PERIPHERAL PROTEINS AND SMOOTH MEMBRANE FROM ERYTHROCYTE GHOSTS

Segregation of ATP-Utilizing Enzymes into Smooth Membrane

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ABSTRACT

Erythrocytes and their isolated membranes display ATP-dependent endocytosis. To localize the enzymes responsible for this phenomenon, the erythrocyte membranes (ghosts) were fractionated under conditions which retained ATPase activity. Fractionation of the ghosts resulted in three fractions: spectrin-actin, the peripheral proteins soluble in high salt, and the smooth membrane containing integral proteins. On the average, 87% of the protein and 88% of the phosphorus of the original ghosts were recovered in these fractions, and all of the kinds of ATP-splitting activities of the membrane were recovered in the smooth membrane. A tiny ATPase activity, detectable by special methodology in spectrin-actin, could have been due to contamination with membranous material. Although the purified spectrin-actin did not have a significant ATPase of its own, it stimulated the Ca²⁺, Mg²⁺-ATPase of the smooth membrane significantly, suggesting a cooperative interaction between these two fractions. This segregation of the ATPase activities into the smooth membrane, combined with the energy dependence of endocytosis, showed that the smooth membrane must be involved in the energy production for endocytosis. The possibility that the spectrin-actin filaments cooperate with a myosinlike ATPase in the membrane to generate membrane movements is discussed.

KEY WORDS ATPase, erythrocyte membranes, spectrin, actin-binding protein, endocytosis

Recent studies on motility of endocytic cells such as macrophage and Acanthamoeba castellanii, have focused on the cytoplasmic components which appear to be responsible for these gross movements of cells, such as pseudopod formation, cytoplasmic flow, and cell motility. These studies have emphasized the presence of a cytoskeletal network of actin, myosin, and various necessary cofactors in such cells (22). For example, when actin, myosin, actin-binding protein, and a protein cofactor from rabbit pulmonary macrophages were mixed, a cooperative interaction was observed which suggested that these components were all involved in cellular motility (30).

Of these components, the actin-binding protein was present in greater amounts in homogenates.
of phagocytizing macrophages than in homogenates of resting macrophages, suggesting its involvement in movements associated with endocytosis. This involvement of actin in cellular movement has also been extended to its interaction with the plasma membrane; association of actin with brush border membranes has been investigated and its points of attachment to the membrane have been observed (17).

However, in such studies on whole cells, it is difficult to separate the cytoplasmically controlled cell movements from those which may be carried out by proteins located on and in the membranes. Mature mammalian erythrocytes and their ghosts provide a unique system for the study of the actual endocytic event; they lack a cytoskeletal network, and do not move in the way that more active cells do, but do still take in membrane and form vacuoles in an endocytic process requiring ATP hydrolysis (3, 10, 13, 26). Since the ghosts are morphologically intact, yet lacking in cytoplasm, they offer an opportunity to study this endocytic event in the absence of the complexities present in the whole cell. Since these membranes do not indulge in gross movement, cytoplasmic flow, or pseudopod formation, our attention is necessarily focused on the endocytic event itself, in the absence of any of the other movements which ordinarily accompany pinocytosis or phagocytosis.

The endocytosis observed in erythrocytes and their ghosts is closely linked to shape control in these cells. As has been previously observed (21), the endocytic event can occur as a natural extension of the echinocyte-discocyte-stomatocyte shape progression in erythrocyte ghosts, with the total curvature of the membrane decreasing smoothly during this progression. Thus, the act of endocytosis in erythrocytes and their ghosts appears to be a result of the action of the same components of the membrane which are responsible for shape control.

Most previous studies have focused attention on the peripheral proteins of erythrocyte membranes as being responsible for shape control (28). For example, the resemblance between the major peripheral proteins of the erythrocyte ghost (spectrin and erythrocyte actin) and the proteins present in the contractile systems of other cells has been pointed out by a number of workers. Spectrin has been said to resemble, to some degree, the actin-binding protein of rabbit pulmonary macrophages (30), and it resembles even more closely the actin-associated proteins of echinoderm sperm (32). The unusually large monomer molecular weight of spectrin, together with its circular dichroism, is similar to that of myosin (25), but it can be isolated in a form which forms only monomers and dimers in solution (11), unlike myosin, which forms much larger aggregates.

