Journal of Cellular Biochemistry 31:1-9 (1986)
Membrane Skeletons and Cytoskeletal-Membrane Associations 167-175

An Actomyosin Contractile Mechanism for Erythrocyte Shape Transformations

Velia M. Fowler

Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, Massachusetts 02115

The membrane skeleton of the human erythrocyte consists of many short actin filaments that are multiply cross-linked by long, flexible spectrin molecules into a continuous network in the plane of the membrane. The mechanical properties expected for this spectrin-actin network can account for the tensile strength of the erythrocyte membrane and for the remarkable deformability of the cells, yet not for their characteristic biconcave shape. Recently, an authentic vertebrate myosin as well as a non-muscle form of tropomyosin have been identified and purified from erythrocytes. The myosin is present with respect to the actin in an amount comparable to actin-myosin ratios in other non-muscle cells, and there is enough tropomyosin to almost completely coat all of the short actin filaments in the membrane skeleton. The implications of these unexpected discoveries for the molecular organization of the cytoskeleton are discussed, and a mechanism is proposed by which myosin could interact with the membrane-associated actin filaments to influence erythrocyte shape and membrane properties.

Key words: erythrocyte, membrane-skeleton, actin, myosin

The unstressed, normal human erythrocyte is a perfectly symmetrical biconcave disc ~ 8 μm in diameter. This shape is by no means a rigid or fixed structure, since the cells assume a variety of changing shapes during their passage through the circulation. Yet, except for the narrowest orifices (eg, the 2 μm splenic endothelial cell slits), these deformations are superimposed upon the basic discoid shape. For example, in a small capillary (5–6 μm), the cells tend to fold or buckle in their middle about the long axis of the capillary, rather than simply compressing and extending to a sausage-like shape [1,2].

The mechanical properties of the human erythrocyte are determined by a cytoskeletal network underlying the membrane that is thought to consist of many short actin filaments (~ 12–20 monomers long) cross-linked by long, flexible spectrin molecules in association with a M_r 80,000 helper protein, band 4.1. The cytoskeletal network is multiply attached to the cytoplasmic surface of the membrane via the interactions of spectrin and band 4.1 with ankyrin and glycophorin, respectively. Ankyrin is a M_r 210,000 protein, which is itself tightly associated with band 3, the anion channel and major integral membrane protein, and glycophorin is the major transmembrane sialoglycoprotein in the erythrocyte membrane (see [3,4] for re-

Received May 8, 1985; revised and accepted December 9, 1985.

views). As currently understood, this cytoskeletal network is proposed to function as a viscoelastic gel in the plane of the membrane, in which the multiple linkages among the component parts are responsible for the tensile strength of the membrane, and the flexibility of the linker molecules for the morphologic plasticity of the cells [5,6]. However, neither the specific features of these molecular interactions, nor the mechanical properties of spectrin-actin networks [7,8] can account in any obvious way for the characteristic biconcave shape of the human erythrocyte.

Recently, an authentic myosin has been identified in human erythrocytes, based on cross-reaction with affinity-purified antibodies to human platelet myosin, and characterization of the structural and functional properties of the purified protein [9,10]. In addition, erythrocytes also contain a non-muscle form of tropomyosin [11], a highly conserved, alpha-helical, rod-like protein that is a constituent of the thin filaments of the contractile apparatus in all muscles [12]. In skeletal muscle, tropomyosin lies along the actin filament in a head-to-tail fashion in each of the two grooves of the actin helix and functions together with troponin to confer calcium sensitivity on the interaction of myosin with actin during contraction [12,13]. In smooth muscle and in non-muscle cells (which do not contain troponin), tropomyosin may play a role in stabilizing the actin filaments and regulating their interactions with other actin-binding proteins [14,15], as well as in participating in the cooperative interaction of the myosin heads with actin during ATP-hydrolysis [12,14].

The purpose of this article is to discuss the possibility that myosin could be organized together with tropomyosin and the erythrocyte actin filaments into a membrane-associated contractile apparatus that is capable of exerting force on the membrane, and thus influencing cell shape. First, I will summarize the properties of the erythrocyte myosin and tropomyosin. Following this, I will discuss how the erythrocyte actin filaments could be organized into the functional equivalent of a Z line and how myosin could attach to these actin filaments in a hypothetical contractile apparatus, and finally, how myosin interactions with the erythrocyte actin filaments could influence cell shape. The discussion will be based on the basic features of the classic actomyosin sliding-filament mechanism for muscle contraction [16,17] and on logical considerations derived from our current understanding of the molecular organization of the erythrocyte membrane skeleton.

