Freeze-Fracture Evidence of Differences between Membranes of Perialgal and Digestive Vacuoles in *Paramecium bursaria*

Renate Meier, Werner Reisser, Wolfgang Wiessner,

Abteilung für Experimentelle Phykologie, Institut für Pflanzenphysiologie, Universität Göttingen, Untere Karspüle 2, D-3400 Göttingen, Bundesrepublik Deutschland

and

Marcelle Lefort-Tran

Cytophysiologie de la Photosynthèse, C.N.R.S., F-91190 Gif-sur-Yvette, France

Z. Naturforsch. **35 c,** 1107–1110 (1980); received October 28, 1980

Paramecium bursaria, *Chlorella* sp., Symbiosis, Membranes, Freeze-Fracture

Measurements of particle densities on the two freeze-fracture faces of digestive and perialgal vacuole membranes in green and aposymbiotic *Paramecium bursaria* Ehrbg. show distinct differences between the P-faces of both membrane types. The distribution of particle densities is more homogenous on the P-faces of perialgal vacuole membranes than on the P-faces of digestive vacuole membranes. Possibly homogeneity among perialgal vacuole membranes reflects the stability of perialgal vacuoles during their life cycle. Perhaps lysosomes cannot fuse with them.

Paramecium bursaria Ehrbg. harbours within its cytoplasm several hundred cells of a certain *Chlorella* species which are each located in individual perialgal vacuoles. Both partners form an endosymbiotic unit [1–8].

The size of the algal population inside the *Paramecium* varies in dependence of environmental conditions as light intensity or external food supply [2, 9, 10]. Probably the number of the symbiotic chlorellae is not regulated by digestion of algae: Acid phosphatase activity was detected only in digestive vacuoles but not in perialgal vacuoles [9, 11]. Perhaps fusion of primary lysosomes with the perialgal vacuole membrane, *e.g.* entry of digestive enzymes into the vacuole is prevented. This inhibition could be reflected by the perialgal vacuole membrane structure [12]. Therefore a freeze-fracture study of digestive and perialgal vacuoles was performed in order to test whether the cytochemical differences between both vacuole types are accompanied by a different organisation of their membranes.

Reprint requests to Renate Meier.

0341-0382/80/1100-1107 $ 01.00/0

Materials and Methods

Paramecium bursaria was cultured as described previously [13].

Experiments were performed with alga-containing (green) *Paramecium bursaria* which had been starved for ten days and therefore contained only perialgal vacuoles and with aposymbiotic *Paramecium bursaria* which had been fed with bacteria for four hours before fixation and therefore contained many digestive vacuoles.

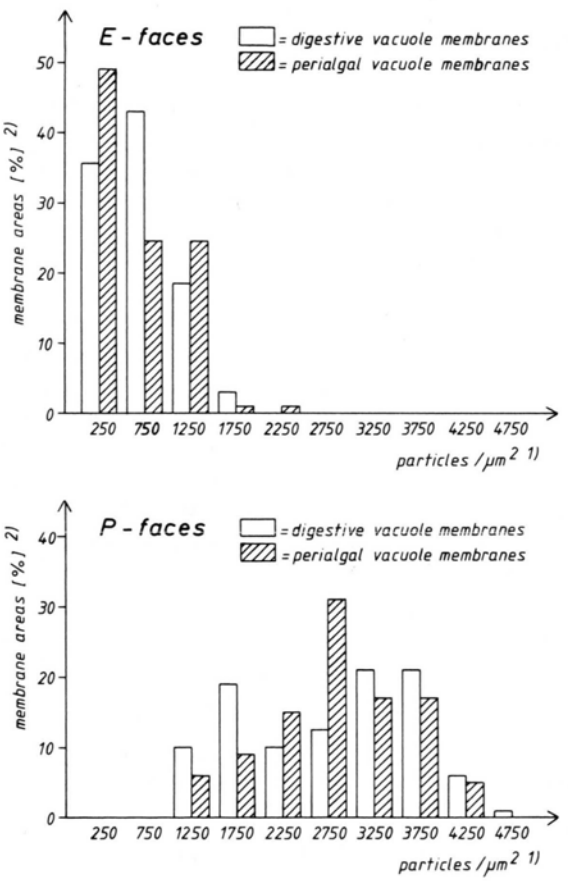


Fig. 1. Particle densities on the E- and P-faces of perialgal and digestive vacuole membranes in *Paramecium bursaria*. [1]) Number of particles/µm² was divided into ten classes (1–500, 501–1000, etc.). The numbers given in the graph represent the middle of each class. [2]) Number of membrane areas with different particle densities is given in per cent of number of totally counted areas of each face.