These important analogies between the spectrin-actin network and contractile systems of other cells must certainly be taken into account in formulating any model for shape control and endocytosis in erythrocyte membranes. However, the very low ATPase activity reported for the spectrin-actin network (23) (nearly three orders of magnitude lower than that reported for myosin [22]) make it unlikely that this network could provide the energy necessary for endocytosis unassisted.

On the other hand, there has been relatively little discussion of a possible role of the integral membrane proteins in erythrocyte shape control. Lateral movement of the intramembranous particles in response to various treatments of the ghosts was first observed by Pinto da Silva (20) and then by Elgsaeter and Branton (6) and Elgsaeter et al. (7). However, the relationship between membrane movement and movement of the intramembranous particles, or between movement of peripheral and integral proteins, has not yet been made clear.

We propose to take a somewhat different approach to determination of the function of the separable parts of the erythrocyte membrane. We report here a separation of peripheral proteins from the erythrocyte membrane with a nearly quantitative recovery of protein and lipid. By an analysis of the localization of the ATP-splitting enzymes (ATPases and protein kinases), we hope to cast some light on the role of the various fractions in the membrane movements which require ATP hydrolysis.

A preliminary report of this work has already appeared (12).

MATERIALS AND METHODS

Preparation of the Membrane Fractions

The starting material for the preparation was 50 ml of intact human erythrocyte ghosts suspended at 5 mg protein/ml in 28 mM TES - Et3N, pH 7.4.

Unusual abbreviations: TES, N-Tris-(hydroxymethyl) methyl-2-aminoethane sulfonyl acid; Et3N, triethanolamine.

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at 0°C. The ghosts were prepared from fresh erythrocytes by the modified Dodge procedure previously described (15). They were prepared from fresh red cells, and smooth membrane was prepared from fresh ghosts.

The ghosts were fractionated into smooth membrane, KCl extract, and spectrin-actin by the procedures shown in Scheme 1. The first dialysis of the ghosts was necessary to remove the buffer in which the ghosts were suspended. If the dialyzing solution was kept below 5°C, no spectrin was released from the membrane by this dialysis. No proteolysis occurred during the 37°C incubation, as is evident from the gels. Even much longer incubations at 37°C yielded intact spectrin as judged by electrophoresis. All centrifugations were carried out in the Beckman JA-20 fixed angle rotor at 20,000 rpm (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) (maximum force, 48,300 g). All solutions were adjusted to pH 7.4 at 5°C unless otherwise indicated, and the resulting fractions were stored at -20°C. After the first centrifugation, it was necessary...

**Scheme 1**

50 ml, 5 mg/ml Human erythrocyte ghosts

↓

Dialyze overnight vs. 1 mM EDTA at 0°C

↓

Centrifuge 30 min.

↓

Pellet resuspended in 200-250 ml 0.1 mM ATP, 0.1 mM EDTA, pH = 8.0

↓

Incubate 30 min at 37°C

↓

Observe by phase-contrast microscopy

↓

Fragments

↓

Centrifuge 40 min

↓

Pellet resuspended in 0.1 mM EDTA

↓

Pellet

↓

Supernate (discard)

↓

Supernate

↓

Centrifuge 40 min

↓

Centrifuge 30 min.

↓

Extract overnight with 0.1 M KCl

↓

Pellet

↓

Supernate

↓

Dialyze vs. 3 × 20 vol H2O

↓

Centrifuge 30 min

↓

KCl extract

↓

Supernate

↓

Concentrate to 5-10 ml by dialysis vs. dry polyethylene glycol 20,000

↓

Dialyze extensively vs. 0.1 mM EDTA, 0.1 mM EGTA

↓

Spectrin-actin

↓

Repeat once, not overnight, discard supernate

↓

Pellet

↓

Wash twice, 5 mM TES-ETN, centrifuge 30 min, store -20°C in 1 mM dithiothreitol

↓

Smooth membrane

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to allow the rotor to coast to a stop without braking; in both the first and second centrifugations the supernatant fluid was removed carefully with a Pasteur pipette. The second pellet could be packed more tightly by spinning for 30 min at 40,000 rpm in the 50 Ti Spinco rotor (Beckman Instruments, Spinco Div.). Both the smooth membrane and the KCl extract formed rather insoluble pellets; in both cases, a brief sonication (2–3 s) was used to homogenize the stored material for further use.

