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Asymmetric distribution of plasma membrane proteins in mouse L-929 cells

(phagolysosome/lactoperoxidase iodination/two-dimensional gel electrophoresis/transmembrane proteins)

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ABSTRACT **The distribution of plasma membrane-associated proteins was studied by using latex-filled phagolysosomes prepared from cultured mouse L-929 cells as a model of "inside-out" membrane. Proteins from ^{131}I/lactoperoxidase-labeled phagolysosomes, phagolysosomes derived from ^{131}I/lactoperoxidase-labeled cells, and phagolysosomes prepared from [^{35}S]methionine metabolically labeled cells were analyzed by high-resolution two-dimensional gel electrophoresis. The gel patterns of iodinated proteins showed specific differences in the availability of membrane proteins to lactoperoxidase labeling between inside-out and right-side-out membranes. However, at least two prominent [^{35}S]methionine-labeled proteins of approximately 60,000 and 100,000 daltons were available for iodination at both sides of the membrane. Partial proteolysis of the 100,000-dalton protein revealed that different peptides were iodinated when the iodination was performed on intact cells or on phagolysosomes, consistent with the idea that this protein spans the plasma membrane.**

There is extensive evidence for a definite topological organization of membrane proteins in nucleated cells (1). However, apart from studies on erythrocytes (2, 3), relatively little is known about the distribution of most plasma membrane-associated proteins or the precise nature of this organization. Recently, studies comparing the properties of intact cell surface with "inside-out" membrane preparations have suggested that some proteins may be restricted to either the external or cytoplasmic side of the membrane whereas others may extend through the membrane and are referred to as transmembrane bridging proteins (4–9). A detailed knowledge of the topological organization of individual membrane proteins is an important step toward a more fundamental understanding of a wide variety of membrane-dependent cellular functions.

It has been reported that latex-filled phagolysosomes can be isolated from cultured cells and used as a source of membrane that has an inside-out orientation (4, 5, 10, 11). Although phagolysosome preparations have been used to study the transmembrane properties of plasma membrane proteins in mouse L-929 cells (4, 5), a detailed analysis of the membrane-associated proteins in these preparations was limited in part by the difficulty encountered in resolving complex mixtures of membrane proteins. The development of high-resolution two-dimensional gel electrophoresis (12) and its application to cell surface proteins (13, 14) provides a sensitive technique for circumventing this limitation.

In the present studies, proteins from phagolysosome membrane that were ^{131}I/lactoperoxidase labeled at either the external or cytoplasmic membrane surfaces were analyzed by two-dimensional polyacrylamide gel electrophoresis. Several individual membrane-associated proteins available to lactoperoxidase were also compared with similar preparations from metabolically labeled cells and membrane prepared by conventional techniques. Some membrane proteins appear to be iodinatable from both sides of the membrane; however, the availability of proteins on the external and cytoplasmic membrane surfaces to lactoperoxidase-catalyzed iodination exhibits a high degree of asymmetry.

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MATERIALS AND METHODS

Cell Culture and Latex-Filled Phagolysosome Preparations. Mouse L-929 cells were grown in suspension culture as described (5). Incubation of cells with 1-μm-diameter latex particles and preparation of latex-filled phagolysosomes were performed by the method of Wetzel and Korn (15) as previously given (5) with the exception that the initial number of latex particles added was reduced to 2×10^3 per cell. In some experiments membrane was also prepared by the two-phase separation technique of Brunette and Till (16).

Metabolic Labeling. Cells were suspended in minimal essential medium without methionine supplemented with 5% calf serum, 0.15% methylcellulose [15 centipoise (1.5 pascal sec)], and 10 μCi (1 Ci = 3.7×10^{10} becquerels) of [^{35}S]methionine per ml. After 4 hr, an equal volume of medium containing, in addition, 0.2 mM methionine was added and the cells were incubated for 5 hr prior to the preparation of phagolysosomes.

