Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2204–2208, April 1989
Biophysics

On the origin and transmission of force in actomyosin subfragment 1

(transduction/energy transfer/proximity mapping)

J. BOTTS*, J. F. THOMASON†, AND MANUEL F. MORALES*

*Cardiovascular Research Institute and †Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143

Contributed by Manuel F. Morales, December 23, 1988

ABSTRACT **A proximity map showing the three-dimensional arrangement of 12 chemically defined points in actomyosin subfragment 1 is developed and roughly correlated with published electron microscope reconstructions of others. Several additional points and topological relationships in the primary polypeptide chain folding are assimilated into this model. Certain crosslinkings and distance change observations are interpreted as indicators of transmission of force/displacement between the nucleotide-binding and an actin-binding site—i.e., as indications of how energy is transduced in this system.**

It is reasonable to think that contractile force arises because the equilibrium geometrical relations between actin and the subfragment 1 (S-1) segment of myosin are different for each enzymatic intermediate occupying an ATPase site of S-1 that is remote from the (S-1)–actin interface(s). Then the operation of the enzymatic cycle compels operation of a cycle of relations between S-1 and actin. If changes in these relations are resisted by (surmountable) external forces, the cycle of relations is a "work cycle" performing external work. In a repetitive system, chemical free energy is thus continuously transduced into mechanical work. One has to say *how* the binding of an enzymatic intermediate determines an actin-binding relation 5 nm away. We have suggested that binding of the intermediate at the ATPase distorts the local S-1 structure and that this distortion is mechanically transmitted to the distant actin-binding site. These ideas have been developed in previous papers (1–3). This paper fleshes out such ideas with recent observations.

Structural Information from Fluorescence Resonance Energy Transfer (FRET) Mapping and Electron Microscopy (EM)

We begin by exploring the structure that may transmit effects between the nucleotide-binding site (N site) and the actin-binding site(s) (A site).‡ Since crystallography is still unavailable, we resort to melding lesser methods. As shown previously, chemically defined points in acto-(S-1) can be pairwise labeled by fluorophores. From FRET between such a pair one can estimate the interpoint distance, and by repetition of the procedure one can build up a lattice of acto-(S-1) points (2, 5). The improvement offered by the Dale *et al.* (6) analysis was previously incorporated (2, 7), but since 1984, other developments have appeared (see below). Though we cite improvements we must also emphasize the limitations of proximity mapping inherent in our results. For example, every point lattice has at least one unresolvable "mirror image" ambiguity. More serious is a shortage of necessary distance measurements. Although $n(n-1)/2$ distances are displayed in a complete lattice of n points, only $4n-10$ distances are required to construct the lattice (2). But in our case even this smaller number has not yet been obtained. The missing distances have been assigned by using extraneous information of variable reliability. For example, chemical information may favor the proximity of two points, sequence locations plus secondary structure predictions may yield an estimate that discriminates between positions, or the known geometry of a ligand may fix points of attachment, etc.

A very useful advance in handling interpoint distance data is the "Distance Geometry Program" (8), designed for NMR data but also applicable to FRET data. The program produces point coordinates referred to an imbedded coordinate system, but this system has no relation to any "laboratory framework." Methods of accomplishing the transformation to the latter framework are among the recent advances. In one of these, Wakabayashi and his colleagues (9) have added to a conventional EM image reconstruction of acto-(S-1) the rough (because avidin is so large) visualization of two chemically defined points (ATP-binding site and Cys-707) and the general interface between S-1 and actin. This permits rotations of the chemical point lattice to bring its N site, Cys-707, and A site into approximate coincidence with the corresponding points in the EM image (figure 4 of ref. 9). The other method of relating the lattice to a laboratory framework stems from recognizing (10) that by combining FRET measurements with actin filament parameters it is possible to calculate the perpendicular distances (radial coordinates) from actin points to the actin filament axis. The radial coordinates of three actin points have recently been measured, and the coordinate of a fourth point has been found to be too large to be measured by FRET (11). One can (physically) construct an axis with radial struts of appropriate length, and then, working on a (physical) model of the lattice in the body-centered framework, one can by trial-and-error estimate how the axis and its struts could be laid so that the tips of the radial struts coincide with the appropriate points. The axis position in the body-centered framework is then defined by its intersection with the x–y plane and two orientational angles. So the translation and Euler transformation required to shift from the body-centered framework to the laboratory framework (in which the z axis is the actin-filament axis) become evident. Finally, there is a new technique of designing fluorophore-labeled peptides ("anti-peptides") complementary to specific sequence stretches (4). Thus it has been possible to locate a definitive contact between S-1 and actin (12).

