REVIEW ARTICLE

The red cell membrane and its cytoskeleton

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Physical character of the cell

The peculiar fascination that the red blood cell has for so long exercised over biophysicists and physiologists derives from the unique mechanical properties [see, for example, Evans & Hochmuth (1978) for a review] that allow it to endure prodigious stresses, to which it is subjected during the several hundred miles that it travels in the course of its 4 month life span. The familiar biconcave discoid shape has evolved to meet the requirement for gross elastic deformation under the influence of high shearing stress*. The cell must be capable of squeezing through capillaries much narrower than its own diameter and reverting rapidly to the discoid state when it emerges. This ability to absorb shearing stresses of a formidable order accounts for the non-Newtonian viscosity of blood: with a rise of shear rate from 0.1 to 100 s^{-1} the viscosity falls by a factor of 10. After fixation with glutaraldehyde this property is lost, and the behaviour of the cell begins to approach that of a rigid body. However, a suspension of entirely rigid particles, each with the volume of a red cell, at a concentration corresponding to the haematocrit of blood would have flow properties not unlike those of well-matured asphalt.

In one sense the red cell membrane behaves as though it were a solid, for it is practically inextensible in respect of surface area. By contrast, linear deformation occurs with great ease, and the

elastic moduli for the two processes differ by a factor of 10^4 or more. The virtue of the discoid shape then lies in its capacity to undergo gross deformation with no significant change in area; a spherical cell, for which the area/volume ratio is already minimal, would be maximally resistant to shape deformations. The mechanical basis for the discoid shape remains an open question. It has been argued that it corresponds to least overall curvature of the geometrical envelope (Canham, 1970), and also that it minimizes the electrostatic free energy associated with the phospholipid surface charge (Adams, 1973). What may now be regarded as certain, however, is that the cell owes the preservation of its integrity, its discoid shape and its elasticity alike to a filamentous structure, covering the cytoplasmic surface of the membrane, and referred to as the cytoskeleton, or sometimes (to distinguish it from the multitude of filaments that traverse the cytoplasm of other, less differentiated cell types) the membrane skeleton.

There is abundant evidence for this conclusion. For example, when red cell ghosts are extracted with media of low ionic strength the protein complex is dissociated, and disintegration of the membrane into vesicles rapidly ensues. Much the same occurs if the proteins are destroyed by proteolysis. More graphic perhaps than these and many related observations is the discovery of several strains of mice, deficient in the preponderant cytoskeletal constituent, the high-molecular-weight protein, spectrin. These mice are afflicted with a spherocytic haemolytic anaemia of a severity that correlates with the extent of the deficiency (Lux *et al.*, 1979). The worst affected have red cells with an immeasurably short survival time, which, when subjected to the slightest mechanical perturbation, disintegrate into vesicles and myelin forms. When the deficit is made good, using a transient haemolysis procedure to introduce spectrin into the cell, the anomalous mechanical properties are to a large extent mitigated (Shohet, 1979). The cytoskeleton appears also to be the agency through which the cell exercises a number of biologically

* This shape and the associated mechanical properties, as well as the composition of the membrane in respect of the major protein constituents, are common to most mammalian red cells. The best-documented exception is the camel, which has cells of elliptical section, containing an exceptionally high proportion of protein and an elevated ratio of integral to peripheral proteins, and exhibiting remarkable resistance to osmotic lysis (Eitan *et al.*, 1976). Avian red cells are different again, most notably in that they possess peripheral microtubules. The major peripheral (cytoskeletal) protein, spectrin, or at least an equivalent, appears to be a feature common to all erythroid cells, even those of an invertebrate (Pinder *et al.*, 1978).

important functions, involving for instance the passage of signals across the membrane, as will be recounted.

Metabolic regulation of cell shape

It has been known these 20 years (Nakao *et al.*, 1960) that metabolic depletion of the red cell is accompanied by a succession of shape changes. Thus when cells are incubated in the absence of glucose, the intracellular ATP is consumed, and at the same time crenations appear on the membrane surface. The cell then begins to lose its discoid profile and becomes spherically symmetrical though still crenated (type III echinocyte). The protuberances become progressively more elongated, form necks and eventually separate as vesicles or microspheres, leaving a smooth spherocyte. Up to the echinocyte (type III) stage at least, these changes are reversible on addition of metabolites to the medium to allow recharging of the intracellular ATP pool. Very similar changes can be produced in resealed ghosts. A superabundance of ATP causes invagination of the membrane, followed by the formation of internal vesicles (Hayashi *et al.*, 1975; Hardy *et al.*, 1979). The mechanism of metabolic control of shape (and with it the mechanical character) of the cell is perhaps the central unsolved question in the field, and its resolution may be expected to throw light on the issue of cytoskeletal function in broader terms.

