# A CLOSELY PACKED ARRAY OF MEMBRANE INTERCALATED PARTICLES AT THE FREE SURFACE OF HYDRA

RICHARD L. WOOD. From the Department of Biological Structure, University of Miami School of Medicine, Miami, Florida 33152

## INTRODUCTION

The freeze-cleave technique has revealed particles on internal aspects of plasma membranes which appear to be glycoprotein and lipoprotein complexes (1, 2, 12, 18, 20). The particle size most commonly observed is 85–90 Å but in certain sites the particles may be as small as 60 Å or as large as 300 Å in diameter (9, 11, 17, 22, 25). For most membrane systems the granules are most numerous on the cytoplasmic fracture face (Face A), but in certain circumstances the polarity is reversed and the granules are more numerous on the external leaflet (Face B) (3, 5).

In the specialized areas of close intercellular contact recognized as gap junctions (14, 21), for which there is good evidence of involvement in intercellular communication (7, 16, 22), the intramembranous granules are arranged in arrays frequently displaying a hexagonal lattice with an interparticle spacing of 90–100 Å (8, 14). It is characteristic of gap junctions that the outer fracture face (Face B) shows a complementary hexagonal lattice of pits corresponding to the granular lattice on the cytoplasmic leaflet (Face A) (14).

Free membrane surfaces may display special aggregation patterns of intramembranous particles associated with specific regions of highly specialized cells such as spermatozoa (6), sites of secretion product discharge (23), forming micropinocytotic vesicles (15) or the bases of motile cilia (27). Also, granule patterns may be altered experimentally in immune reactions (24), by changes in pH and temperature (19), by exposure to low concentrations of proteolytic enzymes (18), and by physiologic activation (6). All of these patterns are distinct from the closely packed hexagonal arrays that characterize gap junctional complexes.

The current study has revealed a specialized configuration of intramembranous particles at the external surface of normal Hydra epithelial cells which closely resembles the arrangement in gap junctions. This specialization appears to be confined to epidermal cells as it has not been observed on gastrodermal cells included in the same replicas. The purpose of this communication is to describe this specialized arrangement and to discuss its possible significance.

## MATERIAL AND METHODS

Brown Hydra *Pelmatohydra oligactis*, were obtained from Carolina Biological Supply Co., Burlington, N. C.,

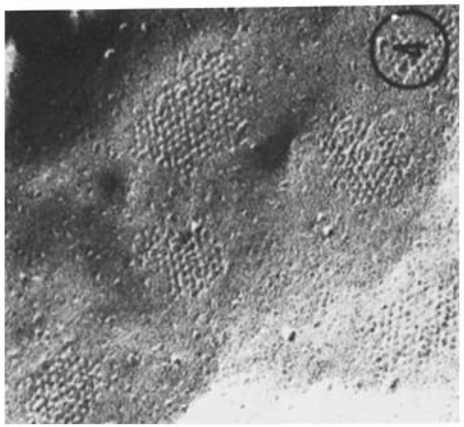
FIGURE 1 Portion of the cytoplasmic leaflet (Face A) of an epidermal cell showing random distribution of typical intramembranous granules. × 92,000.

FIGURE 2 Portion of fracture Face A of an epidermal cell showing arrays of granule aggregates. The apparent smaller size of the aggregate granules is attributable to close packing and less space for shadows. × 100,000.

FIGURE 3 Portion of fracture Face B of an epidermal cell showing regular arrays of pits. × 57,000.

FIGURE 4 Higher magnification of Face B pits demonstrating the hexagonal packing. × 125,000.






and cultured in the laboratory according to the methods of Loomis and Lenhoff (10). Animals were relaxed in culture water by adding a few drops of Nembutal (Abbott Laboratories, North Chicago, Ill.) and fixed 10–30 min in a mixture of 2% glutaraldehyde (highly purified, Ladd Research Industries, Inc., Burlington, Vt.) and 1% formaldehyde in a 0.1 M sodium cacodylate buffer, pH 7.4. The formaldehyde was generated from paraformaldehyde fresh for each fixation. After rinsing in 0.1 M cacodylate buffer to which 5% sucrose was added, the specimens were treated 4 h each in 10% and 20% cacodylate-buffered glycerol and stored in the refrigerator in cacodylate-buffered 30% glycerol. Freeze-fracturing was performed on a Denton apparatus (Denton Vacuum Inc., Cherry Hill, N. J.) at −110°C, and platinum-carbon shadowed replicas were cleaned in Chlorox (Chlorox Co., Oakland, Calif.) and 5 N NaOH before recovery on carbon-coated grids for examination in an AEI-801 electron microscope.