PROPERTIES OF ERYTHROCYTE MYOSIN AND TROPOMYOSIN

Structurally, the purified erythrocyte myosin is a typical vertebrate myosin, with two globular heads at the end of a rod-like tail ~ 150 nm long, as visualized by rotary shadowing of individual molecules. Similar to other non-muscle myosins, the erythrocyte myosin is capable of self-association into bipolar filaments 200 to 300 nm long at physiologic salt concentrations [9,10]. The ATPase activity of erythrocyte myosin is calcium-activated and magnesium-inhibited, and in 0.5 M KCl, is also high in the absence of any divalent cations (EDTA-KCl ATPase), as is characteristic of the enzymatic activity of many non-muscle myosins [9,10]. As isolated, the Mg^{++}-ATPase activity of erythrocyte myosin is not activated by rabbit skeletal muscle F-actin, but Wong et al [10] have shown that phosphorylation of the erythrocyte myosin M_r 19,500 light chain by smooth muscle myosin light chain kinase results in about a six-fold stimulation of the erythrocyte actomyosin Mg^{++}-ATPase activity. Myosin is an endogenous component of mature human erythrocytes, based on immunofluore-

scence localization of myosin in all cells and is present with respect to the actin in the erythrocyte in an amount comparable to actin/myosin ratios in other non-muscle cells (80/1) [9].

Tropomyosin purified from human erythrocytes resembles other non-muscle tropomyosins closely and is a dimer (M_r 60,000) composed of two equivalent $M_r \sim 30{,}000$ polypeptide chains, with an elongate, rod-like shape [11]. The erythrocyte tropomyosin binds to F-actin, and each molecule spans between six and seven monomers along an actin filament at saturation, as is true for other non-muscle tropomyosins [14]. In contrast to most other non-muscle tropomyosins, however, binding of the erythrocyte tropomyosin to F-actin is highly cooperative [11] and may indicate that erythrocyte tropomyosin self-associates in a head-to-tail fashion along the actin filament, as do tropomyosins from muscle [12]. The erythrocyte tropomyosin is localized exclusively on the membrane in cells hemolyzed in the presence of physiologic concentrations of magnesium (1–2 mM), indicating that it may be a heretofore unsuspected component of the membrane-cytoskeletal network. Tropomyosin comprises about 1% of the erythrocyte membrane protein and is present in a ratio of one M_r 60,000 tropomyosin molecule for every 7–8 actin monomers, an amount almost sufficient to coat all of the short actin filaments in the membrane skeleton ([11]; see below).

ORGANIZATION OF ERYTHROCYTE ACTIN FILAMENTS IN A "Z LINE"

A key feature of the organization of the basic contractile unit (the sarcomere) in skeletal muscle is the attachment of actin filaments to the Z lines by their barbed ends only [16,17]. This condition can be satisfied in the erythrocyte by restricting the association of the spectrin molecules only to actin subunits at or near the barbed end of the filaments (Fig. 1). This would lead to a situation in which adjacent actin filaments would have the same polarity with respect to one another, whereas actin filaments distant from one another in the plane of the membrane would be antiparallel in polarity. Therefore, sets of actin filaments at an appropriate distance from one another in the plane of the membrane (see below) would be oriented appropriately to interact productively with bipolar myosin filaments (Fig. 2). Thus, the spectrin network itself would be the functional analogue of the Z line, with the spectrin tetramers playing the role that the alpha-actinin molecules play in cross-linking the actin filaments in the muscle sarcomere. The multiple cross-links between spectrin and the actin filaments in the plane of the membrane would serve to distribute the forces uniformly over the surface of the cell, and the associations of spectrin and band 4.1 with ankyrin-band 3 and glycophorin, respectively, would provide the mechanism by which force exerted on the spectrin-actin network is transmitted to the membrane itself.