For freeze-fracture cells were fixed with glutaraldehyde for 1 h at 22 °C (0.5% in 0.01 M phosphate-buffer, pH 7.1), infiltrated with glycerol, frozen and fractured in a Balzers apparatus (BA 360 M) at −100 °C without etching.

Replicas were examined with a Hitachi HU11A electron microscope at 75 kV.

Intramembrane particle density was measured on micrographs printed at ×100000 by counting the particles within areas of 0.04 μm² and calculating the final value for 1 μm squares. Ten 0.04 μm² areas were counted on each of about ten different micrographs per fracture-face. The size of particles was measured as particle diameter.

Results and Discussion

Vacuole membranes are internally split by freeze-fracture, thus exposing two faces: the protoplasmic face (P-face) and the complementary exoplasmic face (E-face) [14] (Figs. 2–5). Particle density measurements of both perialgal and digestive vacuole membrane faces are presented in Fig. 1. As it was intended to compare the features of both membranes in general, membrane particle densities were measured in vacuoles of all stages of their life-cycle, and the data obtained were summarized.

The E-faces of both vacuole types have less particles than the P-faces, and there are only slight differences between the E-faces of digestive and perialgal vacuole membranes in particle distribution. However, the comparison of the P-faces of the two membranes show distinct differences: The percentage of membrane areas with classes of 1750 as well as 3250 and 3750 particles/μm² (Fig. 1) is higher in digestive vacuole membranes than in perialgal vacuole membranes. The latter have a high percentage of areas with classes of 2750 particles/μm² which is much lower in digestive vacuole membranes.

Preliminary measurements of the particle size on both digestive and perialgal vacuole membranes show no significant differences between the two vacuole types.

The distribution of particle densities on the P-faces of digestive vacuole membranes displays two subpopulations which might reflect an heterogeneity in a digestive vacuole itself or, more probably, an heterogeneity in different digestive vacuoles as observed on thin sections (unpublished results). This heterogeneity could result *f.e.* from fusion with lysosomes. This assumption is in agreement with data from *Paramecium caudatum* and other ciliates where it has been shown that in digestive vacuole membranes changes in the intramembranous particle distribution and number correlate with specific stages of the cyclosis [15–17]. However, the structure of the perialgal vacuole membrane shows a homogenous statistical distribution of frequency in particle density with only one maximum. These data indicate that – in contrast to digestive vacuole membranes – the structure of perialgal vacuole membranes remains more or less constant during the whole lifetime of the vacuole. Perhaps fusion with lysosomes is not possible.

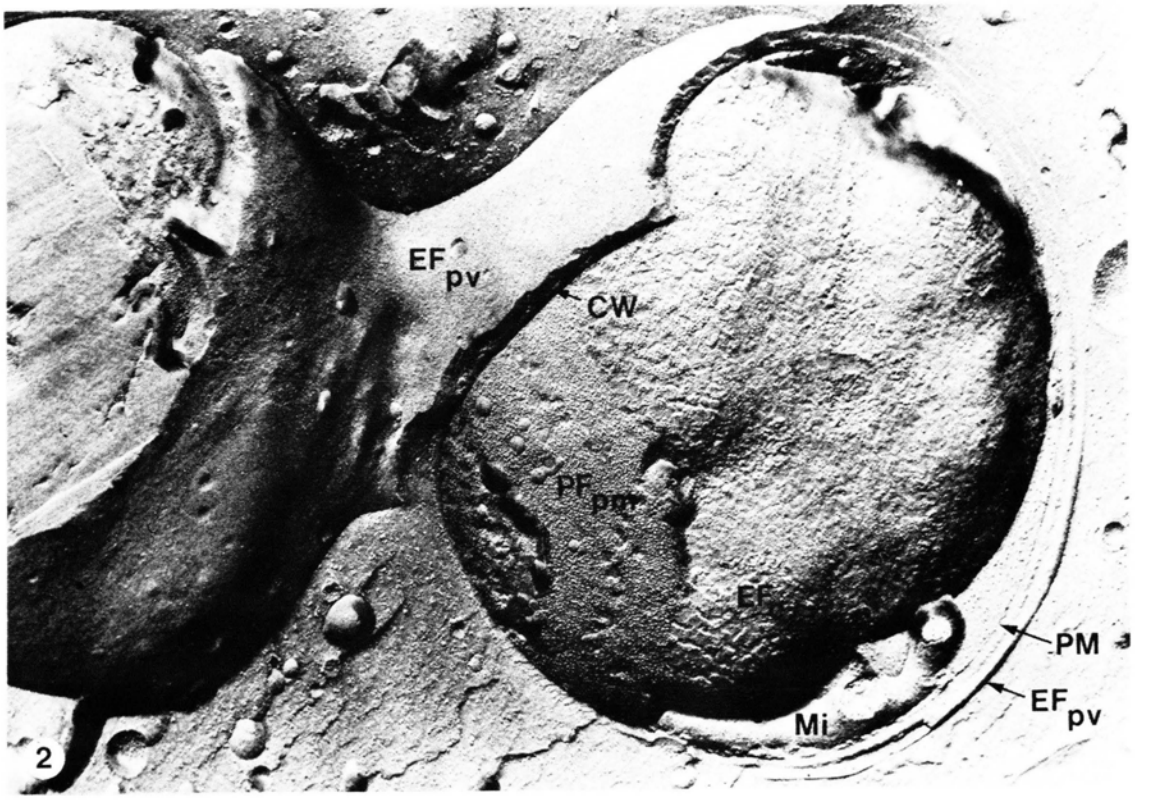
Only few information is available on the formation of perialgal vacuoles in *Paramecium bursaria.* Several experiments suggest that surface components of the algae play an important role, but it is not clear in which way they interfere with the vacuole membrane and thus probably trigger the formation of a special membrane structure [18]. Membrane altera-

