Extraordinary biological membrane structures resulting from different local membrane curvatures

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Summary

The bilayer arrangement of amphiphilic molecules is not only the basic structure of rather flat biological membranes, but also of regularly curved bilayers in most cubic-phase structures. The basis of these cubic-phase structures are infinite periodical minimal surfaces (IPMS). Extraordinary biological membrane structures resembling such IPMS were found as periodically curved bilayers in areas of the plasma membrane in a Streptomyces strain and in liposomes prepared from its extracted lipids. This structure consists of a transition of convex to concave curvatures and vice versa. A structure with curvatures in one direction only was observed in vacuolar membranes of yeast cells with a genetic defect. Our electron microscopical analysis of freeze-fractured membranes of these cells revealed not only fully invaginated but also flat particle-free areas which were mainly circularly shaped, some elongated areas, however, were also present. In addition, sometimes periodical arrangements were detected which obviously are not related to IPMS structures. Both structures, however, indicate a high proportion of wedge-shaped lipid molecules in the bilayer.

Biological membranes consist of a mixture of many constituents, mainly different lipids and different proteins. The amphiphilic molecules of the membrane lipids are arranged in a fluid lamellar phase, thus forming a flat and soft bilayer which is the backbone structure of the normal membrane. Fracturing frozen membranes splits this bilayer since the stabilization by hydrophobic interaction is absent in the frozen state (BRANTON 1966, MEYER and WINKELMANN 1969). Electron microscopy with freeze-fractured specimen is, therefore, especially suitable for the investigation of membranes and other lipid structures. The smooth fracture faces represent the hydrophobic chain ends of the lipid molecules which, in biological membranes, are interspersed with particles representing integral (membrane spanning) proteins.

The amphiphilic molecules in hydrated lipid systems may also adopt liquid-crystalline phase structures (micelles, hexagonal phase structures, cubic-phase structures, sponge-phase structures). All these structures are the result of curvatures: micelles and hexagonal phase structures of the monolayer bend to a spherical or cylindrical geometry, cubic-phase and sponge-phase structures consist of networks of small and regular or larger and irregular bended bilayers. This structural polymorphism is caused by lateral forces (pressure and tension) in different regions of the mono- and bilayer arrangements, according to the "molecular shape concept" (ISRAELACHVILI et al. 1980), where a wedge shape of the lipid molecules by differences in the size between the hydrophilic headgroup and the hydrophobic acyl-chain region results in a spontaneous monolayer curvature (HELFRICH 1973, GRUNER 1985). Curvature is the relief to stress whithin the aggregates and the structure formation is an optimisation between curvature energy of each monolayer and stretching energy of the acyl chains (chain packing energy).

In case of cubic phases the structures are commonly bicontinuous and consist of bilayers arranged in complicated networks topologically equivalent to IPMS = infinite periodic minimal surfaces (LARSSON 1989, LINDBLOM and RILFORS 1989, SEDDON 1990). The basic minimal surfaces (mean curvature = 0) are P-(primitive), D-(diamond), and G-(gyroid) types. The G-type, e.g., is represented by the Ia 3d (Q^{230}) cubic phase. This structure is not rectangularly constructed as the others, but is characterized by angles of 30° and 60° as can be seen from an electronmicroscopical picture of such a freeze-fractured structure (Fig. 1). The bilayer of biological membranes consists of large quantities of lipids which form, after isolation, nonlamellar phases (comonly named H_{II}-lipids). Therefore, both monolayers of the biological membrane bilayer have also the tendency to curve, their curvature, however, is suppressed because both monolayers are hydrophobically coupled. If the deformation stress in both monolayers is complementary, a simple bending lowers the internal stress. If the deformation stress in both halves of the bilayer is counteracted (both curve with the same tendency) an internal frustration is built up (SADOC and CHARVOLIN 1986, SEDDON 1990).

