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ULTRASTRUCTURAL ASPECTS OF MEMBRANE FUSION

Birgit Satir

Department of Physiology-Anatomy, University of California, Berkeley, California 94720

The secretory process represents both a well-known, important biological phenomenon and a problem that has been studied extensively for many years in a diversity of eukaryotic cell systems ranging from protozoa with their discharge of mucocysts and trichocysts (1, 2) to mammalian systems such as synaptic vesicle release (3), pancreatic zymogen release (4), release of neurosecretory material (5), and acrosome formation (6).

Common to all to these systems is the fact that the packing, transport, and storage of the secretory product(s) takes place within membrane-bound vesicles that originate from the endoplasmic reticulum of the respective cells. During maturation the secretory vesicles move from the interior of the cell towards the plasma membrane, where, upon appropriate stimulus, fusion of vesicular and plasma membranes takes place and the secretory product(s) are released. These results were mainly obtained from excellent electron microscopic studies and represented a large portion of our total knowledge of the secretory process until recently.

One of the major stumbling blocks in this area was the lack of any available information that would allow interpretation of and correlation between the electron microscopist's concept of the unit membrane (7) and available biochemical data on membranes (8). However, with the availability of a new technique – the freeze-fracture, freeze-etch technique – it became possible to localize internal membrane components at specific sites. In other words, this technique, in combination with conventional electron microscopy, made it possible to overcome some of the difficulties encountered earlier, and an entirely new dimension and understanding of membrane structure and function emerged. The interpretation of freeze-fracture information has been reviewed extensively in other places; the particles seen in the fracture faces correspond most likely to specific protein, or possibly lipoprotein, arrangements that often occur in extensive special arrays (9).

It now became possible to attack directly several vital links in the chain of events leading to release of a secretory product; we shall here address ourselves to some of these and discuss their general implications for secretion.

The first question pertains to membrane recognition. How do vesicular and plasma

membranes recognize each other as partners in a fusion process? This is a pervasive question in developmental and cell biology and is by no means answered.

In 1957, Del Castillo and Katz (10) put forward a hypothesis concerning the release of transmitter from the synaptic vesicles in the neuromuscular junction. They hypothesized that in order to get release there had to be specific sites on the vesicular membrane and on the axon membrane, and only when the two collided did membrane fusion and transmitter release occur. As later became clear, this hypothesis turned out to be quite predictive for the release of mucocysts in Tetrahymena.

Other questions include the following. Fusion initiation: is there a special trigger for the fusion process, or does it follow spontaneously? Mechanism of product release: does release always require complete membrane reorganization or may special gating mechanisms be involved? Fate of vesicular membrane: after secretion is completed what happens to the vesicular membrane? Does it become incorporated into the existing cell membrane, or does it recycle or become broken down?

In 1972, utilizing a combination of techniques, we illustrated and demonstrated for the first time the intimate details of membrane reorganization that take place within the two partner membranes prior to and during fusion. The system used as a model for the secretory process was mucocyst discharge and release in the ciliated protozoan Tetrahymena pyriformis W. This one-celled organism is especially well suited for such a study, since its secretory vesicles, the mucocysts, are aligned along specific rows, the 2° meridians, running from the anterior to the posterior end of the cell. A further advantage is the presence of cilia, which in this case act as built-in markers for orientation and identification of the different membrane fractures. The cilia are again aligned along rows, 1° meridians, located between the 2° meridians, and mucocysts are also found between adjacent cilia. Prior to release, the vesicle, an elongate sac-like structure 0.3 μm in diameter and 0.96 μm long, has migrated to the plasma membrane, and simultaneously its content has matured and become crystalline. Upon discharge the vesicle changes shape and becomes a sphere of diameter 0.7 μm, and its content changes configuration. Release of the secretory product, presumably a mucoprotein, is reported to take place within milliseconds, and we can induce release under appropriate conditions – by brief fixation with glutaraldehyde, for example. With this extremely well suited system for membrane studies, we have been able to elucidate some of the earlier inaccessible links in the chain of secretory events (13). We shall in this paper address ourselves to some of the questions posed above and attempt to relate and correlate our findings from this model secretory mechanism to some of the higher mammalian systems.