The negatively stained preparations shown in the electron micrographs in Fig. 1 were obtained after the indicated time of incubation, by making the solution 1% in glutaraldehyde to fix the ghosts. To break open the fixed ghosts so that the inner face of the erythrocyte membrane could be seen, the fixed suspension was sonicated. This sonicated suspension was then negatively stained with 1% uranyl acetate.

**Chemical Methods**

The phospholipid phosphorus, sialic acid, and protein concentrations were measured as previously described (13). The ATPase assays were also carried out as previously described (13) except that all the assays were 1 mM in dithiothreitol; all assays not containing NaCl and KCl were 0.1 mM in ouabain and all assays not containing CaCl2 were 0.5 mM in ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetate (EGTA). When proteins from fractions other than ghosts were assayed, the protein concentration varied somewhat, in the range of 1/2–2 mg/ml.

Electrophoresis was carried out using the buffers of Fairbanks et al. (9), and 7.5% gels. The samples were dissolved in 1% SDS, 0.1 M Tris-HCl, pH 8.6, 1% dithiothreitol, 1 mM EDTA, and enough sucrose to dissolve the mixture so that the inner face of the erythrocyte membrane could be seen, the fixed suspension was sonicated. This sonicated suspension was then negatively stained with 1% uranyl acetate.

**RESULTS**

**Properties of the Peripheral Proteins and of the Smooth Membrane Fraction**

The fractionation procedure described in Materials and Methods not only resulted in the preparation of a pure fraction of the peripheral proteins soluble in distilled water (spectrin-actin fraction), such as has been seen by others (9), but in addition it yielded two other fractions, the smooth membrane and the peripheral proteins soluble in high salt (KCl extract). Fig. 1 shows the appearance of negatively stained preparations from ghosts made before and after the separation of peripheral proteins from the smooth membrane. Fig. 1A shows the peripheral proteins on the inner face of the membrane before removal. Fig. 1B shows the appearance of the purified spectrin-actin fraction, while Fig. 1C shows the remaining membranes, devoid of spectrin-actin strands on the surface of the membrane. The fractions from ghosts were analyzed, and a balance sheet was constructed for the recovery of protein and phosphorus in the various fractions. Table I shows that the three fractions recovered account for most of the protein in the starting materials. The average recovery was 87% of the starting protein; this is good recovery, considering the number of steps in the procedure and the opportunities for nonspecific loss of protein and lipid. Analysis of the phosphorus content (generally accepted as a good measure of the amount of phospholipid present, since phosphorus containing metabolites are absent) showed that, on the average, the smooth membrane contained 88% of the total phospholipid of the starting material, in good agreement with the overall recovery of protein in all the fractions. Those results indicated that there was no preferential discarding of protein or of phospholipid, but that both were recovered in good yield by this procedure.

The distribution of the ATPase activities among the various fractions is shown in Table II. As this table illustrates, neither the spectrin-actin fraction nor the KCl extract had an ATPase activity measurable by our normal techniques. However, the use of γ-32P-labeled ATP allowed us to detect a very small Ca2+, Mg2+-ATPase in the spectrin-actin fraction.

The data of Table II also show that all of the different kinds of ATPase activities of the starting ghosts were recovered in the smooth membrane fraction. The amount of ATPase activity recovered also appeared to be good; the second line of the table shows the activity calculated for reconstructed erythrocyte ghosts, assuming that all of the ATPase was recovered in the smooth membrane and that the ghosts contained 54% smooth membrane and 46% other protein material which had no ATPase activity. The specific activity of these hypothetical reconstructed ghosts...
Figure 1. The peripheral proteins and the smooth membrane. (A) Spectrin-actin on the inside of intact ghosts. (B) Spectrin-actin fraction. (C) Smooth membrane after removal of all peripheral proteins. Bars, 100 nm.
was in good agreement with the specific activities observed for the actual red cell ghost preparations. However, calculations such as these must be regarded with caution, since some of the purification procedures may have activated the ATPases, masking a loss of enzyme.

To investigate all of the ATP-splitting processes in ghosts, we also measured the phosphorylation of protein in some of the fractions. The amount of radioactive phosphorus incorporated into the membranes from γ-32P-labeled ATP was measured after washing the membranes. Table III shows that there was a high protein kinase activity in human erythrocyte ghosts, with a somewhat lower activity in the smooth membrane and no detectable activity in the spectrin-actin fraction. The protein kinase observed here was stimulatable by cAMP and inhibited by Ca²⁺, and phosphorylated bands 2, 3, 4.5, and the material migrating with the lipid (L. P. Vaughan, personal communication).