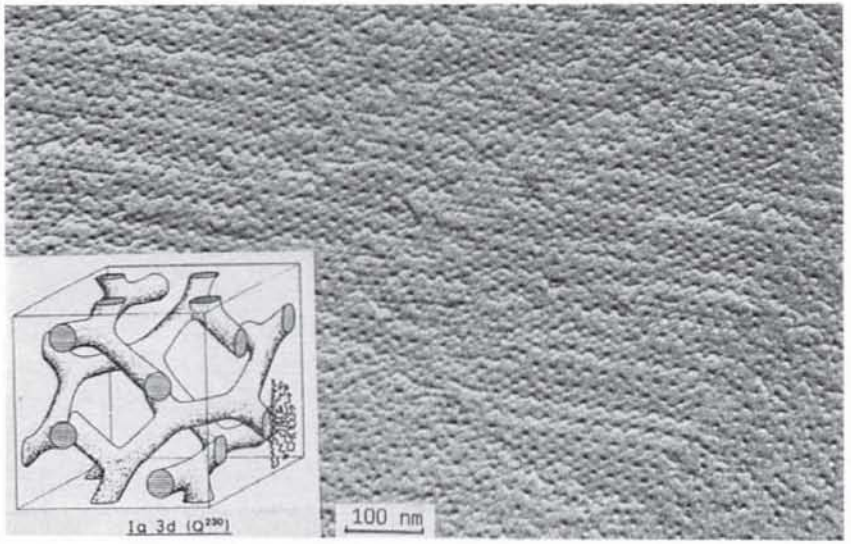

Fig. 1: Freeze-fracture picture of a G-type cubic-phase structure (angles of 30° and 60°) in the system polyoxyethylene-alkyl-ether $C_{12}EO_{10}$ / H_2O / di-butylether (1:1:10) (from investigations in cooperation with Dr. P. MIETHE, Halle). The inset was taken from SEDDON (1990).

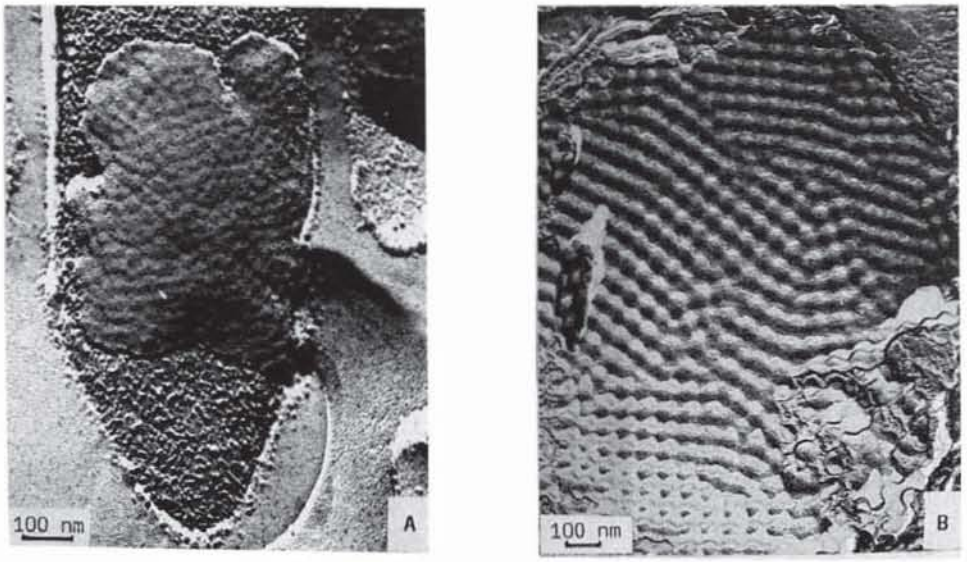

Fig. 2.: Periodically curved bilayer structures in particle- (protein-) free membrane areas of *Streptomyces hygroscopicus*. The repeat distance within the pattern is 30 nm in the hyphal cell (A) and 60 nm in the stable L-form lipid membrane (B).

Normally frustration in biological membranes is reduced by relaxation due to the incorporated membrane proteins. Another and extraordinary way of relaxation is the formation of a periodically curved bilayer structure as was observed in microbial membranes (MEYER et al. 1990). Areas with a regular pattern (Figs. 2a and 2b) are almost particle free, i. e. no integral membrane proteins are present in curved areas. This structure must be attributed to the lipids since the same pattern was detected in liposomes made from the extracted lipids (STERNBERG et al. 1986). The observed structure can be deduced from the Im 3m (Q^{229}) cubic phase structure (on a minimal surface of the P-type, Fig. 3a) by cutting out a bilayer segment and closing the existing holes with bilayer domes (Fig. 3b). The resulting structure resembles an egg tray (Fig. 3c). It is not a real minimal surface since the top of the domes do not fulfil the conditions for IPMS formation, but it is a related structure which also can reduce the frustration within the bilayer.

