

Structure Defining Functions of Cell Membrane Morphologies with Cubic Symmetry

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Cell membranes, particularly the smooth endoplasmic reticulum (SER), have long been recognized to form highly interconnected and convoluted morphologies. The true 3-D structure is, however, yet to be fully understood, and most models describe the SER as an irregular arrangement of tubules. In addition to this traditional model of the SER there are numerous reports dealing with various, hitherto puzzling, membrane morphologies such as the "paracrystalline", "undulating", "honeycomb", "cotte de maile" to mention a few forms of the SER, as well as of other cell membranes. These 3-D membrane morphologies were recently shown to represent the same basic morphology and to be exactly described by the so called periodic cubic structures (PCS) (Landh, in press) well known to mathematicians and physicists in the form of periodic minimal and/or nodal surfaces (Nitché 1990, Dierkes et al. 1992, Schnering and Nesper 1991, Andersson et al. 1995). These membranes were hence named Cubic Membranes (CM), of which, to date, three fundamental forms have been identified (Fig. 1). These intricate continuously folded membranes are, among other things, optimal space partitioners in which capacity they divide space into at least two physically distinct but highly intertwined subspaces. Thus CM's in a closed form are in keeping with the well known barrier function of cell membranes. Most intriguingly, CM's occur in conjunction with numerous cell types, from all kingdoms, and in virtually any cellular membrane, including the SER, inner nuclear membrane (INE), plasma membranes, inner mitochondrial membrane, and Golgi apparatus (Landh, in press). Altogether we have to date cataloged some one thousand published and unpublished transmission TEM electron microscopy micrographs of both normal and pathological tissues in which CM's can be identified

(representative examples are tabulated in ref 1.)

In projection, like those produced in TEM experiments, the patterns of which profiles of CM's often show, due both to their symmetry and the fact that they are necessarily continuous membranes, an extraordinary complexity. While this

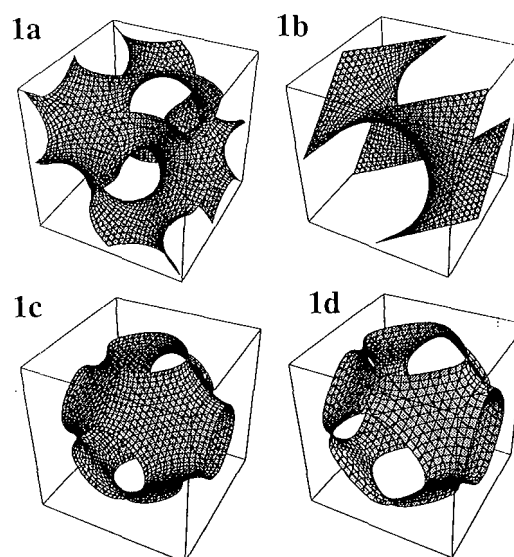


Fig. 1. The three fundamental cubic membranes represented as periodic nodal surfaces with zero potential (a-c). One unit cell is shown. The surfaces describes the mid-membrane surface (see Deng and Landh elsewhere in this volume). (a) The G-PCS with spacegroup $I4_132$. Since each side of the membrane must be, at least, chemically different the space group of the CM's is that of the subgroup of type 2 as compared to the congruent case. Thus the structure of the CM's are conveniently represented by differently colored sides of the membrane. (b) The D-PCS with spacegroup $Fd3m$. (c) The P-PCS with spacegroup $Pm3m$. (d) Example of a periodic equipotential (nonzero) P-surface in which the subspace volume relation is approximately 1:3.

has, on the one hand, made them perplexing and puzzling to researchers for 35 years, it is, on the other, what makes them uniquely identifiable. This can, however, on the basis of information theory, only be realized once we know what we are looking at. CM's thus seems to stand alone since it is the only conserved 3-D continuous cell membrane morphology that can be close to exactly determined and usually easily recognized. Through the knowledge of the base-structure; i.e., its crystallography and the subspace volume relation (potential); of these PCS's we have established a "library" of computer generated projections simulating the appearance of CM's in TEM micrographs. These computer generated theoretical projections are then matched to experimental projections through a direct template-correlative method (Landh in press, Mieczkowski and Landh in press). With this method, exemplified in Figures 2-4, the three fundamental families of PCS's have been identified: the gyroid (G) of Schoen (Schoen 1990) (Figs. 1a and 2), Schwarz' double diamond (D) (Figs. 1b and 3) and primitive (P) (Figs. 1c and 4) surfaces (Schwarz 1890). In addition,