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, subfragment 1 (of myosin); FRET, fluorescence resonance energy transfer; EM, electron microscopy; ε, etheno modification of the adenine ring of ATP; T, trinitrophenyl modification of the ribose of ATP; Me, tightly held divalent cation of actin; N site, nucleotide-binding site; A site, actin-binding site.

‡So far, only one A site has been positively identified (4). We proceed on knowledge of this site, but we know that at least one other site exists and must keep an open mind to revisions that future studies may bring.

Our lattice relates 12 points and displays 66 interpoint distances, but only 38 distances are required for its construction. We considered, however, that we had only 33 distance estimates. From these estimates we constructed a scaled physical model that therefore had "play." By using extraneous information (see above) we resolved the ambiguities thus rigidifying the model. On this model we measured the remainder of the 66 distances. We considered that the uncertainty in experimentally measured distances was ±10% and that in all other distances it was ±20%. Table 1 identifies the 12 points of the lattice; the legend gives the radial coordinate, r (the perpendicular distance to the actin filament axis), of three actin points. The 66 distances and their tolerances were then entered into the Distance Geometry Program (8). When run, the program generated many structures, some enantiomeric to others (2). Of these, one was selected for its low deviation measure, for the likelihood that certain of its points would conform in a general way with the same points in the EM reconstruction (9), and for its dimensions, which should be a length of ≈18.8 and a widest diameter of ≈6 nm (28, 29). The 66 distances from this selected structure are in the lower left sector of Table 1 and can be compared with the measured distances in the upper right sector. As described in the previous paragraph, the selected lattice was translated and rotated so that its point coordinates could be referred to a laboratory framework in which the z axis is the actin–filament axis. The interpoint distances are invariant in this transformation; the new point coordinates (available on request) were used to construct the structure shown in Fig. 1.

Structural Information of Other Kinds

There are additional data that bear on three-dimensional structure but can be incorporated only as approximations or only with the aid of certain conjectures.

The primary sequences of actin (30) and of S-1 heavy chain (31–33) are now known, as are the sequence positions of most lattice points; therefore one may imagine that the chains of actin or of S-1 are "threaded through" the lattice points so that points on the chains coincide with points of the lattice. This operation puts some constraints on the topology of chain folding, and other data cited below constrains folding still more. We subclassify sequence positions into three segments of the S-1 heavy chain (known from their molecular masses as "25 kDa," "50 kDa," and "20 kDa") discovered by Balint *et al.* (34) and two segments of the actin chain ("10 kDa" and "35 kDa") discovered by Konno (35, 36).

The feasibility of crosslinking two residues with a short-length crosslinker indicates interresidue proximity. Since the original experiments on S-1 (37, 38), many authors have reported such crosslinks between certain stretches of 50 kDa and 20 kDa; in two cases the residues joined were identified. Ue (39) showed that a proximity preexists between Cys-522 (50 kDa) and Cys-707 (20 kDa); Chaussepied *et al.* (40) showed a proximity between Cys-540 (50 kDa) and Cys-697 (20 kDa). This information does not place Cys-522 and Cys-540 precisely in the lattice but does show that there is a "loop" of at least 185 residues formed by 50-kDa and 20-kDa strands of S-1 heavy chain. This loop includes the 50-kDa–20-kDa "connector" (residues 633–642), long suspected to be a site at which the N terminus of actin binds (41–43) and now more firmly established as such a site (ref. 4; K. Yamamoto, personal communication). The actin N terminus is thought to bind *alternatively* (44, 45) to another stretch of S-1 (possibly 566–573) in the same critical loop.

It now seems sure that the sequence location of the ATP-binding site of S-1 suggested by Walker *et al.* (46) is correct. Atkinson *et al.* (47) have shown that UTP binds photoactively to this location, and Grammer *et al.* (48) have shown that photoactivation of bound ADP·vanadate cleaves 25 kDa at Ser-180. Between positions 173 and 195 the S-1 sequence seems to conform to the predictions of Bradley *et al.* (49): The initiating β-strand runs from Ser-173 to Gly-178 and is followed by "GXXXXGK" between Gly-178 and Lys-184, which in turn is followed by Thr-185 and an α-helix ending with Phe-195. Speculatively, Mg^{2+} may chelate on the one hand to Asp-168 and Glu-170 and on the other to the γ- (or β-) phosphate of bound ATP; the α-phosphate may bind to (Lys-184)-(Thr-185), whereas the adenosine moiety interacts with the C end of the α-helix. The trinitrophenyl label (attached to the ribose moiety of analog ATP) would be near residues (Lys-189)-(Arg-190) and the ring of a bound ε-nucleotide would be near to Phe-195. As shown in Fig. 1, the ATP-binding region (on 25 kDa) is quite near to Cys-697 (20 kDa), imposing yet another folding constraint. Sutoh and Hiratsuka (50) confirmed this proximity by achieving a crosslink between the vicinity of Lys-189 (25 kDa) and Cys-697 (20 kDa). More recently, Lu and Wong (51) achieved a crosslink between Glu-88, Asp-89, Met-92, and Cys-707. Later we will note that a strand of 50 kDa also runs in this vicinity—i.e., elements of all three sequence segments appear to interact with bound ATPase intermediates.