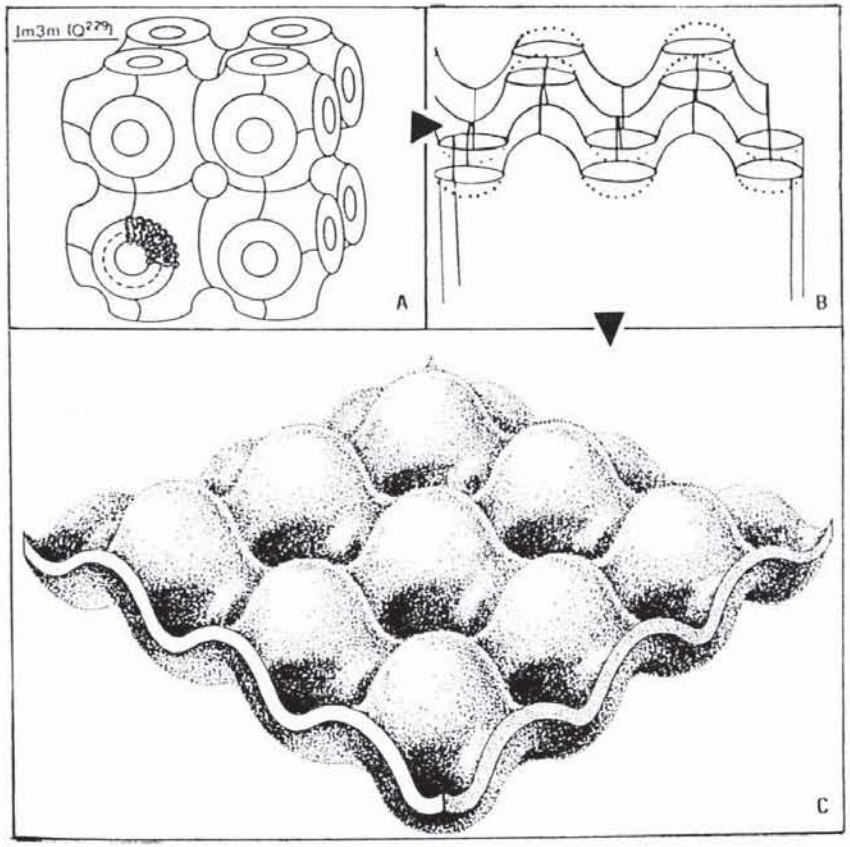

Fig. 3: Interpretation of the periodically curved bilayer pattern as a "two-dimensional cubic phase structure". From the P-type cubic phase (A: taken from SEDDON 1990) a bilayer segment has been cut out (B: was taken from ANDERSON et al. 1988) and the holes were then closed by domes of bilayers (dotted lines). The result is the "egg-tray" structure (C) which is not a true IPMS but a related structure.

The "two-dimensional cubic phase structure" has some pecularities in comparison with the normal three-dimensional cubic phase structures. The size of the unit cell in normal cubic phases is constant for a given system and limited to the range of 20 nm (BRUINSMA 1991). The periodicity in the two-dimensional type is constant in one membrane or one liposome, but repeat distances occur in the range of 30, 45, and 60 nm. It can be estimated that every step of enlargement results in a doubling of the single dome surface. Transition from the 45 to the 60 nm periodicity is visible in Fig. 4. Formation of one greater dome takes place at the expense of two smaller ones. In comparison with the normal three-dimensional cubic phase structure another difference is observed in the degree of curvature. Again, the two-dimensional type is variable but the amplitude is not changed stepwise. Periodicity is either very pronounced (Fig. 2b) or just visible (Fig. 2a). The extremes are exaggerated domes which may bud small vesicles (Fig. 6a) and planar bilayers. The three membranes in Fig. 5 are different with regard to the expression of the periodical curvature pattern. The outer membrane (largest area) is totally planar, the innermost membrane is weakly patterned and the membrane in between reveals a significant pattern. The existing frustration in all three membranes becomes visible by structures in their contact regions. A region in the innermost membrane is strongly deformed, possibly representing a kind of vesicle formation. At the contact region the planar outer membrane also shows an alteration by formation of a line structure in direction to hexagons. Therefore, a mechanism of vesicle formation by two stressed membranes exists, where the vesicles have first the form of hexagonal cushions (Figs. 6b and 6c).

