

## TOPOGRAPHICAL DIFFERENCES IN THE DISTRIBUTION OF SURFACE COAT COMPONENTS AND INTRAMEMBRANE PARTICLES

### A Cytochemical and Freeze-Fracture Study in Culture Forms of *Trypanosoma cruzi*

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The complex relationship between plasma membrane structure, revealed by the freeze-fracture method, and surface coat components detected by electron microscope cytochemical techniques has been shown previously (15). Intramembraneous particles and various surface receptors have a uniform distribution in the pathogenic protozoon *Entamoeba histolytica*. However, when intramembraneous particles are induced to aggregate by glycerol incubation of living amebas, only colloidal iron hydroxide (CIH) binding sites at the outer surface coaggregate, while other receptors remain distributed at random (13, 15). Conversely, redistribution of surface concanavalin A (Con A) receptors to one pole of the cell (cap formation) does not induce capping either of membrane particles or of other surface components. From these results, we have suggested that only certain receptors and antigens at the outer surface of eukaryotic cells are related to membrane particles located within the hydrophobic domain of the plasma lipid bilayer, and that two different levels of membrane fluidity may be considered: integral and peripheral membrane fluidity (15), depending on whether integral or peripheral membrane proteins (17) are involved.

The independent mobility of certain surface determinants relative to membrane particles was demonstrated in *E. histolytica* by means of experimental redistribution of integral or peripheral membrane components. The extent to which actual independence of integral and peripheral constituents is present in untreated living cells could not be evaluated since both constituents have a uniform distribution in the native state. Examination of

another pathogenic protozoon, *Trypanosoma cruzi*, the causative agent of Chagas' disease in man, which has a high prevalence in Central and South America (8), has enabled us to demonstrate not only that regional differentiation may occur at the level of integral and peripheral components of the intact plasma membrane, but also that a third level of differentiation may be found in a localized subplasmalemmal cytoplasmic region.

### MATERIALS AND METHODS

Culture forms of the Y strain of *T. cruzi* maintained in LIT medium (3) were fixed for 1 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at room temperature (24–28°C) and stored in cacodylate buffer at 4°C. All the trypanosomes were in the epimastigote stage (7).

Glutaraldehyde-fixed trypanosomes were slowly infiltrated in glycerol by dropwise addition of 25% glycerol in cacodylate buffer over a period of 30 min and further incubated in 25% glycerol during 1–2 h. The cells were pelleted by centrifugation and frozen in the liquid phase of partially solidified Freon 22 cooled by liquid nitrogen. Freeze-fracture was carried out at –115°C in a Balzers 300 apparatus equipped with a turbomolecular pump (Balzers High Vacuum Corp., Santa Ana, Calif.). Replicas were produced by evaporation from a platinum-carbon source. The specimens were shadowed at  $2 \times 10^{-6}$  mm Hg within 2–5 s of fracturing. The micrographs are mounted with shadow direction from the bottom; shadows are white.

Detection of Con A receptors (1), CIH binding sites (6), and ruthenium red staining (9) was carried out as described earlier (15). After ethanol dehydration and Epon embedding, thin sections were obtained with diamond knives and stained for 5 min with lead citrate.

Replicas and thin sections were observed with a Zeiss EM 10 electron microscope.

## RESULTS

Observation of fracture faces of *T. cruzi* plasma membranes revealed random distribution of membrane particles both on fracture face P (the outer aspect of the inner membrane half) and on fracture face E (inner aspect of the outer membrane half) (2) over most of the membrane covering the cell body. As seen in freeze-fractured plasma membranes of other eukaryotic cells (14, 15), there is a remarkable heterogeneity in the size of the membrane particles.

A prominent differentiation of membrane particles was constantly found in membrane faces of *T. cruzi* fractured near the flagellar pocket, as an oval region ( $0.70\ \mu\text{m} \times 0.52\ \mu\text{m}$ ;  $N = 13$ ) where membrane particles were exceedingly few in number. The particle-poor region in fracture face P was always delimited from the rest of the plasma membrane by a palisade-like row of closely adjacent membrane particles (Figs. 1 and 2). At one side of the particle-poor region, a circular depression with a mean diameter of  $0.13\ \mu\text{m}$  was invariably found. This depression corresponds to the opening of the cytostome, a channel-like depression of the plasma membrane found only in trypanosomes of poikilotherms (20) and in *T. cruzi* (11), but not in salivarian trypanosomes (22).

