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Genetic Control of Membrane Mosaicism

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The mosaic and dynamic character of cellular membranes is illustrated by the specific intramembrane particle array, the fusion rosette, found to be essential for membrane fusion and secretion in the ciliated protozoa, Tetrahymena and Paramecium. The rosette is not a permanent site within the membrane. When secretion of mucocysts is synchronized by treating cells with the local anesthetic dibucaine, all rosettes disappear, only to reassemble as new mucocysts mature. Assembly of the functional rosette is under genetic control. A series of secretory mutants of Paramecium, blocked in various stages of the secretory cycle, has been studied (11). Mutants that do not secrete lack the fusion rosette, although other intramembrane particle components of the fusion site are present. Certain properties of the rosette, in particular its particle partition coefficient, are temperature-dependent, which may affect the ability of the rosette particles to assemble. A temperature-sensitive mutant, nd9, secretes normally, and has rosettes at 18°C, but fails to secrete attached trichocysts at 27°C.

Key words: fusion rosette, membrane fusion, ciliated-protozoa, secretory mutants, particle partition coefficient

INTRODUCTION

The mosaic nature of membranes is well illustrated by the freeze fracture technique that reveals the presence of specific intramembrane particle arrays. In the last decade, several such arrays have been described, including gap, tight and septate junctions, ciliary necklaces and patches (1), particle arrays in connection with neuromuscular junctions (2), and fusion rosettes (3). These all demonstrate the mosaicism inherent both in membranes of diverse cell types and in different zones within one membrane.

These arrays of particulate components probably correspond to integral lipoprotein complexes. Because temporal changes are readily observed in arrays, both with regard to assembly characteristics and with regard to function, they provide proof of the dynamic properties of the membrane and a way of defining important parameters related to these properties (1).

The fusion rosette represents one of the clearest and best understood examples of what role an intramembrane particle array can play in a physiological process – in this case, in membrane fusion and secretion (3, 4). In earlier studies on the ciliated protozoan Tetrahymena, the fusion rosettes, consisting of about eleven particles 15 nm in diameter surrounding a central particle, were found to correspond to sites toward which the secretory organelles of this cell, mucocysts, migrate prior to fusion and release (3, 4). The fusion rosette is a stable unit once it is assembled and it remains in its correct position until

triggered. In this paper I shall use this array to illustrate how local properties of the cell membrane are dependent on physiological state. I shall show that the fusion rosette is a transient arrangement of the cell membrane that is assembled specifically in connection with a fusion event and that disappears on completion of this event. Further, I shall show that certain properties of the rosette are temperature dependent, which may imply that local alterations of membrane fluidity are important to array assembly and function. Finally, I shall show that the assembly of such an array is under genetic control by nuclear genes. This obviously suggests that membrane mosaicism in general is under genetic control, in that the genes control which integral membrane proteins are produced to form specific zones in the membrane that may stay together to perform some physiological function.