Iodination. Cells were ^{131}I-labeled with lactoperoxidase by the method of Hynes (17). Cells from suspension culture were pelleted and resuspended three times in phosphate-buffered saline containing 5 mM glucose prior to labeling. The reaction medium contained approximately 5×10^6 cells per ml, 5 mM glucose, 25 μg of lactoperoxidase (67 units/mg) per ml, 2.5 μg of glucose oxidase (277 units/mg) per ml, and 10 μM NaI containing 2 μCi of $Na^{131}I$ in phosphate-buffered saline. Phagolysosomes prepared from an identical number of unlabeled cells were iodinated under similar conditions except that the concentrations of both lactoperoxidase and glucose oxidase were reduced to 2.5 μg/ml. This modification was made because the rate of iodination in isolated phagolysosomes was found to be much greater than observed with an equivalent amount of intact cells. After a 10-min labeling period 10 vol of phosphate-buffered saline containing 0.5 mM tyrosine was added and the cells or phagolysosomes were centrifuged twice and resuspended in phosphate-buffered saline.

Intact cells were then centrifuged and resuspended in a small volume of a solution containing 9.5 M urea, 2% (vol/vol) Triton X-100, 1.6% carrier pH 5–7 ampholyte, 0.4% carrier pH 3–10 ampholyte, and 2 mM dithiothreitol. This preparation was centrifuged at $10,000 \times g$ for 15 min and the resulting supernatant was recovered for polyacrylamide gel analysis. Iodinated

Abbreviations: $NaDodSO_4$, sodium dodecyl sulfate; IEF, isoelectric focusing.

phagolysosomes or phagolysosomes prepared from iodinated cells were centrifuged at 10,000 × *g* for 15 min and the resulting pellet was resuspended in 100–150 μl of Triton/urea/ampholyte solution. Samples that were not used immediately were quickly frozen. In some experiments, unlabeled membrane preparations prepared by the method of Brunette and Till (16) were added as marker proteins for the ^{131}I-labeled phagolysosomal proteins.

Two-Dimensional Polyacrylamide Gel Electrophoresis. Two-dimensional gel electrophoresis was performed by the method described by O'Farrell (12). Samples were applied to 3 × 130 mm 4% acrylamide cylindrical gels containing 9.2 M urea, 2% Triton X-100, 1.6% carrier pH 5–7 ampholyte, and 0.4% carrier pH 3–10 ampholyte. The first-dimension gels were electrofocused at 350 V for 16 hr then 1000 V for 1.5 hr. The gels were then incubated in a solution containing 0.14 M Tris, 22.3% (vol/vol) glycerol, 6% sodium dodecyl sulfate (NaDodSO$_4$), 2 mM dithiothreitol, and 0.001% bromphenol blue, pH 6.8, for 30 min at room temperature with gentle shaking and placed on top of a discontinuous NaDodSO$_4$/polyacrylamide slab gel. The gel apparatus used in these studies, which allows a 3-mm-diameter cylindrical gel to be electrophoresed into a 0.9-mm-thick slab gel, has been described by Imada (18). NaDodSO$_4$/polyacrylamide gel electrophoresis was carried out by the method of Laemmli (19) as modified by Studier (20). Gels were run at approximately 2 W constant power for 7–8 hr. After electrophoresis the gels were fixed in 46% methanol/8% acetic acid (vol/vol) overnight, rinsed in water, and then infiltrated with 2,5-diphenyloxazole (21) or stained in 46% methanol/8% acetic acid/0.1% Coomassie blue overnight and destained in 5% methanol/10% acetic acid (vol/vol). The gels were then dried onto filter paper and radioautographed at −70°C with Kodak XR film and a Du Pont Hi-Plus image intensifying screen. In some experiments molecular weight markers were run in the second dimension. The following proteins were used as standards for estimation of molecular weights; myosin heavy chain, 205,000; β-galactosidase, 130,000; transglutaminase, 85,000; catalase, 60,000; actin, 42,000.

Limited Proteolysis Peptide Mapping of Phagolysosomal Protein. Preparations of lactoperoxidase/^{131}I-labeled phagolysosomes and phagolysosomes prepared from iodinated cells were mixed with an equal amount of unlabeled L-929 membrane prepared as described by Brunette and Till (16) and electrophoresed in two dimensions as described above. The gels were stained and destained as described above. The Coomassie blue-stained spot of interest was cut from the gel and limited proteolysis peptide mapping was carried out as described by Cleveland *et al.* (22).