In fracture face E, the particle-poor localized region was similar to that seen on P faces, except for the absence of the palisade-like disposition of membrane particles. Instead, a few depressions on E faces were seen at the border of the particle-poor region (Fig. 3). The depressions were probably produced by detachment of the row of face P particles during fracturing.

A second topographical modification was found in regions where the flagellar membrane was exposed by the fracture. On both P and E fracture faces membrane particles were absent, or, less frequently, one or two rows of small particle clusters could be found near the region of flagellar insertion into the cell body (19) (Fig. 4).

Examination of thin sections of *T. cruzi* epimastigotes (Fig. 5) did not reveal a distinct surface coat or the "junctional complex" between body and flagellum described in bloodstream forms of various trypanosomes (21). CIH binding sites were identified over the cell body and over the flagellar membrane (Fig. 6), although the number of sites was considerably lower than that observed in

mammalian red blood cells, or in the nonpathogenic trypanosome *Leptomonas pessoai* (our unpublished results). Concanavalin A (Con A) binding sites revealed by the peroxidase-benzidine reaction and ruthenium red-positive surface material also showed a uniform distribution over most of the cell body and the flagellar membrane (Figs. 7 and 8).

A prominent surface coat with a mean thickness of 22 nm was detected at one side of the cytostome opening, extending over  $0.68\ \mu\text{m}$  ( $0.50\text{--}0.90\ \mu\text{m}$ ,  $N = 32$ ). The value obtained for the diameter of the specialized surface coat in thin sections coincides with the diameter of the particle-poor region detected by freeze-fracture. In addition, the location of the cytostome both at one side of the specialized surface and at the membrane regions renders unambiguous the identification of both aspects as representing the same region.

The specialized surface coat gave a strong reaction with ruthenium red (Fig. 8) and Con A-peroxidase (Fig. 9), whereas no increased binding of CIH was detected (Fig. 10).

An additional finding of interest is the fact that the region of the cytostome was also differentiated at the cytoplasmic region underlying the modified surface coat. As shown in Figs. 11 and 12, the subpellicular microtubules found under the plasma membrane of most of the *T. cruzi* cell body were not present under the specialized surface coat; instead, cross sections of 8–10-nm thick filaments were present in some sections (Fig. 11), while in other sections irregular masses of fibrogranular material were identified (Fig. 12).

## DISCUSSION

The interaction between exogenous macromolecules and pathogenic trypanosomes was first studied by Steinert and Novikoff (20) who found that ferritin molecules do not bind over the entire cell surface but are restricted to the cytostome region. These authors suggested that a specialized outer coat is present at the site of selective membrane binding. The present cytochemical demonstration of a prominent surface coat that is restricted to the cytostome region of *T. cruzi* culture forms fully confirms that suggestion (20). The surface coat at the cytostome region is much thicker than the surface coat located over the rest of the cell body and, in addition, shows a heavier labeling with Con A-peroxidase and ruthenium red.