A specially exciting feature revealed by freeze-fracture studies of Tetrahymena plasma membrane was the presence of specific internal arrays of freeze-fracture particles – rosettes – in places where mucocysts could be expected. Each of these rosettes consists of roughly nine outer 15-nm-diameter particles and one central one; the outer diameter of the rosette is 60 nm (Fig. 1a). Each rosette represents a specialized fusion site towards which the secretory organelle (mucocyst) migrates prior to fusion and release. The fusion rosettes are found along the 1° meridians between adjacent cilia and in rows by themselves along the 2° meridians in exact agreement with the distribution of mucocysts found with conventional electron microscopy. This type of internal membrane particle array has now been found in connection with a number of secretory systems (14) and possibly occurs in endocytic systems as well.

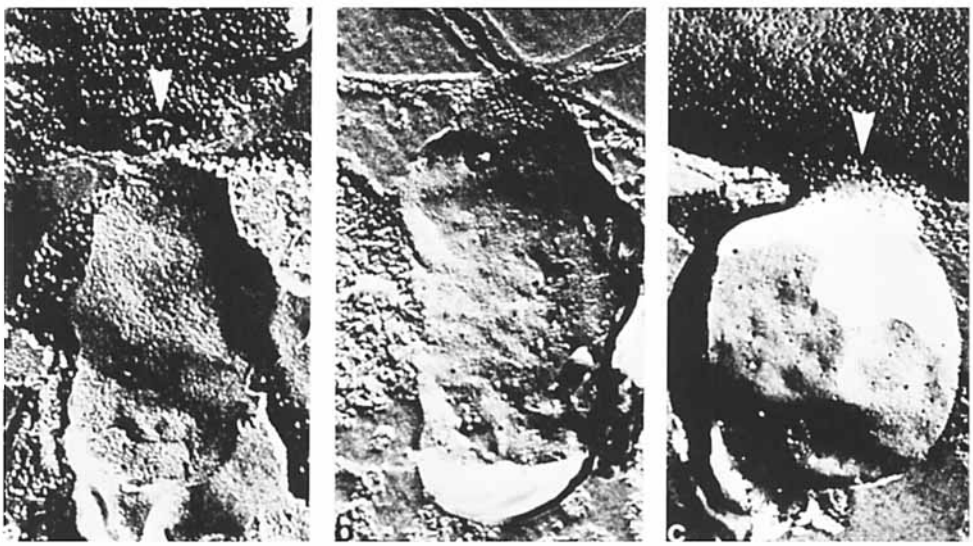

Fig. 1. Summary of membrane events accompanying fusion. (a) Freeze-fracture micrograph of fracture face A of the plasma membrane, showing the presence of the fusion rosette (arrow) and located directly below this the mucocyst – X 91,000. (b) Fracture face A of the mucocyst with its matching array: the annulus already formed at the anterior end of the organelle – X 68,000. (c) Fused A halves of the two partner membranes. Note the presence of the annulus (arrow) and the shape change that has taken place after fusion. × 64,600.

As the secretory organelle comes within a critical distance of the plasma membrane, a corresponding reorganization takes place at the anterior end of the organelle, and an annulus is formed (Fig. 1b) which consists of particles about 10 nm in diameter. The extreme tip of the mucocyst is devoid of any particles in an area of 60 nm and it is into this area that the rosette of the plasma membrane sinks, an event that represents one of the first stages in membrane fusion. Finally, in Fig. 1c the fused partner membranes are shown. The shape change is clearly seen in the replica. Note how the presence of the annulus (arrow) clearly denotes the transition from plasma to vesicular membrane. The role of the annulus appears to be twofold: (1) it acts as a reinforcing ring, hindering membrane rupture during the almost explosive discharge of product, and (2) it acts as a stabilizing barrier boundary when the mucocyst membrane becomes incorporated into the plasma membrane. We shall return to this point in more detail.