Sutoh (52) has reported that antibody directed to the N terminus of S-1 heavy chain locates centrally in S-1. Following Winkelmann and Lowey (53), Miyanishi *et al.* (54)

Table 1. Interpoint distances in acto-(S-1)

	J	LC_1-C_{177}	SH_1	SH_2	ε	T	RLR	A-374	A-N	A-41	A-Me	50/20
≈840		8.0 (13)	13.0 (14)	—	14.0 (17)	—	—	—	—	—	—	—
LC_1-C_{177}	8.69		4.0 (15)	TF	—	5.7 (18)	2.9 (20)	TF	TF	6.6 (24)	—	—
C_{707}	11.66	3.6		2.9 (16)	—	3.8 (19)	2.6 (21)	5.8 (22)	TF	6.3 (24)	—	4.97 (12)
C_{697}	14.06	5.9	2.88		—	—	3.9 (2)	—	—	—	—	4.7 (12)
F_{195}	14.77	6.60	4.21	1.70		—	4.2 (2)	—	—	—	—	4.3, 5.9 (12)
R_{190}	14.84	6.04	3.40	1.20	1.63		—	TF	6.4	—	—	—
K_{83}	11.1	3.20	2.83	4.17	4.60	4.72		4.4 (2)	4.5 (2)	>3.7 (24)	—	—
A-C_{374}	14.36	6.65	5.20	6.43	7.33	7.52	4.53		3.4 (23)	4.36, 4.77 (25)	3.2 (26)	4.79, 5.94 (12)
A-N	16.23	7.66	5.66	5.17	5.30	6.22	4.53	3.65		3.8, 3.9 (25)	<1.0 (27)	4.03 (12)
A-Q_{41}	14.21	6.49	6.68	7.50	7.52	8.26	4.13	4.39	4.20		—	—
A-160	16.58	8.04	5.67	5.16	5.54	6.30	5.05	3.44	1.10	5.06		—
633–642	14.33	5.97	5.50	5.20	4.60	5.67	3.18	5.87	3.84	3.50	4.83	

The column titles are commonly used names of the points (ref. 2; the single-letter amino acid code is used). Row titles refer to the same points but are estimated sequence elements. Elements in the upper right sector are distances (nm) followed by reference. TF (too far for FRET) and the T to A-N site distance are cited in ref. 2 but attributed to R. Cooke and C. G. Dos Remedios (12th International Congress of Biochemistry, Aug. 15–21, 1982, Perth, Australia, abstr. POS 004-230) (T, the trinitrophenyl modification of the ribose of ATP; Me, the tightly held divalent cation of actin; ε, etheno modification of the adenine ring of ATP). Symmetrical elements in the lower left sector are the corresponding distances in the optimized structure generated by the Distance Geometry Program (8). The radial coordinates of points of actin (11) are $r_{374} = 2.0$; $r_N = 3.0$; $r_{41} = 4.1$.