Although all of the kinds of ATPases were recovered in the smooth membrane, the kinetic properties of the Ca²⁺-stimulated ATPase were somewhat altered by the preparation procedure. A comparison of the kinetics of the Ca²⁺-stimulated ATPase of human ghosts and of smooth membrane is shown in Fig. 2. The high and low affinity Ca²⁺-stimulated ATPase activities apparent in the ghosts were converted to an ATPase of a single intermediate Ca²⁺ affinity in the smooth membrane. Similar conversions occur in whole ghosts due to variations in the method of preparation (24).

The protein composition of the various fractions is shown in Fig. 3. These scans of SDS electrophoresis gels show that the smooth membrane was very rich in the intrinsic protein components of bands 3 and 4, while containing <2% spectrin (the numbering system is that of Steck [28] except

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### Table I

| Protein Phospholipid in Smooth Membrane and Peripheral Proteins |
|---------------------------------------------------------------|
| Protein          | Phospholipid |
|------------------|--------------|
| % of that in whole ghost | mg phospholipid/ mg protein |
| Starting material (human ghosts) | 100 | 0.67 ± 0.02 |
| Smooth membrane  | 54 ± 6       | 1.10 ± 0.01 |
| Spectrin-actin fraction | 31 ± 2 | <0.01 |
| KCl extract      | 2.5 ± 0.5    | -            |

The protein recoveries are the mean and standard error of the mean of five samples; the phospholipid, of two samples.

## Table II

### ATPase Activities of Ghosts, Smooth Membrane, and Peripheral Proteins

| ATPase | Mg²⁺ | Na⁺, K⁺, Mg²⁺ | Ca²⁺, Mg²⁺ | Na⁺, K⁺, Ca²⁺, Mg²⁺ |
|--------|------|--------------|------------|---------------------|
|        | nmol, mg⁻¹, min⁻¹ | nmol, mg⁻¹, min⁻¹ | nmol, mg⁻¹, min⁻¹ | nmol, mg⁻¹, min⁻¹ |
| Smooth membrane | 4 | 6.9 ± 0.6 | 15.4 ± 2.1 | 19.5 ± 2.7 | 31.6 ± 3.9 |
| Smooth membrane × 0.54 | - | 3.7 ± 0.3 | 8.3 ± 1.1 | 10.5 ± 1.5 | 17.1 ± 2.1 |
| Human ghosts | 4 | 3.0 ± 0.4 | 6.5 ± 0.5 | 9.6 ± 1.0 | 16.8 ± 1.7 |
| Spectrin-actin | 3 | 0.0 | 0.0 | 0.0* | 0.0 |
| KCl extract | 3 | 0.0 | 0.0 | 0.0 | 0.0 |

The number of different preparations for which values of ATPase activity were averaged was n; activities are reported ± SEM. Since the smooth membrane contained, on average, 54% of the protein of the ghost, the information on the second line represents the theoretical activity of ghosts, assuming that all of the ATPase was recovered in the smooth membrane. Clearly, all of the kinds of ATPase of the ghosts can be accounted for in the smooth membrane.

* When measured by release of 32P from γ-32P-labeled ATP, the ATPase in the presence of 0.5 mM CaCl₂ and 6 mM MgCl₂ was 0.14 ± 0.04 nmol, mg⁻¹, min⁻¹, and in the presence of 0.5 mM CaCl₂ alone, 0.05 ± 0.02 nmol, mg⁻¹, min⁻¹.

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FIGURE 2 Eadie-Hofstee plot with Ca\(^{2+}\) as the variable for the Ca\(^{2+}\)-stimulated component of the ATPase of ghosts (open circles) and smooth membrane (filled circles). The low Ca\(^{2+}\) affinity component of this ATPase (left side), which normally appears in EDTA ghosts, was converted to a higher affinity form by removal of the peripheral proteins. On the horizontal axis, the units of v are the same as shown on the vertical axis, while [Ca\(^{2+}\)] is in nanomolar.

that the recently described band 3.5 [5] is marked separately from band 3). The spectrin-actin fraction consisted mainly of bands 1 and 2 with a minor component of band 5, the "actin" of erythrocyte membranes (33). This composition of this fraction agrees with previous reports (9).