In Fig. 2 is shown a model of some of the molecular events that we hypothesize are occurring during fusion of the two partner membranes. Fig. 2a shows a cross-section of the plasma membrane (PM) containing three of the rosette particles and below it the approaching mucocyst (Mc) with six smaller annulus particles present. As the two membranes approach each other the two particle arrays become apposed to each other, reminiscent of junction formation. As yet we have no information on the position of the membranes, but in other systems microtubules or microfilaments seem to be involved in such alignments. In any case, as the membranes approach each other the cytoplasm between them gets squeezed away and a hinge is formed between surface proteins of the

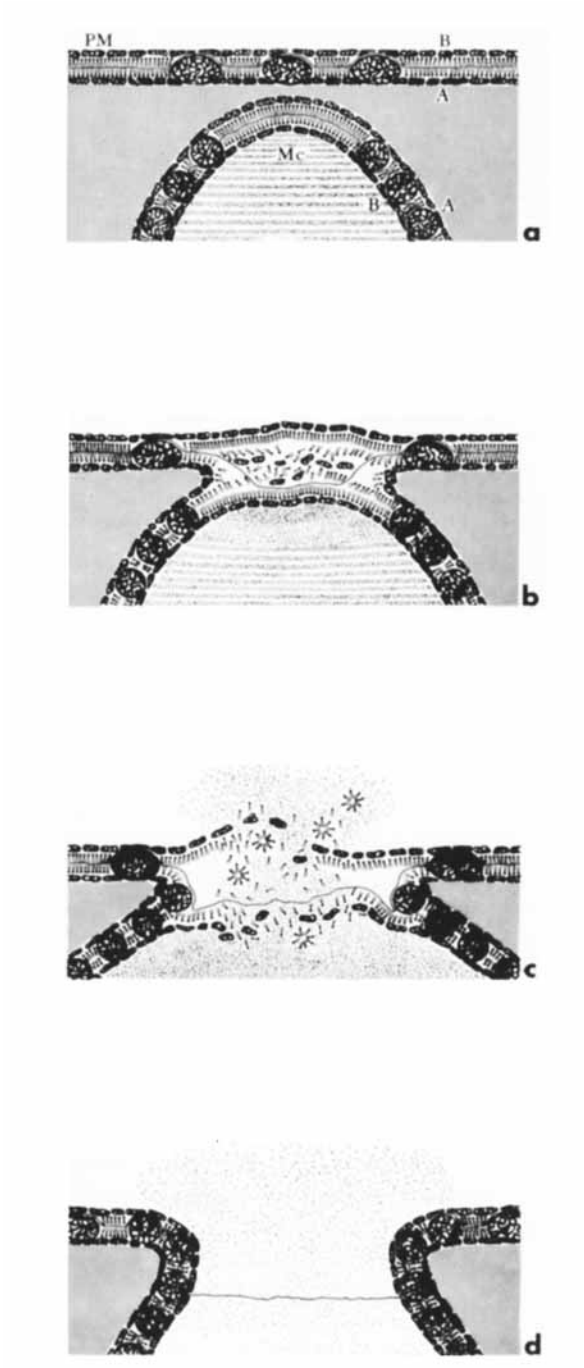

Fig. 2. Diagrammatic representation of membrane events taking place during fusion of plasma and mucocyst membranes. See text for further explanation. (From Satir et al. [13]).

two partner membranes. The first sign of fusion, if the fracture is followed (black line) from face A of the plasma membrane, is the formation of a nonetchable depression in the center of the rosette. This depression corresponds to face B of the mucocyst membrane (Fig. 2b), which is seen as the two A half-membranes begin rearrangement. On the plasma membrane A face rosette particles spread apart, and the annulus particles from the fused mucocyst membrane face A evolute and become visible in the collar of the fusion pocket. Fusion of the two A-half membranes is completed before fusion of the B-half membranes (Fig. 2c). At this stage the depression in the center of the now opened rosette becomes etchable. Because this is an explosive process, we depict loss of a few micelles and proteins; however, this is purely speculative. Finally, in 2d reorganization is completed and a continuous unit membrane has been formed around the fusion lip. At the center of the pocket the fracture would now pass through the mucocyst content.