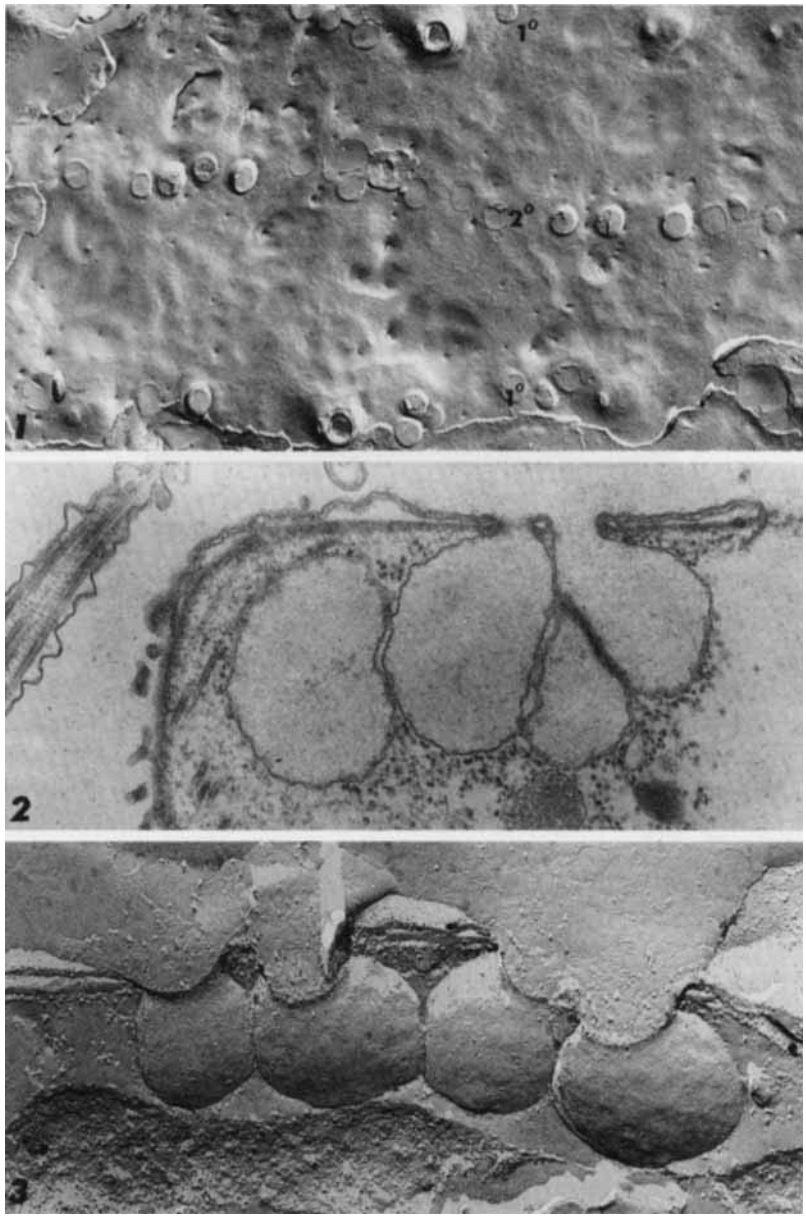
ARE FUSION ROSETTES PERMANENT SITES?

In earlier studies on the Tetrahymena fusion rosette, the sequence of the membrane events taking place when the two partner membranes fuse during secretion is arrived at via a reconstruction of a series of micrographs. Although this sequence indicates that the fusion rosette particles apparently move apart as fusion progresses, it is not conclusive. For one thing, it is dependent on the assumption that when glutaraldehyde fixes logarithmic phase cells, asynchronous release of mucocysts is induced, so that images along a single meridian show different features of the fusion process. One way to demonstrate that this assumption is correct would be to develop a procedure that induces synchronous release of mucocysts. This would enable us to capture a true time sequence of events, since all mature organelles would be secreted upon stimulation, and membrane events would be in phase.

The dibucaine method developed by Thompson et al. (5) for deciliation turns out to be one of the most gentle of its kind; after treatment, it is possible to return the cells to fresh medium and follow regrowth of cilia (6). We have now found that in Tetrahymena, dibucaine first induces rapid massive synchronous release of all mature positioned mucocysts. Mucocyst discharge is extremely sensitive to the addition of dibucaine. It takes place within 60 sec after addition of the drug, while deciliation, which is usually aided by mechanical stimulation, takes 10–15 min under our conditions.

After sedimentation, the released product appears as a white fluffy layer above the cell bodies. In contrast to asynchronous discharge, where the majority of images show intact fusion rosettes, freeze fracture replicas of cells exposed to dibucaine for 60 sec all show open cross-fractured fusion pockets (Fig. 1). In thin section, nearly every mucocyst is seen in the process of either releasing or as already having released its content (Fig. 2). Similar profiles can be observed in freeze fracture of such preparations. Figure 3 is an example from a cell population exposed to dibucaine for 30 sec; a row of four spherical releasing mucocysts can be seen.