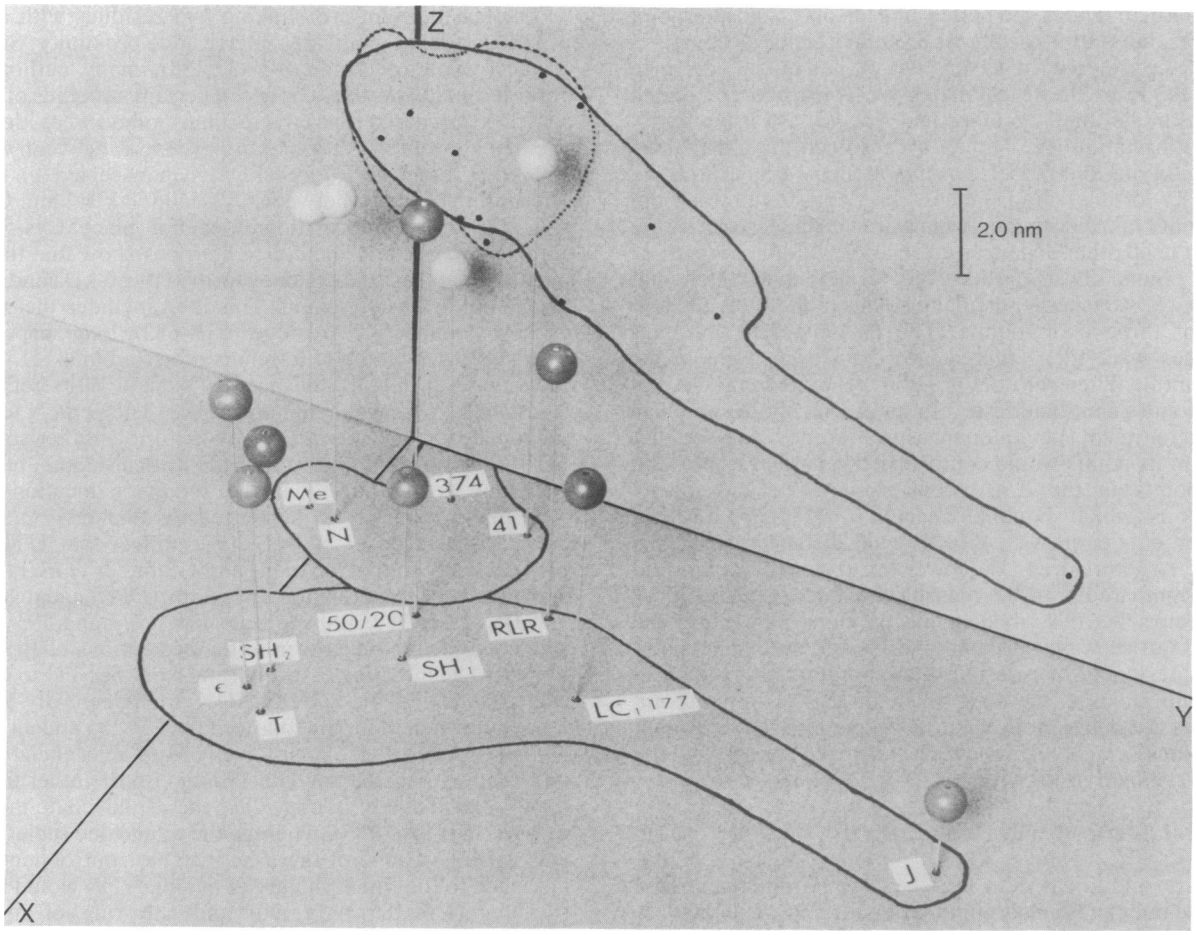

FIG. 1. Spatial arrangement of chemically defined points (spheres) in the actomyosin complex. Names of spheres (depicted on the x–y plane) are explained in Table 1. The z-axis is the actin filament axis, deduced as explained in text. Dark spheres are S-1 (or S-1-attached) points; light spheres are actin points. Projections of acto-(S-1) on the x–y and y–z planes are rough sketches adapted from ref. 9; centers of spheres are projected on these planes as dots; these dots seem always to be on surfaces.

conclude that residues between positions 214 and 301 are near the S-1 tip. This conclusion is reconcilably constrained, by the feasibility of crosslinking position 273 with Cys-697. There may be a discrepancy in the location of the 25-kDa–50-kDa and 50-kDa–20-kDa connectors. Sutoh (55) has reported the former to be 1 nm farther from the tip than the latter. This disagrees with lattice data (Fig. 1), which locate the 50-kDa–20-kDa connector (anti-peptide location) farther from the tip than residue 195 ("C"), which in turn cannot be far from the 25-kDa–50-kDa connector (that spans positions 204–214). However, in harmony with the lattice, Dan-Goor *et al.* (56) locates an antibody directed to position 214 and beyond at the S-1 tip.

Actin has been studied less than S-1, and even conjectures about its substructure are vague. Nevertheless, we know from Konno (35) that 10 kDa comprises the first 44 residues of actin. The N-terminal region (residues 1–4) binds to one of two places on S-1, the 50-kDa–20-kDa connector being one of them, so we can expect the N terminus of actin to be very near to S-1 residues 633–642. According to Mejean *et al.* (57) actin residues 18–28 are also a part of the interface with S-1. Gln-41 is close enough to 44/45 so that most of 10 kDa may be roughly positioned. Sequence segment 10 kDa has some biochemical autonomy, but it is not coextensive with what crystallographers call the "smaller" (N-terminal) domain of actin. Crystallographic studies assign the actin nucleotide to the cleft between the smaller and the "larger" (C-terminal) domain of actin (58). Barden and Dos Remedios (59) report that the tightly held actin metal cation is very close (within 1 nm) to the nucleotide. Recent observations (K. Ue, A. Muhlrad, and M.F.M., unpublished data) place this cation near Thr-160. So, we surmise that the 95 or so residues beyond 10 kDa that are still in the small domain fill the space between the nucleotide–metal cluster and 10 kDa. On this basis the larger domain, which includes Cys-374, would repose lower and be closer to the filament axis.