To be sure, metabolic depletion is by no means the only way in which cell shape changes may be provoked. They can be engendered by most forms of physical abuse, and most of all by lipophilic drugs or other molecules that partition into the membrane. Two types of shape transformation can be recognized: in general (Deuticke, 1968) anionic solutes generate echinocytes, with rather large protuberances distributed over the cell surface, and cationic species give rise to stomatocytes, or smooth cup shapes. Exposure of the cell to two ligands, one of either type, can lead to a cancellation of the two effects, with retention of the discoid shape. Anionic ligands are found to interdigitate mainly in the outer and cationic ligands in the inner leaflet of the membrane. Intuitive expectation is that the cell will react to annul an expansion in area of the outer leaflet by forming crenations and an expansion of the inner leaflet by trying to evert itself and turning into a stomatocyte; this is the basis of the celebrated 'bilayer couple' hypothesis (Sheetz & Singer, 1974). Intuition has been lent the respectability of a theoretical rationalization only for the first part of this proposition (Brailsford *et al.*, 1980), and quantitative evidence for the simple view of the system afforded by the bilayer couple model is lacking. Irregular, spiked cells can be generated (*in vivo* as well as *in vitro*) by increasing the cholesterol

content of the membrane, and it is striking that the spontaneous fragmentation of ghosts from which the cytoskeletal proteins have been extracted yields right-side-out or inside-out vesicles according to whether the cholesterol content is augmented or in the normal range (Lange *et al.*, 1980). The cholesterol content then evidently determines the preferred direction of buckling of the bilayer [as also probably does the disposition of the phospholipids, which are unequally distributed between the leaflets; phosphatidylserine, for example, the only major lipid with a net negative charge, is entirely on the inside (Zwaal *et al.*, 1973)].

Receptor events

The human red cell interacts with many biologically active substances in the nanomolar concentration range. It possesses binding sites for insulin (Herzberg *et al.*, 1980), growth hormone (Rubin *et al.*, 1973), acetylcholine (Huestis & McConnell, 1974), β -adrenergic agents (Rasmussen *et al.*, 1975; Nelson *et al.*, 1979) and prostaglandins (Rasmussen *et al.*, 1975; Kury & McConnell, 1975). In many of these cases, binding at the level of only a few molecules per cell has been reported to result in macroscopically detectable (if not necessarily interpretable) changes, reflected in perturbations of the signals from fluorescent or spin labels in the membrane, as well as in metabolic changes, in particular of phosphorylation levels of intracellular proteins (see below).

The first direct evidence of coupling between surface components and the cell interior (specifically the cytoskeleton) came from Nicolson & Painter (1973), who found that anti-spectrin antibodies sealed into ghosts caused redistribution of heavy-atom electron microscope markers attached to the saccharide residues of the glycoproteins on the outside; Ji & Nicolson (1974) also reported that when lectins were used to cross-link the external saccharides, chemical cross-linking of cytoskeletal proteins on the inside was facilitated. Effects of this nature are now of course accepted as a commonplace in other cells, lymphocytes and fibroblasts for example; in these cases cytoskeletal elements, such as actin filaments, are known to be implicated.

Cytoskeletal constituents

Electrophoretic analysis in sodium dodecyl sulphate/polyacrylamide gels of the total membrane protein reveals the presence of a large number of components. Four of these make up the cytoskeleton. In the standard designation of Fairbanks *et al.* (1971) (numbering from the origin of migration) these are bands 1 and 2 (which together make up spectrin), 4.1 and 5, which is actin. (A very minor