Previously, I had suggested that tropomyosin binding to the short actin filaments in the membrane skeleton restricted the spectrin molecules to the free actin subunits at both ends of the short actin filaments [18]. This idea was based on observations that spectrin and tropomyosin binding to F-actin were mutually exclusive both in the absence [18] or presence [V.M. Fowler, unpublished observations] of band 4.1 and is consistent with the relative stoichiometries of actin, spectrin, and tropomyosin in the membrane skeleton as well as with the ability of erythrocyte tropomyosin to span only 6–7 actin subunits along an actin filament [11]. Since both the spectrin-band 4.1

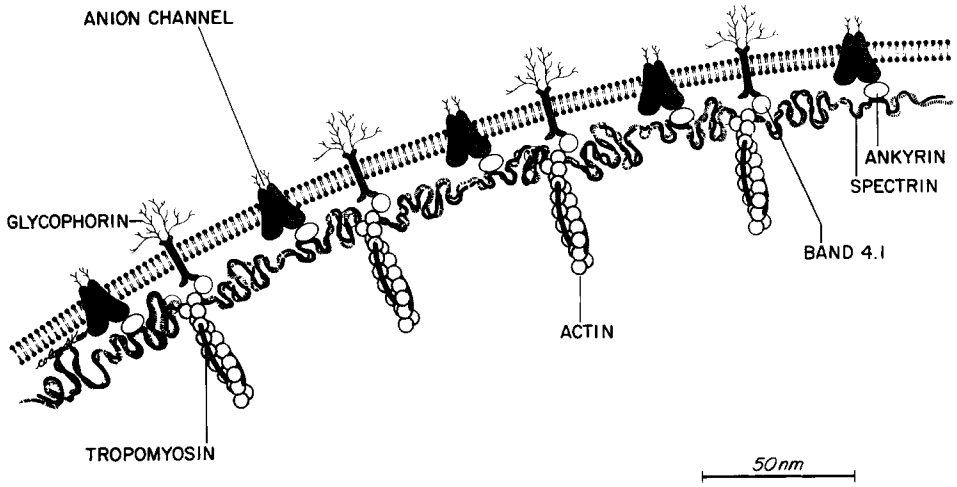

Fig. 1. Organization of the erythrocyte actin filaments in a membrane-associated Z line. Only one spectrin tetramer connects any two adjacent actin filaments in the cross-section of the membrane depicted here, but each actin filament would be attached to 4 to 6 spectrin tetramers in a plane perpendicular to the plane of the paper. The 60-nm spacings between the actin filaments and the excess length of the spectrin tetramers linking the actin filaments are based on recent direct observations of the arrangement of the spectrin-actin network in negatively stained preparations of artificially spread membrane skeletons [23]. The linear end-to-end length of a spectrin tetramer is about 194 nm; see [3,4]. The actin filaments in this illustration are intended to be oriented with their barbed ends attached to the spectrin molecules. The linear dimensions of the cytoskeletal structures are to scale, but the actual sizes of the individual molecules are enlarged for the purposes of illustration.

complex [19,20] and tropomyosin [11,12,14] associate molecularly along the sides of actin filaments, an additional actin-binding protein would presumably be necessary to specify the desired polarity in this system. Alternatively, since one end of the tropomyosin molecule is different from the other [12,14], its binding to actin might itself serve to restrict the spectrin-band 4.1 associations with actin to only one end of the filament. However, associations of spectrin with actin subunits at both ends of the short filaments would not necessarily prevent their interactions with myosin, nor prevent actin filaments at distant locations in the membrane skeleton from being antiparallel with respect to one another. It is also important to point out that this molecular substructure for the actin filaments (Fig. 1) makes no predictions whether one or both of the actin filament ends are blocked to further monomer addition; a point concerning which there is conflicting data in the literature [21,22].

ASSOCIATION OF MYOSIN WITH THE ERYTHROCYTE ACTIN FILAMENTS

If a 300 nm long erythrocyte myosin filament [9,10] were to be attached to the closest two sets of antiparallel actin filaments in the plane of the membrane, it would stretch linearly over a distance occupied by five of the actin filaments (Fig. 2). (Assuming a uniform distribution of the erythrocyte actin filaments in the plane of the membrane, they would be spaced approximately 60 nm apart; see Fig. 1 and [23].) Only two actin filaments are drawn interacting with each end of the myosin filament