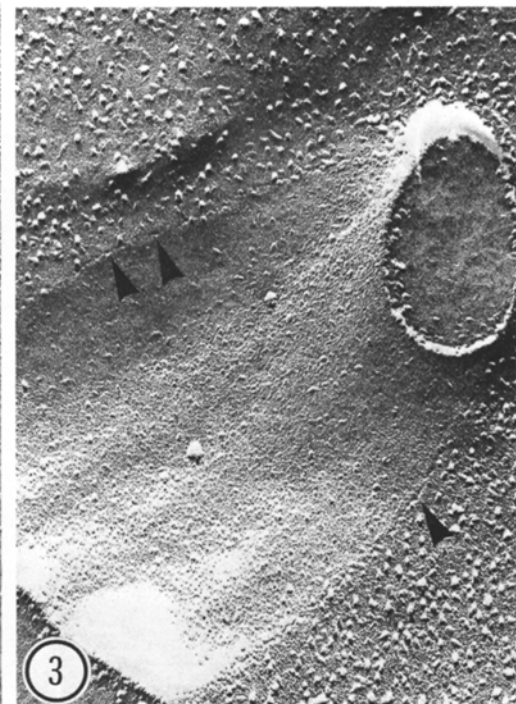
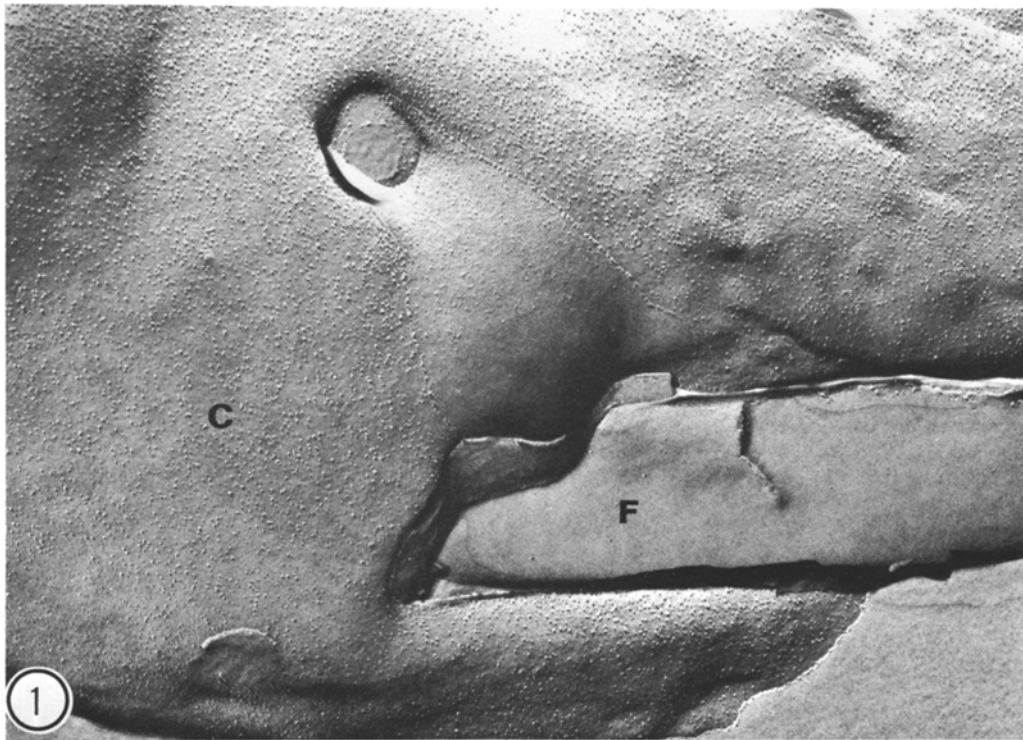


FIGURE 1 Fracture face P of a *T. cruzi* culture form. Numerous membrane particles are randomly distributed, except at a region near the opening of the cytostome (C), and at the flagellar membrane where they are exceedingly few in number (F).  $\times 60,000$ .

FIGURE 2 Fracture face P. A palisade-like row of membrane particles encircles the particle-poor region at one side of the cytostome opening.  $\times 90,000$ .

FIGURE 3 Fracture face E of the cytostome region. A series of membrane depressions (arrowheads) are present at the border of the particle-free region.  $\times 100,000$ .

