Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 2060–2064, April 1984
Biophysics

On the mechanism of energy transduction in myosin subfragment 1

(fluorescence resonance energy transfer/domains/influence transmission)

JEAN BOTTS, REIJI TAKASHI, PETER TORGERSON, TETSU HOZUMI*, ANDRAS MUHLRAD†, DOMINIQUE MORNET, AND MANUEL F. MORALES

Cardiovascular Research Institute, University of California, San Francisco, CA 94143

Contributed by Manuel F. Morales, December 30, 1983

ABSTRACT **It is proposed that the myosin subfragment 1 moiety of the muscle contractile apparatus is a self-contained "engine" whose operational plan is based on the interactive nature of ATP (or degradation intermediate) binding and actin binding, made possible by an intersite communication system. It is suggested that the spatial information required for examining this engine can, at least provisionally, be derived from fluorescence resonance energy transfer measurements interpreted by the Förster equation and that the existence of an intersite communication system can be deduced from piecewise detection of interacting pairs of points.**

A Theory of How the Myosin Subfragment 1 (S-1) Engine Works

It has been thought that myosin S-1 moieties in active muscle fibers, pivoting about their attachments to subfragment 2 (S-2) moieties, deliver mechanical impulses to adjacent actin filaments, thus producing the relative interfilament translation ("slide") that characterizes isotonic muscle contraction (see ref. 1 for a review). Since there is no evidence that the *gross* shape (as distinct from local conformation) of S-1 changes during interaction with nucleotides (2) or actin (3), it is plausible to think that in its "purposeful" motion, as in its thermal motion, the cross-bridge rotates about S-1/S-2 as a quasirigid body. Because S-1 is elongate, a natural positional parameter is the angle that its "long" axis makes with the filament axis (4). There are now investigations showing that during isometric activity (*i*) this angle fluctuates (5) and (*ii*) the stationary value of this angle is quite different from what it is in rigor (6). Also there are investigations showing that (*iii*) in response to a step change in length a positional parameter of the cross-bridge relaxes (7). Furthermore, the motional frequencies observed in *i* and *iii* are what might be expected from independent biochemical (8) and mechanical (9) observations and are magnitudes slower than thermally activated frequencies. Thus points *i–iii* support the idea that during activity cross-bridges *move*. They also suggest that the motion is describable by angles that position S-1 relative to the fiber axis (10), but in what follows it is necessary only that positional parameters relating S-1 to actin change with time repetitively.

It is known that each S-1 bears an ATPase (N) site separate from the site (A) at which it binds actin (11) and that binding at these sites is interactive (12, 13). So it is tempting to think that the observed motions of S-1 are not those of an appendage passively moved by another agency, but rather that S-1 is a self-contained "unitary engine" strategically placed in the muscle apparatus. Previously we have elaborated on this hypothesis (14). We have noted that to examine this hypothesis we must look at common knowledge about S-1 in an uncommon way. For example, instead of considering S-1 motion in a fiber-based coordinate system we should position ligands (actin, nucleotides) in an S-1-based system, and we should think of enzymatic intermediates (15) as sequentially bound N-site ligands. These simple reinterpretations suggest a plan for the S-1 "engine": Because of the intersite communication system, binding of a particular intermediate at the N site determines a particular situation at the A site—namely, the values of the positional parameters describing bound actin (e.g., certain angles, if rigidity is assumed). At the N site the virtually unidirectional temporal sequence of occupants is imposed by the net free energy of ATP hydrolysis; at the A site the virtually invariant sequence of events (attach, move, detach, etc.) that we call a "work cycle" is ensured by the tightness of the communication system. From such a picture certain predictions follow. One is that during an active steady state there should be a high degree of cross-correlation between fluctuations in the concentration of enzymatic intermediates and fluctuations in the orientation of S-1 moieties. Another—to be pursued here—is that there should exist an intersite communication system.

Internal Structure of the Engine

Examining S-1 for the existence of an intersite communication system presupposes knowing the three-dimensional arrangement of the sites and what lies between them. This prerequisite information will ultimately be deduced from crystallography, but a rough version of it, deduced by other means, exists now.