RESULTS

The two-dimensional electrophoretic pattern of phagolysosomes prepared from L-929 cells metabolically labeled with [^{35}S]-methionine is shown in Fig. 1. It can be seen that there are a large number of labeled proteins between 20,000 and 200,000 daltons with isoionic points between pH 5.5 and 8.7. When cells are ^{131}I-labeled by lactoperoxidase and phagolysosomes are subsequently prepared, those proteins available to lactoperoxidase at the external cell surface should be iodinated. Fig. 2 shows the prominent species of iodinatable surface proteins; however, it should be noted that upon longer exposure additional proteins can be detected in the ^{131}I fluorograph. Fig. 3 gives the ^{131}I fluorogram of phagolysosomes that were lactoperoxidase-iodinated after purification, thus indicating those proteins available for iodination in membrane with an inside-out orientation.

It is apparent from comparisons of Figs. 1–3 that most of the prominent [^{35}S]methionine-labeled protein species from phagolysosome preparations appear to be available for lactoperoxidase-catalyzed iodination on either the external or the internal surface and that some proteins are iodinatable from both sides of the membrane. However, these membrane preparations are different in a number of respects. For example, three prominent [^{35}S]methionine-labeled proteins focusing between pH 5.5 and 6.0 at approximately 50,000 daltons are barely detectable in phagolysosome preparations labeled with ^{131}I. Similarly actin (designated protein number 6), a major protein present in [^{35}S]methionine-labeled phagolysosomes, is not detectable after iodination of the cell surface (Fig. 2) and is only lightly labeled in directly iodinated phagolysosomes (Fig. 3). The intensity of iodination of these proteins may reflect a relative lack of accessible tyrosine residues. In addition, there are two protein species labeled with ^{131}I in Figs. 2 and 3 (isoionic point 5.5, approximately 70,000 daltons) that are not detectable in metabolically labeled phagolysosomal preparations (Fig. 1). The presence of these additional proteins in phagolysosomal

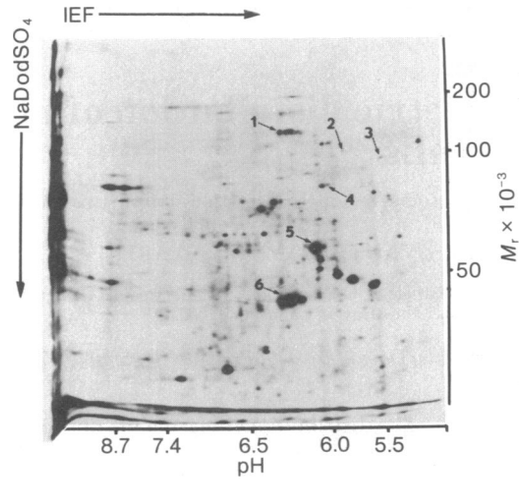

FIG. 1. Two-dimensional gel electrophoresis of [^{35}S]methionine-labeled phagolysosome. Approximately 50,000 cpm of acid-precipitable ^{35}S-labeled material was electrophoresed in two dimensions. IEF, isoelectric focusing. The fluorograph is the result of an 8-day exposure. Arbitrary numbers have been assigned to proteins of interest in order of decreasing molecular weight.

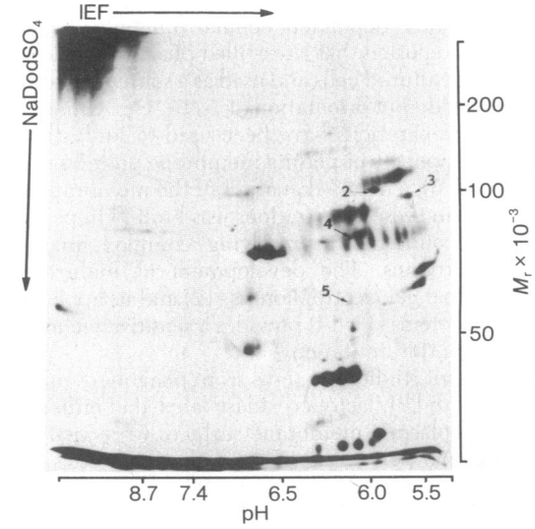

FIG. 2. Two-dimensional gel electrophoresis of phagolysosomes prepared from ^{131}I/lactoperoxidase-labeled cells. Approximately 2.5 × 10^5 cpm of acid-precipitable ^{131}I-labeled material was applied to the first dimension. The fluorograph is the result of a 4-day exposure.