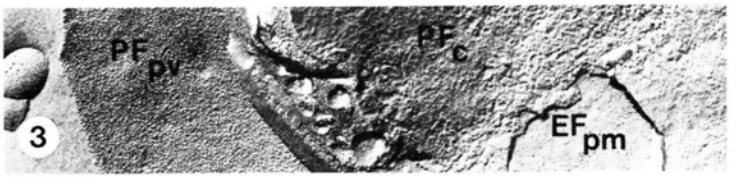
Fig. 2. Green *Paramecium bursaria.* A perialgal vacuole divides after division of the symbiotic *Chlorella.* The vacuole membrane is closely attached to the algal cell wall (CW). Successive membrane fractures are discovered. EF_{pv} = E-face of perialgal vacuole; PF_{pm} = P-face of algal plasma membrane (PM); EF_c = E-face of outer chloroplastic membrane; Mi = Mitochondrion of alga; freeze-fracture; 25000×.

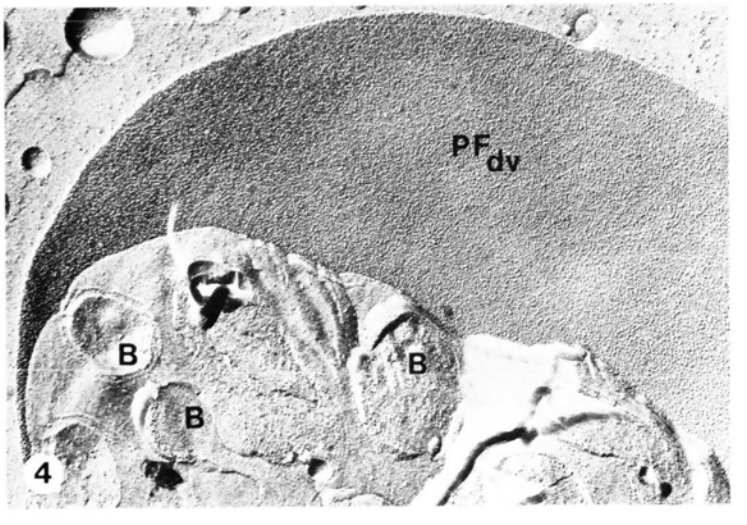
Fig. 3. Green *Paramecium bursaria.* Part of a perialgal vacuole. Particles are more frequent on the membrane's P-face (PF_{pv}) than on the E-face (Fig. 2). EF_{pm} = E-face of plasma membrane; PF_c = P-face of outer chloroplastic membrane; freeze-fracture; 25000×.

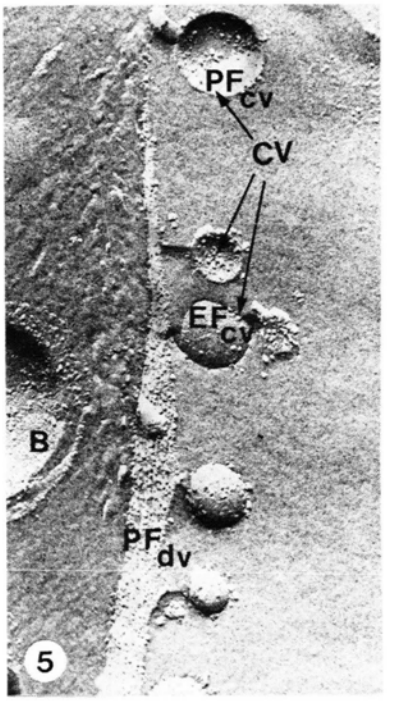
Fig. 4. Aposymbiotic *Paramecium bursaria.* Digestive vacuole with bacteria (B). PF_{dv} = P-face of digestive vacuole; freeze-fracture; 25000×.