Examples for regular periodic structures in biological membranes are rarely found. We have, therefore, concentrated on the vacuolar membrane of a yeast mutant. Investigating the secretory mutant Sec-1 of Saccharomyces cerevisiae, NEČAS and SVOBODA (1986) found regular periodic structures which are somewhat similar to the described "two-dimensional cubic phase structures". Their curvature, however, is unidirectionally concave in a hexagonal arrangement (Fig. 7a). For our electron microscopical analysis we have used the same mutant strain (a gift from Prof. NEČAS, Brno), the same conditions for cultivation at the permissive temperature (24 or 28°C), and induction of regular periodic structures by transfer to the restrictive temperature of 37°C for 2 hours. In addition, we have made some variations in the experimental conditions without remarkable alterations in the resulting effects.

In control cells (cultivation at the permissive temperature) invaginated vacuolar membranes have rarely been found. Sometimes a convex budding of the vacuolar membrane was present and in contrast to the particle-free invaginations these evaginations have the same dotted surface as the surrounding membrane (Fig. 7b). The formation of a protein- (particle-) free lipid domain is a prerequisite for the concave curvature (Fig. 8), but a regular distribution of these invaginated domains is not the rule. The observed regular arrangement of flat circular lipid domains is obviously not connected with the local curvatures but is an effect of the domain formation by separating membrane lipids (Fig. 9a).

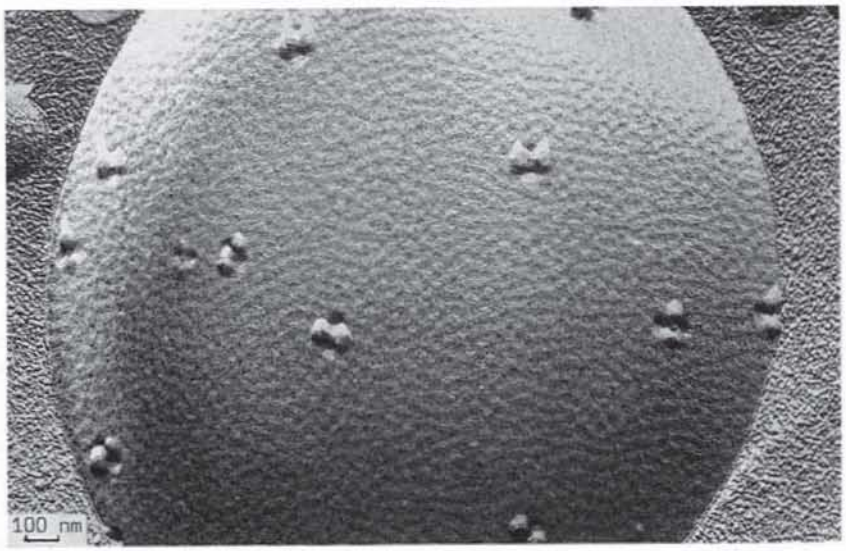

Fig. 4: Lipid membrane from a stable L-form culture of *Streptomyces hygroscopicus*. In some spots the 45 nm-pattern has changed into the 60 nm-pattern. Every such spot consists of two convex and two concave domes.