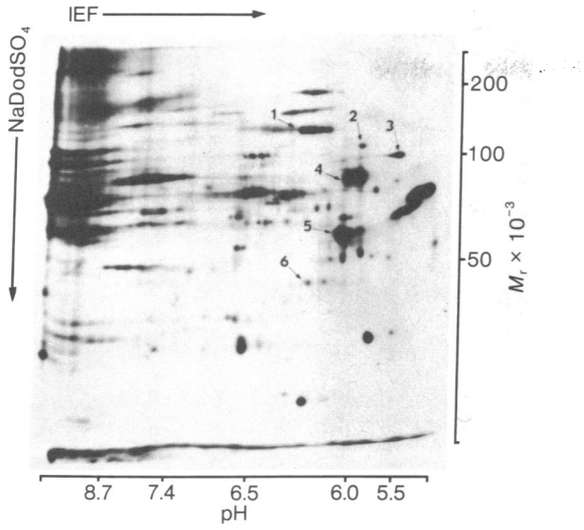

FIG. 3. Two-dimensional gel electrophoresis of ^{131}I/lactoperoxidase-labeled phagolysosomes. Approximately 5 × 10^6 cpm of acid-precipitable ^{131}I-labeled material was applied to the first dimension. The fluorograph is the result of a 12-hr exposure.

preparations labeled by lactoperoxidase could reflect serum contaminants, methionine-deficient proteins, proteins with a low rate of synthesis, or proteins present in small amounts with highly available tyrosine residues. Therefore, in order to compare the distribution of proteins in these membranes, our analysis has been restricted to proteins (designated 1–6 in Figs. 1–3) that are present in both metabolically and ^{131}I/lactoperoxidase labeled preparations.

To convincingly identify individual iodinated proteins and to allow direct comparisons between internally and externally iodinated proteins, unlabeled membrane protein was added to ^{131}I-labeled phagolysosomal preparations. The gels were stained for protein and then radioautographed. A selected portion of a two-dimensional gel of phagolysosomes derived from iodinated cells is shown in Fig. 4. Two prominent proteins, labeled 5 and 6, are seen in the protein staining pattern (Fig. 4*A*). Protein number 5, an acidic 60,000-dalton polypeptide, is iodinated whereas protein 6, actin, is not available for surface labeling with lactoperoxidase.

A higher molecular weight range of a comparable gel is shown in Fig. 5. In addition to the 60,000-dalton protein (number 5) another prominent Coomassie blue-stained protein of approximately 100,000 daltons (number 3) is also available for external iodination (Fig. 5 *A* and *B*). Careful examination of Fig. 5*B* also shows that an 85,000-dalton Coomassie blue-stained protein (number 4) is not available for external labeling. A protein designated 2 (≈110,000 daltons) is obscured by other iodinatable surface protein and cannot be unambiguously identified in this gel. When these proteins are identified in directly iodinated phagolysosomes (Fig. 5 *C* and *D*), proteins 2–5 are all available for ^{131}I/lactoperoxidase labeling.

It has been proposed than an essential criterion to demonstrate that a membrane protein spans the bilayer is to demonstrate that different iodopeptides can be obtained from the protein after it has been iodinated from the external and internal membrane surfaces. Because protein 3 is prominent in Coomassie blue-stained gels and is available for iodination on intact cells (Fig. 5 *A* and *B*) as well as on isolated phagolysosomes (Fig. 5 *C* and *D*), it was subjected to limited proteolysis after differential iodination. Fig. 6 shows the $NaDodSO_4$ gel electrophoretic pattern of the iodopeptides obtained from protein 3 after external cell surface labeling and after lactoperoxidase labeling in isolated phagolysosomes. It is apparent that the major iodopeptides observed in these two protein 3 preparations are different with each of the three proteases used. These results suggest that protein 3 is a true membrane-bridging protein.