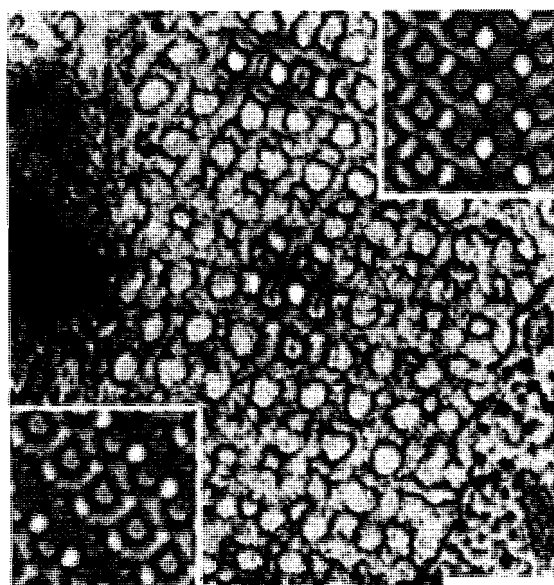


Fig. 2. Example of a G-based CM identified in cultured skeletal muscle cells of thick embryos in which the t-tubule invaginate to form the G-GM. Two areas is matched corresponding to projections of a G-PCS along the [320] (upper) and [331] directions with close to zero potential (minimal surface area) and with a sample thickness of about half a unit cell. The unit cell size is approximately 100 nm. The insets shows projections generated from 5 unit cells. Bar, 200 nm, Adopted from reference 1 and 7 (Whitford & Landh).

we have from biology learned about a new type of PCS's form. Namely their multicon-tinuous forms which are based on multiple parallel membranes in which each membrane is described by a PCS with a particular potential. Thus these morphologies separate space into $n+1$ spaces, where n is the number of membranes in the structure. The multicontinuous surfaces seem to be as widespread in nature as the single PCS's, and all three of the fundamental PCS's above have been found to form them (see Fig. 4 for an example).

The formation of CM's is believed to take place through a intersection-free membrane folding process schematically shown in Fig. 5 (Landh, in press). However, the driving force for this is currently unknown, as is the topology of the initial and final states of the membrane(s). Several additional intriguing questions such as: What governs the selection of a particular CM type? Why are these more than two spaces in conjunc-

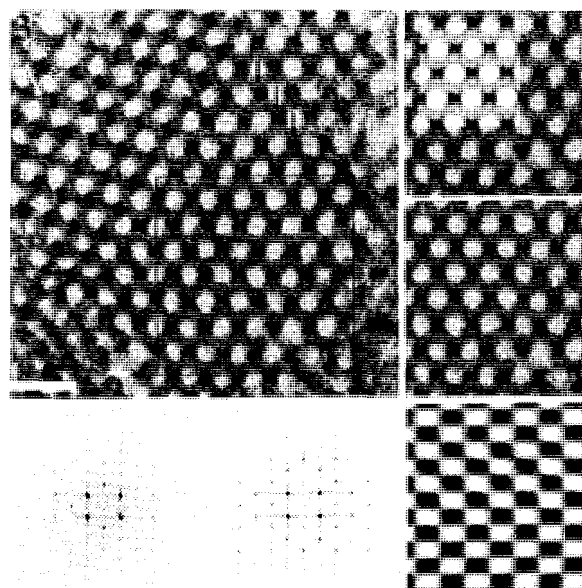


Fig. 3. Example of a D-based CM of the PLB in etiolated leaves of squash. The TEM projection corre[ond] to the projected electron density of the D-PCS along the [110] direction. The computer generated projection is matched to the micrograph before (upper right) and after contrast adjustment and introduction of random noise (mid-right). The power spectrum (calculated on the boxed area in the micrograph (upper left) of the TEM (lower left) and that of the computer computer generated projection (lower center) conrrespond very well as is seen in their cross-correlation (lower right). The specimen thickness is approximately a quarter of a unit cell. The PLB D-PCS has a constant potential corresponding to a subspace volume relation of about 1:4. Bar, 100 nm. Adopted after reference 3.