Freeze fracture studies of the plasma membranes of cells returned to fresh medium indicate that the rosettes are now completely absent from their normal sites. Orientation on these replicas presents no problem since the ciliary rows along 1° meridians are readily recognizable as resealed stubs. The fusion rosettes remain absent until 2–3 hr of recovery; however, prior to this, one can observe bigger particles the size of rosette particles (Fig. 4, circles) spread out among the regular background particles. During recovery, massive synthesis of mucocyst precursors takes place, filling up the cytoplasm (Fig. 5). Fusion rosettes reappear at approximately the same time as new mature mucocysts are found,



Figs. 1–3. Tetrahymena after exposure to dibucaine for 60 sec.
Fig. 1. E fracture face of the plasma membrane. Note along both the 1° and 2° meridians the cross-fractured discharged mucocyst profiles. × 32,000.
Fig. 2. Thin section showing the releasing mucocysts. × 32,000.
Fig. 3. Corresponding row in freeze fracture. × 49,600.

thereby linking the two events together. It is thus clear that the fusion rosettes are not permanent sites within the plasma membrane (7).

The difference in sensitivity of secretion and deciliation to dibucaine might be an indication of a lower threshold level of internal Ca^{++} necessary for the secretory process

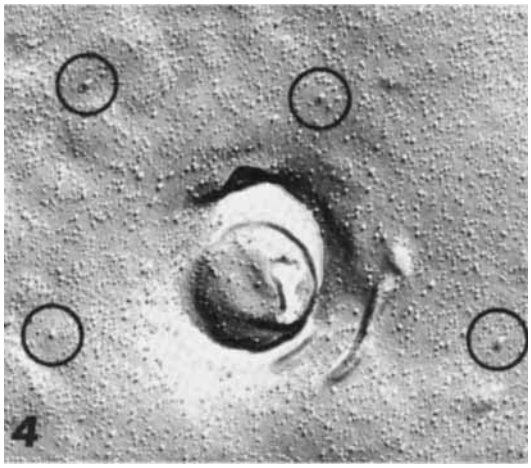


Fig. 4. P fracture face of the plasma membrane after the cell has been returned to fresh medium (approximately 20 min after initial addition of dibucaine). Note the bigger particles (circles) spread out among the smaller regular particles. × 60,000.

than for deciliation. It is well known from other secretory systems (8) that Ca^{++} plays an important, if still unknown, role in the triggering of secretion. The action of dibucaine, a local anesthetic, is generally accepted to be at the cell membrane, where it is capable of reacting with the phosphate groups of acidic phospholipids, to replace Ca^{++}, perhaps locally raising the Ca^{++} concentration. We have also been able to induce mucocyst secretion in Tetrahymena with the divalent cation ionophore A23187, without addition of external Ca^{++} (9). In this case we can vary concentrations of ionophore so as to vary the amount of mucus secreted. This suggests that each separate secretory event requires specific rise in local Ca^{++} concentration. It may be that the fusion rosette forms part of the natural Ca^{++} channel through the membrane. It is interesting that methods that purportedly localize Ca^{++} show significant precipitate at the sites of the fusion rosette (10).

These studies point out the importance of timing in looking for potential fusion sites in other cells. Fusion sites are transient even in Tetrahymena. For 2/3 of the cell's generation time no fusion rosettes can be observed after synchronous release.

IS ARRAY FORMATION UNDER GENETIC CONTROL?

In order to approach this question, I have worked with Dr. J. Beisson, Dr. M. LeFort-Tran, and their colleagues on a unique series of secretory mutants of Paramecium tetraurelia developed by Beisson (11) and Pollack. Earlier studies of Paramecium (12, 13) had confirmed the presence of fusion rosettes within the plasma membrane corresponding to the rosettes in Tetrahymena. These fusion rosettes act similarly in both systems. In Paramecium the corresponding secretory organelle is the trichocyst. In agreement with other observations (14, 15), three distinct zones are found within resting trichocyst sites: (a) a single or double ring of particles, ca. 300 nm in diameter, consisting of about 2 × 40 particles, 11 nm in diameter; (b) a central fusion rosette with an outer diameter of 75 nm, comprised of about 10 particles 15 nm in diameter; and (c) a zone of exclusion between the outer ring and rosette containing only few disperse particles (Fig. 6).