Rabbit myosin S-1 consists of three polypeptide chains of known sequence (16–19): heavy chain, HC, which on its own can bind actin and conduct ATPase (20, 21); a light chain, LC_2, containing a phosphorylatable serine residue (Ser-15) and tightly bound Mg^{2+}; and another light chain, LC_1 or LC_3. LC_3 lacks the first 41 residues of LC_1, and each contains one thiol, Cys-177 and Cys-177′, respectively.

Following the use of trypsin by Balint *et al.* (22), we have shown (23) that proteases of diverse specificity cleave HC to form three fragments, with molecular masses of approximately [within 2 kilodaltons (kDa)] 20, 27, and 50 kDa, termed "20K," "27K," and "50K," respectively. In the intact protein the fragments are arranged as NH_2-27K-50K-20K-COOH. Moreover 20K (and possibly 50K) isolated from, and then rid of, denaturing solvents retains properties of intact S-1 [helicity and ability to bind actin and inhibit acto-(S-1) ATPase] (24). These facts suggest that, in native

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: S-1 and S-2, subfragments 1 and 2; HC, heavy chain; LC, light chain; kDa, kilodalton(s); FRET, fluorescent resonance energy transfer; RLR, reactive lysine residue; TNP, 2,4,6-trinitrophenyl.

*Permanent address: Department of Physiology, Nagoya City University Medical School, Nagoya, Japan.
†Permanent address: Department of Oral Biology, Hebrew University–Hadassah School of Dental Medicine, Jerusalem, Israel.

S-1, 27K, 50K, and 20K are probably sovereign domains joined by thin connectors (I and II) of about 2 kDa each. Several reactive residues and other functionalities have been located on these putative domains.

Bound actin protects HC from proteolysis at connector II (25), and proteolysis at connector II lowers the actin affinity of HC (26). Crosslinking with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide shows that the first (NH_2-terminal) 12 residues of actin (27), but possibly other regions as well (28), bind to either side of connector II. (It remains undecided whether in three dimensions the two binding regions of S-1 form a single site for binding one actin or two sites for different actins.) The carbodiimide-mediated union of actin and S-1 has a natural character, since the Mg^{2+}-ATPase of the complex is even more accelerated than that of the normal complex (29). Binding of nucleotide to the N site permits trypsin [but not other proteases tested (23)] to cleave off a 5-kDa NH_2-terminal segment of 27K and a 5-kDa COOH-terminal segment of 50K (30). Experiments with photoaffinity labels (31, 32) show that at least the adenosine moiety of ATP binds to 27K. Analysis of homologies (33) suggests further that residues 165–193‡ are involved in this binding. The ATP-perturbable tryptophan(s) has been classified spectroscopically to be among the "buried" residues (34) but has not been identified. Tryptophans 112 and 130 are candidates (19).

In the absence of actin, LC_1 (or LC_3) appears to bind HC at both domains 27K and 20K; in the presence of actin the 27K union is lost (35). Fig. 1 summarizes information derived biochemically.