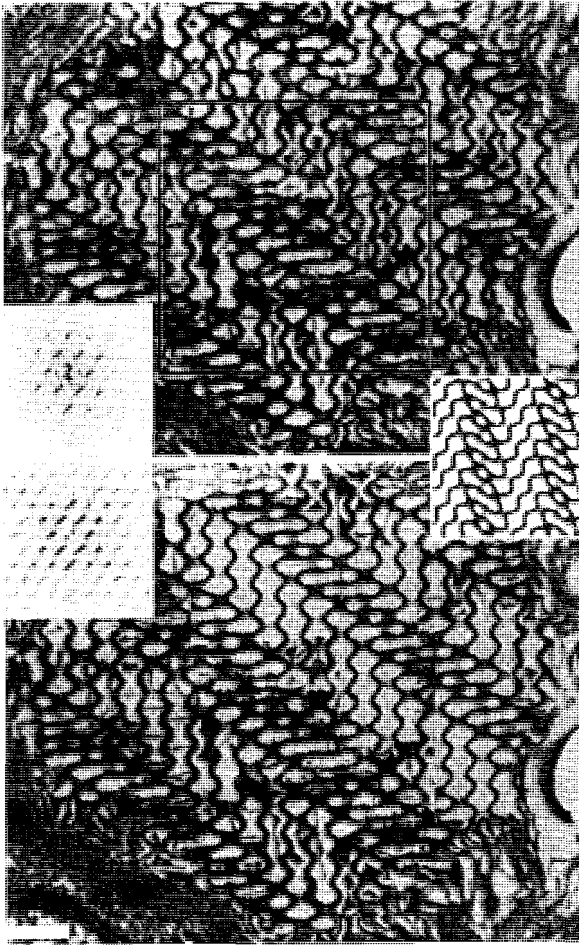


Fig. 4. Example of a P-based multimembranous CM identified in chloroplasts of the green alga *Zygnema*. This complex projected membrane pattern corresponds to the projection of ten individual membranes, hence the large thickness. Its symmetry and pattern can, however, be simulated by a single PCS. The projection can be identified as the [751] projection of P-PCS as is shown in the matched areas in the lower micrograph (note that there are several areas matched), and the specimen thickness corresponds to approximately a quarter of a unit cell which is about 300 nm. The power spectrum (upper) calculated on the boxed area of the micrograph corresponds well to the theoretical (lower). The computer generated projection inset shows 12 unit cells. Bar, 150 nm. Adopted after reference 1.

tion with the multicontinuous CM's, particularly in the ER and mitochondria? How are these physically distinct spaces formed and maintained? etc. are currently being considered/investigated.

The identification of a crystallographically defined 3-D membrane morphology allows for the application of structure-functional relationships. In particular, we have identified several types of

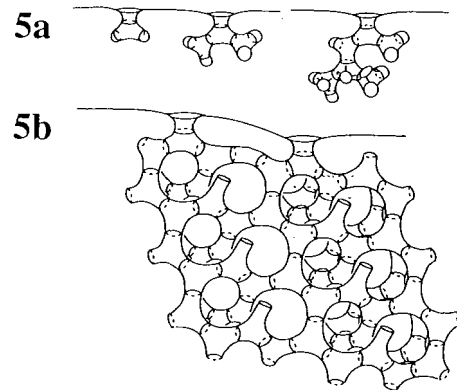


Fig. 5. Schematics of the membrane folding process. (a) The first stages of the development of a cubic membrane with global spherical topology, but with a local topology defined by the specific cubic structure. To the right is shown a tentative model for the continuous cubic-to-cubic (G-to D-PCS) membrane transformation which also is intersection-free. (b) A G-CM which is continuous with two pores. It is thus topologically identical to the torus assuming that its ends are spherically connected. Note that the ends of the gyroid cubic membrane has intentionally been kept open to the interior of the cell, linking the outside with the inside, to show the large number of pores created relative to the number of unit cells. Adopted after reference 1.

CM's which are structurally invariant with respect to species. An example is the prolamellar body (PLB) formed during development or in etiolated states of chloroplast membranes in the leaves of higher plants. Its structure belongs to one of the most studied 3-D membrane morphologies and it has been suggested to exhibit various cubic symmetries by several contributors (Gunning and Seer 1975, Wehrmeyer 1965, Murakami et al 1985, Larsson 1980, Lindstedt and Iijerberg 1990). We have recently shown that the PLB is invariantly described by a D-PCS with a rather constant potential (subspace volume relation) and lattice size (Mieczkowski and Landh). This leads to the suggestion that this particular structure might be selected to fulfill a specific purpose based on the physical properties of the structure and its crystallographic space group. In late 1992 I became aware of the striking similarity between the structure of the PLB and certain experimentally produced dielectrics with cubic symmetry which acts as wave blockers through the existence of band gaps. Thus it was suggested (see e.g. *Science News* 145, 266-268, 1994) that the D-PCS-based morphology of the PLB was selected due to its efficiency in "capturing" certain photons with the wavelength necessary for the conversion of

protochlorophyll to chlorophyll. Even though this theory remains to be experimentally proven, it introduces the concept of crystallographically determined structure-functional aspects of cell membranes which is a new functional attribute of cell membranes. Similar concepts are currently being explored/applied to several other membrane systems in our laboratory (Landh and Whitford, Landh and Deng).

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