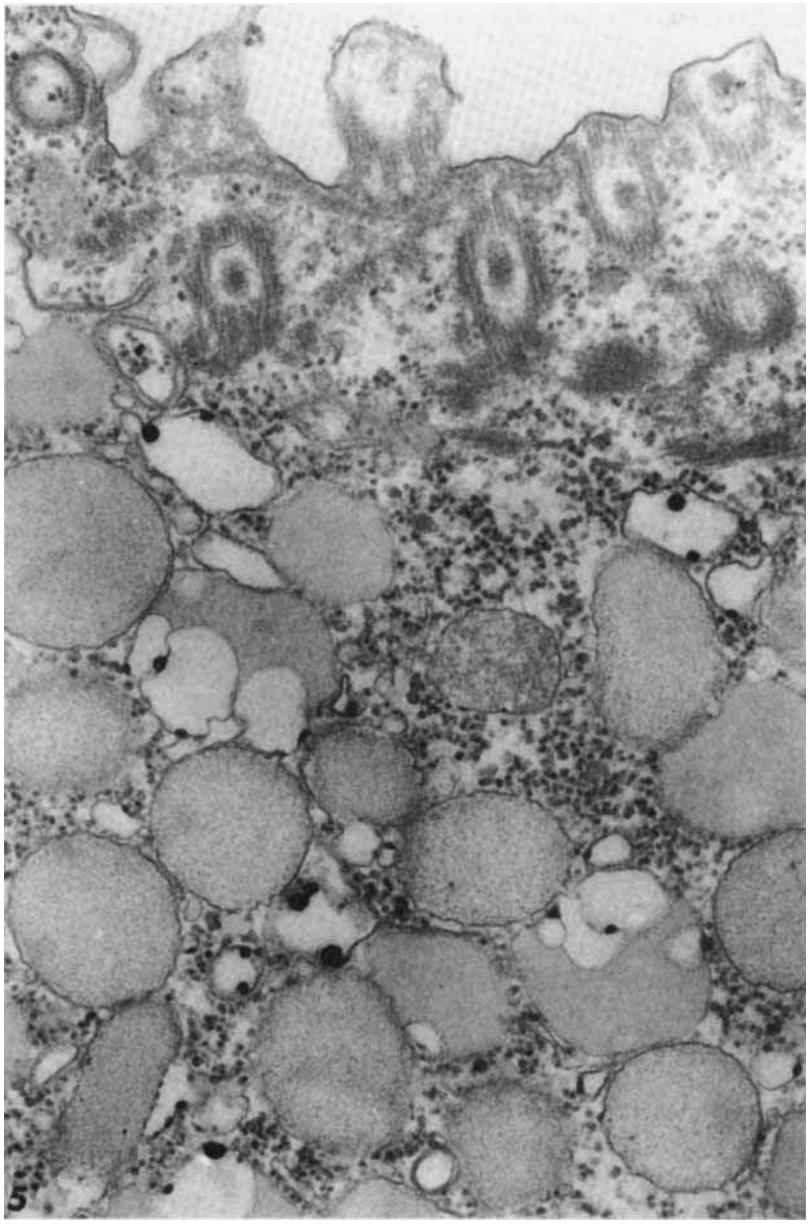


Fig. 5. Tetrahymena cytoplasm after 3 hr of regrowth in fresh medium. The cytoplasm is filled with mucocyst percursors. × 46,400.

One variation in the outer ring is sometimes observed: Instead of being circular it appears flat and this state is referred to as "parenthesis" (Fig. 7).

The trichocyst cycle in Paramecium comprises at least four major steps: 1) morphogenesis in the cytoplasm, 2) migration towards the plasma membrane, 3) attachment of the organelle to the plasma membrane, and 4) release (exocytosis). The latter two steps involve interactions with the plasma membrane that can be visualized by the appearance

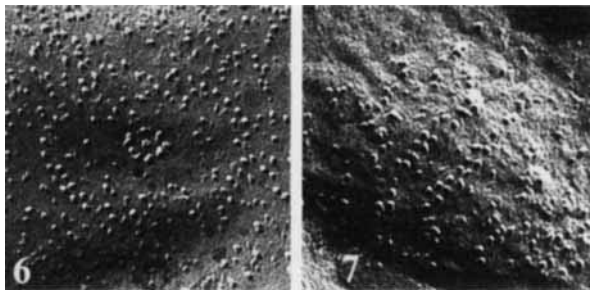


Fig. 6. P face fusion rosette in wt Paramecium surrounded by rings. Note the presence of a central particle in the rosette. × 85,000.
Fig. 7. P face parenthesis as seen in mutant t1 = trichless. Note the absence of the fusion rosette. × 85,000.