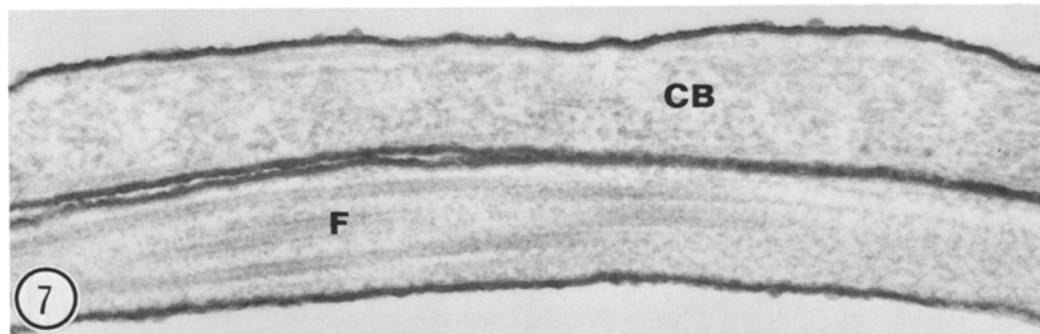
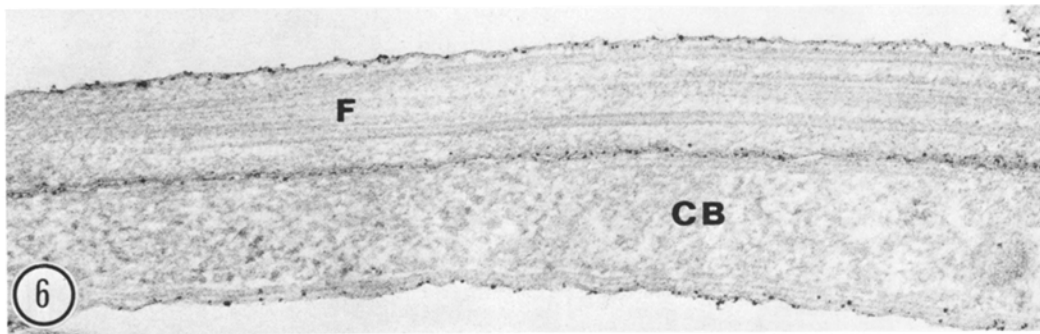
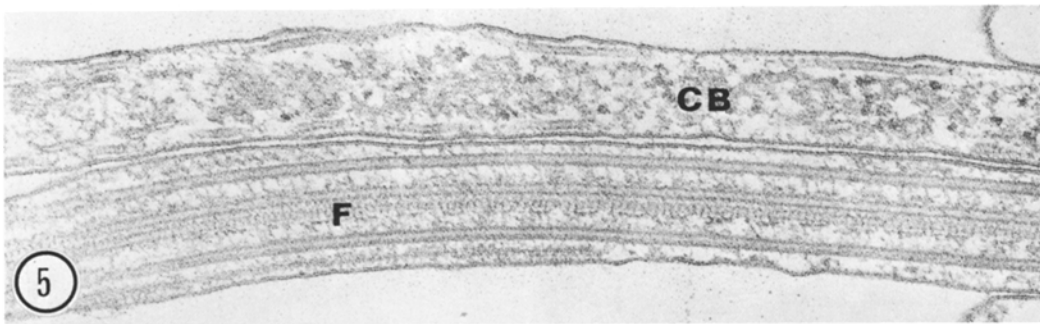
