Membrane-Associated Particles: Increase at Sites of Pinocytosis Demonstrated by Freeze-Etching

Abstract. *An increased number of membrane-associated particles has been demonstrated by freeze-etching in areas of pinocytosis in the intestinal smooth muscle cell. A peculiar distribution of the particles is described, which might be closely related to the formation of pinocytotic vesicles (caveolae).*

One of the major achievements of freeze-etching has been to reveal the presence of particles, about 85 Å in diameter, which are embedded within the membranes of all cell types. These particles are thought to be protein in nature, and it has been assumed that their frequency somehow reflects the metabolic activity of the membrane (*1*). Indeed the myelin sheath membrane, which seems to be rather inert metabolically, has extremely few particles, whereas chloroplast and mitochondrial membranes carry a large number of such particles (*1*). The total number of particles may vary not only from one cell type to another but also within individual cell membranes. For example, the density of intramembranous particles changes during the cell cycle of Chinese hamster cells synchronized in culture (*2*). Similarly, particles aggregate in certain parts of the plasma membrane to form plaques of tightly packed units (*3*). These aggregates occur in regions originally described as gap junctions (*4*), which have been shown to provide metabolic and electric coupling between cells (*5*). All these lines of evidence have favored the idea that membrane particles could be sites in the membrane related to specific functions; as such, they would fit well with models that envision the membrane as a mosaic of lipids and globular proteins (*6*).

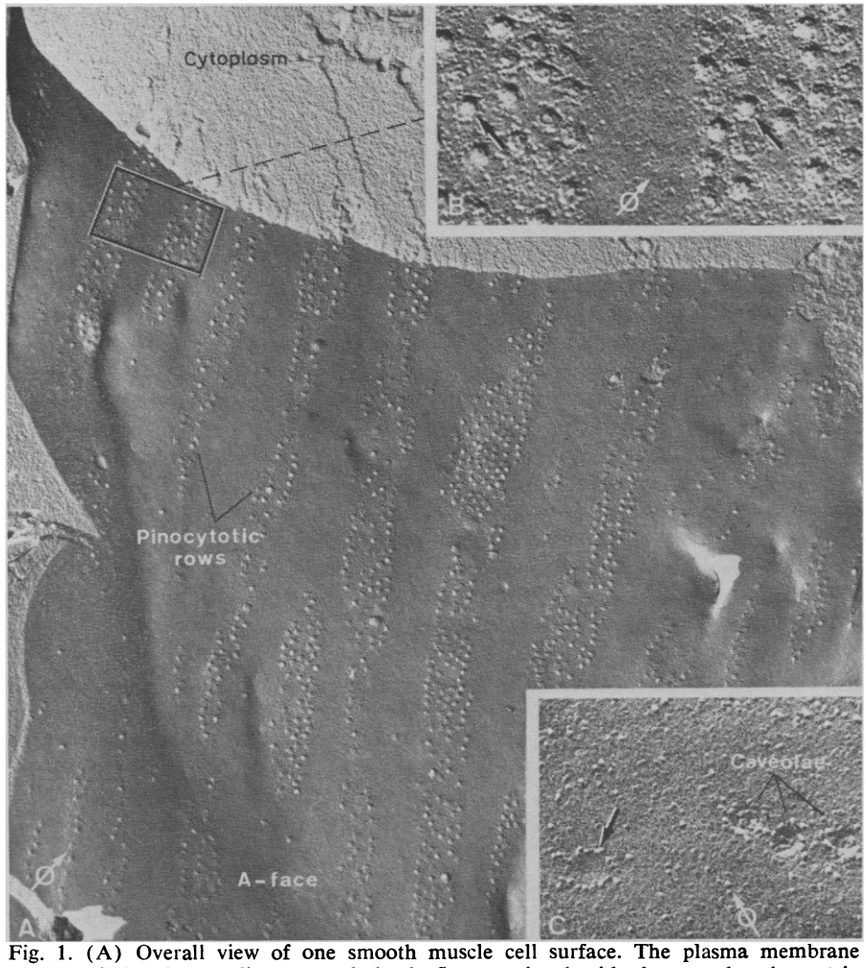


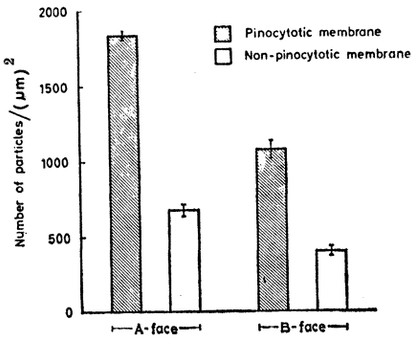
Fig. 1. (A) Overall view of one smooth muscle cell surface. The plasma membrane of the cell has been split to reveal the leaflet associated with the cytoplasmic matrix (A-face). This face presents parallel rows of circular craters, each of which is the neck of a fractured caveola (pinocytotic vesicle). The fracture face appears smooth between the pinocytotic rows. The cytoplasm of the muscle cell, which has been exposed by the fracturing process, can be seen at the top (× 14,000). (B) Higher magnification of the area outlined by a rectangle in (A) reveals the membrane-associated particles. These are numerous in the pinocytotic rows, scarce in the intervening smooth surface. The dark arrows point to the "necklace" disposition of particles around the fractured necks of caveolae (× 55,000). (C) High magnification of an area near the border of one pinocytotic row. In addtion to three caveolar craters, a necklace figure formed by particles (dark arrow) at an early stage in crater formation can be seen (× 65,000). All pictures have been printed to reveal the shadows in white. The encircled arrow in each figure indicates the direction of the platinum-carbon evaporation.