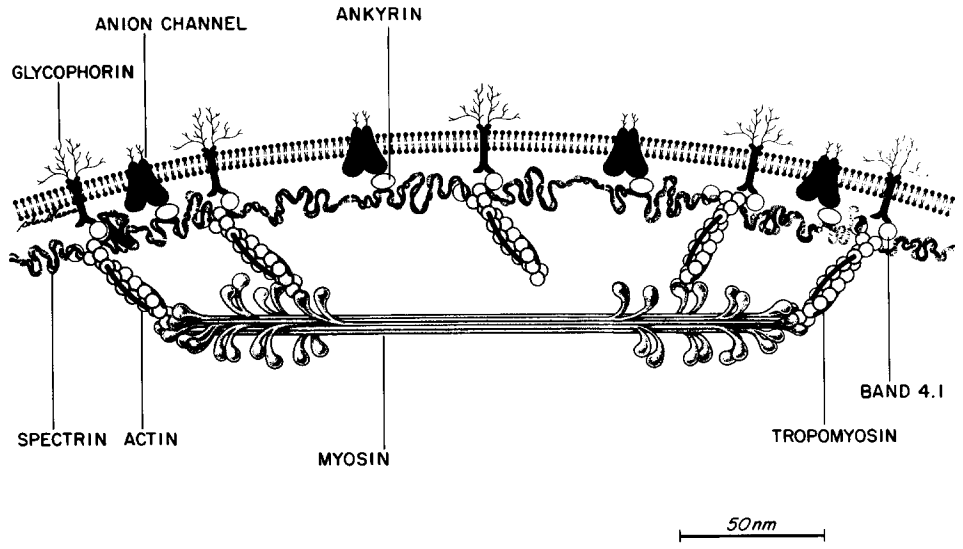

Fig. 2. Association of a bipolar erythrocyte myosin filament with the actin filaments in the membrane skeleton with a minimal effect on the arrangement of the membrane skeleton or on the overall shape of the cell (see text and Fig. 3). The proportions of the myosin filament are taken from the platelet myosin filament depicted in [24], but not all of the individual myosin molecules are indicated in this figure for the sake of clarity.

in Figure 2 because the drawing is in one dimension; in the plane of the membrane, perhaps five or six actin filaments could be associated with each end of the bipolar myosin filament. Since the distance between adjacent actin filaments in the Z line of a skeletal muscle sarcomere is only ~20 nm [16,17], it is likely that binding of the myosin heads to actin would induce the actin filaments immediately surrounding each end of the bipolar myosin filament to become clustered in order to interact optimally with the myosin filament. This would not necessarily result in any net change in the surface area occupied by the spectrin-actin network, because the shortening of the spectrin molecules linking the myosin-associated actin filaments together could be compensated for by the stretching of the spectrin molecules linking them to neighboring, non-myosin-associated actin filaments.

Therefore, although the interaction of myosin with the actin filaments has clearly induced a local rearrangement of the spectrin-actin network in the vicinity of the myosin filament (Fig. 2), it is difficult to imagine how this would translate into an effect on the overall shape of the cell. This difficulty becomes particularly evident when the small amount of myosin in the erythrocyte is taken into account. Assuming that the erythrocyte myosin filaments in vivo are between 200 and 300 nm, then there would be only 200 to 300 myosin filaments per cell (based on a value of 6,000 myosin molecules per cell [9], and 20–30 myosin molecules per filament [24]). This number of filaments would correspond to a density of 1.5 to 2 myosin molecules per μm^2 if evenly spaced over the entire surface of the membrane and contrasts with a density of about 200 to 250 per μm^2 for the short erythrocyte actin filaments [21,23].

ACTOMYOSIN INTERACTIONS AND CELL SHAPE

In Figure 2, a bipolar myosin filament is drawn attached to two sets of actin filaments that are only as far apart from one another as the length of the myosin