## OBSERVATIONS

The epidermal and gastrodermal cells of Hydra may be recognized in freeze-cleave preparations from surface configurations and by the identification of cell types known from standard preparations to be present in each of the two epithelial layers. The gastrointestinal cavity is highly irregular in contour and contains both flagellated and secretory cells. The epidermis is less irregular in surface contour, contains nematocysts, and has no flagella. Septate and gap junctions occur in both layers.

In fractures exposing the cytoplasmic leaflet (Face A) of the free surface of epidermal cells, large areas of membrane may be seen with randomly distributed granules 85–90 Å in diameter (Fig. 1). Patches of aggregated granules appear, however, which display a nearly hexagonal packing with an interparticle distance of approximately 90 Å (Fig. 2). The average diameter of the aggregates is approximately 0.5 $\mu$m although considerable variation in size occurs. External leaflet profiles (Face B) show no evidence of pits in areas of random particle distribution, but in other areas they show a prominent, regular array of pits corresponding in overall dimensions and center-to-center spacing to the A-face particle aggregates (Figs. 3, 4).

Although the sample is limited, the appearance of the specialized particle aggregates does not appear to be correlated with a distinctive cell type, with the exception that no such aggregates have been observed at the surface of any gastrodermal cells. Furthermore, these specialized aggregates bear no close resemblance to gap junctions in Hydra, which consist of a raised plateau bearing particle aggregates (Wood, unpublished observations), similar to the gap junctions of the mollusc *Cominella*, described by Flower (1971) (4). A detailed account of cell-to-cell junctional areas in Hydra disclosed by the freeze-cleave technique will be the subject of a separate communication.

## DISCUSSION

There are several reports in the literature correlating the distribution of intramembranous particles at free cell surfaces with functional changes under various experimental conditions (6, 18, 19, 24), and one recent demonstration that particle aggregation can be induced in lymphocytes by exposure of unfixed cells to glycerol (13). To my knowledge, however, a closely packed array of granules resembling the arrangement at gap junctions has been reported for free cell surfaces previously in only one instance. Plaques of particles within the luminal plasma membrane of transitional epithelial cells described by Staehelin, Chlapowski, and Bonneville (26) bear a superficial resemblance to the granule aggregates in Hydra. However, in transitional epithelium, the intramembranous particles are larger and the polarity is reversed with respect to relative numbers of granules on exposed leaflet faces A and B. It was also noted that the plaques of intramembranous particles in transitional epithelium are prominently associated with subjacent cytoplasmic filaments. Hydra does not have detectable cytoplasmic filaments underlying the free cell surface of epidermal cells. The plaques in transitional epithelial cell membranes are thought to increase surface stability and play some role in decreasing membrane permeability (26).

In other cell types the specialized disposition of intramembranous particles which has been reported at free cell surfaces (1, 6, 9, 15, 18, 19, 23, 24, 27) appears transient in nature. None of these sites has the kind of packed array of intramembranous granules that occurs at the free outer surface of Hydra epidermal cells. Brown Hydra does have granular inclusions in the superficial cytoplasm of the epidermal cells which are presumably responsible for the brown color and which probably also contribute to the glycoprotein cuticular layer found on the external surface of the animal. It is conceivable that the special configuration of intramembranous granules reported here relates to epidermal cell secretion, but there is no direct

evidence to support this concept. In view of the similarity of this configuration to the morphology of gap junctions (7, 8, 11, 14, 17, 25), it seems equally plausible to suggest that the granular aggregates represent sites allowing for direct ionic or metabolic exchange between epidermal cells and their environment. It is possible that the observed particle arrays are a modification limited to the exposed surfaces of neurosensory cells and/or nematocytes, both of which occur in the epidermis of Hydra and both of which must be responsive to direct stimuli; the limited scope of the present observations does not permit a final conclusion on this question. The frequency of observation, however, suggests that this specialization occurs on all epidermal cells. In either case, the implications are sufficiently intriguing to warrant more thorough investigation.

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