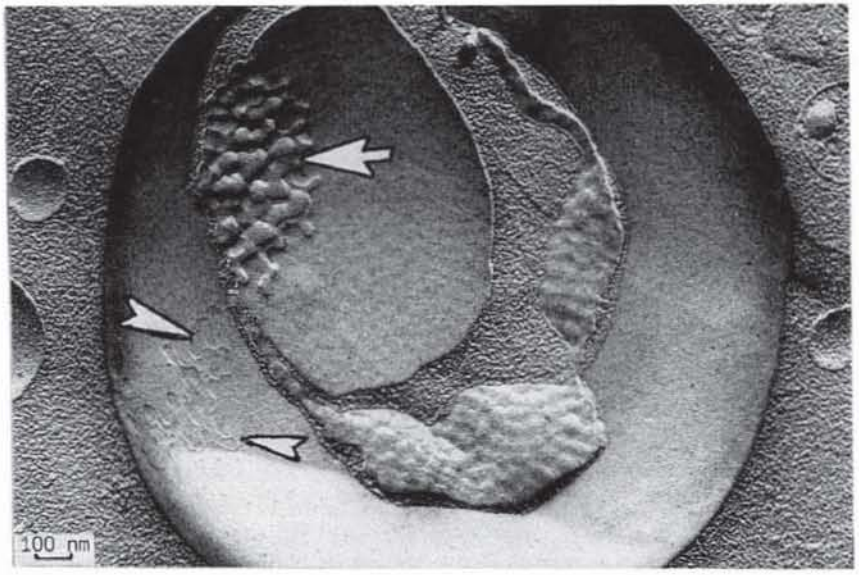

Fig. 5: Three lipid membranes from a stable L-form culture of *Streptomyces hygroscopicus* with different expression of the curvature pattern (in the outermost membrane not perceptible). The regions where the membranes are in close contact are deformed: at higher curvature strong deformation with curling of the size of small vesicles (arrow) occurs and in the flat membrane incomplete hexagons (arrowheads) are formed.

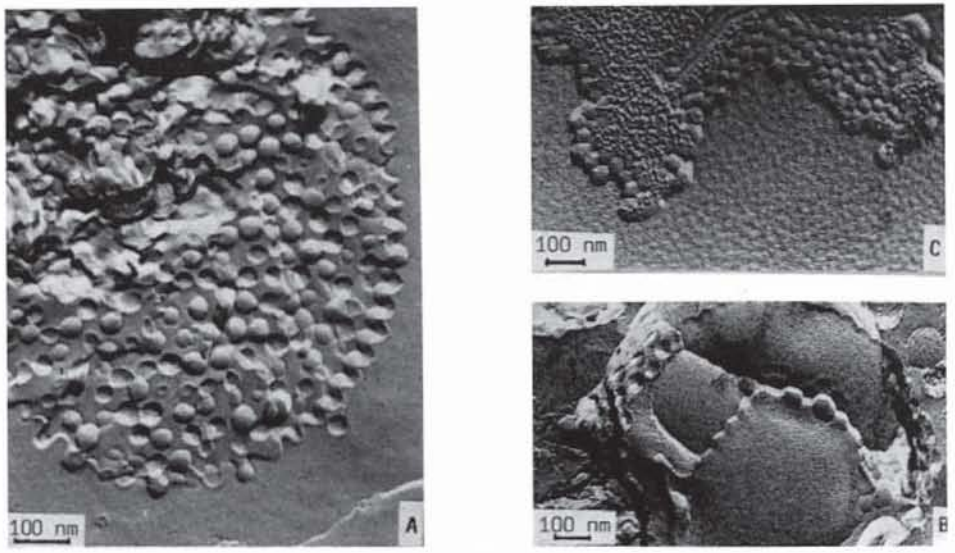

Fig. 6: Two possible mechanisms for formation of small vesicles by lipid membranes from *Streptomyces hygroscopicus*. A: The exaggerated curvature pattern (here in liposomes made from the phospholipid fraction prepared in acidic medium) may lead to small vesicles formed by a budding process. B and C: Between two contacting frustrated membranes small vesicles have primarily the form of hexagonal cushions which are hexagonally arranged. (Picture A from STERNBERG et al. 1987)