The KCl extract represents those peripheral proteins extractable by high salt. The major components of this extract were: band 3.5, band 4.1, and a faster running band which corresponded to band 7 or 8 of whole erythrocyte ghosts. In addition, this fraction was contaminated with some spectrin.

An effect of the interaction of the spectrin-actin fraction and the smooth membrane is the activation of the Ca\(^{2+}\), Mg\(^{2+}\)-ATPase shown in Table IV. This activation, which averaged 4.8 ± 0.5 nmol, mg\(^{-1}\), min\(^{-1}\) for three different ghost preparations, was more than one order of magnitude greater than the intrinsic activity of the spectrin-actin alone. Statistical analysis of the results from three different ghost preparations, using the t test with paired variates, showed that the stimulation of the Ca\(^{2+}\), Mg\(^{2+}\)-ATPase was significant at \(P = 0.02\), while none of the others were significantly stimulated even at \(P = 0.10\). This stimulation of the Ca\(^{2+}\), Mg\(^{2+}\)-ATPase demonstrates a cooperative interaction between the smooth membrane ATPase and the spectrin-actin fraction. Other experiments showed that addition of the KCl extract to the mixture of spectrin-actin and smooth membrane did not cause any further activation.

DISCUSSION
The data presented have shown that all of the kinds of ATPases which could provide energy for this membrane movement are integral to the membrane. Before we draw some general conclu-
TABLE IV
Stimulation of Ca$^{2+}$, Mg$^{2+}$-ATPase of Smooth Membrane by Spectrin-Actin

| Addition       | Mg$^{2+}$, Na$^{+}$, K$^{+}$ | Mg$^{2+}$, Ca$^{2+}$, Na$^{+}$, K$^{+}$ |
|----------------|-----------------------------|---------------------------------------|
| None           | 7                           | 12                                    |
| Spectrin-actin | 5                           | 10                                    |

The ratio of spectrin-actin to smooth membrane protein was 0.3:1.

The table shows that Mg$^{2+}$-ATPase activity was stimulated by Ca$^{2+}$ addition, with a ratio of 5:1 for Mg$^{2+}$:Ca$^{2+}$. 

Avisser et al. (1) prepared a crude erythrocyte membrane extract containing integral proteins and ATPases; from this, they purified an ATPase which required both Ca$^{2+}$ and Mg$^{2+}$ and had a specific activity of 1-6 nmol, mg$^{-1}$, min$^{-1}$.

Weidekamm and Brdiczka (34) extracted a spectrin-like fraction from ghosts which had a very high Ca$^{2+}$, Mg$^{2+}$-ATPase (122 nmol, mg$^{-1}$, min$^{-1}$) and which was detected by an enzymic method which measured the ADP produced instead of inorganic phosphate as in the present report. This fraction contained other protein bands, in addition to bands 1, 2, and 5. They observed a concurrent decrease in the ATPase activity of the extracted ghosts, making it clear that they were extracting material which remains with the smooth membrane in our procedure.

This summary of the literature shows that the purer spectrin-actin preparations had extremely low ATPase activities, and that the ionic requirement reported for the ATPases varied from one laboratory to another. Our present report adds to this variability, since we saw a low activity in the presence of Ca$^{2+}$ which was stimulated by Mg$^{2+}$.

The only reports on the phosphorus content of the spectrin-actin fraction are that of Avisser et al. (23) and the present report. Both have reported <1% (wt/wt) phospholipid, which would correspond to <2.4% (wt/wt) ghost membranes. That level of contamination by membrane fragments would account for a total Ca$^{2+}$, Mg$^{2+}$-ATPase of 0.23 nmol, mg$^{-1}$, min$^{-1}$, comparable with what was found in the samples reported here, when the assay method employing radioactive ATP was used.

However, it is not possible to rule out the possibility that the low level of ATPase detected in the spectrin-actin fraction really belongs to spectrin or actin; ATPase assays on more highly purified protein may be necessary to finally resolve this question.

Energy Requirement for Endocytosis

Because our data confirm the presence of a very tiny ATPase in the spectrin-actin fraction, some light may be cast on the relationship between this ATPase and membrane bending by considering the amount of energy required to

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cause bending in an erythrocyte membrane. Evans (8) has estimated the interfacial free energy change required to cause bending in the erythrocyte membrane; he estimates the energy required to initiate bending to be about $10^{-2}$ erg/cm$^2$. Progressively greater bending of the membrane in hemispherical projections such as occur in crenation of the erythrocyte was estimated by him to require up to 1 erg/cm$^2$ interfacial free energy change.