filament. What if a myosin filament were to be attached to actin filaments that were further away from one another in the plane of the membrane? The consequences of this are illustrated in Figure 3: the regions of membrane in between the two sets of actin filaments to which the myosin filaments are attached must bulge out as a consequence of the viscoelastic properties of the spectrin network. If the myosin filaments were to be uniformly distributed in this configuration, the cell would acquire numerous short protuberances over the entire surface of the membrane. In fact, cells with just this shape (echinocytes) are observed if biconcave erythrocytes are ATP-depleted by incubation at 37°C in the absence of glucose [25,26] or if micromolar calcium is introduced into the cells via the calcium inophore, A23187 [27]. The relationship between these two agents are not clear because intracellular free calcium ion concentrations have not been determined in cells induced to change shape as a result of metabolic depletion. Nevertheless, since actomyosin interactions in non-muscle cells are ATP-dependent and regulated by micromolar calcium (via calcium-calmodulin-dependent regulation of myosin light chain kinase activity [28]), it is tempting to speculate that echinocytes could be produced by the sorts of actomyosin interactions depicted in Figure 3. It may not be fortuitous that the length of a bipolar erythrocyte myosin filament is close to the dimension measured across the base of an individual protrusion on an echinocyte (500 nm, estimated from measurements of scanning electron micrographs; see [29]). However, it is difficult to see what would cause the myosin to associate with the erythrocyte actin filaments in this way rather than as depicted in Figure 2, and it is difficult to imagine how this kind of mechanism

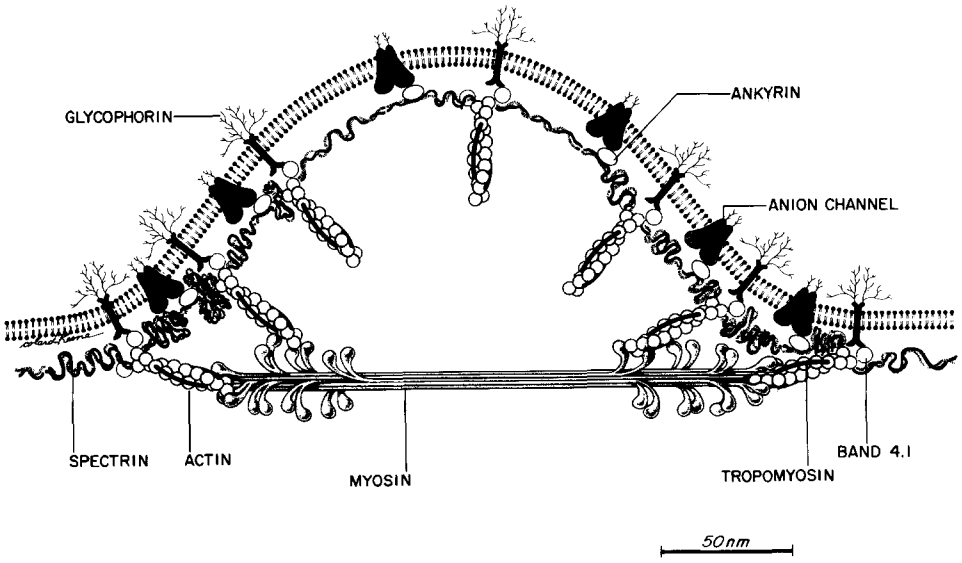

Fig. 3. Effect of actomyosin interactions on cell shape. The strain on the membrane produced by the bipolar myosin filament pulling on antiparallel actin filaments at some distance from one another in the plane of the membrane is anticipated to cause the unattached membrane in between to balloon out. Depending on the distance of the two sets of antiparallel actin filaments from one another and on the lengths of the actin filaments, this unattached region of the membrane could correspond to the protrusions on an echinocyte or the equatorial region of the biconcave erythrocyte (see text).

could generate the observed continuum of transformations in erythrocyte shape from discocytes to echinocytes.

The concept illustrated in Figure 3 also suggests a mechanism whereby actomyosin interactions could account for the biconcave shape of the erythrocyte. If the myosin filaments were to be attached to two sets of actin filaments that are the maximum distance away from one another on the surface of the cell, the myosin filaments would span the dimple of the cell, and the "bulge" would effectively become the equatorial region of the biconcave erythrocyte. However, a 300-nm myosin filament would be unable to attach simultaneously to the 40 nm long actin filaments (see Figs. 1-3) on opposite sides of the cell because the distance across the erythrocyte in this region is 810 nm (±35 nm) [30]. Therefore, either the myosin or actin filaments in this region of the cell would have to be longer. Some support for the latter possibility is the isolation of long actin filaments (up to 100 nm) from phalloidin-treated ghosts [31] or high-salt-extracted detergent cytoskeletons [32]. It may also be significant that erythrocyte tropomyosin may have the somewhat unusual property (for a non-muscle tropomyosin) of self-associating along the actin filament [11]. The absence of such long actin filaments in negatively stained en face preparations of cytoskeletons [23] may simply be due to the fact that they were broken and washed away by the stream of buffer used to remove the upper surface of the cells. Similary, measurements of the lengths of actin filaments in the cells based on the number of actin filament ends would fail to detect a relatively small number of longer actin filaments over the background of the many short ones that function to link the spectrin molecules together over the surface of the membrane. Presumably, spectrin tetramers could still function to attach these hypothetical long actin filaments to the cytoplasmic surface of the membrane as well as to integrate them into the remainder of the cytoskeletal network in the plane of the membrane (Fig. 1 and see above).