Several investigators have, for various purposes, derivatized pairs of groups on S-1 [or acto-(S-1)] with fluorescent reagents or quenchers, measured fluorescent resonance energy transfer (FRET) between attached moieties, and, by application of the Förster equation, inferred the scalar distance between moieties. For us the purpose has been to see whether FRET distance measurements can be assembled in such a way as to provide information about the three-dimensional arrangement of landmark groups (actually, of dye or quencher moieties attached to such groups) in acto-(S-1). This approach to S-1 structure—which is undoubtedly crude and provisional—is fraught with many difficulties, but mention of only two of these seems appropriate in a general report. There is a difficult decision that must be made in each FRET measurement: is it valid to assume that κ^2 (a function of the angles relating the orientation of one dye dipole to the other) is $\frac{2}{3}$? There is also the difficulty of measuring enough distances to determine the spatial arrangement of, say, n points (or attached moieties). Concerning the uncertainty in κ^2, we have thought it best to apply the Dale–Eisinger analysis and make the appropriate time-resolved anisotropy decay measurements required to find the minimum and maximum values that κ^2 could have—i.e., to find the range of uncertainty surrounding the assumption $\kappa^2 = \frac{2}{3}$. These matters are treated elsewhere (36). It has to be conceded, however, that the equipment and programs necessary for the Dale–Eisinger analysis have only recently become available to us, so it is only for our most recent FRET measurements that we know the uncertainty range about $\kappa^2 = \frac{2}{3}$. Of the other authors to be quoted only two groups have reported this experimentally based uncertainty range. Concerning the adequacy of the number of distances that have been measured, authors such as Langer *et al.* (37), or Cantor and Schimmel (38), have stated without proof§ that to determine the space lattice (or its mirror image) formed by n points the requisite number of distances to be measured is $D = 4n - 10$. There are two features of our literature citations that should be considered in gauging the tentativeness of our results. The citations refer to work that we consider reliable, but, while many are full papers, some are preliminary reports (by others) on which there has been no follow-up. Additionally, in this paper we are reporting several new observations that will only later be reported *in extenso*. Finally, it should be noted that in several instances the inability to observe any energy transfer between labeled groups has been taken to mean that the distance between groups is too great (say, >6 or 7 nm). These conclusions are probably correct, but at least logically one should consider that also the distance could be short and the dipole disadvantageously oriented. Despite these many vicissitudes the FRET measurements can be reconciled in a space lattice (Fig. 2). If distances reported only as "too far" are counted, this lattice is nearly determined, since $D = 4(8) - 10 = 22$, and actually 20 distances have been measured. Note that of the 14 measured distances and 6 too-far distances, some have been measured on free S-1 and some on ligated S-1. It is not obvious that distances in S-1 are invariant to ligation, but where tested the changes have usually been small.

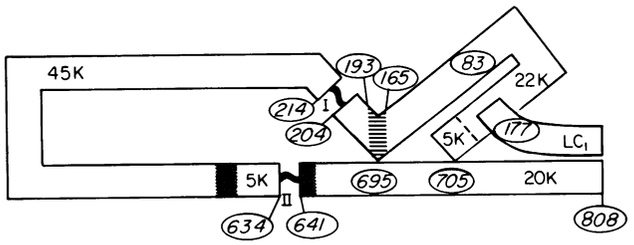

FIG. 1. Chemical organization of S-1. As noted in the text, three putative domains—27K, 50K, and 20K—are joined by two connectors (I and II) of *ca.* 2 kDa each. In the presence of MgADP, trypsin additionally cleaves off *ca.* 5-kDa segments from 27K and 50K, so these domains are shown as 22K + 5K and 45K + 5K. Stippling indicates regions at which actin binds, and hatching indicates the region at which adenosine is expected to bind on the basis of sequence homology. The numeration (circled italic numbers) is due to the sequence determinations of Elzinga and co-workers for HC (18, 19) and of Frank and Weeds for LC_1 (16). Residue 83 is the reactive lysine residue (RLR), residue 695 bears a thiol (SH_2), residue 705 bears another thiol (SH_1), and residue 177 bears the unique thiol of LC_1 (or LC_3).

Even at this stage some structural features are evident. Although the actin-myosin interface (A site) has not yet been labeled, it does not seem likely that it will be "within chemical reaction distance" from the N site. This was very early surmised by Barany and Barany (11). It is probably the feature that holds the clue concerning the coupling mechanism (see below). Another feature of interest is the relation of SH_1 to the N site because modification of the former has a profound effect on catalysis at the latter. We, on the basis of modification experiments (48), and Tao and Lamkin, on the

‡Walker *et al.* (33), comparing the sequences of F_1-ATPase and those of other proteins, associated two sequences, "A" and "B," with adenine binding. The residues indicated in the text above correspond to sequence A. There is some evidence that a sequence homologous to B lies adjacent to A in 27K. The peptide reported by Okamoto and Yount (32) as being labeled by their photoaffinity reagent extends from residue 125 to residue 134.