Fig. 5. Aposymbiotic *Paramecium bursaria.* Part of a digestive vacuole. The P-face of the digestive vacuole (PF_{dv}) is connected with cytoplasmic vesicles (CV), the P- and E-faces of which (PF_{cv} and EF_{cv}) can be observed. B = Bacteria; freeze-fracture; 47000×.


EF pv
CW
PF pm
Ef
PM
EF pv
Mi
2


PF pv
PF c
EF pm
3


PF dv
B
B
B
4


PF cv
CV
EF cv
B
PF dv
5

tions caused by intracellular parasites have been reported, but mechanisms are unknown, too. There is some experimental evidence that certain intracellular parasites are protected against digestion by changing the membrane structure of their surrounding vacuoles in such a way that fusion with lysosomes is inhibited [19–22]. Investigations with antibody-treated parasites suggest that surface components of the parasites are involved in this process [23]. Possibly living symbiotic algae in *Paramecium bursaria* can act on membranes in a closely related way, thus causing membrane alterations which prevent the fusion of lysosomes with the perialgal vacuole. An inactivation of digestive enzymes as a further way to escape from host digestion has also been discussed [11, 24], but in the *Paramecium bursaria – Chlorella*-system the above presented results on the structural differences between perialgal and digestive vacuole membranes rather suggest the prevention of lysosome – perialgal vacuole membrane fusion.

Acknowledgements

This work was supported by the "Stiftung Volks-
membranes rather suggest the prevention of lysosome – perialgal vacuole membrane fusion.

[1] M. W. Karakashian, Symp. Soc. Exp. Biol. **29,** 145 (1975).
[2] S. J. Karakashian, Physiol. Zool. **36,** 52 (1963).
[3] S. J. Karakashian, M. W. Karakashian, and M. A. Rudzinska, J. Protozool. **15,** 113 (1968).
[4] E. G. Pringsheim, Arch. Protistenk. **64,** 289 (1928).
[5] W. Reisser, Arch. Microbiol. **107,** 357 (1976).
[6] W. Reisser, Arch. Microbiol. **111,** 161 (1976).
[7] E. Vivier, A. Petitprez, and A. F. Chivé, Protistologica **3,** 325 (1967).
[8] D. S. Weis, Trans. Amer. Micros. Soc. **93,** 135 (1974).
[9] R. Meier, W. Reisser, and W. Wiessner, Arch. Protistenk. **123,** 333 (1980).
[10] R. Pado, Fol. Biol. **13,** 173 (1965).
[11] M. W. Karakashian and S. J. Karakashian, Exp. Cell Res. **81,** 111 (1973).
[12] D. Gingell and L. Ginsberg, Problems in the Physical Interpretation of Membrane Interaction and Fusion, (G. Poste and G. L. Nicolson, eds.), p. 791, Elsevier, North-Holland Biomedical Press 1978.
[13] W. Reisser, Arch. Microbiol. **125,** 291 (1980).
[14] D. Branton, S. Bullivant, N. B. Gilula, M. J. Karnovsky, H. Moor, K. Mühletahler, D. H. Northcote, L. Packer, B. Satir, P. Satir, V. Speth, L. A. Staehlin, R. L. Steere, and R. S. Weinstein, Science **190,** 54 (1975).
[15] R. D. Allen, Membranes of Ciliates, Ultrastructure, Biochemistry and Fusion, (G. Poste and G. L. Nicolson, eds.), p. 657, Elsevier, North-Holland Biomedical Press 1978.
[16] W. Batz and F. Wunderlich, Arch. Microbiol. **109,** 215 (1976).
[17] Y. Kitajima and G. A. Thompson, J. Cell Biol. **75,** 436 (1977).
[18] W. Reisser, unpublished results.
[19] J. A. Armstrong and P. D'Arcy Hart, J. Exp. Med. **134,** 713 (1971).
[20] P. J. Edelson and Z. A. Cohn, Endocytosis. Regulation of Membrane Interactions, (G. Poste and G. L. Nicolson, eds.), p. 387, Elsevier, North-Holland Biomedical Press 1978.
[21] T. C. Jones and J. G. Hirsch, J. Exp. Med. **136,** 1173 (1972).
[22] Y. Takahashi and I. W. Sherman, Exp. Parasitol. **49,** 233 (1980).
[23] J. A. Armstrong and P. D'Arcy Hart, J. Exp. Med. **142,** 1 (1975).
[24] T. L. O'Brien, J. Exp. Zool. **211,** 343 (1980).