There is some evidence that not all of the spectrin-actin junctions in the membrane skeleton are identical. Oligomeric complexes of actin, spectrin, and band 4.1 have been isolated in which the actin is believed to consist of no more than four or five subunits [32,33]. These actin "protofilaments" would not be expected to bind tropomyosin and could be preferentially localized in the equatorial regions of the biconcave cell, thus freeing up actin for the long filaments proposed here to exist in the dimple region. Self-associations between the spectrin tetramers themselves [34] could also play a similar role in the membrane skeleton. Recently a M_r 48,000 actin-filament bundling protein has been isolated from erythrocyte membranes [35], raising the possibility that some of the erythrocyte actin filaments could be localized in groups at specialized sites in the membrane skeleton. Although the ultrastructural localization of actin and myosin in the intact, biconcave erythrocyte remains to be determined, there are some tantalizing indications that the organization of the membrane skeleton in the region of the dimple might be different from that in the equatorial region of the cell. In 1940, Furchgott [36] observed that the dimple reformed in the same place following restoration of isoosmolarity after the cells had been hypotonically swollen, and Shrivastov and Burton [37] concluded that there were oriented structures across the dimple region on the basis of birefringence measurements on intact biconcave erythrocytes.

FUTURE PROSPECTS

The mechanisms discussed above by which actomyosin interactions could influence cell shape make some very specific predictions concerning the localization of

actin and myosin in the intact, mature, biconcave erythrocyte. First, there would be expected to be two sets of long actin filaments in the region of the dimple in the biconcave erythrocyte, each set originating from the opposite sides of the cell and attached to the cytoplasmic surface of the membrane via their barbed ends. Second, there would be a population of cytoplasmic bipolar myosin filaments in the region of the dimple that interdigitates with and interacts with these actin filaments analogously to the myosin filaments in the skeletal muscle sarcomere. This would not exclude the possibility that there could be myosin filaments elsewhere in the cell, even perhaps associated with the short actin filaments in the membrane skeleton, since interactions of the sort depicted in Figure 2 would not be anticipated to have any effect on the shape of the cell (see above). Third, disruption of this hypothetical actomyosin contractile apparatus localized in the dimple region of the biconcave erythrocyte would be expected to cause the cell to assume a spherical shape, in the absence of any other changes in the membrane skeleton.

If actomyosin interactions are also instrumental in generating an echinocyte, then myosin filaments would be expected to be localized at the bases of the surface protrusions. Additionally, agents that induce the discocyte-echinocyte shape transformations should induce a graded redistribution of myosin from its restricted localization in the dimple region (see above) to a more uniform distribution in the membrane skeleton, and/or changes in the amount or strength of actomyosin interactions (eg, via phosphorylation of the myosin light chain, see [28]) should correspond to the continuum of shapes from the discocyte to the echinocyte.

Alternative mechanisms proposed to account for erythrocyte shape transformations include changes in the degree of assembly or cross-linking of the spectrin-actin network [38,39], changes in the attachment of the spectrin-actin network to the cytoplasmic surface of the membrane [40], changes in the relative surface area of the inner and outer monolayers of the lipid bilayer [41,42], and changes in the degree of hydration and ionization of the spectrin molecules in the two-dimensional spectrin-actin gel underlying the membrane [7]. In the complex, interconnected and interdependent system comprised by the erythrocyte membrane skeleton, bilayer, and hypothetical actomyosin contractile apparatus discussed here, alteration in any of the molecular interactions or linkages is likely to have consequences for cell shape, either directly or indirectly. The challenge is to establish causative relationships between observed effects on specific molecular interactions with defined shapes of the cells.