component, 4.9, is also generally present.) Spectrin, and to a lesser extent the other proteins, are released from the membrane into media of low ionic strength. The cytoskeleton can also be recovered *in toto* by extracting the cells with non-ionic detergent at high salt concentrations. It retains the outline of the cell, but in considerably shrunken form. Lux *et al.* (1978) have found indeed that the cytoskeleton is round if extracted from normal cells, sickle-shaped if from irreversibly sickled cells of sickle-cell anaemia patients, and elliptical from subjects affected by hereditary elliptocytosis. The stoichiometric relation between the constituents is very roughly 1:1:2.5 (molar proportions of either spectrin chain:4.1:actin). As regards the nature of the three proteins, we have the following information. Spectrin is composed of two chains of mol.wt. about 240 000 and 220 000, making up a heterodimer. Electron microscopy (Shotton *et al.*, 1979) shows these to be elongated and laterally associated, apparently adhering to one another at both ends and loosely twisted together. The contour length of the molecule is about 100 nm. Depending on the conditions of extraction, the spectrin is recovered either in this form or as tetramers, consisting of two such dimers associated end-on. Contrary to earlier assertions, spectrin resembles myosin in no significant respect, other than the molecular weight of the dimer. It is different in its structural character, its solubility characteristics, the manner of its interaction with actin and (as can now be stated with some assurance) the lack of ATPase activity. It is a markedly acidic protein, and becomes insoluble in the vicinity of its isoelectric point (between about pH 5 and 3). The two polypeptide chains differ from one another in sequence and in certain physical as well as functional properties (Calvert *et al.*, 1980).

As regards the remaining two cytoskeletal proteins, 4.1 is an apparently globular species of subunit mol. wt. about 80 000. It is probably dimeric in the membrane; the actin (mol.wt. 42 000) is of the β -type (operationally defined in terms of its isoelectric point), which is the prevalent cytoplasmic variant. The minor component, protein 4.9, co-purifies with the cytoskeleton, has a subunit mol.wt. of about 60 000, and is in other respects uncharacterized.

Organization of the cytoskeleton

The interconversion of spectrin heterodimer and tetramer is associated with a high activation energy, and in the cold or even at room temperature is so slow that either form persists essentially uncontaminated by the other for hours or days (Ungewickell & Gratzer, 1978). At low ionic strength the equilibrium strongly favours the dimer. If the extraction from the membrane into low salt is performed at 37°C, dimer alone is recovered. If the

spectrin is extracted at 4°C, the metastable tetramer is obtained. From this it follows that spectrin *in situ* is tetrameric (Ungewickell & Gratzer, 1978; Liu & Palek, 1980). Moreover, both the absence of any end-to-end, linear (isodesmic) self-association beyond the tetramer and the symmetrical disposition of binding sites on the spectrin (see below) show that the two heterodimer components of the tetramer are orientated head-to-head, not head-to-tail. At high protein concentrations cyclic oligomers, in which each chain associates at one end with a partner on a different heterodimer, have been identified by electron microscopy (Tyler *et al.*, 1980; Morrow & Marchesi, 1981).

The state of the actin is also now established beyond reasonable doubt. It is not, as once supposed, monomeric. Its associated nucleotide is largely ADP, not ATP (Pinder *et al.*, 1981); the cytoskeleton has binding sites for cytochalasins, which exhibits specificity for one end (the 'plus' or preferential growing end) of actin filaments (Lin & Lin, 1979), and it also contains elements that will nucleate the polymerization of G-actin (Cohen & Branton, 1979). The inference is that the actin is in the filamentous form. On the other hand, no characteristic filaments can be discerned in the membrane or isolated cytoskeletons in the electron microscope. Moreover, if the actin is to fulfil a linking function in the network, the total amount present in the cell taken together with the number of filaments required dictates that their average length must be very short. A direct measure comes from the titre of cytochalasin binding sites, that is to say of filament ends, present in the membrane (Lin & Lin, 1979; Brenner & Korn, 1980). Dividing this into the number of actin molecules per cell, one obtains a number-average degree of polymerization of 12–18. These short assemblies have been called 'protofilaments' (Brenner & Korn, 1980).

Studies of interactions *in vitro* between the isolated cytoskeletal proteins fulfil the expectations based on these findings. No associations can be detected with the other proteins when the actin is in the monomeric G-form. In the ternary system, containing F-actin, spectrin dimer and 4.1, a complex is formed, which presents the appearance in the electron microscope of isolated actin filaments randomly decorated with thin fringes, plainly spectrin dimers. For an extended network to be formed, the spectrin must be bifunctional and capable of cross-linking the actin filaments, that is to say tetrameric. Electron microscopy of the rapidly sedimenting pellet formed in this system reveals the presence of bridges, uniting the actin filaments (Ungewickell *et al.*, 1979).