FATE OF VESICULAR MEMBRANE

Tetrahymena is a one-celled organism that divides by binary fission. In every cell cycle, Tetrahymena has to generate enough new membrane for a daughter cell. Further, this new membrane must possess a duplicate of the original membrane template with all its arrays maintained in their exact locations. Interestingly, calculations show that approximately the total necessary membrane area could be contributed from mucocyst membrane if each rosette represents a fusion event during one cell cycle (14, 15). One advantage of this hypothesis of membrane addition is the maintenance of a specific membrane template from mother to daughter cell.

It has been possible to follow the spherical vesicles after discharge using high-voltage stereo microscopy (B. Satir [14] and unpublished). Intact vesicles do not appear to pinch off and migrate back into the cell; rather, the vesicular profiles become smaller and smaller. We have interpreted this as follows: the large-diameter vesicular profiles represent the initial fusion event, and small-diameter, shallow vesicular profiles indicate considerable bulk flow into the plasma membrane. Final proof comes from freeze-fracture results, where we have been able to visualize the shallowest vesicular profiles en face, showing the final incorporation step.

Figure 3 shows such a view. We are looking at a freeze-fractured plasma membrane face A and two profiles of exploded mucocysts along a 2° meridian. It should be noted how clearly visible the spreading of the bigger rosette particles is in this case. In the top profile, we recognize the annulus of particles visible in the collar of the fusion pocket. Below it we have a similar image; however, here the fusion pocket is very shallow, so that the A face is unbroken in the replica. The annulus can still be seen and within it a collection of numerous particles can be found. The annulus evidently acts as a boundary between the two partner membranes that allows lipid flow but not particle flow through it. Particle density increases within the remnant of the mucocyst membrane as more and more lipid flows through the annulus into the plasma membrane (compare Figs. 1 and 3). Also, note that surrounding the annulus ring of particles is a zone devoid of particles, as we would expect if this were newly incorporated lipid.

Two other cases should be mentioned in this regard, in which a similar incorporation of bulk membrane takes place. One is in the case of synaptic vesicle release. Here Heuser

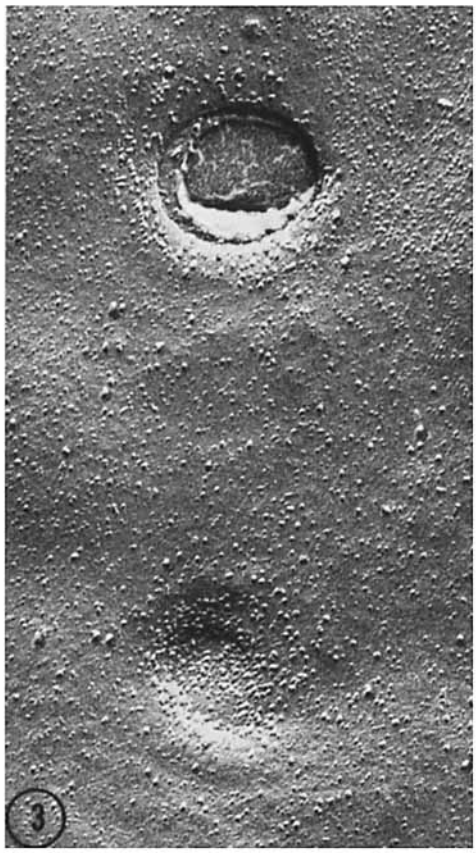

Fig. 3. Fracture face A of the plasma membrane showing two fusing mucocyst profiles en face. The lower profile illustrates the final stage in vesicular membrane incorporation with its shallow fusion pocket and its dense particle concentration. Also note the spreading of the bigger rosette particles clearly visible here. X 78,000.