§The reasoning is instructive because it points up an ambiguity in all "distance mapping": To specify lattices of 1, 2, 3, and 4 points one requires the *additional* measurement of, respectively, 0, 1, 2, and 3 distances—i.e., a total of six distances. In placing the 4th point there is an unresolvable ambiguity (is it "above" or "below" the triangle formed by the previous 3?). After one or the other possibility is accepted, the 5th and all subsequent points require four distances each for placement. To set $D = 4n$ would imply that 16 distances are required to place the first 4 points, but this would be an overestimate of $16 - 6 = 10$, so the correct formula for $n > 4$ is $D = 4n - 10$.

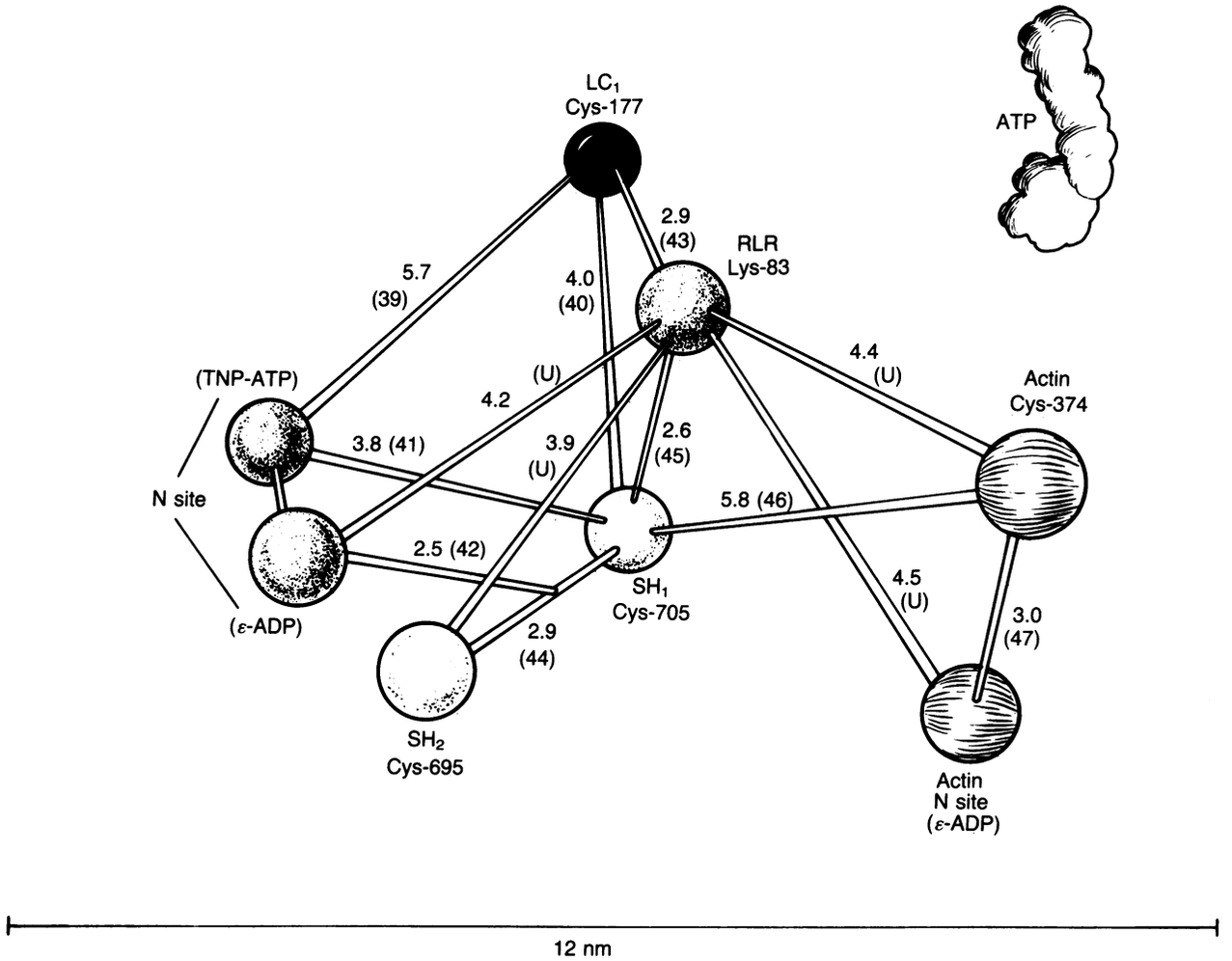

FIG. 2. Space lattice constructed from interpoint distances obtained by FRET measurements. The numbers beside the connecting rods are the distances in nanometers, and the numbers in parentheses are the literature sources (U, unpublished data). For reference we have included, using the same scale, a sketch of ATP and a line 12 nm long; the latter is the maximum chord in S-1 (3). ε-ADP, 1,N^6-ethenoadenosine 5′-diphosphate; TNP-ATP, 2′(3′)-(2,4,6-trinitrophenyl)-ATP. The distance from the ring (ε) modification to the TNP group on the ribose of ATP was estimated on a CPK model. Distances that are known to be too long for FRET measurements are LC_1 Cys-177 to actin N (unpublished data), LC_1 Cys-177 to actin Cys-374 (unpublished data), TNP-ATP to actin N site,* SH_1 to actin N site,* TNP-ATP to actin Cys-374,* and LC_1 Cys-177 to SH_2 (unpublished data).

*Cooke, R. & dos Remedios, C. G., 12th International Congress of Biochemistry, Aug. 15–20, 1982, Perth, Australia, abstr. POS 004-230.

basis of the SH_1–N distance (41), have thought that also these groups are not within chemical reaction distance of each other. The effect of SH_2 reaction on ATPase, however, may well be distance based.

Investigating How the Engine Works