TABLE I. Summary of Mutant Features in Paramecium Trichocyst Cycle

	Trichocyst characteristics			Plasma membrane arrays		
Strain	Morphogenesis	Attachment	Discharge	Parentheses	Rings	Rosettes
wild type	+	+	+	+	+	+
nd9 18°C	+	+	+	+	+	+
nd9 27°C	+	+	–	–	+	–
tam 8	+	–	–	+	–	–
t1	–	–	–	+	–	–

Source: Beisson et al. (11).

of particle arrays and subsequent changes in these arrays. The trichocyst cycle can effectively be dissected and the functional significance of the arrays analyzed by the study of a series of mutants blocked at different steps in the secretory sequence. We have examined three such mutants: 1) t1, blocked at the morphogenetic level which does not form trichocysts; 2) tam 8, which forms the organelles but is blocked either at migration or attachment so that the organelles never arrive at the membrane; and 3) nd9, a temperature-sensitive mutant, blocked at exocytosis at the nonpermissive temperature (Table I).

Freeze fracture studies of these mutants show that t1 (trichless) and tam 8 (unattached trichocysts) have two plasma membrane characteristics in common: Both have only parentheses and in both cases no fusion rosettes are ever seen (Fig. 7). In nd9 mutant cells at the nonpermissive temperature, 27°C, in which trichocysts are attached, nearly all of the rings are circular. Again, the sites lack fusion rosettes, except that sometimes in place of the rosette a few scattered particles can be seen, and these cells do not secrete. At 18°C this mutant secretes normally, and abundant circular rings containing fusion rosettes as well as a few parentheses without rosettes are observed, as in wild type. We therefore conclude (Fig. 8): 1) The parentheses represent a normal precursor form of the circular rings and correspond to unfilled trichocyst attachment sites, whereas rings correspond to filled attachment sites. In nd9 at 27°C nearly every site becomes filled because of failure of normal discharge. 2) The parentheses can assemble in the plasma membrane independently of any interaction with the trichocysts. Trichocyst attachment probably occurs before

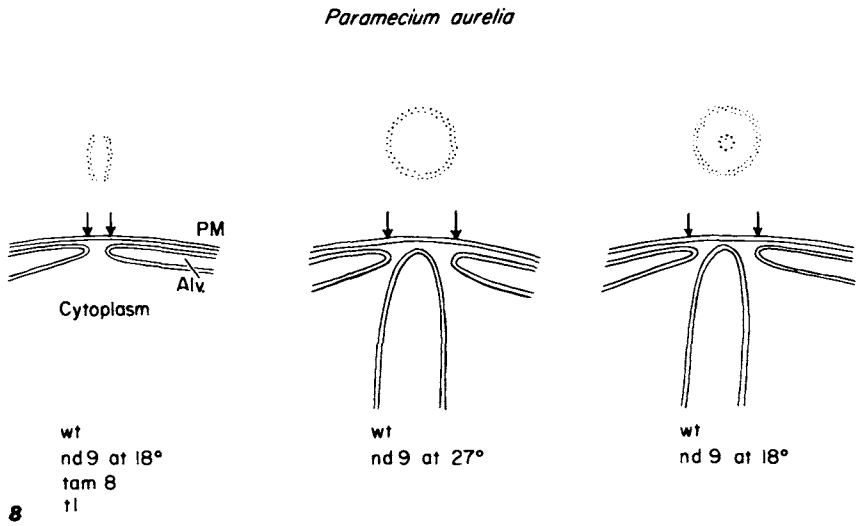


Fig. 8. The sequence of events concerning the various Paramecium mutants are summarized here: As the trichocysts attach to the plasma membrane (PM) the space between alveolar sacs (Alv) enlarges (arrows) concomitantly with the conversion of parenthesis to rings. Finally, the fusion rosette is assembled as secretory competence develops.

formation of the rosette. Therefore, 3) the rosette is not an attachment site for the trichocyst. The rosettes are present only when the trichocysts are attached (wild type and nd9 at the permissive temperature), but trichocyst attachment alone is not sufficient for rosette formation. Most importantly, 4) in contrast to the ring, the rosette appears to be an essential feature of the secretory process of trichocyst release in Paramecium. In the absence of a normal rosette, in nd9 cells at nonpermissive temperature there is no trichocyst discharge, while the same mutant recovers, at the permissive temperature, both its rosette particles and its discharge capacity.