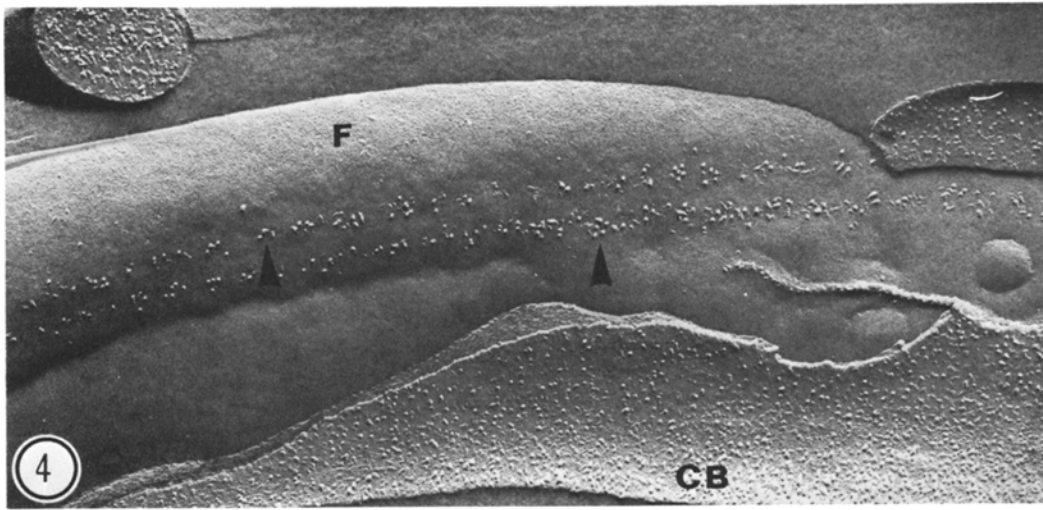
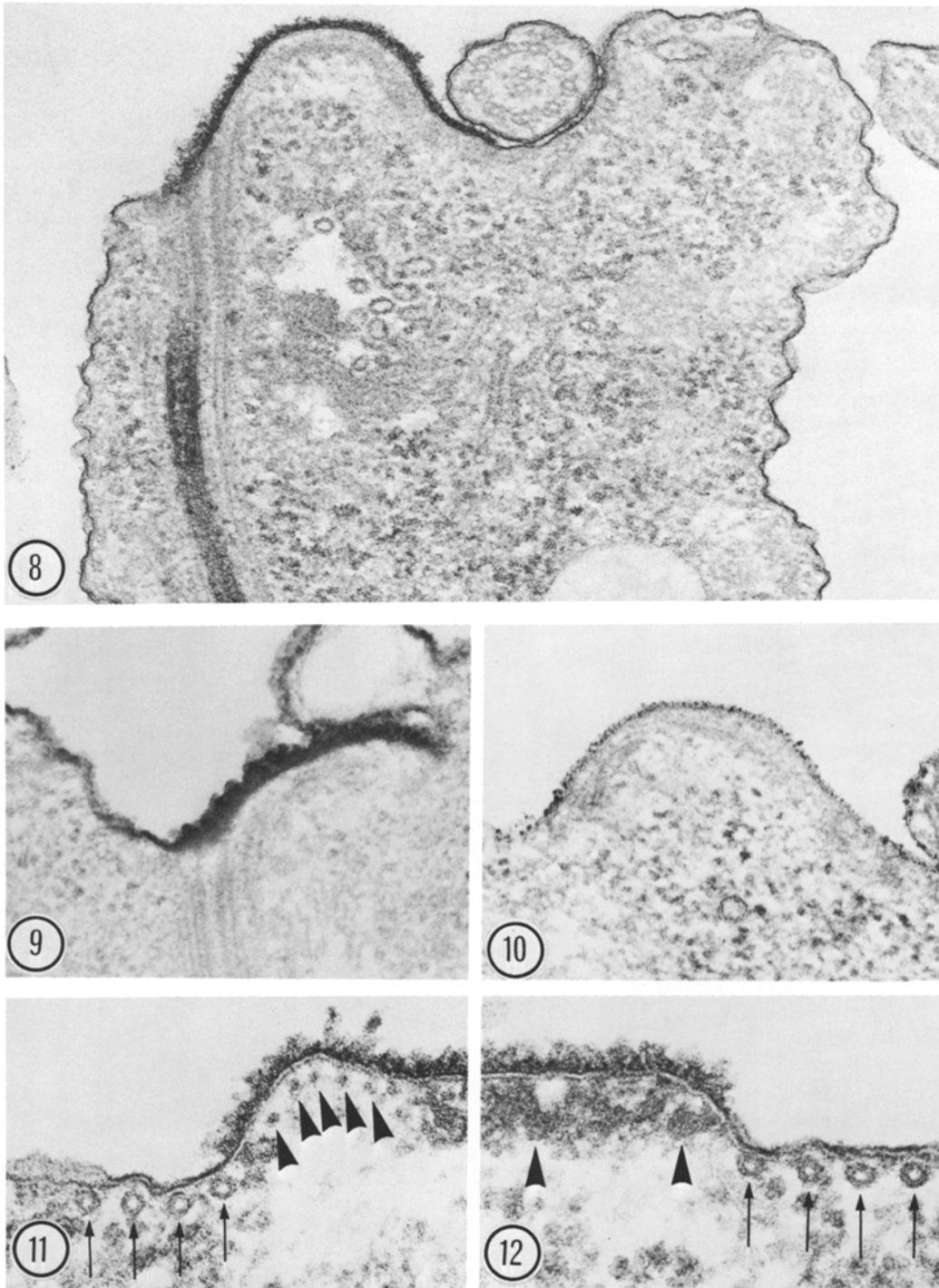