Examining the A ⇄ N communication system and deducing its physical basis would be quite difficult even in the presence of detailed crystallographic knowledge of S-1 (see ref. 49 for an analogous problem). One must therefore be prepared for a mixture of surmise and crude experimental approach. Accepting such evidence as above (or outright assuming) that A and N are not within chemical bond-forming range of each other, but are separated by 1–2 nm, one must consider *a priori* that A events and N events are interactive because (*i*) the fields that they generate are interactive or (*ii*) they are capable of distorting the polypeptide structure intervening between them. Electrostatic fields undoubtedly play important roles in the binding of both the actin and nucleotide ligands, and, at distances of 1 or 2 nm, they could also serve in intersite communications at low ionic strength. However, it is hard to see how such fields could mediate substrate specificity (e.g., the difference between ADP and PP_i binding at the N site). So, at least provisionally, we favor mechanism *ii*. Once alternative *ii* is chosen, the question becomes, can a "path of influence" from one site to the other be demonstrated? Logically, demonstration would consist in being able to detect "effect" along a preferential continuous path joining A and N, when ligand binding occurs at either site. The energy transmitted along this path as a result of a binding event at one of the sites would decay with distance, but the distance dependence of other effects—e.g., displacement—would be unpredictable. Conceivably, disturbances applied to intermediate points of the path might also cause an effect to propagate towards both sites. While crystallographers may in the future be able to detect atomic displacements along such a path, we must for now resort to measuring much less direct effects—indeed, to measuring whatever we can detect. Fortunately, in this generalized sense many effects have been measured, usually as spin-offs from investigations with different objectives. In Fig. 3 we have tried to place this data spatially, guided by the limited knowledge reported in the previous section. Our attempt is handicapped by several shortcomings, especially ignorance about the spatial location of certain proteolytic cuts, but it