DISCUSSION

Differential iodination of inside-out and right-side-out membrane preparations has previously been used as an effective experimental approach to elucidate the disposition of membrane proteins of erythrocytes (23, 24). Although there have been concerns about possible protein rearrangements during membrane preparation (25), labeling studies carried out in intact erythrocytes suggest that this does not occur, at least with the erythrocyte membrane (26). The general asymmetry of plasma membrane glycoproteins has been investigated, and there is considerable evidence that the glycosylated regions of membrane proteins are present only at the external surface (27–29). This strict carbohydrate asymmetry is apparently re-

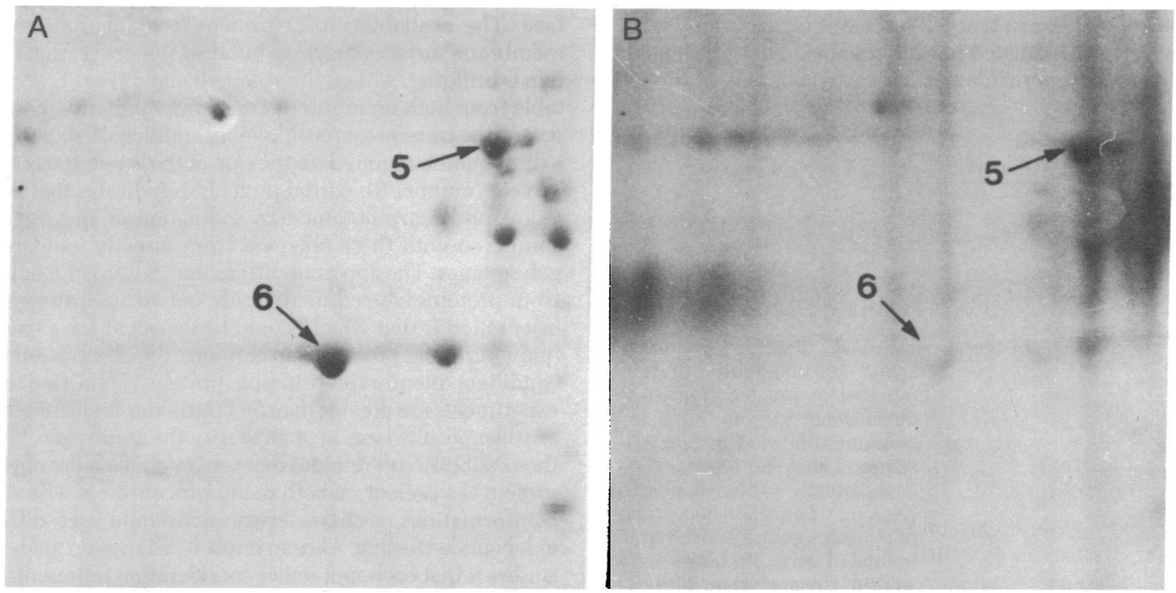

FIG. 4. Enlarged portion of a two-dimensional gel electrophoretic pattern of phagolysosomes prepared from ^{131}I/lactoperoxidase-labeled cells and unlabeled plasma membrane prepared by the method of Brunette and Till (16). (*A*) Protein staining; (*B*) ^{131}I autoradiograph.