In considering the function of 4.1, it should be remarked that the formation of a tight ternary complex implies that binary interactions must exist. Some published experiments show binding of

spectrin to actin in the absence of 4.1, others not. It is plain at all events that the interaction is weak, and whether it manifests itself will therefore depend on the design of the experiment. Brenner & Korn (1979) were the first to describe such an interaction. Its weakness in the absence of 4.1 is illustrated by several observations, for example the failure of a rapidly sedimenting product to appear in mixtures of F-actin and spectrin tetramers (Ungewickell *et al.*, 1979), and dissociation by the mere process of shearing of a viscometrically detected complex (Cohen & Korsgren, 1980). The affinity of spectrin for 4.1 is strong, and electron microscopy reassuringly reveals two binding sites on each tetramer, one at either end (Tyler *et al.*, 1980). We thus arrive at a model for the cytoskeleton in which spectrin tetramers, some 200nm long, constitute the structural members, being attached at their extremities, by way of 4.1 molecules, to multivalent junction points consisting of actin protofilaments (Fig. 1).

Notwithstanding the secure foundations for this model, certain ambiguities remain. For example it has been averred (Fowler & Taylor, 1980; Cohen & Korsgren, 1980) that the ternary interaction is in some manner modulated by calcium ions; other laboratories have found no such effect. The possibility must be considered that it is simply the state of the actin that is affected by calcium (see, e.g., Maruyama, 1981; Borejdo *et al.*, 1981).

Association with other membrane proteins: attachment of cytoskeleton to membrane

Bennett and his co-workers demonstrated the presence in the membrane of a protein with high affinity (dissociation constant 10^{-8} M) for spectrin (see, e.g., Bennett, 1978; Bennett & Stenbuck, 1979). This protein was identified as the electrophoretic component, 2.1 (mol.wt. 210000) and was termed ankyrin. (Other names were floated by other workers, but have proved less durable.) The binding site for spectrin is contained in a globular fragment of mol.wt. 72000, which can be obtained from ghosts by proteolysis. The association becomes weak at low ionic strength, which is why spectrin is liberated from the membrane under such conditions. The binding site for 2.1 is close to the proximal end of the spectrin dimer, that is to say near the middle of the tetramer, or the opposite end to the actin and 4.1 binding site (Tyler *et al.*, 1980; Morrow *et al.*, 1980).

The 2.1 is not an integral membrane protein, for it is solubilized by media of high ionic strength. Recent investigations (Bennett & Stenbuck, 1979; Hargreaves *et al.*, 1980) have established that 2.1 is attached to the membrane by way of band 3, the preponderant integral membrane protein. This species [for a review see Steck (1978)], which has a mol.wt of some 90000, traverses the membrane and

Fig. 1. Schematic depiction of red cell membrane organization (not to scale)

Band 3 is the major integral membrane protein, and is present as dimers or tetramers. To its cytoplasmic domain is attached band 2.1 (ankyrin) and other proteins, such as band 6 (glyceraldehyde 3-phosphate dehydrogenase). The 2.1 is the primary point of attachment of the cytoskeleton by way of two sites on each spectrin tetramer (only one of which on average is occupied). The spectrin tetramers (consisting of two heterodimers associated head-to-head) are attached at their ends to junction points consisting of short filaments of F-actin (protofilaments) and protein 4.1. The cytoskeletal components are shown in black. PAS-1 is the sialoglycoprotein, also known as glycophorin. This occurs as a dimer; there are some 200000 such dimers per cell, about 300000 tetramers of band 3 (if all the band 3 is tetrameric), and 100000 molecules of ankyrin. The carbohydrate chains on PAS-1 and band 3 are represented as whiskers on the extracellular domains. Other proteins (e.g. 4.2 and 4.9) can be inferred from various lines of evidence to be associated with parts of this system; these have been omitted in the interests of clarity, and their functions are in any case unknown.

has an outer, glycosylated domain, and an intracellular domain, which contains the binding site for 2.1, as well as other sites for several proteins, such as glyceraldehyde 3-phosphate dehydrogenase (band 6). Band 3 is also the anion channel. It is self-associated to at least the dimeric and most probably the tetrameric state, and forms the substance of the intramembrane particles, observed in the electron microscope as protuberances on the freeze-fracture faces of the membrane. To it also is bound the transmembrane sialoglycoprotein, PAS-1 or glycophorin, which is itself dimeric (Furthmayr, 1978).