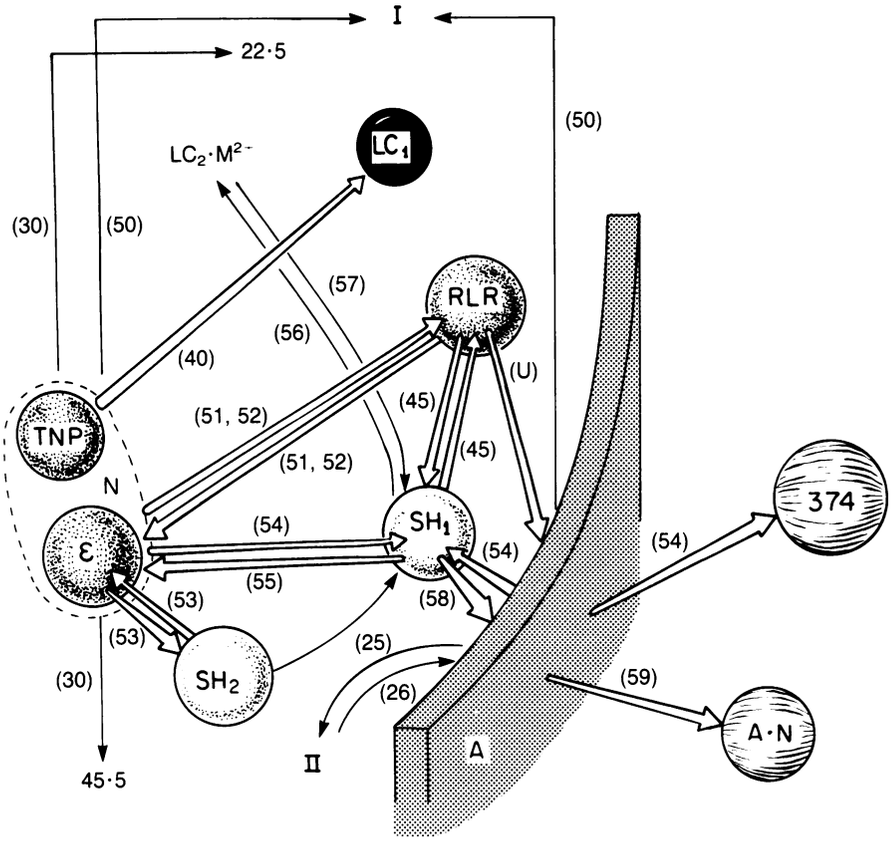

FIG. 3. Map of demonstrated influences. The disposition of labeled sites is as in Fig. 2, and there are other inclusions: An envelope has been drawn about the ε modification and the TNP chromophore; collectively they represent the N site. A surface containing the actin-(S-1) interface (A site) has been included, but its location is only approximate, because this actual surface has not been labeled. Stick arrows indicate that it has been shown (the references are in parentheses) that an event at the arrow tail affects an event at the arrow head. Line arrows have a similar meaning, but involve points not yet located in Fig. 2. The notation "22·5" etc. means the boundary at which a 5-kDa piece is formed by cleavage with trypsin (see Fig. 1 legend). M^{2+}, a cation.

also brings forth a few suggestive features. One is that effect is not transmitted isotropically, but highly anisotropically. For example, the distances from RLR to SH_1 and to LC_1 Cys are rather similar (see Fig. 2), but while the first pair of points is strongly interactive the second pair is not. Anisotropy would be expected, of course, if effect is transmitted through polypeptide chains, since chain folding is unlikely to be isotropic. Fig. 3 reports quite a few instances of transmission of effect, but only one continuous two-way path is evident between A and N sites. This path passes through SH_1. This circumstance could be accidental (resulting from the paucity of points studied), or it could indicate that the path noted is at least near to our hypothetical path of intersite transmission. A related suggestion emerging from Fig. 3 concerns the phenomenon that in the older literature was called "modification"—e.g., the influence on nucleoside triphosphatase activity of chemically reacting SH_1. Numerous experiments—many from our laboratory (60–62)—have indicated that the changes in ATPase do not result directly from the reaction of SH_1 but rather from a regional change in conformation that can be caused, among other ways, by allowing SH_1 to react. Since it is now known that somewhat analogous changes are caused by allowing RLR to react (51, 52), it seems likely that the conformationally sensitive region includes both SH_1 and RLR. Stone and Prevost (63) have called attention to certain suggestive analogies between modification and acceleration of Mg^{2+}-ATPase by actin. Fig. 3 suggests a structural basis for explaining why actin binding and SH_1 or RLR modification might have similar effects at the N site, since the modifiable region could lie astride the intersite communication path.