The information in this section, interpreted in a somewhat speculative way, leads us to the general folding depicted in Fig. 2*A*.§ We reiterate that in the absence of secondary and tertiary structural information, Fig. 2*A* only suggests topological relationships.

How the Internal Structure of Acto-(S-1) May Function in Transduction

For the purposes of this section we focus on a particular region of S-1 structure (Fig. 2*B*) and consider how the binding of an N-site ligand may affect the (S-1)–actin interfaces in the critical loop. In this blowup the lattice points of Fig. 1 and the connectivities of Fig. 2*A* are reproduced, but new points are added in anticipation of the discussion below. The spatial locations of these added points are based on proximities proposed in the previous section or on their sequence proximity to established (open circle) points; because their

§A synthesis of this kind, but incorporating less data, was recently reported by Masaki and his colleagues (33).

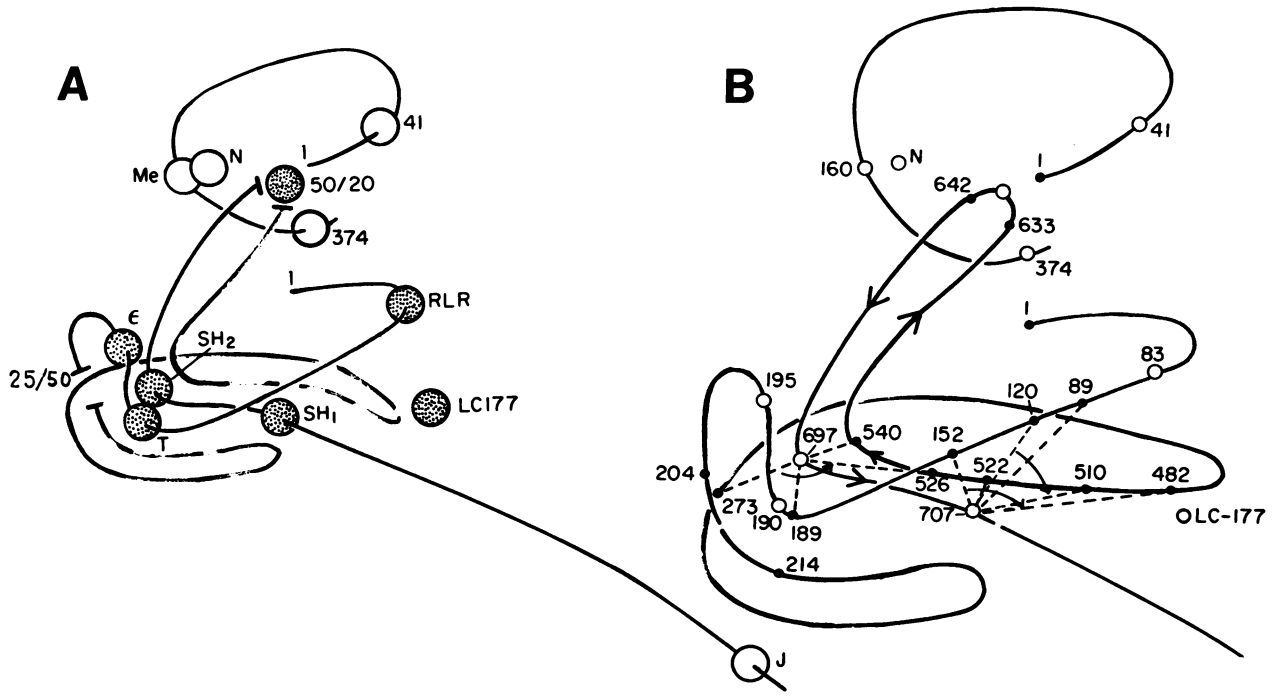

FIG. 2. (*A*) Topological arrangement of the myosin S-1 heavy chain and the actin chain. The spatial arrangement of the points is that in Fig. 1. The chain arrangements shown fit presently known facts but may not be unique. No attempt to show secondary/tertiary structure has been made, so chain lengths have no significance; however, the S-1 heavy chain is shown split into 25-kDa, 50-kDa, and 20-kDa segments. (*B*) Possible mechanical transmission from ATP-binding site to A sites. Points shown as open circles are those depicted in Fig. 1 and *A* and retain the same spatial relationship as in Fig. 1 and *A*. Several additional (closed circle) points have been added on grounds explained in text. Broken lines show crosslinks. Circular arrows show changes from crosslinks made before in relation to those made after nucleotide binding (60, 61). The text explains that these changes would be enabled by displacements (large arrowheads) in 50-kDa and 20-kDa strands. Such displacements would alter (S-1)-actin relations at interfaces—e.g., that between the actin N terminus and stretch 633–642 of the S-1 heavy chain.

positions are less sure than those of lattice points they are depicted in a distinctive way (closed circles).