ACKNOWLEDGMENTS

The author is especially grateful to Sylvia Collard Keene for her skill and patience in translating the ideas expressed in the text into the artwork in the figures and would also like to thank Peter Ley for the photography. I would also like to acknowledge the critical comments of two anonymous reviewers who contributed to the development of the ideas expressed in this paper. This work was supported by NIH grant R01 GM34225 and by a grant from the Whitaker Health Sciences Fund.

REFERENCES

1. Bagge U, Branemark PI, Karlsson R, and Skalak R: Blood Cells 6:231, 1980.
2. Gaehtgens P, Duhrssen C, Albrecht KH: Blood Cells 6:799, 1980.

3. Bennett V: Ann Rev Biochem 54:273, 1985.
4. Marchesi, VT: Ann Rev Cell Biol 1:531, 1985.
5. Evans EA, La Celle PL: Blood 45:29, 1975.
6. Hochmuth RM: Ann Rev Biophys Bioeng 11:43, 1982.
7. Stokke BT, Mikkelsen A, Elgsaeter A: Biochim Biophys Acta 816:111, 1985.
8. Schanus E, Booth S, Hallaway B, Rosenberg A: J Biol Chem 260:3724, 1985.
9. Fowler VM, Davis JQ, Bennett V: J Cell Biol 100:47, 1985.
10. Wong AJ, Kiehart DP, Pollard TD: J Biol Chem 260:46, 1985.
11. Fowler VM, Bennett V: J Biol Chem 259:5978, 1984.
12. Mannherz HG, Goody RS: Ann Rev Biochem 45:427, 1979.
13. Ebashi S: Essays in Biochem 10:1, 1974.
14. Cote GP: Molec Cell Biochem 57:127, 1983.
15. Payne MR, Rudnick SE: Trends in Biochem Sci 9:361, 1984.
16. Huxley HE: Scientific American 213:18, 1965.
17. Squire J: "The Structural Basis for Muscle Contraction." New York: Plenum Press, 1984.
18. Fowler VM, Bennett V: "Erythrocyte Membranes 3: Recent Clinical and Experimental Advances." New York: Alan R. Liss, Inc., 1984, pp 57–71.
19. Cohen CM, Tyler JM, Branton D: Cell 21:875, 1980.
20. Ungewickell E, Bennett PM, Calvert R, Ohanian V, Gratzer WB: Nature (Lond) 280:811, 1979.
21. Pinder JM, Gratzer WB: J Cell Biol 96:768, 1983.
22. Tsukita S, Tsukita S, Ishikawa H: J Cell Biol 96:1102, 1984.
23. Byers T, Branton D: Proc Nat Acad Sci (USA) 82:6153, 1985.
24. Niederman R, Pollard TD: J Cell Biol 67:72, 1975.
25. Nakao M, Nakao T, Yamazoe S, Yoshikawa H: J Biochem (Tokyo) 49:487, 1961.
26. Weed RI, La Celle PL, Merrill EW: J Clin Invest 48:795, 1969.
27. Kirkpatrick FH, Hillman DG, LaCelle PL: Experientia 31:653, 1975.
28. Adelstein RS: Cell 30:349, 1982.
29. Bessis M: "Living Blood Cells and Their Ultrastructure." New York: Springer-Verlag, 1973.
30. Evans E, Fung Y-C: Microvasc Res 4:335, 1972.
31. Atkinson MAL, Morrow JS, Marchesi VT: J Cell Biochem 18:493, 1982.
32. Shen BW, Josephs R, Steck TL: J Cell Biol 99:810, 1984.
33. Liu S-C, Windisch P, Kim S, Palek J: Cell 37:587, 1984.
34. Morrow JS, Marchesi VT: J Cell Biol 88:463, 1981.
35. Siegel D, Branton D: J Cell Biol 100:775, 1985.
36. Furchgott RF: J Exp Biol 17:30, 1940.
37. Shrivastov BB, Burton AC: J Cell Physiol 74:101, 1969.
38. Fowler VM, Taylor DL: J Cell Biol 85:367, 1980.
39. Pinder JC, Bray D, Gratzer WB: Nature (Lond) 270:752, 1977.
40. Anderson RA, Marchesi VT: Nature (Lond) 318:285, 1985.
41. Sheetz MP, Singer SJ: Proc Nat Acad Sci (USA) 71:4457, 1974.
42. Ferrell JE Jr, Huestis WH: J Cell Biol 98:1922, 1984.