In summary: We suggest that the myosin S-1 moiety is a micro-engine that accomplishes chemomechanical transduction because the binding of each nucleotide (hydrolytic) intermediate at the N site imposes a conformation that, transmitted through the polypeptide structure, imposes a corresponding conformation at the remote A site, thus deciding the spatial disposition of the bound actin. In an effort to explore the foregoing hypothesis we have used many FRET measurements, translated into interpoint distances by the Förster equation, to construct a three-dimensional lattice of points in a relevant region of S-1. Finally, we have studied pairwise interpoint interactions to trace the possible path of influence from the N site to the A site. Preliminary study suggests that this path may pass through the thiol of Cys-705 (SH_1) in the 20K domain.

This research was supported by grants from the U.S. Public Health Service (HL-16683), the National Science Foundation (PCM-79-22174), and the American Heart Association (CI-8). P.T. and D.M. are Fellows of the Muscular Dystrophy Association. T.H. and A.M. are Visiting Scientists supported by a grant from the U.S. Public Health Service (HL-16683). M.F.M. is a Career Investigator of the American Heart Association.

1. Morales, M. F., Borejdo, J., Botts, J., Cooke, R., Mendelson, R. A. & Takashi, R. (1982) *Annu. Rev. Phys. Chem.* **33,** 319–351.
2. Mendelson, R. M., Putnam, S. & Morales, M. F. (1975) *J. Supramol. Struct.* **3,** 162–168.
3. Kretzschmar, K. M. & Mendelson, R. M. (1980) *Biochemistry* **19,** 4103–4108.
4. Mendelson, R. M., Morales, M. F. & Botts, J. (1973) *Biochemistry* **12,** 2250–2255.