Before there was any structural information about myosin, Sekine *et al.* (62) showed that Cys-697 ("SH_2") is more reactive when ADP is bound than when it is not, so it could be surmised that Cys-697 is mobile. Such surmises were much enhanced by Reisler *et al.* (63), who discovered the first topographical crosslink—Cys-697 to Cys-707—and showed that it can be fixed at various lengths. More recently, Wells and Yount (64) showed that when this crosslink is made in the presence of bound ADP it retards the escape of the then trapped ADP but that subsequent actin binding releases the trap. That bound nucleotide enters a "pocket" is also shown by the observations of Ando *et al.* (65). Moreover, Yount and co-workers (16) and Cheung and co-workers (66) have shown that as the nucleotide initiates the pocket formation *the distance between Cys-697 and Cys-707 decreases*. However, in this decrease the two thiols may move unsymmetrically. Attaching monofunctional reagents to Cys-697 moderately reduces ATPase (67), but attaching bifunctional (crosslinking) reagents extinguishes ATPase (40). Monofunctional attachment to Cys-707 has a variable effect, depending on substrate conformation; some crosslinkers affect, but do not extinguish, ATPase (39). The emission anisotropy of fluorophores attached to Cys-697 is much lower than that of fluorophores attached to Cys-707 (suggesting that Cys-697 is more mobile) (12, 67). When ADP binds, the distance from the 50-kDa–20-kDa connector to Cys-707 changes little, but that to Cys-697 significantly increases (12). Together, these recent observations suggest that upon nucleotide binding the interthiol distance decreases because *a relatively mobile Cys-697 moves toward a relatively static Cys-707*. In Fig. 2*B* this suggestion is noted by a rightward arrow in the interthiol stretch of 20 kDa.

Next we focus on the important experiments of Lu *et al.* (68) and of Burke (60). In these, one function of a bifunctional crosslinker first reacts with Cys-697 or Cys-707. The other (photoactivatable) function is then caused to react either before or after ATP is added; the stretch of residues with which the photoactive function reacts is then ascertained. We cite only the estimated midpoint of this stretch. Fig. 2*B* shows that, using various reagents, Cys-697 in the absence of ATP crosslinks to positions 189 (50), 273 (60), and 540 (40), but, after ATP, bonding to position 526 intensifies at the expense of bonding to position 273 (60). Also, Cys-707, in the absence of ATP, crosslinks to positions 120 (61), 152 (60), and 522 (39); in the presence of ATP crosslinks to positions 120 and 510 appear together (61) as do crosslinks to positions 152 and 482 (60). It is evident that the changes in crosslinking patterns around both thiols can be explained by a relative leftward displacement of the 50-kDa strand running parallel to the interthiol strand; this is suggested by a leftward arrow on the 50-kDa strand. Burke (60) notes that Cys-707 (unlike Cys-697) seems to retain its position relative to 25 kDa and concludes that Cys-697 may move toward Cys-707, thus reaching a conclusion similar to that above but on independent evidence.

Although we do not know the higher-order chain folding intervening between these strands and the actin-binding segments of the loop (e.g., the 50-kDa–20-kDa connector), it is clear that strains in the strands identified above could be communicated mechanically so as to alter the attitude and affinity of the actin bound to A sites of the loop. Mechanically, one should expect reciprocal effects that may actually have been observed. For example, if forming a pocket ("trap") for binding nucleotide entails strains that remotely affect actin binding, then actin binding should result in undoing the strains ("springing the trap"); actin binding

does, in fact, release trapped nucleotide. Also, it is already known that crosslinking the 50-kDa and 20-kDa strands (Cys-522 to Cys-707) inhibits actin binding, perhaps because it prevents the (reverse) strains required for actin binding (69). Lu *et al.* (70) have found that nucleotide-dependent crosslinks between either Cys-697 or Cys-707 with 50 kDa are inhibited by actin binding. Finally, the approximation of LC_1-177 and Act-374 by shortening the SH_1-SH_2 distance within S-1, by crosslinking or adding nucleotide (71), may be accommodated by supposing a rigid rotation of S-1 in the *x*–*y* plane (Fig. 1) about a vertical axis through the interface.