One may apply the results of Evans to invagination of the same membrane, since the geometry is similar. For a single portion of membrane the size of an average vacuole (1 $\mu$m$^2$ in area), the energy to initiate bending would be $10^{-10}$ erg. Since $kT$ at 37°C is about $4 \times 10^{-18}$ erg, even the initiation of bending would not occur as a result of thermal agitation. Assuming that vacuolation occurs over 40% of the ghost area (15), and converting to joules and milligrams, calculations show that the energy required to initiate bending for invagination would be about $1.3 \times 10^{-2}$ J/mg protein. Just as for crenation, the energy required for full formation of hemispherical invaginations would be up to 100 times as great.

This energy required for bending may be compared with that available from ATP hydrolysis by the spectrin-actin network. Based on the data reported here, the spectrin-actin ATPase could hydrolyze 1.4 nmol ATP/mg spectrin-actin protein over a period of 10 min, or about 0.4 nmol/mg total ghost protein. This would give an energy yield of about $2 \times 10^{-5}$ J/mg, about ten times the amount required to initiate bending, but one-tenth the amount probably required to form hemispherical invaginations. The period of 10 min was chosen because substantial vacuolation normally occurs in ghosts after 10 min of incubation with ATP (15).

The amount of energy available in a span of 10 min from the spectrin-actin ATPase would clearly be enough to carry the process beyond this point. The energy required for completing the invagination and pinching off a vacuole cannot be calculated at present, but one might expect it to be at least comparable to, and probably much greater than, the energy required for the first stages of endocytosis. Thus, even if the ATPase activity of the spectrin-actin fraction is truly a property of the peripheral protein network, energetic considerations show that this ATPase is probably inadequate to provide the energy for vacuolation.

### Locale of Protein Kinase

The study of the location of the protein kinase completes the localization of the known ATP-hydrolyzing proteins on the erythrocyte ghosts. Like the ATPases, protein kinase was found on the smooth membrane, and the amount of phosphorylation of the peripheral protein fraction was small, or more usually zero, as shown in the respective results in Table III. Since spectrin is a good substrate for protein kinase, the absence of phosphorylation in the spectrin-actin fraction suggests the absence of any protein kinase in this fraction.

### General Conclusions

The peripheral proteins have been widely thought of in terms of a structural and/or motile system lying on the inside of the membrane; many workers have emphasized the resemblance of spectrin to myosin (25); the presence of actin together with spectrin and the weak immunological cross-reaction between spectrin and myosin (27) have strengthened this analogy. However, the absence of any significant level of ATP splitting in the peripheral protein fractions, combined with the presence of a number of ATPases and other ATP-splitting activities on the membrane proper, raises interesting possibilities concerning the way in which energy can be put into the spectrin-actin system in order for movement to occur.

If we accept the idea that the isolated peripheral protein fractions have too low a content of ATP-splitting enzymes to support membrane movements, then it must follow that such movements get energy either from the smooth membrane acting alone, or in cooperation with the peripheral proteins. The evidence presented here does not distinguish between these two possibilities. When this evidence is considered together with the body available from the spectrin-actin ATPase would be enough to carry the process beyond this point.
of evidence which exists concerning the nature of spectrin (11, 25, 27), it appears most likely that membrane movement occurs as a result of a cooperation between the smooth membrane, which has the ATP-splitting enzymes to supply the energy, and the spectrin-actin, which has structural properties similar to those which occur in filaments found in other contractile systems.

The relatively small amount of actin on the membrane, combined with the enzymic inactivity of spectrin, suggests that the spectrin-actin network may fulfill a role comparable to that of the actin filaments in skeletal muscle. Because the distances over which movement occurs are relatively small in this system, and because large amounts of energy generation are not required, it may be that the filaments in this system are composed of actin plus an actin-binding protein (the spectrin) which is used in those parts of the filament which do not require the movement generating capabilities of actin. In such a picture, an ATPase on the membrane itself would fulfill the role which is assumed by myosin in the muscle system. It is not clear which ATPases are responsible for endocytosis, although the Ca$^{2+}$-stimulated ATPase is probably involved. It seems to us that a model such as this explains the data well, and retains the role of spectrin and actin in a contractile system, based on the strong analogies which exist between spectrin and actin and the components of the contractile system of muscle.

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