We now have evidence that the number of membrane particles is significantly increased at sites of caveolae formation (pinocytosis) in intestinal smooth muscle cells.

Strips of mouse intestine were fixed with glutaraldehyde, washed several times in isotonic phophate buffer, and soaked for several hours in the same buffer containing 20 percent glycerol as an antifreeze agent. Freeze-etching was performed according to the method of Moor *et al.* (*7*) in a Balzers freeze-etching device. Fracturing was carried out at − 100°C under a vacuum of 10^{-6} torr, and the surfaces obtained were replicated with carbon and platinum without etching. Replicas were cleaned in sodium hypochlorite to remove adherent organic material, rinsed in distilled water, and examined in a Philips EM 300 electron microscope.

An overall view of the surface of the intestinal smooth muscle cell is shown in Fig. 1A. The A-face of the membrane has been exposed by the freeze-etching (*8*) and it appears dotted with numerous circular craters. These craters or dimples are arranged in rows coursing parallel to the longitudinal axis of the cell, and the rows leave between them bands of smooth surface. This orderly disposition of craters, each of which represents the fractured neck of one caveola or pinocytotic vesicle, seems to be peculiar to smooth muscle cells and has already been reported in arterial muscle (*9*). Two rows of craters with an intervening smooth surface are seen at higher magnification in Fig. 1B. This magnification reveals the 85-Å particles on the A-face of the membrane, and shows that particles are more numerous inside the pinocytotic rows than outside. The particles have been counted inside a rectangular grid representing

Fig. 2. Results of counting the membrane-associated particles on fracture faces A and B, within and outside the pinocytotic rows. The vertical bar at the top of each column indicates two standard errors of the mean. For both faces, the difference between the number of particles in the pinocytotic and nonpinocytotic membrane has a probability $P < .005$.



an area of 0.25 nm² on pictures magnified 100,000 times. This grid was placed alternately over pinocytotic and nonpinocytotic membrane, and a total of 2700 particles were counted. The results are presented in Fig. 2, which indicates that there are approximately twice as many particles in regions that show pinocytotic events as in regions that do not. This difference also holds for the B-face of the membrane (*8*), which has fewer particles than the A-face. Within the rows of pinocytosis, many particles are preferentially localized as necklaces around the craters (indicated by the dark arrows in Fig. 1B). This disposition is emphasized in Fig. 1C, which illustrates a zone near the border of one pinocytotic row. In addition, one sees a peculiar "necklace" arrangement of particles which is associated with an incompletely formed crater (dark arrow).

The orderly disposition of pinocytotic caveolae in smooth muscle cell has enabled us to show that (i) membrane-associated particles are increased in pinocytotic regions of the cell membrane compared to nonpinocytotic ones; (ii) in pinocytotic regions the particles often gather in a necklace or rosette formation around the individual caveolae; and (iii) in some cases necklaces of particles can be identified before clear-cut craters are seen. These observations might suggest that the distribution of membrane particles is related in some way to the formation of caveolae. Assuming that the particles represent specific functional sites in the membrane, we propose that the accumulation of such sites in one particular area of the membrane of the smooth muscle cell might be a necessary prelude to pinocytosis. The finding of particle aggregation at specific sites of the membrane (other than the well-known gap junctions) has precedents in other studies of freeze-fractured tissues. For example, longitudinal arrays of particles, the "ciliary necklaces," have been described at the base of the motile cilia in vertebrates and invertebrates (*10*). Even more relevant to our work are the studies by Satir *et al.* (*11*), who reported "rosette" arrangements of particles on the plasma membrane as a prelude to mucocyst discharge in the protozoan *Tetrahymena*. From their results and ours, it seems therefore that rosette figures occur at sites where membrane fragments are to be added to the preexisting plasma membrane (as in mucocyst discharge), or subtracted from it (as in pinocytosis).

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References and Notes

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Natural Abundance Carbon-13 Nuclear Magnetic Resonance Spectra of the Canine Sciatic Nerve

Abstract. *The proton-decoupled natural abundance carbon-13 nuclear magnetic resonance spectrum of the canine sciatic nerve is virtually identical to that of canine adipose tissue and markedly similar to that of liquid triolein. No resonances assignable to cholesterol, glycolipids, or sphingolipids are detectable in the sciatic nerve spectrum despite their abundance in the myelin sheath of this nerve. However, many such resonances are observed in lipid extracts of the nerve. Chromatographic analysis of specimens of canine and rabbit sciatic nerve has revealed that these contain sufficient triglyceride to account quantitatively for the observed spectrum. Proton nuclear magnetic resonance and spin-labeling results for preparations containing myelin, especially those derived from the peripheral nerve, should be critically examined for experimental artifacts reflecting the triglyceride content.*

Recent investigations into the structure and properties of biological membranes have made considerable use of the techniques of proton nuclear magnetic resonance (NMR) and electron spin resonance spectroscopy, particularly in order to study the dynamic properties of the alkyl chains of lipid molecules. Among these studies are several which present proton NMR spectra of myelin-containing preparations from the central nervous system (*1, 2*) and peripheral nerve (*3*). Two of these studies (*2, 3*) conclude, on the basis of line widths of proton resonances, that myelin contains lipids with alkyl chains in a fluid-like or liquid crystalline state. Other physical studies on myelin, however, suggest that the presence of cholesterol in the myelin sheath restricts the segmental motions of the lipid chains, thereby creating a physical state which is intermediate between liquid crystal and gel (*1, 4*). Here we explore the possibility that the reported proton NMR spectra of the myelin-containing preparations may actually reflect a mobile nonpolar lipid fraction rather than the lipids of the myelin sheath itself.