In summary, data of various kinds lead to an acto-(S-1) "structure" that seems to provide a basis for transmission of mechanical effect between binding of ATPase intermediates at one site and actin binding at a remote site. A precise future confirmation of this interpretation would complete a molecular theory of contraction.

Several friends contributed suggestions and helpful criticisms: (alphabetically) T. L. Hill, I. D. Kuntz, A. Muhlrad, R. Takashi, D. R. Trentham, and R. G. Yount. M. Burke made important results known to us prior to publication, and T. A. Webster kindly examined the S-1 sequence for homologies. Grants HL-16683 (U.S. Public Health Service) and CI-8 (American Heart Assocation) supported this research.

1. Morales, M. F. & Botts, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3857–3859.
2. Botts, J., Takashi, R., Torgerson, P., Hozumi, T., Muhlrad, A., Mornet, D. & Morales, M. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2060–2064.
3. Morales, M. F. (1987) in *Perspectives on Energy-Transducing Systems*, ed. Mukohata, Y. (Academic, Tokyo).
4. Chaussepied, P. & Morales, M. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7471–7475.
5. Dos Remedios, C. G., Miki, M. & Barden, J. A. (1987) *J. Muscle Res. Cell Motil.* **8,** 97–117.
6. Dale, R. E., Eisinger, J. & Blumberg, W. E. (1979) *Biophys. J.* **26,** 161–194.
7. Torgerson, P. M. & Morales, M. F. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3723–3727.
8. Kuntz, I. D., Thomason, J. F. & Oshiro, C. M. (1989) *Adv. Enzymol.*, in press.
9. Tokunaga, M., Sutoh, K., Toyoshima, C. & Wakabayashi, T. (1987) *Nature (London)* **329,** 635–638.
10. Taylor, D. L., Reidler, J., Spudich, J. A. & Stryer, L. (1981) *J. Cell Biol.* **89,** 362–367.
11. Kasprzak, A., Takashi, T. & Morales, M. F. (1988) *Biochemistry* **27,** 4512–4522.
12. Kasprzak, A., Chaussepied, P. & Morales, M. F. (1989) *Biophys. J.* **55,** 441a (abstr.).
13. Yamamoto, K., Tokunaga, M., Sutoh, K., Wakabayashi, T. & Sekine, T. (1985) *J. Mol. Biol.* **183,** 287–290.
14. Sutoh, K., Yamamoto, K. & Wakabayashi, T. (1984) *J. Mol. Biol.* **178,** 323–339.
15. Marsh, D. J. & Lowey, S. (1980) *Biochemistry* **19,** 774–784.
16. Dalbey, R. E., Weiel, J. & Yount, R. G. (1983) *Biochemistry* **22,** 4696–4706.
17. Sutoh, K., Yamamoto, K. & Wakabayashi, T. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 212–216.
18. Moss, D. J. & Trentham, D. R. (1983) *Biochemistry* **22,** 5261–5270.
19. Cooke, R. (1982) *Methods Enzymol.* **85,** 574–593.
20. Takashi, R., Torgerson, P. M. & Duke, J. A. (1984) *Biophys. J.* **45,** 223a (abstr.).
21. Takashi, R., Muhlrad, A. & Botts, J. (1982) *Biochemistry* **21,** 5661–5668.
22. Takashi, R. (1979) *Biochemistry* **18,** 5164–5169.
23. dos Remedios, C. G. & Cooke, R. (1984) *Biochim. Biophys. Acta* **188,** 193–205.
24. Takashi, R. (1987) *Biochemistry* **26,** 7471–7477.
25. Takashi, R. (1989) *Biophys. J.* **55,** 281a (abstr.).
26. Barden, J. A. & Dos Remedios, C. G. (1987) *Eur. J. Biochem.* **168,** 103–109.
27. Miki, M. & Wahl, P. (1985) *Biochim. Biophys. Acta* **828,** 188–195.
28. Walker, M. & Trinick, J. (1988) *J. Muscle Res. Cell Motil.* **9,** 359–366.
29. Vibert, P. J. (1988) *J. Muscle Res. Cell Motil.* **9,** 147–155.
30. Elzinga, M., Collins, J. H., Kuehl, W. H. & Adelstein, R. S. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2681–2687.
31. Tong, S. W. & Elzinga, M. (1983) *J. Biol. Chem.* **258,** 13100–13110.
