.L. ed.) pp.413–443, University Park Press, Baltimore.
[60] Górecka, A., Aksoy, M.O. and Hartshorne, D.J. (1976) Biochem. Biophys. Res. Commun. 71, 325–331.
[61] Ikebe, M., Ogihara, S. and Tonomura, Y. (1982) J. Biochem. 91, 1809–1812.
[62] Persechini, A. and Hartshorne, D.J. (1983) Biochemistry 22, 470–476.
[63] Sellers, J.R., Chock, P.B. and Adelstein, R.S. (1983) J. Biol. Chem. 258, 14181–14188.
[64] Sellers, J.R., Eisenberg, E. and Adelstein, R.S. (1982) J. Biol. Chem. 257, 13880–13883.
[65] Suzuki, H., Onishi, H., Takahashi, K. and Watanabe, S. (1978) J. Biochem. 84, 1529–1542.
[66] Trybus, K.M., Huiatt, T.W. and Lowey, S. (1982) Proc. Natl. Acad. Sci. USA 79, 6151–6155.
[67] Trybus, K.M. and Lowey, S. (1984) J. Biol. Chem. 259, 8564–8571.
[68] Ohnishi, H. and Wakabayashi, T. (1984) J. Biochem. 95, 903–905.
[69] Ikebe, M. and Hartshorne, D.J. (1984) J. Biol. Chem. 259, 11639–11647.
[70] Somlyo, A.V., Butler, T.M., Bond, M. and Somlyo, A.P. (1981) Nature 294, 567–570.
[71] Tsukita, S., Tsukita, S., Usukura, J. and Ishikawa, H. (1982) Eur. J. Cell Biol. 28, 195–201.
[72] Sellers, J.R. and Harvey, E.V. (1984) Biochemistry 23, 5821–5826.
[73] Kendrick-Jones, J., Cande, W.Z., Tooth, P.J., Smith, R.C. and Scholey, J.M. (1983) J. Mol. Biol. 165, 139–162.
[74] Sohma, H., Yazawa, M. and Morita, F. (1985) J. Biochem. 98, 569–572.
[75] Chalovich, J.M. and Eisenberg, E. (1982) J. Biol. Chem. 257, 2432–2437.
[76] Chalovich, J.M., Chantler, P.D., Szent-Györgyi, A.G. and Eisenberg, E. (1984) J. Biol. Chem. 259, 2617–2621.