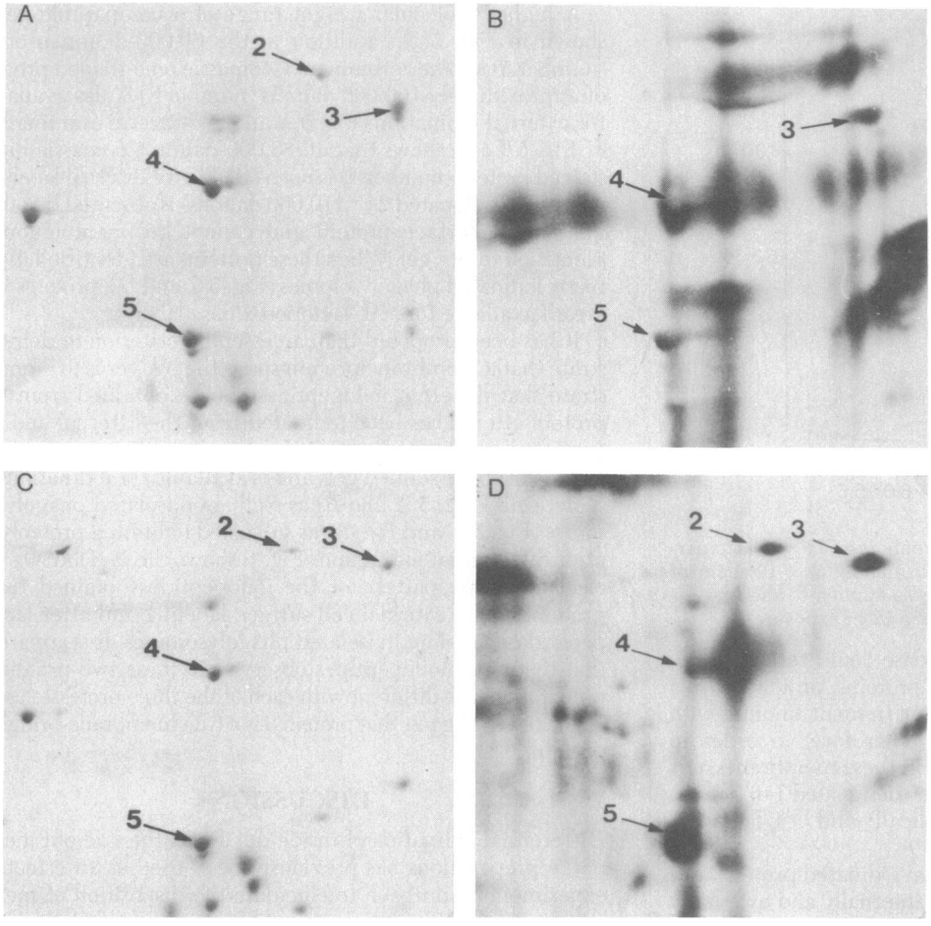

FIG. 5. Enlarged portions of two-dimensional gel electrophoretic patterns of ^{131}I-labeled phagolysosomes and unlabeled plasma membrane prepared by the method of Brunette and Till (16). (*A*) Protein staining of unlabeled membrane and phagolysosomes prepared from iodinated cells. (*B*) ^{131}I autoradiograph of *A*. (*C*) Protein staining of unlabeled membrane and directly iodinated phagolysosomes. (*D*) ^{131}I autoradiograph of *C*.

versed in inside-out lymphocyte membrane preparations (7) and latex-filled phagolysosomes (30). It has also been demonstrated that whereas nearly 90% of the ^{125}I incorporated into cell surface proteins by lactoperoxidase-catalyzed iodination in intact cells is sensitive to trypsin, less than 5% of ^{125}I in phagolysosomes prepared from iodinated cells is sensitive to similar trypsin treatment (4, 5). In the present study, prominent proteins were found to be available to lactoperoxidase-catalyzed iodination only at the external cell surface or on the cytoplasmic surface in directly iodinated phagolysosomes. This differential availability to lactoperoxidase is indicative not only of the sidedness of the preparation but also of an asymmetric distribution of individual membrane proteins.

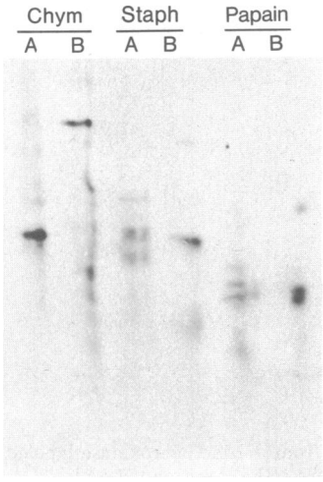

FIG. 6. Peptide maps of an acidic 100,000-dalton protein (number 3) isolated from two-dimensional gels of ^{131}I/lactoperoxidase-labeled phagolysosomes. Lanes A, Iodopeptides from directly iodinated phagolysosomes. Lanes B, iodopeptides of phagolysosomes derived from iodinated cells. Proteases used were α chymotrypsin, *Staphylococcus aureus* V8 protease, and papain, 0.67 μg per sample.