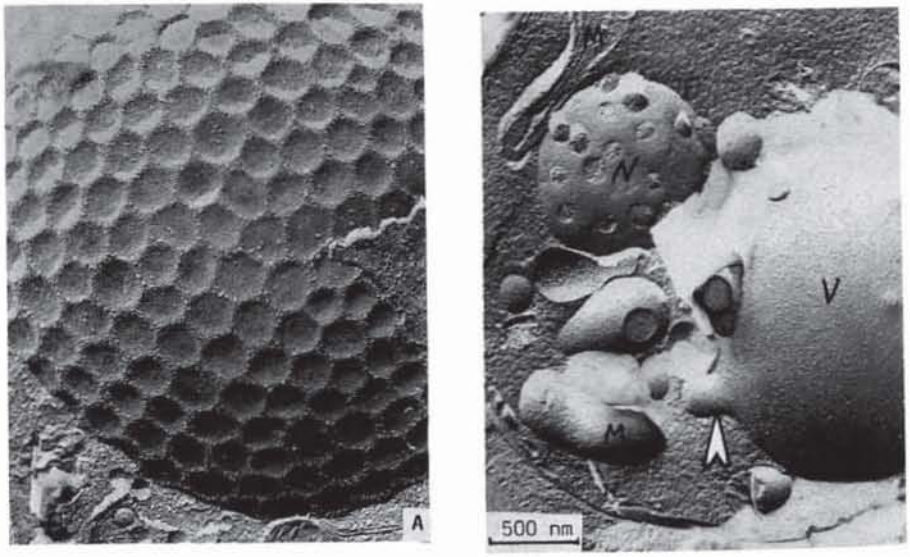

Fig. 7.: Yeast Sec-1, a secretory mutant of *Saccharomyces cerevisiae*. A: regular pattern of circular invaginations of the vacuolar membrane when incubated at the restrictive temperature (37°C for 1 h). B: Incubation at the permissive temperature (24°C) results in vacuolar membranes with normal appearance. Formation of a convex bud (arrowhead) is an exception. V = vacuole, N = nucleus, M = mitochondria.

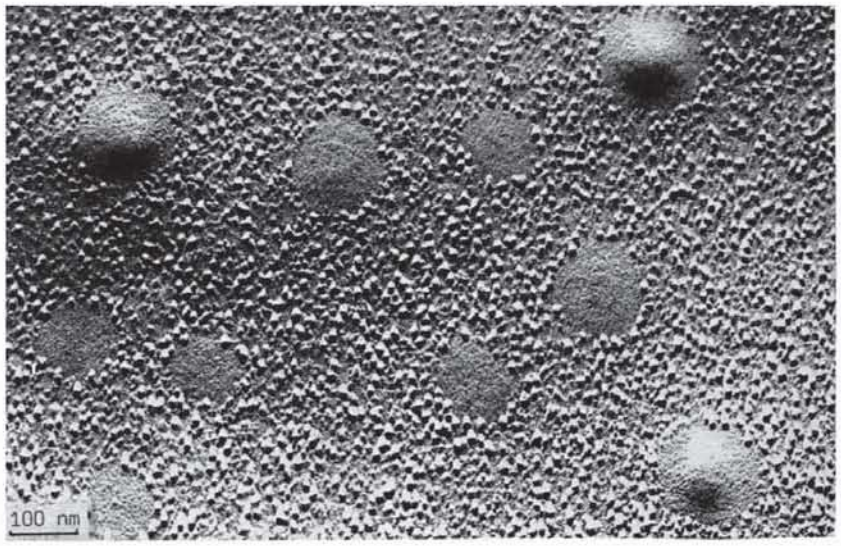

Fig. 8: Vacuolar membrane of yeast Sec-1, incubated 1 h at 37°C, with circular particle- (protein-) free lipid domains. No regular arrangement can be seen, few domains have started with invagination.