The membrane of each red cell contains an estimated 1.2×10^6 molecules of band 3, and 100 000 molecules of 2.1, so that only a minority of the intramembrane particles are encumbered with 2.1 (and for reasons that are far from clear no more 2.1 than corresponds to this ratio is taken up by the band 3 in 2.1-depleted membrane vesicles). Spectrin is present to the extent of 100 000 tetramers, each therefore attached on average to the membrane by only one of its 2.1-binding sites. Clearly the constellations formed by band 3, 2.1 and spectrin afford pathways linking the inside and outside of the cell. A schematic view of the important interactions is shown in Fig. 1. There are very possibly secondary modes of interaction between the cytoskeleton and the membrane; this would seem to follow for example from the observation that when spectrin is extracted at low salt, some of the actin and the bulk of the 4.1 remain with the membrane.

Relation between the cytoskeleton and gross membrane properties

The manner in which the cytoskeleton stabilizes the lipid bilayer is not altogether clear, but in restricting intramembrane particle migration it undoubtedly militates against the formation of bare patches in the membrane, which would constitute sites for incipient exo- and endocytosis, vesiculation or fusion. A direct interaction is not out of the question, for spectrin is evidently capable of some association with phospholipids, and in particular the inner leaflet constituent, phosphatidylserine; there are, moreover, indications of a sort that such an interaction *in situ* may indeed stabilize the bilayer in respect of phospholipid flip-flop and the tendency towards fusion (Haest *et al.*, 1978; Gerritsen *et al.*, 1979).

The manner in which the cytoskeleton affects the movement of intramembrane proteins is an engrossing question. Flash relaxation experiments on cells labelled from the outside, therefore almost entirely on band 3, with a luminophore have shown that the bulk of the band 3 is rotationally unconstrained, and is thus not bound to the cytoskeleton (Cherry *et al.*,

1976); a minor immobilized fraction becomes free when the 2.1 is extracted. Nevertheless the lateral diffusion of band 3 is very slow as long as the cytoskeleton remains in place (Peters *et al.*, 1974; Fowler & Branton, 1977). These observations can most simply be reconciled if one supposes the band 3, projecting inwards into the cytoplasm, to be physically confined within the interstices of the cytoskeletal net, its rate of diffusion being limited by the stability of this structure. This (albeit undoubtedly simplified) view receives support from the results of Schindler *et al.* (1980), who have found that the rate of diffusion is much elevated by the addition of polyvalent anions (polyphosphates, ATP, 2,3-bisphosphoglycerate), each of which has a parallel tendency to solubilize isolated cytoskeletons (Sheetz & Casaly, 1980).

Mechanisms of metabolic shape control

In this regard, the last 3 years have seen an almost unremitting regress in our grasp of the system. Until recently there seemed every reason to be content with the evidence of Birchmeier & Singer (1977) that the shape of the red cell was controlled by the phosphorylation of spectrin under the action of ATP and an endogenous (cyclic AMP-independent) kinase. It is not disputed that spectrin is phosphorylated and that a turnover is maintained by the kinase and a phosphatase. There are four phosphorylation sites, all clustered at the C-terminal end of the smaller subunit (Harris & Lux, 1980). However, Anderson & Tyler (1980) have shown that echinocytosis sets in and indeed is half complete before dephosphorylation of spectrin becomes apparent. Mg-ATP-dependent shape changes in ghosts are unrelated to phosphorylation levels (Patel & Fairbanks, 1981). Moreover, none of the known properties of spectrin, at least when pure, are sensibly perturbed by phosphorylation. These include its association with 2.1 on the membrane (Bennett, 1978), its dimer-tetramer equilibrium (Ungewickell & Gratzer, 1978) and its propensity to form complexes with actin and 4.1 (Ungewickell *et al.*, 1979; Brenner & Korn, 1979).

No satisfactory alternative to the spectrin phosphorylation hypothesis of shape control has yet been formulated. Possibilities to be entertained, and which await exploration are the following. (1) ATP is needed for the phosphorylation of other proteins implicated in cytoskeletal interactions. These include 4.1 and actin, both of which are substrates for a different (cyclic AMP-dependent) kinase (as also indeed are 2.1 and band 3). (2) When ATP gives place to ADP, the critical monomer concentration in equilibrium with actin filaments rises, and short filaments would be expected to become less stable, and might thus tend, by virtue of the co-operativity

of the monomer-polymer equilibrium, to disproportionate to fewer, longer filaments, with loss of cytoskeletal junction points. That this tendency is inherent in actin as it is organized in the red cell is attested by the spontaneous appearance of long F-actin filaments after proteolytic destruction of spectrin (and no doubt 4.1, which is even more labile to proteolysis) (Tilney & Detmers, 1975). (3) The ATP (and for that matter 2,3-bisphosphoglycerate) may be required to maintain the dynamic state of the cytoskeletal complex (Schindler *et al.*, 1980; Sheetz & Casaly, 1980). The disappearance of these ions might allow co-operative clustering of spectrin, with its 4.1, on some protofilaments at the expense of others; such a rearrangement, not altogether unlike the formation of a rigor complex in muscle, would lead to interruptions in the continuity of the cytoskeleton.