and Reese (3) showed that after release of transmitter the synaptic vesicle membrane became incorporated into the axon membrane. However, there appears to be no total gain in membrane area since membrane is concomitantly pinched off further away; so in this case the bulk membrane incorporation may act more in the sense of membrane renewal. The second example is found at release of the acrosomal content of the sperm (6). After the acrosome has released its content, its membrane is added to that of the sperm membrane, in this case as growth in total membrane area like Tetrahymena, since at this point the sperm needs to expand its total membrane area in order to make the projections that later on make contact with the egg.

MECHANISM OF PRODUCT RELEASE

In Fig. 4 I have illustrated two ways in which secretory product release might

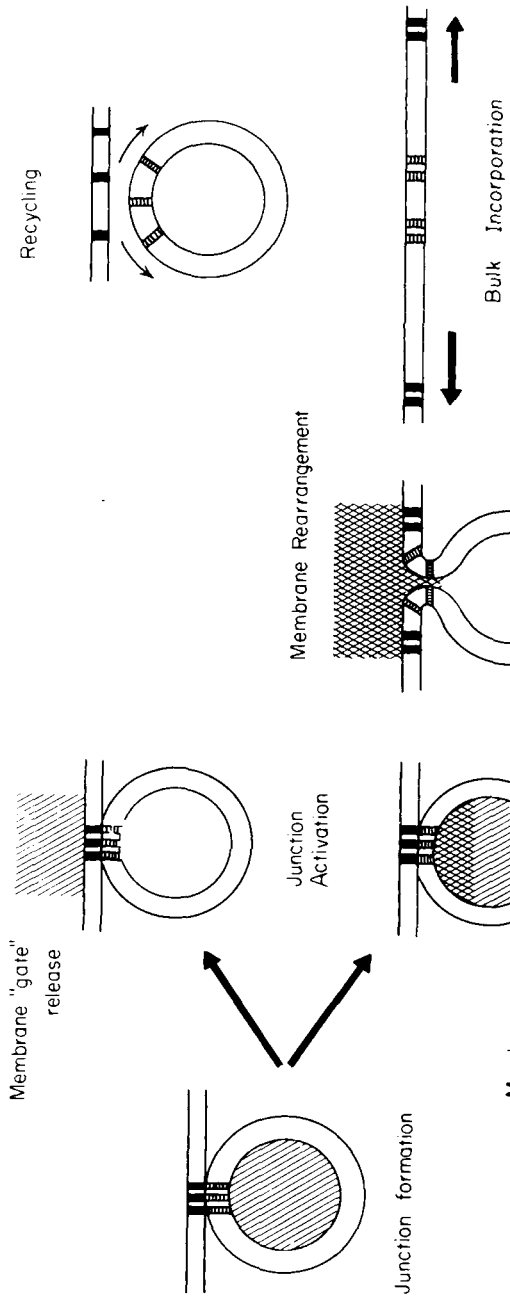

Fig. 4. Models of vesicular release. Matching arrays are formed within the partner membranes (junction formation) prior to discharge. Upon triggering of discharge (junction activation), hydrophilic channels become available through the membranes. (Above) In membrane gate release low-molecular-weight material passes directly through the channel without membrane rearrangement. Vesicles can be recycled after release by uncoupling and detachment. (Below) In membrane fusion release, on junction activation osmotic pressure or other changes within the vesicle lead to membrane rearrangement. High-molecular-weight substances are released after completion of fusion. The vesicular membrane is incorporated into the cell membrane in bulk at the fusion site. (From Satir [14]).