The secretory mutants of Paramecium should allow further dissection of vesicular transport processes, array assembly, and biochemistry as they are studied further. The nonsecretory mutants are not lethal. This is in accord with recent studies (16) that show that after fusion the trichocyst membrane is recaptured by vesiculation and is not completely incorporated into the plasma membrane. Presumably, this means that the major pathway of all membrane growth in Paramecium is independent of bulk secretory vesicle incorporation. In Tetrahymena, however, membrane expansion is seen after dibucaine treatment where massive amounts of vesicle membrane are added by synchronous discharge. Whether this membrane is eventually recaptured elsewhere or is used for site-specific membrane growth or differentiation is yet to be determined. In some higher cells, bulk incorporation is obviously utilized in this manner.

CHARACTERIZATION OF THE FUSION ROSETTE

In order to characterize and compare fusion rosettes in wild type vs mutant cells, we have calculated the "particle partition coefficients" (K_p) of the rosettes where K_p = CP/CE, CP and CE being the concentrations in numbers of particles per unit surface adhering to the P or E faces respectively (1). K_p provides a quantitative measure of particle

association in an array. For some particles, such as the central particle of the fusion rosette in Tetrahymena, K_p is approximately unity. We interpret this to mean that the chance of the fracture plane passing to either side of the particle is essentially the same; so that there is no special association of the particle with either side of the membrane. However, in many arrays K_p differs significantly from unity (1). For example, in Tetrahymena the K_p of the outer ring of particles of the fusion rosette is 7.5 as opposed to 1.25 for the central particle. A K_p greater than unity means that the particles adhere more strongly to the protoplasmic fracture face than to the E face, which may be a function of their interaction or association with submembrane structures, such as microfilaments.

In Paramecium, wild type and nd9 cells at 18°C have, on the average, slightly higher total numbers of particles per fusion rosette, i.e., 8 vs 6 (Table II). Despite the difference, the K_p's of wild type and mutant (nd9) rosettes at 18°C are virtually identical (2.9). We conclude that the rosette particles in wild type and mutant cells at 18°C are identical in bonding strength to either side of the membrane – that is, their insertion into the surrounding lipid is similar. This is not surprising in view of the fact that the physiological function of the rosette is retained. At 27°C, the total number of particles per rosette rises somewhat in wild-type and the partition coefficient changes. Aside from being the first reported variation of K_p as a function of temperature, this may be indicative of changes in the intrinsic environment of the particle. In this mutant, the ability of the rosette particles to assemble to a critical number necessary for function during exocytosis may be vitally altered by such changes.

TABLE II. Partition Coefficient of the Fusion Rosette in Wild-type and nd9 Trichocyst Sites*

Cell type	Fracture face	Mean number of particles per rosette	Kp
Wt 27°	P	8	5
	E	1.6	
Wt 18°	P	6	2.9
	E	2.1	
nd9 27°	P	0.6	*
	E	0.8	
nd9 18°	P	4.4	2.9
	E	1.4	

*Modified from Beisson et al. (11)

CONCLUSIONS

We are beginning to be able to dissect the components of the secretory process in these systems. The mutants are a powerful tool for this dissection. In the first place they have provided a direct demonstration of the necessity of assembling the fusion rosette in order for secretion to proceed. This particle array must come together at a precise site for a short time in the life cycle of the ciliate and, when triggered, perform some function, perhaps related to regulation of internal Ca^{2+} concentration across the cell membrane, and disappear. Clearly, this is an exceptional example of both mosaic and dynamic properties of membranes, and of how membrane events are under direct genetic control.

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