In evaluating the transmembrane disposition of individual proteins on the basis of availability to lactoperoxidase iodination, certain limitations must be acknowledged. The failure of a protein to be available to ^{131}I/lactoperoxidase labeling at one surface may suggest an asymmetric distribution but it cannot be considered proof that this protein is not exposed at this surface. The availability of a protein to iodination from both membrane surfaces suggests, but does not prove, that this protein is bridging. Although proteins 3 and 5 (Fig. 1) are iodinatable from both membrane surfaces and thus are likely candidates to be transmembrane proteins, additional evidence of such a disposition is required. In the case of the acidic 100,000-dalton protein (number 3), partial proteolysis indicates that different iodopeptides are obtained by iodination at the cell surface compared with those obtained from directly iodinated phagolysosomes. The apparent differential pattern of iodopeptides from proteins labeled in an inside-out versus a right-side-out orientation is that which would be expected for a true membrane-bridging protein. Furthermore, the observation that the candidate membrane-bridging proteins identified in these experiments are greater than 50,000 daltons indicates that they are theoretically large enough to span the membrane. However, the possibility still remains that separate molecules of the same protein are present on both membrane surfaces with different comformations, a characteristic that could give differential iodopeptide labeling. Also, an implicit assumption made in these studies is that each spot under consideration represents a single peptide and is not two or more comigrating species. Efficient methods to definitively distinguish between a protein that truly

spans the membrane and one that adopts a different conformation on each side of the membrane must be developed.

Consideration must also be given to the possibility that artifacts may be generated during isolation or analysis of phagolysosome preparations. Lactoperoxidase iodination could alter the migration of some proteins in IEF, particularly those proteins with basic isoionic points (24). In this study, only those iodinated proteins from phagolysosome preparations that could also be identified in Coomassie blue-stained noniodinated preparations were evaluated for transmembrane configuration. All these proteins have isoionic points below pH 7, making it unlikely that iodination is a significant factor. Another consideration is the possibility of artifacts generated during IEF, which could include deamidation, ampholyte-induced isomerization (31), pH-dependent conformational changes (32), and ampholyte-induced subunit association or dissociation (33). Actin, a prominent protein isolated in phagolysosome preparations (protein number 6), demonstrates multiple forms that reflect real biochemical differences (34, 35). However, the heterogeneity seen in a basic 90,000-dalton protein (Fig. 1) may reflect posttranslational modification, limited proteolysis, or an IEF artifact. A more prominent example is an 85,000-dalton "string" of acidic proteins that are detected only in external surface lactoperoxidase iodinations (Fig. 2). A similar phenomenon has also been observed in other two-dimensional gel studies (13, 14). It has been suggested that this heterogeneity may reflect differences in glycosylation (14). Indeed, we have observed that the heterogeneity of the 85,000-dalton protein is sensitive to treatment by endoglycosidase or affinity-purified neuraminidase prior to electrophoresis. This pattern is not the result of serum contamination because similar patterns are produced by surface labeling of L-929 cells that have been grown in serum-free medium for many divisions.

Interactions between extracellular or cell surface proteins and cytoplasmic proteins have been proposed to be important in what has been termed "transmembrane signaling" (36). A number of studies have suggested that cell surface proteins (37–42) as well as extracellular proteins such as fibronectin (43) interact with elements of the cell's cytoskeletal network. Although a direct association of this type has been demonstrated in erythrocytes (44), the precise nature of transmembrane interactions in nucleated cells is unknown. It has been speculated that phosphorylation is important in an interaction between actin and histocompatibility antigen (HLA) (45), reportedly a transmembrane protein in human lymphocytes (8). Therefore, it is interesting to note that both the 100,000-dalton (number 3) and 60,000-dalton (number 5) proteins found to be membrane-bridging candidates in the present study are also phosphoproteins (unpublished data).

In summary, the present study demonstrates the asymmetrical distribution of proteins in the plasma membrane of L-929 cells. In addition, we have identified two polypeptides (numbers 3 and 5) that possess characteristics of membrane-bridging proteins.