take place. The first is independent of membrane fusion while in the second membrane fusion is imperative for release. In both postulates, release is dependent on formation of ion-permeability channels through the hydrophobic centers of the membranes. In Tetrahymena, the rosette and annulus could serve this purpose. Because the close apposition of arrays in matching membranes is reminiscent of the structure of cell junctions (9) and because gap junctions in particular are known to provide ion-permeability channels through these matching membranes under conditions where cells are coupled, we have called the first steps in secretory release (1) "junction formation," to indicate that the matching arrays are present, and (2) "junction activation," to indicate that triggering must involve conditions where the junction is freely permeable to appropriate cations. It seems likely that Ca^{++} ion will prove important to the latter step. After activation and channel formation it might be possible for low-molecular-weight secretory products ($<$ 20,000 daltons) to be released via simple diffusion out through the newly formed channels. This is essentially a statement of the "membrane gate" hypothesis. After release this vesicle could easily be recycled if the particles forming the array effectively closed the channels by moving apart.

In the second case, illustrated by the mucocyst, the high-molecular-weight, relatively insoluble secretion product would not pass through the junctional channels. Instead, the shifting environment within the secretory vesicle induced by junction activation would cause water to flow in, swelling the vesicle osmotically. In Tetrahymena, at fusion, the vesicle swells and its shape change is dramatic, as I have already mentioned (Fig. 1). Additionally, our recent experiments indicate that release is blocked by the presence of 0.25 M sucrose in the medium before fixation; we think that the sucrose only provides osmotic protection. The explosive shape change could bring the membranes in close contact and initiate the rearrangements discussed above. Whether the rosette and annular particles also represent special phospholipases or other enzymes that encourage the rearrangements is unknown. After fusion, the cavity of the vesicle is continuous with the exterior of the cell and the high-molecular-weight product is now free. In this case, the newly fused membrane generally is incorporated into the old cell membrane, thus expanding the membrane by bulk addition at specific sites. In dividing cells, this may be useful for membrane growth and specific differentiation, while the older membrane template is retained or slowly modified. Modification or turnover will also occur in nondividing cells since, although net expansion is stopped by compensating pinocytosis, the sites of addition and removal may be spatially separated. In systems such as the mammalian pancreas and parotid, where particle arrays within the membranes are perhaps less elaborately organized, and where osmotic changes are less dramatic, release is slower, as might be expected if mechanisms similar to those described here operated in a less explosive fashion.

ACKNOWLEDGMENTS

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REFERENCES

1. Tokuyasu, K., and Scherbaum, O. H., J. Cell biol. 27:67 (1965).
2. Yusa, A., J. Protozool. 10:253 (1963).
3. Heuser, J. E., and Reese, T. S., J. Cell Biol. 57:315 (1973).
4. Jamieson, J. D., and Palade, G. E., J. Cell Biol. 50:135 (1971).
5. Pfenninger, K., Akert, K., Moor, H., and Sandri, C., J. Neurocytol. 1:129 (1972).
6. Colwin, A. L., and Colwin, L. H., J. Cell Biol. 19:477 (1963).
7. Robertson, J. D., Biochem. Soc. Symp. 16:3 (1959).
8. Poste, G., and Allison, A. C., Biochim. Biophys. Acta 300:421 (1973).
9. Satir, P., and Gilula, N. B., Ann. Rev. Entolmol. 18:143 (1973).
10. Del Castillo, J., and Katz, B., in "Microphysiologie Comparée des Eléments excitables." Coll. Internat. C.W.R.S. Paris 67:246 (1957).
11. Satir, B., Schooley, C., and Satir, P., Nature, London 235:53 (1972).
12. Pitelka, D., "Electron Microscopic Structure of Protozoa." Macmillan Co., New York (1963).
13. Satir, B., Schooley, C., and Satir, P., J. Cell Biol. 56:153 (1973).
14. Satir, B., in Soc. for Exptl. Biol., London XXVIII:391 (1974).
15. Satir, P., and Satir, B., in Control of Cell Proliferation (R. Baserga, and B. Clarkson, eds.) p. 233 (1974).