5. Borejdo, J., Putnam, S. V. & Morales, M. F. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6346–6350.
6. Burghardt, T. P., Ando, T. & Borejdo, J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7515–7519.
7. Huxley, H. E., Simmons, R. M., Faruqi, A. R., Kress, M., Bordas, J. & Koch, M. H. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2297–2301.
8. Takashi, R. & Putnam, S. V. (1979) *Anal. Biochem.* **92,** 375–382.
9. Huxley, A. F. & Simmons, R. M. (1971) *Nature (London)* **233,** 533–538.
10. Morales, M. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 145–149.
11. Barany, M. & Barany, K. (1959) *Biochim. Biophys. Acta* **35,** 293–309.
12. Szent-Gyorgyi, A. (1947) *Chemistry of Muscle Contraction* (Academic, New York).
13. Kiely, B. & Martonosi, A. N. (1968) *J. Biol. Chem.* **243,** 2273–2278.
14. Morales, M. F. & Botts, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3857–3859.
15. Trentham, D. R., Eccleston, J. F. & Bagshaw, C. R. (1976) *Q. Rev. Biophys.* **9,** 217–281.
16. Frank, G. & Weeds, A. G. (1974) *Eur. J. Biochem.* **44,** 317–334.
17. Collins, J. H. (1976) *Nature (London)* **259,** 699–700.
18. Gallagher, M. & Elzinga, M. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39,** 2168 (abstr.).
19. Tong, S. W. & Elzinga, M. (1983) *J. Biol. Chem.* **258,** 13100–13110.
20. Wagner, P. D. & Giniger, E. (1981) *Nature (London)* **292,** 560–562.
21. Sivaramakrishnan, M. & Burke, M. (1982) *J. Biol. Chem.* **257,** 1102–1105.
22. Balint, M., Wolf, I., Tarcsafalvi, A., Gergely, J. & Sreter, F. A. (1978) *Arch. Biochem. Biophys.* **190,** 793–799.
23. Mornet, D., Ue, K. & Morales, M. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 736–739.
24. Muhlrad, A. & Morales, M. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1003–1007.
25. Mornet, D., Bertrand, R., Pantel, P., Audemard, E. & Kassab, R. (1979) *Biochem. Biophys. Res. Commun.* **89,** 925–932.
26. Botts, J., Muhlrad, A., Takashi, R. & Morales, M. F. (1982) *Biochemistry* **21,** 6903–6905.
27. Sutoh, K. (1982) *Biochemistry* **21,** 3654–3661.
28. Mornet, D., Bertrand, R., Pantel, P., Audemard, E. & Kassab, R. (1981) *Biochemistry* **20,** 2110–2120.
29. Mornet, D., Bertrand, R., Pantel, P., Audemard, E. & Kassab, R. (1981) *Nature (London)* **293,** 301–306.
30. Hozumi, T. (1983) *Biochemistry* **22,** 799–804.
31. Szilagyi, L., Balint, M., Sreter, F. A. & Gergely, J. (1979) *Biochem. Biophys. Res. Commun.* **87,** 936–945.
32. Okamoto, Y. & Yount, R. G. (1983) *Biophys. J.* **41,** 298a (abstr.).
33. Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. (1982) *EMBO J.* **1,** 945–951.
34. Torgerson, P. M. (1984) *Biochemistry* **23,** in press.
35. Labbé, J. P., Mornet, D., Roseau, G. & Kassab, R. (1982) *Biochemistry* **21,** 6897–6902.
36. Torgerson, P. M. & Morales, M. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** in press.
37. Langer, J. A., Engelman, D. M. & Moore, P. B. (1978) *J. Mol. Biol.* **119,** 463–485.
38. Cantor, C. R. & Schimmel, P. R. (1980) *Biophysical Chemistry* (Freeman, San Francisco).
39. Moss, D. J. & Trentham, D. R. (1983) *Biochemistry* **22,** 5261–5270.
40. Marsh, D. J. & Lowey, S. (1980) *Biochemistry* **19,** 774–784.
41. Tao, T. & Lamkin, M. (1981) *Biochemistry* **20,** 5051–5055.
42. Perkins, J., Wells, J. A. & Yount, R. G. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39,** 1937 (abstr.).
43. Takashi, R., Torgerson, P. M. & Duke, J. A. (1984) *Biophys. J.* **45,** 223a (abstr.).
44. Dalbey, R. (1983) *Biophys. J.* **41,** 98a (abstr.).
45. Takashi, R., Muhlrad, A. & Botts, J. (1982) *Biochemistry* **21,** 5661–5668.
46. Takashi, R. (1980) *Biochemistry* **18,** 5164–5169.
47. Miki, M. & Mihashi, K. (1978) *Biochim. Biophys. Acta* **533,** 163–172.
48. Botts, J., Ue, K., Hozumi, T. & Samet, J. (1979) *Biochemistry* **18,** 5157–5163.
49. Chotia, C., Lesk, A. M., Dodson, G. G. & Hodgkin, D. C. (1983) *Nature (London)* **302,** 500–505.
50. Muhlrad, A. & Hozumi, T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 958–962.
51. Kubo, S., Tokura, S. & Tonomura, Y. (1960) *J. Biol. Chem.* **235,** 2835–2839.
52. Fabian, F. & Muhlrad, A. (1968) *Biochim. Biophys. Acta* **162,** 596–603.
53. Sekine, T. & Kielly, W. W. (1964) *Biochim. Biophys. Acta* **81,** 336–345.
54. Duke, J., Takashi, R., Ue, K. & Morales, M. F. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 302–306.
55. Kielly, W. W. & Bradley, L. B. (1956) *J. Biol. Chem.* **218,** 653–659.
56. Srivastava, S. & Wikman-Coffelt, J. (1980) *Biochem. Biophys. Res. Commun.* **92,** 1383–1388.
57. Watterson, J. G., Kohler, L. & Schaub, M. C. (1979) *J. Biol. Chem.* **254,** 6470–6477.
58. Mulhern, S. A. & Eisenberg, E. (1978) *Biochemistry* **17,** 4419–4425.
59. Ando, T. & Asai, H. (1979) *J. Mol. Biol.* **129,** 265–277.
60. Duke, J. A., MacKay, R. & Botts, J. (1966) *Biochim. Biophys. Acta* **126,** 600–603.
61. Rainford, P., Hotta, K. & Morales, M. F. (1964) *Biochemistry* **3,** 1213–1220.
62. Warren, J. C., Stowring, L. & Morales, M. F. (1966) *J. Biol. Chem.* **241,** 309–316.
63. Stone, D. B. & Prevost, S. C. (1973) *Biochemistry* **12,** 4206–4211.