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1. Rothman, J. E. & Lenard, J. (1977) *Science* **195,** 743–753.
2. Guidotti, G. (1972) *Annu. Rev. Biochem.* **41,** 731–752.
3. Marchesi, V. T., Furthmayr, H. & Tomita, M. (1976) *Annu. Rev. Biochem.* **45,** 667–698.
4. Hunt, R. C. & Brown, J. C. (1975) *J. Mol. Biol.* **97,** 413–422.
5. Evans, R. M. & Fink, L. M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5341–5344.
6. Zachowski, A., Leliever, L., Aubry, J., Charlemagne, D. & Paraf, A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 633–637.
7. Walsh, F. S., Barber, B. H. & Crumpton, M. J. (1976) *Biochemistry* **15,** 3557–3563.
8. Walsh, F. A. & Crumpton, M. J. (1977) *Nature (London)* **269,** 307–311.
9. Schmidt-Ullrich, R., Mikelsen, R. B. & Wallach, D. F. H. (1978) *J. Biol. Chem.* **253,** 6973–6978.
10. Hubbard, A. L. & Cohn, Z. A. (1975) *J. Cell Biol.* **64,** 461–479.
11. Sandra, A. & Pagano, R. E. (1978) *Biochemistry* **17,** 332–338.
12. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
13. Jones, P. (1977) *J. Exp. Med.* **146,** 1261–1279.
14. Ivarie, R. D. & O'Farrell, P. H. (1978) *Cell* **13,** 41–55.
15. Wetzel, M. G. & Korn, E. D. (1969) *J. Cell Biol.* **43,** 90–104.
16. Brunette, D. M. & Till, J. E. (1971) *J. Membr. Biol.* **5,** 215–224.
17. Hynes, R. O. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3170–3174.
18. Imada, M. (1978) *Anal. Biochem.* **89,** 292–296.
19. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
20. Studier, F. W. (1972) *Science* **176,** 367–376.
21. Lasky, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56,** 335–341.
22. Cleveland, D. W., Fisher, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252,** 1102–1106.
23. Mueller, T. J. & Morrison, M. (1974) *J. Biol. Chem.* **249,** 7568–7573.
24. Litman, G. W., Litman, R. T. & Merz, D. C. (1975) *Biochim. Biophys. Acta* **394,** 348–360.
25. Carraway, K. L. (1975) *Biochim. Biophys. Acta* **415,** 379–410.
26. Staros, J. V., Richards, F. M. & Haley, B. E. (1975) *J. Biol. Chem.* **250,** 8174–8178.
27. Nicolson, G. L. & Singer, S. J. (1974) *J. Cell Biol.* **60,** 236–248.
28. Roos, E. & Temmick, J. H. M. (1975) *Exp. Cell Res.* **94,** 140–146.
29. Nigam, V. N. & Brailowsky, C. A. (1977) *Biochim. Biophys. Acta* **468,** 472–485.
30. Evans, R. M., Grillo, F. G. & Fink, L. M. (1979) *J. Supramol. Struct.*, in press.
31. Cann, J. R. & Stimpson, D. I. (1977) *Biophys. Chem.* **7,** 103–114.
32. Stimpson, D. I. & Cann, J. R. (1977) *Biophys. Chem.* **7,** 115–119.
33. Cann, J. R., Stimpson, D. I. & Cox, D. J. (1978) *Anal. Biochem.* **86,** 34–39.
34. Whalen, R. G., Butler-Browne, G. S. & Gros, F. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2018–2022.
35. Izant, J. G. & Lazarides, E. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1450–1454.
36. Nicolson, G. L. (1976) *Biochim. Biophys Acta* **457,** 57–108.
37. Ash, J. F. & Singer, S. J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4575–4579.
38. Ash, J. F., Louvard, D. & Singer, S. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5584–5588.
39. Gabbiani, G., Chaponnier, C., Zumbe, A. & Vassalli, P. (1977) *Nature (London)* **269,** 697–698.
40. Schlessinger, J., Barak, L. S., Hammes, G. G., Yamada, K. M., Pastan, I., Webb, W. W. & Elson, E. L. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 2909–2913.
41. Flanagan, J. & Koch, G. L. E. (1978) *Nature (London)* **273,** 278–281.
42. Koch, G. L. E. & Smith, M. J. (1978) *Nature (London)* **273,** 274–278.
43. Hynes, R. O. & Destree, A. T. (1978) *Cell* **15,** 875–886.
44. Liu, S. C., Fairbanks, G. & Palek, J. (1977) *Biochemistry* **16,** 4066–4074.
45. Prober, J. S., Guild, B. C. & Strominger, J. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 6002–6006.