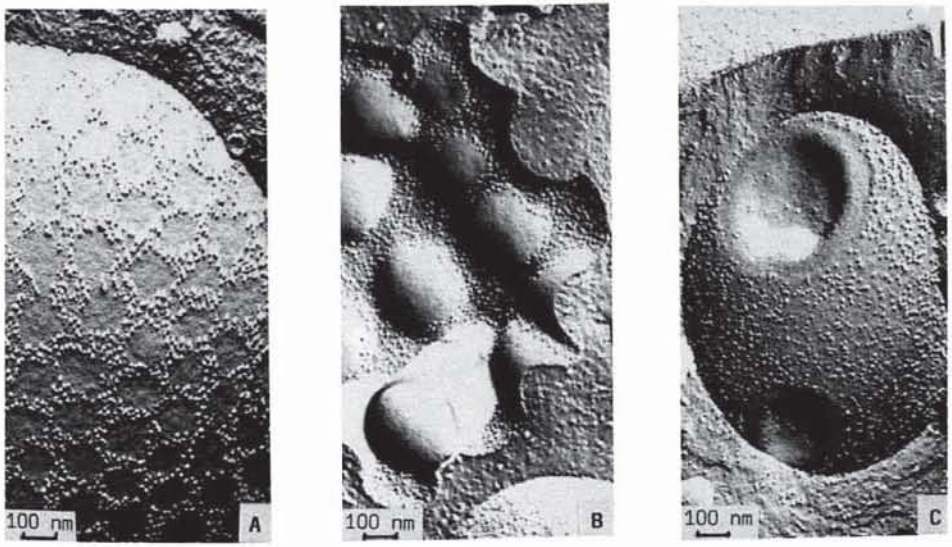

Fig. 9: Vacuolar membranes of yeast Sec-1, incubated 1 h at 37°C, with different structures formed by circular lipid domains. A: Regular hexagonal pattern of small planar lipid domains. B: Highly curved domains without a regular distribution. C: Two very large invaginations.

The reason for the homogeneous size of the single domains, leading to the regular appearance after tight packing, is unknown. The size, however, can differ considerably in different vacuoles (Figs. 9b and 9c). Additionally, not only circular but also elongated forms of domains and invaginations are present (Fig. 10). Sometimes circular and elongated forms co-exist in the same membrane (Figs. 10b and 10c), an effect which may be explained by small differences in the lipid composition of the domains. The diversity of formations is illustrated by regions, where the enlargement of the lipid domains have condensed the particle-bearing areas to small stripes (Fig. 11a). An angular form of invagination can be formed between this framework of stripes (Fig. 11b).

The concave curvature of the domains is probably induced by an asymmetrical distribution of the lipid species in the two monolayers of the membrane. Lipid asymmetry is the rule for biological membranes (DEVAUX 1991), where the resulting frustration will partially be diminished by integral membrane proteins. In case of protein-free lipid domains, however, the internal stress is reduced by curvatures. An invagination could be caused by a pressure difference between cytoplasma and vacuole which, however, is not in accordance with our observations. The spherical form of the vacuoles and the occurence of evaginations (Fig. 7b) rather indicate the same or a slightly higher pressure in vacuoles. Furthermore, biological membranes are soft structures and, therefore, the simultaneous occurence of planar and invaginated domains on the same membrane (Figs. 8 and 10c) excludes a pressure difference as the reason for the observed membrane invaginations.

The "two-dimensional cubic phase" with a convex-concave curved bilayer is a result of the membrane's lipid composition only. The (periodically) concave curved bilayer of the yeast vacuolar membrane, however, needs a framework for its construction. This framework is given by lipids in an eutectic state. In biological membranes integral as well as peripheral membrane proteins are aggregated in these disordered regions (Fig. 9a and 11). Proteins, however, are no prerequisite for the formation of these structures since the same concave curved bilayers are also found in protein free liposomes made of a 1:1 mixture of cholesterol and an amphiphilic polymer (MEYER 1990).