Other cellular events: significance of phosphorylation

It seems likely that processes such as fusion, exocytosis and endocytosis may result from dislocations in the cytoskeleton with consequent local uncoupling from the membrane. The early inference by Lucy and his associates (see for example Vos *et al.*, 1976) that fusion follows on the formation of bare patches in the membrane, devoid of intramembrane particles, has been abundantly borne out. Likewise endocytosis is reportedly presaged by the disappearance of intramembrane particles from the locality in which the initial invagination occurs (Hardy *et al.*, 1979), and at the same time the inner surface becomes depleted of spectrin. This effect is marked in neonatal red cells, which are especially prone to endocytosis (Tokuyasu *et al.*, 1979). Moreover, both Sendai virus-induced fusion (Sekiguchi & Asano, 1978) and endocytosis (Hardy *et al.*, 1979) are blocked by prior introduction of anti-spectrin antibodies. More, fusion is accompanied by considerable dephosphorylation (Loyter *et al.*, 1977). Other structural disturbances are also apparently linked to changes in phosphorylation; for example, echinocytosis, induced by exposure to serum low-density lipoprotein (in the absence of high-density lipoproteins), is accompanied by dephosphorylation of spectrin (Hui & Harmony, 1979), and the binding of β -adrenergic agents to receptors on the cell surface by a rise in phosphorylation (Nelson *et al.*, 1979), as well as a perturbation of the membrane structure, as revealed spectroscopically.

In the latter case it has been argued (Nelson & Huestis, 1980) that the phosphorylation changes are mediated by calcium ions, which may indeed regulate both kinase and phosphatase activities. The introduction of calcium into red cells of itself causes

crenation and other changes. In addition to running down the ATP and thereby, as well as through its effect on the kinase and phosphatases, altering the phosphorylation balance, it can, by activating a phosphodiesterase, cause loss of the minor inner-leaflet phosphoinositide constituents with release of 1,2-diacyl-*sn*-glycerol (Allan & Michell, 1978), activate a calcium-dependent proteinase and (at rather higher concentrations) stimulate the activity of an endogenous transglutaminase, that will irreversibly cross-link cytoskeletal constituents (Siefing *et al.*, 1978).

Abnormal red cells

A number of genetic abnormalities of red cell shape have been recognized, one of which, hereditary spherocytosis, is a common condition, with an incidence of about 1 in 5000 in white populations. There is circumstantial evidence to identify the genetic lesion with an anomaly of the cytoskeleton. In some cases of the rarer and less defined condition, hereditary elliptocytosis, and in pyropoikilocytosis, a spectrin abnormality, revealing itself in an altered dimer-tetramer equilibrium, has been reported (Palek & Liu, 1981). For a survey of the evidence for cytoskeletal abnormalities in genetic conditions affecting the red cell see the definitive review by Lux & Glader (1981).

Relation of red cell cytoskeletal proteins to constituents of other cell types

Whether a membrane-associated cytoskeletal network of the kind that has been discussed here exists in other types of cell is not clear. The evident intrinsic instability of a unit membrane of extended surface area suggests the need for a stabilizing substructure of some description. Spectrin, however, has not been detected in anything other than red cells, although only a restricted search, extending to a few lines of tissue culture cells (Hiller & Weber, 1977) has as yet been carried out. In gross morphological terms, though not as has so far been demonstrated in function, spectrin bears a certain resemblance to some other high-molecular-weight proteins, such as filamin and macrophage actin-binding protein (Tyler *et al.*, 1980). Especially interesting is a report by Bennett (1979) of the immunological identification of 2.1 (ankyrin) in several types of cells, albeit in very low concentrations; a protein designed to bind both spectrin and band 3 with very high affinity must be expected to enter into analogous interactions in other cells in which it occurs. A search for universal principles underlying the construction of filamentous assemblies of cells is probably however at this stage premature.

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