FIGURE 4 Freeze-fracture replica of the junctional region between the cell body (*CB*) and the flagellum (*F*). A row of membrane particles (arrowheads) is present over the fracture face P of the flagellar membrane.  $\times 60,000$ .

FIGURES 5-7 Thin sections of the junctional region between the flagellum (*F*) and the cell body (*CB*). Fig. 5, uranyl acetate and lead staining; Fig. 6, negative sites labeled with CIH; Fig. 7, con A-peroxidase.  $\times 60,000$ .



FIGURES 8-10 Specialized surface coat at the cytostome region of *T. cruzi*. Fig. 8, ruthenium red staining; Fig. 9, con A-peroxidase; Fig. 10, negative sites labeled with CIH.  $\times 80,000$ .

FIGURES 11 and 12 Specialized surface coat; ruthenium red staining. Cross-sectional views of microtubules are seen (arrows); however, under the specialized surface coat, filaments or fibrogranular material are evident (arrowheads).  $\times 160,000$ .

In terms of the interpretation of the structural organization of biological membranes, the localized differentiation of the plasma membrane in *T. cruzi* provides a striking example of the maintenance of a highly differentiated structure at the outer, middle, and inner regions of the plasma membrane. Such a stable differentiation is not expected in a fluid membrane (18) unless special restraints limit the mobility of certain membrane domains, as demonstrated in the plasma membrane of the mammalian spermatozoon (5, 12). The palisade of membrane particles at the border of the cytostome particle-free region may represent one restraint. In this respect, it has been suggested that during amphibian neurulation the orderly disposition of membrane particles to form a specialized gap junction may be insured by limiting tight junctional ridges (4). Membrane particle arrays have also been implicated as barriers to membrane fluidity in the secretion process (16). A second level at which restraints may act to maintain structural organization is the cytoplasmic side of the plasma membrane, where subplasmalemmal fibrils were located exclusively under the specialized surface coat. If these subplasmalemmal components hinder the mobility of the specialized surface coat, a transmembrane interaction (16) not mediated by membrane particles as visualized by freeze-fracturing must operate. This interaction would tend to maintain the segregation of membrane and surface components on the highly differentiated cytostome region.

The almost complete absence of intramembranous particles in the flagellar plasma membrane of *T. cruzi* contrasts with the uniform distribution of CIH-labeled negative sites, Con A receptors, and ruthenium red-positive components present on the flagellar surface coat. From this observation, the inference can be made that in the undisturbed plasma membrane of *T. cruzi*, several surface receptors are not associated with membrane particles, at least in the region of the flagellar membrane and at the cytostome region. Independence between integral (membrane particles) and certain peripheral (surface receptors, antigenic determinants) membrane constituents was suggested previously on the basis of our study of the *E. histolytica* plasma membrane (15). In the latter system, redistribution of integral or peripheral (17) components in the plane of the membrane had to be experimentally induced (10). In *T. cruzi*, however, this independence is inherent in the native plasma membrane.

## SUMMARY

A regional specialization of the cell surface of *T. cruzi* culture forms was found at the cytostome as a localized thick surface coat rich in carbohydrate-containing components. The prominent surface coat was located over a region of the plasma membrane where intramembranous particles were exceedingly low in number. In turn, the particle-poor region was related to specialized submembrane fibrils not present under other regions of the plasma membrane. The cytostome region provides a striking example of a stable regional differentiation of the plasma membrane, involving the outer surface, the membrane interior, and the underlying cytoplasm. In addition, independence of Con A receptors, colloidal iron binding sites, and ruthenium red-stainable surface components from membrane particles was demonstrated at the flagellar membrane.

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