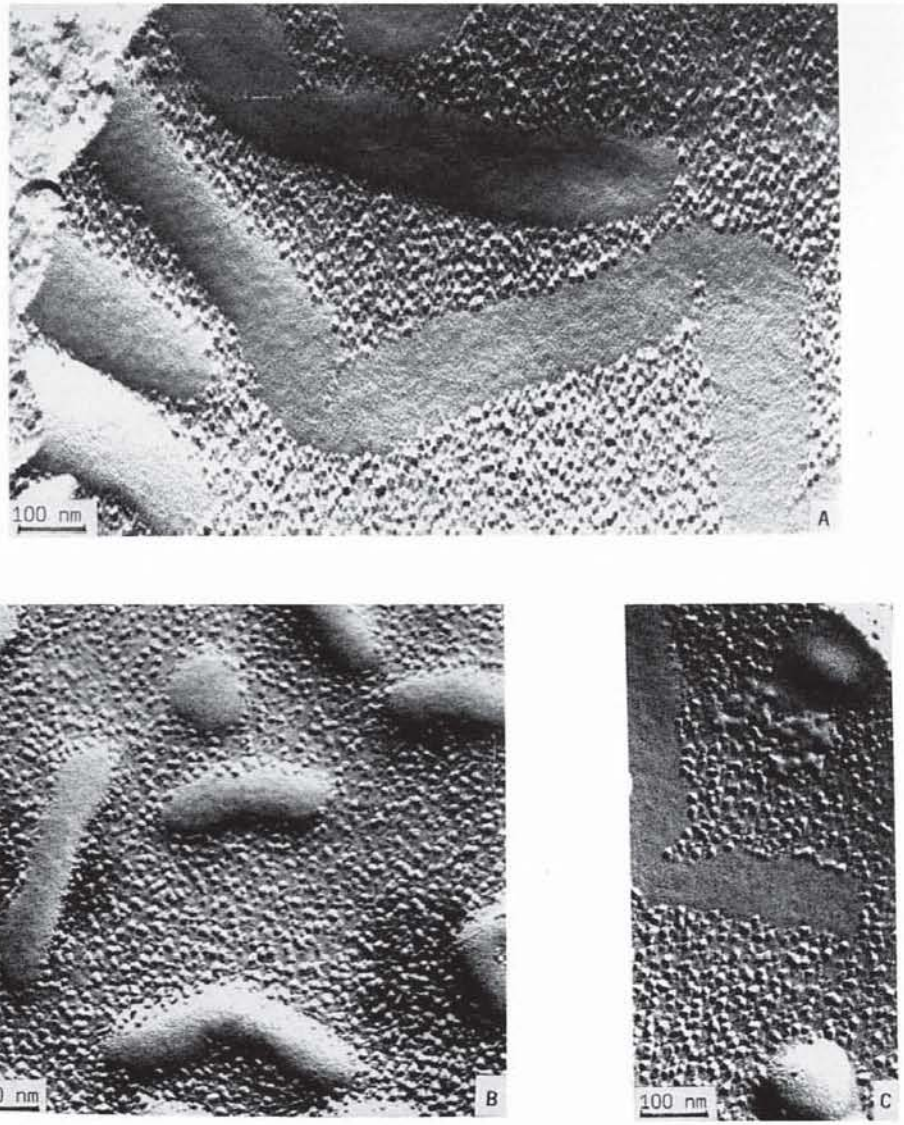

Fig. 10: Vacuolar membrane of yeast Sec-1, incubated 1 h at 37°C, with elongated lipid domains. A: Complex structure of elongated domains, mostly planar, sometimes (on the left side of the picture) partially invaginated. B: Well invaginated elongated domains, one domain is circular. C: Planar elongated and invaginated circular domains on the same membrane.

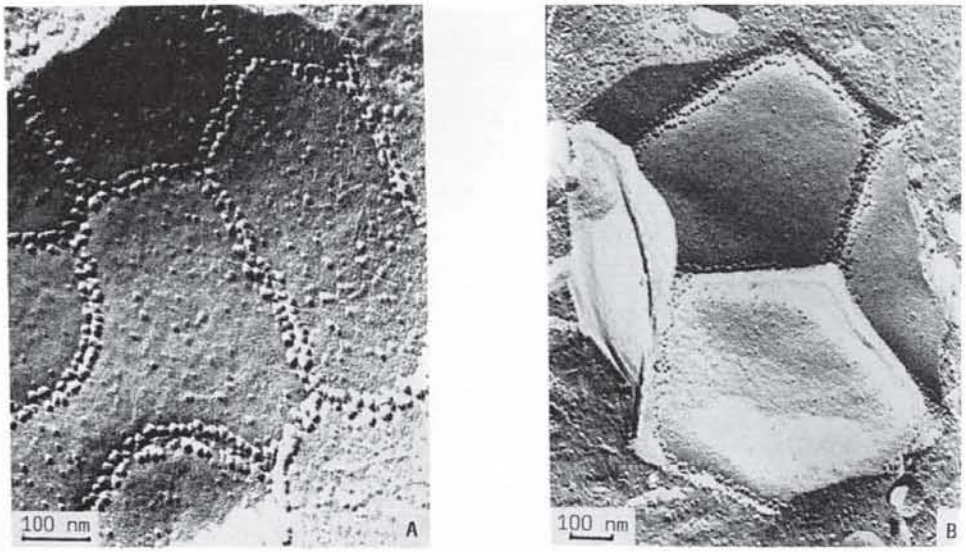

Fig. 11: Vacuolar membrane of yeast Sec-1, incubated 1 h at 37°C. A: Membrane proteins are aggregated in small stripes between large domains and B: outline the framework when the domains invaginate.

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