32. Maita, T., Hayashida, M., Tanioka, Y., Komine, Y. & Matsuda, G. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 416–420.
33. Yanagisawa, M., Hamada, Y., Katsuragawa, Y., Imamura, M., Mikawa, T. & Masaki, T. (1987) *J. Mol. Biol.* **198,** 143–157.
34. Balint, M., Wolf, L., Tarcfalvi, A., Gergely, J. & Sreter, F. A. (1978) *Arch. Biochem. Biophys.* **190,** 793–799.
35. Konno, K. (1987) *Biochemistry* **26,** 3582–3589.
36. Konno, K. (1988) *J. Biochem.* **103,** 386–392.
37. Kosower, N. S., Kosower, E. M., Newton, G. L. & Ranney, H. M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3382–3386.
38. Mornet, D., Ue, K. & Morales, M. F. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1658–1662.
39. Ue, K. (1987) *Biochemistry* **26,** 1889–1894.
40. Chaussepied, P., Morales, M. F., Kassab, R. (1988) *Biochemistry* **27,** 1778–1785.
41. Mornet, D., Pantel, P., Audemard, E. & Kassab, R. (1979) *Biochem. Biophys. Res. Commun.* **89,** 925–932.
42. Sutoh, K. (1982) *Biochemistry* **21,** 3654–3661.
43. Botts, J., Muhlrad, A., Takashi, R. & Morales, M. F. (1982) *Biochemistry* **21,** 6903–6905.
44. Chen, T., Applegate, D. & Reisler, E. (1984) *Biophys. J.* **45,** 222a (abstr.).
45. Mornet, D. & Ue, K. (1985) *Biochemistry* **24,** 840–846.
46. Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. (1982) *EMBO J.* **1,** 945–951.
47. Atkinson, M. A. L., Robinson, E. A., Appella, E. & Korn, E. D. (1986) *J. Biol. Chem.* **261,** 1844–1848.
48. Grammer, J. C., Cremo, C. R. & Yount, R. G. (1988) *Biophys. J.* **53,** 237a (abstr.).
49. Bradley, M. K., Smith, T. F., Lathrop, R. H., Livingston, D. M. & Webster, T. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4026–4030.
50. Sutoh, K. & Hiratsuka, T. (1988) *Biochemistry* **27,** 2964–2969.
51. Lu, R. C. & Wong, A. (1988) *Biophys. J.* **53,** 175a (abstr.).
52. Sutoh, K. (1987) *J. Mol. Biol.* **195,** 953–956.
53. Winkelmann, D. A. & Lowey, S. (1986) *J. Mol. Biol.* **188,** 595–612.
54. Miyanishi, T., Toyoshima, C., Wakabayashi, T. & Matsuda, G. (1988) *J. Biochem.* **103,** 458–462.
55. Sutoh, K. (1989) *J. Mol. Biol.*, in press.
56. Dan-Goor, M., Kessel, M., Silberstein, L. & Muhlrad, A. (1988) *J. Muscle Res. Cell Motil.* **9,** 75–76.
57. Mejean, C., Boyer, M., Labbe, J. P., Derancourt, J., Benyamin, Y. & Roustan, C. (1986) *Biosci. Rep.* **6,** 493–499.
58. Kabsch, W., Mannherz, H. G. & Suck, D. (1985) *EMBO J.* **4,** 2113–2118.
59. Barden, J. A. & Dos Remedios, C. G. (1987) *Eur. J. Biochem.* **168,** 103–109.
60. Burke, M. (1989) *J. Biol. Chem.*, in press.
61. Sutoh, K. & Lu, R. C. (1987) *Biochemistry* **26,** 4511–4516.
62. Sekine, T., Barnet, L. M. & Kielley, W. W. (1962) *J. Biol. Chem.* **273,** 2769–2772.
63. Reisler, E., Burke, M., Himmelfarb, S. & Harrington, W. F. (1974) *Biochemistry* **13,** 3837–3840.
64. Wells, J. A. & Yount, R. G. (1980) *Biochemistry* **19,** 1711–1717.
65. Ando, T., Tonomura, Y., Duke, J. A. & Morales, M. F. (1982) *Biochem. Biophys. Res. Commun.* **109,** 1–6.
66. Aguirre, R., Gonsoulin, F. & Cheung, H. C. (1986) *Biochemistry* **25,** 6827–6835.
67. Miyanishi, T. & Borejdo, J. (1989) *Biochemistry*, in press.
68. Lu, R. C., Moo, L. & Wong, A. G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6392–6396.
69. Mornet, D., Ue, K., Chaussepied, P. & Morales, M. F. (1986) *Eur. J. Biochem.* **159,** 555–561.
70. Lu, R. C., Lyitray, L., Wong, A. & Gergely, J. (1987) *Biophys. J.* **51,** 318a (abstr.).
71. Trayer, H. R. & Trayer, I. P. (1988) *Biochemistry* **27,** 5718–5727.